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Herbivory and nitrogen availability affect performance of an invader *Alternanthera philoxeroides* and its native congener *A. sessilis*

Xiao-Ting Hu, Bi-Cheng Dong*

School of Nature Conservation, Beijing Forestry University, Beijing, 100083, China

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ABSTRACT

Tolerance to herbivory influences the success of invasive species in introduced ranges, and thus a comparison of the difference in herbivory tolerance between invasive and native species may help to understand the mechanisms of plant invasions. We conducted a greenhouse experiment in Beijing, China to examine effects of aboveground herbivory (by a beetle *Agasicles hygrophila* or not) and nitrogen availability (high vs. low) on growth and physiology of the invasive plant *Alternanthera philoxeroides* and its coexisting native congener *A. sessilis*. Herbivory by *A. hygrophila* significantly reduced total mass and aboveground development of both plant species, and the negative effects of herbivory on leaf mass, ramet number, and leaf number were more remarkable at the high than at the low nitrogen level. On the other hand, herbivory did not affect root mass and even increased root to shoot ratio of both plant species. The increase in root to shoot ratio and relative ratio of root mass caused by herbivory was also stronger in *A. philoxeroides* than in *A. sessilis*. Besides, herbivory exerted neutral or positive effects on the concentrations of starch and total non-structural carbohydrates in stems and roots of *A. philoxeroides*, but negative effects on the physiological measures of *A. sessilis*. We conclude that *A. philoxeroides* may possess a stronger tolerance to herbivory than *A. sessilis* and that increased resource allocation to roots could potentially contribute to compensatory responses of *A. philoxeroides* to aboveground herbivory. Our findings also suggest that the control of *A. hygrophila* may be invalid in belowground invasion of *A. philoxeroides*, and that belowground herbivores should be considered in the future management of invasive species.

1. Introduction

Plant species are mostly sessile (Bohnert, 2007), and many of them, including some exotic invasive species, suffer inevitably from the attack by different types of herbivores (Lay et al., 2011; Wilson et al., 2012; Dong et al., 2018). Consequently, these plant species have to develop various defense strategies against herbivory to alleviate the herbivory-induced decline in fitness (Pilson, 2000; Tiffin, 2000; Núñez-Farfán et al., 2007; Turley et al., 2013; Katz et al., 2014). Assessing the defense strategies of invasive plants to herbivory may thus help to understand the mechanisms underlying exotic plant invasions and develop measures to manage invasive plants (Pilson, 2000; Tiffin, 2000; Vergés et al., 2008; Turley et al., 2013; Wei et al., 2016).

Two common types of herbivory defense strategies have been identified: resistance and tolerance (Strauss and Agrawal, 1999). Besides resistance by e.g., producing secondary metabolites such as tannins and phenolics to reduce the preference or performance of herbivores, tolerance is another defense strategy that allows host plants to relieve the direct damage from herbivores (Strauss and Agrawal, 1999; Núñez-Farfán et al., 2007; Robert et al., 2015). Herbivory tolerance occurs via not only the production of primary metabolites in specific organs to directly promote compensatory growth, but also the modification in resource allocation between damaged and undamaged organs (Rosenthal and Kotanen, 1994; Schwachtje et al., 2006; Vergés et al., 2008). For instance, plants damaged by herbivores could accumulate a greater amount of non-structural carbohydrates (water-soluble sugars and starch) in undamaged or storage organs to support their subsequent regrowth (Das et al., 2005; Vergés et al., 2008; Wiley et al., 2013). In addition, nutrient elements such as nitrogen in plants can also be remobilized between different organs following herbivory, either to interfere with the feeding habits of herbivores (Forkner et al., 2004; Andrew et al., 2011) or to enhance compensatory growth of damaged plants (Maschinski and Whitham, 1989; Pinkard et al., 2007).

Herbivory-induced tolerance responses may interact with the availability of external resources (Gao et al., 2008; Sun et al., 2010; Piippo et al., 2011; Gianoli and Salgado-Luarte, 2017). There are two prevailing but opposite hypotheses related to interactions between plant tolerance and resource availability (Hilbert et al., 1981;

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* Corresponding author.
E-mail address: bcdong@bjfu.edu.cn (B.-C. Dong).

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Maschinski and Whitham, 1989; Wise and Abrahamson, 2005). One is that tolerance to herbivory increases in resource-rich conditions compared to resource-poor ones (Belsky et al., 1993; Rosenthal and Kotanen, 1994; Strauss and Agrawal, 1999; Wise and Abrahamson, 2005). This prediction is based on the assumption that damaged plants can uptake and assimilate resources more easily at higher resource levels, and thus grow faster and recover more rapidly from damage (Chapin and McNaughton, 1989). The other hypothesis is that tolerance to herbivory should be greater in low- than in high-resource conditions (Hilbert et al., 1981; Alward and Joern, 1993). The idea is that plants growing in low-resource conditions are normally below their maximum growth rate, and thus require only small changes in growth rate to recover from damage (Hilbert et al., 1981; Hawkes and Sullivan, 2001). In contrast, plants growing in high-resource conditions are likely to reach their maximum growth rate, so that they may not compensate for damage by increasing growth rate further (Hilbert et al., 1981; Hawkes and Sullivan, 2001). Therefore, it is necessary to test herbivory tolerance of target plants at various resource levels.

Herbivory tolerance can influence the invasion success of some exotic plant species in introduced ranges (Mitchell et al., 2006; Ashton and Lerdau, 2008; Liu et al., 2012; Zhang et al., 2018). For lack of specialist enemies, invasive plant species in introduced ranges may undergo a rapid evolution of life-history traits (Keane and Crawley, 2002; Theoharides and Dukes, 2007; Zhang et al., 2018), and exhibit an increased resource utilization efficiency and thus increased growth rate at the expense of reduced defense against herbivory (Rogers and Siemann, 2003; Wolfe et al., 2004; Robert et al., 2015; Stastny and Sargent, 2017). For instance, compared to native populations in China, populations of *Sapium sebiferum* invading the United States had a higher growth rate and a higher reproduction capacity, but a lower chemical compound production to adapt to underground damage caused by simulated herbivory (Rogers and Siemann, 2004). The increase in growth and reproduction can greatly increase the competitive advantage of invasive plant species over native ones, and thus contribute to their invasion success in introduced ranges (Rogers and Siemann, 2004; Stastny et al., 2005; Zou et al., 2008; Stastny and Sargent, 2017).

Alternanthera philoxeroides (Mart.) Griseb, native to South America, is now considered to be one of the most serious invasive species in temperate and subtropical areas throughout the world (Spencer and Coulson, 1976; Sainty et al., 1998; Ma and Wang, 2005). In southern China, *A. philoxeroides* is widespread in waterways and irrigation ditches and in adjacent, open terrestrial areas, including crop fields (Pan et al., 2006). Invasion of *A. philoxeroides* can cause a serious decline in species diversity of native plants in both aquatic and terrestrial habitats (Wang et al., 2017a; Wu et al., 2016, 2017). To control the invasion of *A. philoxeroides*, the leaf-feeding beetle *Agasicles hygrophila* has been introduced into China since 1986 and used widely as a biological control agent (Lu and Ding, 2011). It is thus necessary to explicitly examine the tolerance strategy of *A. philoxeroides* against herbivory by *A. hygrophila.*

We thus conducted a greenhouse experiment to test effects of herbivory by the specialist beetle *A. hygrophila* and nitrogen availability on growth and physiology of the invasive plant species *A. philoxeroides* and its co-occurring, native congener *A. sessilis*. Specifically, we tested the following hypotheses. (1) The two species of *Alternanthera* can tolerate herbivory via changes in resource allocation related to biomass, non-structural carbohydrates, and nitrogen. (2) The invasive species *A. philoxeroides* may exhibit a lower tolerance but greater growth ability compared to its native congener *A. sessilis*. (3) The ability of herbivory tolerance in the two species of *Alternanthera* can be mediated by external nitrogen availability.

2. Materials and methods

2.1. Plant and insect species

Alternanthera philoxeroides is a perennial herb of Amaranthaceae (Holm et al., 1997; Sainty et al., 1998). The species can produce creeping stems that root at the nodes, which thus function as ramets. It occurs in wetlands, riparian habitats, grasslands, and agricultural fields (Sainty et al., 1998; Pan et al., 2006), and exhibits high tolerance of disturbances such as herbivory, mowing, and flooding (Lu and Ding, 2011; Luo et al., 2014; You et al., 2014; Dong et al., 2017, 2018). In southern China, *A. philoxeroides* mainly reproduces vegetatively and forms offspring ramets via stem and root fragments, thus exhibiting extremely low genetic diversity (Xu et al., 2003; Ye et al., 2003).

Alternanthera sessilis (Linn.) DC. is native to China, and its growth habit and ecological range are similar to those of *A. philoxeroides* (Pan et al., 2006; Li et al., 2014; Liu et al., 2016). However, this species can reproduce both by seeds and by stem fragments (Wu and Raven, 2003). It often co-occurs with *A. philoxeroides* in different habitats (Pan et al., 2006).

Agasicles hygrophila Selman & Vogt, 1971 (Coleoptera: Chrysomelidae), native to South America, is a host-specific leaf beetle (Spencer and Coulson, 1976). This beetle normally has six life-history stages, i.e., eggs, the 1 st, 2nd, 3rd larvae, pupa, and adults (Pan et al., 2011). The adult of *A. hygrophila* is about 5.7–7 mm in length, with two yellow stripes on their black elytra. The larvae and adults both feed on aboveground organs (leaves and stem buds) of *A. philoxeroides* and *A. sessilis*, with their chewing mouthparts.

Plants of *A. philoxeroides* were collected in Zhejiang province in May 2011, and those of *A. sessilis* were collected in Guangdong province in the summer of 2009. Adults of *A. hygrophila* were collected in Zhejiang province in May 2017. All plant species were propagated vegetatively in a greenhouse at Forest Science Company, Ltd., of Beijing Forestry University in Beijing, China.

2.2. Experimental design

The experiment employed a three-factorial design, consisting of species identity (*A. philoxeroides* or *A. sessilis*), herbivory (without or with herbivory by *A. hygrophila*), and N availability (high or low). On 11 July 2017, 44 stem fragments of *A. philoxeroides* and 36 stem fragments of *A. sessilis* were selected for use in the experiment. Each stem fragment was cut to 15 cm length with 5 nodes and an apex but no side branch.

Each single stem fragment (thereafter referred to as “plant”) was planted vertically in a pot (14 cm in diameter and 12 cm in height), with two oldest stem nodes buried in the soil. The soil was an even mixture of quartz sand (0.5–1 mm in particle diameter) and peat (Pindstrup Seedling; Pindstrup Mosebrug A/S, Pindstrup, Denmark) at a volume ratio of 1:1. After 20 days of cultivation, plants of both *A. philoxeroides* and *A. sessilis* were subjected to two levels of herbivory (without or with herbivory by *A. hygrophila*) crossed with two levels of N availability (high or low). For the N availability treatments, once every three days, half of the plants of *A. philoxeroides* (22) and *A. sessilis* (18) were added with 500 ml modified Hoagland solution containing 20 mg N L^{-1} (low N level), and the other half with 500 ml modified Hoagland solution containing 60 mg N L^{-1} (high N level). N was supplied as $Ca(NO_3)_2$, and the concentrations of Ca in the two types of nutrient solutions were maintained the same by adjusting the concentration of $CaSO_4$ (Wang et al., 2017b).

For the herbivory treatments, half of the plants of *A. philoxeroides* (11) and of *A. sessilis* (9) at each level of N availability were grown under the herbivory of *A. hygrophila* (density: one male and one female adult per plant), and the other half were not infested and served as the control. To prevent the interference between treatments, each plant was placed in a cage (25 cm long × 25 cm wide × 50 cm high) covered with a 0.25-mm nylon mesh. There were 11 replicates of *A. philoxeroides* and 9 replicates of *A. sessilis* for each of four treatments.

The experiment lasted for 28 days, from 30 July 2017 to 26 August 2017. The short experiment duration was because most leaves of the two species were already consumed by the beetle. The mean temperature and relative humidity during the experiment were 27.8 ± 0.3 °C and 80.8 ± 1.7% (mean ± SE), respectively, using Hobo Temp/RH loggers (HOBO UX100-003; Onset Computer Co., Bourne, MA, USA). On sunny days, the photosynthetic photon flux density at noon was 1032.6 ± 92.3 μmol m^{-2} s^{-1} (mean ± SE).

2.3. Growth and physiological measurements

At harvest, number of ramets and number of leaves were counted, and leaf area was scanned and estimated by ImageJ (Image Processing and Analysis in Java, available at http://rsbweb.nih.gov/ij/). Leaves, stems, and roots of each plant were separated, dried at 70 °C for 48 h, and weighed. The oven-dried stems and roots were ground into powder using a Retsch MM400 Mixer Mill (Retsch GmbH, Haan, Germany) for chemical measurements. Stem and root samples of about 50 mg were analyzed for concentrations of water-soluble sugars and starch using the perchloric acid / anthrone method (John et al., 1950; Luo et al., 2014; Dong et al., 2017; Wang et al., 2018). Briefly, samples were extracted three times in 80% ethanol at 80 °C, and then centrifuged to obtain the supernatant. The supernatant was reacted with anthrone reagent, and measured at 620 nm in a spectrophotometer to calculate the concentration of soluble sugars. Starch in the residue was first reacted with perchloric acid, extracted, and then analyzed using the anthrone reaction with the method described for measuring soluble sugars. The concentration of total non-structural carbohydrates in samples was the sum of the concentrations of soluble sugars and starch. The concentrations of total nitrogen in root and stem samples were determined using a continuous-flow injection auto-analyzer (Technicon AA3-HR; SEAL Analytical, Germany). Six replicates of plants were used for chemical measurements. Leaves were not analyzed for these chemical contents as the amount was insufficient due to grazing by the beetle.

2.4. Data analysis

Three-way ANOVAs were used to test effects of species identity (*A. philoxeroides* or *A. sessilis*), herbivory (without or with herbivory by *A. hygrophila*), and N availability (high or low), and their interactions on growth (total mass, leaf mass, stem mass, root mass, number of ramets, number of leaves, and leaf area), biomass allocation (root to shoot ratio) and physiology (concentrations of soluble sugars, starch, total non-structural carbohydrates and nitrogen in stems and roots) of plants. To determine the relative strength of herbivory in *A. philoxeroides* or *A. sessilis*, relative ratios (RRs) of growth measures were then measured as the equation ($RR = P_{with}/P_{without}$), where P_{with} is the growth measure of each individual of one species with herbivory, and $P_{without}$ is the mean growth measure of one species without herbivory (Strauss and Agrawal, 1999). Data were square-rootedly transformed to meet the assumptions of normality and homogeneity of variances when necessary (see Table 1). All data were analyzed using SPSS 22.0 (SPSS, Inc., Chicago, IL, USA).

3. Results

3.1. Growth responses of Alternanthera

The two species of *Alternanthera* growing at the high nitrogen level produced 45%–64% greater leaf mass, leaf area and ramet number, but had averagely 30% lower root to shoot ratio, compared to those growing at the low nitrogen level (Table 1, Fig. 1). The effects of nitrogen availability on root mass depended on species identity, i.e., increasing nitrogen availability decreased 39% root mass of *A. philoxeroides* but not that of *A. sessilis* (significant effects of S × N in Table 1; Fig. 1d).

Herbivory by *A. hygrophila* imposed detrimental effects on all growth measures of both plant species except root mass (Table 1). The two species of *Alternanthera* had 39%–69% less total mass, leaf mass, stem mass, ramet number, leaf number, and leaf area, but 111% higher root to shoot ratio when herbivory was present than when it was absent (Fig. 1a–c and e–g). In addition, the negative effects of herbivory on leaf mass, ramet number and leaf number became more remarkable at the high than at the low nitrogen level (significant effects of H × N in Table 1; Fig. 1b, e, and f). The positive effects of herbivory on root to shoot ratio was more significant in *A. philoxeroides* than in *A. sessilis* (significant effect of H × S in Table 1; Fig. 1h).

3.2. Physiological responses of Alternanthera

Both plant species had 42%–64% higher concentrations of soluble sugars and nitrogen in stems, and 26% higher concentration of nitrogen in roots at the high than at the low nitrogen level (Fig. 2a, d and h). Plants of *A. sessilis* also had 38%–65% lower concentrations of starch and total non-structural carbohydrates in stems at the high than at the low nitrogen level, whereas such an effect of nitrogen availability was not detected in *A. philoxeroides* (significant effects of S × N in Table 2; Fig. 2b and c).

The effects of herbivory on concentrations of starch and non-structural carbohydrates in stems also depended on species identity (significant effects of S × H in Table 2). Plants of *A. philoxeroides* had significantly or marginally significantly ($P < 0.1$) higher concentrations of starch and total non-structural carbohydrates in stems and roots when herbivory was present than when it was absent (Fig. 2b, c, f, and g). The opposite pattern was found for the herbivory effect on *A. sessilis*.

3.3. Relative responses of Alternanthera to herbivory

There was a significant effect of species identity on the relative ratio of root mass, but not on the other relative ratios of growth measures (Fig. 3). The relative ratio of root mass was significantly greater in *A. philoxeroides* than in *A. sessilis* (Fig. 3d). Besides, there were no effects of nitrogen availability or interactions on any relative ratios (Fig. 3).

4. Discussion

4.1. Growth and physiological responses to herbivory

Our results clearly show the similarity of growth and physiological responses to the aboveground herbivore *A. hygrophila* between the two species of *Alternanthera*. For herbivory-induced growth responses, the short-period herbivory by *A. hygrophila* dramatically reduced the aboveground development (leaf and stem productions) of both *A. philoxeroides* and *A. sessilis*. The removal of photosynthetic structures caused by *A. hygrophila* further reduced the net carbon gain of the two species of *Alternanthera*, thereby resulting in a severe limitation in ramet production and biomass accumulation (Sun et al., 2010; Wei et al., 2016). Besides, both plant species also exhibited the same pattern for resource allocation under aboveground herbivory, i.e., they could maintain root growth and increase the allocation of root mass, functioning as a potential buffer against the direct attack by aboveground herbivores. For herbivory-induced physiological responses, the short-period herbivory basically cannot alter the physiological property of different organs of both plant species, except the concentrations of stem starch and non-structural carbohydrates in *A. sessilis*.

The results of growth and physiological responses combinedly support the first hypothesis, suggesting that the species of *Alternanthera* did not appear to directly compensate for the tissue loss caused by herbivory, but instead, allocated relatively more resources to

Table 1
ANOVAs results for effects of species identity (*Alternanthera philoxeroides* or *A. sessilis*), herbivory (without or with herbivory by *Agasicles hygrophila*), and nitrogen availability (high or low) on growth and root to shoot ratio.

	Species (S)		Herbivory (H)		Nitrogen (N)		S × H		S × N		H × N		S × H × N	
	$F_{1, 72}$	P	$F_{1, 72}$	P	$F_{1, 72}$	P	$F_{1, 72}$	P	$F_{1, 72}$	P	$F_{1, 72}$	P	$F_{1, 72}$	P
Total mass	**4.7**	**0.034**	**34.2**	**< 0.001**	0.3	0.616	1.2	0.278	2.2	0.145	0.3	0.583	0.1	0.809
Leaf mass	**18.3**	**< 0.001**	**53.5**	**< 0.001**	**14.5**	**< 0.001**	0.9	0.347	2.7	0.103	**4.2**	**0.044**	< 0.1	0.937
Stem mass	**17.1**	**< 0.001**	**33.1**	**< 0.001**	0.3	0.611	0.5	0.477	< 0.1	0.899	< 0.1	0.970	< 0.1	0.891
Root mass[1]	**29.5**	**< 0.001**	1.8	0.179	**19.5**	**< 0.001**	3.0	0.089	**5.0**	**0.028**	0.6	0.426	1.0	0.311
Number of ramets[1]	**99.9**	**< 0.001**	**46.1**	**< 0.001**	**17.2**	**< 0.001**	0.1	0.761	3.1	0.084	**5.6**	**0.021**	2.8	0.101
Number of leaves[1]	**39.9**	**< 0.001**	**57.2**	**< 0.001**	3.3	0.075	2.7	0.105	1.6	0.204	**6.9**	**0.011**	2.1	0.150
Leaf area	**20.1**	**< 0.001**	**45.2**	**< 0.001**	**10.7**	**0.002**	0.3	0.598	2.5	0.115	2.8	0.098	< 0.1	0.934
Root to shoot ratio	**51.6**	**< 0.001**	**36.1**	**< 0.001**	**8.4**	**0.005**	**14.2**	**< 0.001**	2.5	0.121	0.2	0.675	0.2	0.638

[1] Data are square-root transformed. Values for which $P < 0.05$ are in bold.

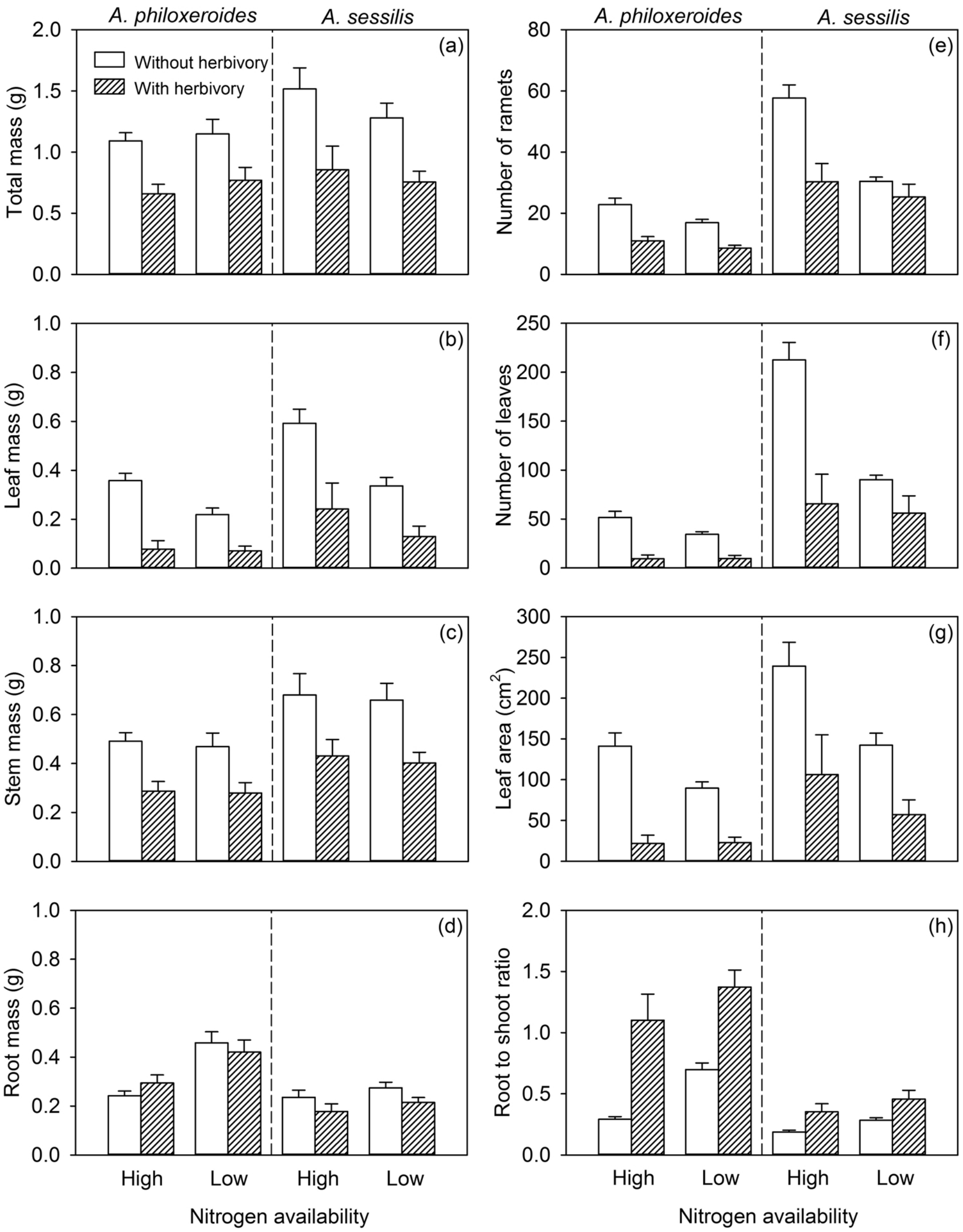

Fig. 1. Effects of species identity (*Alternanthera philoxeroides* or *A. sessilis*), herbivory (without or with herbivory by *Agasicles hygrophila*), and nitrogen availability (high or low) on growth (a–g) and root to shoot ratio (h). Bars and vertical lines show mean + standard error (n = 11 for *A. philoxeroides*, n = 9 for *A. sessilis*).

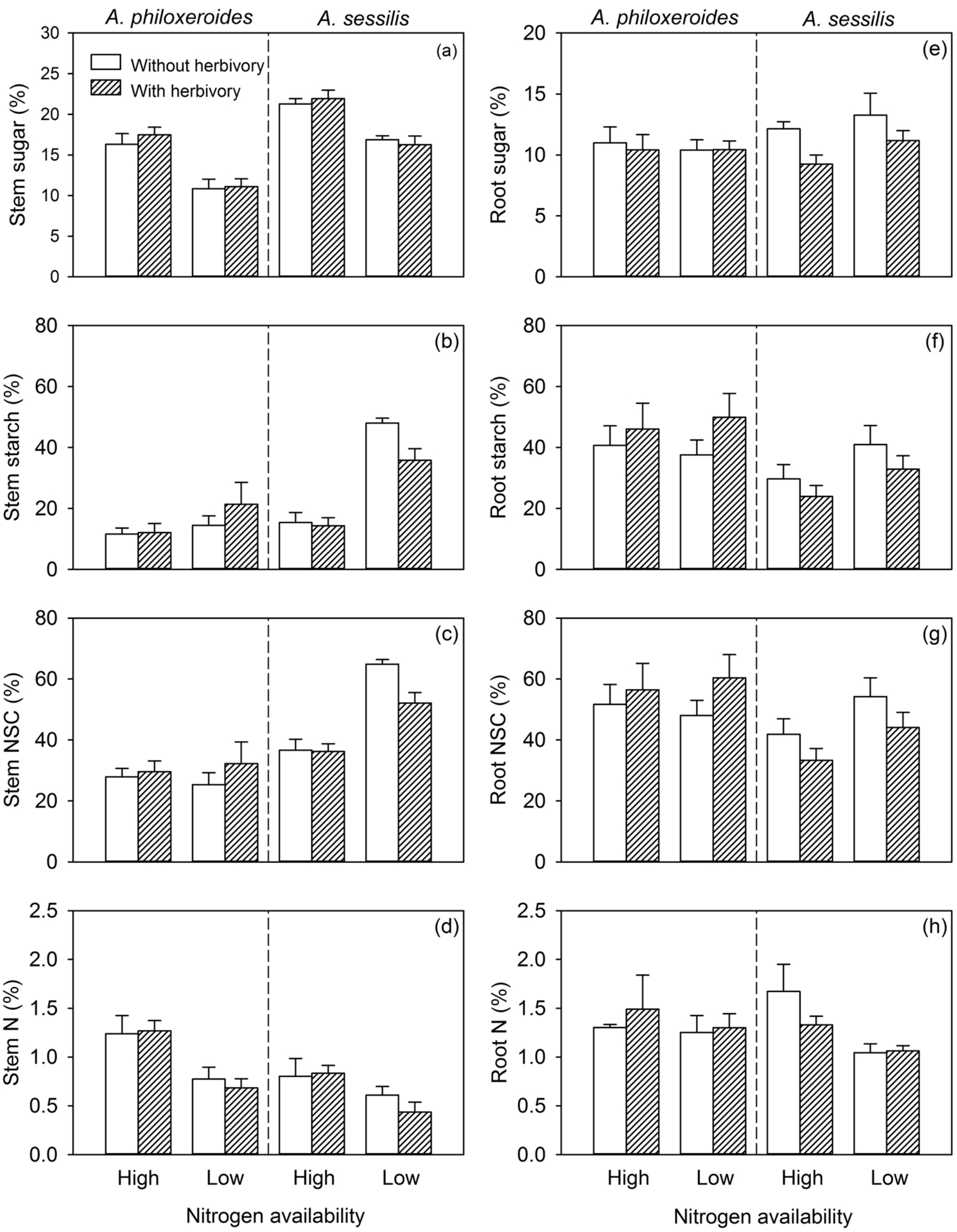

Fig. 2. Effects of species identity (*Alternanthera philoxeroides* or *A. sessilis*), herbivory (without or with herbivory by *Agasicles hygrophila*), and nitrogen availability (high and low) on concentrations of non-structural carbohydrates (NSC, a–c, and e–g) and nitrogen (d and h) in stems and roots. Bars and vertical lines show mean + standard error (n = 6).

Table 2
ANOVAs results for effects of species identity (*Alternanthera philoxeroides* or *A. sessilis*), herbivory (without or with herbivory by *Agasicles hygrophila*), and nitrogen availability (high or low) on concentrations of non-structural carbohydrates (NSC) and nitrogen in stems (a) and roots (b).

	Species (S)		Herbivory (H)		Nitrogen (N)		S × H		S × N		H × N		S × H × N	
	$F_{1,\ 40}$	P	$F_{1,\ 40}$	P	$F_{1,\ 40}$	P	$F_{1,\ 40}$	P	$F_{1,\ 40}$	P	$F_{1,\ 40}$	P	$F_{1,\ 40}$	P
(a) Stems														
Sugars	**54.2**	**< 0.001**	0.3	0.608	**61.1**	**< 0.001**	0.2	0.633	0.4	0.531	0.6	0.446	< 0.1	0.906
Starch[1]	**30.2**	**< 0.001**	0.4	0.556	**45.4**	**< 0.001**	**4.4**	**0.043**	**18.3**	**< 0.001**	0.2	0.634	3.2	0.083
Total NSC[1]	**52.0**	**< 0.001**	0.2	0.660	**18.1**	**< 0.001**	**4.4**	**0.043**	**18.0**	**< 0.001**	0.5	0.499	2.9	0.097
Nitrogen	**13.1**	**0.001**	0.3	0.572	**21.2**	**< 0.001**	0.1	0.817	1.7	0.205	0.8	0.364	0.1	0.811
(b) Roots														
Sugars	1.4	0.248	3.3	0.078	0.7	0.422	2.1	0.154	1.4	0.243	0.2	0.645	< 0.1	0.950
Starch[1]	**7.3**	**0.010**	< 0.1	0.824	1.5	0.234	3.3	0.077	1.2	0.271	< 0.1	0.791	0.3	0.597
Total NSC[1]	**5.9**	**0.020**	< 0.1	0.934	1.7	0.197	4.1	0.050	1.7	0.205	0.1	0.742	0.3	0.606
Nitrogen	0.2	0.653	< 0.1	0.867	**4.8**	**0.035**	1.2	0.286	1.6	0.214	0.2	0.673	0.9	0.342

[1] For the concentrations of starch and total NSC in stems and roots, the degree of freedom of each effect is 1, 39. Values for which $P < 0.05$ are in bold.

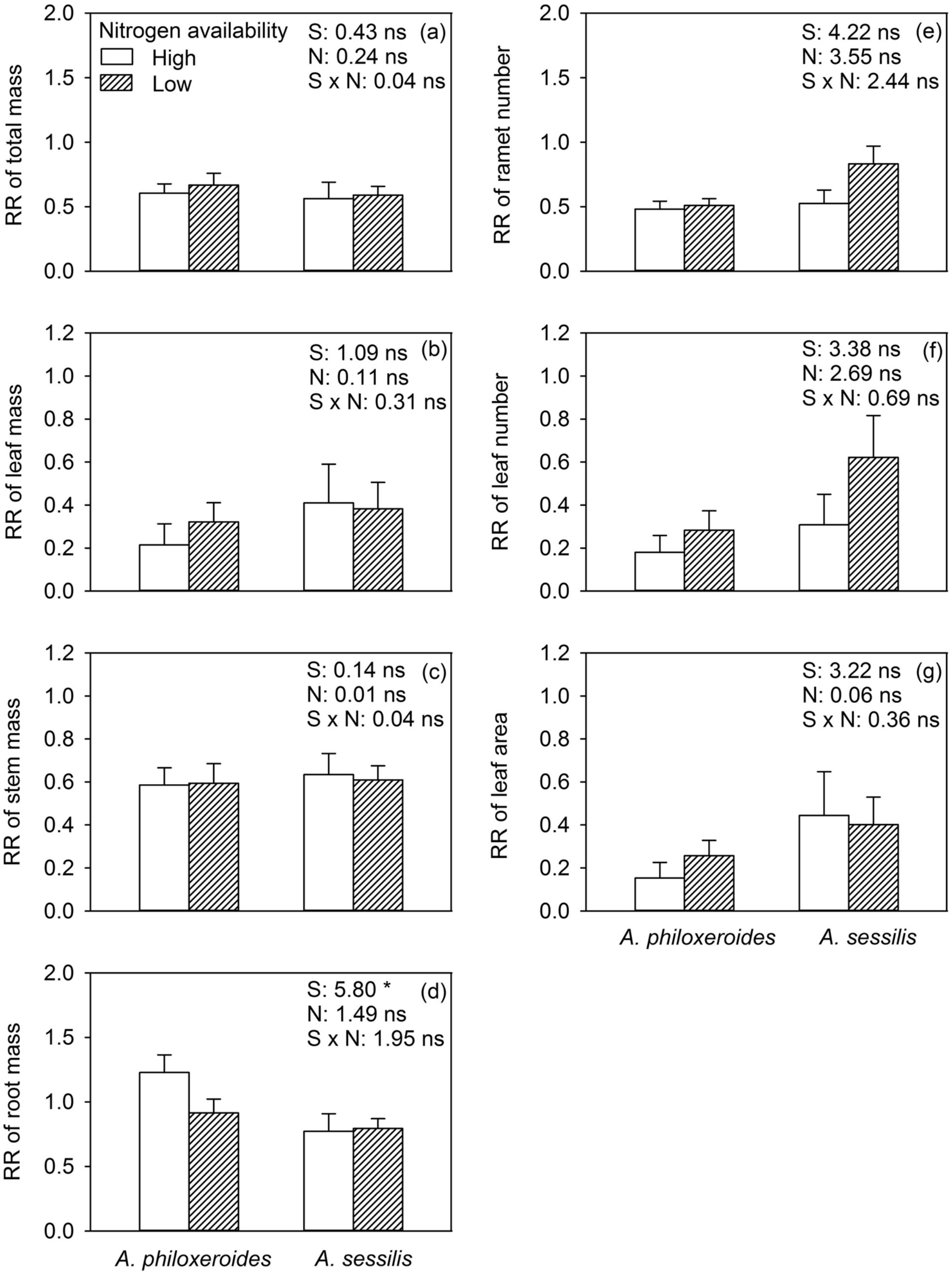

Fig. 3. Effects of species identity (*Alternanthera philoxeroides* or *A. sessilis*), and nitrogen availability (high and low) on relative ratios (RRs) of growth measures (a–g). Bars and vertical lines show mean + standard error. Labels show ANOVAs of effects of species identity (S), nitrogen (N), and S × N, with symbols for *P*: ns > 0.05 and * 0.01–0.05. The degree of freedom of each effect is 1, 36.

underground organs for subsequent growth following herbivory (Wilson et al., 2007; Jia et al., 2009; Robert et al., 2015; Dong et al., 2018). Such resource allocation was also found in *A. philoxeroides* subjected to aboveground herbivory by two other insect herbivores, i.e., *Cassida piperata* (Hui et al., 2016), and *Planococcus minor* (Dong et al., 2017). Nevertheless, You et al. (2014) also reported that the physiological integration between damaged and undamaged ramets of *A. philoxeroides* could to some degree shift the pattern of resource allocation in damaged ramets, and allow them to invest more resources into aboveground organs rather than their belowground part, for the avoidance from aboveground herbivory (You et al., 2014). Therefore, the tolerance to herbivory in clonal plants should be examined in the premise that the influence of physiological integration is confirmed.

In addition, the invasive plant *A. philoxeroides* could exhibit significantly higher biomass allocation and thus accumulate relatively more resources in roots than the native plant *A. sessilis*. The pattern of resource allocation of *A. philoxeroides* under real herbivory were basically consistent with the patterns under simulated herbivory (You et al., 2014). Such differences in herbivory-induced responses between two plant species may be attributed to the variation in life history. More precisely, *A. philoxeroides* has the ability to persistently produce taproots (underground storage organ) during the entire growth period, whereas *A. sessilis* did not. The maintenance and development of taproots allowed *A. philoxeroides* to recover more efficiently from foliar herbivory and other aboveground disturbance (Wilson et al., 2007; Jia et al., 2009; Piippo et al., 2011; Dong et al., 2018). Similarly, because of the maintenance and reallocation of storage resources in roots following herbivory, some invasive plant species such as *Celastrus orbiculata* showed over-compensatory growth and had higher growth rates than their native congeners (Ashton and Lerdau, 2008). Our results thus do not support the second hypothesis, and imply that the specific life-history traits related to root storage may facilitate the invasive plant *A. philoxeroides* to alleviate the fitness decline caused by foliar herbivory, and partly contribute to the potential of invasion under herbivore pressure (Buschmann et al., 2006; Ashton and Lerdau, 2008; Sun et al., 2010; Wei et al., 2016). Furthermore, the results also mirror the fact that the aboveground herbivore *A. hygrophila* could effectively terminate aquatic growth of *A. philoxeroides*, but often fails to control the expansion of *A. philoxeroides* in terrestrial habitats, thereby not being fully successful in all habitats (Sainty et al., 1998; Dong et al., 2018).

4.2. Effects of nitrogen availability on herbivory tolerance

Increasing nitrogen level improved the growth and physiological properties of both *A. philoxeroides* and *A. sessilis*. One the one hand, both plant species growing in the high nitrogen condition obtained more external nitrogen resources to construct aboveground organs (including leaf and ramet productions), and decreased biomass allocation to roots. Such a pattern of biomass allocation is consistent with the optimal partitioning theory predicting that plants allocate relatively more resources to organs that acquire more limiting resources (Bloom et al., 1985; McCarthy-Neumann and Ibáñez, 2013; Tomlinson et al., 2013). On the other hand, increasing nitrogen significantly increased the accumulation of water-soluble sugars in stems of *A. sessilis*, but decreased the accumulation of starch. These results imply that there may be a potential trade-off between different types of non-structural carbohydrates in *A. sessilis*, allowing carbohydrate compounds in stems to switch from storage (starch) to water-soluble sugars to improve resource utilization efficiency for constructing photosynthesis structures in the high nitrogen condition. However, *A. philoxeroides* relied less on the trade-off as a buffer against environmental variability.

Increasing nitrogen availability exacerbated the negative effects of herbivory on ramet and leaf productions of both plant species, supporting the third hypothesis. These results are also consistent with the previous finding, i.e., *A. philoxeroides* demonstrated greater tolerance to simulated herbivory in the nutrient-poor soil than in the nutrient-rich soil (Sun et al., 2009). One plausible reason is that the species of *Alternanthera* growing in the nutrient-poor condition could have a higher priority of root development, which prevents them from the aboveground damage caused by *A. hygrophila* (Alward and Joern, 1993; Hawkes and Sullivan, 2001; Sun et al., 2010). The other plausible reason is that the increase in the availability of non-structural carbohydrates and nitrogen in damaged plants, to some degree elevated the nutritive values of plant tissues and the potential palatability for the herbivores, thereby resulting in leaf over-consumption of herbivores (Agrawal and Weber, 2015; Fan et al., 2016).

5. Conclusions

Our findings suggest that the invasive species *A. philoxeroides* exhibited a stronger tolerance ability in comparison with the native species *A. sessilis*. The tolerance traits of *A. philoxeroides* to herbivory may be strongly attributed to the maintenance and development of underground storage organs (especially taproots) that facilitate a rapid compensatory growth following herbivory, and also to external nitrogen availability. Such knowledge thus provides important implications that the control of aboveground herbivores may become invalid for populations of *A. philoxeroides* with extensive root systems, and that belowground herbivores such as root-knot nematodes should be considered together with aboveground herbivores in the future management of invasive species. Besides, the limitation of our work should be concerned that the present experimental results may only represent a short-term tolerance response of *Alternanthera* to aboveground herbivory, rather than a long-term tolerance strategy (e.g., compensatory growth).

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