

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

L. Díaz-Guerra  · D. Verdaguer · M. Gispert · G. Pardini · J. Font · J. A. González · E. Peruzzi · G. Masciandaro · L. Llorens

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Abstract

Background and aims In the Mediterranean basin, reduction in cloudiness owing to climate change is expected to enhance solar ultraviolet (UV) levels and to decrease rainfall over the coming years, which would be accompanied by more frequent and intense wildfires. The aim of the present study was to investigate the role of solar UV-A and UV-B radiation in C and N pools of a Mediterranean shrubland and whether drier conditions could alter this role before and after a fire.

Methods Over a three-year field experiment, 18 plots of 9 m² were subjected to three UV conditions (UV-A + UV-B exclusion, UV-B exclusion or near-ambient UV-A + UV-B exposure) combined with two rainfall regimes (natural or reduced rainfall). Several parameters related to C and N cycles in the soil and in the leaves and

litter of two dominant plant species (*Arbutus unedo* and *Phillyrea angustifolia*) were measured before and after an experimental fire.

Results UV-A exposure increased soil moisture throughout the study period, as well as respiration before the fire. The additional presence of UV-B decreased β -glucosidase activity at 5–10 cm depth and soil respiration and pH. UV-B exposure also raised leaf C concentration in *P. angustifolia* and $\delta^{15}\text{N}$ values in *A. unedo*. Reduced rainfall often emphasized the opposite effects of UV-A and UV-B on the studied parameters. After the fire, most of the UV and rainfall effects were lost.

Conclusion UV-A exposure seems to stimulate soil biological activity and, thus, C and N turn-over, while the effect of UV-B would be the opposite. At least in the short term, the “homogenizing influence” of fire would

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L. Díaz-Guerra (✉) · D. Verdaguer · J. Font · L. Llorens
Department of Environmental Sciences, Faculty of Sciences,
University of Girona, Campus Montilivi, C/ Maria Aurèlia
Capmany i Famés 69, E-17003 Girona, Spain
e-mail: l.diaz.gue@gmail.com

M. Gispert · G. Pardini
Department of Chemical Engineering, Agriculture and Food
Technology, Polytechnic School, University of Girona, Campus
Montilivi, C/ Maria Aurèlia Capmany i Famés 61,
E-17003 Girona, Spain

J. Font
Faculty of Sciences and Technology, University of Vic – Central
University of Catalonia, E-08500 Vic, Spain

J. A. González
Department of Physics, Polytechnic School, University of Girona,
Campus Montilivi, C/ Maria Aurèlia Capmany i Famés 61,
E-17003 Girona, Spain

E. Peruzzi · G. Masciandaro
Institute of Ecosystem Studies, CNR, via Moruzzi 1, 56124 Pisa,
Italy

probably have a stronger effect on the C and N cycles than the expected changes in UV and rainfall levels.

Keywords Carbon cycle · Drought · Fire · Mediterranean shrublands · Nitrogen cycle · UV radiation

Introduction

As a consequence of climate change, cloudiness reduction in the Mediterranean basin will decrease overall precipitation and increase ultraviolet (UV) radiation fluxes, both UV-B (280–315 nm) and UV-A (315–400 nm), 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 favoring 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 and Peñuelas 2013).

Specifically, increases in UV-B and UV-A 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 plays 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 and Ballaré 2010; Baker and 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 and 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.

The activity of soil enzymes involved in the biological decomposition of organic matter might also be altered by the change in solar UV fluxes (Nannipieri et al. 2002; Caldwell 2005). One of these soil 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). Despite the importance of this enzyme in the C cycle, present knowledge about how UV radiation affects β -glucosidase activity is limited (Gallo et al. 2006; Choudhary et al. 2013), with even less information available in Mediterranean ecosystems (Baker and Allison 2015). 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). Conversely, several studies performed in dryland ecosystems found that β -glucosidase activity in litter samples was unaffected by changes in UV exposure (Gallo et al. 2006; Baker and

Allison 2015). Clearly, more information is needed to disentangle how changes in UV radiation can affect the activity of soil enzymes, and in particular of β -glucosidase.

Carbon and nutrient cycles may also be substantially affected by other components of climate change, such as altered patterns of rainfall, which can interact with UV effects (Erickson 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 and 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, rainfall regime might be an important factor modulating UV effects on C and N levels in Mediterranean ecosystems. Indeed, there is evidence 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 and 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 below-ground organs to ensure post-disturbance nutrient supply (Verdaguer and 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 rainfall 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.

In this context, the main objectives of this study were: 1) to assess 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) to elucidate whether this role can be altered by water availability. To achieve these goals, we performed a field experiment where the levels of UV and rainfall 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 would be supported by changes in related variables, such as 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 rainfall regime on C and N cycles.

Materials and methods

Study area and experimental design

A field experiment involving UV radiation and rainfall reduction was conducted from August 2011 to June 2014 in a Mediterranean shrubland at the Gavarres Massif (41° 53' 57" N, 2° 54' 43" E) near Cassà de la Selva (Girona, NE of the Iberian Peninsula). The study

area was situated at about 250 m above sea level. 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. Climatological variables, such as global solar irradiation, temperature and rainfall, were monitored throughout the study period (Fig. 1) by a meteorological station located at Cassà de la Selva, 3 km away from the study site.

In August 2011, eighteen plots (3×3 m per plot) were distributed over the study area on a south-facing slope to ensure a high solar exposure. All plots had a similar slope, and their distribution and the relatively high plant cover of the soil (64% on average before the beginning of the experiment) minimized the

effects of sporadic runoff and/or leaching. In each of these plots, plastic filters were installed above the vegetation on metallic frames with a 10° slope towards the south and at a height of around 1.5 m at the center of the plot. 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 35 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 tank (310 L) placed next to each plot, allowing to combine the three UV conditions with two different rainfall regimes (see below). Each one of the six different UV x rainfall conditions was replicated three times, with plots being distributed in three blocks (six plots per block). In each plot, several parameters related to C and N levels were analyzed at soil, litter and plant leaf level. Litter and plant leaves were studied from the two dominant species *A. unedo* and *P. angustifolia* (Table 1). In February–March 2013, all the vegetation of the experimental plots was burned in a controlled fire (see below).

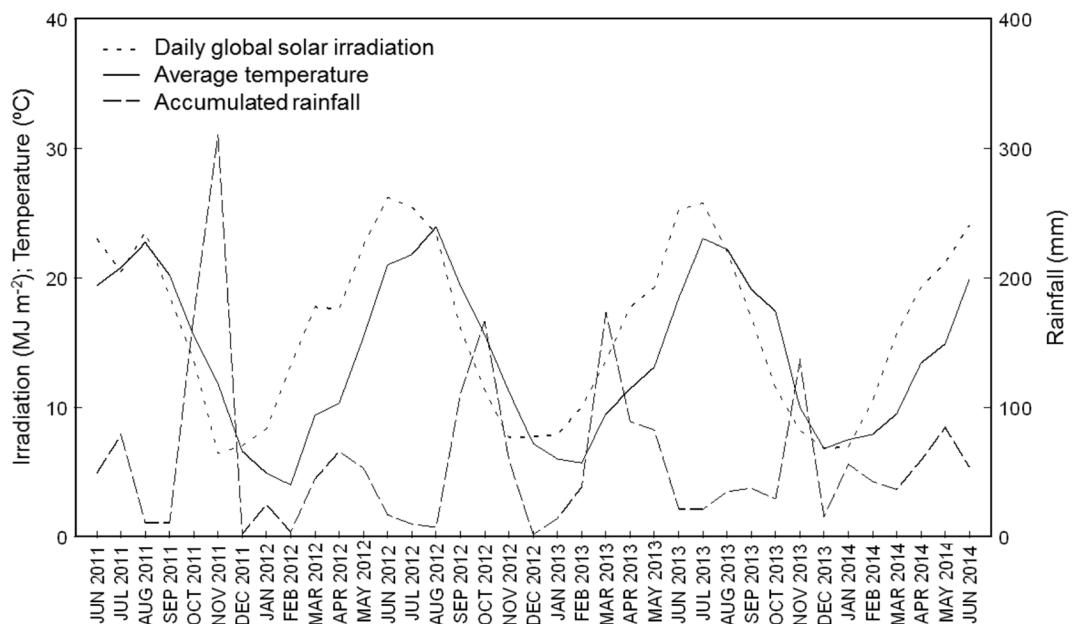


Fig. 1 Monthly averages of daily global solar irradiation (MJ m^{-2}) and temperature ($^\circ\text{C}$), together with accumulated rainfall (mm) for each month, along the study period. Data set was

obtained from the meteorological station of Cassà de la Selva (177 m above sea level, $41^\circ 52' 28''$ N, $2^\circ 55' 37''$ E)

Table 1 Sampling months and parameters analyzed from soil, and from litter and plant leaves of *Arbutus unedo* and *Phillyrea angustifolia* before and after the experimental 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
Moisture (%)										
Temperature (°C)										
Respiration ($\mu\text{mol m}^{-2} \text{s}^{-1}$)										
Organic C (mg g^{-1})										
Soil Total N (mg g^{-1})										
C:N ratio										
pH _{1:2.5}										
EC _{1:5} (dS m^{-1})										
β -glucosidase ($\text{mg pNP kg}^{-1} \text{h}^{-1}$)										
Litter cover (%)										
Plant cover (%)										
Leaf litter (<i>A. unedo</i> and <i>P. angustifolia</i>)	C (mg g^{-1})									*
	N (mg g^{-1})									*
	C:N ratio									*
	$\delta^{13}\text{C}$ (‰)									*
	$\delta^{15}\text{N}$ (‰)									*
Plant leaf (<i>A. unedo</i> and <i>P. angustifolia</i>)	C (mg g^{-1})									
	N (mg g^{-1})									
	C:N ratio									
	$\delta^{13}\text{C}$ (‰)									
	$\delta^{15}\text{N}$ (‰)									

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. 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):

- UV0 plots (i.e., plots where UV-A and UV-B were excluded): This condition was achieved by means of a 2-mm-thick polycarbonate filter (PC0100UV, PolimerTecnica, 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 (i.e., plots where UV-B was excluded): 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 (i.e., plots exposed to near-ambient UV radiation levels): These plots were aimed to provide similar microclimate conditions (degree of shading and temperature) to those found in UV0 and UVA plots. They were covered by a 3-mm-thick methacrylate filter (MC0100XN, PolimerTecnica, Girona, Spain), which transmitted, on average, 80.5 and 85% of UV-B and UV-A radiation, respectively.

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

Table 2 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 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%

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)

^a Plant response action spectrum according to Caldwell (1971)

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

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; 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 and 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.

Rainfall treatment

Half of the plots received 100% of the natural rainfall (NR plots), whereas the other half were watered with 70% of the rainfall throughout the study period, except in winter when they were watered with 90% (reduced rainfall or RR plots). To achieve these two levels of rainfall, the precipitation collected in the tanks placed beside each plot was used to irrigate the plots according to the above rainfall 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 RR plots compared to NR ones (Fig. 2), which confirms that the treatment was properly applied.

Experimental disturbance (fire)

Vegetation of the plots was completely burned by specialist firefighting 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.

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 1). In the post-fire period, monthly measurements of soil moisture were also taken from May 2013 to June 2014 to confirm that the rainfall treatment was properly applied (Fig. 2). 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.

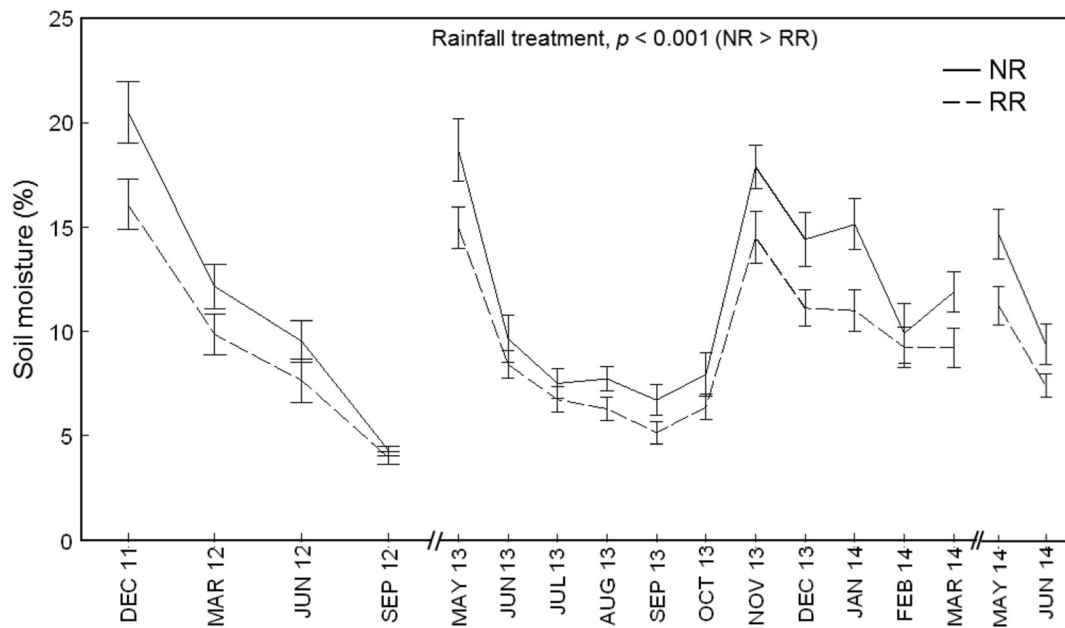


Fig. 2 Mean values of soil moisture (%) throughout the study period under the two experimental rainfall regimes: natural rainfall (NR) and reduced rainfall (RR). Error bars represent the standard error of the mean ($n = 9$). The significance level was set at $p \leq 0.05$

Soil CO_2 fluxes were measured with a portable infrared gas analyzer (IRGA; CIRAS-2, PP-Systems, Amesbury, USA) connected to an SRC-1 soil respiration chamber. Once the closed chamber (10 cm diameter \times 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 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$. 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 and spring before the fire (December 2011 and June 2012) and after the fire (December 2013 and June 2014) (Table 1). Soil was also sampled just before the experimental fire (February 2013). 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 analyzed 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 analyzed for all the samples except for February 2013 (Table 1).

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 g 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 p -nitrophenol (p NP) from the 0.05 M 4-nitrophenyl- β -D-glucopyranoside (p NPG), used as substrate of the enzyme (Hayano and Tubaki 1985). The concentration of p NP released from 0.5 g of dried soil was determined spectrophotometrically at 398 nm (Tabatabai and Bremner 1969). Thus, β -glucosidase activity was expressed as $\text{mg } p\text{NP kg}^{-1} \text{ h}^{-1}$.

Plant and litter cover

Plant and litter cover of each plot was measured by means of the vertical “pin-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 1). 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 litter traps were installed in each plot at the beginning of the experiment. Three of these traps were positioned 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 1). For each sampling date, one sample per plot was obtained by joining the leaf litter accumulated in the four traps. 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 analyzed.

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 1), 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 analyzed 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 analyzer (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 $\delta^{15}\text{N}$ and relative to PDB standard for $\delta^{13}\text{C}$, according to the following equation:

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

where Z is the heavy isotope of either N or C, and R is the ratio of heavier to lighter isotope ($^{15}\text{N}/^{14}\text{N}$ or $^{13}\text{C}/^{12}\text{C}$) 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 normalized 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, and their interactive effects with the two treatments, we performed three-way ANOVAs using fire, UV and rainfall treatments as fixed factors. To analyze the effects of the two treatments, pre- and post-fire data were also analyzed separately. Soil parameters determined from composite samples per plot and depth (organic C, total N, C:N ratio, pH, electrical conductivity and β -glucosidase) were analyzed by means of repeated-measures ANOVAs for each depth, with UV and rainfall treatments as factors. Treatment effects on soil moisture, temperature and respiration, as well as on leaf C, N, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and C:N ratio, were tested by ANOVA analyses, since data for these parameters were obtained from several soil points or leaves per plot. In the case of soil parameters, sampling date, and UV and rainfall

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, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and C:N) for each species, were analyzed within each sampling date by means of two-way ANOVAs (with UV and rainfall treatments as fixed 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 (UVAB, UVA and UV0). 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 analyzed with the Levene's test. For all the statistical tests, the significance level considered was $p \leq 0.05$. PCA was done with PRIMER 6 software (PRIMER-E Ltd., Plymouth, UK) and other statistical analyses were done using SPSS software (IBM SPSS Statistics, Corporation, Chicago, USA).

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, Fig. 3). 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). Although soil moisture also contributed to PC3, soil respiration was the most relevant variable related to this component (Table 3).

PC2 clearly segregated December from June samplings, due to higher soil moisture and lower soil temperature in December than in June months (Fig. 3a, 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. 3a). PC3 separated

Table 3 Principal component solution on six soil variables ($n = 72$)

Parameters	PC1	PC2	PC3
Moisture (%)	-0.019	0.603	0.527
Temperature ($^{\circ}\text{C}$)	-0.056	-0.695	0.014
Respiration ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	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 ($\text{mg pNP kg}^{-1} \text{h}^{-1}$)	0.525	-0.020	-0.034
Variance explained (%)			
Absolute	38.2	30.2	15.6
Cumulative	38.2	68.4	84.0

Factor loadings ≥ 0.50 in absolute value are marked in bold

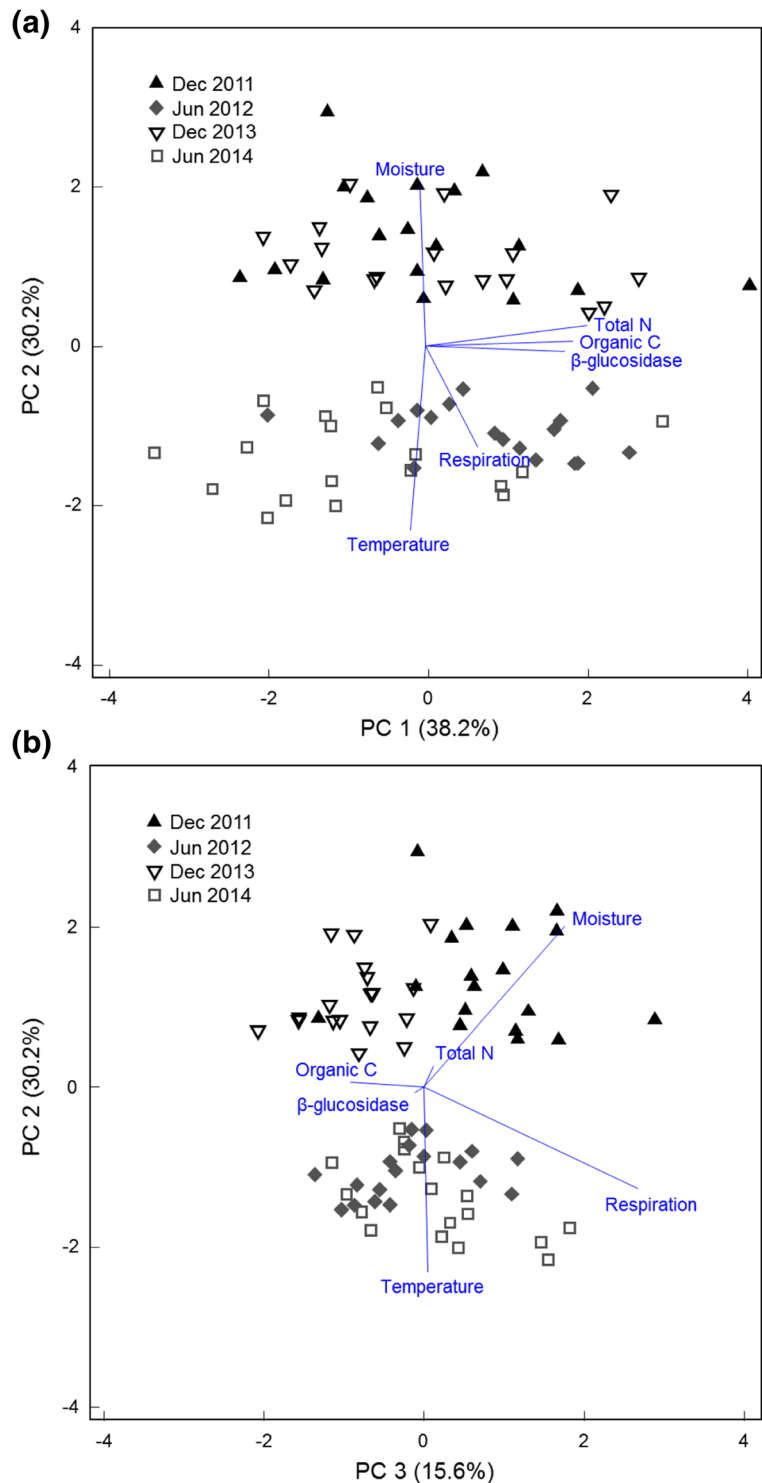
December 2011 from December 2013 data, mainly as a result of higher respiration in December 2011 associated to slightly higher soil moisture (Fig. 3b). No segregation was observed in response to the treatments.

Differences between pre- and post-fire periods

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 S1). 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 S2). In June 2014, vegetation cover already showed similar values to those found before the fire, while litter cover was still lower (Table S2). Regarding the chemical properties of the leaf litter of *A. unedo*, values of $\delta^{15}\text{N}$ 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 S2). Conversely, $\delta^{13}\text{C}$ and C concentration values did not vary among sampling dates. As a consequence, the C:N ratio of *A. unedo* leaf litter in

Fig. 3 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 ($^{\circ}\text{C}$), respiration ($\mu\text{mol m}^{-2} \text{s}^{-1}$), organic C (mg g^{-1}), total N (mg g^{-1}) and β -glucosidase activity ($\text{mg pNP kg}^{-1} \text{h}^{-1}$)



June 2014 was 31.1% lower than in September 2012 (Table S2). In the case of *P. angustifolia*, leaf litter production after the fire was too low to be analyzed.

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 S1). Since 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 $\delta^{13}\text{C}$ values (by 0.4‰) after the fire, while, for both species, $\delta^{15}\text{N}$ values were 1‰ higher in the post-fire period, in accordance with the results found for the leaf litter of *A. unedo*.

Regarding inter-specific differences, despite *P. angustifolia* had higher leaf C concentration than *A. unedo* over the study period ($F_{1,120} = 488.4$, $p < 0.001$), differences in leaf N concentration between the two species varied before and after the fire (Table S1). In the pre-fire period, N concentration in *P. angustifolia* leaves was lower than in *A. unedo* ($F_{1,48} = 7.0$, $p = 0.011$), but, after the fire, the contrary was found ($F_{1,48} = 46.9$, $p < 0.001$). These differences led to a higher C:N ratio of *P. angustifolia* leaves in the pre-fire period ($F_{1,48} = 19.5$, $p < 0.001$) followed by a lower ratio after the fire ($F_{1,48} = 31.0$, $p < 0.001$; Table S1). Throughout the study period, $\delta^{13}\text{C}$ values did not differ between the two species, although *P. angustifolia* showed lower $\delta^{15}\text{N}$ values than *A. unedo* ($F_{1,120} = 105.8$, $p < 0.001$).

Effects of UV radiation and rainfall 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 4). 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 rainfall decreased the soil moisture of RR plots throughout all the study period, being 20% and 23% lower than in NR plots before and after the fire, respectively (Table 4). 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.

Throughout the study period, there was an interactive effect between the two treatments on soil respiration (Table 4). Indeed, before the fire, exposure to UV-B 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. 4). On the other hand, control soils (UVAB plots) always exhibited

greater respiration rates 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. 4).

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 4), 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. 5a). 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. 5b).

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

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 5). Regarding the isotopic composition of litter, $\delta^{13}\text{C}$ values of *P. angustifolia* leaf litter were 0.8‰ higher in UVA plots than in UVAB ones in September 2012 (Table 5). On the other hand, the experimental reduction in rainfall decreased by 1.9‰ the $\delta^{15}\text{N}$ values of *P. angustifolia* leaf litter in June 2012 (Table 5). For *A. unedo* litter, we found a significant interactive effect of the two treatments on $\delta^{15}\text{N}$ values in September 2012 (Table 5). In this sampling date, but only in RR plots, leaf litter of this species showed 2.6 and 2.0‰ lower $\delta^{15}\text{N}$ values in UVA and UV0 plots, respectively, than in UVAB ones ($p = 0.009$; Fig. 6a).

At plant leaf level

UV and rainfall 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 4). Also before the fire, rainfall 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 $\delta^{15}\text{N}$ values

Table 4 Overall mean \pm S.E. for all the studied parameters in the soil (depth A, 0–5 cm; depth B, 5–10 cm) and in the leaves of *Arbutus unedo* and *Phillyrea angustifolia* under the three different UV radiation conditions (UVAB, UVA and UV0) and the two rainfall regimes (natural rainfall, NR; reduced rainfall, RR)

PRE-fire Soil	UV radiation (UV)				Rainfall (R)				Interactions	
	UVAB	UVA	UV0	p-value	NR	RR	p-value	RR	p-value	
	Moisture (%)	10.918 \pm 1.185 a	12.024 \pm 1.389 a	8.569 \pm 1.037 b	< 0.001	11.619 \pm 1.108	9.388 \pm 0.867	0.001		
Temperature (°C)	17.850 \pm 0.698	17.635 \pm 0.671	17.713 \pm 0.664	ns	17.928 \pm 0.567	17.537 \pm 0.537	ns			–
Respiration ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	1.815 \pm 0.095 b	2.136 \pm 0.128 a	1.747 \pm 0.105 b	0.038	1.835 \pm 0.095	1.964 \pm 0.091	ns			UV \times R
<i>Depth A</i>										
pH _{1:2.5}	6.008 \pm 0.098 b	6.273 \pm 0.057 a	6.313 \pm 0.058 a	0.027	6.147 \pm 0.074	6.249 \pm 0.058	ns			–
EC _{1:5} (dS m ⁻¹)	0.064 \pm 0.011	0.059 \pm 0.006	0.066 \pm 0.008	ns	0.060 \pm 0.008	0.066 \pm 0.006	ns			–
Organic C (mg g ⁻¹)	17.191 \pm 0.702	18.683 \pm 0.853	18.084 \pm 1.080	ns	18.269 \pm 0.634	17.703 \pm 0.813	ns			–
Total N (mg g ⁻¹)	1.490 \pm 0.080	1.632 \pm 0.085	1.564 \pm 0.097	ns	1.463 \pm 0.072	1.661 \pm 0.067	ns			–
C:N ratio	12.223 \pm 1.051	11.641 \pm 0.466	11.928 \pm 0.765	ns	13.168 \pm 0.783	10.694 \pm 0.324	0.010			–
β -glucosidase (mg $\rho\text{NP kg}^{-1} \text{h}^{-1}$)	124.639 \pm 10.591	140.743 \pm 11.514	128.745 \pm 9.500	ns	124.091 \pm 7.521	138.660 \pm 9.338	ns			–
<i>Depth B</i>										
pH _{1:2.5}	5.760 \pm 0.062	5.922 \pm 0.104	5.825 \pm 0.139	ns	5.911 \pm 0.062	5.760 \pm 0.104	ns			–
EC _{1:5} (dS m ⁻¹)	0.047 \pm 0.006	0.050 \pm 0.007	0.054 \pm 0.008	ns	0.047 \pm 0.005	0.054 \pm 0.006	ns			–
Organic C (mg g ⁻¹)	10.247 \pm 0.526	10.854 \pm 0.768	10.121 \pm 0.453	ns	10.514 \pm 0.472	10.301 \pm 0.501	ns			–
Total N (mg g ⁻¹)	0.840 \pm 0.051	0.902 \pm 0.054	0.848 \pm 0.048	ns	0.881 \pm 0.044	0.846 \pm 0.039	ns			–
C:N ratio	12.534 \pm 0.657	12.233 \pm 0.739	12.240 \pm 0.459	ns	12.339 \pm 0.605	12.333 \pm 0.393	ns			–
β -glucosidase (mg $\rho\text{NP kg}^{-1} \text{h}^{-1}$)	38.504 \pm 5.405 b	54.119 \pm 3.548 a	45.188 \pm 4.065 ab	0.017	47.430 \pm 4.375	44.444 \pm 3.252	ns			Date \times UV \times R
<i>Plant leaf A. unedo</i>										
C (mg g ⁻¹)	492.004 \pm 2.041	488.030 \pm 1.924	487.910 \pm 1.861	ns	490.783 \pm 1.053	487.847 \pm 1.988	ns			Date \times R
N (mg g ⁻¹)	12.864 \pm 0.622	12.005 \pm 0.330	12.700 \pm 0.550	ns	12.234 \pm 0.347	12.811 \pm 0.480	ns			–
C:N ratio	39.214 \pm 1.860	41.031 \pm 1.258	39.251 \pm 1.775	ns	40.652 \pm 1.125	39.012 \pm 1.508	ns			–
$\delta^{13}\text{C}$ (‰)	-27.306 \pm 0.234	-27.025 \pm 0.190	-27.524 \pm 0.291	ns	-27.313 \pm 0.233	-27.256 \pm 0.163	ns			–
$\delta^{15}\text{N}$ (‰)	-1.713 \pm 0.226	-2.315 \pm 0.271	-2.230 \pm 0.314	ns	-2.131 \pm 0.206	-2.042 \pm 0.248	ns			–
<i>P. angustifolia</i>										
C (mg g ⁻¹)	512.470 \pm 1.429 a	506.282 \pm 2.099 b	509.480 \pm 1.783 ab	0.011	509.654 \pm 1.526	509.167 \pm 1.603	ns			–
N (mg g ⁻¹)	11.823 \pm 0.543	11.717 \pm 0.460	11.755 \pm 0.628	ns	11.255 \pm 0.392	12.276 \pm 0.453	0.020			–
C:N ratio	44.406 \pm 2.127	44.036 \pm 1.930	44.825 \pm 2.580	ns	46.351 \pm 1.814	42.494 \pm 1.647	0.033			–
$\delta^{13}\text{C}$ (‰)	-27.489 \pm 0.126	-27.535 \pm 0.387	-27.398 \pm 0.269	ns	-27.233 \pm 0.111	-27.715 \pm 0.289	ns			–
$\delta^{15}\text{N}$ (‰)	-3.965 \pm 0.383	-3.762 \pm 0.396	-4.069 \pm 0.352	ns	-3.665 \pm 0.324	-4.199 \pm 0.269	ns			–

Table 4 (continued)

	UV radiation (UV)			Rainfall (R)			Interactions	
	UVAB	UVA	UV0	NR	RR	p-value	p-value	
POST-fire Soil								
Moisture (%)	9.823 ± 0.606 a	10.915 ± 0.902 a	7.526 ± 0.643 b	10.609 ± 0.681	8.234 ± 0.518	<0.001	<0.001	–
Temperature (°C)	20.364 ± 0.914	20.535 ± 0.857	20.011 ± 0.799	20.429 ± 0.713	20.180 ± 0.683	ns	ns	–
Respiration (μmol m ⁻² s ⁻¹)	1.746 ± 0.144	1.774 ± 0.110	1.628 ± 0.097	1.661 ± 0.092	1.769 ± 0.099	ns	ns	UV x R
<i>Depth A</i>								
pH _{1:2.5}	6.081 ± 0.088	6.281 ± 0.077	6.318 ± 0.080	6.205 ± 0.077	6.248 ± 0.063	ns	ns	–
EC _{1:5} (dS m ⁻¹)	0.083 ± 0.013	0.082 ± 0.005	0.086 ± 0.009	0.075 ± 0.008	0.092 ± 0.007	ns	ns	–
Organic C (mg g ⁻¹)	15.640 ± 1.593	17.787 ± 1.166	17.298 ± 1.503	17.103 ± 1.089	16.714 ± 1.250	ns	ns	–
Total N (mg g ⁻¹)	1.126 ± 0.135	1.246 ± 0.064	1.240 ± 0.115	1.214 ± 0.085	1.194 ± 0.092	ns	ns	–
C:N ratio	14.439 ± 1.001	14.338 ± 0.675	14.120 ± 0.597	14.387 ± 0.555	14.211 ± 0.689	ns	ns	–
β-glucosidase (mg pNP kg ⁻¹ h ⁻¹)	83.390 ± 5.078	110.333 ± 10.253	116.817 ± 11.118	106.023 ± 8.961	101.004 ± 7.308	ns	ns	–
<i>Depth B</i>								
pH _{1:2.5}	5.916 ± 0.093	5.997 ± 0.094	6.037 ± 0.097	6.004 ± 0.087	5.962 ± 0.066	ns	ns	–
EC _{1:5} (dS m ⁻¹)	0.055 ± 0.004	0.060 ± 0.004	0.070 ± 0.008	0.055 ± 0.003	0.069 ± 0.005	ns	ns	–
Organic C (mg g ⁻¹)	11.347 ± 0.685	13.458 ± 0.597	12.357 ± 0.928	12.969 ± 0.599	11.805 ± 0.646	ns	ns	Date x UV x R
Total N (mg g ⁻¹)	0.828 ± 0.059	0.924 ± 0.063	0.858 ± 0.068	0.919 ± 0.051	0.821 ± 0.049	ns	ns	–
C:N ratio	13.916 ± 0.546	15.091 ± 1.002	14.466 ± 0.346	14.276 ± 0.302	14.706 ± 0.738	ns	ns	–
β-glucosidase (mg pNP kg ⁻¹ h ⁻¹)	59.671 ± 6.508	75.263 ± 8.112	67.442 ± 6.813	68.158 ± 5.554	66.759 ± 6.368	ns	ns	–
Plant leaf <i>A. unedo</i>								
C (mg g ⁻¹)	482.669 ± 1.639	483.505 ± 1.329	479.354 ± 1.482	483.349 ± 1.395	480.336 ± 1.016	ns	ns	–
N (mg g ⁻¹)	11.952 ± 0.652	11.380 ± 0.688	11.865 ± 0.772	11.401 ± 0.588	12.064 ± 0.542	ns	ns	–
C:N ratio	41.711 ± 2.258	44.333 ± 2.822	42.384 ± 2.791	44.348 ± 2.266	41.271 ± 1.931	ns	ns	–
δ ¹³ C (‰)	-27.980 ± 0.211	-27.675 ± 0.201	-27.472 ± 0.279	-27.884 ± 0.203	-27.534 ± 0.175	ns	ns	–
δ ¹⁵ N (‰)	-0.562 ± 0.310 a	-1.257 ± 0.335 b	-1.622 ± 0.245 b	-1.217 ± 0.248	-1.077 ± 0.277	0.011	ns	UV × R
<i>P. angustifolia</i>								
C (mg g ⁻¹)	504.430 ± 1.404	503.235 ± 0.955	501.739 ± 1.307	503.830 ± 1.096	502.440 ± 0.930	ns	ns	–
N (mg g ⁻¹)	15.354 ± 0.758	14.227 ± 0.628	13.843 ± 0.814	13.944 ± 0.582	15.004 ± 0.616	ns	ns	–
C:N ratio	33.802 ± 1.760	36.198 ± 1.715	37.712 ± 2.316	37.231 ± 1.566	34.576 ± 1.601	ns	ns	–
δ ¹³ C (‰)	-27.401 ± 0.254	-27.153 ± 0.182	-27.587 ± 0.177	-27.337 ± 0.150	-27.423 ± 0.192	ns	ns	–
δ ¹⁵ N (‰)	-2.541 ± 0.238	-3.172 ± 0.428	-3.101 ± 0.272	-2.493 ± 0.271	-3.383 ± 0.220	ns	0.027	–

Pre- and post-fire data and analyses are shown separately. Numbers in bold indicate significant differences among the levels of the factor. In the case of UV radiation, significant differences among the UV conditions are also indicated by different letters. For pre-fire and post-fire, *n* = 12 in each UV condition and *n* = 18 in each rainfall regime for all variables, 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* ≤ 0.05. Only significant two-way or three-way interactions were included in the column “interactions”. EC, electrical conductivity; ns, not significant

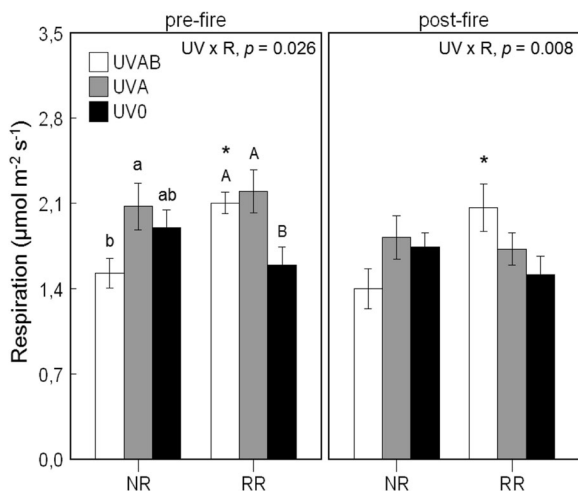


Fig. 4 Soil respiration in plots subjected to three UV radiation conditions (UVAB, UVA and UV0) combined with two rainfall regimes (natural rainfall, NR; reduced rainfall, RR), both before and after the fire. Error bars represent the standard error of the mean ($n = 12$). Asterisks indicate significant differences between NR and RR plots exposed to the same UV condition, whereas different letters indicate significant differences among UV conditions within each rainfall regime. Only significant differences within the same UV or rainfall condition are highlighted. The significance level was set at $p \leq 0.05$

0.9% lower than those from plots receiving natural rainfall (Table 4).

In *A. unedo* leaves, in the pre-fire period, the effect of the rainfall treatment on the C concentration depended on the sampling date (Table 4). 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 rainfall ($p = 0.009$; Fig. 7). After the fire, there was a significant interactive effect between UV and rainfall treatments on the $\delta^{15}\text{N}$ of *A. unedo* leaves (Table 4), since, only under drier conditions, leaves from UVA and UV0 plots showed, respectively, 1.8 and 1.3% lower $\delta^{15}\text{N}$ values than control ones (Fig. 6b). Treatments did not affect leaf $\delta^{13}\text{C}$ values of any of the two species studied.

Discussion

Differences in soil parameters measured in late autumn and late spring

According to the results of the PCA, soil characteristics were only segregated by the season, since soil

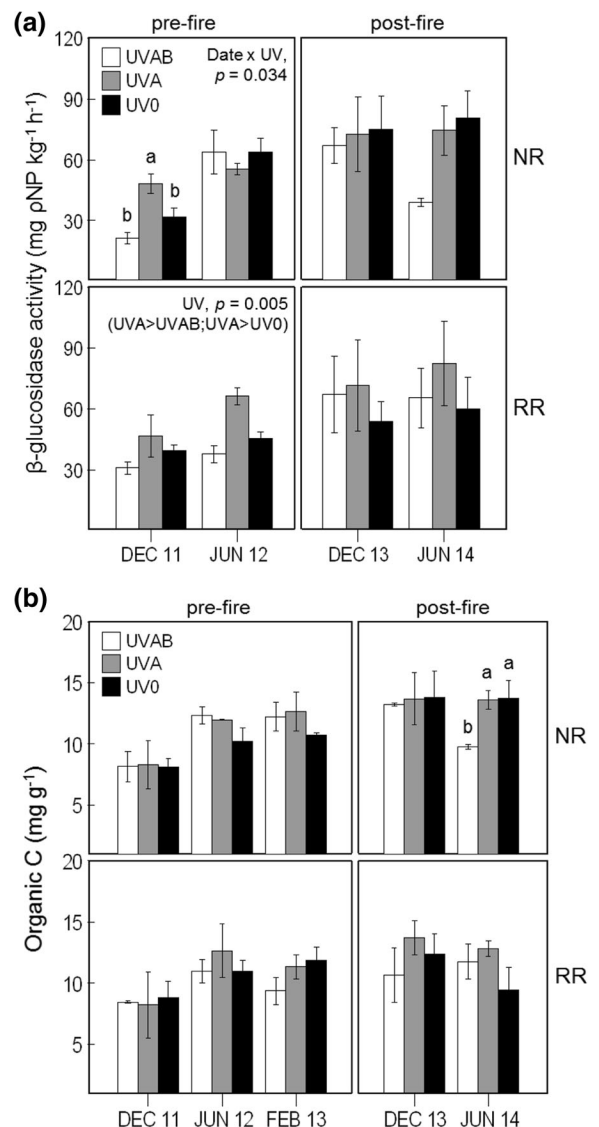


Fig. 5 Soil β -glucosidase activity (a) and organic C (b) at depth B (5–10 cm) from plots subjected to three UV radiation conditions (UVAB, UVA and UV0) combined with two rainfall regimes (natural rainfall, NR; reduced rainfall, RR) along all the sampling dates both before and after the fire. Error bars represent the standard error of the mean ($n = 3$). Since the interaction between UV radiation, rainfall 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 analyzed UV effects within the two levels of rainfall 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 rainfall regime. The significance level was set at $p \leq 0.05$

moisture and temperature were higher and lower, respectively, in December than in June (Fig. 3a).

Table 5 Overall mean ± S.E. for all the studied parameters in the leaf litter of *Arbutus unedo* and *Phillyrea angustifolia* under the three different UV radiation conditions (UVAB, UVA and UV0) and the two rainfall regimes (natural rainfall, NR; reduced rainfall, RR)

	UV radiation (UV)				Rainfall (R)				UV × R	
	UVAB	UVA	UV0	<i>p</i> -value	NR	RR	<i>p</i> -value	<i>p</i> -value		
June 2012										
<i>A. unedo</i>										
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		
δ 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		
δ 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		
<i>P. angustifolia</i>										
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		
δ 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		
δ 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										
<i>A. unedo</i>										
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		
δ 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		
δ 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		
<i>P. angustifolia</i>										
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		
δ ¹³ C (‰)	-28.334 ± 0.213 b	-27.525 ± 0.213 a	-27.804 ± 0.150 ab	0.040	-27.917 ± 0.200	-27.858 ± 0.187	ns	ns		
δ 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 *										
<i>A. unedo</i>										
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		
δ 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		
δ 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		

Data from each sampling date are shown separately. Numbers in bold indicate significant differences among the levels of the factor. In the case of UV radiation, significant differences among the UV conditions are also indicated by different letters. For all sampling dates, *n* = 6 in each UV condition and *n* = 9 in each rainfall regime for all variables. The significance level considered was *p* ≤ 0.05. ns, not significant

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

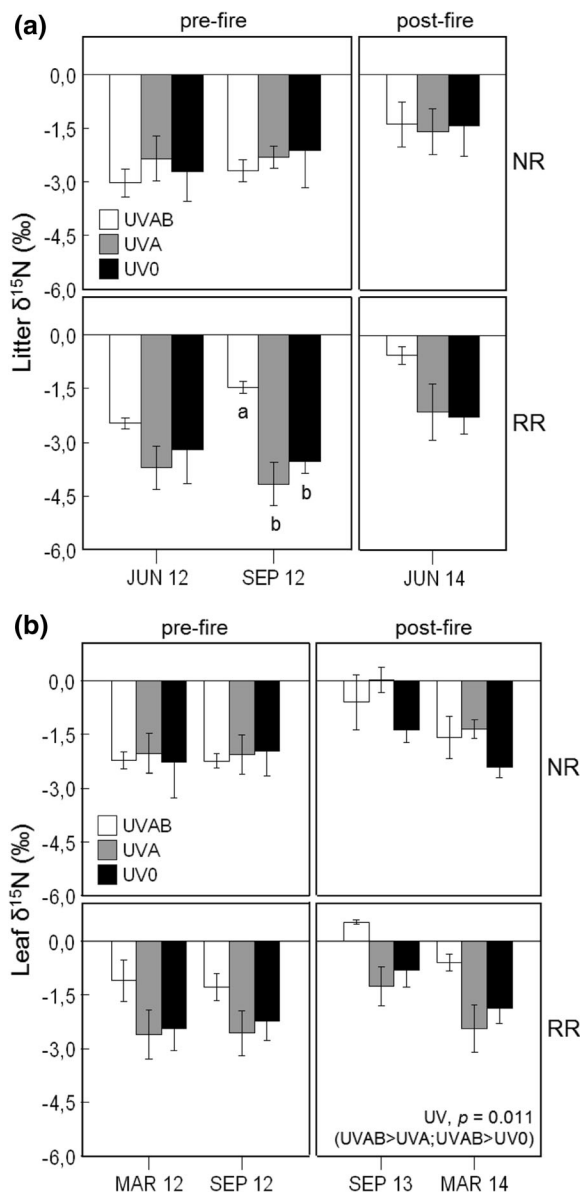


Fig. 6 *Arbutus unedo* $\delta^{15}\text{N}$ in litter (a) and leaves (b) from plots subjected to three UV radiation conditions (UVAB, UVA and UV0) combined with two rainfall regimes (natural rainfall, NR; reduced rainfall, RR) along all the sampling dates both before and after the fire. Error bars represent the standard error of the mean ($n = 3$). Since there was a significant interaction between the effects of the two treatments (UV radiation and rainfall) on $\delta^{15}\text{N}$ values of litter samples collected in September 2012 ($p = 0.034$) and of leaves from the post-fire period ($p = 0.002$), we analyzed the UV effects within the two levels of rainfall for these sampling dates and only significant differences are highlighted. Thus, different letters indicate significant differences among UV conditions within a specific sampling date and rainfall regime. The significance level was set at $p \leq 0.05$

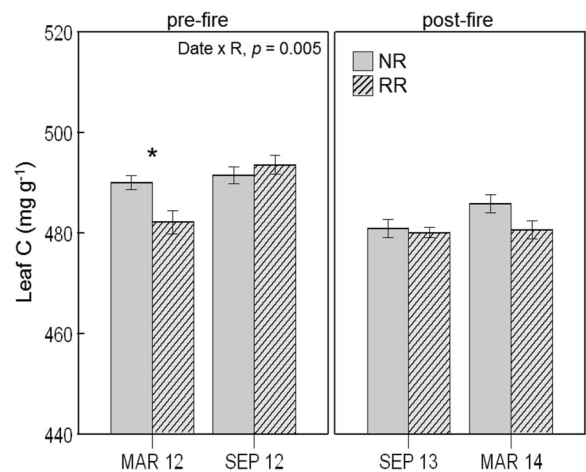


Fig. 7 C concentration in leaves of *Arbutus unedo* from plots subjected to two rainfall regimes (natural rainfall, NR; reduced rainfall, RR) along all the sampling dates both before and after the fire. Error bars represent the standard error of the mean ($n = 9$). Since there was a significant interaction between the sampling date and the rainfall treatment before the fire, we analyzed the rainfall 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 $p \leq 0.05$

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 greater values in December 2011 (before the fire) than in December 2013 (i.e. nine months after the fire) probably due to the slightly higher soil moisture observed in autumn 2011 (Fig. 3b). Regarding spring data, soils in June 2012 (before the fire) tended to have, in general, higher 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. 3a), although these effects varied at the two studied depths. 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 related to the greater litter cover, which might enhance soil water retention and nutrient input, stimulating soil microbial activity (Raich and 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.

Differences between pre- and post-fire periods

There were significant differences before and after the experimental fire in most of the parameters studied, regardless of the UV and rainfall treatments. After the fire, soil respiration was lower, while temperature, electrical conductivity and C:N ratio values were higher, compared to pre-fire values (Table S1). Enhanced soil temperature after the fire would probably be related to the decrease in vegetation and litter cover. It is known that, in semi-arid Mediterranean areas, a reduction in shrub cover can decrease soil moisture, increase solar radiation reaching the soil and thus soil temperature (Sherman et al. 2012), and diminish nutrient inputs through decaying debris, attenuating microbial activity and, as a consequence, soil respiration (Raich and Tufekcioglu 2000; Talmon et al. 2011).

At depth A, a higher soil C:N ratio after the fire might be, at least partially explained, by the observed reduction in soil N concentration, which might be linked to enhanced N losses through volatilization, runoff or leaching (Certini 2005; Hart et al. 2005). Lower soil respiration rates and β -glucosidase activity would also be in agreement with enhanced C:N ratios at depth A, suggesting lower decomposition rates (Geisseler and Horwath 2009; Bengtsson et al. 2012) and a post-fire attenuation of biological activity at the topsoil. Post-fire reductions in β -glucosidase activity have been documented (López-Poma and Bautista 2014), being mostly attributed to enzyme denaturation and temporary soil sterilization (Certini 2005; Knicker 2007). Conversely, at depth B (5–10 cm), the increase in the C:N ratio after the fire seemed to be linked to the enhancement in soil organic C concentration (Table S1). Given that soil surface is more exposed to erosion and nutrient leaching after a fire (Certini 2005), deeper soil layers may become enriched in organic C and nutrients (López-Poma and Bautista 2014), which might also explain the increase in β -glucosidase activity found at this subsurface layer.

After the fire, we found higher $\delta^{15}\text{N}$ values in *A. unedo* and *P. angustifolia* leaves (Table S1), as well as in *A. unedo* litter (Table S2), probably because of a fire-induced ^{15}N enrichment in soil organic matter (Szpak 2014). Post-fire leaves of *P. angustifolia*, as well

as leaf litter of *A. unedo*, showed higher values of N concentration, which might be explained by a concentration phenomenon due to the lower shoot:root ratio of these plants. Finally, the lower foliar C concentration observed in both species after the fire would presumably reflect a higher carbon investment into growth (Savé et al. 1993; El Omari et al. 2003; Bussotti 2008).

Effects of UV radiation

Throughout the whole study period, plots exposed to UV radiation (UVA and UVAB) showed higher soil moisture content than those not receiving this type of radiation (Table 4). This effect would be associated to UV-A exposure, since there were no significant differences between UVA and UVAB plots. The increase in soil moisture in response to UV-A is intriguing, but might, at least partially, be related to a UV-A-induced reduction of plant transpiration, since this effect was detected for *P. angustifolia* plants in a parallel study conducted in the same experiment, although this was only observed under reduced-rainfall conditions (Verdaguer et al., in prep). In addition, before the fire, UV-A exposure increased soil respiration rates under reduced rainfall (Fig. 4), which would be in agreement with the observed rise in β -glucosidase activity at depth B (Fig. 5a), likely as a result of stimulated soil microbial activity. Higher microbial activity might be related with the UV-A-induced enhancement of soil moisture, since soil water content has been positively correlated with microorganism activity in Mediterranean shrublands (Sardans et al. 2008a). Nevertheless, we cannot rule out a UV-A effect also on root respiration, which is largely controlled by solar irradiance through its effects on plant photosynthetic rates (Ferréa et al. 2012) and on the supply of photosynthates to roots (Högberg et al. 2001; Matteucci et al. 2015).

Contrary to the observed UV-A effects, the presence of near-ambient UV-B radiation reduced soil respiration and β -glucosidase activity (at depth B) in the pre-fire period (Table 4), suggesting a negative effect of UV-B radiation on soil biological activity. This negative effect might have been mediated by the observed pH reduction (significant at depth A) in response to UV-B exposure (Table 4), since acidity has been negatively linked to enzyme and microorganism activity, mainly through its effects on the availability of mineral nutrients (Eivazi

and Tabatabai 1990; Sardans et al. 2008a). Based on previous papers (Rinnan et al. 2006, 2008), the significant pH reduction found at depth A in plots exposed to UV-B could be associated to plant chemical changes in root exudates in response to UV-B exposure. The fact that UV-B effects on soil pH, respiration and β -glucosidase activity were mostly observed before the fire, i.e. when plant cover was higher, supports the idea that these parameters might be, at least partially, influenced by plant responses to this type of radiation. It has been shown that plants receiving enhanced UV-B radiation increase root accumulation and secretion of phenolics, which can affect negatively microorganism and β -glucosidase activity in the rhizosphere (Erickson et al. 2000; Castells et al. 2004; Castaldi et al. 2009; Choudhary et al. 2013).

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. 5b). Although not significant, the same tendency was observed for β -glucosidase activity (Fig. 5a). Similarly to the pre-fire results, these effects could be mediated by plant responses to UV-B, such as a 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 $\delta^{13}\text{C}$ values of plant leaves in any of the two studied species, in September 2012, leaf litter of *P. angustifolia* from UVAB plots showed lower $\delta^{13}\text{C}$ values than those found in UVA plots (Table 5). This might reflect a UV-B-induced reduction in the integrated water use efficiency of these leaves while they were alive, which would be in accordance with what it was found in a parallel study, although differences were only significant under reduced-rainfall conditions (Verdaguer et al., in prep.).

Under reduced rainfall, $\delta^{15}\text{N}$ values of *A. unedo* litter and leaves were highest in UVAB plots throughout the study period, although differences were only significant in September 2012 for the leaf litter (Fig. 6a) and in the post-fire period for the leaves (Fig. 6b). Increases in leaf $\delta^{15}\text{N}$ 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). Higher leaf $\delta^{15}\text{N}$ values could also indicate increased nitrification in the soil and, consequently, higher N losses mostly in the form of nitrates (Pardo et al. 2007; Högberg et al. 2014).

Effects of the rainfall regime

The reduction in rainfall, 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 4). In Mediterranean ecosystems, a wide variety of precipitation effects on soil C:N ratio has been reported, although, often, this ratio increases 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 and 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 (Table 4). A higher N content in these leaves might indicate a greater accumulation of leaf soluble protein, 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 $\delta^{15}\text{N}$ values of *P. angustifolia*, suggesting lower N losses at the soil level (Högberg et al. 2014; Ruiz-Navarro et al. 2016) despite we did not detect significant differences in soil total N in response to the rainfall treatment (Table 4). 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 and Peñuelas 2008) or similar (Llorens et al. 2003) $\delta^{15}\text{N}$ values under low rainfall. Differences in the intensity of the drought treatment applied in these experiments might explain, at least partially, these contrasting results.

In *A. unedo*, drier conditions decreased the leaf C concentration in March 2012 (the same tendency was observed in March 2014, although it was not significant), but not at the end of summer (September 2012 and 2013) (Fig. 7), probably due to the scarce rainfall

recorded during summer months (Fig. 1), which would have minimized the differences between the two irrigation levels. Considering that, 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.).

Concluding remarks

Exposure to UV-A radiation appeared to favor soil biological activity and C turn-over before the fire, since we found higher soil respiration rates and β -glucosidase activity. This might be a consequence of the observed increase in soil moisture 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 $\delta^{15}\text{N}$ values in leaves and litter of *A. unedo*, suggesting higher N losses in the soil, particularly in the soil compartment from which these plants took the N, 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 $\delta^{15}\text{N}$ values recorded in *P. angustifolia* plants grown under reduced rainfall points to lower N losses in the soil (at least in the soil compartment from which plants of this species took the N) linked to an ecosystem with a tighter N cycle.

Overall, the experimental reduction in rainfall 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 water supply. Many of the UV effects found were modulated by the rainfall 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 rainfall were not found for *P. angustifolia* plants. Species-specific responses to changes in UV fluxes and rainfall 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 rainfall amount.

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