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Carbon gain optimization in five broadleaf deciduous trees in response to light variation within the crown: correlations among morphological, anatomical and physiological leaf traits

ROSANGELA CATONI[1*], LORETTA GRATANI[1], FRANCESCO SARTORI[2], LAURA VARONE[1], MIRKO U. GRANATA[2]

[1] Sapienza University of Rome, Department of Environmental Biology, P. le A. Moro, 5, 00185 Rome, Italy

[2] University of Pavia, Department of Earth and Environmental Sciences, Via S. Epifanio 14, 27100 Pavia, Italy

Abstract – Leaf trait variations in five deciduous species (*Quercus robur, Corylus avellana*, *Populus alba, Acer campestre*, *Robinia pseudoacacia*) growing in an old broadleaf deciduous forest in response to light variation within the tree crown was analyzed. Net photosynthetic rate (P_N), leaf respiration rate (R) and the photosynthetic nitrogen use efficiency were, on average, more than 100% higher in sun than in shade leaves. *A. campestre* and *C. avellana* sun leaves had the highest specific leaf area (SLA, 156.0 ± 17.9 $cm^2 g^{-1}$) and the lowest total leaf thickness (L, 101.9 ± 8.8 µm) underlining their shade-tolerance. Among the shade-intolerant species (*Q. robur*, *P. alba* and *R. pseudoacacia*), *Q. robur* had the lowest SLA and the highest L in sun leaves (130.6 ± 10.0 $cm^2 g^{-1}$ and 160.8 ± 9.6 µm, respectively) since shade-intolerant species typically have thicker leaves. The higher P_N decrease in respect to R decrease from sun to shade leaves attested the higher sensitivity of P_N than R to light variations within the crown. This determined a 69% lower R/P_N in sun than in shade leaves. This result is further attested by the significant correlation between P_N and the relative photosynthetic photon flux density. The shade-tolerant species have a 76% higher R/P_N ratio than the shade-intolerant ones. The measured leaf phenotypic plasticity (PI = 0.35) was in the range of broadleaf deciduous species. Plasticity is a key trait useful to quantify plant response to environmental stimuli. It is defined as the ability of a genotype to produce different phenotypes depending on the environment. Among the considered species, *Q. robur* showed the highest PI (0.39) and *P. alba* the lowest (0.29). Knowledge on phenotypic plasticity is important in making hypotheses about the dynamics of the studied forest in consideration of environmental stress factors, including invasive species competition and global climate change.

Keywords: deciduous trees, forest, gas exchange, light gradient, shade tolerance, specific leaf area

* Corresponding author, e-mail: rosangela.catoni@uniroma1.it

Abbreviations: P_N – net photosynthesis; g_s – stomatal diffusive conductance to water vapour; E – transpiration rates; R – leaf respiration rates; R/ P_N – ratio of leaf respiration to photosynthesis; PNUE – photosynthetic nitrogen use; N_a – leaf nitrogen content per unit of leaf area; Chl *a* + *b* – chlorophyll *a* + *b*; Chl/Car – ratio of chlorophyll to carotenoid content; Chl *a*/*b* – ratio of chlorophyll *a* to chlorophyll *b* content; Chl/N – ratio of chlorophyll to nitrogen content; LA – projected leaf surface area; DM – leaf dry mass; SLA – specific leaf area.

Introduction

In forest ecosystems, the heterogeneous light environment within a tree crown due to self-shading and shading by neighboring trees (Sack et al. 2006, Wyka et al. 2012) determines leaf trait variations (Ellsworth and Reich 1993, Gratani 1997, Gratani and Foti 1998, Gratani et al. 2006, Yoshimura 2010). Large trees support leaves acclimated to high light intensities (sun leaves) in the upper canopy and may produce leaves acclimated to low light intensities (shade leaves) in lower canopy layers (Hölscher 2004). The range of variations reflects the optimization of whole plant gas exchange and the resource investment strategy (Gratani 1997, Wyka et al. 2012). Most studies of plant response to light have been aimed at underlining the ecological implications of the tolerance to extremes (i.e. tolerance to sun and shade), but noticeably less effort has been invested in the exploration of trends in the plastic response to light (Valladares et al. 2000). Sun leaves with respect to shade leaves generally exhibit a higher photosynthetic rate on a leaf area basis, a higher Chl *a* to Chl *b* ratio, a lower light-harvesting Chl *a*/*b* protein (LHCP), a lower stacking degree of thylakoids (Lichtenthaler et al. 1982), and a higher nitrogen (N) content per unit of leaf area (Hikosaka 2005) since approximately half of N is invested in photosynthetic proteins (Evans 1989). Hirose and Werger (1987) suggest that N varies with light availability in the plant crown in such a way as to optimize daily crown photosynthesis. Differentiation in N distribution patterns in sun and shade leaves within the crown increases the crown photosynthetic nitrogen use efficiency (PNUE) (Hikosaka 2003). PNUE underlines the efficiency with which species utilize N to grow (Garnier et al. 1995). There is a strong relationship between photosynthesis and respiration (R) as respiration relies on photosynthetic substrates (Atkin et al. 2007). Plant respiration accounts for a large fraction of carbon cycling in forest ecosystems and may be of comparable importance to photosynthesis as a determinant of net primary productivity (Ryan et al. 1997). In general, upper-canopy leaves had much higher leaf respiration than lower-canopy leaves, reflecting a greater metabolic activity related to a higher light availability and thus, higher net photosynthetic rates (Gunderson et al. 2002). At a morphological level, the specific leaf area (SLA) varies vertically in the forest canopy (Niinemets 1995) altering the amount of light that can be intercepted per unit of leaf dry mass (Evans and Poorter 2001). In general, plants growing in high light conditions have thicker leaves with a lower SLA than plants growing in shade conditions (Björkman 1981) partially due to longer or extra layers of palisade cells (Hanson 1917).

Species can be classified into obligate or facultative shade plants and obligate or facultative sun plants (Damascos and Rapoport 2002). However, comparative studies indicate that there are few species that are either extremely shade-tolerant or light-demanding, with most species having intermediate and thus, overlapping light preferences (Wright et al. 2003). One of the components of shade tolerance resides in the ability of individuals to optimize carbon gain under low light environments, by maximizing light interception and minimizing carbon loss by respiration (WALTERS and REICH 1999), according to the carbon gain hypothesis (VALLADARES and NIINEMTES 2008). Light competition and inter-specific differences in shade tolerance are frequently important determinants of forest structure and dynamics (GRAVEL et al. 2010). Nevertheless, the potential correlation between plasticity of morphological and physiological leaf traits and the tolerance to shade is still under discussion (ROBAKOWSKI et al. 2003, PORTSMUTH and NIINEMETS 2007). A frequent assumption in literature is that shade-intolerant species display a larger plasticity than shade-tolerant ones (STRAUSS DE BENEDETTI and BAZZAZ 1991), although the reverse is sometimes found. Other authors have observed a large variability in plasticity that depended on the studied traits with no real connection to shade tolerance (VALLADARES et al. 2000).

The main objective of this research was to analyze leaf trait variations in response to the light gradient within the crown of five tree species growing in an old broadleaf deciduous forest characterized by different tolerance to shade. Intra-canopy plasticity has important impacts on many aspects of tree biology, potentially contributing to whole-crown performance via effects on light penetration through the crown and on the energy, carbon, and water balance of individual leaves (HIKOSAKA 2005). Considering that information about the correlation between plasticity and shade tolerance is limited and the evidence is contrasting (PORTSMUTH and NIINEMETS 2007), we tested the relationship between leaf trait plasticity and shade tolerance of the selected trees, assuming that shade-tolerant tree species might have a lower plasticity index than the shade-intolerants.

Material and methods

The study was carried out in the period May – July 2012 in an old broadleaf deciduous forest developing in the Natural Reserve Siro Negri (45°12'39''N; 09°03'26''E, 74 m a. s. l, Italy) extending over 10 ha. The forest largely covered the fluvial valleys along the Ticino river from the 19th century, and no logging has been carried out since the establishment of the reserve in 1970 (SARTORI 1984, CASTAGNERI et. al. 2013). The Reserve represents one of the best conserved relicts of the original alluvial forest which in the past largely covered the banks of the Ticino river, and contains trees that are more than 100-years old (CASTAGNERI et al. 2013).

The Reserve has been reported as part of a Sites of Community Importance (SIC) IT 2080014 »Bosco Siro Negri e Moriano«.

The following broadleaf species were considered for measurements: *Acer campestre* L., *Corylus avellana* L., *Populus alba* L., *Robinia pseudoacacia* L. and *Quercus robur* L.

C. avellana is a shade-tolerant species (KULL and NIINEMETS 1993, GRATANI and FOTI 1998) growing in the understory of European deciduous forests (TUTIN 2001) and able to colonize large gaps (KULL and NIINEMETS 1993). *A. campestre* is a shade-tolerant species growing in the understory of deciduous mixed forests in Europe (MILLS 1996). *P. alba* is a shade-intolerant species (COOPER et al. 1999) with a great geographic distribution, including the Centre and South of Europe, North Africa, Western and Central Asia (FAO 1980) where it grows on the banks of rivers (DELLEDONNE et al. 2001). *R. pseudoacacia* is a shade-intolerant species (MOTTA et al. 2009) originating from the south-eastern United States (FERRARIS et al. 2000). The dispersion of the *R. pseudoacacia* into Italian native forests started in the early 20th century (Motta et al. 2009). It is mainly a threat for nutrient-poor sites based on its ability to fix nitrogen by symbiosis (Roloff et al. 1994). This species can cause an unwanted and long-lasting shift in vegetation composition toward nitrogen rich and species-poor plant communities (Kowarik 2010). *Q. robur* is a predominant European oak species (Scotti-Saintagne et al. 2004) growing in more open habitats; it is a more light–demanding species (Niinemets 1996, Valladares et al. 2002).

Meteorological data

The climate of the area was characterized by a mean annual rainfall of 654 mm, most of it falling in spring and autumn. The mean minimum air temperature (T_{min}) of the coldest month (January) was –0.2 ± 1.8 °C, the mean maximum air temperature (T_{max}) of the hottest month (July) was 30.1 ± 1.3 °C and the mean annual temperature (T_m) was 13.7 ± 8.2 °C. In the period May – July, total rainfall was 136 mm, T_m 22.2 ± 3.1 °C and T_{max} (July) 30.3 ± 1.2 °C (Lombardia Regional Agency for Environmental Protection, Meteorological Station of Pavia, Ponte Ticino SS35, data for the period 2002 to 2012). Floods occurred sporadically every 5–10 years during the last 40 years, with water levels up to 1.50 m height in the forest during exceptional events (Motta et al. 2009, Castagneri et al. 2013). On average, groundwater level was around –4.50 m in winter reaching –3.50 m in summer due to irrigation in the surrounding areas (Sartori, unpublished).

Forest measurements and microclimate

Measurements of forest structure were carried out in 10 representative sample areas (400 m^2 each) randomly selected in the considered forest. Plant traits included: plant height (H) and the diameter at breast height (D). Total forest density and total tree basal area were calculated. Leaf area index (LAI) was estimated at the end of June by the »LAI2000 Plant Canopy Analyzer« (LI-COR Inc., Lincoln, Nebraska, USA).

Microclimate and leaf trait measurements were made by an aerial lift for the selected species (four representative mature plants per species) at the top (sun leaves) and at the bottom (shade leaves) of the crown of each considered tree, according to Sack et al. (2006). The photosynthetic photon flux density (PPFD, μmol photons m^{-2} s^{-1}) was measured at the top and at the bottom of the crown for each sampled tree by a quantum radiometer photometer (LI-189 LI-COR, USA) with the quantum sensor LI-190SA. Simultaneously, PPFD was determined in the open near the forest, in order to provide references irradiance level for calculation of relative irradiance (PPFD$_{\%}$), according to Wyka et al. (2012). Measurements were carried out in June and July on overcast days from 09.00 a. m. to 12.00 p. m to provide a reliable estimate of the average light conditions during the »in-leaf« growing season, according to Tobin and Reich (2009). Air humidity (RH, %) and air temperature (T_a, °C) were measured by thermo-hygrometers (HD8901, Delta Ohm, It).

Leaf morphology and anatomy

Fully expanded leaves (n = 20 per species and per crown position) were collected from the selected trees at the end of June, sealed in plastic bags and transported immediately to the laboratory for measurements. Measurements included leaf surface area (LA, cm^2), obtained by the image analysis system (Delta-T Devices, UK), and leaf dry mass (DM, mg), determined by drying leaves at 80 °C to constant mass. Specific leaf area (SLA, $cm^2 g^{-1}$) was calculated by the ratio of LA and DM.

Fresh leaf sections from fully expanded leaves (n = 10 per species and per crown position) were hand cut and analyzed by light microscopy using an image analysis system (ARKON, A&P, I). The following parameters were measured: total leaf thickness (L, µm), palisade and spongy parenchyma thickness, adaxial and abaxial epidermis and cuticle thickness. All measurements were restricted to vein-free areas.

Gas exchange

Gas exchange measurements were carried out in June – July (five leaves per species per crown position per each sampling occasion), from 09:00 a. m. to 12:00 p. m. to ensure that the maximum rates were measured (REICH et al. 1995). Leaves were retained in their natural position during measurements. Net photosynthetic rate (P_N, µmol CO_2 m^{-2} s^{-1}), stomatal conductance to water vapor (g_s, mol H_2O m^{-2} s^{-1}), leaf transpiration rate (E, mmol H_2O m^{-2} s^{-1}), photosynthetic photon flux density (PPFD, µmol photons m^{-2} s^{-1}) and leaf temperature (T_l, °C) were measured by an infrared gas analyzer (LC-Pro+, ADC, UK) equipped with a leaf chamber (PLC, Parkinson Leaf Chamber, UK).

On each sampling occasion, respiration rates (R, µmol CO_2 m^{-2} s^{-1}) were measured after P_N ones (on the same leaves) as CO_2 efflux, by darkening the leaf chamber with a black paper, according to CAI et al. (2005) for 30 min prior to each measurement, to avoid the release of CO_2 transient post-irradiation bursts (ATKIN et al. 1998). The shown R and P_N rates represented the mean values of three days of measurements per month characterized by the same weather conditions, under clear sky. The ratio between R and P_N was calculated.

Leaf nitrogen and pigment content

Leaf samples were collected on the same occasions as those for gas exchange measurements. Immediately after collection leaf samples were kept cool in the dark and transported immediately to the laboratory.

Leaf nitrogen content per leaf area (N_a, g m^{-2}) was determined by drying leaf samples at 70 °C (6 leaf samples, 0.5 g of leaf dry mass each, per species and per crown position in each sampling occasion) and grinding them into a fine powder. The N content was measured by the Kjeldahl method (MENDES et al. 2001). Photosynthetic nitrogen use efficiency (PNUE, µmol CO_2 g^{-1} N_a s^{-1}) was calculated by the ratio between P_N rates and N_a content.

Chlorophyll content (Chl, mg g^{-1} fresh weight) and carotenoid content (Car, mg g^{-1} fresh weight) were determined after grinding leaves in acetone (6 samples, 1.5 g of leaf fresh weight each, per species and per crown position in each sampling occasion). The homogenates were centrifuged in a refrigerated centrifuge (4237R. A. L. C., I). Absorbance of the supernatants was measured by a Jasco model 7800LCD (Japan) spectrophotometer at the wavelengths of 645, 663, and 440 nm. Chl content was calculated according to MACLACHLAN and ZALIK (1963) and Car content according to HOLM (1954). The Chl *a* + *b* content, the ratio Chl *a*/*b*, the ratio Chl/Car, and the ratio Chl/N were calculated.

Plasticity index

The plasticity index of morphological (PI_m), anatomical (PI_a) and physiological (PI_p) leaf traits for each of the species was calculated by the difference between the minimum and the maximum mean value between sun and shade leaves divided by the maximum mean value, according to Valladares et al. (2000). The mean plasticity index (PI), which had a scale ranging from 0 to 1, was calculated by averaging PI_m, PI_a and PI_p per species, according to Valladares et al. (2000).

Statistics

All statistical tests were performed using a statistical software package (Statistica, Statsoft, USA).

Differences in the considered leaf traits were determined by the analysis of variance (ANOVA) and the Tukey test for multiple comparisons. Kolmogorov–Smirnov and Levene tests were used to verify the assumption of normality and homogeneity of variances, respectively.

Regression analysis was carried out to examine the relationship among the considered leaf traits.

Principal component analysis (PCA) was carried out in order to detect structure in the correlations between the considered leaf traits (P_N, g_s, E, R, R/P_N, N_a, PNUE, Chl/N, Chl *a+b*, Chl *a*/*b*, Chl /Car, SLA, leaf thickness, palisade parenchyma thickness, spongy parenchyma thickness, adaxial epidermis thickness, adaxial cuticle thickness, abaxial epidermis thickness, abaxial cuticle thickness). The analysis was performed on a standardized matrix. The matrix was subjected to a rotated principal component analysis with the objective of summarizing the main factors determining the variation of the analyzed leaf traits.

In order to evaluate the similarity among the species in terms of phenotypic plasticity a correspondence analysis (CA) was carried out. The analysis was performed using the species as group variable and PI as category variable. In particular, three PI categories were considered: physiological, morphological and anatomical PI.

Results

Forest structure and microclimate

The forest structure was characterized by a dominant tree layer consisting of *P. alba*, *Populus nigra* L., *Q. robur* and *R. pseudoacacia*, (H = 30 ± 3 m), a dominated tree layer of *R. pseudoacacia, A. campestre* and *C. avellana* (H = 20 ± 3 m), two shrub layers of *Sambucus nigra* L., *Ulmus minor* Mill. and *C. avellana* (H = 6.0 ± 1.5 m and 1.5 ± 0.5 m, respectively) and a grass layer (Tab. 1). Total tree density was 237 ± 100 stems ha^{-1} and the total basal area 74.5 ± 24.6 m^2 ha^{-1}. LAI was 4.5 ± 0.3.

On average, $PPFD_{\%}$ ranged from 6.1 ± 3.5% at the bottom to 97.4 ± 1.1% at the top of the crown. The T_a and RH ranged from 28.1 ± 0.5 °C and 48.0 ± 0.9%, respectively, at the bottom, to 30.4 ± 0.6 °C and 35.1 ± 2.1%, respectively, at the top of the crown of the trees considered.

Tab. 1. Structural traits of the considered species; H – plant height, D – diameter at breast height.

Species	H (m)	D (cm)
Acer campestre	15 ± 6	33.0 ± 7.9
Corylus avellana	8 ± 2	12.3 ± 3.6
Populus alba	28 ± 5	79.5 ± 26.8
Robinia pseudoacacia	20 ± 6	27.2 ± 8.8
Quercus robur	26 ± 5	75.3 ± 8.0

Leaf morphology

There were significant differences between sun and shade leaves among the species (Tab. 2). On average, SLA was 81% higher in shade than in sun leaves. In particular, *C. avellana* had the highest SLA both in sun and in shade leaves (168.8 ± 15.1 cm^2 g^{-1} and 400.7 ± 60.0 cm^2 g^{-1}, respectively), *Q. robur* the lowest SLA in sun leaves (130.6 ± 10.0 cm^{2}g^{-1}) and *P. alba* the lowest SLA in shade leaves (168.4 ± 16.8 cm^2 g^{-1}).

Tab. 2. Morphological leaf traits of sun and shade leaves of A*cer campestre, Corylus avellana, Populus alba, Quercus robur* and *Robinia pseudoacacia.* Mean values (± SD) are shown (n = 20). Mean values with the same letters are not significantly different between sun and shade leaves (Tukey test, $p \geq 0.05$). LA – projected leaf surface area; DM – leaf dry mass; SLA – specific leaf area.

	A. campestre		*C. avellana*		*P. alba*		*Q. robur*		*R. pseudoacacia*	
	sun	shade	sun	shade	sun	shade	sun	shade	sun	shade
LA (cm^2)	20.2± 4.2a	57.7± 7.7b	31.0± 9.6a	88.5± 17.8b	14.8± 3.2a	26.8± 2.1b	19.8± 3.0a	50.6± 11.1b	130.1± 22.5a	470.4± 73.8b
DM (mg)	157.4± 41.4a	198.4± 31.2b	190.8± 57.5a	227.2± 62.8b	109.6± 24.4a	161± 22.2b	150.9± 33.7a	263.4± 59.4b	932± 168a	1838± 376b
SLA (cm^2 g^{-1})	143.2± 15.1a	305.1± 35.3b	168.8± 15.1a	400.7± 60.0b	135.6± 7.3a	168.4± 16.8b	130.6± 10.0a	192.7± 10.2b	139.9± 4.8a	258.3± 15.4b

Leaf anatomy

On average, L and palisade parenchyma thickness were 23% and 38% higher in sun than in shade leaves, respectively, and spongy parenchyma thickness was 17% higher in sun than in shade leaves (Tab. 3). In particular, *Q. robur* had the highest L thickness in sun leaves (160.8 ± 9.6 µm), *P. alba* in shade leaves (128.9 ± 4.7 µm) and *C. avellana* the lowest L thickness both in sun and in shade leaves (95.7 ± 10.1 µm and 85.3 ± 5.3 µm, respectively). The ratio between palisade parenchyma thickness and L in sun leaves was the highest in *R. pseudoacacia* (55%) and the lowest in *C. avellana* (33%), while in shade leaves it was the highest in *P. alba* (53%) and the lowest in *C. avellana* (30%). *Q. robur* had the highest spongy parenchyma thickness, the highest adaxial and abaxial epidermis thickness and cuticle thickness both in sun and in shade leaves, and *C. avellana* the lowest palisade parenchyma thickness, adaxial and abaxial epidermis thickness and cuticle thickness both in sun and in shade leaves.

Tab. 3. Anatomical leaf traits of sun and shade leaves of *Acer campestre, Corylus avellana, Populus alba, Quercus robur* and *Robinia pseudoacacia.* Mean values (± SD) are shown (n = 10). Mean values with the same letters are not significantly different between sun and shade leaves (Tukey test, $p \geq 0.05$).

Leaf traits	*A. campestre*		*C. avellana*		*P. alba*		*Q. robur*		*R. pseudoacacia*	
	sun	shade	sun	shade	sun	shade	sun	shade	sun	shade
Leaf thickness (μm)	108.2± 6.7a	93.2± 1.4b	95.7± 10.1a	85.3± 5.3a	142.6± 8.3a	128.9± 4.7b	160.8± 9.6a	120.8± 5.9b	146.3± 12.0a	101.9± 2.5b
Palisade parenchyma thickness (μm)	42.0± 2.4a	39.6± 0.9a	31.9± 7.5a	25.6± 5.4a	75.3± 2.4a	68.4± 5.0b	60.7± 3.9a	37.3± 3.5b	80.2± 9.7a	43.4± 5.1b
Spongy parenchyma thickness (μm)	43.2± 5.5a	34.2± 2.5b	46.0± 8.5a	44.4± 4.6a	46.7± 5.3a	42.3± 6.7a	71.7± 4.9a	56.9± 5.0b	51.2± 11.2a	42.6± 4.6a
Adaxial epidermis thickness (μm)	12.4± 1.6a	10.5± 2.8a	9.2± 0.7a	7.6± 2.1a	11.2± 1.7a	10.7± 0.8a	15.2± 1.6a	14.5± 3.0a	8.2± 1.1a	7.9± 1.4a
Adaxial cuticle thickness (μm)	1.0± 0.2a	0.9± 0.1a	0.9± 0.2a	0.8± 0.1a	1.1± 0.3a	1.0± 0.3a	1.3± 0.4a	1.1± 0.4a	1.0± 0.3a	0.9± 0.2a
Abaxial epidermis thickness (μm)	8.7± 1.6a	7.4± 0.9a	7.0± 1.3a	5.7± 0.9a	8.0± 1.9a	5.7± 0.6a	10.9± 2.1a	10.3± 1.4a	7.0± 1.3a	6.2± 0.8a
Abaxial cuticle thickness (μm)	0.9± 0.1a	0.8± 0.1a	0.7± 0.1a	0.6± 0.1a	0.8± 0.2a	0.7± 0.1a	1.0± 0.4a	0.9± 0.2a	0.9± 0.1a	0.8± 0.1a

Gas exchange

On average, P_N was more than twice as high in sun leaves than in shade leaves. P_N ranged between 20.3 ± 0.3 μmol m^{-2} s^{-1} (*P. alba*) and 5.8 ± 0.1 μmol m^{-2} s^{-1} (*C. avellana*) in sun leaves, and between 3.6 ± 0.3 μmol m^{-2} s^{-1} (*P. alba*) and 1.8 ± 0.2 μmol m^{-2} s^{-1} (*A. campestre*) in shade leaves (Tab. 4).

The g_s had the same P_N trend with the highest rates in sun leaves (0.20 ± 0.07 mol m^{-2} s^{-1}) decreasing, on average, by 49% in shade leaves. In particular, *P. alba* had the highest g_s in sun leaves (0.27 ± 0.06 mol m^{-2} s^{-1}) and *C. avellana* the lowest one (0.08 ± 0.01 mol m^{-2} s^{-1}). *R. pseudoacacia* showed the highest g_s in shade leaves (0.19 ± 0.02 mol m^{-2} s^{-1}) and *C. avellana* the lowest (0.02 ± 0.01 mol m^{-2} s^{-1}).

There was a significant ($p < 0.01$) positive correlation between P_N and g_s and between P_N and palisade parenchyma thickness, showing that 46% and 63% of P_N variations were explained by the two parameters, respectively.

The highest R were monitored in sun leaves (1.10 ± 0.33 μmol m^{-2} s^{-1}) decreasing by 36% in shade leaves (Tab. 4). Among the considered species, *R. pseudacacia* had the highest R both in sun and in shade leaves (1.33 ± 0.23 and 1.18 ± 0.06 μmol m^{-2} s^{-1}, respectively), *C. avellana* had the lowest R in sun leaves (0.60 ± 0.07 μmol m^{-2} s^{-1}) and *Q. robur* in shade leaves (0.21 ± 0.06 μmol m^{-2} s^{-1}).

The ratio R/P_N was the highest in shade leaves (more than 100%), *A. campestre* having the highest R/P_N ratio both in sun and in shade leaves (0.17 ± 0.01 and 0.63 ± 0.18, respectively).

Tab. 4. Values of photosynthesis and respiration rates of sun and shade leaves of *Acer campestre, Corylus avellana, Populus alba, Quercus robur* and *Robinia pseudoacacia.* Mean values (± SD) are shown (n = 15). Mean values with the same letters are not significantly different between sun and shade leaves (Tukey test, $p \geq 0.05$). P_N – net photosynthesis; R – leaf respiration rate.

	A. campestre		*C. avellana*		*P. alba*		*Q. robur*		*R. pseudoacacia*	
	sun	shade	sun	shade	sun	shade	sun	shade	sun	shade
P_N (μmol m^{-2} s^{-1})	7.5± 0.2a	1.8± 0.5b	5.8± 0.1a	1.9± 0.6b	20.3± 0.3a	3.6± 0.3b	16.2± 0.3a	2.0± 0.1b	19.5± 1.5a	2.6± 0.2b
R (μmol m^{-2} s^{-1})	1.30± 0.09a	1.12± 0.07b	0.60± 0.07a	0.53± 0.07b	1.32± 0.36a	0.48± 0.05b	0.92± 0.32a	0.21± 0.06b	1.33± 0.23a	1.18± 0.06a

Leaf nitrogen content and pigment content

The N_a was, on an average, 54% higher in sun than in shade leaves, *R. pseudoacacia* having the highest N_a in sun leaves (2.51 ± 0.08 g m^{-2}) and *A. campestre* the lowest one (0.91 ± 0.11 g m^{-2}). *P. alba* had the highest N_a in shade leaves (1.56 ± 0.04 g m^{-2}).

PNUE was 8.4 ± 3.0 μmol g^{-1} s^{-1} in sun leaves, decreasing by 68% in shade leaves, *P. alba* having the highest value in sun leaves (12.7 ± 0.2 μmol g^{-1} s^{-1}) and *C. avellana* the lowest one (4.2 ± 0.2 μmol g^{-1} s^{-1}).

The Chl $a + b$ was 82% higher in shade than in sun leaves. Among the considered species, *Q. robur* and *P. alba* had the highest Chl $a + b$ in sun leaves (1.61 ± 0.26 mg g^{-1}) and *C. avellana* the lowest both in sun and in shade leaves (0.76 ± 0.10 and 1.14 ± 0.20 mg g^{-1}, respectively). *A. campestre* had the highest Chl $a + b$ in shade leaves (3.61 ± 0.39 mg g^{-1}). The ratio Chl a/b was 8% higher in sun than in shade leaves, *P. alba* having the highest ratio both in sun and in shade leaves (2.80 ± 0.03 and 2.72 ± 0.27, respectively), *R. pseudoacacia* the lowest ratio in sun leaves (2.02 ± 0.03) and *Q. robur* in shade leaves (1.85 ± 0.04) (Fig. 1A).

The ratios Chl/Car and Chl/N were 32% and 44% higher in shade than in sun leaves, respectively. In particular, among the species considered, *Q. robur* had the highest Chl/Car in sun leaves (7.84 ± 0.45) and *A. campestre* in shade leaves (9.84 ± 0.87). The ratio Chl/N was the highest in *A. campestre* both in sun and in shade leaves (0.102 ± 0.010 and 0.174 ± 0.014, respectively) (Figs. 1B and 1C).

Plasticity index

The PI for the considered species was, on average, 0.35, *Q. robur* having the highest PI (0.39), followed by *R. pseudoacacia* (0.38), *A. campestre* (0.34), C. *avellana* (0.33) and *P. alba* (0.29) (Tab. 5).

PI_m was, on average, 0.45, *R. pseudoacacia* showing the highest value (0.56) and *P. alba* the lowest (0.32). Among the considered leaf morphological traits, LA had the largest variation (PI_{LA} = 0.62, mean value). The PI_a was, on average, 0.15, *R. pseudoacacia* showing the highest (0.18) and *P. alba* the lowest value (0.12). Among the considered anatomical leaf traits, palisade parenchyma thickness had the largest variation (0.24, mean value). The PI_p was, on an average, 0.44, *Q. robur* having the highest value (0.53). Among the considered physiological traits, P_N had the largest variation (0.79, mean value).

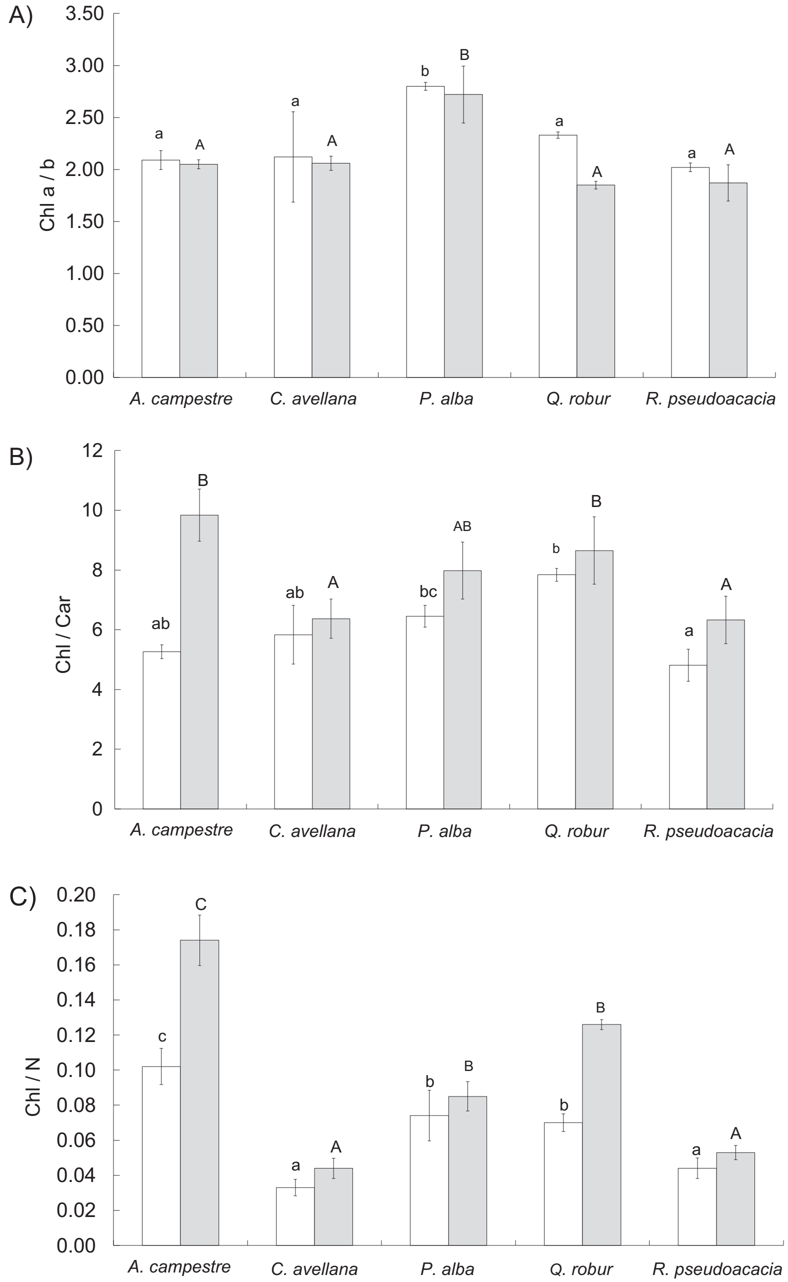

Fig. 1. A) Ratio of chlorophyll *a* to chlorophyll *b* (Chl *a*/*b*), B) ratio of chlorophyll to carotenoid content (Chl /Car) and C) ratio of chlorophyll to nitrogen content (Chl/N) of *Acer campestre, Corylus avellana, Populus alba, Quercus robur* and *Robinia pseudoacacia*. Lowercase and capital letters indicate the inter-specific differences in sun (white bars) and shade (grey bars) leaves, respectively. The means with the same letters are not significantly different (ANOVA, $p \geq 0.05$). Mean values (± SD) are shown ($n = 36$).

Tab. 5. Phenotypic plasticity index for physiological (PI_p), morphological (PI_m) and anatomical (PI_a) leaf traits of *Acer campestre, Corylus avellana, Populus alba, Quercus robur* and *Robinia pseudoacacia.* P_N – net photosynthesis, g_s – stomatal diffusive conductance to water vapour, E – transpiration rates, R – leaf respiration rates, R/ P_N – ratio of leaf respiration to photosynthesis, PNUE – photosynthetic nitrogen use, N_a – leaf nitrogen content per unit of leaf area, Chl *a* + *b* – chlorophyll *a* + *b* content, Chl/Car – ratio of chlorophyll to carotenoid content, Chl *a*/*b* – ratio of chlorophyll a to chlorophyll *b* content, Chl/N – ratio of chlorophyll to nitrogen content, LA – projected leaf surface area, DM – leaf dry mass, SLA – specific leaf area, PI – mean plasticity index.

Plasticity index		*A. campestre*	*C. avellana*	*P. alba*	*Q. robur*	*R. pseudoacacia*
Physiological traits	P_N	0.76	0.65	0.82	0.87	0.86
	g_s	0.29	0.75	0.63	0.51	0.09
	E	0.11	0.73	0.74	0.65	0.60
	R	0.14	0.12	0.64	0.77	0.11
	R/P_N	0.73	0.66	0.50	0.67	0.87
	PNUE	0.68	0.27	0.82	0.90	0.75
	N_a	0.26	0.53	0.03	0.24	0.46
	Chl *a*+*b*	0.66	0.33	0.28	0.53	0.16
	Chl/Car	0.47	0.08	0.19	0.09	0.24
	Chl *a*/*b*	0.02	0.03	0.03	0.21	0.07
	Chl/N	0.41	0.25	0.13	0.44	0.17
	Mean value PI_p	**0.41**	**0.40**	**0.44**	**0.53**	**0.40**
Morphological traits	LA	0.65	0.65	0.45	0.61	0.72
	DM	0.21	0.16	0.32	0.43	0.49
	SLA	0.53	0.58	0.19	0.32	0.46
	Mean value PI_m	**0.46**	**0.46**	**0.32**	**0.45**	**0.56**
Anatomical traits	Leaf thickness	0.14	0.11	0.10	0.25	0.30
	Palisade parenchyma thickness	0.06	0.20	0.09	0.39	0.46
	Spongy parenchyma thickness	0.21	0.03	0.09	0.21	0.17
	Adaxial cuticle thickness	0.10	0.11	0.09	0.15	0.10
	Abaxial cuticle thickness	0.11	0.14	0.12	0.10	0.11
	Adaxial epidermis thickness	0.15	0.17	0.04	0.04	0.04
	Abaxial epidermis thickness	0.15	0.18	0.28	0.06	0.11
	Mean value PI_a	**0.13**	**0.13**	**0.12**	**0.17**	**0.18**
	PI	**0.34**	**0.33**	**0.29**	**0.39**	**0.38**

Regression and multivariate analysis

There was a significant ($p < 0.01$) positive correlation between P_N and N_a and a significant ($p < 0.01$) negative correlation between SLA and L, 52% of P_N variations being explained by N_a, and 51% of SLA variation by L. The regression analysis between P_N and $PPFD_{\%}$ showed a high correlation coefficient between these two variables ($r = 0.81$).

PCA extracted two factors which accounted for 56.5% of the total variance and of which 38.8% was due to factor 1 and 17.8% to factor 2 (Fig. 2). The analysis showed a co-linearity among the considered leaf traits. Factor 1 was significantly correlated to P_N (r = 0.92), total leaf thickness (r = 0.88), E (r = 0.86), SLA (r = –0.84), PNUE (r = 0.82), N_a (r = 0.78), palisade parenchyma thickness (r = 0.77) and R/P_N (r = – 0.71). Factor 2 was significantly correlated to Chl *a* + *b* (r = 0.77), adaxial epidermis thickness (r = 0.73) and Chl/N_a (r = 0.73). Along the factor 1, which explained most of the total variance, sun leaves were completely separated from shade leaves. Among the considered species, *A. campestre* and *C. avellana* sun leaves were closer to shade leaf behavior and *P. alba* shade leaves were closer to sun leaf behavior.

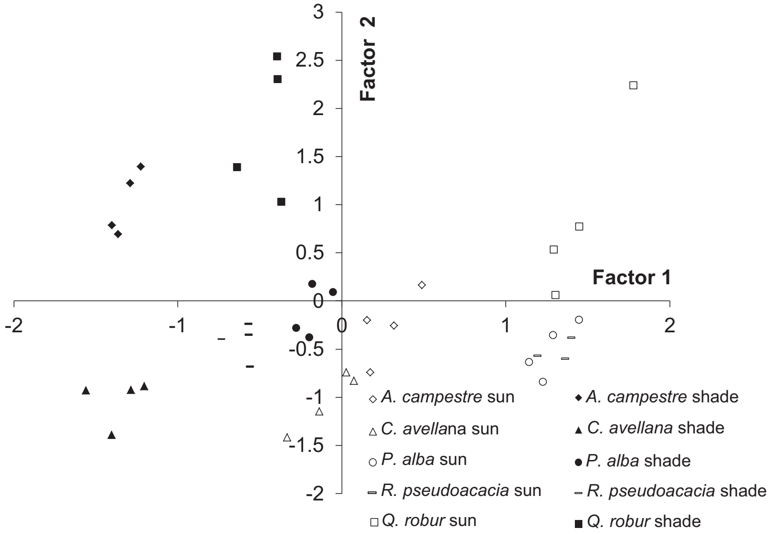

Fig. 2. Principal component analysis (PCA) carried out using the physiological, morphological and anatomical leaf traits. Factor 1 and factor 2 accounts for 38.8% and 17.8% of the total variance, respectively. Open symbols for each species indicate sun leaves and dark symbol indicate shade leaves.

The CA showed a greater similarity between *P. alba* and *Q. robur* because of the similar PI_p while *A. campestre* and *C. avellana* were closer on the base of PI_m. *R. pseudoacia* was further from the other species (Fig. 3).

Discussion

Light gradient within the crown in a forest ecosystem depends on the structure and stage of development of trees (LARCHER 2003). Moreover, the distribution, size and orientation of leaves control such processes as leaf development, leaf water use, and photosynthesis (NORMAN and CAMPBELL 1989). The relationship between canopy structure and the spatial distribution of light availability differs between primary and secondary growth forests (NICOTRA et al. 1999) and light availability in the understory is frequently associated with regeneration processes and the long-term survival of forest tree species (WOODS 2000).

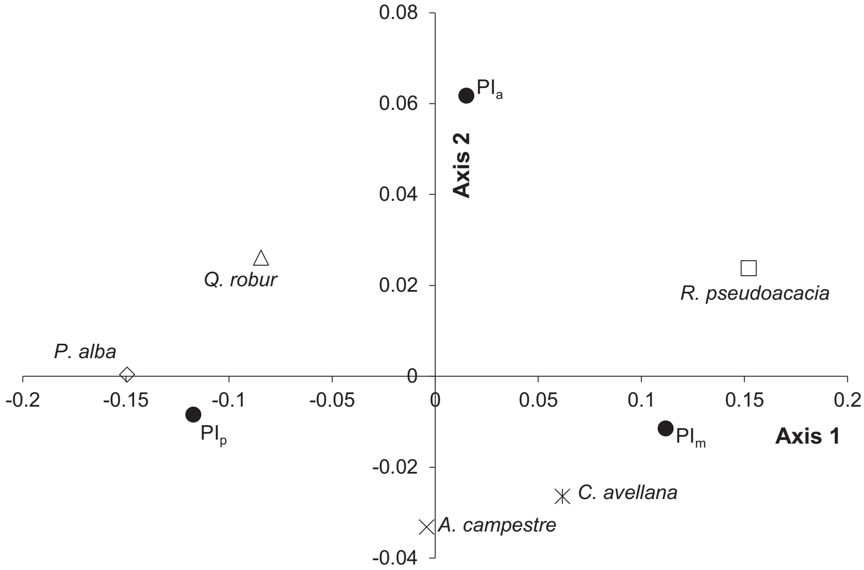

Fig. 3. Correspondence analysis (CA) performed using the species as group variable and plasticity index as category variable, PI_m – morphological plasticity, PI_a – anatomical plasticity, PI_p – physiological plasticity.

Light response of broadleaf deciduous species is of particular interest because regeneration, either natural or via silvicultural practices, is influenced by their shade tolerance (WELANDER and OTTOSSON 2000).

The studied forest is characterized by a tree density of 237 ± 100 stem ha^{-1} and a LAI of 4.5 ± 0.3 which is within the range of broadleaf deciduous forests in Italy (SCHIRONE et al. 1985, PICCOLI and BORELLI 1988, GRATANI and Foti 1998, Gratani and CRESCENTE 2000). LAI is an important variable for characterizing vegetation structure and functioning including estimation of plant productivity and canopy cover density (GARRIGUES et al. 2008). LAI drives both the within- and the below-canopy microclimate since LAI and PPFD absorption are closely related parameters (KULL et al. 1999; PORTE et al. 2000). Any change in forest LAI by frost, storm, defoliation, drought and management practices is accompanied by modifications of plant productivity (BRÉDA 2003). Our results show that the $PPFD_{\%}$ through the considered tree crown decreases, on average, by 94% from the top to the bottom involving a large number of leaf trait variation as confirmed by the PCA that shows a complete separation between sun and shade leaves. In particular, SLA is 81% higher in shade than in sun leaves (mean value of the considered species), according to the results of WITKOWSKI and LAMONT (1991), GRATANI (1997) and GRATANI et al. (2006). The higher SLA of shade leaves is mostly the result of a decreased total leaf thickness, as attested by the significant ($p < 0.01$) negative correlation between SLA and L. In fact, the larger and thinner shade leaves are more advantageous for light capture in low light conditions and, in general, species which grow in shade conditions are characterized by a larger SLA and a lower L than those growing in more open areas (GRATANI and FOTI 1998).

Moreover, SLA differs between shade-tolerant and intolerant species (ABRAMS and KUBISKE 1990, KULL and NIINMETS 1993), and is the trait most strongly correlated to shade tolerance (JANSE-TEN KLOOSTER et al. 2007). The considered shade-tolerant species (*A. campestre* and *C. avellana*) have a 49% higher SLA and a 28% lower L than the shade-intolerant species (*P. alba*, *R. pseudoacacia* and *Q. robur*) as confirmed by the PCA. The analysis shows that sun leaves of *A. campestre* and *C. avellana* are closer in behavior to shade leaves, underlining their ability to tolerate shade in comparison to others species. Among the shade-intolerant species, *Q. robur* has the lowest SLA (130.6 ± 10.0 $cm^2\ g^{-1}$) and the highest L in sun leaves (160.8 ± 9.6 μm), since shade-intolerant species typically have thicker leaves (NIINEMETS et al. 1998). Leaf thickening under higher irradiance can be interpreted as a means for the plant to optimize light use through irradiance attenuation (JACQUEMOUD and BARET 1990, USTIN et al. 2001).

Moreover, the structure of the mesophyll is associated with the photosynthetic performance of leaves via regulation of the internal light and carbon dioxide profiles (EVANS 1999). In particular, the palisade parenchyma reflects the leaf ability to capture solar energy accounting for the photosynthetic efficiency (KUMAR et al. 2012). This is attested by the significant ($p < 0.01$) positive correlation between P_N and palisade parenchyma thickness. On average, P_N is more than 100% higher in sun than in shade leaves, associated with a 38% higher palisade parenchyma thickness, while shade leaves are characterized by a 3% higher spongy parenchyma thickness, that enhances light capture by scattering light (MENDES et al. 2001, SACK et al. 2006), in respect to palisade parenchyma. The higher P_N rates in sun leaves than in shade leaves for the considered species are supported by a more than twice as high g_s value indicating that the larger stomata opening in sun leaves (SCHULZE et al. 1975, FARQUHAR and SHARKEY 1982) determines a higher intercellular CO_2 concentration. This result is supported by the significant ($p < 0.01$) correlation between P_N and g_s. The shade-intolerant species have a greater difference between P_N in sun and shade leaves than shade-tolerant species, depending on the different level of P_N light saturation (KOIKE et al. 2001). Moreover, shade-tolerant species have lower P_N rates both in sun than in shade leaves (6.7 ± 1.2 and 1.9 ± 0.1 $\mu mol\ m^{-2}\ s^{-1}$ respectively) than shade-intolerant species, according to the results of KOIKE et al. (2001), VALLADARES et al. (2002) and VALLADARES and NIINEMETS (2008).

Variations in light availability within the crown also induce changes in CO_2 release through R, this last reflecting the acclimation response to the light variations within the crown (ZHA et al. 2002). High PPFD increases R as a result of higher maintenance costs caused by higher P_N (AMTHOR 1989, NIINEMETS et al. 1998), higher protein turnover and increased need for secondary compounds such as flavonoids or carotenoids (DELAGRANGE et al. 2004). The ratio R/P_N can be considered a simple approach to leaf carbon balance because it indicates the percentage of photosynthates that are respired (LOVEYS et al. 2002). The 69% lower R/P_N ratio in sun than in shade leaves (mean value of the considered species) is mainly due to the higher P_N decrease from sun to shade leaves, with respect to the R decrease, underlining the higher P_N sensitivity to light variations within the crown. This result is further attested by the significant correlation between P_N and $PPFD_{\%}$. Because they have the lowest P_N rates, the shade-tolerant species have a 76% higher R/P_N ratio than those that are shade-intolerant.

The strong and direct effect of light on the photosynthetic capacity is mainly related to light-induced variations in the pool of proteins, pigments and enzymes that supports light capture and CO_2 fixation by leaves (NIINEMETS et al. 1998, LE ROUX et al. 1999). Thus, crown photosynthesis is a function of leaf N_a distribution within the crown profile (GRATANI et al. 2006) which is attested by the significant correlation between these two variables, and may be explained by more than 70% leaf N being allocated to the photosynthetic apparatus (POORTER and EVANS 1998). Sun leaves have a 54% higher N_a than shade leaves which enhances their capacity to convert high PPFD to organic matter (MENDES et al. 2001). Moreover, the higher PNUE (greater than 100%) in sun leaves underlines the efficiency to allocate N to RUBISCO, according to POORTER and EVANS (1998) and GRATANI et al. (2006). The shade-intolerant species have a 21% higher PNUE than the shade-tolerant species, demonstrating different light demands (KULL and NIINEMETS 1993). The acclimation of the photosynthetic pigment apparatus to high irradiance in sun leaves determines a higher Chl *a*/*b* ratio (8%, mean value) and a lower (22%) Chl/Car ratio than in shade leaves, according to the results of LICHTENTHALER et al. (2007). The higher Chl *b* content in shade leaves is indicative of acclimation to low irradiance, because it is usually the main component of the LHCP (KOIKE et al. 2001), while the lower Chl/Car ratio in sun leaves is primarily caused by the highest activity in the xanthophyll cycle (DEMMIG-ADAMS 1998). Moreover, the ratio Chl/N, which is an indicator of leaf N allocation to chlorophyll-protein complexes in the light-harvesting component (KOIKE et al. 2001), is 44% higher in the shade than in the sun leaves of the species considered. The increase in the proportional investment of leaf N in thylakoids, which improves incident light-use efficiency, is a major response of N partitioning within the leaf to decreased irradiance (EVANS 1989). Among the considered species, *A. campestre* has the highest Chl/N both in sun and shade leaves (0.102 ± 0.010 and 0.174 ± 0.014, respectively) with a large increase from sun to shade leaves, thus attesting its shade-tolerance. In fact, there is a wide consensus that a higher increase of Chl/N is even more significant for species of greater shade tolerance (EVANS 1989).

Plasticity is a key trait useful to quantify plant response to environmental stimuli (NICOTRA et al. 2010) and phenotypic plasticity is defined as the ability of a genotype to produce different phenotypes depending on the environment (SULTAN 2000). The analysis of leaf phenotypic plasticity attests the considered species responsiveness to light variation. The PI for the considered species (0.35) is in the range of broadleaf deciduous species (VALLADARES et al. 2002, WYKA et al. 2007, 2012). Nevertheless, there are significant differences among the species. In particular, the shade-intolerant *Q. robur* and *R. pseudoacacia* have the highest PI (0.39 ± 0.01, mean value) while *P. alba* has the lowest (0.29). This last can be explained by the preference of *P. alba* to grow along the edge of forests and on river banks (MADEJÓN et al. 2004) considering that forest edges have a significant influence on the solar radiation pattern on nearby landscape units, in relation to the number and size of openings, the distance of openings from the edge, and the orientation of openings relative to the path of the sun (GALO et al. 1992). The presence of *P. alba* along the edge of forests and its vertically extended crown structure (CHIUSOLI 1991) are typical traits of shade-intolerant species (DELAGRANGE et al. 2004). Moreover, *P. alba* has the lowest PI_m (0.32) and PI_a (0.12), which are mainly explained by the lowest SLA and L variations between sun and shade leaves, due to the lack of deep shade in its crown. The shade-intolerant species have a higher PI_p (0.46 ± 0.07) than the shade-tolerant species, in agreement with other studies (STRAUSS-DEBENEDETTI and BAZZAZ 1996, GRONINGER et al. 1996, VALLADARES et al. 2002, BLOOR 2003, DELAGRANGE et al. 2004, SÁNCHEZ-GÓMEZ et al. 2006, PORTSMUTH and NIINEMETS 2007). This result is also attested by the results of the correspondence analysis (CA). PI_p is also linked to an enhanced capacity to colonize gaps and open areas (Valladares et al. 2002, Niinemets and Valladares 2004) because it ensures adjustments of gas exchange in response to stress factors changes in the short term (Zunzunegui et al. 2009). A high physiological plasticity allows species to achieve rapid growth increasing the capability to colonize early successional habitats (Walters and Reich 1999).

In particular, among the considered physiological traits, the photosynthetic plasticity (PI$_{P_N}$) is significantly higher in shade-intolerant than in shade-tolerant species (Ducrey 1994). Several studied show that that shade-intolerant species have greater photosynthetic acclimation potential than shade-tolerant species (Strauss-Debenedetti and Bazzaz. 1996). This higher photosynthetic plasticity determines a large photosynthetic capacity to use full sunlight, which results in a more efficient avoidance of photo-inhibition (Valladares et al. 2002). The capacity for photosynthetic acclimation to light could be considered species-specific (Duan et al. 2005). *Q. robur* has the highest PI$_p$ (0.53) which is related to its shade-intolerant behavior, according to the results of Valladeres et al. (2002). Moreover, its highest PI$_{P_N}$ (0.87) allows a rapid acclimation of photosynthesis which leads to an increased carbon gain, thereby facilitating growth and competitive ability in high light conditions (Valladares et al. 2002).

The high *R. pseudoacacia* PI confirms that phenotypic plasticity allows invasive species to occupy a wide range of new environments (Callaway et al. 2003, Pigliucci 2005, Rejmanek et al. 2005) and the highest PI$_m$ (0.56) and PI$_a$ (0.18) of *R. pseudoacaia* contribute to its adaptability. The higher PI$_m$ of *A. campestre* and *C. avellana* is linked to an enhanced capacity to survive and grow in the understory (Valladares et al. 2002, Niinemets and Valladares 2004), because it has an important role in resource acquisition (Navas and Garnier 2002, Yamashita et al. 2000).

On the whole, our results support the overall trend that light-demanding species are more plastic than shade-tolerant species (Strauss-Debenedetti and Bazzaz 1991, 1996, Muth and Bazzaz 2002, Longuetaud et al. 2013). However, from this general scheme, *P. alba,* despite its shade intolerance, exhibits the lowest PI which can be related to its crown type and the environment where it grows. Moreover, our results support the trend for which the early-successional species (*R. pseudoacacia* and *Q. robur*) are more plastic than the late successional ones (Strauss-Debenedetti and Bazzaz 1991, 1996). In this context, the presence of large openings could favor both *Q. robur* and *R. pseudoacacia* regeneration.

Knowledge on phenotypic plasticity is important to make hypotheses about the dynamics of the studied forest in consideration of environmental stress factors, including invasive species competition and global climate change. These stress factors might alter the composition of the forest in the long term, influencing the competition of the early-successional species. In particular, the increase of air temperature and CO_2 concentration might act as potent agents of natural selection among species favoring the more phenotypically plastic species (Chown et al. 2007). In particular, air temperature increase might allow the reproduction of *Phythophora cinammomi*, a pathogen largely affecting *Q. robur* in Europe (La Porta et al. 2008) while the CO_2 concentration increase might favor *R. pseudoacacia* because of its high growth rate (Mohan et al. 2007). Considerations can be made for the conservative management of the forest carried out since the establishment of the Reserve, which has probably limited the presence of *R. pseudoacacia* until 1980 when it knowingly became established (Motta et al. 2009). Thus, it is important to maintain this type of management in the future since creating gaps could allow a greater seed regeneration of *R.*

pseudoacacia over *Q. robur* because of its higher growth rate. Consequently, land use is a fundamental determinant in shaping vegetation composition and structure with important implications for forest management. In particular, forests with old-growth characteristics (Bauhus et al. 2009), like the investigated forest, are important reference sites for more natural management approaches involving a broad range of ecosystem functions and services (Wirth et al. 2009). In recent years, interest in understanding the natural processes in forest ecosystems has increased because of a few primary forests remaining in many regions (e.g., Europe, Peterken 1996). Knowledge on the studied species, their plant and leaf traits, shade-tolerance, plasticity and information on the forest history may be important in making hypotheses about the current dynamics of individual trees and of the whole forest considering the increase of environmental stress factors including global climate change.

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References

Abrams, M. D., Kubiske, M. E., 1990: Leaf structural characteristics of 31 hardwood and conifer tree species in central Wisconsin: influence of light regime and shade-tolerance rank. Forest Ecology and Management 31, 245–253.

Amthor, J. S., 1989: Respiration and crop productivity. Berlin, Germany: Springer-Verlag.

Atkin, O. K., Evans, J. R., Ball, M. C., Siebke, K., 1998: Relationships between the inhibition of leaf respiration by light and enhancement of leaf dark respiration following light treatment. Australian Journal of Plant Physiology 25, 43–443.

Atkin, O. K., Scheurwater, I., Pons, T. L., 2007: Respiration as a percentage of daily photosynthesis in whole plants is homeostatic at moderate, but not high, growth temperatures. New Phytologist 174, 367–380.

Bauhus, J., Puettmanna, K., Messier, C., 2009: Silviculture for old-growth attributes. Forest Ecology and Management 258, 525–537.

Björkman, O., 1981: Responses to different quantum flux densities. In: Lange, O. L., Nobel, P. S., Osmond, C. B., Ziegler, H. (eds.), Physiological plant ecology I. Responses to the physical environment encyclopedia of plant physiology, 57–107. New Series, Vol. 12A, Springer-Verlag, Berlin.

Bloor, J. M. G., 2003: Light responses of shade-tolerant tropical tree species in north-east Queensland: a comparison of forest- and shadehouse-grown seedlings. Journal of Tropical Ecology 19, 163–170.

Bréda, N. J. J., 2003: Ground-based measurements of leaf area index: a review of methods, instruments and current controversies. Journal of Experimental Botany 54, 2403–2417.

Cai, Z. Q., Slot, M., Fan, Z. X., 2005: Leaf development and photosynthetic properties of three tropical tree species with delayed greening. Photosynthetica 43, 91–98.

Callaway, R. M., Pennings, S. C., Richards, C. L., 2003: Phenotypic plasticity and interactions among plants. Ecology 84, 1115–1128.

Castagneri, D., Garbarino, M., Nola, P., 2013: Host preference and growth patterns of ivy (*Hedera helix* L.) in a temperate alluvial forest. Plant Ecology 214, 1–9.

Chiusoli, A., 1991: Guida pratica agli alberi e arbusti in Italia. Reader's Digest S. p. A. Milano.

Chown, S. L., Slabber, S., Mcgeoch, M. A., Janion, C., Leinaas, H. P., 2007: Phenotypic plasticity mediates climate change responses among invasive and indigenous arthropods. Proceedings of the royal society b-Biological sciences 274, 2531–2537.

Cooper, D. J., Merritt, D. M., Andersen, D. C., Chimner, R. A., 1999: Factors controlling the establishment of Fremont Cottonwood seedlings on the Upper Green River, USA. Regional Rivers Research Management 15, 419–440.

Damascos, M. A., Rapoport, E. H., 2002: Differences in the herb and shrub flora growing under canopy gaps and under closed canopies in a *Nothofagus pumilio* forest of Argentina. Revista Chilena de Historia Natural 75, 465–472.

Delagrange, S., Messier, C., Lechowicz, M. J., Dizengremel, P., 2004: Physiological, morphological and allocational plasticity in understory deciduous trees: importance of plant size and light availability. Tree Physiology 24, 775–784.

Delledonne, M., Allegro, G., Belenghi, B., Balestrazzi, A., Picco, F., Levine, A., Zelasco, S., Calligari, P., Confalonieri, M., 2001: Transformation of white poplar (*Populus alba* L.) with a novel *Arabidopsis thaliana* cysteine proteinase inhibitor and analysis of insect pest resistance. Molecular Breeding 7, 35–42.

Demmig-Adams, B., 1998: Survey of thermal energy dissipation and pigment composition in sun and shade leaves. Plant Cell and Physiology 39, 474–482.

Duan, B., Lü Y., Yin C., Li, C., 2005: Morphological and physiological of woody plant in response to high light and low light. Chinese Journal of Applied Environmental Biology 11, 238–245.

Ducrey, M., 1994: Influence of shade on photosynthetic gas exchange of 7 tropical rainforest species from Guadeloupe (French West Indies). Annuals of Forest Science 51, 77–94.

Ellsworth, D. S., Reich, P. B., 1993: Canopy structure and vertical patterns of photosynthesis and related leaf traits in a deciduous forest. Oecologia 96, 169–178.

Evans, J. R. 1989: Photosynthesis and nitrogen relationships in leaves of C_3 plants. Oecologia 78, 9–19.

Evans, J. R., 1999: Leaf anatomy enables more equal access to light and CO_2 between chloroplasts. New Phytologist 143, 93–104.

Evans, J. R., Poorter, H., 2001: Photosynthetic acclimation of plants to growth irradiance: the relative importance of specific leaf area and nitrogen partitioning in maximizing carbon gain. Plant Cell and Environment 24, 755–767.

Fao, 1980: Poplars and willows in wood production and land use. FAO Forestry Series No. 10, FAO, Rome.

Farquhar, G. D, Sharkey, T. D., 1982: Stomatal conductance and photosynthesis. Annual Reviews of Plant Physiology 33, 317–345.

Ferraris, P., Terzuolo, P., Brenta, P. P., Palenzona, M. 2000: La robinia. Indirizzi per la gestione e la valorizzazione. Regione Piemonte, Blu Edizioni, Peveragno (CN).

Galo, A. T., Rich, P. M., Ewet, J. J. 1992: Effects efforts edges on the solar radiation regime in a series of reconstructed tropical ecosystems. American Society for Photogrammety and Remote Sensing Annual Meeting, 98–108.

Garnier, E., Gobin, O., Poorter, H. 1995: Nitrogen productivity depends on photosynthetic nitrogen use efficiency and on nitrogen allocation within the plant. Annals of Botany 76, 667–672.

Garrigues, S., Allard, D., Baret, F., Morisette, J. 2008: Multivariate quantification of landscape spatial heterogeneity using variogram models. Remote Sensing of Environment 112, 216–230.

Gratani, L., 1997: Canopy structure, vertical radiation profile and photosynthetic function in a *Quercus ilex* evergreen forest. Photosynthetica 33, 139–149.

Gratani, L., Crescente, M. F. 2000: Map-making of plant biomass and leaf area index for management of protected areas. Aliso 19, 1–12.

Gratani, L., Foti, I., 1998: Estimating forest structure and shade tolerance of the species in a mixed deciduous broad-leaved forest in Abruzzo, Italy. Annales Botanici Fennici 35, 75–83.

Gratani, L., Covone, F., Larcher, W. 2006: Leaf plasticity in response to light of three evergreen species of the Mediterranean maquis. Trees 20, 549–558.

Gravel, D., Canham, C. D., Beaudet, M., Messier, C. 2010: Shade tolerance, canopy gaps and mechanisms of coexistence of forest trees. Oikos 119, 475–484.

Groninger, J. W., Seiler, J. R., Peterson, J. A., Kreh, R. E. 1996: Growth and photosynthetic responses of four Virginia Piedmont tree species to shade. Tree Physiology 16, 773–778.

Gunderson, C. A., Sholtis, J. D., Wullschleger, S. D., Tissue, D. T., Hanson, P. J., Norby, R. J. 2002: Environmental and stomatal control of photosynthetic enhancement in the canopy of a sweetgum (*Liquidambar styraciflua* L.) plantation during 3 years of CO_2 enrichment. Plant Cell and Environment 25, 379–394.

Hanson, H. C. 1917: Leaf-structure as related to environment. American Journal of Botany 4, 533–560.

Hirose, T., Werger, M. J. A. 1987: Maximizing daily canopy photo- synthesis with respect to the leaf allocation pattern in the canopy. Oecologia 72, 520–526.

Hikosaka, K., 2003: A model of dynamics of leaves and nitrogen in a plant canopy: an integration of canopy photosynthesis, leaf life span, and nitrogen use efficiency. American Naturalist1 62, 149–164.

Hikosaka, K. 2005: Leaf canopy as a dynamic system: ecophysiology and optimality in leaf turnover. Annals of Botany 95, 521–533.

Holm, G. 1954: Chlorophyll mutations in barley. Acta Agriculturae Scandinavica 4, 457–471.

Hölscher, D. 2004: Leaf traits and photosynthetic parameters of saplings and adult trees of co-existing species in a temperate broad-leaved forest. Basic and Applied Ecology 5, 163–172.

Jacquemoud, S., Baret, F. 1990: PROSPECT: a model of leaf optical properties spectra. Remote Sensing of Environment 34, 75–91.

Janse-Ten Klooster, S. H., Thomas, E. J. P., Sterck, F. J., 2007: Explaining interspecific differences in sapling growth and shade tolerance in temperate forests. Journal of Ecology 95, 1250–1260.

Koike, T., Kitao, M., Maruyama, Y., Mori, S., Lei, T. 2001: Leaf morphology and photosynthetic adjustments among deciduous broad-leaved trees within the vertical canopy profile. Tree Physiology 21, 951–958.

Kowarik, I. 2010: Biologische Invasionen: Neophyten und Neozoen in Mitteleuropa, third ed. Ulmer (Eugen).

Kull, O., Niinemets, Ü. 1993: Variations in leaf morphometry and nitrogen concentration in *Betula pendula* Roth., *Corylus avellana* L. and *Lonicera xylosteum* L. Tree Physiology 12, 311–318.

Kull, O., Broadmeadow, M., Kruijt, B., Meir, P., 1999: Light distribution and foliage structure in an oak canopy. Trees 14, 55–64.

Kumar, V., Kodandaramaiah, J., Rajan, M. V., 2012: Leaf and anatomical traits in relation to physiological characteristics in mulberry (*Morus* sp.) cultivars. Turkish Journal of Botany 36, 683–689.

La Porta, N., Capretti, P., Thomsen, I. M., Kasanen, R., Hietala, A. M., Von Weissenberg, K. 2008: Forest pathogens with higher damage potential due to climate change in Europe. Canadian Journal of Plant Pathology 30, 177–195.

Larcher, W. 2003: Physiological plant ecology. 4th edn. Springer, Berlin.

Le Roux, X., Sinoquet, H., Vandame, M. 1999: Spatial distribution of leaf dry weight per area and leaf nitrogen concentration in relation to local radiation regime within an isolated tree crown. Tree Physiology 19, 181–188.

Lichtenthaler, H. K., Kuhn, G., Prenzel, U., Meier, D. 1982: Chlorophyll-protein levels and stacking degree of thylakoids in radish chloroplasts from high light, low-light and bentazon-treated plants. Physiologia Plantarum 56, 183–188.

Lichtenthaler, H. K., Babani, F., Langsdorf, G. 2007: Chlorophyll fluorescence imaging of photosynthetic activity in sun and shade leaves of trees. Photosynthesis Research 93, 235–244.

Longuetaud, F., Piboule, A., Wernsdörfer, H., Collet, C. 2013: Crown plasticity reduces inter-tree competition in a mixed broadleaved forest. European Journal of Forest Research 132, 621–634.

Loveys, B. R., Scheurwater, I., Pons, T. L., Fitter, A. H., Atkin, O. K. 2002: Growth temperature influences the underlying components of relative growth rate: an investigation using inherently fast- and slow-growing plant species. Plant Cell and Environment 25, 975–987.

Maclachlan, S., Zalik, S. 1963. Plastid structure, chlorophyll concentration, and free aminoacid composition of a chlorophyll mutant of barley. Canadian Journal of Botany 41, 1053–1062.

Madejón, P., Marañón, T., Murillo, J. M., Robinson, B. 2004: White poplar (*Populus alba*) as a biomonitor of trace elements in contaminated riparian forests. Environmental Pollution 132, 145–155.

Mendes, M. M., Gazarini, L. C., Rodrigues, M. L. 2001: Acclimation of *Myrtus communis* to contrasting Mediterranean light environments–effects on structure and chemical composition of foliage and plant water relations. Environmental Experimental Botany 45, 165–178.

Mills, E., 1996: An appreciation and natural history of the English field maple (*Acer campestre* L.). Arboricultural Journal 20, 405–410.

Mohan, J. E., Clark, J. S., Schlesinger, W. H., 2007: Long-term CO_2 enrichment of a forest ecosystem: implications for forest regeneration and succession. Ecological Applications 17, 1198–1212.

Motta, R., Nola, P., Berretti, R. 2009: The rise and fall of the black locust (*Robinia pseudoacacia* L.) in the »Siro Negri« Forest Reserve (Lombardy, Italy): lessons learned and future uncertainties. Annals of Forest Science 66, 410–9.

Muth, C. C., Bazzaz, F. A. 2002: Tree canopy displacement at forest gap edges. Canadian Journal of Forest Research 32, 247–254.

Navas, M.-L., Garnier, E., 2002: Plasticity of whole plant and leaf traits in *Rubia peregrine* in response to light, nutrient and water availability. Acta Oecologica 23, 375–383.

Nicotra, A. D., Chazdon, R. L., Iriarte, S. V. B. 1999: Spatial heterogeneity of light and woody seedling regeneration in tropical wet forests. Ecology 80, 1908–1926.

Nicotra, A. B., Atkin, O. K., Bonser, S. P., Davidson, A. M., Finnegan. E. J., Mathesius, U., Poot, P., Purugganan, M. D., Richards, C. L., Valladares, F., Van Kleunen, M. 2010. Plant phenotypic plasticity in a changing climate. Trends in Plant Science 15, 684–692.

Niinemets, Ü., 1995: Distribution of foliar carbon and nitrogen across the canopy of *Fagus sylvatica*: adaptation to a vertical light gradient. Acta Oecologia 16, 525–541.

Niinemets, Ü. 1996: Changes in foliage distribution with relative irradiance and tree size: Differences between the saplings of *Acer platanoides* and *Quercus robur*. Ecological Research 11, 269–281.

Niinemets, Ü., Valladares, F. 2004: Photosynthetic acclimation to simultaneous and interacting environmental stresses along natural light gradients: optimality and constraints. Plant Biology 6, 254–268.

Niinemets, Ü., Kull, O., Tenhunen, J. D., 1998: An analysis of light effects on foliar morphology, physiology, and light interception in temperate deciduous woody species of contrasting shade tolerance. Tree Physiology 18, 681–696.

Norman, J. M., Campbell, G. S., 1989: Canopy structure. In: Pearcy, R. W., Ehleringer, J., Mooney, H. A., Rundel, P. W. (eds.), Plant physiological ecology: field methods and instrumentation, 301–325. Chapman and Hall, London and New York.

Peterken, G. F., 1996: Natural woodland. Cambridge University Press.

Piccoli, D., Borelli, S., 1988: Introduzione agli studi fitometrici sulla cerreta di Roccarespampani (VT). Informatore Botanico Italiano 20, 818–815.

Pigliucci, M., 2005: Evolution of phenotypic plasticity: where are we going now? Trends in Ecology & Evolution 20, 481–486.

Porte, A., Bosc, A., Champion, I., Loustau, D., 2000: Estimating the foliage area of Maritime pine (*Pinus pinaster* Aït.) branches and crowns with application to modelling the foliage area distribution in the crown. Annals of Forest Science 57, 73–86.

Poorter, H., Evans, J. R., 1998: Photosynthetic nitrogen-use efficiency of species that differ inherently in specific leaf area. Oecologia 116, 26–37.

Portsmuth, A., Niinemets, Ü. 2007: Structural and physiological plasticity in response to light and nutrients in five temperate deciduous woody species of contrasting shade tolerance. Functional Ecology 21, 61–77.

Ryan, M. G., Lavigne, M. B., Gower, S. T. 1997: Annual carbon cost of autotrophic respiration in boreal forest ecosystems in relation to species and climate. Journal of Geophysical Research 102, 28871–28883.

Reich, P. B., Kloeppel, B. D., Ellsworth, D. S., Walters, M. B. 1995: Different photosynthesis-nitrogen relations in deciduous hardwood and evergreen coniferous tree species. Oecologia 104, 24–30.

Rejmanek, M., Richardson, D. M., Pysek, P., 2005: Plant invasions and invasibility of plant communities. In: Van Der Maarel, E. (ed.), Vegetation ecology, 332–355. Blackwell Publishing, Oxford UK.

Robakowski, P., Montpied, P., Dreyer, E. 2003: Plasticity of morphological and physiological traits in response to different levels of irradiance in seedlings of silver fir (*Abies alba* Mill). Trees 17, 431–441.

Roloff, A., Weisgerber, H., Lang, U., Stimm, B., Schütt, B. 1994: Enzyklopädie der Holzgewächse Handbuch und Atlas der Dendrologie. Wiley-VCH, Weinheim.

Sack, A., Melcher, P. J., Liu, W., Middleton, E., Pardee, T. 2006: How strong is intracanopy leaf plasticity in temperate deciduous trees? American Journal of Botany 93, 829–839.

Sánchez-Gómez, D., Valladares, F., Zavala, M. A. 2006: Functional traits and plasticity in response to light in seedlings of four Iberian forest tree species. Tree Physiology 26, 1425–1433.

Sartori, F. 1984: Les forêts alluviales de la basse vallée du Tessin (Italie du nord). In: Cramer, J. (ed.), Colloques phytosocologiques, la végétation des forêts alluviales, 201–216. Berlin.

Schirone, B., Scarascia Mugnozza, G., Valentini, R., 1985: Osservazioni preliminari sull'indice di area fogliare di *Quercus cerris* L. Monti e Boschi 5, 47–51.

Schulze, E., Lange, O.L., Evenari, M., Kappen, L., Buschbom, U. 1975. The role of air humidity and temperature in controlling stomatal resistance of *Prunus armeriaca* L. under desert conditions. Oecologia 19, 303–314.

Scotti-Saintagne, C., Mariette, S., Porth, I., Goicoechea, P. G., Barreneche, T., Bodénès, C., Burg, K., Kremer, A. 2004: Genome scanning for interspecific differentiation between two closely related oak species [*Quercus robur* L. and *Q. petraea* (Matt.) Liebl.]. Genetics 168, 1615–1626.

Strauss-Debenedetti, S., Bazzaz, F. A. 1991: Plasticity and acclimation to light in tropical moraceae of different successional positions. Oecologia 87, 377–387.

Strauss-Debenedetti, S., Bazzaz, F. A. 1996: Photosynthetic characteristics of tropical trees along successional gradients. In: Mulkey, S. S., Chazdon, R. L., Smith, A. P. (eds.), Tropical forest plant eco-physiology, 162–186. Chapman and Hall, New York.

Sultan, S. E. 2000: Phenotypic plasticity for plant development, function and life history. Trends in Plant Science 5, 537–542.

Tobin, M. F., Reich, P. B. 2009: Comparing indices of understory light availability between hemlock and hardwood forest patches. Canadian Journal of Forest Research 39, 1949–1957.

Tutin, T. G., Heywood, V. H., Burges, N. A., Valentine, D. H., Walters, S. M., Webb, D. A. 2001: Flora Europaea, Volumes 1–5. Cambridge University Press, Cambridge.

Ustin, S., Jacquemoud, S., Govaerts, Y., 2001: Simulation of photon transport in a three-dimentional leaf: implications for photosynthesis. Plant Cell and Environment 24, 1095–1103.

Valladares, F., Niinemets, Ü. 2008: Shade tolerance, a key plant feature of complex nature and consequences. Annual Review of Ecology, Evolution and Systematics 39, 237–57.

Valladares, F., Wright, S. J., Lasso, E., Kitajima, K., Pearcy, R. W. 2000: Plastic phenotypic response to light of 16 congeneric shrubs from a Panamanian rainforest. Ecology 81, 1925–1936.

Valladares, F., Chico, J. M., Aranda, I., Balaguer, L., Dizengremel, P., Manrique, E., Dreyer, E. 2002: The greater seedling high-light tolerance of *Quercus robur* over *Fagus sylvatica* is linked to a greater physiological plasticity. Trees 16, 395–403.

Walters, M. B., Reich, P. B. 1999: Low-light carbon balance and shade tolerance in the seedlings of woody plants: do winter deciduous and broad-leaved evergreen species differ? New Phytologist 143, 143–154.

Welander, N. T., Ottosson, B. 2000: The influence of low light, drought and fertilization on transpiration and growth in young seedlings of *Quercus robur* L. Forest Ecology and Management 127, 1390–151.

Wirth, C., Messier, C., Bergeron, Y., Frank, D., Frankhänel, A. 2009: Old-growth forest definitions: a pragmatic view. Ecological Studies 209, 11–33.

Witkowski, E. T. F., Lamont, B. B. 1991: Leaf specific mass confounds leaf density and thickness. Oecologia 88, 486–493.

Wright, S. J., Muller-Landau, H. C., Conditand R., Hubbell, S. P. 2003: Gap-dependent recruitment, realized vital rates and size distributions of tropical trees. Ecology 84, 3174–3185.

Woods, K. D. 2000: Long-term change and spatial pattern in a late successional hemlock–northern hard wood forest. Journal of Ecology 88, 267–282.

Wyka, T., Robakowsk, P., Ytkowiak, R. 2007: Acclimation of leaves to contrasting irradiance in juvenile trees differing in shade tolerance. Tree Physiology 27, 1293–1306.

Wyka, T. P., Oleksyn, J., Zytkowiak, R., Karolewski, P., Jagodzinski, A. M., Reich, P. B. 2012: Responses of leaf structure and photosynthetic properties to intra-canopy light gradients: a common garden test with four broadleaf deciduous angiosperm and seven evergreen conifer tree species. Oecologia 170, 11–24.

Yamashita N., Ishida, A., Kushima, H., Tanaka, N. 2000: Acclimation to sudden increase in light favouring an invasive over native trees in subtropical islands, Japan. Oecologia 125, 412–419.

Yoshimura, K. 2010: Irradiance heterogeneity within crown affects photosynthetic capacity and nitrogen distribution of leaves in *Cedrela sinensis*. Plant Cell and Environment 33,750–758.

Zha, T., Wang, K.Y., Ryyppö, A., Kellomäki, S. 2002: Needle dark respiration in relation to within-crown position in Scots pine trees grown in long-term elevation of CO_2 concentration and temperature. New Phytologist 156, 33–41.

Zunzunegui, M., Ain-Lhout, F., Díaz Barradas, M. C., Álvarez-Cansino, L., Esquivias, M. P, García Novo, F. 2009: Physiological, morphological and allocation plasticity of a semi-deciduous shrub. Acta Oecologica 35, 370–379.