

# Water regulation dynamics of lichens as functional traits could predict future climate change scenarios in an elevational gradient from Central Argentina

Raúl Díaz Dominguez<sup>1,4</sup>, Daniel Stanton<sup>2</sup>, Mariana Peralta<sup>3</sup> & Juan Manuel Rodriguez<sup>1</sup>

<sup>1</sup> Instituto de Investigaciones Biológicas y Tecnológicas, Centro de Ecología y Recursos Naturales Renovables, Facultad de Ciencias Exactas, Físicas y Naturales. (CONICET – Universidad Nacional de Córdoba. Present address: Av. Velez Sarsfield 1611. Córdoba, Argentina; <sup>2</sup> Department of Ecology, Evolution and Behavior, University of Minnesota, St. Paul, MN 55455, U.S.A. Present address: 1479 Gortner Avenue, St Paul, MN 55108, U.S.A.; <sup>3</sup> Instituto Multidisciplinario de Biología Vegetal. (CONICET – UNC) y Facultad de Ciencias Químicas – UNC. Present address: Medina Allende N°1998. Córdoba, Argentina

**ABSTRACT.** Mountain top environments are particularly vulnerable to climate change effects, given that biological organisms in these systems live at specific temperature conditions. The poikilohydric nature of lichens emphasizes variables like water holding capacity (WHC) and the hydrophobicity of the thallus to understand the species occupational patterns in altitudinal gradients and microsites. WHC and hydrophobicity were measured in 3 saxicolous species with 2 different morphologies in an elevational mountain gradient of Central Argentina: *Usnea amblyoclada*, *Parmotrema reticulatum* and *Parmotrema warmingii*. We measured WHC in three elevations corresponding to the distribution range of the species, and 3 microsite conditions: north (equatorial-facing) / south (polar-facing) aspect with high steep inclination >70° and rock outcrops with low steep inclination <20°. Results show differences between *U. amblyoclada*, *P. reticulatum*, and *P. warmingii* for measured traits. Hydrophobicity and WHC of *U. amblyoclada* showed a significant interaction between microsite and elevation. WHC of *P. reticulatum* was greater at 900 m.a.s.l. while its hydrophobicity was higher at 1800 m.a.s.l. WHC and hydrophobicity of *P. warmingii* are higher in samples from south-facing rocks. Results suggest that *P. reticulatum* can acclimate at microsite level without the ability to cope with more drastic environmental demands, losing the possibility to migrate to higher elevations in a climatic change scenario, while *U. amblyoclada* show higher intraspecific water retention variations, hence a wider potential distribution. *P. warmingii* could migrate to more protected microsites but will tend to disappear in an extreme scenario where temperature will increase.

**KEYWORDS.** Water holding capacity, hydrophobicity, *Parmotrema*, *Usnea*, microsites, South America.



It is well established that macro-climatic patterns in mountain environments are strongly correlated with elevation; these features significantly affect plant life in many contexts determining population structure, altitudinal range limits, and supporting endemism niches (Körner 1999; Spehn et al. 2011). However, the heterogeneity of mountain topography drives several changes at the microclimatic level providing

microsite shelters for alpine life. Existing research recognizes the vital role of microsites for endemic species by buffering the consequences of climate change (Ohler et al. 2020). The close relationship between lichen and their environment makes these organisms particularly vulnerable to the effects of climate change (Hauck 2009). Considering that lichen water content is regulated by morphology (Gauslaa 2014), functional traits in environmental gradient studies may provide a simpler perspective

<sup>4</sup> Corresponding author's e-mail: raulenriquedd@hotmail.com  
DOI: 10.1639/0007-2745-125.3.xxx

to inquire into changed hydration regimens on organisms (Ellis et al. 2021).

Lichen water holding capacity (WHC) is defined as the amount of water that the thallus can hold per area unit and is driven mainly by the specific thallus mass (dry mass per area), although different features can influence internal and external water pools (Gauslaa 2014). Whereas internal water holding capacity ( $WHC_{int}$ ) is determined by internal anatomical characters such as thickness or density of the different tissue layers (Gauslaa & Coxson 2011), external water holding capacity ( $WHC_{ext}$ ) is shaped by morphology and the hydrophobicity of the cortex. Internal water retention defines the time that the thallus is able to remain hydrated with important implications for physiological processes (Gauslaa 2014; Green et al. 2011). In contrast, external water can cause initial diffusion-limitation of photosynthetic activity by suprasaturation depression (Lange et al. 1993). A third trait related to water relations is hydrophobicity, the ability of the thallus to repel water, which we here measure as the time required for a droplet to be absorbed by the thallus (Matos & Rosado 2016).

Lichen water holding capacity varies within and across taxa and environments. For example, the success of *Bryoria* in inhabiting extreme environments has been attributed to WHC responses to microsites (Esseen et al. 2017). However, despite the value of WHC as a functional trait in lichens, there is still uncertainty around how WHC interacts with environmental stress. Previous studies suggest that microsite conditions such as rock aspect and inclination are main factors affecting stress responses of saxicolous lichens (Rutherford & Rebertus 2022). Drawing upon the evidence that WHC is driven mainly by thallus mass and morphology, it is plausible that this trait should respond differently according to the aspect of the substrate: north (equatorial-facing) and south (polar-facing), the inclination (flat and vertical rocks), and that these responses may vary across species differing in altitudinal ranges.

This work aims to analyze the impact of micro- (aspect and inclination) and macro- (elevation) effects on lichen water retention traits in the mountains of Central Argentina. We expect higher stress environments (more exposed microsites, lower elevations) to be associated with greater water absorption and retention capabilities, characterized

as lower hydrophobicity and greater WHC respectively. We furthermore predict different responses between the species with different altitudinal distribution: *Usnea amblyoclada* with a wider distribution will show different trait-responses in microsites at all elevations; the more restricted *Parmotrema reticulatum*, and the endemic *Parmotrema warmingii*, will show no differences considering that differences in microsite should imply higher plasticity to migrate in elevation. These trait-based responses inform the prediction of future climate change responses, both using the information of the lower elevations to infer into the future potential segregations of the species as proposed by Tito et al. (2020), and the potential for climate change mitigation by small shifts in microsite occupation (Rodriguez et al. 2017).

#### MATERIAL AND METHODS

**Study site.** Study was carried out in the southern locations from the mountain systems of Central Argentina on a road known as “Camino a los Linderos” located in Villa Yacanto, Departamento de Calamuchita of Córdoba city. The gradient starts at 900 m.a.s.l. and reaches 2700 m.a.s.l. with main annual temperatures going from 15.7°C at the lowest elevation, and 7.4°C at the highest elevation (Marcora et al. 2008). The landscape is composed by a mosaic of tussock grasslands, grazing lawns, granite outcrops, *Polylepis australis* woodlands, and eroded areas with exposed rock surfaces that occupy approximately 30% of the mountain area according (Cingolani et al. 2004), these areas are the ones that provided the rock outcrops from which the samples were taken.

**Microsite and microsite temperature data.** Three microsite conditions were established as follows: north aspect (equatorial-facing) with steep inclination (slope of the rock  $>70^\circ$  with respect to the ground), south aspect (polar-facing) with steep inclination, and rocks with slope  $<20^\circ$  with respect to the ground, considered as flat and the most exposed microsites. Temperature data were collected in a representative north and a south-facing microsite at 900 m.a.s.l. ( $32^\circ 2' 16.76''S$ ,  $64^\circ 45' 4.86''W$ ); 1800 m.a.s.l. ( $32^\circ 4' 2.46''S$ ,  $64^\circ 52' 28.30''W$ ) and 2700 m.a.s.l. ( $32^\circ 2' 55.96''S$ ,  $64^\circ 56' 6.53''W$ ) using HOBO Pendant MX2201 data loggers with integrated sensors. The data loggers were installed at a height

of 50 cm above the ground on wood-made pieces with surface area of  $20 \times 5$  cm and a thickness of 1 cm inside radiation shields. The shields consist of plastic domes made from 6 L water bottles painted white to reduce the effect of direct sunlight and with their bottoms removed to encourage air circulation within the shields (**Supplementary Fig. S3**). Data loggers were configured to take one measurement each hour. Daily mean temperature was calculated averaging the temperature data of each hour per day ( $n = 24$ ), and then the monthly mean temperature with their corresponding standard errors were calculated by averaging the daily mean temperature per month ( $n =$  number of days of each month). Mean maximum and mean minimum temperatures per month at each orientation were calculated by taking the maximum and minimum temperature of each day and averaging these values per month. Also, mean temperature per elevation at each orientation was calculated by averaging the mean daily temperature per elevation ( $n = 365$ ). The data are summarized in **Figs. 2 & 3** and **Supplementary Tables S1 and S2**.

**The species, their altitudinal distribution and the sampling process.** We collected samples of 3 species growing on rocks: *Usnea amblyoclada*, *Parmotrema reticulatum* and *Parmotrema warmingii*. The species were identified according to Rodríguez & Estrabou (2008) and Rodríguez et al. (2011) for *Usnea* and Spielmann & Marcelli (2020) for *Parmotrema*. *Usnea amblyoclada* altitudinal distribution is from 900 m.a.s.l. to 2100 m.a.s.l., *Parmotrema reticulatum* is from 900 m.a.s.l. to 1800 m.a.s.l. and *Parmotrema warmingii* is present only at 2700 m.a.s.l. Each species altitudinal specialization was taken from Rodríguez et al. (2017) classification: *Usnea amblyoclada* and *Parmotrema reticulatum* are found at lower elevations, while *Parmotrema warmingii* is a mountain-top species. We took 5 samples from each microsite at each elevation according to each species distribution: 900, 1800, 2100 m.a.s.l. for *Usnea amblyoclada*; 900 and 1800 m.a.s.l. for *Parmotrema reticulatum*; and 2700 m.a.s.l. for *Parmotrema warmingii*. However, *P. warmingii* thalli in flat microsites were not found, so we collected samples only from north- and south-facing microsites. Each sample consisted of bags with 1 to 5 thalli fragments totaling approximately 5 g each. The collection was made trying to get the thallus the most entirely possible. The samples were

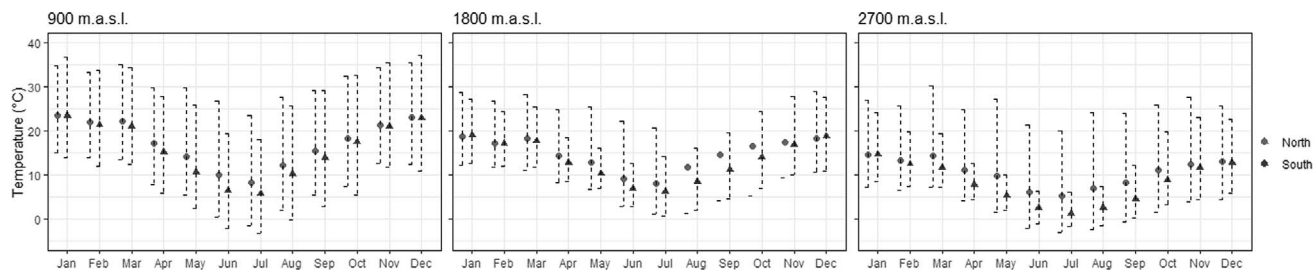
air dried for 24 hours, cleaned under a stereoscopic loupe to make sure of removing all remaining substrate and stored at  $-7^{\circ}\text{C}$  until the measurements were carried out. The total time of measurements of all parameters and samples was 2 weeks.

**Water relations and hydrophobicity measurements.** We measured the following main parameters related to water storage and wettability: total water holding capacity ( $\text{WHC}_{\text{tot}}$ ), internal water holding capacity ( $\text{WHC}_{\text{int}}$ ), external water holding capacity ( $\text{WHC}_{\text{ext}}$ ), and droplet initial absorption time ( $T_{\text{ini}}$ ).

$\text{WHC}_{\text{tot}}$ ,  $\text{WHC}_{\text{int}}$ , and  $\text{WHC}_{\text{ext}}$ : measurements and calculations of water holding capacity were made following established protocols (Esseen et al. 2017; Gauslaa & Coxson 2011). Briefly, each sample was dried at ambient temperature and weighed (DM), sprayed with distilled water for 10 minutes making sure that they got fully saturated, gently shaken and re-weighed to get the total water content ( $\text{WC}_{\text{total}}$ ), and photographed to obtain the hydrated surface area ( $A_{\text{wet}}$ ) using ImageJ, version 1.53e for image analysis process. Fully saturated thalli were gently dried with filter paper and re-weighed ( $\text{WC}_{\text{internal}}$ ). Parameters were calculated as follows:  $\text{WHC}_{\text{tot}} = (\text{DM} - \text{WC}_{\text{total}})/A_{\text{wet}}$ ;  $\text{WHC}_{\text{int}} = (\text{DM} - \text{WC}_{\text{internal}})/A_{\text{wet}}$ ;  $\text{WHC}_{\text{ext}} = (\text{WHC}_{\text{tot}} - \text{WHC}_{\text{int}})$ .

Droplet initial absorption time ( $T_{\text{ini}}$ ): measurements of absorption time are adapted from leaf hydrophobicity measures (Matos & Rosado 2016) with an emphasis on absorption rates rather than contact angles. At least 10 droplets of  $10\mu\text{L}$  distilled water were gently placed on random locations of air-dry thallus samples, choosing an undamaged, dry section, and avoiding soiled areas. The process was recorded using an Olympus TG-3 Tough camera with a 1080P ( $1920 \times 1080$ ) resolution, 30 FPS quality. For highly hydrophobic samples a 90 s cut-off after the application of the last droplet limit after the last droplet was applied. Absorption time was evaluated from video analysis using VLC media player in its 3.0.12 Vetinari version. In this work, initial absorption time is considered as the time between contact and when the water droplet starts to change its initial rounded shape.

**Data analysis.** The temperature means were calculated with their corresponding standard deviation (see supplementary material), while the water relation traits means were computed with the standard error of the mean. Generalized linear



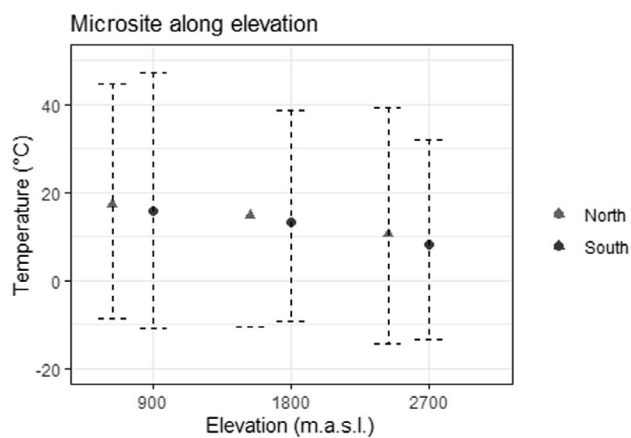
**Figure 1.** Mean monthly temperature on each elevation. Dotted bars indicate the monthly mean maximum and mean minimum temperature ( $n =$  number of days of each month).

mixed models (GLMMs) were used to test whether  $T_{ini}$ ,  $WHC_{tot}$ ,  $WHC_{int}$  and  $WHC_{ext}$  (response variable) depended on elevation, rock aspect (fixed effects), and their interaction using lme4 and glmmTMB packages (Bates et al. 2015; Brooks et al. 2017). Each lichen bag sample was treated as a random factor. To check heteroskedasticity and normality, residual analyses of models were carried out.  $WHC_{tot}$ ,  $WHC_{int}$  and  $WHC_{ext}$  were log-transformed to improve their distribution. Hydrophobicity GLMMs were applied considering a negative binomial distribution of the response variable ( $T_{ini}$ ) with linear parameterization (Hardin & Hilbe 2012). Simplifications and significance of the model terms were carried out by parametric bootstrap using 999 simulations, also Statterwise correction was done to interpret the statistical significance of the fixed effects (Luke 2017). Estimated marginal means plots from the final models were carried out to visualize the response variable patterns in elevation and microsite using the ggeffects package (Lüdtke 2018). Terms with no statistical significance were

not included on the plots, and finally, general linear hypothesis post-hoc tests were carried out to compare all the terms and interactions using the multcomp package (Hothorn et al. 2008).

**RESULTS**

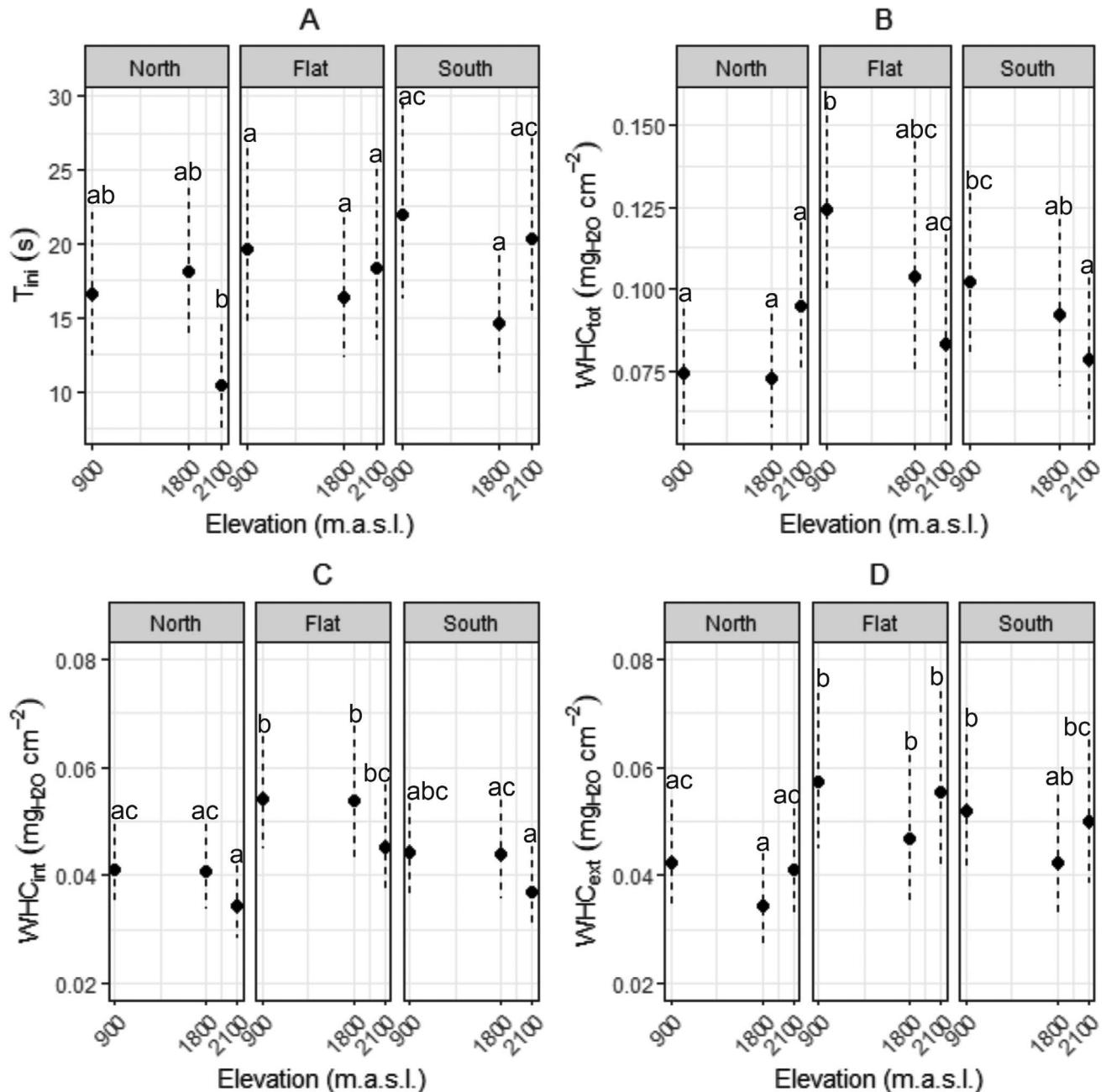
**Microsite temperature.** In general, median temperature tends to decrease with elevation for both the north (equatorial-facing) and south (polar-facing) aspects (Fig. 2). Median temperatures at 900 m.a.s.l. were  $17 \pm 5^\circ\text{C}$  (north) and  $16 \pm 6^\circ\text{C}$  (south), at 1800 m.a.s.l. were  $15 \pm 4^\circ\text{C}$  (north) and  $13 \pm 5^\circ\text{C}$  (south), and at 2700 m.a.s.l. were  $10 \pm 5^\circ\text{C}$  (north) and  $8 \pm 5^\circ\text{C}$  (south). The missing values at 1800 m.a.s.l. in Figs. 2 & 3 are due to the fact that the respective datalogger was found turned around fully exposed to direct sunlight, which produced atypically high measurements; thus, it was decided to eliminate the corresponding values. What is interesting about the data is that the warmest mean maximum ( $39 \pm 7^\circ\text{C}$ ) and coldest mean minimum ( $0 \pm 7^\circ\text{C}$ ) temperatures at 900 m.a.s.l. were registered on the south aspect; however, at 2700 m.a.s.l. the warmest mean maximum ( $34 \pm 3^\circ\text{C}$ ) and minimum ( $-5 \pm 6^\circ\text{C}$ ) values were registered at the north (Fig. 2, see also Supplementary Tables S1 & S2).



**Figure 2.** Mean temperature along the altitudinal gradient. Dotted bars indicate the mean maximum and mean minimum temperature per elevation ( $n = 365$ ).

**Hydrophobicity and water holding capacity response to elevation and microsite in the species.** The traits slightly differed in the species: the  $T_{ini}$  of *Usnea amblyoclada* averaged  $17.3 \pm 1.1$  s ( $n = 448$ ); followed by *Parmotrema reticulatum* with an average of  $15.5 \pm 0.97$  s ( $n = 627$ ), and finally *Parmotrema warmingii* with  $4.3 \pm 0.86$  s ( $n = 167$ ). The total water holding capacity of the species were  $0.098 \pm 0.003$  mg  $\text{H}_2\text{O cm}^{-2}$  for *U. amblyoclada* ( $n = 108$ );  $0.131 \pm 0.005$  mg  $\text{H}_2\text{O cm}^{-2}$  for *P. reticulatum* ( $n = 54$ ); and  $0.151 \pm 0.011$  mg  $\text{H}_2\text{O cm}^{-2}$  for *P. warmingii* ( $n = 34$ ). As to the predictive effects





**Figure 3.** *Usnea amblyoclada* responses to elevation and microsites. A.  $T_{ini}$ . B.  $WHC_{tot}$ . C.  $WHC_{int}$ . D.  $WHC_{ext}$ . Bars indicate 95% confidence intervals of the predicted values by the GLMMs. Lower case letters indicate terms that are significantly different (general linear hypothesis post-hoc test).

(elevation and microsite), all the water related traits changed with elevation in *U. amblyoclada* and *P. warmingii* with high significance  $p$ -values ( $p < 0.001$ ; **Table 1**), while the microsite-related effect was significant in almost all the traits and species (with the exception of the  $T_{ini}$  of *U. amblyoclada*) but with weaker statistical difference (bigger  $p$ -values; see **Table 1**), suggesting a stronger effect of elevation on the studied traits.

The  $T_{ini}$  of *Usnea amblyoclada* was significantly different with elevation ( $p < 0.001$ ); however, this parameter showed lower values in samples from north-facing rocks at 2100 m.a.s.l. in comparison with samples from the same microsite conditions at 900 m.a.s.l. and 1800 m.a.s.l. ( $p = 0.037$ ; **Fig. 3A**; **Table 1**).

The parameters  $WHC_{tot}$ ,  $WHC_{int}$  and  $WHC_{ext}$  of *Usnea amblyoclada* were also different with

**Table 1.** Summary of Generalized linear mixed models (GLMMs). *Pr(>Chi)*: Type II Wald chisquare tests. *Pr(>F)*: *F*-statistics calculated by Satterthwaite’s method. *df*: degrees of freedom for error. *n*: number of observations. Droplet initial absorption time ( $T_{ini}$ ), total water holding capacity ( $WHC_{tot}$ ), internal water holding capacity ( $WHC_{int}$ ) and external water holding capacity ( $WHC_{ext}$ ) are defined in the “water relations and hydrophobicity measurements” section of the methods.

Factor	<i>Usnea amblyoclada</i>						<i>Parmotrema reticulatum</i>						<i>Parmotrema warmingii</i>						
	$T_{ini}$		$WHC_{tot}$	$WHC_{int}$	$WHC_{ext}$	<i>df</i>	$T_{ini}$		$WHC_{tot}$	$WHC_{int}$	$WHC_{ext}$	<i>df</i>	$T_{ini}$		$WHC_{tot}$	$WHC_{int}$	$WHC_{ext}$	<i>df</i>	
	<i>df</i>	<i>Pr(&gt;Chi)</i>	<i>df</i>	<i>Pr(&gt;F)</i>			<i>df</i>	<i>Pr(&gt;Chi)</i>	<i>df</i>	<i>Pr(&gt;F)</i>			<i>df</i>	<i>Pr(&gt;Chi)</i>	<i>df</i>	<i>Pr(&gt;F)</i>			
Predictors																			
Elevation	3	<0.001	3	<0.001	<0.001	<0.001	2	<0.001	2	<0.001	<0.001	<0.001	-	-	-	-	-	-	-
Rock aspect	2	NS	2	0.04	0.03	0.06	2	0.013	2	0.07	0.05	<0.001	2	<0.001	2	<0.001	<0.001	<0.001	<0.001
Elevation × Rock aspect	4	0.037	6	0.04	NS	NS	2	NS	2	0.05	0.05	0.006	-	-	-	-	-	-	-
<i>n</i>	448		108			627		54			167		34						

elevation ( $p < 0.001$ ; **Fig. 3B & C**; **Table 1**)  $WHC_{tot}$  showed higher values at 900 m.a.s.l., lower values at 1800 m.a.s.l. and the lowest values at 2100 m.a.s.l. (**Fig. 3B**). However,  $WHC_{tot}$  of this species changed with elevation at flat microsities and at south-facing microsities while the  $WHC_{tot}$  at north-facing microsities did not change with elevation. This pattern is interpretable as a significant interaction between elevation and microsite ( $p = 0.04$ ; **Fig. 3B**; **Table 1**).

The  $WHC_{int}$  was lower at 2100 m.a.s.l. with respect to the 1800 m.a.s.l. and 900 m.a.s.l. (**Fig. 3C**).  $WHC_{ext}$  was significantly lower at 1800 m.a.s.l. in comparison to the others elevations (**Table 1**). Finally,  $WHC_{tot}$ ,  $WHC_{int}$  and  $WHC_{ext}$  of *Usnea amblyoclada* are higher in samples from flat microsities in comparison to the south- and north-facing microsities ( $p = 0.04$ ;  $p = 0.04$ ;  $p = 0.09$ , respectively; **Fig. 3B, C, D**; **Table 1**).

The  $T_{ini}$  of *Parmotrema reticulatum* changed with elevation ( $p < 0.001$ ; **Table 1**) and with microsite aspect ( $p = 0.013$ ; **Table 1**).  $T_{ini}$  was greater at 1800 m.a.s.l. in comparison to 900 m.a.s.l. while it was higher at flat microsities with respect to north- and south-facing microsities (**Fig. 4A**; **Table 1**).  $WHC_{tot}$  and  $WHC_{int}$  were higher at 900 m.a.s.l. while  $WHC_{ext}$  was higher at 1800 m.a.s.l. (**Fig 4B, C, D**;  $p < 0.001$ ; **Table 1**); however, these parameters responded differently according to microsite and elevation,  $WHC_{tot}$ ,  $WHC_{int}$ ,  $WHC_{ext}$  showed significant interaction between elevation and microsite ( $p = 0.05$ ,  $p = 0.05$  and  $p = 0.006$ , respectively; **Table 1**); in 900 m.a.s.l. higher values of  $WHC_{tot}$  were observed at south-facing microsities in comparison to samples from south-facing microsities in other elevations (**Fig. 4B**). Higher  $WHC_{int}$  values were

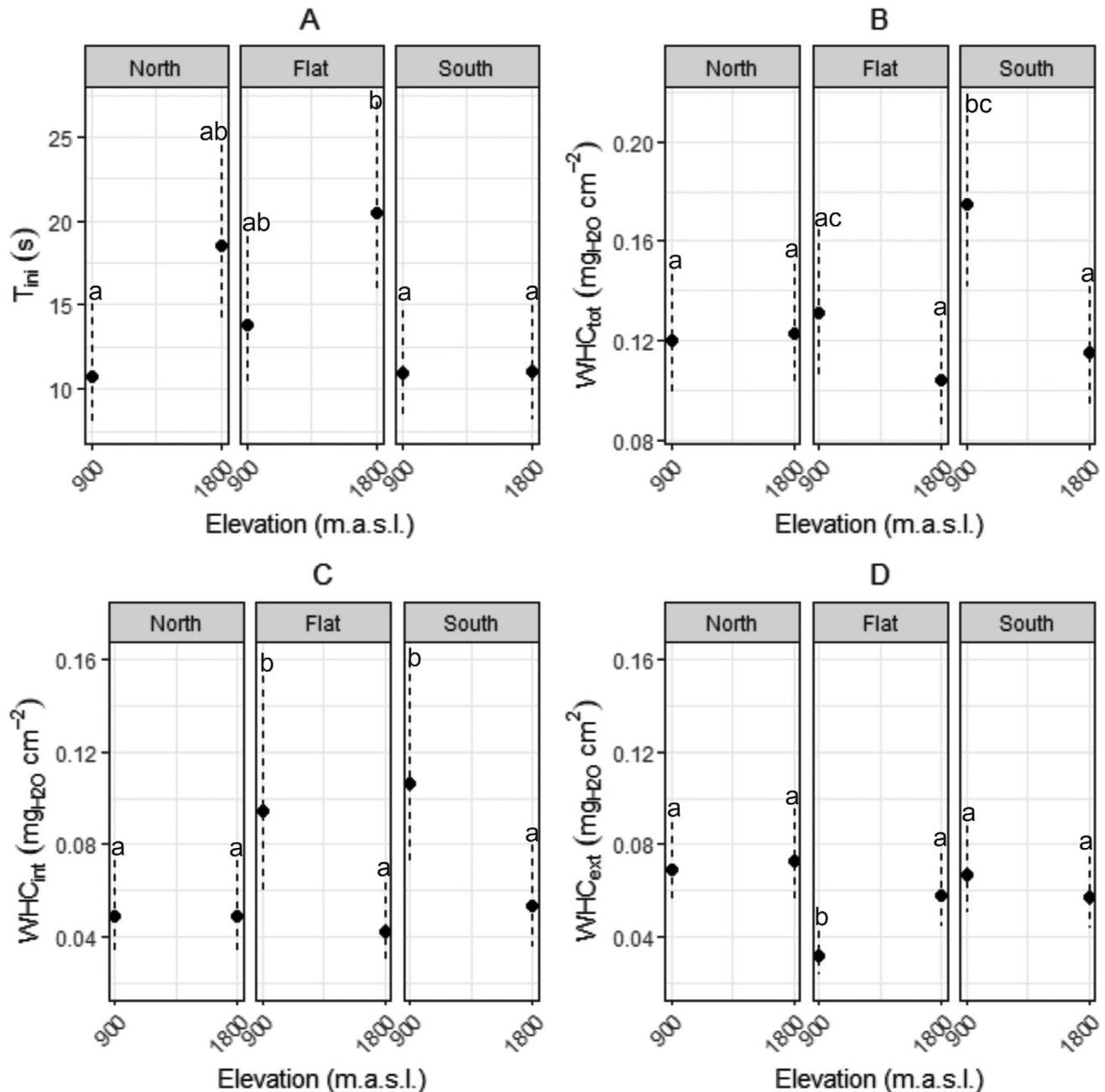
observed at flat and south-facing microsities from 900 m.a.s.l. with respect to north-facing microsities from the same elevation, while  $WHC_{int}$  did not differ between microsities conditions in samples from 1800 m.a.s.l. (**Fig. 4C**).  $WHC_{ext}$  of samples from flat microsities at 900 m.a.s.l. were lower in comparison to samples from 1800 m.a.s.l. ( $p < 0.001$ ; **Fig 4D**; **Table 1**). An interaction between elevation and microsite was observed given that  $WHC_{int}$  is lower at flat microsite from 900 m.a.s.l. with respect to the predicted values at south- and north-facing microsities from the same elevation while these parameters did not differ in north- and south-facing microsities from 900 m.a.s.l. and 1800 m.a.s.l. ( $p = 0.006$ ; **Fig. 4C**; **Table 1**). Interestingly,  $WHC_{tot}$ ,  $WHC_{int}$  and  $WHC_{ext}$  show a stable pattern in microsite without changes in samples from 1800 m.a.s.l. (**Fig. 4B, C, D**; **Table 1**).

The mountain top species *Parmotrema warmingii* showed a single pattern (**Fig. 5**):  $T_{ini}$ ,  $WHC_{tot}$ ,  $WHC_{int}$  and  $WHC_{ext}$  were higher in samples from south-facing microsities in comparison to samples from north-facing microsities ( $p < 0.001$ ; **Table 1**).

**DISCUSSION**

Our study confirms the association between lichen water retention traits and environmental factors. The water retention and wetting parameters of lichens observed in this work were sensitive to both macro- and micro-climatic features of the mountain gradient.

Microclimatic temperature differed with aspect at all elevations, median temperatures tended to be higher on north (equatorial-facing) aspects, however the differences between mean temperatures at north



**Figure 4.** *Parmotrema reticulatum* responses to elevation and microsites. A.  $T_{ini}$ . B.  $WHC_{tot}$ . C.  $WHC_{int}$ . D.  $WHC_{ext}$ . Dotted bars indicate 95% confidence intervals of the predicted values by the GLMMs. Lower case letters indicate terms that are significantly different (general linear hypothesis post-hoc test).

and south exposures seems to be minimal in the hottest months (Fig. 1). Also, from these records, we can see that north aspects reach higher maximum and lower minimum temperatures in comparison to southern microsites, with the exception of 900 m.a.s.l. (Fig. 1), supporting the idea of north-facing microsites as the most exposed microsites in mountain gradients (Deák et al. 2020).

The interactions between  $WHC_{tot}$  of *Usnea amblyoclada*,  $WHC_{tot}$ ,  $WHC_{int}$ ,  $WHC_{ext}$  of *Parmotrema reticulatum* and elevation are due to a different response of the parameters to rock aspect at different elevations: the effects of microsite are strongest at lower elevations (Figs. 3B & 4B, C, D; Table 1). This result seems to be consistent with other studies which found that the effect of micro-

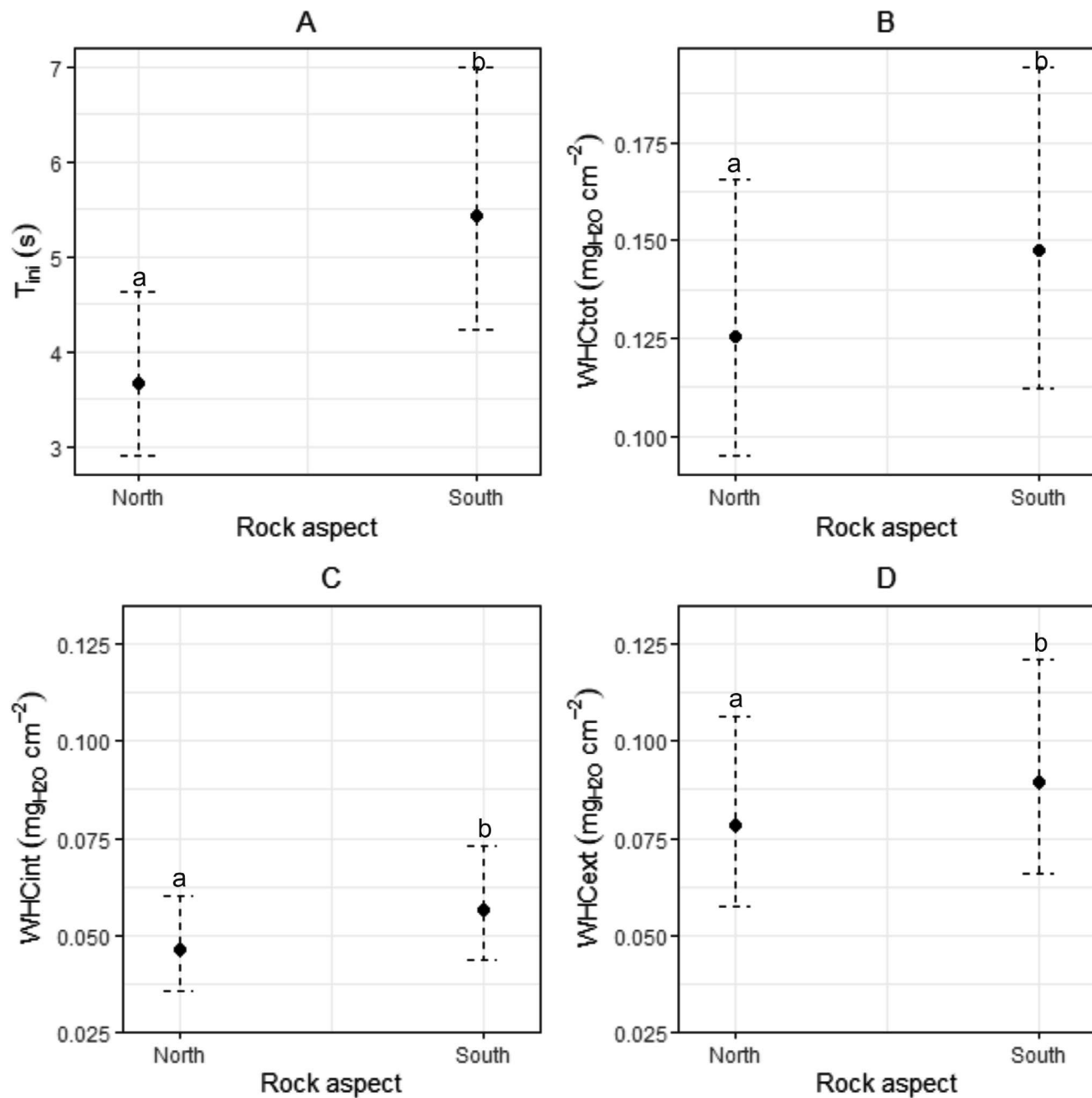


Figure 5. *Parmotrema warmingii* responses to microsities. A.  $T_{ini}$ . B.  $WHC_{tot}$ . C.  $WHC_{int}$ . D.  $WHC_{ext}$ . Bars indicate 95% confidence intervals of the predicted values by the GLMMs. Lower case letters indicate terms that are significantly different (general linear hypothesis post-hoc test).

site on lichen community composition was observable only at lower elevation (Costas et al. 2021; Rodriguez et al. 2017). Similarly, the higher  $WHC_{tot}$  of *Parmotrema reticulatum* at southern microsities from 900 m.a.s.l. suggests that in a climate change scenario this species will reach higher elevations but restricted to southern microsities. We suggest that microclimatic effects should be considered in future

predictions given their effects on the species traits and community composition as is proposed by previous works (Allen & Lendemer 2016; Rodriguez et al. 2017; Rutherford & Rebertus 2022).

Our findings broadly support the work of other studies linking environmental conditions with lichen traits and highlight the importance of considering intraspecific trait variability to explain



general ecological arrays (Hurtado et al. 2020). The strong response of lichen traits to the gradient observed in our results is also a clear indicator of their plasticity, showing their potential resilience in a hypothetical climate change scenario (Roos et al. 2019; van Zuijlen et al., 2021). High values of  $WHC_{int}$  were observed in previous works and attributed to a strategy to take advantage of liquid water (Esseen et al. 2017). The reduced  $WHC_{int}$  of *Usnea amblyoclada* at 2100 m.a.s.l. in comparison to other elevations (Fig. 3C, Table 1) supports this explanation and may be attributable to higher air humidity. Likewise, thallus morphology modifications according to water sources in extreme environments have also been reported (Stanton 2015) and can drive water retention performances in fruticose lichens (Eriksson et al. 2018). The higher ability to retain water internally and externally in flat microsites of *Usnea amblyoclada* (Fig. 3C, D; Table 1) could be supported by the idea of increasing water holding capacity externally to extend hydration periods in exposed microsites, similarly to what Esseen et al. (2017) proposed when they founded that *Bryoria* species growing in upper canopies hold high amounts of external water.

It is interesting to note that that *Usnea amblyoclada* samples showed high hydrophobicity in the elevations in which they showed low  $WHC_{tot}$ ,  $WHC_{int}$  and  $WHC_{ext}$ , a possible explanation for these results may be a strategy of the species to favor rapid uptake of vapor water, a widely available form of water in these environments (Poca et al. 2018). This strategy was observed in other studies in which variability in intraspecific water storage was associated with widespread success in cold climates (Esseen et al. 2017). Moreover, the high values of  $WHC_{ext}$  and low values of  $WHC_{int}$  in *Usnea amblyoclada* samples from 2100 m.a.s.l. could be an important explanation to argue the success of the species in covering its wider distribution along the gradient in comparison to *Parmotrema reticulatum*, considering that the high capacity of holding external water is associated with extended hydration periods (Esseen et al. 2017).

In lower altitudes *Parmotrema reticulatum*  $WHC_{int}$  is higher at the south and flat microsites in comparison to the  $WHC_{int}$  at north-facing microsites, however, as altitude increases the high values of  $WHC_{int}$  at the south and flat rocks decreases, until stabilized in the different micro-

habitats at the highest altitude (Fig. 4C; Table 1). Considering that  $WHC_{int}$  is related to the time that a lichen can be metabolically active, this pattern could indicate a lack of plasticity of the species (Essen et al. 2017). On the other side, the higher  $WHC_{int}$  at flat and south-facing microsites from 900 m.a.s.l. may be explained by a strategy of the thalli to remain active in high humidity conditions (Pintado & Sancho 2002), especially in flat microsites given that they are more exposed to rain, following Gauslaa (2014), suprasaturation depression can easily take place in thalli exposed to liquid water sources and with low hydrophobicity in their surface.

Finally, the higher hydrophobicity and  $WHC$  at south-facing rocks of *Parmotrema warmingii* with respect to the north-facing microsites suggests that the species does not reach its maximum range since the stabilization of  $T_{ini}$  and  $WHC$  occurred in the top ranges of the lowermost species (Figs. 3A & 4A; Table 1).

## CONCLUSIONS

The hydration traits of the studied species change in response to elevation and microsite. The success of *Usnea amblyoclada* in covering a wider distribution is attributed to the variability of the internal traits with respect to the external ones, thus covering a greater range of responses to adverse environmental conditions. We predict that *Parmotrema reticulatum* will disappear in extreme climate change scenarios where the temperature will increase over time, while *Usnea amblyoclada* hydration traits show greater plasticity, suggesting that this species could expand its altitudinal distribution along the gradient. We hypothesize that the mountain top species *Parmotrema warmingii* is able to migrate at more sheltered microsites in the proposed climate change scenario, but it is necessary to develop experiments to observe the response of this species in other macro-climatic contexts.

Lichen community studies are increasingly including hydration traits (water holding capacity, specific thallus mass, etc.), documenting patterns in many environmental contexts at the community level. However, our study reinforces the need to consider these traits at the intra-species level, this viewpoint offers tools to answer questions about distribution and limitation of species and to explain endemism patterns.

This study provides new insights into lichens WHC water retention traits response to different climatic conditions provided by rock outcrops and altitudinal gradients of mountain systems. The findings should make an important contribution to explain lichen species range limits in elevation and how WHC interact to different climatic settings in species with known distribution in the study site improving the WHC potential as functional trait and stating more tools to inquire into lichen ecological patterns in situations in which other responses are unlikely observable (Baniya et al. 2010; Rodriguez et al. 2017; Wan & Ellis 2020).

#### LITERATURE CITED

- Allen, J. L. & J. C. Lendemer. 2016. Climate change impacts on endemic, high-elevation lichens in a biodiversity hotspot. *Biodiversity and Conservation* 25(3): 555–568. <https://doi.org/10.1007/s10531-016-1071-4>
- Baniya, C. B., T. Solhøy, Y. Gauslaa & M. W. Palmer. 2010. The elevation gradient of lichen species richness in Nepal. *The Lichenologist* 42(1): 83–96. <https://doi.org/10.1017/S0024282909008627>
- Bates, D., M. Mächler, B. Bolker & S. Walker. 2015. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* 67(1). <https://doi.org/10.18637/jss.v067.i01>
- Brooks, M. E., K. Kristensen, K. J. Benthem, A. van Magnusson, C. W. Berg, A. Nielsen, H. J. Skaug, M. Mächler & B. M. Bolker. 2017. GlmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *The R Journal* 9(2): 378. <https://doi.org/10.32614/RJ-2017-066>
- Cingolani, A. 2004. Mapping vegetation in a heterogeneous mountain rangeland using landsat data: An alternative method to define and classify land-cover units. *Remote Sensing of Environment* 92(1): 84–97. <https://doi.org/10.1016/j.rse.2004.05.008>
- Costas, S. M., N. Canton & J. M. Rodríguez. 2021. The relative effect of altitude and aspect on saxicolous lichen communities at mountain summits from central-west of Argentina. *Rodriguésia* 72. <https://doi.org/10.1590/2175-7860202172064>
- Deák, B., B. Kovács, Z. Rádai, I. Apostolova, A. Kelemen, R. Kiss, K. Lukács, S. Palpurina, D. Sopotlieva, F. Báthori & O. Valkó. 2020. Linking environmental heterogeneity and plant diversity: The ecological role of small natural features in homogeneous landscapes. *Science of The Total Environment* 763: 144–199. <https://doi.org/10.1016/j.scitotenv.2020.144199>
- Ellis, C. J., J. Asplund, R. Benesperi, C. Branquinho, L. Di Nuzzo, P. Hurtado, I. Martínez, P. Matos, J. Nascimbene, P. Pinho, M. Prieto, B. Rocha, C. Rodríguez-Arribas, H. Thüs & P. Giordani. 2021. Functional traits in lichen ecology: A review of challenge and opportunity. *Microorganisms* 9(4): 766. <https://doi.org/10.3390/microorganisms9040766>
- Eriksson, A., Y. Gauslaa, K. Palmqvist, M. Ekström & P. Esseen. 2018. Morphology drives water storage traits in the globally widespread lichen genus *Usnea*. *Fungal Ecology* 35: 51–61. <https://doi.org/10.1016/j.funeco.2018.06.007>
- Esseen, P., T. Olsson, D. Coxson & Y. Gauslaa. 2015. Morphology influences water storage in hair lichens from boreal forest canopies. *Fungal Ecology* 18: 26–35. <https://doi.org/10.1016/j.funeco.2015.07.008>
- Esseen, P., M. Rönnqvist, Y. Gauslaa & D. S. Coxson. 2017. Externally held water – a key factor for hair lichens in boreal forest canopies. *Fungal Ecology* 30: 29–38. <https://doi.org/10.1016/j.funeco.2017.08.003>
- Gauslaa, Y. 2014. Rain, dew, and humid air as drivers of morphology, function and spatial distribution in epiphytic lichens. *The Lichenologist* 46(1): 1–16. <https://doi.org/10.1017/S0024282913000753>
- Gauslaa, Y. & D. Coxson. 2011. Interspecific and intraspecific variations in water storage in epiphytic old forest foliose lichens. *Botany* 89(11): 787–798. <https://doi.org/10.1139/b11-070>
- Gauslaa, Y., M. Lie, K. A. Solhaug & M. Ohlson. 2006. Growth and ecophysiological acclimation of the foliose lichen *Lobaria pulmonaria* in forests with contrasting light climates. *Oecologia* 147(3): 406–416. <https://doi.org/10.1007/s00442-005-0283-1>
- Green, T. G. A., L. G. Sancho & A. Pintado. 2011. Ecophysiology of desiccation/rehydration cycles in mosses and lichens. Pages 89–120. In: U. Lüttge, E. Beck. & D. Bartels (eds.), *Plant Desiccation Tolerance*. Springer, Berlin Heidelberg. [https://doi.org/10.1007/978-3-642-19106-0\\_6](https://doi.org/10.1007/978-3-642-19106-0_6)
- Hardin, J. W. & J. M. Hilbe. 2012. *Generalized linear models and extensions* (3rd ed). Stata Press, College Station, Texas.
- Hauck, M. 2009. Global warming and alternative causes of decline in arctic-alpine and boreal-montane lichens in North-Western Central Europe: global warming and cold-tolerant lichens. *Global Change Biology* 15(11): 2653–2661. <https://doi.org/10.1111/j.1365-2486.2009.01968.x>
- Hothorn, T., F. Bretz & P. Westfall. 2008. Simultaneous inference in general parametric models. *Biometrical Journal* 50(3): 346–363.
- Hurtado, P., M. Prieto, J. Martínez-Vilalta, P. Giordani, G. Aragón, J. López-Angulo, A. Košuthová, S. Merinero, E. M. Díaz-Peña, T. Rosas, R. Benesperi, E. Bianchi, M. Grube, H. Mayrhofer, J. Nascimbene, M. Wedin, M. Westberg & I. Martínez. 2020. Disentangling functional trait variation and covariation in epiphytic lichens along a continent-wide latitudinal gradient. *Proceedings of the Royal Society B: Biological Sciences* 287(1922): 20192862–20192862. <https://doi.org/10.1098/rspb.2019.2862>
- Körner, C. 1999. *Alpine Plant Life*. Springer, Berlin Heidelberg. <https://doi.org/10.1007/978-3-642-98018-3>
- Lange, O. L., B. Büdel, U. Heber, A. Meyer, H. Zellner & T. G. A. Green. 1993. Temperate rainforest lichens in New Zealand: high thallus water content can severely limit photosynthetic CO<sub>2</sub> exchange. *Oecologia* 95: 303–313.
- Lüdecke, D. 2018. ggeffects: Tidy data frames of marginal effects from regression models. *Journal of Open Source Software* 3(26): 772. <https://doi.org/10.21105/joss.00772>
- Luke, S. G. 2017. Evaluating significance in linear mixed-effects models in R. *Behavior. Research Methods* 49(4): 1494–1502. <https://doi.org/10.3758/s13428-016-0809-y>
- Marcora, P., I. Hensen, D. Renison, P. Seltmann & K. Wesche. 2008. The performance of *Polylepis australis* trees along their entire altitudinal range: Implications of climate change for their conservation. *Diversity and Distributions* 14(4): 630–636. <https://doi.org/10.1111/j.1472-4642.2007.00455.x>
- Matos, I. S. & B. H. P. Rosado. 2016. Retain or repel? Droplet volume does matter when measuring leaf wetness traits. *Annals of Botany* 117(6): 1045–1052. <https://doi.org/10.1093/aob/mcw025>

- Ohler, L., M. Lechleitner & R. Junker. 2020. Microclimatic effects on alpine plant communities and flower-visitor interactions. *Scientific Reports* 10(1): 1366. <https://doi.org/10.1038/s41598-020-58388-7>
- Phinney, N. H., K. A. Solhaug & Y. Gauslaa. 2018. Rapid resurrection of chlorolichens in humid air: specific thallus mass drives rehydration and reactivation kinetics. *Environmental and Experimental Botany* 148: 184–191. <https://doi.org/10.1016/j.envexpbot.2018.01.009>
- Pintado, A. & L. G. Sancho. 2002. Ecological significance of net photosynthesis activation by water vapour uptake in *Ramalina capitata* from rain-protected habitats in central Spain. *The Lichenologist* 34(5): 403–413. <https://doi.org/10.1006/lich.2002.0414>
- Poca, M., A. M. Cingolani, D. E. V. Saur PalmieriGurvich, & G. Bertone. 2018. Water storage dynamics across different types of vegetated patches in rocky highlands of central Argentina: water storage across vegetated patches in rocky highlands. *Ecohydrology* 11(7): e1981. <https://doi.org/10.1002/eco.1981>
- Rodríguez, J. M., C. Estrabou, C. Truong & P. Clerc. 2011. The saxicolous species of the genus *Usnea* subgenus *Usnea* (Parmeliaceae) in Argentina and Uruguay. *The Bryologist* 114(3): 504–525. <https://doi.org/10.1639/0007-2745-114.3.504>
- Rodríguez, J. M., D. Renison, E. Filippini & C. Estrabou. 2017. Small shifts in microsite occupation could mitigate climate change consequences for mountain top endemics: A test analyzing saxicolous lichen distribution patterns. *Biodiversity and Conservation* 26(5): 1199–1215. <https://doi.org/10.1007/s10531-017-1293-0>
- Roos, R. E., K. Zuijlen, T. Birkemoe, K. Klanderud, S. I. Lang, S. Bokhorst, D. A. Wardle & J. Asplund. 2019. Contrasting drivers of community-level trait variation for vascular plants, lichens and bryophytes across an elevational gradient. *Functional Ecology* 33(12): 2430–2446. <https://doi.org/10.1111/1365-2435.13454>
- Rutherford, R. D. & A. Rebertus. 2022. A habitat analysis and influence of scale in lichen communities on granitic rock. *The Bryologist* 125: 43–60. <https://doi.org/10.1639/0007-2745-125.1.043>
- Spehn, E. M., K. Rudmann-Maurer & C. Körner. 2011. Mountain biodiversity. *Plant Ecology & Diversity* 4(4): 301–302. <https://doi.org/10.1080/17550874.2012.698660>
- Spielmann, A. & M. P. Marcelli. 2020. Type studies on *Parmotrema* (Parmeliaceae, Ascomycota) with salazinic acid. *Plant and Fungal Systematics* 65(2): 403–508. <https://doi.org/10.35535/pfsyst-2020-0028>
- Stanton, D. E. 2015. Small scale fog-gradients change epiphytic lichen shape and distribution. *The Bryologist* 118(3): 241–244. <https://doi.org/10.1639/0007-2745-118.3.241>
- Tito, R., H. L. Vasconcelos & K. J. Feeley. 2020. Mountain Ecosystems as Natural Laboratories for Climate Change Experiments. *Frontiers in Forests and Global Change* 38(3): 1–8. <https://doi.org/10.3389/ffgc.2020.00038>
- van Zuijlen, K., K. Klanderud, O. S. Dahle, Å. Hasvik, M. S. Knutsen, S. L. Olsen, S. Sundsbø & J. Asplund. 2021. Community-level functional traits of alpine vascular plants, bryophytes, and lichens after long-term experimental warming. *Arctic Science* 7(1): 1–15. <https://doi.org/10.1139/as-2020-0007>
- Wan, S. & C. J. Ellis. 2020. Are lichen growth form categories supported by continuous functional traits: water holding capacity and specific thallus mass? *Edinburgh Journal of Botany* 77(1): 65–76. <https://doi.org/10.1017/S0960428619000209>

manuscript received April 5, 2022; accepted July 8, 2022.

### Supplementary documents online:

**Supplementary Table S1.** Monthly mean, daily mean maximum and daily mean minimum temperature registered by the dataloggers on each elevation with their corresponding standard error.

**Supplementary Table S2.** Mean, mean maximum and mean minimum values per elevation on north and south-facing microsites.

**Supplementary Fig. S3.** HOBO Pendant MX2201 data logger installation in the field (A) and inside the radiation shield (B).

**Supplementary Table S4.** Estimates values and their confidence intervals of *Usnea amblyoclada* GLMMs.

**Supplementary Table S5.** Estimates values and their confidence intervals of *Parmotrema reticulatum* GLMMs.

**Supplementary Table S6.** Estimates values and their confidence intervals of *Parmotrema warmingii* GLMMs.

Queries for bryo-125-03-08

This article has been typeset from the submitted materials. Check proofs carefully for conversion or other inadvertent errors. Please follow the [Allen Press Guide to PDF Annotation](#) when marking revisions. Do not edit the PDF directly.

If present, queries will be listed below with corresponding numbers in the margins or may appear as PDF comments addressed to the author or editor. If a correction is desired in response to a query, mark the necessary changes directly in the proof using the appropriate annotation tool. If no change is desired, no action is necessary in response.