Bryophyte gas-exchange dynamics along varying hydration status reveal a significant COS sink in the dark and COS source in the light

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SUMMARY

- *Rationale:* Carbonyl sulphide (COS) is a potential tracer of gross primary productivity (GPP), assuming a unidirectional COS flux into the vegetation that scales with GPP. However, carbonic anhydrase (CA), the enzyme that hydrolyses COS, is expected to be light independent, thus plants without stomata should continue to take up COS in the dark.
- *Method:* We measured net CO_2 (A^C) and COS (A^S) uptake rates from two astomatous bryophytes at different relative water contents (RWC), COS concentrations, temperatures and light intensities.
- *Results:* We found large A^S in the dark, indicating that CA activity continues without photosynthesis. More surprisingly, we found a non-zero COS compensation point in light and dark conditions, indicating a temperature-driven COS source with a Q_{10} (fractional change for a 10 °C temperature increase) of 3.7. This resulted in greater A^S in the dark than in the light at similar RWC. The processes underlying such COS emissions are still unknown.
- *Conclusion:* Our results suggest that ecosystems dominated by bryophytes might be strong atmospheric sinks of COS at night and weaker sinks or even sources of COS during daytime. Biotic COS production in bryophytes could result from symbiotic fungal and bacterial partners that could also be found on vascular plants

Keywords: carbohydrates, desiccation, liverwort, *Marchantia polymorpha*, moss, protein, *Scleropodium purum*, respiration.

INTRODUCTION

Carbonyl sulphide (COS) is the most abundant sulphur-containing gas in the troposphere and has the potential to serve as a proxy for estimating gross primary productivity (GPP, Sandoval-Soto *et al.*, 2005; Montzka *et al.*, 2007; Campbell *et al.*, 2008). The foundation for using COS as a GPP tracer is built on the assumption that terrestrial uptake is dominated by, and proportional to, plant photosynthetic activity. This is because COS is taken up by plants following a similar pathway to that of CO_2. COS diffuses into the vegetation through the stomatal pores and is hydrolysed by the enzyme carbonic anhydrase (CA) in the mesophyll cells (Protoschill-Krebs *et al.*, 1996). However, in contrast to CO_2 hydration by CA, COS hydrolysis by CA is irreversible (Notni *et al.*, 2007) and no other leaf-level processes have been identified in the production of COS (Bloem *et al.*, 2015). Thus, COS uptake (A^S) is assumed to be unidirectional and not the net result of two opposed fluxes (photosynthesis and respiration in the case of CO_2). This assumption is key for the calculation of GPP from COS fluxes, together with an estimate of the ratio between CO_2 and COS uptake, the so-called 'leaf relative uptake' (LRU, Campbell *et al.*, 2008). Relying on LRU for estimating GPP requires some important assumptions. Principally, the LRU approach assumes that the consumption of CO_2 and COS diffusing into the leaf is linked to downstream light-dependent reactions. However, CA activity is expected to be light independent (Protoschill-Krebs *et al.*, 1996). Thus, as long as stomata remain open, a sink for COS should be maintained in the dark when CO_2 uptake ceases and A^C becomes dominated by leaf respiration. Indeed an uncoupling of A^C and A^S (i.e. more variable and usually larger LRU values) has been reported at low light intensities in the lab (Stimler *et al.*, 2011) and in the field at night (Berkelhammer *et al.*, 2014; Commane *et al.*, 2015). Furthermore, the utility of COS as a tracer of GPP depends heavily on the assumption that the flux of COS between the atmosphere and the leaf is one-way and driven by CA activity alone. This assumption has been validated at the leaf level for certain species and environmental conditions (Stimler *et al.*, 2010; Sandoval-Soto *et al.*, 2012). However, recent field studies have shown that COS emissions from wheat leaves may occur during senescence and from deciduous forests during periods of high temperature and drought (Maseyk *et al.*, 2014; Commane *et al.*, 2015). Thus it is not entirely clear whether the unidirectional hypothesis holds in plants exposed to stress and whether COS emissions are masked to some extent by the stomata of vascular plants. Currently, the mechanisms underlying these COS emissions also remain unclear.

91 Plants without stomata such as bryophytes are a potentially useful model that could
92 provide important insights on the dynamics of A^S under varying environmental conditions.
93 These dynamics would otherwise be difficult to detect in the presence of stomata that actively
94 impose diffusional limitations. In astomatous plants, the dynamics of leaf COS fluxes should
95 be more closely related to changes in the enzymatic activity and substrate availability.
96 Disentangling diffusional and enzymatic processes for plant COS fluxes may be especially
97 critical since stomata have been shown to open in response to an increase in the atmospheric
98 COS mixing ratio (Stimler *et al.*, 2010; Stimler *et al.*, 2012), creating a potential feedback on
99 leaf COS and CO_2 uptake. Although some bryophytes have tiny pores that facilitate gas
100 exchange, they lack stomatal regulation (Proctor *et al.*, 2007) and seem therefore better suited
101 for assessment of changes in leaf COS fluxes in response to changing environmental
102 conditions.
103 Bryophytes lack active control of transpiration so they rapidly equilibrate with
104 prevailing environmental conditions (Proctor *et al.*, 2007). Desiccation tolerance in these
105 organisms involves a number of biochemical mechanisms such as the accumulation of non-
106 structural carbohydrates and other compatible solutes or the up- or down-regulation of gene
107 expression and protein synthesis (Oliver *et al.*, 2005). To reduce the rate of water loss,
108 bryophytes also deploy morphological adaptations and preserve a thin layer of capillary water
109 on their leafy shoots (Marschall & Proctor, 2004). Capillary water slows tissue desiccation,
110 but it is a barrier to CO_2 diffusion. As a consequence, bryophyte A^C commonly displays a
111 three-phase response to tissue dehydration. Initially, as diffusion-resistance through capillary
112 water decreases with evaporation, A^C increases until a plateau in A^C is reached indicating the
113 optimal hydration status for photosynthetic activity. As evaporation continues, A^C decreases
114 as cells dehydrate and photosynthesis becomes metabolically impaired (Dilks & Proctor,
115 1979; Royles *et al.*, 2013). This layer of capillary water should pose a resistance not only to
116 CO_2, but also to COS, and thus we suggest that a similar optimum-like response of A^S to
117 desiccation should be observed in the light. Alternatively, in bryophytes with carbon
118 concentration mechanisms similar to those of algae and cyanobacteria (Smith & Griffiths,
119 2000), external CA activity could potentially counterbalance the initial expected increase in
120 A^S with desiccation (Rech *et al.*, 2008). In the dark, A^C flux is negative as respiration
121 dominates and the magnitude of the respiratory flux decreases progressively with desiccation.
122 However, in the absence of stomata and because CA is expected to be light-independent
123 (Gries *et al.*, 1994; Protoschill-Krebs *et al.*, 1996), we hypothesise that A^S should continue at
124 similar rates to those observed in the light.

125 Bryophytes should also be a good model to test the assumption that COS emission
126 does not occur, during the day/night cycle or in plants exposed to water or heat stress. Testing
127 this hypothesis on vascular plants would be challenging, as A^S would be strongly limited by
128 stomatal closure. The only previous study estimating the COS compensation point (Γ^S, the
129 COS concentration at which A^S is zero) on non-vascular photoautotrophic organisms (lichens,
130 Kuhn & Kesselmeier, 2000) suggested that Γ^S could be greater than zero. A positive Γ^S
131 would imply that A^S is the net result of simultaneous COS uptake and emission. As far as we
132 are aware no studies looking into the relationship between A^S-C^S have been conducted on
133 astomatous plants to date.

134 Here, we challenge our current understanding of COS uptake by terrestrial plants
135 using astomatous bryophytes as model organisms. Our aims were to provide a first estimate
136 of the COS sink strength in bryophytes and to test some of the assumptions that underlie the
137 proposed relationship between GPP and COS uptake. Specifically we hypothesised that (*1*) in
138 astomatous bryophytes, COS uptake varies with tissue hydration analogously to CO_2 uptake
139 in the light, (*2*) COS uptake dynamics during desiccation would be similar in the light and in
140 the dark and (*3*) the COS compensation point would be zero. To test these hypotheses we ran
141 a series of experiments under controlled conditions to characterise the response of A^S and A^C
142 to desiccation, COS concentration and increasing light intensity and temperature, in two
143 bryophyte species with contrasting life forms and evolutionary origin.

144

145 ## MATERIAL AND METHODS

146

147 *Study species and sampling protocol*

148 We chose two bryophytes with contrasting evolutionary origins and life forms, representative
149 of temperate regions. The mat-forming liverwort *Marchantia polymorpha* L. has
150 gametophytes with a complex thallus structure and occasional static pores to improve
151 ventilation (Meyer *et al.*, 2008). The loosely packed weft-moss *Scleropodium purum* (Hedw.)
152 Limpr. is a desiccation-tolerant slow-growing moss with feather-like shoots and poorly-
153 developed rhizoids (Arroniz-Crespo *et al.*, 2008). Given their abundance and wide-spread
154 distribution, both species have been the subjects of an ample body of literature. *Marchantia*
155 *polymorpha* has served as a model bryophyte for characterizing plant physiology, metabolism
156 and genetics (Bowman, 2016) while *S. purum* physiology and distribution have been widely
157 studied in response to nutrient availability and heavy metal contamination (Arroniz-Crespo *et*
158 *al.*, 2008).

159 Mats of moss and liverwort were collected locally from naturally growing populations
160 at the INRA campuses of 'La Ferrade' (Villenave d'Ornon, France) and 'Pierroton' (Cestas,
161 France). Mats of *M. polymorpha* were occasionally intermingled with *Lunularia cruciata* L.,
162 a liverwort with similar gametophytes to those of *M. polymorpha* in the absence of spore-
163 bearing cups. The mats were collected 1-5 days before the experiments and maintained in
164 ambient external light with regular watering. On the day before each experiment, green tissue
165 was separated and rinsed with deionized (DI) water. Individual samples of 2.5-4 g (fresh
166 mass) of green tissue were placed onto pierced aluminium circular trays (6.5 cm diameter).
167 To fully rehydrate the tissue, sample trays were sprayed with DI water, placed onto moist
168 paper and kept refrigerated in closed glass jars for 12-24 h. Before the start of the experiment,
169 the jars were acclimated to room temperature for one hour. After blotting excess water from
170 the trays, all trays were weighed to the nearest 0.1 mg and placed into gas-exchange
171 chambers, a few minutes before the experiment. All trays were re-weighed at the end of each
172 experiment.
173
174 *Gas-exchange measurements*
175 Experiments were carried out at the facility for online trace gas and stable isotope analyses at
176 INRA-Bordeaux (France). The system comprised a set of gas analysers that measured CO_2,
177 COS and H_2O mixing ratios of the inlet and outlet airstreams from seven multiplexed gas-
178 exchange chambers. Each chamber consisted of a 0.5 L glass jar and a glass top fitted with
179 two stainless steel Swagelok® connectors attached to 0.25 inch (3.175 mm) Teflon™ inlet
180 and outlet lines. The gas-exchange chambers were housed in a climatically-controlled
181 chamber that regulated air temperature, relative humidity and light intensity, outside the
182 chambers (MD1400, Snijders, Tillburg, NL). Inside each chamber, temperature was
183 monitored continuously with self-contained thermocouple data-loggers (Hygrochron
184 Temperature & Humidity iButton, DS1923, Embedded Data Systems, Lawrenceburg, KY,
185 US), placed on the aluminium trays in direct contact with the samples but not completely
186 covered by them. Air pressure inside the chambers was established with a pressure-transducer
187 (BMP180, Bosch GmbH, Gerlingen, Germany), during preliminary experiments with the
188 exact same flow of air and measuring sequence.
189 The airflow into each chamber was set to 250 mL min^{-1} on a dry air basis using
190 individual mass-flow controllers (MFC, EL-Flow® Select, Bronkhorst, Ruurlo, NL). CO_2
191 and COS mixing ratios (C^C and C^S, respectively) of the inlet air were adjusted by mixing
192 synthetic CO_2- and COS-free dry air from a compressor (FM2 Atlas Copto, Nacka, Sweden),

193 coupled to a chemical scrub column (Ecodry K-MT6, Parker Hannifin, Cleveland, OH, US),
194 with two cylinders of commercial gas mixtures (pure CO_2 and 500 nmol mol^{-1} COS). Inlet
195 and outlet C^C and C^S were pre-dried with a Nafion™ dryer (MD-070-24-S-2, Perma Pure
196 LLC, NJ, USA) before being measured with a mid-infrared quantum cascade laser
197 spectrometer (QCLS, Aerodyne Research Inc. Billerica, MA, USA). Flow through the
198 instrument was maintained with a TriScroll 600 pump (Agilent Technolgies, Santa Clara,
199 CA, USA) connected to the QCLS *via* a vacuum line. Instrument drift was corrected with
200 frequent (every 14 minutes) background calibrations (with dry N_2) in all runs. In most runs
201 (75%) a two-point standard calibration was also implemented using dry N_2 (zero) and
202 compressed dry air with a COS concentration of 524.8 ± 2.2 pmol mol^{-1} from an Aculife®-
203 treated cylinder that was prepared and calibrated for COS by the NOAA Global Monitoring
204 Division. The 14-min frequency was based on instrument stability estimated from an Allan
205 variance calculated from a 24h continuous measurement on tank air that indicated a standard
206 deviation at 10 s averaging of 2.1 pmol mol^{-1} for COS, a deviation from pure white noise
207 after more than 400 s and a standard deviation <1 pmol mol^{-1} after 900 s integration time
208 (Supplementary information, Fig. S1).

209 The QCLS alternately measured inlet and outlet C^C and C^S over 120 s and only the
210 mean of the last 10 s was used in further calculations. For each chamber, three consecutive
211 inlet/outlet pairs were measured and the seven chambers were measured sequentially. We
212 calculated CO_2 (A^C) and COS (A^S) net assimilation rates from the inlet and outlet
213 concentration difference as follows:

214 $$A = \frac{f(C_e - C_o)}{M_d} \qquad \text{Eq. 1}$$

215 where f (mol s^{-1}) is the inlet flow rate (dry air basis), C_e and C_o are the CO_2 or COS mixing
216 ratios (mol mol^{-1}) entering and leaving the chamber in dry air and M_d is the sample dry mass
217 (kg). Because mixing ratios were determined on a dry air basis (because of the Nafion dryer
218 upstream of the QCLS) only the flow of dry air on the inlet of the chamber was necessary to
219 perform the mass balance. Net assimilations (A^C and A^S) were calculated from inlet/outlet
220 pairs and then averaged (n = 3) for consecutive pairs of the same chamber. The leaflet
221 relative uptake (LRU) rates of A^C and A^S normalised to their ambient concentrations were
222 then computed as (Stimler *et al.*, 2010):

223 $$\mathrm{LRU} = \frac{A^S}{A^C} \frac{C_o^S}{C_o^C} \qquad \text{Eq. 2}$$

224 Outlet water vapour concentration was measured with an infrared gas analyser
225 (IRGA, LI-6262, LI-COR, Lincoln, NE, USA). Analyser calibration was made prior to the

226 experiment with a dew-point generator (LI-610, LI-COR). Outlet water vapour concentration
227 (W) of each chamber was measured for 240 s, after having flushed the instrument for 600 s
228 and the mean of the last 10 s (W_o, mol mol^{-1}) was used for further calculations. The
229 instantaneous transpiration rate of each chamber was calculated as in Eq. 1, substituting W for
230 C, with $W_e = 0$. We fitted a spline to transpiration over time to derive continuous values of
231 instantaneous transpiration for each sample (E_t in mm s^{-1} kg^{-1}). The estimated cumulative
232 transpiration of each sample (E_{cum} in mm kg^{-1}) was then calculated as:

233 $$E_{\mathrm{cum}} = \sum_{i=0}^{i=n} E_{t,i} \, (t_i - t_{i-1}) \qquad \text{Eq. 3}$$

234 where t is time in seconds since the start of the experiment and n is the experiment duration.
235 We then calculated the fresh mass (M_f) of each sample at any given point in time ($M_{f,\,t}$) as:

236 $$M_{\mathrm{f,t}} = M_{\mathrm{f,end}} + E_{\mathrm{total}} - E_{\mathrm{cum,t}} \qquad \text{Eq. 4}$$

237 where $M_{f,end}$ is the sample mass at the end of the experiment and E_{total} is total transpiration
238 (i.e. maximum E_{cum}). Then, we calculated sample relative water content over time (RWC_t):

239 $$\mathrm{RWC_t} = \frac{M_{\mathrm{f,t}} - M_{\mathrm{d}}}{M_{\mathrm{d}}} 100 \qquad \text{Eq.5}$$

240

241 *Experimental design*

242 We performed four experiments to (*1*) characterize A^C and A^S during desiccation in the light
243 and in the dark, (*2*) determine whether a COS compensation point and a COS source term
244 could be detected, (*3*) evaluate the temperature sensitivity of any COS source term and (*4*)
245 test for the effect of light intensity and temperature on COS uptake. Metabolite
246 concentrations and gas-exchange dynamics during desiccation in the light and in the dark
247 were characterised for both the moss, *S. purum*, and the liverwort, *M. polymorpha*,
248 (Experiment 1), whilst COS, temperature and light curves were performed only for the
249 liverwort (Experiments 2-4), with larger uptake rates per unit of dry mass.

250 *1. Desiccation curves*

251 We measured A^C and A^S during desiccation for 10-13 h, in moss and liverwort samples, in the
252 light and in the dark (Supplementary information, Fig. S2). We ran desiccation curves
253 separately for each bryophyte (moss and liverwort) and light/temperature regime (light
254 on/21°C and light off/16°C). During each desiccation experiment, six trays with fully
255 hydrated samples were placed into gas-exchange chambers whilst one empty tray (also
256 containing a temperature data-logger) was placed into a seventh (blank) chamber to check for
257 any COS contamination during the experiment. During the desiccation experiment the
258 chambers were placed in a light regime with a photosynthetic photon flux density (PPFD) of

259 580 µmol m^{-2} s^{-1} supplied by fluorescent lamps (BriteGro 2084, Sylvania, BioSystems,
260 Wageningen, NL). According to the manufacturer, the spectral power distribution of these
261 lamps was 400-700 nm, with only two minor peaks detected below 400 nm (UV), of a
262 magnitude ten times smaller than the peaks at all other wavelengths. The temperature of the
263 climatically-controlled chamber was 16°C in the dark and 21°C in the light. Temperature
264 inside the blank chamber was 1-2°C higher than that of the climatically-controlled chamber
265 (Supplementary information, Fig. S3). Sample chamber temperature varied with hydration
266 status and transpiration rate (Fig. S2). CO_2 and COS mixing ratios (C^C and C^S) of the inlet air
267 were set to: 410 µmol mol^{-1} CO_2 and 540 pmol mol^{-1} COS.

268 *2. COS curves for determination of the COS-compensation point (Γ^S)*

269 To test for the existence of a COS-compensation point (Γ^S) in the liverwort, we measured A^C
270 and A^S at varying COS concentrations, in the light at 21°C and in the dark at 16°C. To
271 minimise the effect of drying, COS curves were limited to the plateau of the *A*-RWC curve.
272 We sequentially measured A^C and A^S at five C^S (510, 285, 385, 105 and 5-10 pmol mol^{-1}) on
273 four liverwort samples, C^C was kept constant (410 µmol mol^{-1}). The same four samples were
274 measured in the light and in the dark. We then estimated Γ^S as the C^S at which $A^S = 0$.

275 *3. Temperature response curves*

276 To assess the effect of temperature on the COS source term (P^S), we performed additional
277 COS curves at three chamber temperatures (16, 21 and 25°C), in the light and in the dark. We
278 measured four liverwort samples for each combination of temperature set point and light
279 regime (for 25 and 16°C in the light and 16°C in the dark, only three). We measured A^C and
280 A^S at four C^S (120, 200, 400 and 520 pmol mol^{-1}) while C^C was kept constant
281 (410 µmol mol^{-1}). For each sample, including those from Experiment 3, we estimated P^S as
282 the intercept of the linear regression between A^S and C^S, i.e. A^S at $C^S = 0$.

283 *4. Light and temperature curves*

284 To assess the effect of light intensity and temperature on COS uptake and emission, we
285 measured A^C and A^S whilst gradually increasing light intensity and temperature. Similar to the
286 COS curves, to minimise the effect of RWC, we successively measured A^C and A^S at five
287 PPFD levels (0, 90, 255, 420 and 580 µmol m^{-2} s^{-1}), within the plateau of the A^S-RWC curve.
288 Light curves were performed under ambient C^C and C^S (410 µmol mol^{-1} and 510 pmol mol^{-1},
289 respectively) on four liverwort samples and under ambient C^C and near-zero C^S
290 (5-10 pmol mol^{-1}) on four different samples. The temperature inside the gas-exchange
291 chamber increased with light intensity (Fig. S3).
292

293 *Biochemical assays*

294 To assess the change in total protein and non-structural carbohydrate (NSC) content during
295 desiccation we performed additional desiccation curves, under similar conditions as described
296 above, in the light and in the dark, with successive sampling. We collected three replicate
297 samples (per species and light level) consisting of 1-3 g of tissue at five points in time. For
298 the liverworts, tissue RWC at different points in time was estimated from three independent
299 samples (for each light level) that were conserved intact along the whole desiccation curve.
300 For the mosses, tissue RWC was measured individually on a separate sub-sample at the time
301 of collection.

302 Quantification of total protein content and NSC was performed following enzymatic
303 digestion as in Biais *et al.* (2014) at the HitMe platform of the INRA-Bordeaux Metabolome
304 Facility (France). Briefly, ~20 mg aliquots of frozen sample were powder-homogenised and
305 fractionated three times at 95°C for 15 minutes with 250 and 150 µL (80 % v/v) and 250 µL
306 (50% v/v) ethanol, 10 mM Hepes/KOH (pH 6). Glucose, fructose and sucrose concentrations
307 were quantified from the ethanolic supernatant following an adapted procedure from Jelitto *et*
308 *al.* (1992). Aliquots of 50 µL of ethanolic extract were added to 160 µL of a mix of 100 mM
309 HEPES–KOH buffer (pH 7), 3 mM $MgCl_2$, 3 mM ATP, 1.3 mM NADP and 5 units of
310 glucose-6-phosphate dehydrogenase. Then, 1 unit of hexokinase, 1 unit of phosphoglucose
311 isomerase, and 30 units of invertase were added successively. Glucose, fructose and sucrose
312 content were quantified from the difference in absorbance between successive steps. Total
313 protein content and starch were determined from the pellet resuspended in 100 mM NaOH
314 and heated at 95°C for 30 minutes (Hendriks *et al.*, 2003). Total protein content was
315 quantified using Bradford reagent (Bradford, 1976). Analyses were run in duplicate.
316 Extractions and assays were performed using a robotised Starlet platform (Hamilton,
317 Villebon sur Yvette, France) and absorbencies were read at 340 nm for NSC and 600 nm for
318 protein in MP96 readers (SAFAS, Monaco). All chemicals were purchased from Sigma-
319 Aldrich Ltd. (Gillingham, UK) and enzymes from Roche Applied Science (Meylan, France)

320

321 *Statistical analyses*

322 To test that A^S would show an optimum-like response to desiccation similar to that of A^C in
323 the light, and that A^S during desiccation would not differ between the light and the dark, we
324 fitted general additive mixed models (GAMMs). GAMMs were fitted to A^C and A^S with
325 RWC as a predictor and taking into account the random sample-to-sample variability (Wood,
326 2006). Significant differences (α = 0.05) between the light and the dark were assessed

327 graphically based on non-overlapping 95% CI's. We used package *mgcv*, in R version 3.3.1
328 (R Development Core Team, 2014). To quantify the effect of C^S on liverwort A^C and A^S, we
329 fitted linear mixed models (LMMs) with C^S and light and temperature regime (dark/cool vs.
330 light/warm) as fixed predictors and sample as random factor, using packages *lme4* and
331 *lmerTest* and *investr*, to calculate Γ^S (Greenwell & Schubert Kabban, 2014). We obtained the
332 COS source term (P^S) as the intercept of the A^S-C^S relationships measured at varying
333 temperatures, in the light and in the dark. Since P^S increased exponentially with temperature,
334 we fitted a linear relationship between ln-transformed P^S and temperature and then calculated
335 the relative increase per 10°C increase (Q_{10}) as in Eq. 3 in Zaragoza-Castells *et al.* (2007). To
336 analyse the effect of PPFD and COS availability on A^S we fitted a LMM with PPFD and
337 source air (ambient vs. near-zero COS) as fixed predictors and the sample as a random factor.
338 In our experimental setup, the increase in light intensity was coupled to a 5°C increase (Fig.
339 S3), potentially affecting both COS uptake and emission, irrespective of light intensity. To
340 partially disentangle the effects temperature on COS uptake and emission, we first estimated
341 the P^S for each measurement from temperature and the Q_{10} and then calculated the gross COS
342 uptake (U^S) as the sum of the net uptake and the source. Finally, we performed a similar
343 LMM on estimated U^S with PPFD and source air as fixed predictors. For photosynthesis, the
344 A^C shows a response to PPFD of the form:

345 $$A^C = R_d + A^C_{max}(1 - e^{-k\text{PPFD}}) \qquad \text{Eq. 6}$$

346 where R_d is the net CO_2 emission in the dark, A_{max} is the asymptote of the curve and k is a
347 constant, such that $A_{max}k$ is the slope of the initial part of the curve (Marschall & Proctor,
348 2004). We fitted Eq. 6 to A^C-PPFD measurements and tested for differences between ambient
349 and near-zero C^S by comparing the 95% confidence interval of the fitted parameters. Finally,
350 to assess the effect of light on total protein and NSC during desiccation, we performed linear
351 model analyses with RWC, light and their interaction on metabolite concentrations. Prior to
352 analyses, we checked for normality and ln-transformed metabolite concentrations and RWC
353 (the latter for mosses only).

354

355 **RESULTS**

356

357 *COS uptake dynamic along desiccation in the light and in the dark and LRU*

358 The typical optimum-like response of the net CO_2 uptake (A^C) during dehydration in the light,
359 characteristic of bryophytes, was clearly observed in liverwort, *M. polymorpha* (Fig. 1c),

360 while in moss, *S. purum*, the increase in A^C upon initial desiccation from maximum RWC
361 was less evident (Fig. 1a). Maximum A^C in the light was similar between the moss and the
362 liverwort (15.9 and 22.7 µmol kg^{-1} s^{-1}, respectively), in contrast maximum A^S was four times
363 higher in *M. polymorpha*, the liverwort, compared to *S. purum*, the moss (20.7 ± 3.6 and
364 5.2 ± 2.3 pmol kg^{-1} s^{-1}, estimated maximum from fitted GAMM ± 95% CI). In the dark, at a
365 cooler temperature (16°C), respiration (A^C < 0) decreased progressively during tissue
366 desiccation until it reached zero (Fig. 1 a,c), whilst A^S followed an optimum-like response to
367 desiccation similar to that observed for A^C in the light, in both bryophytes (Fig. 1 b,d). These
368 data support our assumption that COS uptake by astomatous plants continues in the dark.
369 Contrary to our expectations, however, A^S in the dark was higher under the dark and cooler
370 conditions along the whole desiccation curve, in both the liverwort, *M. polymorpha*, and the
371 moss, *S. purum* (Fig. 1b,d). Furthermore, towards the end of the desiccation curves in the
372 light (and at 21°C), A^S shifted from net uptake to net emission in both species, while in the
373 dark (and at 16°C) A^S remained positive, or not significantly different from zero, during the
374 entire desiccation curve (Fig. 1 b,d). It is worth noting that during the desiccation
375 experiments our blank chambers showed no signs of COS or CO_2 uptake or release
376 throughout. Collectively, the above results led to negative values of leaflet relative uptake
377 (LRU) not only in the dark, but also in the light when RWC fell below its optimum value for
378 gas exchange (Table 1). At optimum RWC in the light, the calculated LRU was 0.22 for the
379 moss and 0.89 for the liverwort.
380

381 *COS compensation point (Γ^S) and temperature sensitivity of source term (P^S) in the liverwort*
382 Both CO_2 photosynthetic uptake in the light and CO_2 respiratory release in the dark were
383 unaffected by C^S (Fig. 2a, Supplementary information Table S1). In contrast, and as
384 expected, A^S increased linearly with C^S ($P < 0.001$, Fig. 2b) regardless of the light and
385 temperature regimen. LMM revealed that the rate of increase of A^S with C^S did not differ
386 between the two light and temperature regimes ($P = 0.526$, Fig. 2b), but predicted P^S (A^S
387 extrapolated at zero C^S) was significantly ($P = 0.005$) greater in the light at 21°C
388 (21 ± 2.7 pmol mol^{-1}) than in the dark at 16°C (12 ± 1.9 pmol mol^{-1}). For *M. polymorpha*, we
389 estimated a COS compensation point (Γ^S) of 345 ± 68 pmol mol^{-1} in the light at the warmer
390 temperature and 199 ± 74 pmol mol^{-1} in the dark at the cooler temperature. During all
391 experiments manipulating COS concentrations, light intensity and temperature in our blank
392 chamber remained constant indicating that chamber artefacts such as COS emission or uptake

393 were minimised in our experimental set-up despite large changes in environmental
394 conditions.
395 Our estimates of P^S (A^S at zero C^S) represent a COS emission rate coming from the
396 liverwort. We found that P^S increased exponentially with temperature ($t = 6.5$, $P < 0.001$),
397 regardless of the light/dark regime with a Q_{10} of 3.7 (Fig. 3).
398
399 *Effect of light and temperature on net* (A^S) *and gross* (U^S) *COS uptake*
400 In *M. polymorpha*, A^C increased with increasing light intensity and temperature, until it
401 reached a plateau (Fig. 4) according to Eq. 4. We found that the A^C-PPFD relationship did not
402 change between ambient and near-zero COS supply (Fig. 4 a); demonstrated by the overlap
403 of the 95% confidence interval of the parameter estimates (Table S1). In contrast, A^S
404 decreased with increasing light intensity and temperature, both under ambient and near-zero
405 COS supply, but the rate of change with light intensity was lower under near-zero than
406 ambient C^S (Fig. 4 b, Table S1). This decrease in A^S with light and temperature is partly
407 explained by an increase in P^S with temperature (see above). However, even after accounting
408 for the temperature effect on P^S, we found that the gross COS uptake ($U^S = A^S + P^S$) still
409 decreased with light intensity under ambient COS (Fig. 3 c, Table S1). This could suggest an
410 inhibitory effect of light intensity on COS uptake. However, we cannot completely discard
411 the influence of an experimental artefact potentially biasing our observations. For example,
412 given our experimental sequence, uptake rates at the highest light intensities were measured
413 towards the end of the experiment, when decreasing RWC could have negatively affected
414 uptake.
415
416 *Total protein and NSC accumulation in the light and in the dark along desiccation*
417 Total protein content decreased with desiccation in both the liverwort (*M. polymorpha*) and
418 the moss (*S. purum* Table 2). In the moss, non-structural carbohydrates (NSC) and all its
419 components (glucose, fructose, sucrose and starch) also decreased with desiccation (Fig. 5,
420 Table 2). The decrease rate with RWC of protein and NSCs (in the moss) with desiccation
421 did not differ between the light and the dark (Table 2).
422
423
424
425
426

427 **DISCUSSION**

428

429 *Challenging the unidirectional flux assumption for the vegetation COS flux*

430 Here, we aimed to critically assess some of the key assumptions underlying the relationship
431 between COS and CO_2 uptake, in bryophytes. We hypothesised that COS uptake rates in
432 bryophytes would be light independent. Our results on two astomatous bryophytes (one moss
433 and one liverwort) partially agreed with this prediction as we found that net COS uptake (A^S)
434 in the dark remained positive, and this supports the current idea that COS hydrolysis is
435 dominated by the enzyme carbonic anhydrase (CA), which is assumed to be light-
436 independent (Protoschill-Krebs *et al.*, 1996). However, our results also showed that A^S may
437 be affected by light in an unexpected way. We found that for an equivalent hydration status,
438 A^S was significantly greater in the dark than in the light. A plausible explanation for this
439 observation is that bryophyte A^S is the net result of two opposing fluxes, uptake and emission,
440 with COS emission being of greater magnitude at warmer temperatures in the light than in
441 cooler dark conditions. All our other results are compatible with this explanation. Firstly, we
442 observed that below an optimal hydration status, A^s shifted from net uptake to net emission in
443 the light, but not in the dark. Also A^S decreased with increasing light intensity and warmer
444 temperatures. Finally, we found a non-zero compensation point (Γ^S) that was greater in the
445 light than in the dark. Previously, Kuhn & Kesselmeier (2000) had suggested the existence of
446 a non-zero Γ^S in lichens. Non-zero Γ^S have also been observed in some higher plants
447 (Kesselmeier & Merk, 1993) but its influence on the overall COS uptake rate seemed small
448 (Seibt *et al.*, 2010; Stimler *et al.*, 2010). These observations, together with other studies
449 conducted on plants senescing, under fungal attack or under heat and drought stress (Bloem
450 *et al.*, 2012; Maseyk *et al.*, 2014; Commane *et al.*, 2015), suggest that plant COS emissions
451 may be more ubiquitous than previously assumed. Our results encourage further studies
452 revisiting COS fluxes from vascular plants, for example performing A^S-C^S curves at different
453 temperatures, to determine whether COS emissions can be detected with the new generation
454 of commercially available COS analysers offering much higher precision (~5ppt). In
455 particular, experiments using mutants (Costa *et al.*, 2015) that maintain stomata open in the
456 dark could provide a novel approach to detect COS emissions from vascular plants and how
457 the gross COS fluxes respond to different environmental drivers.

458 The existence of a bi-directional COS flux contradicts our initial expectation for COS
459 uptake by astomatous bryophytes, since there are currently no described leaf-level processes

460 that result in COS as a by-product (Protoschill-Krebs *et al.*, 1996; Bloem *et al.*, 2015).
461 Previously, Fried *et al.* (1993) measured COS emissions in the light from a peat soil and moss
462 microcosm, but they ascribed the emissions to the soil component. Indeed, Whelan & Rhew
463 (2015) demonstrated that soils can emit COS and that the rate of COS emission increases in
464 the light and with warmer temperatures. Whelan & Rhew (2015) suggested that COS
465 originated from abiotic photo-degradation of dead organic matter by UV light, similar to COS
466 emissions measured from sea, lake and precipitation water (Zepp & Andreae, 1994; Mu *et al.*,
467 2004; Du *et al.* 2016). However, based on the manufacturers specifications for our light
468 source (see Methods) and because our chambers were not made of UV-transparent quartz
469 glass, we assume that our bryophyte samples were not exposed to high intensity UV
470 radiation. Thus it is unlikely that our COS emissions in the light would have been strongly
471 affected by UV-driven organic matter degradation. Our results suggest that an additional
472 light-independent process of biological origin underlies observed COS emission from
473 bryophytes. We argue that this process is likely to be of biological origin because in the dark,
474 COS emission at minimum hydration status (below 70% RWC) was not detectable, while our
475 A^S-C^S curve demonstrated that COS emission still occurred in the dark at optimal hydration
476 status. Despite uncertainties in the mechanistic driver of the emissions, in our study, we
477 observed protein degradation during desiccation in the moss and below optimal hydration in
478 the liverwort that could have led to the liberation and eventual catabolism of sulphur-
479 containing amino acids (cysteine and methionine), potential precursors for COS production
480 (Bloem et al. 2004; Du *et al.* 2016; Zepp & Andreae, 1994; Mu *et al.*, 2004). This protein
481 degradation would have occurred while the leaflet tissues were metabolically active and was
482 accompanied by a significant decrease in NSC content. This result alongside the gas
483 exchange data suggest that as the leaflets became progressively water-stressed, protein
484 degradation affected the photosynthetic machinery, including CA, and probably caused the
485 increased apparent COS emission by reducing gross COS uptake. This is consistent with
486 results in the literature on vascular plants that have shown that during water stress the total
487 protein content decreases rapidly and is accompanied by a strong reduction in the activity of
488 key enzymes involved in carbon assimilation (Majumdar *et al.*, 1991; Khanna-Chopra, 2012).
489 The metabolic activity of accompanying microorganisms, sensitive to water stress too
490 (Vacher *et al.*, 2016), could also be contributing to the observed COS emission in bryophytes.
491 Bryophytes, like any other plants, host rich microbial communities and both liverworts and
492 feather-like mosses are known to form symbiotic associations with fungi and bacteria
493 (DeLuca *et al.*, 2002; Humphreys *et al.*, 2010; Davey *et al.*, 2012). It has recently been shown

494 that some fungal and bacterial enzymatic reactions produce COS (Masaki *et al.*, 2016; Ogawa
495 *et al.*, 2016); hence it is plausible that natural symbionts could also be contributing to the net
496 COS fluxes in bryophytes.
497
498 *Assessing the climatic sensitivity of COS uptake and emission*
499 In bryophytes, tissue water content is the main driver of net CO_2 exchange (A^C, Wagner *et al.*,
500 2013) and we expected the same for net COS uptake (A^S). We indeed observed that A^S was
501 strongly driven by tissue water content, but our results also showed that A^S was sensitive to
502 temperature. Our observations on liverworts in the dark showed that A^S at optimal water
503 content was lower at 21°C than at 16 °C, while CO_2 respiratory release was greater at the
504 warmer temperature (Supplementary information, Fig. S5). This seems to contradict the
505 increase of A^S observed in lichens by Kuhn & Kesselmeier (2000) for the same temperature
506 range. Since a thermal optimum below 21°C for enzymatic COS hydrolysis is not within the
507 range of published data (Burnell & Hatch, 1988), we argue that lower net A^S at a warmer
508 temperature (within our measurement range) is caused by higher COS emissions rather than
509 by reduced COS uptake. In fact, here we demonstrated that the COS source term (P^S,
510 estimated from A^S-C^S curves) is very sensitive to temperature, with a Q_{10} of 3.7. Our Q_{10}
511 estimate for COS emissions is higher than those reported for net COS fluxes in soils (Maseyk
512 *et al.*, 2014) but within the range of respiratory Q_{10} for several moss species (Wagner *et al.*,
513 2013).
514 Our results also seem to indicate a small, but statistically significant, decrease of the
515 gross COS uptake (U^S) with increasing light intensity. Given our current knowledge of the
516 temperature sensitivity of CA activity (Burnell & Hatch, 1988), it is unlikely that COS
517 hydrolysis was inhibited by the warming experienced inside the gas-exchange chamber
518 during our light curves. It could be argued that this decrease in U^S with light intensity was
519 simply driven by a reduction in tissue RWC along the experiment. In our experiment, for
520 light intensities above 400 µmol m^{-2} s^{-1} the mean RWC was 535 ± 25%, *i.e.*, close to the
521 point beyond which A^S starts to decrease. Yet, such reduction in RWC should have also
522 negatively affected A^C and we did not detect a major drop in A^C towards the end of the light
523 curve. Alternatively, other biological reasons may explain this decrease of U^S with light
524 intensity in bryophytes. For example, carbon concentration mechanisms (CCM) that
525 incorporate CA as a key constituent have evolved in some bryophyte lineages (specifically in
526 the Anthocerophyta, the hornworts), but there is no conclusive evidence from previous gas
527 exchange measurements for an active CCM in either mosses or liverworts (Smith & Griffiths,

528 2000; Badger, 2003; Meyer *et al.*, 2008). However, the presence of CAs of different types
529 associated to a basal CCM has been hypothesised in all C_3 plants (Zabaleta *et al.*, 2012), thus
530 one hypothesis that might deserve future study would be to test whether the observed
531 decrease in A^S with light could be explained by activity of a light-sensitive CA (Rech *et al.*,
532 2008). However, in one of our study species (*M. polymorpha*, the liverwort), the
533 photosynthetic CO_2 compensation point and carbon isotope discrimination did not respond to
534 the addition of a CA-inhibitor, indicating that most likely it lacks any external CA or CCM
535 activity (Smith & Griffiths, 2000).
536
537 *The unexpected contribution of bryophytes to the COS budget*
538 The quantification of COS fluxes in bryophytes is not only relevant to better understand the
539 drivers of the net leaf COS flux in other species, but also to help constrain the global COS
540 budget, as bryophytes are key constituents of many ecosystems (DeLuca *et al.*, 2002). The
541 estimated leaflet relative uptake rates (LRU of 0.2 for the moss, *S. purum* and 0.9 for the
542 liverwort, *M. polymorpha*, in the light and at optimal hydration status) found in our study,
543 were lower than current LRU estimates for vascular plants, which range between 1.4 and 2
544 (Seibt *et al.*, 2010; Stimler *et al.*, 2010). If we were to estimate the contribution of
545 cryptogamic covers to the global COS budget from their current estimates of CO_2 uptake
546 (3.9 Pg C y^{-1} according to Elbert *et al.*, 2012) following the same LRU approach as proposed
547 for vascular plants (Campbell *et al.*, 2008; Sandoval-Soto *et al.*, 2005), with atmospheric
548 mixing ratios of 400 µmol CO_2 mol^{-1} and 540 pmol COS mol^{-1}, we would arrive at an
549 estimate between 0.005 and 0.024 Tg COS y^{-1} (for LRU of 0.2 and 0.9, respectively). This
550 flux is within the same order of magnitude as the estimate of COS uptake for swamps and
551 marshes and is larger than current estimates for tundra, alpine and desert scrublands (see
552 Table 3 of Sandoval-Soto *et al.*, 2005). These values could serve as first approximations for
553 high latitudes in the summer, where extensive regions are dominated by uniform bryophyte
554 carpets, daylight is continuous and the evaporative demand is low (Lindo *et al.*, 2013).
555 However, bryophytes are also commonly found in areas where day-night cycles alternate and
556 physiological activity is strongly constrained by tissue hydration (Elbert *et al.*, 2012). In these
557 areas, during the day, when temperatures are high and air moisture is low, bryophytes would
558 tend to dehydrate and CO_2 and COS uptake would be metabolically limited, whilst warmer
559 temperatures and incident radiation would enhance unexpected COS emission. In contrast, at
560 night, when the temperature is cooler and the evaporative demand is low, bryophytes would
561 rehydrate towards full turgor and act as strong COS sinks, but CO_2 sources. Our estimated

562 Q_{10} constitutes a first step towards quantifying the contribution of the COS emissions.
563 However, further work is required to understand the sensitivity of this parameter (P^S) to
564 additional environmental constraints, particularly changes in tissue hydration and light
565 regimes with seasons or ontogeny (Porada *et al*., 2013).
566
567 *Conclusions*
568 Here, using bryophytes as model organisms, we have demonstrated that net COS uptake
569 continues in the dark, but is also progressively decreased as irradiance and temperature
570 increase, mostly because of an unexpected, temperature-driven COS emission. Together, our
571 results challenge a key underlying assumption for quantifying GPP from COS fluxes that
572 vegetation COS uptake is unidirectional. Obviously, we cannot immediately extrapolate our
573 findings to other terrestrial vascular plants; however our results should encourage further
574 studies to revisit the unidirectional flux assumption in vascular plants making use of the
575 improved laser spectrometers now available.
576
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588
589 **AUTHOR CONTRIBUTIONS**
590
591 TEG, LW, JO and JR conceived and designed the experiment. TEG, SJ, RB, SW, JS and
592 JBW designed and took the gas-exchange measurements. TEG, CB and YG performed and
593 interpreted the biochemical analyses. TEG, JO, JBW and LW analysed the data. TEG wrote
594 the first manuscript draft. All authors commented and contributed to the final version.
595

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761

SUPPORTING INFORMATION

Figure S1. Allan variance plot showing the standard deviation for the QCLS.

Figure S2. Tissue relative water content and sample temperature along desiccation.

Figure S3. Sample temperature inside the gas-exchange chamber during light curves.

Figure S4. Individual metabolite (protein and non-structural carbohydrate) concentrations.

Figure S5. CO_2 and COS net uptake rates in the dark along desiccation at two temperatures.

Table S1. Estimated regression coefficients and summary statistics of the linear mixed models performed to assess the effects of COS concentration in the light and in the dark, and light intensity under ambient and near-zero COS mixing rations on CO_2 and COS uptake rate.

777 **FIGURE LEGENDS**

778

779 **Figure 1.** CO_2 (A^C, a and c) and COS (A^S, b and d) net assimilation in the light at 21 °C (red)
780 and in the dark at 16 °C (blue) along decreasing tissue relative water content (RWC) in the
781 moss (a and b) and in the liverwort (c and d). Each symbol is an individual sample, the lines
782 are smooth curves (fitted with a generalized additive model) and the blue and red areas
783 denote the 95% confidence interval for A^C and A^S in the light (red) or in the dark (blue).
784 Areas where the confidence interval do not overlap denote a significant effect at $\alpha = 0.05$.
785 The non-RWC dependent black regions (at approximately 0) denote the mean (± sd) fluxes of
786 the blank chamber.

787

788 **Figure 2.** Mean (± se, $n = 4$) CO_2 (A^C, a) and COS (A^S, b) net assimilation along a COS
789 mixing ratio (C^S) gradient in the light at 21 °C (open symbols and red areas) and in the dark
790 at 16 °C (closed symbols and blue areas), in the liverwort. Lines and areas represent linear
791 fits ($P < 0.001$, $R^2 = 0.9$ in b), and their 95% confidence intervals. Black regions denote the
792 mean (± sd) fluxes of the blank chamber.

793

794 **Figure 3**. Relationship between mean measurement temperature (T, $n = 4$ measurements) and
795 estimated (± se) COS source (P^S) from the intercept of A^S-C^S curves (A^S is net COS
796 assimilation and C^S is the COS mixing ratio). Open and closed symbols represent estimated
797 P^S from A^S-C^S curves performed in the light or in the dark, respectively, in the liverwort. The
798 lines represent the modelled common temperature response (continuous line) and its
799 uncertainty (dashed lines) over the entire experimental temperature range: $P^S_{\mathrm{modelled}} =$
800 $e^{\frac{\log(Q_{10})}{10}}$ with $Q_{10} = 3.7$

801

802 **Figure 4** Relationship between light intensity (PPFD) and mean (± se, $n = 4$) net measured
803 CO_2 (A^C, a) and COS assimilation (A^S, b, closed symbols and solid lines) and gross estimated
804 assimilation (U^S, c, open symbols and dashed lines) under ambient COS mixing ratio (Amb
805 COS, squares) and near-zero COS mixing ratio (Zero COS, circles), in the liverwort. Lines
806 and grey areas correspond to the exponential (in a) and linear (in b and c) fits and their 95%
807 confidence interval. Black regions denote the mean (± sd) fluxes of the blank chamber.

808

809 **Figure 5.** Protein and total non-structural carbohydrates (NSC) content, per unit of dry
810 weight (DW), in the moss (upper panels) and the liverwort (lower panels) measured along
811 decreasing relative water content (RWC) in the dark (filled bars) and in the light (open bars).
812 Bars are the means (+se, n = 1-5) for grouped data according to four categorical levels of
813 RWC (very high, high, intermediate and low), RWC values on the x-axis are the overall
814 means for the light and dark treatments for each RWC level. In the liverwort, (glucose),
815 fructose, sucrose, (total NSC) and protein content were (marginally) higher in the light than
816 in the dark (F = 2.98, 4.41, 4.97, 3.93 and 4.94; P = 0.098, 0.047, 0.036, 0.06 and 0.037,
817 respectively; according to the results of a two-way ANOVA of light and RWC, the latter
818 included as a categorical variable with four levels).

819 **Tables**

820

821 **Table 1.** Estimated net uptake (se) of CO_2 (A^C in µmol kg^{-1} s^{-1}), COS (A^S in pmol kg^{-1} s^{-1})
822 and leaflet relative uptake (LRU), in the light at 21 °C and in the dark at 16 °C, at the optimal
823 (when A^S was maximal) and minimal tissue relative water content (RWC in %) in the moss
824 (*Scleropodium purum*) and the liverwort (*Marchantia polymorpha*).

			RWC	A^C	A^S	A^C	**LRU**
Moss	Optimum	Light	887	15.3 (0.6)	4.3 (1.0)	15.3 (0.6)	0.22
		Dark	661	-4.9 (0.2)	18.0 (1.1)	-4.9 (0.2)	-3.08
	Minimum	Light	1	-0.3 (0.3)	-6.9 (0.5)	-0.3 (0.3)	15.12
		Dark	1	-0.3 (0.1)	-1.3 (0.6)	-0.3 (0.1)	3.43
Liverwort	Optimum	Light	762	18.8 (1.7)	20.2 (1.8)	18.8 (1.7)	0.89
		Dark	598	-10.7 (0.3)	42.9 (1.9)	-10.7 (0.3)	-3.04
	Minimum	Light	42	-1.5 (1.8)	-7.5 (1.8)	-1.5 (1.8)	4.14
		Dark	1	-0.4 (0.1)	-2.1 (0.7)	-0.4 (0.1)	4.24

825

826 **Table 2.** Results of the linear model (*t* and *P*) to assess the effect of relative water content
827 (RWC), light and temperature regime (light/21 °C and dark/16 °C) and their interaction on
828 different metabolite concentrations (proteins and non-structural carbohydrates, NSC) in the
829 liverwort and the moss.

		RWC		Light		RWC x Light	
	Metabolite	**t**	***P***	**t**	***P***	**t**	***P***
Liverwort	Glucose	-0.597	0.556	0.319	0.752	0.529	0.601
	Fructose	-0.591	0.56	0.084	0.934	0.932	0.36
	Sucrose	-0.416	0.681	0.008	0.994	1.047	0.305
	Starch	-0.626	0.536	-0.095	0.925	0.661	0.514
	Total NSC	-0.533	0.599	0.161	0.873	0.798	0.432
	Protein	2.267	**0.032**	1.036	0.31	-0.241	0.812
Moss	Glucose	2.485	**0.022**	0.28	0.782	-0.336	0.74
	Fructose	2.752	**0.013**	0.282	0.781	-0.081	0.936
	Sucrose	3.953	**<0.001**	0.381	0.708	0.241	0.812
	Starch	10.077	**<0.001**	0.842	0.41	-0.3	0.767
	Total NSC	5.458	**<0.001**	0.512	0.615	-0.115	0.91
	Protein	5.262	**<0.001**	0.18	0.859	-0.383	0.706

830