

“The effect of water potential on the water balance of lichens and mosses – distribution patterns of internal and external water”

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**“The effect of water potential on the water balance of lichens and mosses – distribution patterns
of internal and external water”**

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1 Abstract:

2 Lichen-forming fungi and mosses form a major part of the terrestrial non-vascular vegetation and are
3 thought to have a significant impact on global biogeochemical cycles, such as the nitrogen and carbon
4 cycle. However, in order to draw quantitative conclusions about their ecosystem functions, it is
5 essential to understand the metabolic processes underlying their growth. The dynamic water balance
6 of lichens and mosses is a crucial factor in this regard since the metabolic processes of the organisms
7 can only occur in phases with sufficient water saturation. Water can occur inside the cells (internal) in
8 the symplast and in pores in the cell wall (apoplast water), and also externally in the capillaries of the
9 intercellular space. It is poorly known, however, how atmospheric demand for water, related to water
10 potential (ψ), affects the dynamic distribution of internal and external water in lichens and mosses and
11 which consequences this may have for their water balance. Here, we examined water absorption of
12 *Pleurozium schreberi*, *Cladonia portentosa* and *Peltigera rufescens* under a gradient of ψ in the

13 laboratory. Results show that for all species, relative water content decreased with decreasing ψ . Both
14 internal and external water contents thereby showed a consistent pattern across the range of ψ -values
15 tested here. This indicates that, although a proportion of the internal water has already evaporated
16 and therefore the turgor pressure is altered, in all three species a proportion of external water is
17 retained by capillary forces even at low ψ .

18

19 **Key words:**

20 hydration; internal and external water; lichens; mosses; water potential

21

22 **Introduction:**

23 Lichen-forming fungi and mosses are globally distributed organisms that show a wide range of
24 adaptations to, often extreme, environmental conditions. They form a major part of the terrestrial
25 non-vascular vegetation, and they account for a large proportion of total vegetation in multiple
26 ecosystems (e.g., drylands with biological soil crusts, tundra, parts of boreal forests) (Ahmadjian, 1995;
27 Weber et al., 2016). They may play a key role in local and global biogeochemical cycles of carbon,
28 nutrients, and water (Cornelissen et al., 2007; Porada et al., 2014; 2019; Weber et al., 2015). In order
29 to draw quantitative conclusions about their ecosystem functions, however, it is crucial to understand
30 the metabolic processes underlying their growth, especially with regard to their dynamic water
31 balance.

32 Lichens and mosses, as poikilohydric organisms, can survive long periods of drought in inactive
33 state (Proctor, 1998). Thus, the periods when they are water-saturated are essential for metabolic
34 processes, as growth can only occur in these phases. The basic processes of water uptake by lichens
35 (e.g., Blum, 1973; Kershaw, 1985; Rundel, 1988) and mosses (e.g., Bayfield, 1973; Hébant, 1977;
36 Proctor, 1982) have been extensively studied. Uptake of water may occur in liquid form or from
37 atmospheric moisture, usually over the entire surface. The ability to reactivate from air humidity alone,
38 however, is species-dependent, and some groups need liquid water for activation, such as
39 cyanolichens, for instance (Jonsson et al., 2008). For lichens, morphological and physiological
40 parameters such as the water-holding capacity (Green & Lange, 1991), growth form (Kershaw, 1985),
41 type of photobiont (Lange & Kilian, 1985), the cortex (Pardow et al., 2010) and the hydrophobicity of
42 lichen surfaces (Lakatos et al., 2006) have been found to influence water relations (Gauslaa, 2014). In
43 addition, species-specific internal factors, such as the presence of secondary metabolites, affect the
44 responses of lichens after hydration events (Jonsson et al., 2008; Gauslaa, 2014). Mosses conduct
45 water mostly externally, but vascular tissue has been found in some species (Brodrribb et al., 2020).
46 Species-specific water uptake can additionally occur via the rhizoids, with water being transferred
47 through the stems. This process is not sufficient to cover water needs but can optimise water supply
48 for drought-tolerant species (Bayfield, 1973).

49 Since lichens and mosses lack active means of controlling water loss, such as stomata, for
50 instance, they ultimately equilibrate with the relative humidity of the surrounding air. As a
51 consequence, their water potential (ψ) becomes negative when exposed to unsaturated air. The
52 organisms react differently to desiccation depending on the species. Hygrophyte moss species are
53 sensitive, whereas mesophytes and especially xerophytes are adapted to drought. Xerophytes show
54 special repair mechanisms and can resume their metabolic functions after years of desiccation
55 (Proctor, 2000).

56 While factors which affect the total dynamic water content of lichens and mosses have been
57 assessed in many studies, less attention has been directed towards the dynamics of different pools of
58 water in the organisms. Water can occur both as internal water content inside the cells (mostly
59 symplast, but also in the cell wall pores), and also as external water in the intercellular space
60 (capillaries) (Beckett, 1996; 1997; Proctor, 2000). Only few studies quantify both internal and external
61 water in lichens and mosses (Green et al., 1985; Green & Lange, 1991; Gauslaa, 2014) and it is largely
62 unknown how these two fractions are distributed under different water potentials. However, the
63 dynamic changes of relative fractions of internal and external water during water uptake and loss may
64 be highly relevant for the ecophysiology of lichens and mosses, due to their differing physiological
65 implications.

66 The internal water pool provides some means of resistance against evaporation (Larson, 1979)
67 but requires the build-up of cell tissue which comes at a certain carbon cost. The external water pool
68 may thus serve as an alternative, as it extends the duration of hydration and therefore metabolic
69 activity (Esseen et al., 2015). The higher rate of evaporation from this pool compared to internal water
70 may be compensated, from a carbon economy viewpoint, by the lower carbon costs of creating the
71 water storage capacity. However, external water often leads to supersaturation and thus limitation of
72 photosynthesis by low diffusivity for CO₂ (Green et al., 2011), while internal water is likely to have a
73 weaker effect on depression of photosynthesis (Esseen et al., 2015). These differences may affect the
74 carbon balance and growth of lichens and mosses. If all external water was depleted first during
75 desiccation, for instance, CO₂-diffusivity would increase and may allow for higher rates of

76 photosynthesis before the organism becomes entirely inactive, compared to the opposite case where
77 internal water content decreases first.

78 Further uncertainty exists regarding the interpretation of the transition point of water
79 potential (ψ) from zero to negative values (Beckett, 2002). Since the cell water potential consists of
80 the sum of turgor pressure and osmotic potential, it will usually become negative once the cells stop
81 being fully turgid; at the turgor loss point (*TLP*), turgor pressure has then reached a value of zero
82 (Proctor, 2000). Since capillary forces in the intercellular space act on the external water, however, the
83 overall water potential ψ may show negative values already while the cells are still fully turgid.

84 The aim of this study is to determine how different values of water potential ψ affect the relative
85 fractions of internal and external water in different species of lichens and mosses, and which
86 implications this has for the interpretation of the transition point of ψ from zero to negative values. In
87 addition to these physiological considerations, the distribution of water content into different pools
88 and its effect on turgor loss is a relevant factor in modelling water uptake and loss by lichens and
89 mosses. The process-based non-vascular vegetation model *LiBry*, which simulates a large number of
90 different physiological strategies at the phenotype level (Porada et al., 2013; 2019), has so far assumed
91 that ψ is zero as long as external water is stored and becomes negative at the onset of evaporation of
92 the internal water (Baldauf et al., 2020). However, a negative potential could already occur at external
93 water evaporation due to capillary forces. This would not only affect the simulated dynamic water
94 balance, but also the growth of lichens and mosses estimated by the *LiBry* model. Our study will thus
95 provide a basis for developing further quantitative process models of non-vascular vegetation.

96

97 **Material and Methods:**

98 **Species selection**

99 This study involved *Pleurozium schreberi* (Hedw.) as a moss, *Peltigera rufescens* (Weiss) Humb. as a
100 foliose lichen and *Cladonia portentosa* (Dufour) Coem. as a fruticose lichen. All three species are
101 common at the study site (Hamburg, Germany) and differ morphologically and in their ecological
102 preferences. *P. schreberi* is a common forest moss, which prefers light-rich areas and is widespread on

103 acid soils (Heaths, semi-humid coniferous forests), and is especially common in the northern
104 hemisphere (Düll & Düll-Wunder, 2008). *C. portentosa* is an erect, fruticose chlorolichen, that has a
105 wide site amplitude but prefers light-rich sites and nutrient-poor soils, mainly due to its lower
106 competition from vascular plants. *P. rufescens* is a broad-lobed, foliose cyanolichen that prefers open,
107 base-rich sites and is widespread in northern to southern Europe. (Wirth & Kirschbaum, 2017)

108

109 **Sampling**

110 Sampling took place at three sites in Hamburg, Germany, in December 2021. *P. schreberi* and *C.*
111 *portentosa* were collected at two sites in NSG Fischbeker Heide (53°27'N, 9°50"E), a nature reserve in
112 the outer part of the city, which is characterised by sandy, nutrient-poor podzols and dominated by
113 heath vegetation, such as *Calluna vulgaris* in the understory, and trees of the families *Betula* and *Pinus*.
114 *P. rufescens* was collected from a semi-shaded meadow at Ohlsdorf cemetery, in the inner-city area
115 (53°37'N 10°02"E). The samples were stored outside the research institute for four weeks under
116 natural conditions until the measurements took place.

117

118 **Sample preparation**

119 All samples were taken from the exterior on the day of measurement and cleaned from soil particles
120 and interfering organisms. Visible water on the surfaces of the organisms was removed by delicate
121 task wipes (Hájek & Beckett, 2007). Subsequently, the samples (approx. 200-700 mg) were sprayed
122 evenly with artificial rainwater - H₂O dist. with low nutrient concentrations (8.8 mg l⁻¹ K₂CO₃, 4.6 mg l⁻¹
123 Na₂CO₃, 5 mg l⁻¹ CaCO₃, 4.4 mg l⁻¹ Fe₂SO₄7H₂O and 0.6 mg l⁻¹ MnSO₄H₂O), in order to avoid osmotic
124 stress for the organisms (Johansson et al., 2011). The samples were pre-dried under room temperature
125 for 3 hours after spraying to ensure a semi-moist condition, (Beckett, 1995; 1997; 2002) and
126 immediately transferred to the experimental setup.

127

128 **Measurement**

129 In order to measure the water absorption of the samples under a gradient of ψ , we generated a relative
130 humidity (RH), corresponding to a specific ψ , in desiccators by using specific saturated salt solutions
131 (Table 1) (Kutzelnigg, 1963; Scheidegger et.al, 1995). Additionally, a relative humidity of 98.5% was
132 created by adding 7.0 g/100ml NaCl and a humidity of 99.5% by H₂O dist. The desiccators were filled
133 with 1 l of the solution, the samples were placed on the grid and subsequently hydrated for 48 h under
134 the specific relative humidity in a climate chamber with 15°C and a day and night rhythm of 12/12 h.
135 The relative humidity was controlled by hygrometers (*TFA Dostmann*, Thermo-Hygrometer, 45.2027).
136 These were calibrated beforehand by wrapping them in humidified tissues for a few hours. In one run,
137 the same potential was generated in three desiccators, with one sample of each species placed in each
138 desiccator (three species per desiccator). This resulted in seven experimental runs representing seven
139 different solutions, yielding three replicates per species (7x3x3).

140

141 [Place Table 1 here]

142

143 Directly after each run the samples were gently shaken in order to remove surface water and weighed
144 (wet mass, external water included, WM_E) (*Sartorius* Entris II, analytical scale, accuracy: 0.1 mg).
145 Subsequently, the external water was removed by slightly blotting them in filtration paper and the
146 samples were weighed again (only internal water included, WM_I) (Kershaw, 1985; Proctor et al., 1998;
147 Esseen et al., 2017). Afterwards, the samples were dehydrated in a desiccator with Silica Gel in the
148 climatic chamber (15°C and a day and night rhythm of 12/12 h) for 48 h (Scheidegger et al., 1995) and
149 immediately weighed afterwards (dry mass, DM). We further measured the water content under
150 maximum water saturation as the water holding capacity (WHC) of all species by generously irrigating
151 five samples of each species with H₂O dist. After gently shaking the samples, the samples were weighed
152 and afterwards dried in the climatic chamber.

153

154 **Data analysis**

155 Parameters of water uptake (Table 2) were determined based on the results of the measurements.
156 Mean and standard deviation of all parameters were calculated. W_{total} against ψ was plotted. rW_e was
157 plotted and statistically tested for correlation for each species separately and combined (Pearson
158 residual). The statistical analysis was carried out with the software R (R Studio, version 1.4.1717).

159

160 [Place Table 2 here]

161

162 **Results:**

163 **Water content**

164 W_{total} increased for all species with increasing ψ (Fig. 1 B, Table 3). The highest increase was observed
165 for *P. schreberi*, which did not show the highest water contents in the lower potentials but achieved
166 higher values than the other species as ψ increased. *C. portentosa* and *P. rufescens* showed a similar
167 increase, with *P. rufescens* showing the lowest mean water content for all potentials (Fig 1 A, B; Table
168 3).

169 For all species, the shape of the curve changed towards higher ψ . This is particularly apparent through
170 the representation of the reciprocal of ψ (Fig. 1 A). Since the relationship between saturation and ψ is
171 non-linear and amounts to approximately $1/x$, the non-linearity can be dissolved through the
172 transformation to $-1/\psi$, thus facilitating the visual detection of the values. The change of mean values
173 of W_{total} increased with an increasing water potential up to -3.7 MPa for *P. schreberi*, and -2.0 MPa for
174 *P. rufescens* and *C. portentosa* (Fig. 1 B). Both lichens even showed a decrease in mean water content
175 above a potential of -2.0 MPa. (Fig. 1 A).

176

177 [Place Figure 1 here]

178

179 **External and internal water**

180 rW_e increased for all species with increasing ψ (Fig. 2 A). Statistical analysis by Pearson's test showed
181 a positive correlation between rW_e and ψ for all species (Fig. 2 A) and combined (Fig. 2 B).

182

183 [Place Figure 2 here]

184

185 rW_i achieved higher values than rW_e for all three species. The ratio between rW_i and rW_e decreased
186 with increasing ψ in all species up to a up to a ψ of -2.0 MPa for *P. schreberi* and *C. portentosa* and a
187 ψ of -3.7 MPa for *P. rufescens*. (Table 3; Fig. 3 A-C). *C. portentosa* showed the widest ratio (28.1:1) at
188 the lowest ψ . However, rW_e showed an increase up to a ψ of -2.0 MPa (*P. schreberi* and *C. portentosa*)
189 and decreased under higher ψ . For *P. rufescens* rW_e increases until a ψ of -3.7 MPa (Fig. 3 A-C).

190

191 [Place Figure 3 here]

192

193 **Discussion:**

194 The effect of ψ on the uptake and release of water by lichens and mosses has been rarely studied.
195 Using desiccators and generating specific water potentials is an easily applicable and convenient
196 method to address direct questions, such as comparing external and internal water content. The
197 method also provides a good opportunity to compare effects on different species. Nonetheless, it
198 cannot deliver a direct course of the effect of ψ , as it is possible with other methods, e.g., with a
199 *Thermocouple Psychrometer*, which can directly measure the water potential of the tissue (Beckett,
200 1995; 1997, 2002; Hájek & Beckett, 2007). However, the results of the measurements of rW_e and rW_i
201 seem reasonable and consistent with comparable studies. Scheidegger et al. (1995) examined the
202 influence of different ψ on three fruticose lichens (*Ramalina maciformis*, *Pseudoevernia furfuracea*,
203 *Teloschistes laconosus*) and one foliose lichen (*Lobaria pulmonaria*) with the desiccator method.
204 Results of water content varied between species in a range of 16-26% at a water potential of -30.0
205 MPa and 38%-60% at a potential of -2.7 MPa which is consistent with the results of W_{total} presented
206 here for *P. rufescens* and *C. portentosa* (at -37.5 MPa: 8.2% and 16.5%; at -3.7 MPa: 32.0% and 51.0%).

207 The values of W_{total} of the moss *P. schreberi* are clearly higher (91.0% at -3.7 MPa) but achieve lower
208 values than mosses analysed in comparable studies (e.g. Hájek & Beckett, 2007).

209 Overall, the cyanolichen *P. rufescens* showed lower water uptake than the chlorolichen *C.*
210 *portentosa* and the feather moss *P. schreberi* (Fig. 1; Table 3). This can be attributed to differences in
211 the photobiont and in morphology. The three-dimensional growth of *C. portentosa* and *P. schreberi*
212 and the resulting larger surface-area-to-mass ratio promote both water uptake and evaporation
213 (Kershaw, 1985; Hartard et al., 2009). Nevertheless, cyanolichens are known to have a higher *WHC*
214 compared to chlorolichens (Gausslaa et al., 2012), which partly compensates for the dependence of
215 cyanolichens on rain or dew events (Lange et al., 1986) versus activation in humid air by chlorolichens
216 (Lange, 2003). This factor may explain lower responses of *P. rufescens* to the gradient of humidity.
217 This is confirmed by the fact that *P. rufescens* did not show water saturation under 99.5% humidity.
218 Water saturation through direct irrigation with distilled water resulted in an averaged W_{total} of 43.4%
219 (32.5% under 99.5% humidity). In contrast, *C. portentosa* appeared to be nearly saturated under a
220 humidity of 99.5%, as direct irrigation generated comparable values of *WHC* to W_{total} (1.9% higher).

221 Other factors mentioned in the literature are anatomy and colour (Palmqvist, 2000), which is
222 not addressed in this study. Nevertheless, Scheidegger et al. (1995) observed differences when
223 comparing the responses of three lichens that all showed a fruticose growth form under a gradient of
224 ψ . Thus, growth form cannot be considered as the sole factor. *P. schreberi*, the only moss species
225 examined, showed the highest water uptake, which is in line with comparable studies of mosses.
226 However, as with lichens, there seem to be differences between the species. For example, Hájek and
227 Beckett (2007) showed higher values in water uptake in *Sphagnum* species which are known to keep
228 water within dead hyaline cells (Hájek & Beckett, 2007). *P. schreberi* did not appear to be water
229 saturated under the highest humidity of 99.5% but revealed higher amounts of *WHC* compared to
230 W_{total} through direct irrigation with distilled water (14.3% higher).

231 So far, there are few studies that directly address the relation between external and internal
232 water of mosses and lichens. External and internal water under a gradient of ψ has not been examined

233 yet to our knowledge. Esseen et al. (2017) assessed the content of internal and external water of the
234 fruticose hair lichen *Bryoria* under water-saturated condition and recorded much higher proportions
235 of external water (79-84%) compared to the presented study (>20.8%). Longinotti et al. (2017)
236 examined the external water-holding capacity (*WHC*) of the foliose lichen *Lobaria amplissima* and
237 reported a similar relation between $WHC_{internal}$ and $WHC_{external}$ as rW_I and rW_E in the present study.
238 Esseen et al. attribute the high proportion of external water to the specific morphology of the lichen,
239 with thin branches and a large surface-area-to-mass ratio. The fruticose lichen *C. portentosa* and the
240 foliose lichen *P. rufescens* showed similar ratios at higher ψ , whereas *C. portentosa* showed the lowest
241 amount of external water in non-saturated water conditions. In contrast to *Bryoria* sp., the secondary
242 thallus of *C. portentosa* is scabrous and therefore appears tomentose (Cezanne et al., 2009).
243 Nevertheless, although both *Cladonia* and *Bryoria* belong to the fruticose lichens, *Bryoria* has a larger
244 surface area due to the thinner and hair-like branches. Hájek and Beckett (2007) showed higher values
245 of external water (54% up to 77% of total water) in *Sphagnum* species. In sphagnum mosses, however,
246 hyalocytes probably contribute to a large proportion of the external water content (Hájek & Beckett,
247 2007). In our study we considered the relation of the different water pools of the three species under
248 a gradient of ψ . *P. rufescens* and *P. schreberi* appeared not be fully saturated through humidity, which
249 explains lower results of external water in comparison to other studies.

250 Resulting pressure-volume (*PV*) curves were expected to be initially concave, but beyond the
251 region where turgor is lost (i.e., where turgor no longer contributes to ψ) they should assume a linear
252 shape. Accordingly, TLP (turgor loss point) can be deduced from the graph by determining the point at
253 which the curve is no longer concave but becomes linear (Beckett, 1995; 1997, Hájek & Beckett, 2007).
254 However, the curves generated in this study (Fig. 1 A, B) provide a good impression of how ψ affects
255 the water content of the individual species, but do not allow the exact determination of the TLP. This
256 cannot be clearly deduced as the number of measuring points with -3.7, -2.0 and -0.5 MPa in the
257 specific range (-5.0 to 0 MPa) is not sufficient. Nevertheless, according to our findings the approximate
258 position of the TLP of the three respective species would be located at -3.7 MPa. This would be in
259 agreement with values from previous literature. Hájek and Beckett (2007) determined the TLP for

260 various *Sphagnum* species at a water potential of -1.40 to -3.98 MPa. Due to the low resolution of our
261 method, the question if the TLP is already reached before the transition between the evaporation of
262 the external and internal water is reached, cannot be answered with certainty by considering the
263 course of the curve, although the values would indicate that *P. rufescens* reaches its TLP earlier than
264 *P. schreberi* and *C. portentosa* (Fig. 3). It is also uncertain whether the transition between the
265 evaporation of the external and the internal water approximately corresponds to the transition to a
266 negative water potential. It is, however, notable that rW_e decreases under high ψ of -2.0 and -0.5 MPa
267 while the amount of rW_i increases. This can be explained by the fact that the internal water becomes
268 predominant in the region of saturated turgor. For *P. rufescens* a decrease in the internal water content
269 can be observed in the range between -2.0 and -0.5 MPa, indicating a loss of turgor. Furthermore, it is
270 notable that a clear content of external water was measured even at low ψ (Table 3, Fig. 2). This
271 indicates that in all three species a proportion of external water is retained by capillary forces even at
272 low ψ , although a proportion of the internal water has already evaporated and therefore the turgor
273 pressure is altered. In that case, the assumption that upon desiccation first the external water
274 evaporates completely before reaching the water in the cytoplasm and finally the apoplastic water
275 cannot be confirmed. Our findings have important implications for the mechanistic modelling of water
276 relations in lichens and mosses. The results suggest that an independent consideration of both the
277 external and internal water pools is more realistic than the simpler sequential model.

278 In the present study, the removal of external water was performed by gently blotting the
279 samples. This method is common in the literature (Kershaw, 1985; Proctor et al., 1998; Esseen et al.,
280 2015, 2017; Loginotti et al., 2017), but has some potential for error. Removal of external water requires
281 practice. Either too much water can be removed, therefore some of the internal water is extracted, or
282 too little, therefore not all the external water is captured. Moreover, blotting can result in loss of plant
283 material. While measuring the samples after removal from the desiccators, a rapid adjustment of the
284 thalli to the room humidity was observed. Hence, the results of the external and internal water must
285 be considered with caution. In addition, it should be noted that cleaning the thalli of *P. rufescens*
286 proved difficult and damage could not be avoided, which could have affected potential water uptake.

287 The insertion of organisms into the set-up is associated with further uncertainties. The application of
288 semi-moist organisms into the desiccators might lead to a distortion of the water content in the
289 experiment. Drying the organisms would most likely cause a delayed and impeded water uptake, which
290 would reduce the potential water uptake of the organisms (hysteresis effect) (Jonsson, 2010). In the
291 present study, it proved difficult to ensure the same semi-moist condition for all species, as they differ
292 in morphology and physiological processes.

293 It would be worthwhile to examine the ratio of external and internal water under a gradient
294 of ψ in more detail in further studies. The variation between replicates of the same species could be
295 reduced by increasing the number of samples. Also, the number of potentials generated could be
296 increased in order to prescribe the course of the curve, especially in the relevant range of TLP between
297 -5 MPa and 0 MPa. Furthermore, it would be of interest to include other species in the study and to
298 consider other factors besides growth form, such as differences in anatomy and colour, that might
299 account for the species-specific response to different ψ . Another interesting extension of the study
300 would be to include measurements of photosynthetic activity to see how different ψ and resulting
301 changes in water content (internal and external) affect the activity of different species.

302 To summarize, our aim was to ascertain how atmospheric water demand, related to ψ , affects
303 the dynamic distribution of internal and external water in lichens and mosses and which consequences
304 this may have for their water balance. Results indicate that, although a proportion of the internal water
305 has already evaporated and therefore the turgor pressure is altered, in all three species a proportion
306 of external water is retained by capillary forces even at low ψ . In this sense, internal water pools
307 showed a parallel dynamic in their response to a gradient of ψ .

308

309

310 **Declarations:**

311 **Funding:** Not applicable

312 **Conflicts of interest/Competing interests:** Not applicable

313 **Ethics approval:** Ethics approval was not required for this study according to local legislation.

314 **Consent to participate:** Not applicable

315 **Consent for publication:** Not applicable

316 **Availability of data and material:** The datasets used and/or analysed during the current study are
317 available from the corresponding author on request.

318 **Code availability:** Not applicable

319

320 **References:**

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Table 1: Salt Concentration for saturation in H₂O (c_{sat}) [g] (15°C), the generated relative humidity (RH) [%] and the corresponding water potential (ψ) [MPa] of the respective salts at 15°C.

Salt	c_{sat} [g/100ml]	RH [%]	ψ [MPa]
NaCl	35.8	75.6	-37.5
KCl	33.0	85.85	-21.4
MgSO ₄ •7H ₂ O	71.0	92.2	-10.5
KNO ₃	31.5	94.6	-7.2
Na ₂ SO ₄ •10H ₂ O	32.2	96.7	-3.7

Table 2: Parameters of water content with unit, symbol, and formula.

Parameter	Unit	Symbol	Formula
external water	g	R_e	$WM_E - WM_I$
internal water	g	R_i	$WM_I - DM$
total water content	g	R_{total}	$WM_E - DM$
total water content/ DM	%	W_{total}	$\frac{WM_E}{DM} * 100$
internal water content/ R_{total}	%	rW_i	$\frac{R_i}{R_{total}} * 100$
external water content/ R_{total}	%	rW_e	$\frac{R_e}{R_{total}} * 100$
water holding capacity	%	WHC	$\frac{WC_{sat}}{DM} * 100$

Table 3: Mean values (\bar{X}) of the proportions of W_{total} , rW_i , $rW_e \pm$ standard deviation (SD), $rW_i:rW_e$.

Species	ψ [MPa]	\bar{X} W_{total}	$\pm SD$	\bar{X} rW_i	$\pm SD$	\bar{X} rW_e	$\pm SD$	ratio $rW_i:rW_e$
<i>C. portentosa</i>	-37.5	16.5	2.8	3.4	1.3	96.6	1.3	28.7:1
	-21.4	21.8	4.0	8.3	3.4	91.7	3.4	11.1:1
	-10.5	25.5	7.1	12	3.8	88.0	3.8	7.3:1
	-7.2	37.2	7.9	12.4	5.7	87.6	5.7	7.1:1
	-3.7	51.0	15.0	16.4	1.7	83.6	1.7	5.1:1
	-2.0	59.4	17.0	19.3	1.4	80.7	1.4	4.2:1
	-0.5	49.1	12.0	19.3	3.3	80.7	3.3	4.2:1
<i>P. rufescens</i>	-37.5	8.2	2.1	6.8	1.7	93.2	1.7	13.6:1
	-21.4	14.3	2.4	7.5	2.7	92.5	2.7	12.3:1
	-10.5	18.3	5.6	11.7	3.6	88.3	3.6	7.6:1
	-7.2	26.7	9.2	16.1	2.3	83.9	2.3	5.2:1
	-3.7	32.0	4.9	17.6	4.5	82.4	4.5	4.7:1
	-2.0	34.4	7.8	20.8	2.0	79.2	2.0	3.8:1
	-0.5	29.9	7.6	20.2	3.9	79.8	3.9	3.9:1
<i>P. schreberi</i>	-37.5	12.3	3.9	5.8	1.6	94.2	1.6	16.4:1
	-21.4	25.2	3.0	6.6	1.3	93.4	1.3	14.2:1
	-10.5	48.8	6.0	11.2	2.3	88.8	2.3	7.9:1
	-7.2	61.3	10.0	11.2	2.9	88.8	2.9	7.9:1
	-3.7	91.0	21.0	15.7	3.7	84.3	3.7	5.4:1
	-2.0	95.7	13.0	12.2	2.3	87.8	2.3	7.2:1
	-0.5	96.4	18.0	12.6	1.5	87.4	1.5	6.9:1

Figure captions

Figure 1: W_{total} plotted against ψ as (A) $-1/\psi$ [MPa⁻¹] and (B) ψ [MPa].

Figure 2: rW_e against ψ for (A) all species separately (N=21), (B) all species combined (N=63). With regression line, p-value, and r^2 (Pearson).

Figure 3: rW_i and rW_e plotted against ψ for all species.

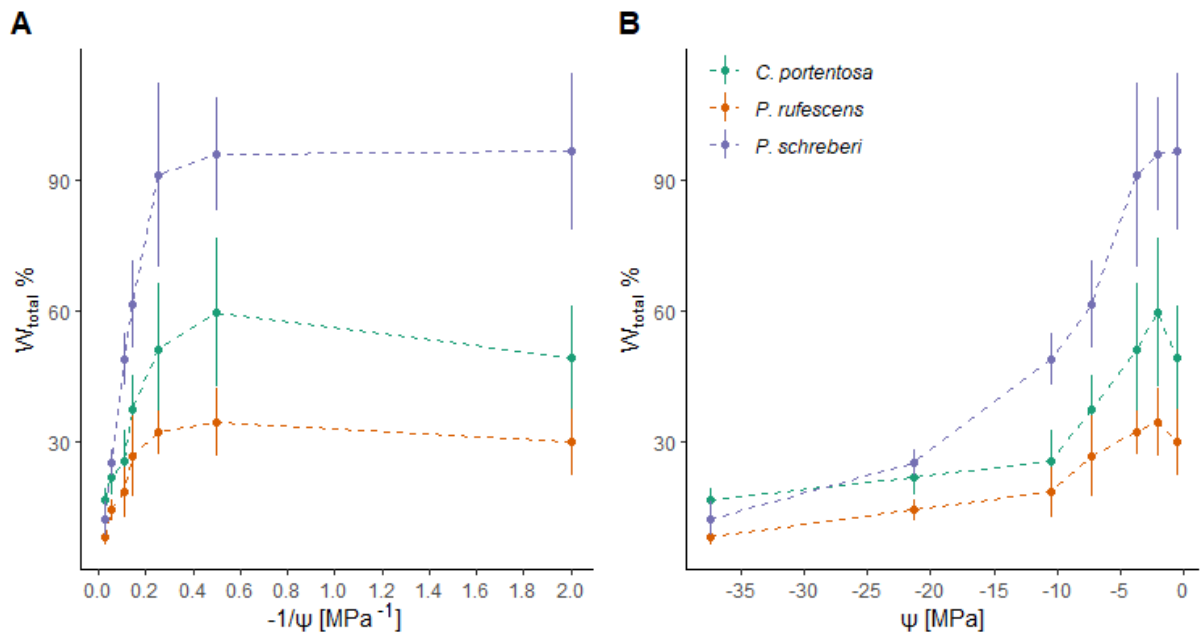


Figure 1

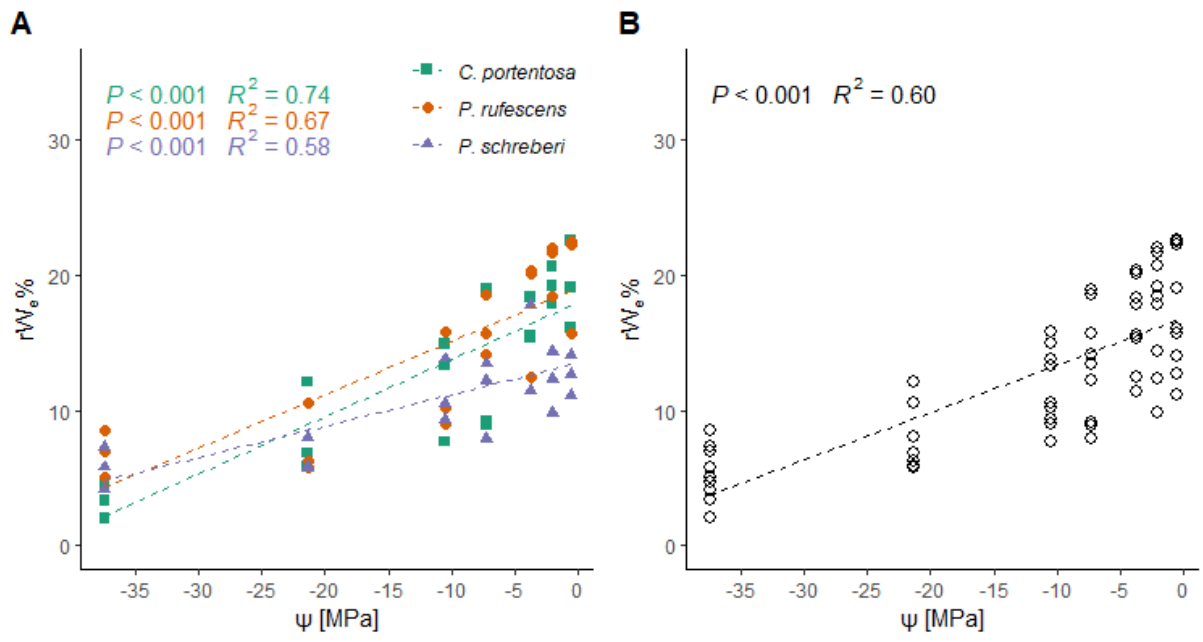


Figure 2

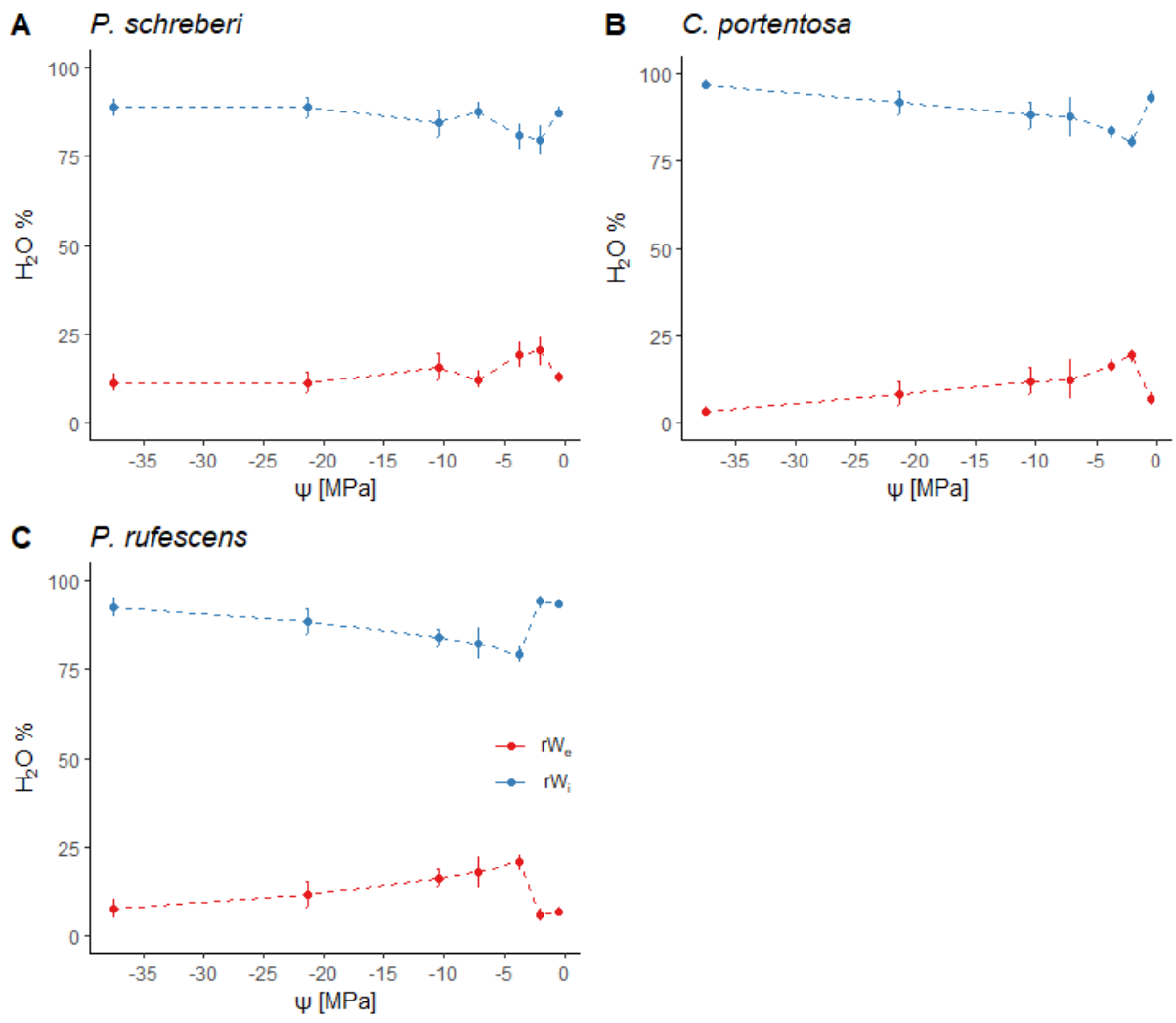


Figure 3