CHAPTER 2

A three-stage symbiosis forms the foundation of seagrass ecosystems

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ABSTRACT

Seagrasses evolved from terrestrial plants into marine foundation species around 100 million years ago. Their ecological success, however, remains a mystery as natural organic matter accumulation within the beds should result in toxic sediment sulfide levels. Using a meta-analysis, a field study and a laboratory experiment, we reveal how an ancient three-stage symbiosis between seagrass, lucinid bivalves and their sulfide-oxidizing gill-bacteria reduces sulfide stress for seagrasses. We found that the bivalve-sulfide-oxidizer symbiosis reduced sulfide levels and enhanced seagrass production as measured by biomass. In turn, the bivalves and their endosymbionts profit from organic matter accumulation and radial oxygen release from the seagrass roots. These findings elucidate the long-term success of seagrasses in warm waters and offer new prospects for seagrass ecosystem conservation.

Published in *Science* **336**: 1432-1434, 2012

Seagrass meadows are important ecological and thus economic components of coastal zones worldwide (Larkum et al. 2006, Waycott et al. 2009). In many areas, coral reefs and seagrass meadows are tightly linked habitats that form the basis for marine biodiversity (Nagelkerken 2009). Seagrasses serve as keystone habitat for migrating coral reef species, thousands of other animals including waterbirds, fish, dugongs, manatees and turtles, are important carbon and nutrient sinks, and are important to fisheries and coastline protection (Larkum et al. 2006, Nagelkerken 2009, Waycott et al. 2009). Dense seagrass meadows attenuate currents and waves and trap pelagic and benthic organic matter in the sediment (Larkum et al. 2006, van der Heide et al. 2007, van der Heide et al. 2011). Owing to a lack of oxygen in many coastal marine sediments, an important fraction of organic matter is decomposed by bacteria that use the abundant sulfate in seawater as an electron acceptor instead of oxygen, and produce toxic sulfide as a metabolic end product (Jorgensen 1982). Although seagrasses transport oxygen into their roots and the surrounding rhizosphere (radial oxygen release) (Larkum et al. 2006, Calleja et al. 2007), sulfide production outpaces oxygen release under warmer conditions, resulting in sulfide accumulation and seagrass mortality (Larkum et al. 2006, Calleja et al. 2007, Koch et al. 2007). Seagrass beds tend to accumulate organic matter and so it is expected that seagrass beds would build up toxic sulfides and hence have a limited productivity and diversity (Larkum et al. 2006). But this is not the observed case and the underlying reason for the long-term persistence of seagrass ecosystems is an enigma (Appendix Figure A2.1A).

We tested the hypothesis that a three-stage symbiosis between seagrasses, associated burrowing lucinid bivalves and their symbiotic gill-bacteria contribute to reducing the cyclic build-up of sulfide (Appendix Figure A2.1B-D). Paleo-records suggest that the Lucinidae and their endosymbiotic relation date back to the Silurian (Liljedahl 1991, Distel 1998, Taylor and Glover 2000), but that they increasingly diversified since the evolutionary emergence of seagrasses in the late Cretaceous (Stanley 1977, Larkum et al. 2006, Taylor et al. 2011). Seagrass communities later became widespread in the Eocene and lucinid remains frequently occur in association with their deposits since (Taylor et al. 2011, Vermeij 2011). Lucinids and their gill-inhabiting bacteria have a symbiosis in which the bivalves transport sulfide and oxygen to their gills (Appendix Figure A2.1D) where the bacteria oxidize sulfide for synthesizing sugars that fuel growth of both organisms (Cavanaugh 1983, Johnson et al. 1994, Anderson 1995, Reynolds et al. 2007, Childress and Girguis 2011). We hypothesized that seagrass meadows may provide an optimal habitat for these bivalves and their symbionts by indirectly stimulating sulfide production by high organic matter input, and by providing oxygen through radial oxygen release from the roots. In turn, lucinids remove sulfide, which could relieve any stress caused to seagrass growth by sulfide accumulation as organic matter is degraded (Appendix Figure A2.1A & B).

Indirect support for our hypothesis was provided by a worldwide meta-analysis of 84 studies describing the fauna of seagrass beds in 83 sites covering the entire climatic distribution of seagrasses, combined with a 110-point field survey that we conducted at Banc

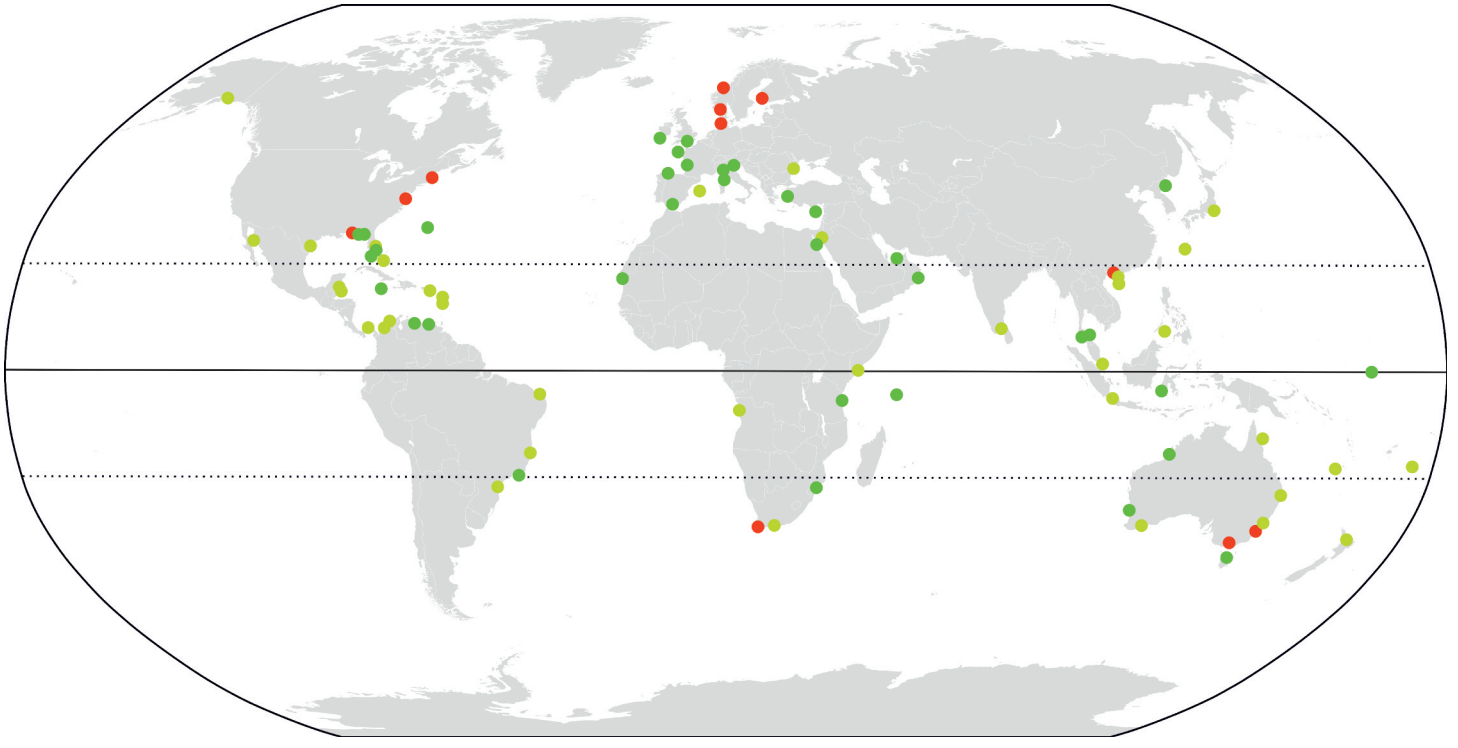

Figure 2.1 Presence (green; dark points are quantitative, light points are qualitative) and absence (red) of lucinids in seagrass ecosystems based on our meta-analysis. The bivalves were present in 97% (93% of the quantitative sites) of all tropical seagrass beds, 90% (83% of the quantitative sites) of the subtropical beds and 56% (50% of the quantitative sites) of the temperate seagrass meadows. The seagrass-lucinid association spans six out of seven continents, at least 18 genera of lucinids and 11 out of 12 seagrass genera (and *Ruppia spp.*). Only meadows of *Phyllospadix spp.*, a seagrass genus that grows on bare rock, did not contain Lucinidae. The analyzed ecosystems generally contained high (~100 ind. m^{-2}) to extremely high densities (>1000 ind. m^{-2}) of lucinids (Table A2.1).

d'Arguin, Mauritania (see Appendix A2, Materials and methods). The meta-analysis reveals a relationship that covers 11 out of 12 seagrass genera (and *Ruppia spp.*) and at least 18 genera of Lucinidae (Figure 2.1 & Appendix Table A2.1). Only meadows of *Phyllospadix spp.*, a seagrass genus that grows on bare rock, do not associate with Lucinidae. The association spans six out of seven continents, with bivalve densities ranging from 10 to over 1000 individuals per m^2. The bivalves were present in 97% of the tropical seagrass sites, 90% of the subtropical meadows and 56% of the temperate seagrass beds surveyed, indicating that the association may be dependent on temperature-related sulfide production (Koch et al. 2007). Furthermore, results from our field study showed a positive correlation between seagrasses and lucinids that explained 42% of their respective variation (Pearson's $r = 0.65$; Appendix Figure A2.2).

To experimentally test our hypothesis (Appendix Figure A2.1B), we investigated the effects of sulfide oxidation by the lucinid bivalve *Loripes lacteus* on the production of the seagrass species *Zostera noltii* and the potential reciprocal benefits for *Loripes* in a full factorial experiment under controlled conditions (see Appendix A2, Materials and methods). We set up *Zostera*, *Loripes*, *Zostera-Loripes* and bare sediment treatments in the top sections of 40 two-compartment columns (Appendix Figure A2.3), which were placed in a large seawater basin. The lower compartment of each column contained anaerobic seawater and an injection tube through which sulfide was added twice a week in half of the columns. The injected sulfide was allowed to diffuse into the top section through a porous membrane.

The presence of *Loripes*, and to a lesser extent of *Zostera* decreased sediment sulfide levels. After five weeks, pore water sulfide concentrations in the top sections of the sediment controls reached about 400 μM, while the semi-weekly addition of sulfide caused levels to increase to nearly 2700 μM (Figure 2.2A). The presence of *Zostera* decreased sulfide levels to around 200 μM in the controls and 2200 μM in the sulfide addition treatments. In contrast, sulfide levels remained low when *Loripes* was present ($\sim$15 μM), even in the sulfide addition treatments. As expected, the oxygen detection depth was reduced when sulfide was added, but increased when only *Loripes*, but not *Zostera* was present, due to sulfide-oxidation and intake of surface water (Figure 2.2B). *Zostera* alone did not significantly affect sediment oxygen conditions. Strikingly, the joint presence of *Zostera* and *Loripes* enhanced oxygen detection depth beyond that of their separate effects.

Our experiment showed that *Zostera* production is facilitated by *Loripes*; both in the control and in the sulfide addition treatments. In the treatments without *Loripes*, sulfide addition reduced *Zostera* shoot biomass to 50% of the controls (Figure 2.3A). Reduced shoot biomass was accompanied by decreased root biomass (Figure 2.3B) and impaired phosphate uptake (see Appendix A2, Materials and methods). In contrast, the addition of *Loripes* increased *Zostera* shoot biomass 1.9-fold and root weight 1.5-fold seen in the sulfide addition treatments. In the treatments without additional sulfide, the presence of *Loripes* increased both shoot and root weight by 1.4-fold and 1.3-fold respectively.

Loripes condition, expressed as the flesh/shell dry weight ratio, was positively affected by sulfide addition (Figure 2.3C). Furthermore, the addition of *Zostera* did not affect

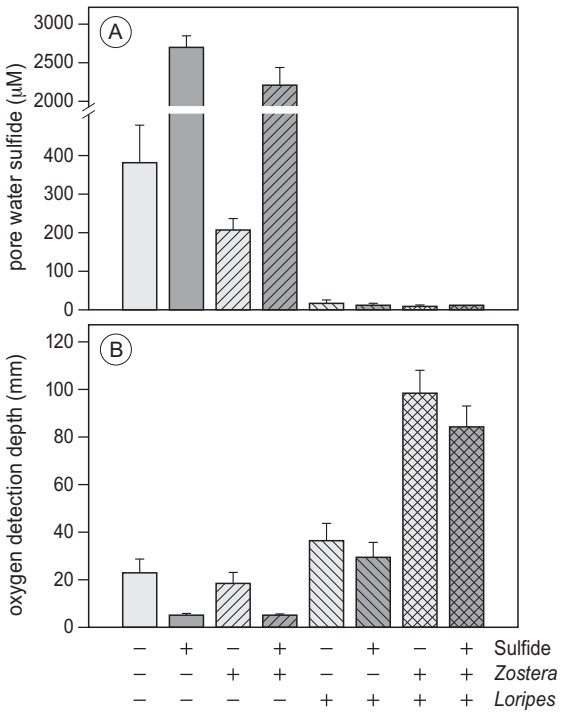

Figure 2.2 (A) Pore water sulfide concentrations and (B) oxygen detection depth after five weeks; error bars represent SEM ($n = 5$). Oxygen detection depth decreased as sulfide was added (ANOVA: v8.9, $P < 0.006$). The presence of *Loripes* reduced sulfide levels (RM-ANOVA: $F_{1,32} = 268.8$, $P < 0.001$) and increased oxygen detection depth ($F_{1,32} = 125.0$, $P < 0.001$). Reduction of the sulfide concentration by *Zostera* alone was less, but still significant ($F_{1,32} = 6.8$, $P = 0.014$). That interactions occured between *Zostera* and *Loripes* was apparent in the oxygen measurements ($F_{1,32} = 48.3$, $P < 0.001$), but was also significant in the sulfide data ($F_{1,32} = 7.8$, $P = 0.009$). The interaction between *Loripes* and sulfide was significant for the sulfide measurements ($F_{1,32} = 102.7$, $P < 0.001$), but not for the oxygen data ($F_{1,32} = 0.3$, $P = 0.578$).

Loripes in the units where no sulfide was added, but improved the bivalve's condition in the sulfide treatments. As hypothesized, the positive effect of *Zostera* on *Loripes* seems to result from radial oxygen release from the seagrass roots (Appendix Figure A2.1B). Although sulfide was almost completely removed in all *Loripes* treatments (Figure 2.2A), the bivalve was less able to profit from the addition of sulfide in the absence of *Zostera* (Figure 2.3C). This indicates that at least in the *Loripes* units without seagrass, sulfide was not completely oxidized by the symbiotic bacteria because of oxygen limitation.

Overall, our results confirm our hypothesis that a three-stage symbiosis between seagrass, lucinids and sulfide-oxidziing bacteria reduces sulfide stress in seagrass meadows. Even though radial oxygen release by *Zostera noltii* and of seagrasses in general is limited (Caffrey and Kemp 1991, Sand-Jensen et al. 2005), *Loripes* in our experiment clearly benefitted from the increased oxygen input in the sediment. In the field, the positive

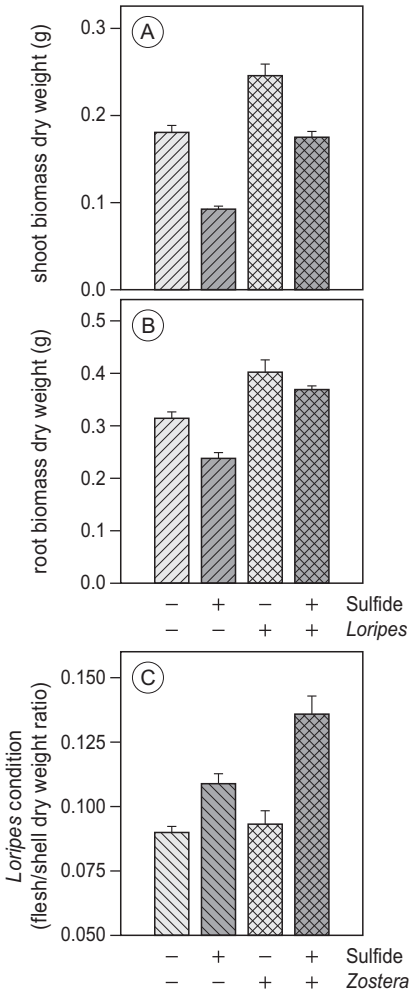

Figure 2.3 (A) *Zostera* shoot and (B) root dry weight biomass per column and (C) *Loripes* condition expressed as the dry weight flesh/shell ratio after five weeks; error bars represent SEM ($n = 5$). *Zostera* biomass was reduced by sulfide addition (ANOVA: shoots $F_{1,16} = 72.6$, $P < 0.001$; roots $F_{1,16} = 12.0$, $P = 0.003$), whereas the presence of *Loripes* had a positive effect on both shoot ($F_{1,16} = 61.3$, $P < 0.001$) and root biomass ($F_{1,16} = 50.2$, $P < 0.001$). We found no significant effects on rhizome biomass. *Loripes* condition was positively affected by both sulfide addition (ANOVA: $F_{1,16} = 37.3$, $P < 0.001$) and *Zostera* presence ($F_{1,16} = 9.0$, $P = 0.008$). We also found a significant positive combined effect of the presence of *Zostera* and sulfide on *Loripes* condition ($F_{1,16} = 5.4$, $P = 0.034$).

effects of seagrasses on lucinids are not confined to sediment oxygenation alone, but also by indirectly stimulating sulfide production and releasing dissolved organic molecules (Larkum et al. 2006, Reynolds et al. 2007). The positive effects of *Loripes* on *Zostera* in our experiment could not be explained by differences in nutrient availability (see Appendix A2, Materials and methods) Plants were not nutrient limited, but both *Zostera* and

Loripes significantly lowered dissolved ammonium and phosphorus in the sediment pore water, whereas sulfide addition increased nutrient availability (Appendix Figure A2.4). We found that in our experiment, the negative effects of sulfide addition on *Zostera* biomass could not fully be prevented by *Loripes* addition (Figure 2.3A), despite the removal of almost all sulfide by *Loripes* after three days. As the observed experimental effects could not be attributed to differences in nutrient availability, this is most likely caused by the pulsed nature of our sulfide supply. This may have led to short periods of exposure of *Zostera* to toxic sulfide levels.

Coastal ecosystems, and seagrass meadows in particular, are currently declining at an alarming and increasing rate worldwide, leading to loss of biodiversity (Waycott et al. 2009). Extensive restoration efforts have had little success so far (< 30%), despite their extremely high costs (± $ 100,000 per ha) (Fonseca et al. 2001). Similar to the function of mycorrhizae, pollinators or seed dispersers in terrestrial systems (van der Heijden et al. 1998, Bascompte and Jordano 2007, Bastolla et al. 2009), our findings indicate that restoration efforts should not only focus on environmental stressors like eutrophication, sediment run-off or high salinity as a cause of decline, but should also consider internal ecological interactions such as the presence and vigor of symbiotic or mutualistic relations. Breakdown of symbiotic interactions can affect ecosystem functioning, with bleaching events in coral reefs as a clear example (Carpenter et al. 2008). Similar to the well-known symbiosis between corals and their unicellular algal endosymbionts (Baker 2003), we conclude that symbioses, rather than one defining species forms the foundation of seagrass ecosystems.

ACKNOWLEDGMENTS

We thank G. Quaintenne and H. Blanchet for their help with the collection of *Loripes*; J. Eygensteyn and E. Pierson for technical assistance; and G.J. Vermeij, H. de Kroon, T.J. Bouma, E.J. Weerman and C. Smit for their comments on the manuscript. TvdH was financially supported by the "Waddenfonds" programme; MvdG and TP by the NWO-WOTRO Integrated Programme grant W.01.65.221.00 awarded to TP; and JdF and JvG by the NWO-VIDI grant 864.09.002 awarded to JvG. BS was supported by an NSF CAREER award, the Andrew Mellon Foundation and the Royal Netherlands Society Visiting Professorship. The authors declare no conflict of interest. A detailed description of all materials and methods, sources as well as supplementary information are available as Supporting Online Material.

Appendix A2

MATERIALS & METHODS

Meta-analysis

To test the seagrass-lucinid association, we performed an extensive, worldwide meta-analysis that covered the entire climatic distribution of seagrasses. Criteria for including a study were: (1) seagrasses were present at the site, and (2) when Lucinidae were present, they were found inside the seagrass bed. In total, we analyzed 84 studies that sampled the fauna of seagrass beds in a total of 83 areas (temperature range = 1 to 33ºC, mean = 22ºC). Overall, 36 sites were from tropical areas, 31 from subtropical and 16 from temperate areas; quantitative data were available for 46 out of 83 sites. Apart from the geographical location of each site, and the seagrass and lucinid families found, we also report the annual seawater temperature range. These were obtained from freely available satellite imagery of the long-term monthly means (1971 – 2000) of the sea surface temperature (NOAA/OAR/ESRL/PSD 2011).

Field study

We conducted a field survey at Banc d'Arguin (Mauritania) to test the strength of the relation between seagrass biomass and lucinid density. Banc d'Arguin consists of about 500 km^2 of intertidal flat dominated by mixed meadows of *Zostera noltii*, *Halodule wrightii* and *Cymodocea nodosa* that are inhabited by the lucinid bivalve *Loripes lacteus* (Wolff et al. 1993). In total, we sampled 110 stations across seven intertidal flats. *Loripes* was sampled up to a depth of 20 cm using a cylindrical 15-cm diameter PVC core sampler and seagrass was sampled with a 7-cm diameter corer. Each sample was sieved over a 1-mm mesh sieve. Next, *Loripes* was counted and seagrass biomass was determined after drying for 24-h at 70ºC. Prior to linear regression analysis, *Loripes* counts and seagrass dry weight from the cores were transformed with the Box-Cox procedure to achieve normality and homoscedasticity (Box and Cox 1964).

Laboratory experiment

Organisms and sediment for the experiment were collected in Arcachon Bay (southwest France) and transported at 15ºC to the laboratory, where both species were separately acclimatized for three weeks in 100-L polyethylene tanks. *Zostera* units contained 15 cm of sediment and 20 cm of surface water; *Loripes* tanks contained 30 cm of sediment and 5 cm of surface water. We used artificial seawater (33-35 PSU Tropic Marin at 20ºC) throughout the acclimatization period and during the experiment; pH was kept at 8.1 to 8.3 by CO_2 aeration. Light period was 16 h day^{-1}; intensity at the leaf surface was 300 μmol m^{-2} s^{-1}, similar to growing season conditions in the field (Isaksen and Finster 1996). During this three-week period, we did not observe any bivalve mortality, and seagrasses exhibited healthy vegetative growth.

Experimental setup

The lower 6-cm tall sections of 40 two-compartment PVC columns (diameter 8.4 cm) were filled with anaerobic seawater (Figure A2.3). These 330-ml sections contained an injection tube and were separated from their upper compartments through a porous 0.1-mm membrane. Sediment was passed through a 1-mm sieve and transferred to the upper 12-cm tall sections (surface area: 0.0055 m^2). Depending on the treatment, each unit then received either 1) *Loripes*, 2) *Zostera*, 3) both *Zostera* and *Loripes*, or 4) no further treatment. Nine *Loripes* specimens were added to each *Loripes* treatment (~1600 ind. m^{-2}; mean shell length ~9 mm) and 5 seagrass ramets with 2 or 3 shoots (12 shoots in total) were planted in each unit containing *Zostera* (~2200 sh. m^{-2}; ~0.12 g shoot, ~0.06 g rhizome and ~0.03 g DW root biomass per column). Each ramet contained one apical shoot to allow vegetative growth. Pilot experiments showed that this approach ensured consistent colonization of the units within the two-week adjustment period, with no detectable mortality of the plants. Densities of both species were well within reported ranges of densities in the field (up to 23000 sh. m^{-2} for *Zostera* and 3700 ind. m^{-2} for *Loripes*) (Vermaat and Verhagen 1996, Johnson et al. 2002, van der Geest et al. 2011).

A full factorial experiment was designed with eight treatments and five replicates per treatment. The columns were randomly placed in a 40-cm high 250-L polyethylene basin where water flow and oxygen saturation (measured with a 556 Multi Parameter Sampler, Yellow Springs Instruments) were maintained by two aquarium water pumps, and pH was kept constant (8.1-8.3) by CO_2 aeration. After setup, the units were allowed to adjust for two weeks. During this period, sulfide levels in the treatments containing *Loripes* stabilized at ~7 μM, while sulfide in treatments without *Loripes* increased to ~233 μM. Following the adjustment period, the experiment was performed for five weeks. Sulfide levels in the lower compartments of the sulfide addition treatments were increased twice a week by 3.3-ml injections of 100 mM Na_2S solution with pH adjusted to sediment conditions (pH 7.5) with HCl, while control treatments were injected with anaerobic water. Before each injection, we used 5 cm Rhizon samplers to extract 3 ml of pore water from the main root zone (top 6 cm) of each upper compartment into vacuumized 30 ml flasks containing 3 ml Sulfide Anti-Oxidation Buffer (SAOB). After each sampling, columns were re-randomized in the basin to minimize possible differences in light levels and water flow velocities between units. Sulfide concentrations were determined immediately with an ion selective silver/sulfide electrode (Thermo Scientific (USA), Orion 9416 BN; reference electrode: Orion 900200). Oxygen detection depth was measured after five weeks with an oxygen-sensitive microelectrode (Microscale Measurements, 1-mm tip). Ammonium, nitrate and total dissolved phosphorus in the sediment pore water were also measured after five weeks. We used 5 cm Rhizon samplers to extract 10 ml of pore water from the main root zone (top 6 cm) of each upper compartment into vacuumized 30 ml flasks. Ammonium and nitrate concentrations were determined colorimetrically. Ammonium was measured with salicylate (Lamers et al. 1998) and nitrate was determined by sulfanilamide after reduction of nitrate to nitrite in a cadmium column (Wood et al. 1967). Dissolved phosphorus was measured on an Inductively Coupled Plasma emission spec-

trophotometer (ICP; Spectroflame, Spectro). Total nitrogen concentration in *Zostera* leaves was measured in freeze-dried tissues by a CNS analyzer (type NA1500; Carlo Erba Instruments, Milan, Italy) (Lamers et al. 1998). Total phosphorus was measured by ICP after digestion with nitric acid (Lamers et al. 1998). *Zostera* shoot, root and rhizome biomass and *Loripes* flesh were measured as dry weight after 24 h of freeze-drying. *Loripes* shell weight was measured after drying for 24 h at 70ºC. *Loripes* condition was expressed as flesh/shell dry weight ratio, which is a commonly used size-and-age independent measure of fitness in bivalves (Lucas and Beninger 1985). Sulfur contents in the *Loripes* tissues were measured on ICP, following nitric acid digestion.

Statistical analyses

Data were tested for normality prior to analysis. Sulfide data were analyzed with Repeated-Measures three-factor ANOVA. All other variables were analyzed by two- or three-factor ANOVA. All relevant and/or significant effects and interactions are mentioned in the figure legends or supporting text. A complete overview of the statistical output for Figures 2, 3 and A2.4 is provided in Table A2.2.

SUPPORTING TEXT

Both *Zostera* and *Loripes* significantly lowered dissolved ammonium and phosphorus in the sediment pore water, while sulfide addition increased their availability (Figure A2.4). Nitrate concentrations were 0.8 ±0.9 μM (mean ±SD) on average with no significant differences between treatments. Mean leaf nitrogen and phosphorus content were 1.78 ±0.26 and 0.15 ±0.02 % dry weight respectively, which is around reported median values from the field for both (1.8 and 0.2 % DW respectively) (Duarte 1990). None of the treatments had any significant effect on leaf nitrogen. Leaf phosphorus content was unaffected by *Loripes*, but decreased significantly in the sulfide addition and sulfide addition with *Loripes* treatments (from 0.17 ±0.01 to 0.13 ±0.01 % DW; ANOVA: $F_{1,16} = 29.0$, $P < 0.001$). Apparently, high sulfide levels impaired phosphorus uptake by *Zostera* in the sulfide addition treatment, leading to decreased leaf phosphorus content, despite high dissolved phosphorus availability in the pore water (Figure A2.4). Our pulsed sulfide addition also seemed to impair phosphorus uptake in the sulfide addition with *Loripes* treatment, which, by interacting with the reduced dissolved phosphorus pool may have limited growth of *Zostera* under our conditions (Figure 2.3).

Sulfide addition resulted in a significant increase in the relative (ANOVA: $F_{1,16} = 13.8$, $P = 0.002$) and absolute sulfur content (ANOVA: $F_{1,16} = 24.1$, $P < 0.001$) in the flesh of the bivalves. Relative sulfur content was 2.0 ±0.2 % (g:g) in the control treatments and 3.0 ±0.9 % in the sulfide addition treatments. The total amount of sulfur stored in *Loripes* tissues per unit was 1.3 ±0.2 mg in the control treatments and 3.0 ±1.1 mg in the sulfide addition treatments. These results suggest that the increased sulfide availability led to increased storage of sulfur in the tissues of the bivalves, for instance as sulfur granules in the gills (Anderson 1995). We found no significant effects of *Zostera* on *Loripes* sulfur content.

SUPPORTING FIGURES

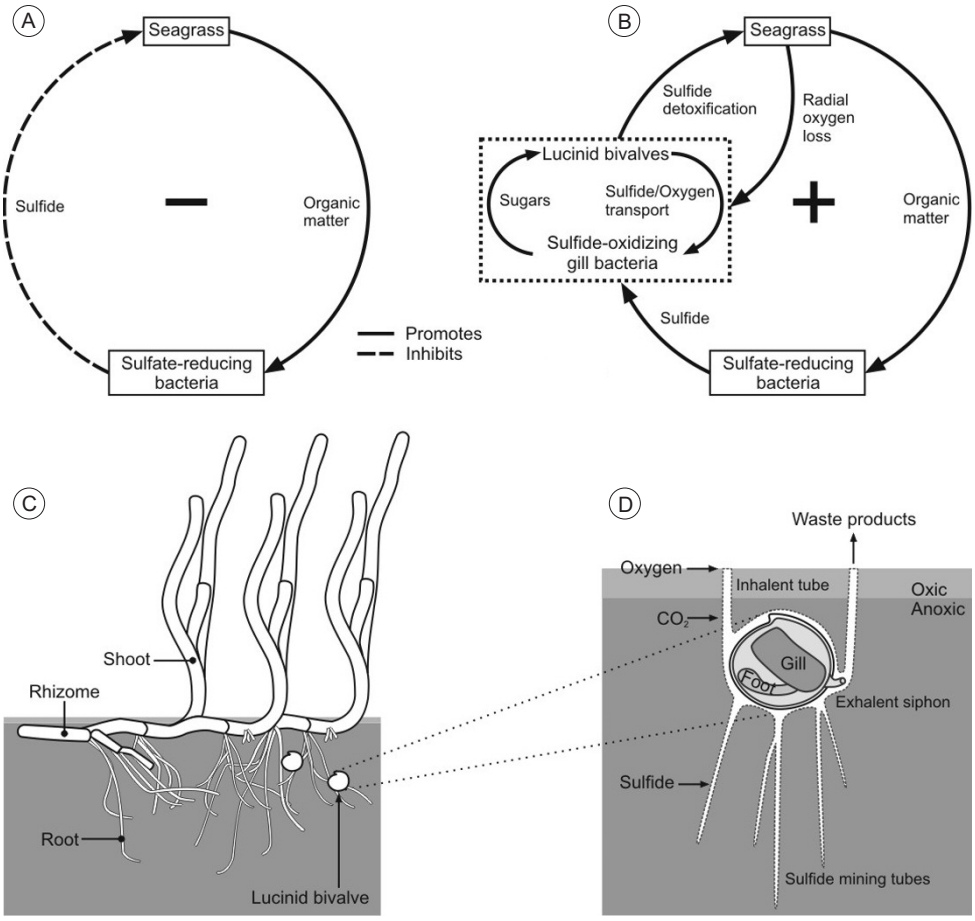

Figure A2.1 (A) Seagrasses generally create a negative feedback on their own growth through organic matter accumulation, which stimulates production of toxic sulfide by heterotrophic sulfate-reducing bacteria. (B) We propose in this study that the presence of lucinid bivalves and their sulfide-oxidizing gill-symbionts breaks the negative feedback, resulting in a network of positive interactions. (C) The bivalves are found in high abundances in the root zones of seagrass meadows in warmer, mild temperate to tropical regions where sulfide production rates are high. (D) They occur in the anoxic zone of the sediment and use their highly extensile foot to create tubes for sulfide mining, export of waste products and import of oxygen and CO_2 from the sediment pore water and surface water (Anderson 1995, Reynolds et al. 2007). Both sulfide and oxygen are transported to the gills where chemoautotrophic bacteria oxidize sulfide for synthesizing sugars that fuel growth of both the bacteria and the bivalve (Johnson et al. 1994, Anderson 1995, Reynolds et al. 2007, Childress and Girguis 2011).

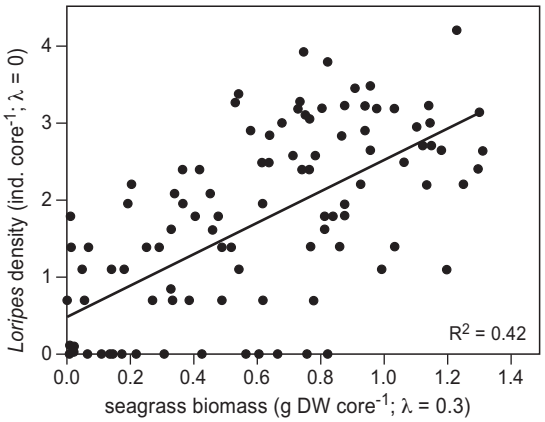

Figure A2.2 Positive correlation (Pearson's r = 0.65) between seagrass biomass and *Loripes* density on Banc d'Arguin. *Loripes* counts and seagrass dry weight from the cores were transformed using the Box-Cox procedure prior to plotting and the regression analysis (see Materials & Methods).

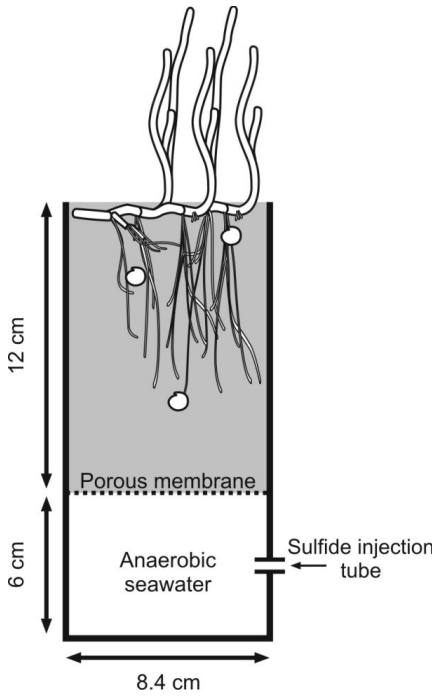

Figure A2.3 Schematic drawing of the setup of an experimental unit. The dimensions of the top section were chosen to fit the organisms and to resemble field conditions. The lower section was kept large enough to allow rapid mixing and upward diffusion. Sulfide was injected twice a week in the sulfide addition treatments and allowed to diffuse from the lower compartment into the upper section through a 0.1-mm porous membrane.

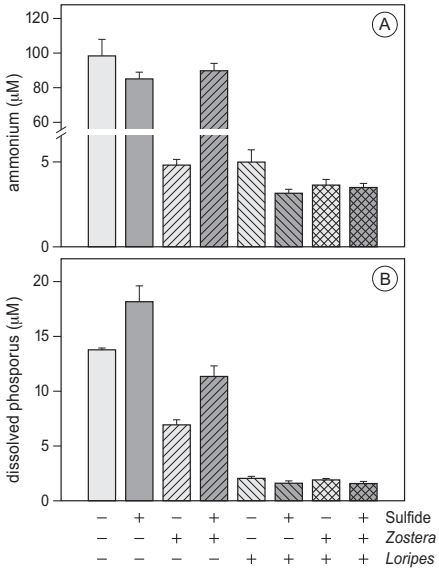

Figure A2.4 Pore water ammonium and dissolved phosphorus contents after five weeks; error bars represent SEM ($n = 5$). Ammonium (A) was lowered significantly by *Zostera* (ANOVA: $F_{1,32} = 59.7$, $P < 0.001$) and *Loripes* ($F_{1,32} = 505.9$, $P < 0.001$), while sulfide addition caused an increase ($F_{1,32} = 35.2$, $P < 0.001$). We found significant interactions between all treatments (Z*L: $F_{1,32} = 57.1$, $P < 0.001$; Z*S: $F_{1,32} = 73.3$, $P < 0.001$; L*S: $F_{1,32} = 39.3$, $P < 0.001$; Z*L*S: $F_{1,32} = 68.5$, $P < 0.001$). The treatment effects on dissolved phosphorus (B) were similar to ammonium, with significant effects of *Zostera* ($F_{1,32} = 58.2$, $P < 0.001$), *Loripes* ($F_{1,32} = 562.1$, $P < 0.001$) and sulfide addition ($F_{1,32} = 19.6$, $P < 0.001$). We found significant interactions of *Zostera* and *Loripes* ($F_{1,32} = 55.1$, $P < 0.001$), and *Loripes* and sulfide addition ($F_{1,32} = 28.2$, $P < 0.001$).

SUPPORTING TABLES

Table A2.1 Lucinid bivalve densities found in seagrass beds. These data provide a basic indication of the association between seagrasses and lucinids worldwide.

Area (source)	Temp.	Clim.	Seagrass genus	Lucinid genus	Density
North America					
Alaska (Jewett et al. 1999, Dean and Jewett 2001)	5 – 13	Temp.	*Zostera*	Lucinidae	p
Boston Harbor (Leschen et al. 2009)	3 – 18	Temp.	*Zostera*		0
Chesapeake Bay (Orth 1973)	1 – 23	Temp.	*Zostera*		0
Apalachee Bay, Florida (Lewis and Stoner 1981)	18 – 29	Subtr.	*Syringodium, Thalassia*	*Codakia*	+
Biscayne Bay, Florida (Moore et al. 1968)	24 – 30	Subtr.	*Halodule, Syringodium, Thalassia*	*Anodontia, Codakia, Lucina*	++/+++
Florida Bay, Florida (Reynolds et al. 2007)	24 – 30	Subtr.	*Halodule, Syringodium, Thalassia*	*Anodontia, Codakia, Lucinesca*	++/+++
Indian River lag., Florida (Mikkelsen et al. 1995)	23 – 29	Subtr.	*Thalassia*	*Lucina*	p
St. Joseph's Bay, Florida (Fisher and Hand 1984)	18 – 29	Subtr.	*Thalassia*	*Lucina*	++/+++
Pensacola Bay, Florida (Stoner et al. 1983)	18 – 29	Subtr.	*Halodule*		0
Redfish Bay, Texas (Center for Coastal Studies 1996)	19 – 29	Subtr.	*Halodule, Thalassia*	*Anodontia, Lucina, Phacoides*	p
Gulf of California, Mexico (Torra Cosio and Bourillón 2000)	19 – 30	Subtr.	*Zostera, Halodule, Ruppia*	*Codakia, Divalinga*	p
Bahia de Chetumal, Mexico (Quesada et al. 2004)	27 – 29	Trop.	*Syringodium, Thalassia*	*Codakia, Lucina*	p
Turneffe Islands, Belize, Mexico (Hauser et al. 2007)	27 – 29	Trop.	*Thalassia*	*Codakia, Parvilucina*	p
Bocas del Toro, Panama (Continental Shelf Associates 1995)	27 – 29	Trop.	*Halodule, Syringodium, Thalassia*	*Codakia, Diplodonta Lucina, Phacoides*	p
Bahama's (Brissac 2009)	24 – 29	Trop.	*Thalassia*	*Codakia*	p
Jamaica (Jackson 1972, Greenway 1995)	27 – 29	Trop.	*Thalassia*	*Anodontia, Codakia, Ctena, Divaricella, Lucina, Parvilucina*	+++/++++
St Croix, Virgin Islands (Ferguson and Miller 2007)	26 – 29	Trop.	*Halodule, Syringodium, Thalassia*	*Codakia, Divalinga, Lucina, Parvilucina*	p
Guadeloupe (Gros et al. 2003)	26 – 29	Trop.	*Thalassia*	*Anodontia, Codakia*	p
Martinique (Brissac 2009)	26 – 29	Trop.	*Thalassia*	*Lucina*	p
Bermuda (Aurelia 1969, Schweimanns and Felbeck 1985)	19 – 28	Subtr.	*Thalassia*	*Codakia, Ctena*	++/+++

Table A2.1 Continued.

Area (source)	Temp.	Clim.	Seagrass genus	Lucinid genus	Density
South America					
Bahia de Neguange, Columbia (Diaz 2003)	26 – 29	Trop.	*Thalassia*, *Syringodium*	*Codakia*, *Lucina*, *Anodontia*	p
Santiago de Tolú, Columbia (Otero Otero and Romani Lobo 2009)	27 – 29	Trop.	*Thalassia*	*Lucina*	p
Morrocoy, Venezuela (Bitter-Soto 1999)	26 – 28	Trop.	*Thalassia*	*Codakia*	+
Mochima Bay, Venezuela (Díaz and Liñero-Arana 2004)	25 – 28	Trop.	*Thalassia*	*Codakia*	+++
Parracho de Maracajaú, Brazil (Martinez 2008)	26 – 28	Trop.	*Halophila*, *Halodule*	*Codakia*, *Divaricella*	p
Abrolhos Bank, Bahia Brazil (Dutra et al. 2005)	25 – 28	Trop.	*Halodule*, *Halophila*	*Codakia*, *Ctena*, *Parvilucina*	p
Ilha do Japonês, Brazil (Marques and Creed 2000, Creed and Kinupp 2011)	23 – 27	Trop.	*Halodule*	*Codakia*, *Divaricella*	++++
Ilha do Mel, Paranaguá, Brazil (Couto and Savian 1998)	18 – 26	Trop.	*Halodule*	*Lucina*	p
Europe					
Western Atlantic, Norway (Fredriksen et al. 2010)	6 – 13	Temp.	*Zostera*		0
Skagerrak, Atlantic, Norway (Fredriksen et al. 2010)	4 – 17	Temp.	*Zostera*		0
Baltic, Finland (Bostrom and Bonsdorff 1997)	1 – 16	Temp.	*Zostera*		0
Sylt, Wadden Sea (Reise 1985)	4 – 18	Temp.	*Zostera*		0
South England (Dando et al. 1986)	8 – 17	Temp.	*Zostera*	*Lucinoma*	+
South Ireland (Dale et al. 2007)	9 – 17	Temp.	*Zostera*	*Lucinoma*	+++
Brittany, France (Monnat 1970, Hily and Bouteille 1999)	10 – 17	Temp.	*Zostera*	*Loripes*, *Lucinoma*, *Lucinella*	+++/++++
Arcachon, France (Blanchet et al. 2004)	12 – 21	Temp.	*Zostera*	*Loripes*	++
Eo estuary, Atlantic coast, Spain (de Paz et al. 2008)	13 – 19	Temp.	*Zostera*	*Loripes*	++/+++
Mediterranean, Spain (Rueda and Salas 2008)	15 – 23	Subtr.	*Zostera*	*Lucinella*	+++
Mallorca, Spain (Centeno 2008)	14 – 25	Subtr.	*Posidonia*	*Ctena*, *Loripes*, *Lucinella*	p

Table A2.1 Continued.

Area (source)	Temp.	Clim.	Seagrass genus	Lucinid genus	Density
Europe					
Corsica, France (Johnson et al. 2002)	13 – 24	Subtr.	*Cymodocea*	*Loripes*	+++/++++
Prelo Bay, Ligurian Sea (Harriague et al. 2006)	13 – 23	Subtr.	*Posidonia*	*Lucinella*	++/+++
Venice lag., Italy (Pranovi et al. 2000, Sfriso et al. 2001)	10 – 26	Subtr.	*Cymodocea*, *Zostera*	*Loripes*	+++/++++
Izmir Bay, Turkey (Cinar et al. 1998)	15 – 23	Subtr.	*Zostera*	*Loripes*	++
Cyprus (Argyrou et al. 1999)	17 – 28	Subtr.	*Posidonia*	*Loripes*, *Myrtea*	+
Black Sea, Romania (Nicolaev and Zaharia 2011)	6 – 24	Temp.	*Zostera*	*Loripes*, *Lucinella*	p
Africa					
Banc d'Arguin, Mauritania (van der Geest et al. 2011)	18 – 26	Subtr.	*Cymodocea*, *Halodule*, *Zostera*	*Loripes*	+++/++++
Baia da Corimba, Angola (Van-Dunem do Sacramento Neto dos Santos 2007)	22 – 29	Trop.	*Halodule*	*Loripes*	p
Kismayo, Somalia (Chelazzi and Vannini 1980)	25 – 29	Trop.	*Halodule*, *Thalassia*	*Codakia*, *Lucina*	p
Zanzibar, Tanzania (Eklof et al. 2005)	25 – 29	Trop.	*Cymodocea*, *Thalassia*, *Enhalus*, *Thalassodendron*	Lucinidae	++/++++
Mahé, Seychelles (Taylor and Lewis 1970)	26 – 30	Trop.	*Thalassia*	*Anodontia*, *Codakia*, *Ctena*	++
Inhaca, Mozambique (de Boer and Prins 2002)	23 – 27	Trop.	*Cymodocea*, *Halodule*, Zostera	*Anodontia*, *Cardiolucina*, *Loripes*, *Lucina*, *Pillucina*	++
Langebaan lag., South-Africa (Siebert and Branch 2005)	15 – 19	Subtr.	*Zostera*		0
Swartvlei estuary, South-Africa (Whitfield 1989)	17– 22	Subtr.	*Zostera*	*Loripes*	p
Asia/Pacific					
Jordan, Red Sea (Taylor et al. 2005)	21 – 28	Subtr.	*Halodule*, *Halophila*	*Rasta*	p
Egypt, Red Sea (Zuschin and Hohenegger 1998)	22 – 29	Subtr.	*Cymodocea*, *Halodule*, *Halophila*	*Cardiolucina*, *Divaricella*, *Pillucina*, *Wallucina*	++++
United Arab Emirates (Feulner and Hornby 2006)	21 – 33	Subtr.	*Halodule*, *Halophila*	*Anodontia*, *Pillucina*	++++
Oman (this study)	25 – 28	Trop.	*Halodule*, *Halophila*	*Pillucina*	++++
Palk Bay, India (Gophinadha-Pillai and Appukuttan 1980)	27 – 30	Trop.	*Cymodocea*, *Halodule*, *Syringodium*, *Thalassodendron*	*Codakia*, *Lucina*	p

Table A2.1 Continued.

Area (source)	Temp.	Clim.	Seagrass genus	Lucinid genus	Density
Asia/Pacific					
Posyet Bay, Sea of Japan (Kharlamenko et al. 2001)	2 – 21	Temp.	*Zostera*	*Pillucina*	+++
Tokyo, Bay of Japan (Whanpetch 2011)	16 – 26	Subtr.	*Zostera*	Luncinidae	p
Okinawa, Japan (Yamaguchi 1999)	22 – 29	Subtr.	*Cymodocea*, *Enhalus*, *Halodule*, *Halophila*, *Thalassia*	*Codakia*, *Epicodakia*	p
Guangxi, China (Huang 2008)	20 – 29	Trop.	*Halodule*, *Halophila*, *Zostera*		0
Guangdong, China (Huang 2008)	21 – 29	Trop.	*Halodule*, *Halophila*	*Pillucina*	p
Hainan, China (Huang 2008)	22 – 29	Trop.	*Cymodocea*, *Enhalus*, *Halodule*, *Thalassia*	*Pillucina*	p
Tubbataha Reefs, Philipines (Yamaguchi 1999)	27 – 30	Trop.	*Halodule*, *Halophila*, *Thalassia*	*Epicodakia*	p
Kungkrabaen Bay, Thailand (Meyer et al. 2008)	28 – 30	Trop.	*Halodule*	*Anodontia*, *Indoaustriella*, *Pillucina*	++++
Had Chao Mai, Thailand (Nakaoka et al. 2002)	28 – 30	Trop.	*Cymodocea*, *Enhalus*, *Halodule*, *Halophila*, *Thalassia*	*Pillucina*	++++
Pulau Semakau, Singapore (Tan and Yeo 2010)	28 – 29	Trop.	*Cymodocea*, *Enhalus*, *Halodule*, *Halophila*, *Syringodium*, *Thalassia*	*Anodontia*	p
Bone Batang, Indonesia (Vonk et al. 2008)	28 – 30	Trop.	*Cymodocea*, *Enhalus*, *Halodule*, *Halophila*, *Thalassia*	Lucinidae	+++
Banten Bay, Indonesia (Kuriandewa 2008)	28 – 30	Trop.	*Cymodocea*, *Enhalus*, *Halodule*, *Halophila*, *Syringodium*, *Thalassia*	*Anodontia*, *Codakia*	p
Tongapatu, Tonga (Yamaguchi 1999)	23 – 27	Trop.	*Halodule*	*Codakia*, *Epicodakia*	p
Tarawa Atoll (Paulay 2000)	28 – 29	Trop.	*Thalassia*	*Codakia*, *Wallucina*	++/+++
Oceania					
Roebuck Bay, Australia (this study, Piersma et al. 2006)	25 – 30	Trop.	*Halodule*, *Halophila*	*Anodontia*, *Ctena*, *Divaricella*	+++
Lizard Island, Australia (Taylor and Glover 2008)	25 – 29	Trop.	*Halophila*	*Anodontia*, *Chaviana*, *Wallucina*	p
Moreton Bay, Australia (Taylor and Glover 2008)	21 – 26	Subtr.	*Cymodocea*, *Halodule*, *Halophila*, *Zostera*	*Anodontia*, *Pillucina*	p
Rottnest Island, Australia (Barnes and Hickman 1999)	19 – 23	Subtr.	*Posidonia*	*Wallucina*	+++/++++
South-West Australia (Hutchings et al. 1991)	16 – 20	Subtr.	*Amphibolis*, *Posidonia*	*Anodontia*	p

Table A2.1 Continued.

Area (source)	Temp.	Clim.	Seagrass genus	Lucinid genus	Density
Oceania					
New South-Wales, Australia (Gibbs et al. 1984)	19 – 24	Subtr.	*Halophila*	*Wallucina*	p
New South-Wales, Australia (McKinnon et al. 2009)	17 – 23	Subtr.	*Halophila*, *Zostera*		0
Western Port, Victoria, Australia (Watson et al. 1984, Edgar et al. 1994)	13 – 18	Temp.	*Halophila*, *Zostera*		0
Tasmania (Edgar et al. 1999a, Edgar et al. 1999b)	12 – 16	Temp.	*Heterozostera*, *Ruppia*, *Zostera*	*Wallucina*	++/+++
New Caledonia (Glover and Taylor 2007)	24 – 28	Subtr.	*Cymodocea*, *Halodule*, *Thalassia*	*Anodontia*, *Codakia*, Ctena	p
Slipper Island, New Zealand (Schwarz et al. 2006)	15 – 21	Subtr.	*Zostera*	*Divaricella*	p

Temp. depicts the mean annual temperature range based on the sea surface temperature (ºC);
Clim. indicates type of climate (tropical, subtropical or temperate);
Lucinid density (spatial average): + = 1–10; ++ = 11–100; +++ = 101-1000; ++++ = >1000 ind/m^2
p = present (no abundance data); u = uncertain; 0 = absent.

Table A2.2 Overview of the statistical output from the analyses of the data presented in Figures 2, 3, and S4.

Treatment	df	F	p
Sulfide measurements (Figure 2.2A; repeated measures ANOVA)			
Zostera	1	6.8	0.014
Loripes	1	268.8	<0.001
Sulfide	1	109.7	<0.001
Zostera * Loripes	1	7.8	0.009
Zostera * Sulfide	1	2.2	0.150
Loripes * Sulfide	1	102.7	<0.001
Zostera * Loripes * Sulfide	1	2.4	0.127
Error	32		
Oxygen measurements (Figure 2.2B; ANOVA)			
Zostera	1	39.3	<0.001
Loripes	1	125.0	<0.001
Sulfide	1	8.9	0.006
Zostera * Loripes	1	48.3	<0.001
Zostera * Sulfide	1	0.0	0.862
Loripes * Sulfide	1	0.3	0.578
Zostera * Loripes * Sulfide	1	0.5	0.505
Error	32		
Zostera shoot biomass (Figure 2.3A; ANOVA)			
Loripes	1	61.3	<0.001
Sulfide	1	72.6	<0.001
Loripes * Sulfide	1	0.9	0.348
Error	16		
Zostera root biomass (Figure 2.3B; ANOVA)			
Loripes	1	50.2	<0.001
Sulfide	1	12.0	0.003
Loripes * Sulfide	1	1.7	0.211
Error	16		
Loripes fitness (Figure 2.3C; ANOVA)			
Sulfide	1	37.3	<0.001
Zostera	1	9.0	0.008
Sulfide * Zostera	1	5.4	0.034
Error	16		
Ammonium (Figure A2.4A; ANOVA)			
Zostera	1	59.7	<0.001
Loripes	1	505.9	<0.001
Sulfide	1	35.2	<0.001
Zostera * Loripes	1	57.1	<0.001
Zostera * Sulfide	1	73.3	<0.001
Loripes * Sulfide	1	39.3	<0.001
Zostera * Loripes * Sulfide	1	68.5	<0.001
Error	32		
Phosphorus (Figure A2.4B; ANOVA)			
Zostera	1	58.2	<0.001
Loripes	1	562.1	<0.001
Sulfide	1	19.6	<0.001
Zostera * Loripes	1	55.1	<0.001
Zostera * Sulfide	1	0.0	0.888
Loripes * Sulfide	1	28.2	0.000
Zostera * Loripes * Sulfide	1	0.0	0.965
Error	32		