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Construction costs, chemical composition and payback time of high- and low-irradiance leaves

Hendrik Poorter[1,2,3,*], Steeve Pepin[2,4], Toon Rijkers[5], Yvonne de Jong[1], John R. Evans[3] and Christian Körner[2]

[1] *Plant Ecophysiology, Utrecht University, PO Box 800.84, 3508 TB Utrecht, The Netherlands*

[2] *Institute of Botany, Schönbeinstrasse 6, 4056 Basel, Switzerland*

[3] *Environmental Biology, Research School of Biological Sciences, Australian National University, GPO Box 475, Canberra, ACT 2601, Australia*

[4] *Département des Sols et de Génie Agroalimentaire, Université Laval, Québec G1K 7P4, Canada*

[5] *Centre for Ecosystem Studies, Forest Ecology and Forest Management, Wageningen University, PO Box 47, 6700 AA Wageningen, The Netherlands*

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Abstract

The effect of irradiance on leaf construction costs, chemical composition, and on the payback time of leaves was investigated. To enable more generalized conclusions, three different systems were studied: top and the most-shaded leaves of 10 adult tree species in a European mixed forest, top leaves of sub-dominant trees of two evergreen species growing in small gaps or below the canopy in an Amazonian rainforest, and plants of six herbaceous and four woody species grown hydroponically at low or high irradiance in growth cabinets. Daily photon irradiance varied 3–6-fold between low- and high-light leaves. Specific leaf area (*SLA*) was 30–130% higher at low light. Construction costs, on the other hand, were 1–5% lower for low-irradiance leaves, mainly because low-irradiance leaves had lower concentrations of soluble phenolics. Photosynthetic capacity and respiration, expressed per unit leaf mass, were hardly different for the low- and high-light leaves. Estimates of payback times of the high-irradiance leaves ranged from 2–4 d in the growth cabinets, to 15–20 d for the adult tree species in the European forest. Low-irradiance leaves had payback times that were 2–3 times larger, ranging from 4 d in the growth cabinets to 20–80 d at the most shaded part of the canopy of the mixed forest. In all cases, estimated payback times were less than half the life span of the leaves, suggesting that even at time-integrated irradiances lower than 5% of the total seasonal value, investment in leaves is still fruitful from a carbon-economy point of view. A sensitivity analysis showed that increased *SLA* of low-irradiance leaves was the main factor constraining payback times. Acclimation in the other five factors determining payback time, namely construction costs, photosynthetic capacity per unit leaf mass, respiration per unit leaf mass, apparent quantum yield, and curvature of the photosynthetic light-response-curve, were unimportant when the observed variation in each factor was examined.

Key words: Chemical composition, construction costs, payback time, photosynthesis, respiration, shade leaf, soluble phenolics, specific leaf area, sun leaf

Introduction

Irradiance and leaf morphology

Light forms a highly dynamic factor in a plant's life. Light intensity as experienced by a leaf can change within seconds due to clouds covering the sun or because of leaf flapping, in hours due to the diurnal cycle, and in months because of changes in the maximal inclination of the sun. On top of these changes comes variation in irradiance induced by the presence of neighbouring plants. Neighbours can either cast shade continuously throughout the

* To whom correspondence should be addressed in Utrecht. Fax: +31 30 2518366. E-mail: H.Poorter@bio.uu.nl

year, such as in an evergreen forest, or develop their leaves during the growing season, such as in a deciduous forest or in herbaceous stands. Finally, part of the shading can take place within an individual, with top leaves of a given plant shading their own lower leaves. Ample research has investigated the adjustments a plant can make in its photosynthetic apparatus, both in the short term during light-flecks (Valladares *et al*., 1997; Leaky *et al*., 2005) as well as in the long term (Björkman, 1981; Evans *et al*., 1988). At the physiological level, long-term shading of isolated plants generally results in leaves that have a lower photosynthetic capacity and leaf nitrogen content per unit area, and a larger investment of total available nitrogen in chlorophyll and light-harvesting compounds (Evans, 1996; Hikosaka and Terashima, 1996; Evans and Poorter, 2001). Structural adjustments involve decreases in leaf thickness, especially due to decreases in the number of layers and the thickness of palisade parenchyma (Chabot *et al*., 1979; Lee *et al*., 2000). Older leaves play a special role, as they determine at least partly anatomical characteristics such as stomatal density and leaf thickness of the newly-developing leaves (Yano and Terashima, 2001; Lake *et al*., 2002). Apart from the decrease in leaf thickness, shading also causes a decrease in leaf density, the net result being a higher amount of leaf area formed per unit of biomass invested in leaves (Specific Leaf Area, *SLA*). At the whole plant level, a shift in allocation occurs, with low-light plants investing more biomass in leaves and especially stems, and less in roots (Brouwer, 1963; Corré, 1983; Poorter and Nagel, 2000).

Plants that form part of a canopy experience much stronger light gradients over the vertical axis than isolated plants. Leaves of herbaceous plants generally develop at high light, but get shaded during the course of the growing season. Anatomical adjustment in that case is difficult, but physiological acclimation and an increase in *SLA* will often take place (Pons and Pearcy, 1994). In deciduous woody species, lowermost leaves experience much lower light intensities than top leaves almost immediately after flushing. For at least a number of species it has been found that leaf characteristics such as leaf size are influenced by the light climate experienced by the leaves of the previous year (Eschrich *et al*., 1989; Uemura *et al*., 2000). In all cases, in both herbaceous and woody canopies, there is a strong gradient in leaf anatomy, nitrogen content per unit area, and photosynthetic capacity (DeJong and Doyle, 1985; Hollinger, 1989; Kull and Niinemets, 1998; Frak *et al*., 2002), which scales with the light gradient. Such scaling allows for an efficient use of the invested nitrogen (Field, 1983; Evans, 1993; Pons and Anten, 2004).

Construction costs

Compared with the effect of light on leaf physiology and anatomy, only little is known about the effect of irradiance on chemical composition and construction costs. Construction costs are defined as the amount of glucose required to produce 1 g of biomass out of glucose and minerals. Part of the glucose will serve to provide for the carbon skeletons of the organic material, part will be used to form the ATP and NAD(P)H that drives the anabolic reactions necessary in biosynthesis (Penning de Vries *et al*., 1974; Griffin, 1994). Using different short-cut methods to estimate this parameter, some papers report the construction costs to be lower at high light (Sims and Pearcy, 1994), others show higher values at high light (Niinemets, 1999; Baruch *et al*., 2000). However, no systematic investigation has been made across a wider range of plant species. Therefore, the first question is to find out how growth irradiance affects the construction costs of leaves.

Chemical composition

The advantage of short-cut methods to assess construction costs is that they provide a quick and easy estimate of the glucose costs of biomass. However, they do not provide an insight into the underlying reasons for possible changes in construction costs. Understanding these changes requires knowledge of the chemical composition of the plants. Such an analysis necessarily has to be a proximate one, as it is not possible to quantify the full array of compounds present in leaves (Chapin, 1989). Following Poorter and Villar (1997), the range of chemical constituents was classified into eight different classes. Four of these classes are relatively reduced and therefore expensive to produce. Using glucose as a starting point and taking into account the relevant biosynthetic pathways, the costs to produce lignin, protein, soluble phenolics, and lipids have been calculated to be 2–3 g of glucose g^{-1} compound (Penning de Vries *et al*., 1974). Three other classes of compounds (total structural carbohydrates [TSC; cellulose, hemi-cellulose, and pectin], total non-structural carbohydrates [TNC; soluble sugars plus starch] and organic acids) are relatively oxidized and require close to 1 g of glucose to produce 1 g of end-product. The eighth group is that of the inorganic compounds, which are termed 'minerals' throughout this paper. They are considered to bear no glucose costs for the plant, except for root respiratory costs necessary to take up these nutrients. Variation in glucose costs between different constituents within a given class of compounds is minor compared with variation between classes. Therefore, the eight different classes provide a relatively simple way to characterize plant material and enables an appropriate integration level to understand why the costs to construct a leaf, stem or root differ between species or treatments.

Some of the above-mentioned classes of compounds have been reported to vary with the light environment experienced by the plant. Leaves of low-light-grown plants, for example, have lower concentrations of non-structural carbohydrates (TNC, Waring *et al*.; 1985, Mooney *et al*., 1995). The soluble sugars that are used as an osmoticum in the vacuole, are replaced by nitrate (Blom-Zandstra *et al*.,

1988), resulting in higher mineral concentrations at low light. Moreover, lower concentrations of lignin have been found in low-irradiance leaves (Waring *et al*., 1985; Niinemets, 1999) as well as lower concentrations of soluble phenolics (McKee, 1995; Yamasaki and Kikuzawa, 2003). Protein concentrations, on the other hand, have been found to increase to some extent (Evans and Poorter, 2001). However, it requires insight into the absolute changes in all of these compounds concurrently, to analyse what the effect on construction costs are. The second focus of this paper is therefore on the quantitative importance of differences in chemical composition in explaining the observed difference in construction costs of high- and low-irradiance leaves.

Payback time

Knowing the costs in terms of glucose that a plant has to invest to produce 1 g of leaf opens up a whole avenue of other interesting topics which relate to the carbon economy of the plant and the realized returns on given investments. One example of such a question is what additional costs in terms of chemical investments a plant has to make to increase its leaf life span, and what the expected returns are for such an investment, given the leaf's biotic and abiotic environment (Chabot and Hicks, 1982; Diemer *et al*., 1992). A question that is pertinent to the current research is how long sun and shade leaves need to function to contribute at least as much glucose to the plant as it cost the plant to produce that leaf (Miller and Stoner, 1979). This time period that a leaf requires to amortize its costs is called the 'payback time' of a leaf. One of the first to use this concept in relation to the light environment were Jurik and Chabot (1986), who measured photosynthesis, respiration, light climate, and construction costs in leaves of *Fragaria*. They found the payback time of leaves of shaded plants to be *c*. 40 d, whereas leaves of sun plants had payback times around 20 d. Williams *et al*. (1989), carrying out similar research for different *Piper* shrubs in the understorey of a Meso-American forest, found that payback times for shaded plants could surpass 3000 d. This exceeded the lifespan of these leaves, implying that such plants would show negative carbon balance in the long term and would die unless the light climate improved. As far as the authors are aware, payback times in relation to the light environment have not received much attention after Williams *et al*. (1989). Therefore, the third question posed here is to what extent do payback times differ between low- and high-irradiance leaves, and which of the components of the carbon budget of a leaf are essential in minimizing the payback time at low irradiance.

To allow for more general conclusions, this paper reports the construction costs, chemical composition, and payback times of high- and low-irradiance leaves from plants growing in three separate systems. In the first, the top and most-shaded leaves of 10 mature tree species that are growing in a Western European forest close to Basel, were analysed. This site harbours the Swiss Canopy Crane, which enabled us to access not only the lower but also the top branches of these species, three of which were evergreen, and seven deciduous. Second, saplings and subordinate individuals of two evergreen broad-leaved species growing in an Amazonian rainforest, either in gaps or under a closed vegetation were analysed. Third, an experiment was carried out where six herbaceous and four woody species were grown hydroponically in growth cabinets in a low- and a high-light environment. In all cases the light climate and leaf morphology of the high- and low-irradiance leaves were quantified. The effect of the light environment on construction costs was determined and, for the first study, the underlying chemical composition was measured. Finally, photosynthesis and respiration were determined for leaves at different light conditions and an estimate was made of the payback time of these leaves. For the two field studies, these payback times were compared with the measured lifespan of the leaves.

Materials and methods

Growth conditions and experimental design

Study 1 was carried out at the Swiss Canopy Crane site in the Jura Region in the vicinity of Hofstetten, close to Basel. Annual precipitation there is 800–1000 mm, mean temperature during the growing season 16.5 °C. The site is 550 metres above sea level (masl), the soil is calcareous, and the forest is *c*. 100 years old. In reach of the crane were 10 tree species, which are listed in the first part of Table 1. All individuals were over 30 m tall, with the top of the crown fully exposed to light, except for one of the *Acer* trees and the only individual of *Abies*, which were slightly subdominant. The canopy has a very high surface roughness and therefore the vertical light profile is highly variable in space. For eight individuals per tree species, or fewer if not that many individuals were in reach of the crane, the light-exposed part of the tree was marked, generally the south-facing part at the top, and the location where leaves of those trees were growing in deepest shade, least exposed to gaps in the canopy. Leaf sampling was carried out twice during the growing season: in the middle of June and the middle of August, 1999. The number of leaves sampled from each individual depended on the amount of trees of that species present and the weight of their leaves, and varied between 10 (*Quercus*) and a few hundred (*Larix*). In the case of the evergreen conifers equal weight samples were taken from the current-year needles and the one-year-old ones. Because only one individual of *Abies* was present, it was decided to harvest two branches that were wide apart. Before the start of the chemical processing, the biomass of the June and August harvests, and of different individuals, were mixed to obtain two independent bulk samples of biomass for each species and the two leaf positions. A more detailed description of the site is given in Körner *et al*. (2005) and Zotz *et al*. (2005).

Study 2 was carried out at the research station Nouragues in an undisturbed lowland tropical rainforest in French Guiana, 100 masl. Mean annual rainfall is 3000 mm, and the average temperature 26 °C. There is a dry season from August to November, and sometimes a shorter one around March. For two shade-tolerant evergreen species with compound leaves (Table 1), plants were selected that were either growing in gaps or under a closed canopy. A range of subordinate individuals for each species were sampled, varying in height from 1 m to 20 m. Hemispherical photographs were taken to estimate the

Table 1. *Species used in the three studies described in this paper, functional type of species and the number of individuals sampled*

In case the number of individuals were different for the low and high irradiance classes, values between brackets give the number of high-irradiance plants.

Study	Species	Type	No. of individuals
1	*Abies alba* Mill.	Evergreen conifer	1
	Picea abies (L.) Karsten	Evergreen conifer	8
	Pinus sylvestris L.	Evergreen conifer	5
	Larix decidua Mill.	Deciduous conifer	8
	Acer campestre L.	Deciduous broad-leaved	3
	Carpinus betulus L.	Deciduous broad-leaved	8
	Fagus sylvatica L.	Deciduous broad-leaved	8
	Prunus avium L.	Deciduous broad-leaved	3
	Quercus robur L.	Deciduous broad-leaved	8
	Tilia platyphyllos Scop.	Deciduous broad-leaved	3
2	*Dicorynia guianensis* Amshoff	Evergreen broad-leaved	13 (8)
	Vouacapoua americana Aubl.	Evergreen broad-leaved	7 (11)
3	*Eucalyptus goniocalyx* F. Muell. ex Miq.	Evergreen broad-leaved	8
	Eucalyptus macrorhyncha F. Muell.	Evergreen broad-leaved	8
	Nerium oleander W.	Evergreen broad-leaved	8
	Radyera farragei (Fryxell and Hashmi)	Deciduous broad-leaved	8
	Datura stramonium L.	Herbaceous dicot	8
	Echium plantagineum L.	Herbaceous dicot	8
	Nicotiana tabacum L.	Herbaceous dicot	8
	Physalis peruvianum L.	Herbaceous dicot	8
	Plantago major L. ssp. *pleiosperma*	Herbaceous dicot	8
	Raphanus sativus L.	Herbaceous dicot	8

light availability of each individual. In all cases leaves were collected that were positioned in the upper part of the trees. More details are given in Rijkers *et al*. (2000) and Bongers *et al*. (2001).

In study 3, six herbaceous and four woody species were used, listed in the bottom part of Table 1. Seeds of the species were germinated in sand and transferred to hydroponics, with nitrate as the only nitrogen source at a concentration of 2 mM. Plants were placed in a growth cabinet, with 1000 W HPI lamps as a light source. The growth cabinet was partitioned into two halves, in one half the irradiance at plant level was 1000 μmol m^{-2} s^{-1}, in the other part neutral shade cloth reduced the irradiance to 200 μmol m^{-2} s^{-1}. The length of the light period was 11 h. Plants were grown for 3–6 weeks, depending on their growth rate, and four plants were harvested for both treatments. The experiment was repeated independently in a second growth chamber. For a more detailed description see Poorter and Evans (1998) and Evans and Poorter (2001).

Measurements

In study 1, photosynthetic photon irradiance was determined continuously during the growing season on top of the crane or in an open field nearby, for the years 1997–1999 and 2001–2003. Relative irradiance for sun-exposed top leaves and the most-shaded lower leaves of the different trees was determined with an 80 light sensor ceptometer (Decagon Devices, Pullman, WA, USA), relating the irradiance in the 400–700 nm waveband measured just above the leaves to the irradiance above the canopy. This procedure was carried out three times during the growing season, on two bright and one overcast day, between 10.00 h and 15.00 h.

A total of 40 light-response-curves and 220 estimates of photosynthetic capacity were made throughout the growing seasons of both 1999 and 2000 with a portable IRGA (Li-Cor 6400, Li-Cor, USA) on broad-leaved species only. Gas exchange was determined at the locations previously marked for sampling, but leaves were not harvested after the measurements. Light-response-curves of sun leaves were measured at eight different irradiances ranging from 0–2000 μmol m^{-2} s^{-1}; for shade leaves light intensities from 0–1200 μmol m^{-2} s^{-1} were used. Photosynthetic capacity of sun leaves was taken as the maximum value of measurements carried out at 1500 and 2000 μmol m^{-2} s^{-1}, for shade leaves the capacity was derived from the maximum of three measurements determined at 400, 800, and 1200 μmol m^{-2} s^{-1}. For the analysis of leaf morphology and chemical composition, leaves were collected at the marked locations during the two sampling periods between 10.00 h and 15.00 h, and stored overnight in a refrigerator at 4 °C between wet tissue. The next day, fresh mass was determined as well as leaf area. For *Larix*, the projected leaf area was derived from length and width measurements on a subsample of leaves, assuming the leaf to be a cylinder. For the other species, leaf area was measured with a Li-Cor 3100 leaf area meter. Thereafter, leaves were dried at 80 °C and weighed again. In all cases only the leaf blades, and not the petioles, were processed.

In study 2, photosynthetic photon irradiance was determined from sunrise to sunset for 54 d in the dry seasons of 1996 and 1997. Relative irradiance was estimated from hemispherical photographs just above the plant with a 7.5 mm fish-eye lens under a standard overcast sky, using the program of Ter Steege (1994). For the current comparison, plants were classified as growing in 'low light' when the total photosynthetic photon irradiance integrated over the day (*DPI*) above the plant was between 0.5 and 4 mol photons m^{-2} d^{-1}, and as 'high light' plants when *DPI* was between 6 and 18 mol photons m^{-2} d^{-1}. A total of 80 photosynthetic light-response curves were measured with a portable IRGA (CIRAS-1, PP-system, Hitchin, UK) at eight light intensities ranging from 0 to 1420 μmol photons m^{-2} s^{-1}.

In study 3, the largest full-grown unshaded leaf of eight plants per species and treatment was measured for photosynthesis at growth light conditions, and for leaf respiration after leaves had experienced darkness for 30 min. As plants were grown at a constant irradiance throughout the day, these values suffice to estimate payback times. However, for the sensitivity analysis carried out at the end of this paper a light-response curve is required. Therefore, gas exchange was determined at saturating light conditions, and the shape of the light-response curve (slope and curvature) was derived from fluorescence measurements at light intensities ranging from 50–2500 μmol m^{-2} s^{-1}. All leaves of the plant were collected for chemical analysis and combined into two independent bulk samples per light intensity and species.

All chemical determinations were carried out on two independent bulk samples that were ground to pass a 0.08 mm sieve, and redried. The full procedure is given in Poorter and Villar (1997). In short, C and N concentrations were measured with an elemental analyser (Carlo Erba, Italy), and the ash content was determined by combustion of the plant material in a muffle furnace. Ash contains not only minerals, but also carbonates formed from organic acids and nitrate during the combustion process. To correct for this, ash alkalinity was determined by titration in studies 1 and 3 and the nitrate content determined separately according to Cataldo *et al*. (1975). Organic acid concentration was estimated by subtracting the nitrate content (in meq g^{-1}) from the ash alkalinity, and multiplying by an average molecular weight of 62.5. In studies 1 and 3, the mineral concentration was calculated by multiplying the ash alkalinity (in meq g^{-1}) by 30 g eq^{-1} (mass of carbonate), subtracting this value from the total ash, and adding the weight of nitrate. In study 2, the mineral content was taken as 0.67× the ash content (Vertregt and Penning de Vries, 1987). Leaf material of study 1 was subsequently analysed for a range

of other compounds. Lipids were determined gravimetrically in the chloroform fraction of a chloroform–methanol–water extract. Soluble phenolics were determined in the methanol–water phase, using the Folin–Ciocalteu reagent. Protein concentration was calculated by subtracting nitrate-N from total N, and multiplying by 6.25. Soluble sugars were determined in the methanol–water phase, the insoluble sugars after boiling with 3% HCl, with the anthrone method described by Fales (1951). The residue left over after the chloroform–methanol–water extraction and the 3% HCl treatment was considered to consist of (hemi)cellulose, lignin, precipitated protein and cell wall protein as well as some silica. From the C and N content of this residue the concentration of lignin was determined, after correcting for the protein and silica fractions that were still present in this residue. This was done assuming a C concentration in lignin of 640 mg g^{-1}, and a C concentration in the (hemi-)cellulose complex of 444 mg g^{-1} (Poorter and Villar, 1997). Total structural carbohydrates were assumed to be the remainder of the residue.

Calculations and statistics

Construction costs (*CoCo*) were calculated following the approach of Vertregt and Penning de Vries (1987), slightly modified by Poorter (1994):

$$CoCo = (-1.041 + 5.077C_{om})(1 - M) + (5.235N_{org}) \quad (1)$$

where *CoCo* are the construction costs (g glucose g^{-1} DW), C_{om} the C content of the organic material (g g^{-1}), and M and N_{org} the mineral and organic N concentration of the total dry mass (in g g^{-1}), respectively.

Estimates of payback times are necessarily rough, and therefore two conservative calculations were used. The first assumes that all sugars fixed throughout a leaf's life have equal value to the plant, and that no leaf respiration is involved in growth processes (Williams *et al*., 1989):

$$PBT = \frac{CoCo}{(\int A_m - \int R_m) \times 12 \times 180/172} \quad (2)$$

where *PBT* is the payback time (expressed in days) and $\int A_m$ and $\int R_m$ are the mass based instantaneous rates of net CO_2 fixation, integrated over the day period, and leaf respiration, integrated over the night period (mol C g^{-1} DW d^{-1}). Mass-based daily gas exchange was derived from *SLA* values and area-based momentary photosynthesis, and depends on light intensity as:

$$A_m = \left\{ \frac{\phi I + A_{max} - \sqrt{\{(\phi I + A_{max})^2 - 4\theta\phi A_{max} I\}}}{2\theta} - R_d \right\} SLA \quad (3)$$

where ϕ is the apparent quantum yield, I is the irradiance, A_{max} is the light-saturated gross photosynthetic rate per unit leaf area, θ is the curvature of the non-rectangular hyperbola, and R_d is the area-based respiration. The daily rate of CO_2 fixation in studies 1 and 2 were estimated by taking the average time between sunrise and sunset during the growing season (14.4 h in study 1, 12.2 h in study 2), the frequency of different light intensities throughout this period as measured above the canopy, and the relative irradiance as measured for leaves at each location, in combination with equation 3. Respiration was estimated by integrating the measured dark respiration rate over the average period during the growing season between sunset and sunrise. The numbers at the right side of equation 2 convert moles of C into grams of glucose.

The second estimate of payback time is also based on equation 2, but with different parameters for the light-response curves. The reason for this is that the use of small cuvettes in portable gas exchange systems yield proper estimates of photosynthetic capacity, but may give rise to substantial measurement errors at low rates of CO_2 exchange (Pons and Welschen, 2002). Therefore, the instantaneous rate R_m was estimated as being 7% of the instantaneous light-saturated A_m. This value was derived from the woody species of experiment 3, with only small differences between low-light and high-light plants, and was also found by Givnish (1988) in a compilation of experiments on 20 different species. Furthermore, a common apparent quantum yield (ϕ=0.05 mol C mol^{-1} photons) and curvature (θ=0.75) was used for all species.

Data were analysed with the statistical package SPSS. Photosynthetic light-response curves were fitted with a non-rectangular hyperbola. Most other data were analysed in a 2-way ANOVA, with Species and Light as independent variables. The Sum of Squares due to species was further partitioned by contrasting evergreen conifers with the broad-leaved deciduous species in study 1, and the herbaceous and woody seedlings in study 3.

Results

Irradiance and leaf morphology

Daily photosynthetic photon irradiance (*DPI*) above the Swiss forest, averaged over six growing seasons (19 April to 6 October), was 30.3 mol m^{-2} d^{-1}. Light environment within the forest differed strongly for the leaves sampled at the top and the most-shaded location of the adult trees. Relative irradiance (irradiance incident on a leaf relative to the irradiance above the forest), averaged over all individuals of a species, varied between 75% and 92% for leaves at the top and 4–23% at the lowermost part of the canopy. Consequently, the most-shaded leaves experienced a *DPI* which was on average one-sixth of that of the top leaves (Fig. 1A, all significance values given in Table 2). *DPI* values at the top were somewhat lower for *Abies* and *Acer* because some individuals were not completely dominant. There were clear differences between species in irradiance level of the most-shaded leaves. For *Prunus*, *Pinus,* and *Larix*, the lowest relative irradiance was 28–32%, whereas *Abies*, *Tilia*, and especially *Fagus* and *Carpinus* still had leaves at a relative irradiance of 4–5%. There was no systematic difference between the evergreen gymnosperms and the deciduous broad-leaved species in this respect (Table 2). In study 2, *DPI* values above the canopy were only available for 54 d in the dry season, measured over two different years. From 15-year-long *DPI* data available for Barro Colorado Island, it was calculated that *DPI* in the wet season is 28% lower than during the dry season. Given that the dry season at the site of study 2 lasts *c*. 120 d, it was derived that the average *DPI* throughout the year is 30.6 mol m^{-2} d^{-1} above the canopy. For the two species under study, the average *DPI* differed 2–3-fold between individuals of gaps and understorey (Fig. 1B), with differences in irradiance being lower than in study 1. *DPI* in the growth chamber study 3 was 39.6 mol m^{-2} d^{-1} for the unshaded plants and 80% lower in the shaded treatment (Fig. 1C).

Specific leaf area varied widely between species, and was significantly higher for low-irradiance leaves in all three studies (Fig. 1D–F). In study 1, the gymnosperms had

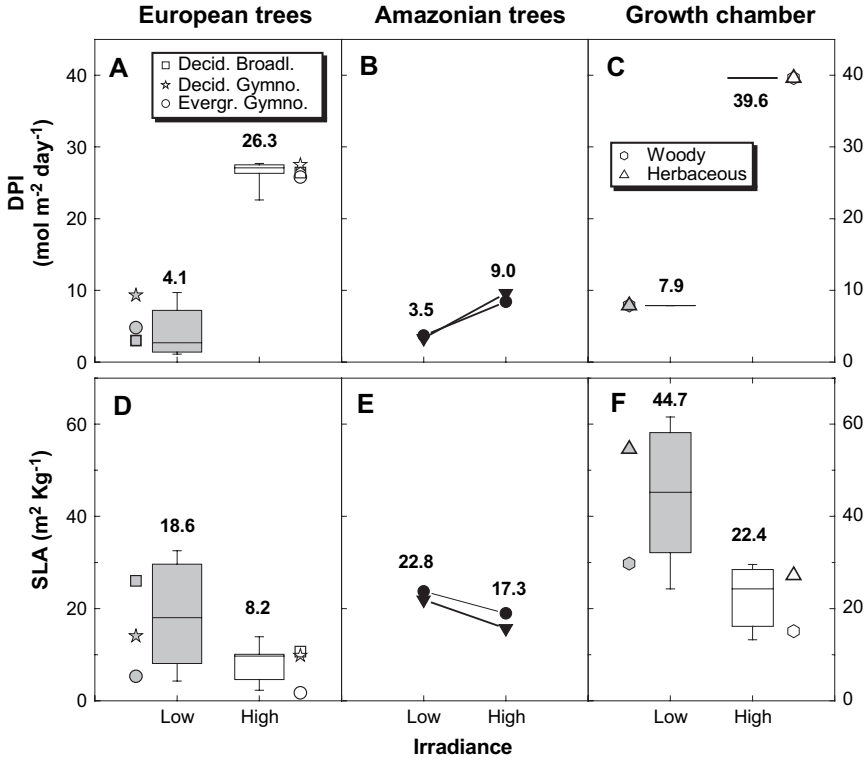

Fig. 1. (A) Daily photosynthetic photon irradiance (*DPI*) during the growing season above the sun and shade leaves of ten Western European tree species (study 1), (B) at the top of subdominant individuals of *Dicorynia guianensis* (triangles) and *Vouacapoua americana* (circles) growing in an Amazonian forest at different locations, divided into a low-irradiance and a high-irradiance category (study 2), (C) during growth in growth cabinets of six herbaceous and four woody species (study 3). (D, E, F) Specific Leaf Area (*SLA*, leaf area per unit dry mass) of low- and high-irradiance leaves in the three studies. For studies 1 and 3, box plots are used to indicate the range of values across species: the lower and the upper part of the box indicate the 25th and 75th percentile of the overall distribution, the line in the middle of the box is the median value. The bars at the end indicate the lowest and highest average values observed for the range of species. In study 1, squares indicate the average value for the six deciduous broad-leaved trees, circles the average value for the three evergreen gymnosperm trees, and stars the value for 1 deciduous gymnosperm tree. In study 3, the triangles indicate the value of six herbaceous species and hexagons indicate the average of four evergreen broad-leaved woody species. In all panels the numbers indicate the averages across all species in a given study for low-irradiance or high-irradiance leaves.

the lowest *SLA*, and increased in *SLA* at low light relative to high light less (40–140%) than did the deciduous species (70–230%). In study 3, woody species had much lower *SLA* than herbaceous species (Table 2). Species from both groups roughly doubled *SLA* in low light. *SLA* in the Amazonian study differed less between the high- and low-light leaves than in the other two studies, which will, at least partly, be caused by the smaller differences in irradiance.

Construction costs

Construction costs were estimated from the concentration of C, organic N, and minerals. Carbon concentration was close to 500 mg g^{-1} for the evergreen conifers (study 1; Fig. 2A) and the broad-leaved evergreens (study 2, Fig. 2B), with only marginally lower values (<1%) in low light. Differences between high- and low-light leaves were more apparent for the broad-leaved deciduous species in study 1 (3.5% lower at low light, Table 2), and the plants in study 3 (4% lower values). Leaves of woody species characteristically have low concentrations of minerals, both in the field (Fig. 2D, E) as well as in hydroponics (Fig. 2F). Herbaceous species showed much higher concentrations of minerals, and 10–30% higher values at low light. Higher mineral concentrations in low light were seen in all cases, except in study 2, where values were low anyway (Fig. 2E).

Construction costs were consistently and significantly lower in the low-irradiance leaves, but the differences were small, ranging from 1–5% (Fig. 2G–I). Coinciding with their lower C and higher mineral concentration, plants from study 3 had lower construction costs than those from the other two studies. This was not only true for herbaceous species, but also for the woody ones.

Chemical composition

To understand the differences in construction costs between low-light and high-light leaves, insight into the chemical composition of the leaves is required. Are the differences with light modest because differences in chemical composition are minor, or are the changes in concentration large but the net effect on construction costs small? A full analysis of the eight classes of compounds is only available for the 10 species in study 1. Lipid concentration was quite

Table 2. *Percentage of the total sum of squares in the ANOVA explained by the effect of light, species and the light×species interaction for the three studies, as well as the significance values*

The values between brackets indicate the significance of the contrast between the three evergreen gymnosperms and the six deciduous broad-leaved species in study 1, and the contrast between six herbaceous and four woody species in study 3. ns, Non-significant; +, 0.05 <*P* <0.10; *, *P* <0.05; **, *P* <0.01; ***, *P* <0.001).

	Study 1			Study 2			Study 3		
	Light (position)	Species	Light×Species	Light (location)	Species	Light×Species	Light (treatment)	Species	Light×Species
Irradiance (mol m^{-2} d^{-1})	94***	4*** (^{ns})	2*** (**)	68***	0^{ns}	1^{ns}	–	–	–
SLA (m^2 kg^{-1})	28***	53*** (***)	15*** (***)	51***	11*	1^{ns}	48***	39*** (***)	6*** (***)
[C] (mg g^{-1})	17***	59*** (***)	14* (**)	1^{ns}	40***	2^{ns}	5***	91*** (***)	2^{ns} (^{ns})
C/N (g g^{-1})	1**	93*** (***)	3* (*)	5^{+}	25***	27***	3***	91*** (***)	3^{+} (*)
Construction costs (g glucose g^{-1})	17***	47*** (*)	18^{+} (*)	4^{ns}	27***	1^{ns}	7***	79*** (***)	7^{+} (^{ns})
Lipids (mg g^{-1})	1^{+}	85*** (***)	8* (***)	–	–	–	–	–	–
Soluble phenolics (mg g^{-1})	16***	67*** (***)	16*** (***)	–	–	–	–	–	–
Protein (mg g^{-1})	2**	92*** (***)	2^{ns} (*)	1^{ns}	15**	24***	1^{ns}	76*** (***)	12^{+} (**)
Lignin (mg g^{-1})	4***	84*** (***)	8** (*)	–	–	–	–	–	–
TNC (mg g^{-1})	25***	45*** (*)	18* (^{ns})	–	–	–	–	–	–
TSC (mg g^{-1})	27***	62*** (***)	5^{ns} (^{ns})	–	–	–	–	–	–
Organic acids (mg g^{-1})	8**	71*** (***)	10^{ns} (*)				1^{ns}	88*** (***)	5^{ns} (^{ns})
Minerals (mg g^{-1})	11***	68*** (***)	9^{ns} (**)	0^{ns}	29***	1^{ns}	4***	94*** (***)	1^{ns} (*)
A_{max} (nmol g^{-1} s^{-1})	9*	45*	22^{ns}	27**	0^{ns}	0^{ns}	72***	9*** ($^{+}$)	5** (^{ns})
Respiration (nmol g^{-1} s^{-1})	1^{ns}	58*	10^{ns}	6^{ns}	0^{ns}	14^{+}	31***	25*** (***)	5^{ns} ($^{+}$)
Payback time (d)	88***	4^{+}	6*	83***	15***	1^{ns}	30***	54***(***)	11***(*)

similar between species, with the exception of *Pinus*, which had much higher values than the rest of the species (Fig. 3). This will at least partly be due to the high resin content in their needles. Lipid concentration was 2–9% lower in the low-light leaves of the gymnosperms than in the high-light ones, but consistently higher (4–30%) in the low-light deciduous leaves and, consequently, the light × species interaction was strong (Table 2). Protein concentration was somewhat higher in the low-light leaves in study 1 (Fig. 3, 7% on average) and in the woody species of study 3 (7%), but 7% lower in the herbaceous plants of that experiment. Relative changes in study 1 were larger for minerals and organic acids, with increases at low light of 30% and 41%, respectively. Similar observations were made for the mineral concentration in study 3.

Non-structural carbohydrates, like soluble sugars and starch, can be expected to be lower in a low light environment, and this is what was observed, with, on average, 13% lower values for the most-shaded leaves (Fig. 3). These differences were mainly due to the soluble sugars (data not shown), both for the evergreen and the deciduous species. Interestingly, it was found that the total structural carbohydrates to increased at lower light levels, whereas lignin behaved in the opposite way and increased at higher light levels. At both light levels, TSC and lignin concentrations were significantly higher in the evergreen than in the deciduous species (Table 2). Soluble phenolic concentration was variable among species, with the highest concentrations in *Acer campestre* and *Carpinus betulus*. It was the class of compounds that changed most dramatically with irradiance, with larger decreases at low light in the deciduous species (44–97%) than in the gymnosperms (3–22%).

Payback time

Photosynthetic capacity expressed per unit leaf area ranged from 13–18 μmol CO_2 m^{-2} s^{-1} in the high-irradiance leaves at the top of the trees of the different species and was 40–60% lower in the most-shaded leaves (study 1, *P* <0.001). Note that in this study gas exchange data are available for broad-leaved species only. In study 2, photosynthetic capacity of the high-irradiance category in both species was around 7 μmol m^{-2} s^{-1} and was 30% lower for trees of the most shaded category. In study 3, photosynthetic capacity was much higher, ranging from 20 to 47 μmol m^{-2} s^{-1}, with low-light grown plants having a 25–55% lower capacity (*P* <0.001). These differences scaled strongly with the biomass investment per unit leaf area, such that most of the difference in photosynthetic capacity between low- and high-irradiance leaves disappeared when values were expressed per unit leaf mass (Fig. 4A–C). Area-based respiration was strongly positively correlated with area-based photosynthesis, but did not differ between low- and high-irradiance leaves when expressed on a leaf mass basis (Fig. 4D–F). In study 1, apparent quantum yield was not significantly different between the top and lowermost leaves (0.066 versus 0.067 mol CO_2 mol^{-1} photons; *P* >0.8), whereas the curvature parameter of the light-response curve was higher for the lowest leaves (0.50

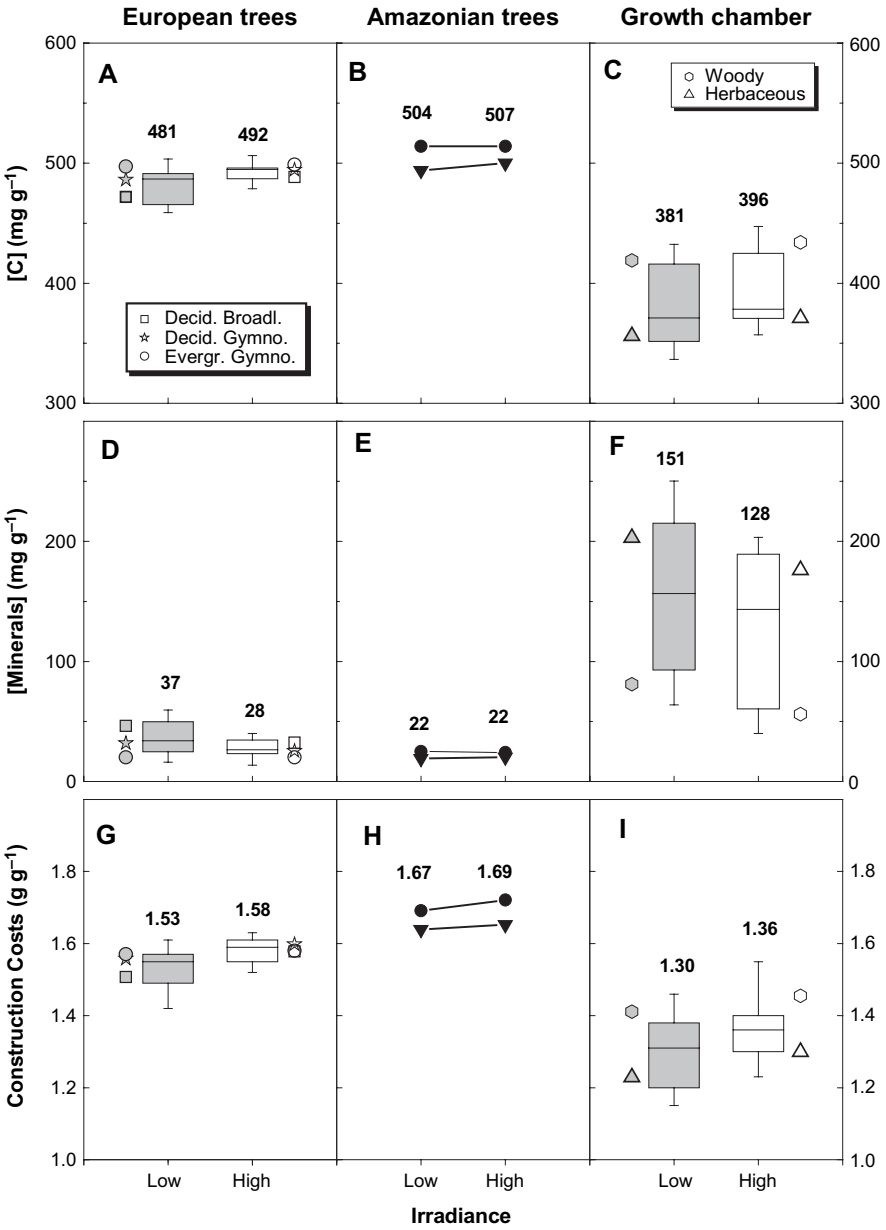

Fig. 2. Concentration of carbon (A, B, C), minerals (D, E, F), and construction costs (G, H, I) in low-irradiance and high-irradiance leaves in study 1 (A, D, G), 2 (B, E, H), and 3 (C, F, I). All values are expressed on a leaf dry mass basis. More information is in the legend of Fig. 1.

versus 0.31, $P < 0.05$). From these data, and the light distribution data observed throughout the day and integrated over the whole growing season, the payback time of the leaves was calculated. This was done in two different ways, as described in the Materials and methods. For each species and light condition, the most conservative estimate of the two was selected and these values were plotted in Fig. 4G–I. Payback times were very low in the growth room with leaves fixing as much carbon as they had cost in 3–5 d (Fig. 4I). Leaves in the upper crown of the deciduous tree species took longer, but still had payback times in the order of 15–20 d (Fig. 4G). Shade leaves of trees with an open crown, such as *Prunus*, had a payback time of *c*. 20 d, whereas the highest values (60–75 d) were found for *Carpinus* and *Fagus*, which position their lowest leaves in deep shade. With a growing season of *c*. 170 d, it can be concluded that even these leaves show positive carbon balances. Data for the lightly shaded evergreen trees in study 2 showed payback time estimates of 25 d, those in deeper shade had payback times of 40 d (Fig. 4H).

Discussion

Irradiance and leaf morphology

In this paper, an attempt was made to bring together the carbon costs and revenues of leaves that experience

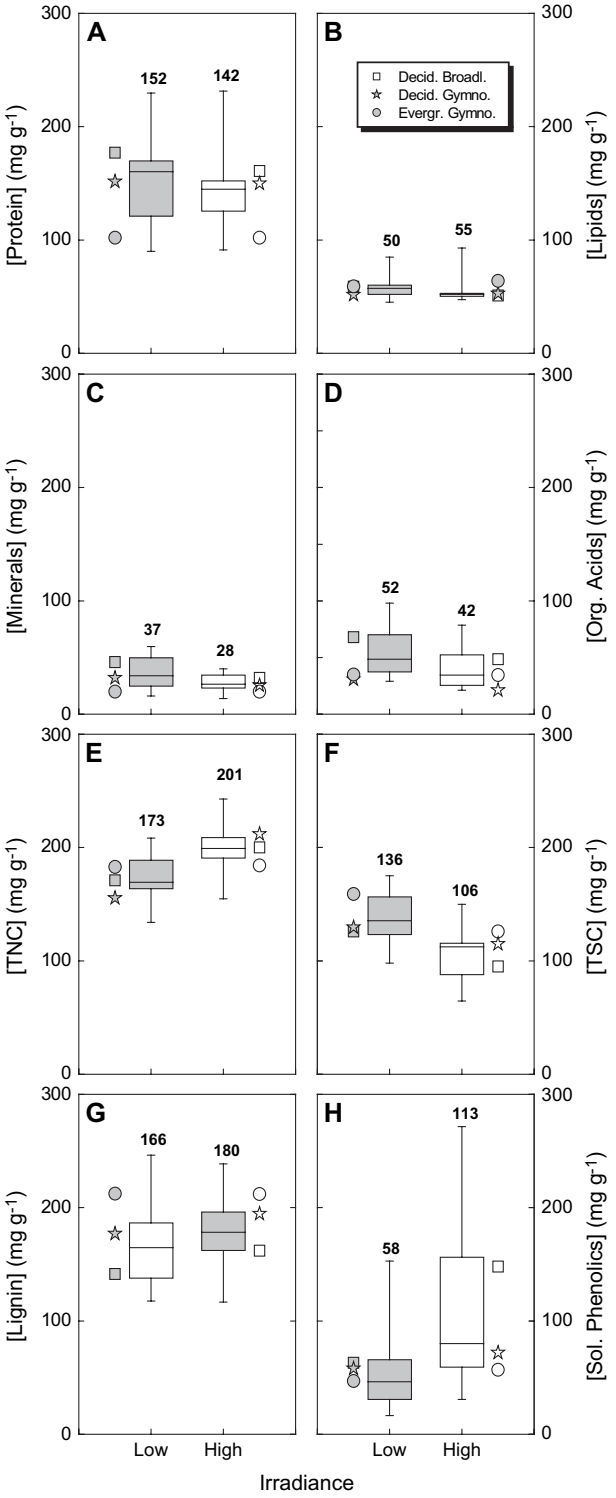

Fig. 3. Concentration of the various chemical constituents for high- and low-irradiance leaves of ten woody species from study 1. (A) Protein, (B) lipids, (C) minerals, (D) organic acids, (E) total non-structural carbohydrates, (F) total structural carbohydrates, (G) lignin, (H) soluble phenolics. All values are expressed on a leaf dry mass basis. More information is in the legend of Fig. 1.

different light climates. In the field, this is a daunting task, which already shows its complications if one tries to characterize the light climate of different leaves. In the tops of the trees, leaves are often positioned at an angle with the horizontal (Kull *et al*., 1999, but see Kitajima *et al*., 2005), and the leaf lamina will change position continuously as wind induces movements of branches and leaves (Roden and Pearcy, 1993). Moreover, even in the upper layer of the tree, leaves will not experience full sunlight continuously (Fig. 1A), as changes in the solar position during the day will cause leaves that were sun-lit in the morning to be shaded by other leaves in the crown during the afternoon. Leaves low in the tree crown will generally show leaf angles closer to the horizontal (Kull *et al*., 1999), with less leaf movement than in the top due to lower wind speed. Sunflecks will play an important role here, which are difficult to integrate throughout the season. Finally, light penetration throughout the canopy is different for sunny and overcast days (Goudriaan, 1977). We are aware of these limitations when characterizing the light environment of the leaves, but are confident that differences in low- and high-irradiance were large enough to analyse its effects on leaf morphology, chemistry and physiology. It may pose a problem for the exact estimate of the payback times, a point which will be dealt with later.

Differences in leaf morphology are strongly driven by the integrated photosynthetic irradiance over the day (Chabot *et al*., 1979). Variation in *DPI* between leaves of different position (study 1) or different light treatments (study 3) was 5–6-fold (Fig. 1A, C), and consequently the specific leaf area was also different, variation being 2-fold on average. Similar differences in *SLA* are reported by others investigating leaf characteristics within trees (DeJong and Doyle, 1985; Niinemets and Kull, 1998; Meir *et al*., 2002) or herbaceous canopies (Evans, 1993; Anten *et al*., 1998). There were small but significant light × functional group interactions when the absolute change in *SLA* was considered, but these differences between evergreen conifers and deciduous trees in study 1, or woody and herbaceous species in study 3 largely disappeared when the relative differences were considered. There were large differences, though, in the position of the lowest leaves. Species like *Pinus sylvestris*, and *Prunus avium* have their most-shaded leaves still at relatively high light levels, whereas species such as *Picea abies*, *Fagus sylvatica*, and *Carpinus betulus* have leaves in positions that receive less than 5% of the daily irradiance at the top. Although the light climate in this mixed forest is far more heterogeneous at any given height, differences between species compare with those observed in monostands (Monsi and Saeki, 1953). The individuals of the two evergreen species studied in study 2 ranged from 1 m tall saplings to sub-dominant trees, and were all grown in small gaps or under the tree canopy. Therefore, they all experienced relatively low levels of light (Fig. 1B). This is reflected in their *SLA*

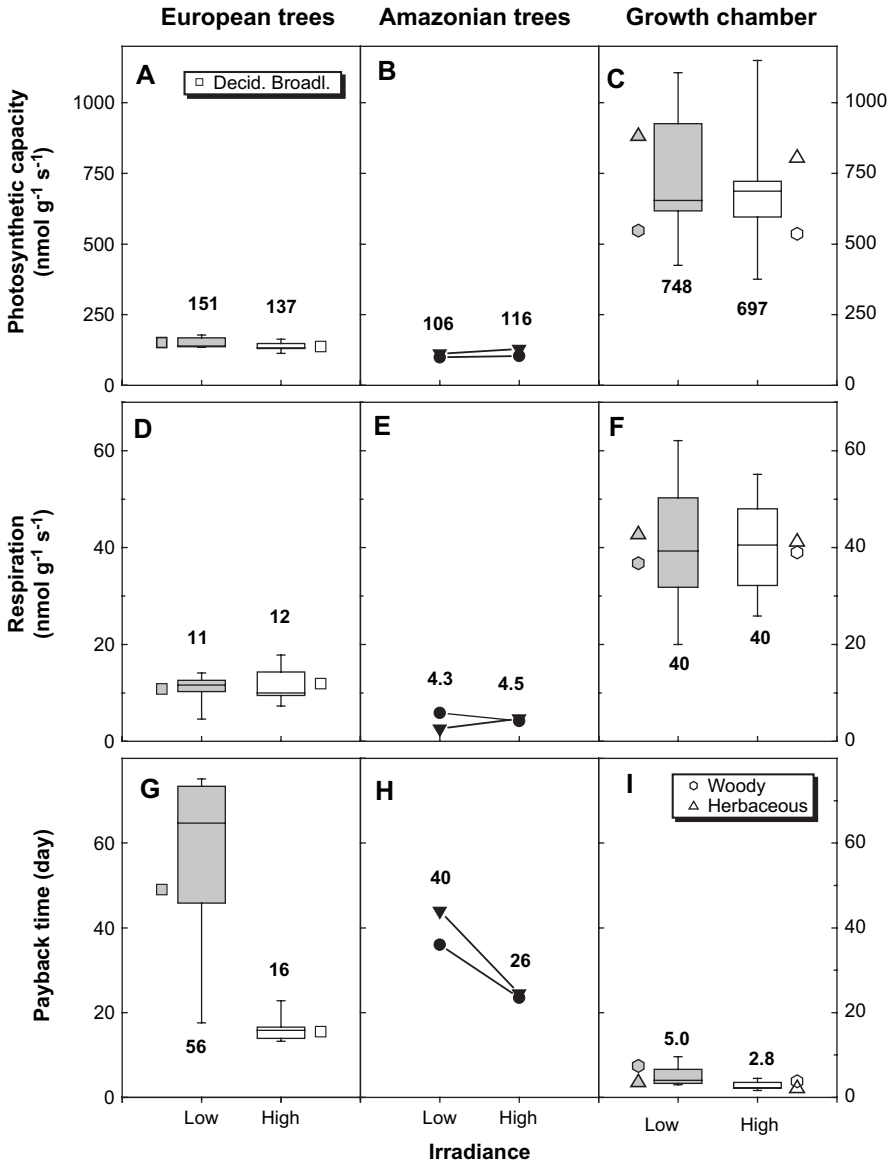

Fig. 4. Mass-based photosynthetic capacity (A, B, C), mass-based respiration rate (D, E, F), and payback times (G, H, I) of low-irradiance and high-irradiance leaves in study 1 (A, D, G), 2 (B, E, H), and 3 (C, F, I). Note that the photosynthetic data in study 1 are only available for the deciduous species. Payback times shown are the maximum values per species obtained with two different calculations, as explained in the Materials and methods. For more information see the legend of Fig. 1.

range, which differed only 30%, less than in the other two studies. Another reason for the *SLA* difference being modest in this case is that these climax species, compared to pioneer species, show relatively less variation in *SLA* with *DPI* anyway (Poorter, 1999; Rijkers *et al.*, 2000).

Construction costs

Construction costs of a plant organ can be estimated from the proximate chemical composition, but such determinations are tedious, and the total amount of plant material accounted for generally does not add up to 100%. Therefore, simpler methods have been developed, which can estimate construction costs more easily and quickly. In one of these, construction costs are derived from the carbon concentration of the plant, as well as the concentration of minerals and protein (Vertregt and Penning de Vries, 1987; Poorter, 1994). In the present studies, which comprise field-grown and controlled environment-grown plants, and includes both herbaceous and woody species, almost the full concentration range in C and minerals that is generally found in nature was covered (Fig. 2). Therefore, almost the full range in construction costs that is generally observed for leaves (1.2–1.8 g glucose g^{-1}; Poorter and Villar, 1997) was also found. In all three studies, low-irradiance leaves have slightly lower C-concentrations, and (for almost all species) higher concentrations of minerals and, consequently, construction costs are a few per cent lower in low-irradiance leaves. Data in the literature are scarce.

Contrary to our observations, Sims and Pearcy (1994) found low light leaves to have higher construction costs. The same trend as reported here was found both for tree leaves across the crown (Niinemets, 1999), for field-grown plants experiencing different light levels (Williams *et al*., 1989) and for individual plants grown in controlled conditions in high and low light (Baruch *et al*., 2000). In all cases, however, changes are rather small (Table 3), with low-irradiance leaves being, on average, 4% cheaper. Therefore, it is concluded that changes in construction costs do occur, but that these changes are relatively modest. This is very similar to the variation found for plants grown at different levels of CO_2 (Poorter *et al*., 1997; Wullschleger *et al*., 1997), nutrients (Peng *et al*., 1993), water (Baruch *et al*., 2000) and across herbaceous vegetation growing along a fertility gradient (Poorter and DeJong, 1999).

Chemical composition

To understand the underlying reasons for the lower construction costs, the proximate chemical composition of the leaves was determined in study 1, classifying constituents into eight groups of compounds. Concentrations of all eight classes of compounds changed significantly with light (Table 2). However, most changes were only modest. Low-light leaves generally had lower levels of total non-structural carbohydrates (TNC), lignin, and soluble phenolics, which is in accordance with most of the literature data (Waring *et al*., 1985; McKee, 1995; Mooney *et al*., 1995; Niinemets, 1999). In the leaves of study 1, TNC surprisingly changed only because the soluble sugars decreased. This was not anticipated, as starch values have been found to decrease as well in low-light-grown plants (Mooney *et al*., 1995). However, comparing TNC along a light gradient in five deciduous tree species, a similar trend was found by Niinemets and Kull (1998). The lower lignin content was compensated by an increase in total structural carbohydrates (TSC), as was also observed by Niinemets and Kull (1998). They suggest that increased soluble sugars and lignin concentrations may be caused by the higher water stress found at the top of the tree.

To what extent can the decrease in construction costs at low light be explained by the underlying chemical composition? A shift in chemical composition has an impact that depends on two factors. First, how large is the change in concentration, in absolute terms. And second, to what extent do the specific costs for that compound differ from the construction costs of the whole leaf. If these differences are large, as in the case of minerals or lipids, then a modest change in concentration may still have an impact. However, if the costs of a class of compounds are closer to the average (like TSC), then it requires much larger differences in concentration to affect leaf construction costs. The procedure to analyse the contribution of each constituent is described in more detail in the appendix of Poorter and DeJong (1999). In total, it was possible to account for 85% of the total dry mass of the leaf material. For the analysis of the effect of different compounds on total construction costs, the rest of the dry mass was assumed to be TSC. On average, the sensitivity analysis showed that most differences only have a small impact on construction costs (Fig. 5). Although the percentage changes in minerals and organic acids were large, they only have small effects because they represent small fractions of the plant biomass. The lower concentration of TNC and higher

Table 3. *Compilation of literature data on the effect of light on construction costs for a range of woody and herbaceous species*

Data are only given where there was at least a 3-fold change in daily irradiance. The average change at the bottom of the table includes all listed values as well as those of the present studies. The average is based on observations for 37 species, and is significantly different from zero ($P < 0.001$).

Species	System	Range in light conditions (*DPI* or relative irradiance)	Change in construction costs in low light relative to high light (%)	Reference
Woody				
Clidemia hirta	Glasshouse	3–70%	−5	Baruch *et al*. (2000)
Corylus avellana	Within field-grown trees	8–78%	−8	Niinemets (1999)
Fagus sylvatica	Within field-grown trees	20–93%	−1	Niinemets (1999)
Fraxinus excelsior	Within field-grown trees	10–90%	−5	Niinemets (1999)
Miconia calvescens	Glasshouse	3–70%	−3	Baruch *et al*. (2000)
Piper hispidum	Field-grown	4–28 mol m^{-2} d^{-1}	−8	Williams *et al*. (1989)
Piper umbellatum	Field-grown	4–28 mol m^{-2} d^{-1}	−15	Williams *et al*. (1989)
Populus tremula	Within field-grown trees	20–95%	−1	Niinemets (1999)
Tilia cordata	Within field-grown trees	4–99%	−15	Niinemets (1999)
Herbaceous				
Alocasia macrorrhiza	Glasshouse	0.5–24 mol m^{-2} d^{-1}	+6	Sims and Pearcy (1994)
Arabidopsis thaliana	Growth chamber	2.9–12 mol m^{-2} d^{-1}	−2	Poorter and DeJong (unpublished results)
Arthostema ciliatum	Glasshouse	18–70%	−5	Baruch *et al*. (2000)
Helianthus annuus	Growth chamber	4.5–18 mol m^{-2} d^{-1}	−9	Poorter and DeJong (unpublished results)
Hordeum spontaneum	Growth chamber	4.5–18 mol m^{-2} d^{-1}	−8	Poorter and DeJong (unpublished results)
Tibouchina herbacea	Glasshouse	18–70%	+3	Baruch *et al*. (2000)
Overall average			−4	

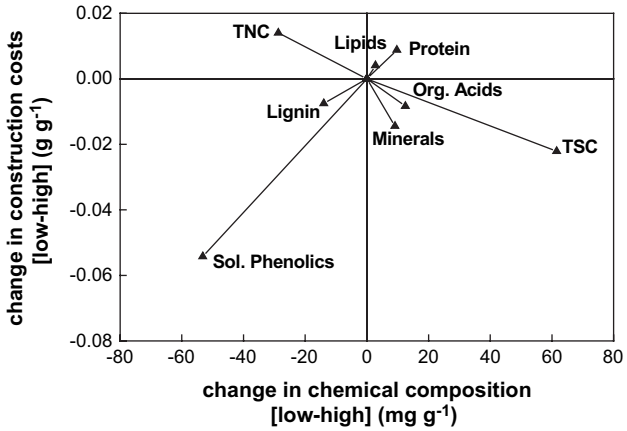

Fig. 5. Change in leaf construction costs, when for each of the eight classes of compounds the concentrations observed for the low-irradiance leaves is replaced by that of the top leaves.

level of TSC have a somewhat stronger effect because they are more abundant in the plant material. The strongest effect, on average, is due to the soluble phenolics. These were present in much lower concentrations in low-light leaves and have high specific construction costs relative to that of the average leaf. The lower concentration of soluble phenolics at low light reduced the calculated construction costs by 4%, close to the actual value of 3%. Soluble phenolics comprise a wide range of compounds, including flavonoids. A number of these compounds are involved in the protection from UV-B damage at high irradiance (Li *et al*., 1993).

Payback time calculations and assumptions

The payback time of a leaf is the ratio of the cost to produce that leaf, and the daily net carbon gain that a leaf realized (equation 2). It is, in fact, a somewhat peculiar parameter, as a leaf cannot function without roots that take up water and nutrients, and a stem to position the leaf in the proper light environment. As such, it is more relevant to consider the payback time of a unit of total plant biomass (Givnish, 1988; Poorter, 1994). However, in the case of trees growing in a forest, the payback time of a leaf is an appropriate parameter to understand the return on an investment by the plant at different canopy positions. It seems self-evident that top leaves have short payback times, but the question is whether this is also the case for the most-shaded leaves. This is especially interesting, as game-theory models predict that it may be an evolutionary stable strategy to increase the amount of leaf area per unit ground area, even if this has a negative consequence for total canopy carbon gain (Schieving and Poorter, 1999). Could it be that species such as *Fagus sylvatica* and *Picea abies* have more leaf area on display than is actually profitable? To evaluate this question insight is required into the payback times of leaves at different positions.

To estimate carbon gain of the different leaves, light-response-curves throughout two growing seasons were characterized in study 1. This is necessary as photosynthetic capacity in trees may decrease during the growing season (Kitajima *et al*., 2002), especially for high-irradiance leaves (Sims and Pearcy, 1991; Miyaji *et al*., 1997). In study 2, there are only light-response curves obtained during the dry season, but for these shaded evergreen plants with leaf longevities ranging from 2–6 years (Sterck, 1999), it is expected that capacities will not show strong decreases over time (Thomas and Winner, 2002). Maximal photosynthetic rates were very different for low- and high-irradiance leaves when expressed on an area basis, a general finding both within tree canopies (DeJong and Doyle, 1985; Frak *et al*., 2002; Meir *et al*., 2002) and for plants grown individually at high and low light (Pons, 1977; Thompson *et al*., 1992). However, on a mass basis differences largely disappear in all three studies (Fig. 4A–C, Ellsworth and Reich, 1993). Respiration scales with photosynthesis (Givnish, 1988), and while respiration rates for low- and high-irradiance leaves differed on an area basis, they were similar on a mass basis (Fig. 4D–F). This is a finding that simplifies the analysis to a large extent (Rosati *et al*., 2000).

From continuous measurements for a period of six years throughout the growing season, a good idea of the 'average' distribution of irradiance above the canopy of the Swiss forest was obtained. Assuming, for simplicity, a mean day length over the growing season, the C-gain of top and most-shaded leaves was integrated, combining the parameters of the light-response curve and the relative irradiance at that position with the observed irradiance distribution. As mentioned above, these estimates will be prone to a certain amount of error, because it is difficult to quantify light levels for each leaf position continuously, especially as leaves are continuously moving in the wind. Moreover, there will be problems with the determination of gas exchange values close to the light compensation point (Pons and Welschen, 2002), which will lead to an overestimation of the effect of respiration. Thirdly, diurnal variation in leaf temperature could result in an overestimation of respiration, if left uncorrected. Fourth, light-flecks have been found to have a substantial positive effect on the carbon balance of the leaf (Valladares *et al*., 1997; Leaky *et al*., 2005). Relative irradiance on both sunny and overcast days was determined, but will probably have missed out on most sun-flecks. Fifth, it was assumed that all sugars fixed throughout the life of a leaf have equal value for the plant. It can be argued, though, that the first sugars translocated out of the leaf could be invested in other leaves and could have a compounding effect when invested in other leaves (Harper, 1989; Poorter, 1994). Sixth, in principle, growth respiration should be deducted from the total respiration measured, as growth respiration is already accounted for in the construction cost values (Poorter, 1994). Seventh, in this analysis leaf blades only were considered, whereas petioles have to be constructed as well,

which form 2–12% of the biomass of these leaves, depending on species. Lastly, photosynthesis and respiration were not measured during the period between bud break and completion of leaf expansion, when gas exchange rates are likely to differ from those of mature leaves. However, most of the above-mentioned simplifications will cause the calculated payback times to be overestimated, which means that these values are likely to be on the conservative side.

Payback times

To account for the different processes that play a role, payback times were calculated in two different and conservative ways, not taking into account most of the points mentioned above. The most conservative estimate was then included in Fig. 4G-I. Estimated payback times for the ten species grown in a growth room at high-irradiance ranged from 2–4 d. How realistic are these values? Poorter (1994) calculated payback times for growth cabinet-grown plants and arrived at quite similar values of 1.5–3.5 d. These data could be verified by considering the payback times of whole plants. By definition, they should equal the doubling time of plants, which is in fact another expression for relative growth rate. Relative growth rates of whole plants as determined from sequential harvests were very close to those estimated from payback times, giving support to the approach followed. Payback time estimates for the leaves of the herbaceous species in study 3 are somewhat longer than those found by Poorter (1994). Given that the *DPI* in study 3 was 2.5-fold higher than that of Poorter (1994), it might be expected that the plants were growing faster and, therefore, that payback times were shorter. As this is not the case, it seems likely that our current approach to calculate payback times is indeed at the conservative side.

Payback times of sun leaves in field-grown woody plants have been estimated before by Saeki and Nomoto (1958). They found values for a deciduous species to be 9–15 d, and for evergreens 30 d. Their values should actually be *c*. 50% higher, because they implicitly assumed construction costs to be 1.0 g glucose g^{-1} biomass, whereas 1.5 is a more realistic estimate for leaves of woody species (Villar and Merino, 2001). Jurik and Chabot (1986) estimated values for herbaceous *Fragaria* to be 22 d, and Williams *et al*. (1989) found values for sun leaves of different *Piper* species to range from 5–20 d. The estimates from this work for the sun leaves of the six adult tree species vary from 15–20 d. Notwithstanding different environmental conditions, and different approaches, these values all fall in the same range, and suggest that sun leaves amortize their cost in very short time spans.

How much longer is the payback time of low-irradiance leaves? Are they able to contribute positively to the plant's carbon budget within their lifespan? Williams *et al*. (1989), using conservative equation 2, but only assuming days with bright sunshine, calculated payback times of over 3000 d, whereas maximal leaf longevity was estimated to be 800 d. This would suggest that these *Piper* shrubs at the time of measurements were running a negative carbon balance. However, this is clearly not the norm for the low-irradiance leaves studied here. The estimates from this study for the payback time of the most-shaded leaves of the trees in the European forest range from 20–80 d. Leaf census data at the site show that *Fagus* leaves have a leaf life span of 190 d, *Carpinus* leaves 200 d and *Quercus* leaves 220 d (R Asshoff, personal communication). Allowing for 10 d of leaf expansion, a period for which there is no physiological data, and 15 d of senescence, this would make the leaves physiologically mature and active for *c*. 165–180 d. In that case, even the most shaded *Fagus sylvatica* leaves can still contribute carbon to the rest of the plant for at least half of their life span. A similar conclusion is reached with regard to the payback times in the Amazonian forest, which were estimated to be around 40 d. The leaf longevities for these evergreen species have been found to be 350–1500 d (Sterck, 1999). Payback times for these tree leaves are remarkably similar to the 43 d estimate calculated for shaded leaves of *Fragaria* reported by Jurik and Chabot (1986). In the growth cabinet, payback times were found to be much shorter, even at a similar daily photon input, which most likely reflects the better nutrient and water supply, and/or the lower mechanical stress on these plants.

Sensitivity analysis

To obtain more insight into the different parameters that dominate the payback time of shade leaves, a sensitivity analysis was carried out for each of the three studies described above, assessing which of the parameters in equations 2 and 3 has the strongest impact on payback time. A simple sensitivity analysis would change each of the parameters in the equation by 10% and evaluate its effect on payback time. However, some plant parameters have higher phenotypic plasticity than others. Consequently, the average values of *SLA*, construction costs, Apparent Quantum Yield, and the curvature of the light-response curve, as well as the mass-based photosynthetic capacity and respiration, were taken for the low-irradiance leaves and the 'average' payback time of these shade leaves was calculated. The mass-based rates of photosynthesis and respiration were used because they are virtually independent of *SLA*, a prerequisite for such an analysis. Then, by turn, each of the values of the six parameters of the shade leaves was replaced by the average value of the high-irradiance leaves, and its effect on the payback time calculated. Changes in payback time differed between studies due to differences in light and nutrient levels, but when all values per experiment were scaled relative to the largest change observed, the results were remarkably similar across studies. In all three studies, differences in construction costs between high- and low-irradiance leaves had marginal effects on the payback

time, and this was also the case for the apparent quantum yield of the light-response curve and dark respiration (Fig. 6). Photosynthetic capacity and the curvature of the light-response curve had some effect in study 2, but by far the most important difference in payback time between the low-light and high-light leaves in all the systems was the difference in *SLA*. This is in line with the analysis of Evans and Poorter (2001), who showed that for shade leaves acclimation with respect to *SLA* was more important than differential allocation of N within the photosynthetic apparatus.

Bearing the above conclusion in mind, what constrains the functioning of leaves in the lower part of the tree crown? Simplifying the results of the sensitivity analysis by assuming that only *SLA* differs for high- and low-irradiance leaves (cf. Evans, 1998), the payback time for each combination of *DPI* and *SLA* can be calculated. If irradiance halves, then a leaf can maintain its carbon gain per unit mass by doubling its *SLA*. This yields hyperbolic relationships as given in Fig. 7, where each curve indicates a given payback time, in this case ranging from 10 d to 180 d. Circles indicate the observed data obtained in study 1. Payback times at high *DPI* vary around 15 d. With decreasing *DPI*, plants respond by increasing *SLA*, but the change is not large enough to maintain fully the rather short payback times of the sun leaves: they move towards a curve with a longer payback time. These changes are not dramatic as long as the *DPI* is above 4 mol m^{-2} d^{-1}. However, if irradiance decreases below 2 mol m^{-2} d^{-1}, *SLA* has to increase strongly to maintain reasonably short payback times. There have been some reports of shaded plants with *SLA* as high as 150 m^2 kg^{-1} (Corré, 1983), but these values are obtained for glasshouse-grown plants. Higher wind speeds, lower water availability, and lower temperatures will keep field values of *SLA* below 60 m^2 kg^{-1}, even more so for trees than for herbs (Elias, 1979). With *SLA* constrained to such a maximum value, payback time increases strongly as irradiance decreases just marginally. Given the constraint of a growing season of 170 d, species with a large amount of leaf area per unit ground area, such as *Fagus* and *Carpinus*, therefore, have only limited possibilities to form additional layers of leaves in the lower part of the crown. This will be especially true in growing seasons with a low *DPI*. From these calculations we derived that the lowest leaves of these two species have payback times longer than the growing season if *DPI* drops below 23 mol m^{-2} d^{-1}.

In this paper, the focus was on irradiance as the main environmental factor. Quite likely, other factors change with irradiance as well: leaves will be warmer, and especially at higher locations in the tree may be exposed to higher vapour pressure deficits and hence transpire more water, but will also see earlier stomatal closure (Niinemets and Kull, 1998). However, given the similar responses between the top and the most-shaded leaves in the Western European forest, contrasting gap environments in the understorey of an Amazonian forest, as well as herbs and woody seedlings grown under controlled environments, there is reasonable confidence that most of the observed changes will be predominantly driven by light.

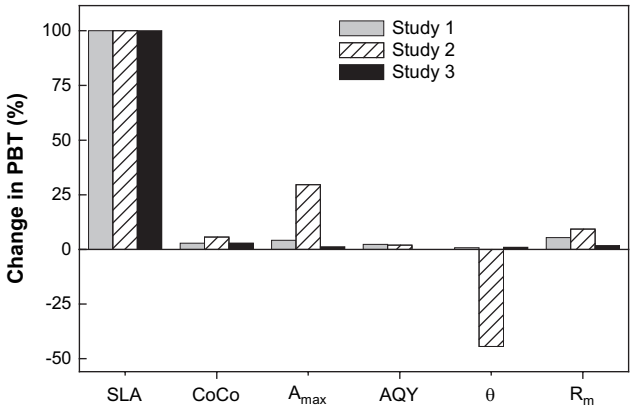

Fig. 6. Sensitivity analysis of the six factors involved in the payback time of the shade leaves for the three different studies. All parameters were taken as averages from the low-irradiance leaves in each study and the pertaining payback time was calculated. Values for each of the six parameters were subsequently replaced by those from the high-irradiance leaves, averaged across all species in a given study. The changes in payback time were calculated and for each study, these changes were scaled to that of the largest positive value which was set to 100%. Positive values indicate that for a given parameter, replacement of the value of the shade leaf by that of the sun leaf increases the payback time. *SLA*, specific leaf area (m^2 kg^{-1} leaf dry mass); CoCo, construction costs (g glucose g^{-1} leaf dry mass); A_{max}, photosynthetic capacity per unit leaf dry mass (nmol g^{-1} s^{-1}); AQY and θ, apparent quantum yield and curvature parameters, respectively, in the non-rectangular hyperbola of the photosynthesis:irradiance curve; R_m, leaf respiration rate per unit leaf dry mass. The absolute change (increase) in PBT due to the change in *SLA* from low-light to the high-light value was 39 d in study 1, and 5 d in studies 2 and 3.

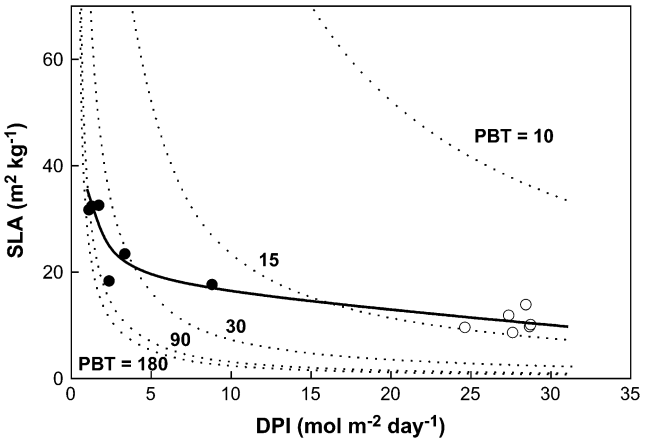

Fig. 7. Payback time curves as a function of the daily photosynthetic photon irradiance (*DPI*) and *SLA* of leaves, assuming that all other parameters that affect the carbon economy (construction costs, shape of the light-response curve of photosynthesis, mass-based rates of light-saturated photosynthesis, and respiration) are exactly equal. Different lines indicate payback times of 10, 15, 30, 90, and 180 d, respectively. Average *SLA* values are shown for high-irradiance (open circles) and low-irradiance (solid circles) trees of the six broad-leaved species of study 1.

Conclusions

Low-irradiance leaves have slightly lower construction costs, on average 3%, than high-irradiance leaves. These differences are mainly caused by the lower concentration of soluble phenolics, at least in adult trees. Photosynthetic capacity and dark respiration of low- and high-light leaves are remarkably constant when expressed per unit dry mass. *SLA*, therefore, turns out to be the most important parameter in determining the capability of plants to acclimate to a low light environment. However, given that there is a mechanical upper limit to *SLA*, payback times at low daily photon irradiance (<2 mol m^{-2} d^{-1}) quickly rise to values so high that it is not possible to amortize the cost of investment within the life span of most leaves.

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