Why does the leaf of Japanese Cypress in the temperate region experience transient leaf reddening under winter excessive light stress

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Background

February May

November August

- ***Winter leaf reddening***

  Transient and **recoverable** phenomenon that Japanese cypress leaf changes color from green to red before or during winter due to the **accumulation of red pigment （rhodoxanthin)**. *(Hughes, 2011)*

2019/11/1 2019/11/11 2019/11/18

Leaf reddening during senescence

Winter leaf reddening (last months) ≠ leaf reddening during senescence or development (last weeks)

Photoprotection Function

Why leaf reddening happens?

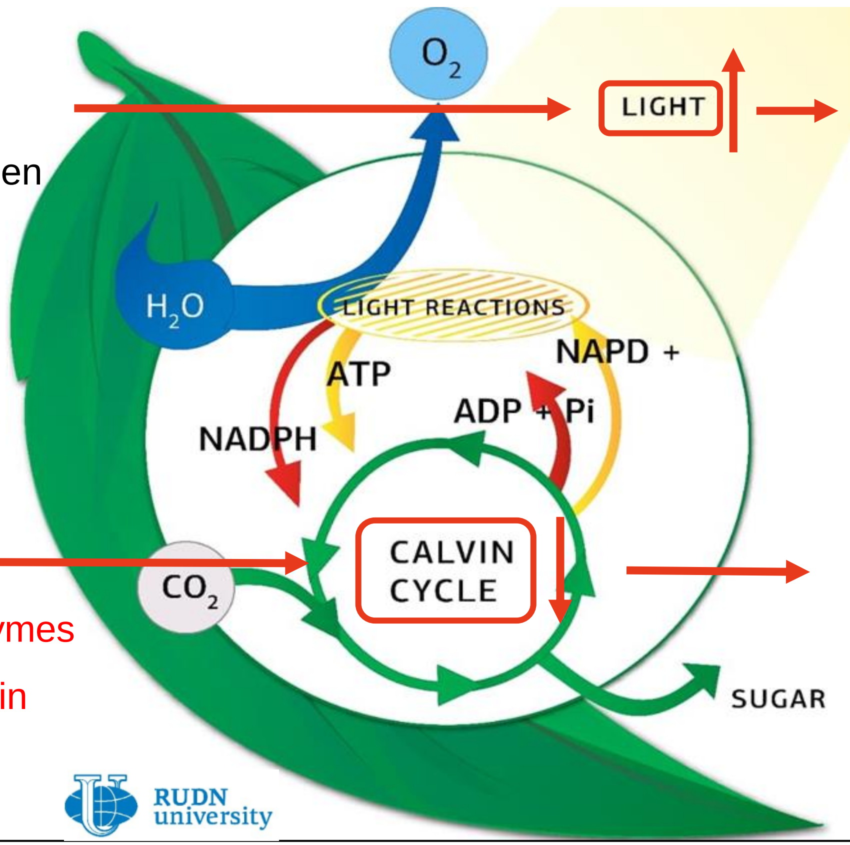
A classical explanation: the winter leaf reddening phenomenon is a **photoprotection** strategy, which is the biochemical process that helps organisms cope with molecular damage caused by sunlight. *(*Hughes et al., 2005, 2007).*

- **High solar radiation**

Causes an imbalance between light energy absorption and light energy utilization

- **Low temperature**

Reduces the activity of enzymes involved in the Calvin cycle in photosynthesis.



light energy absorption ↑

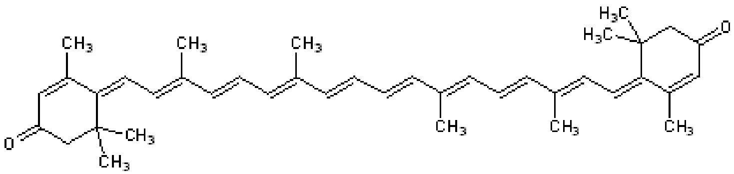
Imbalance

light energy utilization ↓

The joint role of Rhodoxanthin and Xanthophyll cycle

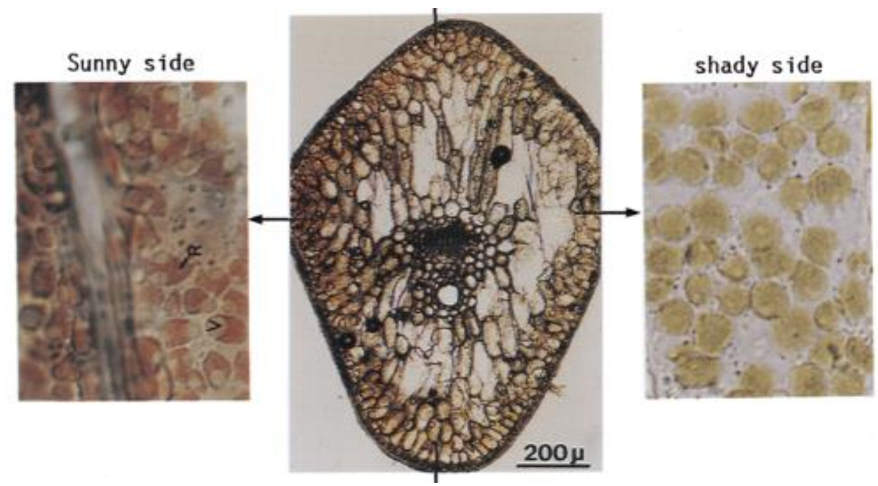
Rhodoxanthin (in gymnosperms)

- A xanthophyll pigment with a purple color



Japanese cypress

- The photodamage defense mechanism of rhodoxanthin is **screening and trapping of solar radiation.**
- Reddish particles of rhodoxanthin are localized in ***chromoplasts*** and most of the rhodoxanthin is located **nearer to the surface of the sunny side of a leaf than is Chlorophyll** *(*Ida et al., 1991)*



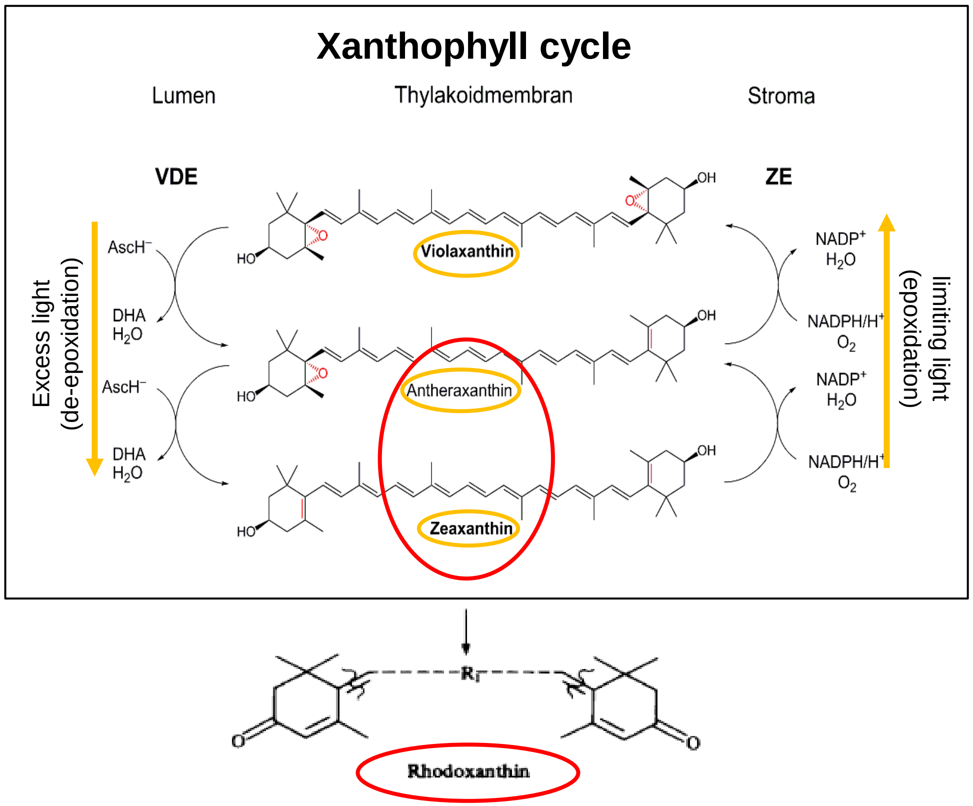
Microphotographs of cross section of Cryptomeria leaf and chromoplasts in mesophyll cells in a full daylight plot (Ida et al., 1991)

- ***The Red-Green vegetation index (RGVI) can be used to reflect leaf redness.***

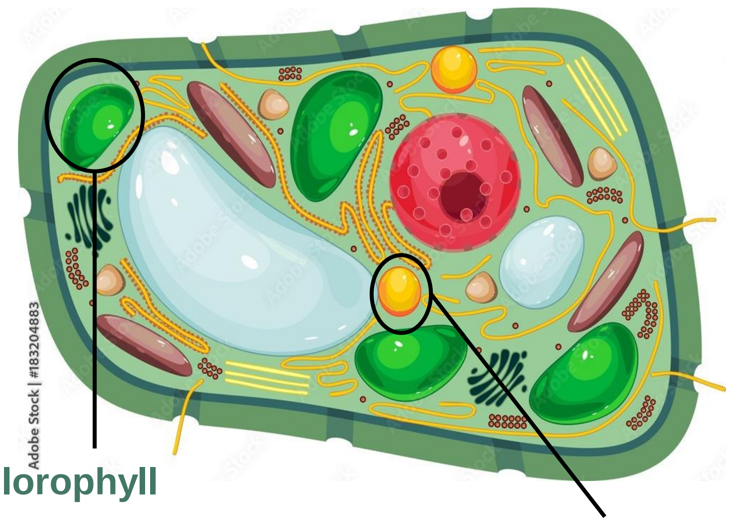
The joint role of Rhodoxanthin and Xanthophyll cycle

Xanthophyll cycle (VAZ cycle)

- Xanthophyll cycle is a **non-photochemical quenching mechanism** that can efficient **thermal dissipation** of the excess energy.



- ☐ *The* ***joint role of these two processes*** *in regulating canopy photosynthesis under winter excess light stress has not been further studied.*



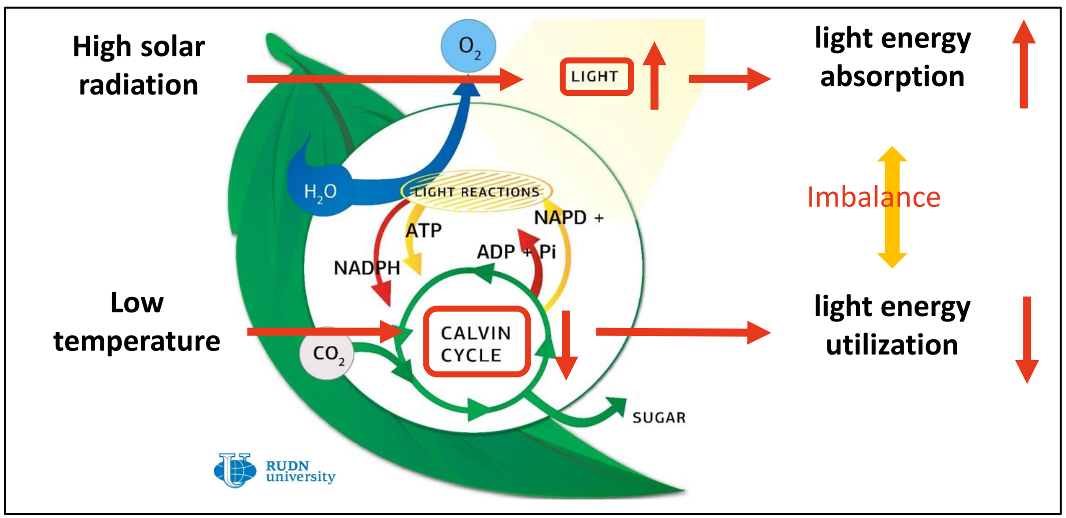
- ***The Photochemical reflectance index (PRI) can be used to reflect xanthophyll cycle dynamic.***

Hypothesis

Green → **Red**

Redness
Indicator: RGVI

Photoprotection mechanism activated under excess light stress



Xanthophyll cycle

Indicator: PRI

Rhodoxanthin

Indicator: RI

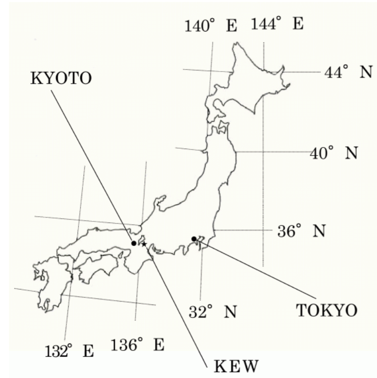
Questions：

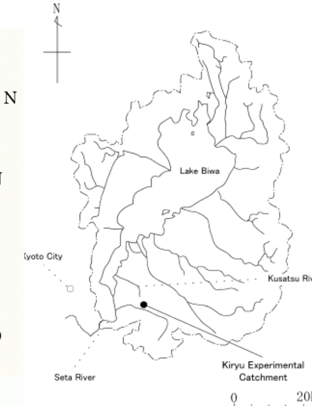
1. *How the* ***canopy gas exchange****,* ***vegetation indices****, and* ***environmental conditions*** *change during this phenomenon?*
2. *What is the* ***specific excess light intensity conditions*** *trigger this phenomenon to occurs?*
3. *Is the winter leaf reddening in Japanese cypress leaves caused by* ***photoprotection mechanisms****?*

Study site

Kiryu Experimental Watershed (code: KEW)

- Location: south Shiga, Japan(34°58’N, 136°00’E)
- Mean annual air temperature: 13.5℃
- Mean annual precipitation: 1630mm
- LAI: 4.5-5.5
- Canopy height: approximately 20m
- Vegetation: Japanese cypress *(gymnosperm)*





Spectroradiometer

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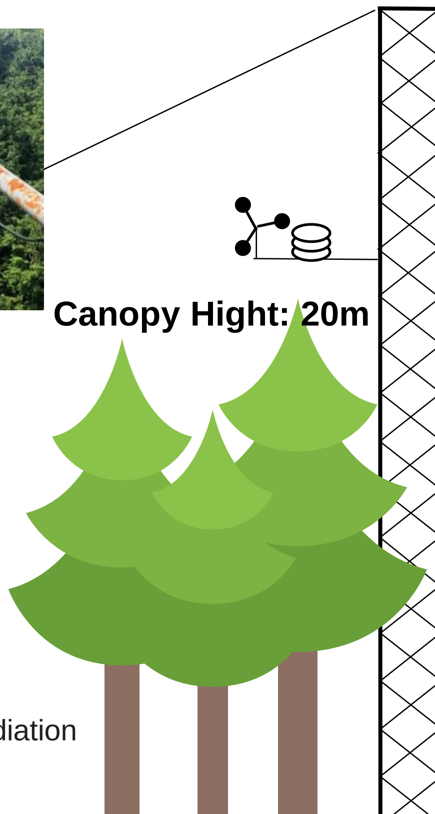
PRI (Photochemical reflectance index)



Tower Hight: 29m

Canopy Hight: 20m

Eddy covariance system

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GPP & LUE (Light-use efficiency)

Meteorological data

Photosynthetically active radiation and Temperature

Sensors in 1m,10m,20m,26m,29m

Phenology monitor

Digital camera (3h intervals)

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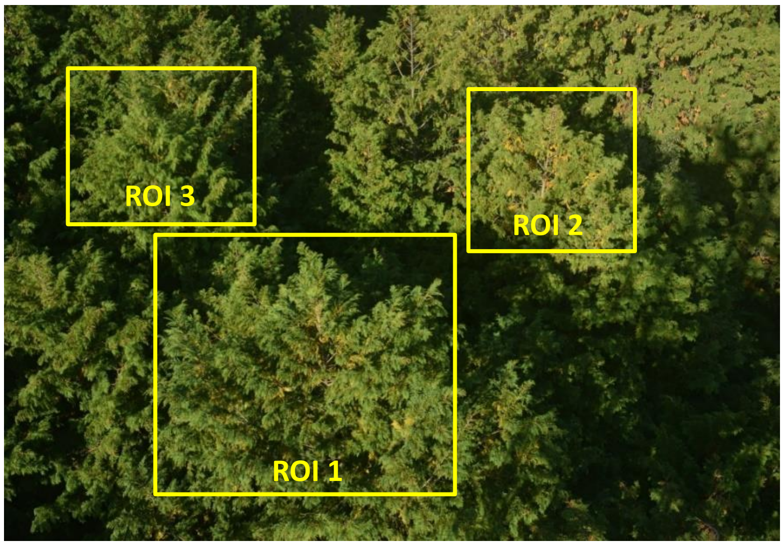
RGVI (Red-Green vegetation index)

RGVI (Red – Green vegetation index)

ROI	1	2	3
Area (pixels)	2250*2000	1250*1250	1400*1200



- RGB color have integer values between 0 and 255. Image processing software can help extract RGB channels and calculate RGVI.

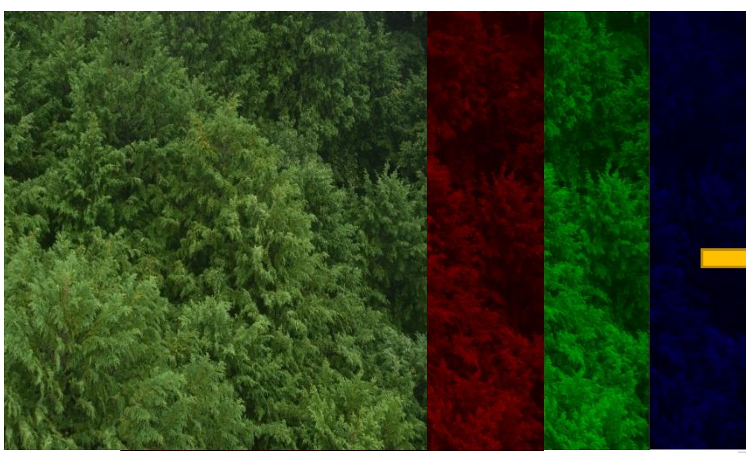
RGVI (Red-Green vegetation index)

$$RGVI = (Rcc - Gcc)/(Gcc + Rcc)$$

RGBcc (RGB chromatic coordinates)

$$RGBcc = \frac{R/G/B}{R + G + B}$$

- Region of interest (ROI) were selected to reduce the influence of the shadow of the canopy gap and the non-canopy part.

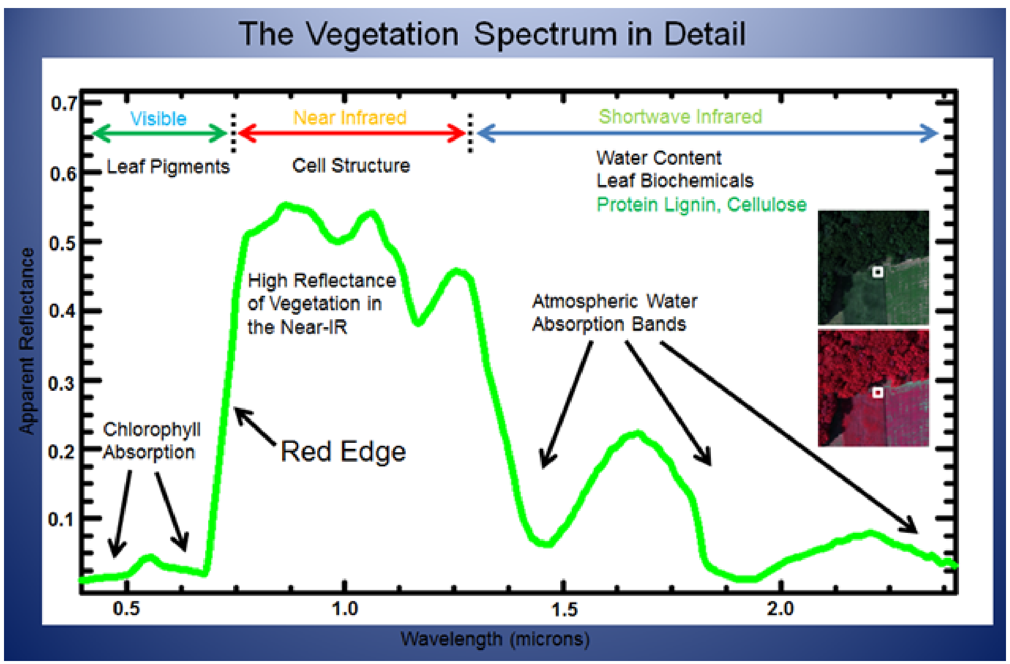


	ROI 1			ROI 2			ROI 3		
	R	G	B	R	G	B	R	G	B
2016/2/25 9:00	112.322	104.558	62.763	148.718	139.698	87.034	112.515	106.684	59.533
2016/2/25 12:00	63.17	62.714	40.932	68.939	67.248	42.4	49.513	51.383	33.436
2016/2/25 15:00	39.866	37.268	17.318	42.455	39.209	17.44	33.605	32.715	14.89
2016/2/26 9:00	109.059	100.836	56.694	141.523	130.375	75.064	106.452	100.59	53.091
2016/2/26 12:00	118.67	111.502	66.537	130.048	120.673	71.481	99.654	96.58	55.811
2016/2/26 15:00	32.734	32.508	20.253	34.326	33.452	20.006	28.887	29.826	18.135
2016/2/27 9:00	98.031	90.156	46.024	117.532	106.152	54.348	93.209	87.698	42.433
2016/2/27 12:00	105.918	99.645	59.163	119.057	110.557	64.905	87.156	85.117	50.008
2016/2/27 15:00	71.027	65.628	32.528	71.571	65.477	32.109	59.365	56.759	27.921
2016/2/28 9:00	106.559	97.949	51.871	135.199	123.24	67.045	104.388	97.832	48.901
2016/2/28 12:00	106.168	99.772	60.06	120.228	111.773	66.585	86.405	84.088	50.035
2016/2/28 15:00	61.214	58.921	38.078	62.914	60.505	38.55	47.588	48.787	34.789

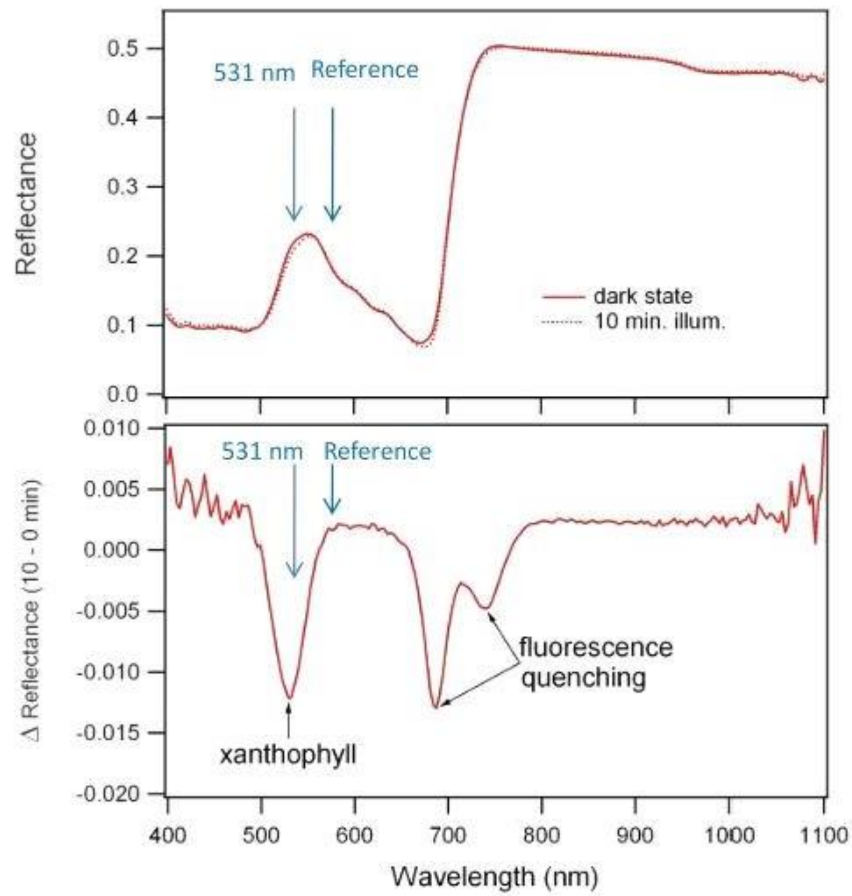
PRI (Photochemical reflectance index)

- PRI (Photochemical reflectance index) is a carotenoid-based vegetation index that can remotely track the invisible phenology of photosynthesis by assessing carotenoid pigment dynamics.
- The PRI is a useful index to track the xanthophyll cycle.

$$PRI = \frac{R_{531} - R_{570}}{R_{531} + R_{570}}$$



By GIS resources



Gamon & Qiu 1999

*RI (Redness index)

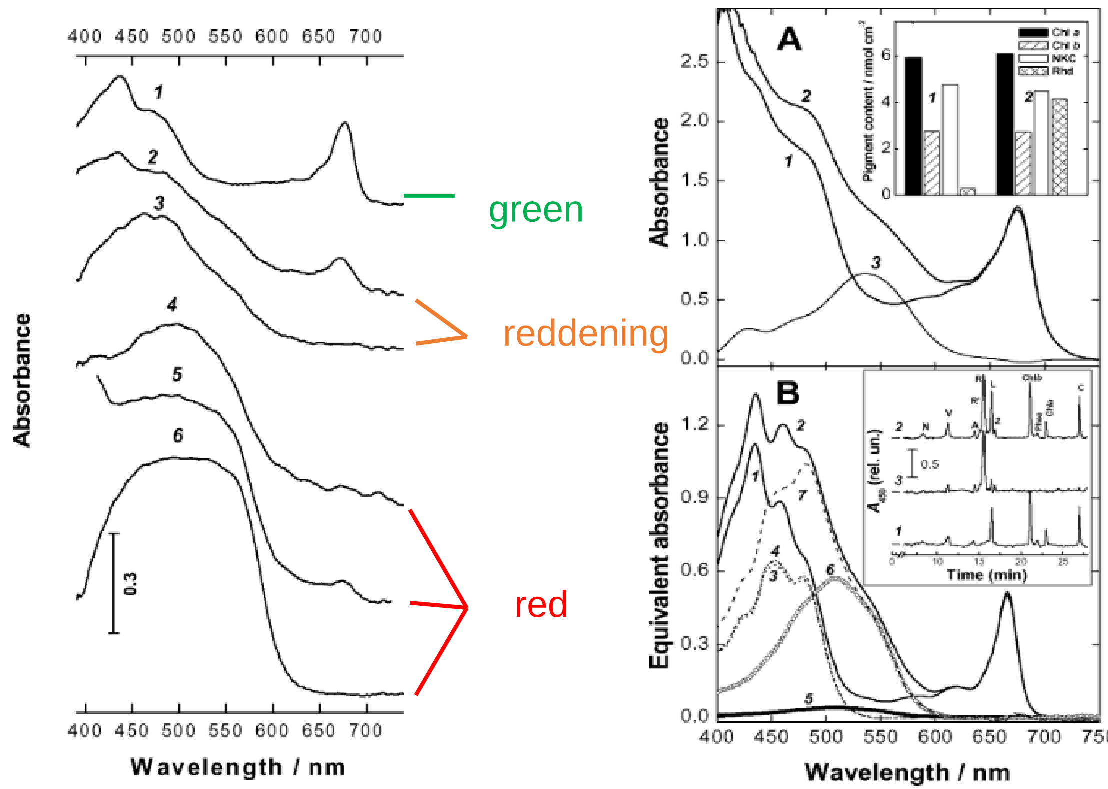
- This study developed the RI (Redness index) based on *Merzlyak's* study on red pigment rhodoxanthin. Compared with camera data analysis, remote sensing method is more accurate and can monitor whether there is a change in leaf pigment within a day. We expect RI to become a more convenient index for monitoring rhodoxanthin dynamic.

- Due to the maximum of Rhodoxanthin absorption in aloe leaves is located near 540 nm.

  (Mark Merzlyak et al., 2005)

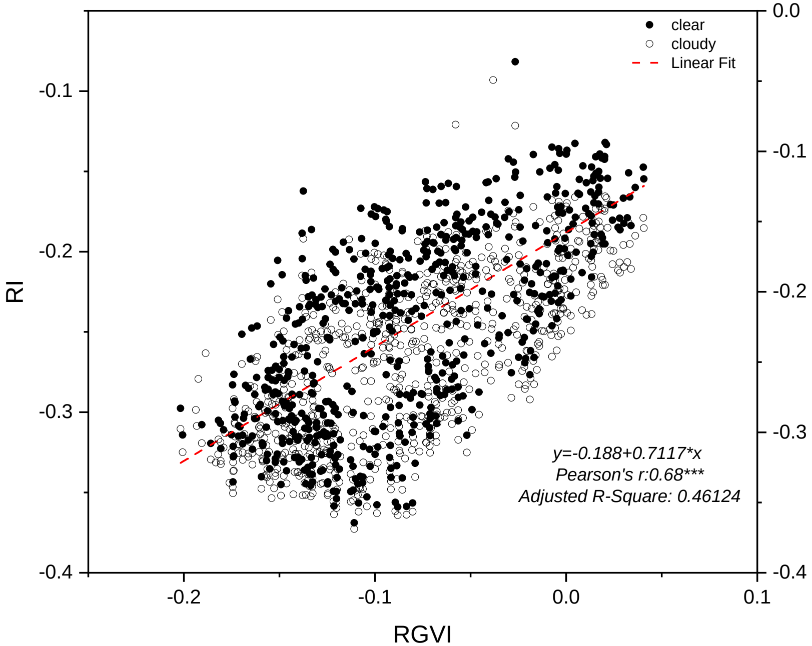
- The RI may be a useful index to track the rhodoxanthin accumulation.

$$RI = \frac{R_{470}-R_{540}}{R_{470}+R_{540}}$$

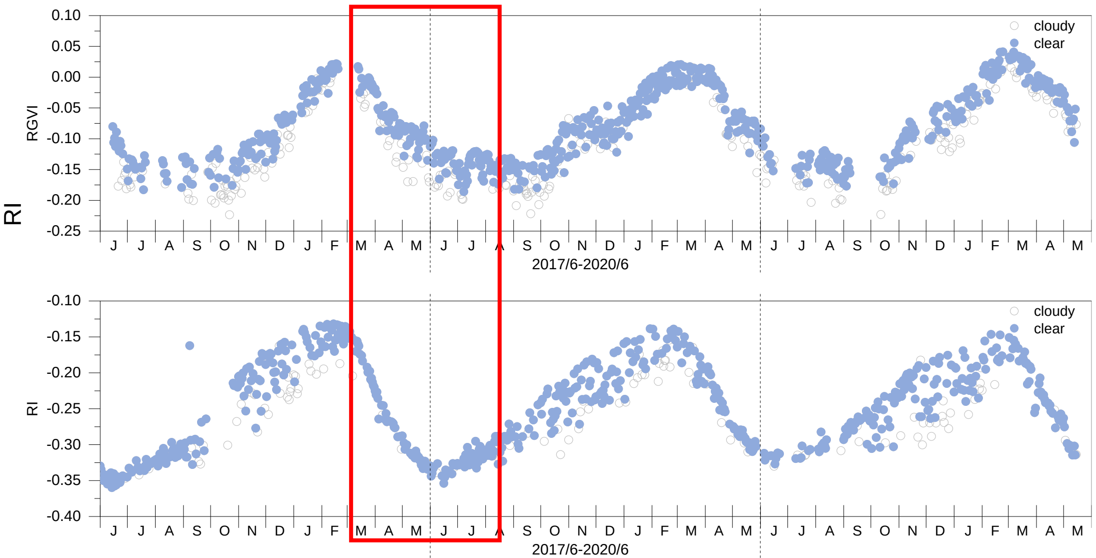


Absorption spectra and pigment analysis of green and red aloe adaxial chlorenchyma (AC) tissues (Mark Merzlyak et al., 2005)

*RI (Redness index)



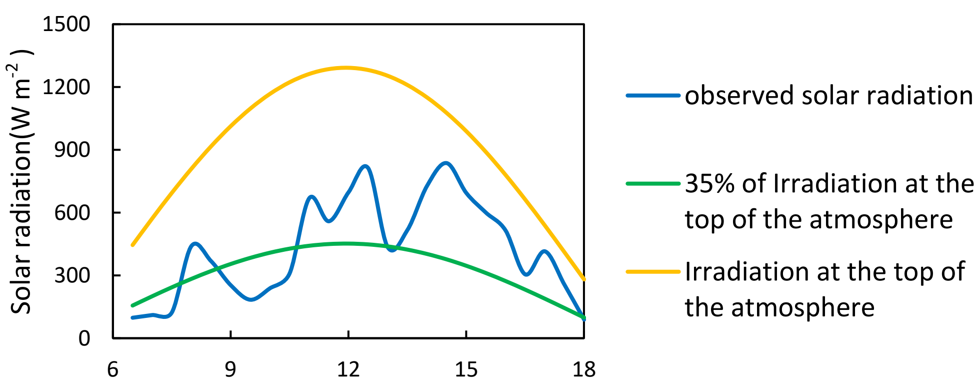
- The correlation between RI and RGVI was high. However, the difference was evident during the redness fading process, indicating that the change in RI may be faster than expected



Clear & Cloudy days

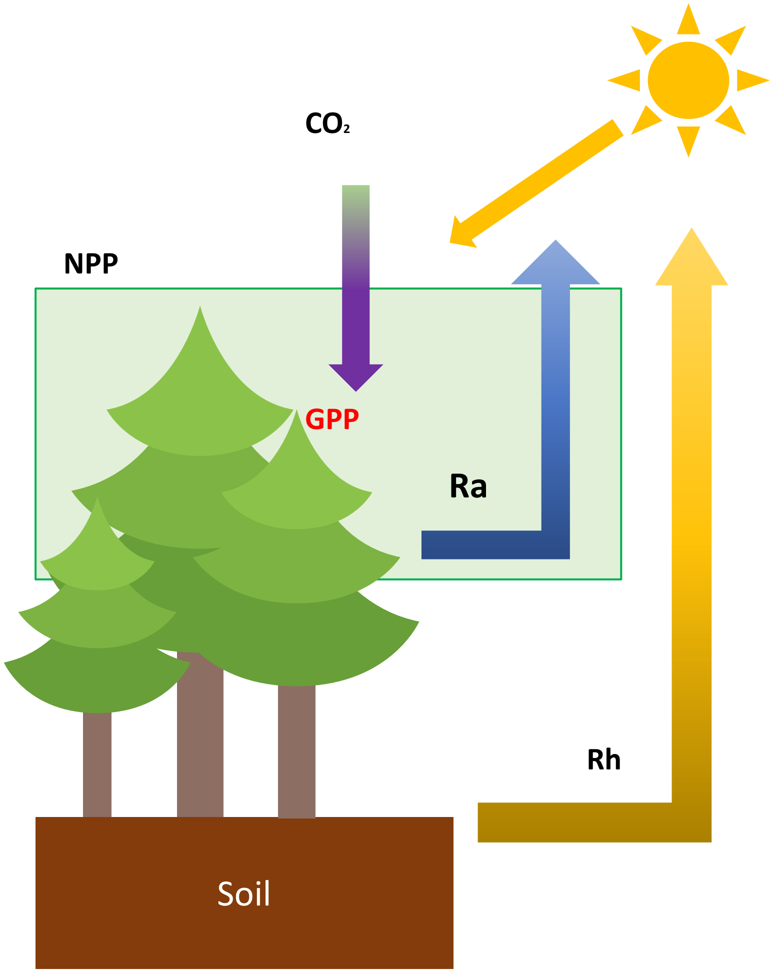
$$\cos\theta = \sin\phi\sin\delta + \cos\phi\cos\delta\cosh$$

$$S_0^{\downarrow} = I_{00}\left(\frac{d_0}{d}\right)^2 \cos\theta \qquad \left(\frac{d_0}{d}\right)^2 = 1.00011 + 0.034221\cos\eta + 0.00128\sin\eta + 0.000719\cos 2\eta + 0.000077\sin 2\eta$$



- The cloud has a great influence on the duration of solar illumination and radiation intensity.
- The data when the observed solar radiation amount was 35% or more of the solar radiation amount at the upper space atmosphere was regarded as clear day data. Higher than 60% is sunny day data. *(水環境の気象学, 1999)*

GPP& Light-use efficiency *(LUE)*



Fc : Canopy CO_2 flux

Sc : Variations in CO_2 storage

RE: Daytime ecosystem respiration

PAR: photosynthetically active radiation

APAR: Absorbed photosynthetically active radiation

FAPAR: A fraction of APAR

GPP (gross primary production)

- Total primary production produced by plants in an ecosystem. GPP presents total ecosystem photosynthesis.

$$GPP = -(F_C + S_C) + RE$$

LUE (light-use efficiency)

- The light-use efficiency is one of the most essential parameters in production estimation models for terrestrial ecosystems.

$$LUE = \frac{GPP}{APAR}$$

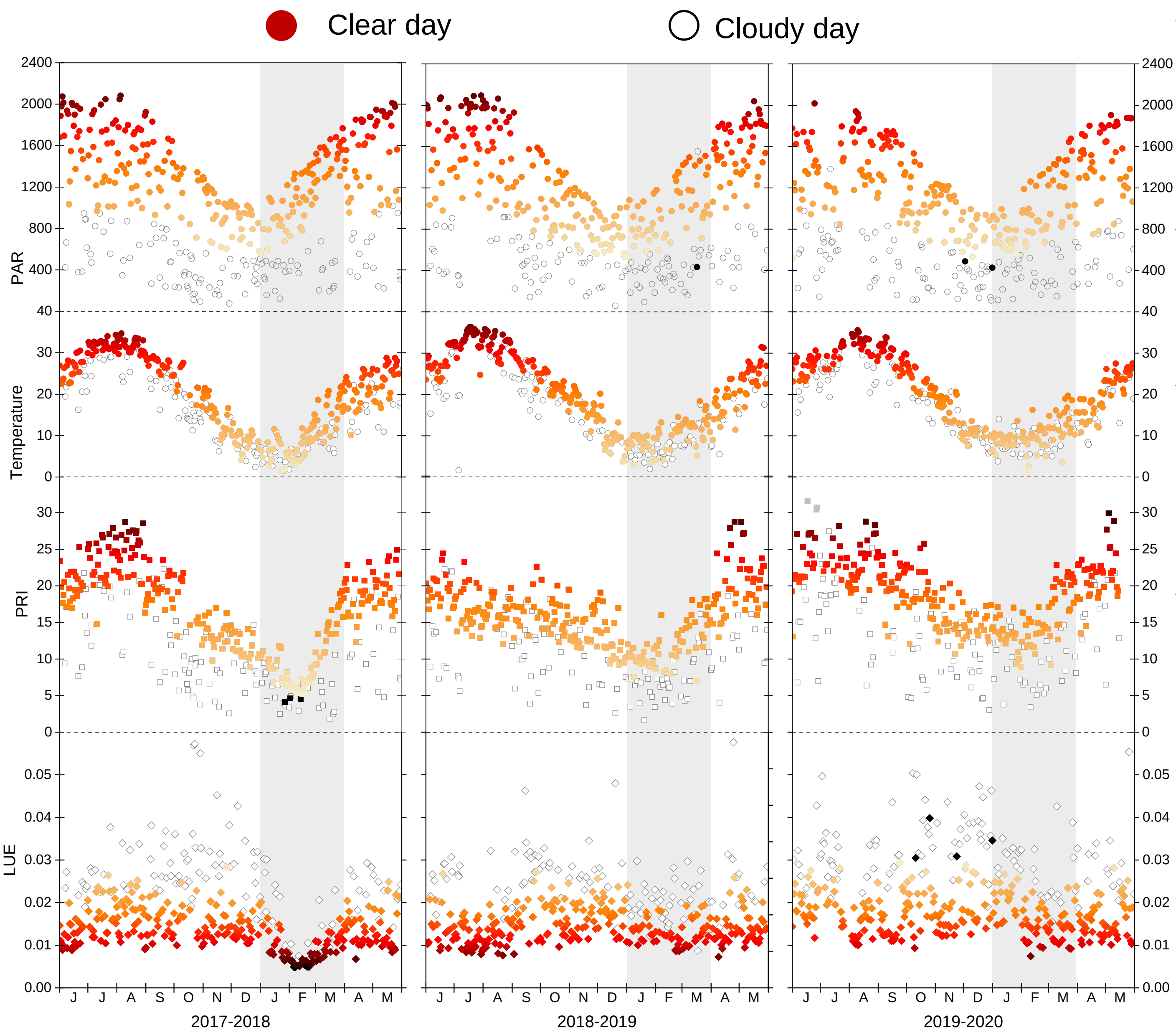
$$APAR = FAPAR * PAR$$

Leaf gas exchange

- Multi-Layer Model was used to assess the importance of leaf physiology on canopy scale gas exchange.

Result & discussion

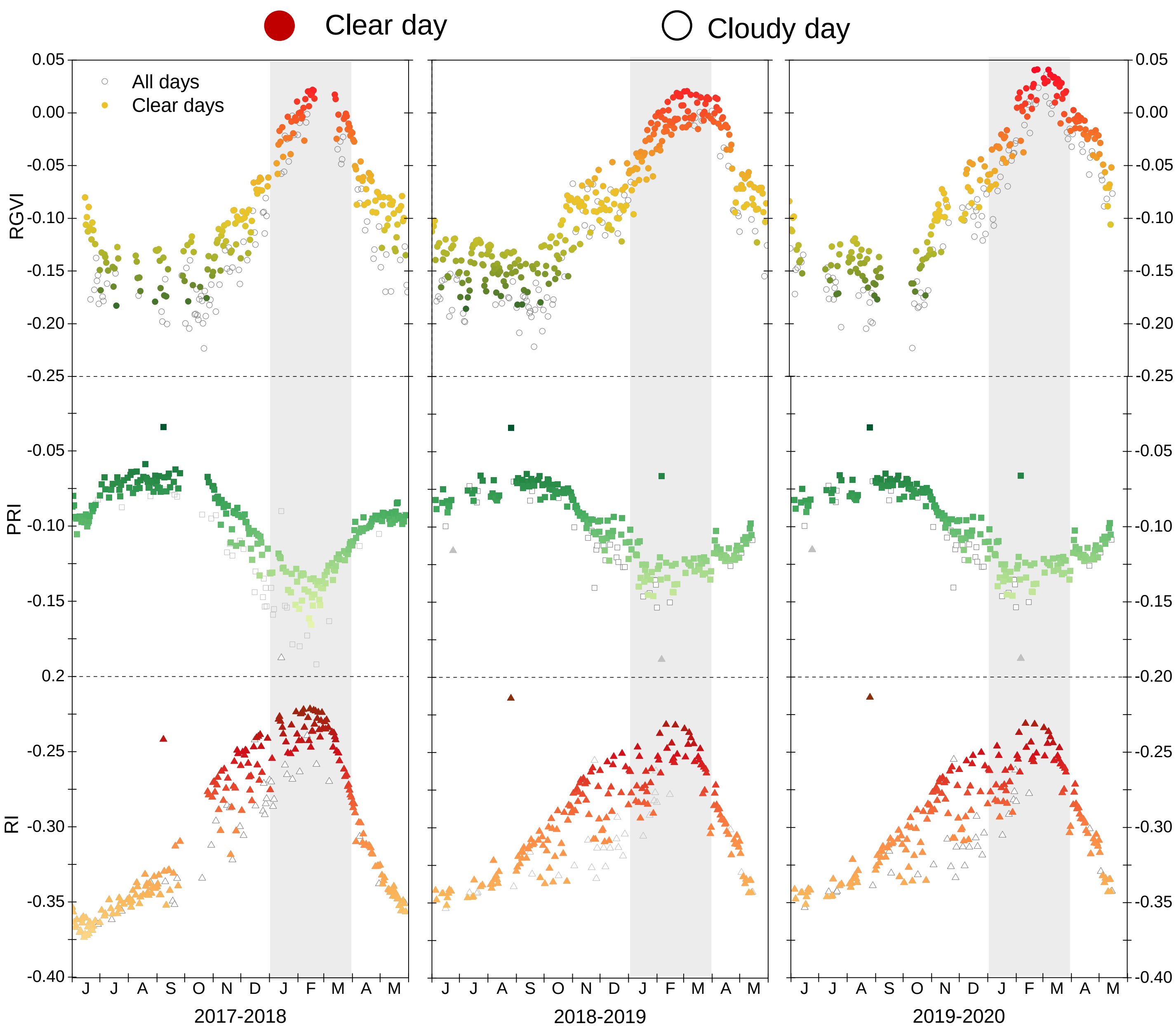
◻ The inter-annual changes of PAR, Ta, GPP and LUE *(2017/6-2020/6)*



- Clear seasonal changes in environmental factors were observed during 2017/6-2020/6.
- PAR, Ta, GPP and LUE reaches its trough during winter season (1-3).
- There are distinct difference between the clear day and cloudy day. Especially in PAR, GPP and LUE.
- LUE on clear days is considerably lower than that on cloudy days. It may due to the scattered sunlight from a cloudy sky can enhance the LUE of compared to direct incident sunlight *(Zheng Yuan, 2011).*

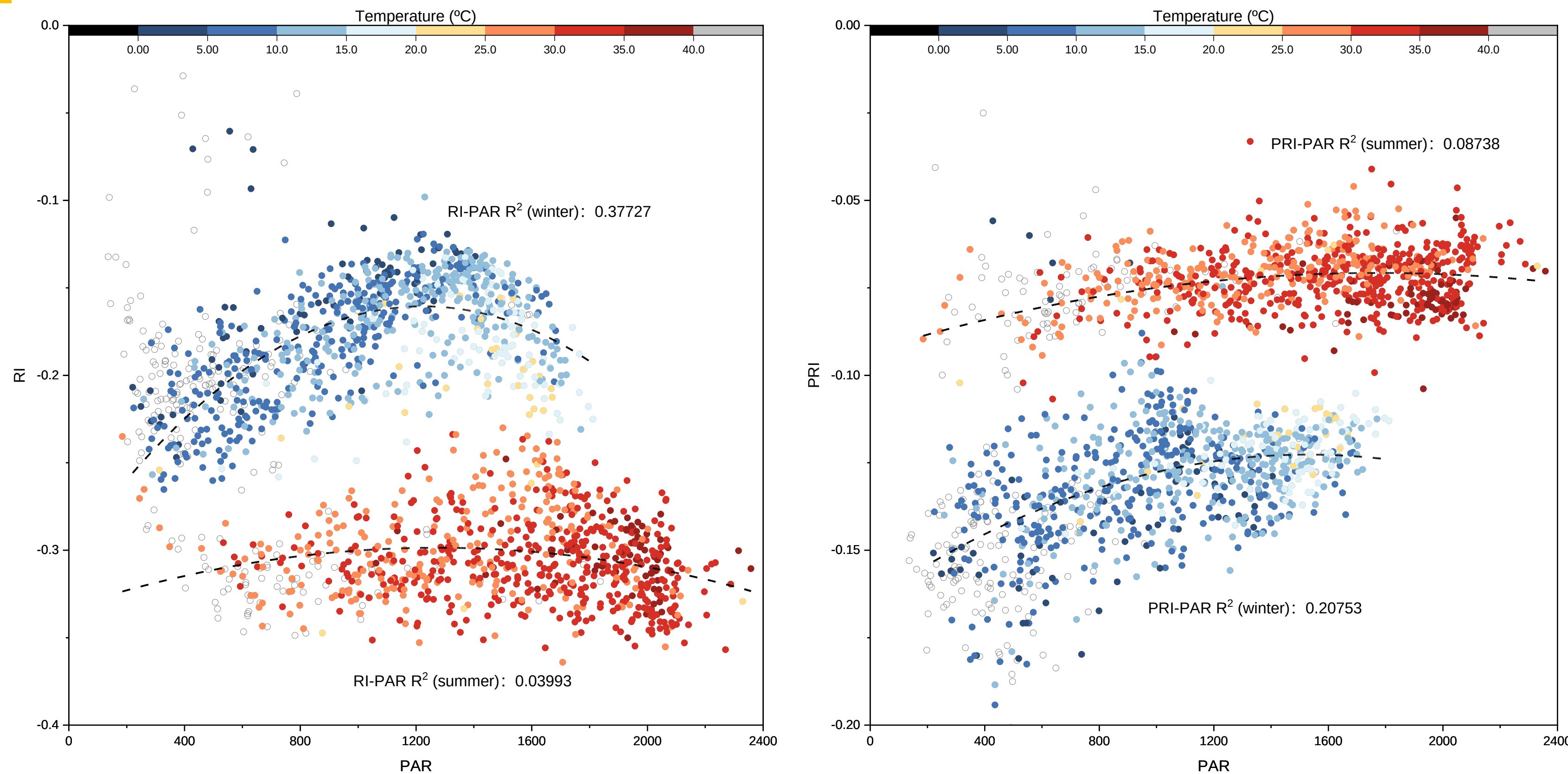
Result & discussion

☐ The inter-annual changes of Vis *(2017/6-2020/6)*



- Clear seasonal changes in vegetation indices were observed during 2017/6-2020/6.
- Environmental factors and PRI reaches its trough during winter season and the accumulation of rhodoxanthin lead to a maximum level on leaf redness.
- The red pigment synthesis (RGVI & RI) accompanied by the Ta, PAR, and PRI declines during winter season (1-3).
- ***The winter leaf reddening phenomenon occur along with the lower level of Ta, PAR, PRI, GPP and LUE.***

◻ Non-Linear regression between Vis and LUE during winter and summer *(30mins)*



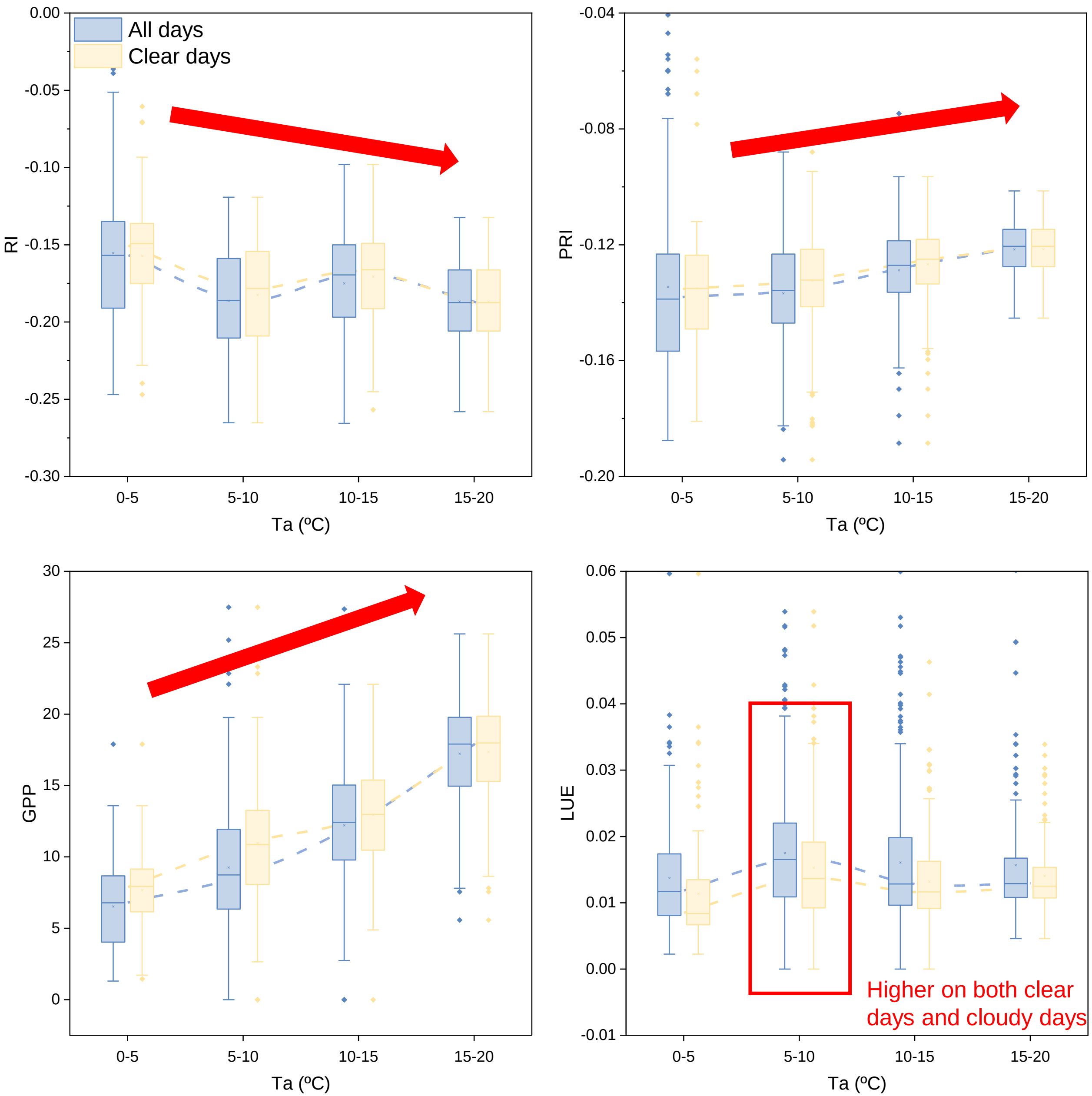
- RI and PRI have a higher correlation with PAR during winter.
- During winter when Ta reached 5-10 range, RI and LUE showed highest correlation while PRI showed lowest.
- ***During winter, when Ta reached 5-10 ℃, Rhodoxanthin may play a more important role than xanthophyll cycle.***

R-Square between Vis and LUE during winter

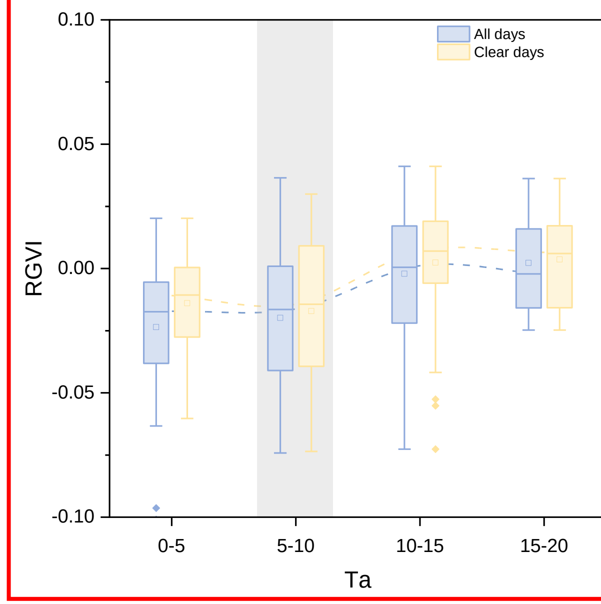
R-Square	*Ta (℃)*	*0-5*	***5-10***	*10-15*	*15-20*	ALL
RI-LUE	*Clear*	*0.25****	***0.68*****	*0.438****	*0.242****	*0.498****
PRI-LUE	*Clear*	*0.04ns*	***0.011ns***	*0.03**	*0.296****	*0.035****

*Asterisk indicates significance of correlation: ***P < 0.001; **P < 0.01; *P < 0.05; ns: not significant*

RI, PRI, GPP, LUE variation during winter in different Ta ranges *(30mins)*



Daily redness variation



- The synthesis of rhodoxanthin may also change rapidly within days, but there is a certain time lag in the accumulation of red pigment to the obvious phenological phenomenon.
- LUE showed higher level in 5-10 ℃ on both clear days and cloudy days

➢ ***During winter, 5-10 ℃ may be a threshold range for Ta***

Conclusion

1. The winter leaf reddening occur along with the lower level of Ta, PAR, PRI, GPP and LUE.
2. During winter, 5-10°C may be a threshold for Ta
   - *In 5-10℃ ,due to the lower temperature, the enzyme activity is limited and the imbalance is caused by the light energy utilization capacity is restricted.*
3. Winter leaf reddening is a common phenomenon that occurs due to the photoprotection function. The accumulation of rhodoxanthin and VAZ cycle played a collaborative role to adapt a excess light stress condition during winter.
4. Rhodoxanthin dynamic is more rapid than expected, whether there is a clearly diurnal variation needs further verification.