Diurnal dynamics of nonphotochemical quenching in Arabidopsis \textit{npq} mutants assessed by solar-induced fluorescence and reflectance measurements in the field

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Summary

- Solar-induced fluorescence (SIF) is highly relevant in mapping photosynthesis from remote-sensing platforms. This requires linking SIF to photosynthesis and understanding the role of nonphotochemical quenching (NPQ) mechanisms under field conditions. Hence, active and passive fluorescence were measured in \textit{Arabidopsis} with altered NPQ in outdoor conditions.
- Plants with mutations in either violaxanthin de-epoxidase (\textit{npq1}) or PsbS protein (\textit{npq4}) exhibited reduced NPQ capacity. Parallel measurements of NPQ, photosystem II efficiency, SIF and spectral reflectance ($\rho$) were conducted diurnally on one sunny summer day and two consecutive days during a simulated cold spell.
- Results showed that both \textit{npq} mutants exhibited higher levels of SIF compared to wild-type plants. Changes in reflectance were related to changes in the violaxanthin–antheraxanthin–zeaxanthin (VAZ) cycle. Nonphotochemical quenching (NPQ) mechanisms under field conditions. Hence, active and passive fluorescence were measured in \textit{Arabidopsis} with altered NPQ in outdoor conditions.
- Plants with mutations in either violaxanthin de-epoxidase (\textit{npq1}) or PsbS protein (\textit{npq4}) exhibited reduced NPQ capacity. Parallel measurements of NPQ, photosystem II efficiency, SIF and spectral reflectance ($\rho$) were conducted diurnally on one sunny summer day and two consecutive days during a simulated cold spell.
- Results showed that both \textit{npq} mutants exhibited higher levels of SIF compared to wild-type plants. Changes in reflectance were related to changes in the violaxanthin–antheraxanthin–zeaxanthin cycle and not to PsbS-mediated conformational changes. When plants were exposed to cold temperatures, rapid onset of photoinhibition strongly quenched SIF in all lines.
- Using well-characterized \textit{Arabidopsis npq} mutants, we showed for the first time the quantitative link between SIF, photosynthetic efficiency, NPQ components and leaf reflectance. We discuss the functional potential and limitations of SIF and reflectance measurements for estimating photosynthetic efficiency and NPQ in the field.

Introduction

The global mapping of ecosystem functions largely relies on an understanding of the functional meaning of solar-induced fluorescence (SIF) and plant spectral reflectance ($\rho$). Solar-induced fluorescence signal has been successfully measured at leaf level (van Wittenbergh \textit{et al.}, 2013; Magney \textit{et al.}, 2017; Vilfan \textit{et al.}, 2019), at the top of the canopy (Pinto \textit{et al.}, 2016), in airborne systems (Zarco-Tejada \textit{et al.}, 2000; Rascher \textit{et al.}, 2015) and from satellite platforms (Frankenberg \textit{et al.}, 2011; Joiner \textit{et al.}, 2011). There are therefore increasing opportunities for understanding plant photosynthesis in natural environments on different scales (Rascher \textit{et al.}, 2009). Several studies have demonstrated the sensitivity of SIF to major abiotic stress conditions (Ač \textit{et al.}, 2015), absorbed photosynthetically active radiation (APAR; Yang \textit{et al.}, 2015) and photosynthetic efficiency (Rossini \textit{et al.}, 2015; Pinto \textit{et al.}, 2016). As a passive signal, the potential mapping of SIF on a large scale is clearly a novel advantage for global studies. Several existing satellites, such as GOSAT (Frankenberg \textit{et al.}, 2011), MERIS (García-Plazaola \textit{et al.}, 2007), GOME-2 (Joiner \textit{et al.}, 2011), Sentinel-5 Precursor, TROPOMI (Kohler \textit{et al.}, 2018), Tansat (Du \textit{et al.}, 2018) and OCO-2 (Frankenberg \textit{et al.}, 2011; Sun \textit{et al.}, 2018) are capable of providing SIF at global scales by spatially and temporally aggregating existing satellite data. Because of the potential of SIF to deliver information about actual photosynthesis, Fluorescence Explorer (FLEX) was selected as the 8th Explorer satellite mission of the European Space Agency, to provide timely maps of SIF and $\rho$ to understand terrestrial photosynthesis on a global scale (Drusch \textit{et al.}, 2016).

Because the fundamental relationships between Chl fluorescence, photochemistry and heat dissipation are dynamic, linking SIF to photosynthesis (or photochemistry in the context of this study) requires a holistic understanding of the interplay between nonphotochemical quenching (NPQ) mechanisms operating in field conditions. Nonphotochemical quenching involves thermal dissipation of excess absorbed energy. When leaves experience an increase in light intensity, thylakoid lumen becomes acidic, which protonates PsbS protein and activates violaxanthin de-epoxidase (VDE) to convert violaxanthin (V) to zeaxanthin (Z) via antheraxanthin (A) in the VAZ cycle. Violaxanthin de-epoxidase is also capable of converting lutein epoxide (Lx) to lutein (L) in species in which the lutein epoxide–lutein cycle operates (García-Plazaola \textit{et al.}, 2007). Under these conditions, conformational change...
in the major light-harvesting antenna complex (LHCII) of photosystem II (PSII) are induced, switching the functional mode from light harvesting to heat dissipation (for a review, see Goss & Lepetit, 2015; Ruban, 2016). Different components of NPQ are defined by their relaxation kinetics upon darkening, namely energy-dependent quenching (qE), zeaxanthin-dependent quenching (qZ) and photoinhibitory quenching (qi). Rapidly inducible and reversible qE is the predominant form of NPQ in higher plants. Energy-dependent quenching is activated and deactivated within seconds to minutes and thus responds to light fluctuations (Müller et al., 2001). Arabidopsis mutants without qE were found to lack PsbS protein (npq4) and apparently also conformational change in LHCII (Li et al., 2000). In addition, NPQ is dependent on Z (Demmig-Adams, 1990). The part of NPQ which is solely dependent on Z and does not require acidic lumen was eventually termed qZ (Jahns & Holzwarth, 2012). It has been speculated that Z is either capable of directly quenching the singlet excited state of Chl (Owens et al., 1992) or altering the conformation of LHCII (Horton et al., 1991). The qZ component relaxes more slowly than qE, taking several minutes to an hour in the dark. Arabidopsis npq1 mutants lacking VDE (Niyogi et al., 1998) are deficient in light-induced Z formation and thus have less photoprotective capacity (Havaux & Niyogi, 1999). The third type, qi, is a slowly reversible component of NPQ, also known as sustained NPQ, and is associated with Z retention (Demmig-Adams & Adams, 2006) and takes several hours to relax. Photoinhibitory quenching is characterized by reduced maximal photochemical efficiency (F/Fm, Krause, 1988) and increased energy dissipation within PSII (Krause & Weis, 1991). Reduction and recovery of F/Fm were shown to be associated with inactivation and repair of PSII, respectively (Greer et al., 1991).

The mechanistic interplay between fluorescence yield (Fyield), NPQ and the effective quantum yield of PSII (ΦPSII) can be quantified using a pulse-amplitude modulation (PAM) fluorometer (Krause & Weis, 1991; Maxwell & Johnson, 2000). At leaf level, there is a clear negative relationship between NPQ and ΦPSII over the course of a day, protecting plants from excess light (i.e. photoprotection). Studies carried out in nonstressful conditions have shown that ΦPSII decreases while NPQ increases with increasing light intensity. This relationship is tightly linked to the de-epoxidation state of the VAZ cycle (Demmig-Adams et al., 1995). In winter, sustained NPQ predominates, especially in overwintering evergreen plants (Öquist & Huner, 2003; Verhoeven, 2014).

NPQ is quantified from the maximal fluorescence in dark-adapted (Fm) and light-adapted (Fm′) states measured during short strong light pulses which saturate photochemical quenching (qP, see Eqn 3; Bilger & Björkman, 1990). While Magne et al. (2019) have shown that spectral changes in SIF at leaf level were attributed to a rather small contribution of NPQ, the effects of different NPQ components (qE, qZ and qi) have not been resolved. In remote sensing, the photochemical reflectance index (PRI) is used to track changes in the composition of the VAZ-cycle pigments (Gamon et al., 1990). In drought-stressed tobacco, Alonso et al. (2017) showed how diurnal dynamics of NPQ are related to changes in PRI and SIF. Zeaxanthin formation increases absorbance at 505 nm, and changes in LHCII conformation alter the absorbance at 535 nm (Krause, 1973; Bilger & Björkman, 1994). This creates opportunities to measure ρ in leaves and thereby monitor the interconversion between V and Z (Gamon & Surfus, 1999). Correlation between PRI and NPQ was validated in situ using Arabidopsis npq1 and npq4 mutants. Using these mutants, Kohzuma & Hikosaka (2018) found that PRI is capable of tracking qZ but not the total NPQ activity. Notably, they proposed that PRI is sensitive to changes in luminal pH. However, the limitation of PRI in tracking interconversion between V and Z, which is relevant for the changes in NPQ, lies in its sensitivity to long-term or seasonal changes in carotenoid : Chl ratio (Wong & Gamon, 2014), leaf albedo (Busch et al., 2009; Wong & Gamon, 2014) and canopy structure (Barton & North, 2001; Garbulsky et al., 2011). Nevertheless, Nichol et al. (2006) proposed that PRI can potentially account for 70% of the total NPQ, assuming that 70% of the total NPQ is dependent on Z accumulation. Furthermore, van Wittenberge et al. (2019) suggested that changes in light absorbance (a fast response for 500–570 nm and an occasional slow response at c. 550 nm and c. 750 nm) upon illumination at leaf level may be attributable to structural adjustments of photosynthetic membranes involving both carotenoids and Chls. If PRI and ρ can detect changes in the luminal pH and LHCII conformation at leaf level, it may be possible to monitor the qE component of NPQ remotely.

Because SIF reflects both photochemical and nonphotochemical quenching events, it does not linearly correlate with photosynthesis. Using the Soil-Canopy Observation, Photosynthesis and Energy Balance (SCOPE) model, van der Tol et al. (2014) demonstrated the effect of relative light saturation on the relationship between Fyield and photochemical yield (Φp). In low light, the relationship between Fyield and Φp is negative. Maguire et al. (2020) recently confirmed that this negative relationship between Fyield and Φp under low light was determined by photosynthetic induction at the canopy scale under field conditions. In high light, on the other hand, Fyield and Φp can decrease concurrently due to the activation of NPQ. Under severe stress, however, they showed that Fyield may increase as Φp decreases. Despite these complex relationships between Fyield and Φp, the power of the SCOPE model has been demonstrated by predicting the net canopy photosynthesis using SIF retrieved at O2A and O2B bands (Verrelst et al., 2016).

Atherton et al. (2016) showed that combining SIF with PRI can predict the dynamics of photochemical and nonphotochemical activities at leaf level. Although the integration of leaf-level gas-exchange parameters has led to great progress and improvements in modelling, its application is still limited to healthy and not chronically photoinhibited leaves (Hikosaka & Noda, 2018). Recent efforts to link active and passive fluorescence signals with CO2 uptake have led to the development of tools which help us advance our understanding of the relationship between SIF and the photosynthetic processes in leaves (Magne et al., 2017; Vilfan et al., 2019). Also, Pinto et al. (2016) recently provided a proof of concept demonstrating that SIF images captured at the top of the canopy can visualize spatial and temporal variation in
photosynthetic efficiency \( \Phi_{\text{PSII}} \). Since canopy photosynthesis occurs via a totality of individual leaf parts, an understanding of spatio-temporal variation of photosynthesis at leaf level is of fundamental importance. In the context of the FLEX satellite mission (Drusch et al., 2016), it is necessary to understand the influence of NPQ on SIF and \( \rho \) in order to correctly estimate photosynthesis or gross primary productivity (GPP). However, quantitative data describing the link between photosynthesis \( \Phi_{\text{PSII}} \), SIF, NPQ and \( \rho \) are still scarce and inconclusive, mainly due to the composite nature of fluorescence quenching (i.e. different NPQ components and qP).

The objective of this study is to provide a mechanistic understanding of the link between photochemical/non-photochemical fluorescence quenching and remote-sensing properties. We used well-established Arabidopsis \( npq \) mutants (\( npq4 \) and \( npq1 \)) to evaluate the effects of qE, qZ, qI and the formation of Z on SIF and \( \rho \). The experiments were conducted on a sunny summer day (qE and qZ dominate NPQ) and two consecutive days during a simulated cold spell (also, qI influences NPQ). Specifically, the following questions were asked: How do different NPQ components (qE, qZ and qI) affect the emission of SIF as well as leaf \( \rho \)? How much does \( \Phi_{\text{PSII}} \) and NPQ contribute to diurnal SIF variations under conditions of high light and sudden change to cold temperatures (i.e. a simulated cold spell)? And what NPQ components can be quantitatively estimated by diurnal measurements of leaf \( \rho \) and SIF emission? Our results show, for the first time, the link between active and passive fluorescence signals along with plant \( \rho \) during NPQ variations under high light and cold stress in outdoor conditions.

Materials and Methods

Arabidopsis mutants and growth conditions

\textit{Arabidopsis thaliana} Col-0 ecotype seeds, VDE-deficient (\( npq1 \); Niyogi et al., 1998) and PsbS-deficient (\( npq4 \); Li et al., 2000) mutant seeds (derived from mutagenesis using either ethyl methanesulfonate or fast-neutron bombardment) were sown and transplanted into small pots (0.34 l) containing Dachstaudensubstrat soil (Hawita, Vechta, Germany). The seedlings were grown in a growth chamber with a 12 h : 12 h, light : dark photoperiod (100 \( \mu \text{mol photon m}^{-2} \text{s}^{-1} \)), with the temperature set to 20°C during the day and 15°C during the night. Relative humidity was maintained at 60% (Supporting Information Fig. S1). Plants were transferred to the glasshouse 20 d after sowing. In summer, plants were placed outside for at least two consecutive days before measurements were taken. In winter, plants were directly exposed to the elements outside, and diurnal measurements were conducted on two consecutive days (keeping the plants in the glasshouse at night).

Active fluorescence measurement using PAM and LIFT

Fluorescence yield \( \Phi_{\text{PSII}} \), NPQ and \( \rho \) were measured in five plants of each plant type using the Imaging-PAM M-Series (Walz, Effeltrich, Germany) and LIFT instrument (LIFT-REM version, Soliense Inc., New York, NY, USA). During light induction, actinic light was set to c. 370 \( \mu \text{mol photon m}^{-2} \text{s}^{-1} \), while in PAM, the measuring light and saturating pulse were at < 1 \( \mu \text{mol photon m}^{-2} \text{s}^{-1} \) and > 5000 \( \mu \text{mol photon m}^{-2} \text{s}^{-1} \) for 800 ms, respectively.

All plants were dark-adapted for at least 1 h when minimal fluorescence \( F_{0} \) and \( F_{m} \) were measured. A light-induction curve was determined by illuminating the plant continuously for 5 min while \( F_{m}' \) was recorded every 20 s. All fluorescence images were analysed using IMAGINGWIN Software (Walz, Germany), and the mean value was derived as the average of all selected pixels of the plant image. (See Table S1 for a list of abbreviations).

The LIFT instrument was equipped with a pulse-controlled blue LED (445 nm) excitation source, which was focused on a 2-cm measuring spot. Fluorescence was detected at 685 (±10) nm in the coaxial optical path of the instrument. Measurement was based on the fast repetition-rate principle (Kolber et al., 1998) (capable of saturating the primary electron acceptor of PSII (Q\( A \))) and is called the Q\( A \) flash protocol (Osmond et al., 2017). Our measurement protocol was 209.75 ms, which produced 300 flashlets from 0.75 ms of the induction phase and 127 flashlets from 209 ms of the relaxation phase. Fluorescence emission was calculated by subtracting the signal for the inter-flashlet periods from the in-flashlet fluorescence signal. Finally, \( F_{\text{yield}} \) \( ( F_{\text{LIFT}} \) was derived by normalizing the fluorescence emission against the constant excitation power.

The LIFT sensor was situated at a distance of 60 cm, approximately in the nadir position relative to the plant target. In the laboratory, LIFT parameters were traced every 5 s for a duration of 5 min using the Q\( A \) flash protocol while actinic illumination was turned on. Values for traced parameters were averaged every 40 s. During outdoor measurements, an average of the two simultaneous Q\( A \) flashes was used to quantify \( \Phi_{\text{PSII}} \) and NPQ of light-adapted plants.

Measurement of SIF spectra, F687 and F760

Full-SIF spectra were measured in three detached leaves of each plant type using a FieldSpec 4 Wide-Res Spectroradiometer (Analytical Spectral Devices (ASD), Boulder, CO, USA) coupled to a FluoWat leaf clip (van Wittenberge et al., 2013), integrated with a short-pass band filter (> 650 nm). The ASD FieldSpec device has a wavelength range of 350–2500 nm, with a resolution of 3 nm at 700 nm and 30 nm at 1400/2100 nm. A scanning time of 100 ms was used, and 10 readings were averaged per log. The measurement protocol for detecting SIF spectra was based on that described in a study by van Wittenberge et al. (2013). In contrast, SIF at 687 nm (F687) and 760 nm (F760) were measured using a FLOX device (JB Hyperspectral Devices, Düsseldorf, Germany) and retrieved using an improved Fraunhofer line depth (FLD) method (Alonso et al., 2008).

Measurement of plant reflectance

Diurnal changes in plant \( \rho \) were measured with a point spectrometer (Flame; Ocean Optics, FL, USA) integrated...
within the FLOX device. The FLOX fibre optics were set orthogonally to the plant at a distance of 5 cm (for the measurement setup, see Fig. S2). The upwelling and downwelling fibre-optic channels had a field of view of 180° and 25°, respectively.

Measuring the diurnal response of Arabidopsis plants in outdoor conditions on a summer day and during a simulated cold spell

To quantify the diurnal changes, magnitudes and relationships among SIF, ΦPSII and NPQ, parallel measurements of active and passive fluorescence parameters were conducted outdoors under a clear sky on a summer day and on two consecutive winter days.

Four plants of each type were measured in the summer (28 August 2017) from 07:34 to 16:04 h with a daytime temperature range from 19.7°C (minimum) to 31.5°C (maximum). In winter, three plants were measured from 09:37 to 16:50 h on day 1 (22 February 2018, −0.8 to 4.1°C) and from 07:00 to 16:30 h on day 2 (23 February 2018, −3.9 to 3.3°C). All plants were randomized for each measurement window to remove the bias associated with temporal changes that may affect both optical and physiological plant responses. Outdoor measurements were performed at the Plant Science Department of the Research Center, Jülich, Germany (lat 50°54′35.4″N, long 6°24′45.5″E). Recorded diurnal light intensity and temperature for both summer and winter conditions are summarized in Fig. S3.

Tracing recovery of PSII efficiency in glasshouse conditions

Recovery of $F_v/F_m$ (Eqn 1) was traced in glasshouse plants exposed to the cold. Three plants of each type were transferred to the glasshouse the day after measurements during a winter spell. Plants were dark-adapted for at least 30 min before $F_v/F_m$ measurement, and measurements were taken every c. 45 mins for a duration of 3.5 h. As in the laboratory and outdoor measurements, the QA flash protocol was used for the LIFT device to estimate the $F_v/F_m$ parameter.

Calculation of active fluorescence parameters and remote-sensing signals

Active fluorescence parameters derived from PAM were calculated using Eqns 1–3, based on Maxwell & Johnson (2000). In the case of LIFT measurements, $F_{0,LIFT}$ was equal to the first fluorescence transient recorded upon the first excitation flashlet of the QA protocol, while $F_{m,LIFT}$ was the average $F_{LIFT}$ recorded in 301 and 302 flashlets. All LIFT data were collected and analysed based on the method described by Keller et al. (2019).

$$F_v/F_m = \frac{(F_m - F_0)}{F_m}$$  \hspace{1cm} Eqn 1

$$\Phi_{PSII} = \frac{F'_m - F'_0}{F'_m}$$  \hspace{1cm} Eqn 2

$$NPQ = \frac{F_m - F'_m}{F'_m}$$  \hspace{1cm} Eqn 3

Passive fluorescence was quantified using either the FluoWat device or the FLOX system. Solar-induced fluorescence and SIF$_{yield}$ from the FluoWat device were derived as described previously (van Wittenbergh et al., 2013), and F687 and F760 derived from FLOX measurements were normalised to the photosynthetically active radiation (PAR) to estimate solar-induced fluorescence yield at 687 nm (F687$_{yield}$) and 760nm (F760$_{yield}$), respectively.

Reflectance from 400 to 750 nm was calculated from the FLOX diurnal measurements by normalizing reflected radiance to the incoming irradiance. Reflectance values were further adjusted based on the minimum and maximum $\rho$ values for each measurement:

$$\rho_{adj} = \frac{\rho_k - \rho_{min}}{\rho_{max} - \rho_{min}}$$  \hspace{1cm} Eqn 4

Using the adjusted $\rho$, the photochemical reflectance index (PRI) was calculated based on the equation given in a study by Gamon et al. (1992).

$$PRI_{570} = \frac{\rho_{531} - \rho_{570}}{\rho_{531} + \rho_{570}}$$  \hspace{1cm} Eqn 5

Statistical analysis

A completely randomized design was used in the laboratory setup, and a repeated measures design was used in the diurnal outdoor setup. The models were fitted using the lmer function in R (R Core Team, 2013), where genotypes and the time of measurements were denoted as fixed effects and replicates as random effects. Pairwise mean comparisons were made using the lsmeans function.

For cluster analysis, the adjusted $\rho$ data were pooled, consisting of individual measurements from four plants of each plant type from all measurement points taken throughout the entire day in the summer. Dissimilarity between data points was computed in R using Pearson’s $R^2$ in the dist function. Hierarchical clustering was computed using the hclust function (Ward’s method). The correlation coefficient ($r$) values for average points between SIF and NPQ, SIF and PRI, and SIF and $\Phi_{PSII}$ were calculated using the cor.test function in R using Pearson’s method.

Results

Effect of reduced NPQ on active Chl-fluorescence parameters

To characterize the photochemical and nonphotochemical responses of npq mutants, active fluorescence parameters were
traced on dark-adapted plants during photosynthetic induction. Fluorescence yield in both npq mutants was higher than in the Arabidopsis wild-type (WT) which is more pronounced in the steady-state condition (Fig. 1a). By contrast, \( \Phi_{\text{PSII}} \) was similar in the mutant and WT plants (Fig. 1b), while the degree of NPQ for both mutants was lower (Fig. 1c). Moreover, the level of NPQ in the npq1 mutants increased abruptly, while npq4 showed a gradually increasing trend. Absolute values of PAM and LIFT were different (see discussion in Pieruschka et al., 2014 on this methodological difference); however, relative changes showed the same dynamics. Despite the difference in \( F_{\text{yield}} \) no morphological differences in size or greenness were observed between the WT and mutants (data not shown).

Diurnal trend of PSII efficiency, NPQ and SIF in summer and winter conditions

To investigate the link between SIF, \( \Phi_{\text{PSII}} \) and NPQ in the field, active and passive fluorescence were simultaneously measured in an outdoor scenario throughout the course of the day. During the day in summer, for all plant types, \( \Phi_{\text{PSII}} \) was negatively correlated with incoming PAR, whereas NPQ was positively correlated with incoming PAR (Fig. 2a, b). The lowest value of \( \Phi_{\text{PSII}} \) was observed at midday, when the light intensity was at its peak, while NPQ was highest at the end of the day. The effective quantum yield of PSII for both mutants was significantly lower than that of the WT (Table S2). Almost invariably throughout the day, NPQ was lower for both mutants than it was for the WT. Like NPQ, SIF followed the diurnal course of PAR; that is, \( F_{760} \) was highest at midday, and lowest in the early morning and late afternoon. While this trend was found to apply to all the plant types, the npq mutants had higher SIF emission than the WT (Fig. 2c). Conversely, \( F_{760,\text{yield}} \) in the WT showed high values in the morning and late afternoon, and the lowest values at midday (Fig. 2d). \( F_{760,\text{yield}} \) in mutants was higher than the WT but tended to decrease towards the end of the day (Fig. 2d). At the end of the day, this decrease coincided with significantly lower \( \Phi_{\text{PSII}} \) in the mutants, compared to the WT (Table S2).

During the winter spell, \( \Phi_{\text{PSII}} \) for all plant types immediately decreased as soon as the plants were exposed to sudden cold treatment (Fig. 3a). At the end of the day, \( F/F_{\text{m}} \) decreased to less than half its initial value (Fig. 3a, marked with an asterisk). While \( \Phi_{\text{PSII}} \) dropped rapidly after exposure to the cold, NPQ gradually increased over the course of the day (Fig. 3b). In addition, npq mutants showed different levels of NPQ to those of the WT. Accordingly, differences in \( F_{760} \) were also observed between mutants and the WT, but these were only evident between the initial hours of exposure to the cold and midday (Fig. 3c). Thereafter, \( F_{760} \) was quenched in line with the other plant types. Interestingly, \( F_{760} \) emission followed a similar diurnal trend to \( F_{760,\text{yield}} \), with both being independent of the diurnal PAR (Fig. 3c,d). Notably, the quenched state was sustained until the second day of cold treatment (Fig. S4e,f). When plants were transferred back to the glasshouse, \( F/F_{\text{m}} \) slowly increased for all types, suggesting recovery of PSII efficiency (Fig. S5).

Fig. 1 Fluorescence dynamics after turning on the actinic light (370 \( \mu \text{mol} \text{ m}^{-2} \text{s}^{-1} \)), measured in dark-adapted Arabidopsis wild-type (WT) and nonphotochemical quenching (NPQ)-deficient (npq) mutants, using active fluorescence measurement techniques. Lines show 4.5-min induction of (a) the fluorescence yield from the pulse-amplitude modulation technique (\( F_{\text{PAM}} \)) or light-induced fluorescence transients (LIFT), (b) the effective quantum yield of photosystem II (\( \Phi_{\text{PSII}} \)), and (c) nonphotochemical quenching (NPQ) of the Columbia 0 (Col-0) ecotype (circles), and npq1 (violaxanthin de-epoxidase (VDE)-deficient; triangles) and npq4 (PsbS-deficient; squares) mutants. Measurements were taken using either pulse-amplitude modulation (PAM, closed symbols) or light-induced fluorescence transients (LIFT, open symbols) techniques. Points indicate the average \( \pm \) SE of five plants from each type, measured at room temperature with controlled illumination.
Effect of NPQ mutation in spectrally- and iFLD-resolved SIF

To characterise the effect of PsbS- and Z-deficiency on SIF, spectrally-resolved SIF data for npq mutants and WT were compared, and the correlation between iFLD-derived F687 and F760 was calculated. At leaf level, npq mutants showed consistently higher SIF$_{yield}$ at the two fluorescence peaks across the spectrum (Fig. 4a). In outdoor conditions, a strong correlation between F687 and F760 was observed (Fig. 4b).
Diurnal measurement of PRI and NPQ in summer showed a clear negative relationship in both the WT and npq mutants, although the WT plants reached higher NPQ levels than the mutants (Fig. 6a). By contrast, the negative PRI–NPQ relationship became less distinct in measurements from the winter spell. While the WT had higher NPQ and a lower PRI, the mutants had lower NPQ and a higher PRI (Fig. 6b).

Analysis of spectral reflectance across the visible spectral window

To further investigate the NPQ mechanisms that can be related to changes in ρ signal, a comparison of ρ in WT and npq mutants was made, and cluster analysis of diurnal ρ in summer was conducted. There was a clear difference in ρ between the WT and npq1, characterized by two distinct peaks – one broadband signal peaking at 520 nm and a narrow range peaking at 700 nm (Fig. 7g). By contrast, there was almost no difference in ρ between the WT and npq4 plants (Fig. 7h). After cluster analysis, ρ in the WT and npq4 was almost inseparable, while most npq1 mutants were clustered together (Fig. 8). The major clustering found was from the WT and npq4 mutants measured from c. 10:30 h.

Measuring diurnal dynamics of PRI in the WT and npq mutants

To determine how PRI is linked to different NPQ components, we calculated PRI from the reflectance index was higher in the morning, which then decreased in the late afternoon (Fig. 5b). Photochemical reflectance index was higher in npq1 than in the WT in the morning, then gradually decreased in the afternoon, while npq4 had an intermediate value between that of the WT and npq1. On the second day, all plants showed an identical PRI trend, which followed the diurnal course of PAR (Fig. 5c).

and F760 was observed among all plant types in summer ($r = 0.95$; Fig. 4b). Although we found a similar relationship during the winter spell experiment, more residuals were observed on the first day, with an $r$ of only 0.88 (Fig. 4c), while the correlation was weakest on the second day ($r = 0.71$; Fig. 4d).

Fig. 4 Relationship between the two fluorescence peaks as affected by the mutation in nonphotochemical quenching (NPQ), presented in full spectra of solar-induced fluorescence yield (SIFyield), as well as solar-induced fluorescence retrieved in O2 A (F760) and O2 B (F687) absorption bands using the improved Fraunhofer Line Depth (FLD) method. The graphs show the following: (a) total SIFyield spectra, measured in upwelling and downwelling emissions of a single leaf for each plant type; (b) correlation between F687 and F760, measured diurnally during the summer; (c) correlation between F687 and F760, measured diurnally during the cold winter on the first day of exposure; and (d) the second day of exposure. Values are the average ± SE of four plants from each plant type. Pearson’s correlation coefficients ($r$) are displayed at the top of each graph (***, $P < 0.001$).
onwards (branch A, Fig. 8). Interestingly, the clustering also resolved \( \rho \) collected at different times of the day. In particular, there was a clear separation of \( \rho \) data measured during the morning, at midday and in the late afternoon in branch D (npq1 cluster) as well as in branch E (Col-0/npq4 cluster).

Notably, the difference in \( \rho \) between branches A and C (Fig. 8) was identical to the pattern of change in \( \rho \) (\( \Delta \rho \)), resolved as the difference between the WT and npq1 (Fig. 7g). The spectra depicted in Fig. 7(d,f) compare the diurnal changes of \( \rho \) from midday to late afternoon; both curves show a broad peak centralized at 560 nm and a narrow peak at 700 nm. Furthermore, the 700 nm peak observed was consistent, yet the magnitude was variable. By contrast, this peak was not observed in the difference between the WT and npq4 (Fig. 7h). The \( \Delta \rho \) at different times of the day, relative to the first measurement in the morning, peaked at 520 nm and 700 nm, while the region from 520 to 650 nm was variable (Fig. S6). It is also worth noting that the 700-nm peak consistently increased between the morning and the afternoon for all plant types.

Relationship of \( F760_{\text{yield}} \) to \( \Phi_{\text{PSII}}, \text{NPQ} \) and PRI

To quantify the changes in remote-sensing signals that can be related to photochemical and NPQ events, correlations among SIF, PRI, \( \Phi_{\text{PSII}} \), and NPQ were computed. For the WT plants, \( F760_{\text{yield}} \) was found to be positively correlated to \( \Phi_{\text{PSII}} \) but negatively correlated to NPQ during the summer (Figs. 9a, c). The correlation between \( F760_{\text{yield}} \) and \( \Phi_{\text{PSII}} \) was slightly stronger than that between \( F760_{\text{yield}} \) and NPQ (Table 1). In mutants, \( F760_{\text{yield}} \) was directly correlated to \( \Phi_{\text{PSII}} \) and negatively correlated to NPQ, but with lower \( r \) than in the WT, with a p-value < 0.05. By contrast, during sudden cold treatment \( F760_{\text{yield}} \) in
all plant types was positively correlated to $\Phi_{\text{PSII}}$ only in the morning and then became negatively correlated in the afternoon (Fig. 9b). On the other hand, $F_{760,\text{yield}}$ was found to have a strong but nonlinear negative relationship with NPQ ($r = 0.79$) throughout the whole day during the winter spell, for all plant types (Fig. 9d).

Fig. 7 Spectral characteristics of the difference in spectral reflectance ($\rho$) between two distinct sub-groupings resolved in the cluster analysis of summer data, as well as the $\rho$ difference between the Arabidopsis wild-type (WT) and nonphotochemical quenching (NPQ)-deficient $npq1$ mutants. The data show two distinct peaks at c. 520 nm and 700 nm, and are presented as follows: (a) measurement points associated with strong-minus-weak violaxanthin-antheraxanthin-zeaxanthin cycle (VAZ-cycle) activity; (b) midday minus late afternoon measurement within the violaxanthin de-epoxidase deficient $npq1$ mutant cluster; (c) combined Columbia 0 (Col-0, WT) and PsbS-deficient $npq4$ mutant minus $npq1$ measurements, within the morning cluster; (d) measurements for midday minus late afternoon within the strong VAZ activity cluster; (e) second to third measurements minus the first measurement within the morning cluster from both Col-0 and $npq4$; (f) morning cluster minus $npq1$ cluster within the weak VAZ activity cluster; (g) all measurements in summer, during the day, for the WT minus mutant $npq1$; (h) all measurements in summer, during the day, for the WT minus mutant $npq4$. Horizontal dashes indicate reflectance used to calculate photochemical reflectance index (PRI) and solar-induced fluorescence (SIF) retrieval in the O$_2$B absorption band.

Fig. 8 Cluster analysis of spectral reflectance from 400 nm to 700 nm diurnally, measured during the day in summer, differentiating the Arabidopsis violaxanthin de-epoxidase (VDE)-deficient $npq1$ mutant from the wild-type (WT) but not the PsbS-deficient $npq4$ mutant. Temporal groupings within plant types were also resolved from morning, mid-day and afternoon measurements. The time range of each measurement window and average light intensity are shown on the upper right-hand side of the figure. Letters indicate the point of separation of spectral reflectance, which was used as the basis for calculating the difference in reflectance previously shown in Fig. 7.
The correlation between F760yield and PRI was stronger during the day in summer than during the winter spell (Table 1). F760yield was found to be positively related to PRI in WT plants but less distinct in both mutants during summer (Fig. 10a). During sudden cold treatment, F760yield and PRI had a direct but nonlinear relationship, and the degree of saturation differed across plant types (Fig. 10b). For correlation coefficients, see Table 1.

**Discussion**

To understand the link between NPQ, photosynthesis, and SIF under field conditions, we combined active and passive fluorescence techniques along with changes in $p$. We used Arabidopsis due to its simple canopy structure and mutants with well-defined NPQ alterations. Firstly, we showed that npq1 and npq4 mutants exhibit a similar increase in SIF, indicating that a lack of either PsbS or Z has a similar effect on SIFyield at leaf level (Figs 2c,d, 4a). Secondly, we demonstrated the gradual quenching of SIF during the onset of q1 in a winter spell, masking the consequence of NPQ deficiency (Figs 3c,d). Furthermore, the correlation displayed a better fit between SIF and $\Phi_{PSII}$ in summer but a closer relationship between SIF and NPQ during the winter spell (Table 1; Fig. 9). Thirdly, cluster analysis of $p$ differentiated npq1 but not npq4 from the WT (Fig. 8). We therefore conclude that changes in diurnal $p$ are modulated only by the VAZ cycle, eliminating the effect of PsbS-mediated conformational changes of the LHCII to $p$ in an outdoor setup. These results are vital for understanding the link between NPQ, photosynthesis, and SIF under field conditions.

**Table 1** Correlation coefficients for solar-induced fluorescence yield at 760 nm (F760yield) and nonphotochemical quenching (NPQ), and effective quantum yield of photosystem II from the light-induced fluorescence transients (LIFT) technique ($\Phi_{PSII}$LIFT) and photochemical reflectance index (PRI).

|              | Summer       | Winter spell |          |
|--------------|--------------|--------------|----------|
|              | F760yield vs NPQ | F760yield vs $\Phi_{PSII}$ | F760yield vs PRI |
| Col-0        | $-0.74^*$    | 0.88**       | 0.83**   |
| npq1         | $-0.58^*$ns  | 0.55**       | 0.67*    |
| npq4         | $-0.64^*$ns  | 0.55**       | 0.65**   |
| All points   | $-0.83^{***}$| 0.29*        | 0.67^{***} |
|              | F760yield vs NPQ | F760yield vs $\Phi_{PSII}$ | F760yield vs PRI |
| Col-0        | $-0.78^{***}$| 0.05^ns      | 0.80^{***} |
| npq1         | $-0.86^{***}$| 0.14^ns      | 0.72^{***} |
| npq4         | $-0.87^{***}$| 0.13^ns      | 0.75^{***} |
| All points   | $-0.79^{***}$| $-0.05^{**}$ | 0.72^{***} |

Test of significance: ^ns, not significant ($P > 0.05$); ^*, $P < 0.05$; ^**, $P < 0.01$; ^***, $P < 0.001$.

The correlation between F760yield and PRI was stronger during the day in summer than during the winter spell (Table 1). F760yield was found to be positively related to PRI in WT plants but less distinct in both mutants during summer (Fig. 10a). During sudden cold treatment, F760yield and PRI had a direct but nonlinear relationship, and the degree of saturation differed across plant types (Fig. 10b). For correlation coefficients, see Table 1.
limitations of SIF and ρ in terms of the remote sensing of NPQ and photosynthesis. Furthermore, they aid our understanding of how to use PRI to estimate the contribution of NPQ to the SIF signal, and how to combine ρ and SIF measurements to understand dynamic photosynthesis under field conditions.

Influence of NPQ deficiency on active and passive fluorescence signals

We showed the consequences of PsbS- and Z-deficiency for active and passive fluorescence signals at leaf level in both a controlled indoor setup and diurnal field conditions in the summer and during a winter spell. PsbS and Z play key roles in the onset of rapidly inducible and reversible qE, and in sustained NPQ in winter (Verhoeven, 2014). We characterized the kinetics of NPQ induction in low light. Absence of Z in the npq1 mutant reduced the extent of NPQ, but quick induction of NPQ upon illumination was observed as a result of functional PsbS protein (Fig. 1c). Conversely, the npq4 mutant showed a slower increase in NPQ which was the effect of VAZ interconversion in the absence of PsbS protein. Our results consistently showed a similar increase in FYIELD for both npq mutants measured in indoor and outdoor conditions (Figs 1a, 2c, 3c), indicating that the extent of NPQ (but not the kinetics) affected diurnal SIF emission. This increase was also consistently visible at the two fluorescence peaks, suggesting that the F687 : F760 ratio is unaffected by changes in NPQ (Fig. 4). Although state transitions may affect this ratio, this was not included in our study as it only occurs under low light levels, and this dynamic is considered less relevant in a remote-sensing context (sensu Porcar-Castell et al., 2014). Nevertheless, in winter, the strength of the correlation between the two fluorescence peaks decreased, which was probably due to a smaller signal-to-noise ratio when SIF was fully quenched (Fig. 4c).

The diurnal observations in summer revealed a dynamic relationship between NPQ, ΦPSII and SIFyield which is consistent with controlled measurements made in a laboratory setting. This coherence also supports the concept that changes in diurnal SIF (in this setup and possibly at larger scales) are not affected by other cellular or leaf level events (e.g. we consider the effect of chloroplast movement on ρ to be negligible on the canopy scale). Fig. 2(d) shows that aside from an absolute increase in SIFyield values in both mutants in the morning, SIFyield was found to be relatively lower in the mutants at the end of the day, and this finding was also coupled with lower ΦPSII (Table S2). This confirms that npq mutants with reduced photoprotection are more susceptible to high light stress (Havaux & Niyogi, 1999; Külheim et al., 2002; Li et al., 2002). Thus, SIFyield for the WT was found to recover, while the mutants had a reduced SIFyield when compared to the initial value in the morning (Table S2). We postulate that the qI exhibited by the mutants resulted in a decrease in SIFyield. To test this hypothesis, we controlled the development of qI by exposing the plants to high light and a cold spell during winter. In doing so, SIF quenched equally for all plant types, indicating the onset of qI (Fig. 3), which continued on the second day (Fig. S7). The reduced Fv/Fm and its slow recovery in the glasshouse (Fig. S5) strongly suggest that PSII sustained damage during the winter spell, as well as its corresponding repair.

We have also shown in our setup that a decrease in SIF is always related to an increase in NPQ but is also associated with a decrease in ΦPSII; however, the coupling of the three processes is not straightforward. We thus developed a conceptual model taking all our measurements into account (Fig. 11, and see Notes S1 and Table S3 on how we translated the measurements into the conceptual model). It is noteworthy that the contribution of basal nonradiative decay is not depicted in the model. The relationship between the three pathways mostly depends on the prevailing environmental conditions. In the WT, SIF was found to be slightly more strongly correlated with ΦPSII than NPQ during the diurnal measurement in the summer condition (Table 1). Conversely, SIF was found to be more strongly correlated with NPQ than ΦPSII during the winter spell, which is predominantly
Fig. 11 Schematic representation illustrating the major quenching pathways of absorbed light energy in Arabidopsis wild-type (WT) plants and in the violaxanthin de-epoxidase (VDE)-deficient npq1 and PbS-deficient npq4 mutants under low and high light in summer or during a cold spell. The relative size of each pathway is either based on the observations during the diurnal field experiment (for SIF<sub>yield</sub> – solar-induced fluorescence yield, and Φ<sub>PSII</sub> – effective quantum yield of PSII) or estimated by comparison between WT and the mutants (for PbS, PbS-dependent quenching; qI, photoinhibitory quenching; Z, zeaxanthin-dependent quenching). Since the quantum yield is not known for the nonphotochemical quenching (NPQ)-related pathways, the width of the pathways does not correspond to their relative contributions. For simplicity, nonregulated energy dissipation is assumed to be similar in the three genotypes under all conditions, and thus is not depicted in this summary.

How can PRI be used as a tool to remotely quantify NPQ contributions to the SIF–photosynthesis relationship?

Photochemical reflectance index is related to NPQ at leaf level (Peñuelas et al., 1995; Gamon et al., 1997; Evain et al., 2004). In remote sensing, changes in ρ can indicate changes in Z accumulation, which is an important component in evaluating de-epoxidation state in the VAZ cycle. We showed that SIF is linearly related to PRI in summer (more importantly in the case of WT plants which naturally exist in the field) (Fig. 10). A weak and nonlinear correlation between SIF and PRI was only found during the cold spell, when qI, accompanied by changes in other leaf pigments, is suspected to influence the diurnal trend. An overall, albeit uneven, decline in PRI was observed during the cold spell, which is likely due to pigment breakdown (Fig. 5b). It has been shown that seasonal changes in PRI are sensitive to both short and long-term changes in pigment composition (Gamon & Berry, 2012; Wong & Gamon, 2014). Thus, the use of PRI during winter conditions still needs further investigation in order to link with sustained NPQ when Z accumulation is compounded by changes in other pigments in overwintering species (Busch et al., 2009). Kohzuma & Hikosaka (2018) previously showed that PRI in leaf discs changes upon infusion with a pH-controlled buffer. It seems worth exploring how to better retrieve the pH-dependent signal from the ρ measurements, taking into account the pigment and structural changes in canopies. Wong & Gamon (2014) showed that in seasonal observations PRI is strongly related to carotenoid : Chl pigment ratios and minimally attributed to the xanthophyll cycle. In Fig. 5a, the slight decline in PRI observed in the npq1 mutants was probably due to a build-up of luminal pH as PAR increases. Despite the fact that low luminal pH and PbS trigger NPQ, we have shown (by tracking in outdoor conditions) a strong correlation between PRI and NPQ (Fig. 6), while SIF and NPQ were found to have a negative relationship (Fig. 9c,d). Since our study was limited to the VAZ cycle, studying the effect of the lutein cycle on SIF and ρ will give a more holistic understanding of the relative Δρ that can be attributed to the NPQ of SIF. Although there are various NPQ mechanisms, the VAZ cycle still plays a major role in regulating the extent of NPQ (Jahns & Holzwarth, 2012).
Clustering clearly separated npq1 from both WT and npq4 mutants, which strongly suggests that plant ρ is only sensitive to VAZ activity and not to PsbS-mediated heat dissipation (Fig. 8). While this observation may seem to contradict the report of van Wittenberghe et al. (2019), this discrepancy might be due to differences in how Δρ was traced. In particular, they traced Δρ for a maximum of 10 min after actinic illumination, while we monitored Δρ throughout the entire day at c. 45 min intervals. Furthermore, diurnal Δρ that is independent of VAZ activity was also resolved in clustering (Fig. 8, branches B and D) which appears to be due to changes at 700 nm, probably resulting from Chl breakdown (Fig. S6). We acknowledge that the lack of pigment data in our study limits further interpretation of this result.

Here, we have shown that reflectance at 531 nm is sensitive to changes occurring during the VAZ interconversion (Fig. 7a). By comparing PRI calculated in other spectral regions (Fig. S8), 570 nm showed itself to be a fairly reliable reference band for PRI (first shown by Gamon et al., 1990). PRI on the second day in winter followed light intensity, which was probably a consequence of the changing angle of the sun (Fig. 5c). This suggests that some consideration needs to be given to the effect of leaf albedo and pigment pools when scaling up PRI both temporally and spatially (Wong & Gamon, 2014). Changes in PRI associated with dark conditions or low levels of light would facilitate quantification of pigment changes in a fixed space for remote-sensing applications.

Towards remote sensing of photosynthesis

Changes in SIF due to a dynamic environment are linked to both physical and biological events. Fig. 2(c) shows that SIF is strongly dependent on light intensity. To accurately separate function-related traits from light dependency, emission should be normalized to the fraction of aPAR (fAPAR). Although we only calculated SIFyield by the incoming PAR, it accords with PAM and FluoWat due to the close measurement distance and simple canopy of our target. In remote sensing, SIFyield is compounded by structural effects as well as factors that can influence the ρ-based estimates for light absorption. Future research should, therefore, focus on improving fAPAR estimation from leaf to canopy level, including the escape probability of fluorescence (Zeng et al., 2019).

At canopy level, fAPAR is driven by the complexity of the leaf-area index, leaf angle, leaf clumping, and the pigment composition of the single leaves, which all affect canopy ρ. We have previously shown Δρ at leaf level to be influenced by pigment regulation and that such changes are short-term (diurnal) and long-term (seasonal) responses. This, however, does not directly equate to canopy level as structural effects may have a bigger influence on PRI than physiological dynamics (Gamon et al., 1992; Gitelson et al., 2017). We suggest that changes in PRI can be quantified by removing the canopy effect, as proposed by Hilker et al. (2008) and Wu et al. (2015). PRI can then be combined with SIF and fAPAR within a spatial domain, and the leaf signal can be scaled-up to the total canopy signal. Pinto et al. (2016) was first to report a proof of concept that high-resolution imaging spectrometers can provide the spatial pattern of SIF in order to quantify photosynthetic functions at canopy level. More recently, Pinto et al. (2020) have provided more convincing evidence at a larger spatial scale that the dynamics in PRI and SIF can potentially be linked to photochemical and nonphotochemical events in plants during stress-induced scenarios. The maturity of SIF and ρ measurements at higher spatial and temporal resolutions will enable the scientific community to generate a vast amount of data in order to provide more dynamic empirical correlations between the light-dependent signals and biological events. Our study provides evidence that improves our fundamental understanding of NPQ mechanisms and their contributions, at leaf level, to regulating SIF and PRI during diurnal changes in photosynthesis. Investigation of the influence of Z on PSII efficiency and its consequences for SIF emission is a promising direction for future research, and will create new opportunities to test hypotheses related to the functional role of NPQ in canopy photosynthesis and productivity.

It is well established that SIF can improve estimates of photosynthetic carbon uptake rates and GPP (Guanter et al., 2014; Gu et al., 2019). With the assumption that a homogenous canopy is a single big leaf, modelling photosynthesis using SIF would require understanding how NPQ is linked to this relationship. While the focus of this paper was to relate SIF to the yield of PSII photochemistry, it is equally important to understand the feedback effect of the Calvin cycle on the rate of electron transport, PSII yield and thus Fyield (e.g. stomatal response, carboxylation efficiency, photorespiration, substrate regeneration, etc.). Although ρ reflects only partial NPQ, a parallel survey of light and temperature can be added to numerical simulations to ascertain both the component and mechanism of NPQ involved. We therefore propose that the conceptual model summarized in Fig. 11 should be tested at canopy level and on different species, employing various NPQ strategies.

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Author contributions
KA, SM, CJ, OM and UR conceptualized the experiments. KA performed the experiments and analysed the data. DE performed the cluster analysis. KA, SM, OM and UR interpreted the results. KA, UR and SM structured the manuscript and wrote with contributions from OM, DE and CJ.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

**Fig. S1** Growing conditions of the Arabidopsis WT and *npq* mutants before field measurement in summer and winter conditions.

**Fig. S2** Illustration of diurnal measurement combining both active and passive fluorescence measurements and spectral reflectance.

**Fig. S3** Recorded diurnal light intensity and temperature during field measurement on summer and winter days.

**Fig. S4** Diurnal trend of F687 in summer and winter field conditions.

**Fig. S5** Recovery of $Fv/Fm$ after exposure to a cold spell for 2 d.

**Fig. S6** Diurnal change in spectral reflectance in the WT, *npq1* and *npq4* mutants, relative to the first measurement.

**Fig. S7** Diurnal trends for active and passive fluorescence parameters measured during the second day of exposure to outdoor winter conditions.

**Fig. S8** Diurnal pattern of two different PRI calculations measured in summer and a simulated cold spell during winter.

**Notes S1** Considerations for developing quantitative values for the conceptual model in Fig. 11.

**Table S1** List of abbreviations used in this manuscript.

**Table S2** $SIF_{yield}$ and $\Phi_{PSII}$ during the morning, midday and afternoon, measured for all Arabidopsis plant types.

**Table S3** Final input values for the Sankey diagram shown in Fig. 11.

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