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High-light acclimation in *Quercus robur* L. seedlings upon over-topping a shaded environment

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ABSTRACT

High developmental plasticity at the seedling-level during acclimation to the light environment may be an important determinant of seedling establishment and growth in temperate broadleaf forests, especially in dense understories where spatial light availability can vary greatly. Pedunculate oak (*Quercus robur* L.) seedlings were raised beneath a range of artificial light environments (high light, partial high light and low light) to examine morphological and photosynthetic acclimation to vertically stratified light availability. Acclimation observed at the seedling level included changes in proportional distribution of biomass and leaf area ratio to enhance either light gathering under low light availability or reduction of moisture stress under high light availability. Seedling-level acclimation was partially driven by plasticity at the flush level, but plasticity of traits determining flush morphology, such as leaf number, area, and mass, was largely controlled during bud formation rather than during shoot development. Therefore, flush-level acclimation was restricted when shoots elongated from a shaded environment into a high light environment. In contrast, traits influencing leaf-level acclimation, such as leaf thickness, specific leaf area, and pigment concentrations appeared to be driven primarily by the prevailing light environment during leaf development. The plastic response in leaf traits to light environments during shoot development enabled immediate acclimation of photosynthetic capacity to the prevailing light environment. In conclusion, oak seedlings displayed a large phenotypical plasticity on multiple levels that maximized whole seedling performance.

1. Introduction

Light availability near the floor of temperate forests varies greatly and has a strong impact on establishment and growth of tree seedlings (van Hees, 1997; Domke et al., 2007; Niinemets, 2007; Barbier et al., 2008). Presence of understory vegetation may further increase the complexity of the light environment experienced by subordinate seedlings (Löf, 2000). Seedlings able to over-top other understory vegetation experience a high degree of spatial light heterogeneity (Collet et al., 1998). Accordingly, a number of morphological, physiological and biochemical changes can be induced in individual leaves and seedlings to maximize and sustain photosynthetic performance in a heterogeneous light environment (Givnish, 1988; Walters, 2005).

Research on photosynthetic acclimation in herbaceous and woody species has shown a high degree of plasticity in response to gradual and abrupt change in light availability (e.g. Terashima and Evans, 1988; Naidu and DeLucia, 1997, 1998; Rosati et al., 1999; Fownes and Harrington, 2004; Niinemets, 2007). Acclimation responses may be local or involve high integration between different parts of the same plant (Novoplansky, 2002). To gain a more complete understanding of acclimation to light availability by tree seedlings will require investigations of the relevant mechanisms at different functional levels, e.g. at the levels of individual leaves, cohorts of leaves (leaves emerging at the same time), and the whole seedling.

Oak (*Quercus* spp.) seedlings growing under favorable conditions can develop several shoot flushes during a growing season (e.g. Collet et al., 1997; Welander and Ottosson, 2000). After the first flush expands and matures, terminal growth of the shoot

Abbreviations: PPFD, photosynthetic photon flux density; R/FR, red to far-red; C_a, atmospheric CO_2 concentration; C_i, internal CO_2 concentration; A/Q, photosynthetic light response; A/C_i, photosynthetic CO_2 response; A_{max}, maximum gross CO_2 assimilation rate; R_D, apparent dark respiration; I_c, light compensation point; Φ, apparent quantum efficiency; g_{smax}, maximum stomatal conductivity; $V_{c,max}$, maximum rate of carboxylation by Rubisco; J_{max}, light saturated rate of electron transport; N, nitrogen; Chl_{total}, total chlorophyll; $Chl_{a/b}$, chlorophyll a to b; SLA, specific leaf area; LMR, leaf mass ratio; R:S, root to shoot; LAR, leaf area ratio.

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enters a quiescent or 'lag' phase prior to elongation of the next terminal bud (Hanson et al., 1986). During early flush development, newly formed tissues lack photosynthetic development and are thus a net carbon sink (Dickson, 1991; Keel and Schädel, 2010; Landhäusser, 2011). Therefore, the capacity to acclimate to a high-light environment may be restricted by insufficient light availability to lower flush leaves. Leaf morphological characteristics determined during bud formation and early expansion may depend on the light environment of lower flush leaves, whereas biochemical and physiological processes such as pigment content and photosynthetic capacity may be influenced more by the newly exploited environment (e.g. Eichelmann et al., 2005; Laisk et al., 2005; Rodríguez-Calcerrada et al., 2008).

Detailed knowledge about acclimation responses at different functional levels of seedlings would contribute to our understanding of regeneration development in temperate deciduous forests. One tree species for which acclimation responses may be particularly crucial is Pedunculate oak (*Quercus robur* L.), a species that can produce multiple shoot flushes during a growing season. *Q. robur* is an ecologically and economically important component of mixed temperate broadleaved forests of Europe (e.g. Berg et al., 1994; Ranius and Jansson, 2000). This species exhibits intermediate light requirements, and management recommendations for regenerating *Q. robur* generally call for maintaining 30–50% light availability in the forest understory (Diekmann, 1996; Götmark, 2007).

To our knowledge no study has identified the mechanisms and quantified constraints involved in the high-light acclimation as oak seedlings over-top a shady environment. Most earlier studies addressing growth and acclimation to high light availability by oaks have used constant or homogeneous light environments and often focused on responses at a single flush level (e.g. Welander and Ottosson, 2000; Valladares et al., 2002; Rodríguez-Calcerrada et al., 2008). However, a constant light regime may poorly reflect understory light availability and spatial variation in the light environment would seemingly create a range of acclimation requirements for the developing seedling.

Against this background, we studied the effects of a heterogeneous light environment on morphological and photosynthetic acclimation in Pedunculate oak, particularly those changes that occur as a seedling over-tops a low light environment. The objectives of this research were to (i) identify the mechanisms that contribute to or restrict morphological and photosynthetic acclimation at the seedling, flush, and leaf levels, and to (ii) quantify the plasticity of these acclimation responses.

2. Materials and methods

2.1. Experimental design

This experiment was conducted in a greenhouse and growth chamber facility located at the Center for Bottomland Hardwoods Research, USDA Forest Service, Stoneville, MS, USA. On 26 August 2008, *Q. robur* acorns purchased from Sheffield's Seed Company (New York, USA) were removed from cold storage, soaked in water for 24 h, then sown directly into 5-L pots filled with a mixture of peat moss and commercial potting soil enriched with an Osmocot® time released fertilizer (The Scotts Miracle Grow Company, OH, USA). Pots were placed in the greenhouse where they received either high light (HL) or low light (LL). The pots assigned to HL were placed under a canopy of 130 Clear Gel Filter (Lee Filters, CA, USA) and received ambient sunlight. Pots assigned to LL were placed under a canopy of neutral density shade cloth (PAK Unlimited, Inc., GA, USA) and 138 Pale Green Gel Filter (Lee Filters, CA, USA). Potting substrate was watered daily to maintain a high moisture capacity that promoted acorn germination. Developing seedlings were raised in one of the two assigned light environments up to maturity of the first flush.

Forty-five randomly chosen seedlings in the 1-Lag stage of development (Hanson et al., 1986) were transferred to a Conviron PGR15 growth chamber (Conviron, Winnipeg, Canada) programmed to maintain a 16 h diurnal period with a 30 min sunrise and sunset and a constant air temperature at 25 °C. The climate chamber was partitioned to provide three distinct light environments (HL, LL, and partial high light, PHL). A total of 15 seedlings were assigned to each light environment and were maintained in their respective environments through maturity of their third flush. Seedlings assigned to the HL environment received a photosynthetic photon flux density (PPFD) of about 1050 $\mu mol\ m^{-2}\ s^{-1}$ with a red to far-red (R/FR) ratio of 1.14 during the diurnal period. As in the greenhouse, neutral density shade cloth and green gel filter were used to create the LL environment in the growth chamber. Seedlings assigned this light environment received a PPFD of about 115 $\mu mol\ m^{-2}\ s^{-1}$ with a R/FR ratio of 0.84. The PHL treatment was designed so that seedlings initially receiving LL grew into a HL environment: the first-flush of seedlings in the PHL treatment level was kept in LL (PPFD of 115 $\mu mol\ m^{-2}\ s^{-1}$ with R/FR ratio of 0.84), while the second flush grew through a 2-cm hole in the canopy to experience the HL environment (PPFD of 1050 $\mu mol\ m^{-2}\ s^{-1}$ with R/FR ratio of 1.14). Therefore, seedlings assigned to the HL environment in the growth chamber were raised under HL in the greenhouse, whereas seedlings assigned to the LL or PHL environment in the growth chamber were raised under LL in the greenhouse. The HL seedlings completed all three flushes under high light and LL seedlings under low light. The PHL seedlings were exposed to a heterogeneous light environment so that their entire first flush and their second-flush buds developed under LL, but their second-flush leaves and third flush developed under the HL environment. All seedlings were kept well watered during the experiment and remained free of pests and pathogens.

2.2. Photosynthetic response

Between 4 and 26 November 2008, leaf gas exchange was measured to construct photosynthetic light response (A/Q) and CO_2 response (A/C_i) curves. We sampled a mature leaf from each of three flushes from seven seedlings growing under each light environment (total of 63 leaves). The evening prior to gas exchange measurements, sample plants were moved from the climate chamber to a dark room in order to limit photosynthetic activity before measurements. Pots were watered to soil saturation and seedlings remained in the dark room prior to and during sampling so that leaf moisture status was optimal during measurements. For each seedling, gas exchange measurements were conducted on a fully developed median leaf from each of the three flushes with a Ciras-2 portable gas analyzer and automatic leaf cuvette (PP-Systems, MA, USA) that provided a reference CO_2 concentration of 374.9 ± 0.1 ppm. Measurements began at a PPFD level of 0 then proceeded to 1600, 800, 400, 200 and 50 $\mu mol\ m^{-2}\ s^{-1}$. Net assimilation typically stabilized within 30 min of leaf exposure to the maximum light level, and within 10 min of leaf exposure at the other light levels. Without moving the cuvette, A/C_i curves were generated at a PPFD of 800 $\mu mol\ m^{-2}\ s^{-1}$ by increasing the reference CO_2 concentration (C_a) from 0 to 1600 ppm (0, 100, 200, 400, 800 and 1600 ppm) to generate a broad range of intercellular CO_2 concentrations (C_i). Leaking CO_2 was insignificant and a new leaf was selected if inhibition was observed during any part of A/Q or A/C_i measurements. Relative humidity was 50% of ambient and leaf temperature was kept at 25 ± 0.4 °C during all measurements.

2.3. Seedling foliage and biomass characteristics

At the end of the experiment, gas exchange sample leaves were harvested to quantify their pigment concentrations, nitrogen (N) concentration and leaf thickness. Fresh weight of the sample leaf was measured, and a Li-3100 Area Meter (Li-Cor Inc., USA) was used to measure leaf area. Pigment concentrations were determined using a protocol modified from Barnes et al. (1992) and Lichtenthaler (1987). Three leaf disks (0.5 cm^2) void of major veins were collected from each sample leaf and incubated overnight in 65 °C DMSO to extract pigments. Extract solutions were measured on a Milton Ray, Spectronic 21 D spectrometer at λ = 664.9, 648.2 and 407.0 nm. Total chlorophyll (Chl_{total}), chlorophyll a, chlorophyll b and carotenoid concentrations were determined with equations presented by Barnes et al. (1992).

One seedling per treatment combination was randomly selected for leaf anatomy sampling. Three leaf disks (0.5 cm^2) were sampled from 1 leaf in each flush and were fixed in 6% (v/v) glutaraldehyde in 0.05 M PIPES buffer (pH 7.2) supplemented with 1% caffeine to agglutinate the phenols (Vaughn and Wilson, 1981) at room temperature for 2 h. The samples were then washed in two exchanges of PIPES buffer, 15 min each. Ethanol dehydration was carried out with 25, 50 and 75% steps at 4 °C and 100% ethanol at −20 °C. After a day at −20 °C, the samples were gradually infiltrated (25% each day) with LR White resin (Polysciences Inc., PA, USA). After the samples had reached 100% resin, they were transferred to a rocking platform and agitated for 24 h. Samples were then transferred to flat embedding pucks (Ted Pella Inc., CA, USA), and the resin and samples were covered with Aclar® film to ensure that minimal air bubbles were trapped. Polymerization was carried out at 55 °C for 2.5 h. To obtain cross-sections, samples were sawed from the pucks with a jeweler's saw and mounted on acrylic stubs so that the long axis of the leaf was perpendicular to the cutting face of the block. Semi-thin (0.35 μm) sections were cut with a HistoKnife (Delaware Diamond Knives, DE, USA) and mounted on chrome-alum coated glass slides. Sections were stained ~30 s with 1% (w/v) Toluidine blue in 1% (w/v) sodium borate. After thorough water rinsing, the samples were dried with compressed air and mounted with Permount (Fisher Scientific, TN, USA). Cross sections were photographed digitally with a Zeiss Axioskop light microscope (Zeiss, NY, USA). For each treatment combination, leaf thickness was measured at 18 locations on 2400× cross-sectional images with ImageJ 1.43n (National Institute of Health, USA).

Remaining sample leaf tissue was dried at 70 °C, weighed and ground in a ball mill to pass a 100-mesh sieve. Total N (%) of the ground leaf tissue was measured with a dry combustion technique using a PE 2400 Series II CHNS/O Analyzer (Perkin-Elmer Corporation, CT, USA). The rest of the seedling was harvested and separated into root, shoot, and leaf biomass. Total leaf area for each seedling was measured as above and all tissues were dried at 70 °C for measurement of dry weight.

2.4. Calculations and statistical analysis

A/Q and A/C_i curves were modeled using Photosyn Assistant Software version 1.2 (Dundee Scientific, Scotland, UK). The software uses the standard method of Prioul and Chartier (1977) to estimate maximum photosynthesis at saturating light (A_{max}) under ambient CO_2, apparent dark respiration (R_D), the light compensation point (I_c) and apparent quantum efficiency (Φ). Maximum stomatal conductivity (g_{smax}) was generated from A/Q curves under light saturation (1600 μmol m^{-2} s^{-1}). Empirical A/C_i curves were analyzed using the Michaelis–Menten constant to estimate maximum carboxylation rate ($V_{c,max}$) and maximum electron transport rate (J_{max}) (Brooks and Farquhar, 1985; Harley et al., 1992). C_i concentrations below 200 ppm were not used in model fitting. Root to shoot (R:S) ratio was calculated as root dry weight over the total above-ground biomass including leaves (g g^{-1} DW). Specific leaf area (SLA) and leaf mass ratio (LMR) were calculated as leaf area over leaf mass (m^2 g^{-1} DW) and total leaf mass over total seedling mass (g g^{-1} DW), respectively. Leaf area ratio (LAR) was calculated as total leaf area over above-ground mass.

Light environment effects on seedling-level variables (height, basal diameter, total biomass, leaf mass, leaf area, R:S ratio, LMR and LAR) were analyzed using an one-way ANOVA with a least squares mean separation. Light environment and flush effects on flush-level variables (leaf number, area and mass) and leaf-level variables (SLA, pigment, N concentration, A/Q and A/C_i response variables) were tested using a mixed model procedure with light environment and flush as fixed effects, and with a least squares mean separation. The model estimated light environment (3 levels) and flush (3 levels) means and their interactions (4 levels). Effects of light environment and flush on leaf thickness were tested using a similar model but with leaf cross-sections as the random factor. Mean separations were conducted using Tukey's adjustment at a significance level of 0.05. Distribution assumptions were tested using the Kolmogorov–Smirnov normality-test and Bartlett's test. Variables (total biomass, R:S ratio, leaf number, leaf area and J_{max}) that did not meet these assumptions were normalized using a Box-Cox transformation. A Satterthwaite-approximation was added to the model for heteroscedasticity-consistent standard errors if needed (J_{max}). Linear regression was used to model the relationships between g_{smax} and A_{max}, and $V_{c,max}$ and J_{max}. All statistical analyses were conducted using SAS 9.2 (SAS Institute Inc., USA).

3. Results

3.1. Seedling-level acclimation

Height of *Q. robur* seedlings was not responsive to the light environment, whereas basal diameter and subsequently total biomass accumulation was greatest for seedlings assigned to HL and least for seedlings assigned to LL (Table 1). Along with the increase in biomass accumulation, leaf mass and area were greatest for seedlings raised under HL (Table 1). In addition to biomass accumulation, the light environment also influenced proportional distribution of biomass within seedlings as R:S ratio was greatest for seedlings grown under HL, and LMR was greatest for seedlings grown under LL. Likewise, seedlings assigned to LL developed a LAR nearly twice that of seedlings that received either PHL or HL (Table 1).

3.2. Flush-level acclimation

Relative to seedlings raised under LL, successive flushes on seedlings that received HL showed an increasing number of leaves, leaf area and leaf mass (Fig. 1A–C and Table 2). Seedlings raised in the PHL environment developed a similar number of second-flush leaves and leaf area as those assigned to LL, but their third flush produced the same leaf number and leaf area as seedlings raised under HL (Fig. 1A and B). In contrast to leaf number and area, third-flush leaf mass of seedlings in the PHL environment was lower than third-flush leaf mass of seedlings raised under HL (Fig. 1C).

Total leaf mass of seedlings raised under the LL environment was equally proportioned (33%) between the first, second and third shoot flushes (Fig. 1D). In contrast, the proportion of leaf mass increased with subsequent flushes for seedlings assigned to HL and PHL environments. For these seedlings, the proportion of leaf mass in the third flush averaged nearly 15 percentage points greater than that observed on seedlings raised in the LL environment (Fig. 1D).

Table 1
Oak seedling biomass and morphology when grown under high-light (HL), partial high-light (PHL) and low-light (LL) environments. Values are means ± 1 SE of seven oak seedlings per light environment. *F*-Values and significance levels ($P < 0.05$ (*), $P < 0.01$ (**) and $P < 0.001$ (***)) were generated using an one-way ANOVA. Significant difference ($P < 0.05$) between light environment means is indicated with different letters in a column.

Treatment	Height (cm)	Basal diameter (mm)	Total biomass (g)	Leaf mass (g)	Leaf area (m^2)	R/S ratio ($g\,g^{-1}$)	LMR ($g\,g^{-1}$)	LAR ($cm^2\,g^{-1}$)
HL	65.4 ± 3.0a	8.2 ± 0.2a	23.7 ± 1.0a	6.4 ± 0.5a	0.134 ± 0.014a	0.71 ± 0.07a	0.32 ± 0.02b	56.5 ± 5.2a
PHL	59.6 ± 1.9a	6.7 ± 0.2b	14.1 ± 0.6b	4.4 ± 0.3b	0.097 ± 0.005b	0.54 ± 0.04ab	0.35 ± 0.01ab	69.5 ± 3.6a
LL	63.3 ± 2.4a	4.9 ± 0.3c	6.5 ± 0.4c	2.5 ± 0.1c	0.087 ± 0.004b	0.37 ± 0.03b	0.41 ± 0.02a	135.7 ± 5.9b
F-Value	1.43	49.2***	143.5***	34.3***	7.3***	10.9***	7.1***	72.4***

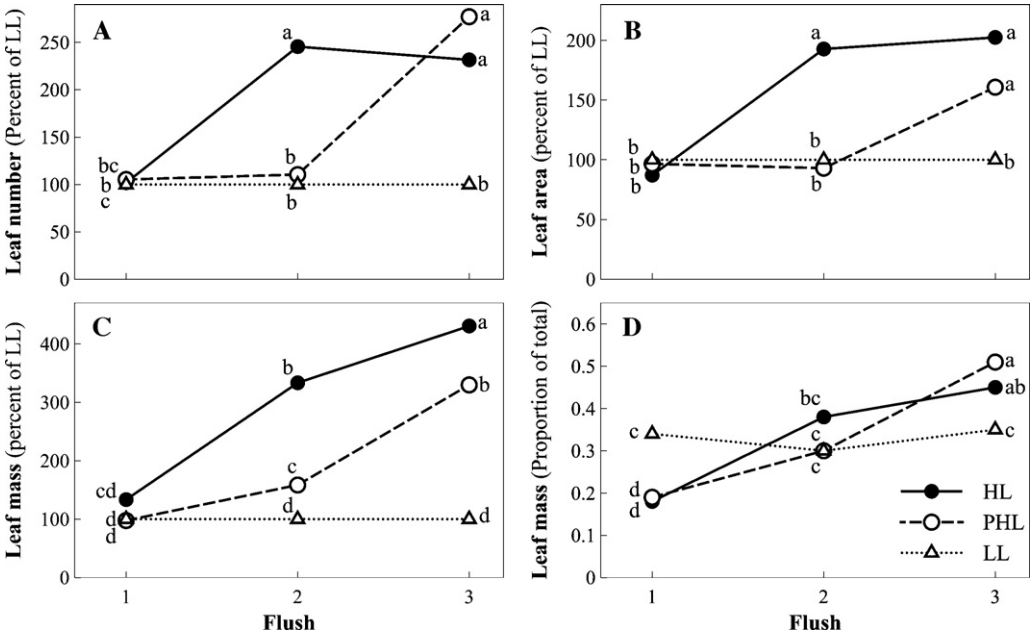

Fig. 1. (A) Total leaf number, (B) total leaf area and (C) total leaf mass relative to seedlings raised in the low-light (LL) environment and (D) proportion of total leaf mass by flush. Different letters indicate significant difference between light environment and flush combination means ($P < 0.05$). The symbol key illustrated in panel D is appropriate for all panels.

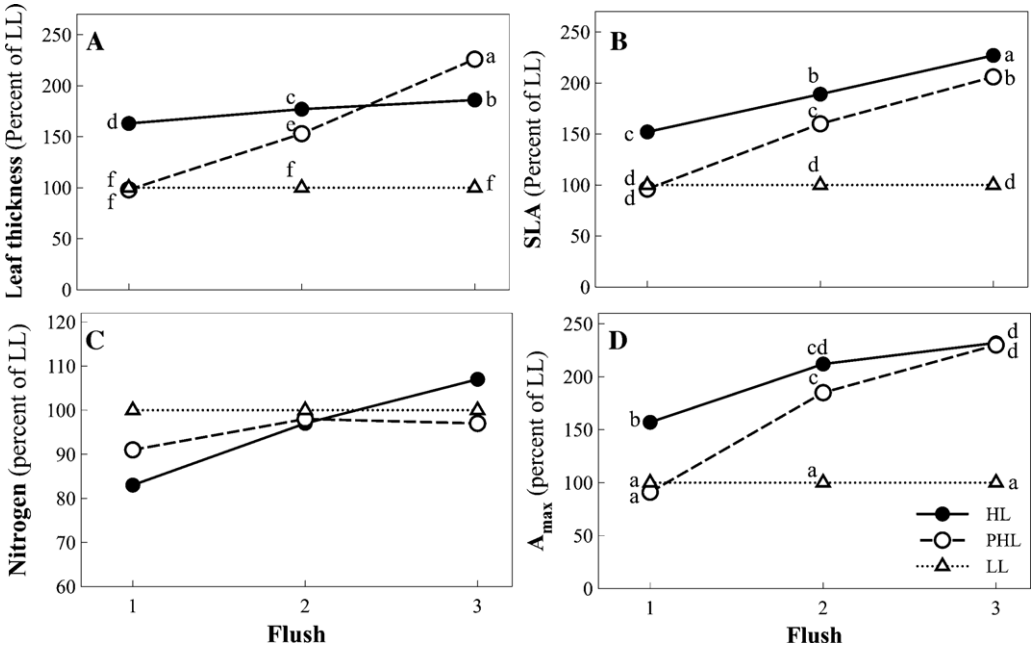

Fig. 2. (A) Leaf thickness, (B) SLA, (C) nitrogen and (D) A_{max} in three successive flushes of oak seedlings grown under high-light (HL) and partial high-light (PHL) environments. Values are relative to seedlings raised in the low-light (LL) environment. Different letters indicate significant difference between light environment and flush combination means ($P < 0.05$). The symbol key illustrated in panel D is appropriate for all panels.

Table 2
Statistics resulting from the analysis of flush-level and leaf-level response variables. *F*-Values are presented with the significance level indicated by asterisks ($P < 0.05$ (*), $P < 0.01$ (**) and $P < 0.001$ (***)). Means for selected response variables are shown in Supplemental Tables S1 and S2.

Variable	Light environment (df = 2)	Flush (df = 2)	Light environment × Flush (df = 4)
Flush-level traits			
Leaf number (number)	6*	13***	4*
Leaf area (m^2)	7**	30***	26***
Leaf mass (g)	45***	152***	58***
Leaf mass (proportion of total)	0	37***	11***
Leaf-level traits			
Leaf thickness (mm)	1683***	569***	412***
SLA ($m^2\ g^{-1}$)	147***	82***	28***
Chl_{total} ($\mu g\ mg^{-1}$)	30***	80***	12***
Carotenoid ($\mu g\ mg^{-1}$)	20***	57***	16***
$Chl_{a/b}$ ratio ($\mu g\ mg^{-1}$)	1	7**	2
Nitrogen ($mg\ mg^{-1}$)	1	21***	2
A_{max} ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$)	63***	73***	14***
R_D ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$)	16***	42***	4**
I_c ($\mu mol\ photons\ m^{-2}$)	5*	22***	1
Φ	1	14***	1
g_{smax} ($mmol\ CO_2\ m^{-2}\ s^{-1}$)	19***	45***	8***
$V_{c,max}$ ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$)	14***	59***	6**
J_{max} ($\mu mol\ electrons\ m^{-2}\ s^{-1}$)	14***	38***	7***

3.3. Leaf-level acclimation

Leaves in successive flushes of seedlings raised in the HL environment showed an increasing thickness ($P < 0.001$) and SLA ($P < 0.001$) relative to those from seedlings raised in the LL environment (Fig. 2A and B). Leaf thickness and SLA increased as the second flush of seedlings raised in the PHL environment over-topped shade, but these leaves did not achieve the thickness ($P < 0.001$) and SLA ($P < 0.001$) of those from the second flush of seedlings raised in the HL environment. Third-flush leaves were 126% thicker ($P < 0.001$) for seedlings assigned to PHL and 86% thicker ($P < 0.001$) for seedlings assigned to HL relative to corresponding leaves produced under LL (Fig. 2A and B).

Chl_{total} concentrations were affected by the interaction of light environment and flush (Table 2). First-flush leaves from seedlings grown in the LL and PHL environments, and second-and third-flush leaves from seedlings grown in the LL environment maintained the highest chlorophyll concentrations with means ranging from 23.7 to 29.7 $\mu g\ mg^{-1}$ (see supplemental Table S1). Second- and third-flush leaves of seedlings assigned to PHL maintained a Chl_{total} concentration similar to that in second- and third-flush leaves from the HL environment, 36% and 45% lower than the average concentration in second- and third-flush leaves that developed under LL. In all light environments, Chl_{total} concentrations of first-flush leaves were lower than in third-flush leaves (18, 56, and 40% lower for LL, PHL and HL, respectively). Carotenoid concentrations followed a pattern similar to Chl_{total} concentrations, while the chlorophyll a to b ratio ($Chl_{a/b}$) differed ($P < 0.001$) between flush one and flush three, but was not affected by the light environment (Table 2 and Table S1).

Relative to seedlings raised under LL, leaf N concentration increased with subsequent flushes in seedlings assigned HL ($P < 0.01$). This trend was not observed for seedlings that developed under PHL, as N concentration remained similar for all three shoot flushes (Fig. 2C).

A_{max} ranged greatly between flush position and light environment; first-flush leaves from seedlings in the PHL environment had an average A_{max} of 7.2 $\mu mol\ CO_2\ m^{-2}\ s^{-1}$, and third-flush leaves from seedlings in the HL environment had an average A_{max} of 21.0 $\mu mol\ CO_2\ m^{-2}\ s^{-1}$ (see supplemental Table S2). For all light environments, first-flush leaves generally maintained the lowest A_{max} rates, but the increase observed for A_{max} between first- and third-flush leaves in the HL and PHL environments was not observed for seedlings raised under LL (Fig. 2D). R_D was lowest in first-flush leaves averaging −0.4 $\mu mol\ CO_2\ m^{-2}\ s^{-1}$ regardless of light environment. R_D generally increased for second- and third-flush leaves sampled on PHL and HL seedlings (Table S2). I_c was lowest for leaves of seedlings raised under LL, but increased with subsequent flushing across all light environments (Table 2 and Table S2). Φ decreased 68% between the first and third flushes across all light environments (Table 2 and Table S2). g_{smax}, $V_{c,max}$ and J_{max} were influenced by light environment, flush position, and their interaction (Table 2).

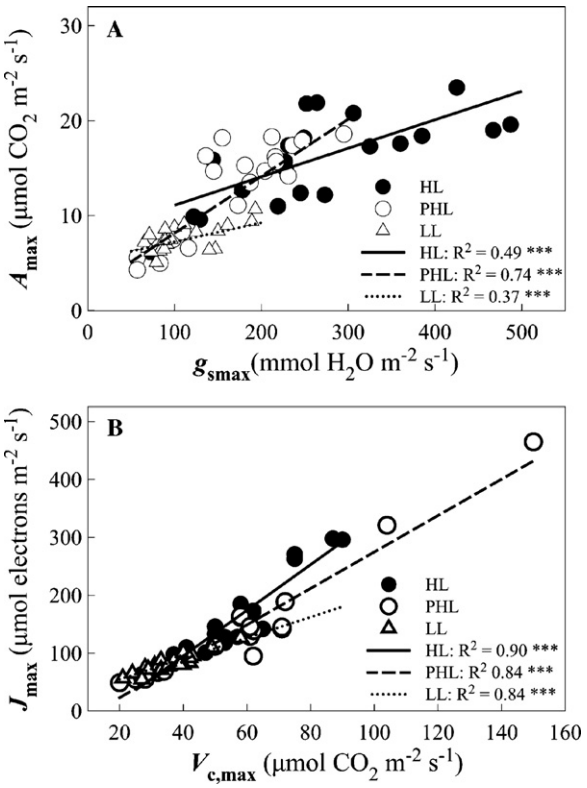

Fig. 3. Relationship between (A) g_{smax} and A_{max} and (B) $V_{c,max}$ and J_{max} estimated from photosynthetic response curves of oak leaves grown in high-light (HL), partial high-light (PHL) and low-light (LL) environments. Lines are from simple linear regressions conducted by light environment.

A positive linear relationship was found between g_{smax} and A_{max} with slopes and intercepts differing among light environments ($P < 0.001$) (Fig. 3A). The relationship between $V_{c,max}$ and J_{max} was linear for all light environments and flushes, but with significantly different slopes ($P < 0.001$) (Fig. 3B).

4. Discussion

The understory light environment in temperate broadleaved forests, as in many forest types around the world, is characteristically heterogeneous as spatial variation in light quantity and quality is driven by stand structure. Mature forest stands of mixed broadleaved species including *Q. robur* maintain overstory canopy cover that ranges between 60 and 90% (Brunet et al., 1997; Härdtle et al., 2003). The overstory structure modifies the understory light environment by significantly reducing light availability and depleting the photosynthetically active spectrum of red wavelengths (Wagner et al., 2011). Thus, light availability in the understory of temperate broadleaved forests can be less than 10% of above canopy levels (Dobrowolska, 2008), and light quality is typically shifted

towards a reduced R/FR ratio (Balandier et al., 2006). Understory vegetation adds additional heterogeneity to the light environment by further reducing light availability and further decreasing the R/FR ratio (Domke et al., 2007). Artificial light environments assigned in this experiment were designed to approximate a relatively high and a relatively low light environment found in the understory of temperate broadleaved forest of northern Europe in order to identify acclimation mechanisms of *Q. robur*, as seedlings over-top a deeply shaded environment.

Acclimation to the light environment by *Q. robur* seedlings involved a variety of mechanisms operating at the seedling, flush and leaf levels. Seedling acclimation to LL included biomass distribution and leaf area relationships that resulted in whole-plant morphology indicative of favoring light gathering. *Q. robur* seedlings raised under LL developed a high proportion of leaf biomass and the greatest ratio of leaf area among seedlings of the various light environments; findings consistent with earlier reports on acclimation to limited light availability by oaks (Jarvis, 1964; van Hees, 1997). Seedlings raised under HL developed a phenology in contrast to those raised under LL as evidenced by their comparatively low LMR and low LAR. The relatively high R:S ratio of these seedlings indicated that acclimation to HL favored root development, a response that lead to a phenology consistent with reduction of moisture stress (Kozlowski and Pallardy, 2002). Though seedlings in the PHL environment were initially established under LL, they responded to the increased light received by their second and third flush with a proportional shift in biomass accumulation away from leaf area. Upon maturation of the third flush, seedlings raised in the PHL environment had developed a morphology characterized by a low LAR and indicative of acclimation to a high light environment (Givnish, 1988). These findings indicate that LAR of *Q. robur* seedlings is quite plastic to the light environment, and that proportional distribution of biomass to leaf area is an important seedling-level mechanism by which this species initiates morphological acclimation to light resources.

The mechanism of periodic shoot flushing under favorable environmental conditions is the primary driver of seedling-level acclimation, and potentially provides *Q. robur* seedlings with opportunities to develop cohorts of foliage acclimated to spatial variation in the light environment. In this study, a shift in flush morphology was not obvious between successive flushes of seedlings raised in the LL environment. Flushes produced by these seedlings were isomorphic in terms of leaf number, total leaf area, total leaf mass and proportional leaf mass. This finding indicates that sequential flushing advanced seedling height growth, but evidence of plasticity in flush morphology was minimal in the homogeneous shade of the LL environment.

In contrast to seedlings raised in the LL environment, those raised in HL and PHL environments exhibited plasticity in leaf number, leaf area, leaf mass and proportional leaf mass between successive flushes. The second and third flushes of seedlings assigned to the HL environment developed a greater number of leaves, leaf area and leaf mass than in their first flush. For seedlings in both the HL and PHL environments, the third flush accounted for more than 45% of the seedlings leaf mass. However, the trajectory of development to the third flush differed between HL and PHL environments in that leaf number and leaf area were restricted in the second flush of seedlings raised in the PHL environment.

While these results illustrate that periodic flushing can enable *Q. robur* seedlings to acclimate to a spatially stratified light environment, most traits contributing to plasticity of flush morphology appear to be influenced during an early phase of shoot ontogeny. For example, the number of leaves in a flush appeared to be determined by the prevailing light regime during bud formation. This finding is consistent with an earlier report by Welander and Ottosson (1998) who demonstrated that leaf number in *Q. robur* was determined by light availability of the previous growing season. Because the number of leaf primordia is apparently determined during bud formation rather than during shoot development, there is a limit to the number of leaves that can form on shoots which elongate from a shaded environment into a high-light environment.

Though plasticity of most variables associated with flush-level acclimation appeared to be determined primarily by the prevailing environment of the forming bud, plasticity of leaf mass within a flush was also influenced by the prevailing light environment of the developing shoot. This is apparent from the moderate increase in total leaf mass observed in the second flush of seedlings raised in the PHL environment as compared to those from the LL environment. Dickson (1991) showed that a *Quercus rubra* flush in the early stage of elongation is dependent upon the preceding flush for as much as 90% of its photosynthates. However, the developing flush switches from a carbon sink to a carbon source as it matures (Dickson, 1991; Keel and Schädel, 2010). In this study, the increase in leaf mass observed in the second flush of seedlings raised in the PHL environment could have resulted from photosynthate production by leaves acclimated to high light. This is a finding that may indicate that *Q. robur* leaves rapidly become autotrophic.

In support of the previous finding for leaf mass, the prevailing light environment of the developing shoot appeared to have a more significant role in determining leaf-level acclimation than it did for flush-level acclimation. For example, leaf thickness increased in sequential flushes of seedlings raised under HL and PHL. Third-flush leaves from seedlings raised under PHL were thicker than corresponding leaves in the HL and LL environment, suggesting that leaf development in the third flush responded to stratified light availability. *Quercus petraea* leaves have earlier been observed to increase in thickness upon transfer from low to high light before flushing (Rodríguez-Calcerrada et al., 2008).

Leaf pigments responded to stratified light availability as noted by decreases in mass-based Chl_{total} and carotenoid contents with increased light availability. Our findings for Chl_{total} were consistent with the literature and suggest vertical acclimation to an environment where photon availability did not limit leaf carbon assimilation (Terashima and Hikosaka, 1995; Ammer, 2003). However, the response we observed for carotenoid content was in contrast to other reports in the literature that typically note a positive relationship between carotenoid content and light availability (e.g. Matsubara et al., 2009).

In addition to morphological and chemical acclimation, leaves also exhibited functional acclimation to the prevailing light environment. Area-based photosynthetic variables (A_{max}, R_D, I_c, $V_{c,max}$ and J_{max}) were similar in second-flush leaves of seedlings raised in the PHL and HL environments indicating that lower flushes did not impose restrictions on development of photosynthetic capacity. This finding is in line with several earlier studies on *Q. robur* and other oaks confirming that photosynthetic capacity of these species is highly responsive to the light environment (Niinemets, 1996; Naidu and DeLucia, 1998; Rosati et al., 1999; Rodríguez-Calcerrada et al., 2008). The higher A_{max} of third-flush leaves compared to second-flush leaves from seedlings assigned to the PHL environment may have reflected enhanced photosynthetic capacity with subsequent flush development as observed by Masarovičová (1991). However, if this were the case such a pattern should also have been observed in seedlings raised under the other light environments. Since nitrogen concentrations did not differ between second- and third-flush leaves, we interpret the higher A_{max} of the third-flush leaves as a product of increased leaf thickness.

The steep relationship between A_{max} and g_{smax} for leaves that developed in the PHL environment indicates that stomatal conductance of second-flush leaves was not restricted by light available to the first flush. This relationship may have resulted from the

increased thickness of leaves receiving high light. At the seedling level, the steeper regression line between $V_{c,max}$ and J_{max} in HL seedlings compared to PHL and LL seedlings, suggests that the apparent rate of electron transport was greater at lower values of $V_{c,max}$. This may be a result of a greater Rubisco content on a leaf-area basis. However as N concentration was similar between second- and third-flush leaves in PHL seedlings, the increased photosynthetic activity by these leaves may be explained more by increased stomatal conductance than increased enzymatic activity (Grassi et al., 2005).

5. Conclusion

Q. robur exposed to vertically heterogeneous light availability exhibits a range of plastic responses that support morphological and physiological acclimation at the seedling, flush and leaf levels. At the seedling level, light availability altered proportional distribution of leaf area and biomass such that plants grown in a shaded environment developed a morphology consistent with enhanced light gathering, and those grown in a high-light environment developed a morphology consistent with reduction of moisture stress. This seedling-level acclimation to the prevailing light environment was partially driven by plasticity of developing traits at the flush level. However, plasticity of traits determining flush morphology, such as leaf number, area, and mass, was largely controlled during bud formation rather than during shoot development. Therefore, flush-level acclimation was limited when shoot elongation proceeded from a shaded environment to an environment of higher light availability. In contrast, the prevailing light environment of the developing shoot largely determined morphological and physiological development of leaves. The photosynthetic mechanism of *Q. robur* exhibited high plasticity to light availability enabling immediate acclimation of photosynthetic capacity to the prevailing light environment of the developing leaf. Overall, *Q. robur* seedlings were able to acclimate to a vertically stratified light environment through integration of photosynthetic and morphological plasticity at multiple levels. It is probable that other temperate species that exhibit periodic shoot flushing during a growing season also utilize a range of mechanisms at multiple levels to acclimate to heterogeneous light environments. In particular, seedlings of the many other oak species that range across the northern temperate zone likely acclimate to vertically stratified light environments with comparable strategies involving leaf-, flush- and seedling-level mechanisms.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.envexpbot.2011.12.020.

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