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Low humidity and hypersalinity reduce cold tolerance in mangroves

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ABSTRACT

Macroclimatic changes are expected to radically alter coastal wetland ecosystems in the coming century. The trajectory of the response to climate warming may differ based on other concomitantly changing abiotic variables such as soil salinity and relative humidity. Thus, understanding plant responses to multiple interacting stressors is required to accurately predict coastal wetland shifts under climate change. The ongoing poleward shift of mangrove range limits has been linked with a reduction in freeze events, yet interactions between low temperature and other abiotic stressors remain underexplored. We grew two common mangroves (*Avicennia germinans* and *Rhizophora mangle*, n = 1222) from propagules for 10 months in environmental growth chambers under experimentally manipulated temperature, salinity, and relative humidity treatments that reflected the range of conditions these species experience in the field. We measured variation in growth and physiological characteristics before, during, and after low temperature exposure. For both species, resistance and resilience to low temperature stress were mediated by salinity and relative humidity conditions. Chronic chilling at 10 °C caused widespread reduction in seedling stem elongation rate, altered leaf gas exchange rates, and increased mortality, particularly under high salinity and low humidity conditions. Additional exposure to an overnight freeze (−4 °C) had relatively minor impacts. Five months after exposure to low temperatures, some *R. mangle* exhibited the capacity to recover from severe cold damage, but only under optimal humidity and salinity conditions. Although *A. germinans* were generally more resistant to low temperature stress, severely damaged plants did not recover, even in low salinity and high humidity conditions. We contend that current and future mangrove range limits are the result of interactions between multiple abiotic stressors including temperature, salinity, and relative humidity. Consequently, future modelling approaches to predicting range shifts under climate change need to consider multiple concomitantly changing abiotic variables and their interactions.

1. Introduction

Macroclimatic changes are expected to radically alter vegetated coastal wetlands in the coming century (Gabler et al., 2017). Ongoing changes in the global distribution and productivity of mangrove and saltmarsh ecosystems have in fact already been linked with anthropogenic climate change (Saintilan et al., 2014; Gedan et al., 2009; Osland et al., 2013; Whitt et al., 2020). Given the importance of coastal wetlands for carbon sequestration, fisheries, mitigating sea-level rise, and other key ecosystem services (Kelleway et al., 2017), predicting climate driven shifts in coastal vegetation has become increasingly necessary (Cavanaugh et al., 2015).

To date, a considerable body of research has assessed the role of climate warming as a driver of macroecological change in coastal wetlands (e.g. Coldren et al., 2019; Noyce et al., 2019; Strain et al., 2017), and the poleward expansion of mangrove forests into temperate saltmarsh habitats has been linked to a reduction of winter chilling (Ross et al., 2009) and freeze events (Saintilan et al., 2014; Cavanaugh et al., 2014). Low temperature as a principal range limiting factor for the global distribution of mangrove ecosystems is well established (Osland et al., 2017a; Lovelock et al., 2016) - it has previously been proposed that global mangrove range limits are generally located at a 20 °C isotherm of winter sea surface temperatures (SST), beyond which conditions become too cold for mangrove trees to persist (Duke et al., 1998). However, the increasing availability of higher resolution and more accurate datasets has revealed a relatively wide range in global temperature thresholds at which mangrove range limits occur (Osland et al., 2016; Cavanaugh et al., 2018; Quisthoudt et al., 2012). For example, the

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mean winter SST at latitudinal range limits of mangroves has been shown to vary from as high as 22 °C in southern Japan to as low as 12.5 °C in eastern Australia (Quisthoudt et al., 2012).

Recent studies have highlighted that temperature-based limits on mangrove distribution interact with other abiotic factors such as rainfall (and associated soil salinity) and relative humidity (Osland et al., 2016; Cavanaugh et al., 2018; Lovelock et al., 2016). Quisthoudt et al. (2012) demonstrated that minimum winter air temperature thresholds associated with widely distributed mangrove genera (*Avicennia* and *Rhizophora*) varies with aridity - wetter/humid regions such as New Zealand and eastern Australia support mangrove forests at average winter air temperatures <8 °C, whereas at arid range limits such as Mauritania and Peru mangroves persist only to winter air temperatures of >20 °C. Yet, although numerous experimental studies have explored how single abiotic stressors alter mangrove growth and physiology (MCMillan and sherrod, 1986; Chen et al., 2017; Pickens and hester, 2011; Cavanaugh et al., 2015; Ball, 1988; Nguyen et al., 2015), and some studies have addressed interactive effects (e.g. Ball and Farquhar, 1984; Ball, 2002; Reef et al., 2015), the additive effects of three or more stressors have rarely been investigated experimentally.

Recent studies on climate driven species range shifts have emphasized the need to consider multiple interacting climate variables such as temperature, precipitation, and relative humidity (Lenoir and Svenning, 2015; Mchenry et al., 2019; Bradie and Leung, 2017). For example, modeling approaches have demonstrated that changing vapor pressure deficit (a function of both air temperature *and* relative humidity and a key determinant of stomatal conductance and water-use efficiency in plants) is more important than temperature alone in causing tree mortality in Australian dry forests (Eamus et al., 2013). For mangroves, although research has addressed the effect of soil salinity on growth and establishment (e.g. Devaney et al., 2020; Ball, 1988; Sobrado, 2005), relatively few studies have experimentally tested the additional impact of varying humidity conditions (but see Ball and Farquhar, 1984; Ball et al., 1997). Consequently, improved understanding of the interactive effects of minimum temperature, humidity, and salinity stress on mangrove growth and development is required to inform predictions of mangrove range shifts.

Experimental studies of mangrove responses to climate-related variables have generally been conducted over relatively short time periods (mostly between 8 and 22 weeks), with little time to monitor recovery following exposure to stress. This is particularly relevant for mangroves which are known to exhibit physiological adaptations for recovery following severe stress events (Snedaker et al., 1992; Chen et al., 2017; Feller et al., 2010; Osland et al., 2015). Thus, accurate predictions of how climate change will alter mangrove distribution globally is further limited by our lack of understanding of species resistance and longer-term resilience to multiple interacting abiotic stressors.

Using the mangroves *Avicennia germinans* and *Rhizophora mangle* as model species, we assessed how resistance and resilience of mangroves to low temperature stress is influenced by relative humidity and salinity conditions. Specifically, we assessed the additive effects of chilling, freezing, salinity, and relative humidity stress on growth and survival of mangrove propagules and young seedlings. *Avicennia* and *Rhizophora* are the most geographically widespread mangrove genera globally, are representative of latitudinal limits of mangroves globally, and possess divergent traits for coping with freeze, aridity, and salinity stress (Cook-Patton et al., 2015, Hayes et al., 2019; Reef and Lovelock, 2014; Hayes et al., 2019, Hayes et al., 2020). We hypothesized that chilling (10 °C) and freezing (−4 °C) stress in mangroves is mediated by concomitant relative humidity and salinity conditions. Using *A. germinans* and *R. mangle* propagules collected in northern Florida, we conducted a 10-month study in controlled environmental growth chambers under manipulated humidity and salinity conditions. We measured variation in seedling growth and physiological characteristics before, during, and after low temperature exposure. Our findings are discussed in the context of shifting global mangrove range limits under climate change.

2. Materials and methods

2.1. Experimental design

In October 2015, we collected propagules of *A. germinans* from multiple parent trees at North Peninsula State Park, Volusia County, Florida, USA (29°25′08″N, 81°06′14″W). Monthly average temperature lows range from 8.5 °C (January) to 23 °C (July), with average highs ranging from 20.2 °C (January) to 32.3 °C (July). Average monthly relative humidity (afternoon) ranges from 53% (April) to 67% (September). Average porewater salinity at the site was 25 and yearly precipitation is approximately 1260 mm. We collected *R. mangle* from multiple parent trees at both Sebastian Inlet State Park (27°51′26″N 80°27′28″W) and Avalon State Park (38 km to the south; 27°32′46″N 80°19′52″W). Average porewater salinity at *R. mangle* sites was 35 and 31, respectively, and yearly precipitation for the region (Vero Beach, 27°39′N 80°21′W) is approximately 1326 mm. Monthly average temperature lows for the region range from 10.2 °C (January) to 22.7 °C (July), with highs ranging from 22.7 °C (January) to 32.5 °C (July). Average monthly relative humidity (afternoon) ranges from 42% (January) to 60% (August). Eastern Florida represents the northernmost continental range limit of both *A. germinans* and *R. mangle*, where distribution is principally limited by low winter temperatures (Cavanaugh et al., 2014; Cook-Patton et al., 2015, Cavanaugh et al., 2015).

Following collection, propagules were placed in plastic bags and transported to the Smithsonian Environmental Research Center, Maryland, USA (38°53′N, 76°33′W). Average fresh mass (±1 S.E.) of *A. germinans* and *R. mangle* propagules was 2.5 ± 0.03 g and 14.14 ± 0.19 g respectively. Propagules (total n = 1222) were placed in individual Ray Leach Cone-tainers (Stuewe and Sons, 2.5 cm diameter, 12.1 cm length, 49 ml volume), using cotton wool to secure them in Cone-tainers. Cone-tainers were placed in holding frames in plastic containers filled with deionized water and assigned to salinity and relative humidity treatments (n = 34 per container, 17 of each species). Using propagules (i.e. prior to shoot and root development) allowed us to investigate the likelihood of mangrove establishment under treatment conditions, rather than first establishing seedlings to a common set of conditions and then testing their ability to adapt to a range of imposed treatments. After one week, we removed two propagules that exhibited evidence of mold damage and excluded them from subsequent analysis (no further mold damage was observed). For salinity treatments, three levels of saline sea water solutions were used; 15 (estuarine), 35 (sea water), and 60 (hypersaline), representing a typical range of field porewater salinities experienced by mangroves (Smith, 1992). Saline solutions were amended by adding Instant Ocean® aquarium salt to the deionized water solution. To minimize effects of differential evaporation, all treatments were maintained at equivalent volume by adding deionized water to coolers when required. Salt concentrations were checked weekly using a conductivity meter and adjusted if necessary. Water solutions were completely changed every four weeks. We replicated treatments by using twelve coolers at each salinity level, placing 17 propagules of each species in each cooler, giving a total of n = 204 for each species in each salinity treatment.

Coolers were evenly distributed among two environmental growth chambers where relative humidity was maintained at either low (42% relative humidity) or high humidity (87% relative humidity) resulting in six coolers (n = 102 propagules per species) in each humidity treatment for each salinity level. Humidity levels were selected to represent approximate minimum and maximum limits of humidity at which *Avicennia* and *Rhizophora* genera occur based on global mangrove distributions (Hamilton and Casey, 2016; GIRI et al., 2011) in conjunction with climate datasets (Kriticos et al., 2012). The positions of coolers within growth chambers were rotated weekly to account for spatial variation in conditions within growth chambers. Light intensity was

maintained at 300 μmol m^{-2} s^{-1} on a 16:8 light:dark cycle throughout, which is approximate to understory light conditions in the field (Devaney et al., 2017). Growth chamber temperature was maintained at 25 °C for three months. At 25 °C, low and high humidity treatments corresponded to leaf-to-air vapor pressure deficits of 0.44 kPA and 2.2 kPA respectively.

Following three months of growth in salinity and relative humidity conditions, we tested plant growth and survival responses to chronic chilling and short-term freeze treatments, similar to conditions at cold mangrove range limits. To chill mangroves, we reduced growth chamber temperatures by 1 °C per day for 14 days until a temperature of 10 °C was reached. Mangroves at the northern edge of the range in Florida (Ft. George Inlet, Jacksonville, 30.41°N, −81.42°W) typically experience 73 ± 1.5 days per year where minimum temperature reaches 10 °C or below (chill days), and 14 ± 0.6 consecutive chill days per year (Fig. S1). The most severe continuous cold spell in the last 100 years occurred in 1940, when average temperatures remained below 10 °C for 21 consecutive days. Thus, to simulate a chronic winter chill event, we exposed plants to 21 days of chilling at 10 °C. Ten °C also represents approximate average air temperatures of the coldest month below which mangroves do not occur based on global datasets (Quisthoudt et al., 2012). After three weeks, we exposed half of the plants to an additional freeze treatment in an unlit growth chamber at −4 °C for 4 h which simulated an overnight freeze event. Control coolers remained in unlit growth chambers at 10 °C throughout. Finally, in order to test long-term resilience, we gradually increased growth chamber temperatures back to 25 °C over a period of two weeks and maintained this temperature for five months, maintaining humidity and salinity at treatment levels throughout (Fig. S2).

2.2. Measurements

After three months of growth in humidity and salinity treatments at 25 °C, we measured stomatal conductance (mol m^{-2} s^{-1}) of the uppermost fully expanded leaf from five established seedlings of each species per cooler per treatment using a diffusion porometer (Leaf Porometer, model SC-1; Decagon Devices Inc., Pullman, WA, USA). To quantify the response of conductance to chilling, we re-measured conductance on the same leaves following 21 days of growth at 10 °C. To assess long-term physiological impact of chronic chilling and freezing, we re-measured the same leaves five months after temperatures had returned to 25 °C. The presence of leaf trichomes can have strong effects on leaf gas exchange (Schuepp, 1993), therefore we recorded the percent cover of trichomes on the abaxial surface of *A. germinans* leaves for all stomatal conductance measurements (*R. mangle* do not have leaf trichomes). To non-destructively quantify growth throughout the experiment, we measured stem elongation (i.e. height from root collar to the highest living apical bud) of all established seedlings after three months in salinity and humidity treatments (n = 816), after 21 days of chilling at 10 °C (n = 842), and five months after temperatures were restored to 25 °C (n = 290).

We assessed freeze-induced damage to photosynthetic function by comparing chlorophyll fluorescence of frozen and non-frozen leaves. The quantum yield of fluorescence (Φ_{II}) has been shown to be a useful metric for detecting freezing injury to leaf tissue and is a common freeze tolerance metric (Cavender-Bares et al., 2005, Pérez et al., 2014; Cook-Patton et al., 2015, Cavanaugh et al., 2015). We measured dark-adapted Φ_{II} of all established seedlings (n = 842) 24 h after freeze treatments with a mini-PAM (Photosynthetic Yield Analyzer, Walz, Germany). The yield of a light-adapted sample is (Fm'-F)/Fm', where F is the fluorescence before a saturation pulse is applied and Fm' is the maximal fluorescence of a light-adapted sample with all photosystem II centers closed (Genty et al., 1989). Measurements were taken in the early morning before the light photoperiod in growth chambers had resumed, therefore plants experienced at least 6 h of dark adaption.

We recorded functional mortality of plants throughout the experiment. We defined functional mortality as seedlings/propagules with no obvious photosynthetic tissue (green leaves or stems) and non-pliable, blackened, dried stems/propagules. Consequently, at the end of the experiment, if propagules did not produce leaves, but still had green, pliable, healthy propagules, these individuals were recorded as alive, but with no stem elongation or biomass accumulation. Because some mangrove species can recover from severe freeze damage (Tomlinson, 2016; Osland et al., 2015), functional mortality did not necessarily indicate absolute mortality; seedlings can re-sprout following loss of all photosynthetic tissue. Consequently, we continued to monitor all plants for recovery for an additional five months after chilling and freeze treatments. We measured the final biomass of all living plants at the end of the experiment (n = 390) as the fresh weight of root tissue and above-ground growth, excluding the mass of the original propagule.

2.3. Analyses

Due to inherent differences in their growth rate and physiology, and because *A. germinans* and *R. mangle* propagules were collected from different locations, we conducted separate analyses for each species. For established seedlings, we first tested the responses of stomatal conductance and stem elongation rates after three months of growth in salinity and humidity treatments using ANOVA, with averaged values per cooler as replicates (n = 6 coolers per salinity*humidity treatment for each species). We also used ANOVA to test treatment differences in pre-versus post-chilling induced proportional changes in stomatal conductance and stem elongation rates of established seedlings after 21 days of growth at 10 °C. A minority (<10%) of sampled leaves of *A. germinans* had no leaf trichomes. Given the potential impact of leaf trichomes on stomatal conductance values (Schuepp, 1993), we only included *A. germinans* seedlings that had leaf trichomes in analyses of stomatal conductance. To test freeze induced damage to photosynthetic function, we tested humidity and salinity treatment related differences in the quantum yield of fluorescence (Φ_{II}) measured 24 h after freeze treatments using ANOVA, again using averaged values per cooler as replicates (n = 3 coolers per salinity*humidity*freeze treatment for each species).

Using generalized linear models (GLMs), we assessed differences in functional mortality of plants in humidity and salinity treatments after three months of growth, using survival per cooler (no. alive/no. dead) as our response variable. Analysis of functional mortality included all 1222 propagules/seedlings, regardless of whether they established roots or shoots. Of 1222 initial propagules, 842 produced shoots and roots (i.e. established seedlings) at some stage during the experiment. A further 101 propagules did not produce shoots and roots but remained green, pliable, and healthy at the end of the experiment and were therefore recorded as alive, but with no stem elongation or biomass accumulation. Two hundred and seventy-eight propagules did not produce shoots or roots and became progressively dry and blackened during the experiment - 85% of these were in the hypersaline treatment and we attributed their mortality to treatment conditions.

To assess differences in functional mortality following exposure to low temperatures, we monitored seedlings daily following chill and freeze treatments and used survival per cooler 16 days post-treatment (when clear chilling and freeze exposure effects stabilized) as our response, and salinity, humidity and freeze as predictor variables in GLMs. Significance of each variable was tested using likelihood-ratio tests of reduced versus full models.

To assess long-term recovery, we used ANOVAs to test differences in proportion change in stomatal conductance and stem elongation rates of seedlings following return to original temperature (25 °C) and relative humidity (42% or 87%) conditions for five months after exposure to chronic chilling and short-term freeze. We also assessed long-term recovery of vitality following exposure to chronic chilling and short-term freeze, defining recovery of vitality as any plant that had been recorded as functionally dead 16 days after exposure to low temperatures but was recorded as living and healthy at the end of the experiment (five months later). Thus, we only included plants that were recorded as functionally

dead post low temperature treatments and assessed survival per cooler (no. alive/no. dead) at the end of the experiment with humidity, salinity, and freeze treatments as our predictor variables in GLMs. Recovery of functionally dead *A. germinans* was negligible (8 out of 356 plants), therefore we only conducted our analysis of recovery for *R. mangle*. As previously, significance of each variable was tested using likelihood-ratio tests of reduced versus full models. Lastly, we tested biomass change (calculated as fresh mass at the end of the experiment – initial propagule mass) of all surviving plants (n = 390) using ANOVA. We again used averaged values per cooler as single replicates (n = 3 coolers per salinity*humidity*freeze treatment for each species). All statistical analyses were conducted using R software (v3.3.2, R Foundation for Statistical Computing).

3. Results

3.1. Initial response to variable humidity and salinity

After three months of growth at 25 °C, no *R. mangle* propagules growing in hypersaline (60) conditions had developed into seedlings. Similarly, no *A. germinans* propagules in combined hypersaline and low humidity treatments developed into seedlings. Measured after three months, stomatal conductance of *A. germinans* and *R. mangle* was 169% and 125% higher in high humidity compared to low humidity, respectively (*A. germinans*; $F_{(1,27)} = 91.51$, $p < 0.001$, *R. mangle*; $F_{(1,27)} = 37.76$, $p < 0.001$, Fig. 1). Conductance did not differ with salinity for either species (*A. germinans*; $F_{(2,27)} = 0.09$, $p = 0.76$, *R. mangle*; $F_{(1,20)} = 1.12$, $p = 0.30$, Fig. 1), although no *R. mangle* propagules in combined high salinity and low humidity treatments produced leaves during the experiment (Fig. 1). After three months of growth in humidity and salinity treatments, lower conductance at low humidity was associated with 30% and 45% slower stem elongation rates in low humidity relative to high humidity in *A. germinans* and *R. mangle*, respectively (*A. germinans*; $F_{(1,32)} = 27.45$, $p < 0.001$, *R. mangle*; $F_{(1,34)} = 82.11$, $p < 0.001$, Fig. 2). During the same period, stem elongation declined with increasing salinity for both species (*A. germinans*; $F_{(2,32)} = 82.36$, $p < 0.001$, *R. mangle*; $F_{(2,32)} = 496.43$, $p < 0.001$, Fig. 2). After three months, functional mortality was higher in low humidity compared to high humidity for both species (*A. germinans*; $\text{deviance}_{(63.06)} = 34.75$, $p < 0.001$, *R. mangle*; $\text{deviance}_{(34.99)} = 19.84$, $p < 0.001$, Fig. 3). Salinity had no effect on mortality of *A. germinans* after three months ($\text{deviance}_{(31.84)} = 3.53$, $p > 0.05$), but mortality of *R. mangle* decreased with increasing salinity ($\text{deviance}_{(22.87)} = 7.72$, $p = 0.005$, Fig. 3).

3.2. Response to low temperature stress under variable humidity and salinity

At low humidity, chilling of air temperatures from 25 °C to 10 °C led to a reduction in VPD from 2.2 kPa to 0.85 kPa, whereas at high humidity, VPD decreased from 0.44 kPa to 0.15 kPa (Fig. S2). Chilling induced reduction in VPD at low humidity resulted in stomatal opening for *A. germinans* and *R. mangle*, with conductance increasing by 79% and 19%, respectively (Fig. 1). In contrast, the chilling-induced reduction of VPD in high humidity corresponded with a 12% decrease in stomatal conductance of *A. germinans* (ANOVA for responses of *A. germinans* stomatal conductance to chilling at different humidity; $F_{(1,25)} = 56.55$, $p < 0.001$, Fig. 1). Similar responses were observed for *R. mangle* in high humidity, with stomatal conductance decreasing 50% relative to pre-chilled conditions (ANOVA for responses of *R. mangle* stomatal conductance to chilling at different humidity; $F_{(1,20)} = 39.19$, $p < 0.001$, Fig. 1). The response of stomatal conductance to chilling did not differ between salinity treatments for either species (*A. germinans*; $F_{(1,25)} = 0.393$, $p = 0.679$, *R. mangle*; $F_{(1,20)} = 0.01$, $p = 0.976$, Fig. 1). Chilling resulted in a decline in stem elongation rates for both species, with larger declines in high humidity treatments for both *A. germinans* ($F_{(1,32)} = 23.59$, $p < 0.001$, Fig. 2) and *R. mangle* ($F_{(1,32)} = 51.80$, $p < 0.001$, Fig. 2). Measured 16 days after exposure to low temperature treatments (when chilling and freeze treatment effects had stabilized), functional mortality of *A. germinans* increased by 52% relative to measurements taken prior to the chilling period (Fig. 3). Chilling-induced functional mortality of *A. germinans* was interactively affected by salinity and humidity treatments ($\text{deviance}_{(101.79)} = 24.44$, $p < 0.001$, Fig. 3) - the largest declines were observed in hypersaline and low humidity conditions (Fig. 3). No differences in functional mortality were observed

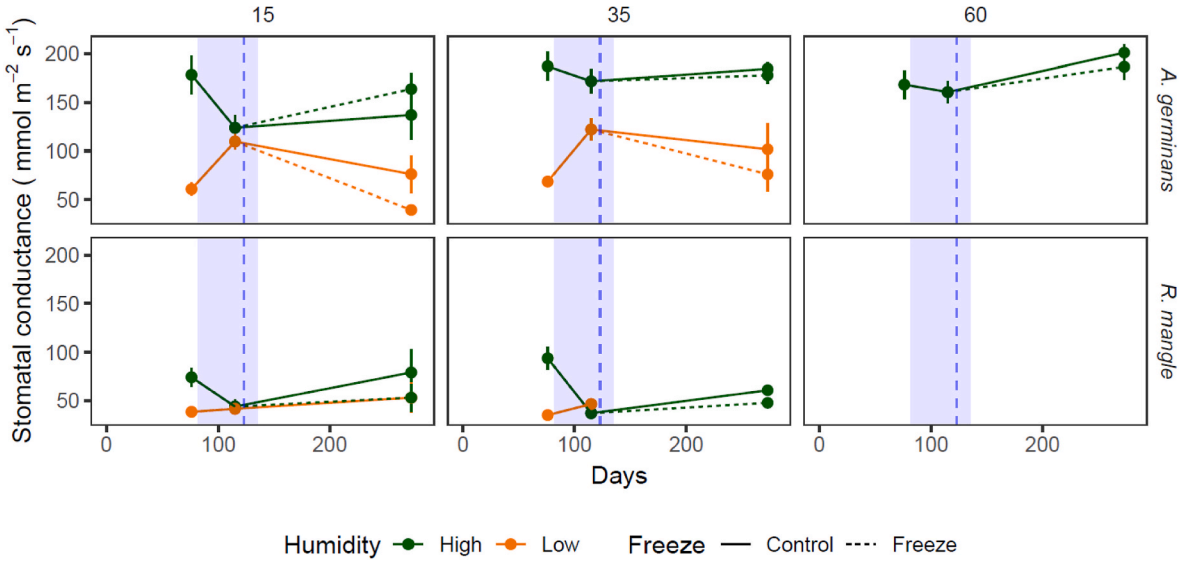

Fig. 1. Changing stomatal conductance (±1 S.E.; mmol m^{-2} s^{-1}) of leaves of *A. germinans* (top panels) and *R. mangle* (bottom panels) seedlings over 273 days in salinity (15, 35, 60) and relative humidity (green = high humidity 87%, orange = low humidity 42%) treatments. The shaded blue area indicates the period when temperatures in experimental growth chambers were reduced from 25 °C to 10 °C and the dashed vertical blue line represents the freeze treatment which half of all seedlings received. Dashed lines represent seedlings that were exposed to an overnight freeze event at −4 °C and solid lines indicate control seedlings. No *R. mangle* propagules growing in hypersaline (60) conditions produced leaves during the experiment, so could not be measured for stomatal conductance. Similarly, no *A. germinans* mangrove propagules in combined hypersaline and low humidity treatments produced leaves during the experiment so could not be measured for stomatal conductance. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

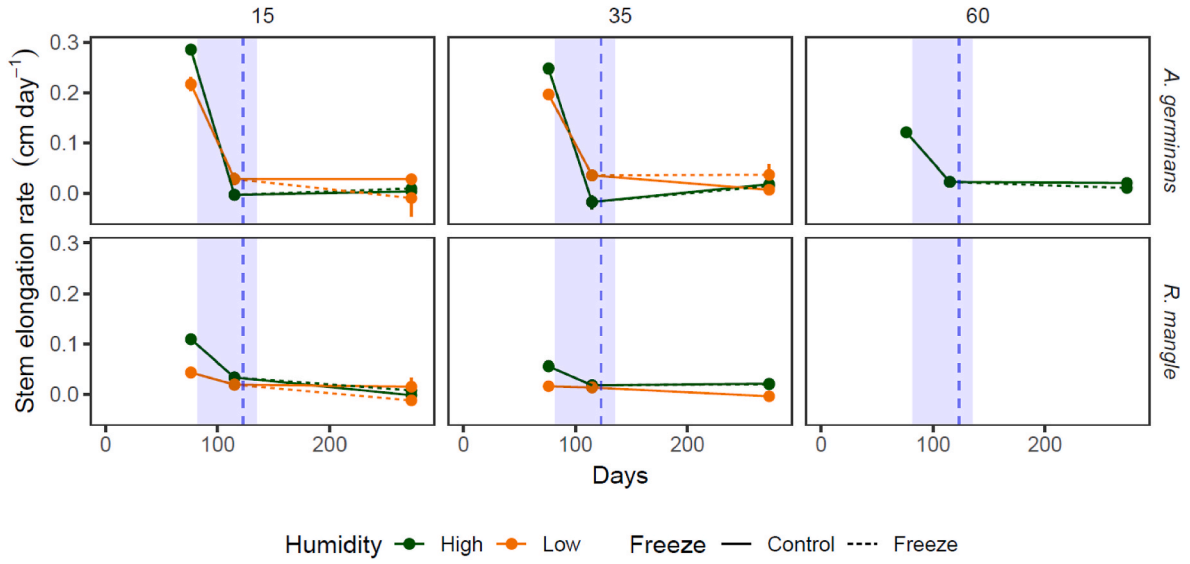

Fig. 2. Relative stem elongation rates (cm day^{-1}) of *A. germinans* (top) and *R. mangle* (bottom) seedlings over 273 days in salinity (15, 35, 60) and relative humidity (green = high humidity 87% humidity, orange = low humidity 42%) treatments. The shaded blue area indicates the period when temperatures in experimental growth chambers were reduced from 25 °C to 10 °C and the dashed vertical blue line represents the freeze treatment which half of all seedlings received. Dashed lines represent seedlings that were exposed to an overnight freeze event at −4 °C and solid lines indicate control seedlings. By the last measurement date (after 273 days) no seedlings in combined hypersaline (60) and low humidity treatments survived. As in Fig. 1, no *R. mangle* propagules growing in hypersaline (60) conditions produced leaves during the experiment. Similarly, no *A. germinans* propagules in combined hypersaline and low humidity treatments produced leaves during the experiment. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

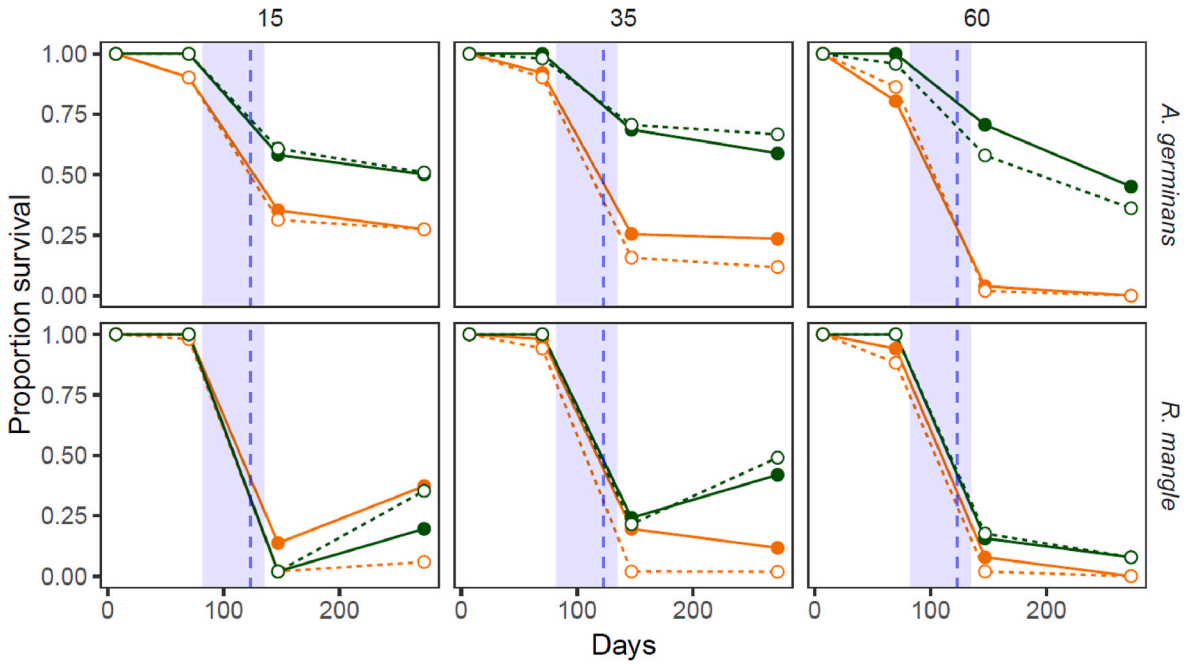

Fig. 3. Proportion survival of *A. germinans* (top) and red (bottom) mangrove plants over 273 days in salinity (15, 35, 60) and relative humidity (green = high humidity 87% humidity, orange = low humidity 42%) treatments. Errors bars are not shown for clarity. The shaded area represents the period when temperatures in experimental growth chambers were reduced from 25 °C to 10 °C and the dashed line represents the freeze treatment which half of all plants received. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

between chilled *A. germinans* and chilled plants that received an additional 8-h freeze at −4 °C (deviance$_{(101.79)}$ = 1.28, p = 0.256, Fig. 3). However, the quantum yield of fluorescence in *A. germinans* mangroves exposed to −4 °C was reduced relative to plants that did not receive an overnight freeze, indicating freeze induced reduction of photosynthetic efficiency ($F_{(1,25)}$ = 5.41, p = 0.028, Fig. 4). For *R. mangle*, chilling also led to widespread functional mortality (88% of healthy pre-chill plants, Fig. 4). Functional mortality of *R. mangle* exposed to −4 °C for 8 h was higher compared to plants not exposed to sub-zero temperatures (deviance$_{(66.62}$ = 5.78, p = 0.016, Fig. 3). As with *A. germinans*, the quantum yield of fluorescence was reduced in *R. mangle* exposed to −4 °C relative to plants in the no-freeze treatment ($F_{(1,20)}$ = 9.43, p = 0.006, Fig. 4).

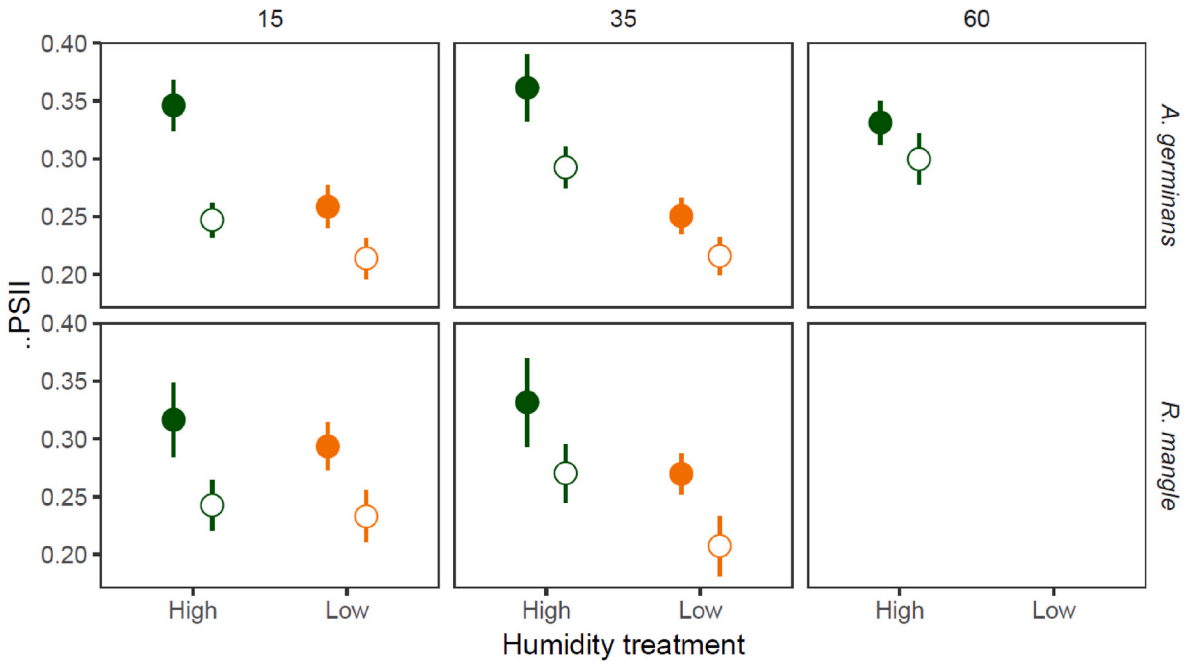

Fig. 4. Quantum yield of fluorescence (±1 S.E, ΦII) of chilled only (filled circles) and chilled and frozen (unfilled circles) *A. germinans* (top) and *R. mangle* (bottom) seedlings in salinity (15, 35, 60) and relative humidity (green = high humidity 87% humidity, orange = low humidity 42%) treatments. No *A. germinans* mangrove propagules in combined hypersaline and low humidity treatments produced leaves during the experiment. Similarly, no *R. mangle* propagules in hypersaline (60) conditions produced leaves during the experiment. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3.3. Recovery from low temperature stress

After exposure to chilling and freezing temperatures, plants were returned to their initial treatment conditions at 25 °C for a further five months (Fig. S2). Stomatal conductance of both species recovered to pre-low temperature treatment levels, with no significant differences across salinity or freeze treatments (Fig. 1). For both species, stem elongation rates did not recover after growth chamber conditions were restored from 10 °C to 25 °C (Fig. 2). Returning temperatures to 25 °C (with original humidity treatment conditions maintained throughout) did not influence overall functional mortality of *A. germinans* (Fig. 3). In contrast, 13% of *R. mangle* plants recorded as functionally dead (i.e.,

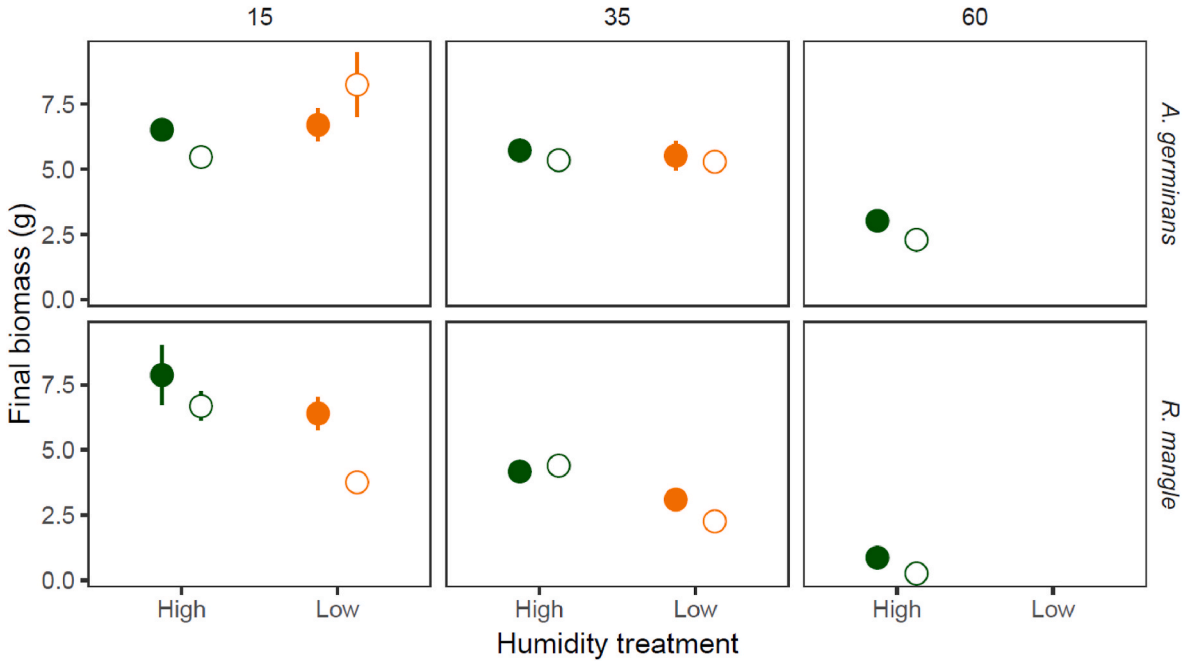

Fig. 5. Final fresh biomass (±1 S.E, g) of chilled only (filled circles) and chilled plus frozen (unfilled circles) *A. germinans* (top) and *R. mangle* (bottom) plants in salinity (15, 35, 60) and relative humidity (green = high humidity 87% humidity, orange = low humidity 42%) treatments. For both species, no propagules in combined hypersaline and low humidity treatments survived until the end of the experiment. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

having lost all photosynthetic tissue either through severe browning or loss of leaves, and having non-pliable stems) following chronic chilling had recovered after five months in 25 °C (Fig. 3). Effects of humidity, salinity, and freeze treatments on *R. mangle* recovery following chilling were interactive (deviance$_{(85.81)} = 23.01$, $p < 0.001$, Fig. 3). In 15 and 35 salinity treatments, 20% of *R. mangle* recovered from severe chilling damage at high humidity, whereas at low humidity, only non-frozen plants in low salinity (15) exhibited recovery (Fig. 3). No plants in hypersaline treatments recovered from chilling damage (Fig. 3).

Final biomass declined with increasing salinity for both *A. germinans* ($F_{(1,24)} = 39.86$, $p < 0.001$, Fig. 5) and *R. mangle* ($F_{(1,25)} = 70.79$, $p < 0.001$, Fig. 5). For *R. mangle*, final biomass was also affected by humidity ($F_{(1,25)} = 15.45$, $p < 0.001$), freeze ($F_{(1,25)} = 4.25$, $p = 0.04$), and their interaction ($F_{(1,16)} = 9.87$, $p = 0.006$, Fig. 5), with plants growing in low humidity and exposed to −4 °C having lower biomass compared to plants growing at high humidity and not exposed to freezing temperatures.

4. Discussion

We found that resistance and resilience to low temperature in two widely distributed mangrove species *Avicennia germinans* and *Rhizophora mangle* is mediated by salinity and relative humidity. Chronic chilling at 10 °C resulted in widespread reduction in growth rate and mortality in both species, with additional short-term exposure to sub-zero temperatures resulting in lower biomass of *R. mangle* but not *A. germinans*. Under combined hypersaline and low humidity conditions, prolonged exposure to 10 °C resulted in almost 100% mortality of both species. In contrast, under combined low salinity and high relative humidity, chilling resulted in <50% mortality of *A. germinans* but did result in widespread mortality of the less cold tolerant *R. mangle*. Few previous studies have explored the additive effects of three or more stressors, yet, our results suggest that mangrove growth and survival are strongly influenced by interactive effects of temperature, salinity, and relative humidity. Consequently, future modelling approaches to predicting range shifts under climate change need to consider multiple concomitantly changing abiotic variables and their interactions.

4.1. Mangrove responses to low temperature stress

Tolerance to low air temperatures, particularly freeze events, is hypothesized to be a key determinant of the poleward extent of mangroves globally (Stuart et al., 2007; Cook-Patton et al., 2015, Osland et al., 2020). In our study, *A. germinans* plants were more resistant to low temperatures than *R. mangle*. One limitation of our study however is that interpreting interspecific differences in freeze tolerance is difficult given that *A. germinans* and *R. mangle* were collected at different latitudes (29°N and 27°N respectively), which may have conferred differential adaptation to low temperatures - latitudinal variation in cold tolerance has previously been demonstrated for mangroves in Florida (Cook--Patton et al., 2015). Nevertheless, greater cold tolerance of *A. germinans* follows previously documented patterns in experimental studies (Cook-Patton et al., 2015, Cavanaugh et al., 2015; Coldren and Proffitt, 2017) and reflects the global distribution of both genera (Quisthoudt et al., 2012). Quantum yield of fluorescence following exposure to freezing temperatures was reduced in both species. However, differences in final biomass and survival between “freeze” and “no freeze” seedlings were only apparent for *R. mangle*. Differential freeze tolerance between these species has previously been attributed to a suite of adaptive traits such as smaller, drier leaves, and smaller xylem diameter in *A. germinans* compared to *R. mangle* (Cook-Patton et al., 2015, Stuart et al., 2007). Interestingly, the additive effects of freezing were relatively minor compared to the widespread mortality of mangrove seedlings induced by exposure to chronic chilling at 10 °C. For many sub-tropical plants, exposure to temperatures below 12 °C can lead to water loss, wilting and declines in growth due to a range of chill sensitive mechanisms (Allen and ORT, 2001). In mangroves, both MCMillan and Sherrod (1986) and Ross et al. (2009) documented physiological dysfunction and chilling injury to North American mangrove species following exposure to temperatures of 2–3 °C. Similarly, a study of mangrove response to chilling stress in southern China reported that 35% of *A. marina* seedlings died following exposure to 5 °C for five days (Peng et al., 2015). Previous studies have also highlighted the importance of duration of low temperature exposure on mangrove injury and recovery (Pickens and Hester, 2011). Kao et al. (2004) showed that exposure to chilling temperatures (15 °C) for 10 days led to reduced light-saturated rates of photosynthesis, potential quantum yields, and total leaf chlorophyll concentration in *A. marina* seedlings in Taiwan. Although such extended periods of low temperature stress are rare at range limits, our study is the first to show that prolonged chilling, even at temperatures as high as 10 °C, can have significant impacts on growth and survival in common mangrove species.

4.2. Low temperature stress is mediated by salinity and humidity

Although mangrove species exhibit a range of adaptations for coping with growth in saline substrates (Reef and Lovelock, 2014), the drought-like symptoms of cold stress (e.g., reduced hydraulic conductivity and xylem embolism [Stuart et al., 2007, Madrid et al., 2014]) can be exacerbated by high sodium content in the soil because water acquisition is more difficult and ions (mainly Na+ and Cl−) can accumulate at toxic concentrations in plant tissues (Reef et al., 2015). To overcome the combined effects of cold temperature and salinity stress, some mangroves have narrow xylem vessel diameters, thereby reducing embolism risk. These narrow xylem vessels, however, also reduce hydraulic conductivity capacity. This may contribute to lower carbon fixation capacity in mangroves from relatively arid and cold environments which may result in dwarf stature of mangroves in such regions (Madrid et al., 2014; Dahdouh-guebas and Koedam, 2001; Ezcurra et al., 2016; Almahasheer et al., 2017). In our study, while effects of salinity on stomatal conductance were relatively minor, increasing salinity did result in reduced biomass and survival of plants, suggesting that the negative impacts of increasing salinity may be more related to ion toxicity than to gas exchange, as has been found previously for *A. germinans* (Reef et al., 2015).

At high humidity, lowering growth room temperatures from 25 °C to 10 °C resulted in a reduction in VPD from 0.44 kPa to 0.15 kPa. This was associated with a decrease in conductance across both species and all salinity treatments. Many plant species actively close stomata during chilling to conserve water (Melkonian et al., 2004) - this can be attributed to a direct effect of low temperature on guard cell function or an indirect effect caused by a chill-induced reduction of Rubisco activity. During periods of high VPD, most plant species (including mangroves) close stomata to slow transpiration and conserve water, thereby decreasing CO_2 supply and reducing photosynthetic rates and productivity (Merilo et al., 2018; Yuan et al., 2019; Ball and Farquhar, 1984). At low humidity in our experiment, a chill-induced decrease in VPD from 2.2 kPa to 0.85 kPa resulted in stomatal opening, which corresponded to greater mortality rates relative to high humidity, particularly for *A. germinans*. Thus, for plants growing in already water-stressed conditions where evaporative demand is high, a reduction in VPD may result in maladaptive stomatal opening. Indeed, many tropical and sub-tropical species lack the ability to respond normally to chill-induced leaf water deficit, with stomata appearing locked open for extended periods resulting in subsequent water loss (Allen and Ort, 2001). We speculate that low water temperature and associated low root hydraulic conductance (Allen and Ort, 2001) coupled with relatively high evaporative demand may have driven water loss and subsequent mortality following chilling at low humidity (Figs. 1 and 3). Our findings suggest a potential mechanistic basis for observations that arid mangrove range limits also correspond to higher minimum sea surface temperatures (Quisthoudt et al., 2012). At range limits controlled by winter

temperatures, cold SSTs may result in low hydraulic conductivity and water intake, but simultaneous low relative VPD may allow mangroves to maintain water balance and persist at winter SSTs of 8 °C or even less. In contrast, at range limits where minimum SSTs are much lower than minimum air temperatures (i.e. relatively cold SSTs coupled with high VPD - e.g. Mauritania, Peru [Quisthoudt et al., 2012]), root hydraulic conductivity may be too low to keep up with the high evaporative demand, resulting in water loss and mortality.

4.3. Mangrove recovery following abiotic stress

Mangroves are known to exhibit physiological adaptations for recovery following severe stress events (Snedaker et al., 1992; Chen et al., 2017; Feller et al., 2010; Osland et al., 2015). Five months after chilling and freeze treatments, 13% of *R. mangle* plants recorded as having lost all photosynthetic tissue (either through severe browning of leaves or loss of leaves altogether) and having non-pliable stems (i.e. functional mortality) had recovered vitality. Although resistance to low temperature stress was much higher for *A. germinans* (52% functional mortality compared to 88% in R. mangle), for *A. germinans* plants that did exhibit severe temperature induced damage, recovery was negligible. Interspecific differences in long-term resilience may have an ontogenetic component, however, as recovery of *Avicennia* has been reported at older life-stages (Snedaker et al., 1992). Osland et al. (2015) documented widespread stem and basal resprouting of mature *A. germinans* five months after a severe freeze damage event. Although *Avicennia* was more resistant to low temperature damage in our study, our results suggest that *Rhizophora* may be more resilient to low temperature stress than previously considered, at least at the seedling stage. We focused on the seedling stage given its importance in the context of mangrove establishment and range shifts under climate change, however, more research is required to understand how resistance and resilience of mangroves to abiotic stress varies across different life-stages.

4.4. Implications for mangrove responses to climate change

As with the overall decline in freeze events in Florida (Cavanaugh et al., 2014; Devaney et al., 2017), the number of consecutive "chill" days (when temperatures drop below 10 °C) is also decreasing (Fig. S1). The chill-induced changes in stomatal conductance, growth rates, and widespread mortality in both species suggests that a reduction in the intensity and duration of prolonged chilling associated with climate warming may further accelerate the rate of poleward expansion in mangrove range limits. Additionally, warming will also likely impact ecosystem structure and functioning - Feher et al. (2017) suggest that warming at temperature-controlled mangrove range limits will likely lead to increased canopy height and aboveground biomass. However, our data indicates that the rate at which these changes occur will be strongly related to simultaneous changes to relative humidity and precipitation regimes.

More broadly, it has been proposed that low temperatures coupled with low rainfall and/or high salinity is limiting poleward expansion of mangroves in a number of regions (Osland et al., 2017b). We found that while salinity strongly affected mangrove productivity, long-term survival of seedlings was more influenced by changes in VPD. As a component of aridity, relative humidity may play at least as an important role as salinity in altering mangrove range limits in arid regions. Thus, it is important to consider potential changes to both temperature and relative humidity (which together determine VPD) in future predictions of mangrove range shifts under climate change (Grossiord et al., 2020). Moreover, other global change factors such as increasing atmospheric CO_2 (McKee and Rooth, 2008), nitrogen enrichment (Dangremond et al., 2019), sea-level-rise (Ellison, 2015), and the increasing frequency of extreme weather events such as hurricanes and droughts (Feher et al., 2020; Mafi-Gholami et al., 2020) have all been shown to influence mangrove growth and distribution, adding further complexity to predictions of range shifts under climate change. For example, negative impacts of increasing VPD may be offset by increased water-use efficiency under elevated atmospheric CO_2 (Lovelock et al., 2016), although evidence on how this may influence range expansion is lacking. Further still, the impact of changing climate variables on mangrove distribution will also be mediated by biotic factors, most notably competitive interactions with marsh grasses at mangrove-saltmarsh ecotones (McKee and Rooth, 2008; Saintilan et al., 2014). Indeed, Pickens et al. (2019) demonstrated that while mangrove seedling growth was reduced when grown under intact saltmarsh grass canopies, the canopies also created microclimates that alleviated low temperature damage, indicating a possible positive effect of saltmarsh vegetation on mangrove range expansion. Thus, future modelling approaches to predicting mangrove range shifts under climate change need to consider multiple concomitantly changing abiotic and biotic variables and their interactions.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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References

Cavender Bares, J., Cortes, P., Rambal, S., Joffre, R., Miles, B., Rocheteau, A., 2005. Summer and winter sensitivity of leaves and xylem to minimum freezing temperatures: a comparison of co-occurring Mediterranean oaks that differ in leaf lifespan. New Phytol. 168, 597–612.

Cook Patton, S.C., Lehmann, M., Parker, J.D., 2015. Convergence of three mangrove species towards freeze-tolerant phenotypes at an expanding range edge. Funct. Ecol. 29, 1332–1340.

Allen, D.J., Ort, D.R., 2001. Impacts of chilling temperatures on photosynthesis in warm-climate plants. Trends Plant Sci. 6, 36–42.

Almahasheer, H., Serrano, O., Duarte, C.M., Arias-Ortiz, A., Masque, P., Irigoien, X., 2017. Low carbon sink capacity of red sea mangroves. Sci. Rep. 7, 9700.

Ball, M.C., 1988. Salinity tolerance in the mangroves Aegiceras corniculatum and Avicennia marina. I. Water use in relation to growth, carbon partitioning, and salt balance. Funct. Plant Biol. 15, 447–464.

Ball, M.C., 2002. Interactive effects of salinity and irradiance on growth: implications for mangrove forest structure along salinity gradients. Trees (Berl.) 16, 126–139.

Ball, M.C., Farquhar, G.D., 1984. Photosynthetic and stomatal responses of two mangrove species, Aegiceras corniculatum and Avicennia marina, to long term salinity and humidity conditions. Plant Physiol. 74, 1–6.

Ball, M.C., Cochrane, M.J., Rawson, H.M., 1997. Growth and water use of the mangroves Rhizophora apiculata and R. stylosa in response to salinity and humidity under ambient and elevated concentrations of atmospheric CO2. Plant Cell Environ. 20 (9), 1158–1166.

Bradie, J., Leung, B., 2017. A quantitative synthesis of the importance of variables used in MaxEnt species distribution models. J. Biogeogr. 44, 1344–1361.

Cavanaugh, K.C., Kellner, J.R., Forde, A.J., Gruner, D.S., Parker, J.D., Rodriguez, W., Feller, I.C., 2014. Poleward expansion of mangroves is a threshold response to decreased frequency of extreme cold events. Proc. Natl. Acad. Sci. Unit. States Am. 111, 723–727.

Cavanaugh, K.C., Parker, J.D., Cook-Patton, S.C., Feller, I.C., Williams, A.P., Kellner, J.R., 2015. Integrating physiological threshold experiments with climate modeling to project mangrove species' range expansion. Global Change Biol. 21, 1928–1938.

Cavanaugh, K.C., Osland, M.J., Bardou, R., Hinojosa-Arango, G., López-Vivas, J.M., Parker, J.D., Rovai, A.S., 2018. Sensitivity of mangrove range limits to climate variability. Global Ecol. Biogeogr. 27, 925–935.
Chen, L., Wang, W., Li, Q.Q., Zhang, Y., Yang, S., Osland, M.J., Huang, J., Peng, C., 2017. Mangrove species' responses to winter air temperature extremes in China. Ecosphere 8, e01865.
Coldren, G.A., Proffitt, C.E., 2017. Mangrove seedling freeze tolerance depends on salt marsh presence, species, salinity, and age. Hydrobiologia 803, 159–171.
Coldren, G.A., Langley, J.A., Feller, I.C., Chapman, S.K., 2019. Warming accelerates mangrove expansion and surface elevation gain in a subtropical wetland. J. Ecol. 107, 79–90.
Dahdouh-Guebas, F., Koedam, N., 2001. Are the northernmost mangroves of west africa viable?–a case study in banc d'Arguin national Park, Mauritania. Hydrobiologia 458, 241–253.
Dangremond, E.M., Simpson, L.T., Osborne, T.Z., Feller, I.C., 2019. Nitrogen enrichment accelerates mangrove range expansion in the temperate–tropical ecotone. Ecosystems 23, 703–714.
Devaney, J.L., Lehmann, M., Feller, I.C., Parker, J.D., 2017. Mangrove microclimates alter seedling dynamics at the range edge. Ecology 98, 2513–2520.
Devaney, J.L., Marone, D., Mcelwain, J.C., 2020. Impact of soil salinity on mangrove restoration in a semi-arid region: a case study from the Saloum Delta, Senegal. Restor. Ecol. https://doi.org/10.1111/rec.13186.
Duke, N., Ball, M., Ellison, J., 1998. Factors influencing biodiversity and distributional gradients in mangroves. Global Ecol. Biogeogr. Lett. 7, 27–47.
Eamus, D., Boulain, N., Cleverly, J., Breshears, D.D., 2013. Global change-type drought-induced tree mortality: vapor pressure deficit is more important than temperature per se in causing decline in tree health. Ecology and evolution 3, 2711–2729.
Ellison, J.C., 2015. Vulnerability assessment of mangroves to climate change and sea-level rise impacts. Wetl. Ecol. Manag. 23 (2), 115–137.
Ezcurra, P., Ezcurra, E., Garcillán, P.P., Costa, M.T., Aburto-Oropeza, O., 2016. Coastal landforms and accumulation of mangrove peat increase carbon sequestration and storage. Proc. Natl. Acad. Sci. Unit. States Am. 113, 4404–4409.
Feher, L.C., Osland, M.J., Anderson, G.H., Vervaeke, W.C., Krauss, K.W., Whelan, K.R.T., Balentine, K.M., Tiling-Range, G., Smith III, T.J., Cahoon, D.R., 2020. The long-term effects of hurricanes Wilma and Irma on soil elevation change in Everglades mangrove forests. Ecosystems 23 (5), 917–931.
Feher, L.C., Osland, M.J., Griffith, K.T., Grace, J.B., Howard, R.J., Stagg, C.L., Enwright, N.M., Krauss, K.W., Gabler, C.A., Day, R.H., Rogers, K., 2017. Linear and nonlinear effects of temperature and precipitation on ecosystem properties in tidal saline wetlands. Ecosphere 8 (10), e01956.
Feller, I.C., Lovelock, C.E., Berger, U., Mckee, K.L., Joye, S.B., Ball, M., 2010. Biocomplexity in mangrove ecosystems. Annual review of marine science 2, 395–417.
Gabler, C.A., Osland, M.J., Grace, J.B., Stagg, C.L., Day, R.H., Hartley, S.B., Enwright, N. M., From, A.S., Mccoy, M.L., Mcleod, J.L., 2017. Macroclimatic change expected to transform coastal wetland ecosystems this century. Nat. Clim. Change 7, 142.
Gedan, K.B., Silliman, B., Bertness, M., 2009. Centuries of human-driven change in salt marsh ecosystems. Annu. Rev. Mar. Sci 1, 117–141.
Genty, B., Briantais, J.-M., Baker, N.R., 1989. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochim. Biophys. Acta Gen. Subj. 990, 87–92.
Giri, C., Ochieng, E., Tieszen, L.L., Zhu, Z., Singh, A., Loveland, T., Masek, J., Duke, N., 2011. Status and distribution of mangrove forests of the world using earth observation satellite data. Global Ecol. Biogeogr. 20, 154–159.
Grossiord, C., Buckley, T.N., Cernusak, L.A., Novick, K.A., Poulter, B., Siegwolf, R.T., Sperry, J.S., Mcdowell, N.G., 2020. Plant responses to rising vapor pressure deficit. New Phytol. https://doi.org/10.1111/nph.16485.
Hamilton, S.E., Casey, D., 2016. Creation of a high spatio-temporal resolution global database of continuous mangrove forest cover for the 21st century (CGMFC-21). Global Ecol. Biogeogr. 25, 729–738.
Hayes, M.A., Shor, A.C., Jesse, A., Miller, C., Kennedy, J.P., Feller, I., 2019. The role of glycine betaine in range expansions; protecting mangroves against extreme freeze events. J. Ecol.
Hayes, M.A., Chapman, S., Jesse, A., O'Brien, E., Langley, J.A., Bardou, R., Devaney, J., Parker, J.D., Cavanaugh, K.C., 2020. Foliar water uptake by coastal wetland plants: a novel water acquisition mechanism in arid and humid subtropical mangroves. J. Ecol.
Kao, W.-Y., Shih, C.-N., Tsai, T.-T., 2004. Sensitivity to chilling temperatures and distribution differ in the mangrove species Kandelia candel and Avicennia marina. Tree Physiol. 24, 859–864.
Kelleway, J.J., Cavanaugh, K., Rogers, K., Feller, I.C., Ens, E., Doughty, C., Saintilan, N., 2017. Review of the ecosystem service implications of mangrove encroachment into salt marshes. Global Change Biol. 23, 3967–3983.
Kriticos, D.J., Webber, B.L., Leriche, A., Ota, N., Macadam, I., Bathols, J., Scott, J.K., 2012. CliMond: global high-resolution historical and future scenario climate surfaces for bioclimatic modelling. Methods in Ecology and Evolution 3, 53–64.
Lenoir, J., Svenning, J.C., 2015. Climate-related range shifts–a global multidimensional synthesis and new research directions. Ecography 38, 15–28.
Lovelock, C.E., Krauss, K.W., Osland, M.J., Reef, R., Ball, M.C., 2016. The Physiology of Mangrove Trees with Changing Climate. Tropical Tree Physiology. Springer.
Madrid, E.N., Armitage, A.R., López-Portillo, J., 2014. Avicennia germinans (black mangrove) vessel architecture is linked to chilling and salinity tolerance in the Gulf of Mexico. Front. Plant Sci. 5, 503.
Mafi-Gholami, D., Zenner, E.K., Jaafari, A., 2020. Mangrove regional feedback to sea level rise and drought intensity at the end of the 21st century. Ecol. Indicat. 110, 105972.
Mchenry, J., Welch, H., Lester, S.E., Saba, V., 2019. Projecting marine species range shifts from only temperature can mask climate vulnerability. Global Change Biology.
McKEE, K.L., Rooth, J.E., 2008. Where temperate meets tropical: multi-factorial effects of elevated CO2, nitrogen enrichment, and competition on a mangrove-salt marsh community. Global Change Biol. 14 (5), 971–984.
Mcmillan, C., Sherrod, C.L., 1986. The Chilling Tolerance of Black Mangrove, Avicennia Germinans, from the Gulf of Mexico Coast of Texas. Louisiana and Florida.
Melkonian, J., Yu, L.-X., Setter, T.L., 2004. Chilling responses of maize (Zea mays L.) seedlings: root hydraulic conductance, abscisic acid, and stomatal conductance. J. Exp. Bot. 55, 1751–1760.
Merilo, E., Yarmolinsky, D., Jalakas, P., Parik, H., Tulva, I., Rasulov, B., Kilk, K., Kollist, H., 2018. Stomatal VPD response: there is more to the story than ABA. Plant Physiol. 176, 851–864.
Nguyen, H.T., Stanton, D.E., Schmitz, N., Farquhar, G.D., Ball, M.C., 2015. Growth responses of the mangrove Avicennia marina to salinity: development and function of shoot hydraulic systems require saline conditions. Ann. Bot. 115, 397–407.
Noyce, G.L., Kirwan, M.L., Rich, R.L., Megonigal, J.P., 2019. Asynchronous nitrogen supply and demand produce nonlinear plant allocation responses to warming and elevated CO2. Proc. Natl. Acad. Sci. Unit. States Am. 116 (43), 21623–21628.
Osland, M.J., Enwright, N., Day, R.H., Doyle, T.W., 2013. Winter climate change and coastal wetland foundation species: salt marshes vs. mangrove forests in the southeastern United States. Global Change Biol. 19, 1482–1494.
Osland, M.J., Day, R.H., From, A.S., Mccoy, M.L., Mcleod, J.L., Kelleway, J.J., 2015. Life stage influences the resistance and resilience of black mangrove forests to winter climate extremes. Ecosphere 6, 1–15.
Osland, M.J., Feher, L.C., Griffith, K.T., Cavanaugh, K.C., Enwright, N.M., Day, R.H., Stagg, C.L., Krauss, K.W., Howard, R.J., Grace, J.B., 2016. Climatic controls on the global distribution, abundance, and species richness of mangrove forests. Ecol. Monogr.
Osland, M.J., Day, R.H., Hall, C.T., Brumfield, M.D., Dugas, J.L., Jones, W.R., 2017a. Mangrove expansion and contraction at a poleward range limit: climate extremes and land-ocean temperature gradients. Ecology 98, 125–137.
Osland, M.J., Feher, L.C., Griffith, K.T., Cavanaugh, K.C., Enwright, N.M., Day, R.H., Stagg, C.L., Krauss, K.W., Howard, R.J., Grace, J.B., 2017b. Climatic controls on the global distribution, abundance, and species richness of mangrove forests. Ecol. Monogr. 87, 341–359.
Osland, M.J., Day, R.H., Hall, C.T., Feher, L.C., Armitage, A.R., Cebrian, J., Dunton, K.H., Hughes, A.R., Kaplan, D.A., Langston, A.K., Macy, A., 2020. Temperature thresholds for black mangrove (Avicennia germinans) freeze damage, mortality and recovery in North America: refining tipping points for range expansion in a warming climate. J. Ecol. 108 (2), 654–665.
Peng, Y.-L., Wang, Y.-S., Fei, J., Sun, C.-C., Cheng, H., 2015. Ecophysiological differences between three mangrove seedlings (Kandelia obovata, Aegiceras corniculatum, and Avicennia marina) exposed to chilling stress. Ecotoxicology 24, 1722–1732.
Pérez, F., Hinojosa, L.F., Ossa, C.G., Campano, F., Orrego, F., 2014. Decoupled evolution of foliar freezing resistance, temperature niche and morphological leaf traits in Chilean Myrceugenia. J. Ecol. 102, 972–980.
Pickens, C.N., Hester, M.W., 2011. Temperature tolerance of early life history stages of black mangrove Avicennia germinans: implications for range expansion. Estuar. Coast 34, 824–830.
Pickens, C.N., Sloey, T.M., Hester, M.W., 2019. Influence of salt marsh canopy on black mangrove (Avicennia germinans) survival and establishment at its northern latitudinal limit. Hydrobiologia 826 (1), 195–208.
Quisthoudt, K., Schmitz, N., Randin, C.F., Dahdouh-Guebas, F., Robert, E.M., Koedam, N., 2012. Temperature variation among mangrove latitudinal range limits worldwide. Trees (Berl.) 26, 1919–1931.
Reef, R., Lovelock, C.E., 2014. Regulation of water balance in mangroves. Ann. Bot. 115, 385–395.
Reef, R., Winter, K., Morales, J., Adame, M.F., Reef, D.L., Lovelock, C.E., 2015. The effect of atmospheric carbon dioxide concentrations on the performance of the mangrove Avicennia germinans over a range of salinities. Physiol. Plantarum 154, 358–368.
Ross, M.S., Ruiz, P.L., Sah, J.P., Hanan, E.J., 2009. Chilling damage in a changing climate in coastal landscapes of the subtropical zone: a case study from south Florida. Global Change Biol. 15, 1817–1832.
Saintilan, N., Wilson, N.C., Rogers, K., Rajkaran, A., Krauss, K.W., 2014. Mangrove expansion and salt marsh decline at mangrove poleward limits. Global Change Biol. 20, 147–157.
Schuepp, P., 1993. Tansley review No. 59. Leaf boundary layers. New Phytol. 477–507.
Smith, T.J., 1992. Forest Structure. Tropical mangrove ecosystems, 41. American Geophysical Union, pp. 101–136.
Snedaker, S.C., Brown, M.S., Lahmann, E.J., Araujo, R.J., 1992. Recovery of a mixed-species mangrove forest in south Florida following canopy removal. J. Coast Res. 919–925.
Sobrado, M., 2005. Leaf characteristics and gas exchange of the mangrove Laguncularia racemosa as affected by salinity. Photosynthetica 43, 217–221.
Strain, E.M., Van Belzen, J., Comandini, P., Wong, J., Bouma, T.J., Airoldi, L., 2017. The role of changing climate in driving the shift from perennial grasses to annual succulents in a Mediterranean saltmarsh. J. Ecol. 105 (5), 1374–1385.
Stuart, S.A., Choat, B., Martin, K.C., Holbrook, N.M., Ball, M.C., 2007. The role of freezing in setting the latitudinal limits of mangrove forests. New Phytol. 173, 576–583.

Tomlinson, P.B., 2016. The Botany of Mangroves. Cambridge University Press.
Whitt, A.A., Coleman, R., Lovelock, C.E., Gillies, C., Ierodiaconou, D., Liyanapathirana, M., Macreadie, P.I., 2020. March of the mangroves: drivers of encroachment into southern temperate saltmarsh. Estuar. Coast Shelf Sci., 106776
Yuan, W., Zheng, Y., Piao, S., Ciais, P., Lombardozzi, D., Wang, Y., Ryu, Y., Chen, G., Dong, W., Hu, Z., 2019. Increased atmospheric vapor pressure deficit reduces global vegetation growth. Science advances 5, eaax1396.