Increases in multiple resources promote plant invasion

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

Abstract

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

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

Introduction

The rapid accumulation of alien species is one of the characteristics of the Anthropocene[1,2]. Because some alien species can threaten native species and ecosystem functioning[3], it has become an urgent quest to understand the mechanism whereby aliens outcompete natives. One widely considered mechanism is proposed by the fluctuating resource hypothesis[4], which poses that 'a plant community becomes more susceptible to invasion whenever there is an increase in the amount of unused resources'. Numerous studies have shown that resource increases can favor alien plants over natives[5,6]. However, while plants need different types of resources (e.g. nutrients and light), most studies investigated only one single resource, mainly nutrients. Therefore, it remains largely unknown whether multiple resources will interact to affect competitive outcomes between alien and native plants.

How resources affect competition has long fascinated and puzzled ecologists[7,8]. Resource-competition theory predicts that if multiple species are competing for resources, coexistence of all species is possible when each species is limited by a different resource[9]. A classic example comes from algae, where *Asterionella formosa* and *Cyclotella meneghiniana* were able to coexistence when *A. formosa* was limited by phosphate and *C. meneghiniana* was limited by silicate[10]. Resource addition (e.g. phosphate) will decrease the number of limiting resources and will thus favor dominance of one species (known as the niche-dimension hypothesis *sensu* Harpole & Tilman[11]). Although it remains challenging to identify limiting resources for more complex species (e.g. vascular plants), a few follow-up experiments have shown that coexistence of multiple plant species was less likely with addition of multiple resources[11,12]. The explanation behind this is straightforward: the more types of resources are added, the more likely it is that the previously limiting resources are no longer limiting. A next step in this field of research is to predict which type of species will be favored with addition of multiple resources.

One group of species that might benefit from addition of multiple resources is alien species. First, most alien plants origin from human-associated habitats[13], which are frequently rich in resources due to disturbance. Consequently, successful aliens are those that are pre-adapted to high resource availabilities and thus are favored by resource addition[14,15]. Second, aliens might be limited by fewer factors than natives are, because their evolutionary history differs from the one of natives[16,17]. Such advantage of aliens over natives may be invisible when both types of species suffer from resource limitation, especially from the limitation of multiple resources. However, the advantage will become noticeable when resource limitation is removed by resource addition. Although resource-competition theory offers a potential mechanistic explanation of the success of alien species, empirical test remains rare.

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

Methods and Materials

Study species

To increase our ability to generalize the results, we conducted multispecies experiments[18]. For the experiment in China, we selected 8 species that are either native or alien in China (Table S1). For the experiment in Germany, we selected 16 species that are either native or alien in Germany (Table S1). All 24 species, representing seven families, are common in their respective regions. All alien species are naturalized (*sensu* Richardson *et al*[19]) in the country where the respective experiment was conducted. We classified the species as naturalized alien or native to China or Germany based on the following databases: (1) "The Checklist of the Alien Invasive Plants in China"[20], (2) the Flora of China (www.efloras.org) and (3) BiolFlor (www.ufz.de/biolflor). Seeds or stem fragments of the study species were obtained from botanical gardens, commercial seed companies, or from wild populations (Table S1).

Experimental set-up

The experiment in China

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

On 13 July 2020, we transplanted the seedlings into 2.5-L pots filled with a mixture of sand and vermiculite (1:1 v/v). Three competition treatments were imposed: 1) no competition, in which plants were grown alone; 2) intraspecific competition, in which two 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 × 4.05 × 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 no-competition × 7 replicates + 8 intraspecific-competition × 7 replicates + 28 interspecific-competition × 2 replicates] × 2 nutrient treatments × 2 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□ 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 × 2 × 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 × 2 replicates + 16 intraspecific-competition + 32 interspecific-competition] × 2 nutrient treatments × 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□ for 96h to constant weight, and then weighed to the nearest 0.1 mg.

Statistical analyses

All analyses were performed using R version 3.6.1[21]. 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* package[22]. 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 pseudoreplication[23], 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* functions[24]. 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 variables[27] 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 —irrespectively 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 plants[6]. 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 competition[29], 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 enemies[30]. 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 habitats[13], 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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References

1 van Kleunen, M. *et al.* Global exchange and accumulation of non-native plants. *Nature* **525**, 100-103 (2015).

2 Lewis, S. L. & Maslin, M. A. Defining the Anthropocene. *Nature* **519**, 171-180 (2015).

3 Vilà, M. *et al.* Ecological impacts of invasive alien plants: a meta-analysis of their effects on species, communities and ecosystems. *Ecology Letters* **14**, 702-708 (2011).

4 Davis, M. A., Grime, J. P. & Thompson, K. Fluctuating resources in plant communities: a general theory of invasibility. *Journal of Ecology* **88**, 528-534 (2000).

5 Parepa, M., Fischer, M. & Bossdorf, O. Environmental variability promotes plant invasion. *Nature communications* **4**, 1604 (2013).

6 Liu, Y., Zhang, X. & van Kleunen, M. Increases and fluctuations in nutrient availability do not promote dominance of alien plants in synthetic communities of common natives. *Functional Ecology* **32**, 2594-2604 (2018).

7 Hutchinson, G. E. The Paradox of the Plankton. *The American Naturalist* **95**, 137-145 (1961).

8 Chesson, P. Mechanisms of maintenance of species diversity. *Annual Review of Ecology and Systematics* **31**, 343-366 (2000).

9 Tilman, D. *Resource competition and community structure*. (Princeton University Press, 1982).

10 Tilman, D. Resource Competition between Plankton Algae: An Experimental and Theoretical Approach. *Ecology* **58**, 338-348 (1977).

11 Harpole, W. S. & Tilman, D. Grassland species loss resulting from reduced niche dimension. *Nature* **446**, 791-793 (2007).

12 Harpole, W. S. *et al.* Addition of multiple limiting resources reduces grassland diversity. *Nature* **537**, 93-96 (2016).

13 Kalusová, V. *et al.* Naturalization of European plants on other continents: The role of donor habitats. *Proceedings of the National Academy of Sciences* **114**, 13756-13761 (2017).

14 Seabloom, E. W. *et al.* Plant species' origin predicts dominance and response to nutrient enrichment and herbivores in global grasslands. *Nature communications* **6**, 7710 (2015).

15 Liu, Y. & van Kleunen, M. Responses of common and rare aliens and natives to nutrient availability and fluctuations. *Journal of Ecology* **105**, 1111-1122 (2017).

16 Darwin, C. *On the origin of species*. (J. Murrary, 1859).

17 Saul, W.-C., Jeschke, J. & Heger, T. The role of eco-evolutionary experience in invasion success. *NeoBiota* **17**, 57-74 (2013).

18 van Kleunen, M., Dawson, W., Bossdorf, O. & Fischer, M. The more the merrier: Multi-species experiments in ecology. *Basic and Applied Ecology* **15**, 1-9 (2014).

19 Richardson, D. M. *et al.* Naturalization and invasion of alien plants: concepts and definitions. *Diversity and Distributions* **6**, 93-107 (2000).

20 Ma, J. & Li, H. *The checklist of the alien invasive plants in China*. (Higher Education Press, 2018).

21 *R: A language and environment for statistical computing* (R Foundation for Statistical Computing, Vienna, Austria. Available at: http://www.R-project.org/. 2019).

22 Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D. & R Core Team. nlme: Linear and nonlinear mixed effects models. *R package version* **3**, 57 (2018).

23 Hurlbert, S. H. Pseudoreplication and the design of ecological field experiments. *Ecological Monographs* **54**, 187-211 (1984).

24 Zuur, A., Ieno, E., Walker, N., Saveliev, A. & Smith, G. Mixed effects models and extensions in ecology with R. Gail M, Krickeberg K, Samet JM, Tsiatis A, Wong W, editors. *New York, NY: Spring Science and Business Media* (2009).

25 Gibson, D., Connolly, J., Hartnett, D. & Weidenhamer, J. Designs for greenhouse studies of interactions between plants. *Journal of Ecology* **87**, 1-16 (1999).

26 Zhang, Z., Liu, Y., Brunel, C. & van Kleunen, M. Soil-microorganism-mediated invasional meltdown in plants. *Nature Ecology & Evolution* **4**, 1612-1621 (2020).

27 Schielzeth, H. Simple means to improve the interpretability of regression coefficients. *Methods Ecol. Evol.* **1**, 103-113 (2010).

28 Liu, Y. *et al.* Do invasive alien plants benefit more from global environmental change than native plants? *Glob Chang Biol* (2016).

29 Hautier, Y., Niklaus, P. A. & Hector, A. Competition for light causes plant biodiversity loss after eutrophication. *Science* **324**, 636-638 (2009).

30 Keane, R. M. & Crawley, M. J. Exotic plant invasions and the enemy release hypothesis. *Trends in Ecology & Evolution* **17**, 164-170 (2002).

31 Coley, P. D., Bryant, J. P. & Chapin, F. S. Resource availability and plant antiherbivore defense. *Science* **230**, 895-899 (1985).

32 Blumenthal, D. M. Interactions between resource availability and enemy release in plant invasion. *Ecology Letters* **9**, 887-895 (2006).

Figures

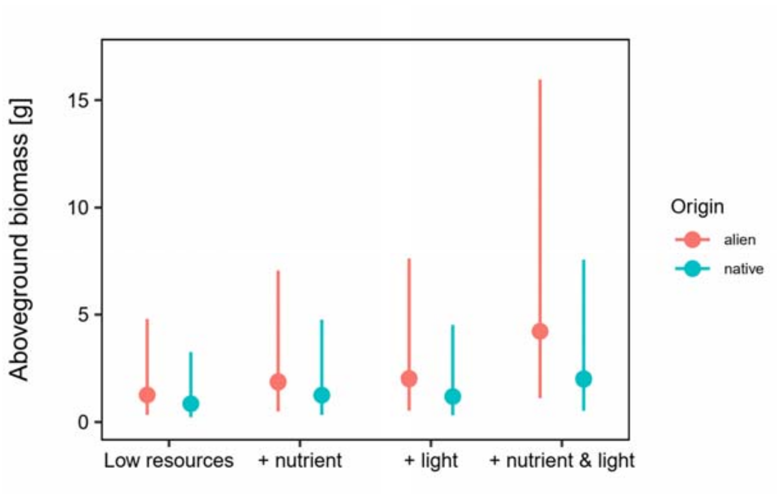

Figure 1 Effects of nutrient and light availabilities on competitive outcomes between alien (red) and native (blue) plants. Competitive outcome is indicated by the difference in average biomass production. For example, a higher biomass production of alien plants indicates that aliens outcompete natives.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

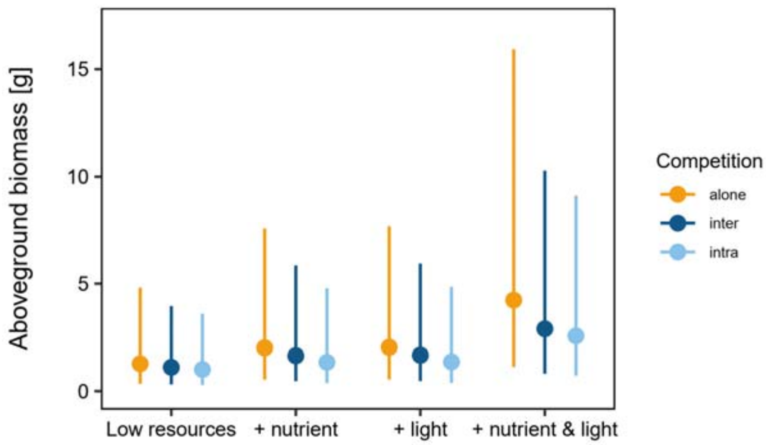

Figure 2 Effects of nutrient and light availabilities on competition. Yellow, dark blue and light blue lines represent plants without competition, and with inter- and intraspecific competition, respectively. Error bars indicate 95% CIs.