

## Research



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## Conservation biology

# Increased nitrogen input enhances *Kandelia obovata* seedling growth in the presence of invasive *Spartina alterniflora* in subtropical regions of China

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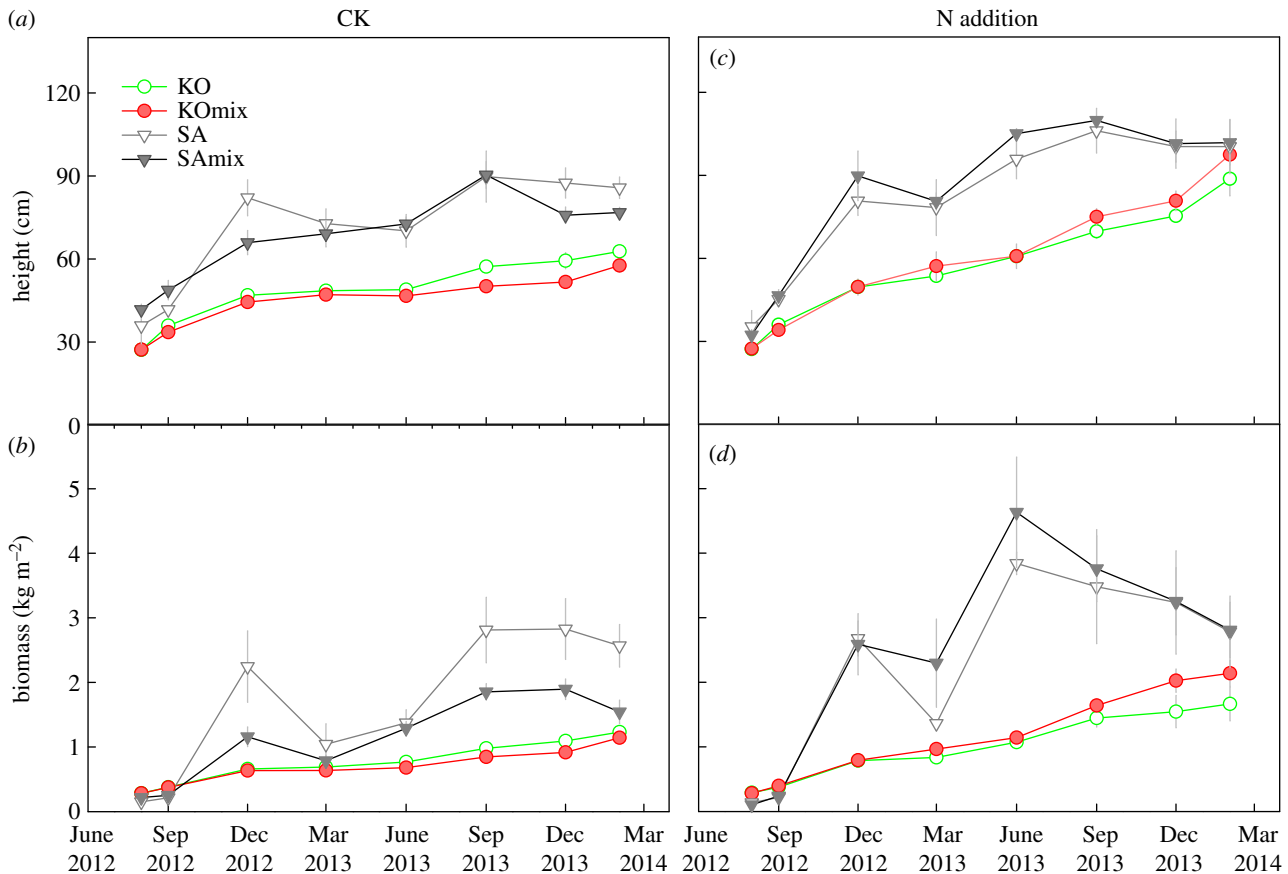
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Mangroves in China are severely affected by the rapid invasion of the non-native species *Spartina alterniflora*. Although many studies have addressed the possible impacts of *S. alterniflora* on the performance of mangrove seedlings, how excessive nitrogen (N) input due to eutrophication affects the interactions between mangrove species and *S. alterniflora* remains unknown. Here, we report the results from a mesocosm experiment using seedlings of the native mangrove species *Kandelia obovata* and the exotic *S. alterniflora* grown in monoculture and mixed culture under no nitrogen addition and nitrogen (N) addition treatments for 18 months. Without N addition, the presence of *S. alterniflora* inhibited the growth of *K. obovata* seedlings. Excessive N addition significantly increased the growth rate of *K. obovata* in both cultures. However, the positive and significantly increasing relative interaction intensity index under excessive N input suggested that the invasion of *S. alterniflora* could favour the growth of *K. obovata* under eutrophication conditions. Our results imply that excessive N input in southeastern China can increase the competitive ability of mangrove seedlings against invasive *S. alterniflora*.

## 1. Introduction

Located along coastlines throughout the tropical and subtropical regions of the world, mangrove wetlands are recognized as one of the most biologically important ecosystems [1]. However, many mangrove ecosystems are severely disturbed and threatened by human activities, causing many biological and environmental changes, particularly in China and other Southeast Asian countries [2]. Worldwide, mangrove forests are lost at an annual rate of 1–2% [3,4]; however, this rate is even higher in Southeast Asia [2].

*Spartina alterniflora*, commonly known as smooth cordgrass in the USA, was introduced to China in 1979. Since then, it has aggressively spread and invaded native mangrove habitats in China [5]. Although extensive studies have evaluated the possible impacts of *S. alterniflora* on native mangroves, the interactions between *S. alterniflora* and native mangrove seedlings have long been a subject of great debate. For example, some studies have shown that by stabilizing the sediment and ameliorating soil properties, *S. alterniflora* could play a 'nursing' role for mangrove seedlings [6]. By contrast, other studies reported that *S. alterniflora* inhibited the establishment and growth of mangrove seedlings by occupying their niche and shading their leaves owing to its fast growth [7–9].



**Figure 1.** Changes in height and biomass of *K. obovata* (KO: monoculture; KOmix: mixed culture) and *S. alterniflora* (SA: monoculture; SAMix: mixed culture) under no nitrogen addition (CK, *a* and *b*) and nitrogen addition (N addition, *c* and *d*). (Online version in colour.)

Mangrove wetlands in China have seriously suffered from increasing eutrophication due to sewage discharge and aquaculture activities [10]. The results from a previous study suggest that eutrophication caused by nitrogen (N) enrichment can increase the survival and growth of mangroves, particularly young seedlings, because they are highly sensitive to changes in soil physico-chemical conditions [11,12]. However, whether excessive N can change the impact of *S. alterniflora* invasion on mangrove seedlings is still unclear. On the northeastern coast of Mexico, where mangroves have severely invaded *S. alterniflora* marshes, excessive N input had no effect on the interactions between mangroves and *S. alterniflora* [7]. In mangrove wetlands of China invaded by *S. alterniflora*, the effect of excessive N input on the interactions between *S. alterniflora* and mangrove seedlings is in urgent need of better understanding.

In this study, we examined the responses of *Kandelia obovata* (a common native mangrove species in China) seedlings and *S. alterniflora* grown in monoculture and mixed culture to excessive N loading typical of coastal regions in southeast China. The objectives of this study were to evaluate (i) how excessive N affects the growth of *K. obovata* seedlings with or without neighbouring *S. alterniflora* and (ii) the ability of excessive N to alleviate the negative impacts of *S. alterniflora* on mangrove seedlings.

## 2. Material and methods

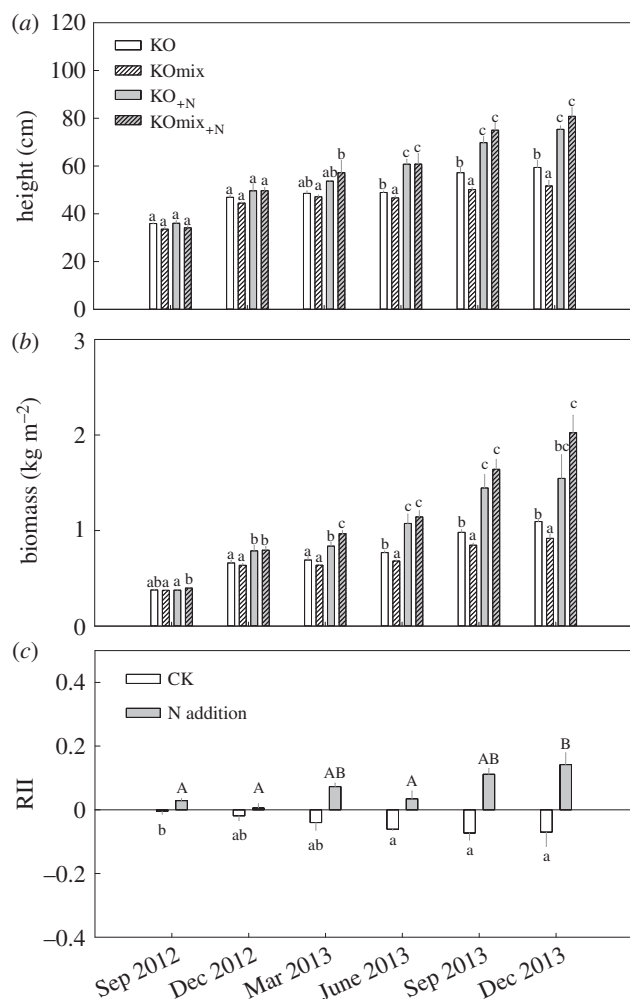
### (a) Experimental design

A mesocosm experiment was conducted in a greenhouse at the Graduate School at Shenzhen, Tsinghua University, People's

Republic of China (22°59' N, 113°97' E). The mesocosm system consisted of 18 cement tanks (1.2 m width × 2.0 m length and 0.5 m depth) as experimental mesocosms and two cement tanks (0.5 m width × 3.0 m length and 1.2 m depth) as seawater reservoirs. One seawater reservoir tank was connected to nine control mesocosms with no N addition (hereafter 'CK'). The other was connected to nine N addition treatment mesocosms (hereafter 'N addition'). For the N addition treatment, 450 g NH<sub>4</sub>Cl (NH<sub>4</sub><sup>+</sup>-N was the dominant N form in eutrophication-seawater column in coastal regions of southeast China) was added to the 3000 l seawater reservoir to create a high concentration of approximately 150 mg l<sup>-1</sup> NH<sub>4</sub><sup>+</sup>-N, which was similar to the concentration occurring in the coastal seawater of southeast China. Healthy seedlings of *K. obovata* and young ramets of *S. alterniflora* approximately 15 cm in height were transplanted into each mesocosm in August 2012. Each *K. obovata* seedling and *S. alterniflora* ramet covered an area of 0.15 × 0.15 m and 0.25 × 0.25 m, respectively. This mimics typical seedling densities of these two species according to our observations in the field. We simulated three types of vegetation compositions: (i) *K. obovata* monoculture, (ii) *S. alterniflora* monoculture, and (iii) mixed culture of the two species. Each vegetation type was tested with and without N addition ( $n = 3$ ). For the monoculture mesocosms, there were 140 seedlings or ramets per tank, while for the mixed culture mesocosms there were 70 seedlings and 70 ramets cross-planted per tank. A schematic representation of the experimental mesocosms is presented in the electronic supplementary material, figure S1, and more detailed information is available in [13].

### (b) Measurements of plant height and biomass

We randomly sampled one plant of the two species per mesocosm one month after planting and every three months thereafter. We measured the stem height and then harvested the aboveground biomass. The roots were sampled by the soil



**Figure 2.** Seasonal dynamics in stem height (a) and biomass (b) of *K. obovata* seedlings under the four treatments, and the mean relative interaction intensity (RII, c) of neighbouring *S. alterniflora* on *K. obovata* seedlings under the two nitrogen addition treatments.

core method. Four soil cores (10 cm diameter and 30 cm depth) around the sampled plants were systematically taken and sieved to extract the total root biomass of each plant. All samples were oven-dried and weighed. The dry weights of the above-ground plant and roots were summed for the total biomass. There were in total seven plants per species per tank sampled during the experiment periods. Though this sampling would have changed the density and interaction of the two species, we believe that the data were credible considering the large number of plants in each tank.

### (c) Calculation of the relative interaction intensity index

To examine whether *K. obovata* growth was significantly affected by *S. alterniflora*, we calculated the relative interaction intensity index (RII) [14] for each mesocosm as follows:

$$\text{RII} = \frac{(B_{\text{mixed}} - B_{\text{mono}})}{(B_{\text{mixed}} + B_{\text{mono}})}, \quad (2.1)$$

where  $B_{\text{mixed}}$  is the *K. obovata* biomass in the mixed culture, and  $B_{\text{mono}}$  is that in the monoculture.

### (d) Statistical analyses

A three-way ANOVA was performed to examine the effects of N treatment, *S. alterniflora* invasion, sampling month and their possible interactions on *K. obovata* seedling growth. One-way

**Table 1.** *p*-Values of three-way ANOVA for the effects of *S. alterniflora* invasion (I), nitrogen addition treatment (N), sampling time (T), and their interactions on height and biomass of *K. obovata* seedlings.

	d.f.	height	biomass
I	1	0.828	0.132
N	1	<0.001	<0.001
T	4	<0.001	<0.001
I × N	1	<0.001	0.006
I × T	4	0.551	0.723
N × T	4	<0.001	<0.001
I × N × T	4	<0.001	0.057

ANOVA was used to examine significant differences in height and biomass for each sampling time and in the RII for *K. obovata* under the two N addition treatments. All data analyses were performed using SPSS v. 19.0.

## 3. Results

The height and biomass of *K. obovata* seedlings gradually increased in both mono- and mixed cultures under the CK treatment (figure 1*a,b*). There was no significant difference in the height or biomass of *K. obovata* between the mono- and mixed cultures during the period from September 2012 to March 2013, but the *K. obovata* in monoculture showed a greater height and biomass than those in mixed culture from June 2013 until the experiment ended (figure 2*a,b*). During the experimental period, the RII was significantly negative ( $p < 0.01$ , figure 2*c*), indicating that competition by *S. alterniflora* invasion suppressed the growth of *K. obovata* seedlings.

Nitrogen addition significantly increased the growth rates of *K. obovata* and *S. alterniflora* in both mono- and mixed cultures ( $p < 0.001$ , table 1; figure 1*c,d*). The interactive effects of N addition and *S. alterniflora* invasion on the height and biomass of *K. obovata* seedlings were significant ( $p < 0.001$ ; table 1). There was no difference in the height or biomass of *K. obovata* between the mono- and mixed cultures under the N addition treatment ( $p > 0.05$ ; figure 2*a,b*). The RII was positive and increased significantly during the experiment ( $p < 0.01$ ; figure 2*b*), indicating that the presence of *S. alterniflora* favoured the growth of *K. obovata* seedlings.

## 4. Discussion

Without N addition, *S. alterniflora* inhibited the growth of *K. obovata* seedlings, which could be due to the following reasons. First, the high root uptake capacity of fast-growing *S. alterniflora* could deplete nutrient pools (electronic supplementary material, figure S2), resulting in a low growth rate of *K. obovata*. Second, the fast increase in the height of *S. alterniflora* could have inhibited photosynthesis in *K. obovata* by shading [15].

The addition of N significantly increased the growth rate of *K. obovata* in both mono- and mixed cultures, implying its growth is N-limited (electronic supplementary material, figure S3). To our surprise, N addition led to a positive

and increasing RII value, suggesting that the invasion of *S. alterniflora* could facilitate rather than inhibit the growth of *K. obovata* under excessive N input.

It has been theorized that biological interactions between plant species are affected by changes in nutrient availability and the environment [16] and that competition between plant species is more intense under high fertility conditions [17]. Thus, under high soil N conditions, competition for N resources between *K. obovata* seedlings and *S. alterniflora* could increase significantly, favouring the growth of *S. alterniflora* because of its higher growth rate and nutrient uptake capacity. However, in this study, excessive N input reversed the suppressive effects of *S. alterniflora* on *K. obovata*, suggesting that other factors may be involved in the changes in *K. obovata* seedling growth under excessive N inputs. Previous studies have indicated that, under some extreme conditions, the survival and growth of mangrove seedlings are facilitated by the presence of herbaceous species because they can ameliorate soil properties [18]. In this study, the regular loadings of excessive N in the form of  $\text{NH}_4^+$ -N might have exceeded the carrying capacity of the soils in the mesocosms, resulting in detrimental effects to the growth of both species due to physical pressure and/or ammonia toxicity [19,20]. Therefore, we hypothesize that the effects of *S. alterniflora* on *K. obovata* are likely to change from competition to facilitation under excessive N input, leading to a higher growth rate of *K. obovata* in the mixed culture than in the monoculture.

Our study provides direct experimental evidence that excessive N input due to eutrophication provides some hope for the survival and spread of *K. obovata* seedlings in southeastern China, where *S. alterniflora* invasions have

become progressively more serious. More studies are needed to validate whether this phenomenon also occurs in other native mangrove species in China. Furthermore, the Chinese government has made great effects in mangrove afforestation during the last few decades, and more than 2000 ha of mangrove forests have been successfully restored [21]. Our results might shed some light on how we can govern and protect the restored mangrove forests under the influences of *Spartina* invasion. In addition, this study lasted only 18 months and examined only one scenario of possible interactions between *S. alterniflora* and *K. obovata*, so more experimental studies with longer experimental period and a greater number of scenarios of mangrove–*Spartina* interactions are needed to better understand the eutrophication effects on the interactions between mangrove seedlings and *S. alterniflora*.

**Data accessibility.** Raw data are available from Dryad: <http://dx.doi.org/10.5061/dryad.j5148> [22].

**Authors' contributions.** G.L. conceived and designed the experiment. J.F., D.J., J.G., Z.W., H.W. and F.Q. conducted the experiment and collected data. X.C., W.S., J.F., J.L. and G.L. analysed the data and wrote the initial draft of the manuscript. All authors contributed to the editing and revising of the final version of the manuscript. The authors approved the final version for publication and agree to be held accountable for the content therein.

**Competing interests.** We have no competing interests.

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