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Research article

A facultative mutualism facilitates European seagrass meadows

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Coastal ecosystem functioning often hinges on habitat-forming foundation species that engage in positive interactions (e.g. facilitation and mutualism) to reduce environmental stress. Seagrasses are important foundation species in coastal zones but are rapidly declining with losses typically linked to intensifying global change-related environmental stress. There is growing evidence that loss or disruption of positive interactions can amplify coastal ecosystem degradation as it compromises its stress mitigating capacity. Multiple recent studies highlight that seagrass can engage in a facultative mutualistic relationship with lucinid bivalves that alleviate sulphide toxicity. So far, however, the generality of this mutualism, and how its strength and relative importance depend on environmental conditions, remains to be investigated. Here we study the importance of the seagrass-lucinid mutualistic interaction on a continental-scale using a field survey across Europe. We found that the lucinid bivalve *Loripes orbiculatus* is associated with the seagrasses *Zostera noltii* and *Zostera marina* across a large latitudinal range. At locations where the average minimum temperature was above 1 °C, *L. orbiculatus* was present in 79% of the *Zostera* meadows; whereas, it was absent below this temperature. At locations above this minimum temperature threshold, mud content was the second most important determinant explaining the presence or absence of

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L. orbiculatus. Further analyses suggest that the presence of the lucinids have a positive effect on seagrass biomass by mitigating sulphide stress. Finally, results of a structural equation model (SEM) support the existence of a mutualistic feedback between *L. orbiculatus* and *Z. noltii.* We argue that this seagrass-lucinid mutualism should be more solidly integrated into management practices to improve seagrass ecosystem resilience to global change as well as the success of restoration efforts.

Keywords: conservation, European coastline, Lucinidae, mutualism, positive interactions, seagrass meadows

Introduction

Coastal ecosystems make up only 4% of the Earth's surface but are of great social, economic and ecological importance (Costanza et al. 1997, Barbier et al. 2011). Because almost half of the human population lives in coastal areas, with numbers still increasing (Halpern et al. 2008), there is growing concern that coastal ecosystems are increasingly negatively affected by human-induced global change (e.g. eutrophication, climate change, infrastructure developments, fisheries). Indeed, numerous studies show that (human-induced) disturbance is associated with a rapid decline of coastal ecosystems such as seagrass meadows, coral reefs, salt-marshes and mangrove forests (Orth et al. 2006, Waycott et al. 2009, De los Santos et al. 2019, Halpern et al. 2019, Dunic et al. 2021). Despite many protection and restoration efforts, these decline continue (Bayraktarov et al. 2016, Saunders et al. 2020), which may suggest that certain fundamental processes or interactions may be overlooked.

Classically, declines of coastal ecosystems are often directly linked to intensifying environmental stressors. However, growing evidence suggests that environmental stressors may also drive or amplify degradation through disruption of positive biotic interactions between species (e.g. facilitation and mutualism) (Bruno et al. 2003, de Fouw et al. 2016a, Maxwell et al. 2017). Over the last two decades, the importance of positive interactions has become increasingly recognized in marine systems such as salt marshes, mangroves, coral reefs and seagrass meadows (Bruno et al. 2003, Maxwell et al. 2017, Gagnon et al. 2020, Valdez et al. 2020, van der Heide et al. 2021). Seagrass meadows are archetypical examples of ecosystems shaped by positive interactions (Maxwell et al. 2017, Valdez et al. 2020), which can be both intra- and interspecific. An example of the former, is the positive interaction in which seagrasses increase water clarity through suspended particle trapping and sediment stabilization, yielding a self-facilitating feedback on their own growth (van der Heide et al. 2007, Fourqurean et al. 2012, Hansen and Reidenbach 2012). An example of the latter is the engagement of seagrasses in positive interactions with bivalves, yielding for example increased nutrient availability, drought resistance, or alleviation of anoxia (Peterson and Heck Jr 2001, Gagnon et al. 2020, Meysick et al. 2020).

Over the last decade, several studies have shown the importance of a recently discovered positive interaction between seagrass and bivalve of the Lucinidae family for functioning and stability of seagrass meadows (van der Heide et al. 2012, Stanley 2014, de Fouw et al. 2018, Fales et al. 2020, Martin et al. 2020, van der Geest et al. 2020, Chin et al. 2021). Although seagrasses generate a positive feedback by increasing light and nutrient availability through suspended particle trapping, this process can simultaneously create a negative feedback as accumulating organic matter in the sediment is decomposed anaerobically by sulphate-reducing bacteria to yield toxic sulphides (van der Heide et al. 2012, Lamers et al. 2013, Holmer and Hasler-Sheetal 2014). While seagrasses release oxygen through their roots in the surrounding rhizosphere (radial oxygen loss) to detoxify sulphide (Pedersen et al. 2004), its production can outpace oxygen release in organic matter-rich sediments, particularly under warmer temperature conditions. As a consequence, sulphide concentrations can reach toxic levels, reducing seagrass productivity and increasing mortality (Borum et al. 2014, Holmer and Hasler-Sheetal 2014, Hasler-Sheetal and Holmer 2015). To mitigate sulphide accumulation, however, seagrasses can engage in a facultative mutualistic interaction with lucinid bivalves and their gill-inhabiting, sulphide-oxidizing bacteria. In return, the bivalves and their endosymbionts profit not only from sulphide that is indirectly provided by the seagrasses, but also from oxygen released by seagrass roots and protection from predation due it's complex root and rhizome network (van der Heide et al. 2012, de Fouw et al. 2016b, de Fouw et al. 2018, van der Geest et al. 2020, Chin et al. 2021, de Fouw et al. 2022).

Current understanding is that the seagrass-lucinid mutualism could be a key interaction mediating both seagrass productivity and ecosystem stability (van der Heide et al. 2012, de Fouw et al. 2016a, Gagnon et al. 2020, Valdez et al. 2020, Chin et al. 2021). Even though lucinids occur in many seagrass meadows worldwide (van der Heide et al. 2012, Stanley 2014), it remains unknown how the strength and importance of the mutualism depends on abiotic conditions. As anaerobic degradation and related sulphide production are strongly temperature-dependent (Jørgensen 1977), it is likely that mutualism strength depends on temperature. Sediment characteristics may also affect the mutualism. For instance, particulate trapping by seagrass may increase mud content potentially negatively influencing lucinid occurrence (Anderson 2008, Ellis et al. 2017, Douglas et al. 2019). In addition, organic matter-rich sediments like those commonly found in eutrophic systems and in wave sheltered areas, may have an inherently higher sulphide production, increasing the importance of the detoxification mutualism. Furthermore, mutualism strength may also depend on the iron content of the sediment. In general, iron-rich sediments have a high chemical sulphide binding capacity by

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forming non-toxic iron sulphide precipitates (e.g. FeS, FeS_2). Therefore, compared to more iron-rich sand- or clay- based sediments, seagrasses growing on carbonate-based sediments may more often experience harmful sulphide levels as these sediments are typically iron-poor (Holmer et al. 2005, Ruiz-Halpern et al. 2008). Because it is currently unclear how the detoxification mutualism depends on these environmental conditions, it is also challenging to predict the consequences of future environmental changes for this mechanism and its importance for conservation and restoration.

To explore the conditional generality of this facultative mutualism for seagrass meadows, and hence its importance for conservation and restoration, we investigated 1) under what environmental conditions lucinid bivalves are associated with seagrass meadows (i.e. what determines their presence/absence), 2) if they facilitate seagrasses when present, and 3) if there is evidence for the generic mutualistic feedback at the continental scale. To this end, we conducted a continent-wide survey of seagrasses, lucinids, sediments and environmental conditions in seagrass meadows at 31 *Zostera* meadows along European coastal zones stretching 17 degrees latitude. We hypothesize that 1) sediment characteristics (e.g. mud content, organic matter, dissolved carbon, sulphur- and iron content), temperature and seagrass biomass will show a strong positive relation with lucinid presence/absence, 2) lucinids in turn will promote seagrass biomass by decreasing sulphide stress and thus 3) effectively result in a generic mutualistic feedback. Finally, we used structural equation modelling (SEM) to find evidence for the mutualistic seagrass-lucinid feedback (Fig. 1).

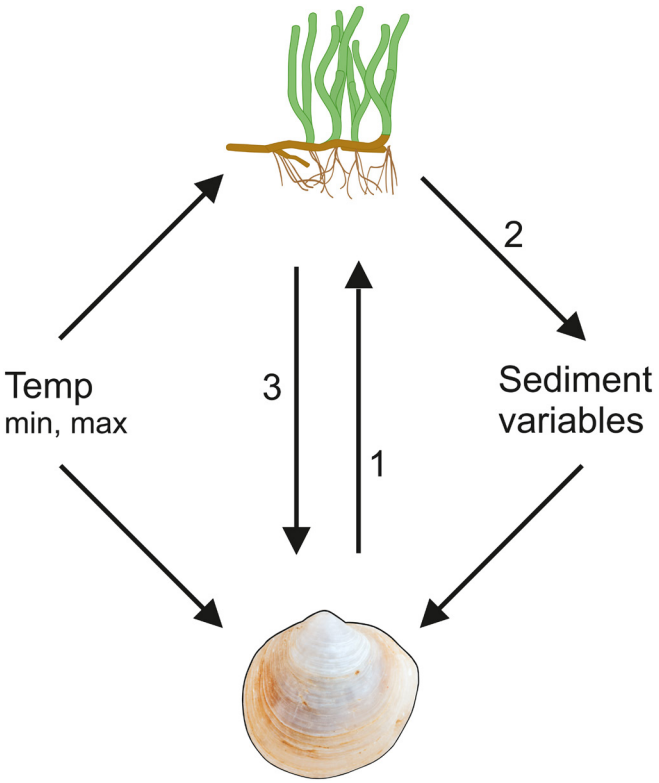

Figure 1. Conceptual diagram for the structural equation model describing the feedback between lucinids and seagrass and other variables which potentially influence the feedback. Numbered lines represent the potential effect of the direct effect between seagrass on lucinids (1) or indirect by the influence of seagrass on sediment characteristics (2), and the effect of lucinids on seagrass biomass, either direct or indirect through sediment stress indicator (SSI) in the plant (3).

Material and methods

Study area and data collection

Sampling was done in 14 *Zostera noltii* and 17 *Zostera marina* meadows across western Europe during the growing season (August–October) in 2018–2019 (Fig. 2). At almost each location ten sampling points where randomly selected within the seagrass meadow about 5–10 m apart, however, due to logistic circumstances at a some locations less than 10 samples were taken (Supporting information).The seagrass meadows covered a broad range of sediment types from low to high organic matter content (min–max: 0.6–8.5%) and mud content (particles < 63 µm; min–max: 2.5–66.3%), which was representative for general sediment conditions in seagrass meadows (Kennedy et al. 2010). Sampling followed a strict protocol to standardize data collection across all locations. Lucinid bivalves and seagrasses were sampled with sediment cores (ø: 10–15 cm) to a depth of about 20 cm and then sieved over a 1 mm mesh. Live seagrass and lucinids where sorted and frozen for further determination in the laboratory. In the laboratory lucinids were counted and total dry weight (above- and belowground) of the seagrass was determined after drying (48 h at 60°C). Sediment samples were taken with a small core (ø: 1.5–3.6 cm) to a depth of 5 cm, frozen after collection and weighed in the laboratory. Temperature variables were derived from the monthly values from WorldClim (Hijmans et al. 2005). For the analyses we used annual minimum and maximum air temperature for each location (temperature of the coldest and warmest month). Air temperature is expected to be the most biologically meaningful variable as the seagrass meadows in our study are intertidal and shallow subtidal, potentially showing latitudinal limits for lucinid bivalves and effects on seagrass growth (Larkum et al. 2006, van der Heide et al. 2012).

Sample analysis

Dried above ground seagrass tissue was analysed for sulphur isotope ratio ($\delta^{34}S$) and total sulphur (TS) using dynamic flash combustion ratio mass spectroscopy. The stable isotope signatures were reported relative to the international Vienna Caňon Diablo Troilite standard for $\delta^{34}S$. Indication for sulphide sediment stress in the plant was calculated as sediment stress index (SSI) with the ratio of $\delta^{34}S+30/TS$, where 30 was added for each $\delta^{34}S$ to ensure positive values, following Kilminster et al. (2014). Reduced sulphate by bacteria results in fractionated sulphide with more negative $\delta^{34}S$ values in

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the sediment porewater. As a consequence, plant tissues also exhibit more negative $\delta^{34}S$ value under sulphide stress with assimilation of sulphur (TS) (Holmer and Kendrick 2013, Kilminster et al. 2014). SSI positively correlates with seagrass growth as the high proportion of TS and high $\delta^{34}S$ in seagrass leaves results in high SSI, which indicates low sulphide intrusion and increased plant growth (Kilminster et al. 2014, Timbs and Durako 2021).

Sediment samples were freeze-dried, after which mud content (particles < 63 µm; Wentworth 1922) was determined using a particle size analyser with auto sampler (Coulter LS 13 320), and organic matter as loss on ignition (LOI; 5 h at 550°C). Total iron (Fe) and sulphur (S) was determined after nitric acid digestion of dried sediment and analysed using an inductively coupled plasma emission spectrophotometer (ICP-OES iCAP 6000). The fraction of labile carbon in the organic matter pool relates to the sulphate reduction rate which increases the sulphide concentrations in the sediment (Holmer and Nielsen 1997, Frederiksen et al. 2007). As a proxy of labile carbon, we measured dissolved organic carbon (DOC) in the sediment. DOC was determined in a two-step water extraction procedure following Ghani et al. (2003) on 3 mg of dried sediment in 50 ml polypropylene centrifuge tubes by adding 30 ml distilled water. The first step involved a cold-water extraction at 20°C (DOC-c) for the removal of the readily soluble carbon from the sediment. The second step involved a warm-water extraction of labile components of sediment carbon at 80°C for 16 h (DOC-w) (Ghani et al. 2003). After the extraction procedure the tubes where centrifuged at 3500 rpm for 15 min and supernatant was filtered through a 0.45 µm filter and transferred to separate vials for DOC analyses. DOC for both cold and warm water extraction was determined on a Shimadzu total organic carbon (TOC-L) analyser.

Statistics

To investigate the first hypothesis – i.e. the occurrence (presence/absence) of lucinid bivalves in seagrass meadows in relation to environmental variables (seagrass biomass, temperature, DOC, mud- and organic matter content, sediment sulphur and -iron) – we fitted mixed logistic regression models (i.e. binary generalized linear mixed models). We used these models with a stepwise backward elimination of variables procedure to predict the presence or absence of lucinid bivalves. We included the explanatory variables as fixed effects and sampling location as random effect. To detect multicollinearity between explanatory variables, variance inflation factors (VIF) were computed with the car R package (www.r-project.org) (Fox and Weisberg 2019) in combination with Pearson correlation coefficient. Total sediment sulphur content was highly correlated with mud content (Pearson rho = 0.78) and was therefore dropped before starting the stepwise procedure. This resulted in a VIF of 2.8, indicating that we successfully reduced collinearity (Zuur et al. 2009). Logistic regression models in the stepwise procedure were compared according Akaike's information criterion (AIC). The model with all variables being significant and the lowest AIC (Burnham and Anderson 2002) was considered as best support. As a proxy for reliability, we determined the explanatory potential by calculating the predicted probability of presence or absence for the final model and compared this with observed presence or absence (Jongman et al. 1995). Next, we calculated percentage of correct model predictions (following van der Heide et al. 2009).

To investigate the second hypothesis – i.e. the potential facilitating effect of lucinids on seagrass – we used a linear mixed effect model with a stepwise backward procedure, with all explanatory variables as fixed effect and sampling location as random effect. To detect multicollinearity between explanatory variables we followed the same procedure as above. Mud content was highly correlated with DOC-w (Pearson rho = 0.88) and organic matter (Pearson rho = 0.92) in the data for *Z. noltii*. Dropping mud content and DOC-w before starting the stepwise procedure for the *Z. noltii* analysis resulted in VIF 2.2. *Z. noltii* biomass and lucinid densities were transformed with the Box-Cox procedure to achieve normality and homoscedasticity (Box and Cox 1964). Sediment sulphur was highly correlated with mud content (Pearson rho = 0.91) in the data for *Z. marina* and dropping sulphur resulted in VIF 2.3. The effect of location and seagrass species on plant tissue sulphur parameters (TS and $\delta^{34}S$) were tested in two-way analyses of variance. We used Nakagawa and Schielzeth's (2013) method to obtain the conditional R^2 for linear mixed-effect models. We tested if the independent variables had a significant effect at a significance level of $p = 0.05$. p-values of linear mixed effect models with gaussian distribution were computed by using Satterthwaite approximation for denominator degrees of freedom from the lmerTest R package (www.r-project.org) (Kuznetsova et al. 2017). All statistical analysis were performed with R statistical and programming environment (www.r-project.org).

Finally, we used structural equation modelling (SEM) with the AMOS software (Arbuckle 2011) to explore the generality and significance of the mutualistic seagrass-lucinid feedback, taking the abiotic environmental variables into account. Based on the outcomes of the analyses in this study and our previous work, we constructed a conceptual model showing the potential relationships in our study system (Fig. 1). We expect a positive effect of seagrass on the lucinid bivalves by oxygen release and protection for predation both from the seagrass roots (van der Heide et al. 2012, de Fouw et al. 2016b). In addition, the bivalves (and their endosymbionts) profit from the sulphide that is indirectly provided by the seagrasses due to the accumulation of organic matter (Fig. 1). Apart from a positive effect, seagrass may also increase mud content potentially negatively influencing lucinid bivalve densities (Anderson 2008, Ellis et al. 2017, Douglas et al. 2019). In turn, we expect the lucinids positively affect seagrass health due to sulphide detoxification (van der Heide et al. 2012, de Fouw et al. 2022). However, as we do not have data of porewater sulphide levels and such measurements typically only reflect recent conditions, we used biomass and SSI as long-term proxy for seagrass health for

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Z. noltii and *Z. marina*, respectively. Furthermore, we expect temperature and sediment characteristics (organic matter and DOC) to affect lucinid densities (van der Heide et al. 2012), seagrass growth (Terrados et al. 1999, Olivé et al. 2009) and/or sediment sulphide production which in turn effect SSI or plant biomass (Fig. 1). As a SEM with direct feedbacks cannot cope with random effects (Lefcheck 2015), we averaged variables per location for *Z. noltii* and *Z. marina* separately. These averaged sediment characteristics (organic matter, mud content and DOC) were highly collinear and therefore tested in three separate SEMs including the feedback between *L. orbiculatus* and *Z. noltii* or *Z. marina* (Supporting information). For each SEM we used the option '*specification search*' in AMOS to optimise each separate SEM and choose best model according Akaike's information criterion (AIC) and χ^2 test. Next, we ranked the three optimised SEMs based on R^2 and significant relations ($p < 0.05$).

Results

Under what conditions do lucinids occur in European seagrass meadows?

In our field survey of 14 locations with *Z. noltii* and 17 with *Z. marina* we found on that total biomass per location on average varied from 10.1 to 397.4 g DW m^{-2} and 46.9 to 835.9 g DW m^{-2} for *Z. noltii* and *Z. marina,* respectively. We found lucinids in 54% of the seagrass meadows; with a strong latitudinal shift in presence/absence at 53° (Fig. 2, Supporting information). Densities were up to 3096 m^{-2}, all of the species *Loripes orbiculatus* (Supporting information). When explanatory variables were included in the stepwise backward logistic regression procedure, the occurrence of *L. orbiculatus* was best explained by a model including minimum temperature ($\chi^2 = 11.48$, $p < 0.001$), mud content ($\chi^2 = 12.37$, $p < 0.001$) and total seagrass biomass ($\chi^2 = 4.19$, $p < 0.05$). This model correctly predicted 86% of all presence–absence data (Fig. 3a, c). None of the other variables (e.g. DOC, organic matter and sediment iron content) had any significant explanatory value regarding the occurrence of *L. orbiculatus*. When comparing seagrass meadows where the minimum temperature remains above 1°C throughout the year, mud content was on average 66% lower in the meadows where *L. orbiculatus* was present (Fig. 3b). Total seagrass biomass was on average 63% higher in the meadows where *L. orbiculatus* was present (Fig. 3d).

Do lucinids facilitate seagrass?

To investigate the facilitative effect of *L. orbiculatus* on seagrass we analysed the relationship with seagrass total biomass, and SSI, as proxy for sulphide intrusion in the plant. Results of the stepwise backward linear regression procedure showed that for *Z. noltii* total biomass there were significant positive effects of *L. orbiculatus* density ($F_{1,68.88} = 9.00$, $p < 0.01$, Fig. 4a) and maximum air temperature ($F_{1,13.92} = 16.33$, $p < 0.01$). For *Z. marina* biomass, however, we found no significant effect of *L. orbiculatus* density or of the other variables (Fig. 4b). Analysis of plant tissue revealed that leaf TS differed significantly between locations ($F_{1,27} = 36.13$, $p < 0.001$) and seagrass species ($F_{1,28} = 15.08$, $p < 0.001$). Mean $\delta^{34}S$ also differed significant between locations ($F_{1,27} = 68.09$, $p < 0.001$) and seagrass species ($F_{1,27} = 33.78$, $p < 0.001$) (Supporting information). SSI for *Z. noltii* – which was calculated from the above sulphur parameters – was positively and significantly related to *L. orbiculatus* density ($F_{1,128.93} = 7.24$, $p < 0.01$; Fig. 4c). For *Z. marina* SSI, we also found a positive

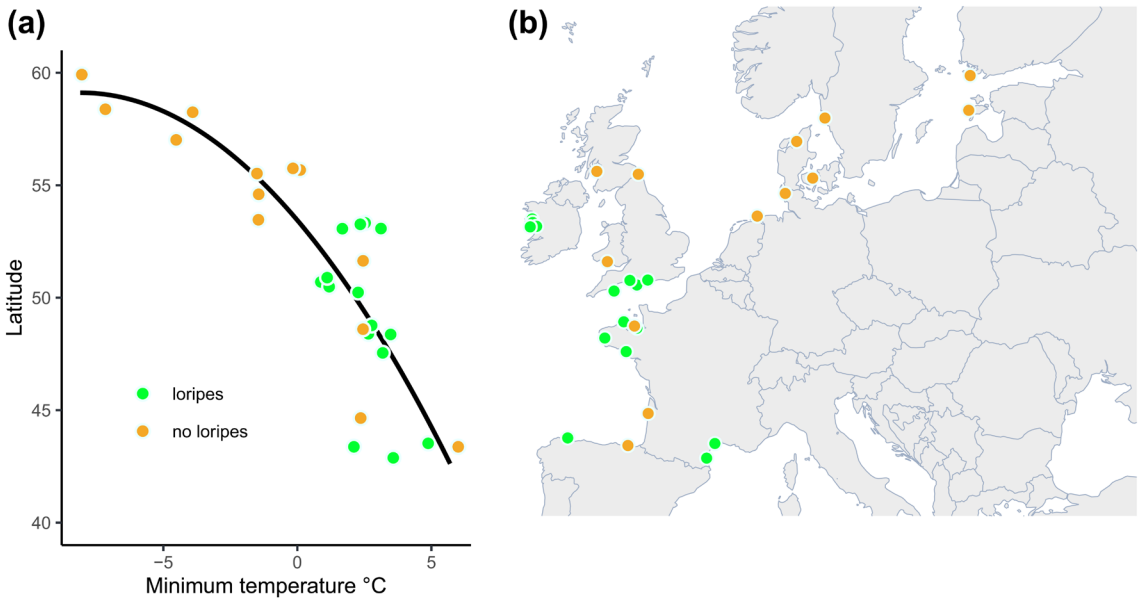

Figure 2. Latitudinal pattern for minimum temperature for locations (a) where bivalves of the family Lucinidae occurred in European survey and map of the 28 studied locations (b).

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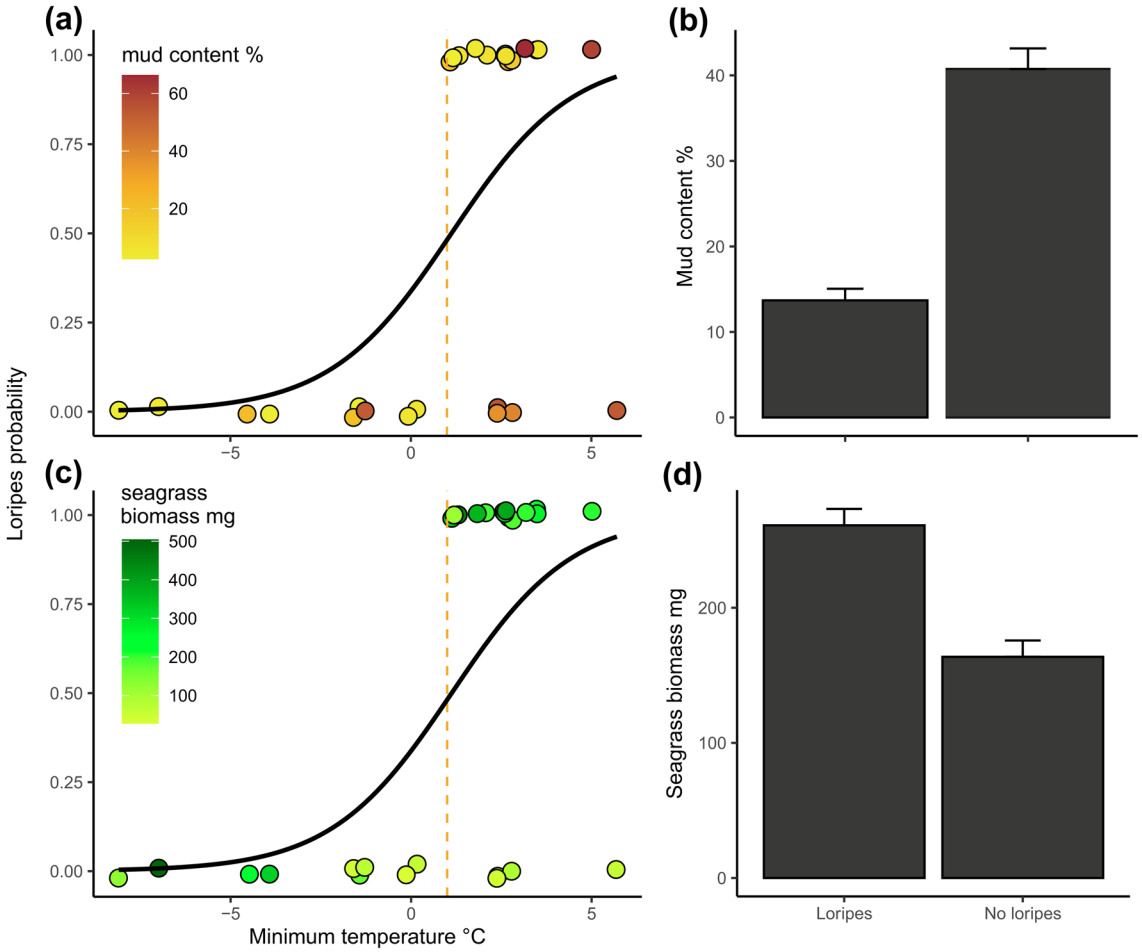

Figure 3. Probability of absence (0) or presence (1) of *L. orbiculatus* plotted against minimum temperature with dots filled according to a low and high percentage mud content (a) and total seagrass biomass (d). Percentage of mud content on locations with and without *L. orbiculatus* at the studied locations with minimum temperature above 1°C (b) and total seagrass with and without lucinids at the studied locations (d). Orange line at 1°C. Bars represent mean and standard errors.

relationship with *L. orbiculatus* density ($F_{1,145.35} = 4.00$, $p < 0.05$) and maximum air temperature ($F_{1,13.53} = 10.90$, $p < 0.01$; Fig. 4d), indicating reduced sulphide intrusion in plant tissues with increasing *L. orbiculatus* densities for both *Zostera* species.

Is there evidence for a generic mutualistic feedback?

Based on the outcomes of the linear analyses, we used biomass and SSI as the strongest proxies for *Z. noltii* and *Z. marina* health, respectively. The best SEM for *Z. noltii* explained 80% of the variation of *Z. noltii* biomass and 85% of *L. orbiculatus* density (Fig. 5, Supporting information). We found support for a direct positive feedback between *Z. noltii* biomass and *L. orbiculatus* density. The feedback was mediated by temperature, with a positive effect of minimum temperature on *L. orbiculatus* density and of maximum temperature on *Z. noltii* biomass. In addition to this temperature effect, organic matter negatively influenced *L. orbiculatus* density. We did not find evidence for an indirect effect of *Z. noltii* on *L. orbiculatus* through organic matter, indicating that other unknown variables influenced organic matter (Fig. 5, Supporting information). In contrast to *Z. noltii*, our SEM analyses did not find support for a feedback between *Z. marina* SSI and *L. orbiculatus* density (Supporting information).

Discussion

Our field study provides the first continental-scale evidence that the lucinid bivalve *L. orbiculatus* occurs in *Zostera* meadows across a large latitudinal gradient. Specifically, we found *L. orbiculatus* to be present in 79% of the *Zostera* meadows at locations where the annual minimum air temperature remains above 1°C and mud content is relatively low. Simultaneously, *Zostera* biomass had an overall positive relationship with *L. orbiculatus* presence (Fig. 3c, d). Furthermore, our analyses suggest that *L. orbiculatus* mitigates sediment sulphide stress in *Zostera* meadows, but that the strength of this association

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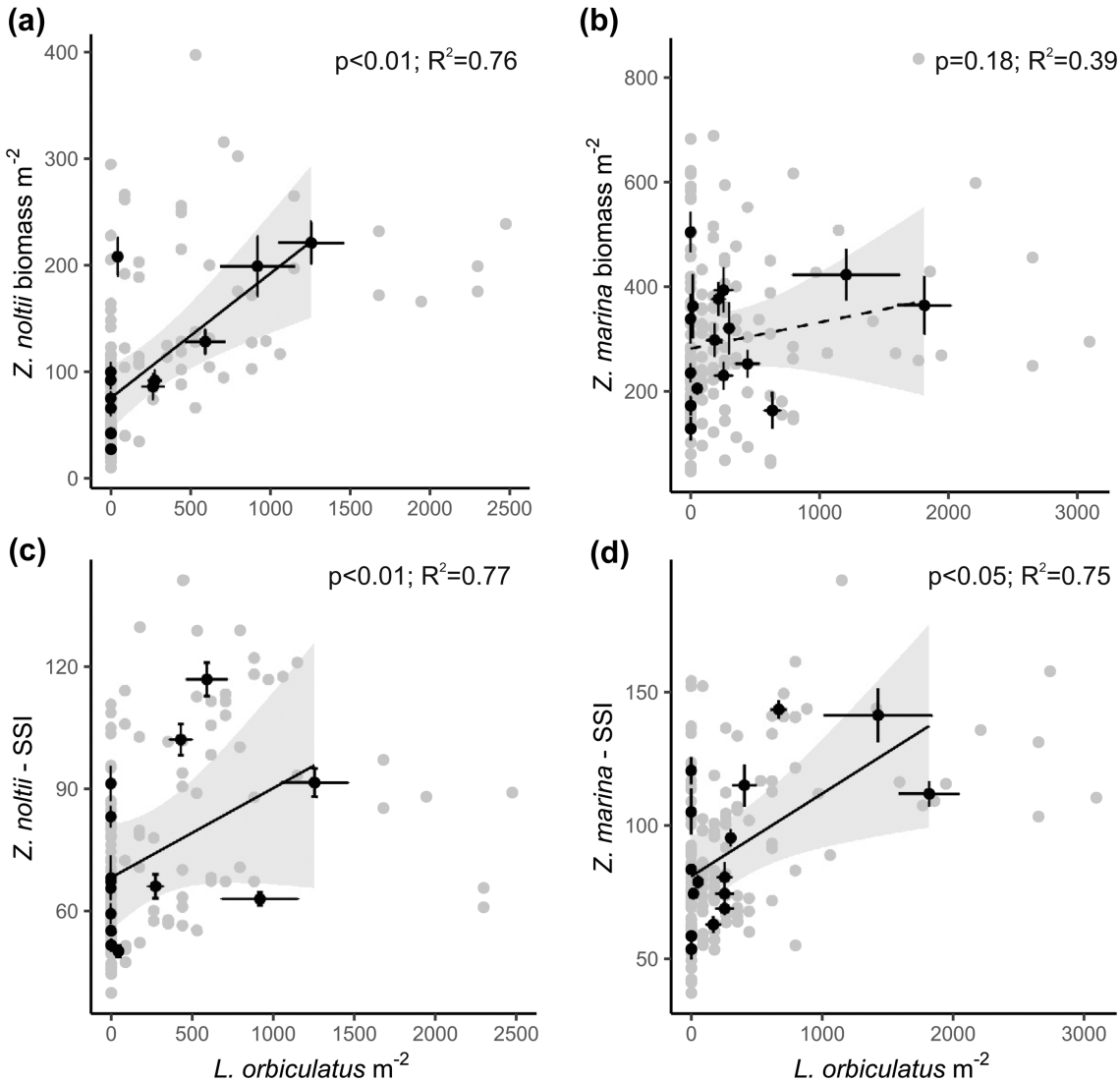

Figure 4. Total seagrass biomass versus *L. orbiculatus* densities, mean values per location for *Z. noltii* (a) and *Z. marina* (b). Relation between the sulphide sediment stress indicator (SSI) versus *L. orbiculatus* densities for *Z. noltii* (c) and *Z. marina* (d). p-values reflect significance level of *L. orbiculatus* and the conditional R^2 for linear mixed effect model. Grey points are all individual samples for all locations. Error bars depict standard errors.

is conditional to temperature and sediment characteristics as *L. orbiculatus* presence depends on these variables. Finally, our SEM analysis supports the existence of the mutualistic feedback between the *L. orbiculatus* and *Z. noltii*, showing the potential importance of the mutualistic seagrass-lucinid interaction for seagrass systems.

Lucinid occurrence

Our data indicates that the occurrence of *L. orbiculatus* is temperature dependent; no bivalves were found below an annual minimum temperature of 1°C. Hence, the distribution of *L. orbiculatus* seems to be physiologically limited to minimum temperatures below 1°C. Temperature-driven limitation of the distribution is observed in many bivalve species, particularly species originating from (sub)tropical regions as these typically have a lower cold-tolerance compared to boreal and temperate species (Stevens 1989, Compton et al. 2007). As the Lucinidae have greatly diversified during the warmer Cretaceous–Eucene period, and still have the highest diversity in the tropics (Stanley 2014, Taylor and Glover 2021), it is likely that lucinids also have such a limited cold-tolerance.

A second potential explanation could be that *L. orbiculatus* becomes sulphide-limited in colder regions. Although lucinids can be mixotrophic, they depend to a large extent on sulphide that serves as the energy source for their endosymbionts. The bacterial endosymbionts in turn provide sugars to the bivalves (Rossi et al. 2013, van der Geest et al. 2014). In marine sediments, bacterial sulphate reduction rates increase with higher temperatures (Jørgensen 1977, Koch et al. 2007), with higher sediment sulphide concentrations as a result. Hence, it is possible that lucinids become sulphide starved

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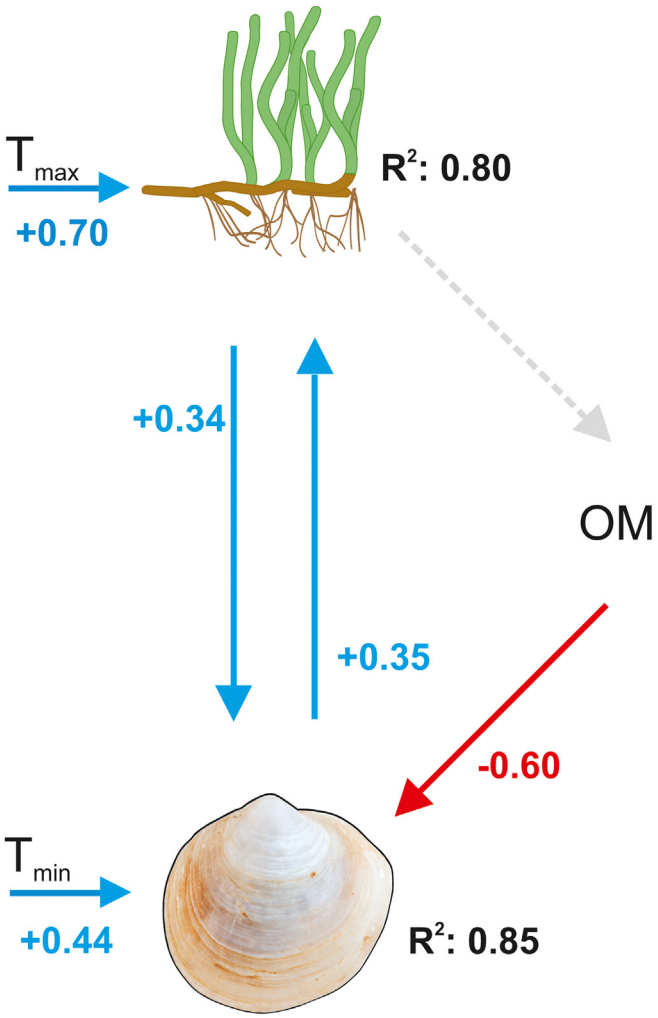

Figure 5. Best structural equation model for *Z. noltii* including a positive feedback with *L. orbiculatus*. Temperature positively affects the feedback and organic matter had negative effect on *L. orbiculatus*. Values besides the arrows indicate the standardised regression weights, all positive (blue) and negative (red) solid lines are significant, and dotted grey line represent non-significant path. R^2 values indicate the explained variance.

in colder sediments at higher latitudes in temperate and boreal regions. The exact mechanism behind the temperature effect on the occurrence of *L. orbiculatus* in European *Zostera* meadows remains to be tested experimentally.

Mud content was the second most important determinant for the occurrence of *L. orbiculatus* in European *Zostera* meadows. It is well-known that sediment mud content plays an important role in the distribution of macro zoobenthos (Anderson 2008, Ellis et al. 2017, Douglas et al. 2019). In our study, we found that *L. orbiculatus* presence correlates negatively with mud content. We argue that in the case of *L. orbiculatus*, and lucinids in general, this is determined by thixotrophicity (i.e. soupiness) and/or the instability of finer sediments (Dashtgard et al. 2008, Dufour 2018). Specifically, we hypothesize that in muddy sediments, similar to findings for the lugworms *Arenicola* spp. (Longbottom 1970), the bivalves may be unable to keep their delicate mucous-lined inhalant tube intact, which they need to obtain oxygen-rich surface water (Taylor and Glover 2000).

Apart from mud content and minimum temperature, we also found a positive association with seagrass biomass. The combination of these biotic and abiotic variables best predicts the optimal niche for *L. orbiculatus*. Their regulating role is supported by the fact that the four locations above the average annual minimum temperature of 1°C where *L. orbiculatus* was absent, have low seagrass biomass and high mud content (Fig. 3). There are also two locations with high mud content where *L. orbiculatus* is present in low densities, but at these locations *Z. noltii* biomass is relatively high. This suggests that by providing enough oxygen through their roots, seagrass can extend the realised niche of *L. orbiculatus* beyond the predicted fundamental niche towards higher mud content as suggested by the stress gradient hypothesis (Bertness and Callaway 1994, Bruno et al. 2003).

Notably, other sediment characteristics (OM, iron content, DOC) did not correlate with the occurrence of the lucinids. For example, organic matter and DOC are known to relate to sulphate reduction increasing sulphide concentrations in the sediment (Holmer and Nielsen 1997, Frederiksen et al. 2007, Jørgensen et al. 2019). However, sulphide concentration in the sediment depends on several interacting variables (e.g. microbial community, sediment oxygen permeability) making the relationship with organic matter and/or labile carbon not sufficiently straightforward. In its oxidized form, iron(III) can buffer sulphide toxicity via oxidation and subsequently also by forming iron(II)-sulphide precipitates (Chambers et al. 2001, Holmer et al. 2005, Jørgensen et al. 2019). However, iron is predominately already present in its reduced sulphide-bound form in anoxic marine sediments. In such cases, sulphide iron-mediated buffering can only take place when iron is oxidized first, for instance by oxygen released into the rhizosphere by seagrass roots. In this study, however, we did not find any support for an iron sulphide buffering mechanism.

Effects of lucinids on seagrass

Our analyses show that when lucinids are present in *Zostera* meadows, they promote plant growth. For *Z. noltii,* there was a positive relationship with *L. orbiculatus,* resulting in a plant biomass increase of 84% with a density increase of 540 lucinids bivalves m^{-2}, the average densities in *Z. noltii* meadows (Fig. 4a). The nature and strength of this association is in line with experimental mesocosm results from other studies showing a positive effect on seagrass growth due to sulphide detoxification by the lucinids (van der Heide et al. 2012, Chin et al. 2021, de Fouw et al. 2022). In addition, we found a positive effect of higher temperatures (i.e. annual maximum temperature) on *Z. noltii* biomass. A possible, indirect explanation for this temperature effect is that southern *Z. noltii* meadows can reach higher photosynthetic production due to higher year round solar irradiation (Touchette and Burkholder 2000). Increased photosynthetic production also increases the

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capacity to release oxygen through the roots into the surrounding rhizosphere where it detoxifies sulphide (Pedersen et al. 2004, Hasler-Sheetal and Holmer 2015). For *Z. marina* we did not found a significant relation, however there was a positive trend of 12% biomass increase with a density increase of 465 lucinids bivalves m^{-2}, the average densities in a *Z. marina* meadow (Fig. 4b). Interestingly, for *Z. marina* there was a significant effect of *L. orbiculatus* densities on SSI, indicating that *L. orbiculatus* reduced plant sulphide intrusion.

Evidence for a generic mutualistic feedback

Our SEM analyses supports the hypothesis that the observed linear correlation between *L. orbiculatus* and *Z. noltii* in this continent-wide study result from a generic facultative mutualistic feedback. These findings support previous experimental laboratory studies that found a positive effect of *L. orbiculatus* on seagrass biomass (van der Heide et al. 2012, de Fouw et al. 2022). Based on the logistic regression analysis on the presence–absence of the bivalves, we expected a negative effect of mud content on *L. orbiculatus* density in our SEM. However, the feedback was not supported by the model (Supporting information). Instead, we found a significant negative effect of organic matter content (Fig. 5, Supporting information), which is counterintuitive as organic matter is important for anaerobic sulphide production and thus for sulphide consuming lucinids. We suggest that this is due to the high correlation between mud content and organic matter (Supporting information). Overall, the model containing organic matter performed slightly better when predicting the feedback between *L. orbiculatus* density and *Z. noltii* biomass.

We found no evidence for a mutualistic feedback in our SEM analysis between *L. orbiculatus* and *Z. marina* SSI. However, our linear models supported a facilitative (one-way) positive influence of *L. orbiculatus* on *Z. marina* SSI. There may be several reasons why we did not find evidence for the feedback and why sulphide alleviation by lucinids is less apparent in *Z. marina*. First, *Z. marina* has substantially larger leave biomass and therefore a larger photosynthetic capacity to release oxygen through their roots in the surrounding rhizosphere to sufficiently detoxify sulphide without the help from lucinids (Pedersen et al. 2004, Hasler-Sheetal and Holmer 2015). Second, contrary to the intertidal *Z. noltii*, *Z. marina* grows mostly subtidal where sediments are less influenced by high temperatures; a factor strongly influencing porewater sulphide concentrations.

Overall, mutualism dependency could potentially increase under stress conditions (e.g. eutrophication, temperature increase). For example, the facilitation strength between *Thallasia testudinum* and *Codakia orbicularis* which became more important under a combination of reduced light and sulphide stress conditions (Chin et al. 2021). This is consistent with the stress gradient hypothesis, which predicts that facilitation strength becomes more important under stress conditions (Bertness and Callaway 1994, He et al. 2013).

Although our observational, correlative approach was not designed to test facilitation strength perse, our study adds to the understanding of the context-dependency of the lucinid-seagrass interaction. We suggest that future studies could further elucidate this context dependency – also in relation to the stress gradient hypothesis – by including field experiments in which lucinid densities are manipulated at several locations along the latitudinal gradient based our field study results (Bertness and Leonard 1997).

Implications

Our results show that lucinids naturally occur in the majority of the southern *Zostera* meadows with a warmer climate where the risk of toxic sulphide levels and thus the potential importance of the mutualism are the highest. In the future, both the occurrence of the lucinids and the importance of the mutualism may expand towards regions that will become warmer due to climate change. In the intertidal, desiccation is one of the major factors responsible for the depth zonation of seagrass meadows (Leuschner et al. 1998, van der Heide et al. 2010). Increased temperatures due to climate change may thus lower the upper limits of intertidal seagrass. Decreased seagrass health caused by desiccation stress may lead to decreased photosynthesis-driven oxygen release by the roots, required to mitigate sulphide production (de Fouw et al. 2016a). This may imply an increase vulnerability of the mutualism, while their importance may also increase. Recent studies in North American salt marshes have shown the importance of a positive interaction on a large scale where cordgrass *Spartina alterniflora* and ribbed mussels *Geukensia demissa* are mutualistically associated (Angelini et al. 2016). In this mutualistic association, cordgrass serves as a settlement substrate for mussels and reduces heat stress by providing shading (Angelini et al. 2016). In return, mussel aggregations deposit nutrients and decrease sulphide concentrations (Derksen-Hooijberg et al. 2018a,b). During recent heat waves, the mussels were found to prevent cordgrass die-off by lowering salinity levels and generally increasing plant health (Angelini et al. 2016), highlighting its increasing importance as temperature extremes are globally predicted to increase in frequency and intensity. At the same time, however, mutualisms may also become more vulnerable to collapse as a result of an increase in climatic extremes. This phenomenon has been observed in a west-African intertidal seagrass meadows where drought disrupted the seagrass-lucinid mutualism, which in turn amplified ecosystem degradation (de Fouw et al. 2016a, de Fouw et al. 2018). Overall, recent findings suggest that facultative mutualisms supporting coastal foundation species may increase in importance in the face of global warming, but simultaneously become more vulnerable, increasing the risk of ecosystem collapse (van der Heide et al. 2021).

Our results and those from other recent studies suggest that facultative mutualistic interactions can be key interactions for the functioning of in coastal ecosystems and should thus be integrated into conservation and restoration management approaches (Derksen-Hooijberg et al. 2018a, Gagnon et al. 2020, Valdez et al. 2020, Donaher et al. 2021). In a broader perspective, it is increasingly recognised that mutualisms in

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general are indispensable for maintaining biodiversity and ecosystem functioning (see Bronstein 2015). For example, inoculation of plant-specific microbes, adding mycorrhizal fungi, reintroducing seed dispersers and pollinators has been very successful to restore biodiversity or even whole ecosystems (Holguin et al. 2001, Bashan and Holguin 2002, Swarts and Dixon 2009, Winfree 2010, Bustamante-Sánchez and Armesto 2012). For seagrass conservation, an important first step would be to include the seagrass-lucinid mutualism more routinely into monitoring schemes by assessing Lucinidae abundance as an indicator for seagrass ecosystem health. In restoration projects, it may be beneficial to consider co-transplanting lucinids with seagrasses, at least in warmer areas with sulphide-rich sediments, an approach that has been successfully employed for the cordgrass-ribbed mussel mutualism in salt marshes (Derksen-Hooijberg et al. 2018).

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Data availability statement

Data are available from the Dryad Digital Repository: https://doi.org/doi:10.5061/dryad.7sqv9s4wr (de Fouw et al. 2023).

Supporting information

The Supporting information associated with this article is available with the online version.

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