A Three-Stage Symbiosis Forms the Foundation of Seagrass Ecosystems

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A Three-Stage Symbiosis Forms the Foundation of Seagrass Ecosystems

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Seagrasses evolved from terrestrial plants into marine foundation species around 100 million years ago. Their ecological success, however, remains a mystery because 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 in 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.

Seagrass meadows are important ecological and thus economic components of coastal zones worldwide (*1*, *2*). In many areas, coral reefs and seagrass meadows are tightly linked habitats that form the basis for marine biodiversity (*3*). Seagrasses serve as a keystone habitat for migrating coral reef species as well as 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 (*1*–*3*). Dense seagrass meadows attenuate currents and waves and trap pelagic and benthic organic matter in the sediment (*2*, *4*, *5*). 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 (*6*). Although seagrasses transport oxygen into their roots and the surrounding rhizosphere (radial oxygen release) (*2*, *7*), sulfide production outpaces oxygen release under warmer conditions, resulting in sulfide accumulation and seagrass mortality (*2*, *7*, *8*). 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 (*2*). But this is not the observed case, and the underlying reason for the long-term persistence of seagrass ecosystems is an enigma (fig. S1A).

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 (fig. S1, B to D). Paleo records suggest that the Lucinidae and their endosymbiotic relation date back to the Silurian (*9*–*11*), but that they increasingly diversified since the evolutionary emergence of seagrasses in the late Cretaceous (*2*, *12*, *13*). Seagrass communities later became widespread in the Eocene, and lucinid remains frequently occur in association with their deposits since (*13*, *14*). Lucinids and their gill-inhabiting bacteria have a symbiosis in which the bivalves transport sulfide and oxygen to their gills (fig. S1D), where the bacteria oxidize sulfide for synthesizing sugars that fuel growth of both organisms (*15*–*19*). We hypothesized that seagrass meadows may provide an optimal habitat for these bivalves and their symbionts by indirectly stimulating sulfide production through 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 (fig. S1, A and 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

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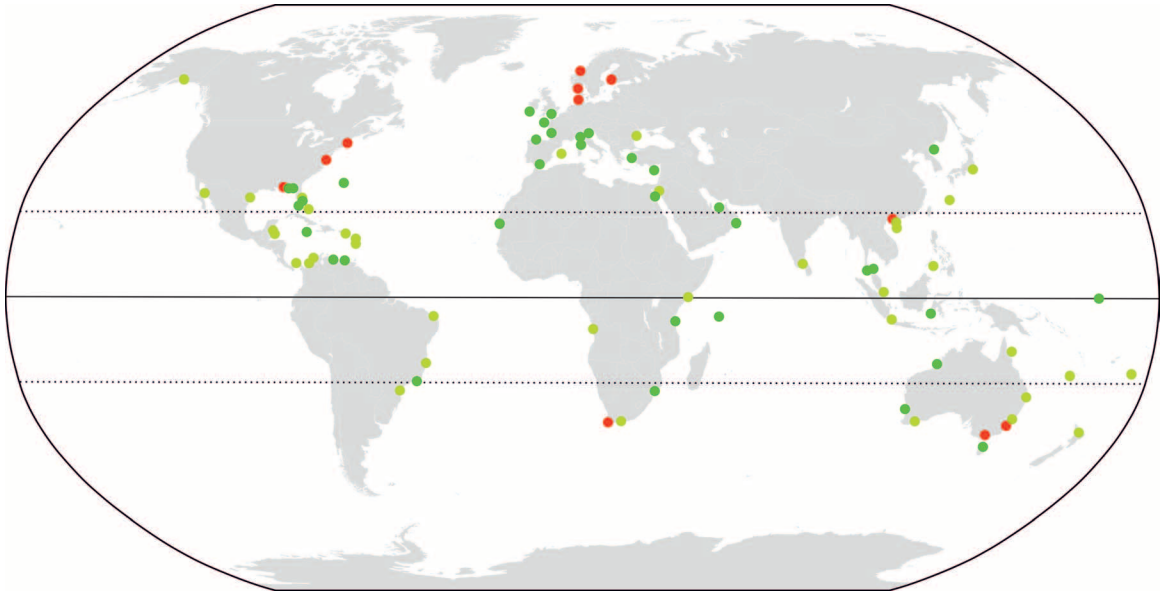

Fig. 1. Presence (green; dark points are quantitative, light points are qualitative) and absence (red) of lucinids in seagrass ecosystems based on our meta-analysis. The bivalves were present in 97% (93% of the quantitative sites) of all tropical seagrass beds, 90% (83% of the quantitative sites) of 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 individuals per square meter) to extremely high densities (>1000 individuals per square meter) of lucinids (table S1).

that we conducted at Banc d'Arguin, Mauritania (*20*). The meta-analysis reveals a relationship that covers 11 out of 12 seagrass genera (and *Ruppia* spp.) and at least 18 genera of Lucinidae (Fig. 1 and table S1). 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 square meter. 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 (*8*). Furthermore, results from our field study showed a positive correlation between seagrasses and lucinids that explained 42% of their respective variation [Pearson's correlation coefficient (r) = 0.65] (fig. S2).

To experimentally test our hypothesis (fig. S1B), 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 (*20*). We set up *Zostera*, *Loripes*, *Zostera-Loripes*, and bare sediment treatments in the top sections of 40 two-compartment columns (fig. S3), 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 5 weeks, pore water sulfide concentrations in the top sections of the sediment controls reached about 400 μM, whereas the semiweekly addition of sulfide caused levels to increase to nearly 2700 μM (Fig. 2A). The presence of *Zostera* decreased sulfide levels to ~200 μM in the controls and 2200 μM in the sulfide addition treatments. In contrast, sulfide levels remained low when *Loripes* was present (~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 because of sulfide-oxidation and intake of surface water (Fig. 2B). *Zostera* alone did not significantly affect sediment oxygen conditions. 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 (Fig. 3A). Reduced shoot biomass was accompanied by decreased root biomass (Fig. 3B) and impaired phosphate uptake (*20*). In contrast, the addition of *Loripes* increased *Zostera* shoot biomass 1.9-fold and root weight 1.5-fold, as 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 (Fig. 3C). Furthermore, the addition of *Zostera* did not affect *Loripes* in the units to which 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 (fig. S1B). Although sulfide was almost completely removed in all *Loripes* treatments (Fig. 2A), the bivalve was less able to profit from the addition of sulfide in the absence of *Zostera* (Fig. 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-oxidizing bacteria reduces sulfide stress in seagrass meadows. Even though radial oxygen release by *Zostera noltii* and of seagrasses in general is limited (*21*, *22*), *Loripes* in our experiment clearly benefitted from the increased oxygen input in the sediment. In the field, the positive effects of seagrasses on lucinids are not confined to sediment oxygenation alone but also by indirectly stimulating sulfide production and releasing dissolved organic molecules (*2*, *18*). The positive effects of *Loripes* on *Zostera* in our experiment could not be explained by differences in nutrient availability (*20*). 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 (fig. S4). We found that in our experiment, the negative effects of sulfide addition on *Zostera* biomass could not fully be prevented by *Loripes* addition (Fig. 3A), despite the removal of almost all sulfide by *Loripes* after 3 days. As the observed experimental effects could not be attributed to differences in nutrient availability, this is

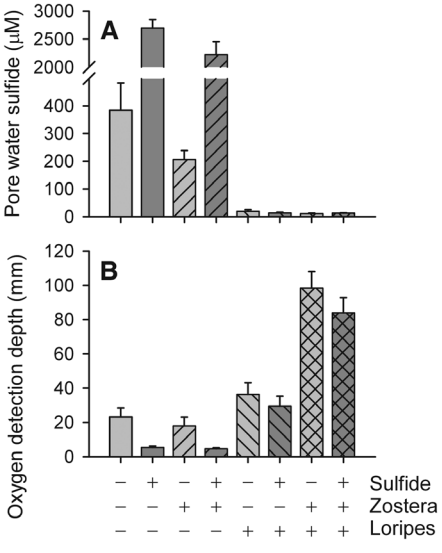

Fig. 2. (**A**) Pore water sulfide concentrations and (**B**) oxygen detection depth after 5 weeks; error bars represent SEM (n = 5 replicates). Oxygen detection depth decreased as sulfide was added [analysis of variance (ANOVA) $F_{1,32}$ = 8.9, P < 0.006]. The presence of *Loripes* reduced sulfide levels (repeated measures 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 occurred 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).

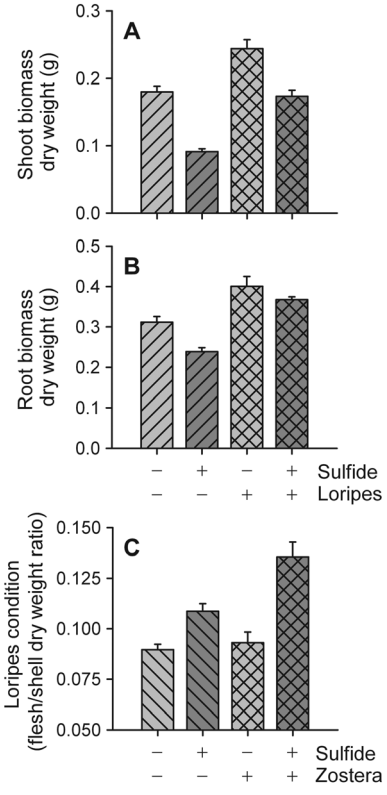

Fig. 3. (**A**) *Zostera* shoot and (**B**) root dry weight biomass per column and (**C**) *Loripes* condition expressed as the dry weight flesh/shell ratio after 5 weeks; error bars represent SEM (n = 5 replicates). *Zostera* biomass was reduced by means of sulfide addition (ANOVA: shoots $F_{1,16}$ = 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).

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 (*1*). Extensive restoration efforts have had little success so far (<30%), despite their extremely high costs (±$100,000 per hectare) (*23*). Similar to the function of mycorrhizae, pollinators, or seed dispersers in terrestrial systems (*24*–*26*), our findings indicate that restoration efforts should not only focus on environmental stressors such as eutrophication, sediment runoff, 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 (*27*). Similar to the well-known symbiosis between corals and their unicellular algal endosymbionts (*28*), we conclude that symbioses, rather than one defining species, forms the foundation of seagrass ecosystems.

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Supplementary Materials

www.sciencemag.org/cgi/content/full/336/6087/1432/DC1
Materials and Methods
Supplementary Text
Figs. S1 to S4
Tables S1 and S2
References (*29*–*119*)

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Fear of Predation Slows Plant-Litter Decomposition

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Aboveground consumers are believed to affect ecosystem functioning by regulating the quantity and quality of plant litter entering the soil. We uncovered a pathway whereby terrestrial predators regulate ecosystem processes via indirect control over soil community function. Grasshopper herbivores stressed by spider predators have a higher body carbon-to-nitrogen ratio than do grasshoppers raised without spiders. This change in elemental content does not slow grasshopper decomposition but perturbs belowground community function, decelerating the subsequent decomposition of plant litter. This legacy effect of predation on soil community function appears to be regulated by the amount of herbivore protein entering the soil.

The quantity and quality of detrital inputs to soil regulate the rate at which microbial communities perform ecosystem processes such as decomposition, nitrogen (N) mineralization, and carbon (C) sequestration (*1*, *2*). Because uneaten plant litter makes up the majority of detritus (*3*), it is assumed that these belowground ecosystem processes are only marginally influenced by biomass inputs from higher trophic levels in aboveground food webs, such as herbivores themselves (*4*). We provide evidence here, however, that predators may influence the decomposition of plant litter via a legacy effect of predation risk. Specifically, a physiological stress response to the risk of predation changes the elemental content of herbivore biomass. In turn, the decomposition of these stressed herbivores alters the function of belowground communities, leading to an overall decrease in the decomposition of plant litter.

Our work addresses whether food web structure (especially the existence of predators) influences ecosystem functioning via changes in the nutritional contents of prey (*5*, *6*). The prevailing view is that food web structure does not influence prey body C-to-N (C:N) contents, because to survive and reproduce, prey must maintian relatively constant body C:N ratios (*7*). However, this view assumes that predator effects on prey are entirely consumptive (*5*). Instead the presence of predators generates fear, leading to physiological stress responses in prey, such as elevated metabolism and the synthesis of heat shock proteins (*8*). Together, these stress responses increase basal energy demands (*9*–*12*) that, in nutrient-limited systems, reduce the energy available for the competing demands of production (that is, reproduction and growth) (*13*). Thus, to meet heightened maintenance-energy demands, stressed herbivores divert energy from production, as well as increase their consumption of energy-rich carbohydrates (*12*). Given that the amount of energy used for production correlates positively with N demand, and that herbivores have limited ability to store excess nutrients, stressed herbivores should also excrete more N (*8*, *14*). N excretion is further enhanced because chronically heightened stress hormone levels increase the breakdown of body proteins to produce glucose (*15*). Ultimately, prey stressed by predation risk should increase their body C:N ratio (*8*), and this is observed in field and laboratory experiments (*12*, *16*).

In this study we asked whether predators can regulate plant-litter decomposition through

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