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## Increase in nutrient availability promotes success of invasive plants through increasing growth and decreasing anti-herbivory defenses — [Source link](#)

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1     **Increase in nutrient availability promotes success of invasive plants through increasing**  
2                                     **growth and decreasing anti-herbivory defenses**

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14

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25 **Summary:**

26 Invasive plant species often exhibit greater growth and lower anti-herbivory defense than  
27 native plant species. However, it remains unclear how nutrient enrichment of invaded habitats  
28 may interact with competition from resident native plants to affect growth and defense of  
29 invasive plants.

30 In a greenhouse experiment, we grew five congeneric pairs of invasive and native plant  
31 species under two levels of nutrient availability (low vs. high) that were fully crossed with  
32 simulated herbivory (clipping vs. no-clipping) and competition (alone vs. competition).

33 Invasive plants produced more gibberellic acid, and grew larger than native species. Nutrient  
34 enrichment caused a greater increase in total biomass of invasive plants than of native plants,  
35 especially in the absence of competition or without simulated herbivory treatment. Nutrient  
36 enrichment decreased leaf flavonoid contents of invasive plants under both simulated  
37 herbivory conditions, but increased flavonoid of native plants under simulated herbivory  
38 condition. Nutrient enrichment only decreased tannins production of invasive species under  
39 competition. For native species, it enhanced their tannins production under competition, but  
40 decreased the chemicals when growing alone.

41 The results indicate that the higher biomass production and lower flavonoids production in  
42 response to nutrient addition may lead to competitive advantage of invasive species than  
43 native species.

44

45 **Key words:** competition, exotic, interactions, nutrient, phytohormone, secondary metabolites

46

## 47 **1. Introduction**

48 Understanding the physiological and ecological processes underlying invasion success of  
49 alien plant species is an important topic in ecology (Jia *et al.*, 2016; Reilly *et al.*, 2020).  
50 Invasive plants commonly experience herbivory in their native ranges (Keane & Crawley,  
51 2002; Wolfe, 2002), but because plant defense against herbivory incurs significant  
52 physiological and ecological costs (Cipollini *et al.*, 2014), plants often have to trade off  
53 defense against growth and reproduction (Herms & Mattson, 1992). Therefore, theory  
54 predicts that alien plants that become successful invaders are those that have escaped from  
55 their own herbivores and re-allocated limited resources into greater growth and reproduction  
56 at the expense of defense (Keane & Crawley, 2002). In support it, several studies have  
57 reported that invasive plants interact with fewer herbivore species, and thus exhibit less  
58 defense and greater growth in the exotic range than in the native range (Colautti *et al.*, 2004;  
59 Oduor *et al.*, 2011; Meijer *et al.*, 2016; Zhang *et al.*, 2018). Therefore, alien plant species that  
60 become successful invaders may trade-off high growth and reproductive output with low  
61 investments in anti-herbivory defenses.

62 Observational studies have found that low-resource environments are generally less  
63 prone to invasion (Chytrý *et al.*, 2008). Experimental studies also suggest that increased  
64 availability of resources for plant growth can confer invasive species with growth advantage  
65 over native species (D'Antonio & Vitousek, 1992; Bobbink *et al.*, 1998; Davis *et al.*, 2000;  
66 Tilman *et al.*, 2001), because many native plant species are adapted to conditions of soil  
67 low-nutrient and water availability in their natural habitats (Bobbink *et al.*, 1998; Dukes &  
68 Mooney, 1999). In fact, meta-analyses have found that nutrient enrichment is more beneficial  
69 to growth of invasive plant species than of native plant species (González *et al.*, 2010; Liu *et*  
70 *al.*, 2017). Following this logic, nutrient enrichment might also affect defense differently  
71 between invasive plant species and native plant species due to the trade-off between plant

72 growth and defense (Herms & Mattson, 1992). However, how nutrient availability impacts  
73 growth-defense trade-offs of invasive and native plants remains little tested empirically.

74 Competition is important to determine plant invasion success (Levine *et al.*, 2004;  
75 Petruzzella *et al.*, 2020). On the one hand, strong competition from invasive plants often  
76 reduces diversity of native plant species, and results in mono-specific stands of invaders  
77 (Gaertner *et al.*, 2009). Invasive plants exert strong competitive effects on native plants  
78 because invasive plants often have disproportionately higher demand for resources (Leishman  
79 & Thomson, 2005; Funk, 2013). Consequently, nutrient enrichment could confer invasive  
80 plants greater competitive advantage relative to native plants in communities (Seabloom *et al.*,  
81 2015). On the other hand, given that competition from other plants could create stressful  
82 environments, costs of plant defense against herbivory in such environment may also increase  
83 when competition is present (Herms & Mattson, 1992; Siemans *et al.*, 2002). In other words,  
84 competition may amplify the growth-defense trade-offs of plants. However, it remains  
85 unclear whether competition affect trade-offs of invasive and native plants differently.  
86 Therefore, studies testing effects of tests of whether nutrient availability enrichment on  
87 growth-defense trade-offs of invasive and native plants, should also consider whether the  
88 plants grow alone or with competition.

89 Plant growth and defense are generally regulated by different types of hormones. For  
90 example, as the major hormones that stimulate plant growth and development (Ross & Reid,  
91 2010), gibberellic acids (GA) stimulate seed germination, trigger stem elongation, leaf  
92 expansion, flowering and seed development (Yang *et al.*, 2012; Gupta & Chakrabarty, 2013).  
93 However, expression of defense hormones can suppress expression of plant  
94 growth-promoting hormones, because these two type hormones often have negative  
95 cross-talks within the plants (Ross & Reid, 2010; Yang *et al.*, 2012; Vos *et al.*, 2015). For  
96 example, herbivory-induced production of a defense-regulating hormone jasmonic acid (JA)

97 can constrain plant growth by antagonizing production of GA (Machado *et al.*, 2017).  
98 Therefore, invasive plants that escape intense herbivores may produce high concentrations of  
99 growth-promoting hormones (e.g., GAs) and low concentrations of hormones that regulate  
100 anti-herbivore defenses (Liu *et al.*, 2021). However, this prediction has not been tested  
101 empirically.

102 Here, we conducted a greenhouse experiment with five congeneric pairs of invasive and  
103 native plant species to test the following hypotheses: (i) Nutrient enrichment induces invasive  
104 plants to produce greater total biomass and lower concentrations of anti-herbivore defense  
105 compounds than native plants; (ii) Invasive plants express a lower concentration of a defense  
106 hormone JA and a higher concentration of a growth-promoting hormone GA.

## 107 **2. Methods**

### 108 **2.1 Plant species**

109 We used five congeneric pairs of native and invasive clonal plant species from three families  
110 that co-occur naturally in the field in China (**Table S1**). We raised plantlets/seedlings of the  
111 test plant species using seeds and asexual reproductive organs that were collected in the field  
112 (**Table S1**). For asexual species, we first selected intact rhizomes and stolons and cut them  
113 into single-node/bud fragments, and then cultivated the fragments in trays. For the sexually  
114 reproducing species, we directly sowed seeds in trays filled with potting soil (Pindstrup Plus,  
115 Pindstrup Mosebrug A/S, Denmark). The resultant plantlets/seedlings were then raised under  
116 uniform conditions for one month in a greenhouse (temperature: 22-28 °C; natural lighting  
117 with an intensity of *c.* 75% of the light outdoors; and *c.* 60% relative humidity). We then  
118 selected similar-sized plantlets /seedlings of each species for use in the experiment described  
119 below.

### 120 **2.2 Experimental set up**

121 To test whether native and invasive plants differed in their responses to competition and

122 herbivory at different levels of nutrient availability, we performed a fully-crossed factorial  
123 experiment with three factors: simulated herbivory (clipping vs. no-clipping), competition  
124 (alone vs. competition), and nutrient availability (low vs. high). Each treatment combination  
125 was replicated six times, which resulted in a total of 480 pots (i.e. 5 congeneric pairs of plants  
126  $\times$  2 levels of invasion status per plant pair  $\times$  2 simulated herbivory levels  $\times$  2 competition  
127 levels  $\times$  2 nutrient treatment levels  $\times$  6 replicates). The plants were grown in 2.5-L circular  
128 plastic pots (top diameter  $\times$  bottom diameter  $\times$  height: 18.5  $\times$  12.5  $\times$  15 cm) that had been  
129 filled with a 1:1 mixture of sand and fine vermiculite. On 24<sup>th</sup> March 2020, we transplanted  
130 48 similar-sized individuals of each species into the center of the pot individually as target  
131 plants. For pots that received plant competition treatments, we planted three similar-sized  
132 seedlings of *Taraxacum mongolicum* around the target individual plants. We used *T.*  
133 *mongolicum* as a competitor because it commonly co-occurs with all the study species in  
134 various habitats in China (Chen *et al.*, 2015). Plastic dishes were placed underneath each pot  
135 to hold water and nutrients that had been applied to the pots. All pots were randomly assigned  
136 to positions on four benches in a greenhouse, with a temperature range of 22 °C to 30 °C and  
137 natural light/darkness cycle.

138 We started the nutrient treatment on the second week following transplant. To impose  
139 low and high nutrient treatments, we added 0.2 and 1 g L<sup>-1</sup> of a fertilizer solution (Peters  
140 Professional 20-20-20 General Purpose Fertilizer, Everris NA Inc., Dublin, OH, USA),  
141 respectively. Fifty milliliters of the respective nutrient solutions were supplied to the soil in  
142 the pots every week for 14 weeks. As all the experimental plant species were prone to  
143 herbivory by generalist above-ground herbivores in the natural habitats (Dang *et al.*, 2012;  
144 Hu & Dong, 2019), we simulated above-ground herbivory effect on 17<sup>th</sup> June 2020 (i.e., on  
145 the 12<sup>th</sup> week following transplant). To remove *c.* 50% of leaf biomass of target plants as  
146 would happen under natural herbivory in the field, we clipped each leaf once by half across

147 the midvein using a pair of scissors (for a similar approach, see Lurie *et al.*, 2017; Kempel *et*  
148 *al.*, 2020). The plants were watered once every day until harvest. The experiment was  
149 conducted in a greenhouse of Northeast Institute of Geography and Agroecology, Chinese  
150 Academy of Sciences (125°24'30"E, 43°59'49"N).

### 151 **2.3 Harvest and measurements**

152 On 27<sup>th</sup> March 2020 (i.e. three days after transplanting), we measured initial height of each  
153 target individual plant. Two hours after clipping treatment (i.e. simulated herbivory  
154 application) on 17<sup>th</sup> June 2020, we randomly selected three replicates of each target species  
155 under each treatment combination, and collected leaf samples for the measurement of GA3  
156 (i.e. one of the principal gibberellic acids) and JA (Gupta & Chakrabarty, 2013; Camara *et al.*,  
157 2018). For the collection of leaves, we collected most recently matured leaves (the first fully  
158 expanded leaves, about 3-6 leaves below the shoot apex), froze them immediately in liquid  
159 nitrogen, and then stored the frozen samples at -80 °C until chemical analyses were performed  
160 as described below. Twelve days after the clipping treatment had been applied, we collected  
161 all leaf samples from the remaining three replicates of each target species under each  
162 treatment combination for the measurement of two groups of anti-herbivore defense  
163 compounds flavonoids and tannins. We used different replicates for plant hormones and  
164 defense compounds measurements, because we also cut leaves for plants under no-clipping  
165 condition when collecting samples for hormones measurements, which could induce changes  
166 of defense compounds. All leaves were dried at 40 °C to a constant biomass before being  
167 subjected to flavonoid and tannin analysis. We then harvested the left above-ground and  
168 below-ground biomass separately for each target plant immediately. The fresh biomass was  
169 then dried at 65 °C to constant weight and then weighed. As target or competitor plants died in  
170 six pots during the experiment, we harvested plants in 474 pots out of the original 480 pots.  
171 We calculated total biomass by summing the all above-ground and below-ground biomass.



172 We also calculated the root mass fraction of each target plant as the ratio between the  
173 below-ground biomass and the total biomass.

174 For analysis of JA and GA3, all leaf samples were subjected to enzyme-linked  
175 immunosorbent assay (ELISA) procedure following previous studies (Dai *et al.*, 2016). For  
176 analysis of flavonoid (quercetin, isoquercetin, quercetin glycoside, kaempferitrin, and  
177 kaempferol) and tannin (gallic acid, catechin, tannic acid, and ellagic acid) compounds, all  
178 leaf samples were firstly ground to a fine powder (< 0.25 mm) using a ball mill (MM400;  
179 Retsch, Haan, Germany), and then subjected to a high-performance liquid chromatography  
180 (HPLC) analysis (Wang *et al.*, 2012). To quantify concentrations of individual flavonoids, 50  
181 mg of ground leaf tissue samples were extracted with a 100% methanol-0.4% phosphoric acid  
182 (48:52, v:v) solution for 24 h. Next, we used 0.22- $\mu$ m membrane to filter the extract, and then  
183 injected the filtrate (40  $\mu$ L) into HPLC system for quantification. To quantify concentrations  
184 of tannins in plant leaves, 50 mg of ground leaf tissue samples were extracted in a 50%  
185 aqueous methanol solution for 30 min. Next, we used 0.45- $\mu$ m membrane to filter the extract,  
186 and then injected the filtrate (40  $\mu$ L) into HPLC system for quantification of individual  
187 tannins.

## 188 **2.4 Statistical analyses**

189 All statistical analyses were performed in R v4.0.2 (R Core Team, 2020). To test for main  
190 and interactive effects of species status, simulated herbivory, competition, and nutrient  
191 addition treatments on growth performance (i.e. total biomass production and root biomass  
192 allocation), concentrations of hormones (i.e. GA3 and JA) and chemical defense compounds  
193 (flavonoids and tannins) of target plants, we fitted linear mixed-effects models with the *lme*  
194 function in the *nlme* package (Pinheiro *et al.*, 2020). In the models, total biomass, root mass  
195 fraction, leaf concentrations of GA3, JA, flavonoids, and tannins of target species were  
196 specified as response variables, while species status (native vs. invasive), simulated herbivory

197 (clipping vs. no-clipping), competition (alone vs. competition), nutrient availability (low vs.  
198 high) treatments and their all interactions were specified as fixed-effect-independent  
199 variables. To meet the assumptions of normality of variance, total biomass and GA3  
200 concentrations were square-root-transformed, while concentrations of flavonoids, tannins,  
201 and JA were natural-log-transformed. As initial size of plantlets/seedlings can influence the  
202 final growth performance of a species, we also added initial plant height as scaled  
203 natural-log-transformed\_covariates in the models for total biomass and root mass fraction.

204 To account for non-independence of replicates of the same species and for phylogenetic  
205 relatedness among target species, we also included target species nested within genus as  
206 random effects in all models. In addition, we also included reproduction modes as random  
207 factor in all models to account for the non-independence of replicates of the same  
208 reproduction modes. As the data did not fulfill homoscedasticity assumption, for the analyses  
209 of total biomass, root mass fraction and flavonoids, we included variance structures to allow  
210 for variance among species using the “*varIdent*” function in the R package “*nlme*” (Pinheiro  
211 *et al.*, 2020). In the linear mixed-effect models described above, we assessed the significance  
212 of fix-effect independent variables (i.e., species status, competition, simulated herbivory,  
213 nutrient availability treatment and interactions among the four factors) using likelihood-ratio  
214 tests. The variance components were estimated using the restricted maximum-likelihood  
215 method of the full model (Zuur *et al.*, 2009).

### 216 **3. Results**

#### 217 **3.1 Biomass production and allocation**

218 Nutrient enrichment enhanced total biomass more for invasive species than for native species,  
219 in particular when plants grew alone (i.e., significant three-way interaction between species  
220 status, nutrient enrichment, and competition in Table 1; Fig. **1a**). Specially, the biomass  
221 increase of invasive species under competition was +127.0%, native species under

222 competition +118.5%, invasive species growing alone +134.6%, and native species growing  
223 alone +124.3%. Moreover, nutrient enrichment caused a greater increase in total biomass of  
224 invasive plants than of native plants, especially in the absence of simulated herbivory  
225 condition (i.e. significant three-way interaction between species status, nutrient enrichment,  
226 and simulated herbivory in Table 1; Fig. **1b**). Specifically, the absolute biomass increase of  
227 invasive species under clipping treatment was +4.9 g, native species under clipping treatment  
228 +2.9 g, invasive species under no-clipping treatment +6.0 g, and native species under  
229 no-clipping treatment +3.3 g.

230 Total biomass production was also influenced significantly by two-way interactive  
231 effects of species status and nutrient enrichment, species status and competition, nutrient  
232 enrichment and competition, and competition and simulated herbivory (Table 1). When  
233 plants grew alone, simulated herbivory reduced the mean total biomass of target plants by  
234 -14.2%, and under competition, it reduced the total biomass by -13.0% (Fig. **1c**). Total  
235 biomass produced by the target plants was also influenced significantly by the main effects of  
236 plant invasion status, nutrient enrichment treatment, simulated herbivory, and competition  
237 (Table 1). Competition increased (Fig. **1d**), but nutrient enrichment decreased (Fig. **1e**) the  
238 proportion of total biomass that was allocated to the roots (i.e., root mass fraction). Simulated  
239 herbivory increased root mass fraction of the test plant species (Fig. **1f**).

### 240 **3.2 Defense compounds**

241 Simulated herbivory (i.e. leaf clipping) and nutrient enrichment had different effects on  
242 flavonoids concentrations in the leaves of invasive and native species (significant three-way  
243 interaction between plant invasion status, simulated herbivory, and nutrient availability  
244 treatment in Table 1; Fig. **2c**). Specially, for invasive plants, nutrient enrichment decreased  
245 concentrations of flavonoids similarly under both simulated herbivory treatments (clipping vs.  
246 no-clipping: -9.3% vs. -11.8%; Fig. **2c**). In contrast, for native plant species, nutrient

247 enrichment increased flavonoid concentrations by +62.2 % under simulated herbivory (leaf  
248 clipping), whereas the reverse was true in the absence of simulated herbivory (no-clipping)  
249 treatment (-35.5%; Fig. **2c**). Flavonoids concentrations were also influenced significantly by  
250 a two-way interaction between plant invasion status and nutrient availability treatments, and  
251 by main effects of nutrient availability and competition treatments (Table 1). Competition  
252 increased the leaf concentrations of flavonoids by +27.8 % (Fig. **2a**) and of tannins by  
253 +13.2 % (Fig. **2b**). Leaf tannin concentrations were influenced by interactive effects of  
254 species status, competition, and nutrient enrichment (Table 1; Fig. **2d**). Specifically, for  
255 invasive plants, nutrient enrichment decreased tannin concentrations by -19.9% under  
256 competition, while it had little effect in the absence of competition (+0.7%; Fig. **2d**). For  
257 native species, nutrient enrichment enhanced tannin content under competition (+23.5%),  
258 whereas the reverse was true in absence of competition (Fig. **2d**).

### 259 **3.3 Plant hormones**

260 Invasive plants expressed higher (+24.9%) GA3 content in the leaves than native plants  
261 (Table 1; Fig. **3a**). The JA content in leaves was not significantly influenced by the separate  
262 effects of species status, nutrient enrichment, simulated herbivory, competition, and  
263 interactions among them (Table 1). Nevertheless, invasive plants tended to produce a greater  
264 amount of JA than native plants ( $p = 0.064$ ; Fig. **3b**).

## 265 **4. Discussion**

### 266 **4.1 Biomass production and allocation**

267 We found that nutrient enrichment enhanced total biomass production of invasive plant  
268 species more than it did for native plant species, more so in the absence of competition (Fig.  
269 **1a**). These results support those of other studies, which found that nutrient enrichment often  
270 promoted growth of invasive plant species over natives (Dawson *et al.*, 2012; Parepa *et al.*,  
271 2013; Liu *et al.*, 2017). Invasive plant species generally benefit more from an increase in

272 resource availability than non-invasive species because invaders often have inherent fast  
273 growth strategies and the ability to rapidly exploit high-resource conditions (Dukes &  
274 Mooney, 1999; van Kleunen *et al.*, 2010; Dawson *et al.*, 2011). Moreover, high  
275 resource-use-efficiency can promote growth of some invasive species (Funk & Vitousek,  
276 2007). Future studies may unravel which of these physiological mechanisms underlie the  
277 capacity of the current test invasive species to have greater total biomass than native species  
278 under nutrient enrichment. Overall, the present results support the idea that nutrient  
279 enrichment could enhance invasiveness of alien plant species that are already invasive.

280 Competition from a common native *T. mongolicum* lowered the beneficial effects of high  
281 nutrient enrichment for both native and invasive plants although the suppressive effect of the  
282 native competitor was higher on the invasive species (Fig. 1a). These results suggest that the  
283 current invasive species are generally more sensitive to competition from *T. mongolicum* than  
284 the native plant species. As the native plants have co-existed with *T. mongolicum* longer than  
285 the invasive species have, it is likely that the native plant species are more strongly adapted to  
286 competition from *T. mongolicum* than the invasive species. Indeed, empirical studies show  
287 that native plants can evolve adaptation to competition from invasive plant species, and that  
288 native plants can also exert strong competitive effects on invasive plants (Oduor, 2013,  
289 2021).

290 Our results complement those of other studies that tested the effects of nutrient  
291 enrichment on competitiveness of invasive plants, with variable outcomes. For example,  
292 Zhang *et al.* (2017) found that nutrient enrichment enhanced the competitive dominance of an  
293 invader *Alternanthera philoxeroides* over two natives *Oenanthе javanica* and *Iris*  
294 *pseudacorus*. In contrast, high nutrients diminished the competitiveness of an invasive herb  
295 *Hydrocotyle vulgaris* against a native plant community (Liu *et al.*, 2016). Moreover, the  
296 invasive grass *Agrostis capillaris* suppressed growth of two co-occurring native grasses *Poa*

297 *cita* and *Poa colensoi* in New Zealand regardless of nitrogen availability (Broadbent *et al.*,  
298 2018). The mix findings may because that these studies did not test the potential interactive  
299 effects of nutrient enrichment and herbivory on competitiveness of invasive and native plants.  
300 Our present study tested it, and found that nutrient enrichment caused a greater increase in  
301 total biomass of invasive plants than of native plants, especially in absence of simulated  
302 herbivory treatment (Fig. **1b**). In other words, nutrient enrichment may act dependently of  
303 herbivory to affect competitiveness of invasive and native plants. Additional studies in other  
304 invasion systems may help to clarify the relative and combined effects of herbivory and  
305 nutrient enrichment on competitive interactions between invasive and native plant species.

306 Simulated herbivory caused a slightly greater decline in total biomass of the target plants  
307 in the absence of competition than in the presence of competition against *T. mongolicum* (Fig.  
308 **1c**), which suggests that simulated herbivory stimulated compensatory growth in the target  
309 plants. Herbivory can induce compensatory growth of the host plants specie through various  
310 mechanisms including increased rates of photosynthesis, increased growth rate, and increased  
311 allocation of biomass to the roots (Strauss & Agrawal, 1999; Stowe *et al.*, 2000). We found  
312 that compensatory growth for invasive plants tended to be greater under competition than that  
313 in absence of competition (Fig. **S1**), which supports findings from other studies that  
314 simulated herbivory can stimulate compensatory growth in various invasive plants when  
315 grown in competition with other plants. For instance, leaf clipping of the invaders *Centaurea*  
316 *solstitialis* (Callaway *et al.*, 2006) and *C. melitensis* (Callaway *et al.*, 2008) and herbivory by  
317 a community of insects on invasive individuals of *Brassica nigra* (Oduor *et al.*, 2013)  
318 enhanced growth of the host plants under interspecific competition. As the current focal  
319 plants had a greater root mass fraction under simulated herbivory and competition treatments  
320 (Fig. **1d**), it is likely that the focal plants deploy increased allocation of biomass to the roots  
321 as strategy to cope with simultaneous herbivory and competition. Future studies may unravel

322 the other mechanisms of compensatory growth in the current study species.

## 323 **4.2 Plant defense compounds**

324 Invasive plants did not express a significantly lower concentration of the two classes of  
325 defense compounds tannins and flavonoids than native plants across the different treatment  
326 levels, which does not support a prediction of the idea that invaders should invest less in  
327 defense compounds (Keane & Crawley, 2002). As invaders escape from the specialist  
328 enemies but still face generalist enemies in the exotic range (Liu & Stiling, 2006; Meijer *et*  
329 *al.*, 2016), it is plausible that invasive plants maintain a high concentrations of anti-herbivory  
330 chemicals to deter generalist herbivores. Indeed, Ni *et al* (2020) found that the invasive  
331 species *Alternanthera philoxeroides*, *Mikania micrantha* and *Wedelia trilobata* expressed  
332 significantly higher tannin concentrations than congeneric native species *A. sessilis*, *P.*  
333 *scandens* and *W. chinensis*. Similarly, total phenolic content of nine invasive plant species  
334 was 2.6-fold higher than that of nine native species in East Asia (Kim & Lee, 2011).

335 The finding that flavonoids and tannins concentrations were higher under competition  
336 treatment suggests that these metabolites may additionally have been involved in allelopathic  
337 interactions (Enge *et al.*, 2012; Qi *et al.*, 2020) between the target plants and the competitor  
338 species *T. mongolicum*. Invasive species produced greater concentrations of tannins than  
339 native species across the two nutrient treatments and in the absence of competition, although  
340 this trend was reversed by both high nutrient enrichment and competition (Fig. **2d**). This  
341 indicates that plant defense compounds tannins may play a greater allelopathic role in the  
342 native species than in the invasive species. In fact, flavonoids and tannins have been  
343 implicated in allelopathic interference in natural soils (Putnam & Duke, 1978; Weston &  
344 Mathesius, 2013), and it is likely to increase production of such allelopathic chemicals when  
345 stressed by other competitor plants (Lankau & Kliebenstein, 2009). This has been  
346 demonstrated for other groups of plant compounds. For example, *B. rapa* expressed higher

347 concentrations of glucosinolate compounds when grown in interspecific competition  
348 (Siemens *et al.*, 2002). In another study, the North American invader *B. nigra* increased  
349 production of glucosinolate compounds when grown in interspecific competition with three  
350 species (*Amsinckia menziesii*, *Malva parviflora*, and *Sonchus oleraceus*) (Lankau & Strauss,  
351 2008). Future studies may test the relative allelopathic effects of tannins and flavonoids of the  
352 current test invasive and native plants against their natural competitors.

353 Nutrient treatment caused a decline in leaf flavonoid concentrations of invasive plants  
354 under both levels of simulated herbivory treatments, while it caused an increase in leaf  
355 flavonoid concentrations of native plants under simulated herbivory treatment and decreased  
356 the concentration under condition without simulated herbivory (Fig. 2c). These results  
357 suggest that native plants rather than invasive plants may invest more in flavonoid-based  
358 defense against herbivores, and consequently grew slower than invasive plants in response to  
359 nutrient enrichment. On the other hand, under the condition without simulated herbivory,  
360 native plants would invest less in flavonoid-based defense against herbivores in response to  
361 nutrient addition. This is why native plants, in response to nutrient enrichment, increased  
362 growth stronger under condition without simulated herbivory than under simulated herbivory  
363 condition. Therefore, our study indicates that the increased investment in biomass and  
364 simultaneous decreased investment in flavonoid production by the focal invasive species in  
365 response to nutrient addition may facilitate their invasion success under scenarios of  
366 increased nutrient enrichment of invaded habitats.

### 367 **4.3 Plant hormones**

368 We found that invasive plants expressed a higher concentration of GA3 than native plants  
369 (Fig. 3a). This supports our second hypothesis that invasive species often grow larger than  
370 native species because invaders produce greater amount of growth-promoting hormones  
371 (including GA3) than native species. However, contrary to our hypothesis, invasive plants did



372 not express a lower concentration of JA than native plants (Fig. **3b**). Simulated herbivory  
373 induced a slightly higher amount of GA under high nutrient condition, but a lower amount of  
374 GA under low nutrient condition (Fig. **S2**). As the plants produced a lower amount of total  
375 biomass under simulated herbivory (Fig. **1b**), it is likely that an increase in GA stimulated a  
376 compensatory growth in the focal plants under high nutrient condition. Gibberellic acids can  
377 increase nutrient uptake, which is necessary for increased growth. For instance, exogenous  
378 application of GA on *Cicer arietinum* caused the plant to accumulate higher concentrations of  
379 the macronutrients nitrogen, phosphorus, and potassium (Rafique *et al.*, 2021). Therefore,  
380 nutrient enrichment may have synergistic effects with GA on plant growth. The invasive  
381 plants expressed a slightly higher concentration of JA than native plants (Fig. **3b**). As the  
382 invasive plants produced higher concentrations of flavonoids under simulated herbivory  
383 (especially under low nutrient condition), it is likely that the hormone stimulated a higher  
384 production of such defense compounds. In fact, exogenous spray of JA on plants has been  
385 shown to induce a high concentration of flavonoids (War *et al.*, 2014). The higher  
386 concentrations of JA and the flavonoids content in invasive plants suggest that the plants may  
387 be more strongly defended against herbivores than native plants.

### 388 **Conclusion**

389 Our results indicate that the higher expression of growth-related hormones could contribute  
390 the larger growth of invasive species than native species. Moreover, the lower investment of  
391 invasive species in the anti-herbivory chemicals when they grew under high nutrient  
392 conditions could lead to their stronger growth response to nutrient enrichment. Our study  
393 provides the evidence that higher investment of growth and lower investment of  
394 anti-herbivory chemicals, at least flavonoids in response to nutrient enrichment would lead to  
395 competitive advantage of invasive alien species than native species.

396

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402

403 **Author contributions**

404 YL conceived the idea and designed the experiment. LS performed the experiment. LS and  
405 YL analyzed the data. LS and AO wrote the first draft of the manuscript, with major inputs  
406 from YL and further inputs from WH.

407

408 **Data accessibility**

409 Should the manuscript be accepted, the data supporting the results will be archived in Dryad  
410 and the data DOI will be included at the end of the article.

411

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596 **Figure legends**

597 **Figure 1** Mean ( $1 \pm$  SE) biomass production and allocation of five congeneric pairs of  
598 invasive and native plant species that were grown in a common greenhouse condition. The  
599 panels show effects of: **(a)** three-way interaction between plant invasion status (invasive vs.  
600 native), nutrient enrichment (low vs. high), and competition (alone vs. competition [comp])  
601 on total biomass; **(b)** three-way interaction between plant invasion status, nutrient enrichment,  
602 and simulated herbivory (clipping [clip] vs. no-clipping [no-clip]) on total biomass; **(c)**  
603 two-way interaction between competition and simulated herbivory on total biomass; **(d)** main  
604 effects of competition on plant root mass fraction; **(e)** main effects of nutrient enrichment on  
605 plant root mass fraction; **(f)** main effects of simulated herbivory on plant root mass fraction.  
606 The figure here only presents the significant main and interactive effects.

607 **Figure 2** Mean ( $1 \pm$  SE) concentrations of defense compounds (flavonoids and tannins) of  
608 five congeneric pairs of invasive and native plant species that were grown in a common  
609 greenhouse condition. The panels show effects of: **(a)** main effects of competition (alone vs.  
610 competition [comp]) on concentrations of flavonoids; **(b)** main effects of competition on  
611 concentrations of tannins; **(c)** three-way interaction between plant invasion status (invasive vs.  
612 native), nutrient enrichment (low vs. high), and simulated herbivory (clipping [clip] vs.  
613 no-clipping [no-clip]) on flavonoids concentrations; **(d)** three-way interaction between plant  
614 invasion status, competition and simulated herbivory on tannins concentrations. The figure  
615 here only presents the significant main and interactive effects.

616 **Figure 3** Mean ( $1 \pm$  SE) concentrations of growth- and defense-enhancing hormones (GA3  
617 and JA) of five congeneric pairs of invasive and native plant species that were grown in a  
618 common greenhouse condition. The panels show effects of main effect of plant invasion  
619 status (invasive vs. native) on **(a)** GA3 concentrations and **(b)** JA concentration.

620 **Tables**

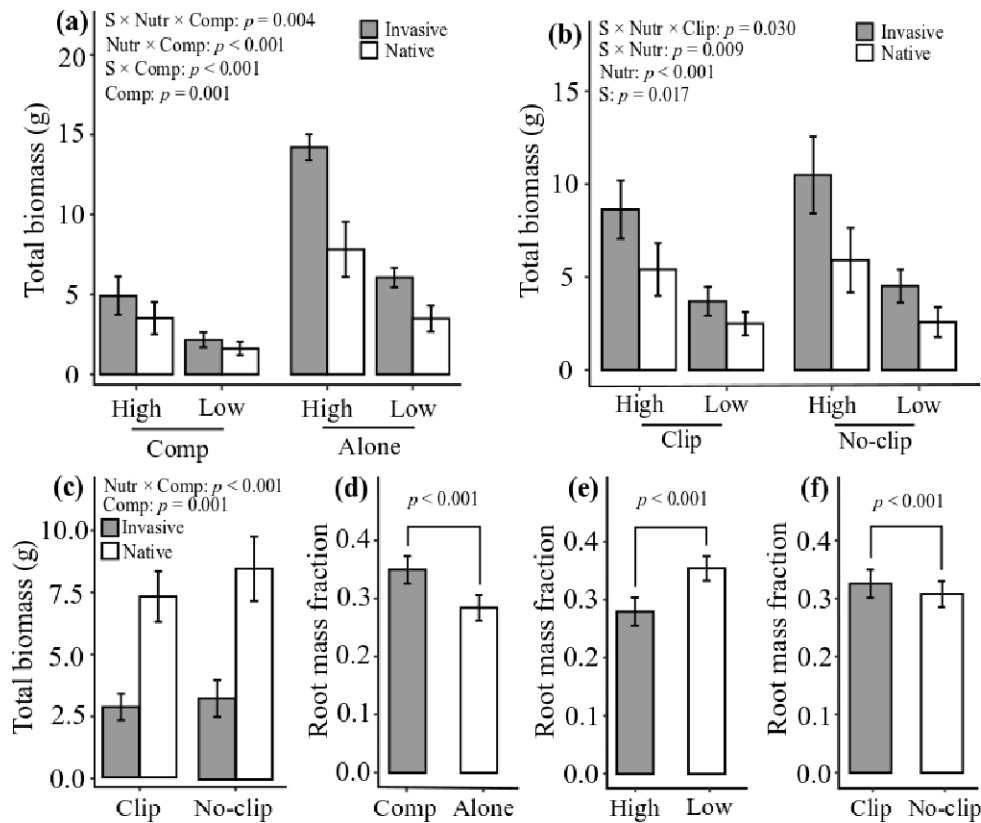
621 **Table 1** Results of linear mixed-effect models that tested main and interactive effects of plant invasion status (invasive vs. native), nutrient  
 622 enrichment (low vs. high), simulated herbivory (clipping vs. no-clipping), and competition (alone vs. competition) on growth performance of  
 623 five congeneric pairs of plant species. Significant effects ( $P < 0.05$ ) are in bold, while marginally significant effects ( $0.05 \leq P < 0.1$ ) are underlined  
 624 and in bold.

	Total biomass (g) (square root transformed)		Root mass fraction (logit- transformed)		Flavonoids (% dw) (ln- transformed)		Tannins (% dw) (ln- transformed)		GA3 (mg/g) (square root-transformed)		JA (mg/g) (ln- transformed)	
	$\chi^2(df = 1)$	<i>p</i>	$\chi^2(df = 1)$	<i>p</i>	$\chi^2(df = 1)$	<i>p</i>	$\chi^2(df = 1)$	<i>p</i>	$\chi^2(df = 1)$	<i>p</i>	$\chi^2(df = 1)$	<i>p</i>
<b>Fixed effects</b>												
Initial Height	0.10	0.75	7.21	<b>0.007</b>	-	-	-	-	-	-	-	-
Invasion status (S)	5.66	<b>0.017</b>	3.77	<u>0.052</u>	1.15	0.284	1.57	0.211	4.69	<b>0.030</b>	3.44	<b>0.064</b>
Nutrient enrichment (Nutr)	304.52	<b>&lt;0.001</b>	113.46	<b>&lt;0.001</b>	5.22	<b>0.022</b>	0.07	0.786	0.36	0.547	2.74	<u>0.098</u>
Clipping (Clip)	15.56	<b>&lt;0.001</b>	14.16	<b>&lt;0.001</b>	2.99	<u>0.084</u>	<0.01	0.980	0.73	0.392	0.16	0.692
Competition (Comp)	391.29	<b>0.001</b>	24.13	<b>&lt;0.001</b>	7.04	<b>0.008</b>	4.47	<b>0.035</b>	1.32	0.251	1.50	0.221
S × Nutr	6.82	<b>0.009</b>	2.78	<u>0.096</u>	5.84	<b>0.016</b>	1.52	0.218	0.45	0.501	0.03	0.867
S × Clip	2.62	0.106	1.27	0.260	<0.01	0.958	0.17	0.679	1.61	0.204	0.26	0.609
S × Comp	59.36	<b>&lt;0.001</b>	0.19	0.660	1.61	0.205	1.31	0.252	0.07	0.797	0.11	0.746
Nutr × Clip	0.83	0.363	-0.34	1.000	0.09	0.770	0.62	0.431	2.78	<u>0.095</u>	0.23	0.634
Nutr × Comp	49.56	<b>&lt;0.001</b>	<0.01	0.952	2.41	0.120	0.26	0.611	1.59	0.208	0.10	0.756
Comp × Clip	4.04	<b>0.044</b>	0.08	0.783	<0.01	0.952	0.03	0.864	0.32	0.571	0.13	0.724
S × Clip × Comp	2.75	<u>0.097</u>	0.13	0.714	0.03	0.873	1.12	0.289	1.32	0.252	0.13	0.720
S × Nutr × Clip	4.74	<b>0.030</b>	0.05	0.818	5.72	<b>0.017</b>	0.52	0.473	1.34	0.247	0.75	0.386
S × Nutr × Comp	8.28	<b>0.004</b>	0.29	0.588	0.93	0.336	4.35	<b>0.037</b>	1.11	0.292	1.39	0.239
Nutr × Clip × Comp	0.29	0.591	1.65	0.199	2.27	0.132	1.46	0.227	0.49	0.483	0.37	0.542
S × Nutr × Clip × Comp	1.92	0.166	0.59	0.442	0.02	0.889	0.22	0.643	0.03	0.854	2.17	0.141
<b>Random effects</b>	<i>SD</i>		<i>SD</i>		<i>SD</i>		<i>SD</i>		<i>SD</i>		<i>SD</i>	
Genus	0.555		<0.001		<0.001		0.531		0.107		0.196	
Species	0.349*		0.535*		0.735*		<0.001		0.060		0.154	
Sowing	0.004		0.531		0.226		0.264		0.168		0.242	
Residual	0.288		0.641		1.296		0.586		0.209		0.473	
	$R^2_m$	$R^2_c$	$R^2_m$	$R^2_c$	$R^2_m$	$R^2_c$	$R^2_m$	$R^2_c$	$R^2_m$	$R^2_c$	$R^2_m$	$R^2_c$
$R^2$ of the model	0.574	0.931	0.141	0.640	0.039	0.289	0.035	0.523	0.094	0.546	0.085	0.405

625 \* Standard deviations for individual species random effects for the saturated model are found in Table S2.  $R^2_m$ : marginal  $R^2$ ,  $R^2_c$ : conditional  $R^2$ .

626

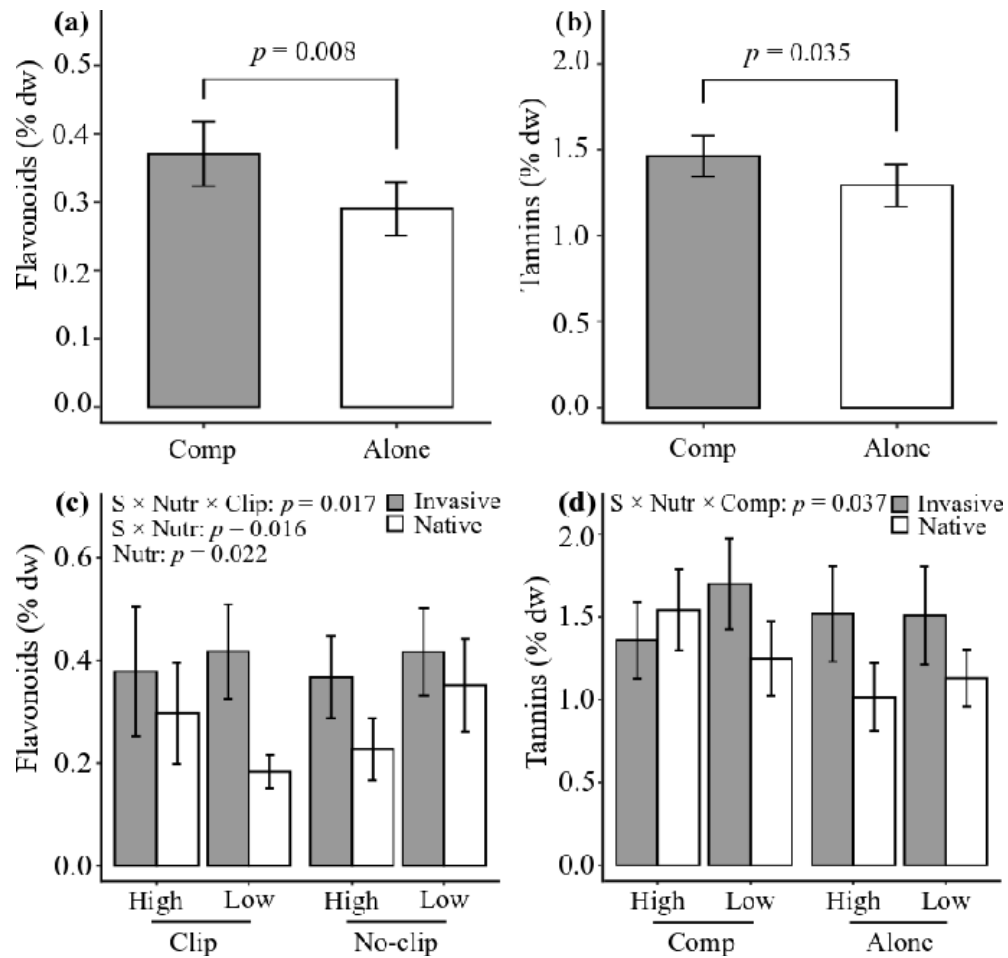
627 **Figures**



628

629 **Figure 1** Mean ( $1 \pm \text{SE}$ ) biomass production and allocation of five congeneric pairs of  
 630 invasive and native plant species that were grown in a common greenhouse condition. The  
 631 panels show effects of: **(a)** three-way interaction between plant invasion status (invasive vs.  
 632 native), nutrient enrichment (low vs. high), and competition (alone vs. competition [comp])  
 633 on total biomass; **(b)** three-way interaction between plant invasion status, nutrient enrichment,  
 634 and simulated herbivory (clipping [clip] vs. no-clipping [no-clip]) on total biomass; **(c)**  
 635 two-way interaction between competition and simulated herbivory on total biomass; **(d)** main  
 636 effects of competition on plant root mass fraction; **(e)** main effects of nutrient enrichment on  
 637 plant root mass fraction; **(f)** main effects of simulated herbivory on plant root mass fraction.  
 638 The figure here only presents the significant main and interactive effects.

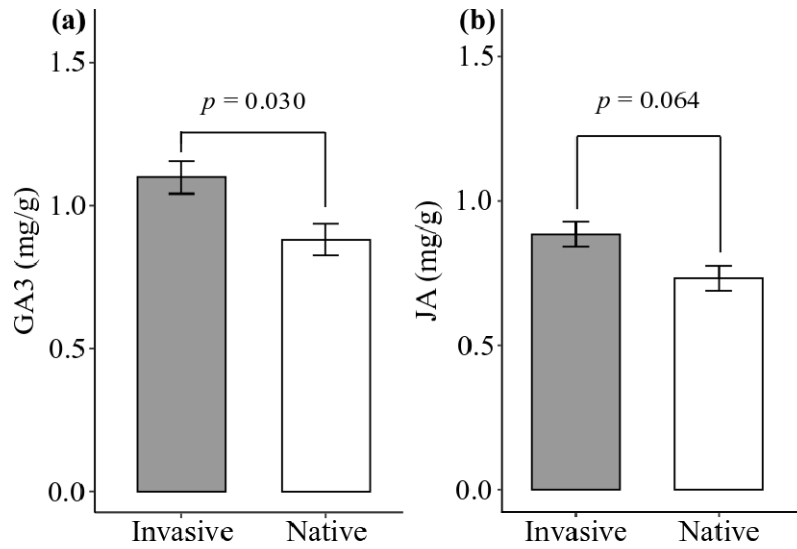
639



640

641 **Figure 2** Mean ( $1 \pm$  SE) concentrations of defense compounds (flavonoids and tannins) of  
 642 five congeneric pairs of invasive and native plant species that were grown in a common  
 643 greenhouse condition. The panels show effects of: **(a)** main effects of competition (alone vs.  
 644 competition [comp]) on concentrations of flavonoids; **(b)** main effects of competition on  
 645 concentrations of tannins; **(c)** three-way interaction between plant invasion status (invasive vs.  
 646 native), nutrient enrichment (low vs. high), and simulated herbivory (clipping [clip] vs.  
 647 no-clipping [no-clip]) on flavonoids concentrations; **(d)** three-way interaction between plant  
 648 invasion status, competition and simulated herbivory on tannins concentrations. The figure  
 649 here only presents the significant main and interactive effects.

650



651

652 **Figure 3** Mean ( $1 \pm$  SE) concentrations of growth- and defense-enhancing hormones (GA3  
653 and JA) of five congeneric pairs of invasive and native plant species that were grown in a  
654 common greenhouse condition. The panels show effects of main effect of plant invasion  
655 status (invasive vs. native) on (a) GA3 concentrations and (b) JA concentration.

656



657 **Supporting information**658 **Table S1** Information on the five congeneric pairs of invasive and native plant species that were used in the current experiment

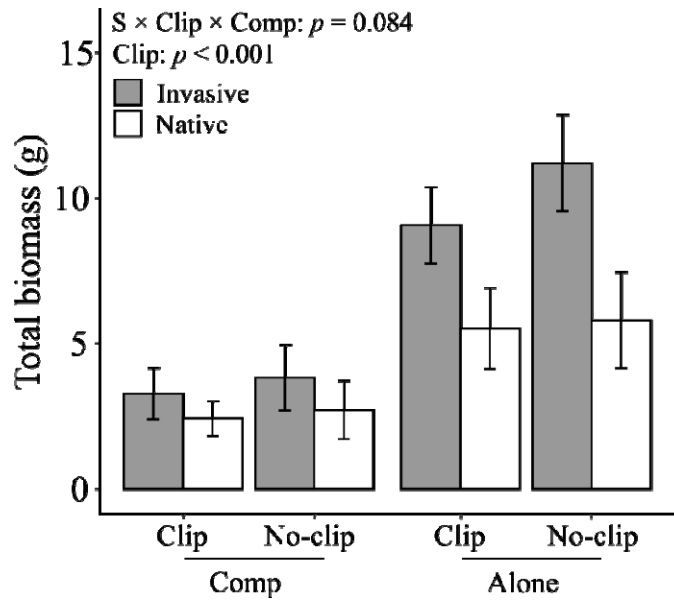
Species	Family	Invasion status in China	Clonal organ	Province of plant collection	Year of field collection	Reproduction modes	Sowing date
<i>Alternanthera Sessilis</i>	Amaranthaceae	Native	Stolon	Zhejiang	2017	Stolon	15/2/2020
<i>Alternanthera philoxeroides</i>	Amaranthaceae	Invasive	Stolon	Zhejiang	2018	Stolon	15/2/2020
<i>Hydrocotyle sibthorpioides</i>	Araliaceae	Native	Stolon	Hubei	2014	Stolon	14/2/2020
<i>Hydrocotyle verticillata</i>	Araliaceae	Invasive	Stolon	Hubei	2014	Stolon	14/2/2020
<i>Solidago decurrens</i>	Asteraceae	Native	Rhizome	Zhejiang	2018	Seed	20/11/2019
<i>Solidago canadensis</i>	Asteraceae	Invasive	Rhizome	Zhejiang	2018	Seed	20/11/2019
<i>Sphagneticola calendulacea</i>	Asteraceae	Native	Stolon	Guangdong	2014	Stolon	14/2/2020
<i>Sphagneticola trilobata</i>	Asteraceae	Invasive	Stolon	Fujian	2018	Stolon	14/2/2020
<i>Paspalum orbiculare</i>	Poaceae	Native	Rhizome	Zhejiang	2018	Stolon	15/2/2020
<i>Paspalum notatum</i>	Poaceae	Invasive	Rhizome	Hubei	2014	Seed	3/1/2020

659

660 **Table S2** Standard deviations for individual species random effects for metrics analyzed with models with a Gaussian error distribution. The  
 661 standard deviations given refer to the first species. For each species, these should be multiplied by the multiplication factors. The names of the  
 662 species in the table are abbreviated using the first and second letters of the genus and the first letter of species epithet.

Metric	Species Standard Deviation	PA_N	PA_O	AL_P	AL_S	HY_S	HY_V	SO_C	SO_D	SP_C	SP_T
Total biomass production	0.349	1.000	1.999	1.923	1.225	1.399	1.191	1.078	1.989	1.123	1.337
Root mass fraction	0.539	1.000	1.321	0.356	0.434	0.978	0.765	0.517	1.362	0.556	0.792
Flavonoids	0.751	1.000	0.745	0.363	0.311	0.425	0.348	0.327	0.583	0.703	0.412

663 **Figure S1**

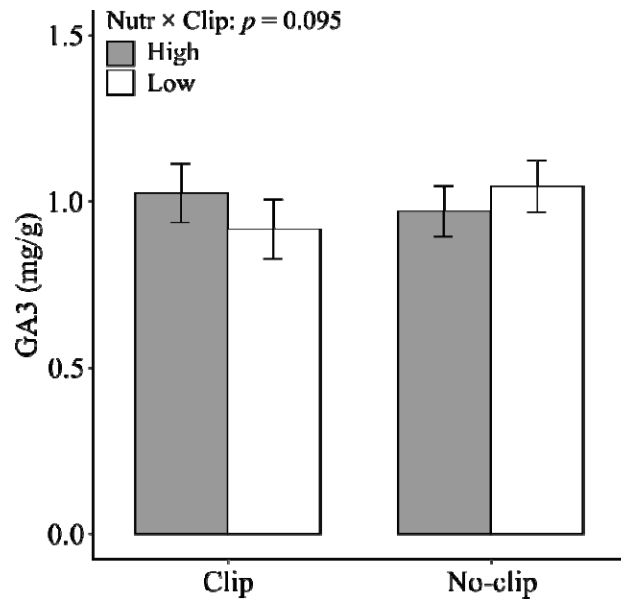


664

665 **Figure S1** Mean ( $1 \pm$  SE) total biomass of five congeneric pairs of invasive and native plant  
666 species that were grown in a common greenhouse condition. The panels show effects of  
667 three-way interaction between plant invasion status (invasive vs. native), competition (alone  
668 vs. competition [comp]), and simulated herbivory (clipping [clip] vs. no-clipping [no-clip])  
669 on total biomass.

670

671 **Figure S2**



672

673 **Figure S2** Mean ( $1 \pm$  SE) concentrations of GA3 of five congeneric pairs of invasive and  
674 native plant species that were grown in a common greenhouse condition. The panels show  
675 effects of two-way interaction between nutrient enrichment (low vs. high) and simulated  
676 herbivory (clipping [clip] vs. no-clipping [no-clip]) on GA3 concentrations.

677