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1 **Biochemical traits enhance the trait concept in *Sphagnum* ecology**

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32 **Abstract**

33 *Sphagnum* mosses are key to northern peatland carbon sequestration. They have a range of
34 morphological and anatomical characteristics that allow them to cope with environmental stress.
35 *Sphagnum* also produces a plethora of biochemicals that may prevent stress-induced cell-damage.
36 However, the linkages between *Sphagnum* anatomical, morphological and biochemical traits (*i.e.*
37 metabolites, pigments and antioxidant enzyme activities) are poorly known, neither are their joint
38 responses to environmental change. Here, we quantify and link an array of *Sphagnum* anatomical,
39 morphological and biochemical traits in five *Sphagnum*-dominated peatlands distributed along a
40 latitudinal gradient in Europe, covering a range of regional and local environmental conditions.
41 *Sphagnum* morphological and anatomical traits were intrinsically linked to *Sphagnum* metabolites and
42 enzyme activities, and these relationships were driven by shared responses to local and regional
43 environmental factors. More particularly, we found that *Sphagnum* traits can be grouped into four
44 clusters related to growth, biomass, defense and water stress tolerance. We used regional and local
45 environmental conditions data to further show that biochemicals and their specific linkages with some
46 morphological traits describe dimensions of physiology not captured by anatomical and morphological
47 traits alone. These results suggest that *Sphagnum* morphology and function is rooted in the metabolome,
48 and that incorporating biochemicals into the functional trait space concept can enhance our mechanistic
49 understanding and predictive power in *Sphagnum* ecology.

50

51 **Key words:** *Sphagnum*, peatlands, metabolites, antioxidant enzyme activities, bryophytes,
52 climate change.

53 **Introduction**

54 Recent decades have seen an explosion of research into the use of plant traits to determine how
55 individual plants cope with changing environments (Díaz et al., 2004; Kattge et al., 2011; Reich et al.,
56 2003), and how they can affect ecosystem processes in return (Chelli et al., 2019; Lavorel and Garnier,
57 2002). Despite their relevance for generalizing predictions across organizational and spatial scales
58 (Adler et al., 2013), the plant traits which are commonly used by ecologists (*e.g.* plant height, seed
59 mass, and specific leaf area; Díaz et al., 2016) only moderately explain ecosystem properties (Plas et
60 al., 2020), thus leaving a large fraction of ecosystem processes unexplained. This indicates that there
61 are some limits to which extent classical morphological traits can predict ecosystem processes, so that
62 additional drivers, more closely embodied in plant physiology such as primary and secondary
63 metabolites, may be required to improve predictions of plant form and function (Walker et al., 2019).

64 Plants produce thousands of metabolites that collectively determine their fitness *via* their effects
65 on survival, growth and/or reproduction (Weng et al., 2021). For example, plants respond to drought,
66 temperature, herbivore or pathogen stress by producing specialized metabolites, such as alkaloids,
67 terpenoids, and phenolic compounds (Cipollini et al., 2012; Defosse et al., 2021; Fernandez-Conradi
68 et al., 2021; Fernandez et al., 2016; Peters et al., 2019; van Dam and Bouwmeester, 2016; Van der
69 Putten et al., 2013; Weckwerth and Kahl, 2013). Therefore, plant metabolites can be key for
70 understanding the ecosystem functioning as they may drive ecosystem processes such as decomposition
71 and/or carbon cycling (Chomel et al., 2016; Whitham et al., 2006). As classical morphological traits,
72 plants metabolites are determined by the same suite of evolutionary and ecological processes (Defosse
73 et al., 2021; Sedio et al., 2017). However, they may provide better mechanistic understanding in
74 ecological context as they lie at the interface between genes and environment (Ahuja et al., 2010). The
75 identification and quantification of plant metabolites can therefore provide a snapshot of the overall
76 plant performance and adaptation success when facing changing environmental conditions (Ahanger et
77 al., 2018).

78 While many attempts have been done to characterize the diversity and the dynamic of
79 metabolites within vascular plants (see examples in Bakhtiari et al., 2021; Defossez et al., 2021; Obata
80 and Fernie, 2012; Skoneczny et al., 2018; Turner et al., 2016), much less has been done for non-vascular
81 plants such as mosses. Yet mosses make a significant contribution to aboveground biomass and soil
82 carbon sequestration, particularly in peatlands (Tan and Pócs, 2000; Turetsky, 2003). *Sphagnum* mosses
83 effectively facilitate wet, anoxic and acidic conditions that inhibit decomposition (Clymo and Hayward,
84 1982; Rydin and Jeglum, 2006; Turetsky, 2003). They also produce a recalcitrant litter and release
85 antimicrobial compounds in their surrounding environment that further hampers the decomposition of
86 dead organic matter (Fudyma et al., 2019; Hamard et al., 2019; Turetsky, 2003; van Breemen, 1995).
87 *Sphagnum* mosses have therefore an extraordinary impact on the global C cycle by modulating peatland
88 C uptake and storage. However, despite these general properties we still lack a clear understanding of
89 the species-specific characteristics, or traits, controlling *Sphagnum* growth and/or survival at global and
90 local scale (Bengtsson et al., 2020a). *Sphagnum* species exhibit high interspecific variability in their
91 inherited anatomical, morphological and biochemical traits (Bengtsson et al., 2020b, 2018, 2016;
92 Chiapusio et al., 2018; Dorrepaal et al., 2005; Gong et al., 2019). While *Sphagnum* anatomical and
93 morphological traits are increasingly used to explain how *Sphagnum* species interact with
94 environmental conditions (Bengtsson et al., 2020b; Jassey and Signarbieux, 2019; Laine et al., 2021),
95 we lack knowledge on interspecific differences in *Sphagnum* biochemical traits, their relationship with
96 anatomical and morphological trait, and how they are affected to local and regional changes.

97 *Sphagnum* mosses produce numerous metabolites which can be released in the environment
98 (Fudyma et al., 2019; Hamard et al., 2019; Verhoeven and Liefveld, 1997). These metabolites facilitate
99 *Sphagnum* growth, defense and competitiveness *via* interaction with the environment and/or with other
100 organisms in the environment (Erb and Kliebenstein, 2020; Khare et al., 2020; Massalha et al., 2017;
101 Wink, 2010). These metabolites have been suggested to be involved in specific or multiple functions,
102 but most likely they play a role in moss fitness and stress tolerance (Isah, 2019; Tissier et al., 2014;
103 Yang et al., 2018). More particularly, a wide and important class of primary (carbohydrates,
104 carotenoids) and specialized metabolites (phenols, proline, flavonoids, tannins, proline) could play

105 crucial roles in the growth, photosynthesis, litter-resistance to decomposition and tolerance to abiotic
106 stresses in bryophytes (Cornelissen et al., 2007; Xie and Lou, 2009). For instance, the production of
107 carbohydrates and polyphenols can support the maintenance of the integrity of plant cell-structure and
108 the internal regulation of plant cell physiology (Ferrer et al., 2008; Roberts et al., 2012; Rudolph and
109 Samland, 1985; Tissier et al., 2014). To protect their cells against oxidative damages (Davies, 1986;
110 Dizdaroglu, 1994; Leprince et al., 2000; Sharma et al., 2012), mosses— like vascular plants— further
111 accumulate antioxidants such as flavonoids and/or activate antioxidant enzymes such as catalase (CAT),
112 glutathione reductase (GR), peroxidases (POX)/ascorbate peroxidases (APX) and superoxide dismutase
113 (SOD) (Choudhury et al., 2013; Das and Roychoudhury, 2014; Guan et al., 2016; Minibayeva and
114 Beckett, 2001; Noctor et al., 2018). The regulation of the production of *Sphagnum* metabolites and of
115 the antioxidant enzyme activities are likely important for *Sphagnum* morphology, growth, physiology
116 and the tolerance to environmental changes (Ahanger et al., 2018). Hitherto, only a limited number of
117 studies have focused on *Sphagnum* metabolites (Chiapusio et al., 2018; Fudyma et al., 2019; Hamard
118 et al., 2019; Klavina et al., 2018; Sytiuk et al., 2021), while antioxidant enzyme activities remains
119 largely unexplored. In addition, the linkages among *Sphagnum* anatomical and morphological traits,
120 metabolites and antioxidant enzyme activities are virtually unknown, while resource allocation trade-
121 offs among these different *Sphagnum* traits could have important ramifications for peatland carbon
122 cycling.

123 In this work, we aim to explore the potential of biochemicals (*i.e.*, metabolites and enzyme
124 activities) to provide deeper mechanistic and predictive power of *Sphagnum* traits space across
125 environmental gradients. To address this aim, we quantified the contribution and the role of each trait
126 group to the functional trait space of *Sphagnum* mosses. Our study was conducted in five European
127 *Sphagnum*-dominated peatlands distributed along a latitudinal gradient in Europe, representing a range
128 of local and regional environmental conditions. We collected one dominant *Sphagnum* species per site,
129 to observe species performance at their site of origin. With this design we assumed that species were
130 not facing stress conditions. Specifically, our aims were to: (i) gather new *Sphagnum* traits information
131 relevant to further ecological and environmental studies, (ii) test the strength of the linkages among

132 *Sphagnum* anatomical/morphological traits and metabolites/antioxidant enzyme activities, and (iii)
133 assess the extent to which local and regional environmental factors influence these linkages.

134

135

136 Materials and methods

137 Study sites

138 Five sites were selected along a latitudinal gradient spanning different local and regional environmental
139 conditions, from northern Sweden to southern France. Overall, these peatlands covered a range of mean
140 annual temperature from -0.1 °C to 7.9 °C, and a range of precipitation from 419 mm to 1027 mm
141 (Table 1, WorldClim v2 (Fick and Hijmans, 2017)). Details of these five sites follow.

142 (1) Counozouls is a moderately rich fen in southwestern France that belongs to the Special Area of
143 Conservation Natura 2000 site “Massif du Madres Coronat” (42°41'19.7"N 2°14'02.4"E) in the
144 Pyrenees mountains. The moss layer was dominated by *Sphagnum capillifolium*, *S. warnstorffii* and *S.*
145 *palustre* while vascular plants were dominated by *Molinia caerulea*, *Carex rostrata*, *Ranunculus*
146 *polyanthemoides*. and *Potentilla anglica*.

147 (2) Kusowo Bagno is an open bog in northern Poland (53°48'47.9"N 16°35'12.1"E) in a nature reserve
148 and is a part of the Special Area of Conservation Natura 2000 site “Lake Szczecineckie”. The peat moss
149 layer was dominated by *S. magellanicum* and *S. fallax*, while *Eriophorum vaginatum*, *Andromeda*
150 *polifolia*, *Vaccinium oxycoccos*, and *Drosera rotundifolia* were the most common vascular plants at the
151 sampling location.

152 (3) The Männikjärve bog (58°52'26.4"N 26°15'03.6"E) is in Central Estonia in the Endla mire system.
153 The ground layer was dominated by *S. rubellum*, *S. fuscum* and *S. magellanicum* whereas *Drosera*
154 *rotundifolia*, *Eriophorum vaginatum*, *Andromeda polifolia*, and *Vaccinium oxycoccos* characterized the
155 vascular plant community.

156 (4) Siikaneva (61°50'41.6"N 24°17'17.5"E) is an ombrotrophic bog complex located in Orivesi,
157 southern Finland. The *Sphagnum* carpet was dominated by *S. fuscum* in hummocks and *S. papillosum*,
158 *S. majus*, and *S. rubellum* in lawns-hollows. The vascular plant layer was composed of dwarf shrubs
159 (mainly *Vaccinium oxycoccos* and *Andromeda polifolia*), ombrotrophic sedges (*Eriophorum vaginatum*
160 and *Carex limosa*) and herbs (*Scheuchzeria palustris* and *Drosera rotundifolia*).

161 (5) Abisko (sub-arctic Sweden; 68°20'43.1"N 19°03'58.7"E) is a gently sloping aapa mire surrounded
162 by tundra. *S. balticum* was the numerically dominant peat moss species, whereas vascular plants such
163 as *Carex sp.*, *Andromeda polifolia*, *Rubus chamaemorus* and *Empetrum nigrum* were scarce.

164 Sample collection

165 *Sphagnum* samples were collected at every site within the same week in early-July 2018. At each site,
166 we selected five homogeneous plots of the same microtopography comprising of lawns (50 x 30 cm
167 each; 5 plots x 5 sites = 25 plots in total, Table 1) with a single dominant *Sphagnum* species as based
168 on a vegetation survey (Table S1): *S. warnstorffii* (France), *S. magellanicum* (Poland), *S. rubellum*
169 (Estonia), *S. papillosum* (Finland) and *S. balticum* (Sweden). Dominant *Sphagnum* species were site
170 specific, potentially creating a confounding effect with environmental conditions. However, it has been
171 recently demonstrated that such potential confounding effect between species and site was not an
172 important issue when referring to *Sphagnum* biochemical traits because of the decoupling between
173 *Sphagnum* biochemical traits and phylogeny (see Material and Methods and Figures S1-S3 in Sytiuk et
174 al., 2021). In addition, we provide in Fig. S1 the ranges of variations of the different biochemical traits
175 analyzed in the five *Sphagnum* species based on data from a reciprocal transplant experiment on the
176 same *Sphagnum* species dispatched along our latitudinal gradient. The results show that the variability
177 within the species is higher than between the species and thus that environment has stronger effect on
178 the *Sphagnum* biochemical traits than *Sphagnum* taxonomy. Nevertheless, we acknowledge that
179 anatomical and morphological traits are used to identify *Sphagnum* moss to species level (Isoviita,
180 1966), and that in our study anatomical and morphological traits cannot be disentangled from *Sphagnum*
181 taxonomy, and hence sites. We therefore used site as fixed effect in all statistical analyses (see numerical
182 analysis section) to avoid any further confusion.

183 In each plot, we collected 15-20 *Sphagnum* shoots around 10 marked spots. Upon sampling,
184 the living part of *Sphagnum* shoots (top 3 cm from the capitula) were cut immediately, pooled,
185 homogenized and then distributed in plastic bag/tubes for different lab analyses. Samples were kept in
186 a cooler (4 °C) while travelling back to the laboratory. Upon arrival, samples were frozen at -20 °C or
187 kept cold at 4 °C depending on the type of analysis.

188 Characterizing local environmental conditions

189 Water-table depth (WTD) and porewater pH were measured directly in the field using a ruler and a
190 portable multimeter Elmetron CX742, respectively. 50 mL of porewater was collected at each plot to
191 analyze water chemistry. Additionally, water-extractable organic matter (WEOM) was extracted from
192 the *Sphagnum* shoots (0-3 cm height) collected in each plot following (Jassey et al., 2018). Briefly,
193 *Sphagnum* shoots were soaked in 30 mL of demineralized water and then shaken for 90 min at 150 rpm.
194 *Sphagnum* shoots were then dried at 60°C for 48 hours and weighted to obtain dry mass (mg/g DW).
195 Nutrients and ions were measured in porewater and WEOM as follow (Table S2): dissolved organic
196 carbon and nitrogen and phosphorous (measured by combustion on a Shimadzu TOC-L) and ions (SO_4^{2-}
197 , Cl^- , NO_3^- , Ca^{2+} , Mg^{2+} , K^+ , Na^+ , Fe^{3+} ; measured on Dionex Ics-5000+ and Dionex DX-120). Cluster
198 analysis (Ward method) on porewater and WEOM chemistry was used to group the five peatlands
199 according to similarity in their ‘nutrient status’. As pore water in Polish site was impossible to collect
200 (too dry), we based our analysis on the four other sites. We found similar clusters between the pore
201 water and WEOM (Fig. S2). This shows that both the pore water and WEOM can be used to define the
202 ‘nutrient status’ of our sites. Subsequently, we used WEOM chemistry to classify the five sites
203 according to their nutrient richness and pH.

204 We assessed the dynamic of plant species cover at each date in each of the five sites using
205 pictures taken in each plot (Table S1, Fig S3). Following Buttler *et al.* (2015), we took two high-
206 resolution images in each plot (25 x 15 cm). On each picture, we had a grid of 336 points laid and
207 quantified species overlaying the grid intersects. This technique did not support an estimation of vertical
208 biomass distribution and possibly underestimated the frequency of certain species (Jassey et al., 2018).
209 However, the potential bias was similar across plots, making species frequencies comparable among
210 sites. We especially distinguished the dwarf-shrubs cover from the non-woody herbaceous cover as
211 these two plant types differ in energy and nutrient allocation and litter production, and as such may have
212 different effects on ecosystem C dynamic (Kruk and Podbielska, 2018).

213 *Sphagnum* anatomical and morphological traits

214 We characterized an architectural variability of five *Sphagnum* species using a suite of five anatomical
215 and morphological traits following Jassey and Signarbieux (2019): volume of the capitulum (height x
216 diameter of capitulum), water-holding capacity of the capitulum and shoot, number of hyaline cells per
217 leaf area (*i.e.* dead cells storing water), the surface area of hyaline cell (length x width), and width of
218 chlorocystes (photosynthetic cells surrounding hyaline cells). We randomly selected 125 *Sphagnum*
219 shoots (25 per site) to estimate the volume of the capitula (mm^3) by measuring their height and diameter
220 using a ruler. Then, the same samples were used to quantify the water-holding capacity of the capitula
221 at water saturation. Capitula were submerged in water until saturated, then the plant was suspended
222 horizontally and allowed water to flow naturally after removing capitula from water for two minutes.
223 Then, every capitula and stems were weighed as water-saturated and dried out after 3 days at 60 °C.
224 The net water content at water saturated of each capitula was expressed in grams of water per gram of
225 dry mass ($\text{g H}_2\text{O/g DW}$). For anatomical traits analyses, five *Sphagnum* capitula from every plot (125
226 capitula in total) were carefully deconstructed to separate *Sphagnum* leaves. Then, all *Sphagnum* leaves
227 were pooled, homogenized, and three leaves (per plot) were taken from that pool to prepare microscope
228 slide from each plot (in total, 375 leaves). We measured the number of hyaline cells per leaf area
229 (number of hyaline cells per mm^2), their surface (μm^2), as well as the width of chlorocystes (μm) using
230 a light microscope connected to a camera (LEICA ICC50 HD) and the size analytic tools (LEICA suite
231 software).

232

233 *Sphagnum* biochemical traits: metabolites, pigments, proteins and antioxidant enzyme
234 activities

235 Figure 1 summarizes the different extractions pathways used to quantify the various *Sphagnum*
236 biochemical traits: pigments, metabolites and enzyme activities. *Sphagnum* mosses were previously
237 frozen, lyophilized and stored at -20 °C before performing any biochemical analysis. All details about
238 biochemical extraction and quantification can be found in Supplementary method.

239 Statistical analyses

240 All statistical analyses were performed in *R* 3.5.3 (R Core Team, 2019) using specific packages, as
241 specified below. Linear mixed effects models (LMEs) were used to assess the effect of site (fixed effect)
242 on *Sphagnum* traits (anatomical, morphological and biochemical traits), as well as the effect of the site
243 on local and regional environmental conditions and relative plant cover. The models were fitted with
244 plot nested within site as a random effect on the intercept (Pinheiro and Bates, 2000). Tukey's multiple
245 comparison test was used for *post hoc* analyses of differences among the levels of the fixed effects in
246 the final model. Assumptions of normality and homogeneity of the residuals were checked visually
247 using diagnostic plots and a Shapiro test. \log_{10} -transformation was used when necessary in order to
248 meet these assumptions. As the distributions of distribution of water-extractable organic matter data are
249 not normal, non-parametric Friedman tests were performed to assess the differences between sites.

250 To represent differences of *Sphagnum* trait space, including anatomical, morphological and
251 biochemical traits, we performed principal coordinate analysis (PCoA) using Gower's distance
252 (Villéger et al., 2008). A standardization of *Sphagnum* anatomical, morphological and biochemical
253 traits was applied on the matrices beforehand (Legendre and Legendre, 2012). To assess which
254 proportion of *Sphagnum* moss functional space was actually occupied by *Sphagnum* anatomical and
255 morphological and biochemical traits among overall traits, we calculated the volume of the observed
256 multidimensional convex hull in the selected ordination space (Barber et al., 1996). The volumes of
257 observed hulls were based on the three first axes of principal component analysis and was quantified
258 using *geometry R* package.

259 To identify the linkages among *Sphagnum* traits under changing environmental conditions, we
260 used a joint species distribution modelling approach (JSDM, (Pollock et al., 2014). JSDM takes
261 hierarchical approach, which combines abundances and similarities in trait responses to latent
262 parameters such as environmental and edaphic variables (*i.e.* traits co-occurrence with environmental
263 parameters). The model begins with a trait joint distribution analysis and builds on inverse prediction
264 that evaluates how environmental parameters affect the distribution of co-occurring traits (Pollock et
265 al., 2014). Then, traits correlations can be divided into two groups: (1) environmental correlations

266 among traits due to similar response of traits to environmental conditions and (2) residual correlations,
267 *i.e.* correlation between traits that are not due to environmental conditions. JSDM was performed with
268 the Bayesian Ordination and Regression AnaLysis as implemented in *boral* R package and using two
269 latent variables to estimate the residual correlations among traits. The trait matrix was spatially
270 detrended beforehand to avoid any confounding effect with regional climate. We used package *igraph*
271 to build networks of either trait's linkages due to shared environmental responses or traits linkages not
272 due to environmental responses. We considered traits co-response to be robust when the correlation
273 between traits due to shared environmental response (or residual correlation) was significant at $P < 0.05$.
274 The analysis was performed with *Sphagnum* traits, regional (annual precipitations and the mean
275 temperature of the wettest quarter) and local (pH, WEOM chemistry and plant cover) environmental
276 parameters.

277 Furthermore, we tested whether combining biochemicals to anatomical and morphological
278 traits improves the characterization of '*Sphagnum* form and function' in the context of species
279 environmental tolerances. To this end, we built a structural equation model (SEMs; Fig. S4) that tested
280 for associations between the regional and local variables and a latent variable capturing "*Sphagnum*
281 form and function" – that is, an unmeasured variable described by the product of the metabolites,
282 enzymes and anatomical/morphological traits (Fig. S4). Specifically, we constructed SEM using the
283 package "lavaan" (Rosseel, 2012), which drew from a hypothesis-driven *a priori* model based on our
284 expectation that biochemicals enhances mechanistic understanding of how environmental change affect
285 *Sphagnum* form and function (Fig. S4; hypotheses: Table S3). We diagnosed model fits using chi-
286 squared statistics ($P > 0.05$), root-mean squared errors for approximation and residual indices (≤ 0.1)
287 and comparative fit indices (≥ 0.95), and included variables and paths in the final model based on chi-
288 squared statistics ($P < 0.05$) and AIC values.

289

290 Results

291 In this section we refer to changes in sites (see Table 1 for site acronyms), which nevertheless
292 are confounded with *Sphagnum* species identity. We advise to check Fig S1 and Sytiuk et al. (2021),
293 where the potential confounding effect between species and site has been addressed into details,
294 especially for biochemical traits.

295 Regional and local environmental conditions across the five sites

296 Climatic conditions varied along the latitudinal gradient. Whilst mean annual temperature and annual
297 precipitation together increased from SE to FR (Table 1), we found that *Sphagnum* field water content
298 was the lowest in PL with 4.1 gH₂O/g DW and the highest in SE with 13.96 gH₂O/g DW ($F_{4,16}=9.1$, P
299 < 0.0001 ; Table 1). In addition to climatic and water content availability variability, both *Sphagnum*
300 and vascular plant relative cover differed among the five sites (*Sphagnum*: $F_{4,16}=25.9$, $P < 0.001$;
301 vascular plants: $F_{4,16}=31.8$, $P < 0.001$; Table 1, Table S1). In the warmest site (FR), moss relative cover
302 was the lowest (~32%), and the vascular plant cover the highest (moss: $F_{1,19}=5.14$, $P =0.03$, vascular
303 plants: $F_{1,19}=9.02$, $P =0.007$; Table 1). The highest moss cover was observed in EST (~78 %) and FI
304 (~73 %), while vascular plant cover was $< 6\%$ (moss: $F_{1,19}=20$, $P <0.001$, vascular plants: $F_{1,19}=40.8$, P
305 <0.0001 ; Table 1). The PCoA analysis showed somewhat contrasting plant community patterns. Three
306 groups emerged from the first and second axes (Fig. S3): one group composed of FR and SE, another
307 group composed of FI and EST and a last one composed of PL only. These patterns strongly related to
308 shrub and herbaceous covers, which were negatively correlated. While shrub cover was the highest in
309 EST, FI and in a lesser extent PL, herbaceous cover peaked in FR and SE sites (Fig. S3). These patterns
310 further accounted for the nutrient status of the different sites. A clustering analysis (Ward method) on
311 the physicochemical properties of the *Sphagnum* WEOM (DOC, DON, DP, pH and ions, see method
312 and Table S2) revealed the exact same three groups than for vegetation composition: FR-SE, EST-FI
313 and PL (Fig. S3). PL differed from other sites because of higher concentrations of dissolved organic
314 carbon, potassium and chlorine (11.8, 0.74 and 1.35 mg/g DW, respectively; Table S2), while SE-FR
315 differed from PL and EST-FI because of higher pH and dissolved phosphorous concentrations (Table 1
316 and Tables S2).

317 Characteristic of *Sphagnum* anatomical, morphological and biochemical traits

318 *Sphagnum* moss from PL had the highest capitulum diameter, capitulum height and capitulum
319 volume with 1 cm², 0.424 cm and 0.337 cm³ respectively, while the lowest values were observed in
320 EST with 0.524 cm², 0.25 cm and 0.006 cm³, respectively (diameter: $F_{4,16}=27.8$, $P < 0.001$; height:
321 $F_{4,16}=8.53$, $P < 0.01$; volume: $F_{4,16}=23.15$, $P < 0.001$; Fig. 2). *Sphagnum* from EST had the highest
322 number of hyaline cells (on average 693.2 mm⁻¹) ($F_{4,16}=21.53$, $P < 0.001$), but the highest water holding
323 capacity was found in FI (Fig. 2; $F_{4,16}=17.61$, $P < 0.001$). The width of chlorocystes was the highest in
324 FI with 9.86 μm, while the other species had about two-fold lower width (Fig. 2; $F_{4,16}=26.41$, $P < 0.001$).

325 For biochemicals, total carbohydrates and proteins concentrations had a maximum
326 concentration in moss from PL (229.08 mg/g DW and 0.17 mg/ml, respectively), and a minimum in FI
327 (102.07 mg/g and 0.072 mg/ml, respectively; Fig. 3; carbohydrates: $F_{4,16}=18.6$, $P < 0.01$ and proteins:
328 $F_{4,16}=5.33$, $P=0.0063$). Chlorophyll (*a+b*) and carotenoids concentrations followed similar trends with
329 the highest values in PL and the lowest in FI (Fig. 3; chl: $F_{4,16}=3.1$, $P=0.04$; car: $F_{4,16}=2.9$, $P=0.052$).
330 Water soluble phenolic concentration was the highest in FR (5.61 mg/g DW) and the lowest in FI (0.49
331 mg/g DW; Fig. 4; $F_{4,16} = 18.9$, $P < 0.001$). Total phenols peaked in FR, while the lowest concentration
332 was observed in SE (Fig. 3; $F_{4,16}=5.26015$, $P=0.0067$). Total flavonoids also differed among sites (Fig.
333 3; $F_{4,16} = 3.1$, $P = 0.046$), with the lowest values found in *Sphagnum* from SE, PL and EST and the
334 highest in FR. Finally, concentrations of tannins and proline did not reveal any significant variation in
335 their concentrations (Fig. 3; $P > 0.05$).

336 For *Sphagnum* antioxidant enzyme activities, *Sphagnum* from FI showed the highest APX
337 activity with 27.94 mmol min⁻¹ mg⁻¹, while mean activity for the four other species was similar and
338 around 12.1 ± 2.37 mmol min⁻¹ mg⁻¹ (Fig. 3; $F_{4,16} = 6.9$, $P = 0.002$). CAT and SOD showed similar
339 trends with maximum activities in FI (Fig. 3; CAT: $F_{4,16} = 5.9$, $P = 0.0039$; SOD: $F_{4,16} = 9.52$, $P <$
340 0.001). The POX activity was the highest in *Sphagnum* from EST with 11.26 mmol min⁻¹ mg⁻¹,
341 following by FI with 6.87 mmol min⁻¹ mg⁻¹, while almost no activity have been observed in other sites
342 (Fig. 3; $F_{4,16}=14.9$, $P < 0.001$).

343 When all *Sphagnum* traits (*i.e.* the three data sets: (1) metabolites, proteins and pigments
344 (hereafter ‘metabolites’), (2) antioxidant enzyme activities, and (3) anatomical and morphological
345 traits) were merged together, the five species formed distinct groups (Fig. 4A). However, the separate
346 PCoAs on anatomical and biochemical parameters showed contrasting patterns (Fig. 4B and 4C). For
347 *Sphagnum* anatomical and morphological traits, the ordination space showed more or less well separate
348 groups composed of each site/species (Fig. 4B), while the PCoA on biochemical parameters showed
349 two distinct groups on the second axis: one with *Sphagnum* from PL and SE and second with FR, EST
350 and FI.

351

352 Linkages between *Sphagnum* traits, regional and local environmental conditions

353 Using JSDM approach, we interrogated the biochemical features observed to covary with anatomical
354 and morphological traits (Fig. 5) to determine whether the metabolites and antioxidant enzyme activities
355 provide deeper mechanistic insight into *Sphagnum* functional trait variation across environmental
356 gradients. We found that *Sphagnum* traits grouped into four distinct clusters of co-occurring
357 metabolites, enzyme activities and anatomical/morphological traits (Fig. 5A); each cluster showing
358 shared responses to local and regional environmental conditions (*i.e.*, annual precipitation, the mean
359 temperature of the wettest quarter, nutrient status of the sites and herbaceous plant cover). Cluster 1 was
360 a mix of traits with total tannins, total phenols, POX, water-soluble phenols and proline— some of them
361 being involved in ‘*Sphagnum* defense’. Cluster 2 was composed of traits involved in antioxidant
362 activities and water-holding capacity (hereafter ‘water stress tolerance’) including flavonoids, APX,
363 CAT, SOD, capitulum water-holding capacity, area of hyaline cell and the width of chlolocyste. Cluster
364 3 referred to ‘*Sphagnum* biomass’ and was composed of morphological traits only with capitulum
365 diameter, capitulum volume and capitulum height. Cluster 4 referred to ‘*Sphagnum* growth’ with total
366 proteins, total carbohydrates, carotenoids and total chlorophyll. Only the number of hyaline cells did
367 not relate to any of these four main clusters.

368 Posterior distribution of the trait coefficients (Fig. 5B) showed that cluster 1 (defense) and 2
369 (water stress tolerance) were responded negatively to mean temperature of the wettest quarter and
370 herbaceous cover, and positively to annual precipitation. Cluster 2 further responded negatively to
371 WEOM characteristics (the first and second PCA axis Fig. S5). Cluster 1 was mostly positively related
372 to the first PCA axis of WEOM, *i.e.* mostly dissolved C and N, and pH (Fig. S5). Cluster 3 ('capitulum
373 biomass') and 4 ('*Sphagnum* growth') showed somewhat opposite patterns to cluster 1 & 2. They
374 positively correlated with temperature, herbaceous relative cover, and WEOM PC1, but negatively with
375 annual precipitation (Fig. 5B). When the effect of local and regional environmental factors on
376 *Sphagnum* traits linkages (residual correlations) was filtered, some co-occurrences among traits
377 remained, the most significant being the one among *Sphagnum* capitulum morphological traits (Fig.
378 5C). Residual correlations among *Sphagnum* traits further showed that non-clustered traits
379 corresponded mostly to traits from cluster 1 & 2.

380

381 *Sphagnum* trait space and contribution of trait-clusters to *Sphagnum* forms and
382 functions

383 The hypervolume of the *Sphagnum* trait space was four times bigger when biochemical traits were
384 included (trait space = 159.41) than when they were excluded (trait space = 40.34) for every *Sphagnum*
385 species but *Sphagnum papillosum* (FI; Table 2). *S. papillosum* showed a stronger contribution of
386 anatomical and morphological traits to its trait space than other species. Furthermore, we tested which
387 of the different cluster of *Sphagnum* traits (Fig. 5A) better define '*Sphagnum* form and function' in the
388 context of environmental variations using a SEM analysis (see method for details on our rationale). Our
389 SEM model revealed that the different clusters of *Sphagnum* traits describe unique aspects of *Sphagnum*
390 form and function (Fig. 6). Standardized coefficients from the different clusters to the latent variable
391 '*Sphagnum* form and function' were statistically significant for only cluster 2 ('water stress tolerance',
392 coefficient = 0.50) and cluster 4 ('growth'; coefficient = 0.72; Fig. 6). We further found strong
393 relationships between local and regional environmental factors and the latent variable '*Sphagnum* form

394 and function'. More particularly, we found that annual precipitation (coefficient = 0.89, $P < 0.01$),
395 temperature (coefficient = -0.69, $P < 0.01$) and herbaceous cover (coefficient = -0.94, $P < 0.01$) were
396 important determinants of '*Sphagnum* form and function' latent variable, and in a lesser extent nutrient
397 status of the sites (WEOM PC1, coefficient = -0.45, $P = 0.068$).

398 Discussion

399 Our results show previously unreported linkages among anatomical, morphological and
400 biochemical traits in *Sphagnum* mosses. We demonstrate that *Sphagnum* traits can be grouped into four
401 clusters which show similar response to local and regional environmental factors. *Sphagnum* traits from
402 the same cluster have very similar responses to environmental factors, while traits from different
403 clusters have the opposite response to those factors. Overall, we found that *Sphagnum*
404 anatomical/morphological and biochemical traits had relatively poor shared responses to environmental
405 conditions. Only *Sphagnum* water holding capacity, chlorocyste width and hyaline cell size were linked
406 with antioxidants like flavonoids and enzyme activities. Our findings also showed that *Sphagnum*
407 biochemicals were regulated by external forces, such as climate, nutrient availability and plant cover,
408 that were not well represented by anatomical and morphological traits. This suggests that these
409 biochemical features (metabolites and enzyme activities) may describe aspects of *Sphagnum* physiology
410 not captured by classical functional traits such as defense and water stress tolerance (*e.g.* antioxidants).
411 Moreover, when local and regional environmental effects were filtered (*i.e.*, residual correlations; Fig.
412 5C), we found that the clusters of traits involving biochemicals (*e.g.* defense and water stress tolerance
413 clusters) were disbanded, whilst the cluster with classical traits (capitulum size related traits) remained.
414 We interpret this result as an evidence that the degree of environmental stability determines the plant
415 performance by regulating the interplay between biochemical (metabolites and enzyme activities) and
416 phenotypical (morphological and anatomical) traits (Falster et al., 2011; Guo et al., 2017; Mazziotta et
417 al., 2018).

418 The plant metabolome is composed of thousands of biochemicals (or metabolites), which are
419 present in a plant at a given moment, and often referred to as the chemical phenotype of an organism
420 (Fiehn, 2002). Plant metabolites are indeed the ultimate expression agents of genetic changes and the
421 first responders to environmental changes (Handakumbura et al., 2019; Peñuelas and Sardans, 2009).
422 In our study, we found that local and regional environmental factors dispatched traits into four clusters
423 which are involved in *Sphagnum* growth, biomass, defense and water stress tolerance. Our SEM
424 analysis revealed that these different clusters of *Sphagnum* traits describe unique aspects of *Sphagnum*

425 form and function, especially those related to ‘water stress tolerance’ and ‘growth’ (Fig. 6). Our results
426 further showed that the functional trait space of *Sphagnum* mosses expanded when biochemicals were
427 taken into account. This suggests that *Sphagnum* biochemical traits capture key differences in foliar
428 chemistry and responses to environmental variability which are complementary to the variation
429 captured by anatomical and morphological traits. We further interpret this result as an evidence that
430 biochemicals offer an effective way to assess variation among *Sphagnum* phenotypes associated to
431 environmental tolerance (Bakhtiari et al., 2020; Callis-Duehl et al., 2017; Chiapusio et al., 2018;
432 Defossez et al., 2021).

433 We highlighted the importance of vascular plant functional types in driving *Sphagnum*
434 biochemical traits, as showed by the response of traits involved in defense (*i.e.* total tannins, total
435 phenols, water soluble phenols) and growth (*i.e.* carbohydrates, pigments and proteins) along with
436 herbaceous cover. This suggests that *Sphagnum* mosses modulate their whole biochemical machinery
437 in response to increasing competition with vascular plants, agreeing with previous findings on phenolic
438 metabolism (Chiapusio et al., 2018). Indeed, *Sphagnum* mosses can compete with vascular plants
439 through the release of specialized metabolites that inhibit vascular plant germination and radicle growth
440 (Chiapusio et al., 2013). In parallel, the increase in carbohydrates, proteins and photosynthetic pigments
441 suggest that *Sphagnum* produce structural and non-structural compounds to maintain its growth and
442 enhance its stress tolerance to cope with vascular plant competition (Liu et al., 2019). In short, we
443 suggest that vascular plant encroachment over *Sphagnum* causes alterations to the moss metabolites
444 production that directly impact its physiology. Our results further revealed higher phenols and tannins
445 concentrations in *Sphagnum* tissues along with increasing precipitation and nutrients (Fig. 5B). Such
446 increases could reflect a lesser nutrient limitation on *Sphagnum* growth in warmer and wetter sites
447 (Bragazza and Freeman, 2007; Dorrepaal et al., 2005). This further suggests that *Sphagnum* traits
448 evolved to have specific physiological and defense functions in the adaptation of *Sphagnum* mosses to
449 their growth environment, as showed for vascular plants (Yan et al., 2013). Thus, we could assume that
450 environmental filtering drives not only the taxonomic composition of *Sphagnum* mosses in peatlands
451 (Robroek et al., 2017), but also selects (or counter selects) on their traits.

452 Our results showed that *Sphagnum* with high water holding capacity exhibited the highest
453 antioxidant capacity, as evidenced by the high enzymatic activities and flavonoid content in their cells
454 (Nakabayashi et al., 2014). The ability of *Sphagnum* to transport water to the capitula and retain
455 cytoplasmic water is a key for its physiological activity such as photosynthesis (Bengtsson et al., 2020a;
456 Gong et al., 2019; Hájek, 2020; Jassey and Signarbieux, 2019; Robroek et al., 2009). As *Sphagnum*
457 mosses lack stomata and water-conducting tissues, the ability to maintain moist in their active apical
458 parts (capitula) rely on the water transporting efficiency from water table to the capitula and the
459 desiccation avoidance strategy (Clymo and Hayward, 1982). The desiccation avoidance strategy of
460 *Sphagnum* mosses, is mediated by large, dead and empty hyaline cells, which requires strong cell walls
461 to avert the collapse of large capillarity spaces (Hájek and Vicherová, 2014). Bordering chlorocystes
462 (living photosynthetic cells) contain chloroplasts that participate in photosynthesis and thus support
463 *Sphagnum* performance and functioning. We found a positive correlation between the width of
464 chlorocystes and antioxidant enzyme activities (Fig. 5A), suggesting that larger photosynthetic cell
465 requires more efficient activities of antioxidant enzymes. The production of antioxidant enzymes, as
466 protection of cell components from oxidative cell damages, is an important feature of desiccation
467 tolerance in plants, and particularly in drought-hardening (Cruz De Carvalho et al., 2012; Minibayeva
468 and Beckett, 2001; Proctor, 1990). Photosynthetic cells are under constant oxidative stress resulting
469 from the accumulation of reactive oxygen species (ROS) during various metabolic processes (Eltner,
470 1982). To fight against oxidative damages, cells have efficient antioxidative systems (e.g. activation of
471 certain enzymes) (Eltner, 1982). Synchronized action of major antioxidant enzymes (APX, CAT, SOD,
472 GR) and specialized metabolites such as flavonoids outcome in detoxification of ROS and limit
473 oxidative stress in plants (Choudhury et al., 2013; Das and Roychoudhury, 2014; Noctor et al., 2018).
474 Our findings demonstrate that the degree of desiccation tolerance of *Sphagnum* mosses is not only
475 limited to its morphological traits (Bengtsson et al., 2020a), but also rely on specific biochemical traits
476 to avoid cell-damages.

477 To sum up, our findings show that *Sphagnum* growing in different local environmental
478 (vegetation composition, nutrients content) and regional (temperature, precipitation) conditions possess

479 a suite of diverse biochemical traits that supports *Sphagnum* form and function as well as its resistance
480 to environmental changes. Besides, anatomical and morphological traits (*i.e.* width of chlorocyste), in
481 addition to local and regional environmental conditions, could explain some aspects of biochemical
482 traits distribution, and thus potentially revealed unreported linkages among different traits. Shifts in
483 *Sphagnum* metabolites, pigments, proteins and antioxidant enzyme activities indicate that *Sphagnum*
484 biochemical traits underpin *Sphagnum* niche differentiation through their role in specialization towards
485 biotic stressors (*e.g.* plant competitors) and abiotic stressors (*e.g.* temperature and water availability),
486 which both are important factors governing *Sphagnum* growth (Weston et al., 2015). We suggest that
487 these relationships represent previously unreported trade-offs of resource allocation by mosses,
488 whereby *Sphagnum* mosses devote resources to maintain their growth to cope with environmental
489 changes or resist to increasing competition. Even though our study reports new linkages among
490 anatomical, morphological and biochemical traits, which can be important for further studies on
491 peatland functioning, some limitations have to be acknowledged and considered for further
492 experiments. The confounding effect between dominant *Sphagnum* species and climate (sampled single
493 per site) and our observations were undertaken at a single date, did not allow us to compare the variation
494 of the responses of *Sphagnum* traits for each species when transferred to different condition (*e.g.* a
495 latitudinal gradient, seasonal variability). However, it didn't prevent us to assess traits interactions with
496 local and regional environmental conditions, as well as linkages among *Sphagnum* traits. Further studies
497 are needed to test the generality of the trade-offs detected here and their importance for peatland carbon
498 cycling. Nevertheless, this study provided evidence that measurements of the metabolites and
499 antioxidant enzyme activities, once properly incorporated with classical morphological and anatomical
500 traits expand our understanding of the coupling between physiology and fitness in *Sphagnum* trait-based
501 studies.

502

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Table 1. Characteristic of regional and local conditions of the five study sites. Mean annual temperature and annual precipitation data are averaged over the period 1960-2018 (WorldClim v2); water table depth and pore water pH were measured directly at the field conditions in July 2018; *Sphagnum* field water content and relative plant/moss cover are presented as mean±SE (n=5). Sites ordered along the south-north latitudinal gradient.

	France	Poland	Estonia	Finland	Sweden	
Site	FR	PL	EST	FI	SE	833
Coordinates	42°41'19.7"N	53°48'47.9"N	58°52'26.4"N	61°50'41.6"N	68°20'43.1"N	834
	2°14'02.4"E	16°35'12.1"E	26°15'03.6"E	24°17'17.5"E	19°03'58.7"E	
Altitude (m asl)	1374	145	82	160	281	835
Microtopography of sampling	lawns	lawns	lawns	lawns	lawns	836
Mean annual temperature (°C)	7.9	7.3	4.9	2.9	-0.1	
Annual precipitation (mm)	1027	656	623	611	418	837
Water table depth (cm)	-16.5	-60	-20	-8	-10	
Pore water pH	4.89	3.56	4.10	3.86	3.83	838
<i>Sphagnum</i> field water content (gH ₂ O/g DW)	11.4±0.6	4.1±1.1	9.8±1.7	10.6±0.7	13.96±1.8	839
Relative moss cover (%)	32.1±4.6	63.3±3.6	77.3±2.6	73.2±3.2	62±3.2	840
Relative vascular plant cover (%)	50.2±4.6	19.3±2.2	14.8±1.0	13.1±2.5	18.3±2.9	

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Table 2. The generalized volume of the hull representing the functional trait space of *Sphagnum* mosses based on three first axes of principal component analysis (PCA) for standardized values of all *Sphagnum* traits (biochemical, anatomical and morphological traits) and only *Sphagnum* traits without biochemical traits.

<i>Sphagnum</i> species	All <i>Sphagnum</i> traits	<i>Sphagnum</i> traits without biochemical traits
All species together	159.41	40.34
<i>S. warnstorffii</i> (FR)	2.24	0.11
<i>S. magellanicum</i> (PL)	1.36	0.43
<i>S. rubellum</i> (EST)	0.68	0.29
<i>S. papillosum</i> (FI)	2.56	1.34
<i>S. balticum</i> (SE)	0.23	0.038

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845 **Figure captions**

846 **Figure 1.** A summary of the extraction methods for quantification of *Sphagnum* pigments, metabolites,
847 antioxidant enzyme activities and proteins. Abbreviations are APX (EC 1.11.1.11) - ascorbate peroxidase
848 activity, CAT (EC 1.11.1.6) – catalase activity, POX (EC 1.11.1.7) – peroxidase activity, SOD activity (EC
849 1.15.1.1) - superoxide dismutase.

850 **Figure 2.** Barplot (n=5) of anatomical and morphological traits of dominant *Sphagnum* mosses in five sites.
851 Data represents means and standard errors (SE). Letters indicate significant differences among *Sphagnum*
852 species at $P < 0.05$ (linear mixed effect models). *Sphagnum* species are indicated by letters FR=France,
853 PL=Poland, EST=Estonia, FI=Finland, SE=Sweden.

854 **Figure 3.** Barplot (n=5) of *Sphagnum* proteins (A), enzymatic activities (B-E), metabolites and pigments
855 (F-M) of dominant *Sphagnum* mosses in five sites. Data represents means and standard errors (SE). Letters
856 indicate significant differences among the sites along a latitudinal gradient at $P < 0.05$ (linear mixed effect
857 models). *Sphagnum* species are indicated by letters FR=France, PL=Poland, EST=Estonia, FI=Finland,
858 SE=Sweden.

859 **Figure 4.** Principle coordinates analysis (PCoA) on the Gower dissimilarity matrix of (A) all *Sphagnum*
860 traits, (B) anatomical and morphological traits, (C) biochemical traits. Groups (hulls) are coloured
861 according to sites spanning from south to north.

862 **Figure 5.** Co-response clusters representing species similarity in *Sphagnum* traits-environment
863 relationships: A) shared responses of *Sphagnum* traits to environmental conditions, B) Bayesian coefficient
864 of each trait pooled into their respective clusters to every environmental parameter, C) residual correlations
865 among *Sphagnum* traits. Colors represent four distinct co-response clusters, while non-connected (purple)
866 points represent cluster-unrelated traits. Black lines indicate positive correlations between traits, while red
867 – negative (with $P < 0.05$). WEOM is site scores from the two first axes of PCA performed on WEOM
868 physicochemical parameters.

869 **Figure 6.** Structural equation model (SEM) combining the biochemical and anatomical/morphological
870 traits and environmental factors. SEM showing relationships between global (blue) and local (green)
871 environmental conditions and an unmeasured latent variable named “*Sphagnum* form and function”,
872 defined as the product of the four clusters of *Sphagnum* traits identified in Fig. 5A. Trait-clusters were
873 scores from the first two axes of PCAs performed on traits that belong to each cluster from Fig. 5A. WEOM
874 is site scores from the two first axes of PCA performed on WEOM physicochemical parameters. Thin solid
875 arrows show contributions of trait-clusters to the latent variable (latent variable r-squared also displayed),
876 thick solid arrows show effects of local and global parameters on the latent variable and grey arrows
877 illustrate non-significant path coefficients ($P > 0.05$). Numbers indicate the strength and direction of path
878 coefficients. The global fit of the model was very good: $\chi^2 = 8.95$, $df = 14$, $P = 0.83$; CFI = 1, RMSEA =
879 0, SRMR = 0.067, AIC = 409.2.

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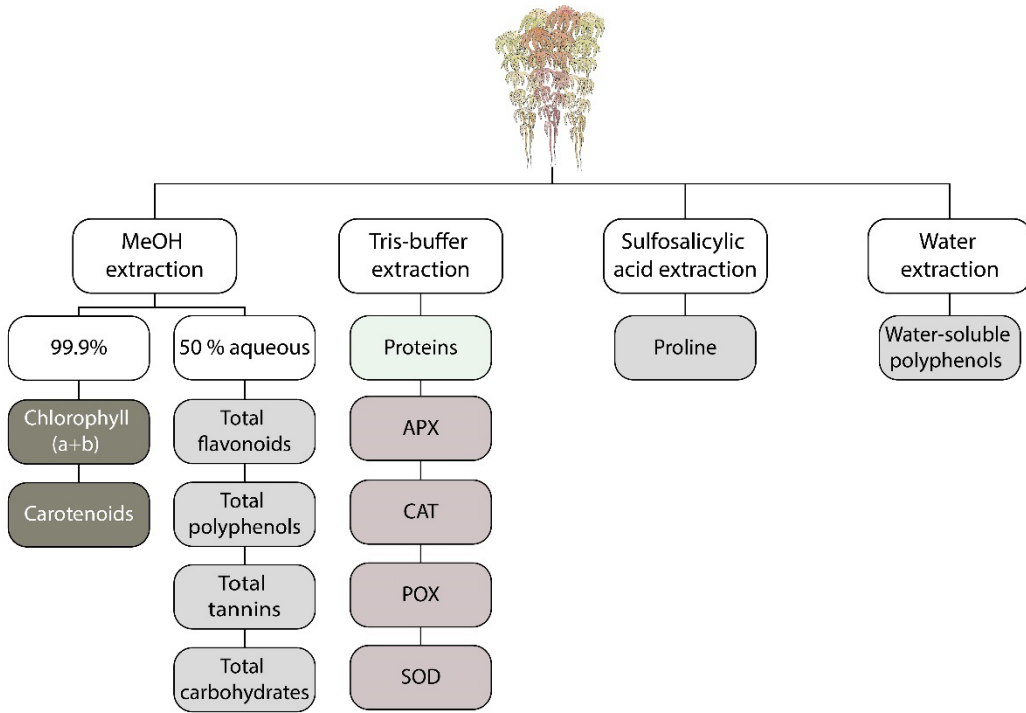
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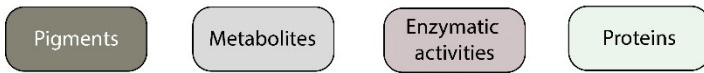
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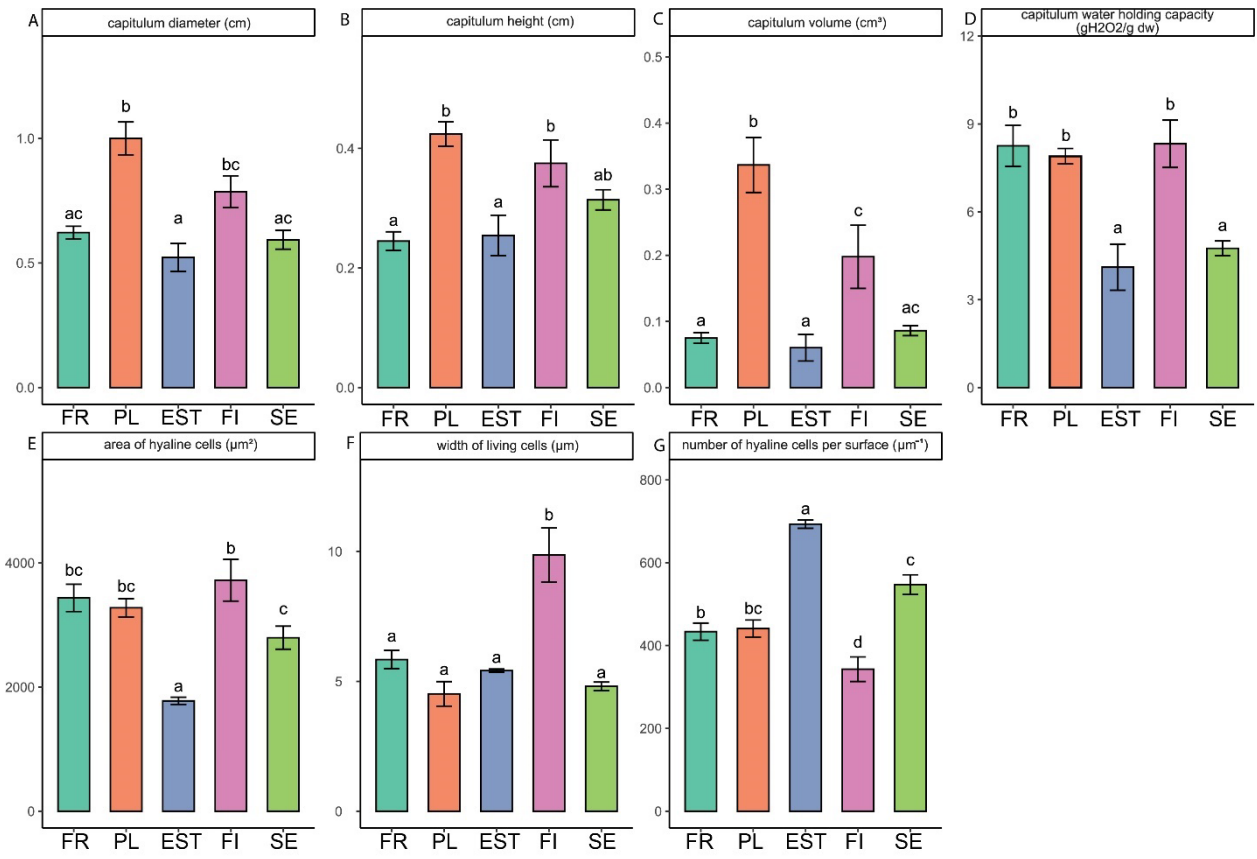
888 Figure 1



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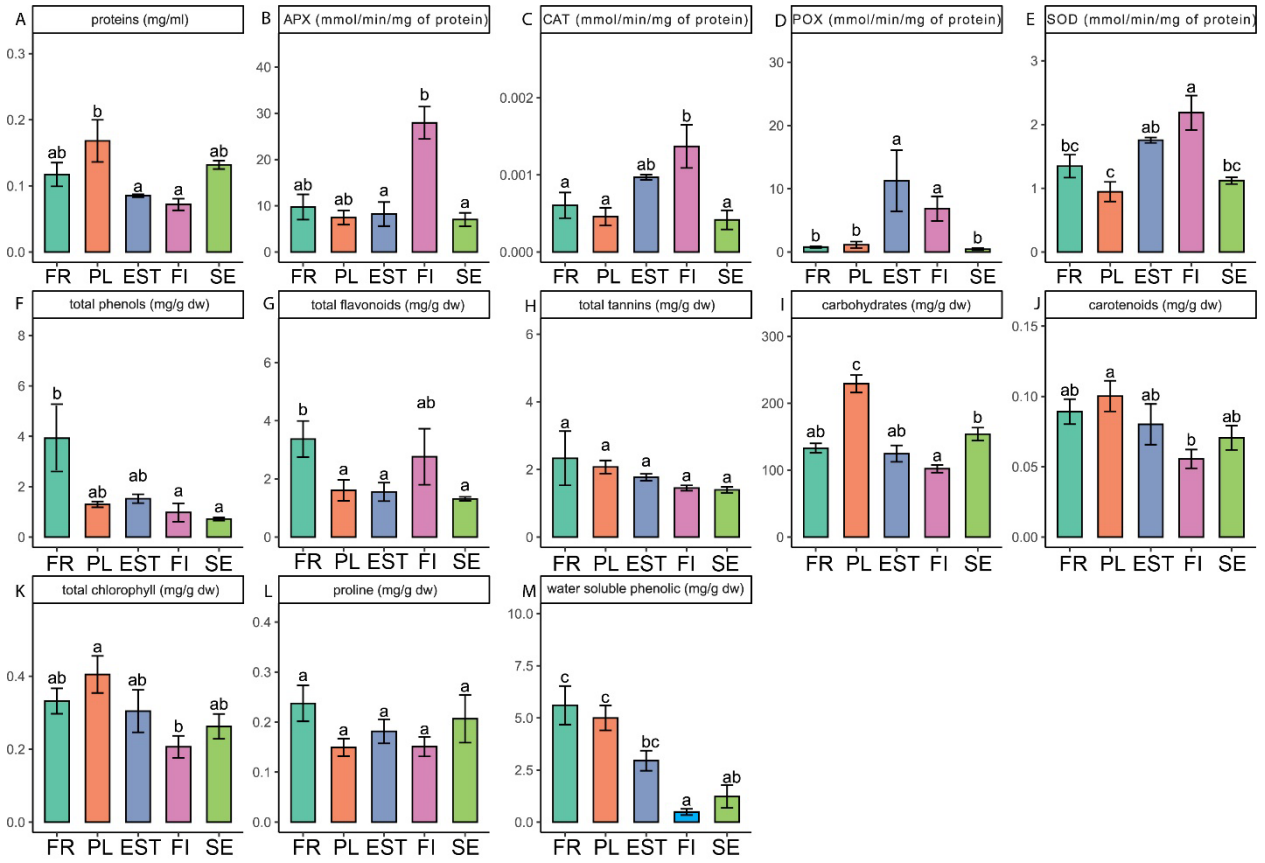
890 Figure 2



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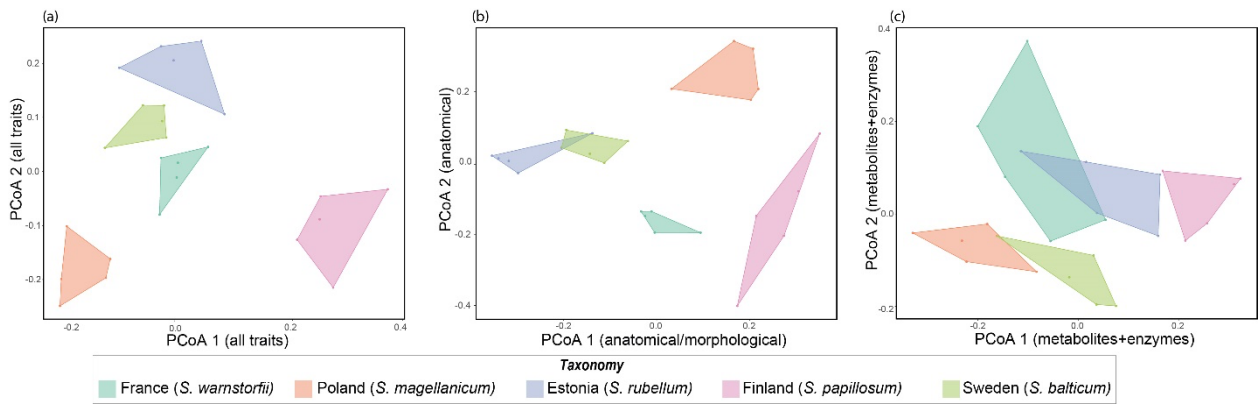
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893 Figure 3



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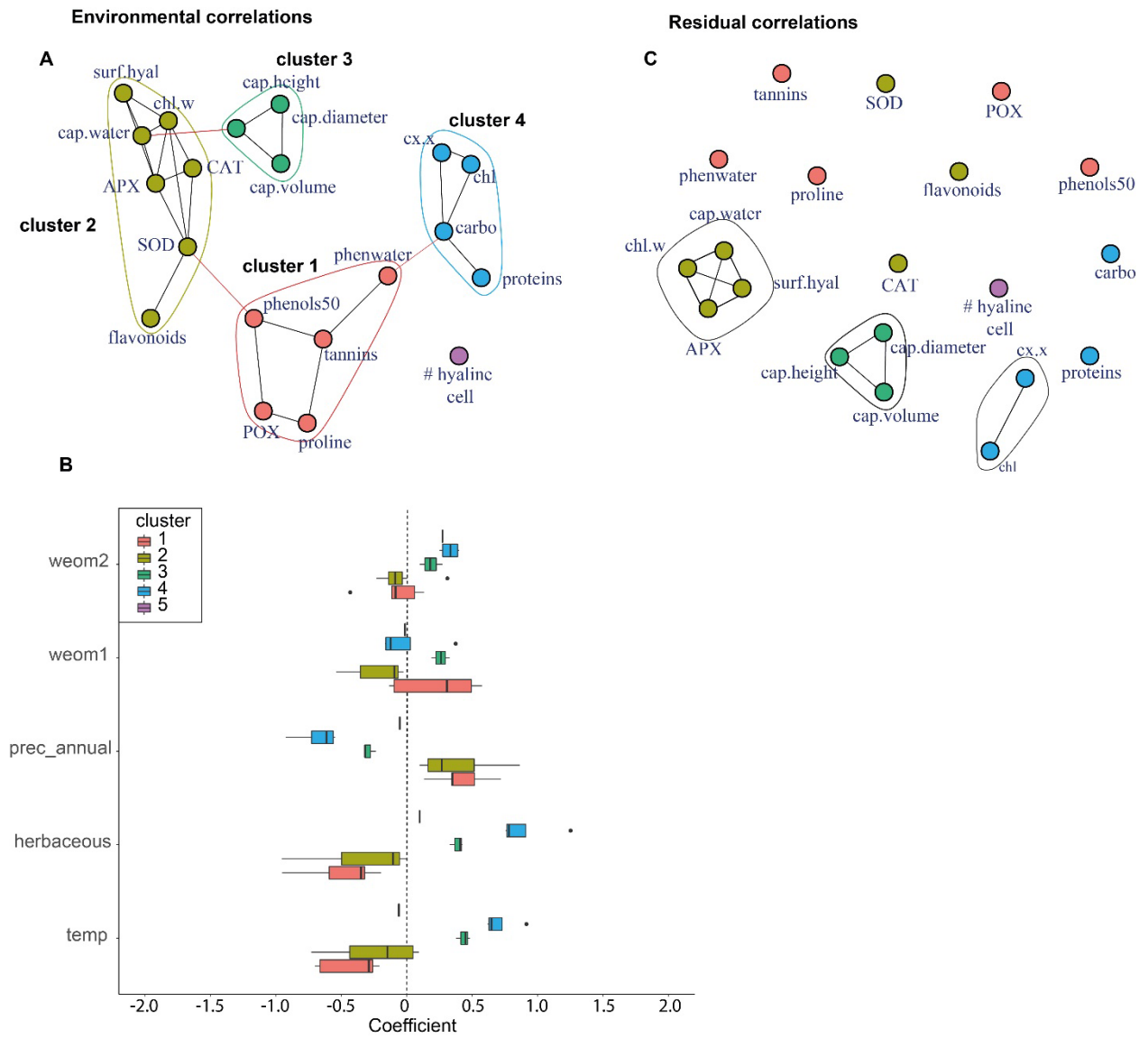
895 Figure 4



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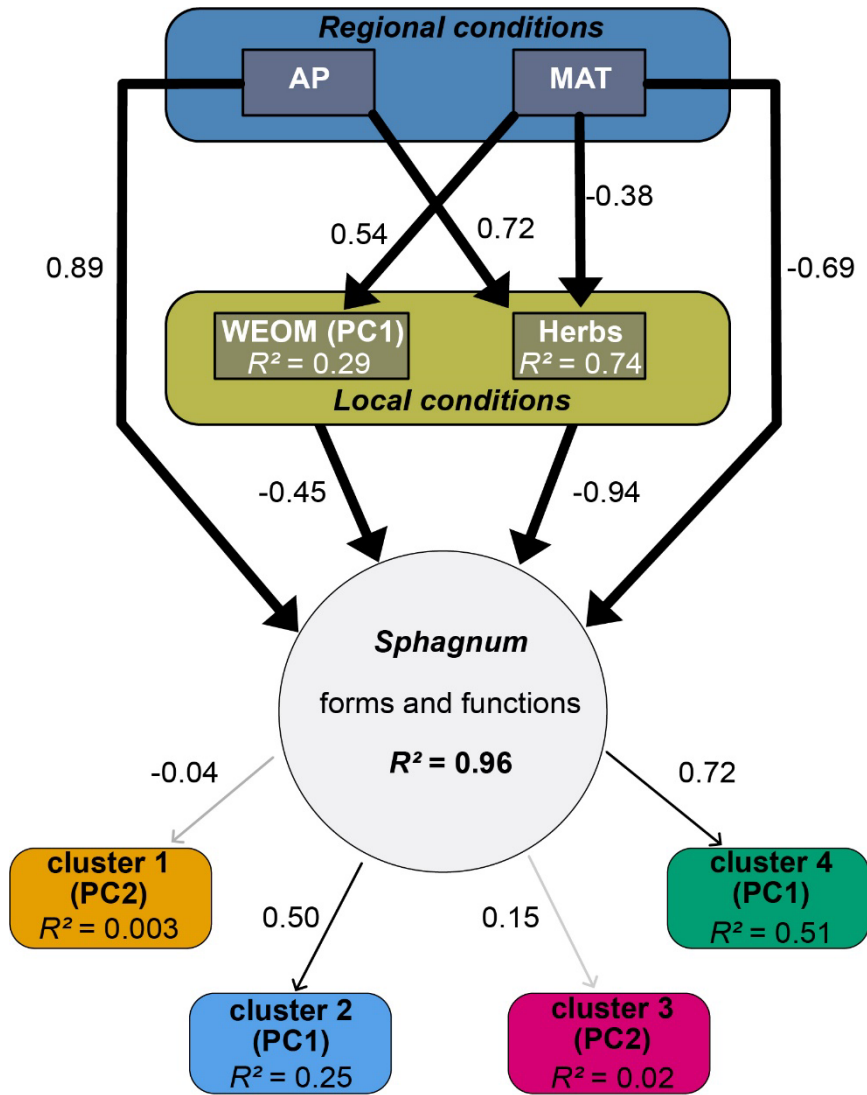
898 Figure 5



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909 Figure 6

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