Effects of oil exposure, plant species composition, and plant genotypic diversity on salt marsh and mangrove assemblages

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Abstract. Climate change is causing shifts in the distribution and abundance of many species. Because species vary in the rate and degree of these shifts, novel transition zones have developed where new combinations of species overlap. If climate-mediated range shifts result in greater diversity, transition communities could have enhanced resistance and/or resilience, particularly if the resident and colonizing species differ in their response to environmental change. The range expansion of the tropical black mangrove, *Avicennia germinans*, into salt marshes dominated by the temperate cordgrass, *Spartina alterniflora*, provides an opportunity to examine the responses of climate-mediated transition zones to disturbance. We conducted a yearlong mesocosm experiment testing the effects of plant species identity and composition (*A. germinans*, *S. alterniflora*), as well as plant genotypic diversity (*S. alterniflora* only), on the response of coastal wetlands to oiling disturbance. Oil negatively impacted *S. alterniflora* and *A. germinans* both above- and belowground, though the timing of these effects varied, with *S. alterniflora* showing more immediate declines than *A. germinans*. As hypothesized, the magnitude of the oil effect was reduced in the mixed plant species treatment compared to the single species treatment for *A. germinans* survival (12% vs. 21% reduction) and belowground biomass (19% vs. 71% reduction). In addition, when exposed to oil, *A. germinans* crown area and volume were greater in the mixed species treatment compared to the single species treatment at the end of the experiment. However, we did not detect any benefit of mixed species communities or *S. alterniflora* genotypic diversity for the *S. alterniflora* response to oil. Our results suggest that transition habitats in the northern Gulf of Mexico where *A. germinans* and *S. alterniflora* co-occur will be negatively impacted by future oiling events, but that they are no more susceptible, and perhaps slightly less so, than habitats dominated by either individual species.

Key words: *Avicennia germinans*; Deepwater Horizon; disturbance; mesocosm; primary production; range shift; *Spartina alterniflora*.

Received 23 January 2018; revised 15 March 2018; accepted 21 March 2018. Corresponding Editor: Julie C. Zinnert.

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INTRODUCTION

Global change is causing shifts in the distribution and abundance of many species, both on land and in the sea (Chen et al. 2011, Poloczanska et al. 2013, Pecl et al. 2017). The direction of climate-driven range shifts across latitude and elevation is often predictable (Pecl et al. 2017);
for instance, terrestrial taxa have moved poleward an average of 17 km per decade (Chen et al. 2011). However, species vary in the rate and degree of climate-induced range shifts, blurring the boundaries between formerly distinct communities and resulting in novel transition zones where new combinations of species overlap (Gilman et al. 2010, Alexander et al. 2015, Pecl et al. 2017). The effects of changes in community composition and species overlap on ecosystem processes are often unknown, but impacts on function are possible, particularly if range expansions involve habitat-forming species (HilleRisLambers et al. 2013, Christiaen et al. 2016, Pecl et al. 2017).

Results from studies on the effects of biodiversity on ecosystem function (Cardinale et al. 2012, Duffy et al. 2017) suggest that climate-mediated range shifts could enhance ecosystem function if they result in greater species and/or functional diversity (Pecl et al. 2017). Importantly, given the increasing extent of anthropogenic disturbance (Buma 2015), more diverse transition communities resulting from climate-induced expansions may have enhanced resistance and/or resilience, particularly if the colonizing species differs from the resident species in its response to environmental change (Yachi and Loreau 1999, Elmqvist et al. 2003). Understanding the response of climate-induced mixed species transition zones to disturbance, and whether they are more or less susceptible than either species separately, is critical to inform the trajectory of change, as well as to devise appropriate conservation and restoration practices (Pecl et al. 2017).

The influence of biodiversity on ecosystem function and disturbance response is not limited to variation among species; intraspecific genetic and trait variation, especially within habitat-forming species, can also have community- and ecosystem-level effects (Hughes et al. 2008, Bolnick et al. 2011). For example, seagrass genetic diversity enhances the ecosystem resistance and resilience to disturbances in both natural and experimental assemblages (Hughes and Stachowicz 2004, 2011, Reusch et al. 2005). Genetic variation can also affect the strength and direction of species interactions (Booth and Grime 2003, Crawford and Rudgers 2012, Hughes et al. 2014). For instance, interactions between pine trees and their mycorrhizal fungi are strongly controlled by plant genotype, and these genotype-specific associations in turn have a large influence on drought tolerance of the trees (Gehring et al. 2017). Thus, genetic variation within species could influence the functional outcome of climate-mediated range shifts among habitat-forming species.

The range expansion of the tropical black mangrove, *Avicennia germinans*, into salt marshes dominated by *Spartina alterniflora* along the Atlantic and Gulf Coasts of the United States (Perry and Mendelssohn 2009, Cavanaugh et al. 2014) provides an opportunity to examine the responses of climate-mediated transition zones to disturbance. The northern extent of this mangrove species’ distribution is controlled primarily by cold intolerance (McMillan and Sherrod 1986, Duke et al. 1998, Cavanaugh et al. 2014), with *A. germinans* populations limited by freeze intensity and duration (Sherrod and McMillan 1985, Cook-Patton et al. 2015, Osland et al. 2017). Thus, winter warming due to climate change is surmised to be driving the observed encroachment of mangroves into marsh systems in the northern Gulf of Mexico and along the Atlantic Coast (Cavanaugh et al. 2014, Osland et al. 2017). Similar poleward expansions of mangroves into marsh ecosystems are also happening in other regions of the world (e.g., Australia, Africa; Saintilan et al. 2014, Ward et al. 2016). Changes in the distribution and abundance of habitat-providing plant species such as salt marsh plants and mangroves can have important cascading effects on both ecological functions (Ellison et al. 2005, Altieri et al. 2007, Bishop et al. 2013) and the human communities that depend on their ecosystem services (Martinez et al. 2007, Mcleod et al. 2011). Encroachment of mangroves into marsh habitat is likely to increase the provision of some services, while reducing others (Johnston and Caretti 2017, Kelleway et al. 2017, Scheffel et al. 2017a; A. Macy et al., unpublished manuscript). Thus, there is a pressing need to better understand ecosystem processes and functions in marsh–mangrove transition zones, as well as the responses of these new communities to other anthropogenic disturbances. In addition, given that genetic variation in the dominant marsh
Oiling effects in salt marshes

It is well documented that oil exposure has negative effects on coastal vegetation, causing reduced photosynthesis, transpiration, shoot height, stem density, and biomass, as well as impaired growth and regrowth (Pezeshki et al. 2000, Lin and Mendelssohn 2012). Sublethal effects on mangroves include inhibited respiration, with particularly negative effects on mangrove propagules and seedlings (Grant et al. 1993, Proffitt et al. 1995, Shapiro et al. 2016). As a result of these detrimental effects, oil exposure can reduce vegetation cover and destabilize the soil due to the loss of living tissue belowground (Shapiro et al. 2016). However, marsh response to and recovery from oil spills can vary greatly (Mendelssohn et al. 2012, Shapiro et al. 2016). For instance, heavy oiling of marsh vegetation during the DWH spill caused almost complete mortality of *Spartina alterniflora*, but moderate oiling had no significant effect relative to reference marshes (Lin and Mendelssohn 2012). The response to oil can also vary among dominant plant species (Pezeshki et al. 2000, Lin and Mendelssohn 2012, Mendelssohn et al. 2012), suggesting that mixed species communities may exhibit greater response diversity to this type of disturbance. Further, plant sensitivity can differ among populations within a species (Pezeshki et al. 2000), indicating the potential importance of intraspecific genetic variation in the response to oiling.

The type and condition of oil can also influence its impact on vegetation. Although initially toxic, oil loses its volatile components quickly upon dispersion into the marine environment, and it continues to undergo further compositional changes due to weathering that make it less toxic, more viscous, and more likely to coagulate (Mendelssohn et al. 2012). In fact, most of the oil from DWH that reached coastal areas had been extensively weathered and consisted of emulsions of crude oil that were depleted of the more volatile and toxic components (Lin and Mendelssohn 2012, Mendelssohn et al. 2012, Judy et al. 2014). Thus, in keeping with past manipulations testing the effects of oiling on coastal vegetation (Lin and Mendelssohn 2012, Mendelssohn et al. 2012, Judy et al. 2014), we...
used a repetitive soil oiling manipulation with weathered crude oil in our experiment.

The specific plant tissues exposed to oiling can also contribute to the observed variation in response. When oil coats leaf surfaces, it leads to reduced gas exchange via stomata, causing oxygen-stressed roots or pneumatophores (Shapiro et al. 2016). However, oiling that only covers aboveground tissues does not cause as lasting damage as soil oiling (Mendelssohn et al. 2012, Judy et al. 2014). In fact, the most important determinant of plant damage is whether oil penetrates the soil and comes into contact with roots and rhizomes (Mendelssohn et al. 2012). Because soil oiling is one of the most common oiling scenarios (Lin and Mendelssohn 2012), we chose to simulate it in our study.

**Experimental design**

We conducted a one-year outdoor mesocosm experiment at Dauphin Island Sea Lab (DISL) to examine the effects of plant species identity and composition (A. germinans, S. alterniflora), as well as plant genotypic diversity (S. alterniflora only), on the response of coastal wetlands to oiling disturbance. We used a split-plot design, with oiling treatments as the whole-plot factor randomly assigned to each of four 19,000-L tanks (two oiled and two non-oiled tanks). Our plant treatments were randomly assigned to twenty-two 75.7-L mesocosms within each tank (see Appendices S1, S2 for additional details and figures of the experimental design). We included three plant composition treatments: S. alterniflora only (hereafter, SA only), A. germinans only (hereafter, AG only), and S. alterniflora and A. germinans mix (hereafter, SA+AG). In addition, within the treatments containing S. alterniflora, we examined the effects of plant genotypic identity and diversity using one genotype (monoculture) or three genotypes (three genotypes randomly selected from a pool of six genotypes; polyculture). These genotypic diversity treatments are within the range of values observed in the northern Gulf of Mexico (Hughes and Lotterhos 2014). Each of the S. alterniflora genotypes was grown in SA only monoculture in each tank (see Appendix S1: Table S1 for details).

**Spartina alterniflora** used in the experiment came from a stock of known genotypes that we propagated in a greenhouse at the Florida State University Coastal and Marine Laboratory. These genotypes were collected from St. Joseph Bay, Florida, prior to the DWH oil spill, and to our knowledge had no exposure to oil or to mangroves (which are present in St. Joseph Bay, but not at the sites where these genotypes were collected) before this experiment. These genotypes were confirmed to be genetically distinct using eight DNA microsatellite loci specific to this species (Hughes 2014, Hughes and Lotterhos 2014). Replicate transplants of each genotype (mean [standard error, SE] live density = 4.06 [0.10] stems per transplant) were grown in individual 4-L pots in the greenhouse and transported to DISL in June 2015. Due to the difficulty and potential disturbance of collecting sufficient numbers of A. germinans from natural populations, we purchased one-year-old seedlings (mean [SE] stem height = 61.3 [0.8] cm) from a commercial nursery in Louisiana and brought them to DISL in July 2015.

In mid-July 2015, we drilled three equally spaced holes along the bottom of each mesocosm to facilitate drainage. We filled each mesocosm with a 5 cm deep layer of gravel, topped with a 25 cm deep layer of commercial sand. Mesh screens (0.5 mm) were placed on the inside of the tubs where the holes were drilled to prevent gravel or sand from passing through. We then planted S. alterniflora and A. germinans using a substitutive experimental design, with six plants in each mesocosm: six S. alterniflora transplants, six A. germinans seedlings, or three S. alterniflora transplants and three A. germinans seedlings (Appendix S1: Table S1, Fig. S1). Mesocosms were exposed to ambient light, temperature, and precipitation. In addition, we irrigated them with freshwater twice per week for two months prior to oil exposure to allow the plants to acclimate.

**Oiling procedure**

A 30-L sample of surrogate Louisiana sweet crude oil, similar to Macondo source oil, was acquired from BP Exploration and Production (Houston, Texas, USA). To simulate the condition of oil that reached the shorelines post-DWH disaster, we weathered the oil outdoors using bubbler aeration for five days prior to the experiment following published methods (Lin and Mendelssohn 2012). To confirm that weathering occurred, we measured the change in
density of the oil over the course of the weathering process (N = 3 samples post-weathering; Mortazavi et al. 2013). The observed increase (from 0.84 g/cm³ to 1.02 ± 0.05 g/cm³) indicates the loss of low molecular weight hydrocarbons to volatilization. The original amount of oil was reduced by approximately 17% by volume.

Following weathering, we used a 5-d repetitive dosage procedure for the oiling treatment, with each mesocosm receiving 1 L/m² of a 1:1 oil : water mixture (350:350 mL). We used an oiling concentration (8 L/m²) and drainage procedures as described in the soil oiling treatment in Lin and Mendelssohn (2012). For the oiling treatment, we applied the emulsified, weathered oil : water mixture over a standing water layer in each mesocosm, and then, the water/oil was allowed to contact and migrate through the sediment column via the suction produced as the mesocosm drained. This procedure ensured that the plant belowground tissues were exposed to oil. The drained water and associated oil were collected via a drainage tube at the bottom of the mesocosm and returned into the mesocosm onto the soil surface. All drainage holes were then plugged and the mixture kept in the mesocosm for approximately 12 h before being drained and re-oiled. This process was repeated daily for four more days. In the two non-oiled tanks, we added 700 mL seawater with no oil and repeated the same exact procedure as in the oiled mesocosms.

We applied our oiling treatment in October 2015, after the plants had time to establish and acclimate. At that time, we also began simulating a diurnal tidal regime in each tank using an Arduino Uno microcontroller system. Each large experimental tank was paired with a smaller tank (volume = 3785 L) that housed water for tidal exchange and prevented oiled and non-oiled water from mixing. Each tank was equipped with one submersible bilge pump that was plumbed with 1.27-cm PEX tubing. The system controlled the inflow : outflow rate of seawater transported between each pair of tanks. We established a high tide of approximately 50 cm depth in each tank (7–10 cm above the sediment surface in each mesocosm; flooding = 12 h) and a low tide of 40 cm depth in each tank (sediment surface exposed in each mesocosm; ebbing = 12 h) to simulate conditions along the north-central Gulf Coast (Appendix S2: Figs. S2, S3). Water changes and salt additions were performed throughout the experiment to remove excess precipitation from the tanks and keep salinity stable (mean [SE] salinity over the course of experiment = 15.0 [0.7] ppt).

Response variables

We measured a suite of S. alterniflora and A. germinans morphological and growth traits to estimate plant production and examine the impacts of oiling disturbance through time. For S. alterniflora, these included live stem density (number of live stems per mesocosm); stem height (distance from sediment surface to tip of tallest leaf on each stem); and leaf growth. For A. germinans, we included survival (proportion of live seedlings per mesocosm); stem height (distance from sediment surface to tip of tallest stem); crown area (calculated using the equation for an ellipse and measurement of crown diameter in two perpendicular directions; Osland et al. 2014); crown volume (calculated as crown area × height; Osland et al. 2014); leaf number (number of live leaves per individual); and leaf area. Leaf area for A. germinans was estimated using a leaf tagging technique developed by Onuf et al. (1977). We tagged and photographed five leaves from each of two seedlings per mesocosm in September 2015. Leaves were re-photographed in December 2015, July 2016, and September 2016. All images were processed with ImageJ (Abramoff et al. 2004) to estimate leaf area. A similar tagging technique developed by Hopkinson et al. (1980) was used to estimate leaf growth for S. alterniflora from September to December 2015 and June to September 2016. To account for potential genotypic variation, replication in each mesocosm varied depending on the number of S. alterniflora genotypes present: In monoculture treatments, only two shoots were tagged, whereas polyculture treatments contained 3–6 tagged shoots. These plant responses were measured before the oiling treatment was applied (September 2015) and on four dates post-oil (December 2015, April 2016, June 2016, and September 2016). The initial plant measurements did not vary by treatment for any response variable and thus are not presented here.

We also quantified S. alterniflora flower and seed production per mesocosm in each year of the experiment (1 December 2015 and 20 September 2016).
2016). Flower production was estimated as the number of flowering stems present on each sampling date. Seed production was estimated by collecting all flowers per mesocosm and counting the total number of seeds. At the end of the experiment (20 September 2016), we measured sediment oxygen availability (i.e., redox) and temperature at a depth of 5 cm using a Thermo Fisher Scientific Orion Star Series A321 Portable pH meter (ThermoFisher Scientific, Waltham, Massachusetts, USA). In addition, we took two sediment cores (5 cm diameter and 10 cm deep) in the center of each mesocosm to quantify living belowground biomass by species. The contents of the core were rinsed over a mesh sieve (7.1 mm) to remove any excess sediment. For *S. alterniflora*, we separated belowground biomass into roots and rhizomes. All samples were dried at 60°C for 72 h prior to weighing. Sediment cores were processed at the Marine Science Center at Northeastern University.

**Analyses**

We used a repeated-measures (RM) split-plot analytical approach for all plant responses measured across multiple sampling dates: *S. alterniflora* live stem density (standardized by the number of transplants per mesocosm); *S. alterniflora* stem height; *A. germinans* leaf number and area; *A. germinans* crown area and volume; and *A. germinans* stem height. Oiling treatment was our whole-plot effect, with species composition, genotypic diversity (*S. alterniflora* responses only), and date as the within-plot effects. We also included a fixed effect of mesocosm nested in tank to account for measuring the same plants in each mesocosm through time. Genotypic diversity was not analyzed for *A. germinans* responses, since it was only manipulated for *S. alterniflora*. When there were significant interactions with time, we then tested separate split-plot models for each date. We also conducted separate split-plot analyses for each individual date for all responses measured at only one or two time points: *S. alterniflora* flowering and seed production in each year; and *S. alterniflora* leaf growth in December 2015 and September 2016; as well as *S. alterniflora* belowground biomass, *A. germinans* belowground biomass, and sediment redox and temperature at the end of the experiment.

**RESULTS**

*Spartina alterniflora* responses

*Spartina alterniflora* live stem density was affected by oiling treatment and plant species composition, but the degree of these impacts varied through time (RM: oil × date $F_{4, 256} = 14.99$, $P < 0.001$; species composition × date $F_{4, 256} = 41.52$, $P < 0.001$; Fig. 1; Table 1). Immediately post-oil (December 2015) and through the middle of the next growing season (April and June 2016), live stem density was lower in oiled than unoiled treatments regardless of species composition (December $F_{1, 2} = 26.33$, $P = 0.03$; April $F_{1, 2} = 244.70$, $P = 0.004$; June $F_{1, 2} = 86.82$, $P = 0.01$; Fig. 1). In the middle of the growing season (June 2016) and at the end of the experiment (September 2016), there was an effect of plant species composition regardless of oiling (June $F_{1, 62} = 15.14$, $P < 0.001$; Sept $F_{1, 62} = 42.13$, $P < 0.001$; Fig. 1), with more stems in the mixed species treatment. *S. alterniflora* genetic diversity did not significantly affect live stem density in the repeated measures analysis or at any individual date (Table 1).

*Spartina alterniflora* stem height differed across oiling treatments through time (RM: oil × date...
Table 1. Statistical results for the effects of experimental treatments on *Spartina alterniflora* (SA) responses.

<table>
<thead>
<tr>
<th>Factor</th>
<th>SA live density per transplant</th>
<th>SA avg stem height</th>
<th>SA leaf growth</th>
<th>SA root biomass</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date</td>
<td>December 2015</td>
<td>April 2016</td>
<td>June 2016</td>
<td>September 2016</td>
</tr>
<tr>
<td>Oil</td>
<td>ns</td>
<td>*</td>
<td>***</td>
<td>ns</td>
</tr>
<tr>
<td>Species composition</td>
<td>ns</td>
<td>ns</td>
<td>***</td>
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</tr>
<tr>
<td>Genetic diversity</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
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<tr>
<td>Oil × Species composition</td>
<td>ns</td>
<td>ns</td>
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<tr>
<td>Oil × Genetic diversity</td>
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<td>Species composition × Genetic diversity</td>
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<td>Oil × Species composition × Genetic diversity</td>
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</tr>
</tbody>
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Notes: ns, not significant. Separate analyses were conducted for each sampling date for stem density and stem height because there were significant treatment interactions with time. There were no significant treatment effects on flower or seed production, so they are not shown. 

\[ F_{4, 256} = 16.90, P < 0.001; \text{Appendix S2: Fig. S1a}, \]

with reduced height in oiling treatments in the early growing season (April 2016; Table 1). There was also an interactive effect of plant species composition and time on stem height (RM: species treatment × date \( F_{4, 256} = 2.45, P = 0.05 \), but plant species composition did not significantly affect stem height at any individual date when analyzed separately (Appendix S2: Fig. S1b; Table 1).

*Spartina alterniflora* leaf growth was marginally reduced in oiling treatments in December 2015 \( (F_{1, 2} = 13.69, P = 0.06) \); oil mean [SE] cm/d = 0.02 [0.01], no oil mean [SE] cm/d = 0.09 [0.02]). In September 2016, leaf growth varied marginally by plant species composition, with a tendency for higher rates in the mixed species treatment \( (F_{1, 62} = 3.69, P = 0.06); \) SA only mean [SE] cm/d = 1.10 [0.10]; SA+AG mean [SE] cm/d = 1.47 [0.16].

At the end of the experiment, nearly one year after oiling, there was a negative effect of oil on *S. alterniflora* root biomass \( (F_{1, 2} = 17.96, P = 0.05) \); Fig. 2a). There was also a marginal reduction in rhizome biomass \( (F_{1, 2} = 6.97, P = 0.11) \), as well as total *S. alterniflora* belowground biomass (roots and rhizomes combined; \( F_{1, 2} = 9.93, P = 0.09 \)). When we adjusted the belowground biomass to account for the initial number of *S. alterniflora* transplants (three or six per mesocosm), there was a marginal negative effect of oil \( (F_{1, 2} = 11.33, P = 0.08) \) and a significant effect of plant species composition \( (F_{1, 62} = 5.4, P = 0.02; \) Appendix S2: Fig. S2), with 74% more *S. alterniflora* belowground biomass in the mixed species treatment.

Neither *S. alterniflora* flower production nor total seed production was affected by oiling or our plant treatments in 2015 or 2016. Similarly, neither sediment temperature nor redox varied significantly by any experimental treatment at the end of the experiment.

**Avicennia germinans responses**

*Avicennia germinans* survival differed interactively by oiling and date (RM: oil × date \( F_{3, 132} = 22.43, P < 0.001 \)); Survival was reduced by exposure to oil, but not until June and September 2016 (Fig. 3a). In the repeated-measures analysis, there was also a significant species treatment-by-oil interaction (RM: species treatment × oil \( F_{1, 132} = 5.23, P = 0.02 \)). Oilling caused a greater reduction in survival when *A. germinans* grew alone (21% reduction) vs. with *S. alterniflora* (12% reduction) over the course of the experiment (Fig. 3b).

There was a significant effect of oiling on *A. germinans* leaves that was consistent over the course of the experiment (RM: oil \( F_{1, 2} = 22.19, P = 0.04 \); Table 2), with fewer leaves per seedling.

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\[ P < 0.10, * P < 0.05, ** P < 0.01, *** P < 0.001. \]
Species composition and oiling, and these effects were consistent across the duration of the experiment (RM: species treatment × oil $F_{1, 132} = 5.33, P = 0.02$; Fig. 4b). Leaf area was generally higher in the AG only treatment than the SA+AG treatment in the absence of oil, but there was no significant difference between species treatments in the presence of oil.

*Avicennia germinans* crown area varied interactively with plant species composition, oiling, and time (RM: species treatment × oil × date $F_{3, 130} = 8.03, P < 0.001$; Fig. 4c, Table 2). When each time point was analyzed individually, there was a significant interaction between plant species composition and oiling only at the end of the experiment (September 2016; $F_{1, 41} = 17.85, P = 0.001$). At this point, crown area was greater in the AG only treatment than in the SA+AG.
treatment in the absence of oil, but the mixed species treatment had higher crown area than the AG only treatment in the presence of oil (Fig. 4c).

Avicennia germinans stem height decreased slightly over the course of the experiment (RM: date $F_{4, 176} = 9.02$, $P < 0.001$; $y = -0.01x + 643.29$, $R^2 = 0.65$), but it was not affected by oiling or species treatments.

The effects of our experimental treatments on crown volume (calculated as crown area $\times$ stem height) were similar to those for crown area, and varied interactively with oiling and plant species composition over time (RM: species treatment $\times$ oil $\times$ date $F_{3, 130} = 7.11$, $P < 0.001$; Table 2), with lower crown volume in the AG only treatment in the absence of oil than in the other treatments, but only at the final sampling date (Appendix S2: Fig. S1c).

Avicennia germinans belowground biomass was reduced by oiling, but only substantially in the AG only treatment (71%; species treatment $\times$ oil $F_{1,42} = 12.27$, $P = 0.001$; Fig. 2b). This result was consistent if we accounted for the initial number of A. germinans seedlings per mesocosm (three or six; species treatment $\times$ oil $F_{1,42} = 3.78$, $P = 0.05$).

**DISCUSSION**

Consistent with prior studies (Pezeshki et al. 2000, Lin and Mendelssohn 2012, Mendelssohn et al. 2012), oil negatively impacted both S. alterniflora and Avicennia germinans in our yearlong mesocosm experiment. These negative oiling effects were apparent both above- and belowground, with belowground effects evident even a year after the original exposure for both species. As hypothesized, the magnitude of the oil effect was reduced in the mixed plant species treatment compared to the single species treatment for A. germinans survival (12% vs. 21% reduction) and belowground biomass (19% vs. 71% reduction). However, the biomass result was due in part to reduced A. germinans belowground biomass in the mixed species treatment even in the absence of oil. Further, we did not detect any benefit of mixed species communities or S. alterniflora genotypic diversity for the S. alterniflora response to oil; stem density was higher in mixed species treatments at the end of the experiment, but this result did not vary by oiling treatment. Our results suggest that transition habitats in the northern Gulf of Mexico where A. germinans and S. alterniflora co-occur will be negatively impacted by future oiling events, but that they are no more susceptible, and perhaps slightly less so, than habitats dominated by either individual species.

The negative effects of oil exposure on S. alterniflora were evident at our first sampling event that occurred two months post-oiling, indicating low resistance to this disturbance. However, the absence of significant oiling effects on...
S. alterniflora aboveground traits (density, stem height, leaf growth) at the end of the experiment suggests that this species does have some resilience to oil exposure, which is consistent with studies finding that S. alterniflora has a high capacity for disturbance recovery (Hanley et al. 2017). This aboveground recovery likely occurred due to a translocation of resources from below-ground tissues, as evidenced by reduced below-ground biomass in the oiled treatments at the end of the experiment. Thus, although there were no visible signs of oiling damage to S. alterniflora one year following exposure, these plants likely had reduced ability to withstand any future stresses or disturbances. Further, the reduction in below-ground biomass suggests that key ecosystem functions such as erosion prevention and carbon storage were likely also compromised (Macreadie et al. 2013).

In contrast to S. alterniflora, we did not detect negative effects on A. germinans survival until 6–12 months post-oiling, and mangrove leaf area, crown area, and crown volume showed only modest responses to oil over the course of our experiment, suggesting that A. germinans has greater resistance to oil exposure. There were significant interactions of oil and species composition on A. germinans traits, with AG only treatments in the absence of oil having the highest performance. While the presence of S. alterniflora reduced the magnitude of the oiling effect on A. germinans, but S. alterniflora also led to lower A. germinans below-ground biomass, number of leaves, and leaf area overall, regardless of oiling. These results are consistent with prior evidence for competition between S. alterniflora and A. germinans seedlings (Patterson et al. 1993, McKee and Rooth 2008, Guo et al. 2013, Simpson et al. 2013) and suggest that both oil exposure and interactions with S. alterniflora can independently lead to decreased A. germinans performance.

Fig. 4. Effects of oiling and plant species composition on Avicennia germinans morphology over the course of a 1-yr mesocosm experiment. (a) A. germinans leaves per seedling by plant species composition. Red lines are A. germinans only (AG) treatments averaged across oiling treatments, and purple lines are Spartina alterniflora–A. germinans (SA+AG) treatments averaged across oiling treatments. Error bars are present but smaller than the symbols in September 2016. (b) A. germinans leaf area (cm²) by oiling and plant species composition. (c) A. germinans crown area (cm²) per seedling by oiling and plant species composition. Red diamonds represent A. germinans only (AG) treatments; purple squares represent S. alterniflora–A. germinans (SA+AG) treatments; solid black lines are oiled treatments; and dashed lines are unoiled treatments. Error bars represent ±1 standard error.
Spartina alterniflora did not experience reductions in the impact of oiling in the mixed species treatments as expected. In the six months post-oiling when negative impacts on S. alterniflora were most evident, both SA only and SA+AG treatments had similarly low density. In addition, the negative effects of oil on S. alterniflora leaf growth in December 2015, as well as belowground biomass at the end of the experiment, were consistent regardless of the presence of A. germinans. Interestingly, by the end of the experiment S. alterniflora stem density and leaf growth, as well as the belowground biomass per transplant, were higher in the mixed species treatments, regardless of oiling. These results suggest that although A. germinans seedlings did not mediate the effects of oil on S. alterniflora, there may be some facilitative interactions between the two species that benefit S. alterniflora production on a per plant basis. However, the direction and magnitude of these interactions likely change across A. germinans life history stages (Callaway and Walker 1997, Guo et al. 2013). For example, A. germinans seedlings do not influence S. alterniflora above- or belowground growth (Patterson et al. 1993, McKee and Rooth 2008), yet the cover of salt marsh plants such as S. alterniflora declines as the cover or height of A. germinans increases (Guo et al. 2017, Weaver and Armitage 2018). Ultimately, the replacement of low-stature forbs such as S. alterniflora by taller, woody vegetation such as adult A. germinans is a common process across ecosystems (Van Auken 2000) and has already been documented in some areas of the northern Gulf of Mexico (Armitage et al. 2015).

We also found little evidence that genotypic diversity of S. alterniflora influenced the response to oiling, in contrast to our hypotheses, as well as prior results showing strong effects of genotypic identity and diversity in this system (Wang et al. 2012, Hughes 2014, Hughes et al. 2014). We used S. alterniflora genotypes that had no prior documented exposure to oil, which may have enhanced the negative effects of oil on S. alterniflora while also limiting the likelihood that genotypes would vary in their response. In addition, the levels of genotypic diversity tested in our experiment (one genotype vs. three genotypes) were relatively low, potentially contributing to the absence of an effect. However, these levels of diversity are realistic for marshes in the northern Gulf of Mexico (Hughes and Lotterhos 2014), suggesting that the effects of S. alterniflora genotypic diversity on resistance and resilience to oiling may be limited.

The closed nature of our experimental mesocosm system may have exacerbated the effects of oiling, since there was no mechanism for oil to physically leave the system (e.g., via currents). However, our mesocosms were exposed to ambient light and weather conditions, which allowed further weathering and degradation. In addition, the timing of our oil exposure may have underestimated the negative effects (Mendelsohn et al. 2012). Spills during colder periods have a reduced impact relative to exposure during warmer seasons, because of plant dormancy and/or reduced metabolism in the non-growing season (Mendelsohn et al. 2012). Such dormancy could have also contributed to the delayed impacts of the oil on A. germinans vs. S. alterniflora. Finally, our mesocosms excluded the effects of macrofauna such as fiddler crabs whose bioturbation activities could have influenced the distribution and impacts of oil (Culbertson et al. 2007).

There is interest in the outcome of S. alterniflora and A. germinans interactions, even in the absence of oiling, as A. germinans expands its range northward, with implications for ecosystem functions and services (Guo et al. 2017) and the composition of associated faunal communities (Diskin and Smee 2017, Scheffel et al. 2017b, Smee et al. 2017). The magnitude of the impact that salt marsh plants have on the survival and establishment of mangrove seedlings can vary depending on environmental conditions and stressors, such as salinity and the frequency and duration of freezing events (Coldren and Proffitt 2017), as well as mangrove species identity (Devaney et al. 2017). In our experiment, S. alterniflora had negative effects on A. germinans seedlings in the absence of oil, consistent with prior field experiments examining S. alterniflora interactions with A. germinans seedlings (Patterson et al. 1993, McKee and Rooth 2008). However, S. alterniflora mediated the negative effects of oil on A. germinans seedling survival and belowground biomass. This benefit may have resulted from S. alterniflora trapping more oil on its leaves and roots than A. germinans,
particularly early in the experiment. Although the exact mechanisms underlying the interaction are uncertain, this benefit for A. germinans in the presence of oil, combined with the lack of a similar benefit for S. alterniflora, suggests that oiling could shift the balance of local interactions in mixed assemblages in favor of A. germinans. Furthermore, our mesocosm environment precluded additional mechanisms by which S. alterniflora can facilitate A. germinans seedlings (e.g., through positive effects on seedling recruitment; Peterson and Bell 2012). Although the success of A. germinans at a local scale is highly site- and environment-dependent (Guo et al. 2013, Armitage et al. 2015), disturbances such as oil may allow it to establish and dominate in sites where it would otherwise be at a competitive disadvantage. Taken together, these results suggest the continued expansion of A. germinans at the expense of marshes dominated by S. alterniflora within the marsh–mangrove ecotone.

Climate-mediated range expansion of species such as A. germinans is leading to the creation of novel transition communities consisting of co-occurring foundation species. Our results indicate that these mixed communities can mediate the negative effects of disturbance for at least one of the interacting species. As multiple disturbances become more frequent (Buma 2015), complementarity in species responses may be even more likely, which could facilitate species coexistence in the transition zone. However, disturbance may also alter the rate of climate-induced range shifts by impeding or facilitating colonization by expanding species (Moran and Ormond 2015, Liang et al. 2017). Additional work is needed to examine these possibilities, as well as to consider the impacts of these novel transition communities on a range of different ecosystem processes (e.g., ecosystem multifunctionality; Hector and Bagchi 2007). Such information is critical for informing the conservation and restoration in the face of climate change.

ACKNOWLEDGMENTS

We would like to thank the DISL Technical Support and Facilities staff for their help setting up and maintaining the experiment. Staff and students at the Dauphin Island Sea Lab, especially B. Troast and A. Rodriguez, helped with experimental setup, breakdown, and system maintenance. B. Mortazavi and A. Ortmann assisted with the oiling treatment. A. Knott and A. Weiss also assisted with data collection and sample processing. This research was made possible by a grant from The Gulf of Mexico Research Initiative through the Alabama Center for Ecological Resilience Consortium administered by the Dauphin Island Sea Lab to KH, JC, and RH. Data will be publicly available through the Gulf of Mexico Research Initiative Information & Data Cooperative (GRIIDC) at https://data.gulfresearchinitiative.org (https://doi.org/10.7266/n7k93629; https://doi.org/10.7266/N7ZG6QS6). This is contribution 366 from the Northeastern University Marine Science Center.

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**Supporting Information**

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