



## STUDIES

# Photochemical performance of reproductive structures in Great Basin bunchgrasses in response to soil-water availability

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## Abstract

Active restoration, especially seeding, is necessary in sagebrush steppe rangelands degraded by the spread and dominance of exotic invasive annual grasses, in part due to low, episodic seed production of native perennial bunchgrasses. In contrast, the widespread exotic bunchgrass, crested wheatgrass, readily produces viable seed cohorts. How soil-water availability affects the ecophysiology of the reproductive structures that may underlie these differences are unclear. To address this, we measured pre- and post-anthesis chlorophyll fluorescence parameters of optimal ( $F_v/F_m$ ) and light-adapted PSII quantum yield ( $\phi_{PSII}$ ) and  $\phi_{PSII}$ -derived electron transport rate (ETR) response to photosynthetic photon flux density (PPFD) in seed heads and flag leaves of watered and unwatered crested wheatgrass and squirreltail wild rye. Watering increased  $F_v/F_m$  in the sampled structures of both species, but  $\phi_{PSII}$  was similar between watering treatments. Pre- to post-anthesis  $F_v/F_m$  levels were maintained in crested wheatgrass seed heads but declined in flag leaves, with the opposite pattern apparent in squirreltail. Watering did not affect the ETR–PPFD response, but crested wheatgrass seed heads maintained higher ETR across saturating PPFD than did squirreltail. These findings suggest (i) photochemical efficiency is expressed in structures most closely associated with reproductive effort, and (ii) documented differences in seed head photosynthetic characteristics likely include some degree of allocation to individual floret photosynthetic capacity in addition to structural characteristics. We concluded that these physiological and structural differences may contribute to the differential ability of these species to establish from seed, and may help in effective plant material selection needed to improve restoration and conservation success in sagebrush steppe rangelands.

**Keywords:** aridland; bunchgrass; chlorophyll fluorescence; photosynthesis; reproduction; restoration.

## Introduction

Establishing self-sustaining bunchgrass populations is critical to the successful restoration and improved resiliency of the Great Basin sagebrush steppe degraded by invasive grass-driven altered fire regimes (Davies *et al.* 2010; Morris *et al.* 2011; Chambers *et al.* 2014; Svejcar *et al.* 2017). Sagebrush steppe bunchgrass population dynamics are driven primarily by sexual reproduction, and plant establishment from high-quality seed to overcome emergence, a major demographic bottleneck (Liston

*et al.* 2003; James *et al.* 2011; Hamerlynck and Davies 2019). It has long been recognized that variation in photosynthesis of reproductive structures such as fruits and seed heads is important to the reproductive effort and success of plant (Bazzaz *et al.* 1979; Raven and Griffiths 2015), especially in cereal grasses, where seed head and flag leaf photosynthesis are the principal determinants of overall grain yield (Evans and Rawson 1970; Ziegler-Jöns 1989; Wechsung *et al.* 2001; Tambussi *et al.* 2007).

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Therefore, increasing our basic understanding of reproductive ecophysiology will provide insights that could be used to improve plant material development or to mechanistically inform restoration strategies to improve sagebrush steppe restoration (Hamerlynck and Boyd 2021).

A recent series of studies have compared the reproductive ecophysiological characteristics of an exotic, widely planted bunchgrass, crested wheatgrass (*Agropyron cristatum*) to those of native Great Basin bunchgrasses. Compared to native bunchgrasses, crested wheatgrass seed heads had: (i) higher light-saturated photosynthetic rates and light-use efficiency (Hamerlynck and Ziegenhagen 2020), (ii) higher maximum photosynthetic capacity ( $A_{\max}$ ), carboxylation efficiency (CE) and lower relative stomatal limitation (RSL) than native bunchgrasses, while  $A_{\max}$ , CE and RSL were similar to native bunchgrass flag leaves (Hamerlynck et al. 2019) and (iii) seed head carbon contributions were greater than those of flag leaves to overall reproductive effort compared to native bunchgrasses (Hamerlynck and O'Connor 2021). These characteristics were consistent with crested wheatgrass's ability to produce viable seed cohorts under conditions that limit the success of native perennial grasses (Wilson and Pärtel 2003; Hamerlynck and Davies 2019).

Valuable as these studies have been, there are substantial uncertainties that need to be addressed. First, all were performed in single growing seasons, and therefore provide limited information regarding inter-annual variation in reproductive ecophysiological performance. Inter-annual precipitation variation is critical to successful restoration across semi-arid and aridland systems (Hardegee et al. 2018, 2020), and bunchgrass reproduction is associated with inter-annual precipitation and features that affect soil-water availability (Coffin and Lauenroth 1992; Vega and Montaña 2004). Crested wheatgrass has leaf-level and whole-plant attributes that likely impart higher drought tolerance compared to most native grasses (Anderson and Toft 1993; Meays et al. 2000; Hamerlynck et al. 2016; Denton et al. 2018). However, how variation in soil-water availability is related to reproductive ecophysiological performance in these aridland grasses is currently unknown. Secondly, these studies explicitly acknowledged the surface area corrections applied to photosynthetic gas exchange measurements oversimplified the structural complexity of seed heads (Hamerlynck et al. 2019; Hamerlynck and Ziegenhagen 2020). Thus, we do not know the obtained results reflect changes in architectural display, which can affect carbon assimilation ability in annual cereal grasses (Ziegler-Jöns 1989; Tambussi et al. 2007), or the ecophysiological characteristics of individual florets within the seed head itself.

To address these uncertainties, we implemented an experimental field study assessing chlorophyll fluorescence responses of seed heads and flag leaves of watered and unwatered control crested wheatgrass and a native bunchgrass, squirreltail wild rye (*Elymus elymoides*, here after squirreltail). While chlorophyll fluorescence techniques have been applied to leaf-level studies of arid and semi-arid species across extensive soil moisture and temperature conditions (Epron and Dryer 1990, 1993; Loik and Harte 1996; Barker and Adams Iii 1997; Huxman et al. 1998; Hamerlynck et al. 2000; Loik et al. 2000; Ogaya and Peñuelas 2003; Hamerlynck and Huxman 2009; Xu and Zhou 2011), they have yet to be applied to assess bunchgrass reproductive ecophysiological performance. While absolute chlorophyll fluorescence yield (F) depends on the total concentration of chlorophyll per area, F-derived ratios such as optimal PSII quantum yield ( $F/F_m$ ), light-adapted PSII quantum yield ( $\phi_{\text{PSII}}$ ) and  $\phi_{\text{PSII}}$ -derived electron transport rate (ETR) are

leaf-area independent (Kraus and Weis 1984; Baker 2008) and therefore ideal to assess individual floret performance in the aggregate. In addition to measuring watered and unwatered plants, we measured  $F/F_m$ ,  $\phi_{\text{PSII}}$  and ETR during pre- and post-anthesis periods, as previous research showed the latter is when differences in seed head photosynthetic parameters in crested wheatgrass and squirreltail are most apparent (Hamerlynck et al. 2019). We specifically expect higher soil moisture to increase  $F/F_m$  and  $\phi_{\text{PSII}}$  in seed heads and flag leaves of both species, and that these responses would be higher in the more drought-tolerant crested wheatgrass, consistent with leaf-level studies of similar semi-arid Central Asian bunchgrasses (Xu and Zhou 2011). However, if species differences in seed head performance were lacking, we could then attribute most of the differences in seed head ecophysiological performance previously observed to variation in reproductive biomass and seed head display, as postulated by Hamerlynck et al. (2019).

## Materials and Methods

Research was performed on the USDA Agricultural Research Service Northern Great Basin Experimental Range (NGBER; 119°43'W, 43°29'N), located ~70 km west of Burns, OR. The site is situated at 1402 m ASL, with a mean annual temperature of 14.8 °C, ranging from average daily maximums of 28.7 °C in July to -7.1 °C in January. Mean annual precipitation is 278.4 mm, primarily as rain, with ~71 % of this distributed evenly across the cool season period (November–May), with occasional snowfall over the coldest months. The plants sampled were selected from adult individuals established in a 14 × 145 m (2030 m<sup>2</sup>) plot used in plant demography studies from 2006 to 2011 (James et al. 2011). Soils at the site are classified as a Vil-Decantel-Ratto complex, consisting of well-drained loamy to sandy-loam Aridic Durixerols with an indurated illuvial silica duripan ( $B_{\text{qm}}$ ) beginning at 30–50 cm (Lentz and Simonson 1986). The plot was tilled and all grasses and shrubs removed prior to planting, and all weeds, annuals and volunteer remnant grasses removed for 3 years within the plot following seedling emergence and establishment. A perimeter fence has kept the area free of lagomorphs and livestock herbivory since its establishment. Seed sources were: squirreltail wild rye (*E. elymoides*) var. Toe Jam Creek from North Basin Seed, Yakima, WA, USA (Lot# NBS-CF5-TJC-1) and Granite Seed, Lehi, UT, USA (Lot# SIHY-39289), and crested wheatgrass (*A. cristatum*) var. Hycrest (Granite Seed, Lehi, UT, USA, AGDE Lot# 33426) and Hycrest II (Bruce Seed Farm, Townsend, MT, USA, Lot# 1480-BBHY9A).

Since October 2018 rooting zone volumetric soil moisture ( $\theta$ ; mol<sup>3</sup> mol<sup>-3</sup>) at 10 cm soil depth has been measured every 4 h for 20 plants for each species used in this study using 5TM probes, with data recorded on Em50 dataloggers (Decagon Devices, Pullman, WA, USA). At the time of installation, probes were inserted into the wall of a slit-trench extending into rooting zone of individual plants. Starting 15 May 2021, half of the plants monitored for  $\theta$  were randomly assigned watering treatments. Hand watering occurred weekly (every Friday), with water first applied carefully to the crown of the plant, and halted when run-off from the crown was visible on the soil surface. After this initial application was allowed to infiltrate, the application was repeated within the wetted area until soil surface water was noted and halted again; this was repeated until 5.5 L of water per plant had been applied. This protocol was followed to reduce surface run-off and maximize infiltration into the plant rooting zone.

Concurrent with watering, we noted when seed head emergence occurred and subsequently commenced twice-weekly chlorophyll fluorescence measures starting 15 June 2021. For each sampling date, we measured all plants with emergent seed heads to insure comparison under similar light and temperature conditions. Pre-anthesis measurements for squirreltail spanned 15 June to 25 June, and 15 June to 2 July for crested wheatgrass. Due to rapid anther shedding, post-anthesis sampling for squirreltail was limited to two sampling dates, 25 June and 29 June, with the bulk of post-anthesis plants sampled on 25 June. Post-anthesis sampling for crested spanned 6 July and 9 July.

Chlorophyll fluorescence ( $F$ ) measurements were made on fully emergent seed heads and flag leaves with a Li-600 porometer/fluorometer (Li-COR Instruments, Lincoln, NE, USA). Tissue was enclosed in a 0.75-cm<sup>2</sup> aperture clamp, held in its natural orientation to incident sunlight, with incident photosynthetic photon flux density (PPFD in  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) serving as the actinic light source, with flux density measured with a Si-photodiode quantum sensor integrated into the Li-600. A modulated  $F$ -measuring beam was provided by two LED light sources focused on the cuvette aperture, filtered by a 750 + 40 nm bandpass filter, with fluorescence detected from 700 to 780 nm. Initial determination of steady-state fluorescence ( $F_s$ ) used a sub-saturating modulated beam pulsed at 4–8 Hz over 2–5 s, followed by a saturating flash of 10 000  $\mu\text{mol m}^{-2} \text{s}^{-1}$  intensity with a pulse width of 667 ns and 250–750 kHz frequency to determine light-adapted maximum fluorescence yield ( $F_m'$ ). From these, light-adapted PSII ( $\phi_{\text{PSII}}$ ) yield was determined as  $(F_m' - F_s)/F_m'$ .  $\phi_{\text{PSII}}$  was used to determine ETR as  $\text{ETR} = \phi_{\text{PSII}} * \text{PPFD} * \text{abs} * \text{PS2/1}$ , leaf absorbance (abs) and fraction of PSII absorbance (PS2/1) assumed to be 0.8 and 0.5, respectively (Li-COR Biosciences 2020). As absorbance can differ between species and with tissue water relations (Larcher 1995; Ustin and Jacquemoud 2020), we hereafter refer to ETR as 'apparent ETR'. Sampled flowering culms were marked with a tag at the base of the culm. Once all marked, seed heads and flag leaves measured for  $\phi_{\text{PSII}}$  were wrapped with a ca. 20 cm strip of reflective mylar film and dark-adapted for ca. 45–60 min. To minimize breakage, the wrapped tissue was gently attached to a pin flag with one or two twist ties. After dark adaptation, chlorophyll fluorescence measurements were repeated in a 'dark chamber' provided by enclosing the operator under a multilayer covering of space blankets sealed with bean-bags around the base until no external light could be detected [see Supporting Information—Fig. S1]. Seed heads were sampled first; prior to this, flag leaves were inserted into a plastic straw wrapped in electrical tape to avoid inducing any photochemical activity by the saturating flash made while measuring the seed head. Dark-adapted measurements provided baseline fluorescence ( $F_0$ ; equivalent to  $F_s$ ) and maximum fluorescence yield ( $F_m$ ; equivalent to  $F_m'$ ) to determine optimal PSII yield ( $F_v/F_m$ ) as  $F_v/F_m = (F_m - F_0)/F_m$ .

We used a split-plot two-way repeated-measures analysis of variance (RM-ANOVA; Statistix v8.0, Analytical Software, Tallahassee, FL, USA) to test for differences in  $\theta$ ,  $F_v/F_m$  and  $\phi_{\text{PSII}}$  between species-watering treatment combination and pre- and post-anthesis phenological stages. As pre- and post-anthesis periods were of differing length and the number of observations varied considerably between the species, we averaged all pre- and post-anthesis observations for each replicate plant, then performed the RM-ANOVA using these results. The species-by-watering treatment combination was used as the whole-plot term, using the species-treatment by replicate plant interaction as the  $F$ -test error term. The subplot, repeated-measure effects

was phenological stage and the species-treatment combination by phenological period interaction effect. We analysed  $\theta$  averaged for entire day preceding and up to 12:00 PM of the chlorophyll fluorescence sampling day. As all the measured variables were proportional, data were arcsine transformed to meet ANOVA data distribution assumptions. Post hoc means testing for species-watering combination and phenological stages were made using  $\alpha$ -adjusted least significant difference (LSD); general linear contrasts (Scheffe's  $F$ ) were used to make full pairwise comparisons to determine significant differences contributing to any significant two-way species-watering treatment  $\times$  phenological stage interactions.

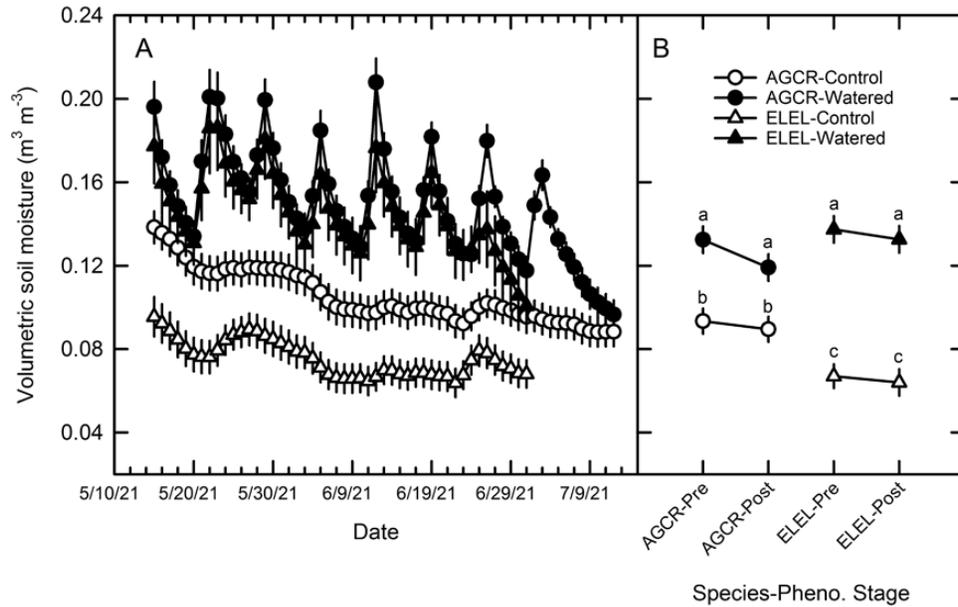
To simplify our analysis, we did not directly compare seed head and flag leaf photochemical dynamics. Rather, we used a one-way ANOVA (Statistix v8.0) to compare species-watering treatment combinations pooled across pre- and post-anthesis periods, using the species-watering treatment by replicate plant interaction effect as the  $F$ -test error term, as per Hamerlynck et al. (2019). Pairwise comparisons were made using  $\alpha$ -adjusted LSD. This allows us to ascertain how 'leaf-like' the seed heads were between the species and in response to increased soil-water availability.

We also assessed the  $\phi_{\text{PSII}}$  and  $\phi_{\text{PSII}}$ -derived apparent ETR responses to PPFD of crested wheatgrass and squirreltail seed heads pooled across the entire study. A curvilinear regression model was fit which included PPFD as a quadratic polynomial, with species and watering treatment included as independent variables. This was compared to a linear regression model including the same independent variables fit to the data. The linear and curvilinear models were tested against one another using ANOVA model selection (car package, version 3.0-11; Fox and Weisberg 2019); based on a  $P$ -value  $< 0.05$  the quadratic model was chosen for its best fit. All model assumptions were met, and model significance was set *a priori* at 0.05. The model selection, assumptions and analysis were done using RStudio (version 3.6.3) and the tidyverse (version 1.3.0) package (R Core Team 2020; Wickham et al. 2019).

## Results

Crested wheatgrass unwatered control plants had consistently higher  $\theta$  than squirreltail counterparts throughout the study period, and our weekly watering treatment reduced these differences to similar high  $\theta$  for the two species (Fig. 1A). The decline in rooting zone  $\theta$  in watered plants marks when irrigation ceased after the final post-anthesis measurement for each species (Fig. 1A). For the chlorophyll fluorescence study period,  $\theta$  significantly differed between the species-by-watering treatments, with no other significant effects (Table 1). Irrigation resulted similar high  $\theta$  in irrigated soils under crested wheatgrass and squirreltail plants, with significantly lower  $\theta$  in control plants, where crested wheatgrass soil  $\theta$  was significantly higher than soils under squirreltail controls (Fig. 1B).

Seed head  $F_v/F_m$  significantly differed between species-by-watering treatment combinations, pre- and post-anthesis stages, with a significant species-treatment by phenological stage interaction (Table 1). Post hoc pairwise LSD means comparisons showed watered squirreltail seed head  $F_v/F_m$  ( $0.75 \pm 0.016$  SE) was equal to crested wheatgrass counterparts ( $0.72 \pm 0.016$  SE), and that these were significantly higher than levels in unwatered control crested wheatgrass ( $0.61 \pm 0.015$  SE) and squirreltail ( $0.56 \pm 0.015$  SE), which were statistically indistinguishable. Overall, post-anthesis  $F_v/F_m$  ( $0.63 \pm 0.0156$  SE) was lower than  $F_v/F_m$



**Figure 1.** Volumetric soil moisture ( $\theta$ ;  $\text{mol}^3 \text{mol}^{-3}$ ) of rooting zones under watered (closed symbols) and unwatered control (open symbols) crested wheatgrass (AGCR; circles) and squirreltail wild rye (ELEL; triangles) (A) over the course of the entire 2021 irrigation application, and (B) over pre- and post-anthesis chlorophyll fluorescence measurement periods. Letters differ significantly (LSD  $P < 0.05$ ; from two-way ANOVA, Table 1).

**Table 1.** Two-way RM-ANOVA F-test results for rooting zone volumetric soil moisture ( $\theta$ ), and seed head and flag leaf optimal ( $F_v/F_m$ ) and ambient-light PSII quantum yields ( $\phi_{\text{PSII}}$ ) of watered and unwatered crested wheatgrass and squirreltail wild rye (Spp  $\times$  Water Combo) across pre- and post-anthesis sampling periods (Stage). F-test degrees of freedom (df) are presented parenthetically; \* indicate significant effects at  $P < 0.05$ ; all results are from arcsine transformed data.

Effect <sub>(df)</sub>	$\theta$	Seed head $F_v/F_m$	Seed head $\phi_{\text{PSII}}$	Flag leaf $F_v/F_m$	Flag leaf $\phi_{\text{PSII}}$
Spp $\times$ Water Combo <sub>(3, 17)</sub>	11.00*	14.36*	2.66 <sup>ns</sup>	1.88 <sup>ns</sup>	0.57 <sup>ns</sup>
Stage <sub>(1, 43)</sub>	1.87 <sup>ns</sup>	8.28*	86.69*	12.22*	14.09*
Combo $\times$ Stage <sub>(3, 43)</sub>	0.30 <sup>ns</sup>	3.09*	3.92*	8.07*	0.45 <sup>ns</sup>

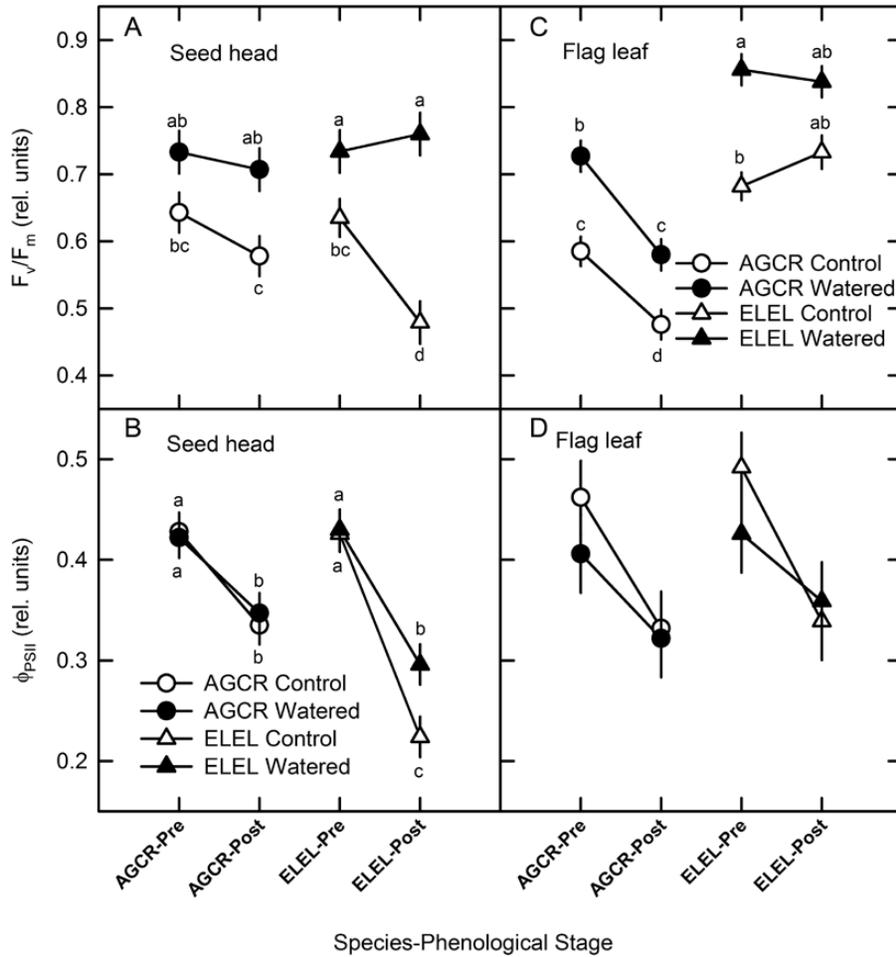
$F_m$  over pre-anthesis periods ( $0.69 \pm 0.0152$  SE; LSD  $< 0.05$ ). The two-way interaction effect in seed head  $F_v/F_m$  was likely due to (i) a lack of significant species differences and pre- and post-anthesis differences in irrigated plants, and (ii) more marked pre- to post-anthesis declines in  $F_v/F_m$  in squirreltail controls compared to crested wheatgrass counterparts (Fig. 2A). In contrast to seed head  $F_v/F_m$ , seed head  $\phi_{\text{PSII}}$  only significantly differed between phenological stages, with a significant species-treatment by phenological stage interaction (Table 1). As in  $F_v/F_m$  pre-anthesis  $\phi_{\text{PSII}}$  ( $0.43 \pm 9.61 \times 10^{-3}$  SE) exceeded post-anthesis levels ( $0.30 \pm 9.90 \times 10^{-3}$  SE; LSD  $< 0.05$ ). The two-way interaction was likely due to significant  $F_v/F_m$  differences between watered and unwatered in post-anthesis squirreltail that were not apparent in crested wheatgrass seed heads (Fig. 2B).

Flag leaf  $F_v/F_m$  differed between phenological stages, with a significant species-treatment by phenological stage interaction (Table 1). As with  $F_v/F_m$ ,  $\phi_{\text{PSII}}$  declined significantly from pre-anthesis ( $0.71 \pm 0.011$  SE) to post-anthesis stages ( $0.66 \pm 0.012$  SE; LSD  $< 0.05$ ). The two-way interaction likely arose from a lack of pre- to post-anthesis differences in both watered and control squirreltail flag leaves, while post-anthesis declines in flag leaf  $F_v/F_m$  were significantly more pronounced in crested wheatgrass (Fig. 2C). In addition, similar pre- and post-anthesis  $F_v/F_m$  in watered crested wheatgrass and unwatered control

squirreltail plants (Fig. 2C) may have contributed to the two-way interaction. Flag leaf  $\phi_{\text{PSII}}$  significantly differed only between pre- and post-anthesis stages (Table 1), and was higher in over the pre-anthesis ( $0.45 \pm 0.018$  SE) compared to post-anthesis levels ( $0.35 \pm 0.019$  SE; LSD  $< 0.05$ ; Fig. 2D).

Seed head to flag leaf  $F_v/F_m$  ratios significantly differed between species-by-watering treatment combinations ( $F_{3, 17} = 24.62$ ;  $P < 0.001$ ), while those for  $\phi_{\text{PSII}}$  did not ( $F_{3, 17} = 1.43$ ;  $P = 0.269$ ). Crested wheatgrass had similar high seed head to flag leaf  $F_v/F_m$  ratios between water and unwatered controls, while squirreltail ratios, though lower than those in crested wheatgrass, increased significantly with watering (LSD  $\leq 0.05$ ; Table 2).

The full curvilinear PPF-response model relationship was highly significant for seed head  $\phi_{\text{PSII}}$  (adjusted  $R^2 = 0.73$ ;  $F_{4, 196} = 138.0$ ;  $P < 0.001$ ) and  $\phi_{\text{PSII}}$ -derived apparent ETR (adjusted  $R^2 = 0.69$ ;  $F_{4, 196} = 110.2$ ;  $P < 0.001$ ). Watering treatment did not contribute significantly to the overall model ( $t = 0.191$ ,  $P = 0.849$  and  $t = -0.739$ ,  $P = 0.461$  for  $\phi_{\text{PSII}}$  and apparent ETR, respectively), while species identification did ( $t = -2.93$ ;  $P = 0.004$  for both  $\phi_{\text{PSII}}$  and apparent ETR, respectively). These follow overall higher  $\phi_{\text{PSII}}$  in crested wheatgrass seed heads at lower PPF ( $< 250 \mu\text{mol m}^{-2} \text{s}^{-1}$ ; Fig. 3A) and higher  $\phi_{\text{PSII}}$  and attendant ETR in at higher, saturating PPF ( $> 1500 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) compared to those in squirreltail (Fig. 3B).



**Figure 2.** Dark-adapted optimal PSII yield ( $F_v/F_m$ ) and ambient light-adapted PSII quantum yield ( $\phi_{PSII}$ ) of seed heads (A and B) and flag leaves (C and D) for watered (closed symbols) and unwatered control (open symbols) plants of crested wheatgrass (AGCR; circles) and squirreltail wild rye (ELEL; triangles) over pre- and post-anthesis periods. Letters differ significantly (general linear contrasts  $P < 0.05$ ; from two-way ANOVA, Table 1).

**Table 2.** Ratio of seed head to flag leaf optimal ( $F_v/F_m$ ) and light-adapted ( $\phi_{PSII}$ ) quantum yields of irrigated and unirrigated crested wheatgrass (AGCR) and squirreltail wild rye (ELEL) pooled across pre- and post-anthesis stages. Letters differ at  $P < 0.05$ , LSD from one-way ANOVA.

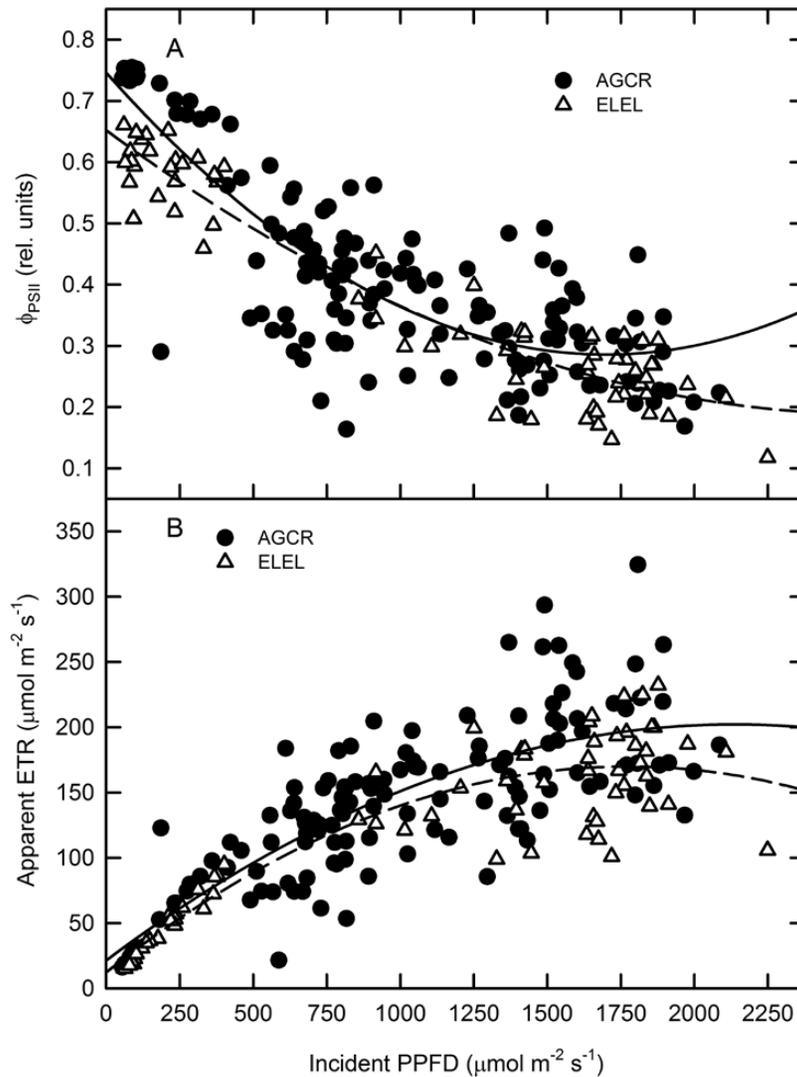
Species × watering combination	$F_v/F_m$ ratio	$\phi_{PSII}$ ratio
AGCR-Watered	1.15 <sup>a</sup> (0.026)	1.09 <sup>a</sup> (0.066)
AGCR-Control	1.10 <sup>a</sup> (0.025)	0.97 <sup>a</sup> (0.062)
ELEL-Watered	0.91 <sup>b</sup> (0.026)	1.01 <sup>a</sup> (0.066)
ELEL-Control	0.77 <sup>c</sup> (0.026)	0.86 <sup>a</sup> (0.062)

## Discussion

Contrary to our expectations, watering only improved  $F_v/F_m$  of both seed heads and flag leaves, while  $\phi_{PSII}$  was largely unaffected in both structures (Fig. 2). Watering imparted less increased available soil moisture in crested wheatgrass rooting zones, which had higher  $\theta$  in unwatered control treatments compared to those of squirreltail. Subsequently, crested wheatgrass had a more muted increased  $F_v/F_m$  in response to watering compared to squirreltail (Fig. 2A; Table 2). Given the considerable effort in homogenizing soils prior planting (James et al. 2011), it is

unlikely higher  $\theta$  under crested wheatgrass reflects variation in soil texture or some other microsite difference that could affect soil  $\theta$ . Crested wheatgrass's more extensive canopy could reduce evaporative losses compared to more the more compact canopy of squirreltail. Bunchgrass architecture is thought to concentrate limiting resources to the grass (Smith et al. 1997), and canopy structure can affect soil moisture and attendant whole-plant gas exchange in bunchgrasses (Hamerlynck et al. 2010). Therefore, as the two species had markedly different starting and control  $\theta$  conditions, it is difficult to directly ascertain from our results if crested wheatgrass is more drought-tolerant—or if squirreltail is more drought-resilient—at the organelle membrane level resolution that chlorophyll fluorescence measurements provide (Baker 2008).

The expression of pre- to post-anthesis  $F_v/F_m$  in reproductive structures differed markedly between crested wheatgrass and squirreltail. In crested wheatgrass,  $F_v/F_m$  did not vary significantly from pre- to post-anthesis sampling in seed heads, while declines in flag leaf  $F_v/F_m$  were pronounced; this pattern was essentially reversed in squirreltail (Fig. 2). Hamerlynck and O'Connor (2021) suggested that photosynthetic activity of the seed head itself was the primary carbon source for reproductive effort in crested wheatgrass compared to a more equal degree contributions by the seed head and flag leaf in squirreltail. It may be, therefore, our results show evidence of



**Figure 3.** Incident photon flux density (PPFD) responses of (A) light-adapted PSII quantum yields ( $\phi_{\text{PSII}}$ ) and (B)  $\phi_{\text{PSII}}$ -derived apparent ETR for seed heads of crested wheatgrass (AGCR; closed circles, solid line) and squirreltail wild rye (ELEL; open triangles, dashed line) pooled across watering treatments and reproductive phenological stages.

greater allocation to photochemical efficiency in the structure most likely contributing to reproductive effort in these two species. The marked declines in post-anthesis seed head  $F_v/F_m$  in squirreltail also suggest strongly that flag leaf contributions in this species may be more important than previously realized (Hamerlynck and O'Connor 2021).

While seed head  $\phi_{\text{PSII}}$  did not strongly differ between species (Fig. 2), the higher recovery of  $F_v/F_m$  in response to dark-adapting in crested wheatgrass seed heads may indicate variation in photoprotective mechanisms from the native grass. Squirreltail could be engaging PSII antennae-based, thermal dissipative mechanisms to a greater degree, or relaxing these quenching mechanisms more slowly (Baker and Adams 1997; García-Plazaola et al. 2012). Alternatively, squirreltail may have engaged more slowly reversing reaction-centre-based photoprotective mechanisms (Osmond 1994). Unfortunately, as our sampling protocol could not insure measuring exactly the same tissue sampled under ambient light, we could not consistently attain reliable absolute maximum and minimum  $F$  yields between dark- and light-adapted samples needed to determine

the quenching coefficients to discriminate between these mechanisms (Baker 2008; see Supporting Information—Excel file (S1)). Regardless, the end result is that ETR, a proxy for gross photosynthetic rate (Baker 2008), was higher in crested wheatgrass than squirreltail across the full range of environmental and phenological conditions of this study (Fig. 3). Higher  $F_v/F_m$  and higher sustained ETR are consistent with higher photosynthetic light-use efficiency and light-saturated photosynthetic rates (Hamerlynck and Ziegenhagen 2020) and higher  $A_{\text{max}}$  and CE (Hamerlynck et al. 2019) observed in seed heads of this species. Thus, variation in these photosynthetic characteristics may, in some degree, reflect higher allocation to individual floret photosynthetic capacity. However, we should note the apparent ETR reported here assume identical leaf and PSII absorptance characteristics; as these could be affected by intrinsic species differences in photosynthetic pigment concentrations, pigment ratios or by variation in tissue water relation responses to soil-water availability (Larcher 1995; Ustin and Jacquemoud 2020), our apparent ETR may not fully reflect the absolute magnitude of electron transport attained by these structures.

Following Hamerlynck et al. (2019), we did not directly compare seed head and flag leaf performance, but rather compared the ratio of seed head to flag leaf results, which provides an index of how 'leaf-like' attained seed head performance was. Hamerlynck et al. (2019) found that  $A_{\max}$  and CE seed head/flag leaf ratios in crested wheatgrass consistently exceeded 1.0, but never approached 1.0 in native species, including squirreltail wild rye, indicating seed heads of the exotic grass were functionally more 'leaf-like' than native grasses. Consistent with Hamerlynck et al. (2019), crested wheatgrass  $F_v/F_m$  seed head/flag leaf ratios were consistently near 1.0, and markedly higher than those of squirreltail (Table 2). In addition, the significant increase in seed head/flag leaf  $F_v/F_m$  ratios in squirreltail with watering (Table 2) may indicate a functional shift, with squirreltail seed heads having the potential to physiologically contribute more in more favourable soil moisture conditions (Table 2). In contrast, the relatively invariant  $F_v/F_m$  ratio in crested wheatgrass suggests it has the capacity to maintain more consistent reproductive photosynthetic activity across years or landscape features with contrasting soil moisture conditions. Our findings and those of Hamerlynck et al. (2019) are consistent with crested wheatgrass's well-documented ability to more regularly produce viable seed cohorts across a wide range of environmental conditions than native bunchgrasses (Wilson and Pärtel 2003; Hamerlynck and Davies 2019). However, we should note that seed head/flag leaf ratios obtained for  $F_v/F_m$  are considerably lower than those derived from gas exchange measurements (Hamerlynck et al. 2019), as are light-adapted  $\phi_{\text{PSII}}$  seed head/flag leaf ratios which were consistently similar between the two species (Table 2). These suggest that much of the enhanced seed head photosynthetic gas exchange performance documented by Hamerlynck et al. (2019) most likely followed variation in seed head structure rather than dramatic differences in photosynthetic capacity and ecophysiological characteristics of individual florets.

In summary and in conclusion, this study shows allocation to photochemical functioning is differentially expressed between seed heads and flag leaves in these two grasses, and that soil-water availability modulates the degree to which these differences are expressed. Paired chlorophyll fluorescence and gas exchange studies will need to be made to fully discriminate between structural and physiological contributions to variation in seed head photosynthetic dynamics. These should be done in conjunction with experimentally controlled soil moisture across a range of similar low to high levels to fully ascertain tissue-level drought tolerance, as well as structural and physiological contributions to seed head photosynthesis and attendant reproductive effort. Information from such studies is needed to develop truly effective cultivars, which frequently do not possess the functional attributes needed for successful restoration of aridland ecosystems (Blumenthal et al. 2021; Garbowski et al. 2021).

## Supporting Information

The following additional information is available in the online version of this article—

**Excel file (S1).** raw data.

**Figure S1.** Sequence of photographs showing preparations to sample dark-adapted chlorophyll fluorescence measurements of bunchgrasses under a multilayered space blanket.

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## Contributions by the Authors

E.P.H. and R.C.O. conceived, designed, and implemented the experiment. E.P.H. performed the RM-ANOVA and ANOVA analyses, prepared all figures, and led writing the initial and final versions of the MS. R.C.O. performed the curvilinear regression analysis, and contributed to writing the initial and final versions of the MS.

## Conflicts of Interest

None.

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## Data Availability

All data used for analysis are provided in the accompanying **Supporting Information—Excel file (S1)**.

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