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3 1 **Constitutive changes in pigment concentrations: Implications for**  
4 **estimating isoprene emissions using the photochemical reflectance index**  
5 **(PRI)**  
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30 13 **Abstract**  
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32 14 The photochemical reflectance index (PRI), through its relationship with light use efficiency (LUE)  
33 15 and xanthophyll cycle activity, has recently been shown to hold potential for tracking isoprene  
34 16 emissions from vegetation. However, both PRI and isoprene emissions can also be influenced by  
35 17 changes in carotenoid pigment concentrations. Xanthophyll cycle activity and changes in carotenoid  
36 18 concentrations operate over different timescales but the importance of constitutive changes in pigment  
37 19 concentrations for accurately estimating isoprene emissions using PRI is unknown. To clarify the  
38 20 physiological mechanisms behind the PRI-isoprene relationship, the light environment of potted *Salix*  
39 21 *viminalis* (dwarf willow) trees was modified to induce acclimation in photosynthetic rates,  
40 22 phytopigments, isoprene emissions and PRI. Acclimation resulted in differences in pigment  
41 23 concentrations, isoprene emissions and PRI. Constitutive changes in carotenoid concentration were  
42 24 significantly correlated with both isoprene emissions and PRI, suggesting that the relationship  
43 25 between PRI and isoprene emissions is significantly influenced by constitutive pigment changes.  
44 26 Consequently knowledge regarding how isoprene emissions are affected by both longer term changes  
45 27 in total carotenoid concentrations and shorter term dynamic adjustments of LUE is required to  
46 28 facilitate interpretation of PRI for monitoring isoprene emissions.  
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57 29 **Abbreviations**  
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3 30 A, antheraxanthin;  $\beta$ C,  $\beta$ -carotene; BVOC biogenic volatile organic compounds; Car, carotenoids;  
4 31 EPS, epoxidation state; L, lutein; LUE, light use efficiency; N, neoxanthin; PRI, photochemical  
5 32 reflectance index; V, violaxanthin; Z, zeaxanthin.  
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## 10 34 **Introduction**

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13 35 Biogenic volatile organic compounds (BVOCs) are a chemically reactive carbon flux and thus play an  
14 36 important role in global atmospheric chemistry. BVOCs affect the distribution and residence time of  
15 37 short-lived radiatively active trace gases such as tropospheric ozone ( $O_3$ ) and methane ( $CH_4$ ; Fiore et  
16 38 al., 2012). Land-based vegetation returns about 1 PgC of total BVOC emissions to the atmosphere  
17 39 each year (Guenther et al., 2012). The reasons why plants invest in BVOC emissions remains unclear,  
18 40 although ecological and physiological roles of emissions are thought to include the ability to attract  
19 41 pollinators and decrease pathogen attacks and herbivory (Gershenson, 1994; Michelozzi, 1999;  
20 42 Niinemets et al., 2013); to increase leaf thermotolerance (Singsaas et al., 1997); and to protect the  
21 43 plant against oxidative stress (Vickers et al., 2009).

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27 44 Isoprene is the most dominant BVOC emitted by plants, representing almost half of the total annual  
28 45 flux of reactive carbon (Guenther et al., 2012). Whilst it is generally accepted that anthropogenic and  
29 46 natural perturbations to isoprene emissions are likely to have an important influence on regional  
30 47 climates and feedbacks to global climate (Pitman et al., 2012), there is a lack of quantitative  
31 48 understanding of the mechanisms controlling patterns of emissions over long timescales (weeks to  
32 49 months; Porcar-Castell et al., 2009) and across regions (Foster et al., 2014), which makes modelling  
33 50 emissions challenging. Furthermore, many isoprene emission models, both empirical and process-  
34 51 based, base estimations on linkages between isoprene emissions and plant primary productivity  
35 52 (Arneth et al., 2007; Foster et al., 2014; Guenther et al., 1993), even though it is known that isoprene  
36 53 and photosynthetic activity can become decoupled under conditions such as high temperatures (Unger  
37 54 et al., 2013); during drought stress (Monson et al., 2007; Niinemets et al., 2010); under increasing  
38 55 atmospheric  $CO_2$  concentration (Monson et al., 2007; Rosenstiel et al., 2003); and due to the presence  
39 56 of time lags between the seasonal onset of photosynthesis and isoprene emissions (Monson et al.,  
40 57 1994; Pressley et al., 2005). As a consequence, there is a need to base isoprene estimations on  
41 58 fundamental links between emissions and the biological processes that affect these emissions.  
42 59 Emission models such as the Model of Emissions of Gases and Aerosols from Nature (MEGAN;  
43 60 Guenther et al., 2006), go some way towards achieving this aim, but they are increasingly complex  
44 61 and uncertainty in their estimations can be high (Guenther et al., 2006).

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56 62 Recently Peñuelas *et al.* (2013) suggested a simple approach for estimating isoprenoid (i.e. isoprene  
57 63 and monoterpene) emissions using remotely sensed data. Unlike many previous attempts at using  
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3 64 remote sensing to estimate isoprenoid emissions, which focus on the detection of formaldehyde (an  
4 65 isoprenoid oxidation product) in the atmosphere (Barkley et al., 2008; Foster et al., 2014; Palmer et  
5 66 al., 2003), Peñuelas et al. (2013) showed that a simple spectral index (the photochemical reflectance  
6 67 index; Gamon et al. 1992) that is indicative of changes in plant light use efficiency (LUE), when  
7 68 combined with basal emission factors, was as good a predictor for isoprenoid emissions as some  
8 69 standard emission models (Peñuelas et al., 2013). Furthermore, the high temporal resolution and  
9 70 spatially extensive nature of remotely sensed data can help capture some spatial and temporal  
10 71 variability in emissions that other models may miss (Peñuelas et al., 2013).

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16 72 The use of LUE as an indicator of isoprene emissions is based on the idea that when LUE is low (e.g.  
17 73 under conditions of high irradiance), photosynthesis is reduced and thus more reducing power  
18 74 (NADPH) is available for isoprene production (Morfopoulos et al., 2013). Previous studies have also  
19 75 shown strong links between LUE and the photochemical reflectance index (PRI) due to changes in the  
20 76 levels of photoprotective xanthophyll cycle pigments in response to excess irradiance, which can be  
21 77 detected through changes in leaf reflectance (Gamon et al., 1992; Peñuelas et al., 1995). In the short  
22 78 term (seconds to hours), conversions of xanthophyll cycle pigments between their epoxidised and de-  
23 79 epoxidised states (e.g. conversion of violaxanthin to zeaxanthin via antheraxanthin; Demmig-Adams  
24 80 and Adams, 1992) results in rapid, and typically temporary, facultative PRI changes that scale with  
25 81 LUE (Gamon et al., 1992; Peñuelas et al., 1995). Whilst at these time scales, PRI and LUE are  
26 82 closely correlated at the leaf-scale, at longer time scales (weeks to months) correlations between LUE  
27 83 and PRI are often variable as other factors besides xanthophyll pigment conversion may be driving the  
28 84 PRI signal (Filella et al., 2009; Gamon et al., 1997; Porcar-Castell et al., 2012; Wong and Gamon,  
29 85 2014). Similarly, at longer time scales, isoprene emissions are also thought to be influenced by  
30 86 environmental factors other than temperature and light (Geron et al., 2000; Harley et al., 1996;  
31 87 Pressley et al., 2005). In a series of unrelated studies, at longer time scales changes in the carotenoid  
32 88 concentration has been shown to influence both PRI, through reflectance changes caused by changes  
33 89 in the chlorophyll to carotenoid pigment ratio (Gamon and Berry, 2012; Wong and Gamon, 2014),  
34 90 and isoprene emissions, thought to be caused by either substrate availability or complementary  
35 91 functionality (Owen and Peñuelas, 2013).

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49 93 The correlations between PRI and isoprene emissions observed by Peñuelas *et al.* (2013), which are  
50 94 based on LUE (through relationships between xanthophyll pigment conversions and LUE), are thus  
51 95 likely to be influenced by facultative changes in xanthophyll cycle pigments. The extent to which  
52 96 constitutive pigment concentrations also influence the PRI-isoprene emission relationship has not  
53 97 been explicitly studied, but may be important for seasonal monitoring of emissions, and under  
54 98 conditions where changes in LUE and pigment concentrations vary asynchronously. The main aim of  
55 99 this study is thus to investigate the effect of constitutive changes in pigment pools on the PRI –

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3 100 isoprene emission relationship. In doing so we assess some of the physiological mechanisms behind  
4 101 the relationship, primarily through understanding the influences of constitutive differences in pigment  
5 102 concentrations, and facultative differences in xanthophyll pigment conversions, on both isoprene  
6 103 emissions and the PRI reflectance signal.  
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## 11 105 **Materials and methods**

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14 106 The relationships between PRI, isoprene emissions, photosynthetic rates and phytopigments were  
15 107 investigated in the tree species *Salix viminalis* (Dwarf Willow). Willows were chosen as they are  
16 108 known to be strong emitters of isoprene (Kesselmeier and Staudt, 1999) and are also widely  
17 109 recognised as suitable bioenergy crops (Karp and Shield, 2008; Keoleian and Volk, 2005; Larsson,  
18 110 1998). Consequently, Willows play a potentially important role in the future global rate of isoprene  
19 111 emissions (Lathiere et al., 2010).  
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### 25 113 *Plant material and sampling strategy*

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28 114 The experiment was performed at the Centre for Ecology & Hydrology (CEH) Edinburgh, UK, during  
29 115 the end of July 2014 when natural daylight extends from ~ 05:15 to 21:15. Potted *S. viminalis* plants  
30 116 (approximately 1-2 years old) were obtained from a commercial nursery  
31 117 (<http://www.treesbypost.co.uk>) at the beginning of June 2014. The saplings were potted into compost  
32 118 in 6.5 litre pots. To widen the range of isoprene emissions and pigment concentrations tested, each of  
33 119 the twenty four plants were subsequently transferred to one of three natural light environments in the  
34 120 grounds of CEH, Edinburgh. Eight plants were kept against a south-facing wall, which received full  
35 121 sun (SUN), eight were kept against a south-east facing wall, which was shaded for half of the day  
36 122 (HALFSHADE) and eight were kept in a naturally full shaded location, with the addition of a double  
37 123 layer tent of horticultural netting to produce deep shade (SHADE). During the eight week period,  
38 124 generally sunny conditions prevailed and 30% of the continuous 30-minute PAR measurements were  
39 125 greater than 500  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . All plants were kept well-watered until the measurements commenced  
40 126 approximately 8 weeks later.  
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50 127 Sampling was undertaken over the course of a three day period i.e. one sampling day for plants  
51 128 exposed to each of the three different light environments. Leaves of equivalent size and maturity were  
52 129 selected for each plant. Prior to sampling, each plant in a given treatment was transferred to a dark  
53 130 room and covered with a black shade cloth for ~ 40 minutes to ensure that leaves were in a dark-  
54 131 adapted state prior to reflectance sampling. PRI from dark-adapted leaves has previously been shown  
55 132 to relate to constitutive changes in pigment concentrations and thus by using PRI values obtained  
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3 133 from dark-adapted leaves we minimise confusion of the PRI interpretation caused by facultative  
4 134 changes in xanthophyll pigment pools, which occur as a consequence of diurnal acclimatisation  
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6 135 (Gamon and Berry 2012; Porcar-Castell et al., 2012).  
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8 136 Measurements for each plant leaf were undertaken in the following order: 1) spectral reflectance, 2)  
9 137 isoprene emission measurements and 3) pigment analyses. All sampling was undertaken between the  
10 138 hours of 09:00 – 17:30.  
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16 140 *Spectral reflectance measurements*  
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18 141 Leaf reflectance was measured using a spectroradiometer (FieldSpec Jr; ASD, Boulder, CO, USA)  
19 142 equipped with a fibre optic, a leaf clip and contact probe (ASD), which enabled all reflectance  
20 143 measurements to be collected under fixed geometric and illumination conditions. The contact probe  
21 144 probe's light source is a halogen–krypton bulb with peak irradiance at a wavelength of ~ 966 nm. The  
22 145 spot size was 10 mm, and the sampling interval and spectral resolution of the instrument was 1.4 nm  
23 146 and 3 nm; respectively. The integration time was 68 ms and to avoid any light acclimatisation during  
24 147 measurements, spectra were averaged over 5 measurements only. To calculate reflectance, each leaf  
25 148 spectra was divided by a white reference measurement obtained from a calibrated Spectralon®  
26 149 (Spectralon, LabSphere, North Sutton, NH, USA) reference panel immediately prior to each set of  
27 150 leaf measurements. The Photochemical Reflectance Index (PRI) was calculated as follows:  
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$$PRI = (R_{531} - R_{570}) / (R_{531} + R_{570})$$

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37 151 Where  $R$  is reflectance and the subscript indicates the wavelength (nm; Gamon et al., 1992; Peñuelas  
38 152 et al., 1995).  
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43 154 *Isoprene emission sampling and analysis*  
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45 155 Isoprene emission measurements were made using an ADC LCpro leaf cuvette. The flow rate through  
46 156 the cuvette was  $200 \text{ } \mu\text{mol s}^{-1}$  (~300 ml min<sup>-1</sup>). The temperature inside the cuvette was set to 25 °C and  
47 157 the PAR was  $1000 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$ . On each occasion, the sampled leaf was installed in the cuvette and  
48 158 equilibrated for 20-30 minutes to allow dynamic equilibrium of photosynthesis and isoprene emission  
49 159 rates within the cuvette (Geron et al., 2006). A sample of the air exiting the cuvette was drawn  
50 160 through a stainless steel tube containing Tenax and Carbotrap to trap emitted volatile organic  
51 161 compounds (VOCs), using an SKC mass flow controlled pocket pump at  $200 \text{ ml min}^{-1}$ .  
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3 162 At the end of the sampling, the leaf area inside the cuvette was marked and leaf samples of 4 mm  
4 163 diameter were extracted and frozen in liquid nitrogen for pigment analysis using high performance  
5 164 liquid chromatography (HPLC; see section *Pigment analyses*). The outline of the leaf was  
6 165 subsequently traced onto paper, cut out, and the leaf area determined by weighing the paper template  
7 166 after calibration of the paper. Due to the simple structure of the leaves, there was < 5 % uncertainty  
8 167 associated with this method of determining leaf area.

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12 168 Isoprene emission samples were desorbed using a Perkin Elmer automatic thermodesorption device  
13 169 (Turbomatrix™ ATD), into a gas chromatograph-mass spectrometer (Perkin Elmer GC-MS) with  
14 170 Helium as carrier gas. Compounds were desorbed at 280 °C for 6 minutes onto a Tenax-TA cold trap,  
15 171 which was maintained at -30 °C. The trap was then flash-heated to 280 °C for 5 minutes secondary  
16 172 desorption onto the GC column. The GC column was held at 35 °C for 2 minutes, then heated to 160  
17 173 °C at 4 °C min<sup>-1</sup>, followed by a final heating to 300 °C at 45 °C min<sup>-1</sup>. The temperature was held at 300  
18 174 °C for 10 minutes. Isoprene quantification was achieved by injecting and analysing 30 ml of 0.7 ppm  
19 175 gaseous isoprene standard (Air Products) onto adsorbent tubes and analysed in the same way as the  
20 176 samples. Quality assurance standards were analysed at the start of the batch, and then for every 5  
21 177 samples.

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31 179 *Pigment analyses*

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33 180 Phytopigments in the frozen (-80 °C) leaf discs from the sampled willow leaves were extracted into  
34 181 acetone (buffered with CaCO<sub>3</sub>), after grinding under liquid nitrogen in a mortar and pestle. Extracts  
35 182 were centrifuged (5000 rpm for 5 minutes) and the supernatant filtered through a 0.2 micron PTFE  
36 183 syringe filter. Prior to HPLC analysis, extracts were diluted 3:7 with the aqueous component (70 %  
37 184 MeOH plus 30% 28 mM Tetra butyl ammonium acetate TBAA) of the HPLC mobile phase to achieve  
38 185 a good chromatographic peak shape. A variable aliquot (20-200 µl) was injected into the HPLC  
39 186 (Agilent 1100), using a reverse phase chromatographic Agilent Zorbax Eclipse XDB 8 (3.0 x 150  
40 187 mm, 3.5 µm particle size @ 60 °C).

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46 188 Absorbance was measured at: 440 nm, 450 nm, 470 nm, 480 nm and 665 nm depending on the  
47 189 pigment. The complete spectrum of photosynthetic pigments in the 370-750 nm ranged was carried  
48 190 out to confirm identification

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52 191 External standards (DHI Lab products, Høersholm, Denmark), duplicates and blanks were used for  
53 192 Identification and quantification. The linear gradient of solvent elution for quantification is shown in  
54 193 Table 1.

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3 195 Xanthophyll cycle pigment pools (VAZ) were calculated as the sum of violaxanthin (V),  
4 196 antheraxanthin (A) and zeaxanthin (Z) concentrations. Total carotenoid concentration (Car) was  
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6 197 calculated as the sum of neoxanthin (N), V, A, Z, lutein (L) and  $\beta$ -carotene ( $\beta$ C). Total chlorophyll  
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8 198 (Chl) was calculated as the sum of Chlorophyll *a* and *b* concentrations. Pigments were expressed  
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10 199 individually on an area basis ( $\mu\text{mol m}^{-2}$ ) and carotenoids were also normalised to total chlorophyll  
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12 200 concentration ( $\text{mmol mol}^{-1}$ ). The epoxidation state of the xanthophyll cycle, which is an expression of  
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14 201 the non-photoprotective pigment composition of the xanthophyll cycle, was calculated as:

$$EPS = \frac{V + 0.5A}{V + A + Z}$$

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20 203 *Statistical analyses*

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22 204 Differences between the three light environment groups were analysed using one-way ANOVA with  
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24 205 Tukey's honestly significant difference (HSD) post-hoc test. We calculated the Pearson's correlation  
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26 206 coefficient (*r*) to evaluate relationships between variables. All statistical analyses were undertaken in  
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28 207 the R statistical software package (R Development Core Team 2012).

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32 209 **Results**

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34 210 *Isoprene emission potentials, phytopigments and PRI of leaves acclimatised to sun, shade and half-*  
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36 211 *shade*

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38 212 Leaves acclimatised to different illumination conditions showed significant differences in their  
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40 213 pigment pools, isoprene emissions, photosynthetic rates and recorded PRI values (Table 2).

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44 215 SUN leaves exhibited higher concentrations of total carotenoids ( $F_{(2,21)} = 33.11$ ,  $P < 0.001$ ), and  
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46 216 xanthophyll cycle pigments ( $F_{(2,21)} = 118$ ,  $P < 0.001$ ) per area than SHADE leaves. Chlorophyll  
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48 217 concentration per area was highly variable within leaves of a given treatment (data not shown) and  
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50 218 thus observed differences were only significant between leaves in the HALFSHADE and SUN  
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52 219 treatments ( $F_{(2,21)} = 7.908$ ,  $P < 0.01$ ), where chlorophyll levels were highest in the leaves exposed to  
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54 220 full sun. The Chl *a/b* ratio was significantly higher in SUN leaves than those grown in shaded  
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56 221 conditions ( $F_{(2,21)} = 95.84$ ,  $P < 0.001$ ). In addition, per leaf area, SUN leaves also possessed  
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58 222 significantly greater pools of both  $\beta$ -carotene ( $F_{(2,21)} = 21.61$ ,  $P < 0.001$ ), and lutein ( $F_{(2,21)} = 13.03$ ,  $P$   
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60 223  $< 0.001$ ) than SHADE leaves, whereas leaves in both SUN and SHADE treatments exhibited similar  
224 concentrations of neoxanthin. These findings were largely mirrored when pigments were expressed on



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3 225 a chlorophyll basis (Table 2). The most notable differences between the leaves in the SUN and  
4 226 SHADE treatments were the greater levels of  $\beta$ -carotene, and particularly the greater concentration of  
5 227 xanthophyll cycle pigments, in the SUN leaves. Consequently, the increase in the sum of all  
6 228 carotenoids in leaves from the SUN treatment primarily reflected increases in these two pigment  
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8 229 pools.

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11 230 Isoprene emissions ( $F_{(2,21)} = 12.29, P < 0.001$ ) and photosynthetic assimilation rates ( $F_{(2,21)} = 23.05, P$   
12 231  $< 0.001$ ) were highest in leaves from the SUN treatment, compared to those acclimatised to the  
13 232 SHADE or HALFSHADE treatments (Table 2), whereas PRI of SUN leaves was significantly lower  
14 233 than for leaves in either of the other two treatments ( $F_{(2,21)} = 34.17, P < 0.001$ ). Lowest values of EPS  
15 234 were observed in SHADE leaves ( $F_{(2,21)} = 4.07, P < 0.05$ ).

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### 21 22 236 *Relationships between isoprene emissions, phytopigments and PRI*

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24 237 Isoprene emissions were significantly positively related to carotenoid and chlorophyll pigments per  
25 238 leaf area, in response to differences in the light acclimatisation treatments (Table 3; Fig. 1). Total  
26 239 carotenoid concentration (Fig. 1a),  $\beta$ -carotene and lutein were the carotenoids most strongly  
27 240 correlated with isoprene emissions ( $r = 0.8; P < 0.0001$ ). Isoprene emissions were also well  
28 241 correlated with xanthophyll cycle pigment concentration ( $r = 0.75, P < 0.0001$ ; Fig. 1b) but not with  
29 242 the epoxidation state of these pigments (EPS; Table 3). When carotenoid concentrations were  
30 243 expressed on a chlorophyll basis, as opposed to per leaf area, the relationship with isoprene emissions  
31 244 was significantly weaker (Table 3). Isoprene emissions were only moderately correlated with the  
32 245 photosynthetic assimilation rate ( $r = 0.60 P < 0.01$ ; Fig. 1c).

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35 246 PRI was most strongly correlated with the size of the xanthophyll cycle pigment pool ( $r = -0.82, P <$   
36 247  $0.0001$ ; Fig. 2a) and was also strongly correlated with carotenoid concentration (Fig. 2b). The strength  
37 248 of the correlations was similar regardless of whether carotenoids or the xanthophyll pigment  
38 249 concentration was expressed per leaf area or on a chlorophyll basis (Table 3). PRI was also  
39 250 moderately well correlated with isoprene emissions ( $r = -0.66$ ; Fig. 2c) even though PRI and isoprene  
40 251 emissions were found to correlate best with different biochemical variables (Table 3). EPS was not  
41 252 significantly correlated with PRI ( $r = -0.1, P > 0.05$ ; Table 3).

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## 51 52 53 254 **Discussion**

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55 255 *Effect of sun, shade and half-shade acclimation on leaf chemistry, physiology, isoprene emissions*  
56 256 *and spectral reflectance*

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3 257 Phytopigments, isoprene emissions, photosynthetic assimilation rates and recorded PRI values of  
4 258 willow leaves acclimatised to different illumination conditions are similar to those observed in other  
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6 259 studies and across a range of species.  
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10 260 The higher Chl *a/b* ratio for leaves acclimatised to a higher growth irradiance reported in the current  
11 261 study, agree with those reported previously (e.g. Dale and Causton, 1992; Niinemets, 2007) and  
12 262 suggests that leaves acclimatised to shady conditions have greater levels of Chl *b*, and a larger antenna  
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14 263 size than sun-exposed leaves, both of which help shaded leaves gather more light (Hallik et al., 2012).  
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16 264 When plants experience a large range of light availabilities, there should also be a strong relationship  
17 265 between leaf area-based total carotenoid concentrations and growth irradiance or leaf mass area  
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19 266 (LMA; Hallik et al., 2012). The results from our study indicate that the effect of light regime on total  
20 267 carotenoid concentration was significant, with larger total carotenoid pools observed in plants adapted  
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22 268 to full sunlight. These findings are similar to a number of other studies that have reported higher  
23 269 carotenoid concentrations in plants transferred from shade to sun conditions, than in plants remaining  
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25 270 shaded (Porcar-Castell et al., 2009). Differences in carotenoid concentrations have also been reported  
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27 271 along vertical light gradients within natural canopies. For example, Gamon and Berry (2012) observed  
28 272 larger carotenoid concentrations (relative to chlorophyll) in leaves of three conifer species (*Tsuga*  
29 273 *heterophylla*, *Pinus ponderosa* and *Pinus banksiana*), which were exposed to full sun at the top of the  
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31 274 canopy, than those located further down in the shade. Similarly, Hallik et al. (2012) also reported an  
32 275 increase in carotenoid concentration along an increasing vertical light availability gradient in natural  
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34 276 canopies of two herbaceous species (*Inula salicina*, *Centaurea jacea*) and two woody species  
35 277 (*Populus tremula*, *Tilia cordata*).  
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39 278 Acclimation to low light tends to enhance the pools of light-harvesting carotenoids (lutein and its  
40 279 precursor  $\alpha$ -carotene; Hallik et al., 2012), which improves light harvesting in deep shade (Krause et  
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42 280 al., 2001; Matsubara et al., 2009). Our results show no difference in lutein concentration in *S.*  
43 281 *viminalis* leaves based on leaf dry mass between shade and sun treatments (data not shown) and  
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45 282 slightly higher (9%) lutein concentration (on a chlorophyll basis) in sun-adapted plants. Demmig-  
46 283 Adams (1998) also observed a small (5%) increase in lutein (on a chlorophyll basis) in some sun-  
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48 284 exposed leaves compared to shaded leaves. Leaf acclimation to high light tends to increase the pool of  
49  
50 285 carotenoids associated with the efficiency of photosystem I (PSI) and II (PSII), and photoprotection  
51 286 (i.e.  $\beta$ -carotene, its derivatives and the xanthophyll pigments, Z, A and V; Hallik et al., 2012). In our  
52 287 study,  $\beta$ -carotene concentrations in *S. viminalis* leaves, were indeed highest in sun-adapted plants (leaf  
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54 288 area and chlorophyll basis). Light-dependent conversion between V, A and Z plays a central role in  
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56 289 photoprotection, dissipating excess light energy as heat (Demmig-Adams and Adams, 2006; Muller et  
57 290 al., 2001). Our results show a significantly greater VAZ concentration in *S. viminalis* leaves grown in  
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3 291 full sun conditions compared to shade-adapted leaves (leaf area and chlorophyll basis). Our findings  
4 292 are consistent with other studies, which suggest that sun-exposed leaves invest more in  
5 293 photoprotection relative to those growing in the shade (e.g. Demmig-Adams, 1998; Filella et al.,  
6 294 2009; Gamon and Berry, 2012; Porcar-Castell et al., 2009). As expected, there was a significant effect  
7 295 of shading on isoprene emissions from *S. viminalis*. The higher isoprene emissions observed for plants  
8 296 in full sun compared to those growing in the shade agrees with several other published results (e.g.  
9 297 Harley et al., 1996; Sharkey et al., 1996). Differences in isoprene emissions between sun and shaded  
10 298 leaves can, in part, be attributed to differences in the biochemical and/or physiological properties that  
11 299 influence emission potential. For example due to differences in the proportion of photosynthate  
12 300 allocated to isoprene emissions (Litvak et al., 1996).

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20 301 PRI values were lower in leaves that were acclimated to full sunlight than those leaves that were  
21 302 subject to either of the shaded treatments (Table 2). PRI was also strongly correlated with total  
22 303 carotenoids and specifically the size of the xanthophyll cycle pigment pool, but not with EPS. These  
23 304 findings are consistent with previous reports that leaf-level PRI, over seasonal timescales, is strongly  
24 305 influenced by constitutive changes in photoprotective pigment concentrations (Gamon and Berry,  
25 306 2012; Porcar-Castell et al., 2012; Stylinski et al., 2002; Wong and Gamon, 2014).

### 307 *Relationships between leaf chemistry, physiology, isoprene emissions and spectral reflectance*

308 Our results show a significant positive correlation between isoprene emissions and photosynthesis  
309 (Table 3). Similar, correlations between isoprene emissions and photosynthetic light response, has  
310 been reported in a range of species (e.g. Kuhn et al., 2004a; Kuhn et al., 2004b; Litvak et al., 1996),  
311 suggesting a close relationship between isoprene biosynthesis and carbon (Litvak et al., 1996; Sharkey  
312 and Singsaas, 1995). However, our results also show that photosynthesis explains only ~36 % of the  
313 variability in isoprene emissions (Fig. 1). Differences in leaf temperature and variations in CO<sub>2</sub>  
314 concentration in the leaf cuvette air may contribute to some of the variability. It is possible that some  
315 of the observed variability in isoprene emissions may be due to different leaf densities, and perhaps to  
316 emission samples being taken at different times of the day with a possible underlying circadian effect  
317 on the emissions (Litvak et al., 1996; Loivamaki et al., 2007; Wilkinson et al., 2006), though this has  
318 not been demonstrated for *Salix* species. Furthermore, 10-30 % of isoprene production is not directly  
319 linked to photosynthesis, but is associated with older carbon sources (Unger et al., 2013). The  
320 relationship between photosynthesis and isoprene emissions may also break down under a range of  
321 different environmental conditions (see *Introduction*). Consequently, isoprene emissions are not  
322 expected to correlate strongly with photosynthesis rate in all situations and conditions (Sanadze,  
323 2004).

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3 324 As anticipated, dark adaptation of the leaves prior to commencement of the experiment resulted in a  
4 325 non-significant correlation between EPS and isoprene emissions, indicating that short-term facultative  
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6 326 changes in the xanthophyll cycle pigment pool (i.e. pigment conversions) were not related to isoprene  
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8 327 emissions in our study.  
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11 328 Our results show that isoprene emissions from *S. viminalis* at standard conditions were significantly  
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13 329 correlated with total carotenoid concentration, as well as  $\beta$ -carotene, lutein and total xanthophyll  
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15 330 pigment concentration. The correlations between carotenoid concentration and isoprene emissions  
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17 331 observed in our study could be due to biochemical or functional (anti-oxidant) relationships, or a  
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19 332 combination of both. This supports the “Opportunistic hypothesis” (Owen and Peñuelas, 2005) and  
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21 333 also concurs with previous studies that report relationships between volatile isoprenoid emission  
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23 334 potential and carotenoid pools for light-dependent monoterpene and isoprene emissions from a range  
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25 335 of different species (Owen and Peñuelas, 2005; Porcar-Castell et al., 2009).

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27 336 PRI values were significantly correlated with isoprene emissions (Table 3), which agrees with the  
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29 337 results reported by Peñuelas *et al.* (2013). However, the strength of the correlation reported in our  
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31 338 study is slightly lower than those reported by Peñuelas *et al.* (2013) for *Populus nigra*, which may in  
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33 339 part be due to the narrower range of isoprene emissions produced by *S. vimarlix*, which was  
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35 340 approximately half of that observed by Peñuelas *et al.* (2013).

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37 341 The lack of correlation between PRI and EPS indicates that under the conditions of this experiment,  
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39 342 short term facultative adjustments in the epoxidation state of the xanthophyll cycle pigments did not  
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41 343 influence PRI. Since isoprene emissions were also strongly correlated with total carotenoid  
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43 344 concentrations, and to a slightly lesser extent the size of the xanthophyll cycle pigment pool, but not  
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45 345 with EPS; our results strongly support the hypothesis that at longer time scales (weeks to months), the  
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47 346 relationship between isoprene emissions and the PRI signal is influenced by constitutive adjustments  
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49 347 in carotenoid concentration.

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51 348 Our results complement those of Peñuelas *et al.* (2013) who suggested that isoprene-PRI relationships  
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53 349 under naturally varying illumination conditions were a function of changes in LUE, and thus at least  
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55 350 in part, thought to be associated with short-term facultative xanthophyll cycle pigment conversions in  
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57 351 response to irradiance. However, we also show that at longer timescales the isoprene-PRI relationship  
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59 352 is also likely to be driven by constitutive adjustments in the size of carotenoid pigment pools.  
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3 353 If PRI is to be used as an estimator of isoprenoid emissions or incorporated into isoprenoid emission  
4 354 models, then knowledge of how isoprene emissions are influenced by both the longer term effects of  
5 355 growth irradiance on carotenoid pigment concentrations observed in our study, and the dynamic  
6 356 adjustments of xanthophyll cycle pigments suggested by Peñuelas *et al.* (2013), have important  
7 357 implications for interpreting the PRI-isoprene relationship. This may be especially true where  
8 358 facultative and constitutive changes in pigments are out of phase (Gamon and Berry, 2012; Sims et  
9 359 al., 2006; Wong and Gamon, 2014). Consequently, further work is needed to isolate and understand  
10 360 the relative influence of short term facultative and longer term constitutive changes in carotenoid  
11 361 pigment pools on the relationship between isoprenoid emissions and PRI, across a wider range of  
12 362 species and in field conditions.  
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#### 364 **Author contributions**

365 AH and SMO conceived, designed and performed the experiment. DS and MGP generated the  
366 pigment data. AH and SMO analysed the data and wrote the manuscript.  
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#### 372 **References**

- 373 Arneth A, Niinemets U, Pressley S, Back J, Hari P, Karl T, Noe S, Prentice IC, Serca D, Hickler T,  
374 Wolf A and Smith B (2007) Process-based estimates of terrestrial ecosystem isoprene  
375 emissions: Incorporating the effects of a direct CO<sub>2</sub>-isoprene interaction. *Atmos Chem Phys*  
376 **7**:31-53.
- 377 Barkley MP, Palmer PI, Kuhn U, Kesselmeier J, Chance K, Kurosu TP, Martin RV, Helmig D and  
378 Guenther A (2008) Net ecosystem fluxes of isoprene over tropical south america inferred  
379 from global ozone monitoring experiment (GOME) observations of HCHO columns. *J*  
380 *Geophys Res* **113**.
- 381 Dale MP and Causton DR (1992) Use of the chlorophyll a-b ratio as a bioassay for the light  
382 environment of a plant. *Funct Ecol* **6**:190-196.
- 383 Demmig-Adams B (1998) Survey of thermal energy dissipation and pigment composition in sun and  
384 shade leaves. *Plant Cell Physiol* **39**:474-482.
- 385 Demmig-Adams B and Adams WW, III (1992) Photoprotection and other responses of plants to light  
386 stress. *Annu Rev Plant Physiol Plant Mol Biol* **43**:599-626.
- 387 Demmig-Adams B and Adams WW, III (2006) Photoprotection in an ecological context: The  
388 remarkable complexity of thermal energy dissipation. *New Phytol* **172**:11-21.

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3 389 Filella I, Porcar-Castell A, Munne-Bosch S, Back J, Garbulsky MF and Peñuelas J (2009) Pri  
4 390 assessment of long-term changes in carotenoids/chlorophyll ratio and short-term changes in  
5 391 de-epoxidation state of the xanthophyll cycle. *Int J Remote Sens* **30**:4443-4455.
- 7 392 Fiore AM, Naik V, Spracklen DV, Steiner A, Unger N, Prather M, Bergmann D, Cameron-Smith PJ,  
8 393 Cionni I, Collins WJ, Dalsoren S, Eyring V, Folberth GA, Ginoux P, Horowitz LW, Josse B,  
9 394 Lamarque J-F, MacKenzie IA, Nagashima T, O'Connor FM, Righi M, Rumbold ST, Shindell  
10 395 DT, Skeie RB, Sudo K, Szopa S, Takemura T and Zeng G (2012) Global air quality and  
11 396 climate. *Chem Soc Rev* **41**:6663-6683.
- 15 397 Foster PN, Prentice IC, Morfopoulos C, Siddall M and van Weele M (2014) Isoprene emissions track  
16 398 the seasonal cycle of canopy temperature, not primary production: Evidence from remote  
17 399 sensing. *Biogeosciences* **11**:3437-3451.
- 20 400 Gamon JA and Berry JA (2012) Facultative and constitutive pigment effects on the photochemical  
21 401 reflectance index (pri) in sun and shade conifer needles. *Isr J Plant Sci* **60**:85-95.
- 23 402 Gamon JA, Peñuelas J and Field CB (1992) A narrow-waveband spectral index that tracks diurnal  
24 403 changes in photosynthetic efficiency. *Remote Sens Environ* **41**:35-44.
- 26 404 Gamon JA, Serrano L and Surfus JS (1997) The photochemical reflectance index: An optical indicator  
27 405 of photosynthetic radiation use efficiency across species, functional types, and nutrient levels.  
28 406 *Oecologia* **112**:492.
- 30 407 Geron C, Guenther A, Sharkey T and Arnts RR (2000) Temporal variability in basal isoprene  
31 408 emission factor. *Tree Physiol* **20**:799-805.
- 33 409 Geron C, Owen S, Guenther A, Greenberg J, Rasmussen R, Bai JH, Li QJ and Baker B (2006)  
34 410 Volatile organic compounds from vegetation in southern yunnan province, china: Emission  
35 411 rates and some potential regional implications. *Atmos Environ* **40**:1759-1773.
- 38 412 Gershenson J (1994) Metabolic costs of terpenoid accumulation in higher-plants. *J Chem Ecol*  
39 413 **20**:1281-1328.
- 41 414 Guenther A, Karl T, Harley P, Wiedinmyer C, Palmer PI and Geron C (2006) Estimates of global  
42 415 terrestrial isoprene emissions using MEGAN (Model of Emissions of Gases and Aerosols  
43 416 from Nature). *Atmos Chem Phys* **6**:3181-3210.
- 45 417 Guenther AB, Jiang X, Heald CL, Sakulyanontvittaya T, Duhl T, Emmons LK and Wang X (2012)  
46 418 The Model of Emissions of Gases and Aerosols from Nature version 2.1 (MEGAN 2.1): An  
47 419 extended and updated framework for modeling biogenic emissions. *Geosci Model Dev*  
48 420 **5**:1471-1492.
- 51 421 Guenther AB, Zimmerman PR, Harley PC, Monson RK and Fall R (1993) Isoprene and monoterpene  
52 422 emission rate variability - model evaluations and sensitivity analyses. *J Geophys Res*  
53 423 **98**:12609-12617.
- 56  
57  
58  
59  
60

- 1  
2  
3 424 Hallik L, Niinemets Ü and Kull O (2012) Photosynthetic acclimation to light in woody and  
4 425 herbaceous species: A comparison of leaf structure, pigment content and chlorophyll  
5 426 fluorescence characteristics measured in the field. *Plant Biology* **14**:88-99.
- 7 427 Harley P, Guenther A and Zimmerman P (1996) Effects of light, temperature and canopy position on  
8 428 net photosynthesis and isoprene emission from sweetgum (*liquidambar styraciflua*) leaves.  
9 429 *Tree Physiol* **16**:25-32.
- 11 430 Karp A and Shield I (2008) Bioenergy from plants and the sustainable yield challenge. *New Phytol*  
12 431 **179**:15-32.
- 14 432 Keoleian GA and Volk TA (2005) Renewable energy from willow biomass crops: Life cycle energy,  
15 433 environmental and economic performance. *Critical Reviews in Plant Sciences* **24**:385-406.
- 17 434 Kesselmeier J and Staudt M (1999) Biogenic volatile organic compounds (voc): An overview on  
18 435 emission, physiology and ecology. *J Atmos Chem* **33**:23-88.
- 20 436 Krause GH, Koroleva OY, Dalling JW and Winter K (2001) Acclimation of tropical tree seedlings to  
21 437 excessive light in simulated tree-fall gaps. *Plant Cell and Environment* **24**:1345-1352.
- 23 438 Kuhn U, Rottenberger S, Biesenthal T, Wolf A, Schebeske G, Ciccioli P, Brancaleoni E, Frattoni M,  
24 439 Tavares TM and Kesselmeier J (2004a) Seasonal differences in isoprene and light-dependent  
25 440 monoterpene emission by amazonian tree species. *Glob Change Biol* **10**:663-682.
- 27 441 Kuhn U, Rottenberger S, Biesenthal T, Wolf A, Schebeske G, Ciccioli P and Kesselmeier J (2004b)  
28 442 Strong correlation between isoprene emission and gross photosynthetic capacity during leaf  
29 443 phenology of the tropical tree species *hymenaea courbaril* with fundamental changes in  
30 444 volatile organic compounds emission composition during early leaf development. *Plant Cell*  
31 445 *Environ* **27**:1469-1485.
- 33 446 Larsson S (1998) Genetic improvement of willow for short-rotation coppice. *Biomass Bioenergy*  
34 447 **15**:23-26.
- 36 448 Lathiere J, Hewitt CN and Beerling DJ (2010) Sensitivity of isoprene emissions from the terrestrial  
37 449 biosphere to 20th century changes in atmospheric CO<sub>2</sub> concentration, climate, and land use.  
38 450 *Global Biogeochem Cycles* **24**.
- 40 451 Litvak ME, Loreto F, Harley PC, Sharkey TD and Monson RK (1996) The response of isoprene  
41 452 emission rate and photosynthetic rate to photon flux and nitrogen supply in aspen and white  
42 453 oak trees. *Plant Cell Environ* **19**:549-559.
- 44 454 Loivamaki M, Louis S, Cinege G, Zimmer I, Fischbach RJ and Schnitzler JP (2007) Circadian  
45 455 rhythms of isoprene biosynthesis in grey poplar leaves. *Plant Physiol* **143**:540-551.
- 47 456 Matsubara S, Krause GH, Aranda J, Virgo A, Beisel KG, Jahns P and Winter K (2009) Sun-shade  
48 457 patterns of leaf carotenoid composition in 86 species of neotropical forest plants. *Funct Plant*  
49 458 *Biol* **36**:20-36.
- 51 459 Michelozzi M (1999) Defensive roles of terpenoid mixtures in conifers. *Acta Bot Gall* **146**:73-84.  
52  
53  
54  
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56  
57  
58  
59  
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- 1  
2  
3 460 Monson RK, Harley PC, Litvak ME, Wildermuth M, Guenther AB, Zimmerman PR and Fall R  
4 461 (1994) Environmental and developmental controls over the seasonal pattern of isoprene  
5 462 emission from aspen leaves. *Oecologia* **99**:260-270.
- 7 463 Monson RK, Trahan N, Rosenstiel TN, Veres P, Moore D, Wilkinson M, Norby RJ, Volder A,  
8 464 Tjoelker MG, Briske DD, Karnosky DF and Fall R (2007) Isoprene emission from terrestrial  
9 465 ecosystems in response to global change: Minding the gap between models and observations.  
10 466 *Philos Trans A Math Phys Eng Sci* **365**:1677-1695.
- 13 467 Morfopoulos C, Prentice IC, Keenan TF, Friedlingstein P, Medlyn BE, Peñuelas J and Possell M  
14 468 (2013) A unifying conceptual model for the environmental responses of isoprene emissions  
15 469 from plants. *Ann Botany* **112**:1223-1238.
- 18 470 Muller P, Li XP and Niyogi KK (2001) Non-photochemical quenching. A response to excess light  
19 471 energy. *Plant Physiol* **125**:1558-1566.
- 21 472 Niinemets U (2007) Photosynthesis and resource distribution through plant canopies. *Plant Cell*  
22 473 *Environ* **30**:1052-1071.
- 24 474 Niinemets U, Arneth A, Kuhn U, Monson RK, Peñuelas J and Staudt M (2010) The emission factor of  
25 475 volatile isoprenoids: Stress, acclimation, and developmental responses. *Biogeosciences*  
26 476 **7**:2203-2223.
- 29 477 Niinemets U, Kannaste A and Copolovici L (2013) Quantitative patterns between plant volatile  
30 478 emissions induced by biotic stresses and the degree of damage. *Fron Plant Sci* **4**.
- 32 479 Owen SM and Peñuelas J (2005) Opportunistic emissions of volatile isoprenoids. *Trends Plant Sci*  
33 480 **10**:420-426.
- 35 481 Owen SM and Peñuelas J (2013) Volatile isoprenoid emission potentials are correlated with essential  
36 482 isoprenoid concentrations in five plant species. *Acta Physiol Plant* **35**:3109-3125.
- 38 483 Palmer PI, Jacob DJ, Fiore AM, Martin RV, Chance K and Kurosu TP (2003) Mapping isoprene  
39 484 emissions over north america using formaldehyde column observations from space. *J*  
40 485 *Geophys Res* **108**.
- 42 486 Peñuelas J, Fiella I and Gamon JA (1995) Assessment of photosynthetic radiation-use efficiency with  
43 487 spectral reflectance. *New Phytol* **131**:291-296.
- 45 488 Peñuelas J, Marino G, Llusia J, Morfopoulos C, Farre-Armengol G and Filella I (2013)  
46 489 Photochemical reflectance index as an indirect estimator of foliar isoprenoid emissions at the  
47 490 ecosystem level. *Nat Commun* **4**:2604.
- 50 491 Pitman AJ, Arneth A and Ganzeveld L (2012) Regionalizing global climate models. *Int J Climatol*  
51 492 **32**:321-337.
- 53 493 Porcar-Castell A, Garcia-Plazaola J, Nichol C, Kolari P, Olascoaga B, Kuusinen N, Fernández-Marín  
54 494 B, Pulkkinen M, Juurola E and Nikinmaa E (2012) Physiology of the seasonal relationship  
55 495 between the photochemical reflectance index and photosynthetic light use efficiency.  
56 496 *Oecologia* **170**:313-323.
- 59  
60



- 1  
2  
3 497 Porcar-Castell A, Peñuelas J, Owen SM, Llusia J, Munne-Bosch S and Back J (2009) Leaf carotenoid  
4 498 concentrations and monoterpene emission capacity under acclimation of the light reactions of  
5 499 photosynthesis. *Boreal Environ Res* **14**:794-806.
- 7 500 Pressley S, Lamb B, Westberg H, Flaherty J, Chen J and Vogel C (2005) Long-term isoprene flux  
8 501 measurements above a northern hardwood forest. *J Geophys Res* **110**.
- 10 502 R Core Development Team (2012) R: A language and environment for statistical computing. R  
11 503 Foundation for Statistical Computing, Vienna, Austria
- 13 504 Rosenstiel TN, Potosnak MJ, Griffin KL, Fall R and Monson RK (2003) Increased CO<sub>2</sub> uncouples  
14 505 growth from isoprene emission in an agriforest ecosystem. *Nature* **421**:256-259.
- 16 506 Sanadze GA (2004) Biogenic isoprene - (a review). *Russ J Plant Physiol* **51**:729-741.
- 18 507 Sharkey TD and Singsaas EL (1995) Why plants emit isoprene. *Nature* **374**:769-769.
- 20 508 Sharkey TD, Singsaas EL, Vanderveer PJ and Geron C (1996) Field measurements of isoprene  
21 509 emission from trees in response to temperature and light. *Tree Physiol* **16**:649-654.
- 23 510 Sims DA, Luo HY, Hastings S, Oechel WC, Rahman AF and Gamon JA (2006) Parallel adjustments  
24 511 in vegetation greenness and ecosystem CO<sub>2</sub> exchange in response to drought in a southern  
25 512 california chaparral ecosystem. *Remote Sens Environ* **103**:289-303.
- 27 513 Singsaas EL, Lerdau M, Winter K and Sharkey TD (1997) Isoprene increases thermotolerance of  
28 514 isoprene-emitting species. *Plant Physiol* **115**:1413-1420.
- 30 515 Stylinski CD, Gamon JA and Oechel WC (2002) Seasonal patterns of reflectance indices, carotenoid  
31 516 pigments and photosynthesis of evergreen chaparral species. *Oecologia* **131**:366-374.
- 33 517 Unger N, Harper K, Zheng Y, Kiang NY, Aleinov I, Arneth A, Schurgers G, Amelynck C, Goldstein  
34 518 A, Guenther A, Heinesch B, Hewitt CN, Karl T, Laffineur Q, Langford B, McKinney KA,  
35 519 Misztal P, Potosnak M, Rinne J, Pressley S, Schoon N and Seraca D (2013) Photosynthesis-  
36 520 dependent isoprene emission from leaf to planet in a global carbon-chemistry-climate model.  
37 521 *Atmos Chem Phys* **13**:10243-10269.
- 41 522 Vickers CE, Possell M, Cojocariu CI, Velikova VB, Laothawornkitkul J, Ryan A, Mullineaux PM and  
42 523 Hewitt CN (2009) Isoprene synthesis protects transgenic plants from oxidative stress. *Plant*  
43 524 *Cell Environ* **32**:520-531.
- 45 525 Wilkinson MJ, Owen SM, Possell M, Hartwell J, Gould P, Hall A, Vickers C and Nicholas Hewitt C  
46 526 (2006) Circadian control of isoprene emissions from oil palm (*Elaeis guineensis*). *Plant J*  
47 527 **47**:960-968.
- 50 528 Wong CYS and Gamon JA (2014) Three causes of variation in the photochemical reflectance index  
51 529 (pri) in evergreen conifers. *New Phytol*. doi:10.1111/nph.13159.
- 53 530  
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534 **Figure Legends**

535 Fig. 1 Correlations between isoprene emissions and a) total carotenoids, b) xanthophyll cycle pigment  
536 pool (VAZ) and c) photosynthetic assimilation rates. Lines are fitted for reasons of clarity only. ns =  
537 not significant; \*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ ; \*\*\*\*  $P < 0.0001$ .

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539 Fig. 2 Correlations between the photochemical reflectance index (PRI) and a) xanthophyll cycle  
540 pigment pool (VAZ), b) total carotenoids and c) isoprene emissions. Lines are fitted for reasons of  
541 clarity only. ns = not significant; \*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ ; \*\*\*\*  $P < 0.0001$ .

Table 1 Analytical gradient protocol for elution of pigments using HPLC

| Time (mins) | % Solvent A | % Solvent B |
|-------------|-------------|-------------|
| 0           | 95          | 5           |
| 22          | 30          | 70          |
| 35          | 95          | 5           |
| 36          | 0           | 100         |
| 42.5        | 0           | 100         |
| 43          | 95          | 5           |
| 50          | 95          | 5           |

Solvent A - 70% MeOH plus 30% 28mM Tetra butyl ammonium acetate TBAA; solvent B - methanol

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Table 2 Differences in pigment composition, isoprene emissions, photosynthetic assimilation rates, epoxidation state of the xanthophyll cycle pigments, and the photochemical reflectance index from pooled data for SHADE, HALFSHADE and SUN treatment leaves.

|   | SHADE            |       | HALFSHADE       |       | SUN             |       | SUN, % of SHADE |
|---|------------------|-------|-----------------|-------|-----------------|-------|-----------------|
|   | Mean             | SD    | Mean            | SD    | Mean            | SD    |                 |
| Chl <i>a/b</i> ratio  | 3.5 <i>a</i>     | 0.1   | 3.6 <i>b</i>    | 0.1   | 4.0 <i>c</i>    | 0.1   | 16.0 %          |
| <i>Pigment concentration on a leaf area basis</i>                         |                  |       |                 |       |                 |       |                 |
| Chl ( $\mu\text{mol m}^{-2}$ )  | 1171.4 <i>ab</i> | 157.8 | 1038.4 <i>a</i> | 113.5 | 1363.3 <i>b</i> | 220.9 | -               |
| Car ( $\mu\text{mol m}^{-2}$ )  | 274.9 <i>a</i>   | 36.8  | 264.5 <i>a</i>  | 24.8  | 409.0 <i>b</i>  | 52.2  | 48.8 %          |
| VAZ ( $\mu\text{mol m}^{-2}$ )  | 45.1 <i>a</i>    | 5.9   | 51.8 <i>a</i>   | 4.5   | 112.8 <i>b</i>  | 14.7  | 150.1 %         |
| $\beta$ - Carotene ( $\mu\text{mol m}^{-2}$ )                             | 71.8 <i>a</i>    | 11.8  | 64.6 <i>a</i>   | 7.5   | 100.5 <i>b</i>  | 14.7  | 39.9 %          |
| Lutein ( $\mu\text{mol m}^{-2}$ )   | 116.9 <i>a</i>   | 14.6  | 110.9 <i>a</i>  | 10.2  | 147.8 <i>b</i>  | 19.7  | 26.4 %          |
| Neoxanthin ( $\mu\text{mol m}^{-2}$ )                                     | 41.1 <i>ab</i>   | 5.8   | 37.1 <i>b</i>   | 4.7   | 47.8 <i>a</i>   | 7.7   | -               |
| <i>Pigment concentration on a chlorophyll basis</i>                       |                  |       |                 |       |                 |       |                 |
| Car Chl <sup>-1</sup> ( $\text{mmol mol}^{-1}$ )                          | 234.9 <i>a</i>   | 9.1   | 255.4 <i>b</i>  | 14.8  | 301.9 <i>c</i>  | 20.4  | 28.5 %          |
| VAZ Chl <sup>-1</sup> ( $\text{mmol mol}^{-1}$ )                          | 38.6 <i>a</i>    | 3.2   | 50.2 <i>b</i>   | 4.4   | 83.7 <i>c</i>   | 10.8  | 116.6 %         |
| $\beta$ -Carotene Chl <sup>-1</sup> ( $\text{mmol mol}^{-1}$ )            | 61.2 <i>a</i>    | 3.7   | 62.3 <i>a</i>   | 4.3   | 73.9 <i>b</i>   | 2.8   | 20.9 %          |
| Lutein Chl <sup>-1</sup> ( $\text{mmol mol}^{-1}$ )                       | 100.0 <i>a</i>   | 4.2   | 107.1 <i>b</i>  | 6.7   | 109.0 <i>b</i>  | 5.6   | 9.0 %           |
| Neoxanthin Chl <sup>-1</sup> ( $\text{mmol mol}^{-1}$ )                   | 35.1 <i>a</i>    | 2.1   | 35.8 <i>a</i>   | 2.8   | 35.3 <i>a</i>   | 4.0   | -               |
| Isoprene emission potential ( $\text{nmol m}^{-2} \text{s}^{-1}$ )        | 2.7 <i>a</i>     | 2.6   | 0.8 <i>a</i>    | 1.0   | 7.3 <i>b</i>    | 3.6   | 172.6 %         |
| Photosynthetic assimilation rate ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) | 7.7 <i>a</i>     | 1.4   | 9.8 <i>b</i>    | 0.9   | 12.2 <i>c</i>   | 1.5   | 58.0 %          |
| PRI   | 0.05 <i>a</i>    | 0.01  | 0.05 <i>a</i>   | 0.00  | 0.03 <i>b</i>   | 0.01  | -45.0 %         |
| EPS   | 0.61 <i>a</i>    | 0.07  | 0.77 <i>b</i>   | 0.16  | 0.75 <i>ab</i>  | 0.13  | -               |

The Tukey test compared differences between treatments at the 5% level of significance ( $P < 0.05$ ). Same letters indicate mean values are not significantly different in the horizontal direction. Percentage differences (SUN, % of SHADE) are not calculated when there is no statistically significant difference between SHADE and SUN treatments. Abbreviations: Chl, sum of Chl *a* + *b*; Car, sum of all carotenoids; VAZ, sum of violaxanthin (V) + antheraxanthin (A) + zeaxanthin (Z); PRI, photochemical reflectance index; EPS: epoxidation state of xanthophyll cycle pigments.

Table 3 Correlation matrix showing the Pearson's correlation coefficient ( $r$ ) between variables.

|                       | PRI       | EPS   | Isoprene | A        | Chl      | Car      | VAZ      | $\beta$ C | L        | N    | Car Chl <sup>-1</sup> | VAZ Chl <sup>-1</sup> |
|-----------------------|-----------|-------|----------|----------|----------|----------|----------|-----------|----------|------|-----------------------|-----------------------|
| PRI                   | 1         |       |          |          |          |          |          |           |          |      |                       |                       |
| EPS                   | -0.10     | 1     |          |          |          |          |          |           |          |      |                       |                       |
| Isoprene              | -0.66***  | 0.11  | 1        |          |          |          |          |           |          |      |                       |                       |
| A                     | -0.50*    | 0.33  | 0.60**   | 1        |          |          |          |           |          |      |                       |                       |
| Chl                   | -0.47*    | 0.02  | 0.83**** | 0.52**   | 1        |          |          |           |          |      |                       |                       |
| Car                   | -0.75**** | 0.23  | 0.84**** | 0.71***  | 0.86**** | 1        |          |           |          |      |                       |                       |
| VAZ                   | -0.82**** | 0.28  | 0.75**** | 0.77**** | 0.67***  | 0.94**** | 1        |           |          |      |                       |                       |
| $\beta$ C             | -0.70**** | 0.16  | 0.89**** | 0.66***  | 0.98**** | 0.98**** | 0.86**** | 1         |          |      |                       |                       |
| L                     | -0.64***  | 0.16  | 0.81**** | 0.63*    | 0.97**** | 0.97**** | 0.85**** | 0.97****  | 1        |      |                       |                       |
| N                     | -0.46*    | 0.29  | 0.73***  | 0.36     | 0.84**** | 0.83**** | 0.65***  | 0.87****  | 0.84**** | 1    |                       |                       |
| Car Chl <sup>-1</sup> | -0.77**** | 0.42* | 0.45*    | 0.62**   | 0.20     | 0.67***  | 0.83**** | 0.53**    | 0.51*    | 0.35 | 1                     |                       |
| VAZ Chl <sup>-1</sup> | -0.80**** | 0.36  | 0.53**   | 0.72***  | 0.32     | 0.74**** | 0.91**** | 0.60**    | 0.59**   | 0.38 | 0.97****              | 1                     |

Abbreviations: PRI, photochemical reflectance index; EPS, epoxidation state of the xanthophyll cycle pigments; Isoprene, isoprene emissions ( $\text{nmol m}^{-2} \text{s}^{-1}$ ); A, photosynthetic assimilation rate ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ); Chl, sum of chl  $a + b$  ( $\mu\text{mol m}^{-2}$ ); Car, sum off all carotenoids ( $\mu\text{mol m}^{-2}$ ); VAZ, sum of violaxanthin (V) + antheraxanthin (A) + zeaxanthin (Z) ( $\mu\text{mol m}^{-2}$ );  $\beta$ C,  $\beta$ -carotene ( $\mu\text{mol m}^{-2}$ ); L, lutein ( $\mu\text{mol m}^{-2}$ ); N, neoxanthin ( $\mu\text{mol m}^{-2}$ ); Car Chl<sup>-1</sup>, total carotenoids expressed on a chlorophyll basis ( $\text{mmol mol}^{-1}$ ); VAZ Chl<sup>-1</sup>, sum of violaxanthin (V) + antheraxanthin (A) + zeaxanthin (Z) expressed on a chlorophyll basis ( $\text{mmol mol}^{-1}$ ); Significance levels: \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ ; \*\*\*\*,  $P < 0.0001$ .

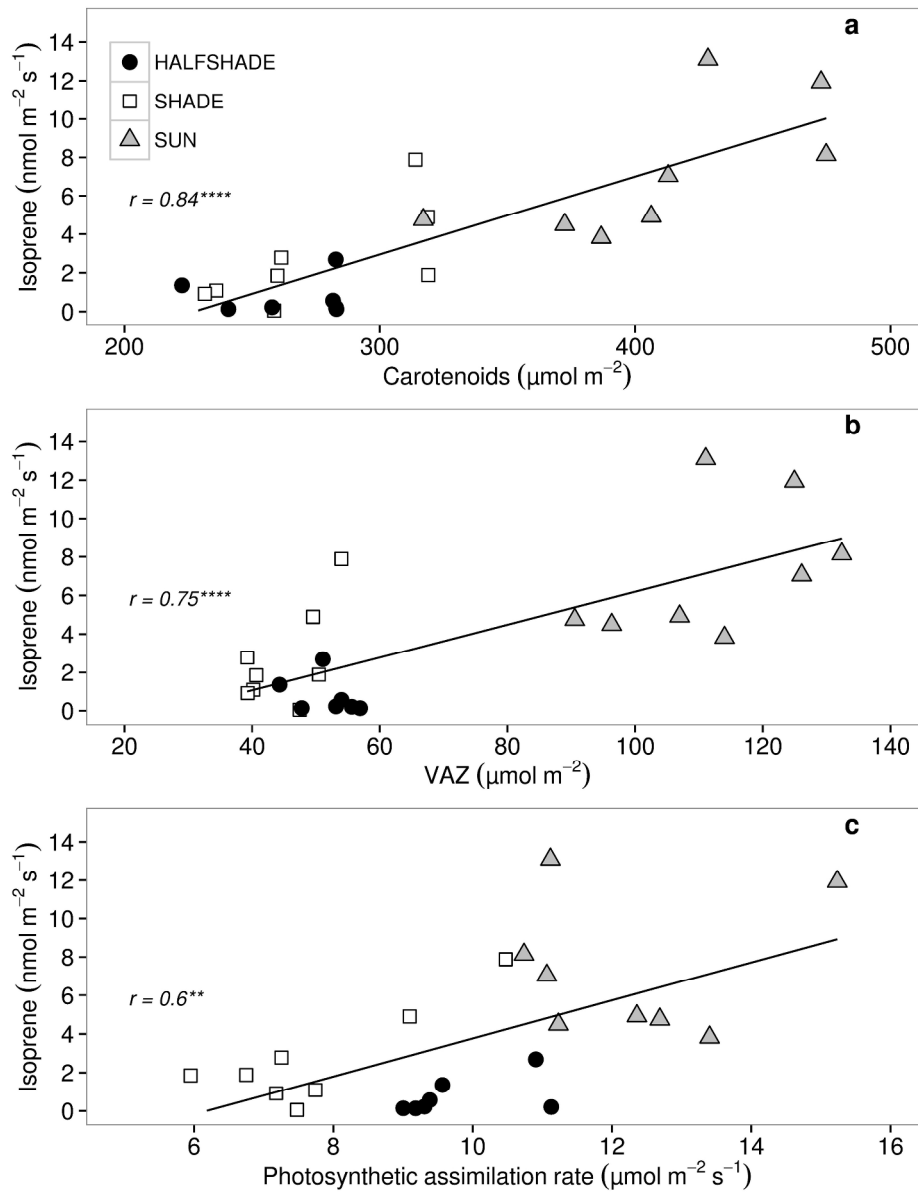


Fig. 1 Correlations between isoprene emissions and a) total carotenoids, b) xanthophyll cycle pigment pool (VAZ) and c) photosynthetic assimilation rates. Lines are fitted for reasons of clarity only. ns = not significant; \*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ ; \*\*\*\*  $P < 0.0001$ .  
250x312mm (300 x 300 DPI)

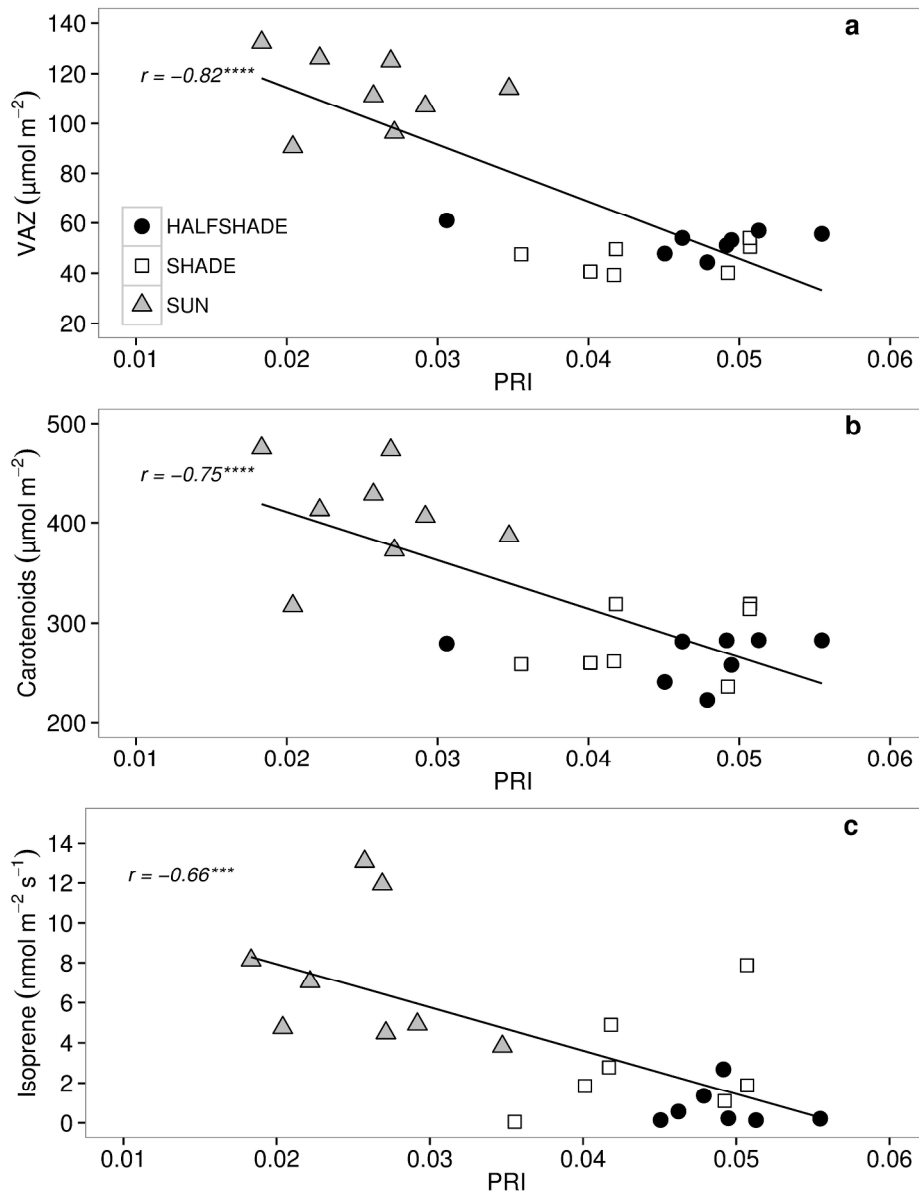


Fig. 2 Correlations between the photochemical reflectance index (PRI) and a) xanthophyll cycle pigment pool (VAZ), b) total carotenoids and c) isoprene emissions. Lines are fitted for reasons of clarity only. ns = not significant; \*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ ; \*\*\*\*  $P < 0.0001$ .  
250x312mm (300 x 300 DPI)