CLIMATE CHANGE AND MEDITERRANEAN ECOSYSTEMS: PLANT AND SOIL RESPONSES TO UV RADIATION AND WATER AVAILABILITY BEFORE AND AFTER A PERTURBATION

Laura Díaz Guerra

Universitat de Girona

Doctoral Thesis

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Supervised by

Dra. Dolors Verdaguer Murla **Dra. Laura Llorens Guasch**

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Universitat de Girona

La Dra. Dolors Verdaguer Murla i la Dra. Laura Llorens Guasch, de la Universitat de Girona

DECLAREM:

Que el treball titulat "**Climate change and Mediterranean ecosystems: plant and soil responses to UV radiation and water availability before and after a perturbation**", que presenta **Laura Díaz Guerra** per a l'obtenció del títol de doctora, ha estat realitzat sota la nostra direcció i que compleix els requisits per poder optar a Menció Internacional.

I, perquè així consti i tingui els efectes oportuns, signem aquest document.

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Girona, abril del 2017

A mis padres,

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Contents

Resum

A la conca del Mediterrani, tot i la recuperació de la capa d'ozó, s'espera que la reducció de la nuvolositat, com a conseqüència del canvi climàtic, incrementi els nivells de radiació UV solar (UV-A i UV-B) que arriben a la Terra en els pròxims anys, especialment durant els mesos d'estiu. Alhora, degut a les condicions més seques i més càlides que es donaran, es preveu que els incendis forestals siguin més freqüents i intensos. Dosis més elevades d'UV i una reducció en la disponibilitat d'aigua podrien afectar els trets bioquímics, morfològics i fisiològics de les plantes, modificant el seu creixement, amb la qual cosa es podria veure alterada la capacitat de regeneració d'aquestes després d'una pertorbació, fet que seria especialment rellevant per a les espècies rebrotadores. Una reducció en la capacitat de rebrotar de les plantes en resposta a nivells més elevats d'UV i a l'escassetat d'aigua podria tenir implicacions en la resiliència dels ecosistemes arbustius, un dels ecosistemes terrestres mediterranis més extensos d'Europa. A més, el paper de la radiació UV en el funcionament dels matollars mediterranis, en particular els seus efectes sobre els nivells de C i N en plantes i sòls, segueix essent poc clar, no existint informació sobre els possibles efectes interactius de la radiació UV amb la precipitació i/o els incendis. El present estudi té com a objectiu aportar informació sobre els efectes que els canvis projectats en la radiació UV i la precipitació podrien tenir sobre les espècies rebrotadores mediterrànies i sobre els ecosistemes arbustius en un futur pròxim, tenint en compte el possible impacte modulador de l'eliminació de la biomassa aèria de les plantes a causa d'una pertorbació (com podria ser un incendi).

En aquest context, els objectius específics d'aquesta tesi són: examinar els efectes de l'augment de la radiació UV (UV-A i UV-B) i de la disminució de la disponibilitat hídrica, abans i després d'una pertorbació que implica l'eliminació de la biomassa aèria, sobre i) el contingut foliar de fenols i components antioxidants, i ii) la morfologia i fisiologia de les fulles, creixement de les plantes, contingut en midó i fenols de les arrels i, finalment, la capacitat de rebrotar de dues espècies rebrotadores mediterrànies; i, per altra banda, iii) investigar l'efecte de la radiació UV (UV-A i UV-B) sobre els nivells de C i N del sòl i de les espècies dominants d'un matollar mediterrani abans i després d'un incendi, i veure si aquests efectes poden variar al reduir-se la precipitació. Per assolir els objectius 1 i 2, es va realitzar un experiment a l'aire lliure de suplementació de radiació UV en combinació amb dos nivells de reg utilitzant plàntules d'*Arbutus unedo* i *Quercus suber*, dues espècies esclerofil·les rebrotadores. A meitat de l'experiment, es va eliminar la part aèria de les plàntules i es va determinar el número de plantes rebrotades. Tant en plàntules com en rebrots, es van mesurar diversos paràmetres, en fulles i arrels, biomètrics, morfomètrics, fisiològics i bioquímics. Per assolir l'objectiu 3, es va realitzar un experiment en una comunitat arbustiva natural en què es van modificar els nivells de radiació UV i precipitació que arribaven a l'ecosistema. A la meitat del període d'estudi, tota la zona es va cremar de forma experimental. Es va mesurar la cobertura vegetal i de fullaraca, i diferents paràmetres relacionats amb els nivells de C i N en el sòl, en la fullaraca i en les fulles d'*A. unedo* i *Phillyrea angustifolia*, les dues espècies dominants de la comunitat.

Com a resultats principals, les plantes d'*A. unedo* van incrementar el conjunt de compostos antioxidants, especialment els nivells de quercetines, ascorbat, glutatió i l'activitat catalasa (CAT) en resposta a l'augment de la radiació UV, especialment UV-B. En canvi, *Q. suber*, per a fer front a l'increment d'UV-B, va augmentar els compostos que s'associen amb l'absorció i/o reflexió de la radiació UV, com els kaempferols, evitant la penetració de la radiació UV-B en els teixits de la fulla. Aquesta espècie també va respondre a l'augment d'UV-A, però en aquest cas la resposta va implicar ajustos morfològics per endurir les fulles (més petites i amb més massa per àrea (LMA)), i canvis en l'assignació de carboni, augmentant proporcionalment la biomassa destinada a arrels en relació a la destinada a fulles. Pel que fa a la disponibilitat hídrica, mentre *A. unedo* hauria contrarestat l'escassetat d'aigua incrementant l'activitat antioxidant, de forma similar al descrit en resposta a la radiació UV-B, *Q. suber* disminuiria l'assignació de biomassa a les fulles en relació a les arrels i a la biomassa total, minimitzant la pèrdua d'aigua per transpiració i augmentant l'absorció d'aigua per les arrels. Tot i que diferents, les estratègies presentades per les dues espècies per a fer front a la radiació UV i/o a la sequera serien, aparentment, efectives, ja que no van disminuir les reserves a nivell radicular (midó), ni el número de plantes rebrotades de cap de les dues espècies. No obstant això, la sensibilitat de les plàntules i dels rebrots a la radiació UV i a la disponibilitat hídrica va ser notablement diferent segons l'espècie. Així, els rebrots d'*A. unedo* varen ser més sensibles als tractaments que els de *Q. suber*. A nivell d'ecosistema, la radiació UV-A i UV-B van tenir efectes oposats sobre els nivells de C i N, essent aquests efectes en algun cas intensificats per la reducció de la precipitació. Abans de l'incendi, les parcel·les exposades a la radiació UV-A van presentar valors més elevats d'humitat del sòl, respiració i activitat β-glucosidasa, la qual cosa suggereix una major activitat biològica del sòl i un major reciclatge de C i N. En canvi, la presència addicional d'UV-B va fer disminuir la respiració del sòl, el pH i l'activitat β-glucosidasa, i va augmentar la concentració de C en les fulles de *P. angustifolia*, cosa que indica una menor activitat dels microorganismes del sòl, alentint, molt probablement, els cicles del C i del N. Una menor precipitació, juntament amb l'exposició a la radiació UV-B, també va fer augmentar els valors de $\delta^{15}N$ de les fulles i la fullaraca d'*A. unedo*, suggerint una major nitrificació i pèrdues de N en el sòl per lixiviació. La majoria dels efectes descrits prèviament només es van trobar abans de l'incendi, la qual cosa suggereix que el foc hauria tingut un efecte homogeneïtzador sobre aquests.

En resum, en les pròximes dècades, els canvis en els cicles del C i del N en les comunitats arbustives mediterrànies dependrien, entre d'altres factors, del balanç entre els efectes oposats de l'augment de la radiació UV-A i UV-B sobre els processos del sòl, essent modulats per la disponibilitat hídrica així com per les respostes específiques de les espècies a aquests factors, que alhora segons l'espècie podrien accentuar-se o atenuar-se després d'una pertorbació com ara el foc o la tala.

Resumen

En la cuenca mediterránea, a pesar de la recuperación de la capa de ozono, se espera que la reducción de la cobertura de nubes como consecuencia del cambio climático incremente los niveles de radiación UV solar (UV-A y UV-B) en los próximos años, especialmente durante los meses de verano. Al mismo tiempo, condiciones más secas y más cálidas favorecerán la aparición de incendios forestales más frecuentes e intensos. A nivel de planta, los efectos de mayores dosis de radiación UV y una baja disponibilidad hídrica sobre los rasgos bioquímicos, morfológicos, fisiológicos y de crecimiento podrían alterar la capacidad que presentan las especies rebrotadoras del Mediterráneo de regenerarse después de una perturbación. En última instancia, los cambios en la capacidad de rebrotar de las especies vegetales que coexisten en respuesta a un aumento de los niveles de UV y disminución del agua disponible, podrían tener implicaciones en la resiliencia de los matorrales, uno de los ecosistemas terrestres mediterráneos más extensos de Europa. Además, el papel de la radiación UV en el funcionamiento de los matorrales mediterráneos, en particular sus efectos sobre los niveles de C y N en plantas y suelos, sigue siendo desconocido, no habiendo información sobre sus posibles efectos en combinación con una disminución de la precipitación y/o los incendios. El presente estudio tiene como objetivo contribuir a dilucidar los efectos que los cambios proyectados en la radiación UV y la precipitación podrían tener sobre las especies rebrotadoras mediterráneas y los ecosistemas arbustivos en un futuro próximo, teniendo en cuenta el posible impacto de la eliminación de la biomasa aérea como consecuencia de una perturbación (como podría ser un incendio) modulando estos efectos.

En este contexto, los objetivos específicos de esta tesis fueron: examinar los efectos del aumento de la radiación UV (UV-A y UV-B) y de la disminución de la disponibilidad hídrica, antes y después de una perturbación que implicara la eliminación de la biomasa aérea, sobre i) el contenido foliar de fenoles y de ciertos elementos antioxidantes, y ii) la morfología y fisiología de las hojas, el crecimiento, las reservas radiculares y, finalmente, la capacidad de rebrotar de dos especies rebrotadoras mediterráneas; y, por otro lado, iii) investigar el efecto de la radiación UV (UV-A y UV-B) sobre los niveles de C y N del suelo y de las especies vegetales dominantes de un matorral mediterráneo antes y después de un incendio, y si estos efectos pueden ser alterados por una reducción en la precipitación. Para lograr los objetivos 1 y 2, se realizó un experimento al aire libre de suplementación de radiación UV combinado con dos niveles de riego utilizando plántulas de *Arbutus unedo* y *Quercus suber*, dos especies esclerófilas y rebrotadoras, cuya biomasa aérea fue eliminada durante el período de estudio. En plántulas y rebrotes se midieron varios parámetros asociados a la bioquímica, morfología y fisiología de las hojas, la biomasa vegetal y la bioquímica de las raíces y también se midió el éxito del rebrote. Para lograr el objetivo 3, se realizó un experimento de campo en una comunidad arbustiva natural, donde se modificaron los niveles de UV y disponibilidad de agua que llegaban al ecosistema, realizando un fuego experimental hacia la mitad del período de estudio. Además de la cobertura vegetal y de hojarasca, se midieron diferentes parámetros relacionados con los niveles de C y N en el suelo, y en la hojarasca y las hojas de *A. unedo* y *Phillyrea angustifolia*, dos de las especies dominantes en la comunidad mediterránea.

Como resultados principales, las plantas de *A. unedo* respondieron al aumento en la radiación UV, en especial UV-B, reforzando la maquinaria antioxidante a través de incrementos en los niveles de quercetinas, ascorbato y glutatión junto con una estimulación de la actividad catalasa (CAT). Por el contrario, *Q. suber*, para hacer frente al incremento de UV-B, aumentó su capacidad de reflejar este tipo de radiación, evitando su penetración en los tejidos foliares a través de la acumulación de kaempferoles. Esta especie también fue sensible al aumento en la radiación UV-A, pero en este caso la respuesta implicó ajustes morfológicos para endurecer las hojas (hojas más pequeñas con mayor masa por área (LMA)), junto con una asignación de carbono proporcionalmente mayor a las raíces (menor proporción de biomasa foliar respecto a la biomasa radicular). Con respecto a la disponibilidad hídrica, *A. unedo* contrarrestó las limitaciones de agua mediante la estimulación de la actividad antioxidante foliar, al igual que se observó en respuesta al aumento de la radiación UV-B. En cambio, la respuesta principal de *Q. suber* a la disminución del contenido hídrico fue una reducción de la biomasa foliar en relación a la biomasa radicular y total, probablemente como una estrategia para minimizar la pérdida de agua por transpiración y aumentar la absorción de agua por las raíces. Las estrategias protectoras presentadas por ambas especies fueron aparentemente efectivas, ya que no se encontró una reducción en las sustancias de reserva de las raíces o, en el éxito del rebrote. Sin embargo, la sensibilidad de los rebrotes a la radiación UV y a los niveles de agua fue notablemente diferente en relación a la de las plántulas, siendo más sensibles los rebrotes que las plántulas en *A. unedo*, y al contrario en *Q. suber*. En relación al ecosistema, se observaron efectos opuestos de la exposición a la radiación UV-A y UV-B sobre los niveles de C y N, efectos que a menudo fueron enfatizados por una disminución de la precipitación. Durante el período previo al incendio, la exposición a la radiación UV-A aumentó la humedad, la respiración y la actividad β-glucosidasa del suelo, lo que sugiere una mayor actividad biológica y un mayor reciclaje de C. La presencia adicional de radiación UV-B disminuyó la respiración, el pH y la actividad β-glucosidasa del suelo, aumentando la concentración de C en las hojas de *P. angustifolia*, lo que indica una menor actividad de los microorganismos del suelo probablemente ralentizando los ciclos del C y del N. En condiciones de reducida precipitación, la exposición a la radiación UV-B también aumentó los valores de $\delta^{15}N$ en hojas y hojarasca de *A. unedo*, cosa que sugiere una mayor nitrificación y mayores pérdidas de N por lixiviación en el suelo. La mayoría de los efectos descritos previamente sólo se encontraron antes del incendio, indicando una influencia homogeneizadora de esta perturbación sobre estos efectos.

En resumen, en las próximas décadas, los cambios inducidos por el incremento de la radiación UV y por la disminución de la precipitación sobre los niveles de C y N de los matorrales mediterráneos estarían en parte determinados por los efectos opuestos de las radiaciones UV-A y UV-B sobre los procesos del suelo, siendo modulados por la disponibilidad hídrica así como por las respuestas especie-específicas a estos factores, las cuales podrían acentuarse o atenuarse después de una perturbación (incendio, herbivoría, tala) dependiendo de la especie.

Abstract

In the Mediterranean basin, despite the current recovery of the ozone layer, reduction in cloud cover as a consequence of climate change is expected to enhance solar UV levels (UV-A and UV-B) decreasing precipitation amount over the coming years, especially during summer months. At the same time, drier and warmer conditions would favor the occurrence of more frequent and intense wildfires. At plant level, the potential effects of higher UV doses and diminished water availability on the biochemical, morphological, physiological and growth traits could alter the regeneration ability after a disturbance of Mediterranean resprouter species. Ultimately, variations in resprouting success among plant species in response to UV and water levels could have implications on the resilience of shrubland ecosystems, one of the most extensive Mediterranean-type terrestrial community in Europe. In addition, the role of UV radiation in the functioning of Mediterranean shrublands, particularly its effects on C and N pools in plants and soils, remains still unclear, with null information about its possible interactive effects with precipitation amounts and/or fire. The present study aims to contribute to the elucidation of the effects that projected changes in UV radiation and precipitation could have on Mediterranean resprouter species and shrubland ecosystems in the near future, taking into account the possible impact of the aerial plant biomass removal due to a disturbance (such as a fire) on modulating these effects.

In this context, the specific objectives pursued in this thesis were: to examine the effects of enhanced UV radiation (UV-A and UV-B) and diminished water supply, before and after a disturbance implying aerial biomass removal, on i) leaf content of phenols and antioxidant components, and ii) leaf morphology and physiology, plant growth, root reserves and, finally, resprouting capacity of two common Mediterranean resprouter species; and iii) to investigate the role of UV radiation (UV-A and UV-B) on C and N pools of the soil and dominant plant species of a Mediterranean shrubland before and after a fire, and whether these UV effects can be altered by the expected reduction in precipitation. To achieve objectives 1 and 2, an outdoor experiment involving UV supplementation combined with two levels of irrigation was conducted using seedlings of *Arbutus unedo* and *Quercus suber*, two sclerophyllous resprouter species, whose aerial biomass was removed during the study period. Several parameters associated to leaf biochemistry, morphology and physiology, plant biomass, root biochemistry and resprouting success were measured in seedlings and resprouts. To accomplish objective 3, a field experiment was conducted in a natural Mediterranean shrub community, where the levels of UV and water availability reaching the ecosystem were modified, performing an experimental fire around the middle of the study period. In addition to plant and litter cover, different parameters related to C and N pools were measured in the soil, and in the litter and leaves of *A. unedo* and *Phillyrea angustifolia*, two resprouter species largely dominant in the shrubland community.

As main results, responses of *A. unedo* plants to enhanced UV, especially UV-B, were mainly addressed to the reinforcement of the antioxidant machinery through increases in the levels of quercetins, ascorbate and glutathione coupled with an upregulation of catalase (CAT) activities. In contrast, *Q. suber* augmented UV-screening to face UV-B enhancement, avoiding UV-B penetration into leaf tissues via accumulation of kaempferols. This species was also sensible to enhanced UV-A, but, in this case, the response implied morphological adjustments to harden the leaves (smaller leaves with greater leaf mass per area (LMA)) coupled with a proportionally greater carbon allocation to roots (lower leaf to root biomass ratio). Concerning water supply, *A. unedo* appears to counteract water constraints by a stimulated antioxidant activity, as in response to enhanced UV-B, while the main response in *Q. suber* was a reduction in biomass allocation to leaves in relation to roots and to total biomass, likely as a strategy to minimize water loss by transpiration and increase water absorption by roots. The protective strategies displayed by both species were apparently efficient, since no reductions in root reserves or in resprouting success were found. However, the sensitivity of resprouts to UV radiation or water levels was notably different in relation to that of seedlings, being higher in *A. unedo* but lower in *Q. suber* resprouts. At ecosystem level, the effects of UV-A and UV-B exposure on C and N pools were contrasted and often exacerbated by reduced levels of precipitation. During the pre-fire period, UV-A exposure increased soil moisture, respiration and β-glucosidase activity, suggesting increased soil biological activity and C turn-over. The additional presence of UV-B decreased soil respiration, pH and β-glucosidase activity, and increased C concentration in *P. angustifolia* leaves, pointing to a lower soil microorganism activity probably slowing down C and N cycling. Under reduced rainfall, UV-B exposure also enhanced $\delta^{15}N$ values in leaves and litter of *A. unedo*, suggesting higher nitrification and N losses by leaching in the soil. Most of the effects were only found before the fire, indicating a homogenizing influence of this perturbation.

To sum up, over the coming decades, changes induced by enhanced UV doses and diminished precipitation levels on C and N dynamics in Mediterranean shrublands would be in part driven by opposite effects of UV-A and UV-B on soil processes, modulated by low water availability in combination with plant species-specific responses to these factors, which, depending on the species, could be emphasized or mitigated after a disturbance (fire, clear-cut, grazing) occurs.

Chapter I

INTRODUCTION

1. Climate change in the Mediterranean basin

Expected UV radiation and precipitation changes

Solar radiation spectrum is distributed in different wavelength intervals, including ultraviolet (UV; 100-400 nm), visible (400-700 nm) and infrared region (> 700 nm). UV radiation represents a highly energetic part of the electromagnetic spectrum reaching the terrestrial atmosphere, being subdivided into three bands: UV-C (100-280 nm), UV-B (280-315 nm) and UV-A (315-400 nm). While UV-C is blocked effectively by oxygen, ozone and nitrogen molecules in the upper atmosphere and most UV-A passes right through the ozone layer in the stratosphere, the UV-B radiation is only partially absorbed by the stratospheric ozone (Bais *et al.*, 2007), being the shortest wavelength reaching the Earth's surface.

More than 30 years ago, it was discovered a decline in the concentration of stratospheric ozone as a consequence of the emission of ozone-depleting substances, such as chlorofluorocarbons (CFC), which increased the UV-B levels reaching the Earth's surface during the 1980s and 1990s, especially at high latitudes (McKenzie *et al.*, 2011). This fact caused a considerable concern about the possible impact of enhanced UV-B radiation on organisms, as well as on the whole ecosystem, stimulating the research into this topic. CFC emissions were banned by the Montreal Protocol in 1987, leading to a gradual recovery of the ozone layer in the last decades (Bais *et al.*, 2015). Despite this, changes in other factors that influence solar radiation penetration through the atmosphere, such as cloudiness, could alter the UV levels reaching the Earth's surface. In particular, it is expected a reduction in cloudiness in the Mediterranean basin due to climate change, which could increase the levels of UV radiation (UV-B and UV-A) reaching Mediterranean ecosystems over the coming years (IPCC, 2013; Bais *et al.*, 2015; Sanchez-Lorenzo *et al.*, 2017) (Fig. 1.1). Reduced cloudiness in the Mediterranean region will also modify the seasonal distribution of precipitation and its frequency, resulting in longer dry periods (IPCC, 2013). Indeed, according to climatic models, the Mediterranean basin is expected to be one of the regions with the most drastic decrease in precipitation in the future (Bussotti *et al.*, 2014).

Specifically, models estimate that the annual number of precipitation days will decrease and the rainfall amount could be between 4% and 27% lower over the next decades (Fig. 1.2). Hence, Mediterranean terrestrial ecosystems will probably have to cope with higher UV doses and lower water availability in the coming years, especially in summer months.

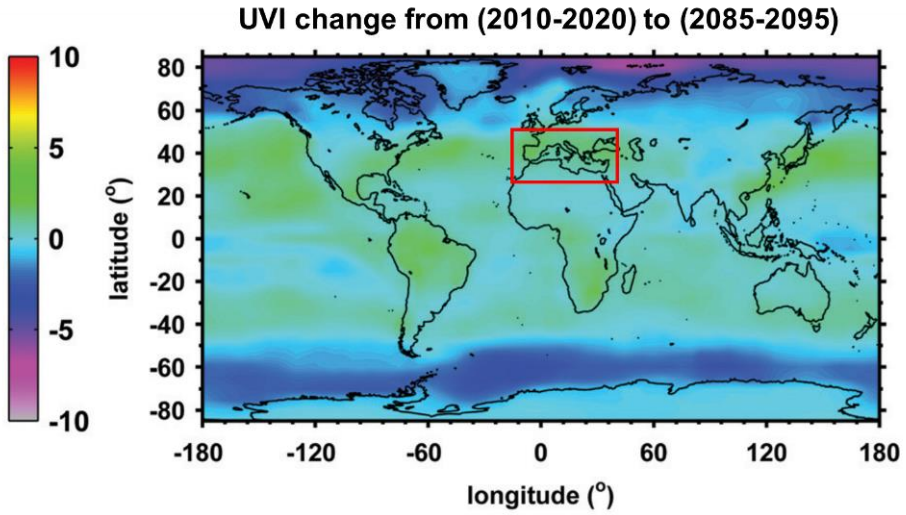


Fig. 1.1 Simulated annually average percentage of changes expected in noontime UVI (or erythemally-weighted UV irradiance) from the present (i.e. 2010–2020) to the period 2085–2095, as a consequence of the reduction in cloud cover. The Mediterranean basin is framed in red. Adapted from Bais *et al.*, 2015.

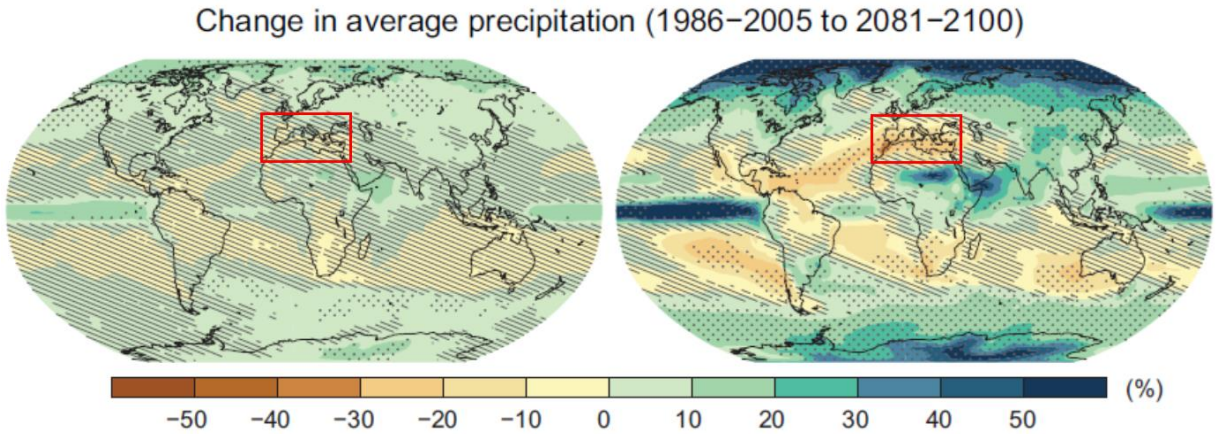


Fig. 1.2 Maps of CMIP5 multi-model mean results for the scenarios RCP2.6 and RCP8.5 in 2081–2100 of average percent change in annual mean precipitation. Changes are shown relative to 1986–2005. The Mediterranean basin is framed in red. Adapted from IPCC, 2013.

Changes in fire intensity and frequency

Fires are inherent to Mediterranean ecosystems (Lloret *et al.*, 2002; Paula & Pausas, 2006), but, during the last decades, larger and more intense fires have already been recorded mainly due to warmer and drier conditions as a consequence of climate change (Pausas, 2004; Moreno *et al.*, 2014). This trend is thought to continue over the coming years (IPCC, 2013; Sousa *et al.*, 2015).

Loss of vegetation due to a fire increase the risk of erosion processes and nutrient leaching in the soil (Certini, 2005), also increasing soil temperature and reducing soil moisture (Sherman *et al.*, 2012). In addition, nutrient input is often high just after a fire (Certini, 2005), and there are evidences of an increase in soil N mineralization (Goberna *et al.*, 2012; Caon *et al.*, 2014), leading to higher N concentrations and lower C:N ratios (De la Rosa *et al.*, 2008). Nevertheless, over the longer term, reduced plant cover provides a lesser amount of nutrients to the soil, consequently, decreasing the decomposer activity (Raich & Tufekcioglu, 2000; Talmon *et al.*, 2011). Moreover, enzyme denaturation and temporary soil sterilization due to the fire (Certini, 2005; Knicker, 2007) can slow down the C cycle through the reductions in the activity of key enzymes such as β-glucosidase (López-Poma & Bautista, 2014), which is involved in the breakdown of labile cellulose and other carbohydrate polymers (Sardans *et al.*, 2008a). Fires can also promote N losses through leaching, preferentially in the form of nitrates because of their negative charge, resulting in ^{15}N enrichment in soil organic matter and then increasing the $\delta^{15}N$ values in soil and plants (Szpak, 2014).

Considering the projected evolution of wildfires in the Mediterranean region, as well as its large effects on the whole ecosystem, future fires could alter plant and soil responses to changes in other environmental factors, such as UV radiation and precipitation; nevertheless, this topic has not yet been extensively investigated. The current study aims to shed light on this issue, focusing on possible shifts induced by fire in the sensitivity of Mediterranean shrublands to UV radiation and precipitation, which are expected to also be modified as a consequence of climate change.

Shrubland ecosystems and resprouter species

Given the increase in the frequency and magnitude of fires projected for the Mediterranean basin, it is expected a change in landscape structure, modifying vegetation patterns towards communities highly resilient, such as shrublands (Acácio *et al.*, 2009; Arnan *et al.*, 2013). In the last decades, the increase in wildfire occurrence together with the abandonment of agricultural lands have favored the persistence and expansion of these communities (Díaz-Delgado *et al.*, 2002; Lloret *et al.*, 2002). Thus, areas formerly covered by forests have been replaced by shrublands which have been spreading in Spain and other parts of southern Europe (Acácio *et al.*, 2009). The success of shrub communities is due to the dominance of species that are able to regenerate by sprouting after being top-killed or by germination of fire-protected seeds stored in the soil or in the canopy bank (Arnan *et al.*, 2013), resulting in a fast recovery of plant cover after a disturbance, such as a fire or logging.

The capacity of some plant species to resprout is a functional trait based on the accumulation of reserves in underground storage organs to support the regrowth of their aerial biomass from a set of protected dormant buds after a disturbance (Canadell & López-Soria, 1998; Paula *et al.*, 2016). Resprouter species allocate a great percentage of assimilates to roots, increasing root biomass and carbohydrate reserves in form of starch (Zeppel *et al.*, 2015), which provides an important carbon source for resprouting after a disturbance and also for plant respiration during periods of resource shortage (Verdaguer & Ojeda, 2002; Sanz-Pérez *et al.*, 2007). Roots of resprouter plants often present lignotubers, with a large bud bank where meristematic tissues are protected and with a great quantity of starch (Molinas & Verdaguer, 1993; Canadell & López-Soria, 1998; Paula *et al.*, 2016). Resprouting capacity makes possible the survival of individuals after a fire as well as the persistence of populations.

Mediterranean shrublands represent nowadays one of the most extensive Mediterranean-type terrestrial community in Europe, and especially in the Iberian Peninsula (Riera *et al.*, 2007). Nevertheless, changes projected in fire regime along with those in UV irradiance and precipitation could alter root biochemical and anatomical traits of resprouter

species, affecting the success of resprouting and thus the resilience of some Mediterranean shrublands.

2. UV effects on plants

Biochemical responses

Despite the UV band (UV-B and UV-A) represents only about 8% of total solar irradiance, it is a highly energetic radiation with a substantial effect on the biosphere. In plants, high UV levels reaching the leaf surface can result in photodamage due to the accumulation of reactive oxygen species (ROS) (Jansen *et al.*, 1998; Caldwell *et al.*, 2007). ROS are partially reduced forms of atmospheric oxygen (O_2) that act as signal molecules controlling many plant processes like growth, development, cell cycle and abiotic stress responses (Bettini *et al.*, 2008); however, they are also able to trigger an unspecific oxidation of different cellular components (damaging nucleic acids, oxidizing proteins and causing lipid peroxidation) and even the oxidative destruction of the cell (Mittler, 2002; Gill & Tuteja, 2010). ROS are generated by reactions involved in the primary metabolism of plants, such as photosynthesis and respiration (Mittler, 2002), being their accumulation controlled by various antioxidative systems maintaining the balance between production and scavenging (Foyer & Noctor, 2005; Gill & Tuteja, 2010). Thus, under steady-state conditions, ROS can be produced by oxygen generated during photosynthesis in the chloroplasts. However, a photon flux largely greater than that used for CO_2 assimilation can induce ROS overproduction and perturb their equilibrium within the cell (Asada, 2006). ROS production occurs mainly in the reaction centres of photosystems I and II in chloroplast thylakoids, but also in the peroxisomes and other cell organelles (Gill & Tuteja, 2010).

To prevent UV oxidative damage, plants have developed different protection and repair mechanisms, which involve the production of certain secondary metabolites (A-H-Mackerness, 2000; Jansen *et al.*, 2012; Bussotti *et al.*, 2014). UV radiation, especially UV-B, promotes the expression and activity of enzymes, such as chalcone synthase (CHS) and

phenylalanine ammonia lyase (PAL) that are involved in the synthesis of phenylpropanoids (A-H-Mackerness, 2000). The phenylpropanoid pathway is responsible for the production of phenolic compounds, which represent the most abundant secondary metabolites in plants (Fig. 1.3). The group of phenols include a wide range of specific compounds, being broadly divided in two classes: simple phenols, such as phenolic acids; and polyphenols, such as tannins and flavonoids. The basic molecular structure of phenols consists of at least one hydroxyl group bounded to an aromatic hydrocarbon group (Fig. 1.3). Nevertheless, these compounds present variations in their molecular structures (such as hydroxylation, glycosylation, acylation, prenylation, sulfation and methylation) (Dixon & Paiva, 1995), which determine their different functions within the plant. Indeed, the capacity of phenolic compounds to scavenge ROS is linked to the hydroxylation level of their molecular structure (Winkel-Shirley, 2002). Moreover, phenols are also considered a first line of protection against UV radiation, since they occur in the wall and the vacuole of epidermal cells as well as in external surface organs more susceptible to UV light (Winkel-Shirley, 2002; Agati *et al.*, 2013), contributing to absorb the most energetic solar wavelengths before they penetrate within the tissues (Julkunen-Tiitto *et al.*, 2005; Caldwell *et al.*, 2007; Li *et al.*, 2010).

Flavonoids are one of the most important classes of polyphenols (Treutter, 2006), being mainly attributed to monohydroxy B-ring substituted flavonoids, such as kaempferols, the function of UV screening. Dihydroxy B-ring substituted flavonoids, such as quercetins, have a catechol group in the B-ring, which confers them effective antioxidant properties (Agati & Tattini, 2010; Hideg *et al.*, 2013). These flavonoids are confined in the vacuoles of mesophyll cells and in the chloroplasts, near or within the sites of ROS production (Agati & Tattini, 2010). Thus, antioxidant flavonoids can inhibit ROS generation as well as quench excess ROS once they are formed, representing a second line of protection aimed at diminishing the immediate impact of UV-mediated oxidative stress within the tissues (A-H-Mackerness, 2000; Agati *et al.*, 2012). In plants exposed to severe stress conditions, flavonoids are thought to constitute a secondary component of ROS-scavenging system,

since they can be upregulated following depletion of primary antioxidants (Fini *et al.*, 2011; Agati *et al.*, 2013).

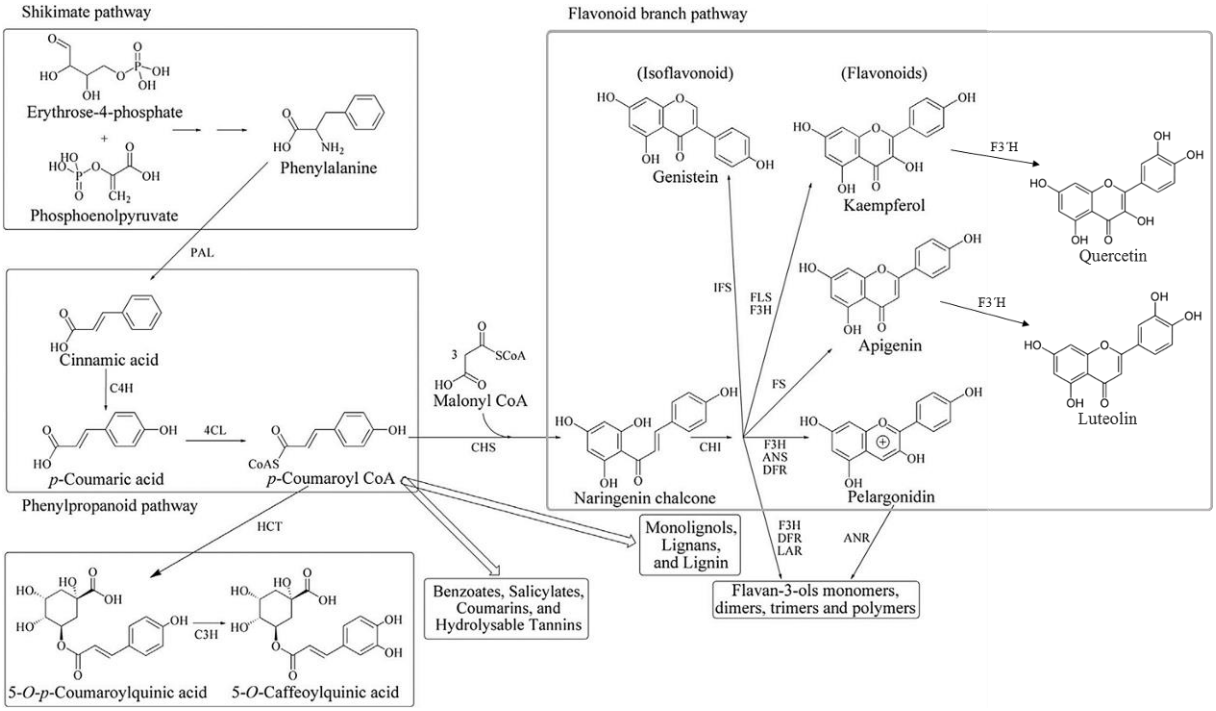


Fig. 1.3 Schematic representation of the major branch pathways of (poly)phenol biosynthesis. PAL, phenylalanine ammonia-lyase; C4H, cinnamate-4-hydroxylase; 4CL, 4-coumaroyl-CoA ligase; HCT, hydroxycinnamoyl transferase; C3H, p-coumarate-3-hydroxylase; CHS, chalcone synthase; CHI, chalcone isomerase; ANS, anthocyanidin synthase; DFR, dihydroflavonol reductase; FS, flavone synthase; FLS, flavonol synthase; F3H, flavanone 3-hydroxylase; IFS, isoflavone synthase; ANR, anthocyanidin reductase; LAR, leucoanthocyanidin reductase; F3´H, flavonoid 3´-hydroxylase. Modified from Cheynier *et al.*, 2013.

Primary ROS-scavenging mechanisms comprise both enzymatic and non-enzymatic antioxidants (Zlatev *et al.*, 2012; Hideg *et al.*, 2013). The first involves the activation of antioxidant enzymes, including ascorbate peroxidase (APX) and catalase (CAT); whilst the non-enzymatic components encompass other well-known antioxidants like ascorbate and glutathione (Ueda & Nakamura, 2011; Lidon *et al.*, 2012). Ascorbate is able to donate electrons in numerous enzymatic and non-enzymatic reactions, likely being the most powerful ROS scavenger; whereas glutathione plays a key role maintaining the normal reduced state of cells (Gill & Tuteja, 2010). Both compounds, together with APX, are essential in the detoxification of superoxide radicals and hydrogen peroxide through the ascorbate-glutathione cycle (Noctor & Foyer, 1998). In this metabolic pathway, APX reduces

H_2O_2 to water using two molecules of ascorbate, which is regenerated via glutathione that acts as the reducing substrate. Alternatively, H_2O_2 can be directly converted to water and molecular oxygen without the need of a reductant, by means of CAT, which are enzymes with extremely high turnover rates, being indispensable for ROS detoxification during stress conditions (Mittler, 2002; Gill & Tuteja, 2010). Thus, plants often respond to UV oxidative stress by upregulation of enzymatic antioxidant activities coupled with increases in both the reduction state and pool-size of key non-enzymatic antioxidants (i.e. ascorbate and glutathione) (Agarwal, 2007; Jansen *et al.*, 2012).

Mediterranean species generally present natural adaptations to cope with harsh climatic conditions, including high levels of photoprotective and antioxidant compounds, being often resistant to high UV doses (Paoletti, 2005; Bussotti *et al.*, 2014). Hence, while the increase in the total amount of leaf phenols is considered a general response of plants to cope with enhanced UV levels (Searles *et al.*, 2001; Bassman, 2004; Julkunen-Tiitto *et al.*, 2005; Li *et al.*, 2010), this would not be the case in Mediterranean species. Indeed, most of the studies performed with Mediterranean species have not found changes in the total pool of leaf phenols in response to enhanced UV radiation levels (Kyparissis *et al.*, 2001; Bernal *et al.*, 2015; Nenadis *et al.*, 2015). Despite this, in some species UV-induced changes in leaf biochemistry, particularly in the leaf phenolic content (Grammatikopoulos *et al.*, 1998; Tattini *et al.*, 2005; Bernal *et al.*, 2013) and in the phenolic profile (Tattini *et al.*, 2004; Agati *et al.*, 2009; Nenadis *et al.*, 2015), have been detected. For instance, in a study with six Mediterranean species, UV exposure induced variations in the leaf phenolic composition of *Pistacia lentiscus*, but not in the remaining five species (Bernal *et al.*, 2013). In another Mediterranean species, *Arbutus unedo*, Nenadis *et al.* (2015) found contrasting UV-B effects on the leaf flavonoids, since the overall concentration of flavanols decreased while the concentration of quercetins, mainly quercitrin (the most abundant quercetin identified), increased in response to UV-B enhancement. Other studies have also reported variations in the amount of specific phenols in response to UV radiation, which were associated to differences in their potential antioxidant capacities (Tattini *et al.*, 2004; Agati *et al.*, 2009).

However, until now, the disparity of biochemical responses observed makes difficult to understand the mechanisms behind these responses, and, thus, in Mediterranean species, this aspect clearly needs much more research.

Changes in leaf morphology, physiology and plant growth

Changes in UV radiation, especially UV-B, can also lead to variations in leaf morphology and physiology. Regarding foliar morphology, plants have developed different protective strategies against excessive UV levels, such as the generation of leaf hairs or thicker and smaller leaves (Skaltsa *et al.*, 1994; Newsham *et al.*, 1999; Barnes *et al.*, 2005), addressed to screen out UV in order to prevent photodamage to the photosynthetic apparatus.

At the physiological level, UV radiation can inhibit photosynthesis by UV-induced stomatal closure, and subsequent reduction in CO_2 uptake (Musil & Wand, 1993), and/or by an excessive accumulation of ROS, which can prompt deleterious effects on photosystem II (PSII) components and reduce the Rubisco content and activity (Teramura & Sullivan, 1994; Zlatev *et al.*, 2012). In addition, leaf content in photosynthetic pigments, chlorophylls and carotenoids, can be reduced by UV-B wavelengths via inhibition of their synthesis (Kataria *et al.*, 2014). To avoid damage to the photosynthetic apparatus, excess excitation energy may be dissipated through non-photochemical quenchers (Lidon *et al.*, 2012). One of the main routes of excess excitation energy dissipation is the xanthophyll cycle, whose activity is linked to parameters widely used like the non-photochemical quenching coefficient (NPQ), which quantifies the dissipation of excess energy as heat.

A reduction in carbon assimilation as a consequence of enhanced UV would entail a subsequent decline in the synthesis of carbohydrates and thus in the starch and sugar levels, all of which may eventually restrict plant growth and development. In fact, enhanced UV-B radiation frequently reduces elongation rates of the main stem and branches, resulting in shorter plants (Reddy *et al.*, 2013; Kataria *et al.*, 2014). Belowground development can also be altered, although the direction of this effect seems to differ among species, with the root

growth being stimulated (Bernal *et al.*, 2015) or attenuated (Zaller *et al.*, 2002; Sharma & Guruprasad, 2012) in response to UV exposure.

Mediterranean plant species are generally adapted to high solar UV fluxes thanks to various morphological adaptations, such as a high leaf dry mass per area (LMA), which would be a proxy of leaf sclerophylly. Despite this, Mediterranean plants exposed to enhanced UV levels have been found to increase even more their LMA (Verdaguer *et al.*, 2012; Bernal *et al.*, 2013) and leaf thickness (Grammatikopoulos *et al.*, 1998; Bernal *et al.*, 2015). More sclerophyllous and thicker leaves imply a reinforcement of the foliar tissues that can attenuate UV penetration into the leaf, protecting the photosynthetic apparatus. In fact, variations in photosynthetic performance are not often found in Mediterranean plants exposed to enhanced UV levels (Paoletti, 2005; Bussotti *et al.*, 2014), although there are some evidences of species-specific responses. For instance, seedlings of *Pinus pinea* grown under supplemented UV-B increased needle cuticle thickness resulting in improvements of leaf water relations and photosynthetic rates (Manetas *et al.*, 1997). Conversely, in a study with six Mediterranean species, UV-B promoted thicker leaves but did not affect photosynthesis (Verdaguer *et al.*, 2012). In another study with three *Erica* species, enhanced UV-B levels reduced CO_2 assimilation rates (Musil & Wand, 1993). Reductions in photosynthetic rates might be particularly relevant in Mediterranean resprouter species, since a reduction of photoassimilates could diminish the amount of reserves stored belowground (such as starch), which, as mentioned above, support plant regeneration after a disturbance (Canadell & López-Soria, 1998; Paula & Pausas, 2006). Root reserves can also decrease as a result of a greater allocation of photosynthetic products and nutrients to the biosynthesis of photoprotective compounds, such as phenolics (Sumbele *et al.*, 2012). Therefore, enhanced plant investment in protective mechanisms to cope with increased UV levels might alter the resprouting capacity and persistence of Mediterranean resprouter species. However, no information is currently available regarding this important issue.

3. UV effects on soils and C and N cycles

Dynamics of C and N cycles greatly control the functioning of terrestrial ecosystems. At the soil level, expected changes in UV radiation may impact on C and N cycles, both indirectly (through its effects on vegetation) and directly. Among indirect effects, variations in plant production of polyphenols in response to altered UV doses can affect litter quality and/or leachates from leaves, shoots and roots, and thus modify nutrient cycling (Hättenschwiler & Vitousek, 2000; Castaldi *et al.*, 2009) (Fig. 1.4). In the soil, polyphenols can alter N availability by forming recalcitrant complexes with proteins from litter or with extracellular enzymes from microorganisms, slowing down the decomposition process (Hättenschwiler & Vitousek, 2000). Moreover, phenolic compounds can attenuate the activity of decomposer organisms and extracellular enzymes in the soil, further delaying organic matter decomposition and mineralization, and inhibiting nitrification (Castells *et al.*, 2004; Castaldi *et al.*, 2009; Formánek *et al.*, 2014). One of the enzymes involved in the C cycle is β-glucosidase, which takes part in the breakdown of labile cellulose and other carbohydrate polymers, facilitating nutrient release of organic compounds and thus microbe metabolism (Sardans *et al.*, 2008a). Consequently, attenuated β-glucosidase activity could decrease plant-available soil N, which in part controls plant development. Thus, in addition to direct UV effects on photosynthesis (see previous section), plant growth can be indirectly reduced through UV-induced lower N availability in the soil. A decline in vegetal cover would increase the direct incidence of solar UV on ground surface (Hughes *et al.*, 2006), with its concomitant effects on chemical and biological properties of the soil (Garcia *et al.*, 2002).

Soil direct exposure to high UV doses, both UV-B and UV-A, may stimulate photodegradation, i.e. the photochemical mineralization of organic matter, associated to a UV-induced decline in lignin concentration of plant litter (Day *et al.*, 2007; Henry *et al.*, 2008; Dirks *et al.*, 2010), reducing the structural and chemical limitations imposed by lignin in secondary cell walls and facilitating the enzymatic degradation and the microbial access to cell wall polysaccharides (Austin & Ballaré, 2010; Baker & Allison, 2015; Austin *et al.*, 2016)

(Fig. 1.4). However, the positive UV impact on litter photodegradation can be counteracted by the detrimental effects of direct sunlight on soil microorganisms (Hughes *et al.*, 2003), impairing the C and N release by decomposition (Zepp *et al.*, 2007) (Fig. 1.4). Therefore, the overall UV impact on C and N cycles in the soil may result from a balance between positive and negative UV effects.

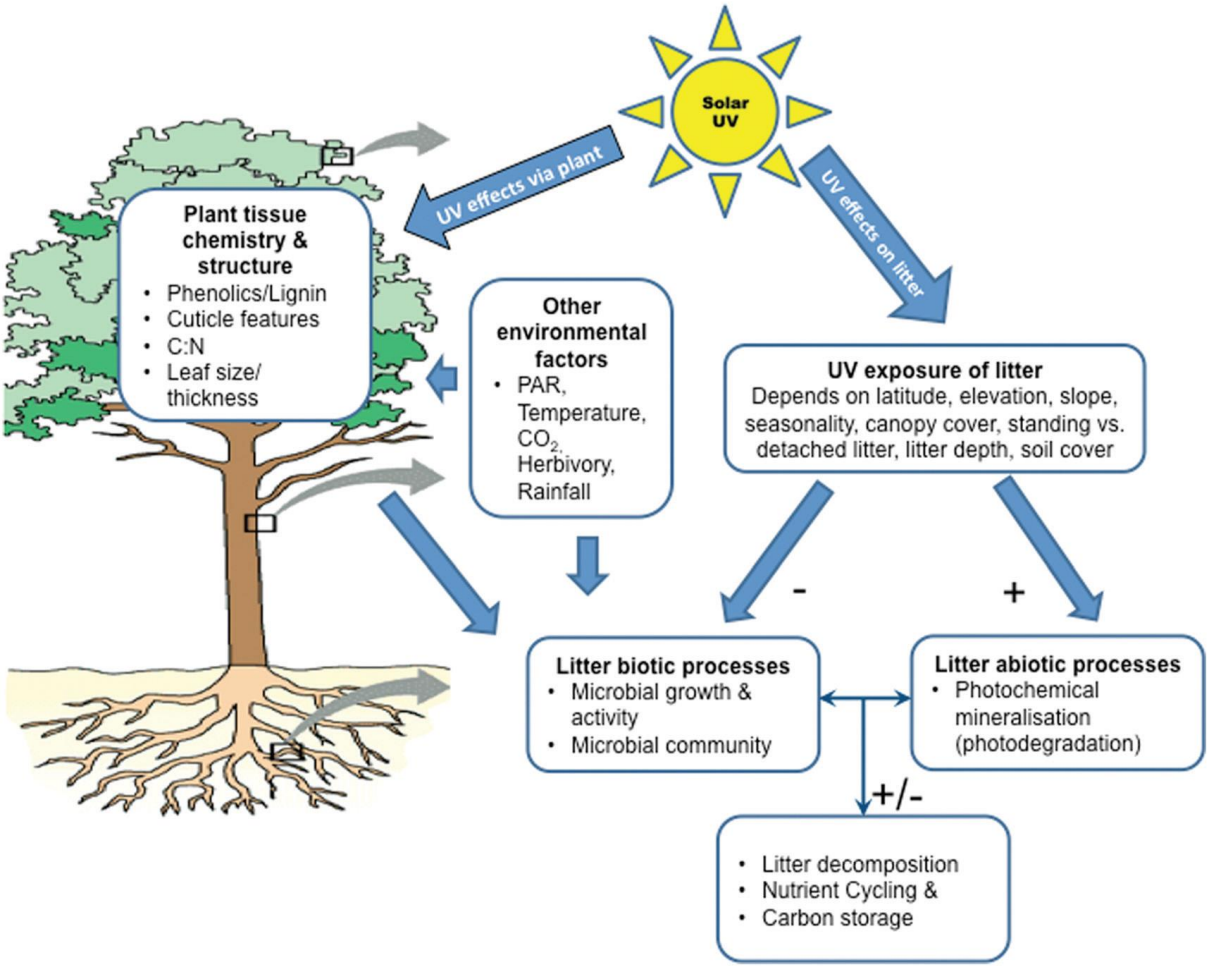


Fig. 1.4 Conceptual model of the direct and indirect effects of solar UV radiation (280–400 nm) on the decomposition of terrestrial leaf litter, including potential interactions with other environmental factors (from Bornman *et al.*, 2015).

Although there are several studies about the UV effects on plant litter degradation in Mediterranean terrestrial ecosystems (Henry *et al.*, 2008; Dirks *et al.*, 2010; Almagro *et al.*, 2015; Baker & Allison, 2015), it is still unclear the overall UV impact on soil and plant C and N pools, with null information about the interactive effects between UV and other co-occurring abiotic factors, such as a fire and/or water shortage.

4. Interactive effects between UV and water availability

Water constraints can lead to similar plant responses as high UV levels. As UV radiation, water deficit can alter the equilibrium between the generation and the scavenging of ROS, inducing also the activation of the detoxification machinery (Reddy *et al.*, 2004; Selmar & Kleinwächter, 2013). Thereby, plants under water constraints often respond by increasing the accumulation of phenolic compounds, particularly flavonoids with antioxidant properties (Hofmann *et al.*, 2003; Caldwell *et al.*, 2007). In addition, plants control water loss through stomatal closure, avoiding extensive desiccation that could result in cell dehydration, xylem cavitation and death (Chaves *et al.*, 2003). Stomatal closure together with drought-mediated lower biochemical capacity for carbon assimilation can lead to a decline of photosynthetic performance, with a subsequent reduction in plant growth and productivity (Reddy *et al.*, 2004). Moreover, plants under low water availability can increase carbon allocation to roots as well as modify the usage of recent photosynthates from metabolic activity to storage pools (Hasibeder *et al.*, 2015; Lavinsky *et al.*, 2015). At morphological level, many plant species under water shortage, similarly to plant UV responses, present smaller leaves with a high sclerophyllous index as a mechanism to diminish the loss of water through transpiration (Bussotti, 2008; Sardans & Peñuelas, 2013).

At the soil level, it is known that drought tends to attenuate soil microbial activity, leading to reduced respiration rates (Rey *et al.*, 2002) and enzyme activities (Gallo *et al.*, 2006), along with increases in soil C concentration (Sardans *et al.*, 2008a). In turn, higher C:N ratios can delay mineralization and eventually the transformation of organic N into plant-available forms (Bengtsson *et al.*, 2012). Decomposition process can be further slowed down due to the increase in plant phenolic compounds in response to drier conditions (Castells *et al.*, 2004). With lower plant N uptake, C:N ratio tends to further increase in plants and, consequently, the soil is enriched with hardly mineralizable organic debris.

Therefore, soil water availability is related to many variables and processes in plants and soils that can also be affected directly or indirectly by UV radiation. It is well known that

primary exposure to a single stress can alter responses to other stresses (cross-tolerance effects) (Stratmann, 2003; Poulson *et al.*, 2006; Jansen *et al.*, 2012). In this context, drought-induced changes in plant biochemistry, particularly increases in phenolic compounds, could modify plant tolerance to UV radiation and vice versa (Agati *et al.*, 2012; Di Ferdinando *et al.*, 2014). Enhancements in the amount of phenols, such as flavonoids, in response to UV radiation may mechanically strengthen the tissues that, along with chemical-related functions, improve the water-stress tolerance (Di Ferdinando *et al.*, 2014). Moreover, there is evidence that low water supply can counteract the effects of UV radiation, resulting in attenuated or null variations in leaf morphology (Verdaguer *et al.*, 2012), leaf relative water content (Bernal *et al.*, 2015) and photosynthetic activity (Nogués & Baker, 2000; Poulson *et al.*, 2006; Feng *et al.*, 2007) in response to UV radiation changes. In other cases, UV radiation, especially UV-B, in combination with low irrigation can interact synergistically leading to emphasized plant responses, specifically increasing the concentration of leaf phenols (Hofmann *et al.*, 2003; Caldwell *et al.*, 2007).

At the soil level, the degree of photodegradation can depend on soil water content (Gallo *et al.*, 2006; Brandt *et al.*, 2007). In addition, metabolic activity of soil microbiota may be strongly limited by both high UV fluxes (Hughes *et al.*, 2003) and low soil moisture (Sherman *et al.*, 2012). Interactive effects between UV and water supply on the activity of soil microorganisms can also be indirectly modulated by plant responses to both factors. Given that terrestrial ecosystems, particularly those within the Mediterranean basin, are often simultaneously subjected to both conditions, i.e. high UV and low water levels, it is essential to study their interactive effects on plants and soils. Currently, there is limited information about how interactions between UV radiation and water availability changes could affect the functioning of plants, along with the C and N pools in vegetation and soil, in Mediterranean ecosystems. The present work aims to contribute to the elucidation of the impacts that projected changes in UV radiation and precipitation could have on Mediterranean resprouter species and shrubland ecosystems in the next future, including the possible role of fire modulating these impacts.

5. Resprouter species of study

The species selected, *Arbutus unedo*, *Quercus suber* and *Phillyrea angustifolia*, are long-lived co-occurring evergreen sclerophyllous species (Fig. 1.5), commonly widespread in Mediterranean shrublands and forests. *Arbutus unedo* L. (strawberry tree; family *Ericaceae*) is a broadleaved shrub (up to 7 m of height) characterized by simple leaves with serrated margins, hairy pinkish stems and a long taproot with dual root systems, i.e. active roots both in the upper and lower soil layers (Filella & Peñuelas, 2003). *Quercus suber* L. (cork oak; family *Fagaceae*) is a slow-growing tree that grows up to 20 m, with curved and coarsely toothed leaf margins and with a deep well-developed root system (Verdaguer *et al.*, 2000). *Phillyrea angustifolia* L. (family *Oleaceae*) is a shrub of up to 2-3 m of height with lanceolate and hairless leaves, and a well-developed root system (Paula *et al.*, 2016). The three species also have in common a high capacity of resprouting after a disturbance, being able to regenerate the aboveground biomass from reserves stored in the lignotuber, which is characterized by a high starch content and a large bank of protected buds (Canadell & López-Soria, 1998; Paula *et al.*, 2016). In *Q. suber*, lignotubers have been observed particularly in the early stages of plant development, while mature plants show an elongated hypocotyl with an abundant cluster of buds (Molinas & Verdaguer, 1993).

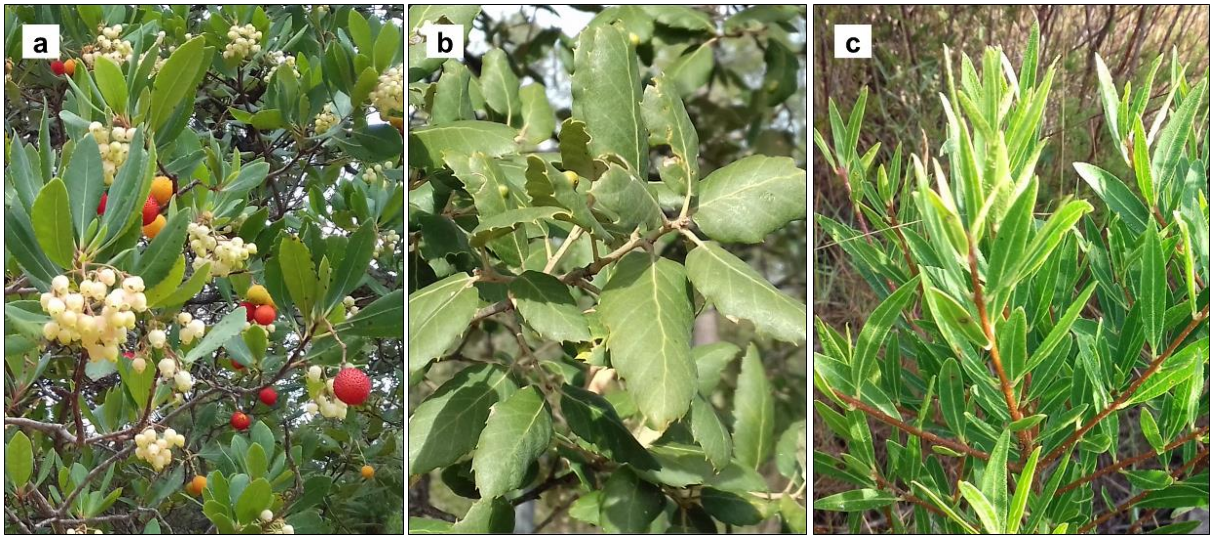


Fig. 1.5 The three evergreen Mediterranean species of study: *Arbutus unedo* (**a**), *Quercus suber* (**b**) and *Phillyrea angustifolia* (**c**).

6. Main objectives and structure of the thesis

Taking into account the expected changes in solar UV levels, precipitation and fire regime in the Mediterranean basin, the main goal of this thesis was to evaluate the effects of UV radiation (UV-A and UV-B) and diminished water availability on the biochemical, morphological, physiological and growth traits of common Mediterranean resprouter species, as well as on C and N pools of a Mediterranean shrubland, before and after a perturbation. In particular, the specific objectives pursued in this thesis were:

1) To examine the effects of enhanced UV radiation (UV-A and UV-B) and diminished water availability on the leaf content of phenols and antioxidant components of two Mediterranean common resprouter species, *Arbutus unedo* and *Quercus suber*, before and after a disturbance that removes all plant aerial biomass (such as fire, clear-cut, herbivory).
2) To assess the effects of enhanced UV radiation (UV-A and UV-B) and diminished water availability on leaf morphology and physiology, plant growth, root biochemistry (reserve storage and phenol content) and, ultimately, on the resprouting capacity of *A. unedo* and *Q. suber*, before and after a disturbance that removes all plant aerial biomass (such as fire, clear-cut, herbivory).
3) To investigate the role of UV radiation (UV-A and UV-B) on C and N pools in soil and plants of a Mediterranean shrubland before and after a fire, and whether these UV effects can be altered by changes in water availability.

To achieve the objectives 1 and 2, an outdoor experiment involving UV supplementation combined with two irrigation conditions was conducted using seedlings of the two Mediterranean sclerophyllous resprouter species (*A. unedo* and *Q. suber*) grown in pots, whose aerial biomass was removed during the study period (similar to the effects caused by intense fires). Several parameters were measured in seedlings and resprouts associated to leaf biochemistry, morphology and physiology, plant growth and biomass allocation, root biochemistry and resprouting capacity. In relation to objective 3, we carried out a field

experiment in a natural Mediterranean shrub community where the levels of UV and water availability reaching the ecosystem were modified, and an experimental fire was performed around the middle of the three years of the study period. In addition to plant and litter cover, different parameters related to C and N levels were measured in the soil, and in the litter and leaves of *A. unedo* and *Phillyrea angustifolia*, two of the dominant species in the community.

The thesis is composed mainly by four chapters, as follows: Introduction (Chapter I), which includes the main concepts and background of this work; Methodology (Chapter II) detailing the experimental designs and all the methods applied throughout the two experiments performed; Results (Chapter III) showing the main data obtained in the two experiments; Discussion (Chapter IV), where the most relevant findings have been integrated and, finally, Conclusions. A last section with all the References is also included, along with an annex with Supplementary Material.

Chapters III and IV are subdivided in three sections in concordance with the three main objectives and with the three scientific publications that will be produced as a result of this thesis, which are:

- Leaf biochemical adjustments in two Mediterranean resprouter species facing enhanced UV level and reduced water availability before and after pruning (objective 1)
- Growth and physiological responses of two Mediterranean resprouter species exposed to enhanced UV radiation and reduced water availability before and after pruning (objective 2)
- Effects of UV radiation and rainfall reduction on leaf and soil parameters related to C and N cycles of a Mediterranean shrubland before and after a controlled fire (objective 3)

Chapter II

METHODOLOGY

EXPERIMENT I. Effects of enhanced UV radiation and low water supply on two resprouter species, *Arbutus unedo* and *Quercus suber*, before and after the loss of all the aerial biomass

Plant material and experimental design

An outdoor experiment using two Mediterranean woody resprouter species, *Arbutus unedo* L. and *Quercus suber* L., was established in an area (Can Vilallonga; 41º 52' 48" N, 2º 54' 33" E), near Cassà de la Selva (Girona, NE of the Iberian Peninsula) situated at about 150 m a.s.l. (see Fig. S1 available as Supplementary Material). Weather variables, i.e. global solar irradiation, air temperature, air relative humidity and precipitation (Fig. 2.1), were obtained from the nearest meteorological station located 3 km away from the experimental site.

Seedlings of *A. unedo* and *Q. suber* (n = 144 plants per species) were cultivated under natural environmental conditions and subjected to three UV radiation conditions combined with two watering regimes. Briefly, one-year-old seedlings of both species were planted in pots (2 L volume; 11.3 cm wide x 21.5 cm deep) with 775 g of a growth medium fertilised with 8 g of fertilizer (Osmocotex; 4 kg m^{-3}), basal dressing (1 kg m^{-3}) and dolomite (4 kg m^{-3}) to prevent nutritional deficiencies during the experimental period. Potted seedlings were placed in plots made with 1.3 m x 1.2 m metallic frames and equipped with four UV lamps installed above plants (1.2 m of distance from the UV lamps to the top of the pots) (Fig. 2.2; see also Fig. S2 available as Supplementary Material). A total of nine plots (n = 16 plants per species and plot) were organized in three blocks, with each block having one plot of each one of the three UV radiation conditions (see below). Within each plot, two watering regimes were applied (n = 8 plants per species and watering condition). Thus, seedlings of the two species were subjected to three UV radiation conditions combined with two irrigation regimes, with each UV x watering combination replicated three times in a randomized complete block design. Plants were rotated every 2 weeks to minimise environmental, shading and border effects.

The experiment started in June 2012 and continued until October 2013. In February

2013, 8 months after the start of the experiment, all seedlings were pruned removing all the aerial biomass to simulate the effects of an intense disturbance, such as a severe fire. Samples were taken at two time points: 5 months after the start of the experiment, i.e. before pruning (October 2012), and 8 months after pruning (October 2013). Sampled plants were chosen randomly from each species on sunny days and around midday.

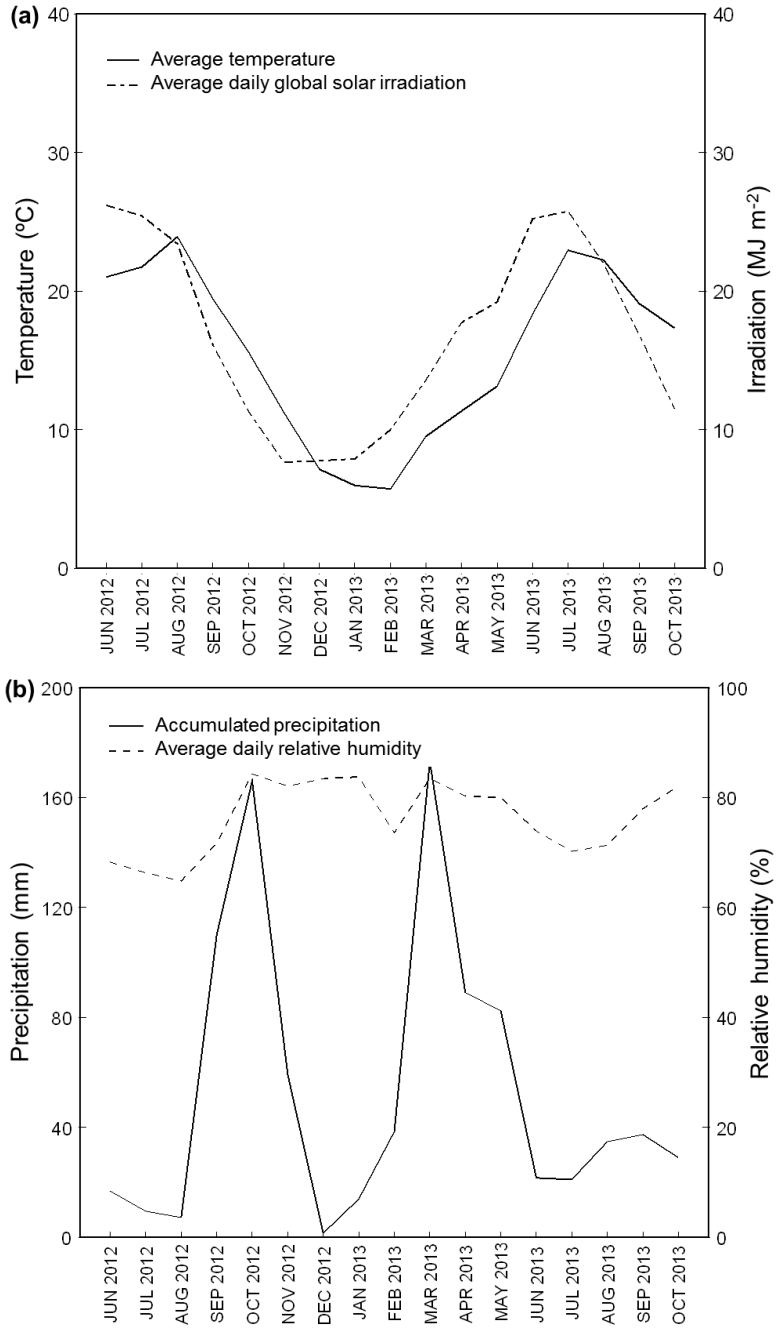


Fig. 2.1 (**a**) Monthly average of temperature (ºC) and daily global solar irradiation (MJ m^{-2}), and (**b**) monthly accumulated precipitation (mm) and monthly average of daily relative humidity (%), for the study period. Data set was obtained from the meteorological station of Cassà de la Selva (177 m a.s.l., 41º 52' 28" N, 2º 55' 37" E).

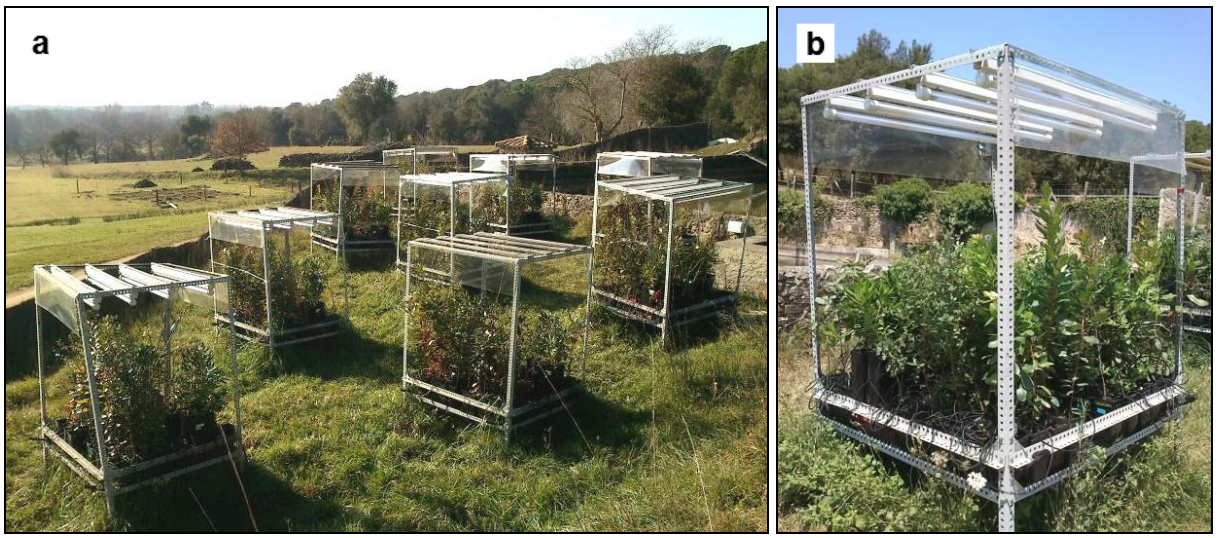


Fig. 2.2 Pictures of Experiment I showing: (**a**) the nine study plots, which were made with metallic frames and equipped with four UV lamps (UVA and UVAB plots) or four wood strips (control plots); and (**b**) detail of one study plot (1.56 m^2 of area). Note the drip irrigation system used.

UV-radiation treatment

As detailed in Bernal *et al.* (2015), solar UV radiation was supplemented by means of four 40 W fluorescent lamps (TL 40W/12 RS, with a peak at 313 nm; Philips, Spain) in six of the nine plots. The lamps were wrapped with filters of different materials to provide the following UV conditions:

- Enhanced UV-A+UV-B radiation (UVAB plots): Fluorescent lamps were wrapped with cellulose diacetate filters (Ultraphan URT, 0.1 mm; Digefra GmbH, Munich, Germany) to exclude UV-C radiation emitted by the lamps (wavelengths <280 nm). Cellulose diacetate films were pre-burned for 3 h prior to use.
- Enhanced UV-A radiation (UVA plots): Supplementation with UV-A was achieved by wrapping the fluorescent lamps with polyester film (Melinex, 0.25 mm; Ponscosta, Valencia, Spain) to block UV-B and UV-C radiation emitted by the lamps, transmitting only irradiance ≥ 315 nm. These plots served as a control for the UV-A effect in UVAB plots.
- Ambient UV radiation (control plots): Plants in these plots did not receive additional UV radiation. These plots were equipped with wood strips instead of fluorescent lamps to ensure that plants were exposed to similar shading conditions as those under UV supplementation conditions.

Monthly averages of daily UV doses (Table 2.1) and percentages of UV enhancement in UVAB (Fig. 2.3a-c) and UVA plots (Fig. 2.3d) were estimated from erythemal UV irradiance data (UVE; *Commission International de l'Éclairage*, CIE) in combination with spectral measurements and radiative modelling. UV supplementation was applied daily at solar noon for 0.5–3.5 h, depending on the period of the year, to achieve simulated increases according to those expected as a consequence of cloudiness reduction (IPCC, 2013). Filters were replaced after 36 h of use to avoid spectral changes. To prevent UV contamination among plots, two clear polycarbonate filters (transmission ≥ 400 nm) of 120 cm x 30 cm were placed parallel to the UV lamps along the two sides of the top part of each plot.

Table 2.1 Monthly means of natural daily UV doses (kJ m^{-2} day^{-1}) reaching the experimental plots throughout the study period expressed as unweighted UV-B (280-315 nm) and UV-A (315-400 nm) irradiances, and plant growth weighting function (PG; Flint & Caldwell, 2003). Daily doses were estimated considering: measured data (UVE and photosynthetic photon flux density, PPFD, as in Nenadis *et al.*, 2015) for clear-sky and cloudy days, measured data only for clear-sky days, and simulated data for clear-sky days. Gaps in UV estimations correspond to periods of calibration of the UVE sensor.

Month	clear-sky and cloudy days			clear-sky days (measured data)			clear-sky days (simulated data)		
	PG[a]	UV-B	UV-A	PG[a]	UV-B	UV-A	PG[a]	UV-B	UV-A
June 2012	31.8	34.4	1318	38.5	42.1	1592	41.8	46.2	1708
July 2012	30.2	32.4	1260	37.9	40.9	1578	40.5	44.4	1661
August 2012	-	-	-	-	-	-	35.1	36.8	1460
September 2012	-	-	-	-	-	-	26.5	25.0	1139
October 2012	13.9	11.6	619	15.2	10.1	700	17.6	13.7	788
November 2012	9.0	5.6	417	12.6	7.9	583	10.7	6.3	496
December 2012	7.9	4.1	375	9.8	4.5	469	7.8	3.7	366
January 2013	8.6	4.3	405	11.3	4.9	538	8.9	4.7	419
February 2013	11.5	7.1	536	14.9	8.7	698	14.4	10.0	656
March 2013	17.3	13.3	779	22.8	15.6	1041	22.4	19.4	979
April 2013	23.1	20.1	1015	35.8	32.5	1560	31.8	32.1	1340
May 2013	27.1	25.5	1173	35.6	31.7	1555	38.7	41.8	1595
June 2013	34.2	34.5	1450	41.3	44.8	1716	41.8	46.2	1708
July 2013	32.9	35.2	1373	35.8	36.8	1512	40.5	44.4	1661
August 2013	28.4	29.7	1196	36.2	38.9	1506	35.1	36.8	1460
September 2013	22.0	20.8	953	25.7	24.5	1109	26.5	25.0	1139
October 2013	14.4	12.0	642	18.2	15.1	812	17.6	13.7	788

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

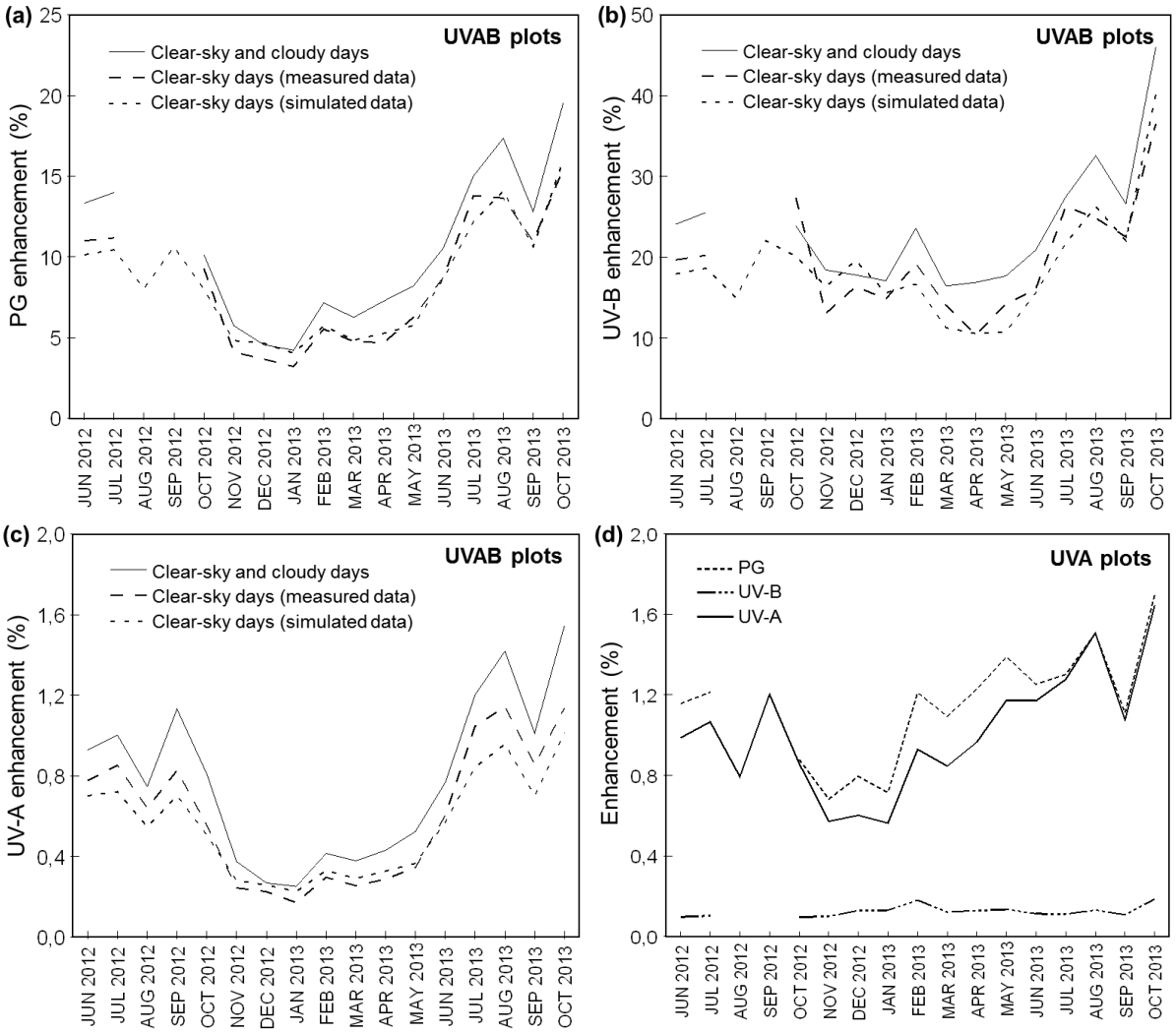


Fig. 2.3 (**a**) Percentages of UV enhancement applied to UVAB plots according to the plant growth weighting function (PG), (**b**) percentages of UV-B (280-315 nm) enhancement in UVAB plots, (**c**) percentages of UV-A (315-400 nm) enhancement in UVAB plots, and (**d**) percentages of UV (according to PG), UV-B and UV-A enhancement in UVA plots, throughout all the experiment. Percentages in UVA plots (**d**) were calculated taking into account measured data for clear-sky and cloudy days, while, in UVAB plots (**a**-**c**), three different approaches are shown, using: 1) measured data for both, clear-sky and cloudy days, 2) measured data only for clear-sky days, and 3) simulated data for clear-sky days. Gaps in UV estimations correspond to periods of calibration of the UVE sensor.

Watering treatment

In addition to natural rainfall, plants were watered twice daily by means of an automatic system of drip irrigation. The drip-irrigation system was programmed according to monthly rainfall and the two experimental watering regimes. In each plot, half of the plants were irrigated to field capacity ("well-watered", WW), while the other half received a smaller proportion of the water supplied to well-watered plants ("low-watered", LW). Specifically, WW

plants received 667 ml per day by irrigation from mid-June 2012 to January 2013 and 200 ml from February 2013 until the end of the experiment; while LW plants received 60% of the water given to WW ones during the first 40 days (from mid-June to late-July 2012), 40% from the end of July 2012 to January 2013 and 33% from February 2013 until the end of the experiment. As an indication, the average soil water content measured in pots in October 2013 was 9.4% lower in low-watered plants than in well-watered ones. Watering of plants was necessary, even in the case of low-watered plants, to ensure plant survival.

1.1 Leaf biochemistry (phenolic compounds and antioxidant components)

Determination of the leaf concentration of phenolic compounds by HPLC

For each species, plot and watering regime, four fully-developed mature leaves located at the top of plant canopy and exposed to sunlight, were taken from four seedlings in October 2012 and from 2 to 4 resprouts (depending on the number of plants that resprouted after pruning) in October 2013. For *A. unedo*, a disc of 9 mm of diameter from each leaf was collected with a cork-borer. All leaf samples were frozen with liquid nitrogen in the field and stored at -80 ºC until being analysed in the laboratory.

Leaf discs of *A. unedo* from the same plot and watering conditions were pooled, whereas leaves from *Q. suber* were analysed separately. Between 5 and 10 mg of fresh material were mixed and homogenized (Homogenizer Precellys 24, Bertin Technologies, Montigny-le-Bretoneux, France) with 0.6 ml of cold methanol into Precellys-vials for 25 s. Then, samples were incubated in an ice bath for 15 min (at 4 ºC), homogenized again for 25 s, and centrifuged (13,000 rpm) for 3 min (Centrifuge 5415R, Eppendorf, Hamburg, Germany). The supernatant was collected into a 6 ml glass tube. The extraction was repeated 3 times more, adding to the remaining pellet 0.6 ml of methanol and leaving the extracts for 5 min on ice. The combined supernatants were evaporated under nitrogen and then stored at 4 ºC until their analysis.

Dried samples were dissolved in 300 µl of methanol plus 300 µl of MilliQ-water (1:1) and analysed by means of a high performance liquid chromatography (HPLC) system (1100 Series, Agilent, Waldbroon, Germany), which consisted of a binary pump (G1312A), an autosampler (G1329A), vacuum degasser (G1322A), a diode array detector (G1315B), a column oven (G1316A), and a C_{18} reverse-phase column (Zorbax SB-C18, 4.6 x 75 mm, particle size 3.5 µm). The column and injector temperatures were kept at 30 and 22 ºC, respectively. The injection volumes for *A. unedo* and *Q. suber* samples were 20 µl and 15 µl, respectively. For both species, the eluent flow was 2 ml/min and the HPLC solvents were A (aqueous 1.5% tetrahydrofuran and 0.25% *o*-phosphoric acid) and B (100% methanol). The 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; 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 min, 50% of B in A; and from 45 to 60 min, 50 to 100% of B in A. Runs were monitored at 220, 270 and 320 nm (see Fig. S3 available as Supplementary Material). The identification of the detected phenolic compounds was performed by comparison of the UV-spectra characteristics and retention times obtained in the chromatograms with the spectral libraries available in the Natural Product Research Laboratory (University of Eastern Finland, Joensuu, Finland). The quantification of each compound (mg g^{-1} DW) was based on following standards: (+)-catechin for (+)-catechin, gallocatechin, epigallocatechin and epigallocatechin gallate; quercetin 3-galactoside (hyperin) for quercetin 3-galactoside, quercetin 3-arabinoside, quercetin 3-glucoside, quercetin 3-rhamnoside, quercetin-glycoside, two rutin derivatives and four “unknown” flavonols; kaempferol 3-glucoside (astragalin) for kaempferol 3-glucoside, kaempferol glycoside and two monocoumaroyl-astragalins; kaempferol 3-rhamnoside (afzelin) for kaempferol 3-rhamnoside; myricetin 3-rhamnoside (myricitrin) for myricetin 3-rhamnoside, myricetin 3-galactoside and myricetin 3-glucoside; gallic acid for gallic acid, galloylglucose, two digalloylglucoses, seven gallotannins and other four hydrolyzable tannins; pentagalloylglucose for pentagalloylglucose; ellagic acid for ellagic acid, helasogalloylglucoside and two ellagitannins; and arbutin for arbutin, galloylarbutin and digalloylarbutin.

From the dissolved methanol extract used in HPLC test, the concentration of condensed tannins was also determined, by means of the butanol-HCl test (Hagerman, 1995). Briefly, 1 ml of sample (300 µl of dissolved extract plus 700 µl of methanol) was added to a 20 ml vial with 6 ml of acid butanol-reagent and 200 µl of Fe-reagent. The sample was vortexed thoroughly using a vial mixer and then hydrolysed in a boiling bath for 50 min. After this, the vial was cooled and the absorbance was measured at 550 nm (20 Genesys Spectrophotometer, Thermo Spectronic, Rochester, USA). The amount of condensed tannins in the sample was calculated from a standard curve created from purified tannins of aspen leaves (*Populus tremula*), expressing its concentration as mg g^{-1} DW.

Leaf antioxidant concentration in resprouts

In October 2013, 8 months after pruning, 2-4 fully-developed and sun-exposed leaves per plot and watering regime were collected from 2 to 4 resprouts of *A. unedo* and *Q. suber* (depending on the number of plants that resprouted after pruning). Leaves were frozen in liquid nitrogen and, once in the laboratory, stored at -80 ºC until biochemical analysis. To determine leaf total ascorbate, reduced ascorbate (ASC), dehydroascorbate (DHA), total glutathione, reduced glutathione (GSH) and oxidized glutathione (GSSG) content, 100 mg of plant material was mixed with 1.5 ml of 3% perchloric acid and then centrifuged (5000 rpm, for 20 min) at 4°C. The supernatant was collected and its pH adjusted to 7 by adding 300–400 µl of sodium carbonate. This solution was used as the leaf extract for the following analyses.

Total ascorbate, ASC and DHA were determined following the method of Arakawa *et al.* (1981). This assay is based on the reduction of ferric to ferrous ion with ascorbic acid in acid solution followed by the formation of a red chelate between ferrous ion and the a,a´-dipyridyl, used as reagent to develop colour. The determination of ascorbic acid is performed by using the stoichiometric relationship between the ascorbic acid in the sample and the formation of the chelate compound. Total ascorbate was determined in a reaction mixture to reduce DHA to ASC consisting of 200 µl of supernatant, 500 µl of 150 mM KH_2PO_4 buffer

(pH 7.4) containing 5 mM EDTA, and 100 µl of 10 mM dithiothreitol (DTT). After 10 min at room temperature, 100 µl of 0.5% (w/v) N-ethylmaleimide was added to remove excess DTT. ASC was assayed in a similar manner to DHA except that DTT was substituted for 200 µl of deionized H_2O. Colour was developed in both reaction mixtures with the addition of 400 µl of 10% (w/v) trichloroacetic acid (TCA), 400 µl of 44% (v/v) *o*-phosphoric acid, 400 µl of a,a´-dipyridyl in 70% (v/v) ethanol and 200 µl of 30g L^{-1} $FeCl_3$. The reaction mixtures were incubated at 40 °C for 1 h and quantified spectrophotometrically at 525 nm. Ascorbate standards were between 1 and 50 mmol ascorbate in 3% perchloric acid. DHA was estimated from the difference between total ascorbate and ASC. The concentrations of total ascorbate, ASC and DHA were expressed as µg g^{-1} DW.

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

To measure ascorbate peroxidase (APX; EC 1.11.1.11) and catalase (CAT; EC 1.11.1.6) activities, 100 mg of frozen leaf samples were homogenized with 0.1 M phosphate

buffer (pH 7.8) in a pre-chilled mortar. The homogenate was centrifuged at 4°C for 20 min at 5000 rpm. APX activity was determined spectrophotometrically by a decrease in absorbance of ASC at 265 nm (ε = 14 mM cm^{-1}) (Nakano & Asada, 1987). The reaction mixture contained 50 mM of potassium phosphate buffer (pH 7), 5 mM of ascorbic acid, 0.5 mM of H_2O_2 and the enzyme extract. Addition of H_2O_2 started the reaction. APX activity was expressed as µmol ASC min^{-1} mg^{-1} protein. CAT activity was determined by the consumption of H_2O_2 (Dhindsa *et al.*, 1981). The reaction mixture contained 50 mM of potassium phosphate buffer (pH 7), 15 mM of H_2O_2 and 20 µl of the enzyme extract. The consumption of H_2O_2 was monitored spectrophotometrically at 240 nm (ε = 0.0435 mM cm^{-1}). CAT activity was expressed as µmol H_2O_2 min^{-1} mg^{-1} protein.

Statistical analysis

Treatment effects on the leaf concentrations of phenolic compounds were analysed by means of three-way ANOVAs using pruning, UV radiation and watering regime as fixed factors. In the case of the resprouts, treatment effects on the leaf concentrations of antioxidants were analysed using two-way ANOVAs with UV radiation and irrigation as fixed factors. Analyses were always performed separately for *A. unedo* and *Q. suber*. Mean values of each parameter per plot and watering condition were used for all the statistical tests. In the case of significant UV effects, Fisher's LSD *post-hoc* pairwise comparisons were applied to determine differences among UV conditions. When the interaction between factors was significant, we assessed the treatment effects of one of the factors within the levels of the other factor/s by one- or two-way ANOVAs. The Kolmogorov–Smirnov test was used to analyse normality while the homogeneity of variances was tested with the Levene's statistic. For all the statistical tests, the significance level considered was $p \leq 0.05$. Statistical analyses were done using SPSS software (IBM SPSS statistics, Corporation, Chicago, USA).

1.2 Leaf morphology and physiology, plant growth and biomass allocation, root biochemistry and resprouting capacity

Plant growth and biometric parameters

Three plants of *A. unedo* and *Q. suber* from each UV and watering treatment were harvested in October 2012 (before pruning) and in October 2013 (after pruning). Leaves, stems and roots were separated for each plant and oven-dried at 45 ºC for 5 days to determine leaf, stem and root biomass (g). For both sampling times, height and basal diameter of the main stem were measured for all sampled plants. Basal diameter of the main stem (D) was obtained by averaging two perpendicular basal diameter measurements (large diameter, D1, and short diameter, D2).

Relative growth rates (RGR) of the stems and roots of seedlings (before pruning) were calculated as: RGR (mg g^{-1} d^{-1}) = (B_f – B_0) / (B_0 x t); where B_f was the stem or root biomass measured in October 2012, B_0 was the estimated stem or root initial biomass and *t* was the time in days since the start of the experiment (June 2012). To estimate B_0, allometric relationships between stem or root biomass (dry weight) and initial stem height (H_0) and/or initial basal diameters (D_0, $D1_0$ or $D2_0$) were calculated for both species, using a subsample of 10 seedlings per species, which were measured and harvested just before the start of the experiment (May 2012). By means of multiple linear regression analyses, the following allometric equations were obtained for *A. unedo* seedlings:

B_0 stems = 0.038 x H_0 + 0.159 (R^2 = 0.433; *p* = 0.039)

Ln (B_0 roots + 1) = 1.497 x $D1_0$ + 0.146 (R^2 = 0.599; *p* = 0.009)

The allometric equations used for *Q. suber* seedlings were:

B_0 stems = 0.088 x H_0 – 2.056 (R^2 = 0.599; *p* = 0.009)

B_0 roots = 17.568 x $D2_0$ – 1.946 (R^2 = 0.610; *p* = 0.008)

The initial biomass of leaves (B_0 leaves) could not be estimated due to non-significant linear relationships with stem height or diameter. In the case of the resprouts, stem RGR was calculated dividing the final stem dry biomass (B_f) by the number of days since the

appearance of the first resprout of each species after pruning, since all the aerial biomass of the seedlings was eliminated and, thus, B_0 was equal to zero. Root RGR of the resprouting plants was calculated as: Root RGR = $(B_f - B_0) / (B_0 \times t)$, with B_f being the final root biomass, B_0 the estimated root biomass at the beginning of the experiment and *t* the time in days since the start of the experiment. Hence, root RGR of resprouting plants represented the accumulated rate of root growth throughout the experiment (i.e. before and after the pruning), while the root RGR of seedlings only refers to the root growth rate between the start of the experiment and the first sampling in October 2012 (before pruning).

The resprouting success of the two study species was determined as the percentage of plants that regenerated their aerial biomass after the pruning in relation to the total number of plants in each treatment after the first sampling (*n* = 5 plants per species).

Leaf morphological traits and water status

One fully-developed leaf was collected from four individual plants per species, plot and watering regime in October 2012 and from 2 to 4 individuals per species, plot and watering regime in October 2013, depending on the number of plants that resprouted after pruning. In each sample, leaf thickness (LT) was measured using a portable digital micrometer (4000DIG model, Baxlo, Barcelona, Spain). Leaves were scanned (Epson Perfection 1250, USA) and from these images leaf area (LA) was calculated by means of an image processing software (ImageTool version 3.00, University of Texas, San Antonio, USA). Leaves were weighed to obtain leaf fresh mass (FM) and stored in distilled water at 4 ºC in darkness for 24 h to determine leaf turgid mass (TM). Leaves were then dried at 45 ºC for 5 days and reweighed to obtain leaf dry mass (DM). Based on these data, leaf mass per area (LMA) of each species was calculated as: LMA (mg cm^{-2}) = DM / LA; and the leaf tissue density (LTD) as: LTD (g cm^{-3}) = DM / (LA x LT). The leaf water status of *A. unedo* and *Q. suber* plants was assessed by means of two parameters, leaf water content (LWC) and relative water content (RWC), calculated as: LWC (%) = (FM – DM) x 100 / FM; and RWC (%) = (FM – DM) x 100 / (TM – DM).

Leaf gas-exchange measurements

Rates of leaf gas exchange for both species were determined using three plants per plot and watering regime at both pre- and post-pruning sampling times. Measurements were taken at midday on clear-sky days using fully-expanded leaves located at the top of the canopy and exposed to sunlight. Rates of leaf gas exchange were measured using a portable CO_2/H_2O infrared gas analyser (CIRAS-2, PP-Systems, Amesbury, USA) connected to a PLC6 leaf gas-exchange cuvette of 18 mm diameter (2.5 cm^2). Leaf photosynthesis (A, µmol CO_2 m^{-2} s^{-1}), transpiration (E, mmol H_2O m^{-2} s^{-1}) and stomatal conductance (g_s, mmol m^{-2} s^{-1}) were measured. Instantaneous water use efficiency (WUE, µmol CO_2 $mmol^{-1}$ H_2O) was calculated for both species as the quotient between rates of photosynthesis and transpiration.

Leaf chlorophyll fluorescence

Components of foliar chlorophyll fluorescence were measured in both sampling dates using four fully-developed sun-exposed leaves from four different plants per species, plot and watering regime. Measurements were taken with a portable chlorophyll fluorometer (PAM-2100, Heinz Walz GmbH, Effeltrich, Germany). Minimum (F_0) and maximum (Fm) dark-adapted fluorescence were measured and used to calculate Fv (Fv = Fm − F_0) and the potential (or maximum) photochemical efficiency of photosystem II (Fv/Fm). Actual photochemical efficiency of photosystem II in the light-adapted state was determined as: ΔF/Fm' = (Fm' − F) / Fm' where F was the steady-state fluorescence yield under the given environmental conditions, and Fm' was the maximum level of fluorescence obtained during a saturating flash of light. From these measurements, we calculated the apparent electron transport rate (ETR) as: ETR = ΔF/Fm' x PAR x 0.84 x 0.5, where PAR (in µmol m^{-2} s^{-1}) was the incident photosynthetically active radiation; 0.84 was the assumed coefficient of absorption of the leaves; and 0.5 was the assumed distribution of absorbed energy between the two photosystems (Galmés *et al.*, 2007). In addition, non-photochemical quenching coefficient (NPQ) was determined using the following equation: NPQ = (Fm – Fm') / Fm'.

Root biochemical traits

For both sampling periods, dried root samples from three individuals of *A. unedo* and *Q. suber* per UV and watering condition were ground by means of a ball mill (Mixer Mill MM 400, Retsch GmbH, Haan, Germany) and used to determine concentrations of starch, sugars and total phenols (TP).

Root starch concentration was analysed following the method of Bellasio *et al.* (2014) based on the release of glucose as a product of starch enzymatic hydrolysis and determined by spectrophotometry at 530 nm. Briefly, samples (approximately 40 mg of root powder), together with a sample blank with no powder, were extracted three times with 80% ethanol by stirring for 5 min and centrifuged (9000 g for 5 min), collecting the supernatant for sugar analysis. The pellet of ethanol-moistened root powder was digested with α-amylase (E-BLAAM, Megazyme International, IR) in buffer (sodium acetate buffer 0.1 M pH 5.0 plus $CaCl_2$ 5 mM) at 100 ºC for 12 min. A second digestion was done using amyloglucosidase (E-AMGDF, Megazyme International, IR) at 50 ºC for 45 min and centrifuged (9000 g for 5 min). From the supernatant, the glucose assay was done using 60 µl of sample (S) and the sample blank (SAB). Tubes with 40 µl of distilled water plus 20 µl of glucose standards (20 µg of glucose solution in 20 µl of benzoic acid 0.1%; GS) and a standard blank with 60 µl of distilled water (STB) were included in the assay. Following this, 540 µl of distilled water and 2 ml of a reagent composed of glucose oxidase and peroxidase in 0.1 M phosphate buffer pH 7.0 (P7119, Sigma, USA) plus *o*-dianisidine (D3252, Sigma, USA) were added to all tubes. The samples were incubated in a water bath at 37 ºC for 45 min and the reaction was stopped by adding 400 µl of 75% H_2SO_4. Absorbance was read at 530 nm in a spectrophotometer (UVmini-1240, Shimadzu, Japan). From this data, the starch concentration (SC) was calculated as: $SC\ (g / 100\ g) = ((A_S - A_{SAB}) \times m_{GS} \times V_T \times 0.9) / ((A_{GS} - A_{STB}) \times m_S \times V_S)$, where A indicates the absorbance values of samples (A_S), sample blank (A_{SAB}), glucose standards (A_{GS}) and standard blank (A_{STB}); m_{GS} is the amount of glucose added to glucose standards (20 µg); V_T is the final volume to which solutions were adjusted

(10 ml); m_S is the amount of root powder used (mg); V_S is the supernatant volume used in the glucose assay (60 µl); and 0.9 converts glucose-to-starch mass.

Determination of total soluble-sugar concentrations from supernatants collected from ethanol starch extraction were done using the method described by Chow and Landhäusser (2004). Specifically, 0.5 ml of ethanol extract was mixed with 1 ml of a 2% phenol solution and 2.5 ml of H_2SO_4 to allow hydrolysis of oligosaccharides forming monomers (glucose, fructose and galactose; GFG). After 10 min of colour development in the dark and 30 min of cooling, absorbance was measured at 490 nm using a spectrophotometer (UVmini-1240, Shimadzu, Japan). A solution of GFG (1:1:1 glucose, fructose, galactose) was used as the reference standard. Total soluble-sugar concentration was expressed as g of GFG equivalent per 100 g of leaf dry weight.

The determination of total phenolic concentration in roots was conducted using the method described in Bernal *et al.* (2015). Briefly, 10 mg of root powder was extracted with 50% methanol. The extract was shaken for 1 h and centrifuged at 2500 rpm for 5 min. A 50 µl aliquot of extract was mixed with 3.5 ml of distilled water and 250 µl of Folin Ciocalteu reagent (Panreac, Barcelona, Spain). After 8 min, 750 µl of 20% Na_2CO_3 was added. After 2 h, the absorbance of the mixture was determined spectrophotometrically at 760 nm (Genesys 6, Thermo Spectronic, Rochester, USA). Total phenolic concentration was calculated based on a gallic acid (GA) standard curve prepared from 50 µl of GA standard solution (40, 80, 150, 200, 400, 600, 800 and 1000 mg L^{-1}). Total phenol concentration (TP) was expressed as g of GA equivalent per 100 g of leaf dry weight.

Statistical analysis

Principal component analysis (PCA) was conducted using data for seedlings and resprouts of *A. unedo* and *Q. suber* for four biometric variables (stem height and diameter, and leaf and stem biomass), two leaf morphological traits (thickness and area) and three root biochemical characteristics (concentrations of starch, total soluble-sugar and total phenols), with the nine variables being normalised before use. Given the remarkable differences found in the PCA

between *A. unedo* and *Q. suber*, all the parameters studied were analysed separately for each species by means of three-way ANOVA using pruning, UV radiation and watering regime as fixed factors. Treatment effects on stem basal diameter and root biomass were tested by three-way ANCOVA including the pre-treatment data collected in May 2012 and the initial root biomass estimated as covariables, respectively. To analyse the treatment effects on stem height, leaf and stem biomass, three-way ANCOVA were also applied using the initial plant height as the covariable. To avoid pseudoreplication (Hurlbert, 1984), mean values of each parameter per treatment (UV x irrigation) were used for all statistical tests. In the case of significant UV effects, Fisher's LSD *post-hoc* pairwise comparisons were applied to determine differences among UV radiation conditions. When the interaction between factors was significant, we assessed the effects of the factor/s within the levels of the other factor/s by one- or two-way ANOVA. For certain variables, ANOVA within the treatments or before and after pruning were also done separately. Correlations among root traits (root biomass and concentrations of starch, sugar and total phenols) and the biometric and biochemical parameters measured were also explored for each species before and after pruning using Pearson's correlation tests. The Kolmogorov–Smirnov test was applied to analyse normality, while homogeneity of variance was determined using Levene's test. The level of significance considered was $p \leq 0.05$ for all the statistical analyses, except for the correlation tests ($p \leq 0.001$). Principal component analysis was done with PRIMER 6 software (PRIMER-E Ltd, Plymouth, United Kingdom) and other statistical analyses were done using SPSS software (IBM SPSS statistics, Corporation, Chicago, USA).

EXPERIMENT II. Effects of UV radiation and rainfall reduction on C and N pools in soil and plants of a shrubland ecosystem, before and after a controlled fire

Study area and experimental design

A field experiment involving UV radiation and precipitation reduction was conducted from August 2011 to June 2014 in a Mediterranean shrubland at the Gavarres Massif (41º 53' 57" N, 2º 54' 43" E) near Cassà de la Selva (Girona, NE of the Iberian Peninsula) (see Fig. S1 available as Supplementary Material). The study area was situated at about 250 m above sea level on a south-facing slope. The vegetation was dominated by *Arbutus unedo*, *Erica scoparia* and *Phillyrea angustifolia* whose relative abundances in the study site just before the experiment (spring 2011) were around 12%, 36% and 17%, respectively. Other woody Mediterranean species present were *Quercus suber*, *Pinus pinaster*, *Calluna vulgaris*, *Viburnum tinus*, *Daphne gnidium*, *Ulex parviflorus* and *Cistus salviifolius*, along with an herbaceous layer composed mainly of *Brachypodium retusum* and *Carex oedipostyla*. The soils of the study area were mostly Inceptisols, classified as Typic Haploxerept according to Soil Taxonomy System (Soil Survey Staff, 2010), with A, B, C/R horizon development over a Palaeozoic granitic parent material (see Table S1 available as Supplementary Material). Climatological variables, such as global solar irradiation, temperature and precipitation, were monitored throughout the study period (Fig. 2.4) by a meteorological station located at Cassà de la Selva, 3 km away from the study site.

In August 2011, eighteen plots (3 x 3 m per plot) were distributed over the study area. In each of these plots, plastic filters were installed above the vegetation on metallic frames with a 10º slope towards the south and at a height of around 1.5 m at the centre of the plot (Fig. 2.5; see also Fig. S4 available as Supplementary Material). These filters were made of different materials, which excluded or transmitted solar UV-A and/or UV-B radiation, allowing the establishment of three different UV conditions (see below). At the south-face side of each plot, a 40 cm-wide filter made of the same type of plastic that covered the plot was also placed in order to prevent plant exposure to unfiltered solar radiation. Filters covering the

plots also stopped the rainfall, which was collected in a deposit (310 L) settled next to each plot, allowing to combine the three UV conditions with two different precipitation regimes (see below). Each one of the six different UV x precipitation conditions was replicated three times, with plots being distributed in three blocks (six plots per block) (see Fig. S5 available as Supplementary Material). In each plot, several parameters related to C and N cycles were analysed at soil, litter and plant leaf level. Litter and plant leaves were studied from the two dominant species *A. unedo* and *P. angustifolia* (Table 2.2). In February-March 2013, all the vegetation of the experimental plots was burned in a controlled fire (see below).

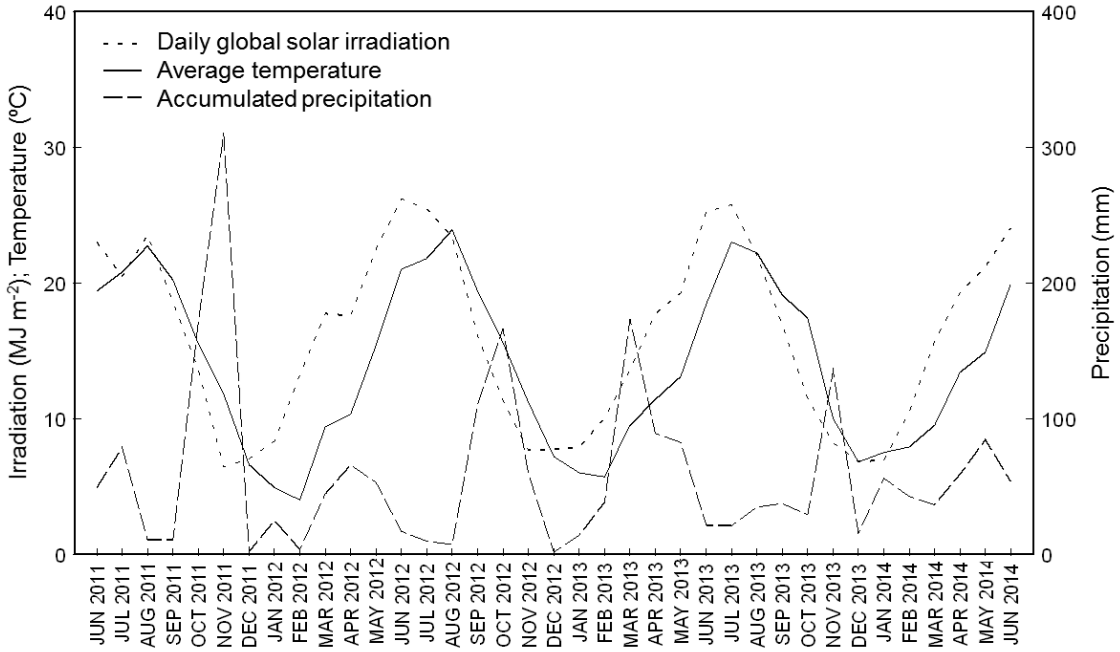


Fig. 2.4 Monthly averages of daily global solar irradiation (MJ m^{-2}) and temperature (ºC), together with accumulated precipitation (mm) for each month, along the study period. Data set was obtained from the meteorological station of Cassà de la Selva (177 m above sea level, 41º 52' 28" N, 2º 55' 37" E).

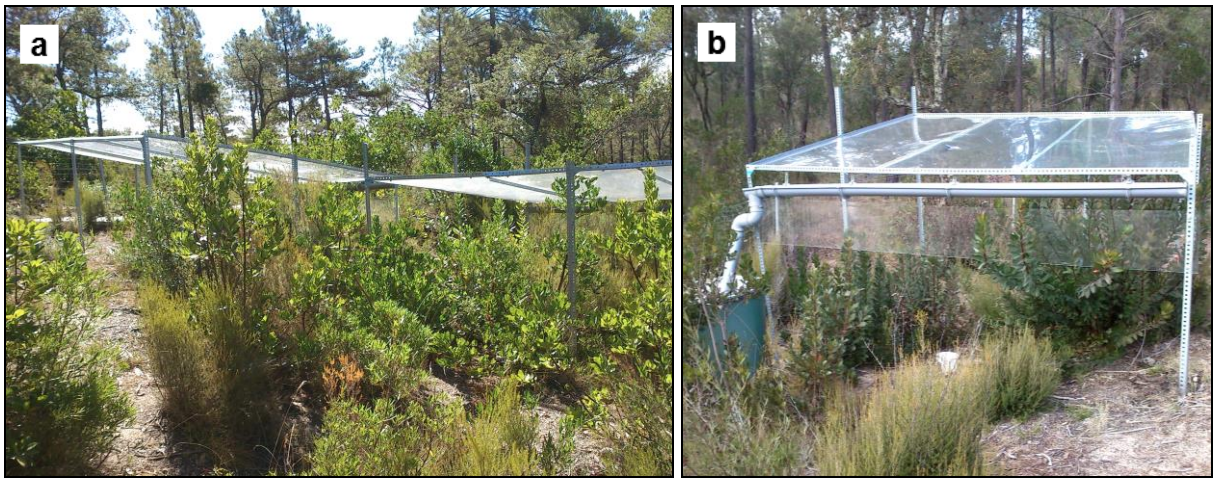


Fig. 2.5 Pictures of Experiment II showing: (**a**) a couple of the 18 study plots, which were distributed over a natural shrub community; and (**b**) detail of one plot (9 m^2 of area) made with metallic frames and equipped with UV filters and a water deposit.

Table 2.2 Sampling months and parameters analysed from soil, and from litter and plant leaves of *Arbutus unedo* and *Phillyrea angustifolia* before and after the experimental fire. Grey colour indicates sampling months, which correspond to the end of the different seasons, except in the case of February 2013, which is a sampling performed just before the fire.

Parameters		PRE-fire					POST-fire				
		Dec. 2011	Mar. 2012	Jun. 2012	Sep. 2012	Feb. 2013	Jun. 2013	Sep. 2013	Dec. 2013	Mar. 2014	Jun. 2014
Soil	Moisture (%)	■	■	■	■			■	■	■	■
	Temperature (ºC)	■	■	■	■			■	■	■	■
	Respiration (µmol m^{-2} s^{-1})	■	■	■	■			■	■	■	■
	Organic C (mg g^{-1})	■		■		■			■		■
	Total N (mg g^{-1})	■		■		■			■		■
	C:N ratio	■		■		■			■		■
	$pH_{1:2.5}$	■				■			■		■
	$EC_{1:5}$ (dS m^{-1})	■				■			■		■
	β-glucosidase (mg ρNP kg^{-1} h^{-1})	■		■					■		■
Litter cover (%)				■			■				■
Plant cover (%)				■			■				■
Leaf litter (*A. unedo* and *P. angustifolia*)	C (mg g^{-1})			■	■						*
	N (mg g^{-1})			■	■						*
	C:N ratio			■	■						*
	$\delta^{13}C$ (‰)			■	■						*
	$\delta^{15}N$ (‰)			■	■						*
Plant leaf (*A. unedo* and *P. angustifolia*)	C (mg g^{-1})		■		■			■		■	
	N (mg g^{-1})		■		■			■		■	
	C:N ratio		■		■			■		■	
	$\delta^{13}C$ (‰)		■		■			■		■	
	$\delta^{15}N$ (‰)		■		■			■		■	

EC: electrical conductivity.

* In June 2014, we only collected litter from *A. unedo*, since production of *P. angustifolia* litter was too low.

UV-radiation treatment

As detailed in Nenadis *et al.* (2015), the three UV conditions applied were (Table 2.3):

- UV0 plots (UV-A and UV-B exclusion): This condition was achieved by means of a 2-mm-thick polycarbonate filter (PC0100UV, PolimerTecnic, Girona, Spain) which allowed the transmission, on average, of only 5% of UV-B (280-315 nm) and 6% of UV-A (315-400 nm) solar radiation.
- UVA plots (UV-B exclusion): Plots under this condition did almost not receive UV-B radiation (3% on average), whereas average transmission of UV-A radiation was 52%. To accomplish this, a 0.25-mm-thick polyester filter (Melinex, Ponscosta, Valencia, Spain) was used.
- UVAB or control plots (near-ambient UV radiation): These plots were aimed to provide similar environmental conditions (degree of shading and temperature) as those under UV radiation exclusion. They were covered by a 3-mm-thick methacrylate filter (MC0100XN, PolimerTecnic, Girona, Spain), which transmitted, on average, 80.5 and 85% of UV-B and UV-A radiation, respectively.

Table 2.3 Percentage of UV radiation and photosynthetic photon flux density (PPFD) transmitted through the filter in each UV condition in the field (UVAB, UVA and UV0). UV radiation fluxes were expressed as unweighted UV-A and UV-B radiation and also using the plant response action spectrum (GEN) and the new plant growth weighting function (PG).

	UV radiation treatment		
	UVAB plots	UVA plots	UV0 plots
Filter type	methacrylate	polyester	polycarbonate
UV-A radiation	84-86 %	49-55 %	5-7 %
UV-B radiation	79-82 %	2-4 %	4-6 %
GEN[a]	81-83 %	3-4 %	4-6 %
PG[b]	83-84 %	35-46 %	4-5 %
PPFD	88-94 %	82-87 %	77-90 %

[a] *Plant response action spectrum according to Caldwell (1971)*

[b] *Plant growth weighting function according to Flint & Caldwell (2003)*

Spectral transmittances of filter materials in the UV and visible bands were assessed and verified periodically in the laboratory using a deuterium/halogen lamp and a CCD spectrometer (Avantes; The Netherlands). Effective *in situ* reduction in UV radiation and photosynthetic photon flux density (PPFD) under filters were determined using a double

monochromator spectroradiometer (SR9910, Irradian Ltd., UK). Since spectral measurements could not be taken continuously, we measured erythemal irradiance to assess the UV doses by means of two UV-S-E-T Kipp & Zonen sensors (The Netherlands): the first one was placed at the experimental site during several days each season; and the second one was located at the radiometric station of the Environmental Physics Group (EPG) at the University of Girona (41° 97' N, 2° 82' E, 115 m above sea level), 16 km far from the study site, where it was taking measurements continuously. The erythemal UV irradiance data (UVE; *Commission International de l'Éclairage*, CIE) in combination with the spectral measurements and radiative modelling allowed obtaining continuous series of unweighted UV irradiances. Series of irradiances weighted according to the generalized plant action spectrum (GEN) from Caldwell (1971) and to the new plant growth response spectrum (PG) from Flint & Caldwell (2003) were also obtained (Nenadis *et al.*, 2015).

UV-A doses were estimated from PPFD measurements in combination with the radiative model. PPFD was determined using continuous measurements of a quantum sensor (Li-190SA, Li-cor, USA, located at the EPG station) which was verified against spectroradiometric measurements. Also, PPFD measurements were performed seasonally at different points of the plots and at vegetation canopy level to confirm that filters reduced or transmitted PPFD levels adequately. Filters were periodically cleaned and they were replaced when radiation transmittance characteristics were not optimal or when they were damaged by strong winds.

Precipitation treatment

Half of the plots received 100% of the natural precipitation (natural rainfall condition, NR), whereas the other half (reduced rainfall condition, RR) were watered with 70% of the precipitation throughout the study period, except in winter when they were watered with 90%. To achieve these two levels of precipitation, the rainfall collected in the deposits settled beside each plot was used to irrigate the plots according to the above precipitation conditions. Percentages of reduced rainfall were established based on the changes in

precipitation expected for the Mediterranean basin in the near future as a consequence of climate change (IPCC, 2013). Throughout the study period, soil moisture was significantly lower in reduced rainfall plots compared to natural rainfall ones (Fig. 2.6), which confirms that the treatment was properly applied.

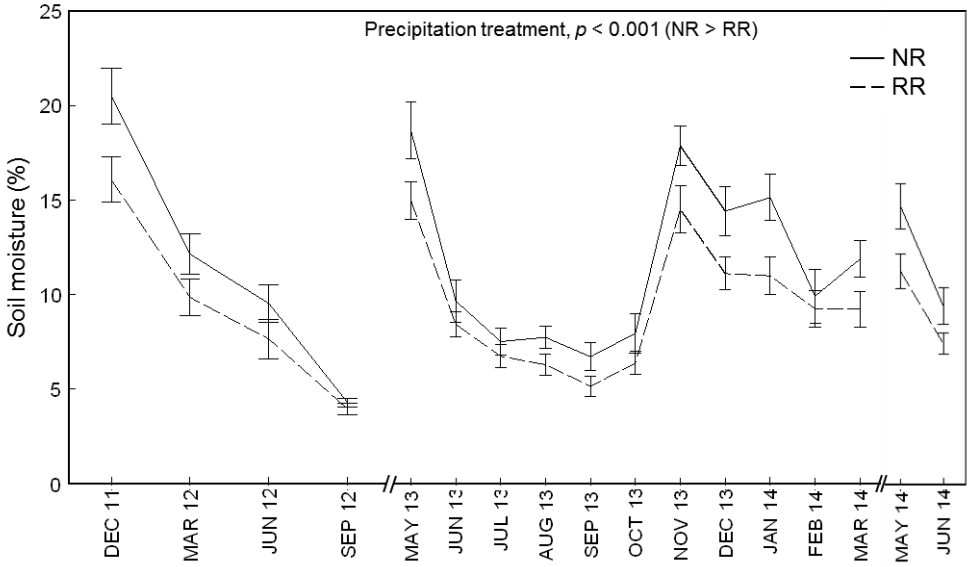


Fig. 2.6 Mean values of soil moisture (%) throughout the study period under the two experimental precipitation regimes: natural rainfall (NR) and reduced rainfall (RR). Error bars represent the standard error of the mean (n = 9). The significance level was set at $p \leq 0.05$.

Experimental disturbance (fire)

Vegetation of the plots was completely burned by specialized firefighter personnel in February-March of 2013. Just before the fire, the entire experimental infrastructure was removed, being rebuilt after the fire. The experimental setup was fully functional again by the end of March 2013.

At soil level

Soil parameters measured in situ

Measurements of soil moisture, temperature and respiration rates were performed *in situ* at midday, on sunny days, in five points distributed over each plot area. These parameters were measured at the end of each season throughout one year before the fire, and another year

after the fire (Table 2.2). In the post-fire period, monthly measurements of soil moisture were also taken from May 2013 to June 2014 to confirm that the precipitation treatment was properly applied (Fig. 2.6). Soil moisture was determined as the percentage of volumetric water content by means of a time domain reflectometer (FieldScout TDR 300 Soil Moisture Meter, Spectrum Technologies, Inc., Aurora, USA), with two 20-cm probe rods, providing instantaneous readings.

Soil CO_2 fluxes were measured with a portable infrared gas analyser (CIRAS-2, PP-Systems, Amesbury, USA) connected to an SRC-1 soil respiration chamber. Once the closed chamber (10 cm diameter x 15 cm height) was placed on the soil surface, the flux of CO_2 was measured by the IRGA for one minute. Carbon dioxide concentration was then calculated and expressed as μmol CO_2 m^{-2} s^{-1}. Data were calibrated according to soil temperature, which was determined just before the respiration measurements using a thermometer with a 10-cm probe rod (HANNA Instruments, Woonsocket, USA).

Soil parameters measured in the laboratory

For each plot, soil was sampled at two depths (A: 0-5 cm, and B: 5-10 cm) at the end of autumn (December 2011), spring (June 2012) and winter (February 2013) before the fire, and at the end of autumn (December 2013) and spring (June 2014) after the fire (Table 2.2). At each sampling date, and for each depth, samples were collected from five points distributed over the plot area and then mixed and homogenized in order to have one representative sample per plot and depth. In the laboratory, samples were air dried and sieved to 2 mm before the analyses. Soil organic C and total N were analysed for each one of the five seasons. Soil pH and electrical conductivity were measured for all the samples except for June 2012, while β-glucosidase enzyme activity was analysed for all the samples except for February 2013.

Organic C was quantified by the dichromate wet oxidation method in presence of concentrated sulphuric acid (Forster, 1995). The concentration of total N was determined by means of the Kjeldahl method (Forster, 1995). Briefly, 1 gram of soil was digested with 98%

H_2SO_4 for 1 h at 175 °C and 1.5 h at 370 °C for organic N mineralization. Ammonium was then distilled with a Kjeldahl Distiller Pro-nitro I (J.P. Selecta, Instrumentación Científica Técnica S.L., La Rioja, Spain).

Soil pH was determined using 1:2.5 soil water ratios and a Crison 20 pH meter, and electrical conductivity with a 1:5 soil water ratios and a Crison micro CM 2200 conductivity meter (Crison Instruments S.A., Barcelona, Spain).

The determination of β-glucosidase activity was conducted using the method of Masciandaro *et al.* (1994), which is based on the release of ρ-nitrophenol (ρNP) from the 0.05 M 4-nitrophenyl-β-D-glucopyranoside (ρNPG), used as substrate of the enzyme (Hayano & Tubaki, 1985). The concentration of ρNP released from 0.5 g of dried soil was determined spectrophotometrically at 398 nm (Tabatabai & Bremner, 1969). Thus, the β-glucosidase activity was expressed as mg ρNP kg^{-1} h^{-1}.

At plant level

Plant and litter cover

Plant and litter cover of each plot was measured by means of the vertical “pint-point” method (Arévalo *et al.*, 2011) just before the start of the treatments (May 2011) and, then, annually throughout the experimental period, in June 2012, 2013 and 2014 (Table 2.2). In each plot, data were collected from 5 parallel 3-m transects oriented east-west, along which 30 measuring points (one each 10 cm) were taken; hence, in total, 150 data points were considered per plot. For all these points, plant presence or absence was determined, as well as soil cover (which was classified as bare or covered with litter). Then, the percentage of points with vegetation presence, as well as those with soil litter, were calculated in relation to the total number of points sampled per transect, obtaining 5 values of plant and litter cover per plot.

Litter and leaf parameters

Four collectors of litter were installed in each plot at the beginning of the experiment. Three of these collectors were settled below the three dominant species (*A. unedo*, *P. angustifolia* and *E. scoparia*) whereas the fourth was placed in an area without vegetation. Leaf litter was sampled before (in June and September 2012) and after (in June 2014) the fire (Table 2.2). For each sampling date, one sample per plot was obtained by joining the leaf litter accumulated in the four collectors. After collection, leaf litter of *A. unedo* and *P. angustifolia* were separated for subsequent analysis. In June 2014, leaf litter of *P. angustifolia* was too scarce to be analysed.

Samplings of *A. unedo* and *P. angustifolia* leaves were always conducted at the end of winter and summer before and after the fire (i.e., March and September 2012, September 2013 and March 2014) (Table 2.2), and always on sunny days during hours of maximum solar irradiation. Leaves of both species were taken from the top of the canopy of each plant, selecting always south-facing fully-developed leaves exposed to solar radiation. For each plot and sampling date, we collected three leaves from three different plants of *P. angustifolia*, and four leaves from one or two plants of *A. unedo* (always from different branches).

Once in the laboratory, litter and leaf samples of both species were dried in an oven at 45 °C for 5 days and grounded using a ball mill (Mixer Mill MM 400, Retsch GmbH, Haan, Germany). From each litter sample, three subsamples of 3-4 mg of powder were encapsulated into tin (Sn) capsules to have replicas of each analysis. In the case of leaves, the different samples were analysed separately. Analyses of C and N concentrations, as well as of ^{15}N and ^{13}C, were performed at the University of California (UC Davis Stable Isotope Facility, Davis, USA), using an elemental analyser (PDZ Europa ANCA-GSL, Sercon Ltd., Cheshire, UK) linked to a continuous flow isotope ratio mass spectrometer (IRMS; PDZ Europa 20-20 IRMS, Sercon Ltd., Cheshire, UK). The final delta values were expressed relative to atmospheric nitrogen for $\delta^{15}N$ and relative to PDB standard for $\delta^{13}C$, according to the following equation:

$$\delta Z = (R_{sample} / R_{standard} - 1) * 1000$$

where Z is the heavy isotope of either N or C, and R is the ratio of heavier to lighter isotope ($^{15}N/^{14}N$ or $^{13}C/^{12}C$) for the sample and the standard. The long term standard deviation was 0.3‰ for ^{15}N and 0.2‰ for ^{13}C.

Statistical analysis

A Principal Component Analysis (PCA) was performed using data for six soil variables (moisture, temperature, respiration, organic C, total N and β-glucosidase activity) determined in four sampling dates (December 2011, June 2012, December 2013 and June 2014). The six variables were previously normalised and mean values were used in the case of those variables measured at two soil depths.

To evaluate the differences between pre- and post-fire data for soil and plant leaf parameters, as well as the possible interactive effects between fire and the two treatments, we performed three-way ANOVAs using fire and UV and precipitation treatments as factors. For those variables with at least two sampling dates both before and after the fire, pre- and post-fire data were also analysed separately. Among these variables, soil parameters determined from composite samples per plot and depth (organic C, total N, C:N ratio, pH, electrical conductivity and β-glucosidase) were analysed by means of repeated-measures ANOVAs for each depth, with UV and precipitation treatments as factors. Treatment effects on soil moisture, temperature and respiration, as well as on leaf C, N, $\delta^{13}C$, $\delta^{15}N$ and C:N ratio, were tested by ANOVA analyses, since data for these parameters were obtained from several soil points or leaves per plot. For soil parameters, sampling date, and UV and precipitation treatments were used as factors, while, for leaf parameters, plant species was also included as a factor. To avoid pseudoreplication (Hurlbert, 1984), mean values of each parameter per plot were used for all these statistical tests.

Treatment effects on plant and litter cover, as well as on litter quality variables (C, N, $\delta^{13}C$, $\delta^{15}N$ and C:N) for each species, were analysed within each sampling date by means of

two-way ANOVAs (with UV and precipitation treatments as factors). For plant and litter cover, pre-treatment data (May 2011) was also included in the statistical tests as a co-variable.

In the case of significant UV effects, Fisher's LSD *post-hoc* pairwise comparisons were applied to determine differences among UV conditions. When the interaction between factors was significant, treatment effects were assessed within the levels of the other factor. The Kolmogorov–Smirnov test was used to test normality, while the homogeneity of variances was analysed with the Levene's test. For all the statistical tests, the significance level considered was $p \leq 0.05$.

Chapter III

RESULTS

1. Leaf biochemical adjustments in two Mediterranean resprouter species facing enhanced UV level and reduced water availability before and after pruning

State-of-the-art

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

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

The first line of protection against UV radiation at biochemical level consists of minimizing UV exposure by means of UV-induced accumulation of phenylpropanoid

compounds in superficial plant tissues. The phenylpropanoid pathway is responsible for the synthesis of phenolics, such as tannins and flavonoids, that can occur mainly in the cuticle and epicuticular materials, cell walls and vacuoles of epidermis, and also in other external surfaces, such as leaf hairs (Paoletti, 2005; Agati *et al.*, 2013). Among other functions, phenolic compounds contribute to screen out the most energetic solar wavelengths reaching the leaf, avoiding its penetration into the tissues (Julkunen-Tiitto *et al.*, 2005; Caldwell *et al.*, 2007; Li *et al.*, 2010). Some flavonoids can also act as antioxidant compounds. Indeed, while the function of UV screening is mainly attributed to monohydroxy B-ring substituted flavonoids (e.g. kaempferols), flavonoids, such as quercetins, having a catechol group in the B-ring of the flavonoid skeleton (dihydroxy B-ring substituted flavonoids) show effective antioxidant properties (Agati & Tattini, 2010; Hideg *et al.*, 2013). Dihydroxy B-ring substituted flavonoids are confined near or within the sites of ROS production, such as chloroplasts (Agati & Tattini, 2010). Hence, flavonoids with antioxidant activity can inhibit the generation of ROS, but they can also reduce ROS once formed, representing a second line of defense against UV radiation (A-H-Mackerness, 2000; Agati *et al.*, 2012).

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

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

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

Mediterranean plant species have to face high solar irradiance together with other environmental stresses, such as water deficit, especially during summer (Bussotti *et al.*, 2014). However, interactive effects between UV and water availability levels on plant biochemical parameters, particularly phenolic compounds, are often not found in Mediterranean species, since many of them do not appear to be sensitive to UV doses (Paoletti, 2005; Bussotti *et al.*, 2014), although some studies have reported species-specific UV effects (Grammatikopoulos *et al.*, 1998; Bernal *et al.*, 2013). In a study with six Mediterranean species, despite there was not a general UV effect on the total leaf content of phenols, leaf phenolic composition of one of the species, *Pistacia lentiscus*, varied in response to UV exposure (Bernal *et al.*, 2013). In addition, it is known that UV radiation can modify the plant phenolic profile of some species. In a study conducted with *Arbutus unedo*, Nenadis *et al.* (2015) reported contrasting UV-B effects on leaf flavonoids, since while the concentration of flavanols decreased, the content of a flavonol derivative, quercetin 3-rhamnoside, increased. Differences in the behaviour of phenolic compounds might be associated to their different antioxidant capacities (Tattini *et al.*, 2004; Agati *et al.*, 2009), as mentioned above. Therefore, the responses of these photoprotective compounds to UV

levels in Mediterranean species are highly variable, often being dependent on the species and the specific compounds.

Apart from high UV radiation and water deficit, Mediterranean terrestrial ecosystems are usually exposed to periodical perturbations that reduce or remove the aerial plant biomass (fires, clear-cuts, grazing). Therefore, the persistence of these ecosystems strongly depends on the success of vegetation regeneration mechanisms, such as plant resprouting capacity. This strategy is based on the carbon reserve storage in roots which supports energetically the regrowth process of the aerial biomass after a disturbance (Canadell & López-Soria, 1998; Paula *et al.*, 2016). Since resprouter species allocate a much greater percentage of assimilates to roots in comparison with non-resprouter ones (Verdaguer & Ojeda, 2002), they could be especially sensitive to resource allocation changes in response to UV radiation. A higher investment of assimilated carbons into biochemical mechanisms involved in UV-protection could diminish the reserve storage belowground, impairing the regeneration capacity and, subsequently, the survival of resprouting plants. Taking into account the expected changes in solar UV levels, precipitation and fire frequency over the coming years, it is essential to increase our understanding of the biochemical adjustments involved in Mediterranean plant responses to these factors.

In this context, our objective was to examine the effects, before and after the removal of plant aerial biomass, of enhanced UV radiation (both UV-A and UV-B) and diminished water supply on the leaf concentration of phenols (and other antioxidants in the case of resprouts) of two sclerophyllous resprouter species, *Arbutus unedo* L. and *Quercus suber* L. that co-occur widely in Mediterranean shrublands. To achieve this goal, an outdoor experiment involving UV supplementation combined with controlled irrigation conditions was conducted in seedlings grown in pots of these two species, which, during the study period, were pruned removing all of the aerial biomass. Main leaf phenolic compounds and antioxidants were first analysed in the seedlings and, later, in their resprouts. We hypothesized that: (i) there will be interactive effects between UV radiation and water deficit on the leaf phenol and antioxidant profiles of these species; (ii) leaf antioxidant compounds,

including phenolics, would be mainly responsiveness to reduced water supply, while enhanced UV doses would primarily favour UV-screening responses; and (iii) in comparison with seedlings, resprouting plants would be more sensitive to enhanced UV due to their earlier stage of aerial biomass development, but less sensitive to low water supply due to their improved water status through the lower shoot to root biomass ratio and thus higher water availability.

Results

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

A similar number of phenolic compounds were detected in *A. unedo* and *Q. suber* leaves (30 and 26, respectively) (Tables 3.1 and 3.2). Despite this, the total concentration of identified phenols was 2.7-fold higher in *A. unedo* than in *Q. suber*. Detected phenols were grouped into the following classes: tannins (condensed and hydrolyzable), flavonoids (flavanols and flavonols) and phenolic acids, although, in *A. unedo*, hydroquinones were also found.

In *A. unedo*, identified phenols were: 14 hydrolyzable tannins (2 ellagitannins, 7 gallotannins and 5 galloyl derivatives); 2 flavanols ((+)-catechin and gallocatechin); 8 flavonols, including 4 quercetins (quercetin 3-galactoside, quercetin 3-arabinoside, quercetin-glycoside and quercetin 3-rhamnoside), 3 kaempferols (kaempferol 3-glucoside, kaempferol 3-rhamnoside and kaempferol glycoside) and the myricetin 3-rhamnoside; 3 hydroquinones (arbutin and 2 arbutin derivatives); and 2 phenolic acids (gallic acid and ellagic acid) (Table 3.1). Identified phenols in *Q. suber* leaves were: 4 hydrolyzable tannins; 4 flavanols ((+)-catechin, gallocatechin, epigallocatechin and epigallocatechin gallate); 16 different flavonols, including 4 quercetins (quercetin 3-galactoside, quercetin 3-glucoside, quercetin-glycoside and quercetin 3-rhamnoside), 4 kaempferols (kaempferol 3-glucoside, kaempferol glycoside and 2 monocoumaroyl-astragalin), 2 myricetins (myricetin 3-galactoside and myricetin 3-glucoside), 2 rutin derivatives and 4 unknown flavonols; and 1 phenolic acid (ellagic acid) (Table 3.2).

Table 3.1 Overall mean ± S.E. for the concentration (mg g DW^{-1}) of the identified phenolic compounds in leaves of *Arbutus unedo* under three UV radiation conditions (control, UVA and UVAB) and two watering regimes (well-watered, WW, and low-watered, LW). Numbers in bold indicate significant differences between the levels of the factor. n = 12 in each UV level and n = 18 for each watering and pruning condition. The significance level considered was $p \leq 0.05$. Only significant two-way or three-way interactions among UV radiation (UV), 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	237.292 ± 11.135	240.376 ± 13.688	246.993 ± 10.600	ns	239.082 ± 8.139	244.025 ± 10.851	ns	**212.299 ± 6.484**	**270.809 ± 6.479**	**<0.001**	-
Tannins	89.957 ± 6.122	88.082 ± 6.007	87.434 ± 4.274	ns	85.022 ± 3.478	91.960 ± 5.122	ns	**77.542 ± 2.960**	**99.439 ± 4.109**	**<0.001**	-
Condensed tannins	74.398 ± 5.951	72.961 ± 6.548	70.837 ± 4.638	ns	70.174 ± 3.741	75.290 ± 5.342	ns	**61.900 ± 3.332**	**83.563 ± 4.289**	**0.001**	-
Hydrolyzable tannins	15.559 ± 0.515	15.121 ± 1.196	16.597 ± 1.095	ns	14.848 ± 0.693	16.670 ± 0.846	ns	15.642 ± 0.763	15.876 ± 0.842	ns	-
ellagitannin 1	0.132 ± 0.015	0.102 ± 0.012	0.116 ± 0.012	ns	**0.095 ± 0.008**	**0.138 ± 0.011**	**0.002**	0.115 ± 0.007	0.118 ± 0.013	ns	W x P
ellagitannin 2	1.516 ± 0.118	1.387 ± 0.203	1.286 ± 0.203	ns	**1.188 ± 0.092**	**1.605 ± 0.171**	**0.020**	**1.213 ± 0.095**	**1.580 ± 0.172**	**0.038**	W x P
gallotannin 1	0.377 ± 0.074	0.331 ± 0.075	0.402 ± 0.075	ns	0.355 ± 0.049	0.385 ± 0.070	ns	**0.497 ± 0.070**	**0.243 ± 0.022**	**0.002**	-
gallotannin 2	1.445 ± 0.097	1.388 ± 0.171	1.485 ± 0.126	ns	**1.255 ± 0.068**	**1.623 ± 0.123**	**0.016**	1.519 ± 0.112	1.360 ± 0.102	ns	-
gallotannin 3	1.039 ± 0.104	1.273 ± 0.184	1.224 ± 0.155	ns	1.087 ± 0.094	1.271 ± 0.145	ns	1.333 ± 0.139	1.024 ± 0.093	ns	W x P
gallotannin 4	0.630 ± 0.100	0.948 ± 0.161	0.810 ± 0.145	ns	0.837 ± 0.121	0.755 ± 0.108	ns	0.801 ± 0.103	0.791 ± 0.126	ns	W x P
gallotannin 5	0.603 ± 0.125	0.666 ± 0.145	0.659 ± 0.138	ns	0.641 ± 0.100	0.644 ± 0.118	ns	**0.461 ± 0.069**	**0.824 ± 0.124**	**0.023**	-
gallotannin 6	0.276 ± 0.047	0.254 ± 0.054	0.256 ± 0.039	ns	0.287 ± 0.033	0.238 ± 0.042	ns	**0.327 ± 0.019**	**0.198 ± 0.045**	**0.019**	-
gallotannin 7	1.848 ± 0.151	1.789 ± 0.143	1.874 ± 0.297	ns	**1.528 ± 0.078**	**2.146 ± 0.199**	**0.003**	1.692 ± 0.109	1.982 ± 0.206	ns	W x P
galloylglucose	0.371 ± 0.084	0.461 ± 0.133	0.356 ± 0.098	ns	0.419 ± 0.088	0.373 ± 0.085	ns	0.413 ± 0.091	0.379 ± 0.082	ns	-
digalloylglucose 1	0.795 ± 0.107	1.116 ± 0.427	1.017 ± 0.176	ns	0.827 ± 0.100	1.126 ± 0.294	ns	0.675 ± 0.076	1.277 ± 0.288	ns	-
digalloylglucose 2	0.123 ± 0.020	0.123 ± 0.025	0.201 ± 0.028	ns	0.148 ± 0.022	0.150 ± 0.022	ns	0.162 ± 0.017	0.136 ± 0.025	ns	-
pentagalloylglucose	6.130 ± 0.341	4.962 ± 0.592	6.525 ± 0.637	ns	5.828 ± 0.460	5.917 ± 0.465	ns	6.114 ± 0.438	5.631 ± 0.479	ns	-
helasogalloylglucoside	0.273 ± 0.031	0.321 ± 0.055	0.386 ± 0.053	ns	0.354 ± 0.038	0.300 ± 0.041	ns	0.320 ± 0.034	0.334 ± 0.045	ns	-
Flavonoids	47.836 ± 3.711	46.710 ± 4.624	49.968 ± 3.539	ns	48.859 ± 3.329	47.484 ± 3.105	ns	**36.420 ± 1.537**	**59.923 ± 1.474**	**<0.001**	-
Flavanols	15.881 ± 2.118	15.322 ± 2.258	17.676 ± 2.608	ns	17.123 ± 1.965	15.464 ± 1.800	ns	**9.176 ± 0.553**	**23.410 ± 0.959**	**<0.001**	-
(+)-catechin	14.362 ± 1.876	13.593 ± 1.891	15.106 ± 2.259	ns	15.029 ± 1.710	13.678 ± 1.526	ns	**8.323 ± 0.406**	**20.385 ± 0.929**	**<0.001**	-
gallocatechin	1.519 ± 0.313	1.729 ± 0.482	2.570 ± 0.832	ns	2.093 ± 0.498	1.785 ± 0.463	ns	**0.853 ± 0.303**	**3.025 ± 0.485**	**0.002**	-
Flavonols	31.954 ± 1.776	31.388 ± 2.504	32.292 ± 1.696	ns	31.736 ± 1.702	32.021 ± 1.556	ns	**27.244 ± 1.154**	**36.512 ± 1.210**	**<0.001**	UV x P
Quercetins	21.862 ± 1.533	20.475 ± 2.013	21.092 ± 1.400	ns	20.906 ± 1.293	21.380 ± 1.402	ns	**17.340 ± 0.973**	**24.946 ± 0.998**	**<0.001**	UV x P
quercetin 3-galactoside	1.875 ± 0.293	1.497 ± 0.295	1.597 ± 0.287	ns	1.583 ± 0.213	1.730 ± 0.259	ns	**1.057 ± 0.148**	**2.256 ± 0.222**	**<0.001**	-
quercetin 3-arabinoside	2.289 ± 0.246	1.877 ± 0.238	2.222 ± 0.363	ns	1.974 ± 0.155	2.285 ± 0.290	ns	1.831 ± 0.162	2.427 ± 0.273	ns	UV x W
quercetin 3-rhamnoside	14.918 ± 0.854	14.548 ± 1.262	14.153 ± 0.648	ns	15.125 ± 0.792	13.954 ± 0.725	ns	**12.730 ± 0.634**	**16.349 ± 0.638**	**<0.001**	UV x P
quercetin-glycoside	2.781 ± 0.635	2.554 ± 0.632	3.119 ± 0.526	ns	**2.225 ± 0.389**	**3.411 ± 0.529**	**0.055**	**1.722 ± 0.226**	**3.913 ± 0.529**	**0.001**	-
Kaempferols	3.365 ± 0.142	3.498 ± 0.325	3.613 ± 0.167	ns	3.597 ± 0.209	3.387 ± 0.149	ns	3.330 ± 0.135	3.655 ± 0.214	ns	-
kaempferol 3-glucoside	0.495 ± 0.055	0.532 ± 0.073	0.454 ± 0.047	ns	0.559 ± 0.050	0.429 ± 0.041	ns	0.473 ± 0.045	0.514 ± 0.051	ns	-
kaempferol 3-rhamnoside	1.837 ± 0.216	1.874 ± 0.271	2.026 ± 0.191	ns	1.823 ± 0.236	2.002 ± 0.109	ns	1.671 ± 0.133	2.154 ± 0.209	ns	-
kaempferol glycoside	1.033 ± 0.107	1.092 ± 0.155	1.133 ± 0.173	ns	1.215 ± 0.097	0.956 ± 0.131	ns	1.186 ± 0.094	0.986 ± 0.136	ns	-
Myricetins											
myricetin 3-rhamnoside	6.727 ± 0.332	7.415 ± 0.587	7.587 ± 0.473	ns	7.232 ± 0.479	7.254 ± 0.282	ns	**6.574 ± 0.284**	**7.912 ± 0.420**	**0.018**	-
Hydroquinones	97.295 ± 4.219	103.284 ± 6.793	107.306 ± 7.818	ns	102.942 ± 5.210	102.314 ± 5.364	ns	96.152 ± 4.222	109.105 ± 5.760	ns	-
arbutin	50.858 ± 3.213	52.444 ± 4.159	54.051 ± 4.250	ns	51.228 ± 2.760	53.674 ± 3.480	ns	**45.504 ± 1.715**	**59.398 ± 3.360**	**0.001**	W x P
galloylarbutin	32.359 ± 2.819	35.138 ± 4.069	37.424 ± 4.712	ns	36.269 ± 3.150	33.679 ± 3.245	ns	33.734 ± 2.850	36.213 ± 3.514	ns	-
digalloylarbutin	14.079 ± 1.085	15.701 ± 1.865	15.831 ± 1.484	ns	15.446 ± 1.143	14.961 ± 1.311	ns	16.913 ± 0.977	13.493 ± 1.317	ns	-
Phenolic acids	2.204 ± 0.128	2.300 ± 0.194	2.285 ± 0.173	ns	2.260 ± 0.137	2.267 ± 0.133	ns	2.184 ± 0.079	2.342 ± 0.172	ns	-
gallic acid	1.766 ± 0.117	1.839 ± 0.181	1.913 ± 0.177	ns	1.836 ± 0.138	1.843 ± 0.123	ns	1.776 ± 0.087	1.903 ± 0.161	ns	-
ellagic acid	0.438 ± 0.027	0.460 ± 0.026	0.372 ± 0.027	ns	0.423 ± 0.020	0.424 ± 0.026	ns	0.408 ± 0.023	0.439 ± 0.023	ns	-
TAN:TP	0.377 ± 0.013	0.367 ± 0.016	0.357 ± 0.016	ns	0.358 ± 0.012	0.376 ± 0.012	ns	0.366 ± 0.011	0.368 ± 0.014	ns	-
FLAV:TP	0.199 ± 0.009	0.190 ± 0.011	0.202 ± 0.011	ns	0.202 ± 0.010	0.192 ± 0.007	ns	**0.172 ± 0.005**	**0.223 ± 0.006**	**<0.001**	W x P
Que:Kae	6.522 ± 0.419	6.071 ± 0.633	5.972 ± 0.507	ns	5.984 ± 0.430	6.392 ± 0.420	ns	**5.250 ± 0.255**	**7.126 ± 0.444**	**0.002**	-
Hq:TP	0.414 ± 0.016	0.433 ± 0.017	0.432 ± 0.021	ns	0.431 ± 0.016	0.422 ± 0.013	ns	**0.452 ± 0.011**	**0.401 ± 0.015**	**0.014**	-
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**	**0.019**	W x P

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

Table 3.2 Overall mean ± S.E. for the concentration (mg g DW^{-1}) of the identified phenolic compounds in leaves of *Quercus suber* under three UV radiation conditions (control, UVA and UVAB) and two watering regimes (well-watered, WW, and low-watered, LW). Numbers in bold indicate significant differences among the levels of the factor. In the case of the UV treatment, significant differences among UV conditions are also indicated by different letters. n = 12 in each UV level and n = 18 for each watering and pruning condition. The significance level considered was $p \leq 0.05$. Only significant two-way or three-way interactions among UV radiation (UV), 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	90.450 ± 7.702		88.434 ± 3.992		92.763 ± 7.338	ns	89.329 ± 4.674	91.768 ± 5.834	ns	**80.213 ± 4.886**	**100.885 ± 4.429**	**0.006**	-
Tannins	60.833 ± 6.426		59.722 ± 3.250		62.213 ± 6.421	ns	59.479 ± 4.141	62.366 ± 4.784	ns	55.347 ± 4.462	66.499 ± 4.088	ns	-
Condensed tannins	55.668 ± 6.392		53.951 ± 3.228		56.139 ± 6.416	ns	53.403 ± 4.143	57.103 ± 4.735	ns	50.834 ± 4.386	59.671 ± 4.295	ns	-
Hydrolyzable tannins	5.165 ± 0.468		5.771 ± 0.519		6.074 ± 0.417	ns	**6.076 ± 0.377**	**5.264 ± 0.375**	**0.029**	**4.513 ± 0.241**	**6.827 ± 0.294**	**<0.001**	-
hydrolyzable tannin 1	0.814 ± 0.048		0.837 ± 0.037		0.902 ± 0.055	ns	0.890 ± 0.045	0.812 ± 0.029	ns	**0.786 ± 0.042**	**0.916 ± 0.029**	**0.021**	-
hydrolyzable tannin 2	2.841 ± 0.289		3.019 ± 0.337		2.801 ± 0.283	ns	3.023 ± 0.252	2.751 ± 0.234	ns	**2.095 ± 0.112**	**3.679 ± 0.185**	**<0.001**	-
hydrolyzable tannin 3	**1.078 ± 0.135**	**b**	**1.421 ± 0.159**	**ab**	**1.870 ± 0.215**	**a** **0.009**	1.643 ± 0.172	1.270 ± 0.132	ns	1.310 ± 0.160	1.603 ± 0.151	ns	-
hydrolyzable tannin 4	0.434 ± 0.060		0.494 ± 0.072		0.501 ± 0.065	ns	0.522 ± 0.054	0.430 ± 0.051	ns	**0.323 ± 0.028**	**0.629 ± 0.047**	**<0.001**	-
Flavonoids	29.263 ± 1.757		28.382 ± 1.442		30.223 ± 1.684	ns	29.506 ± 1.319	29.072 ± 1.332	ns	**24.566 ± 0.607**	**34.012 ± 0.725**	**<0.001**	-
Flavanols	17.839 ± 1.277		17.231 ± 0.944		17.287 ± 1.185	ns	17.618 ± 0.920	17.286 ± 0.923	ns	**14.405 ± 0.549**	**20.499 ± 0.555**	**<0.001**	-
(+)-catechin	10.012 ± 1.084		9.454 ± 0.497		9.187 ± 0.908	ns	9.407 ± 0.713	9.696 ± 0.687	ns	8.892 ± 0.682	10.210 ± 0.683	ns	-
gallocatechin	1.968 ± 0.260		1.932 ± 0.176		2.454 ± 0.346	ns	**2.420 ± 0.256**	**1.816 ± 0.156**	**0.058**	1.879 ± 0.209	2.357 ± 0.225	ns	-
epigallocatechin	3.735 ± 0.517		3.524 ± 0.633		3.670 ± 0.660	ns	3.720 ± 0.474	3.566 ± 0.501	ns	**2.111 ± 0.215**	**5.176 ± 0.392**	**<0.001**	-
epigallocatechin gallate	2.220 ± 0.271		2.321 ± 0.237		1.975 ± 0.236	ns	2.136 ± 0.203	2.208 ± 0.203	ns	**1.588 ± 0.151**	**2.756 ± 0.140**	**<0.001**	-
Flavonols	**11.425 ± 0.692**	**b**	**11.150 ± 0.607**	**b**	**12.936 ± 0.569**	**a** **0.006**	11.888 ± 0.581	11.786 ± 0.490	ns	**10.161 ± 0.377**	**13.513 ± 0.324**	**<0.001**	-
Quercetins	4.194 ± 0.317		3.900 ± 0.340		4.409 ± 0.403	ns	4.170 ± 0.301	4.165 ± 0.279	ns	**3.220 ± 0.127**	**5.115 ± 0.216**	**<0.001**	-
quercetin 3-galactoside	1.463 ± 0.114		1.403 ± 0.116		1.449 ± 0.145	ns	1.423 ± 0.109	1.453 ± 0.094	ns	**1.151 ± 0.043**	**1.725 ± 0.095**	**<0.001**	-
quercetin 3-glucoside	2.090 ± 0.189		1.908 ± 0.194		2.239 ± 0.216	ns	2.092 ± 0.167	2.067 ± 0.161	ns	**1.541 ± 0.096**	**2.618 ± 0.102**	**<0.001**	-
quercetin 3-rhamnoside	**0.316 ± 0.026**	**ab**	**0.269 ± 0.023**	**b**	**0.362 ± 0.033**	**a** **0.036**	0.320 ± 0.025	0.311 ± 0.022	ns	**0.268 ± 0.017**	**0.363 ± 0.025**	**0.002**	-
quercetin-glycoside	0.324 ± 0.030		0.320 ± 0.029		0.359 ± 0.038	ns	0.335 ± 0.028	0.333 ± 0.025	ns	**0.260 ± 0.009**	**0.408 ± 0.026**	**<0.001**	-
Kaempferols	**1.293 ± 0.091**	**b**	**1.382 ± 0.087**	**b**	**1.802 ± 0.090**	**a** **0.001**	1.559 ± 0.089	1.426 ± 0.087	ns	1.452 ± 0.091	1.533 ± 0.087	ns	-
kaempferol 3-glucoside	**0.659 ± 0.081**	**b**	**0.759 ± 0.064**	**b**	**1.053 ± 0.085**	**a** **0.004**	0.884 ± 0.074	0.763 ± 0.071	ns	0.846 ± 0.072	0.801 ± 0.075	ns	W x P
kaempferol glycoside	0.298 ± 0.023		0.278 ± 0.024		0.306 ± 0.028	ns	0.290 ± 0.019	0.298 ± 0.021	ns	**0.264 ± 0.020**	**0.324 ± 0.018**	**0.024**	W x P
monocoumaroyl-astragalin 1	**0.183 ± 0.012**	**b**	**0.200 ± 0.010**	**b**	**0.262 ± 0.019**	**a** **0.001**	0.227 ± 0.015	0.203 ± 0.012	ns	**0.198 ± 0.014**	**0.232 ± 0.013**	**0.041**	-
monocoumaroyl-astragalin 2	**0.153 ± 0.011**	**ab**	**0.145 ± 0.010**	**b**	**0.181 ± 0.016**	**a** **0.051**	0.158 ± 0.009	0.161 ± 0.012	ns	**0.144 ± 0.009**	**0.176 ± 0.011**	**0.012**	W x P
Myricetins	0.152 ± 0.027		0.211 ± 0.038		0.262 ± 0.039	ns	0.200 ± 0.034	0.217 ± 0.026	ns	0.219 ± 0.030	0.199 ± 0.031	ns	-
myricetin 3-galactoside	0.059 ± 0.020		0.090 ± 0.014		0.085 ± 0.017	ns	0.063 ± 0.015	0.093 ± 0.013	ns	0.082 ± 0.013	0.074 ± 0.016	ns	-
myricetin 3-glucoside	0.094 ± 0.013		0.121 ± 0.026		0.178 ± 0.032	ns	0.137 ± 0.027	0.124 ± 0.016	ns	0.137 ± 0.020	0.125 ± 0.023	ns	-
Rutins	1.878 ± 0.081		1.812 ± 0.092		2.062 ± 0.090	ns	1.916 ± 0.086	1.919 ± 0.062	ns	**1.791 ± 0.084**	**2.043 ± 0.049**	**0.018**	-
rutin derivative 1	1.633 ± 0.086		1.566 ± 0.081		1.817 ± 0.081	ns	1.664 ± 0.081	1.681 ± 0.060	ns	**1.537 ± 0.077**	**1.808 ± 0.046**	**0.007**	-
rutin derivative 2	0.245 ± 0.012		0.246 ± 0.022		0.244 ± 0.017	ns	0.251 ± 0.016	0.238 ± 0.011	ns	0.254 ± 0.014	0.235 ± 0.014	ns	-
unknown flavonols	3.923 ± 0.289		3.845 ± 0.288		4.401 ± 0.276	ns	4.053 ± 0.254	4.059 ± 0.218	ns	**3.489 ± 0.205**	**4.623 ± 0.180**	**0.001**	-
flavonol 1	**0.589 ± 0.046**	**b**	**0.700 ± 0.074**	**ab**	**0.752 ± 0.057**	**a** **0.026**	0.710 ± 0.057	0.650 ± 0.044	ns	**0.543 ± 0.036**	**0.818 ± 0.042**	**<0.001**	-
flavonol 2	1.658 ± 0.182		1.631 ± 0.193		1.853 ± 0.172	ns	1.677 ± 0.149	1.751 ± 0.149	ns	**1.314 ± 0.094**	**2.114 ± 0.129**	**<0.001**	-
flavonol 3	0.054 ± 0.008		0.062 ± 0.008		0.069 ± 0.006	ns	0.063 ± 0.006	0.061 ± 0.006	ns	0.056 ± 0.007	0.068 ± 0.005	ns	-
flavonol 4	1.622 ± 0.123		1.452 ± 0.102		1.726 ± 0.138	ns	1.603 ± 0.120	1.597 ± 0.080	ns	1.576 ± 0.111	1.624 ± 0.091	ns	-
Phenolic acids													
ellagic acid	0.350 ± 0.023		0.330 ± 0.020		0.328 ± 0.022	ns	0.342 ± 0.016	0.330 ± 0.019	ns	**0.298 ± 0.016**	**0.374 ± 0.014**	**0.004**	-
TAN:TP	0.654 ± 0.013		0.664 ± 0.011		0.637 ± 0.016	ns	0.644 ± 0.012	0.659 ± 0.010	ns	0.660 ± 0.012	0.644 ± 0.010	ns	-
FLAV:TP	0.341 ± 0.013		0.332 ± 0.011		0.359 ± 0.016	ns	0.351 ± 0.012	0.337 ± 0.010	ns	0.336 ± 0.012	0.352 ± 0.010	ns	-
Que:Kae	3.683 ± 0.318		3.280 ± 0.385		3.030 ± 0.511	ns	3.223 ± 0.352	3.440 ± 0.321	ns	**2.638 ± 0.199**	**4.024 ± 0.364**	**0.005**	-
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.

Comparing the percentages of the different classes of phenols in *A. unedo* and *Q. suber* leaves in relation to total phenols (TP), it is remarkable that, whereas the percentage of hydrolyzable tannins is similar in both species (by 6%), there were important differences in the leaf content of condensed tannins, flavonoids and hydroquinones. Indeed, in *A. unedo* leaves, hydroquinones represent the major group of phenols (42.5% of TP), followed by condensed tannins (30.1% of TP) and flavonoids (19.9% of TP). On the contrary, in *Q. suber* leaves, condensed tannins were the most abundant group of phenols (61.0% of TP) being flavonoids the second one (32.4% of TP), while hydroquinones were not detected. Thus, despite *A. unedo* leaves had the highest amount of phenols, the percentage of condensed tannins in *Q. suber* was double that in *A. unedo*.

Regarding the differences between seedlings and resprouts, in both species, the leaf total pool of phenols increased by more than 25% in resprouting plants (by 27.6% in *A. unedo* and by 25.8% in *Q. suber*) (Tables 3.1 and 3.2). The groups of phenols showing the largest increases in leaves of *A. unedo* and *Q. suber* resprouts were flavanols (by 155% and 42%, respectively) and quercetins (by 44% and 59%, respectively). In *A. unedo* leaves, tannins, mainly the condensed ones, myricetins, and the hydroquinone arbutin were also higher in the resprouts (by 35%, 20% and 30%, respectively) compared to seedlings. In *Q. suber* leaves, the total amount of tannins did not differ between seedlings and resprouts, although hydrolyzable tannins were significantly higher in the resprouting plants (by 51%). For this species, other phenols that were more abundant in the resprouts were rutins (by 14%), the unknown flavonols (by 32%) and the ellagic acid (by 25%) (Table 3.2). In both species, we found a greater quercetin to kaempferol ratio (Que:Kae) in the leaves of the resprouting plants due to their enhanced content of quercetins. In *A. unedo* leaves, resprouts also exhibited higher FLAV:TP and lower Hq:TP and PA:TP ratios in relation to seedlings (Table 3.1).

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

UV supplementation affected the concentration of two quercetins in *A. unedo* leaves, the quercetin 3-arabinoside and the quercetin 3-rhamnoside (quercitrin), being these effects modulated, respectively, by the watering treatment and the pruning (Table 3.1). In particular, under enhanced UV-A+UV-B radiation, the level of quercetin 3-arabinoside was higher (almost twice) in low-watered than in well-watered plants ($F_{1,10}$ = 4.791, p = 0.053; Fig. 3.1), while no irrigation effects were found in plants grown in UVA and control plots.

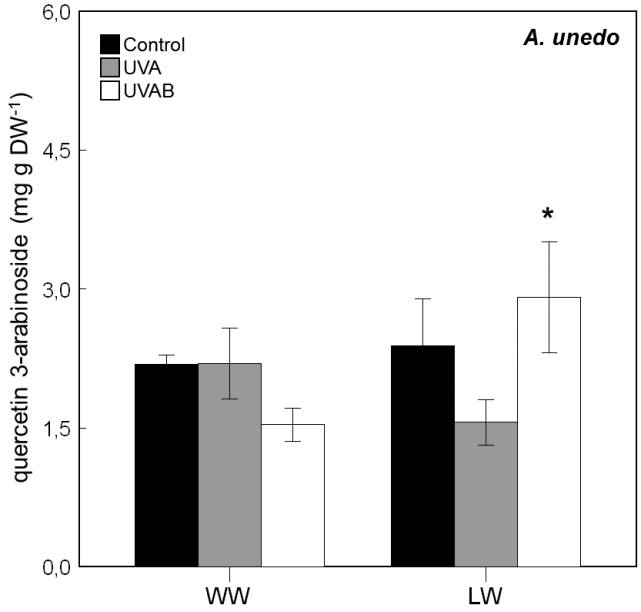


Fig. 3.1 *Arbutus unedo* leaf concentration of quercetin 3-arabinoside in plants subjected to three UV radiation conditions (control, UVA and UVAB) combined with two watering regimes (well-watered, WW, and low-watered, LW). Error bars represent the standard error of the mean (n = 6). The asterisk indicates a significant difference between WW and LW plants exposed to the same UV condition. The significance level was set at $p \leq 0.05$.

Regarding quercetin 3-rhamnoside, we found a 67.4% higher leaf concentration of this compound in the resprouts, but only when plants were grown under enhanced UV-A radiation ($F_{1,10}$ = 33.044, p < 0.001; Fig. 3.2c). This effect was also found for the total amount of quercetins and flavonols ($F_{1,10}$ = 90.806, p < 0.001; $F_{1,10}$ = 82.422, p < 0.001, respectively; Fig. 3.2a,b). Resprouts also had a higher concentration of flavonols than seedlings when grown under control conditions ($F_{1,10}$ = 6.241, p = 0.032; Fig. 3.2b). The significant interactive effect between UV and pruning on the total amount of quercetins and flavonols (Table 3.1) is also explained by the fact that the effect of the UV treatment was only significant for the

seedlings (Fig. 3.2a,b). Indeed, seedlings exposed to enhanced UV-A+UV-B had a 33.3% and a 26.9% greater concentration of quercetins ($F_{2,15}$ = 3.512, p = 0.056) and flavonols ($F_{2,15}$ = 3.535, p = 0.055), respectively, compared to plants grown under enhanced UVA (Fig. 3.2a,b). Control seedlings also showed a higher concentration of quercetins than UVA ones (Fig. 3.2a).

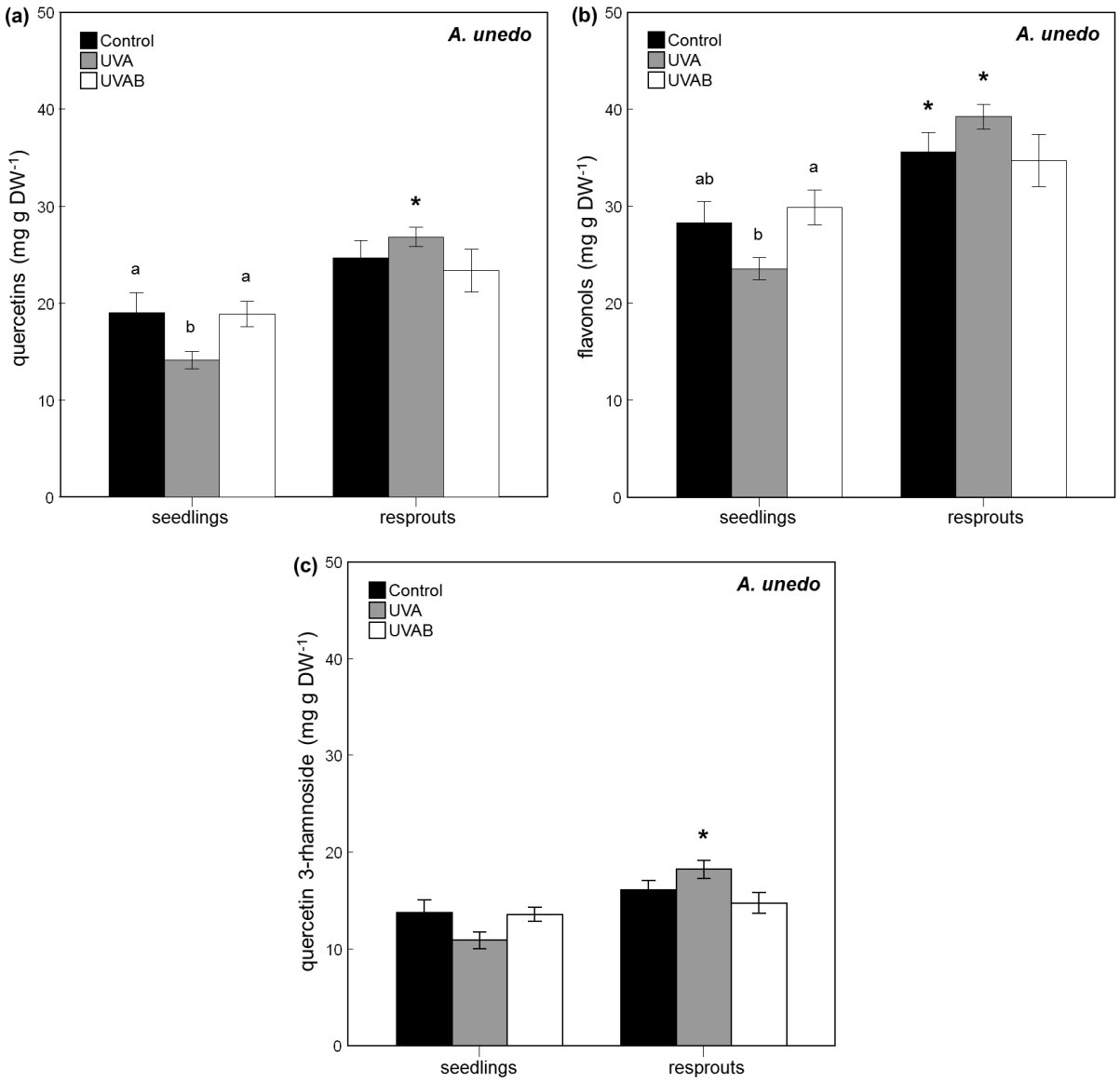


Fig. 3.2 *Arbutus unedo* leaf concentration of quercetins (**a**), flavonols (**b**) and quercetin 3-rhamnoside (**c**) in plants subjected to three UV radiation conditions (control, UVA and UVAB), both before and after pruning (seedlings and resprouts, respectively). Error bars represent the standard error of the mean (n = 6). The asterisk indicates a significant difference between seedlings and resprouting plants exposed to the same UV condition, whereas different letters indicate significant differences among the UV conditions within seedlings or resprouts. The significance level was set at $p \leq 0.05$.

There was a general effect of the irrigation regime (irrespective of the UV treatment or pruning) on the leaf concentration of gallotannin 2, a hydrolyzable tannin, and quercetin-glycoside, being the levels of both compounds greater in the leaves of low-watered plants (Table 3.1). The watering regime also affected the leaf concentration of arbutin and five hydrolyzable tannins, but the effect was different in seedlings and resprouts (Table 3.1). Indeed, in seedlings, the leaf concentrations of arbutin and gallotannin 3 were 13.54% lower ($F_{1,16}$ = 4.468, p = 0.051; Fig. 3.3a) and 69.70% higher ($F_{1,16}$ = 8.827, p = 0.009; Fig. 3.3b), respectively, under drier conditions. Conversely, after pruning, low-watered resprouts had greater leaf concentrations of ellagitannin 1 ($F_{1,16}$ = 10.949, p = 0.004; Fig. 3.3c), ellagitannin 2 ($F_{1,16}$ = 12.211, p = 0.003; Fig. 3.3d) and gallotannin 7 ($F_{1,16}$ = 21.879, p < 0.001; Fig. 3.3e) and a lower leaf concentration of gallotannin 4 ($F_{1,16}$ = 6.105, p = 0.025; Fig. 3.3f) compared to well-watered ones.

Regarding the ratios between the different groups of compounds, we found effects of the watering regime on the FLAV:TP and PA:TP ratios, which were different in seedlings than in resprouts (Table 3.1). Indeed, while there was no effect of the irrigation treatment on the FLAV:TP ratio of seedlings, well-watered resprouts showed a 12.8% higher ratio than low-watered ones ($F_{1,16}$ = 6.308, p = 0.023; data not shown). In contrast, the differences in PA:TP ratio were significant only in seedlings, being 13.7% lower in well- than in low-watered ones ($F_{1,16}$ = 4.655, p = 0.047; data not shown).

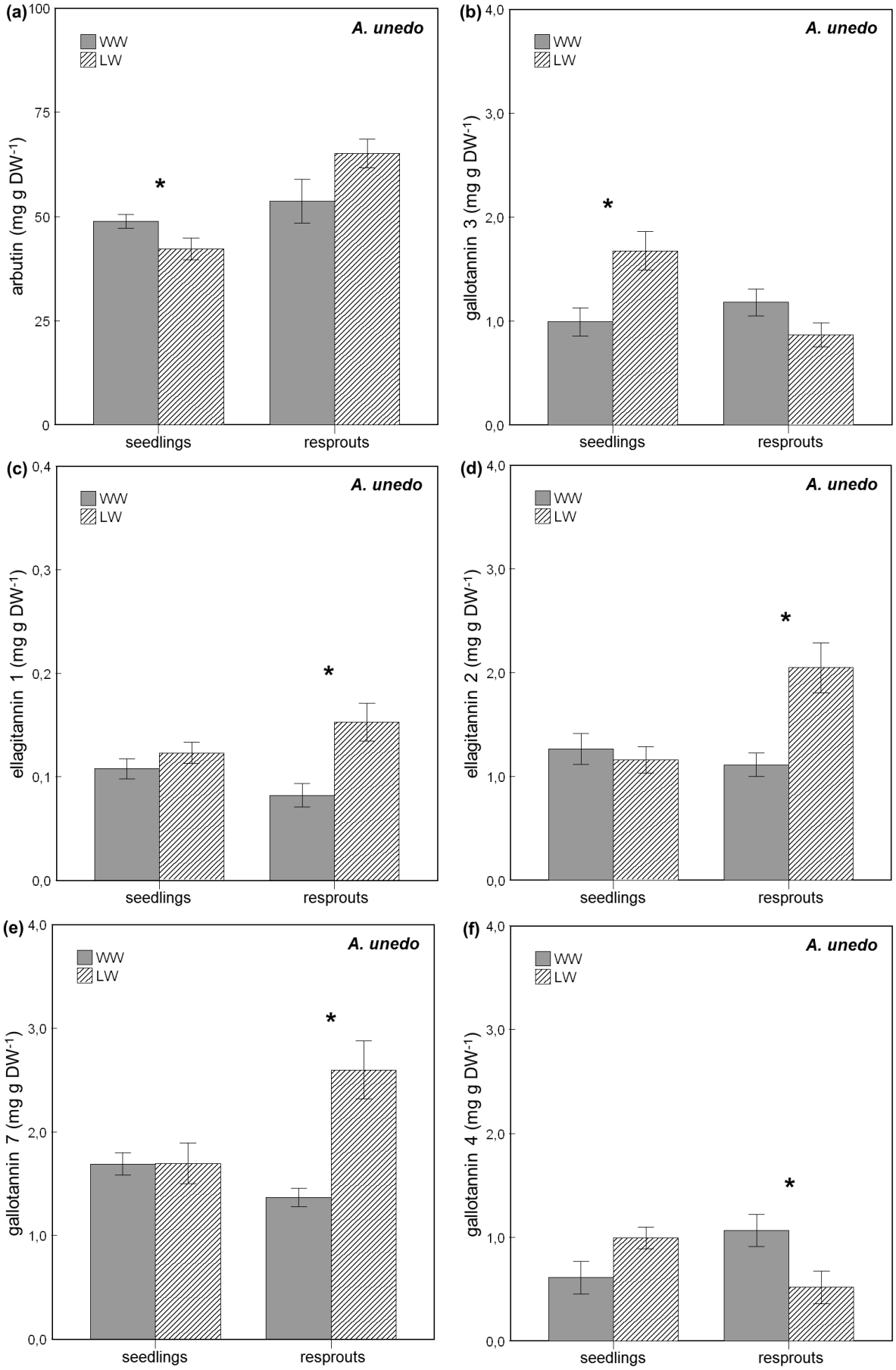


Fig. 3.3 *Arbutus unedo* leaf concentrations of arbutin (**a**), gallotannin 3 (**b**), ellagitannins 1 (**c**) and 2 (**d**), and gallotannins 7 (**e**) and 4 (**f**) in seedlings and resprouts subjected to two watering regimes (well-watered, WW, and low-watered, LW). Error bars represent the standard error of the mean (n = 9). Asterisks indicate significant differences between WW and LW plants within seedlings or resprouts. The significance level was set at $p \leq 0.05$.

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

UV radiation affected one fourth of the phenolic compounds identified in *Q. suber* leaves, although it did not modify the total concentration of phenols (TP) or flavonoids (Table 3.2). The UV-sensitive phenols, with the exception of the hydrolyzable tannin 3, were five flavonols (Table 3.2). These flavonols responded similarly to the UV treatment, showing the highest levels in leaves under enhanced UV-A+UV-B radiation. As a result, UVAB plants showed the highest overall amount of flavonols. For some of these flavonols, differences were only significant between UVAB and UVA plants (this is the case of the kaempferol monocoumaroyl-astragalin 2 and the quercetin 3-rhamnoside), whereas for others (kaempferol 3-glucoside and monocoumaroyl-astragalin 1) differences were also significant in relation to control plants. As a consequence, the leaf total concentration of kaempferols was a 30% and a 39% greater in plants under enhanced UV-A+UV-B than in those under enhanced UV-A or control conditions, respectively (Table 3.2). The concentration of the unknown flavonol 1 was also higher in UVAB plants, but in this case only in relation to controls, similar to the results obtained for the leaf concentration of the hydrolyzable tannin 3.

Regarding the effects of the watering treatment, we found a 13% lower overall amount of hydrolyzable tannins in low- than in well-watered leaves (Table 3.2). Low-watered plants also showed the lowest levels of gallocatechin (a flavanol), although differences were only marginally significant (Table 3.2). Moreover, two of the four identified kaempferols varied their leaf concentrations in response to watering, but only in the resprouts (Table 3.2, Fig. 3.4). Indeed, the concentration of kaempferol 3-glucoside was around 32% lower ($F_{1,16}$ = 5.232, p = 0.036; Fig. 3.4a) and the concentration of kaempferol glycoside 24% higher ($F_{1,16}$ = 4.948, p = 0.041; Fig. 3.4b) in low- than in well-watered resprouts. Under low irrigation, the leaf concentrations of kaempferol glycoside and monocoumaroyl-astragalin 2 were greater in resprouts than in seedlings ($F_{1,16}$ = 15.809, p = 0.001; $F_{1,16}$ = 7.890, p = 0.013, respectively; data not shown).

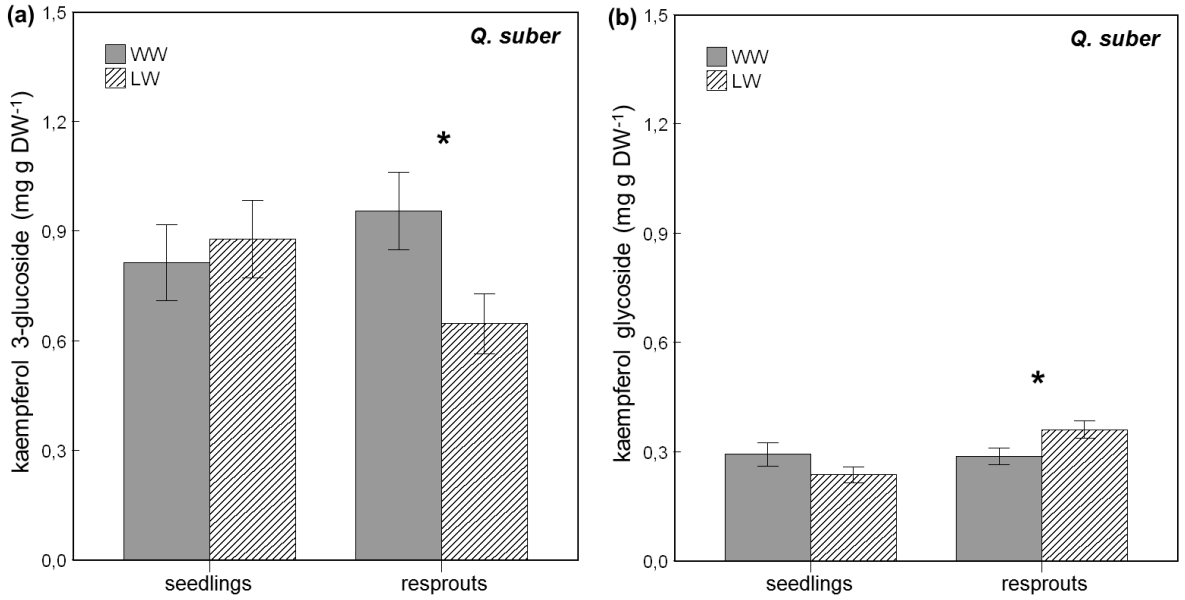


Fig. 3.4 *Quercus suber* leaf concentration of kaempferol 3-glucoside (**a**) and kaempferol glycoside (**b**) in seedlings and resprouts subjected to two watering regimes (well-watered, WW, and low-watered, LW). Error bars represent the standard error of the mean (n = 9). Asterisks indicate significant differences between WW and LW plants within seedlings or resprouts. The significance level was set at $p \leq 0.05$.

UV and watering treatment effects on the leaf antioxidants of resprouts

In general, the studied parameters related to the leaf antioxidant activity of *A. unedo* resprouts responded to UV radiation and/or water supply, being some of the responses the result of both treatments (Table 3.3). Conversely, leaf antioxidants of *Q. suber* resprouts were only affected by the watering regime (Table 3.3).

In the case of *A. unedo*, the leaf concentration of total ascorbate, reduced ascorbate (ASC), total glutathione and reduced glutathione (GSH), as well as the activity of catalase enzyme (CAT), were substantially enhanced (by more than 25%) in leaves under UV-A+UV-B supplementation in comparison to those growing only under UV-A supplementation (Table 3.3). However, the increase in total glutathione and GSH in response to enhanced UV-A+UV-B was only significant in low-watered resprouts ($F_{2,6}$ = 6.227, p = 0.034; $F_{2,6}$ = 16.653, p = 0.004, respectively; Fig. 3.5a,b), which would explain the significant interaction found between UV radiation and watering for these parameters (Table 3.3). In contrast, the leaf

ascorbate peroxidase (APX) activity of the resprouts was a 44.5% and a 34.3% higher under enhanced UV-A compared to UVAB and control plants, respectively (Table 3.3).

The watering treatment affected the leaf concentration of total ascorbate, ASC and dehydroascorbate (DHA) in *A. unedo,* total glutathione and GSH in *Q. suber*, and APX and CAT activities in both species. For both species, whereas the concentration of antioxidant compounds sensitive to irrigation increased when water availability decreased, the contrary was found for the activities of APX and CAT (Table 3.3). ASC:DHA and ASC:total ascorbate ratios, as well as GSH:GSSG ratio and the leaf concentration of oxidized glutathione (GSSG), did not vary significantly as a result of the treatments in any of the two species (Table 3.3).

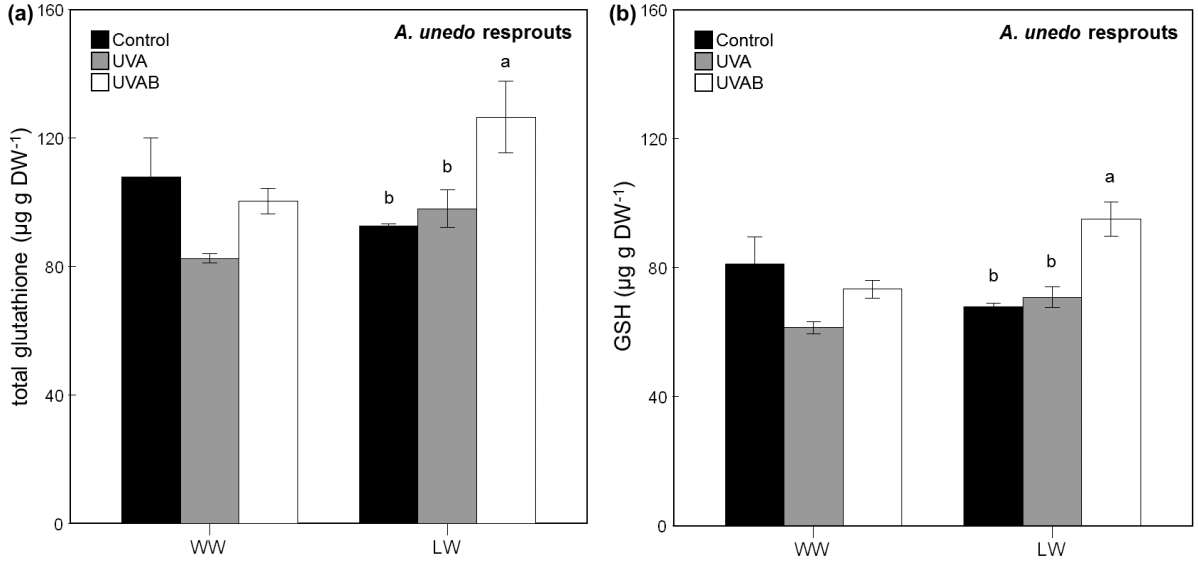


Fig. 3.5 *Arbutus unedo* leaf concentrations of total glutathione (**a**) and reduced glutathione (GSH) (**b**) in resprouts subjected to three UV radiation conditions (control, UVA and UVAB) combined with two watering regimes (well-watered, WW, and low-watered, LW). Error bars represent the standard error of the mean (n = 3). Different letters indicate significant differences among the UV conditions within the same watering regime. The significance level was set at $p \leq 0.05$.

Table 3.3 Overall mean ± S.E. for different parameters related to the antioxidant activity in leaves of *Arbutus unedo* and *Quercus suber* resprouts under three UV radiation conditions (control, UVA and UVAB) and two watering regimes (well-watered, WW, and low-watered, LW). 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. n = 6 in and n = 9 for each UV and watering condition, respectively. The significance level considered was $p \leq 0.05$. Only significant interactions between the two factors were included in the column "UV x W", being highlighted in bold.

	UV radiation (UV)							Watering (W)			UV x W
	control		UVA		UVAB		*p*-value	WW	LW	*p*-value	*p*-value
A. unedo resprouts											
Total ascorbate (µg g DW^{-1})	**819.520 ± 56.213**	**ab**	**739.551 ± 100.489**	**b**	**932.199 ± 118.544**	**a**	**0.044**	**647.289 ± 37.930**	**1013.558 ± 55.834**	**<0.001**	ns
ASC (µg g DW^{-1})	**673.673 ± 46.427**	**ab**	**621.799 ± 77.728**	**b**	**788.669 ± 97.687**	**a**	**0.044**	**551.759 ± 34.137**	**837.668 ± 48.299**	**<0.001**	ns
DHA (µg g DW^{-1})	145.848 ± 19.065		117.752 ± 26.440		144.386 ± 24.594		ns	**95.53 ± 13.577**	**176.461 ± 11.948**	**0.001**	ns
ASC:DHA	6.987 ± 1.385		6.555 ± 1.246		6.859 ± 1.280		ns	8.019 ± 1.241	5.581 ± 0.474	ns	ns
ASC:Total	0.831 ± 0.019		0.846 ± 0.020		0.848 ± 0.016		ns	0.854 ± 0.018	0.829 ± 0.010	ns	ns
Total glutathione (µg g DW^{-1})	**100.165 ± 6.456**	**ab**	**90.152 ± 4.386**	**b**	**113.423 ± 7.903**	**a**	**0.027**	96.865 ± 5.309	105.628 ± 6.414	ns	**0.041**
GSH (µg g DW^{-1})	**74.492 ± 4.789**	**ab**	**66.144 ± 2.698**	**b**	**84.195 ± 5.541**	**a**	**0.006**	71.960 ± 3.858	77.928 ± 4.677	ns	**0.007**
GSSG (µg g DW^{-1})	25.673 ± 1.910		24.007 ± 2.001		29.228 ± 2.951		ns	24.906 ± 1.581	27.700 ± 2.239	ns	ns
GSH:GSSG	2.999 ± 0.155		2.840 ± 0.169		3.091 ± 0.274		ns	3.017 ± 0.093	2.936 ± 0.216	ns	ns
APX (µmol ASC min^{-1} mg^{-1} protein)	**1.459 ± 0.161**	**b**	**1.959 ± 0.290**	**a**	**1.356 ± 0.155**	**b**	**0.025**	**1.957 ± 0.151**	**1.226 ± 0.130**	**0.001**	ns
CAT (µmol H_2O_2 min^{-1} mg^{-1} protein)	**239.643 ± 16.743**	**ab**	**211.942 ± 11.308**	**b**	**272.713 ± 10.341**	**a**	**0.007**	**255.917 ± 13.451**	**226.949 ± 11.643**	**0.042**	ns
Q. suber resprouts											
Total ascorbate (µg g DW^{-1})	3550.269 ± 178.944		3136.034 ± 191.110		3102.577 ± 206.468		ns	3087.250 ± 138.678	3438.670 ± 172.444	ns	ns
ASC (µg g DW^{-1})	3043.792 ± 121.056		2764.988 ± 155.941		2719.714 ± 207.268		ns	2678.740 ± 123.201	3006.923 ± 131.765	ns	ns
DHA (µg g DW^{-1})	506.476 ± 91.160		371.045 ± 42.002		382.863 ± 36.091		ns	408.51 ± 34.814	431.747 ± 66.576	ns	ns
ASC:DHA	10.057 ± 2.299		10.669 ± 1.708		8.469 ± 1.635		ns	8.844 ± 1.486	10.619 ± 1.531	ns	ns
ASC:Total	0.858 ± 0.020		0.882 ± 0.009		0.873 ± 0.014		ns	0.865 ± 0.009	0.878 ± 0.015	ns	ns
Total glutathione (µg g DW^{-1})	251.138 ± 6.254		247.301 ± 10.892		238.722 ± 14.820		ns	**228.079 ± 6.262**	**263.363 ± 6.852**	**0.003**	ns
GSH (µg g DW^{-1})	194.335 ± 5.886		194.870 ± 8.172		184.980 ± 12.832		ns	**175.481 ± 5.220**	**207.309 ± 5.046**	**0.001**	ns
GSSG (µg g DW^{-1})	56.803 ± 4.180		52.431 ± 4.257		53.743 ± 3.898		ns	52.598 ± 2.917	56.053 ± 3.571	ns	ns
GSH:GSSG	3.843 ± 0.390		4.123 ± 0.437		3.646 ± 0.364		ns	3.775 ± 0.355	3.966 ± 0.283	ns	ns
APX (µmol ASC min^{-1} mg^{-1} protein)	1.187 ± 0.127		1.520 ± 0.234		1.174 ± 0.158		ns	**1.571 ± 0.111**	**1.016 ± 0.122**	**0.005**	ns
CAT (µmol H_2O_2 min^{-1} mg^{-1} protein)	214.437 ± 20.763		240.458 ± 22.989		233.866 ± 22.280		ns	**262.501 ± 14.161**	**196.673 ± 12.877**	**0.009**	ns

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

2. Growth and physiological responses of two Mediterranean resprouter species exposed to enhanced UV radiation and reduced water availability before and after pruning

State-of-the-art

Levels of solar ultraviolet-B radiation (UV-B, 280–315 nm) reaching the surface of the Earth have increased in the last few decades due to the depletion in stratospheric ozone (McKenzie *et al.*, 2007; Bais *et al.*, 2015). Despite recovery of the ozone layer (Bais *et al.*, 2015), decreases in cloudiness associated with climate change have the potential to increase UV radiation fluxes (UV-B and UV-A, 315–400 nm) impacting on terrestrial ecosystems (Sanchez-Lorenzo *et al.*, 2017), as it is predicted to occur in the Mediterranean basin (IPCC, 2013). In this region, vegetation will also be exposed to a decrease in precipitation with longer dry periods in the coming years (IPCC, 2013). The effect of higher UV levels coupled with lower water supply can be particularly relevant for Mediterranean sclerophyllous woody plants which, because of their long lifespan, will have to adapt to these environmental constraints.

Higher levels of solar UV radiation, particularly UV-B, can lead to photooxidative damage in plants and alterations in their development (A-H-Mackerness, 2000; Frohnmeyer & Staiger, 2003; Li *et al.*, 2010). Plants have evolved different mechanisms to tolerate UV radiation such as changes in leaf morphology and biochemistry. Indeed, most plants exposed to enhanced UV radiation develop smaller and thicker leaves (Newsham *et al.*, 1999; Barnes *et al.*, 2005) with higher leaf mass per area (LMA) (Verdaguer *et al.*, 2012; Bernal *et al.*, 2013). More sclerophyllous and thicker leaves imply a reinforcement of the foliar tissues that can attenuate UV penetration into the leaf. Many species also exhibit increases in leaf UV-absorbing compounds (UACs) and free radical scavengers, such as flavonoids (Searles *et al.*, 2001; Bassman, 2004; Julkunen-Tiitto *et al.*, 2005; Li *et al.*, 2010).

In a study encompassing 49 species, Sumbele *et al.* (2012) detected a negative relationship between the concentration of phenolic compounds in leaves and photosynthetic

rates which, in turn, were associated with thicker leaves and reduced growth. This indicates a functional integration between carbon gain and phenol accumulation, suggesting a trade-off between growth and defense/protection demands (Sumbele *et al.*, 2012; Ballaré, 2014). In some species, enhanced UV-B radiation can increase the phenolic content in leaves and roots with a concomitant decrease in shoot and root biomass (Choudhary *et al.*, 2013). Given the investment of resources into defense mechanisms, plant carbon reserves, especially those in roots, can potentially be altered in response to enhanced UV radiation (Ballaré, 2014). For Mediterranean plants, this trade-off might be particularly threatening for resprouter species which, in comparison with non-resprouters, need to allocate more assimilates to roots (e.g. higher biomass and starch content) (Verdaguer & Ojeda, 2002) to ensure plant regrowth after disturbance (Canadell & López-Soria, 1998; Paula & Pausas, 2006). Thus, changes in plant resource allocation due to protection against UV radiation might alter regeneration capacity and, subsequently, survival after a disturbance, such as fire, drought or herbivory.

Plant survival in Mediterranean ecosystems also depends to a large extent on precipitation regime. The effects of low water availability on Mediterranean woody species have been extensively investigated (Sardans & Peñuelas, 2012; Bussotti *et al.*, 2014; Matesanz & Valladares, 2014). In general, a reduction in water availability is associated with changes at the leaf level similar to those triggered by enhanced UV radiation, i.e. increased levels of phenols (Selmar & Kleinwächter, 2013) and production of smaller and thicker leaves with higher LMA (Bussotti *et al.*, 2002; Valladares & Sánchez-Gómez, 2006; Sardans & Peñuelas, 2013). In plants under water shortage, the increase in sclerophyllous index (i.e. LMA) represent an efficient mechanism to diminish the loss of water through transpiration (Bussotti, 2008; Sardans & Peñuelas, 2013). Moreover, water deficits are often found to reduce photosynthetic activity associated with lower leaf relative water content (RWC) (Reddy *et al.*, 2004) and a subsequent reduction in plant growth (Ogaya *et al.*, 2003; Llorens *et al.*, 2004). A decrease in the shoot to root mass ratio under water deficit has also been

reported to prevent water loss and improve water uptake in some Mediterranean species (Verdaguer *et al.*, 2011).

It is well known that primary exposure to a single stress can alter plant responses to other stresses (cross-tolerance effects) (Stratmann, 2003; Poulson *et al.*, 2006; Jansen *et al.*, 2012). In this context, several studies have shown that the combination of enhanced UV radiation and low water supply can attenuate or suppress the changes induced by these factors individually on leaf morphology (Verdaguer *et al.*, 2012), leaf RWC (Bernal *et al.*, 2015) and photosynthetic activity (Nogués & Baker, 2000; Poulson *et al.*, 2006; Feng *et al.*, 2007). Thus, previous studies using Mediterranean woody species have shown that, despite leaf RWC often decrease under low water supply, the hydric status improved in response to the combined action of reduced water and enhanced UV radiation (Bernal *et al.*, 2015). In addition, UV exposure, specifically UV-A radiation, stimulated root growth under water shortage while aboveground biomass responses to low water availability were species-dependent (Bernal *et al.* 2013; 2015).

To date, there are no studies that have evaluated the interactive effects of enhanced UV radiation and reduced water availability on the regeneration capacity of resprouting Mediterranean woody species after the loss of aerial biomass, which often happens after intense fires. Thus, our goal was to assess the effects of higher doses of UV radiation (both UV-A and UV-B) and lower water availability on seedlings of two Mediterranean resprouter species, *Arbutus unedo* and *Quercus suber*, both widespread evergreens with a similar sclerophyllous index, before and after the loss of aerial biomass. To achieve this, we established a UV supplementation experiment using seedlings of *A. unedo* and *Q. suber* grown in pots under controlled watering conditions. Biomass production together with a range of morphological, physiological and biochemical parameters were determined both in seedlings before pruning and in the resprouts appeared after pruning. We hypothesized that: (i) higher UV levels and water shortage would trigger cross-tolerance effects leading to attenuated changes in leaf morphology, physiology and water status compared to the individual effects of these two abiotic factors; (ii) enhanced UV radiation, especially

enhanced UV-A, combined with low water supply would benefit belowground plant biomass production, altering also root biochemical characteristics, such as root starch and phenolic content; (iii) changes in root growth and biochemistry induced by the experimental treatments before pruning would affect the growth and development of resprouts after pruning; and (iv) since the two studied species have similar leaf sclerophyllous index, we would expect similar responses to the experimental treatments.

Results

Principal component analysis of measured plant traits

Principal component analysis created two “principal components” (PCs) explaining 84% of the variance (Fig. 3.6). According to factor loadings for the different variables (Table 3.4), the first principal component axes (PC1) accounted for about 49% of the variance and was mainly associated with leaf biomass and leaf area which had negative loadings. Plant height and stem biomass were the variables with the highest contribution, also negative, to the second principal component axes (PC2), which accounted for 35% of the total variance. To a lesser extent (factor loadings < $|0.40|$), root biochemical traits contributed to both PC axes although in opposite directions, with the concentrations of starch and TP having maximum positive loadings to PC1, while sugar concentration to PC2.

Graphical representation of the two PC axes segregated four sets of samples according to the species and the pruning condition (Fig. 3.6). Thus, *A. unedo* and *Q. suber* were separated mainly by root biochemical traits, with *A. unedo* having higher sugar, and lower starch and total phenol concentrations than *Q. suber*. In addition, seedlings and resprouting plants were also segregated as a result of differences in biometric parameters with seedlings having greater stem height and diameter, and leaf and stem biomass than resprouting plants. No segregation was found in response to UV or watering treatments.

Table 3.4 Factor loadings of the nine plant variables (*n* = 72) on the two principal component axes (PC1 and PC2). Factor loadings ≥ 0.35 in absolute value are marked in bold.

Parameters	PC1	PC2
Height (cm)	-0.214	**-0.494**
Diameter (mm)	-0.337	**-0.386**
Leaf biomass (g)	**-0.425**	-0.185
Stem biomass (g)	-0.280	**-0.434**
LT (µm)	0.127	-0.214
LA (cm^2)	**-0.424**	0.146
Root starch (g $100g^{-1}$)	**0.368**	-0.310
Root sugar (g $100g^{-1}$)	-0.331	**0.370**
Root TP (g $100g^{-1}$)	**0.376**	-0.293
Variance explained (%)		
Absolute	49.30	34.60
Cumulative	49.30	83.90

LT, leaf thickness; LA, leaf area; TP, total phenols.

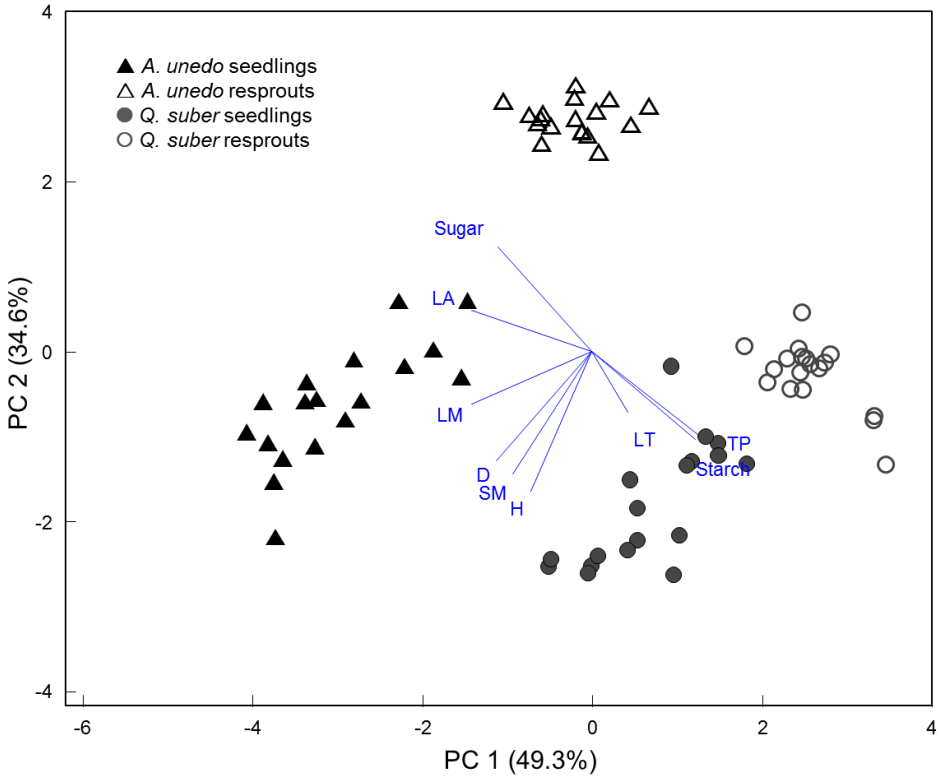


Fig. 3.6 Score plot of principal component analysis (PCA) for *Arbutus unedo* and *Quercus suber* before and after pruning (seedlings and resprouts, respectively) according to data on plant height (H; cm), basal diameter (D; mm), leaf biomass (LM; g), stem biomass (SM; g), leaf thickness (LT; µm), leaf area (LA; cm^2), root starch (g 100 g^{-1}), root total soluble-sugar (g 100 g^{-1}) and root total phenols (TP; g 100 g^{-1}). Variation explained by each one of the two principal components (PC1 and PC2) is indicated in parenthesis.

Differences between seedlings and resprouting plants

The percentage of seedlings that resprouted after the aerial biomass removal (i.e. resprouting success) was 68% for *A. unedo* and 82% for *Q. suber* plants (Tables 3.5 and 3.6). For *A. unedo* resprouts, values of stem height and diameter, leaf and stem biomass, and stem RGR were more than 50% lower than those of seedlings (Table 3.5). Conversely, for *Q. suber* resprouts values of these parameters, except for stem biomass, were comparable with those of seedlings indicating faster recovery after pruning (Table 3.6). Nevertheless, considering the leaf:stem ratio, it is noteworthy that both species allocated more biomass to leaves than to stems after pruning. Compared to seedlings, resprouts of *A. unedo* increased by 174% the portion of biomass assigned to leaves while, in *Q. suber*, the increase in the leaf:stem ratio was 85%.

Concerning leaf morphology, resprouts of both species had smaller leaves than seedlings. Leaves of *A. unedo* resprouts were also thinner, with lower LMA and higher tissue density compared to seedling leaves. The opposite pattern was found for resprouting plants of *Q. suber* (Tables 3.5 and 3.6). As expected, root biomass of both species and, thus, the biomass allocated to roots in relation to total biomass, was higher after pruning. The RGR of roots decreased by 48% in *A. unedo* and by 32% in *Q. suber* resprouts in relation to seedlings (Tables 3.5 and 3.6).

Regarding physiological measurements, resprouting plants of *A. unedo* showed lower leaf water content, with leaf transpiration (E) and stomatal conductance (g_s) being 1.6-fold and 2.0-fold higher, respectively, and photosynthetic rates (A) 25% lower than in seedlings (Table 3.5). For resprouting plants of *Q. suber*, both leaf water content and RWC were lower than in seedlings (Table 3.6). However, *Q. suber* resprouts had 5-fold greater rates of E and g_s than seedlings, whereas A did not show significant differences between the two plant types (Table 3.6). In both species, increases in E explain the lower (75% in *A. unedo* and 90% in *Q. suber*) instantaneous water use efficiency (WUE) found for resprouts compared to seedlings. Maximum photochemical efficiency of PSII (measured as Fv/Fm) was lower in *A. unedo* and higher in *Q. suber* resprouts compared to seedlings (Tables 3.5 and 3.6).

Root starch and total soluble-sugar concentrations in *A. unedo* did not differ between seedlings and resprouts, although the starch:sugar ratio was significantly lower in the resprouts (Table 3.5). Conversely, in *Q. suber* roots, starch and total soluble-sugar concentrations were higher after pruning by 17 and 22%, respectively, but the starch:sugar ratio did not differ (Table 3.6). Total phenol content in roots was higher after pruning in *A. unedo*, but not for *Q. suber*. Hence, the total phenol:starch ratio was higher in roots of *A. unedo* resprouts compared to seedlings while the opposite pattern was found for *Q. suber*.

Table 3.5 Overall means ± S.E. for all the studied parameters in plants of *Arbutus unedo* under the three UV radiation conditions (control, UVA and UVAB), the two watering regimes (well-watered, WW, and low-watered, LW) and before and after pruning (seedlings and resprouts, respectively). Numbers in bold indicate significant differences among the levels of the factor. n = 12 in each UV level and n = 18 in each watering and pruning condition for all parameters, except for the resprouting success (n = 6 and n = 9, respectively). The significant level considered was $p \leq 0.05$. Only significant two-way or three-way interactions 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	
Resprouting success (%)	66.667 ± 8.433	63.333 ± 8.028	73.333 ± 8.433	ns	64.444 ± 6.479	71.111 ± 6.759	ns	-	67.778 ± 4.613	-	-
Height (cm)	63.187 ± 11.356	61.811 ± 11.108	56.423 ± 9.792	ns	62.307 ± 8.879	58.640 ± 8.484	ns	**94.722 ± 3.474**	**26.225 ± 1.072**	**<0.001**	-
Diameter (mm)	9.437 ± 1.167	9.649 ± 1.354	9.438 ± 1.178	ns	9.843 ± 0.993	9.173 ± 0.989	ns	**13.490 ± 0.289**	**5.526 ± 0.170**	**<0.001**	-
Leaf biomass (g)	17.903 ± 2.750	18.314 ± 3.326	18.287 ± 2.449	ns	18.286 ± 2.352	18.050 ± 2.257	ns	**26.285 ± 1.523**	**10.051 ± 0.747**	**<0.001**	-
Stem biomass (g)	15.931 ± 3.938	15.827 ± 4.157	14.876 ± 3.630	ns	15.568 ± 3.171	15.521 ± 3.131	ns	**27.494 ± 1.739**	**3.596 ± 0.187**	**<0.001**	-
Root biomass (g)	18.192 ± 1.434	18.061 ± 1.506	19.618 ± 1.807	ns	17.912 ± 0.909	19.336 ± 1.564	ns	**15.329 ± 0.711**	**21.919 ± 1.245**	**<0.001**	-
leaf:total	0.331 ± 0.016	0.326 ± 0.024	0.336 ± 0.022	ns	0.335 ± 0.017	0.328 ± 0.016	ns	**0.383 ± 0.009**	**0.279 ± 0.013**	**<0.001**	-
stem:total	0.260 ± 0.047	0.246 ± 0.045	0.237 ± 0.042	ns	0.245 ± 0.035	0.250 ± 0.037	ns	**0.392 ± 0.011**	**0.104 ± 0.005**	**<0.001**	-
root:total	0.409 ± 0.057	0.427 ± 0.067	0.427 ± 0.059	ns	0.420 ± 0.050	0.423 ± 0.049	ns	**0.225 ± 0.007**	**0.618 ± 0.016**	**<0.001**	-
leaf:root	1.100 ± 0.188	1.172 ± 0.229	1.080 ± 0.189	ns	1.162 ± 0.175	1.072 ± 0.151	ns	**1.756 ± 0.066**	**0.479 ± 0.035**	**<0.001**	-
leaf:stem	1.950 ± 0.316	1.845 ± 0.276	1.989 ± 0.296	ns	1.907 ± 0.221	1.950 ± 0.256	ns	**1.029 ± 0.052**	**2.827 ± 0.128**	**<0.001**	-
SRGR (mg g^{-1} d^{-1})	42.753 ± 8.953	42.440 ± 9.837	41.866 ± 8.896	ns	42.374 ± 7.473	42.332 ± 7.391	ns	**70.148 ± 4.360**	**14.557 ± 0.757**	**<0.001**	-
RRGR (mg g^{-1} d^{-1})	26.617 ± 3.459	25.567 ± 3.576	28.697 ± 3.643	ns	24.862 ± 2.417	29.060 ± 3.200	ns	**35.522 ± 2.391**	**18.399 ± 1.503**	**<0.001**	-
LT (µm)	411.354 ± 12.302	408.466 ± 14.691	399.486 ± 14.199	ns	409.029 ± 8.763	403.843 ± 13.068	ns	**445.947 ± 5.342**	**366.924 ± 6.014**	**<0.001**	W x P
LA (cm^2)	11.828 ± 0.924	11.520 ± 1.184	11.602 ± 0.945	ns	11.945 ± 0.927	11.354 ± 0.700	ns	**13.906 ± 0.698**	**9.393 ± 0.522**	**<0.001**	-
LMA (mg cm^{-2})	14.024 ± 0.335	14.319 ± 0.466	13.821 ± 0.393	ns	13.845 ± 0.277	14.264 ± 0.363	ns	**14.652 ± 0.292**	**13.457 ± 0.295**	**0.014**	-
LTD (g cm^{-3})	0.343 ± 0.010	0.352 ± 0.009	0.347 ± 0.008	ns	0.339 ± 0.006	0.355 ± 0.008	ns	**0.328 ± 0.006**	**0.367 ± 0.006**	**<0.001**	-
LWC (%)	57.917 ± 0.608	57.479 ± 0.647	58.039 ± 0.778	ns	**58.387 ± 0.383**	**57.236 ± 0.650**	**0.039**	**59.313 ± 0.363**	**56.310 ± 0.459**	**<0.001**	W x P
RWC (%)	81.284 ± 0.956	80.577 ± 1.169	80.053 ± 0.961	ns	80.369 ± 0.779	80.907 ± 0.892	ns	79.758 ± 0.883	81.518 ± 0.736	ns	UV x W
E (mmol H_2O m^{-2} s^{-1})	1.207 ± 0.220	1.071 ± 0.121	1.202 ± 0.241	ns	1.162 ± 0.171	1.158 ± 0.153	ns	**0.637 ± 0.057**	**1.683 ± 0.131**	**<0.001**	-
g_s (mmol m^{-2} s^{-1})	65.396 ± 13.389	60.479 ± 8.380	70.646 ± 15.493	ns	65.486 ± 10.570	65.528 ± 10.073	ns	**32.366 ± 3.061**	**98.648 ± 8.638**	**<0.001**	-
A (µmol CO_2 m^{-2} s^{-1})	7.573 ± 0.638	7.138 ± 0.593	7.876 ± 0.636	ns	7.059 ± 0.447	7.999 ± 0.535	ns	**8.579 ± 0.428**	**6.480 ± 0.447**	**0.001**	UV x P
WUE (µmol CO_2 $mmol^{-1}$ H_2O)	11.197 ± 3.217	8.399 ± 1.585	9.983 ± 2.059	ns	9.022 ± 1.526	10.698 ± 2.263	ns	**15.734 ± 1.851**	**3.985 ± 0.214**	**<0.001**	-
ETR	126.969 ± 6.445	123.090 ± 7.612	146.461 ± 5.376	ns	127.483 ± 5.925	136.864 ± 5.477	ns	130.743 ± 4.855	133.604 ± 6.624	ns	-
Fv/Fm	0.780 ± 0.008	0.781 ± 0.010	0.791 ± 0.011	ns	**0.777 ± 0.009**	**0.792 ± 0.006**	**0.017**	**0.810 ± 0.004**	**0.759 ± 0.006**	**<0.001**	W x P
NPQ	3.346 ± 0.267	3.206 ± 0.195	2.863 ± 0.227	ns	3.118 ± 0.204	3.159 ± 0.180	ns	3.219 ± 0.203	3.059 ± 0.179	ns	W x P
Root starch (g 100 g^{-1})	4.854 ± 0.326	4.189 ± 0.570	4.769 ± 0.418	ns	**3.967 ± 0.234**	**5.241 ± 0.411**	**0.019**	4.944 ± 0.209	4.263 ± 0.463	ns	-
Root sugar (g 100 g^{-1})	26.010 ± 1.084	24.534 ± 1.081	26.348 ± 1.139	ns	25.627 ± 0.806	25.634 ± 0.997	ns	24.581 ± 0.696	26.680 ± 1.015	ns	-
Root TP (g 100 g^{-1})	2.308 ± 0.161	2.418 ± 0.126	2.117 ± 0.205	ns	2.199 ± 0.085	2.362 ± 0.173	ns	**2.109 ± 0.101**	**2.453 ± 0.156**	**0.034**	W x P
starch:sugar	0.196 ± 0.018	0.172 ± 0.022	0.192 ± 0.020	ns	**0.160 ± 0.011**	**0.213 ± 0.018**	**0.023**	**0.209 ± 0.014**	**0.163 ± 0.017**	**0.048**	-
TP:starch	0.628 ± 0.081	0.946 ± 0.226	0.635 ± 0.095	ns	0.802 ± 0.098	0.671 ± 0.146	ns	**0.501 ± 0.046**	**0.972 ± 0.151**	**0.008**	-
TP:sugar	0.090 ± 0.005	0.104 ± 0.010	0.085 ± 0.009	ns	0.089 ± 0.004	0.097 ± 0.009	ns	0.089 ± 0.005	0.097 ± 0.008	ns	-

SRGR, stem relative growth rate; RRGR, root relative growth rate; LT, leaf thickness; LA, leaf area; LMA, leaf mass area; LTD, leaf tissue density; LWC, leaf water content; RWC, leaf relative water content; E, leaf transpiration rate; g_s, stomatal conductance; A, photosynthetic rate; WUE, water-use efficiency; ETR, apparent electron transport rate; NPQ, non-photochemical quenching; TP, total phenols; ns, not significant.

Table 3.6 Overall means ± S.E. for all the studied parameters in plants of *Quercus suber* under the three UV radiation conditions (control, UVA and UVAB), the two watering regimes (well-watered, WW, and low-watered, LW) and before and after the pruning (seedlings and resprouts, respectively). Numbers in bold indicate significant differences among the levels of the factor. In the case of UV radiation, significant differences among the UV conditions also are indicated by different letters. *n* = 12 in each UV level and *n* = 18 in each watering and pruning condition for all parameters, except for the resprouting success (*n* = 6 and *n* = 9, respectively). The significant level considered was $p \leq 0.05$. Only significant two-way or three-way interactions 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	
Resprouting success (%)	73.333 ± 9.888	83.333 ± 8.028	90.000 ± 6.831	ns	80.000 ± 6.667	84.444 ± 7.286	ns	-	82.222 ± 4.821	-	-
Height (cm)	72.123 ± 8.430	68.242 ± 6.956	74.763 ± 7.355	ns	72.015 ± 5.795	71.404 ± 6.489	ns	**94.831 ± 3.268**	**48.588 ± 1.458**	**<0.001**	-
Diameter (mm)	8.745 ± 0.757	8.189 ± 0.762	8.607 ± 0.763	ns	8.430 ± 0.565	8.597 ± 0.659	ns	**10.752 ± 0.373**	**6.275 ± 0.161**	**<0.001**	-
Leaf biomass (g)	10.159 ± 1.632	8.611 ± 0.872	9.236 ± 0.940	ns	9.680 ± 1.106	8.990 ± 0.817	ns	**12.400 ± 0.863**	**6.271 ± 0.234**	**<0.001**	-
Stem biomass (g)	16.096 ± 3.556	14.512 ± 3.036	16.829 ± 3.495	ns	14.528 ± 2.333	17.097 ± 3.028	ns	**25.131 ± 2.127**	**6.494 ± 0.264**	**<0.001**	-
Root biomass (g)	26.650 ± 2.354	27.352 ± 2.532	28.439 ± 2.366	ns	26.674 ± 1.952	28.286 ± 1.936	ns	**21.285 ± 1.225**	**33.675 ± 1.272**	**<0.001**	-
leaf:total	0.181 ± 0.018	0.172 ± 0.013	0.168 ± 0.011	ns	**0.186 ± 0.012**	**0.162 ± 0.010**	**0.003**	**0.214 ± 0.008**	**0.133 ± 0.004**	**<0.001**	-
stem:total	0.274 ± 0.044	0.273 ± 0.041	0.283 ± 0.041	ns	0.269 ± 0.030	0.284 ± 0.038	ns	**0.412 ± 0.010**	**0.140 ± 0.006**	**<0.001**	W x P
root:total	0.545 ± 0.059	0.556 ± 0.053	0.549 ± 0.050	ns	0.546 ± 0.040	0.554 ± 0.046	ns	**0.374 ± 0.007**	**0.726 ± 0.010**	**<0.001**	-
leaf:root	**0.428 ± 0.078 a**	**0.372 ± 0.058 b**	**0.368 ± 0.053 b**	**0.038**	**0.409 ± 0.054**	**0.369 ± 0.049**	**0.052**	**0.589 ± 0.023**	**0.189 ± 0.008**	**<0.001**	UV x P
leaf:stem	0.888 ± 0.117	0.777 ± 0.078	0.729 ± 0.078	ns	0.823 ± 0.060	0.773 ± 0.090	ns	**0.560 ± 0.049**	**1.036 ± 0.051**	**<0.001**	W x P
SRGR (mg g^{-1} d^{-1})	33.249 ± 4.058	32.087 ± 3.763	40.397 ± 5.997	ns	32.635 ± 3.111	37.853 ± 4.454	ns	**44.197 ± 4.444**	**26.292 ± 1.067**	**0.001**	-
RRGR (mg g^{-1} d^{-1})	8.932 ± 0.990	9.905 ± 0.730	9.296 ± 0.832	ns	8.688 ± 0.640	10.067 ± 0.710	ns	**11.197 ± 0.663**	**7.558 ± 0.375**	**<0.001**	-
LT (µm)	426.934 ± 22.081	458.054 ± 18.031	466.162 ± 18.146	ns	455.355 ± 15.677	445.412 ± 16.661	ns	**401.102 ± 10.969**	**499.665 ± 10.964**	**<0.001**	-
LA (cm^2)	**3.983 ± 0.240 a**	**3.523 ± 0.295 ab**	**3.137 ± 0.155 b**	**0.024**	3.475 ± 0.234	3.620 ± 0.178	ns	**3.987 ± 0.206**	**3.108 ± 0.148**	**0.001**	-
LMA (mg cm^{-2})	**13.289 ± 0.549 b**	**14.322 ± 0.364 a**	**14.452 ± 0.371 a**	**0.040**	14.079 ± 0.408	13.963 ± 0.333	ns	**13.080 ± 0.302**	**14.962 ± 0.287**	**<0.001**	-
LTD (g cm^{-3})	0.315 ± 0.006	0.316 ± 0.006	0.317 ± 0.008	ns	0.313 ± 0.005	0.319 ± 0.006	ns	**0.329 ± 0.005**	**0.303 ± 0.004**	**0.001**	-
LWC (%)	44.995 ± 1.055	44.889 ± 1.162	44.783 ± 0.921	ns	**45.696 ± 0.780**	**44.082 ± 0.863**	**0.007**	**47.901 ± 0.390**	**41.878 ± 0.459**	**<0.001**	-
RWC (%)	67.503 ± 1.450	68.069 ± 1.484	69.258 ± 1.223	ns	68.594 ± 1.096	67.959 ± 1.163	ns	**70.091 ± 0.868**	**66.462 ± 1.193**	**0.027**	-
E (mmol H_2O m^{-2} s^{-1})	1.698 ± 0.509	1.326 ± 0.373	1.641 ± 0.394	ns	1.411 ± 0.312	1.699 ± 0.378	ns	**0.433 ± 0.050**	**2.677 ± 0.303**	**<0.001**	-
g_s (mmol m^{-2} s^{-1})	102.118 ± 31.565	76.501 ± 19.931	96.135 ± 22.131	ns	84.168 ± 18.151	99.000 ± 22.150	ns	**26.104 ± 3.133**	**157.064 ± 17.671**	**<0.001**	-
A (µmol CO_2 m^{-2} s^{-1})	11.203 ± 0.984	11.782 ± 1.159	13.711 ± 0.938	ns	11.874 ± 0.821	12.591 ± 0.907	ns	12.764 ± 0.747	11.701 ± 0.960	ns	-
WUE (µmol CO_2 $mmol^{-1}$ H_2O)	18.728 ± 5.364	28.451 ± 10.697	21.540 ± 8.429	ns	19.528 ± 4.871	26.284 ± 8.326	ns	**40.963 ± 7.476**	**4.850 ± 0.354**	**<0.001**	-
ETR	158.333 ± 12.123	160.710 ± 9.821	186.781 ± 10.973	ns	157.477 ± 8.468	179.740 ± 9.497	ns	164.890 ± 8.804	172.326 ± 9.867	ns	-
Fv/Fm	**0.776 ± 0.005 b**	**0.784 ± 0.004 b**	**0.796 ± 0.003 a**	**0.004**	0.782 ± 0.004	0.789 ± 0.004	ns	**0.779 ± 0.005**	**0.792 ± 0.003**	**0.010**	UV x P
NPQ	1.740 ± 0.142	1.673 ± 0.166	1.827 ± 0.143	ns	1.757 ± 0.131	1.737 ± 0.113	ns	1.643 ± 0.108	1.851 ± 0.130	ns	-
Root starch (g 100 g^{-1})	13.179 ± 0.613	12.542 ± 0.428	14.001 ± 0.660	ns	13.230 ± 0.477	13.251 ± 0.493	ns	**12.192 ± 0.262**	**14.289 ± 0.522**	**0.002**	-
Root sugar (g 100 g^{-1})	9.459 ± 0.477	8.920 ± 0.449	8.381 ± 0.385	ns	9.167 ± 0.411	8.673 ± 0.310	ns	**8.029 ± 0.235**	**9.811 ± 0.351**	**<0.001**	-
Root TP (g 100 g^{-1})	5.725 ± 0.235	5.666 ± 0.210	5.687 ± 0.375	ns	5.977 ± 0.219	5.408 ± 0.215	ns	5.603 ± 0.196	5.782 ± 0.253	ns	-
starch:sugar	1.473 ± 0.074	1.464 ± 0.072	1.805 ± 0.152	ns	1.544 ± 0.070	1.617 ± 0.112	ns	1.590 ± 0.057	1.571 ± 0.120	ns	-
TP:starch	0.446 ± 0.015	0.457 ± 0.021	0.423 ± 0.032	ns	0.460 ± 0.017	0.423 ± 0.021	ns	**0.472 ± 0.020**	**0.412 ± 0.016**	**0.019**	-
TP:sugar	0.651 ± 0.026	0.665 ± 0.055	0.748 ± 0.089	ns	0.704 ± 0.042	0.672 ± 0.059	ns	0.736 ± 0.046	0.640 ± 0.054	ns	-

SRGR, stem relative growth rate; RRGR, root relative growth rate; LT, leaf thickness; LA, leaf area; LMA, leaf mass area; LTD, leaf tissue density; LWC, leaf water content; RWC, leaf relative water content; E, leaf transpiration rate; g_s, stomatal conductance; A, photosynthetic rate; WUE, water-use efficiency; ETR, apparent electron transport rate; NPQ, non-photochemical quenching; TP, total phenols; ns, not significant.

UV effects and interactions with watering and pruning

In the two species, the experimental treatments did not alter the percentage of resprouting success after aerial biomass removal (Tables 3.5 and 3.6). Nevertheless, UV enhancement affected some of the parameters analysed, although this effect was generally dependent on the watering treatment or the sampling date. Indeed, while UV did not affect the leaf RWC of *A. unedo* plants under low watering, well-watered plants had lower leaf RWC under enhanced UV-A+UV-B than under enhanced UV-A alone ($F_{2,15}$ = 3.557, p = 0.054; Fig. 3.7a). Moreover, *A. unedo* plants exposed to enhanced UV-A+UV-B radiation showed greater leaf RWC (about 5.3%) ($F_{1,10}$ = 7.483, p = 0.021; Fig. 3.7a) and marginally higher A ($F_{1,10}$ = 4.942, p = 0.057; Fig. 3.7b) under low- than under well-watered conditions, whereas this effect was not found in plants under UVA or control conditions. In line with this, leaves of *A. unedo* resprouts exposed to enhanced UV-A+UV-B radiation tended to have the highest Fv/Fm and NPQ values when grown under drier conditions (data not shown). Resprouts of *A. unedo* grown under supplemented UV-A+UV-B also showed greater A compared to those under ambient UV or supplemented UV-A radiation alone ($F_{2,15}$ = 3.627, p = 0.052; Fig. 3.8a), which would explain the interaction found between UV and pruning (Table 3.5).

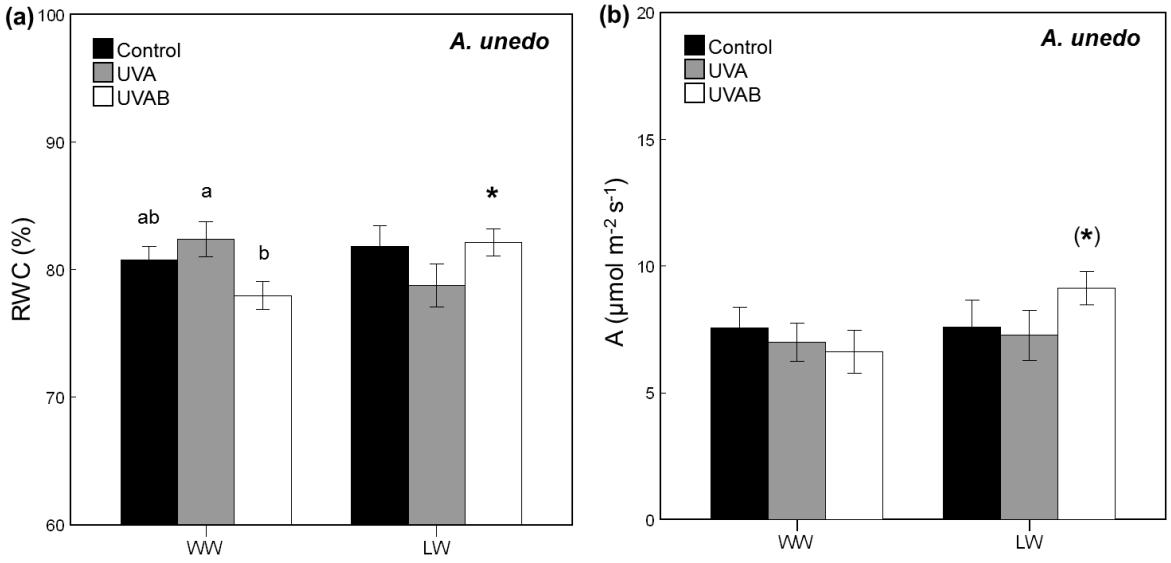


Fig. 3.7 (**a**) Leaf relative water content (RWC) and (**b**) photosynthetic rates (A) of *Arbutus unedo* plants (seedlings and resprouts) subjected to three UV radiation conditions (control, UVA and UVAB) combined with two watering regimes (well-watered, WW, and low-watered, LW). Error bars represent the standard error of the mean (n = 6). Asterisks indicate significant differences between WW and LW plants exposed to the same UV condition, whereas different letters indicate significant differences among UV conditions within the same watering regime. The significance level was set at $p \leq 0.05$.

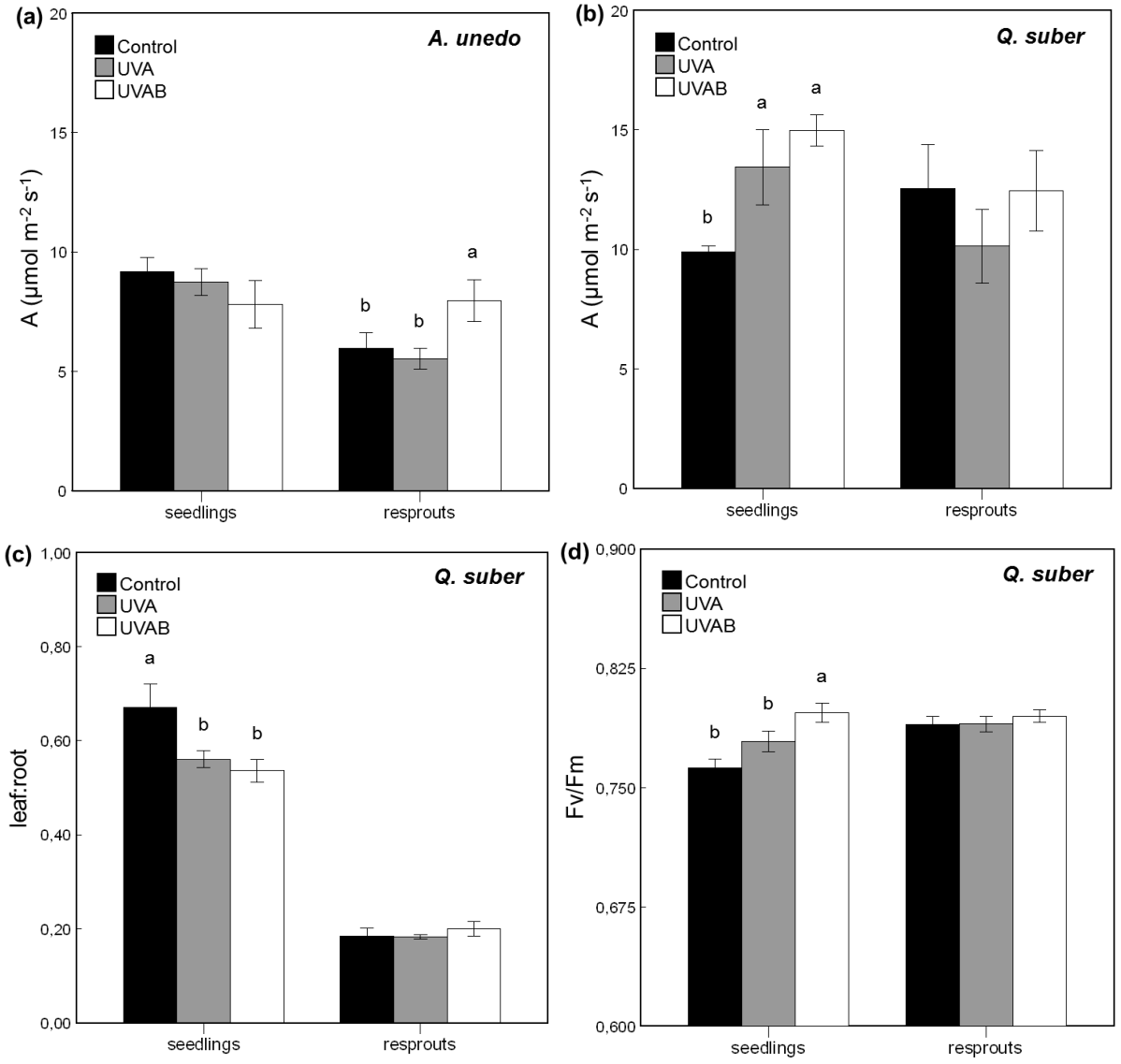


Fig. 3.8 (**a, b**) Leaf photosynthetic rates (A), (**c**) leaf to root mass ratio (leaf:root) and (**d**) maximum photochemical efficiency of PSII (Fv/Fm) in *Arbutus unedo* (**a**) and *Quercus suber* (**b**-**d**) seedlings and resprouts subjected to three UV radiation conditions (control, UVA and UVAB). Error bars represent the standard error of the mean (n = 6). Different letters indicate significant differences among UV conditions within seedlings or resprouts. The significance level was set at $p \leq 0.05$.

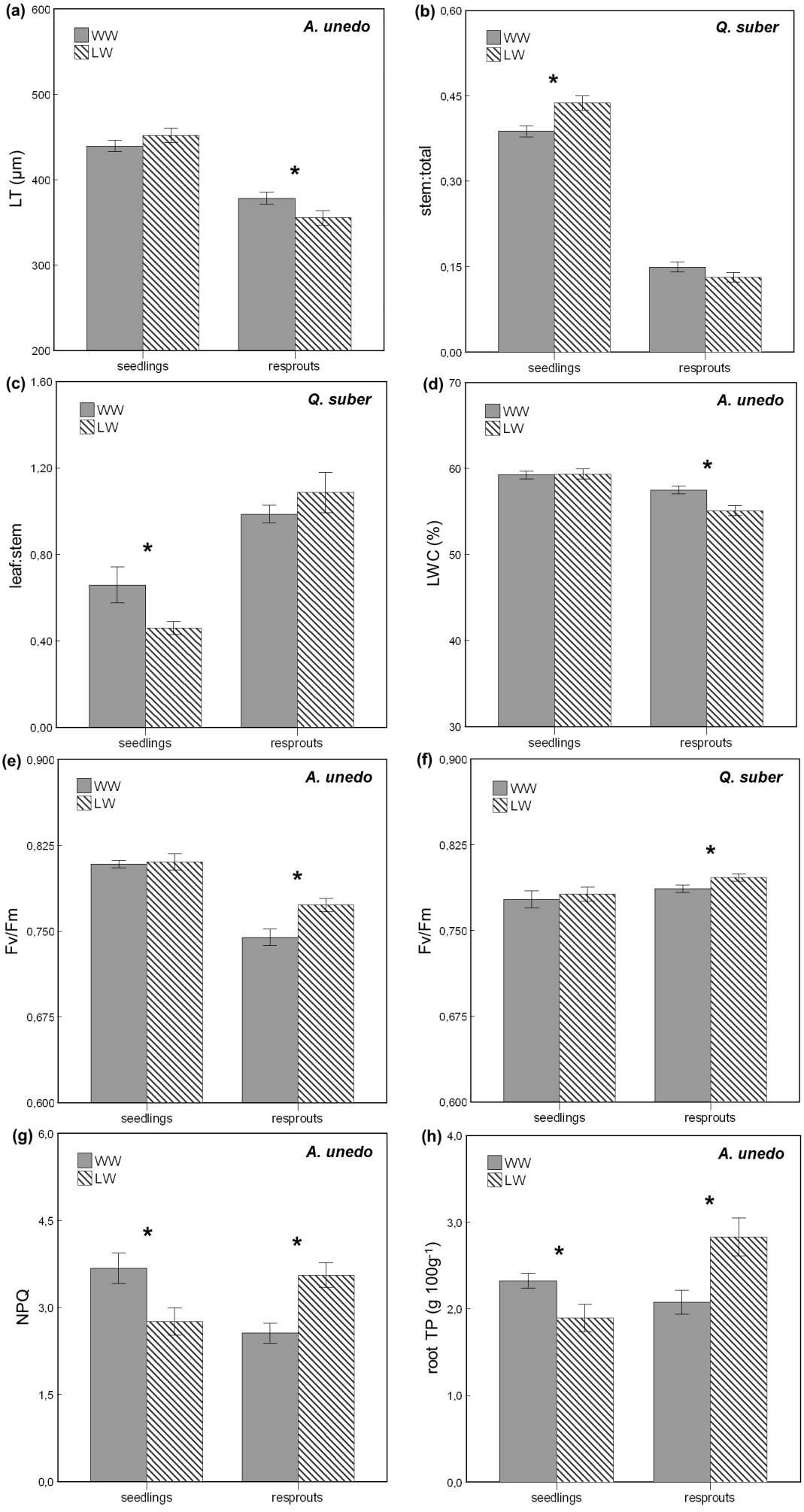
In the case of *Q. suber*, leaves exposed to enhanced UV-A+UV-B radiation were significantly smaller (21%), and consequently showed a higher LMA (~ 9%), than those grown under ambient UV (Table 3.6). LMA of plants supplemented with UV-A radiation also increased by nearly 8% compared to controls, suggesting an UV-A-mediated effect on LMA. The leaf:root ratio of seedlings (but not of resprouts) was sensitive to the UV treatment, since plants grown under enhanced UVA and UVA+UVB showed a lower ratio than controls ($F_{2,15}$ = 4.406, p = 0.031; Fig. 3.8c). This could be related to the higher A in *Q. suber* seedlings exposed to UVA

and UVAB enhancement ($F_{2,12}$ = 6.243, p = 0.014; Fig. 3.8b). Seedlings of this species grown under UVAB conditions also had higher Fv/Fm values than those grown in UVA and control plots ($F_{2,15}$ = 8.303, p = 0.004; Fig. 3.8d).

Watering effects

In the case of *A. unedo*, water availability only affected the leaf thickness of the resprouts. Indeed, leaves from *A. unedo* resprouts grown under optimal irrigation were thicker (by around 6%) than those developed under drier conditions ($F_{1,16}$ = 4.330, p = 0.054; Fig. 3.9a). In the case of *Q. suber,* leaf:total and leaf:root biomass ratios were higher in well-watered than in low-watered plants, regardless of the sampling date (Table 3.6). An interactive effect between watering and pruning was detected for stem:total and leaf:stem biomass ratios (Table 3.6), since, only before pruning, well-watered *Q. suber* plants allocated less biomass to stems ($F_{1,16}$ = 9.822, p = 0.006; Fig. 3.9b) having a higher leaf:stem ratio than low-watered ones ($F_{1,16}$ = 5.171, p = 0.037; Fig. 3.9c).

At the physiological level, as expected, leaf water content of both species was lower in low- than in well-watered plants (Tables 3.5 and 3.6). However, in *A. unedo*, differences were only significant for the resprouts ($F_{1,16}$ = 10.760, p = 0.005; Fig. 3.9d). Effects of water availability on Fv/Fm and NPQ values of *A. unedo* leaves were also dependent on the sampling date (Table 3.5; Fig. 3.9e,g). Indeed, before pruning, Fv/Fm values of *A. unedo* seedlings were not affected by the watering regime, while, after pruning, low-watered resprouts showed higher Fv/Fm values than well-watered ones ($F_{1,16}$ = 9.292, p = 0.008). A similar effect of the watering treatment was found on Fv/Fm values of *Q. suber* resprouts ($F_{1,12}$ = 4.907, p = 0.047; Fig. 3.9f). Regarding NPQ, low-watered seedlings of *A. unedo* had lower values than well-watered ones ($F_{1,16}$ = 6.820, p = 0.019), while the opposite was found after pruning ($F_{1,16}$ = 13.428, p = 0.002).


(a)
A. unedo
WW
LW
LT (µm)
600
500
400
300
200
seedlings
resprouts
*
(b)
Q. suber
WW
LW
stem:total
0,60
0,45
0,30
0,15
0,00
seedlings
resprouts
*
(c)
Q. suber
WW
LW
leaf:stem
1,60
1,20
0,80
0,40
0,00
seedlings
resprouts
*
(d)
A. unedo
WW
LW
LWC (%)
70
60
50
40
30
seedlings
resprouts
*
(e)
A. unedo
WW
LW
Fv/Fm
0,900
0,825
0,750
0,675
0,600
seedlings
resprouts
*
(f)
Q. suber
WW
LW
Fv/Fm
0,900
0,825
0,750
0,675
0,600
seedlings
resprouts
*
(g)
A. unedo
WW
LW
NPQ
6,0
4,5
3,0
1,5
0,0
seedlings
resprouts
*
*
(h)
A. unedo
WW
LW
root TP (g 100g-1)
4,0
3,0
2,0
1,0
0,0
seedlings
resprouts
*
*

◀◀ **Fig. 3.9** (**a**) Leaf thickness (LT), (**b**) stem to total mass ratio (stem:total), (**c**) leaf to stem mass ratio (leaf:stem), (**d**) leaf water content (LWC), (**e, f**) Fv/Fm, (**g**) NPQ and (**h**) root total phenol concentration (TP) in *Arbutus unedo* (**a, d, e, g, h**) and *Quercus suber* (**b, c, f**) seedlings and resprouts under two watering regimes (well-watered, WW, and low-watered, LW). Error bars represent the standard error of the mean (n = 9). Asterisks indicate significant differences between WW and LW plants within seedlings or resprouts. The significance level was set at $p \leq 0.05$.

Root biochemical traits of *Q. suber* were not affected by the reduction in water availability. Conversely, starch concentration in *A. unedo* roots increased by 31% under drier conditions, and, as a consequence, the starch:sugar ratio increased (Table 3.5). The effect of watering on total phenol concentration of *A. unedo* roots differed between seedlings and resprouts (Table 3.5). Indeed, while low-watered seedlings had lower root phenol concentration than well-watered ones ($F_{1,16}$ = 5.833, p = 0.028; Fig. 3.9h), the opposite was found in the resprouts ($F_{1,16}$ = 8.533, p = 0.010). Despite this, the phenol:starch ratio of *A. unedo* roots was not affected by water availability.

Correlations

For seedlings of *Q. suber*, a number of significant correlations between root biomass and other biometric parameters were found, while very few significant correlations were found in the case of *A. unedo* seedlings (Table 3.7). Regarding the UV treatment, all the significant correlations found were detected in seedlings of both species grown in UVA plots. Under enhanced UV-A, root biomass of *A. unedo* seedlings was positively correlated with leaf biomass, while, in *Q. suber*, root biomass was strongly correlated with stem basal diameter, stem biomass and root RGR (Table 3.7). Considering the watering treatment, the root biomass of *A. unedo* seedlings was positively correlated with the stem RGR under low water supply (Table 3.7). Conversely, the root biomass of *Q. suber* seedlings was significantly correlated with the stem diameter and biomass under the two irrigation levels and with the stem height in well-watered plants (Table 3.7). In any case, the root biomass of seedlings was correlated with the parameters analysed for the resprouts (data not shown). The correlations between root biochemical traits (concentrations of starch, soluble sugar and total

phenols) and the remaining variables were not significant, either for seedlings (Table 3.7) or resprouts (data not shown).

Table 3.7 Pearson's correlation coefficients (*r*) between root biomass and the studied biometric and biochemical parameters for *Arbutus unedo* and *Quercus suber* seedlings within the different UV (control, UVA and UVAB) and watering conditions (well-watered, WW, and low-watered, LW). The significance level considered was $p \leq 0.001$. Only significant correlations are shown, being highlighted in bold.

		A. unedo seedlings (root biomass)					*Q. suber* seedlings (root biomass)				
		UV radiation			Watering		UV radiation			Watering	
		control	UVA	UVAB	WW	LW	control	UVA	UVAB	WW	LW
seedlings	Height (cm)	ns	ns	ns	ns	ns	ns	ns	ns	**0.91**	ns
	Diameter (mm)	ns	ns	ns	ns	ns	ns	**0.98**	ns	**0.93**	**0.97**
	Leaf biomass (g)	ns	**0.99**	ns	ns	ns	ns	ns	ns	ns	ns
	Stem biomass (g)	ns	ns	ns	ns	ns	ns	**0.99**	ns	**0.94**	**0.96**
	SRGR (mg g^{-1} d^{-1})	ns	ns	ns	ns	**0.95**	ns	ns	ns	ns	ns
	RRGR (mg g^{-1} d^{-1})	ns	ns	ns	ns	ns	ns	**0.98**	ns	ns	ns
	Root starch (g 100 g^{-1})	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Root sugar (g 100 g^{-1})	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Root TP (g 100 g^{-1})	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

SRGR, stem relative growth rate; RRGR, root relative growth rate; TP, total phenols; ns, not significant.

3. Effects of UV radiation and rainfall reduction on leaf and soil parameters related to C and N cycles of a Mediterranean shrubland before and after a controlled fire

State-of-the-art

As a consequence of climate change, cloudiness reduction in the Mediterranean basin will decrease overall precipitation and increase ultraviolet radiation (UV; 280-400 nm) fluxes reaching terrestrial ecosystems in the near future (IPCC, 2013; Sanchez-Lorenzo *et al.*, 2017). Models also predict that Mediterranean ecosystems will be exposed to an increase in fire frequency over the coming years (IPCC, 2013), which could trigger changes in plant communities favouring the persistence and expansion of highly resilient communities such as Mediterranean shrublands (Acácio *et al.*, 2009). Shrub ecosystems have been spreading in Spain and other parts of Europe in the last decades (Tárrega *et al.*, 2001; Riera *et al.*, 2007), as a result of the increase in wildfire occurrence together with agricultural abandonment (Díaz-Delgado *et al.*, 2002; Lloret *et al.*, 2002). Mediterranean-type terrestrial communities deserve special attention for its role in fuel potential, plant variety and soil quality. Despite this, current knowledge about the UV effects on the functioning, and in particular on the biogeochemical cycles, of Mediterranean shrublands is limited, with even less information being available about the interactive effects between UV levels and other environmental factors, such as water availability or fire (Zepp *et al.*, 2007; Sardans & Peñuelas, 2013).

Specifically, increases in UV radiation, both UV-B (280-315 nm) and UV-A (315-400 nm), may directly alter C and N cycles of Mediterranean shrublands through the stimulation of photodegradation of plant litter and its phototransformation into soil microorganism-available forms. In arid and semi-arid environments, photodegradation by direct sunlight exposure play an important role in the breakdown of organic matter, particularly because of a UV-induced decline in the lignin concentration of the soil litter (Day *et al.*, 2007; Henry *et al.*, 2008; Dirks *et al.*, 2010). Enhanced lignin degradation in plant litter leaves the N easily available to microbes (Foereid *et al.*, 2010) facilitating the enzymatic degradation and the

microbial access to labile C compounds (Austin & Ballaré, 2010; Baker & Allison, 2015). However, direct sunlight exposure in the UV range can also be harmful for soil microorganisms (Hughes *et al.*, 2003), somewhat hindering the C and N release by biological decomposition (Zepp *et al.*, 2007).

UV radiation effects on litter decomposition and, thus, on C and N cycles may also be mediated by UV-induced chemical responses in plants which can vary depending on the species (Caldwell *et al.*, 2007; Austin *et al.*, 2016). Exposure to enhanced UV radiation can increase plant production of phenylpropanoid compounds, such as phenols (Searles *et al.*, 2001; Bassman, 2004; Julkunen-Tiitto *et al.*, 2005; Li *et al.*, 2010), which are used as UV-absorbing compounds (UACs) and free radical scavengers in leaves (Agati & Tattini, 2010). Higher amounts of phenolic compounds in the litter can delay soil organic matter decomposition and mineralization (Castells *et al.*, 2004), and inhibit nitrification due to their harmful effects on soil microorganisms and enzyme activities (Erickson *et al.*, 2000; Castells *et al.*, 2004; Castaldi *et al.*, 2009; Formánek *et al.*, 2014), thus decreasing available soil N. Enhanced UV-B exposure during plant growth may also directly increase (Yue *et al.*, 1998) or decrease (Pancotto *et al.*, 2005), depending on the species (Zepp *et al.*, 1998), leaf N concentration.

Chemical and biological properties of the soil can also be modified by increases in the amount of solar UV radiation reaching the soil surface due to UV-B-induced reductions in plant growth (Ballaré *et al.*, 2001; Garcia *et al.*, 2002; Hughes *et al.*, 2006). Among other effects, altered solar UV fluxes can change the activity of soil enzymes involved in the biological decomposition of organic matter (Nannipieri *et al.*, 2002; Caldwell, 2005). One of these enzymes is β-glucosidase, which controls the C cycle through the breakdown of labile cellulose and other carbohydrate polymers, enhancing nutrient release from organic compounds and thus facilitating microbe metabolism (Sardans *et al.*, 2008a). However, present knowledge about UV radiation effects on soil β-glucosidase activity is limited (Gallo *et al.*, 2006; Choudhary *et al.*, 2013) with even less information available in Mediterranean ecosystems (Baker & Allison, 2015). In dryland ecosystems, several studies found that β-

glucosidase activity in litter samples was unaffected by changes in UV exposure (Gallo *et al.*, 2006; Baker & Allison, 2015). Conversely, in a field experiment with mung bean cultivars, enhanced UV-B radiation stimulated root accumulation and secretion of phenolic compounds, which depleted microbial biomass of the rhizosphere leading to a reduction of β-glucosidase activity; on the contrary, at the non-rhizosphere soil, reduced root activity resulted in nutrient accumulation, increasing the microbial population and thus β-glucosidase activity (Choudhary *et al.*, 2013).

Carbon and nutrient cycles may also be substantially affected by other components of climate change, such as altered patterns of precipitation, which can interact with UV effects (Erickson III *et al.*, 2015). Unlike what happens with UV radiation, the effects of drought on the biogeochemical cycles of Mediterranean ecosystems have been extensively investigated, especially in relation to soil microbial activity and litter decomposition (Incerti *et al.*, 2011; Sardans & Peñuelas, 2013). Drier conditions tend to attenuate soil microbial activity, leading to reduced respiration rates (Rey *et al.*, 2002) and enzyme activities (Gallo *et al.*, 2006), along with increases in soil C concentration (Sardans *et al.*, 2008a). In turn, higher C:N ratios would delay the mineralization process and eventually the transformation of organic N into plant-available forms (Bengtsson *et al.*, 2012). With lower plant N uptake, the C:N ratio tends to increase in plants and, consequently, the soil is enriched with hardly mineralizable organic debris. Under water shortage, plants often increase their content of phenolic compounds (Hofmann *et al.*, 2003), which become an additional factor that can further slowdown decomposition rates (Castells *et al.*, 2004). Therefore, soil water availability is related to many variables and processes that may also be affected directly or indirectly by UV radiation. Because of that, precipitation regime might be an important factor modulating UV effects on C and N levels in Mediterranean ecosystems. For instance, there are evidences that the degree of photodegradation can vary with soil water content (Gallo *et al.*, 2006; Brandt *et al.*, 2007). In addition, metabolic activity of soil microbiota can be strongly limited by both high UV fluxes (Hughes *et al.*, 2003) and low soil moisture (Sherman *et al.*, 2012). Interactive effects between UV and water supply on litter decomposition can also be modulated by plant

responses to both factors. At plant level, enhanced UV radiation in combination with low soil moisture conditions have been reported to increase plant production of phenolic compounds being this effect dependent on plant species (Hofmann *et al.*, 2003; Ren *et al.*, 2007). In Mediterranean species, the direction of these effects can vary among specific phenols, despite the total pool of phenols not being changed (Nenadis *et al.*, 2015).

The evolution and dynamics of most Mediterranean-type ecosystems are also linked to wildfires (Lloret *et al.*, 2002; Paula & Pausas, 2006), with many species showing post-fire regeneration mechanisms, such as resprouting (Pausas *et al.*, 2004). Plant resprouting capacity is associated to storage of resources in belowground organs to ensure post-disturbance nutrient supply (Verdaguer & Ojeda, 2002). In soils of Mediterranean shrublands, decreases in organic C and increases in total N have been reported in the short-term after a fire, being dependent on factors such as soil moisture, vegetation type and climatic conditions (Caon *et al.*, 2014). Therefore, effects of UV fluxes and precipitation regime on the biogeochemical cycles of Mediterranean shrublands could be modulated after a fire by changes in soil C and nutrients and the reduction in plant aerial biomass. Moreover, the post-fire regeneration of the vegetation could also be affected by the levels of UV radiation and soil water availability, for instance, through their effects on the capacity of plants to store resources.

The main objectives of this study were to assess: 1) the role of UV radiation (UV-A and UV-B) on the C and N cycles of a Mediterranean shrubland, before and after a fire, and 2) whether these UV radiation effects can be altered by water availability. To achieve these goals, we performed a field experiment where the levels of UV and water availability reaching the ecosystem were modified. Different parameters related to C and N cycles were measured at soil, litter and plant level before and after an experimental fire. We hypothesized that: (i) UV exposure will affect soil C and N levels through effects on litter decomposition, which will be reflected in changes in soil respiration rates and β-glucosidase activity; (ii) UV-induced changes in soil C and N levels will be mediated by alterations in C and N concentrations of plant leaves and litter; (iii) UV effects will be modulated by the amount of

rainfall; and (iv) fire-induced changes in soil C and N and/or in plant cover will alter the interactive effects between UV fluxes and precipitation regime on C and N cycles.

Results

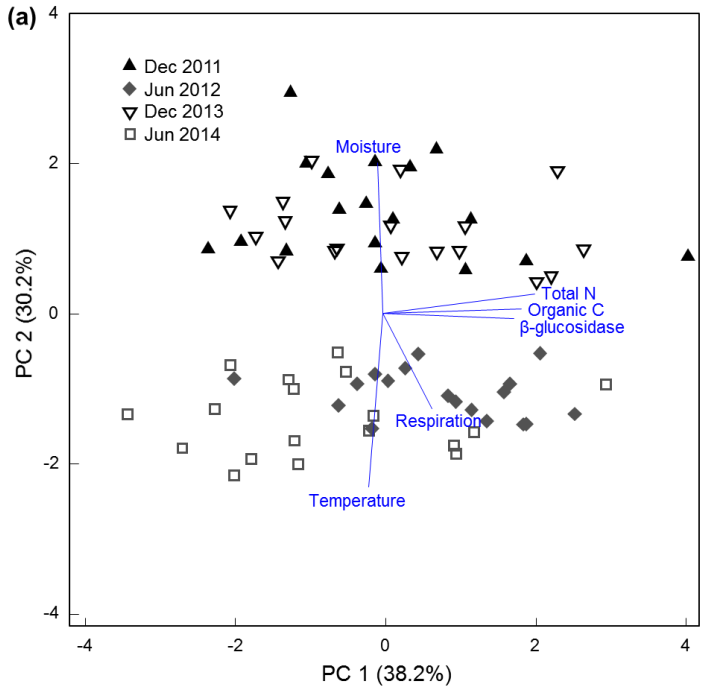
PCA on soil parameters

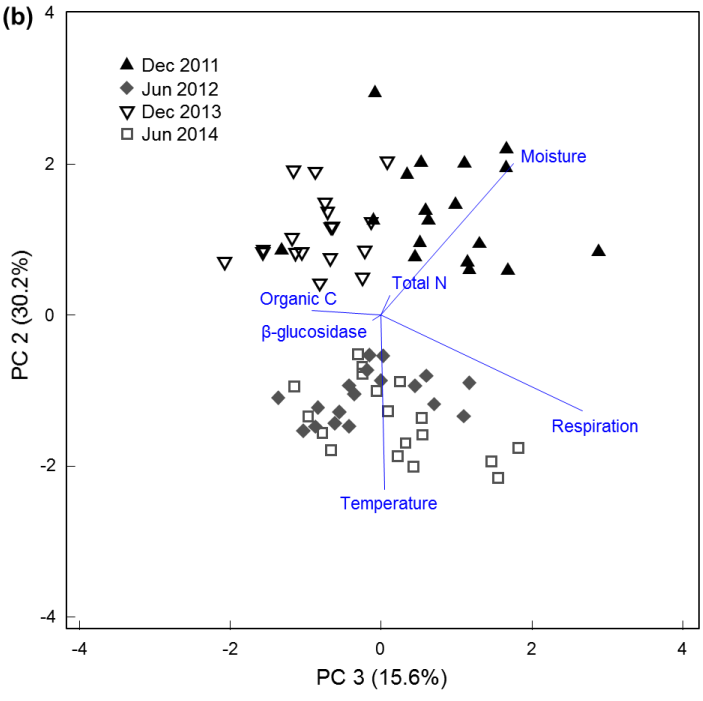
Three “principal components” (PCs) were obtained from the PCA performed with the six soil variables determined in four sampling dates, explaining 84.0% of the variance of the data set (Table 3.8, Fig. 3.10). Soil organic C, total N and β-glucosidase activity were the most important parameters related to PC1 (factor loadings > 0.50) while soil moisture and temperature showed the highest contribution (positive and negative, respectively) to PC2 (Table 3.8). Although soil moisture also contributed to PC3, soil respiration was the most relevant variable related to this component (Table 3.8).

PC2 clearly segregated December from June samplings, due to higher soil moisture and lower soil temperature in December than in June months (Fig. 3.10a,b). No clear separation was observed along PC1, although values obtained in June 2012 tended to be more positive than those obtained in June 2014, indicating higher overall values of organic C, total N and β-glucosidase activity in the first sampling date (Fig. 3.10a). PC3 separated December 2011 from December 2013 data, mainly as a result of higher respiration in December 2011 associated to slightly higher soil moisture (Fig. 3.10b). No segregation was observed in response to the experimental treatments.

Table 3.8 Principal component solution on six soil variables (*n* = 72). Factor loadings ≥ 0.50 in absolute value are marked in bold.

Parameters	PC1	PC2	PC3
Moisture (%)	-0.019	**0.603**	**0.527**
Temperature (ºC)	-0.056	**-0.695**	0.014
Respiration (µmol m^{-2} s^{-1})	0.200	-0.382	**0.802**
Organic C (mg g^{-1})	**0.556**	0.018	-0.275
Total N (mg g^{-1})	**0.610**	0.080	0.034
β-glucosidase (mg ρNP kg^{-1} h^{-1})	**0.525**	-0.020	-0.034
Variance explained (%)			
Absolute	38.2	30.2	15.6
Cumulative	38.2	68.4	84.0





◀◀ **Fig. 3.10** Ordination plot by principal component analysis (PCA) of the studied experimental plots along four sampling dates, representing PC1 vs. PC2 (**a**) and PC3 vs. PC2 (**b**), according to soil data of moisture (%), temperature (ºC), respiration (µmol m^{-2} s^{-1}), organic C (mg g^{-1}), total N (mg g^{-1}) and β-glucosidase activity (mg ρNP kg^{-1} h^{-1}).

Effects of the experimental fire

Significant differences were found in most of the studied parameters between pre- and post-fire periods regardless of the treatments. At the soil level, temperature, electrical conductivity (at the two studied depths) and organic C at depth B were significantly higher in the post-fire period, while respiration and total N at depth A decreased by 9.7% and 23%, respectively, in relation to pre-fire values (Table 3.9). As a consequence, soil C:N ratio was 19.8% and 17.5% higher at depth A and B, respectively, after the fire. Contrasting differences were obtained for the β-glucosidase activity between the two depths studied, since the activity of this enzyme declined by 21% at depth A whereas it increased by 47% at depth B in the post-fire period compared to pre-fire values. Soil moisture and pH showed similar values before and after the fire.

As expected, there were significant differences in soil cover by litter and plants before (June 2012) and after the fire (June 2013) (Table 3.10). In June 2014, vegetation cover already showed similar values to those found before the fire, while litter cover was still lower (Table 3.10).

Regarding the chemical properties of the leaf litter of *A. unedo*, values of $\delta^{15}N$ and N concentration were significantly higher after the fire (1.34‰ and 30.7%, respectively, compared to June 2012 and 1.14‰ and 55.1%, respectively, compared to September 2012) (Table 3.10). Conversely, $\delta^{13}C$ and C concentration values did not vary among sampling dates. As a consequence, the C:N ratio of *A. unedo* leaf litter in June 2014 was 31.1% lower than in September 2012 (Table 3.10). In the case of *P. angustifolia*, leaf litter production after the fire was too low to be analysed.

In the two studied species, leaf C concentration was significantly lower after the fire (by 1.5% in *A. unedo* and 1.2% in *P. angustifolia*) (Table 3.9). Since the N concentration of *P. angustifolia* leaves was a 23% higher after the fire, the leaf C:N ratio of this species was a

19.2% lower in the post-fire period. *A. unedo* leaves also showed a decrease in $\delta^{13}C$ values (by 0.4‰) after the fire, while, for both species, $\delta^{15}N$ values were 1‰ higher in the post-fire period, in accordance with the results found for the leaf litter of *A. unedo*.

Table 3.9 Overall mean ± S.E. for the studied parameters at two soil depths (A: 0-5 cm; B: 5-10 cm) and for the leaves of *Arbutus unedo* and *Phillyrea angustifolia*, before and after the experimental fire. Numbers in bold indicate significant differences before and after the fire. For all variables n = 36 (18 plots x 2 sampling dates), except for soil moisture, temperature and respiration (n = 72; 18 plots x 4 sampling dates) and for pre-fire values of soil organic C, total N and C:N ratio (n = 54; 18 plots x 3 sampling dates). The significance level considered was $p \leq 0.05$. Fire effects did never interact with UV radiation and/or precipitation effects on the studied parameters.

				PRE-fire	POST-fire	*p*-value
Soil			Moisture (%)	10.504 ± 0.711	9.421 ± 0.447	ns
			Temperature (ºC)	**17.732 ± 0.678**	**20.303 ± 0.857**	**0.025**
			Respiration (µmol m^{-2} s^{-1})	**1.900 ± 0.066**	**1.715 ± 0.068**	**0.036**
	Depth A		$pH_{1:2.5}$	6.198 ± 0.047	6.226 ± 0.049	ns
			$EC_{1:5}$ (dS m^{-1})	**0.063 ± 0.005**	**0.084 ± 0.005**	**0.009**
			Organic C (mg g^{-1})	17.986 ± 0.512	16.908 ± 0.818	ns
			Total N (mg g^{-1})	**1.562 ± 0.050**	**1.204 ± 0.062**	**<0.001**
			C:N ratio	**11.931 ± 0.453**	**14.299 ± 0.437**	**<0.001**
			β-glucosidase (mg ρNP kg^{-1} h^{-1})	**131.376 ± 6.036**	**103.513 ± 5.714**	**0.001**
	Depth B		$pH_{1:2.5}$	5.836 ± 0.061	5.983 ± 0.054	ns
			$EC_{1:5}$ (dS m^{-1})	**0.050 ± 0.004**	**0.062 ± 0.003**	**0.031**
			Organic C (mg g^{-1})	**10.407 ± 0.341**	**12.387 ± 0.445**	**0.001**
			Total N (mg g^{-1})	0.863 ± 0.029	0.870 ± 0.036	ns
			C:N ratio	**12.336 ± 0.357**	**14.491 ± 0.395**	**<0.001**
			β-glucosidase (mg ρNP kg^{-1} h^{-1})	**45.937 ± 2.698**	**67.458 ± 4.166**	**<0.001**
Plant leaf	*A. unedo*		C (mg g^{-1})	**489.315 ± 1.136**	**481.842 ± 0.888**	**<0.001**
			N (mg g^{-1})	12.523 ± 0.296	11.732 ± 0.398	ns
			C:N ratio	39.832 ± 0.937	42.809 ± 1.490	ns
			$\delta^{13}C$ (‰)	**-27.285 ± 0.140**	**-27.709 ± 0.135**	**0.026**
			$\delta^{15}N$ (‰)	**-2.086 ± 0.159**	**-1.147 ± 0.183**	**<0.001**
	P. angustifolia		C (mg g^{-1})	**509.410 ± 1.091**	**503.135 ± 0.718**	**<0.001**
			N (mg g^{-1})	**11.765 ± 0.307**	**14.474 ± 0.427**	**<0.001**
			C:N ratio	**44.422 ± 1.251**	**35.904 ± 1.126**	**<0.001**
			$\delta^{13}C$ (‰)	-27.474 ± 0.158	-27.380 ± 0.120	ns
			$\delta^{15}N$ (‰)	**-3.932 ± 0.213**	**-2.938 ± 0.188**	**0.001**

EC, electrical conductivity; ns, not significant.

Table 3.10 Overall mean ± S.E. (n = 18) for litter and plant covers and for the studied parameters in the leaf litter of *Arbutus unedo* in each sampling date, before and after the experimental fire. Significant differences among sampling dates are indicated in bold and by different letters. For litter and plant covers, pre-treatment data (May 2011) was included in the statistical test as a co-variable. The significance level considered was $p \leq 0.05$.

		PRE-fire		POST-fire		p-value
		Jun. 2012	Sep. 2012	Jun. 2013	Jun. 2014	
Litter cover (%)		**88.749 ± 1.348 a**	-	**70.703 ± 4.036 b**	**72.079 ± 3.782 b**	**0.001**
Plant cover (%)		**75.815 ± 1.695 a**	-	**62.106 ± 3.149 b**	**81.103 ± 2.837 a**	**<0.001**
A. unedo litter	C (mg g^{-1})	484.951 ± 1.469	482.797 ± 1.713	-	481.063 ± 3.133	ns
	N (mg g^{-1})	**6.916 ± 0.416 b**	**5.829 ± 0.434 b**	-	**9.036 ± 0.955 a**	**0.004**
	C:N ratio	**74.504 ± 4.463 ab**	**91.107 ± 6.850 a**	-	**62.780 ± 5.786 b**	**0.006**
	$\delta^{13}C$ (‰)	-29.039 ± 0.112	-28.874 ± 0.158	-	-28.494 ± 0.262	ns
	$\delta^{15}N$ (‰)	**-2.906 ± 0.248 b**	**-2.710 ± 0.289 b**	-	**-1.564 ± 0.257 a**	**0.001**

ns, not significant

Effects of UV radiation and precipitation regime

At soil level

Regardless of the watering regime and along all the experimental period, soil moisture was significantly higher in UVA and UVAB plots than in UV0 ones (Table 3.11). In addition, at depth A (0-5 cm), pre-fire soils from UVA and UV0 plots showed around 5% higher values of pH than those from UVAB plots.

As it was expected, the reduction in precipitation decreased the soil moisture of reduced rainfall plots throughout all the study period, being 20% and 23% lower than in natural rainfall plots before and after the fire, respectively (Table 3.11). Before the fire, the reduction in rainfall also decreased by 19% the soil C:N ratio at depth A, but this effect was lost after the fire.

Overall the study period, there was an interactive effect between the two treatments on soil respiration (Table 3.11). Indeed, before the fire, exposure to UV-B radiation reduced soil respiration rates (UVAB < UVA, p = 0.051) under natural rainfall, while exposure to UV-A increased soil respiration rates (UVA > UV0, p = 0.010) under reduced rainfall (Fig. 3.11). On the other hand, control soils (UVAB plots) always exhibited greater respiration under drier conditions (pre-fire: p = 0.001; post-fire: p = 0.018), whereas water supply did not significantly affect soil respiration of UVA and UV0 plots (Fig. 3.11).

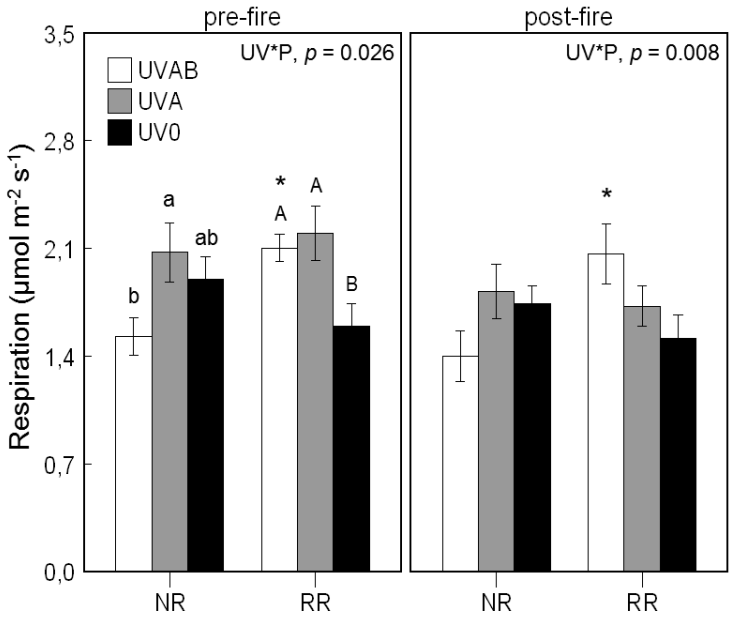


Fig. 3.11 Soil respiration in plots subjected to three UV radiation conditions (UVAB, UVA and UV0) combined with two precipitation regimes (natural rainfall, NR; reduced rainfall, RR), both before and after the fire. Error bars represent the standard error of the mean (n = 12). Asterisks indicate significant differences between NR and RR plots exposed to the same UV condition, whereas different letters indicate significant differences among UV conditions within each precipitation regime. Only significant differences within the same UV or precipitation condition are highlighted. The significance level was set at $p \leq 0.05$.

At depth B (5-10 cm), the effects of the two treatments on β-glucosidase activity showed a significant interaction with the sampling date before the fire (Table 3.11), since UVA plots showed significantly higher β-glucosidase activity than UV0 and UVAB plots under reduced rainfall and in December 2011, but not in June 2012, under natural rainfall (p = 0.010) (Fig. 3.12a). After the fire, the effects of the two treatments on organic C at depth B also differed between sampling dates, since, under natural rainfall, soils of UVA and UV0 plots showed higher organic C values than UVAB plots in June 2014 (p = 0.047), but not in December 2013 (Fig. 3.12b).

Finally, plant and litter cover did not show significant differences as a result of the treatments (data not shown).

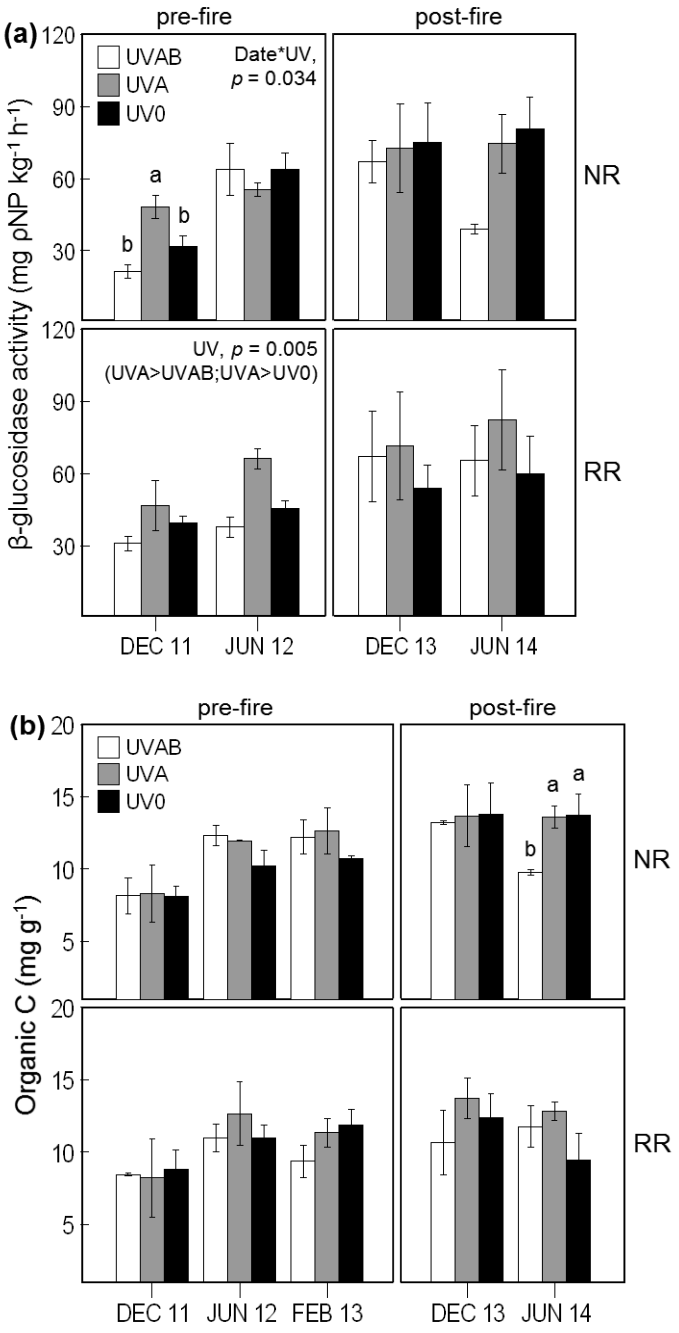


Fig. 3.12 Soil β-glucosidase activity (**a**) and organic C (**b**) at depth B (5-10 cm) from plots subjected to three UV radiation conditions (UVAB, UVA and UV0) combined with two precipitation regimes (natural rainfall, NR; reduced rainfall, RR) for all the sampling dates both before and after the fire. Error bars represent the standard error of the mean (n = 3). Since the interaction between UV radiation, precipitation and sampling date was significant in the pre-fire period for β-glucosidase activity (p = 0.009) and in the post-fire period for organic C (p = 0.012), we analysed UV effects within the two levels of precipitation for each of these periods and only significant differences are highlighted. Thus, different letters indicate significant differences among UV conditions within a specific sampling date and precipitation regime. The significance level was set at $p \leq 0.05$.

At leaf litter level

Treatments did not affect leaf litter C and N concentrations or C:N ratio of any of the two studied species (Table 3.12). Regarding the isotopic composition of litter, in September 2012, $\delta^{13}C$ values of *P. angustifolia* leaf litter were 0.8‰ higher in UVA plots than in UVAB ones (Table 3.12). On the other hand, the experimental reduction in precipitation decreased by 1.9‰ the $\delta^{15}N$ values of *P. angustifolia* leaf litter in June 2012 (Table 3.12). For *A. unedo* litter, we found a significant interactive effect of the two treatments on $\delta^{15}N$ values in September 2012 (Table 3.12). In this sampling date, but only in plots submitted to reduced rainfall, leaf litter of this species showed 2.6 and 2.0‰ lower $\delta^{15}N$ values in UVA and UV0 plots, respectively, compared to UVAB ones (p = 0.009; Fig. 3.13a).

Table 3.11 Overall mean ± S.E. for all the studied parameters at two soil depths (A, 0-5 cm; B, 5-10 cm) and for the leaves of *Arbutus unedo* and *Phillyrea angustifolia* under three UV radiation conditions (UVAB, UVA and UV0) and two precipitation regimes (natural rainfall, NR; reduced rainfall, RR). Pre- and post-fire data and analyses are shown separately. Numbers in bold indicate significant differences among the levels of the factor. Significant differences among the UV conditions are also indicated by different letters. For all the variables, n = 12 in each UV condition and n = 18 in each precipitation regime, except for soil moisture, temperature and respiration (n = 24 and n = 36, respectively) and for pre-fire values of soil organic C, total N and C:N ratio (n = 18 and n = 27, respectively). The significance level considered was $p \leq 0.05$. Only significant two-way or three-way interactions were included in the column "interactions".

				UV radiation (UV)				Precipitation (P)			Interactions
				UVAB	UVA	UV0	*p*-value	NR	RR	*p*-value	
PRE-fire	Soil	Depth A	Moisture (%)	**10.918 ± 1.185 a**	**12.024 ± 1.389 a**	**8.569 ± 1.037 b**	**<0.001**	**11.619 ± 1.108**	**9.388 ± 0.867**	**0.001**	-
			Temperature (ºC)	17.850 ± 0.698	17.635 ± 0.671	17.713 ± 0.664	ns	17.928 ± 0.567	17.537 ± 0.537	ns	-
			Respiration (µmol m^{-2} s^{-1})	**1.815 ± 0.095 b**	**2.136 ± 0.128 a**	**1.747 ± 0.105 b**	**0.038**	1.835 ± 0.095	1.964 ± 0.091	ns	UV x P
			$pH_{1:2.5}$	**6.008 ± 0.098 b**	**6.273 ± 0.057 a**	**6.313 ± 0.058 a**	**0.027**	6.147 ± 0.074	6.249 ± 0.058	ns	-
			$EC_{1:5}$ (dS m^{-1})	0.064 ± 0.011	0.059 ± 0.006	0.066 ± 0.008	ns	0.060 ± 0.008	0.066 ± 0.006	ns	-
			Organic C (mg g^{-1})	17.191 ± 0.702	18.683 ± 0.853	18.084 ± 1.080	ns	18.269 ± 0.634	17.703 ± 0.813	ns	-
			Total N (mg g^{-1})	1.490 ± 0.080	1.632 ± 0.085	1.564 ± 0.097	ns	1.463 ± 0.072	1.661 ± 0.067	ns	-
			C:N ratio	12.223 ± 1.051	11.641 ± 0.466	11.928 ± 0.765	ns	**13.168 ± 0.783**	**10.694 ± 0.324**	**0.010**	-
			β-glucosidase (mg ρNP kg^{-1} h^{-1})	124.639 ± 10.591	140.743 ± 11.514	128.745 ± 9.500	ns	124.091 ± 7.521	138.660 ± 9.338	ns	-
		Depth B	$pH_{1:2.5}$	5.760 ± 0.062	5.922 ± 0.104	5.825 ± 0.139	ns	5.911 ± 0.062	5.760 ± 0.104	ns	-
			$EC_{1:5}$ (dS m^{-1})	0.047 ± 0.006	0.050 ± 0.007	0.054 ± 0.008	ns	0.047 ± 0.005	0.054 ± 0.006	ns	-
			Organic C (mg g^{-1})	10.247 ± 0.526	10.854 ± 0.768	10.121 ± 0.453	ns	10.514 ± 0.472	10.301 ± 0.501	ns	-
			Total N (mg g^{-1})	0.840 ± 0.051	0.902 ± 0.054	0.848 ± 0.048	ns	0.881 ± 0.044	0.846 ± 0.039	ns	-
			C:N ratio	12.534 ± 0.657	12.233 ± 0.739	12.240 ± 0.459	ns	12.339 ± 0.605	12.333 ± 0.393	ns	-
			β-glucosidase (mg ρNP kg^{-1} h^{-1})	**38.504 ± 5.405 b**	**54.119 ± 3.548 a**	**45.188 ± 4.065 ab**	**0.017**	47.430 ± 4.375	44.444 ± 3.252	ns	Date x UV x P
	Plant leaf	*A. unedo*	C (mg g^{-1})	492.004 ± 2.041	488.030 ± 1.924	487.910 ± 1.861	ns	490.783 ± 1.053	487.847 ± 1.988	ns	Date x P
			N (mg g^{-1})	12.864 ± 0.622	12.005 ± 0.330	12.700 ± 0.550	ns	12.234 ± 0.347	12.811 ± 0.480	ns	-
			C:N ratio	39.214 ± 1.860	41.031 ± 1.258	39.251 ± 1.775	ns	40.652 ± 1.125	39.012 ± 1.508	ns	-
			$\delta^{13}C$ (‰)	-27.306 ± 0.234	-27.025 ± 0.190	-27.524 ± 0.291	ns	-27.313 ± 0.233	-27.256 ± 0.163	ns	-
			$\delta^{15}N$ (‰)	-1.713 ± 0.226	-2.315 ± 0.271	-2.230 ± 0.314	ns	-2.131 ± 0.206	-2.042 ± 0.248	ns	-
		P. angustifolia	C (mg g^{-1})	**512.470 ± 1.429 a**	**506.282 ± 2.099 b**	**509.480 ± 1.783 ab**	**0.011**	509.654 ± 1.526	509.167 ± 1.603	ns	-
			N (mg g^{-1})	11.823 ± 0.543	11.717 ± 0.460	11.755 ± 0.628	ns	**11.255 ± 0.392**	**12.276 ± 0.453**	**0.020**	-
			C:N ratio	44.406 ± 2.127	44.036 ± 1.930	44.825 ± 2.580	ns	**46.351 ± 1.814**	**42.494 ± 1.647**	**0.033**	-
			$\delta^{13}C$ (‰)	-27.489 ± 0.126	-27.535 ± 0.387	-27.398 ± 0.269	ns	-27.233 ± 0.111	-27.715 ± 0.289	ns	-
			$\delta^{15}N$ (‰)	-3.965 ± 0.383	-3.762 ± 0.396	-4.069 ± 0.352	ns	-3.665 ± 0.324	-4.199 ± 0.269	ns	-
POST-fire	Soil	Depth A	Moisture (%)	**9.823 ± 0.606 a**	**10.915 ± 0.902 a**	**7.526 ± 0.643 b**	**<0.001**	**10.609 ± 0.681**	**8.234 ± 0.518**	**<0.001**	-
			Temperature (ºC)	20.364 ± 0.914	20.535 ± 0.857	20.011 ± 0.799	ns	20.429 ± 0.713	20.180 ± 0.683	ns	-
			Respiration (µmol m^{-2} s^{-1})	1.746 ± 0.144	1.774 ± 0.110	1.628 ± 0.097	ns	1.661 ± 0.092	1.769 ± 0.099	ns	UV x P
			$pH_{1:2.5}$	6.081 ± 0.088	6.281 ± 0.077	6.318 ± 0.080	ns	6.205 ± 0.077	6.248 ± 0.063	ns	-
			$EC_{1:5}$ (dS m^{-1})	0.083 ± 0.013	0.082 ± 0.005	0.086 ± 0.009	ns	0.075 ± 0.008	0.092 ± 0.007	ns	-
			Organic C (mg g^{-1})	15.640 ± 1.593	17.787 ± 1.166	17.298 ± 1.503	ns	17.103 ± 1.089	16.714 ± 1.250	ns	-
			Total N (mg g^{-1})	1.126 ± 0.135	1.246 ± 0.064	1.240 ± 0.115	ns	1.214 ± 0.085	1.194 ± 0.092	ns	-
			C:N ratio	14.439 ± 1.001	14.338 ± 0.675	14.120 ± 0.597	ns	14.387 ± 0.555	14.211 ± 0.689	ns	-
			β-glucosidase (mg ρNP kg^{-1} h^{-1})	83.390 ± 5.078	110.333 ± 10.253	116.817 ± 11.118	ns	106.023 ± 8.961	101.004 ± 7.308	ns	-
		Depth B	$pH_{1:2.5}$	5.916 ± 0.093	5.997 ± 0.094	6.037 ± 0.097	ns	6.004 ± 0.087	5.962 ± 0.066	ns	-
			$EC_{1:5}$ (dS m^{-1})	0.055 ± 0.004	0.060 ± 0.004	0.070 ± 0.008	ns	0.055 ± 0.003	0.069 ± 0.005	ns	-
			Organic C (mg g^{-1})	11.347 ± 0.685	13.458 ± 0.597	12.357 ± 0.928	ns	12.969 ± 0.599	11.805 ± 0.646	ns	Date x UV x P
			Total N (mg g^{-1})	0.828 ± 0.059	0.924 ± 0.063	0.858 ± 0.068	ns	0.919 ± 0.051	0.821 ± 0.049	ns	-
			C:N ratio	13.916 ± 0.546	15.091 ± 1.002	14.466 ± 0.346	ns	14.276 ± 0.302	14.706 ± 0.738	ns	-
			β-glucosidase (mg ρNP kg^{-1} h^{-1})	59.671 ± 6.508	75.263 ± 8.112	67.442 ± 6.813	ns	68.158 ± 5.554	66.759 ± 6.368	ns	-
	Plant leaf	*A. unedo*	C (mg g^{-1})	482.669 ± 1.639	483.505 ± 1.329	479.354 ± 1.482	ns	483.349 ± 1.395	480.336 ± 1.016	ns	-
			N (mg g^{-1})	11.952 ± 0.652	11.380 ± 0.688	11.865 ± 0.772	ns	11.401 ± 0.588	12.064 ± 0.542	ns	-
			C:N ratio	41.711 ± 2.258	44.333 ± 2.822	42.384 ± 2.791	ns	44.348 ± 2.266	41.271 ± 1.931	ns	-
			$\delta^{13}C$ (‰)	-27.980 ± 0.211	-27.675 ± 0.201	-27.472 ± 0.279	ns	-27.884 ± 0.203	-27.534 ± 0.175	ns	-
			$\delta^{15}N$ (‰)	**-0.562 ± 0.310 a**	**-1.257 ± 0.335 b**	**-1.622 ± 0.245 b**	**0.011**	-1.217 ± 0.248	-1.077 ± 0.277	ns	UV x P
		P. angustifolia	C (mg g^{-1})	504.430 ± 1.404	503.235 ± 0.955	501.739 ± 1.307	ns	503.830 ± 1.096	502.440 ± 0.930	ns	-
			N (mg g^{-1})	15.354 ± 0.758	14.227 ± 0.628	13.843 ± 0.814	ns	13.944 ± 0.582	15.004 ± 0.616	ns	-
			C:N ratio	33.802 ± 1.760	36.198 ± 1.715	37.712 ± 2.316	ns	37.231 ± 1.566	34.576 ± 1.601	ns	-
			$\delta^{13}C$ (‰)	-27.401 ± 0.254	-27.153 ± 0.182	-27.587 ± 0.177	ns	-27.337 ± 0.150	-27.423 ± 0.192	ns	-
			$\delta^{15}N$ (‰)	-2.541 ± 0.238	-3.172 ± 0.428	-3.101 ± 0.272	ns	**-2.493 ± 0.271**	**-3.383 ± 0.220**	**0.027**	-

EC, electrical conductivity; ns, not significant.

Table 3.12 Overall mean ± S.E. for all the studied parameters in the leaf litter of *Arbutus unedo* and *Phillyrea angustifolia* under three UV radiation conditions (UVAB, UVA and UV0) and two precipitation regimes (natural rainfall, NR; reduced rainfall, RR). Data from each sampling date are shown separately. Numbers in bold indicate significant differences among the levels of the factor. Significant differences among the UV conditions are also indicated by different letters. For all sampling dates and variables, n = 6 in each UV condition and n = 9 in each precipitation regime. The significance level considered was $p \leq 0.05$.

			UV radiation (UV)				Precipitation (P)			UV x P
			UVAB	UVA	UV0	*p*-value	NR	RR	*p*-value	*p*-value
June 2012	*A. unedo*	C (mg g^{-1})	488.756 ± 2.635	484.806 ± 2.373	481.291 ± 1.967	ns	486.173 ± 2.266	483.728 ± 1.915	ns	ns
		N (mg g^{-1})	6.803 ± 0.594	6.336 ± 0.629	7.610 ± 0.920	ns	6.645 ± 0.734	7.188 ± 0.421	ns	ns
		C:N ratio	74.741 ± 6.715	80.532 ± 8.269	68.240 ± 8.635	ns	79.874 ± 7.855	69.134 ± 3.968	ns	ns
		$\delta^{13}C$ (‰)	-28.908 ± 0.243	-29.006 ± 0.194	-29.202 ± 0.153	ns	-28.965 ± 0.204	-29.112 ± 0.105	ns	ns
		$\delta^{15}N$ (‰)	-2.747 ± 0.223	-3.022 ± 0.493	-2.950 ± 0.573	ns	-2.691 ± 0.334	-3.121 ± 0.373	ns	ns
	P. angustifolia	C (mg g^{-1})	507.117 ± 4.025	508.839 ± 4.635	509.320 ± 3.772	ns	510.662 ± 3.298	506.594 ± 3.037	ns	ns
		N (mg g^{-1})	9.462 ± 1.371	7.444 ± 0.331	6.546 ± 0.438	ns	8.265 ± 1.177	7.552 ± 0.643	ns	ns
		C:N ratio	59.873 ± 8.776	68.814 ± 3.497	79.277 ± 4.465	ns	68.055 ± 7.389	70.419 ± 5.098	ns	ns
		$\delta^{13}C$ (‰)	-28.450 ± 0.429	-28.456 ± 0.174	-28.561 ± 0.251	ns	-28.375 ± 0.239	-28.585 ± 0.269	ns	ns
		$\delta^{15}N$ (‰)	-3.436 ± 0.865	-3.502 ± 1.097	-3.042 ± 0.671	ns	**-2.249 ± 0.627**	**-4.126 ± 0.536**	**0.032**	ns
September 2012	*A. unedo*	C (mg g^{-1})	480.609 ± 3.866	485.788 ± 2.093	481.992 ± 2.782	ns	484.803 ± 3.014	480.790 ± 1.544	ns	ns
		N (mg g^{-1})	6.672 ± 0.672	5.051 ± 0.487	5.764 ± 0.982	ns	5.968 ± 0.734	5.690 ± 0.506	ns	ns
		C:N ratio	75.842 ± 7.721	100.156 ± 8.607	97.324 ± 16.560	ns	92.922 ± 12.293	89.293 ± 6.892	ns	ns
		$\delta^{13}C$ (‰)	-28.714 ± 0.386	-28.776 ± 0.175	-29.131 ± 0.237	ns	-28.613 ± 0.228	-29.134 ± 0.194	ns	ns
		$\delta^{15}N$ (‰)	-2.080 ± 0.315	-3.232 ± 0.518	-2.817 ± 0.590	ns	-2.366 ± 0.343	-3.053 ± 0.456	ns	**0.034**
	P. angustifolia	C (mg g^{-1})	505.620 ± 4.364	504.880 ± 4.521	499.444 ± 4.780	ns	503.584 ± 2.983	503.046 ± 4.353	ns	ns
		N (mg g^{-1})	6.127 ± 0.380	6.207 ± 0.230	6.000 ± 0.228	ns	5.811 ± 0.149	6.411 ± 0.247	ns	ns
		C:N ratio	84.070 ± 5.072	81.912 ± 3.201	83.852 ± 3.278	ns	87.158 ± 2.474	79.398 ± 3.134	ns	ns
		$\delta^{13}C$ (‰)	**-28.334 ± 0.212 b**	**-27.525 ± 0.213 a**	**-27.804 ± 0.150 ab**	**0.040**	-27.917 ± 0.200	-27.858 ± 0.187	ns	ns
		$\delta^{15}N$ (‰)	-3.586 ± 0.551	-3.177 ± 0.792	-3.890 ± 0.158	ns	-3.080 ± 0.565	-4.022 ± 0.214	ns	ns
June 2014 *	*A. unedo*	C (mg g^{-1})	480.776 ± 2.942	479.314 ± 4.184	483.099 ± 8.516	ns	483.452 ± 5.434	478.674 ± 3.283	ns	ns
		N (mg g^{-1})	8.078 ± 1.612	7.786 ± 1.220	11.246 ± 1.932	ns	10.087 ± 1.470	7.985 ± 1.198	ns	ns
		C:N ratio	69.968 ± 11.122	69.572 ± 10.321	48.801 ± 7.253	ns	55.749 ± 7.449	69.811 ± 8.628	ns	ns
		$\delta^{13}C$ (‰)	-28.777 ± 0.406	-28.770 ± 0.364	-27.934 ± 0.556	ns	-28.024 ± 0.433	-28.964 ± 0.222	ns	ns
		$\delta^{15}N$ (‰)	-0.969 ± 0.354	-1.864 ± 0.471	-1.859 ± 0.471	ns	-1.471 ± 0.354	-1.658 ± 0.392	ns	ns

ns, not significant

* In June 2014, we only collected litter from *A. unedo* , since production of *P. angustifolia* litter was too low.

At plant leaf level

Despite *P. angustifolia* had higher leaf C concentration than *A. unedo* over the study period ($F_{1,120}$ = 488.412, p < 0.001), differences in leaf N concentration between the two species varied before and after the fire (Table 3.9). In the pre-fire period, N concentration in *P. angustifolia* leaves was lower than in *A. unedo* ($F_{1,48}$ = 7.003, p = 0.011), but, after the fire, the contrary was found ($F_{1,48}$ = 46.920, p < 0.001). These differences led to a higher C:N ratio of *P. angustifolia* leaves in the pre-fire period ($F_{1,48}$ = 19.520, p < 0.001) followed by a lower ratio after the fire ($F_{1,48}$ = 30.971, p < 0.001; Table 3.9). Throughout the study period, $\delta^{13}C$ values did not differ between the two species, although *P. angustifolia* showed lower $\delta^{15}N$ values than *A. unedo* ($F_{1,120}$ = 105.767, p < 0.001).

UV and precipitation treatments had different effects on the leaf parameters studied depending on the species. In the case of *P. angustifolia*, leaves from UVA plots had a 1.2% lower C concentration than those from UVAB plots before the fire (Table 3.11). Also before the fire, precipitation reduction increased foliar N concentration of this species by 9.0%, reducing, as a consequence, the C:N ratio by 8.3%. After the fire, *P. angustifolia* leaves grown in plots under reduced rainfall had $\delta^{15}N$ values 0.9‰ lower than those from plots receiving natural rainfall (Table 3.11).

In *A. unedo* leaves, in the pre-fire period, the effect of the precipitation treatment on the C concentration depended on the sampling date (Table 3.11). Indeed, in March 2012 (but not in March 2014 despite the same tendency was observed), leaves of this species had a 2% lower C concentration in plots under reduced rainfall than in those receiving natural precipitation (p = 0.009; Fig. 3.14). After the fire, there was a significant interactive effect between UV and precipitation treatments on the $\delta^{15}N$ of *A. unedo* leaves (Table 3.11), since, only under drier conditions, leaves from UVA and UV0 plots showed, respectively, 1.8 and 1.3‰ lower $\delta^{15}N$ values than control ones (Fig. 3.13b). Treatments did not affect leaf $\delta^{13}C$ values of any of the two species studied.

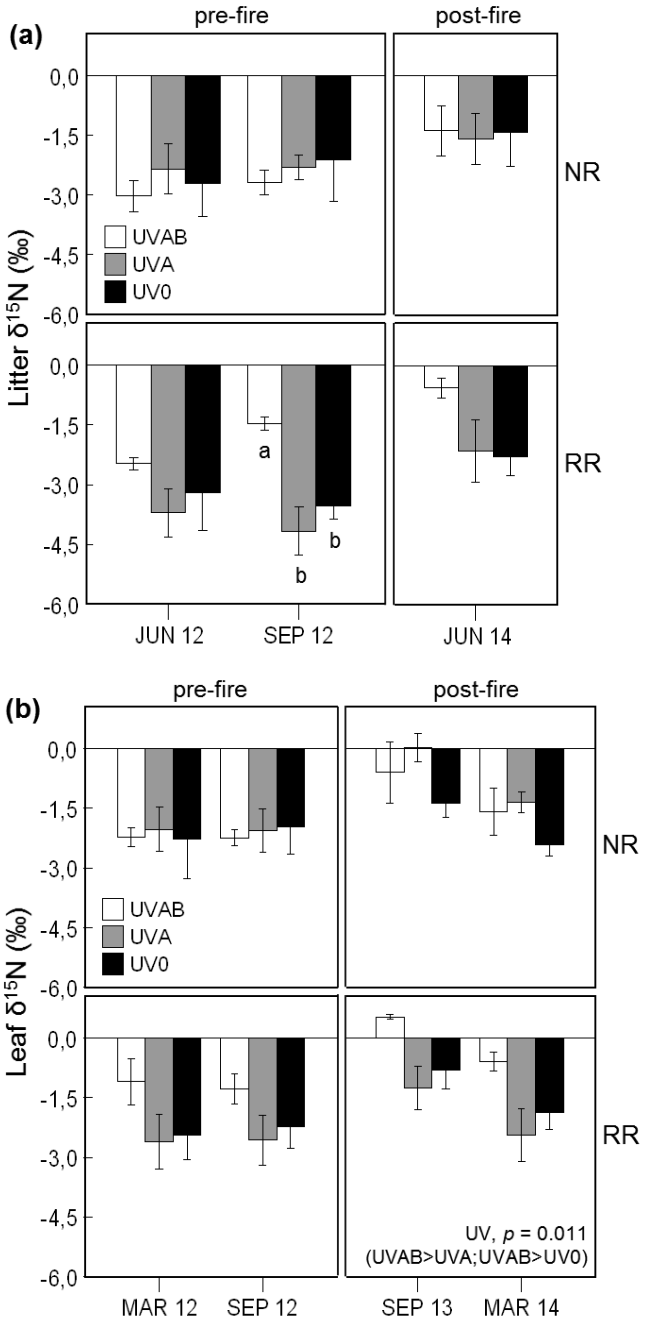


Fig. 3.13 *Arbutus unedo* $\delta^{15}N$ in litter (**a**) and leaves (**b**) from plots subjected to three UV radiation conditions (UVAB, UVA and UV0) combined with two precipitation regimes (natural rainfall, NR; reduced rainfall, RR) for all the sampling dates both before and after the fire. Error bars represent the standard error of the mean (n = 3). Since there was a significant interaction between the effects of the two treatments (UV radiation and precipitation) on $\delta^{15}N$ values of litter samples collected in September 2012 (p = 0.034) and of leaves from the post-fire period (p = 0.002), we analysed the UV effects within the two levels of precipitation for these sampling dates and only significant differences are highlighted. Different letters indicate significant differences among UV conditions within a specific sampling date and precipitation regime. The significance level was set at $p \leq 0.05$.

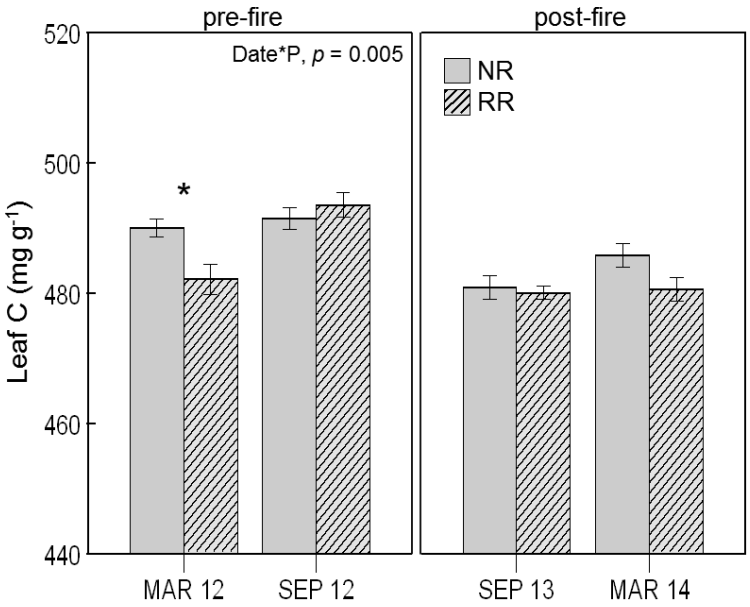


Fig. 3.14 C concentration in leaves of *Arbutus unedo* from plots subjected to two precipitation regimes (natural rainfall, NR; reduced rainfall, RR) for all the sampling dates both before and after the fire. Error bars represent the standard error of the mean (n = 9). Since there was a significant interaction between the sampling date and the precipitation treatment before the fire, we analysed the precipitation effects within the two sampling dates and only significant differences are highlighted. Asterisk indicates significant differences between NR and RR plots within a specific sampling date. The significance level was set at $p \leq 0.05$.

Chapter IV

DISCUSSION

1. Leaf biochemical adjustments in two Mediterranean resprouter species facing enhanced UV level and reduced water availability before and after pruning

Both *A. unedo* and *Q. suber* are sclerophyllous woody species with similar foliar traits that widely co-occur in Mediterranean terrestrial ecosystems, thus, being adapted to similar environmental conditions. Despite this, the investment in leaf phenols was substantially greater in *A. unedo*, while *Q. suber* appears to allocate more resources to the accumulation of leaf antioxidants, suggesting different protective strategies to face stress conditions.

Effects of treatments on leaf phenols of A. unedo

Changes in UV levels led to variations in the overall leaf amounts of quercetins and flavonols in *A. unedo* plants before pruning (Table 3.1). In particular, results suggest a UV-A-induced decrease of quercetins in *A. unedo* seedlings together with a UV-B-mediated increase in the levels of both quercetins and flavonols. A decrease in the content of quercetins in *A. unedo* leaves in response to UV-A was also reported in a previous study (Nenadis *et al.*, 2015) and suggests a lower oxidative stress, which might be explained by the enhancement of photoprotective mechanisms, such as the thermal dissipation of excess light energy in the antenna of photosystem II (Bernal *et al.*, 2015). This would also be in accordance with the positive effect of UV-A radiation on plant growth detected in previous studies (Bernal *et al.*, 2013, 2015). The increase in quercetins in response to enhanced UV-B appears to counteract the effect of enhanced UV-A resulting in similar leaf concentrations of quercetins in UVAB and control plants. This UV-B effect suggests the activation of antioxidant systems in this species in response to high UV-B doses. Taking into account that the contribution of quercetins to UV-B screening is similar to the contribution of other flavonoids, such as kaempferols (Di Ferdinando *et al.*, 2014), which were not affected by the UV treatment, the UV-B response of quercetins must be associated to their superior capacity to scavenge ROS in *A. unedo* (Agati *et al.*, 2012), as in other species such as *Betula pendula* (Tegelberg *et al.*,

2001), *Salix myrsinifolia* (Nybakken & Julkunen-Tiitto, 2013) and *Populus tremula* (Randriamanana *et al.*, 2015). Similar results were reported by Nenadis *et al.* (2015) in *A. unedo* plants exposed to UV-B, which showed an increased leaf content of quercetin 3-rhamnoside, the most abundant quercetin derivative identified in this species, while kaempferols remained practically unaffected. Thus, quercetins act as electron or hydrogen donating molecules, diminishing the oxidative damage induced by excessive UV levels (Hernández *et al.*, 2009).

Under enhanced UV-A exposure, the leaf concentration of quercetin 3-rhamnoside and, as a consequence, of total quercetins and flavonols were substantially higher in resprouts than in seedlings (Fig. 3.2). Under ambient UV, resprouts also showed a greater amount of flavonols than seedlings (Fig. 3.2). Apart from a possible pruning effect by itself, these differences might be explained by environmental differences between the two sampling dates, since, in October 2013 (after pruning), UV-A doses and mean temperature were greater, while precipitation was substantially lower, compared to October 2012 (Fig. 2.1). Therefore, a higher content of flavonols, especially quercetins, in UVA and control resprouts in comparison to seedlings might be the consequence of reduced photosynthetic rates presumably induced by drier conditions, as we found in a parallel study (see next section). Low photosynthetic capacity can be associated to a greater ROS accumulation (Lidon *et al.*, 2012; Kataria *et al.*, 2014), leading to an increased production of flavonols aimed to strengthen the antioxidant machinery.

UV radiation also modulated the changes in quercetin 3-arabinoside in response to the watering regime, since, only under enhanced UV-A+UV-B exposure, the concentration of this compound was almost twice in low- than in well-watered plants (Fig. 3.1). This result seems to indicate a synergistic effect between high UV (mainly UV-B) and low water availability on the leaf quercetin 3-arabinoside levels. Taking into account that, as mentioned above, quercetins are antioxidant flavonoids with high capacity to act as ROS scavengers (Agati *et al.*, 2012), an accumulation of these compounds would be expected under environmental conditions that induce oxidative stress, such as high UV-B levels and/or water

constraints, being even higher when both factors co-occur (Hofmann *et al.*, 2003; Caldwell *et al.*, 2007).

Irrespective of the UV conditions, reduced water supply led to an increase in the leaf concentrations of quercetin-glycoside and gallotannin 2, an hydrolyzable tannin (Table 3.1). Numerous studies have shown that secondary metabolites in plants, such as phenolic compounds, accumulate in response to drought (e.g. Selmar & Kleinwächter, 2013). Since water-stress is also associated to a higher risk of ROS generation (Reddy *et al.*, 2004; Krieger-Liszkay, 2005), the increase in quercetins, as well as of hydrolyzable tannins, would be expected due to their function as ROS-scavengers, contributing to avoid oxidative damage (Close & McArthur, 2002; Sumbele *et al.*, 2012).

Leaf concentrations of other five hydrolyzable tannins were also modified by the watering treatment, usually increasing in response to low irrigation, although the effect varied between seedlings and resprouts, being the resprouts much more sensitive to water availability (Table 3.1, Fig. 3.3). The fact that the increases in hydrolyzable tannins in response to drier conditions have been mostly detected in resprouts could be at least partially related to their lower foliar thickness (see next section), probably associated to specific features of foliar ontogenic development since young leaves of this species are often soft and rich in phenols as a defense mechanism to face herbivory (Kouki & Manetas, 2002). In addition, thinner leaves can favour the loss of water through transpiration (Bussotti, 2008; Sardans & Peñuelas, 2013), being more vulnerable to water stress. This could have stimulated the reinforcement of the antioxidant machinery via increased accumulation of hydrolyzable tannins (Close & McArthur, 2002; Barbehenn & Constabel, 2011). Finally, high levels of hydrolyzable tannins in resprouts may be partly due to the fact that often hydrolyzable tannins are more abundant in juvenile organs, such as leaves, being later replaced by other phenolics (e.g. Keinänen *et al.*, 1998).

Arbutin, the most abundant phenolic compound identified in *A. unedo* leaves, showed a diminished concentration in low-watered seedlings compared to well-watered ones (Fig. 3.3a). This was contrary to what it was expected, since arbutin can contribute to the

protection of membrane components due to its potential antioxidant capacity (Oliver *et al.*, 2001; Pavlović *et al.*, 2009). In accordance with the higher levels of phenolic compounds detected in the resprouts, a much higher amount of arbutin after pruning could be associated to its potential toxicity to organisms (Jurica *et al.*, 2015), since it might contribute to the protection of the thinner leaves of the resprouts (Kouki & Manetas, 2002). Moreover, drier conditions in October 2013 (Fig. 2.1) might have further emphasized the increase in arbutin, mainly due to its antioxidant capacity.

***Effects on leaf phenols of* Q. suber**

In *Q. suber*, the concentration of total phenols was not significantly modified by the experimental UV enhancement, according to the results found in other Mediterranean species (Bernal *et al.*, 2013, 2015; Nenadis *et al.*, 2015; Verdaguer *et al.*, 2017). However, increased UV radiation changed the phenolic profile of *Q. suber* leaves. In particular, higher UV levels significantly altered the leaf concentration of one hydrolyzable tannin and five flavonols (one quercetin, three kaempferols and one unidentified flavonol; Table 3.2). All these flavonols exhibited the highest concentrations in leaves grown with supplemented UV-A+UV-B, and, since three of them were kaempferols, the concentration of total kaempferols was also substantially increased in response to enhanced UV-B (Table 3.2). Monohydroxy B-ring-substituted flavonoids, such as kaempferols, are mainly distributed in the epidermal cells and, despite their lower concentrations compared to quercetins, they are highly effective in UV attenuation, in addition to protect leaf tissues from pathogens (Agati & Tattini, 2010). Hence, the higher UV-B-induced accumulation of kaempferols in *Q. suber* leaves, accompanied by no global differences in quercetins and hydrolyzable tannins, and neither in the antioxidant compounds and enzyme activities of the resprouts (Table 3.3), suggest that this species copes with enhanced UV-B levels by improving UV screening via higher concentration of kaempferols instead of stimulating ROS-scavenging mechanisms (Hideg *et al.*, 2013; Majer *et al.*, 2014).

Regarding the irrigation treatment, reduced water supply decreased the overall leaf

concentration of hydrolyzable tannins accompanied by a slight decrease in gallocatechin (Table 3.2). Similarly, although only after pruning, the kaempferol 3-glucoside also reduced its concentration under low water supply, while the level of the kaempferol glycoside increased (Fig. 3.4a,b). As mentioned above, antioxidant phenols such as hydrolyzable tannins are expected to increase in response to oxidative stress (Close & McArthur, 2002; Sumbele *et al.*, 2012) mediated by drought-induced stomatal closure (Reddy *et al.*, 2004). In our study, decreases in hydrolyzable tannins and in kaempferol 3-glucoside, in response to low water availability could be related to other drought avoiding strategies in *Q. suber* plants, such as adjustments in plant architecture addressed to minimize water losses and optimize belowground water uptake. This assumption would be supported by the lower leaf to root biomass ratio observed in low-watered *Q. suber* plants (see next section), which was accompanied by a slight increase (mainly in resprouts) in maximum photochemical efficiency of PSII (measured as Fv/Fm) and maintained values of stomatal conductance and photosynthesis.

Effects on leaf antioxidants of resprouts

In *A. unedo*, analysed leaf antioxidants exhibited a higher sensitivity to supplemented UV radiation than phenolic compounds, whereas the opposite was found in *Q. suber* leaves. Specifically, in *A. unedo* resprouts, the foliar concentration of reduced ascorbate, and as a consequence of total ascorbate, was greatly increased in plants grown with supplemented UV-A+UV-B in relation to those exposed to enhanced UV-A alone (Table 3.3), suggesting a UV-B-mediated increase in the antioxidative response of these leaves. Previous studies found that plants try to counteract UV-B oxidative stress through increases in the reduction state and the pool-size of ascorbate (Agarwal, 2007; Zlatev *et al.*, 2012; Hideg *et al.*, 2013), which acts as a powerful ROS scavenger minimizing photodamage in plant tissues, mainly via the ascorbate-glutathione cycle (Noctor & Foyer, 1998; Gill & Tuteja, 2010). However, in our study, the concentration of oxidized ascorbate (i.e. dehydroascorbate) was not higher in UVAB resprouts, probably because of the lower activity of ascorbate peroxidase (APX)

observed in these plants (Table 3.3). Hence, other protective mechanisms should be operating, which is consistent with the increased activity of catalase enzymes (CAT) detected in these plants in response to enhanced UV-B (Table 3.3). CAT enzymes have high turnover rates acting as a highly efficient pathway to quench ROS, particularly when stress conditions are severe or prolonged (Asada, 2006; Gill & Tuteja, 2010). When there is massive ROS generation in response to an excess of excitation energy in the chloroplast, decreases in the APX activity (Agati *et al.*, 2013) can induce CAT production, since CAT enzymes are considered insensitive to the redox status of the cells because they do not require a reducing substrate, and, thus, they are able to maintain their activity under stress conditions (Mittler, 2002). Because of this, stimulated CAT activity has been commonly reported in studies with plants exposed to enhanced UV-B radiation (A-H-Mackerness, 2000; Jansen *et al.*, 2012; Zlatev *et al.*, 2012). Overall, UV-B effects on the studied antioxidant compounds and enzymes of *A. unedo* resprouts are consistent with the scarce UV effects observed on the leaf amounts of phenols, since phenolic compounds, especially flavonoids, are thought to constitute a secondary component of the ROS-scavenging system, which is upregulated following depletion of primary antioxidants under severe stress conditions (Fini *et al.*, 2011; Agati *et al.*, 2013).

In accordance with the UV-B-induced increase in the leaf concentration of ascorbate, reduced glutathione (and consequently total glutathione) was also accumulated in *A. unedo* resprouts receiving supplemental UV-B radiation, although this effect was only significant under low watering (Table 3.3, Fig. 3.5). Therefore, this result points to an amplification of the UV-B effect by water shortage. Indeed, the combined action of both stresses might have led to an excess in ROS production, being necessary a larger pool of glutathione which, along with ascorbate, contribute to maintain the normal reduced state of plant cells (Meyer, 2008; Rouhier *et al.*, 2008).

The fact that leaf antioxidant responses observed in UVAB resprouts of *A. unedo* were usually significant only when compared with plants grown under supplemental UV-A alone (Table 3.3), suggests a tendency towards opposite UV-B and UV-A effects. Although

not significant, enhanced UV-A tended to decrease the leaf concentrations of ascorbate and glutathione, as well as CAT activity. Conversely, APX activity was significantly higher in UVA plants, suggesting higher rates of ROS detoxification by means of the ascorbate-glutathione cycle rather than via CAT enzymes in these plants, and again may indicate a lower degree of oxidative stress in the resprouts exposed to enhanced UV-A alone than in those grown with additional UV-B supplementation, according to previous reports (Bernal *et al.*, 2013; Nenadis *et al.*, 2015).

Regarding the irrigation treatment, reduced water supply significantly increased the leaf concentrations of reduced and total ascorbate, along with dehydroascorbate, in *A. unedo* resprouts, while it raised the leaf concentrations of reduced glutathione and, subsequently, total glutathione in *Q. suber* resprouts (Table 3.3). In contrast, antioxidant activities of APX and CAT declined in both species in response to drier conditions. In the two studied species, the accumulation of key antioxidant components, such as ascorbate or glutathione, in response to low water availability might indicate a reinforcement of the antioxidant machinery, improving drought resistance of these plants. Reduced APX and CAT activities contrast with previous studies reporting increased enzymatic detoxification under drought conditions (Reddy *et al.*, 2004; Sánchez-Díaz *et al.*, 2007; Selmar & Kleinwächter, 2013). Considering that particularly CAT activity is associated to ROS detoxification during stress conditions (Mittler, 2002; Gill & Tuteja, 2010), our results suggest no severe levels of oxidative stress probably associated to a high resistance of the two species to water deficit, but especially of *Q. suber*.

Concluding remarks

In both species, UV-induced responses were only observed in the amounts of some phenolic compounds, without any change in the total pool of phenols, which indicates a differential UV regulation of the identified compounds, probably because of dissimilarities in their contributions to leaf photoprotection and/or antioxidant activity. While *A. unedo* appears to invest more resources in leaf phenols than *Q. suber*, *Q. suber* leaves accumulate larger

amounts of antioxidants, suggesting inter-specific variations in their strategies to counteract environmental stress. Indeed, in *A. unedo*, enhanced UV-B increased the amount of quercetins in seedlings, as well as of key antioxidants (ascorbate and glutathione), stimulating also CAT activity, in resprouts. Conversely, in *Q. suber*, UV-B supplementation led to a greater accumulation of various flavonoids, mostly kaempferols, suggesting an improved capacity for UV screening to avoid UV penetration into the tissues.

While reduced water supply did not modulate UV-B effects on *Q. suber* leaves, the interactive effect of both factors in *A. unedo* resulted in higher concentration of the antioxidant glutathione (total glutathione and GSH), probably reflecting an amplified antioxidant response. Apart from this, low irrigation in *A. unedo* increased the levels of some hydrolyzable tannins (both ellagitannins and gallotannins) mostly in resprouts, while, in *Q. suber*, it decreased the leaf content of hydrolyzable tannins and of kaempferol 3-glucoside in resprouts. These findings point to lesser degree of oxidative stress in *Q. suber* plants probably associated to a greater tolerance to water constraints compared to *A. unedo*.

Taking into account the broad range of functions of the studied compounds, the notable inter-specific differences in the biochemical adjustments in response to higher UV levels and decreased water availability might imply alterations in the competitive ability of these species under the expected near-future climatic changes.

2. Growth and physiological responses of two Mediterranean resprouter species exposed to enhanced UV radiation and reduced water availability before and after pruning

Differences between seedlings and resprouts

A. unedo and *Q. suber* are two co-occurring Mediterranean evergreen species that resprout after the loss of aerial biomass. In this sense, despite no differences were found between the two species in the proportion of plants that resprouted after the removal of aboveground biomass, plant growth response after pruning was distinct. Indeed, differences in stem height, diameter, leaf biomass and stem RGR for seedlings compared to resprouts of both species indicate that *Q. suber* recovered faster than *A. unedo* (Tables 3.5 and 3.6).

In relation to the physiological traits studied, resprouts of both species showed higher leaf E and g_s than seedlings, with a subsequent reduction in WUE in resprouts, probably in response to the milder mean temperature (2 ºC higher) in October 2013 compared to October 2012, and the greater water availability experienced by the resprouts due to their lower leaf:root ratio (Tables 3.5 and 3.6). The pronounced increase in E and g_s in *Q. suber* resprouts could explain their lower leaf water content and RWC. It is also remarkable that, while in *Q. suber* A did not differ between seedlings and resprouts, it was lower in *A. unedo* after pruning, in agreement with the lower Fv/Fm values found in *A. unedo* resprouts (Table 3.5). Conversely, Fv/Fm values of resprouting plants of *Q. suber* were higher than those of seedlings (Table 3.6). A reduction in photochemical efficiency of *A. unedo* linked to an attenuated level of photosynthesis could be related to the smaller and thinner leaves detected in the resprouts of this species (Gratani & Ghia, 2002). Conversely, the increase in Fv/Fm values, along with the unchanged A, for resprouting plants of *Q. suber* can be attributed to the thicker leaves with larger LMA found in the resprouts compared to seedlings. Similar A values between seedlings and resprouts of *Q. suber*, together with lower stem (SRGR) and root (RRGR) growth in the resprouts, could explain the higher concentrations of starch and sugar found in the roots of the resprouts of this species after pruning (Table 3.6),

i.e., a decreased demand of assimilates for growth in the resprouting plants might favour the accumulation of sugar and starch reserves belowground (Sulpice *et al.*, 2009; Hasibeder *et al.*, 2015). Conversely, the lower starch to sugar ratio found in *A. unedo* resprouts compared to seedlings suggests greater mobilisation of starch stores to keep the sugar supply necessary to maintain plant growth and functioning (Smith & Stitt, 2007; Heldt & Piechulla, 2011). This would be in accordance with the lower A found in the resprouts of this species (Table 3.5) and with the findings reported by Canadell & Lopez-Soria (1998) who detected a depletion of root starch in *A. unedo* plants after clipping. The greater concentration of total phenols in the roots of *A. unedo* resprouts compared with seedlings could reflect a trade-off between protection and growth demands (Ballaré, 2014), since plant secondary metabolites, such as TP, are synthesized from primary metabolites, including sugars and starch, and they are often associated with protective mechanisms against stress conditions (Lavinsky *et al.*, 2015).

Overall, our findings highlight interspecific morphological, physiological and biochemical differences between *A. unedo* and *Q. suber* that could explain their distinct responses to UV supplementation, watering regime and/or pruning. Specifically, lower leaf thickness and LMA together with decreases in A and Fv/Fm in *A. unedo* resprouts in comparison to seedlings could indicate higher vulnerability to variations in water supply or UV fluxes which is consistent with the numerous treatment effects found in *A. unedo* resprouts. In contrast, changes occurring in *Q. suber* after pruning (i.e. thicker leaves with higher LMA, maintained A and increased Fv/Fm values together with enhanced accumulation of root reserves) would confer greater protection to the resprouts against higher UV doses and/or greater resistance to lower water availability, which would explain why treatment effects were detected mainly in the seedlings of this species.

UV effects and interactions with watering and/or pruning

Different responses to UV radiation were detected in *A. unedo* and *Q. suber*. Indeed, whereas biometric and leaf morphological parameters of *A. unedo* were not affected by the

UV treatment, they were significantly modified in *Q. suber*. Moreover, in both species, some physiological parameters were UV sensitive, but physiological responses to UV were always modulated by water availability or disturbance.

For *A. unedo*, our results suggest that plants receiving low water supply were less sensitive to changes in UV levels, at least in relation to water status, since UV effects on leaf RWC were found only under optimal irrigation. Interestingly, under enhanced UV-A+UV-B, low-watered plants had higher leaf RWC than well-watered ones (Fig. 3.7a). In fact, water limitation has been frequently shown to decrease UV-B sensitivity (Hofmann *et al.*, 2003). In turn, plants exposed to enhanced UV-B radiation have been found to be more tolerant to drought conditions showing attenuated drought effects on leaf RWC and photosynthetic activity (Poulson *et al.*, 2006; Feng *et al.*, 2007). In our study, plants growing under low-water conditions and receiving UVAB also tended to exhibit higher rates of photosynthesis compared to well-watered ones in accordance with their higher leaf RWC (Fig. 3.7b). UV-B-mediated improvements in leaf water economy and photosynthetic performance are often associated to thicker leaves (Manetas *et al.*, 1997); however, in *A. unedo,* UV radiation did not affect leaf morphological traits related to UV protection (i.e. foliar thickness and LMA). Alternatively, the higher tolerance of UVAB plants of *A. unedo* to drought might be associated to UV-B-induced increases in leaf antioxidant compounds which has been corroborated in a parallel study (see previous section). A greater allocation of resources aimed to strengthening cell antioxidant machinery could partly explain why the improvements detected in water status and photosynthesis in UVAB plants grown under a low water supply were not accompanied by increases in either plant biomass or growth. Indeed, in agreement with this hypothesis, the accumulation of phenolic compounds in leaves of *A. unedo* was twice that of *Q. suber* (see previous section) indicating greater carbon investment in these compounds.

For *Q. suber*, our results suggest that LMA increased in response to UV-A enhancement since no differences were found between plants subjected to enhanced UV-A and those exposed to enhanced UV-A+UV-B radiation (Table 3.6). Changes in LMA could be

explained by a reduction in leaf area or an increase in leaf thickness and/or leaf density (Poorter *et al.*, 2009). Accordingly, leaf area decreased and leaf thickness tended to increase in UV supplemented plants. These morphological responses in *Q. suber* could be the result of various UV-induced changes in leaf tissues, such as increased thickness of the palisade parenchyma and/or adaxial epidermis (Verdaguer *et al.*, 2017). In our study, enhanced UV-A radiation increased photosynthetic rates in the seedlings of *Q. suber* (Fig. 3.8b). Fv/Fm values also increased slightly, although differences were only evident between UVAB and control plants indicating a stronger effect of enhanced UV-B radiation (Fig. 3.8d). Interestingly, the leaf to root biomass ratio of *Q. suber* seedlings decreased in response to supplemented UV-A alone (Fig. 3.8c). UV-A-mediated increases in LMA could have improved protection of the photosynthetic apparatus and/or increased the amount of photosynthetic tissue resulting in greater carbon assimilation and greater allocation of carbon to roots. Like *Q. suber*, other *Quercus* resprouter species are characterized by a high investment in root biomass in comparison to leaves (Villar *et al.*, 2014) which confer to this species a great capacity to resprout and recover after a disturbance (Pausas, 1997).

The physiological and biometrical results presented here for *Q. suber* are consistent with the results of the correlation analyses performed, since, while the root biomass of UVAB and control seedlings of this species did not correlate with any of the other plant biometric parameters considered, root biomass of seedlings grown under enhanced UV-A alone correlated with several of these variables (stem diameter and biomass, and root RGR) (Table 3.7). Hence, seedlings of *Q. suber* seem to be very sensitive to supplemented UV-A radiation, since we detected changes in leaf morphology and physiology and in plant biomass allocation. Accordingly, when correlation analyses for root biomass and the other biometric parameters were done within each one of the two watering regimes, similar results were obtained regardless of irrigation conditions. This suggests that the soil water content does not strongly influence biomass allocation pattern. After pruning, correlations between root biomass and the other plant traits were not significant. Nevertheless, because of the regenerative capacity of these plants, it is likely that, after some more time, allometric

relationships would become similar to those found before the disturbance (Eshel *et al.*, 2001; Poorter *et al.*, 2012).

Watering effects

The effect of water availability was also species-specific, with *A. unedo* being more sensitive to reduced water supply than *Q. suber* particularly in the case of the resprouts. As expected, low-watered plants of *A. unedo* had a lower leaf water content, accompanied by lower thickness than well-watered ones (Fig. 3.9d,a), while LMA tended to increase in response to low irrigation, although only in resprouts. An increase in LMA has been shown in many Mediterranean species under conditions of low water availability (Sardans & Peñuelas, 2013; Bussotti *et al.*, 2014) leading to more sclerophyllous leaves and, thus, limiting the loss of water through transpiration (Bussotti, 2008; Sardans & Peñuelas, 2013). This agrees with our values of leaf transpiration and RWC. Interestingly, resprouts of *A. unedo* receiving limited water showed greater Fv/Fm and NPQ values than well-watered ones (Fig. 3.9e,g), indicating no photoinhibitory damage and greater thermal dissipation of excess energy in response to water shortage. In contrast, *A. unedo* resprouts under low water supply also showed a higher pool of total phenols in roots (Fig. 3.9h), as found in a previous study with *Vitis vinifera* (Weidner *et al.*, 2009). Increases in the amount of root phenols might be associated with an accumulation of wall-linked phenolic substances that can result in a higher lignification of cell walls in roots as a protection mechanism to face water constraints (Bandurska *et al.*, 2012). Hence, in resprouting plants of *A. unedo*, an increase in root phenolic compounds as a result of water shortage might have ameliorated drought effects on these plants. Conversely, for seedlings of *A. unedo*, NPQ and root phenols decreased under low water supply, while no differences in leaf water content were evident in response to the irrigation treatment. Despite the reduction in water availability, the less stressful environmental conditions prior to pruning may have allowed seedlings of *A. unedo* to cope with the low water supply. Instead, resprouts are often found to be less conservative in the use of water than mature plants, mainly due to higher water availability and juvenile features

(e.g. thin epidermis and leaves with low LMA) (Donovan & Ehleringer, 1992; Castell & Terradas, 1994).

Regardless of the sampling time, starch concentration and, in consequence, the starch to sugar concentration ratio increased in roots of *A. unedo* under drier conditions (Table 3.5). Previous studies have found that low water supply enhances the allocation of several primary metabolites, such as starch, to roots indicating a drought-induced shift in the usage of recent photosynthates from metabolic activity to storage pools (Hasibeder *et al.*, 2015; Lavinsky *et al.*, 2015).

In *Q. suber*, reduced water supply decreased the biomass allocated to leaves in relation to total biomass and to root biomass (Table 3.6). This could be a protection mechanisms against hydric stress in order to reduce water loss and, at the same time, increase water absorption by roots (Reddy *et al.*, 2004; Verdaguer *et al.*, 2011; Pivovaroff *et al.*, 2016). For *Q. suber* seedlings, the stem to total biomass ratio increased under low water supply leading to a reduction in the leaf:stem ratio (Fig. 3.9b,c), which would suggest a higher amount of vascular tissue to transport water from roots to a relatively lower amount of leaves. In contrast, the biochemical parameters determined for roots of *Q. suber* did not show significant changes in response to the watering treatment, probably associated with the lack of irrigation effects on the photosynthetic capacity of these plants. Previous studies with *Quercus* also found no effects on carbon reserves in roots in response to low water supply (Sanz-Pérez *et al.*, 2009; Rosas *et al.*, 2013), supporting the notion that higher plants accumulate large pools of non-structural carbohydrates which are not easily depleted by water stress (Millard *et al.*, 2007; Gruber *et al.*, 2012).

Concluding remarks

Plant responses to the experimental treatments were mostly species-specific. Compared to seedlings, *A. unedo* resprouts were more sensitive to the treatments. Reprouts of this species had thinner leaves with lower LMA together with reduced A and Fv/Fm values compared to seedlings, which may have increased their vulnerability to variations in water

supply or UV levels. Conversely, resprouting plants of *Q. suber* exhibited thicker leaves with higher LMA, similar A values, higher Fv/Fm values, and a greater pool of root reserves compared to seedlings which would confer protection to these plants against UV radiation and water stress and, as a consequence, treatment effects were mostly found in seedlings.

Effects of UV radiation on *A. unedo* were limited, with only leaf RWC and A being modified. Conversely, *Q. suber* plants were more sensitive to UV radiation and changes in leaf morphology, physiology and biomass allocation were found in this species in response to the UV treatment. For *A. unedo*, supplemented UV-B resulted in a reduction of leaf water status under well-watered conditions, which would partially explain why plants exposed to UVAB showed higher leaf RWC and photosynthetic rates under low watering. In contrast to *A. unedo*, *Q. suber* seems to respond to higher doses of UV-A radiation by increasing LMA and UV-screening in order to prevent photodamage to the photosynthetic apparatus (Verdaguer *et al.*, 2012). In *Q. suber* seedlings, this leaf morphological adjustment was accompanied by higher photosynthetic rates and lower leaf to root biomass ratio in response to enhanced UV-A, suggesting that a greater proportion of assimilated carbon was allocated to the root system.

Contrary to what happened with the UV treatment, *A. unedo* was more sensitive to the watering regime than *Q. suber*, which only exhibited changes in biomass allocation. Indeed, reduced water supply affected root biochemistry along with leaf morphology and physiology of *A. unedo* plants. In particular, roots of *A. unedo* had greater amounts of starch in response to drier conditions, pointing to a shift in the allocation of photosynthates from metabolic activity to root storage. Resprouts of *A. unedo* under low irrigation showed thinner leaves with higher Fv/Fm and NPQ values coupled with increased concentration of root phenols compared to well-watered plants suggesting that thermal dissipation of excess excitation energy and a reinforcement of root cell walls acted as effective protection mechanisms against water deficit effects. In contrast, *Q. suber* responded to reduced water supply mainly by decreasing the amount of biomass allocated to leaves in relation to that allocated to roots, suggesting a protective strategy to avoid loss of water by leaf transpiration

and to increase water absorption by roots. In this sense, the higher stem:total biomass ratio, along with the lower leaf:stem ratio, found in low-watered seedlings could indicate a relative increase in vascular tissue to improve the transport of water from roots to leaves.

Altogether, our results highlight remarkable differences in the responses to the experimental treatments between *A. unedo* and *Q. suber*. Notwithstanding the above-mentioned effects, UV enhancement or water shortage did not alter plant biomass production nor resprouting capacity of any of the two species, suggesting that these species have efficient protective mechanisms to cope with the changes in UV levels and precipitation amounts expected for the near future in the Mediterranean basin.

3. Effects of UV radiation and rainfall reduction on leaf and soil parameters related to C and N cycles of a Mediterranean shrubland before and after a controlled fire

Differences in soil parameters measured in late autumn and late spring

According to the results of the PCA, soil characteristics from late autumn and late spring were only segregated by the season (there were no effects of the treatments), since soil moisture and temperature were higher and lower, respectively, in autumn than in spring (Fig. 3.10a). Other studies in Mediterranean shrublands have also reported seasonal patterns for these soil variables (Gispert *et al.*, 2013). Considering only autumn data, soil respiration exhibited higher values in December 2011 (before the fire) than in December 2013 (i.e. nine months after the fire) probably due to slightly increased soil moisture in autumn 2011 (Fig. 3.10b), since soil respiration reaches the maximum at intermediate water contents when the balance between moisture and aeration is optimal (Robertson & Groffman, 2007). In relation to spring values, soils in June 2012 (before the fire) tended to have, in general, higher mean values of total N, organic C and β-glucosidase activity than in June 2014 (i.e. one year and three months after the fire) (Fig. 3.10a), although these effects varied at the two studied depths (Table 3.8). Higher values of these parameters before the fire (respiration and moisture in December 2011, and organic C, total N and β-glucosidase activity in June 2012) could be associated to greater litter cover that might favour a higher soil water availability coupled with enhanced nutrient input and, thus, stimulated microbial activity (Raich & Tufekcioglu, 2000; Talmon *et al.*, 2011). In addition, higher litter cover would be expected to diminish soil UV exposure, avoiding potential harmful effects of UV on soil microorganisms and, thus, favouring their activity.

Effects of the experimental fire

There were significant differences before and after the experimental fire in most of the parameters studied, regardless of the UV and precipitation treatments. After the fire,

respiration, together with β-glucosidase activity, and total N concentration at depth A (0-5 cm) were lower compared to pre-fire values, while temperature, electrical conductivity and C:N ratio values were higher (Table 3.9). Post-fire reductions in β-glucosidase activity have been documented (López-Poma & Bautista, 2014), being mostly attributed to enzyme denaturation and temporary soil sterilization (Certini, 2005; Knicker, 2007). Although it has been shown that fire can increase N mineralization in the short-term (Goberna *et al.*, 2012; Caon *et al.*, 2014) leading to higher soil N concentrations and lower soil C:N ratio (De la Rosa *et al.*, 2008), in our study, the observed post-fire reduction in soil N concentration at depth A might be linked to enhanced N losses through volatilization, runoff or leaching (Certini, 2005; Hart *et al.*, 2005). As a consequence, a higher soil C:N ratio after the fire would be in agreement with the lower soil respiration and β-glucosidase activity found at depth A, suggesting lower decomposition rates (Geisseler & Horwath, 2009; Bengtsson *et al.*, 2012) and a post-fire attenuation of the biological activity at the topsoil.

The observed increase in soil temperature after the fire might be related to the decrease in vegetation and litter cover (Garcia *et al.*, 2002; Hart *et al.*, 2005). In semi-arid Mediterranean areas, shrub cover can prolong moisture availability, decrease radiation reaching the soil, and, thus, moderate temperature (Sherman *et al.*, 2012), providing a larger source of nutrients through decaying debris and favouring microbial activity, and, thus, soil respiration (Raich & Tufekcioglu, 2000; Talmon *et al.*, 2011). In our study, despite all the aerial biomass was burned, vegetation cover recovered very fast, showing in June 2014 (one year and three months after the fire) similar values as those found in the pre-fire period (June 2012) (Table 3.10), in accordance with the fast vegetal succession found in other Mediterranean shrublands dominated by resprouting species (Acácio *et al.*, 2009; Arnan *et al.*, 2013). However, the recovery of soil litter cover was slower, still being a 17% lower in June 2014 compared to pre-fire values.

In deeper soil (5-10 cm), β-glucosidase activity was higher after the fire, together with organic C concentration and C:N ratio (Table 3.9). Given that the soil surface is more exposed to erosion processes and nutrient leaching after a fire (Certini, 2005), deeper soil

layers may become enriched in organic C and nutrients (López-Poma & Bautista, 2014). Higher organic C concentration is consistent with an increase in β-glucosidase activity, pointing to a higher biological activity in the subsurface layer after the fire.

After the fire, we also found higher $\delta^{15}N$ values in *A. unedo* and *P. angustifolia* leaves (Table 3.9), as well as in *A. unedo* litter (Table 3.10). Other studies have reported higher foliar $\delta^{15}N$ values in post-fire vegetation because of a fire-induced ^{15}N enrichment in soil organic matter, which is mineralized and taken up by plants (Szpak, 2014). Higher $\delta^{15}N$ values have been associated to increased rates of nitrification and, consequently, higher N losses mostly in the form of nitrates (Pardo *et al.*, 2007; Högberg *et al.*, 2014) that are preferentially leached because of their negative charge. This fact would be expected to reduce mineral N availability in the soil. In our study, the lower values of soil total N found at depth A after the fire would be in agreement with a loss of soil nitrogen as a consequence of the fire. Despite this, post-fire leaf litter of *A. unedo* and *P. angustifolia* leaves showed higher values of N concentration, which might be explained by a concentration phenomenon due to a lower shoot:root ratio that could enhance N uptake and accumulation. Increased water availability due to an extensive root system associated to a diminished aerial biomass might explain the decrease in foliar C concentration of both species after the fire, presumably reflecting a low accumulation of structural carbon-related compounds (i.e. sclerophylly) as a consequence of improved water status (Savé *et al.*, 1993; El Omari *et al.*, 2003; Bussotti, 2008). In *P. angustifolia* leaves, these results were accompanied by a lower post-fire C:N ratio.

Effects of UV radiation

Throughout the whole study period, soils of plots exposed to UV radiation (UVA and UVAB plots) showed higher moisture content than those not receiving UV radiation (Table 3.11). This effect can be attributed to UV-A radiation, since there were no significant differences between UVA and UVAB plots. A possible explanation for this UV-A effect might be an UV-A-induced increase in vegetation and/or litter cover, which would attenuate the amount of solar

irradiance reaching the soil surface reducing soil temperature and water loss through evaporation (Rutigliano *et al.*, 2009; Sherman *et al.*, 2012). However, litter and plant cover were not affected by the UV treatment. Alternatively, an UV-A-induced reduction in plant transpiration might also cause an increase in soil moisture. This would be in agreement with the decrease in leaf transpiration observed in *P. angustifolia* plants exposed to UV-A and reduced rainfall (Verdaguer *et al.*, in prep.).

Before the fire, reduced-rainfall plots exposed to UV-A or UV-A+UV-B radiation also showed higher soil respiration rates (Fig. 3.11), suggesting a positive UV-A effect on soil biological activity and, thus, on decomposition rates under drier conditions. In accordance with this, pre-fire β-glucosidase activity at depth B was significantly higher in UVA plots, especially under reduced rainfall (Table 3.11, Fig. 3.12a). This UV-A-induced increase in respiration rates and β-glucosidase activity might be a consequence of the higher moisture found in these soils, since, in arid or semiarid soils of Mediterranean shrublands, soil water content has been positively correlated with microorganism activity (Sardans *et al.*, 2008a).

Our findings also reveal an opposite effect of UV-A and UV-B radiation on soil respiration and β-glucosidase activity (at depth B) along the pre-fire period (Table 3.11), suggesting a negative effect of UV-B radiation on soil biological activity. This negative effect might be mediated by the observed pH reduction in response to UV-B exposure (Table 3.11), since acidity has been negatively linked to enzyme and microorganism activity, mainly through its effects on the availability of mineral nutrients (Eivazi & Tabatabai, 1990; Sardans *et al.*, 2008a). The fact that these UV-B effects were observed only before the fire, i.e. when plant cover was higher, also suggests that they could be influenced by plant responses to this type of radiation, such as UV-B-induced changes in root exudates. It has been shown that plants receiving enhanced UV-B radiation increase root accumulation and secretion of phenolics, affecting negatively β-glucosidase activity in the rhizosphere (Choudhary *et al.*, 2013). These compounds are able to inhibit microorganism activity and, subsequently, organic N mineralization and nitrification in the soil (Erickson *et al.*, 2000; Castells *et al.*, 2004; Castaldi *et al.*, 2009). Accordingly, in our study, values of total N concentration tended

to be lower in soils exposed to UV-B radiation, although differences were not statistically significant (Table 3.11).

Unlike what has been observed for the pre-fire period, we have found only punctual UV effects on the studied soil parameters after the fire. Apart from the effect on soil moisture commented above, plots exposed to UV-B radiation showed lower values of soil organic C concentration at depth B in June 2014, but only under natural rainfall (Fig. 3.12b). Although not significant, the same tendency was observed for β-glucosidase activity (Fig. 3.12a). Similarly to the pre-fire results, these effects could be mediated by plant responses to UV-B, such as UV-B-induced increase in root exudation of phenolic compounds (Choudhary *et al.*, 2013), which would reduce soil microorganism activity (Castaldi *et al.*, 2009).

The studied litter and plant parameters responded differently to the UV treatment depending on the species. Despite the UV treatment did not affect significantly the leaf $\delta^{13}C$ values of any of the two studied species, leaf litter of *P. angustifolia* from UVAB plots showed lower $\delta^{13}C$ values compared to that from UVA plots in September 2012 (Table 3.12). This would suggest that UV-B exposure reduced the integrated water use efficiency of these leaves probably via changes in leaf stomatal conductance and/or carboxylase activity (Feng *et al.*, 2003). According to what has been observed in a parallel study, the reduction in leaf water use efficiency in *P. angustifolia* plants grown in UVAB plots would be a consequence of enhanced transpiration rates (although not significant) rather than decreased photosynthesis (Verdaguer *et al.*, in prep.), which would be further supported by the higher pre-fire C concentration observed in *P. angustifolia* leaves exposed to UV-B radiation (Table 3.11).

Throughout the study period, under reduced rainfall, $\delta^{15}N$ values of *A. unedo* litter and leaves were highest in UVAB plots, although differences were only significant in September 2012 for the leaf litter (Fig. 3.13a) and in the post-fire period for the leaves (Fig. 3.13b). Increases in leaf $\delta^{15}N$ values have been correlated with greater biomass allocation to roots versus shoots, allowing plants to exploit more efficiently soil systems and thus increasing water and N uptake (Llorens *et al.*, 2003). Moreover, as discussed earlier, higher $\delta^{15}N$ values

in the leaves could indicate increased nitrification in the soil coupled with higher N losses in the form of nitrates (Pardo *et al.*, 2007; Högberg *et al.*, 2014).

Effects of the precipitation regime

The reduction in precipitation, apart from the expected decrease in soil moisture, also decreased the pre-fire C:N ratio at the topsoil, which would be explained by the tendency of organic C to decrease and of total N to increase in these soils (Table 3.11). In Mediterranean ecosystems, a wide variety of precipitation effects on soil C:N ratio has been reported, although, often, this ratio increase in soils under drought due to the input of plant material with a higher proportion of structural carbon-related compounds in leaves (i.e. more sclerophyllous leaves) (Bussotti, 2008; Sardans *et al.*, 2012; Sardans & Peñuelas, 2013). On the contrary, the lower soil C:N ratio we found under drier conditions might be, at least partially, related to the higher N concentration and, thus, the lower C:N ratio observed in *P. angustifolia* leaves. A higher N content in these leaves could indicate a greater accumulation of leaf soluble protein which would represent a nitrogen stock to be used during the recovery from drought, as it has been reported in wet-temperate ecosystems under moderate drought (Lu *et al.*, 2009).

After the fire, the reduction in water availability led to a decrease in the foliar $\delta^{15}N$ values of *P. angustifolia*, suggesting lower N losses at the soil level (Högberg *et al.*, 2014) despite we did not detect significant differences in soil total N in response to the precipitation treatment (Table 3.11). These results contrast with other studies performed with Mediterranean plant species that have found lower leaf N concentration (Sardans *et al.*, 2008b), and higher (Ogaya & Peñuelas, 2008) or similar (Llorens *et al.*, 2003) $\delta^{15}N$ values under low precipitation.

In *A. unedo*, drier conditions decreased the leaf C concentration at the end of winter (although differences were only significant in March 2012), but not at the end of summer (September 2012 and 2013) (Fig. 3.14). This was probably due to the scarce precipitation recorded during summer months, which would have minimized the differences between the

two irrigation levels. Since, in a parallel study, we found higher photosynthetic rates in this species under drier conditions (Verdaguer *et al.*, in prep.), the observed reduction in leaf C concentration could reflect an enhanced C investment in growth, which is supported by the results of another study (Llorens *et al*., in prep.). A lower C concentration in *A. unedo* leaves in March 2012 in response to rainfall reduction would also be in accordance with the lower soil C:N ratio found in these plots before the fire (Table 3.11). Given that lower C:N ratios in plant leaves have been associated with higher rates of litter decomposition (Bengtsson *et al.*, 2012; Sardans *et al.*, 2013), our findings point to a higher C and N turn-over in response to reduced rainfall.

Concluding remarks

Exposure to UV-A radiation appears to favour soil biological activity, since we found higher soil respiration and β-glucosidase activity (mainly before the fire). This might be a consequence of the observed increase in soil moisture (probably associated to a lower transpiration in *P. angustifolia* plants), suggesting a higher rate of C turn-over in response to UV-A exposure. Also before the fire, the additional presence of UV-B radiation (i.e. UV-A+UV-B exposure) decreased the rate of soil respiration along with soil pH and β-glucosidase activity in relation to UVA plots, increasing C concentration in *P. angustifolia* leaves. This would suggest an attenuated soil microorganism activity coupled with lower rates of decomposition and C turn-over, which would lead to a slowdown of the C cycle in response to UV-B radiation. Under reduced rainfall, the presence of UV-B radiation also resulted in greater $\delta^{15}N$ values in leaves and litter of *A. unedo*, probably indicating higher N losses in the soil which might affect negatively N cycling in the ecosystem.

The reduction in soil moisture due to reduced rainfall was coupled with a decrease in the C:N ratio at the topsoil before the fire, likely related to the higher N concentration and the lower C concentration found in *P. angustifolia* and *A. unedo* leaves, respectively. Therefore, our results suggest increased decomposition rate and, consequently, a faster C and N cycling in response to drier conditions. In addition, the lower foliar $\delta^{15}N$ values recorded in *P.*

angustifolia plants grown under reduced precipitation points to lower N losses in the soil linked to an ecosystem with a tighter N cycle.

Overall, the experimental reduction in precipitation exerted a greater effect on the studied parameters related to N cycle, while the biogeochemical cycle of C was more sensitive to UV radiation, alone or in combination with the water supply. Many of the UV effects found were modulated by the precipitation regime; in particular, UV-induced changes in soil respiration and β-glucosidase activity along with UV responses in *A. unedo* plants were emphasized by rainfall reduction. Unlike *A. unedo*, interactive effects of UV radiation and precipitation were not found for *P. angustifolia* plants. Species-specific responses to changes in UV fluxes and precipitation may induce modifications in the competitive ability of these species, ultimately altering their distribution in the next decades. Taking into account the fundamental role of the vegetation on biogeochemical cycles, these changes might affect the evolution and dynamics of Mediterranean shrublands in the future.

Apart from this, the fact that most UV and water effects were observed only before the fire would indicate a homogenizing influence of this perturbation. Thus, given the predicted increase in fire occurrence over the coming years, this factor might play a more important role modulating C and N cycles of Mediterranean shrublands than the projected changes in UV fluxes and precipitation amount.

4. GENERAL DISCUSSION

Species-specific responses to UV enhancement and low water supply in two Mediterranean resprouter species before and after pruning

A. unedo and *Q. suber* co-occur in the Mediterranean basin and, in general, both species are notably resistant to increases in UV doses, as well as to reduced water availability, although they present distinct strategies to cope with such environmental stresses. Specifically, *A. unedo* response to enhanced UV-B was mainly addressed to minimize the oxidation state of cells through the reinforcement of the antioxidant machinery by means of increases in the levels of foliar quercetins, ascorbate and glutathione, coupled with an upregulated catalase (CAT) activity (Fig. 4.1a). In agreement with previous studies, the antioxidant response of *A. unedo* plants would be a highly sensitive defense mechanism to cope with abiotic stress factors (Munné-Bosch & Peñuelas, 2004). Indeed, the UV-B-induced stimulation of *A. unedo* antioxidant system would have been very efficient especially in the resprouts, since they showed the highest photosynthetic rates under UV-B supplementation (Fig. 4.1a). Nevertheless, *A. unedo* plant growth and biomass allocation were not affected by enhanced UV-B radiation, which suggests that the greater carbon assimilated via photosynthesis in the resprouts would have been invested in meeting the defense/protection demands (Sumbele *et al.*, 2012; Ballaré, 2014). *A. unedo* apparently did not require the activation of further protective strategies typically reported in other Mediterranean species, such as the increase in leaf thickness and/or leaf sclerophylly (Verdaguer *et al.*, 2012; Bernal *et al.*, 2013, 2015).

A. unedo would also have counteracted the negative effects of water constraint by the stimulation of the antioxidant system, although, in this case, this was mainly achieved via increases in ascorbate and hydrolyzable tannins, such as ellagitannins and gallotannins (Fig. 4.1b). In parallel, the reduction in the activities of the antioxidant enzymes ascorbate peroxidase (APX) and CAT in response to drier conditions (Fig. 4.1b) suggests that low irrigation did not cause a severe oxidative stress. The fact that, under enhanced UV-B, low-watered plants showed an improved water status and photosynthetic capacity in relation to

well-watered ones points to the existence of cross-tolerance effects between the two stress factors (Fig. 4.1b). This could in part explain the general increase observed in the amount of plant root starch under water reduction (Fig. 4.1b), which, given that the watering regime did not affect plant biomass, suggests that photosynthates were basically diverted to belowground storage carbon pools (Hasibeder *et al.*, 2015; Lavinsky *et al.*, 2015).

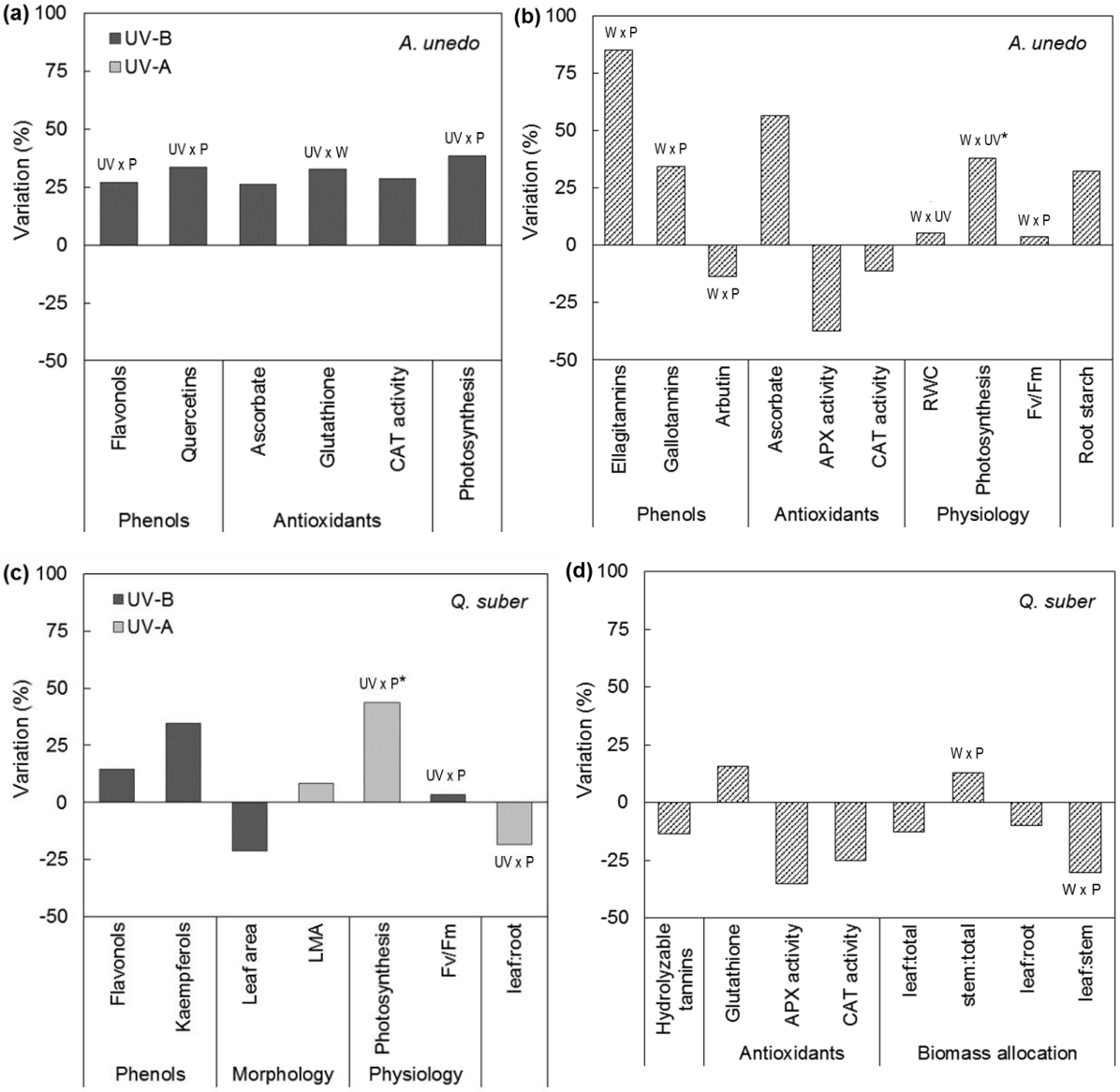


Fig. 4.1 Percentages of variation due to UV-A and UV-B supplementation (**a**, **c**) and reduced irrigation (**b**, **d**) estimated in relation to control values (Experiment I). Only the main parameters significantly affected in *Arbutus unedo* (**a**, **b**) and *Quercus suber* (**c**, **d**) are shown. Interactive effects among treatments (UV radiation, UV; water availability, W; and pruning, P) are indicated above or under the bars. Asterisks indicate an irrigation effect only observed in UVAB condition (**b**) and a UV effect only detected in seedlings (**c**). Abbreviations: LMA, leaf mass per area; APX, ascorbate peroxidase enzyme; CAT, catalase enzyme.

Contrary to what happened in *A. unedo*, the antioxidant system of *Q. suber* leaves was not substantially stimulated in response to increased UV-B radiation. Instead, this species faced UV-B enhancement by means of the activation of UV-screening mechanisms like the accumulation of kaempferols (Fig. 4.1c). This response was accompanied by a reduction in leaf area, further minimizing the incidence of UV-B radiation on leaf photosynthetic tissues. Unlike *A. unedo, Q. suber* was also substantially sensitive to UV-A radiation, despite the low UV-A doses supplemented in our experiment in relation to ambient levels. Enhanced UV-A induced morphological adjustments to harden the leaves of *Q. suber*, which produced smaller leaves with a greater leaf sclerophylly or LMA (Fig. 4.1c), being this a mechanism that can help plants to prevent photodamage to the photosynthetic apparatus. Interestingly, enhanced UV-A also promoted a reduction in the leaf to root biomass ratio of *Q. suber* seedlings, likely associated to the increase in the photosynthetic rates (Fig. 4.1c). A proportionally higher carbon investment in root growth might imply a greater ability to regenerate the aerial biomass after a perturbation (Pausas, 1997).

Low water supply did not trigger a general antioxidant response in *Q. suber,* which suggests that this species experienced a lower degree of cellular oxidative state compared to *A. unedo*. In fact, despite the increase in the leaf concentration of glutathione, the amount of hydrolyzable tannins was reduced, and APX and CAT activities were down-regulated in response to low irrigation (Fig. 4.1d). In addition, low water supply did not induce variations in the leaf morphology or physiology of *Q. suber* plants. These results could be explained by the plant biomass allocation changes observed in this species in response to low irrigation. Indeed, low-watered *Q. suber* plants showed a lower biomass allocation to leaves in relation to roots and total biomass (Fig. 4.1d), which suggests that this would be a strategy to increase water absorption in relation to water loss. Furthermore, the proportion of stem biomass increased in relation to total biomass, suggesting a proportionally greater amount of vascular tissue to transport water from roots to leaves.

The fact that root reserves, i.e. starch and soluble-sugars, of the two species studied were not reduced by UV and watering treatments would suggest that both species displayed

effective strategies to cope with enhanced UV levels and drier conditions with no changes in their resprouting capacity. The low-water-mediated increase in the amount of starch stored in *A. unedo* roots was especially pronounced in seedlings, but, contrary to expectations, it did not result in a higher resprouting capacity. Despite this, the sensitivity of resprouts to the UV or watering levels assayed was notably different in relation to that of seedlings, being higher in *A. unedo* but lower in *Q. suber* resprouts. Indeed, most treatment effects on *A. unedo* were found in the resprouts, while the opposite was found for *Q. suber*.

Role of UV radiation (UV-A and UV-B) and precipitation levels in the modulation of C and N pools of the soil and the dominant plant species of a Mediterranean shrubland

UV-A and UV-B exposure produced opposite effects on many of the parameters studied related to C and N pools, being these effects often modulated by reduced levels of precipitation and mainly detected before the experimental fire. Indeed, before the fire, UV-A exposure increased the levels of soil respiration and β-glucosidase activity (Fig. 4.2a), pointing to a UV-A-induced stimulation of soil biological activity, probably associated to the higher soil moisture of these plots, as found in other studies conducted in Mediterranean shrublands (Sardans *et al.*, 2008a). Since higher biological activity is usually associated to increased organic matter decomposition rates (Geisseler & Horwath, 2009), our findings suggest a positive impact of UV-A exposure on C and N cycling at the soil level. On the contrary, UV-B exposure slowed down soil respiration rates and β-glucosidase activity (at depth B), which would suggest an attenuation of soil biological activity, likely mediated by the observed reduction in soil pH (Eivazi & Tabatabai, 1990; Sardans *et al.*, 2008a) (Fig. 4.2a). Plant exposure to UV-B also increased C concentration of *P. angustifolia* leaves, which could indicate a higher foliar content of structural carbon-related compounds (Sardans & Peñuelas, 2013), further contributing to UV-B-induced deceleration of C and N turn-over in the ecosystem. Furthermore, UV-B exposure raised $\delta^{15}N$ values of *A. unedo* litter and leaves (Fig. 4.2a), suggesting increased soil N losses in the form of nitrates (Högberg *et al.*, 2014). UV effects on soil respiration and β-glucosidase activity, as well as on $\delta^{15}N$ values of *A.*

unedo, were different depending on the precipitation regime, being generally emphasized by reduced rainfall.

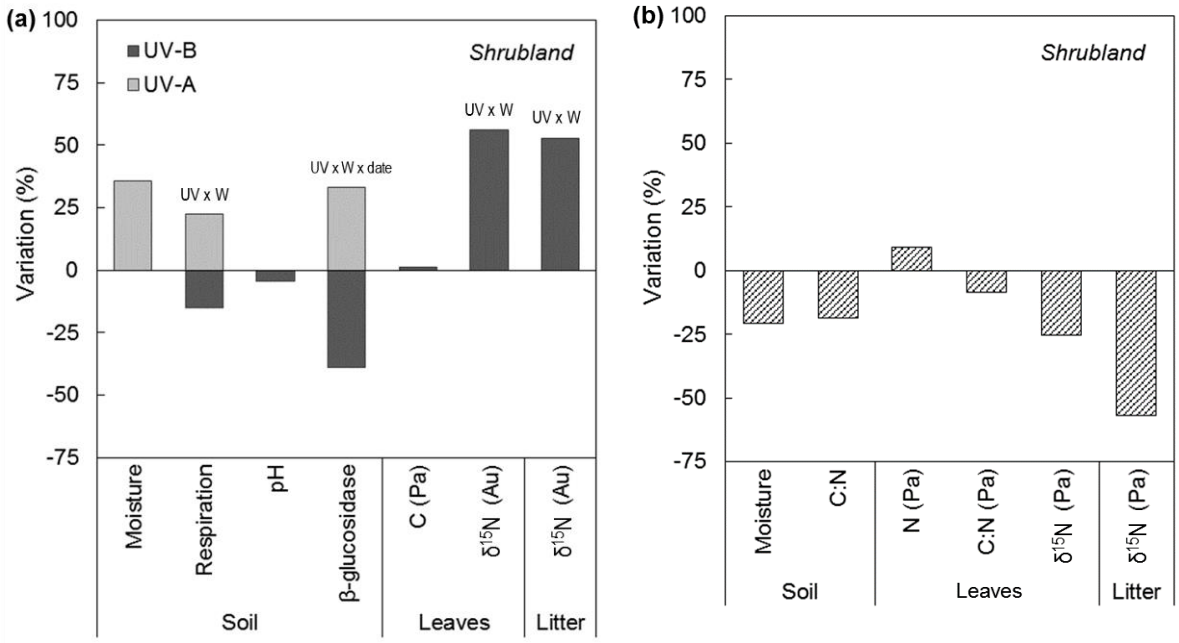


Fig. 4.2 Percentages of variation due to UV-A and UV-B exposure (**a**) and reduced water availability (**b**) estimated in relation to UV0 plots (Experiment II). Only the main parameters significantly affected in soil and plants of *Arbutus unedo* (Au) and *Phillyrea angustifolia* (Pa) are shown. Interactive effects among factors (UV radiation, UV; water availability, W; and sampling date, "date") are indicated above the bars.

The concomitant decrease in soil moisture associated to the experimental reduction in precipitation led to a higher N concentration in *P. angustifolia* leaves before the fire (Fig. 4.2b). Because of this, the C:N ratio of *P. angustifolia* leaves declined and, probably as a consequence, the C:N ratio of the soil was also lower under drier conditions, which might have favoured soil biological activity and litter decomposition (Bengtsson *et al.*, 2012; Sardans *et al.*, 2013). However, we did not find any significant effect of the precipitation regime on soil β-glucosidase activity. The decline in the $\delta^{15}N$ values of *P. angustifolia* leaves (after the fire) and litter (in June 2012) in response to reduced precipitation (Fig. 4.2b) would suggest a tighter N cycle with less N losses in drier soils.

Given that mean temperature and accumulated precipitation values were similar between pre- and post-fire periods, the fact that most of the UV and water availability effects were recorded before the fire points to a homogenizing influence of this perturbation. In

addition, the lack of treatment effects on many of the variables studied involved in C and N cycles suggests a high resilience of shrubland ecosystems against changes in UV and precipitation levels, at least in the short term.

The future of two resprouter species, **Arbutus unedo** *and* **Quercus suber,** *and of shrubland ecosystems under the expected changes in UV and precipitation levels in the Mediterranean basin*

In accordance with other studies in plant Mediterranean species (Paoletti, 2005; Bussotti *et al.*, 2014), *A. unedo* and *Q. suber* have developed efficient mechanisms to cope with high UV doses along with water constraints, at least at the short term, whereby their resprouting ability after a disturbance would remain unaltered. Nevertheless, from a longer-term perspective, the species-specific responses observed could have implications in terms of plant development, species competitive ability and lastly ecosystem functioning. Indeed, the UV-induced increases in quercetins and kaempferols found in *A. unedo* and *Q. suber* leaves, respectively, could modulate plant architecture through their role as key regulators of auxin transport (Jansen, 2002; Agati & Tattini, 2010). Moreover, in *A. unedo* leaves, the raise in hydrolyzable tannins in response to drier conditions might alter plant-animal interactions owing to the functions of tannins as defense compounds against herbivory (Kouki & Manetas, 2002; Barbehenn & Constabel, 2011). In addition, a higher accumulation of tannins in leaves would modify leaf litter chemistry in the soil, probably inhibiting nitrification (Castells *et al.*, 2004; Castaldi *et al.*, 2009; Formánek *et al.*, 2014) as well as diminishing N availability due to the formation of recalcitrant complexes with proteins from litter and/or with extracellular enzymes from microorganisms (Hättenschwiler & Vitousek, 2000).

At ecosystem level, all the above-mentioned changes in response to enhanced UV and reduced water availability would act in combination with the contrasting direct effects of UV-A and UV-B, as well as of reduced precipitation, on C and N pools. Thus, the net impact of UV and precipitation changes on the C and N cycling of Mediterranean shrublands would result from the balance between all these direct and indirect effects. In addition, given the

pronounced seasonal variability in solar UV-B fluxes (Seckmeyer *et al.*, 2008; Verdaguer *et al.*, 2017) and precipitation levels, the result of this balance will also depend on the time of the year. UV-B effects could be more relevant in summer, while UV-A effects could predominate in the other seasons, being modulated by water availability. In this scenario, an environmental perturbation, such as a fire, would have an “homogenizing influence” on UV and water effects on C and N pools at the short term, leading also to species-dependent changes in the sensitivity of plants (resprouts) to UV and water availability levels. Particularly, after a disturbance, the higher and lower sensitivity to enhanced UV radiation observed in *A. unedo* and *Q. suber* resprouts, respectively, in relation to pre-disturbance plants could potentially modify species competitive interactions and ultimately the vegetal structure of the ecosystem.

To sum up, over the coming decades, the changes induced by enhanced UV doses on C and N pools in Mediterranean shrublands would be in part driven by opposite effects of UV-A and UV-B radiation on soil processes, with these effects being modulated by water availability and plant species-specific responses to these factors.

CONCLUSIONS

- Enhanced UV-B doses induced an upregulation of leaf antioxidants or of UV-screening compounds depending on the species. Indeed, while *Arbutus unedo* increased foliar levels of antioxidants, in particular, quercetins, ascorbate and glutathione, stimulating also catalase activity, *Quercus suber* increased foliar UV-screening via accumulation of kaempferols in response to higher UV-B doses.

- Very low increases in UV-A doses over ambient levels promoted changes in leaf morphology (smaller leaves with greater LMA) coupled with a proportionally greater carbon allocation to roots (lower leaf to root biomass ratio) in *Q. suber* plants, while *A. unedo* did not respond to UV-A enhancement.

- *A. unedo* and *Q. suber* presented distinct strategies to counteract the effects of low water availability. Indeed, while water constraints stimulated foliar antioxidant activity in *A. unedo*, the main response of *Q. suber* was a reduction in the biomass allocated to leaves in relation to roots and to total biomass, probably as a strategy to minimize water loss by transpiration increasing water absorption by roots.

- Neither enhanced UV doses nor reduced water availability affected the percentage of plants of *A. unedo* and *Q. suber* that resprouted after aerial biomass removal. However, the sensitivity of resprouts to both treatments was notably different in relation to that of seedlings, increasing in *A. unedo* but decreasing in *Q. suber* resprouts.

- In the shrubland ecosystem, UV-A and UV-B exposure triggered opposite effects on the parameters studied related to C and N pools, being these effects often emphasized by reduced levels of precipitation and mainly detected before the experimental fire.

- Before the fire, UV-A exposure increased soil moisture, respiration and β-glucosidase activity, which points to a UV-A-induced stimulation of soil biological activity that would favour C and N cycling.

- UV-B exposure slowed down soil respiration rates and β-glucosidase activity, while increased C concentration of *P. angustifolia* leaves, altogether pointing to a deceleration of C and N turn-over in the ecosystem. In addition, UV-B exposure raised $\delta^{15}N$ values of *A. unedo* litter and leaves, suggesting higher nitrification and N losses by leaching in the soil.

- In the near future, UV-induced changes on C and N cycles of Mediterranean shrublands will depend, among other factors, on the balance between the opposite effects of UV-A and UV-B radiation on soil processes, modulated by the water availability in combination with plant species-specific responses to these factors, which in turn will depend on whether a perturbation, such as a fire, occurs.

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Supplementary Material

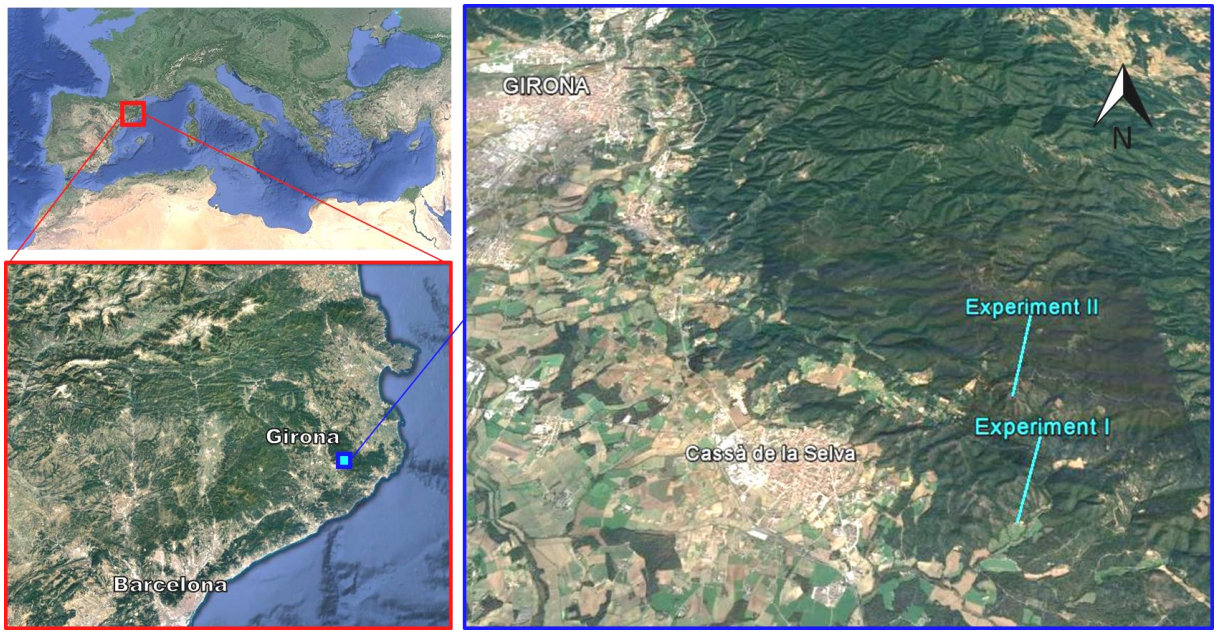
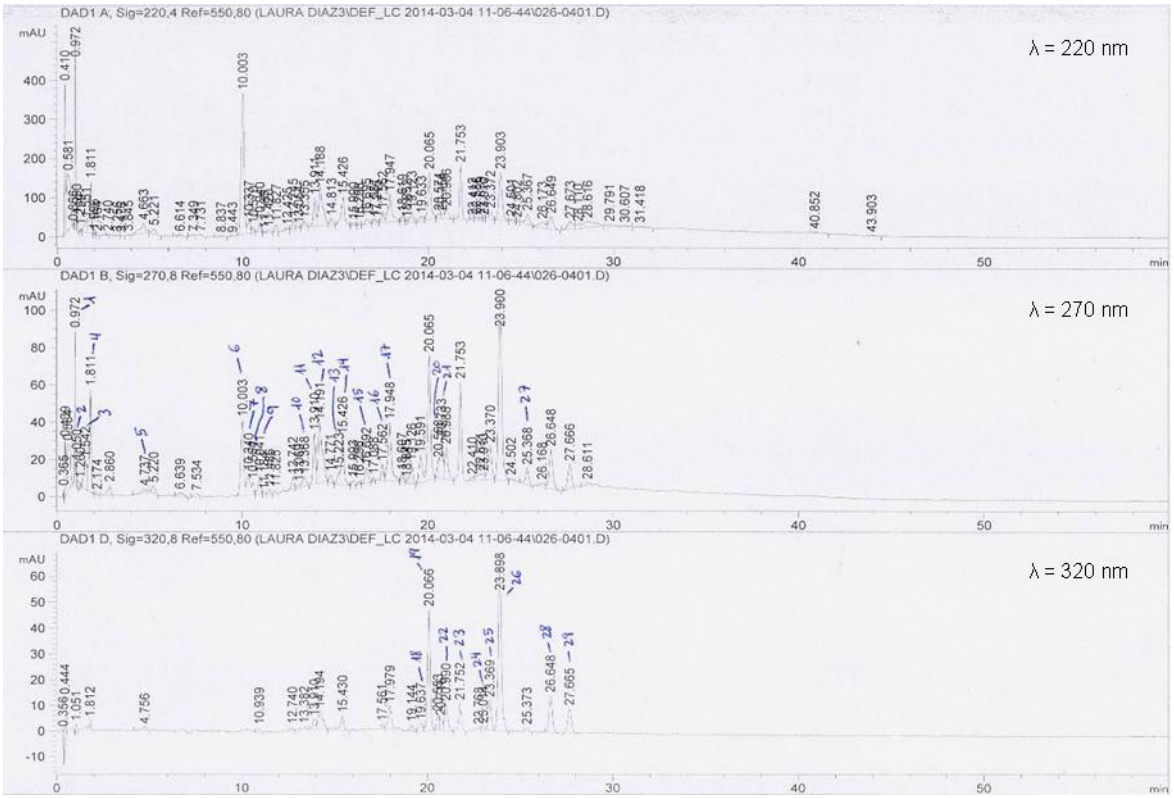


Fig. S1 Locations of the Experiment I (Can Vilallonga; 41º 52' 48" N, 2º 54' 33" E) and the Experiment II (41º 53' 57" N, 2º 54' 43" E), which were established at the Gavarres Massif, near Cassà de la Selva (Girona, NE of the Iberian Peninsula).

Fig. S2 Pictures showing a study plot of the Experiment I (9 plots in total) with an UVE sensor used to estimate the percentages of UV enhancement in UVAB and UVA plots. In addition, it is showed the fluorescent lamps for UV supplementation on, and resprouting plants of both *Arbutus unedo* (photo above) and *Quercus suber* (photo below) a couple of months after the removal of all the aerial biomass (i.e. pruning).

(a) *Arbutus unedo*



(b) *Quercus suber*

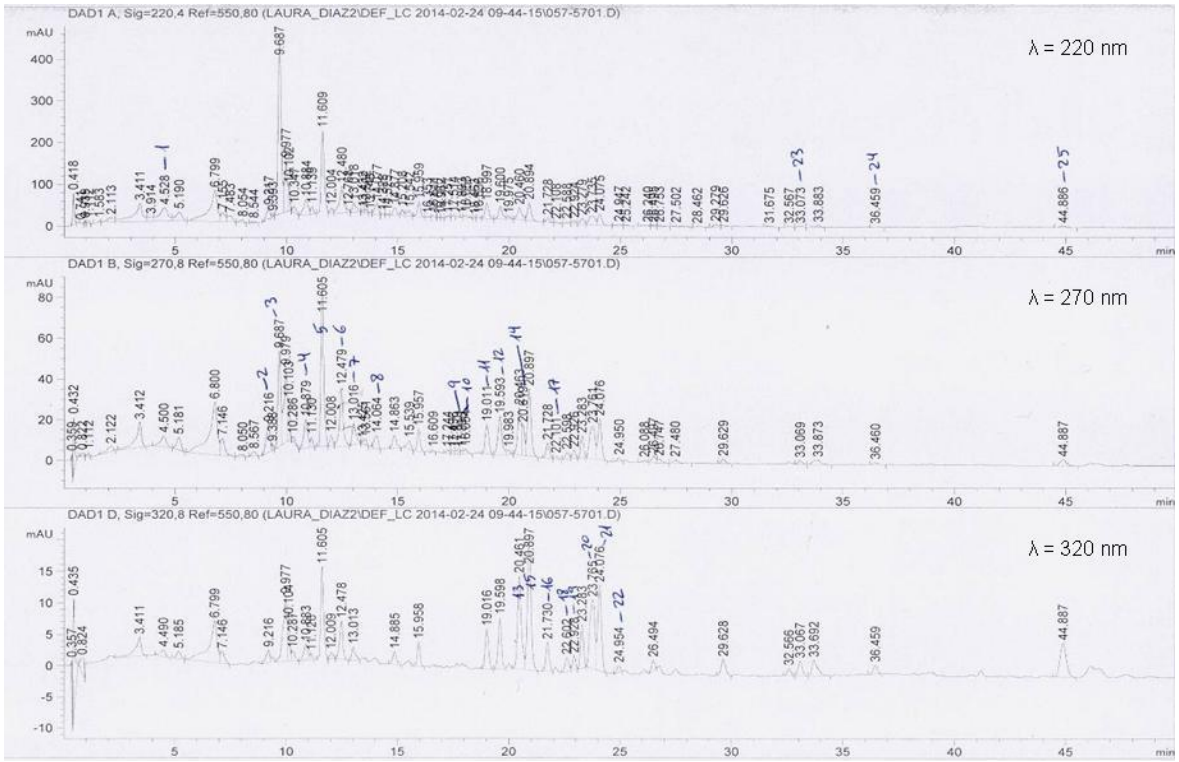


Fig. S3 Examples of chromatograms obtained from HPLC analyses at three different wavelengths (220, 270 or 320 nm) for one leaf sample of *Arbutus unedo* (**a**) and *Quercus suber* (**b**) (Experiment I). Numbers in blue indicate the 29 and 25 different phenolic compounds identified in *Arbutus unedo* and *Quercus suber* samples, respectively. Numbers in black are the retention times of each one of the peaks.

Table S1 Description of the soil in the study area of the Experiment II, composed mainly by three horizons (A, B and C/R) with different mean values of several soil parameters.

Horizon	Depth (cm)	EG >2mm (%)	$pH_{1:2.5}$	$EC_{1:5}$ (dS m^{-1})	SOM (%)	SOC (mg g^{-1})	TN (mg g^{-1})	C:N ratio	CEC ($cmol^{+}$ kg^{-1})
A	0-20	17.21	6.83	0.148	2.31	13.41	1.30	10.35	16.50
B	20-50	25.95	6.54	0.055	0.39	2.24	0.39	5.69	14.20
C/R	> 50	30.20	6.54	0.036	0.14	0.79	0.34	2.36	14.40

EG>2mm, percentage of grains greater than 2 mm; EC, electrical conductivity; SOM, soil organic matter; SOC, soil organic carbon; TN, total nitrogen; CEC, cation-exchange capacity.

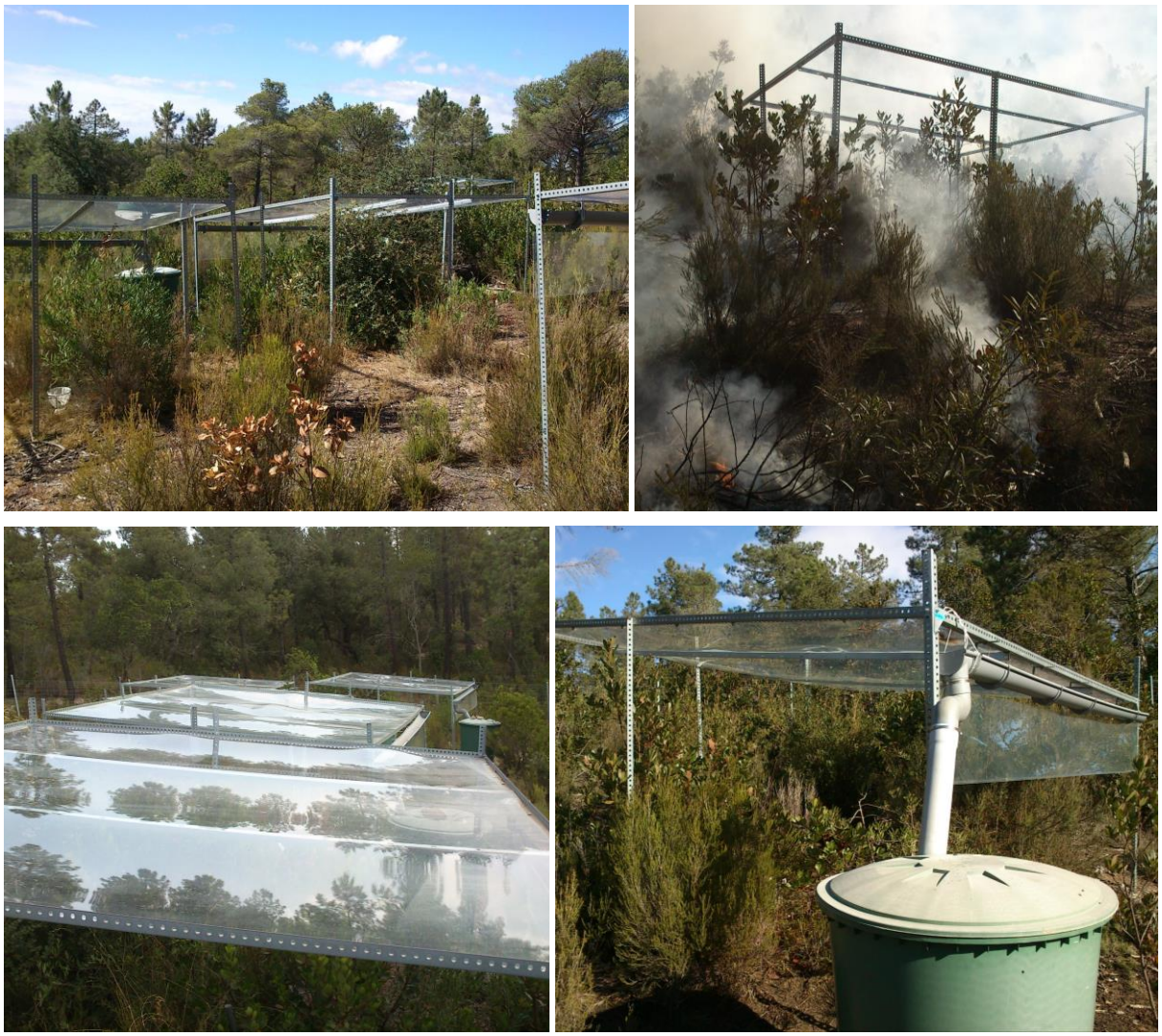


Fig. S4 Pictures of Experiment II showing a number of the 18 study plots, the controlled fire applied, the filters used to exclude solar UV radiation and the deposits where the natural precipitation was collected.

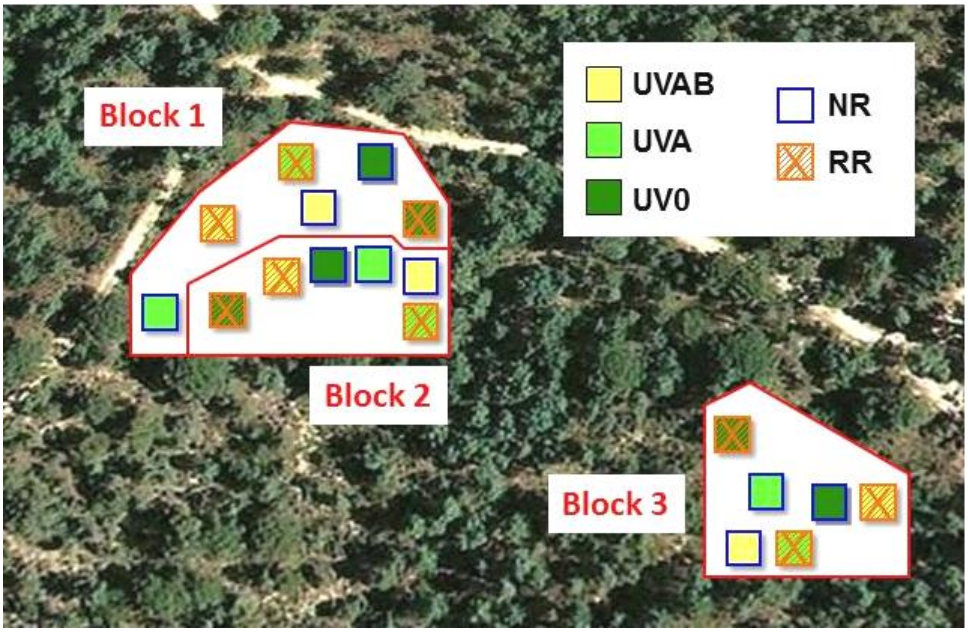


Fig. S5 Distribution of the 18 plots in the study area of the Experiment II, subjected to three UV radiation conditions (UVAB, UVA and UV0) in combination with two precipitation regimes (natural rainfall, NR; reduced rainfall, RR). Each one of the six different UV x precipitation conditions was replicated three times, with plots being distributed in three blocks (six plots per block).