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Research Article

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

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Abstract

Non-native foundation species occasionally invade habitats occupied by native foundation species. Little is known, however, about reciprocal effects of native and non-native foundation species and cascading effects on organisms that depend on foundation species. In a 3-factorial field experiment, we tested for: (1) reciprocal effects between the invasive red alga *Gracilaria vermiculophylla* and two native co-occurring foundation species, the mussel *Mytilus edulis* and the seagrass *Zostera marina*; and (2) effects on mobile macro-invertebrates associated with the three foundation species. We found a negative effect of *G. vermiculophylla* on the above-ground biomass of *Z. marina* and a positive effect of *M. edulis* on *Z. marina* below-ground biomass, but no other significant effects between the three foundation species. Both *M. edulis* and *G. vermiculophylla* had positive effects on invertebrate richness and diversity, but *Gracilaria* also had positive effects on densities of most invertebrate taxa. Additional correlation analyses showed that the abundance of invertebrates increased with the biomass of *G. vermiculophylla*, indicating density-mediated habitat cascades in invaded seagrass beds. The strong facilitation by *G. vermiculophylla* could be related to its complex morphology that creates a 3D mosaic of structures and interstitial spaces for different sizes of invertebrates to occupy. Although these results are constrained by the experimental design (one month duration, small plot size, embedded in seagrass meadow, relatively low invader density, invader physically attached with pegs) we suggest that our findings are typical when an invader is structurally complex and mainly occurs in localized patches.

Key words: facilitation cascade; habitat cascade; ecosystem engineer; seaweeds; seagrass; mussels, non-native species, co-existence

Introduction

Invasive non-native species typically have a negative impact on the abundance and diversity of native species and community structure (Vilà et al. 2011). However, non-native species can also be foundation species (Dayton 1972) that increase biodiversity by creating and modifying habitats for other organisms (Rodriguez 2006; Wallentinus and Nyberg 2007). Impacts of non-native foundation species are likely to depend on the habitat type that is invaded. For example, non-native foundation species that invade habitats that lack functionally similar native foundation species are likely to cause positive effects on associated invertebrates, because these organisms may use the non-native species as food, habitat, for stress amelioration, and protection from grazers and predators (Rodriguez 2006; Wallentinus and Nyberg 2007; Thomsen et al. 2010).

However, most non-native foundation species probably invade habitats that are already dominated by one or more foundation species (Staehr et al. 2000; Levin et al. 2002; Ward and Ricciardi 2010; Hammann et al. 2013), and net impact on the local communities in these cases are more complex. For example, if the invasive and native foundation species have negative effects on each other (e.g., competing for limited resources), facilitation of organisms that depend on the invasive species could be offset by loss of organisms that depend on the negatively-affected native foundation

species. Predicting invasion impacts on native foundation species and associated communities will be even more complicated in habitats where multiple native foundation species co-exist. For example, in mixed seagrass-mollusc habitats, these native foundation species may have both positive and negative effects on each other, depending on densities, environmental conditions, and species involved (Reusch and Chapman 1995; Reusch and Williams 1998; Peterson and Heck 2001a; Peterson and Heck 2001b; Vinther et al. 2008; Vinther et al. 2012). Currently, few field experiments have tested how marine invaders affect multiple foundation species and their associated communities, making it difficult to understand and predict invasion impacts in many natural systems.

Gracilaria vermiculophylla (Ohmi) Papenfuss (hereafter *Gracilaria*) is a coarsely-branched red alga that originates from the northwest Pacific. This species, like most other seaweeds, modifies the local abiotic environment (e.g., sedimentation, anoxia, light levels), provides habitat for numerous sessile and mobile species (Thomsen et al. 2010), and can therefore be considered a foundation species. *Gracilaria* has spread to shallow-water wave-protected estuaries and coastal lagoons along 1000s of km of coastline in the East Pacific, West Atlantic, East Atlantic, and Mediterranean Sea, making it one of the world's most successful marine invasive species (Kim et al. 2010; Sfriso et al. 2010). Within these ecosystems, *Gracilaria* is common on 'barren sediments' (Nejrup and Pedersen 2010; Sfriso et al. 2012) as well as habitats occupied by native foundations species, like salt marshes (Thomsen et al. 2009), seagrass meadows (Cacabelos et al. 2012; Hernández Cordero et al. 2012), fucoid seaweed beds (Weinberger et al. 2008; Hammann et al. 2013), polychaete reefs (Thomsen and McGlathery 2005; Byers et al. 2012) and bivalves reefs (Thomsen and McGlathery 2006). Impacts of *Gracilaria* have been documented on some of these native foundation species and/or their associated communities, typically demonstrating context-dependency. For example, accumulations of unattached *Gracilaria* have negative effects on oysters (and sessile species living on the oyster reefs) (Thomsen and McGlathery 2006), but appear to have less impact on functionally similar mussels, as *Gracilaria* is often found attached to the byssal threads of the blue mussel *Mytilus edulis* (hereafter *Mytilus*) (Weinberger et al. 2008; Thomsen et al. 2010). Furthermore, *Gracilaria* has no effect on the seagrass *Zostera marina* (hereafter *Zostera*) in low densities and at low temperature, but negative effects when occurring in high densities under high temperatures (Hoeffle et al. 2011). However, we are not aware of any studies that have tested for effects of *Gracilaria* on two native foundation species, and potential cascading effects on the communities that are associated with native foundation species.

We therefore tested, in a factorial field experiment, the hypotheses that *Gracilaria* has (1) negative effects on the co-occurring native foundation species *Zostera* and *Mytilus*, but (2) positive effects on invertebrate communities by providing more and/or different habitat structure in mixed *Zostera-Mytilus* beds.

Methods

Study site

The study was conducted in a seagrass bed at 2 m depth in the northern part of Odense Fjord, Denmark (55.52659 °N, 10.531254 °E). *Mytilus*, *Zostera* and *Gracilaria* are all common in this estuary, but *Mytilus* and *Gracilaria* are sparse at this specific site making it easier to conduct 'addition-experiments' and avoid colonization from settling or drifting *Mytilus* and *Gracilaria* into control plots. During the experiment, water temperature varied from 16 to 20°C. At the study site salinity varies seasonally between 13 and 22, and turbidity is high with Secchi depth varying between 1-6 m but reduced to near 0 m following periods of strong east-southeast winds (abiotic data from the Danish National Aquatic Monitoring and Assessment Program; DNAMAP, https://oda.dk).

Field methods

We conducted a 3-factorial orthogonal experiment to test for reciprocal effects between '*Gracilaria'* (G±), '*Zostera'* (Z±) and '*Mytilus'* (M±). Each of the three foundation species was manipulated as a 'presence' ('+') vs. 'absence' ('-') treatment in a 2×2×2 design. The abundance of each foundation species was manipulated in 40 0.4×0.4 m plots, i.e. with 5 replicates for each of the eight treatment-combinations. We did not use cages, thereby avoiding cage-artefacts, including changes to hydrodynamics, light conditions, sediment/seaweed-trapping and attracting animals.

Zostera biomass was manipulated by removing all above-ground biomass from the 20 Z- plots by cutting all leaves with scissors at the

sediment surface (Herkül and Kotta 2009). Seagrass in the Z+ plots were disturbed with hands simulating scissors disturbances without removing any biomass.

Mytilus biomass was manipulated by adding four live individuals to the 20 M+ plots (average size = 37.0 ± 8.1 g wet weight or 6.3 ± 0.5 cm shell length; based on 20 randomly chosen specimens; all reported values are means ± SE). The mussels were collected from a nearby site (<1 km away). We gently scraped off large attached sessile species, e.g., barnacles, as well as seaweed fragments incorporated into byssal threads. The mussels were then carefully added to the sediment surface around the seagrass stems without breaking any leaves.

Gracilaria biomass was manipulated by adding c. 3 kg wet weight (WW) m^{-2} to the 20 G+ plots. This *Gracilaria* was collected from the nearby Holckenhavn Fjord (55°17.8′N, 10°46.2′E) because the alga at this site has little epiphyte cover and few clinging invertebrates. The collected *Gracilaria* was brought ashore, shaken to release the few mobile macro-invertebrates, and further inspected for clinging invertebrates which were then removed by hand. *Gracilaria* was fixed to the substratum in the 20 G+ plots by inserting 10 u-bent thin metal pegs flush with the sediment surface (Thomsen et al. 2012). A similar number of pegs were inserted into the 20 G- plots flush with the sediment surface to control for peg-artefacts (so that any peg-induced disturbances were similar between plots).

The experiment was initiated on 28 August 2012 and ran for 4 weeks. This is a common time period for such experiments where unattached seaweeds persist in a specific seagrass patch and a common time interval to run seaweed-seagrass impact studies (e.g., Nelson and Lee 2001; Holmer and Nielsen 2007; Martínez-Lüscher and Holmer 2010; Höffle et al. 2011; Holmer et al. 2011). The experiment was conducted in late summer/early fall because key invertebrate species produce recruits during this period and could be facilitated by the three foundation species (Thomsen 2010).

Collections and laboratory methods

At the end of the experiment we collected a 290 cm^2 circular core (with sharp edges) from each plot centre. A mesh-bag (1-mm mesh size) covered the top of the core to ensure mobile animals did not escape. We approached each plot slowly before inserting the core over the centre. The core was hammered through the seagrass rhizomes and 10 cm into the sediment. A small shovel was used to dig up sediments into the mesh-bag attached to the core, to ensure all infaunal animals were collected. All core content was pushed into the bottom of the attached mesh-bag together with a plot-marker tag. The mesh-bag was then closed with a string and detached from the core. A new mesh-bag was attached to the core before approaching the next plot. Two of the 40 plots were lost (probably from fishing, vandalism from local snorkelers, or storms) resulting in two of the treatments (Z+M-G-; Z+M-G+) having 4 replicates. The 38 mesh-bags were shaken gently in the field to remove sediments and to 'stress' the collected invertebrates to avoid animal loss due to predation during transport to the laboratory.

Mesh-bags were kept cool until arrival at the laboratory where we immediately separated *Gracilaria*, *Zostera*, *Mytilus* and macro-invertebrates from any remaining sediments by sieving through a 2-mm mesh sieve. Foundation species and invertebrates were then separated from each other, and *Zostera* biomass was further separated into above- (leaves) and below-ground (root and rhizomes) biomass. The biomass of each foundation species was measured after drying at 60° C until no further biomass loss occurred (g DW per core). Invertebrates were immediately conserved in 70% alcohol and, over the following weeks, identified to the lowest practical taxonomic level (usually species) and counted. Amphipods were grouped together as a single taxonomic unit. Sedentary polychaetes were omitted from the analysis because many of these small fragile animals were lost or broken through sieving (data were therefore deemed unreliable).

Data analysis

Tests were conducted as factorial permutation-based ANOVA ('PERANOVA') on univariate responses and permutation-based MANOVA ('PERMANOVA') on multivariate community structure, where *Gracilaria*, *Mytilus* and *Zostera* treatments were considered fixed factors (Anderson et al. 2008). Both univariate and multivariate analyses were conducted with the PERMANOVA add-on to Primer v6 software package, using 4999 permutations (Clarke and Gorley 2006; Anderson et al. 2008). We also compared sum of square values to discuss what test factors explained most of the data variability (Levine and Hullet 2002).

We conducted univariate factorial analyses on the above and below ground biomass of *Zostera*, biomass of *Gracilaria*, biomass of *Mytilus*, on densities of gastropods, bivalves, crustaceans, echinoderms, errant polychaetes (these taxa together constituted >95% of sampled individuals) and total invertebrates density, as well as on invertebrate richness, diversity (Shannon index), and evenness (Pielou's index). All these univariate analyses were conducted using Euclidian distances on untransformed data under a reduced model (Anderson et al. 2008). Most univariate variables had homogeneous variances, although variances for invertebrate densities for the *Gracilaria* treatment were slightly hetero-geneous (Levines test, p > 0.005). However, we did not transform these responses, in part because ANOVA is relatively robust to variance heterogeneity in balanced replicated designs (Underwood 1997).

We did not include the G- and M- plots in the analysis of *Gracilaria* and *Mytilus* biomass, respectively, because these species had virtually no chance of colonizing their control plots (i.e., we found zero biomass in these controls). *Gracilaria* and *Mytilus* biomass were therefore analyzed with 2-factorial tests (Appendix 1C, D, Figure 1C, D). By contrast, biomass of *Zostera* were (like the invertebrate analyses) analyzed with 3-factorial tests because the below-ground biomass was not manipulated and because the above-ground biomass in the Z- treatments could potentially recover through (a) horizontal growth from seagrass adjacent to the plots, (b) vertical growth from cut leaves within the plots, and (c) seed germination (Herkül and Kotta 2009). We conducted tests on both total *Mytilus* biomass and *Mytilus* density (2–4 shells per core). The statistical results between *Mytilus* biomass and *Mytilus* density were similar and we therefore only present the biomass data here (this biomass can then be compared directly to the biomass of the two other foundation species).

We conducted multivariate 3-factorial analysis on the invertebrate species-sample matrix, using Bray Curtis similarity coefficient, and square-root transformed densities to downplay the importance of the dominant taxa (Anderson and Ter Braak 2003; Clarke et al. 2006). Note, however, that we found similar results when data were analyzed without transformation and with a more severe log (x+1) transformation (unpubl. data). The multivariate pattern was visualized with a 2D-PCO plot (Anderson et al. 2008).

Results

Impacts on foundation species

Not surprisingly, we found a highly significant effect of *Zostera* removals on its own above-ground biomass (Appendix 1A), with > 5 times more biomass in the Z+ plots (3.88 ± 0.65 g DW core^{-1}; all reported values are means ± SE) compared to the Z- plots (0.80 ± 0.15 g DW core^{-1}). Furthermore, adding *Gracilaria* significantly reduced *Zostera* above-ground biomass (G- = 2.85 ± 0.73 vs. G+ = 1.68 ± 31 g DW core^{-1}) although graphical comparisons suggested that most of the negative effects occurred in the presence of *Mytilus* (Figure 1A; compare Z+M+G- vs. Z+M+G+). We found near-significant effects (p < 0.1) of *Mytilus* on *Zostera* above-ground biomass (indicating that mussels have a positive net effect on seagrass biomass) and on the Z×G interaction (indicating that negative effects of *Gracilaria* were smaller in Z- than Z+ plots, Figure 1A).

Effects were less pronounced on the below-ground *Zostera* biomass (Appendix 1B, Fig. 1B); *Mytilus* had significant positive effects (M- = 6.78 ± 0.44 vs. M+ = 8.83 ± 0.66 g DW core^{-1}) and there was a near-significant M×G interaction (indicating that positive effects of *Mytilus* on *Zostera* was stronger in G- than G+ plots).

As expected, we found *Gracilaria* in all the G+ plots but none in G- plots (Figure 1C). ANOVA did not detect any effects of *Zostera* or *Mytilus* treatments on *Gracilaria* biomass (5.97 ± 1.35 g DW core^{-1}, all p-values > 0.35, Appendix 1C), even though there appeared to be more *Gracilaria* in the Z+M+ treatment.

We found 2–4 (all alive) *Mytilus* in the M+ but none in M- plots. 'Lost' mussels from the M+ plots had probably re-positioned themselves outside of the plot center (we found no empty shells to indicate predation). However, there was no pattern regarding what treatment-combinations had lost most mussels and this lack of pattern was reflected in the ANOVA on mussel biomass, which did not detect any effects of *Zostera* or *Gracilaria* treatments (53.53 g DW ± 3.44 core^{-1}, all p-values > 0.19, Appendix 1D, Figure 1D).

A comparison of the dry weight between the three foundation species (Figure 1A-C, Appendix 2) showed that *Mytilus* was dominant; however, *Gracilaria* and *Zostera* dominated 'visually' in the field (due to high volume-to-dry weight
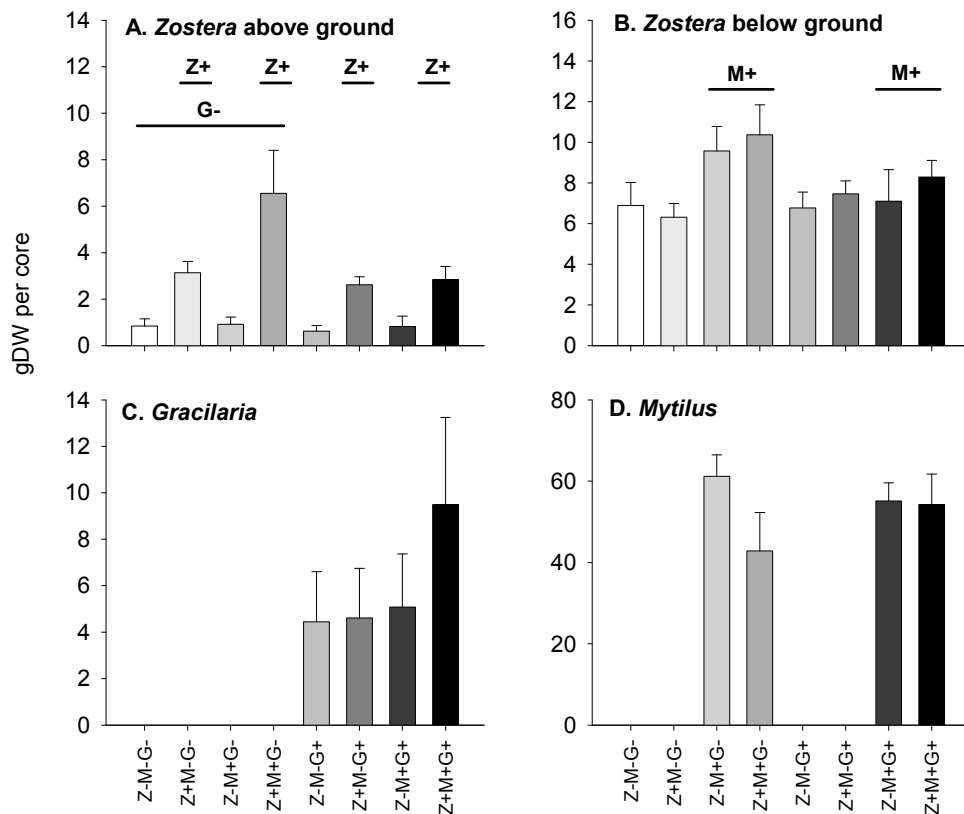
Figure 1. Effects of the three foundation species *Gracilaria vermiculophylla* (G±), *Zostera marina* (Z±), and *Mytilus edulis* (M±) on their own and each other's biomass. The experiment was conducted in a seagrass bed, removing the above-ground Zostera leaves and adding *Gracilaria* and *Mytilus* in all 2×2×2 treatment-combinations (N = 5 for most treatment-combinations, except N = 4 for Z+M-G- and Z+M-G+). We found no significant interaction effects; horizontal bars represent significant single-factor effects (see Appendix 1 and the result section for details). 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).

ratios of *Gracilaria* and *Zostera*; see Appendix 6 for examples of differences in species traits between the three foundation species).

Impact on associated invertebrates

A total of 22 taxa were identified in the 38 samples, dominated by gastropods and crustaceans (including 3 shrimp species - *Crangon crangon*, *Palaemon adspersus* and *Hippolyte varians*). The most common taxa were amphipods, the sea star *Asterias rubens* (recruits only), the crab *Carcinus maenas* (adults and recruits), the snails *Rissoa membranacea* and *Littorina littorea* (adults and recruits), and the isopod *Idotea baltica*. We also found 2 fish species (*Pomatoschistus microps*, *Pholis gunnellus*).

Statistical results were identical for all the taxonomic density responses (Figure 2), with significant positive effects of *Gracilaria* for gastropods (G+ = 15.16 ± 3.23 vs. G- = 8.12 ± 1.04 individuals core^{-1}; Figure 2A, Appendix 1E), bivalves (G+ = 2.31 ± 0.73 vs. G- = 0.32 ± 0.17 individuals core^{-1}; Figure 2B, Appendix 1F, note a near-significant effect of *Mytilus*), crustaceans (G+ = 34.79 ± 12.18 vs. G- = 0.89 ± 0.21 individuals core^{-1}; Figure 2C, Appendix 1G), errant polychaetes (G+ = 4.26 ± 0.74 vs. G- = 1.05 ± 0.34 individuals core^{-1}, Fig. 2D, Appendix 1H), echinoderms (G+ = 3.62 ± 0.82 vs. G- = 0.71 ± 0.29 individuals core^{-1}; Figure 2E, Appendix 1I) and all taxa combined (G+ = 61.01 ± 16.94 vs. G- = 11.68 ± 1.48 individuals core^{-1}; Figure 2F, Appendix 1J).
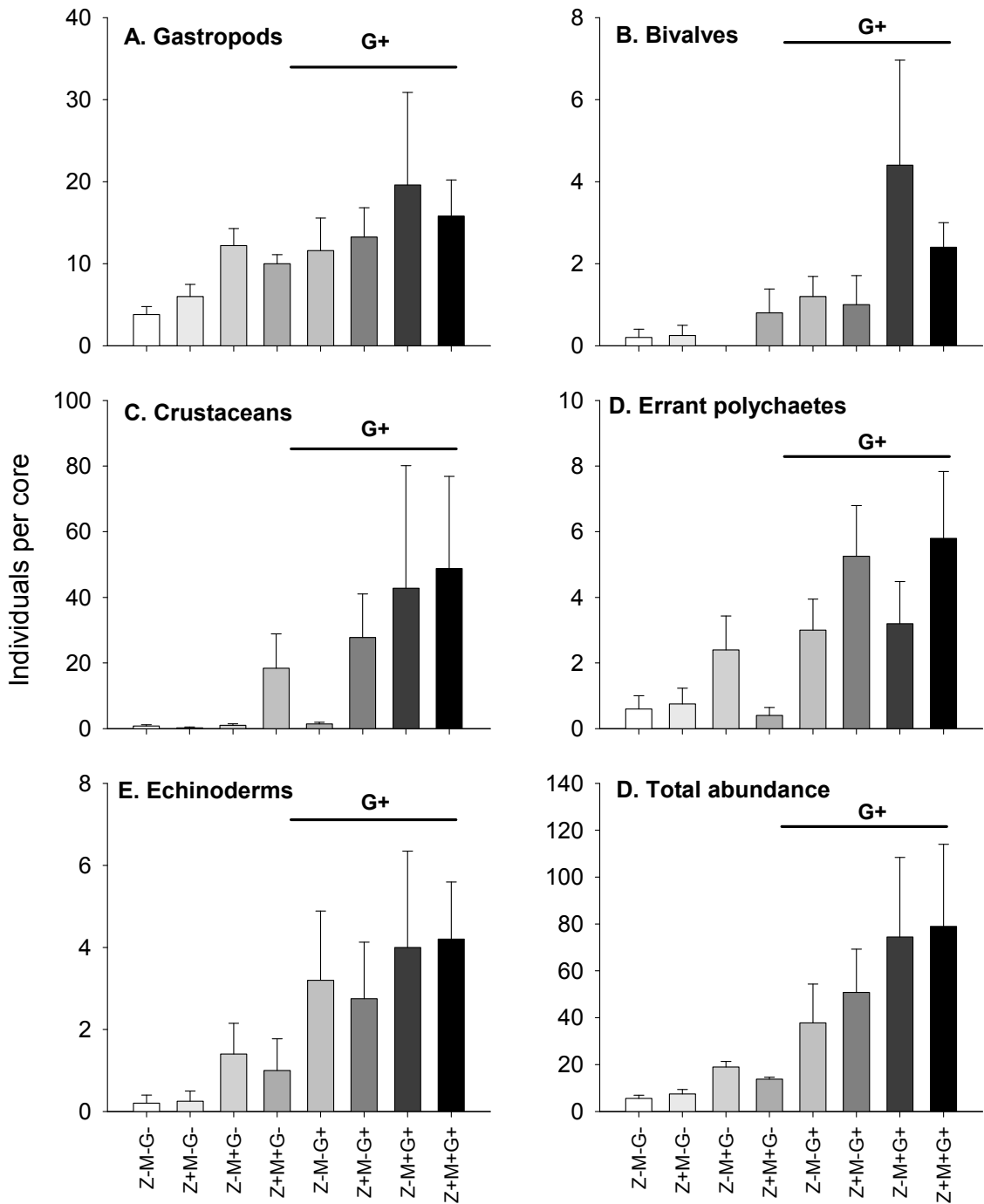
Figure 2. Effects of the three foundation species *Gracilaria vermiculophylla* (G±), *Zostera marina* (Z±), and *Mytilus edulis* (M±) on the density of invertebrates. The experiment was conducted in a seagrass bed, removing above-ground *Zostera* leaves and adding *Gracilaria* and *Mytilus* in all 2×2×2 treatment-combinations (N = 5 for most treatment-combinations, except N = 4 for Z+M-G- and Z+M-G+). We found no significant interaction effects; horizontal bars represent significant single-factor effects (see Appendix 1 and the result section for details).
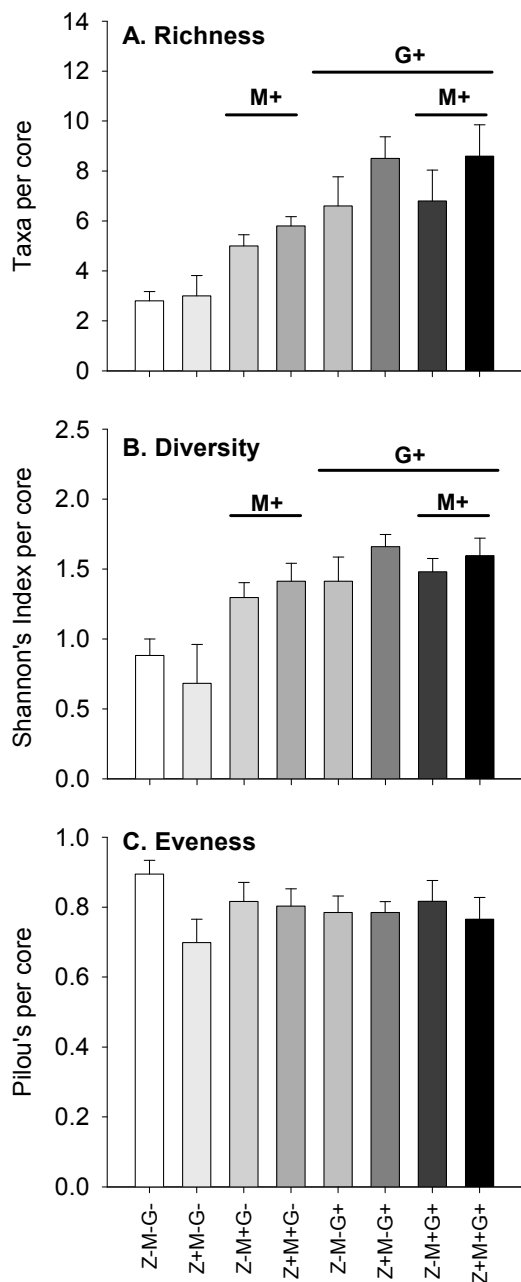
Figure 3. Effects of the three foundation species *Gracilaria vermiculophylla* (G±), *Zostera marina* (Z±) and *Mytilus edulis* (M±) on the taxonomic richness, Shannon's diversity, and Pilou's evenness of invertebrates. The experiment was conducted in a seagrass bed, removing above-ground *Zostera* leaves 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+). Horizontal bars represent significant single-factor effects (see Appendix 1 and results for details). There was a significant G×M interaction on diversity; *Mytilus* had a positive effect in the absence (M+G- = 1.35 vs. M-G- = 0.79) but not presence (M+G+ = 1.52 ± vs. M-G+ = 1.54) of *Gracilaria*.

We found similar results on taxonomic richness (Figure 3A, Appendix 1K), being significantly higher in treatments with *Gracilaria* (G+ = 7.57 ± 0.57 vs. G- = 4.21 ± 0.38 taxa core-1) and *Mytilus* (M+ = 6.67 ± 0.69 vs. M- = 5.11 ± 0.53 taxa core^{-1}) compared to controls.

The analysis on diversity was the only test with a significant interaction term. In this test, we found a significant G×M interaction on diversity (Figure 3B, Appendix 1L) as well as significant G and M single factor effects, where most sums-of-squares data variability was accounted for by *Gracilaria*. This G×M interaction showed that *Mytilus* had a positive effect on invertebrate diversity in the absence (M+G- = 1.35 ± 0.08 vs. M-G- = 0.79 ± 0.13) but not presence M+G+ = 1.52 ± 0.11 vs. M-G+ = 1.54 ± 0.07) of *Gracilaria*.

Evenness (Pielou's index) was the only invertebrate response not affected by any of the treatments (Figure 3C, Appendix M).

Finally, we found significant effects of *Gracilaria* and *Mytilus* on the multivariate community structure, and a near-significant G×M interaction, with *Gracilaria* treatments explaining three times more of the data variability than *Mytilus* (Appendix 1N). This multivariate results result was visualized with a 2D-PCO plot (Figure 4) showing that communities with *Gracilaria* were quite similar (as demonstrated by a relatively tight sample cluster).

Discussion

Non-native foundation species often invade habitats already occupied by native foundation species but little is known about how they interact with each other (Ward and Ricciardi 2010) and possible cascading effects on the organisms that depend on these foundation species (Thomsen et al. 2010). Here we documented that the invasive seaweed *Gracilaria* had relatively low impact on two co-occurring native foundation species, and vice versa, but that it had a strong positive effect on habitat-associated invertebrates. Although our results are constrained by the experimental set-up (i.e., a short time period, relatively small plot sizes, embedded in seagrass meadow, with relatively low invader density) we hypothesize that these findings are typical when invaders are structurally more complex than the native species and when they occur in localized patches over space and time.

Figure 4. PCO-plot of the multivariate community structure of mobile invertebrates associated with the foundation species *Gracilaria vermiculophylla* (G±), *Zostera marina* (Z±), and *Mytilus edulis* (M±). Legend abbreviations correspond to 8 treatment-combinations; n=Z-M-G-; Z=Z+M-G-; M=Z-M+G-; ZM=Z+M+G-; G=Z-M-G+; ZG=Z+M-G+; MG=Z-M+G+; ZMG=Z+M+G+ (N = 5 for most treatment-combinations, except N = 4 for Z+M-G- and Z+M-G+).
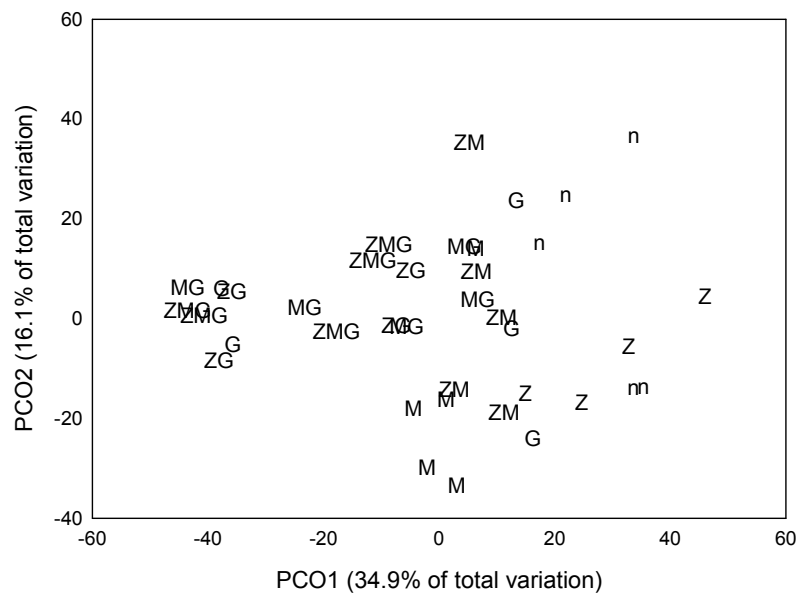
Impact on foundation species

The relatively small effects of the three foundation species on each other are perhaps not surprising. Previous laboratory experiments testing for impacts of *Gracilaria* on *Zostera* done over a similar time period also showed small negative effects (Martinez-Luscher and Holmer 2010; Hoeffle et al. 2011). These findings were supported by our field experiment as *Gracilaria* had negative effect on above-ground, but no effect on below-ground, biomass of *Zostera*. The negative effect of *Gracilaria* on *Zostera* is likely associated with reduced levels of light, nutrients, oxygen and water currents around the basal seagrass leaves and meristem in the presence of the seaweed (Holmer and Nielsen 2007; Holmer et al. 2011).

In contrast to *Gracilaria*, *Mytilus* facilitated *Zostera,* perhaps because bivalves through their filtering capacity and metabolic activities can increase nutrients and decrease turbidity (Reusch et al. 1994; Peterson and Heck 2001b), and their byssal threads may stabilize and protect the rhizomes (Reusch and Chapman 1995; Peterson and Heck 2001b). Interactions between bivalves and seagrasses can, however, be both positive and negative depending on spatial locations, eutrophication levels, wave exposure, and abundance (Reusch et al. 1994; Reusch and

Chapman 1995; Vinther et al. 2008; Vinther et al. 2012). For example, Vinther et al. (2012) found correlative evidence for a threshold of co-existence, as *Zostera* was never found when *Mytilus* was present at more than 1.6 kg WW m^{-2}.

Although we did not find any effects of *Mytilus* on *Gracilaria* we noted that *Gracilaria* was incorporated into the mussels byssal threads in all M+ plots (a few *Zostera* leaves and rhizomes were also incorporated; see Appendix 4). We therefore expect that this bivalve can be important in stabilizing *Gracilaria* populations, as unattached *Gracilaria* is susceptible to removals during storms and by tidal currents (Reusch and Chapman 1995; Thomsen 2004). Therefore, if we had not used pegs to stabilize *Gracilaria,* it might have occurred in lower abundances in the M- than M+ plots.

Finally, the *Mytilus* itself was not significantly affected by either *Gracilaria* or *Zostera*, which is consistent with observations that this mussel often is found together with *Gracilaria* (Weinberger et al. 2008; Thomsen et al. 2010) and *Zostera* (Vinther et al. 2012). Again, it is possible that high densities of *Gracilaria* over spatially extensive areas may cause negative impacts on *Mytilus* through oxygen limitation, water current reductions, and by interfering with bivalve filtration capacity (Norkko and Bonsdorff 1996; Tyler 2007; Vinther et al. 2008; Vinther et al. 2012).

From our experiment it appears that the three foundation species can co-exist when they occur in low to medium abundances and over small/short time scales. However, if *Gracilaria* and *Mytilus* are found in high abundance, in large areas, and over longer time frames, *Zostera* could be dramatically inhibited, particular under stressful conditions, such as high temperatures, excessive nutrient levels, and low light levels (Huntington and Boyer 2008; Vinther et al. 2008; Hoeffle et al. 2011; Vinther et al. 2012).

Impacts on associated invertebrates

The three foundation species differed in their habitat suitability for the associated invertebrates; *Gracilaria* provided better habitat than *Mytilus,* which was more important than *Zostera* (Figure 2–3, Sum-of-Squares in Appendix 1).

It may appear surprising that we, in contrast to established theory (Heck et al. 2003; Boström et al. 2006), found no clear facilitation from the seagrass on invertebrates. However, the experimental habitat (a seagrass bed) likely causes strong spill-over edge effects of seagrass-associated invertebrates into removal plots. Furthermore, above-ground removals were not 100% efficient and showed partial recovery causing all plots to have some seagrass above-ground biomass (Figure 1A). Finally, below-ground biomass was not manipulated, leaving a relatively large and similar below-ground biomass in all plots. We expect stronger facilitation of seagrass on invertebrates if we instead add *Zostera* to *Mytilus* or *Gracilaria* beds or to unvegetated sediments.

Perhaps more surprisingly, adding *Mytilus* only indicated weak (non-significant) positive effects on invertebrate densities. Again, *Mytilus* has, like *Zostera*, been shown to facilitate mobile invertebrates compared to 'barren' sediments (Markert et al. 2010). The lack of a significant effect on invertebrate densities by *Mytilus* could be caused by large data variability - perhaps being swamped by strong *Gracilaria* effects. Still, graphical inspection suggests that *Mytilus* do facilitate invertebrates because mean values were higher across the M+ than the M- treatments for all taxonomic groups (Figure 2), thereby supporting the pattern observed by Markert et al. (2010). Indeed, we did find significant effects on multivariate community structures and increases in taxonomic richness and diversity (Appendix 1), leading us to

conclude that adding *Mytilus* to seagrass beds increased invertebrate biodiversity through provision of additional and/or different habitat.

Gracilaria had a much stronger positive impact on invertebrate densities than did *Zostera* and *Mytilus*, and we found 38, 5, 5, 4, 4 and 2 times more crustaceans, total invertebrates, echinoids, bivalves, errant polychaetes and gastropods, respectively, when *Gracilaria* was present (= 'Magnification Ratios', see Thomsen et al. 2010). Furthermore, *Gracilaria* had positive effect on taxonomic richness and diversity, and modified the multivariate community structure causing invertebrate communities to be relatively more homogenous in the presence of *Gracilaria*. This strong across-the-board facilitation by *Gracilaria* is not surprising because its coarsely branched fronds provide a complex 3-dimensional habitat which is characterized by different-sized interstitial spaces for different species in different ontogenetic phases to occupy, a large attachment space for bivalves to recruit onto, and likely also high protection from predators. Similar facilitation of invertebrates has been found in other habitats (Nyberg et al. 2009; Byers et al. 2012; Johnston and Lipcius 2012; Hammann et al. 2013) and on different *Gracilaria* species (Thomsen et al. 2012), and many studies have found invertebrate densities to be high on algae with complex thalli as opposed to those with simples forms (Hacker and Steneck 1990; Taylor 1994; Chemello and Milazzo 2002; Wernberg et al. 2004).

In this study, we tested for impacts of foundation species using a planned presence-absence approach because we wanted to test for interaction effects (having multiple densities of foundation species in 3-factorial designs requires a very high number of total plots). However, we found few interaction effects and we therefore supplemented the ANOVA with non-parametric correlation and step-wise multivariate linear regression analyses (Appendix 4 and 5). In these analyses we used the biomass per core of each foundation species as predictor variables. These tests confirmed the key importance of *Gracilaria* over *Mytilus* and *Zostera* (almost no additional variation was explained by adding *Mytilus* or *Zostera* to *Gracilaria* in the regression models) and suggested that the facilitation process was density-dependent for all the significant responses detected in the ANOVA. Similar types of correlation analysis have previously been used to show positive continuous effects of foundation

species (Bishop et al. 2012; Byers et al. 2012; Gribben et al. 2013) and density-dependent impacts of invasive species appears to be universal across taxa and ecosystems (Parker et al. 1999; Thomsen et al. 2011).

Perspectives, study limitations and future studies

Our study provides a rare experimental test of how an invasive foundation species can interact with multiple native foundation species with cascading effects on associated invertebrates. We suggest that this scenario – where invasive and native foundations species co-occur with positive effects on associated fauna - is a relatively common phenomenon. This process represent 'cascading habitat formation' (a habitat/facilitation cascade, Altieri et al. 2007; Thomsen et al. 2010) where primary/basal habitat-formers (or modifiers/facilitators - here *Zostera* or *Mytilus*) have positive effects on secondary/inter-mediate habitat-formers (or modifiers/facilitators - here *Gracilaria* that become entangled around stems and byssal threads) to indirectly facilitate end-users (here invertebrates) through trait- or density-mediated interactions (Cruz-Angon et al. 2009; Byers et al. 2010; Thomsen 2010; Bishop et al. 2012).

There are numerous opportunities to expand our experiment to identify mechanistic links between invasions and cascading impacts on communities and ecosystem function. We here documented that most invertebrate end-users were facilitated by the invasive foundation species, but future studies should also test *how* these invertebrates are facilitated. For example, end-users may benefit from habitat-forming foundation species by escaping enemies (e.g., competitors, parasites, predators) and environmental stress (e.g., waves, heating, desiccation), or by finding friends (e.g., mating partners, mutualist, schooling benefits, allee effects) and resources (e.g., nesting/resting space or food, but note that *Gracilaria* is a poor food resource for some grazers (Nylund et al. 2011; Nejrup et al. 2012; Rempt et al. 2012)). These mechanisms will likely differ between environmental conditions (*Gracilaria* may provide predation refugia in the subtidal zone but ameliorate desiccation stress in the intertidal zone), life histories (a juvenile crab may use *Gracilaria* to avoid predators whereas the adult crab may use it as feeding ground), and species characteristics (a bivalve may use it as substrate for attachment, whereas a snail may use it for grazing).

More specifically, we found strong facilitation of herbivorous end-users, like amphipods, isopods and snails, suggesting that this trophic level graze on *Gracilaria* (the secondary habitat-former) or on associated microscopic epiphytes. Many studies have shown that herbivores can have positive indirect effects on primary habitat-formers by preferentially consuming secondary habitat-formers (e.g., Boström and Mattila 1999; Worm and Sommer 2000; Jones and Thornber 2010). This type of 'keystone consumption' (Thomsen et al. 2010) is thereby a mirror-process of cascading habitat formation/facilitation, but where research focus is top-down enemy- vs. bottom-up facilitation- processes, respectively. Understanding these direct and indirect mechanisms and how they vary in space, time and across invaders and invaded habitats is vital to provide better assessments of impacts from invasive foundation species.

Our experiment was, like all experiments, constrained in space, time, and across taxa. Future experiments should therefore explore different spatio-temporal conditions and different invasive vs. native foundation species and end-user taxa. We expect that the positive effects on invertebrates have cascading impacts on higher-order predators, in particular small fish, and, if the invasion occurs on larger scales, also on larger top-predatory fish, birds and mammals. Similarly, we focused on mobile species (including slow moving mollusks), but facilitation of sessile species that depend on hard substratum for attachment can also be important (Thomsen et al. 2010). For example, we noted that filamentous brown and red seaweeds were attached to *Zostera*, spirorbid polychaetes were attached to *Gracilaria*, and barnacles, bryozoa and hydrozoa were attached to *Mytilus*. Future studies should therefore also quantify sessile end-users (as well as smaller meiofauna and fragile sedentary polychaetes) to better understand facilitation cascades involving foundation species.

In addition, we conducted our experiment as a pulse treatment, that is, as a single initial manipulation. We purposely did not maintain treatment levels throughout the experiment because our first emphasis was to test if the foundation species had a strong impact on each other (which they did not). This approach also represents a typical local invasion scenario when invaders are seasonally and spatially patchy distributed, such as when unattached *Gracilaria* clumps drift into a seagrass patch and become

entangled for 1–2 months before disappearing again (Sfriso et al. 2012). Indeed, changes in seaweed biomass over the course of a field experiment are common and can be caused by decay and fragmentation, growth, grazing, biotic disturbances, and hydrodynamic stress (e.g., Nelson and Lee 2001; Huntington and Boyer 2008). However, if the foundation species have strong effects on each other, invertebrate responses may co-vary with biomass changes of the foundation species. Thus, if stronger effects are expected between foundation species and the main aim is to quantify responses to associated species, 'press treatments' with repeated manipulations are more suitable.

In summary, effects of invasive foundation species on native foundation species and associated communities should be tested with pulse and press experiment, with and without stabilizing pegs/cages, with multiple densities, and in different seasons (to reflect temperature and recruitment patterns), locations (e.g. of different salinities), spatio-temporal scales (larger and longer), habitats (e.g., on 'neutral' sediments, in *Mytilus* reef, in *Gracilaria* bed), as well as including novel manipulations to control predation, resources, and abiotic stress (e.g., using mimics).

Acknowledgements

References

Altieri AH, Silliman B, Bertness MD (2007) Hierarchical organization via a facilitation cascade in intertidal cordgrass bed communities. *The American Naturalist* 169: 195–206, http://dx.doi.org/10.1086/510603

Anderson MJ, Gorley RN, Clarke KR (2008) PERMANOVA for PRIMER: guide to software and statistical methods. PRIMER-E Ltd., Plymouth, United Kingdom

Anderson MJ, Ter Braak CJF (2003) Permutation tests for multifactorial analysis of variance. *Journal of statistical computation and simulation* 73: 85–113, http://dx.doi.org/10.1080/00949650215733

Bishop MJ, Byers JE, Marcek BJ, Gribben PE (2012) Density-dependent facilitation cascades determine epifaunal community structure in temperate Australian mangroves. *Ecology* 93: 1388–1401, http://dx.doi.org/10.1890/10-2296.1

Boström C, Jackson EL, Simenstad CA (2006) Seagrass landscapes and their effects on associated fauna: A review. *Estuarine, Coastal and Shelf Science* 68: 383–403, http://dx.doi.org/10.1016/j.ecss.2006.01.026

Boström C, Mattila J (1999) The relative importance of food and shelter for seagrass-associated invertebrates: a latitudinal comparison of habitat choise by isopod grazers. *Oecologia* 120: 162–170, http://dx.doi.org/10.1007/s004420050845

Byers J, Gribben P, Yeager C, Sotka E (2012) Impacts of an abundant introduced ecosystem engineer within mudflats of the southeastern US coast. *Biological Invasions* 14: 2587–2600, http://dx.doi.org/10.1007/s10530-012-0254-5

Byers J, Wright JT, Gribben PE (2010) Variable direct and indirect effects of a habitat-modifying invasive species on mortality of native fauna. *Ecology* 91: 1787–1798, http://dx.doi.org/10.1890/09-0712.1

Cacabelos E, Engelen AH, Mejia A, Arenas F (2012) Comparison of the assemblage functioning of estuary systems dominated by the seagrass *Nanozostera noltii* versus the invasive drift seaweed *Gracilaria vermiculophylla*. *J. of Sea Research* 72: 99–105, http://dx.doi.org/10.1016/j.seares.2012.02.003

Chemello R, Milazzo M (2002) Effect of algal architecture on associated fauna: some evidence from phytal molluscs. *Marine Biology* 140: 981–990, http://dx.doi.org/10.1007/s00227-002-0777-x

Clarke KR, Gorley RN (2006) Primer v6: user manual/tutorial. PRIMER-E, Plymouth

Clarke KR, Somerfield PJ, Chapman MG (2006) On resemblance measures for ecological studies, including taxonomic dissimilarities and a zero-adjusted Bray-Curtis coefficient for denuded assemblages. *Journal of Experimental Marine Biology and Ecology* 330: 55–80, http://dx.doi.org/10.1016/j.jembe.2005.12.017

Cruz-Angon A, Baena ML, Greenberg R (2009) The contribution of epiphytes to the abundance and species richness of canopy insects in a Mexican coffee plantation. *Journal of Tropical Ecology* 45: 453–463, http://dx.doi.org/10.1017/S0266467409990125

Dayton PK (1972) Towards an understanding of community resilience and the potential effects of enrichment to the benthos of McMurdo Sound, Antarctica. Proceedings of the Colloquium on Conservation Problems in Antartica, pp 81–96

Gribben PE, Byers JE, Wright JT, Glasby TM (2013) Positive versus negative effects of an invasive ecosystem engineer on different components of a marine ecosystem. *Oikos* 122: 816–824, http://dx.doi.org/10.1111/j.1600-0706.2012.20868.x

Hacker S, Steneck RS (1990) Habitat architecture and the abundance and body-size-dependent habitat selection of a phytal amphipod. *Ecology* 7: 2269–2285, http://dx.doi.org/10.2307/1938638

Hammann M, Buchholz B, Karez R, Weinberger F (2013) Direct and indirect effects of *Gracilaria vermiculophylla* on native *Fucus vesiculosus*. *Aquatic Invasions* 8, in press

Heck JKL, Hays G, Orth RJ (2003) Critical evaluation of the nursery role hypothesis for seagrass meadows. *Marine Ecology Progress series* 253: 123–136, http://dx.doi.org/10.3354/meps253123

Herkül K, Kotta J (2009) Effects of eelgrass (*Zostera marina*) canopy removal and sediment addition on sediment characteristics and benthic communities in the northern Baltic Sea. *Marine Ecology* 30: 74–82, http://dx.doi.org/10.1111/j.1439-0485.2009.00307.x

Hernández CA, Seitz R, Lipcius R, Bovery C, Schulte D (2012) Habitat affects survival of translocated bay scallops, *Argopecten irradians concentricus* (Say 1822), in Lower Chesapeake Bay. *Estuaries and Coasts*: 35: 1340–1345, http://dx.doi.org/10.1007/s12237-012-9510-2

Hoeffle H, Thomsen MS, Holmer M (2011) High mortality of *Zostera marina* under high temperature regimes but minor effects of the invasive macroalgae *Gracilaria verminculophylla*. *Estuarine, Coastal and Shelf Science* 92: 35–46, http://dx.doi.org/10.1016/j.ecss.2010.12.017

Holmer M, Nielsen RM (2007) Effects of filamentous algal mats on sulfide invasion in eelgrass (*Zostera marina*). *Journal of Experimental Marine Biology and Ecology* 353: 245–252, http://dx.doi.org/10.1016/j.jembe.2007.09.010

Holmer M, Wirachwong P, Thomsen MS (2011) Negative effects of stress-resistant drift algae and high temperature on a small ephemeral seagrass species. *Marine Biology* 158: 297–309, http://dx.doi.org/10.1007/s00227-010-1559-5

Huntington B, Boyer KE (2008) Effects of red macroalgal (*Gracilariopsis* sp.) abundance on eelgrass *Zostera marina* in Tomales Bay, California, USA. *Marine Ecology Progress Series* 367: 133–142, http://dx.doi.org/10.3354/meps07506

Johnston CA, Lipcius RN (2012) Exotic macroalga *Gracilaria vermiculophylla* provides superior nursery habitat for native blue crab in Chesapeake Bay. *Marine Ecology Progress Series* 467: 137, http://dx.doi.org/10.3354/meps09935

Jones E, Thornber CS (2010) Effects of habitat-modifying invasive macroalgae on epiphytic algal communities. *Marine Ecology Progress Series* 400: 87–100, http://dx.doi.org/10.3354/meps08391

Kim SY, Weinberger F, Boo SM (2010) Genetic data hint at a common donor region for invasive Atlantic and Pacific populations of *Gracilaria vermiculophylla* (Gracilariales, Rhodophyta). *Journal of Phycology* 46: 1346–1349, http://dx.doi.org/10.1111/j.1529-8817.2010.00905.x

Levin PS, Coyer JA, Petrik R, Good TP (2002) Community-wide effects of nonindigenous species on temperate rocky reefs. *Ecology* 83: 3182–3193, http://dx.doi.org/10.1890/0012-9658(2002)083[3182:CWEONS]2.0.CO;2

Levine TR, Hullet CR (2002) Eta squared, partial eta squared, and misreporting of effect size in communication research. *Human Communication Research* 28: 612–625, http://dx.doi.org/10.1111/j.1468-2958.2002.tb00828.x

Markert A, Wehrmann A, Kroncke I (2010) Recently established *Crassostrea*-reefs versus native *Mytilus*-beds: differences in ecosystem engineering affects the macrofaunal communities (Wadden Sea of Lower Saxony, southern German Bight). *Biological Invasions* 12: 15–32, http://dx.doi.org/10.1007/s10530-009-9425-4

Martinez-Luscher J, Holmer M (2010) Potential effects of the invasive species *Gracilaria vermiculophylla* on *Zostera marina* metabolism and survival. *Marine Environmental Research* 69: 345–349, http://dx.doi.org/10.1016/j.marenvres.2009.12.009

Nejrup L, Pedersen M, Vinzent J (2012) Grazer avoidance may explain the invasiveness of the red alga *Gracilaria vermiculophylla* in Scandinavian waters. *Marine Biology* 159: 1703–1712, http://dx.doi.org/10.1007/s00227-012-1959-9

Nejrup LB, Pedersen MF (2010) Growth and biomass development of the introduced red alga *Gracilaria vermiculophylla* is unaffected by nutrient limitation and grazing. *Aquatic Biology* 10: 249–259, http://dx.doi.org/10.3354/ab00281

Nelson TA, Lee A (2001) A manipulative experiment demonstrates that blooms of the macroalga *Ulvaria obscura* can reduce eelgrass shoot density. *Aquatic Botany* 71: 149–154, http://dx.doi.org/10.1016/S0304-3770(01)00183-8

Norkko A, Bonsdorff E (1996) Population responses of coastal zoobenthos to stress induced by drifting algal mats. *Marine Ecology Progress Series* 140: 141–151, http://dx.doi.org/10.3354/meps140141

Nyberg CD, Thomsen MS, Wallentinus I (2009) Flora and fauna associated with the introduced red alga *Gracilaria vermiculophylla*. *European Journal of Phycology* 44: 395–403, http://dx.doi.org/10.1080/09670260802592808

Nylund GM, Weinberger F, Rempt M, Pohnert G (2011) Metabolomic Assessment of Induced and Activated Chemical Defence in the Invasive Red Alga *Gracilaria vermiculophylla*. *PLoS ONE* 6: e29359, http://dx.doi.org/10.1371/journal.pone.0029359

Parker IM, Simberloff D, Lonsdale WM, Goodell K, Wonham MJ, Kareiva PM, Williamson MH, Von Holle B, Moyle PB, Byers JL, Goldwasser L (1999) Impact: toward a framework for understanding the ecological effects of invaders. *Biological Invasions* 1: 3–19, http://dx.doi.org/10.1023/A:1010034312781

Peterson BJ, Heck KL (2001a) An experimental test of the mechanism by which suspension feeding bivalves elevate seagrass productivity. *Marine Ecology-Progress Series* 218: 115–125, http://dx.doi.org/10.3354/meps218115

Peterson BJ, Heck KL (2001b) Positive interactions between suspension-feeding bivalves and seagrass - a facultative mutualism. *Marine Ecology-Progress Series* 213: 143–155, http://dx.doi.org/10.3354/meps213143

Rempt M, Weinberger F, Grosser K, Pohnert G (2012) Conserved and species-specific oxylipin pathways in the wound-activated chemical defense of the noninvasive red alga *Gracilaria chilensis* and the invasive *Gracilaria vermiculophylla*. *Beilstein Journal of Organic Chemistry* 8: 283–289, http://dx.doi.org/10.3762/bjoc.8.30

Reusch TBH, Chapman ARO (1995) Storm effects on eelgrass (*Zostera marina* L.) and blue mussel (*Mytilus edulis* L.) beds. *Journal of Experimental Marine Biology and Ecology* 192: 257–271, http://dx.doi.org/10.1016/0022-0981(95)00074-2

Reusch TBH, Chapman ARO, Groger JP (1994) Blue mussels *Mytilus edulis* do not interfere with eelgrass *Zostera marina* but fertilize shoot growth through biodeposition. *Marine Ecology Progress Series* 108: 265–282, http://dx.doi.org/10.3354/meps108265

Reusch TBH, Williams SL (1998) Variable response of native eelgrass *Zostera marina* to a non-indigenous bivalve *Musculista senhousia*. *Oecologia* 113: 428–441, http://dx.doi.org/10.1007/s004420050395

Rodriguez LF (2006) Can invasive species facilitate native species? Evidence of how, when, and why these impacts occur. *Biological Invasions* 8: 927–939, http://dx.doi.org/10.1007/s10530-005-5103-3

Sfriso A, Maistro S, Andreoli C, Moro I (2010) First record of *Gracilaria vermiculophylla* (Gracilariales, Rhodophyta) in the Po Delta lagoons, Mediterranean Sea (Italy). *Journal of Phycology* 46: 1024–1027, http://dx.doi.org/10.1111/j.1529-8817.2010.00893.x

Sfriso A, Wolf MA, Maistro S, Sciuto K, Moro I (2012) Spreading and autoecology of the invasive species *Gracilaria vermiculophylla* (Gracilariales, Rhodophyta) in the lagoons of the north-western Adriatic Sea (Mediterranean Sea, Italy). *Estuarine, Coastal and Shelf Science* 114: 192–198, http://dx.doi.org/10.1016/j.ecss.2012.07.024

Staehr PA, Pedersen MF, Thomsen MS, Wernberg T, Krause-Jensen D (2000) Invasion of *Sargassum muticum* in Limfjorden (Denmark) and its possible impact on the indigenous macroalgal community. *Marine Ecology Progress Series* 207: 79–88, http://dx.doi.org/10.3354/meps207079

Taylor RB, Cole RG (1994) Mobile epifauna on subtidal brown seaweeds in northeastern New Zealand. *Marine Ecology Progress series* 115: 271–282, http://dx.doi.org/10.3354/meps115271

Thomsen MS (2004) Species, thallus size and substrate determine macroalgal break forces and break places in a low-energy soft-bottom lagoon. *Aquatic Botany* 80: 153–161, http://dx.doi.org/10.1016/j.aquabot.2004.08.002

Thomsen MS (2010) Experimental evidence for positive effects of invasive seaweed on native invertebrates via habitat-formation in a seagrass bed. *Aquatic Invasions* 5: 341–346, http://dx.doi.org/10.3391/ai.2010.5.4.02

Thomsen MS, de Bettignies T, Wernberg T, Holmer M, Debeuf B (2012) Harmful algae are not harmful to everyone. *Harmful Algae* 16: 74–80, http://dx.doi.org/10.1016/j.hal.2012.01.005

Thomsen MS, McGlathery K (2005) Facilitation of macroalgae by the sedimentary tube forming polychaete *Diopatra cuprea. Estuarine, Coastal and Shelf Science* 62: 63–73, http://dx.doi.org/10.1016/j.ecss.2004.08.007

Thomsen MS, McGlathery K (2006) Effects of accumulations of sediments and drift algae on recruitment of sessile organisms associated with oyster reefs. *Journal of Experimental Marine Biology and Ecology* 328: 22–34, http://dx.doi.org/10.1016/j.jembe.2005.06.016

Thomsen MS, McGlathery KJ, Schwarzschild A, Silliman BR (2009) Distribution and ecological role of the non-native macroalga *Gracilaria vermiculophylla* in Virginia salt marshes. *Biological Invasions* 11: 2303–2316, http://dx.doi.org/10.1007/s10530-008-9417-9

Thomsen MS, Olden JD, Wernberg T, Griffin JN, Silliman BR (2011) A broad framework to organize and compare ecological invasion impacts. *Environmental Research* 111: 899–908, http://dx.doi.org/10.1016/j.envres.2011.05.024

Thomsen MS, Wernberg T, Altieri AH, Tuya F, Gulbransen D, McGlathery KJ, Holmer M, Silliman BR (2010) Habitat Cascades: The conceptual context and global relevance of facilitation cascades via habitat formation and modification. *Integrative and Comparative Biology* 50: 158–175, http://dx.doi.org/10.1093/icb/icq042

Tyler R (2007) Effects of coverage by benthic seaweed mats on (northern quahog= hard clam) *Mercenaria mercenaria* in a eutrophic estuary. *Journal of Shellfish Research* 26: 1021-1028, http://dx.doi.org/10.2983/0730-8000(2007)26[1021:EOCBBS]2.0.CO;2

Underwood AJ (1997) Experiments in ecology - their logical design and interpretation using analysis of variance. Cambridge University Press, 504 pp

Vilà M, Espinar JL, Hejda M, Hulme PE, Jarošík V, Maron JL, Pergl J, Schaffner U, Sun Y, Pyšek P (2011) Ecological impacts of invasive alien plants: a meta-analysis of their effects on species, communities and ecosystems. *Ecology Letters* 14: 702–708, http://dx.doi.org/10.1111/j.1461-0248.2011.01628.x

Vinther HF, Laursen JS, Holmer M (2008) Negative effects of blue mussel (*Mytilus edulis*) presence in eelgrass (*Zostera marina*) beds in Flensborg fjord, Denmark. *Estuarine, Coastal and Shelf Science* 77: 91–103, http://dx.doi.org/10.1016/j.ecss.2007.09.007

Vinther HF, Norling P, Kristensen PS, Dolmer P, Holmer M (2012) Effects of coexistence between the blue mussel *Mytilus edulis* and eelgrass *Zostera marina* on sediment biogeochemistry and plant performance. *Marine Ecology Progress Series* 447: 139–149, http://dx.doi.org/10.3354/meps09505

Wallentinus I, Nyberg CD (2007) Introduced marine organisms as habitat modifiers. *Marine Pollution Bulletin* 55: 323–332, http://dx.doi.org/10.1016/j.marpolbul.2006.11.010

Ward JM, Ricciardi A (2010) Community-level effects of co-occurring native and exotic ecosystem engineers. *Freshwater Biology* 55: 1803–1817, http://dx.doi.org/10.1111/j.1365-2427.2010.02415.x

Weinberger F, Buchholz B, Karez R, Wahl M (2008) The invasive red alga *Gracilaria vermiculophylla* in the Baltic Sea: adaptation to brackish water may compensate for light limitation. *Aquatic Biology* 3: 251–264, http://dx.doi.org/10.3354/ab00083

Wernberg T, Thomsen MS, Staerh PA, Pedersen MF (2004) Epibiota communities of the introduced and indigenous macroalgal relatives *Sargassum muticum* and *Halidrys siliquosa* in Limfjorden (Denmark). *Helgoland Marine Research* 58: 154–161, http://dx.doi.org/10.1007/s10152-004-0180-8

Worm B, Sommer U (2000) Rapid direct and indirect effects of a single nutrient pulse in a seaweed-epiphyte-grazer system. *Marine Ecology Progress Series* 202: 283–288, http://dx.doi.org/10.3354/meps202283

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.

This material is available as part of online article from:
http://www.aquaticinvasions.net/2013/Supplements/AI_2013_Thomsen_etal_Supplement.pdf