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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 CO_2 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

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* 2010), 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.
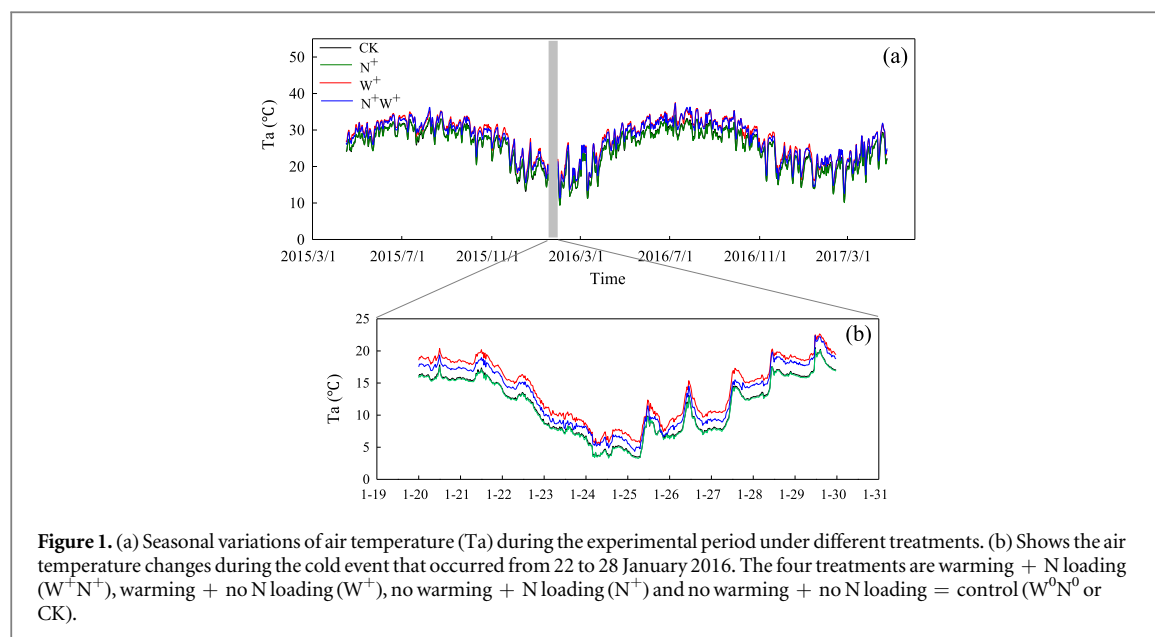
The experiment was conducted as a split-split-plot design with four blocks, which also served as true experimental replication (N = 4) arranged to account for environmental variability among the 16-tank arrangement. Each block consisted of two whole plots differing in temperature—warming (W^+) and an ambient control (W^0). Each of the whole-plots was split into excessive nitrogen (N^+) and ambient (N^0), 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 (W^0N^0 or CK), (2) Warming + no N loading (W^+N^0 or W^+), (3) No warming + N loading (W^0N^+ 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 N^0 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 NH_4Cl and 91 g $NaNO_3$ were added to the N^+ reservoir which contained 3000 l of artificial seawater to make a concentration of 20 mg l^{-1} NH_4^+ and 5 mg l^{-1} NO_3^-, respectively. The concentrations of NH_4^+ and NO_3^- 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, Kalglo 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 compared to a control plot (continuously day and night). In each W^0 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.

Figure 1. (a) Seasonal variations of air temperature (Ta) during the experimental period under different treatments. (b) Shows the air temperature changes during the cold event that occurred from 22 to 28 January 2016. The four treatments are warming + N loading (W^+N^+), warming + no N loading (W^+), no warming + N loading (N^+) and no warming + no N loading = control (W^0N^0 or CK).

2.3. Plant growth and net ecosystem exchange of CO_2 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: RGR = (ln h2 − ln h1)/(t2 − t1), where ln is the natural logarithm, h is the growth variable at times 1 and 2 for each mesocosm, t2 and t1 are the first sampling time and the last sampling time during each experiment period, respectively.

Net ecosystem exchange (NEE) of CO_2 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. CO_2 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 CO_2 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 CO_2 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).
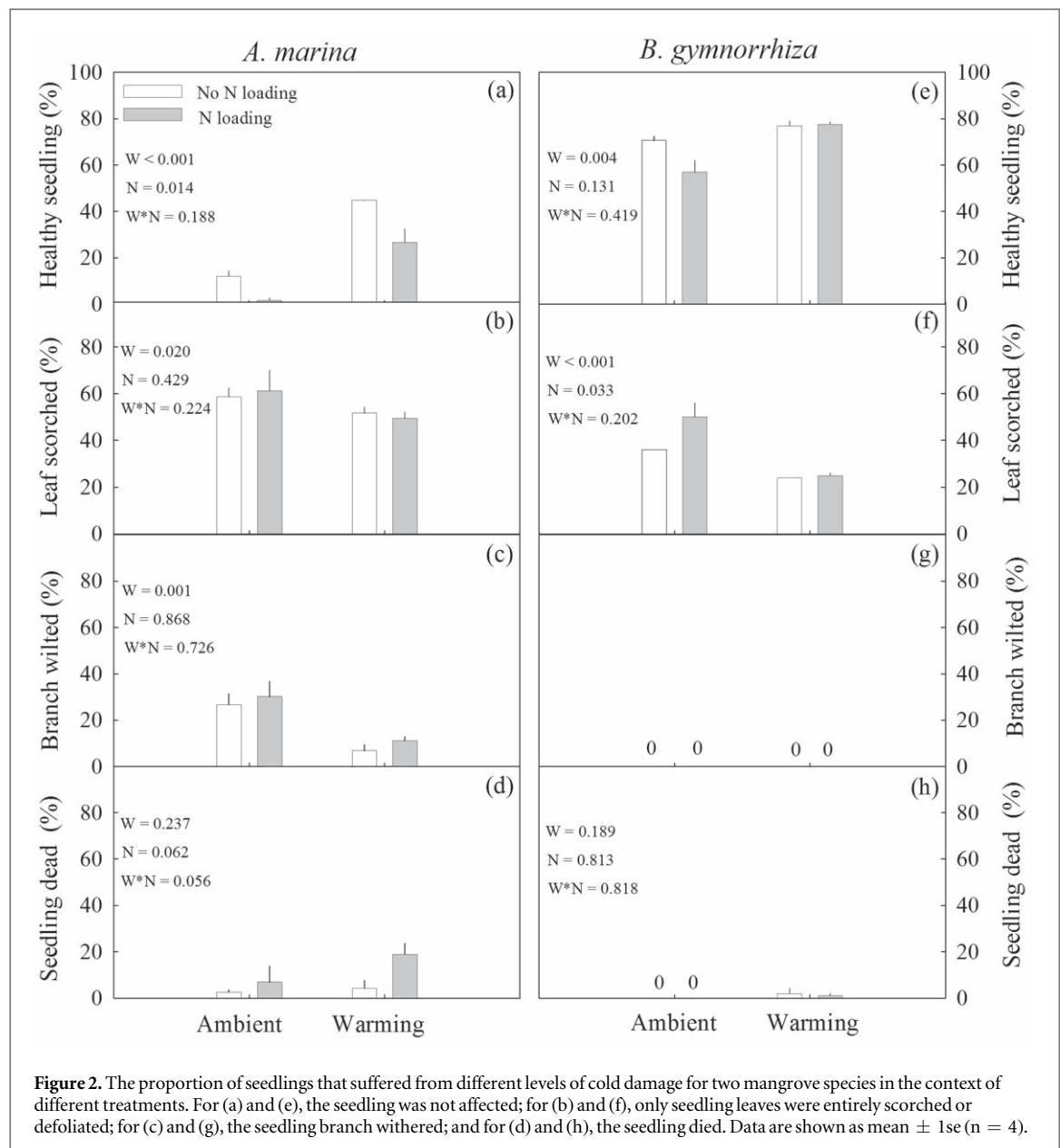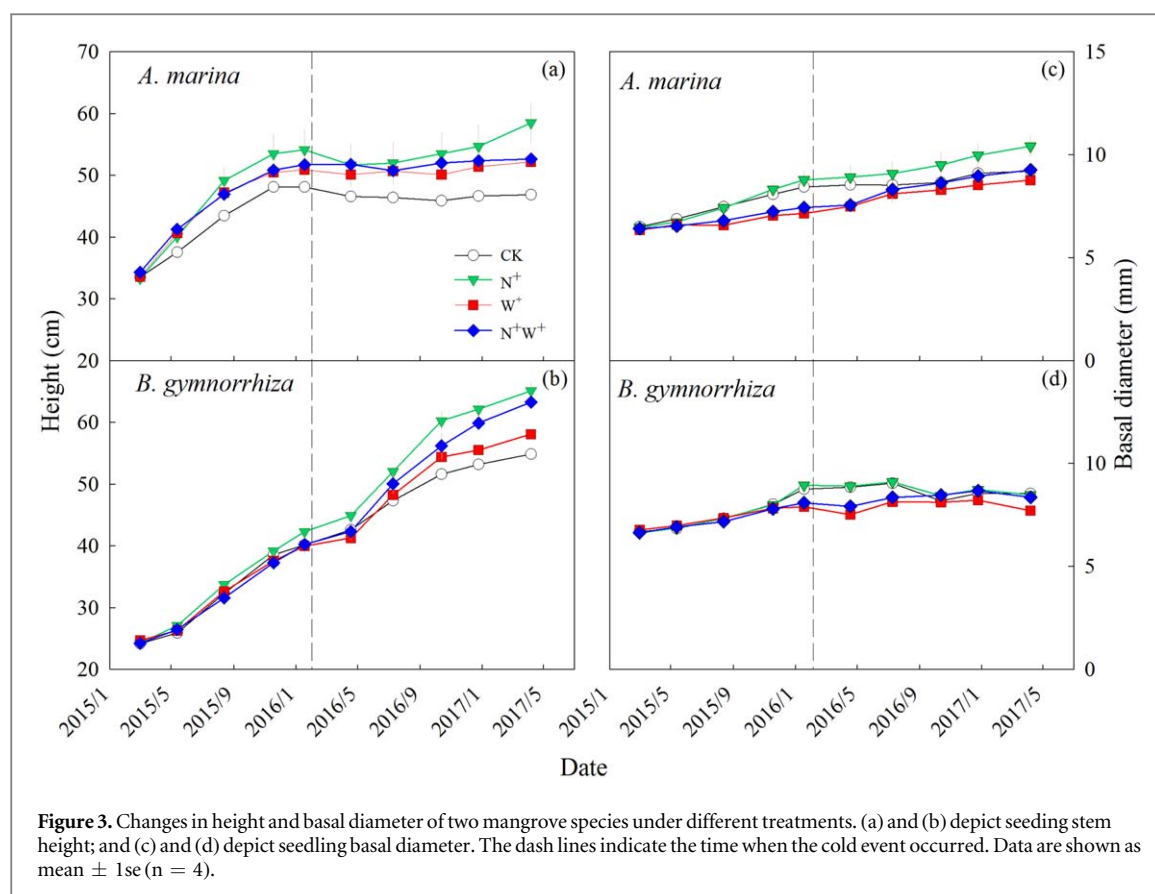
Figure 2. The proportion of seedlings that suffered from different levels of cold damage for two mangrove species in the context of different treatments. For (a) and (e), the seedling was not affected; for (b) and (f), only seedling leaves were entirely scorched or defoliated; for (c) and (g), the seedling branch withered; and for (d) and (h), the seedling died. Data are shown as mean $\pm$ 1se (n = 4).

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

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 seedling basal diameter. The dash lines indicate the time when the cold event occurred. Data are shown as mean $\pm$ 1se (n = 4).

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) (Koštál *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.

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. gymnorrhiza* 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 CO_2 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 CO_2 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.
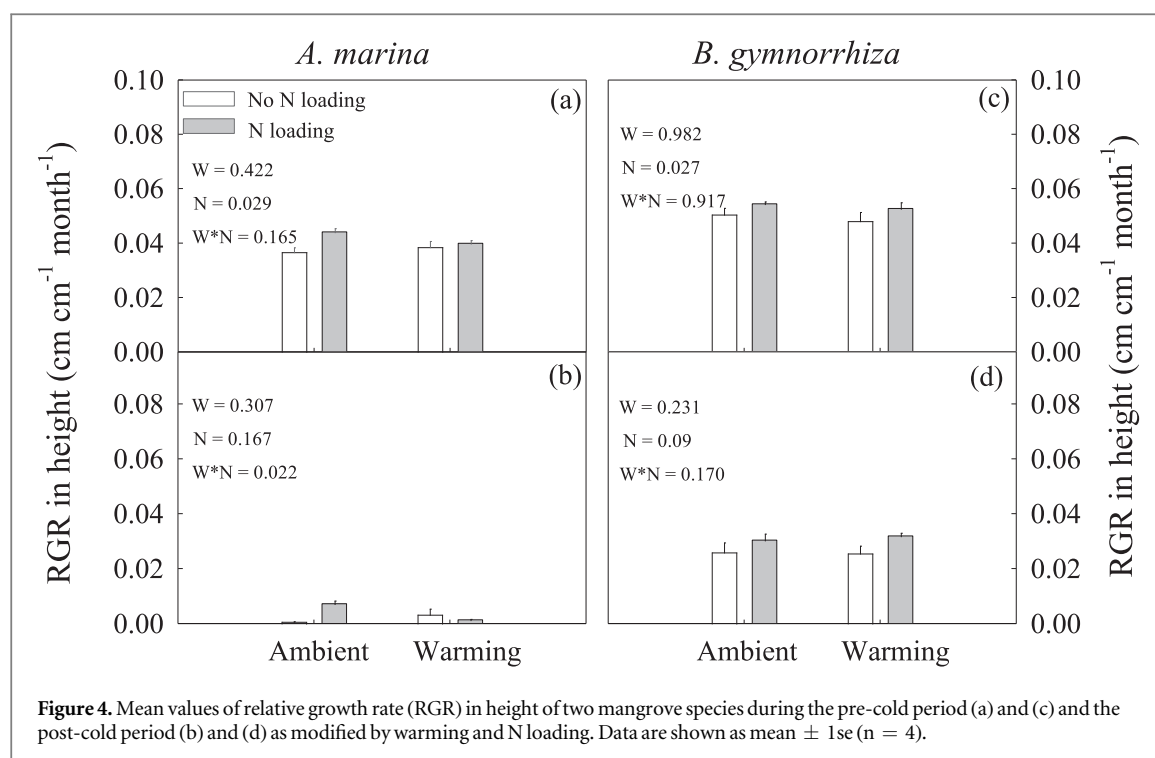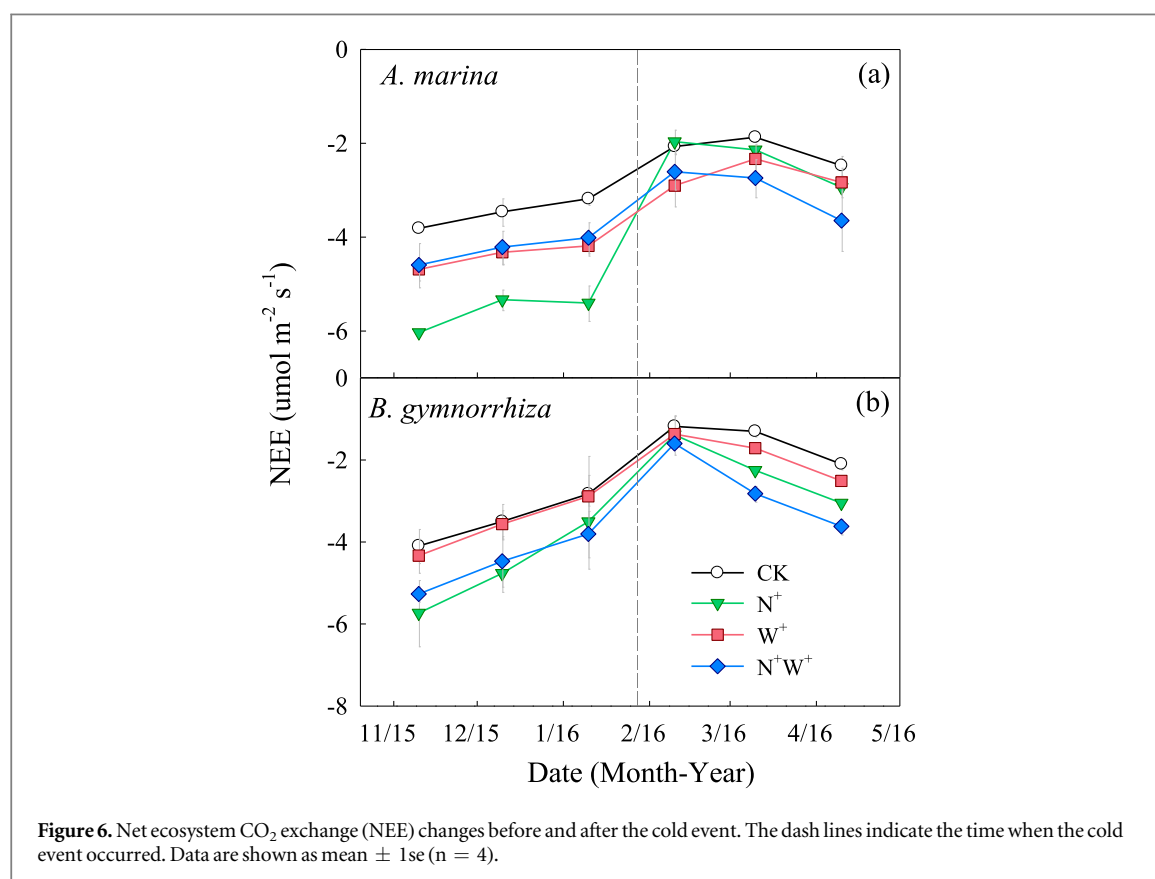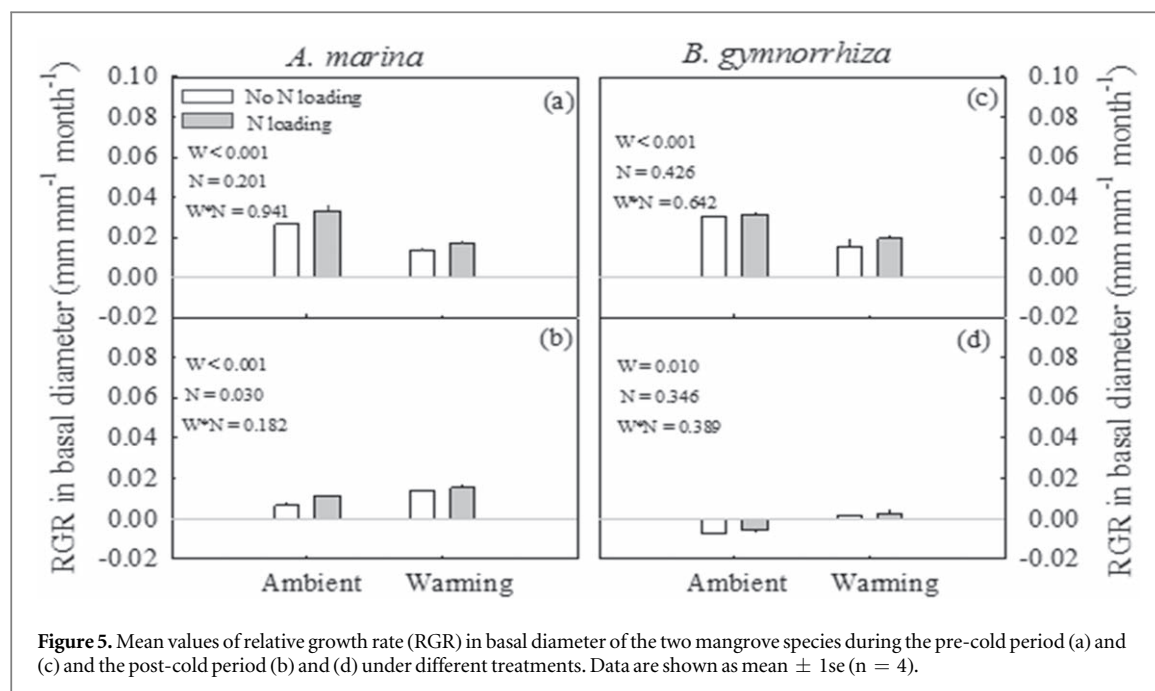
Figure 4. Mean values of relative growth rate (RGR) in height of two mangrove species during the pre-cold period (a) and (c) and the post-cold period (b) and (d) as modified by warming and N loading. Data are shown as mean ± 1se (n = 4).

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.
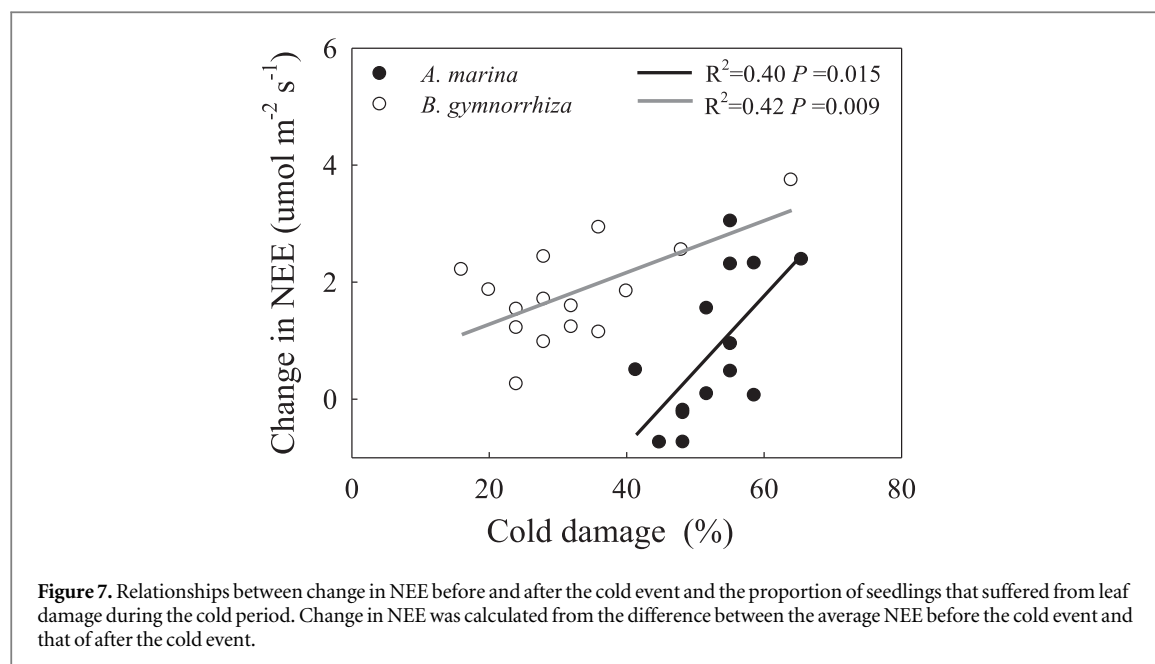
		Height	Basal diameter	NEE
A.marina	W	0.286	0.006	0.115
	N	0.018	0.002	<0.001
	T	<0.001	<0.001	<0.001
	W × N	0.011	0.159	<0.001
	T × W	0.861	0.444	<0.001
	T × N	0.407	<0.001	0.006
	T × N × W	0.664	0.954	<0.001
B.gymnorrhiza	W	0.708	0.107	0.090
	N	0.023	0.269	<0.001
	T	<0.001	<0.001	<0.001
	W × N	0.749	0.748	0.411
	T × W	0.512	<0.001	0.840
	T × N	0.778	0.746	0.383
	T × N × W	0.878	0.608	0.195

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.

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 CO_2 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).

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 7. Relationships between change in NEE before and after the cold event and the proportion of seedlings that suffered from leaf damage during the cold period. Change in NEE was calculated from the difference between the average NEE before the cold event and that of after the cold event.

Before the cold event, warming significantly increased CO_2 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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