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Investigating the Impacts of Drought on Turfgrass (*Festuca arundinacea*)
Chlorophyll-a Fluorescence Emission

An Honors Project for the Program of Biochemistry

By Ayanna S. Hatton

Bowdoin College, 2024

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ABSTRACT

When photons from sunlight are absorbed by plants, they can take paths of photosynthesis, fluorescence, or energy dissipation. Instruments to quantify fluorescence have expanded in scale to allow measurements from satellites and flux towers using Solar-Induced Chlorophyll Fluorescence (SIF). Studies have found a positive correlation between SIF and gross primary productivity (GPP; representative of photosynthesis), suggesting SIF is a proxy for GPP. This conclusion encourages the use of SIF to inform decisions about carbon budgets and responding to climate change. Studies of fluorescence on the single-leaf scale have revealed that SIF measurements do not account for all variables nor is there an understanding of the impact of environmental factors, such as drought, on these measurements.

In this project, tall fescue turfgrass was placed in one of four differing drought severities for 19 days. Leaf-level measurements of photosynthesis and pulse-amplitude modulated fluorescence were made, demonstrating stomatal closure and inhibition of photosynthesis. This physiological change caused greater photon allocation to energy dissipation. Changes in greenness and the utilization of photoprotective mechanisms such as senescence and anthocyanin accumulation were observed. This study has provided an understanding of the temporal, physiological, and visible impacts of drought on turfgrass to inform interpretations of SIF in future experiments. Caution is crucial in utilizing SIF as a proxy for GPP before further research into the impact of drought on SIF is completed.

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INTRODUCTION

Introduction to solar-induced chlorophyll fluorescence

As droughts are becoming more severe with global change, it is growing crucial to understand the impacts they will have on plants. Decisions about carbon budgets, responding to climate change, and the severities of droughts are informed by new methods measuring Solar-Induced Chlorophyll Fluorescence (SIF; Porcar-Castell et al., 2021). Photons that are absorbed by and excite chlorophyll-a molecules—but are not utilized in photosynthesis—can be emitted as SIF (Sun et al., 2017). Measurements of SIF on a large scale, from flux towers and satellites, are utilized to estimate gross primary productivity (GPP), i.e., photosynthetic CO₂ assimilation on a ground area basis (Sun et al., 2017). SIF measurements were recently implemented, with some of the first satellite studies reported in 2011 (Frankenberg et al., 2011b; Joiner et al., 2011). While multiple satellites have been utilized for this, the Orbiting Carbon Observatory-2 (OCO-2) launched in 2014 by NASA (Crisp et al., 2017) has created better-resolution images than had been produced in the past (Sun et al., 2017). OCO-2 produces hundreds of thousands of measurements daily that are transmitted to Earth (Crisp et al., 2017), measuring atmospheric [CO₂] and now SIF. SIF measurements from OCO-2 are used to quantify carbon uptake for application to carbon budgets, among other uses.

The formation of a method for quantitative analysis of fluorescence in 1975 by Plascyk et al. has allowed for this expansion in the scale of measurements. Fluorescence is a small fraction of absorbed light reemitted by photosystem (PS) II chlorophyll-a molecules (Sun et al., 2017). Plascyk et al. (1975) found that sunlight (and reflected solar light) had a specific ratio of energy or depth of Fraunhofer lines, which are regions of absorption of the solar spectrum.

Measurements of SIF are quantified with spectrometers by calculating solar light and fluorescence Fraunhofer depth ratios—depth of the Fraunhofer lines relative to outside of the lines—in the fluorescence spectrum (660-850 nm; Sun et al., 2017). A lower ratio of depth of the Fraunhofer lines relative to outside of the lines is representative of fluorescence. Frankenberg et al. (2011a) and Joiner et al. (2011) improved measurements of SIF with the Fraunhofer line method that allowed for the separation of the fluorescence signal from atmospheric scattering. As tools have improved in resolution, more accurate measurements of SIF have been acquired.

To establish SIF's usefulness, researchers have published studies attempting to show that SIF is a proxy for GPP measured from flux towers and satellites. Utilizing their novel approach, Frankenberg et al. (2011b), found correlations between SIF and GPP. Six years later, Sun et al. (2017) conducted research measuring SIF and GPP from OCO-2. OCO-2 provided better resolution, which demonstrated stronger correlations between SIF and GPP than past methods. GPP tower measurement sites in or near the path of OCO-2 allowed them to compare stand-scale GPP with landscape-scale SIF. They asserted that SIF “reflect[s] photosynthetic dynamics in real time” and found a positive linear relationship between SIF and GPP, suggesting SIF is a proxy for GPP (Fig. 1). In the past decade, research relating to SIF has focused on improving these tools to increase accuracy and to examine relationships. As studies continue to argue for the validity of the proxy relationship, they have suggested utilizing SIF to make decisions about carbon budgets and responses to climate change. In this research, I measured photosynthesis and fluorescence in turfgrasses impacted by differing levels of drought on a small scale to ultimately be compared to larger scale SIF measurements.

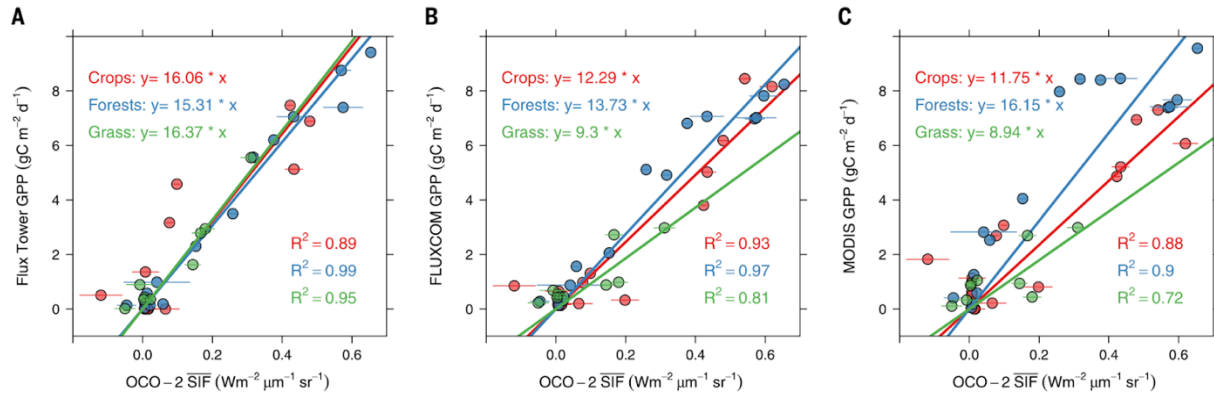


Figure 1. SIF positively correlates to GPP with multiple measurement tools.

The correlation between GPP and OCO-2 SIF (daily mean value) at three environments of crops, forests, and grass. GPP was measured daily in 2015 using “(A) eddy covariance measurements, (B) FLUXCOM products, and (C) MODIS products, sampled at these three flux sites. Error bars represent the standard error of the OCO-2 SIF retrieval” (From Sun, Y., Frankenberg, C., Wood, J. D., Schimel, D. S., Jung, M., Guanter, L., Drewry, D. T., Verma, M., Porcar-Castell, A., Griffis, T. J., Gu, L., Magney, T. S., Köhler, P., Evans, B., & Yuen, K. (2017). OCO-2 advances photosynthesis observation from space via solar-induced chlorophyll fluorescence. *Science*, 358(6360). <https://doi.org/10.1126/science.aam5747>, Figure 3 / Reprinted with permission from AAAS).

An understanding of fluorescence is necessary to interpret SIF data and appreciate the impact that different stresses and environmental changes may have on the measurement. To interpret measurements of fluorescence and photosynthesis, one must comprehend photosynthetic electron transport.

Electron transport in plants

Plants use sunlight as an energy source by utilizing photosynthetic pigments to power the creation of glucose. Photons are critical for photochemistry and aid in creating an electrochemical proton gradient that drives the ATP synthase to generate energy (Eberhard et al., 2008). However, photons can take alternative paths of either fluorescence or non-photochemical quenching through energy dissipation (ED; Fig. 2; Maxwell et al., 2000). When there is an

excess of photons, depending on other conditions, ED and fluorescence may increase (Porcar-Castell et al., 2021). Chlorophyll does not hang on to these extra photons because they can cause uncontrolled redox reactions and the creation of reactive oxygen species (ROS) that are harmful to cells (Eppel et al., 2013).

Energy dissipation is a mechanism utilized for photoprotection (factors that prevent photons from damaging plants by reducing absorption or siphoning surplus energy to other processes). Instead of driving photochemistry, the photon energy is released as heat through the actions of the xanthophyll cycle (Maxwell et al., 2000). When there is excess light, the carotenoid violaxanthin is converted to zeaxanthin (Demmig-Adams & Adams, 1996), a molecule able to accept excitation energy from chlorophyll, which it discharges thermally (Blommaert et al., 2021). When light intensity diminishes, zeaxanthin is reconverted to violaxanthin to lower levels of ED so that only excess light is dissipated (Demmig-Adams & Adams, 1996). The partitioning of energy through ED is regulated over short time scales to manage potentially harmful excess light without disrupting the productive use of light in photosynthesis.

Fluorescence is a biophysical feature of chlorophyll that can be measured at multiple scales utilizing different instruments. Chlorophyll-a fluorescence (ChlaF) is energy—from photons—reemitted as light (at longer wavelengths; Joiner et al., 2011) instead of moving through photosystems and creating energy—this is not directly regulated by chlorophyll. Spectrometer measurements of ChlaF intensity at larger scales (e.g., towers and satellites), using sunlight as a photon source, are referred to as SIF (Sun et al., 2017). ChlaF can also be measured at smaller scales using pulse-amplitude-modulated (PAM) fluorometers (pulses of light). Measurements

with PAM fluorometers can additionally measure aspects of energy partitioning, revealing the siphoning of photons to photosynthesis or ED. Measurements of fluorescence with spectrometers and PAM fluorometers have allowed for a greater understanding of fluorescence at different scales and fluorescence's relationship to other processes.

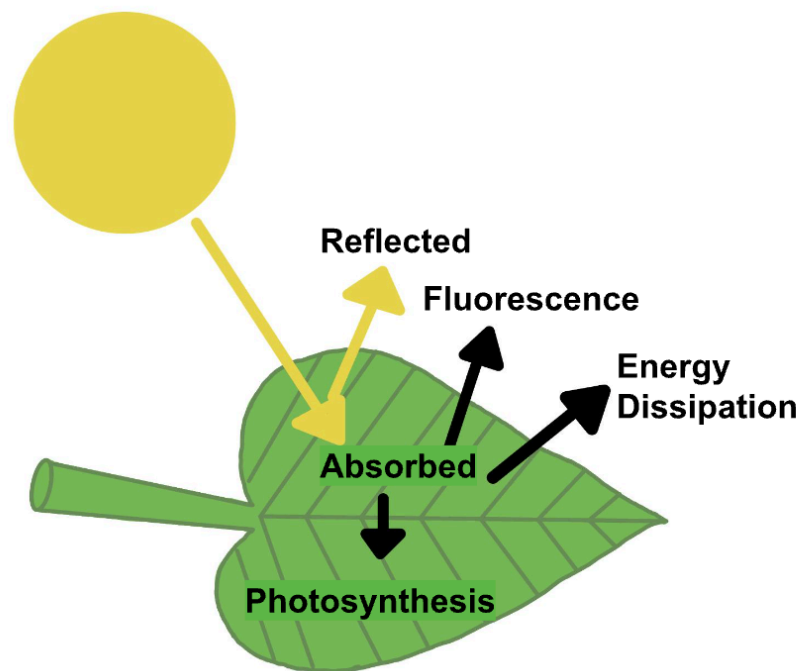


Figure 2. Solar energy can be utilized in multiple ways. Solar energy can be reflected or absorbed. Absorbed photons can be siphoned to photosynthesis or released as fluorescence or energy dissipation. There are additional paths photons can take that have been omitted for simplicity.

It is a fundamental tenet of energy partitioning that if more photons are utilized for one process—photosynthesis, ChlaF, or ED— then the photons siphoned by the other processes must necessarily decrease. In other words, if photosynthesis increases, then energy dissipation and/or fluorescence will decrease. While fluorescence and photosynthesis are related, one cannot be used to directly predict the other (by proxy relationship) because this neglects the partitioning of energy to ED and outside environmental impacts such as changing water and sunlight. PAM

fluorometers are valuable instruments because they can measure photosynthesis, ED, and fluorescence at the same time.

What measurements of fluorescence at different scales reveal

While SIF measurements are novel and innovative, ChlaF measurements on the single-leaf scale (using PAM) demonstrate that the SIF-photosynthesis relationship may vary at different scales. Scientists have suggested that SIF may be seen as a proxy of GPP because of averaging over time, the large spatial scales of the measurements, and differing measurement sites, in addition to other factors (Porcar-Castell et al., 2021). More research is needed to understand the best measurement methodologies and the interpretations that can be drawn from SIF. It is critical to build knowledge of the impact of environmental and physiological processes on fluorescence on a small scale to inform the interpretation of large-scale SIF measurements.

Marrs et al. (2020), a study on which Bowdoin plant ecophysiologicalists collaborated, reported on a two-day study to determine if SIF would respond to experimentally imposed inhibition of photosynthesis. They altered instantaneous leaf photosynthetic carbon fixation by driving the closure of stomata through abscisic acid (ABA) treatment or application of a shoot pressure cuff. ABA is a hormone that signals for stomatal closure, and pressure cuffs cause xylem emboli to form; emboli simulate water stress through the formation of an air cavity blocking the flow of water, which then stimulates stomatal closure. They compared single-leaf-scale measurements of photosynthesis to branch-scale measurements of SIF collected using a rooftop-mounted telescope connected to spectrometers. Measurements took place pre- and post-treatment. They found that photosynthesis declined greatly because the treatments led to stomatal closure. SIF, however, did

not exhibit a statistically significant decrease (Fig. 3). If SIF were a proxy for photosynthesis at this scale, a change in the same direction *and* magnitude would be expected. This challenges if SIF can be utilized as a proxy for photosynthesis and encourages exercising caution in drawing such conclusions under all circumstances.

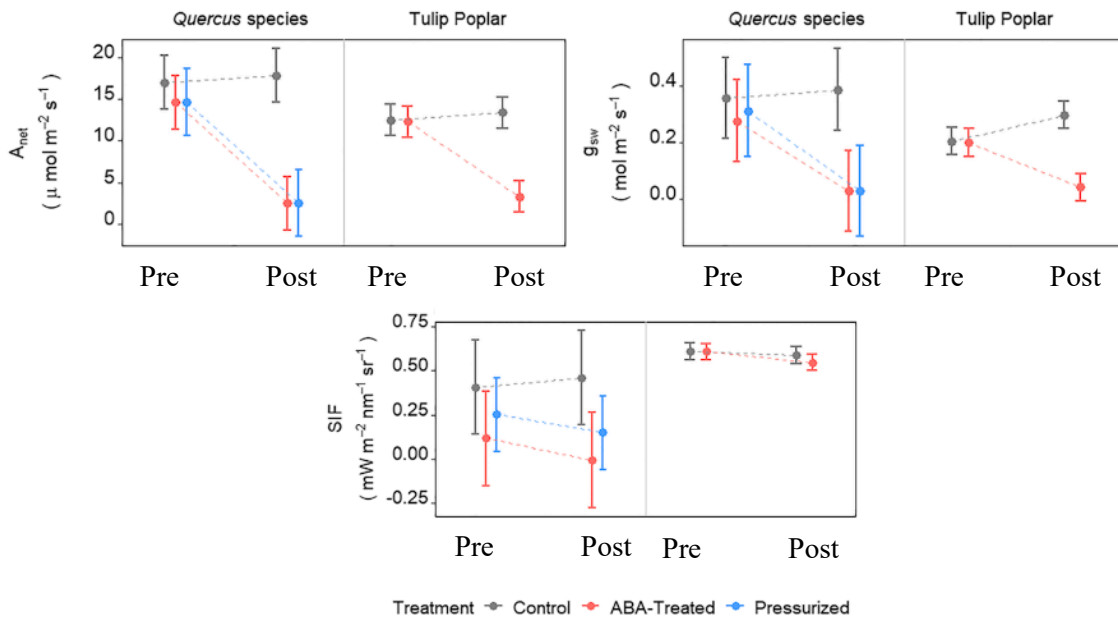


Figure 3. Fluorescence is not correlated with photosynthesis at a small scale. The plot points represent averaged values. Net carbon assimilation (A_{net} , photosynthesis), stomatal conductance, and SIF were measured. “Error bars represent 95% confidence intervals determined from LME models” (Adapted and used with permission, Marrs et al., 2020).

Other studies have demonstrated the impacts of water stress on SIF in tall fescue turfgrass. Jones et al. (2023), another study on which Bowdoin plant ecophysiology collaborators collaborated, conducted a four-day experiment inhibiting photosynthesis using two treatments, applying ABA or withholding water. In the ABA condition, the canopy was sprayed with ABA at the beginning of day one. Water was withheld from the grass pallets for two days before rewatering in the water stress condition. Measurements of SIF—utilizing the same rooftop set up as Marrs et al.

(2020)—were made 18.4 m from the grass samples, and measurements of CO₂ assimilation (i.e., photosynthesis) and ChlaF were made on the blade level (these latter measurements were used to quantify the level of energy dissipation [ED]). Over the days, the turfgrass in the ABA treatment exhibited an increase in ED and a decrease in SIF and photosynthesis. In the water stress condition, they observed no changes to ED and a decrease in photosynthesis *before* rewatering. However, SIF and NDVI (a reflectance greenness index) were significantly lower only *after* rewatering, suggesting leaf coloration had a greater effect on SIF than photosynthesis. In the absence of a response in ED to the short-termed water stress, SIF measurements were unable to recognize the magnitude or direction of photosynthetic decline. This study raises questions about the spatiotemporal scale at which SIF correlates with GPP in plants experiencing stress and encourages the acknowledgement of ED.

The decoupling of SIF and GPP can be caused by a variety of environmental and physiological aspects that weaken or complicate their correlation. During these times, SIF may fail to track a change in fluorescence, hindering SIF as a tool to measure stress. Leaves differ in shape, impacting the overall canopy architecture and interaction with photons. The canopy can inhibit SIF from detecting and representing full ChlaF emission (Porcar-Castell et al., 2021). In addition, visual signs of drought (often photoprotective) such as anthocyanin accumulation (the buildup of anthocyanin to protect chlorophyll), a decrease in chlorophyll, senescence, wilting, and leaf-blade folding can impact the SIF signal and decouple it from GPP. For example, if a senescing blade is blocking a new blade below from measurement, SIF will not be representative of the whole plant but only what is visible to the instrument. Atmospheric gasses and other particles can absorb or cause scattering of photons emitted as ChlaF, causing a lower

measurement of SIF than ChlaF emitted (Porcar-Castell et al., 2021). There are multiple outside impacts that have great effects on measurements of SIF and the decoupling from GPP.

Averaging of data encourages the coupling of SIF and GPP, allowing for the observation of a proxy relationship. Frequently, satellite studies (in general) collect data over a whole day or for multiple days. Over the days, weather and sun exposure change, which can cause lower or higher measurements. This is because both photosynthesis and SIF are tightly associated with sunlight intensity. So, as sunlight intensity fluctuates, one can expect SIF and GPP to fluctuate with it, and, thus, be correlated. Averaging of SIF data over large time scales smooths changes that might occur on a physiological level from SIF measurements (Marrs et al., 2020). This does not often occur with leaf-scale measurements because they tend to be taken consistently in bright sun, in the middle of the growing season, and are nearly instantaneous. This eliminates sunlight intensity as a factor that can aid in correlating ChlaF and photosynthesis. There are fewer changing variables in the small-scale measurements than in the larger-scale measurements, and more subtle physiological trends can predominate.

In addition to data averaging over large sections of time, there also is not consistency in GPP and SIF measurement sites. GPP and SIF measurements are both dependent on vegetation density—greater vegetation density will typically produce high GPP and high SIF, whereas a site with sparse vegetation will display lower values. Often, data from multiple sites (with differing vegetation density) are averaged. When averaged, trends appear between GPP and SIF displaying both as high in greater density and both low in sparser density—this allows large-scale correlations to be established. Averaging site differences adds the variable of site without

acknowledging the impact of that on the measurements. At smaller-scale measurements, controls for the density of vegetation are made, which minimizes variables—this allows for more direct comparison.

The importance of scale is a compelling dimension of the study of SIF. Large-scale correlations between SIF and GPP appear to be innovative and robust. However, further studies have questioned the reason for the correlations and variables in SIF measurements that have not been considered. These measurements still contain a wealth of information, but one cannot assume that SIF is a proxy for photosynthesis at all spatiotemporal scales. Therefore, great care should be exercised when employing SIF into analysis of environments to be applied to policies. We hope that our smaller-scale measurements help to build a mechanistic understanding of the relationship between photosynthesis and SIF to inform the boundaries of where and why one can assume that SIF may serve as a proxy for photosynthesis.

Visual signs of drought

Water is necessary in plants for transpiration, nutrient movement, cell structure, and photosynthesis. Many plants utilize mechanisms to tolerate drought that can be detected visually and may impact SIF. One photoprotective measure against drought that plants employ is the accumulation of anthocyanin (red/blue pigments common in plants). Turfgrasses can accumulate anthocyanins during environmental stress such as drought (Petrella et al., 2016). The increase of anthocyanin protects layers of chlorophyll-rich cells (Logan et al., 2015), limits photosynthesis, reduces stress on the xanthophyll cycle (Cooney et al., 2018), and aids in water retention (Cirillo et al., 2021). In addition, there is evidence that anthocyanins detoxify the build-up of reactive

oxygen species (ROS; Cirillo et al., 2021) that occur because CO₂ is not utilized in the Calvin Cycle during drought (Eppel et al., 2013). Overall, anthocyanin accumulation is an advantageous photoprotective mechanism utilized by plants that can be measured qualitatively and quantitatively.

Senescence, leaf yellowing, is a visual indication of drought and a photoprotective mechanism which appears in grasses more than other plants. Senescence of leaves is a species-specific response which can be caused by drought to decrease the demand for water (Munne-Bosch & Alegre, 2004; Bittman et al., 1988). During drought, ABA can increase, which induces senescence by degrading chlorophyll, causing higher visibility of carotenoids (Munne-Bosch & Alegre, 2004). Senescence allows plants to encourage root growth and hinder shoot growth by limiting water and nutrient consumption by older blades that contribute less to the grass overall, thus decreasing photosynthesis (Finkelstein, 2013; Sarath et al., 2014). When senescence is no longer reversible, cell and blade death occur, allowing grass to decrease the use of a scarce water supply, encouraging survival through a drought. When water becomes available again, grass will regrow its canopy, which may be shielded by senesced blades from instruments positioned above the canopy. As a result, fluorescence emitted by these new leaves may be masked and not measured by SIF.

Leaf folding and wilting are other mechanisms plants employ to preserve themselves. Wilting is a common plant response to drought and loss of turgor that is reversible but can cause death if prolonged (Van Alfen, 1989). Wilting is observed in many plant species, but leaf folding is a feature specific to grasses. When there is limited water, cells on the surface of grass blades lose

turgor and collapse, allowing leaves to fold. Then, when water returns, the turgor recovers, and the leaf reopens. This occurs to limit transpiration—thus, further loss of water (Grigore & Toma, 2017)—and the area of the leaves available for absorbing photons, causing photoprotection (Latif et al., 2023). All of these processes—both visual and those only measurable—are important to the survival and resiliency of grasses. Wilting and leaf folding can decrease the amount of blade considered in SIF measurements while not necessarily decreasing fluorescence by the same proportion.

Physiological signs of drought

Plants react physiologically to water stress, and most of these processes can be measured before they can be visually detected. Stomatal conductance decreases in drought as stomata close to control water loss. This step is one of the earliest responses to drought (Munne-Bosch & Alegre, 2004). When the stomata close, plants reduce transpiration, which can impact photosynthesis because CO₂ diffusion into the leaves is diminished (Hu et al., 2010). In addition, this can limit evaporative cooling, further intensifying thermal stress (Leksungnoen et al., 2012). During this reduction in stomatal conductance, plants may turn to photorespiration to create CO₂, which is less beneficial as this process does not yield energy and produces a limited amount of CO₂ (Eppel et al., 2013). While photorespiration is utilized during times of drought, it is an energetically unfavorable and wasteful side pathway—photosynthesis, when possible, is far more beneficial. The severity of a drought, therefore, can be quantified by utilizing measurements of photosynthesis and stomatal conductance.

Past studies have found that photosynthesis, transpiration, and stomatal conductance were decreased by drought (Hu et al., 2010). During a drought, depending on photoprotective mechanisms utilized by plants, fluorescence may also decrease due to damage of the PSII complex (Ge et al., 2011). In a boreal grass (*Phalaris arundinacea* L.), ChlaF and photosynthesis have been shown to decrease as soil moisture declines regardless of the temperature (Ge et al., 2011). In Eastern Cottonwoods (*Populus deltoides*; Helm et al., 2020), photosynthesis and SIF have also shown decreases at different scales. Though drought can cause a decrease in photosynthesis and SIF, SIF declines at a much smaller magnitude (or not at all), decoupling SIF and photosynthesis. Studies demonstrate favorability of leaf-scale over canopy-scale ChlaF measurements during drought because variables—and their impacts on ChlaF—can be better accounted for. Further leaf-scale measurements of ChlaF may help us better understand the mechanisms driving stress responses of SIF at the canopy scale.

Creating tools that can measure fluorescence and shifts within ecosystems can be beneficial as a measurement of global change. Drought is the most common abiotic cause of growth reduction in crops (Lovell et al., 2018) and can have great impacts on natural ecosystems due to its effect on many species. Turfgrass makes up a large percentage of the Earth's vegetation and is utilized to create greener cities. Turfgrass, as opposed to plants in general, reacts to water stress with species-specific mechanisms such as anthocyanin accumulation, senescence, and leaf curling. Grass is important as a carbon sink (through photosynthesis), for soil retention, and as a food source for animals (Craine et al., 2013). Understanding tolerance mechanisms to drought that grasses employ can aid in studying them and their potential for carbon sequestration. Quantifying photosynthesis in grasses is important for creating carbon budgets and policies. Therefore, it is

crucial that there is an in-depth understanding of the tools and methodologies utilized for the measurement of turfgrass carbon uptake. Measuring the relationships between fluorescence and photosynthesis on a smaller scale can inform larger scale studies using flux towers and satellites. Recognizing the bounds of SIF's usefulness is needed as SIF is utilized for predictions and the creation of carbon budgets (Marrs et al., 2020). My research is intended to define conditions that lead to leaf-scale changes in fluorescence and photochemistry to inform the design of experiments incorporating SIF measurements.

METHODS

Grass preparation

Tall fescue turfgrass seeds (*Festuca arundinacea*; Top Choice fine bladed fescue blend, Fulton, MD, USA; certified by Oregon State University, Corvallis, Oregon, USA) were grown in flats (17 cm x 12.5 cm x 6 cm: l x w x h) in a greenhouse environment (Bowdoin College, Brunswick, ME, USA; Fig. 4). Mid-day temperatures during the measurement period ranged from 13°C to 27°C with an average of 20°C. The position of the flats was rotated daily to account for unequal sun exposure and temperature. Artificial overhead lighting was utilized to create 14 uninterrupted daily hours of light. The soil was kept moist initially, and, after the emergence of blades, grasses were watered to pot capacity. After four weeks of growth, grasses (seven replicates) were put in one of four conditions (day 0): control (90-100%), mild drought (50-60%), moderate drought (35-40%), or severe drought (20-30%) where the percent represented the fraction of flat weight compared to the fully water-saturated flat weight (pot capacity; Turner, 2019). Flats were weighed daily and watered—if lower than the condition limit—to the top of their range. Flats were watered in a secondary containment to allow for adequate soil uptake.

These conditions were maintained until day 19 when all flats were watered daily to pot capacity until the conclusion of the experiment (day 29; Fig. 6).

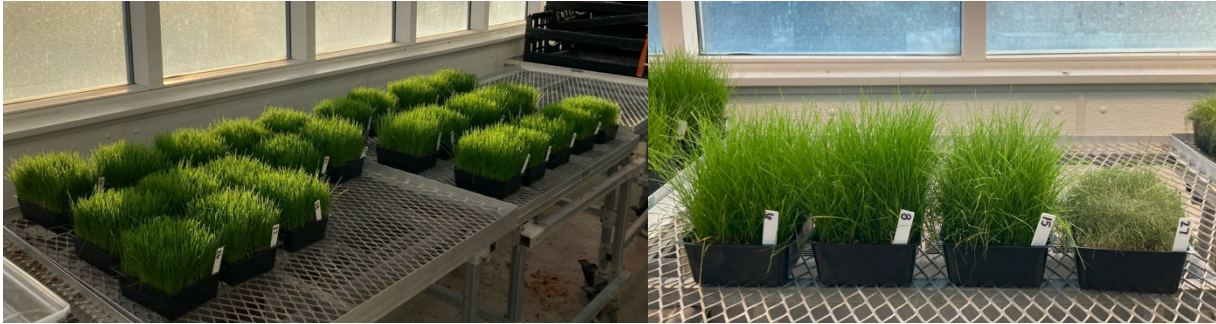


Figure 4. Tall fescue turfgrass on day 0 and day 15 of experiment. Tall fescue turfgrass was grown for four weeks in a greenhouse environment before beginning the experiment. The position of the flats was rotated daily to allow even growth. Left) The photo was taken on January 4, 2024 (day 0). Right) The photo was taken on January 19, 2024 (day 15). Each flat represents a different condition. Control, mild drought, moderate drought, and severe drought from left to right.

Leaf-level measurements

On day 0 of the experiment, measurements of the grass began. Measurements of photosynthetic CO_2 assimilation and pulse-amplitude modulated (PAM) chlorophyll fluorescence were made using the Licor LI-6800 photosynthesis analyzer (Lincoln, NE, USA). Three grass blades (visually representative of the grasses in each flat) were isolated and fed into the center of the measurement chamber. Average leaf width was determined prior to the experiment and utilized throughout in the determination of leaf area measured in the chamber. Leaf-level measurements were made using an internal lamp set to $1200 \mu\text{mol m}^{-2} \text{s}^{-1}$ and a reference CO_2 concentration of $410 \mu\text{mol mol}^{-1}$. The temperature of the chamber was set to 24°C , and the relative humidity was kept at 40%. The light activated photosystem II (PSII) photochemical yield (ϕ_{PSII}) was calculated as $(F'_m - F_s) / F'_m$ where F'_m represents maximum PAM fluorescence emission and F_s represents steady-state fluorescence emission. The light-activated light use efficiency of PSII (F'_v / F'_m)

was calculated as $(F'_m - F'_0) / F'_m$ and is inversely related to the level of energy dissipation (Jones et al., 2023).

Photo analysis

Digital photos of the flats were taken each measurement day by an iPad and analyzed using ImageJ to determine the green chromatic coordinate (G_{cc}). The lighting, position of camera, and flat position were kept consistent throughout the experiment (Fig. 5). When uploaded to ImageJ, photos were separated into red (R), green (G), and blue (B) composite images. Analysis of the color fractions within a region of interest centered on the flat was run to determine the

G_{cc} : $G_{CC} = \frac{G}{(R+G+B)}$ (Sonntag et al., 2012). This value provides a quantitative measurement of visible changes in greenness.



Figure 5. Digital photo set-up for turfgrass image analysis. Digital photos were taken with an iPad from the same position, lighting, and height each day to ensure consistency.

Chlorophyll and carotenoid analysis

Blade samples (0.4 cm²) were collected on each measurement day and stored at -80°C until analysis, using methods from de Villier et al. (2017). On day 16, there was visible yellowing and a decrease in photosynthesis measurements in the severe drought (Fig. 7)—only blades from this day were analyzed. To extract the leaf pigments, each sample was macerated in a mechanical ball mill (SPEX CentriPrep 8000D, Metuchen, NJ, USA) for five minutes in an Eppendorf tube with a stainless-steel ball bearing and 450 µl of 80% chilled acetone. Then, the tubes were spun in a chilled micro-centrifuge at 13,000 rpm for five minutes. The supernatant was transferred, and the pellet was resuspended in 200 µl of 100% chilled acetone. The supernatant was kept in

the dark and on ice. The tubes were centrifuged additionally and the remaining supernatant was pooled. The supernatant was filtered through a 0.2 µm nylon syringe-tip filter into high-performance liquid chromatography (Agilent series 1100 HPLC, Santa Clara, CA, USA) sample vials and stored at -20°C before HPLC analysis. A gradient method and a diode array detector were utilized; absorption was measured at 445 nm (Dr. Maisch Allsphere ODS-1 5µm column, Ammerbuch, Germany).

Anthocyanin analysis

Blade samples collected from day 16 and stored identically to “Chlorophyll and carotenoid analysis” were macerated in a mechanical ball mill (SPEX CentriPrep 8000D, Metuchen, NJ, USA) for five minutes in an Eppendorf tube with a stainless-steel ball bearing in 1 ml of 3M HCl:H₂O:MeOH (1:3:16, v:v:v). Then, the tubes were spun in a chilled micro-centrifuge at 10,000 rpm for five minutes. The absorbance of the solution at 530 nm and 653 nm with a spectrophotometer (Beckman Coulter DU 640, Brea, CA, USA) was measured. Anthocyanin levels were calculated as $A_{530} - (0.24 \times A_{653})$ (Gould et al., 2000).

RESULTS

Photosynthesis decreases due to drought

Flats of tall fescue turfgrass were placed under conditions of control (90-100%), mild drought (50-60%), moderate drought (35-40%), or severe drought (20-30%) where the percent represented the fraction of flat weight compared to the fully water-saturated flat weight (pot capacity). On day 16 (pre-watering), on average the control, mild, moderate, and severe droughts were 85%, 45%, 28%, and 23% of their pot capacity respectively (Fig. 6). The flats reached their

drought conditions around day 6 and then were rewatered on day 19 and recovered control pot capacities by day 21 (Fig. 6). By day 8 there was a decrease in stomatal conductance (g_{sw}) and photosynthetic CO_2 assimilation (A) in the severe drought condition. On day 16 (pre-watering), on average A was 9%, 37%, and 98% lower than the control in the mild, moderate, and severe drought conditions respectively (Fig. 7 upper panel). On the same day, on average g_{sw} was 21%, 57%, and 100% lower than the control in the mild, moderate, and severe drought conditions respectively (Fig. 7 lower panel). Both g_{sw} and A recovered following rewatering. These findings suggest that the most severe drought may cause the most severe decrease in photosynthesis.

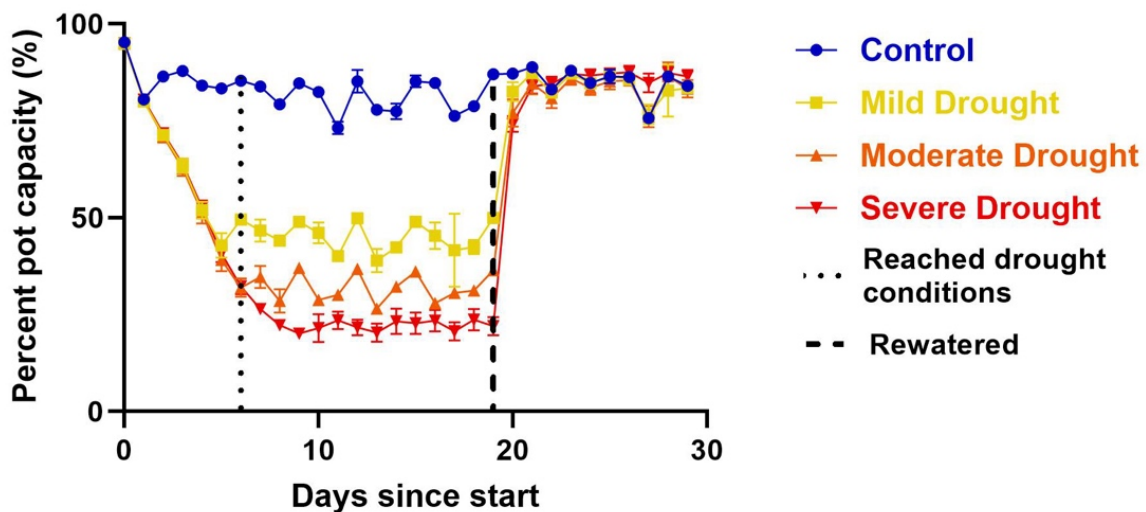


Figure 6. Tall fescue turfgrass was watered (or withheld from water) to achieve varying drought conditions. Flats of tall fescue turfgrass were placed under conditions of control (90-100%), mild drought (50-60%), moderate drought (35-40%), or severe drought (20-30%). Flats reached the conditions around day 6 (dotted line). Daily rewatering to pot capacity began on day 19 (dashed line).

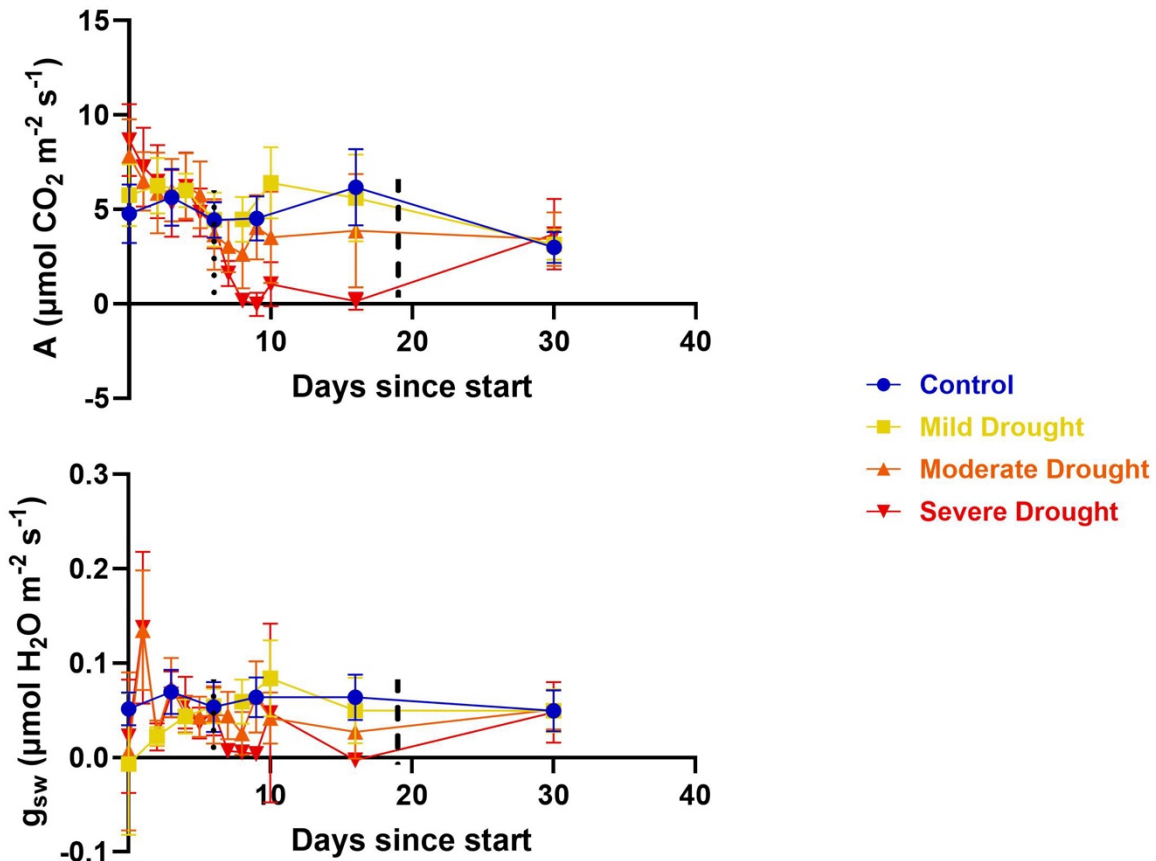


Figure 7. Drought conditions caused a decrease in stomatal conductance (g_{sw}) and photosynthetic CO_2 assimilation (A).

Measurements of photosynthetic CO_2 assimilation (upper panel) and stomatal conductance (lower panel) were made using the Licor LI-6800 photosynthesis analyzer. Flats reached the conditions around day 6 (dotted line). The severe drought was the only condition with a statistically significant effect on A ($p=0.0126$) and g_{sw} ($p=0.0011$; mixed model ANOVA). Daily rewatering to pot capacity began on day 19 (dashed line). The g_{sw} and A (in all conditions) recovered after rewatering.

Drought may lead to an increase in energy dissipation

The severe drought condition caused a decrease in light activated PSII photochemical yield (Φ_{PSII}) seen greatly at day 16 with a recovery following rewatering (Fig. 8 upper panel). The severe drought also caused a decrease in light use efficiency of PSII (F'_v / F'_m ; inversely proportional to energy dissipation), which also recovered following rewatering (Fig. 8 lower panel). There was no significant effect of mild or moderate drought on these measurements. The

negative ϕ_{PSII} values (upper panel) reported in the severe drought are measurement artifacts commonly observed when actual values are very near zero.

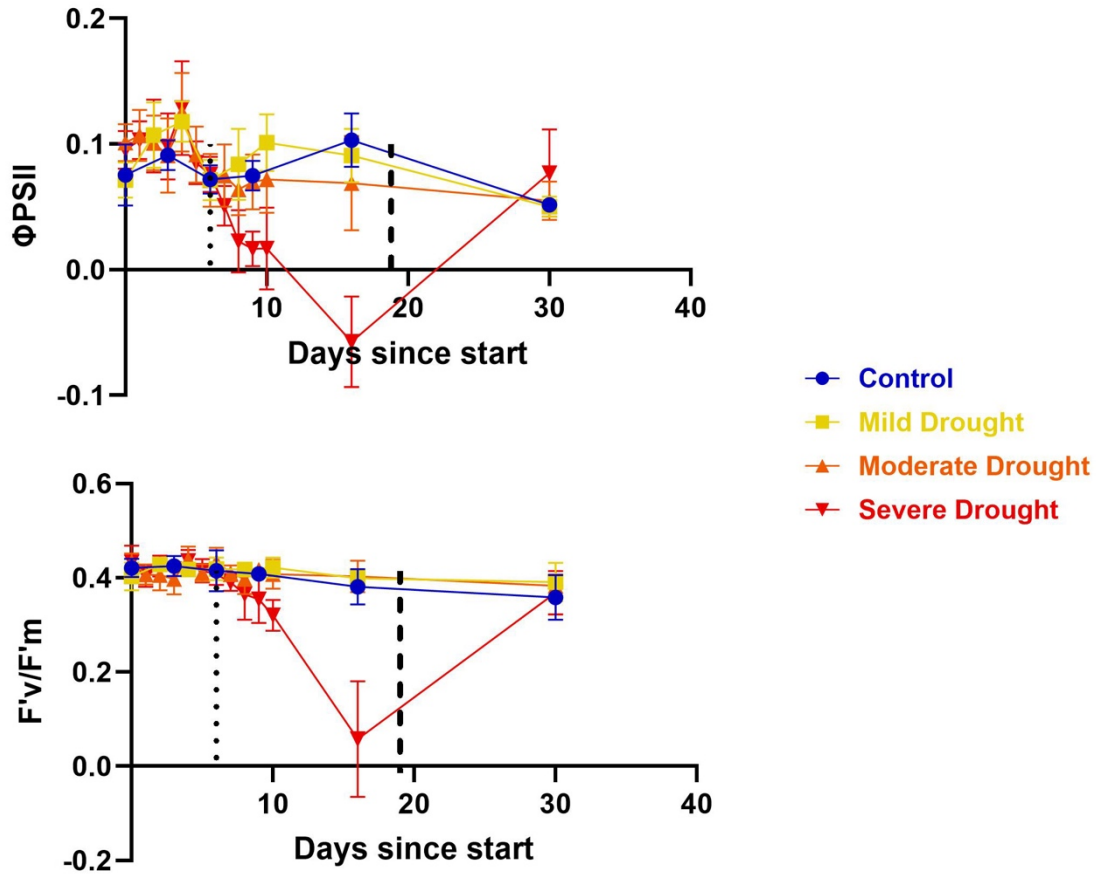


Figure 8. Light activated PSII photochemistry yield (ϕ_{PSII}) and light use efficiency of PSII (F'_v / F'_m) decreased due to severe drought.

The severe drought condition caused a decrease in ϕ_{PSII} (upper panel; $p=0.0052$, mixed model ANOVA). The severe drought was the only condition with a significant decrease in F'_v / F'_m (lower panel, inversely proportional to energy dissipation; $p<0.0001$, mixed model ANOVA). Both the ϕ_{PSII} and F'_v / F'_m recovered following rewatering in all conditions. Flats reached the conditions around day 6 (dotted line). Daily rewatering to pot capacity began on day 19 (dashed line).

Drought causes visible and physiological changes in chlorophyll content but not accompanying anthocyanin accumulation.

Photos were taken daily of the flats, and the G_{cc} was calculated using ImageJ. The severe drought caused a decrease in G_{cc} (greenness; $p < 0.0001$, mixed model ANOVA) which did not recover after watering in the severe drought (Fig. 9 upper panel). Blade samples from day 16 were analyzed utilizing HPLC, and total chlorophyll and total carotenoid content were determined. Severe drought led to a significant decrease in total chlorophyll and total carotenoids as compared to the control (Fig. 9 lower panel). This result demonstrates both a visible (G_{cc}) and physiological (chlorophyll and carotenoid) decrease of greenness due to severe drought. Blade samples from day 16 were analyzed using spectrophotometry to determine the anthocyanin levels. There was no significant difference in anthocyanin level due to drought (Fig. 10).

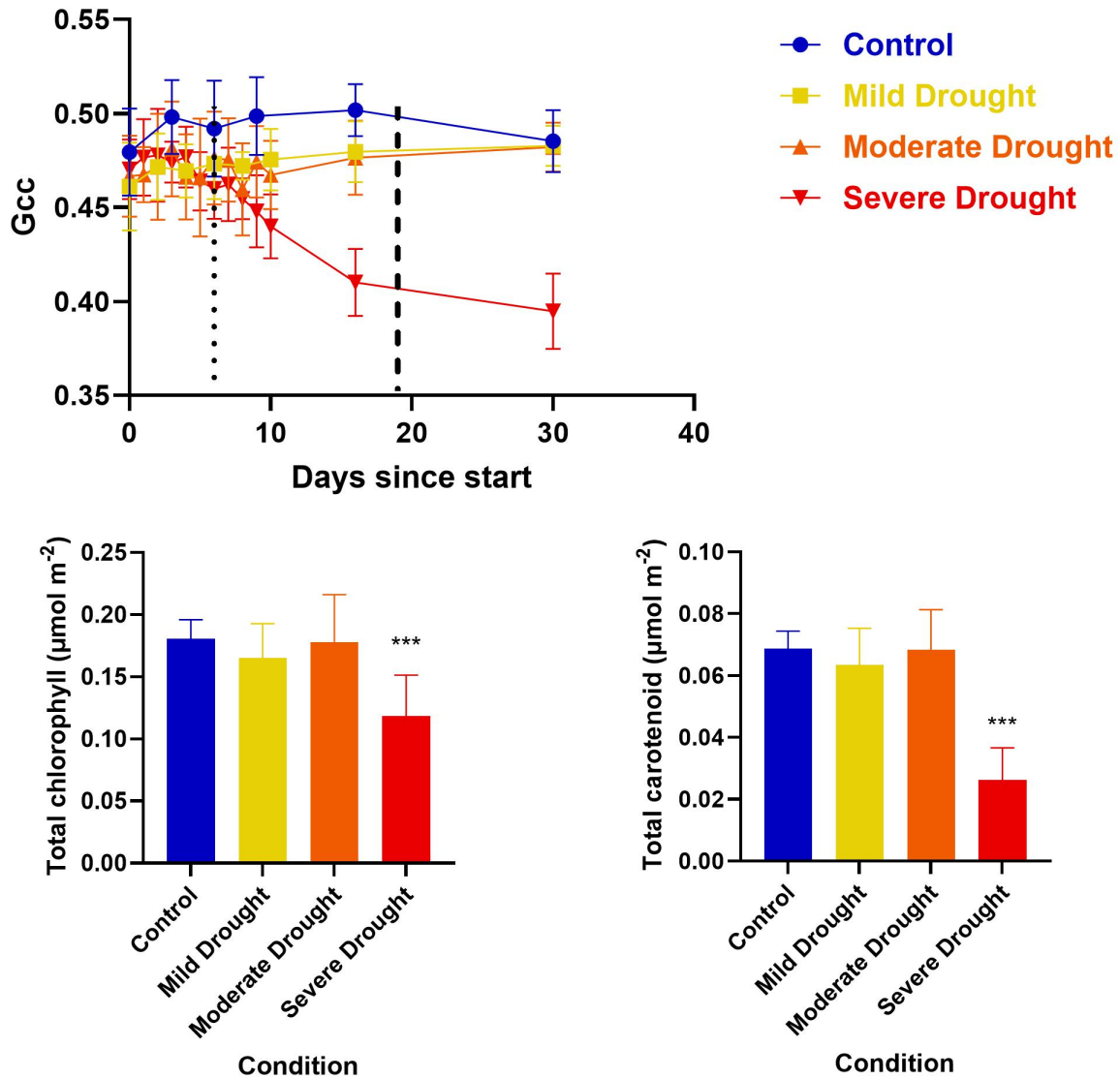


Figure 9. Severe drought caused visible and physiological decreases in “greenness.” Photos of the grass were taken on each measurement day and analyzed utilizing ImageJ. Drought caused a decrease in G_{cc} of the severe drought condition (upper panel). This recovered in the control, mild drought, and moderate drought but not in the severe drought condition. Flats reached the conditions around day 6 (dotted line). Daily rewatering to pot capacity began on day 19 (dashed line). Blade samples were collected from day 16 of the experiment and stored at -80°C . HPLC analysis of the blades allowed for measurement of total chlorophyll (chlorophyll-a and chlorophyll-b) and total carotenoid (violaxanthin, neoxanthin, antheraxanthin, lutein, zeaxanthin, and beta-carotene; lower panel). p-value levels indicating the significance of the treatment effects are shown with stars (***) $p < 0.001$, t-test).

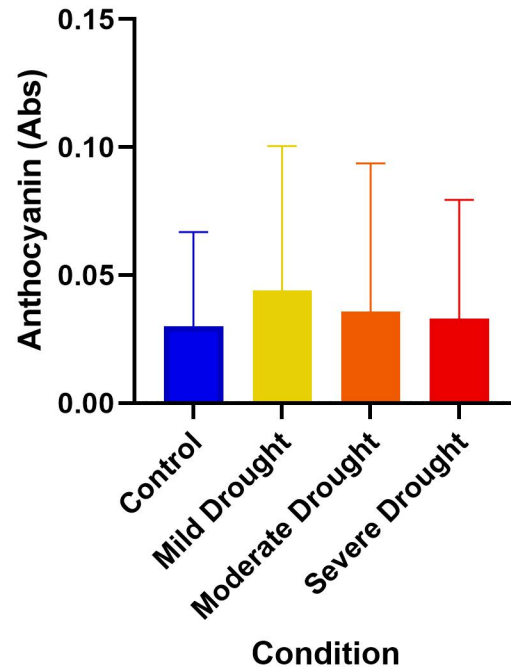


Figure 10. Drought levels caused no change in anthocyanin level. Blade samples were collected from day 16 of the experiment and stored at -80°C . Anthocyanin levels were calculated with $A_{530} - (0.24 \times A_{653})$. No significant difference ($p < 0.05$, t-test) was found in anthocyanin levels between the conditions.

DISCUSSION

In this project, tall fescue turfgrass was placed in a 19-day drought followed by 10 days of rewatering. Leaf-level measurements of photosynthetic CO_2 assimilation and PAM chlorophyll fluorescence were made using a photosynthesis analyzer. This research aimed to build an understanding of the impacts of drought severity and duration on photosynthesis and fluorescence in turfgrass to be applied to future studies of SIF. This can aid in the recognition of SIF limitations as a proxy for photosynthesis.

Severe drought caused stomatal closure, photosynthetic inhibition, enhanced energy dissipation, and senescence

The blade-level measurements revealed physiological impacts of drought, and daily image collection created a greater understanding of visible changes due to drought. The mild and moderate droughts were not stressful enough to invoke significant changes in any measurements—only the severe drought caused a significant water stress and physiological changes. Photosynthesis declined due to drought, which was exhibited as a decrease in A (photosynthetic CO_2 assimilation) and g_{sw} (stomatal conductance). ϕ_{PSII} and F'_v / F'_m (inversely proportional to ED) decreased due to drought, thus a decrease in the photons siphoned to photochemistry and an increase in energy dissipation were observed during drought (Maxwell et al., 2000; Porcar-Castell et al., 2021). Therefore, when one partitioning pathway declines (photosynthesis), another partitioning pathway compensates by increasing photon usage. While g_{sw} , A , and ϕ_{PSII} indicate a decrease in photosynthesis, they do not specify the partitioning of light to fluorescence.

Changes in the visible and measured greenness and anthocyanin accumulation were measured. The severe drought condition caused a decrease in chlorophyll and carotenoid content, suggesting the photoprotective mechanism of senescence (Munne-Bosch & Alegre, 2004; Bittman et al., 1988). A decline in chlorophyll content (through senescence) and leaf area in the field of view (through leaf folding and wilting) lead to a decrease of G_{cc} in the severe drought. The decrease in G_{cc} lead to an increase in B_{cc} without the photoprotective mechanism of anthocyanin accumulation. Because R, B, and G must sum to unity, the decrease in G_{cc} will lead to an increase in B or G without necessarily increasing blue or red pigments in the grass. Thus,

turfgrass experiencing severe drought may utilize photoprotective mechanisms of senescence without anthocyanin accumulation to limit the amount of shoot growth and encourage survival (Sarath et al., 2014).

Predictions

Past studies have measured the impacts of different photosynthesis inhibition assays (mainly through inhibiting g_{sw}). A 2-day study by Marrs et al. (2020), utilizing ABA and a pressure cuff, detected decreases in A and g_{sw} on the leaf scale. In addition, no significant change in SIF was measured, suggesting that photosynthesis and SIF may not be correlated. The withholding of water in my study led to a decrease in A and g_{sw} , thus photosynthesis. Because similarly this was observed in Marrs et al. (2020), it could be assumed that our conditions may have caused no change in SIF early in the experiment. However, our study had a prolonged drought, so there is a possibility that a change in SIF would come later than can be predicted from Marrs et al. (2020).

Jones et al. (2023), in a 4-day study, measured the difference between ABA and withholding water on photosynthetic measurements. In the ABA condition, a decrease in photosynthesis and SIF and an increase in ED were measured. While in the watering condition there was a decrease in photosynthesis, a decrease in SIF and NDVI (greenness index) were *not* detected until after rewatering (day 3). In my study, a decrease in photosynthesis and increase in ED were found in the severe drought condition. But, like Jones et al. (2023), in the early implementation of drought, there was no change in ED nor greenness (G_{cc}). Thus, if my data followed the same trend as Jones, at the beginning of the experiment, no change in SIF may be detected. But, later in the study (day 16), when an increase in ED and decrease in greenness (chlorophyll content and

G_{cc}) was detected, I expect SIF may decrease. Their work suggests that SIF is less correlated to photosynthesis and instead impacted when changes in ED, greenness, and leaf area—through folding—are observed.

The studies of Marrs and Jones were 2 and 4 days respectively. A study of my length (29 days) provides further information for future work, especially with the knowledge that measurements at day 16 differ from measurements on day 2 and 4. Because there are few studies of drought on SIF measurements of this length, it is important to build further research to create an understanding of the temporal changes of SIF. Knowledge on when recovery is expected and for what conditions and measurements will be helpful in designing these future experiments.

Conclusion and further directions

My study has provided an understanding of the temporal, physiological, and visible impacts of drought on turfgrass. This is valuable information to be applied to future studies and SIF research. SIF measurements as an inclusion to this study would give further information about the coupling or lack thereof of SIF and photosynthesis. An understanding of the impact of leaf curling and senescence on SIF would be beneficial to inform policy decisions. It is crucial that further research into the impact of drought on SIF is completed and considered when utilizing this novel data to build awareness of carbon uptake and the regulation of carbon budgets.

REFERENCES

- Banks, J. M. (2018). Chlorophyll fluorescence as a tool to identify drought stress in *Acer* genotypes. *Environmental and Experimental Botany*, *155*, 118–127.
<https://doi.org/10.1016/j.envexpbot.2018.06.022>
- Bittman, S., Simpson, G. M., & Mir, Z. (1988). Leaf senescence and seasonal decline in nutritional quality of three temperate forage grasses as influenced by drought. *Crop Science*, *28*(3), 546–552. <https://doi.org/10.2135/cropsci1988.0011183X002800030025x>
- Blommaert, L., Chafai, L., & Bailleul, B. (2021). The fine-tuning of NPQ in diatoms relies on the regulation of both xanthophyll cycle enzymes. *Scientific Reports*, *11*(1).
<https://doi.org/10.1038/s41598-021-91483-x>
- Brown, W. V. (1958). Leaf anatomy in grass systematics. *Botanical Gazette*, *119*(3), 170–178.
<https://doi.org/10.1086/335974>
- Carrow, R. N. (1996). Drought avoidance characteristics of diverse tall fescue cultivars. *Crop Science*, *36*(2), 371–377. <https://doi.org/10.2135/cropsci1996.0011183X003600020026x>
- Chaffey, N. (2000). Physiological anatomy and function of the membranous grass ligule. *New Phytologist*, *146*(1), 5–21. <https://doi.org/10.1046/j.1469-8137.2000.00618.x>
- Chai, Q., Jin, F., Merewitz, E., & Huang, B. (2010). Growth and physiological traits associated with drought survival and post-drought recovery in perennial turfgrass species. *Journal of the American Society for Horticultural Science*, *135*(2), 125–133.
<https://doi.org/10.21273/JASHS.135.2.125>
- Cirillo, V., D'Amelia, V., Esposito, M., Amitrano, C., Carillo, P., Carputo, D., & Maggio, A. (2021). Anthocyanins are key regulators of drought stress tolerance in tobacco. *Biology*, *10*(2), 139. <https://doi.org/10.3390/biology10020139>

- Cooney, L. J., Logan, B. A., Walsh, M. J. L., Nnatubeugo, N. B., Reblin, J. S., & Gould, K. S. (2018). Photoprotection from anthocyanins and thermal energy dissipation in senescing red and green *Sambucus canadensis* peduncles. *Environmental and Experimental Botany*, *148*, 27–34. <https://doi.org/10.1016/j.envexpbot.2017.12.019>
- Craine, J. M., Ocheltree, T. W., Nippert, J. B., Towne, E. G., Skibbe, A. M., Kembel, S. W., & Fargione, J. E. (2013). Global diversity of drought tolerance and grassland climate-change resilience. *Nature Climate Change*, *3*(1), 63-67. <https://doi.org/10.1038/nclimate1634>
- Crisp, D., Pollock, H. R., Rosenberg, R., Chapsky, L., Lee, R. A. M., Oyafuso, F. A., Frankenberg, C., O'Dell, C. W., Bruegge, C. J., Doran, G. B., Eldering, A., Fisher, B. M., Fu, D., Gunson, M. R., Mandrake, L., Osterman, G. B., Schwandner, F. M., Sun, K., Taylor, T. E., ... Wunch, D. (2017). The on-orbit performance of the Orbiting Carbon Observatory-2 (OCO-2) instrument and its radiometrically calibrated products. *Atmospheric Measurement Techniques*, *10*(1), 59–81. <https://doi.org/10.5194/amt-10-59-2017>
- de Villier, J. A., Reblin, J. S., & Logan, B. A. (2017). Needle properties of host white spruce (*Picea glauca* [Moench] Voss) experiencing eastern dwarf mistletoe (*Arceuthobium pusillum* Peck) infections of differing severity. *Botany*. *95*(3): 295-305. <https://doi.org/10.1139/cjb-2016-0254>
- Demmig-Adams, B., & Adams, W. W. (1996). The role of xanthophyll cycle carotenoids in the protection of photosynthesis. *Trends in Plant Science*, *1*(1), 21-26. [https://doi.org/10.1016/S1360-1385\(96\)80019-7](https://doi.org/10.1016/S1360-1385(96)80019-7)
- Des Marais, D. L., Lasky, J. R., Verslues, P. E., Chang, T. Z., & Juenger, T. E. (2017). Interactive effects of water limitation and elevated temperature on the physiology,

- development and fitness of diverse accessions of *Brachypodium distachyon*. *New Phytologist*, 214(1), 132–144. <https://doi.org/10.1111/nph.14316>
- Eberhard, S., Finazzi, G., & Wollman, F.-A. (2008). The dynamics of photosynthesis. *Annual Review of Genetics*, 42(1), 463–515. <https://doi.org/10.1146/annurev.genet.42.110807.091452>
- Eppel, A., Keren, N., Salomon, E., Volis, S., & Rachmilevitch, S. (2013). The response of *Hordeum spontaneum* desert ecotype to drought and excessive light intensity is characterized by induction of O₂ dependent photochemical activity and anthocyanin accumulation. *Plant Science*, 201–202, 74–80. <https://doi.org/10.1016/j.plantsci.2012.12.002>
- Finkelstein, R. (2013). Abscisic Acid Synthesis and Response. *The Arabidopsis Book*, 11. <https://doi.org/10.1199/tab.0166>
- Frankenberg, C., Butz, A., & Toon, G. C. (2011a). Disentangling chlorophyll fluorescence from atmospheric scattering effects in O₂ A-band spectra of reflected sun-light. *Geophysical Research Letters*, 38(3). <https://doi.org/10.1029/2010GL045896>
- Frankenberg, C., Fisher, J. B., Worden, J., Badgley, G., Saatchi, S. S., Lee, J.-E., Toon, G. C., Butz, A., Jung, M., Kuze, A., & Yokota, T. (2011b). New global observations of the terrestrial carbon cycle from GOSAT: Patterns of plant fluorescence with gross primary productivity. *Geophysical Research Letters*, 38(17). <https://doi.org/10.1029/2011GL048738>
- Ge, Z. M., Zhou, X., Kellomäki, S., Wang, K. Y., Peltola, H., & Martikainen, P. J. (2011). Responses of leaf photosynthesis, pigments and chlorophyll fluorescence within canopy position in a boreal grass (*Phalaris arundinacea* L.) to elevated temperature and CO₂ under

varying water regimes. *Photosynthetica*, 49(2), 172–184. <https://doi.org/10.1007/s11099-011-0029-8>

Grigore, M.-N., & Toma, C. (2017). Bulliform cells. In *Anatomical Adaptations of Halophytes* (pp. 325-338). Springer International Publishing. https://doi.org/10.1007/978-3-319-66480-4_8

Guo, J., Han, W., & Wang, M.-H. (2008). Ultraviolet and environmental stresses involved in the induction and regulation of anthocyanin biosynthesis: A review. *African Journal of Biotechnology*, 7(25), 4966-4972.

Helm, L. T., Shi, H., Lerda, M. T., & Yang, X. (2020). Solar-induced chlorophyll fluorescence and short-term photosynthetic response to drought. *Ecological Applications*, 30(5). <https://doi.org/10.1002/eap.2101>

Hoekstra, F. A., Golovina, E. A., & Buitink, J. (2001). Mechanisms of plant desiccation tolerance. *Trends in Plant Science*, 6(9), 431–438. [https://doi.org/10.1016/S1360-1385\(01\)02052-0](https://doi.org/10.1016/S1360-1385(01)02052-0)

Hu, L., Wang, Z., & Huang, B. (2010). Diffusion limitations and metabolic factors associated with inhibition and recovery of photosynthesis from drought stress in a C₃ perennial grass species. *Physiologia Plantarum*, 139(1), 93–106. <https://doi.org/10.1111/j.1399-3054.2010.01350.x>

Huang, B., Duncan, R. R., & Carrow, R. N. (1997). Drought-resistance mechanisms of seven warm-season turfgrasses under surface soil drying: I. shoot response. *Crop Science*, 37(6), 1858–1863. <https://doi.org/10.2135/cropsci1997.0011183X003700060032x>

- Joiner, J., Yoshida, Y., Vasilkov, A. P., Yoshida, Y., Corp, L. A., & Middleton, E. M. (2011). First observations of global and seasonal terrestrial chlorophyll fluorescence from space. *Biogeosciences*, 8(3), 637–651. <https://doi.org/10.5194/bg-8-637-2011>
- Jones, E. A. L., Contreras, D. J., & Everman, W. J. (2021). *Digitaria ciliaris*, *Digitaria ischaemum*, and *Digitaria sanguinalis*. In B. S. Chauhan (Eds.), *Biology and management of problematic crop weed species* (pp. 173-195). Academic Press. <https://doi.org/10.1016/B978-0-12-822917-0.00014-8>
- Jones, T. S., Logan, B. A., Reblin, J. S., Bombard, D. M., Ross, B. P., Allen, D. W., Marrs, J. K., & Hutrya, L. R. (2023). Stress-induced changes in photosynthesis and proximal fluorescence emission of turfgrass. *Environmental Research Communications*, 5. <https://doi.org/10.1088/2515-7620/ad0b29>
- Krause, G. H., Weis, E. (1991). Chlorophyll fluorescence and photosynthesis: The basics. *Annual Review of Plant Physiology and Plant Molecular Biology*, 42, 313-349. <https://doi.org/10.1146/annurev.pp.42.060191.001525>
- Latif, A., Ying, S., Cuixia, P., & Ali, N. (2023). Rice curled its leaves either adaxially or abaxially to combat drought stress. *Rice Science*, 30(5), 405–416. <https://doi.org/10.1016/j.rsci.2023.04.002>
- Leksungnoen, N., Johnson, P. G., & Kjelgren, R. K. (2012). Physiological responses of turfgrass species to drought stress under high desert conditions. *HortScience*, 47(1), 105–111. <https://doi.org/10.21273/HORTSCI.47.1.105>
- Logan, B. A., Stafstrom, W. C., Walsh, M. J. L., Reblin, J. S., & Gould, K. S. (2015). Examining the photoprotection hypothesis for adaxial foliar anthocyanin accumulation by revisiting

- comparisons of green- and red-leafed varieties of coleus (*Solenostemon scutellarioides*). *Photosynthesis Research*, 124(3), 267–274. <https://doi.org/10.1007/s11120-015-0130-0>
- Lovell, J. T., Jenkins, J., Lowry, D. B., Mamidi, S., Sreedasyam, A., Weng, X., Barry, K., Bonnette, J., Campitelli, B., Daum, C., Gordon, S. P., Gould, B. A., Khasanova, A., Lipzen, A., MacQueen, A., Palacio-Mejía, J. D., Plott, C., Shakirov, E. V., Shu, S., ... Juenger, T. E. (2018). The genomic landscape of molecular responses to natural drought stress in *Panicum hallii*. *Nature Communications*, 9(1). <https://doi.org/10.1038/s41467-018-07669-x>
- Marrs, J. K., Reblin, J. S., Logan, B. A., Allen, D. W., Reinmann, A. B., Bombard, D. M., Tabachnik, D., & Hutryra, L. R. (2020). Solar-induced fluorescence does not track photosynthetic carbon assimilation following induced stomatal closure. *Geophysical Research Letters*, 47(15). <https://doi.org/10.1029/2020GL087956>
- Maxwell, K., & Johnson, G. N. (2000). Chlorophyll fluorescence—A practical guide. *Journal of Experimental Botany*, 51(345), 659-668. <https://doi.org/10.1093/jexbot/51.345.659>
- Meroni, M., Busetto, L., Guanter, L., Cogliati, S., Crosta, G. F., Migliavacca, M., Panigada, C., Rossini, M., & Colombo, R. (2010). Characterization of fine resolution field spectrometers using solar Fraunhofer lines and atmospheric absorption features. *Applied Optics*, 49(15), 2858-2871. <https://doi.org/10.1364/AO.49.002858>
- Munné-Bosch, S., & Alegre, L. (2004). Die and let live: Leaf senescence contributes to plant survival under drought stress. *Functional Plant Biology*, 31(3), 203-216. <https://doi.org/10.1071/FP03236>
- Passioura, J. B. (2006). The perils of pot experiments. *Functional Plant Biology*, 33(12), 1075-1079. <https://doi.org/10.1071/FP06223>

- Petrella, D. P., Metzger, J. D., Blakeslee, J. J., Nangle, E. J., & Gardner, D. S. (2016). Anthocyanin production using rough bluegrass treated with high-intensity light. *HortScience*, 51(9), 1111–1120. <https://doi.org/10.21273/HORTSCI110878-16>
- Plascyk, J. A., & Gabriel, F. C. (1975). The Fraunhofer line discriminator MKII—an airborne instrument for precise and standardized ecological luminescence measurement. *IEEE Transactions on Instrumentation and Measurement*, 24(4), 306–313. <https://doi.org/10.1109/TIM.1975.4314448>
- Porcar-Castell, A., Malenovský, Z., Magney, T., Van Wittenberghe, S., Fernández-Marín, B., Maignan, F., Zhang, Y., Maseyk, K., Atherton, J., Albert, L. P., Robson, T. M., Zhao, F., Garcia-Plazaola, J.-I., Ensminger, I., Rajewicz, P. A., Grebe, S., Tikkanen, M., Kellner, J. R., Ihalainen, J. A., ... Logan, B. (2021). Chlorophyll a fluorescence illuminates a path connecting plant molecular biology to Earth-system science. *Nature Plants*, 7(8), 998–1009. <https://doi.org/10.1038/s41477-021-00980-4>
- Puértolas, J., Larsen, E. K., Davies, W. J., & Dodd, I. C. (2017). Applying ‘drought’ to potted plants by maintaining suboptimal soil moisture improves plant water relations. *Journal of Experimental Botany*, 68(9), 2413–2424. <https://doi.org/10.1093/jxb/erx116>
- Qian, Y., & Fry, J. D. (1997). Water relations and drought tolerance of four turfgrasses. *Journal of the American Society for Horticulture Science*, 122(1), 129-133. <https://doi.org/10.21273/JASHS.122.1.129>
- Sarath, G., Baird, L. M., & Mitchell, R. B. (2014). Senescence, dormancy and tillering in perennial C4 grasses. *Plant Science*, 217–218, 140–151. <https://doi.org/10.1016/j.plantsci.2013.12.012>

- Sonnentag, O., Hufkens, K., Teshera-Sterne, C., Young, A. M., Friedl, M., Braswell, B. H., Milliman, T., O’Keefe, J., & Richardson, A. D. (2012). Digital repeat photography for phenological research in forest ecosystems. *Agricultural and Forest Meteorology*, *152*, 159–177. <https://doi.org/10.1016/j.agrformet.2011.09.009>
- Sun, Y., Frankenberg, C., Wood, J. D., Schimel, D. S., Jung, M., Guanter, L., Drewry, D. T., Verma, M., Porcar-Castell, A., Griffis, T. J., Gu, L., Magney, T. S., Köhler, P., Evans, B., & Yuen, K. (2017). OCO-2 advances photosynthesis observation from space via solar-induced chlorophyll fluorescence. *Science*, *358*(6360). <https://doi.org/10.1126/science.aam5747>
- Turner, N. C. (2019). Imposing and maintaining soil water deficits in drought studies in pots. *Plant and Soil*, *439*(1–2), 45–55. <https://doi.org/10.1007/s11104-018-3893-1>
- Van Alfen, N. K. (1989). Reassessment of plant wilt toxins. *Annual Review of Phytopathology*, *27*, 533-550. <https://doi.org/10.1146/annurev.py.27.090189.002533>
- Xu, S., Atherton, J., Riikonen, A., Zhang, C., Oivukkamäki, J., MacArthur, A., Honkavaara, E., Hakala, T., Koivumäki, N., Liu, Z., & Porcar-Castell, A. (2021). Structural and photosynthetic dynamics mediate the response of SIF to water stress in a potato crop. *Remote Sensing of Environment*, *263*. <https://doi.org/10.1016/j.rse.2021.112555>