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2 **Geographic variation in Sundew (*Drosera*) leaf colour: plant-plant interactions**
3 **counteract expected effects of abiotic factors.**

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16

17 **Abstract**

18 *Aim*

19 To identify geographical patterns in leaf colour of Roundleaved Sundew (*Drosera*
20 *rotundifolia*) growing on ombrotrophic (rain fed) bogs across Europe and establish
21 the controls over these patterns.

22 *Location*

23 North-west Europe.

24 *Taxon*

25 Angiosperms, *Drosera rotundifolia*

26 *Methods*

27 We measured leaf colour of *D. rotundifolia* plants growing on 24 ombrotrophic bogs
28 across north-west Europe covering 26.4 degrees of longitude, and 21.1 degrees of
29 latitude. We measured the height and cover of co-occurring vascular plant vegetation
30 and the amount of incident light intercepted by the vegetation canopy. We
31 determined the role of abiotic variables in controlling the patterns found. In a
32 separate experimental study, we manipulated plant-plant interactions with *D.*
33 *rotundifolia* by removing above-ground vascular plant vegetation and monitoring leaf
34 colour over a single summer.

35 *Results*

36 *Drosera rotundifolia* leaf colour varied between bogs. Leaves were redder in northern
37 latitudes and eastern longitudes, and in sites/plots with lower canopy influence, lower
38 nutrient deposition, and a more continental climate. Canopy influence was greater on
39 sites in southern latitudes, eastern longitudes, and with higher nutrient deposition,
40 longer growing seasons and a more maritime climate. Nutrient deposition was higher
41 at more southerly latitudes, eastern sites had a more continental climate, southern
42 and western sites had warmer and longer growing seasons. In the *in-situ* experiment
43 leaves became more red when canopy light transmission was increased by removing
44 vegetation, but not when shade net was subsequently added to reduce light
45 transmission.

46 *Main conclusion*

47 Geographic variation in *Drosera rotundifolia* leaf colour is strongly affected by its light
48 environment, mediated by plant-plant interactions, but leaf colour is also affected by
49 other abiotic factors. The relative importance of biotic and abiotic factors in
50 determining geographic patterns in traits, and also species responses to
51 environmental change, might depend on the growth form and competitive ability of a
52 species.

53 **Keywords:** anthocyanin, *Drosera*, environmental gradients, leaf colour, light,
54 nutrients, plant-plant interactions, sundew, trait variation

55

56

57 **Introduction**

58 A major goal in ecology is to understand how biotic and abiotic processes influence
59 species distributions, and how individuals respond to these factors. Plant species
60 with broad geographic distributions experience a wide range of biotic and abiotic
61 conditions and plant traits can vary in response. Understanding the patterns and
62 causes of this within-species geographic variability in traits can, therefore, provide
63 insight into the links between plant function, biotic and abiotic interactions (Gaston *et al.*,
64 2008). It can also help us to understand the impacts on ecosystems of
65 anthropogenic drivers such as altered precipitation patterns and increased
66 temperature (Millien *et al.*, 2006; Zeuss *et al.*, 2014), and deposition of atmospheric
67 pollutants such as nitrogen and sulphur (Ellison & Gotelli, 2002; Millett *et al.*, 2015).
68 For example, phenotypic variability along latitudinal gradients reflects the
69 combination of adaptive and plastic effects, which can inform understanding of the
70 effects of warming on plant distributions (De Frenne *et al.*, 2013); latitudinal
71 gradients in nitrogen deposition have also been used to better understand species
72 responses to atmospheric nitrogen deposition (e.g., Millett *et al.*, 2012)

73 Biotic interactions vary geographically—it is predicted that the intensity of biotic
74 interactions will be greater closer to the equator (Schemske *et al.*, 2009)—and play
75 an important role in maintaining biodiversity (Brooker, 2006). Considerable
76 uncertainty remains, however, about the existence and shape of these geographical
77 patterns (Moles *et al.*, 2011; Ollerton *et al.*, 2011; Poore *et al.*, 2012; Dalrymple *et al.*,
78 2015), and many different patterns might actually exist. Geographic patterns of
79 plant-plant interactions might be particularly important because of their key role in
80 structuring plant communities. These interactions are predicted by the stress-
81 gradient hypothesis (SGH; Connell, 1983) to be more intense in more productive
82 environments. This has important implications for plant community responses to
83 global environmental change because many of the key drivers, such as
84 eutrophication and warming, will increase productivity in many systems (Brooker,
85 2006). Experimental evidence from altitudinal and latitudinal gradients supports the
86 SGH (Callaway *et al.*, 2002; Kikvidze *et al.*, 2011). These studies, however, are
87 limited in number and are manipulative so might suffer from artefacts of the
88 experimental approach. Another approach would be to investigate variability in plant
89 traits relating to plant-plant interactions along these productivity gradients, but this
90 relies on the identification of appropriate traits.

91 Carnivorous plants trap and digest animal prey, assimilating the nutrients into their
92 tissues. *Drosera rotundifolia* L. is a small carnivorous herb, which occupies
93 ombrotrophic (rain fed) bogs and catches prey with sticky mucilage secreted on the
94 end of leaf trichomes (tentacles). Like many carnivorous plants the leaves of *D.*
95 *rotundifolia* have a distinctive red colouration, due to the presence of anthocyanins
96 (Egan & der Kooy 2013). This red colour was thought to serve a prey attraction
97 function (Lloyd, 1942; Ichiishi *et al.*, 1999), but experimental studies have shown this
98 not to be the case (Foot *et al.*, 2014). Alternative hypotheses for the functional role of

99 leaf reddening are as a protection against excess light and UV-B radiation, herbivory
100 defence or osmotic adjustment. Aboveground interactions with other plants might be
101 crucial to the ecology of carnivorous plants (Ellison *et al.*, 2003) because the costs of
102 carnivory mean that a net benefit should only occur in high-light environments
103 (Givnish *et al.*, 1984). Aboveground interactions with shrubs might be particularly
104 important for the ecology of *D. rotundifolia* because of its rosette form, with leaves
105 close to the bog surface, meaning it is usually subordinate in the plant canopy.

106 We used both a correlative and experimental approach to explore in ombrotrophic
107 (rain fed) bogs the relationship between latitude, vegetation structure and leaf colour
108 of the carnivorous plant *Drosera rotundifolia*. The aim of the correlative study was to
109 identify geographic patterns in *D. rotundifolia* leaf colour, vascular plant cover and
110 canopy characteristics; the aim of the experimental study was to determine whether
111 the red leaf pigmentation of *D. rotundifolia* leaves is light sensitive and influenced by
112 aboveground plant-plant interactions. We hypothesised that 1. the red colour of *D.*
113 *rotundifolia* leaves is light responsive; 2. the intensity of above-ground interactions
114 between *D. rotundifolia* and other vegetation decreases towards the poles, due to
115 decreased productivity; and 3. these changing interactions are indicated by a change
116 in *D. rotundifolia* leaf colour, with leaves becoming redder due to increased light
117 transmission through the vegetation canopy. An alternative hypothesis is that leaves
118 will become less red towards the poles because, in the absence of plant-plant
119 interactions, a strong latitudinal gradient of solar radiation (due to differences in the
120 solar angle) reduces the requirement for photoprotective adaptation at higher
121 latitudes. This alternative hypothesis has been demonstrated for photoprotective
122 metabolites in other plant species (Willis *et al.*, 2011; Koski & Ashman, 2015).

123 **Materials and Methods**

124 *Correlative study*

125 For the correlative study 25 ombrotrophic bogs were chosen across western and
126 northern Europe covering 26.4 degrees of longitude (-7.638 – 18.810 °E), and 21.1
127 degrees of latitude (47.239 – 68.356 °N) (site details are in Appendix S1 in
128 Supporting Information, locations are in Fig. 1). To reduce variability due to habitat
129 differences we only measured plants growing on *Sphagnum* spp. hummocks. At
130 each site, hummocks (c. 4 m²) were selected on the intact ombrotrophic parts of the
131 bog. The number of hummocks varied depending on the size of the bog and
132 available time for sampling (median = 6, range 4 – 10). To reduce the possibility of
133 seasonal differences, sampling took place in a four-week period from late July –
134 early August; because of the work involved, we sampled bogs over three years
135 (2011 – 2013) to keep sampling within this time window.

136 On each hummock we recorded the percent cover and height of vascular plants.
137 Cover was based on the average of independent estimates of at least two people;
138 height was based on the average of ten randomly located measurements. To

139 determine the impact of the vegetation canopy on the light environment at the bog
140 surface we measured photosynthetically active radiation (PAR) above the plant
141 canopy and on the bog surface at five points using a PAR quantum sensor (SKP
142 215, Skye Instruments, Powys, UK). PAR was always measured between 10:00 and
143 15:00. One fully formed leaf was removed from each of ten *D. rotundifolia* plants per
144 plot and kept chilled at below 10°C but above 0°C before colour analysis. As soon as
145 possible, and within 4 hours, we measured the spectral reflectance of the leaves
146 within the wavelength range 400-800 nm using a VIS-NIR spectrometer (USB4000,
147 Ocean Optics Inc.). The probe was held 15 mm from the leaf giving a measurement
148 area of approximately 35 mm². *Drosera rotundifolia* leaf spectrometry was carried
149 out for all sites where *D. rotundifolia* was present ($n = 24$) but vegetation
150 characteristics only for a sub-set of sites ($n = 18$ for vegetation height, cover and light
151 interception).

152 Canopy light transmission was calculated as PAR at the bog surface as a proportion
153 of above-canopy PAR. This removed the impact of differences in incident light due to
154 meteorological conditions or site differences. Canopy photographs were analysed
155 using the software integrated into the CI-110 system. Sky and vegetation were
156 differentiated in the photographs using a threshold determined manually, based on a
157 visual assessment. From this the canopy transmission coefficient was calculated,
158 this is the proportion of the photograph that is sky (rather than vegetation). We
159 carried out the initial data analyses with and without these canopy photograph data.
160 The results of both approaches were qualitatively identical; because of this, and
161 because these canopy photography data were only available for a smaller sub-set of
162 sites, we excluded these data from the analyses. At all sites, no shading of plots was
163 present due to rocks or trees. Leaf reflectance data were used to calculate leaf
164 colour using SPECTRASUITE 2.0 (Ocean Optics 2010). We determined the colour of
165 the leaves using multiple colour measures: a^* (a measure of red – green colour) in
166 CIE 1976 ($L^*a^*b^*$) colour space (CIELAB), ‘dominant wavelength’ (the dominant
167 wavelength of the colour in CIE 1976 colourspace, removing differences in saturation
168 or intensity), mean red (600-699 nm), green (500-599 nm) reflectance and the ratio
169 of red:green reflectance. The choice of measures is non-exhaustive and was driven
170 by a wish to define the redness or greenness of the leaves, balancing the number of
171 measures used against simplicity. Further details are in appendix S2 in Supporting
172 Information.

173 *Experimental study*

174 The experimental study was carried out in 2012 at Thorne Moor, part of the
175 Humberhead Peatlands National Nature Reserve, in the UK (Lat: 53.6385°, Long: -
176 0.9009°). We carried out this experiment within a single trench in the peat, c. 20 x
177 100 m. The trench was originally formed by hand cutting of the peat in the 19th
178 century and has since revegetated with *Sphagnum* spp. (with a developing hummock
179 hollow topography), small shrubs and *D. rotundifolia*, such that the surface of the
180 *Sphagnum* is now level with the surface of surrounding uncut baulks. We chose five

181 plots, each on a different *Sphagnum* hummock. At each hummock, we established
182 three 0.5 x 0.5 m sub-plots. Each sub-plot was randomly allocated to one of three
183 treatments: 1. 'natural' – vegetation left intact; 2. 'removed' – all aboveground
184 vegetation, except *D. rotundifolia*, removed; and 3. 'removed+shaded' – all
185 aboveground vegetation, except *D. rotundifolia*, removed and a 0.5 x 0.5 m square
186 wooden frame covered in neutral shade cloth (to give a reduction in light intensity of
187 approximately 50%) placed over the plot at a height of approximately 20 cm. The
188 *removed+shaded* treatment was included to determine the extent to which changes
189 due to vegetation removal (*removed*) could be attributed to impacts of vegetation on
190 the aboveground light environment.

191 We applied the treatments on 6th June 2012. The light environment on the
192 *Sphagnum* surface was measured by measuring PAR above the plant canopy/shade
193 frame (or at a height of c. 30 cm for *removed* plots) and at the *Sphagnum* surface.
194 Canopy light transmission was calculated as for the correlative study. On 15th
195 August one fully formed leaf was removed from each of ten *D. rotundifolia* plants in
196 each sub-plot. Spectral reflectance of each leaf was determined using the approach
197 detailed for the correlative study.

198 *Environmental variables data sets*

199 We compiled a set of abiotic variables for each site, chosen to reflect the potential to
200 impact on vegetation or leaf colour characteristics. These variables covered
201 meteorological, nutrient deposition and UV radiation data. We used UV-B dose as an
202 indicator of likely radiative dose because in plants UV is responsible for
203 approximately one third of photodamage (Takahashi & Badger, 2011). UV-B
204 exposure data for each plot were obtained from gIUV (Beckmann *et al.*, 2014). This
205 dataset provides satellite-derived estimates of global surface UV-B dose at a 15 arc-
206 minute resolution. We used the UVB5 layer, which provides the sum of monthly
207 mean (2004-2013) UV-B during the highest quarter. Annual atmospheric N (total N,
208 NO₃, NH₄ and the ratio of NO₃:NH₄) and total S deposition was modelled using a
209 higher resolution national model for the UK (Smith *et al.*, 2000; NEG-TAP, 2001) and
210 for non-UK sites using the lower resolution European Monitoring and Evaluation
211 Program (EMEP)-based Integrated Deposition Model (IDEM) (Pieterse *et al.*, 2007).
212 Meteorological data comprised the following variables: mean annual (July – June)
213 temperature, minimum January and maximum July temperature, temperature
214 variability (CV for temperature in Fahrenheit), growing degree days (base 5), and
215 mean annual precipitation for the previous five years for each site (with a 0.25
216 degree latitude-longitude resolution). The variables were calculated using an
217 interpolated data set from E-OBS (Haylock *et al.*, 2008) accessed through the KNMI
218 climate explorer (KNMI, 2013). For these variables, the mean values for the five-
219 years prior to plot measurements were used to represent the likely time-scale of
220 impacts on *D. rotundifolia*, which has an average life span of six years, while
221 balancing the likely larger influence of more recent abiotic conditions.

222 *Data analysis*

223 Data were analysed in IBM STATISTICS 22.0 (IBM Corp., 2013). Some data reduction
224 was carried out before analysis. Measurements within each plot were averaged to
225 give a mean per plot. These data were used to compare the relationship between
226 canopy characteristics and leaf colour. For all other analyses comparisons were
227 required at the site level ($n = 14 - 26$ sites), so were based on means at each site
228 (i.e. means of plot means, $n = 10$ plants per plot and $5 - 10$ plots per site). There
229 were correlations among abiotic variables, so we performed principal components
230 analysis (PCA) with Varimax rotation on these data. We also used PCA to reduce
231 the five leaf colour measurements to a single index of colour and to reduce the
232 canopy characteristics measures to a single index of canopy influence. These last
233 two PCAs were carried out separately for the site-level and plot-level data analysis.

234 To determine the relationships between variables we used either simple linear
235 regression (for single independent variables) or, where there were multiple
236 independent variables, stepwise ordinary least squares (OLS) regression with
237 forward and backward inclusion, an inclusion cut-off at $P = 0.05$ and an exclusion
238 cut-off at $P = 0.1$. We first determined any systematic geographic variability in the
239 data by using the PCA axes for the environmental, canopy and colour characteristics
240 as dependent variables and latitude and longitude as independent variables (sites
241 west of 0° were coded as negative). We also separately analysed geographical
242 variability in vegetation height, because plant height has previously been shown to
243 vary systematically with latitude (Moles *et al.*, 2009). To do this we used the 'curve
244 estimation' module in IBM Statistics. Appropriateness of the fitted model was
245 assessed by inspecting plots of residuals and the model with the highest r^2 value
246 was chosen.

247 Next, we used the three environmental PCA axes as independent variables and the
248 indices (i.e., PCA axis score) of vegetation influence and *D. rotundifolia* colour
249 characteristics as dependent variables to determine the relationship between the
250 abiotic environment and biotic variables. We also analysed the relationship between
251 UV-B and leaf colour separately because UV-B has been previously implicated in
252 differences in plant colour along latitudinal gradients (Koski & Ashman, 2015).

253 To determine the relationship between canopy characteristics and leaf colour, we
254 used a linear mixed model on plot-level data. We used a two-level model to account
255 for relationships between and within sites. We used plot as a random effect, nested
256 within site, and canopy influence as a fixed effect. We compared four models: 1. null
257 single-level model, 2. null multi-level model, 3. random intercepts model and 4.
258 random slopes and intercepts model. Differences in the appropriateness of each
259 model was tested with a 'log likelihood ratio test', comparing with the previous, less
260 complex model.

261 For the experimental study the impact of the three was tested by comparing
262 differences in the ground-level light environment after treatments were established
263 and the colour (CIE Lab a*) of *D. rotundifolia* leaves at the end of the experiment.
264 We did this tested using a randomised block design ANOVA with hummocks as
265 blocks and subplots as treatments within blocks. Differences between the three
266 treatments were tested using *post-hoc* comparisons (Fisher's LSD).

267 **Results**

268 *PCA results*

269 For the bioclimatic variables, the first three PCA axes explained total of 85.5% of
270 variation (29.5, 29.3 and 26.7% for axes 1, 2 & 3 respectively), and were successful
271 in differentiating between the different groups of environmental variables (Table 1).
272 Axis 1 reflected nutrient deposition differences among the sites, with high scores on
273 this axis representing sites with a high N and S deposition load (higher total N, total
274 S, NO₃ and NH₄ deposition). Axis 2 reflected differences among sites in summer
275 meteorological conditions, sites with high scores on this axis had warmer summers
276 and a longer growing season (higher mean summer temperature, high maximum
277 July temperature and high GDD). Axis 3 reflected the continentality of the climate,
278 sites with high scores on this axis had a more maritime, less continental climate
279 (high January minimum temperature, mean annual temperature, and precipitation,
280 and low monthly temperature variance).

281 We also used PCA to reduce multiple indices of leaf colour and multiple measures of
282 canopy influence to single principle components. For canopy influence and leaf
283 colour, only a single PCA axis was extracted. For canopy influence the first PCA axis
284 explained 79.8% of between-site variability (Table 2). Sites with high scores on this
285 axis had high vegetation cover, tall vegetation and high interception of incident PAR.
286 The PCA axis for leaf colour explained 85% of the variability and differentiated on the
287 basis of how red or green the leaves were (Table 2). Sites in which plants had redder
288 leaves had high values on this axis, which indicated high CIE LAB a*, high values for
289 the dominant wavelength reflected, red reflectance and the ratio of red:green
290 reflectance, and low values for green reflectance.

291 *Patterns*

292 There are some clear geographic patterns in the three data components (leaf colour,
293 vegetation influence, abiotic variables), and some clear patterns of variation between
294 these different components (full analysis results are in Appendix S3 in Supporting
295 Information). The multiple regression analysis showed that geographic variability in
296 *D. rotundifolia* leaf colour was best explained by a linear regression model which
297 included both latitude and longitude. This explained 65.7% of between-site variability
298 in leaf colour (Table 3). Canopy influence and vegetation height varied with only
299 latitude, which explained 81.6% and 84.7% of variability respectively. Abiotic PCA
300 axis 1 (nutrient deposition) varied with only latitude; abiotic axis 2 (summer growing

301 conditions) varied with both latitude and longitude; abiotic axis 3 (continentality)
302 varied with only longitude. (See appendix S3.)

303 When considering only abiotic variables, variability in canopy influence and
304 vegetation height was best explained by a model which included abiotic PCA axes 3,
305 1 and 2 (continentality, nutrient deposition and summer climate respectively). This
306 model explained 86.5% of the variability in canopy influence and 89.5% of the
307 variability in vegetation height (Table 3). However, a model including only PCA axis 1
308 and 3 best explained variability in leaf colour, explaining 72.1% of variability in leaf
309 colour. There was no significant relationship between summer temperature/growing
310 degree days and leaf colour. (See appendix S3.)

311 Canopy influence (vegetation height, cover and light interception) was higher in more
312 northerly and easterly sites (Fig. 2 a & b), though variation with longitude was not
313 statistically significant. *Drosera rotundifolia* leaves were redder in more northerly and
314 easterly sites (Fig. 2c & d). More easterly sites experienced a more continental
315 climate (lower precipitation, lower winter temperature and higher climate variability
316 (Fig. 3a) and higher summer temperatures/longer growing seasons (Fig. 3b). More
317 northerly sites received lower levels of nutrient deposition (Fig. 3c) and lower
318 summer temperatures/longer growing seasons (Fig. 3d).

319 Canopy influence was greater on sites with a more maritime climate than on sites
320 with a more continental climate (Fig. 4a). *Drosera rotundifolia* on sites with a more
321 continental climate had redder leaves than those with a more maritime climate (Fig.
322 4b). Sites with higher nutrient deposition had higher canopy influence than those with
323 lower nutrient deposition (Fig. 4c). *Drosera rotundifolia* in these higher nutrient
324 deposition sites had greener leaves than those in the lower nutrient deposition sites
325 (Fig. 4d). Sites with high summer temperatures and longer growing seasons had
326 greater canopy influence and taller vegetation (Fig. 4e).

327 For the relationship between canopy influence and leaf colour, the model which
328 included both random intercept and slope was the highest ranked based on the -2log
329 likelihood score (comparison is shown in appendix S4 in Supporting Information).
330 This indicates differences between sites in both the relationship between canopy
331 influence and leaf colour, and in the expected leaf-colour at average canopy
332 influence (Fig. 5, appendix S5 in Supporting Information). For this model, overall
333 there was a statistically significant relationship between canopy influence and leaf
334 colour (LMM: d.f.=19.272, $t=-3.778$, $P=0.001$); leaves were less red when canopy
335 influence was higher. The extent of this relationship varied between sites. A strong
336 negative correlation existed between the intercept and slope of the fitted lines
337 (Pearson's Correlation Coefficient: -0.85, $P<0.001$); this indicates that, for sites with
338 a lower intercept (i.e. indicating greener leaves when canopy influence was at the
339 mean of all plots), the slope of the relationship between canopy influence and leaf
340 colour was less steep (i.e. leaf colour varied less with canopy influence).

341 We used UV-B radiation dose as an indicator of the potential for incident radiation to
342 influence leaf colour. The above-canopy UV-B dose received by the sites was
343 strongly dependent on latitude, decreasing towards the poles (linear regression: $F_{1,23} = 46.18$, $r^2 = 0.67$, $P < 0.001$), but the leaves of *D. rotundifolia* growing at sites
344 which received a higher UV-B dose had greener leaves than those at sites with a
345 lower UV-B dose (Linear regression: $F_{1,23} = 10.364$, $P = 0.004$, $r^2 = 0.311$). These
346 were also the sites with higher canopy influence scores (Pearson correlation
347 coefficient for UV-B vs canopy influence = 0.613, $P = 0.005$).
348

349 In our *in-situ* experimental study *D. rotundifolia* leaf colour changed as a result of
350 interactions with co-occurring plants, due to impacts on the light environment. We
351 doubled light transmission through the canopy (Fig. 6, ANOVA: $F_{2,8} = 48.94$, P
352 < 0.001) by removing all aboveground vegetation. After ten weeks, the leaves of *D.*
353 *rotundifolia* plants in these vegetation removal plots were redder than the leaves of
354 plants in control plots (Fig. 6, ANOVA: $F_{2,8} = 11.93$, $P = 0.004$). We also controlled
355 for possible impacts of plant removal independent of their shading effect by adding
356 shade cloth to vegetation removal plots – intercepting approximately the same
357 amount of light as the plant canopy. In these shaded plots, the change in *D.*
358 *rotundifolia* leaf colour was not present (Fig. 6).

359 Discussion

360 We demonstrate systematic geographic variability in plant leaf colour for the first
361 time. *Drosera rotundifolia* leaves were redder in more northerly and easterly sites.
362 Our results provide evidence that this pattern may be controlled to some extent by
363 geographic variability in plant-plant interactions, which are themselves influenced by
364 variation in climate and nutrient deposition. While geographic variation in animal
365 pigmentation has been well-studied (Gloger, 1883; Jablonski & Chaplin, 2000; Lai *et*
366 *al.*, 2008; Alho *et al.*, 2010; Tuomaala *et al.*, 2012; Zeuss *et al.*, 2014), geographic
367 variability in plant colour is poorly understood. Plants are, however, subject to
368 different evolutionary and environmental constraints to animals (Bradshaw, 1972;
369 Huey *et al.*, 2002). For example, the sessile nature of plants might result in more-
370 pronounced stress resistance mechanisms (Huey *et al.*, 2002) such as pigmentation.
371 Plants might also be influenced by biotic interactions to a greater extent. Moles *et al.*
372 (2011) showed that plant height across ecosystems decreased as a function of
373 latitude. We found the same pattern within a single ecosystem, ombrotrophic bogs.
374 We also found that this, in association with an associated decrease in vegetation
375 cover, reduced canopy light interception altering the light environment of *D.*
376 *rotundifolia* plants.

377 The results of our experimental study show that *D. rotundifolia* leaf colour can be
378 directly influenced by vegetation impacts on the light environment. Leaf colour was
379 light sensitive, changing from green to red in response to short-term increases in
380 light intensity due to a reduction in aboveground plant-plant interactions. Increased
381 leaf redness of *D. rotundifolia* indicates higher concentrations of anthocyanins (Egan

382 & der Kooy 2013)—though this was not measured directly. These experimental
383 results suggest that variability in the influence of aboveground vegetation is a
384 plausible explanation for the geographic pattern of leaf colour. This is supported by
385 the clear and strong relationship between canopy measurements and *D. rotundifolia*
386 leaf colour in our correlative study. We suggest that these changes are at least
387 partially responsible for the observed leaf colour changes, and as far as we are
388 aware, this is the first direct, non-manipulative evidence of such a pattern.

389 The abiotic conditions for plant growth can vary significantly geographically. Within a
390 species, adaptive and plastic responses of plants to these different and variable
391 conditions result in geographic variability in traits. This phenotypic variability can
392 itself have important impacts on ecological dynamics (Bolnick *et al.* 2011). Our data
393 suggest that biotic interactions might also play a significant role in geographical
394 patterns of plant traits, and can be more important than changes in abiotic
395 conditions—reversing their impact even. Biotic interactions are thought to increase in
396 importance towards the equator (Schemske *et al.*, 2009), and are considered to be
397 one of the key drivers of latitudinal biodiversity gradients (Mittelbach *et al.*, 2007).
398 There is, however, little evidence of broad geographical patterns in plant-plant
399 interactions. Our results suggest that photosensitive plant secondary metabolites
400 might provide a novel way to test for variability in aboveground plant-plant
401 interactions.

402 Disentangling the correlative patterns in this study was challenging. Leaf-colour,
403 canopy influence and abiotic variables all covaried. Leaf-colour varied with latitude
404 and longitude; canopy influence only varied with latitude. Canopy influence varied in
405 response to all three abiotic PCA axes; leaf colour did not respond to PCA axis 2,
406 which related to growing season temperature. These results might indicate that
407 canopy influence is not the only factor controlling geographic variation in leaf colour
408 in our dataset. For example, the longitudinal gradient in leaf colour might be due to
409 the corresponding impact of increasing continentality of the climate. Leaves were
410 redder in sites which experienced larger extremes of temperature. Low temperatures
411 have been previously suggested to influence anthocyanin synthesis (Close & Beadle
412 2003), and this could be the case here. The between-site differences in the
413 relationship between canopy influence and leaf-colour support this. It is clear that
414 further, experimental, studies are required to determine causation.

415 The adaptive function of *D. rotundifolia* leaf reddening was not tested, but has been
416 implicated in photoprotective and stress response roles in other species (Mendez *et al.*
417 1999; Close & Beadle 2003; Boldt *et al.* 2014). While anthocyanin synthesis in
418 response to light intensity does not directly demonstrate a photoprotective function
419 *per se*, we consider it a plausible explanation. Were this the case, the latitudinal
420 gradient in leaf colour is opposite to expected patterns—photoprotective pigments
421 are predicted to increase towards the equator and higher altitudes, due to the
422 increased intensity of solar radiation. This has been shown to be the case—UV
423 absorbing pigments in *Argentina anserina* flowers (Koski & Ashman, 2015) and

424 concentrations of the UV-B absorbing compound *para*-coumaric acid in *Pinus*
425 *sylvestris* (Willis *et al.*, 2011), for example, increase towards the equator.
426 Conversely, Åkerström *et al.*, (2010), found that anthocyanin concentrations in the
427 fruit of Bilberry (*Vaccinium myrtillus*) increased towards the pole in Europe. No clear
428 mechanism for this unexpected pattern has yet been demonstrated, though
429 decreased temperature at very high latitudes has been suggested as a possible
430 mechanism. We also found that red colouration (and likely anthocyanin synthesis)
431 increased towards the pole. The difference between these two groups of species is
432 canopy position. *Argentina anserina* grows in relatively open habitats, *P. sylvestris*
433 tends to be dominant or co-dominant in forest canopies; the *V. myrtillus* studied by
434 Åkerström *et al.*, (2010) were growing under a forest canopy, and the *D. rotundifolia*
435 in our study were growing under a shrub canopy. These observations and our study
436 present a potential mechanism for this difference—plant-plant interactions. In plants,
437 UV is responsible for approximately one third of photodamage (Takahashi & Badger,
438 2011), so incident UV dose would be expected to be positively correlated with
439 photoprotective metabolites (Willis *et al.*, 2011; Koski & Ashman, 2015). We found
440 the opposite. We therefore predict that, for plants that are subordinate in the
441 vegetation canopy, geographic variability in plant-plant interactions, especially
442 competition for light, are a more important determinant of the synthesis of
443 photoprotective pigments than variability in above-canopy radiation dose. This is
444 intuitive, but has not been previously suggested.

445 An alternative and intriguing hypothesis is that leaf redness in *Drosera rotundifolia* is
446 an anti-herbivory adaptation—either providing crypsis, or as an aposematic warning.
447 There is some evidence for this in other plant species (Manetas 2006). For *D.*
448 *rotundifolia* in sites with more vegetation cover, the need to deter herbivores might
449 be reduced because the plants will be ‘hidden’ by the vegetation canopy. In more
450 open areas the plants might be more apparent and so more likely to be seen by
451 herbivores. Foot *et al.* (2012) found that, in a study of prey capture by *D. rotundifolia*,
452 fewer insects were attracted to red artificial leaves than to green artificial leaves. This
453 might indicate that insects are deterred by red colouration. This hypothesis warrants
454 further investigation in carnivorous plants.

455 In conclusion, understanding geographic variability in other traits of this widespread
456 species and their underlying genetic basis would provide a useful model system for
457 understanding how climate change might impact on species distributions. Biotic
458 interactions and local adaptation are poorly represented in species distribution
459 models (Gotelli & Stanton-Geddes, 2015); we suggest that plant-plant interactions
460 with *D. rotundifolia* vary strongly with latitude and longitude so it might represent a
461 species where current distribution models would be particularly unreliable. It is not
462 unlikely that this is also true for other species. In addition, between-population
463 variability in other traits such as nitrogen nutrition of *D. rotundifolia* has also been
464 demonstrated (Millett *et al.*, 2012, 2015) and might warrant further investigation of
465 the potential for local adaptation. Our data also raise a number of further questions.

466 The extent to which geographic variability in leaf colour is a result of adaptive vs
467 plastic variability cannot be determined, though we present evidence that leaf colour
468 can clearly exhibit phenotypic plasticity. Furthermore, it is still not clear why *Drosera*
469 are red, and more widely, what the adaptive function (if any) is of leaf/trap redness in
470 carnivorous plants. The commonness of leaf redness in carnivorous plants, across
471 unrelated organisms, might provide a useful system for studying the adaptive
472 significance of leaf reddening in plants.

473 **Acknowledgements**

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479 drawing Fig. 1. The comments of three reviewers were invaluable and their input is
480 gratefully acknowledged.

481 **Appendices**

482 **Appendix S1** Characteristics of study sites.

483 **Appendix S2** Leaf colour measurement methods

484 **Appendix S3** Multiple regression results.

485 **Appendix S4** Linear mixed model results.

486 **Appendix S5** Data used in the study.

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627 **Data accessibility**

628 Data are available in supplementary material.

629 **Biosketch**

630 Jonathan Millett is a senior lecturer in the Centre for Hydrological and Ecosystem
631 Sciences, in the Department of Geography in Loughborough University. His work is
632 focussed on linking plant community responses to environmental variability with plant
633 physiological responses. He works mainly on carnivorous plants, bogs, forest and
634 sand dunes. www.drjonmillett.net Author contributions: J.M and B.S. conceived the
635 ideas; J.M., G.W.F. and J.C.T. collected the data; J.M., G.W.F. and J.C.T. analysed
636 the data; and J.M. and B.S. led the writing.

637 Editor: W. Daniel Kissling

638

639 **Tables**

640

641 **Table 1** Results of principle components analysis of between site variability in 13
642 environmental variables for sites across north-western Europe. Presented are the
643 loadings for each variable on the first three axes.

644

Variable	Axis 1	Axis 2	Axis 3
Total N deposition	0.895	0.235	0.321
Total S deposition	0.836	0.431	0.084
NO ₃ deposition	0.951	0.052	0.237
NH ₄ deposition	0.762	0.376	0.365
July maximum temperature	0.162	0.908	-0.142
Growing Degree Days (base 5)	0.388	0.813	0.400
Mean summer temperature	0.292	0.912	0.127
NH ₄ :NO ₃	-0.158	0.542	0.502
UV-B	0.368	0.630	0.132
January minimum temperature	0.349	0.242	0.887
Variance in monthly temperature	-0.349	-0.207	-0.888
Annual precipitation	0.139	-0.216	0.773
Mean annual temperature	0.403	0.523	0.727
Cumulative proportion of variance explained	0.295	0.593	0.855

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647 **Table 2** Results of principle components analysis of between site variability in three
 648 measures of vegetation influence, and *Drosera rotundifolia* leaf colour
 649 characteristics, for plants growing on bogs in north-western Europe. Presented are
 650 the loadings for each variable on the first axes.

651

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Vegetation influence		Leaf colour	
Variable	Axis 1	Variable	Axis 1
Vegetation cover	0.870	CIE LAB a*	0.996
Vegetation height	0.949	Dominant wavelength	0.991
% light transmitted through canopy	-0.960	Red:green	0.974
		Red	0.774
		Green	-0.853
% of variance explained			
	85.97		85.01

653

654

655 **Table 3** Results of stepwise regression for relationship between *Drosera rotundifolia* leaf colour,
 656 vegetation characteristics and environmental variation with geographic location, and between leaf
 657 colour and vegetation characteristics with environmental variation, for sites across north-western
 658 Europe.

Dependent variable	Stepwise selected independent variables	r^2	Adjusted r^2	F	P
	Geographic variables				
Leaf colour	Latitude, longitude	0.686	0.657	23.976	<0.001
Canopy influence	Latitude	0.816	0.806	75.583	<0.001
Vegetation height	Latitude	0.847	0.838	94.346	<0.001
Abiotic PCA axis 1	Latitude	0.291	0.262	9.873	0.004
Abiotic PCA axis 2	Latitude, Longitude	0.551	0.512	14.126	<0.001
Abiotic PCA axis 3	Longitude	0.749	0.738	71.472	<0.001
	Abiotic variables				
Leaf colour	PCA Axis 1 & 3	0.744	0.721	32.008	< 0.001
Canopy influence	PCA axis 3, 1, 2	0.865	0.838	32.116	< 0.001
Vegetation Height	PCA axis 3, 1, 2	0.895	0.874	42.486	<0.001

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667 **Figure Legends**

668 **Figure 1**

669 Location of study sites (site details in Table S1). The colour of the markers is
670 indicative of the average colour of the leaves of *Drosera rotundifolia* growing on
671 *Sphagnum* hummocks. The white marker at site UK10 is because no *D. rotundifolia*
672 were present. The markers for SW1, 2 & 3 are offset to make them visible.

673 **Figure 2**

674 Geographic variation in vascular plant canopy influence and *Drosera rotundifolia* leaf
675 colour, for plants growing across north-western Europe. Presented are the mean site
676 values (circles) for variation with longitude (a and b) or latitude (c and d) in the axis 1
677 scores from a principle components analysis (PCA) of measures of canopy influence
678 (a and b) and *D. rotundifolia* leaf colour (c and d), with the fitted regression line
679 (where statistically significant). The simple linear regression model is shown in b; c
680 and d are partial regression plots where the axes and regression line are adjusted
681 (corrected) to remove variability due to the other variables included in the full multiple
682 regression model, to allow the bivariate relationship to be visualised. Individual leaf
683 colour and vegetation influence measures are shown in table 2.

684 **Figure 3**

685 Geographic variation in the abiotic environment on bogs in north-weestern Europe.
686 Presented are the mean site values (circles) for variation with longitude (a and b) or
687 latitude (c and d) in the first three axes from a principle components analysis (PCA)
688 of environmental variables, with the fitted regression line. The simple linear
689 regression model is shown in a and c; b and d are partial regression plots where the
690 axes and regression line are adjusted (corrected) to remove variability due to the
691 other variables included in the full multiple regression model, to allow the bivariate
692 relationship to be visualised. Individual environmental measures are shown in table
693 1.

694 **Figure 4**

695 Relationship between the abiotic environment on bogs in north-western Europe and
696 vascular plant canopy influence and *Drosera rotundifolia* leaf colour. Presented are
697 the mean site values (circles) for variation in the axis 1 scores from a principle
698 components analysis (PCA) of measures of canopy influence (a, c, and e) or leaf
699 colour (b and d), and variation in the first three axes from a principle components
700 analysis (PCA) of environmental variables. All graphs are partial regression plots,
701 where the axes and regression line are adjusted (corrected) to remove variability due
702 to the other variables included in the full multiple regression model, to allow the
703 bivariate relationship to be visualised. Individual environmental, leaf colour and
704 vegetation influence measures are shown in tables 1 and 2.

705 **Figure 5**

706 The relationship between canopy influence and *Drosera rotundifolia* leaf colour on
707 bogs across north-western Europe. Presented are the values (circles) for the axis 1
708 scores from a principle components analysis (PCA) of measures of canopy influence
709 and leaf colour for individual plots within bogs. The lines represent the fitted
710 regression line for the plots on each bog, based on a multi-level linear mixed model
711 analysis. The equation for each line is presented in appendix S7, site codes are
712 explained in appendix S1. Individual leaf colour and vegetation influence measures
713 are shown in table 2.

714 **Figure 6**

715 The impact of vegetation removal and vegetation removal plus shade addition
716 treatments on canopy light transmission and *Drosera rotundifolia* leaf colour at the
717 Humberhead Peatlands. Presented are the mean (\pm se) for measurements of three
718 different treatments: no vegetation removal (*'natural'*), all above-ground vegetation
719 removed (*'removed'*), all above-ground vegetation removed and shade cloth added
720 (*'removed+shaded'*). Treatments that are significantly different from each other ($P <$
721 0.05 , based on Fisher's LSD) are indicated by different letters. Leaf/trap colour is
722 presented in CIE 1976 ($L^*a^*b^*$) colour space (CIE LAB), where a^* represents a
723 green – red axis of colour. Positive values represent red colours while negative
724 values represent green colours.

Figure 1

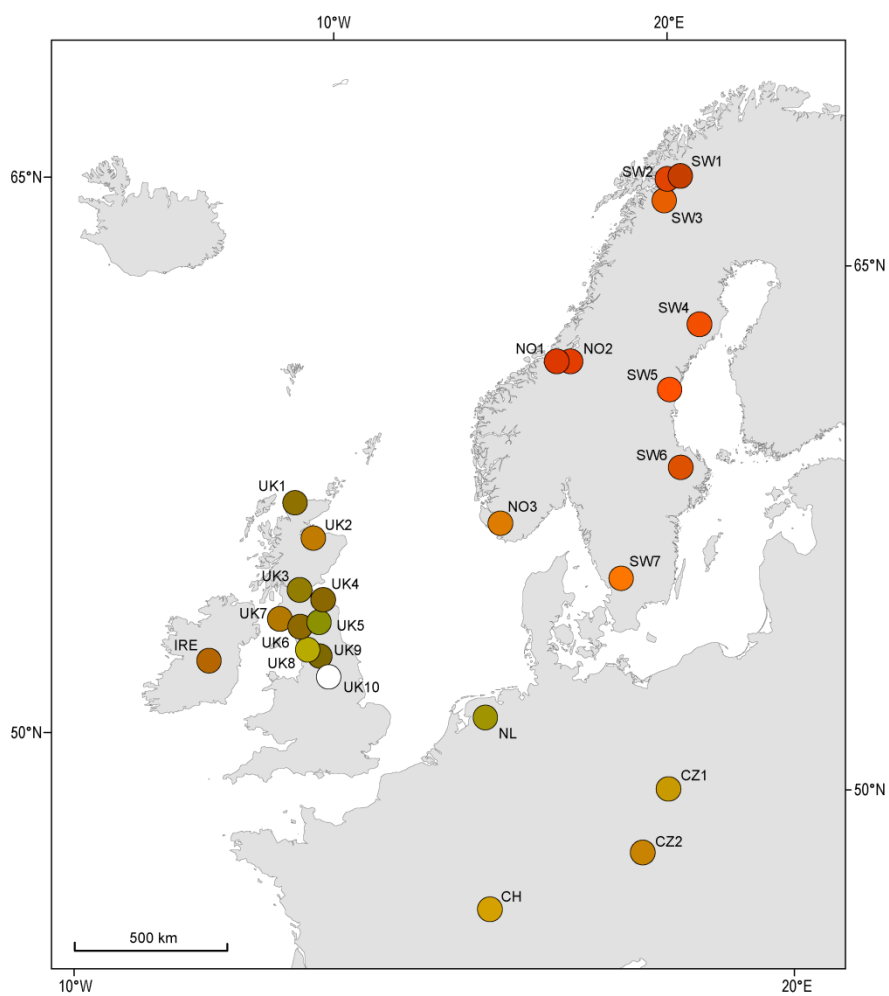


Figure 2

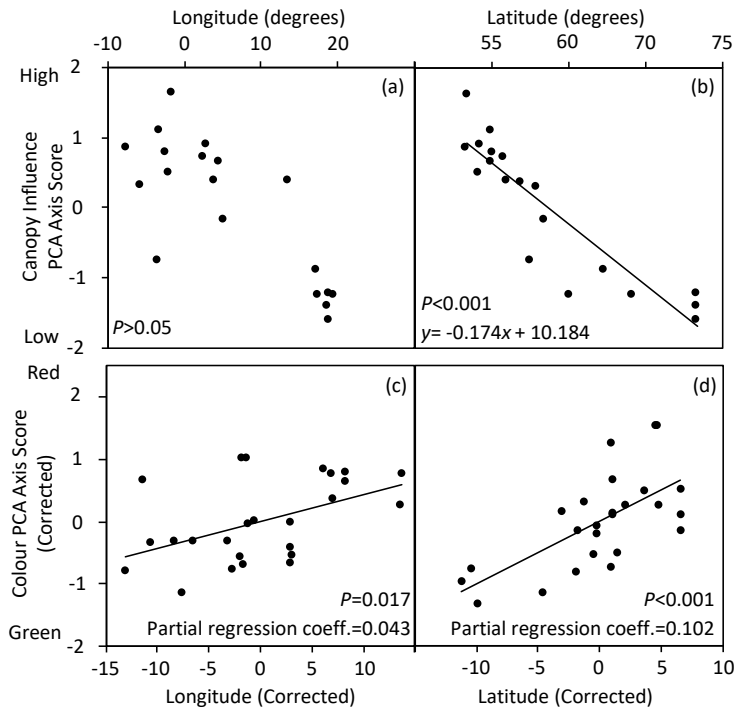


Figure 3

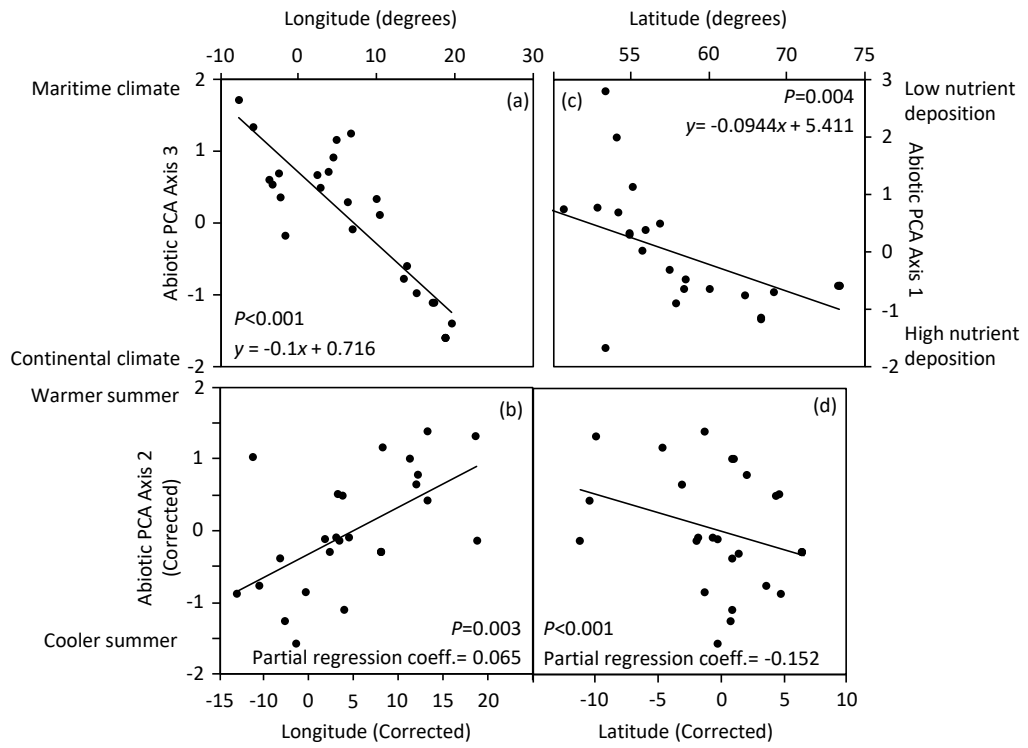


Figure 4

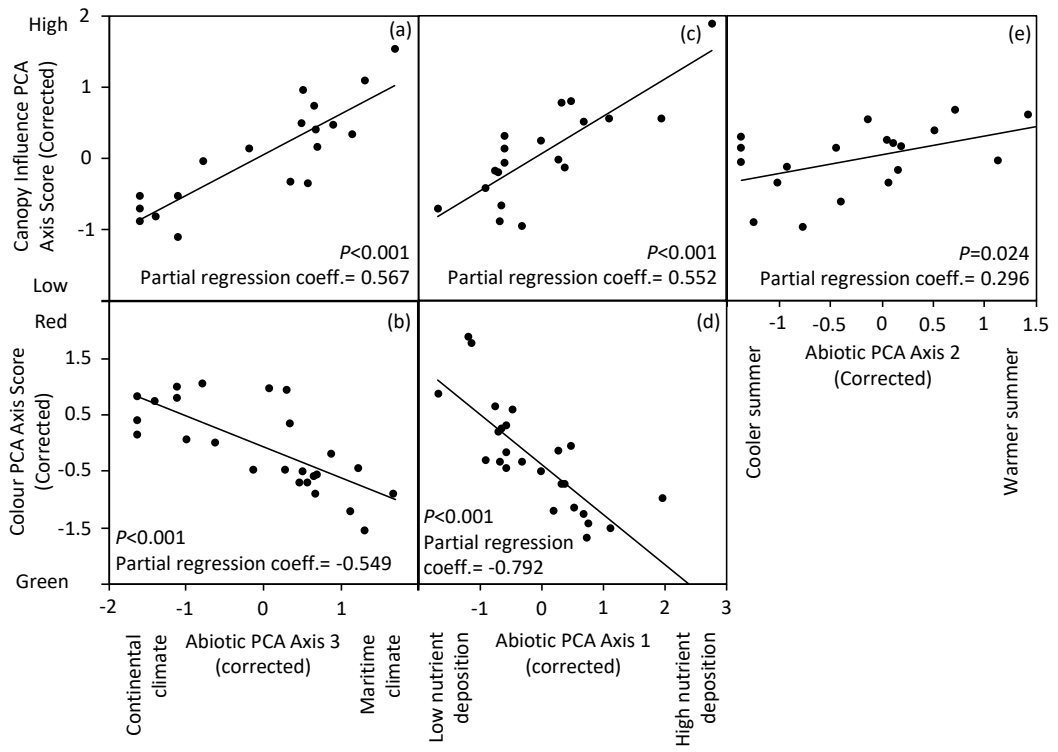


Figure 5

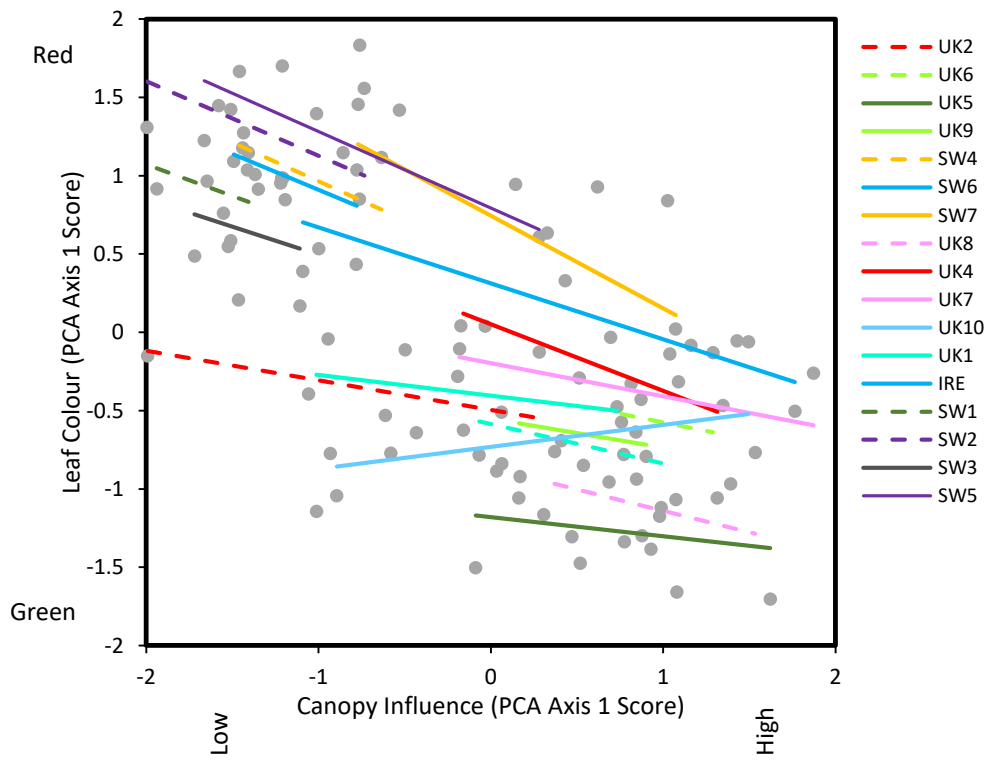
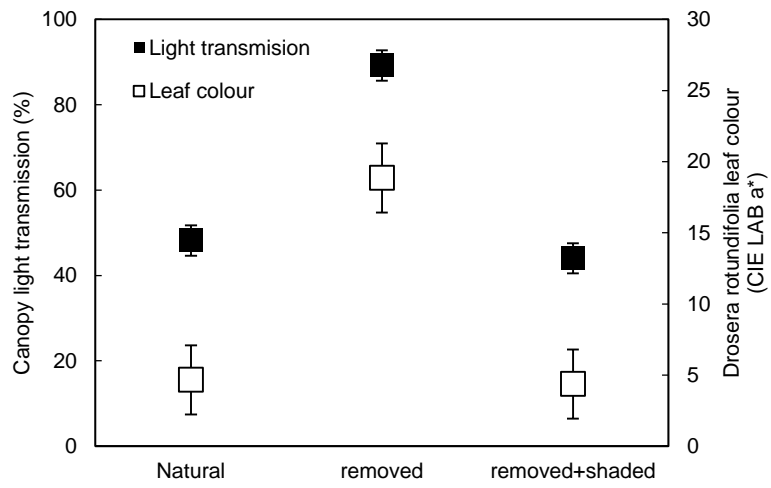


Figure 6



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SUPPORTING INFORMATION

Geographic variation in Sundew (*Drosera*) leaf colour: plant-plant interactions counteract expected effects of abiotic factors.

Jonathan Millett, George W Foot, Juila C Thompson, Brita Svensson

Appendix S1 Characteristics of study sites.

Table S1.1 Characteristics of study sites.

Site	Name	Longitude (°E)	Latitude (°N)	Measurement date	Total atmospheric S deposition (mg m ⁻² yr ⁻¹)	Total atmospheric NO ₃ deposition (mg m ⁻² yr ⁻¹)	Total atmospheric NH ₄ deposition (mg m ⁻² yr ⁻¹)	Total atmospheric N deposition (mg m ⁻² yr ⁻¹)	Mean annual precipitation (mm) *	Mean annual temp. (°C)*
SW1	Abisko 1	18.810	68.355	20.07.2013	0.030	0.030	0.014	0.044	517	-0.86
SW2	Abisko 2	18.797	68.355	23.07.2013	0.030	0.030	0.014	0.044	517	-0.86
SW3	Abisko 3	18.778	68.341	25.07.2013	0.030	0.030	0.014	0.044	517	-0.86
SW4	Lappmyran	19.583	64.164	17.08.2013	0.114	0.138	0.082	0.220	634	2.37
NO1	Skjetnmyra	9.962	63.319	20/21.08.2011	0.069	0.092	0.090	0.182	1135	6.07
NO2	Lysklett-Myran	10.493	63.315	17/18.08.2011	0.069	0.092	0.090	0.182	1065	5.16
SW5	Sundsvall	17.156	62.326	19.08.2013	0.137	0.163	0.101	0.264	659	4.00
SW6	Åkerlänna Römossen	17.354	60.016	21.08.2013	0.183	0.262	0.174	0.436	630	5.54
NO3	Bervamyra	6.748	58.519	23/24.08.2011	0.239	0.300	0.191	0.492	2269	5.59
UK1	Laxford Bridge	5.017	58.371	06.08.2013	0.121	0.302	0.334	0.637	1118	8.15
UK2	Moidach More	-3.614	57.457	9/10.08.2012	0.153	0.407	0.352	0.759	905	7.13
SW7	Saxnäs Mosse	13.508	56.904	23.08.2013	0.522	0.631	0.555	1.186	878	6.40
UK3	Black Loch Moss	3.835	55.904	03.08.2013	0.381	0.630	0.823	1.453	1291	8.37
UK4	Dogden Moss	2.504	55.740	01.08.2013	0.228	0.534	0.702	1.237	1039	8.11
UK5	Butterburn Flow	-2.507	55.072	23/24.08.2012	0.258	1.078	0.919	1.997	1474	7.45
UK6	Bowness Common	-3.265	54.923	31.07.2013	0.373	0.678	0.498	1.176	1158	8.49
UK7	Caresgowan Moss	4.455	54.899	08.08.2013	0.316	0.642	0.830	1.472	1418	8.8
UK8	Meathop Moss	2.856	54.228	30.07.2013	0.428	0.729	0.901	1.629	1093	9.65
UK9	Malham Tarn	-2.175	54.097	29.07.2013	0.502	1.319	1.094	2.413	1310	7.25
UK10	Ringinglow Bog	-1.597	53.348	20/21.08.2012	0.943	1.493	1.271	2.764	956	8.86
IRE	Clara Bog	-7.638	53.322	09.08.2013	0.207	0.157	0.716	0.873	975	9.23
NL	t Groot Veen	6.448	52.834	20/21.07.2011	0.619	0.611	1.635	2.246	876	10.02
CZ1	Chiadla	15.249	50.680	7/8.08.2011	0.762	0.667	0.671	1.338	749	8.82
CZ2	Mrtvy' luh	13.878	48.870	10/11.08.2011	0.442	0.617	0.681	1.298	963	6.65
CH	Etang de la Gruere	7.043	47.239	26/28.07.2011	0.381	0.545	0.806	1.351	1105	8.3

*Mean annual (JUL-JUN) precipitation and temperature for the 5 years before sampling

1 **Appendix S2** Leaf colour measurement methods

2 Leaf reflectance was measured using an Ocean Optics USB4000 VIS-NIR miniature spectrometer. This
3 spectrometer measures reflectance in the range 350-1000 nm in steps of 25 nm. Because plant
4 pigments degrade over time, we measured colour in the field. We therefore used the sun as the light
5 source and adjusted using a Kodak white balance card (90% reflectance). We held the sensor 2 cm
6 from the leaf surface, data were captured using the Ocean optics SpectraSuite software. One single
7 measure of reflectance was used for each leaf based on the mean of 20 measurements. Data were
8 smoothed using a boxcar width of 20 data points. Once captured we calculated colour measures
9 using the SpectraSuite software assuming a D75 illuminant and a 2 degree observer. We calculated
10 the location of the colour of the leaf in CIE 1976 ($L^*a^*b^*$) colour space (CIELAB). This standard colour
11 space includes all colours perceivable to humans. Each colour is defined by its position in the colour
12 space based on the 'Lightness' (L^*) of a colour, its position between red and green (a^* , negative =
13 green, positive = red) and its position between blue and yellow (b^* , negative=blue, positive = yellow).
14 Thus CIELAB enables assessment of colour difference and how red or green an object is by the value
15 of the a^* axis. We also used the Dominant Wavelength from the SpectraSuite analysis. This is the
16 dominant wavelength of the colour in CIE 1976 colour space, removing differences in saturation or
17 intensity. It is calculated by We used the reflectance spectrum to calculate mean red reflectance
18 (average reflectance in the range: 600-699 nm) and mean green reflectance (average reflectance in
19 the range 500-599 nm). We then used these values to calculate the ratio of red:green reflectance.

20

21 **Appendix S3** Results of stepwise multiple regression.

22 **Table S3.2** Results of stepwise multiple regression analysis of geographical variation in a. *Drosera*
 23 *rotundifolia* leaf colour, b. canopy influence of vascular plant vegetation in which the *D. rotundifolia*
 24 are growing, and c. the height of vascular plant vegetation in which the *D. rotundifolia* are growing.

25 **a. *Drosera rotundifolia* Leaf colour.**

Variable	Model 1			Model 2		
	<i>B</i>	<i>SE B</i>	β	<i>B</i>	<i>SE B</i>	β <i>P</i>
Constant	-7.759***	1.365		-6.151***	1.371	
Latitude	0.135***	0.023	0.768	0.102***	0.025	0.578
Longitude				0.043*	0.017	0.363
R ²		0.590			0.686	
F for change in R ²		33.098***			6.680*	

26 **P*<0.005, ****P*<0.001

27 **b. Canopy influence**

Variable	Model 1		
	<i>B</i>	<i>SE B</i>	β
Constant	10.814***	1.176	
Latitude	-0.174***	0.020	-0.904
Longitude†	-0.146		
R ²		0.816	
F for change in R ²		75.583***	

28 †excluded from model, ****P*<0.001

29 **c. Vegetation height**

Variable	Model 1		
	<i>B</i>	<i>SE B</i>	β
Constant	91.779***	7.922	
Latitude	-1.308***	0.135	-0.921
Longitude†	0.004		
R ²		0.847	
F for change in R ²		94.346***	

30 ****P*<0.001

31 **Table S3.3** Results of stepwise multiple regression analysis of geographic variation in principle component axis scores from principle components analysis of abiotic
 32 conditions at each site. a. Axis 1, which reflects nutrient deposition differences among the sites, with high scores on this axis representing sites with a high N and S
 33 deposition load (higher total N, total S, NO₃ and NH₄ deposition); b. axis 2 which reflects differences among sites in summer meteorological conditions, sites with high
 34 scores on this axis had warmer summers and a longer growing season (higher mean summer temperature, high maximum July temperature and high GDD); c. axis 3 which
 35 reflects the continentality of the climate, sites with high scores on this axis had a more maritime, less continental climate (high January minimum temperature, mean
 36 annual temperature, and precipitation, and low monthly temperature variance).

37 **a. Abiotic PCA axis 1**

Variable	Model 1		
	<i>B</i>	<i>SE B</i>	<i>β</i>
Constant	5.41	1.730	
Latitude	-0.094**	0.030	-0.540
longitude			
R ²		0.291	
F for change in R ²		9.873**	

38 ***P*<0.001

39 **b. Abiotic PCA axis 2**

Variable	Model 1			Model 2		
	<i>B</i>	<i>SE B</i>	<i>β</i>	<i>B</i>	<i>SE B</i>	<i>β</i>
Constant	5.760	1.682		8.300	1.596	
Latitude	-0.100**	0.029	-0.575	-0.152***	0.029	-0.874
longitude				0.065**	0.019	0.557
R ²		0.330			0.551	
F for change in R ²		11.839**			11.321**	

40 ***P*<0.001, ****P*<0.001

41 **c. Abiotic PCA axis 3**

Variable	Model 1		
	<i>B</i>	<i>SE B</i>	<i>β</i>
Constant	0.716	0.131	
Latitude	-0.100***	0.012	-0.865
longitude			
R ²		0.749	
F for change in R ²		71.472***	

42 ****P*<0.001

43

44 **Table S3.4** Results of stepwise multiple regression analysis of relationship between environmental variation and a. *Drosera rotundifolia* leaf colour, b. canopy influence of
 45 vascular plant vegetation in which the *D. rotundifolia* are growing, and c. the height of vascular plant vegetation in which the *D. rotundifolia* are growing. Axis 1, 2 and 3 are
 46 principle component axis scores from principle components analysis of abiotic conditions at each site. Axis 1 reflects nutrient deposition differences among the sites, with
 47 high scores on this axis representing sites with a high N and S deposition load (higher total N, total S, NO₃ and NH₄ deposition); axis 2 reflects differences among sites in
 48 summer meteorological conditions, sites with high scores on this axis had warmer summers and a longer growing season (higher mean summer temperature, high
 49 maximum July temperature and high GDD); axis 3 reflects the continentality of the climate, sites with high scores on this axis had a more maritime, less continental climate
 50 (high January minimum temperature, mean annual temperature, and precipitation, and low monthly temperature variance).

51 **a. *Drosera rotundifolia* leaf colour**

Variable	Model 1			Model 2		
	<i>B</i>	<i>SE B</i>	β	<i>B</i>	<i>SE B</i>	β
Constant	-0.036	0.157		-0.030	0.109	
Axis 1	-0.809***	0.189	-0.666	-0.792***	0.131	-0.653
Axis 2†						
Axis 3				-0.549***	0.108	-0.548
R ²		0.444			0.744	
F for change in R ²		18.352***			25.843***	

52 †excluded from model, ****P*<0.001

53 **b. Canopy influence**

Variable	Model 1			Model 2			Model 3		
	<i>B</i>	<i>SE B</i>	β	<i>B</i>	<i>SE B</i>	β	<i>B</i>	<i>SE B</i>	β
Constant	0.010	0.161		-0.006	0.106		0.061	0.096	
Axis 1				0.490***	0.103	0.524	0.296*	0.118	0.252
Axis 2				0.620***	0.099	0.685	0.552***	0.089	0.591
Axis 3	0.664***	0.150	0.732				0.567***	0.089	0.626
R ²		0.536			0.809			0.865	
F for change in R ²		19.676***			22.810***			6.278*	

54 ****P*<0.001

55

56 **c. Vegetation height**

Variable	Model 1			Model 2			Model 3		
	<i>B</i>	<i>SE B</i>	β	<i>B</i>	<i>SE B</i>	β	<i>B</i>	<i>SE B</i>	β
Constant	15.189***	1.071		15.097***	0.830		15.784***	0.628	
Axis 1	5.286***	0.997	0.789	5.036***	0.775	0.752	4.493***	0.580	0.671
Axis 2				2.811**	0.799	0.407	3.446***	0.603	0.499
Axis 3							3.017**	0.771	0.348
R ²		0.623			0.787			0.895	
F for change in R ²		28.080***			12.368**			15.300**	

57 ***P*<0.01, ****P*<0.001

58

59

60 **Appendix S4** Linear mixed model results

61 **Table S4.5** Characteristics of Linear Mixed Models for the relationship between vascular vegetation canopy influence and *Drosera rotundifolia* leaf colour. Presented are
62 characteristics for four models in ranked order based on -2log likelihood.

63

Model ranking	Model	Included parameters	-2log likelihood	-2log likelihood (difference from next ranked model)	P-vale for comparison with next ranked model
1	Random slopes and intercepts	Site, intercept, slope	192.647	0	<0.05
2	Random intercepts	Site, intercept.	201.407	8.76	<0.001
3	Null multi-level	Site	361.475	160.068	<0.001
4	Null single-level	None	560.897	229.422	<0.001

64

65

66 **Table S4.6** Equations of fitted regression lines for the relationship between valcular plant canopy
 67 influence (x) and *Droserea rotundifolia* leaf colour (y) for each of 16 site around Europe. Fitted lines
 68 are derived from a multi-level Linear Mixed Model analysis. Site codes are in table S1.

69

70

site	Fitted regression equation ⁷¹
UK10	$y = 0.1394x - 0.7313$
UK5	$y = -0.122x - 1.1802$
UK1	$y = -0.1331x - 0.4048$
UK9	$y = -0.1852x - 0.5511$
UK2	$y = -0.189x - 0.4951$
UK7	$y = -0.2123x - 0.1967$
UK8	$y = -0.2733x - 0.8668$
IRE	$y = -0.357x + 0.3118$
SW3	$y = -0.3591x + 0.1357$
SW1	$y = -0.4062x + 0.2608$
UK4	$y = -0.4265x + 0.052$
SW6	$y = -0.4598x + 0.4499$
SW2	$y = -0.4755x + 0.6513$
SW5	$y = -0.4889x + 0.7929$
SW4	$y = -0.4955x + 0.4686$
SW7	$y = -0.5921x + 0.7443$

72