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# *Interactive comment on* "Assessing shadow effects on Photochemical Reflectance Index (PRI) for the water stress detection in winter wheat" *by* Xin Yang et al.

**Anonymous Referee #2**

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General Comments: This is a paper that unfortunately suffers from too many flaws to be publishable, in my opinion. The reasons are thus: 1. This study collected PRI data within a window between 1000 and 1400h. Unfortunately, wheat PRI can change dramatically between 1000 and 1400h. Magney et al. (2016) demonstrated that PRI can vary by a factor of 4 between 1000 and 1400h, particularly later in the growing season when water stress is at its peak. This is likely problematic for the current study. It would have been helpful for the authors to conduct an experiment of how the PRI in their wheat plots changes over the course of the data collection period. This information is fundamental to determining whether it is valid to group data across 1000-1400h, or whether the data must be binned in a more time-specific manner before analyzed.



2. Because foliar deepoxidation state will relate to the instantaneous level of non-photosynthetic quenching (NPQ), and NPQ relates to the instantaneous amount of PAR striking a leaf, what is being defined as a sunlit leaf? In figure 1, some leaves are normal to the camera lens, then curve away. There is more of a continuum of light values, rather than two distinct classes of sunlit canopy vs. shaded canopy. As a result, the analysis is flawed because it is trying to capture a process that responds to a continuum (of incident PAR, specifically) using a binary shadow/non-shadow classification. The biological process in question is nonlinear, and the method is oversimplified. 3. The variants of PRI selected for this study are influenced by both long term (constitutive) and short term (facultative) plant physiological processes, and the influences of long term vs. short term pigment pools cannot be isolated from each other. See Gamon and Berry (2012) for more detail. 4. The light (PAR) incident on the plants was not measured or considered in the analyses. This, combined with the fact that the authors are treating a continuous variable of light intensity as a categorical variable (i.e. sunlit vs. shaded) unfortunately are fundamental omissions that make the results of this study invalid in my opinion. Specific Comments: Title: Please remove "the" in the title to make the title more readable. Abstract Line 7: PRI can correlate with several types of plant ecophysiological functions depending on the timeframe of analysis. As a result, several different types of plant stress (nutrient, water, pathogen) can influence the de-epoxidation state of a plant as it responds to excess light. It would also be helpful to indicate here that the PRI is a remotely sensed spectral vegetation index. I therefore suggest that the authors remove 'water' from this first line and rather state "...a pre-visual remotely sensed indicator of plant stress." Line 8: "...whether variations the shadow fraction, which can be influenced by varying view angle and crop density..." Line 11: Three different variants of PRI are presented here, without any indication that the different formulations of PRI can be interpreted in different ways, based on several considerations (e.g. timeframe of analysis, phenological responses within the growth cycle, etc.). Specifying how many formulations were tested would be helpful. For example, "...using 6 different formulations..." (line 10) and then "Results demonstrated

that three of the PRI formulations (PRI570, PRI1, and PRI2)...” Abstract in general: in a paper looking at the PRI to assess plant stress under different light levels (i.e. shadow fractions) I think it imperative that the authors mention the core mechanism driving changes in PRI at short timescales, which is related to the relationship between excess light and non-photochemical quenching. NPQ is the process by which the plant shunts excess light from a cell's light harvesting complex in the form of heat. Introduction Page 1, Line 23: Suggest new paragraph starting with “Remote sensing...” Line 24: “...assess water status...” Page 2, line 4: Please see first comment I made on the abstract. As I read further in this section, it appears that the authors do a pretty good job of overviewing the connection between PRI and stress. It would be useful to still include some mention of non-water-related stressors here in this section (e.g. nutrient availability, pathogens) and also structure the argument to explain to the reader that the xanthophyll cycle serves to protect the light harvesting complex from excess light, and that the threshold for a plant to deal with excess light varies according to a multitude of environmental factors (water availability being one, yes, but not the only one). Line 25: I respectfully disagree that the impacts of shadowing on PRI are generally ignored. This is a widely recognized phenomenon. The authors should re-word this statement. Line 30: The more mechanistically appropriate way to explain this is that the xanthophyll cycle status is affected by incident PAR, which is in turn affected by the level of self-shading (i.e. shadow fraction) within a canopy. Intro in general: Magney et al. (Remote Sensing of Environment, 2016, 173: 84-97) reported relationships between derivations of the PRI and various environmental conditions (including VPD) in three different portions of a wheat field. This paper should be briefly overviewed in the introduction and potentially the discussion due to the goals of the study being closely related to the current paper. Page 3, Line 2: “...the PRI's capability...” The PRI does not have agency or capability. Scientists have a capability to interpret PRI data to detect stress. Please reword. Materials and Methods Page 3, Line 13: “rectangular” Page 3, Line 16: “plots” should be “pots” Line 20: remove “a” Line 22: Were all pots located in the rainout shelter? Because plant light harvesting complexes and pigments



can change as a result of the ambient light environment in which they are growing, it is important to know whether the rain-out shelters reduced the PAR striking the canopy. What was the influence of these shelters on the ambient light condition? Line 23 and 26: “control” is more specific than “reference” Page 4, Line 20: Due to a number of factors such as VPD and air temperature, the PRI of wheat can change dramatically between 1000 and 1400h. See Magney et al. 2016, who demonstrated that PRI can vary by a factor of 4 between 1000 and 1400, particularly later in the growing season. This could be very problematic for the current study. Did the authors conduct an experiment of how the PRI in their wheat plots changes over the course of the data collection period? This information is fundamental to determining whether it is valid to group data across 1000-1400h, or whether the data must be binned in a more time-specific manner before analyzed. Line 30: Depending on the specs of this journal, you probably need to specify the ENVI manufacturer, version, etc. Figure 1: This classified image highlights a question for me. Because the deepoxidation state will relate to the instantaneous level of NPQ, and NPQ relates to the instantaneous amount of PAR striking a leaf, what is being defined as a sunlit leaf? In figure 1, some leaves are normal to the camera lens, then curve away. There is more of a continuum of light values, than two distinct classes of sunlit canopy vs. shaded canopy. As a result, the analysis may be flawed because it is trying to capture a process that responds to a continuum (of incident PAR, specifically) using a binary shadow/non-shadow classification. Table 1: Unfortunately, of all of the PRI calculations used in this study, the two variants of PRI that have been shown to correlate most strongly with water status and other diurnally changing physiological variables, the deltaPRI and the PRIo, (Magney et al. 2016) were not calculated or used in this study. The various variants of PRI used in this particular study are influenced strongly by longer-term chlorophyll:carotenoid ratios that will mask the instantaneous effects of changing light or VPD conditions (Gamon and Berry, 2012). (Note: the deltaPRI calculations shown in Table 2 are different from deltaPRI in the literature and will not remove the seasonal effect of pigment phenology).

Ref: Gamon, J.A., & Berry, J.A. (2012). Facultative and constitutive pigment effects

on the photochemical reflectance index (PRI) in sun and shade conifer needles. Israel Journal of Plant Sciences, 60(1), 85–95.

Final comment: in my reading of this paper, the light (PAR) incident on the plants was not measured or considered in the analyses. This, combined with the fact that the authors are treating a continuous variable of light intensity as a categorical variable (i.e. sunlit vs. shaded) unfortunately are fundamental omissions that make the results of this study invalid in my opinion.

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