FLEX-Fluorescence 2026 Workshop

03 – 06 March | Bonn University, Germany

Exploring the Nonlinear Relationship Between Photosynthesis and Chlorophyll Fluorescence Dynamics for Early Stress Detection

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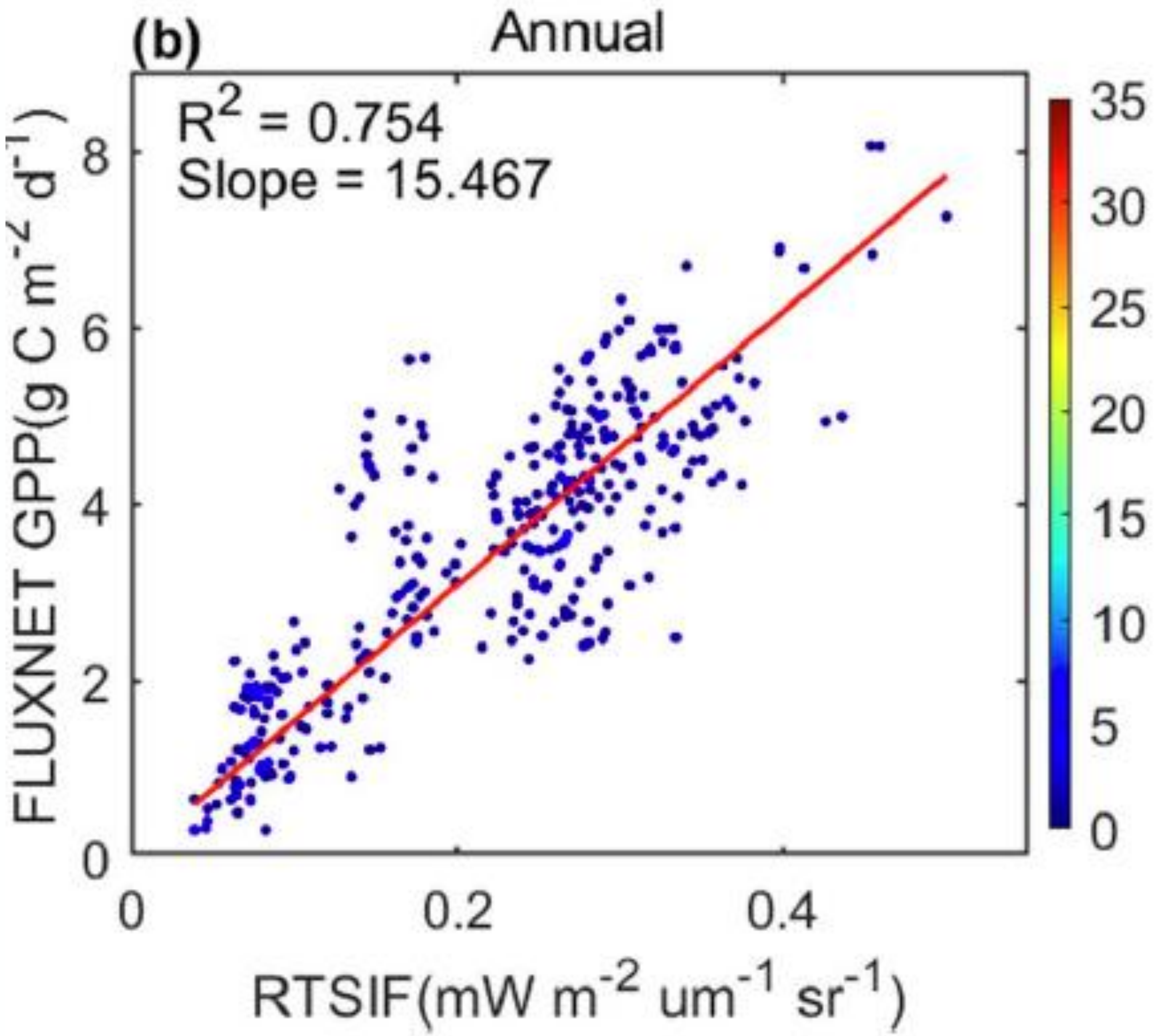
Chlorophyll Fluorescence



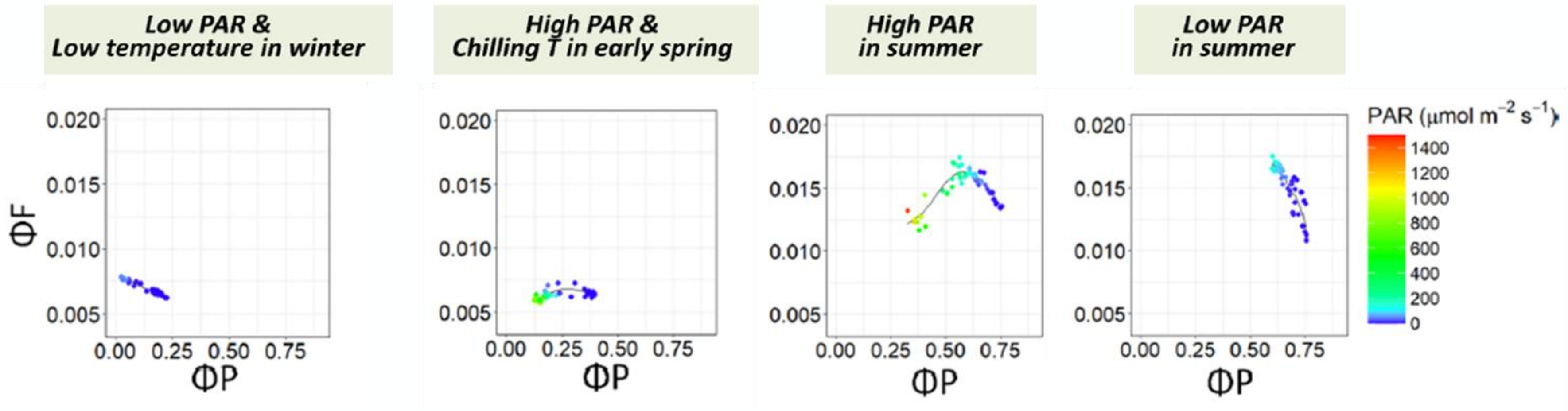
Linear relationship

- Annual
- Driven by **long-term dynamics** link to pigment pools.

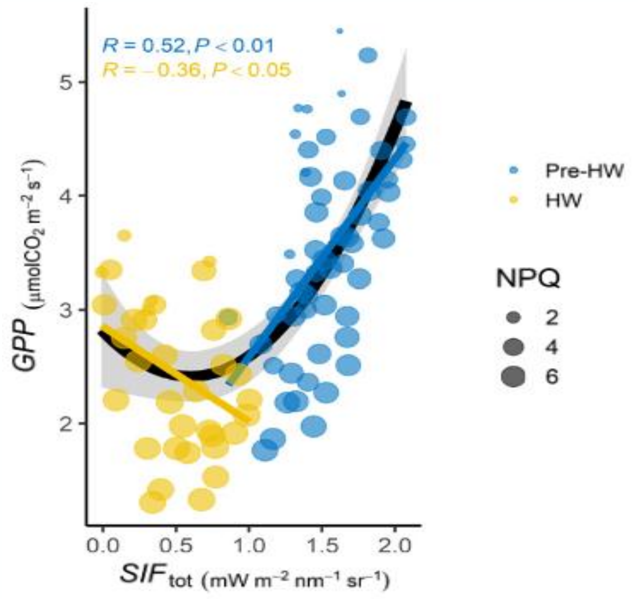
Ref: Chen et al.2022.Scientific data

Non-Linear relationship

- Seasonal-Daily.
- Driven by dynamic changes in the **light reaction** energy dissipations pathways.
- Link to **short term dynamics** in PAR, temperature, and water/nutrients availability.
- **Early stress** detection.

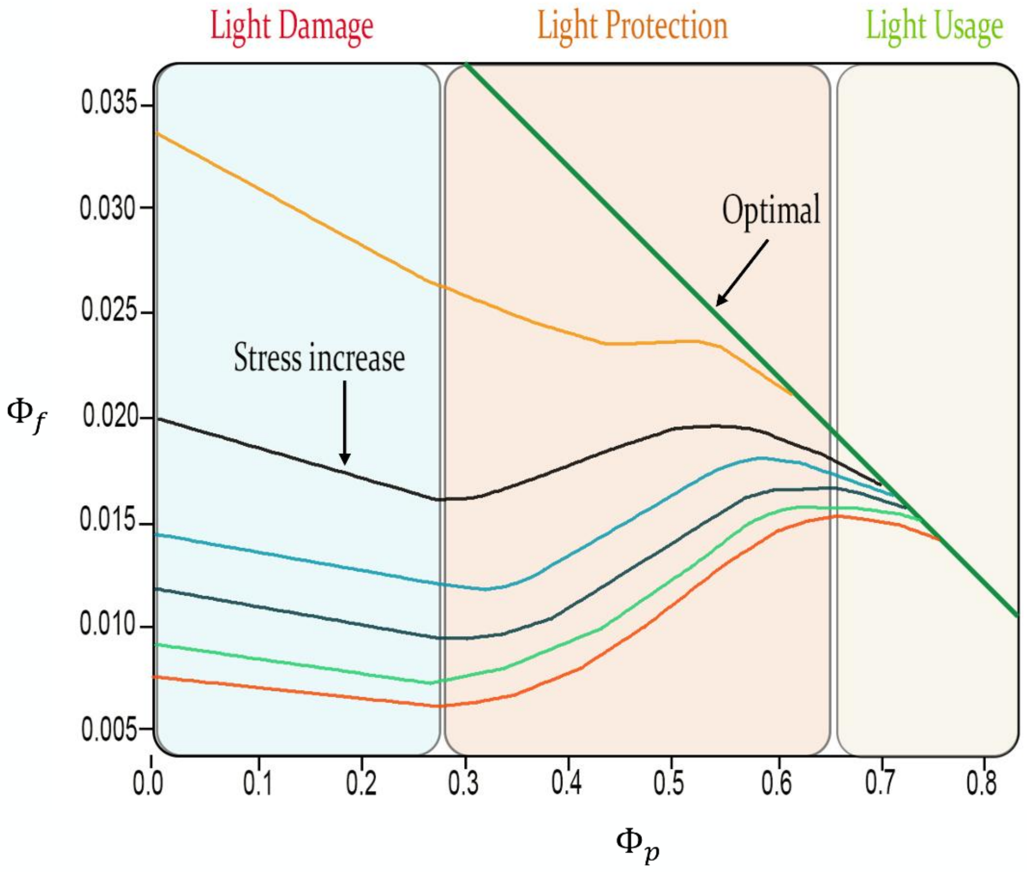


Ref: Zhang et al.2025.Photosynthesis Research



Ref: Martini et al.2022. New Phytologist

Research questions



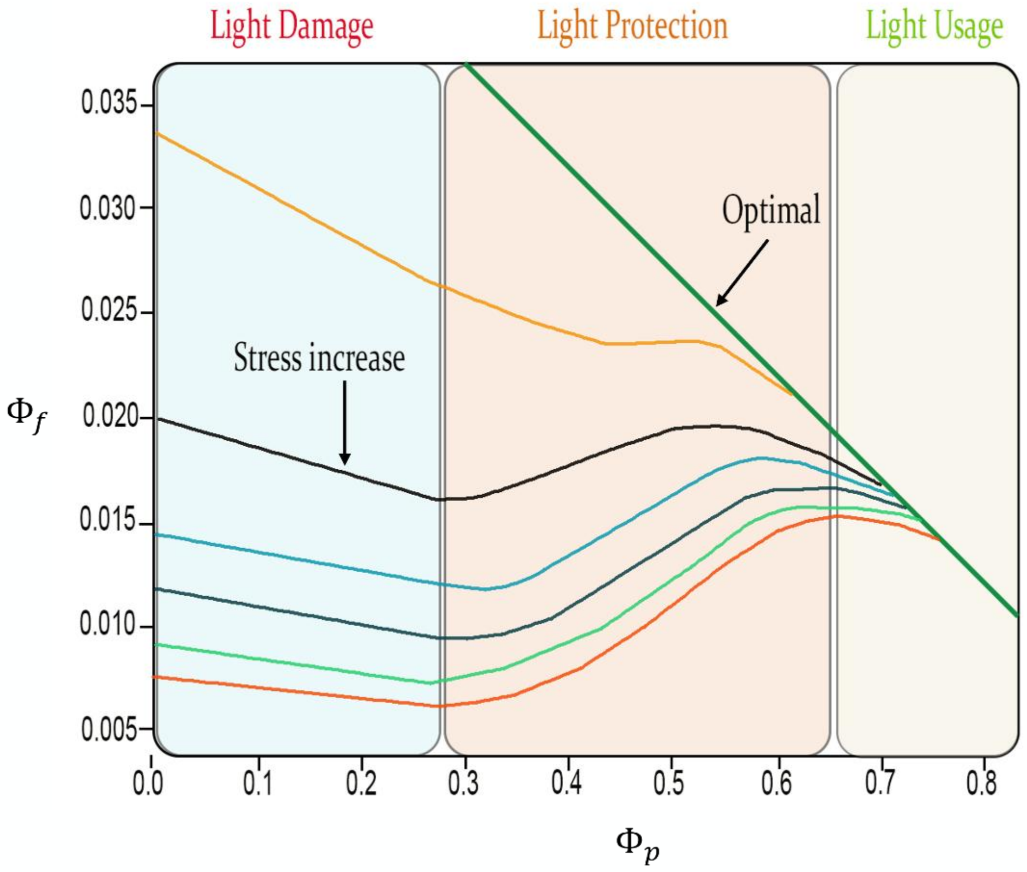
(1) What is the **contribution of ChlF** to **linear and nonlinear light-use efficiency-based models** for the remote estimation of plant **photosynthesis** under **stress**?

(2) How does **growth light** and **stress influence phase duration**?

(3) How do **prevailing environmental conditions affect the dynamics of ChlF and NPQ** under **stress**?

Adapted from Moreno, 2017

Research questions



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Experiment description

Setup

- wheat plants (Triticum durum cv. Orita)
- **low**, **medium**, and **high** nitrogen.
- February 24th to April 27th, 9 days in total
- **PAR intensity 1200-1500 mmol m^{-2} s^{-1}**

Measurements (flag leaf)

Meteorological conditions

- Photosynthetic active radiation (PAR)
- Temperature || rain || irrigation

Passive fluorescence (Fluowat)

- FQE (SIF_{tot}/APAR-Chla)
- Pigment Absorbance beta-Carotene and xanthophyll (APAR-CarbXan) (Van Wittenberghe et al., 2024).

Active fluorescence (Li6400)

- Steady state fluorescence (Fs)
- PSII photochemical yield (**ΦPSII** = (Fm'-Fs)/Fm')

Gas exchange (Li6400)

- Net photosynthesis (A_{net}, ambient light and temperature)

Vegetation indices (Fluowat)

- NDVI (Normalized difference vegetation index)
- PRI (photochemical reflectance index)
- Red edge
- Chlorophyll/carotenoid (CCI)

Light Curves (middle season, Li6400)

- Anet || ΦPSII || Fs || NPQ

https://www.licor.com/

ΦPSII = the fraction of absorbed photons by PSII used for photochemistry (Genty et al 1989).

Linear modelling : Monteith's light-use-efficiency (LUE)

$$A_{net} = LUE \cdot fAPAR \cdot PAR$$

Meteorology-driven Methods (MM)

$$A_{net} = LUE_{max} \cdot f(meteo) \cdot (a_0 + NDVI \cdot a_1) \cdot PAR$$

$$f(meteo) = \left[1 - \left(\frac{Vpd - Vpd_{min}}{Vpd_{max} - Vpd_{min}}\right)\right]$$

fAPAR proxy (fraction of absorbed PAR)
- ▶ NDVI

f(meteo)
- ▶ Limiting factor LUE_{max}

Fitting parameters
- ▶ a0 | a1

(Monteith 1972)

Remote Sensing based methods (RSM)

$$A_{net} = (a_0 \cdot Ph + a_1) \cdot (a_2 \cdot St + a_3) \cdot PAR$$

LUE proxy ~ Physilogical-related proxy (Ph)
- ▶ PRI | APAR-CarbXan | FQE

fAPAR proxy ~ Structural-related proxy (Ph)
- ▶ NDVI | Red edge index

Fitting parameters
- ▶ b0 | b1 | b2 | b3

(Rossini et al., 2010)

Berry-based method (Berry)

$$A_{net} = b_0 \cdot (LUE \cdot fAPAR \cdot PAR)$$

$$A_{net} = b_0 \cdot (\Phi PSII \cdot Opt \cdot PAR)$$

LUE proxy
- ▶ ΦPSII (photochemical yield)

fAPAR proxy ~ Optical proxies (Opt)
- ▶ NDVI | Red edge index | APAR-CarbXan | FQE

Fitting parameters
- ▶ b0

(Berry et al., 2012)

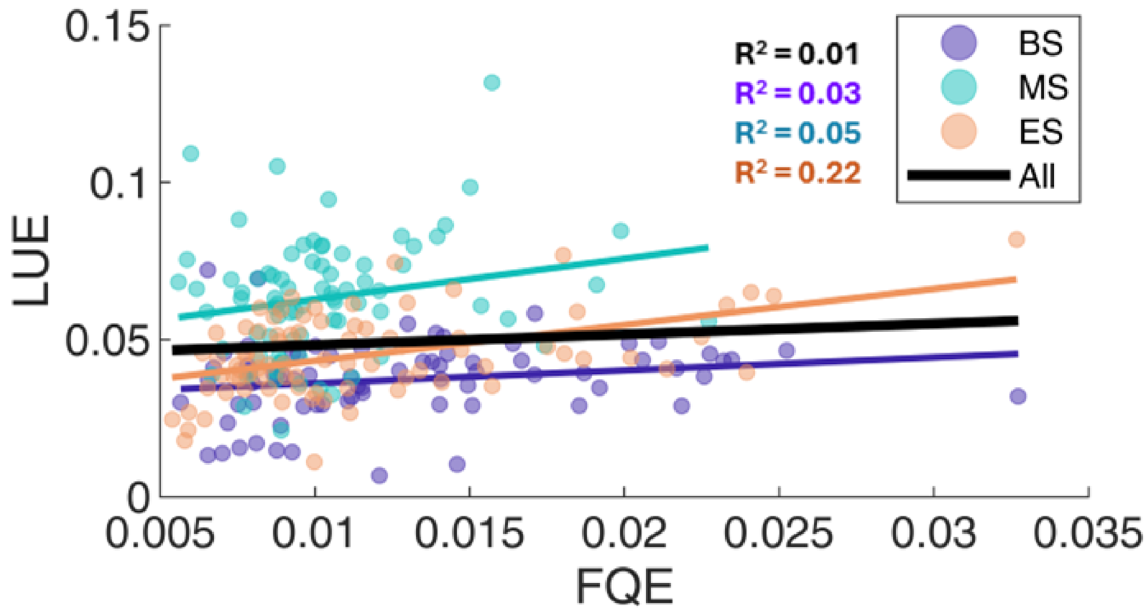
Non-linear modelling: Gaussian process regression (GPR) - LUE based models

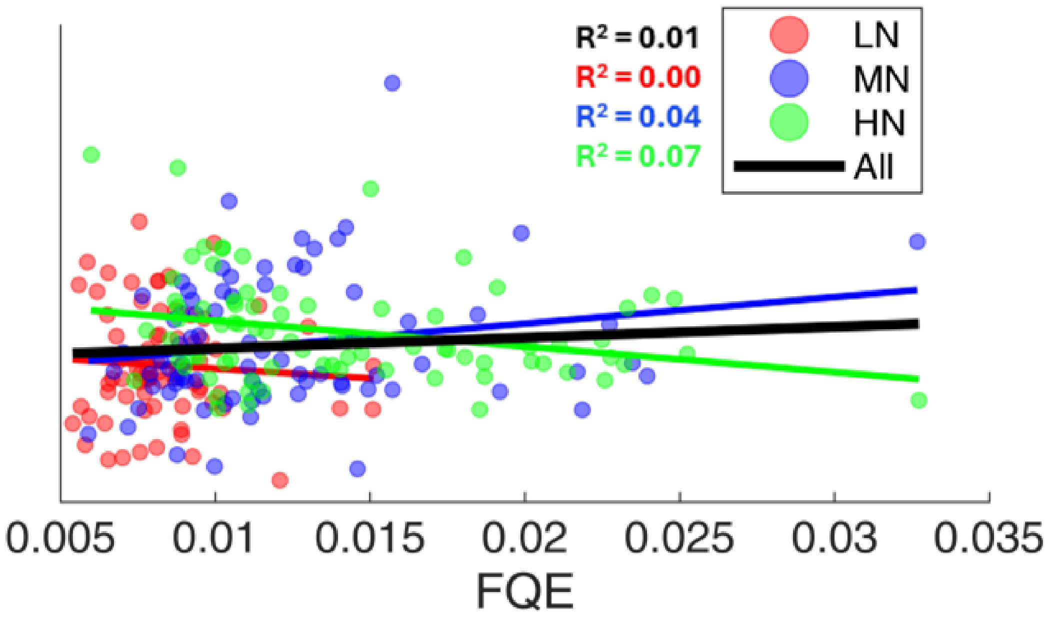
Model	#	R^2	R^2_{Stdv}	Bias	Variance	p-value	AIC
A_{net}=f(PhiPS2,PAR,Vpd,Tair)	4	0.73	0.05	0.10	3.56	0.00	224
A_{net}=f(PhiPS2,RedEdge,Vpd,Tair)	4	0.73	0.07	-0.30	3.48	0.00	220
A_{net}=f(PhiPS2,RedEdge,PAR,Vpd,Tair)	5	0.73	0.05	-0.11	3.51	0.00	220
A_{net}=f(PhiPS2,APARChl,Vpd,Tair)	4	0.72	0.05	-0.26	3.71	0.00	234
A_{net}=f(PhiPS2,NAOC,PAR,Vpd,Tair)	5	0.71	0.06	0.23	3.50	0.00	220
A_{net}=f(PhiPS2,Chla,PAR,Vpd,Tair)	5	0.71	0.09	0.04	3.65	0.00	233
A_{net}=f(PhiPS2,NAOC,Vpd,Tair)	4	0.71	0.07	0.17	3.63	0.00	226
A_{net}=f(FQE,PRI,RedEdge,Vpd,Tair)	5	0.71	0.08	0.00	3.83	0.00	240
A_{net}=f(PhiPS2,NDVI,PAR,Vpd,Tair)	5	0.70	0.10	-0.28	3.81	0.00	246
A_{net}=f(APAR-CarbXan,RedEdge,PAR,Vpd,Tair)	5	0.70	0.04	-0.01	3.69	0.00	234
A_{net}=f(PhiPS2,RedEdge,Vpd,Tair)	4	0.63	0.06	0.22	4.18	0.00	233
A_{net}=f(FQE,RedEdge,Vpd,Tair)	4	0.63	0.07	0.22	4.18	0.00	236
A_{net}=f(RedEdge,PAR,Vpd,Tair)	4	0.62	0.09	-0.33	4.24	0.00	237
A_{net}=f(PhiPS2,RedEdge,Tair)	3	0.62	0.05	-0.24	4.28	0.00	240
A_{net}=f(NAOC,Vpd,Tair)	3	0.61	0.05	0.03	4.40	0.00	248
A_{net}=f(APAR-CarbXan,RedEdge,Vpd,Tair)	4	0.61	0.07	-0.11	4.45	0.00	257
A_{net}=f(PRI,RedEdge,Vpd,Tair)	4	0.61	0.08	-0.10	4.34	0.00	245
A_{net}=f(PhiPS2,NAOC,Vpd,Tair)	4	0.61	0.06	-0.23	4.33	0.00	244
A_{net}=f(PhiPS2,RedEdge,PAR,Vpd,Tair)	5	0.60	0.10	-0.24	4.42	0.00	249
A_{net}=f(FQE,RedEdge,PAR,Vpd,Tair)	5	0.60	0.07	-0.29	4.46	0.00	259

- Based on the LUE input parameters, we used **GPR** as a **tool** to **infer** the **amount of information** that each **combination of optical vegetation indices**, **FQE**, **photoprotection pigments**, and **meteorological parameters contains** about A_{net}.
- We fit a different non-parametric model for each possible combination of input variables.
- A total of **308 models were evaluated**

(Verrelst et al., 2012 and Lázaro-Gredilla et al., 2014)

Relationship between SIF and Anet



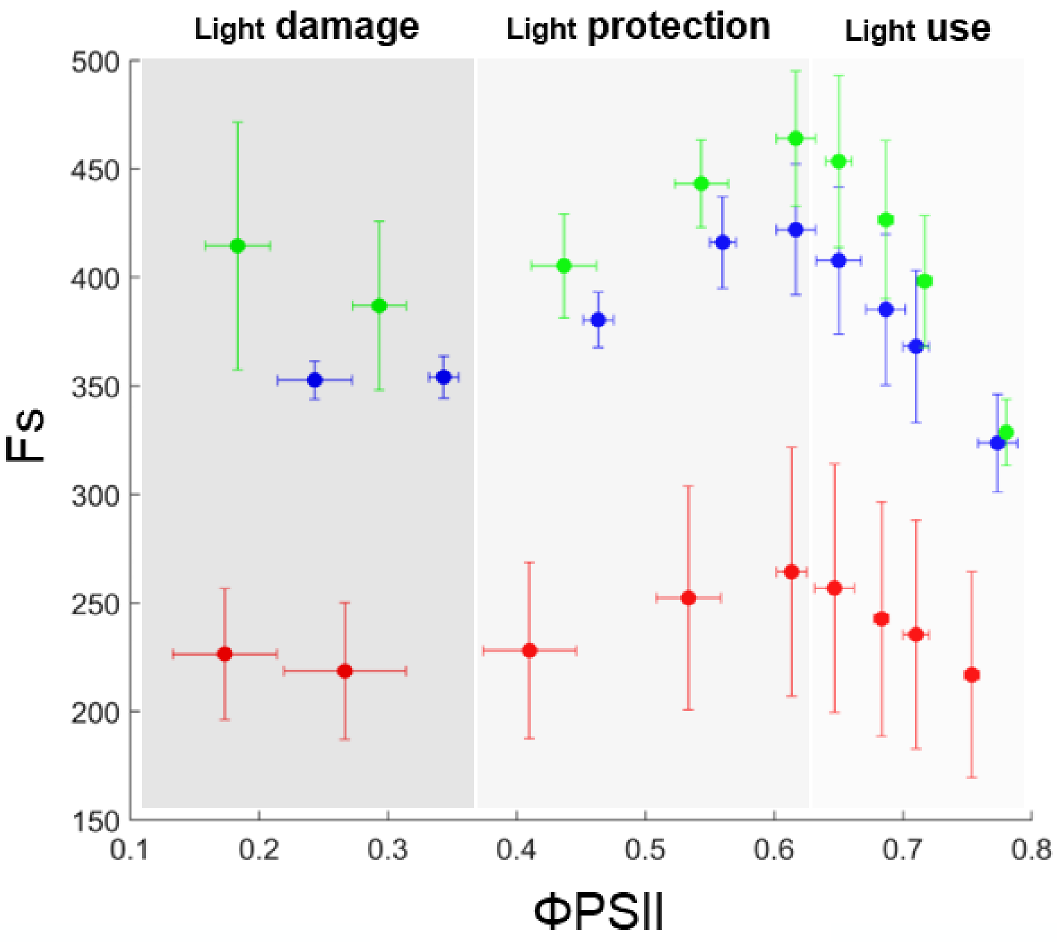


- In this study the relationship between **FQE** and **A_{net}** is not univocal.
- **Linear models meteorological** and **remote sensing based models R^2 < 0.4**

Cendrero-Mateo et al., 2025 IEEE

Light Curve Data Set

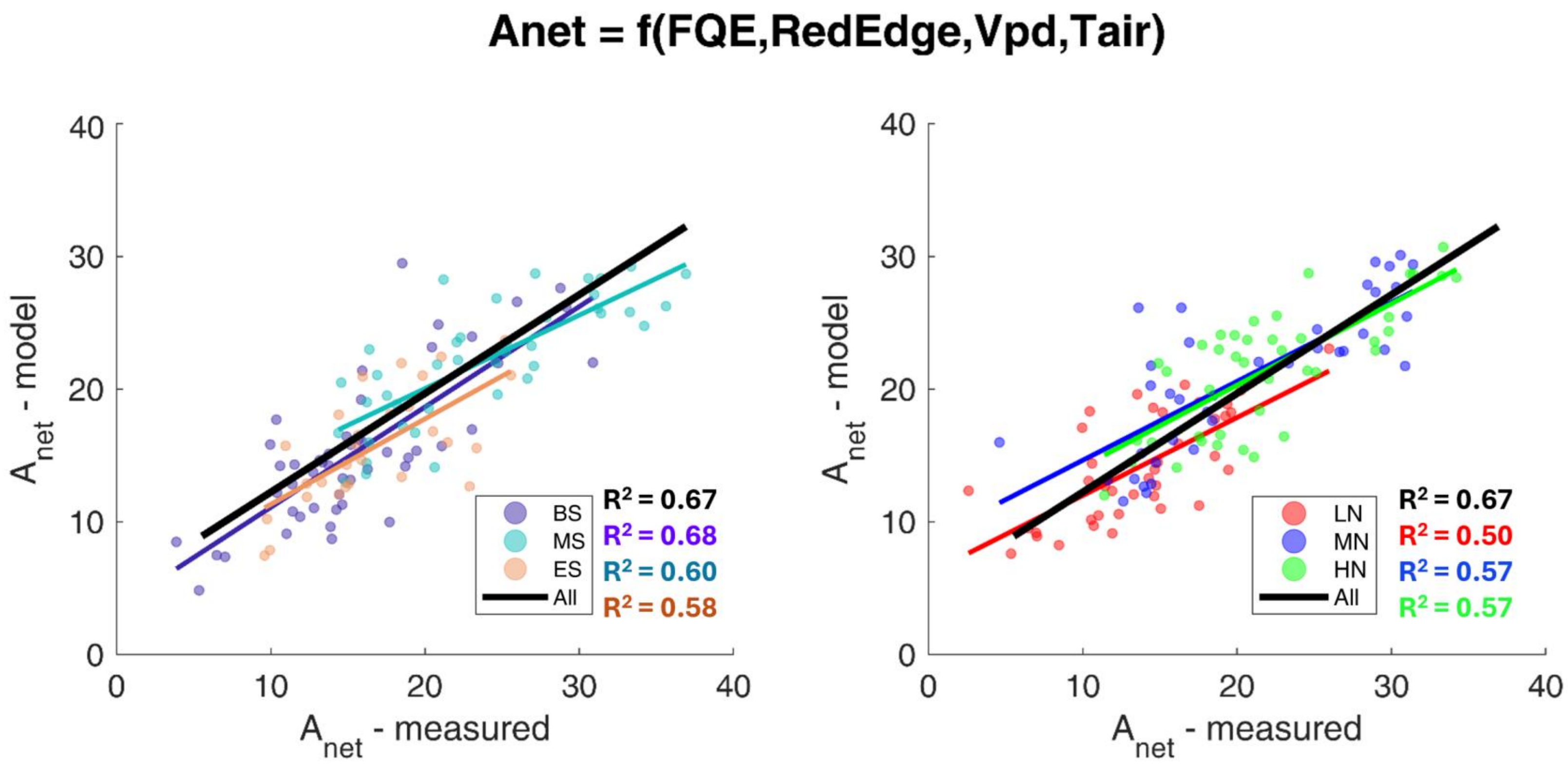
N-Hight | N-Medium | N-Low



- No linear relationship between **Fs** and **ΦPSII**
- Seasonal measurements ΦPSII = 0.2-0.3 (~light damage)

Cendrero-Mateo et al., 2025 IEEE

Non-linear modelling: Gaussian process regression (GPR) - LUE based models

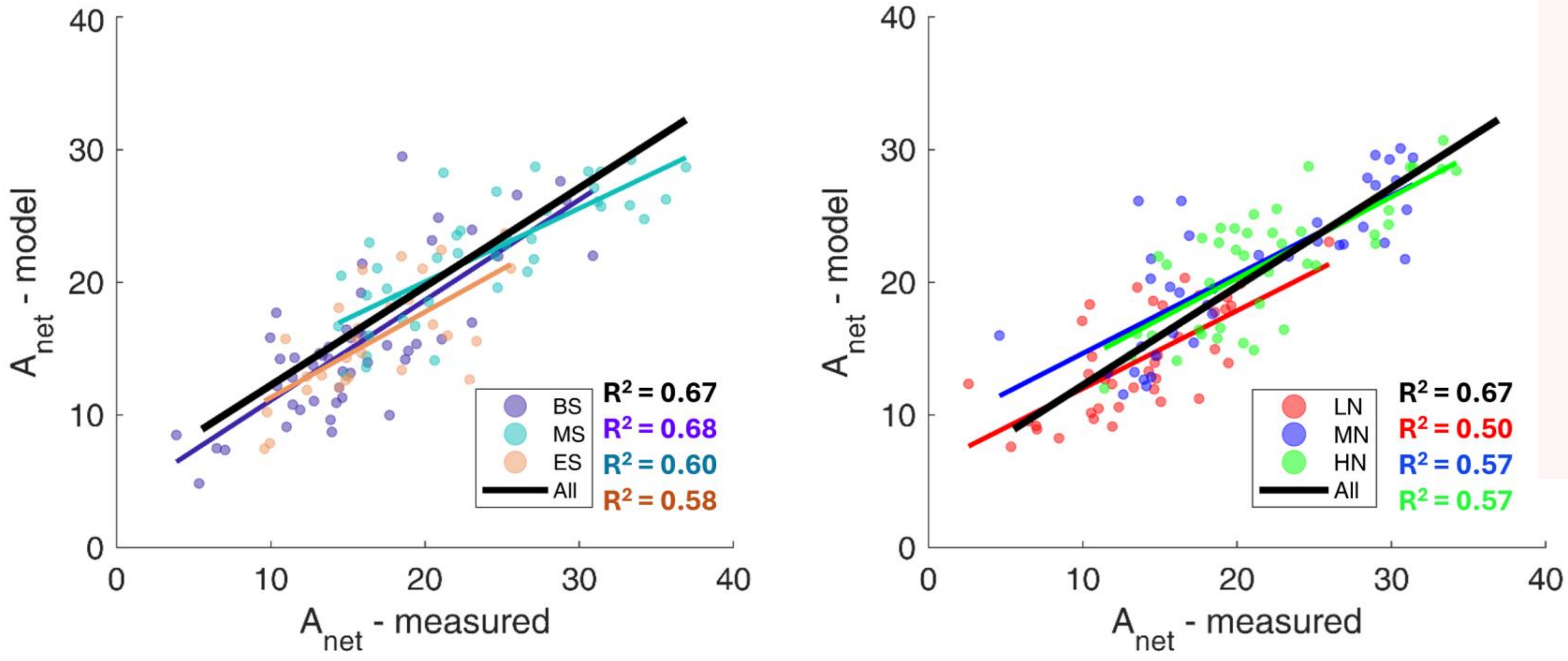


- **Non-linear models improve A_{net} estimation**
- **FQE** and **RedEdge** played a **pivotal** role in inferring $\mathbf{A_{net}}$ variability
- RedEdge indicates a downregulation of light-harvesting for photoprotection

Cendrero-Mateo et al., 2025 IEEE

Non-linear modelling: Gaussian process regression (GPR) - LUE based models

Anet = f(FQE,RedEdge,Vpd,Tair)



Session| Carbon Cycle
Today 11:00

Monitoring photosynthetic quantum yield through non-photochemical quenching, from laboratory to field: integrating canopy fluorescence, reflectance, and GPP.

ERC project PhotoFlux
Lead by Dr. Shari van Wittenberghe
https://leoipl.uv.es/photoflux/

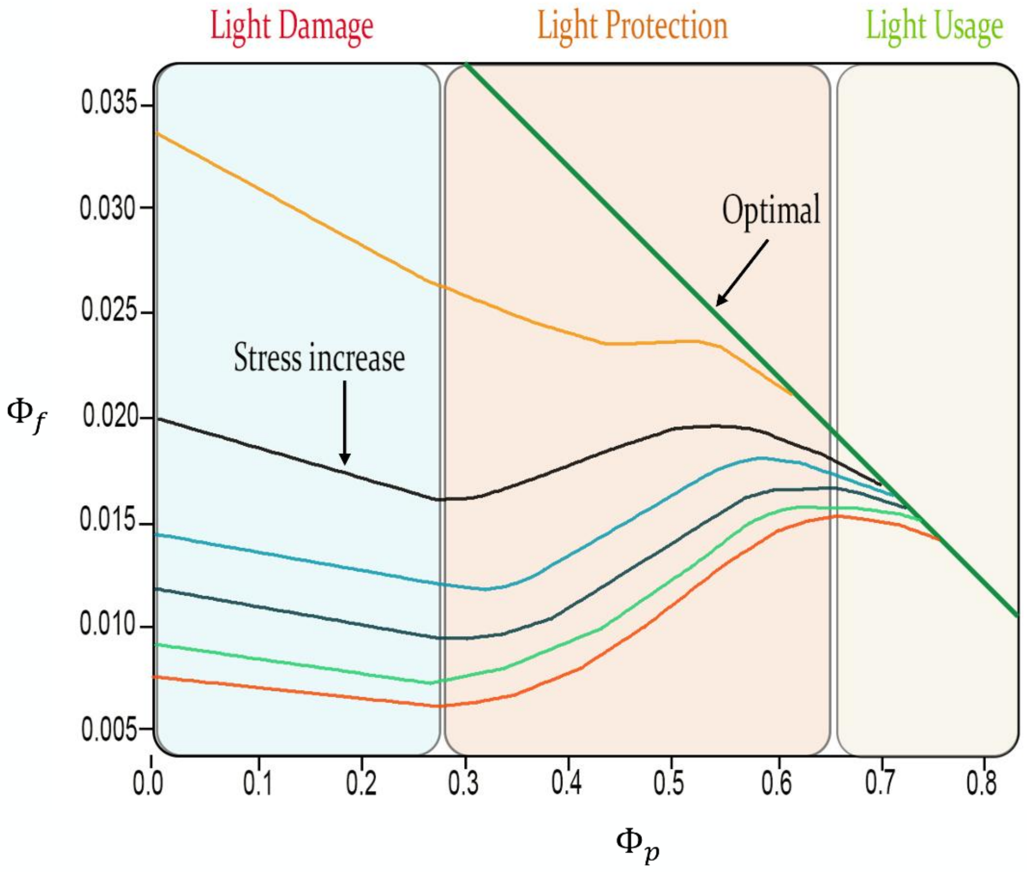
- **Non-linear models improve A_{net} estimation**
- **FQE** and **RedEdge** played a **pivotal** role in inferring A_{net} variability
- RedEdge indicates a downregulation of light-harvesting for photoprotection

Cendrero-Mateo et al., 2025 IEEE

Take Home Message

- Need for **dynamic non-linear models** to **account for** the **contribution of FQE** and **NPQ** to the **carbon** assimilation **models.**

Research questions



Adapted from Moreno, 2017

(1) What is the **contribution of ChlF** to **linear and nonlinear light-use efficiency-based models** for the remote estimation of plant **photosynthesis** under **stress?**

(2) How does **growth light** and **stress influence phase duration**?

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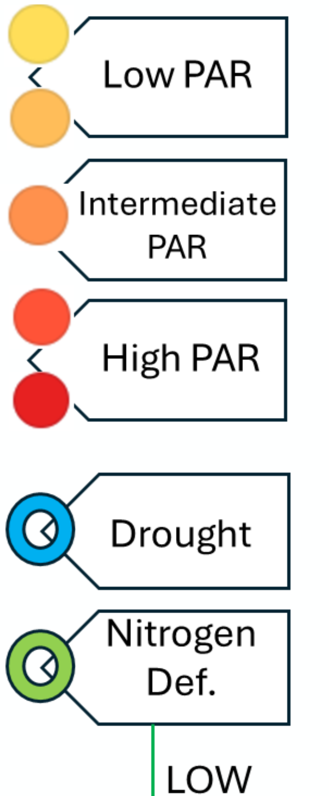
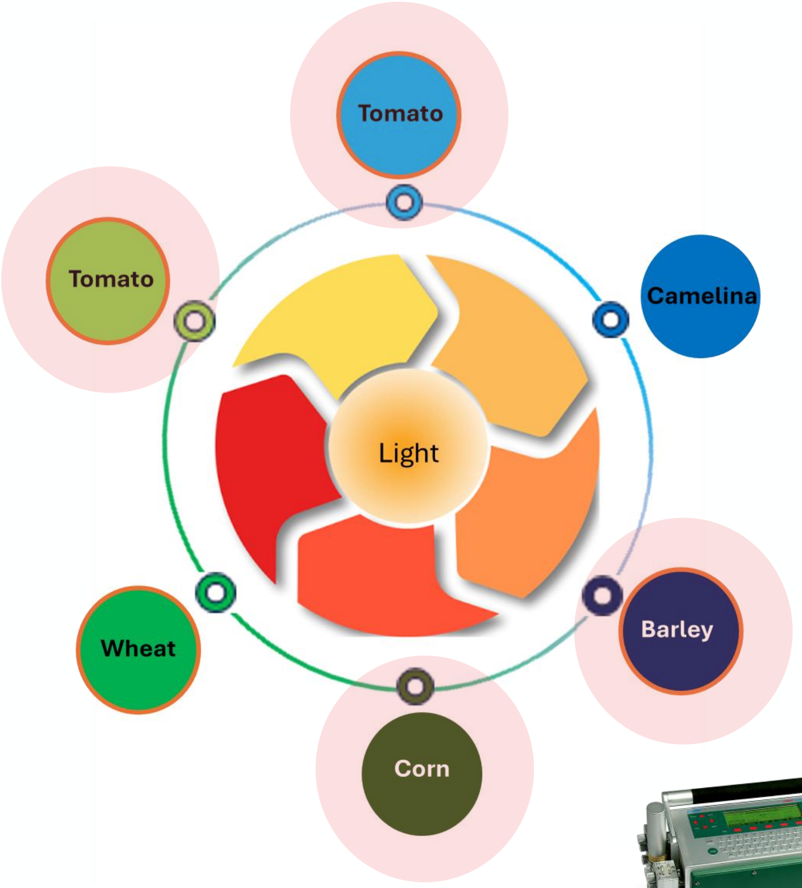
Experiment description

PAR (Photosynthetic Active Radiation)





Chardi-Raga et al. Under Revision || **Poster Session**

Light Curves (0-1800 µmol $m^{-2}s^{-1}$)

Measured parameters

- **Fs:** steady-state fluorescence
- **Fm-Fm'**: maximum fluorescence (dark adapted and light adapted)
- **Fo-Fo'**: minimum fluorescence (dark adapted and light adapted)

Derived parameters

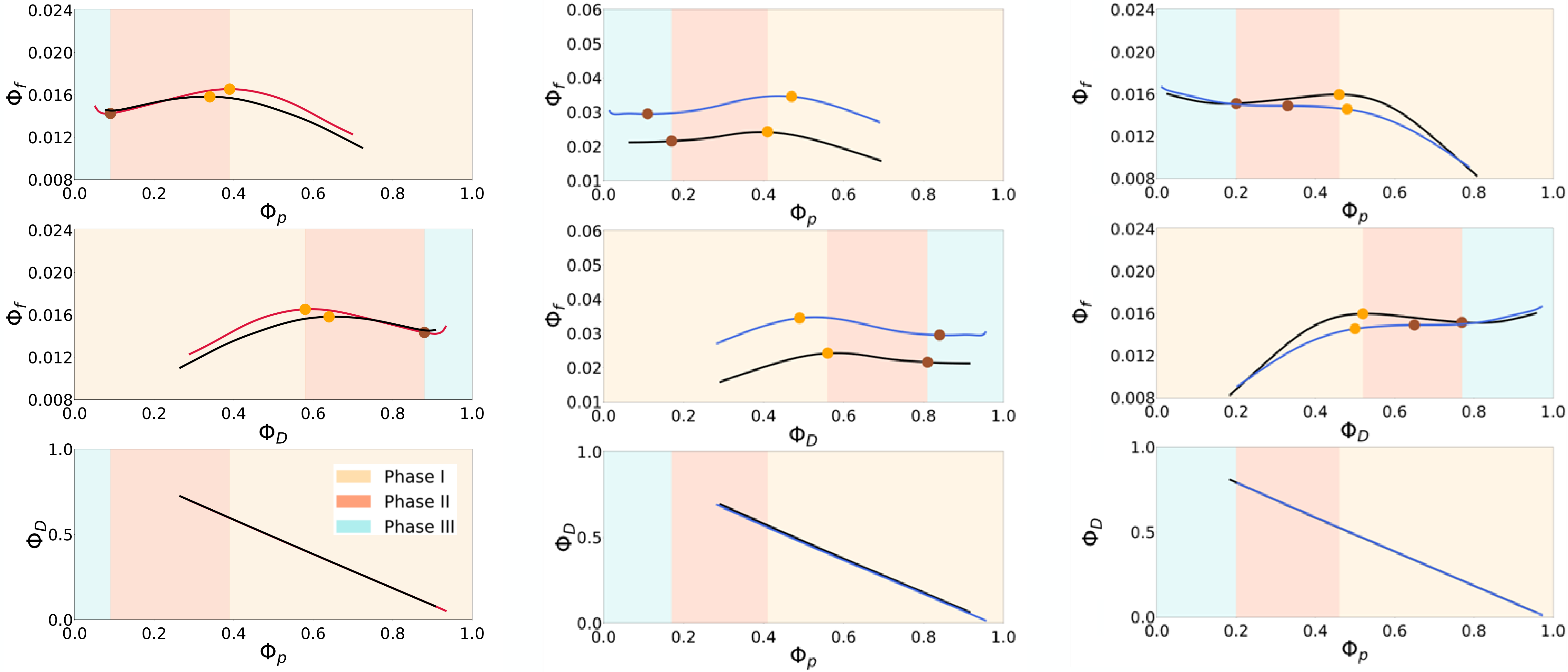
- **Photosynthetic quantum yield** (Φ_p)
- **Fluorescence quantum yield** (Φ_f)
- **NPQ quantum yield** $(\Phi_D = NPQtotal)$

Phases characterization

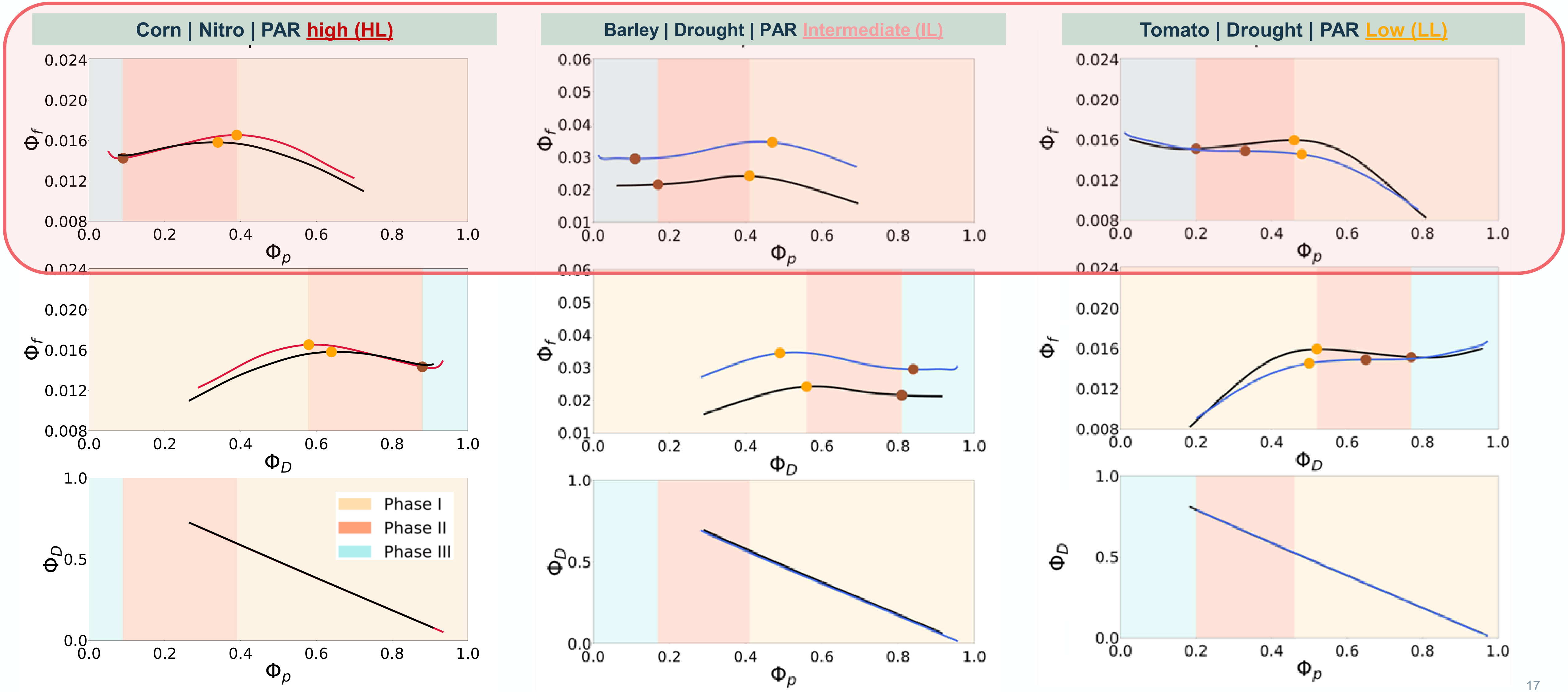
- First derivative

Light Curves

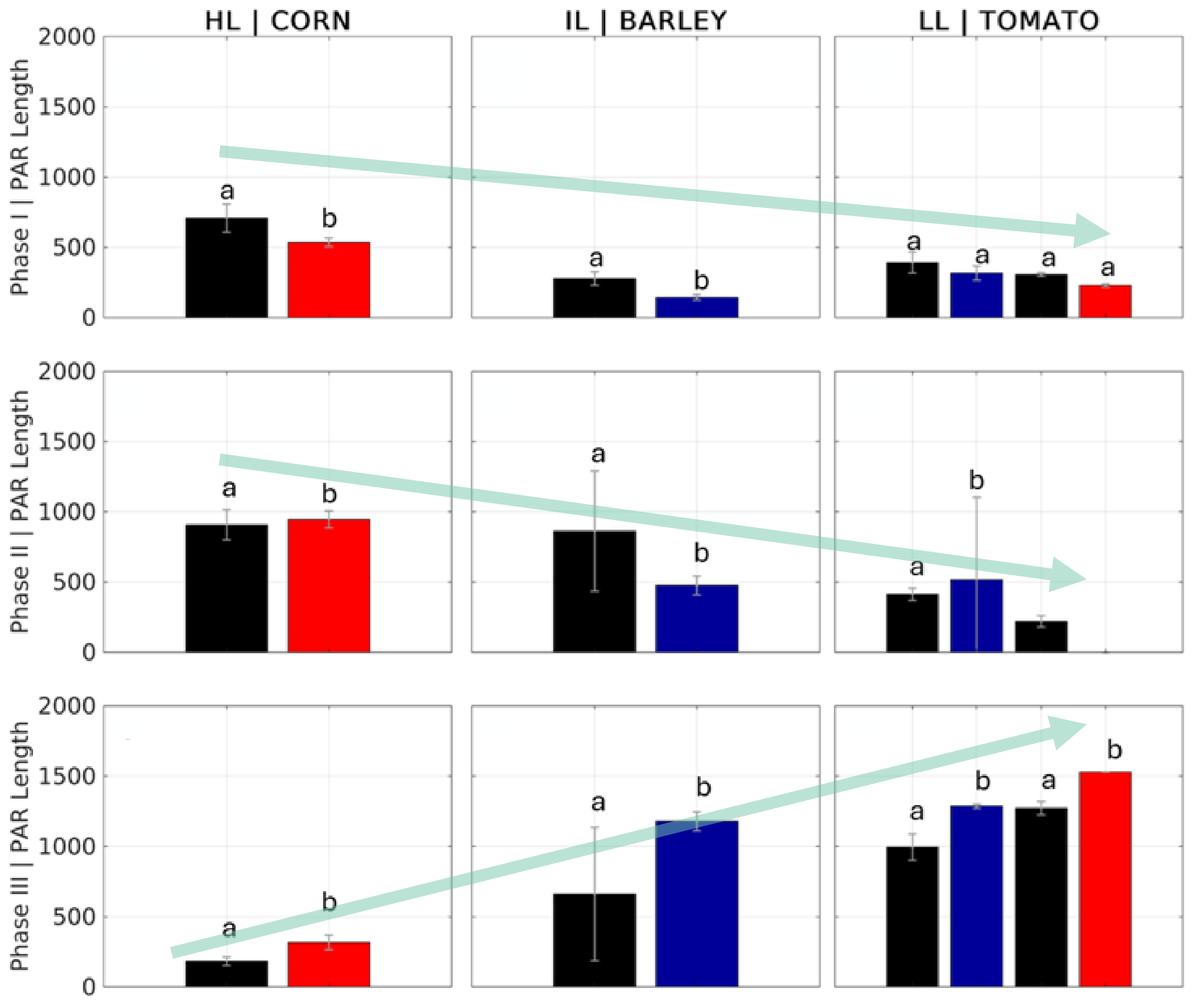
Corn | Nitro | PAR high (HL)	Barley | Drought | PAR Intermediate (IL)	Tomato | Drought | PAR Low (LL)



Light Curves



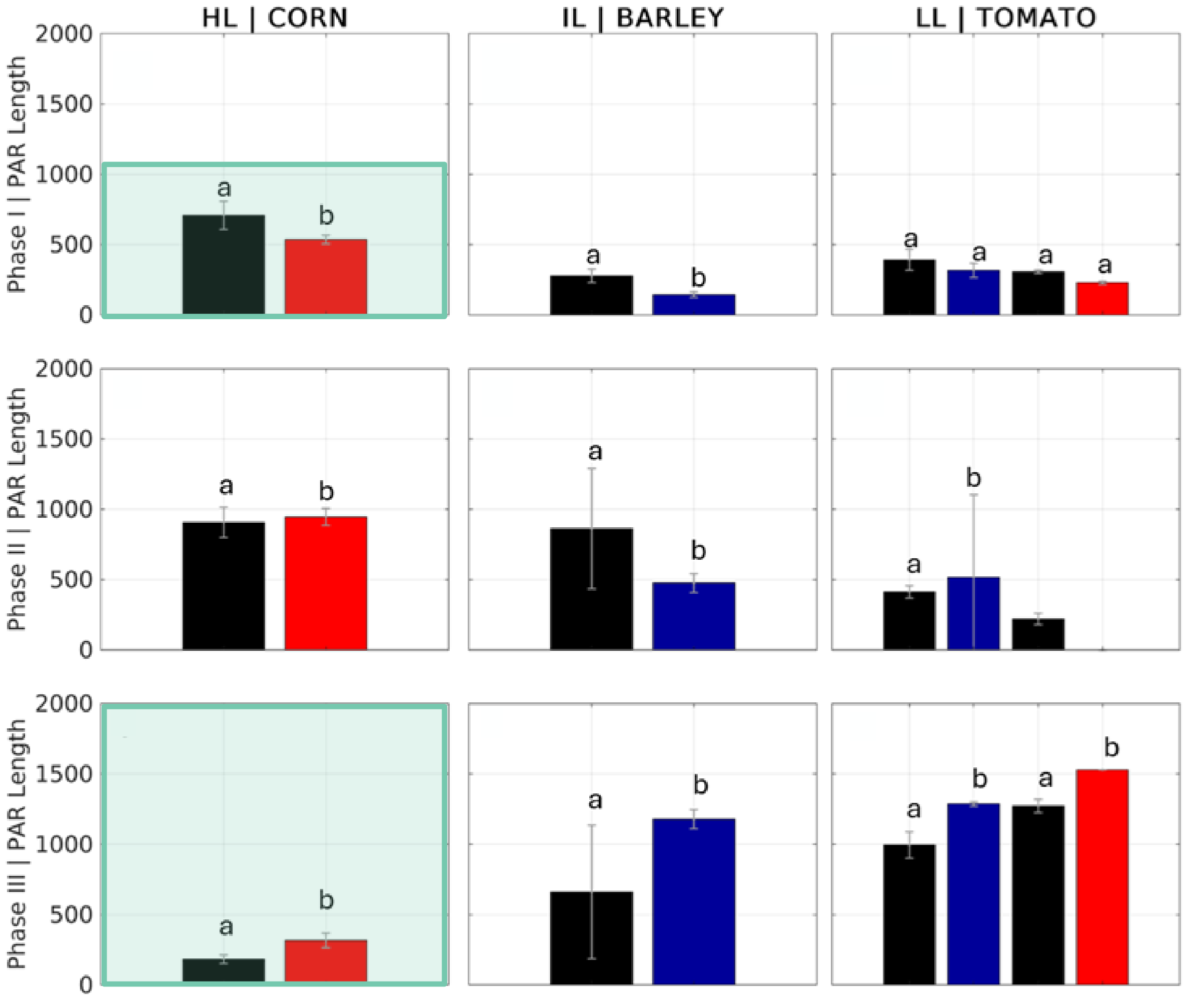
PAR length



PAR length: total PAR accumulated within a specific phase.

- **Growth light**: lower growth light **triggered earlier transitions** through **Phase I** and **Phase II** but led to a **more persistent Phase III** compared to high-light conditions.

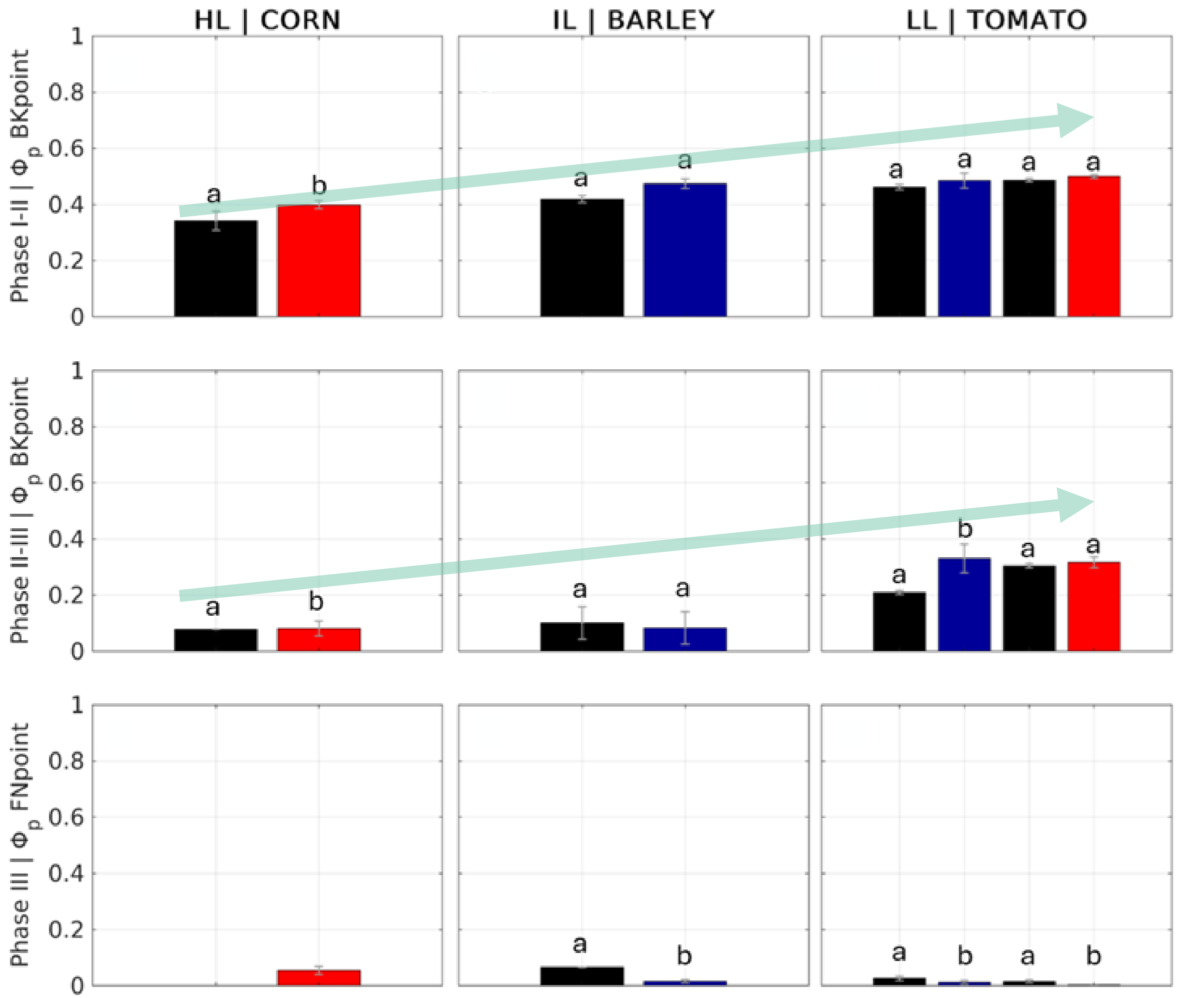
PAR length



PAR length: total PAR accumulated within a specific phase.

- **Growth light**: **lower growth light** **triggered earlier transitions** through **Phase I** and **Phase II** but led to a **more persistent** **Phase III** compared to high-light conditions.
- **Treatment:** under stress, **Phase I** was **shortened**, while **Phase III** became **prolonged**. Not a clear pattern was observed in Phase II.

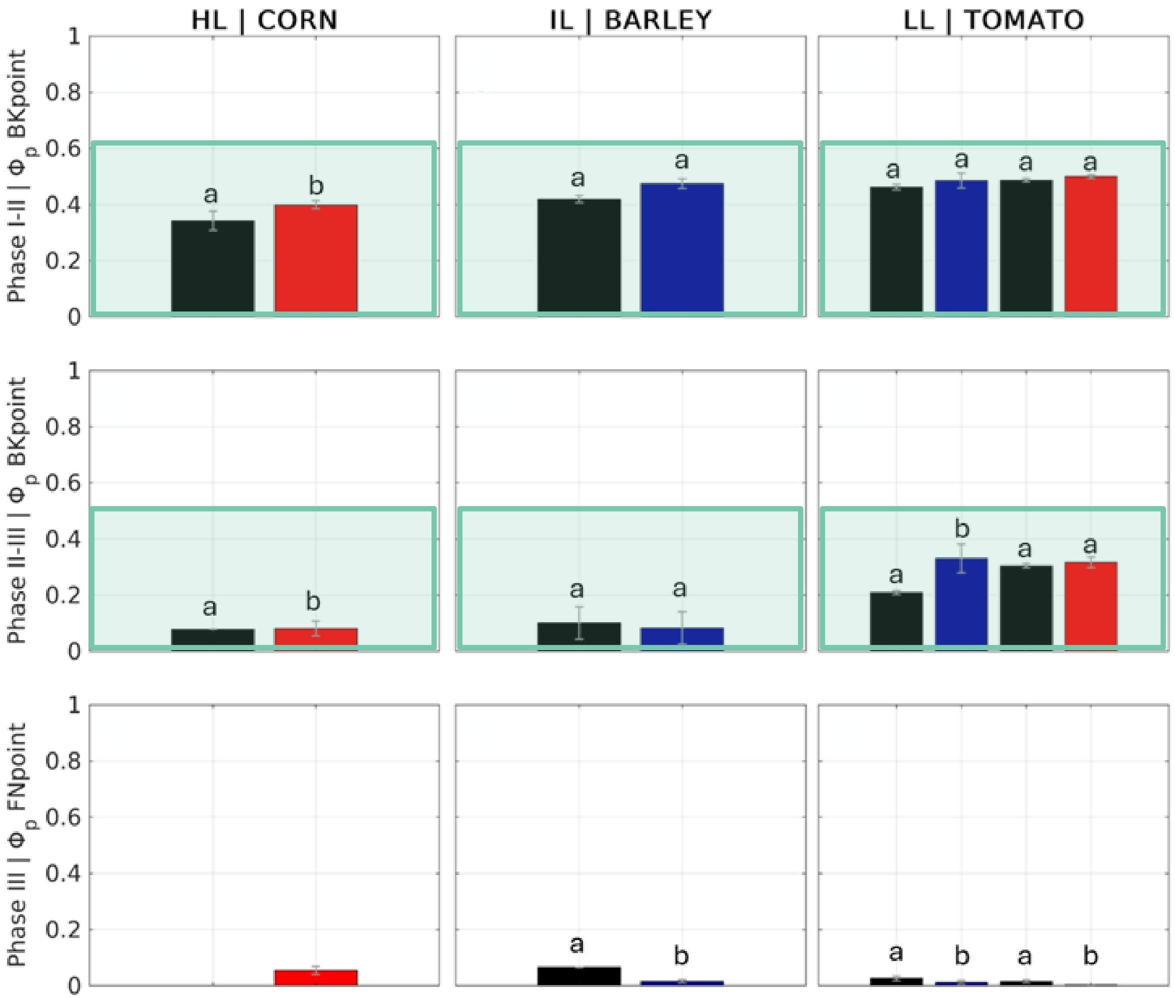
Φ_p BreakingPoint



Φ_p Breaking Point (BKpoint): marks the threshold value for phase transition.

- ► **Growth light**: **lower growth light** **triggered earlier transitions** (**higher Φ_p values**) through **Phase I** and **Phase II.**

Φ_p BreakingPoint



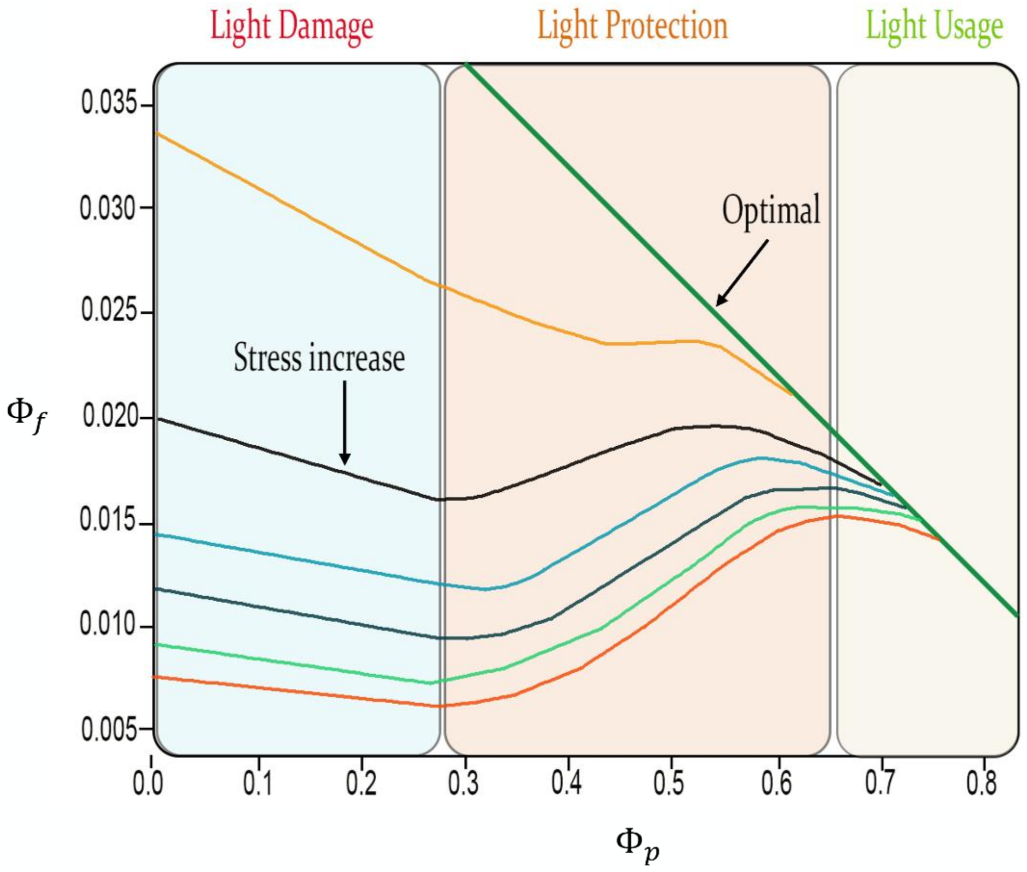
Φ_p Breaking Point (BKpoint): marks the threshold value for phase transition.

- **Growth light**: **lower growth light** **triggered earlier transitions** (**higher Φ_p values**) through **Phase I** and **Phase II.**
- **Treatment**: **stress conditions**, **triggered earlier transitions** (**higher Φ_p values**) through **Phase I** and **Phase II.** Not always statistically different.
- **The opposite trend was observed in Φf and ΦD Breaking Point.** Chardi-Raga || **Poster Session**

Take Home Message

- **Growth light** determined light-curve **shape**; **drought and/or nitrogen deficit shortened** and **shifted** nonlinear **phase duration**.

Research questions



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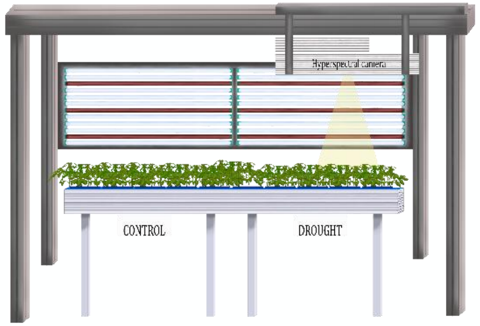
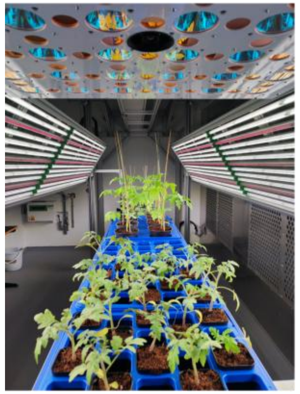
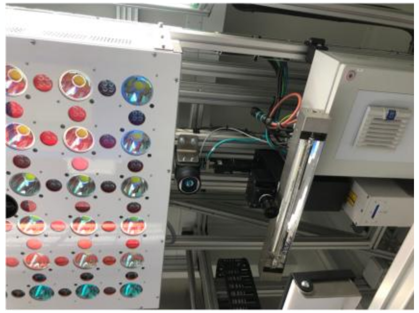
Adapted from Moreno, 2017

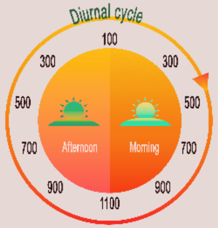
Experiment description





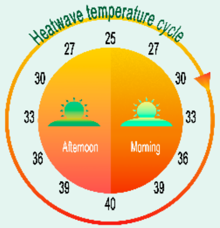


- Active fluorescence imaging system.
- Hyperspectral imaging scanning camera.
- Thermal camera.
- 3D reconstruction system.



Experiment: Diurnal cycle

- Tomato | High light
- Control and drought.
- PAR: 0 – 1100 µmol $m^{-2}s^{-1}$



Experiment: Heatwave

- Tomate | Low light (500 µmol $m^{-2}s^{-1}$)
- Control and drought.
- Temperature :
  Normal : 18 – 25 ºC and Heatwave : 25 – 40 ºC

Measured parameters

- Steady-state fluorescence (**Fs**)
- **Fm-Fm'**: Maximum fluorescence (dark adapted and light adapted)
- **Fo-Fo'**: Minimum fluorescence (dark adapted and light adapted)

Derived parameters | Experiment yield parameters

- Maximun photochemical efficiency (Φ_{Pmax})
- Quantum yield of sustained NPQ (ΦNPQ_s)
- Quantum yield of constitutive thermal dissipation (Φ_D)
- Quantum yield of fluorescence quantum yield (Φ_F)

Derived parameters | Daily yield parameters

- Quantum yield of photochemistry (Φ_P)
- Quantum yield of total NPQ (ΦNPQ_t)
- Quantum yield of reversible NPQ (ΦNPQ_r)
- Quantum yield of constitutive thermal dissipation (Φ_D)
- Quantum yield of fluorescence quantum yield (Φ_F)

Complementary measurements (to be analyzed)

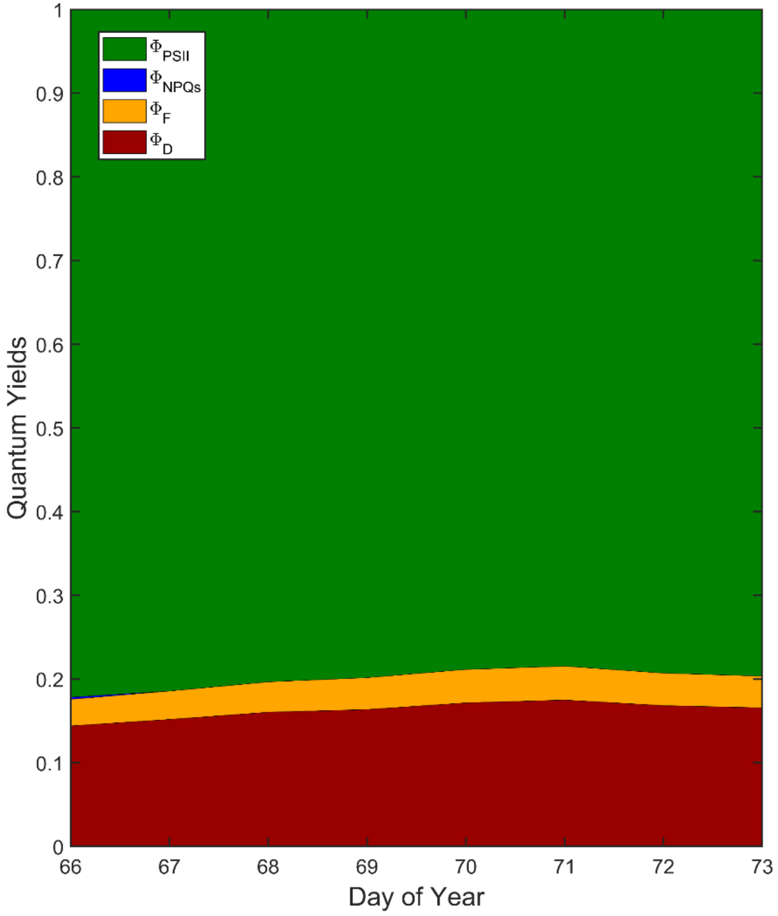
- Leaf reflectance, absorbance, and fluorescence.
- TOC reflectance
- Pigments analysis

Ref: Porcar-Castell (2011).Physiologia Plantarum

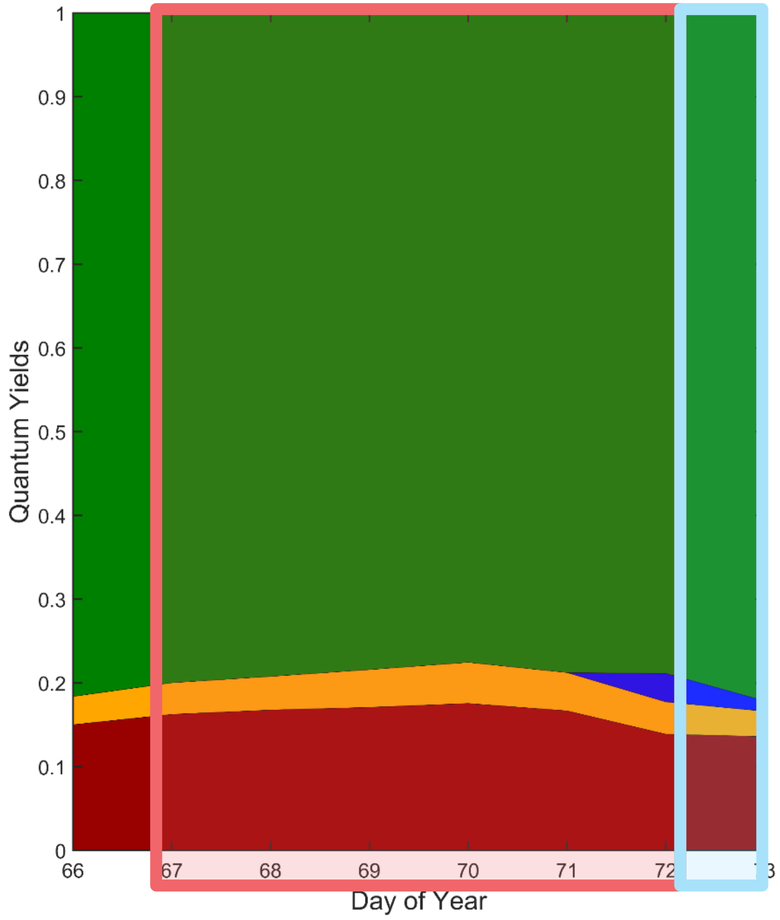
Chardi-Raga et al. In Preparation || **Poster Session**

Diurnal Cycle | Experiment Yield

Control

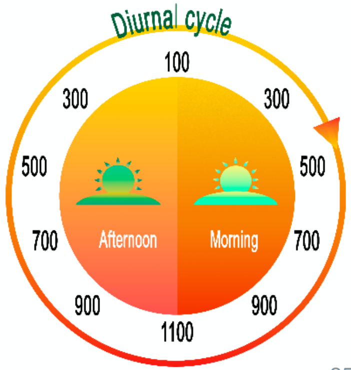


Drought

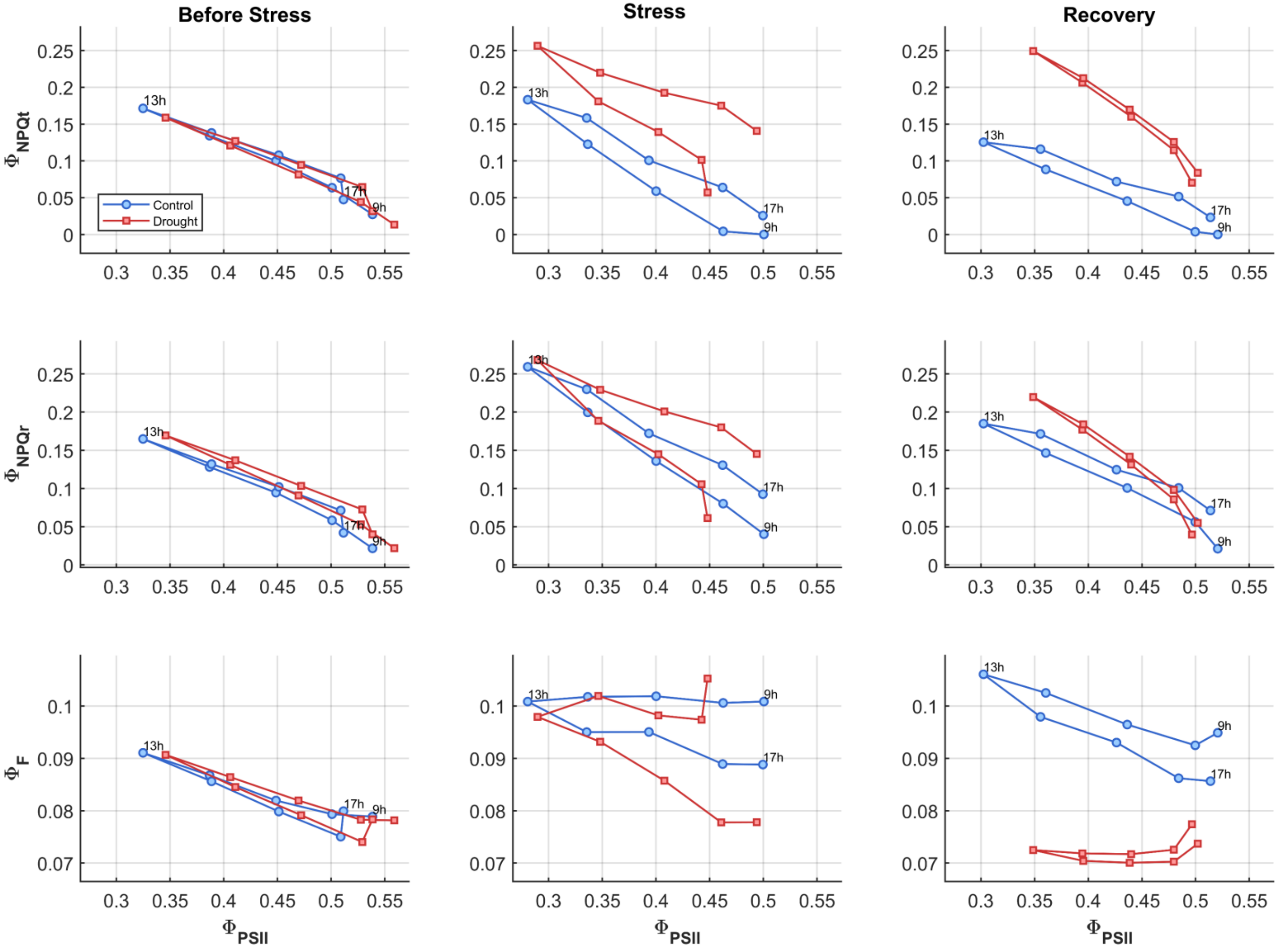


Drought | ReWater

- **Experiment yield** dynamics are **modulated** by the upregulation of **NPQs**.
- NPQs levels began to rise **on the fifth day** of drought stress.



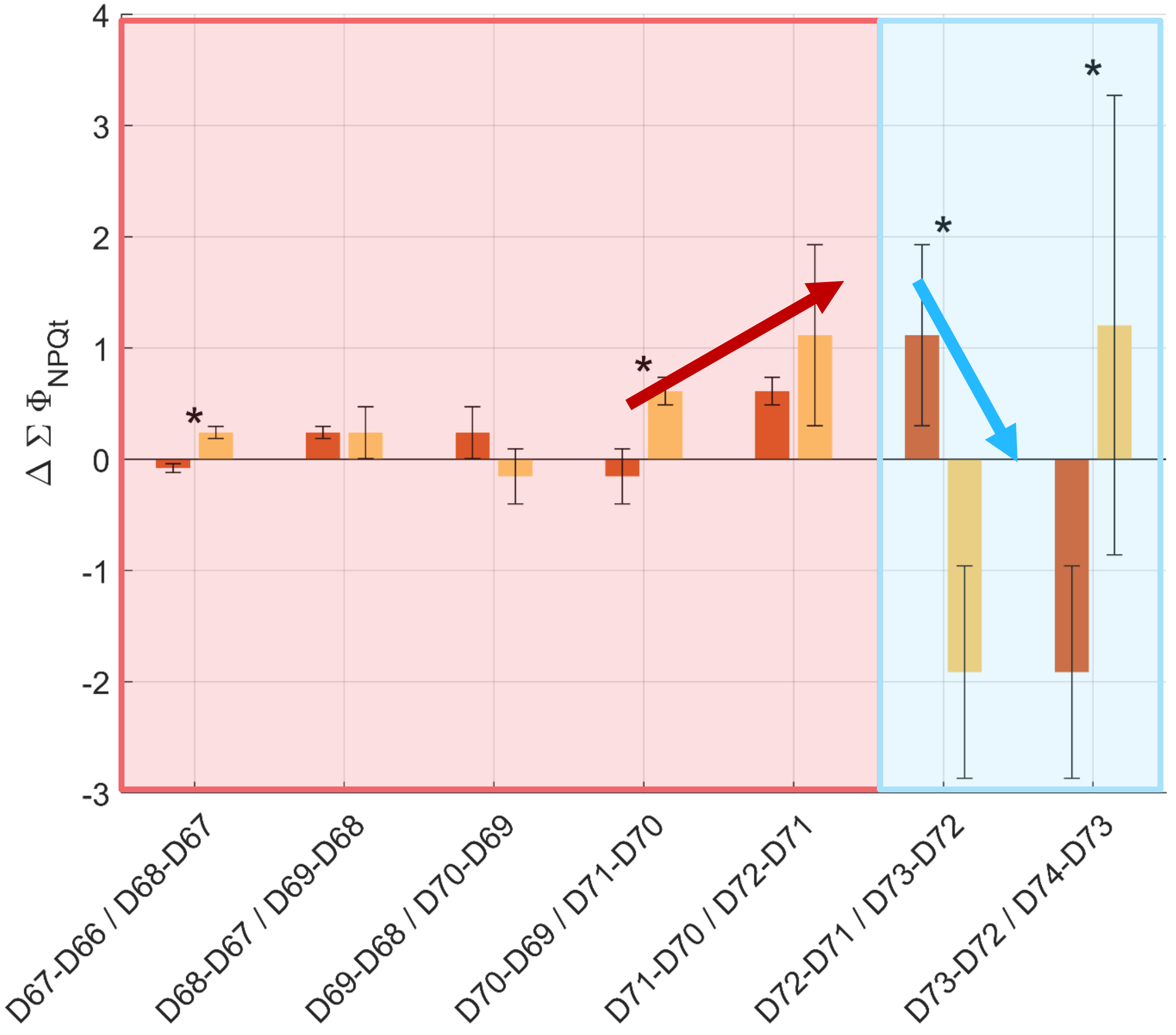
Diurnal Cycle | Diurnal Yield

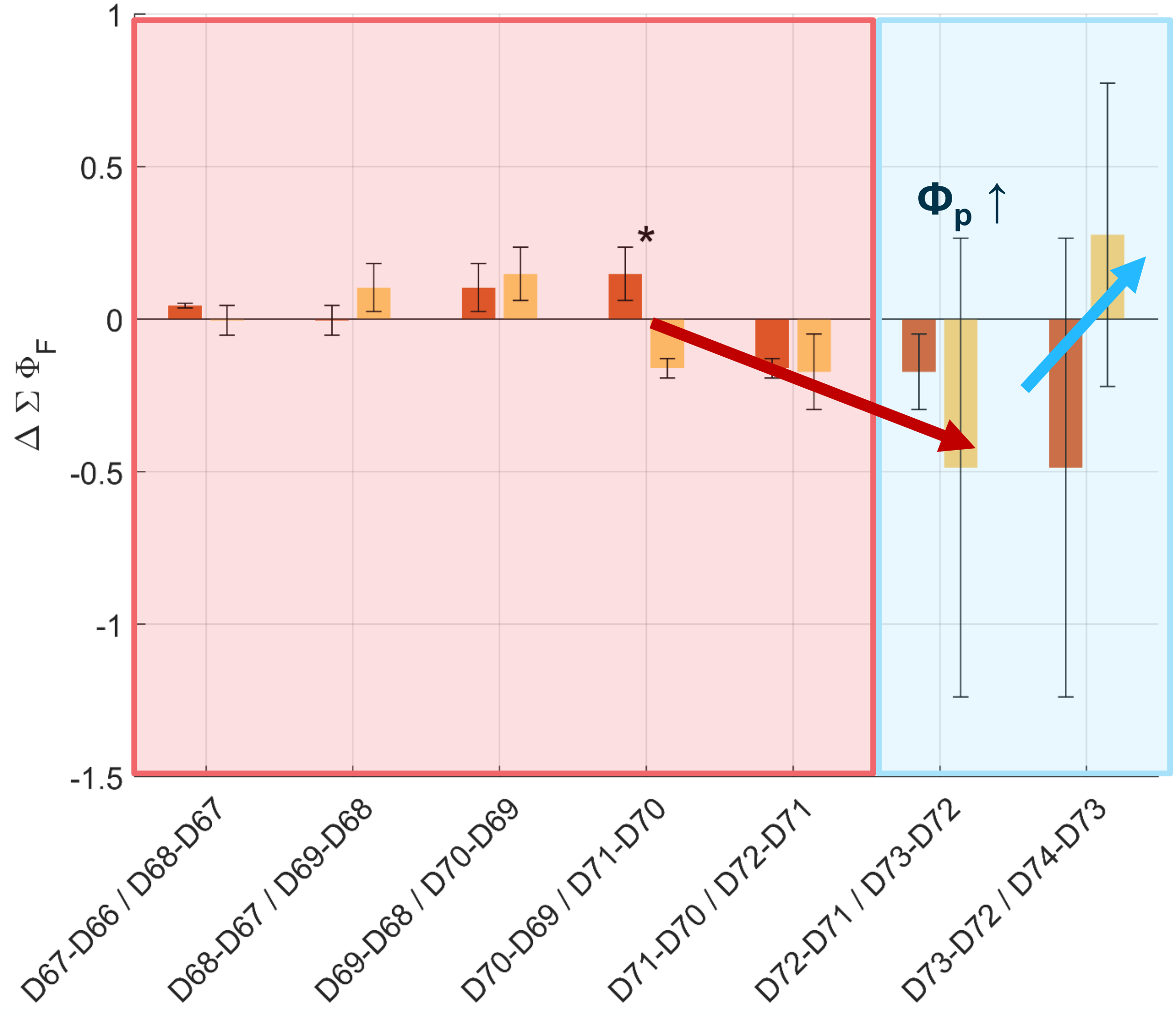


- **Pre-stress:** Daily dynamics of ΦNPQt, ΦNPQr, and Φf are consistent across treatments.
- **Under stress:** ΦNPQt and Φf (Phase III) shows wider variation between treatments than ΦNPQr.
- **Recovery Phase:** ΦNPQt remains elevated, whereas Φf decline due to a recovery in ΦP.

Diurnal Cycle | Stress Detection

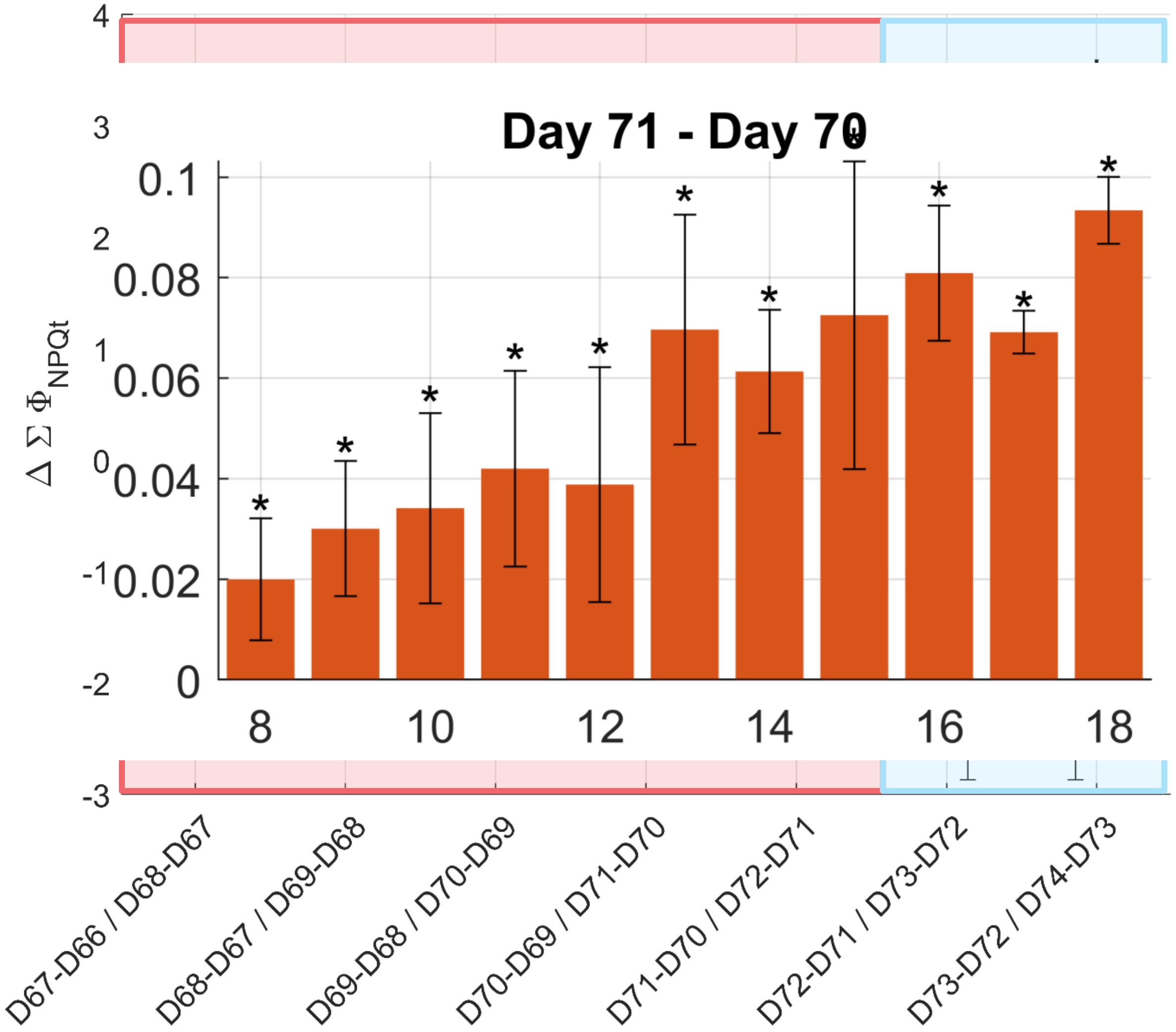
Drought ReWater

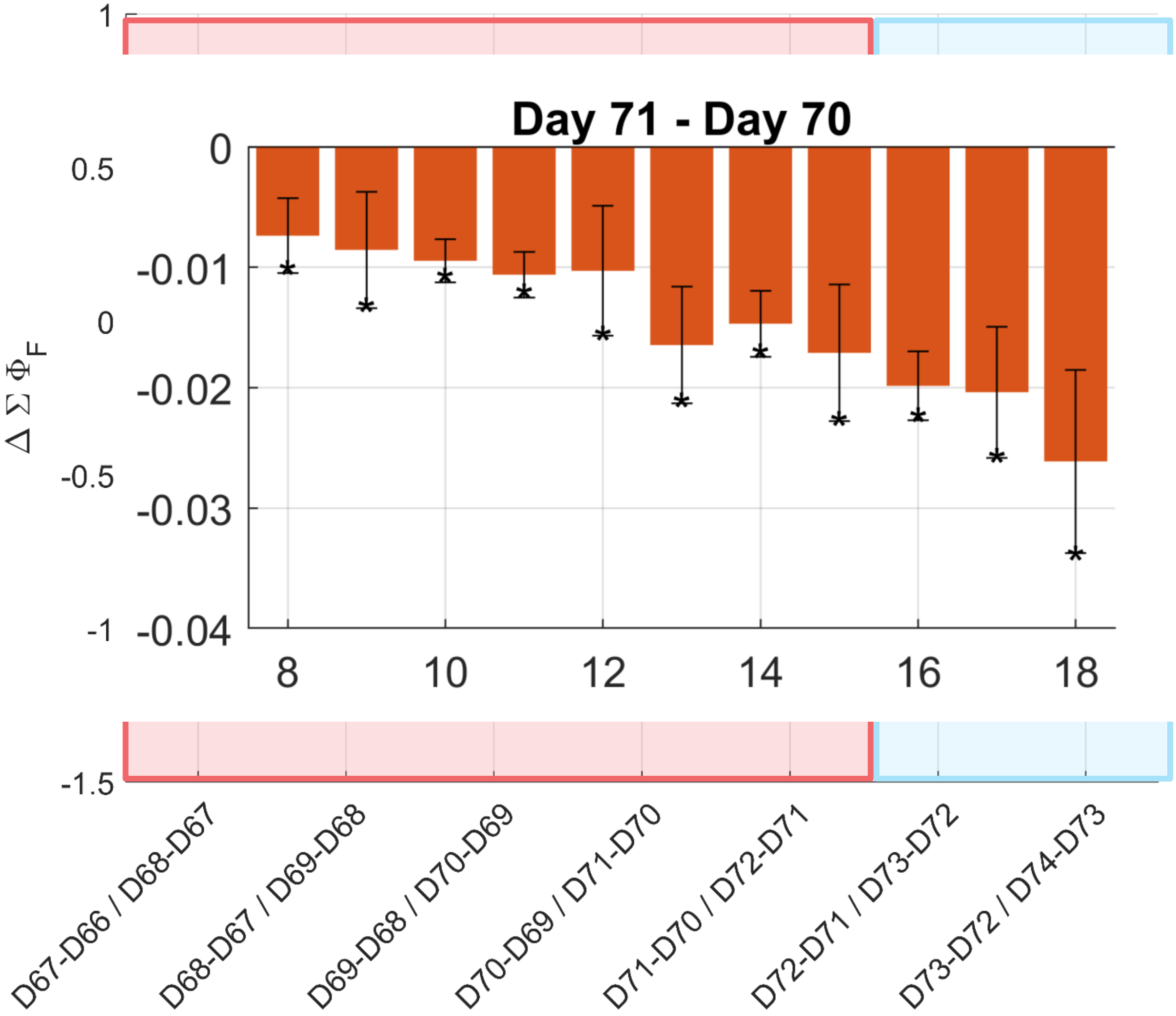




Diurnal Cycle | Stress Detection

Drought ReWater





Take Home Message

- **ΦNPQs** and **Φf emerged as the most sensitive indicators of treatment** variation **compared** to the more stable **ΦNPQr**.

- The onset of **stress** is identified by consecutive observations of an **increase in ΦNPQs** coupled with a **decline in Φf**, which serves as a marker for the shift into stress-induced yield dynamics.

Conclusions

- **Non-Linearity in photosynthetic modeling:** Carbon assimilation (A_{net}) variability is best captured by nonlinear models, in which **fluorescence quantum efficiency (FQE)** and **red-edge** emerge as the primary drivers. This highlights the need to disentangle the contributions of fluorescence and non-photochemical quenching.

- **Growth environmental conditions: Growth light** serves as the baseline for the shape of the light curve, but **environmental stressors**, such as drought and nitrogen deficiency, shorten and shift the duration of the nonlinear phases of photosynthesis.

- **Diagnostic markers for stress**: The **consecutive rise in sustain nonphotochemical quenching (NPQs)** coupled with a **decline in fluorescence yield (Φf)** is an indicator of the transition from photoprotection to light damage, identifying **NPQs** and **Φf** as the most sensitive predictors of physiological strain.

FLEX-Fluorescence 2026 Workshop

03 – 06 March | Bonn University, Germany

~ Thanks for your attention ~

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IMAGE PROCESSING LABORATORY

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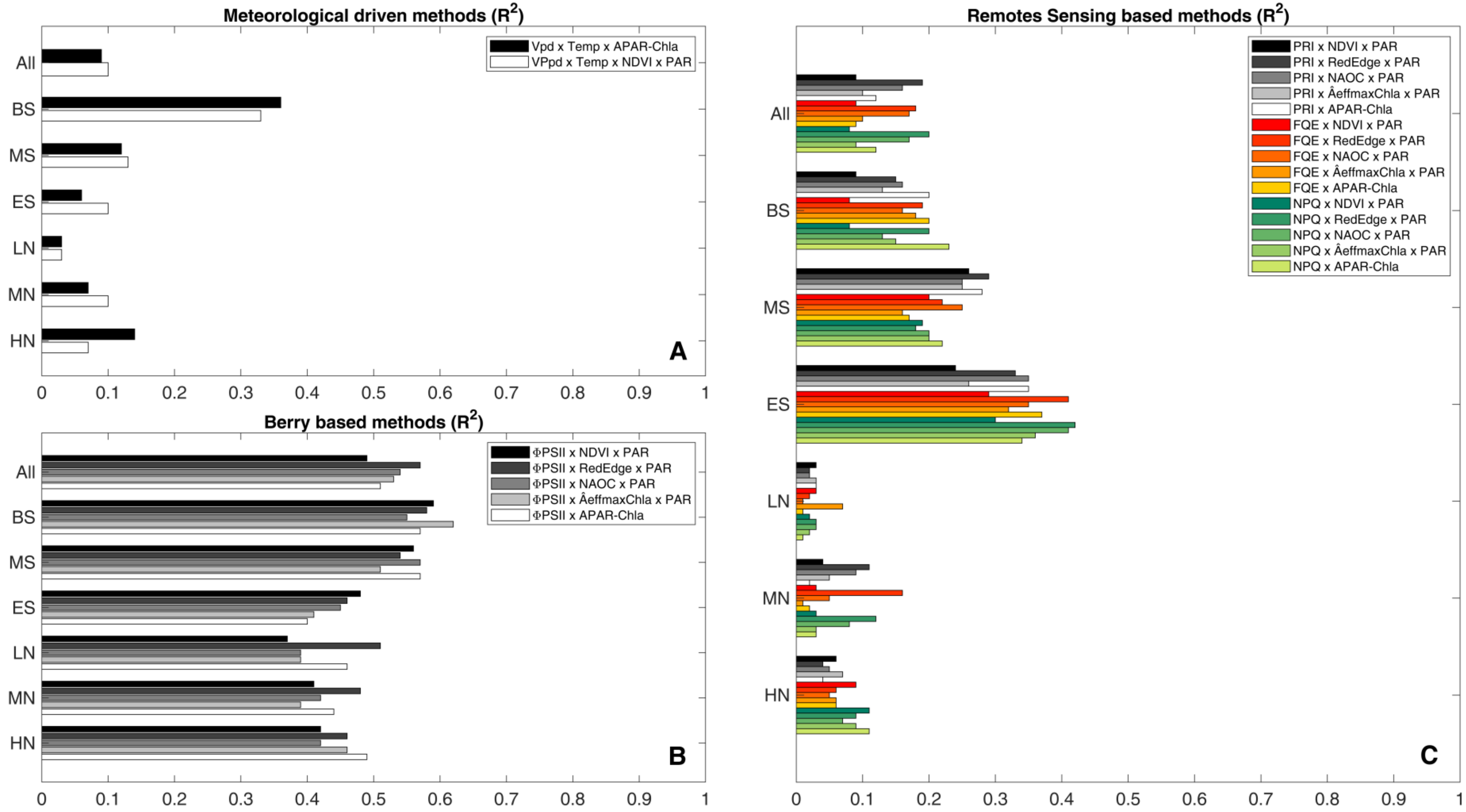
GOBIERNO DE ESPAÑA

UNIÓN EUROPEA

SUPPLEMENTARY MATERIAL

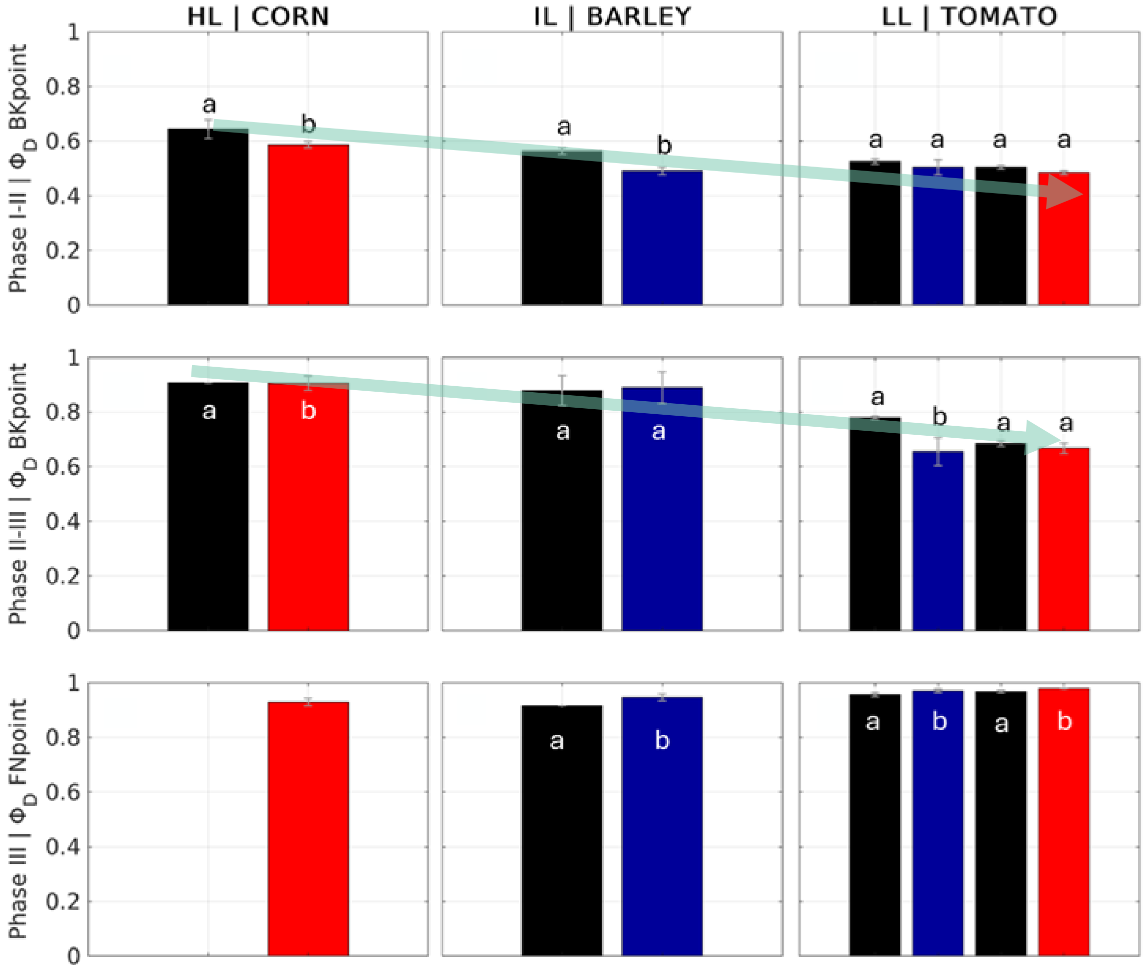
Linear modelling : Monteith's light-use-efficiency (LUE)

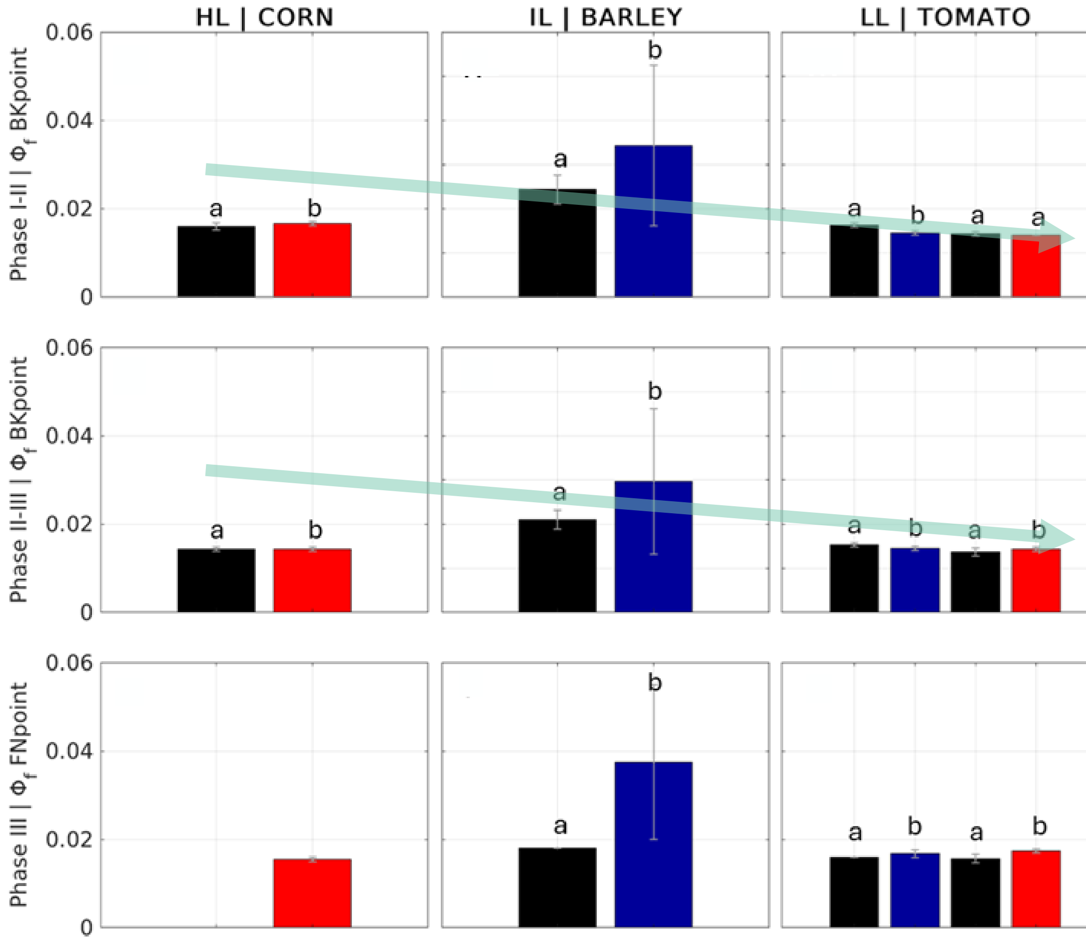


- Linear models **meteorological** and **remote sensing** based models $R^2 < 0.4$

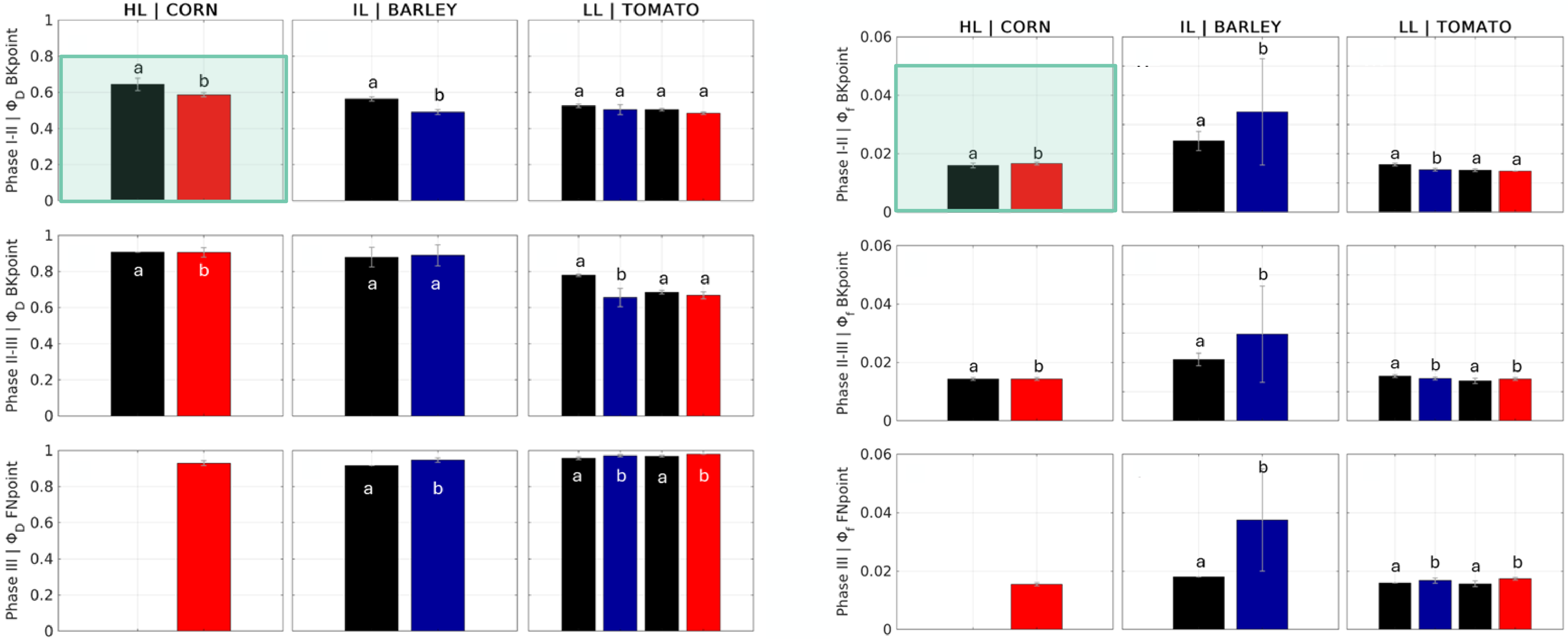
Cendrero-Mateo et al., 2025 IEEE

Φ_f and Φ_D BreakingPoint

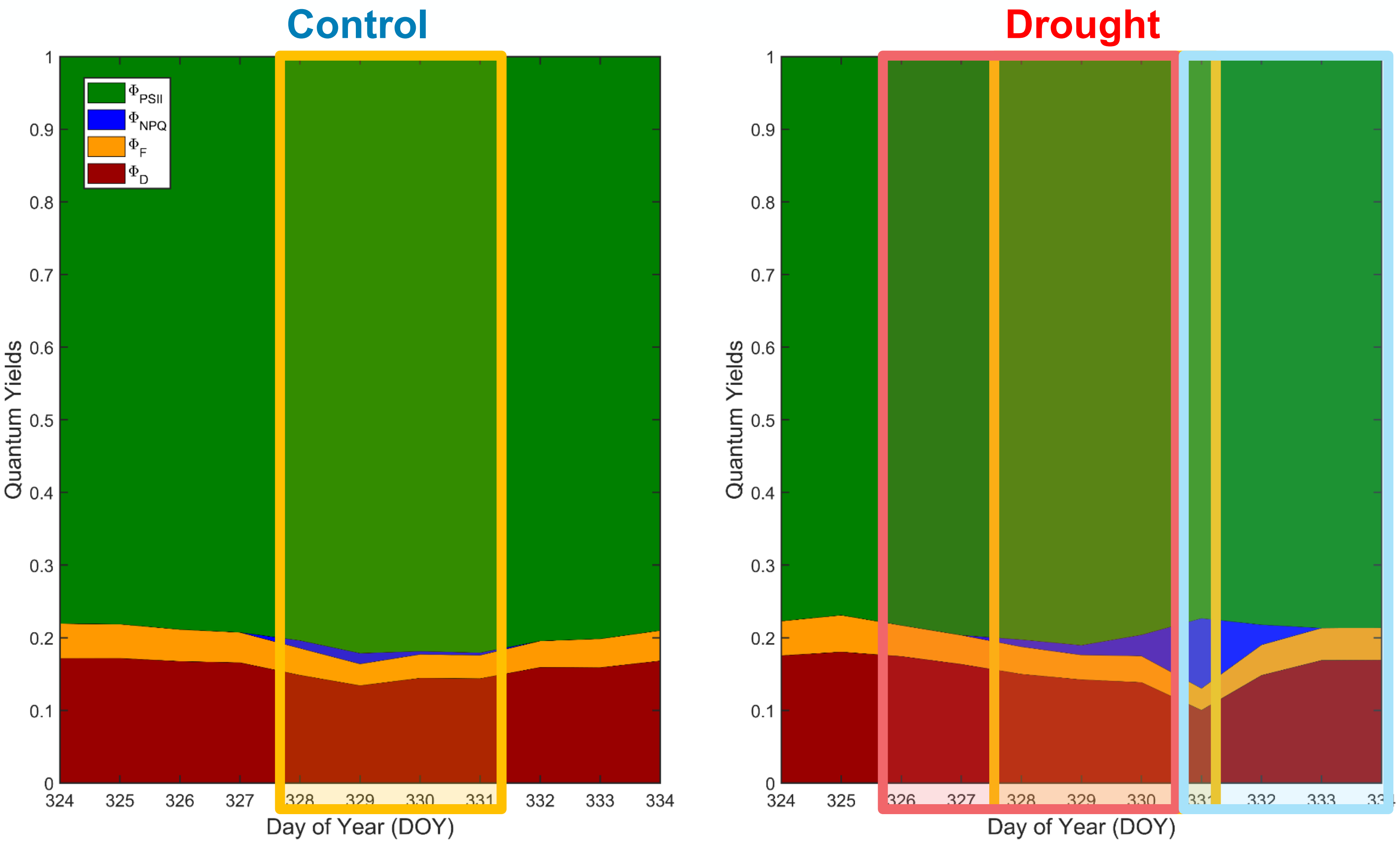




Φ_f and Φ_D BreakingPoint

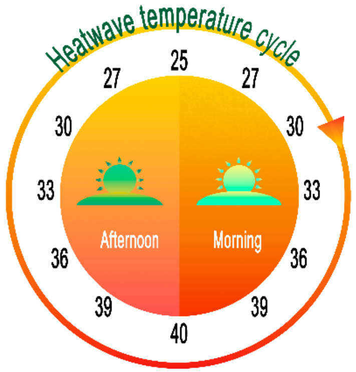


Heatwave | Experiment Yield

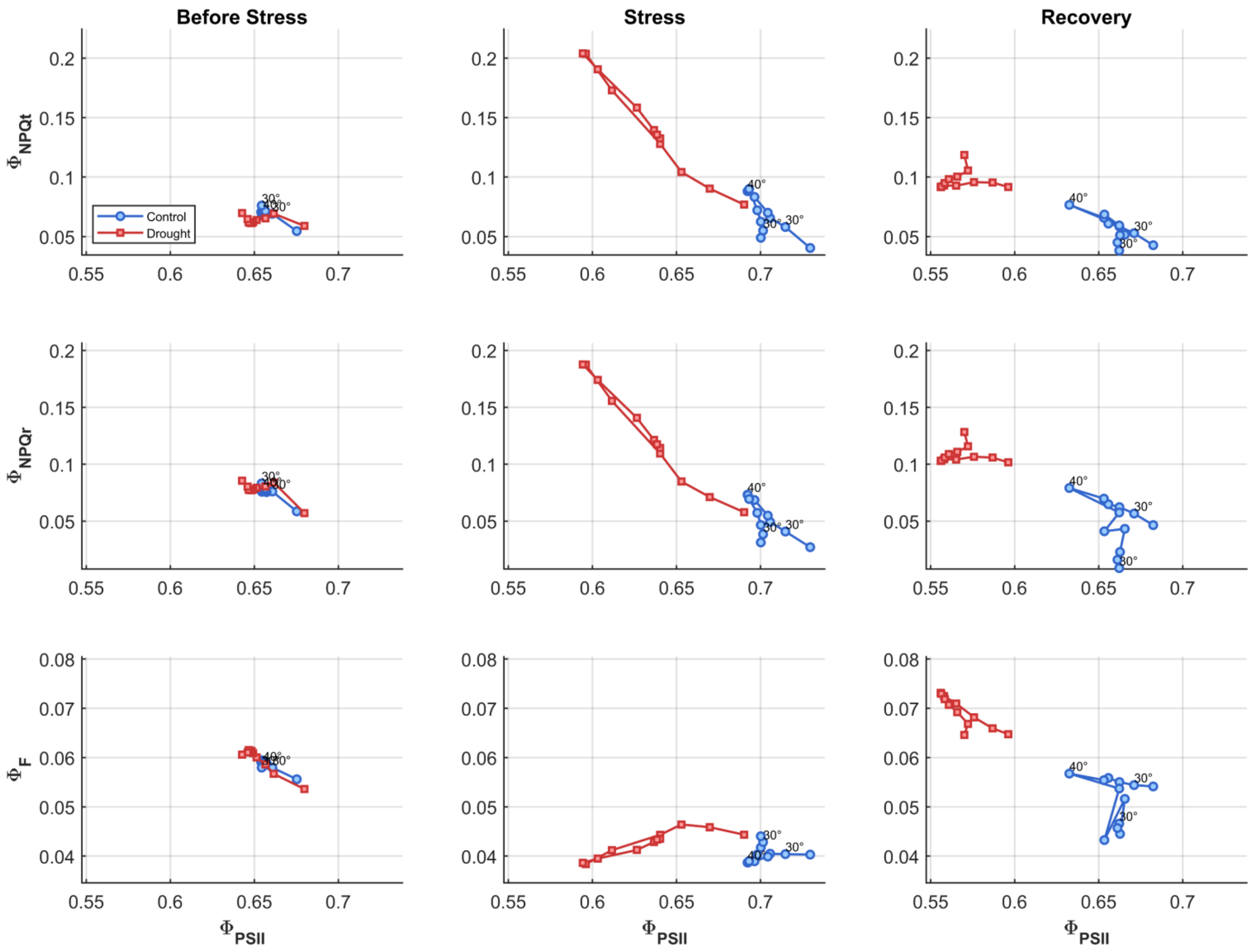


HeatWave | Drought | ReWater

- **Experiment yield** dynamics are **modulated** by the upregulation of **NPQs**.
- NPQs levels began to rise **on the first day** of the heatwave.



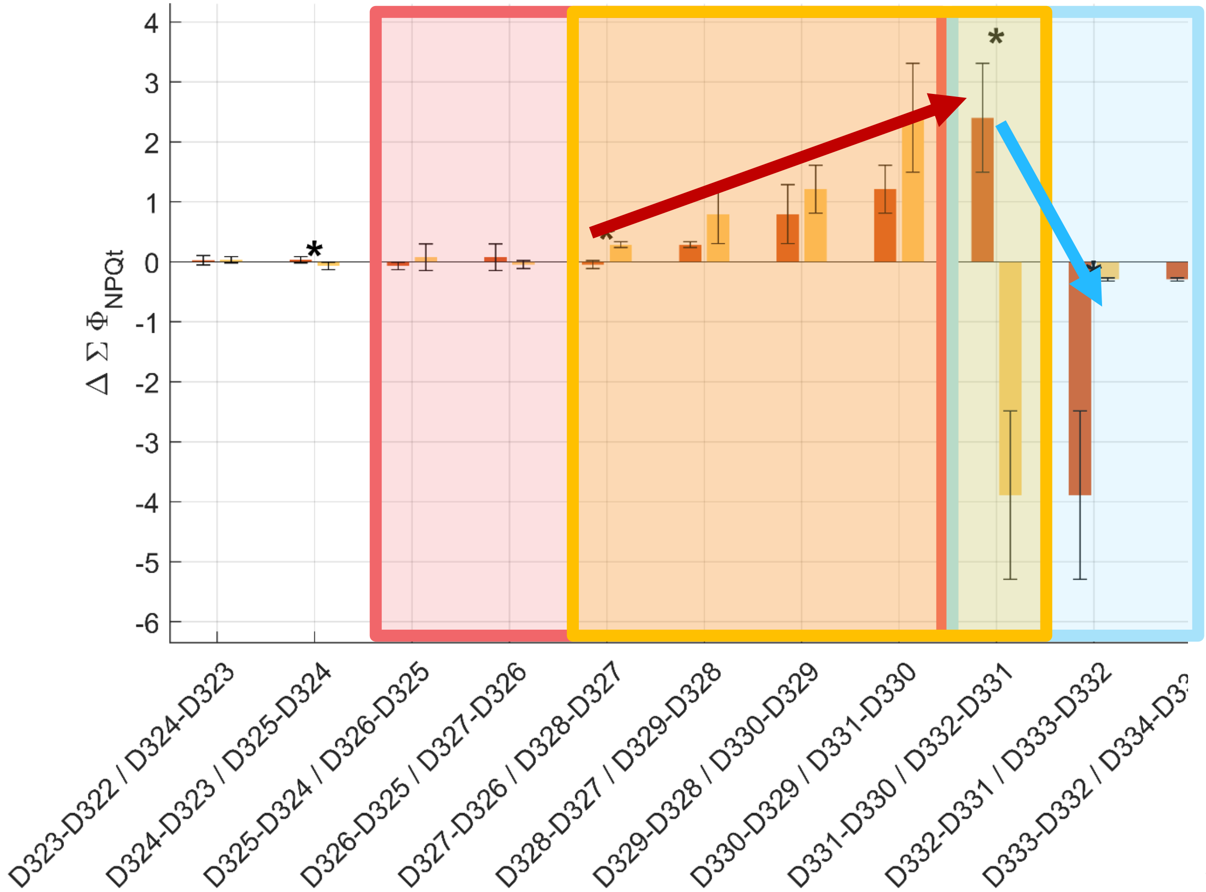
Heatwave | Diurnal Yield

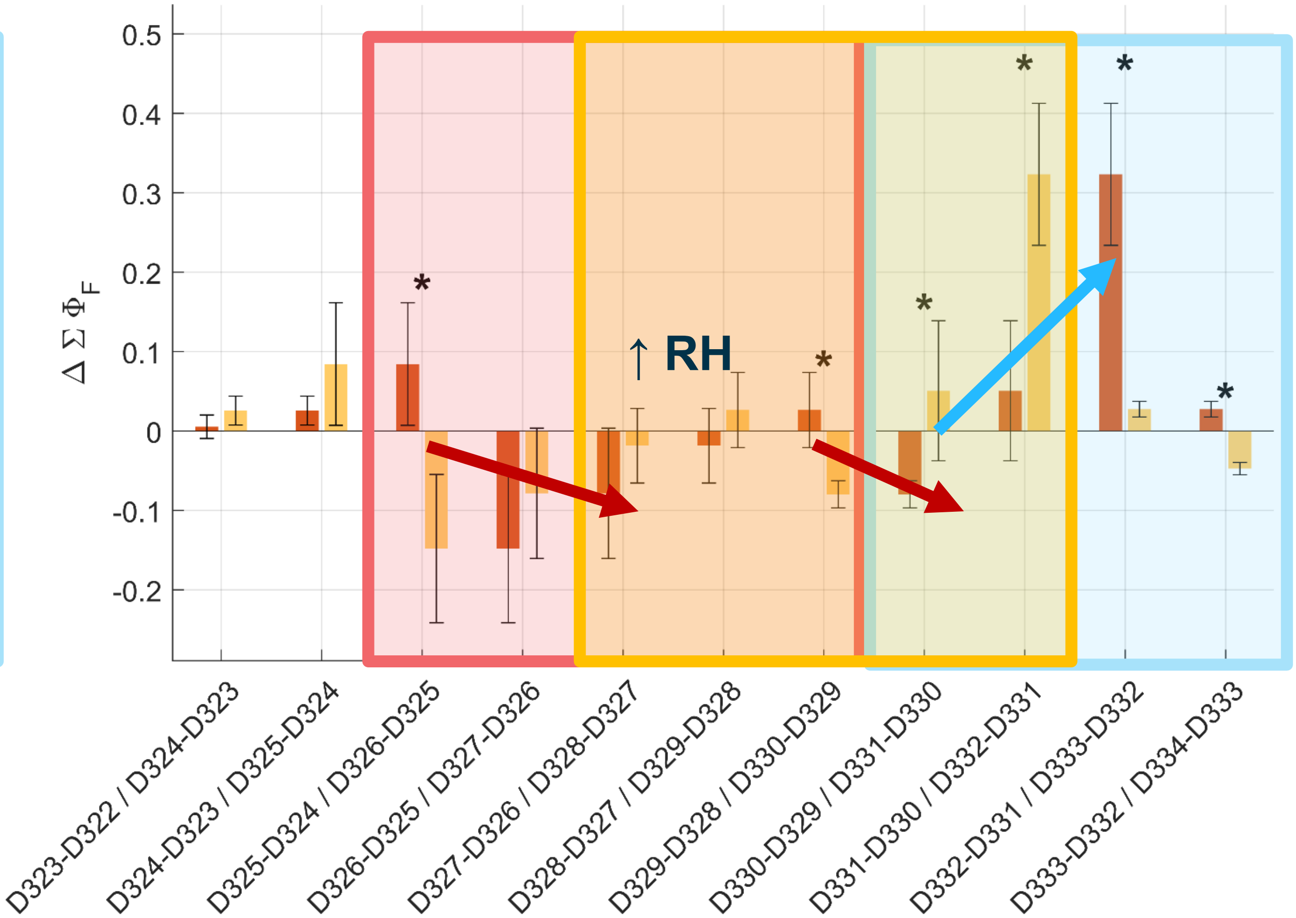


- **Pre-stress:** Daily dynamics of ΦNPQt, ΦNPQr, and Φf are consistent across treatments.
- **Under stress:** ΦNPQt and ΦNPQr rise while Φf declines (Phase II).
- **Recovery Phase:** ΦNPQt and NPQr declines while Φf rises.

Heatwave | Stress Detection

HeatWave Drought ReWater





Heatwave | Stress Detection

HeatWave | Drought | ReWater