Estuaries and Coasts
DOI 10.1007/s12237-015-9999-2

Changes in Coastal Benthic Algae Succession Trajectories and Assemblages Under Contrasting Nutrient and Grazer Loads

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Received: 17 February 2015 / Revised: 22 May 2015 / Accepted: 27 May 2015

Abstract Eutrophication plays a crucial role in coastal systems, driving changes in the composition and abundance of flora and fauna with consequent effects for the entire ecosystem. Sensitive to nutrient levels, micro- and macroalgal blooms serve as valuable indicators of eutrophication. The San Antonio Bay (Northern Argentinean Patagonia, 40° 43′ S, 64° 56′ W) provides an appropriate system to study in situ eutrophication processes on coastal communities. In a multiscale approach, using two different kind of settlement substrates (micro: polyethylene terephthalate, and macro: ceramic), the present study followed benthic algal dynamics over one year, distinguishing changes in natural succession and seasonality. Strong differences were found in the biofilm assemblages after three days, marked by tube dwelling diatoms and *Cocconeis* spp. under high nutrient-grazer conditions and needle like diatoms (e.g. *Nitzschia* spp., *Tabularia* spp.) under lower nutrient-grazer loads. The succession continued by the colonization of macroalgae, with a higher recruitment rate in the nutrient and grazer rich environment with a concomitant higher diversity. Our results show that under higher nutrient-grazer conditions natural benthic succession not only differs in trajectory but in its final taxa composition promoting higher biodiversity and biomass accumulation. In addition, taxa specific substrate preferences interfere with the observed eutrophication pattern, suggesting substrate dependant interrelations between the bloom forming taxa. These findings provide evidence that nutrient enrichment can not only affect an established assemblage but also affect the early succession stages, changing the succession trajectory and thus the final assemblage.

Keywords Nutrients · Grazers · Epibenthos · Algae · Succession · Intertidal

Communicated by James L. Pinckney

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Published online: 13 June 2015

Introduction

Human activities often contribute to increased nutrient loads in coastal waters, promoting elevated growth of primary producers, leading to eutrophication close to populated or agricultural areas (e.g. Cloern 2001; Valiela et al. 1997). The process of eutrophication typically starts with a pulse of nutrients, followed by a bloom of primary producers such as phytoplankton and benthic algae (Nixon 1995), which modify the entire coastal ecosystem (Duarte 1995). Benthic algae react to variations in nutrient levels (Teichberg et al. 2008) by changing their composition and abundance (Worm et al. 1999). Furthermore, nutrients have a direct effect on algal tissue composition, changing C/N or N/P ratios, affecting higher trophic levels and altering top-down and bottom-up interactions (Burkepile and Hay 2006).

To understand benthic dynamics within coastal ecosystems, it is essential to investigate the development of benthic algal assemblages under different environmental scenarios. Generally, the succession of marine benthic communities starts within hours, with the formation of a biofilm (e.g. bacteria, fungi, diatoms, Cuba and Blake 1983; O'Toole et al. 2000), which provides the base for the settlement of early forms (e.g. filamentous algae and cyanobacteria, within days), and it is followed by the colonization of complex organisms (e.g. fleshy macroalgae; Aleem 1957; Fricke et al. 2008, 2011b). In particular, early settlers play a crucial role as they settle under certain environmental conditions (Callow et al. 2002) and either facilitate or inhibit the settlement of later species (e.g. Raimondi 1988). Biofilm and early successional forms have been observed to respond rapidly to increases in nutrients (Littler 1980). Thus, a change in nutrient concentrations during early succession can potentially structure the whole assemblage (Littler et al. 2010), and consequently alter its functional role within the benthic ecosystem (Osterling and Pihl 2001; Valiela et al. 1997).

The Argentinean Patagonian coast is composed of a mosaic of several pristine sites mixed with few places subject to locally increased anthropogenic nitrogen (N) loading (Martinetto et al. 2011; Piriz et al. 2003). The San Antonio Bay (40° 43′ S, 64° 56′ W, Argentina) is a good example of recent changes related to increases in human population along the coastline. This system shows high nutrient concentrations similar to those found in highly eutrophic sites, such as the central basin of the Italian Venice lagoon (Teichberg et al. 2010). The bay experiences a large daily water movement, as a consequence of the macrotidal (up to 9 m) semidiurnal regime present in the area. This considerable water flushing partially relieves the land-derived N loads, as well as the accumulation of biological products (Martinetto et al. 2010, 2011). Despite the large water movement, nutrient concentration pulses during low tide remain in the system long enough to support high biomass and diversity of macroalgae near the town of San Antonio Oeste (Martinetto et al. 2010). Moreover, the growth velocity of *Ulva lactuca* in this area is 20-25 % d^{-1}, which is 2 to 5-fold faster than in other eutrophic sites such as Mondego River estuary in Portugal, Venice Lagoon, and Urias estuary in Mexico (Teichberg et al. 2010). The large water movement also prevents some negative effects associated with eutrophication such as anoxia. In this case, the large macroalgae biomass supports high densities of herbivores by increasing food availability and nutritional content rather than negatively affecting the survival of organisms (Martinetto et al. 2010, 2011). In fact, San Antonio Bay is inhabited by a large abundance of consumers associated with areas where macroalgae blooms are common (Iribarne et al. 2003; Martinetto et al. 2010). There, herbivores can reduce macroalgae biomass up to 60 % (Martinetto et al. 2011), which is quite high compared to other eutrophic sites (e.g., Lotze and Worm 2000). Thus, it is possible that under these extreme conditions of high nutrient levels, large water movement and high levels of herbivory, benthic succession could differ from what has been reported in other eutrophic sites.

In the present study, we investigated benthic succession in two tidal channels with contrasting nutrient and grazer loads at San Antonio Bay over a one year period. Using the same arrangement of different settlement substrates, we evaluated differences between tidal channels in terms of 1) the identity of early settlers, 2) the colonization process of micro- and macroalgal assemblages through time and, 3) the final benthic algae assemblage.

Material and Methods

Study Area

Field work was conducted in two tidal channels of San Antonio Bay (Fig. 1). These channels experience contrasting nutrient and grazer loads (Table 1) and have been used in previous studies to evaluate the effects of eutrophication (Martinetto et al. 2010, 2011; Teichberg et al. 2010). One channel runs nearby and along the town of San Antonio Oeste (hereafter SAO channel) and is characterized by high land-derived nutrient loads that consequently support frequent algal blooms (Table 1, Martinetto et al. 2010, 2011). In contrast, the second channel runs parallel to the SAO channel but is distant from human population (hereafter CONTROL channel) and shows much lower nutrient loads with algal blooms never reported (Table 1, Martinetto et al. 2010). Along with the higher nutrient loads, the SAO channel presents higher invertebrate abundance than the control channel (Martinetto et al. 2010, 2011). The large abundance of herbivorous, such as amphipods, snails, chitons and limpets exert a strong top-down pressure in the SAO channel (Martinetto et al. 2011).

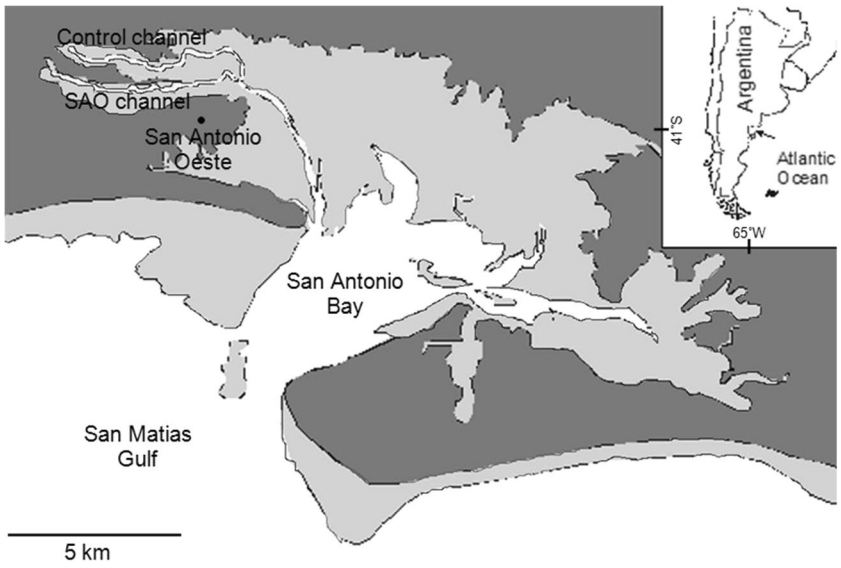

Fig. 1 *Map of San Antonio Bay* showing the flooded area during high tide in light grey and the underwater area during low tide in white. The SAO channel passes through the town of San Antonio Oeste while the CONTROL channel is situated farther away from human activities

Sampling design using contrasting natural conditions, although extensively used in ecological studies (e.g., Geertz-Hansen et al. 1993; Hauxwell et al. 2003, 2006; Martinetto et al. 2010; McClelland and Valiela 1998), results imperfect to draw comparisons given the lack of natural replications of treatment and control. However, by choosing this design we did a compromise between the limitations on the inference to other systems and the benefit of working under natural realistic conditions.

Both channels are subjected to similar variations in temperature and salinity (Martinetto et al. 2011). The substrate in the two channels consists of cobbles, pebbles and shells of mussels and snails where sessile invertebrates, diatoms and macroalgae grow attached. The benthic species composition differed strongly between the CONTROL and SAO channels (Martinetto et al. 2010). At the beginning of the study, the CONTROL channel was covered by few macroalgae attached to pebbles buried in the sediment; *Polysiphonia* was the most common macroalgal genus. The SAO channel had a high abundance of Ulvales, forming a standing bloom (see Martinetto et al. 2010).

Water Motion

In order to investigate potential differences in water motion between the different sites, clod cards (2.5×4×1.5 cm) made of plaster of Paris (Doty 1971), were exposed for three days in both channels (n=3 at each), and in an aquarium (n=2) filled with ambient seawater, which served as control treatment. Relative difference in flow rates (C) between the different channels were calculated by C=*te*/*me*, where *te* refers to the measured weight loss of the clod cards deployed in the

Table 1 Macroalgal biomass and diversity, *Ulva lactuca* N isotopic signature, nutrient concentrations, and herbivore (chitons and limpets) abundances found in the two tidal channels (SAO and CONTROL) at San Antonio Bay. Data showing mean±SE reported in the literature (*: Martinetto et al. 2010; †: Martinetto et al 2011) and from this study ([a]). H': Shannon diversity index, LT: low tide, HT: high tide

	SAO	CONTROL
*Macroalgal biomass (g m^{-2})	125.333±1.623	62.515±1.009
*Macroalgal diversity (H')	0.48	0.22
**Ulva lactuca* $\delta^{15}N$ signature (‰)	15.4±1.8	5.2±0.7
DIN(μM)	98.8±16.0 (LT)[a]	37.8±9.4 (LT)[a]
	88.6±6.2 (LT)†	24.9±5.6 (LT)†
	34.9±1.5 (HT)†	
DIP(μM)	6.9±2.0[a]	1.6±0.2[a]
	14.8±5.0 (LT)†	2.6±0.4 (LT)†
	2.4±0.4 (HT)†	
†pH	8.989±0.049 (LT) 9.186±0.012 (HT)	8.620±0.087 (LT)
†O_2 (mg l^{-1})	5.843±0.471 (LT) 12.847±0.757 (HT)	6.8±1.657 (LT)
†Salinity (ppt)	37.080±0.778	37.267±0.895
†Herbivore abundance (ind m^{-2})	22.930±5.082	0.530±0.363

channels and *me* refers to the measured weight loss of the aquarium control treatment.

Nutrient Analyses

To investigate differences in nutrient composition between the two channels during the study period, samples were taken for nutrient analyses at four days (16, 19, 23 and 26) in March 2013 (autumn season) during the experimental run. At each sampling day three replicates were collected from each channel at ~10 cm below low tide level, using a sterile (60 ml) syringe, extended with a plastic tube (1.5 m long). Water samples were filtrated (Whatmann GF/F) right after taken and kept frozen (below 4 °C) in 50 ml PE bottles for later standard colorimetric measurements (Kattner 1999). Total DIN and DIP values were determined for 24 and 23 samples respectively (one DIP replicate from SAO was lost).

Settlement Substrata and Colonization Set-Up

To evaluate algal succession under contrasting levels of eutrophication, artificial substrates were set in the SAO and CONTROL channels (21 October 2012). Since substrate characteristics play a crucial role in benthic community development and often vary with the environment, artificial settlement units (SU) are commonly used in benthic ecology for the direct comparison of different sites (Fricke et al. 2011b; Wahl et al. 2004). To study the benthic communities at different scales, we generated a bivalent substrate composed of two different materials allowing the investigation of micro- and macrophytobenthos at the same time. Based on our experience with prior settlement studies (Fricke et al. 2008, 2011a), we constructed SU using unglazed ceramic tiles (3×6 cm) as macrosubstrate with a piece (1.5×2 cm) of polyethylene terephthalate (PET, Melinex ®) attached as microsubstrate. Thus, each SU in our study consisted of two different substrates. At each channel a total of 20 SU were exposed horizontally to the water surface~30 cm below low tide water level and 20 cm above sediment.

Sampling of Benthic Assemblages

In order to investigate successional differences under contrasting eutrophic conditions, we analyzed stepwise succession by conducting paired samplings in the two channels. The SU (n= 4 per time and channel) were destructively sampled after 4 (S1: October 25, 2012), 8 (S2: October 29, 2012), 11 (S3: November 1st, 2012), 40 (S4: November 29, 2012) and 357 days (one year, S5: September 19, 2013). The first three samplings (S1, S2, S3, hereafter early succession) were analyzed only on microsubstrates, while the last samplings (S4 and S5, hereafter late succession) were analyzed on both micro- and macrosubstrates. For S3, two microsubstrates were lost for the SAO channel during sampling, but the remaining replicates were included in the analyses.

Micro- and macrosubstrates were separated and treated independently for different analyses. Entire macrosubstrates were fixed in 4 % formaldehyde, while microsubstrates were divided in two pieces: one piece was fixed as a semi-permanent slide (SLIDE) using 50 % Karo® corn-syrup and preserved with 4 % formaldehyde, and the other piece (SCAN) was fixed using 2.5 % glutaraldehyde in 0.05 M cacodilate buffer for later scanning electron microscopic analysis. The different substrates were scanned by different magnifications to investigate algae of different size classes. To investigate macroalgal growth, macrosubstrates were analyzed using a stereomicroscope (Nikon SMZ 1500, 1-3X magnification), while subsamples of microsubstrates (SLIDES) were examined under regular microscope (Nikon Eclipse 80i, 4X-100* magnification) to study smaller macroalgal stages (hereafter "mesoalgae"). Both microscopes were equipped with an ocular grid (100 divisions) to allow quantitative measurement of individual taxa cover within each visual field (VF). In addition, subsamples of microsubstrates (SCANs) were investigated with a scanning electron microscope.

Early Succession

To determine changes in total cover, each early succession SLIDE (S1 to S3) from both channels was scanned for 25 visual fields (VFs) (20X, 8.5 mm^2). To quantify differences in the biofilm assemblage, we distinguished between diatoms, colored cell aggregations ("green cells"), colorless detritus and filaments grown out from the cell aggregations, and macroalgal recruits.

In addition, diatom assemblages were analyzed separately. For this, each SLIDE corresponding to S1 was scanned twice, for 15 VFs (40X, 2.55 mm^2) to determine individual taxa cover and for 10 VFs (20X, 6.8 mm^2) to avoid underestimation of colony forming diatoms. As in the following time the abundance of diatoms increased strongly (see results), S2 and S3 SLIDEs were scanned for 15 VFs (20X, 5.1 mm^2) to capture the abundant taxa in the growing three dimensional assemblage. To identify the diatom species and potentially identify other microorganisms on the microsubstrates, two randomly chosen SCANs of S3 were prepared for scanning electronic microscopy, following the protocol described by Parodi and Cao (2003).

To compare micro- and macrosubstrate, and to determine the diatom species composition, a subsample (2×3 cm) of macrosubstrate was scraped with a sterile razor blade from the S3 SAO and CONTROL assemblages. Samples were transferred to glass vials and boiled 2 h in 30 % H_2O_2 to remove all organic material keeping the cleaned frustules. From each subsample, a minimum of 400 valves were

analyzed and species were identified. To compare diatom composition between the two substrates and techniques (percentage cover vs. cell counts), taxa were ranked by abundance.

Late Succession

To evaluate differences in the late successional assemblages (S4 and S5) at different scales, we analyzed the microsubstrates for filamentous stages and macroalgal germlings (mesoalgal assemblage), and the macrosubstrates for macroalgal composition (macroalgal assemblage). Due to the macroscopic colony size of the tube dwelling diatoms (TDD) we also included this taxon in the analyses for later successional stages.

For S4 and S5 each microsubstrate was scanned for 15 VFs (11.5X, 0.36 mm^2) and each macrosubstrate was scanned for 6 VFs (3X, 4.41 mm^2) to investigate for individual taxa cover. In addition, for each VF of macrosubstrate a subsample of algal material was fixed as a semi-permanent slide (SLIDE), using 50 % Karo® corn-syrup, preserved with 4 % formaldehyde and investigated under higher magnification (4-100X) for taxa identification. For S5, larger macroalgae (>1 cm) were removed from the edge of the macrosubstrate and preserved on a herbarium sheet. The coverage was calculated for every taxon.

Biodiversity Measurements

Taxa richness (S), evenness (J') and the Shannon diversity index (H') were calculated for each assemblage stage using percentage cover data (Magurran 1988). In addition, single taxon cover was added up for each assemblage and total taxon cover (COVER) was compared between channels for each stage. Taxa richness was used instead of species richness due to the lack of essential morphological features (e.g. sexual structures) in macroalgal germlings, and to the presence of organic material on preserved diatom frustules.

Statistical Analysis

Differences in nutrients (DIN and DIP), biodiversity (S, J', H) and total taxa cover between channels and among times were analyzed using a 2-way ANOVA with site and time as factors. Tukey's and Duncan's post-hoc tests were used in all cases when one of the factors or their interaction was significant. Homogeneity of variances was tested with Cochran's test. For one case (the evenness in the diatom assemblage), data could not be transformed to meet homogeneity of variance, nevertheless, ANOVA was used because it is still robust and better than other non-parametric analyses (e.g. Kruskall Wallis test, see Underwood 1997).

Differences in taxa composition between channels (SAO and CONTROL) and over time (S1, S2 and S3 for early and S4 and S5 for late succession), were evaluated using permutational multivariate analysis of variance (PERMANOVA). PERM ANOVA was based on Bray-Curtis similarity indices calculated from percentage cover prior to square root transformations in order to scale-down the importance of highly abundant taxa.

Results

Environmental Conditions

No differences were found in the hydrodynamic environment, as clod card comparisons showed no differences between sites, but a 2.7 times elevated water motion was registered in the channels than in the aquarium control (ANOVA: $F_{2,7}$= 6.95, p=0.01). DIN showed higher concentrations in the SAO channel ($F_{1,23}$=11.555, p=0.004) with no significant effect of time ($F_{3,23}$=1.291, p=0.312) nor interaction ($F_{3,23}$= 1.253, p=0.324). The same pattern was found for DIP with higher concentrations in the SAO channel ($F_{1,22}$=12.593, p= 0.003) and no significant effect of time ($F_{3,22}$=2.983, p= 0.065) nor interaction ($F_{3,22}$=3.328, p=0.058). Average nutrient concentrations were within the range reported in former studies (Table 1).

Benthic Assemblages

We distinguished 38 different taxa, containing 18 Bacillariophyta (Diatoms), including 3 functional groups (i.e. needle like diatoms=all diatoms showing a needle or stick like frustules, centric diatoms=all circulate diatoms, nano diatoms=all micro diatoms, indistinguishable in their valve shapes at scanning magnification). Furthermore we distinguished 20 mainly filamentous macroalgal taxa including 6 Phaeophyceae, 6 Chlorophyta, 7 Rhodophyta, as well as 1 Cyanobacteria (Fig. 2, Table 2).

Benthic Succession

Following the benthic recruitment and further succession, the two tidal channel assemblages differed from each other. These differences were not only observable in the magnitude of recruits but also in the identity of early settlers and the composition of later successional stages. While a general more gradual shift was observed in the CONTROL channel, the SAO assemblages showed stronger over time changes in composition and diversity, which exceeded the CONTROL assemblages at later stages. Opportunistic species were responsible for these changes alternating subsequently in their presence and abundances. Below we describe in detail the alterations in the benthic assemblage successions.

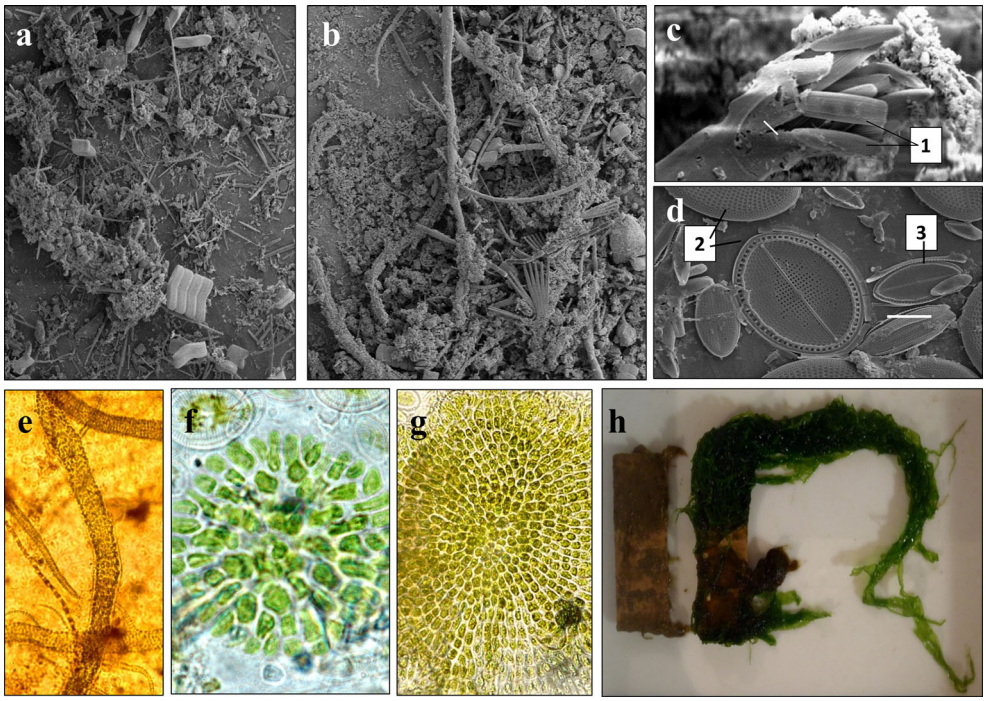

Fig. 2 *Benthic algal communities*. A-d) 40 days (S3) old diatom communities grown in the CONTROL (**a**) and SAO (**b-d**) channels. Scale bars: **a** and **b**= 100 μm, **c** and **d**=10 μm. 1: *Parlibellus* sp., (TDD) *2: Cocconeis scutellum* var. *scutellum*; 3: *Cocconeis euglypta*. **e-g**) *Mesoalgal assemblages grown on microsubstrates*: turbolose Ulvales, composed by *Ulva prolifera* (**e**), crustose Ulvales, composed by *Ulvella lens* (f) and crustose Ectocarpales, formed by *Dermatocelis* sp (**g**); **h**) *Macroalgal assemblages* grown on ceramic tiles at the CONTROL (left) and SAO (right) channels during late succession (S4= 40 days)

Early Succession: Tube Dwelling Diatoms, *Cocconeis* spp. and Macroalgal Recruitment Rates

In general, we observed high colonization and rapid growth of the biofilm with a significant increase in total cover over the first 8 days (S1-S2) at both channels, but higher values were registered at the SAO channel (Fig. 3, Table 3). The composition of the biofilm showed clear differences between channels (Fig. 3, Table 4). The biofilm in SAO showed more aggregated cells containing chlorophyll. A portion of the possibly Chlorophycean cells produced outgrowths, which were identified as *Ulva* spp. at later stages. In contrast, the biofilm in the CONTROL channel was mainly composed by diatom cells that efficiently aggregated more colorless detritus and sediments over time (Fig. 3, Table 4).

The diatom assemblage showed an increase in cover within the first 8 days (S1-S2) in both channels (Table 3). Although no differences were found in species richness and diversity, we observed a lower evenness and higher variance in the CONTROL channel (Fig. 4, Table 3). Furthermore, the composition of the diatom assemblages differed strongly between channels depending on the successional stage (Fig. 3, Table 4). The diatom assemblage in the SAO channel was dominated by *Cocconeis scutellum* var. *scutellum, C. euglypta* and tube dwelling diatoms (TDDs) (Fig. 2, Table 4). A strong increase in these taxa caused a change in the assemblage composition in the SAO channel over the first week of succession (S1-S2, Table 4). In contrast, the diatom assemblage in the CONTROL channel was mainly dominated by the group of needle like diatoms (e.g. *Nitzschia* spp., *Tabularia* spp.) and *Licmophora* (Fig. 3). Overall, differences in the species present resulted in distinct microstructure in the two channels, recognizable in the individual laying (*Nitzschia* spp.) and radiate attached members of needle like diatoms (e.g. *Tabularia* sp.), giving a sprinkled appearance to the CONTROL channel assemblage (Fig. 2 a). In contrast, the erect mucilaginous tubes of TDDs (Fig. 2b-c) and plaster-forming *C. scutellum* and *C. euglypta* (Fig. 2d) dominated the structure of the SAO channel assemblage.

Comparing the different substrates, we found differences in the diatom assemblage after 11 days. The diversity seemed to be slightly increased by the macrosubstrate, as we found 12 diatom taxa on the microsubstrate in both channels and 14 and 13 diatom taxa on the macrosubstrate in SAO and CONTROL channels respectively. In the SAO channel, the most dominant taxa on the microsusbtrate were *C. scutellum*, followed by TDD and *C. euglypta*, whereas the most dominant taxa of the macrosubstrate were found to be the TDDs, followed by the chain forming taxa *Grammatophora* and *Melosira.*

After colonization by Chlorophycean and Phaeophycean cells, rapid recruitment of the red algae Ceramiales, including members of the Ceramiaceae and Rhodomelaceae, was observed in the assemblages of both channels. Germlings were present after three days within the CONTROL assemblage (mean (± SE) CONTROL: 0.5±1 recruits/sample, Ceramiales). Following succession, the recruitment number increased in the SAO channel from 1.25±0.96 recruits/sample (40 % Ceramiales, 40 % Ceramiacae and 20 % Rhodomelacea) after 6 days to 2.5±2.12 recruits/sample after 11 days (60 % Ceramiales and 40 % Rhodomelaceae). In

Table 2 Average percentage cover per taxa in the assemblages at the CONTROL and SAO channels over different times of succession (S1: 3 days, S2: 6 days, S3: 11 days, S4: 40 days, and S5: 357 days=1 yr) grown on microsubstrates (1.5 x 2 cm, polyethylene terephthalate) and (underlined) macrosubstrates (3 x 6 cm, unglazed ceramic tiles). x indicates average cover<1 %. LF indicates life form following Cattaneo (1990) for diatoms: *A*=forms that grow oppressed on the substratum, *St*=comprised forms forming stalks or cushion like aggregations to attach on the substrate, *Td*=comprises colony forming forms living in mucilaginous partly branching tubes, *M*=comprising all motile pennate forms, and *D*=comprising suspended or trapped centric forms; and following Steneck and Dethier (1994) for meso- and macroalgae: *si*=siphonal, single cell tube, *ff*=fine filamentous uniseriate, main axis one cell thick, *f*=filamentous main axis multiseriate, *fo*=foliose single or bilayered, forming sheet, *sac*=saccate, foliose, inflated, bilayeredthallus, *cor*=corticated, multiple cell layers with partly different growth directions, *corfol=corticated* foliose multiseriate, *cru*=crust forming, *calc crus=calcerouscrustous*, partly calcified crust forming, *artcalc=articulated calcerous*calcified upright growing forms. *Nano diatoms*=comprises all micro diatoms, indistinguishable in their valve shapes at scanning

			CONTROL							SAO						
TAXA	LF	SUCCESSION/SUBSTRATE SPECIES/ TIME (days)	S1 3	S2 6	S3 11	S4 40	S4* 40	S5 1 yr	S5* 1 yr	S1 3	S2 6	S3 11	S4 40	S4* 40	S5 1 yr	S5* 1 yr
Bacillariophyta																
Needle like diatoms	M/A	*Nitzschia* spp., *Tabularia fasciculata, Tabularia gaillonii*	9	22	22					2	5	9				
Cocconeis scutellum	A	*Cocconeis scutellum* var. *scutellum*		x	1					1	6	19				
Cocconeis euglypta	A	*Cocconeis euglypta*		x	x					1	12	14				
Licmophora	St	*Licmophora flabellata*	3	7	8					x	1	2				
TDD	Td	*Parlibellus* sp.	1	2	2	2	2	x	1	1	3	15	16	18	x	2
Achnanthes	St	*Achnanthes longipes*	x	2	2					p	2	7				
Diploneis	A	*Diploneis papula*	2	2	3					2	1	x				
Grammatophora	St	*Grammatophora marina*	1	5	2					1	4	3				
Melosira	St	*Melosira nummuloides*	1	1	1					1	1	3				
Amphora	M	*Halamphora* sp.,*Amphora* sp.	1	x	1					1	x	x				
Gyrosigma	M	*Gyrosigma* sp.	x	1	x					x	x	x				
Odontella	St	*Odontella aurita*	x	1	1					x	x	1				
Fragillaria	St	*Striatella unipunctata*	1	2	2					x	x					
Terpsinoe	St	*Terpsinoë americana*	x	x	x					x	x					
Centric diatoms	D	*Auliscus sculptus*		x	x							x				
Odontella	St										x					
Amphitetras	St				x											
Nano diatoms		*Rhopalodia, Opephora*	x	x	x					3	x	x				
Chlorophyta			S1	S2	S3	S4	S4*	S5	S5*	S1	S2	S3	S4	S4*	S5	S5*
turbulose Ulvaceae	f	*Ulva prolifera, Ulva flexuosa, Blidingia* aff. *minima*				2	4		7				13		7	12
foliose Ulvaceae	fo	*Ulva lactuca, Ulva* sp.							2				5	97		22
Cladophora	ff	*Cladophora* cf.*laetevirens*				x	44	2	36						x	7
Chaetomorpha	ff	*Chaetomorpha* sp.					x		3							11
Crustous Chlorophyta	crus	*Ulvella lens, Ulva* spp. *(initial cells)*				2		51					x		10	
Derbesia	si	*Derbesia* sp					x									
Phaeophyta			S1	S2	S3	S4	S4*	S5	S5*	S1	S2	S3	S4	S4*	S5	S5*
Ectocarpales	ff	*Feldmannia* aff. *simplex, Hincksia* sp.					5	21	1				7	2	17	3
Crustous Phaeophyceae	crus	*Myrionema* sp.,Ectocarpales (initial cells)				17		35					x		62	
Scytosiphon	cor	*Scytosiphon* sp.				17	1		19				1			
Punctaria	cor	*Punctaria* sp.							4						10	
Dictyota	corfol	*Dictyota* cf. *dichotoma*							1							5
Sphacelaria	f	*Sphacelaria* sp.														x
Rhodophyta			S1	S2	S3	S4	S4*	S5	S5*	S1	S2	S3	S4	S4*	S5	S5*
Polysiphonia	f	*Polysiphonia* aff. *argentinica, Polysiphonia* aff. *abcissa, Polysiphonia* sp.				19	6	2	3				2	x	6	9
Ceramiun	f	*Ceramium* spp.											x		x	5
Erythrotrichia	ff	*Eryitrotrichia* cf. *carnea*						1	1				x		2	5
Anotrichium	f	*Anotrichium* sp.						x							1	

Table 2 (continued)

			CONTROL							SAO						
TAXA	LF	SUCCESSION/SUBSTRATE SPECIES/ TIME (days)	S1 3	S2 6	S3 11	S4 40	S4* 40	S5 1 yr	S5* 1 yr	S1 3	S2 6	S3 11	S4 40	S4* 40	S5 1 yr	S5* 1 yr
Porphyra	fol	*Porphyra* sp.													x	
Corallina	artcalc	*Corallina officinalis*														
Hydrolithon	calccrus	*Hydrolithon*cf. *farinosum*						9								
Cyanobacteria			S1	S2	S3	S4	S4*	S5	S5*	S1	S2	S3	S4	S4*	S5	S5*
Fil. cyanos	ff	*Lyngbya* sp.							2							x

contrast, the recruitment numbers for the CONTROL channel stayed lower, although the germlings identity changed over time, indicating a loss or replacement of the early stages (6 days: 0.25±0.5, 100 % Rhodomelaceae, 11 days: 0.25± 0.5, 100 % Ceramiaceae).

Late Succession: Alterations in Bloom Forming Chlorophytes and Substrate Differences

At later successional stages clear differences between the assemblages grown on different settlement substrates were observed. The composition of mesoalgal assemblages (grown on microsubstrates) and macroalgal assemblages (grown on macrosubstrate) differed in terms of the presence and abundance of different taxa. For the mesoalgal assemblage 17 different taxa were distinguished: 1 Bacillariophyta, 4 Phaeophyceae, 5 Chlorophyta, 6 Rhodophyta, 1 Cyanobacteria. For the macroalgal assemblages we distinguished 15 different taxa: 1 Bacillariophyta, 5 Phaeophyceae, 5 Chlorophyta, 3 Rhodophyta, 1 Cyanobacteria (Table 2). The discrepancies in taxa numbers were partly due to the presence of minute taxa like crustose Ulvales, crustose Ectocarpales, *Hydrolithon*, and *Anotrichium*, and early life stages of *Ulva* (germlings) and *Porphyra*, which required higher magnification for identification. Next to these cryptic taxa found on the microsubstrates, other taxa were detected and were

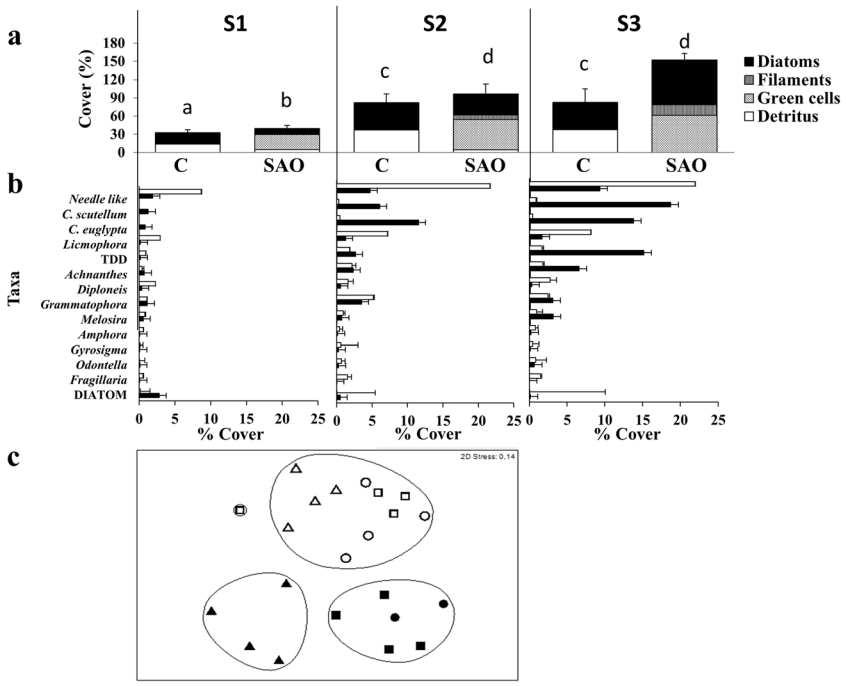

Fig. 3 *Early succession* at CONTROL and SAO channels during the first eleven days of succession (S1=4 days, S2=8 days, S3=11 days). **a** Changes in total biofilm percentage cover composed by diatoms, cells containing chlorophyll (Chl cells) and filamentous algae (filaments), aggregating different amounts of detritus and sediment (detritus). Superimposed letters indicate differences identified by ANOVA. **b** Changes in taxa composition and percentage cover of diatom assemblage at CONTROL (*white bars*) and SAO (black bars) channels. Only individual taxa percent cover>5 % in a treatment is shown. Graph shows mean (±SE). **c** Non-metric multi-dimensional scaling (nMDS) plot, showing differences in the benthic assemblages grown on microsubstrates in the CONTROL (*white*) and SAO (*black*) channels over 4 (*triangle*), 8 (*square*) and 11 (*circle*) days. Superimposed clusters (*lines*) identified by sequence of SIMPROF tests (p-0.05) on dendrograms at similarity levels of 60 %. Taxa abundance was square root transformed before converting to Bray-Curtis similarities

Table 3 ANOVA results for differences in percent cover, total number (COVER/ Number), species richness (S) evenness (J') and diversity (H') between assemblages exposed to contrasting nutrient conditions (SAO, CONTROL) over different times (S1-S5). Significant differences are marked in bold. Conclusions indicate results of Tukey and Duncan's post-hoc test. Italics indicate square root transformation of data,* indicates no homogeneity of data could be achieved by transformation

Type	Factors	COVER/ Number			S		J'		H'		Conclusions
		df	F	p	F	p	F	p	F	p	
a) Early succession (S1-S3)											
Biofilm	Site (S)	1	7.68	0.02							CONTROL<SAO
	Time (T)	2	19.38	0.00							S1<S2, S3
	S x T	2	n.s.								
	Error	16									
Diatoms	Site (S)	1	n.s.		n.s.		9.20*	0.01*	n.s.		J': CONTROL<SAO
	Time (T)	2	24.25	<0.000	n.s.		n.s.		n.s.		Cover: S1<S2, S3
	S x T	2	n.s.		n.s.		n.s.		n.s.		
	Error	16									
Recruits N°	Site (S)	1	*4.86*	*0.04*							
	Time (T)	2	*n.s.*								
	S x T	2	*3.76*	*0.04*							*SAO: S1<S3 *S3: CONTROL<SAO
	Error	16									
b) Late succession (cover)											
Mesoalgae	Site (S)	1	n.s.		n.s.		n.s.		n.s.		
	Time (T)	1	24.85	0.001	n.s.		n.s.		n.s.		Cover: S4<S5
	S x T	1	n.s.		n.s.		n.s.		n.s.		
	Error	12									
Macroalgae	Site (S)	1	8.45	0.01	n.s.		n.s.		n.s.		
	Time (T)	1	n.s.		17.64	0.001	27.99	<0.001	39.69	<0.001	J': S4<S5
	S x T	1	8.49	0.01	25.00	<0.001	n.s.	n.s.	10.96	0.010	Cover: S4_ CONTROL<SAO S: SAO_S4<S5 H': SAO_S4<S5; S4_SAO<CONTROL; S5_CONTROL<SAO
	Error	12									

exclusively found on the macrosubstrates: *Chaetomorpha, Derbesia, Dictyota,* and *Sphacelaria* (Table 2).

The macroalgal assemblage, grown on the macrosubstrate, differed strongly in cover and species richness (S) between the two channels, being higher in SAO, with an increase in diversity values within the SAO channel at the end of the study (Fig. 6, Table 3). In contrast, evenness (J), increased with time at both channels (Fig. 6), indicating a similar change in the relative abundance of different taxa through succession. For the mesoalgal assemblages, grown on the microsubstrates, no differences were found between the SAO and CONTROL channels in S, J', and H' indexes, or in cover, although the cover significantly increased in both channels at the end of the study (after one year) (Fig. 5, Table 3).

Overall, both meso- and macroalgal assemblages differed between channels in their composition (Table 4). These differences were mainly caused by crust forming Ulvales (e.g. *Ulvella lens*) and crustose Ectocarpales (e.g. *Myrionema* sp.) in the mesoalgal assemblage, and by foliose *Ulva* and *Cladophora* in the macroalgal assemblage (Fig. 2, Table 4). For the mesoalgal assemblages, differences between channels at the end of the study (Table 4) were due principally to the increase of crustose Ulvales (e.g. *Ulvella lens*) in the CONTROL and crustose Ectocarpales (e.g. *Myrionema* sp.) in the SAO assemblage (Fig. 5). For the macroalgal assemblages taxa composition differed between channels throughout the study period (Table 4). Differences were mainly driven by the Ulvales (Foliose Ulva and Turbulose Ulvales), which sequentially bloomed in the SAO channel. Thus, after 40 days, the SAO assemblage was mainly composed of tubular Ulvales (*Ulva prolifera*, *U. flexuosa, Blidingia* sp.), whereas the CONTROL channel showed a high abundance of the

Table 4 PERMANOVA results showing differences in taxa composition during a) early succession and b) late succession of different algal assemblages (Biofilm, Diatoms, Mesoalgae and Macroalgae) in the CONTROL and SAO channels. Results of SIMPER (similarity percentage analysis) are given, showing percentage contribution of single or grouped taxa to total dissimilarity between channels or times. Only taxa causing major differences (cut-off level 60 %) are presented. The channel or succession stage showing higher abundance of taxa is in parenthesis. Bold values are statistically significant. Asterisk indicates lower replicate number for 40 days old control Mesoalgal assemblages (n=2)

PERMANOVA						SIMPER		
Type	Factor	df	MS	Pseudo-F	p	Pairwise	%Av.diss	% Contrib.
a) Early succession								
Biofilm	Site (S)	1	11092	18.01	0.001	SAO≠CONTROL	59	52 % "Green cells" (SAO) 26 % Detritus (CONTROL) 19 % Diatoms (CONTROL)
	Time (T)	2	5081.5	8.29	0.001	S1≠S2	46	45 % Diatoms (S2) 26 % Detritus (S2) 24 % "Green cells" (S2)
						S1≠S3	61	43 % Diatoms (S3) 28 % "Green cells" (S3) 22 % Detritus (S3)
	S x T	1	924.11	1.5082	0.209			
	Res	15						
Diatoms	Site (S)	1	6505	13.14	0.001	CONTROL≠SAO	49	14 % needle like diatoms (CONTROL) 13 % *C. euglypta* (SAO) 12 % *Licmophora* (CONTROL) 11 % *C. scutellum* (SAO)
	Time (T)	2	2337.3	4.7213	0.001	S1≠S2	43	14 % *C. euglypta* (S2) 11 % needle like diatoms (S2) 10 % *Licmophora* (S2) 9 % *Grammatophora* (S2) 9 % TDD (S2)
						S1≠S3	44	14 % TDD (S3) 12 % needle like diatoms (S3) 12 % *C. scutellum* (S3) 11 % *C. euglypta* (S3) 9 % *Achnanthes* (S3)
	S x T	1	958.3	1.9357	0.046	CONTROL: n.s.		
	Res	14				SAO: S1≠S2	47	21 % *C. euglypta* (S2) 11 % *C. scutellum* (S2) 10 % TDD (S2) 9 % DIATOM (S1)
						S1: CONTROL≠SAO	49	16 % needle like (CONTROL) 12 % *Licmophora* (CONTROL) 12 % Diatom (SAO) 10 % *C. scutellum* (SAO)
						S2: CONTROL≠SAO	49	17 % *C. euglypta* (SAO) 14 % needle like (CONTROL) 12 % *C. scutellum* (SAO) 11 % *Licmophora* (CONTROL)
						S3: n.s.		

Cladophora. After a year, the number of tubular Ulvales decreased in the SAO channel accompanied by a decline in percentage cover (Fig. 6), and an increase in diversity (Fig. 7).

The SAO assemblage strongly changed its composition over time while differences in the CONTROL assemblage decreased at the end of the study (Fig.7, Table 2). Despite the lack of differences in the analyzed assemblages, differences became evident when observing the edge of the macrosubstrates, characterized by four different grown out macroalgae. Thus, *Ulva lactuca* and *Polysiphonia* aff. *argentinica* dominated the SAO channel, whereas

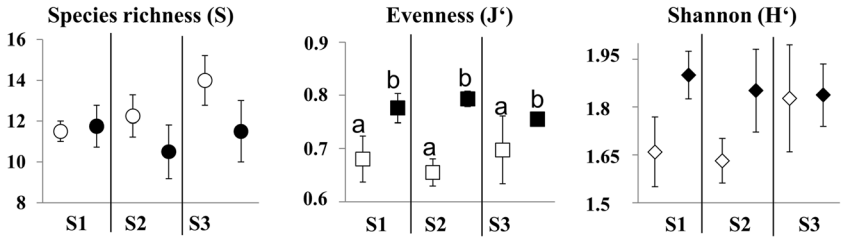

Fig. 4 *Changes during early succession* in species richness (*dots*), Evenness J' (*squares*) and Shannon diversity H'(*diamonds*) during early succession of benthic assemblages grown in the CONTROL (*white*) and SAO (*black*) channels over different times (S1=4 days; S2=8 days, S3=11 days). Different letters indicate differences between channels (p=0.01). Data show means (±SE)

Scytosiphon sp. and *Punctaria* sp. were more abundant in the CONTROL channel (Fig. 6).

Discussion

Our results show that natural benthic succession under different nutrient and grazing conditions not only differs in trajectory but in the final taxonomic composition. Under high nutrient-grazing conditions, the benthic assemblage was dominated by tube dwelling diatoms (TDDs) and *Cocconeis* spp. at early successional stages while under lower nutrient-grazer loads motile needle-like diatoms (e.g. of the genus *Nitzschia*, *Tabularia*) were the most dominant species. Succession continued with macroalgal colonization, e.g., Ceramiales, which showed a higher recruitment rate in the nutrient rich environment. After 40 days, differences were mainly due to the presence of turbulose Ulvales blooming at SAO channel, and a more diverse macroalgal assemblage in the CONTROL channel. Following up the succession, the assemblages in terms of biodiversity did not change in the control channel while in the SAO channel biodiversity increase exceeding that found in the

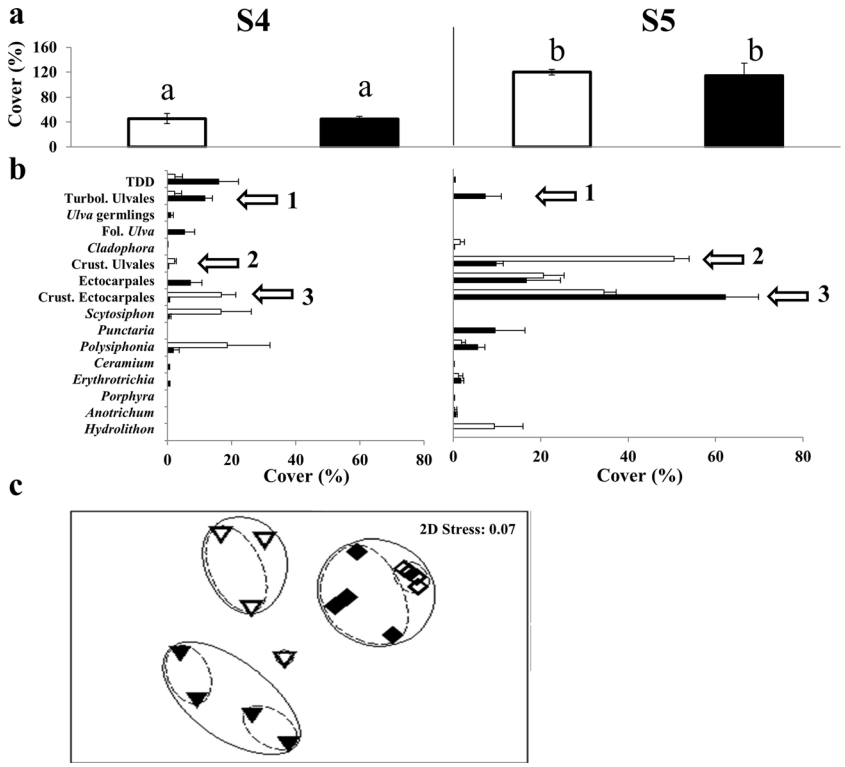

Fig. 5 *Mesoalgal assemblages* at CONTROL and SAO channels during late succession (S4=40 days and S5=365 days). **a** Changes in total percentage cover. Superimposed letters indicate differences identified by ANOVA. Graph shows mean (±SE). **b** Changes in taxa composition and percentage cover of mesoalgal assemblage at CONTROL (*white bars*) and SAO (*black bars*) channels. Only individual taxa percentage cover>5 % in a treatment is shown. Graph shows mean (±SE). *Arrows* highlight the presence of (1) tubular Ulvales (e.g. *Ulva prolifera*) (2) crustose Ulvales (e.g. *Ulvella lens*) and (3) crustose Ectocarpales (e.g. *Dermatocelis* sp), responsible for the differences found between the mesoalgal assemblages grown at CONTROL and SAO channels. **c** Non-metric multi-dimensional scaling (nMDS) plot showing differences in the CONTROL (*white*) and SAO (*black*) benthic assemblages over 40 (*triangle*) and 356 (*diamond*) days. Superimposed clusters (*lines*) identified by sequence of SIMPROF tests (p-0.05) on dendrograms at similarity levels of 51 % (*line*) and 61 % (*dashed line*). Taxa abundance was square root transformed before converting to Bray-Curtis similarities

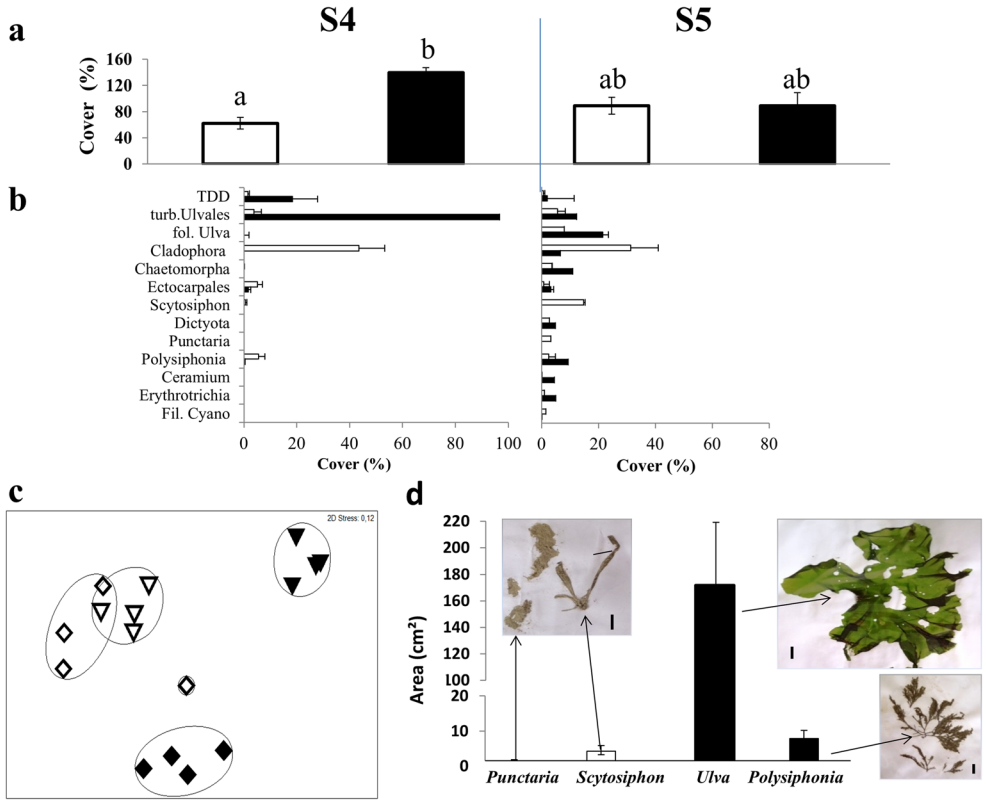

Fig. 6 *Macroalgal assemblages* at CONTROL and SAO channels during late succession (S4=40 days and S5=365 days). **a** Changes in total percentage cover. Superimposed letters indicate differences identified by ANOVA. Graph shows mean (±SE). **b** Changes in taxa composition and percentage cover of macroalgal assemblage at CONTROL (*white bars*) and SAO (*black bars*) channels. Only individual taxa percentage cover>5 % in a treatment is shown. Graph shows mean (±SE). **c** Non-metric multi-dimensional scaling (nMDS) plot showing differences in the benthic assemblages grown in the CONTROL (*white*) and SAO (*black*) channels over 40 (*triangle*) and 356 (*diamond*) days. Superimposed clusters (*line*) identified by sequence of SIMPROF tests (p-0.05) on dendrograms at similarity levels of 60 %. Taxa abundances were square root transformed before converting to Bray-Curtis similarities; d) Area (cm^2) of different macroalgae grown on the tile edge of 365 days (S5) old assemblages at the CONTROL (*white*) and SAO (*black*) channels. Scale bar=1 cm

control. These findings suggest that nutrient enrichment can not only alter an established assemblage, as has been extensively described in the literature, but also affect the benthic assemblage from the very early succession stages, changing the succession trajectory and concomitantly, the resulting final assemblage. Below we discuss the possible mechanisms that could drive the observed differences at different scales.

Early settlers already differed between channels after three days. Despite an observed similar increase in cover, differences were found in the diatom assemblages, with TDD and *Cocconeis* spp. in the SAO and needle-like diatoms in the CONTROL channel. Because the two channels were similar in terms of light, current, tide, and substrate, the differences found could be the result of the more than two fold higher nutrient concentration in the SAO channel. In fact, the observed formation of mucilaginous diatom tubes (TDDs), which bloomed in the eutrophic SAO assemblage, resembled a pattern observed in the North Sea (Hillebrand et al. 2000), where the tube forming *Berkeleya rutilans* strongly increased under artificial N and phosphorus (P) enrichment. This study supports our findings suggesting that the difference found is probably due to the difference in nutrient availability between channels.

The interpretations of the differences in the abundances of other diatom taxa are less obvious, as other taxa observed in the less polluted CONTROL channel, like the pennate genera *Nitzschia* and *Tabularia*, were also commonly found in nutrient-rich habitats (Hillebrand et al. 2000; Michels-Estrada 1998), and are even classified as highly tolerant to organic pollution (Kelly and Whitton 1995). In general, nutrient uptake rates of diatoms strongly depend upon characteristic nanostructures (Mitchell et al. 2013). The observed discrepancies in nutrient tolerant taxa might therefore underline the need of better species knowledge to identify suitable indicators for water quality measurements in the area.

The presence of crustacean tubes, already attached to the different substrates in both channels after a short time, would indicate high grazing (Fricke pers. obs.). In fact, the oxygenated nutrient rich SAO channel provides a unique environment favoring the development of abundant invertebrate fauna (Martinetto et al. 2010, 2011) which might strongly affect the

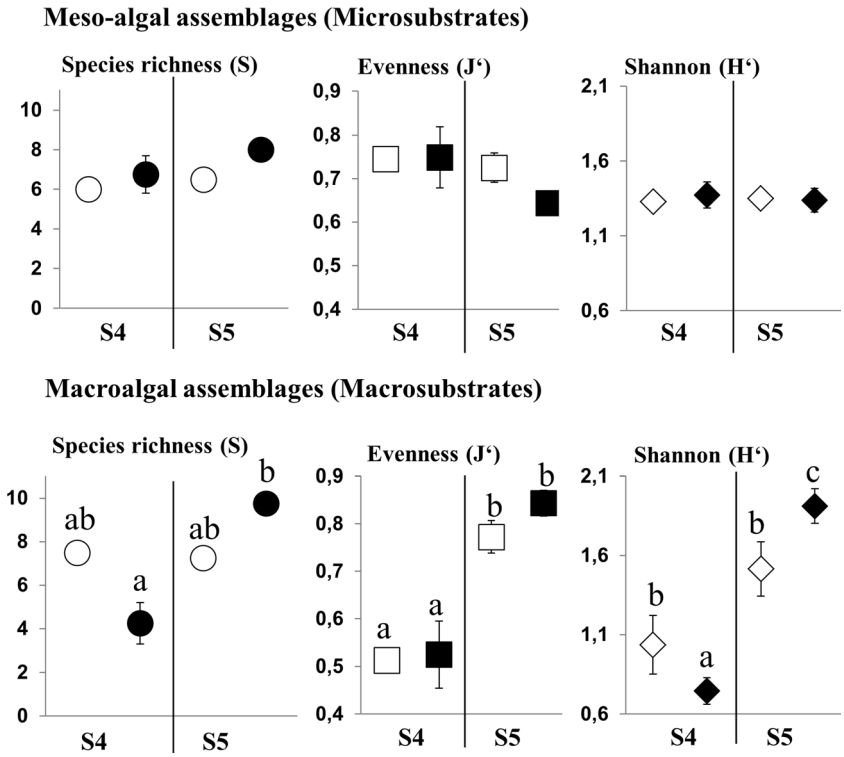

Fig. 7 *Biodiversity changes during later succession*. Graphs showing changes in Species richness (S), Evenness (J') and Shannon diversity H' in the Meso- and Macroalgal assemblages grown on micro- and macrosubstrates in the CONTROL (*white*) and SAO (*black*) channels over 40 (S4) and 365 days (S5). Different letters indicate differences between the different treatments. Data shows means (±SE)

colonization process. Testing the impact of grazing pressure on diatom communities, Hillebrand et al. (2000) found that adnate-growing *Cocconeis scutellum* were resistant to grazing, whereas other more loosely laying taxa like *Melosira moniliformis* were easily grazed away. The observed discrepancies in our study, showing low abundances of loose needle like diatoms but high numbers of *Cocconeis* spp. in the SAO channel, seem to support this hypothesis.

In general, members of the genus *Cocconeis* have been reported as common early colonists after disturbances in several studies (e.g. Patrick 1976; Jones 1978; Robinson and Rushforth 1987). Interestingly, *Cocconeis* was only sparsely observed in the CONTROL channel. This difference may suggest that factors other than nutrient concentration or grazer abundance play an important role. Microstructures play a crucial role in diatom attachment (e.g. Wu et al. 2013), which consequently alter the benthic assemblage composition at later successional stages (Schneck et al. 2011). Accordingly, in our study we observed strong substrate specific differences, with *Cocconeis* dominating in the smoother microsubstrate surfaces, where its firmly attachment mode presents a greater resistance to the drag forces of the flow (Gari and Corigliano 2007). Potential differences in substrate availabilities and qualities might be responsible for the observed differences in *Cocconeis* abundances between channels. In fact, the SAO channel has a closer connection to the adjacent town of San Antonio Oeste and thus it exhibits a higher availability and diversity of substrates derived from anthropogenic activities (e.g. pieces of concrete, bricks, etc).

Close macroalgae-diatom interactions have been observed in other studies. Diatoms play a crucial role for macroalgal settlement (Davis 2009), and thus might facilitate or inhibit their settlement (Connell and Slatyer 1977). Mucilaginous diatom tubes (TDD) can alter the microstructure and three-dimensionality of the early benthic assemblages, through production of extracellular polymers that can facilitate settlement (Lam et al. 2005) and support a variety of epiphytic species (Round et al. 1990). However, given the opportunistic life style of tube dwelling diatoms characterized by seasonal blooms (Minzuno 1989), it is unclear how far this taxon facilitates benthic succession (e.g. supports epiphytic macroalgae over time). However, the composition of epiphytic diatoms depends on the host identity (Al-Handal and Wulff 2008) and is related to different interspecific interactions such as grazing pressure and sloughing (Liess et al. 2009). Thus, it is not clear if there is a closer connection between the observed abundant and bloom forming taxa of macroalgae and the diatoms (e.g. *Ulvales*, *Cocconeis* and tube dwelling diatoms), which consequently would affect not only the benthic but also the planktonic system of the research area. Macroalgae, in turn, provide suitable substrate to different diatom groups (Cejudo-Figueiras et al. 2010). Thus, the abundant and sediment free thalli of macroalgae reported in the SAO channel (Martinetto et al. 2010) might play an important role as suitable substrate for diatom settlement as has been found in other studies (e.g. Cejudo-Figueiras et al. 2010). Therefore, it is possible that micro-macroalgal interactions might impact development of the different macroalgal assemblages in the two channels.

Our results indicate that the succession of benthic algal assemblages follows different trajectories, resulting in different taxonomic compositions in the two channels. As moderate successional and seasonal changes were observed in the CONTROL channel, strong alterations in the eutrophic SAO channel were mainly driven by the formation of different micro- and macroalgal blooms. Interestingly, substrate characteristics provided by the first settlers significantly interfered with the observed assemblage patterns, changing the habitat diversity (β-diversity, Whittaker 1972). Thus, the observed decrease in tubular shaped bloom formers in the SAO channel led to a significant increase in the biodiversity in the macroalgal assemblage, whereas no changes were found in the mesoalgal assemblages. This difference might be related to a specific pattern, as grown-out thalli of foliose Ulvales were exclusively found at the borders of settlement tiles. The observed spatial discrepancy might be explained by spore settlement preferences (Callow et al. 2002), inter-specific relations like settlement inhibition (Connell and Slatyer 1977) and grazing pressure (Kamermans et al. 2002), or more likely by increasing vulnerability to shearing forces, removing grown individuals, as we commonly found loose algal material drifting in the area. As a consequence, the newly bare substrate allows the colonization and development of new taxa, provoking a restructuring in the SAO assemblage on the settlement tile that interestingly resembled the CONTROL assemblage at the end of the study. In addition, other substrate specific differences of *Chaetomorpha* and *Dictyota* and the sparse presence of *Sphacelaria* and *Derbesia* shaped the benthic pattern of the area. Overall, the observed differences between different substrates (habitats, therefore β-diversity, Whittaker 1972) may play a crucial role in the formation and duration of benthic algal blooms in eutrophic areas, and might become crucial in environmental assessment as well as in the evaluation of future pollution scenarios.

The benthic assemblages established after one year differ between channels. In the SAO channel, the assemblage was characterized by the presence of species typically associated with eutrophic systems, like the foliose Ulvales, but also by high diversity, which is not usually associated with eutrophication. The frequent tidal flushing seems not only to allow the development of a diverse grazing fauna (Martinetto et al. 2010), but also to prevent anoxic events in this system (Martinetto et al. 2011), which otherwise would favor the growth of anaerobic bacteria and lead to the degradation of benthic organisms as commonly observed in many eutrophic systems (Diaz and Rosenberg 2013).

Conclusion

In conclusion, our work suggests that even at the very early succession stages, nutrients play an important role in driving succession of benthic species, and along with other factors (e.g. grazers, availability of spores and early settlers) establish the succession pathways and the final composition of the algal assemblage. In addition, settlement substrate seems to play a crucial role by supporting favoring taxa specific characteristics and leading to different assemblages under equal environmental conditions. These findings might play a crucial role for the management of such eutrophied systems. In fact the traditional approach using the presence of certain indicator species might be extended by integrate information on substrate composition of the area and/or vice versa be simplified by using comparable monitoring substrates and fast settling indicators, like specific diatoms and macroalgal at early stages (e.g. germlings).

Daily oxygenation through the macrotidal cycle and the herbivory pressure exhibited in this area may lead to a more diverse assemblage in the nutrient-rich area avoiding the typical domination by few taxa typically described in nutrient-rich environments.

Acknowledgments This work was supported by a fellowship (AF, D/11/46030) within the Postdoc-program of the German Academic Exchange Service (DAAD); grant FONCYT (PICT-2010-0467) to R.J.L., grant PGI-24/Q060 (Universidad Nacional del Sur) to A.M., G.A.K., A.F.; and grants from CONICET, ANPCYT and Universidad Nacional de Mar del Plata to OI. Additional support for this study came from the and PICT CONAE-CONICET 04-2010 and Universidad Nacional del Comahue to M.N. We thank Joanna York (University of Delaware) for useful suggestion and comments.

References

Aleem, A.A. 1957. Succession of marine fouling organisms on test panels immersed in deep-water at la Jolla, California. *Hydrobiologia* 11: 40–58.

Al-Handal, A.Y., and A. Wulff. 2008. Marine epiphytic diatoms from the shallow sublittoral zone in Potter Cove, King George Island, Antarctica. *Botanica Marina* 51(5): 411–435.

Burkepile, D.E., and M.E. Hay. 2006. Herbivore vs. nutrient control of marine primary producers: Context-dependent effects. *Ecology* 87: 3128–3139.

Callow, M.E., A.R. Jennings, B. A:B:, C.E. Seegert, G. Amy, W. Leslie, A. Feinberg, R. Baney, and J.A. Callow. 2002. Microtopographic cues for settlement of zoospores of the green fouling alga *Enteromorpha*. *Biofouling* 18: 237–245.

Cattaneo, A. 1990. The effect of fetch on periphyton spatial variation. *Hydrobiologia* 206(1): 1–10.

Cejudo-Figueiras, C., I. Álvarez-Blanco, E. Bécares, and S. Blanco. 2010. Epiphytic diatoms and water quality in shallow lakes: the neutral substrate hypothesis revisited. *Marine Freshwater Research* 61: 1457–1467.

Cloern, J.E. 2001. Our evolving conceptual model of the coastal eutrophication problem. *Marine Ecological Progress Series* 210: 223–253.

Connell, J.H., and R.O. Slatyer. 1977. Mechanisms of succession in natural communities and their role in community stability and organization. *The American Naturalist* 111: 1119–1144.

Cuba, T.R., and N.J. Blake. 1983. The initial development of a marine fouling assemblage on a natural substrate in a subtropical estuary. *Botanica Marina* XXVI: 259–264.

Davis, A.R. 2009. The role of mineral, living and artificial substrata in the development of subtidal assemblages. In *Marine Hard Bottom Communities*, ed. M. Wahl, 19–37. Dordrecht: Springer.

Diaz, R.J., and R. Rosenberg. 2013. Overview of anthropogenically-induced hypoxic effects on marine benthic fauna, coastal hypoxia: consequences for living resources and ecosystems. In Coastal Hypoxia: Consequences for Living ressources and Ecosystems. American Geophysical Union: pp 129–145.

Doty, M.S. 1971. Measurement of water movement in reference to benthic algal growth. *Botanica Marina* 14: 32–35.

Duarte, C.M. 1995. Submerged aquatic vegetation in relation to different nutrient regimes. *Ophelia* 41: 87–112.

Fricke, A., M. Molis, C. Wiencke, N. Valdivia, and A.S. Chapman. 2008. Natural succession of macroalgal-dominated epibenthic assemblages at different water depths and after transplantation from deep to shallow water on Spitsbergen. *Polar Biology* 31: 1191–1203.

Fricke, A., M. Molis, C. Wiencke, N. Valdivia, and A.S. Chapman. 2011a. Effects of UV radiation on the structure of Arctic macrobenthic communities. *Polar Biology* 1-15.

Fricke, A., M. Teichberg, S. Beilfuss, and K. Bischof. 2011b. Succession patterns in algal turf vegetation on a Caribbean coral reef. *Botanica Marina* 54: 111–126.

Gari, E.N., and M.C. Corigliano. 2007. Spatial and temporal variations of *Cocconeis euglypta* (Ehrenb.) 1854 Grunow, 1884 in drift and periphyton. *Brazilian Journal of Biology* 67.

Geertz-Hansen, O., K. Sand-Jensen, D.F. Hansen, and A. Christiansen. 1993. Growth and grazing control of abundance of the marine macroalga Ulva lactuca L. in aeutrophic Danish estuary. *Aquatic Botany* 46: 101–109.

Hauxwell, J., J. Cebrian, and I. Valiela. 2003. Eelgrass *Zostera marina* loss intemperate estuaries: Relationship to land-derived nitrogen loads and effect of light limitation imposed by algae. *Marine Ecology Progress Series* 247: 59–73.

Hauxwell, J., J. Cebrian, and I. Valiela. 2006. Light dependence of Zostera marina annual growth dynamics in estuaries subject to different degrees of eutrophication. *Aquatic Botany* 84: 17–25.

Hillebrand, H., B. Worm, and H.K. Lotze. 2000. Marine microbenthic community structure regulated by nitrogen loading and grazing pressure. *Marine Ecological Progress Series* 204: 27–38.

Iribarne, O., P. Martinetto, E. Schwindt, F. Botto, A. Bortolus, and P.G. Borboroglu. 2003. Evidences of habitat displacement between two common soft-bottom SW Atlantic intertidal crabs. *Journal of Experimental Marine Biology and Ecology* 296: 167–182.

Jones, J.G. 1978. Spatial variation in epilithic algae in a stony stream (Wilfin Beck) with particular reference to *Cocconeis placentula. Freshwater Biology* 8: 539–546.

Kamermans, P., M. Erik-Jan, J.M. Verschuure, L. Lonneke Schrijvers, L. Franca, and A.T.A. Lien. 2002. Effect of grazing by isopods and amphipods on growth of Ulva spp. (Chlorophyta). *Aquatic Ecology* 36: 425–433.

Kattner, G. 1999. Storage of dissolved inorganic nutrients in seawater: Poisoning with mercuric chloride. *Marine Chemistry* 67(1–2): 61–66.

Kelly, M.G., and W. Brian Allan. 1995. The Trophic Diatom Index: a new index for monitoring eutrophication in rivers. *Journal of Applied Phycology* 7: 433–444.

Lam, C., T. Harder, and P.-Y. Qian. 2005. Growth conditions of benthic diatoms affect quality and quantity of extracellular polymeric larval settlement cues. *Marine Ecological Progress Series* 294: 109–116.

Liess, A., K. Lange, F. Schulz, J.J. Piggott, C.D. Matthaei, and C.R. Townsend. 2009. Light, nutrients and grazing interact to determine diatom species richness via changes to productivity, nutrient state and grazer activity. *Journal of Ecology* 97(2): 326–336.

Littler, M.M. 1980. Morphological form and photosynthetic performances of marine macroalgae: Tests of a functional / form hypothesis. *Botanica Marina* 22: 161–166.

Littler, M.M., D.S. Littler, and B.L. Brooks. 2010. The effects of nitrogen and phosphorus enrichment on algal community development: Artificial mini-reefs on the Belize Barrier Reef sedimentary lagoon. *Harmful Algae* 9: 255–263.

Lotze, H.K., and B. Worm. 2000. Variable and complementary effects of herbivores on different life stages of bloom-forming macroalgae. *Marine Ecological Progress Series* 200: 167–175.

Magurran, A.E. 1988. *Ecological diversity and its measurements*. USA: Princeton University Press. **179pp**.

Martinetto, P., P. Daleo, M. Escapa, J. Alberti, J.P. Isacch, E. Fanjul, F. Botto, M.L. Piriz, G. Ponce, G. Casas, and O. Iribarne. 2010. High abundance and diversity of consumers associated with eutrophic areas in a semi-desert macrotidal coastal ecosystem in Patagonia, Argentina. *Estuarine, Coastal and Shelf Science* 88: 357–364.

Martinetto, P., M. Teichberg, I. Valiela, D. Montemayor, and O. Iribarne. 2011. Top-down and bottom-up controls in a high nutrient-high herbivory coastal environment. *Marine Ecological Progress Series* 69-82.

McClelland, J.W., and V. Ivan. 1998. Linking nitrogen in estuarine producers to land-derived sources. *Limnology and Oceanography* 43: 577–585.

Michels-Estrada, A. 1998. Effects of sewage water on diatoms (Bacillariophyceae) and water quality in two tropical streams in Costa Rica. *Revista de Biología Tropical* 46: 153–175.

Mitchell, J.G., L. Seuront, M.J. Doubell, M. Dusan Losic, N.H. Voelcker, J. Seymour, and R. Lal. 2013. The role of diatom nanostructures in biasing diffusion to improve uptake in a patchy nutrient environment. *PLoS ONE*. doi:10.1371/journal.pone.0059548.

Mizuno, M. 1989. Autecological studies on the marine tube-dwelling diatom *Berkeleya obtusa* (GREV) GRUNOW. Scientific papers of the Institute of Algological Research, Faculty of Science, Hokkaido University 8(2): 63-115.

Nixon, S.W. 1995. Coastal marine eutrophication: A definition, social causes, and future concerns. *Ophelia* 41: 199–219.

Osterling, M., and L. Pihl. 2001. Effects of filamentous green algal mats on benthic macrofaunal functional feeding groups. *Journal of Experimental Marine Biology and Ecology* 263: 159–183.

O'Toole, G., H.B. Kaplan, and R. Kolter. 2000. Biofilm formation as microbial development. *Annual Review of Microbiology* 54: 49–79.

Parodi, E.R., and S.B. Cao. 2003. Benthic microalgal communities in the inner part of the Bahía Blanca estuary (Argentina): A preliminary qualitative study. *Oceanologica Acta* 25: 279–284.

Patrick, R. 1976. The formation and maintenance of benthic diatom communities. *Proceedings of the American Philosophical Society* 120: 475–484.

Piriz, M.L., M. Cecilia Eyras, and C.M. Rostagno. 2003. Changes in biomass and botanical composition of beach-cast seaweeds in a disturbed coastal area from Argentine Patagonia. *Journal of Applied Phycology* 15: 67–74.

Raimondi, P.T. 1988. Settlement cues and determination of the vertical limit of an intertidal barnacle. *Ecology* 69: 400–407.

Robinson, C.T., and S.R. Rushforth. 1987. Effects of physical disturbance and canopy cover on attached diatom community structure in an Idaho stream. *Hydrobiologia* 154: 49–59.

Round, F.E., R.M. Crawford, and D.G. Mann. 1990. The diatoms: biology & morphology of the Genera. Cambridge: Cambridge University Press.

Schneck, F., A. Schwarzbold, and A.S. Melo. 2011. Substrate roughness affects stream benthic algal diversity, assemblage composition, and nestedness. *Journal of the North American Benthological Society* 30: 1049–1056.

Steneck, R.S., and M.N. Dethier. 1994. A functional group approach to the structure of algal dominated communities. *Oikos* 69(3): 476–498.

Teichberg, M., S.E. Fox, C. Aguila, Y.S. Olsen, and I. Valiela. 2008. Macroalgal responses to experimental nutrient enrichment in shallow

coastal waters: Growth, internal nutrient pools, and isotopic signatures. *Marine Ecological Progress Series* 368: 117–126.

Teichberg, M., S.E. Fox, Y.S. Olsen, V. Ivan, M. Paulina, I. Oscar, M. Elizabeti Yuriko, P. Monica AV, T.N. Corbisier, S.-J. Martín, P.-O. Federico, C. Paulo, F. Helena, Z. Andreina, C. Massimo, and T. davide. 2010. Eutrophication and macroalgal blooms in temperate and tropical coastal waters: nutrient enrichment experiments with *Ulva* spp. *Global Change Biology* 16: 2624–2637.

Underwood, A.J. 1997. *Their logical design and interpretation using analyses of variance*. Experiments in ecology. Cambridge: Cambridge University Press.

Valiela, I., J. McClelland, J. Hauxwell, P.J. Behr, D. Hersh, and K. Foreman. 1997. Macroalgal blooms in shallow estuaries: Controls and ecophysiological and ecosystem consequences. *Limnology and Oceanography* 42: 1105–1118.

Wahl, M., M. Molis, A. Andrew Davis, S. Dobretsov, S.T. Dürr, J. Johansson, J. Kinley, D. Kirugara, M. Langer, H.K. Lotze, M. Thiel, J.C. Thomaso, B. Worm, and D.Z. Ben-Yosef. 2004. UVR effects that come and go: A global comparison of marine benthic community level impacts. *Global Change Biology* 10: 1962–1972.

Whittaker, R.H. 1972. Evolution and measurement of species diversity. *Taxon* 21: 213–251.

Worm, B., H.K. Lotze, C. Boström, R. Engkvist, V. Labanauskas, and U. Sommer. 1999. Marine diversity shift linked to interactions among grazers, nutrients and propagule banks. *Marine Ecological Progress Series* 185: 309–314.

Wu, A.H.-F., K.N. Wu, K.L. Cho, and R. Lamb. 2013. Diatom attachment inhibition: Limiting surface accessibility through air entrapment. *Biointerphases* 8: 1–10.