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Mutualistic interactions amplify saltmarsh restoration success

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Abstract

1. Mounting evidence shows that the functioning and stability of coastal ecosystems often depends critically on habitat-forming foundation species such as seagrasses, mangroves and saltmarsh grasses that engage in facultative mutualistic interactions. However, although restoration science is now gradually expanding its long-standing paradigm of minimizing competition to including intraspecific, or within species, facilitation in its designs, the potential of harnessing mutualistic interactions between species for restoration purposes remains uninvestigated.

2. Here, we experimentally tested whether a previously documented mutualism between marsh-forming *Spartina alterniflora* (cordgrass) and *Geukensia demissa* (mussels) can increase restoration success in degraded US saltmarshes.

3. We found that co-transplanted mussels locally increased nutrients and reduced sulphide stress, thereby increasing cordgrass growth and clonal expansion by 50%. We then removed above-ground vegetation and mussels to simulate a disturbance event and discovered that cordgrass co-transplanted with mussels experienced three times greater survival than control transplants.

4. *Synthesis and applications.* Our findings indicate that mussels amplify cordgrass re-colonization and resilience over spatial and temporal scales that exceed those of their actual mutualistic interaction. By experimentally demonstrating that mutualistic partners can enable foundation species to overcome stress barriers to establish and persist, we highlight that coastal restoration needs to evolve beyond the sole inclusion of intraspecific-positive interactions. In particular, we suggest that integrating mutualisms in restoration designs may powerfully enhance long-term restoration success and ecosystem resilience in the many coastal ecosystems where mutualisms involving foundation species are important ecosystem-structuring interactions.

KEYWORDS
coastal ecosystems, cordgrass, facilitation, foundation species, *Geukensia demissa*, mussels, positive interactions, resilience, saltmarsh, *Spartina alterniflora*

1 | INTRODUCTION

Coastal ecosystems are rapidly declining world-wide (Lotze et al., 2006). At present, anthropogenic forcing including climate change, eutrophication and overfishing have severely degraded 19%–85% of all coral and shellfish reefs, seagrass meadows, mangroves and salt-marshes, and these losses still continue (Beck et al., 2011; Gedan & Silliman, 2009; Millennium Ecosystem Assessment, 2005; Waycott et al., 2009; Wilkinson, 2008). Importantly, this habitat loss is accompanied by a reduction in vital ecosystem services such as coastal flood protection, nutrient cycling, carbon storage and maintenance of biodiversity (Brisson, Coverdale, & Bertness, 2014; Hopkinson, Cai, & Hu, 2012; Lee et al., 2014). To halt and reverse this degradation, over 1 billion US$ has been spent to date world-wide on conservation and restoration efforts (Silliman et al., 2015). Despite these investments, restoration attempts often result in failure (Cunha et al., 2012; Tanner et al., 2010), or in only partial recovery of the ecosystem (Benayas, Newton, Diaz, & Bullock, 2009; Suding, 2011). This low return on investment for many restoration projects (Silliman et al., 2015) emphasizes the need to develop novel approaches to not only improve initial success of restored habitats but also to enhance their resilience and long-term persistence.

Increasing evidence suggests that positive interactions play an essential role in the functioning and stability of coastal wetlands by reducing physical stress, increasing resource availability or both (Bruno, Stachowicz, & Bertness, 2003; Hay et al., 2004; Stachowicz, 2001). Such interactions can be intraspecific—for example, seagrass and marsh vegetation increasingly attenuate currents and trap sediment and detritus with higher shoot density (Maxwell et al., 2017; van de Koppel, van der Wal, Bakker, & Herman, 2005)—or interspecific. Examples of the latter are the facilitation of cockles by intertidal mussel beds in the Wadden Sea (Donadi et al., 2013) and bidirectional facilitation in coral-zooxanthellae and seagrass-lucinid clam mutualisms (de Fouw et al., 2016; Hay et al., 2004; van der Heide et al., 2012). Despite their ecological importance, the consideration of positive interactions for coastal restoration has thus far remained limited. Halpern, Silliman, Olden, Bruno, and Bertness (2007) provided a theoretical framework based on terrestrial restoration for the systematic inclusion of positive interactions into aquatic restoration. Recent experimental work has supported the potential utility of this theory by showing that integrating intraspecific interactions through "clumped" transplant designs may offer a simple and low-cost solution to enhance saltmarsh restoration success (Silliman et al., 2015). Nevertheless, a recent survey among restoration agencies in the United States revealed that only 1 of 25 was implementing designs that could harness intraspecific facilitation (Silliman et al., 2015). In a similar survey of 20 US restoration agencies conducted for this study, we found that 85% of the surveyed practitioners do not currently include positive interspecific interactions in saltmarsh restorations (Appendix S1), despite clear indications that these interactions may yield high benefits for restoration success (Angelini et al., 2015, 2016).

In this study, we test the general hypothesis that interspecific-positive interactions can enhance coastal ecosystem restoration success. We focus on southeastern US saltmarshes where sudden dieback events are known to cause vegetation mortality, resulting in marsh areas of several m^2 to several km^2 being converted to bare mud-flat (Alber, Swenson, Adamowicz, & Mendelssohn, 2008; Angelini et al., 2016; Silliman, van de Koppel, Bertness, Stanton, & Mendelssohn, 2005). In these saltmarshes, *Spartina alterniflora* (hereafter cordgrass) is the primary habitat-forming foundation species, supporting a diverse faunal community (Angelini et al., 2015; Bertness, 1991; Dayton, 1972; Paterson & Whitfield, 2000). Recent work demonstrated that in northeastern and southeastern US saltmarshes, *Geukensia demissa*, (hereafter mussels) functions as a secondary foundation species that depends on cordgrass (see Figure 1a for cordgrass and mussels overlapping distributions). The base of cordgrass stems provide settlement substrate to facilitate the establishment of clumped mussel aggregations (Bertness & Grosholz, 1985) and cordgrass leaves provide shade and reduce otherwise lethal temperatures (Angelini et al., 2015). Once established, mussels further enhance biodiversity and ecosystem functioning (Angelini et al., 2015) and engage in a mutualistic interaction with cordgrass, by enhancing cordgrass' growth and drought resistance (Angelini et al., 2016; Bertness, 1984; Bertness, Brisson, & Crotty, 2015). However, whether this mutualism could aid in increasing restoration success is unclear.

To test the hypothesis that reciprocal facilitation may enhance saltmarsh restoration success, we experimentally manipulated the interactions between cordgrass transplants and mussels in a 29-month full-factorial field experiment in a southeastern US saltmarsh. Naturally, the long-term success of restoration does not merely depend on initial survival and outgrowth of the restored vegetation but also on the ability of the restored vegetation to withstand newly emerging stressors. In the present study we therefore measure "success" by two complementary criteria: (1) the relative growth of the restored biotic material and (2) the ability of the restored vegetation to recover from disturbance. To test the first criterion, we manipulated the presence of cordgrass and mussels in a bare, former dieback site and investigated how these species influenced each other's growth and survival for 16 months. After this period, we tested the second criterion, the resilience of the cordgrass transplants, by simulating a physical disturbance through clipping of all above-ground cordgrass biomass and removal of mussels. We then measured cordgrass survival and regrowth 10 days and 13 months after this disturbance. This study provides first empirical evidence that mutualistic interactions can contribute significantly to coastal restoration success and ecosystem resilience.

2 | MATERIALS AND METHODS

2.1 | Study site

This study was conducted in the National Estuarine Research Reserve on Sapelo Island, Georgia, USA (latitude 31.40749167, longitude −81.28987601). The study area is located on a high marsh platform characterized by short-form *S. alterniflora* and is regularly flooded through tidal inundation. Mussel mounds—here defined as

aggregations consisting of more than three mussels—are scattered but abundant, and are generally composed of approximately 5–70 individuals (Appendix S2). We found a number of dieback areas in our study area that formed during a drought event in the summer of 2012 (see Angelini et al., 2016 for dieback area size and spatial distribution information). We selected a dieback mudflat formed during this event of approximately 2,400 m² for the experiment.

2.2 | Experimental set-up

In April 2013, we established 48 plots spaced >3 m apart. We then assigned one of four experimental treatments: control, cordgrass-only, mussels-only and cordgrass + mussels (Figure 1b; N = 12 replicates/treatment). Into control and mussel-only plots, we transplanted bare-soil plugs from an adjacent dieback site. Into cordgrass-only and cordgrass + mussel plots, we transplanted healthy cordgrass transplants. Transplants were standardized for stem density (14–16 stems) and for root-soil volume (25 × 15 cm, diameter × depth, transplant cores). To mimic natural mussel distributions, we assessed mussel size and abundance at our research site prior to the experiment. Five hundred mussels were haphazardly collected, measured (maximum length: 99.5 mm) and categorized in 10-mm size classes (Appendix S2). We excluded the smallest and largest size classes from the experiment as these mussels were found to be most sensitive to handling and their

collection sometimes resulted in mortality. We therefore selected five size classes from the mid size range (Appendix S2). We placed 20 mussels per plot; a frequently occurring natural aggregation size (Angelini et al., 2015). Mussels were inserted vertically, with half of their shells protruding from the sediment, mimicking natural conditions. All mussels were individually marked with shellfish tags (Hallprint, Australia) and shell length was measured prior to installation in the field.

2.3 | Cordgrass colonization and resilience

To investigate marsh colonization, clonal outgrowth of each cordgrass transplant was measured after 16 months. We measured the maximum diameter of each patch and the diameter perpendicular to it, from which we calculated the colonized surface area (ellipse shaped). After 16 months, in August 2014, we simulated a disturbance to assess cordgrass resilience. All mussels were removed and all above-ground biomass of cordgrass clipped. For each plot, cordgrass shoot biomass was collected and divided into biomass located on the original transplant plot (25 cm diameter), and the total biomass composed of biomass on the transplant and the clonal outgrowth into the surrounding mudflat. Root and rhizome biomass was quantified in the initial transplant area by taking four, 20 cm deep cores (4 cm diameter) which we divided into four depth intervals: 0–5, 5–10, 10–15 and 15–20 cm. In plots with mussels, an additional depth interval that
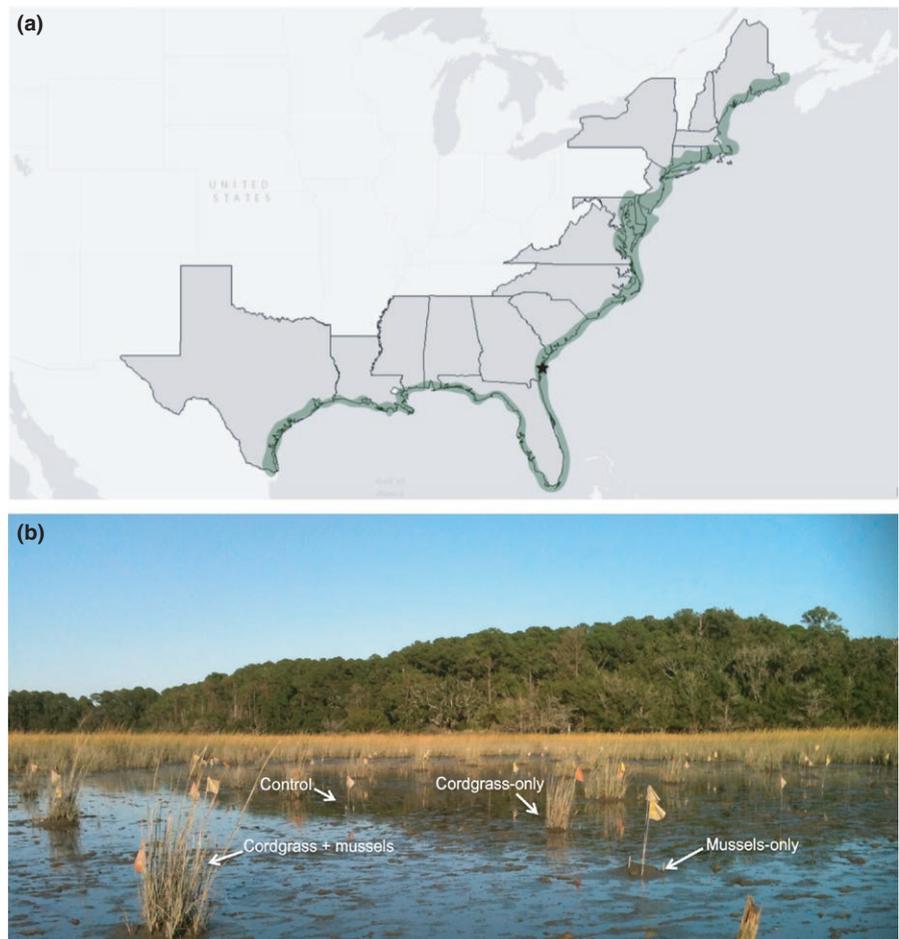

FIGURE 1 (a) Map showing coastal marsh regions in the Eastern and Southern US where the cordgrass-mussel mutualism can be found (green); the star depicts the location of the experimental site. (b) Photograph showing experimental units in the dieback area after the first growing season

spanned the top of the pseudofaeces layer to the marsh surface (0 cm) was taken for each core. Below-ground cordgrass tissue was washed and divided into live and dead fractions by testing through floatation and by visually assessing the colour and texture (living tissue floats and is easily distinguishable by its white coloration and firm texture). Live tissue was then further divided into root and rhizome portions. Shoots, roots and rhizomes were oven-dried at 60°C for at least 48 hr until no further decrease in weight occurred and weighed. Finally, we monitored cordgrass plot survival and measured regrowth by counting the number of shoots that emerged per transplant 10 days and 13 months following the simulated disturbance.

2.4 | Chemical analyses of sediment porewater and vegetation

At the end of the first growth season in August 2013, porewater was collected anaerobically by vertically inserting 5-cm rhizon soil moisture samplers (Rhizosphere Research Products, the Netherlands) connected to vacuumized 60-ml syringes into the upper part of the sediment. Directly after collection, part of each sample was fixated with sulphide anti-oxidant buffer solution (SAOB) (HI4015-00; Hanna Instruments, USA). After fixation, total sulphide concentrations (composed of H_2S, HS^- and S^{2-}) were measured in the laboratory with a sulphide combination electrode (HI4115, Hanna Instruments) within 6 hr upon collection. A second part of each sample was stored at −20°C for further analyses.

Porewater, shoot and root samples were further processed in the laboratory in Nijmegen, the Netherlands, to determine nutrient and elemental contents. Porewater concentrations of ammonium (NH_4^+) and nitrate (NO_3^-) were analysed colorimetrically on Auto Analyzer III systems (Bran and Luebbe, Norderstedt, Germany), using ammonium molybdate and salicylate for NH_4^+ (Lamers, Tomassen, & Roelofs, 1998) and sulphanilamide after reduction of nitrate to nitrite in a cadmium column for NO_3^- (Wood, Armstrong, & Richards, 1967). Dried shoots and the upper 5 cm layer of the roots were homogenized with a ball mill (MM301; Haan, Germany) and digestion was conducted in 4 ml HNO_3 (65%) and 1 ml H_2O_2 (30%) in a digestion microwave (Milestone type MLS 1200; Sorisole Lombardy, Italy). The concentrations of iron (Fe), phosphorus (P) and sulphur (S) in the digested samples and in the porewater were measured on an inductively coupled plasma emission spectrophotometer (ICP-OES, model Iris Intrepid II; Thermo Fisher Scientific, Waltham, MA, USA). Porewater samples were, upon defrosting, immediately acidified by adding 1% nitric acid (HNO_3) and diluted three times with Milli-Q water (ELGA LabWater PURELAB flex, Lane End, UK) prior to measurement on ICP-OES. Carbon (C) and nitrogen (N) contents (%) in cordgrass shoots were determined with an elemental analyzer (Carlo Erba NA1500; Thermo Fisher Scientific).

2.5 | Mussels

After 16 months, mussels were collected, washed, categorized as either new recruits or retrieved co-transplanted mussels based on the presence of shellfish tag numbers, and identified as dead, alive

or missing. Length was again measured to determine shell growth for each surviving mussel. Mussel tissue was then separated from the shell and both were dried for at least 72 hr at 60°C prior to weighing. To compare mussel growth rates across different initial sizes, growth data were fit by means of the Von Bertalanffy (1938) growth curve, in which growth rate declines with increasing size. The equation was adapted for mark–recapture data (Ramón, 2003; Equation 1) and rearranged to estimate growth constant k for each mussel (Equation 2);

$$L_{t2} = L_{t1} + (L_\infty - L_{t1})\left(1 - e^{-k(t2-t1)}\right) \tag{1}$$

and therefore:

$$k = \frac{\left(\frac{\ln(L_{t2} - L_\infty)}{(-L_\infty + L_{t1})}\right)}{(t_1 - t_2)} \tag{2}$$

where k is the growth constant (per year), L_{t1} is mussel shell length (mm) in April 2013, L_{t2} is mussel shell length (mm) in August 2014, L_∞ is the maximum length mussels can attain in this system, $t_1 = 0$ and $t_2 = 1.33$ year (April 2013–August 2014). L_∞ was set at 99.5 mm as this was the maximum mussel shell length found in our survey.

2.6 | Statistical analyses

Data were analysed with ʀ version 3.1.2 (R Development Core Team, 2014). Independent samples t tests or two-way factorial ANOVAs were conducted to assess main effects and interactive effects of cordgrass and mussels when appropriate. Normality of the residuals and heterogeneity of variances were checked prior to data analyses and, if found to be unsatisfactory, data were Box-Cox transformed to best fit the assumptions. Cordgrass survival was analysed with a binary logistic model. Count data were first analysed with a Poisson distribution by means of the ʟᴍᴇ4 package in R. All Poisson models were checked for overdispersion, and, if found to be unsatisfactory, a negative binomial model was fit to the data instead.

3 | RESULTS

3.1 | Cordgrass restoration

Clonal outgrowth into the dieback mudflat was 45% higher in cordgrass + mussel than in cordgrass-only plots after 16 months (4.2 vs. 2.9 m²; $p = .047$; Figure 2a). Above- and below-ground biomass in the initial transplant area (25 cm diameter) was not significantly affected by mussel addition ($p = .325$ and $p = .226$). However, total above-ground cordgrass biomass (i.e. shoots on the initial transplant plus adjacent, colonized mudflat) was 53% higher in cordgrass + mussel treatments compared to cordgrass-only plots (749 vs. 488 g; $p = .025$; Figure 2b).

Mussel addition also augmented the ability of cordgrass transplants to withstand and recover from the simulated disturbance. Ten days after above-ground biomass was clipped and mussels were removed, we observed the emergence of shoots in only 50% of the cordgrass-only plots, whereas 100% of the cordgrass plots that originally had mussels contained new shoots ($p = .001$; Figure 3a). This
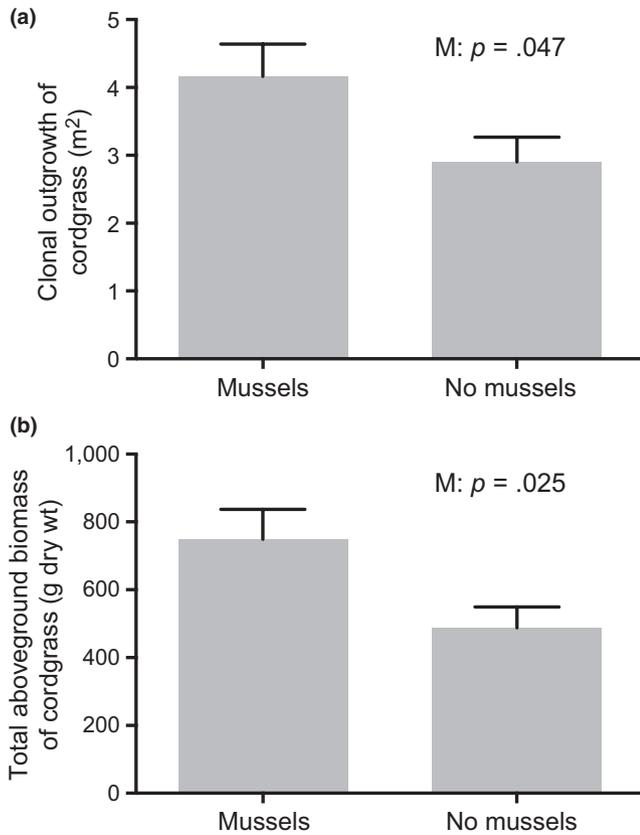
FIGURE 2 Cordgrass recovery after 16 months: (a) Clonal outgrowth of cordgrass in m^2. (b) The total colonized above-ground biomass of cordgrass. Clonal outgrowth and above-ground biomass were higher in mussel plots than in plots without mussels. M represents main effect of mussels, error bars represent + SE
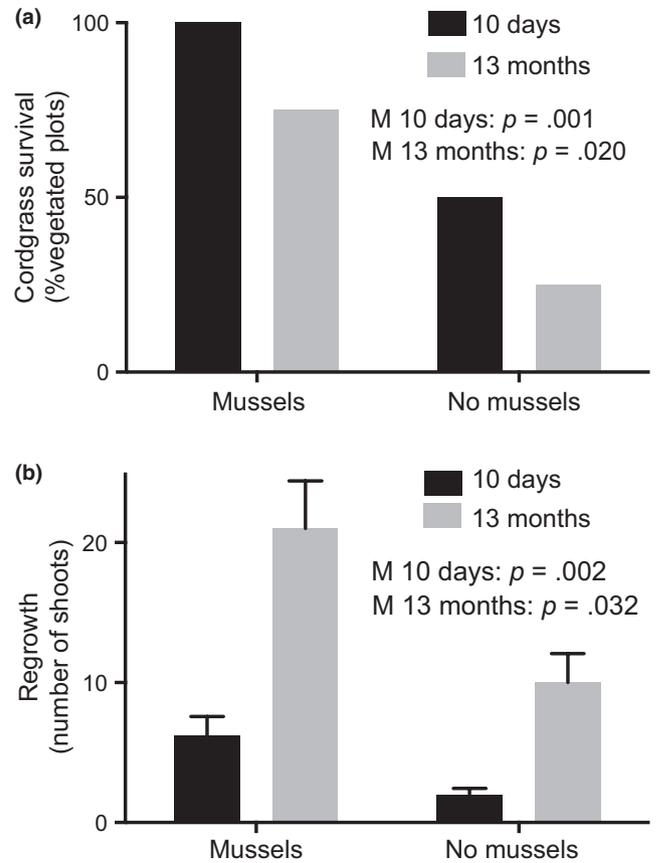

FIGURE 3 Cordgrass resilience: (a) Cordgrass survival—percentage of plots still harbouring vegetation 10 days and 13 months after clipping of all above-ground biomass and the removal of all mussels. (b) Regrowth of the number of cordgrass stems 10 days and 13 months after clipping and mussel removal. Cordgrass survival and regrowth after clipping was significantly higher in plots that received a mussel treatment in the past. M represents main effect of mussels, error bars represent + SE

effect was still prominent after 13 months (25% vs. 75%; $p = .020$; Figure 3a). Mussels also enhanced the number of shoots that emerged on the plots 10 days after the disturbance (6.25 vs. 2 shoots; $p = .002$; Figure 3b) and after 13 months (21 vs. 10 shoots, $p = .032$). Thus, mussels continued to enhance cordgrass survival and growth more than a year after they were removed.

3.2 | Chemical analyses of sediment porewater and vegetation

At the end of the first growing season, sulphide concentrations in the porewater of the top 5 cm of sediment were three times higher, on average, in plots with cordgrass (cordgrass-only and cordgrass + mussels) compared to plots without cordgrass (mussels-only and control) (255 vs. 700 μmol/L; $p < .001$). Similarly, sulphide concentrations were 73% lower in mussel (mussel-only and cordgrass + mussels) than in no-mussel plots (cordgrass-only and control) (749 vs. 205 μmol/L; $p < .001$; Figure 4e). The lower porewater sulphide levels in mussel plots were accompanied by a 23% decrease in total sulphur concentrations in cordgrass shoots (190 vs. 245 μmol/g; $p = .008$) and 19% decrease in cordgrass roots (321 vs. 394 μmol/g; $p = .023$; Figure 4f).

Porewater nutrient levels were enhanced by mussels and lowered by cordgrass. NO_3^- was undetectable in all samples, but we found lower NH_4^+ concentrations in the porewater from cordgrass plots compared to plots without cordgrass (7 vs. 227 μmol/L; $p > .001$; Figure 4a). In mussel addition plots (mussels-only and cordgrass + mussels) a nearly two-fold increase in NH_4^+ levels in the porewater was found (149 vs. 78 μmol/L; $p < .001$), compared to plots without mussels (cordgrass-only and control). In addition, we found an interaction between mussels and cordgrass, in which the highest NH_4^+ concentrations were observed in mussel plots without cordgrass ($p = .002$). The increases in NH_4^+ availability were also visible in shoot nitrogen contents in mussel addition plots: cordgrass displayed a 17% higher N concentration in shoots in cordgrass + mussels vs. cordgrass-only treatments ($p = .003$; Figure 4b). Similar to NH_4^+, total P concentrations in the porewater were 34% lower in cordgrass plots (34 vs. 52 μmol/L; $p = .034$; Figure 4c) and 58% higher in mussel plots (53 vs. 33 μmol/L; $p = .021$). Concomitantly, mussel addition resulted in a higher P concentration in cordgrass roots (34.1 vs. 10.5 μmol/g; $p < .001$; Figure 4d), but this effect was not visible in the shoots ($p = .371$). N:P ratios in cordgrass
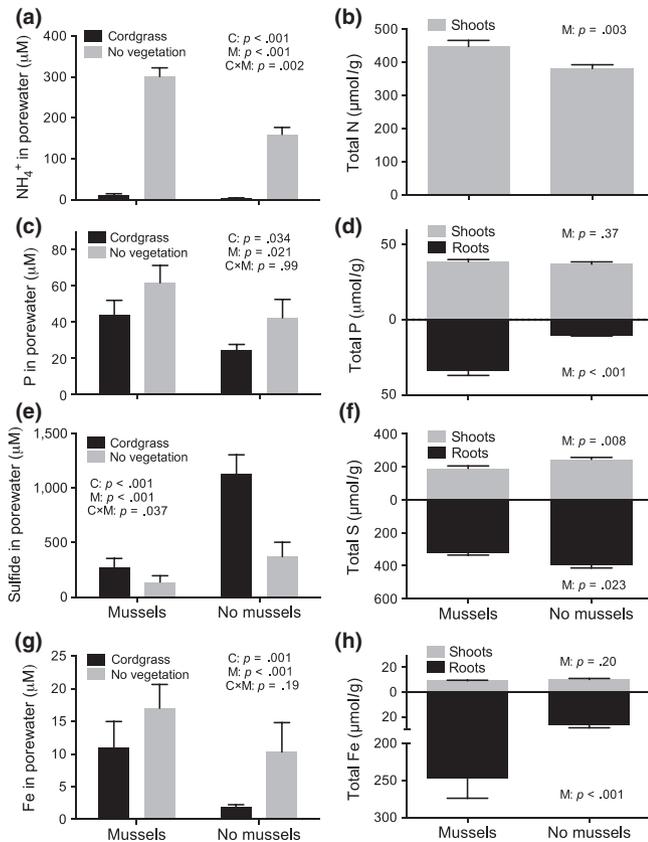
FIGURE 4 (a), (c), (e), (g) NH$_4^+$, P and sulphide and Fe concentrations in the porewater of the top 5 cm of the sediment. (b) (d), (f), (h) Total N, P, S and Fe concentrations in cordgrass shoots and roots. In mussel plots N, P and Fe was enhanced—and sulphide reduced—in the porewater, and N was increased in shoots, P and Fe increased in roots and S reduced in shoots and roots. C, M and C×M represent main effects of cordgrass, mussels and their interactions respectively. Error bars represent + SE

FIGURE 5 (a) Mussel condition after 16 months: live, missing and dead mussels. >90% of all mussels survived. (b) Mussel shell growth expressed in growth constant K (per year). Mussel growth was significantly higher in cordgrass plots. C represents main effect of cordgrass. Error bars represent + SE

shoots varied between 9.3 and 10.9. Finally, porewater Fe concentrations were 50% lower in cordgrass plots (6 vs. 14 μmol/g; $p = .001$) and were two times higher in mussel plots (14 vs. 6 μmol/g; $p < .001$; Figure 4g). The latter effect was even more pronounced in cordgrass roots, resulting in a nine-fold higher root Fe concentration in mussel plots (247 vs. 26 μmol/g; $p < .001$; Figure 4h). No increase in Fe concentration in cordgrass shoots was found, however (9 vs. 10 μmol/g; $p = .196$; Figure 4h). Across all plots, root Fe and root P concentrations were strongly and positively correlated ($r = .96$; $p < .001$).

3.3 | Mussel survival and growth

More than 90% of all mussels survived the experiment (Figure 5a), and absolute mussel shell growth decreased with increasing size (Appendix S4). Mussel growth was 22% higher in cordgrass plots (0.24 vs. 0.29 per year; $p = .008$; Figure 5b). All mussel addition plots harboured new mussel recruits with densities ranging from 2 to 26 individuals per plot, whereas only three recruits in total were found in the 24 plots that did not contain mussels at the start of the experiment (11.3 vs. 0.1 recruits per transplant; $p < .001$; Mann–Whitney
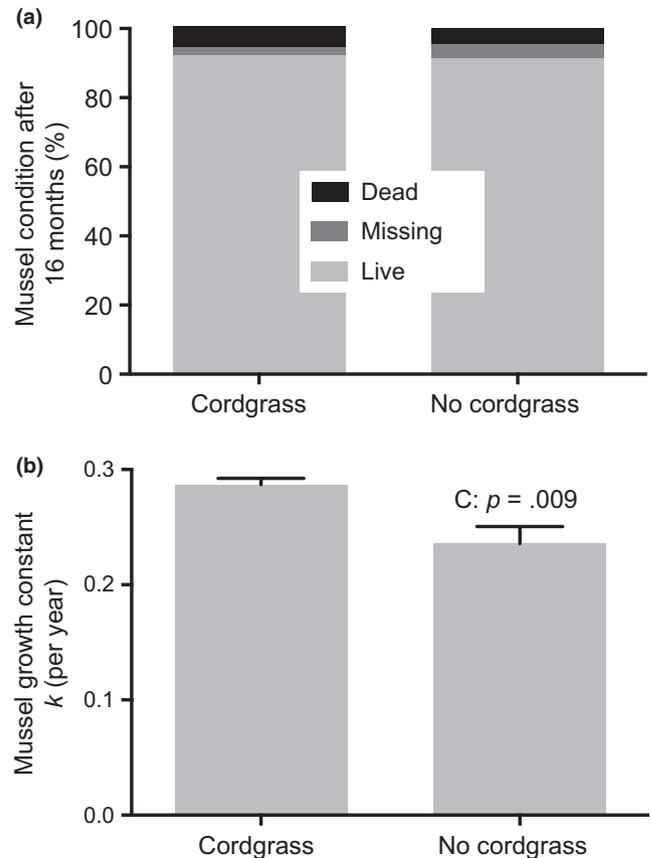
U test). Moreover, a clear bimodal distribution in the shell length of the recruits enabled us to distinguish between recruits that settled in 2013, the year of transplantation, and 2014 (Appendix S3). Analyses showed that recruitment was low and not significantly different between treatments in 2013 (2 vs. 4 recruits per plot; $p = .124$), but that recruitment was higher in cordgrass + mussel than in mussel-only plots in 2014 (11 vs. 6 recruits per plot; $p = .013$).

4 | DISCUSSION

Our experimental results show that the integration of positive interspecific interactions in restoration design can be a powerful and easy-to-incorporate approach for promoting coastal restoration success. In this saltmarsh system, we found that co-transplantation of mussels with cordgrass greatly enhanced short- and long-term restoration success as defined by: (1) enhancement of the relative growth of both cordgrass and mussels and (2) enhanced resilience of the primary foundation species—cordgrass—to disturbance. The effects of the co-transplantation of mussel mutualists extended to adjacent mudflats, far exceeding the spatial scale of the actual interaction. Impressively, mussel addition resulted in a 45% increase in clonal outgrowth in our

experiment, which corresponds to an average additional colonized surface area of 1.3 m^2 for each 0.05 m^2 cordgrass transplant. These results indicate that restoration practitioners may regain vegetation faster with established transplant array designs, or even reduce the number of transplants needed per unit marsh area by integrating the mussel-cordgrass mutualism. Importantly, cordgrass transplants that were originally associated with mussels also proved to be much more resilient to disturbance, resulting in enhanced biomass production and survival over 1 year after the disturbance. These findings suggest that inclusion of mutualism in restoration could also reduce the cost of maintaining restored marshes as it powerfully enhances the resistance of the transplanted material to future disturbances.

4.1 | Mechanisms facilitating cordgrass restoration

Mussels gave cordgrass a substantial initial boost in growth when the transplants were in their most vulnerable state—that is, lowest in biomass and stressed from transplantation. The mechanisms underpinning this initial stimulation of growth and their subsequent resistance to disturbance are at least two-fold. First, mussels increase the concentration of key limiting nutrients, providing the plants with a pool of resources to sustain rapid and vigorous growth. Second, mussels prevent the accumulation of sulphide, an important chemical stressor in saltmarsh systems that inhibits ammonium uptake and disrupts cordgrass root respiration (Lamers et al., 2013; Mendelssohn & Morris, 2002), and has been linked to cordgrass dieback (Koch, Mendelssohn, & McKee, 1990; Mendelssohn & McKee, 1988).

Evidence for the first mechanism is provided by higher nutrient concentrations in porewater in mussel addition plots and associated increases in cordgrass biomass and higher nutrient levels in shoots (N) and roots (P). Similarly, total N in above-ground cordgrass tissue was increased in mussel addition plots, illustrating that the increase in above-ground biomass was not merely a dilution effect. Although cordgrass N:P ratios at our study site were around 10, indicating nitrogen limitation (Koerselman & Meuleman, 1996), we found higher P levels in cordgrass roots collected from mussel plots where porewater P concentrations were much higher. As P-levels were not enhanced in the shoots and those in the roots strongly correlated with Fe-levels, we suspect that formation of external P-containing Fe plaques, that were visible around the roots within the oxidized rhizosphere (Appendix S5), is the most likely explanation for this response (Mendelssohn & Morris, 2002; Mendelssohn & Postek, 1982).

Support for the second proposed mechanism comes from the observation of a 73% reduction of sulphide concentrations in the porewater of mussel addition plots, which was associated with a lowering of sulphur concentrations in cordgrass roots and shoots. Mussels could lower sulphide levels in a number of ways. First, mussels increased porewater Fe concentrations—*perhaps by the accretion of iron-rich pseudofeces*—and Fe can bind and precipitate sulphide as iron-sulphides (e.g. FeS and FeS_2), and may catalyse chemical sulphide oxidation in the rhizosphere when oxygen is available (Hughes, Centelles, & Moore, 2009). Second, high numbers of fiddler crab burrows in mussel mounds (Angelini et al., 2015) and the increased elevation through excretion of pseudofaeces likely increase infiltration of oxygen-rich surface water and drainage on the mussel mounds, lowering sulphide levels in the process (Angelini et al., 2015; Bertness, 1985; Wilsey, McKee, & Mendelssohn, 1992). And finally, active sulphide removal by the mussels may also occur as ribbed mussels have been found to oxidize sulphide in their gill mitochondria (Lee, Kraus, & Doeller, 1996; Parrino, Kraus, & Doeller, 2000). The ability of mussels to reduce sulphide exposure in the field underlines the potential of this mutualist to facilitate cordgrass resilience and recovery not merely by enhancing nutrient availability, but through multiple co-occurring biogeochemical pathways.

4.2 | Co-transplantation of mussels for restoration

To harness these positive effects of mussels in saltmarsh restoration, initial co-transplantation will be required as we found that mussel recruits occurred almost exclusively on experimental plots already containing mussels. This observation is in agreement with earlier experiments (Bertness & Grosholz, 1985; Nielsen & Franz, 1995) and is important because the lack of recruitment on plots without mussels suggests that beneficial effects can only be obtained on restoration time-scales with active co-transplantation. Moreover, mussel survival after transplantation was high in our experiment (>90% after 16 months) and cordgrass facilitated the maintenance of mussel aggregations by enhancing recruitment in 2014. Cordgrass had no significant effect on recruitment in 2013 (i.e. the larger sized recruits), presumably because the experiment captured only part of the spawning, settlement and post-settlement movement of this year's mussel recruits as a result of its start in April (Nielsen & Franz, 1995). In agreement with earlier work on northeastern US marshes and cobble beaches (Altieri, Silliman, & Bertness, 2007; Bertness, 1984; Bertness & Grosholz, 1985; Bertness et al., 2015), we found that cordgrass also enhanced mussel growth. Collectively, our results that cordgrass facilitates mussels, mussels experience high post-transplantation survival, and mussels have strong positive legacy effects on cordgrass recovery and resilience support the conclusion that mussels should be proactively included in saltmarsh restoration designs as an easy-to-implement means of elevating restoration success. However, harvesting of mussels should be done with care and large-scale harvesting should be avoided to prevent excessive damage to healthy saltmarshes. Perhaps a larvae collection method similar to, for instance, the rope spat collectors used in the Wadden Sea with the mussel *Mytilus edulis* (Puente-Rodríguez, Swart, Middag, & van der Windt, 2015) could, combined with mussel breeding programmes, provide a sustainable supply for mussel co-transplantation.

4.3 | Positive interactions as a tool in coastal ecosystem restoration

Currently, the basic premise of many coastal restoration efforts is to foster the establishment of a single foundation species with the expectation that these dominant, habitat-forming organisms will facilitate the colonization of diverse, abundant assemblages of associated

species. Our results highlight that this view might be too limited, as relatively mature species assemblages may be needed to mitigate multiple stressors acting on the foundation species. In southeastern US saltmarshes, the diverse community of burrowing fauna, crab predators, snail grazers, and bivalves play a critical role in increasing nutrient capture (Bertness, 1984), alleviating soil salinity stress (Angelini et al., 2016), providing protection from overgrazing (Angelini et al., 2016; Bertness et al., 2015) and reducing sulphide stress (this study). As mussels contribute substantially to these benefits, our results hence point towards the necessity of a shift in restoration paradigm from one that emphasizes a single foundation pioneer species as a facilitator of community assembly, to one that promotes a species assemblage better suited to alleviate potential stressors. The re-establishment of the mutualistic feedback loop that forms between these partner species may be vital in enabling primary foundational species to overcome initial thresholds for establishment (Angelini et al., 2016; de Fouw et al., 2016) and in establishing biogeochemical processes that promote the long-term persistence of the transplanted species and their associated communities (Angelini et al., 2015).

Our results contribute to a restoration toolbox that includes a suite of strategies that coastal managers and policymakers can use as standalone measures or in concert. Depending on the environmental conditions, that is, the type and level of external stressors and available resources, managers may choose to include positive interactions by choosing a clumped transplant design, by incorporating mutualistic feedbacks through co-transplantation, or by applying both strategies. For example, in low-energy saltmarsh locations were sulphide stress poses a major threat to new transplants, a design including mussels and cordgrass may be most efficient. The mutualism alleviates this stress, and allows for transplanting cordgrass in widely spaced arrays, thereby maximizing the contribution of these patches to mudflat re-colonization via clonal expansion (Angelini et al., 2016). However, in more hydrodynamically active locations, a clumped design, which is more effective in attenuating waves and currents than widely spaced arrays (Silliman et al., 2015), may yield high success rates. In cases where multiple stressors pose a risk to transplants, a combination of both options may be optimal. Overall, we argue that co-transplantation of mussels may be an effective tool to enhance saltmarsh restoration success in any location where mussel and cordgrass distributions overlap (Figure 1a), whether it is a newly created restoration site or a recent dieback site.

In this study, we provide the first experimental evidence that the integration of a mutualism into restoration designs not only boosts local production and re-colonization rates but also enhances the resilience of the restored habitat to disturbance. Because positive interspecific interactions involving habitat-forming foundation species are similarly important in many other ecosystems such as mutualisms among seagrasses and lucinids (van der Heide et al., 2012), corals and sponges (de Goeij et al., 2013), plants and insect pollinators (Potts et al., 2010), and trees with large vertebrate dispersers (Terborgh et al., 2008), we predict that practitioners may derive similar gains in restoration return on investment from the inclusion of these interactions in many other ecosystems. We conclude that our findings lay the foundation for a new line of research studying how reciprocal positive interactions can be utilized in the restoration of heavily degraded aquatic and terrestrial ecosystems where habitat-forming foundation species engage in mutualistic interactions.

AUTHORS' CONTRIBUTIONS

M.D.H., T.v.d.H., C.A., L.P.M.L., and A.S. designed the study. M.D.H., T.v.d.H., C.A., J.R.H.H., A.B., L.P.M.L., H.d.P., B.R.S., J.v.d.K., and A.S. conducted the field experiment. M.D.H and T.v.d.H. analysed the data. M.D.H. wrote the first draft of the manuscript, all authors contributed substantially to revisions.

DATA ACCESSIBILITY

The data supporting the results of this manuscript are archived in Data Archiving and Networked Services (DANS) EASY: https://doi.org/10.17026/dans-x9j-atxa (Derksen-Hooijberg et al., 2017).

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