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# Multiple drivers of seasonal change in PRI: Implications for photosynthesis 2. Stand level

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# Multiple drivers of seasonal change in PRI: Implications for photosynthesis 2. Stand level

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## Abstract

The goal of this study was to explore the relationships between stand-level photochemical reflectance index (PRI) and canopy structure/pigment pools, as well as light use efficiency (LUE) of photosynthetically active vegetation focusing on seasonal or ontogenetic time frames. PRI was originally designed as a means of assessing the xanthophyll cycle and LUE over short (e.g. diurnal) time frames, and few studies have explored the drivers of PRI over longer, seasonal time frames, particularly in crops having different photosynthetic pathways or canopy structures. Consequently, our purpose was to understand and quantify the drivers of PRI responses over seasonal time scales for two crops, maize ( $C_4$ ) and soybean ( $C_3$ ), contrasting in photosynthetic pathway, leaf structure and canopy architecture. In both crops, PRI was very closely related to green LAI ( $R^2 > 0.90$ ) and stand chlorophyll (Chl) content ( $R^2 > 0.93$ ). The slopes of the relationships in different phenological stages, vegetative and reproductive, were substantially different (3-fold smaller in the vegetative stage). The main cause of this disparity was the high PRI value of soil/residue background. While PRI was a sensitive indicator of the changes in stand green LAI and stand Chl content over the full growing season, it was not sensitive to LUE; LUE explained below 12% of PRI variation in maize and 19% in soybean. Unlike leaf-level PRI, stand-level PRI was not clearly related to the Car/Chl ratio, presumably because the large changes in canopy structure (affecting stand Chl and green LAI) had a dominant influence on PRI over this time frame. The strong relationship between PRI and stand Chl content as well as between PRI and Chl-related vegetation index over a growing cycle allowed us to subtract the stand Chl content effect from measured PRI to reveal the component of PRI most likely related to periods of stress. However, for accurate subtraction of the Chl effect from long-term PRI records, thoughtful study of uncertainties related to “natural” variation of PRI-stand Chl relationships, and stand Chl content estimation for different varieties of the same species and for different species is required. The findings of a strong link between stand-level PRI and stand green LAI and Chl content and the lack of a clear relationship between PRI and LUE over seasonal and ontogenetic time spans suggest the need 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 to clarify the different mechanisms involved at different temporal and spatial scales. These findings have important implications for attempts to monitor photosynthetic phenology from remote sensing, many of which have relied on PRI as an indicator of photosynthetic activity.

**Keywords:** Chlorophyll, Carotenoids, Xanthophyll cycle, Leaf area

## 1. Introduction

Much of the foundational work on the photochemical reflectance index (PRI) was done on leaves and closed-canopy stands, demonstrating a strong link between PRI, xanthophyll cycle activity, and photosynthetic light-use efficiency (LUE) over diurnal time scales (Gamon et al., 1992, Peñuelas et al., 1995, Gamon et al., 1997). Expanding this interpretation of PRI to larger spatial scales and longer temporal scales has been a challenge. Several studies have compared leaf-level to canopy-level PRI and have found a close

relationship between the two for dense monocultural stands suggesting that a closed-canopy stand approximates a “big leaf” in terms of the PRI signal (e.g. Styliniski et al., 2002, Gamon and Qiu, 1999, Wong and Gamon, 2015b, Gamon, 2015). However, when expanding to the full seasonal time scale, the interpretation of PRI often remains unclear because few long-term studies explicitly compare PRI to many factors that can affect this signal (see Barton and North (2001) for examples of these potentially confounding factors). Over seasonal time scales, especially for annual vegetation that undergoes large changes in canopy structure, greening

and senescence, the seasonal change in green canopy display can have a dominant influence on the PRI signal. While relatively few long-term remote sensing studies of PRI explicitly link this index to xanthophyll cycle activity, many studies have reported a correlation between PRI and LUE (Nichol et al., 2002, Rahman et al., 2004, Drolet et al., 2005, Goerner et al., 2011, Garbulsky et al., 2011), but the exact reasons remain obscure due to the many factors that affect the PRI signal at these scales (Barton and North, 2001) and due to the different operational definitions of LUE (Gitelson and Gamon, 2015). Understanding the underlying reasons for these correlations between LUE and PRI from aircraft or satellite data is critical to implementing defensible LUE models from remote sensing that incorporate PRI.

Modeling studies (Barton and North, 2001) suggests many potentially complicating factors when trying to apply PRI to whole stands in a remote sensing context. Among them, the effects of canopy structure, including green leaf area index (LAI), the degree of canopy closure and soil background contribution to the reflectance signal are known to strongly affect PRI. Additionally, angular effects, including leaf angle distribution and sun-target-sensor sampling geometry can have a significant influence on the PRI signal and its interpretation (Barton and North, 2001, Drolet et al., 2005, Gamon, 2015). As predicted by modeling (Barton and North, 2001), previous empirical studies have found a strong correlation between PRI and green canopy cover as measured by NDVI (Gamon et al., 1995), indicating a strong influence of green canopy structure on the PRI signal. Because these structural effects also influence the overall stand photosynthetic rate, they can potentially influence the PRI-photosynthesis relationship independently of the xanthophyll cycle activity, creating an ill-conditioned situation when interpreting PRI. There are few published, long-term studies examining how PRI is affected by canopy structure over the annual growth cycle of a vegetation stand, leaving this topic relatively unexplored.

In addition to canopy structure, leaf pigmentation, which can change gradually with leaf development and senescence, clearly affects PRI (Gamon et al., 2001, Sims and Gamon, 2002, Garrity et al., 2011, Gitelson et al. — the companion paper in review). In an attempt to discern the short-term effects from the longer-term effects, Gamon and Berry (2012) classified PRI responses to pigmentation in evergreens into “facultative” (xanthophyll cycle-driven effects operating over the diurnal time scale) and “constitutive” (changing pigment pool sizes over seasonal time scales, e.g. due to ontogeny and senescence and/or in response to resource levels). Several studies have now confirmed that long-term (seasonal) PRI responses at the leaf level are primarily driven by constitutive pigment pool size changes, and not facultative xanthophyll cycle activity (Stylinski et al., 2002, Sims and Gamon, 2002, Filella et al., 2009, Gamon and Berry, 2012, Wong and Gamon, 2015a, 2015b). These studies have primarily considered evergreen responses, leaving long-term PRI responses less-well characterized

for deciduous and annual vegetation (e.g. crops). Because pigment content can also be tied to photosynthetic capacity and LUE, there may be multiple reasons why PRI often correlates with photosynthetic activity (Garbulsky et al., 2011, Gamon, 2015), creating an overdetermined situation that easily leads to misunderstanding of mechanism. Further clarification of these different pigment effects against a background of changing canopy structure is an essential step toward understanding PRI responses in a long-term monitoring campaign, particularly for deciduous and annual plants (e.g. crops) where long-term responses have been less-well studied.

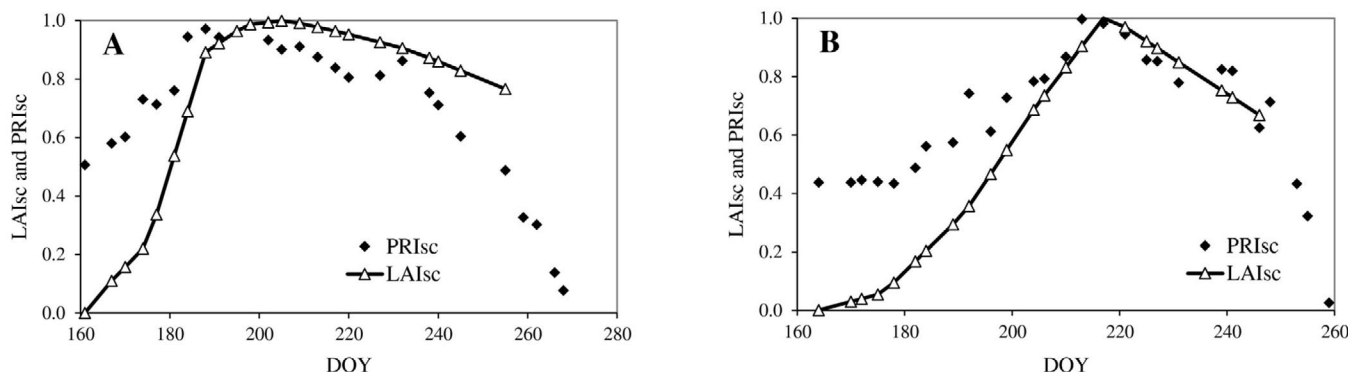
Given the potential for PRI to provide a useful metric of LUE, a full understanding of multiple confounding variables is needed. In a companion paper, we established relationships between PRI and foliar pigment content and composition (Gitelson et al., 2017). The goal of this current study at a larger scale was to explore the relationships between stand-level PRI and canopy structure/pigment pools, as well as LUE of photosynthetically active vegetation focusing on seasonal or ontogenetic time frames. The purpose was to understand and quantify the drivers of PRI responses over seasonal time scales for two crops ( $C_3$  and  $C_4$ ), contrasting in photosynthetic pathway, leaf structure and canopy architecture. A key point was to evaluate how the PRI signal over the growing cycle is influenced by changes in canopy structure and pigment pools associated with changing crop phenology and physiology and compare it with seasonal change in LUE.

**2. Methods**

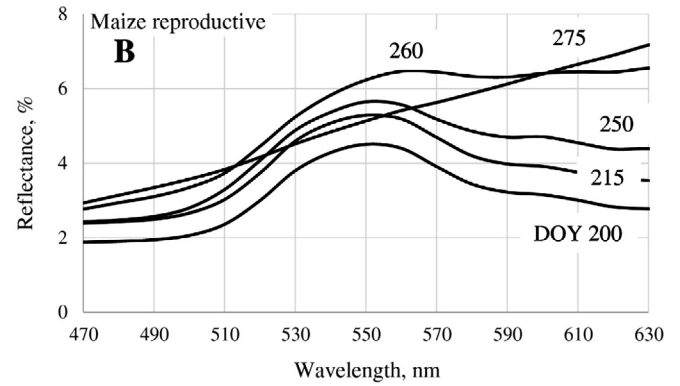
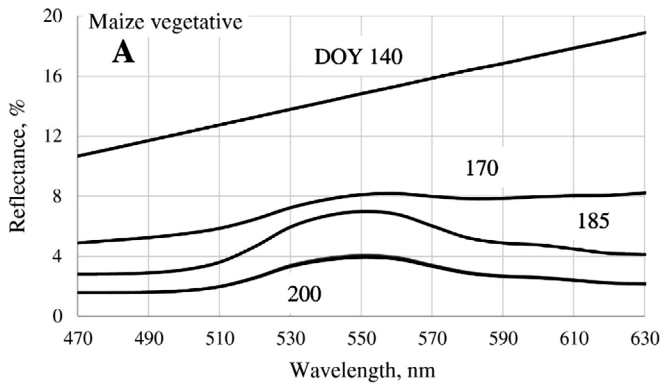
The study site was located at the University of Nebraska-Lincoln Agricultural Research and Development Center near Mead, Nebraska. This study site consists of three 65-ha fields. Each field was managed as either continuous irrigated maize, irrigated maize/soybean rotation, or rain-fed maize (*Zea mays*)/soybean (*Glycine max*) rotation following the best management practices (e.g. fertilization, herbicide/pesticide treatment) for eastern Nebraska for its respective planting cycle. There were a total of 24 field-years for maize and soybean. Maximal green LAI values ranged from 4.3 to 6.5  $m^2 m^{-2}$  for maize and 3.0 to 5.5  $m^2 m^{-2}$  for soybean (details are in Verma et al., 2005 and Viña et al., 2011).

*2.1. Incoming PAR and fraction of radiation absorbed by photosynthetically active vegetation*

In each study site quantum sensors were placed to collect hourly incoming PAR ( $PAR_{in}$ ), PAR reflected by the canopy and soil ( $PAR_{out}$ ), PAR transmitted through the canopy ( $PAR_{transm}$ ) and PAR reflected by the soil ( $PAR_{soil}$ ).  $PAR_{in}$  was measured 6m above the surface by point quantum sensors (Model LI-190, Li-Cor Inc., Lincoln, Nebraska) pointing toward the sky. Daytime  $PAR_{in}$  were calculated by integrating the hourly measurements during a day from sunrise to sunset (period when  $PAR_{in}$  exceeding  $1 \mu mol m^{-2} s^{-1}$ ).



**Figure 1.** Temporal behavior of scaled (between 0 and 1) green LAI and PRI of maize (A) and soybean (B).



**Figure 2.** Canopy reflectance spectra of maize in vegetative (A) and reproductive (B) stages.

PAR<sub>out</sub> was measured at 6 m above the ground by point quantum sensors aimed downward; PAR<sub>transm</sub> was measured at about 2 cm above the ground with line quantum sensors (Model LI-191, Li-Cor Inc., Lincoln, Nebraska) pointing upward. PAR<sub>soil</sub> was measured about 12 cm above the ground with line quantum sensors pointing downward. All daily values of radiation were computed by integrating the hourly measurements during a day when hourly PAR<sub>in</sub> exceeded 1 μmol m<sup>-2</sup> s<sup>-1</sup>. Daily values of the fraction of PAR absorbed by the whole canopy (fAPAR<sub>total</sub>) were then calculated as (Viña and Gitelson, 2005):

$$fAPAR_{total} = (PAR_{in} - PAR_{out} - PAR_{transm} + PAR_{soil}) / PAR_{in}$$

To obtain a measure of the fAPAR absorbed solely by the photosynthetic component of the vegetation, fAPAR<sub>green</sub> was calculated as (Hall et al., 1992):

$$fAPAR_{green} = fAPAR_{total} \times (LAI_{green} / total\ LAI)$$

**2.2. Gross primary production, absorbed PAR and light use efficiency**

GPP was measured by the eddy covariance method. Each site was equipped with an eddy covariance tower and meteorological sensors, with which measurements of CO<sub>2</sub> fluxes, water vapor, and energy fluxes were obtained continuously. Daytime net ecosystem exchange (NEE) values were calculated by integrating hourly CO<sub>2</sub> fluxes when PAR<sub>in</sub> exceeded 1 μmol m<sup>-2</sup> s<sup>-1</sup>. Daytime ecosystem respiration (Re) was obtained from the night CO<sub>2</sub> exchange-temperature relationship (e.g., Xu and Baldocchi, 2003). GPP was then obtained by subtracting Re from NEE as: GPP=NEE - Re.

Daytime PAR absorbed by the canopy (aPAR<sub>total</sub>) was calculated as the product of fAPAR<sub>total</sub> and daytime PAR<sub>in</sub>: aPAR<sub>total</sub> = fAPAR<sub>total</sub> × PAR<sub>in</sub>. PAR absorbed by the photosynthetic component of the vegetation was calculated as aPAR<sub>green</sub> = fAPAR<sub>green</sub> × PAR<sub>in</sub>.

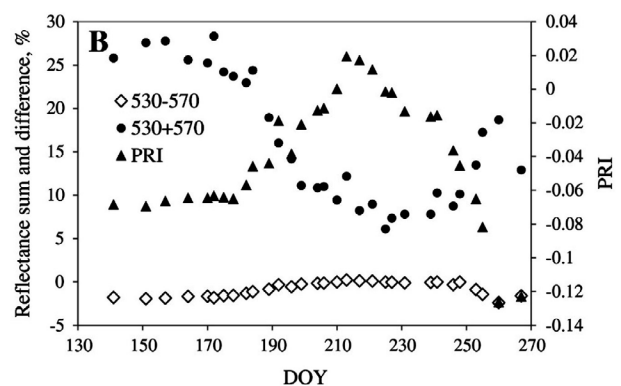
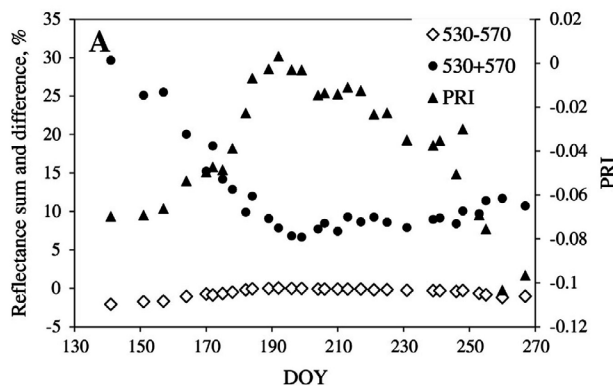
LUE of photosynthetically active vegetation (LUE<sub>green</sub>), which is a quantitative measure of the efficiency of conversion of aPAR<sub>green</sub> into fixed carbon (Gitelson and Gamon, 2015) was calculated as (Monteith, 1972; Monteith and Moss, 1977):

$$LUE_{green} = GPP / aPAR_{green}$$

**2.3. Green LAI**

Within each of three fields, six small plot areas (20 m × 20 m) were established. They represented major occurrences of soil and crop production zones within each field (Verma et al., 2005). LAI was measured from destructive samples at 10–14 day intervals during the growing seasons 2001 through 2008. On each sampling date, plants from a 1-m length of either of two rows within each plot were collected and the total number of plants recorded. Plants were kept on ice and transported to the laboratory where they were separated into green leaves, dead leaves, and litter components. All leaves were run through an area meter (Model LI-3100, Li-Cor, Inc., Lincoln, Nebraska) and the leaf area per plant was determined. For each plot, the leaf area per plant was multiplied by the plant density to obtain a total LAI. Total LAI values for the six plots were then averaged as a site-level value (details in Viña et al., 2011). Green leaves were measured in the same way to obtain the green LAI.

Total canopy Chl content was estimated as Chl = Chl<sub>leaf</sub> × total LAI, where Chl<sub>leaf</sub> is the Chl content of the upper-most collared or ear leaf in maize and upper leaf in soybean. Adaxial reflectance of the crop leaves was measured in the spectral range from 400 nm to 900 nm using a Mini Leaf Clip (UNI501, PP Systems, Amesbury MA, USA) with a 2.3-mm-diameter bifurcated fiber-optic cable attached to both a spectroradiometer (USB2000, Ocean Optics, Dunedin FL, USA) and to a tungsten halogen light source (LS-1, Ocean Optics). A 99% reflectance standard (Spectralon, LabSphere, North Sutton NH, USA) was scanned before and after each leaf



**Figure 3.** Temporal behavior of PRI and sum and difference of reflectances at 530 and 570 nm of maize (A) and soybean (B).

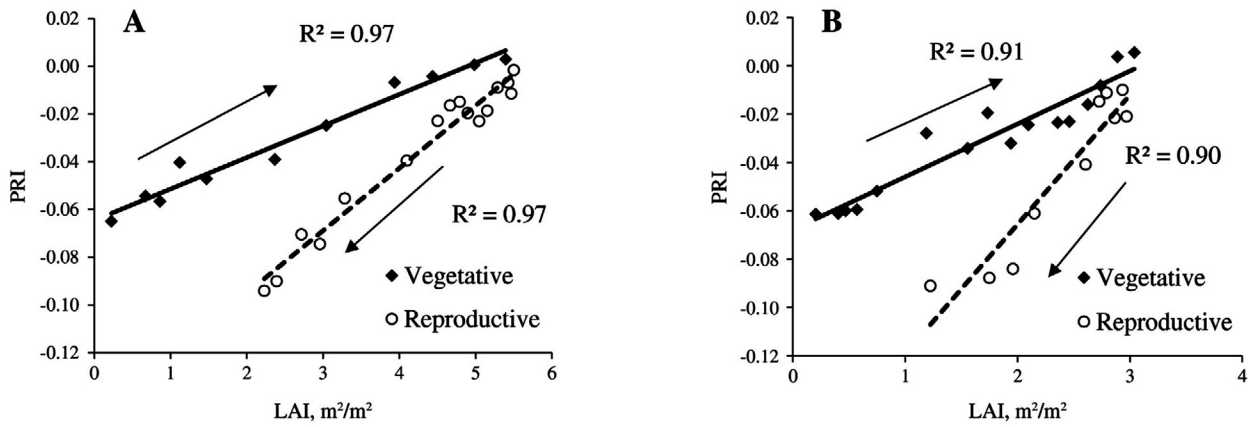


Figure 4. Relationship between PRI and green LAI in maize (A) and soybean (B).

measurement. The reflectance was calculated as the ratio of upwelling leaf radiance to the upwelling radiance of the standard (details are in Gitelson et al., 2005, Ciganda et al., 2009).

A red-edge chlorophyll index  $Cl_{red\ edge} = (\rho_{NIR} / \rho_{red\ edge} - 1)$ , where  $\rho_{NIR}$  and  $\rho_{red\ edge}$  are reflectance in the range 770–800 nm and 720–730 nm, respectively, was used for estimating leaf Chl content. RMSE of Chl content prediction by  $Cl_{red\ edge}$  was 38 mg m<sup>-2</sup> in the range of Chl content 22–880 mg m<sup>-2</sup> (details in Gitelson et al., 2003, 2006; Ciganda et al., 2009). The stand reflectance measurements and ground measurements were not always concurrent and, since LAI and leaf Chl content changed gradually, spline interpolations were taken between destructive LAI sampling dates for each field in each year using Matlab (V. 7.9.0.529, The MathWorks, Massachusetts, USA) (Nguy-Robertson et al., 2012).

The hyperspectral data were collected from 2001 to 2008 using an all-terrain sensor platform (Rundquist et al., 2004, 2014). Two spectroradiometers (USB2000 Ocean Optics, Inc., Dunedin FL, USA) were used to collect reflectance in the range 400 to 1100 nm with about 1.5 nm resolution. The down-welling fiber was fitted with a cosine diffuser to detect incident irradiance; the upwelling fiber-measured canopy radiance. A field of view of the upwelling fiber was approximately 2.4m in diameter; the distance between the fiber and the top of canopy was constant throughout the growing season. The mean of 36 reflectance spectra collected in each field was used as the stand-level reflectance measurement. A total of 278 spectra for maize and 145 for soybean were acquired in 2001 through 2008 (Viña et al., 2011; Nguy-Robertson et al., 2012, 2014).

### 3. Results and discussion

The relationships between PRI and green LAI (PRI-LAI), and PRI and stand Chl content (PRI-Chl) were investigated for two crops (maize and soybean) contrasting in photosynthetic pathways, leaf structure, and canopy architecture. The temporal behavior of PRI (scaled between 0 and 1) and green LAI for maize and soybean is shown in Figure 1. In the beginning of the growing season PRI was around 40 to 50% of its maximal value in both crops and increased as the crop developed, reaching its maximal value at the time of maximal green LAI. The main drivers of PRI in the vegetative stage appeared to be the increasing green LAI, green vegetation cover and leaf Chl content, causing a sharp decrease in reflectance at both wavelengths used in PRI,  $\rho_{530}$  and  $\rho_{570}$ , and, thus, decrease in denominator of PRI, ( $\rho_{530} + \rho_{570}$ ) (Figures 2A and 3, DOY 140 - beginning of the season, DOY 200 - top of the season). The numerator of PRI, ( $\rho_{530} - \rho_{570}$ ), played a minor role in PRI performance at this stage (Figure 3). Importantly, in both crops during canopy development in the vegetative stage the dynamic range of PRI was narrower than the dynamic range of green LAI (Figure 1). The main reason for that was the high PRI value for soil/residue (as evidenced by the reflectance values at day 140 in Figure 2A on the PRI wave bands), which contributed to the stand reflectance signal in the early growth stages. Modeling studies have also indicated high sensitivity of PRI to soil background (Barton and North, 2001).

In the reproductive stage, when leaf Chl content decreased but leaf area remained virtually invariant,  $\rho_{530}$  and  $\rho_{570}$  increased almost synchronously in accord with a decrease of foliar Chl content

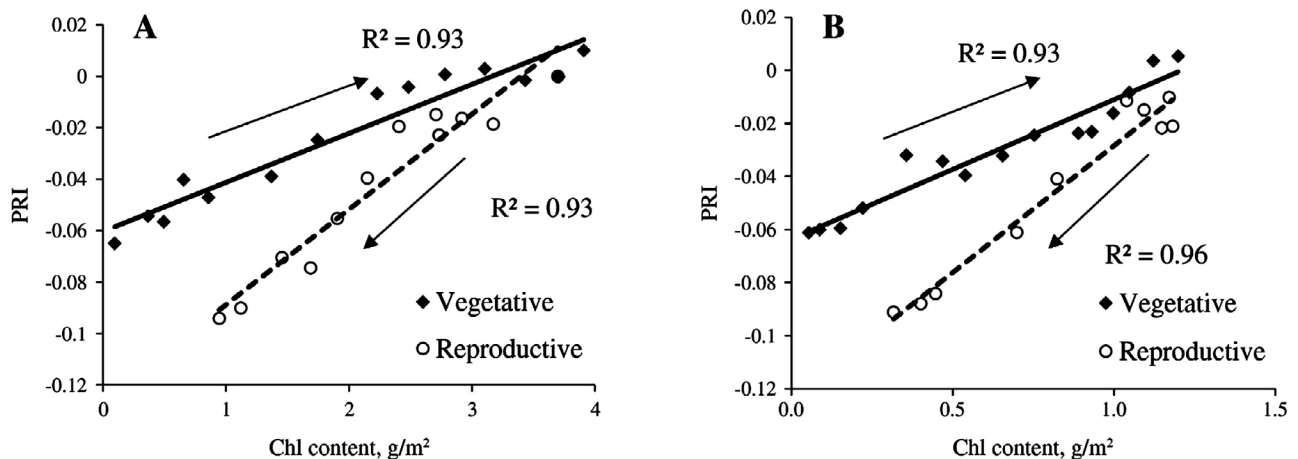
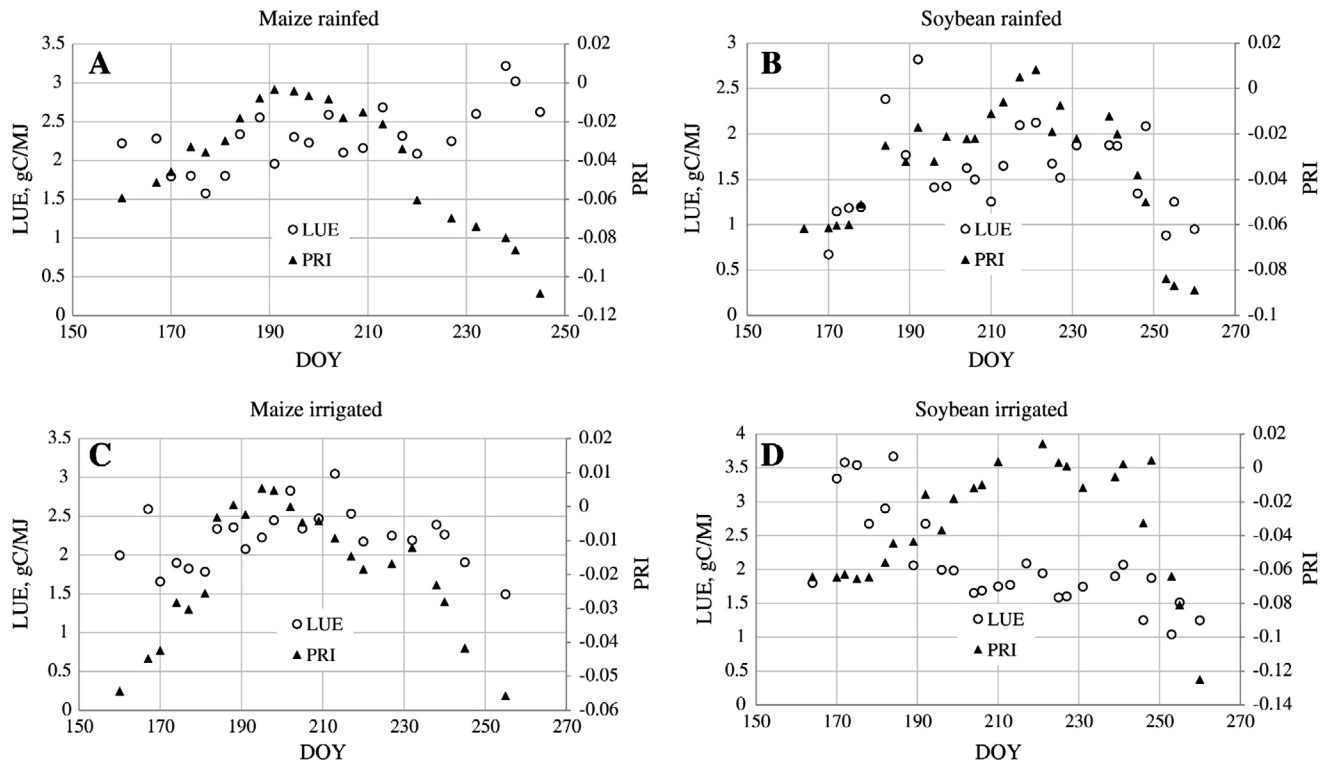


Figure 5. Relationship between PRI and stand chlorophyll content in maize (A) and soybean (B).





**Figure 6.** Temporal change in LUE and PRI at irrigated and rain-fed maize and soybean sites.

and green LAI (Figure 2B, DOY 200 through 250). So the PRI numerator ( $p530-p570$ ) remained virtually invariant while the PRI denominator ( $p530 + p570$ ) increased, decreasing PRI (Figures 1 and 3). Thus, in contrast to the vegetative stage, in the reproductive stage, the decrease of PRI was almost synchronous with a decrease of green LAI (Figure 1). In late senescence, when foliar Chl dropped sharply, carotenoids became the primary leaf pigments (Figure 2B, DOY from 250 through 270). This restricted the rate of  $p530$  increase comparable to that of  $p570$  due to a significant absorption by carotenoids at 530 nm and negligible absorption of Car at 570 nm (Lichtenthaler, 1987). Only at this stage did the numerator of PRI play a significant role in the PRI temporal behavior (Figure 3).

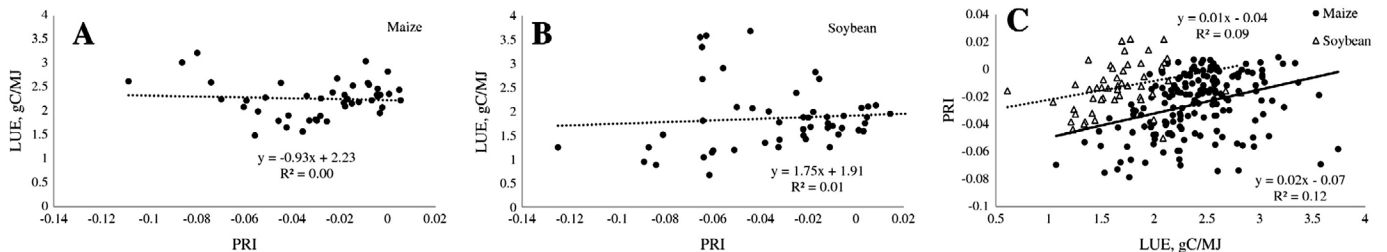
The contrasting LAI and PRI behaviors in the vegetative and reproductive stages led to hysteresis when the two were plotted against each other (Figure 4). While the PRI-LAI relationships were very close, the slope of the PRI increase in the vegetative stage was much smaller than the slope of the green LAI increase in the reproductive stage (Figures 1 and 4).

The main reason for the hysteresis was the high PRI value of soil/residue in the beginning of growing season (Figures 1, 3, 4). Soil/residue PRI was about  $-0.06$ , which corresponded to 40–50% of its maximal value (0.006), reached at the top of the season in

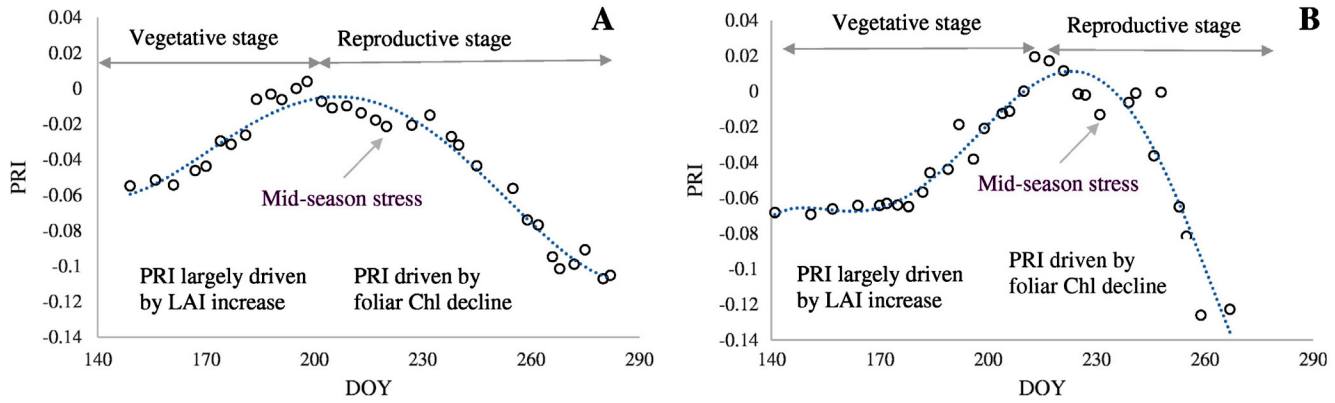
the end of vegetative stage. The minimal value of PRI at the end of the reproductive stage was around  $-0.1$ – $-0.12$ , which is much smaller than in the beginning of the season. Thus, in the vegetative stage, the PRI change was between 40 and 50% and 100% of its maximal value while, in the reproductive stage, PRI changed between 100% and 0%. Hence, the dynamic range of PRI in reproductive stage was 100% and only 50–60% in vegetative stage, while the dynamic range of green LAI was 100% in each stage. This resulted in a strong hysteresis of the PRI-green LAI relationship with the slope of PRI-LAI relationship in vegetative stage 3-fold smaller than in reproductive stage and senescence (Figure 4).

This hysteresis may be also in part due to hysteresis in the green LAI vs. stand Chl content relationship (Ciganda et al., 2008; Gitelson et al., 2014). Green LAI has been traditionally determined using a visual subjective attribute of leaf “greenness” (Ciganda et al., 2008; Viña et al., 2011). While a strong linear relationship exists between stand Chl content and green LAI obtained using this subjective procedure (Ciganda et al., 2008; Gitelson et al., 2014), the relationship leads to hysteresis between stand Chl and green LAI; for the same green LAI, stand Chl content may be much higher in the vegetative stage than in the reproductive stage.

The stand Chl content is an objective, quantitative measure of vegetation vigor that avoids the problem of subjective



**Figure 7.** LUE vs. PRI for maize and soybean irrigated and rain-fed sites during growing season 2003 for maize (A), 2002 for soybean (B). PRI vs. LUE for irrigated and rain-fed maize and soybean sites in 2001–2005 (C).



**Figure 8.** Seasonal course of PRI for irrigated maize (A) and soybean (B) showing different drivers of PRI change during the vegetative and reproductive stages. Pronounced minima in the middle of the season when pigment pools and leaf area were not variable probably indicate crop stress.

determination of “greenness”. The PRI-stand Chl relationships for maize and soybean were very close (Figure 5). Although the hysteresis was less than that of PRI-green LAI relationships (Figure 4), it still was substantial. While PRI-LAI and PRI-Chl relationships were very close, PRI-LUE relationships were weak in both species under different water availability (irrigated and rain-fed) – Figures 6 and 7. Stand Chl content explained >90% variability of PRI, and LUE explained below 0.12% in maize and 9% in soybean (Figure 7C).

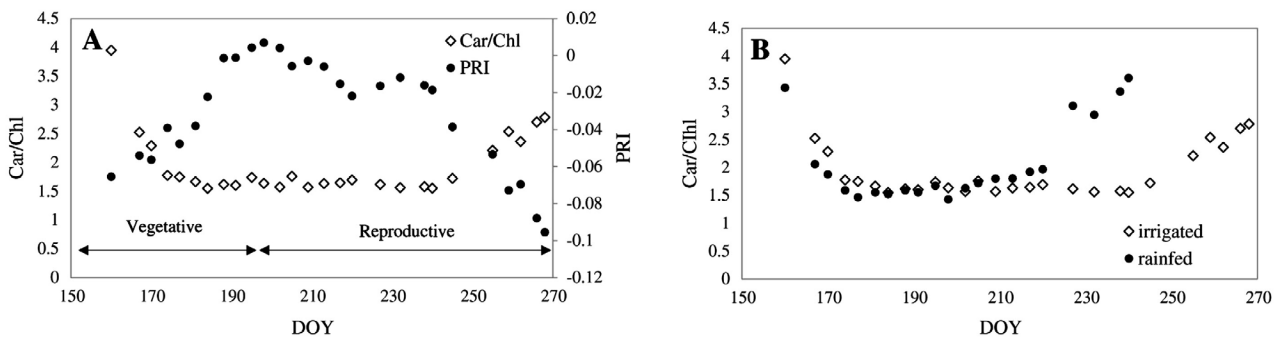
In this case, over seasonal time scales, PRI emerged more as an index of photosynthetic capacity or activity (Figures 4 and 5) than LUE (Figures 6–7) as originally discussed in the early PRI literature focusing on facultative (short-term) PRI variation associated with diurnal xanthophyll cycle activity. However, the slopes of the relationships in different phenological stages, vegetative and reproductive, were substantially different from each other, indicating that additional factors besides pigment levels alone, specifically the effect of soil/residue background during canopy development in the vegetative stage, were influencing PRI. In the vegetative stage, PRI was largely driven by increasing vegetation cover, green LAI and foliar Chl. In the reproductive and senescence stages, where canopy structure changed relatively little compared to the vegetative stage (Figure 1), PRI was strongly affected by the foliar Chl decline (Figures 1 and 8). This decline in PRI without much change in total LAI was especially pronounced in the end of the season at the senescence stage, manifesting the senescence-induced decline of leaf-level pigment pools, which was not as pronounced as in the beginning of reproductive stage in both crops (Figure 8).

In our accompanying paper, we have found that in these annual crops leaf-level PRI is strongly related to the carotenoid-to-chlorophyll ratio, Car/Chl (Gitelson et al., 2017), similar to findings in evergreen vegetation (Stylinski et al., 2002, Sims and Gamon, 2002, Filella et al., 2009, Gamon and Berry, 2012, Wong and Gamon, 2015A and B). To understand whether and how the stand level PRI relates to the

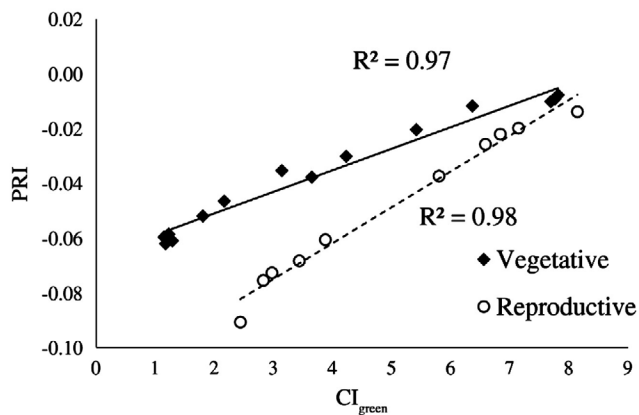
Car/Chl ratio in crops, the stand Chl content was estimated by  $Cl_{red\ edge}$  and the stand Car content by the modified carotenoid reflectance index,  $mCRI = (\rho_{510}^{-1} - \rho_{red\ edge}^{-1}) \times \rho_{NIR}$  (Gitelson et al., 2006). Then the ratio of these indices was presented as the stand Car/Chl ratio. Both indices have been shown to be accurate measures of Chl and Car content, respectively (Gitelson et al., 2005, 2006).

The temporal PRI change was affected by reflectance of soil/residue background that was attenuated as green vegetation fraction increased (Figure 9A). Maximal PRI value was achieved when crop density was the highest. Along with the decrease of foliar Chl content at the reproductive stage, PRI decreased sharply in the course of senescence. PRI mimicked very closely the temporal profile of the stand Chl content and green LAI (examples in Figure 1). Thus, the main factor governing seasonal change in stand PRI during senescence was the stand Chl content.

The temporal change pattern in stand-level Car/Chl was very different from that of PRI (Figure 9A and B). The Car/Chl ratio expectedly decreased in the beginning of the season, illustrating the maturation of photosynthetic apparatus and the deployment of the light-harvesting antenna in the crop leaves, and then remained virtually invariant in the reproductive stage, with a sharp increase during senescence corresponding to the decrease of Chl content (Figure 9B). Importantly, the stand Car/Chl was very clearly different between irrigated and rain-fed sites. The difference was especially pronounced in the reproductive and senescence stages when Chl sharply decreased in the rain-fed crop reflecting a higher degree of stress more affected by water shortage and, thus, Car/Chl increased substantially. Thus, in contrast to PRI at the leaf level, crop stand PRI relates primarily to the stand Chl content and green LAI, and did not relate strongly to the leaf-level Car/Chl ratio, unlike previous leaf-level studies of PRI (e.g., Gitelson et al., 2017). These findings reflected the strong influence of canopy closure and changing vegetation/soil cover on PRI at the stand scale in these



**Figure 9.** Temporal behavior of PRI and Car/Chl ratio for irrigated maize in arbitrary units (A) and Car/Chl in irrigated and rain-fed maize (B). Car/Chl was calculated as a ratio of modified carotenoid reflectance index, mCRI (Gitelson et al., 2002, 2006), to red edge chlorophyll index,  $Cl_{red\ edge}$  (Gitelson et al., 2003, 2006).



**Figure 10.** Relationship between PRI and green chlorophyll index,  $CI_{\text{green}} = (\rho_{\text{NIR}}/\rho_{570}) - 1$  (Gitelson et al., 2006), in maize.

annual crops, illustrating the importance of considering spatial and temporal scale as well as vegetation type when using PRI as an indicator of photosynthetic activity.

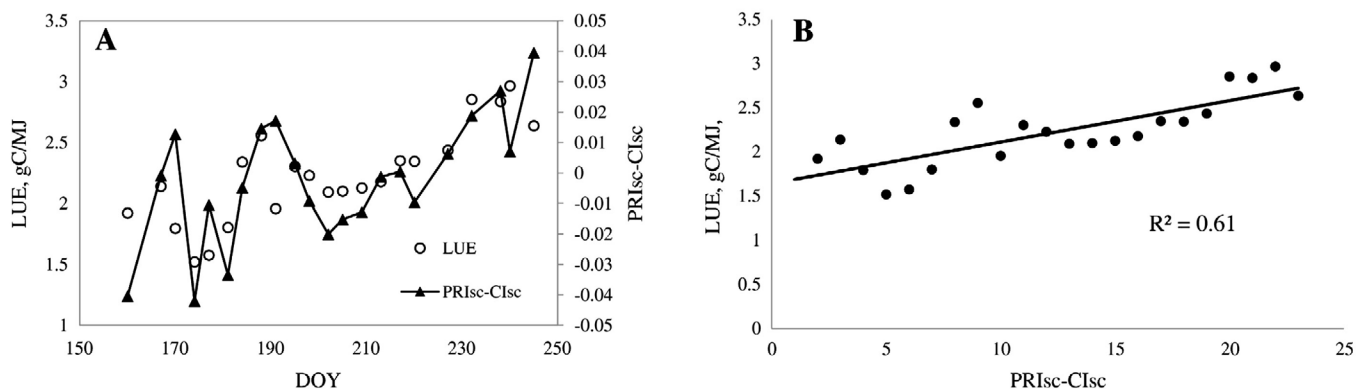
To reveal facultative changes in PRI associated with short-term periods of stress, the original intent of the PRI index (Gamon et al., 1992, Peñuelas et al., 1995, Gamon et al., 1997), the larger background of seasonal change in PRI due to changing canopy structure and constitutive pigment effects must first be considered. To illustrate this, we used the close relationships between the stand Chl vs.  $CI_{\text{green}}$  (Figure 10) and subtracted the stand Chl content effect from PRI. The slopes of PRI vs. stand Chl as well as PRI vs. Chl relationships were different in vegetative and reproductive stages (Figure 5). So to estimate stand Chl content over the whole season, we used two equations relating PRI and the stand Chl content, one for the vegetative stage and another for the reproductive and senescence stages. Then, we scaled PRI and the stand Chl content between 0 and 1 and subtracted scaled stand Chl from scaled stand PRI. The difference,  $PRI_{\text{sc}} - Chl_{\text{sc}}$ , was plotted together with LUE (presented in Gitelson et al., 2015) for rain-fed maize (Figure 11). The variability of residuals was at about 7%, which corresponds well to the expected facultative change in LUE for maize examined in this study (Gitelson et al., 2015) and the temporal behaviors of residuals,  $PRI_{\text{sc}} - Chl_{\text{sc}}$ , and LUE were in agreement (Figure 11B). The main reason for the wide variation of LUE in the beginning of the season was related to uncertainties of  $f_{\text{APAR}}$  measurement at sparse canopy (Gitelson et al., 2015). This method revealed two noticeable dips in PRI and LUE, one around days 170–180, and a second dip between the days 200 and 230. Those were

due to dry weather condition and decline in soil moisture in the rain-fed maize in 2003 (Peng et al., 2013; Gitelson et al., 2015).

Despite the encouraging result for this particular case, it is premature to say whether the suggested approach, subtraction of stand Chl content from stand PRI, would be able to accurately assess LUE using seasonal PRI data and stand Chl-related vegetation indices in all cases. There are several challenges that must be first overcome. The primary challenge is the uncertainty caused by variability in the PRI-Chl relationships, which may be both intraspecific (with variation of canopy architecture) and interspecific. Different slopes of PRI-Chl relationships for vegetative and reproductive stages revealed in this study require accurate remote estimation of the time window when maximal values of either green LAI or stand Chl content appear indicating the changeover of vegetative and reproductive stages. Further, we note that the calculation of LUE depends upon the assumptions made and definitions used (Gitelson and Gamon, 2015). In an attempt to remove any effects of changing green canopy structure on apparent LUE, in this case we based the LUE calculation on radiation absorbed by photosynthetically active “green” vegetation defined as  $f_{\text{APARgreen}} = f_{\text{APAR}} \times (\text{green LAI} / \text{total LAI})$ , as described in Hall et al. (1992), Viña and Gitelson (2005), not total absorbed or incident radiation. Without doing this, a very different seasonal pattern of LUE would result (see comparison in Figure 4, Gitelson and Gamon, 2015).

The hypothesis that LUE can be assessed after correction for the larger seasonal change in PRI due to canopy structure and pigmentation requires very thoughtful testing across a range of species and conditions, with close attention to the issues discussed here. We note that we would not expect all species and vegetation types to have similar PRI responses (Garbulsky et al., 2011, Gamon, 2015). For example, evergreen versus annual species would presumably yield contrasting seasonal patterns of PRI, canopy structure, and photosynthetic activity. In evergreens, large changes in Car/Chl occur with the spring activation and winter deactivation of photosynthesis, reflecting a very clear role for constitutive pigment changes in the seasonal PRI signal that can be closely tied to seasonal patterns of photosynthetic activity and LUE (Wong and Gamon, 2015b). Garbulsky et al. (2011) and Gamon (2015) have suggested that the PRI-photosynthesis (or LUE) relationship would vary for different types of vegetation. They hypothesized that for annual plants (e.g., crops) the changing canopy structure (e.g. LAI or canopy cover) would be a strong driver of the PRI signal. This has not been fully tested yet.

Notably, the changes in PRI indeed reflecting changes in LUE modulated by the xanthophyll cycle are relatively small in magnitude and occur on a much faster (minute to hours) time scale (Gamon et al., 1992; Solovchenko, 2010) than the changes associated with seasonally changing canopy structure and pigment pools



**Figure 11.** Temporal behavior of light use efficiency (LUE) and difference between scaled PRI and red edge vegetation index,  $CI_{\text{red edge}}$  (Gitelson et al. 2003, 2006) in rain-fed maize (A) and (B) relationship LUE vs.  $PRI_{\text{sc}} - CI_{\text{sc}}$ .



(Gamon and Berry, 2012; Wong and Gamon, 2015a, 2015b). LUE-related PRI changes can be tightly linked not only with the Car/Chl ratio per se but with (dis)engagement of energy-dependent photoprotective mechanisms also apparent as non-photochemical quenching of Chl fluorescence (Horton, 2014). In view of these circumstances, interpretation and comparison of PRI should be done with caution, particularly when made across species or growth conditions. It should also take into account the plant ontogenetic stage and stress acclimation state, very much the same as for Chl-fluorescence-based LUE estimation, representing an alternative method of estimating LUE (Maxwell and Johnson, 2000) that can be tightly linked to PRI (Gamon et al., 1997; Solovchenko, 2010).

#### 4. Conclusions

Analysis of seasonal changes in the stand chlorophyll content and green leaf area index in two crops, maize and soybean, lead to the following conclusions.

- In both crops, maize and soybean, PRI related very closely to stand Chl content ( $R^2 > 0.93$ ) and green LAI ( $R^2 > 0.9$ ). PRI was not clearly related to LUE of photosynthetically active vegetation that explained below 12% of PRI variation. Thus, canopy structure exerted a dominant influence at both vegetative and reproductive stages.
- The dynamic range of PRI at vegetative stage was restricted by high PRI value of soil/residue and, the slopes of PRI-LAI relationships were significantly different for vegetative and reproductive stages. At the reproductive stage, PRI was about 3-fold more sensitive to Chl content and green LAI than at vegetative stage.
- Unlike leaf-level responses, PRI was less clearly related to the stand level Car/Chl ratio in crops, likely due to the confounding effect of changing canopy structure.
- PRI was closely related to green and red edge chlorophyll indices that were found to be accurate measures of green LAI and stand Chl content. The strong relationship between PRI and stand Chl content/chlorophyll indices over a growing cycle allowed us to subtract the stand Chl effect from measured PRI to reveal the facultative PRI change related to short periods of stress.
- For accurate subtraction of Chl effect from long-term PRI records, thoughtful study of uncertainties related to "natural" variation of PRI-stand Chl relationships, and stand Chl estimation for different varieties of the same species as well as between different species is required. A better understanding of these relationships and their variation for different types of vegetation is a key for understanding the PRI-LUE relationship.
- In light of other recent studies, these findings have important implications for attempts to monitor photosynthetic activity from satellite platforms, and indicate careful consideration of sampling context (including vegetation type, temporal scale, and spatial scale) and operational definitions (e.g. LUE formulation) is needed when using PRI remotely.

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