



Photosynthetic characteristic and leaf traits variations along a natural light gradient in *Acer campestre* and *Crataegus monogyna*

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ABSTRACT

Variations in leaf traits at anatomical, morphological and physiological level were assessed in *Acer campestre* L. and *Crataegus monogyna* Jacq. plants in a forest understory-gap-edge light gradient inside a broadleaf deciduous forest. The relative changes of the considered parameters monitored in the two species were compared in terms of intraspecific plasticity. Overall, both species were able to acclimatize to the natural light gradient with different light regime (*i.e.* low light, medium light and high light), through an integrated response at the three analyzed levels. However, some differences in intraspecific plasticity were detected related to the different shade-tolerance of these species. In particular, the investigated species showed significant differences in plasticity at morphological level (*i.e.* leaf area and specific leaf area) with *A. campestre* having a greater morphological plasticity related to its greater shade-tolerance compared to *C. monogyna*. At the physiological level, differences in intraspecific plasticity have been identified for those parameters describing a higher drought susceptibility in high light regime (*i.e.* dark respiration rate, maximum PSII photochemical efficiency and actual quantum yield of photosynthesis) suggesting that *A. campestre* was less capable to acclimatize to these conditions, thus confirming the trade-off between drought and shade-tolerance.

1. Introduction

The heterogeneous light environment of forest ecosystems requires the acclimation to different light regimes, achieved through adjustments at both the leaf and the whole-plant level (Givnish, 1988). Beyond the intra-canopy vertical light gradient (Cano et al., 2013), an understory-gap-edge light gradient is present in the complex forest ecosystem, characterized by a gradual increase of the photosynthetic photon flux density (PPFD). Therefore, distinct physiological and morphological traits have evolved among plant species in response to a range of environmental stressors (Letts et al., 2012). In particular, adjustments at the leaf level are of utmost importance in the forest understory, where light is usually the most limiting resource (Pearcy and Sims, 1994), and in the canopy gaps, where the ability to utilize high PPFD efficiently plays a key role (Bazzaz, 1996). Otherwise, in the edge with full sun light the capacity to reduce the photo-inhibition effect is crucial to avoid severe damage to the photosynthetic machinery (Letts et al., 2012). From this, it is expected that leaves developed under low, moderate and high light condition differ predictably in a number of functional traits (Bongers and Popma, 1988). For instance, several parameters at the morpho-anatomical level (*e.g.* leaf area, fresh leaf thickness, specific leaf area, stomatal size and density) and at the

physiological level (*e.g.* net photosynthetic rates, intrinsic water-use efficiency) can be used as representative indices to evaluate plant adaptation to different light regimes (Guo et al., 2019). Among these traits, specific leaf area (SLA, the projected leaf area per unit leaf dry mass) is one of the main morphological traits that change in response to light variations (Puglielli et al., 2017), being a measure of the amount of leaf area for light capture per unit biomass invested (Rozendaal et al., 2006). Thus, on one hand, generally, plants grown in high light environments have thicker leaves with a low SLA associated with increased investment in structural components and, consequently, associated with greater foliar longevity and resistance to desiccation and herbivory (Wright et al., 2004). On the other hand, a higher SLA under shade condition is necessary to intercept more light (Granata et al., 2020).

Moreover, compared to sun-grown leaves, leaves from plants growing in low light environment typically maintain lower rates of photosynthesis at saturating light levels associated with reduced rates of dark leaf respiration (Letts et al., 2012). This is also linked to a lower carboxylation efficiency (Givnish et al., 2004) and results in low light compensation points (Craine and Reich, 2005). Otherwise, leaves from plants in high light environment are able to keep higher rates of photosynthesis linked to higher dark leaf respiration rates (Granata et al.,

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2020) and higher electron transport rates (Rodríguez-Calcerrada et al., 2007). The high rates of photosynthesis are supported by a higher stomatal density and stomatal conductance to maximize CO₂ absorption (Sack et al., 2006). At the same time, to cope with the stress due to a higher PPFD, these plants show, generally, a lower effective quantum yield of PSII photochemistry (i.e. Φ_{PSII}) compared to plants under low light condition, indicative of a higher capacity to dissipate the excess of the excitation energy as heat (Björkman and Demmig-Adams, 1994). This ability of an organism to alter its morpho-anatomical and physiological traits in response to environmental changes is defined as phenotypic plasticity (Schlichting, 1986), which results an important means by which individual plants may adjust to environmental heterogeneity (Bradshaw and Hardwick, 1989).

In this research, morphological, anatomical, and physiological leaf traits were measured along a natural understory-gap-edge light gradient in *Acer campestre* L. (maple) and *Crataegus monogyna* Jacq. (hawthorn) in order to develop greater insight into the physiological and morphological acclamatory changes of these two common deciduous trees under contrasting light regimes. These two species are often dominant understory shrubs or trees in forest understory, canopy gap and in edge under full-light conditions. However, between them, *A. campestre* results to be the more adapted to low light conditions, although a high shade-tolerance is mainly associated with other species of this genus with a sub-mediterranean distribution, young individuals of this species are generally considered shade-tolerant being indeed really widespread in the understory of broadleaf deciduous forests (Mills, 1996); while *C. monogyna* results to be more shade-intolerant (Aranda et al., 2004). From these light preferences we can introduce the widely recognized interspecific trade-off between shade and drought tolerances, according to which species with greater shade-tolerance are more vulnerable to drought than more light-demanding species (Aranda et al., 2005). Indeed, shade and drought tolerance involve conflicting requirements for biomass investment in leaves and branches for an efficient light capture enhanced at anatomical, morphological and physiological level against biomass investment in roots for efficient water uptake, and reduction in total leaf area to reduce evaporation (Cescatti and Niinemets, 2004). Therefore, our research hypotheses were: (i) that the considered leaf traits at morphological, anatomical and physiological level would change in relation to light availability in the three light regimes, (ii) that the degree of variation would differ between the species, and (iii) that due to a trade-off between drought and shade-tolerance, *A. campestre* would show both a higher capacity to tolerate shade and a higher sensitivity under high light regime. In order to test these hypotheses, the responses to a natural light gradient were studied in *A. campestre* and *C. monogyna* during one growing season. Moreover, the relative changes of the analyzed leaf traits of the two species were compared in terms of intraspecific plasticity by means of the slope of the regression equation between the dependent variable at leaf level and the environmental gradient according to Valladares et al. (2006). In this context the topic of the present work is relevant since it aims to understand through a field study the mechanisms by which two common deciduous species are able to acclimatize to contrasting light regime, explaining their dynamism inside a broadleaf forest ecosystem also according to the general trade-off between drought and shade tolerance.

2. Material and methods

2.1. Study area and plant material

The study was carried out in the period May–September 2019 in the broadleaf deciduous forest inside ‘Bosco Siro Negri’ Reserve (45° 12′ 39″ N, 09° 03′ 26″ E; 74 m a.s.l., temperate eco-region, Italy) belonging to the University of Pavia and established in 1970, currently extending over 10 ha. From the date of its establishment the Reserve has been unmanaged and the last important human disturbances date back to the

two world wars, due to an elevated demand for firewood (Motta et al., 2009). The forested area represents one of very few relicts of the original alluvial forest in northern Italy, and is characterized by a tree layer dominated by *Quercus robur* L., *Robinia pseudoacacia* L., *Ulmus minor* Mill., *Populus nigra* L. and *Populus alba* L., with many of them being more than 100 years old (Castagneri et al., 2013). A sub-dominant tree layer is characterized by younger individuals of the dominant species and also by *A. campestre*, *Corylus avellana* L., *Prunus padus* L., *C. monogyna*. Due to a high tree density the considered forest is characterized by a great light extinction at soil level, but is also interspersed with canopy gaps of variable size (Granata et al., 2016).

The climate of the area is characterized by a total annual rainfall of 637 mm, with 132 mm distributed in winter and 123 mm in summer months. During the year, the coldest month is January with a mean minimum air temperature (T_{min}) of 0.30 ± 2.0 °C, the hottest month July, with a mean maximum air temperature (T_{max}) of 30.2 ± 1.5 °C, and an average mean yearly temperature (T_m) of 13.9 ± 8.1 °C is recorded in this study area (Lombardia Regional Agency for Environmental Protection, Meteorological Station of Pavia, Ponte Ticino SS35; data for the period 2002–2018).

Throughout the experimental period a total rainfall of 201 mm was recorded, associated to a T_m of 21.1 ± 4.8 °C and a T_{max} in July of 31.2 ± 2.9 °C (Lombardia Regional Agency for Environmental Protection, Meteorological Station of Pavia, Ponte Ticino SS35; data for the period May–September 2019).

Fifteen well-grown mature plants of *A. campestre* and *C. monogyna* were selected as experimental objects under different light regimes along a natural light gradient, being two of the most common woody plant species in the forest understory, forest gap and in the edge. The understory-gap-edge light gradient covered a length of approximately 100 m, and the sandy soil showed a pH, soil water content and soil organic matter values ranging from 5.50 ± 0.05 , $25.2 \pm 3.3\%$ and $5.2 \pm 0.3\%$, respectively, to 5.15 ± 0.03 , $17.0 \pm 1.0\%$ and $3.3 \pm 0.3\%$, respectively (Granata et al., 2020). In particular, five plants of each species were selected in the forest understory under low light (LL), five plants were selected in the forest gap under medium light (ML), and five plants in the open under high light (HL) regime. The selected plant height ranged from 2 to 5 m for *A. campestre* and from 1.5 to 4 m for *C. monogyna*, with an average age for these species of 5 years.

To assess light availability in each site (i.e. HL, ML and LL), the photosynthetic photon flux density (PPFD) was measured by a quantum radiometer photometer (LI-189 LI-COR, USA) with the quantum sensor LI-190SA. Measurements were taken at the site of leaf photosynthesis measurements and compared to above-canopy value obtained above a canopy walk platform in the open to calculate relative intercepted PPFD (%PPFD). Measurements were carried out 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 for each of the three sites per species, according to Tobin and Reich (2009).

2.2. Morphological and anatomical measurements

Leaf blade area (LA, cm²) and leaf dry mass (DM, g) were measured on fully expanded leaves (excluding petiole) collected in June from the external medium portion of the crown of the considered plants ($n = 5$ leaves per plant in each light regime per species). LA was obtained by an image analysis system (Winfolia Software) and DM determined by drying leaves at 80 °C to constant mass. The specific leaf area (SLA, cm² g⁻¹) was calculated as leaf blade area per unit leaf dry mass.

For anatomical measurements, fully expanded leaves ($n = 5$ per plant in each light regime per species) collected in June were hand cut using a double-edged razor blade. The obtained cross sections of leaf blades, restricted to vein-free areas (Chabot and Chabot, 1977), were cleared using a 50% solution of bleach and water. Anatomical data were quantified using an image analysis program (Axiovision AC

software) where the images were acquired using a camera attached to a Carl Zeiss light microscope (Carl Zeiss Microscopy, Jena, Germany). The following anatomical data were then assessed at 20 \times and 40 \times magnification: total leaf thickness (L, μm), palisade and spongy parenchyma thickness (PP and SP, μm , respectively), thickness of the adaxial and abaxial cuticle and epidermis (CET_{ad} and CET_{ab}, μm , respectively). From abaxial epidermal peels, obtained with nail varnish, stomatal length (mm) and stomatal density (SD, n° stomata mm^{-2}) were determined at 40 \times and 20 \times magnification, respectively, and taken on separate impressions of the lamina (each of 0.5 cm \times 1.0 cm). Then, stomatal area index (SAI) was calculated by taking the product of the mean stomatal length and SD according to Ashton and Berlyn (1994) and expressed in mm stomata \times number of stomata mm^{-2} leaf.

2.3. Gas-exchange measurements

Leaf gas-exchange performances in the three light regimes were estimated through the study period by measuring net CO₂ assimilation rate (P_N), stomatal conductance (g_s), leaf transpiration (E) and sub-stomatal CO₂ concentration (C_i) using an infrared gas analyzer in an open system (LCPro+, ADC, BioScientific Ltd., Hoddesdon, UK) equipped with a Parkinson broad leaf chamber (window area of 6.25 cm², cuvette window made in polyethylene terephthalate film with \approx 90% light transmission in the range of photosynthetically active radiation). CO₂ concentration in the leaf chamber (C_a) was set at 400 $\mu\text{mol CO}_2 \text{mol}^{-1}$ air, the relative humidity of the incoming air ranged between 40 and 60% and leaf temperature was maintained to match the outdoor environmental conditions, ranging from 25 to 33 °C. In particular, measurements in high light were carried out when PPFD was \geq 1200 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ to ensure that the maximum rates were measured. On each sampling occasion, leaf respiration (R_D) was measured after P_N measurements (on the same leaves) as CO₂ 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 CO₂ transient post irradiation bursts. Moreover, apparent carboxylation efficiency (C_E) was determined by the ratio between P_N and C_i (Flexas et al., 2001). All measurements were carried out on cloud-free days in the morning from 9.00 a.m. to 11.00 a.m. on three attached fully expanded leaves per plant in each light regime per species.

2.4. Chl fluorescence measurements

Chlorophyll fluorescence measurements were carried out by a portable modulated fluorometer (OS5p, Opti-Sciences, USA) on the same leaves used simultaneously for gas exchange measurements for each light regime (three leaves per each light regime per species). The Chl *a* fluorescence parameters included maximum PSII photochemical efficiency (F_v/F_m), actual quantum yield of photosynthesis of light-adapted leaves (Φ_{PSII}) and electron transport rate (ETR). In particular, F_v/F_m was

determined after 30 min of dark adaptation in leaf clips. F_0 was obtained with a light pulse of \approx 0.1 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and F_M with a light-saturating pulse (\approx 8000 $\mu\text{mol m}^{-2} \text{s}^{-1}$) for 0.8 s. Then, F_v/F_m was calculated as $(F_M - F_0)/F_M$. The Φ_{PSII} was calculated on light-adapted leaves, in the three light regimes, as $\Phi_{PSII} = (F_M - F_S)/F_M$, where F_S was the fluorescence emission of the leaf under ambient light and F_M was the maximal light-adapted fluorescence yield where a light-saturating pulse (\approx 8000 $\mu\text{mol m}^{-2} \text{s}^{-1}$) is superimposed on the prevailing light intensity (Genty et al., 1989). Finally, the ETR ($\mu\text{mol e}^{-} \text{m}^{-2} \text{s}^{-1}$) was calculated according to Krall and Edwards (1992) as: $(\Phi_{PSII}) \times \text{PPFD} \times 0.5 \times 0.84$, where PPFD was the light intensity during measurement, 0.84 the fraction of light absorbed by leaves, and 0.5 the fraction of excitation energy distribution in photosystem II (Melis et al., 1987).

2.5. Statistical analysis

Differences in morphological and anatomical leaf traits were analyzed per species by one-way analysis of variance (ANOVA) at $p \leq 0.05$. Multiple comparisons were analyzed by a Tukey's test, while a repeated measure ANOVA (ANCOVA) was performed on physiological variables. Both normality and homogeneity of variances were checked before performing the analysis. Simple regression analysis was carried out between SLA as dependent variable and total leaf thickness, and between g_s as dependent variable and SD. Moreover, the light measurement in each site expressed as relative intercepted photosynthetic photon flux density (%PPFD) was used as the independent variable for the analyzed leaf traits. According to Schlichting and Pigliucci (1998), the magnitude of intraspecific plasticity was evaluated as the slope of the considered traits along the environmental gradient (i.e. light regime expressed as %PPFD). The slopes of regression equations were compared by tests of parallelism (ordinary least square regression) to elucidate if there are significant differences between the species in terms of plasticity of a given trait (Gianoli, 2001). All statistics were performed with Past, statistics software (Hammer et al., 2001). Results are presented as means \pm standard error.

3. Results

3.1. Morphological and anatomical measurements

Morphological and anatomical changes were observed at the leaf level in response to decreasing light availability (Table 1). In particular, in both species, LA and SLA decreased from HL to LL; both slopes of these relationships were significantly greater in *A. campestre* than *C. monogyna* ($p < 0.05$) (Fig. 1 A, B). On the contrary, total leaf thickness and PP gradually decreased with decreases in light availability from HL to LL, with *C. monogyna* HL showing the highest values of both parameters (174.8 μm and 82.3 μm , respectively). The same trends were

Table 1

Morphological and anatomical leaf traits in *Acer campestre* and *Crataegus monogyna* under three different light regimes (%PPFD = intercepted photosynthetic photon flux density) HL – high light (%PPFD > 70%); ML – medium light (%PPFD 20–50%); LL – low light (%PPFD < 10%). Mean values \pm standard error are shown ($n = 5$). Different letters indicate significant differences among the three light regimes within each species (ANOVA, $p < 0.05$).

	<i>Acer campestre</i>			<i>Crataegus monogyna</i>		
	HL	ML	LL	HL	ML	LL
Leaf blade area (cm ²)	17.7 \pm 1.1b	27.0 \pm 5.5b	46.3 \pm 3.6a	5.8 \pm 0.5c	13.8 \pm 0.9b	19.0 \pm 1.2a
Specific leaf area (cm ² g ⁻¹)	132.1 \pm 13.5b	255.5 \pm 6.5a	298.8 \pm 15.9a	111.5 \pm 4.9b	171.4 \pm 10.7a	183.4 \pm 14.9a
Total leaf thickness (μm)	121.4 \pm 4.0a	116.8 \pm 2.0a	86.1 \pm 3.8b	174.8 \pm 9.3a	134.6 \pm 5.0b	104.4 \pm 2.5c
Palisade parenchyma thickness (μm)	59.3 \pm 2.0a	49.8 \pm 1.5b	37.4 \pm 1.8c	82.3 \pm 1.0a	54.8 \pm 1.4b	45.9 \pm 1.2c
Spongy parenchyma thickness (μm)	40.4 \pm 1.0a	33.1 \pm 0.7b	43.7 \pm 1.2a	62.0 \pm 0.9a	39.2 \pm 1.1c	56.2 \pm 1.1b
Adaxial cuticle and epidermis thickness (μm)	12.0 \pm 0.3a	8.5 \pm 0.3b	7.8 \pm 0.3b	13.9 \pm 0.6a	12.3 \pm 0.2b	10.3 \pm 0.2c
Abaxial cuticle and epidermis thickness (μm)	8.5 \pm 0.2a	8.4 \pm 0.2a	7.1 \pm 0.1b	11.5 \pm 0.1a	10.3 \pm 0.6a	8.8 \pm 0.2b
Stomatal density (n° mm^{-2})	296 \pm 18a	272 \pm 18a	203 \pm 12b	408 \pm 29a	267 \pm 13b	251 \pm 15b
Stomatal area index	5.3 \pm 0.5a	4.7 \pm 0.2a	3.4 \pm 0.3b	15.4 \pm 2.0a	11.2 \pm 0.6ab	10.5 \pm 0.6b

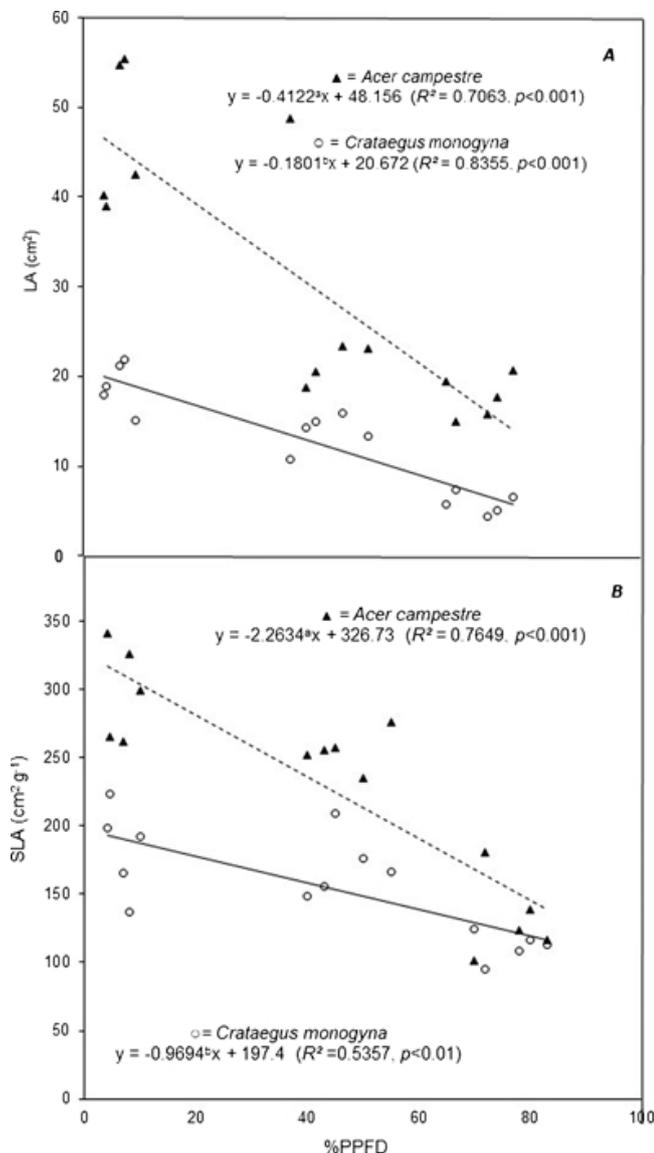


Fig. 1. Relationship between (A) leaf area (LA); (B) specific leaf area (SLA) and relative photosynthetic photon flux density (%PPFD) in *Acer campestre* (▲) and *Crataegus monogyna* (○) trees. Regression equation, coefficient of determination (R^2) and p -value are shown. Different letters in the equations indicate significant differences in the slopes between species ($p < 0.05$).

observed in SD and SAI, increasing from LL to HL (Fig. 2 A, B); however, both slopes of the regression lines did not differ between the species ($p > 0.05$). A significant negative relationship was found between SLA and total leaf thickness both in *A. campestre* ($y = -2.948x + 547.36$, $R^2 = 0.45$, $p < 0.01$) and *C. monogyna* ($y = -0.8798x + 276.83$, $R^2 = 0.52$, $p < 0.01$).

3.2. Gas-exchange and Chl fluorescence measurements

The average P_N value, over the entire study period, was higher in *C. monogyna* compared to *A. campestre*, with the greater difference observed in HL regime (Fig. 3A, B). A significant decrease by 76% and 49% was monitored in July in *A. campestre* and *C. monogyna*, respectively. Stomatal conductance showed the same trend as for P_N with the lowest values monitored in July ($0.036 \pm 0.002 \text{ mol m}^{-2} \text{ s}^{-1}$ and $0.083 \pm 0.008 \text{ mol m}^{-2} \text{ s}^{-1}$ in *A. campestre* and *C. monogyna*, respectively) (Fig. 3C, D). In both species, P_N increased sharply with light availability (Fig. 4 A), with the regression slopes not significantly

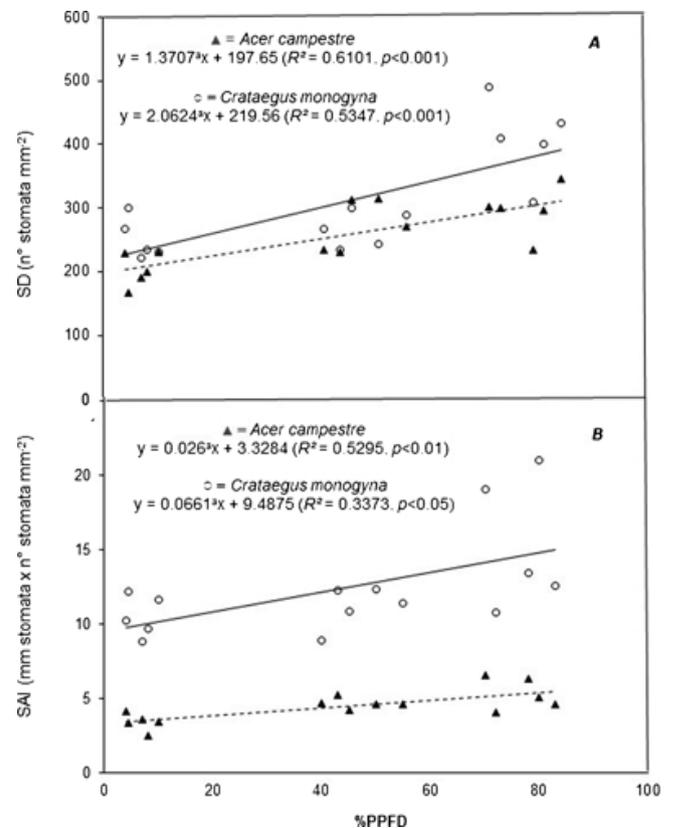


Fig. 2. Relationship between (A) stomatal density (SD); (B) stomatal area index (SAI) and relative photosynthetic photon flux density (%PPFD) in *Acer campestre* (▲) and *Crataegus monogyna* (○) trees. Regression equation, coefficient of determination (R^2) and p -value are shown. Different letters in the equations indicate significant differences in the slopes between species ($p < 0.05$).

different between species ($p > 0.05$). For R_D , no significant difference was observed in the average value between the two species, with R_D increasing linearly with light availability (Fig. 4 B), and the regression slopes significantly differing between the two species ($p < 0.05$). The same trend was observed in C_E , which showed, on an average, 14% higher values, although not significant, in *C. monogyna* than *A. campestre* and a gradual increase with light availability (Fig. 4 C). The regression slopes did not differ between species ($p > 0.05$). A significant positive relationship was found between g_s in June and SD, both in *A. campestre* ($y = 0.00101x - 0.1527$, $R^2 = 0.5532$, $p < 0.01$) and *C. monogyna* ($y = 0.000331x + 0.0241$, $R^2 = 0.436$, $p < 0.01$).

The values of Φ_{PSII} decreased with increasing PPFD in both species, reaching the lowest values at PPFD above $1200 \mu\text{mol m}^{-2} \text{ s}^{-1}$ in the HL environment (Fig. 5). Thus, both Φ_{PSII} and F_V/F_M negatively correlated with %PPFD, and for both variables, slopes were significantly different between species ($p < 0.05$) (Fig. 6 A, B). In particular, for Φ_{PSII} , significant ($p < 0.05$) differences were detected between the species mainly in HL regimes, where both under favorable (May and June) and constraint period (July and August), *A. campestre* showed a 36% lower Φ_{PSII} compared to *C. monogyna*. The same was detected for F_V/F_M during the favorable period, where *A. campestre* had a significantly lower value (by 9%) than *C. monogyna*, while under more constraint period the species did not differ significantly ($p > 0.05$). ETR was positively correlated with %PPFD, and slopes were significantly different between the species ($p < 0.05$) (Fig. 6 C). The slopes of the regression lines for these traits (Φ_{PSII} , F_V/F_M and ETR) resulted significantly higher in *A. campestre* than *C. monogyna*, except for ETR.

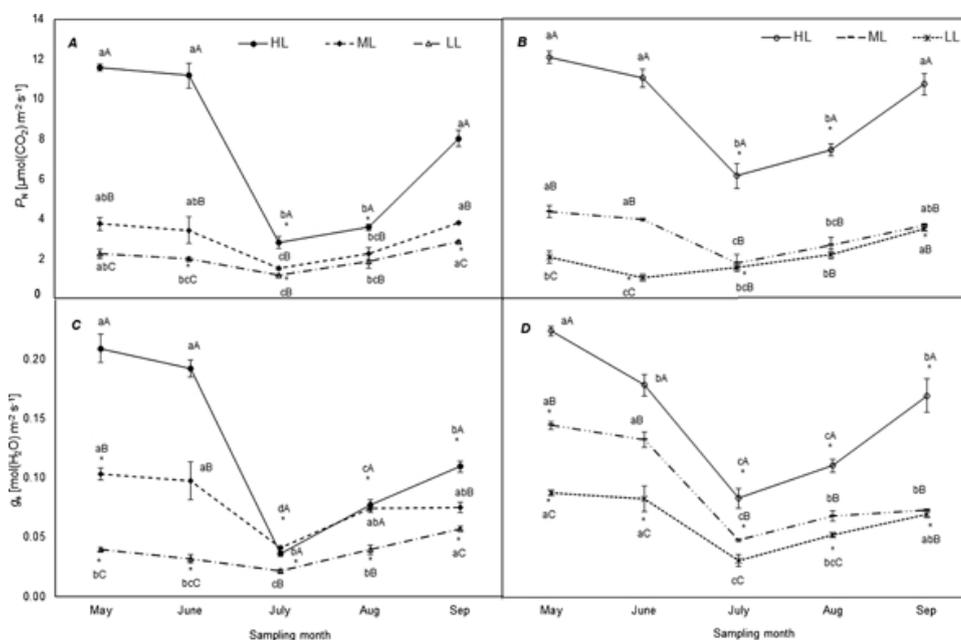


Fig. 3. Trends of net photosynthetic rates (P_n) in *Acer campestre* (A) and *Crataegus monogyna* (B) and stomatal conductance (g_s) in *A. campestre* (C) and *C. monogyna* (D) during the study period (May–September) under the three light regimes: high light (HL), medium light (ML) and low-light (LL). Each value denotes the mean (\pm SE) of five plants ($n = 5$) under the same regimes. Mean values with the same letters are not significantly different. Capital letters indicate the differences among the three light regimes in each month per species; lowercase letters indicate the differences during the study period. For the two variables, asterisk denotes significant inter-specific differences between each month under the same light regime (repeated measure ANOVA, $p \geq 0.05$).

4. Discussion

Overall, strong correlations between the considered morphological (LA and SLA), anatomical (SD and SAI) and physiological (P_n , R_D , C_E , Φ_{PSII} , F_V/F_M and ETR) leaf traits and the relative irradiance (%PPFD) along the natural light gradient were observed in both maple and hawthorn. These results stress the importance of the adjustments of the three analyzed levels (*i.e.* morphological, anatomical, and physiological) as an integral feature in the process of acclimation to contrasting light environments (Delgado-Sánchez et al., 2013). Nevertheless, different behavior has been observed in the three analyzed levels in terms of intraspecific plasticity.

Starting from the morphological level, both species showed a significant degree of correlation of LA vs %PPFD ($R^2 = 0.706$, $p = 0.0000856$ in *A. campestre* and $R^2 = 0.836$, $p = 0.0000018$ in *C. monogyna*; Fig. 1A) and SLA vs %PPFD ($R^2 = 0.765$, $p = 0.0000193$ in *A. campestre* and $R^2 = 0.536$, $p = 0.0019651$ in *C. monogyna*; Fig. 1B). These results underlined that the acclimation process to contrasting light regimes is related to SLA variations, which in turn happen through modification of leaf anatomy (Evans and Poorter, 2001; Rodríguez-Calcerrada et al., 2008). In fact, as confirmed by the regression analysis carried out between SLA and total leaf thickness (LT), a negative correlation between these variables was observed according to Torres-Leite et al. (2019) due to an increase in leaf area and reduction in leaf thickness, with, in our case, a significant portion equal to 48% (mean value for both species) of SLA variations dependent on changes in LT. Specifically, the gradual increase in total lamina thickness from LL to HL was mainly the result of an increase in palisade parenchyma thickness, according to the results of Rodríguez-Calcerrada et al. (2008) for two oak species, and is usually interpreted as a way of developing internal cell surface to facilitate efficient packaging of increased numbers of chloroplasts (Oguchi et al., 2003). When comparing the two species, significant differences in plasticity in both LA and SLA were detected, with maple showing about twofold steeper slopes than hawthorn (LA vs %PPFD, slope = -0.412 and intercept = 48.156 in *A. campestre*, slope = -0.180 and intercept = 20.672 in *C. monogyna*; SLA vs %PPFD, slope = -2.263 and intercept = 326.7 in *A. campestre*, slope = -0.969 and intercept = 197.4 in *C. monogyna*). These results were consistent with the greater shade tolerance of maple compared to hawthorn since a higher plasticity at the morphological level is linked to an enhanced capacity to survive under low-light regimes, having an

important role in resource acquisition (Niinemets and Valladares, 2004), although it leads to less capacity to cope with drought (Smith and Huston, 1989).

The response of stomatal density against light gradient showed a higher slope in hawthorn than in maple (slope = 1.37 and 2.06 in *A. campestre* and *C. monogyna*, respectively). The sign of the slopes clearly indicated that leaves developed under high light environments possessed more densely distributed stomata than leaves under more shade conditions according to the results of Sack et al. (2006) for other deciduous species. The higher SD in HL regime drove the higher stomatal conductance in maple and hawthorn as expressed by the regression analysis between these two variables, and maximizing CO_2 absorption resulted in higher net photosynthetic rates in HL compared to ML and LL. Moreover, stomatal conductance depends on both stomatal size (also defined as guard cell length) and stomatal density, here combined by stomatal area index (*i.e.* SAI) which also increased significantly with light availability according to the results of van Wittenberghe et al. (2012) for beech. In particular, we found a similar stomatal size from LL to HL in maple, while a decrease in stomatal size from LL to HL was observed in hawthorn. Hence, the same or lower stomatal size associated to a high number of stomata per unit of leaf area contributed to increase stomatal conductance from LL to HL according to Franks and Beerling (2009). The comparison of the slopes between the two species for the parameters SD, SAI, P_n and C_E did not show significant differences suggesting comparable amounts of intraspecific plasticity for these leaf traits. The slope in the response of R_D against %PPFD (slope = 0.009 and 0.005 in *A. campestre* and *C. monogyna*, respectively) suggested an R_D increase across the forest understorey to the high light environment, according to the results of Rodríguez-Calcerrada et al. (2010) for beech. The observed trend was a possible result of higher maintenance cost caused by higher P_n (Amthor, 1986), higher protein turnover and increased need for secondary compounds such as flavonoids or carotenoids (Delgrange et al., 2004). Otherwise, the lower respiratory rates in ML and LL allowed the plants to maintain the balance between carbon gain and energy expenditure (de Souza Lopes et al., 2019). In this case, significant differences in plasticity were found between maple and hawthorn, and the steeper slope in maple was mainly driven by the higher R_D rates measured in July and August in HL regime [$1.39 \pm 0.05 \mu\text{mol}(\text{CO}_2) \text{m}^{-2} \text{s}^{-1}$, mean value] compared to the same in hawthorn. In this environment, the higher amount of PPFD in summer months associated with the highest air temperature and a

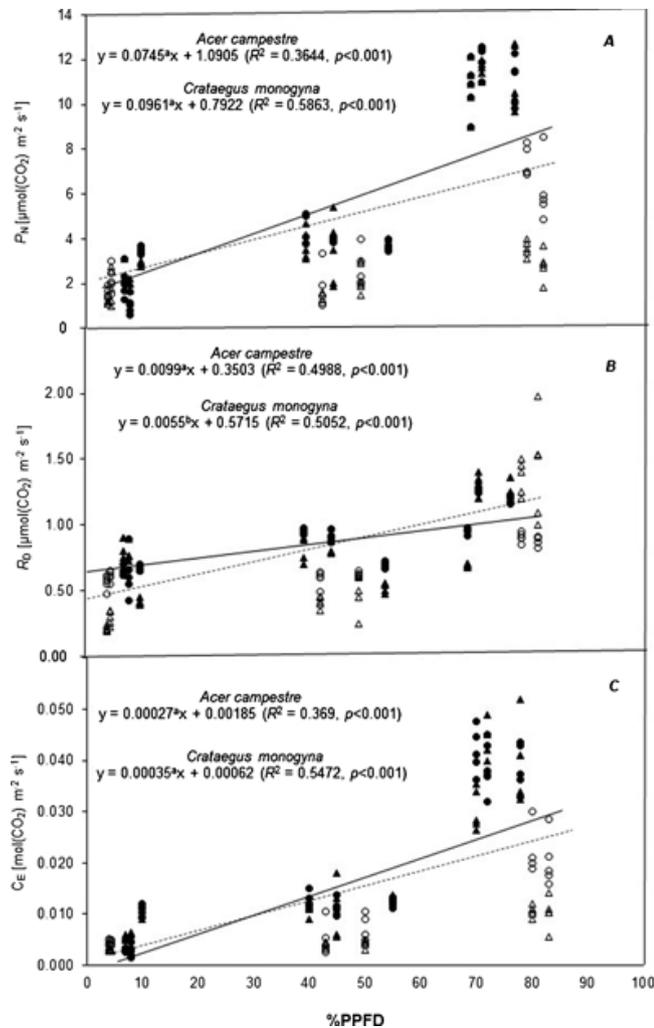


Fig. 4. Relationship between (A) net photosynthetic rates (P_N), (B) dark respiration rates (R_D) and (C) apparent carboxylation efficiency (C_E) and relative photosynthetic photon flux density (%PPFD) in *Acer campestre* (dashed lines, symbols \blacktriangle and \triangle for non-drought and drought stress period: July–August, respectively) and *Crataegus monogyna* (continuous lines, symbols \bullet and \circ for non-drought and drought stress period: July and August, respectively) trees. Regression equation, coefficient of determination (R^2) and p -value are shown. Different letters in the equations indicate significant differences in the slopes between species ($p < 0.05$).

lower soil water content can result in drought condition to which maple seems to be more susceptible (Leuzinger et al., 2005) compared to the more drought-tolerant hawthorn (Buse-Dragomir and Ion, 2012). An increase in R_D rates under constraining drought condition was observed in other species (Zaragoza-Castells et al., 2008; Catoni and Gratani, 2013; Catoni et al., 2017), and it was indicative of a higher proportion of carbon respired for the mobilization of the stored material (i.e. sucrose and starch) in response to plant requirements for maintenance in drought (Atkin and Macherel, 2009), underlining the strong correlation between the process of respiration and photosynthesis (Cano et al., 2014). Hence, the 72% lower P_N in maple, compared to the maximum value in May, necessitated the monitored higher R_D rates. In the same period, the P_N decrease in hawthorn resulted 40% lower compared to that of maple and, the comparison between the monitored g_s rates in these months [$0.057 \pm 0.021 \text{ mol(H}_2\text{O)} \text{ m}^{-2} \text{ s}^{-1}$ and $0.096 \pm 0.014 \text{ mol(H}_2\text{O)} \text{ m}^{-2} \text{ s}^{-1}$ mean value July and August, in *A. campestre* and *C. monogyna*, respectively] showed a greater stomatal closure in maple which limited transpiration rates, but in the same time resulted in the biggest constrain to P_N by restricting inward diffusion of

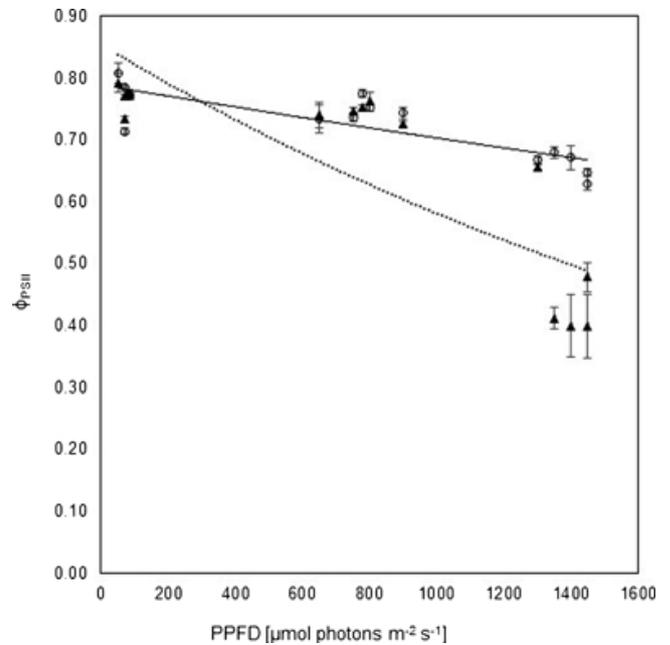


Fig. 5. Actual quantum yield of photosynthesis of light-adapted leaves (ϕ_{PSII}) with photosynthetic photon flux density (PPFD) during each measurement [$\phi_{\text{PSII}} = 0.8544e^{-3.851E-04\text{PPFD}}$, $R^2 = 0.645$ in *Acer campestre*, dashed line, symbol \blacktriangle ; $\phi_{\text{PSII}} = 0.7887e^{-1.150E-04\text{PPFD}}$, $R^2 = 0.712$ in *Crataegus monogyna*, continuous line, symbol \circ]. Values are means ($n = 5$) \pm SE.

CO_2 (Medrano et al., 2002; Chaves et al., 2009). Otherwise, the not significant differences in C_E between the two species suggested that mesophyll processes were not limiting.

The lower P_N decrease observed in hawthorn compared to maple, during drought, suggested a greater capacity of this species to exploit high irradiance more efficiently minimizing the risk of photoinhibition (Baker and Oxborough, 2004; Rodríguez-Calcerrada et al., 2008). Accordingly, we found a lower decline of ϕ_{PSII} with increasing PPFD in hawthorn than in maple, which returned a significant different amount of plasticity between the species in this leaf trait (slope = -0.0041 and -0.0015 in *A. campestre* and *C. monogyna*, respectively). Overall, for both species, this mechanism proved sufficient for avoiding photo-inhibition as judged for the quite constant F_V/F_M from LL to HL, particularly in hawthorn, highlighting an efficient functioning of the photo-protective mechanism without oxidative damage to the photosynthetic machinery (Thiele et al., 1998). Moreover, the significantly steeper slope (regression F_V/F_M vs %PPFD) in maple than hawthorn (slope = -0.0014 and -0.0007 in *A. campestre* and *C. monogyna*, respectively) showed a higher plasticity also for this trait in maple. Finally, the response of ETR against change in irradiance showed a positive slope in both the species, with a significant steeper slope in hawthorn than in maple (slope = 3.29 and 4.92, in *A. campestre* and *C. monogyna*, respectively). Hence, on one hand, the ETR increase across PSII from LL throughout ML to HL suggested a high capacity of ETR to adjust in response to changes in light availability in both species (Hjelm and Ögren, 2004), with the ETR increase from low to high light regimes in accordance with the results of Cano et al. (2011, 2013) for beech and oak species. Nevertheless, on the other hand, the 50% significantly higher ETR in HL in hawthorn than in maple was indicative of the maintenance of a higher ETR capacity in high light environment and thus a lower susceptibility to photo-inhibition, suggesting a better adaptation to take advantage in the %PPFD increase in hawthorn compared to maple. One possible drawback of this study is the lack of other parameters to assess the plant water status in response to drought conditions, confirming the co-occurrence of high temperature and soil water deficit in stressing these plants, but anyway the observed

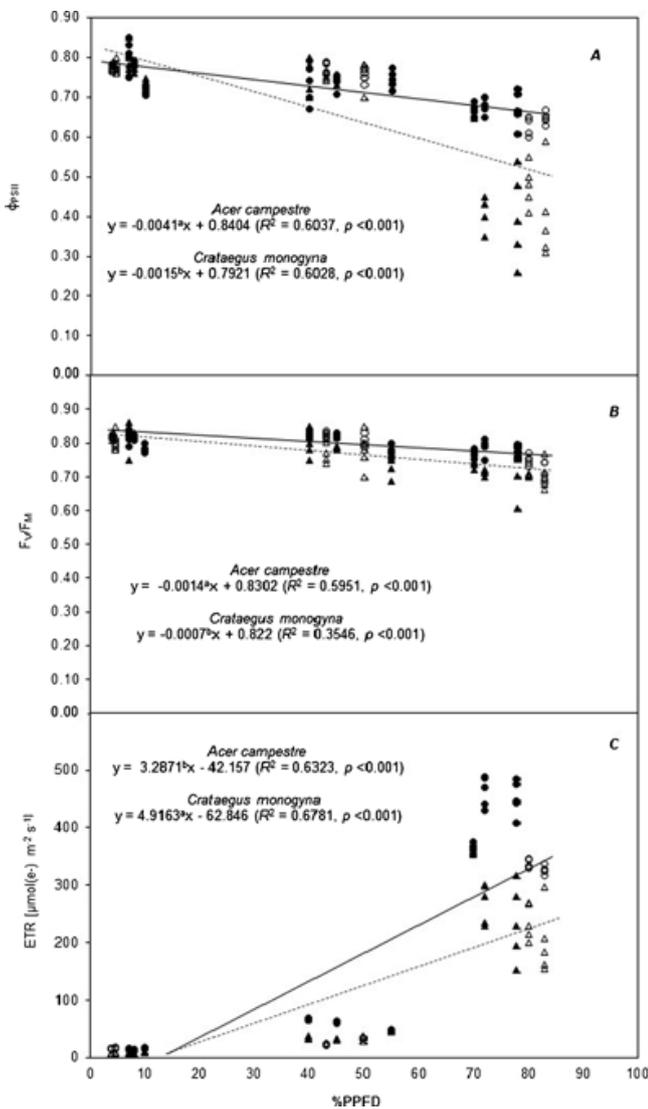


Fig. 6. Relationship between A) actual quantum yield of photosynthesis of light-adapted leaves (Φ_{PSII}), B) maximum PSII photochemical efficiency (F_v/F_m), and C) electron transport rate (ETR) and relative photosynthetic photon flux density (%PPFD) in *Acer campestre* (dashed lines, symbols \blacktriangle and \triangle for non-drought and drought stress period: July–August, respectively) and *Crataegus monogyna* (continuous lines, symbols \bullet and \circ for non-drought and drought stress period: July and August, respectively). Regression equation, coefficient of determination (R^2) and p -value are shown. Different letters in the equations indicate significant differences in the slopes between species ($p < 0.05$).

variations of the analyzed parameters clearly suggest a greater susceptibility of maple than hawthorn to these conditions.

5. Conclusion

According to our first research hypothesis all the considered leaf traits changed with the different light availability in the three light regimes and, particularly, six out of ten analyzed leaf traits (i.e. LA, SLA, R_D , Φ_{PSII} , F_v/F_m and ETR) showed differences in plasticity between maple and hawthorn. Overall, maple had higher morphological plasticity than hawthorn, primarily linked to its higher shade-tolerance. Except for ETR, maple showed also significantly higher plasticity in these traits: R_D , Φ_{PSII} and F_v/F_m , mainly related to its more stress-sensitive performance in the high-light regime, especially during summer months, and reflecting a lower capacity, compared to hawthorn, to cope with drought. This supports the hypothesized trade-offs in the stress-

tolerance mechanisms preclude that a species can be both shade and drought tolerance.

CRediT authorship contribution statement

Mirko U. Granata: Conceptualization, Investigation, Methodology, Writing - original draft. **Francesco Bracco:** Funding acquisition, Resources, Conceptualization, Supervision, Validation. **Paola Nola:** Conceptualization, Supervision, Validation. **Rosangela Catoni:** Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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