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Multiple drivers of seasonal change in PRI: Implications for photosynthesis 1. Leaf level

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Abstract

The goal of this study was to explore the relationships between the photochemical reflectance index (PRI) at the leaf level and pigment pools, focusing on the constitutive role of pigments in influencing PRI over seasonal or ontogenetic time frames. The purpose was to re-evaluate the role of PRI as an indicator of seasonally shifting pigment (chlorophyll, carotenoid and anthocyanin) contents, and hence photosynthetic activity, across a range of tree and crop species. We studied natural vegetation — three tree species (maple, chestnut and beech) and two managed irrigated and rain-fed crop species (maize and soybean), contrasting in photosynthetic pathway and leaf structure, and having wide variation of pigment content and composition. In anthocyanin-free leaves, PRI related to both total chlorophyll (Chl) and carotenoid (Car) contents, however, much closer relationships were found between PRI and Car to Chl ratio (Car/Chl). The sensitivity of PRI to Car/Chl varied widely in tree species with the degree of secondary carotenoids. In crop leaves where the Car vs. Chl relationship was very close, the slopes of PRI vs. Car/Chl relationships for maize and soybean were almost identical. PRI vs. Car/Chl relationships for leaves of different tree species formed a significant, uniform relationship with PRI. Two crops also formed a significant, uniform PRI vs. Car/Chl relationship with a slope half the value found for trees. In anthocyanin-containing leaves, PRI did not clearly relate to any pigment content because reflectance values at both PR wavebands are affected by anthocyanin content. The findings of a strong link between leaf level PRI and Car/Chl over seasonal and ontogenetic time spans supports recent findings calling for a more careful evaluation of the relationship between PRI and either LUE or photosynthetic activity. In particular, studies that contrast short-term (e.g. diurnal) vs. long-term (e.g. seasonal) pigment, PRI, and photosynthetic responses in contrasting vegetation types are needed.

Keywords: Chlorophyll, Carotenoids, Anthocyanin, Xanthophyll cycle

1. Introduction

The photochemical reflectance index (PRI) was originally derived as an optical indicator of xanthophyll cycle activity and photosynthetic light-use efficiency (LUE) over diurnal time scales (Gamon et al., 1992, 1993; Peñuelas et al., 1995). More recently, a large body of research has demonstrated that PRI is also strongly influenced by pigment pool sizes, particularly in studies spanning seasons (Stylinski et al., 2002; Filella et al., 2009), multiple species (Sims and Gamon, 2002; Garrity et al., 2011; Gamon and Berry, 2012) or canopy positions (Gamon et al., 2001; Gamon and Berry, 2012). These two facets of PRI that reflect the rapid xanthophyll cycle activity and the slower, seasonal or ontogenetic pigment pool size adjustments have been termed the “facultative” and “constitutive” responses, respectively (Gamon and Berry, 2012; Wong and Gamon, 2015A, 2015B). Both are of interest because they represent physiological adjustments of photosynthesis to prevailing environmental conditions, one over short (minutes or diurnal) time spans, and

another over long (ontogenetic or seasonal) time spans. Both have been shown to correlate with light-use efficiency, but the mechanistic explanations of these correlations are likely to be different (Sims and Gamon, 2002; Solovchenko, 2010; Gamon, 2015; Gitelson et al., 2017). This dual nature of PRI along with different adjustments of the photosynthetic pigments occurring over disparate time scales has led to considerable confusion in the PRI literature. Most remote sensing studies of PRI have not directly tested mechanism at the level of pigment responses, assuming implicitly that the xanthophyll cycle operation is the primary driver of the changing PRI signal.

The issue of the underlying mechanism(s) driving PRI responses has a larger significance. While the majority of studies report positive PRILUE correlations for individual circumstances (Garbulsky et al., 2011), some studies suggest that the PRI-LUE relationship is not constant across all locations (Nichol et al., 2002; Goerner et al., 2011) and ontogenetic stages (Solovchenko et al., 2010), and it is likely that the different underlying mechanisms discussed above

could be partly responsible. Understanding the mechanistic relationships between PRI, LUE, and photosynthetic activity across a range of time scales is clearly important for developing accurate, predictive photosynthesis models from remote sensing approaches involving pigment-based reflectance indices.

Much of the recent carotenoid (Car) research in higher plants has focused on the role of the Car involved in operation of xanthophyll cycle pigments (specifically, violaxanthin, antheraxanthin and zeaxanthin) in the regulation of light energy conversion and photoprotection under stress. This “downregulation” of photosynthesis is balanced against the need for light harvesting and photochemical utilization, served predominantly by lutein and, to a certain extent, β -carotene. Another photoprotective function is served by Car and other pigments that are not directly involved in the xanthophyll cycle or core photosynthetic light reactions but operate mainly via light screening (for reviews, see Solovchenko, 2010, 2013). All of these pigments can influence the vegetation reflectance spectra (and hence PRI). It is conceivable that the PRI response would reflect the diversity of functional roles of Car in plants, particularly in the context of different plant types having different pigment responses.

Recently, Garbulsy et al. (2011) and Gamon (2015) have proposed that the PRI-LUE relationship should vary not only with time scale, but also with vegetation type. According to this view, particularly in annual or deciduous species where green canopy display drives the seasonal photosynthetic response, PRI should be driven primarily by pigment transformations associated with ontogeny and senescence as modulated by environmental conditions, and not the xanthophyll cycle per se. On the other hand, over short time scales in evergreens, where green canopy display changes little, and the chlorophyll (Chl) content is relatively stable, PRI is likely to more closely follow changes in photosynthetic activity mediated in part by rapid operation of xanthophyll cycle. However, across seasons, the PRI changes are more likely to reflect Car/Chl ratio shifts (Wong and Gamon, 2015a and 2015b). This predicted complementary behavior of functionally different vegetation types recently called the “complementarity hypothesis” (Gamon, 2015) offers a framework for evaluating PRI responses across different vegetation types, but has not yet been fully tested. Since it suggests that the role of pigment pool sizes in driving PRI should vary with vegetation type, condition, and time scale in ecologically significant ways, it provides an ecological and evolutionary framework for testing our understanding of PRI responses across multiple ecosystems and biomes.

One way to examine these different perspectives is through the concept of pigment stoichiometry. In many cases, the ratios of Car/Chl pigment pools seem to be largely invariant suggesting accessory light harvesting and its regulation as a predominant functional role for the Car closely associated with the photosynthetic apparatus (Féret et al., 2011). On the other hand, stresses that limit photosynthesis often increase Car/Chl ratios (see Solovchenko, 2010 and references therein). Thus temporal changes in the stoichiometry of plant pigments can give an insight into their functional roles, and stoichiometric variation can be detectable with PRI (Wong and Gamon, 2015A and B). In the context of this study, “stress” refers to any condition that leads to an excess of radiation beyond what can be used for photosynthesis. Conditions leading to such stress can include temperature extremes, drought, or nutrient stress. A well-documented short-term response of leaves to stress is the conversion of violaxanthin to zeaxanthin affecting PRI. In the long term, leaves respond to stress by changing the pigment levels and ratios that we refer to as constitutive changes.

A further reason for examining the PRI-pigment relationship is that the LUE model (a primary modeling framework for relating PRI to photosynthetic activity) can be parameterized or defined in multiple ways, and this has implications for the interpretation of PRI. For example, some authors have defined the LUE based on incident radiation, and some on absorbed radiation, while others define it

solely on the radiation absorbed by green (photosynthetic) canopy material (see references in Gitelson and Gamon, 2015). These different definitions can have significant consequences for the conclusions that can be drawn regarding the function of PRI, a topic that has added to the confusion in the PRI literature. Changes in canopy structure and/or pigmentation occurring over a growing season, can lead to the conclusion that LUE, defined or measured via PRI, is changing seasonally, when actually these changes in *apparent LUE* are being driven by gradual changes in canopy structure and pigmentation associated with plant development, stress acclimation or senescence, among other factors. Functionally, these changes in PRI due to pigment pools and canopy structure are distinct from activity of the xanthophyll cycle that is associated with PSII LUE or non-photochemical quenching (NPQ; Horton, 2014), and they may or may not coincide with this activity. Without further information to constrain the problem, PRI by itself cannot resolve these underlying mechanisms.

The goal of this study was to quantify the relationships between leaf level PRI and pigment pools, focusing on the constitutive role of pigments in influencing PRI over seasonal or ontogenetic time frames. A growing body of evidence suggests a primary role for pigment pools in driving the seasonal PRI responses. However, most studies have been done in evergreens and very few (if any) papers have explored the link between pigment (chlorophyll, carotenoid and anthocyanin) contents and their composition in deciduous species and managed crops (see Garbulsy et al., 2011 for review). The purpose was to re-evaluate the role of PRI as an indicator of pigment (chlorophyll, carotenoid and anthocyanin) pools, and hence photosynthetic activity, across a range of tree and crop species.

2. Methods

2.1. Plant material

Juvenile, mature and senescent leaves of Norway maple (*Acer platanoides* L.) and horse chestnut (*Aesculus hippocastanum* L.) were collected in a park at Moscow State University campus in the spring, summer and fall of 1992–2000 (Table 1). Second-flush beech (*Fagus sylvatica* L.) leaves grown on the University of Karlsruhe campus were taken in August and September 1996. Leaves were visually selected according to their difference in color. Leaves healthy and homogeneous in color without anthocyanin (AnC) pigmentation or visible symptoms of damage were used in the experiments (Table 1, details are in Gitelson and Merzlyak, 1997; Gitelson et al., 2003).

Table 1. Pigment content (in mgm^{-2}) and Car/Chl ratio in tree leaves studied; *n* is the number of leaves in each data set.

	Min	Max	Mean
Chestnut <i>n</i> = 27			
Chl	117.90	562.50	346.39
Car	30.85	83.20	55.30
Car/Chl	0.09	0.46	0.20
Beech <i>n</i> = 37			
Chl	144.45	675.00	431.62
Car	43.51	137.18	91.55
Car/Chl	0.15	0.44	0.24
Maple (<i>AnC-free</i>) <i>n</i> = 61			
Chl	3.00	570.00	189.16
Car	14.60	82.60	41.18
Car/Chl	0.09	0.50	0.16
Maple (<i>AnC-containing</i>) <i>n</i> = 23			
Chl	103.2	399.8	268.7
Car	29.81	101.9	55.67
AnC	10.73	298.34	130.1
Car/Chl	0.11	0.69	0.21

Anthocyanin-containing leaves of Norway maple were collected in the park at Moscow State University campus (1992–2000) in spring and fall. In maple, the red pigmentation determined by the presence of anthocyanins was especially expressed during cold seasons. The anthocyanin pigmentation was normally observed in sunlit leaves whereas shaded leaves lacked the anthocyanin pigmentation being green to yellow in color (details are in Gitelson et al., 2001). Growing conditions of tree species were typical for these species in the regions - Moscow, Russia (maple and chestnut), and Karlsruhe, Germany (beech).

Maize (*Zea mays* L.) and soybean (*Glycine max* L.) leaves (Table 2) were collected in irrigated and rain-fed sites at Mead Nebraska (USA) in 2003 through 2005 with varying weather conditions. Leaves were collected across the growing season in maize and in the middle of the reproductive stage in soybean when wide variability of pigment content occurred (Ciganda et al., 2009). To study temporal change in leaf pigment content, leaves were collected across the growing season at the rain-fed maize site in 2003 with a period of drought lasted from the 190th through 220th day of year, DOY (Peng et al., 2011). For more detail see Gitelson et al. (2005, 2006).

2.2. Pigment analysis

The leaf pigment content was determined from the same leaf samples that were used for reflectance measurement. Leaf disks were cut from the leaves and extracted with 100% acetone or methanol by grinding the disks in a mortar and a pestle. The pigment homogenates were centrifuged for 3–5 min at 3000g in glass tubes to obtain the fully transparent supernatant which was immediately assayed spectrophotometrically. Specific absorption coefficients of Chl *a*, Chl *b* and total carotenoids reported by Lichtenthaler (1987) were used. The accepted average molecular weight of carotenoids was 540 g mol⁻¹.

Anthocyanin content was determined after extract acidification with concentrated HCl. Absorbance at 530 nm was corrected for pheophytin contribution: pheophytins *a* and *b* were obtained from corresponding chlorophylls (Fluka Chemie AG) and their absorption coefficients at 530 nm in acid methanol were found to be 8.17 and 6.35 mM⁻¹ cm⁻¹, respectively. Anthocyanin absorption coefficient of 30 mM⁻¹ cm⁻¹ at 530 nm (Strac and Wray, 1989) was used (details in Gitelson et al., 2001). Pigment content was expressed on leaf area basis in mg m⁻² (Tables 1 and 2).

2.3. Reflectance measurements

Adaxial reflectance (ρ) spectra of the leaves were taken in a spectral range between 400 and 800 nm with a spectral resolution of 2 nm with a Hitachi 150-20 spectrophotometer equipped with a 150-mm integrating sphere for reflectance measurements (maple and chestnut leaves), a Shimadzu 2101 PC spectrophotometer equipped for reflectance measurements with an integrating sphere (beech leaves). Maize and soybean leaf reflectance spectra were measured using an Ocean Optics radiometer (US92000, Ocean Optics, Dunedin FL, USA) with an Ocean Optics LS-1 light source with a Mini Leaf Clip (UNI501, PP Systems, Amesbury MA, USA) with a 2.3-mm diameter bifurcated fiber-optic attached to

Table 2. Pigment content (in mg m⁻²) and ratio in the crop leaves studied; *n* is the number of leaves in data set.

	Min	Max	Mean
Maize <i>n</i> = 20			
Chl	2.80	775.21	229.21
Car	45.53	143.16	64.61
Car/Chl	0.18	18.84	0.24
Soybean <i>n</i> = 20			
Chl	105.33	859.05	424.01
Car	43.81	174.46	88.30
Car/Chl	0.19	0.45	0.21

both radiometer and light source. In each leaf at least five reflectance spectra were measured and median value of reflectance was used for analyses. A 99% reflectance standard (Spectralon, Lab-Sphere, North Sutton NH, USA) was scanned before and after each leaf measurement. The reflectance was calculated as the ratio of upwelling leaf radiance to the upwelling radiance of the standard.

3. Results and discussion

3.1. Chlorophyll vs. carotenoid content relationship

Three main pigment groups, Chl, Car, and AnC, affect reflectance at the two wavelengths used for PRI. To quantify the effect of the pigments on PRI, we firstly analyzed PRI-Chl and PRI-Car relationships in AnC-free leaves. It is often difficult to discern the specific behavior of these relationships against the background of an inherently close correlation between Chl and Car content, because Chl and primary (photosynthetic) Car are bound in the pigment-protein complexes of the photosynthetic apparatus according to a conserved stoichiometry that is often species-specific. At the same time, absolute amounts of these pigments and their ratios can be adjusted within genetically programmed limits in response to environmental stimuli. By contrast, pigments that do not participate in assimilation of light energy (exemplified by secondary Car) are not so strictly controlled genetically and may vary over a much wider range e.g. during senescence and/or under stress (Green and Durnford, 1996; Young, 1993).

For the species studied, the Chl-Car relationships were very close ($p < 0.001$), with R^2 above 0.7 for leaves of the three tree species and above 0.94 for both crops (Fig. 1 for beech and maize). A similar close relationship ($R^2=0.86$) was found in seventeen independent datasets including a wide range of leaf spectral, chemical, and structural properties collected all over the world (Féret et al., 2011). This largely conserved stoichiometry has led to the perspective of an invariant Chl/Car ratio in many modeling studies (Jacquemoud and Baret, 1990).

PRI uses reflectance at 531 nm in the so-called green edge and at 570 nm in the green range of the spectrum. The green edge is a transition region where in situ absorption by Chl *a* and *b* and different Car drops sharply from a high value at 480 nm to an almost negligible amount above 531 nm (Gitelson et al., 2002). Reflectance in the green edge range is governed by Chl *a*, Chl *b*, Car and AnC absorption. In some studies, the reflectance at 570 nm was invariant with respect to Car content due to very small (if any) Car absorption (Lichtenthaler, 1987; Gitelson et al., 2002; Féret et al., 2008, 2011). Thus, it was expected that PRI in AnC-free leaves would be affected by both Car and Chl pigments, and we found significant PRI-Chl and PRI-Car relationships in all tree and crop leaves studied (Fig. 2 for beech and maize). PRI-Chl relationships were closer than PRI-Car (R^2 was 0.73 for Chl vs. 0.47 for Car in beech, and 0.62 vs. 0.53 in maize, respectively; $p < 0.001$). Multiple

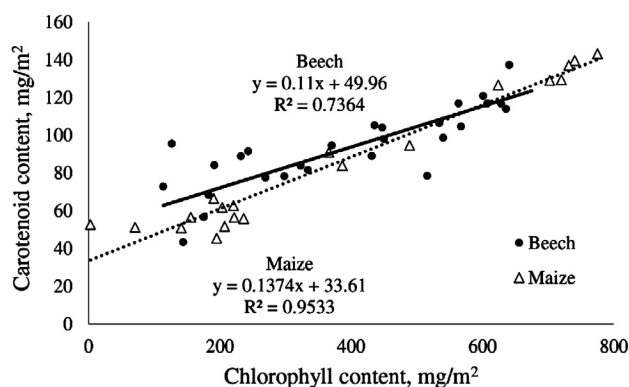


Fig. 1. Relationships between carotenoid and chlorophyll contents in beech and maize leaves.

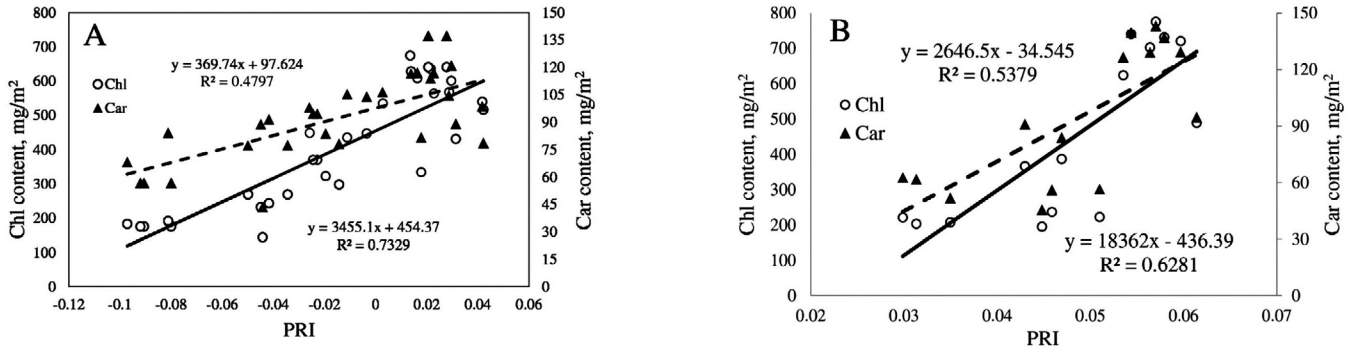


Fig. 2. Relationships between PRI and pigment contents in beech (A) and maize (B) leaves.

regression analysis of PRI vs. Car and Chl relationships showed that both pigments together were responsible for >74% of PRI variation in maize and 76% in beech.

To illustrate how the deviation of points from Chl-PRI and Car-PRI relationships (Fig. 2) is affected by the close Chl-Car relationships (Fig. 1), we plotted residuals of the PRI-Chl relationship versus residuals of the PRI-Car relationship (Fig. 3). The relationships between residuals were very close for both tree and crop leaves and, as for the Chl-Car relationship (Fig. 1), R² was higher for crop than for tree leaves. Relationships for tree and crop leaves were very close to each other with R² above 0.79 for tree leaves and above 0.95 for crops.

The reasoning for such close relationships may be the tightly related Chl and Car contents that often vary synchronously during ontogeny and senescence. Since both pigments affect reflectance at 530 nm, PRI-pigment relationships are quite scattered (Fig. 2), but the deviations from best-fit functions (i.e., the residuals) of Chl-PRI and Car-PRI relationships are inherently linked. In other words, if a certain Chl-PRI data point departs from a linear trend, the corresponding Car-PRI data point has a high probability of diverging from its corresponding linear trend by a certain value which is ‘hardcoded’ by the close Car-Chl relationship. The absolute deviation of these departures may be different, but their ratios show a similar strength as the Chl-Car relationship. Thus, the close relationship shown in Fig. 3 is an inevitable consequence of close Car-Chl relationship for these particular species and growing conditions (Fig. 1). However, a close Car-Chl relationships might not necessarily hold for the wide range of leaf traits, species, or conditions existing in nature or under all cultivation conditions. As could be seen in Fig. 1, the tree leaves studied did not show as tight a stoichiometry as crop leaves, suggesting that ontogenetic and environmental factors may be at play (Wright et al., 2004), suggesting that pigment ratios were responding in a different way to the tight stoichiometric ratios seen in crops, as further discussed below.

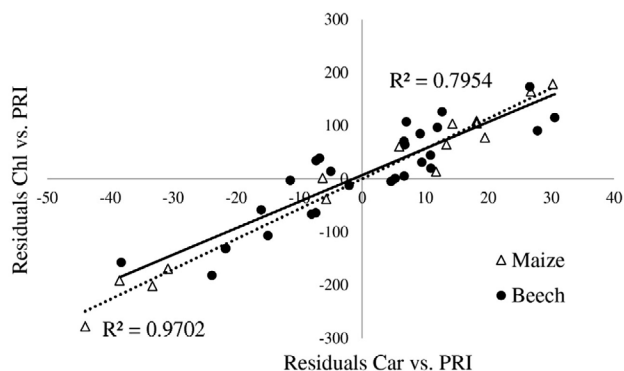


Fig. 3. Relationships between residuals of Chl vs. PRI and Car vs. PRI relationships for beech and maize leaves.

3.2. Departure from a strong stoichiometric relationship

Reasons for deviations from the main Car/Chl trend could be divided, based on time scale, into long-term and short-term responses, and many of these reflect a leaf’s response to the amount of excess energy (Bilger et al., 1989; Björkman and Demmig-Adams, 1994; Gamon, 2015). Over longer periods, acclimation to elevated solar irradiances have been known to increase the Car/Chl ratio due to decline in Chl content against the background of Car retention (Young, 1993). Shifts in the Car/Chl ratio over long time scales in response to environmental conditions (Wong and Gamon, 2015A), or during senescence-induced Car transformation (Merzlyak et al., 1999; Gamon et al., 2001), lead to gradual spectral changes that influence the PRI signal. Furthermore, differences in the amount of excess radiation can influence the composition of distinct Car having slightly different spectral properties (Britton, 1995) and thus affect the PRI-Car relationship. Thus, the change in the proportion between light-harvesting (e.g. lutein) and photoprotective Car (e.g. violaxanthin cycle xanthophylls) might also displace both the Car/Chl ratio and PRI.

Over long time spans, additional disturbing factors include the accumulation of so called “secondary carotenoids” and/or acclimatory rearrangements of the pigment apparatus. In contrast to “primary” or photosynthetic Car, which are tightly linked with photosynthetic apparatus (PSA), being involved in light harvesting and photoprotection, secondary Car are structurally and functionally uncoupled from PSA (Young, 1993; Solovchenko, 2010). As a result, secondary Car do not obey the strict stoichiometry linking the primary Car and Chl contents, and this appears to be the case for the deciduous tree leaves. In contrast, very close Car-Chl relationships evident in crops (Fig. 1) indicate leaves with a negligible content of secondary Car. This situation is clearly illustrated by the temporal behavior of Chl content, Car/Chl ratio and PRI as a function of day of year, DOY (Fig. 4) in rain-fed maize site during 2003 with period of drought around DOY 180-200 (Peng et al., 2013). The PRI closely followed the increase in Chl early in the growing season as well as the decrease of Chl in the reproductive stage beyond DOY 210. However, in a period of decrease in Chl content due to water deficiency (DOY 180-205) PRI did not follow Chl content. Due to the very close relationship between Chl and Car (R² above 0.97, $p < 0.0001$), the Car/Chl ratio in this period of stress increased very slightly. It underlines the limitation of using seasonal PRI changes as an indicator of plant stress in cases where there is a strict stoichiometry of Chl and Car.

In contrast to crops, where the Car-Chl ratio is conserved, we observed a much weaker Car-Chl relationship in tree leaves (Fig. 1), most likely due to an increased accumulation of secondary Car. The chloroplasts of beech leaf cells (Steinmüller and Tevini, 1985; Tevini and Steinmüller, 1985) as well as number of other plant species (Merzlyak et al., 2005a, 2005b, Han et al., 2003, Hormaetxe et al., 2005) often contain lipid inclusions serving as a depot for secondary Car.

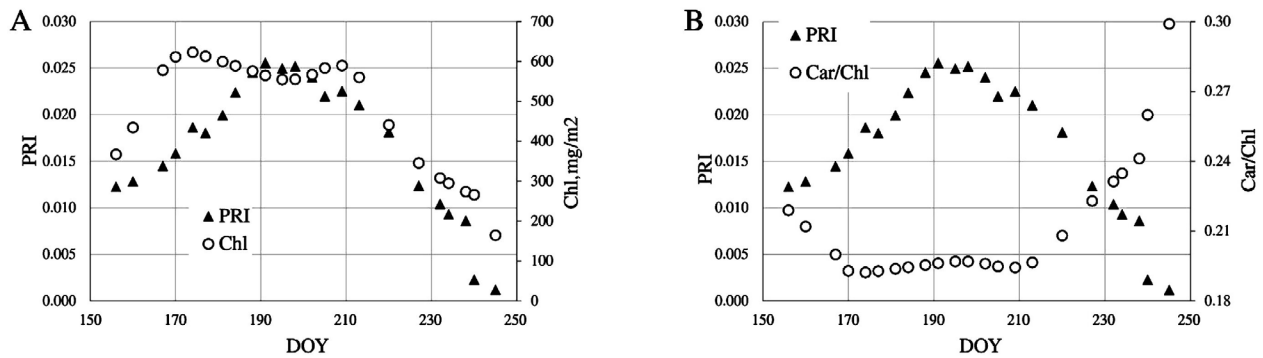


Fig. 4. Temporal change of PRI and Chl content (A) and PRI and Car/Chl ratio (B) in leaves collected at maize rain-fed site in year 2003 with drought event between day of year (DOY) 190-220.

Due to the close relationship between the residuals (Fig. 3), it is not surprising that PRI was found to be closely related to the Car/Chl ratio (Fig. 5). As a result of the strong stoichiometric relationship between Chl and Car for crops (Fig. 1), the range of PRI variation was narrow and the slope of the PRI vs. Car/Chl relationship was very small (Fig. 5A). By contrast, the PRI vs. Car/Chl relationship for tree leaves illustrates how the departure from this strong stoichiometric relationship affects the sensitivity of PRI to Car/Chl ratio (Fig. 5B). The PRI sensitivity to Car/Chl in tree leaves was higher than in crops, which emerges as a steeper slope of the PRI vs. Car/Chl relationship.

In two crops (soybean and maize) having different photosynthetic pathways (C3 vs. C4) and related leaf structure, the slopes of PRI vs. Car/Chl relationships were quite close to each other. In contrast, the slopes of PRI vs. Car/Chl relationships for three unrelated tree species were different depending on the closeness of Car vs. Chl relationships and the dynamic range of each pigment content (Fig. 5B). Despite these differences, a significant uniform the PRI vs. Car/Chl relationship was formed for three tree species with PRI explaining >73% ($p < 0.05$) of Car/Chl variation (Fig. 5C). The same was the case for crop leaves also forming a uniform (but distinct from the trees leaves with twofold smaller slope) significant PRI vs. Car/Chl relationship where PRI explained about 50% ($p < 0.05$) of Car/Chl variation (Fig. 5C).

Rahimzadeh-Bajgiran et al. (2012) evaluated PRI for assessing plant photosynthetic performance throughout the plant life cycle in eggplant. PRI was significantly correlated with plant pigments estimated via vegetation indices (VI) retrieved from leaf reflectance. They found that photosynthetic capacity and steady-state PRI could be better explained by Chl content than by xanthophylls, arguing that PRI reflects pigment content over long time spans. To test how the VIs used in their study relate to leaf pigment content, we calculated the VIs (Gitelson et al., 2001, 2002, 2003) using measured reflectance and compared them to analytically determined pigment contents. The relationships were very close with $p < 0.0001$ (Fig. S1). Our findings on the dominant role of pigment

pools over long time spans closely correspond to Rahimzadeh-Bajgiran et al. (2012) results taken at different species.

Thus, in AnC-free tree and crop leaves PRI was related to both Chl and Car contents, but much more closely to the Car/Chl ratio. The sensitivity of PRI to Car/Chl depends strongly on the fidelity (i.e. stoichiometry) of the Chl/Car relationship. These processes might not all be immediately or equally linked with LUE and this may confound the interpretation of PRI in remote sensing studies that have often assumed a single mechanism of PRI response linked to the xanthophyll cycle. These findings illustrate the importance of assessing leaf-level pigment levels when evaluating PRI or other similar vegetation indices. Variation in PRI-LUE responses in remote sensing studies spanning dates or species could in fact reflect different functional roles of the pigments influencing PRI, leading to misinterpretation of the underlying function. Profound changes in Car/Chl ratio and Car composition take place during ontogenesis during the maturation and senescence of the leaf photosynthetic apparatus (Merzlyak et al., 1998; Lichtenthaler and Babani, 2004). This trend of Car/Chl ratio is genetically programmed and further modulated by acclimation to long-term stresses (Solovchenko et al., 2006; Lichtenthaler and Babani, 2004). Similarly, Car/Chl ratios, and hence PRI values, vary with canopy position and irradiance (e.g., Hilker et al., 2008; Gamon and Berry, 2012). These processes reflect ontogenetic and acclimatory responses to prevailing conditions, and obviously exert a dominant contribution to the changes in PRI observed over seasonal timescales. What is less clear (and requires further study) is how closely PRI reflects underlying photosynthetic activity or LUE across this range of pigment responses, and to what degree the varying PRI-LUE relationships often reported in the literature (Nichol et al., 2002; Goerner et al., 2011) reflect these different mechanisms.

Behavior of PRI in AnC-containing leaves was very different from that in AnC-free leaves. Anthocyanins absorb in situ in a wide range around 550 nm (Gitelson et al., 2001; Peters and Noble, 2014) affecting reflectance at both wavelengths used in PRI. In AnC-containing leaves, reflectances at 530 and 570 nm are

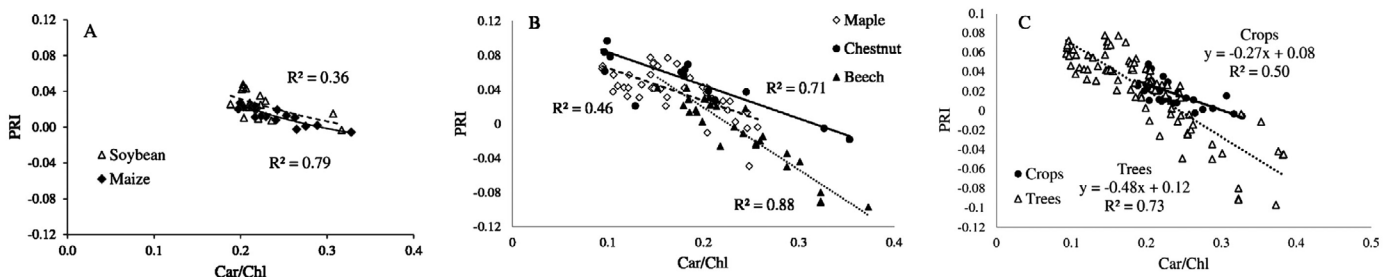


Fig. 5. Relationships between PRI and Car to Chl ratio in crop (A) and tree (B) leaves. Also shown are summary relationships comparing all crops and trees investigated (C) where PRI explained 73% of Car/Chl variation in crops and 50% in trees ($p < 0.05$).

governed mainly by AnC absorption (i.e., AnC content). When AnC and Chl contents did not relate closely (as in our data), *there was no significant relationship between PRI and any pigment (Chl, Car and AnC) content as well as with Car/Chl* (Fig. S2). The disturbance of the close Car/Chl relationship upon appearance of AnC can be explained by a strong photoprotective effect of anthocyanins (Hattier and Gould, 2009; Gould, 2004). When AnC accumulated in appreciable amounts, it takes over the photoprotective function of Car resulting in a weakening of its tight relationships with PRI. The presence of anthocyanins led to a strong interference of AnC absorption with the PRI-Car/Chl relationship, and indicates an additional leaf photoprotective strategy from the xanthophyll cycle that is available to some species (e.g. *Quercus* spp. Gamon and Surfus, 1999). Additional studies are needed to further elucidate alternative leaf strategies for photoprotection over different time scales and their overall effects on leaf optical signals.

4. Conclusions

Analysis of *seasonal constitutive changes* in foliar pigment content and composition of three tree species and two crop species lead to the following conclusions.

- In anthocyanin-free leaves, PRI related to both total Chl and Car content. Each pigment (Chl and Car) content explained from 43 to 73% of PRI variation. Both pigments together were responsible for >74% of PRI variation.
- In the tree and crop leaves, PRI was closely related to the Car/Chl ratio over long time spans, most likely affecting physiological and ontogenetic factors.
- PRI vs. Car/Chl relationships for leaves of three different tree species formed a significant uniform relationship with PRI explaining >73% of Car/Chl variation.
- The crop leaves displayed a strict stoichiometric relationship between Chl and Car, and also formed a significant PRI vs. Car/Chl relationship (distinct from that observed in the trees) where PRI explained about 50% of Car/Chl variation.
- The sensitivity of PRI to Car/Chl in the tree leaves was at least 2-fold higher than in the crops. It was boosted by less close Chl vs. Car relationships in the tree leaves stemming likely from the increased contribution of secondary carotenoids.
- In anthocyanin-containing leaves, PRI did not clearly relate to any pigment content due to the effect of AnC absorption on reflectance at both PRI wavelengths. This indicates both a confounding effect of anthocyanins on reflectance and an alternative leaf photoprotective strategy.

These findings are consistent with other recent research in other vegetation types indicating that constitutive pigment effects, not the xanthophyll cycle, are the primary cause of PRI variation over long time spans, and illustrate the importance of assessing leaf pigments when evaluating vegetation indices. Finally, the strong link between leaf-level PRI and Car/Chl, as well as PRI and separate Chl and Car contents suggest the need for a more careful evaluation of the relationship between PRI and LUE and photosynthetic activity. In particular, studies that clarify PRI, pigments, and photosynthetic responses over short-term (e.g. diurnal) vs. long-term (e.g. seasonal) time periods for contrasting vegetation types are needed.

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Appendix A. Supplementary data — Supplementary data to this article follows the References.

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Multiple drivers of seasonal change in PRI: Implications for photosynthesis

1. Leaf level

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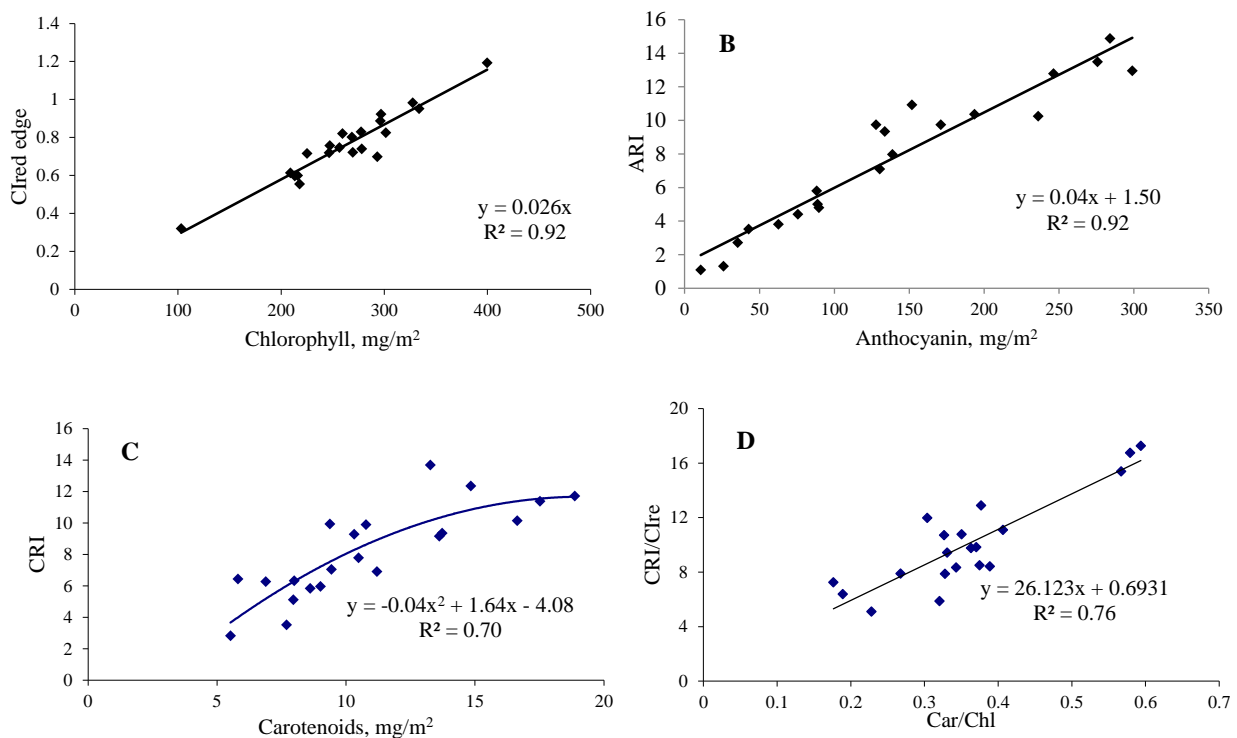


Figure S1. Relationships between leaf pigment contents (A) chlorophyll, (B) anthocyanin, (C) carotenoids, and (D) Car/Chl ratio and vegetation indices used for their non-destructive estimation. Clred edge is red edge chlorophyll index (Gitelson et al., 2003), ARI is anthocyanin reflectance index (Gitelson et al., 2001) and CRI is carotenoid reflectance index (Gitelson et al., 2002).

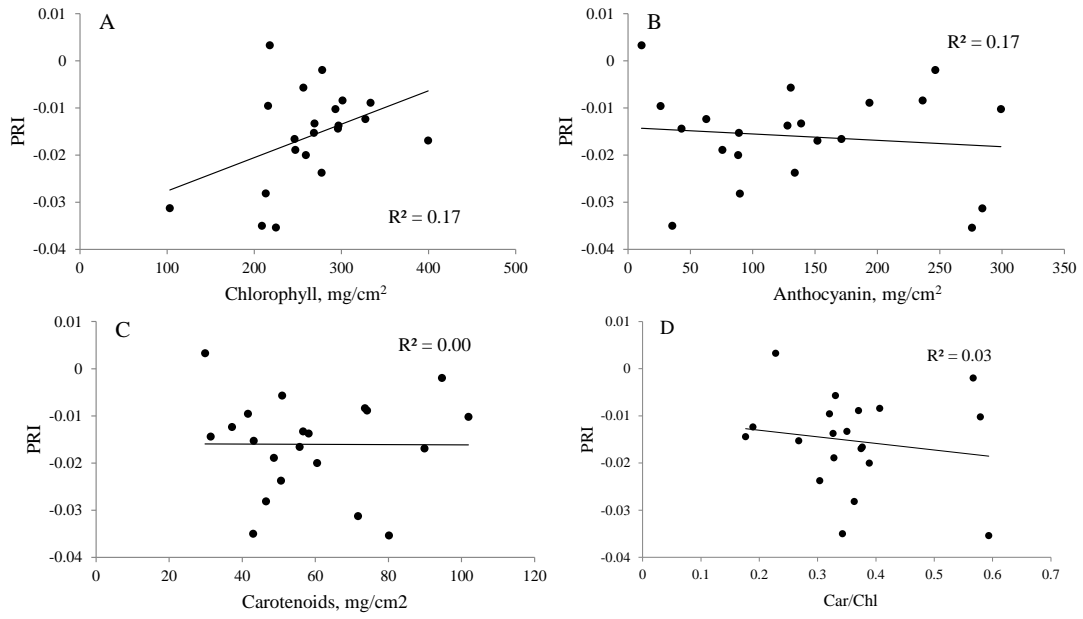


Figure S2. Relationships between PRI and pigment content (A) chlorophyll, (B) anthocyanin, (C) carotenoids, and (D) Car/Chl ratio in anthocyanin-containing maple leaves.