

Variation in reproductive photosynthetic compensation of distinct germplasm varieties of a native rangeland grass, *Pseudoroegneria spicata*, following floral defoliation

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Understanding plant ecophysiological functioning is critical in formulating effective ecologically based strategies to conserve and enhance resiliency and resistance in sagebrush steppe, as well as improving their restoration following degradation by interactive effects of climate change, wildland fire and invasive annual grasses. Recent research has shown increased reproductive photosynthesis following floral defoliation can be important to reproductive potential, yet how this is expressed in plant material selected for different functional attributes is unknown. To address this, we measured photosynthetic gas exchange in clipped and unclipped basal florets and flag leaves of two germplasms of the native perennial bunchgrass, bluebunch wheatgrass, var. Anatone and var. Columbia, selected for higher reproductive culm production. Clipping induced a stronger direct compensatory reproductive photosynthetic response in basal florets of Anatone compared to Columbia germplasm individuals, with no indirect compensatory response apparent in unaffected distal florets of either germplasm. Flag-leaf photosynthesis did not differ between the germplasm lines, but Columbia flag leaves did show evidence of increased photosynthesis on culms with clipped basal florets. These findings suggest selection for increased flowering culms may alter reproductive herbivory tolerance, a feature important in the convergence of herbivory and drought tolerance traits. Such information could help in planning effective seed mixes to enhance population stability across highly variable sagebrush steppe ecosystems, as well as directing future plant material selection to improve restoration success in these economically important rangelands.

Lay summary: Plant restoration in semi-arid rangelands requires species to survive stressors like drought and herbivory, while also provisioning viable seed. Here, we show that two germplasms (Columbia, Anatone) of bluebunch wheatgrass can have different reproductive responses to seedhead defoliation. Columbia, bred for spike production, had lower reproductive physiological outcomes than Anatone.

Key words: Bluebunch wheatgrass, rangelands, restoration, sagebrush steppe, water use efficiency

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Introduction

The North American sagebrush steppe has been degraded to approximately half of its historical extent, necessitating novel tools and approaches for restoration of vital rangeland habitat. The ‘sagebrush sea’ encapsulates a wide range of climate extremes and is subject to a variety of ecological disturbances, namely wildland fire exacerbated by extensive spread of invasive annual grasses. Annual grasses like cheatgrass (*Bromus tectorum*) have invaded >28 million hectares in the Intermountain West in the last century, and cheatgrass-dominated communities have increased by ~8-fold since 1990 (Smith *et al.*, 2022). Land management agencies in sagebrush steppe ecosystems are therefore faced with serious conservation and restoration challenges, and formulating effective ecologically based strategies is critical to conserving and protecting resiliency and resistance in ‘core’ sagebrush steppe, as well as restoring these in degraded sagebrush steppe plant communities (Doherty *et al.*, 2022; Johnson *et al.*, 2022). As seed-based strategies (SBS) are and likely will continue to be the most economically viable way to attain conservation and restoration goals, Larson *et al.* (2023) proposed exploring and developing Grubb’s regeneration niche (Grubb, 1977) as a way to optimize conservation and restoration success. Larson *et al.* noted that such an approach will require developing plant material capable of maintaining the regeneration niche under future climate conditions. This may be especially important for conserving perennial bunchgrass populations in sagebrush steppe ecosystems whose population dynamics are determined principally by sexual reproduction and consistent production of viable seed cohorts (Smith *et al.*, 1997; Liston *et al.*, 2003; Hamerlynck and Davies, 2019). Moreover, establishment of self-sustaining perennial bunchgrass population is of principal importance for successful restoration of degraded sagebrush steppe (Chambers *et al.*, 2014), and is often limited by low establishment success of native grasses (James *et al.*, 2011; Clements *et al.*, 2017). Thus, understanding the ecophysiological mechanisms underlying reproductive effort in native arid and semi-arid land bunchgrasses is critical to informing management decisions and strategies aimed at conserving population viability, as well as improving success of seed-based restoration efforts across the wide range of environmental variability typical of sagebrush steppe.

There is growing evidence that reproductive effort in rangeland bunchgrasses is supported largely by photosynthetic activity within the seed head (spike) itself (Hamerlynck *et al.*, 2019; Hamerlynck and O’Connor, 2021, 2022). Recently, Hamerlynck *et al.* (2023) showed experimental clipping induced compensatory photosynthetic upregulation in affected basal florets of crested wheatgrass, a Eurasian exotic bunchgrass widely planted and naturalized across the Intermountain sagebrush steppe (Davies *et al.*, 2020). Moreover, compensatory floral photosynthesis was coincident with increased floret size and specific mass in the intact distal florets compared to the clipped basal florets,

suggesting that clipped florets provided carbon to enhance reproductive potential in those distal florets (Hamerlynck *et al.*, 2023). Previous work has shown that variation in sexual reproductive effort is an important expression of convergent drought- and grazing-tolerance traits, and convergent trait development is a critical feature in plant population and community stability in arid and semi-arid ecosystems (Adler *et al.*, 2004; Quiroga *et al.*, 2010). However, these studies have examined convergent responses only to vegetative herbivory; as such, Hamerlynck *et al.* (2023) suggested compensatory reproductive photosynthetic dynamics in response to floral herbivory may (i) underly crested wheatgrass’s ability to consistently produce viable seed cohorts under conditions that limit reproductive success of native bunchgrass species, and (ii) reflect a novel aspect in the development of convergent drought and grazing tolerance at the site of reproductive effort itself.

Despite the value of the Hamerlynck *et al.* (2023) study, their assertions were drawn from a single variety of a single species, and acknowledged that further testing was needed to support their proposed theory. Furthermore, there has been no assessment of the importance of genetic variation to reproductive photosynthesis within a bunchgrass species. Such intraspecific variation may be important in developing plant materials to match current or future environmental conditions. Despite their widespread use, bunchgrass germplasm varieties are not necessarily tested for performance to in response to the stressful conditions for which they are supposedly developed (Garbowski *et al.*, 2021). Varieties or cultivars of the same species can also exhibit a wide range of intrinsic performance as well as vary in their abilities to tolerate specific stressors, *e.g.* salt stress tolerance of rice cultivars (Tsai *et al.*, 2019). Thus, it is possible that reproductive photosynthesis could also vary considerably among germplasm varieties selected for distinct functional attributes.

Here, we present the results of a study assessing the compensatory reproductive photosynthetic responses of two germplasm lines of the native sagebrush steppe perennial bunchgrass, bluebunch wheatgrass (*Pseudoroegneria spicata* [Pursh] A. Löve). Bluebunch wheatgrass is a widespread native bunchgrass in sagebrush steppe plant communities and is considered an ideal candidate for genomic selection for performance-based plant material development (Jones *et al.*, 2022). Plant material development over the past 60+ years has yielded several widely planted *P. spicata* cultivars, henceforth referred to as ‘germplasm’. Others have investigated performance of native rangeland bunchgrass in both field and laboratory settings, including vegetative compensatory photosynthesis in bluebunch wheatgrass (Caldwell *et al.*, 1981; Nowak and Caldwell, 1983; Anderson and Toft, 1993; Smith *et al.*, 1997; Mukherjee *et al.*, 2015, 2019; Zheng *et al.*, 2019), intraspecific differences in reproductive photosynthetic performance of widely used germplasm varieties have yet to be investigated. In this study, we sampled two relatively recent releases, both of which

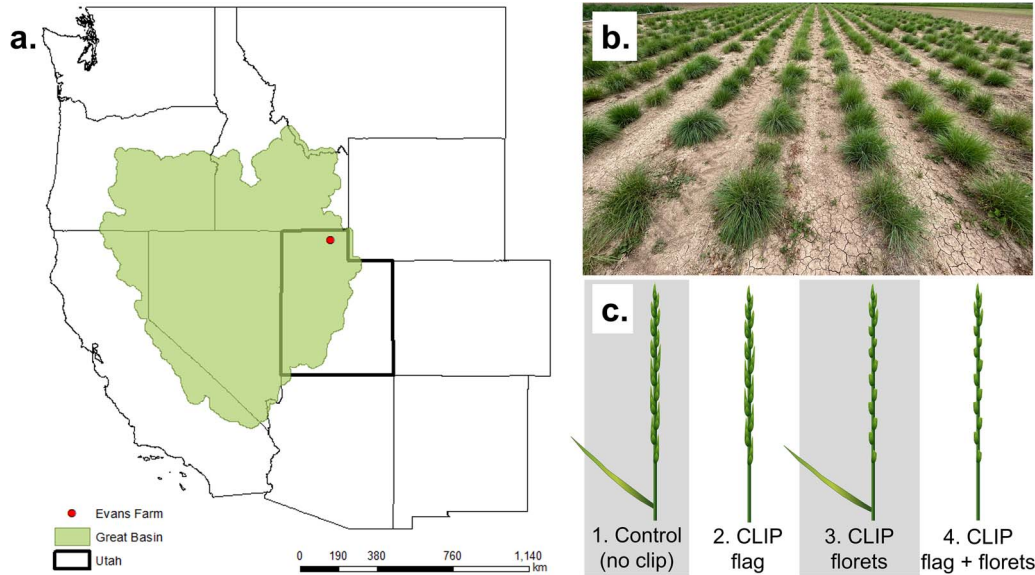


Figure 1: Study site and experimental treatments. This study took place at Evans Research Farm, Utah, within the Great Basin ecosystem of the western United States (a). The study plants were grown in a 750-m² nursery with 1-m spacing between adjacent rows and 0.5 m between individuals within a row (b). Four flowering culms were marked on each plant, and each culm was assigned to a control or clipping treatment group, including flag leaf removal, basal floret removal or flag + basal floret removal (c). The modified Great Basin extent boundary in panel a was obtained from the USGS Western Ecological Research Center via the USGS ScienceBase-Catalogue.

originated from populations in semi-arid eastern Washington state: ‘Anatone’, an unmodified accession originating from more mesic sites in eastern Washington (~508 mm mean annual precipitation (MAP); *Monsen et al., 2003*), and ‘Columbia’, which originated from the K68 population at a more xeric location (~250 mm MAP) (*Jones and Mott, 2016*). While drawn from populations exposed to distinct MAP, both germplasms have been successfully planted in locations across the Intermountain West, including those with MAP similar to the other’s type location, and are suited to the full environmental range of bluebunch wheatgrass habitats (*Monsen et al., 2003*; *USDA-NRCS, 2012*; *Jones and Mott, 2016*). The principal difference between these germplasms, therefore, is that Anatone is an unmodified germplasm, while Columbia development included five cycles of recurrent selection for high reproductive culm production (*Jones and Mott, 2016*). *Hamerlynck et al. (2023)* hypothesized that a combination of natural and artificial selective pressures resulted in crested wheatgrass becoming more similar to agronomic species developed for higher reproductive effort. Based on previous knowledge of bunchgrass compensatory photosynthesis and the selection criteria of each germplasm variety, we hypothesized that: (1) both germplasm lines would exhibit compensatory photosynthesis in response to floral defoliation, (2) Columbia germplasm, developed for higher reproductive effort, would display greater compensatory photosynthetic responses than the unmodified Anatone germplasm and (3) Columbia germplasm would exhibit greater overall investment in reproductive structures (*i.e.* spikes and seeds) than Anatone individuals.

Materials and Methods

Study site and field sampling

Our study took place at the Utah State University Evans Research Farm, located near Millville, Utah (*Fig. 1a*; 41.6945° N, 111.8332° W). This location is ecologically classified as Semi-wet Fresh Meadow, a type of high-yielding range site (*NRCS, 2023*). Local topography is level (slope <3%), and soils are somewhat poorly drained silty clay loams developed in lacustrine terraces, further classified as mesic Aquic Argixerolls (Nibley series). Evans Farm lies in the semi-arid Great Basin (elevation = 4535 m) and is characterized by hot and dry summers with most precipitation occurring in winter months as snowfall. MAP from 1980 to 2011 was 47 cm, and the minimum, mean and maximum daily temperature during July 2023 were approximately 13, 23 and 33°C, respectively (*Utah Climate Center, 2023*).

Field-based measurements were made on mature bluebunch wheatgrass (*P. spicata* [Pursh] A. Löve) plants during June and July of 2023. The full ‘training population’ at Evans farm spans a 750 m² nursery plot consisting of ~1300 individuals arranged in a 77 x 17 grid, with 0.5 m spacing between individuals within a row and 1 m spacing between neighbouring rows (*Fig. 1b*). The training population was developed by breeding three commercially available germplasm source populations: Anatone, Columbia and P-7. Maternal parents were determined by pedigree (*i.e.* seed source), and paternity was determined via a genotype-by-sequencing approach, allowing us to know the maternal and paternal germplasm source of

each plant (Poland *et al.*, 2012; Crain *et al.*, 2020). From this population, we selected five healthy individuals in a similar boot stage for both Anatone × Anatone (AA) and Columbia × Columbia (CC) germplasm crosses for inclusion in this study. Both Anatone and Columbia germplasm originated from low-precipitation sites (MAP <500 mm) in the western extent of the species' range (Washington, USA), but Columbia germplasm was developed through selection cycles specifically for increased spike number (Jones and Mott, 2016).

In early June of 2023, on each of the 10 selected plants, we basally tagged four flowering culms of similar phenological stage and randomly assigned each to one of four treatment groups: 1) control (not clipped), 2) flag leaf removed, 3) basal florets removed and 4) flag leaf + basal florets removed. We tracked the length of each culm (from flag leaf to base of spike) from the beginning of the study until culms were fully expanded (*i.e.* zero change in length between measurement periods). Clipping treatments were initiated once plants reached the anthesis stage and took place on 21 June 2023; flag leaves were removed by clipping fully expanded flag leaves at the ligule (Treatment groups 2 and 4), and basal florets were manually excised to remove approximately half of the floret biomass occurring below the distal 2 cm of each spike (Treatment groups 3 and 4). A visual representation of the experimental treatments is provided in Fig. 1c. During each measurement date, we also took three readings of volumetric soil moisture in the upper 5 cm of topsoil (Theta Probe ML2x meter and HH2 data logger, Delta-T Devices, Cambridge, UK) under the canopy of each plant to track changes in soil moisture throughout the study. These values, along with precipitation data from the Utah Climate Center, are presented in Supplementary Fig. S1.

Ecophysiological measures

Physiological measurements were recorded on flag leaves and the basal and distal portions of spikes of each of the four culms on each selected plant using a portable photosynthesis system (LI-6800, LiCoR Instruments, Lincoln NE, USA) at five time points: one pre-anthesis (June 8), and four subsequent measurements spanning anthesis and post-anthesis (June 23, June 29, July 5 and July 11). Sampling occurred between 0800 and 1300 local time and measurement order was randomized each sampling day to minimize any diurnal effects. Each spike section or leaf was enclosed inside of a round leaf chamber with a 2-cm² aperture while maintaining its natural orientation and using the following chamber conditions: 1500 μmol m⁻² s⁻¹ (40 μmol blue light, 1460 red) photosynthetic photon flux density (PPFD) supplied by an LED light array, 400 ppm CO₂ supplied at a flow rate of 200 μmol sec⁻¹, 40% relative humidity and 25°C Peltier-exchange block temperature at a 0.1 kPa pressure differential. Light-adapted PSII photochemical yield (ϕ_{PSII}) was determined by measuring chlorophyll fluorescence (F) with a multi-phase fluorimeter integrated with the cuvette. A beam of 5.0 μmol m⁻² s⁻¹ intensity modulated at 50 kHz

was applied for 5 s to determine steady-state fluorescence yield (Fs) under the incident PPFD of 1500 μmol m⁻² s⁻¹. This was followed by exposure to three successive flashes of a saturating actinic beam of 10 000 μmol m⁻² s⁻¹, each of 300-ms pulse width and a ~97% red/3% blue light balance, modulated at 250 kHz with data gathered at 100 Hz to determine maximum light-adapted fluorescence yield (Fm'); ϕ_{PSII} was calculated as $\phi_{PSII} = (Fm' - Fs) / Fm'$. Before initiating a point measurement, each sample was allowed to equilibrate to chamber conditions and reach steady-state for net photosynthesis (A_{net}), stomatal conductance (g_{sw}) and intercellular [CO₂], as indicated by stability parameters. The widths of each leaf blade and spike were estimated to the nearest millimetre at the exact measurement location prior to enclosure in the chamber using electronic callipers, allowing the instrument to calculate basic area-based gas exchange measurements. Further adjustments were made to the basal and distal spike area calculations assuming a basic open-ended cylindrical geometry. For clipped basal florets, the cylindrical area was reduced by half to account for the 50% biomass removal in those treatment groups. This approach assumes negligible contribution of the rachis to the photosynthetic surface area and has been applied in previous studies of rangeland grass physiology (Hamerlynck *et al.*, 2019). Response variables of interest included net photosynthesis (A_{net}), stomatal conductance to water (g_{sw}) and ϕ_{PSII} . Due to sample loss resulting from broken culms and damaged flag leaves, our final sample size for floret measurements was $n = 9$ individuals (5 Columbia and 4 Anatone), and the final sample size for flag leaves was $n = 5$ individuals (3 Columbia and 2 Anatone).

Within the clipped treatment groups (2 and 4), flag leaves were returned to the lab, scanned on a flatbed scanner (Epson Expression 12000XL) and digital images were analysed for leaf area using analytical software (WinRhizo Pro 2021, Regent Instruments Inc., Quebec, CA); leaves were then oven-dried at 65°C to constant mass and weighed to the nearest 0.1 mg using a Metler Toledo XSR64 microbalance. At the end of the study, all culms were collected for analysis of remaining flag leaves and spikes. Each sample was assessed for biomass and projected area, and spike and leaf specific mass were calculated as the mass per unit area (g cm⁻²).

Statistical analyses

For basal and distal florets, we pooled measurements across time points and used three-way ANOVAs to test for main and interactive effects of germplasm source, basal floret clipping and flag leaf clipping on floret physiology (A_{net} , g_{sw} , and ϕ_{PSII}). In each model, we allowed for an initial interaction among germplasm, basal clipping and distal clipping, and included an error structure to account for pseudoreplication at the plant level (*i.e.* four culms sampled on each plant). ANOVAs were conducted in the R statistical computing environment (R Core Team, 2022) using the 'anova' command in base R, and *post hoc* pairwise comparisons were conducted

using Tukey's tests to identify groups that differed in a statistically significant manner via the 'HSD.test' command in the agricolae package (Mendiburu, 2023).

Flag leaf physiological response to basal floret defoliation was assessed using two-way ANOVAs testing for the effects of germplasm and floret clipping, pooled across time points. We again used Tukey's *post hoc* multiple comparisons to identify differences among treatment groups. For flag leaf responses, we also investigated the two-way interaction between time point and floret clipping for Columbia germplasm using a repeated-measures ANOVA. Flag leaves of Anatone could not be statistically evaluated via repeated-measures ANOVA due to more extensive leaf senescence in this group.

The time required in making ~100 gas exchange measurements per day necessitated the relatively low sample sizes for these analyses, and we recognize these constraints on the statistical power of our tests. We also recognize that ANOVA is robust to small sample size effects to data distribution assumptions, and that low *P*-values themselves are indicative of sufficient statistical power to confidently reject null hypotheses (Hoenig and Heisey, 2001). Moreover, we are confident in the validity of any statistically significant test results because: i) we are highly confident in the distinct germplasm identities of the selected plants, and ii) that these plants were distributed randomly in a common soil and climate condition.

Finally, to assess relative investment in foliar versus reproductive structures between the two germplasm varieties, we also used *t*-tests to compare leaf mass per area (LMA; a metric of leaf tissue quality) and seed head specific mass.

Results

Basal floret physiology

Basal floret A_{net} of both Anatone and Columbia germplasm was significantly impacted by basal floret clipping, regardless of whether flag leaves were clipped (Fig. 2a; $F_{15, 123} = 9.5$, $P < 0.01$). Pooled across time points and flag clipping treatments, floret-clipped Anatone A_{net} was $11.42 \mu\text{mol m}^{-2} \text{s}^{-1}$ (± 0.53 s.e.) vs 5.21 ± 0.53 in the control group, and A_{net} of floret-clipped versus control groups for Columbia germplasm were 7.88 ± 0.48 and 4.39 ± 0.49 , respectively. RM-ANOVA also revealed a significant two-way interaction between germplasm and floret clipping. Unclipped Anatone and Columbia did not differ in terms of A_{net} , but floret-clipped Anatone showed a greater relative increase in A_{net} than Columbia (2.2-fold increase in Anatone vs 1.8-fold in Columbia). Stomatal conductance (g_{sw}) also consistently increased in response to basal floret clipping, regardless of flag leaf clipping treatment and germplasm identity (Fig. 2b; $F_{15, 123} = 16.2$, $P < 0.01$). Similar to A_{net} , basal floret g_{sw} showed a slightly greater increase in Anatone germplasm than Columbia germplasm (2-fold vs 1.8-fold change) in response to basal floret clipping. PSII yield showed a subtle decline after floret clipping, but three-way ANOVA and

subsequent *post hoc* tests revealed only a main effect of floret clipping pool across flag treatments and germplasm (Fig. 2c; $F_{15, 123} = 6.5$, $P < 0.01$).

Distal floret physiology

Pooled across measurement dates, distal florets did not exhibit any consistent physiological response to clipping treatments and did not vary systematically by germplasm source. A_{net} (Fig. 3a), stomatal conductance (Fig. 3b) and PSII yield (Fig. 3c) were all remarkably consistent across all combinations of germplasm and clipping treatments.

Flag leaf physiology

When pooled across time points, flag leaves did not show any significant physiological differences between germplasm varieties or among floret clipping treatments (Supplementary Fig. S2a–c). However, the two-way ANOVA (time point \times floret clipping) for Columbia germplasm indicated that all three response variables showed a general pattern of decline over the study period (Supplementary Fig. S3). Furthermore, although not significant at the $\alpha = 0.05$ level, flag leaf net photosynthesis and g_{sw} both showed a compensatory response to basal floret clipping.

Flag leaf versus spike biomass and tissue quality

Pooled across all treatment groups, flag leaf mass and LMA were similar between the two germplasm varieties (Anatone flag mass = 23.5 ± 3.3 mg (s.e.), Columbia flag mass = 18.8 ± 1.9 ; Anatone LMA (mg cm^{-2}) = 7.2 ± 0.3 , Columbia LMA = 7.3 ± 2.5) (Supplementary Fig. S4a, b). There was a marginally significant (*i.e.* $P < 0.10$) flag-clipping \times floret-clipping interaction on flag leaf mass ($F_{15, 20} = 4.3$, $P < 0.01$) and a significant three-way interaction of Germ \times Flag-Clip \times Floret-Clip on LMA ($F_{15, 20} = 2.9$, $P = 0.01$), but no significant pairwise differences were observed among the full factorial.

The mass and specific mass of unclipped spikes (green boxes, Supplementary Fig. S4c) were also similar between the two germplasm varieties. Control (unclipped) Anatone spikes had an average mass of 124 mg (± 19.4 s.e.), and average unclipped Columbia spike mass was 117 mg (± 15.0 s.e.). Spike mass generally ranged from 50 to 170 mg, and there was one very large spike mass outlier in the Anatone flag-clip \times spike-control group (dry mass = 237 g). Despite an absence of pairwise differences among groups for spike mass and for spike specific mass, three-way ANOVA revealed that our basal floret clipping significantly reduced spike mass ($F_{14, 20} = 2.9$, $P = 0.02$).

Discussion

As expected, we observed compensatory photosynthesis in both Anatone and Columbia germplasm of *P. spicata*,

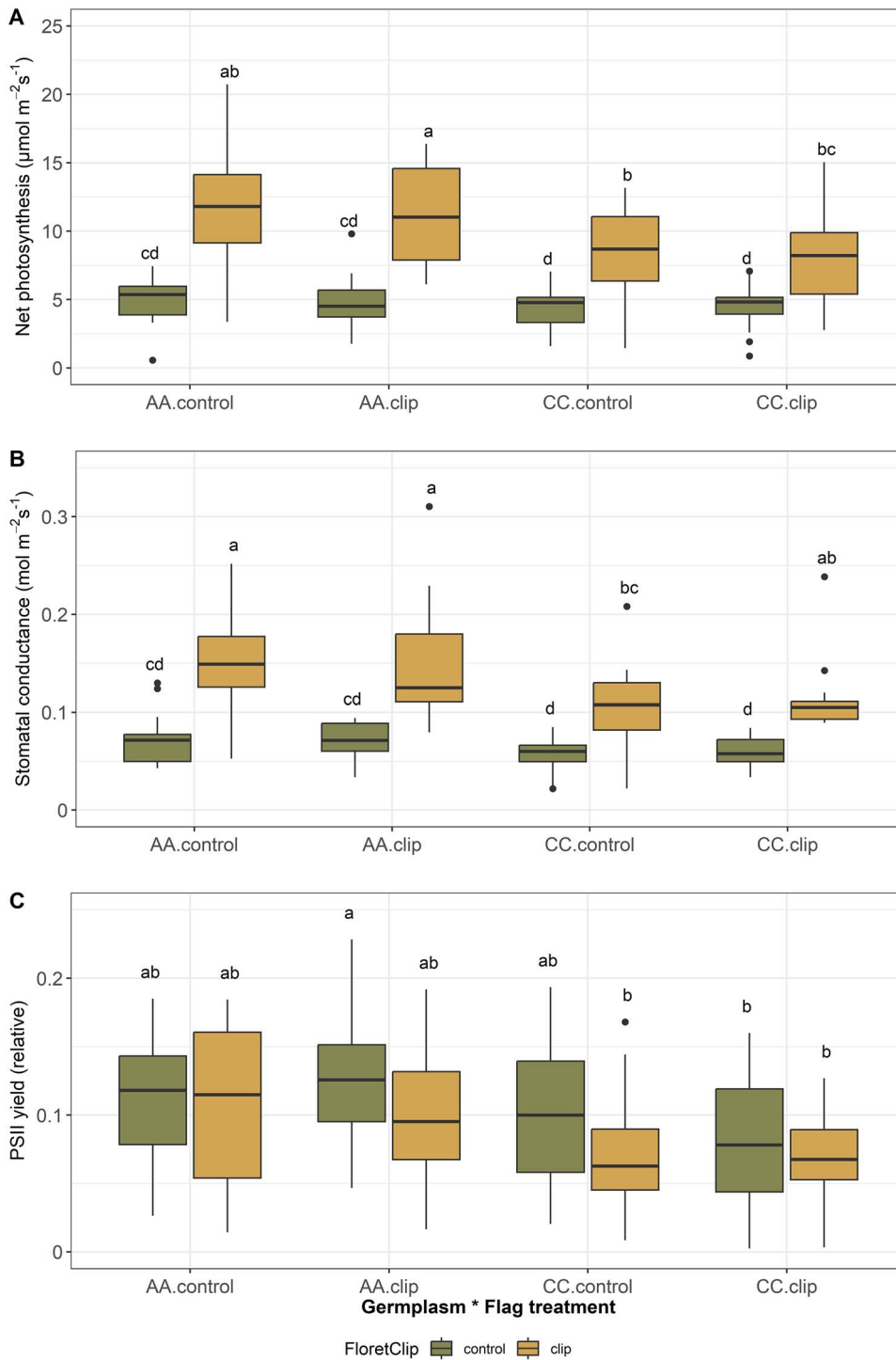


Figure 2: Basal floret photosynthetic responses to flag (x-axis) and basal floret (fill colour) clipping treatments for Anatone (AA) and Columbia (CC) germplasm. Significant treatment effects of the flower and flag-by-flower removal were observed for net photosynthesis (a) and stomatal conductance (b), but no treatment effects were observed for PSII yield (c). There was no main effect of flag leaf removal on the measured basal floret physiological parameters. Lowercase letters above boxplots indicate pairwise differences indicated by a Tukey's test at $\alpha = 0.05$.

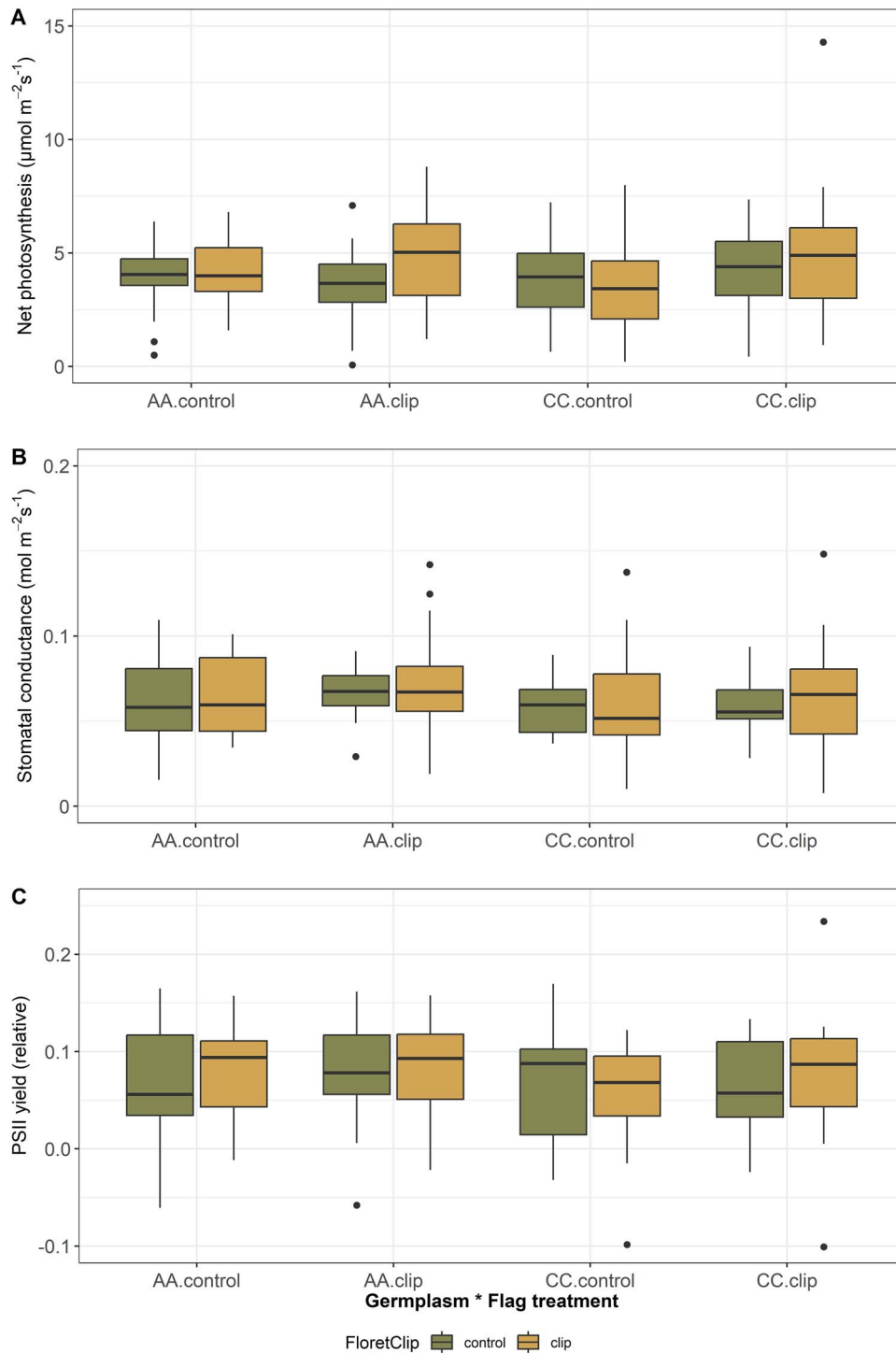


Figure 3: Distal floret photosynthetic responses to flag (x-axis) and basal floret (fill colour) clipping treatments for Anatone (AA) and Columbia (CC) germplasm. Although the flag-by-flower clipping treatment appeared to induce a slight compensatory response in distal floret A_{net} , effects of clipping were not statistically significant for A_{net} (a), g_{sw} (b) or ψ_{PSII} (c).

specifically at the basal florets (Fig. 2), suggesting that this could be a common trait in semi-arid perennial bunchgrasses, as conjectured by Hamerlynck *et al.* (2023). Contrary

to our expectations for intra-specific variation, however, reproductive compensatory photosynthesis was not expressed to a greater extent in Columbia germplasm individuals,

the germplasm selected for increased reproductive effort (Jones and Mott, 2016). Hamerlynck *et al.* (2019) noted the exotic bunchgrass crested wheatgrass (*Agropyron cristatum* L.) is distinct from native grasses in both reproductive photosynthetic capacity and in per-unit investment to reproductive structures, and further study has shown this is accompanied by higher filled seed specific mass, which could enhance seedling establishment (Hamerlynck and O'Connor, 2021; Quigley *et al.*, 2023). Despite these advantages, *A. cristatum* can have similar total reproductive culm production compared to native species (Hamerlynck and Ziegenhagen, 2020). It may be, therefore, that while selection for more reproductive culms in Columbia germplasm was not accompanied by enhanced photosynthetic compensatory ability, this did not alter basic per unit allocation to reproductive tissue. This is supported by the lack of differences in total spike mass or spike specific mass between Anatone and Columbia germplasm (Supplementary Fig. S4).

Understanding the differences and similarities in ecophysiological performance of native versus introduced lineages can help land managers predict community change under future environmental conditions and in scenarios of exotic plant invasion (Mozdzer *et al.*, 2013). Novel physiological traits have been shown to confer competitive advantages; *e.g.* success of a widespread exotic desert annual, *Erodium cicutarium*, relative to a native congener (*Erodium texanum*) was driven by differences in water use efficiency (Kimball *et al.*, 2014). Among rangeland bunchgrasses, such differences in physiological performance may cascade to further influence traits like persistence and reproductive success (Anderson and Toft, 1993; Meays *et al.*, 2000; Hamerlynck *et al.*, 2010, 2016; Mukherjee *et al.*, 2015). The two *P. spicata* varieties tested also showed some notable differences relative to the Eurasian exotic, *A. cristatum*. For instance, clipped basal *P. spicata* florets did not significantly decrease ϕ_{PSII} yield in response to floral defoliation, as observed for *A. cristatum* (Hamerlynck *et al.*, 2023). Hamerlynck *et al.* (2023) conjectured altered ϕ_{PSII} reflected physiological adjustment to changes in light interception resulting from defoliation. It may be the invariant ϕ_{PSII} apparent in *P. spicata* was due to its distinct spike morphology that resulted in little change in floret light interception following clipping, or because *P. spicata* is more limited in its ability to adjust PSII light-harvesting and photoprotective mechanisms in response to defoliation compared to *A. cristatum*. We also note that ϕ_{PSII} exhibits a strong diurnal response (Demmig-Adams and Adams, 1992; Hamerlynck and Huxman, 2009) and although they were randomized, our measurements were taken over a 4-h measurement period each day. We therefore suggest that simply implementing a more rapid measurement approach may minimize among-sample drift over the course of the day and make it easier to discern fluorescence response to clipping treatments.

Widespread senescence of Anatone flag leaves resulted in insufficient sample sizes to test for germplasm varieties or for an overall (pooled) compensatory response of flag

leaves to floret defoliation (Supplementary Fig. S2). However, there were noticeable time point-specific elevated A_{net} and g_s in Columbia individuals after basal floret clipping, even as overall photosynthetic rates declined (Supplementary Fig. S3). This could indicate an indirect photosynthetic response in Columbia individuals to clipped basal, but without concurrent flag leaf measurements in the Anatone group, this remains conjectural. Additional studies of flag leaf response to floral defoliation are needed as performance of flag leaves following reproductive defoliation has not been assessed in past studies (Hamerlynck *et al.*, 2023) and, unlike responses reported for *A. cristatum*, distal florets of neither germplasm showed evidence of indirect compensatory photosynthesis in florets distal to clipped basal sections (Fig. 3). Moreover, recent studies have shown flag leaves of perennial bunchgrasses can either energetically supply the seed head or compete with it for allocated resources or carbon (Guo *et al.*, 2020; Hamerlynck *et al.*, 2024). These studies and our results here suggest the interaction between floret and flag leaf physiological functioning in response to genetic or environmental variation may be a novel avenue for future research. Most arid systems are associated with highly variable, pulsed precipitation/soil moisture regimes (Noy-Meir, 1973; Huxman *et al.*, 2004). Compensating for floral tissue loss under such challenging conditions by shifting resources to increasing reproductive photosynthesis makes sense, since any loss of reproductive effort could have much more negative consequences compared to more mesic, less variable ecosystems. The greater compensatory photosynthetic response of Anatone compared to Columbia germplasm was unexpected and has implications in understanding the development of convergent drought- and herbivory-tolerance traits. Though both germplasms had similar A_{net} in unclipped control florets (Fig. 2) and in all distal florets (Fig. 3), the similar A_{net} attained under ambient conditions may not reflect differences in floret photosynthetic capacity or in reproductive photosynthetic regulatory processes, such as Hamerlynck *et al.* (2019) found across native sagebrush steppe bunchgrasses. Thus, it may be selection for increased spike production in Columbia germplasm plants was concurrent with decreased reproductive photosynthetic capacity, thereby limiting the magnitude of compensatory activity following defoliation. In Anatone germplasm plants, lower proportions of reproductive culms may have allowed allocation to higher reproductive photosynthetic capacity, facilitating greater compensatory photosynthesis. If so, these germplasms may display different patterns of reproductive effort. Hamerlynck and O'Connor (2021) showed that while photosynthetic contributions of the seed head itself were important to reproductive effort, the species with greater reproductive photosynthetic capacity produced proportionally fewer filled seeds, but these were ~2-fold greater in seed specific mass. Thus, selection for more reproductive culms may also, in addition to altering palatability of vegetative biomass to maintain sexual reproduction (Quiroga *et al.*, 2010), induce shifts to reproductive photosynthetic capacity associated with overall seed production and seed energetic quality traits (*i.e.* seed-specific

mass) important to seedling survival (Hamerlynck *et al.*, 2023; Quigley *et al.*, 2023).

Seed mass has been identified as a critical feature in dryland restoration success globally (Shackelford *et al.*, 2021). Hence, understanding and quantifying the mechanisms contributing to variation maternal energetic provisioning within and between species is of vital interest in arid land restoration and conservation efforts. Traditional plant material selection criteria based around biomass and seed production can lose critical trait expression from original accessions to field in a few generations (Dryer *et al.*, 2016) or does not produce plants with the requisite trait characteristics needed to meet management goals (Leger and Baughman, 2015; Garbowski *et al.*, 2021). With this in mind, Larson *et al.* (2023) recently proposed selecting seed mix species based on their specific recruitment strategies and mechanisms of adaptation as a means of grounding seed-based restoration within a recruitment niche framework. We take this assertion one step further by proposing that within a candidate seed mix species, carefully selecting for germplasm varieties that have greater physiological reproductive efforts may further improve restoration success. By identifying differences in the intrinsic physiological performance of different native perennial bunchgrass germplasms, we can leverage specific traits that provide improved adaptation to specific environmental filters, like drought, or select for general indicators of improved ability to acquire resources and compete with widespread invasives. While our study was limited to comparing only two germplasm varieties, subtle differences between these two similar varieties suggest that a wide range of physiological trait expression may be observed among the greater scope of genetically distinct original population clusters as well as newer potential sources (Larson *et al.*, 2004; St Clair *et al.*, 2013) or among other earlier released *P. spicata* plant materials like Whitmar, Goldar and P-7. Furthermore, our finding that Anatone had a greater compensatory response to floret clipping than Columbia germplasm may suggest that selection for increased culm production may not always confer improved reproductive success. Thus, specific germplasm varieties may display performance-based traits beyond, or contrary to, what was intended or expected during plant material development. Previous studies of cultivated rice varieties have identified genome-wide associations of physiological traits, including PSII efficiency (Tsai *et al.*, 2019). Moving forward, we suggest that researchers continue to investigate intraspecific physiological differences in bunchgrass performance, as well as work to identify genetic signals of adaptive traits to enable genomic-based plant material selection and, ultimately, improve the success of rangeland restoration and conservation (Whalley *et al.*, 2013; Jones *et al.*, 2022).

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Author contributions

K.Q. and E.H. designed the experiment. K.Q. implemented the experiment and collected data. T.M. provided lab resources and processed plant samples. K.Q. processed and analysed Licor data. K.Q. and E.H. wrote the initial manuscript draft. K.Q., E.H., R.C. and T.M. each made substantial contributions to interpreting results and writing the final manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

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Data availability

All data used in this study is available on the Dryad data repository, doi:10.5061/dryad.4b8gthpf.

Supplementary Material

Supplementary Material is available at *Conservation Physiology* online.

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