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**Determining which mechanisms underlie facilitation by tussocks in tropical high mountains and their relative importance**

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18 **ABSTRACT**

19 Facilitation by tussocks is common in high-altitude tropical environments. It is thought that  
20 facilitation results from stress amelioration, but it is unclear which of the many stressors acting in  
21 these environments is ameliorated. We aimed at determining the relative importance of different  
22 stressors as drivers of facilitation by the tussock *Festuca toluensis* in Mexico. We manipulated  
23 five stressors in the field: minimum temperatures by using electric radiators that kept plants  
24 warm; maximum temperatures by means of reflective sand that precluded temperature build-up  
25 during the day; UV radiation by using screens opaque to UV; poor soil properties by comparing  
26 soils from beneath tussocks and from bare ground; and low water availability by adding  
27 vermiculite to the soil. The performance (survival and growth) of *Mexerion sarmentosum* (a  
28 plant usually associated with *Festuca*) in these treatments was compared to that recorded under  
29 tussocks and in bare ground. Amelioration of extreme temperatures had the largest positive  
30 effects on *Mexerion* survival. UV radiation and increased soil humidity did not affect survival,  
31 although humidity increased growth rates. Nevertheless, tussocks reduced the growth of  
32 *Mexerion*, which is consistent with observations of competition between plants and soil  
33 microorganisms favoured by tussocks. Our results highlight the importance of the extreme daily  
34 fluctuations in temperature that characterise tropical mountains as fundamental drivers of their  
35 dynamics.

36

37 **KEY WORDS:** Extreme temperatures; Iztaccíhuatl Volcano, Mexico; Nurse plant; Soil  
38 improvement; Stress amelioration; Thermal buffering; UV radiation.

39

40

## 41 INTRODUCTION

42 HIGH-ALTITUDE ENVIRONMENTS ARE HARSH. UV radiation is very intense due to the  
43 comparatively thin atmospheric layer above mountains (Billings 1974, Luteyn 1999). Soils are  
44 frequently sandy, young and undeveloped, and thus may be deficient in nutrients and have a  
45 reduced water-holding capacity (Sarmiento 1986, Luteyn 1999, Körner 2003). Moreover, in  
46 contrast with extratropical alpine environments, páramos (high-altitude tropical environments  
47 above the treeline) face extreme and rapid changes in temperature every day: freezing  
48 temperatures at night and very high soil-surface temperatures during the day are common  
49 throughout the year (Sarmiento 1986, Smith and Young 1987, Rundel et al. 1994). Thus, it is  
50 said that páramos experience summer every day and winter every night (Hedberg 1964). Plants  
51 living in páramos must face this suite of harsh and often rapidly changing stressors.

52 One way plants may cope with such hostile conditions is through interactions with  
53 neighboring individuals (Pugnaire and Luque 2001, Callaway et al. 2002, He et al. 2013). Some  
54 páramo plants ameliorate abiotic stress in their vicinity (Anthelme et al. 2012, Anthelme and  
55 Dangles 2012) resulting in strong facilitation, i.e., a non-trophic interaction in which at least one  
56 species is favoured by the presence of another (Choler et al. 2001, Callaway et al. 2002). There is  
57 evidence that high-altitude plant communities, such as páramos, are in fact one of the systems in  
58 which plant-plant facilitation is strong and most frequent worldwide (Anthelme and Dangles  
59 2012).

60 Tussock grasses are common benefactors in páramos (Smith and Young 1987, Patty et al.  
61 2010, Anthelme et al. 2014, Malatesta et al. 2016). In fact, tussocks have been considered to be  
62 ecosystem engineers (Malatesta et al. 2016), and are sometimes the most important facilitators in  
63 páramos (Catorci et al. 2011). Several studies from páramos throughout the world have found

64 large numbers of species facilitated by tussocks (see Anthelme and Dangles 2012 and references  
65 therein). Tussocks are said to reduce the intensity of multiple stressors. They may buffer extreme  
66 temperatures and lessen UV incidence because they produce dense shade (Coe 1969, Chapin III  
67 et al. 1979, Hedberg and Hedberg 1979, Körner 2003, Monteiro et al. 2011, Malatesta et al.  
68 2016). They also improve soil properties increasing fertility, reducing the proportion of sands in  
69 the soil, and producing organic matter, which results in increased humidity and water-holding  
70 capacity (Monteiro et al. 2011, Anthelme and Dangles 2012, Malatesta et al. 2016). The intensity  
71 of facilitation has been shown to decrease from the canopy center outwards (Moro et al. 1997,  
72 Monteiro et al. 2011), suggesting that the close spatial associations observed between species at  
73 high altitudes (Nuñez et al. 1999, Choler et al. 2001) are related to stress reduction near the  
74 tussock. However, no studies have aimed to test how the protégés are affected by the  
75 environmental changes induced by the tussocks. We also ignore which of the multiple stressors  
76 that occur in páramos is most important in driving facilitation by tussocks. Given the importance  
77 of tussocks both in terms of their abundance in páramos and the large number of species  
78 associated to them, these questions deserve attention.

79         In this contribution, we test in the field five stress-amelioration mechanisms to determine  
80 which (if any) drive facilitation by the tussock *Festuca toluensis*, and evaluate which are more  
81 important. We hypothesise that facilitation is the result of the amelioration of at least one of the  
82 five stressors manipulated in our experiment: maximum and minimum daily temperatures, UV  
83 radiation, unfavourable soil properties, and low water availability. Given the large variations in  
84 temperature in páramos (Sarmiento 1986, Smith and Young 1987), it seems likely that the  
85 buffering of maximum and minimum temperatures is the most important driver of facilitation by  
86 *Festuca*. We analyse the effects of the amelioration of each of the five stressors on the survival

87 and growth of recently-germinated individuals of *Mexerion sarmentosum*, a small rosette plant  
88 that is positively associated to *Festuca* at the study site (Tovar-Romero 2010). Our results were  
89 compared with the performance of *Mexerion* growing under tussocks and in full-stress conditions  
90 on bare ground. If the amelioration of a given stressor operates in our system, we expect  
91 performance to improve compared to bare ground.

92

### 93 **METHODS**

94

95 The study was conducted at the Iztaccíhuatl volcano, Mexico, (19.12° N, 98.65° W), at 3980 m  
96 a.s.l. Climatic reports at Paso de Cortés, located 320 m below our study site, indicate a mean  
97 annual temperature of 5.5 C and weak seasonality (NOAA n/d). However, temperature  
98 undergoes wide fluctuations throughout the day (mean annual maximum air temperature is 13.9  
99 °C and the average minimum is -2.8 °C), and thus may act as an important stressor. The study  
100 site is dominated by the tussock *Festuca tolucensis* (Poaceae). Many species grow under these  
101 tussocks, out of the 24 species found at our study site, 63 % showed significant positive  
102 association with *Festuca*. This trend is especially strong in rosette species, as 80 % of them were  
103 positively associated to the tussock (Tovar-Romero 2010). One of such species was *Mexerion*  
104 *sarmentosum* (Asteraceae), a perennial herb that remains a small (< 4 cm in diameter) rosette for  
105 much of its life cycle, but becomes a decumbent herb as large as 30 cm tall when reproductive.  
106 In our experiment we used recently germinated plants with a mean diameter of 1.51 cm, and a  
107 height < 0.5 cm. In small individuals, all the leaves in the rosette are appressed to the ground.  
108 *Mexerion* is found associated with *Festuca* four times more frequently than expected by chance  
109 ( $P < 0.001$ ) (Tovar-Romero 2010).

110

111 *Stress amelioration experiment*

112 On September 2008, we collected 500 recently-germinated *Mexerion* rosettes and transplanted  
113 them to peat pots (7 cm side, 8 cm tall) filled to the top with soil from bare ground where no  
114 other plants were growing (except when stated otherwise, see below) taken from the study site.  
115 These pots were chosen because, when buried in the ground, they allow the movement of water.  
116 As a result, the hydric potential of the soil in the pot matches that of the surrounding  
117 environment since the materials inside and outside the pot have similar hydraulic properties (Day  
118 and Skoupy 1971, Heiskanen 1999). The plants were kept for one month on the roof of a  
119 building located at Iztaccíhuatl Park at 3980 m a.s.l. where they were protected from the wind  
120 but exposed to direct sunlight. Plants were watered at least once a week. After one month,  
121 survivors were relocated in the field along with their pots. Pots were buried so the level of the  
122 soil in them matched that of the surrounding ground. Because pots were almost completely full,  
123 the portion of the pot that protruded from the soil was minimal, which promotes evaporation and  
124 reduces water availability (Day and Skoupy 1971). Relocating the plant altogether with the pot  
125 also minimises transplant shock (Schrader 2000), which would have obscured our results. Pots  
126 may in principle preclude the roots of *Mexerion* from spreading and interacting with *Festuca*'s.  
127 There is some evidence for root competition for water between tussocks and herbs in arid  
128 environments (Maestre and Cortina 2004). Nevertheless, our *Mexerion* individuals were very  
129 small and it seems unlikely that their roots would have spread beyond the limits imposed by the  
130 pot even if it were absent. Plants were randomly assigned to eight treatments designed to test the  
131 effect of different stressors and analyse the mechanisms underlying facilitation by tussocks:

132 Tussock treatment (TUS): *Mexerion* individuals grown in pots with soil from beneath tussocks  
133 were placed under a tussock canopy >80 cm in diameter. In this positive control all the stressors  
134 analysed in this study are expected to be alleviated.

135 Fully exposed treatment (EXP): As in the remainder of the treatments, plants were placed in  
136 areas without vegetation present in a radius of at least 1.5 m. *Mexerion* in this negative control  
137 experienced all the stressors analysed in this study.

138 Soil conditions treatment (SOI): As EXP, but pots were filled with soil from beneath tussocks  
139 prior to transplant. This soil was expected to have more nutrients and greater water-holding  
140 capacity than that from bare areas (Mizuno 1998, Anthelme and Dangles 2012). This procedure  
141 allows an evaluation of the effects that tussocks have by changing soil properties, but it does not  
142 provide direct evidence of which properties are changed (nutrients, soil texture, organic matter,  
143 etc.).

144 Reduced hydric stress treatment (HYD): As EXP, but pots contained a mixture of three-parts soil  
145 per one of vermiculite before the *Mexerion* seedlings were transplanted. Vermiculite absorbs  
146 large amounts of water and releases it gradually to the soil (Libardi et al. 1983, Okada et al.  
147 2008). Thus, the plants in this treatment were expected to have access to water for longer  
148 periods, as it may happen under tussocks. Results from this treatment must be interpreted  
149 carefully, as vermiculite may also affect pH and nutrient availability after some time in the soil  
150 (Libardi et al. 1983).

151 Minimum temperature amelioration treatment (MIN): Temperature under tussock during the  
152 night has been reported to be higher than in bare areas (Coe 1969; Hedberg & Hedberg 1979). To  
153 keep *Mexerion* warm during this low-temperature time of the day we used electric radiators.  
154 These consisted of a 700 W, 120 V electric resistance contained in 15 × 4 cm metal cases and

155 connected to a thermostat that could be regulated. This equipment was designed and  
156 manufactured specially for the experiment by KinTel S.A. de C.V. (Mexico City, Mexico). After  
157 some preliminary tests, we found that the best option to keep a relatively constant temperature  
158 throughout the night was to place the radiator 5 cm away from the plants. We then regulated the  
159 thermostat so that the mean night temperature 5 cm away from the radiator equalled that  
160 recorded under tussocks. The measurements of temperature and calibration of the radiators were  
161 conducted in December.

162 Maximum temperature amelioration treatment (MAX): Tussocks also keep the daytime  
163 temperatures milder than those in bare areas (Coe 1969, Hedberg and Hedberg 1979). In high  
164 mountains, soil surface temperatures are high enough to be detrimental to plants (Körner 2003).  
165 As a way of lowering soil temperatures near the soil surface without interfering with  
166 photosynthetically active radiation, we covered the soil with a thin layer (~2 mm) of marble  
167 sand. This product is white, so we expected it to reduce soil temperature during the day by  
168 increasing the albedo. The diameter of the particles was similar to that of the sandy soils at the  
169 study site in order to minimise differences in texture that could affect water movement. Plants  
170 were placed at the centre of a 0.25 m<sup>2</sup> square covered with white marble sand.

171 No ultraviolet radiation treatment (UV-): We set 1 × 1 m Mylar screens 0.4 m above ground. The  
172 space between the soil and the screen was left open to allow the movement of air and minimize  
173 the screen's effect on temperature and air humidity. Mylar is opaque to UV radiation below  
174 0.314 μm (UV-B), but is transparent to the rest of the spectrum (Robson et al. 2003). The screens  
175 were perforated in a 5 cm grid to allow rainfall to pour in. This treatment intended to resemble  
176 the reduction in UV radiation caused by tussocks.



177 Ultraviolet radiation treatment (UV+): The presence of a screen in the UV- treatment may affect  
178 temperature, wind, humidity and precipitation, which in turn can affect *Mexerion* performance.  
179 This could result in confounded effects that would make it impossible to attribute the effects of  
180 the screen to UV reduction *per se*. As a control for the UV- treatment, we used Tedlar screens  
181 which are optically similar to Mylar but do not interfere with UV radiation (Day and Neale 2002,  
182 Prado et al. 2012). UV-opaque screens also affect several environmental variables, but they do so  
183 in ways identical to UV-transparent screens. Thus, the differences between UV+ and UV-  
184 treatments can be safely ascribed to UV radiation (Day and Neale 2002, Robson et al. 2003,  
185 Robson et al. 2005).

186 We set the experiment in a 0.25 ha area near the Altzomoni high-mountain refuge, which  
187 was the highest place where electricity was available to power the MIN treatment. The study site  
188 seems pretty homogeneous spatially, so our data are likely to be representative of the overall  
189 conditions. We used a randomized complete-block design. Blocks were areas < 6 m in diameter,  
190 and that thus may experience similar conditions. In total we set 10 blocks, each having the eight  
191 treatments represented once. In each experimental unit (area affected by a screen, tussock,  
192 radiator, patch of marble sand, or spot on bare ground) we placed two plants (= two adjacent  
193 pots), serving as subsamples to increase the precision of our results (Selwyn 1996). Therefore,  
194 we have ten replicates (ten experimental units) in our experiment with two subsamples, meaning  
195 that 20 plants were subject to each treatment. The mean distance between each pair of pots was  
196 1.10 m, and the mean distance between blocks was 9.24 m. Plant diameter and survival were  
197 measured at the beginning of the experiments and in four subsequent occasions until the end of  
198 the experiment six months later, where only four *Mexerion* individuals were still alive.

199

200 *Measurements of environmental variables*

201 We used HOBO Pro temp/external temp data loggers to monitor temperature every minute in  
202 TUS, MAX, MIN, UV+ and EXP from 12 December 2008 to 25 December 2008 and 28 April  
203 2009 to 11 May 2009. These dates were selected because they correspond to the coldest  
204 (minimum temperature in December = -7 °C, the coldest of the year) and the warmest (minimum  
205 temperature in May = 0 °C, the second warmest) seasons of the year. We did not measure  
206 temperatures in UV- because it has been shown that there is no difference in temperature below  
207 screens that filter or transmit UV radiation (Robson et al. 2005). We thus assumed that the  
208 temperature in UV- was identical to that in UV+. We have no data for the MAX treatment in  
209 April because coyotes chewed on the data-logger cables. We have two measurements for each  
210 treatment.

211 The data-loggers have two sensors: one for air temperature and a thermistor enclosed in a  
212 small metal pipe on the tip of a cable. Air temperature sensors and thermistors were placed  
213 immediately above the ground. Because the air temperature sensor is housed in a relatively large  
214 plastic case, measurements provide an averaged temperature representative of an area of about  
215 30 cm<sup>2</sup>. This is appropriate for most treatments, because preliminary measurements showed that  
216 there were only weak horizontal temperature gradients. In contrast, steep gradients were  
217 observed in MIN and MAX, so measuring temperatures slightly away from the plant would  
218 result in large errors. Thus, we used the thermistor in these two treatments because, due to their  
219 small size, thermistors allowed for measurements in the close vicinity of plants without  
220 interfering with them. For EXP we used both sensors, so we could compare the results of each.  
221 We found that the thermistor attains air temperature at night (difference between air sensor and  
222 thermistor  $\approx$  0.5 °C), but gets several degrees ( $>$  10 °C) above air temperature during daytime.  
223 This precludes a direct comparison between MIN, MAX and the other treatments during the day.

224 To have daytime data that are comparable between MAX, EXP and TUS treatments (the ones  
225 expected to differ in temperature during daytime) we measured soil-surface temperature with a  
226 Fluke 62 mini infrared thermometer. These measurements were conducted in March 2017 using  
227 15 replicates.

228 The validity of the HYD treatment depends on vermiculite actually increasing soil hydric  
229 potential. We were unable to measure soil desiccation rates in the field due to unpredictable bad  
230 weather (sudden rainfall, heavily overcast days with nearly no evaporation) every time we tried  
231 to. Instead, we conducted an experiment filling five peat pots as the ones described above with  
232 soil collected from the study site, and five with a 3:1 soil:vermiculite mixture. In each pot we  
233 placed a Delmhorst GB-1 gypsum blocks, which allowed us to determine the water potential  
234 with a Delmhorst KS-D1 moisture meter. Water was added to all pots until the content was fully  
235 saturated. Pots were then allowed to drain in a dark room for 48 h, after which the soil water  
236 potential was near zero in both treatments. The pots were then placed in a greenhouse for 72 h,  
237 recording the water potential at ~8 h intervals.

238

### 239 *Statistical analyses*

240 Plant longevity was defined as the number of days it survived. When an individual died between  
241 two observations, its longevity was recorded as the midway point between observations  
242 (Crawley 2007). Plant life expectancy (i.e. mean longevity) was calculated using package  
243 ‘survival’ (Therneau and Grambsch 2013), by regressing each plant’s longevity on initial plant  
244 size and treatment using a model with data censoring, a Weibull distribution and within-block  
245 variations accounted by a frailty term (Crawley 2007). Package ‘gamm4’ (Wood and Scheipl  
246 2014) was used to analyse the change in plant size via a generalized additive mixed-effect model  
247 with Gaussian error (Crawley 2007). For this model, fixed effects were: treatment, logged plant-

248 size at the beginning of each observation period — *i.e.*, time between two consecutive size  
249 measurements—, and their interaction. The response variable was the logged size at the end of  
250 each observation period. Random components were individual plants nested in blocks crossed  
251 with the effect of time.

252 For all analyses significance was calculated from log-likelihood ratio tests. To determine  
253 differences between pairs of experimental treatments, we pooled all data for each possible pair of  
254 treatments and repeated the analysis to determine whether pooling caused a significant increase  
255 in unexplained deviance (Crawley 2007). Minimum temperatures were analysed via mixed-  
256 effects linear models using ‘lme4’ (Bates et al. 2014) package for R (R-Core-Team 2015). Data  
257 logger and date were set as random crossed factors, and treatments as a fixed variable. Error was  
258 normal. For high temperatures an ANOVA was conducted because a single measurement was  
259 obtained from each experimental unit. Soil hydric potentials were compared by a Mann-Whitney  
260 *U* test because of lack of normality.

261

## 262 **RESULTS**

263 Minimum temperatures differed between treatments (December:  $P < 0.001$ , April:  $P < 0.001$ ).  
264 MIN, UV+ and UV- increased temperature compared with EXP, whereas MAX had virtually no  
265 effect on nighttime temperatures (Fig. 1). The same patterns were observed in April, with the  
266 exception of MIN, which did not differ from EXP (Fig. 1) because temperatures did not drop low  
267 enough to activate the radiators. Maximum soil-surface temperatures in May differed greatly  
268 between TUS, MAX and EXP ( $P < 0.001$ ). In bare soil, temperatures were even  $> 75$  °C, while  
269 tussocks kept soil much cooler (Fig. 1, compare EXP with TUS maximum temperature

270 measurements). Marble sand prevented soil overheating, reaching temperatures only ~10 °C  
271 above those observed under tussocks (Fig. 1).

272 Soils from the study site lost moisture very rapidly in the greenhouse. After three days  
273 they were nearly dry. In contrast, soil with vermiculite retained much of the water it had initially  
274 (Fig. 2). After 72 h, soils with vermiculite had significantly less negative hydric potential ( $P =$   
275 0.008)

276 Life expectancy differed between treatments ( $P < 0.001$ ; Fig. 3), increasing with their  
277 minimum temperatures (Spearman correlation between mean minimum temperature and life  
278 expectancy:  $\rho = 0.71$ ,  $P = 0.048$ ). The only prominent exception to this trend was MAX  
279 (Temperature-life expectancy correlation after removing MAX:  $\rho = 0.95$ ,  $P < 0.001$ ), which had  
280 a much larger survival than expected from its minimum temperature. Screens had positive effects  
281 on survival. However, because survival did not differ between UV+ and UV-, the increase in  
282 survival cannot be attributed to changes in UV radiation. Instead, this was likely the result of  
283 screens ameliorating minimum temperatures. SOI did not differ significantly from EXP.

284 Initial size had a strong effect on growth ( $P < 0.001$ ), which was also affected by  
285 treatments ( $P < 0.001$ ) but not their interaction. In most cases, plants shrank. Plants in HYD had  
286 the smallest reductions in size. However, HYD had also the lowest survival. In contrast, MIN  
287 caused the largest reductions in size, and did not differ from SOI and MAX (Fig. 2). As before,  
288 no differences in growth were observed between treatments UV+ and UV-, indicating no effects  
289 from UV radiation (Fig. 3).

290

291 **DISCUSSION**

292

293 Most of the treatments had some positive effects on *Mexerion*, although none was as effective as  
294 the tussock. However, the regulation of extreme temperatures seems to be the most important  
295 factor. Growth rates revealed some negative effects caused by tussocks, which were seemingly  
296 related to soil conditions. In contrast, UV radiation had no effect on *Mexerion*'s performance,  
297 and the role of water in the plant-plant interaction was unclear.

298 As in other páramos (Diemer 1996), we recorded a large difference between daily  
299 minimum and maximum temperatures. The lowest minimum temperatures were recorded in EXP  
300 and the highest under TUS. Tussocks also had large effects on maximum temperatures, which  
301 were 40 °C lower than in EXP (Fig. 1). Thus, tussocks are able to ameliorate both low and high  
302 temperatures, supporting the notion that they act as thermal buffers (Coe 1969, Chapin III et al.  
303 1979, Rundel et al. 1994).

304 Life expectancy increased with minimum temperature (Fig. 2). This indicates that  
305 extreme minimum-temperatures were a major driver of mortality in this high-altitude  
306 environment. Furthermore, the minimum temperatures (around -10 °C in December, and -4 °C in  
307 April) were close to the those expected to cause freezing damage to plants (Pearce 2001).  
308 Therefore, the amelioration of minimum temperatures by tussocks appears to be a key driver of  
309 facilitation by preventing freezing, as previously suggested (Anthelme and Dangles 2012). This  
310 idea is supported by the fact that plants in MIN had the second largest life expectancy, and did  
311 not differ significantly from TUS.

312 A notable exception to the observed correlation between survival and minimum  
313 temperatures was MAX. This suggests that, unlike other treatments such as UV+ or UV-,  
314 increased minimum temperatures were not responsible of the relatively good performance of  
315 *Mexerion* individuals in the MAX treatment. This is what was expected. Whereas this treatment

316 was incapable of reducing soil temperatures at noon as much as tussocks, it still caused a  
317 decrement of about 30 °C in comparison with bare soil. The large positive effect of MAX on  
318 survival highlights the importance of maximum-temperature amelioration in páramos. While the  
319 effects of minimum temperatures on plants have been widely studied, maximum temperatures  
320 have been largely neglected in páramos. In our study, the maximum soil temperatures observed  
321 in EXP are high enough to cause irreversible damage to plant growth (Salvucci and Crafts-  
322 Brandner 2004), whereas in TUS and MAX, temperatures may at most inactivate photosynthesis  
323 for short periods of time (Wahid et al. 2007).

324 UV radiation is strongest at high altitudes near the equator (Caldwell et al. 1980). Thus,  
325 plants living in páramos are expected to experience high levels of potentially lethal radiation.  
326 Excessive UV radiation has negative effects on plant life, damaging DNA, membranes and the  
327 photosynthetic apparatus (Rozema et al. 1997). In our experiment, screens had positive effects on  
328 plants, though this was not due to UV radiation, as survival and growth in UV+ and UV-  
329 treatments did not differ (Fig. 2). Instead, the effect may be attributed to low temperature  
330 buffering under the screens.

331 The effects of water availability on *Mexerion* were unclear. Our results show that  
332 vermiculite increases water potential in the soil, as expected. Using models for soil desiccation  
333 based on soil temperature, it can be estimated that the difference between the hydric potentials in  
334 bare ground and under tussocks, increases at the same rate as that between soils with and without  
335 vermiculite (Appendix 1). This suggests that the addition of vermiculite is an acceptable  
336 surrogate for the effects of tussocks. However, plants in HYD had the lowest survival. A lack of  
337 positive effects of vermiculite would be expected if moisture were not limiting. We consider that  
338 this is likely, because the removal of the topmost layers (~3-5 mm) of the soil revealed a very

339 humid substrate during the first weeks of the experiment. Perhaps if *Mexerion* individuals had  
340 not died so rapidly in HYD, surviving into the drier months, positive effects of increased water  
341 availability on survival would have become apparent. Changes in soil chemistry due to the  
342 addition of vermiculite may also have obscured our data. Vermiculite tends to increase nutrient  
343 availability, more so if we consider that it neutralizes pH (Libardi et al. 1983), and thus could be  
344 mobilising nutrients in the acidic soils (pH 5 - 6) of the Iztaccíhuatl volcano (Miguel 2013). This  
345 would not account for the reduced survival in the HYD treatment, although it may explain why  
346 growth rates observed there were the highest.

347 Tussocks had not only positive, but negative effects on *Mexerion*, as evinced by the  
348 analyses of growth. Such negative effects may be caused by a reduction of photosynthetic  
349 radiation under the shade of *Festuca* (Callaway 1995), but also seem to be related to soil  
350 conditions and biota. Plants in SOI also had low growth rates in our experiment The use of soil  
351 from *Festuca* in SOI probably affected nutrients and soil biota, which is expected to be very  
352 abundant under tussocks (Chapin III et al. 1979). In alpine environments, plants compete  
353 strongly for nutrients with soil microbes (Jonasson et al. 1999). Nutrient-rich soils, such as those  
354 found under tussocks (Chapin III et al. 1979), favour microorganisms over plants, enhancing  
355 competition (Jonasson et al. 1999, Dunn et al. 2006) and ultimately leading to large reductions in  
356 plant growth (Schmidt et al. 1997, van der Heijden et al. 2008). The idea that competition with  
357 microbes affects plants negatively is further supported by the fact that MIN and MAX had the  
358 most negative effects on *Mexerion* growth. Just as both treatments strongly promoted *Mexerion*  
359 survival, they may have favoured *Mexerion*'s microbial competitors by providing a more  
360 thermally-stable environment (Margesin et al. 2008) and competition, leading to large reductions  
361 in plant size.



362           This study highlights the importance of considering the simultaneous effects of multiple  
363 stressors on facilitation. None of our treatments had positive effects as large as those observed  
364 under tussocks. This may in part be expected because our treatments were imperfect mimics of  
365 the ameliorating effects of tussocks. However, it would be surprising that, given the high  
366 intensity of different sources of stress, only one of them determines plant performance. Consider  
367 temperature: both extreme maximum and minimum temperatures had strong negative effects on  
368 plants growing on bare soil. However, tussocks ameliorate both of these adverse effects by  
369 maintaining protégées warmer during the night and cooler during the day. This joint effect may  
370 explain why plants in the TUS treatment had the largest observed life expectancies. The effects  
371 of tussocks on other factors may also contribute to making tussocks the most favourable  
372 environment in terms of survival. A full-factorial experiment would be required to analyse  
373 formally the joint effects of many stressors, but it would have been impossible to conduct (given  
374 6 experimental forms of manipulation, we would have required  $2^6 = 64$  experimental treatments).

375           Our results suggest a scenario in which the benefactor species exerts positive and  
376 negative, direct and indirect effects on its protégé through a multiplicity of environmental  
377 modifications. Such complex effects probably depend on the benefactor's identity: whereas  
378 facilitation by *Festuca* seemed independent of soil properties (although our data are not  
379 conclusive), these are important when cushion plants are considered (Anthelme and Dangles  
380 2012, Hupp et al. 2017). In turn, the protégé's tolerance to different stressors may determine its  
381 responses to the benefactor (Liancourt et al. 2005). For instance, the negligible effect of UV  
382 radiation on *Mexerion* performance probably arises from its dense, reflective pubescence, which  
383 may confer resistance to UV radiation (Rozema et al. 1997). This interplay between amelioration

384 of and tolerance to multiple stressors may explain why facilitative interactions are highly  
385 species-specific in nature (Callaway 1998, Hupp et al. 2017).

386

## 387 **ACKNOWLEDGMENTS**

388

389 This work was funded by Programa de Apoyos a Proyectos de Investigación e Innovación  
390 Tecnológica-Universidad Nacional Autónoma de México IN217607. D. Montañana assisted in  
391 the field. Parque Nacional Izta-Popo-Zoquiapan provided logistic support.

392

## 393 **LITERATURE CITED**

394

- 395 Anthelme, F., B. Buendia, C. Mazoyer, and O. Dangles. 2012. Unexpected mechanisms  
396 sustain the stress gradient hypothesis in a tropical alpine environment. **23**:62-72.
- 397 Anthelme, F., L. A. Cavieres, and O. Dangles. 2014. Facilitation among plants in alpine  
398 environments in the face of climate change. *Frontiers in plant science* **5**:387.
- 399 Anthelme, F., and O. Dangles. 2012. Plant-plant interactions in tropical alpine  
400 environments. *Perspectives in Plant Ecology, Evolution and Systematics* **14**:363-  
401 372.
- 402 Bates, D., M. Mächler, B. Bolker, and S. Walker. 2014. Fitting linear mixed-effects models  
403 using lme4. arXiv preprint arXiv:1406.5823.
- 404 Billings, W. 1974. Adaptations and origins of alpine plants. *Arctic and Alpine Research*  
405 **6**:129-142.
- 406 Caldwell, M. M., R. Robberecht, and W. Billings. 1980. A steep latitudinal gradient of solar  
407 ultraviolet-B radiation in the arctic-alpine life zone. *Ecology* **61**:600-611.
- 408 Callaway, R. M. 1995. Positive interactions among plants. *The Botanical Review* **61**:306-  
409 349.
- 410 Callaway, R. M. 1998. Are positive interactions species-specific? *Oikos*:202-207.
- 411 Callaway, R. M., R. Brooker, P. Choler, Z. Kikvidze, C. J. Lortie, R. Michalet, L. Paolini, F. I.  
412 Pugnaire, B. Newingham, and E. T. Aschehoug. 2002. Positive interactions among  
413 alpine plants increase with stress. *Nature* **417**:844.
- 414 Catorci, A., S. Cesaretti, J. L. Velasquez, and H. Zeballos. 2011. Plant-plant spatial  
415 interactions in the dry Puna (southern Peruvian Andes). *Alpine botany* **121**:113.
- 416 Chapin III, F. S., K. Van Cleve, and M. C. Chapin. 1979. Soil temperature and nutrient cycling  
417 in the tussock growth form of *Eriophorum vaginatum*. *The Journal of Ecology*:169-  
418 189.

- 419 Choler, P., R. Michalet, and R. M. Callaway. 2001. Facilitation and competition on gradients  
420 in alpine plant communities. *Ecology* **82**:3295-3308.
- 421 Coe, M. 1969. Microclimate and animal life in the equatorial mountains. *Zoologica Africana*  
422 **4**:101-128.
- 423 Crawley, M. J. 2007. *The R Book*. J. Willey, Chichester.
- 424 Day, R., and J. Skoupy. 1971. Moisture storage capacity and postplanting patterns of  
425 moisture movement from seedling containers. *Canadian Journal of Forest Research*  
426 **1**:151-158.
- 427 Day, T. A., and P. J. Neale. 2002. Effects of UV-B radiation on terrestrial and aquatic primary  
428 producers. *Annual Review of Ecology and Systematics* **33**:371-396.
- 429 Diemer, M. 1996. Microclimatic convergence of high-elevation tropical páramo and  
430 temperate-zone alpine environments. *Journal of Vegetation Science* **7**:821-830.
- 431 Dunn, R. M., J. Mikola, R. Bol, and R. D. Bardgett. 2006. Influence of microbial activity on  
432 plant-microbial competition for organic and inorganic nitrogen. *Plant and Soil*  
433 **289**:321-334.
- 434 He, Q., M. D. Bertness, and A. H. Altieri. 2013. Global shifts towards positive species  
435 interactions with increasing environmental stress. *Ecology Letters* **16**:695-706.
- 436 Hedberg, I., and O. Hedberg. 1979. Tropical-alpine life-forms of vascular plants. *Oikos*:297-  
437 307.
- 438 Hedberg, O. 1964. Features of afroalpine plant ecology. *Acta Phytogeographica Suecica*  
439 **49**:297-307.
- 440 Heiskanen, J. 1999. Hydrological properties of container media based on sphagnum peat  
441 and their potential implications for availability of water to seedlings after  
442 outplanting. *Scandinavian journal of forest research* **14**:78-85.
- 443 Hupp, N., L. D. Llambí, L. Ramírez, and R. M. Callaway. 2017. Alpine cushion plants have  
444 species-specific effects on microhabitat and community structure in the tropical  
445 Andes. *Journal of Vegetation Science* **28**:928-938.
- 446 Jonasson, S., A. Michelsen, and I. K. Schmidt. 1999. Coupling of nutrient cycling and carbon  
447 dynamics in the Arctic, integration of soil microbial and plant processes. *Applied Soil*  
448 *Ecology* **11**:135-146.
- 449 Körner, C. 2003. *Alpine plant life: functional plant ecology of high mountain ecosystems*.  
450 Springer-Verlag, Berlin.
- 451 Liancourt, P., R. M. Callaway, and R. Michalet. 2005. Stress tolerance and competitive-  
452 response ability determine the outcome of biotic interactions. *Ecology* **86**:1611-  
453 1618.
- 454 Libardi, P. L., E. Salati, and K. Reichardt. 1983. Use of expanded vermiculite as a soil  
455 conditioner in the tropics. Pages 301-308 *in* IAEA, editor. *Isotope and Radiation*  
456 *Techniques in Soil Physics and Irrigation Studies*. International Atomic Energy  
457 Agency, Vienna.
- 458 Luteyn, J. 1999. *Páramos: a checklist of plant diversity, geographical distribution, and*  
459 *botanical literature*. The New York Botanical Garden Press, New York.
- 460 Maestre, F. T., and J. Cortina. 2004. Do positive interactions increase with abiotic stress? A  
461 test from a semi-arid steppe. *Proceedings of the Royal Society of London B:*  
462 *Biological Sciences* **271**:S331-S333.

- 463 Malatesta, L., F. M. Tardella, K. Piermarteri, and A. Catorci. 2016. Evidence of facilitation  
464 cascade processes as drivers of successional patterns of ecosystem engineers at the  
465 upper altitudinal limit of the dry puna. *PLoS ONE* **11**:e0167265.
- 466 Margesin, R., M. Jud, D. Tschirko, and F. Schinner. 2008. Microbial communities and  
467 activities in alpine and subalpine soils. *FEMS Microbiology Ecology* **67**:208-218.
- 468 Miguel, J. D. 2013. Cobertura vegetal, materia orgánica y pH en suelo y su relación con el  
469 índice de vegetación de diferencia normalizada (NDVI) en el Parque Nacional  
470 Iztaccíhuatl-Popocatepetl. Universidad Nacional Autónoma de México, Mexico.
- 471 Mizuno, K. 1998. Succession processes of alpine vegetation in response to glacial  
472 fluctuations of Tyndall Glacier, Mt. Kenya, Kenya. *Arctic and alpine research*:340-  
473 348.
- 474 Monteiro, J. A. F., E. Hiltbrunner, and C. Körner. 2011. Functional morphology and  
475 microclimate of *Festuca orthophylla*, the dominant tall tussock grass in the Andean  
476 Altiplano. *Flora-Morphology, Distribution, Functional Ecology of Plants* **206**:387-  
477 396.
- 478 Moro, M., F. Pugnaire, P. Haase, and J. Puigdefábregas. 1997. Effect of the canopy of *Retama*  
479 *sphaerocarpa* on its understorey in a semiarid environment. *Functional Ecology*  
480 **11**:425-431.
- 481 Nuñez, C. I., M. A. Aizen, and C. Ezcurra. 1999. Species associations and nurse plant effects  
482 in patches of high-Andean vegetation. *Journal of Vegetation Science* **10**:357-364.
- 483 Okada, K., S. Matsui, T. Isobe, Y. Kameshima, and A. Nakajima. 2008. Water-retention  
484 properties of porous ceramics prepared from mixtures of allophane and vermiculite  
485 for materials to counteract heat island effects. *Ceramics international* **34**:345-350.
- 486 Patty, L., S. R. Halloy, E. Hiltbrunner, and C. Körner. 2010. Biomass allocation in herbaceous  
487 plants under grazing impact in the high semi-arid Andes. *Flora-Morphology,*  
488 *Distribution, Functional Ecology of Plants* **205**:695-703.
- 489 Pearce, R. S. 2001. Plant Freezing and Damage. *Annals of Botany* **87**:417-424.
- 490 Prado, F. E., M. Rosa, C. Prado, G. Podazza, R. Interdonato, J. A. González, and M. Hilal. 2012.  
491 UV-B radiation, its effects and defense mechanisms in terrestrial plants. Pages 57-83  
492 *Environmental Adaptations and Stress Tolerance of Plants in the Era of Climate*  
493 *Change*. Springer.
- 494 Pugnaire, F. I., and M. T. Luque. 2001. Changes in plant interactions along a gradient of  
495 environmental stress. *Oikos* **93**:42-49.
- 496 R-Core-Team. 2015. R: A language and environment for statistical computing. R Foundation  
497 for Statistical Computing, Vienna.
- 498 Robson, T. M., V. A. Pancotto, S. D. Flint, C. L. Ballaré, O. E. Sala, A. L. Scopel, and M. M.  
499 Caldwell. 2003. Six years of solar UV-B manipulations affect growth of *Sphagnum*  
500 and vascular plants in a Tierra del Fuego peatland. *New Phytologist* **160**:379-389.
- 501 Robson, T. M., V. n. A. Pancotto, A. L. Scopel, S. D. Flint, and M. M. Caldwell. 2005. Solar UV-B  
502 influences microfaunal community composition in a Tierra del Fuego peatland. *Soil*  
503 *Biology and Biochemistry* **37**:2205-2215.
- 504 Rozema, J., J. van de Staaij, L. O. Björn, and M. Caldwell. 1997. UV-B as an environmental  
505 factor in plant life: stress and regulation. *Trends in Ecology & Evolution* **12**:22-28.
- 506 Rundel, P. W., A. P. Smith, and F. C. Meinzer. 1994. Tropical alpine environments: plant form  
507 and function. Cambridge University Press, Cambridge.

- 508 Salvucci, M. E., and S. J. Crafts-Brandner. 2004. Inhibition of photosynthesis by heat stress:  
509 the activation state of Rubisco as a limiting factor in photosynthesis. *Physiologia*  
510 *plantarum* **120**:179-186.
- 511 Sarmiento, G. 1986. The climatic background. Pages 9–41 *in* F. Vuilleumier and M.  
512 Monasterio, editors. *High altitude tropical biogeography*. Oxford University Press,  
513 Oxford.
- 514 Schmidt, I. K., A. Michelsen, and S. Jonasson. 1997. Effects of labile soil carbon on nutrient  
515 partitioning between an arctic graminoid and microbes. *Oecologia* **112**:557-565.
- 516 Schrader, W. L. 2000. *Using transplants in vegetable production*. UCANR Publications.
- 517 Selwyn, M. R. 1996. *Principles of experimental design for the life sciences*. CRC Press.
- 518 Smith, A. P., and T. P. Young. 1987. Tropical alpine plant ecology. *Annual Review of Ecology*  
519 *and Systematics* **18**:137-158.
- 520 Therneau, T. M., and P. M. Grambsch. 2013. *Modeling survival data: extending the Cox*  
521 *model*. Springer Science & Business Media, New York.
- 522 Tovar-Romero, H. A. 2010. Reglas de ensamblaje entre plantas de distinta forma en un  
523 ambiente estresante. MSc thesis. Universidad Nacional Autónoma de México.
- 524 van der Heijden, M. G., R. D. Bardgett, and N. M. Van Straalen. 2008. The unseen majority:  
525 soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems.  
526 *Ecology Letters* **11**:296-310.
- 527 Wahid, A., S. Gelani, M. Ashraf, and M. R. Foolad. 2007. Heat tolerance in plants: an  
528 overview. *Environmental and experimental botany* **61**:199-223.
- 529 Wood, S., and F. Scheipl. 2014. gamm4: Generalized additive mixed models using mgcv and  
530 lme4. R package version 0.2-3.
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533 **FIGURE LEGENDS**

534 FIGURE 1. Extreme temperatures recorded in the experiment. See text for treatment  
535 abbreviations. We have no data for the MAX treatment in April because coyotes chewed on the  
536 thermistor. Shared letters indicate no significant differences ( $\alpha = 0.05$ ).

537 FIGURE 2. Soil hydric potential of soil with (dashed line) and without (solid line) vermiculite  
538 over three days. Mean  $\pm$  SE are shown.

539 FIGURE 3. Performance of *Mexerion* individuals in different treatments (see text for  
540 abbreviations). Life expectancy and plant growth correspond to mean-sized plants. Shared letters  
541 indicate no significant differences ( $\alpha = 0.05$ ).

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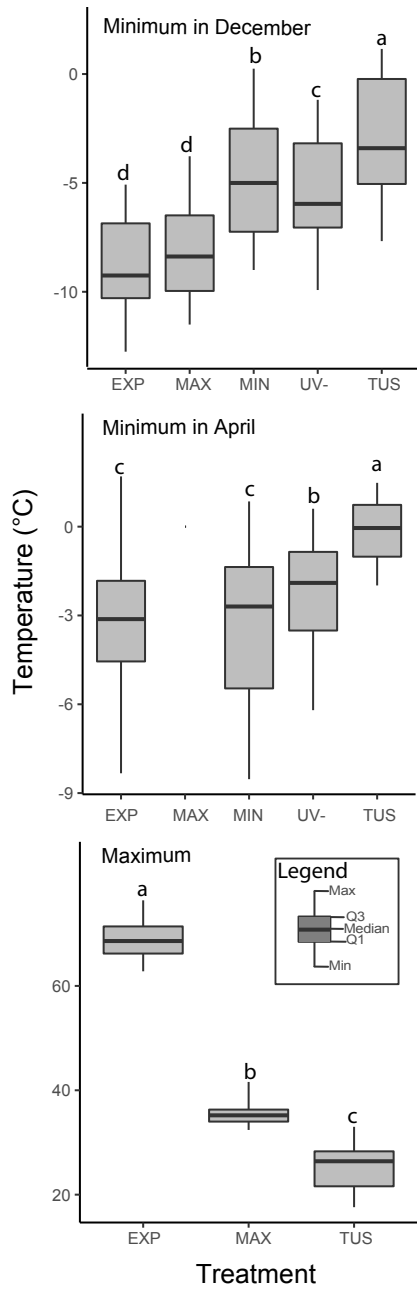
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557 **FIGURES**

558 **FIGURE 1**



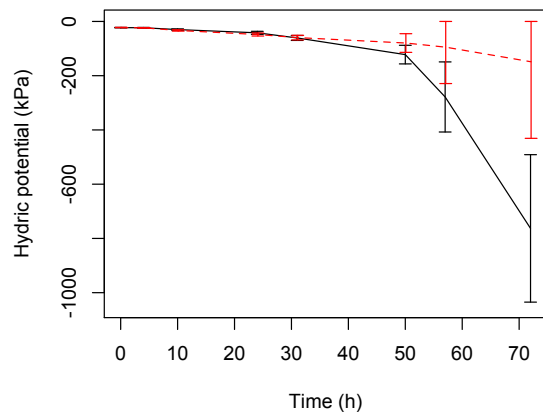
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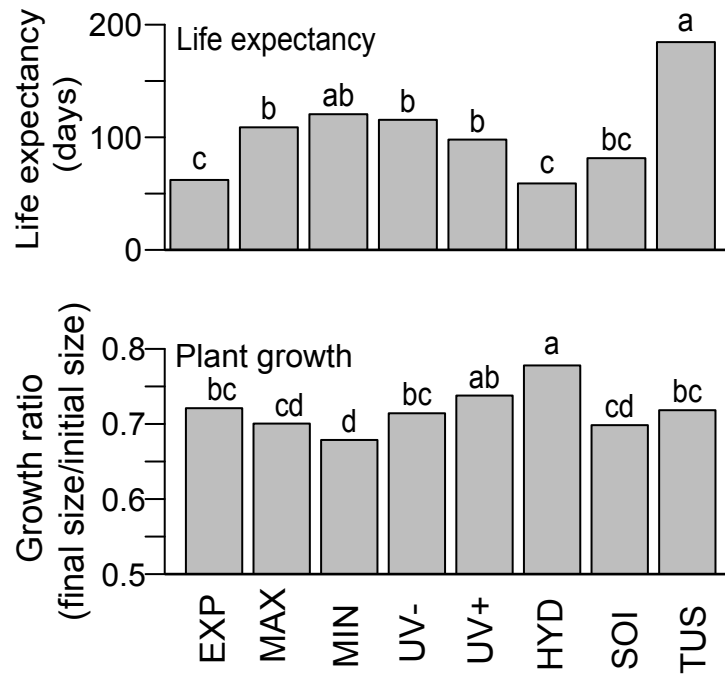
563 FIGURE 2



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565 FIGURE 3





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