

1 **Long-term physiological and morphological acclimation by the evergreen shrub *Buxus***  
2 ***sempervirens* L. to understory and canopy gap light intensities**

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20

21 **Abstract**

22 Physiological and morphological plasticity are essential for growth and reproduction in  
23 contrasting light environments. In dry forest ecosystems, light generalists must also cope  
24 with the trade-offs involved in synchronous acclimation to light availability and drought. To  
25 understand how the broadleaf evergreen tree-shrub *Buxus sempervirens* L. (common box)  
26 inhabits both understory and successional terrain of Mediterranean forest, we measured  
27 photosynthesis-fluorescence light response, morphological traits and architectural  
28 characteristics across a light gradient. Our results show that *B. sempervirens* exhibits stress  
29 resistance syndrome, with little change in net photosynthesis rate across a light availability  
30 gradient, due to compensatory physiological and morphological acclimation. Light energy  
31 processing and dissipation potential were highest in leaves of well-illuminated plants, with  
32 higher electron transport rate, fraction of open Photosystem II reaction centres, non-  
33 photochemical quenching, photorespiration and dark respiration. By contrast, traits reducing  
34 light capture efficiency were observed in high light shrubs, including higher leaf mass per unit  
35 area, leaf clumping, leaf inclination and branch inclination. We suggest that both  
36 physiological and morphological plasticity are required for *B. sempervirens* to survive across  
37 a light gradient in a dry forest ecosystem, while exhibiting homeostasis in photosynthetic gas-  
38 exchange. We further speculate that the low growth rate of *B. sempervirens* is effective in full  
39 sun only due to a lack of competition in low resource microsites.

40

41 **Key words:** chlorophyll fluorescence, irradiance, leaf economics spectrum, phenotypic  
42 plasticity, self-shading, stable carbon isotope

43

#### 44 **Introduction**

45 Distinct physiological and morphological traits have evolved among plant species in  
46 understory and full sun environments. These traits represent the net effect of natural selection  
47 in response to a range of environmental stressors, in balance with the need to facilitate  
48 adequate carbon gain in low light and to avoid photoinhibition in full sun. At the leaf scale,  
49 understory species have evolved low light compensation points (Walters and Reich 1996;  
50 Craine and Reich 2005), carboxylation efficiency (Givnish et al. 2004) and leaf nitrogen  
51 content per unit area (Sack et al. 2003), resulting in a tendency for reduced dark respiration  
52 rates ( $R_d$ ), but also lower net photosynthesis rates at light saturation ( $A_{max}$ ). By contrast, high  
53 light specialists exhibit high electron transport rates (Rodríguez-Calcerrada et al. 2007), leaf  
54 nitrogen content per unit area (Gratani et al. 2006) and  $A_{max}$ , but also high  $R_d$ . To cope with  
55 the stresses associated with strong irradiance, including photoinhibition and desiccation, high  
56 light specialists also show higher leaf inclination angles, leaf clumping and turnover rates than  
57 shade specialists (Valladares and Niinemets 2008), at the expense of light interception  
58 efficiency (Falster and Westoby 2003) and leaf lifespan (Hikosaka 2003). Because of the  
59 opposing benefits of sun and shade plant adaptations, species that exhibit rapid growth rates  
60 in full sun generally show low understory survivorship (Kobe et al. 1995). Hence, species  
61 that grow, compete and reproduce effectively in both sun and shade environments must  
62 exhibit phenotypic plasticity in physiological or morphological characteristics related to light  
63 harvesting and use. In this study, we measure the physiological and morphological  
64 acclimation of the slow-growing, broadleaf evergreen tree-shrub *Buxus sempervirens* to  
65 understory, canopy gap and full sun light regimes.

#### 66 Phenotypic plasticity in response to contrasting irradiance

67       Within individual species, phenotypic plasticity is required for growth, reproduction  
68 and survival in distinct irradiance regimes.. This entails orchestrated changes at the whole-

69 plant level that help to maintain a balance between light energy harvesting and use. Plants that  
70 grow in full sun reduce investment in nitrogen for leaf chlorophyll, but increase the  
71 production of pigments involved in photoprotection, including xanthophylls (Bilger and  
72 Bjorkman 1990; Demmig-Adams 1990; Galmés et al. 2007; Matsubara et al. 2009). Under  
73 long-term exposure to high irradiance, carbon allocation is directed toward high leaf dry mass  
74 per unit area (LMA; Lusk et al. 2008; Poorter et al. 2009; Coste et al. 2010), dense branching  
75 and leaf clumping (Ali and Kikuzawa 2005). Leaf inclination also tends to increase, resulting  
76 in lower radiation interception per unit leaf area when solar zenith angles are small (Falster  
77 and Westoby 2003). These architectural and biochemical adjustments help to prevent  
78 photoinhibition, by reducing light capture efficiency (Kern et al. 2004), defined broadly as the  
79 ability of leaves to intercept a high amount of radiation per unit carbon allocation at the  
80 branch scale. When light absorption still exceeds the capacity of chloroplasts to process light  
81 energy, permanent photoinhibition of Photosystem II (PSII) is mitigated through  
82 photorespiration (Krömer 1995), mitochondrial respiration (Padmasree et al. 2002) and non-  
83 photochemical quenching of chlorophyll fluorescence (Ort 2001; Coopman et al. 2010).

84 Species that grow and reproduce successfully in both low and high light sites often  
85 exhibit high phenotypic plasticity (Valladares et al. 2002; Matos et al. 2009). Light plasticity  
86 is thought to be related to plant form and function (Valladares and Niinemets 2008), but  
87 similar plasticity of leaf traits has been observed in co-occurring tropical evergreens differing  
88 in successional status and LMA (Coste et al. 2010). Physiological plasticity tends to be  
89 higher in species tolerant of high light, while higher morphological plasticity has been  
90 observed in certain shade-tolerant species at the leaf (Feng et al. 2004), shoot (He and Dong  
91 2003) and whole-plant scales (Kitajima 1994).

92 Light plasticity is also affected by resource availability, with lower plasticity observed  
93 where plants are exposed to drought stress or low nutrient availability (Chapin 1993). In

94 resource-rich environments, plasticity might be enhanced by selective pressures favouring  
95 high photosynthetic capacity and other traits that facilitate rapid growth under strong  
96 irradiance. Conversely, in environments constrained by limiting resources, stress adaptation  
97 may reduce the potential for plasticity in response to environmental heterogeneity (Lortie and  
98 Aarssen 1996). For example, in drought-prone regions, enhanced photoprotection and  
99 reduced light capture efficiency are essential for evergreen species to tolerate periods of low  
100 moisture availability. Consequently, low plasticity to light was observed in the Mediterranean  
101 evergreen oak *Quercus ilex* L. (Valladares et al. 2000a), and in European holly (*Ilex*  
102 *aquifolium* L.; Valladares et al. 2005; Aranda et al. 2008), reflecting its conservative resource-  
103 use strategy.

#### 104 Assessment of long-term light acclimation

105         Physiological and morphological acclimation to environmental conditions occurs at  
106 varying time scales (Külheim et al. 2002; Schurr et al. 2006; Rodríguez-Calcerrada et al.  
107 2008a). Photosynthetic gas exchange characteristics, including stomatal conductance to water  
108 vapour ( $g_s$ ), transpiration ( $E$ ) and  $A_{max}$ , rapidly respond to changes in light intensity,  
109 temperature, vapour pressure deficit (VPD) and leaf water potential ( $\psi_L$ ), but can also  
110 acclimate to distinct environmental conditions more slowly, as a result of long-term changes  
111 in LMA and leaf chemistry (Brooks et al. 1996). In response to contrasting light availability,  
112 changes in photosynthetic capacity occur over periods of several days or weeks in woody  
113 plants, due to gradual adjustments of maximum electron transport rate ( $ETR_{max}$ ) and  
114 maximum rate of Rubisco-mediated carboxylation ( $V_{cmax}$ ), which increase with light  
115 availability (Frak et al. 2001). Changes in branch morphology develop over a period of years,  
116 while differences in leaf morphology occur within a single growth season. Unfavourable  
117 growth conditions, including soil moisture stress, can cause large changes in rapidly  
118 responding variables that can mask long-term light acclimation. To isolate long-term

119 physiological acclimatory responses to contrasting light regimes in a field setting, it is, thus,  
120 important to assess differences in fully-developed leaves and under optimal growth  
121 conditions, including moderate temperature, high soil moisture availability, similar  $\psi_L$  and  
122 controlled leaf chamber conditions. Inevitably, long-term acclimatory responses to  
123 understory and canopy gap light regimes will also reflect slow acclimatory responses to other  
124 abiotic and biotic conditions that might differ between these contrasting environments, such  
125 as drought stress, herbivory and competition. Therefore, any long-term morphological and  
126 physiological differences observed between contrasting light regimes must be interpreted  
127 within a broader ecological context, as a net response to multiple environmental stressors  
128 representative of understory and canopy gap sites.

129 The common box: *Buxus sempervirens* L.

130 *Buxus sempervirens* L. is a broadleaf evergreen tree-shrub, prevalent throughout warm  
131 temperate and northern Mediterranean climates of Europe (Quézel and Médail 2003). It is  
132 found in both the evergreen and deciduous forest understory, in canopy gaps, and in low-  
133 shrub secondary successional areas with full sunlight. Previous research on *B. sempervirens*  
134 in deciduous forest regions of northern Spain has demonstrated higher chlorophyll content,  
135 but lower antioxidant and photoprotective mechanisms in understory specimens (Hormaetxe  
136 et al. 2005, 2007), including lower ratios of xanthophyll cycle to chlorophyll pigments  
137 (García-Plazaola et al. 2008). These results demonstrate specific mechanisms of  
138 physiological adjustment to light conditions. However, little is known regarding the impact  
139 of long-term acclimation to distinct light regimes on photosynthetic gas-exchange or  
140 morphological characteristics, including LMA, leaf inclination angle and leaf clumping.

141 Objectives and hypotheses

142 In this study, photosynthetic gas-exchange, chlorophyll fluorescence and  
143 morphological characteristics are measured along a light gradient in *B. sempervirens*, to

144 develop greater insight into the physiological and morphological acclimatory changes that  
145 enable the growth and reproduction of a sclerophyllous evergreen shrub in contrasting light  
146 regimes. Given the shade tolerance, drought tolerance and high leaf longevity of *B.*  
147 *sempervirens* (García-Plazaola et al. 2008), we hypothesized that this shrub would exhibit  
148 only modest physiological acclimation to differences in light availability, including higher  
149  $A_{\max}$ , dark respiration rate ( $R_d$ ), maximum quantum yield of Photosystem II ( $F_v/F_m$ ) and  
150 electron transport rate ( $ETR_{\max}$ ) in shrubs of full sun environments. We further anticipated  
151 that morphological acclimation to understory, gap and full sun light regimes would exhibit  
152 greater plasticity than physiological acclimation, with full sun shrubs exhibiting higher LMA,  
153 self-shading and leaf inclination angles.

## 154 **Materials and methods**

### 155 Site description

156 The study was carried out at the Puéchabon Experimental Site, a forested plateau, located 35  
157 km north-west of Montpellier, Hérault, France (3°35'45"E, 43°44'29"N, 270 m.a.s.l.). The  
158 site was managed as a coppice for centuries, but has not been logged since 1942. The forest is  
159 dominated by *Quercus ilex* L., which provides a partially-closed canopy of about 5.5 m in  
160 height, but the forest is also interspersed with canopy gaps of variable size. The tree-shrub *B.*  
161 *sempervirens* is the most common woody plant in the understory and in open areas, achieving  
162 heights of up to three metres. Soils at Puéchabon contain a very high stone fraction,  
163 composed primarily of Jurassic limestones. Mean volumetric stone fraction within 50 cm of  
164 the surface is 0.75, increasing to 0.90 at greater depths (Rambal et al. 2003).

165 Mean annual temperature at Puéchabon was 13.2°C during the period from 1984 –  
166 2009, ranging seasonally from 5.5°C in January to 22.9°C in July. Mean annual precipitation  
167 was 907 mm, with 80% occurring between September and April (Misson et al. 2010).

168 Meteorological data were obtained from April 18 to May 25, using a weather station located

169 in a large forest gap (Allard et al. 2008). Predawn leaf water potential ( $\psi_L$ ) was measured  
170 with a pressure chamber (PMS 1000, PMS Instruments, Corvallis, Oregon, USA) on May 19,  
171 2010, to coincide with the photosynthesis-fluorescence measurement period (May 7 – May  
172 25). Soil water content was determined at 0 – 15 cm, with a time-domain reflectometry  
173 (TDR) probe (MiniTrase 6050X3K1B, Soil Moisture Equipment Corp., USA) on April 27  
174 and May 18, 2010.

#### 175 Plant sampling and light treatment design

176 Thirty-three mature *B. sempervirens* shrubs were chosen for morphological,  
177 physiological and radiation regime analysis along a light gradient. All shrubs were apparently  
178 healthy and at least 1.5 m in height. Mean stem diameter of the study specimens was 4.39 cm,  
179 with no significant difference along the light gradient. To obtain a complete and continuous  
180 light gradient, 21 plants were selected from three 140 m<sup>2</sup> forest understory plots in variable  
181 light conditions, while six plants were chosen from two large gaps with full sun exposure and  
182 six plants were sampled from a small canopy gap with intermediate light conditions. The  
183 understory plots contain inverted PVC gutters, because they also serve as a control plot in an  
184 ongoing drought acclimation experiment. The gutters have no effect on soil moisture  
185 (Limousin et al. 2010), but provide additional shading. We chose 9 of 21 understory branches  
186 below the gutters to expand the lower end of the light intensity range. Plants in full sun  
187 exhibited new (2010) growth in most branches, but many understory branches were devoid of  
188 new shoots with growth (see Results). To ensure an adequate sample of 2010 leaves for  
189 morphological analyses, we restricted our branch selection to those containing at least one  
190 shoot with new growth. Sampled leaves and branches were selected exclusively from the  
191 outer crown of individuals. To ensure long-term acclimation to the contrasting light regimes  
192 of understory and canopy gap environments, all measurements were made on previous-year  
193 leaves (second growth unit) from branches with new growth. Current-year leaves were not



194 studied, because they had not fully developed. While this selection scheme was designed to  
195 reduce ontogenetic effects resulting from differential leaf age, it is possible that not all *B.*  
196 *sempervirens* leaves flushed in spring, and that not all branches flushed each year.  
197 Relationships between light availability and leaf physiological and morphological traits must,  
198 therefore, be considered with regard to the possibility that some leaves may have developed in  
199 seasons differing in abiotic conditions, including light and drought intensity.

200 To assess light availability, we calculated percent above-canopy photosynthetic photon  
201 flux density (%PPFD) during completely overcast periods on April 18 and 20, 2010.  
202 Measurements were taken with an LI-250 Light Meter (LI-COR Biosciences, Lincoln, NB,  
203 USA) at the site of leaf photosynthesis measurements and compared to above-canopy values  
204 obtained above a permanent canopy walk platform. The instantaneous percent above-canopy  
205 PPFD method effectively estimates light levels in understory environments beneath  
206 continuous canopies (Machado and Reich 1999). For the discontinuous canopy of  
207 Puéchabon, we caution that this method does not account for the timing and duration of light  
208 flecks, which can also impact the light acclimation response.

#### 209 Photosynthesis-fluorescence light response curves

210 Simultaneous chlorophyll fluorescence and photosynthetic gas-exchange were  
211 measured with two LI-COR 6400XTR Portable Photosynthesis Systems (LI-COR  
212 Biosciences), equipped with a LI-COR 6400-40 Leaf Chamber Fluorometer. An assessment  
213 of the equilibration period required to achieve  $A_{\max}$  was carried out from April 27 to May 5,  
214 2010. Measurements were then taken on twelve dates between May 7 and May 25, 2010,  
215 alternating among understory, gap and high light specimens, to prevent measurement  
216 condition effects, including developmental, time of day and day of year (DOY) bias. Before  
217 each fluorescence-light curve was produced, leaves were allowed to equilibrate to PPFD  
218 intensity of  $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$ , air temperature near  $25^{\circ}\text{C}$  and relative humidity near 50% for

219 45 minutes. A long equilibration period was required, because  $A_{\max}$  and  $g_s$  usually did not  
220 reach asymptotic maxima, assessed visually from the graphic display, for 30 to 45 minutes.  
221 Atmospheric conditions on the measurement dates caused moderate deviation from chamber  
222 microclimate target conditions, but mean leaf-to-atmosphere vapour pressure deficit (VPD)  
223 and chamber air temperature ( $T_a$ ) were similar at PPFD-saturation in leaves from high light  
224 ( $1.63 \pm 0.10$  kPa,  $26.3 \pm 0.6$  °C), intermediate light ( $1.81 \pm 0.10$  kPa,  $25.6 \pm 0.5$  °C) and low  
225 light shrubs ( $1.79 \pm 0.07$  kPa,  $24.8 \pm 0.5$  °C), with these three categories defined as leaves with  
226 ambient %PPFD in the 60 - 90 %, 30 - 60 % and 0 - 30 % ranges, respectively (one-way  
227 ANOVA tests;  $P > 0.2$ ). Most leaves did not fill the 2.0 cm<sup>2</sup> LI-6400-40 chamber. Projected  
228 leaf area was, thus, determined with a light table system (MK2 Area Meter; Delta-T devices  
229 Ltd., Cambridge, UK), after cutting out the measured portion with a sharpened 2.0 cm<sup>2</sup> borer.  
230 Gas exchange parameters were corrected for the actual leaf area enclosed in the chamber.

231         Photosynthetic gas-exchange and fluorescence parameters were measured at PPFD  
232 intensities of 1500, 1000, 650, 400, 200, 120, 80, 50, 20 and 0  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . For each leaf,  
233 twenty photosynthesis readings were taken at each level, with the mean value used for the  
234 production of the light curves. Chlorophyll fluorescence was determined after the last record  
235 of gas exchange at each of the target PPFD intensities. The leaves were allowed to equilibrate  
236 for a minimum of five minutes between each light level, to ensure steady state conditions of  
237 net photosynthesis ( $A$ ). Dark respiration ( $R_d$ ) measurements were taken after dark acclimation  
238 for 30 minutes, accompanied by a chlorophyll fluorescence measurement. While the use of  
239 ambient initial PPFD is recommended for light response curves, we elected to use identical  
240 sequences of descending PPFD intensity with long equilibration times, to minimize among-  
241 specimen differences in hysteretic effects resulting from changing PPFD. Photosynthetic gas-  
242 exchange characteristics examined included  $A_{\max}$ , stomatal conductance of water vapour ( $g_s$ ),  
243 transpiration rate ( $E$ ), apparent quantum yield ( $\alpha$ ), light compensation point (LCP), and  $R_d$ .

244 Apparent quantum yield was estimated by non-linear regression, after fitting the Mitscherlich  
245 equation to individual photosynthesis light response curves (Peek et al. 2002). Light  
246 compensation points were calculated as the intercepts of the initial, linear portions of the  
247 photosynthesis-light curves with the light axis.

248 Fluorescence parameters determined included the dark-adapted maximum quantum  
249 yield of photosystem II ( $F_v/F_m = (F_m - F_o) / F_m$ ) and, at each PPFD level, the fraction of open  
250 PSII reaction centres ( $q_L$ ), efficiency of open reaction centres ( $\phi q_L$ ), quantum yield of PSII  
251 ( $\phi_{PSII} = (F_m' - F_s) / F_m'$ ), non-photochemical quenching ( $NPQ = (F_m - F_m') / F_m'$ ) and electron  
252 transport rate (ETR). The parameters  $q_L$  and  $\phi q_L$  were calculated assuming all open reaction  
253 centres compete for excitons, according to Kramer et al. (2004):  $q_L = ((F_m' - F_s) \cdot F_o') / ((F_m' -$   
254  $F_o') \cdot F_s)$  and  $\phi q_L = ((F_m' - F_o') \cdot F_s) / (F_m' \cdot F_o')$ . In these equations,  $F_m$  and  $F_m'$  represent the  
255 fluorescence values of leaves in darkness and illumination, respectively, during a pulse of  
256 saturating light,  $F_o$  is the minimum value of fluorescence in darkness, and  $F_o'$  is the minimum  
257 value of fluorescence in light, obtained after supplying leaves with a far-red light pulse.

258 Electron transport rate was determined using the Krall and Edwards (1992) formulation:

259 (1) 
$$ETR = 0.5 \cdot PPFD \cdot 0.84 \cdot \phi_{PSII}$$

260 In the absence of calibration curves, an average absorptivity value of 0.84 is commonly  
261 applied in light gradient studies involving a wide range of species, including Mediterranean  
262 evergreens (eg. Peguero-Pina et al. 2009, Puértolas et al. 2010). However, in some species,  
263 this value varies among specimens from microsites differing in PPFD availability (Evans,  
264 1989). Therefore, we caution that ETR,  $\phi q_L$  and photorespiration ( $R_p$ ) estimates are subject to  
265 errors introduced by deviation from the estimated absorptivity value.

266 The responses of each parameter to %PPFD were fitted to non-mechanistic, non-linear  
267 models, described in Rodríguez-Calcerrada et al. (2007). Photorespiration ( $R_p$ ) was calculated  
268 from photosynthesis and ETR data, as follows (Valentini et al. 1995):

269 (2) 
$$R_p = 1/12 \cdot [ETR_{\max} - 4(A_{\max} + R_d)]$$

270 Leaf chlorophyll, stable carbon isotope ( $^{13}\text{C}/^{12}\text{C}$ ) and nitrogen content

271 A surrogate measure of chlorophyll content was obtained with a SPAD chlorophyll  
272 meter (SPAD-502, Konica Minolta Sensing Co., Japan), as the mean value of three leaves  
273 adjacent to the measured leaf, taken from the same branch and growth unit. Leaves used for  
274 photosynthesis-fluorescence curves were collected following measurement, dried for 96 hours  
275 at 60°C, and then weighed to determine leaf mass per unit area (LMA). Leaf stable carbon  
276 isotope composition ( $\delta^{13}\text{C}$ ) was measured, to determine whether water stress might have  
277 differed during leaf development and subsequent carbon turnover at sites of high,  
278 intermediate and low light availability. Leaf  $\delta^{13}\text{C}$  serves as an integrative measure of  
279 photosynthetic water-use efficiency ( $\text{WUE} = A/E$ ; Farquhar et al. 1982), which tends to  
280 increase in response to soil moisture stress (Meinzer et al. 1992). To prepare foliar material  
281 for  $\delta^{13}\text{C}$  and leaf N analysis, dry leaf samples (petioles excluded) were placed into centrifuge  
282 tubes and crushed into a fine powder using a ball mill (2000 Retsch, Haan, Germany). The  
283  $\delta^{13}\text{C}$  composition and total N concentration of the leaf tissue were then determined by  
284 combustion of leaf samples in an elemental analyzer (Flash EA1112 Series, ThermoFinnigan,  
285 Milan, Italy), coupled to a gas isotope ratio mass spectrometer (Delta S, Finnigan, San Jose,  
286 CA, USA). All analysis was carried out at the CNRS Central Analysis Centre in Solaze,  
287 France. Stable carbon isotope composition was calculated from the ratio R, ( $^{13}\text{CO}_2/^{12}\text{CO}_2$ ):

288 (3) 
$$\delta^{13}\text{C} = \left[ \frac{R_{\text{sample}}}{R_{\text{std}}} - 1 \right] \cdot 1000,$$

289 where  $R_{\text{std}}$  is the molar ratio of the international standard Pee Dee Belemnite (Craig 1957).

290 Architectural and morphological characteristics

291 Architectural traits, morphological characteristics and fruit production were  
292 determined in the same tree-shrub specimens, to assess plasticity along the light gradient. The

293 percentage of terminal shoots with new growth was calculated on each of ten randomly  
294 selected, southeast-oriented branches. The oldest growth unit with at least one apparently  
295 healthy leaf was counted on four of these branches, to estimate relative leaf lifespan. In the  
296 youngest three growth units of the same four branches, mean stem and leaf inclination were  
297 measured relative to the horizontal, using a protractor. Maximum proportional midday light  
298 interception was approximated for south-facing leaves as the cosine of the difference between  
299 the solar declination at solar noon on May 16, 2010 and the mean leaf inclination. Solar  
300 declination was calculated for Puéchabon, using the Iqbal (1983) formulation.

301 One of the four branches of each sampled specimen was severed at the base of the  
302 eighth growth unit, taken to the laboratory, and refrigerated at 4°C. Mean growth unit length  
303 was determined by dividing total branch length by the number of growth units. Leaves were  
304 removed to determine total projected leaf area (TLA) with an area meter (MK2, Delta-T  
305 devices Ltd., Cambridge, UK). The stem diameter of the eighth growth unit was measured  
306 before and after bark removal, to determine stem cross-sectional area and the Huber value  
307 (stem area / TLA). Archimedes' principle was used to determine the volume of a 2 cm-long  
308 stem segment stripped of bark. The volume displaced by the stem was determined using an  
309 analytical balance (Sartorius CP224S, Germany) and density determination kit (Sartorius  
310 YDK01, Germany) to determine the displacement weight, which was divided by the density  
311 of water at the measurement temperature ( $\rho = 0.998 \text{ g cm}^{-3}$  at 20°C) to determine stem segment  
312 volume. Wood density ( $\rho_{\text{wood}}$ ) was determined by dividing the dry weight of the 2-cm stem  
313 segment by its volume,

314 All plant material was oven-dried for 72 hours at 60 °C, and weighed. The branch leaf  
315 area ratio (LAR) was determined in every specimen, by dividing TLA by the total branch dry  
316 weight, including all leaves and stems. Leaf mass fraction (LMF) was calculated by dividing  
317 total leaf dry weight by total branch dry weight. A leaf clumping index (LCI) was defined as

318 the ratio of TLA to total branch length. Lastly, all fruits in the south half of the sampled  
319 individuals were collected, counted and weighed. Total fruit production was expressed on a  
320 dry weight basis.

#### 321 Data analysis

322 Scatterplots were produced with PPFD availability as the independent variable, and 32  
323 physiological and morphological characteristics as dependent variables. To assess the  
324 strength of the resulting relationships, best-fit regression lines and curves were produced, as  
325 appropriate, to determine correlation coefficients ( $R^2$ ) and associated *P*-values. Sample means  
326 and standard errors were also tabulated for all variables in three distinct light regimes: low  
327 light (<30% above-canopy per-cent PPFD; N = 15), intermediate light (30% – 60% PPFD; N  
328 = 12) and high light (>60% PPFD; N = 6). To facilitate further analysis, the full dataset used  
329 in this study is provided as online supplementary material (Supplement I).

330 A standard phenotypic plasticity index (PI; see Valladares et al. 2000b) was calculated  
331 as the difference between the highest and lowest means among the three light treatments,  
332 divided by the highest mean. To account for the full range of plasticity along the continuous  
333 light gradient, a modified PI was also calculated as the difference between the best-fit values  
334 at 10% and 80% PPFD availability, divided by the higher of these two means ( $PI_r$ ). Plasticity  
335 indices were not calculated for  $\delta^{13}C$ , because of its relative scale. We caution that PI is more  
336 useful for meta-analysis than for comparison of plasticity among variables, because of non-  
337 linear responses and among-variable differences in the proportional ranges observed in  
338 response to light availability.

### 339 **Results**

#### 340 Environmental conditions

341 The first four months of 2010 were 66 % wetter than normal, with 447 mm of precipitation  
342 observed between January and April at the Puéchabon Experimental Site. An additional 76

343 mm of rain fell during the photosynthesis measurement period in May. In response to above-  
344 average precipitation, mean soil water content of the 0–15 cm soil layer was  $19.1 \pm 4.5\%$  on  
345 April 27 and  $19.9 \pm 3.4\%$  on May 18, 2010, which is equivalent to 92% field capacity. Daily  
346 mean and maximum air temperature ( $T_a$ ) were  $15.0 \pm 3.4$  °C and  $19.6 \pm 4.7$  °C at Puéchabon  
347 from May 7 - 25, while corresponding values of vapour pressure deficit (VPD) were  $0.7 \pm 0.5$   
348 kPa and  $1.4 \pm 0.8$  kPa. The highest and lowest air temperatures recorded during the study  
349 period were 27.6 °C (May 24) and 5.1 °C (May 19), while the highest ambient VPD recorded  
350 was 2.33 kPa (May 24). As a result of high soil moisture, moderate temperature and low  
351 atmospheric demand, predawn leaf water potential ( $\psi_L$ ) was high in all specimens, though a  
352 trend was observed toward slightly lower values at high light sites (Table 1).

#### 353 Physiological acclimation to light conditions

354 Net PPFD-saturated photosynthesis rates ( $A_{max}$ ) were low relative to other  
355 sclerophyllous evergreens of the Mediterranean (Gulías et al. 2011) and increased slightly  
356 with light availability on an area basis (Figure 1), but not on a mass basis ( $R^2 = 0.01$ ,  $P =$   
357 0.60). Dark respiration ( $R_d$ ) and  $R_p$  were also very low, but increased sharply with light  
358 availability (Figure 1), so that LCP was highest at open sites (Table 1). Maximum electron  
359 transport rate ( $ETR_{max}$ ), the fraction of open PSII reaction centres ( $q_L$ ) and non-photochemical  
360 quenching (NPQ) each increased with light availability (Figure 1). No change was observed  
361 in the dark-adapted maximum quantum yield of Photosystem II ( $F_v/F_m$ ; Table 1), but PPFD-  
362 saturated  $\phi_{qL}$  ( $\phi_{qL\ sat}$ ) decreased linearly across the light gradient (Figure 1). Leaf N increased  
363 with light availability on an area basis, while chlorophyll content decreased (Table 1).

364 Water-use efficiency ( $WUE = A_{max}/E$ ) did not differ significantly among low ( $5.9 \pm$   
365  $0.3$  mmol mol<sup>-1</sup>), intermediate ( $6.0 \pm 0.5$  mmol mol<sup>-1</sup>) and high ( $6.5 \pm 0.7$  mmol mol<sup>-1</sup>) light  
366 sites in May 2010 ( $P = 0.63$ ). However, stable carbon isotope composition ( $\delta^{13}C$ ) increased

367 with both PPFD availability ( $R^2 = 0.42$ ;  $P < 0.0001$ ) and leaf mass per unit area (LMA;  $R^2 =$   
368  $0.49$ ,  $P < 0.0001$ ; Figure 2).

#### 369 Morphological acclimation to light conditions

370 Morphological changes were observed at the leaf and branch scale in response to  
371 increasing light availability. At the leaf scale, LMA increased linearly, while leaf inclination  
372 angles increased logarithmically with light availability (Figure 3), resulting in lower  
373 maximum light interception (Table 2). Branch inclination also increased, rising linearly from  
374 near  $0^\circ$  at low light sites, to about  $40^\circ$  in high light. The leaf clumping index (LCI) doubled  
375 from the low to high extremes of the light gradient, while branch LAR decreased (Figure 3).  
376 Whereas factors directly related to light capture efficiency differed significantly along the  
377 light gradient, other morphological variables showed little or no change. Mean dry wood  
378 density ( $\rho_{\text{wood}}$ ) remained near 0.85 throughout the light gradient. The number of successive  
379 growth units with green leaves decreased with PPFD exposure, but there was a tendency for a  
380 greater percentage of shoots to exhibit new (spring 2010) growth (Figure 3). No significant  
381 differences in fruit size or production were observed across the PPFD gradient (Table 2).

#### 382 Mass-based leaf nitrogen and photosynthesis

383 Relationships among mass and area-based measures of leaf nitrogen and  
384 photosynthesis reflect the combined effect of physiological and morphological acclimation to  
385 light availability. Mass-based leaf nitrogen content ( $N_{\text{mass}}$ ) was weakly correlated with LMA  
386 (Figure 4). Consequently, area-based leaf nitrogen content ( $N_{\text{area}}$ ) increased in proportion  
387 with LMA. Consistent with the weakly positive effect of light availability on  $A_{\text{max}}$  (Figure 1)  
388 and the strong correlation between LMA and light availability (Figure 3), the relationship  
389 between  $A_{\text{max}}$  and LMA was significant on an area basis ( $R^2 = 0.13$ ,  $P = 0.04$ ), but not on a  
390 mass basis ( $R^2 = 0.05$ ,  $P = 0.22$ ; Figure 4).

#### 391 Phenotypic plasticity



392 Physiological acclimation to irradiance was observed in  $A_{\max}$ ,  $R_d$ ,  $R_p$ , LCP,  $ETR_{\max}$ ,  
393  $q_{L\text{sat}}$ , NPQ and  $\phi q_{L\text{sat}}$ . The phenotypic plasticity indices (PI and  $PI_r$ ) were 0.46 - 0.71 in the  
394 respiration-related variables, 0.34 - 0.45 in those related to photosynthetic capacity ( $ETR_{\max}$ ,  
395  $q_{L\text{sat}}$  and  $\phi q_{L\text{sat}}$ ) and 0.36 for non-photochemical quenching (NPQ; Table 1). Light plasticity  
396 was comparatively low for leaf gas-exchange ( $E$ ,  $g_s$  and  $A_{\max}$ ), with no significant effect of  
397 %PPFD on  $E$ ,  $g_s$  or mass-based  $A_{\max}$ . Low plasticity was observed for leaf  $N_{\text{area}}$  and  
398 chlorophyll content (0.14 - 0.24), while  $N_{\text{mass}}$  showed no difference among light classes.  
399 Strong morphological acclimation to irradiance was observed, with increasing branch  
400 inclination, leaf inclination, leaf clumping and LMA, but lower branch LAR and maximum  
401 light interception. The number of successive growth units with green foliage decreased with  
402 %PPFD availability, while the percentage of shoots with new growth increased (PI = 0.16 -  
403 0.34; Table 2).

#### 404 Discussion

405 Phenotypic plasticity can be constrained by stress adaptations that confer tolerance of limiting  
406 resources (Chapin 1993; Lortie and Aarsen 1996). Given that *B. sempervirens* is a  
407 sclerophyllous, woody evergreen tree-shrub known for its tolerance of severe drought  
408 (García-Plazaola et al. 2008), it was hypothesized that only limited leaf physiological  
409 plasticity would be observed across a light gradient. Despite the conservative resource-use  
410 strategy of this species, phenotypic plasticity was observed in a number of leaf photochemical  
411 and morphological variables that can influence photosynthetic gas-exchange rates. However,  
412 light regime had only modest impact on  $A_{\max}$  (area), and no significant impact on  $A_{\max}$  (mass),  
413  $g_s$ , or  $E$ . The lack of light plasticity observed in photosynthetic gas-exchange characteristics  
414 was the result of a co-ordinated acclimatory response at different levels of organization,  
415 including leaf physiology, leaf and branch morphology and leaf chemistry. Long-term

Commented [HAC1]: Need to discuss potential causes of low  $F_v/F_m$ .

416 acclimation to high irradiance was achieved through (i) less effective light interception, (ii)  
417 higher LMA, and (iii) higher potential for light energy dissipation.

418 Co-ordination of physiological and morphological light acclimation

419 Self-shading is a key mechanism used to reduce light capture efficiency (Pearcy et al.  
420 2005). Our data suggest that this was achieved at the canopy scale in high light individuals of  
421 *B. sempervirens*, through high branch inclination and leaf clumping, which increase the ratio  
422 of leaf area to silhouette area (Domingo et al. 2000). Higher leaf inclination causes light to be  
423 received more obliquely at low solar zenith angles. This serves a photoprotective role, by  
424 reducing the intensity of intercepted solar radiation, but at the expense of potential carbon  
425 gain (Falster and Westoby 2003). In *B. sempervirens*, leaf inclination was  $48.7^\circ \pm 25.3^\circ$   
426 (mean  $\pm$  standard deviation), with more vertically-oriented leaves in high light than in low  
427 light environments causing lower proportional light interception at high light sites near  
428 midday (Table 2). Similar leaf inclination has been observed in a variety of Mediterranean  
429 evergreen sclerophylls, with vertically-oriented leaves showing higher  $F_v/F_m$  than horizontal  
430 leaves during the summer months (Werner et al. 2001).

431 The invariability of  $F_v/F_m$  across the light gradient suggests that *B. sempervirens*  
432 adjusts its branch and leaf morphology to alter light capture efficiency, thus preventing crown  
433 photoinhibition in high light environments, but increasing light capture in understory sites. In  
434 a winter study of ten Mediterranean evergreens in the Basque region of Spain, García-  
435 Plazaola et al. (2003) found that *B. sempervirens* had much lower  $F_v/F_m$  ( $0.63 \pm 0.01$ ) than all  
436 other species during a mild winter period of 1999, but higher  $F_v/F_m$  ( $0.66 \pm 0.03$ ) than all  
437 other species following an episode of intense cold in 2001. The low  $F_v/F_m$  observed in this  
438 study during spring ( $0.69 \pm 0.01$ ) is consistent with what García-Plazaola et al. (2003)  
439 describe as a high 'constitutive level of photoprotection,' and suggests slow and limited  
440 relaxation of photoprotection in response to cold and irradiance stress.

441 At sites of high light availability, higher LMA may cause mass gain to be diverted  
442 away from photosynthetic enzymes and toward structural investments, thus reducing the  
443 efficiency of light use by the chloroplasts (Niinemets and Valladares 2004). At Puéchabon,  
444 the LMA of *B. sempervirens* leaves increased from 150 to 250 g cm<sup>-2</sup> from low to high light  
445 sites, with only a slight increase in  $A_{\max}$  (area). Given that increasing LMA resulted in larger  
446 increases in leaf  $N_{\text{area}}$  than  $A_{\max}$  (area), while chlorophyll content decreased, it is possible that  
447 a substantial fraction of leaf nitrogen may be present in cell wall proteins of the  
448 sclerophyllous foliage, found at high light sites (see Loomis 1997). We further speculate that  
449 low mesophyll conductance ( $g_m$ ) might have limited  $A_{\max}$  in leaves acclimated to high light  
450 intensity. Although greater leaf thickness, as observed in high light plants, tends to increase  
451 LMA and  $A_{\max}$  without restricting  $g_m$ , higher leaf density can reduce internal conductance of  
452 CO<sub>2</sub>, lower its concentration within the chloroplast and increase the photorespiration rate,  
453 thereby reducing  $A_{\max}$  (Niinemets and Kull 1998; Mediavilla et al. 2001; Niinemets and Kull  
454 2001, Limousin et al. 2010). Along with differences in antecedent drought exposure during  
455 leaf development, a negative relationship between  $g_m$  and light availability could help explain  
456 why higher foliar  $\delta^{13}\text{C}$  was observed in high light plants (Figure 2), even though WUE  
457 ( $A_{\max}/E$ ) was not significantly higher. To address these questions, there is a need for further  
458 leaf physiological and morphological measurement during drought, including an assessment  
459 of the impact of light availability, leaf thickness and leaf density on mesophyll conductance.

460 To balance light input and utilization, leaves of high light specimens of *B.*  
461 *sempervirens* exhibited lower chlorophyll concentration and a higher capacity for light energy  
462 dissipation. Non-photochemical quenching (NPQ),  $R_d$  and  $R_p$  increased with light availability  
463 (Table 1). The exponential increase in dark respiration resulted in a 161% increase in  
464  $R_d/A_{\max}$ , from sites of low light ( $5.9 \pm 0.7$  %) to high light availability ( $15.4 \pm 3.5$  %;  $P=$   
465 0.001). These acclimatory changes resulted in a higher fraction of open PSII reaction centres

466 ( $q_{L, sat}$ ) in leaves exposed to high light, which can prevent photoinhibition (Ögren and  
467 Rosenqvist 1992). Effects of long-term exposure to contrasting light regimes on  $R_d$  and  $R_p$   
468 may explain the greater light plasticity of  $ETR_{max}$  relative to  $A_{max}$  (Table 1).

469 *Buxus sempervirens* is often a dominant understory tree-shrub in forest understory,  
470 edge, canopy gap and full sun environments. High plasticity might be expected in species  
471 that are prevalent in contrasting light regimes, as observed for several morphological (eg.  
472 branch inclination, leaf inclination and leaf clumping) and physiological variables (eg.  $q_L, R_d$ ,  
473 LCP and  $R_p$ ) in this study. However, this coordinated response resulted in low plasticity of  
474 photosynthetic gas-exchange across the light availability gradient. The ability of *B.*  
475 *sempervirens* to thrive in full sun, despite low  $A_{max}$ , is likely explained by the absence of  
476 strong interspecific competition and by its high drought tolerance.

#### 477 Light plasticity in drought-prone ecosystems

478 When assessing long-term acclimation to environmental stressors in a natural  
479 environment, it is important to consider that multiple stressors operate simultaneously in  
480 resource-limited environments. In summer-dry forest ecosystems of the Mediterranean,  
481 drought is common. The tree-shrubs examined in this study are acclimated both to long-term  
482 exposure to distinct light regimes and to frequent drought stress, which can be severe in both  
483 high light and understory environments (Rodríguez-Calcerrada et al. 2008a). *B. sempervirens*  
484 displayed a number of physiological, morphological and architectural traits that serve to  
485 enhance drought tolerance, including high LMA, WUE and  $\rho_{wood}$ , as well as low  $A_{max}$ ,  $g_s$  and  
486 shoot growth increment. These characteristics also imply low resource use and turnover rates.  
487 For example, high LMA and  $\rho_{wood}$  reduce leaf and stem respiration, respectively (Larjavaara  
488 and Muller-Landau 2010). High  $\rho_{wood}$  also decreases xylem cavitation vulnerability, which is  
489 of critical importance to long-term survival in drought-prone ecosystems (Hacke et al. 2000).

490 It has been suggested that there are both physiological and morphological trade-offs  
491 that prevent effective and simultaneous adaptation to irradiance and water stress (Smith and  
492 Huston 1989). For example, at the low end of the PPFD availability gradient, shade plants  
493 tend to maximize leaf display to ensure effective light capture, but this reduces drought  
494 tolerance (Aranda et al. 2005). There are other adaptations, however, such as high wood  
495 density, that clearly serve to promote both shade and drought tolerance. At the high end of  
496 the PPFD availability gradient, leaves that are well-adapted to withstand drought tend to  
497 possess a greater fraction of non-assimilative tissues to help resist collapse when exposed to  
498 strongly negative leaf water potentials (Oertli et al. 1990), and *B. sempervirens* is known to  
499 withstand low leaf water potential without embolism (Aussenac and Valette 1982).  
500 Allocation of mass gain to non-assimilative tissues can reduce photosynthetic capacity  
501 (Niinemets and Valladares 2004), illustrating an important trade-off in terms of simultaneous  
502 acclimation to irradiance and drought. In the case of *B. sempervirens*, successful occupation  
503 of both low and high light sites in a drought-prone ecosystem required (i) coordinated  
504 physiological and morphological acclimation in selected traits and (ii) the absence of strong  
505 competition in high light.

506 Chapin (1993) suggested that natural selection for traits promoting tolerance to  
507 stressors in low resource environments can produce a “stress resistance syndrome,” in which  
508 productivity remains low in resource-limited environments, to avoid excessive growth or  
509 resource allocation to leaf tissues, even during temporary periods of favourable environmental  
510 conditions. Our results support the hypothesis that *B. sempervirens* exhibits this sort of  
511 obligate conservatism. Very low  $A_{\max}$ ,  $g_s$  and  $E$  were observed, even in specimens acclimated  
512 to full sun and presented with optimal temperature and moisture availability. This likely  
513 reflects the trade-off between light and drought acclimation in a woody evergreen species that  
514 is limited in its capacity for rapid resource use, due to the natural selection of traits conferring

515 stress tolerance. However, the hypothesis that morphological plasticity would exceed  
516 physiological plasticity is not fully supported by our findings. Instead, numerous  
517 physiological and morphological variables showed strong acclimation to changing light  
518 conditions, which were compensatory in terms of photosynthetic gas-exchange. Additional  
519 research is needed to elucidate the complex relationships between drought and light plasticity  
520 in species occupying wide fractions of the light availability gradient

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754 *Oecologica* 22:129-138

755 Table 1. Means, standard errors and plasticity indices (PI) of *Buxus sempervirens* L. physiological characteristics in low (0 - 30 % PPFD; N =  
756 15), intermediate (30 - 60 % PPFD; N = 12) and high light (60 - 90 % PPFD; N = 6) sites at Puéchabon. A second plasticity index is  
757 based on best-fit regression values at 10% vs. 80% PPFD availability (PI<sub>r</sub>), and is shown in bold for variables exhibiting regression  
758 slopes differing significantly from 0 at  $P < 0.05$ . Abbreviations:  $\psi_L$  = leaf water potential;  $A_{\max}$  = net photosynthesis at light saturation;  
759  $R_d$  = dark respiration,  $\alpha$  = apparent quantum yield;  $R_p$  = photorespiration; LCP = light compensation point;  $g_s$  = stomatal conductance;  
760  $ETR_{\max}$  = light-saturated electron transport rate;  $q_{L\ sat}$  = fraction of open PSII reaction centres at light saturation;  $NPQ$  = non-  
761 photochemical quenching;  $\phi_{q_{L\ sat}}$  = efficiency of open reaction centres at light saturation;  $F_v/F_m$  = maximum quantum yield of PSII;  
762  $\delta^{13}C$  = stable carbon isotope composition.  
763

Category	Variable	Means $\pm$ standard error			PI	PI <sub>r</sub>	R <sup>2</sup>	P
		Low light	Intermediate	High light				
Leaf water status	$\psi_L$ (MPa)	-0.64 $\pm$ 0.02	-0.68 $\pm$ 0.02	-0.73 $\pm$ 0.02				
Leaf gas-exchange	$A_{\max}$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	4.1 $\pm$ 0.3	4.6 $\pm$ 0.3	5.1 $\pm$ 0.5	0.20	<b>0.26</b>	<b>0.16</b>	<b>0.02</b>
	$A_{\max}$ ( $\text{nmol g}^{-1} \text{s}^{-1}$ )	25.6 $\pm$ 1.6	23.1 $\pm$ 1.6	24.1 $\pm$ 2.4	0.10	0.07	0.01	0.60
	$g_s$ ( $\text{mmol m}^{-2} \text{s}^{-1}$ )	41.1 $\pm$ 3.7	45.1 $\pm$ 5.3	52.7 $\pm$ 7.5	0.22	0.39	0.06	0.15
	$E$ ( $\text{mmol m}^{-2} \text{s}^{-1}$ )	0.73 $\pm$ 0.05	0.82 $\pm$ 0.09	0.89 $\pm$ 0.16	0.18	0.22	0.03	0.33
	$R_d$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	0.22 $\pm$ 0.02	0.39 $\pm$ 0.06	0.76 $\pm$ 0.16	0.71	<b>0.70</b>	<b>0.42</b>	<b>&lt;0.0001</b>
	$R_d$ ( $\text{nmol g}^{-1} \text{s}^{-1}$ )	1.41 $\pm$ 0.12	1.95 $\pm$ 0.25	3.56 $\pm$ 0.81	0.61	<b>0.54</b>	<b>0.24</b>	<b>0.004</b>
	$\alpha$ ( $\text{g mol}^{-1}$ )	0.28 $\pm$ 0.02	0.31 $\pm$ 0.04	0.27 $\pm$ 0.03	0.12	0.10	0.01	0.51
	$R_p$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	1.94 $\pm$ 0.23	2.50 $\pm$ 0.21	3.59 $\pm$ 0.53	0.46	<b>0.57</b>	<b>0.28</b>	<b>0.002</b>
Leaf photochemistry	LCP ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	8.7 $\pm$ 1.1	12.6 $\pm$ 2.3	23.5 $\pm$ 3.8	0.63	<b>0.66</b>	<b>0.37</b>	<b>0.0002</b>
	$ETR_{\max}$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	40.8 $\pm$ 2.7	50.2 $\pm$ 2.4	67.0 $\pm$ 7.9	0.38	<b>0.45</b>	<b>0.39</b>	<b>0.0001</b>
	$q_{L\ sat}$	0.16 $\pm$ 0.01	0.18 $\pm$ 0.01	0.27 $\pm$ 0.04	0.42	<b>0.45</b>	<b>0.29</b>	<b>0.001</b>
	NPQ	1.96 $\pm$ 0.07	2.03 $\pm$ 0.06	3.08 $\pm$ 0.24	0.36	<b>0.36</b>	<b>0.39</b>	<b>0.0001</b>
	$\phi_{q_{L\ sat}}$ *	0.45 $\pm$ 0.02	0.39 $\pm$ 0.01	0.32 $\pm$ 0.03	0.30	<b>0.34</b>	<b>0.47</b>	<b>&lt;0.0001</b>
Leaf chemistry	$F_v/F_m$ dark	0.69 $\pm$ 0.01	0.68 $\pm$ 0.01	0.69 $\pm$ 0.01	0.01	0.03	0.03	0.31
	leaf N ( $\text{mg g}^{-1}$ )	15.6 $\pm$ 0.42	14.8 $\pm$ 0.64	14.2 $\pm$ 0.73	0.09	0.11	0.09	0.10
	leaf N ( $\text{g m}^{-2}$ )	2.51 $\pm$ 0.11	2.97 $\pm$ 0.15	3.07 $\pm$ 0.21	0.18	<b>0.24</b>	<b>0.24</b>	<b>0.004</b>
	chlorophyll (SPAD units)**	72.5 $\pm$ 1.0	70.1 $\pm$ 1.9	62.2 $\pm$ 4.5	0.14	<b>0.14</b>	<b>0.19</b>	<b>0.01</b>
Isotopic composition	leaf $\delta^{13}C$ (‰)	-29.0 $\pm$ 0.2	-27.6 $\pm$ 0.3	-27.3 $\pm$ 0.5			<b>0.42</b>	<b>&lt;0.0001</b>

764 \* one intermediate light outlier removed; \*\* one missing value

765



766 Table 2. Means, standard errors and plasticity indices (PI) of *Buxus sempervirens* L. morphological characteristics in low (0 - 30 % PPFD; N =  
767 15), intermediate (30 - 60 % PPFD; N = 12) and high light (60 - 90 % PPFD; N = 6) sites at Puéchabon. A second plasticity index is based on  
768 best-fit regression values at 10% and 80% PPFD availability, and is shown in bold for variables exhibiting regression slopes differing  
769 significantly from 0 at  $P < 0.05$ . Maximum light interception refers to the theoretical maximum intensity of spectral light that would be  
770 absorbed by south-facing leaves at solar noon, relative to a leaf plane oriented perpendicular to the sun. A forced-origin regression line was used  
771 to calculate the  $PI_r$  for branch inclination. Abbreviations: LMA = leaf mass per unit area; LAR = branch leaf area ratio; LMF = leaf mass  
772 fraction; and  $\rho_{\text{wood}}$  = wood density. \*Measures of reproductive allocation refer to the dry weight of fruit present on the south half of the tree-shrub  
773 canopy on the date of measurement, and do not account for herbivory and senescence. Mean fruit mass was calculated only for shrubs with fruit  
774 present, including ten low, seven intermediate and six high light shrubs.  
775

Category	Variable	Means $\pm$ standard error			PI	$PI_r$	$R^2$	$P$
		Low light	Intermediate	High light				
Leaf morphology	LMA ( $\text{g m}^{-2}$ )	161 $\pm$ 5	202 $\pm$ 7	215 $\pm$ 6	0.25	<b>0.32</b>	<b>0.61</b>	<b>&lt;0.0001</b>
	leaf inclination ( $^\circ$ )	38 $\pm$ 6	47 $\pm$ 6	73 $\pm$ 6	0.48	<b>0.48</b>	<b>0.33</b>	<b>0.0005</b>
	max. light intercept. (12h00, %)	87 $\pm$ 3	83 $\pm$ 4	58 $\pm$ 9	0.34	<b>0.26</b>	<b>0.34</b>	<b>0.0004</b>
Branch morphology	branch inclination ( $^\circ$ )	0.2 $\pm$ 3.2	13.6 $\pm$ 4.4	39.4 $\pm$ 8.2	1.00	<b>0.88</b>	<b>0.41</b>	<b>&lt;0.0001</b>
	branch LAR ( $\text{cm}^2 \text{g}^{-1}$ )	42.4 $\pm$ 1.9	38.6 $\pm$ 1.7	36.1 $\pm$ 2.2	0.15	<b>0.23</b>	<b>0.23</b>	<b>0.005</b>
	LMF ( $\text{g g}^{-1}$ )	0.67 $\pm$ 0.02	0.71 $\pm$ 0.01	0.75 $\pm$ 0.02	0.09	0.08	0.07	0.14
	Huber ( $\text{cm}^2 \text{m}^{-2}$ )	2.40 $\pm$ 0.18	2.33 $\pm$ 0.13	2.31 $\pm$ 0.25	0.04	0.03	0.00	0.78
	leaf clumping ( $\text{cm}^2 \text{cm}^{-1}$ )	6.4 $\pm$ 0.4	9.7 $\pm$ 1.4	12.3 $\pm$ 1.8	0.48	<b>0.51</b>	<b>0.26</b>	<b>0.002</b>
	# growth units with foliage	6.4 $\pm$ 0.3	5.5 $\pm$ 0.4	5.3 $\pm$ 0.5	0.16	<b>0.24</b>	<b>0.14</b>	<b>0.03</b>
	growth unit length (cm)	3.98 $\pm$ 0.28	3.81 $\pm$ 0.32	2.81 $\pm$ 0.16	0.29	0.24	0.08	0.10
	% shoots with new growth	52 $\pm$ 8	63 $\pm$ 7	78 $\pm$ 11	0.34	<b>0.34</b>	<b>0.15</b>	<b>0.03</b>
$\rho_{\text{wood}}$ ( $\text{g cm}^{-3}$ )	0.85 $\pm$ 0.01	0.85 $\pm$ 0.01	0.84 $\pm$ 0.02	0.01	0.01	0.00	0.75	
Reproductive allocation*	fruit mass (mg, south)	104 $\pm$ 7	102 $\pm$ 11	96 $\pm$ 14	0.12	0.11	0.02	0.50
	fruit production (mg, south)	414 $\pm$ 182	584 $\pm$ 243	489 $\pm$ 183	0.29	0.35	0.01	0.57

776 Figure captions

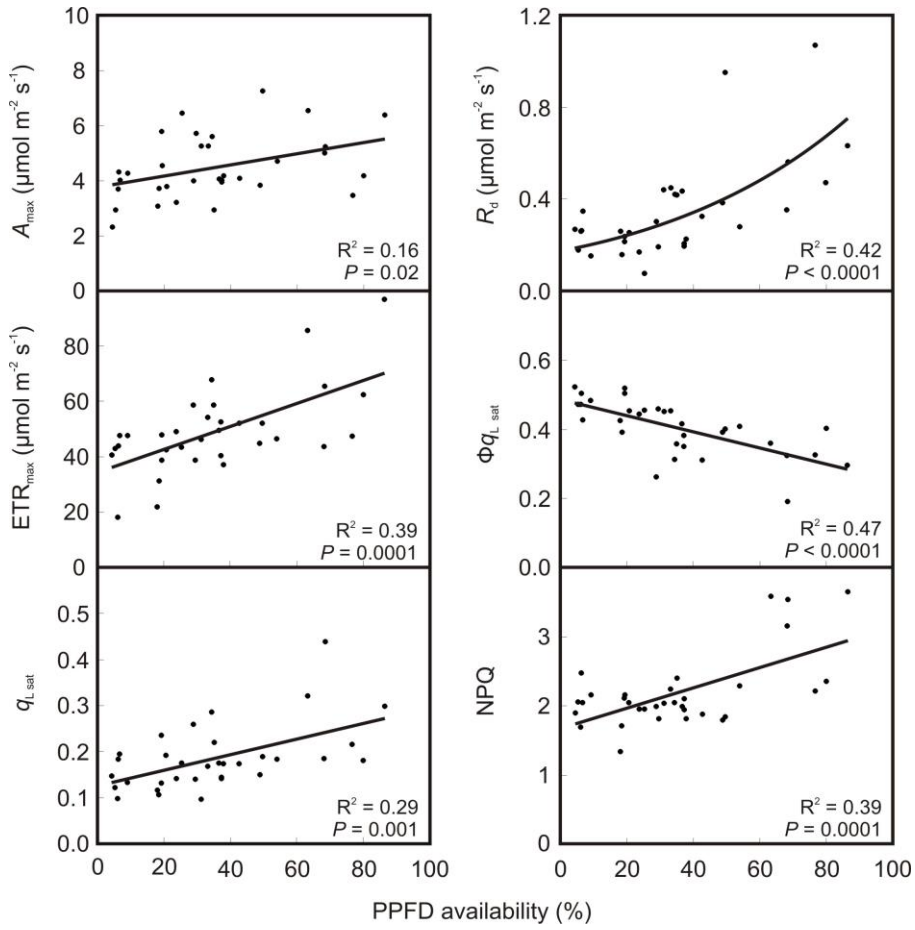
777 Figure 1. Relationships between per-cent PPFD availability and leaf physiological  
778 characteristics on *B. sempervirens*, including net photosynthesis rate at light saturation ( $A_{\max}$ ),  
779 dark respiration rate ( $R_d$ ), maximum photosynthetic electron transport rate ( $ETR_{\max}$ ), quantum  
780 efficiency of open Photosystem II (PSII) reaction centres at a saturating PPFD of  $1500 \mu\text{mol}$   
781  $\text{m}^{-2} \text{s}^{-1}$  ( $\phi q_{L \text{ sat}}$ ), fraction of open PSII centres ( $q_{L \text{ sat}}$ ), and non-photochemical quenching  
782 (NPQ).

783 Figure 2. Stable carbon isotopic composition of *B. sempervirens* leaves ( $\delta^{13}\text{C}$ ), as a function  
784 of leaf dry mass per unit area (LMA), in shrubs exposed to low light (0 - 30% PPFD),  
785 intermediate light (30 - 60% PPFD) and high light (PPFD > 60%).

786 Figure 3. Relationships between per-cent photosynthetic photon flux (PPFD) availability and  
787 morphological characteristics of *B. sempervirens*, including leaf mass per unit area (LMA),  
788 branch inclination, leaf inclination, leaf clumping index, branch leaf area ratio (LAR) and the  
789 percentage of shoots with new growth.

790 Figure 4. Relationships between leaf mass per unit area (LMA) and area and mass-based leaf  
791 nitrogen content ( $N_{\text{area}}$  and  $N_{\text{mass}}$ ) and net photosynthesis rates ( $A_{\max}$ ).

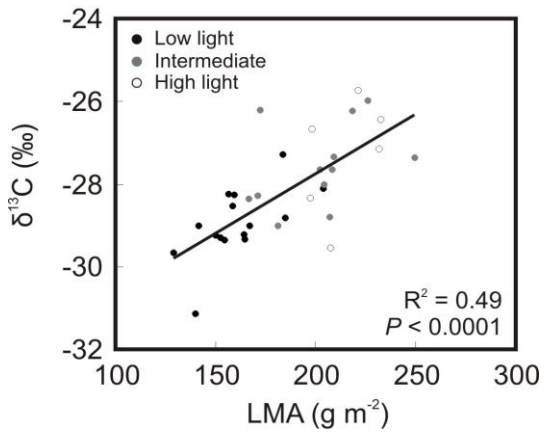
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794 Figure 1.

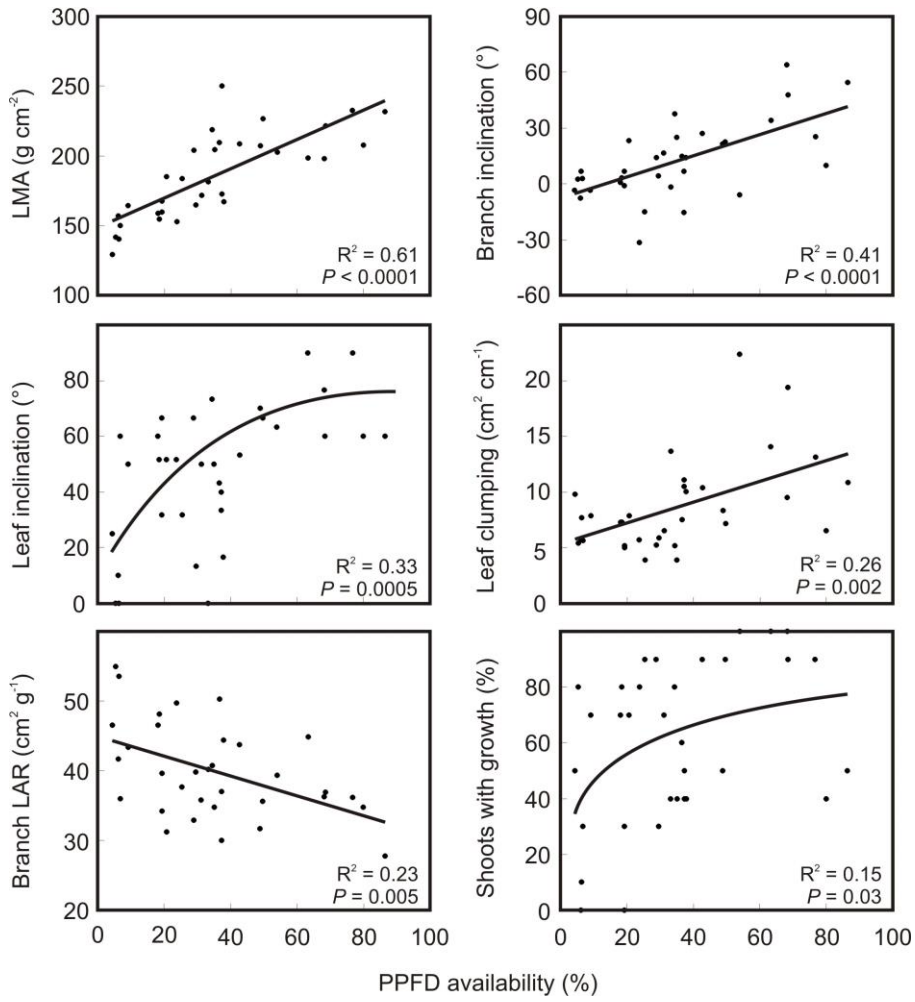
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797 Figure 2.

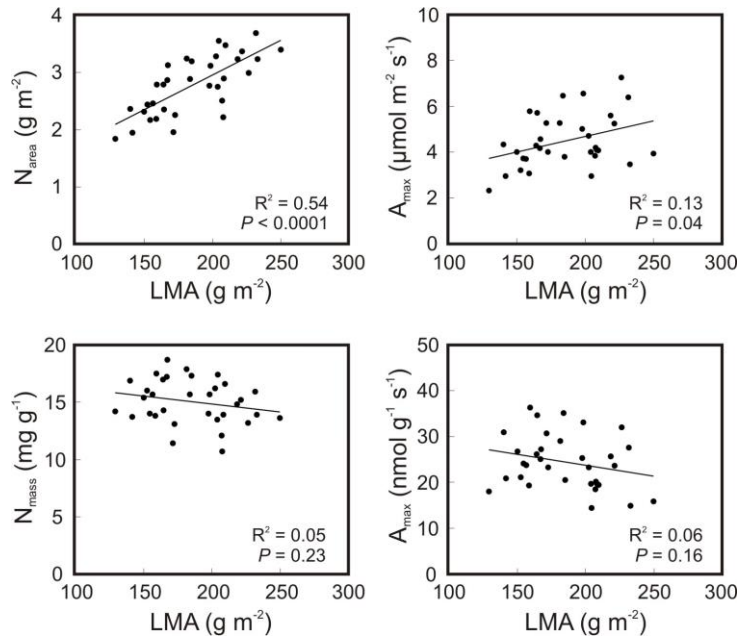
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800 Figure 3.

801



802

803 Figure 4.