1 Title: UV-screening and springtime recovery of photosynthetic capacity in leaves of

2 *Vaccinium vitis-idaea* above and below the snow pack

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15 **Declaration of Authorship**: TMR SMH conceived & designed the experiments. TS, SN, SMH, MP TMR,

16 did the majority of the field work and sampling, TS, MP, AS, PJA TMR analysed the data, JAPC JA

17 provided site-specific help, and access to meteorological data integral to the experiment, TS, PJA,

18 TMR wrote the manuscript, and all authors provided editorial input.

19   Abstract (limit 250/250 Words)

20   Evergreen plants in boreal biomes undergo seasonal hardening and dehardening adjusting their

21   photosynthetic capacity and photoprotection; acclimating to seasonal changes in temperature and

22   irradiance. Leaf epidermal ultraviolet (UV)-screening by flavonols responds to solar radiation,

23   perceived in part through increased ultraviolet-B(UV-B) radiation, and is a candidate trait to provide

24   cross-photoprotection. At Hyytiälä Forestry Station, central Finland, we examined whether the

25   accumulation of flavonols was higher in leaves of *Vaccinium vitis-idaea* L. growing above the

26   snowpack compared with those below the snowpack. We found that leaves exposed to colder

27   temperatures and higher solar radiation towards the top of hummocks suffered greater

28   photoinhibition than those at the base of hummocks. Epidermal UV-screening was highest in upper-

29   hummock leaves, particularly during winter when lower leaves were beneath the snowpack. There

30   was also a negative relationship between indices of flavonols and anthocyanins across all leaves

31   suggesting fine-tuning of flavonoid composition for screening vs. antioxidant activity in response to

32   temperature and irradiance. However, the positive correlation between the maximum quantum

33   yield of photosystem II photochemistry (F_v/F_m) and flavonol accumulation in upper hummock leaves

34   did not confer on them any greater cross-protection than would be expected, from the relationship

35   of F_v/F_m with temperature and irradiance during dehardening present among leaves throughout the

36   hummocks. Irrespective of timing of snow-melt, photosynthesis fully recovered in all leaves,

37   suggesting that *V. vitis-idaea* has the potential to exploit the continuing trend for longer growing

38   seasons in central Finland without incurring significant impairment from reduced duration of snow

39   cover.

40

41 **Graphical Abstract**: Schematic representing the changes in leaf level processes moderated by the

42 snowpack, sunlight and temperature after different heights on hummocks of *V. vitis-idaea* during

43 spring dehardening

44

45 **Keywords** *(7 max)*: spring dehardening; flavonoids; anthocyanins; understorey; spectral quality;

46 photoprotection; Arctic browning

47

1. Introduction

An expected outcome of ongoing human-induced climate change is a shift in seasonal precipitation and temperature affecting growing season length (Harsch and HilleRisLambers 2016; IPCC 2014). This has contributed to a reduction in annual snow-cover in the Arctic by as much as 5 days each decade since 1979 (Derksen et al. 2016). At high northern latitudes, less of the winter precipitation is expected to fall as snow and more as rain (Mellander et al. 2007; Rasmus et al. 2004) and temperatures favourable for growth are forecast to occur earlier in the spring (Henry 2008; Jylhä et al. 2008). In Finland, mean annual temperature is expected to increase by 4-11°C and precipitation (and hence cloudiness) by 10-60 % by the end of 21st century, with most increases occurring during the winter (Finland's Seventh National Communication under the United Nations Framework Convention on Climate Change, 2017). The benefit of this change for plant growth and photosynthesis is uncertain because warmer mean temperatures together with reduced precipitation as snow may lead a smaller snow pack that melts earlier (Kreyling et al. 2012). The absence of snow cover will imply greater temperature fluctuations close to the ground, exposing plants to colder minimum, and warmer maximum, temperatures when they are starting to deharden at the end of winter (Blume-Werry et al. 2016; Preece and Phoenix, 2013).

Plants possess several photoprotective mechanisms which mitigate abiotic stresses under harsh conditions: (1) when reaction centres are open photochemical quenching ($_qP$) entails activation of the enzymes and metabolites involved in charge separation to produce energy via photosynthesis; (2) excess light energy, causing reaction centres to close, is dissipated through xanthophyll-cycle pigments (carotenoids) zeaxanthin and antheraxanthin in a process regulated by the pH the thylakoid lumen (Adams et al. 2004; Murchie and Lawson 2013); (3) additionally antioxidants such as phenolic compounds are accumulated to quench reactive oxygen species (ROS) (Agati et al. 2013; Hideg et al. 2013; Salter et al. 2017). It is reasonable to assume that the pressure on these mechanisms is increased when a plant is subject to both fluctuating temperatures and irradiances above the snowpack rather than the more stable conditions offered under snow (Loik et al. 2004; Preece and

74   Phoenix, 2013). Low temperatures dampen physiological processes meaning that even moderate

75   irradiances cause photoinhibition through photoinactivation via the Mn_4CaO_5 cluster or inhibition of

76   the photosystem II (PS_{II}) electron acceptors (Neuner et al. 1999; Saarinen et al. 2011). Sunlight can

77   penetrate the snowpack, where temperature fluctuations are small, potentially allowing some

78   photosynthesis at low irradiances (Saarinen et al. 2016). Some studies have found the relative

79   transmittance of different regions of the spectrum by snow to be quite even (Gorton and Vogelmann,

80   2003) while others have reported spectrally-differential attenuation through the snowpack (Curl Jr et

81   al. 1972; Richardson and Salisbury, 1977). Although solar irradiance, particularly ultraviolet-B (UV-B:

82   280-315 nm) radiation, is low in winter, the ratio of ultraviolet (UV-B plus UV-A: 280-400 nm) to

83   photosynthetically active radiation (PAR: 400-700 nm) in forest understorey shade is enriched (Jansen

84   et al. 2017; Hartikainen et al. 2018).

85   Flavonoids such as flavonols and anthocyanins are the most-common class of phenolic

86   compounds produced in response to UV-radiation and blue light (Searles et al. 2001; Sullivan et al.

87   2007; Taulavuori et al. 2016). Flavonols accumulate in the leaf adaxial epidermis absorbing UV-

88   radiation so screening the photosynthetic apparatus in the mesophyll below (Barnes et al. 2017;

89   Caldwell et al. 1983; Nybakken et al. 2004). Although their absorption spectrum is predominately in

90   the UV region, it has been proposed that flavonoids provide cross-protection to high solar irradiance

91   in general (Jansen et al. 2017), and a positive correlation is sometimes reported between flavonoid

92   accumulation and the yield of PS_{II} (Robson et al. 2014; Wargent et al. 2011). This relationship may be

93   attributable to protection against PS_{II} photodamage or enhanced ROS scavenging conferred by

94   flavonoids (reviewed by Takahashi and Badger, 2011). The accumulation of flavonoids in response to

95   UV-radiation is moderated by temperature, being less pronounced in warm than in cool temperatures

96   (Bilger et al. 2007; Coffey et al. 2017; Petridis et al. 2016) where flavonoids can be important in

97   mitigating against oxidative stress (Lütz et al. 2005) and can increase frost hardiness (Chalker-Scott

98   and Scott 2004; Shulze et al. 2016). Hence, the accumulation of flavonoids could provide a feasible

99 strategy whereby evergreen plants ameliorate photoinhibition, imposed by increasing irradiance and

100 temperature fluctuations, as they deharden over the spring.

101 Boreal evergreen and wintergreen plants adjust light harvesting complexes to optimise

102 quenching processes at the expense of the light harvesting and photosynthetic capacity of their leaves

103 during winter when light and temperature conditions are unfavourable (Adams et al. 2004).

104 Subsequently, they undergo a recovery period in preparation for more favourable growth conditions

105 in late spring and summer (Muller et al. 2005). During this period of dehardening, full functionality

106 returns to the photosynthetic apparatus of leaves and this can be measured using chlorophyll

107 fluorescence as an increase in the yield of PS_{II} (Murchie and Lawton, 2013; Saarinen et al. 2011).

108 Monitoring leaf-level chlorophyll fluorescence of evergreen trees (Ensminger et al. 2004; Porcar-

109 Castell et al. 2008) and field-layer plants (Starr and Oberbauer, 2003) through spring in the boreal

110 forest, has revealed that recovery of F_v/F_m is modulated by temperature and light.

111 Here, we aimed to compare leaf epidermal flavonoids (flavonols and anthocyanins), chlorophyll

112 content and the PS_{II} yield at different positions on hummocks relative to the snowpack, through a

113 seasonal time-course of measurements of evergreen-shrub *Vaccinium vitis-idaea* L. During the spring

114 dehardening period, the over-wintering leaves of this species are adapted to take advantage of

115 favourable early-spring conditions by photosynthesizing when still under the snowpack (Lundell et al.

116 2008; Saarinen et al. 2011; Starr and Oberbauer, 2003). Our hypothesis was that leaves at the top of

117 the hummock above the snowpack during winter and early spring would suffer greater photoinhibition

118 because they encounter fluctuating temperatures and higher irradiance than those at the base of

119 hummocks receiving lower irradiance, of both PAR and UV radiation, and more stable temperatures

120 remaining closer to 0°C under the snow. However, we also expected higher accumulation of

121 epidermal UV-absorbing compounds (flavonols and anthocyanins) in leaves above the snow, and for

122 this to partially ameliorate the photoinhibition they suffered relative to those at equivalent

123 temperatures beneath the snowpack.

124   2.   Methods

125   2.1. Study site and species

126   The study was performed under an open *Pinus sylvestris* L. canopy, basal area 23 m^{2}ha^{-1}, on

127   level ground at Hyytiälä Forestry Station of the University of Helsinki in central Finland (61.84741°N

128   24.29477°E 181 m asl). The hummock-forming evergreen shrub *V. vitis-idaea* covers vast areas of

129   peatland and boreal forest at high latitudes in the Northern Hemisphere (Karlsson, 1985; Lundell et

130   al. 2008). At our site, the understorey comprises almost exclusively *V. vitis-idaea* in hummocks of up

131   to 75 cm in height intermingled with *Vaccinium myrtillus* L. Four hummocks, positioned at least a

132   metre from the nearest pine tree, were selected at random within a 10-by-10-m area of understorey.

133   These hummocks were divided vertically into three height categories on 14-02-2017: the "upper"

134   hummock being the uppermost shoots, most exposed to the wind and sun, the "middle" hummock

135   being the outer shoots on the south side of the hummock, somewhat sheltered from the wind but just

136   below the snow pack through the winter, and the "lower" hummock being shoots at the very base of

137   the hummock on the south side, below the snow pack during the whole winter and early spring.

138   The snow depth relative to the lower, middle and upper hummocks, and the leaf phenology of

139   the *Vaccinium* species, was monitored throughout the study concomitantly with leaf traits, and these

140   data were compared against a continuous monitoring by Phenocam time-lapse images nearby in the

141   stand (Kolari et al. 2017). By 19-06-2017, plants of both *V. vitis-idaea* and *V. myrtillus* produced new

142   leaves. From this date onwards, until our final measurement date, 08-11-2017, a set of measurements

143   was also made on these new *V. vitis-idaea* leaves (unfurling in June 2017) in same way as those on

144   leaves produced in June 2016. This allowed us to obtain data for leaves from the time of their unfurling

145   through their first and second years of growth. To allow this continuum of leaf age to be visualised in

146   the figures we have combined these two set of leaf traits in a single time series; with 2017 leaves

147   plotted from their unfurling until 08-11-2017 (day 312 of the year, leaf age 152 days) and 2016 leaves

148   from 14-02-2017 onwards (day 45 of the year, leaf age 250 days).

149 2.2. Characterisation of the forest canopy by hemispherical photograph

150 Plant area index (PAI)(m^2m^{-2}), was estimated at each of the four hummocks by taking

151 hemispherical photographs (at 40 cm height from the ground) through a Sigma 4.5mm f2.8 EX DC HSM

152 circular Fisheye lens (Sigma Corporation of America, Ronkonkoma, NY) on a Nikon D7000 camera

153 (Nikon corporation, Tokyo, Japan) levelled on a tripod. Measurements were done on a completely

154 overcast day in mid-July (20-07-2017) to ensure homogeneous grey sky and a good contrast between

155 the sky and the canopy (Fig S1). All photos were analysed following the procedure detailed in

156 Hartikainen et al. (2018).

157 2.3. Measurement of spectral irradiance at the study site

158 The solar spectral irradiance was measured close to solar noon, directly to the south of each of

159 the four hummocks on 07-03-2017 and 19-06-2017 in sunflecks and canopy shade on completely

160 clear-sky days. These data were compared with corresponding measurements in an open field next

161 to the forest stand earlier on the same days. Spectral irradiance recordings were made using a Maya

162 2000 Pro (Ocean Optics, Dunedin, FL, USA) array spectrometer. The cosine diffuser (Bentham

163 Instruments Ltd. Reading UK) was levelled on the tripod 40-cm high and attached with a fibre-optic

164 cable to the spectrometer. Measurements were corrected for dark-noise and stray light in the UV

165 range (<400 nm) using a protocol detailed by Hartikainen et al. (2018). The spectrometer had been

166 calibrated against a Bentham scanning spectroradiometer for outdoor measurements of solar spectral

167 irradiance, over the wavelength range 290-900 nm. Pre-processing of the data was performed using

168 functions from the R packages Ooacquire and Photobiology in R version 3.4.3 (R Core Team, 2018).

169 2.4. Optical measurements of *V. vitis-idaea* leaf pigments and photosynthetic capacity

170 On each measurement date, starting 14-02-2017, then 07-03-2017, and at 2-week intervals

171 subsequently until 08-07-2017, with an additional final measurement date of 08-11-2017, leaf optical

172 properties and photosynthetic capacity were measured. Ten leaves (remaining attached to the plants)

173 were measured *in situ* from each height (upper, middle, lower) on four hummocks. Sun-exposed

174 leaves of equivalent size were measured (or leaves which would have been sun exposed had it not

175 been cloudy on overcast days), all from different shoots of each *V. vitis-idaea* hummock, avoiding the

176   three distal leaves of a shoot. Lower and middle hummock leaves were, when necessary, excavated

177   from beneath the snow to be measured and subsequently the snow was replaced over these leaves.

178   Leaves that were damp from dew, rain or melting snow were quickly but gently dried with a tissue

179   prior to measurement.

180   Dualex Scientific[+] (Force-A, University Paris-Sud, Orsy, France) was used to optically assess the

181   epidermal flavonol concentration (epidermal absorption in the UV-A region at 375 nm) and

182   anthocyanin concentration (absorption at 515 nm), from both the adaxial and abaxial sides of each

183   leaf, as well as the leaf chlorophyll concentration (assessed optically by transmittance in the red at

184   650 nm vs. near-infra red at 710 nm, Cerovic et al. 2012). We recorded the maximum quantum yield

185   of PS_{II} photochemistry (F_v/F_m; where F_v [variable fluorescence] is the maximal fluorescence, F_m, minus

186   the minimal fluorescence, F_0, of dark-adapted leaves, as defined by Murchie and Lawson, 2013)

187   measured with a mini-PAM (Heinz Walz GmbH, Effeltrich, Germany) following 30 min of dark-

188   adaptation with darkening clips. According to Bokhorst et al. (2010), 30 min in darkness should be

189   sufficient for full dark adaptation of *V. vitis-idaea* leaves year 'round. We also recorded the effective

190   quantum yield of PS_{II} photochemistry (φPS_{II} calculated as $F_q`/F_m`$; where $F_q`$ is the maximal

191   fluorescence, $F_m`$, minus the steady-state fluorescence, $F`$, of leaves in ambient sunlight, as defined by

192   Murchie and Lawson, 2013) of leaves in the sun and shade in the mid-to-late morning. Where possible,

193   the same ten leaves were used for chlorophyll fluorescence and Dualex measurements on any given

194   day, but it was not feasible to measure the same ten leaves from one measurement date to another,

195   so from day to day leaves meeting the above criteria were selected at random.

196   2.5. Harvest of leaves for trait measurements
197   On 08-07-2017, leaves of age 29 days and 394 days were harvested. From each age cohort 8-

198   10 leaves were sampled from each of the four hummocks at each of the three heights (2 epidermis ×

199   10 leaves × 3 heights × 4 hummocks). Fresh leaves were placed in zip-lock plastic bags, weighed and

200   digitally photographed against an area reference on the day of sampling, then dried at 40°C for 9 days

201   in a drying oven with forced ventilation before reweighing to obtain their dry weight. Leaf areas were

202   calculated from the photographs using ImageJ (US National Institute of Health, Bethesda, Maryland,

203   USA: following Wang, 2017) and used to calculate the leaf mass area (LMA mg mm^{-2}). The leaf area

204   covering the optical window of the Dualex Scientific $^+$ was 47.7 mm^2 (Area = πr^2, r = 3.90 mm), used

205   with LMA, to calculate per area and per mass values of pigment concentrations on the harvest dates.

206   2.6. Environmental monitoring of temperature during the experiment
207   At the time of each measurement of leaf optical properties, the temperature of the leaves of *V.*

208   *vitis-idaea* at each height on all four hummocks was measured at close range with a high precision

209   infra-red thermometer with special macro optics (LS laser-sight Optris GmbH, Berlin, Germany).

210   Measurements were also recorded at the tree base and at 1 m 50 cm height on the trunks (emissivity

211   *c* 0.98) of the four nearest trees to the hummocks at opposite compass points (North and South at

212   midday) in the sun and shade, and on the snow surface (emissivity of tree trunks and snow *c* 0.98).

213   Meteorological data for the experimental period were obtained from the SMEAR II ICOS Hyytiälä

214   station through the AVAA data research portal (https://avaa.tdata.fi/). Temperature in the

215   understorey was recorded every minute with a PT100 sensor (Pentronic, Västervik, Sweden) mounted

216   on a mast at 4 m height and soil surface temperature was recorded every minute with a KTY 81-110

217   sensor (NXP Philips, Amsterdam, Netherlands). Understorey photosynthetically active radiation (PAR)

218   was measured with an array of four Li190SZ PAR sensors (LI-COR, Lincoln, NE) at 0.6m height next to

219   the hummocks. These time series of meteorological data were used to calculate averages over a range

220   of time periods preceding each measurement (1 hour, 1 day, 2 days, and 2 weeks) and the relationship

221   between temperature and PAR over these periods and leaf trait data was plotted. Of these four time

222   integrals the best fit to the trait data was retained.

223   2.7. Data Analysis
224   Differences due to hummock height among optically-assessed leaf traits were tested using a

225   two-way ANOVA with hummock height, and leaf age (days) as fixed factors, or a three-way-ANOVA

226   for the responses of flavonols and anthocyanins which were measured in both the adaxial and abaxial

227   epidermis of each leaf (with leaf side as a nested third fixed factor). One measurement date (Leaf Age:

228   250 days), when only the adaxial epidermis was measured with the Dualex, was excluded from the

229   three-way ANOVA. The equivalent single-factor ANOVA was used to test the effect of hummock height

230   on traits measured only once, e.g. from harvested leaves at the end of the experiment. The four

231   hummocks were the experimental units of replication and height was nested within each hummock.

232   When significance differences were produced by the ANOVA, pairwise differences were identified

233   between the responses across three levels of hummock height on either leaf side on each

234   measurement date (function glht in the package Multicomp). Holm's adjustment was used to account

235   for multiple pairwise comparisons. The relationship of F_v/F_m with temperature and PAR was assessed

236   using linear regression (function lm()). All the analyses were performed using R version 3.4.3 (R core

237   development team, 2018).

238   3. Results
239   3.1. Forest cover, snow depth and temperature
240   The snow depth was *c*. 40 cm at the first winter measurement (14-02-2017), meaning that the

241   upper shoots of hummocks (upper hummocks) were above the snow, the middle of the hummocks

242   were just below the top of the snowpack, and lower shoots of hummocks (lower hummocks) were

243   entirely beneath the snowpack. From 14-02-2017 onwards, the snow depth didn't increase from this

244   maximum and remained above the middle-hummock shoots until 27-03-2017 and above the lower-

245   hummock shoots until snow melt was complete on 10-05-2017.

246   Soil temperatures under the snow were relatively stable, starting to fluctuate only when snow

247   melt was almost complete and the lower hummock leaves above the snow (Fig. 1; Table 1). From the

248   time series of snow depth and air temperature data, compared with IR-thermometer measurements

249   of snow-pack temperature (Fig. 1), we were able to estimate that the upper-hummock leaves were

250   subject to 111 freeze-thaw cycles, the middle-hummock leaves 86 and lower-hummock leaves 38 such

251   cycles, during the period between New Year and mid-summer 2017 which encompasses full

252   dehardening (Table 1).

253 3.2. Spectral irradiance at the study site

254   The solar spectral irradiance was measured on three dates during the winter and spring (14-02-

255 2017, 07-03-2017 and 19-06-2017). On all three dates, the normalised spectra show that PAR was

256 depleted in the under-canopy sunflecks and shade compared with the open field, and the relative

257 contribution of UV radiation was similar in the sunflecks and open sun but enriched in the shade (Figs.

258 2 and S2, Table S1). The spectral composition of the sunflecks varied with sunfleck size as well as time

259 of year, and the relative contribution to shade by needles and branches also affected the shape of

260 these spectra. The Plant Area Index (PAI) and sunfleck duration on 20-07-2017 were 2.14 ± 0.05 m^2m^{-}

261 ^{2}and 169.6 ± 11.27 min day^{-1} respectively, and was similar among the four hummocks (Table S2).

262   The reflectance (up to 90%) and transmittance of sunlight by the snowpack modifies amount

263 and composition of radiation received by leaves above and below the snowpack at different

264 hummocks heights. We found the snowpack to transmit about 10% of incident PAR irradiance to a

265 depth of 10 cm, and a similar or slightly higher percentage of UV radiation to reach the same depth

266 (Robson unpublished data) in line with comparable measurements elsewhere (Richardson and

267 Salisbury, 1977; Gorton and Vogelmann, 2003; Perovich 2007).

268 3.3. Seasonal changes in leaf pigments at different heights on the *V. vitis-idaea* hummocks

269   Leaf flavonoid concentration (i.e. both flavonols and anthocyanins), measured optically and

270 presented as an index of absorption on a per leaf-area basis, was most similar directly after leaf

271 unfolding both among hummocks and between leaf sides. As the leaves matured, their flavonol

272 concentration increased in the adaxial epidermis and initially decreased in the abaxial epidermis

273 between the first and second measurement of new leaves during the summer (Leaf Age 10-29 days,

274 Fig. 3A). During this period, UV-screening by flavonols was greater in the upper-, and middle-,

275 hummock leaves than the lower leaves. Accumulation of flavonols continued until the start of winter

276 in the adaxial-, and to a lesser extent the abaxial-, epidermis, and only started to definitely decline

277 with age following the spring and summer of their second year (Leaf Age 271 days; Fig. 3A). Leaves of

278   *V. vitis-idaea* can remain productive for 5 years or more, but after their first growing season maximum

279   photosynthesis declines sharply (Karlsson, 1985).

280   Adaxial epidermal flavonol concentration in *V. vitis-idaea* leaves transiently decreased during

281   winter in lower- and mid-hummock leaves when they were beneath the snowpack, but started to

282   increase again from 24-03-2017 (Leaf Age 288 days) coinciding with temperatures rising above

283   freezing.   In sequence, the upper, then middle, then lower hummock leaves' adaxial flavonol

284   concentration increased around the time that the middle hummocks leaves emerged from the

285   snowpack (Fig. 3A, from 27-03-2017, Leaf Age 291 days).   This phase of flavonol accumulation

286   continued until the end of the snow melt period (16-05-2017 Leaf Age 341 days), after which flavonol

287   concentration was stable and eventually started to decline from 19-06-2017 Leaf Age 375 days (Fig.

288   3A). Flavonol accumulation in the adaxial epidermis during spring was greater than that in the abaxial

289   epidermis, leading the adaxial-to-abaxial flavonol ratio to increase until 08-07-2017, Leaf Age 394

290   days, but it returned to the equivalent of pre-spring ratios later in the year (08-11-2017, Leaf Age 517;

291   Figs. 3A and S3A).

292   Leaf anthocyanin concentration is typically highest in young leaves, and higher in the abaxial

293   than the adaxial epidermis, declining once leaves are mature (Landi et al. 2015): our results were

294   consistent with this pattern until late winter, when anthocyanin accumulation rebounded (07-03-

295   2017, Leaf Age 271 days, Fig. 3B). Initially, there were no differences in anthocyanin concentration

296   with respect to height on the hummocks, and this remained the case until 24-03-2017 (Leaf Age 313

297   days) after the middle-hummock had emerged but while the lower hummock was still beneath the

298   snow pack. During this period (24-03-2017 to 18-04-2017), adaxial epidermal anthocyanins continued

299   to accumulate in the upper hummock leaves (Fig. 3B) and the difference in adaxial epidermal

300   anthocyanin concentration with height was maintained, with higher values in upper hummock leaves

301   compared with the others (Fig. 3B). In the abaxial epidermis, a corresponding difference with height

302   occurred later during the 6-week spring dehardening period (19-04-2017 to 30-05-2017: Leaf Age 313-

303   355 days), persisting until abaxial anthocyanin concentration was maximal in the upper and mid

304   hummock leaves between (17/30-05-2017, Leaf Age 341-355 days). By this time, the abaxial

305   epidermal anthocyanins had already started to decline in the lower-hummock (Fig. 3B). By mid-

306   summer, both adaxial and abaxial epidermal anthocyanins had converged to similar values across the

307   three height classes (19-06-2017: Leaf Age 375) (Fig. 3B). The ratios of adaxial-to-abaxial epidermal

308   anthocyanins all declined throughout the spring, but differed among the three heights, being highest

309   in the upper-hummock leaves and lowest in the lower-hummock leaves (Fig. S3B).

310   Chlorophyll concentration per leaf area increased in young leaves until 14-02-2017 (Leaf Age

311   250 days), by which time it was lower in the lower-hummock leaves than the middle-and-upper-

312   hummock leaves (Fig. 3C). This difference persisted until the upper and middle hummocks were above

313   the snowpack but the lower hummock beneath the snowpack (18-04-2017, Leaf Age 313 days), at

314   which time chlorophyll concentration of the lower-hummock leaves increased beyond that of the

315   exposed upper and middle hummock leaves where it declined (Fig. 3C). Lower-hummock leaves

316   retained the highest chlorophyll concentration from then until midsummer by which time chlorophyll

317   values at the three heights converged again (19-06-2017, Leaf Age 394 days) (Fig. 3C).

318   The ratio leaf flavonols vs. anthocyanins (sum of both epidermis) was higher in the upper

319   hummocks than the lower hummocks through the winter (Fig. 4A). In the adaxial epidermis, the rate

320   of flavonol accumulation was faster than that of anthocyanins during the dehardening period so this

321   ratio rose, particularly in the lower hummock leaves (due to increasing flavonol accumulation as

322   described above - Fig 4A). There was negative relationship between flavonols and anthocyanins in the

323   adaxial epidermis when data are plotted for all leaves and all dates (Fig. 4B). This relationship may be

324   confounded by leaf age but nevertheless persisted for the adaxial epidermis when the relationships

325   were calculated separately for each measurement date (Table S5).

326   3.4. Seasonal changes in photosynthetic yield of PS_{II} in leaves at different heights on the *V.*

327   *vitis-idaea* hummocks

328   The F_v/F_m of leaves across the three heights on the hummocks attained their maximal values of

329   about 0.82 on reaching maturity on their second measurement following unfurling (09-07-2017, Leaf

330   Age 30 days) and F_v/F_m remained high prior to snow cover in the winter (Fig. 5A). During the winter

331   and spring, F_v/F_m was depressed most in the upper hummocks, then middle hummocks and least

332   depressed in the lower hummocks (Fig. 5A). This ranking of F_v/F_m according to height on the

333   hummocks was retained throughout the spring dehardening period (until 30-05-2017: Leaf Age 355

334   days), but by midsummer one-year old leaves at all three heights had attained similar maximal F_v/F_m

335   again (19-06-2017, Leaf Age 375 days) (Fig. 5A).

336   The φPS_{II}, measured *in situ* under ambient sunlight, varied with the solar irradiance, i.e. on clear-

337   sky measurement days φPS_{II} was very low during the dehardening period (Fig. 5B). At sub-zero

338   temperatures (e.g. -5.5 °C on 07-03-2017, Leaf Age 313 days) with clear-sky irradiance during early

339   spring, the φPS_{II} was lowest and approached zero. Though bear in mind that it was necessary to

340   remove the snow from covered leaves during winter to take the measurement, exposing them briefly

341   to higher PAR than they would otherwise have received and thus probably caused greater

342   photoinhibition than when under the snow. Nevertheless, in early spring on days 83 and 96 (leaf age

343   288 and 301 days), φPS_{II} of almost 0.2 was reached in the lower hummock leaves (Fig. 5B). Both

344   measures of photosynthetic capacity (F_v/F_m and φPS_{II}) responded to day-to-day differences in

345   environmental conditions, as evident from differences between F_v/F_m values from consecutive daily

346   measurements made on three occasions during the spring dehardening period (Fig. 5B, Leaf Age 313-

347   314, 327-328, 341-342 days).

348   3.5. The relationship between *V. vitis-idaea* photosynthetic yield*,* leaf flavonoids and
349   environmental conditions
350   We tested the relationship between temperature and F_v/F_m for leaves from the upper, middle,

351   and lower hummocks, identifying their position relative to the snowpack prior to each F_v/F_m

352   measurement. The air temperature (T_{aw}) or soil temperature (T_{sw}) at the site were positively

353   correlated with the average F_v/F_m of leaves above or below the snowpack respectively, and in order

354   to make a direct comparison with other studies of *V. vitis-idaea* (Bokhorst et al. 2010; Saarinen et al.

355   2016), we tested a range of different time periods to find the closest relationship. Of these time

356   periods, the temperature over the 2 weeks prior to each F_v/F_m measurement gave the best fit; for

357   leaves above the snowpack vs. T_{aw} (adj. R^2=0.91, Table S4), and leaves below the snowpack vs. T_{sw} (adj.

358   R^2 = 0.81, Table S4). These relationships with temperature were stronger than F_v/F_m vs. PAR on the

359   day of measurement for leaves above the snowpack (adj. R^2 = 0.74, Table S4).

360   Individual leaf measurements of F_v/F_m were also positively correlated with leaf temperature

361   (Fig. 6A) and PAR incident on the leaf (Fig. 6B) at the time of measurements, and likewise with adaxial-

362   epidermal flavonol concentration on the day of measurement (Fig. 6C). However, these relationships

363   were strongly conditioned by measurement day and held only during the period from 24-03-2017 to

364   17-05-2017: Leaf Age 281-342 days (Fig. 6). The relationships broke down once spring recovery of

365   F_v/F_m was almost complete (30-05-2017 Leaf Age 355 days – not shown) and in winter when leaf

366   temperatures were consistently around 0°C. During dehardening, all three relationships were

367   strongest in the upper hummocks and remained strong in the middle hummocks, where F_v/F_m recovery

368   coincided with an increase in adaxial epidermal flavonols during this time period, but were but much

369   less strong in the lower hummocks (Fig. 6C). The same relationship was not evident for anthocyanins

370   in either epidermis (not shown).

371   4. Discussion

372   4.1 Consistent pattern of increasing flavonoids with hummock height throughout the year

373   Differences in exposure to harsh weather conditions among adjacent plants or shoots may be

374   particularly pronounced in the understorey of open canopies typical of the boreal forest which

375   unevenly intercept solar radiation (both incoming and reflected or emitted radiation) and snow

376   (Hedstrom and Pomeroy, 1998). The undulating topography created by hummock-forming plants,

377   adds to the heterogeneity of irradiance, temperature, and snow cover (Essery et al. 2008; Pomeroy et

378 al. 2002; Sturm et al. 2001). Accordingly, a large proportion of their leaves can remain above the

379 snowpack over winter where they are subject to greater fluctuations in temperature and irradiance

380 than those beneath the snowpack. In our study, this was the case for the upper leaves of *V. vitis-idaea*

381 hummocks, which accumulated more epidermal UV-screening (attributable to flavonols) than lower

382 hummock leaves. Using a Dualex to optically detect UV-screening allowed us to repeatedly and non-

383 destructively follow changes over time on the same plant but not to gather biochemical information

384 on flavonoid composition.

385   The results of studies based on optical properties of the leaf epidermis *in vivo* and

386 measurements of whole-leaf extracts can be difficult to reconcile because the two methods are not

387 equivalent. In *V. vitis-idaea*, flavonoids can be found in the trichomes and bound to the epidermal

388 cell walls (Semerdjieva et al. 2003a, 2003b) where they will affect UV-screening, whereas they are

389 spread throughout the leaf including chloroplasts in the mesophyll and cuticular waxes in species

390 like *V. myrillus* (Semerdjieva et al. 2003b). Despite a certain lack of equivalency, it is worth noting

391 that the relative differences we found in UV-screening within *V. vitis-idaea* hummocks, among leaves

392 differing only in the time spent above and below the snowpack (Fig. 1), were larger than those

393 produced in the same species by a 25% UV supplementation (Semerdjieva et al. 2003b). A strong

394 negative correlation between accumulation of leaf flavonoids and increasing temperature can

395 obscure the effects of UV-B radiation (Coffey et al. 2017), and consequently, the differences in

396 temperature across hummock height related to differences in seasonal snow cover may partially

397 explain the vertical gradient in flavonols accumulation we report. It has not yet been elucidated

398 whether both temperature and UV-B radiation may regulate flavonoid accumulation via the uvr8

399 pathway, but this would constitute an interesting future avenue of research.

400   The response of *V. vitis-idaea* leaves to UV radiation at high latitudes has already been

401 extensively studied in the context of northern-hemisphere stratospheric ozone depletion in the late

402 1990s and 2000s (e.g. Johansson et al. 1995; Phoenix et al. 2001; Semerdjieva et al. 2003a). The

403   leaves of evergreen species emerge from snow cover in spring when seasonal UV-B exposure can be

404   periodically high, but a meta-analysis generally found evergreen species like *V. vitis-idaea* to be

405   resilient against such seasonal increases in UV radiation (Newsham and Robinson, 2009). The

406   modest flavonoid induction caused by increased UV-B radiation could help protect from

407   photodamage to PS_{II} and improve antioxidant capacity during dehardening at the start of the

408   growing season when high irradiance and cold temperatures in unison exacerbate photoinhibition

409   (Takahashi and Badger 2011).

410   　　　Most studies from the 1990s-early-2000s have presented these data on a per mass basis

411   which is not as easily interpretable as leaf-area based measures in terms of screening. Differences in

412   LMA among the one-year old leaves in our experiment caused the effect of hummock height on

413   flavonols and anthocyanins per-leaf-area to be reduced when considered on a per-mass basis (Table

414   2). Likewise, the effect of UV-B radiation on flavonol accumulation is sometimes smaller on a per-

415   leaf-mass than per-leaf-area basis in understorey shrubs, because UV-B exposure causes mature

416   leaves to be thicker and tougher (Newsham and Robinson, 2009, Semerdjieva et al. 2003a).

417   　　　The increase in the ratio of adaxial to abaxial epidermal flavonols in the lower hummock

418   leaves (Fig. S3A) during snow melt suggests that accumulation of adaxial flavonols could aid in

419   photoprotection in addition to amelioration of cold temperature stress as antioxidants (Chalker-

420   Scott and Scott, 2004). Comparison of the UV screening on either side of a leaf show this

421   relationship to be highly species specific and environmentally dependent: sometimes UV screening

422   by the adaxial and abaxial epidermises are strongly positivity correlated (Bartlod et al. 2007, Siipola

423   et al. 2014) sometimes this relationship is weak (Kolb and Pfündel 2005, Morales et al. 2011), as was

424   the case in our study. The accumulation of epidermal flavonoids is commonly attributed to exposure

425   to UV-B radiation, or high irradiance of UV-A radiation and blue light (Barnes et al., 2017), although

426   flavonoid accumulation is also known to increase in response to cold temperatures (Agati et al,

427   2013). Temporal differences in flavonoid (both flavonol and anthocyanin) accumulation and their

428   localization in the planophile leaves of *V. vitis-idaea* might reflect differences in the allocation of

429   resources based on differences between the adaxial and abaxial epidermis in their relative exposure

430   to cold and high irradiance.

431   Over the whole dataset, there was an inverse correlation between the anthocyanin and

432   flavonol concentrations in the adaxial epidermis, even though during spring dehardening both

433   tended to increase (Fig. 4). A similar pattern showing a trade-off between these two phenolic

434   compounds was reported in *Arabidopsis thaliana* under controlled conditions, where the ratio of

435   flavonoids to anthocyanins was higher when they received either blue light or UV-A radiation

436   (Brelsford et al, 2018). Anthocyanins are typically found in greater amounts in young leaves and

437   have a specific role as antioxidants involved in cold and high-light tolerance (Havaux and Kloppstech,

438   2001; Landi et al. 2015). Although anthocyanins only infrequently respond to elevated UV-B

439   radiation (Searles et al. 2001), both flavonols and anthocyanins are phenolic compounds of the

440   flavonoid pathway and it is possible that regulation of this pathway shifts during spring to emphasize

441   the photoprotective function of flavonols driven by seasonally-increasing irradiance of blue light and

442   UV radiation at the expense of anthocyanins whose antioxidant function would be more important

443   during winter (Agati et al. 2013).

444   4.2 Prolonged snow cover accelerates recovery of photosynthetic capacity during spring
445   dehardening
446   The timing of spring dehardening in our study was better correlated with temperature than

447   irradiance, and the relationship with temperature was maintained over 2 weeks prior to measurement

448   (Fig 6), in agreement with Karlsson (1985). Several studies have found a consistent relationship

449   between the ambient temperature and F_v/F_m of *V. vitis-idaea* leaves across a range of latitudes

450   (Bokhorst et al. 2010; Saarinen et al. 2016; Table S6 and S7) and in *Pinus sylvestris* at our Hyytiälä site

451   (Porcar-Castell et al. 2011). Saarinen et al. (2016) report that F_v/F_m in leaves of *V. vitis-idaea* plants

452   was depressed by snow removal or even when covered by only a thin layer of snow. In our study,

453   upper-hummock leaves had the lowest F_v/F_m and mid-hummock leaves, which were also only thinly

454   snow-covered, had lower F_v/F_m than lower-hummock leaves. Prior to their emergence from under the

455   snow, the F_v/F_m of lower-hummock leaves was already increasing. It is conceivable this recovery was

456   aided by the low to moderate irradiance that penetrates the upper cm's of the snowpack, potentially

457   activating photoprotective mechanisms during snowmelt (Richardson and Salisbury, 1977). This could

458   partially explain why the difference in F_v/F_m was maintained between leaves at different heights on

459   the hummocks for several weeks following snow melt rather than the lower leaves exhibiting a cold-

460   shock response upon exposure.

461   Large fluctuations in temperature and sustained winter warming can both prematurely activate

462   dehardening creating the potential to bring forward the growing season but also increasing the risk of

463   damage if cold winter temperatures return (Bokhorst et al. 2008). Photoinhibition of exposed shoots

464   was high in our study, and leaf chlorophyll concentration dropped in the upper and middle hummock

465   leaves during the spring dehardening period but remained stable in the lower hummock leaves

466   (surpassing that in the upper hummocks) following emergence from under the snowpack. The

467   regulation of leaf chlorophyll may be a means of acclimation, to reduce photon capture under high

468   irradiance to balance the limited carboxylation capacity at low temperature in the upper and middle

469   hummocks when photoprotective mechanisms were surpassed.

470   Winter- and spring-time depression of F_v/F_m and φPS_{II} was largely attributable to a decrease in

471   maximal fluorescence (F_m and F_m` respectively) rather than an increase in $F_0$ or $F_0$` (Fig. S5). This

472   suggests that the reduction in photosynthetic capacity is the result of decreased light harvesting by

473   PS_{II} lowering the F_m and F_m`. Such a result has previously been attributed to smaller antenna size or

474   sustained NPQ associated with decreased epoxidation of xanthophylls or PS_{II} reaction centre

475   quenching under cold temperatures (Ivanov et al. 2008), rather than damage or disassembly of the

476   PS_{II} LHC_{II} core subunit super-complexes which would be reflected in higher F_0 or F_0` (Öquist and Huner,

477   2003). The positive correlation between epidermal flavonols and F_v/F_m found in the upper and middle,

478   but not lower, hummock leaves during dehardening suggests that these leaves are acclimating to the

479 more-exposed conditions. This is consistent with some mechanistic studies which report flavonoids

480 to determine of cold acclimation and frost tolerance of *Arabidopsis thaliana* (Havaux and Kloppstech,

481 2001; Shulz et al. 2016). However, knowledge of the contribution of xanthophyll cycle pigments at

482 different hummock heights, which was not quantified in this study, would allow us to better describe

483 this response (Adams et al. 2004).

484 4.3 Ecological consequences of changing snow cover for *V. vitis-idaea*

485   In our study in central Finland, all leaves achieved full functionality following dehardening.

486 Likewise, Saarinen et al. (2016), found that exposed leaves of *V. vitis-idaea*, subjected to experimental

487 removal of the snow pack during winter, fully recovered PS_{II} capacity during the spring in southern

488 Finland, abet later than those from which snow was not removed. This result is consistent with other

489 Arctic shrub species, e.g. *Rhododendron ferrugineum* L, whose leaves above the snow in winter have

490 transiently reduced F_v/F_m compared with those that are covered by snow (Neuner et al. 1999). These

491 findings suggest that *V. vitis-idaea* could benefit from the expected warmer winters and earlier snow

492 melt in Finland, and much of the rest of its range (Rasmus et al. 2004 Henry 2008; Jylhä et al. 2008),

493 by dehardening earlier and contributing more in the future to photosynthetic carbon gain in the

494 understorey (Gehrmann et al., 2018; Larsen et al. 2007). However, this does not exclude the possibility

495 that environmental changes involving reduced duration or size of snowpack can also be detrimental

496 to understorey plants. Further north than our study, across a range of habitats in the Arctic where

497 winters are colder than in central Finland, a reduced snow pack can cause sufficient cold stress to

498 damage the leaves and shoots of shrubs including *V. vitis-idaea* (Preece and Phoenix 2013). Reduced

499 snow cover can also lead to colder soils and prolonged freezing temperatures which can be a problem

500 over a wider geographical range across Europe causing root mortality and affecting biogeochemical

501 cycling (Groffman et al. 2001; Helama et al. 2011).

502   The link between increasing temperature and irradiance, and F_v/F_m recovery in *V. vitis-idaea*

503 during dehardening reinforces established knowledge of this and other species (Ensminger et al.

504 2004; Havaux and Kloppstech 2001; Muller et al. 2005; Neuner et al. 1999; Porcar-Castell et al. 2008;

505   Saarinen et al. 2016). At our site in central Finland, winter temperatures are relatively mild (-7.37 $\pm$

506   1.15 °C; 10-year mean $\pm$ 1 SD January temperature, 2008-2018), and are expected to increase by 4-

507   11 °C by the end of the century (Jylhä et al. 2008; Finland's Seventh National Communication under

508   the United Nations Framework Convention on Climate Change 2017). Winter warming is forecast to

509   increase productivity at the stand level (Porcar-Castell et al. 2011). However, the expected 30-40%

510   reduction in snow cover (Jylhä et al. 2008; Finland's Seventh National Communication under the

511   United Nations Framework Convention on Climate Change 2017) may lead to increased soil freezing

512   or freeze thaw cycles which may partially negate the increase in productivity (Taulavuori et al. 2011).

513   New estimates of ecosystem carbon assimilation aim to include the contribution of understorey

514   vegetation in addition to that by tree species (Nikopensius et al. 2015, Starr and Oberbauer, 2003).

515   To estimate understorey photosynthesis during spring a better understanding of how it is affected

516   by uneven snow cover under patchy canopies will be needed (Blume-Werry et al. 2016; Kreyling et

517   al. 2012).

518   5.   Conclusions (preferably 10-15 lines & no references)

519   In our study, there was a positive correlative relationship during the winter and spring

520   between UV-screening by epidermal flavonols and F_v/F_m in leaves that were largely above the

521   snowpack. More flavonols and anthocyanins accumulated in these upper-hummock leaves

522   compared to leaves below the snowpack. The cross-protection provided by epidermal flavonols to

523   cold and high light is likely to be regulated by solar radiation and temperature, but further research

524   is needed to disentangle the roles of these two cues. Recent evidence that leaves can rapidly adjust

525   their UV-screening to acclimate to light conditions (Barnes et al. 2017) suggests that a high-degree of

526   fine tuning of these responses is possible, and our finding that flavonoids (flavonols and

527   anthocyanins) accumulate in the upper hummocks even in cold conditions when metabolic activity is

528   low supports this assertion. Leaves with a higher index of epidermal flavonols tended to have a

529   lower epidermal anthocyanin index, and a compositional shift in this ratio towards flavonols during

530   spring suggests fine-tuning of acclimation in response to increasing irradiance and a less cold

531   temperatures. Finally, on hummocks at our site in central Finland, leaves that remained under snow

532   cover longer surpassed exposed leaves in their F_v/F_m directly following snowmelt despite having

533   lower epidermal flavonols and anthocyanins but all leaves eventually reached a similar maximum

534   photosynthetic capacity.

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738

739 Tables

740 Table 1: Maximum and Minimum Air and Soil Temperatures, and maximum PAR (PPFD) on the days

741 of chlorophyll fluorescence and leaf pigments measurements. Freeze-thaw cycles are calculated for

742 the period between measurements (usually 2 weeks, including 14-02-2017) and represent the

743 number of times that the temperature passed above and returned below freezing during this period.

744 Tree trunk temperatures were recorded at breast height on the completed shaded north side of the

745 tree during winter and spring.

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749 upper and middle hummocks from lower hummock values are given in parentheses. ANOVA results

750 given in Table S5

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756 measurements. B. Daily time course of understorey photosynthetically active radiation (PAR) at 0.6

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758 November 2017), and including photosynthetic recovery period (June 2017 to November 2017) and

759 subsequent new leaf unfurling in June 2017.

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761 a sunfleck and canopy shade, and in an adjacent open field, in clear sky conditions during the

762 measurement period on (A) 14-02-2017, (B) 07-03-2017 and (C) 19-06-2017. Spectral photon

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769   side) leaf epidermis, at the 3 heights on the hummock, and the period under the snowpack marked

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781   Fig. 6: The relationship between F_v/F_m and (A) leaf temperature, (B) PAR and (C) epidermal flavonols

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784   2017 to 18-05-2017) when the temperature is above freezing but leaves have not yet recovered to

785   maximal F_v/F_m of 0.82.

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787   Online Supplemental Material

788   Table S1: Spectral photon irradiance at the *V. vitis-idaea* hummocks. Spectral integrals and photo

789   ratios calculated from spectral irradiance (Fig S2) measured in shade and sunflecks on three different

790   dates (Feb, 14-02-2017; Mar 07-03-2017; Jun 19-06-17). The mean ± 1SE for the four hummocks are

791   given plus the % decrease compared with adjacent open full sun measurements.

792   Table S2: Plant area index (PAI) and sunfleck duration on 20-07-2017 at the four hummocks sampled

793   in the study, calculated from the hemispherical image analysis.

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795   side, leaf age, and their interactions on leaf pigments (Figs 3 and 4), chlorophyll fluorescence (Fig. 5),

796   quenching (Figs S4 and S5), Leaf area and mass-based pigment values (Table 2) (df = degrees of

797   freedom)

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800   below the snowpack were tested against air and soil temperature respectively (2 weeks only). The

801   mean of F_v/F_m over 4 hummocks on each of 6 measurement dates during the period of spring

802   dehardening was used (leaf age 228-375, 24-03-2017 to 19-06-2017).

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804   epidermis separately. Each measurement date is tested separately for leaves at each hummock

805   height.

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807   F_v/F_m of *V. vitis-idaea* leaves at the time of measurement on date 2009-03-23.

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809   F_v/F_m of *V. vitis-idaea* leaves at the time of measurement in 2008 and 2009.

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811   with IrfanView 4.44 for all the original hemispherical photos on the hummocks sampled at the

812   measuring site on 20-07-2017.

813   Fig. S2: Solar spectral Irradiance at midday measured at the experimental site under a pine canopy in

814   a sunfleck and canopy shade, and in an adjacent open field, in clear sky conditions during the

815   measurement period on 14-02-2017, 07-03-2017 and 19-06-2017. (as Fig. 2 before normalisation).

816   Presented according to date (left) and according to canopy position (right). Spectral integrals and

817   photon ratios in Table S1.

818   Fig. S3: Ratio of adaxial to abaxial epidermal (A) flavonol and (B) anthocyanin indices, measured by

819   Dualex. Ratios calculated from the data presented in Fig. 3, over the period from leaf emergence

820   until leaves of *V. vitis-idaea* were one-and-a-half years old. Mean $\pm$ 1 SE of the 4 hummocks (n=4)

821   are plotted at 3 hummock heights, and the period under the snowpack marked with vertical lines. *

822   indicates a significant difference with height on the adjacent date, ANOVA summaries in Table S3.

823   Fig. S4: The parameters F_m and F_0 (A & B) are used to calculate F_v/F_m, the maximum quantum yield of

824   photosystem II photochemistry (Fig 5A), and F_m` and F (C & D) are used to calculate φPSII the

825   effective quantum yield of photosystem II photochemistry (Fig 5B). The *ETR* (E), electron transfer

826   rate is calculated from φPSII and PAR incident on the leaf (F). The period covers leaf emergence until

827   leaves of *V. vitis-idaea* were one-and-a-half years old is given. Mean $\pm$ 1 SE of the 4 hummocks (n=4)

828   are plotted, and the period under the snowpack marked with vertical lines. Quenching coefficients

829   calculated from these data in Fig. S5.

830   Fig. S4: A. The photochemical ($_qP$) and non-photochemical quenching (NPQ) and fraction of PSII

831   centers that are 'open' ($_qL$) calculated from chlorophyll fluorescence measurements, defined

832   according to Baker, 2008 (Chlorophyll Fluorescence: A Probe of Photosynthesis In Vivo, Annu. Rev.

833   Plant Biol. 2008. 59:89–113 doi:10.1146/annurev.arplant.59.032607.092759). Time series over the

834   period from leaf emergence until leaves of *V. vitis-idaea* were one-and-a-half years old. Mean $\pm$ 1 SE

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838 of times that the temperature passed above and returned below freezing during this period. Tree trunk temperatures were recorded at breast height on the
839 completed shaded north side of the tree during winter and spring.

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| Date of Measurement	DOY	Leaf Age Days	Max-Air Temperature °C	Min-Air Temperature °C	Max-Soil Temperature °C	Min-Soil Temperature °C	Max-PAR µmol m^{-1}s^{-1}	Freeze-thaw cycles	Snow Temperature °C	Tree Trunk Temperature (North)°C
14-02-2017	45	250	5.3	-1.3	0.5	0.3	112.9	34	--	--
07-03-2017	66	271	-0.4	-10.4	0.0	-1.2	289.5	8	-7.9	-6.6
24-03-2017	83	288	1.8	-4.4	0.4	0.2	474.7	14	-10.0	-4.2
06-04-2017	96	301	8.6	-0.3	0.5	0.4	764.4	15	-3.5	0.8
18-04-2017	108	313	3.7	-7.3	0.3	-0.2	677.0	--	-2.6	-1.4
19-04-2017	109	314	5.5	-5.9	0.4	-0.1	519.8	14	-2.7	2.2
02-05-2017	122	327	12.2	0.2	0.6	-0.4	828.9	--	-3.0	8.7
03-05-2017	123	328	10.9	0.6	0.8	0.5	868.8	17	-2.8	8.1
16-05-2017	136	341	13.5	-2.7	3.4	1.1	919.6	--	--	7.7
17-05-2017	137	342	10.4	1.6	3.7	2.1	271.1	16	--	6.6
30-05-2017	150	355	13.6	0.1	7.6	4.7	290.0	--	--	9.8
19-06-2017	170	375	20.9	9.7	11.9	9.9	932.3	3	--	--
08-07-2017	189	394/29	20.3	7.2	12.7	9.2	836.8	1	--	--
09-07-2017	190	395/30	21.9	9.9	13.9	10.3	899.6	--	--	--
08-11-2017	312	517/152	5.2	2.7	4.3	0.4	51.7	34	--	--

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843 Table 2: Comparison of leaf pigment measurements and leaf morphological traits on per area and per mass basis. Final harvest of one-year old leaves (leaf
844 age 394 days) and young leaves (leaf age 29 days) leaves of *V.vitis-idaea* in July 2017. Mean of 4 hummocks ± 1 SE. Percentage changes of upper and
845 middle hummocks from lower hummock values are given in parentheses. ANOVA results given in Table S5.

| Leaf Trait	Young leaves (Leaf Age 29 days old)			One-year old leaves (Leaf Age 394 days old)		
	Lower Hummock	Middle Hummock	Upper Hummock	Lower Hummock	Middle Hummock	Upper Hummock
Morphological Trait						
Leaf Area (mm^2)	240 ± 9	240 ± 15 (0%)	246 ± 12.3 (+3%)	174 ± 11	206 ± 27 (+18%)	188 ± 20.6 (+8%)
Leaf Dry Matter Content (mg)	14.5 ± 1.9	13.6 ± 1.5 (-6%)	13.7 ± 1.6 (-5%)	28.9 ± 2.4	35.8 ± 4.8 (+24%)	34.9 ± 3.9 (+21%)
Leaf Mass Area (mg mm^{-2})	0.060 ± 0.006	0.056 ±0.003 (-6%)	0.055 ±0.005 (-8%)	0.166 ± 0.005	0.174 ± 0.01(+5%)	0.187 ± 0.01(+12%)
Dualex (per area)						
Chlorophyll index per leaf area (Dualex A mm^{-2})	78.6 ± 5.8	74.6 ± 6.4 (-5%)	77.8 ± 4.8 (-1%)	122.6 ± 5.6	136.8 ± 23.4 (+12%)	121.7 ± 17 (-1%)
Epidermal flavonol index per leaf area (Dualex A mm^{-2})	5.6 ± 0.7	6.8 ± 0.4 (+22%)	8.0 ± 0.7 (+43%)	5.7 ± 0.1	7.2 ± 0.9 (+25%)	6.8 ± 0.9 (+19%)
Epidermal anthocyanin index per leaf area (Dualex A mm^{-2})	0.89 ± 0.01	1.03 ± 0.07 (+16%)	1.07 ± 0.08 (+20%)	0.26 ± 0.03	0.36 ± 0.03 (+39%)	0.34 ± 0.02 (+29%)
Dualex (per mass)						
Chlorophyll index per leaf mass (Dualex A mg^{-2})	1346 ± 162	1322 ± 52 (-2%)	1445 ± 173 (+7%)	739 ± 14	798 ± 154 (+8%)	657 ± 99 (-11%)
Epidermal flavonol index per leaf mass (Dualex A mg^{-2})	93 ± 6	120 ± 4 (+30%)	146 ± 13 (+57%)	35 ± 1	42 ± 6 (+20%)	37 ± 5 (+6%)
Epidermal anthocyanin index per leaf mass (Dualex A mg^{-2})	15.2 ± 1.3	18.3 ± 0.6 (+20%)	19.4 ± 0.3 (+27%)	1.6 ± 0.2	2.1 ± 0.3 (+34%)	1.8 ± 0.1 (+15%)

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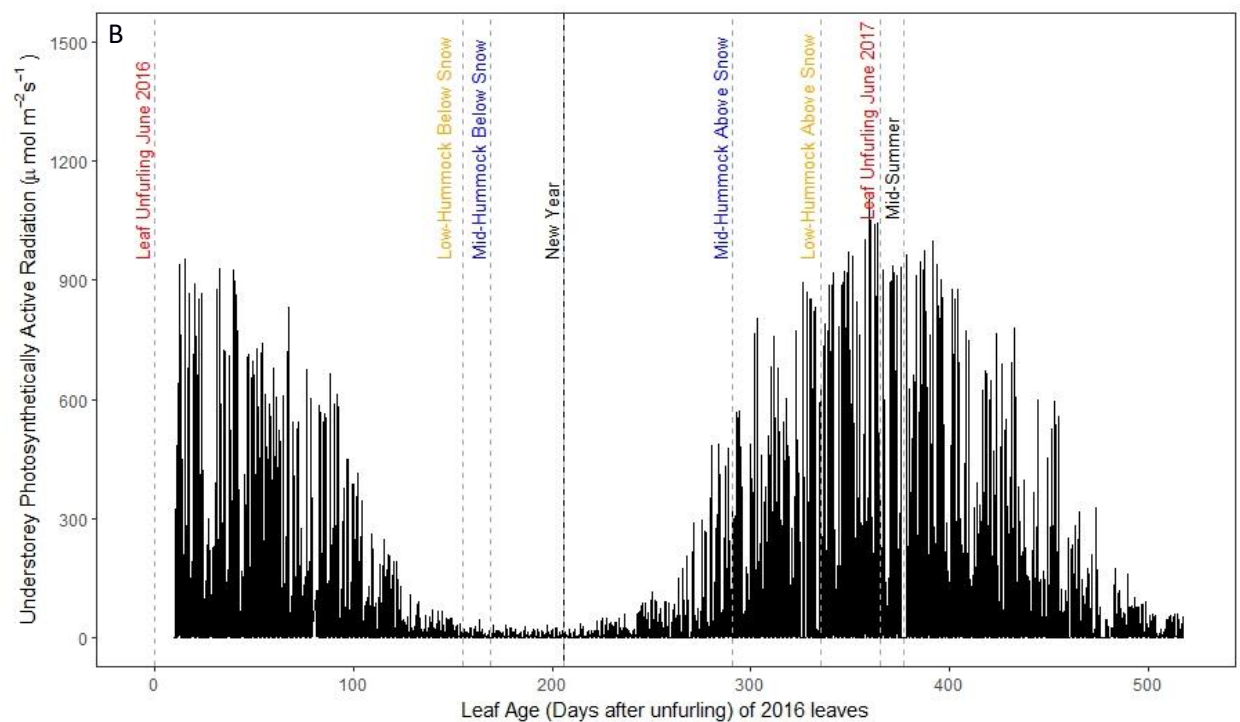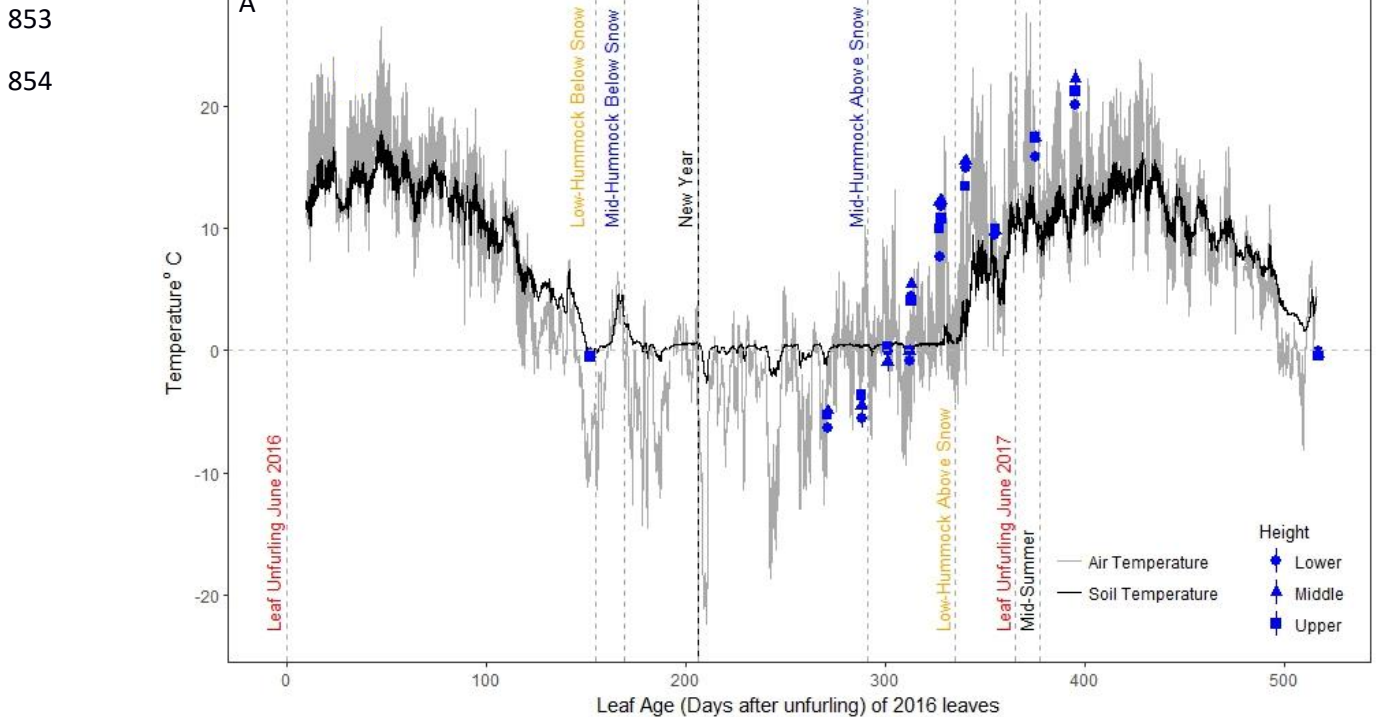
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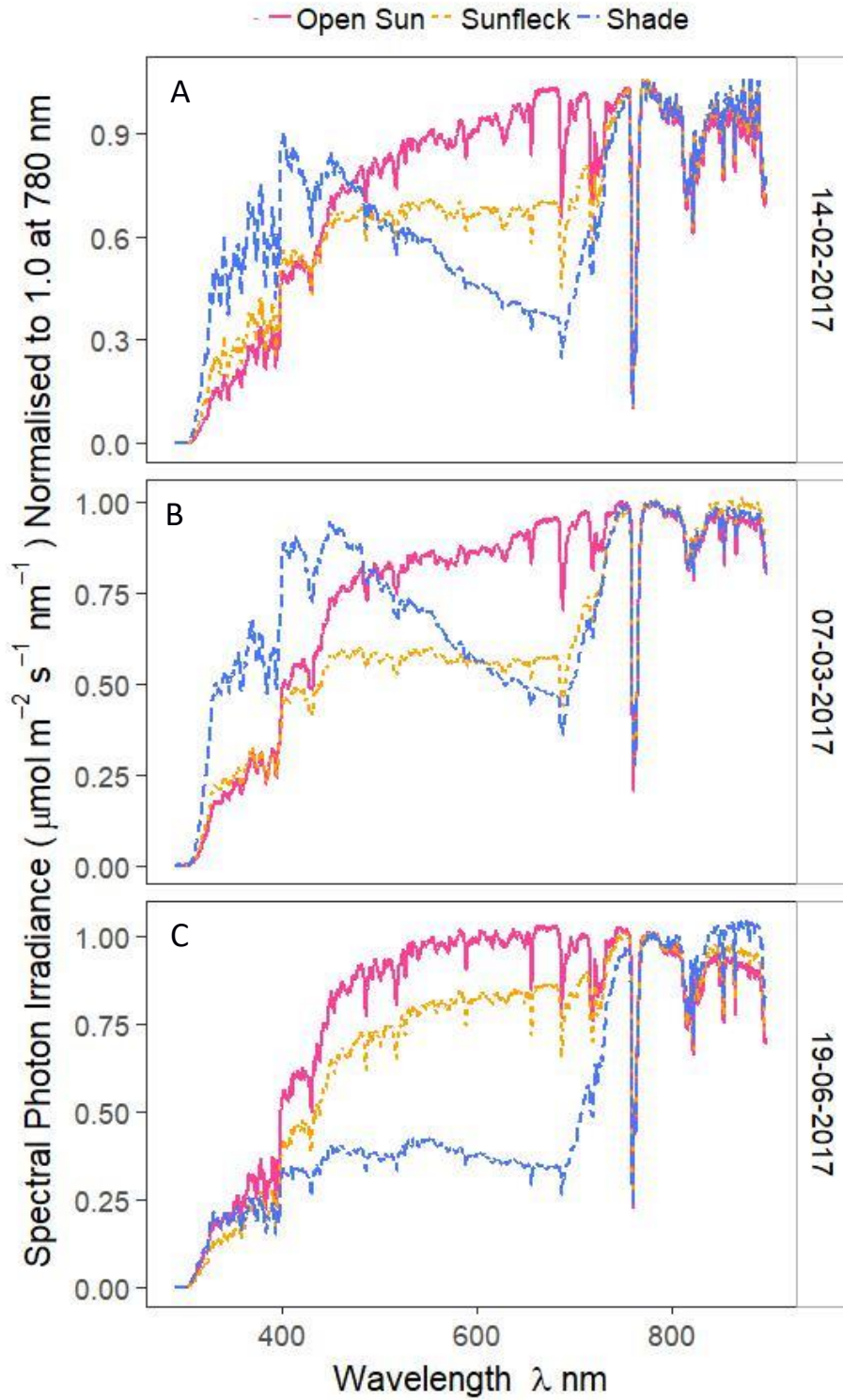
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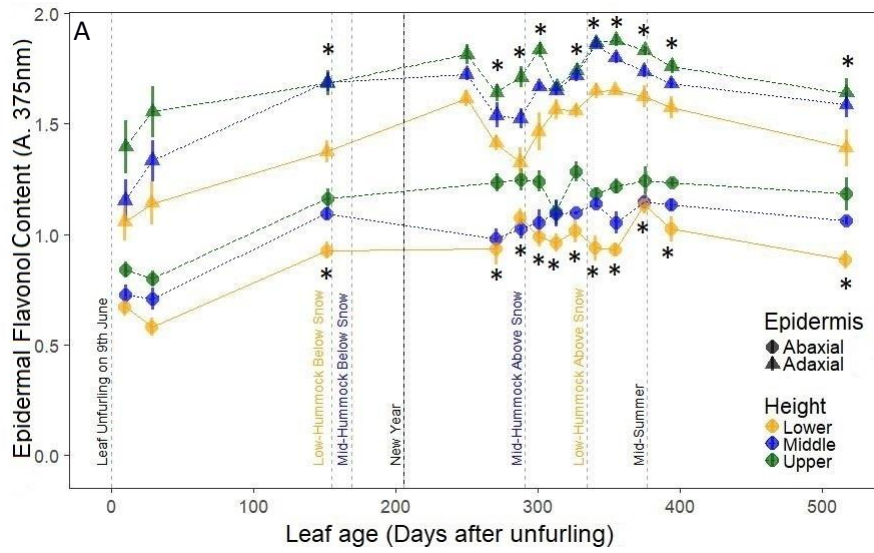
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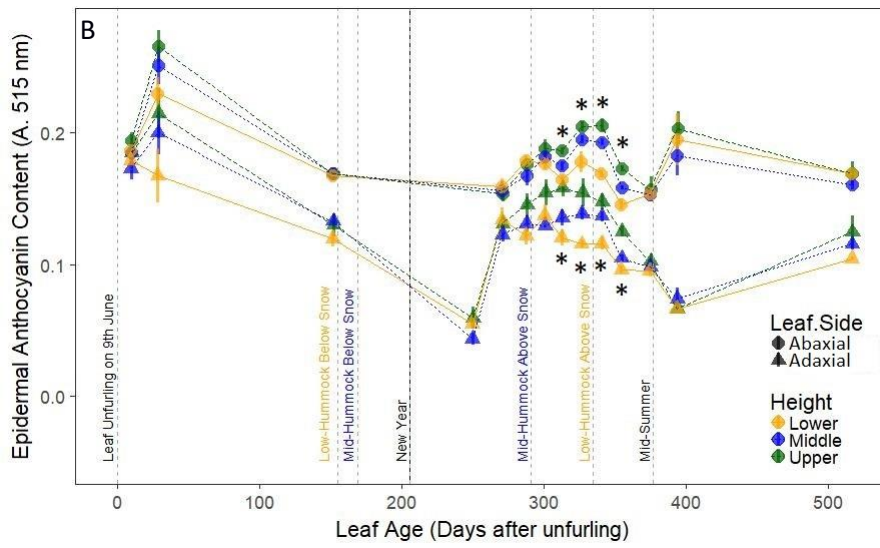
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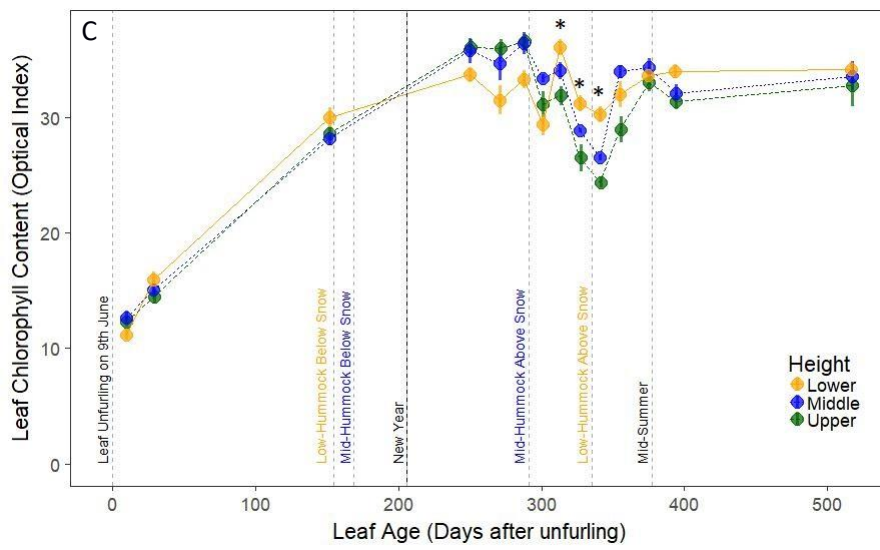
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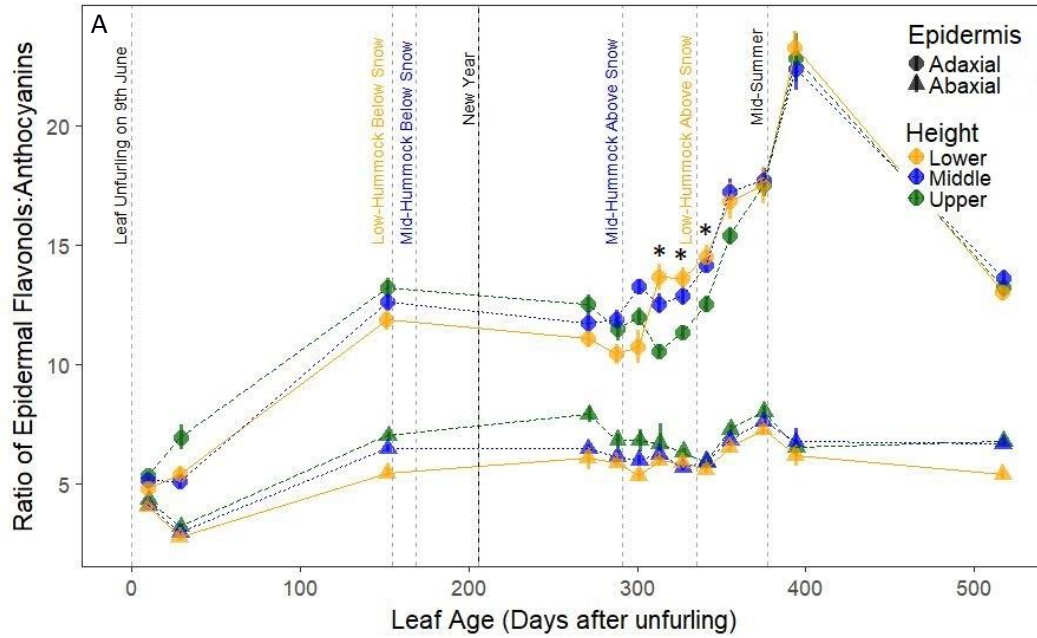
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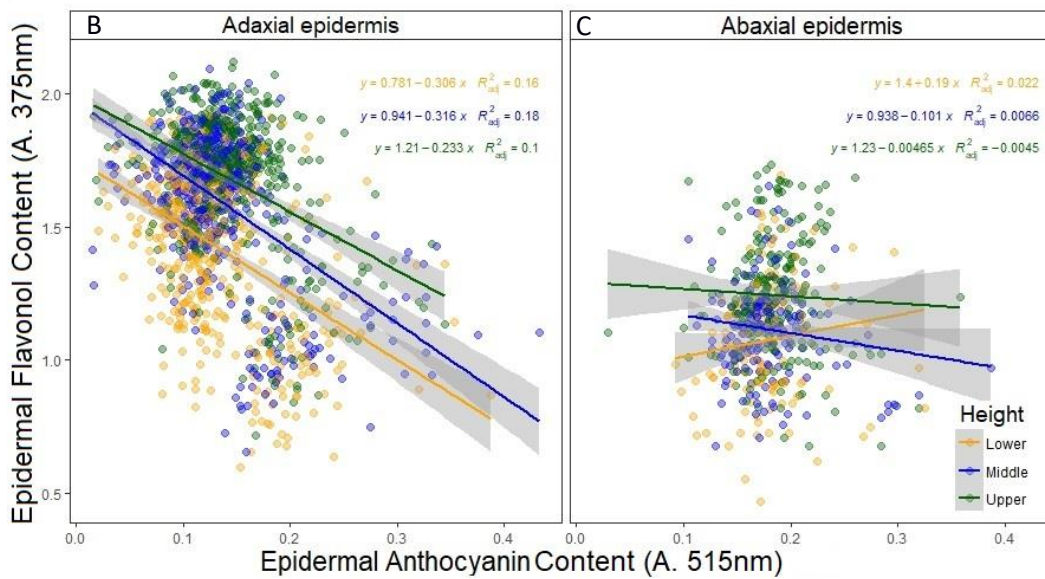
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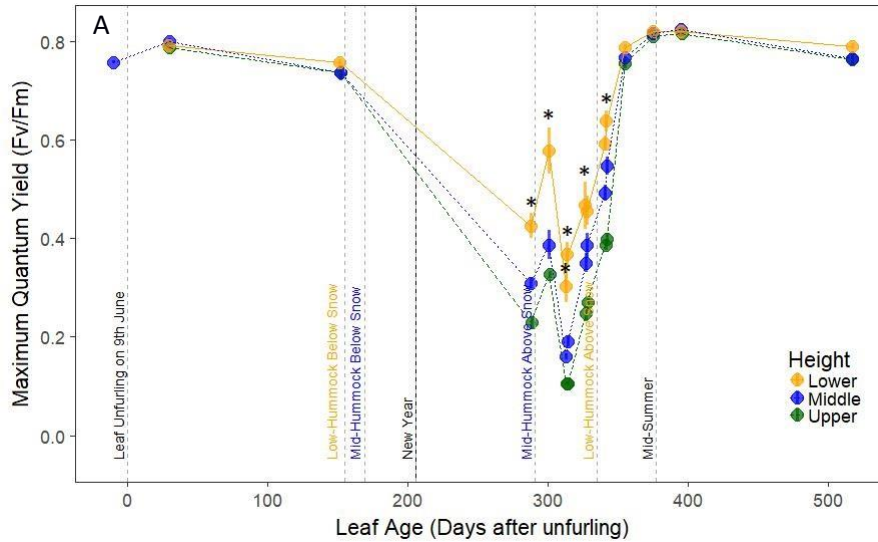
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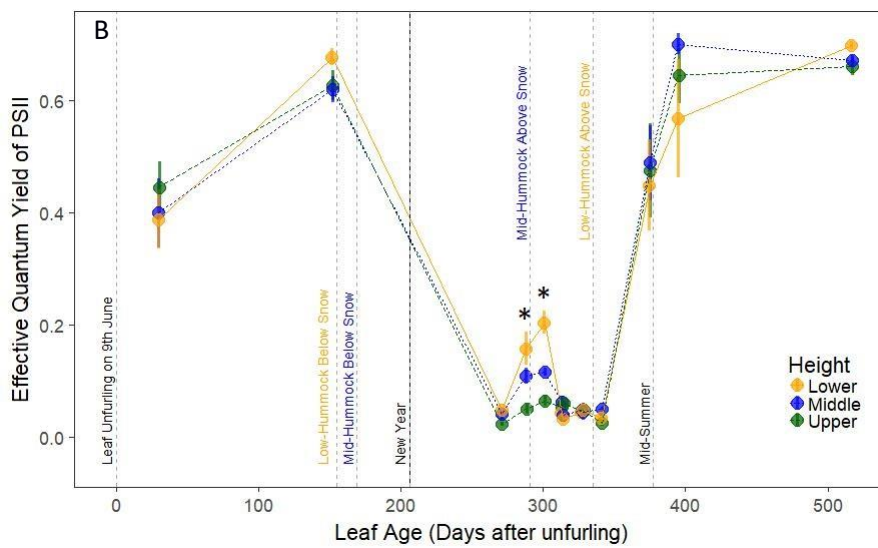
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