

RESEARCH ARTICLE

# Survival of *Xanthoria parietina* in simulated space conditions: vitality assessment and spectroscopic analysis

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## Abstract

*Xanthoria parietina* is a widespread foliose lichen growing on barks and rocks showing a broad spectrum of tolerance to air pollutants such as NO<sub>x</sub> and heavy metals, and resistance to UV-radiation because of the screening properties provided by the secondary metabolite parietin. The aim of this study was to evaluate the ability of this lichen species to survive in the following simulated space conditions, UV-radiation in N<sub>2</sub> atmosphere and UV-radiation in vacuum. The efficiency of the photosynthetic apparatus was used as an indicator of vitality, and was expressed in terms of chlorophyll *a* fluorescence ( $F_V/F_M$ ) and Normalized Difference Vegetation Index (NDVI), which were measured within 72 h from the exposure. Additionally, during the irradiation, the IR reflectance spectrum of the lichen was monitored *in situ* to assess changes in spectral bands. The results showed significant differences in physiological recovery trends between the treatments, highlighting that UV-radiation in vacuum causes stronger effects on  $F_V/F_M$  values. The IR analysis revealed several spectral band changes in the fingerprint region. The most visible variation was the 5200 cm<sup>-1</sup> water band that disappeared in the overtone region. Nevertheless, *X. parietina* was able to survive UV-radiation in N<sub>2</sub> atmosphere and in vacuum, and for this reason it may be considered a candidate for further evaluations on its survival capacity in extreme conditions.

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## Introduction

One of the main topics of astrobiology research is the study of life limits in stressful environments. The aim of this research field is to explore the physiological and biochemical effects of extreme conditions – such as space – on unprotected biological samples (de la Torre Noetzel *et al.*, 2020). At the ecological and physiological level, the study of organisms in extreme environments might give an indication about their potential adaptive plasticity, in the view of a climate change perspective, the terrestrial geological past (Wassmann *et al.*, 2010) and future scenarios, as well as extra-terrestrial habitats such as Mars surface. Several experiments have been run in Low Earth Orbit (LEO) with satellites (LICHENS experiment performed on the BIOPAN-5 facility on the FOTON-M2 satellite, see e.g. Sancho *et al.*, 2007; Raggio *et al.*, 2011) and on the International Space Station (ISS), specifically on the EXPOSE facilities (Onofri *et al.*, 2012), to test organism survival capacity in space conditions, characterized by high vacuum, solar radiation and galactic ionizing radiation, solar UV-radiation and extreme temperature range (de la Torre Noetzel *et al.*, 2018; de Vera *et al.*, 2019). Studies were conducted on different eukaryotic and prokaryotic organisms. Bacteria such as *Bacillus subtilis* and *Deinococcus radiodurans* can survive space radiation and vacuum (Horneck *et al.*, 2001; de la Vega *et al.*, 2007). Rotschild and Mancinelli (2001) reported that 25–40% of halophilic *Archea* tested samples were able to survive 2 weeks on the FOTON-9 spacecraft (BIOPAN-1 mission), exposed to the unfiltered Sun spectrum. It has been discovered that akinetes of Cyanobacteria were tolerant to extra-terrestrial conditions and survived in dried state for long periods (Billi, 2009; Billi *et al.*, 2019). Furthermore, it has been shown that tardigrades (*Metazoa*) in a desiccated state can survive space condition, while organisms belonging to other *phyla* of *Metazoa*, such as nematodes, die when exposed directly to solar radiation (Bertolani *et al.*, 2001; Jönsson *et al.*, 2008).

Several studies focused on lichens, which are pioneer species – organisms able to colonize extreme environments first – proved to be resistant in space conditions too. Lichens are able to colonize almost all terrestrial habitats, including the most extreme ecological niches (Green *et al.*, 2012; Zedda and Rambold, 2015). The symbiotic association of two bionts, represented by a mycobiont and a photobiont (this last an alga and/or a cyanobacterium), allows them to survive in a wide range of conditions, tolerating dry environments, cold habitats, salt spray, water immersion, from high latitudes to the tropics (Kappen, 1973; Nash, 1996; de Vera *et al.*, 2004). This ability to survive and thrive under very stressful conditions is determined by their poikilohydry and anhydrobiosis, allowing long periods in desiccated state, and to the high variety of synthesis pathways due to the symbiotic association. The products of lichen secondary metabolism mostly determine the tolerance of these organisms to stressful conditions such as high doses of UV-radiation and rapid variations in the environmental context (de Vera *et al.*, 2004). The BIOPAN experiments showed that 70–100% of *Rusavskia elegans* (Link) S.Y. Kondr. & Kärnefelt subsp. *elegans* (synonym of *Xanthoria elegans* (Link) Th. Fr. var. *elegans*), *Rhizocarpon geographicum* (L.) DC. s. l. and *Aspicilia fruticulosa* (Eversm.) Flagey samples survived for 10–14 days in space conditions, being metabolically active and able to grow after the exposure (de la Torre Noetzel *et al.*, 2007; Sancho *et al.*, 2007, 2008; Raggio *et al.*, 2011; de Vera, 2012). The LiFE experiment onboard the EXPOSE-E facility was performed exposing different biological samples for 1.5 years to space conditions. The tested lichen species were *R. elegans* and *R. geographicum* and were exposed to space vacuum with a UV-shield (0.1% transmission neutral density filter) and without it, receiving 100% space insolation (Onofri *et al.*, 2012; Brandt *et al.*, 2016). *Rusavskia elegans* showed a high recovery in post-flight fluorescence measurements of photosystem II (PSII) activity. In particular, *R. elegans* showed the highest post-flight viability compared to the other tested species. UV-shielded samples showed fluorescence value of 99% of pre-flight control, while 100% transmission insolated samples showed 45% of the pre-flight control (Onofri *et al.*, 2012).

The main difference between space-exposing experiments and ground-based studies is that the latter allow to simulate extreme conditions for the biological sample and to immediately test viability parameters after the exposure (de Vera *et al.*, 2003, 2004, 2010). In the light of many different studies in literature about lichen astrobiological experiments, in this paper we decided to select *Xanthoria parietina* (L.) Th.

Fr. as test organism owing to its close phylogenetical relation to *R. elegans*. The choice of this species was also related to its ecological features: *X. parietina* is a cosmopolitan foliose lichen growing on barks and rocks, which can colonize almost all habitats from the seashore up to the treeline (Nimis, 2016). This species is also frequent in anthropized environments, being tolerant to air pollutants such as NO<sub>x</sub> and heavy metals (Silberstein *et al.*, 1996; Bačkor *et al.*, 2003) and by virtue of these features, it is often used as a bio-accumulator of persistent airborne pollutants (Loppi *et al.*, 2006). *Xanthoria parietina* is tolerant to UV-radiation because of the orange pigment parietin, a product of its secondary metabolism (Gauslaa and Ustvedt, 2003). This anthraquinone protects the photosystem from high light influx (Solhaug and Gauslaa, 1996) and its production is stimulated by UV-B radiation (Solhaug *et al.*, 2003; Solhaug and Gauslaa, 2004). In the astrobiological context, *X. parietina* has been investigated by de Vera *et al.* (2004) in a ground-based experiment, along with *R. elegans* and *Gyalolechia bracteata* (Hoffm.) A. Massal. (synonym of *Fulgensia bracteata* (Hoffm.) Räsänen). In particular, the lichens ascospores germination capacity was evaluated after simulated space conditions in different culture soil media. Generally, 50% of the recovered ascospores were viable and *R. elegans* ascospores showed the highest survival (de Vera *et al.*, 2004). Also *X. parietina* showed a remarkable germination rate of irradiated ascospores.

The aim of this study was to examine *X. parietina* recovery through eco-physiological analysis after exposure to simulated space conditions. The tested treatments were UV-radiation in N<sub>2</sub> atmosphere and UV-radiation in vacuum. These two conditions – simulated in laboratory – can be associated to different space environments. Vacuum condition at room temperature is analogous to the environmental situation found in the proximity of Earth orbit and other planetary bodies with a comparable distance from the Sun. Saturated nitrogen atmosphere, as an extreme environment, may be related to several planetary conditions (i.e. early Earth, Mars, possible altered terrestrial scenarios, exoplanets). Chlorophyll *a* fluorescence and reflectance were evaluated within 72 h from treatments to assess the recovery capacity of the photosynthetic apparatus. To contribute to a deeper understanding of the lichen response to the simulated space conditions, the Fourier-transform infrared reflectance (FTIR) spectra of lichen samples were monitored *in situ* during treatments. In this way, it was possible to appreciate in real time changes in spectral bands, providing information about chemical bonds involved in photodegradation too. Consequently, the main purposes of the current study were: (i) to evaluate and verify *X. parietina* recovery capacity after exposure to simulated space conditions, (ii) to obtain the lichen FTIR reflectance spectrum in not-irradiated conditions and (iii) to monitor the lichen FTIR reflectance spectrum during treatment to highlight eventual changes in the spectrum itself.

## Materials and methods

### Lichen material

For this study, thalli of *X. parietina* were randomly collected in a remote area of Florence province (Tuscany, Italy 43°59'25.09" N 11°13'24.65" E, at 300 m a.s.l.) in June and July 2020. Thalli were dehydrated for 24 h at room temperature (25°C) and stored at –18°C until treatment, as this procedure ensures that thalli remain healthy for later physiological measurements (Paoli *et al.*, 2013). Three days before the treatment, thalli were allowed to slowly recover their normal metabolic conditions in a growth chamber at 25°C and 70 μmol m<sup>-2</sup> s<sup>-1</sup> PAR photons; overnight, thalli were covered with a black cotton cloth and kept moistened by spraying with distilled water (Paoli *et al.*, 2019; Bianchi *et al.*, 2019a).

### Experimental design

Six samples (replicates) of about 1 cm<sup>2</sup> (the size was determined by the sample holder in the UV-radiation apparatus, Fig. S1) were used for each experimental condition: (i) UV-radiation in nitrogen atmosphere at room temperature and atmospheric pressure (UV N<sub>2</sub>); (ii) UV-radiation in vacuum

( $10^0$ – $10^{-2}$  Pa) at room temperature (UV VAC). Six unexposed samples were used as control, kept in a growth chamber at 25°C, under  $70 \mu\text{mol m}^{-2} \text{s}^{-1}$  PAR photons. The UV irradiation time periods were 1 s, 2 s, 5 s, 10 s, 15 s, 30 s, 60 s, 90 s, 120 s, 5 min, 10 min and 15 min. Between one irradiation time period and another one, it was possible to perform *in situ* spectroscopic analyses (see Spectroscopic analysis section). The time recovery for one spectrum was of 60 s. The total UV irradiation period was ~36 min. The total UV irradiance was  $1.34 \text{ MJ m}^{-2}$  for each exposed sample in the lamp wavelength range 185–2000 nm. We simulated UV space radiation using a Newport 300 W Ozone Free Xe-enhanced UV lamp with a sun-like emission spectrum (wavelength range 185–2000 nm, Fig. S2). Because of its sun-like emission spectrum, the Xenon lamp is used for simulating solar radiation outside the atmosphere. The above described atmospheric conditions ( $\text{N}_2$  and VAC) were chosen to set up an extreme and dehydrating environment for the lichen. The conditions of irradiation *in situ* did not allow to reach lower pressure values.

Air-dried samples were weighed before and immediately after the treatments. To evaluate sample viability, photosynthetic efficiency measurements (NDVI and  $F_V/F_M$ , see further) were carried out at different times: before treatment (pre\_exp), immediately after (post\_exp) and 24, 48 and 72 h after the exposure. After UV irradiation samples were kept in a growth chamber at 25°C and  $70 \mu\text{mol m}^{-2} \text{s}^{-1}$  PAR photons, covering overnight the thalli with a black cotton cloth and keeping them moist by spraying with distilled water (Bianchi *et al.*, 2019a; Paoli *et al.*, 2019).

### Photosynthetic efficiency

The efficiency of the photosynthetic system of the lichen *X. parietina* was assessed in terms of spectral reflectance and chlorophyll *a* fluorescence emission. The spectral reflectance of chlorophyll pigments was expressed in terms of Normalized Difference Vegetation Index (NDVI). The NDVI was evaluated with a portable reflectance-based device (Plant Pen NDVI 310 – Photon System Instruments, Czech Republic, 2016). NDVI is directly related to PSII integrity, being associated with the difference in plant reflectance in the visible and near-infrared wavelengths. These differences were used to calculate the NDVI as follows:  $\text{NDVI} = ((\text{NIR}_{740} - \text{VIS}_{660}) / (\text{NIR}_{740} + \text{VIS}_{660}))$ . VIS and NIR are the spectral reflectance measurements acquired respectively in the visible and near-infrared regions (Miloš *et al.*, 2018).

The analysis of the chlorophyll *a* fluorescence was carried out with a portable fluorimeter (Plant Efficiency Analyzer – Handy PEA, Hansatech Ltd, Norfolk, UK). In all experiments, samples were hydrated and dark-adapted for 10 min before fluorescence measurements (Bianchi *et al.*, 2019b). Samples were exposed to a flashing light for 1 s with an excitation pulse ( $3000 \mu\text{mol s}^{-1} \text{m}^{-2}$ ) of red light (650 nm) and the maximum quantum yield of PSII was determined with the parameter  $F_V/F_M = ((F_M - F_0) / F_M)$  where  $F_V$  is the variable fluorescence yield,  $F_M$  is the maximal fluorescence yield and  $F_0$  is the minimal fluorescence yield (van Kooten and Snel, 1990). The maximum quantum yield and variations in  $F_0$ , related to the chlorophyll *a* content of the light harvesting complex (Baruffo and Tretiach, 2007), were evaluated to quantify lichen viability and PSII efficiency.

### Spectroscopic analysis

Spectroscopic analyses were performed *in situ* without moving the samples. IR spectra have been recorded after each period of UV irradiation to monitor changes in the IR spectrum (see Experimental design section). The time required to scan one IR spectrum was 60 s. The irradiation setup consisted of the Harrick High Temperature Reaction Chamber (vacuum chamber, Fig. S1) inserted in the Harrick Praying Mantis™ Diffuse Reflectance Accessory, interfaced to the Bruker VERTEX 70v FTIR (Fornaro *et al.*, 2020), where lichen samples were irradiated and monitored *in situ* with infrared spectroscopy. The technique used to obtain the infrared spectra was the FTIR – Fourier-transform infrared spectroscopy. Spectra were acquired in the wavenumber range from 8000 to  $400 \text{ cm}^{-1}$  with the resolution of  $4 \text{ cm}^{-1}$ . Spectroscopic analysis was performed with the OPUS

software. The UV source was a Newport Xe-enhanced UV Ozone Free 300 W lamp with purified Xe at 5–20 bar (wavelength range 185–2000 nm, Fig. S2). Xe arc lamps have a relatively smooth emission curve in the UV-VIS spectrum, with sun-like emission spectrum and 5800 K colour temperature. The Xe lamp is hosted in the Newport Research Arc Lamp Housing 50–500 W (Corazzi *et al.*, 2020). The UV-radiation emitted by the lamp is collimated through an optical system consisting of a first mirror that reflects the radiation coming from the lamp to a grade fused silica collimating condenser lens (type Fused Silica Asphere, f-number  $f/2.2$ ), which directs UV-radiation towards an optical fibre with an aperture of 800  $\mu\text{m}$  (Poggiali *et al.*, 2020).

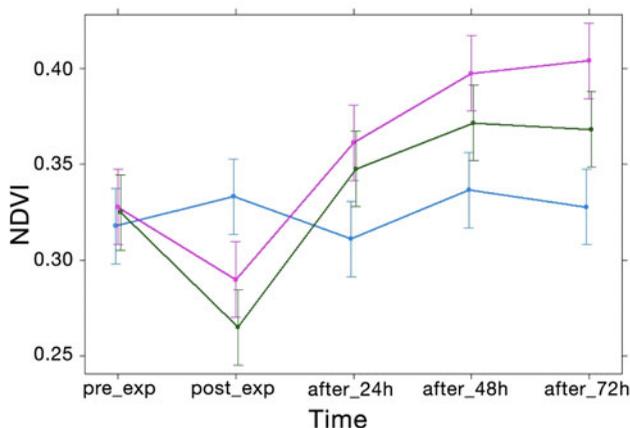
### Data analysis

The data of lichen physiological parameters (NDVI and  $F_V/F_M$ ) were analysed fitting linear mixed-effects models (LMMs) in a repeated measurement ANOVA design, using thallus identity as a random effect factor to account for the temporal correlation of observations. Time was used as an ordinal variable because the relationship between NDVI or  $F_V/F_M$  values and time was not a simple linear regression. The physiological effects of the treatment were investigated using NDVI and  $F_V/F_M$  values as response variables and the two treatment conditions explored (UV N<sub>2</sub> and UV VAC) and time of recovery as explanatory variables in a full factorial design. ANOVA type III table, with Satterthwaite's method was used to verify the significance of the fixed effects and of associated interaction factors. The analyses were run with the free software R. LMM computations were performed using the *lmer* function of the *lme4* package version 1.1–12 for fitting the models. Significant changes in lichen mass at the end of the experiment by different treatment conditions were also examined by means of ANOVA with Tukey's post-hoc test (with at least  $p < 0.05$  as the significance level). For each analysis, data normality was checked with the Shapiro–Wilk test.

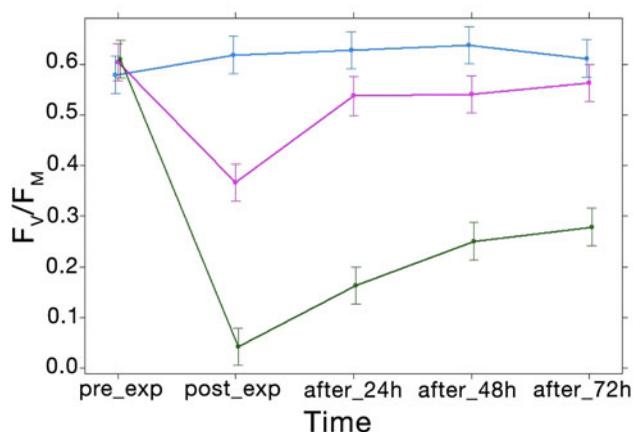
## Results

### Physiological response

The results of the physiological response of *X. parietina* samples under the different experimental conditions are shown in Fig. 1 (NDVI) and Fig. 2 ( $F_V/F_M$ ). Once samples were removed from the Harrick High Temperature Reaction Chamber, they showed significantly lower NDVI values (mean  $\pm$  SD:  $0.2898 \pm 0.07$  UV N<sub>2</sub> and  $0.2647 \pm 0.01$  UV VAC) compared with the respective pre-exposure values ( $0.3278 \pm 0.06$  UV N<sub>2</sub>,  $0.3251 \pm 0.08$  UV VAC) for both treatments ( $p < 0.001$ ) (Tables S1 and S2). After 72 h in the recovery conditions, samples that reached significantly higher values ( $0.4041 \pm 0.07$ ) were those treated in UV N<sub>2</sub>, compared to UV VAC samples ( $0.3682 \pm 0.06$ ) and control ones ( $0.3277 \pm 0.02$ ) ( $p < 0.001$ ) (Tables S1 and S2). The maximum quantum yield of primary photochemistry ( $F_V/F_M$ ) changed significantly after the treatment in both the experimental conditions ( $p < 0.001$ ) (Table S3).  $F_V/F_M$  values of the samples treated in UV N<sub>2</sub> showed a significant decrease compared to pre-exposure ones ( $0.607 \pm 0.02$ , pre\_exp and  $0.375 \pm 0.1$ , post\_exp) ( $p < 0.001$ ) (Fig. 2 and Table S3). The same trend was observed for the UV VAC samples, which reached lower values ( $0.042 \pm 0.02$ ) than the initial ones ( $0.611 \pm 0.07$ , with  $p < 0.001$ ) (Tables S3 and S4), crossing the threshold of 0.200, which is considered consequent to photoinhibition (Long *et al.*, 1994). Once the recovery period began,  $F_V/F_M$  values increased significantly depending on the treatments ( $p < 0.001$ ) (Table S3). Samples exposed to UV N<sub>2</sub> recovered already after 24 h, reaching values comparable to the starting ones after 72 h (Fig. 2). On the other hand, the efficiency of the samples exposed to UV VAC showed a gradually increasing trend, reaching  $0.163 \pm 0.1$  after 24 h,  $0.251 \pm 0.1$  after 48 h and  $0.273 \pm 0.1$  after 72 h, without reaching initial values (Fig. 2 and Table S3). Control samples were daily hydrated and kept in dark-adapted conditions.  $F_V/F_M$  of control values ranged during the experiment between  $0.579 \pm 0.05$  and  $0.638 \pm 0.03$  (Fig. 2 and Table S4).  $F_0$  values changed significantly after the exposure in both the experimental conditions ( $p < 0.001$ ) (Tables S5, S6 and Fig. S3). A relative increase in  $F_0$



**Fig. 1.** Normalized Difference Vegetation Index (NDVI) of *Xanthoria parietina* before (*pre\_exp*), immediately after (*post\_exp*) and 24 h, 48 h and 72 h after the exposure. Cyan line = control; magenta line = UV N<sub>2</sub>; green line = UV VAC. Error bars stand for confidence intervals. See Table S1 for ANOVA results (Supplemental Information).



**Fig. 2.** Variation in *Xanthoria parietina* of the photosystem II ( $F_v/F_m$ ) efficiency before (*pre\_exp*), immediately after (*post\_exp*) and 24 h, 48 h and 72 h after the treatment. Cyan line = control; magenta line = UV N<sub>2</sub>; green line = UV VAC. Error bars stand for confidence intervals. See Table S3 for ANOVA results (Supplemental Information).

followed the exposure of UV N<sub>2</sub> until the initial values were reached after 72 h of recovery.  $F_0$  values strongly decreased in samples exposed to UV VAC compared with those treated with UV N<sub>2</sub> treatment and control ones. UV VAC  $F_0$  values declined even in the recovery period reaching significantly lower values ( $225 \pm 32$ ) (Table S8 and Fig. S3). The samples were also weighed before and after the treatments (Table S7) and it turned out that there is no statistical difference in mass variation between treatments (Table S8).

### IR analysis: band assignments

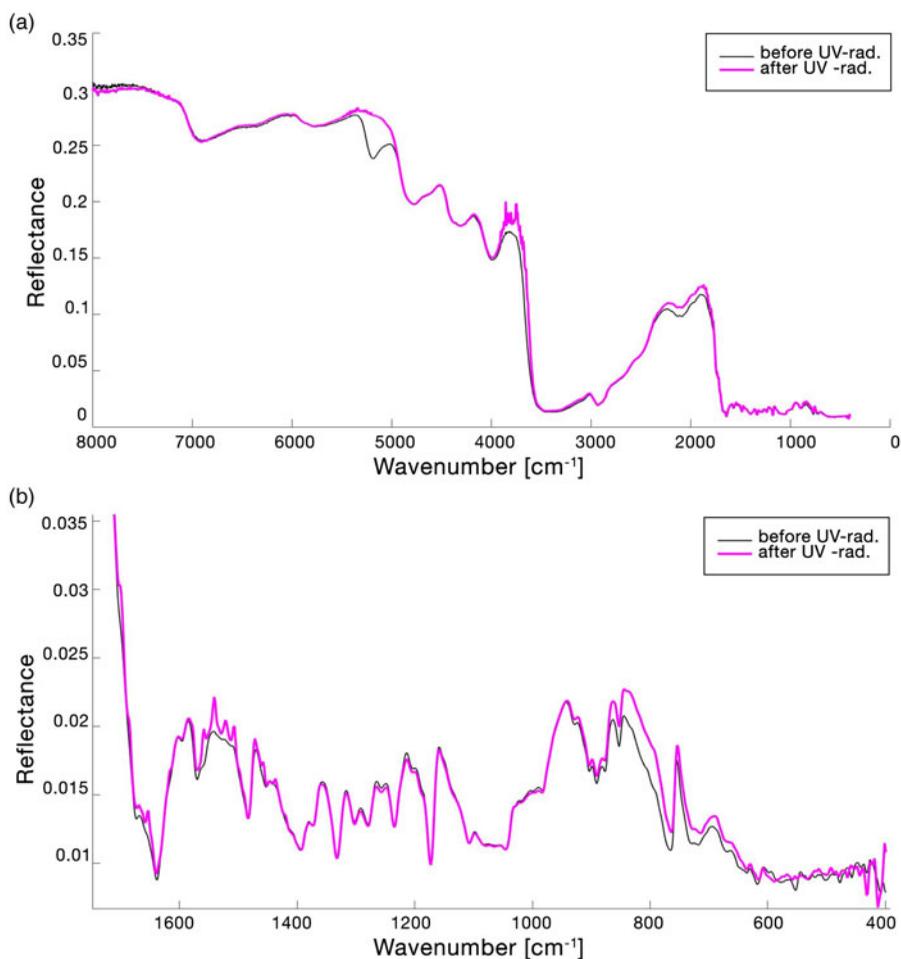
During the experiment, the FTIR spectrum was evaluated for each lichen sample. Slight differences in bands' peak intensities were noticed between samples spectra but all the mentioned bands were noticed in all the lichens spectra. Band assignments of *X. parietina* spectrum were based on theoretical data

**Table 1.** Band assignments for *Xanthoria parietina* FTIR reflectance spectrum

Peak position [cm <sup>-1</sup> ]	Band assignments	Reference
5200	$\nu(\text{H}_2\text{O}) + \delta(\text{H}_2\text{O})$ ( $\text{H}_2\text{O}_m$ )	Bishop <i>et al.</i> (2013); Gerber <i>et al.</i> (2011); Granlund <i>et al.</i> (2018); McIntosh <i>et al.</i> (2017)
3500	$\nu(\text{H}_2\text{O}) + \nu(\text{OH})$	Bishop <i>et al.</i> (2013); Coates (2000); McIntosh <i>et al.</i> (2017); Stuart (2004)
2930	$\nu(-\text{CH})$ asym./sym. + $\nu(=\text{CH}_2)$ asym./sym.	Coates (2000); Stuart (2004)
1730	$\nu(\text{C}=\text{O})$	Stuart (2004)
1675	$\nu(\text{C}=\text{O})$ conjugated, free	Edwards <i>et al.</i> (2003); Stuart (2004)
1630	$\delta(\text{H}_2\text{O})$ ( $\text{H}_2\text{O}_m$ )	Bishop <i>et al.</i> (2013); McIntosh <i>et al.</i> (2017); Stuart (2004)
1595–1570–1560	$\nu(\text{C}=\text{C})$ aromatic	Coates (2000); Edwards <i>et al.</i> (2003); Stuart (2004)
1483	Ring stretch, in plane	Edwards <i>et al.</i> (2003)
1395–1373	$\nu(\text{C}-\text{O})$ phenyl	
1333	Ring stretch, in plane	
1172	$\delta(\text{OH})$ phenyl, free, in plane	
1107	$\nu(\text{C}-\text{CH})$ in plane	
1045	$\nu(\text{C}-\text{O})$ aromatic ether	Coates (2000); Edwards <i>et al.</i> (2003)
983	Ring breathing, $\delta(\text{C}-\text{H})$ out of plane	Coates (2000); Edwards <i>et al.</i> (2003); Stuart (2004)
928	$\delta(\text{C}-\text{H})$ out of plane	
765	$\nu(\text{C}-\text{H})$ aromatic, out of plane	Edwards <i>et al.</i> (2003)

Vibrational modes legend:  $\nu$ , stretching;  $\delta$ , bending.  $\text{H}_2\text{O}_m$  stands for molecular water.

from the literature (Stuart, 2004; Coates, 2000) and experimental data from Edwards *et al.* (2003) IR spectroscopy characterization of the lichen substance parietin, extracted from *R. elegans* and *X. parietina* samples. Gerber *et al.* (2011) and Granlund *et al.* (2018) were used as reference to assign 5200 cm<sup>-1</sup> band to H<sub>2</sub>O. Band assignments are reported in Table 1. The lichen FTIR spectrum can be seen in Fig. S4 and ranged between 8000 and 400 cm<sup>-1</sup> wavenumbers. The reported vibrational modes ‘ $\nu$ ’ and ‘ $\delta$ ’ stand for stretching and bending. Bands at 5200 and 1630 cm<sup>-1</sup> were assigned to H<sub>2</sub>O<sub>m</sub> (molecular water). In particular, 5200 cm<sup>-1</sup> was assigned to the stretch and the bend of H<sub>2</sub>O modes (Gerber *et al.*, 2011; Bishop *et al.*, 2013; McIntosh *et al.*, 2017; Granlund *et al.*, 2018). The band at 1630 cm<sup>-1</sup> was referred to the H<sub>2</sub>O bend mode (Stuart, 2004; McIntosh *et al.*, 2017) and the 3500 cm<sup>-1</sup> band is characteristic for bend H<sub>2</sub>O and OH mode. The band at 2930 cm<sup>-1</sup> was assigned to the  $\nu(-\text{CH})$  and  $\nu(=\text{CH}_2)$  antisymmetric and symmetric modes. Furthermore, it was noticed that there were vibrational modes distributed in a particular region from 1750 and 400 cm<sup>-1</sup> (Fig. S5). From Edwards *et al.* (2003), it comes out that the aforementioned region is particularly relevant for parietin vibrational modes. The band at 1675 cm<sup>-1</sup> was assigned to the  $\nu(\text{C}=\text{O})$  conjugated and free vibrational modes (Stuart, 2004; Edwards *et al.*, 2003) and the 1595, 1570 and 1560 cm<sup>-1</sup> bands were referred to the aromatic (C=C) stretching mode (Stuart, 2004; Edwards *et al.*, 2003; Coates, 2000). The two bands at 1483 and 1333 cm<sup>-1</sup> were both assigned to the ring stretch, the first one coupled with OH and the other one with in-plane bending mode (Edwards *et al.*, 2003). Bands at 1395 and 1373 cm<sup>-1</sup> are due to the phenyl  $\nu(\text{C}-\text{O})$  (Edwards *et al.*, 2003). A strong band at 1172 cm<sup>-1</sup> was referred to the phenyl, free and in plane OH bending (Edwards *et al.*, 2003). 1107 cm<sup>-1</sup> band was specified for (C–CH)



**Fig. 3.** (a) FTIR reflectance spectrum of the lichen *Xanthoria parietina* in the range 8000–400  $\text{cm}^{-1}$  and (b) in the range 1750–400  $\text{cm}^{-1}$  before and after UV exposure in  $\text{N}_2$ . Band changes were evaluated normalizing the pre-exposure spectrum and the after-exposure spectrum at 7000  $\text{cm}^{-1}$ .

stretching in plane (Edwards *et al.*, 2003) and 1045  $\text{cm}^{-1}$  band was assigned to aromatic ether (C–O) stretching (Edwards *et al.*, 2003; Coates, 2000). The band at 983  $\text{cm}^{-1}$  was referred to the ring breathing and the out of plane (C–H) bending mode (Stuart, 2004; Edwards *et al.*, 2003; Coates, 2000), while 928 and 765  $\text{cm}^{-1}$  were assigned respectively to the out of plane  $\delta(\text{C–H})$  (Stuart, 2004; Edwards *et al.*, 2003; Coates, 2000) and the out of plane and aromatic  $\nu(\text{C–H})$  vibrational modes (Edwards *et al.*, 2003).

### IR analysis: spectral changes in UV $\text{N}_2$

The full FTIR spectrum of the lichen *X. parietina*, before and after irradiation for UV  $\text{N}_2$  treatment, is reported in Fig. 3(a) and the change in the spectrum in the range 1750–400  $\text{cm}^{-1}$  is shown in Fig. 3(b). Table 2 reports the peak shifting ( $\Delta\tilde{\nu}$ ) and the qualitative assessments of the changes of the assigned band features due to UV  $\text{N}_2$  treatment (~36 min irr.). The changes in the spectrum *continuum* were evaluated normalizing the pre-exposure spectrum and the post-exposure spectrum at 7000  $\text{cm}^{-1}$ . Figure 4 shows the details of UV  $\text{N}_2$  spectral changes. As it is reported in Figs 3(a) and 4(a), there is a remarkable variation of the peak reflectance in 5200  $\text{cm}^{-1}$  band ( $\nu(\text{H}_2\text{O}) + \delta(\text{H}_2\text{O})$ ), which disappears completely after treatment. A general spectrum upshifting is observed. Bands at 3500  $\text{cm}^{-1}$

**Table 2.** FTIR reflectance spectrum of *Xanthoria parietina* after ~36 min of UV irradiation in N<sub>2</sub>

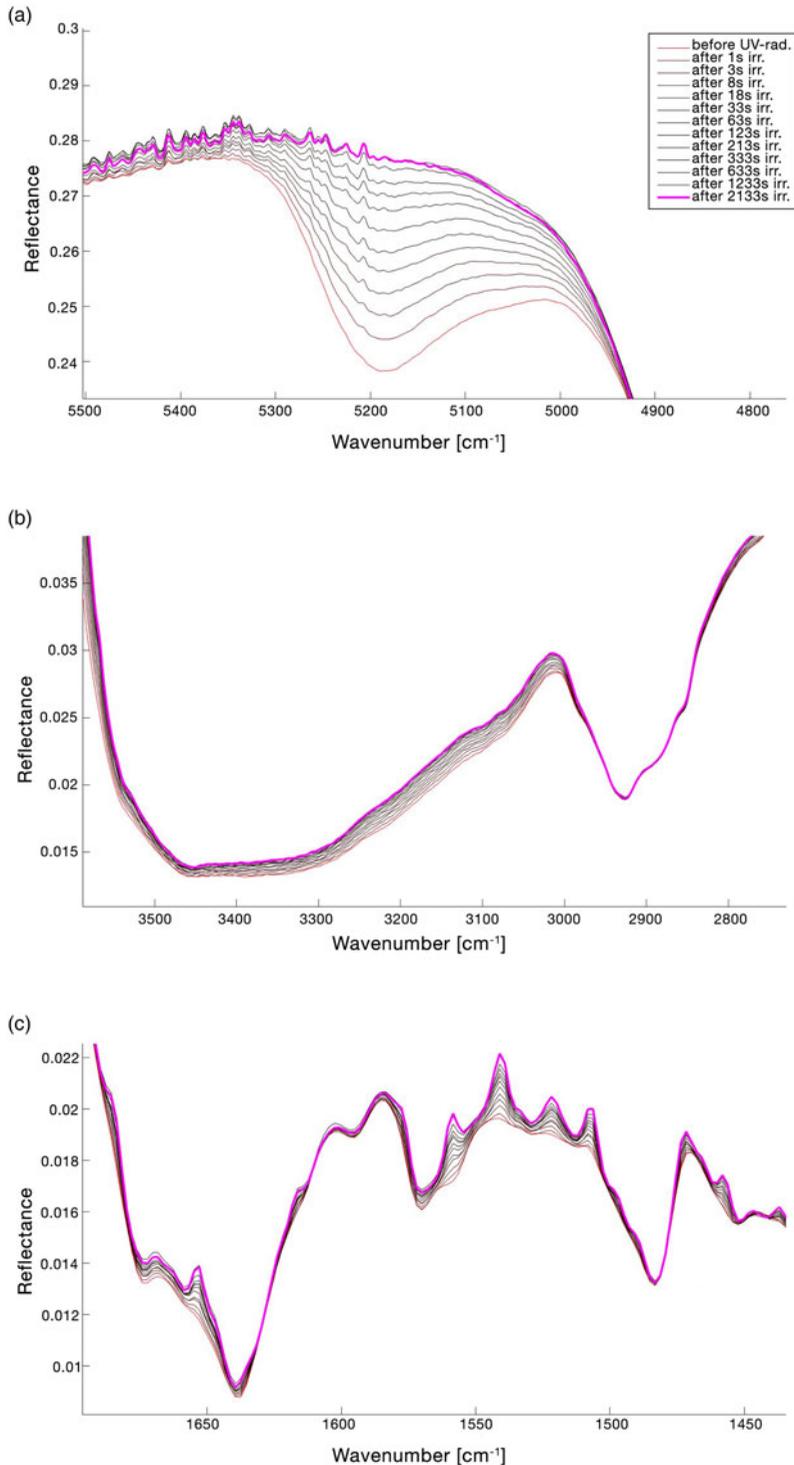
Peak position [cm <sup>-1</sup> ] before UV-irr.	$\Delta\tilde{\nu}$ [cm <sup>-1</sup> ]	Qualitative bands changes assessment	Band assignments
5200		Complete disappearance	$\nu(\text{H}_2\text{O}) + \delta(\text{H}_2\text{O})$ (H <sub>2</sub> O <sub>m</sub> )
3500	-8	Intensity decrease	$\nu(\text{H}_2\text{O}) + \nu(\text{OH})$
2930	-2	Slight intensity decrease	$\nu(-\text{CH})$ asym./sym. + $\nu(=\text{CH}_2)$ asym./sym.
1730	-	Intensity decrease	$\nu(\text{C}=\text{O})$
1675	-2	Intensity decrease	$\nu(\text{C}=\text{O})$ conjugated, free
1630	+2	Intensity decrease	$\delta(\text{H}_2\text{O})$ (H <sub>2</sub> O <sub>m</sub> )
1595	-	Slight intensity decrease	$\nu(\text{C}=\text{C})$ aromatic
1570	-	Intensity decrease	
1560	-3	Intensity decrease	
1483	-	Slight intensity decrease	Ring stretch, in plane
1395	-1	Slight intensity decrease	$\nu(\text{C}-\text{O})$ phenyl
1373	-	Slight intensity decrease	
1333	-	Slight intensity increase	Ring stretch, in plane
1172	-	Slight intensity increase	$\delta(\text{OH})$ phenyl, free, in plane
1107	-	Slight intensity decrease	$\nu(\text{C}-\text{CH})$ in plane
1045	-	Slight intensity decrease	$\nu(\text{C}-\text{O})$ aromatic ether
983	-	Slight intensity increase	Ring breathing, $\delta(\text{C}-\text{H})$ out of plane
928	-	Intensity decrease	$\delta(\text{C}-\text{H})$ out of plane
765	-2	Highly intensity decrease	$\nu(\text{C}-\text{H})$ aromatic, out of plane

Band positions were evaluated normalizing the pre-exposure spectrum and the after-exposure spectrum at 7000 cm<sup>-1</sup>. Vibrational modes legend:  $\nu$ , stretching;  $\delta$ , bending. H<sub>2</sub>O<sub>m</sub> stands for molecular water.  $\Delta\tilde{\nu}$  stands for wavenumber shift with respect to the same peak before UV irradiation. Blank cells stand for too weak differences to evaluate or null variations

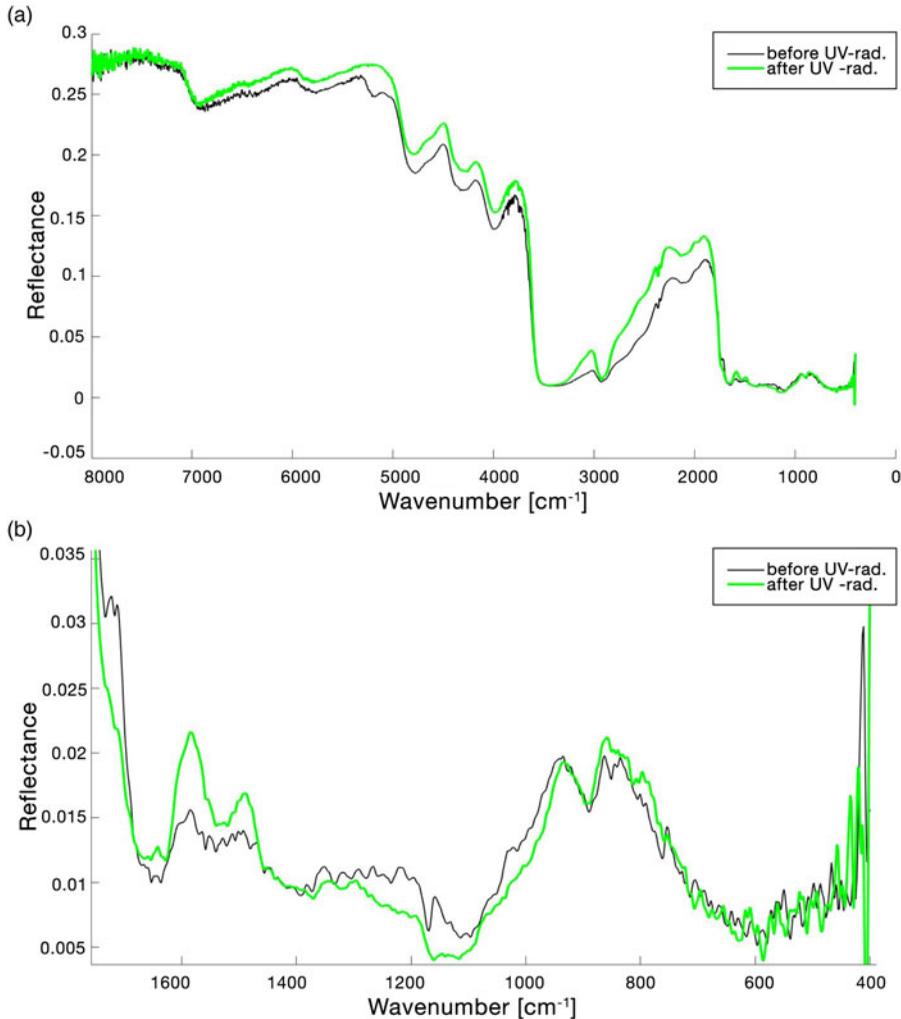
( $\nu(\text{H}_2\text{O}) + \nu(\text{OH})$ ) and 2930 cm<sup>-1</sup> ( $\nu(-\text{CH})$  asym./sym. +  $\nu(=\text{CH}_2)$  asym./sym.) show respectively -8 and -2 cm<sup>-1</sup> variation in the wavenumber peak. In Fig. 4(c), the formation of new bands in the range 1550–1450 cm<sup>-1</sup> is observed. The new observed bands are 1529 and 1512 cm<sup>-1</sup> (already recognized as shoulders in the pre-exposure spectrum), 1501, 1493, 1462, 1441 and 1423 cm<sup>-1</sup>. Furthermore, the 1400–900 cm<sup>-1</sup> spectral region shows a general down peak shifting (Table 2 and Fig. 3(b)).

### IR analysis: spectral changes in UV VAC

The full FTIR spectrum of the lichen *X. parietina* before and after UV VAC treatment is shown in Fig. 5(a) and the global change in spectrum in the range 1750–400 cm<sup>-1</sup> is reported in Fig. 5(b). Table 3 reports the peak shifting ( $\Delta\tilde{\nu}$ ) and qualitative assessment of the identified band changes after treatment. Changes in the spectrum *continuum* were evaluated normalizing the pre-exposure spectrum and the post-exposure spectrum at 7000 cm<sup>-1</sup>. Differently from UV N<sub>2</sub> treatment, it was decided that UV irradiation in vacuum should start when pressure reached 0.60 Pa to normalize the starting conditions for each sample. The UV-waiting time period necessary to reach 0.60 Pa could be 20–60 min since the pump was turned on. The sample reported in Table 3, Figs 5 and 6 had a UV-waiting period of 24 min. As it is shown in Fig. 6(a), 5200 cm<sup>-1</sup> band ( $\nu(\text{H}_2\text{O}) + \delta(\text{H}_2\text{O})$ ) disappeared before starting to UV-irradiate the sample. Bands at 3500 cm<sup>-1</sup> ( $\nu(\text{H}_2\text{O}) + \nu(\text{OH})$ ) and 2930 cm<sup>-1</sup> ( $\nu(-\text{CH})$  asym./sym. +  $\nu(=\text{CH}_2)$  asym./sym.) showed respectively null and -6 cm<sup>-1</sup> variation in wavenumber peak shifting with a visible change in bands area. It is noticeable that the upshifting of the *continuum* is higher than the UV N<sub>2</sub> one (Fig. 5(a)). Observing the range 1700–1450 cm<sup>-1</sup> (Fig. 6(c)), from the UV-waiting period spectra to the first 10–20 s of UV-radiation spectra, a down peak shifting trend



**Fig. 4.** Details of the lichen IR spectrum changes for UV N<sub>2</sub> treatment. The legend of the lines' colours showed in (a) is also referred to (b) and (c). (a) Band at 5200 cm<sup>-1</sup> ( $\nu(\text{H}_2\text{O}) + \delta(\text{H}_2\text{O})$ ); (b) band at 3500 cm<sup>-1</sup> ( $\nu(\text{H}_2\text{O}) + \nu(\text{OH})$ ) and band at 2930 cm<sup>-1</sup> ( $\nu(-\text{CH})$  asym./sym. +  $\nu(=\text{CH}_2)$  asym./sym.); (c) general changes in the spectral range 1700–1450 cm<sup>-1</sup>. Band changes were evaluated normalizing the pre-exposure spectrum and the after-exposure spectrum at 7000 cm<sup>-1</sup>.



**Fig. 5.** (a) FTIR reflectance spectrum of the lichen *Xanthoria parietina* in the range 8000–400  $\text{cm}^{-1}$  and (b) in the range 1800–400  $\text{cm}^{-1}$  before and after UV exposure in VAC. Band changes were evaluated normalizing the pre-exposure spectrum and the after-exposure spectrum at 7000  $\text{cm}^{-1}$ .

was noticeable, then the spectrum continued to upshift. Furthermore, in the range 1400–400  $\text{cm}^{-1}$ , a general wavenumber peak shifting is observed, as it is reported in Table 3 and Fig. 5(b). In the range 1400–950  $\text{cm}^{-1}$ , a down peak shifting in reflectance was recorded (Fig. 5(b)).

## Discussion

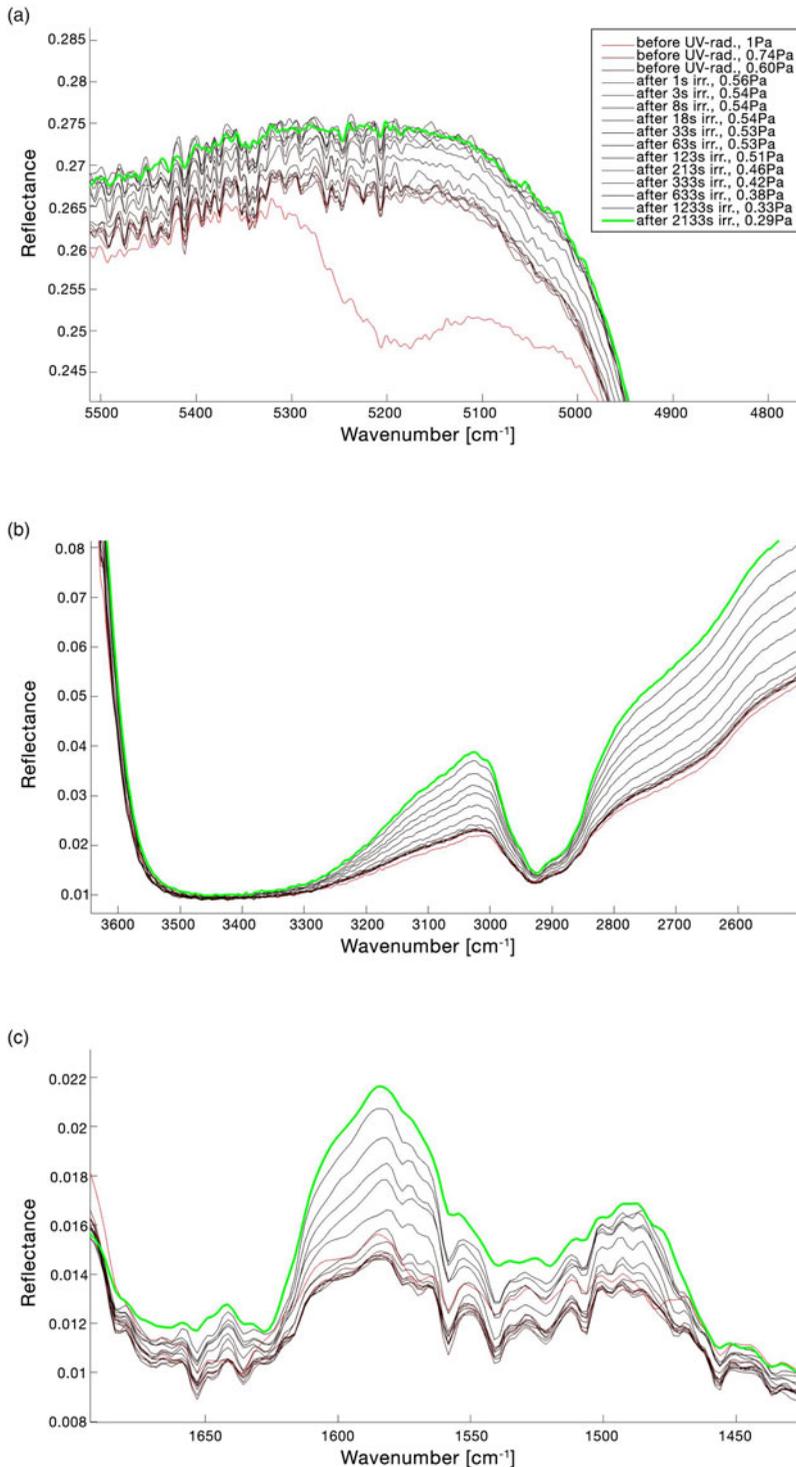
The lichen *X. parietina* survived the simulated extreme conditions used in this study. UV-radiation, able to photo-dissociate chemical bonds, and high vacuum, first cause of extreme dehydration (Sancho *et al.*, 2007), are two of the most lethal factors that make outer space an inhospitable environment. The two simulated treatments (UV N<sub>2</sub> and UV VAC) affected differently the photosynthetic parameters, showing remarkable differences in *X. parietina* recovery trends. UV N<sub>2</sub> treatment affected post-exposure values of the photobiont metabolic activity. Noteworthy, in the 72 h of recovery period, NDVI values recovered more than the 100%, overcoming the starting ones. Actually, the recovery conditions – offering daily water spraying – increased thallus hydration and consequently increased NDVI

**Table 3.** FTIR reflectance spectrum of *Xanthoria parietina* after ~36 min of UV irradiation in VAC

Peak position [cm <sup>-1</sup> ] before UV-irr.	$\Delta\tilde{\nu}$ [cm <sup>-1</sup> ]	Qualitative bands changes assessment	Approximate band assignments
5200		Complete disappearance	$\nu(\text{H}_2\text{O}) + \delta(\text{H}_2\text{O})$ ( $\text{H}_2\text{O}_m$ )
3500	–	Slight intensity decrease and high decrease in band area	$\nu(\text{H}_2\text{O}) + \nu(\text{OH})$
2930	–6	Intensity decrease and high decrease in band area	$\nu(-\text{CH})$ asym./sym. + $\nu(=\text{CH}_2)$ asym./sym.
1730	–2	Intensity increase	$\nu(\text{C}=\text{O})$
1675	Not evaluable		$\nu(\text{C}=\text{O})$ conjugated, free
1630	–8	Intensity decrease	$\delta(\text{H}_2\text{O})$ ( $\text{H}_2\text{O}_m$ )
1595	–	Highly intensity decrease	$\nu(\text{C}=\text{C})$ aromatic
1570	+6	Highly intensity decrease	
1560	+2	Highly intensity decrease	
1483	–	Highly intensity decrease	Ring stretch, in plane
1395	Not evaluable		$\nu(\text{C}-\text{O})$ phenyl
1373	–2	Slight intensity increase	
1333	–8	Slight intensity increase	Ring stretch, in plane
1172	–8	Intensity increase	$\delta(\text{OH})$ phenyl, free, in plane
1107	Not evaluable		$\nu(\text{C}-\text{CH})$ in plane
1045	Not evaluable		$\nu(\text{C}-\text{O})$ aromatic ether
983	–12	Intensity increase	Ring breathing, $\delta(\text{C}-\text{H})$ out of plane
928	Not evaluable		$\delta(\text{C}-\text{H})$ out of plane
765	–3	Intensity decrease	$\nu(=\text{C}-\text{H})$ aromatic, out of plane

Band positions were evaluated normalizing the pre-exposure spectrum and the after-exposure spectrum at 7000 cm<sup>-1</sup>. Vibrational modes legend:  $\nu$ , stretching;  $\delta$ , bending.  $\text{H}_2\text{O}_m$  stands for molecular water.  $\Delta\tilde{\nu}$  stands for wavenumber shift with respect to the same peak before UV irradiation. Blank cells stand for too weak differences to evaluate or null variations. ‘Not evaluable’ stands for those bands that show too high noise or peak overlapping.

values, as reported in Barták *et al.* (2018) for the lichen, *Dermatocarpon polyphyllizum* (Nyl.) Blomb. & Forssell. According to Miloš *et al.* (2018), NDVI variation related to thallus water content is species-specific, such as the positive correlation noticeable in *Physconia muscigena* (Ach.) Poelt, while in *R. elegans*, the NDVI showed constant values for different thallus water contents. In UV VAC conditions, the same NDVI recovery pattern was observed. Nevertheless, significant differences between treatments were recorded in chlorophyll *a* fluorescence value. UV N<sub>2</sub> treatment produced a decrease in samples  $F_v/F_M$  post-exposure values, recovering 86% of the starting ones in 24 h and 93% in 72 h. In this study, the integrity of the photobiont belonging to the lichen symbiosis was evaluated, but it is well known that the mycobiont is involved in the protection of the photobiont from UV-radiations (see e.g. Bianchi *et al.*, 2019b and references therein). In the case of *X. parietina*, this protection is mainly achieved by the production of the anthraquinone parietin, a compound secreted by the fungus as a secondary lichen metabolite (Solhaug and Gauslaa, 2004). Photosynthetic activity recovery may be explained considering also the thallus structure, playing an efficient role in UV rays screening and avoiding damages to the photobiont layer (Collins and Farrar, 1978; Solhaug *et al.*, 2003; Solhaug and Gauslaa, 2004). On the other hand, samples treated in UV VAC conditions were strongly photo-inhibited reaching only 7% of the starting  $F_v/F_M$  values. After that, lichens recovered 45% of the initial values after the 72 h recovery period. This treatment proved to be more impacting on lichen photosynthetic performance, likely because of the combined effect of UV-radiation and vacuum application, as suggested by de Vera *et al.* (2004). A possible explanation of the difference between the recovery



**Fig. 6.** Details of the lichen IR spectrum changes for UV VAC treatment. The legend of the lines' colours showed in (a) is also referred to (b) and (c). (a) Band at  $5200\text{ cm}^{-1}$  ( $\nu(\text{H}_2\text{O}) + \delta(\text{H}_2\text{O})$ ); (b) band at  $3500\text{ cm}^{-1}$  ( $\nu(\text{H}_2\text{O}) + \nu(\text{OH})$ ) and band at  $2930\text{ cm}^{-1}$  ( $\nu(-\text{CH})\text{ asym./sym.} + \nu(=\text{CH}_2)\text{ asym./sym.}$ ); (c) general changes in the spectral range  $1700\text{--}1450\text{ cm}^{-1}$ . Band changes were evaluated normalizing the pre-exposure spectrum and the after-exposure spectrum at  $7000\text{ cm}^{-1}$ .

trends in the physiological parameters (NDVI and  $F_V/F_M$ ) may be that NDVI is usually correlated to chlorophyll content (Garty *et al.*, 2001). The intra-thalline water content in lichens (Granlund *et al.*, 2018) is the reason why NDVI can be used to make an indirect evaluation of primary photosynthesis (Bednaříková *et al.*, 2020). On the contrary,  $F_V/F_M$  is more related to chlorophyll damage (Zhang *et al.*, 2017). Hence, we can suppose that, while carotenoids are similarly affected by both treatments, UV VAC may affect more deeply the chlorophyll content inducing a delay in photosynthesis reactivation, with a consequent longer recovery time, as proposed by Kranner *et al.* (2008). The longer recovery time may be related to the  $F_0$  decreasing values after exposure in UV VAC samples (Fig. S3). These findings suggest that some lichen species may be considered extremotolerant, representing excellent test-organism for space survival studies. Their ability to regulate water content depending on the environmental water availability (poikilohydric organisms) allow them to tolerate wet–dry cycles including thallus water fluctuations from 20 to 160% water per thallus dry weight (Kappen, 1988). These features may have contributed to the survival capability of samples in the extreme dehydration conditions such as the simulated ones here. On the spectroscopical side, the IR band at  $5200\text{ cm}^{-1}$  ( $\nu(\text{H}_2\text{O}) + \delta(\text{H}_2\text{O})$ ) disappeared in the two performed treatments, suggesting a water loss from the lichen. Lichens are able to resist to dehydration cycles, longer desiccation periods may produce permanent cellular collapses and consequently affect the metabolic activity (Brandt *et al.*, 2016). The treatments of nitrogen and vacuum are considered as the main cause of dehydration process in the samples here analysed. For this reason, it can be assumed that the  $5200\text{ cm}^{-1}$  band decrease, associated to thalli water release, is more strictly related to the Reaction Chamber atmosphere than to the UV-radiation. In both the treatments, it is also noticeable a general upshifting of the spectral continuum due to the UV-radiation effects, which is higher in vacuum conditions. A particular band change trend is observed in UV VAC spectral range  $1400\text{--}950\text{ cm}^{-1}$ , where the band peak intensities become deeper, apparently suggesting an increase related to those vibrational modes during the exposure. Lichen samples were not able to biosynthesize any secondary metabolic substances during both exposures because they were presumably inactivated and dehydrated by the extreme simulated conditions. Despite the increasing of peak intensities, the spectrum showed a decreasing spectral contrast for the overall bands suggesting a decreasing of the bands area linked to the number of chemical bonds associated to that particular frequency. This fact may be interpreted as a direct damage of molecular bonds due to UV irradiation. Physiological parameters suggested that vacuum application may cause stronger damages due to a higher dehydration effect. According to Gauslaa and Solhaug (1999) air-dried lichen thalli exposed to high light exposure show a time-lag in fluorescence recovery trends, as observed in this study. As a consequence of the more intense UV-radiation effects in vacuum, we observed several spectral band changes related to damage and extensive photodegradation of the thallus. Moreover, we observed in both treatments a decrease in peak intensity of those bands related to  $-\text{CH}$  groups on aromatic rings, which are probably related to parietin. Further studies may be performed with the aim of confirming the possibility of thallus morphological and ultrastructural damages and parietin photodegradation under UV-radiation.

## Conclusions

Our work analysed two different environmental conditions: (i) vacuum and room temperature and (ii) saturated nitrogen atmosphere at room temperature and atmospheric pressure. These two conditions – simulated in laboratory – can be associated to different space environments, such as those found in the proximity of Earth orbit for vacuum simulation and saturated nitrogen atmosphere may be comparable to several planetary conditions (i.e. early Earth, Mars, possible altered terrestrial scenarios, exoplanets). The examination of *X. parietina* recovery through eco-physiological analysis revealed the capability of this lichen species to survive in extreme conditions such as those simulated in this investigation. It has been highlighted the significant difference between treatments about the PSII efficiency ( $F_V/F_M$ ) and PSII integrity (NDVI) recovery trends, finding that UV-radiation in vacuum produces more intense effects on  $F_V/F_M$  values than UV N2. After 72 h, UV N2 fluorescence mean values recovered up to

93% of the starting ones, while UV VAC fluorescence recovered up to 45% of the pre-exposure values. Moreover, the study introduces the first scanned FTIR reflectance spectrum of the lichen species *X. parietina*. Besides, the experiment comprised a spectral monitoring during the treatment phase in order to evaluate band changes due to the exposure conditions. Spectral analysis suggests that the relation between mass variation and H<sub>2</sub>O 5200 cm<sup>-1</sup> band disappearance after treatment is strictly linked to the thalli dehydration due to the atmospheric simulated conditions represented by N<sub>2</sub> insufflation and high vacuum application. As stated in the introduction, one of the main objects of this study was to increase our knowledge about the potential adaptive capability of organisms in extreme environments. Our results, for the first time, have investigated the survival of *X. parietina* with multiple analytical techniques and in particular we performed IR spectroscopy *in situ* during treatments. The study highlighted that the recovery performances of the lichen – kept in a growth chamber at 25°C with 12 h dark and 12 h light 70 μmol m<sup>-2</sup> s<sup>-1</sup> PAR photons, daily moistened – were better after the exposure to solar-like UV-radiation in conditions close to extreme planetary environments (i.e. saturated nitrogen atmosphere in our ground-based experiment) than those in pure space conditions (i.e. vacuum).

**Supplementary material.** The supplementary material for this article can be found at <https://doi.org/10.1017/S1473550422000076>.

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**Conflict of interest.** None.

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