Seedling growth and biomass allocation in relation to leaf habit and shade tolerance among 10 temperate tree species

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Received May 5, 2014; accepted May 18, 2015; published online June 26, 2015; handling Editor Sean Thomas

Initial growth of germinated seeds is an important life history stage, critical for establishment and succession in forests. Important questions remain regarding the differences among species in early growth potential arising from shade tolerance. In addition, the role of leaf habit in shaping relationships underlying shade tolerance-related differences in seedling growth remains unresolved. In this study we examined variation in morphological and physiological traits among seedlings of 10 forest tree species of the European temperate zone varying in shade tolerance and leaf habit (broadleaved winter-deciduous species vs needle-leaved conifers) during a 10-week period. Seeds were germinated and grown in a controlled environment simulating an intermediate forest understory light environment to resolve species differences in initial growth and biomass allocation. In the high-resource experimental conditions during the study, seedlings increased biomass allocation to roots at the cost of leaf biomass independent of shade tolerance and leaf habit. Strong correlations between relative growth rate (RGR), net assimilation rate (NAR), leaf area ratio (LAR), specific leaf area (SLA) and leaf mass fraction (LMF) indicate that physiology and biomass allocation were equally important determinants of RGR as plant structure and leaf morphology among these species. Our findings highlight the importance of seed mass- and seed size-related root morphology (specific root length—SRL) for shade tolerance during early ontogeny. Leaf and plant morphology (SLA, LAR) were more successful in explaining variation among species due to leaf habit than shade tolerance. In both broadleaves and conifers, shade-tolerant species had lower SRL and greater allocation of biomass to stems (stem mass fraction). Light-seeded shade-intolerant species with greater SRL had greater RGR in both leaf habit groups. However, the greatest plant mass was accumulated in the group of heavy-seeded shade-tolerant broadleaves. The results of our study suggest that the combinations of plant attributes enhancing growth under high light vary with shade tolerance, but differ between leaf habit groups.

Keywords: biomass partitioning, ontogeny, photosynthesis, relative growth rate.

Introduction

A variety of tree species coexist in forests of the temperate zone. However, not all species coexist within the same spatio-temporal settings. Differences in requirements for light, water and nutrients, and in life-history traits, as well as plant morphology and physiology give rise to variation in species’ successional status and abundance. The seedling stage is critical in forest trees, because survival and performance at this phase will affect composition, structure and functioning of future forest ecosystems. While the light environment often serves as an abiotic filter during establishment, even shade-tolerant species may utilize forest gaps to promote regeneration from seed (Yamamoto 2000, Bilek et al. 2014, Zhu et al. 2014). Comparisons of seedling development of contrasting species in uniform light conditions can help elucidate underlying morphological and...
physiological traits important for growth and survival. Understanding how seedlings of tree species perform relative to each other in a common environment will contribute to the knowledge of mechanisms that maintain species diversity in forest communities and to inform forest management practices that emphasize multifunctional and biodiversity-oriented objectives.

To this end it is important to recognize variation among species in traits affecting seedling carbon balance and growth, particularly for trees regenerating from seed. Biomass allocation patterns may affect the carbon status of a developing seedling through shifts in photosynthetic ‘source’ relative to the respiratory ‘sink’ within a plant. Biomass allocation also affects capture of light, nutrients and water, and thus growth. In terms of classical plant growth analysis theory, plant relative growth rate (RGR) is divided into two components: net assimilation rate (NAR, change in plant mass per unit leaf area per unit time) and leaf area ratio (LAR, leaf area per unit plant mass). Leaf area ratio is further defined as a product of specific leaf area (SLA, leaf area per unit leaf mass) and leaf mass fraction (LMF, ratio of leaf mass to total plant mass) (Evans 1998, Poorter and Garnier 1999, Poorter et al. 2009). Variation in RGR among woody plant species more strongly correlates with SLA than with LMF (Cornelissen et al. 1998, Wright and Westoby 2000). Thus, knowledge of variation in leaf morphology and seedling biomass allocation patterns may help elucidate sources of variation in early growth of tree species.

Variation in RGR and associated traits may be related to species’ successional status, leaf habit or shade tolerance (Valladares and Niinemets 2008). In broad comparisons among woody plants, high RGR is often associated with high values of SLA, leaf nitrogen concentrations and leaf-level carbon assimilation rates (Reich et al. 1998b, 1998c, Wright et al. 2004). Trait complexes such as leaf morphology, leaf lifespan, seed mass and plant physiology are interrelated when interpreted for species’ shade tolerance or growth (Reich et al. 1998b, Niinemets 2006, Baltzer and Thomas 2007b, Hallik et al. 2009). Early successional shade-intolerant species typically are small-seeded, have high growth rates and have high values of RGR-related traits (Reich et al. 1998b, Walters and Reich 2000, Baltzer and Thomas 2007b). However, the view that shade-intolerant species trade off between growth potential in high light and survival in low light conditions (Kitajima 1994, Walters and Reich 2000) has been challenged by the observation that RGR crosses over between light environments in species of contrasting shade tolerances (Sack and Grubb 2001, 2003, Baltzer and Thomas 2007b). In very young seedlings in both light and shade, variation in RGR is driven initially by seed size-related differences in SLA (and thus LAR), whereas NAR becomes a stronger determinant of RGR in later stages (Sack and Grubb 2001). Because of inherent differences in leaf morphology between temperate evergreen needle-leaved conifers and deciduous broadleaved angiosperms, additional traits and mechanisms may be responsible for variation in growth. In the first growing season from seed, differences arising from direct effect of leaf lifespan are not immediately realized, but may reflect other correlated traits that influence leaf-lifetime return on carbon investment, namely, SLA and leaf photosynthetic rates (Reich et al. 1997, Hallik et al. 2009). These traits will also affect species’ ability to persist in the shade beyond the seedling stage. It is thus of interest to determine how variation in growth and biomass allocation patterns and trait complexes between groups of species of contrasting leaf habit and shade tolerance affects their relative competitive ability during early ontogeny.

The relative allocation of resources belowground is an important trait related to shade tolerance. Shade-tolerant species typically exhibit higher root/shoot ratios compared with shade-intolerant ones (Kitajima 1994, Walters and Reich 2000, Day et al. 2014). Building large root systems that may also serve as storage organs allows the establishment of seedlings that are less susceptible to resource limitations and stresses associated with low-light environments. However, root morphology has received much less attention (Reich et al. 1998b). Root morphology may be defined by the specific root length (root length per unit root mass)—SRL. Even with a similar level of plant biomass investment belowground, species with finer roots (i.e., greater SRL) may capture belowground resources more effectively than those with lower SRL. This advantage may allow species to better compete in overall resource-limited environments. Thus, knowledge of variation in root structure in addition to relative partitioning of plant growth may contribute to a better understanding of mechanisms of shade tolerance.

In this study we compared growth and biomass allocation, and growth-related leaf and root morphology of 10 forest tree species of the European temperate zone that are important both ecologically and commercially (Table 1). Among the studied tree species three are stand-forming monodominants—Fagus sylvatica [(L.), Picea abies [(L.) H.Karst.] and Pinus sylvestris (L.)—but may also grow as an admixture with other species. The other species included in our study occur mainly as admixtures in various proportions in forest stands, depending on the stage of succession. Betula pendula (Roth), Larix decidua (Mill.) and P. sylvestris are early successional species, whereas Abies alba (Mill.), F. sylvatica, Acer pseudoplatanus (L.), Acer platanoides (L.), Tilia cordata (Mill.) and P. abies are late successional. For the purpose of this study, species were assigned to four groups based on their shade tolerance class and leaf habit (Table 1).

We were interested in exploring to what extent growth and biomass allocation vary in relation to shade tolerance and leaf habit in seedlings of the 10 examined species during the first growing season after germination in uniform environmental conditions, and how biomass partitioning patterns, and leaf and root traits were linked to whole-plant growth. An important aim of our study was to quantify the temporal dynamics of the traits investigated over the first weeks of growth where seed mass and
Species with seeds requiring cold stratification. Needles-leaved conifers, and shade-tolerant broadleaves and conifers (Table 1) followed standard procedures for each species (Table 1) followed standard procedures for each species (Table 1) followed standard procedures for each species (Table 1). Species were ranked according to decreasing shade tolerance within each of the two leaf habit groups.

| Species                | Leaf habit | Shade tolerance class | 1000 seed mass (g) | Duration of experiment (days) |
|------------------------|------------|-----------------------|--------------------|-------------------------------|
| Fagus sylvatica        | Broadleaved| 3 (t)                 | 274.36±0.74        | 61                            |
| Acer platanoides       | Broadleaved| 4 (t)                 | 143.64±0.74        | 60                            |
| Acer pseudoplatanus    | Broadleaved| 4 (t)                 | 239.91±0.74        | 76                            |
| Tilia cordata          | Broadleaved| 5 (t)                 | 27.49±0.74         | 98                            |
| Betula pendula         | Broadleaved| 7 (int)               | 0.14±0.74          | 68                            |
| Abies alba             | Needle-leaved | 3 (t)           | 46.12±0.74        | 60                            |
| Picea abies            | Needle-leaved | 5 (t)           | 6.79±0.74         | 67                            |
| Pinus nigra            | Needle-leaved | 7 (int)           | 25.03±0.74        | 68                            |
| Pinus sylvestris       | Needle-leaved | 7 (int)           | 7.14±0.74         | 68                            |
| Larix decidua          | Needle-leaved | 8 (int)           | 6.15±0.74         | 68                            |

1Species shade tolerance class scales from 1—very shade-tolerant to 9—completely intolerant to shade (Ellenberg 1978). In this study species with shade tolerance class ≤5 are considered shade-tolerant (t), and those with a class ≥6 are considered shade-intolerant (int).

2Species with seeds requiring cold stratification.

Initial differences in biomass partitioning patterns may influence early ontogeny. We hypothesized that differences among species in first season growth from seed and initial biomass allocation will be related to leaf habit (needle-leaved vs broadleaved) and shade tolerance. Specifically we hypothesized that (i) compared with shade-tolerant species, seedlings of shade-intolerant species will have greater RGR within a particular leaf habit type, and greater values of traits typically associated with high RGR—LAR, SLA and photosynthetic rates; (ii) broadleaved species will have faster growth than needle-leaved conifers; and (iii) variation in RGR will be related more to plant structure and leaf morphology (LAR, SLA) than physiology (NAR, photosynthesis) or biomass allocation during early ontogeny.

Materials and methods

Plant material

Seeds of 10 tree species used in the study (Table 1) were collected from mature stands in Poland. Storage and stratification for seeds requiring cold stratification (Table 1) followed standard procedures for each species (Suszka et al. 1996). The seeds of Tilia were scarified before stratification (Tylkowski 1998). Stratification was applied in the Institute of Dendrology in Kórnik, Poland, and after it was completed, the seeds were shipped to the Department of Ecosystem Science and Management, Texas A&M University (College Station, TX, USA). Species were grouped a priori based on their leaf habit and shade tolerance into four categories: shade-intolerant broadleaves and conifers, and shade-tolerant broadleaves and conifers (Table 1). This grouping was used in the subsequent analyses. The shade tolerance estimates for our set of species were based on those given in Ellenberg (1978), but correspond well with estimates given in Niinemets and Valladares (2006). Although the term ‘conifer’ does not exactly reflect the needle-leaved habit, these two terms will be used interchangeably hereafter for brevity. The conifers in the study included both evergreen and deciduous species (Larix), whereas all broadleaves were winter deciduous. The class of shade-intolerant broadleaves was represented by a single species (B. pendula).

Seeds were sown into a mixture (v/v) of vermiculite (55–65%) and sphagnum peat moss (35–45%), and covered with 1.5 cm of substrate or 0.5 cm of washed sand, except for seeds of Betula. The substrate in germination trays was kept moist all the time (sprayed with deionized water), and the trays were covered with transparent plastic lids. Species with stratified seeds required alternating temperatures during germination (+20 °C/8 h day and +4 °C/16 h night); therefore, their trays were moved between a growth chamber (Conviron, BDR 16; Winnipeg, MB, Canada) during the day and a cold room (Environmental Growth Chambers, Chagrin Falls, OH, USA) at night. Trays with the five other species were kept at constant conditions of +20 °C, 16 h day/8 h night in a growth chamber.

The first seedlings for most species appeared within 10 days of sowing, but full germination occurred 15–30 days after sowing; thus, the goal to obtain seedlings of all 10 species at about the same time was achieved. Germination success varied between 13% for Fagus and 93% for P. sylvestris. After germination, the trays were placed for 5–8 days under natural light −12 h/day; −20 °C and −70% relative humidity. Subsequently, individual germinated seedlings were transplanted to 1.6 l plastic pots (24 cm tall, 10 × 10 cm at the top, 7 × 7 cm at the bottom) filled with a mixture (v/v) of vermiculite (50–60%), sphagnum peat moss (20%), perlite (10–20%) and washed sand (10%).

Experimental conditions and measurements

The experiment was conducted in two (90 × 180 × 200 cm) walk-in plant growth chambers (Environmental Growth Chambers, M18). Conditions inside the chambers were set at 75% relative humidity, 24/18 °C (day/night) temperature and 16 h/8 h (day/night) photoperiod. Light was supplied by fluorescent tubes and incandescent bulbs above a clear Plexiglas barrier. Mean (±SE) light intensity (photosynthetic photon flux density (PPFD), 400–700 nm) at the seedling level was 443 ± 1.0 and 438 ± 5.9 µmol m−2 s−1 in the two chambers, which is equivalent to ∼25 mol m−2 day−1 daily photon irradiance. This light intensity represents ∼30% of full sunlight during summer at latitude −52°N and is comparable to light levels experienced at stand edges or large canopy openings.

Between 37 and 44 days after sowing (6–12 days after transplanting), individual plants were randomly assigned to the two growth chambers. The two chambers were treated as two replications (i.e., blocks), as each chamber contained all 10 species. Within each chamber the 12 individuals of each species...
were randomly arranged. The pots were watered every day for the first week after randomization, subsequently every second day for 4 weeks and then twice a week until final harvest. Seedlings were not fertilized throughout the experiment.

Five destructive harvests (H₁–H₅) were conducted throughout the course of the experiment. The first harvest (H₁) was conducted simultaneously with transplanting seedlings into pots over a 10-day period. At that time nine individuals from each species were randomly sampled from the sowing trays. For each subsequent harvest, three seedlings of each species per chamber (replications within chambers) were sampled for a total of six plants per species. The second harvest (H₂) was completed 26–33 days after transplanting. The three subsequent harvests occurred at intervals of 10–14 days. The sequence of species at harvests was kept constant to keep the intervals between harvests as even as possible within the species; however, the total duration of the experiment varied from 60 to 70 days depending on the species (Table 1).

At harvest, whole seedlings were collected and divided into biomass components—cotyledons, leaves, hypocotyl, stem above hypocotyl and roots. All leaves and cotyledons, and entire root systems were scanned with a calibrated scanner and analysed with WinRHIZO image analysis software (Version 2001a, Regent Instruments, Québec City, QC, Canada) for projected area (foliage without petioles) and length (roots). Roots were scanned in water-filled trays. In cases where it was too large to fit into a tray, the root system was divided into sections that were scanned separately and the results were summed. Subsequently, all plant parts were dried at 65 °C to a constant weight, and dry mass was measured. Because species and individual plants within species varied in retention of cotyledons, the leaves and cotyledons were pooled for the analysis and are referred to as leaves hereafter. Similarly, hypocotyl and stem data were pooled and are referred to as stem hereafter.

Relative growth rate (RGR; g g⁻¹ plant day⁻¹) between the five consecutive harvests (from H₁ to H₅) was calculated for each species within a chamber based on total (aboveground plus belowground) plant biomass (W) as (lnW₂ – lnW₁)/(H₂ – H₁), where H₁ and H₂ are the consecutive harvests for each of the four intervals (Hunt 1982). Mean values over those same time intervals for the net assimilation rate (NAR; gₙ₉₆ μmol m⁻² s⁻¹) were also calculated according to a formula given in Hunt (1982). At each harvest, leaf area ratio (LAR; cm² g⁻¹ plant), specific leaf area (SLA; cm² g⁻¹ leaf), specific root length (SRL; cm g⁻¹ root), leaf mass fraction (LMF; ratio of leaf mass to plant mass), stem mass fraction (SMF; ratio of stem mass to plant mass) and root mass fraction (RMF; ratio of root mass to plant mass) were calculated for each species per chamber.

Before the third (H₃) and fifth harvests (H₅), light-saturated photosynthesis rate (Aₘₕₐₓ; expressed on a leaf area basis) was measured on attached leaves of three seedlings of each species per growth chamber with the LI-COR 6400 portable photosynthesis system (LI-COR Biosciences, Lincoln, NE, USA). The Aₘₕₐₓ of needle-leaved species was measured using a conifer chamber (LICOR). The PPFD (mean ± SE) was 1600 ± 0.10 μmol m⁻² s⁻¹ for broadleafes (2 × 3 chamber and red/blue LED light source) and 947 ± 1.83 μmol m⁻² s⁻¹ for conifers (external light in the growth chamber), representing light-saturated conditions based on independent checks. Photosynthesis was determined at a reference CO₂ concentration of 385 ± 0.05 μmol mol⁻¹, measurement temperature (block temperature) of 24 °C and ambient humidity. Leaves present in the measurement chamber were collected and processed as described above. Leaf samples at the final harvest (H₅) were dried, ground to fine powder and analysed for foliar nitrogen (N) and carbon (C) concentration using the ECS 4010 CHNS-O analyzer (Costech Analytical Technologies, Inc., Cernusco S/Nav.- MI, Italy).

**Analysis**

Analysis of variance (ANOVA) was used to test for the effect of species, chambers (blocks), time (either at harvests (H₁–H₅) or time intervals between harvests—T₁–T₄) and for significance of interactions among these factors (see Table 2). Observed values at harvests for individual trees were analysed. However, for RGR and NAR mean interval-based values between consecutive harvests for species within replicate chambers were used. The data were In-transformed before analysis where appropriate, except for RGR, NAR, LMF, SMF, RMF, Aₘₕₐₓ and nitrogen expressed on a leaf mass basis (N₉₈₆). A similar analysis was performed for the four groups of species (shade-tolerant and -intolerant within broadleaved and needle-leaved groups, see Table 1), where groups were used instead of species in the model. In cases of statistically significant differences between species or groups of species, means were compared using the Tukey–Kramer HSD test. For each trait, a contrast analysis was performed to test for differences between consecutive harvests within a group of species. Separate ANOVAs were used to test for differences among groups of species within each harvest. However, because species differed in total biomass and likely in ontogenetic stage at each harvest, we also used allometric analysis to investigate variation in growth and allocation.

We used the standardized major axis procedure in the (S) MATR program (Warton et al. 2006) to analyse allometric relationships between biomass components (In-transformed). We hypothesized isometry (slope = 1) for the relationships of aboveground biomass components and belowground mass vs total plant mass. Although plotting leaf, stem and root mass vs plant mass should be equivalent to analysis of LMF, SMF and RMF, respectively, we also plotted those ratios against plant mass (Foorter and Nagel 2000).

First we tested for heterogeneity of slopes among species groups for each relationship. The Bonferroni correction was applied to adjust the probability level for multiple comparisons among slopes. We found significant differences in slopes among...
species groups, but for most relationships we also found some species groups sharing common slopes (see Results). Subsequently, we tested only those groups for shifts in elevation (intercept) or shifts along a common slope (Warton et al. 2006).

Bivariate relationships between traits were analysed using Pearson correlation analysis. We used principal component analysis (PCA) on correlations followed by factor analysis to determine interrelationships among traits explaining variation among groups of species in our experiment. The traits included in the PCA were total plant biomass, seed mass, LMF, SMF, RMF, SLA, SRL, RGR and NAR. For the RGR and NAR the average value per species per chamber between the two consecutive harvests was used, and for the other traits the average values per species per chamber at the later of those two harvests were used. The variable $A_{\text{max}}$ was not included in the PCA, because it was measured only at two harvests and would reduce the number of items in the analysis. The interpretation of rotated factor pattern was carried out after using the varimax rotation method. The trait was deemed to load on a component if the factor loading was $\geq 0.41$. The ANOVA and PCA analysis were performed in the JMP 9.0.0 (SAS Institute Inc., Cary, NC, USA) statistical package.

### Results

#### Variation in growth and biomass allocation

Species differed significantly in all examined traits (Table 2). At the final harvest after 60–70 days of growth, seedling biomass varied more than 77-fold among species (mean $\pm$ SE: $A. \text{pseudoplatanus}$ 5.55 $\pm$ 0.25 g; $P. \text{abies}$ 0.07 $\pm$ 0.006 g), leaf area varied more than 170-fold ($T. \text{cordata}$ 412.82 $\pm$ 18.27 cm$^2$; $P. \text{abies}$ 2.37 $\pm$ 0.18 cm$^2$) and root length more than 18-fold ($T. \text{cordata}$ 1782.48 $\pm$ 145.49 cm; $A. \text{alba}$ 97.51 $\pm$ 16.13 cm), see Table S1 available as Supplementary Data at Tree Physiology Online. Similarly, in the analysis of species’ shade tolerance and leaf habit groups, significant differences among groups were found for all traits (Table 2). However, the species $\times$ time or group $\times$ time interaction component was significant for most traits, except for RGR and NAR (Table 2), as a consequence of growth rate differences among species or species groups. Because of differences in growth, for a given harvest, species differed in size and ontogenetic stage. For example, shade-tolerant conifers only at $H_3$ accumulated a comparable plant mass to that of tolerant broadleaves at $H_1$, and at $H_2$ attained a leaf area comparable to that of both shade-intolerant groups at $H_1$ (Figure 1). Compared with other groups, shade-tolerant conifers produced less new growth throughout the experiment (Figure 1), and maintained a significant proportion of cotyledons in their foliage (>50% both in mass and in area) until the last harvest (not shown). Therefore, allometric analysis was used to resolve growth traits and biomass allocation trends against plant size.

The SMA regressions generally fitted well to groups of species when component biomass was plotted against plant mass.
Slopes differed among species groups. Some species groups shared a common slope of regression lines, but in general, the groups of common slope did not correspond to either leaf habit or shade tolerance groupings. For example, intolerant broadleaves shared a common slope with tolerant conifers, and tolerant broadleaves shared a slope with intolerant conifers for regressions of aboveground and belowground biomass vs plant mass (Table 3). However, for some relationships, especially those involving belowground and leaf biomass, the intercepts of regression lines differed between species groups that shared common slopes. For most other cases where species groups shared regression slopes, shifts along a common slope were significant due to variation in plant size among groups (Table 3).

Biomass of all components increased with plant size, but at different rates (Table 3). In all groups of species, the slopes of aboveground mass vs plant mass relationships were significantly lower from hypothesized isometry, and for belowground mass vs plant mass the slopes were >1. This indicates that root growth was relatively faster than that of aboveground parts in all groups (increasing root : shoot ratio with plant size), which was confirmed by slopes of aboveground vs belowground mass significantly smaller than 1.0 (Table 3).

Because of different rates of increase for various plant parts, the proportional biomass allocation varied with plant size depending on the component (Figure 2). The LMF decreased and RMF increased with plant size. The SMF increased for shade-tolerant broadleaves and decreased for shade-intolerant conifers. The changes in SMF were smaller than for the other components, but SMF separated species by shade tolerance group (Figure 2). For the proportional biomass allocation where groups of species shared a common slope, there were also significant shifts in elevation and shifts along a common slope. At a comparable plant size both groups of shade-tolerant species had greater SMF, and tended to have smaller LMF than shade-intolerant ones. The group of shade-tolerant conifers had the fastest rates of increase in RMF and decrease in LMF among the compared groups (Figure 2), but it also accumulated the least biomass. The groups that shared common slopes for proportional allocation (Figure 2) were not equivalent to those for analysis of biomass components (Table 3).

Declining trends in SLA, LAR and SRL with increasing plant size were found in all four groups of species (Figure 3). There was a clear distinction between broadleaves and conifers in terms of SLA and LAR. Although shade-intolerant conifers shared a common slope with both groups of broadleaves for SLA, the conifers had lower values of both traits than broadleaves across a range of common plant sizes (Figure 3). At a given harvest the group of intolerant broadleaves had greater LAR than tolerant broadleaves (see Table S2 available as Supplementary Data at Tree Physiology Online). However, when compared at the same plant size, the tolerant broadleaves had greater values of LAR and SLA. Within the conifers, the group of shade-intolerant species had greater SLA and LAR than shade-tolerant species at a given plant size, but also a slower rate of decrease of both traits with plant size (Figure 3).

The pattern of variability in SRL was dominated by very high values in the group of shade-intolerant broadleaves (comprising only B. pendula) compared with other groups. At the first harvest, SRL in that group was 7.5–13 times greater than in the other groups, and even at the end of the experiment the groups varied from three- to over fivefold (Figure 3). Within a given leaf habit group, shade-intolerant species had finer roots (greater

Figure 1. Time course of total seedling biomass (g), leaf area (cm²) and root length (cm) for four plant functional groups of temperate tree species. At each harvest data from all seedlings per group were averaged (n varies from 9 to 36 at harvest 1, and from 6 to 24 at harvests 2–5); error bars represent the standard error of the mean. The time scale starts at the day of the first harvest. A solid line connecting consecutive points denotes significant difference between harvests within a group at P ≤ 0.05; a dashed line denotes lack of significance. Note the logarithmic scale of the y-axis.
SRL) than shade-tolerant species. The group of shade-tolerant conifers had the lowest SRL and the steepest decline in this trait with plant size. However, except for intolerant broadleaves, the SMA regressions fitted rather poorly to the SRL vs plant size relationships for the examined groups (Figure 3).

The RGR and NAR represent mean values for a group of species, and thus we did not use the regression approach in this analysis, because there were too few points to obtain a meaningful regression. In all groups there was an overall declining trend in RGR with increasing plant size (Figure 4). On average, RGR of shade-intolerant species (both broadleaves and conifers) was greater than for shade-tolerant conifers (Tukey-Kramer HSD test $\alpha = 0.05$; Figure 4). However, when compared at individual time intervals, significant differences (contrast analysis $P \leq 0.0191$) in RGR between shade tolerance groups was found only on one occasion within each leaf habit group—at $T_{2.3}$ for broadleaves, and at $T_{3.4}$ for conifers (Figure 4). Similarly, there was a tendency for broadleaves to have greater RGR than conifers within both shade tolerance groups (Figure 4), but the difference between broadleaves and conifers was statistically significant only in the shade-tolerant group, and only on one occasion (at $T_{3.4}$ $P \leq 0.0237$). At the end of the experiment all groups of species had comparably low RGR, but at varying plant size (Figure 4).

Although NAR varied through time (Table 2), the changes observed with increasing plant size were not consistent among groups of species (Figure 4). On average, the NAR of shade-intolerant conifers was greater than in all other groups of species. Within a given leaf habit group, the only significant differences ($P \leq 0.0291$) were found between shade-intolerant and -tolerant conifers at $T_{2.3}$ and $T_{3.4}$: NAR was a slightly better correlate of RGR ($r = 0.67$, $P < 0.0001$) than was LAR ($r = 0.64$, $P < 0.0001$) across all the species and time intervals. However, the strength of those correlations changed during the experiment. At $T_{1.2}$ the correlation was not significant for NAR, but at each consecutive time interval it was a stronger correlate of RGR ($r = 0.84$, $P < 0.0001$ at $T_{4.5}$) than was LAR.

Photosynthesis rates and leaf $N$

$A_{\text{max}}$ differed among individual species and among species groups ($P < 0.0001$). Photosynthesis rates were generally higher at the earlier harvest ($H_3$; after 36–45 days) than at the last harvest ($H_5$; after 60–70 days, Figure 5). Significant time × species ($P < 0.0001$) and time × group of species ($P = 0.0360$) interaction terms resulted mostly from variation among species or groups of species in the magnitude of differences in $A_{\text{max}}$ between the two sampling dates. Shade-intolerant conifers had a greater $A_{\text{max}}$ than other groups at both harvests; in addition, at the last harvest $A_{\text{max}}$ of shade-tolerant conifers was greater than that of tolerant broadleaves (Figure 5).

At the last harvest both individual species and groups of species varied in leaf $N$ expressed on an area and mass basis.

Table 3. The slope and intercept estimates from the SMA regression analysis between biomass variables (ln-transformed) in examined shade tolerance/leaf habit groups of species. Slope values for a given relationship followed by the same lower-case letter do not differ significantly ($P \leq 0.05$) among groups. Asterisks indicate significant differences ($P \leq 0.05$) in the intercepts between groups sharing a common slope. $^1$

| Relationship | Group | Slope (95% CI) | Intercept (95% CI) | N | $R^2$ |
|--------------|-------|--------------|-------------------|---|-------|
| Aboveground vs Plant mass | int. broadl. | 0.9145 (0.894/0.936) a | −0.5622 (−0.630/−0.494) | 33 | 0.996 |
| | int. conif. | 0.9637 (0.951/0.977) b | −0.3977 (−0.428/−0.367) | 99 | 0.996 |
| | tol. broadl. | 0.9640 (0.942/0.986) b | −0.4184 (−0.458/−0.379) | 132 | 0.983 |
| | tol. conif. | 0.9114 (0.878/0.946) a | −0.6109 (−0.722/−0.500) | 66 | 0.978 |
| Leaf mass vs Plant mass | int. broadl. | 0.9101 (0.886/0.934) ab | −0.7719 (−0.849/−0.695)* | 33 | 0.995 |
| | int. conif. | 0.9821 (0.968/0.996) c | −0.5186 (−0.551/−0.486) | 99 | 0.995 |
| | tol. broadl. | 0.9194 (0.883/0.957) b | −0.9086 (−0.976/−0.841)* | 132 | 0.946 |
| | tol. conif. | 0.8728 (0.835/0.912) a | −1.0870 (−1.213/−0.962)* | 66 | 0.969 |
| Stem mass vs Plant mass | int. broadl. | 0.9546 (0.902/1.010) b | −2.2070 (−2.381/−2.032) | 33 | 0.976 |
| | int. conif. | 0.8750 (0.843/0.908) a | −2.5450 (−2.622/−2.468) | 99 | 0.965 |
| | tol. broadl. | 1.0816 (1.056/1.108) c | −1.4280 (−1.476/−1.380) | 132 | 0.980 |
| | tol. conif. | 1.0841 (0.983/1.196) c | −1.3500 (−1.669/−1.000) | 66 | 0.846 |
| Belowground vs Plant mass | int. broadl. | 1.3380 (1.266/1.413) b | −0.536 (−0.774/−0.298)* | 33 | 0.977 |
| | int. conif. | 1.1400 (1.103/1.179) a | −1.0713 (−1.161/−0.982)* | 99 | 0.973 |
| | tol. broadl. | 1.1700 (1.121/1.220) a | −1.1586 (−1.249/−1.068)* | 132 | 0.940 |
| | tol. conif. | 1.3380 (1.263/1.417) b | −0.3013 (−0.554/−0.049)* | 66 | 0.947 |
| Aboveground vs Belowground mass | int. broadl. | 0.6836 (0.635/0.736) a | −0.1959 (−0.436/0.045)* | 33 | 0.960 |
| | int. conif. | 0.8451 (0.807/0.885) b | 0.5077 (0.368/0.647) | 99 | 0.948 |
| | tol. broadl. | 0.8242 (0.774/0.878) b | 0.5365 (0.388/0.686) | 132 | 0.867 |
| | tol. conif. | 0.6813 (0.622/0.747) a | −0.4056 (−0.669/−0.112)* | 66 | 0.865 |

$^1$The intercepts shown are those for the separate slopes models for each of the four groups. The asterisks are for tests conducted in a subsequent analysis for which neither the slopes nor intercepts are shown.
Leaf N varied among the individual species more than fivefold on a leaf area basis ($N_{area}$; mean ± SE from 0.46 ± 0.03 g m$^{-2}$ in *B. pendula* to 2.67 ± 0.39 g m$^{-2}$ in *...
P. nigra), and more than twofold on a leaf mass basis ($N_{mass}$; from 7.62 ± 1.15 mg g$^{-1}$ in *P. abies* to 17.52 ± 0.91 mg g$^{-1}$ in *P. sylvestris*). In terms of $N_{area}$, all four groups of species were statistically separated, but $N_{area}$ of conifers was greater than for broadleaves (Figure 5). In contrast, $N_{mass}$ did not differentiate species groups based on either leaf habit or shade tolerance (Figure 5), suggesting that SLA differences between conifers and broadleaves underpinned differences in $N_{area}$. Across all 10 species a significant positive correlation ($r = 0.84$, $P < 0.0001$) was found between $A_{max}$ and leaf N on a leaf area basis ($N_{area}$).

**How was the whole-plant growth related to morphology, physiology and biomass partitioning patterns?**

The PCA incorporating growth, morphological and physiological traits revealed that the first three components had eigenvalues above 1 and accounted for 81% of the total variance. These components were retained for rotation. The three rotated factors explained a significant portion of variation in the underlying traits as determined by the final communality values (Table 4). Although LMF, RGR and SRL showed some cross-loading on the factors, they were retained as variables for the interpretation of rotated factors.

Seed mass and SRL had the most influence on factor 1, but in opposite directions (Table 4, Figure 6). In addition, plant mass and SMF loaded positively, and LMF loaded negatively on the first factor. Thus, the first factor was associated with seed size-related morphology and biomass allocation (RL, SMF and LMF), which together affect total plant biomass. The first factor separated the studied species more or less according to shade tolerance groups, although the separation by shade tolerance was not as effective for conifers as in broadleaves. For example, *P. abies* was separated (had negative values of factor 1) from *A. alba* within the group of shade-tolerant conifers, because it had lower seed and plant mass and SMF, and much greater SRL. In the group of

Figure 4. Relative growth rate (RGR) and net assimilation rate (NAR) vs plant mass for the four examined groups of tree species. Each point represents an average for the group between the two consecutive harvests. Biomass at the later of these two harvests is represented on the x-axis. Error bars represent the standard error of the mean.

Figure 5. (a) Mean (±SE) values of area-based maximum photosynthetic rates measured at the third and fifth harvests, and (b) mass- ($N_{mass}$) and area-based ($N_{area}$) leaf nitrogen measured at the fifth harvest in four groups of species. Values sharing the same letter are not statistically different at $\alpha = 0.05$ in a Tukey–Kramer HSD test for each harvest in (a) and for each trait in (b).
Table 4. Values of rotated factor loadings and final factor communality estimates from the factor analysis, and the percent of variance explained by each factor. Bold values indicate loadings which were considered valid for the factor.

| Trait          | Factor 1 | Factor 2 | Factor 3 | Final communality |
|----------------|----------|----------|----------|-------------------|
| Plant biomass  | 0.7569   | 0.1227   | 0.1901   | 0.6240            |
| Seed mass      | 0.8537   | -0.1935  | -0.1022  | 0.7766            |
| RGR            | -0.0667  | 0.8041   | 0.4683   | 0.8703            |
| NAR            | 0.0444   | 0.8340   | -0.2056  | 0.7398            |
| LMF            | -0.5366  | 0.7703   | -0.1308  | 0.8985            |
| SMF            | 0.7163   | -0.3053  | 0.3555   | 0.7326            |
| RMF            | 0.0756   | -0.7987  | -0.1565  | 0.6681            |
| LAR            | -0.1327  | 0.3103   | 0.9152   | 0.9515            |
| SLA            | 0.2039   | -0.1801  | 0.9525   | 0.9813            |
| SRL            | -0.8298  | 0.0367   | 0.4390   | 0.8827            |
| % variance explained | 28.63 | 28.49 | 24.14 |

Some correlations from those presented above, as well as some correlations with variables which did not enter the PCA, are worth mentioning. No significant correlation was found between $A_{\text{max}}$ and RGR or seedling mass across all species. However, when analysed within leaf habit groups, the correlation was significant and positive between $A_{\text{max}}$ and RGR for both broadleaves and conifers, and for conifers between $A_{\text{max}}$ and plant mass. The $A_{\text{max}}$ correlated positively with NAR across the species ($r = 0.48$, $P = 0.0016$). Throughout the experiment, except for the first harvest, the correlation between NAR and RGR was stronger than between LAR and RGR. For conifers NAR was a stronger correlate of RGR ($r = 0.88$, $P < 0.0001$) than was LAR ($r = 0.66$, $P < 0.0001$), whereas in broadleaved species both traits showed similar correlations ($r = 0.77$, $r = 0.73$, $P < 0.0001$ for NAR and LAR, respectively). The LMF and RMF also showed significant correlations with RGR across all species ($r = 0.51$, $r = -0.61$, $P < 0.0001$, respectively), and these correlations were stronger when analysed for groups of species, except for shade-intolerant conifers.

Across all the species the correlation between LAR and SLA ($r = 0.85$, $P < 0.0001$) was stronger than between LAR and LMF ($r = 0.38$, $P = 0.0003$). The same trend was found for conifers independently of their shade tolerance groupings. However, for both groups of broadleaves the correlations of LAR were equally strong with SLA and LMF ($r = 0.84$, $r = 0.88$, $P < 0.0001$, respectively).

A significant negative correlation was found between RGR and seed mass ($r = -0.25$, $P = 0.0239$), and this correlation was largely a result of the presence of a light-seeded broadleaved species—$B. pendula$. In contrast, seed mass correlated positively with seedling biomass, but the strength of this relationship diminished over time from $r = 0.86$ at $H_1$ to $r = 0.39$ at $H_5$.

**Discussion**

The aim of the experiment was to compare early growth responses of seedlings of 10 temperate species of contrasting leaf habit and shade tolerance in the uniform, high-resource conditions of growth chambers representing the light environment of an intermediate-sized forest gap. The set of 10 tree species in our experiment constitute a significant group, owing to their common occurrence and dominance in many temperate forest community types throughout central Europe. While our group classifications were composed of relatively few species, which perhaps limits broader inferences, they nonetheless represent the key plant functional groups as well as genera found in northern temperate forests in many regions worldwide. Findings derived from controlled-environment studies (e.g., Reich et al. 1998b) along with studies of shade tolerance and growth traits...
in forest communities in the field (Niinemets and Kull 1994, Baltzer and Thomas 2007a, Janse-ten Klooster et al. 2007, Markesteijn and Poorter 2009) are necessary to better understand suites of traits underpinning shade tolerance and growth potential.

The uniform conditions of our experiment allowed the expression of the inherent variation in a suite of traits among the investigated species during early ontogeny. In this short-term study we expected to find a greater RGR and traits associated with high RGR in the shade-intolerant than in the shade-tolerant species within a given leaf habit group (broadleaved or needle-leaved). We also expected broadleaved species to have faster growth than needle-leaved conifers, and variation in RGR to be related more to plant structure and leaf morphology than to physiology or biomass allocation. In the course of the study we found large variation among species in the initial seedling growth, biomass allocation, leaf-level photosynthesis and root morphology that was related to leaf habit and shade tolerance. These differences among seedlings during their first season may have implications for further development and the success of recruitment.

**Do shade-intolerant species have greater RGR than shade-tolerant ones?**

The hypothesis that seedlings of shade-tolerant species trade off growth potential in high light with survival in low light (Kitajima 1994, Walters and Reich 2000) has led us to hypothesize a greater RGR in shade-intolerant than in shade-tolerant species in our study. Our findings partially confirmed that hypothesis. The RGR values tended to be greater in seedlings of shade-intolerant species than in shade-tolerant ones within both leaf habit groups. However, due to large variation in total seedling mass, it was difficult to compare groups of species at a similar plant size. Nonetheless, the contrasts in RGR values between tolerant and intolerant species occurred later in the study and not immediately following germination and early growth, suggesting that differences in growth rate were only fully realized following new leaf and seedling growth beyond the cotyledon stage. At the end of the experiment, all groups of species exhibited slowing growth rates as they showed similarly low RGR.

Variation in RGR may result from differences in whole-plant dry mass accumulation rate per unit leaf area (NAR), plant structure and leaf morphology (LAR, SLA), and biomass allocation patterns (LMF). Thus, comparing a set of temperate tree species varying both in leaf habit and shade tolerance as in this experiment, we were able to investigate whether determinants of RGR vary due to those functional attributes. In accordance with our first hypothesis, shade-intolerant species had higher LAR and SLA than tolerant species, but only within the group of needle-leaved conifers. In broadleaves, at a comparable plant size, the LAR and SLA were greater in shade-tolerant than in shade-intolerant species. This contrasts with the notion that species adapted to shade show lower SLA and LAR than shade-intolerant species (Kitajima 1994, Reich et al. 1998b). However, more species within shade-intolerant broadleaves would be needed to confirm the generality of the observed trend.

Positive correlations between RGR, LAR and SLA have often been found (Saverimutu and Westoby 1996, Reich et al. 1998b, Wright and Westoby 1999, 2000, Poorter et al. 2009), as in our experiment. However, given the trends we found in RGR, SLA and LAR, other traits likely come into play when it comes to explaining variation in the final seedling biomass. Biomass allocation provides part of this explanation. Maintaining a larger LMF allowed for greater final growth of shade-intolerant conifers, despite having lower LAR and RGR, compared with shade-intolerant broadleaves. Thus, our findings suggest that leaf and plant attributes enhancing leaf area display and light interception promoted growth in these uniform conditions independent of shade tolerance, but the combination of morphology and biomass allocation that lead to increased light interception may vary by leaf habit.

High RGR does not necessarily correspond to high absolute growth rates and seedling biomass shortly after germination, owing to differences in seed size (Niinemets 2006). Likewise, the almost 2000-fold variation in seed mass among the species in our experiment corresponded to a 77-fold difference in the final plant mass, whereas differences in RGR were only up to 19-fold. Seed mass was an important determinant of variation between the shade tolerance groups of species, as shown by the factor analysis (see below). Trends we found in seedling growth were associated with differences in seed mass, because early successional shade-intolerant species tend to have lighter seeds and greater RGR (Reich et al. 1998b, Niinemets 2006, Baltzer and Thomas 2007b). In fact, seed mass and RGR were negatively correlated in our dataset, as in other studies (Reich et al. 1998b, Walters and Reich 2000, Seiwa 2007). We also found a positive correlation between seed mass and seedling biomass, indicating that large-seeded species may have an initial size advantage over small-seeded ones. In many instances large-seeded species tend to perform better under hazards associated with seedling establishment (Westoby et al. 2002). Thus, seedlings arising from larger seeds have a greater biomass and potential to survive and recruit.

The effect of seed mass on seedling biomass diminished with time in our experiment, indicating that the stage of seedling development is important for such comparisons. It may be partly related to when the seedlings become independent of seed reserves, which likely varied in timing among our study species. All the compared species had photosynthetic cotyledons that contributed to carbon acquisition. However, it is extremely difficult to definitively confirm when the plant is no longer mobilizing its seed reserves (Fenner 1987). In addition to seed mass, the...
amount of neofomed shoot growth may contribute to differences in the final seedling biomass (Greenwood et al. 2008). We did not quantify the length of time to bud set, but we observed bud development at the last harvest; the group of shade-tolerant conifers had a higher proportion of seedlings with set buds than the other groups (not shown). Thus, they produced less biomass, perhaps as a result of earlier growth cessation, and not because of their seed mass. However, seed mass is an element of a suite of plant traits including plant size and longevity, time to maturity, seed output and seedling survival that together determine plant reproductive success (Moles and Westoby 2004, 2006). In addition RGR may be related to seed mass through its secondary correlations with other traits affecting those plant attributes.

In conclusion, in a comparatively high-light environment, small-seeded shade-intolerant species had greater RGR in both leaf habit groups, but it was the group of heavy-seeded shade-tolerant broadleaves that realized the greatest absolute growth and accumulated the greatest plant mass at the end of the experiment. The suite of traits that lead to successful growth may vary by shade tolerance group and depend upon the leaf habit.

**What traits determined variation in growth?**

We hypothesized that variation in RGR among groups of species in our experiment would be related more to plant structure and leaf morphology (LAR, SLA) than to physiology (NAR, photosynthesis) or biomass allocation during early ontogeny. Variation in light-saturated net photosynthesis and leaf N was strongly associated with leaf habit (i.e., needle-leaved vs broadleaved), and only partially with shade tolerance (see Figure 5). The area-based estimates of photosynthesis and leaf N showed a strong positive correlation in our study. This type of association has been found in other studies both across (Evans 1989, Bond et al. 1999, Chmura and Tjoelker 2008) and within species (Ellsworth and Reich 1992), but for broad comparisons among species, such correlations on an area basis are typically weaker than on a mass basis (Peterson et al. 1999, Wright et al. 2004). Most of leaf N is associated with photosynthetic proteins, and thus may also reflect the differences in photosynthetic potential between shade-intolerant and -tolerant species. However, the differences we observed were largely the result of differences in SLA between the two leaf habit groups (see Figure 3), because SLA serves as a conversion factor between area- and mass-based estimates (Niinemets 1997). Consequently, the lower photosynthetic rates at the last harvest in our study also resulted from the decreasing trend in SLA over time.

The leaf-level photosynthetic rates were positively correlated with seedling growth, but a stronger association was found when rates of carbon gain were integrated at the whole-plant level as NAR. Contrary to our hypothesis, the NAR was a stronger correlate of RGR than was LAR. In a study with seedlings of nine boreal tree species, Reich et al. (1998b) found that LAR explained a greater share of variation in RGR than did NAR, and that among-species variation in LAR was driven by differences in SLA, but not in LMF. On the contrary, we found significant among-species variation in LMF, but SLA was still a stronger determinant of LAR among our species than was LMF. Biomass allocation to leaves and roots also showed strong correlations with RGR within the leaf habit/shade tolerance groups. All these findings failed to support our hypothesis, and suggest that physiology and biomass allocation were at least equally important determinants of RGR as plant structure (LAR) and leaf morphology among temperate tree species differing in leaf habit and shade tolerance.

**How was variation in growth, biomass allocation and physiology related to leaf habit and shade tolerance?**

We hypothesized that broadleaved species would have faster growth than needle-leaved conifers, and that shade-intolerant species would grow faster within each leaf habit group. The allometric analysis of our data revealed that growth trajectories, i.e., slopes of relationships between component biomass and plant mass, in general, did not reflect the groupings of species based on leaf habit or shade tolerance. When the groups shared a common slope of regression, the differences of the intercepts among groups or shifts along a common slope indicated only differences in scale, arising from variation in total biomass, and not from fundamentally different ontogenetic trajectories.

Overall, LMF decreased and RMF increased with plant size, indicating that seedlings invested in root biomass at the cost of leaves. This finding suggests that irrespective of shade tolerance classification, under high-light conditions the building of roots for acquisition of belowground resources is important for initial seedling establishment. The trends we observed for LMF in all groups of species and RMF in broadleaves were similar to the ontogenetic trends described for tree seedlings in Poorter et al. (2012) over the same range of plant sizes. However, most of the shifts in allocation we observed were between LMF and RMF, and not between LMF and SMF, in contrast to Poorter et al. (2012). The SMF in our experiment showed less ontogenetic variation compared with leaves and roots, but it also separated the groups according to shade tolerance. This suggests that accounting for shade tolerance may help in interpreting ontogenetic trends in allocation to stems between contrasting leaf habit groups during early ontogeny.

Correlations of SLA, LAR and SRL with plant mass were consistent in sign among the groups of species. However, the patterns of leaf and plant morphology seem to differ for shade tolerance groups between broadleaves and conifers. Including more species within the group of shade-intolerant broadleaves would allow testing of the generality of those trends. Nonetheless, our findings indicate that there is no single growth trait conferring shade tolerance. In order to understand
the mechanistic basis for shade tolerance within leaf habit groups, it is necessary to address variation in a complex of traits simultaneously.

The factor analysis provided the basis for such a comparison. The first factor that separated the species well according to shade tolerance was related to seed mass, SRL, plant mass and allocation to stems and leaves. Seed mass appeared again as an important factor explaining variation among our species, not only through its influence on plant mass and growth, but also by affecting biomass allocation and plant morphology. Sack and Grubb (2001) argue that in very young seedlings, independent of their shade tolerance, seed size-related differences in SLA, and thus LAR, drive the initial variation in RGR, whereas the traits related to NAR become more important in the longer term. In our study we found that both NAR and LAR were strong determinants of RGR across the species. However, NAR did not provide a clear distinction among groups, and LAR and SLA separated species more by leaf habit than by shade tolerance (factor 3), and showed a different pattern of variation between shade tolerance groups depending on leaf habit (see Figure 3). In addition, not only leaf morphology, but also seed size-related root morphology helped explain differences in shade tolerance among these temperate tree species. Few studies have addressed variation in SRL in relation to shade tolerance (Reich et al. 1998b, Paz 2003). Our study provides new evidence that not only biomass allocation to roots, but also root morphology may be an important element of the shade tolerance syndrome.

The SRL is not directly related to light harvesting, but is associated with adaptation to the low soil resource conditions or more efficient uptake, because finer roots maximize the surface for belowground resource capture. In our study the SRL in broad-leaves was greater than in conifers, similar to the study by Withington et al. (2006). However, this difference between leaf habit groups was mainly due to the particularly high values of SRL in Betula—an early successional, light-demanding pioneer broadleaf, which often occupies low-resource sites (Jaworski 2011). Within a given leaf habit group, finer roots were associated with lower tolerance to shade. This distinction, however, applied to a lesser degree in the shade-tolerant conifers. Although P. abies had greater SRL than more shade-tolerant A. alba (see Table 1), its SRL values were comparable to those of shade-intolerant conifers: L. decidua and P. sylvestris. This may be related, in part, to its relatively lower seed mass, but also to species ecology. Picea abies is a shade-tolerant species when growing under optimal temperature and soil conditions; however, it also occupies marginal high-altitude sites with poor soils where its light requirements increase (Jaworski 2011). Higher SRL may promote capture of resources that are most limiting under such conditions. Our findings suggest that the role of root morphology in shade tolerance may differ between broadleaves and conifers, but comparative studies involving more species are needed to resolve the relationship between the SRL and tolerance to shade.

Traits that maximize energy conservation allow seedlings to persist in shaded habitats where carbon balance is unfavourable (Kitajima 1994, Walters and Reich 2000). Shade-tolerant species in our study not only had lower SRL, but also higher SMF than shade-intolerant species. Root systems built of coarser roots may better serve as storage organs and persist longer than finer roots. A greater allocation to stem biomass perhaps also reflects increased ability to grow taller and store carbohydrates in stem tissues than in shade-intolerant species. Thus, root morphology and biomass allocation to stems of shade-tolerant species constitute traits favourable for survival in shade.

In conclusion, shade tolerance is not governed by a single trait, but by the whole suite of traits that may vary by leaf habit. In high-resource settings shortly after germination, building a root system was a priority at the cost of leaf biomass, independent of shade tolerance classification. During early ontogeny, seed mass correlated with plant biomass, biomass allocation and plant morphology. The seed size-related root morphology (SRL) and differences in allocation to stems provided a functional basis for shade tolerance among the studied species.

Seed germination and seedling establishment are an initial phase of forest stand development. The ability to outgrow competitors at this stage is just one aspect of seedling survival and recruitment, and may require a different set of traits from those important to withstand other hazards that young trees are exposed to during later stages. Longer-term studies in varying light conditions would help resolve how traits identified in this study would contribute to later survival through sapling stage to maturity.

Supplementary data
Supplementary data for this article are available at Tree Physiology Online.

Acknowledgments
The authors thank Dr Sean Thomas and the two anonymous reviewers for comments that helped improve the early version of the manuscript. We also thank Dr Pawel Chmielarz from the Institute of Dendrology in Kórnik, Poland, for providing and preparing seeds for the experiment.

Conflict of interest
None declared.

Funding
The study was supported by the Department of Ecosystem Science and Management, Texas A&M University. J.M. was supported by the Polish State Committee for Scientific Research, Grant No. 4 T08A 017 15.
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