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LETTER

Non-freezing cold event stresses can cause significant damage to mangrove seedlings: assessing the role of warming and nitrogen enrichment in a mesocosm study

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Abstract

Mangroves are expanding poleward along coastlines globally as a response to rising temperatures and reduced incidence of freezing under climate change. Yet, knowledge of mangrove responses to infrequent cold events in the context of climate warming and regional nitrogen (N)-enriched eutrophication is limited. We conducted a mesocosm experiment in which the seedlings of two mangrove species (A. marina and B. gymnorrhiza) were grown either at ambient temperature or under warming with and without nitrogen (N) loading. During a short winter period, an unusually severe cold event occurred with the lowest temperature of 2 °C in the experimental region. We took this unique opportunity to assess possible response of these mangrove species to the cold stress under various environmental conditions. The cold event caused various degrees of damage to the seedlings of both mangrove species, with the warming treatment seemingly protecting leaves and branches from the cold damage. However, the warming treatment did not buffer mangroves to mortality from the low temperature stress in either species. The cold event resulted in significant decreases in seedling growth rates and net ecosystem CO2 uptake in the post-cold period relative to the pre-cold period, although the cold event did not alter the effects of warming treatment on these parameters of both mangrove species. The cold event differentially altered physiological responses of the two species growing under N loading, with the seedlings of A. marina growing in higher N concentrations having a reduced growth response after the cold event, whereas those of B. gymnorrhiza showed no change in post-cold period versus pre-cold period growth. Our results suggest that cold events may play a pivotal role in regulating mangrove survival and growth even under future warming scenarios. Two mangrove species exhibited differential survival and growth responses to the cold event at different N concentrations, which may have implications for how we can restore and conserve mangroves among the world’s eutrophied sub-tropical estuaries and with future warming.

1. Introduction

With the intensification of climate change, increasing concerns have been raised for understanding and modeling ecosystem structure and function under future climate scenarios, especially increasing temperatures

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and changes in precipitation regimes (e.g., Weltzin et al 2003, Tylianakis et al 2008, Smith et al 2009). While, climatic feedbacks of ecosystems often go beyond gradual shifts in modal conditions because of the present of variations in climatic extremes (e.g., freezing, drought, flooding) (Jentsch et al 2007, IPCC 2013, Bailey and van de Pol 2016). Therefore, elucidating ecological implications of changes in the frequency and intensity of climatic extremes is vital for our better understanding the ecological consequences of changing climatic conditions.

Growing in the narrow inter-tidal zone of tropical, subtropical and warm temperate coastlines, mangroves are a favorable ecosystem for investigating trait-driven responses to climate change (Cook-Patton et al 2015). In response to recent climate change, the range of mangroves is gradually expanding poleward across the globe (Saintilan et al 2014, Hickey et al 2017, Coldren et al 2019), which may have significant consequences (e.g., soil carbon storage) for these new mangrove-occupied regions (Comeaux et al 2012, Doughty et al 2016). However, mangroves are highly susceptible to temperature variations as they oscillate their distributions into warm temperature regions (Tomlinson 1986, Pickens and Hester 2011). For example, Cavanaugh et al (2015) investigated the historic range of mangroves on the east coast of Florida, USA, and suggested that extreme cold temperature controls the distribution of mangroves in these coastal regions. Therefore, low air temperatures associated with extreme cold events are recognized as a determinant factor constraining the survival, distribution and growth of mangroves at latitudinal extremes globally (Soares et al 2012, Cavanaugh et al 2014). Accordingly, understanding how mangroves respond to cold events, not necessarily low enough to cause freeze damage, has implications for both restoration and conservation under climate change scenarios.

While improving, our current understanding of mangrove responses to the frequency, duration and intensity of extreme cold events is still limited because of the relatively uncommon and unpredictable nature of cold events (Osland et al 2019), and the difficulty in replicating the exact conditions of specific interest using natural experiments (Pickens and Hester 2011, Cook-Patton et al 2015), though limited associated studies have been conducted by using remote sensing images and in situ surveys (Cavanaugh et al 2014, Chen et al 2017). Furthermore, warmer temperatures globally have decreased the intensity and frequency of ‘extreme’ cold events in warm temperate and subtropical regions, which may have contributed to the range expansion of mangrove species through enhanced physiological performance (Cavanaugh et al 2014, Osland et al 2016). However, whether persistent climate warming can alter the response of mangroves to infrequent cold events remains unknown.

As one of the most pressing environmental concerns, eutrophication caused by excessive nitrogen (N) input have increased in coastal regions worldwide as a direct consequence of human activities, particularly in China and many Southeast Asian countries (Valiela et al 2001, Cao et al 2011). Considering that mangroves are typically adapted to lower nutrient environments where they occur naturally (Reef et al 2016), the periodic input of excessive N would affect growth strategies of mangroves such as changing seedling morphology, increasing leaf growth and decreasing root biomass allocation, and therefore affect the survival, growth and competition of mangroves there (LoveLock et al 2009, Simpson et al 2013, Cui et al 2017). For example, Feller et al (2007) found that N addition shifted the grow strategies of A. germinans trees by increasing investment in wood relative to leaf biomass and stem length relative to lateral growth, and thereby promoted A. germinans trees grow out of their stunted form caused by nutrient deficiency. As such, we surmise that excessive N loading into coastal regions may change the physical and physiological responses of mangroves to cold stresses, but our current knowledge of this potential influence is limited to individual studies on freezing compared with separate studies on N loading, none of which were conducted in combination. In terrestrial ecosystems, many studies have examined the relationship between N deposition and damage incurred from cold stresses, but no uniform conclusions have been drawn, which could be associated with the duration of N treatment and plant species (Power et al 1998, Vankoughnett and Henry 2014).

We conducted a 3-year mesocosm experiment in March 2015 to study the effects of climate warming and regional N loading on mangrove wetlands. An extreme cold event occurred during the period of 20–28 January 2016, during which the air temperature at our experimental site quickly dropped as low as 2 °C for up to 9 h. We conducted an intensive survey of cold damage to the seedlings of these mangrove species within the mesocosms in the context of both climate warming and N loading. Our objectives were (1) to assess the possible responses of mangrove seedlings to the cold stress, (2) to study the responses of seedling survival and growth with increasing temperature and N loading, and (3) to evaluate the differential responses between the two mangrove species.

2. Materials and methods

2.1. Experimental design

The mesocosm experiment was carried out at the Graduate School at Shenzhen, Tsinghua University, Shenzhen, China (22°59′N, 113°97′ E). Avicennia marina and Bruguiera gymnorrhiza were chosen because they are the pioneer mangrove species and the late succession mangrove species, respectively, and are the widely distributed
true mangrove species of southern China. One-year-old seedlings of *A. marina* and *B. gymnorrhiza* were acquired from a field nursery managed by the Gaoqiao Mangrove Natural Reserve (21°20′N, 103°03′E), just to the south of our mesocosm experiment. Seedlings represent collections from multiple parent trees. Healthy *A. marina* and *B. gymnorrhiza* seedlings approximately 25 cm in height with usually 5–8 green leaves (no cotyledons exist) were selected for this study. The seedlings of the two mangrove species were arranged in a large cement tank (2.60 m × 1.10 m × 0.50 m in volume), with half of each individual tank planted with *A. marina* and half planted with *B. gymnorrhiza*. There were 25 seedlings of *A. marina* and 25 seedlings of *B. gymnorrhiza* in each tank. Each seedling covered an area of 0.25 m × 0.25 m, which mimics typical seedling densities of these two species according to our field observations. A total of 16 cement tanks were used as experimental mesocosms. The soil in each mesocosm was derived from sugarcane plantations near the Gaoqiao Mangrove Natural Reserve. After stones, benthic animals and plant residues in the soil were carefully sampled and removed, the soil was then used to fill each mesocosm to a depth of 30 cm.

The experiment was conducted as a split-split-plot design with four blocks, which also served as true experimental replication (N = 4) arranged to account for environmental variability among the 16-tank arrangement. Each block consisted of two whole plots differing in temperature—warming (W*) and an ambient control (W0). Each of the whole-plots was split into excessive nitrogen (N+) and ambient (N0), and within each of those split-plots, the experiment was further split between the two species. The combination of treatments resulted in the following four treatments: (1) No warming + no N loading (W0N0 or CK), (2) Warming + no N loading (W+N0 or W0), (3) No warming + N loading (W+N+ or N+), and (4) Warming + N loading (W+N+).

To simulate natural tidal circulation, we constructed two seawater reservoirs with one reservoir connected to the N+ mesocosms and the other connected to all the N0 mesocosms. The simulated tidal system consisted of a water pump, water meter, and water inlet and outlet pipe network system (figure S1). The high tide was set to occur at the same time every day between 14:00 and 20:00 of local time (6 h per 24-h period), simulating the diurnal tidal cycle of the middle intertidal location of many mangrove forests in southern China. The seawater rose gradually and the water table maintained at +5 cm above the soil surface during high tide period. For the N loading treatment, 229 g NH4Cl and 91 g NaNO3 were added to the N+ reservoir which contained 3000 l of artificial seawater to make a concentration of 20 mg l−1 NH4+ and 5 mg l−1 NO3−, respectively. The concentrations of NH4+ and NO3− in the seawater we applied were within the range of concentrations in estuarine waters with severe eutrophication in southeastern China for which we were simulating (Wu et al. 2014). The artificial seawater was prepared by dissolving natural sea salts in tap water and had a salinity of 15 g l−1, which represented an average salinity in a typical mangrove environment of southeastern China. Because the seawater may be lost by evaporation and evapotranspiration, which may increase the salinity of remaining seawater. The artificial seawater in the two reservoirs was used for 1 week, then drained and replenished by freshly prepared artificial seawater throughout the experiment.

The warming treatment was carried out on 1 March 2015, and was achieved by infrared heaters (55 cm length, 1 cm width, Kalgoor Electronics Inc., Bethlehem, USA). Two infrared heaters were suspended in parallel at 1 m above the treatment tanks, and were set to produce a 3 °C increment in the air temperature near the canopy top of an associated warming plot compare to a control plot (continuously day and night). In each W0 mesocosm, one ‘dummy’ heater with the same shape, size and height of the functional heater was installed to simulate the shading effects of the heating elements identically as for W+. During the experimental period, the in situ increased air temperature in the warming plot often varied because of the heat loss due to such as wind and rain (figure 1(a)). Warming significantly increased air temperature during the experimental period, with the air temperature near the canopy top was increased by about 2.4–2.6 °C compared to the control (Yang et al. 2018). As desired, N loading did not change air temperature during the study period (P = 0.40).

### 2.2. Cold damage analysis

During the period of 20–28 January 2016, an extreme cold event associated with a cold front (current) from the north affected southeastern China. The air temperature quickly dropped, with the lowest mean hourly temperature of 2 °C on 25 January (figure 1(b)). Warming increased the air temperature near the canopy top of 2.1 °C and 2.0 °C in W+ mesocosm and W+N+ mesocosm, respectively, compared to the CK mesocosm during this period (figure S2). We conducted an intensive survey one week after the cold event and assessed cold damage of the two mangrove species (figure S4). Furthermore, to evaluate the degree of cold damage of seedlings, we divided the cold damaged seedlings into four levels based on apparent stress impact from this event according to Chen et al (2017) and Wang et al (2011): Level 1: seedling was not affected; Level 2: leaf scorched and/or abnormal defoliated; Level 3: branch wilted; Level 4: seedling dead or dying. The evaluation criteria for seedling dying was that all the leaves of the seedling fall off or wither, and there was no new shoots grow out within a month after the cold event.
2.3. Plant growth and net ecosystem exchange of CO2 measurements

To monitor the seedling growth of the two mangrove species, we measured stem height and basal diameter (at 1 mm above the soil surface in A. marina and 1 mm above the hypocotyl in B. gymnorrhiza) of all seedlings of the two mangrove species by using a caliper from the first month after plantation, and then repeated measurements at 3 month intervals. Seedlings that appeared to be dead at each measurement time were excluded from the growth analyses. In addition, to analyze if mangrove growth performances were affected by the cold event, we divided the experiment into two periods: pre-cold period and post-cold period. The changes in seedling height and basal diameter of the two mangrove species during each experiment period were determined by calculating relative growth rate (RGR) with the formulation: \[ \text{RGR} = \frac{\ln h_2 - \ln h_1}{t_2 - t_1}, \] where \( \ln \) is the natural logarithm, \( h \) is the growth variable at times 1 and 2 for each mesocosm, and \( t_2 \) and \( t_1 \) are the first sampling time and the last sampling time during each experiment period, respectively.

Net ecosystem exchange (NEE) of CO2 was conducted monthly from November 2015 to April 2016. All measurements were conducted at 09:30–11:30 local time. NEE was measured using a closed static chamber method (Weston et al 2014). The chamber was 60 cm × 60 cm at the base and 70 cm in height, which can cover four seedlings during one measurement. Chamber walls were constructed of clear polyethylene, which allows 90% of photosynthetically active radiation (PAR) to pass through. During the measurement, the chamber was sealed to the surface of an aluminum frame, which was inserted into the soil to a depth of about 3 cm at the center of each plot to prevent gas loss and ensure a closed system during sampling. CO2 gas exchange was measured using a Li-Cor 6400 portable photosynthesis system (LiCor Inc., Lincoln, NE, US) attached directly to the chamber. Consecutive recordings of CO2 concentrations at full PAR were taken during a 90-s period after steady state conditions were achieved within the chamber for 20 s. By convention, net CO2 uptake by the ecosystem is reported as negative NEE values.

2.4. Statistical analyses

A split–split plot analysis of variance (ANOVA) was used to analyze effects on seedling cold damage, relative growth rate of height, relative growth rate of basal diameter and NEE. Whole plots in a randomized complete block design were used, with warming as the whole plot factor, N loading as the subplot factor, and species and time (pre-cold period versus post-cold period) as the sub-subplot factor. All statistical analyzes were performed with the General Linear Models. The normality of data was confirmed with Shapiro-Wilk normality test (\( P > 0.05 \)), the homogeneity of variance with Levene’s test (\( P > 0.05 \)). Seedling cold damage data were transformed with an inverse sine conversion. All statistical analyses were carried out using SPSS 16.0 for Windows (SPSS, Chicago, Illinois, USA).
3. Results and discussion

3.1. The cold event significantly affected seedling survival and growth of mangroves

The cold event dramatically affected the seedlings of the two mangrove species and resulted in 88% of *A. marina* seedlings (figure 2(a)) and 30% of *B. gymnorrhiza* seedlings (figure 2(e)) damaged during this period in the control plots. This result confirms that the seedlings of these two mangrove species are highly sensitive to cold stress in this region. The low temperature during a cold event period can induce lesions in biomembranes that interrupt energy and material supply to cells, as well as tissue injuries such as vascular embolism, dehydration, and/or cellular rupture (Larcher 2001, Krauss et al 2008), which could contribute to the leaf and branch injury as well as seedling sudden death in mangroves during cold periods of various intensity (Urli et al 2013, Cavanaugh et al 2014). Similarly, researchers have studied the impacts of extreme cold events on mangroves in the field and documented that cold events could result in large areas of mangrove damage and mortality along coastlines such as in USA (Ross et al 2009, Osland et al 2013) and China (Kao et al 2004, Chen et al 2017). The lowest temperature during the cold period experienced by mangrove seedlings was 2.0 °C, which suggests that non-freeze cold events can cause great damage to mangrove seedlings.

*A. marina* and *B. gymnorrhiza*, two widely abundant native mangrove species in the subtropical coastal regions in China, were identified to have a relatively high cold resistance (Chen et al 2017). However, the responses of the two species were significantly different, with *A. marina* having a higher mortality ratio than *B. gymnorrhiza*. *A. marina* seedlings suffered various forms of damage from the cold event, including leaf scorch...
and/or defoliation, branch wilt and plant mortality (figures 2(b)–(d)); by comparison the cold event only resulted in leaf injury in the form of wilting for *B. gymnorrhiza* seedlings in the control plots (figures 2(f)–(h)). The different cold responses may be attributed to differential cold adaptation strategies of the two mangrove species as expressed by their physical (i.e., leaf and xylem tissue structure) (Stuart et al 2007) and physiological (i.e., osmolality) (Koštal et al 2011) traits. Alternatively, the two species may differ in phenotypic plasticity and/or genetic differences, such as the observation that the hypocotyl of *B. gymnorrhiza* have larger energy reserves than those rooted seedlings of *A. marina* (Krauss et al 2008), which could have protected them against cold damage.

Figure 3. Changes in height and basal diameter of two mangrove species under different treatments. (a) and (b) depict seeding stem height; and (c) and (d) depict seeding basal diameter. The dash lines indicate the time when the cold event occurred. Data are shown as mean ± 1se (n = 4).

The cold event significantly affected seedling growth dynamics for both mangrove species (figure 3 and table 1). The *A. marina* seedlings showed a rapid height increase during the pre-cold period, while the cold event significantly inhibited seedling height growth, resulting in a small RGR during the post-cold period (figures 3(a), 4(a) and (b)). By comparison, the seedling height of *B. gymnorrhiza* steadily increased during both periods (figures 3(b), 4(c) and (d)). The seedling basal diameter of *A. marina* increased during both periods (figures 3(c), 5(a) and (b)), whereas the basal diameter of *B. gymnorrhiza* exhibited different dynamics in pre- and post-cold period, with a positive RGR during the pre-cold period and a negative RGR for the post-cold period (figures 3(d), 5(c) and (d)). Alongi (2011) demonstrated that intrinsic growth traits such as leaf photosynthetic rates and photosynthetic allocation to different tissues in the early growth of mangrove can lead to different growth responses to environmental changes among species. In this study, the cold event resulted in serious seedling damage to *A. marina*, particularly for leaves and branch tips. We speculate that *A. marina* seedlings may subsequently have changed the growth strategies and have invested relatively more energy in root production for tolerance (Chapin 1991, Carroll et al 2010). While, because the cold event led to a relatively small damage to *B. gymmrhiza* seedlings, which might have favored the fast growth and increase in seedling height during the post-cold period. Therefore, when attempting to understand the impacts of cold events on mangrove growth, researchers should account for the species-specific traits.

Large negative values of NEE occurred for the two experimental mangrove systems suggesting a high CO2 uptake capacity of both systems during the pre-cold period (figure 6). In addition, we found that the proportion of seedlings suffering from leaf injury was significantly correlated to changes in NEE (figure 7), suggesting a mechanism of reduced CO2 uptake for the two mangrove systems during the post-cold period was dramatically reduced leaf photosynthetic rates, and indicating that cold stress can affect the capacity of mangroves to store C if repetitive events occur.
3.2. Warming played a limited role in protecting mangroves against the cold stress

By increasing the leaf surface temperature, warming can decrease injury to biomembranes and prevent plant tissues from experiencing vascular embolism (Larcher 2001), thereby dramatically alleviating the negative effect of the cold event on mangrove seedlings. As expected, warming protected A. marina seedlings from leaf and branch damage during the cold period, leading to the proportion of A. marina seedlings that suffered from leaf and branch injury during the cold period significantly lower than in the control (figures 2(b)–(c)). While, warming did not reduce A. marina seedling mortality during the cold event (figure 2(d)), highlighting that mild warming could have played a limited role in protecting mangroves against the cold stress. Similar results have been reported in terrestrial ecosystems (e.g. Augspurger 2009, Richardson et al. 2018). For instance, by investigating the plant frost damage caused by an extreme cold event in the spring of 2007 in USA, Gu et al. (2008) warned that even under constant warming condition due to climate change, plants are still at risk of serious damage from sudden temperature extremes.

Table 1. Results (P-values) of the analysis of variance of warming (W), N (nitrogen) addition, and time (pre-cold event period and the post cold event period, T) effect on seedling height, basal diameter and net ecosystem carbon exchange (NEE) of the two mangrove species. The relative growth rate of seedling height and basal diameter during the pre- and post-cold event period were used for the ANOVA analysis.

![Figure 4. Mean values of relative growth rate (RGR) in height of two mangrove species during the pre-cold period (a) and (c) and the post-cold period (b) and (d) as modified by warming and N loading. Data are shown as mean ± 1se (n = 4).](image-url)
We found warming had no effect on seedling height growth but significantly inhibited seedling basal diameter growth for both species during the pre-cold period (figures 4 and 5). The cold event did not affect the response of seedling height growth to warming, but changed the warming response to basal diameter growth, resulting in an increased rate of basal diameter growth for the two mangrove species with warming (table 1 and figure 5). Because stem diameter has been shown to be a good indicator of the growth and stand biomass of a tree (Rosell and Olson 2007), the cold-induced change in allocation to lateral growth compared to vertical growth may have implications for the morphology of surviving trees once they become mature.

Figure 5. Mean values of relative growth rate (RGR) in basal diameter of the two mangrove species during the pre-cold period (a) and (c) and the post-cold period (b) and (d) under different treatments. Data are shown as mean ± 1se (n = 4).

Figure 6. Net ecosystem CO₂ exchange (NEE) changes before and after the cold event. The dash lines indicate the time when the cold event occurred. Data are shown as mean ± 1se (n = 4).
Before the cold event, warming significantly increased CO₂ uptake (more negative NEE) of the *A. marina* system but had no effect on NEE of the *B. gymnorrhiza* system (figure S5). The different responses of the two mangrove species could be attributed to differences in their ability to quickly implement physiological and morphological changes to adapt to the warmer environment (Krauss et al. 2008). Though the cold event did not change the warming response of NEE, there was a marginal effect (\( P = 0.053 \)) of warming on NEE of *B. gymnorrhiza*. Because warming significantly protected mangrove seedlings from leaf damage during the cold period, the two mangrove species should have had a higher photosynthetic rate and thereby a higher NEE in warming condition compared to control plots.

### 3.3. The cold event changed the growth responses of mangroves to N loading

Generally, plants exposed to high levels of nutrient availability have greater susceptibility to environmental stressors such as drought and cold because they invest less in roots, which are often required in order to tolerate stress in general (Chapin 1991, Carroll et al. 2010). In this study, N loading had no significant effect on the three levels of cold damage (figures 2(b)–(d)) for *A. marina* seedlings, though there was a marginal effect on seedling mortality that was visual more than statistical (\( P = 0.062 \)). While, N loading significantly increased the number of *B. gymnorrhiza* seedlings that suffered from leaf injury, but N loading had no effect on branch damage and seedling mortality during the cold period in both warming and ambient conditions (figures 2(f)–(h)). Therefore, the limited role of N loading in regulating the resistance of mangrove seedlings to cold stresses in this study might be attributed to the reason that N loading might not have changed the ratio of root and aboveground production before the cold event. Similarly, Naidoo (1987) demonstrated that three levels of N loading did not change the shoot/root ratio of *Avicennia marina* seedlings at high salinity. Additionally, species specificity of mangroves would also be apparent in their responses to N loading because of their different early physiological ability (Alongi 2011).

Before the cold event, N loading significantly increased seedling height growth of the two mangrove species (figures 4(a), (b)), which supported previous findings that N loading can increase the emergent growth of young mangrove seedlings because N is a principal limiting nutrient to growth for many mangroves (Reef et al. 2016, Hayes et al. 2017). Surprisingly, the cold event affected the growth responses of mangroves to N loading and resulted in different seedling response patterns of the two mangrove species. During the post-cold period, N loading had no effect on height growth (figure 4(c)) but significantly increased basal diameter growth of *A. marina* seedlings (figure 5(c)); by comparison *B. gymnorrhiza* seedlings showed a large increase in height (figure 4(d)) but exhibited no changes in basal diameter (figure 5(d)). Previous studies have demonstrated that the interaction between N loading and cold damage exhibited high geographic and species variability in terrestrial ecosystems (Power et al. 1998, Vankoughnett and Henry 2014). The different response patterns between the two mangrove species after the cold event in this study may be attributed to the following two reasons. First, the cold event resulted in more serious seedling damage to *A. marina* relative to *B. gymnorrhiza*. The loss of healthy leaves from *A. marina* seedlings after the cold event may have decreased plant photosynthetic capacity, an observation that is partly supported by the lack of an NEE response in *A. marina* to N loading during
the post-cold period (figure S5). Second, the cold stress could have changed the growth strategies of *A. marina* seedlings under N loading, which subsequently may have invested relatively more energy in root production for tolerance (Lovelock et al 2004), resulting in a significant increase in basal diameter observed in this study. In addition, the different NEE response of the two mangrove species to N loading after the cold event suggests that the effect of N-enriched eutrophication on C exchange in mangroves can be modified by cold event stresses.

4. Conclusions and implications

In this study, we for the first time studied the impact of a cold event on mangrove survival and growth in the context of warming and N-based eutrophication using a mesocosm experiment. We found that the cold stress, in lieu of freezing, causes various types of damage to mangrove seedlings. Warming strongly protected mangrove seedlings from leaf and branch damage during the cold period, but not from seedling mortality. Although warming has allowed mangroves to expand poleward around the globe, our results from this mesocosm study suggest that occasional cold events, sometimes above freezing, may slow the progression of mangrove dominance of these coastal wetlands. Furthermore, N loading had no significant effect on cold damage of these mangrove species, but the responses of mangrove seedling growth to N loading were affected by the cold event. These two mangrove species exhibited seemingly large differences in cold response and growth performances. Nowadays, many countries including China have made great effects in mangrove afforestation during last few decades. Our results might shed some light on how we can govern and protect the restored mangrove forests under the influences of cold stresses with global and regional environmental changes. In addition, in terrestrial ecosystems, previous studies suggested that resistance of forest trees to cold damage was age-dependent (Lafon 2004, Zhu et al 2015), but the responses exhibited high geographic variability, which were also species-specific. In this study, we focused only on mangrove seedlings, whether mature mangroves showed similar cold sensitivity under both warming and N loading conditions remains unclear, thus research on cold stress responses of mangroves should focus on all age types in the future. This study conducted the warming experiment using mesocosm systems, so caution is also suggested when applying our conclusion in the field because of the occurrence of artificial conditions caused by mesocosm experiments (Carpenter 1996).

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References

Alongi D M 2011 Early growth responses of mangroves to different rates of nitrogen and phosphorus supply J. Exp. Mar. Biol. Ecol. 397 85–93

Augspurger C K 2009 Spring 2007 warmth and frost: phenology, damage and refoliation in a temperate deciduous forest Funct. Ecol. 23 1031–9

Bailey L D and van de Pol M 2016 Tackling extremes: challenges for ecological and evolutionary research on extreme climatic events J. Anim. Ecol. 85 85–96

Cao L, Diana J S, Keoleian G A and Lai Q 2011 Life cycle assessment of chinese shrimp farming systems targeted for export and domestic sales Environ. Sci. Technol. 45 6531–8

Carpenter S R 1996 Microcosm experiments have limited relevance for community and ecosystem ecology Ecology. 77 677–80

Carroll J A, Caporn S J M, Cowley L, Read D J and Lee J A 2010 The effect of increased deposition of atmospheric nitrogen on Calluna vulgaris in upland Britain New Phytol. 144 423–31

Cavanaugh K C et al 2014 Poleward expansion of mangroves is a threshold response to decreased frequency of extreme cold events PNAS 111 723–7

Cavanaugh K C et al 2015 Integrating physiological threshold experiments with climate modeling to project mangrove species’ range expansion Global Chang. Biol. 21 1928–38

Chapin F S 1991 Integrated responses of plants to stress: a centralized system of physiological responses Bioscience 41 29–36

Chen L et al 2017 Mangrove species’ responses to winter air temperature extremes in China Ecosphere 8 e01865
Coldren G A, Langley J A, Feller I C and Chapman A K 2019 Warming accelerates mangrove expansion and surface elevation gain in a subtropical wetland J. Ecol. 107 79–90

Comeaux R S, Allison M A and Bianchi T S 2012 Mangrove expansion in the gulf of mexico with climate change: implications for wetland health and resistance to rising sea levels Estuar. Coast. Shelf Sci. 96 81–95

Cook-Patton S C, Lehmann M and Parker J D 2015 Convergence of three mangrove species towards freeze-tolerant phenotypes at an expanding range edge Funct. Ecol. 29 1332–40

Cui X et al 2017 Increased nitrogen input enhances Kandelia obovata seedling growth in the presence of invasive Spartina alterniflora in subtropical regions of China Biol. Letters. 13 20160760

Doughty C L et al 2016 Mangrove range expansion rapidly increases coastal wetland carbon storage Estuar. Coast. 39 1–12

Feller I C et al 2007 Nutrient addition differentially affects ecological processes of Avicennia germinans in nitrogen versus phosphorus limited mangrove ecosystems Ecosystems. 10 347–59

Gu L et al 2008 The 2007 Eastern US spring freeze: Increased cold damage in a warming world Bioscience 58 253–62

Hayes M A et al 2017 The contrasting effects of nutrient enrichment on growth, biomass allocation and decomposition of plant tissue in coastal wetlands Plant Soil. 416 193–204

Hickey S M et al 2017 Is climate change shifting the poleward limit of mangroves? Estuar. Coast. 40 1–12

IPCC 2013 Summary for policymakers

Jentsch A et al 2007 A new generation of climate–change experiments: events, not trends Front. Ecol. Environ. 5 365–74

Jones S F, Stagg C L, Krauss K W and Hester M W 2018 Flooding alters plant-mediated carbon cycling independently of elevated atmospheric CO2 concentrations J. Geophys. Res.-Biogeosci. 123 1976–87

Kao W Y et al 2004 Sensitivity to chilling temperatures and distribution differ in the mangrove species Kandelia candel and Avicennia marina Tree Physiol. 24 859–64

Koizumi M et al 2011 Physiological and biochemical analysis of overwintering and cold tolerance in two Central European populations of the spruce bark beetle. Ips typographus. J. Insect Physiol. 57 1136–46

Krauss K W et al 2008 Environmental drivers in mangrove establishment and early development: A review Aquat. Bot. 89 105–27

Lafon C W 2004 Ice–storm disturbance and long-term forest dynamics in the Adirondack Mountains J. Veg. Sci. 15 267–76

Larcher W 2001 Physiological Plant Ecology (Berlin: Springer)

Lovelock C E, Ball M C, Martin K C and Feller I C 2004 The effect of nutrient enrichment on growth, photosynthesis and hydraulic conductance of dwarf mangroves in Panama Funct. Ecol. 18 25–33

Naidee G 1987 Effects of salinity and nitrogen on growth and water relations in the mangrove, Avicennia marina (Forsk.) Vierh New Phytol. 107 317–25

Osaland M J et al 2016 Beyond just sea-level rise: considering macroclimatic drivers within coastal wetland vulnerability assessments to climate change Global Chang. Biol. 22 1–11

Osaland M J et al 2019 Microclimate influences mangrove freeze damage: Implications for range expansion in response to changing macroclimate Estuar. Coast. 42 1–13

Osaland M J, Enwright N, Day R H and Doyle T W 2013 Winter climate change and coastal wetland foundation species: salt Marshes vs. mangrove forests in the southeastern United States Global Chang. Biol. 19 1482–94

Pickens C N and Hester M W 2011 Temperature tolerance of early life stages of black mangrove Avicennia germinans: Implications for range expansion Estuar. Coast. 34 824–39

Powell S A et al 1998 Effects of nitrogen addition on the stress sensitivity of Calluna vulgaris New Phytol. 138 663–73

Reef R et al 2016 The effects of CO2 and nutrient fertilisation on the growth and temperature response of the mangrove, Avicennia germinans Photosynth. Res. 129 159–70

Reef R, Feller I C and Lovelock C E 2010 Nutrition of mangroves Tree Physiol. 30 1148–60

Richardson A D et al 2018 Ecosystem warming extends vegetation activity but heightens vulnerability to cold temperatures Nature 560 368–71

Rosell J A and Olson M E 2007 Testing implicit assumptions regarding the age vs. size dependence of stem biomechanics using Pittocaulon Senecio (Asteraceae) Am. J. Bot. 94 161–72

Ross M S et al 2009 Chilling damage in a changing climate in coastal landscapes of the subtropical zone: a case study from south Florida Global Chang. Biol. 15 1817–32

Saintilan N, Wilson N C, Rogers K, Rajkaran A and Krauss K W 2014 Mangrove expansion and salt Marsh decline at mangrove poleward limits Global Chang. Biol. 20 147–57

Simpson L T et al 2013 Effects of competition and nutrient enrichment on Avicennia germinans in the salt Marsh–mangrove ecotone Aquat. Bot. 104 55–9

Smith M D et al 2009 A framework for assessing ecosystem dynamics in response to chronic resource alterations induced by global change Ecology. 90 3278–89

Soares M L G, Estrada G C D, Fernandez V and Tognella M M P 2012 Southern limit of the Western South Atlantic mangroves: Assessment of the potential effects of global warming from a biogeographical perspective Estuar. Coast. Shelf Sci. 101 44–53

Stuart S A, Choat B, Martin K C, Holbrook N M and Ball M C 2007 The role of freezing in setting the latitudinal limits of mangrove forests New Phytol. 173 576–83

Tomlinson P B 1986 The Botany of Mangroves (Cambridge: Cambridge University Press)

Tylianakis J M et al 2008 Global change and species interactions in terrestrial ecosystems Ecol. Letters. 11 1351–63

Ueki M et al 2013 Xylem embolism threshold for catastrophic hydraulic failure in angiosperm trees Tree Physiol. 33 672–83

Valiela I, Bowen J L and York J K 2001 Mangrove forests: One of the world’s threatened major tropical environments Bioscience 51 807–15

Vankoughnet M R and Henry H A L 2014 Soil freezing and N deposition: transient vs multi-year effects on plant productivity and relative species abundance New Phytol. 202 1277–85

Wang W et al 2011 Influence of frost on nutrient resorption during leaf senescence in a mangrove at its latitudinal limit of distribution Plant Soil. 342 105–15

Weltzin J F et al 2003 Assessing the response of terrestrial ecosystems to potential changes in precipitation Bioscience 53 941–52

Weston N B et al 2014 Net ecosystem carbon exchange and the greenhouse gas balance of tidal Marshes along an estuarine salinity gradient Biogeochemistry 120 163–89
Wu H et al 2014 Mariculture pond influence on mangrove areas in south China: Significantly larger nitrogen and phosphorus loadings from sediment wash-out than from tidal water exchange. *Aquaculture* 426 204–12
Yang Z 2018 Differential responses of litter decomposition to regional excessive nitrogen input and global warming between two mangrove species. *Estuarine, Coastal and Shelf Science* 214 141–8
Zhu L et al 2015 How does tree age influence damage and recovery in forests impacted by freezing rain and snow? *Sci. China Life Sci.* 58 472–9