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Effects of the invasive macroalgae *Gracilaria vermiculophylla* on two co-occurring foundation species and associated invertebrates

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Supplementary material

The following supplementary material is available for this article.

Appendix 1. Results from factorial permutation based ANOVA and MANOVA testing for reciprocal impact of three foundation species on 'their own biomass' and densities and univariate and multivariate community structures of associated invertebrates.

Appendix 2. Effects of the three foundation species *Gracilaria vermiculophylla*, *Zostera marina*, and *Mytilus edulis* on their combined biomass.

Appendix 3. Incorporations of *Gracilaria* and *Zostera* into *Mytilus* byssal threads.

Appendix 4. Pearsons correlation coefficient R between univariate variables.

Appendix 5. Stepwise linear regressions on community structure and densities of invertebrate associated with *Zostera marina*, *Mytilus edulis* and *Gracilaria vermiculophylla*.

Appendix 6. Examples of architectural, morphological, anatomical and physiological differences between three co-occurring foundation species.

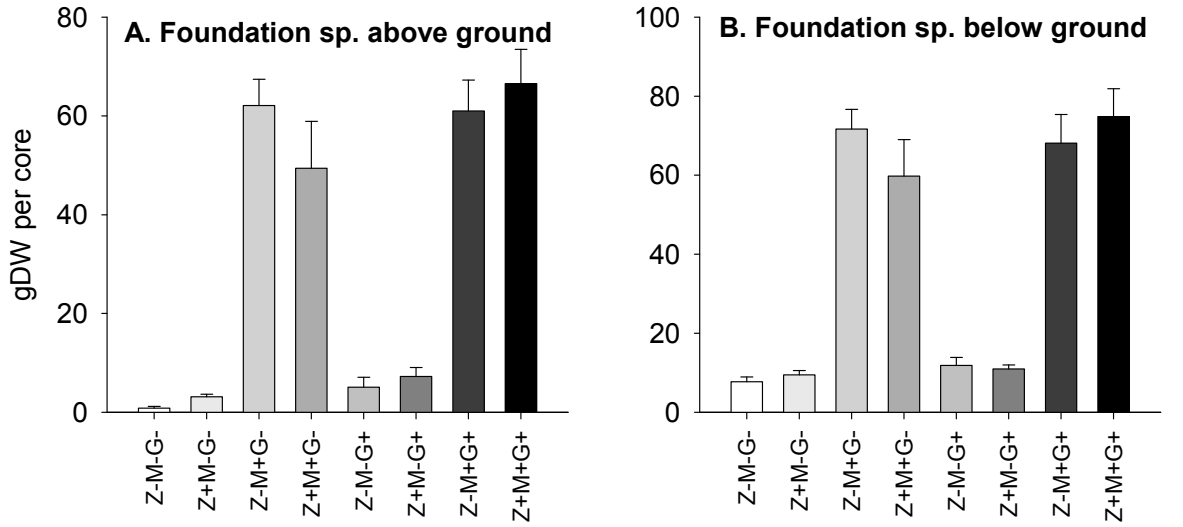
Appendix 1. Results from factorial permutation based ANOVA (A-M) and MANOVA (N) testing for reciprocal impact of three foundation species on 'their own biomass' (A-D) and densities (E-J) and univariate (K-M) and multivariate (N) community structures of associated invertebrates. Treatment; Z = *Zostera marina* removal (native seagrass), M = *Mytilus edulis* addition (native mussel), G = *Gracilaria vermiculophylla* addition (invasive red seaweed). The G- treatments was excluded from analysis of *Gracilaria* biomass and the M- treatment from *Mytilus* biomass, because these foundation species had little opportunity to colonize control plots (i.e., with zero biomass at the end of the experiment).

Response	Treatment	Df	SS	F	P
A. *Zostera* Above-ground	M	1	9.057	3.061	0.0892
	Z	1	83.952	28.371	**0.0002**
	G	1	12.168	4.112	**0.0426**
	M × Z	1	6.653	2.248	0.1384
	M × G	1	5.537	1.871	0.1850
	Z × G	1	9.073	3.066	0.0812
	M × Z × G	1	6.490	2.193	0.1542
	Residual	30	88.771		
B. *Zostera* Below-ground	M	1	36.658	6.113	**0.0178**
	Z	1	2.562	0.427	0.5126
	G	1	7.308	1.219	0.2804
	M × Z	1	2.040	0.340	0.5676
	M × G	1	18.298	3.052	0.0888
	Z × G	1	1.626	0.271	0.6076
	M × Z × G	1	0.436	0.073	0.7818
	Residual	30	179.890		
C. *Gracilaria*	M	1	32.6	0.914	0.3542
	Z	1	27.5	0.770	0.3939
	M × Z	1	21.2	0.593	0.4531
	Residual	15	534.7		
D. *Mytilus*	Z	1	463	1.908	0.1861
	G	1	35	0.145	0.7080
	Z × G	1	380	1.567	0.2286
	Residual	16	3880		
E. Gastropods	M	1	309.830	2.614	0.1210
	Z	1	2.719	0.023	0.9050
	G	1	469.450	3.961	**0.0490**
	M × Z	1	57.072	0.482	0.5560
	M × G	1	2.013	0.017	0.9220
	Z × G	1	2.719	0.023	0.8990
	M × Z × G	1	0.649	0.005	0.9470
	Residual	30	3555.600		
F. Bivalves	M	1	10.13	3.249	0.0762
	Z	1	0.18	0.057	0.8292
	G	1	28.41	9.112	**0.0026**
	M × Z	1	0.04	0.012	0.9146
	M × G	1	7.00	2.245	0.1522
	Z × G	1	2.98	0.955	0.3564
	M × Z × G	1	1.80	0.578	0.4728
	Residual	30	93.55		
G. Crustaceans	M	1	1288	0.806	0.4150
	Z	1	136	0.085	0.7850
	G	1	10610	6.640	**0.0080**
	M × Z	1	3	0.002	0.9740
	M × G	1	1144	0.716	0.4370
	Z × G	1	141	0.088	0.7530
	M × Z × G	1	11	0.007	0.9280
	Residual	30	47934		
H. Errant polychaetes	M	1	2.85	0.448	0.4910
	Z	1	5.29	0.833	0.3780
	G	1	100.95	15.881	**0.0020**
	M × Z	1	1.91	0.300	0.5620
	M × G	1	0.29	0.045	0.8370
	Z × G	1	26.41	4.154	0.0580
	M × Z × G	1	3.68	0.578	0.4550
	Residual	30	190.70		

Appendix 1 (continued).

Response	Treatment	Df	SS	F	P
I. Echinoderms	M	1	10.376	1.230	0.2926
	Z	1	0.212	0.025	0.8792
	G	1	75.112	8.903	**0.0050**
	M × Z	1	0.024	0.003	0.9618
	M × G	1	0.053	0.006	0.9334
	Z × G	1	0.006	0.001	0.9826
	M × Z × G	1	0.712	0.084	0.7706
	Residual	30	253.100		
J. All organisms	M	1	4205	1.360	0.2794
	Z	1	119	0.039	0.8540
	G	1	22609	7.311	**0.0036**
	M × Z	1	140	0.045	0.8494
	M × G	1	1199	0.388	0.5740
	Z × G	1	256	0.083	0.7854
	M × Z × G	1	1	0.000	0.9884
	Residual	30	92778		
K. Richness	M	1	16.52	4.281	**0.0480**
	Z	1	12.99	3.366	0.0718
	G	1	113.65	29.444	**0.0002**
	M × Z	1	0.15	0.038	0.8500
	M × G	1	12.99	3.366	0.0802
	Z × G	1	4.29	1.111	0.2968
	M × Z × G	1	0.29	0.075	0.7908
	Residual	30	115.80		
L. Diversity	M	1	0.775	7.897	**0.0106**
	Z	1	0.047	0.475	0.4936
	G	1	2.069	21.095	**0.0002**
	M × Z	1	0.020	0.200	0.6480
	M × G	1	0.766	7.809	**0.0076**
	Z × G	1	0.116	1.186	0.2874
	M × Z × G	1	0.118	1.199	0.2982
	Residual	30	2.943		
M. Evenness	M	1	0.001	0.054	0.8126
	Z	1	0.020	1.353	0.2488
	G	1	0.011	0.726	0.3976
	M × Z	1	0.002	0.123	0.7180
	M × G	1	0.002	0.155	0.7044
	Z × G	1	0.004	0.269	0.6224
	M × Z × G	1	0.015	0.999	0.3248
	Residual	30	0.447		
N. Community	M	1	3506	2.719	**0.0126**
	Z	1	1837	1.424	0.2034
	G	1	11616	9.007	**0.0002**
	M × Z	1	924	0.717	0.6448
	M × G	1	2590	2.008	0.0634
	Z × G	1	1323	1.026	0.4314
	M × Z × G	1	1150	0.892	0.5186
	Residual	30	38687		

Appendix 2. Effects of the three foundation species *Gracilaria vermiculophylla* (G±), *Zostera marina* (Z±), and *Mytilus edulis* (M±) on their combined biomass. The experiment was conducted in a seagrass bed, removing above-ground *Zostera* leaves and adding *Gracilaria* and *Mytilus* in all 2×2×2 treatment-combinations (N = 5 for most treatment-combinations, except N = 4 for Z+M-G- and Z+M-G+).



Appendix 3. Incorporations of *Gracilaria* (left) and *Zostera* (right) into *Mytilus* byssal threads. Incorporation may stabilize the plants, in particular *Gracilaria* that is only loosely entangled in and around seagrass leaves. However, in the present experiment pegs were used to stabilize *Gracilaria* and physical facilitation through byssal incorporation did therefore not influence our results.

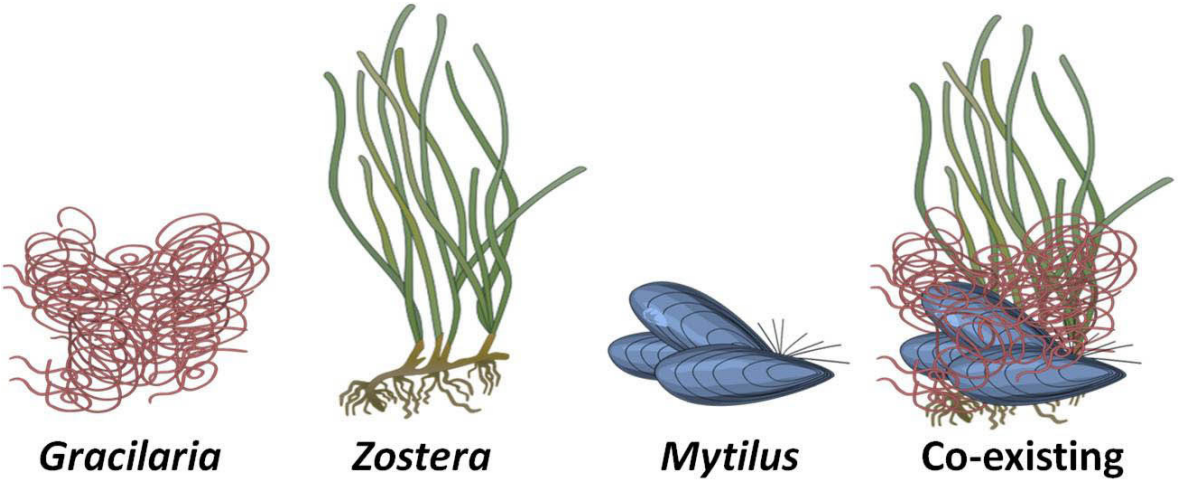
Appendix 4. Pearsons correlation coefficient R between univariate variebles. Significant results are in bold. FS-Tot = total above and below ground biomass of all 3 foundation species, FS-Abo = total above ground biomass of all 3 foundation species, Z = *Zostera marina* (native seagrass), M = *Mytilus edulis* (native mussel), G = *Gracilaria vermiculophylla* (invasive red seaweed). RICH, EVE, DIV, ALL, GAS, BIV, CRU, ERR = taxonomic richness, Pilou's evenness, Shannon's diversity, densities of all, gastropods, bivalves, crustaceans and errant polychaets associated with foundation species.

		FS-TOT	FS-Abo	Z-Bel	Z-Abo	G	M	RICH	EVE	DIV	ALL	GAS	BIV	CRU	ERR
HFABO	R	0.974													
	p	**0.000**													
ZOSR	R	0.456	0.310												
	p	**0.004**	0.058												
ZOSL	R	0.136	0.164	0.190											
	p	0.414	0.324	0.254											
GRA	R	0.270	0.294	-0.108	-0.241										
	p	0.102	0.073	0.518	0.144										
MYTTOT	R	0.928	0.930	0.341	0.052	0.066									
	p	**0.000**	**0.000**	**0.036**	0.756	0.692									
RICH	R	0.437	0.472	-0.012	-0.015	0.739	0.242								
	p	**0.006**	**0.003**	0.945	0.927	**0.000**	0.143								
PILE	R	-0.207	-0.230	-0.027	0.078	-0.255	-0.133	-0.304							
	p	0.212	0.165	0.874	0.642	0.122	0.426	0.064							
SHAH	R	0.278	0.317	-0.037	0.045	0.583	0.133	0.849	0.143						
	p	0.091	0.052	0.827	0.790	**0.000**	0.427	**0.000**	0.391						
ABUTOT	R	0.614	0.629	0.167	-0.095	0.775	0.406	0.826	-0.430	0.599					
	p	**0.000**	**0.000**	0.316	0.571	**0.000**	**0.012**	**0.000**	**0.007**	**0.000**					
GAS	R	0.486	0.496	0.183	-0.102	0.499	0.299	0.682	-0.558	0.430	0.854				
	p	**0.002**	**0.002**	0.273	0.541	**0.001**	0.068	**0.000**	**0.000**	**0.007**	**0.000**				
BIV	R	0.190	0.235	-0.278	-0.148	0.616	0.121	0.660	-0.051	0.632	0.489	0.294			
	p	0.252	0.156	0.091	0.376	**0.000**	0.468	**0.000**	0.759	**0.000**	**0.002**	**0.074**			
CRU	R	0.399	0.431	-0.151	-0.121	0.846	0.230	0.831	-0.287	0.650	0.772	0.545	0.602		
	p	**0.013**	**0.007**	0.365	0.470	**0.000**	0.166	**0.000**	0.080	**0.000**	**0.000**	**0.000**	**0.000**		
ERR	R	0.356	0.351	0.257	-0.120	0.668	0.170	0.531	-0.223	0.386	0.657	0.447	0.182	0.492	
	p	**0.028**	**0.031**	0.119	0.472	**0.000**	0.307	**0.001**	0.178	**0.017**	**0.000**	**0.005**	0.275	**0.002**	
ECH	R	0.335	0.353	0.028	-0.334	0.695	0.171	0.658	-0.379	0.445	0.759	0.707	0.521	0.541	0.464
	p	**0.040**	**0.030**	0.866	**0.040**	**0.000**	0.305	**0.000**	**0.019**	**0.005**	**0.000**	**0.000**	**0.001**	**0.000**	**0.003**

Appendix 5. Stepwise linear regressions on community structure (A-C) and densities (D-I) of invertebrate associated with *Zostera marina* (Z; native seagrass – separated into above- vs. below-ground biomass), *Mytilus edulis* (M, native mussel) and *Gracilaria vermiculophylla* (G, invasive red seaweed). Responses were treated as continuous variables using dry weight of each species per core. Models were evaluated from step-wise regression on untransformed data. *Gracilaria* biomass explained most of the data variability. Violations of linear regression assumptions imply that P-values should be interpreted with caution.

Response	Predictors	R	R^2	R^2 Adjusted	P
A. Richness	M, Z-Above, G, Z-Below	0.825	0.681	0.642	**0.0000**
	M, Z-Above, G	0.824	0.680	0.651	**0.0000**
	Z-Above, G	0.818	0.669	0.650	**0.0000**
	G	0.801	0.642	0.632	**0.0000**
B. Evenness	M, Z-Above, G, Z-Below	0.435	0.189	0.091	0.1298
	M, Z-Above, G	0.435	0.189	0.117	0.0651
	M, G	0.428	0.183	0.136	**0.0290**
	G	0.420	0.176	0.153	**0.0087**
C. Diversity	M, Z-Above, G, Z-Below	0.488	0.238	0.146	0.0555
	M, Z-Above, G	0.484	0.234	0.166	**0.0269**
	M, G	0.452	0.205	0.159	**0.0182**
	G	0.401	0.161	0.138	**0.0126**
D- All organims	M, Z-Above, G, Z-Below	0.856	0.733	0.700	**0.0000**
	M, G, Z-Below	0.855	0.731	0.708	**0.0000**
	M, G	0.854	0.730	0.714	**0.0000**
	G	0.842	0.710	0.702	**0.0000**
E. Gastropods	M, Z-Above, G, Z-Below	0.772	0.596	0.547	**0.0000**
	M, G, Z-Below	0.768	0.590	0.554	**0.0000**
	G, Z-Below	0.758	0.574	0.550	**0.0000**
F. Bivalves	M, Z-Above, G, Z-Below	0.651	0.423	0.354	**0.0009**
	M, G, Z-Below	0.647	0.418	0.367	**0.0003**
	M, G	0.618	0.382	0.347	**0.0002**
	G	0.590	0.348	0.330	**0.0001**
G. Crustaceans	M, Z-Above, G, Z-Below	0.830	0.689	0.652	**0.0000**
	M, Z-Above, G	0.830	0.689	0.662	**0.0000**
	M, G	0.830	0.689	0.671	**0.0000**
	G	0.822	0.676	0.667	**0.0000**
H. Errantia	M, Z-Above, G, Z-Below	0.769	0.591	0.541	**0.0000**
	Z-Above, G, Z-Below	0.769	0.591	0.555	**0.0000**
	G, Z-Below	0.767	0.588	0.564	**0.0000**
I. Echinoderms	M, Z-Above, G, Z-Below	0.858	0.736	0.704	**0.0000**
	Z-Above, G, Z-Below	0.855	0.731	0.707	**0.0000**
	G, Z-Below	0.846	0.716	0.700	**0.0000**
	G	0.839	0.705	0.696	**0.0000**

Appendix 6. Examples of architectural, morphological, anatomical and physiological differences between three co-occurring foundation species. Top figure; physiognomy of *Gracilaria vermiculophylla* (invasive red seaweed), *Zostera marina* (native seagrass) and *Mytilus edulis* (native mussel). Bottom table; qualitative above ground characteristics associated with the three foundation species. The list is by no means exhaustive – but highlight that different mechanisms can cause different communities to be associated with different foundation species.



#	Characteristics (above ground)	*Gracilaria*	*Zostera*	*Mytilus*
1	Dry weight (g/plot)	Low (4)	Low (3)	High (15)
2	Wet weight	High	Medium	Low
3	Volume	High	Medium	Low
4	Interstitial spacing	High	Medium	Low
5	Key building materials	Alginates-Cellulose	Lignin-Cellulose	Calcium carbonate
6	Rigidity	Low	Medium	Very high
7	Colour	Red	Green	Blue
8	Activity	Compression with waves	Compression with waves	Constant filtering
9	Branching pattern	Complex	None	None
10	Cover of sessile epibiota	Low	Low	High
11	Sessile epibiota species	Polychaetes (*Spirobis*)	Filamentous alga (*Ectocarpus*, *Callithamnium*)	Various animals (barnacles, bryozoa, hydrozoa)
12	Resource acquisition	Uptake of nutrients from water	Uptake of nutrients from water and sediments	Filter suspended particles
13	Release products	Branches break	Old leaves are shed	Organic faecal particles
14	Canopy height under calm condition (cm)	20	30	10
15	Canopy height under wavy conditions (cm)	10	10	10
16	Morphological complexity	Cylindrical branches	Sheet leaves	Ellipsoid cylinder
17	Perimeter	High	Medium	Low
18	Consumers	Grazers	Grazers	Predators
19	Resistance to consumers	Induced Eicosanoids	Lignin/cellulose	$CaCO_3$ shell