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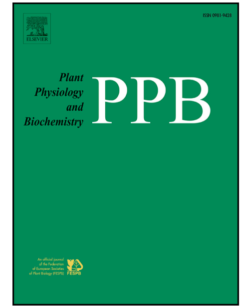

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Leaf biochemical adjustments in two Mediterranean resprouter species facing enhanced UV levels and reduced water availability before and after aerial biomass removal

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25 **Abstract**

26 Effects of supplemented UV radiation and diminished water supply on the leaf concentrations
27 of phenols and antioxidants of two Mediterranean resprouter species, *Arbutus unedo* and
28 *Quercus suber*, were assessed before and after entire aerial biomass removal. Potted seedlings
29 of both species were grown outdoors for 8 months with enhanced UV-A+UV-B, enhanced
30 UV-A or ambient UV, in combination with two watering conditions (field capacity or
31 watering reduction). After this period, all aerial biomass was removed and new shoots
32 (resprouts) developed for a further 8 months under the two treatments. In general, the
33 investment in leaf phenols was substantially greater in *A. unedo* than in *Q. suber*, while *Q.*
34 *suber* allocated more resources to non-phenolic antioxidants (ascorbate and glutathione). In
35 response to enhanced UV-B radiation, *Q. suber* leaves rose their UV-screening capacity
36 mainly via accumulation of kaempferols, accompanied by an increased concentration of
37 rutins, being these effects exacerbated under low-watering conditions. Conversely, *A. unedo*
38 leaves responded to UV-B radiation reinforcing the antioxidant machinery by increasing the
39 overall amount of flavonols (especially quercetins) in seedlings, and of ascorbate and
40 glutathione, along with catalase activity, in resprouts. Nevertheless, UV effects on the
41 amount/activity of non-phenolic antioxidants of *A. unedo* resprouts were modulated by water
42 supply. Indeed, the highest concentration of glutathione was found under the combination of
43 enhanced UV-B radiation and reduced watering, suggesting an enlargement of the antioxidant
44 response in *A. unedo* resprouts. Different biochemical responses to enhanced UV and drier
45 conditions in seedlings and resprouts of these two species might modulate their competitive
46 interactions in the near future.

47

48 **Key words**: Mediterranean resprouter species, ultraviolet (UV) radiation, rainfall reduction,
49 disturbance, phenolic compounds, antioxidants.

50

51 **INTRODUCTION**

52 In the Mediterranean basin, higher levels of solar ultraviolet radiation (UV; 280-400 nm)
53 reaching terrestrial ecosystems are predicted to occur in the next decades owing to decreases
54 in the cloudiness associated with climate change (IPCC, 2013; Sanchez-Lorenzo et al., 2017;
55 UNEP, 2016). Reduced cloudiness is expected to also modify the precipitation regime
56 resulting in longer dry periods over the coming years (IPCC, 2013). Higher UV levels
57 coupled with lower water supply are likely to affect Mediterranean vegetation. In the case of
58 sclerophyllous woody plants, these effects may be particularly relevant due to their high
59 abundance in Mediterranean shrublands, one of the most extensive terrestrial communities in
60 Europe and particularly in the Iberian Peninsula (Acácio et al., 2009; Arnan et al., 2013).

61 UV radiation (UV-B, 280-315 nm; UV-A, 315-400 nm) represents a small fraction of
62 the solar spectrum reaching the ground surface. Nevertheless, enhanced plant exposure to UV
63 can stimulate the generation of reactive oxygen species (ROS), which are able to induce
64 oxidative damage to DNA and other cell compounds, affecting negatively the development of
65 the whole organism (Caldwell et al., 2007; Jansen et al., 1998). In leaves, ROS production
66 takes place mainly in the reaction centers of photosystem I and II in chloroplast thylakoids,
67 and this production increases when light energy is absorbed above the capacity of
68 photosynthetic and photoprotective mechanisms (Asada, 2006). One of these photoprotective
69 mechanisms would be the biochemical changes associated with the production of secondary
70 metabolites (A-H-Mackerness, 2000; Bussotti et al., 2014; Jansen et al., 2012).

71 The first line of protection against UV radiation at biochemical level consists of
72 minimizing UV exposure by means of UV-induced accumulation of phenylpropanoid
73 compounds in superficial plant tissues. The phenylpropanoid pathway is responsible for the
74 synthesis of phenolics, such as tannins and flavonoids, that are abundant in the walls and
75 vacuoles of epidermal cells, in the cuticle and epicuticular materials, and in other external

76 surfaces, such as leaf hairs (Agati et al., 2013; Paoletti, 2005). Among other functions,
77 phenolic compounds contribute to screen out the most energetic solar wavelengths reaching
78 the leaf, reducing its penetration into the tissues (Caldwell et al., 2007; Julkunen-Tiitto et al.,
79 2005; Li et al., 2010). Some flavonoids can also act as antioxidant compounds. Indeed, while
80 the function of UV screening is mainly attributed to monohydroxy B-ring substituted
81 flavonoids (e.g. kaempferols), those having a catechol group in the B-ring of the flavonoid
82 skeleton (dihydroxy B-ring substituted flavonoids), such as quercetins, show effective
83 antioxidant properties (Agati and Tattini, 2010; Hideg et al., 2013; Tegelberg and Julkunen-
84 Tiitto, 2001). Dihydroxy B-ring substituted flavonoids are confined near or within the sites of
85 ROS production, such as chloroplasts (Agati and Tattini, 2010). Hence, flavonoids can inhibit
86 the generation of ROS and/or reduce ROS once formed, being also part of the second line of
87 defense against UV radiation (A-H-Mackerness, 2000; Agati et al., 2012).

88 In addition to phenolic compounds, other molecules such as ascorbate and glutathione
89 can counteract the toxic effects of ROS (Lidon et al., 2012; Ueda and Nakamura, 2011). Both
90 compounds are essential in the detoxification of superoxide radicals and hydrogen peroxide
91 through the ascorbate-glutathione cycle (Foyer and Noctor, 2011). Enzymatic antioxidant
92 systems are also crucial to reduce ROS. For instance, catalases (CAT) have extremely high
93 turnover rates, being indispensable for ROS detoxification during stress conditions (Gill and
94 Tuteja, 2010; Mittler, 2002). Ascorbate peroxidase (APX) enzymes use ascorbate as the
95 electron donor, being also essential in the scavenging of H_2O_2 in water-water and ascorbate-
96 glutathione cycles (Ahmad et al., 2010; Asada, 2006). Thus, plants often respond to UV
97 oxidative stress by an upregulation of enzymatic antioxidant activities coupled with increases
98 in both the reduction state and pool-size of key antioxidants (i.e. ascorbate and glutathione)
99 (Agarwal, 2007; Jansen et al., 2012).

100 Apart from UV radiation, other abiotic stresses, such as water deficit, can alter the

101 equilibrium between the generation and the scavenging of ROS, inducing the oxidative
102 detoxification machinery (Reddy et al., 2004; Selmar and Kleinwächter, 2013). In plants
103 under water constraints, an accumulation of phenolics, particularly flavonoids with potential
104 antioxidant properties, has been described (Caldwell et al., 2007; Hofmann et al., 2003). For
105 this reason, drought-induced changes in plant biochemistry can also modify plant tolerance to
106 enhanced UV levels and vice versa (Agati et al., 2012; Bandurska et al., 2013). Taking into
107 account the role of flavonoids in the secondary cell wall thickening (Agati et al., 2012), the
108 UV-induced flavonoid increase may mechanically strengthen the tissues, which, along with
109 chemical-related functions, could improve water-stress tolerance (Di Ferdinando et al., 2014).
110 In other cases, UV radiation, especially UV-B, and low water supply have been found to
111 interact synergistically increasing the concentration of leaf phenols (Caldwell et al., 2007;
112 Hofmann et al., 2003) or even affecting differentially specific phenolic compounds (Turtola et
113 al., 2005).

114 Mediterranean plant species have to face high solar irradiance together with other
115 environmental stresses, such as water deficit, especially during summer (Bussotti et al., 2014).
116 However, currently available evidences on possible interactions between these environmental
117 factors are scarce. In the few studies conducted in Mediterranean species, an interactive effect
118 between UV and water availability levels on plant biochemical parameters, particularly
119 phenolic compounds, were not found (Bussotti et al., 2014; Paoletti, 2005), although species-
120 specific UV effects have been reported (Bernal et al., 2013; Grammatikopoulos et al., 1998).
121 In a study with six Mediterranean species, despite there was no general UV effect on the leaf
122 total concentration of phenols, the leaf phenolic composition varied in response to UV
123 exposure in *Pistacia lentiscus* (Bernal et al., 2013). In *Arbutus unedo*, Nenadis et al. (2015)
124 reported contrasting UV-B effects on leaf flavonoids. Indeed, while the concentration of
125 flavanols decreased in response to UV-B, the concentration of the flavonol quercetin 3-

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126 rhamnoside increased. Differences in the behavior of individual phenolic compounds might
127 be associated to their different antioxidant capacities (Agati et al., 2009; Tattini et al., 2004),
128 as mentioned above. Therefore, the responses of these photoprotective compounds to UV
129 levels in Mediterranean species are highly variable, often being dependent on the species and
130 the specific compound.

131 Apart from high UV radiation and water deficit, Mediterranean terrestrial ecosystems
132 are usually exposed to frequent disturbances that reduce or remove the aerial plant biomass
133 (fires, clear-cuts, grazing). Therefore, the persistence of these ecosystems strongly depends on
134 the success of vegetation regeneration mechanisms, such as plant resprouting capacity. In the
135 resprouting strategy, the amount of carbon stored in roots is basic to support the growth of
136 new sprouts after a disturbance (Canadell and López-Soria, 1998; Paula et al., 2016). Since
137 resprouter species allocate a much greater percentage of assimilates to roots in comparison
138 with non-resprouter ones (Verdaguer and Ojeda, 2002), they could be especially sensitive to
139 resource allocation changes in response to UV radiation. A higher investment of assimilated
140 carbons into biochemical mechanisms involved in UV-protection could diminish the reserves
141 stored belowground, impairing the regeneration capacity and, subsequently, the survival of
142 resprouting plants.

143 Taking into account the expected changes in UV levels, precipitation and fire
144 frequency over the coming years, it is essential to improve our understanding of the
145 biochemical adjustments involved in Mediterranean plant responses to these factors. In
146 addition, more information is needed about the responses of phenolic compounds to UV
147 radiation, since highly variable responses have been found till now, usually depending on the
148 specific properties of each compound and the plant species. In this context, our objective was
149 to examine the effects, before and after the removal of plant aerial biomass, of enhanced UV
150 radiation and low water availability on the leaf concentration of phenols of two sclerophyllous

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151 resprouter species, *Arbutus unedo* L. and *Quercus suber* L., that co-occur widely in
152 Mediterranean shrublands. In addition, we aimed to study the effects of these two abiotic
153 factors on the leaf amount of ascorbate and glutathione, as well as on the activities of
154 ascorbate peroxidase and catalase enzymes, in resprouting plants of these two species. To
155 achieve these goals, an outdoor experiment involving UV supplementation combined with
156 two levels of irrigation was conducted using seedlings of these two species, which were
157 pruned, removing all the aerial biomass, during the study period. Hence, we hypothesized
158 that: (i) there will be interactive effects between UV radiation and water deficit on the leaf
159 phenolic profile and antioxidants of these species; (ii) leaf antioxidant compounds would be
160 mainly responsive to reduced water supply, while leaf phenolics would be primarily favored
161 to face enhanced UV doses; and (iii) in comparison with seedlings, resprouting plants would
162 be more sensitive to enhanced UV due to their earlier stage of shoot development, but less
163 sensitive to low water supply due to their improved water status through the lower shoot to
164 root biomass ratio (i.e. higher water availability).

165

166 **MATERIALS AND METHODS**

167 ***Plant material and experimental design***

168 An outdoor experiment of UV supplementation and reduced watering was carried out in Can
169 Vilallonga (150 m above sea level, 41º 52’ 48” N, 2º 54’ 33” E), near Cassà de la Selva
170 (Girona, NE of the Iberian Peninsula). Seedlings of *A. unedo* and *Q. suber* were grown in
171 their natural environment being subjected to three UV radiation levels combined with two
172 watering regimes. Specifically, 144 one-year-old seedlings per species were planted in pots (2
173 L volume; 11.3 cm wide x 21.5 cm deep) with 775 g of a growth medium with 8 g of fertilizer
174 (Osmocotex; 4 kg m^{-3}), basal dressing (1 kg m^{-3}) and dolomite (4 kg m^{-3}) to prevent
175 nutritional deficiencies during the experimental period. Seedlings were distributed in 9 plots

176 (16 plants per species and plot) made with 1.3 m x 1.2 m metallic frames and equipped with
177 four UV lamps installed above the plants (1.2 m of distance from the UV lamps to the top of
178 the pots). The 9 plots were organized in 3 blocks, each block having one plot of each one of
179 the three UV radiation conditions assayed. Within each plot, half of the plants were watered
180 to field capacity, while the other half received a lower amount of water (see below). Thereby,
181 each UV x watering combination was replicated three times in a split-plot randomized
182 complete block experimental design. Within each half plot with the same UV and watering
183 conditions, plants were rotated every two weeks throughout the study period to minimize
184 environmental, shading and border effects. Weather variables were obtained from the nearest
185 meteorological station to the experimental site, 3 km away (Table 1).

186 The experiment was carried out from June 2012 to October 2013. In February 2013, 8
187 months after the start of the experiment, all seedlings were pruned, being all their aerial
188 biomass completely removed, to simulate the effects of an intense disturbance, such as a
189 severe fire. The pruning was done in February, just before the growing season, in order to
190 allow the regrowth of the plants during the spring months. Along the experiment, two
191 samplings were conducted: the first one was performed in seedlings before pruning, 4 months
192 after the start of the experiment (October 2012); while the second one took place in
193 resprouting plants, 8 months after pruning (October 2013).

194 At the beginning of the experiment (June 2012), the height of all plants of each species
195 was similar (mean height of *A. unedo* plants was 42.92 ± 0.48 cm and for *Q. suber* plants was
196 53.02 ± 0.60 cm), as well as in October 2012, before pruning (mean height of *A. unedo* plants
197 was 94.7 ± 3.5 cm and for *Q. suber* plants was 94.8 ± 3.3 cm). The total biomass (below- and
198 above-ground) was also similar (mean biomass of *A. unedo* plants was 69.1 ± 3.7 g and for *Q.*
199 *suber* plants was 58.8 ± 3.9 g). However, in October 2013 (after pruning), while the mean
200 height and biomass for *A. unedo* resprouts were 26.2 ± 1.1 cm and 35.6 ± 1.9 g, respectively;

201 for *Q. suber*, they were 48.6 ± 1.5 cm and 46.4 ± 1.6 g, respectively. On the other hand, the
202 mean percentage of plants that resprouted was 68% for *A. unedo* and 82% for *Q. suber*. No
203 significant differences in response to the levels of UV radiation and/or watering were
204 observed regarding the percentage of plants that resprouted for any of the two species (Díaz-
205 Guerra et al., in prep). Sampled leaves from both species were randomly chosen on sunny
206 days and around midday.

207

208 ***UV-radiation treatment***

209 As detailed in Bernal et al. (2015), solar UV radiation was supplemented using four 40 W
210 fluorescent lamps (TL 40W/12 RS, with a peak at 313 nm; Philips, Spain) installed above the
211 plants and wrapped with filters of different materials to achieve the following UV conditions
212 (Table 1): a) enhanced UV-A+UV-B radiation (UVAB plots): in these plots, fluorescent
213 lamps were wrapped with 3 h pre-burned cellulose diacetate filters (Ultraphan URT, 0.1 mm;
214 Digefra GmbH, Munich, Germany) to exclude the UV-C radiation emitted by the lamps
215 (wavelengths < 280 nm); b) enhanced UV-A radiation (UVA plots): here, lamps were
216 wrapped with polyester film (Melinex, 0.25 mm; Ponscosta, Valencia, Spain) in order to
217 block the UV-B and UV-C radiation emitted by the lamps, transmitting only irradiance ≥ 315
218 nm; and c) ambient UV radiation (control plots): these plots were equipped with wood strips
219 instead of fluorescent lamps to ensure similar shading conditions as in the other plots.

220 Monthly averages of daily UV doses and percentages of UV enhancement in UVAB
221 and UVA plots were estimated from erythemal UV irradiance data (UVE; *Commission*
222 *International de l'Éclairage*, CIE) in combination with spectral measurements and radiative
223 modeling (Table 1), taking into account the mean height of the study plants. Along all the
224 experiment, we tried to keep the top of plant canopies at a similar height, raising those pots
225 with smaller individuals. Plants were daily irradiated with supplemental UV for 0.5–3.5 h

226 (depending on the period of the year), centered at solar noon, to simulate the potential
227 increases in UV radiation doses that might occur in the next decades as a consequence of
228 cloudiness reduction (IPCC, 2013). Filters were replaced after 36 h of use to avoid spectral
229 changes. To prevent UV contamination among plots, two clear polycarbonate filters
230 (transmission ≥ 400 nm) of 120 cm (width) x 30 cm (height) were positioned parallel to the
231 UV lamps along the two sides of the top part of each plot.

232

233 ***Watering treatment***

234 In addition to the natural precipitation, seedlings and resprouts (before and after pruning,
235 respectively) of *A. unedo* and *Q. suber* were irrigated twice daily using an automatic system
236 of drip-irrigation, which was programmed according to the monthly rainfall and the watering
237 regime of each plant (Table 1). Plants were subjected to two watering regimes: half of the
238 plants of each plot were watered to field capacity ("well-watered", WW), while the other half
239 received on average 45% of the water supplied to well-watered plants ("low-watered", LW).
240 Specifically, before pruning (from mid-June 2012 to January 2013), well-watered plants were
241 irrigated with 667 ml per day, while, after pruning (from February to October 2013), the
242 amount of supplied water was 200 ml per day. Low-watered plants received 60% of this water
243 during the first 40 days (from mid-June to 26th of July 2012), 40% from the end of July 2012
244 to January 2013 and 33% from February 2013 till the end of the experiment. The watering
245 reduction was applied gradually to these plants in order to allow their acclimation and thus to
246 avoid an excessive drought stress that could damage them. As an indication, the average soil
247 water content measured in the plant pots in October 2013, using a time domain reflectometer
248 (FieldScout TDR 300 Soil Moisture Meter, Spectrum Technologies, Inc., Aurora, USA), was
249 9.4% lower in low-watered plants than in well-watered ones (WW: 36.42 ± 0.77%; LW:
250 33.01 ± 0.83%).

251

252 **Table 1** Monthly means of UV supplementation applied to UVAB and UVA plots throughout the
253 study period, expressed as the percentage increase relative to ambient using the plant growth
254 weighting function (PG; Flint and Caldwell, 2003) or the unweighted UV-B (280-315 nm) and UV-A
255 (315-400 nm) irradiances. Monthly averages of ambient UV doses (kJ m^{-2} day^{-1}), temperature (ºC) and
256 accumulated precipitation (mm) are also shown. UV doses and percentages of UV enhancement were
257 estimated considering measured data (erythemal UV irradiance, UVE, and photosynthetic photon flux
258 density, PPFD, as in Nenadis et al., 2015) for clear-sky and cloudy days. Gaps in UV estimations
259 correspond to periods of calibration of the UVE sensor. Temperature and precipitation data were
260 obtained from the meteorological station of Cassà de la Selva (177 m above sea level, 41º 52' 28" N,
261 2º 55' 37" E).

Month	UV supplementation (%)						Ambient data				
	UVAB plots			UVA plots			UV doses (kJ m^{-2} day^{-1})			Temperature (ºC)	Precipitation (mm)
	PG[a]	UV-B	UV-A	PG[a]	UV-B	UV-A	PG[a]	UV-B	UV-A		
June 2012	13	24	0.9	1.2	0.1	1.0	31.8	34.4	1291	21.0	16.8
July 2012	14	26	1.0	1.2	0.1	1.1	30.2	32.4	1195	21.7	9.5
August 2012	-	-	0.7	-	-	0.8	-	-	1070	23.9	7.2
September 2012	-	-	1.1	-	-	1.2	-	-	706	19.5	110.1
October 2012	10	24	0.8	0.9	0.1	0.9	13.9	11.6	494	15.6	166.1
November 2012	6	18	0.4	0.7	0.1	0.6	9.0	5.6	372	11.2	59.3
December 2012	5	18	0.3	0.8	0.1	0.6	7.9	4.1	353	7.2	1.5
January 2013	4	17	0.3	0.7	0.1	0.6	8.6	4.3	377	6.0	13.9
February 2013	7	24	0.4	1.2	0.2	0.9	11.5	7.1	522	5.7	38.4
March 2013	6	16	0.4	1.1	0.1	0.8	17.3	13.3	753	9.5	173.2
April 2013	7	17	0.4	1.2	0.1	1.0	23.1	20.1	1020	11.4	89.1
May 2013	8	18	0.5	1.4	0.1	1.2	27.1	25.5	1115	13.1	82.4
June 2013	11	21	0.8	1.3	0.1	1.2	34.2	34.5	1270	18.4	21.6
July 2013	15	27	1.2	1.3	0.1	1.3	32.9	35.2	1164	23.0	21.0
August 2013	17	33	1.4	1.5	0.1	1.5	28.4	29.7	986	22.2	34.8
September 2013	13	27	1.0	1.1	0.1	1.1	22.0	20.8	790	19.1	37.4
October 2013	20	46	1.5	1.7	0.2	1.6	14.4	12.0	517	17.3	29.0

262 [a] *Plant growth weighting function according to Flint and Caldwell (2003)*

263

264 ***Determination of the leaf concentration of phenolic compounds by HPLC***

265 For each plot, species, and watering regime, one fully-developed leaf per plant, located at the
266 top of the plant canopy and exposed to sunlight, was taken from four different seedlings in
267 October 2012, and from 3-4 (exceptionally, two) different resprouting plants (depending on
268 the number of plants that resprouted after pruning) in October 2013. All leaf samples were
269 frozen with liquid nitrogen in the field and stored at -80 ºC until being analyzed in the
270 laboratory.

271 For *A. unedo*, one disc of 9 mm of diameter was obtained with a cork-borer from each
272 leaf, and then discs from plants belonging to the same plot and watering condition were
273 pooled for the analyses. Composite sample was used for this species because for some sub-

274 plots (5 out of 18) we had only 2 resprouting plants. In the case of *Q. suber*, whole leaves
275 (without leaf petioles) were always analyzed separately in order to have replicates within each
276 plot and watering condition. For both species, between 5 and 10 mg of fresh material
277 (avoiding leaf midrib and margins) were mixed and homogenized (Homogenizer Precellys 24,
278 Bertin Technologies, Montigny-le-Bretoneux, France) for 25 s with 0.6 ml of cold methanol
279 in Precellys-vials. Then, samples were incubated in an ice bath for 15 min, homogenized
280 again for 25 s, and centrifuged (13,000 rpm) for 3 min (Centrifuge 5415R, Eppendorf,
281 Hamburg, Germany). The supernatant was collected into a 6 ml glass tube. The extraction
282 was repeated 3 times more, adding to the remaining pellet 0.6 ml of methanol and leaving the
283 extracts on ice for 5 min. The combined supernatants were evaporated under nitrogen and
284 then stored at 4 ºC until their analysis.

285 Dried samples were dissolved in 300 µl of methanol plus 300 µl of MilliQ-water (1:1)
286 and analyzed by means of a high performance liquid chromatography (HPLC) system (1100
287 Series, Agilent, Waldbroon, Germany), which consisted of a binary pump (G1312A), an
288 autosampler (G1329A), vacuum degasser (G1322A), a diode array detector (G1315B), a
289 column oven (G1316A), and a C_{18} reverse-phase column (Zorbax SB-C18, 4.6 x 75 mm,
290 particle size 3.5 µm). The column and injector temperatures were kept at 30 and 22 ºC,
291 respectively. The injection volumes for *A. unedo* and *Q. suber* samples were 20 µl and 15 µl,
292 respectively. For both species, the eluent flow was 2 ml/min and the HPLC solvents were A
293 (aqueous 1.5% tetrahydrofuran and 0.25% *o*-phosphoric acid) and B (100% methanol). The
294 elution gradient used was: from 0 to 5 min, 0% of B in A; from 5 to 10 min, 0-20% of B in A;
295 from 10 to 20 min, 20-30% of B in A; from 20 to 40 min, 30-50% of B in A; from 40 to 45
296 min, 50% of B in A; and from 45 to 60 min, 50 to 100% of B in A. Runs were monitored at
297 220, 270 and 320 nm. The identification of the detected phenolic compounds was performed
298 by comparison of the UV-spectra characteristics and retention times obtained in the

299 chromatograms with the spectral libraries available in the Natural Product Research
300 Laboratory (University of Eastern Finland, Joensuu, Finland). The quantification of each
301 compound was based on the following standards: (+)-catechin for (+)-catechin, gallocatechin,
302 epigallocatechin and epigallocatechin gallate; quercetin 3-galactoside (hyperin) for quercetin
303 3-galactoside, quercetin 3-arabinoside, quercetin 3-glucoside, quercetin 3-rhamnoside,
304 quercetin-glycoside, two rutin derivatives and four "unknown" flavonols; kaempferol 3-
305 glucoside (astragalin) for kaempferol 3-glucoside, kaempferol glycoside and two
306 monocoumaroyl-astragalins; kaempferol 3-rhamnoside (afzelin) for kaempferol 3-
307 rhamnoside; myricetin 3-rhamnoside (myricitrin) for myricetin 3-rhamnoside, myricetin 3-
308 galactoside and myricetin 3-glucoside; gallic acid for gallic acid and hydrolyzable tannins;
309 ellagic acid for ellagic acid; and arbutin for arbutin, galloylarbutin and digalloylarbutin. The
310 concentration of each phenolic compound initially expressed as mg g^{-1} of fresh weight was
311 converted to mg g^{-1} of dry weight using the relationship between leaf fresh and dry weight
312 obtained for each sub-plot, species and sampling date (Díaz-Guerra et al., in prep.).

313 The concentration of condensed tannins (CT) was determined by means of the
314 butanol-HCl test (Hagerman, 1998), from the dissolved methanol extract obtained for the
315 HPLC analyses. Briefly, 1 ml of sample (300 µl of dissolved extract plus 700 µl of methanol)
316 was added to a 20 ml vial with 6 ml of acid butanol-reagent and 200 µl of Fe-reagent. The
317 sample was vortexed thoroughly using a vial mixer and then hydrolysed in a boiling bath for
318 50 min. After this, the vial was cooled and the absorbance was measured at 550 nm (20
319 Genesys Spectrophotometer, Thermo Spectronic, Rochester, USA). The amount of condensed
320 tannins in the sample was calculated from a standard curve (mg of CT = (Abs_{550nm} -
321 0.043467) / (0.0036 x 1000)) created from purified tannins of aspen leaves (*Populus tremula*),
322 expressing its concentration as mg g^{-1} DW.

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324 ***Non-phenolic leaf antioxidants in resprouts***

325 In October 2013, 8 months after pruning, one fully-developed and sun-exposed leaf per plant
326 was collected from 3 to 4 (exceptionally, two) different individuals of *A. unedo* and *Q. suber*
327 (depending on the number of plants that resprouted after pruning) in each plot and watering
328 regime. Leaves were frozen in liquid nitrogen and, once in the laboratory, stored at -80 ºC
329 until biochemical analysis. To determine leaf total ascorbate, reduced ascorbate (ASC),
330 dehydroascorbate (DHA), total glutathione, reduced glutathione (GSH) and oxidized
331 glutathione (GSSG) concentration, 100 mg of plant material was mixed with 1.5 ml of 3%
332 perchloric acid and then centrifuged (5000 rpm, for 20 min) at 4°C. The supernatant was
333 collected and its pH adjusted to 7 by adding 300–400 μl of sodium carbonate. This solution
334 was used as the leaf extract for the following analyses.

335 Total ascorbate, ASC and DHA were determined following the method of Arakawa et
336 al. (1981). This assay is based on the reduction of ferric to ferrous ion with ascorbic acid in
337 acid solution followed by the formation of a red chelate between ferrous ion and the a,a´-
338 dipyridyl, used as reagent to develop color. The determination of ascorbic acid is performed
339 by using the stoichiometric relationship between the ascorbic acid in the sample and the
340 formation of the chelate compound. Total ascorbate was determined in a reaction mixture to
341 reduce DHA to ASC consisting of 200 μl of supernatant, 500 μl of 150 mM KH_2PO_4 buffer
342 (pH 7.4) containing 5 mM EDTA, and 100 μl of 10 mM dithiothreitol (DTT). After 10 min at
343 room temperature, 100 μl of 0.5% (w/v) N-ethylmaleimide was added to remove excess DTT.
344 ASC was assayed in a similar manner to DHA except that DTT was substituted for 200 μl of
345 deionized H_2O. Color was developed in both reaction mixtures with the addition of 400 μl of
346 10% (w/v) trichloroacetic acid (TCA), 400 μl of 44% (v/v) *o*-phosphoric acid, 400 μl of a,a´-
347 dipyridyl in 70% (v/v) ethanol and 200 μl of 30g L^{-1} $FeCl_3$. The reaction mixtures were
348 incubated at 40 °C for 1 h and quantified spectrophotometrically at 525 nm. Ascorbate

349 standards were between 1 and 50 mmol ascorbate in 3% perchloric acid. DHA was estimated
350 from the difference between total ascorbate and ASC. The concentrations of total ascorbate,
351 ASC and DHA were expressed as μg g^{-1} DW.

352 Leaf total glutathione (GSSG plus GSH) was determined enzymatically. The reaction
353 mixture contained: 50 μl of leaf extract solution, 1 mM reagent 5,5´-dithiobis-2-nitrobenzoic
354 acid (DTNB), 100 mM phosphate buffer (pH 7.4), 5 mM EDTA and 0.5 mM NADPH. After 3
355 minutes at 25°C, the reaction was started by adding 2 units of glutathione reductase that
356 reduces GSSG to GSH. Then, the formation of 2-nitro-5-thiobenzoic acid from the reaction of
357 the DTNB with the GSH was continuously recorded at 412 nm with a UV-vis
358 spectrophotometer (Lambda Bio 20, Perkin-Elmer, Norwalk, CT, USA) (Tietze, 1969). The
359 total amount of glutathione in the samples (which is proportional to the rate of 2-nitro-5-
360 thiobenzoic acid formation) was determined from a standard curve obtained by plotting the
361 rate of change of absorbance at 412 nm (change in absorbance of the sample at 412 nm over 1
362 min of measurement) versus the known amount of glutathione (0.125-4 μM). For the
363 determination of GSSG, 1000 μl of leaf extract was incubated for 1 h at room temperature
364 with 20 μl of 4-vinyl pyridine. Incubation with 4-vinyl pyridine conjugates any GSH present
365 in the sample and, thus, GSSG is converted to GSH without interference by GSH. GSH was
366 estimated from the difference between total glutathione and GSSG. Leaf concentrations of
367 total glutathione, GSH and GSSG were expressed as μg g^{-1} DW.

368 To measure ascorbate peroxidase (APX; EC 1.11.1.11) and catalase (CAT; EC
369 1.11.1.6) activities, 100 mg of frozen leaf samples were homogenized with 0.1 M phosphate
370 buffer (pH 7.8) in a pre-chilled mortar. The homogenate was centrifuged at 4°C for 20 min at
371 5000 rpm. APX activity was determined spectrophotometrically by a decrease in absorbance
372 of ASC at 265 nm (ε = 14 mM cm^{-1}) (Nakano and Asada, 1987). The reaction mixture
373 contained 50 mM of potassium phosphate buffer (pH 7), 5 mM of ascorbic acid, 0.5 mM of

374 H_2O_2 and the enzyme extract. Addition of H_2O_2 started the reaction. APX activity was
375 expressed as μmol ASC min^{-1} mg^{-1} protein. CAT activity was determined by the consumption
376 of H_2O_2 (Dhindsa et al., 1981). The reaction mixture contained 50 mM of potassium
377 phosphate buffer (pH 7), 15 mM of H_2O_2 and 20 μl of the enzyme extract. The consumption
378 of H_2O_2 was monitored spectrophotometrically at 240 nm (ε = 0.0435 mM cm^{-1}). CAT
379 activity was expressed as μmol H_2O_2 min^{-1} mg^{-1} protein. Protein concentrations were
380 determined spectrophotometrically using Coomassie brilliant blue R-250 (Bradford, 1976),
381 with bovine serum albumin as a protein standard.
382

383 ***Statistical analyses***

384 To avoid pseudoreplication (Hurlbert, 1984; Searles et al., 2001), mean values of the different
385 studied parameters were calculated per species, plot and watering condition and were used for
386 all the statistical tests. Analyses were always performed separately for *A. unedo* and *Q. suber*.
387 For each species, treatment effects on the leaf concentration of phenolic compounds were
388 analyzed by means of repeated measures (split-plot) ANOVAs using pruning (with two
389 levels, seedlings and resprouts) as the within-subject factor, and UV radiation (with three
390 levels, enhanced UV-A+UV-B, enhanced UV-A and ambient UV) and watering (with two
391 levels, field capacity and watering reduction) as between-subject factors. Watering was
392 considered a factor nested within the UV treatment. Since leaf concentrations of ascorbate and
393 glutathione antioxidants, as well as the activities of ascorbate peroxidase and catalase
394 enzymes, were only determined in the resprouts, treatment effects on these parameters were
395 analyzed for each species using two-way ANOVAs with UV radiation as factor and watering
396 as a factor nested within UV.

397 For all the analyses, when the interaction between factors (UV x pruning and/or
398 watering x pruning) was significant, we assessed the effects of one of the factors within the

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399 levels of the other factor by one-way ANOVAs. Since the interaction UV x watering could
400 not be tested in the split-plot ANOVAs, we used one-way ANOVAs to analyze the UV effects
401 on the variables studied within each one of the two levels of the watering treatment. In the
402 case of significant UV effects, Fisher's LSD *post-hoc* pairwise comparisons were applied to
403 determine differences among UV conditions. Effects found in UVA plots were used as a
404 control for the effects of UV-A in UVAB plots. The Kolmogorov–Smirnov test was used to
405 analyze normality while the homogeneity of variances was tested with the Levene's statistic.
406 For all the statistical tests, the significance level was set at $p \leq 0.05$. Statistical analyses were
407 performed using SPSS software (IBM SPSS statistics, Corporation, Chicago, USA).

408

409 **RESULTS**

410 ***Leaf concentration of phenolic compounds in* A. unedo *and* Q. suber**

411 A similar number of phenolic compounds were detected in *A. unedo* and *Q. suber* leaves (17
412 and 23, respectively) (Tables 2 and 3). Despite this, the sum of the concentrations of the
413 identified phenols was 2.7-fold higher in *A. unedo* than in *Q. suber*. Detected phenols were
414 grouped into the following classes: tannins (condensed and hydrolyzable), flavonoids
415 (flavanols and flavonols) and phenolic acids. In *A. unedo*, hydroquinones were also found.

416 Comparing the amount of the different classes of phenols in *A. unedo* and *Q. suber*
417 leaves in relation to total phenol concentration (TP), it is remarkable that, whereas the
418 percentage of hydrolyzable tannins was similar in both species (around 6%), there were
419 important differences in the relative leaf concentration of condensed tannins, flavonoids and
420 hydroquinones. Indeed, in *A. unedo* leaves, hydroquinones represent the major group of
421 phenols (42.6 ± 1.0%), followed by condensed tannins (30.0 ± 1.0%) and flavonoids (19.7 ±
422 0.6%). On the contrary, in *Q. suber* leaves, hydroquinones were not detected, being the
423 condensed tannins the most abundant group of phenols (60.1 ± 1.1%) followed by flavonoids

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424 (33.1 ± 0.8%).

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Table 2 Overall mean ± S.E. for the concentration (mg g^{-1} DW) of the identified phenolic compounds in leaves of *Arbutus unedo* for each level of the three studied factors separately (UV radiation: ambient UV = control, enhanced UV-A = UVA and enhanced UV-A+UV-B = UVAB; Watering regime: well-watered = WW and low-watered = LW; Pruning: seedlings = plants sampled in October 2012, before pruning, and resprouts = plants sampled in October 2013, after pruning). For each UV level n = 12 and for each watering and pruning condition n = 18. Numbers in bold indicate significant differences between the levels of the factor. The significance level considered was $p \leq 0.05$. Only significant two-way interactions between UV radiation (UV) and pruning (P) and/or watering (W) and pruning (P) were included in the column "interactions".

	UV radiation (UV)				Watering (W)			Pruning (P)			Interactions
	control	UVA	UVAB	*p*-value	WW	LW	*p*-value	seedlings	resprouts	*p*-value	
Total phenols	237.29 ± 11.14	240.38 ± 13.69	246.99 ± 10.60	ns	239.08 ± 8.14	244.02 ± 10.85	ns	**212.30 ± 6.48**	**270.81 ± 6.48**	**<0.01**	-
Tannins	89.96 ± 6.12	88.08 ± 6.01	87.43 ± 4.27	ns	85.02 ± 3.48	91.96 ± 5.12	ns	**77.54 ± 2.96**	**99.44 ± 4.11**	**<0.01**	-
Condensed tannins	74.40 ± 5.95	72.96 ± 6.55	70.84 ± 4.64	ns	70.17 ± 3.74	75.29 ± 5.34	ns	**61.90 ± 3.33**	**83.56 ± 4.29**	**<0.01**	-
Hydrolyzable tannins	15.56 ± 0.51	15.12 ± 1.20	16.60 ± 1.10	ns	14.85 ± 0.69	16.67 ± 0.85	ns	15.64 ± 0.76	15.88 ± 0.84	ns	-
Flavonoids	47.84 ± 3.71	46.71 ± 4.62	49.97 ± 3.54	ns	48.86 ± 3.33	47.48 ± 3.11	ns	**36.42 ± 1.54**	**59.92 ± 1.47**	**<0.01**	-
Flavanols	15.88 ± 2.12	15.32 ± 2.26	17.68 ± 2.61	ns	17.12 ± 1.97	15.46 ± 1.80	ns	**9.18 ± 0.55**	**23.41 ± 0.96**	**<0.01**	-
(+)-catechin	14.36 ± 1.88	13.59 ± 1.89	15.11 ± 2.26	ns	15.03 ± 1.71	13.68 ± 1.53	ns	**8.32 ± 0.41**	**20.39 ± 0.93**	**<0.01**	-
gallocatechin	1.52 ± 0.31	1.73 ± 0.48	2.57 ± 0.83	ns	2.09 ± 0.50	1.79 ± 0.46	ns	**0.85 ± 0.30**	**3.03 ± 0.48**	**<0.01**	-
Flavonols	31.95 ± 1.78	31.39 ± 2.50	32.29 ± 1.70	ns	31.74 ± 1.70	32.02 ± 1.56	ns	**27.24 ± 1.15**	**36.51 ± 1.21**	**<0.01**	UV x P
Quercetins	21.86 ± 1.53	20.47 ± 2.01	21.09 ± 1.40	ns	20.91 ± 1.29	21.38 ± 1.40	ns	**17.34 ± 0.97**	**24.95 ± 1.00**	**<0.01**	UV x P
quercetin 3-galactoside	1.88 ± 0.29	1.50 ± 0.30	1.60 ± 0.29	ns	1.58 ± 0.21	1.73 ± 0.26	ns	**1.06 ± 0.15**	**2.26 ± 0.22**	**<0.01**	-
quercetin 3-arabinoside	2.29 ± 0.25	1.88 ± 0.24	2.22 ± 0.36	ns	1.97 ± 0.16	2.28 ± 0.29	ns	**1.83 ± 0.16**	**2.43 ± 0.27**	**0.03**	-
quercetin 3-rhamnoside	14.92 ± 0.85	14.55 ± 1.26	14.15 ± 0.65	ns	15.13 ± 0.79	13.95 ± 0.73	ns	**12.73 ± 0.63**	**16.35 ± 0.64**	**<0.01**	-
quercetin-glycoside	2.78 ± 0.64	2.55 ± 0.63	3.12 ± 0.53	ns	2.22 ± 0.39	3.41 ± 0.53	ns	**1.72 ± 0.23**	**3.91 ± 0.53**	**<0.01**	-
Kaempferols	3.37 ± 0.14	3.50 ± 0.32	3.61 ± 0.17	ns	3.60 ± 0.21	3.39 ± 0.15	ns	3.33 ± 0.14	3.65 ± 0.21	ns	-
kaempferol 3-glucoside	0.50 ± 0.06	0.53 ± 0.07	0.45 ± 0.05	ns	0.56 ± 0.05	0.43 ± 0.04	ns	0.47 ± 0.05	0.51 ± 0.05	ns	-
kaempferol 3-rhamnoside	1.84 ± 0.22	1.87 ± 0.27	2.03 ± 0.19	ns	1.82 ± 0.24	2.00 ± 0.11	ns	**1.67 ± 0.13**	**2.15 ± 0.21**	**0.03**	-
kaempferol glycoside	1.03 ± 0.11	1.09 ± 0.16	1.13 ± 0.17	ns	1.22 ± 0.10	0.96 ± 0.13	ns	1.19 ± 0.09	0.99 ± 0.14	ns	-
Myricetins											
myricetin 3-rhamnoside	6.73 ± 0.33	7.42 ± 0.59	7.59 ± 0.47	ns	7.23 ± 0.48	7.25 ± 0.28	ns	**6.57 ± 0.28**	**7.91 ± 0.42**	**0.03**	-
Hydroquinones	97.30 ± 4.22	103.28 ± 6.79	107.31 ± 7.82	ns	102.94 ± 5.21	102.31 ± 5.36	ns	**96.15 ± 4.22**	**109.10 ± 5.76**	**0.04**	-
arbutin	50.86 ± 3.21	52.44 ± 4.16	54.05 ± 4.25	ns	51.23 ± 2.76	53.67 ± 3.48	ns	**45.50 ± 1.71**	**59.40 ± 3.36**	**<0.01**	-
galloylarbutin	32.36 ± 2.82	35.14 ± 4.07	37.42 ± 4.71	ns	36.27 ± 3.15	33.68 ± 3.25	ns	33.73 ± 2.85	36.21 ± 3.51	ns	-
digalloylarbutin	14.08 ± 1.09	15.70 ± 1.86	15.83 ± 1.48	ns	15.45 ± 1.14	14.96 ± 1.31	ns	16.91 ± 0.98	13.49 ± 1.32	ns	-
Phenolic acids	2.20 ± 0.13	2.30 ± 0.19	2.29 ± 0.17	ns	2.26 ± 0.14	2.27 ± 0.13	ns	2.18 ± 0.08	2.34 ± 0.17	ns	-
gallic acid	1.77 ± 0.12	1.84 ± 0.18	1.91 ± 0.18	ns	1.84 ± 0.14	1.84 ± 0.12	ns	1.78 ± 0.09	1.90 ± 0.16	ns	-
ellagic acid	0.44 ± 0.03	0.46 ± 0.03	0.37 ± 0.03	ns	0.42 ± 0.02	0.42 ± 0.03	ns	0.41 ± 0.02	0.44 ± 0.02	ns	-
TAN:TP	0.38 ± 0.01	0.37 ± 0.02	0.36 ± 0.02	ns	0.36 ± 0.01	0.38 ± 0.01	ns	0.37 ± 0.01	0.37 ± 0.01	ns	-
FLAV:TP	0.20 ± 0.01	0.19 ± 0.01	0.20 ± 0.01	ns	0.20 ± 0.01	0.19 ± 0.01	ns	**0.17 ± 0.01**	**0.22 ± 0.01**	**<0.01**	-
Que:Kae	6.52 ± 0.42	6.07 ± 0.63	5.97 ± 0.51	ns	5.98 ± 0.43	6.39 ± 0.42	ns	**5.25 ± 0.25**	**7.13 ± 0.44**	**<0.01**	-
Hq:TP	0.41 ± 0.02	0.43 ± 0.02	0.43 ± 0.02	ns	0.43 ± 0.02	0.42 ± 0.01	ns	**0.45 ± 0.01**	**0.40 ± 0.01**	**0.02**	-
PA:TP	0.0094 ± 0.0006	0.0097 ± 0.0007	0.0093 ± 0.0006	ns	0.0095 ± 0.0005	0.0095 ± 0.0006	ns	**0.0104 ± 0.0004**	**0.0086 ± 0.0005**	**0.03**	-

TP, total phenols; TAN, tannins; FLAV, flavonoids; Que, quercetins; Kae, kaempferols; Hq, hydroquinones; PA, phenolic acids; ns, not significant.

Table 3 Overall mean ± S.E. for the concentration (mg g^{-1} DW) of the identified phenolic compounds in leaves of *Quercus suber* for each level of the three studied factors separately (UV radiation: ambient UV = control, enhanced UV-A = UVA and enhanced UV-A+UV-B = UVAB; Watering regime: well-watered = WW and low-watered = LW; Pruning: seedlings = plants sampled in October 2012, before pruning, and resprouts = plants sampled in October 2013, after pruning). For each UV level n = 12 and for each watering and pruning condition n = 18. Numbers in bold indicate significant differences between the levels of the factor. In the case of the UV treatment, significant differences among UV conditions are also indicated by different letters. The significance level considered was $p \leq 0.05$. Only significant two-way interactions between UV radiation (UV) and pruning (P) and/or watering (W) and pruning (P) were included in the column "interactions".

	UV radiation (UV)							Watering (W)			Pruning (P)			Interactions
	control		UVA		UVAB		*p*-value	WW	LW	*p*-value	seedlings	resprouts	*p*-value	
Total phenols	90.45 ± 7.70		88.43 ± 3.99		92.76 ± 7.34		ns	89.33 ± 4.67	91.77 ± 5.83	ns	**80.21 ± 4.89**	**100.88 ± 4.43**	**0.01**	-
Tannins	60.83 ± 6.43		59.72 ± 3.25		62.21 ± 6.42		ns	59.48 ± 4.14	62.37 ± 4.78	ns	55.35 ± 4.46	66.50 ± 4.09	ns	-
Condensed tannins	55.67 ± 6.39		53.95 ± 3.23		56.14 ± 6.42		ns	53.40 ± 4.14	57.10 ± 4.73	ns	50.83 ± 4.39	59.67 ± 4.30	ns	-
Hydrolyzable tannins	5.17 ± 0.47		5.77 ± 0.52		6.07 ± 0.42		ns	6.08 ± 0.38	5.26 ± 0.38	ns	**4.51 ± 0.24**	**6.83 ± 0.29**	**<0.01**	-
Flavonoids	29.26 ± 1.76		28.38 ± 1.44		30.22 ± 1.68		ns	29.51 ± 1.32	29.07 ± 1.33	ns	**24.57 ± 0.61**	**34.01 ± 0.72**	**<0.01**	-
Flavanols	17.84 ± 1.28		17.23 ± 0.94		17.29 ± 1.18		ns	17.62 ± 0.92	17.29 ± 0.92	ns	**14.41 ± 0.55**	**20.50 ± 0.55**	**<0.01**	-
(+)-catechin	10.01 ± 1.08		9.45 ± 0.50		9.19 ± 0.91		ns	9.41 ± 0.71	9.70 ± 0.69	ns	8.89 ± 0.68	10.21 ± 0.68	ns	-
gallocatechin	1.97 ± 0.26		1.93 ± 0.18		2.45 ± 0.35		ns	2.42 ± 0.26	1.82 ± 0.16	ns	1.88 ± 0.21	2.36 ± 0.22	ns	-
epigallocatechin	3.74 ± 0.52		3.52 ± 0.63		3.67 ± 0.66		ns	3.72 ± 0.47	3.57 ± 0.50	ns	**2.11 ± 0.21**	**5.18 ± 0.39**	**<0.01**	-
epigallocatechin gallate	2.22 ± 0.27		2.32 ± 0.24		1.98 ± 0.24		ns	2.14 ± 0.20	2.21 ± 0.20	ns	**1.59 ± 0.15**	**2.76 ± 0.14**	**<0.01**	-
Flavonols	**11.42 ± 0.69**	**b**	**11.15 ± 0.61**	**b**	**12.94 ± 0.57**	**a**	**0.05**	11.89 ± 0.58	11.79 ± 0.49	ns	**10.16 ± 0.38**	**13.51 ± 0.32**	**<0.01**	-
Quercetins	4.19 ± 0.32		3.90 ± 0.34		4.41 ± 0.40		ns	4.17 ± 0.30	4.16 ± 0.28	ns	**3.22 ± 0.13**	**5.11 ± 0.22**	**<0.01**	-
quercetin 3-galactoside	1.46 ± 0.11		1.40 ± 0.12		1.45 ± 0.15		ns	1.42 ± 0.11	1.45 ± 0.09	ns	**1.15 ± 0.04**	**1.73 ± 0.10**	**<0.01**	-
quercetin 3-glucoside	2.09 ± 0.19		1.91 ± 0.19		2.24 ± 0.22		ns	2.09 ± 0.17	2.07 ± 0.16	ns	**1.54 ± 0.10**	**2.62 ± 0.10**	**<0.01**	-
quercetin 3-rhamnoside	0.32 ± 0.03		0.27 ± 0.02		0.36 ± 0.03		ns	0.32 ± 0.03	0.31 ± 0.02	ns	**0.27 ± 0.02**	**0.36 ± 0.02**	**<0.01**	-
quercetin-glycoside	0.32 ± 0.03		0.32 ± 0.03		0.36 ± 0.04		ns	0.33 ± 0.03	0.33 ± 0.02	ns	**0.26 ± 0.01**	**0.41 ± 0.03**	**<0.01**	-
Kaempferols	**1.29 ± 0.09**	**b**	**1.38 ± 0.09**	**b**	**1.80 ± 0.09**	**a**	**0.01**	1.56 ± 0.09	1.43 ± 0.09	ns	1.45 ± 0.09	1.53 ± 0.09	ns	-
kaempferol 3-glucoside	**0.66 ± 0.08**	**b**	**0.76 ± 0.06**	**b**	**1.05 ± 0.08**	**a**	**0.03**	0.88 ± 0.07	0.76 ± 0.07	ns	0.85 ± 0.07	0.80 ± 0.08	ns	-
kaempferol glycoside	0.30 ± 0.02		0.28 ± 0.02		0.31 ± 0.03		ns	0.29 ± 0.02	0.30 ± 0.02	ns	**0.26 ± 0.02**	**0.32 ± 0.02**	**0.04**	-
monocoumaroyl-astragalin 1	**0.18 ± 0.01**	**b**	**0.20 ± 0.01**	**b**	**0.26 ± 0.02**	**a**	**0.01**	0.23 ± 0.02	0.20 ± 0.01	ns	**0.20 ± 0.01**	**0.23 ± 0.01**	**0.01**	-
monocoumaroyl-astragalin 2	**0.15 ± 0.01**	**ab**	**0.15 ± 0.01**	**b**	**0.18 ± 0.02**	**a**	**0.05**	0.16 ± 0.01	0.16 ± 0.01	ns	**0.14 ± 0.01**	**0.18 ± 0.01**	**0.03**	-
Myricetins	0.15 ± 0.03		0.21 ± 0.04		0.26 ± 0.04		ns	0.20 ± 0.03	0.22 ± 0.03	ns	0.22 ± 0.03	0.20 ± 0.03	ns	-
myricetin 3-galactoside	0.06 ± 0.02		0.09 ± 0.01		0.08 ± 0.02		ns	0.06 ± 0.01	0.09 ± 0.01	ns	0.08 ± 0.01	0.07 ± 0.02	ns	-
myricetin 3-glucoside	0.09 ± 0.01		0.12 ± 0.03		0.18 ± 0.03		ns	0.14 ± 0.03	0.12 ± 0.02	ns	0.14 ± 0.02	0.12 ± 0.02	ns	-
Rutins	1.88 ± 0.08		1.81 ± 0.09		2.06 ± 0.09		ns	1.92 ± 0.09	1.92 ± 0.06	ns	**1.79 ± 0.08**	**2.04 ± 0.05**	**0.05**	-
rutin derivative 1	**1.63 ± 0.09**	**ab**	**1.57 ± 0.08**	**b**	**1.82 ± 0.08**	**a**	**0.05**	1.66 ± 0.08	1.68 ± 0.06	ns	**1.54 ± 0.08**	**1.81 ± 0.05**	**0.02**	-
rutin derivative 2	0.24 ± 0.01		0.25 ± 0.02		0.24 ± 0.02		ns	0.25 ± 0.02	0.24 ± 0.01	ns	0.25 ± 0.01	0.24 ± 0.01	ns	-
unknown flavonols	3.92 ± 0.29		3.84 ± 0.29		4.40 ± 0.28		ns	4.05 ± 0.25	4.06 ± 0.22	ns	**3.49 ± 0.20**	**4.62 ± 0.18**	**<0.01**	-
flavonol 1	0.59 ± 0.05		0.70 ± 0.07		0.75 ± 0.06		ns	0.71 ± 0.06	0.65 ± 0.04	ns	**0.54 ± 0.04**	**0.82 ± 0.04**	**<0.01**	-
flavonol 2	1.66 ± 0.18		1.63 ± 0.19		1.85 ± 0.17		ns	1.68 ± 0.15	1.75 ± 0.15	ns	**1.31 ± 0.09**	**2.11 ± 0.13**	**<0.01**	-
flavonol 3	0.05 ± 0.01		0.06 ± 0.01		0.07 ± 0.01		ns	0.06 ± 0.01	0.06 ± 0.01	ns	0.06 ± 0.01	0.07 ± 0.01	ns	-
flavonol 4	1.62 ± 0.12		1.45 ± 0.10		1.73 ± 0.14		ns	1.60 ± 0.12	1.60 ± 0.08	ns	1.58 ± 0.11	1.62 ± 0.09	ns	-
Phenolic acids														
ellagic acid	0.35 ± 0.02		0.33 ± 0.02		0.33 ± 0.02		ns	0.34 ± 0.02	0.33 ± 0.02	ns	**0.30 ± 0.02**	**0.37 ± 0.01**	**<0.01**	-
TAN:TP	0.65 ± 0.01		0.66 ± 0.01		0.64 ± 0.02		ns	0.64 ± 0.01	0.66 ± 0.01	ns	0.66 ± 0.01	0.64 ± 0.01	ns	-
FLAV:TP	0.34 ± 0.01		0.33 ± 0.01		0.36 ± 0.02		ns	0.35 ± 0.01	0.34 ± 0.01	ns	0.34 ± 0.01	0.35 ± 0.01	ns	-
Que:Kae	3.68 ± 0.32		3.28 ± 0.38		3.03 ± 0.51		ns	3.22 ± 0.35	3.44 ± 0.32	ns	**2.64 ± 0.20**	**4.02 ± 0.36**	**0.01**	-
PA:TP	0.0047 ± 0.0007		0.0039 ± 0.0002		0.0044 ± 0.0006		ns	0.0046 ± 0.0004	0.0041 ± 0.0005	ns	0.0047 ± 0.0006	0.0040 ± 0.0002	ns	-

TP, total phenols; TAN, tannins; FLAV, flavonoids; Que, quercetins; Kae, kaempferols; PA, phenolic acids; ns, not significant.

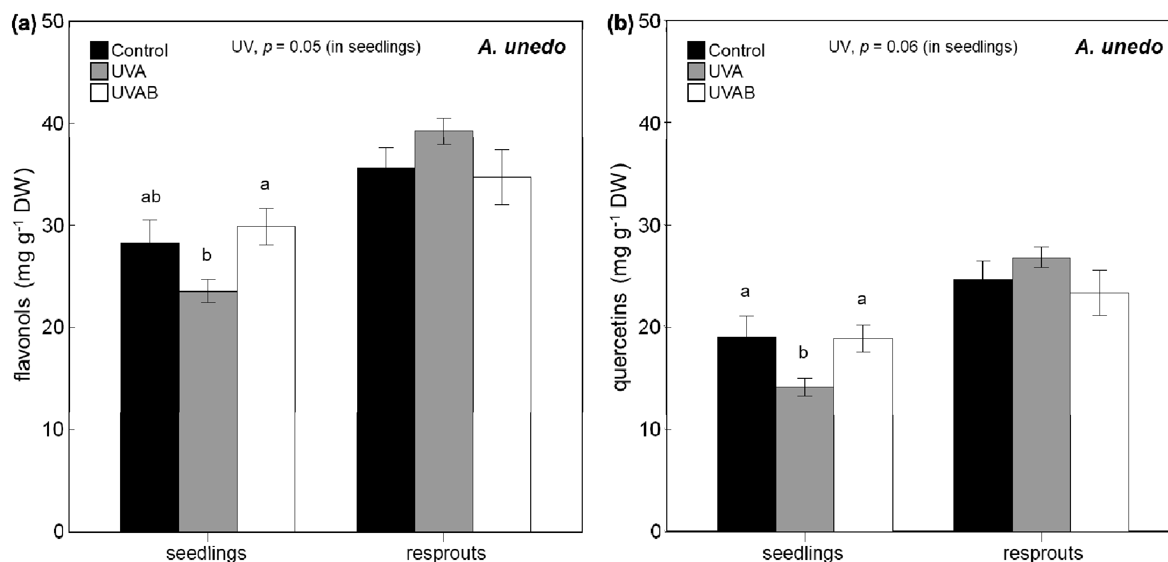
443 The leaf total pool of phenols was around 28% and 26% higher in the resprouts than in the
444 seedlings of *A. unedo* and *Q. suber*, respectively (Tables 2 and 3). The groups of phenols
445 showing the largest increases after the pruning were flavanols (by 155% and 42% for *A.*
446 *unedo* and *Q. suber*, respectively) and quercetins (by 44% and 59% for *A. unedo* and *Q.*
447 *suber*, respectively). In *A. unedo* leaves, tannins, mainly the condensed ones, myricetins and
448 the hydroquinone arbutin were also higher in the resprouts (by 35%, 20% and 30%,
449 respectively) compared to seedlings. In *Q. suber* leaves, the total amount of tannins did not
450 differ between seedlings and resprouts, although hydrolyzable tannins were significantly
451 higher in the resprouting plants (by 51%). For this species, other phenols that were more
452 abundant in the resprouts were rutins (by 14%), the unknown flavonols (by 32%) and the
453 ellagic acid (by 25%) (Table 3). In both species, we found a greater quercetin to kaempferol
454 ratio (Que:Kae) in the leaves of the resprouts due to their enhanced concentration of
455 quercetins. In *A. unedo* leaves, resprouts also exhibited higher flavonoids to TP ratio
456 (FLAV:TP) and lower hydroquinones and phenolic acids in relation to TP (Hq:TP and PA:TP,
457 respectively) compared to seedlings (Table 2).

458

459 ***UV and watering treatment effects on leaf phenols of* A. unedo**

460 In general, UV and watering treatments did not affect significantly the content of phenols of
461 *A. unedo* leaves (Table 2). The only effects observed were an interactive influence of UV and
462 pruning on the foliar concentration of flavonols and quercetins (Table 2). Indeed, only in
463 seedlings, the UV treatment affected the total amount of flavonols and quercetins, although
464 the overall effect on quercetins was only marginally significant (Fig. 1a,b). Seedlings exposed
465 to enhanced UV-A+UV-B had a 27% greater concentration of flavonols compared to plants
466 grown under enhanced UV-A (Fig. 1a). Similarly, the content of quercetins was a 33% greater
467 in UVAB seedlings in relation to UVA ones, with the same tendency being observed for

468 control seedlings (Fig. 1b).



469

470 **Fig. 1** *Arbutus unedo* leaf concentrations of flavonols (**a**) and quercetins (**b**) in seedlings (sampled in
471 October 2012, before pruning) and resprouts (sampled in October 2013, after pruning) subjected to
472 three UV radiation conditions (ambient UV = control, enhanced UV-A = UVA, and enhanced UV-
473 A+UV-B = UVAB). Error bars represent the standard error of the mean (n = 6). Letters indicate
474 significant differences among UV conditions within seedlings or resprouts. The significance level was
475 set at $p \leq 0.05$.

476

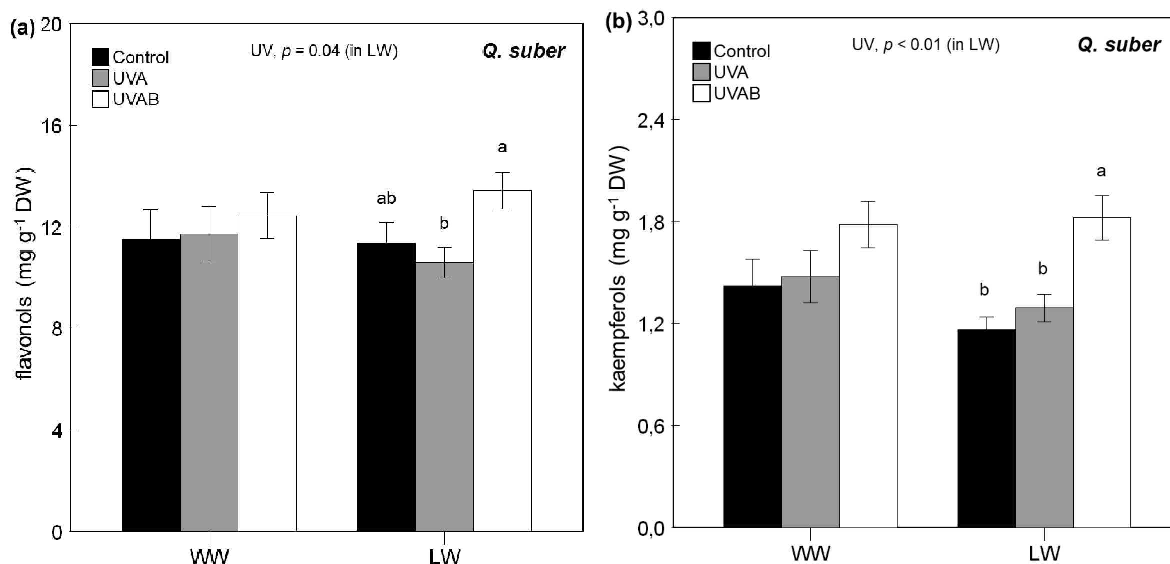
477 ***UV and watering treatment effects on leaf phenols of* Q. suber**

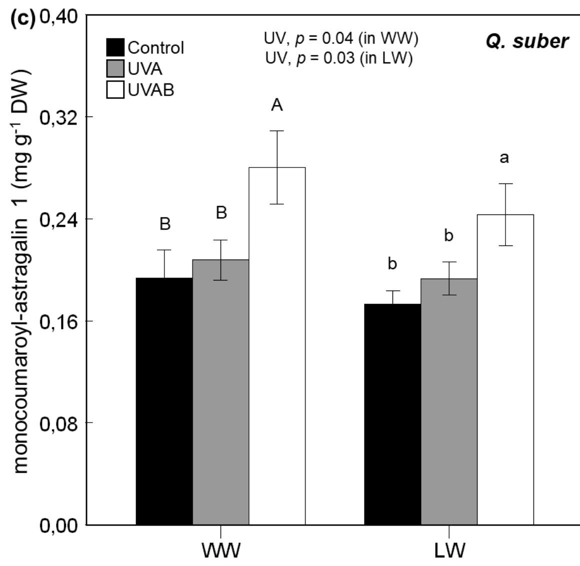
478 The UV radiation treatment affected one sixth of the phenolic compounds identified in *Q.*
479 *suber* leaves, although it did not modify the total concentration of flavonoids or phenols
480 (Table 3). The UV-sensitive phenols were four flavonols, three of them being kaempferols
481 and the fourth being a rutin (Table 3). These four flavonols responded similarly to the UV
482 treatment, with leaves under enhanced UV-A+UV-B radiation showing the highest levels. As
483 a result, UVAB plants had the highest overall amount of kaempferols, as well as of total
484 flavonols (Table 3).

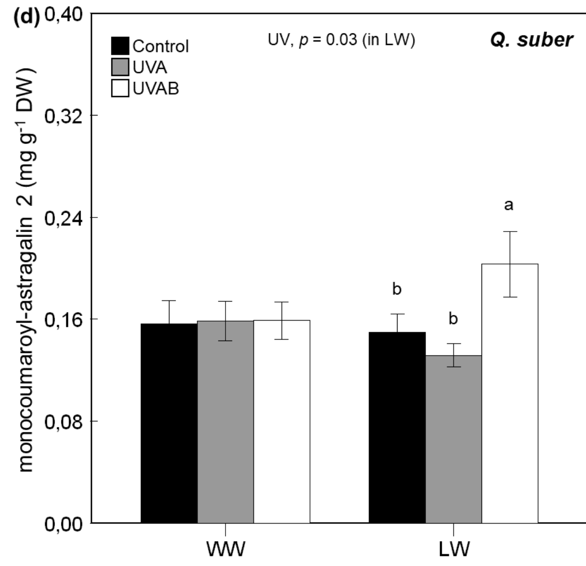
485 Analyzing the UV effects within each watering regime, we found that low-watered
486 plants were more sensitive to UV supplementation than well-watered ones. Indeed, the
487 concentration of total flavonols (Fig. 2a) and kaempferols (Fig. 2b) were significantly higher

488 in plants subjected to enhanced UV-A+UV-B only under low watering. Compared to UVA
489 and control plants, UVAB plants showed higher leaf concentrations of five flavonols: three
490 kaempferols, the monocoumaroyl-astragalin 1 (Fig. 2c) and 2 (Fig. 2d), and the kaempferol 3-
491 glucoside (Fig. 2e); one myricetin, the myricetin 3-glucoside (Fig. 2f); and one rutin, the rutin
492 derivative 1 (Fig. 2g). Under low watering, the total concentration of rutins was also greater in
493 plants exposed to enhanced UV-A+UV-B (Fig. 2h). Only the monocoumaroyl-astragalin 1
494 was affected by the UV treatment under optimal watering conditions, showing similar
495 responses to UV than those observed under low watering (Fig. 2c).

496 The watering treatment *per se* did not affect significantly the concentration of any of
497 the different phenols found in *Q. suber* leaves (Table 3).

498 





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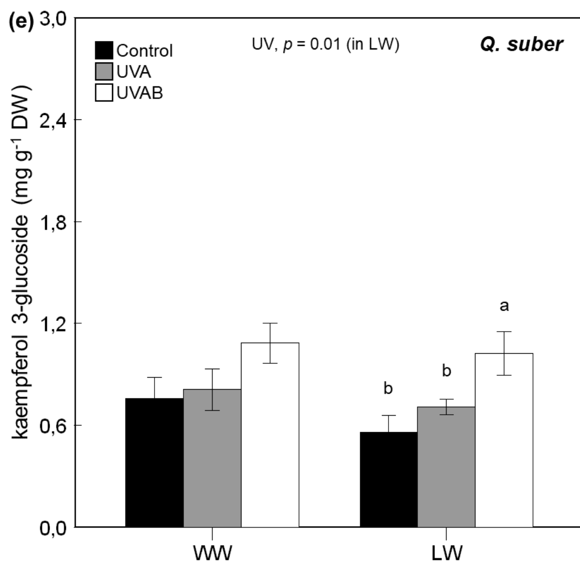

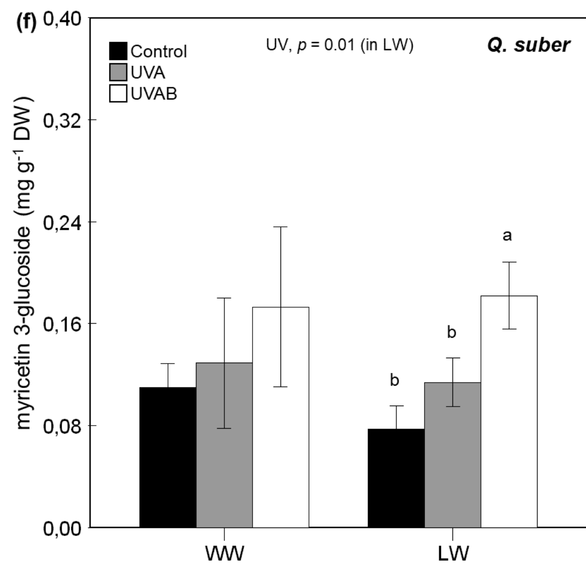

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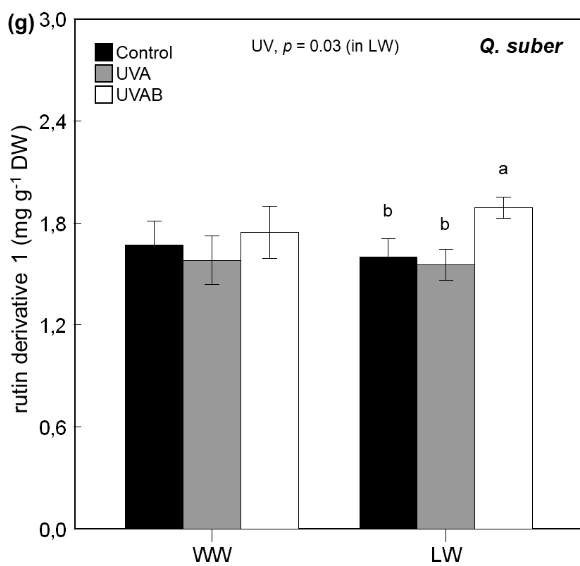

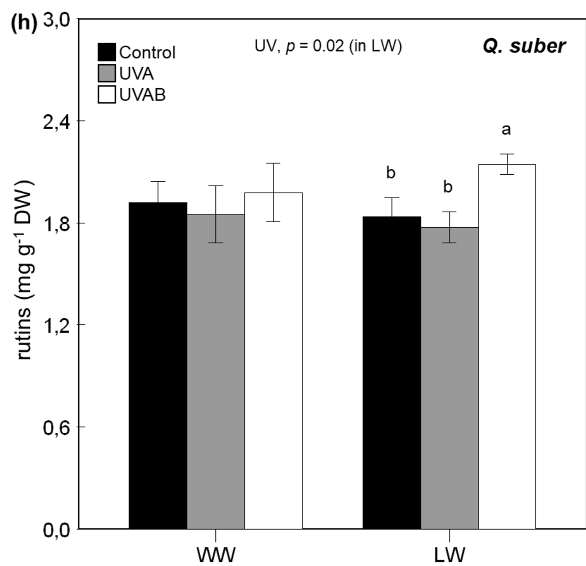

501

502 **Fig. 2** *Quercus suber* leaf concentration of flavonols (**a**), kaempferols (**b**), monocoumaroyl-astragalin

503 1 (**c**) and 2 (**d**), kaempferol 3-glucoside (**e**), myricetin 3-glucoside (**f**), rutin derivative 1 (**g**) and rutins
504 (**h**) in plants (data on seedlings and resprouts have been pooled) subjected to three UV radiation
505 conditions (ambient UV = control, enhanced UV-A = UVA, and enhanced UV-A+UV-B = UVAB)
506 combined with two watering regimes (well-watered = WW and low-watered = LW). Error bars
507 represent the standard error of the mean (n = 6). Letters indicate significant differences among UV
508 conditions within the same watering regime. The significance level was set at $p \leq 0.05$.

509

510 ***UV and watering treatment effects on the leaf non-phenolic antioxidants of resprouts***

511 Many of the studied parameters related to the leaf non-phenolic antioxidant activity of *A.*
512 *unedo* resprouts responded to UV radiation, to water supply or to both treatments (Table 4).
513 Conversely, this was not the case of *Q. suber* resprouts, since, for this species, only a few of
514 these parameters were affected by the watering regime, while UV radiation did not have any
515 significant effect (Table 4).

516 In the case of *A. unedo* leaves, the concentrations of total ascorbate and reduced
517 ascorbate (ASC), total glutathione and reduced glutathione (GSH), as well as the activity of
518 catalase enzyme (CAT), were substantially enhanced (by more than 25%) under UV-A+UV-B
519 supplementation in comparison to plants grown under UV-A supplementation alone (Table 4).
520 In contrast, leaf ascorbate peroxidase (APX) activity was about 45% and 34% higher under
521 enhanced UV-A compared to UVAB and control plants, respectively (Table 4). The analyses
522 of the UV effects within each irrigation level showed that most of these effects were
523 dependent on the watering treatment. Indeed, leaf concentrations of total glutathione and
524 reduced glutathione (GSH) were the highest in response to enhanced UV-A+UV-B only in
525 low-watered resprouts (Fig. 3a,b). Although UV conditions tended to have the same effect on
526 the total leaf concentration of ascorbate and on the activity of catalase enzyme (CAT) under
527 low watering, differences for these parameters were not significant (Fig. 3c,d). In contrast,
528 under well-watering conditions, the concentrations of total ascorbate and CAT activity were
529 significantly lower under enhanced UV-A compared to UVAB and control plants (Fig. 3c,d),
530 while the contrary was observed for the ascorbate peroxidase (APX) activity (Fig. 3e).

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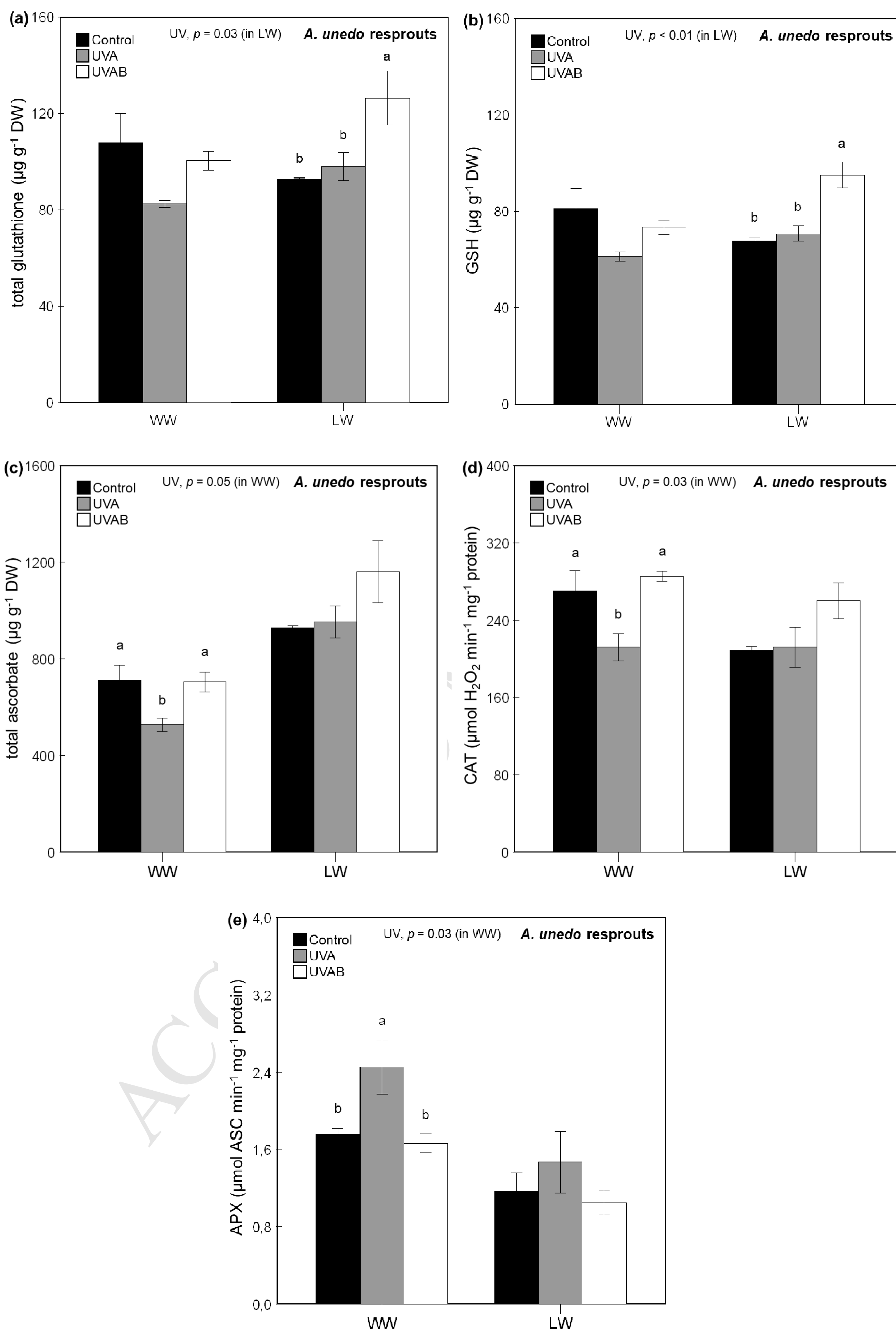

531

532

533

534 **Fig. 3** *Arbutus unedo* leaf concentrations of total glutathione (**a**), reduced glutathione (GSH) (**b**), total

535 ascorbate (**c**), catalase activity (CAT) (**d**) and ascorbate peroxidase activity (APX) (**e**) in resprouts
536 (sampled in October 2013, after pruning) subjected to three UV radiation conditions (ambient UV =
537 control, enhanced UV-A = UVA, and enhanced UV-A+UV-B = UVAB) combined with two watering
538 regimes (well-watered = WW and low-watered = LW). Error bars represent the standard error of the
539 mean (n = 3). Different letters indicate significant differences among UV conditions within the same
540 watering regime. The significance level was set at $p \leq 0.05$.

541 The reduction of watering enhanced the leaf concentrations of total ascorbate, ASC and
542 dehydroascorbate (DHA) in *A. unedo*, as well as of total glutathione and GSH in both species.
543 On the contrary, the activity of the APX enzyme was reduced by drier conditions in *A. unedo*
544 and in *Q. suber* (Table 4). ASC:DHA and ASC:total ascorbate ratios, as well as the
545 GSH:GSSG ratio and the leaf concentration of oxidized glutathione (GSSG), did not vary
546 significantly as a result of the treatments in any of the two species (Table 4).

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Table 4 Overall mean ± S.E. for different parameters related to the antioxidant activity in leaves of *Arbutus unedo* and *Quercus suber* resprouts (sampled in October 2013, after pruning) for each level of the studied factors separately (UV radiation: ambient UV = control, enhanced UV-A = UVA and enhanced UV-A+UV-B = UVAB; Watering regime: well-watered = WW and low-watered = LW). For each UV level n = 6 and for each watering condition n = 9. Numbers in bold indicate significant differences among the levels of the factor. In the case of UV radiation, significant differences among UV conditions are also indicated by different letters. The significance level considered was $p \leq 0.05$.

	UV radiation							Watering		
	control		UVA		UVAB		*p*-value	WW	LW	*p*-value
A. unedo resprouts										
Total ascorbate (μg g^{-1} DW)	**819.52 ± 56.21**	**ab**	**739.55 ± 100.49**	**b**	**932.20 ± 118.54**	**a**	**0.04**	**647.29 ± 37.93**	**1013.56 ± 55.83**	**<0.01**
ASC (μg g^{-1} DW)	**673.67 ± 46.43**	**ab**	**621.80 ± 77.73**	**b**	**788.67 ± 97.69**	**a**	**0.04**	**551.76 ± 34.14**	**837.67 ± 48.30**	**<0.01**
DHA (μg g^{-1} DW)	145.85 ± 19.07		117.75 ± 26.44		144.39 ± 24.59		ns	**95.53 ± 13.58**	**176.46 ± 11.95**	**0.01**
ASC:DHA	6.99 ± 1.39		6.56 ± 1.25		6.86 ± 1.28		ns	8.02 ± 1.24	5.58 ± 0.47	ns
ASC:Total	0.83 ± 0.02		0.85 ± 0.02		0.85 ± 0.02		ns	0.85 ± 0.02	0.83 ± 0.01	ns
Total glutathione (μg g^{-1} DW)	**100.16 ± 6.46**	**ab**	**90.15 ± 4.39**	**b**	**113.42 ± 7.90**	**a**	**0.03**	**96.87 ± 5.31**	**105.63 ± 6.41**	**0.05**
GSH (μg g^{-1} DW)	**74.49 ± 4.79**	**ab**	**66.14 ± 2.70**	**b**	**84.20 ± 5.54**	**a**	**0.01**	**71.96 ± 3.86**	**77.93 ± 4.68**	**0.01**
GSSG (μg g^{-1} DW)	25.67 ± 1.91		24.01 ± 2.00		29.23 ± 2.95		ns	24.91 ± 1.58	27.70 ± 2.24	ns
GSH:GSSG	3.00 ± 0.16		2.84 ± 0.17		3.09 ± 0.27		ns	3.02 ± 0.09	2.94 ± 0.22	ns
APX (μmol ASC min^{-1} mg^{-1} protein)	**1.46 ± 0.16**	**b**	**1.96 ± 0.29**	**a**	**1.36 ± 0.16**	**b**	**0.02**	**1.96 ± 0.15**	**1.23 ± 0.13**	**0.01**
CAT (μmol H_2O_2 min^{-1} mg^{-1} protein)	**239.64 ± 16.74**	**ab**	**211.94 ± 11.31**	**b**	**272.71 ± 10.34**	**a**	**0.01**	255.92 ± 13.45	226.95 ± 11.64	ns
Q. suber resprouts										
Total ascorbate (μg g^{-1} DW)	3550.27 ± 178.94		3136.03 ± 191.11		3102.58 ± 206.47		ns	3087.25 ± 138.68	3438.67 ± 172.44	ns
ASC (μg g^{-1} DW)	3043.79 ± 121.06		2764.99 ± 155.94		2719.71 ± 207.27		ns	2678.74 ± 123.20	3006.92 ± 131.77	ns
DHA (μg g^{-1} DW)	506.48 ± 91.16		371.05 ± 42.00		382.86 ± 36.09		ns	408.51 ± 34.81	431.75 ± 66.58	ns
ASC:DHA	10.06 ± 2.30		10.67 ± 1.71		8.47 ± 1.64		ns	8.84 ± 1.49	10.62 ± 1.53	ns
ASC:Total	0.86 ± 0.02		0.88 ± 0.01		0.87 ± 0.01		ns	0.87 ± 0.01	0.88 ± 0.02	ns
Total glutathione (μg g^{-1} DW)	251.14 ± 6.25		247.30 ± 10.89		238.72 ± 14.82		ns	**228.08 ± 6.26**	**263.36 ± 6.85**	**0.02**
GSH (μg g^{-1} DW)	194.34 ± 5.89		194.87 ± 8.17		184.98 ± 12.83		ns	**175.48 ± 5.22**	**207.31 ± 5.05**	**<0.01**
GSSG (μg g^{-1} DW)	56.80 ± 4.18		52.43 ± 4.26		53.74 ± 3.90		ns	52.60 ± 2.92	56.05 ± 3.57	ns
GSH:GSSG	3.84 ± 0.39		4.12 ± 0.44		3.65 ± 0.36		ns	3.78 ± 0.36	3.97 ± 0.28	ns
APX (μmol ASC min^{-1} mg^{-1} protein)	1.19 ± 0.13		1.52 ± 0.23		1.17 ± 0.16		ns	**1.57 ± 0.11**	**1.02 ± 0.12**	**0.03**
CAT (μmol H_2O_2 min^{-1} mg^{-1} protein)	214.44 ± 20.76		240.46 ± 22.99		233.87 ± 22.28		ns	262.50 ± 14.16	196.67 ± 12.88	ns

ASC, ascorbate; DHA, dehydroascorbate; GSH, reduced glutathione; GSSG, oxidized glutathione; APX, ascorbate peroxidase; CAT, catalase; ns, not significant.

555 **DISCUSSION**

556 Both *A. unedo* and *Q. suber* are woody species with sclerophyllous leaves that co-occur in
557 Mediterranean terrestrial ecosystems, and thus they have to face similar environmental
558 conditions. Despite this, the investment in leaf phenols was substantially greater in *A. unedo*,
559 while *Q. suber* appears to allocate more resources to the accumulation of ascorbate and
560 glutathione, suggesting different protective strategies to face stress conditions. In accordance
561 with previous results in Mediterranean species (Bernal et al., 2013, 2015; Nenadis et al.,
562 2015; Verdaguer et al., 2018), the concentration of total phenols was not significantly
563 modified by the experimental UV enhancement in any of the two studied species, although
564 increased UV radiation changed the leaf phenolic profile of plants, especially in the case of
565 *Q. suber*.

566

567 ***Effects of treatments on the leaf phenols of* A. unedo**

568 Changes in UV levels led to significant variations in the overall leaf amounts of flavonols of
569 *A. unedo* seedlings (i.e. before pruning). Even though the percentage of UV-A enhancement
570 was very low compared to ambient UV-A doses (Table 1), seedlings showed the lowest
571 concentration of total flavonols under enhanced UV-A (Fig. 1a), which was mainly due to a
572 tendency to have less quercetins (Fig. 1b). A decrease in the concentration of quercetins in
573 response to UV-A exposure was previously reported in leaves of wild mature *A. unedo* plants
574 (Nenadis et al., 2015). This fact might indicate a lower oxidative stress, which could be
575 related to a UV-A enhancement of photoprotective mechanisms, such as the thermal
576 dissipation of excess light energy (Bernal et al., 2015). In the present study, the trend
577 observed for quercetins in UVA plants appeared to be counteracted by enhanced UV-B (Fig.
578 1b). Taking into account that the contribution of quercetins to UV-B screening is similar to
579 the contribution of other flavonoids, such as kaempferols (Di Ferdinando et al., 2014), which

580 were not affected by the UV treatment, the UV-B response of quercetins in *A. unedo* must be
581 associated to their superior capacity to scavenge ROS (Agati et al., 2012; Hernández et al.,
582 2009), as it has been found in other species (Nybakken and Julkunen-Tiitto, 2013;
583 Randriamanana et al., 2015; Tegelberg et al., 2001). Similar results were reported by Nenadis
584 et al. (2015) in wild mature *A. unedo* plants, which showed an increase in the leaf
585 concentration of quercetin 3-rhamnoside, the most abundant quercetin derivative identified in
586 this species, in response to ambient UV-B radiation, while kaempferols remained practically
587 unaffected.

588 Overall, the resprouts of *A. unedo* showed significantly higher amounts of phenols
589 than seedlings (Table 2, Fig. 1). Previous studies have shown that young leaves of this
590 species are rich in phenols as a defense mechanism to face herbivory (Kouki and Manetas,
591 2002). In accordance with this, a much higher amount of arbutin (the most abundant phenolic
592 compound identified in the leaves of this species) after pruning could be associated to its
593 potential toxicity to organisms (Jurica et al., 2015), contributing to the protection of the
594 thinner leaves of *A. unedo* resprouts compared to seedlings (Díaz-Guerra et al., in prep.).
595 However, these differences might also be explained by environmental differences between
596 the two sampling dates, since, in October 2013 (after pruning), UV doses and mean
597 temperature were greater, while precipitation was substantially lower, compared to October
598 2012 (Table 1). Therefore, a higher concentration of flavonols, especially quercetins, in the
599 resprouts in comparison to the seedlings might be consequence of reduced photosynthetic
600 rates presumably induced by drier conditions in October 2013, as we found in a parallel study
601 (Díaz-Guerra et al., in prep.). Low photosynthetic capacity can be associated to a greater ROS
602 accumulation (Kataria et al., 2014; Lidon et al., 2012), leading to an increased production of
603 flavonols aimed to strengthen the antioxidant machinery.

604 Nevertheless, no drought-induced changes in the leaf concentration of phenols were

605 found for this species, presumably indicating that other protective mechanisms should be
606 operating in *A. unedo* plants, such as the production of non-phenolic antioxidants (see below).
607

608 ***Effects of treatments on the leaf phenols of* Q. suber**

609 Increased UV radiation changed the phenolic profile of *Q. suber* leaves; specifically,
610 enhanced UV-B increased the concentrations of five flavonols, especially under low water
611 supply. Three of these flavonols were kaempferols, and, in consequence, the total
612 concentration of kaempferols was also substantially increased by supplemented UV-A+UV-B
613 (Table 3). Monohydroxy B-ring-substituted flavonoids, such as kaempferols, are mainly
614 distributed in the epidermal cells and, despite their low concentrations, they are highly
615 effective in UV attenuation, in addition to protect leaf tissues from pathogens (Agati and
616 Tattini, 2010). Hence, the higher UV-B-induced accumulation of kaempferols in *Q. suber*
617 leaves, along with no overall differences in quercetins, and neither in the non-phenolic
618 antioxidant compounds and enzyme activities of the resprouts (Table 4), suggest that this
619 species copes with enhanced UV-B levels mainly by improving UV screening via higher
620 concentration of kaempferols instead of stimulating ROS-scavenging mechanisms (Hideg et
621 al., 2013; Majer et al., 2014).

622 The UV-B-induced increase in the concentration of kaempferols was detected mostly
623 in plants under low water availability (Fig. 2b-e), in agreement with previous reports
624 (Caldwell et al., 2007; Hofmann et al., 2003), suggesting an emphasized UV-B-screening
625 response when both factors, high UV-B levels and water constraints, co-occurred. Like UV-B
626 radiation, water stress is also associated to a higher risk of ROS generation mediated by
627 drought-induced stomatal closure (Krieger-Liszkay, 2005; Reddy et al., 2004). Therefore, in
628 plants exposed to both factors, it could be expected an overproduction of antioxidant phenols
629 in order to avoid oxidative damage (Bandurska et al., 2013). Accordingly, higher UV-B doses

630 increased the leaf concentrations of myricetin 3-glucoside and rutins in *Q. suber* plants grown
631 under low water supply (Fig. 2f-h), probably indicating a slight activation of the antioxidant
632 mechanisms (Masuoka et al., 2012; Tsurunaga et al., 2013; Zvezdanović et al., 2012). These
633 mechanisms coupled with the improved UV-B-screening capacity might contribute to face
634 these environmental conditions.

635 The lack of significant overall effects of the watering treatment on the concentration
636 of phenols of *Q. suber* leaves (Table 3) suggests that this species has other drought-avoiding
637 strategies. One of these strategies would be the adjustment of plant architecture in order to
638 minimize water loss and optimize belowground water uptake, since a lower leaf to root
639 biomass ratio was observed in low-watered *Q. suber* plants. This change in plant architecture
640 in response to drier conditions was accompanied by maintained values of leaf stomatal
641 conductance and photosynthesis in comparison to well-watered plants (Díaz-Guerra et al., in
642 prep.).

643

644 ***Effects of treatments on the leaf non-phenolic antioxidants of* A. unedo *and* Q. suber**
645 ***resprouts***

646 In *A. unedo* resprouts, non-phenolic antioxidants were much more sensitive to supplemented
647 UV radiation than phenolic compounds, whereas the opposite was found for *Q. suber*.
648 Specifically, in *A. unedo*, UV-B induced an increase in the foliar concentration of reduced
649 ascorbate and reduced glutathione and, as a consequence, of total ascorbate and total
650 glutathione, suggesting an enhanced antioxidative response (Table 4), in accordance with
651 previous studies (Agarwal, 2007; Hideg et al., 2013; Zlatev et al., 2012). The increase in the
652 reduction state and the pool-size of ascorbate and glutathione is a powerful ROS scavenger
653 mechanism to minimize photodamage in plant tissues, mainly via the ascorbate-glutathione
654 cycle (Gill and Tuteja, 2010; Noctor and Foyer, 1998). In our study, the lower activity of

655 ascorbate peroxidase (APX) found in UVAB resprouts could explain why the concentration
656 of oxidized ascorbate (i.e. dehydroascorbate) was not higher in these plants (Table 4).
657 Conversely, *A. unedo* resprouts showed an increased activity of catalase (CAT) enzymes in
658 response to enhanced UV-B. CAT enzymes have high turnover rates acting as a highly
659 efficient pathway to quench ROS, particularly when stress conditions are severe or prolonged
660 (Asada, 2006; Gill and Tuteja, 2010). When there is massive ROS generation in response to
661 an excess of excitation energy in the chloroplast, decreases in the APX activity (Agati et al.,
662 2013) can induce CAT production, since CAT enzymes are considered insensitive to the
663 redox status of the cells because they do not require a reducing substrate, and, thus, they are
664 able to maintain their activity under stress conditions (Mittler, 2002). Because of this,
665 stimulated CAT activity has been commonly reported in studies with plants exposed to
666 enhanced UV-B radiation (A-H-Mackerness, 2000; Jansen et al., 2012; Zlatev et al., 2012).
667 Overall, UV-B effects on the studied antioxidant compounds and enzymes of *A. unedo*
668 resprouts are consistent with the scarce UV effects observed on the leaf amounts of phenols,
669 since phenolic compounds, especially flavonoids, are thought to constitute a secondary
670 component of the ROS-scavenging system, which is upregulated following depletion of
671 primary antioxidants under severe stress conditions (Agati et al., 2013; Fini et al., 2011).

672 When we analyzed the effect of UV within each watering regime, we found different
673 UV effects in low- and well-watered *A. unedo* resprouts. Under low water supply, reduced
674 and total glutathione were accumulated in response to supplemental UV-B radiation, pointing
675 to an amplification of the UV-B effect by water shortage (Fig. 3a,b). Indeed, the combined
676 action of both factors (enhanced UV-B and drought) might have raised ROS production,
677 being necessary a larger pool of glutathione to maintain the normal reduced state of plant
678 cells (Meyer, 2008; Rouhier et al., 2008). On the other hand, resprouts under optimal
679 irrigation showed a reduction in the total amount of ascorbate and CAT activity together with

680 an enhanced APX activity under supplemental UV-A alone (Fig. 3c-e). These results suggest
681 higher rates of ROS detoxification by means of the ascorbate-glutathione cycle rather than
682 via CAT enzymes in UVA plants. A lower CAT activity under enhanced UV-A may also
683 indicate a lower degree of oxidative stress in accordance with previous reports (Bernal et al.,
684 2013; Nenadis et al., 2015), which might be associated to a UV-A-mediated activation of
685 photoprotective mechanisms (Bernal et al., 2015). The different responses observed in UVAB
686 and UVA resprouts of *A. unedo* indicate opposite UV-B and UV-A effects on these
687 parameters. Under drier conditions, UV-A effects on the studied non-phenolic antioxidants
688 were not significant, which suggests that they were counteracted by the detrimental effects of
689 water deficit.

690 The accumulation of key antioxidant components under drier conditions such as
691 ascorbate (DHA, ASC and total) in *A. unedo* and glutathione (GSH and total) in both species
692 indicates a reinforcement of the antioxidant machinery (Table 4), which is in accordance with
693 previous reports (Close and McArthur, 2002). Nonetheless, the reduction of the APX activity
694 along with the unaffected CAT activity in both species in response to reduced water
695 availability contrast with previous studies reporting increased enzymatic detoxification under
696 drought conditions (Reddy et al., 2004; Sánchez-Díaz et al., 2007; Selmar and Kleinwächter,
697 2013). Considering that particularly CAT activity is associated to ROS detoxification during
698 hard stress conditions (Gill and Tuteja, 2010; Mittler, 2002), our results suggest no severe
699 levels of oxidative stress in low-watered plants probably associated to a high resistance of
700 these two species to water deficit.

701

702 ***Concluding remarks***

703 Our results suggest that the two studied species have different strategies to counteract
704 environmental stress, since while *A. unedo* has a higher amount of leaf phenols, *Q. suber*

705 leaves show larger amounts of non-phenolic antioxidants. In both species, UV-induced
706 responses were only observed in the concentration of some phenolic compounds, without any
707 change in the total pool of phenols. This differential UV regulation of individual phenolic
708 compounds was probably due to dissimilarities in their contribution to leaf photoprotection
709 and/or in their antioxidant activity. Specifically, *A. unedo* would mainly respond to enhanced
710 UV-B stimulating the antioxidant response through the increase of quercetins in seedlings
711 and of key antioxidants (ascorbate, glutathione and CAT activity) in resprouts. Conversely, in
712 *Q. suber*, UV-B supplementation led to a greater accumulation of kaempferols, suggesting an
713 improved capacity for UV screening to avoid UV penetration into cells. These UV-B effects
714 on the amount of kaempferols and other flavonols of *Q. suber* leaves were mainly found
715 under water constraint. In *A. unedo* resprouts, the combination of enhanced UV-B and low
716 irrigation resulted in a higher concentration of glutathione (total glutathione and GSH),
717 probably reflecting an amplified antioxidant response. In these species, opposite UV-B and
718 UV-A effects on the levels of ascorbate and on APX and CAT activities were observed under
719 optimal irrigation. Low watering by itself favored the accumulation of key antioxidant
720 components such as ascorbate (DHA, ASC and total) and/or glutathione (GSH and total) in *A.*
721 *unedo* and *Q. suber* resprouts, but reduced and did not affect APX and CAT activities,
722 respectively.

723 Taking into account the broad range of functions of the studied compounds, the
724 notable inter-specific differences in the biochemical adjustments in response to higher UV
725 levels and decreased water availability in seedlings and resprouts of *A. unedo* and *Q. suber*
726 might imply alterations in the competitive ability of these two species under the expected
727 near-future climatic changes.

728

729

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737

738

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Month	UV supplementation (%)						Ambient data				
	UVAB plots			UVA plots			UV doses (kJ m-2 day-1)			Temperature (^0C)	Precipitation (mm)
	PGa	UV-B	UV-A	PGa	UV-B	UV-A	PGa	UV-B	UV-A		
June 2012	13	24	0.9	1.2	0.1	1.0	31.8	34.4	1291	21.0	16.8
July 2012	14	26	1.0	1.2	0.1	1.1	30.2	32.4	1195	21.7	9.5
August 2012	-	-	0.7	-	-	0.8	-	-	1070	23.9	7.2
September 2012	-	-	1.1	-	-	1.2	-	-	706	19.5	110.1
October 2012	10	24	0.8	0.9	0.1	0.9	13.9	11.6	494	15.6	166.1
November 2012	6	18	0.4	0.7	0.1	0.6	9.0	5.6	372	11.2	59.3
December 2012	5	18	0.3	0.8	0.1	0.6	7.9	4.1	353	7.2	1.5
January 2013	4	17	0.3	0.7	0.1	0.6	8.6	4.3	377	6.0	13.9
February 2013	7	24	0.4	1.2	0.2	0.9	11.5	7.1	522	5.7	38.4
March 2013	6	16	0.4	1.1	0.1	0.8	17.3	13.3	753	9.5	173.2
April 2013	7	17	0.4	1.2	0.1	1.0	23.1	20.1	1020	11.4	89.1
May 2013	8	18	0.5	1.4	0.1	1.2	27.1	25.5	1115	13.1	82.4
June 2013	11	21	0.8	1.3	0.1	1.2	34.2	34.5	1270	18.4	21.6
July 2013	15	27	1.2	1.3	0.1	1.3	32.9	35.2	1164	23.0	21.0
August 2013	17	33	1.4	1.5	0.1	1.5	28.4	29.7	986	22.2	34.8
September 2013	13	27	1.0	1.1	0.1	1.1	22.0	20.8	790	19.1	37.4
October 2013	20	46	1.5	1.7	0.2	1.6	14.4	12.0	517	17.3	29.0

a Plant growth weighting function according to Flint and Caldwell (2003)

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	UV radiation (UV)				Watering (W)			Pruning (P)			Interactions
	control	UVA	UVAB	*p*-value	WW	LW	*p*-value	seedlings	resprouts	*p*-value	
Total phenols	237.29 ± 11.14	240.38 ± 13.69	246.99 ± 10.60	ns	239.08 ± 8.14	244.02 ± 10.85	ns	**212.30 ± 6.48**	**270.81 ± 6.48**	**<0.01**	-
Tannins	89.96 ± 6.12	88.08 ± 6.01	87.43 ± 4.27	ns	85.02 ± 3.48	91.96 ± 5.12	ns	**77.54 ± 2.96**	**99.44 ± 4.11**	**<0.01**	-
Condensed tannins	74.40 ± 5.95	72.96 ± 6.55	70.84 ± 4.64	ns	70.17 ± 3.74	75.29 ± 5.34	ns	**61.90 ± 3.33**	**83.56 ± 4.29**	**<0.01**	-
Hydrolyzable tannins	15.56 ± 0.51	15.12 ± 1.20	16.60 ± 1.10	ns	14.85 ± 0.69	16.67 ± 0.85	ns	15.64 ± 0.76	15.88 ± 0.84	ns	-
Flavonoids	47.84 ± 3.71	46.71 ± 4.62	49.97 ± 3.54	ns	48.86 ± 3.33	47.48 ± 3.11	ns	**36.42 ± 1.54**	**59.92 ± 1.47**	**<0.01**	-
Flavanols	15.88 ± 2.12	15.32 ± 2.26	17.68 ± 2.61	ns	17.12 ± 1.97	15.46 ± 1.80	ns	**9.18 ± 0.55**	**23.41 ± 0.96**	**<0.01**	-
(+)-catechin	14.36 ± 1.88	13.59 ± 1.89	15.11 ± 2.26	ns	15.03 ± 1.71	13.68 ± 1.53	ns	**8.32 ± 0.41**	**20.39 ± 0.93**	**<0.01**	-
gallocatechin	1.52 ± 0.31	1.73 ± 0.48	2.57 ± 0.83	ns	2.09 ± 0.50	1.79 ± 0.46	ns	**0.85 ± 0.30**	**3.03 ± 0.48**	**<0.01**	-
Flavonols	31.95 ± 1.78	31.39 ± 2.50	32.29 ± 1.70	ns	31.74 ± 1.70	32.02 ± 1.56	ns	**27.24 ± 1.15**	**36.51 ± 1.21**	**<0.01**	UV x P
Quercetins	21.86 ± 1.53	20.47 ± 2.01	21.09 ± 1.40	ns	20.91 ± 1.29	21.38 ± 1.40	ns	**17.34 ± 0.97**	**24.95 ± 1.00**	**<0.01**	UV x P
quercetin 3-galactoside	1.88 ± 0.29	1.50 ± 0.30	1.60 ± 0.29	ns	1.58 ± 0.21	1.73 ± 0.26	ns	**1.06 ± 0.15**	**2.26 ± 0.22**	**<0.01**	-
quercetin 3-arabinoside	2.29 ± 0.25	1.88 ± 0.24	2.22 ± 0.36	ns	1.97 ± 0.16	2.28 ± 0.29	ns	**1.83 ± 0.16**	**2.43 ± 0.27**	**0.03**	-
quercetin 3-rhamnoside	14.92 ± 0.85	14.55 ± 1.26	14.15 ± 0.65	ns	15.13 ± 0.79	13.95 ± 0.73	ns	**12.73 ± 0.63**	**16.35 ± 0.64**	**<0.01**	-
quercetin-glycoside	2.78 ± 0.64	2.55 ± 0.63	3.12 ± 0.53	ns	2.22 ± 0.39	3.41 ± 0.53	ns	**1.72 ± 0.23**	**3.91 ± 0.53**	**<0.01**	-
Kaempferols	3.37 ± 0.14	3.50 ± 0.32	3.61 ± 0.17	ns	3.60 ± 0.21	3.39 ± 0.15	ns	3.33 ± 0.14	3.65 ± 0.21	ns	-
kaempferol 3-glucoside	0.50 ± 0.06	0.53 ± 0.07	0.45 ± 0.05	ns	0.56 ± 0.05	0.43 ± 0.04	ns	0.47 ± 0.05	0.51 ± 0.05	ns	-
kaempferol 3-rhamnoside	1.84 ± 0.22	1.87 ± 0.27	2.03 ± 0.19	ns	1.82 ± 0.24	2.00 ± 0.11	ns	**1.67 ± 0.13**	**2.15 ± 0.21**	**0.03**	-
kaempferol glycoside	1.03 ± 0.11	1.09 ± 0.16	1.13 ± 0.17	ns	1.22 ± 0.10	0.96 ± 0.13	ns	1.19 ± 0.09	0.99 ± 0.14	ns	-
Myricetins											
myricetin 3-rhamnoside	6.73 ± 0.33	7.42 ± 0.59	7.59 ± 0.47	ns	7.23 ± 0.48	7.25 ± 0.28	ns	**6.57 ± 0.28**	**7.91 ± 0.42**	**0.03**	-
Hydroquinones	97.30 ± 4.22	103.28 ± 6.79	107.31 ± 7.82	ns	102.94 ± 5.21	102.31 ± 5.36	ns	**96.15 ± 4.22**	**109.10 ± 5.76**	**0.04**	-
arbutin	50.86 ± 3.21	52.44 ± 4.16	54.05 ± 4.25	ns	51.23 ± 2.76	53.67 ± 3.48	ns	**45.50 ± 1.71**	**59.40 ± 3.36**	**<0.01**	-
galloylarbutin	32.36 ± 2.82	35.14 ± 4.07	37.42 ± 4.71	ns	36.27 ± 3.15	33.68 ± 3.25	ns	33.73 ± 2.85	36.21 ± 3.51	ns	-
digalloylarbutin	14.08 ± 1.09	15.70 ± 1.86	15.83 ± 1.48	ns	15.45 ± 1.14	14.96 ± 1.31	ns	16.91 ± 0.98	13.49 ± 1.32	ns	-
Phenolic acids	2.20 ± 0.13	2.30 ± 0.19	2.29 ± 0.17	ns	2.26 ± 0.14	2.27 ± 0.13	ns	2.18 ± 0.08	2.34 ± 0.17	ns	-
gallic acid	1.77 ± 0.12	1.84 ± 0.18	1.91 ± 0.18	ns	1.84 ± 0.14	1.84 ± 0.12	ns	1.78 ± 0.09	1.90 ± 0.16	ns	-
ellagic acid	0.44 ± 0.03	0.46 ± 0.03	0.37 ± 0.03	ns	0.42 ± 0.02	0.42 ± 0.03	ns	0.41 ± 0.02	0.44 ± 0.02	ns	-
TAN:TP	0.38 ± 0.01	0.37 ± 0.02	0.36 ± 0.02	ns	0.36 ± 0.01	0.38 ± 0.01	ns	0.37 ± 0.01	0.37 ± 0.01	ns	-
FLAV:TP	0.20 ± 0.01	0.19 ± 0.01	0.20 ± 0.01	ns	0.20 ± 0.01	0.19 ± 0.01	ns	**0.17 ± 0.01**	**0.22 ± 0.01**	**<0.01**	-
Que:Kae	6.52 ± 0.42	6.07 ± 0.63	5.97 ± 0.51	ns	5.98 ± 0.43	6.39 ± 0.42	ns	**5.25 ± 0.25**	**7.13 ± 0.44**	**<0.01**	-
Hq:TP	0.41 ± 0.02	0.43 ± 0.02	0.43 ± 0.02	ns	0.43 ± 0.02	0.42 ± 0.01	ns	**0.45 ± 0.01**	**0.40 ± 0.01**	**0.02**	-
PA:TP	0.0094 ± 0.0006	0.0097 ± 0.0007	0.0093 ± 0.0006	ns	0.0095 ± 0.0005	0.0095 ± 0.0006	ns	**0.0104 ± 0.0004**	**0.0086 ± 0.0005**	**0.03**	-

TP, total phenols; TAN, tannins; FLAV, flavonoids; Que, quercetins; Kae, kaempferols; Hq, hydroquinones; PA, phenolic acids; ns, not significant.

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	UV radiation (UV)							Watering (W)			Pruning (P)			Interactions
	control		UVA		UVAB		*p*-value	WW	LW	*p*-value	seedlings	resprouts	*p*-value	
Total phenols	90.45 ± 7.70		88.43 ± 3.99		92.76 ± 7.34		ns	89.33 ± 4.67	91.77 ± 5.83	ns	**80.21 ± 4.89**	**100.88 ± 4.43**	**0.01**	-
Tannins	60.83 ± 6.43		59.72 ± 3.25		62.21 ± 6.42		ns	59.48 ± 4.14	62.37 ± 4.78	ns	55.35 ± 4.46	66.50 ± 4.09	ns	-
Condensed tannins	55.67 ± 6.39		53.95 ± 3.23		56.14 ± 6.42		ns	53.40 ± 4.14	57.10 ± 4.73	ns	50.83 ± 4.39	59.67 ± 4.30	ns	-
Hydrolyzable tannins	5.17 ± 0.47		5.77 ± 0.52		6.07 ± 0.42		ns	6.08 ± 0.38	5.26 ± 0.38	ns	**4.51 ± 0.24**	**6.83 ± 0.29**	**<0.01**	-
Flavonoids	29.26 ± 1.76		28.38 ± 1.44		30.22 ± 1.68		ns	29.51 ± 1.32	29.07 ± 1.33	ns	**24.57 ± 0.61**	**34.01 ± 0.72**	**<0.01**	-
Flavanols	17.84 ± 1.28		17.23 ± 0.94		17.29 ± 1.18		ns	17.62 ± 0.92	17.29 ± 0.92	ns	**14.41 ± 0.55**	**20.50 ± 0.55**	**<0.01**	-
(+)-catechin	10.01 ± 1.08		9.45 ± 0.50		9.19 ± 0.91		ns	9.41 ± 0.71	9.70 ± 0.69	ns	8.89 ± 0.68	10.21 ± 0.68	ns	-
gallocatechin	1.97 ± 0.26		1.93 ± 0.18		2.45 ± 0.35		ns	2.42 ± 0.26	1.82 ± 0.16	ns	1.88 ± 0.21	2.36 ± 0.22	ns	-
epigallocatechin	3.74 ± 0.52		3.52 ± 0.63		3.67 ± 0.66		ns	3.72 ± 0.47	3.57 ± 0.50	ns	**2.11 ± 0.21**	**5.18 ± 0.39**	**<0.01**	-
epigallocatechin gallate	2.22 ± 0.27		2.32 ± 0.24		1.98 ± 0.24		ns	2.14 ± 0.20	2.21 ± 0.20	ns	**1.59 ± 0.15**	**2.76 ± 0.14**	**<0.01**	-
Flavonols	**11.42 ± 0.69**	**b**	**11.15 ± 0.61**	**b**	**12.94 ± 0.57**	**a**	**0.05**	11.89 ± 0.58	11.79 ± 0.49	ns	**10.16 ± 0.38**	**13.51 ± 0.32**	**<0.01**	-
Quercetins	4.19 ± 0.32		3.90 ± 0.34		4.41 ± 0.40		ns	4.17 ± 0.30	4.16 ± 0.28	ns	**3.22 ± 0.13**	**5.11 ± 0.22**	**<0.01**	-
quercetin 3-galactoside	1.46 ± 0.11		1.40 ± 0.12		1.45 ± 0.15		ns	1.42 ± 0.11	1.45 ± 0.09	ns	**1.15 ± 0.04**	**1.73 ± 0.10**	**<0.01**	-
quercetin 3-glucoside	2.09 ± 0.19		1.91 ± 0.19		2.24 ± 0.22		ns	2.09 ± 0.17	2.07 ± 0.16	ns	**1.54 ± 0.10**	**2.62 ± 0.10**	**<0.01**	-
quercetin 3-rhamnoside	0.32 ± 0.03		0.27 ± 0.02		0.36 ± 0.03		ns	0.32 ± 0.03	0.31 ± 0.02	ns	**0.27 ± 0.02**	**0.36 ± 0.02**	**<0.01**	-
quercetin-glycoside	0.32 ± 0.03		0.32 ± 0.03		0.36 ± 0.04		ns	0.33 ± 0.03	0.33 ± 0.02	ns	**0.26 ± 0.01**	**0.41 ± 0.03**	**<0.01**	-
Kaempferols	**1.29 ± 0.09**	**b**	**1.38 ± 0.09**	**b**	**1.80 ± 0.09**	**a**	**0.01**	1.56 ± 0.09	1.43 ± 0.09	ns	1.45 ± 0.09	1.53 ± 0.09	ns	-
kaempferol 3-glucoside	**0.66 ± 0.08**	**b**	**0.76 ± 0.06**	**b**	**1.05 ± 0.08**	**a**	**0.03**	0.88 ± 0.07	0.76 ± 0.07	ns	0.85 ± 0.07	0.80 ± 0.08	ns	-
kaempferol glycoside	0.30 ± 0.02		0.28 ± 0.02		0.31 ± 0.03		ns	0.29 ± 0.02	0.30 ± 0.02	ns	**0.26 ± 0.02**	**0.32 ± 0.02**	**0.04**	-
monocoumaroyl-astragalin 1	**0.18 ± 0.01**	**b**	**0.20 ± 0.01**	**b**	**0.26 ± 0.02**	**a**	**0.01**	0.23 ± 0.02	0.20 ± 0.01	ns	**0.20 ± 0.01**	**0.23 ± 0.01**	**0.01**	-
monocoumaroyl-astragalin 2	**0.15 ± 0.01**	**ab**	**0.15 ± 0.01**	**b**	**0.18 ± 0.02**	**a**	**0.05**	0.16 ± 0.01	0.16 ± 0.01	ns	**0.14 ± 0.01**	**0.18 ± 0.01**	**0.03**	-
Myricetins	0.15 ± 0.03		0.21 ± 0.04		0.26 ± 0.04		ns	0.20 ± 0.03	0.22 ± 0.03	ns	0.22 ± 0.03	0.20 ± 0.03	ns	-
myricetin 3-galactoside	0.06 ± 0.02		0.09 ± 0.01		0.08 ± 0.02		ns	0.06 ± 0.01	0.09 ± 0.01	ns	0.08 ± 0.01	0.07 ± 0.02	ns	-
myricetin 3-glucoside	0.09 ± 0.01		0.12 ± 0.03		0.18 ± 0.03		ns	0.14 ± 0.03	0.12 ± 0.02	ns	0.14 ± 0.02	0.12 ± 0.02	ns	-
Rutins	1.88 ± 0.08		1.81 ± 0.09		2.06 ± 0.09		ns	1.92 ± 0.09	1.92 ± 0.06	ns	**1.79 ± 0.08**	**2.04 ± 0.05**	**0.05**	-
rutin derivative 1	**1.63 ± 0.09**	**ab**	**1.57 ± 0.08**	**b**	**1.82 ± 0.08**	**a**	**0.05**	1.66 ± 0.08	1.68 ± 0.06	ns	**1.54 ± 0.08**	**1.81 ± 0.05**	**0.02**	-
rutin derivative 2	0.24 ± 0.01		0.25 ± 0.02		0.24 ± 0.02		ns	0.25 ± 0.02	0.24 ± 0.01	ns	0.25 ± 0.01	0.24 ± 0.01	ns	-
unknown flavonols	3.92 ± 0.29		3.84 ± 0.29		4.40 ± 0.28		ns	4.05 ± 0.25	4.06 ± 0.22	ns	**3.49 ± 0.20**	**4.62 ± 0.18**	**<0.01**	-
flavonol 1	0.59 ± 0.05		0.70 ± 0.07		0.75 ± 0.06		ns	0.71 ± 0.06	0.65 ± 0.04	ns	**0.54 ± 0.04**	**0.82 ± 0.04**	**<0.01**	-
flavonol 2	1.66 ± 0.18		1.63 ± 0.19		1.85 ± 0.17		ns	1.68 ± 0.15	1.75 ± 0.15	ns	**1.31 ± 0.09**	**2.11 ± 0.13**	**<0.01**	-
flavonol 3	0.05 ± 0.01		0.06 ± 0.01		0.07 ± 0.01		ns	0.06 ± 0.01	0.06 ± 0.01	ns	0.06 ± 0.01	0.07 ± 0.01	ns	-
flavonol 4	1.62 ± 0.12		1.45 ± 0.10		1.73 ± 0.14		ns	1.60 ± 0.12	1.60 ± 0.08	ns	1.58 ± 0.11	1.62 ± 0.09	ns	-
Phenolic acids														
ellagic acid	0.35 ± 0.02		0.33 ± 0.02		0.33 ± 0.02		ns	0.34 ± 0.02	0.33 ± 0.02	ns	**0.30 ± 0.02**	**0.37 ± 0.01**	**<0.01**	-
TAN:TP	0.65 ± 0.01		0.66 ± 0.01		0.64 ± 0.02		ns	0.64 ± 0.01	0.66 ± 0.01	ns	0.66 ± 0.01	0.64 ± 0.01	ns	-
FLAV:TP	0.34 ± 0.01		0.33 ± 0.01		0.36 ± 0.02		ns	0.35 ± 0.01	0.34 ± 0.01	ns	0.34 ± 0.01	0.35 ± 0.01	ns	-
Que:Kae	3.68 ± 0.32		3.28 ± 0.38		3.03 ± 0.51		ns	3.22 ± 0.35	3.44 ± 0.32	ns	**2.64 ± 0.20**	**4.02 ± 0.36**	**0.01**	-
PA:TP	0.0047 ± 0.0007		0.0039 ± 0.0002		0.0044 ± 0.0006		ns	0.0046 ± 0.0004	0.0041 ± 0.0005	ns	0.0047 ± 0.0006	0.0040 ± 0.0002	ns	-

TP, total phenols; TAN, tannins; FLAV, flavonoids; Que, quercetins; Kae, kaempferols; Hq, hydroquinones; PA, phenolic acids; ns, not significant.

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	UV radiation							Watering		
	control		UVA		UVAB		*p*-value	WW	LW	*p*-value
A. unedo resprouts										
Total ascorbate (μg g^{-1} DW)	**819.52 ± 56.21**	**ab**	**739.55 ± 100.49**	**b**	**932.20 ± 118.54**	**a**	**0.04**	**647.29 ± 37.93**	**1013.56 ± 55.83**	**<0.01**
ASC (μg g^{-1} DW)	**673.67 ± 46.43**	**ab**	**621.80 ± 77.73**	**b**	**788.67 ± 97.69**	**a**	**0.04**	**551.76 ± 34.14**	**837.67 ± 48.30**	**<0.01**
DHA (μg g^{-1} DW)	145.85 ± 19.07		117.75 ± 26.44		144.39 ± 24.59		ns	**95.53 ± 13.58**	**176.46 ± 11.95**	**0.01**
ASC:DHA	6.99 ± 1.39		6.56 ± 1.25		6.86 ± 1.28		ns	8.02 ± 1.24	5.58 ± 0.47	ns
ASC:Total	0.83 ± 0.02		0.85 ± 0.02		0.85 ± 0.02		ns	0.85 ± 0.02	0.83 ± 0.01	ns
Total glutathione (μg g^{-1} DW)	**100.16 ± 6.46**	**ab**	**90.15 ± 4.39**	**b**	**113.42 ± 7.90**	**a**	**0.03**	**96.87 ± 5.31**	**105.63 ± 6.41**	**0.05**
GSH (μg g^{-1} DW)	**74.49 ± 4.79**	**ab**	**66.14 ± 2.70**	**b**	**84.20 ± 5.54**	**a**	**0.01**	**71.96 ± 3.86**	**77.93 ± 4.68**	**0.01**
GSSG (μg g^{-1} DW)	25.67 ± 1.91		24.01 ± 2.00		29.23 ± 2.95		ns	24.91 ± 1.58	27.70 ± 2.24	ns
GSH:GSSG	3.00 ± 0.16		2.84 ± 0.17		3.09 ± 0.27		ns	3.02 ± 0.09	2.94 ± 0.22	ns
APX (μmol ASC min^{-1} mg^{-1} protein)	**1.46 ± 0.16**	**b**	**1.96 ± 0.29**	**a**	**1.36 ± 0.16**	**b**	**0.02**	**1.96 ± 0.15**	**1.23 ± 0.13**	**0.01**
CAT (μmol H_2O_2 min^{-1} mg^{-1} protein)	**239.64 ± 16.74**	**ab**	**211.94 ± 11.31**	**b**	**272.71 ± 10.34**	**a**	**0.01**	255.92 ± 13.45	226.95 ± 11.64	ns
Q. suber resprouts										
Total ascorbate (μg g^{-1} DW)	3550.27 ± 178.94		3136.03 ± 191.11		3102.58 ± 206.47		ns	3087.25 ± 138.68	3438.67 ± 172.44	ns
ASC (μg g^{-1} DW)	3043.79 ± 121.06		2764.99 ± 155.94		2719.71 ± 207.27		ns	2678.74 ± 123.20	3006.92 ± 131.77	ns
DHA (μg g^{-1} DW)	506.48 ± 91.16		371.05 ± 42.00		382.86 ± 36.09		ns	408.51 ± 34.81	431.75 ± 66.58	ns
ASC:DHA	10.06 ± 2.30		10.67 ± 1.71		8.47 ± 1.64		ns	8.84 ± 1.49	10.62 ± 1.53	ns
ASC:Total	0.86 ± 0.02		0.88 ± 0.01		0.87 ± 0.01		ns	0.87 ± 0.01	0.88 ± 0.02	ns
Total glutathione (μg g^{-1} DW)	251.14 ± 6.25		247.30 ± 10.89		238.72 ± 14.82		ns	**228.08 ± 6.26**	**263.36 ± 6.85**	**0.02**
GSH (μg g^{-1} DW)	194.34 ± 5.89		194.87 ± 8.17		184.98 ± 12.83		ns	**175.48 ± 5.22**	**207.31 ± 5.05**	**<0.01**
GSSG (μg g^{-1} DW)	56.80 ± 4.18		52.43 ± 4.26		53.74 ± 3.90		ns	52.60 ± 2.92	56.05 ± 3.57	ns
GSH:GSSG	3.84 ± 0.39		4.12 ± 0.44		3.65 ± 0.36		ns	3.78 ± 0.36	3.97 ± 0.28	ns
APX (μmol ASC min^{-1} mg^{-1} protein)	1.19 ± 0.13		1.52 ± 0.23		1.17 ± 0.16		ns	**1.57 ± 0.11**	**1.02 ± 0.12**	**0.03**
CAT (μmol H_2O_2 min^{-1} mg^{-1} protein)	214.44 ± 20.76		240.46 ± 22.99		233.87 ± 22.28		ns	262.50 ± 14.16	196.67 ± 12.88	ns

ASC, ascorbate; DHA, dehydroascorbate; GSH, reduced glutathione; GSSG, oxidized glutathione; APX, ascorbate peroxidase; CAT, catalase; ns, not significant.

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Leaf biochemical adjustments in two Mediterranean resprouter species facing enhanced UV levels and reduced water availability before and after aerial biomass removal

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Highlights:

- UV induced different leaf biochemical responses in two co-occurring woody species

- *Quercus suber* improved the leaf UV-screening capacity to face enhanced UV-B

- Over-ambient UV-B levels stimulated the antioxidant defences of *Arbutus unedo* leaves

- Water shortage exacerbated some of the UV-B-induced responses in both species

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Leaf biochemical adjustments in two Mediterranean resprouter species facing enhanced UV levels and reduced water availability before and after aerial biomass removal

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Contributions

L. Díaz-Guerra, D. Verdaguer and L. Llorens designed and performed the field experiment, as well as the samplings and analyses of plants, the processing of the results and the writing of the manuscript; L. Díaz-Guerra and R. Julkunen-Tiitto analyzed the content of phenolic compounds in leaves; I. Nogués carried out the analyses of non-phenolic antioxidants in leaves; J. Font collaborated in the samplings and contributed to maintain the field experiment; and J.A. González performed the spectral measurements and verified periodically the UV doses applied throughout the experiment. All authors contributed to the manuscript writing and gave final approval for its publication.

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