1 **Increases in multiple resources promote plant invasion**

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21 **Authorship:** ZZ and YL conceived the idea. ZZ, YL and MvK designed the study. AH and
22 HJ performed the experiment. ZZ led the analyses and writing, with inputs from YL and
23 MvK.

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- 25 **Data and code accessibility:** Should the manuscript be accepted, the data and code of the
- 26 study will be archived in Figshare and the DOI will be included at the end of the article.

27 **Abstract**

28 Invasion by alien plants is frequently attributed to increased resource availabilities.
 29 Still, our understanding is mainly based on effect of single resource. Despite the fact that
 30 plants rely on many resources, little is known about how multiple resources affect success of
 31 alien plants. Here, with two common garden experiments, one in China and one in Germany,
 32 we tested whether nutrient and light availabilities affected the competitive outcomes between
 33 alien and native plants. We found that under low resource availabilities or with addition of
 34 only one type of resources aliens were not more competitive than natives. However, with a
 35 joint increase of nutrients and light intensity, aliens outcompeted natives. Our finding
 36 indicates that addition of multiple resources could greatly reduce the number of limiting
 37 factors (i.e. niche dimensionality), and that this favors the dominance of alien species. It also
 38 indicates that habitats experiencing multiple global changes might be more vulnerable to
 39 plant invasion.

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41 **Keywords:** light, niche, nutrient, plant invasion, resource competition

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44 Introduction

45 The rapid accumulation of alien species is one of the characteristics of the
 46 Anthropocene^{1,2}. Because some alien species can threaten native species and ecosystem
 47 functioning³, it has become an urgent quest to understand the mechanism whereby aliens
 48 outcompete natives. One widely considered mechanism is proposed by the fluctuating
 49 resource hypothesis⁴, which poses that ‘a plant community becomes more susceptible to
 50 invasion whenever there is an increase in the amount of unused resources’. Numerous studies
 51 have shown that resource increases can favor alien plants over natives^{5,6}. However, while
 52 plants need different types of resources (e.g. nutrients and light), most studies investigated
 53 only one single resource, mainly nutrients. Therefore, it remains largely unknown whether
 54 multiple resources will interact to affect competitive outcomes between alien and native
 55 plants.

56 How resources affect competition has long fascinated and puzzled ecologists^{7,8}.
 57 Resource-competition theory predicts that if multiple species are competing for resources,
 58 coexistence of all species is possible when each species is limited by a different resource⁹. A
 59 classic example comes from algae, where *Asterionella formosa* and *Cyclotella meneghiniana*
 60 were able to coexistence when *A. formosa* was limited by phosphate and *C. meneghiniana*
 61 was limited by silicate¹⁰. Resource addition (e.g. phosphate) will decrease the number of
 62 limiting resources and will thus favor dominance of one species (known as the niche-
 63 dimension hypothesis *sensu* Harpole & Tilman¹¹). Although it remains challenging to identify
 64 limiting resources for more complex species (e.g. vascular plants), a few follow-up
 65 experiments have shown that coexistence of multiple plant species was less likely with
 66 addition of multiple resources^{11,12}. The explanation behind this is straightforward: the more
 67 types of resources are added, the more likely it is that the previously limiting resources are no

68 longer limiting. A next step in this field of research is to predict which type of species will be
69 favored with addition of multiple resources.

70 One group of species that might benefit from addition of multiple resources is alien
71 species. First, most alien plants origin from human-associated habitats¹³, which are frequently
72 rich in resources due to disturbance. Consequently, successful aliens are those that are pre-
73 adapted to high resource availabilities and thus are favored by resource addition^{14,15}. Second,
74 aliens might be limited by fewer factors than natives are, because their evolutionary history
75 differs from the one of natives^{16,17}. Such advantage of aliens over natives may be invisible
76 when both types of species suffer from resource limitation, especially from the limitation of
77 multiple resources. However, the advantage will become noticeable when resource limitation
78 is removed by resource addition. Although resource-competition theory offers a potential
79 mechanistic explanation of the success of alien species, empirical test remains rare.

80 Here, we conducted two experiments, one in Germany and one in China, with similar
81 designs. In both locations, we grew multiple alien and native plant species
82 either alone, in monoculture, or in mixture of two species. To vary resource availabilities, we
83 used two levels of nutrients, and two levels of light. We aimed to test whether resource
84 availabilities affected pairwise competitive outcomes between alien and native species. We
85 expected that 1) an increase in resource favors aliens over natives, and 2) that this effect is
86 stronger when two resources instead of one resource is added.

87 **Methods and Materials**

88 **Study species**

89 To increase our ability to generalize the results, we conducted multispecies
 90 experiments¹⁸. For the experiment in China, we selected 8 species that are either native or
 91 alien in China (Table S1). For the experiment in Germany, we selected 16 species that are
 92 either native or alien in Germany (Table S1). All 24 species, representing seven families, are
 93 common in their respective regions. All alien species are naturalized (*sensu* Richardson *et*
 94 *al*¹⁹) in the country where the respective experiment was conducted. We classified the species
 95 as naturalized alien or native to China or Germany based on the following databases: (1)
 96 “The Checklist of the Alien Invasive Plants in China”²⁰, (2) the Flora of China
 97 (www.efloras.org) and (3) BiolFlor (www.ufz.de/biolflor). Seeds or stem fragments of the
 98 study species were obtained from botanical gardens, commercial seed companies, or from
 99 wild populations (Table S1).

100 **Experimental set-up**

101 ***The experiment in China***

102 From 21 May to 27 June 2020, we planted or sowed the eight study species into
 103 plastic trays filled with potting soil (Pindstrup Plus, Pindstrup Mosebrug A/S, Denmark). To
 104 ensure that the species were in similar developmental stages at the beginning of the
 105 experiment, we sowed the species at different times (Table S1). Three species were grown
 106 from stem fragments because they mainly rely on clonal propagation, and the others were
 107 sown as seeds (Table S1).

108 On 13 July 2020, we transplanted the seedlings into 2.5-L pots filled with a mixture of
 109 sand and vermiculite (1:1 v/v). Three competition treatments were imposed: 1) no
 110 competition, in which plants were grown alone; 2) intraspecific competition, in which two
 111 individuals of the same species were grown together; 3) interspecific competition, in which

two individuals, each from another species were grown together. We grew all eight species without competition, in intraspecific competition and in all 28 possible pairs of interspecific competition. For the no-competition and intraspecific-competition treatments, we replicated each species seven times. For the interspecific-competition treatment, for which we had many pairs of species, we replicated each pair two times.

The experiment took place in a greenhouse at the Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences (Changchun, China). The greenhouse had transparent film on the top, which reduced the ambient light intensity by 12%. It was open on the side, so that insects and other organisms can enter. To vary nutrient availabilities, we applied to each pot either 5g (low nutrient treatment) or 10g (high nutrient treatment) of a slow-release fertilizer (Osmocote® Exact Standard, Everris International B.V., Geldermalsen, The Netherlands). To vary light availabilities, we used two cages (size: $9 \times 4.05 \times 1.8$ m). One of them was covered with two layers of black netting material, which reduced the light intensity by 71% (low light-intensity treatment). The other was left uncovered (high light-intensity treatment).

The experiment totaled 672 pots ($[8 \text{ no-competition} \times 7 \text{ replicates} + 8 \text{ intraspecific-competition} \times 7 \text{ replicates} + 28 \text{ interspecific-competition} \times 2 \text{ replicates}] \times 2 \text{ nutrient treatments} \times 2 \text{ light treatments}$). The pots were randomly assigned to positions, and were randomized once on 15 August within the block (low or high light-intensity treatment). We watered the plants daily to avoid water limitation. On 1 September 2020, we harvested aboveground biomass of all plants. The biomass was dried at 65°C for 72h to constant weight, and then weighed to the nearest 1mg.

The experiment in Germany

On 15 June 2020, we sowed seeds of the 16 species into plastic trays filled with potting soil (Topferde, Einheitserde Co). On 6 July 2020, we transplanted the seedlings into

1.5-L pots filled with a mixture of potting soil and sand (1:1 v/v). Like the experiment in China, we imposed three competition treatments: no competition, intraspecific competition and interspecific competition. However, in this experiment, which had two times more species than the experiment in China, we only included 24 randomly chosen species pairs for the interspecific-competition treatment, and all of these pairs consisted of one alien and one native species. For the no-competition treatments, we replicated each species two times. For the competition treatments, we did not use replicates for any of the species combinations, as replication of the competition treatments was provided by the large number of species pairs.

The experiment took place outdoors in the Botanical Garden of the University of Konstanz (Konstanz, Germany). To vary nutrient availabilities, we applied once a week, to each pot either 100 ml of a low-concentration liquid fertilizer (low-nutrient treatment; 0.5‰ Universol ® Blue oxide fertilizer) or 100 ml of a high-concentration of the same liquid fertilizer (high nutrient treatment; 1‰). To vary light availabilities, we used eight metal wire cages (size: $2 \times 2 \times 2$ m). Four of the cages were covered with one layer of white and one layer of green netting material, which reduced the ambient light intensity by 84% (low light-intensity treatment). The remaining four cages were covered only with one layer of the white netting material, which served as positive control (to control for the effect of netting) and reduced light intensity by 53% (high light-intensity treatment). In other words, the low light-intensity treatment received 66% less light than the high light-intensity treatment.

The experiment totaled 320 pots ([16 no-competition $\times$ 2 replicates + 16 intraspecific-competition + 32 interspecific-competition] $\times$ 2 nutrient treatments $\times$ 2 light treatments). The eight cages were random assigned to fixed positions in the botanical garden. The pots were randomly assigned to the eight cages (40 pots in each cage), and were re-randomized once within and across cages of the same light treatment on 3 August. Besides the weekly fertilization, we watered the plants two or three times a week to avoid water limitation. On 7

and 8 September 2020, we harvested aboveground biomass of all plants. The biomass was dried at 70°C for 96h to constant weight, and then weighed to the nearest 0.1 mg.

Statistical analyses

All analyses were performed using R version 3.6.1²¹. To test whether resource availabilities affected competitive outcomes between alien and native species, we applied linear mixed-effects models to analyze the two experiments jointly and separately, using the *nlme* package²². For the model used to analyze the two experiments jointly, we excluded interspecific competition between two aliens and between two natives from the experiment in China, because these combinations were not included in the experiment in Germany. When we analyzed each experiment separately, the results were overall similar to the result of the joint analysis. Therefore, we focus in the manuscript on the joint analysis, and present the results of the separate analyses in Supplement S1.

In the model, we included aboveground biomass as response variable. Because plant mortality was low and mainly happened after transplanting, we excluded pots in which plants had died. We included origin of the species (alien or native), competition treatment (see below for details), nutrient treatment, light treatment and their interactions as fixed effects; and study site (China or Germany), and identity and family of the species as random effects. In addition, we allowed each species to respond differently to the nutrient and light treatments (i.e. we included random slopes). To account for pseudoreplication²³, we also tried to include cages as random block effect and pots as random effect. However, the cages explained very little variation and did not change the results qualitatively, most likely because we re-randomized plants across cages of the same treatment in Germany, and because environmental differences between cages were small. The pots explained very little variation as well. Therefore, we removed cages and pots from the final model to reduce model complexity. To improve normality of the residuals, we natural-log-transformed aboveground

biomass. To improve homoscedasticity of residuals, we allowed the species and competition treatment to have different variances by using the *varComb* and *varIdent* functions²⁴. Significances of the fixed effects were assessed with ANOVA.

A significant effect of origin would indicate that alien and native species differed in their biomass production, across all competition and resource-availability (light and nutrients) treatments. This would tell us the competitive outcome between aliens and natives across different resource availabilities. ‘Competitive outcome’ here refers to which species will exclude or dominate over the other species at the end point for the community^{25,26}. For example, an overall higher level of biomass production of alien species would indicate that aliens would dominate when competing with natives. A significant interaction between resource-availability treatment and origin of the species would indicate that resource availabilities affect the biomass production of alien and native species differently, averaged across all competition treatments. In other words, it would indicate that resource availabilities affect the competitive outcome between aliens and natives. A significant interaction between a resource-availability treatment and the competition treatment would indicate that resource availabilities affect the effect of competition (e.g. no competition *vs.* competition).

In the competition treatment, we had three levels: 1) no competition, 2) intraspecific competition, and 3) interspecific competition between alien and native species. To split them into two contrasts, we created two dummy variables²⁷ testing 1) the effect of competition, and 2) the difference between intra- and interspecific competition.

Results

Overall, biomass production of plants increased with nutrients (+66.6%; Fig. 1; Table S2; $F_{1,1156} = 46.71$, $P < 0.001$), and increased with light intensity (+67.9%; $F_{1,1156} = 9.73$, $P = 0.002$). Moreover, biomass production increased the most with a joint increase of nutrients and light intensity (+79.4%), as indicated by the interaction between nutrient and light treatments ($F_{1,1156} = 23.15$, $P < 0.001$). Across competition treatments and the different light and nutrient treatments, alien and native species did not differ in their biomass production ($F_{1,1156} = 1.95$, $P = 0.163$). This indicates that, overall, aliens did not outcompete natives. However, this competitive outcome between aliens and natives was affected by the interaction between nutrient and light treatments (Fig. 1; Table S2; $F_{1,1156} = 4.30$, $P = 0.038$). More specifically, with a joint increase of nutrients and light intensity, aliens produced more (+110.8%) biomass than natives; whereas this difference was much smaller under low resource availabilities (+48.3%) or with addition of only one type of resources (+48.4% under only high nutrients; +68.9% under only high light intensity).

Competition reduced (-26.0%) biomass production, as indicated by the difference between plants grown without competition and plants with competition (Fig. 2; Table S2; $F_{1,1156} = 2.00$, $P = 0.158$). Although this effect of competition was not statistically significant across different nutrient or light treatments, it became more apparent with increased nutrients ($F_{1,1156} = 5.71$, $P = 0.017$) and increased light intensity ($F_{1,1156} = 4.99$, $P = 0.026$). In addition, we found that plants produced more (+16.4%) biomass when competing with interspecific competitors than with intraspecific competitors (Fig. 2; Table S2; $F_{1,1156} = 20.26$, $P < 0.001$).

Discussion

We found that under low resource availabilities, alien and native plants did not differ in biomass production, indicating that under those conditions aliens will not outcompete natives. Although an increase in one type of resource, either nutrients or light, increased biomass production; it affected aliens and natives similarly, and thus did not change the potential competitive outcome. However, with a joint increase of nutrients and light intensity, aliens produced more biomass than natives, indicating that aliens will outcompete natives under high availabilities of both resources. Our finding thus supports the fluctuating resource hypothesis, which predicts that ‘a plant community becomes more susceptible to invasion whenever there is an increase in the amount of unused resources’. Furthermore, our finding, along with those of others^{14,28}, explains why plant invasion is frequently associated with disturbance. This is because disturbance could increase nutrient availability and create open patches with a higher light intensity, a combination that favors naturalized alien plants.

Our finding that across two experiments, addition of one type of resource did not favor alien plants has several implications. First, it suggests that plants —irrespective of their origin— are limited by multiple factors, such as nutrients, light and herbivory. In other words, niche space has multiple dimensions, each of which is represented by one limiting factor. While addition of one resource removes one dimension from the niche space, the remaining dimensions could still limit both alien and native plants, maintaining coexistence of aliens and natives. Some previous studies, in line with our finding, showed that addition of one type of resource (nutrients) did not favor alien plants⁶. However, others found that addition of only nutrients was sufficient to favor alien plants^{5,15}. One explanation for the apparent discrepancy could be that the latter studies were conducted under high light conditions. This was likely the case as the latter two studies were done in summer, while Liu *et al.*⁶ was done in a greenhouse in winter. With addition of nutrients, their environments

were similar to joint increases of nutrients and light in our study, which reduced more dimensions of the niche space, favoring dominance by one of the two species.

A second implication is that our finding suggests that alien and native species did not strongly differ in their competitive abilities for nutrients or light. As addition of one resource removes one dimension from the niche space, it intensifies competition for other dimensions. For example, nutrient addition can intensify light competition²⁹, which is also indicated by our finding that competition was more severe with nutrient addition (Fig. 2a). Consequently, if alien plants have stronger competitive abilities for light (e.g. have a lower minimum requirement of light) than natives, they will dominate with nutrient addition. However, as this was not the case, we conclude that there was no strong difference in competitive abilities for light between the aliens and natives.

The two implications mentioned above raise the question which factor or factors determine the higher competitiveness of naturalized alien plants with joint increases of nutrients and light. One potential factor could be plant enemies. Because the evolutionary histories of alien plants differ from those of the native plants, alien plants might be released from natural enemies³⁰. This advantage might be stronger when other factors, for example, resource availabilities, are not limiting the plants^{31,32}. An alternative potential factor is preadaptation of naturalized alien plants. Many alien plants occur in human-associated habitats¹³, where resource availabilities are high due to human disturbance. Consequently, of the many alien plants that have been introduced the ones that managed to naturalize or become invasive are most likely the ones that were selected for high growth rates under high resource availabilities. Given that these two explanations are not mutually exclusive, future studies that test their relative importance are needed.

Conclusions

The fluctuating resource hypothesis suggests that a plant community becomes more susceptible to invasion with additional resources. Our study suggests that this is particularly the case with increases of multiple resources, as this could greatly reduce the dimensionality of niche space, leading to competitive exclusion of one of the species. This can also explain why many studies have found that biological invasions are more frequent in disturbed, high resource environments.

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369 Figures

370

371 **Figure 1** Effects of nutrient and light availabilities on competitive outcomes between alien
372 (red) and native (blue) plants. Competitive outcome is indicated by the difference in average
373 biomass production. For example, a higher biomass production of alien plants indicates that
374 aliens outcompete natives. Error bars indicate 95% CIs.

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378 **Figure 2** Effects of nutrient and light availabilities on competition. Yellow, dark blue and
 379 light blue lines represent plants without competition, and with inter- and intraspecific
 380 competition, respectively. Error bars indicate 95% CIs.

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