

# Responses to shading of naturalized and non-naturalized exotic woody species

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• **Background and Aims** Recent studies have suggested that responses to shading gradients may play an important role in establishment success of exotic plants, but hitherto few studies have tested this. Therefore, a common garden experiment was conducted using multiple Asian woody plant species that were introduced to Europe > 100 years ago in order to test whether naturalized and non naturalized species differ in their responses to shading. Specifically, a test was carried out to determine whether naturalized exotic woody species maintained better growth under shaded conditions, and whether they expressed greater (morphological and physiological) adaptive plasticity in response to shading, relative to non naturalized species.

• **Methods** Nineteen naturalized and 19 non naturalized exotic woody species were grown under five light levels ranging from 100 to 7 % of ambient light. For all plants, growth performance (i.e. biomass), morphological and CO<sub>2</sub> assimilation characteristics were measured. For the CO<sub>2</sub> assimilation characteristics, CO<sub>2</sub> assimilation rate was measured at 1200 μmol m<sup>-2</sup> s<sup>-1</sup> (i.e. saturated light intensity, A<sub>1200</sub>), 50 μmol m<sup>-2</sup> s<sup>-1</sup> (i.e. low light intensity, A<sub>50</sub>) and 0 μmol m<sup>-2</sup> s<sup>-1</sup> (A<sub>0</sub>, i.e. dark respiration).

• **Key Results** Overall, the naturalized and non naturalized species did not differ greatly in biomass production and measured morphological and CO<sub>2</sub> assimilation characteristics across the light gradient. However, it was found that naturalized species grew taller and reduced total leaf area more than non naturalized species in response to shading. It was also found that naturalized species were more capable of maintaining a high CO<sub>2</sub> assimilation rate at low light intensity (A<sub>50</sub>) when grown under shading.

• **Conclusions** The results indicate that there is no clear evidence that the naturalized species possess a superior response to shading over non naturalized species, at least not at the early stage of their growth. However, the higher CO<sub>2</sub> assimilation capacity of the naturalized species under low light conditions might facilitate early growth and survival, and thereby ultimately favour their initial population establishment over the non naturalized species.

**Key words:** Alien woody species, carbon loss and gain, exotic species, light gradient, naturalization, photosynthesis, phenotypic plasticity, respiration, shade tolerance.

## INTRODUCTION

Light gradients are ubiquitous in nature and, as a consequence, almost all plants are exposed to a certain degree of shading during their lifetime (Valladares and Niinemets, 2008). Therefore, competition for light and responses to light availability play pivotal roles in determining the distribution and success of plant species (Grubb, 1998; Valladares, 2003; Silvertown, 2004; Zavala *et al.*, 2007). Recent studies showed that many exotic plant species have established self-sustaining populations (i.e. have become naturalized) in shady understoreys of temperate and tropical forests that were formerly regarded as strongly resistant to plant invasions (Rejmánek, 1996; Martin, 1999; Von Holle *et al.*, 2003; Martin *et al.*, 2004, 2009; Gilbert and Lechowicz, 2005; Martin and Marks, 2006). However, it is not yet clear whether and to what extent shade tolerance and the response to shading account for successful establishment and spread of exotic plants in their introduced ranges (Martin *et al.*, 2009; Funk, 2013).

Most plants respond to changing light conditions by adjusting a suite of morphological and physiological characteristics in order to maintain fitness in light-limited environments (Valladares and Niinemets, 2008; Funk, 2013). Therefore,

those exotic plant species that are more capable of dealing with low-light conditions may be more likely to establish and spread into (semi-)natural vegetation (Martin *et al.*, 2009; van Kleunen *et al.*, 2011). For instance, those exotic plants that are more capable of optimizing efficiency of light interception in shade by forming more leaf area, by producing more leaves and/or leaves with a higher specific leaf area (SLA), should be better able to maintain high biomass production (Valladares and Niinemets, 2008). Moreover, an exotic plant species that is better able to achieve a relatively high CO<sub>2</sub> assimilation rate at low light intensity might be more successful (Givnish, 1988; Martin *et al.*, 2009; van Kleunen *et al.*, 2011). While some studies have assessed morphological and CO<sub>2</sub> assimilation characteristics in response to shading for certain naturalized or invasive species (Funk, 2013), we need large comparative multi-species experimental studies to explore general patterns (van Kleunen *et al.*, 2010, 2014).

Adaptive phenotypic plasticity of certain morphological and physiological traits in response to shading might facilitate the establishment of exotic plants by allowing them (1) to maintain a high fitness in stressful environments (Jack of all trades); (2) to take advantage of benign environments (Master of some); or (3) both (Jack and master) (*sensu* Richards *et al.*, 2006).

Alternatively, it can be that such fitness response patterns are not driven by plastic changes in morphology and physiology but by an intrinsic higher tolerance to low-light conditions due to constitutive trait differences (Grubb, 1998; Valladares *et al.*, 2000a, b; Grime and Mackey, 2002; Funk, 2008; van Kleunen *et al.*, 2011). Irrespective of the underlying mechanisms, to test and discern the different fitness response scenarios, one should use more than two treatments along a resource gradient (Richards *et al.*, 2006; Hulme, 2008). This has, however, rarely been done so far.

Woody species (e.g. trees and shrubs) are increasingly reported as established or invasive aliens in many parts of the world (Reichard and Hamilton, 1997; Van Auken, 2000; Eldridge *et al.*, 2011, 2012; Richardson and Rejmanek, 2011; Rejmanek and Richardson, 2013). Furthermore, shrubs are more frequently reported as shade-tolerant invasives than trees, which implies that growth form also plays a role in establishment success of exotic woody plants (Bucharova and van Kleunen, 2009; Martin *et al.*, 2009). In the introduced range, exotic trees and shrubs will inevitably experience shading cast by the resident plants in natural habitats such as forests and shrublands. Even for the exotic woody species that encroach on grasslands, shading by grasses and herbs is highly likely, at least during the early growth period of seedlings. Therefore, shade-induced morphological and physiological responses could be particularly crucial for the naturalization of exotic woody plants (Martin *et al.*, 2009).

Few studies hitherto have been carried out to address the role of responses to shading for establishment of exotic plants (but see van Kleunen *et al.*, 2011), and particularly not for woody plants (Martin *et al.*, 2009; Funk, 2013). Therefore, we conducted a common-garden experiment with 38 exotic woody species (shrubs and trees) that are native to Asia, and have all been introduced to Europe >100 years ago. Nineteen of the study species have become naturalized in at least one European country, while the other 19 species have not. We grew these species outdoors in pots under five different light levels ranging from 100 to 7 % of ambient photosynthetically active radiation (PAR), to test whether, relative to non-naturalized exotic woody species, naturalized species (1) maintain better growth performance under shaded conditions and (2) express greater adaptive plasticity in terms of morphological and physiological responses to shading.

## MATERIALS AND METHODS

### *Study species*

Based on a database with historical information on the introduction of woody species to Europe (Goeze, 1916; also Bärtels, 2001) and the DAISIE database of alien organisms in Europe (<http://www.europe-aliens.org/>), we selected 38 woody species (shrubs and trees), representing 17 families, that have been introduced to Europe from Asia (mainly from China, Korea and Japan) >100 years ago (i.e. median year of introduction 1878, range 1596–1909; see Table 1). Among the species, 19 have established self-sustaining populations in one or more European countries (i.e. have successfully naturalized), while the remaining 19 species have not (DAISIE, last accessed in January 2014; Table 1). The naturalized and non-naturalized

species had a similar residence time, as the year of introduction to Europe was not significantly different (Table 1; naturalized:  $1836.8 \pm 18.9$ , non-naturalized:  $1871.4 \pm 8.3$ ;  $t = 1.6753$ , d.f. = 24.731,  $P = 0.107$ ). Although the naturalized species were on average planted in slightly more countries than the non-naturalized species (10.7 vs. 13.2 countries), this difference was not significant (Welch two-sample  $t$ -test:  $t = 1.73$ , d.f. = 35.9,  $P = 0.092$ ). This suggests that the naturalized and non-naturalized species had similar propagule pressures. We obtained seeds of the 38 study species from botanic gardens in Europe, mainly in Germany (Table 1). We chose botanic gardens as seed sources because many of the naturalized species might have escaped from botanic gardens (Hulme, 2011).

### *Experimental set up*

To test whether performance (biomass production), morphological (leaf) and physiological ( $\text{CO}_2$  assimilation) characteristics, and whether the plasticity of these characteristics in response to shading differ between naturalized and non-naturalized woody species, we set up a common-garden experiment in the botanic garden of the University of Konstanz. On 16 May 2012, we sowed 20 seeds of each study species in each of five pots (height, 10 cm; diameter, 8 cm; totalling 100 seeds per species) filled with commercial potting soil (Standard soil, Gebr. Patzer GmbH & Co. KG, Sinntal, Germany) in a glasshouse (night,  $\geq 20^\circ\text{C}$ ; day, 23–28  $^\circ\text{C}$ ). We randomly repositioned the pots on the tables every 2 weeks.

Approximately 50 d after sowing (on 4 July 2012), we transplanted 15 similarly sized seedlings of each species into 3 L pots (height, 15 cm; diameter, 19 cm) filled with the same substrate as used for the germination, and placed them outdoors. After 1 week of acclimatization, we counted the number of leaves, and measured stem height, leaf area (for three randomly chosen leaves per plant), and diameter at the top and base of the stem, as measures of the initial size of the plants. After this (on 14 July 2012), we randomly assigned the 15 plants of each species to 15 wooden cages (length, 360 cm; width, 110 cm; height, 118 cm). Three of these cages were left uncovered, and the others were covered with one or two layers of green cloth with different mesh sizes. In this way, we created five relative light intensities: 100, 85, 49, 25 and 7 % of ambient sunlight, each with three replicate cages. We estimated the relative light intensities by measuring the light intensity inside and outside each cage (ambient sunlight), using a LI-190SA Quantum PAR sensor (LI-COR, Lincoln, NE, USA). Five cages, each with a different shading intensity, were then randomly placed in each of three rows (blocks). Within each cage, we positioned all 38 plants randomly (one plant per species). One cage (85 % of ambient light) had only 37 plants because *Berberis koreana* did not have enough seedlings. We watered all the plants regularly, sometimes multiple times a day during hotter periods, to make sure that water availability was not limiting to either the shaded or the non-shaded plants.

### *Measurements*

More than 2 months after starting the shading treatments (from 25 September to 1 October), we chose the first fully expanded, healthy leaf counted from the top of each plant for

TABLE 1. Family, growth form, year of introduction, naturalization status and seed source (botanic garden) of the 38 study species

| Species <sup>†</sup>            | Family           | Growth form | Year of introduction <sup>‡</sup> | No. of naturalized countries or regions <sup>†</sup> | Seed source    |
|---------------------------------|------------------|-------------|-----------------------------------|--|----------------|
| <b>*Berberis aggregata</b>      | Berberidaceae    | Shrub       | 1908                              | 1  | Hohenheim      |
| <i>Berberis koreana</i>         | Berberidaceae    | Shrub       | 1899                              | 0  | Hohenheim      |
| <i>Berberis pruinosa</i>        | Berberidaceae    | Shrub       | 1894                              | 0  | Hohenheim      |
| <b>*Lonicera ruprechtiana</b>   | Caprifoliaceae   | Shrub       | 1880                              | 1  | Marseille      |
| <i>Cornus capitata</i>          | Cornaceae        | Shrub       | 1825                              | 0  | Talence        |
| <b>*Cryptomeria japonica</b>    | Cupressaceae     | Tree        | 1842                              | 4  | Bonn           |
| <b>*Platycladus orientalis</b>  | Cupressaceae     | Tree        | 1690                              | 2  | Hohenheim      |
| <i>Chamaecyparis obtusa</i>     | Cupressaceae     | Tree        | 1855                              | 0  | Bonn           |
| <i>Cunninghamia lanceolata</i>  | Cupressaceae     | Tree        | 1804                              | 0  | Bonn           |
| <b>*Weigela florida</b>         | Diervillaceae    | Shrub       | 1845                              | 2  | Hohenheim      |
| <i>Weigela praecox</i>          | Diervillaceae    | Shrub       | 1894                              | 0  | Gießen         |
| <b>*Rhododendron luteum</b>     | Ericaceae        | Shrub       | 1894                              | 3  | Tallinn        |
| <i>R. schlippenbachii</i>       | Ericaceae        | Shrub       | 1875                              | 0  | Tallinn        |
| <i>Indigofera decora</i>        | Fabaceae         | Shrub       | 1844                              | 0  | Frankfurt      |
| <b>*Deutzia scabra</b>          | Hydrangeaceae    | Shrub       | 1882                              | 3  | Tallinn        |
| <i>Philadelphus inodorus</i>    | Hydrangeaceae    | Shrub       | 1896                              | 0  | Ulm            |
| <i>Deutzia discolor</i>         | Hydrangeaceae    | Shrub       | 1897                              | 0  | Tallinn        |
| <i>Deutzia parviflora</i>       | Hydrangeaceae    | Shrub       | 1883                              | 0  | Tallinn        |
| <i>Philadelphus delavayi</i>    | Hydrangeaceae    | Shrub       | 1887                              | 0  | Lublin         |
| <i>Philadelphus schrenkii</i>   | Hydrangeaceae    | Shrub       | 1874                              | 0  | Opava          |
| <b>*Pterocarya stenoptera</b>   | Juglandaceae     | Tree        | 1860                              | 1  | Gießen         |
| <i>Pterocarya sorbifolia</i>    | Juglandaceae     | Tree        | 1782                              | 0  | Lissabon       |
| <b>*Melia azedarach</b>         | Meliaceae        | Tree        | 1864                              | 8  | Lissabon       |
| <b>*Broussonetia papyrifera</b> | Moraceae         | Tree        | 1750                              | 9  | Hohenheim      |
| <b>*Morus alba</b>              | Moraceae         | Tree        | 1596                              | 12   | Talence        |
| <i>Forsythia viridissima</i>    | Oleaceae         | Shrub       | 1844                              | 0  | Hohenheim      |
| <b>*Fraxinus mandshurica</b>    | Oleaceae         | Tree        | 1882                              | 1  | Hohenheim      |
| <i>Syringa patula</i>           | Oleaceae         | Shrub       | 1907                              | 0  | Opava          |
| <i>Syringa villosa</i>          | Oleaceae         | Shrub       | 1888                              | 0  | Jena           |
| <b>*Paulownia tomentosa</b>     | Paulowniaceae    | Tree        | 1840                              | 6  | Talence        |
| <b>*Larix kaempferi</b>         | Pinaceae         | Tree        | 1860                              | 4  | Ulm            |
| <b>*Cotoneaster franchetii</b>  | Rosaceae         | Shrub       | 1895                              | 3  | Strassburg     |
| <b>*Sorbaria kirilowii</b>      | Rosaceae         | Shrub       | 1896                              | 1  | St. Petersburg |
| <b>*Spiraea japonica</b>        | Rosaceae         | Shrub       | 1870                              | 4  | Lublin         |
| <b>*Pyracantha angustifolia</b> | Rosaceae         | Shrub       | 1895                              | 2  | Lissabon       |
| <i>Spiraea rosthornii</i>       | Rosaceae         | Shrub       | 1909                              | 0  | Mailand        |
| <i>Buddleja albiflora</i>       | Scrophulariaceae | Shrub       | 1900                              | 0  | Hohenheim      |
| <b>*Ailanthus altissima</b>     | Simaroubaceae    | Tree        | 1751                              | 25   | Lissabon       |

All species are native to Asia and have been introduced to Europe > 100 years ago. Nineteen of the species (printed in bold and marked with asterisks \*) have become naturalized in one or more European countries, while the remaining species have not.

<sup>†</sup>Naturalization success is based on DAISIE (<http://www.europe.aliens.org/>, last accessed in January 2014).

<sup>‡</sup>Year of introduction to Europe is based on Goeze (1916) and Bärtels (2001).

measurements of CO<sub>2</sub> assimilation characteristics. These measurements were done using an LCpro-SD porometer (ADC BioScientific Limited, Herts, UK), and included light-saturated CO<sub>2</sub> assimilation at 1200 µmol m<sup>-2</sup> s<sup>-1</sup> (A<sub>1200</sub>), CO<sub>2</sub> assimilation at 50 µmol m<sup>-2</sup> s<sup>-1</sup> (i.e. at low light intensity; A<sub>50</sub>) and CO<sub>2</sub> assimilation at 0 µmol m<sup>-2</sup> s<sup>-1</sup> (i.e. dark respiration; A<sub>0</sub>). The different light (PAR) intensities were implemented with a light unit fitted to the leaf chamber, and the conditions in the gas exchange chamber were kept constant (e.g. the temperature was 25 °C). On every measurement day, the CO<sub>2</sub> assimilation measurements were taken between 0800 and 1700h (with a break from 1200 to 1400 h). We calculated the light compensation point (LCP; i.e. the light intensity at which the assimilation rate is zero) from a linear regression based on A<sub>0</sub> and A<sub>50</sub>. The CO<sub>2</sub> assimilation measurements of the species were done in a random order, cage by cage, within each of the three blocks. The different plant species in each cage were also measured in random order. When the leaves were too small to cover the full area of the window (6.25 cm<sup>2</sup>) of the porometer chamber, we took photos of the window and the enclosed leaf, and estimated

actual leaf area with the image analysis software ImageJ (Rasband 1997–2012). The CO<sub>2</sub> assimilation parameters were then recalculated based on the actual leaf area.

From 25 September to 5 October 2012, we made morphological measurements and afterwards harvested all of the plants. After counting the number of leaves per plant, we randomly collected, if possible, three leaves per plant, and digitized them with an Epson scanner (Expression 10000XL, Epson, Suwa, Japan), and calculated individual leaf area using ImageJ (Rasband 1997–2012). In order to determine the SLA, we then weighed the three leaves per plant after drying them at 70 °C for > 72 h. Based on the individual leaf area and the number of leaves, we calculated the total leaf area of each plant. We harvested the above-ground biomass of each plant, and weighed it after drying at 70 °C for > 72 h.

#### Data analysis

We used linear mixed effects models in which performance, morphological traits and CO<sub>2</sub> assimilation characteristics of

the study species were the response variables. As the explanatory variables, we included naturalization status (naturalized or non-naturalized), the continuous covariate relative light intensity (RLI) and the interaction between the two as fixed terms. We accounted for differences in growth form (shrub or tree) by also including this variable and its interaction with RLI as fixed terms. In order to test and account for non-linear responses of traits to the light gradient, we initially also added the quadratic term of relative light intensity ( $RLI^2$ ) and its interactions with naturalization status and growth form as explanatory variables to all the models. However, when the quadratic term and its interactions were not significant, we removed them from the model. For some of the response variables, we also accounted for

initial variation in size, by including the respective initial size measurements (initial stem height, stem volume, total leaf area and number of leaves) as covariates in the fixed part of the models (see Table 2). In all models, we included cage nested within block, and species nested within family as random factors. We applied a natural logarithm or a square root transformation (only for SLA) for the response variables that violated the assumption of normality of the residuals. We also included variance structures in the models in which the assumption of variance homogeneity was violated (Zuur *et al.*, 2009). We used log-likelihood ratio tests to assess significance of the fixed effects of naturalization status, RLI, growth form,  $RLI^2$  and their interactions. The significance of each interaction term

TABLE 2. Results of linear mixed effects models testing the effects of relative light intensity (RLI), growth form and naturalization status and the interactions of the latter two with RLI on performance and morphological traits of the exotic woody species

|        |  |   | Above ground biomass |          | Stem height |          |
|--------|--|---|----------------------|----------|-------------|----------|
|        |  |   | $\chi^2$             | <i>P</i> | $\chi^2$    | <i>P</i> |
|        |  |   | d.f.                 |          |             |          |
| Fixed  | Initial stem height                    | 1 |                      |          | 51.91       | < 0.001  |
|        | Initial stem volume                    | 1 | 0.30                 | 0.584    |             |          |
|        | Initial total leaf area                | 1 | 29.63                | < 0.001  |             |          |
|        | Growth form                            | 1 | 0.04                 | 0.846    | 1.45        | 0.239    |
|        | Naturalization status                  | 1 | 1.55                 | 0.214    | 6.82        | 0.009    |
|        | Relative light intensity (RLI)         | 1 | 29.95                | < 0.001  | 11.86       | < 0.001  |
|        | $RLI^2$                                | 1 | 22.93                | < 0.001  |             |          |
|        | Growth form $\times$ RLI               | 1 | 1.46                 | 0.227    | 6.22        | 0.013    |
|        | Growth form $\times$ $RLI^2$           | 1 | 0.0001               | 0.991    |             |          |
|        | Naturalization status $\times$ RLI     | 1 | 0.28                 | 0.597    | 2.57        | 0.109    |
|        | Naturalization status $\times$ $RLI^2$ | 1 | 0.05                 | 0.816    |             |          |
| Random |  |   | s.d.                 |          | s.d.        |          |
|        | Block                                  |   | 0.00                 |          | 0.00        |          |
|        | Cages                                  |   | 0.15                 |          | 0.03        |          |
|        | Family                                 |   | 0.92                 |          | 0.39        |          |
|        | Species                                |   | 0.62                 |          | 0.59        |          |
|        | Residual                               |   | 0.52                 |          | 0.25        |          |

|  |                                    |   | Total leaf area |          | Specific leaf area |          | No. of leaves |          |
|--|------------------------------------|---|-----------------|----------|--------------------|----------|---------------|----------|
|  |                                    |   | $\chi^2$        | <i>P</i> | $\chi^2$           | <i>P</i> | $\chi^2$      | <i>P</i> |
|  |                                    |   | d.f.            |          |                    |          |               |          |
| Fixed                                  | Initial number of leaves           | 1 |                 |          |                    |          | 23.58         | < 0.001  |
|  | Initial total leaf area            | 1 | 14.10           | < 0.001  |                    |          |               |          |
|  | Growth form                        | 1 | 1.20            | 0.274    | 0.78               | 0.175    | 0.56          | 0.456    |
|  | Naturalization status              | 1 | 0.26            | 0.610    | 0.05               | 0.816    | 2.45          | 0.118    |
|  | Relative light intensity (RLI)     | 1 | 6.17            | 0.013    | 33.36              | < 0.001  | 24.29         | < 0.001  |
|  | $RLI^2$                            | 1 |                 |          | 24.52              | < 0.001  |               |          |
|  | Growth form $\times$ RLI           | 1 | 29.00           | < 0.001  | 0.78               | 0.378    | 14.20         | < 0.001  |
|  | Growth form $\times$ $RLI^2$       | 1 |                 |          | 0.33               | 0.568    |               |          |
|  | Naturalization status $\times$ RLI | 1 | 4.42            | 0.036    | 1.45               | 0.228    | 1.38          | 0.240    |
| Naturalization status $\times$ $RLI^2$ | 1                                  |   |                 | 0.87     | 0.350              |          |               |          |
| Random                                 |                                    |   | s.d.            |          | s.d.               |          | s.d.          |          |
|  | Block                              |   | 0.03            |          | 0.00               |          | 0.00          |          |
|  | Cages                              |   | 0.09            |          | 1.46               |          | 0.03          |          |
|  | Family                             |   | 1.40            |          | 1.31               |          | 0.87          |          |
|  | Species                            |   | 0.65            |          | 1.49               |          | 0.46          |          |
|  | Residual                           |   | 0.43            |          | 1.04               |          | 0.43          |          |

For some of the traits, we also included relevant initial size measurements as covariates in the fixed model. To test and account for non linear effects of RLI, we initially also included the quadratic term of relative light intensity ( $RLI^2$ ) and its interactions with growth form and naturalization status in the models, but removed them when they were not significant. Cage nested within block, and species nested within family were included as random terms.

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

was determined by comparing the full model with the model from which the respective interaction term was removed. The significance of each main effect (i.e. naturalization status, RLI and life form) was determined by comparing the model with all main effects (i.e. without interactions) with the model from which the respective main effect was removed. The variance components were estimated using restricted maximum likelihood (REML), and the log-likelihood ratio tests for the fixed terms were based on maximum likelihood (ML), as recommended by Zuur *et al.* (2009).

In order to visualize patterns in phenotypic plasticity of all the response variables along the light gradient, we plotted the mean values of each species against RLI, and added the (linear or non-linear) regression lines for the naturalized and non-naturalized species based on the estimates from the linear mixed effects models. All analyses and plots were performed in R version 2.15.2 (R Development Core Team, 2012). The linear mixed effects models were implemented with the *nlme* package (Pinheiro *et al.*, 2013).

## RESULTS

### *Performance and morphology of naturalized and non naturalized species in response to shading*

As shading increased (i.e. from 100 to 7 % of ambient light), above-ground biomass of the woody species showed a significant non-linear decrease (Fig. 1A; Table 2), whereas stem height,

on average, increased (Fig. 1B; Table 2). However, it was primarily the shrubs that showed stem elongation in response to shading, and tree species even showed a slight decrease in stem height (significant growth form  $\times$  RLI interaction, Table 2; Supplementary Data Fig. S1). The above-ground biomass was not significantly greater for naturalized species than for non-naturalized species across the whole experimental light gradient (Table 2; Fig. 1A). However, after correction for growth form, stem height was significantly greater for naturalized species than for non-naturalized species across the light gradient (Table 2; Fig. 1B).

As shading increased, plants produced significantly fewer leaves, but they had a greater SLA (Fig. 1D, E; Table 2). The response of the number of leaves to shading was significantly stronger for shrubs than for trees (significant growth form  $\times$  RLI interactions in Table 2; Supplementary Data Fig. S1). On average, total leaf area of plants decreased significantly with shading (Fig. 1C; Table 2), but this effect was mainly accounted for by the naturalized species (significant naturalization  $\times$  RLI interaction in Table 2; Fig. 1C). Moreover, the decrease in total leaf area with shading was significantly stronger for trees than for shrubs (significant growth form  $\times$  RLI interaction in Table 2; Supplementary Data Fig. S1). On average, naturalized and non-naturalized species did not differ significantly in total leaf area, number of leaves or SLA (Fig. 1C, E; Table 2). In summary, although naturalized plants, on average, grew taller than non-naturalized plants, they did not differ greatly in their plastic morphological and performance responses to shading.

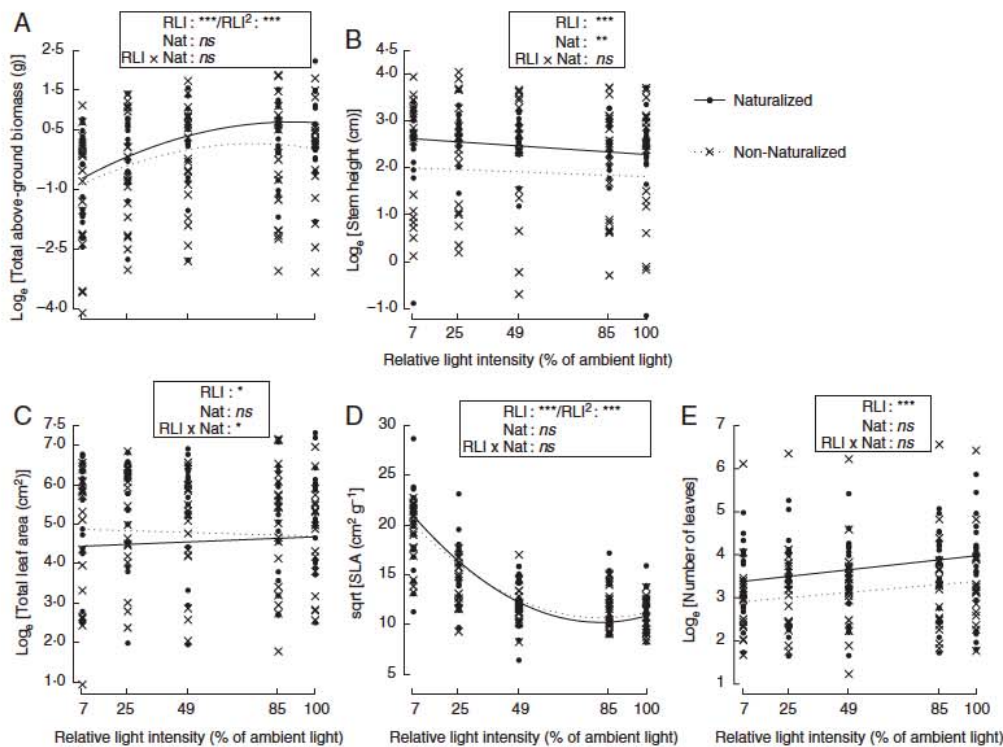
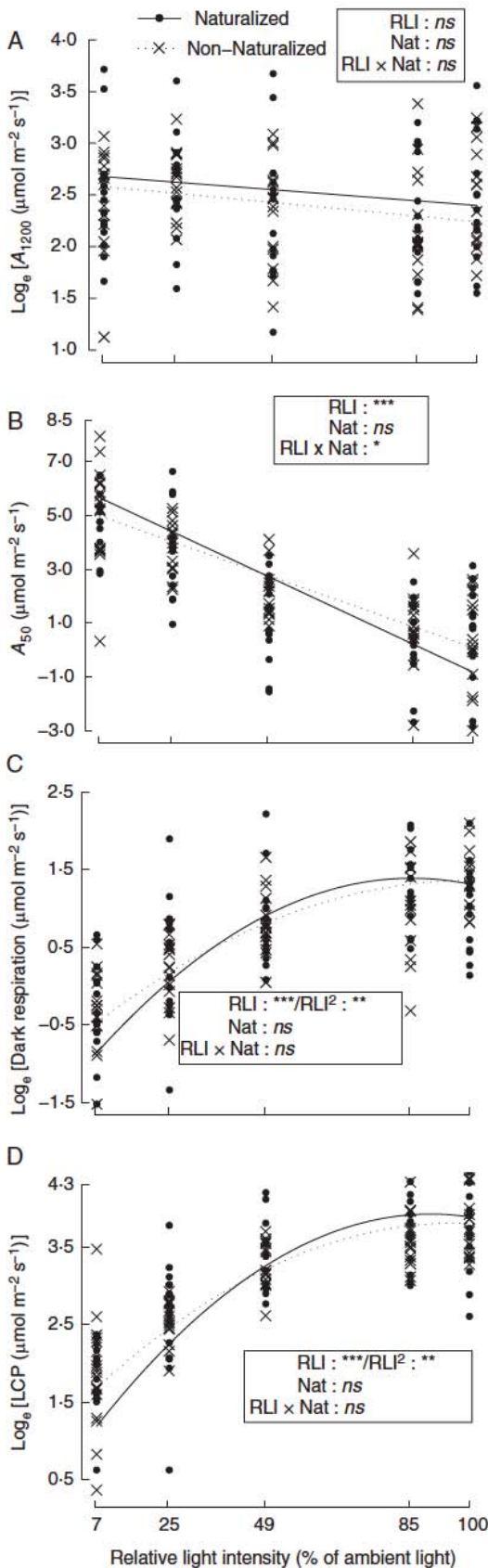


FIG. 1. Mean values of plant performance and morphological characteristics of naturalized and non naturalized exotic woody species in response to shading. The regression lines (linear or non linear) were added according to the estimates from the linear mixed effects models (Table 2), and thus were corrected for all other variables in the model. All response variables, except specific leaf area (SLA), were  $\log_e$ -transformed. SLA was square root transformed. The significances of relative light intensity (RLI), the quadratic term of RLI ( $RLI^2$ ), naturalization status (Nat) and the interaction between relative light intensity and naturalization status (RLI  $\times$  Nat) are indicated as: \*\*\* $< 0.001$ , \*\* $< 0.01$ , \* $< 0.05$ ; ns, not significant.



### CO<sub>2</sub> assimilation characteristics of naturalized and non naturalized species in response to shading

The light-saturated CO<sub>2</sub> assimilation rate ( $A_{1200}$ ) of the woody species was not significantly affected by the RLI at which the plants grew (Fig. 2A; Table 3). However, the CO<sub>2</sub> assimilation rate measured at low light intensity ( $A_{50}$ ) significantly increased, while the dark respiration ( $A_0$ ) and the light compensation point (LCP) significantly decreased in a non-linear way with increasing shade level (Fig. 2B–D; Table 3). The response of  $A_{50}$  to shading was stronger for shrubs than for trees (significant growth form × RLI interaction in Table 3; Supplementary Data Fig. S2). Naturalized and non-naturalized species did not differ, on average, in their CO<sub>2</sub> assimilation characteristics across the light gradient (Table 3; Fig. 2). However, the increase in  $A_{50}$  with shading was, after correction for growth form, significantly greater for naturalized than for non-naturalized species (Fig. 2B; Table 3). As a consequence, naturalized plants were, on average, capable of maintaining a higher CO<sub>2</sub> assimilation rate at low light intensity than non-naturalized species when grown at low light levels (Fig. 2B).

## DISCUSSION

As frequently reported in other studies, our results showed that plants responded to shading by stem elongation and by producing fewer leaves with a higher SLA. While naturalized exotic shrubs and trees were taller than non-naturalized species overall, naturalized species were less capable of maintaining a large total leaf area under shading than non-naturalized species. When grown in shade, plants increased their net CO<sub>2</sub> assimilation rate by decreasing dark respiration and thereby the LCP, whereas they did not adjust their light-saturated assimilation rate ( $A_{1200}$ ). However, compared with non-naturalized species, naturalized plants were more capable of maintaining a high assimilation rate at low light intensity ( $A_{50}$ ) when grown in the low-light treatments. This suggests that naturalized plants have a slightly higher capacity to adjust their assimilation apparatus plastically to shaded conditions.

### Performance and morphology of naturalized and non naturalized plants in response to shading

As expected, the total above-ground biomass of the plants in our study significantly decreased with shading (Table 2; Fig. 1A). Contrary to expectations, however, we found no evidence that naturalized species are more capable than non-naturalized species of maintaining high biomass production in unfavourable low-light environments (Jack-of-all-trades), of taking more advantage of favourable high-light environments

FIG. 2. Mean values of CO<sub>2</sub> assimilation characteristics of naturalized and non naturalized exotic woody species in response to shading. The regression lines (linear or non linear) were added according to the estimates from the linear mixed effects models (Table 3), and thus were corrected for all other variables in the model. All response variables, except CO<sub>2</sub> assimilation rate at low light intensity ( $A_{50}$ ), were log<sub>e</sub> transformed. The significances of relative light intensity (RLI), the quadratic term of RLI (RLI<sup>2</sup>), naturalization status (Nat) and the interaction between relative light intensity and naturalization status (RLI × Nat) are indicated as: \*\*\* < 0.001, \*\* < 0.01, \* < 0.05; ns, not significant.

TABLE 3. Results of linear mixed effects models testing the effects of relative light intensity (RLI), growth form and naturalization status and the interactions of the latter two with RLI on CO<sub>2</sub> assimilation characteristics of the exotic woody species

|        |  | d.f. | Light saturated CO <sub>2</sub> assimilation rate ( $A_{1200}$ ) |              | CO <sub>2</sub> assimilation rate at low light intensity ( $A_{50}$ ) |                  | Dark respiration ( $A_0$ ) |                  | Light compensation point (LCP) |                  |
|--------|--|------|--|--------------|---|------------------|----------------------------|------------------|--------------------------------|------------------|
|        |  |      | $\chi^2$   | <i>P</i>     | $\chi^2$  | <i>P</i>         | $\chi^2$                   | <i>P</i>         | $\chi^2$                       | <i>P</i>         |
| Fixed  | Growth form                              | 1    | 1.15   | 0.285        | 0.22  | 0.640            | 0.71                       | 0.400            | 0.05                           | 0.818            |
|        | Naturalization status                    | 1    | 0.57   | 0.451        | 0.09  | 0.761            | 0.002                      | 0.968            | 0.07                           | 0.793            |
|        | Relative light intensity (RLI)           | 1    | 2.25   | 0.134        | 19.78   | <b>&lt;0.001</b> | 16.93                      | <b>&lt;0.001</b> | 16.73                          | <b>&lt;0.001</b> |
|        | RLI <sup>2</sup>                         | 1    |  |              |   |                  | 9.86                       | <b>0.002</b>     | 9.01                           | <b>0.003</b>     |
|        | Growth form × RLI                        | 1    | 2.76   | <i>0.097</i> | 7.96  | <b>0.005</b>     | 0.25                       | 0.619            | 0.10                           | 0.749            |
|        | Naturalization status × RLI              | 1    | 0.13   | 0.723        | 6.27  | <b>0.012</b>     | 2.36                       | 0.125            | 2.48                           | 0.116            |
|        | Naturalization status × RLI <sup>2</sup> | 1    |  |              |   |                  | 2.29                       | 0.131            | 1.55                           | 0.213            |
|        | Growth form × RLI <sup>2</sup>           | 1    |  |              |   |                  | 0.004                      | 0.949            | 0.05                           | 0.820            |
| Random |  |      | s.d.   |              | s.d.  |                  | s.d.                       |                  | s.d.                           |                  |
|        | Block                                    |      | 0.16   |              | 0.16  |                  | 0.19                       |                  | 0.00                           |                  |
|        | Cages                                    |      | 0.15   |              | 1.10  |                  | 0.28                       |                  | 0.35                           |                  |
|        | Family                                   |      | 0.27   |              | 0.44  |                  | 0.17                       |                  | 0.00                           |                  |
|        | Species                                  |      | 0.28   |              | 0.64  |                  | 0.35                       |                  | 0.28                           |                  |
|        | Residual                                 |      | 0.43   |              | 2.28  |                  | 0.41                       |                  | 0.61                           |                  |

To test and account for non linear effects of RLI, we initially also included the quadratic term of relative light intensity (RLI<sup>2</sup>) and its interactions with growth form and naturalization status in the models, but removed them when they were not significant. Cage nested within block, and species nested within family were included as random terms.

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

(Master-of-some) or a combination thereof (Jack-and-master) (terminology *sensu* Richards *et al.*, 2006; Theoharides and Dukes, 2007; Funk, 2008; Davidson *et al.*, 2011). A previous study found that invasive herbaceous species had more biomass production than congeneric non-invasive (but naturalized) species under either shaded or non-shaded conditions (van Kleunen *et al.*, 2011). The discrepancy with our results could be explained at least partly by the difference in life cycle and growth properties between woody (our study) and herbaceous (van Kleunen *et al.*, 2011) species, and the fact that the studies considered different stages of the invasion process.

On average, both naturalized and non-naturalized species managed to elongate their stems slightly in response to shading (Table 2; Fig 1B). This indicates that despite the resource limitation that reduced overall growth, the plants actively changed allocation of resources towards stem elongation (van Kleunen and Fischer, 2005). After correction for growth form, naturalized species had on average taller stems than non-naturalized species across the whole experimental light gradient (Table 2; Fig. 1B). In our study, this higher stature might not have resulted in increased biomass of naturalized species because the plants could not escape from shade and did not compete. However, the larger stature of naturalized species implies that in nature they might be more capable of projecting their leaves in the higher strata of the vegetation, and thus be more successful in competition for light with neighbouring plants.

Plants growing in low-light environments are likely to maximize the efficacy of whole-plant light capture by producing leaves with lower construction costs (Kitajima, 1994; Walters and Reich, 1996; Reich *et al.*, 2003; Funk, 2013). Indeed, our results showed that the SLA increased with shading for all plants (Fig. 1D). However, as the number of leaves decreased for all study species (Fig. 1F), the total leaf area, on average, decreased with shading, and this was particularly the case for

naturalized species (Fig. 1C). The latter suggests that naturalized species were less capable of maintaining a large light-intercepting surface under shading. However, this did not result in a difference in biomass production between naturalized and non-naturalized species, which suggests that naturalized species might compensate somehow for the reduction in total leaf area. Therefore, future studies are needed to test whether naturalized and non-naturalized species differ in light-harvesting components of photosynthesis in terms of regulation of leaf chlorophyll content under shading (Funk, 2013).

#### *Physiology of naturalized and non naturalized plants in response to shading*

It is well known that plants acclimatize to shading by adjusting leaf physiological traits associated with CO<sub>2</sub> assimilation (Gommers *et al.*, 2013) in order to balance gains and losses of carbon efficiently under shading (Givnish, 1988). We found that the plants tended to decrease dark respiration rather than to increase light-saturated CO<sub>2</sub> assimilation ( $A_{1200}$ ) with shading, irrespective of whether they are naturalized or non-naturalized. This is in line with the revised carbon balance hypothesis positing that minimizing carbon losses is more efficient than enhancing potential carbon gain (Walters and Reich, 2000; Craine and Reich, 2005; Valladares and Niinemets, 2008). As the latter might actually lead to higher respiratory costs (Lambers *et al.*, 2008; Valladares and Niinemets, 2008; Funk, 2013), it was surprising that plants did not decrease the light-saturated CO<sub>2</sub> assimilation when grown in shade. However, in natural vegetation where plants might experience sun flecks (i.e. temporary exposure to high-light patches), the maintenance of a high light-saturated CO<sub>2</sub> assimilation rate might be advantageous (Küppers *et al.*, 1996; Valladares *et al.*, 1997).

The naturalized and non-naturalized species did not differ in the average values of their CO<sub>2</sub> assimilation characteristics. However, the increase in CO<sub>2</sub> assimilation rate measured at low light intensity ( $A_{50}$ ) with shading was stronger for naturalized than for non-naturalized species. As a consequence, naturalized species were better able to maintain a high CO<sub>2</sub> assimilation rate at low light intensity ( $A_{50}$ ) when grown under such low-light conditions (i.e. 7 and 25 % of ambient light) than non-naturalized species. Possibly, this did not result in a higher biomass production of naturalized species under shaded conditions because they also simultaneously reduced the total leaf area. This indicates that naturalized and non-naturalized woody species may have different strategies for dealing with shading.

### Conclusions

The woody plants in our study showed strong morphological and physiological plasticity in response to a shading gradient, and all of them managed to survive and produce biomass, even in the most severe shading treatment (7 % of ambient light). This suggests that all of these plants might have the potential to establish in open habitats as well as in forest understoreys, at least as saplings. Nevertheless, although all the species have been introduced >100 years ago, which should have given most of them enough opportunity to establish naturalized populations (Kowarik, 1995), not all of them have done so. This suggests that there must be some differences between the naturalized and non-naturalized species. Indeed, the naturalized species grew taller than the non-naturalized species. Moreover, the decrease in total leaf area and the increase in CO<sub>2</sub> assimilation measured at low light intensity with shading were stronger for the naturalized species. However, during the growing period of the plants in our experiment (approx. 19 weeks), these differences in trait values and their plasticity did not yet translate into differences in above-ground biomass production at any of the light intensities. Possibly, differences in performance, which would ultimately favour the population establishment of the naturalized species over the non-naturalized species, will only become apparent after a longer time in long-lived woody species.

### SUPPLEMENTARY DATA

Supplementary data are available at [www.aob.oxfordjournals.org](http://www.aob.oxfordjournals.org) and consist of the following. Figure S1: mean values of morphological traits of tree and shrub species in response to shading. Figure S2: mean values of assimilation rate at low light intensity ( $A_{50}$ ) of the tree and shrub species in response to shading.

### ACKNOWLEDGEMENTS

We thank Stefanie Fehrman, Otmar Ficht, Linda Lörcher, Christine Giele, Katya Stift-Mamonova and Demissew Tsigemelak for practical assistance, and Wayne Dawson, Marc Stift, Janosch Sedlacek, Mialy Razanajatovo, Gregor Müller and Samuel Carleial for valuable comments on previous drafts of the manuscript. We thank all the botanic gardens (Table 1) that kindly sent us seeds of the study species, and Veit Dörken for his valuable suggestions for seed sources. Y.H.F. was

supported by the China Scholarship Council (CSC). The data associated with this paper will be archived in the Dryad repository.

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