The importance of phenotypic plasticity for plant success under environmental change

Doctoral thesis for obtaining the academic degree Doctor of Natural Sciences

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Date of the oral examination: 28 July 2017

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To my wife who has supported me during my Ph.D. study

To my son who is the most precious gift I have received until now
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Summary

Environmental factors can change in space and time both in terms of the mean conditions and in terms of variability. Phenotypic plasticity is assumed to be an important mechanism leading to plant success under both natural and/or anthropogenic environmental change conditions. Yet, we still have a limited understanding of whether phenotypic plasticity contributes to the success of alien plant species under environmental change. Moreover, phenotypic plasticity of many functional traits associated with nutrient uptake and light capture are frequently implicitly assumed to be adaptive in many studies. However, the adaptive value has rarely been tested for most of them. Therefore, my thesis aimed to gain insights into how phenotypic plasticity contributes to plant success under environmental change, specifically focusing on plant invasion under anthropogenic global change and nutrient fluctuations. Additionally, I also tested whether phenotypic plastic responses that are assumed to be adaptive are really adaptive, specifically focusing on the plastic response of specific leaf area (SLA) induced by changes in light intensity.

First, I performed a meta-analysis to test whether there is a general pattern in invasive and native plant responses to environmental change in mean conditions. I established a plant database, which included 74 invasive alien plant species and 117 native plant species. I compared plastic responses in performance traits to increasing atmospheric CO₂ concentrations, increasing temperatures, increasing N deposition, and increasing or decreasing precipitation between invasive alien and native plants. I found that invasive alien plant species showed stronger positive responses to favourable environmental changes, particularly global warming and atmospheric CO₂ enrichment. My results suggest that, because of the higher plasticity of invasive alien plant species than native plant species, global environmental change may promote spread of invasive plants in the future.

Second, I performed a multispecies greenhouse experiment to test whether alien and common plant species take more advantage of increases in nutrient levels and fluctuations therein than native and rare species. There were six nutrient-supply treatments that differed in the mean and temporal availability of nutrients. I compared plastic responses in biomass production, root allocation and root morphology to such nutrient treatments among seven common alien, seven rare alien, nine common native and six rare native plant species. I found that the plastic responses of biomass production, root morphology and root allocation to nutrient changes
under mean conditions did not differ between alien and native plant species. However, I found that, compared to a constant high nutrient supply, alien plant species showed positive plastic responses in biomass production to large nutrient pulses, whereas native plant species showed negative plastic responses, possibly as a consequence of differences in plasticity of root traits. My findings suggest alien species might become more dominant when fluctuations in nutrients increase. In this study, I did not find differences in plastic responses to nutrient addition and fluctuations between invasive (i.e. common) and non-invasive (i.e. rare) alien species, possibly, because plants were grown in the absence of competition.

Third, I used a multispecies greenhouse experiment to test whether invasive alien plant species might show higher plastic response and thus take more advantage of nutrient fluctuations than non-invasive alien, when grown in competition with native plant species. I grew ten pairs of invasive and non-invasive alien plant species under the same nutrient-supply treatments as used in the first greenhouse experiment, but this time in the presence of native competitors. I found that invasive alien plant species exhibited a significantly stronger increase in biomass production in response to high nutrient levels than non-invasive alien plant species. This is inconsistent with the findings of the first greenhouse experiment, where plants grew without competition. This suggests that responses to nutrient-supply patterns for single plants might not be representative for plants grown under competition. However, I also found that both groups of alien target species benefited proportionally less from nutrient addition overall than the native competitors. Surprisingly, the alien species, and particularly the invasive ones, suffered from nutrient pulses. These findings strongly suggest that it is not a general phenomenon that environmental variability promotes plant invasion.

Finally, I performed a meta-analysis to test whether a phenotypic plastic response that is widely assumed to be adaptive – the increase in specific leaf area (SLA) induced by shading - is really adaptive. I compiled a database including data from 467 case studies using 32 publications and two unpublished experiments, which measured the responses of biomass and SLA of 280 plant species to shading. I found that the potential higher ability of plants to capture light by increasing SLA under low-light conditions was not associated with the maintenance of biomass homeostasis in plant species, but rather with a greater reduction in biomass. This suggests that plasticity of SLA to shading might not constitute adaptive plasticity. Therefore, I argue that some of the plastic responses of plant species to environmental chang-
es, which are frequently thought to be adaptive, might simply reflect passive responses to the environment, or may reflect indirect responses due to correlations with adaptive plasticity of other traits.

To sum up, my thesis explored the importance of phenotypic plasticity for plant success under environmental change. My findings reveal that phenotypic plasticity could be linked to a certain extent to plant success under environmental change in space and time. The next steps in this field of research should be studies that systematically integrate the indirect influence on phenotypic plasticity of plants from plant species and other trophic levels, such as soil biota, herbivores and pollinators. My findings also suggest that more studies are needed to test explicitly whether the phenotypic plasticity of functional traits in response to a specific environmental cue is really adaptive, and thus contributes to plant success under environmental change in time and space.
Zusammenfassung


Als zweiten Schritt führte ich mit verschiedenen Pflanzenarten ein Gewächshausexperiment durch, um zu untersuchen, ob fremde und verbreitete Arten einen größeren Vorteil aus...

auch heraus, dass beide Gruppen der fremden Zielspezies proportional weniger von zusätzlicher Nährstoffzufuhr profitierten als die nativen Konkurrenten. Überraschenderweise litten die fremden Pflanzenarten, insbesondere die invasiven Spezies, unter der Nährstoffzufuhr in einzelnen Impulsen. Diese Ergebnisse weisen stark darauf hin, dass es kein allgemeines Phänomen ist, dass die Umweltvariabilität Pflanzeninvasionen fördert.


General introduction

Environmental change

Environmental factors can change in space and time in terms of the mean conditions, such as an overall increase or decrease in nutrient availability (Fig. 1a). Most of this variation is due to natural causes. For example, light intensity declines from the top to the bottom of a vegetation. In addition, atmospheric carbon dioxide (CO$_2$) and other greenhouse gases, nitrogen (N) deposition, temperatures, precipitation may also increase due to anthropogenic global change. The environment can also change in terms of variability, involving fluctuations around a mean condition over time (Fig. 1b). For instance, precipitation regimes have become more variable both intra- and inter-annually (Min et al., 2011; Smith, 2011; Coumou & Rahmstorf, 2012), and more frequent extreme rainfall events are increasing the variability in water availability and are linked to increased variability in nutrient supply (Emmett et al., 2004; Matias et al., 2011). Changes in the mean and variability can strongly impact ecosystem structure and function (Knapp et al., 2002; Yang et al., 2010; Wu et al., 2011; Grimm et al., 2013; Luque et al., 2013; Parepa et al., 2013).

![Figure 1 Schematic representation of environmental change: (a) change in mean environmental conditions (e.g. an overall increase from condition A [blue] to condition B [red]); (b) variability in environmental conditions (i.e. A [blue] and B [red] conditions have the same mean but different fluctuations over times).](attachment://figure1.png)
**Biological invasions**

*Plant invasion is one important component of biological invasions*

Due to human activity, an increasing number of species have been transported out of their native range and introduced into a new territory (Seebens *et al.*, 2015; van Kleunen *et al.*, 2015; Seebens *et al.*, 2017). Some of these introduced species have become naturalized (i.e. subsequently established self-sustaining populations), and some of those eventually become invasive (i.e. rapidly spread into multiple sites across a large area in the introduced range; Blackburn *et al.*, 2011). Such human induced biological invasions have become a defining feature of global environmental change in the Anthropocene (Vitousek *et al.*, 1996; Lewis & Maslin, 2015). Biological invasions can disrupt ecosystem functions and services (Vitousek *et al.*, 1997; Ricciardi, 2007; Vilà *et al.*, 2011), and have a huge financial impact (Pimentel *et al.*, 2005). To date, at least 3.9% of all currently known vascular plant species have become naturalized outside their native range (van Kleunen *et al.*, 2015), and more plant species will be introduced with continuing globalization and increasing international traffic and trade (Seebens *et al.*, 2015; van Kleunen *et al.*, 2015; Seebens *et al.*, 2017). Consequently, plant invasion is one important component of global change, and its prevention will be imperative in the future.

*Plant invasion under changes in mean environmental conditions*

Plant invasion may interact with environmental changes in mean conditions. Many case studies have investigated how plant invasion interact with a specific environmental change in its mean conditions such as increased atmospheric CO$_2$ concentrations, increased temperatures, altered precipitation and enhanced N deposition. For example, some invasive plant species responded more positively than native plants to increased water supply (e.g. Cox & Conran, 1996; Baruch & Jackson, 2005), elevated atmospheric CO$_2$ concentrations (e.g. Nagel *et al.*, 2004; Baruch & Jackson, 2005; Lei *et al.*, 2012; Tooth & Leishman, 2013), increased N deposition (e.g. Sigueenza *et al.*, 2006; Lei *et al.*, 2012; Vallano *et al.*, 2012), and climate warming (e.g. Song *et al.*, 2010; Verlinden *et al.*, 2013). However, studies on other species found that invasive and native plants exhibited similar responses (e.g. elevated atmospheric CO$_2$: Lei *et al.*, 2011; increased N deposition: Luo *et al.*, 2014; Osone *et al.*, 2014) or even that native plants had significantly stronger responses than invasive plants (e.g. increased
water supply: Horton & Clark, 2001; Blicker et al., 2003; Domènech & Vilà, 2008; Cuda et al., 2015; elevated atmospheric CO$_2$: Anderson & Cipollini, 2013; increased N deposition: Rao & Allen, 2010; and climate warming: Williams et al., 2007; Anderson & Cipollini, 2013). Due to low numbers of species used, and mixed results produced in most case studies, the general patterns of interactions between plant invasion and changes in mean environmental conditions remain unclear.

*Plant invasion under environmental variability*

In addition to interactions with environmental change in mean conditions, plant invasion may also be affected by variability in environmental change. Several years ago, Davis et al., (2000) proposed that habitats an increased variability in resource availability will be more easily invaded than those with less variable resource conditions. In other words, if an environment experiences a pulse in resource supply, which can be caused by meteorological fluctuations and site-specific events, such as large- or small-scale disturbances and anthropogenic eutrophication, invasive plants may be better able to exploit the unused resources than resident species, resulting successful invasion. The few studies that this suggested that this might indeed be the case (Davis & Pelsor, 2001; White et al., 2001; Koerner et al., 2015; Tognetti & Chaneton, 2015). For example, in experimental plant communities, the invasive Japanese knotweed (*Fallopia* spp.) experienced a two- to four-fold proportional increase in biomass when nutrients were supplied in a single large pulse, or in multiple pulses of different magnitudes, whereas uniform application of nutrients did not result in a significant proportional increase in biomass of the invader (Parepa et al., 2013). Consequently, it is important to test how plants respond to change in environmental variability in addition to how they respond to changes in mean environmental conditions.

*Phenotypic plasticity*

*Plant phenotypic plasticity in response to environmental factors*

Individual organisms can alter their development as an acute response to changes in environmental conditions. Phenotypic plasticity is defined as the capacity of a given genotype to express different phenotypes under different environmental conditions (Bradshaw, 1965; 1973). It is ubiquitous among organisms, and is itself evolved characteristic that varies among genotypes, populations and species (Sultan, 2000). Plants are sessile organisms that
cannot migrate to more favorable patches to avoid stressful conditions, in contrast to animals. Plants have to cope with the condition where they are and/or what comes. Consequently, phenotypic plasticity, which can enable plants to cope with environmental heterogeneity by increasing environmental tolerance (i.e. fitness homeostasis; Valladares et al., 2014), is thought to be very advantageous to plants.

Plants are frequently exposed to heterogeneity within the natural environment. Light is a crucial factor required for the growth and development of plants, and is highly heterogeneous in nature. Therefore, most plants are exposed to a certain degree of shading during their lifetime (Valladares & Niinemets, 2008). Plants can respond to changing light conditions by adjusting their morphological and physiological traits. For example, under shading, most plant species in open habitats elongate their stem and petioles, bend their leaves upwards, and/or reduce branching to attain light (Givnish, 1988; Griffith & Sultan, 2005; Gommers et al., 2013). Conversely, if plants cannot outgrow surrounding plants and adopt a shade tolerance response (e.g. some herbaceous plants from forest understories), they can increase their specific leaf area (SLA), photosystem II: I ratio, or reduce their chlorophyll a:b ratio to optimize carbon gain (Givnish, 1988; Griffith & Sultan, 2005; Gommers et al., 2013).

Soil nutrients and water availability are crucial factors for the growth and development of plants, and are heterogeneously distributed in space and time. Therefore most plants are also exposed to different nutrient and water conditions during their lifetime. One of the primary functions of roots is the uptake of nutrients and water from the soil for plant growth; therefore, roots can exhibit plastic change in response to variations in soil nutrients. For example, plants can allocate more biomass to their root system than to aboveground biomass under limiting nutrient or water conditions (Poorter et al., 2012; Freschet et al., 2015; Poorter & Ryser, 2015). With increasing nutrient limitation, plants generally decrease their root diameter and increase the total length of root per unit root mass, i.e. specific root length (Hill et al., 2006; Ostonen et al., 2007).

Phenotypic plasticity and plant invasion

As phenotypic plasticity allows plant to express advantageous phenotypes in a broader range of environments (i.e. enhances ecological niche breadth), it has long been suggested to promote plant invasion (Daehler, 2003; Rejmanek et al., 2005; Richards et al., 2006; Pyšek &
Richardson, 2007; Davidson et al., 2011). Compared with some native and/or non-invasive plants, successful invasive plants are thought to benefit more from phenotypic plasticity by maintaining fitness homoeostasis under stressful environmental conditions, obtaining a larger increase in fitness in response to favourable environmental conditions, or maintaining and increasing fitness in stressful and favourable environmental conditions, respectively (Richards et al., 2006; Davidson et al., 2011). Consequently, high phenotypic plasticity is considered to increase the chances of an alien plant species to establish in a new environment, and to outcompete resident native plant species (Baker & Stebbins, 1965; Richards et al., 2006).

Two common comparative approaches can be used to test and reveal different insight of the contribution of phenotypic plasticity to plant invasion. One approach involves the comparison between invasive alien plants and native plants, which allows to determine whether phenotypic plasticity plays a key role in competition between invasive and resident native plant species. For example, Funk, (2008) explored trait plasticity in response to variation in light and nutrient availability in five phylogenetically related pairs of native and invasive species occurring in a nutrient-poor habitat, and found that invasive species display higher trait plasticity than native species. Another approach involves the comparison between invasive alien plants and non-invasive alien plants, and can determine whether phenotypic plasticity contributes to the success of alien plants. For example, van Kleunen et al., (2011b) grew 14 congeneric pairs of invasive and non-invasive alien plant species under shaded and non-shaded conditions in a common garden experiment, and found that the shade-induced phenotypic responses did not differ between invasive and non-invasive species.

*Adaptive phenotypic plasticity of plants*

Plants exhibit plasticity in numerous ecologically important traits related to plant function, development and life history (Sultan, 2000; Valladares et al., 2007; Gratani, 2014). Because of the high potential fitness benefit of phenotypic plasticity (when coping with environmental heterogeneity), it is often assumed that phenotypic plasticity of plants has frequently evolved as an adaptation to environmental heterogeneity (Baker, 1974; Richards et al., 2006). In other words, the plastic responses to varying environments are thought to be active and adaptive. However, many of the plastic responses of plant species to contrasting environments may be only passive responses to the environment (e.g. growth reductions due to resource limitation;
Dorn et al., 2000; van Kleunen et al., 2000). Therefore, not all plastic responses to contrasting environments are adaptive, i.e. help to increase the environmental tolerance of plants.

Because adaptive phenotypic plasticity could allow plants perform optimally under contrasting environments, the question arises of why plastic generalist plants do not always evolve. The rarity or absence of plastic generalist plants suggests that there are constraints on the evolution of plasticity. Theoretical studies show that the evolution of adaptive phenotypic plasticity may be constrained by costs and limits of phenotypic plasticity, which lead to fitness decreases when a trait is produced via plasticity (Moran, 1992; Sultan et al., 2002; Ernande & Dieckmann, 2004; van Kleunen & Fischer, 2005; Valladares et al., 2007; Murren et al., 2015). Therefore, the prerequisites of adaptive phenotypic plasticity evolved is that the benefit of phenotypic plasticity could compensate and overcompensate for their cost.

There is wide consensus that plastic responses associated with shade avoidance and tolerance, and nutrient-uptake strategies constitute adaptive phenotypic plasticity. However, only a few case studies have tested it explicitly, i.e. linked the phenotypic plasticity directly to performance maintenance across contrasting environments. Such plastic responses include increases in leaf length, SLA and biomass allocation to shoots in response to shading (Dudley & Schmitt, 1996; Schmitt et al., 1999; van Kleunen & Fischer, 2005; Valladares & Niinemets, 2008), and increases in root length, specific root length, and biomass allocation to the root system in response to nutrient limitation (Hutchings & de Kroon, 1994; de Kroon et al., 2012; Grossman & Rice, 2012; Keser et al., 2014; Keser et al., 2015).

**Research gaps**

Predicting how the spread of invasive plants may change with ongoing global environmental change has become a hot topic in ecology (Dukes & Mooney, 1999; Bradley et al., 2010a). Many case studies have attempted to test the interactions between plant invasion and environmental change in mean conditions, but have produced mixed results (refer to the section Biological invasions). Therefore, a comprehensive analysis is needed to assess whether a general pattern exists. A few years ago, van Kleunen et al., (2010b) performed a meta-analysis on trait differences between invasive and non-invasive (mostly native) plant species under current environmental conditions, and found that invasive plant species had significantly higher values than native plant species for traits reflecting physiology, size, and fitness.
These findings indicate that invasive plants are already tending to outperform native plants. If there is also a general pattern that, for traits reflecting physiology, size, and fitness, invasive plants respond more positively than native plants to global environmental change factors, we can predict that the severity of plant invasion will increase in the future, when global environmental changes continue.

Environmental changes do not only include changes in mean conditions, but also include changes in their variability. However, most empirical studies linking phenotypic plasticity to plant invasion have only assessed the plastic response to environmental changes in mean conditions (Leishman & Thomson, 2005; Funk, 2008; Godoy et al., 2011; Porté et al., 2011; van Kleunen et al., 2011b; Dawson et al., 2012a; Jia et al., 2016). This is also the reason why the abovementioned comprehensive analysis was only limited to the environmental changes in their overall mean conditions. The fluctuating-resource-hypothesis suggests indicates that a habitat with high variability in resource availability will generally lead to a higher invisibility of the habitat compared to one with less variable resource conditions (Davis & Pelsor, 2001). This might occur because invasive plants benefit more than native plants from resource variability due to higher phenotypic plasticity. Therefore, it is important to assess the plastic responses of alien and native plant species to such resource variability, in addition to their responses to changes in mean resource conditions. Moreover, although the fluctuating-resource-hypothesis has become a key theory in invasion biology, it has only been tested and confirmed in a few case studies, so it remains still unclear whether a general pattern exists for both invasive and non-invasive alien plant species.

Many studies trying to link phenotypic plasticity of specific traits to plant success across environmental changes in space and time are based on wide consensus (i.e. the assumption) that such specific plastic responses constitute adaptive plasticity (Leishman & Thomson, 2005; Funk, 2008; Schlaepfer et al., 2010; van Kleunen et al., 2011b; Feng & van Kleunen, 2014). However, few studies have assessed explicitly whether phenotypic plasticity of the specific traits they used are adaptive. Only adaptive phenotypic plasticity can help plants maintain fitness homeostasis across different environmental conditions (van Kleunen & Fischer, 2005). Therefore, it is very important to distinguish between adaptive and non-adaptive phenotypic plasticity. One example of phenotypic plasticity for which there appears to be wide consensus that it is adaptive is the plastic response of specific leaf area (SLA) induced by changes in
light intensity. SLA is measured as the area of a leaf divided by the dry weight, and is related to growth rate and palatability. Plants usually develop a higher SLA when grown under low-light conditions (Reich et al., 2003; Rozendaal et al., 2006; Feng & van Kleunen, 2014). As SLA tends to scale positively with the mass-based light-saturated photosynthetic rate (Pérez-Harguindeguy et al., 2013), higher SLA could help plants to increase the efficiency of light capture and maximize carbon gain under low-light conditions (Evans & Poorter, 2001; Gommers et al., 2013). Therefore, it is generally assumed that the plastic response of SLA enables plants to maintain high performance under shading, and thus constitutes adaptive phenotypic plasticity (Valladares & Niinemets, 2008; van Kleunen et al., 2011b; Feng & van Kleunen, 2014). As light is a crucial factor required for the growth and development of plants, and is highly heterogeneous in nature, many case studies have determined SLA and performance responses of plants to shading. Therefore, using a comprehensive analysis of these studies can directly test whether SLA plasticity in response to shading is adaptive.

**Contribution of this thesis**

My thesis tried to gain insights into how phenotypic plasticity contributes to plant success under environmental change, specifically focusing on plant invasion under global change and nutrient fluctuations, and plant tolerance to shading. I addressed this using two approaches: meta-analysis and greenhouse experiments. First, I used a meta-analysis to assess whether there is a general pattern that invasive plants show higher plasticity than native plants in response to changes in other global environmental factors. Few study tested for plasticity of invasive and native plants in response to fluctuations in environmental factors. Therefore, second, I performed a greenhouse experiment to assess how successful and less successful alien and native plants respond to fluctuating nutrient availability, and whether such responses relate to success of the different plants. Third, together with a Master student, I performed another greenhouse experiment following up on the first greenhouse experiment to test whether invasive plants benefit more than non-invasive alien plants from fluctuating nutrient availability when planted into native communities. Almost all studies trying to link to phenotypic plasticity to plant success are based on the assumption that phenotypic plasticity is adaptive, although few studies have assessed this explicitly (see Section Research gaps). Therefore, in the fourth study, I used a meta-analysis to test whether phenotypic plasticity in SLA to shading is really adaptive.
In the 1\textsuperscript{st} chapter, I tested whether there is a general pattern in invasive and native plant responses to environmental change in mean conditions. I established a plant database, which included the responses in performance traits of invasive alien and native plant species to environmental change. Global environmental change factors were restricted to increasing atmospheric CO\textsubscript{2} concentrations, increasing temperatures, increasing N deposition, and increasing or decreasing precipitation. I used these data to perform a phylogenetically controlled meta-analysis to address the following specific questions: (i) Do invasive alien plant species respond more positively to each component of global environmental change than native plant species? (ii) Which components of global environmental change are likely to favour or inhibit the performance of invasive alien plants over native plants?

In the 2\textsuperscript{nd} chapter, I used a multispecies greenhouse experiment to test whether alien and common plant species take more advantage of increases in resource levels and fluctuations therein than native and rare species. I compared plastic responses in biomass production, root allocation and root morphology to nutrient addition and fluctuations among seven common alien, seven rare alien, nine common native and six rare native plant species. I applied six nutrient-supply treatments that differed in the mean and temporal availability of nutrients. Specifically, I asked the following questions: (i) Do alien and common plant species exhibit stronger plastic responses in root morphology and allocation, and take more advantage of increased mean nutrient levels than native and rare plant species? (ii) Do alien and common plant species exhibit stronger plastic responses in root morphology and allocation, and take more advantage of gradual changes in nutrient availability over time than native and rare plant species? (iii) Do alien and common plant species take more advantage of nutrient pulses than native and rare plant species?

In the 3\textsuperscript{rd} chapter, I used a multispecies greenhouse experiment to test whether there is a general pattern through which invasive alien plant species might show higher plastic response and thus take more advantage of nutrient fluctuations than non-invasive alien and native plant species. We created a nutrient-supply pattern with six different nutrient-supply treatments, as described in the 2\textsuperscript{nd} chapter. We grew ten pairs of closely related invasive and non-invasive species (i.e. 20 species in total) as target species in communities of native competitors. As competitors, 12 native grassland species were grouped into four communities of three species each. Specifically, we asked the following questions: (i) Does increased nutrient availability
promote absolute and relative biomass (compared to the native communities) of both invasive and non-invasive alien plants? (ii) Does higher fluctuations in nutrient availability promote the absolute and relative biomass of both invasive and non-invasive alien plants? (iii) Do invasive alien plants show stronger plastic response and thus take more advantage of changes in mean nutrient conditions and fluctuations than non-invasive alien plant?

In the 4th chapter, I tested whether plasticity of SLA in response to shading is adaptive, i.e. whether it enables plants to maintain their performance under shade conditions. I compiled a database including data from 467 studies using 32 publications and two unpublished experiments, which measured the responses of biomass and SLA of 280 plant species to shading. Specifically, I asked whether greater plastic changes in SLA in response to shading help plants to better maintain performance under shaded conditions (i.e. whether plasticity in SLA is positively related to the maintenance of plant biomass).
Do invasive alien plants benefit more from global environmental change than native plants?

Yanjie Liu, Ayub M. O. Oduor, Zhen Zhang, Anthony Manea, Ifeanna M. Tooth, Michelle R. Leishman, Xingliang Xu, Mark van Kleunen

Abstract

Invasive alien plant species threaten native biodiversity, disrupt ecosystem functions, and can cause large economic damage. Plant invasions have been predicted to further increase under ongoing global environmental change. Numerous case studies have compared the performance of invasive and native plant species in response to global environmental change components (i.e. changes in mean levels of precipitation, temperature, atmospheric CO$_2$ concentration or nitrogen deposition). Individually these studies usually involve low numbers of species and therefore the results cannot be generalized. Therefore, we performed a phylogenetically-controlled meta-analysis to assess whether there is a general pattern of differences in invasive and native plant performance under each component of global environmental change. We compiled a database of studies that reported performance measures for 74 invasive alien plant species and 117 native plant species in response to one of four global environmental change components. We found that elevated temperature and CO$_2$ enrichment increased performance of invasive alien plants more strongly than was the case for native plants. Invasive alien plants tended to also have a slightly stronger positive response to increased N deposition and increased precipitation than native plants, but these differences were not significant (N deposition: $P = 0.051$; increased precipitation: $P = 0.679$). Invasive alien plants tended to have a slightly stronger negative response to decreased precipitation than native plants, although this difference was also not significant ($P = 0.060$). So while drought could potentially reduce invasion, increases in the four other components of global environmental change considered, particularly global warming and atmospheric CO$_2$ enrichment, may further increase the spread of invasive plants in the future.

Keywords: Climate change, effect size, meta-analysis, nitrogen deposition, plant invasion, precipitation, temperature
Introduction

Across the globe, thousands of plant species have been introduced to biogeographic regions where they are not native (van Kleunen et al., 2015). Some of these introduced plants have since become naturalized, and eventually invasive, whereby they displace native plants and hence threaten native diversity, disrupt ecosystem functions and services, and cause large economic damage (Pimentel et al., 2005; Vilà et al., 2011). Consequently, understanding the mechanisms by which invasive alien plant species outperform native plants in the recipient native communities has become a hot topic in ecology (Funk & Vitousek, 2007; Leishman et al., 2010; van Kleunen et al., 2010b; Heberling & Fridley, 2013). With ongoing global environmental change, there is also increasing interest in how the spread of invasive plants may change in the future (Dukes & Mooney, 1999; Bradley et al., 2010a; Jia et al., 2016).

Biotic exchange is itself a major component of global environmental change, but it might be strongly affected by other global change components such as increasing atmospheric CO$_2$ concentrations, increasing temperatures, increasing nitrogen (N) deposition, and increasing or decreasing precipitation. It is thought that these environmental changes are more likely to promote than to inhibit invasive plant performance compared to native plant performance. This is because invasive plants often exhibit broad environmental tolerance and high phenotypic plasticity, which may confer the capacity to survive in altered environmental conditions (Richards et al., 2006; Davidson et al., 2011). Furthermore, the intrinsically high growth rate characteristic of many invasive plant species (Grotkopp et al., 2010; van Kleunen et al., 2010b; Dawson et al., 2011) may enable them to respond more positively to environmental changes that result in increased resource availability (elevated levels of water supply, atmospheric CO$_2$ concentrations, and N deposition) than native plants adapted to low resource conditions (Tilman, 2004). Thus, global environmental change could further promote invasiveness of invasive alien plant species.

The hypothesis that global environmental change may favour performance of invasive plant species more strongly than that of native plants has been subjected to numerous experimental tests. These are usually case studies involving local comparisons of a single pair or a few pairs of invasive and native plant species, and have produced mixed results (Dukes & Mooney, 1999; Bradley et al., 2010a). A few years ago, Sorte et al., (2013) did a meta-analysis on the responses of naturalized alien and native organisms to climate change. Across
different types of organisms and ecosystems, naturalized alien species tended to show stronger responses than natives, but, among terrestrial plants, naturalized alien and native plants showed similar responses. That study, however, was not restricted to invasive alien plant species and did not correct for phylogenetic non-independence of the studied species. Although Sorte et al., (2013) included many different types of organisms and ecosystems, they did not consider responses to N deposition, which is another major component of global environmental change (Holland et al., 2005; Liu et al., 2013). Successful plant species are often associated with a particular suite of traits that enable them to respond more positively to N deposition (Dawson et al., 2012b). Therefore, one could hypothesize that invasive plants are more successful in areas with high N deposition. Indeed, several studies found evidences in support of this hypothesis at a continental or reginal scale (Scherer-Lorenzen et al., 2000; Scherer-Lorenzen et al., 2007; Seabloom et al., 2015). Moreover, a previous meta-analysis also found evidence that in terrestrial plants, invasive species responded more strongly to N deposition than native species (Gonzalez et al., 2010). However, that meta-analysis did not correct for phylogenetic non-independence of the studied species either. Recent studies have shown that inclusion of phylogenetic information can significantly change the outcomes of a meta-analysis (Chamberlain et al., 2012), and hence correction for species relatedness should be an important component of any meta-analysis on variation among species.

Here, we established a database, restricted to plants, with responses of invasive alien and native species to environmental change. We used these data to do a phylogenetically-controlled meta-analysis to address the question: (1) Do invasive alien plant species respond more positively (i.e. benefit more) to each component of global environmental change than native plant species? (2) Which components of global environmental change are likely to favour or inhibit performance of invasive alien plants over native plants? Answering these questions will enable an assessment of whether global environmental change is likely to further increase invasiveness of invasive alien plants, and thereby may exacerbate their impacts on native plants in the future.
Materials and methods

Data compilation

To identify studies on performance responses of both native and invasive alien plants to global change, we conducted a literature search for peer-reviewed publications in ISI Web of Science (http://apps.webofknowledge.com/) and Google Scholar using the following search string: ‘climate change’ OR ‘global change’ OR ‘warm*’ OR ‘temperature’ OR ‘nitrogen’ OR ‘nitrogen deposition’ OR ‘CO₂’ OR ‘carbon dioxide’ OR ‘precipitation’ OR ‘watering’ OR ‘drought’ OR ‘rainfall’ AND ‘invasive’ OR ‘alien’ OR ‘non-native’. All published records from 1980 to 30th June 2015 were included in the search. We found two pre-1980 studies on temperature responses of native and invasive species (i.e. Henry & William, 1958; Ashby & Hellmers, 1959), but, as these studies did not provide measures of variation (standard errors or standard deviations), they could not be used for the meta-analysis. We also included studies published in the Chinese language (www.cnki.net). Our searches were limited to studies on plants and resulted in 1,036 publications.

We then individually assessed each publication, and retained the ones that met each of the three criteria given below. (1) The publication reported effects of manipulating mean values of at least one of the five different components of global environmental change (i.e. increases in temperature, atmospheric CO₂ concentration, N deposition, increased precipitation, or a decrease in precipitation) on performance of invasive alien and native plants. Although global environmental change also entails changes in variability, such as the increased frequency of extremes in temperature and precipitation, we focus on changes in means values because only few studies have manipulated variability in global change components. (2) Publications included at least one invasive alien and one native plant species in the same experiment (origin and invasive status of each species was determined from the respective publications). (3) Publications reported mean values, sample sizes and variances for performance-related traits of each species. The performance-related traits included in our meta-analysis were direct estimates of fitness (i.e. survival and reproduction), of growth (i.e. biomass and size) and physiology (i.e. photosynthetic rate, which is likely to increase the performance of plants). In total, 56 publications met these criteria (see Supplementary Materials and Methods S1), covering 74 invasive alien species and 117 native species. There were a few studies in which it was not clear whether the alien species studied was invasive or not. Such studies
were excluded from the analysis presented in the main text. However, analysis with and without data from such studies gave similar results (Tables S1 and S2; Figs. S1 and S2). We also considered whether seeds of invasive species were sourced from their native range or their invaded range, as this might influence the performance of plants. Although not all studies provided information on this, seeds of the invasive species appear to be generally sourced from the invaded range. Therefore, the effect of seeds source could not be tested.

We extracted mean values of the performance-related traits mentioned above and their corresponding variances (standard deviations, standard errors or 95%-confidence intervals) and sample sizes directly from the text or tables, or from figures using the software Image J 1.47v (Rasband, 2013). For all cases of temperature, atmospheric CO₂ concentration and soil N, we considered the ambient level (i.e. no treatment level) of an environmental change factor as the “control”, and the elevated level of the same factor as the “treatment”. However, as precipitation is likely to decrease in some parts of the world and increase in other parts, some studies imposed a drought treatment whereas others increased watering relative to ambient levels. We considered these as two different types of studies. For studies with decreased water availability relative to ambient, the drought treatment is considered the “treatment”, and for studies with increased water availability relative to ambient, the high water-availability treatment is considered the “treatment”. When performance measures were reported for different time points from the same experiment, we only used the data from the last time point (i.e. we chose the longest duration of the study). When more than one environmental change factor was manipulated in an experiment, we used the performance measures corresponding to manipulation of a single focal global environmental change factor, when the other factors were kept at their ambient levels. When the plants were grown under different levels of competition, we included data for all the competition levels (eight of 56 total publications in our meta-analysis manipulated competition).

**Effect size and variance computation**

To examine the effects of global environmental change on native and invasive alien plant performance, we calculated the log response ratio (ln $R$) as the effect size of response variables for each individual performance-related traits of each species per study, following Hedges et al., (1999):
\[ \ln R = \ln \left( \frac{\bar{X}_t}{\bar{X}_c} \right) = \ln(\bar{X}_t) - \ln(\bar{X}_c). \]

Here, \( \bar{X}_t \) and \( \bar{X}_c \) are the mean values of each individual trait measure in the treatment (t) and control (c), respectively. An \( \ln R \) value < 0 indicates a decrease in plant performance in response to a change in the environmental change factor; a value > 0 indicates an increase in plant performance. The variance of \( \ln R \) was calculated, following Hedges et al., (1999) as

\[ \nu_{\ln R} = \frac{(SD_c)^2}{N_c(\bar{X}_c)^2} + \frac{(SD_t)^2}{N_t(\bar{X}_t)^2}. \]

Here, \( N_t, N_c, SD_t, SD_c, \bar{X}_t, \) and \( \bar{X}_c \) are sample sizes, standard deviations and mean values for traits measured in the treatment and control, respectively. Because some studies reported different measures of performance-related traits for the same plant species, we pooled the multiple effect sizes (weighted by the inverse variance) and corresponding variances per study to avoid pseudo-replication (Leimu et al., 2006). Pooling was done using the fixed-effect model (using the rma function in R package metafor), because we assumed that there is a single, true underlying effect size per species in a study (Borenstein et al., 2009). The resulting 252 effect sizes and corresponding mean variances were used in the analyses described below.

**Data analysis**

All meta-analytical calculations and statistical analyses were performed in R 3.1.3 (R Core Team, 2015) using the package metafor v1.9-7 (Viechtbauer, 2010). First, to test whether the plants, on average, exhibited significant positive or negative responses to environmental change regardless of their invasive status, we performed a general meta-analysis using a random-effects model (i.e. we assumed that there is true random variation among effect sizes, as is thought to be the case for ecological data; Gurevitch & Hedges, 2001). Then, to test whether native and invasive alien plants differed significantly in their performance responses to each of the different components of global environmental change (increases in mean levels of precipitation, temperature, atmospheric CO\(_2\) levels or N deposition, or a decrease in mean levels of precipitation) separately, we constructed mixed-effects multivariate models using the rma.mv function. In the models, plant invasive status was included as a fixed-effects moderator. Other fixed-effects moderators were also considered but either had insufficient data, no variance or did not affect the results and so were not presented in this study. Because
some studies included multiple pairs of invasive alien and native plant species, yielding multiple effect sizes per study, and some plant species were used in multiple studies, we included study (i.e. publications from which we extracted the data) and species identity as random factors in the models above.

To control for possible non-independence of effect sizes from species with shared evolutionary history, we also included phylogenetic relatedness among the study species in the models above by including the variance-covariance matrix of species relatedness as an additional random factor. To get the variance-covariance matrix, we first constructed a base tree using the online program Phylomatic (Webb & Donoghue, 2005). Polytomies within this base tree were then resolved as far as possible using published molecular phylogenies (see Supplementary Materials and Methods S2). The phylogenetic tree was then transformed to an ultrametric tree using the compute.brln function in the package ape v 3.2 (Paradis et al., 2004). Finally, a variance-covariance matrix was calculated from the ultrametric tree, representing phylogenetic relatedness among species, using the vcv function in the package ape v 3.2.

In each model, we computed weighted mean effect sizes and 95% confidence intervals (CIs) for the moderator levels (invasive, native). We considered a mean effect size estimate to be significantly different from zero if the 95% CI around the mean did not include zero. In these models, total heterogeneity (Q_T) in effect sizes can be partitioned into heterogeneity explained by the model structure (Q_M) and unexplained heterogeneity (Q_E). We used the Q_M test (Koricheva et al., 2013) to test for a significant difference in the mean effect size between native and invasive alien plant species for the moderator.

Publication bias

In many research fields there is a bias against publishing negative results (Rosenthal, 1979). Hence, to assess whether there is evidence for a publication bias in our meta-data set, we used a funnel plot and Egger’s regression. A funnel plot graphs effect sizes against standard errors, and assumes that studies with the largest sample sizes will have lower standard errors, and hence will be near the average effect size, while studies with smaller sample sizes will show a larger spread on both sides of the average effect size (Koricheva et al., 2014). Deviations from this expected pattern can indicate publication bias (Koricheva et al., 2014). Positive
asymmetry in a funnel plot is typically taken to indicate bias, in that studies with positive effects are published with a greater frequency than studies with negative effects (Koricheva et al., 2014). We first graphed the funnel plots using the funnel function, and visually inspected funnel plots of standard errors or replicate numbers versus standardized effect sizes for the presence of asymmetry (Egger et al., 1997; Sterne & Egger, 2001). We then formally tested the asymmetry of funnel plots using Egger’s test which is widely used for detecting publication bias (Sterne & Egger, 2006) using the regtest function.

**Results**

In the analysis that did not consider the invasive status of the species, increases in mean levels of atmospheric CO₂ concentration and N deposition had significantly positive effects on average plant performance (Table S3; Fig. S3). Increased temperatures and increased precipitation also had net positive effects on average plant performance, but these effects were not significantly different from zero (Table S3; Fig. S3). On the other hand, a decrease in the mean level of precipitation had a significantly negative effect on average plant performance (Table S3; Fig. S3). In the separate analyses for each component of global environmental change in which we considered the invasive status (invasive vs native) of the plant species, elevated temperature and elevated atmospheric CO₂ concentrations resulted in significantly larger increases in performance for invasive alien plants than for native plants (Table 1; Fig. 1). Invasive alien plants tended to have a slightly stronger positive response to increased N deposition and increased precipitation than native plants, but these differences were only marginally significant for N deposition and not significant for precipitation (Table 1; Fig. 1). On the other hand, invasive alien plants tended to have a slightly stronger negative response to decreased precipitation than native plants, and this difference was marginally significant (Table 1; Fig. 1).
Figure 1 Performance responses (indicated by log response ratio mean effect sizes) of native (blue symbols) versus invasive alien plant species (red symbols) to drivers of global environmental change (increased and decreased precipitation, elevated temperature, elevated atmosphere CO\textsubscript{2} levels and nitrogen deposition). Error bars represent 95% confidence intervals around the mean effect-size estimates, and were derived from a phylogenetically informed meta-analytic model. The asterisk (*) indicates a statistically significant difference between native and invasive plant species (i.e. \( p < 0.05 \)), and † indicates a marginally significant difference (i.e. \( p < 0.1 \)), while ns denotes no significant difference. Sample sizes (i.e. the number of effect sizes) are given in parentheses. The dashed vertical line indicates zero effect of the global-environmental change drivers.

In all analyses, the variance components associated with phylogenetic history were low (Table 1 and Tables S1-S3), indicating that the effect sizes used were largely phylogenetically independent. Visual inspection of the funnel plot and Egger’s test for asymmetry of the funnel plot showed that the results were not significantly affected by a publication bias (\( z = -0.887, p = 0.375; \) Fig. S4).
Table 1 Results of a phylogenetically informed meta-analysis comparing invasive alien and native plant species for differences in response to environmental change (i.e. increased and decreased precipitation, elevated temperature, elevated atmospheric CO$_2$ levels, and nitrogen deposition). The analysis was performed for each component of global change individually. In the analysis, the $Q_M$ statistic and associated $P$ value test for a difference between invasive alien plants and native plants. A significant (or marginally significant) difference between invasive and native plants is marked in bold font.

<table>
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<tr>
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<th>Upper 95% CI</th>
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Discussion

Many invasive alien plant species have a broader environmental tolerance and a higher phenotypic plasticity than native plants (Richards et al., 2006; Davidson et al., 2011). Hence invasive plants have been hypothesized to benefit more from global environmental change than native plants do (Dukes & Mooney, 1999; Davidson et al., 2011). The present synthesized results of a phylogenetically-controlled meta-analysis partly support this hypothesis. The separate analyses of the individual global-change components showed that elevated temperature, elevated atmospheric CO$_2$ concentrations and N deposition might favour performance of invasive plants relative to that of native plants. Decreased precipitation, on the other hand, might inhibit performance of invasive plants more relative to that of native plants. Our results thus suggest that particularly elevated temperature, elevated atmospheric CO$_2$ concentrations and N deposition may further promote invasiveness of the invasive alien plant species, while decreased precipitation (i.e. drought) might inhibit it.

Overall, invasive plants benefited more from increases in the global environmental change components than native plants did. This result is concordant with the finding of a meta-analysis by Davidson et al., (2011) that invasive plants are more phenotypically plastic than co-occurring non-invasive plants across several different types of environmental variation. A previous meta-analysis on trait differences between invasive and non-invasive (mostly native) plant species found that, on average, invasive plant species had significantly higher values than native plants for traits reflecting physiology, size and fitness (van Kleunen et al., 2010b). Combined with our results, this suggests that invasive plants may even more strongly outperform native plants under increases in global environmental changes in the future.

Our findings contrast to some extent with results of a recent meta-analysis by Sorte et al., (2013) who evaluated the responses of alien and native organisms, including both plants and animals, to elevated atmospheric CO$_2$ concentrations, warming and changes in precipitation, in terrestrial, marine and freshwater ecosystems. They found that alien and native organisms, primarily plants, in terrestrial ecosystems did not significantly differ in their responses to environmental changes. Nevertheless, some of the patterns that we found are in line with the patterns that Sorte et al., (2013) found. The results of Sorte et al., (2013) indicate a slight tendency for stronger responses to increases in CO$_2$ and precipitation among alien species than among native species. There are several possible explanations for why the results or the
statistical significances deviate between two studies. First, we used the log response ratio (ln \( R \)) as effect size to quantify the different plastic responses to environmental changes between invasive and native plants, while Sorte et al., (2013) used the ratio of the difference between treatment and control responses to the average of responses across treatment and control conditions. Second, we only evaluated responses of plants to environmental change rather than combining plants and animals. Third, we focused on the comparison between natives and invasive aliens, while Sorte et al., (2013) compared natives with naturalized aliens, which are not necessarily invasive. Fourth, we included studies that were published after 2013, and thus were not included in Sorte et al., (2013). Whatever the exact reason for the discrepancies, in contrast to Sorte et al., (2013), who mainly found differences in the responses of alien and native organisms in aquatic systems, we now provide evidence that similar differences exist for terrestrial plants.

Invasive plants took significantly more advantage of CO\(_2\) enrichment than native plants did. Plants with the C\(_3\) photosynthetic pathway are thought to take more advantage of CO\(_2\) enrichment than plants with a C\(_4\) pathway (Pearcy & Ehleringer, 1984; Poorter, 1993). Thus the present results could also reflect differences in photosynthetic pathways between invasive and native plants in our study. However, because invasive and native plants had similar numbers of species characterized by C\(_3\) (invasive: n = 35; native: n = 35) and C\(_4\) (invasive: n = 4; native: n = 7) photosynthetic pathways in our analysis, the photosynthetic pathway likely played little role in differences between invasive and native plant responses to CO\(_2\) enrichment. Therefore, increased CO\(_2\) concentration likely favoured performance of invasive plants over native plants through direct (enhanced growth rate) and indirect (enhanced resource capture) mechanisms regardless of photosynthetic pathway.

Elevated temperature had stronger positive effects on performance of invasive plant species than of native plant species. Warming can directly affect photosynthesis and resource uptake (Ilorens et al., 2004; Blumenthal et al., 2013), increase the duration of the growth period of a plant (Peñuelas et al., 2002), and could also induce a higher soil-nutrient availability through increased mineralization (Rustad et al., 2001). Generally, native plants have a long evolutionary history under ambient temperatures and thus are adapted to the ambient temperature, whereas they might not be optimally adapted to novel temperature conditions created by global warming. Although invasive plant species are locally adapted as frequently
as native plants are (Oduor et al., 2016), invasive plants may naturally be pre-adapted to a wider range of temperatures (Bradley et al., 2015), and hence warming could enhance invasiveness of these alien plants.

Invasive plant species have often been introduced from more nitrogen-rich habitats, and are thus more likely to be adapted to environments with high nitrogen levels (Dostál et al., 2013). A previous study also showed a positive correlation between N deposition and abundance of invasive plant species at a regional scale (Scherer-Lorenzen et al., 2007). This indicates that increased N deposition could promote plant invasion (Bradley et al., 2010a). Our meta-analysis tentatively supports this, because we found that the response of invasive plants to increased N deposition was marginally significantly higher than that of native plants. Our finding is in line with previous cross-species studies (Scherer-Lorenzen et al., 2000), and also with a previous meta-analysis showing that nitrogen enrichment favoured invasive-terrestrial plant species over native-terrestrial plants (Gonzalez et al., 2010). A recent study showed that in many grasslands, introduced plant species respond more strongly to nitrogen enrichment than native plant species do (Seabloom et al., 2015). Thus, the idea that invasive plants benefit more from increased nitrogen than native plants do seems to find general support, despite the marginal significance of this difference in our meta-analysis.

While atmospheric CO₂ concentration, temperature and N deposition are likely to further increase in most parts of the world, precipitation is likely to increase in some regions and decrease in other regions (Naz et al., 2016). Moreover, there is a high uncertainty around the predictions of future precipitation levels, and it is likely that the frequency of extremely dry and wet years will increase (IPCC, 2013). Therefore, it is important to distinguish studies that increased from those that decreased the water availability relative to ambient levels (Sorte et al., 2013). Our meta-analysis indicated that invasive plant species tended to take more advantage of higher water availability, but that this difference was not significant. On the other hand, invasive plants tended to be slightly less drought tolerant than native plants, although this was only marginally significant. Sorte et al., (2013) found similar patterns for responses to changes in precipitation between alien and native organisms as we did, and the differences in their meta-analysis were also not statistical significant. The patterns revealed by both meta-analyses were quite similar due to the high degree of overlap in publications used for this global change component (13 out of 16 publications used in our study were also
used in Sorte et al., (2013). Generally, invasive plant species tend to use more water than native plant species do (Cavaleri & Sack, 2010). Consequently, increases in precipitation may favour and, conversely, decreases in precipitation could inhibit invasive plant species more so than native plant species (Bradley et al., 2010b). Such patterns are also in line with the results of several field experiments (Levine et al., 2010; Ziska & Dukes, 2014). Our finding thus tentatively suggests that invasiveness of many currently invasive alien plants might decrease when the climate becomes drier.

The present meta-analysis has quantitatively summarized the patterns of invasive and native plant species’ responses to individual components of global environmental change. However, many of these components change simultaneously, and these changes may additively or interactively impact plant performance (Dukes et al., 2005; Bloor et al., 2010; Dieleman et al., 2012). For instance, elevated CO$_2$ can enhance water-use efficiency and thereby increase plant productivity under drier conditions (Blumenthal et al., 2013). On the other hand, warming often reduces soil moisture and increases water-use, thus negating the water-saving effects of elevated CO$_2$ (Cantarel et al., 2013). So, while some of the effects of different individual global change components may act in the same direction (Zavaleta et al., 2003), others may act antagonistically (Williams et al., 2007). Despite the potential importance of co-occurring environmental changes, few studies to date (only eight out of the 56 publications included in our meta-analysis) have examined invasive and native species’ responses to more than one global change component at a time. Therefore, the question as to what is the relative significance as well as the interactive effects of environmental change components on performance of invasive and native plants remains largely unexplored empirically.

In a summary, our meta-analysis revealed that invasive alien plant species benefited from elevated mean temperature and atmospheric CO$_2$ concentrations more so than native plants. There were similar patterns in response to increased N deposition and increases in precipitation (although the results were not significant). Among the native species, there was also wide variation in their responses, suggesting that some of them might benefit and expand their ranges. Similarly, among the invasive species, some species might benefit less than others under increased levels of the different global change components. Despite this variation within groups, overall, our findings suggest that global change drivers that create
favourable environmental conditions, particularly elevated temperature and atmospheric CO$_2$ concentrations, will further increase the invasiveness of invasive alien plants in the future.

**Acknowledgements**

We are very grateful to Dr. Zdravko Baruch Glaser who kindly provided data. We apologize to all those authors whose work we may have missed. Y.J. Liu was funded by a scholarship from the China Scholarship Council (scholarship number 201304910318). A.M.O. Oduor was funded by a Georg Forster Research Fellowship of the Alexander von Humboldt Foundation (grant number 3.4-KEN/1148979 STP). Z. Zhang was supported by a grant from the National Natural Science Foundation of China (grant number 31540051).
Supporting information

Materials and Methods S1 List of published studies from which data were extracted. C, elevated CO\textsubscript{2}; N, nitrogen deposition; P, elevated precipitation; T, elevated temperature.


Anderson L.J. & Cipollini D. (2013) Gas exchange, growth, and defense responses of invasive Alliaria petiolata (Brassicaceae) and native Geum vernum (Rosaceae) to elevated atmospheric CO\textsubscript{2} and warm spring temperatures. American Journal of Botany, 100, 1544-1554. C, T.


Materials and Methods S2 A phylogenetic tree used in this study and a list of published studies used for resolving polytomies within the initial base tree.


Petersen G., Seberg O., Yde M. & Berthelsen K. (2006) Phylogenetic relationships of *Triticum* and *Aegilops* and evidence for the origin of the A, B, and D genomes of


Table S1 Results of a phylogenetically informed meta-analysis of plant species responses to environmental change (i.e. increased and decreased precipitation, elevated temperature, elevated atmospheric CO₂ levels, and nitrogen deposition) regardless of a plant origin (alien or native). The analysis was performed for each component of global change individually. A mean effect size is significantly different from zero if its 95% confidence interval (CI) does not overlap zero. These results were obtained from the analysis of combined data including all non-invasive and invasive alien plants and native plants.

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<td>Elevated nitrogen</td>
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Table S2 Results of a phylogenetically informed meta-analysis comparing alien (included both invasive and non-invasive alien plants) and native plant species for differences in response to environmental change (i.e. increased and decreased precipitation, elevated temperature, elevated atmospheric CO$_2$ levels, and nitrogen deposition). The analysis was performed for each component of global change individually. In the analysis, the $Q_M$ statistic and associated $P$ value test for a difference between alien plants (non-invasive and invasive) and native plants. A significant (or marginally significant) difference between invasive and native plants is marked in bold font.

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<td>0.1962</td>
<td>0.7528</td>
<td>0.1422</td>
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</table>
Table S3 Results of a phylogenetically informed meta-analysis of plant species response to environmental change (i.e. increased and decreased precipitation, elevated temperature, elevated atmospheric CO\textsubscript{2} levels, and nitrogen deposition) regardless of a plant invasive status (invasive alien or native). The analysis was performed for each component of global change individually. A mean effect size is significantly different from zero if its 95% confidence interval (CI) does not overlap zero.

<table>
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Figure S1

Performance responses (indicated by log response ratio mean effect sizes) of plant species to drivers of global environmental change (increased and decreased precipitation, elevated temperature, elevated atmospheric CO₂ levels and nitrogen deposition) regardless of their origin (i.e. alien and native). Error bars represent 95%-confidence intervals around the mean effect-size estimates derived from the phylogenetically informed meta-analytic model. The sample sizes (i.e. the number of effect sizes) are given in parentheses. The dashed vertical line indicates zero effect of global environmental changes. These results were obtained from the analysis for the data including all non-invasive and invasive alien plants and native plants.
Figure S2 Performance responses (indicated by log response ratio mean effect sizes) of native (blue symbols) and alien (invasive and possibly non-invasive) plant species (red symbols) to drivers of global environmental change (increased and decreased precipitation, elevated temperature, elevated atmospheric CO$_2$ levels, and nitrogen deposition). Error bars represent 95%-confidence intervals around the mean effect-size estimates derived from the phylogenetically informed meta-analytic model. The asterisk (*) indicates a statistically significant difference (i.e. $P < 0.05$), and † indicates a marginally significant difference (i.e. $p < 0.1$), while ns denotes no significant difference between native and alien plant species. Sample sizes (i.e. the number of effect sizes) are given in parentheses. The dashed vertical line indicates zero effect of global environmental changes.
Figure S3 Performance response (indicated by log response ratio mean effect sizes) of plant species to drivers of global environmental change (increased and decreased precipitation, elevated temperature, elevated atmospheric CO$_2$ levels, and nitrogen deposition) regardless of a plant invasive status (i.e. invasive alien and native). Error bars represent 95%-confidence intervals around the mean effect-size estimates and were derived from the phylogenetically informed meta-analytic model. The sample sizes (i.e. the number of effect sizes) are given in parentheses. The dashed vertical line indicates zero effect of the global environmental-change drivers.
Figure S4. A test for publication bias

Figure S4 A funnel plot showing the relationship between effect size (ln $R$) and the inverse of the standard error (i.e. a test for publication bias).
Chapter 2

Responses of common and rare aliens and natives to nutrient availability and fluctuations

Yanjie Liu, Mark van Kleunen

Summary

1. Global environmental change not only includes changes in mean environmental conditions but also in temporal environmental fluctuations. Because it is frequently suggested that common species, and particularly invasive alien species, are phenotypically highly plastic, they might benefit more from these fluctuations than rare native and rare alien species. Experimental tests, however, are still lacking.

2. Here, we tested whether alien plant species take more advantage of increases in resource levels and fluctuations therein than native species, and whether common species do so more than rare species. Therefore, we grew seven common alien, seven rare alien, nine common native and six rare native herbaceous plants, in one treatment with constantly low nutrient availability and five treatments with high nutrient availability that differed in temporal availability of nutrients (constant, increasing, decreasing, single large pulse, multiple smaller pulses).

3. We found that all species produced more biomass and longer roots, and had a lower root mass fraction under high nutrient conditions than under low nutrient conditions, irrespective of their origin and commonness. Among the high nutrient treatments, the temporal pattern of nutrient supply also influenced biomass production, root allocation and root thickness, but the magnitude and/or directions of these responses varied among the groups of species. Particularly, we found that alien plant species, irrespective of whether they are common or rare, produced more biomass, and had a higher root mass fraction when nutrients were supplied as a single pulse in the middle of the growth period instead of supplied at a constant rate, whereas the reverse was true for the native species.

4. Synthesis. Our study suggests that species origin does not drive differences in plant biomass production, root morphology and allocation in response to changes in mean environmental nutrient availability. However in our study, alien plant species, in contrast to native plant species, benefited from a large nutrient pulse. This suggests that increased fluctuations in nutrient availability might promote alien plant invasions.

Key-words: biological invasion, commonness, exotic plant, invasiveness, non-indigenous, nutrient pulses, plant origin, plant-environment interactions, resource fluctuations
Introduction

With increasing globalization, more and more plant species have established wild populations in biogeographic regions where they are not native (Seebens et al., 2015; van Kleunen et al., 2015). Some of these naturalized species successfully spread and now occupy large areas in the introduced range (i.e. have become common), and some of these invasive species threaten native diversity, disrupt ecosystem services, and cause large economic damage (Vitousek et al., 1996; Pimentel et al., 2005; Vilà et al., 2011). Consequently, explaining what makes some alien plant species successful and allows them to displace native plants in the introduced region is an important research topic in ecology (Funk & Vitousek, 2007; Leishman et al., 2010; van Kleunen et al., 2010b; Heberling & Fridley, 2013; Funk et al., 2016; Jia et al., 2016).

High phenotypic plasticity is thought to help plants tolerate environmental change and to take advantage of increases in resources (Nicotra et al., 2010; Gioria & Osborne, 2014). Consequently, high plasticity could increase the chances of an alien plant species to establish in a new environment, and even to out-compete resident native plant species (Baker & Stebbins, 1965; Richards et al., 2006). Indeed meta-analyses have shown that invasive species are more plastic (Davidson et al., 2011; but see Palacio-López & Gianoli, 2011) and take more advantage of increased CO₂ levels and temperatures (Liu et al., 2017) than native species. So far, empirical studies tested this hypothesis mainly by assessing plastic responses to changes in the means of environmental factors (Leishman & Thomson, 2005; Funk, 2008; Godoy et al., 2011; Porté et al., 2011; van Kleunen et al., 2011b; Dawson et al., 2012a). Environmental change, however, also affects environmental variability (Stenseth et al., 2002; Smith, 2011; Parepa et al., 2013). As phenotypic plasticity could help species to cope with increasing environmental variability, it is important to test plastic responses of alien and native plant species to such variability, in addition to their responses to changes in mean environmental conditions.

A major global environmental change factor is atmospheric nitrogen deposition (Galloway et al., 2008), which has increased nutrient availability in many parts of the world and may have affected the commonness of alien and native plant species. Moreover, in addition to overall increases in nutrient availability, fertilizer spill over from arable fields and grasslands into natural areas, increasing fluctuations in extreme events (e.g. droughts, floodings, heat waves,
fires) affecting nutrient cycling and changes in land use types and their associated management practices (e.g. grazing, tilling) could lead to increasing temporal variability in nutrient availability (Davis & Pelsor, 2001; Pivello et al., 2010; Chen et al., 2011; Matias et al., 2011; Verma & Jayakumar, 2012). Consequently, the likelihood that nutrients might become available in one or multiple pulses of different magnitudes, and might slowly accumulate or decrease, might increase.

As one of the primary functions of roots is the uptake of nutrients from the soil to enhance plant growth, plastic changes in root allocation and morphology in response to nutrient availability could optimize nutrient acquisition, and thereby plant performance (Hutchings & de Kroon, 1994; de Kroon et al., 2012; Grossman & Rice, 2012). For example, under spatially heterogeneous nutrient supply, plants produce more, longer and thinner roots in the high nutrient patches than in the low nutrient patches, and this increases overall biomass production (Keser et al., 2014; Keser et al., 2015). However, little is known about how different patterns of temporal resource availability affect growth and root morphology, and whether this may differ between alien and native plants.

Plants can have different strategies to adjust to constant and fluctuating environments. There is evidence that, in contrast to plants from constant environments, plants from fluctuating environments have higher nutrient-uptake rates and show a stronger increase in potential uptake rates when nutrient availability increases (Craine, 2009). Plant-strategy theory suggests that only plants possessing characteristics related to competitive ability (e.g. high growth rates) could achieve success under high resource availability while competing with other plants (Grime, 1977; Jabot & Pottier, 2012; Grime et al., 2014). Consequently, highly competitive invasive alien, and also common native, plant species are predicted to be able to take more advantage of resource peaks under temporal nutrient fluctuations. In line with this idea, the fluctuating-resource-availability hypothesis poses that habitats become more susceptible to invasion when resources availability increases (Davis et al., 2000). Indeed, studies have shown that alien plants benefited more than native plants in the community when water availability (Blumenthal et al., 2008) or nutrients increased (Brooks, 2003; Jiménez et al., 2011; Lai et al., 2015; Flores-Moreno et al., 2016). Not only increases in the mean levels of resource availability, but also increases in the variability of resource availability are thus likely to increase plant invasion. Few experiments, however, have tested explicitly whether and to
what extent invasive alien plant species benefit from resource fluctuations (but see Davis & Pelsor, 2001; Parepa et al., 2013; Koerner et al., 2015 for notable exceptions).

Invasive alien species are widespread, but some native species are equally common. Similarly, there are not only rare native species but also rare alien species. It has been suggested that the factors that drive invasion success of alien species are the same as the ones that drive commonness of native species (Thompson & Davis, 2011; also see van Kleunen & Richardson, 2007). While this may seem plausible, it still needs to be explicitly tested (van Kleunen et al., 2011a; Knapp & Kühn, 2012). Dawson et al., (2012a) found that common alien plant species did not differ from common native plant species in their responses to nutrient addition, but that both groups of common species exhibited stronger responses to nutrient addition compared to rare alien and native species. Therefore, commonness might affect the outcomes of comparisons between alien and native plant species in response to nutrient changes, and should be tested.

To test whether alien and common plant species take more advantage of increases in resource levels and fluctuations therein than native and rare species, we did a multi-species, common-garden experiment. We compared the plastic responses in biomass production and in root allocation and morphology to nutrient addition and fluctuations therein among seven common alien, seven rare alien, nine common native and six rare native plant species. We applied six treatments differing in the mean and temporal availability of nutrients. By comparing biomass production, root morphology and allocation of the four groups of plant species in the different treatments, we addressed the following specific questions: 1) Do alien and common plant species exhibit stronger plastic responses in root morphology and allocation, and take more advantage of increased mean nutrient levels than native and rare plant species? 2) Do alien and common plant species exhibit stronger plastic responses in root morphology and allocation, and take more advantage of gradual changes in nutrient availability over time than native and rare plant species? 3) Do alien and common plant species take more advantage of nutrient pulses than native and rare plant species?
Materials and Methods

Study species

To test for differences in plant responses to nutrient availability and temporal fluctuations therein among common alien, rare alien, common native and rare native species, we selected a total of 29 terrestrial herbs belonging to seven families and all occurring in Germany (Methods S1). We classified the species as alien or native to Germany based on information in the BiolFlor database (http://www2.ufz.de/biolflor/index.jsp). All selected alien species are considered to be naturalized (i.e. have established long-term populations in the wild). We classified the species as common and rare based on their frequency of occurrence in Germany according to the FLORKART database, which provides distributional data for the vascular flora of Germany (http://www.floraweb.de/). In the FLORKART database, Germany is divided into 3000 grid-cells of 10 minutes longitude × 6 minutes latitude (corresponding to c. 12 km × 11 km). We assigned a species as common if it has been recorded in more than 1000 grid cells (33% of the total number of grid cells in Germany), and as rare (or less common) if it has been recorded in fewer than 500 grid cells (17% of the total number of grid cells in Germany). To avoid confounding of origin and commonness with taxonomy, we aimed to include at least one common alien, one rare alien, one common native and one rare native species per family. However, because of difficulties in obtaining seeds and insufficient germination of some species, our final species set was not fully balanced with regard to taxonomy; we used seven common alien, seven rare alien, nine common native and six rare native species (Methods S1). Four families were represented by at least one species from each group, and three families lacked representatives of one or two groups. Seeds of the study species were collected in natural populations, or acquired from commercial seed companies or botanical gardens (Methods S1).

Experimental design

To test for differences in growth performance, and root morphology and allocation among the four groups of species, we did a multi-species experiment (van Kleunen et al., 2014) in a greenhouse of the botanical garden of the University of Konstanz (Germany). From 4 to 26 August 2014, we sowed the seeds of each species separately into trays (12 × 12 × 4.5 cm) filled with potting soil (Topferde®, Einheitserde Co., Sinntal-Altengronau, Germany; pH 5.8; 2.0 g/l KCl; 340 mg/l N; 380 mg/l P₂O₅; 420 mg/l K₂O; 200 mg/l S; 700 mg/l Mg). As some
species were known, from previous experiments, to geminate earlier than others, we sowed the species on different dates (Methods S1) to ensure that the seedlings were in a similar developmental stage at the start of the experiment. Sowing dates of rare alien, common alien, rare native and common native species differed on average by less than one day. The trays with sown seeds were kept in a greenhouse at a temperature between 22 and 28 °C, and a light cycle of 16h:8h (day:night). After four weeks, we selected 24 similar-sized seedlings per species, and transplanted them into 2.5-L circular plastic pots filled with a 1:1 mixture of sand and fine vermiculite. We transplanted one seedling into each pot. The resulting 696 pots were assigned to positions across eight greenhouse benches according to a fully randomized experimental design, and watered to saturation. We re-randomized the positions of the pots among and within the benches five weeks after the start of the experiment. We kept the temperature between 22 and 28 °C, and we used supplemental lighting to supply the plants with 14 hours of light each day.

One week after transplanting the seedlings, we started to apply different nutrient treatments. We used a Hoagland solution as fertilizer (Methods S2), which was applied at weekly intervals for a total of 10 weeks. To test the effects of different overall nutrient supply and different temporal patterns of nutrient supply on common alien, rare alien, common native and rare native plant species, we created six different nutrient-supply patterns (Fig. 1): (i) constant low, (ii) constant high, (iii) gradual increase, (iv) gradual decrease, (v) single large pulse and (vi) multiple pulses of different magnitudes. The total (i.e. cumulative) amount of nutrients supplied during the 10 weeks was the same in all treatments except for the constant low treatment. In other words treatments ii to vi were all high-nutrient treatments, but differed in the temporal pattern of nutrient supply (Fig. 1). To increase the statistical power for detecting differences among the four groups of studied species, we had maximized the number of species per group over the number of replicate pots per species (van Kleunen et al. 2014). Therefore, we had only four individuals per species for each of the six nutrient treatments. We added 5 ml of 400% strength Hoagland solution to pots in the constant low nutrient treatment, and 40 ml of the same Hoagland solution to the constant high nutrient treatment each week. To avoid introducing a potential bias because of differences in water supply among the six treatments, we added extra water to the specific amount of 400% strength Hoagland solution of each treatment to ensure that each plant received a total of 100 ml nutrient solution each time.
Figure 1 Graphical illustration of the experimental design (number of species per group, nutrient treatments and number of replicates per species and nutrient-treatment combinations) and data-analysis design for the effects of the nutrient treatments (i.e. dummy variables and their corresponding contrasts). Each bar represents the amount of nutrient solution supplied each week during the ten weeks of the experiment. The total amount of nutrients is the same in all high-mean treatments. In the data-analysis design, dummy variable coding for the scenarios of nutrient supply was used to test the effects of the mean level of nutrient supply (constant low treatment vs the average across the five high treatments; T0), and the effects of different temporal patterns of high nutrient supply vs the constant high supply (T1-T4). For the latter, each contrast of interest was made by fitting the corresponding dummy variable after all other dummy variables (for details on model comparisons, see Methods S4). The two lines next to each dummy coding indicate the contrasts of interest.

Among the high-nutrient treatments in this study, the temporal pattern of nutrient supply might influence plant growth differently because of shifts in the degree of nutrient limitation over time. If nutrient supply is not limiting in the high-nutrient treatments, the effects of fluctuations in nutrient availability over time on plant growth should be similar to when nutrients are supplied at a constant rate. Therefore, to ensure that the high nutrient treatments were still limiting for plant growth, we had previously done a pilot experiment with a wider range of nutrient levels (Methods S3). For the current experiment, we chose the high nutrient level so that plants produced significantly more biomass than at the lowest nutrient level of the pilot experiment, but also still significantly less biomass than at the highest nutrient level of the
pilot experiment (Methods S3). For the gradual-increase treatment, we increased the weekly amount of Hoagland solution per pot during the 10 weeks of the experiment as follows: 5, 5, 21, 21, 40, 40, 58, 58, 76 and 76 ml (Fig. 1). For the gradual-decrease treatment, we applied the opposite sequence of nutrient-solution amounts (Fig. 1). The single-large-pulse treatment started after three weeks, and lasted for four weeks; the sequence was 5, 5, 5, 92.5, 92.5, 92.5, 92.5, 5, 5 and 5 ml (Fig. 1). For the multiple-pulses treatment, we supplied to all replicates the same random order of 5, 76, 40, 40, 21, 58, 76, 58, 21 and 5 ml nutrient solution (Fig. 1).

To ensure that water was not limiting for plant growth, we checked all pots every day during the experiment. We watered the plants when the soil looked dry, and supplied the same amounts of water to all plants.

**Measurements**

To be able to account for variation in initial sizes of plants in the analyses, we counted at the start of the experiment the number of true leaves (i.e. excluding the cotyledons), and measured the length and width of the largest leaf on each plant, and the height of each plant. On 24 November 2014, we began to harvest the plants. On the first day, we harvested the aboveground biomass of all plants, and then started to harvest the belowground biomass. As eight plants died during the experiment (Table S1), the total number of harvested plants was 688 instead of 696. As the entire belowground-biomass harvest took eight days, we stored all pots outside at low temperatures after the aboveground-biomass harvest in order to halt growth or decay of the plants and thus to avoid introducing a potential bias due to different harvesting times. After washing all the roots clean of substrate, we randomly took a representative subsample from each replicate root system, and stored it in a plastic 20 ml tube filled with distilled water in a cold room at 4°C. The remaining root mass and the aboveground biomass of each plant were dried for at least 72 hours at 80°C, and weighed. We stained the root subsamples in a neutral-red solution for 30 minutes. We then determined the total root length, and the mean root diameter of each subsample using a flatbed scanner specifically modified for root scanning (Epson Expression 10000 XL; Regent Instruments, Quebec) and WinRhizo software (2012; Regent Instruments Inc., Quebec, Canada). All root subsamples were then dried for at least 72 hours at 80°C, and weighed.

Based on the initial size measurements, we calculated an estimate of initial leaf area as the length × width of the largest leaf × the number of true leaves. Based on the final biomass and
root morphology measurements, we calculated total belowground biomass (biomass of subsample used for root-morphology measurements + biomass of the rest of the root system), total biomass (aboveground biomass + belowground biomass), root mass fraction (belowground biomass / total biomass), total root length (root length of subsample × total belowground biomass / subsample mass) and specific root length (total root length / total belowground biomass).

**Analyses**

To test for differences in growth performance, and root morphology and allocation among common alien, rare alien, common native and rare native plant species, we fitted linear mixed effect models using the *lme* function in the R package ‘nlme’ (Pinheiro *et al.*, 2015) in R 3.1.3 (R Core Team, 2015). Total biomass, root mass fraction, total root length, root average diameter and specific root length of the plants were the response variables in the models. To meet the assumption of normality, we tried different transformations; and achieved the best residual distributions with a square-root transformation of total biomass and root mass fraction, and a natural-log transformation of the three root morphological traits. We included species origin (alien *versus* native), commonness (rare *versus* common), nutrient level (low *versus* high), nutrient variability (constant high *versus* each of the four scenarios of nutrient pulse), and their interactions, as fixed effects in all models. Because nutrient variability was nested within the high nutrient level, we coded the nutrient supply patterns as dummy variables T0 to T4 (Fig. 1) in all models to obtain the different treatment contrasts required (Schielzeth, 2010). Because initial variation in plant size might contribute to differences in final biomass and biomass allocation, we also added initial plant leaf area and initial plant height as scaled natural-log-transformed covariates in the models for total biomass and root mass fraction. To account for phylogenetic non-independence of species and for non-independence of replicates of the same species, we included species nested within family as random effects in all models.

As the homoscedasticity assumption was violated, we also included variance structures modelling different variances per species in all models using the ‘varIdent’ function in the R package ‘nlme’ (Pinheiro *et al.*, 2015; see also Zuur *et al.*, 2009). As species within each of the four groups of different origin and commonness might vary in their responses to the nutrient treatments, we also ran models with random slopes for family and species with respect
to nutrient treatment. However, because this decreased the AIC-values (i.e. increased model fit) only for the total biomass model, we did not consider the random-slopes models for the other traits. In the linear mixed models described above, we assessed the significance of fixed effects (i.e. species origin, commonness, nutrient level and nutrient variability) and their interactions with likelihood-ratio tests (Zuur et al., 2009; for details, see Methods S4).

Plastic changes in root allocation and morphology in response to nutrient availability are frequently implicitly assumed to constitute adaptive plasticity (i.e. to result in high performance of plants across different nutrient conditions). Ideally, the adaptive value of plasticity should be tested explicitly (van Kleunen & Fischer, 2005). Although there are ways of testing whether plasticity is adaptive based on selection-gradient analysis (see Dudley & Schmitt, 1996; van Kleunen & Fischer, 2001 for one approach, and Keser et al., 2015 for another), these approaches cannot be applied in a straight-forward way to a multi-species dataset with few replicates per species. Instead, we tested how the change in biomass across two nutrient conditions (i.e. log-response ratios of biomass) relates to plasticity in the trait of interest across the same two nutrient conditions, using the 29 species as replicates (see Liu et al., 2016). These analyses revealed some significant relationships between plastic responses of some of the root traits and the plant performance response to nutrients (see Methods S5). However, as we have only 29 species (i.e. data points), which does not provide enough degrees of freedom to include multiple traits and their plasticities in one and the same regression model, this approach is not very powerful and informative. Therefore, we did not interpret these results.

Results

Mean values according to origin and commonness of plant species

Averaged across all six nutrient treatments, common alien, rare alien, common native and rare native plants did not differ in total biomass, root mass fraction (Table 1 and Fig. S1) and root morphological traits (root length, root diameter and specific root length; Table 1 and Fig. S1). In other words, there were no significant main effects of origin and commonness, and no significant interaction between both for any of the measured traits (Table 1).

Responses to mean nutrient availability
Averaged across all 29 species, plants produced significantly more biomass (+160.3%), and had a significantly lower root mass fraction (-18.5%) when growing under high nutrient conditions (i.e. averaged across the treatments constant high, gradual increase, gradual decrease, single pulse and multiple pulses) than under constant low nutrient conditions (Table 1 and Fig. 2). The decrease in root mass fraction was stronger for native species (-22.8%) than for alien species (-14.6%; significant O × T0 interaction in Table 1; Fig. 2).

Averaged across all 29 species, plants produced significantly longer roots (+90.1%) when growing under high nutrient conditions than under constant low nutrient conditions (Table 1 and Fig. 2). Root diameter and specific root length, on the other hand, were not significantly affected by the mean level of nutrient availability (Table 1 and Fig. 2). However, while for alien species the root diameter slightly increased (+6.5%) and the specific root length decreased (-12.5%) with increasing nutrient availability, the reverse tended to be the case for native species (root diameter decreased by 0.4% and specific root length increased by 3.6%; significant O × T0 interactions in Table 2; Fig. S2). The slight decrease in root diameter for native species was driven by the common ones (-3.5%) as the rare native species, just like the rare (+3.6%) and common alien species (+9.9%), also showed a slight increase (+4.2%; Fig. 2). This was reflected in a significant O × C × T0 interaction in Table 1.

**Gradual changes in nutrient supply over time**

When nutrients were gradually increased over time, total biomass and root mass fraction were significantly lower than when nutrients were supplied at a constant high rate (total biomass: -11.3%; root mass fraction: -10.8%; significant T1 effect in Table 1; Fig. 3 and Fig. S1). On the other hand, when nutrients were gradually decreased over time, root mass fraction was significantly higher than when nutrients were supplied at a constant high rate (+9.0%; significant T2 effect in Table 1; Fig. 3). Root diameter was significantly larger when nutrients were gradually decreased instead of supplied at a constant high rate (+4.8%; Table 1, Fig. 3 and Fig. S1). On the other hand, specific root length was significantly lower when nutrient were gradually decreased instead of supplied at a constant high rate (-14.8%; Table 1, Fig. 3 and Fig. S1). Root length was not affected by gradual changes in nutrient supply for any of the four groups of plant species (Table 1; Fig. 3 and Fig. S1). For alien plants, root diameter was slightly higher (+4.7%) when nutrients gradually increased than when they were supplied at a constant high rate, whereas the reverse was true for the native species (-3.2%; significant O ×
T1 interaction in Table 1; Fig. 3 and Fig. S3). Moreover, root diameter tended to be increased more for alien species (+7.5%) than for native species (+2.0%) when nutrients were gradually decreased instead of supplied at a constant high rate (marginal significant O×T2 interaction in Table 1; Fig. 3 and Fig. S3).

**Nutrient pulses**

When nutrients were supplied as a single large pulse in the middle of the 10-week growth period instead of at a constant high rate, alien species increased their biomass (+7.5%) and root mass fraction (+5.7%), while native species did the opposite (biomass: -13.8%, root mass fraction: -11.5%; significant O × T3 interaction in Table 1; Fig. 4 and Fig. S4). Root diameter of alien species was also slightly higher (+3.2%) when nutrients were supplied as a single large pulse instead of at a constant high rate, whereas the reverse was true for the native species (-3.5%; marginal significant O × T3 interaction in Table 1; Fig. 4 and Fig. S4). Biomass production and root mass fraction were not affected by whether the nutrients were supplied as multiple pulses or at a constant rate (Table 1 and Fig. 4).

Root length and specific root length were neither affected by a single nutrient pulse nor by multiple nutrient pulses compared to a constant high supply rate for any of the four groups of plants (Table 1, Fig. 4 and Fig. S1). Root diameter was also not affected by the single nutrient pulse (Table 1, Fig. 4 and Fig. S1). However, root diameter was differently affected by multiple nutrient pulses for the four groups of plant species. Root diameter was strongly increased (+14.2%) for common alien species, while it was only slightly increased for rare alien (+2.4%) and rare native species (+3.1%), and even slightly decreased for common native species (-2.6%; significant O × T4 and O × C × T4 interactions in Table 1; Fig. 4).
Table 1 Results of linear mixed effects models testing the effects of species origin, species commonness, changes in mean nutrient availability (T0) and temporal patterns of variability in nutrient availability (T1-T4), and all interactions thereof, on total biomass, root mass fraction, total root length, average root diameter and specific root length of experimental plants.

<table>
<thead>
<tr>
<th>Fixed effects</th>
<th>Total biomass</th>
<th>Root mass fraction</th>
<th>Root length</th>
<th>Average Root Diameter</th>
<th>Specific root length</th>
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<td></td>
<td>df</td>
<td>$\chi^2$</td>
<td>P</td>
<td>df</td>
<td>$\chi^2$</td>
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<tr>
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<tr>
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<tr>
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<tr>
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<tr>
<td>C × T</td>
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<tr>
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<tr>
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<td>-</td>
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<td>-</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
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<td>0.6998</td>
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Note: *the standard deviation (SD) was only given the intercept of family and species here (for the random slopes of all treatments, please, see Table S1). Significant effects (p < 0.05) are in bold
Discussion

Similar responses to mean nutrient availability among alien rare, alien common, native rare and native common species

As expected, all plants produced more biomass under high mean nutrient supply than under low mean nutrient supply. This coincided with a decrease in the relative allocation of biomass to the root system, which is in line with predications of resource-limitation theory, posing that plants should allocate biomass to structures that help them acquire more of the most limiting resource (Bloom et al., 1985; Poorter & Nagel, 2000). Although both native and alien plant species allocated relatively less biomass to the root system under high nutrient conditions, this decrease was weaker for aliens. However, alien plant species produced thinner roots under low than under high nutrient conditions, whereas native plant species showed the opposite pattern. As thin roots partly result from plants producing more root length with a given amount of dry weight (Pearson r = -0.451, n = 679, p < 0.0001 for root diameter and specific root length), an opposite pattern was found for specific root length. Together, these results suggest that natives and aliens follow partly different strategies in response to low nutrient conditions; natives more strongly increase the allocation of biomass to the roots, whereas aliens do so to a lesser degree but compensate this by producing thinner roots.

Despite these apparently different strategies of native and alien plants, we found similar responses in biomass production to changes in mean nutrient availability among the four groups of species. In another multispecies experiment, Dawson et al., (2012a) compared responses to nutrient addition among the same four types of species in Switzerland, and found that common alien plant species did not differ in their performance response to nutrient addition from common native plant species, but that, irrespective of origin, common species exhibited stronger responses to nutrient addition than rare species. Although we did not find that common species took more advantage of increased nutrient availability, we found, just like Dawson et al., (2012a), that there was no overall difference in biomass production response between natives and aliens. The latter is also supported by findings of Godoy et al., (2011), who compared 20 invasive alien and 20 widespread native congeners in Spain across nutrient gradients, and found that both groups responded to environmental variation with similar levels of plasticity. The consistency of these findings might indicate that the aliens and natives in both studies come from habitats with similar variation in mean nutrient levels, sug-
suggesting that species origin does not drive differences of plant performance in response to changes in mean nutrient availability.

**Figure 2** Modelled means of (a) total biomass, (b) root mass fraction, (c) total root length, (d) root diameter and (e) specific root length of the 7 rare alien and 7 common alien, 6 rare native and 9 common native plant species growing under low and high (averaged across 5 different temporal patterns; corresponding to contrast T0 in Table 2, also see Fig. 1) nutrient conditions. Error bars represent SEs of the means.
It has previously been shown that common alien plant species tend to have stronger adaptive root-foraging responses than rare alien species, which could help common alien plant species capture more nutrients (Keser et al., 2014; Keser et al., 2015). Additionally, common species, irrespective of whether alien or native, appear to frequently occur in widespread nutrient-rich habitats (Thompson et al., 1995; Dawson et al., 2012b; Dostál et al., 2013). There is also evidence that rare species tend to disappear from communities after nutrient enrichment (Suding et al., 2005). Thus, common species usually benefit more strongly from nutrient addition than rare species. Although this was also found by Dawson et al., (2012a), our multispecies experiment does not show this. Flores-Moreno et al., (2016) found that nutrient addition controlled the abundance (percentage cover) of native and alien species, and that the nutrient-addition effect on species richness, in particular for native species, was mediated by climatic conditions. In that study, alien species are likely to be common (invasive) rather than rare; whereas the natives included both common and rare species. Their result that native species declined in abundance but not in richness in response to nutrient addition might indicate a reduction of common rather than rare native species when competing with common alien species. Therefore, the question whether differences in performance to changes in mean environmental conditions could drive commonness of species still needs further research.

**Gradual changes in nutrient supply over time influence plant performance**

Under an equally high cumulative nutrient supply during the 10-week growth period, plants produced less biomass when they grew initially under low nutrient supply that gradually increased than when they grew under constant nutrient supply. This could be because under a gradual increase in nutrient supply over time, nutrient availability was insufficient at the early growth stage when plants needed nutrients the most. There was, on the other hand, no difference in biomass production between when plants grew under gradually decreased nutrient supply and when they grew under constant nutrient supply. Possibly, while nutrient supply decreased over time in this treatment, the nutrient availability might have decreased to a lesser extent due to storage of nutrients in the substrate and/or plant. Alternatively, a possible growth disadvantage at the end of the experiment might not have compensated for the initial growth advantage yet.

Plants allocated more biomass to roots under a gradually decreased nutrient supply than under constant nutrient supply, whereas they showed the opposite pattern in response to a grad-
ually increased nutrient supply compared to a constant nutrient supply. We found that the final root mass fraction was higher in the decreasing treatment, where plants had the lowest nutrient supply at the end of the experiment, than in the constant treatment. Useche & Shipley, (2009) found evidence among 14 plant species that those that increased root mass fraction more strongly under decreasing nutrient availability were less affected in their relative growth rate, suggesting that a plastic increase of root mass fraction under decreasing nutrient availability is adaptive. According to an optimal growth model developed by Iwasa & Roughgarden, (1984), when a plant grows in a constant environment, once both the roots and shoots have begun to grow simultaneously, their balanced growth will continue until the end of the vegetative growth. However, this root-shoot balance is likely to change when nutrient availability changes (Iwasa & Roughgarden, 1984). Our results for the patterns in root allocation, as measured at the end of the experiment, are in line with the predictions of Iwasa & Roughgarden, (1984), and with the predictions of the resource-limitation theory (Bloom et al., 1985), suggesting that plants continuously adjust biomass allocation depending on the actual nutrient availability. This might also happen with regard to the thickness of roots produced. However, at the end of the experiment, plants under a gradually decreased nutrient supply had thicker instead of thinner roots. Possibly, this reflects a legacy of the thick roots produced at the beginning of the experiment when nutrient availability was very high in the decreasing treatment. It would thus be interesting to assess how the thickness of newly produced roots changes over time under changing nutrient supplies.

Root traits of alien and native plants responded overall in a similar way to gradual changes in nutrient supply. However, whereas under a gradual increase in nutrient supply, native plants had in the end slightly thinner roots than under a constant nutrient supply, alien plants had slightly thicker roots. These differences in root thickness responses were apparently not large enough to result in significantly different biomass responses of alien and native plants to gradual changes in nutrient supply. On the other hand, rare plants produced slightly more biomass when nutrient supply gradually decreased instead of remained constant, whereas common plants produced slightly less biomass. Under the scenario of a gradual decrease in nutrient supply over time, nutrient availability was sufficient at the beginning, whereas it was likely insufficient at the end. Rare plants might be better able to deal with a decrease in nutrients as they often have adaptations suited for resource conservation rather than acquisition (Reich et al., 1999).
Figure 3 Modelled means of (a, b) total biomass, (c, d) root mass fraction, (e, f) root length, (g, h) root diameter, and (i, j) specific root length of the 7 rare alien (RA) and 7 common alien (CA), 6 rare native (RN) and 9 common native (CN) plant species growing under conditions of constant high vs a gradual increase (a, c, e, g, i; corresponding to contrast T1 in Table 2) and a gradual decrease (b, d, f, h, j; corresponding to contrast T2 in Table 2) in nutrient supply over time. Note that the constant high treatment is shown twice (in a, c, e, g, i and in b, d, f, h, j) for clarity. Error bars represent SEs of the means.
A nutrient pulse favours alien, but not native plant species

The responses to the nutrient-pulse treatments differed among the four groups of plant species. In response to a single large nutrient pulse, alien plant species showed an increase in total biomass, whereas native plant species showed a decrease. This coincided with an increase and decrease in root allocation for alien and native species, respectively, in response to an increase in the mean nutrient level. Based on the resource-limitation theory (Bloom et al., 1985), one would expect that a decrease in root allocation in response to an increase in nutrients would be beneficial. However, in case of an ephemeral increase in nutrient availability, as during a pulse, having a large root mass fraction has the advantage that it allows a plant to take immediate advantage of the temporary surplus of nutrients. Our finding that a large nutrient pulse favours alien but not native plants is in line with the finding of Parepa et al., (2013) that the biomass of the highly invasive Japanese knotweed (Fallopia spp.) was two- to four-fold increased when nutrients were supplied pulse-wise instead of at a constant rate in experimental plant communities.

When nutrients were supplied in multiple pulses instead of at a constant rate, common alien plants increased their root diameter, whereas the other groups showed negligible changes. These differences in root-diameter responses, however, did not appear to affect biomass responses of the four groups of species. Possibly, the effects of the multiple nutrient-pulses treatment were overall small because this treatment is quite similar to the constant nutrient-supply treatment, which in effect consists of multiple pulses of the same magnitude. Nevertheless, our finding contrasts with the results of Parepa et al., (2013), who found that invasive Japanese knotweed did not just benefit from a single large pulse but even more so from multiple pulses of smaller magnitude. A possible explanation for this is that the plants in our study grew without competition, whereas Parepa et al., (2013) grew the invasive alien in competition with natives. The ability of alien plant species to rapidly take advantage of available resources (Richards et al., 2006) could help them outperform native plants when they compete with native plants.
Figure 4 Modelled means of (a, b) total biomass, (c, d) root mass fraction, (e, f) root length, (g, h) root diameter, and (i, j) specific root length of the 7 rare alien (RA), 7 common alien (CA), 6 rare native (RN) and 9 common native (CN) plant species growing under conditions of constant high vs single pulse (a, c, e, g, i; corresponding to contrast T3 in Table 2) and multiple pulses (b, d, f, h, j; corresponding to contrast T4 in Table 2) in nutrient supply. Note that the constant high treatment is shown twice (in a, c, e, g, i and in b, d, f, h, j) for clarity. Error bars represent SEs of the means.
In a community setting, coexistence of plant species, and thus invasion, is more likely when the species differ in their phenology (Wolkovich & Cleland, 2011; Godoy & Levine, 2014). With fluctuating nutrient availability, it is likely that the degree to which nutrient peaks coincide with a species main growth period might determine which species is likely to survive. The competitive advantages resulting from differences in nutrient uptake ability or phenology between alien and native plants might be less apparent when plants are grown individually. Although our results suggest that it is not a general phenomenon that common alien plants take more advantage of multiple nutrient pulses than native plants do, future studies should also consider competitive environments, like Parepa et al., (2013) did, and species with different phenologies.

**Conclusion**

Irrespective of their origin and commonness, all species in our study produced more biomass under high nutrient conditions than under low nutrient conditions. In addition, plants produced longer roots under high nutrient conditions despite allocating less biomass to roots. Among the five high nutrient treatments, the temporal pattern of nutrient supply also influenced biomass production, root allocation and root thickness, but the magnitude and/or direction of these responses varied among the four groups of species. Particularly, we found that alien plant species, irrespective of whether they are common or rare, produced more biomass, and had a higher root mass fraction when nutrients were supplied as a single pulse in the middle of the growth period instead of supplied at a constant rate, whereas the reverse was true for the native species. This suggests that alien plant species, possibly as a consequence of an increased biomass allocation to the root system, could in contrast to native plant species benefit from large nutrient pulses.

**Acknowledgements**

We thank Otmar Ficht, Claudia Martin, Timo Scheu, Yanhao Feng, Yan Li, Simon Waldher, Diego Ellis-Soto, Liridon Hoxha, Annika Woltjen, Sina Glöckner for practical assistance, and Noëlie Maurel, Wayne Dawson, Anke Stein, Samuel Carleial, Emily Haeuser, Eva Malecore, Jasmin Herden, Yan Li for their valuable comments on a previous draft of the manuscript. Yanjie Liu thanks the China Scholarship Council (CSC) for financial support.
Supporting Information

**Methods S1** Information on status, commonness, native continent(s), number of grid cells, seed source, sowing date, transplanting date and mortality of the 29 study species in Germany. The species are grouped per family.

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<th>Mortality</th>
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<tr>
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<td>Alien</td>
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<td>Rare</td>
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<tr>
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<td>Common</td>
<td>Africa, Asia-Temperate, Tropical, Europe</td>
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<td>01/09/14</td>
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</tr>
<tr>
<td>Bromus squarrosus †</td>
<td>Alien</td>
<td>Rare</td>
<td>Africa, Asia-Temperate, Europe</td>
<td></td>
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</tr>
<tr>
<td>Lolium perenne †</td>
<td>Native</td>
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<td>Africa, Asia-Temperate, Tropical, Europe</td>
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<td>22/08/14</td>
<td>01/09/14</td>
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<tr>
<td>Bromus grossus †</td>
<td>Native</td>
<td>Rare</td>
<td>Africa, Asia-Temperate, Tropical, Europe</td>
<td>155</td>
<td>22/08/14</td>
<td>01/09/14</td>
<td>0</td>
</tr>
</tbody>
</table>


* Numbers of grid cells refers to the number of 12 × 11 km cells in Germany (total: 3000 cells) where each species has been recorded.

* Seeds were acquired from the botanical garden at the University of Konstanz.

* Seeds were collected from field in Switzerland.

* Seeds were acquired from Spicegarden URL (http://www.spicegarden.eu/).

* Seeds were acquired from Rieger-Hofmann GmbH, Blaufelden-Raboldshausen, Germany.
Methods S2 Recipe for 400% strength Hoagland's Complete Nutrient Solution

The preparation of Hoagland nutrient solution essentially followed recommendations by Hoagland and Arnon (1950), with the exception of the form in which iron was added (see below).

We prepared the stock solutions 1-6 and micronutrient-stock solution given below, and used the amounts indicated to prepare 1 liter of nutrient solution:

1. 4 mL of 1.00 M/L KH$_2$PO$_4$
2. 20 mL of 1.00 M/L KNO$_3$ use 20 mL
3. 20 mL of 1.00 M/L Ca(NO$_3$)$_2$
4. 8 mL of 1.00 M/L MgSO$_4$
5. 4 ml of micronutrient stock solution (see recipe below)
6. 10 ml of 1000 mg/liter iron from iron chelate (Fe-EDTA, Fe-DTPA, or Fe-EDDHA)*

Micronutrient-stock solution per liter:

- 2.86 g H$_3$BO$_3$
- 1.81 g MnCl$_2$·4H$_2$O
- 0.22 g ZnSO$_4$·7H$_2$O
- 0.08 g CuSO$_4$·5H$_2$O
- 0.02 g H$_2$MoO$_4$·H$_2$O (Assaying 85% MoO3)

* Hoagland's recipe called for 1 ml of 0.5% iron tartrate stock per liter of nutrient solution but we used iron chelate instead.

Methods S3 Pilot experiment to determine nutrient concentrations to be used in main experiment.

To find a high nutrient concentration that increased plant growth but was still limiting, we randomly selected three plant species from the all species we planned to use in the main experiment. We planted the plants in 2.5-L pots filled with a 1:1 mixture of sand and fine vermiculite, and applied five different nutrient treatments: 10, 20, 30, 40, 50 ml of 400% strength Hoagland solution per week. We replicated each treatment four times per species. To avoid introducing a potential bias because of different amounts of water supplied between the six treatments, we added extra water to the specific amount of the 400% strength Hoagland solution of each treatment, and made sure a total of 100 ml of nutrient solution was added to each pot at each application. We applied the different nutrient treatments to the plants every week for five weeks, and then harvested them. We dried the biomass at 80°C for 72 hours before weighing. We analysed the total biomass with linear mixed models using the lme function in the R package ‘nlme’ (Pinheiro et al., 2015)¹ in R 3.1.3 (R Core Team, 2015)². Nutrient treatment was used as a fixed factor and species was used as a random factor.

The results showed that nutrient treatments significantly affected the biomass of plants (Log likelihood ratio test: $\chi^2 = 35.14103$, df = 4, P < 0.0001; Fig. MS2). The results of post-hoc pairwise comparisons showed that plants growing with 40 ml Hoagland solution per application produced significantly more biomass than plant with 10 ml Hoagland solution per application (nutrient 40 ml – 10 ml mean difference = 0.8155 ± 0.1710, P < 0.001), but less biomass than plants with 50 ml nutrient treatment (nutrient 50 ml – 40 ml mean difference = 0.3807 ± 0.1656, P < 0.05). In other words, the application of 40 ml 400% strength Hoagland solution per week was still limiting biomass production of the plants.
Fig. MS3 Modelled means of biomass across three plant species (*Bidens radiata*, *Bromus grossus*, *Salvia aethiopis*) growing under different nutrient conditions. Error bars represent SEs of the means.


**Methods S4** Details of the models compared in likelihood-ratio tests to assess the significance of fixed effects (i.e. initial-size covariates, species origin, commonness, nutrient level [T0] and temporal patterns in nutrient supply [T1-T4]) and their interactions. All models included as random model species nested with family. To test the significance of a term of interest, the reference model was compared to a model from which the term of interest had been removed. R codes for this test are following the table.

<table>
<thead>
<tr>
<th>Term of interest</th>
<th>Reference model</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Initial leaf area</strong></td>
<td>covariates + O + C + T0</td>
</tr>
<tr>
<td><strong>Initial height</strong></td>
<td>covariates</td>
</tr>
<tr>
<td><strong>Main effects</strong></td>
<td></td>
</tr>
<tr>
<td>Origin (O)</td>
<td>covariates + O + C + T0</td>
</tr>
<tr>
<td>Commonness (C)</td>
<td>covariates + O + C + T0</td>
</tr>
<tr>
<td>Low vs High (T0)</td>
<td>covariates + O + C + T0</td>
</tr>
<tr>
<td>Constant vs Increase (T1)</td>
<td>covariates + O + C + T0 + T1 + T2 + T3 + T4</td>
</tr>
<tr>
<td>Constant vs Decrease (T2)</td>
<td>covariates + O + C + T0 + T1 + T2 + T3 + T4</td>
</tr>
<tr>
<td>Constant vs single (T3)</td>
<td>covariates + O + C + T0 + T1 + T2 + T3 + T4</td>
</tr>
<tr>
<td>Constant vs Multiple (T4)</td>
<td>covariates + O + C + T0 + T1 + T2 + T3 + T4</td>
</tr>
<tr>
<td><strong>2-way interactions</strong></td>
<td></td>
</tr>
<tr>
<td>O×C</td>
<td>covariates + O + C + T0 + T1 + T2 + T3 + T4 + O×C + O×T0 + O×C×T1</td>
</tr>
<tr>
<td>O×T0</td>
<td>covariates + O + C + T0 + T1 + T2 + T3 + T4 + O×C + O×T0 + O×C×T1</td>
</tr>
<tr>
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<td>covariates + O + C + T0 + T1 + T2 + T3 + T4 + O×C + O×T0 + O×T1 + O×T2 + O×T3 + O×T4 + O×C×T0 + O×C×T1 + O×C×T2</td>
</tr>
<tr>
<td>O×T2</td>
<td>covariates + O + C + T0 + T1 + T2 + T3 + T4 + O×C + O×T0 + O×T1 + O×T2 + O×T3 + O×T4 + O×C×T0 + O×C×T1 + O×C×T2 + O×C×T3 + O×C×T4</td>
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<tr>
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</tr>
<tr>
<td>O×T4</td>
<td>covariates + O + C + T0 + T1 + T2 + T3 + T4 + O×C + O×T0 + O×T1 + O×T2 + O×T3 + O×T4 + O×C×T0 + O×C×T1 + O×C×T2 + O×C×T3 + O×C×T4</td>
</tr>
<tr>
<td>C×T0</td>
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<tr>
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<tr>
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<tr>
<td><strong>3-way interactions</strong></td>
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</tr>
<tr>
<td>O×C×T0</td>
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<tr>
<td>O×C×T1</td>
<td>covariates + O + C + T0 + T1 + T2 + T3 + T4 + O×C + O×T0 + O×T1 + O×T2 + O×T3 + O×T4 + O×C×T0 + O×C×T1 + O×C×T2 + O×C×T3 + O×C×T4 + O×C×T5</td>
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<tr>
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</tr>
<tr>
<td>O×C×T4</td>
<td>covariates + O + C + T0 + T1 + T2 + T3 + T4 + O×C + O×T0 + O×T1 + O×T2 + O×T3 + O×T4 + O×C×T0 + O×C×T1 + O×C×T2 + O×C×T3 + O×C×T4 + O×C×T5</td>
</tr>
</tbody>
</table>
R codes for likelihood-ratio tests in Methods S4

#####add packages
library(nlme)

#####read data
data<-read.table(file.choose(),header=T)

#####subset data; Note: we only give the codes here for Total biomass analysis. For analysis of other response variables are similar with total biomass analysis.
Total<-subset(data,Total_biomass!="NA"&Initial_H!="NA"&Total_LA!="NA")

#####Model fitted
vf1<-varIdent(form=~1|Species) #variance differs per species
lme01 <- lme(sqrt(Total_Biomass)~scale(log(Total_LA)) + scale(log(Initial_H)) + ##LA means leaf area, H mean height
  origin+commonness+
  T0+T1+T2+T3+T4+
  origin:commonness+
  origin:T0+commonness:T0+
  origin:T1+commonness:T1+
  origin:T2+commonness:T2+
  origin:T3+commonness:T3+
  origin:T4+commonness:T4+
  origin:commonness:T0+
  origin:commonness:T1+
  origin:commonness:T2+
  origin:commonness:T3+
  origin:commonness:T4, 
  random=~Treatment|Family/Species, #we added random slope in the model 
  data=Total, method="REML",weights=vf1, 
  control=list(maxIter=1000,msMaxIter=1000,niterEM=1000,opt="optim"))
summary(lme01)

#####likelihood ratio test

#####three interaction effects for nutrient variability
lme01vf1 <- lme(sqrt(Total_Biomass)~scale(log(Total_LA)) + scale(log(Initial_H)) +
  origin+commonness+
  T0+T1+T2+T3+T4+
  origin:commonness+
  origin:T0+commonness:T0+
  origin:T1+commonness:T1+
  origin:T2+commonness:T2+
  origin:T3+commonness:T3+
  origin:T4+commonness:T4+
  origin:commonness:T0+
  origin:commonness:T1+
  origin:commonness:T2+
  origin:commonness:T3+
  origin:commonness:T4, 
  random=~Treatment|Family/Species, #this is code for random intercept model 
  data=Total, method="ML",weights=vf1, 
  control=list(maxIter=1000,msMaxIter=1000,niterEM=1000,opt="optim"))

anova(lme01vf1,OCT4)

anova(lme01vf1,OCT3)

anova(lme01vf1,OCT2)

anova(lme01vf1,OCT2)

#### T4:origin:commonness
OCT4<-update(lme01vf1 ,.~.-origin:commonness:T4)
anova(lme01vf1,OCT4)

#### T3:origin:commonness
OCT3<-update(lme01vf1 ,.~.-origin:commonness:T3)
anova(lme01vf1,OCT3)

#### T2:origin:commonness
OCT2<-update(lme01vf1 ,.~.-origin:commonness:T2)
anova(lme01vf1,OCT2)

#### T1:origin:commonness
OCT1 <- update(lme01v1, -.origin:commonness:T1)
anova(lme01v1, OCT1)

### two interaction effects for nutrient variability

lme1COT2 <- lme(sqrt(Total_biomass) ~ scale(log(Total_LA)) + scale(log(Initial_H)) +
origin+commonness+
T0+T1+T2+T3+T4+
origin:commonness+
origin:T0+commonness:T0+
origin:T1+commonness:T1+
origin:T2+commonness:T2+
origin:T3+commonness:T3+
origin:T4+commonness:T4,
random=~Treatment|Family/Species,
data=Total, method="ML", weights=vf1,
control=list(maxIter=1000,msMaxIter=1000,niterEM=1000,opt="optim"))

## T4:commonness
CT4 <- update(lme1COT2, -.~. T4:commonness)
anova(lme1COT2, CT4)

## T4:origin
OT4 <- update(lme1COT2, -.~.origin:T4)
anova(lme1COT2, OT4)

## T3:commonness
CT3 <- update(lme1COT2, -.~. T3:commonness)
anova(lme1COT2, CT3)

## T3:origin
OT3 <- update(lme1COT2, -.~.origin:T3)
anova(lme1COT2, OT3)

## T2:commonness
CT2 <- update(lme1COT2, -.~. T2:commonness)
anova(lme1COT2, CT2)

## T2:origin
OT2 <- update(lme1COT2, -.~.origin:T2)
anova(lme1COT2, OT2)

## T1:commonness
CT1 <- update(lme1COT2, -.~. T1:commonness)
anova(lme1COT2, CT1)

## T1:origin
OT1 <- update(lme1COT2, -.~.origin:T1)
anova(lme1COT2, OT1)

### three interaction effect for nutrient variability

lme1T4 <- lme(sqrt(Total_biomass) ~ scale(log(Total_LA)) + scale(log(Initial_H)) +
origin+commonness+
T0+T1+T2+T3+T4,
random=~Treatment|Family/Species,
data=Total, method="ML", weights=vf1,
control=list(maxIter=1000,msMaxIter=1000,niterEM=1000,opt="optim"))

## T4
T4 <- update(lme1T4, -.~. T4)
anova(lme1T4, T4)

## T3
T3 <- update(lme1T4, -.~. T3)
anova(lme1T4, T3)

## T2
T2 <- update(lme1T4, -.~. T2)
anova(lme1T4, T2)

## T1
T1 <- update(lme1T4, -.~. T1)
anova(lme1T4, T1)

### three interaction effect for nutrient level
lme1OCT0 <- lme(sqrt(Total_biomass) ~ scale(log(Total_LA)) + scale(log(Initial_H)) + origin + commonness +
T0 + T1 + T2 + T3 + T4 +
origin:commonness +
origin:T0 + commonness:T0 +
origin:T1 + commonness:T1 +
origin:T2 + commonness:T2 +
origin:T3 + commonness:T3 +
origin:T4 + commonness:T4 +
origin:commonness:T0,
random =~ Treatment|Family/Species,
data = Total, method = "ML", weights = vf1,
control = list(maxIter = 1000, msMaxIter = 1000, niterEM = 1000, opt = "optim"))

##origin:commonness:T0
OCT0 <- update(lme1OCT0, .~ origin:commonness:T0)
anova(lme1OCT0, OCT0)

####two interaction effect for nutrient level
lme1CT0 <- lme(sqrt(Total_biomass) ~ scale(log(Total_LA)) + scale(log(Initial_H)) +
origin + commonness +
T0 + T1 + T2 + T3 + T4 +
origin:commonness +
origin:T0 + commonness:T0,
random =~ Treatment|Family/Species, data = Total, method = "ML", weights = vf1,
control = list(maxIter = 1000, msMaxIter = 1000, niterEM = 1000, opt = "optim"))

##commonness:T0
CT0 <- update(lme1CT0, .~ . - commonness:T0)
anova(lme1CT0, CT0)

##two interaction effect for origin and commonness
## origin:commonness
OC <- update(lme1CT0, .~ . - origin:commonness)
anova(lme1CT0, OC)

####effect for nutrient level
lme1T0 <- lme(sqrt(Total_biomass) ~ scale(log(Total_LA)) + scale(log(Initial_H)) + origin + commonness + T0,
random =~ Treatment|Family/Species, data = Total, method = "ML", weights = vf1,
control = list(maxIter = 1000, msMaxIter = 1000, niterEM = 1000, opt = "optim"))

##T0
T0 <- update(lme1T0, .~ . - T0)
anova(lme1T0, T0)

####other fixed effects
##commonness
C <- update(lme1T0, .~ . - commonness)
anova(lme1T0, C)

##Origin
O <- update(lme1T0, .~ . - origin)
anova(lme1T0, O)

####effects for covariables
lme1S <- lme(sqrt(Total_biomass) ~ scale(log(Total_LA)) + scale(log(Initial_H)),
random =~ Treatment|Family/Species, data = Total, method = "ML", weights = vf1,
control = list(maxIter = 1000, msMaxIter = 1000, niterEM = 1000, opt = "optim"))

##Height
Height <- update(lme1S, .~ . - scale(log(Initial_H))
anova(lme1S, Height)

##Leaf area
LA <- update(lme1S, .~ . - scale(log(Total_LA))
anova(lme1S, LA)
**Methods S5** Results of analyses testing adaptive plasticity of root mass fraction (RMF), root length (RL), root diameter (RD) and specific root length (SRL). We calculated plasticities for each of the five high-nutrient treatments (constant high [H], gradual increase [I], gradual decrease [D], single pulse [S] and multiple pulses [M]; which were considered to be treatments) vs the constant low-nutrient treatment (L; which was used as the control). We did the same for each of the four fluctuating high-nutrient treatments (I, D, S and M; which were considered to be the treatments) vs the constant high-nutrient treatment (H; which was used as the control). The plasticity of each trait (Trait\_plasticity) was calculated as Trait\_treatment - Trait\_control. We did the regressions of ln(Biomass\_treatment/Biomass\_control) on Trait\_control and Trait\_plasticity using linear regression models. The following table shows the coefficients and their significances of each model. Significant coefficients for the plasticity effects are also plotted in the figures below.

<table>
<thead>
<tr>
<th>Estimate</th>
<th>t value</th>
<th>P-value</th>
<th>Estimate</th>
<th>t value</th>
<th>P-value</th>
<th>Estimate</th>
<th>t value</th>
<th>P-value</th>
<th>Estimate</th>
<th>t value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>L (Intercept)</td>
<td>1.038</td>
<td>4.498</td>
<td>&lt;0.001</td>
<td>(Intercept)</td>
<td>0.951</td>
<td>7.179</td>
<td>&lt;0.001</td>
<td>(Intercept)</td>
<td>0.906</td>
<td>2.826</td>
<td>0.009</td>
</tr>
<tr>
<td>vs RMF_plasticity</td>
<td>-0.192</td>
<td>-0.292</td>
<td>7.73</td>
<td>RL_control</td>
<td>&lt;0.001</td>
<td>-0.536</td>
<td>0.596</td>
<td>RD_control</td>
<td>0.139</td>
<td>0.121</td>
<td>0.904</td>
</tr>
<tr>
<td>H (Intercept)</td>
<td>1.104</td>
<td>6.002</td>
<td>&lt;0.001</td>
<td>(Intercept)</td>
<td>0.977</td>
<td>9.248</td>
<td>&lt;0.001</td>
<td>(Intercept)</td>
<td>0.949</td>
<td>4.244</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>vs RMF_plasticity</td>
<td>-0.312</td>
<td>-0.579</td>
<td>0.567</td>
<td>RL_control</td>
<td>&lt;0.001</td>
<td>-1.009</td>
<td>0.322</td>
<td>RD_control</td>
<td>-0.093</td>
<td>-0.119</td>
<td>0.906</td>
</tr>
<tr>
<td>I (Intercept)</td>
<td>0.596</td>
<td>0.556</td>
<td>0.583</td>
<td>RL_plasticity</td>
<td>&lt;0.001</td>
<td>0.894</td>
<td>0.380</td>
<td>RD_plasticity</td>
<td>4.188</td>
<td>1.868</td>
<td>0.073</td>
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<tr>
<td>vs RMF_plasticity</td>
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<td>0.533</td>
<td>0.599</td>
<td>RL_control</td>
<td>&lt;0.001</td>
<td>-1.029</td>
<td>0.313</td>
<td>RD_control</td>
<td>-0.159</td>
<td>4.594</td>
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<td>0.028</td>
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<tr>
<td>vs RMF_control</td>
<td>0.939</td>
<td>5.122</td>
<td>&lt;0.001</td>
<td>(Intercept)</td>
<td>0.862</td>
<td>8.724</td>
<td>&lt;0.001</td>
<td>(Intercept)</td>
<td>1.012</td>
<td>4.981</td>
<td>&lt;0.001</td>
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<td>S (Intercept)</td>
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<td>RD_plasticity</td>
<td>5.834</td>
<td>2.555</td>
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<tr>
<td>vs RMF_plasticity</td>
<td>0.980</td>
<td>4.329</td>
<td>&lt;0.001</td>
<td>(Intercept)</td>
<td>0.988</td>
<td>9.037</td>
<td>&lt;0.001</td>
<td>(Intercept)</td>
<td>1.007</td>
<td>3.352</td>
<td>0.002</td>
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<td>0.532</td>
<td>RL_control</td>
<td>&lt;0.001</td>
<td>-1.947</td>
<td>0.062</td>
<td>RD_control</td>
<td>-0.422</td>
<td>-0.411</td>
<td>0.684</td>
</tr>
<tr>
<td>vs RMF_control</td>
<td>-0.962</td>
<td>-0.569</td>
<td>0.492</td>
<td>RL_plasticity</td>
<td>&lt;0.001</td>
<td>1.033</td>
<td>0.311</td>
<td>RD_plasticity</td>
<td>1.123</td>
<td>0.479</td>
<td>0.636</td>
</tr>
<tr>
<td>H (Intercept)</td>
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<td>0.585</td>
<td>0.564</td>
<td>(Intercept)</td>
<td>0.017</td>
<td>0.281</td>
<td>0.781</td>
<td>(Intercept)</td>
<td>0.015</td>
<td>0.081</td>
<td>0.936</td>
</tr>
<tr>
<td>vs RMF_control</td>
<td>-0.042</td>
<td>-0.123</td>
<td>0.903</td>
<td>RL_control</td>
<td>&lt;0.001</td>
<td>0.176</td>
<td>0.862</td>
<td>RD_control</td>
<td>-0.082</td>
<td>-0.125</td>
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<td>2.719</td>
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<td>-0.285</td>
<td>-0.105</td>
<td>0.917</td>
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<tr>
<td>vs RMF_plasticity</td>
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<td>-0.580</td>
<td>0.567</td>
<td>(Intercept)</td>
<td>0.010</td>
<td>0.129</td>
<td>0.899</td>
<td>(Intercept)</td>
<td>0.145</td>
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<td>0.472</td>
<td>(Intercept)</td>
<td>0.010</td>
<td>0.206</td>
<td>0.839</td>
<td>(Intercept)</td>
<td>0.056</td>
<td>-0.741</td>
<td>0.465</td>
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<td>0.283</td>
<td>1.151</td>
<td>0.139</td>
<td>(Intercept)</td>
<td>0.010</td>
<td>0.206</td>
<td>0.839</td>
<td>(Intercept)</td>
<td>0.056</td>
<td>-0.741</td>
<td>0.465</td>
</tr>
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<td>RD_control</td>
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<td>0.485</td>
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<tr>
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<td>RL_plasticity</td>
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<td>2.927</td>
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<td>0.864</td>
<td>(Intercept)</td>
<td>0.010</td>
<td>0.126</td>
<td>0.901</td>
<td>(Intercept)</td>
<td>0.119</td>
<td>0.611</td>
<td>0.546</td>
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<tr>
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<td>0.879</td>
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<td>-0.703</td>
<td>0.489</td>
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<td>1.302</td>
<td>0.204</td>
<td>RD_plasticity</td>
<td>1.167</td>
<td>0.626</td>
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</tbody>
</table>
Relationships between changes in plant biomass in response to high nutrient levels (left graphs) and in response to fluctuating nutrient levels (right graphs) and plastic changes in root mass fraction, root length, root diameter and specific root length.
Table S1 The standard deviation (SD) for random effects of the total biomass model, which included random slopes for family and species with respect to nutrient treatment (i.e. constant low, constant high, gradual increase, gradual decrease, single pulse and multiple pulses).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Standard deviation (SD)</th>
</tr>
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<td></td>
<td>Family</td>
</tr>
<tr>
<td>Intercept (i.e. Constant low)</td>
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</tr>
<tr>
<td>Constant high</td>
<td>0.1474</td>
</tr>
<tr>
<td>Gradual increase</td>
<td>0.1236</td>
</tr>
<tr>
<td>Gradual decrease</td>
<td>0.0001</td>
</tr>
<tr>
<td>Single pulse</td>
<td>0.2412</td>
</tr>
<tr>
<td>Multiple pulses</td>
<td>0.2337</td>
</tr>
</tbody>
</table>
Figure S1 Modelled means of (a) total biomass and (b) root mass fraction, (c) root length, (d) root diameter, and (e) specific root length of the 7 rare alien, 7 common alien, 6 rare native and 9 common native plant species growing under six different patterns of nutrient supply. Error bars represent SEs of the means.
Figure S2 Modelled means of (a) root mass fraction, (b) root diameter, and (c) specific root length of the 14 alien and 15 native plant species, irrespective of commonness, growing under low and high (averaged across the five patterns of resource supply) nutrient supply. Error bars represent SEs of the means.
Figure S3 Modelled means of root diameter of the 14 alien and 15 native plant species, irrespective of commonness, growing under a constant high and a gradually changed nutrient supply. Error bars represent SEs of the means.
Modelled means of (a) total biomass and (b) root mass fraction of the 14 alien and 15 native plant species, irrespective of commonness, growing under a constant high and a single-pulse nutrient supply. Error bars represent SEs of the means.

**Figure S4**
Chapter 3

Increases in nutrient availability and fluctuations do not promote dominance of alien plants in native communities

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Submitted to *Journal of Ecology*
Summary

1. The fluctuating-resource-availability hypothesis has become a key theory to explain plant invasion. It predicts that fluctuations in resource availability could promote plant invasion into native resident communities. The few clear-cut tests of this theory are limited to a few successful (i.e. invasive) alien plant species. However, it remains unknown how general this phenomenon is, and whether invasive and non-invasive alien species respond differently to resource fluctuations in a plant community.

2. We grew ten invasive and ten non-invasive species as target species in pot-mesocosms with four different resident communities under six nutrient treatments differing in overall nutrient availability and temporal fluctuations in nutrient availability (constant low, constant high, gradual increasing, gradual decreasing, single large pulse, multiple smaller pulses). With the exception of plants in the constant low treatment, the plants were supplied the same total amount of nutrients during the experiment. We tested whether increases in nutrient availability and fluctuations increased performance of both invasive and non-invasive alien plants within resident communities of native species, and whether invasive ones benefitted more than non-invasive ones.

3. We found that the increase in biomass in response to nutrient addition was stronger for invasive than for non-invasive alien species. However, as the native competitors benefited even more from nutrient addition, the relative biomass of the alien target plant species, particularly the non-invasive ones, decreased. When the nutrient supply gradually increased, biomass of alien target as well as native competitors decreased compared to the plants in the constant nutrient supply treatment. Surprisingly, when nutrients were supplied as a single large pulse, the absolute and relative biomass of the alien target plants decreased. The reduction in relative biomass was even stronger for invasive than for non-invasive alien species, and a similar pattern was found when nutrients were supplied as multiple smaller pulses.

4. Synthesis Our results confirm previous findings that invasive alien species benefit more from nutrient addition than non-invasive alien species. However, in contrast to previous findings, our results suggest that nutrient fluctuations do not promote, but even suppress biomass of alien plants relative to that of natives. This strongly suggests that it is not a general phenomenon that environmental variability promotes plant invasion.
**Key words:** Biological invasions, environmental variability, exotic, global change, native community, non-native, plant-plant interactions
Introduction

With the increase in globalization and trade, a large number of plant species have been introduced to new regions where they are not native (Essl et al., 2011; Seebens et al., 2015; van Kleunen et al., 2015). Some of these introduced alien species have established self-sustaining populations in the wild, and some of these naturalized species successfully spread and now occupy large areas in the introduced range. Such invasive species can reduce native species biodiversity and change ecosystem functions (Vitousek et al., 1997; Ricciardi, 2007; Vilà et al., 2011), and might also have economic impacts (Pimentel et al., 2005). The number of naturalized alien species is still increasing (Seebens et al., 2017), and invasions might further increase due to other global change drivers (Seebens et al., 2015; Haeuser et al., 2017; Liu et al., 2017). Therefore, two major questions in ecology are why some alien plant species become invasive and others not, and how may the success of alien species be affected by other global change drivers (Funk & Vitousek, 2007; van Kleunen et al., 2010b; Dawson et al., 2011; Dawson et al., 2012b; Jia et al., 2016; Haeuser et al., 2017; Liu et al., 2017).

As a major component of global change, plant invasion is likely to interact with other environmental changes (Dukes & Mooney, 1999; Bradley et al., 2010a). For example, a recent meta-analysis showed that invasive alien plants take more advantage of CO₂ enrichment and climate warming than native species do (Liu et al., 2017). On the other hand, many alien plant species that do not pose an invasion risk under current environmental conditions, may become invasive under future conditions with ongoing global environmental change (Walther et al., 2009; Haeuser et al., 2017). Numerous empirical studies tested how invasive plants interact with other environmental changes, but almost all of these studies considered only environmental changes in mean conditions (Leishman & Thomson, 2005; Parepa et al., 2013; Manea & Leishman, 2015; Jia et al., 2016; Liu et al., 2017; Liu & van Kleunen, 2017). Environmental change, however, does not only affect the mean conditions but also also environmental variability (Stenseth et al., 2002; Smith, 2011). For example, while atmospheric nitrogen deposition (Galloway et al., 2008) and agricultural fertilizer spill-over (Didham et al., 2015) have increased nutrient levels world wide, increasing fluctuations in weather and fire events, and changes in land-use management practices could also lead to changes in temporal nutrient variability (Davis & Pelsor, 2001; Certini, 2005; Matias et al., 2011). Consequently, an improved understanding of how plants interact with changes in the temporal variability of resources can help to predict plant invasions.
A major theory explaining interactions between plant invasion and environmental change is the fluctuating-resource-availability hypothesis (Davis et al., 2000). It predicts that habitats with a high variability in resource availability will generally be more easily invaded by alien plants than more constant habitats. Indeed, the few experimental studies that tested this hypothesis found support for it (Davis & Pelsor, 2001; Parepa et al., 2013; Liu & van Kleunen, 2017). For example, Parepa et al., (2013) grew the invasive plant Japanese knotweed (Fallopia spp.) in a mesocosm with a native plant community under one low nutrient treatment and four high-nutrient treatments that differed in variability. In this clear-cut experiment, they found evidence that increased variability in nutrient supply can strongly promote the invasion success of Japanese knotweed. However, as only one invasive alien species and only one native community were considered in their study, it remains to be seen how general this phenomenon is. In order to draw more general conclusions, multispecies experiments are needed (van Kleunen et al., 2014). Recently, Liu & van Kleunen, (2017) did a multispecies experiment to test biomass responses of rare and common alien and native plants to nutrient availability and fluctuations therein, and found that alien plants, in contrast to native plants, benefited from a large nutrient pulse. However, in that study, all plants were grown individually, and it therefore remains to be tested whether this pattern holds under competition.

To explicitly test the fluctuating-resource-availability hypothesis in the context of plant competition, we did a multispecies experiment. We grew ten pairs of a taxonomically related invasive and non-invasive alien species (i.e. 20 species in total) as target species in the center of mesocosm pots with communities of native grassland species. To assess whether the responses of the alien target species depends on the composition of the native communities, we used a total of 12 native grassland species to create four different communities of three species. Each of the 20 alien target species was grown in mesocosm pots with each of these four native communities, and exposed to six nutrient-supply treatments differing in the mean and temporal supply of nutrients (i.e. constant low, constant high, gradual increase, gradual decrease, a large single pulse and multiple small pulses). By comparing the absolute above-ground biomass production of the alien target species as well as their biomass production relative to the biomass production of the native competitors in the different treatments, we addressed the following specific questions: (i) Does an increase in mean nutrient availability promote absolute and relative biomass of both invasive and non-invasive alien plants? (ii) Does variability in nutrient supply promote absolute and relative biomass of both invasive
and non-invasive alien plants? (iii) Do invasive alien plants take more advantage than non-invasive alien plant of increases in mean nutrient availability and fluctuations therein?

Materials and Methods

Studied plant species

Figure 1 Graphical illustration of the experimental design (species, nutrient treatments and replicates) and data-analysis design for the effects of the six nutrient treatments (i.e. coded as dummy variables to create specific contrasts for comparing treatment levels). Each bar in the box of nutrient treatment represents the amount of nutrient solution supplied each week during the 10 weeks of the experiment. The five high-nutrient level treatments (constant high, gradual increase, gradual decrease, single pulse and multiple pulses) received the same total amount of nutrients during the 10 weeks, which was eight times higher than in the constant low-nutrient treatment. In the data-analysis design, dummy variables coding for the scenarios of nutrient supply were used to test the effects of the mean level of nutrient supply (constant low treatment vs. the average across the five high treatments; T0), and the effects of different temporal patterns of high nutrient supply vs. the constant high supply (T1-T4). For the latter, each contrast of interest was made by fitting the corresponding dummy variable after all other dummy variables (for details on model comparisons, see Liu & van Kleunen 2017). The two boxes around each dummy coding indicate the contrasts of interest.

To investigate differences in plant responses to mean nutrient availability and temporal fluctuations therein between invasive and non-invasive alien plants in the presence of native competitors, we chose 20 alien species (i.e. target species) and 12 native plant species (i.e. competitors; Table S1) from the herbaceous flora of Germany based on information in the
BiolFlor database (http://www2.ufz.de/biolflor/index.jsp). The 20 target species were chosen from nine families in such a way that they constituted ten taxonomically related pairs of an invasive (i.e. widespread) and a non-invasive (i.e. less widespread) alien plant. In other words, we avoided that all invasive alien species belong to one taxonomic group and all non-invasive alien species belong to another taxonomic group (Felsenstein, 1985). We assigned the alien species to the categories “invasive” and “non-invasive” based on the number of 130-km² grid cells occupied by the species in Germany (maximum 3000) extracted from the FloraWeb database (http://www.floraweb.de/). All invasive alien species occurred in more than 900 grid cells (median = 1925), and all non-invasive species occurred in fewer than 700 grid cells (median = 81; Table S1). Within each pair of species, the invasive species always occurred in at least four times more grid cells than the non-invasive species. All 12 native species, which included four forbs and eight grasses, occur frequently in German grasslands (Table S1). All seed materials were obtained from commercial seeds companies or the seed collection of the Botanical Garden of the University Konstanz (Table S1).

**Experimental set-up**

We conducted the multi-species experiment in a greenhouse of the Botanical Garden of the University of Konstanz, Germany (N: 47°69′19.56″, E: 9°17′78.42″). From 4 to 19 October 2016, we sowed the seeds of each species separately into trays (12 × 12 × 4.5 cm) filled with potting soil (Topferde®, Einheitserde Co., Sinntal-Altengronau, Germany; pH 5.8; 2.0 g L⁻¹ KCl; 340 mg L⁻¹ N; 380 mg L⁻¹ P₂O₅; 420 mg L⁻¹ K₂O; 200 mg L⁻¹ S; 700 mg L⁻¹ Mg). As species were known, from a previous pilot experiment, to geminate at different times, we sowed them on different dates (Table S1) to ensure that the seedlings were in a similar developmental stage at the start of the experiment. On 25 October 2016, we selected similarly sized seedlings of each of the 32 species and transplanted them into 2.5-L circular plastic pots filled with a 1:1 mixture of sand and fine vermiculite. In order to create four different native grassland communities, we randomly assigned the 12 native species into four different groups of three species (Table S1). We first transplanted two seedlings of each native-community member so that each pot included six individuals of native species growing at equal distance in a circle (diameter = 10 cm) around the center of the pot. The two individuals of the same species were planted at opposite to each other (Fig. 1). In total, we had 480 pots, with 120 pots for each of the four native communities. After planting the native competitors, we transplanted one seedling of an alien target species in the center of each pot. For each of the 20
target species, we transplanted a total of 24 seedlings, which were equally divided over the four different native communities (i.e. six pots per native community). All pots were randomly assigned to positions on four greenhouse benches, and were re-randomized again after five weeks. To avoid leakage of water and nutrient solution, we put a plastic dish under each pot. The temperatures of the greenhouse were set to remain between 22 and 28°C, and additional lighting was provided to extend the daily light period to 16 h.

One week after transplanting the seedlings, we started to apply the different nutrient treatments at weekly intervals for a total of 10 weeks. As this study was built on a previous experiment testing the responses of rare and common aliens and natives to nutrient variability and fluctuations (Liu & van Kleunen, 2017), we used the same nutrient treatments as in that study. In short, to test the effects of different overall nutrient supply and different temporal patterns of nutrient supply on invasive and non-invasive alien plants in the presence of native competitors, we created six nutrient-supply patterns (Fig. 1): (i) constant low, (ii) constant high, (iii) gradual increase, (iv) gradual decrease, (v) single large pulse and (vi) multiple pulses of different magnitudes. Treatment (i) was the low nutrient treatment, which received in total 50ml of a 400%-strength Hoagland solution. Treatments (ii) to (vi) all received in total 400ml of a 400%-strength Hoagland solution during the whole experiment, but differed in the temporal pattern of nutrient supply. There were four replicates per alien target species for each of the six nutrient treatments (one replicate per native community). During the experiment, we checked all pots every day to ensure that water did not limit plant growth. We watered the plants when necessary by pouring equal amounts of water in the dish under each pot.

**Harvest and Measurements**

To be able to account for variation in initial size of alien target plants in the analyses, we counted leaf number (except cotyledons), and measured the length and width of the largest leaf and the height of each alien target plant at the beginning of the experiment, immediately after transplanting. We calculated an estimate of initial leaf area per plant by multiplying the number of leaves and the length and width of the largest leaf. On 11 January 2017, 11 weeks after transplanting, we began to harvest the aboveground biomass for all pots one native community after the other. For each pot, we first harvested the alien target species, and then harvested the three native competitor species. As 23 target plants died during the experiment, we only harvested 457 pots instead of 480 pots at the end of the experiment (Table S1). All
biomass samples were dried at 70°C for 72 h and then weighed. We calculated total aboveground biomass per pot by summing the biomass of the alien target species and the three native competitors. We also calculated the biomass proportion of the alien target species in each pot by dividing the biomass of the alien target species by the total biomass.

**Data Analysis**

To test the effects of target-plant status (invasive vs non-invasive alien) and nutrient treatments on aboveground biomass production of the alien target species, biomass proportion of the alien target species in each pot and total biomass per pot, we fitted linear mixed-effects models using the function `lme` in the R package `nlme` (Pinheiro et al., 2016) in R 3.3.2 (R core Team, 2016). To meet the assumption of normality, biomass of the alien target species and total biomass per pot were natural-log transformed, and biomass proportion of alien target species in each pot was square-root transformed. We included alien target-plant status (invasive vs non-invasive), average nutrient level (low vs high), the different nutrient variability treatments nested within the high nutrient level treatment (constant high vs each of the non-constant scenarios), and their interactions as fixed factors. To test the effects of the different nutrient treatments (i.e. nutrient levels [low vs high], nutrient variability [constant high vs each of the four non-constant scenarios]), we created contrasts by coding the nutrient treatments as dummy variables (Schielzeth, 2010) T0 to T4 (Fig. 1) in all models. Because initial plant size variation might contribute to differences in final biomass of the target species, we included initial leaf area and initial plant height as scaled natural-log-transformed covariates in the analysis of biomass production of the alien target species and biomass proportion of alien target species in each pot. We included identity of the target species nested in family and native community as random factors in all models. As the homoscedasticity assumption was violated in all models, we also included variance structures to model different variances per species using the ‘varIdent’ function in the R package `nlme` (Pinheiro et al., 2016; see also Zuur et al., 2009). We used log-likelihood ratio tests to assess significance of the fixed effects target-plant status, nutrient treatments (i.e. dummy variables T0 to T4) and their interactions (Zuur et al., 2009; also see Liu & van Kleunen, 2017). The log-likelihood ratio tests for fixed terms were based on comparisons of maximum likelihood (ML) models with and without the terms of interest, and the variance components were estimated using the restricted maximum likelihood method (REML) of the full model (Zuur et al., 2009).
Results

Averaged across all six nutrient treatments, invasive and non-invasive alien species did not differ significantly in aboveground biomass production and biomass proportion in each pot (Table 1; Fig. 2). The total biomass per pot was also not affected by status of the alien target species (Table 1; Fig. 2).

Biomass responses to an overall increase in nutrient availability

Averaged across all 20 alien target species, an increase in the mean nutrient supply (averaged over the different temporal nutrient-supply patterns) significantly increased the aboveground biomass production of alien target species (+131.4%; Table 1; Fig. 2). This increase in biomass production was stronger for invasive alien species (+179.6%) than for non-invasive alien species (+86.6%; significant S×T0 interaction in Table 2; Fig. 2). However, as the total aboveground biomass production of each pot also increased in response to the mean nutrient supply (+14.4%; Table 1; Fig. 2), the biomass proportion of the alien target species in each pot decreased (-10.0%; Table 1; Fig. 2). This effect was caused by the non-invasive alien species only, as the biomass proportion strongly decreased for non-invasive alien species (-19.9%) and not for invasive alien species (+0.4%; significant S×T0 interaction in Table 2; Fig. 2). The increase in total biomass production per pot in response to an increase in the mean nutrient supply was not significantly affected by status of the alien target plants (Table 1; Fig. 2).

Biomass responses to temporal variability in nutrient supply

In the high mean nutrient-supply treatments, when nutrients were gradually increased over time, biomass production of alien target species and total biomass per pot significantly decreased by 22.3% and 13.1%, respectively, than when nutrients were supplied at a constant rate (significant T1 effects in Table 1; Fig. 2). There were, however, no significant main effects for any of the measured traits when nutrients were gradually increased over time instead of supplied at a constant rate (Table 1). There were also no significant interaction effects between plant status and any types of gradual change in nutrient supply over time (Table 1).

When nutrients were supplied as a single large pulse in middle of the 10-week growth period instead of at a constant high rate, biomass production of alien target species significantly de-
creased (-30.1%; significant T3 in Table 1; Fig. 2). The total biomass per pot, however, was not affected by whether the nutrients were supplied as a single large pulse or at a constant high rate (Table 1; Fig. 2). Therefore, the biomass proportion of alien target species in each pot significantly decreased (-19.8%) when nutrients were supplied as a single large pulse instead of at a constant high rate (significant T3 in Table 1; Fig. 2). This decrease in biomass proportion was much stronger for invasive alien species (-27.9%) than for non-invasive alien species (-8.6%; significant S×T3 interaction in Table 2; Fig. 3). On the other hand, there were no significant main effects for any of the measured traits when nutrients were supplied as multiple smaller pulses instead of at a constant high rate (Table 1). However, when nutrients were supplied as multiple smaller pulses instead of at a constant high rate, the biomass proportion in each pot increased for non-invasive alien species (+6.7%), whereas the reverse was the case for invasive alien species (-18.8%; significant S×T4 interaction in Table 1, Fig. 2).
**Table 1** Results of linear mixed effects models testing the effects of species status, changes in mean nutrient availability (T0) and temporal patterns of variability in nutrient availability (T1-T4), and all interactions thereof, on aboveground biomass and biomass proportion of alien target plants and total aboveground biomass per pot. The nutrient-treatment contrasts T0-T4 are explained in Fig. 1. We also provide the estimates of the standard deviations for the random effects, and the marginal and conditional R\(^2\) values.

<table>
<thead>
<tr>
<th>Fixed effects</th>
<th>Biomass of alien target plants</th>
<th>Biomass proportion of alien target plant</th>
<th>Total biomass per pot</th>
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<tbody>
<tr>
<td></td>
<td>df</td>
<td>(\chi^2)</td>
<td>P</td>
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<tr>
<td>Initial leaf area</td>
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<td>33.484</td>
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<td>0.0006</td>
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<td>Low vs High (T0)</td>
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<td>0.0012</td>
</tr>
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<td>Constant high vs Gradual decrease (T2)</td>
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<td>0.8597</td>
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<td>Constant high vs Single pulse (T3)</td>
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<td>12.167</td>
<td>0.0005</td>
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<td>Constant high vs Multiple pulses (T4)</td>
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<td>0.3745</td>
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<td>S × T0</td>
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<td>0.0058</td>
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<td>S × T1</td>
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<td>0.411</td>
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<td>S × T2</td>
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<td>S × T3</td>
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<td><strong>Random effects</strong></td>
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<td><strong>R(^2) of the model</strong></td>
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<tr>
<td>Marginal (R(^2)</td>
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<tr>
<td>Conditional (R(^2)</td>
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<td>0.8949</td>
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</tr>
</tbody>
</table>

Note: significant effects (p < 0.05) are in bold.
**Discussion**

It is has been suggested that invasive alien plant species might take more advantage of increased nutrient availability than non-invasive alien species (Richards *et al.*, 2006; Dawson *et al.*, 2012a; Dawson *et al.*, 2012b). Our multispecies experiment corroborates this, as we found that the invasive alien plant species in our study benefited more of an increase in mean nutrient level than non-invasive alien plant species did. However, we also found that alien target plant species, particularly the non-invasive ones, benefitted proportionally less of the additional nutrients than the native competitors. The fluctuating-resource-availability hypothesis predicts that increased variability in resource availability could also promote plant invasion into native resident communities (Davis *et al.*, 2000). The results of our experiment, however, do not support this. Surprisingly, we even found that the alien target species, and particularly the invasive ones, decreased in biomass relative to the resident native competitors.

**Biomass responses to an overall increase in nutrient availability**

Comparison of traits between invasive and non-invasive alien species could provide insights into why some alien species become invasive and others fail to do so (van Kleunen *et al.*, 2010a). Our study showed strong evidence that the difference between invasive and non-invasive species in response to an increase in the mean nutrient supply might be one of the mechanisms behind invasiveness of alien species. Richards *et al.*, (2006) described different scenarios of how environmental responses (phenotypic plasticity) might differ between invasive and non-invasive species. Our results support their Master-of-some scenario, in which the successful invader takes more advantage of favorable environments than less successful species. Our results are also in line with the findings of Dawson *et al.*, (2012a) and Dawson *et al.*, (2012b). Surprisingly, however, the results contrast with the ones of our previous study Liu & van Kleunen, (2017), in which the biomass response to an increase in the mean nutrient level did not differ between invasive and non-invasive alien species. A potential reason for the discrepancy could be that Liu & van Kleunen, (2017) used a partly different set of alien species. To test this, we re-analysed the data of the two studies for the subsets of species that were shared between them (five invasive and six non-invasive species; Table S2 and Fig. S1). Although both studies showed the pattern that invasive alien species responded more strongly to nutrient addition than non-invasive alien species (Fig. S2), this interaction effect was only significant in the current study (Table S2). Therefore, a more likely explanation for
the inconsistency between the two studies might be that in the current study invasive and non-invasive alien species were grown with native competitors, whereas Liu & van Kleunen, (2017) grew them without competitors (Callaway et al., 2003).

An increase in resource availability may promote plant invasion, because invasive alien species may be better at capitalizing on an increased availability of a limiting resource than species in the native communities (Leishman & Thomson, 2005; Funk, 2008). Assessing the change in proportional biomass of an alien species in a native community may thus provide insight into whether the alien species might outcompete the native species or vice versa when the environment changes (van Kleunen et al., 2010a; Parepa et al., 2013). We found that in response to increased nutrient availability, the total biomass per pot increased more strongly than the biomass of the alien target plants. This indicates that the native competitors benefit even more from nutrient addition than the alien species did. This was also reflected in the decrease in biomass proportion of the alien target species in response to nutrient addition. This effect was, however, fully accounted for by the decrease in biomass proportion of the non-invasive alien species, as the proportional biomass of the invasive alien species did not change in response to nutrient addition. Our findings therefore show that invasive alien plant species are not necessarily competitively superior to native plant species, but that non-invasive alien species are.

The lack of evidence for a superior competitive ability of invasive species over native species after nutrient addition could reflect that the native species in our resident communities are also widespread (i.e. successful) in Germany. Indeed, Dawson et al., (2012a) found that common native species capitalized more on nutrient addition than rare native species, and Godoy et al., (2011) found that invasive alien plant species in Spain showed similar responses to nutrient addition as widespread native species. Similarly, a previous meta-analysis revealed that invasive alien plants exhibited a higher performance than native plants overall, but that there was no significant difference between them when the analysis was limited to those native species known to be invasive elsewhere (i.e. which are also successful; van Kleunen et al., 2010b). So, although invasive alien and common native species might both be Master-of-some species (Richards et al., 2006) resulting in a competitive balance, we found evidence that non-invasive alien species are weak competitors in favourable environments.
Figure 2 Modeled means of aboveground biomass production (a) and biomass proportion (b) averaged across the ten invasive and the ten non-invasive alien target species, and total biomass per pot (c) in the six nutrient treatments. Error bars represent standard errors.

Biomass responses to temporal variability in nutrient supply

Very few studies have assessed how the response of plants to nutrient addition depends on the temporal pattern of nutrient supply (Dener et al., 2016; Liu & van Kleunen, 2017). We
found that the biomass of the alien target plants and the total biomass per pot (also including the native competitors) were lower when the nutrient supply gradually increased than when the nutrient supply was kept constant. This is in line with the findings of Liu & van Kleunen, (2017), and most likely reflects that in the gradual-increase scenario the nutrient availability was insufficient at the early growth stage. However, our results contrast with the finding of Parepa et al., (2013), who found that biomass productions of their community did not differ between the scenario where plants grew under gradual increase in nutrient supply and a scenario of constant nutrient supply. A possible explanation for this discrepancy is that the experiment of Parepa et al., (2013) lasted longer than our experiment, and that plant in the gradual-increase treatment of their study thus had more time to compensate for the initial nutrient limitation. In line with the findings of Liu & van Kleunen (2017), we also found no difference in plant performance between when plants grew under gradually decreased nutrient supply and when they grew under constant nutrient supply. Possibly, nutrient availability might have decreased to a lesser extent than the nutrient supply because of storage of nutrients in the substrate and/or plant.

The fluctuating-resource-availability hypothesis has become a key theory to explain plant invasion, and seems to find general support (Davis et al., 2000; Davis & Pelsor, 2001; Parepa et al., 2013; Tognetti & Chaneton, 2015). However, the results of our multispecies experiment showed that in response to a single large nutrient pulse and in response to multiple small nutrient pulses, the biomass proportion of alien plant species decreased. This was particularly the case for the invasive alien species, and non-invasive alien species even slightly benefited from the multiple small nutrient pulses. This means that our findings do not support the fluctuation-resource-availability hypothesis. On the contrary, it indicates that nutrient pulses may inhibit performance of invasive alien species relative to the performance of the native community.

Both the invasive alien target species and the native competitor species in our study are common species in Germany, and may strongly capitalize on an increase in the mean nutrient availability (Dawson et al., 2012b). The non-invasive alien species, on the other hand, are likely to possess a resource-conservation strategy (Richards et al., 2006; Dawson et al., 2012a). Possibly, such a strategy provided them with an advantage during the low-nutrient supply periods in between the nutrient pulses. Consequently, the proportional biomass of non-invasive alien species may have been less affected by nutrient pulses than the invasive
ones. Our finding, however, contrasts with the finding by Parepa et al., (2013) that the biomass proportion of invasive Japanese knotweed (*Fallopia* spp.) in mesocosms with a native community increased two- to four-fold when plants received a single large nutrient pulse or multiple small nutrient pulses instead of a constant nutrient supply. *Fallopia* spp. can be highly dominant, and while our species are all widespread in Germany not all of them may form dominant stands. Possibly, only alien plant species with a superior capacity to acquire nutrients can outcompete others and thus become dominant species (Gioria & Osborne, 2014). Our findings are contradict the findings of our previous study (Liu & van Kleunen, 2017), in which we found that alien species, and not native species, benefited from a large nutrient pulse when plants were grown without competition. Whatever the exact reason for the discrepancy, our results highlight that responses to nutrient-supply patterns for single plants might not be representative for plants grown under competition.

**Conclusions**

Our results contribute to the growing amount of evidence that alien species able to strongly capitalize on an increase in the mean resource availability are likely to become widespread and invasive. However, although invasive alien species in our study capitalized more on additional nutrients than the non-invasive alien species, it did not give them an advantage over the native competitors. Most likely this reflects that the native competitors in our study are also widespread and thus successful because of their ability to capitalize on additional nutrients. This could imply that native communities consisting of rare native species might be much more vulnerable to invasion by alien species when nutrient availability increases. To test this, future studies should consider also using communities of rare native species.

Surprisingly, we found that temporal fluctuations in nutrient supply did not promote, but even suppressed plant invasion. In this study, we defined invasiveness based on how widespread the alien species are. Future studies, however, should test whether other dimensions of invasiveness, in particular local dominance of species (Catford et al., 2017) is related to competitive ability under high nutrient variability, and whether the success of alien and native species is linked to differences in nutrient uptake rate. Furthermore, to gain more insights in how plants respond to nutrient fluctuations, and how this changes the competitive balance between species, multiple samplings during the course of the experiment are needed. In conclusions, although our study shows that the ability to capitalize on an increase in nutrients is related to
invasiveness of alien species, it also shows that it is not a general phenomenon that environmental variability promotes plant invasion.

Acknowledgements

We thank Beate Rüter, Claudia Martin, Otmar Ficht for help with experiment setup and plant harvest. Y.L. thanks the China Scholarship Council (CSC) for financial support.
### Supporting information

#### Table S1 Detailed information on the 20 alien target species and 12 native competitor species used in this study.

<table>
<thead>
<tr>
<th>Alien target species</th>
<th>Family</th>
<th>Status</th>
<th>Number of grid cells</th>
<th>Sowing date</th>
<th>Transplanting date</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
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<td>Bidens connata †</td>
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<td>437</td>
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<td>25.10.2016</td>
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<td>25.10.2016</td>
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*Numbers of grid cells refers to the number of 12 x 11 km cells in Germany (total: 3000 cells) where each species has been recorded.

†Number of plants that died during the experiment.

‡Seeds were obtained from the Botanical Garden of the University of Konstanz, Germany.

‡Seeds were obtained from B and T World Seeds, Sarl, France.

‡Seeds were obtained from Rieger-Hofmann GmbH, Germany.

1,2,3 Allocation of native species to the four native communities; each group included three different native species.
Table S2 Results of linear mixed effects models testing the effects of species status, the change in mean nutrient availability (T0) and temporal patterns of variability in nutrient availability (T1-T4), and all interactions thereof, on aboveground biomass of alien target plants grown without competition in the previous study by Liu & van Kleunen (2017), and with competition in the present study. Both analyses include only the five invasive and six non-invasive alien target species that were used in both studies. To meet the assumption of normality, biomass of the alien target plants were natural-log transformed in both studies. The nutrient-treatment contrasts T0-T4 are explained in Fig. 1. We also provide the estimates of the standard deviations for the random effects, and the marginal and conditional $R^2$ values.

<table>
<thead>
<tr>
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<th>Target aboveground biomass in our present study</th>
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Note: significant effects ($p < 0.05$) are in bold.
Figure S1

Means of biomass production across the five invasive and the six non-invasive alien target species, which were used both in the study by Liu & van Kleunen (2017) and our present study, in the six nutrient treatments. Panel (a) was plotted using raw data. Because plant initial size had a significant effect on biomass in the present study, panel (b) was plotted using modelled means. Error bars represent standard errors.
Chapter 4

Does greater specific-leaf-area plasticity help plants to maintain a high performance when shaded?

Yanjie Liu, Wayne Dawson, Daniel Prati, Emily Haeuser, Yanhao Feng, Mark van Kleunen

Abstract

Background and Aims It is frequently assumed that phenotypic plasticity can be very advantageous for plants, because it may increase environmental tolerance (fitness homeostasis). This should, however, only hold for plastic responses that are adaptive, i.e. increase fitness. Numerous studies have shown shade-induced increases in specific leaf area (SLA), and there is wide consensus that this plastic response optimizes light capture and thus has to be adaptive. However, it has rarely been tested whether this is really the case.

Methods In order to identify whether SLA plasticity does contribute to the maintenance of high biomass of plant species under shaded conditions, we employed a meta-analytical approach. Our dataset included 280 species and 467 individual studies from 32 publications and two unpublished experiments.

Key Results Plants increased their SLA by 55.4% on average when shaded, while they decreased their biomass by 59.9%. Species with a high SLA under high-light control conditions showed a significantly greater ability to maintain biomass production under shade overall. However, in contrast to our expectation of a positive relationship between SLA plasticity and maintenance of plant biomass, our results indicated that species with greater SLA plasticity were less able to maintain biomass under shade.

Conclusions Although a high SLA per se contributes to biomass homeostasis, there was no evidence that plasticity in SLA contributes to this. Therefore, we argue that some of the plastic changes that are frequently thought to be adaptive might simply reflect passive responses to the environment, or result as by-products of adaptive plastic responses in other traits.

Keywords: Adaptive, Functional traits, Phenotypic plasticity, Leaf mass area, LMA, Low light environment, Shade tolerance
**Introduction**

Phenotypic plasticity is the ability of an organism to express different phenotypes in different environments, and is ubiquitous among organisms (Bradshaw, 1965; 1973; Schmid, 1992; West-Eberhard, 2003). Plants exhibit plasticity in numerous ecologically important traits related to plant function, development and life history (Sultan, 2000; Valladares *et al.*, 2007; Gratani, 2014). It is frequently assumed that phenotypic plasticity can be very advantageous for plants (Baker, 1974; Richards *et al.*, 2006), because it is thought to increase environmental tolerance (i.e. fitness homeostasis; Valladares *et al.*, 2014). This should, however, only hold for plastic responses that are adaptive, i.e. increase fitness (van Kleunen & Fischer, 2005; Muth & Pigliucci, 2007; van Kleunen *et al.*, 2011b). Although many studies demonstrated that certain plastic responses of plants to contrasting environments are adaptive (Poorter & Lambers, 1986; Valladares & Pearcy, 1998; Donohue *et al.*, 2001), this is not always the case, as some plastic responses might also be neutral (i.e. do not affect fitness) or even maladaptive (i.e. decrease fitness; van Kleunen & Fischer, 2005; Sánchez-Gómez *et al.*, 2006; Ghalambor *et al.*, 2007). Therefore, it is important to explicitly assess whether the plasticity of a trait is adaptive or not by investigating its contribution to performance of plants in multiple environments.

Light, one of the crucial factors for the growth and development of plants, is a highly heterogeneous environmental resource in nature, and almost all plants are exposed to a certain degree of shading during their lifetime (Valladares & Niinemets, 2008). At low light intensity, photosynthesis, and consequently plant growth, is reduced. Plants respond to changing light conditions by adjusting a suite of morphological and physiological traits, such as specific leaf area (SLA), internode and petiole lengths, leaf size, leaf thickness, leaf mass and chlorophyll content (Rozendaal *et al.*, 2006; Valladares & Niinemets, 2008; Legner *et al.*, 2014). While it is frequently implicitly assumed that these morphological and physiological changes are active plastic response to alleviate the plant of environmental stress, they could also reflect passive plastic responses to reduced resource availability (van Kleunen & Fischer, 2005).

SLA, the ratio of leaf area to leaf dry mass, is a key functional trait of plants underlying variation in growth rate among species (Pérez-Harguindeguy *et al.*, 2013). SLA is also a major trait in the worldwide leaf economics spectrum, which reflects the range of fast to slow returns on nutrient and dry mass investment in leaves among species (Wright *et al.*, 2004;
Flores et al., 2014). Plants usually develop a higher SLA when grown under low light conditions (Reich et al., 2003; Rozendaal et al., 2006; Feng & van Kleunen, 2014). This response could help plants to increase the efficiency of light capture and maximize carbon gain in such environments (Evans & Poorter, 2001; Gommers et al., 2013), because SLA tends to scale positively with mass-based light-saturated photosynthetic rate (Pérez-Harguindeguy et al., 2013). Therefore, it is generally assumed that the plastic response of SLA enables plants to maintain a high performance under shading, and has to constitute adaptive plasticity (Valladares & Niinemets, 2008; van Kleunen et al., 2011b; Feng & van Kleunen, 2014). However, few studies have tested explicitly whether plastic responses to shading in SLA are really adaptive (but see Steinger et al., 2003; Avramov et al., 2006; Sánchez-Gómez et al., 2006; McIntyre & Strauss, 2014 for notable exceptions), and thus result in high performance of plants across different light intensities.

Here, we employed a meta-analytical approach to test whether plasticity of SLA in response to shading is adaptive, i.e. whether it enables plants to maintain their fitness under shade conditions. Fitness is ideally measured in terms of reproductive output; however few studies have quantified this. Biomass is an alternative measure of plant performance, as it is the direct product of growth (e.g. Dawson et al., 2012b), and thus the change in biomass between high- and low-light conditions offers a good proxy for a species’ ability to tolerate shade. We compiled a database of 467 studies from 32 publications and two unpublished experiments that measured the responses of biomass and SLA of 280 plant species to shading to test whether greater plastic changes in SLA in response to shading actually help the plants to better maintain performance under shade (i.e. whether plasticity in SLA is positively related to maintenance of plant biomass).

Materials and methods

Study and data collection

As a basis for the meta-analysis, we used a data set from a previous meta-analysis by Dawson et al., (2012b), which was on the relation between resource use and global naturalization success of plants. This data set included 15 studies on this topic published between 1990 and 2009. To obtain more recent studies (i.e. covering 2010 to 2014) on SLA and performance responses of plants to shading, we conducted a literature search in Web of Science (http://apps.webofknowledge.com/) using the following search string ‘shad*’ OR ‘light*’ OR
‘R:FR’ OR ‘PAR’ AND ‘SLA’ OR ‘LMA’ OR ‘SLM’. In order to ensure that we did not miss any important studies, we also did a similar search in Google Scholar using the same keywords. Our searches resulted in 1055 new records. We then individually assessed each publication, and retained them if the study reported data on both plant biomass and SLA responses to shading. In total, we identified 33 publications that met our criteria (See Supplementary Materials and Methods S1 for all publications used), covering 113 species and 280 individual studies. We also added unpublished data from two of our own experiments (Prati, unpublished data; Haeuser, Dawson and van Kleunen, unpublished data) to the dataset, yielding data on an additional 167 species and 187 individual studies.

We extracted mean values, sample sizes and measures of variance (i.e. standard deviations, standard errors or 95%-confidence intervals) for plant biomass and SLA measures under a high-light control treatment and a shade treatment. We used the high-light treatment as the control treatment because we assumed it to be in the range of light intensities under which photosynthesis is light saturated. We did not consider studies that were done in growth chambers with artificial lighting, because high-light conditions in growth chambers are much lower than in glasshouse and garden environments, and below the light intensity under which photosynthesis is light saturated. When more than one shading level was used for a single species, they were all included in our analyses (and compared to the same high-light control), but we accounted for multiple measurements per species in the analysis (see below). We extracted the data directly from the text or tables, or, when presented in figures, we extracted the data using the software Image J 1.47v (Rasband, 2013). We also extracted data on light intensity of the high-light control and shade treatments, and calculated the relative light intensity of the shade treatment compared to the control high-light treatment. Because light intensity in glasshouses is typically lower than outdoors, we also extracted information on whether a study was conducted in a garden experiment or a glasshouse.

**Effect size and variance**

To examine the effects of shade treatment on SLA and plant biomass, we calculated the log-response ratio ($\ln R$) as an effect size of response variables for each individual study following Hedges *et al.*, (1999) as:

$$\ln R = \ln \left( \frac{\bar{x}_s}{\bar{x}_c} \right) = \ln(\bar{x}_s) - \ln(\bar{x}_c) .$$
Here, $\bar{X}_s$ and $\bar{X}_c$ are the mean values of each individual SLA or biomass observation in the shade (S) and control (C) treatments, respectively. LnR values <0 indicate a decrease in SLA or biomass when shaded, and values >0 indicate an increase in SLA or biomass. The variance of $\ln R$ was, following Hedges et al., (1999), calculated as

$$v_{\ln R} = \frac{(SD_s)^2}{N_s(\bar{X}_s)^2} + \frac{(SD_c)^2}{N_c(\bar{X}_c)^2}$$

Here, $N_s$, $N_c$, $SD_s$, $SD_c$, $\bar{X}_s$, and $\bar{X}_c$ are sample sizes, standard deviations and mean values for SLA or biomass in the shade (S) and control (C) treatments, respectively. As average biomass, and consequently also absolute changes in biomass in response to shading, might vary enormously among species (e.g. an annual herb has a much lower biomass than a tree), we chose the log-response ratio as an effect size as it quantifies the proportional change instead of the absolute change in biomass (Hedges et al., 1999).

**Data analysis**

All meta-analytical calculations and statistical analyses were performed in R 3.1.3 (R Core Team, 2015) using the package Metafor v1.9-5 (Viechtbauer, 2010). To test whether plastic changes in SLA in response to shading actually help the plant to better maintain performance (i.e. biomass) under shade, we selected a multivariate meta-analytic model using the rma.mv function. In the model, we included the effect sizes (LnR) of biomass and their corresponding sampling variances as the response variable. As the main explanatory variable of interest, we included plasticity of SLA in response to shading (i.e. $SLA_{shade} - SLA_{control}$) in the model. Because the change in biomass may also depend on the SLA under high-light control conditions ($SLA_{control}$), we also included this baseline SLA as an explanatory variable in the model. Effectively, by including both $SLA_{control}$ and ($SLA_{shade} - SLA_{control}$), we included both standard parameters (the intercept and slope) of a species linear SLA reaction norm to shading. We chose SLA under high-light conditions as the baseline (intercept) instead of $SLA_{shade}$, because the high-light conditions were likely to be more similar among studies than the low-light conditions. Moreover, while $SLA_{shade}$ was strongly correlated with ($SLA_{shade} - SLA_{control}$) (Pearson $r = 0.812, p < 0.001, n = 467$), resulting in multi-collinearity problems when including both variables in a single analysis, this was not the case for $SLA_{control}$ and ($SLA_{shade} - SLA_{control}$) (Pearson $r = 0.084, p = 0.069, n = 467$), despite a strong correlation between $SLA_{shade}$ and $SLA_{control}$ (Pearson $r = 0.650, p < 0.001, n = 467$). As species varied in life form
and studies varied in the degree of shading imposed, and in whether the study was done outdoors or in a glasshouse, we also included life form (woody vs non-woody), relative light intensity (proportion of light in shade treatment compared to high-light control treatment), and experiment type (garden vs glasshouse) as explanatory variables. The continuous explanatory variables ($SLA_{\text{shade}} - SLA_{\text{control}}$, $SLA_{\text{control}}$, and relative light intensity) were all standardized by subtracting the mean and dividing by the standard deviation for the entire dataset, to facilitate interpretation and comparisons of the estimated model parameters (Schielzeth, 2010).

As effect sizes on the same species and from the same study are not independent, we included species and study as random factors. Moreover, as recent studies have shown that the addition of phylogenetic information could have a significant impact on the effect-size estimates from meta-analysis models (Chamberlain et al., 2012), we also included phylogenetic information as a variance-covariance matrix in the model. We first constructed a base phylogenetic tree of all the species in our dataset using the online program Phylomatic (Webb & Donoghue, 2005). Polytomies within this base tree were then solved as far as possible using published molecular phylogenies (see Supplementary Materials and Methods S2 for all publications used). The phylogenetic tree was transformed to an ultrametric tree using the compute.brlen function in the package ape v 3.2 (Paradis et al., 2004). Finally, a variance-covariance matrix was calculated from the ultrametric tree, representing phylogenetic relatedness among species, using the vcv function in the package ape v 3.2.

The estimates of effect size of biomass may be affected by whether or not the same genetic plant material is used in both the high-light and shading treatments (Gianoli & Valladares, 2012) and by whether neutral shade (reduced light quantity alone) or canopy shade (reduced light quantity with altered spectral quality) is used (Griffith & Sultan, 2005). However, as in our dataset only six studies used the same genetic material in the different treatments and only three studies used canopy shade in high-light and shade treatments, we did not include these two factors in the main meta-analytical model described above. Instead, we did separate analyses to test whether material used in each study (replicated genotype or non-replicated genotype) or shade type (neutral shade or canopy shade) had a significant influence on the estimates of the effect sizes of biomass and SLA in response to shading, using the rma.mv function. We included species and study in the model as random factors, and phylogeny as a variance-covariance matrix. We also did separate analyses to test whether experiment type
(garden or greenhouse) or plant lifeform (woody or non-woody) had a significant influence on estimates of effect size of biomass and SLA in response to shading.

Using the models described above, we calculated a weighted mean effect size for each moderator. We calculated 95% confidence intervals (CI) with 1000 bootstrap replications, using the boot.ci function in the package boot v1.3-15 (Canty and Ripley, 2015). We considered the mean effect size estimate to be significantly different from zero if the 95% CI around the mean did not include zero. In order to visualize the relationship between the plasticity of SLA and the changes in plant biomass in response to shading, we plotted all biomass effect sizes against SLA-plasticity values, and added the regression line based on the predicted values from the main meta-analytical model described above. Total heterogeneity ($Q_T$) in the models used for separate analyses can be partitioned into heterogeneity explained by the model structure ($Q_M$) and unexplained heterogeneity ($Q_E$). We used the $Q_M$ test to determine the significance of the difference in the mean effect size between different levels in the following moderator variables: plant material type (replicated genotype or non-replicated genotype), shade type (neutral shade or canopy shade), experiment type (garden or greenhouse) and plant life form (woody or non-woody). Because residual plots revealed a deviation from the assumption of normality, we used randomization tests to obtain a robust significance level of differences between groups ($Q_M$). By performing 1000 iterations for each model, a frequency distribution of possible $Q_M$ values was generated. We then compared the randomly generated values to the observed $Q_M$ value of each model, and calculated the proportion of randomly generated $Q_M$ values more extreme (equal to or larger) than the observed $Q_M$ values. We used this proportion as the significance level (i.e. $p$-value) for differences between groups.
Results

**Figure 1** Mean effect sizes (lnR) describing the overall responses of biomass and SLA to shading, and how these responses depend on whether the species are woody or non-woody, and whether the study was done in a glasshouse or garden, used the same genetic material in the different light treatments, and used neutral or canopy shading. Error bars represent bias-corrected bootstrapped 95%-confidence intervals around the mean effect-size estimates derived from the phylogenetically corrected meta-analytic model. The sample sizes (i.e. the number of studies) are given in parentheses. The dashed line indicates zero effect of shading.

On average, SLA of plants increased by 55.4% when shaded, while biomass decreased by 59.9% (Fig. 1). The responses of SLA and biomass to shading were not significantly affected by shade types (neutral or canopy), plant-material type (replicated genotype or non-replicated genotype), experiment type (garden or greenhouse), or life form (woody or non-woody) (Fig. 1, Table S1). The level of light in the shade treatment, relative to the high-light control treatment (mean: 41.5%, range: 1-85.3%) had no significant effect on the reduction in biomass (Fig. 2). Species with a greater SLA under control conditions (i.e. high light) showed a significantly smaller decrease in biomass under shade versus control conditions overall (Fig. 2 and 3). However, we found a negative relationship between SLA_{shade} – SLA_{control} and LnR of biomass (Fig. 2 and 3). In other words, the decrease in biomass under shading was significantly
greater for plant species that showed a greater plastic increase in SLA. The variance component associated with phylogenetic history was low (0.0446), indicating that the effect sizes used in the analysis were not strongly phylogenetically related.

**Figure 2** Means of parameter estimates describing the relationship between biomass responses to shading ($\ln(\text{biomass}\_\text{shade}/\text{biomass}\_\text{control})$) and SLA plasticity in response to shading (i.e. $\text{SLA}_\text{shade} - \text{SLA}_\text{control}$), SLA in the high-light control treatment ($\text{SLA}_\text{control}$), relative light intensity (percentage light in shade treatment relative to high-light control treatment) and type of experiment (garden vs glasshouse) on the changes of plant biomass in response to shading. Error bars show the bias-corrected bootstrapped 95%-confidence intervals around the parameter estimates derived from the phylogenetically corrected meta-analytic model. The dashed line indicates zero effect of the respective explanatory variable.

**Discussion**

SLA is considered to be an important functional trait that may affect light interception and leaf longevity (Wright *et al.*, 2004), and is highly plastic in response to shading (Valladares & Niinemets, 2008). Although it is known that not all phenotypic plasticity increases performance (van Kleunen & Fischer, 2005), it is still frequently implied that plasticity in SLA should help plants maintain high performance under varying light conditions (van Kleunen *et al.*, 2011b; Gratani, 2014). Surprisingly, however, we found that greater plasticity of SLA of a species in response to shading was not associated with the maintenance of plant perfor-
mance, but rather with greater reductions in plant biomass. Therefore, the results of our meta-analysis indicate that SLA plasticity to shading might not constitute adaptive plasticity.

Figure 3 Relationship between changes in plant biomass in response to shading, and (a) SLA in the high-light control treatment (SLA\textsubscript{control}: i.e. the intercept of the species’ reaction norm) and (b) the changes in SLA (i.e. the slope of the species’ reaction norm). The regression line is based on the predicted values from the phylogenetically corrected meta-analytic model. The solid line is the fitted line, and the dashed lines are 95%-confidence intervals of the fitted line.

Confirming the results of numerous previous studies on plant responses to shading (Reich \textit{et al.}, 2003; Rozendaal \textit{et al.}, 2006; Gianoli & Saldana, 2013; Feng & van Kleunen, 2014), our meta-analysis showed that most plants produced leaves with a higher SLA when shaded. This plastic response of SLA results in thinner, and relatively larger, leaves, and consequently should enhance light capture per gram of leaf tissue and thus mass-based photosynthesis. Therefore, it is frequently assumed that SLA plasticity represents adaptive shade-tolerance plasticity, maximizing plant performance in the shade (Valladares & Niinemets, 2008; van Kleunen \textit{et al.}, 2011b; Freschet \textit{et al.}, 2015). However, in contrast to support for this general assumption, we found a negative relationship between plant biomass responses to shading and SLA plasticity. In other words, our findings indicate that species that increased their SLA to a larger degree in response to shading were not more but less shade tolerant, compared to species that hardly changed their SLA.

Few other studies have tested explicitly whether shade-induced responses in SLA are adaptive. Avramov \textit{et al.}, (2006) tested the adaptive value of plasticity in SLA of plants from two
populations of *Iris pumila* grown at three light levels, and found evidence that the plastic response in SLA to light availability was in the direction of values favored by selection in one of the two populations (i.e. adaptive). Moreover, McIntyre & Strauss, (2014) investigated patterns of plasticity and selection on SLA of *Claytonia perfoliata* plants grown in an oak canopy understory and an adjacent grassland habitat, and found that *Claytonia perfoliata* exhibited plastic responses in SLA in the same direction as promoted by selection (i.e. selection for a higher SLA in a canopy habitat), suggesting that the plastic response in SLA is adaptive. These two results thus contrast with the findings of our meta-analysis. One possible explanation for the discrepancy might be that these other studies tested for the benefit of plasticity within species, while we tested for the benefit of plasticity among species. Therefore, we clearly need more studies that assess the fitness effects of SLA plasticity in response to shading within species to see whether this plasticity is generally beneficial within species.

Our findings do not just suggest that a strong plastic increase in SLA of a species in response to shading is non-adaptive, but even suggest that it is maladaptive. One possible explanation could be that SLA plasticity is genetically and developmentally linked to plasticity in shade-avoidance traits, such as petiole and internode elongation. In contrast to a shade-tolerance trait, a shade-avoidance trait should help the plants to escape from the shade conditions by overtopping the neighboring plants that impose the shade or by finding gaps in the vegetation. However, as most experiments on shade responses use artificial shading treatments from which the plants cannot escape, elongation responses are futile and might even be costly (Valladares *et al.*, 2007; Valladares & Niinemets, 2008). Another explanation for the negative association between SLA plasticity and biomass homeostasis could be that most studies measure SLA at the end of the experiment. If SLA determines light interception per gram leaf, then plants that are able to plastically adjust SLA early should be able to maintain a high biomass production. However, SLA as measured at the end of an experiment might not be driving the performance of plants but might result from it. In other words, a plant that is not very shade tolerant, and thus shows a strong decrease in biomass in response to shading, will not have the resources (e.g. photo-assimilates) to produce thick leaves with a low SLA. A low SLA might be beneficial, also under shaded conditions, if it results in a greater proportion of incident photon capture per unit leaf area. Alternatively, it could be that plants do not actively increase their SLA in response to low light but instead passively decrease their SLA in response to high light due to accumulation of non-structural carbohydrates (thus increasing dry

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mass per leaf area) when the carbohydrate production exceeds the demand in meristems. Whatever the exact reason is for the negative association between SLA plasticity and biomass homeostasis, we recommend that future studies on this topic should measure SLA not only at the end of an experiment but also early on, and that they should impose more realistic shade treatments that allow shade-avoidance responses to be effective.

While our results indicate that SLA plasticity in response to shading is not adaptive, one could argue that our results indicate that SLA plasticity is adaptive in response to an increase in light intensity. In other words, if one uses the shade environment as the reference instead of the high-light environment, the plant species that have a stronger plastic decrease in SLA in response to high light are better able to take advantage of the high light intensity in terms of biomass production (Fig. S1a). To gain more insight into the underlying cause of the relationship between biomass change and SLA plasticity, we also did a regression of biomass in high- and low-light environments separately against SLA plasticity (Fig. S1b). Plant biomass in high-light environments varied little in relation to SLA plasticity (Fig. S1b), but biomass under low-light environments decreased with increasing SLA plasticity (Fig. S1b). This indicates that species with greater SLA plasticity do not have an advantage under high-light conditions, but are disadvantaged under shade compared to less plastic species. In other words, the reduced ability of plants to produce biomass due to a lack of light in shaded environments is not compensated by increasing SLA to a greater degree, but is rather exacerbated by it.

Although SLA plasticity did not help plants to maintain a high performance when shaded, our results showed that species with greater SLA under high-light control conditions have a significantly smaller decrease in biomass when shaded. So, while plasticity in SLA did not increase biomass homeostasis high SLA values did. Generally, shade intolerant species have a higher light compensation points and light-saturated photosynthetic rates (Givnish, 1988; Kitajima, 1994; Valladares & Niinemets, 2008), thus plants with high SLA values would be more shade tolerant. This finding supports the carbon-gain hypothesis, which states that any trait related to light-use-efficiency that improves carbon gain in plants will increase performance under shade (Givnish, 1988; Valladares & Niinemets, 2008). Our finding is also in line with the many studies that found that species with a greater SLA are more shade tolerant (e.g. Sánchez-Gómez et al., 2006; Janse-Ten Klooster et al., 2007; Gianoli & Saldana, 2013). Although the relationship between the biomass response and SLA_{control} in our meta-analysis was shallow, it raises the question why not all species have evolved greater SLA. Most likely,
this is because some species do not encounter much shading in nature and other selective forces, such as herbivory and drought stress, and environments favoring leaf-longevity (Fig. S2), have resulted in the evolution of species with low SLA. Additionally, while plants with lower SLA are less efficient in terms of metabolic cost per unit leaf area, they might capture a greater proportion of incident photons. When the increased photon capture more than offsets the increased metabolic cost of a lower SLA, the lower SLA should be favored.

As species that naturally occur in shaded habitats are presumably more shade tolerant, it could be that the positive relationship between the change in biomass and SLA arose because species from shade habitats have higher SLA values than species from non-shade habitats. As information on the natural habitats is not available for most of the study species, we could not account for this in the main analysis. However, for 136 of the 280 study species, we had data on their Ellenberg light-indicator values (Ellenberg, 1974), which indicate the light conditions in the natural habitat of the species in Europe. Although this subset of species did not contain species from deep-shade habitats, we did not find evidence that species with different light-indicator values differed in SLA under high-light and under shaded conditions (Fig. S3). Therefore, it is unlikely that our result of a higher biomass homeostasis for species with higher SLA values is confounded by species from shade habitats having higher SLA values.

Surprisingly, our results showed that relative light intensity had no significant effect on the reduction in biomass (Fig. 2). This runs counter to the results of many experiments, where biomass typically declines more or less continuously with declining light levels (e.g. Feng & van Kleunen, 2014; Kumar et al., 2014; Konvalinková et al., 2015). A likely explanation for this apparent discrepancy is that most species in our meta-analysis were not grown under more than two experimental light conditions, and that the light conditions varied among studies. Seventy of the 280 species were grown under more than two light levels, and a post-hoc analysis for this subset of species showed that within species, biomass declines more or less continuously with declining light levels (Fig. S4). However, if we run the full meta-analytical model for this subset of 70 species, the effect of relative light intensity was still not significant and also the other results remained qualitatively the same (Fig. S5). So, while within each species relative light intensity is important for the change in biomass, among species it plays no significant role.
Conclusions

In summary, our meta-analysis suggests that plasticity in the ability of plants to capture more light per gram of leaf mass invested under low-light conditions by increasing SLA does not contribute to shade tolerance of plant species in terms of biomass homeostasis, and thus does not constitute adaptive phenotypic plasticity. This is despite wide consensus that plasticity in SLA and other traits associated with shade avoidance and tolerance, such as leaf length, leaf area, shoot-root ratio, chlorophyll content and photosynthesis, can be adaptive (Dudley & Schmitt, 1996; Schmitt et al., 1999; van Kleunen & Fischer, 2005; Valladares & Niinemets, 2008; van Kleunen et al., 2011b). We argue that some of the plastic responses of plant species to shade that are frequently thought to be adaptive might simply reflect passive responses to the environment, or represent by-products of adaptive plastic responses in other traits. In order to further understand the mechanism of plant shade tolerance, we therefore strongly recommend that future studies should explicitly test whether the plasticity of a trait is adaptive or not.

Acknowledgements

We are very grateful to Dr. Judy Simon who kindly provided data. We also thank to Dr. Liam R. Dougherty and Dr. Dylan Craven for their help in doing multivariate meta-analysis in R. YJL is funded by a scholarship from the China Scholarship Council. We thank editors and two anonymous referees for the valuable comments and suggestions on a previous version of the manuscript.
Supplementary materials

Methods S1 List of published studies from which data on plastic changes in biomass and SLA in response to shading were extracted.


Methods S2 Phylogenetic tree used in this study and list of published studies used for resolving the polytomies within the initial base tree


Table S1 Mean effect size estimates from phylogenetically corrected meta-analytic models performed separately for each factor.

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<td>-1.20264</td>
<td>-1.49</td>
</tr>
<tr>
<td>Lifeform</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-woody</td>
<td>261</td>
<td>-0.91864</td>
<td>-1.219</td>
</tr>
<tr>
<td>Woody</td>
<td>206</td>
<td>-0.91246</td>
<td>-1.0893</td>
</tr>
</tbody>
</table>

Mean effect size estimates, 95%-confidence intervals (CIs), Q_M and P values were calculated using phylogenetically corrected meta-analytic models. CIs for estimates were calculated by bootstrapping 1000 times. P values were calculated by permuting 1000 iterations using a randomization test.
Figure S1. Relationship between plasticity in SLA and the changes in plant biomass when going from shade to high-light control environments (a), and plant biomass under different light conditions (b). The solid line is the fitted line, and the dashed lines are 95%-confidence intervals of the fitted line. The significance of the regression lines in b are indicated in parentheses: not significant (ns) and significant at $p < 0.01$ (*).
**Figure S2.** Means (± SE) of specific leaf area (SLA) in the light-light control treatments as estimated from a linear mixed-effects model for evergreen woody, deciduous woody, and non-woody (herbaceous) species. Different letters above the bars indicate that the means differ significantly between lifeforms at \( \alpha = 0.05 \).
Figure S3. Boxplots of SLA values and biomass response to shading of species of different shade intolerance classes. (a) shows the SLA values of species grown under high-light conditions (SLA\textsubscript{control}), (b) shows the SLA values of species grown under shade conditions (SLA\textsubscript{shade}), and (c) shows the biomass response to shading of species of different shade intolerance classes. The shade intolerance classes are the Ellenberg light-indicator values of each species for Central Europe, obtained from FloraWeb (http://www.floraweb.de/). In our dataset, the indicator values ranged from 6 to 9. Species with a value of 6 naturally occur in semi-shade to semi-light conditions, species with a value of 7 occur in half-light conditions, species with a value of 8 occur in half-light to full-light conditions, and species with a value of 9 occur in full-light conditions. There were no differences between high-light adapted species and shade-adapted species for the mean SLA values under control ($F_{3,161} = 0.792, P = 0.500$) and shaded conditions ($F_{3,161} = 0.816, P = 0.487$). There was also no differences between high-light adapted species and shade-adapted species for the biomass response to shading ($F_{3,161} = 2.191, P = 0.091$). This result was obtained from an analysis using the subset of data that only included the 113 species for which we have information on light conditions in their natural habitats.
Figure S4. The relationship between plant biomass reduction ($\ln(\text{biomass}_{\text{shade}}/\text{biomass}_{\text{control}})$) and relative light intensity for 70 species that were grown under more than two light conditions. A relative light intensity of 0 indicates no light, and a value of 1 indicates full light.
Figure S5. Means of parameter estimates describing the relationship between biomass responses to shading (ln(biomass_{shade}/biomass_{control})) and SLA plasticity in response to shading (i.e. SLA_{shade} – SLA_{control}), SLA in the high-light control treatment (SLA_{control}), relative light intensity (percentage light in shade treatment relative to high-light control treatment) and type of experiment (garden vs glasshouse) on the changes of plant biomass in response to shading. Error bars show the bias-corrected bootstrapped 95%-confidence intervals around the parameter estimates derived from the phylogenetically corrected meta-analytic model. The dashed line indicates zero effect of the respective explanatory variable. This result was obtained from the analysis using a subset of data, which only included the 70 species growing under more than two light conditions.
General discussion

Phenotypic plasticity is assumed to be an important mechanism that allows species to tolerate environmental change. Nevertheless, we still have a limited understanding of how phenotypic plasticity varies among species, and whether it is related to their success in nature under current and future conditions (van Kleunen & Fischer, 2005). Additionally, many studies on phenotypic plasticity and plant success assume that the plasticity measured in their studies constitutes adaptive plasticity, particularly so for functional traits associated with nutrient uptake and light capture. However, for most of them, the adaptive value has rarely been tested (van Kleunen & Fischer, 2005; van Kleunen et al., 2011b). To gain more insights, the four studies described in my thesis aimed to assess how plant species respond to environmental change, and whether plastic responses are related to the success of those species.

Plant invasion under changes in mean environmental conditions

In a meta-analysis, I assessed how invasive alien and native plant species respond to other global environmental change factors (Chapter 1). I found that invasive alien plants showed stronger positive responses to elevated temperature and CO$_2$ enrichment than native plants. Additionally, invasive alien plants tended to have a slightly stronger positive response to increased N deposition and increased precipitation than native plants, although these differences were not significant. Invasive alien plants tended to have a slightly stronger negative response to decreased precipitation than native plants, although this difference was also not significant. Combined with the previous findings that invasive plants species usually have a significantly higher performance than native plants under current environment conditions (van Kleunen et al., 2010b), my findings suggest that while drought could potentially reduce invasion, all other global environmental change factors considered, particularly global warming and atmospheric CO$_2$ enrichment, may promote further spread of invasive plants in the future.

Many case studies have assessed how invasive and native plant species respond to changes in precipitation, temperature, atmospheric CO$_2$ concentration, and N deposition; however, such studies usually involve small numbers of species, and have produced mixed results (Dukes & Mooney, 1999; Bradley et al., 2010a). In the 1$^{st}$ chapter, I compared plastic responses in
performance traits for 74 invasive alien plant species and 117 native plant species. The findings showed a general pattern whereby invasive plant species have stronger response to environment changes than native plant species. Such increased performance could confer them greater advantage over native plant species when the environment becomes more favourable in the future. A previous meta-analysis by Sorte et al., (2013), which assessed the responses of naturalized alien and native organisms (both plant and animal species) to climate change. These authors found that naturalized alien species exhibited stronger responses than natives species in aquatic systems (primarily data on animals), but not in terrestrial systems (primarily data on plants, but also some animal data). I restricted the analysis to invasive alien and native plant species and increased the number of studies included. My meta-analysis provides robust evidence that invasive plant species exhibit stronger responses than native plant species, and thus that plant invasion might be promoted by global environmental change.

Although invasive plant species showed stronger responses to individual factors of environmental change than native plant species in the 1st Chapter reported in my thesis, it remains unclear whether such differences would hold under conditions where multiple environmental change factors interact. Different global environmental change factors could occur simultaneously in nature. For example, elevated atmospheric CO$_2$ could also increase the temperature (IPCC, 2013), and increasing precipitation might also increase the N deposition (IPCC, 2013). The interactive effects between different global environmental change factors could be simply additive, synergistic or antagonistic (Dukes et al., 2005; Bloor et al., 2010; Dieleman et al., 2012; Yue et al., 2017). If the interactive effects between different global environmental change factors are simply additive (i.e. equal to the sum of their individual effects), one would expect that invasive plant species respond more positively than native plant species (Fig. 1a and 1b) when the effects of all individual effects are positive or when the positive individual effects are much larger than the sum of negative effects. However, the joint effect could also be neutral (Fig. 1c) or negative (Fig. 1d) otherwise. The interactive effects are complex when the effects are not simply additive, as synergistic and antagonistic effects are even more difficult to predict. Therefore, it remains also largely unexplored whether the observed differences in plastic response patterns between invasive and native plant species would hold under conditions where multiple environmental change factors interact.
Figure 1 A conceptual diagram illustrating how one plant species responds to two simultaneously individual environmental factors if their interactive effect is only additive. When individual effects of both factors are positive, the interactive effect would also be positive (a). When one individual effect is positive and the other is negative, the interactive effect depends on the intensity of each individual effect. If the positive effect is stronger than the negative effect, the interactive effect would be positive (b); if they are equal, the interactive effect would be neutral (c); and if the negative effect is stronger than the positive effect, the interactive effect would be negative (d).

In the 1st chapter of my thesis, I only focused on comparing the plastic response between invasive alien and native plant species on the individual-level. However, plant invasion is not only a individual-levels process, but als a community-level process. Plastic responses of plants to environmental changes may also depend on the community context. Individual plants could respond (physiologically, biochemically, and morphologically) to environmental changes directly, and indirectly through modified interactions with other members of the community (Adler & HilleRisLambers, 2008; Feng & van Kleunen, 2016). Indeed, a species that responds strongly to an altered environmental factor when grown individually or in monoculture may respond quite differently when grown in competition with other plants (Bazzaz & Mcconnaughay, 1992). For instance, a meta-analysis by Poorter & Navas, (2003) found that fast-growing pioneer species had a greater response to CO₂ enrichment than slow-growing climax species when grown alone, whereas no difference was found when grown in
competition. Moreover, invasive and native plant species may be affected by soil biota (Callaway et al., 2004; Reinhart & Callaway, 2006), herbivory (Inderjit, 2012; Cronin et al., 2015) and pollinators (Vanparys et al., 2008; Vilà et al., 2009; Stout & Tiedeken, 2017). Thus, environmental changes could also indirectly affect the plastic response of invasive alien and native plant species through an direct influence on any member of other trophic levels. Unfortunately, my meta-analysis did not include these abovementioned factors because of the limited number of publication related to them. Thus, community-based experiments are needed in further studies to predict whether invasive plant species will benefit more than native plants from environmental change.

**Plant invasion under nutrient fluctuation**

Most of the publications included in the meta-analysis (Chapter 1) focused on plant responses to environmental changes under mean conditions. However, fluctuations around a mean environmental condition over time might also affect plant responses and the competition balance between plants (Davis et al., 2000). Therefore, I focused the two greenhouse experiments on the variability in environmental changes. In the first greenhouse experiment, I grew common and rare alien, and native plant species under six different nutrient treatments without competition (Chapter 2). I found that the plastic responses of biomass production, root morphology, and root allocation to nutrient changes under mean conditions did not differ between alien plant species and native plant species. This suggests that plant plasticity of the studied traits in response to nutrient changes in mean conditions is similar for native and alien species. However, I found that, compared to a constant high nutrient supply, alien plant species showed positive plastic responses to large nutrient pulses, whereas native plant species showed negative plastic responses, possibly as a consequence of differences in plasticity of root traits. No differences were found in the plastic responses to nutrient addition between invasive and non-invasive alien plant species, possibly, because plants were grown in the absence of competition. In the second greenhouse experiment (Chapter 3), ten pairs of invasive and non-invasive alien plant species were grown under the same nutrient-supply treatments used in the first greenhouse experiment, but this time in the presence of native competitors. I found that invasive plant species exhibited a significantly higher plastic response in biomass production (i.e. benefited more) to high nutrient levels than non-invasive plant species, which is inconsistent with the first greenhouse experiment. However, I also found that both groups of alien target species benefited proportionally less from the nutrient
addition overall than the native competitors. Surprisingly, the alien species, and particularly the invasive ones, suffered from nutrient pulses. These findings do not support the fluctuating-resource-availability hypothesis, which poses that communities become more susceptible to invasion when the availability of resources increases (Davis et al., 2000).

Alien plant species introduced from more nitrogen-rich habitats are often pre-adapted to become invasive (Dostál et al., 2013). This is consistent with the fact that nutrient-rich habitats often experience more invasion than resource-poor habitats (Burke & Grime, 1996; Gross et al., 2005; Seabloom et al., 2015). Therefore, invasive alien plant species might show an increased ability to rapidly take advantage of available nutrients in nature, and thus grow faster and be more successful than non-invasive alien plant species (Richards et al., 2006; Davidson et al., 2011). Although invasive and non-invasive alien plant species might differ in their ability to take up nutrients rapidly, both of them may simply have taken up the total amount of nutrients in the absence of native competitors in the first greenhouse experiment. As a consequence, the changes in biomass production were similar for invasive and non-invasive alien plant species in Chapter 2. Due to the presence of the competition in the second greenhouse experiment (Chapter 3), invasive plant species that might have a higher ability to take up nutrients may grow faster and respond more strongly than non-invasive alien plant species that might have a lower ability to take up nutrients. This could be tested by directly comparing the differences in nutrient-uptake rates between invasive and non-invasive alien plant species. However, to my knowledge, no study has investigated this yet. Therefore, tests are needed to determine the differences in nutrient-uptake rates between invasive and non-invasive alien plant species.

Although a few case studies that tested the fluctuating-resource-availability hypothesis found support for the hypothesis that resource variability could promote plant invasion (Davis & Pelsor, 2001; Parepa et al., 2013), the results of my multi-species experiment suggest that it is not a general phenomenon for alien plant species. Nutrient competition is a key process regulating the dynamics of plant communities (Grime, 1973; 1977; Tilman, 1982; 1988), and is also the major mechanism behind the fluctuating-resource-availability hypothesis (Davis et al., 2000). In a community, only the plant species with a superior competitive ability can suppress other species and thus become dominant (Gioria & Osborne, 2014). Even though several studies have showed that invasive plant species possess a superior capacity to acquire nutrients over native plant species (Daehler, 2003; Leishman & Thomson, 2005; Funk &
Vitousek, 2007; van Kleunen et al., 2010b; Matzek, 2012), alien plant species are not necessarily competitively superior to native plant species. Indeed, successful wide-spread and dominant alien plant species might not be better competitors than wide-spread and dominant native plant species (Seabloom et al., 2003; van Kleunen et al., 2010b; Gioria & Osborne, 2014). Therefore, the fluctuating-resource-availability hypothesis might hold true for the plant species that have a superior competitive ability, irrespective of whether they are an alien or a native plant species. More studies in the future should investigate whether the positive effects of increased resources variability on competitive dominance is a general phenomenon.

**Adaptive phenotypic plasticity**

Despite the wide consensus that plasticity of traits associated with nutrient-uptake strategies and shade tolerance can be adaptive, few studies have assessed this explicitly. Recently, Keser et al., (2015) directly tested the relationship between plasticity of root traits and performance for 24 herbaceous native to Europe but alien to USA, and found that the plants with higher plasticity in root traits had a higher performance both within and among species. This multispecies experiment shows that plasticity of root traits, which is associated with nutrient uptake, are adaptive, and that such plasticity contributed invasiveness (i.e. success) of those species in the USA. Therefore, I also measured the plasticity of root traits in my first greenhouse study (Chapter 2) to test whether this plasticity contribute to plant success in response to fluctuating nutrients. I found some evidence that plasticity in root allocation to better alien plant species might capitalize on large nutrient pulses, in contrast to native plant species. Unfortunately, because of the small sample sizes for each of the species in that study, this conclusion was drawn by comparing the plasticity in root allocation and variation in performance between the two groups of species, rather than by investigating the link between the plasticity in root allocation and performance (i.e. testing for adaptive value of plasticity as done by Keser et al., 2015).

Only a few case studies have tested explicitly whether plasticity of traits associated with shade tolerance is adaptive (Steinger et al., 2003; Avramov et al., 2006; Sánchez-Gómez et al., 2006; McIntyre & Strauss, 2014). Such case studies suggest that SLA plasticity in response to shading is adaptive within a species. However, it remains unclear whether this is also the case among species. As higher SLA could help plants to increase the efficiency of light capture and maximize carbon gain (Evans & Poorter, 2001; Gomers et al., 2013), it is
frequently assumed that the SLA increase from high-light conditions to low-light conditions is adaptive, and relates to plant success across heterogeneous light environments among species (van Kleunen et al., 2011b; Feng & van Kleunen, 2014). Therefore, in my second meta-analysis (Chapter 4), I used a database to test whether the assumption that the SLA plasticity in response to shading is adaptive. I found that the higher ability of plants to capture light by increasing SLA under low-light conditions was not associated with the maintenance of biomass homeostasis in plant species, but rather with greater reduction in biomass of plant species. In other words, the plasticity of SLA to shading among species might not constitute adaptive plasticity. Therefore, I argue that some of the plastic responses of plant species to environmental changes, which are frequently thought to be adaptive, might simply reflect passive responses to the environment, or may reflect responses induced by the adaptive plasticity of other traits.

**Conclusion**

Taken together, the different chapters of this thesis provide insights into the importance of phenotypic plasticity for plant success under environmental change. The results presented in my thesis suggest that the higher plasticity of invasive alien plant species than native plant species in response to favourable environmental changes, particularly global warming and atmospheric CO₂ enrichment, may promote spread of invasive plants in the future. Moreover, invasive plant species showed more positive responses to nutrient addition than non-invasive alien plant species when competing with native plant species. Therefore, my findings suggest that higher plasticity to nutrient addition might lead to success for some alien plants. In contrast, my findings also suggest that the promotion of plant invasion by nutrient fluctuations in resident communities is not a general phenomenon. My findings also suggest that some plastic responses for which there is wide consensus that they are adaptive might not really be adaptive. In other words, these plastic responses might not help plants to maintain a high performance across different environment conditions, as shown for SLA plasticity in response to shading. To sum up, my thesis found that phenotypic plasticity could be linked to a certain extent to plant success under environmental change in space and time.
Author contributions


Chapter 2 Y.L. conceived the experiment. Y.L. and M.v.K designed the experiment. Y.L. performed the experiment and collected the data. Y.L. and M.v.K performed data analyses and wrote the manuscript.

Chapter 3 Y.L. conceived the experiment. Y.L. and M.v.K designed the experiment. X.Z. and Y.L. performed the experiment and collected the data. X.Z., Y.L. and M.v.K performed data analyses and wrote the manuscript.

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Acknowledgements

This thesis would have never been possible without the help of many colleagues, friends and my family. I would like to thank all the people who have helped me during my PhD study.

I would like to thank my supervisor, Dr. Mark van Kleunen, for his excellent guidance, support, encouragement and patience. I also thank my second supervisor Dr. Karl-Otto Rothhaupt for his valuable discussions in our regular meetings to improve my projects.

I would like to thank all my colleagues of the Ecology group at the University of Konstanz for a pleasant working atmosphere, patience, encouragement, discussion and friendships. It is my lucky working in such a nice group.

I would like to thank Dr. Wayne Dawson, Dr. Daniel Prati, Emily Haeuse, Dr. Yanhao Feng, Dr. Ayub M. O. Oduor, Dr. Zhen Zhang, Dr. Anthony Manea, Ifeanna M. Tooth, Dr. Michelle R. Leishman, Dr. Xingliang Xu and Xiaoqi Zhang, Dr. Zdravko Baruch Glaser, Dr Judy Simon, Dr. Liam R. Dougherty and Dr Dylan Craven. Without your contribution and data sharing, I cannot finish my work during four years.

Also, I would like to thank Dr. Noëlie Maurel for reading and commenting on parts of a previous draft of this thesis, and Christa Gommel for her help in the translation of the German summary of this thesis. Although there were too many people to list their names here, I would also like to thank all student helpers and gardeners for help during my PhD study.

I would like to thank the China Scholarship Council (CSC) for the financial support my four-years PhD study in Germany. Also, I would like to thank Dr. Haishan Niu. Without your help eight years ago, I would not have been doing Ecological research.

Finally, a very special “thanks” to my wife for her support and love, and also to my son for calling me “BaBa” with smile.

Liu Yanjie

May 2017

Konstanz, Germany