



# Seagrass-bivalve facilitative interactions: Trait-mediated effects along an environmental gradient

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## ABSTRACT

Facilitative interactions are important forces in shaping community structure and function, and understanding how they respond to environmental changes has become an increasing concern in ecology. Lucinid bivalves play a significant role in seagrass meadows, through a mutualism in which the seagrass provides habitat and oxygen via the roots, while the bivalves and their associated bacteria eliminate sulfides from pore water, improving thus plant performance. In this study, we evaluated how this mutualism is modified along a gradient of organic matter content in the sediment, in a coastal bay dominated by *Cymodocea nodosa* meadows. We used a correlative approach, seeking statistical association between sediment organic matter content, lucinid abundance, and plant traits. Lucinid abundance was higher in vegetated than in bare areas. In vegetated areas, lucinid abundance decreased as organic matter content in the sediment increased, decrease seemingly associated to modification in plant traits, i.e. root abundance and morphology. In organic-rich sediments, roots are less abundant and less branched, reducing the potential habitat for lucinids and suggesting a weakening of the interaction. This finding contributes to our understanding of how facilitative interactions can be modified along human disturbance gradients, and how disturbances can reduce resilience of seagrasses through this modification.

## 1. Introduction

Biological interactions are crucial drivers in shaping the biosphere. Positive interactions (i.e., those benefiting at least one of the species involved and not detrimental to the other), are recognized as playing a critical role in ecosystem function and structure (Brooker et al., 2008; Bruno et al., 2003; McIntire and Fajardo, 2014).

Facilitative interactions are widespread, and their occurrence and strength has been shown to be highly context-sensitive (Eckert et al., 2010; Tylianakis et al., 2008) since environment can alter the biological, ecological, or behavioral traits of the species involved (e.g., Kiers et al., 2010; Schöb et al., 2013), and therefore the outcome of their interaction. Moreover, the behavioral, physiological, and biological traits on which facilitative interactions depend are not built-in software, and can undergo evolutionary modifications as a consequence of their strong influence on fitness. Overall, the nature and strength of these interactions and their effects at the ecosystem level are highly variable in space and time. For instance, facilitative interactions have been hypothesized to increase their frequency, intensity, and importance with increasing environmental stress (stress-gradient hypothesis: Bertness and Callaway, 1994; He and Bertness, 2014), although the generality of this hypothesis remains controversial (He and Bertness,

2014; Maestre et al., 2009; Michalet et al., 2014).

Environmental changes, and specifically those caused by human activities, can alter facilitative interactions with potential consequences for ecosystem function. For example, it has been shown that global change may disrupt critical mutualisms by causing shifts in the phenology of the species involved, as for instance plants and their pollinators (Memmott et al., 2007). Such trait-mediated interactions (TMI) triggered by environmental changes have been shown to exert a major influence on modulating community structure and function in a wide range of ecosystems (Callaway et al., 2003; Coldren, 2013).

In marine ecosystems, positive interactions have been revealed as essential functional components (Bulleri, 2009), however, while in the terrestrial environment the stress-gradient hypothesis (see above) has played a pivotal role in focusing research, much less attention has been paid to it in the marine realm (Bulleri, 2009). Thus, gaining knowledge on how environmental drivers modulate facilitation and its implications at the ecosystem level is essential in order to understand how species fitness, ecological functions, and the associated provision of goods and services will shift in a changing world. This increases in importance when the ecosystem is highly vulnerable to human pressures such as coastal ecosystems (Ruiz-Halpern et al., 2008) and when the species involved are foundation or keystone species (Jones et al., 2008;

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Kwiatkowski et al., 2015).

Seagrasses are foundation species inhabiting subtidal, usually soft bottom habitats (Green and Short, 2003) that host a huge biodiversity, while providing a number of valuable goods and services. As with other coastal water ecosystems (e.g., kelp forests, mangroves, and coral reefs), in recent decades they have undergone significant deterioration caused by human activities. How this has affected their structure and function has been extensively documented, although most of the studies published to date have been aimed at assessing the direct effects of shifts in environmental factors such as light, nutrients, organic matter, and sedimentation on the plant and associated organisms (Cabaço et al., 2008; Cardoso et al., 2008; Daudi et al., 2012). Less frequently, research has focused on evaluating how human impacts alter biotic interactions that are important for meadow resilience, especially top-down effects (de Paz et al., 2008; Tomas et al., 2015; Van Der Heide et al., 2007). However, the role played by positive interactions (other than the obvious habitat provision by the seagrass), and how they are affected by human impacts have very rarely been examined (but see Maxwell et al., 2016).

Nevertheless, such positive interactions can play important roles in seagrass ecosystem persistence and resilience. The best example is probably a complex mutualism involving bacteria, lucinid bivalves, and seagrasses (van der Heide et al., 2012) in which seagrasses provide lucinids with a suitable habitat (the rhizosphere) and oxygen through root radial diffusion, while the bivalves protect the plants against sulfide toxicity by oxidizing pore water sulfide, an oxidation which is mediated by the chemoautotrophic bacteria inhabiting their gills (Pedersen et al., 1998; Reynolds et al., 2007). This mutualism has been postulated as fundamental for seagrass persistence (van der Heide et al., 2012), because seagrass sediment pore water is rich in sulfide, due to the high activity of sulfate-reducing bacteria (Koch et al., 2007). In support of this notion, it has recently been demonstrated that disruption of the mutualism due to drought has worsened the deleterious effects of desiccation on seagrasses (de Fouw et al., 2016).

Eutrophication is one of the most pervasive impacts of human activities worldwide, and the increase in sediment organic matter content is one of its main consequences, involving lower level of oxygen in sediment pore water and, consequently, higher levels of sulfide production (Lamers et al., 2013). While it is clear that sulfide effects can be dampened by the activity of the lucinids and their associated bacteria, the possible modification of the mutualism by the increase in sediment organic matter content remains unexplored.

The aim of our study was to help fill this gap by assessing how seagrass-lucinid mutualism responds to increases in organic matter content of sediment. Such increases can trigger a number of responses, affecting plant traits (e.g., modifying shoot and root morphology and production) (Fertig et al., 2013; Pérez et al., 1994), environmental conditions (e.g., light reaching the plant) (Ralph et al., 2007), and sulfide concentration in pore water (Govers et al., 2014), which can potentially affect the outcome of the mutualism.

Thus, we studied the association between the seagrass *Cymodocea nodosa* (Ucria) Ascherson and the abundance of the lucinid bivalve *Loripes lucinalis* (Lamarck, 1818) (synonym *Loripes lacteus*, sensu Poli, 1791) along a gradient of organic matter content in sediment, considering the abundance of lucinids as an indicator of the importance of the seagrass-bivalves interaction. We first assessed whether or not the presence of the seagrass facilitated the presence of lucinids, by comparing bivalve densities between seagrass meadows and bare sediments. Second, we explored the variability in lucinid abundance along a gradient of organic matter content in vegetated sediments, hypothesizing higher lucinid abundances in organic-richer sediments under the assumption of a facilitation enhanced by increased sulfide content in the sediment. Third, we explored the association between the abundance of lucinids and a variety of plant traits (e.g. root biomass, root architecture) to gain insight on the influence of such traits on bivalve population.

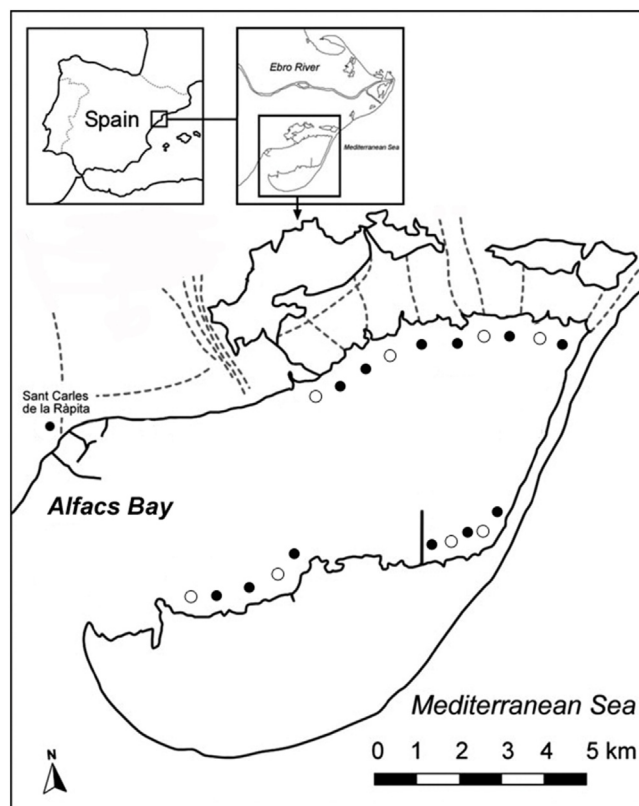


Fig. 1. Sampling locations in Alfacs bay covering the organic matter gradient generated by the freshwater organic-rich inputs from rice fields. Dashed lines represent drainage channels from rice fields. Black spots represent samplings in seagrass meadows and bare sediment (plants, lucinids and sediment). White spots represent the additional samples in seagrass meadows (plants and lucinids).

## 2. Materials and methods

### 2.1. Study site

The study was conducted in Alfacs bay, situated on the southern side of the Ebro river delta (NW Mediterranean) (Fig. 1). This bay is relatively shallow (maximum depth of 6 m) and has a surface area of 50 km<sup>2</sup>. Large *C. nodosa* meadows grow all around the bay, at depths between 0.5 and 1.5 m (Oliva et al., 2012). The area receives nutrient- and organic matter-rich freshwater discharges from rice crop fields, which enter the bay on its northern shore, while the southern shore is influenced by marine waters from the open sea. This generates a marked gradient of eutrophication that has been documented in previous studies based on data of a number of environmental factors (nutrient concentration in water, organic matter content in the sediment, N and P content in seagrass leaves, P in sediment, etc.; see Mascaró et al., 2014; Oliva et al., 2012; Pérez et al., 1994).

### 2.2. Sampling design

To obtain data from conditions encompassing the wider possible range of sediment organic matter content, twelve sampling points at a constant depth (0.5–0.7 m) were selected around the bay (Fig. 1). At each point, we took two samples: one in the seagrass meadow (including above and belowground seagrass parts, plus bivalves and other fauna) and one in bare sediment (including bivalves and other fauna). This was done using a hand-held core sampler (large core thereafter, 15 cm diameter) pushed to a sediment depth of 30 cm. To estimate the sediment organic matter content, at each one of these twelve points, two surface (0–3 cm) sediment samples were taken with hand cores

(small cores thereafter, 4 cm in diameter), one close to the sample in vegetated areas and the other close to the sample in bare areas. Additionally, we took eight additional large core samples in vegetated points along the gradient and at the same depth, to better assess plant traits variability and gain statistical power. Immediately after collection, samples from large cores were rinsed, sieved through a 5 mm mesh to remove sediment and transported chilled to the laboratory. Sampling was conducted in June 2013, since June corresponds to the maximum growth period of *C. nodosa* (Mascaró, 2011) and is close to one of the two annual peaks of reproduction of *L. lucinalis* (Johnson and Le Pennec, 1994).

### 2.3. Sample processing

In the laboratory, live lucinid bivalves from each large core sample were separated from the rest and counted to evaluate their abundance (in terms of density, individuals  $m^{-2}$ ). In addition to this, in samples obtained in seagrass meadows, shoots were counted to estimate shoot density, and plants were sorted into leaves, rhizomes, and roots. These fractions were then rinsed, dried ( $70^{\circ}$  for 24 h) and weighed. We used the root weight ratio (RWR, Reynolds and D'Antonio, 1996) to express the relative abundance of root versus leaves. The RWR was calculated as the biomass of roots divided by the sum of the biomass of roots plus the biomass of leaves. To assess differences in root morphology and branching (viz. root architecture), five roots from each sample were selected at random before drying, and the maximum length of each root was measured and the number of primary and secondary ramifications counted. Then, an index of root branching (BI) was calculated as the total number of ramifications divided by the maximum root length.

Sediment organic matter content was determined as loss on ignition (LOI450) in samples from small cores. Dried sediment samples ( $70^{\circ}C$  until constant weight) were combusted for 4 h at  $450^{\circ}C$ . Organic matter was expressed as percentage of total weight.

### 2.4. Statistical analysis

The significance of the differences in lucinid density between habitats (*C. nodosa* and bare sediment) was assessed using a linear model with habitat as a categorical variable (two levels, seagrass and bare sediment) and organic matter content as continuous variable. Data were square-root transformed to meet the assumptions of normality and homogeneity of variances using the customary tests (Shapiro-Wilks test for normality, Bartlett test for homoscedasticity). Association between plant traits (root biomass, root weight ratio, root branching index, and shoot density), organic matter content in the sediment, and lucinid abundance were assessed by linear correlation analyses using Pearson's correlation. All statistical calculations were conducted using the software program R (R Development Core team 2015) with RStudio (version 0.98.1062).

## 3. Results

Live individuals of *L. lucinalis* were found in all sampled sites, with their abundances displaying a high variability, from 57 to 1981 ind  $m^{-2}$ . Lucinid density was much higher (ca. 5-fold) in vegetated ( $889 \pm 225$  ind  $m^{-2}$ ) than in bare sediments ( $172 \pm 80$  ind  $m^{-2}$ ) (Fig. 2). These differences were highly significant, following the linear model applied ( $p < 0.001$ ). In contrast, and taking all the samples together (from both seagrass and bare areas), organic matter did not influence lucinid abundance ( $p = 0.396$ ).

The abundance of lucinids showed a negative correlation with the organic matter content in vegetated sediments (Fig. 3a), but showed no correlation in bare ones (Fig. 3b).

Seagrass traits varied along the sediment organic matter content gradient. The relative biomass allocation to roots, as indicated by the RWR, clearly decreased with increasing sediment organic matter

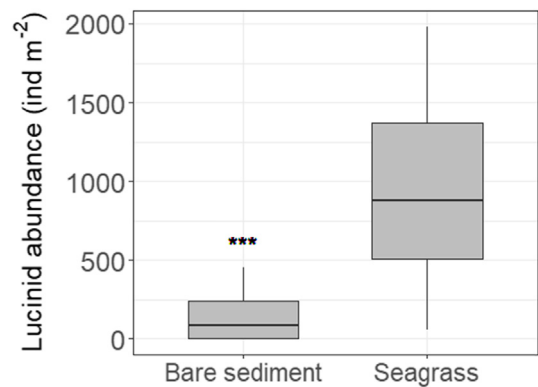


Fig. 2. Abundance of lucinids (ind  $m^{-2}$ ) in bare sediments ( $n = 12$ ) and in seagrass meadows ( $n = 12$ ). Asterisk denote significant differences ( $p < 0.001$ ).

(Fig. 4a). This paralleled the significant changes evidenced along the gradient in root morphology (as indicated by the branching index, BI), with poorly branched roots in organic-rich sediments and densely branched roots in organic-poor ones (Fig. 4b). These changes in root morphology were visually conspicuous, with roots tending to be fibrous (highly branched and without a distinct main axis) in organic-poor sediments, shifting to monopodial roots (a main vertical axis with limited branching) in organic-rich ones. Root biomass was not correlated with sediment organic matter content (Fig. 4c), while shoot density was (Fig. 4d).

Lucinid abundance was significantly and positively correlated to root traits, including root biomass (Fig. 5a), root weight ratio (Fig. 5b), and the root branching index (Fig. 5c). However, it showed no correlation with shoot density (Fig. 5d).

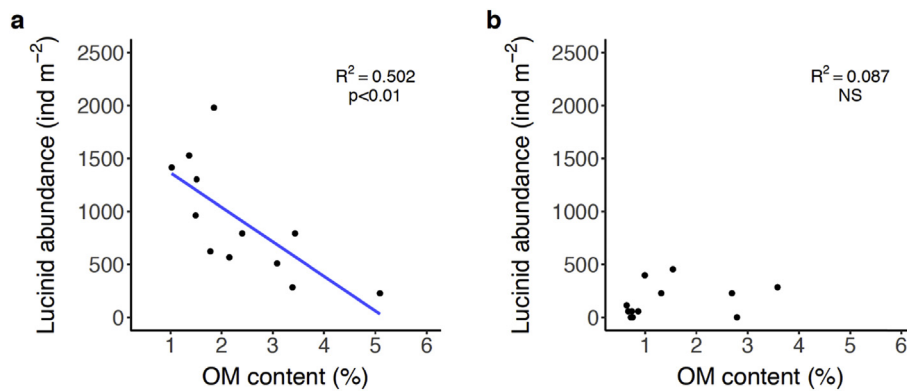
## 4. Discussion

Our results show that an increase in sediment organic matter content is associated to a decline in the abundance of *Loripes lucinalis* (lucinid bivalve) in the *Cymodocea nodosa* meadows studied, which potentially may weaken the mutualism between the two species. This decrease appears to be statistically associated with changes in plant traits, especially in roots, which are less abundant and developed (poorly branched) in the most organic-rich sediments. Albeit not totally conclusive, this suggests an indirect and negative effect of the sediment organic matter content (and, probably, of eutrophication) on the outcome of the facilitative interaction between the seagrass and the bivalves through the modification of plant traits.

Seagrasses present substantial phenotypic plasticity in morphological and physiological traits, which becomes evident under stress conditions or following disturbance events (Maxwell et al., 2014) and may have a strong influence on modulating species interactions. As a fast growing species, *C. nodosa* displays a high variability in morphological, physiological, and reproductive traits in response to environmental stress (Mascaró et al., 2009; Oliva et al., 2014; Sandoval-Gil et al., 2012). In particular, according to previous studies (Oliva et al., 2007; Pérez et al., 1994), root morphology and architecture change from oligotrophic to eutrophic sites, from thinner and densely branched to thicker and monopodial roots, probably as an adaptive response to optimize biomass allocation and exploit the nutrient pools partitioned between the water column and the sediment (Romero et al., 2006; Kiswara et al., 2009). Given the link between lucinids and seagrass roots, this response can influence the facilitative interplay between the plant and the bivalves.

It is well known that seagrass roots can facilitate the presence of lucinid bivalves, either through habitat provision (the roots themselves) or through resource facilitation (radial oxygen release) (Reynolds et al., 2007; van der Heide et al., 2012). This has been also shown for other

How to determine what is a trait? Maybe if it follows after a species or it's possessive?



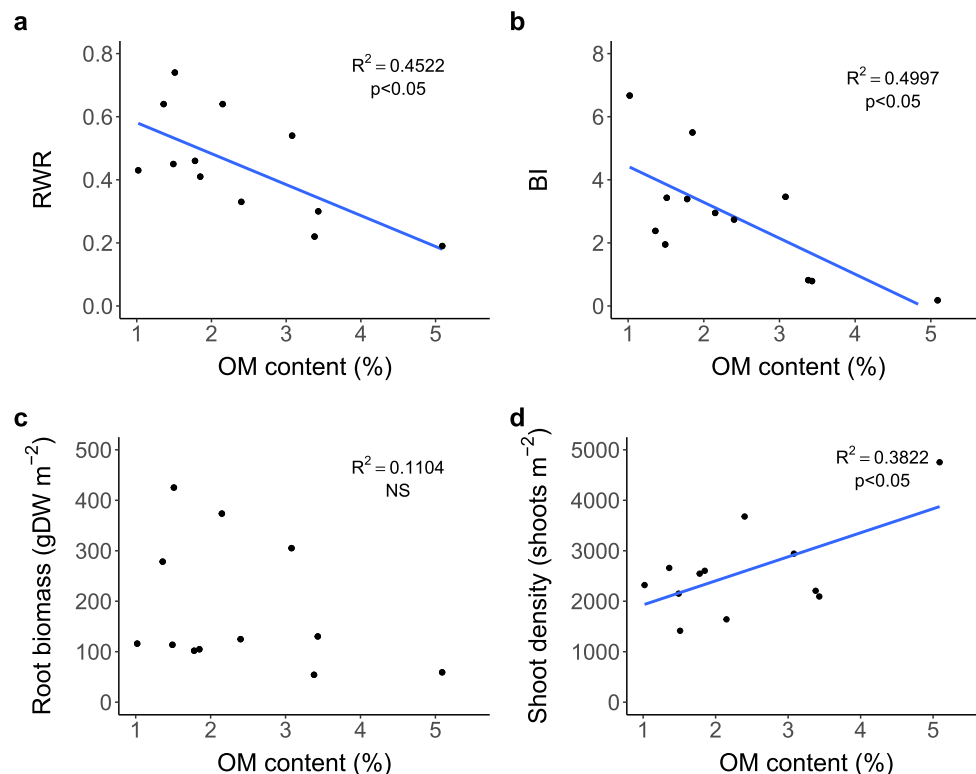
**Fig. 3.** Statistical association, assessed using linear correlation, between the sediment organic matter content and the abundance of lucinid bivalves ( $n = 12$ ) in (a) seagrass meadow and (b) bare sediment.  $R^2$  and  $p$ -values are reported in the plots.

infaunal species (Hemminga and Duarte, 2000; Fredriksen et al., 2010), finding habitat, food, and oxygen in or close to the rhizosphere. Indeed, we found that lucinids were much more abundant in areas with *C. nodosa* vegetation, relative to bare ones. Moreover, in vegetated areas, lucinid abundance was positively associated with the root biomass, the relative abundance of roots (relative to shoots, RWR), and the branching index (BI), but not with the shoot density, suggesting that plant root traits are the major drivers of lucinid abundance in the *C. nodosa* meadows studied.

Our data do not allow us to completely rule out the existence of effects other than those mediated by plant trait modifications, such as direct effects of organic matter content in the sediments (inducing oxygen exhaustion) or other variables associated to the gradient (salinity, sediment grain size). However, these confounding effects are highly unlikely to occur. Thus, on the one hand, the maximum values of sediment organic matter content reported here are similar to or lower than most published values for sediments hosting healthy lucinid populations (Como et al., 2007; de Paz et al., 2008). On the other hand, the effect of variables changing along the transect (direct effect of

organic matter, salinity, grain size) would have affected both to bare and vegetated sediments populations, whereas our data only indicate a decrease in bivalve abundance in the latter. Moreover, the sediment grain size is quite homogenous across the bay (medium-fine sand; from 181 to 298  $\mu\text{m}$  Ø), and the correlation between the grain size obtained in previous works (Romero et al., 2010) and the abundance of lucinids from this work is not significant ( $n = 12$ ,  $r = 0.461$ ,  $p = 0.154$ ). Accordingly, and in spite of the correlative nature of our study, we conclude that the decline in lucinid abundance along the organic matter gradient in vegetated sediments is an indirect effect mediated by the modification of plant traits.

As facilitation plays a major role in the structuring and the functioning of marine ecosystems (Bertness, 1989, 1991; Hacker and Bertness, 1995; Bruno, 2000; Bulleri, 2009; among others), understanding how the strength of these facilitative interactions is altered by increasing levels of stress is critical to assess the resilience of the affected systems. In this respect, and according to the stress-gradient hypothesis, facilitative interactions are more frequent with increasing levels of stress (Bertness and Callaway, 1994; Crotty and Bertness,



**Fig. 4.** Statistical association between the organic matter content and plant variables ( $n = 12$ ) assessed using linear correlation. (a) root weight ratio (RWR), (b) root branching index (BI), (c) root biomass, and (d) shoot density.  $R^2$  and  $p$ -values are reported in the plots.



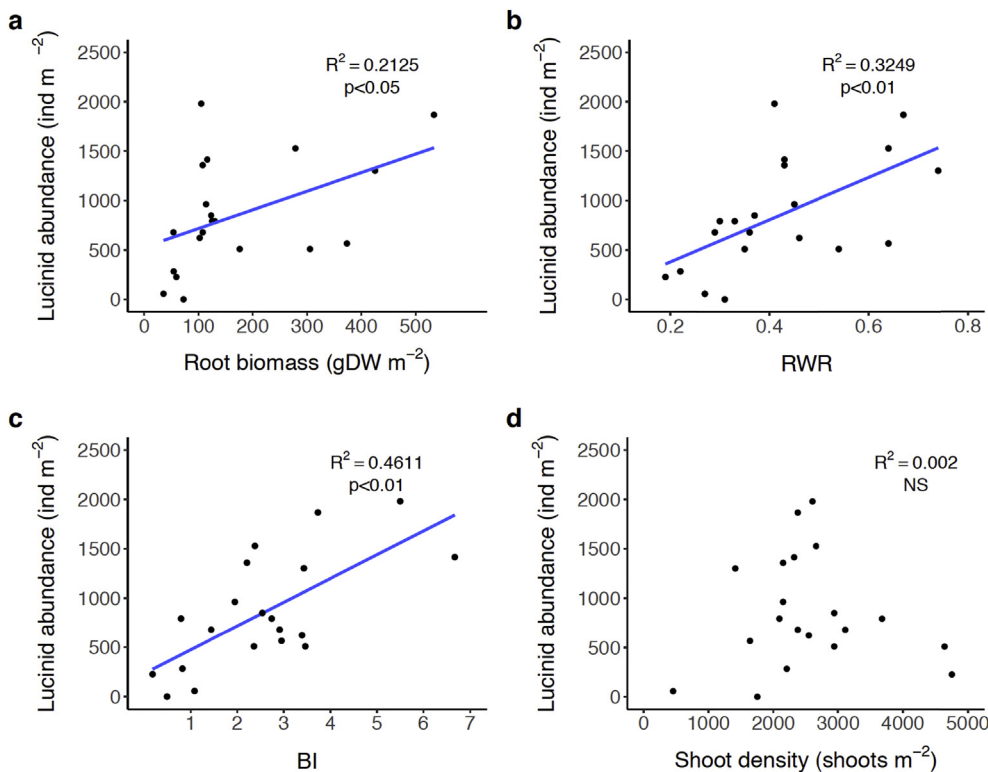


Fig. 5. Statistical association between the abundance of lucinid bivalves and plant variables ( $n = 20$ ), assessed using linear correlation. (a) root biomass, (b) root weight ratio (RWR), (c) root branching index (BI), and (d) shoot density.  $R^2$  and  $p$ -values are reported in the plots.

2011; He et al., 2013). However, our results do not conform to this general pattern. Two opposite influences seem to act simultaneously on our *C. nodosa* meadows. On the one hand, the increase in sediment organic matter increases pore water concentration of sulfide, which constitutes a resource for the symbiotic lucinid bacteria. This, together with higher food availability for bivalves in organic-rich sediments (van der Geest et al., 2014), would eventually lead to increases in lucinid abundance. On the other hand, the modification of *C. nodosa* root traits reduces habitat and resource provision (oxygen) to the lucinid population. Apparently, and in line with our results, the latter influence overrules the former, yielding as a net result a clear decline in lucinid abundance and, consequently, the possible disruption of the facilitative mechanism. Given the importance attributed to the seagrass-lucinid interaction for the persistence of seagrass meadows (van der Heide et al., 2012), the weakening of the interaction can represent a serious threat for these important habitat-forming species, as has been demonstrated for the seagrass *Zostera noltii* (de Fouw et al., 2016). Interestingly, we did not observe signs of deterioration in plant performance or survival (see, for example, the increase in shoot density with organic matter) in the organic-rich sediments, with low lucinid abundances. This suggests that other mechanisms enable this species to resist relatively high levels of organic matter in the sediments (Terrados and Duarte, 1999; Oliva et al., 2012). In conclusion, our findings shed light on how human disturbances can weaken facilitative interactions indirectly through changes in species traits. The consequence of this for ecosystem function and service provision is still uncertain and further research is required to clarify this question. The disruption of mutualism caused by stress can represent an additional challenge to the resilience of key ecosystems (Kiers et al., 2010). Assessing the importance of facilitative interactions and their susceptibility to environmental change is therefore crucial to better predict marine ecosystem persistence in a changing world.

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