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Nitrogen acquisition of Central European herbaceous plants that differ in their global naturalization success

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Abstract

1. It is frequently assumed that species capable of fast nitrogen (N) acquisition under different N-availability conditions should have a higher establishment success after their introduction into new regions. However, few experimental studies have explicitly tested this. Our multispecies experiment tested whether global naturalization success of plant species native to Central Europe is related to a high N-acquisition ability.
2. We selected 41 common herbaceous species native to Germany that have all become naturalized, and thus been introduced, elsewhere. Twenty-two of these species are widely naturalized and 19 are less widely naturalized. We grew the 41 grassland species, sampled in Germany, under low and high N-conditions in a greenhouse experiment, and assessed their N-acquisition abilities.
3. Although the widely naturalized species grew faster on average, they had a significantly lower N-uptake rate than the less widely naturalized ones. The widely naturalized species, however, had a marginally significantly higher root-mass fraction. Despite these differences, the total plant N-content did on average not differ between the two groups of species. However, N-addition tended to increase the total plant N-content more for the widely naturalized species than for the less widely naturalized species. Nitroge addition also increased biomass production and N-uptake rate, and decreased the root-mass fraction of plants, but these responses did not differ between widely and less widely naturalized species.
4. We conclude that although fast-growing species tend to have a higher global naturalization success than slow-growing species, the naturalization success of plants is not necessarily related to a high N-acquisition ability.

KEYWORDS
biological invasion, exotic plant, invasiveness, non-indigenous, plant–environment interactions, species distribution

1 INTRODUCTION

Human activities have resulted in the introduction of many plant species to new regions, where they are not native (van Kleunen, Essl et al., 2018). More than 13,000 of these alien plants have been able to establish self-sustaining wild populations (i.e. have become naturalized; van Kleunen et al., 2015). Some of these naturalized plants spread rapidly (i.e. become invasive; Richardson et al., 2000) and can cause ecological problems (Vilà et al., 2011). Furthermore, naturalization and invasion of alien plants are likely to continue due to increased global trade (Seebens et al., 2015) and ongoing global environmental changes (Jia, Dai, Li, & Liu, 2016; Liu, Oduor et al., 2017). As a consequence, the impacts of plant invasions on ecosystems will further increase in the future. Therefore, assessing what determines the naturalization success of alien plant species is imperative to prevent further invasions.

Comparative studies are frequently used to test for potential determinants of invasiveness (van Kleunen, Dawson, Schlaepfer, Jeschke, & Fischer, 2010). Many studies compared invasive alien and native species to determine whether invasive alien species take advantage over native species due to specific traits or ecological and evolutionary processes (Funk, 2008; Leishman, Haslehurst, Ares, & Baruch, 2007; Strauss, Webb, & Salamin, 2006). In such studies, native species are frequently used as a control group. However, at a global scale, some of these native "control" species might also be introduced and naturalized outside their native range and might even be considered to be invasive there (van Kleunen et al., 2010). IN-addition, some of these native species are also widely distributed within their native range. Thus, if studies compare successful alien species with such successful native species, they might not find any difference (Godoy, Valladares, & Castro-Díez, 2011; van Kleunen et al., 2010). Therefore, the alien–native comparison cannot directly answer why some alien species naturalize and become invasive and other alien species do not. Instead, we require studies that compare species that differ in their invasion success.

Most comparative experimental studies on alien species that differ in invasion success are performed in a specific introduced range of those species (but see Schlaepfer, Glattli, Fischer, & van Kleunen, 2010; van Kleunen, Schlaepfer, Glaettli, & Fischer, 2011; Keser et al., 2015). Such target-area comparisons can be complicated by the fact that the successful and less successful alien species might have different source regions with, for example different climates that might confound the analyses. An alternative, would be to use a source-area approach, that is to use species that are native to the same region but differ in their naturalization or invasion success elsewhere (Pyšek, Richardson, & Williamson, 2004). Such a home-range comparison can reveal whether species have traits that pre-adapt them to becoming invasive elsewhere (i.e. that pre-adapt them to conditions in the new habitats; van Kleunen, Dawson et al., 2010). Studies that use this source-area approach should ideally use species from similar habitats to avoid trait biases, and species that are equally common (or rare) in their native range to make it more likely that they all had equal chances of being picked up and introduced elsewhere.

As nutrient limitation is common across varied ecosystems, the plants' ability to acquire nitrogen (N) might explain the invasion success of alien species. For example, in a nutrient addition experiment in 64 grasslands world-wide, Seabloom et al. (2015) found that nutrient addition increases the cover and richness of exotic species, while decreasing native cover and diversity. Widespread alien plant species are also frequently suggested to have higher phenotypic plasticity (Richards, Bossdorf, Muth, Gurevitch, & Pigliucci, 2006) and to be better able to capitalize on increased nutrient availability than less widespread alien species (Dawson, Fischer, & van Kleunen, 2012; Dawson, Rohr, van Kleunen, & Fischer, 2012). These findings support the fluctuating-resources hypothesis, which suggests that alien species could establish more easily in a community if the nutrient availability is increased (Davis, Grime, & Thompson, 2000). It indicates that alien species with a higher ability to take up nutrients or a higher plasticity therein may be more successful during the invasion process than those that are less able to do so.

The nutrient-acquisition ability of plants is determined by both morphological and physiological traits, and biomass allocation to roots and nutrient-uptake rates are two important ones among them (Casper & Jackson, 1997). Generally, plants with more roots and higher nutrient-uptake rates would exhibit a higher ability to acquire nutrients (Casper & Jackson, 1997). As a different nutrient-acquisition ability could affect plant–plant interactions, it is likely to determine the naturalization success of alien species into native communities. Among the few studies that addressed this by comparing alien species differing in naturalization or invasion success, all of them assessed the plants' nutrient-acquisition ability by comparing morphological traits related to the ability of absorbing nutrients, such as adaptive root-foraging responses (Keseret al., 2014, 2015), root allocation and biomass accumulation (Dawson, Rohr et al., 2012; Liu & van Kleunen, 2017). However, no study has so far directly tested whether the nutrient-uptake rates differ between alien species with different levels of invasion success.

Here, we grew 41 herbaceous grassland species under low and high N-conditions. All species are native and common (widespread) in Germany and naturalized elsewhere in the world. Twenty-two of these species are widely naturalized across the world (i.e. have a high realized invasion success), whereas the other 19 are less widely naturalized. Using the seeds from their native range (i.e. Germany), and comparing their biomass production, root allocation, N-uptake rate and the total N-content of plants when growing under the two different N-conditions, we tested whether a high ability of N-acquisition in their native range can explain the global naturalization success of alien plant species. More specifically, we addressed the following main questions: (a) Do widely naturalized species produce more biomass, allocate more biomass to roots and exhibit a higher N-uptake rate and total plant N-content than less widely naturalized species? and (b) Does a high N-acquisition ability and plasticity therein help increase the performance of widely naturalized species more than that of less widely naturalized species in response to N-addition?

2 MATERIALS AND METHODS

2.1 Study species

To test for differences in N-acquisition ability between widely naturalized and less widely naturalized plants, we initially selected a total of 45 terrestrial herbs from eleven families. All of them are native to Germany, but have become naturalized elsewhere in the world. Because species that are common in their native range are also more likely to have been widely introduced to new ranges elsewhere (van Kleunen, Johnson, & Fischer, 2007), we only selected species that are very common in grasslands of Germany (occur in >1,600 of 3,000 grid cells in Germany; Supporting Information Table S1). As species that naturally occur in N-rich habitats are likely to have a higher invasion success (Dostal, Dawson, van Kleunen, Keser, & Fischer, 2013), we selected the widely and less widely naturalized species to have similar Ellenberg N-indicator values (Kruskal–Wallis test, $\chi^2 = 0.904$, $df = 1$, $p = 0.342$), which indicate the N-conditions in the natural habitat of the species in Europe (Ellenberg, 1974). To determine how widely the species are naturalized outside of Germany, we used the Global Naturalized Alien Flora (GloNAF) database (version 1.2; https://glonaf.org/; van Kleunen, Pyšek et al., 2018), which includes data on naturalization success of 13,939 alien plant species in a total of 1,029 regions. Although the classification of species as being widely naturalized or less widely naturalized is subjective, we a priori selected species to be widely naturalized if they have been recorded in >100 GloNAF regions (median = 180, range = 108–364) and to be less widely naturalized if they have been recorded in <50 GloNAF regions (median = 10, range = 1–31; Supporting Information Table S1). So, we did not select any species in between the two cut-offs. To avoid confounding of naturalization success with taxonomy, we aimed to include at least one widely naturalized and one less widely naturalized species per family. However, because of insufficient germination of some species, our final species set only included 41 (22 widely naturalized and 19 less widely naturalized) of the 45 selected species and was not fully balanced with regard to taxonomy. Eight families were represented by at least one species from the widely naturalized and at least one species from the less widely naturalized group, and three families lacked representatives of one group. The majority of study species are polycarpic (perennial) herbs, but both the widely and less widely naturalized alien species also contained some species that are monocarpic (annual, biennial) or have a mixed strategy (Supporting Information Table S1). The seeds of all but two study species, which were collected in natural populations, were acquired from a commercial seed company that produces these seeds for grassland-restoration and agricultural purposes (Supporting Information Table S1).

2.2 Experimental design

To test for differences in performance (biomass production), biomass allocation (root-mass fraction) and physiological traits (N-uptake rate and total plant N-content) between the widely naturalized and less widely naturalized plants, we did a multispecies greenhouse experiment in the botanical garden of the University of Konstanz (Germany). As we were interested in the differences between the groups of widely naturalized and less widely naturalized species, rather than in differences among the individual species, we maximized the number of species over the number of replicates per species. As some species were known, from previous experiments, to germinate later than others, we sowed the species on different dates (Supporting Information Table S1) to ensure that the seedlings were in a similar developmental stage at the start of the experiment. From 4 March to 22 April 2016, we sowed the seeds of each species separately into trays (12.0 × 12.0 × 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 P_2O_6; 420 mg/L K_2O; 200 mg/L S; 700 mg/L Mg). We kept the trays with seeds in a greenhouse at a temperature between 22 and 28°C, and a day:night cycle of 16:8 hr.

We filled a total of 820 circular 2.5-L plastic pots with a 1:1 mixture of sand and fine vermiculite. On 2 May 2016, we selected 20 similar-sized seedlings per species and transplanted each of them into the centre of each pot (i.e. one individual per pot). Then, we randomly assigned them to positions on eight greenhouse benches and watered to saturation. As we needed a pair of plants for two separate harvests to calculate the N-uptake rate (see the section Measurements for more details), we put two pots of each species next to each other and considered them as pair-units for position randomization and harvests during the experiment. In total, we had ten pair-units for each species. For each of the ten pair-units, we randomly chose one for each of the two harvests. To avoid that the nutrients that leaked from the pots after watering would be lost to the plant, we put a plastic dish underneath each pot. We kept all pots at a temperature between 22 and 28°C, and we used supplemental lighting to supply them with 14 hr of light each day.

To test whether responses to N-addition differ between widely naturalized and less widely naturalized species, we started to apply a low and a high N treatment from the second week after transplanting the seedlings. Five of the ten pair-units of each species (i.e. ten plants per species) were grown under a low soil N-condition, whereas the others were grown under a high soil N-condition. We manipulated the low and high N-conditions using two different modified Hoagland solutions (see Supporting Information Methods S1), which were applied at weekly intervals for a total of nine weeks. The two nutrient solutions only differed in the N-content; that is they contained the same amounts of the other nutrients. Based on the results of a pilot experiment (Supporting Information Methods S2), we supplied 50 ml of each of the nutrient solutions per week, so that 0.3 and 3 mmol of N were provided each time for the low and high N treatment, respectively. As it is difficult to compare the absolute nutrient levels in a pot experiment, where the soil volume is restricted, to those found in the field, we aimed to provide N-conditions that were strongly growth limiting and N-conditions that were not strongly growth limiting. The low and high N levels were therefore chosen based on a pilot experiment (Supporting Information Methods S2) and previous studies (Liu & van Kleunen, 2017; Liu, Zhang, & van Kleunen, 2018). In the seventh week after transplanting, we harvested one plant from each of the pair-units. Twenty-six days after the first harvest, we harvested the remaining plants. So, at each of the two harvests, we harvested five individuals per species for each of the two nutrient treatments. During the week of the first harvest, we did not apply the different N manipulations, but we randomly reassigned the remaining pots to positions on four greenhouse benches. Between the two harvests, we fertilized the remaining plants three times. To ensure that water supply was not limiting plant growth, we checked all the pots every day during the whole experiment, and we filled the dishes underneath each pot with water when the soil looked dry.

2.3 Measurements

To be able to account for variation in initial sizes of the plants in the analyses, we counted the number of true leaves (i.e. excluding the cotyledons) at the start of the experiment and measured the length and width of the longest leaf on each plant and the height of each plant. Based on the initial size measurements, we calculated as proxy of initial leaf area the length × width of the largest leaf × the number of true leaves. On 15 June 2016 and 11 July 2016, we harvested the first and second sets of plants, respectively. For both harvests, we first harvested the above-ground biomass on the first day and then started to harvest the below-ground biomass (i.e. washing all the roots clean of substrate). As the whole below-ground biomass harvest took two and four days, respectively, for the first and second harvest, we stored all pots in a cold room at 4°C in order to stop growth and decay of the plants and thus to avoid introducing a potential bias due to different root-harvesting times. All above-ground and below-ground biomass was dried for at least 72 hr at 70°C and weighed. As some plants had died during the experiment, we only harvested 386 and 404 plants instead of 410 plants at the two harvests, respectively. We calculated root-mass fraction as the ratio between below-ground and total biomass. As the plants were only 2.5 months old at the last harvest, they had not produced any major storage roots yet (Y. Liu, personal observation).

To calculate the N-uptake rate between the two harvests, we basically followed the approach of Moreau et al. (2015). We first measured the N-content of the plants. For this, we mixed and milled the above-ground and below-ground biomass of each individual plant using a cutting mill (SM100; Retsch, Haan, Germany) and then ground a random subsample of each plant into a fine powder using a ball mill (MM 300; Retsch, Haan, Germany). After that, aliquots of c. 2.5 mg of powdered material per plant were weighed into tin capsules (HEKAtech GmbH, Wegberg, Germany), and then, the N-content (i.e. N-weight proportion of each plant) was measured using a CHNSO combustion analyser (Euro EA; HEKAtech GmbH, Wegberg, Germany). Based on the total plant N-content (i.e. total N-weight of each plant) and plant root biomass of the two harvests, the N-uptake rate can be defined as the rate of increase in the whole plant N-content per unit of root biomass (RB) per day (*t*). We calculated the mean N-uptake rate for the time interval between the two harvests (t_2–t_1; i.e. 26 days) as.

$$\text{N- uptake rate} = \frac{(\mathrm{N}_2 - \mathrm{N}_1)(\ln \mathrm{RB}_2 - \ln \mathrm{RB}_1)}{(t_2 - t_1)(\mathrm{RB}_2 - \mathrm{RB}_1)}.$$

Here, N_1 and N_2 are the total plant N-content of the first and second harvest, respectively. RB_1 and RB_2 are the root biomass of the plants of the first and second harvest, respectively. This equation is adapted from Vernon and Allison (1963)'s equation for the net assimilation rate. N-uptake rate can also be calculated using another equation given by Moreau et al. (2015). In their approach, the root biomass is assumed to have a constant daily increase, whereas our approach assumes that the relative rate of root growth is not constant but exponential, which we think is more realistic (Caloin & Yu, 1982). However, we also calculated the N-uptake rate for all plants using the approach of Moreau et al. (2015). The values calculated by our equation were a little larger than the ones calculated according to Moreau et al. (2015), but both are highly correlated (Pearson $r = 0.972$, $p < 0.0001$, $n = 373$; supporting information Methods S3). The latter is not surprising given that both calculations depend on the differences in total N-content and root biomass between the two harvests.

2.4 Statistical analyses

To test for differences in biomass production, root-mass fraction, N-uptake rate and total plant N-content between widely naturalized and less widely naturalized species grown under different N-conditions, we fitted linear mixed effect models using the *lme* function in the package "nlme" (Pinheiro, Bates, DebRoy, & Sarkar, 2015) in R 3.3.2 (R Core Team, 2016). Total biomass, root-mass fraction, total N-content and N-uptake rate of the plants were the response variables in the models. To meet the assumption of normality, total biomass and N-uptake rate were natural-log-transformed, and root-mass fraction and total N-content were square-root-transformed. We included species status (widely naturalized vs. less widely naturalized), nutrient treatment (low vs. high), harvest time (first vs. second) and their interactions, as fixed effects in the models for total biomass and root-mass fraction. Because initial variation in plant size might contribute to differences in the final biomass production, we also added initial plant leaf area and initial plant height as scaled natural-log-transformed covariates in the model for total biomass. As we used the two harvest times to calculate N-uptake rate, and only analysed final total plant N-content (i.e. only for the second harvest), the models for these two response variables did not include the factor harvest time. To account for phylogenetic non-independence of species and for non-independence of replicates of the same species, we included species nested within family (i.e. taxonomy) as random effects in all models. This also accounts for differences among families in biomass production, root-mass fraction, N-uptake rate and total plant N-content.

As the homoscedasticity assumption was violated, we also included variance structures to allow different variances per species in all models using the "*varIdent*" function in the R package "nlme" (Pinheiro et al., 2015; also see Zuur, Ieno, Walker, Saveliev, & Smith, 2009). Moreover, as species might differ in their responses to nutrient treatments, we also included random slopes for family and species with respect to nutrient treatments in all models. In the linear mixed models described above, we assessed the significance of fixed effects (i.e. species status, nutrient level and harvest time) and their interactions with likelihood-ratio tests (Zuur et al., 2009). As we defined the plant status (i.e. widely naturalized or less widely naturalized) based on the number of regions where a species is naturalized, we also ran all the models including the number of regions of each species as a continuous variable instead of as a categorical factor (i.e. plant status). The results of these models are very similar to the results of the models including the categorical factor "status" (Supporting Information Table S2).

TABLE 1 Results of linear mixed effects models testing the effects of species status (widely vs. less widely naturalized), nitrogen treatment, harvest time and all interactions thereof, on total biomass, root-mass fraction, N-uptake rate and total plant N-content at the end of the experiment

	Total biomass (ln transformation)			**Root-mass fraction (sqrt transformation)**			**N-uptake rate (ln transformation)**			**Total plant N (sqrt transformation)**		
	df	χ^2	*p*	*df*	χ^2	*p*	*df*	χ^2	*p*	*df*	χ^2	*p*
Fixed effects												
Initial leaf area	1	3.8936	**0.0485**	—	—	—	—	—	—	—	—	—
Initial height	1	7.425525	**0.0064**	—	—	—	—	—	—	—	—	—
Status (S)	1	1.6211	0.2029	1	2.8291	0.0926	1	5.0087	**0.0252**	1	0.3077	0.5791
Nitrogen treatment (N)	1	18.3778	**<0.0001**	1	18.0480	**<0.0001**	1	17.8500	**<0.0001**	1	32.7735	**<0.0001**
Harvest time (H)	1	993.9896	**<0.0001**	1	73.1127	**<0.0001**	—	—	—	—	—	—
S × H	1	7.7174	**0.0055**	1	0.1182	0.7310	—	—	—	—	—	—
S × N	1	0.7226	0.3953	1	1.0461	0.3064	1	0.2617	0.6089	1	3.5460	0.0597
N × H	1	64.2523	**<0.0001**	1	0.4632	0.4961	—	—	—	—	—	—
S × H × N	1	0.0069	0.9336	1	3.3244	0.0683	—	—	—	—	—	—
Random effects	*SD*			*SD*			*SD*			*SD*		
Family	0.4633 (0.0842)[a]			0.0576 (0.0304)[a]			0.4739 (0.4082)[a]			0.6078 (0.3398)[a]		
Species within Family	0.7115 (0.3561)[a]			0.0609 (0.0213)[a]			0.6891 (0.3547)[a]			0.8462 (0.6361)[a]		
Residual	0.7194			0.0611			0.3887			0.5434		

Significant effects ($P < 0.05$) are in bold.

[a] Here, the standard deviations (*SD*) are given for the random intercepts (corresponding to the values in the high N treatment) and random slopes (corresponding to the effects of the low N treatment) of family and species. The values in brackets represent the *SD*s of the random slopes.

3 RESULTS

Averaged across the two N treatments and the early and late harvests, the widely naturalized species and the less widely naturalized species did not differ in total biomass (Table 1 and Figure 1). The widely naturalized species tended to have a slightly higher root-mass fraction (mean [*SE*] = 0.352 [0.013]) than the less widely naturalized ones (mean [*SE*] = 0.302 [0.013]), but this difference was only marginally significant ($p = 0.0926$; Table 1; Figure 1). The widely naturalized species, however, had a significantly lower N-uptake rate (mean [*SE*] = 3.580 [0.605] mg [N]/g [root]/day) than the less widely naturalized ones (mean [*SE*] = 4.843 [0.798] mg [N]/g [root]/day; Table 1; Figure 2). Nevertheless, the total plant N-content at the end of the experiment did not differ between widely naturalized and less widely naturalized species (Table 1 and Figure 2). All mean values of total biomass, root-mass fraction, plant N-content and N-uptake rate for each species separately under each treatment combination are given in Supporting Information Table S3.

Averaged across all 41 species, plants produced significantly more biomass (+139%), had a significantly lower root-mass fraction (−24%; Table 1 and Figure 1) and exhibited a higher N-uptake rate (+180%) and a higher total plant N-content (+258%; Table 1 and Figure 2) when growing under high N-conditions than under low N-conditions. In response to N-addition, the widely naturalized species tended to increase total plant N-content slightly more strongly (mean [*SE*] = +0.193 [0.016] g) in absolute terms than the less widely naturalized ones (mean [*SE*] = +0.162 [0.019] g; marginal significant S × N interaction: $p = 0.0597$; Table 1; Figure 2). Plants also produced significantly more biomass (+374%) and had a significantly higher root-mass fraction (+18%) at the second harvest than at the first one (Table 1 and Figure 1). Between the two harvests, the widely naturalized species gained absolutely more biomass (mean [SE] = +9.37 [0.898] g) than the less widely naturalized ones (mean [*SE*] = +6.70 [0.891] g; S × H interaction: $p = 0.0055$; Table 1; Figure 1; also see Supporting Information Figure S1). Moreover, the increase in total biomass between the two harvests was significantly stronger for plants growing under high N-condition (+442%) than for plants growing under low N-condition (+257%; significant N × H interaction: $p < 0.0001$; Table 1; Figure 1).

4 DISCUSSION

Our study is the first multispecies experiment to compare the abilities of N-acquisition between widely naturalized and less widely naturalized species using the source-area approach (i.e. using plant material from the same native range). Our results show that a high growth rate pre-adapts common Central European grassland species to becoming widely naturalized elsewhere. Surprisingly, although it is frequently thought that a high ability of nutrient acquisition might

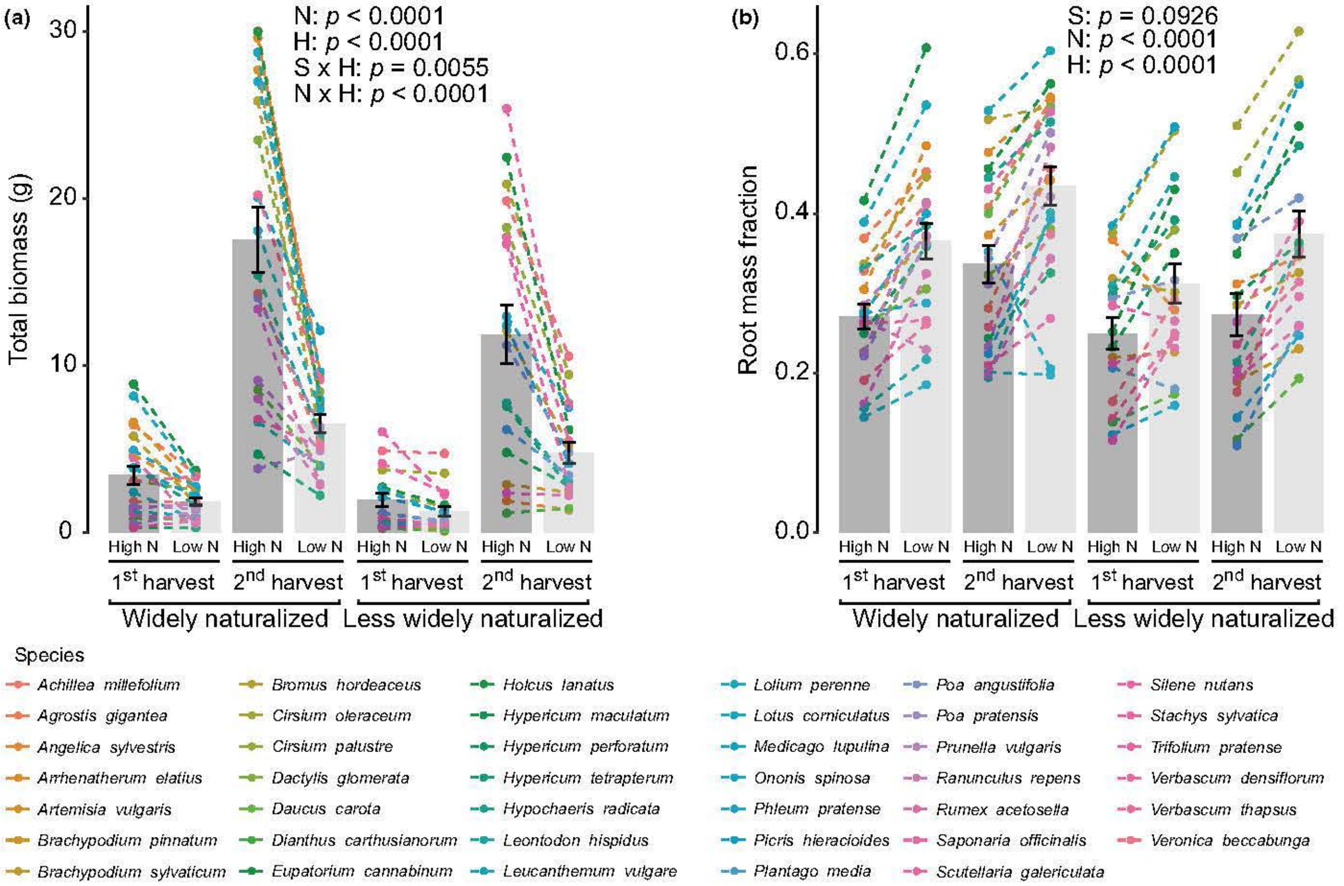

FIGURE 1 Mean (± *SE*) values of total biomass (a) and root-mass fraction (b) of the widely naturalized and the less widely naturalized species growing under low and high N-conditions at the two harvest points. The coloured individual points indicate the mean values per species in each treatment combination. The dashed lines between points indicate the responses of each species to N-addition at the two harvest points. S: species status, N: nitrogen treatment, H: harvest time. For the details of the statistical results, see Table 1

benefit the alien species and thus allow them to establish and become invasive (Davis et al., 2000; Dawson, Rohr et al., 2012), we found that the N-uptake rate was actually lower for the widely naturalized species than for the less widely naturalized ones. However, as the widely naturalized species grew faster (Figure 1, Supporting Information Figure S1) and tended to allocate more biomass to their root system, and thus had overall more roots (Supporting Information Figure S2), than the less widely naturalized species, the total plant N-content did not differ between the two groups of species. Our results also indicate that N-addition increased the total plant N-content more strongly in the widely naturalized species than in the less widely naturalized species. However, the increases in N-uptake rate and biomass production in response to N-addition were similar.

It has been reported that fast-growing species are usually more successful as aliens at a global scale than slow-growing species (Dawson, Fischer, & van Kleunen, 2011; Grotkopp & Rejmánek, 2007). Generally, fast-growing species tend to have a greater resource-capture ability (Wright et al., 2004) and higher reproductive output than slow-growing species (Rose, Atkinson, Turnbull, & Rees, 2009), which might explain their success. Moreover, many fast-growing species are typically disturbance-associated pioneer species. In the current Anthropocene epoch, natural systems have more and more been impacted by human activity, and thus, disturbed habitats that suit fast-growing species are more common than undisturbed habitats (Hannah, Carr, & Landerani, 1995). Consequently, fast-growing alien species are globally more widely naturalized and invasive. Indeed, Dawson et al. (2011), who assessed the relationship between global invasiveness and maximum relative growth rate of 105 plant species commonly occurring in the UK, found that the maximum relative growth rate of species is positively associated with their global invasiveness. Our study adds more evidence that species with a higher mean growth rate (see significant S × H interaction for total biomass analysis in Figure 1a and marginally significant S effect in Supporting Information Figure S1) in their native range usually have a high invasion potential elsewhere.

Besides fast growth, the ability to rapidly exploit available resources is also frequently considered as a potential determinant of invasion success (Davis et al., 2000; Richards et al., 2006). However, we found that the overall abilities of N-acquisition by plants (i.e. the total plant N-content in our study) did not differ between widely naturalized and less widely naturalized species. Although the widely naturalized species unexpectedly exhibited a lower N-uptake rate (per unit root) than the less widely naturalized species, the widely naturalized species probably compensated this by producing larger

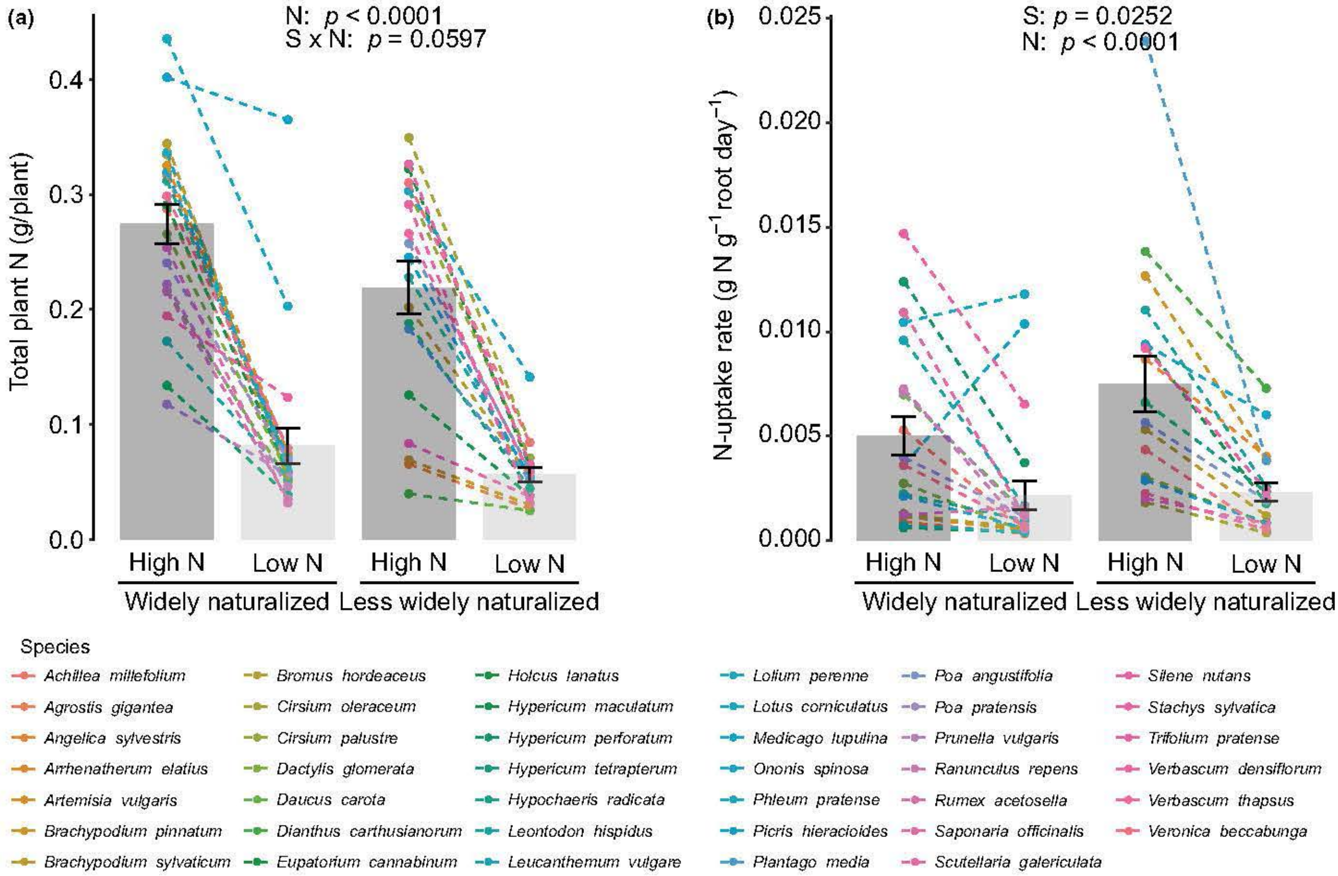

FIGURE 2 Mean (± SE) values of total plant N-content at the end of experiment (a) and mean N-uptake rate between the two harvests (b) of the widely naturalized and the less widely naturalized species growing under low and high N-conditions. The coloured individual points indicate the mean values per species in each treatment. The dashed lines between points indicate the response of each species to N-addition. S: species status, N: nitrogen treatment. For the details of the statistical results, see Table 1

root systems overall (see Supporting Information Figure S2). It should be noted, however, that the N-uptake rate in the present study is an average value of N-uptake rate calculated across a 26-day period. As the increase in root biomass between the two harvests is included in the N-uptake rate equation, a large increase in root biomass between the harvests would decrease the N-uptake rate estimate more. In other words, if widely naturalized plants take up the same amount of N as less widely naturalized plants, but use it more efficiently in biomass production, this might result in an apparently lower N-uptake rate for the widely naturalized ones. Therefore, future studies should also measure the instantaneous rate of N-uptake using an isotope-labelling approach (e.g. Bueno, Greenfield, Pritsch, Schmidt, & Simon, 2018).

Not surprisingly, all plants produced significantly more biomass under high N-conditions than under low N-conditions. We also found a decrease in the relative allocation of biomass to the root system under high N-conditions. This is in line with predictions of resource-limitation theory, posing that plants should allocate biomass to structures that help them acquire more of the most limiting resource (Bloom, Chapin, & Mooney, 1985; Poorter & Nagel, 2000). In line with the findings of Poorter et al. (2012), we found that plants allocated relatively more biomass to roots with time as plants developed, confirming that shoots of herbaceous plants generally have lower growth rates than roots. IN-addition, we found that N-addition also increased the N-uptake rate, which is in line with a recent meta-analysis by Liu, van Groenigen, Dijkstra, and Hungate (2017). This indicates that under low N-conditions, the N-uptake rate is limited by the availability of N. Generally, nutrient addition could stimulate root growth and density, resulting in an increase in absolute root biomass and an extension of rooting depth (Ashraf, Mahmood, Azam, & Qureshi, 2004). These changes in root morphology and size increase the soil volume explored by the plants' root system and root surface area for the uptake of nutrients (Kurdali, 2004), thereby enhancing plant uptake of soil N (Azam, Simmons, & Mulvaney, 1993). As both biomass production and N-uptake rate increased, it is thus no surprise that the total plant N-content also increased with N-addition in our study.

Responses of root allocation, N-uptake rate and total biomass to N-addition were similar between the widely naturalized species and the less widely naturalized species. However, the widely naturalized species tended to increase total N-content more strongly in response to N-addition (although this effect was only marginally significant). Our study thus provides limited support for the idea that higher phenotypic plasticity may drive the invasion success of alien species (Dawson, Fischer et al., 2012; Richards et al., 2006; Schlaepfer et al., 2010). It could be that plastic responses might be less important for becoming widely naturalized at the global scale than they are for other dimensions of invasiveness, such as local abundance and occurrence in many different habitats (Catford et al., 2016; van Kleunen, Bossdorf, & Dawson, 2018).

The trend for a stronger increase in plant N-content in response to N-addition for widely naturalized than for less widely naturalized species did not appear to affect biomass-production responses of the two groups of species in our greenhouse experiment. However, having a stronger plastic increase in plant N-content might benefit the widely naturalized species in the field in their introduced range. Nitrogen is required for plant growth (Onoda, Hikosaka, & Hirose, 2004; Takashima, Hikosaka, & Hirose, 2004) as well as for defence compounds against herbivores and pathogens (Mur, Simpson, Kumari, Gupta, & Gupta, 2017; Schultz, Appel, Ferrieri, & Arnold, 2013). According to the enemy-release hypothesis (Keane & Crawley, 2002), most specialist enemies of the alien plant species will be absent from their introduced range, and thus, both widely naturalized and less widely naturalized species should be attacked less in their introduced range than in their native range. Consequently, alien species could afford to allocate more N to growth and less to defence in their introduced ranges than in their native ranges (Feng et al., 2009). Therefore, stronger increases in plant N-content in response to N-addition might have made the widely naturalized plants grow larger than less widely naturalized species in their introduced range and thereby might have increased their naturalization likelihood.

4.1 Recommendations for future work

Rapid evolution can commonly occur in the invaded range of alien species (Colautti & Barrett, 2013; Oduor, Leimu, & van Kleunen, 2016). Although a high N-acquisition ability did not confer a pre-adaptation for species' world-wide naturalization success, it might differ between widely naturalized and less widely naturalized species, when it would be assessed using plant material from the introduced ranges. IN-addition, other factors such as temperature (Boczulak, Hawkins, & Roy, 2014), soil micro-organisms (Kuzyakov & Xu, 2013) and competition from neighbours (Ashton, Miller, Bowman, & Suding, 2010), as well as varied N-conditions in the field, can also affect N-uptake rate of plants. Therefore, future studies should also assess the role of N-uptake rate on plant invasion in the field, in the non-native range.

Plants can take up different forms of N, such as nitrate, ammonium and amino acids (Ashton et al., 2010; Xu et al., 2011). Therefore, although we did not find a difference in overall N-acquisition ability between widely and less widely naturalized species, the widely naturalized species may be able to use forms of N that the less widely naturalized or native species do not use or to a lesser extent (Funk, 2013). Moreover, widely and less widely naturalized species might also differ in their acquisition of other nutrients. For example, the availability of phosphorus in soil might become limiting to plant growth with a high *N*-input, and thus, the phosphorus acquisition strategy could also affect the success of species in plant communities (Ceulemans et al., 2017). Therefore, future studies should test the roles of different N-niches and the acquisition strategies for other nutrients in plant invasion.

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AUTHORS' CONTRIBUTIONS

Y.L. conceived the idea for 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.

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