

Photosynthetic regulation in seed heads and flag leaves of sagebrush-steppe bunchgrasses

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Native sagebrush-steppe bunchgrass populations are threatened by the spread and dominance of exotic invasive annual grasses, in part due to low, episodic seed production. In contrast, the widespread exotic bunchgrass, crested wheatgrass, readily produces viable seed cohorts. The mechanisms underlying these differences are unclear. To address this, we measured seed head specific mass (g m^{-2}) and net photosynthetic assimilation (A_{net}) as a function of internal $[\text{CO}_2]$ (A/C_i curves) in pre- and post-anthesis seed heads and flag leaves of crested wheatgrass and four native bunchgrasses to determine if differences in allocation and photosynthetic characteristics of seed heads was consistent with differential reproductive success. Crested wheatgrass seed heads had 2-fold greater specific mass compared to the native grasses, concurrent with greater CO_2 -saturated photosynthesis (A_{max}), mesophyll carboxylation efficiency (CE), and higher intrinsic water-use efficiency (WUE_i ; $A_{\text{net}}/\text{stomatal conductance } (g_s)$), but with similar relative stomatal limitations to photosynthesis (RSL). Post-anthesis seed head A_{max} , CE, RSL and g_s decreased in native grasses, while crested wheatgrass RSL decreased and CE increased dramatically, likely due to tighter coordination between seed head structural changes with stomatal and biochemical dynamics. Our results suggest native sagebrush-steppe bunchgrasses have greater stomatal and structural constraints to reproductive photosynthesis, while the exotic grass has evolved seed heads functionally similar to leaves. This study shows elucidating reproduction-related ecophysiological mechanisms provide understanding of plant attributes that underlie restoration success and could help guide the development of native plant materials with functional attributes needed to overcome demographic bottlenecks that limit their restoration into degraded sagebrush-steppe.

Key words: A/C_i curves, A_{max} , carboxylation efficiency, crested wheatgrass, relative stomatal limitation, sagebrush-steppe

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Introduction

Conservation of ecosystem function is critical in the face of ongoing climate change, and a major challenge in ecosystem conservation is maintaining viable plant populations and diverse plant communities (Schwartz *et al.* 2000; Luck *et al.* 2003). This is very much the case for North American

sagebrush-steppe rangeland ecosystems, which face increasingly extensive degradation due to the combined effects of invasive annual grasses and ongoing climate change (Davies *et al.* 2011; Svejcar *et al.* 2017). Over the last century, invasive annual grasses, especially cheatgrass (*Bromus tectorum* L.), have invaded over 28 million ha in the Intermountain West

region of Idaho, Oregon, Nevada, Utah and Washington (Meinke *et al.* 2009). Invasive annuals greatly reduce the diversity and productivity of sagebrush-steppe rangelands by forming stronger competitive regimes, altering nutrient cycling and accelerating fire frequency and severity (Bradley *et al.* 2006; James *et al.* 2008; Davies 2011; Perkins and Nowak 2012; Chambers *et al.* 2014). In sagebrush-steppe, the rate invasive annual spread and accelerated fire frequency so outstrips the natural rate of recovery, and restoration is effectively the only means of conservation (Davies *et al.* 2011). Therefore, elucidating the differences in the biophysical and physiological mechanisms between successful and less successful restoration species is critical to formulating effective ecosystem conservation strategies in these ecologically and economically important rangelands (Davies *et al.* 2011; Madsen *et al.* 2014; Svejcar *et al.* 2017).

Establishing stable perennial bunchgrass populations is the most economical and effective method to halt and reverse the spread of invasive annual grasses and restoring sagebrush-steppe, but its success rates are very low (ca. 3%), and limited by low seedling emergence and establishment success (Morris *et al.* 2011; James *et al.* 2011; Clements *et al.* 2017). It has become increasingly apparent that there is a need to identify and exploit mechanisms that can maximize seed production to enhance restoration and conservation success (Broadhurst *et al.* 2008; Godefroid *et al.* 2011). While establishing native bunchgrass species from seed is frequently problematic, the introduced bunchgrass, crested wheatgrass (*Agropyron cristatum* (L.) Gaertn.), readily establishes from seed and has proven effective in reducing the establishment and spread of invasive annual grasses (Davies *et al.* 2010; Clements *et al.* 2017). Though crested wheatgrass does compete with native bunchgrasses, it does so to a lesser degree than invasive annuals (Perkins and Nowak 2012), dominance by which it has highly negative effects on the integrity of sagebrush-steppe plant community diversity and ecosystem function (Bradley *et al.* 2006; Davies 2011; Chambers *et al.* 2014). In addition to ecophysiological and growth-allocation patterns that impart greater seedling grazing and drought tolerance (Hamerlynck *et al.* 2016a; Denton *et al.* 2018), crested wheatgrass's success is attributed to its ability to regularly produce viable seeds, even when exposed to stringent levels of control (Bakker *et al.* 2003; Wilson and Pärtel 2003; Fansler and Mangold 2011; Hamerlynck and Davies 2019). The mechanisms by which crested wheatgrass consistently produces viable seed crops are poorly understood, and improving our understanding of them could give valuable insights to guide native plant material selection needed to enhance the success of establishing native grasses or augmenting vulnerable extant native plant populations (Elstrand and Elam 1993; Turnbull *et al.* 2003).

Allocation to photosynthetically active reproductive structures can contribute to overall reproductive effort and success (Bazzaz *et al.* 1979; Raven and Griffiths 2015). In annual cereal crop grasses, photosynthetic contributions from the flag leaf are typically the principal carbon source for seed fill-

ing, though photosynthetic fixation within the seed head can equal or exceed these (Evans and Rawson 1970; Austin *et al.* 1982; Ziegler-Jöns 1989; Wechsung *et al.* 2001; Tambussi *et al.* 2007; Rangan *et al.* 2016; Sanchez-Bragado *et al.* 2016; Taylor and Long 2017). Compared to cereal crops, reproductive photosynthesis in perennial grasses is not well studied. Only perennial rye grass (*Lolium perenne* L.) has received some attention; here, carbon from within the seed head—i.e. glumes, florets and the supporting culm—typically contribute more carbon to seed production than the flag leaf (Ong *et al.* 1978; Warringa and Kreuzer 1996; Warringa and Marinissen 1997; Warringa *et al.* 1998). To date, there have been no studies assessing the photosynthetic capacity of reproductive structures in perennial grasses in natural ecosystems. Given the importance of establishment from seed in bunchgrass population dynamics (Liston *et al.* 2003; Hamerlynck and Davies 2019), such information could provide valuable mechanistic insights into the differential success and persistence of bunchgrasses in sagebrush-steppe ecosystems.

Here, we present a study comparing allocation to photosynthetically active seed heads by quantifying seed head-specific mass (g m^{-2} surface area) and attendant photosynthetic characteristics in the exotic bunchgrass, crested wheatgrass and four native bunchgrass species. We measured net photosynthesis (A_{net}) as a function of internal CO_2 concentration (A/C_i response curves) to estimate CO_2 -saturated net photosynthesis as an indicator of maximum photosynthetic capacity (A_{max}) and mesophyll carboxylation efficiency (CE) and relative stomatal limitation (RSL) of emergent seed heads over pre- and post-anthesis periods for all five species. In addition, we measured A_{net} and stomatal conductance to water vapour (g_s) and determined intrinsic water use efficiency ($\text{WUE}_i = A_{\text{net}}/g_s$) at ambient atmospheric CO_2 to gauge performance under prevailing field conditions. We also determined these photosynthetic parameters in flag leaves. We did so for two reasons: first, comparing seed heads to leaves provides a meaningful benchmark to compare the ecophysiological characteristics of different species seed heads. Secondly, it is not clear whether the reproductive ability of crested wheatgrass is due to greater photosynthetic capacity of its flag leaves, as in high-yield cereal hybrids (Evans and Rawson 1970; Austin *et al.* 1982; Taylor and Long 2017), or higher seed head photosynthetic capacity, as in the one perennial grass studied (Ong *et al.* 1978; Warringa and Kreuzer 1996; Warringa and Marinissen 1997). Given that the ability of crested wheatgrass consistently produces viable seed cohorts (Bakker *et al.* 2003; Wilson and Pärtel 2003; Fansler and Mangold 2011), we hypothesized seed heads of this exotic bunch grass to have (i) greater seed head-specific mass and (ii) reproductive photosynthetic characteristics distinct from native bunchgrasses. We postulated three potential ways reproductive photosynthesis in the exotic grass could differ from native grasses:

(i) Crested wheatgrass flag leaves would have higher photosynthetic capacity and performance, while seed head characteristics would be similar to native species.

(ii) Crested wheatgrass seed heads would have higher capacity and performance, but with flag leaf characteristics similar to native grasses.

(iii) Higher capacity and performance would be attained in both flag leaves and seed heads than in native grasses.

While this approach does not partition flag leaf and the seed head contributions to reproductive effort, it does allow us to determine if photosynthetic characteristics of the two main plant features associated with reproductive investment and effort is consistent with the ability of the exotic grass to better produce viable seed cohorts compared to native sagebrush-steppe bunchgrasses.

Materials and methods

The study took place from 15 May to 3 July 2018 on the USDA Agricultural Research Center 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 and has 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. The mean annual precipitation is 278.4 mm, primarily as rain, with ca. 71% of this distributed evenly across the November to May cool season period, with occasional snowfall over the coldest months. Grasses were sampled in a large-level area of intact sagebrush-steppe, enclosed and protected from livestock grazing following completion of construction of five rainout shelters in 1994 (Svejar *et al.* 1999). The total plant cover at the site is 31.1%, with 29.5% perennial plant cover, with the bulk of this in the form of perennial grasses (15.0%) and shrubs (9.6%, primarily sagebrush, *Artemisia tridentata*); the exotic annual grass, *Bromus tectorum*, is infrequent and sparsely distributed (0.01% cover; Hamerlynyck *et al.* 2016b).

We gathered one seed head from 7–14 individual plants per species to assess allocation to reproductive biomass. The four native grasses were squirreltail (*Elymus elymoides*; ELEL), prairie junegrass (*Koeleria macrantha*; KOMA), basin wildrye (*Leymus cinereus*; LECI) and bluebunch wheatgrass (*Pseudoroegneria spicata*; PSSP) and the exotic bunchgrass, crested wheatgrass (*Agropyron cristatum*; AGCR). The four native species are desirable forage grasses (USDA 2019) and were selected to provide as great a diversity of native grasses that could be sampled in sufficient numbers as possible. All plants in the study area were established from existing local seed sources; as such, we could not ascertain what cultivar of crested wheatgrass is present in the study area. Seedheads were scanned on a flat-bed scanner. Images were used to determine the total seed head area, estimating the total area on a projected area basis for crested wheatgrass and on a cylindrical basis for native grasses, with the diameters and lengths of individual elements automatically determined and summed by WinRhizo image analysis software (Regent Instruments, Quebec, QE, Canada). Samples were dried at 48°C for at least

72 h, weighed to the nearest 0.0001 g to determine specific mass (g m^{-2}).

Five plants of each species in the study area were randomly selected for emergent seed head and flag leaf gas exchange measurements. Plants were selected prior to seed head emergence, and we visited the site on a regular basis until such emergence was noted. For each sampling date, we measured all species with emergent seed heads to ensure comparison of gas exchange dynamics under similar soil moisture and temperature conditions. Pre-anthesis measurements for crested wheatgrass, squirreltail, prairie junegrass and bluebunch wheatgrass were made between 29 May and 6 June 2018. Pre-anthesis measurements for basin wildrye were made on 12 June and from 20 to 22 June 2018. We were able to complete two pre-anthesis samples for all species except squirreltail (ELEL), which displayed very rapid emergence and anthesis, permitting only one pre-anthesis sample. Post-anthesis measures made after anthers had exerted then dried and fell off; we were able to do only one sample date for post-anthesis sampling, as drying soil conditions resulted in the senescence of most of the species flag leaves. Post-anthesis measurements for crested wheatgrass, squirreltail, prairie junegrass and bluebunch wheatgrass were made from 19 June to 21 June 2018 and on 2 July 2018 for basin wildrye.

On each sampling date, gas exchange measurements were made from 0830 to 1600, with a random sampling order to avoid any confounding diurnal-species effects. Three-centimetre lengths of flag leaf or seed head were enclosed in the cuvette of a LiCOR 6800 portable photosynthesis system (LiCOR Instruments, Lincoln NE, USA). We took care to orient and stabilize the cuvette such that all enclosed samples were as close to their natural orientation as possible. Saturating light ($1500 \mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetic photon flux density) was supplied by red/blue LED light source attached to cuvette, set to a default red: blue ratio of 9:1. Relative humidity in the cuvette was maintained at 25% by automatically passing a portion of the airstream through a column of Stuttgart-masse saturated with de-ionized water, with the temperature of a Peltier-exchange temperature control block set to 25.0°C. This resulted in leaf temperatures measured with a fine-wire thermocouple of 24.0 to 30.0°C, and leaf-to-air vapour pressure deficits of 1.5 to 3.0 kPa, depending on the time of day. To minimize leak effects, a high-speed fan speed was set to maintain a pressure difference of 0.1 kPa between cuvette and the outside atmosphere.

Reference cell CO_2 concentration was initially set to 400 ppm, and reference and sample cells allowed to stabilize ($[\text{CO}_2]$ and $[\text{H}_2\text{O}]$ slope vs time less than $1.0 \mu\text{mol min}^{-1}$, with a standard deviation less than 0.1), then matched to a common air stream to eliminate reference and sample cell infrared gas analyzer (IRGA) differences. Reference cell $[\text{CO}_2]$ was then reduced to 50 ppm, then increased to 100, 200, 300, 400, 600, 800, 1200 and 2000 PPM set points,

with net photosynthetic rate (A_{net}), stomatal conductance to water vapour (g_s) and internal CO_2 (C_i) recorded at each following IRGA matching after a 1-min minimum stabilizing period. Prior to enclosure, the width of leaf blades and seed heads was measured to the nearest 0.5 mm with a ruler. If the structure was flat, the width was multiplied by the cuvette length (3 cm) to area correct gas exchange measurements; if curled or round, the area was determined as half that of an open-ended cylinder of the measured diameter and length. Only crested wheatgrass had flat seed heads; all native grasses had cylindrical seed heads.

Each A/C_i curve was analyzed using non-linear regression (SigmaPlot v12.3, Systat Software, San Jose, CA, USA). Data was fitted to the function $A = y_0 + a(1 - e^{-b \cdot C_i})$ to determine the maximum photosynthetic assimilation rate ($A_{\text{max}} = a + y_0$), an indicator of photosynthetic capacity, and mesophyll carboxylation efficiency (CE), estimated as first-order derivative of the function at $A = 0$; $dA/dC_i = A_{\text{max}} \cdot b$ (Jacob *et al.* 1995). Relative stomatal limitation (RSL) was calculated as $\text{RSL} = (1 - A_a/A_o) \cdot 100$, where A_a is the photosynthetic rate when the reference cell was at ambient atmospheric CO_2 concentrations (C_a ; 400 ppm) and A_o is

the photosynthetic rate when C_i is the same as C_a (Tissue *et al.* 2005). We used the A_{net} and g_s data recorded at C_a to determine the intrinsic water use efficiency ($\text{WUE}_i = A_{\text{net}}/g_s$) of seed heads and flag leaves.

Statistical analyses

Species differences in seed head specific mass were analyzed using one-way analysis of variance (ANOVA, Statistix v8.0; Analytical Software, Tallahassee, FL), with α -adjusted means comparisons made using LSD. We used two-way analysis of variance (ANOVA; Statistix v8.0) to test for differences in flag leaf and seed head A_{max} , CE, RSL and WUE_i between species and pre- and post-anthesis sampling periods, using the species-by-period-by-replicate interaction as the F test error term. To simplify our analysis, we did not directly compare seed head to flag leaf photosynthetic characteristics. We did test for species differences in the ratios of seed head to flag leaf levels of the six photosynthetic parameters using one-way analysis of variance (Statistix v8.0) to ascertain how 'leaf-like' the seed heads were across the species. In all our analyses, post-hoc, α -adjusted means tests were made using LSD.

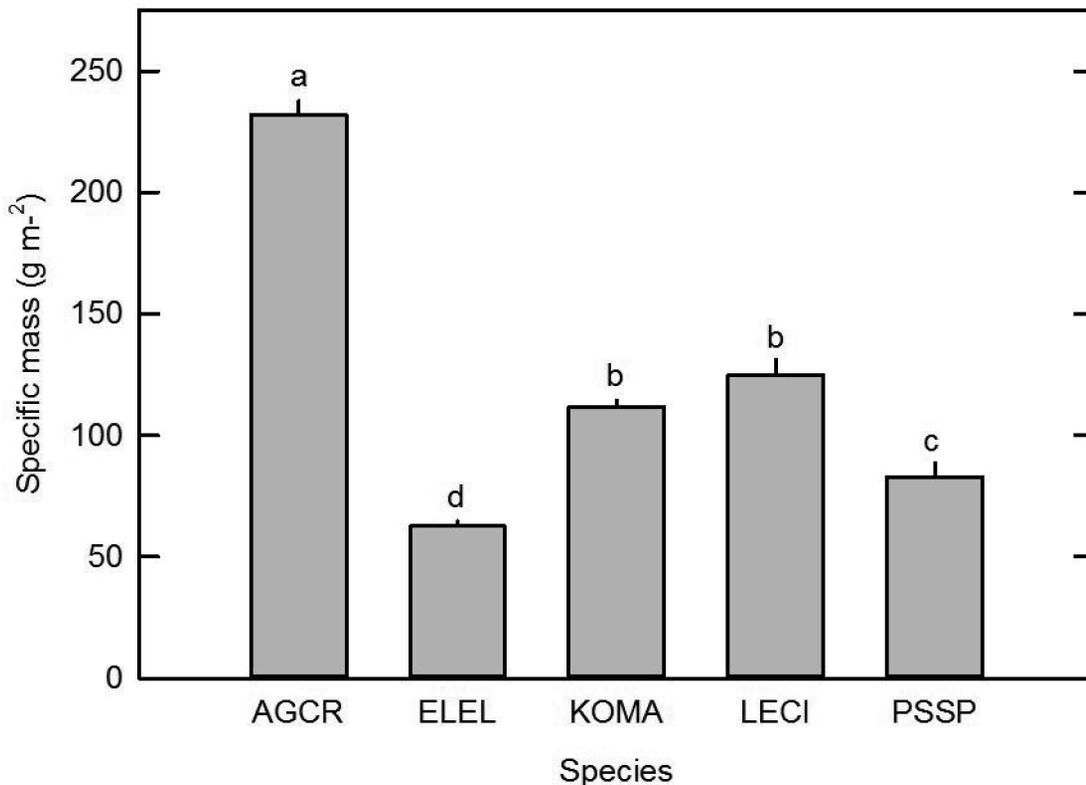


Figure 1: Specific mass (g m^{-2}) of sagebrush-steppe bunchgrass seed heads; error bars are \pm one SE of the mean; letters differ significantly at $P \leq 0.05$ (LSD from one-way ANOVA). Species abbreviations are the exotic crested wheatgrass (AGCR), and the native grasses squirreltail (ELEL), prairie junegrass (KOMA), basin wildrye (LECI) and bluebunch wheatgrass (PSSP)

Results

Seed head-specific mass differed between the species ($F_{4,53} = 216.5$; $P < 0.05$). Crested wheatgrass seed heads had the highest specific mass ($231.8 \text{ g m}^{-2} \pm 5.92 \text{ SE}$), significantly higher than in basin wildrye ($124.8 \text{ g m}^{-2} \pm 6.54 \text{ SE}$) and prairie junegrass ($111.8 \text{ g m}^{-2} \pm 2.67 \text{ SE}$), which were statistically indistinguishable ($\text{LSD} < 0.05$; Fig. 1). These in turn were significantly greater than in bluebunch wheatgrass seed heads ($83.0 \text{ g m}^{-2} \pm 5.73 \text{ SE}$), which had greater seed head-specific mass than in squirreltail ($62.7 \text{ g m}^{-2} \pm 2.27 \text{ SE}$; $\text{LSD} < 0.05$; Fig. 1).

Flag leaf and seed head A_{max} differed significantly between species, phenological periods, with a significant species-by-period interaction in A_{max} in both structures (Table 1). Pooled across periods, flag leaf A_{max} was greatest in prairie junegrass ($33.3 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1} \pm 4.26 \text{ SE}$), significantly higher than in squirreltail ($24.9 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1} \pm 3.86 \text{ SE}$), crested wheatgrass ($24.9 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1} \pm 2.01 \text{ SE}$) and basin wildrye ($22.9 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1} \pm 2.42 \text{ SE}$). Bluebunch wheatgrass A_{max} ($29.7 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1} \pm 4.11 \text{ SE}$) was intermediate between these two groups ($\text{LSD} < 0.05$). Seed head A_{max} was highest in crested wheatgrass ($18.5 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1} \pm 2.95 \text{ SE}$), significantly greater than A_{max} of prairie junegrass ($11.7 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1} \pm 1.20 \text{ SE}$), bluebunch wheatgrass ($11.5 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1} \pm 1.98 \text{ SE}$) and

squirreltail ($9.2 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1} \pm 1.71 \text{ SE}$), which in turn were greater than A_{max} of basin wildrye seed heads ($4.4 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1} \pm 0.53 \text{ SE}$; $\text{LSD} < 0.05$). Pre-anthesis A_{max} of flag leaves ($33.5 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1} \pm 2.14 \text{ SE}$) and seed heads ($14.5 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1} \pm 1.53 \text{ SE}$) pooled across species were greater than levels attained post-anthesis ($20.8 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1} \pm 1.51 \text{ SE}$ and $7.2 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1} \pm 0.75 \text{ SE}$ for flag leaves and seed heads, respectively; $\text{LSD} < 0.05$). The species-by-period interaction in flag leaf A_{max} was due to marked declines from pre- to post-anthesis levels in squirreltail, prairie junegrass and bluebunch wheatgrass, which were not strong in crested wheatgrass, and absent in basin wildrye (Fig. 2a). In addition, species differences were more distinct pre-anthesis compared to very similar post-anthesis flag leaf A_{max} (Fig. 2a). The species-by-period interaction in seed head A_{max} interaction was due to post-anthesis A_{max} in crested wheatgrass being similar to the highest pre-anthesis rates in native grass seed heads, as well as more muted pre- to post-anthesis A_{max} changes in prairie junegrass and basin wildrye seed heads (Fig. 2a).

Flag leaf carboxylation efficiency (CE) did not differ significantly between species, but did between pre- ($0.133 \text{ mol m}^{-2} \text{ s}^{-1} \pm 0.0081 \text{ SE}$) and post-anthesis periods ($0.101 \text{ mol m}^{-2} \text{ s}^{-1} \pm 0.0126 \text{ SE}$), with no significant interaction effect (Table 1). Pre- and post-anthesis differences in flag leaf CE was driven by stronger declines in crested wheatgrass, squirreltail and prairie junegrass, which offset very similar CE in pre- and post-anthesis basin wildrye and bluebunch wheatgrass flag leaves (Fig. 2b). In marked contrast, seed head CE differed significantly between species, phenological periods, with a significant species-by-period interaction (Table 1). Seed head CE was highest in crested wheatgrass ($0.127 \text{ mol m}^{-2} \text{ s}^{-1} \pm 0.0084 \text{ SE}$), which was significantly greater than prairie junegrass ($0.029 \text{ mol m}^{-2} \text{ s}^{-1} \pm 0.0034 \text{ SE}$), which in turn exceeded basin wildrye seed head CE ($0.016 \text{ mol m}^{-2} \text{ s}^{-1} \pm 0.0023 \text{ SE}$) ($\text{LSD} < 0.05$). CE of squirreltail ($0.026 \text{ mol m}^{-2} \text{ s}^{-1} \pm 0.0065 \text{ SE}$) and bluebunch wheatgrass seed heads ($0.024 \text{ mol m}^{-2} \text{ s}^{-1} \pm 0.0051 \text{ SE}$) were intermediate to prairie junegrass and basin wildrye levels, but significantly less than in crested wheatgrass seed heads ($\text{LSD} < 0.05$). Pooled across species, post-anthesis seed head CE ($0.036 \text{ mol m}^{-2} \text{ s}^{-1} \pm 0.0101 \text{ SE}$) was significantly lower than pre-anthesis ($0.049 \text{ mol m}^{-2} \text{ s}^{-1} \pm 0.0074 \text{ SE}$; $\text{LSD} < 0.05$). The species-by-period interaction in seed head CE was due to the marked increase in crested wheatgrass seed head CE from pre- to post-anthesis, while seed heads of squirreltail, prairie junegrass and bluebunch wheatgrass showed significant declines in CE while levels in basin wildrye did not change significantly (Fig. 2b).

Flag leaf and seed head relative stomatal limitation (RSL) differed between pre- and post-anthesis periods, with no species differences, and no species-by-period interaction effect for flag leaf RSL, but with a significant interaction effect in seed head RSL (Table 1). Pooled across species, flag leaf RSL increased significantly pre-anthesis ($47.6\% \pm 1.50 \text{ SE}$) to post-anthesis ($55.4\% \pm 2.17 \text{ SE}$), while pooled seed head RSL

Table 1: Two-way analysis of variance (ANOVA) *F* test results comparing estimates of flag leaf and seed head CO_2 -saturated net photosynthesis (A_{max} ; $\mu\text{mol m}^{-2} \text{ s}^{-1}$), mesophyll carboxylation efficiency (CE; $\text{mol m}^{-2} \text{ s}^{-1}$), relative stomatal limitation (RSL; %) and net photosynthetic assimilation (A_{net} ; $\mu\text{mol m}^{-2} \text{ s}^{-1}$), stomatal conductance to water vapour (g_s ; $\text{mmol m}^{-2} \text{ s}^{-1}$) and intrinsic water use efficiency (WUE_i ; $\mu\text{mol mmol}^{-1}$) at ambient atmospheric $[\text{CO}_2]$ of five sagebrush-steppe bunchgrass species over pre- and post-anthesis periods

Structure-parameter	Species _(4,40)	Period _(1,40)	Species × Period _(4,40)
Flag- A_{max}	2.94*	32.54**	3.75**
Head- A_{max}	14.47**	40.26**	3.23*
Flag-CE	0.61	4.38*	0.76
Head-CE	117.40**	6.59*	6.86*
Flag-RSL	0.20	8.22**	0.96
Head-RSL	1.98	8.89**	3.64*
Flag- A_{net}	0.41	21.85**	1.50
Head- A_{net}	31.71**	21.33**	2.72*
Flag- g_s	1.84	16.43**	0.99
Head- g_s	14.50**	17.39**	1.59
Flag- WUE_i	1.73	4.10*	2.17
Head- WUE_i	7.41**	4.88*	4.29**

F test results with * are significant at $P \leq 0.05$; ** at $P \leq 0.01$; degrees freedom for each effect test are presented parenthetically.

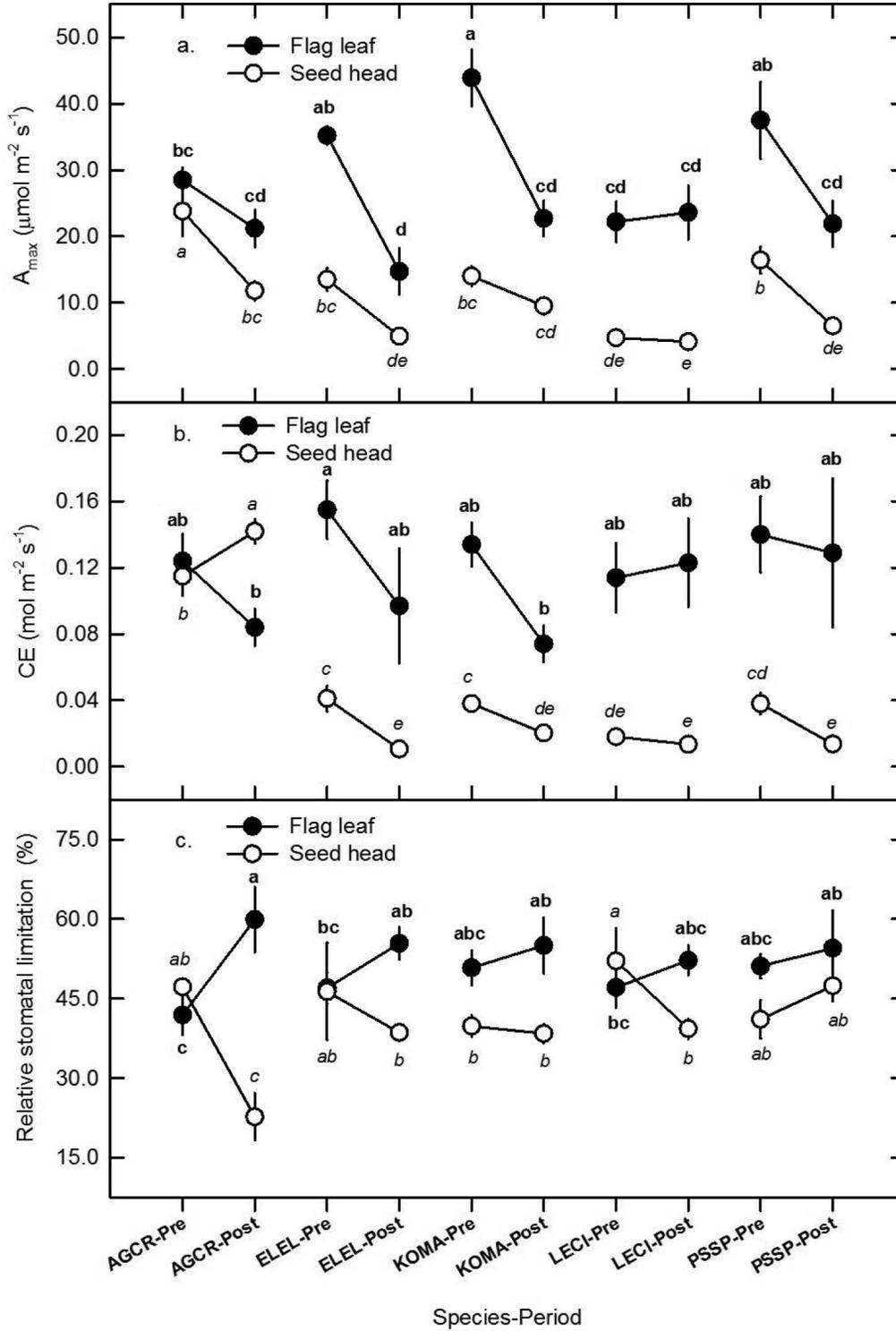


Figure 2: Pre- and post-anthesis (a) maximum photosynthetic capacity (A_{max}), (b) mesophyll carboxylation efficiency (CE) and (c) relative stomatal limitation to photosynthesis (RSL) and (d) intrinsic water use efficiency (WUE_i) of flag leaves (solid symbols) and seed heads (open symbols) of the exotic crested wheatgrass (AGCR), and the native sagebrush-steppe grasses squirreltail (ELEL), prairie junegrass (KOMA), basin wildrye (LECI) and bluebunch wheatgrass (PSSP). Each symbol is the mean of five independent measurements; error bars are \pm one SE of the mean. Letters differ at $P < 0.05$; bold letters compare within flag leaves, italics within seed heads (LSD from two-way ANOVA, Table 1).

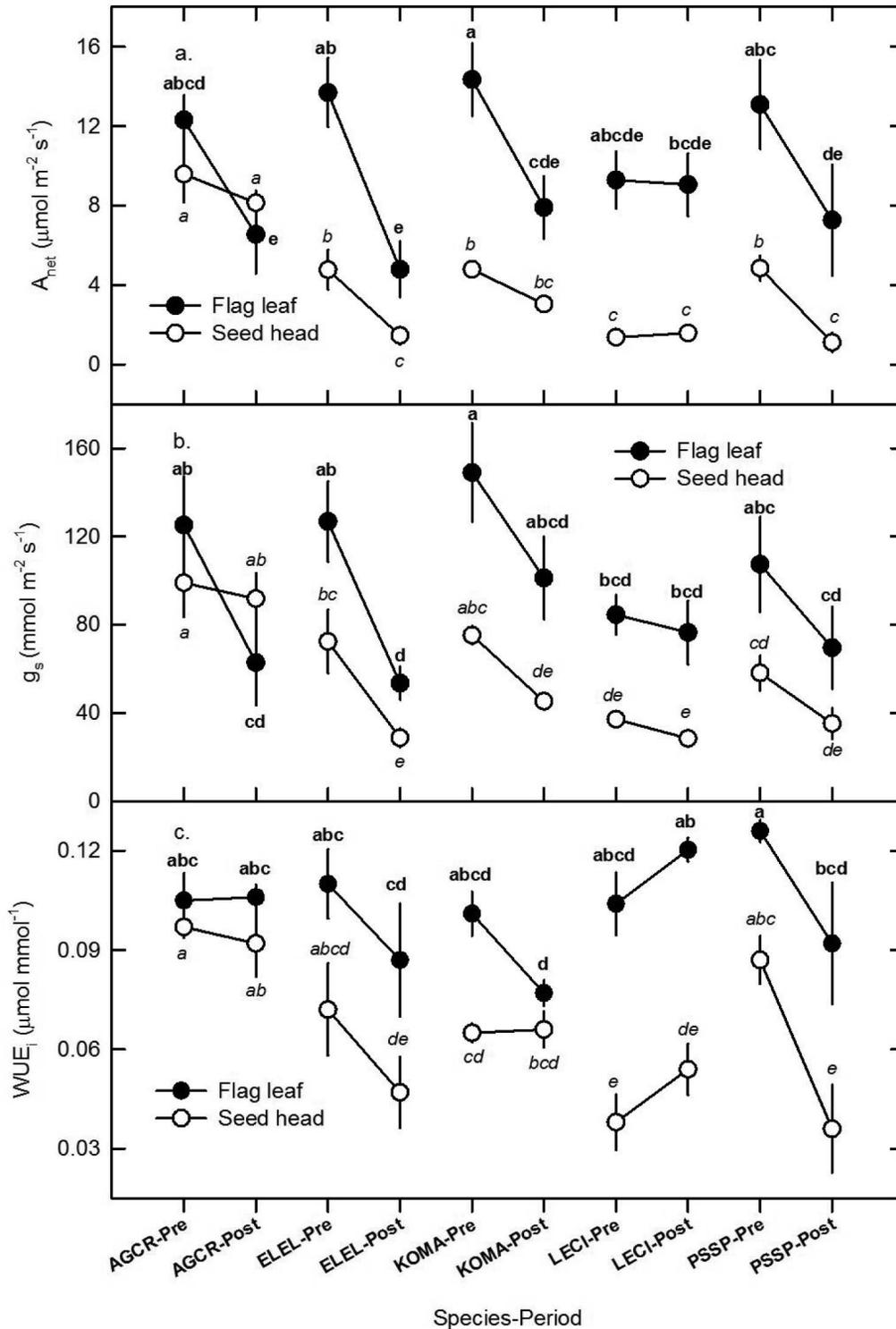


Figure 3: Pre- and post-anthesis (a) net photosynthesis (A_{net}), (b) stomatal conductance to water vapour (g_s) and (c) intrinsic water use efficiency (WUE_i) of flag leaves (solid symbols) and seed heads (open symbols) of the exotic crested wheatgrass (AGCR), and the native sagebrush-steppe grasses squirreltail (ELEL), prairie junegrass (KOMA), basin wildrye (LECI) and bluebunch wheatgrass (PSSP). Each symbol is the mean of five independent measurements; error bars are \pm one SE of the mean. Letters differ at $P < 0.05$; bold letters compare within flag leaves, italics within seed heads (LSD from two-way ANOVA).

declined significantly between the two phenological periods (45.3% ±2.36 SE and 37.9% ±1.89 SE for pre- and post-anthesis, respectively) (LSD < 0.05). The species-by-period interaction in seed head RSL was due to pre-anthesis RSL in seed heads being similar between species, while post-anthesis crested wheatgrass RSL was significantly lower than in all native grasses (Fig. 2c). In addition, there was little change between pre- and post-anthesis RSL in prairie junegrass and bluebunch wheatgrass seed heads, while basin wildrye seed head RSL decreased pre- to post-anthesis (Fig. 2c).

Leaf A_{net} , g_s and WUE_i differed significantly only between pre- and post-anthesis periods, while seed head A_{net} , g_s and WUE_i differed between species and phenological periods, with seed head WUE_i having a significant species-by-period interaction effect (Table 1). Pre-anthesis flag leaf A_{net} (12.5 $\mu\text{mol m}^{-2} \text{s}^{-1} \pm 0.79$ SE) was higher than post-anthesis rates (7.1 $\mu\text{mol m}^{-2} \text{s}^{-1} \pm 0.84$ SE; LSD < 0.05). Crested wheatgrass seed head A_{net} (8.9 $\mu\text{mol m}^{-2} \text{s}^{-1} \pm 0.84$ SE) was significantly greater than in prairie junegrass (3.9 $\mu\text{mol m}^{-2} \text{s}^{-1} \pm 0.37$ SE), squirreltail (3.1 $\mu\text{mol m}^{-2} \text{s}^{-1} \pm 0.76$ SE) and bluebunch wheatgrass (3.0 $\mu\text{mol m}^{-2} \text{s}^{-1} \pm 0.72$ SE), with these three significantly higher than A_{net} of basin wildrye seed heads (1.5 $\mu\text{mol m}^{-2} \text{s}^{-1} \pm 0.20$ SE; LSD < 0.05). Pre-anthesis seed head A_{net} (5.1 $\mu\text{mol m}^{-2} \text{s}^{-1} \pm 0.64$ SE) was greater than post-anthesis (2.9 $\mu\text{mol m}^{-2} \text{s}^{-1} \pm 0.54$ SE), despite relatively small changes in crested wheatgrass and basin wildrye seed head A_{net} (Fig. 3a). Pre-anthesis seed head stomatal conductance (118.6 $\text{mmol m}^{-2} \text{s}^{-1} \pm 9.02$ SE) was greater than post-anthesis g_s (72.7 $\text{mmol m}^{-2} \text{s}^{-1} \pm 7.45$ SE; LSD < 0.05). Crested wheatgrass seed head g_s (95.8 $\text{mmol m}^{-2} \text{s}^{-1} \pm 9.58$ SE) was significantly higher than in prairie junegrass (60.3 $\text{mmol m}^{-2} \text{s}^{-1} \pm 5.47$ SE) and squirreltail (50.5 $\text{mmol m}^{-2} \text{s}^{-1} \pm 10.12$ SE), which in turn had greater g_s than basin wildrye seed heads (32.8 $\text{mmol m}^{-2} \text{s}^{-1} \pm 2.06$ SE) (LSD < 0.05). Stomatal conductance of bluebunch wheatgrass seed heads (46.7 $\text{mmol m}^{-2} \text{s}^{-1} \pm 6.27$ SE) was intermediate between the two latter groups and lower than crested wheatgrass (LSD < 0.05).

Flag leaf WUE_i declined significantly from pre-anthesis (0.109 $\mu\text{mol mmol}^{-1} \pm 0.0037$ SE) to post-anthesis (0.096 $\mu\text{mol mmol}^{-1} \pm 0.0057$ SE; LSD < 0.05). Seed head

WUE_i was greatest in crested wheatgrass (0.095 $\mu\text{mol mmol}^{-1} \pm 0.0046$ SE), greater than in prairie junegrass WUE_i (0.066 $\mu\text{mol mmol}^{-1} \pm 0.0061$ SE), which in turn was greater than basin wildrye (0.046 $\mu\text{mol mmol}^{-1} \pm 0.0061$ SE); seed head WUE_i of bluebunch wheatgrass (0.061 $\mu\text{mol mmol}^{-1} \pm 0.0111$) and squirreltail (0.060 $\mu\text{mol mmol}^{-1} \pm 0.0093$ SE) were intermediate between junegrass and basin wildrye levels, and significantly lower than crested wheatgrass seed head WUE_i (LSD < 0.05). As with leaves, seed head WUE_i declined from pre- (0.072 $\mu\text{mol mmol}^{-1} \pm 0.0053$ SE) to post-anthesis (0.058 $\mu\text{mol mmol}^{-1} \pm 0.0055$ SE; LSD < 0.05). The species-by-period interaction was due to pre-anthesis seed heads having high and similar WUE_i between crested wheatgrass, squirreltail, and bluebunch wheatgrass compared to lower WUE_i in prairie junegrass and basin wildrye (Fig. 3c). Post-anthesis seed head WUE_i of crested wheatgrass was similar to pre-anthesis levels, and was consistently higher than in natives (Fig. 3c). Prairie junegrass pre- and post-anthesis seed head WUE_i also did not vary markedly (Fig. 3c).

Seed head to flag leaf ratios significantly differed between the species for A_{max} ($F_{4,44} = 11.56$; $P < 0.0001$), CE ($F_{4,44} = 31.29$; $P < 0.0001$), A_{net} ($F_{4,44} = 14.75$; $P < 0.0001$), g_s ($F_{4,44} = 7.39$; $P = 0.0001$) and WUE_i ($F_{4,44} = 6.89$; $P = 0.0002$), but not RSL ($F_{4,44} = 0.47$; $P = 0.7604$). In all significant species differences, crested wheatgrass had higher seed head:flag leaf ratios pooled across pre- and post-anthesis periods compared to native bunchgrasses (Table 2).

Discussion

For all five species, seed head A_{max} was high, approaching levels observed in ears of high-yielding wheat cultivars (Wechsung *et al.* 2001). Photosynthetic competence in reproductive structures would be advantageous in arid and semi-arid ecosystems, especially cool-season rainfall systems such as sagebrush-steppe with short springtime growing seasons bracketed by early-season low-temperature limitations and increasing water limitations as temperatures warm (Smith *et al.* 1997; Hamerlynck *et al.* 2016b; Svejcar *et al.* 2017). Even if seasonal conditions do not facilitate seed filling and

Table 2: Ratios of seed head to flag leaf photosynthetic parameters of five sagebrush-steppe bunchgrasses

Species	A_{max} ratio	CE ratio	RSL ratio	A_{net} ratio	g_s ratio	WUE_i ratio
Crested wheatgrass	0.76 ^a (0.075)	1.40 ^a (0.201)	0.80 ^a (0.148)	1.28 ^a (0.227)	1.45 ^a (0.277)	0.91 ^a (0.055)
Squirreltail	0.37 ^b (0.041)	0.22 ^b (0.054)	0.86 ^a (0.123)	0.34 ^b (0.062)	0.60 ^b (0.091)	0.61 ^{bc} (0.090)
Prairie junegrass	0.39 ^b (0.052)	0.31 ^b (0.042)	0.76 ^a (0.043)	0.43 ^b (0.083)	0.55 ^b (0.077)	0.76 ^{ab} (0.060)
Basin wildrye	0.21 ^c (0.030)	0.16 ^b (0.035)	0.93 ^a (0.074)	0.18 ^b (0.028)	0.44 ^b (0.050)	0.41 ^c (0.050)
Bluebunch wheatgrass	0.41 ^b (0.041)	0.21 ^b (0.044)	0.86 ^a (0.058)	0.26 ^b (0.090)	0.69 ^b (0.144)	0.49 ^c (0.103)

Parameters are defined in Table 1; values are means pooled across pre- and post-anthesis phenological periods ($n = 10$ per species, total $n = 50$) with standard errors presented parenthetically. Letters differ significantly at $P < 0.05$ (LSD from one-way ANOVA).

production, allocation to such activity insures that these grasses would gain back some of the carbon invested to reproductive effort (Raven and Griffiths 2017). The observed low RSL and WUE_i suggest photosynthetic activity in seed heads is aimed at rapidly producing the inflorescence and seed filling; rather than optimize the trade-off between carbon uptake and water loss, these grasses likely minimize diffusive limitations to drive rapid reproductive carbon uptake and growth. The lower diffusive limitations were not due to greater stomatal opening, as these were markedly lower than in flag leaves (Fig. 3). Rather, lower inherent g_s may limit the dynamic range of stomatal opening, which can reduce RSL and WUE_i (Tissue *et al.* 2005; Gomes Soares *et al.* 2015).

There was considerable variation in seed head-specific mass in the four native grasses (Fig. 1), while seed head A_{max} and CE in these were remarkably similar (Fig. 2). This is interesting, as the four natives had distinct reproductive timing, with prairie junegrass, squirreltail and bluebunch wheatgrass flowered concurrently with each other and crested wheatgrass, with basin wildrye flowering last (personal observation, consistent with Krall *et al.* 1971). The similar A_{max} and greater range of seed head-specific mass suggests that the native grasses vary considerably in their per unit allocation to reproductive photosynthetic capacity. However, our data cannot determine the photosynthetic characteristics of individual florets within the seed head, and variation in stomatal density and photosynthetic capacity of the different components within these could be critical determinants of reproductive gas exchange and yield (Wechsung *et al.* 2001; Tambussi *et al.* 2007; Sanchez-Bragado *et al.* 2016). Basin wildrye is relatively deeply rooted (USDA 2019) and flowered when soils were likely drier than for the other species. CE reflects photosynthetic performance at low C_i , as when CO_2 is limiting, as when stomatal conductance is low (Smith *et al.* 1997). It is likely that the more marked declines in CE and g_s apparent in the earlier flowering species reflect a greater change from moister to drier soil conditions between pre- and post-anthesis periods, compared to drier and less variable soil water conditions which resulted in low and invariant CE and g_s in basin wildrye seed heads, much as has been observed in response to seasonal reductions in soil moisture in vegetative basin wildrye leaves (Anderson *et al.* 1995).

As hypothesized, crested wheatgrass had greater energetic allocation to photosynthetically active reproductive structures, as evidenced by a nearly 2-fold higher specific mass than seed heads of native grasses (Fig. 1). Higher specific mass could be due to greater specific mass per floret, via allocation to glumes or awns, greater numbers of florets per seed head or both. Concurrent with greater seed head-specific mass, and as per our second postulated response, A_{max} , CE, A_{net} and g_s in crested wheatgrass seed heads attained significantly higher levels than in the native grasses, while flag leaves were similar to native grasses in their photosynthetic characteristics (Fig. 2). This suggests that the ability of crested wheatgrass to produce consistent viable seed cohorts may reflect the photosynthetic characteristics of its seed heads more than of

its flag leaves, as occurs in some wheat cultivars (Austin *et al.* 1982). High seed head-specific mass in crested wheatgrass would also increase the amount of photosynthetic pigments and enzymes per unit area, which would result in greater A_{max} and CE, much as in leaves with high specific mass (Poorter and Evans 1998).

Pre- to post-anthesis photosynthetic behaviour of crested wheatgrass seed heads was also distinct from native grass seed heads. Crested wheatgrass had a marked increase in seed head CE and decline in RSL (Fig. 2), with no change in g_s (Fig. 3). Typically, increased biochemical demand for CO_2 , as would occur with higher CE, incurs stronger stomatal limitations and higher RSL (Tissue *et al.* 2005). In all five species, we observed a considerable degree of post-anthesis seed head opening (Hamerlynck, personal obs.). Post-anthesis ears of wheat open during grain filling, which increases light penetration into the seed head and enhances carbon uptake even with declining photosynthetic capacity (Ziegler-Jöns 1989). It may be that similar post-anthesis structural changes in the seed heads of crested wheatgrass resulted in an apparent relaxation of RSL. In contrast, the native species whose pre- or post-anthesis periods overlapped to some degree with crested wheatgrass (i.e. prairie junegrass, squirreltail and bluebunch wheatgrass) showed marked declines in CE and g_s , while RSL was relatively constant, suggesting stomatal closure may have offset any benefit of any structural changes. These differences resemble how differences in leaf mesophyll structure affect covariation of stomatal dynamics, mesophyll conductance and photosynthetic enzyme kinetics (Flexas *et al.* 2008; Buckley and Warren 2014). Thus, a consequence of greater investment in higher seed head-specific mass is a better ability to coordinate stomatal behaviour, photosynthetic enzyme kinetics and physical changes in seed head structure.

Photosynthetically, crested wheatgrass seed heads are to a remarkable extent more similar to leaves than those of native grasses (Table 2). This is may be an important feature of how crested wheatgrass succeeds in establishing from seed when native bunchgrasses fail (Bakker *et al.* 2003; Wilson and Pärtel 2003; Fansler and Mangold 2011; Hamerlynck and Davies 2019). One distinct advantage of having 'leaf-like' seed heads for crested wheatgrass is greater WUE_i compared to native species' seed heads, especially in the post-anthesis, grain-filling period (Fig. 3). In a geo-referenced co-planting study, Hamerlynck and Davies (2019) found that 80–87% of individuals occurred outside of the species original planting locations; they suggested total seed production and establishment from seed drove all population trajectories and that these allowed crested wheat grass to better persist and spread through prolonged drought compared to native grasses. It could be that having seed heads with high WUE_i could facilitate consistent production of seeds capable of germinating and establishing under such dry soil moisture conditions. In addition, seedlings of crested wheatgrass and bluebunch wheatgrass up-regulate photosynthesis in response to defoliation, but this resulted in reduced WUE_i in bluebunch wheatgrass and increased WUE_i in crested wheat-

grass, a unique response that could enhance seedling survival, especially under drying soil conditions that typically occur following seedling emergence (Hamerlynck *et al.* 2016a; Denton *et al.* 2018). The results of these and our current study paint an emerging picture showing that crested wheatgrass has functional features that enhance WUE_i throughout its demographic development (Read *et al.* 1991), allowing it to thrive in a wider range of environmental conditions than do native North American sagebrush-steppe bunchgrasses.

Compared to crested wheatgrass, our results suggest that the seed heads of native sagebrush-steppe bunchgrasses have characteristics that constrain reproductive photosynthesis. Low seed head WUE_i and lower A_{max} and CE, probably limit quality seed production to years of sufficient spring-time soil moisture. Over the long term, native grass populations rely more on sexual recombination than vegetative mechanisms to persist (Liston *et al.* 2003), even over periods of population decline (Hamerlynck and Davies 2019). Our results support findings that reproductive effort in bunchgrasses is episodic, and they infrequently produce seed cohorts of sufficient quality and size for successful establishment of seedlings (Hamerlynck *et al.* 2016b; Hamerlynck and Davies 2019). To conserve threatened bunchgrass populations, seed augmentation of extant populations would be viable way to address this constraint (Turnbull *et al.* 2003). However, as found in restoration efforts, invasive annual grass densities can impose a strong limitation on successful native grass establishment with increased seeding rates or alterations to seeding timing (Schantz *et al.* 2016). Additional methods aimed at improving native plant materials should be explored. Drawing from seed sources from localized populations with traits associated with better survival holds considerable promise (Baughman *et al.* 2019). There is also growing evidence that native bunchgrasses are rapidly evolving better competitive capability in response to invasive annual dominance (Leger and Espeland 2010). Inherited seedling growth traits related to aboveground/belowground allocation and rooting pattern in response to competitive pressure imposed by cheatgrass have been found (Rowe and Leger 2011), but vary markedly between locations and accessions, which has been attributed to epigenetic effects and variation in parental plant performance (Goergen *et al.* 2011; Espeland and Hammond 2013). Possibly, there is population-level, localized variation in parental reproductive photosynthetic characteristics of native sagebrush-steppe bunchgrasses that underlies this variation in seedling competitive success. Greater reproductive photosynthesis could increase maternal contributions to seedling energetic reserves, which can affect seed quality and subsequent post-germination seedling growth and survival (Huxman *et al.* 1998, 1999).

Building on our current study, carefully assessing sources of variation in reproductive photosynthesis and associated reproductive success in native bunchgrasses will not only address a basic knowledge gap in the functional ecology of these plants but also help in selecting and developing native plant lines with the characteristics that natural and

human selection has imparted to the successful exotic crested wheatgrass. Crested wheatgrass provides a functional roadmap for this selection and improvement of native grass plant materials. Following this roadmap, we need to develop lines of native grasses that have (i) growth and allocation patterns that improve the competitive ability of seedlings and adults (Espeland and Hammond 2013; Bell *et al.* 2019) that are capable of (ii) producing substantial numbers of high-quality seeds to overcome demographic bottlenecks (James *et al.* 2011) and establish greater numbers of seedlings with functional attributes that increase both stress tolerance and competitive ability (Rowe and Leger 2011; Hamerlynck *et al.* 2016a). Even then, it is likely that any potential gains from improved native plants that produce seeds consistently may be outstripped by predicted increases in climate variation and associated intensity of competition and disturbance imposed by invasive annuals. Therefore, improving the accuracy of short-term climate prediction to improve the probability of seeding success is critical to preserving sagebrush-steppe ecosystem integrity (Hardegee *et al.* 2018), both with currently available plant materials and those arising from the improvements that biological and agronomical processes will provide.

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E.H. and E.D. conceived and designed the research. E.H. gathered and analyzed the data. E.H., E.D., K.D. and C.B. wrote and edited the manuscript.

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