#927

Rangeland Ecology & Management 71 (2018) 25–34

Contents lists available at ScienceDirect

Rangeland Ecology & Management

journal homepage: http://www.elsevier.com/locate/rama

Original Research

Seedling Defoliation and Drought Stress: Variation in Intensity and Frequency Affect Performance and Survival☆

Elsie M. Denton [a,*], Brenda S. Smith [b], Erik P. Hamerlynck [c], Roger L. Sheley [c]

[a] Range Technician, US Department of Agriculture (USDA)—Agricultural Research Service (ARS), Eastern Oregon Agricultural Research Center, Burns, OR 97720, USA
[b] Executive Director, High Desert Partnership, Burns, OR 97720, USA.
[c] Research Ecologist, US Department of Agriculture (USDA)—Agricultural Research Service (ARS), Eastern Oregon Agricultural Research Center, Burns, OR 97720, USA

ARTICLE INFO

Article history:
Received 20 December 2016
Received in revised form 13 June 2017
Accepted 24 June 2017

Key Words:
defoliation
drought
establishment
herbivory
perennial grass
seedlings

ABSTRACT

Our ability to restore rangelands is limited, and it is unknown if seedling herbivory on its own, or in interaction with other stressors, is a major contributor to restoration failure. To address this, we conducted two experiments: a No Defoliation (ND) experiment ($n = 48$), in which seedlings from three perennial grasses (crested wheatgrass [*Agropyron cristatum* {(L.} Gaertn.], bluebunch wheatgrass [*Psuedoroegnaria spicata* {Pursh} Á. Love], Sandberg bluegrass [*Poa secunda* J Presl]) were subjected to wet and dry water regimes for 4 mo, and a concurrent Defoliation (D) experiment ($n = 95$), in which seedlings were factorially assigned to two defoliation treatments—frequency (LOW, HIGH) and intensity (30% vegetation removal, 70% vegetation removal). Indicators of seedling performance were aboveground and belowground biomass (AGB and BGB), root:shoot ratio, tillering, and mortality. The effect size statistic, Hedge's g, allowed for comparisons between performance measures. Water stress induced reductions in most performance measures: BGB ($g =$ ND: –1.3; D: –1.6), root:shoot ratio ($g =$ ND: n.s.; D: –0.2), and tillering ($g =$ ND: –1.7; D: –1.2), though not significantly for all species. For ABG, water stress interacted with defoliation, reducing performance less at an intensity of 70% ($g = –2.0$) as opposed to 30% ($g = –3.0$), but not always significantly in the former. Water stress also caused less reduction in AGB when no defoliation occurred (ND: –0.8; $g =$ D: –2.5). Intensity and frequency of defoliation interacted; seedlings were generally resistant to reductions in performance except at high frequency, 70% defoliation. *Agropyron cristatum* and *P. spicata* displayed similar sensitivity to treatments, mostly in terms of changes in AGB and BGB, while *P. secunda* also experienced increased mortality and reduced tillering. If these differences in sensitivity result in differential survival, herbivory could impact species postrestoration population demographics.

Published by Elsevier Inc. on behalf of The Society for Range Management.

Introduction

Rangelands are extensive and diverse ecosystems covering around 40% of the world's land area (White et al., 2000). Like many ecosystems on earth, rangelands are threatened. Upper estimates indicate that 20% of these regions are already degraded (Adeel et al., 2005). While net zero land degradation is one of the Millennium Development Goals, unsuccessful restoration projects are common. Most projects result in no change from prevailing conditions whether they are large-scale revegetation after disturbance, such as fire (Dalzell, 2004; Arkle et al., 2014), mine restorations (Herrick et al., 2006), or smaller-scale tests of concept (Bleak et al., 1965; Wilson et al., 2004). Identifying the mechanisms that cause restoration projects to fail may allow us to develop more successful methods.

A key goal of successful restoration is the formation of self-sustaining and recruiting populations (Hardegree et al., 2016). However, this means that individuals must survive from seed, to seedling, to adult plant. Various ecological conditions, processes, and mechanisms control the ability of a seed to produce a mature adult (James et al., 2013). First, a seed must germinate successfully, which requires breaking potential seed dormancy (Monsen and Stevens, 2004), avoiding seed predation (Barberá et al., 2006), and appropriate light and moisture levels (Isselstein et al., 2002; Barberá et al., 2006; Bailey et al., 2012; Fehmi et al., 2014). A germinated seed must then emerge (Larson et al., 2015) after enduring freeze/thaw cycles, avoiding fungal infection and penetrating soil crusts (James et al., 2011). An emerged seedling further must withstand drought (Leishman and Westoby, 1994; Asay et al., 2001; Engelbrecht et al., 2005) and herbivory (Moles and Westoby, 2004) before it can proceed to future stages and finally become a mature adult.

☆ Funding for this work came from the US Department of Agriculture. The USDA is an equal opportunity employer.

* Correspondence: Elsie M. Denton, Range Technician, USDA-ARS Eastern Oregon Agricultural Research Center, 67826-A Hwy 205, Burns, OR 97720, USA.

E-mail address: elsie.denton@ars.usda.gov (E.M. Denton).

http://dx.doi.org/10.1016/j.rama.2017.06.014
1550-7424/Published by Elsevier Inc. on behalf of The Society for Range Management.

Herbivory of newly emerged seedlings has been documented, but the extent to which it is a dominant ecological process during seedling establishment is largely unknown for most ecosystems. In a limited literature synthesis focusing on the tropics, Moles and Westoby (2004) found that herbivory was among the top three causes of seedling mortality, accounting for 38% of explainable deaths. The seedling stage is vulnerable because survival relies on limited cotyledon nutrient and energy reserves to produce sufficient leaf area to sustain net carbon gain (Hanley et al. 2004). Additionally, establishing plants are particularly susceptible to herbivory as plant palatability changes with age, with strong consequences to community structure (Hanley et al., 1995; Barton and Hanley, 2013).

In this study we aimed to determine if defoliation, as a proxy for herbivory, occurring at the seedling stage in perennial grasses could be an important contributing factor to restoration failure. We were particularly interested in determining if the intensity and frequency of defoliation influence seedling success. Previous work with grass seedlings indicates frequency of defoliation may influence survival but has not addressed intensity (Roundy et al., 1985; Pyke, 1987). Adult grasses have been found to respond negatively to frequent grazing but are more insensitive to the intensity of that grazing (Ferraro and Oesterheld, 2002; Brewer et al., 2007); however, seedlings, that are still reliant on their coleoptile energy and nutrient reserves may be more sensitive to intensity of defoliation (Hanley and Fegan, 2007). Additionally, stressors rarely occur in isolation in natural environment; water stress may interact with defoliation frequency and intensity to influence seedling growth and survival. Seedlings may display greater sensitivity to defoliation, reduced sensitivity to defoliation, or no change in sensitivity to defoliation under water stress depending on what resources are limiting plant growth (Wise and Abrahamson, 2007). Wise and Abrahamson (2007) indicated that increased tolerance to herbivory under water stress might be most likely; however, Hawkes and Sullivan (2001) found that mature monocots frequently were better able to withstand herbivory at high resource levels, and the same may be true of monocot seedlings.

Also of concern when investigating how seedlings respond to defoliation are potential differences between native and introduced species. Native plants are desirable in restoration projects (Monsen and Stevens, 2004; US Department of Interior, 2004), but since the goal of restoration is often to improve or restore ecosystem services, desirable exotics are often used instead because they establish more reliably and are less expensive (Monsen and Stevens, 2004), particularly at drier sites (Asay et al., 2001). In studies of adult plants, introduced species have been found to be more resistant to defoliation than natives (Kimball and Schiffman, 2003; Ralphs, 2009). It is not yet known if the same holds true for seedlings as the few studies that have investigated this issue have found conflicting results (Huber-sannwald and Pyke, 2005; James et al., 2011).

We hypothesized that the intensity and frequency of defoliation would interact to determine the performance and survivorship of seedlings, with repeated, higher-intensity defoliation leading to the lowest performance in terms of tillering, biomass, and survival. We proposed two competing hypotheses for how defoliation will interact with water stress. Either seedlings would be better able to recover from leaf removal when water is abundant, as has been found in monocots previously, or seedlings would be more resistant to defoliation when drought stressed, as has been found across a range of plant functional types. Since the non-native species we selected has evolved under intense grazing pressure, we hypothesized that the non-native species would be more capable of tolerating intense and frequent defoliation.

Methods

Model System

The sagebrush steppe is one of the most extensive ecosystems in North America, but it is under threat from conifer encroachment, invasive species, and human development (Noss et al., 1995; Salvo, 2008; Davies et al., 2011). Sagebrush steppe vegetation once covered >62 million ha, but only around 30 − 40 million ha of this ecosystem remain (Knick et al., 2003; Miller et al., 2011). Even under current best management practices, models suggest that, if left unchecked, altered fire cycles and invasive annual grasses will destroy >100 000 hectares of sagebrush steppe each year (Hemstrom et al., 2002). As this rangeland system is both under threat and frequently targeted for restoration (Dalzell, 2004; Pyke, 2011; Pyke et al., 2015), it provided a good model system in which to test drivers of restoration success.

Additionally, a variety of herbivores have been documented in the sagebrush steppe, including invertebrates, birds, and small mammals such as rodents, rabbits, and hares (Larrison and Johnson, 1973; McAdoo et al., 2006). Further, rodents may play an important role in limiting seedling establishment on sagebrush steppe (Pyke, 1986, 1987). Native, large herbivores, such as deer and antelope, can be found in the sagebrush system (Verts and Carraway, 1998), and the ecosystem is extensively grazed by the cattle industry (Young and Sparks, 2002).

Experimental Design

This study consisted of two concurrent experiments. The first was a randomized block design with four treatments, three species × two watering regimes × two defoliation intensities × two defoliation frequencies, all factorially arranged in four blocks ($n = 96$). This will be referred to as the Defoliation experiment hereafter. Additionally, a control experiment that was not defoliated was conducted simultaneously; it had two treatments, three species × two watering regimes, with two replicates of each treatment combination within each of four blocks ($n = 48$). This will be referred to as the No Defoliation experiment hereafter. The same watering treatments were applied to both the Defoliation and No Defoliation experiments. Watering regimes consisted of a well-watered treatment (water = WET) and dry treatment (water = DRY). Both experiments were conducted in the same place and time, with pots intermixed; however, due to the differing number of treatments that were applied (species and water regime, in the No Defoliation Experiment; species, watering regime, defoliation intensity, and defoliation frequency in the Defoliation Experiment), the data from these experiments could not be analyzed together.

In the Defoliation experiment, defoliation treatments were applied once plants reached the two-leaf stage when individuals are no longer dependent on their cotyledons (Hanley and Fegan, 2007) but have not yet become established or begun to develop their mature root systems (Ries and Svejcar, 1991; Defossé et al., 1997; Moser and Smart, 1997). Either 30% of the leaf blade length (intensity = 30) or 70% of the leaf blade length (intensity = 70) was removed by clipping. Of those plants originally defoliated, half were defoliated a second time 4 wk after the initial defoliation at the same intensity (frequency = HIGH). The other half was not defoliated a second time (frequency = LOW).

Study

This study was conducted from 20 March to 31 July, 2014 at the Eastern Oregon Agricultural Research Center near Burns, Oregon (43°31'06.7"N, 119°01'18.3"W) in a minimally temperature-controlled hoop house structure. The hoop house allowed ventilation from both sides and one end wall; when temperatures reach 32°C, a fan engages to promote further ventilation. Temperatures in the hoop house mirrored ambient outside temperature over the course of the study.

Two widespread, native, perennial grasses of the Wyoming big sagebrush steppe ecosystem were chosen to serve as model species: bluebunch wheatgrass (*Psuedoroegnaria spicata* [Pursh] Á. Love, var Anatone) and Sandberg bluegrass (*Poa secunda* J Presl). In addition, we chose an exotic, perennial grass used frequently for rangeland revegetation, crested wheatgrass (*Agropyron cristatum* [L.] Gaertn., var. Hycrest II), for comparison. Crested wheatgrass seed were obtained from Maple Leaf Seed, Ephraim, Utah and Sandberg bluegrass and bluebunch wheatgrass seed was obtained from Western Reclamation Inc., Eltopia, Washington.

Pots (5.4 cm in width and 10.8 cm in depth) were used as experimental units. Mesh was placed on the bottom of the pots to prevent soil loss, while permitting water drainage. Pots were filled with a soil mix. Soils for use in experimental pots were collected from the Northern Great Basin Experimental Range, 16 km southeast of Riley, Oregon (43°27'58.37"N, 119°41'49.15"W). Soils were Gradon gravelly fine sandy loam. Collected soil was mixed in a 1:1 ratio with a Dogmountain gravelly loam collected 24 km south of Burns, Oregon (43°21′36.15″N, 119°6′6.77″W) to prevent soil from solidifying once natural structure was disturbed.

Water capacity for each pot was determined by weighing the dry pot, watering until it dripped, letting the pot stand overnight, and weighing it again. The difference between the dry and next-day weight of each pot was recorded as pot capacity. The average pot capacity was 0.29 kg water weight, and pot capacity ranged from 0.20 to 0.39 kg water weight. Soil moisture content was measured daily with a CS620 Hydrosense soil moisture probe (Campbell Scientific, Logan, UT). Water of equivalent weight to pot capacity was added to the WET treatment pots when the moisture level read 10%. Pots in the DRY treatment were allowed to dry down until the moisture level read 5% before water was added back to pot capacity.

Soils in each pot were planted with 10 seeds of the single species. Seeds were pregerminated on a moist blotter paper in a warming tray for 24 − 72 hours depending on species. Pot surfaces were kept uniformly moist until the grasses reached the two true-leaf stage, and then each pot was thinned to three uniform plants and the watering treatments described earlier were instituted. These three seedlings were subplots whose response values were averaged for each experimental unit (i.e., pot) to reduce stochastic variation.

Data Collection

Four different performance metrics were measured, all of which have been found to correlate with establishment success and survival: AGB (Gross, 1981; Tsakaldimi et al., 2013), BGB (Roundy et al., 1985; Ries and Svejcar, 1991; Tsakaldimi et al., 2013), tiller number (Ries and Svejcar, 1991; Zhang and Romo, 1995; Lollato, 2015) and root:shoot ratios (Evetts and Burnside, 1973; Lloret et al., 1999; Karcher et al., 2008). AGB included not only the vegetation present at final harvest but also vegetation removed during each defoliation period. Final AGB, which did not include biomass removed during defoliation treatments, was noted as well. Additionally, plant mortality was recorded when it occurred.

The first round of defoliation occurred on 14 May for bluebunch wheatgrass and crested wheatgrass and on 21 May for Sandberg bluegrass. For the subset of pots to be defoliated at HIGH frequency, the second defoliation occurred on 12 June for bluebunch wheatgrass and crested wheatgrass and 20 June Sandberg bluegrass. Defoliated biomass was collected, dried, and weighed. Final sampling was conducted on 8 July for bluebunch wheatgrass and crested wheatgrass and 14 July for Sandberg bluegrass. During final sampling, the total number of living and dead individuals in each pot was recorded, and the total number of tillers for all individuals counted. All live plant material was removed and separated into AGB and BGB. BGB was washed to remove soil. All plant biomass was dried at 65°C for 48 hours and then weighed. Root:shoot ratios were calculated by dividing BGB by AGB.

Data Analysis

For the Defoliation experiment, AGB, BGB, root:shoot ratio, number of tillers, and final AGB were examined separately using a mixed model with species, water, defoliation intensity, defoliation frequency, and their interactions as fixed factors; block was included as a random factor. For the No Defoliation experiment, response variables were fit using a mixed model with species, water, and their interaction as fixed factors and block and block × water × species as random factors. Data analysis was done in SAS 9.4 (SAS Institute, Cary, NC).

Since substantial die-off was only observed in Sandberg bluegrass, a simplified version of the model above was used to examine mortality within that species. Percent mortality was calculated as the number of dead seedlings within an experimental unit at the end of the experiment divided by number of total seedlings, and then these values were logit transformed (Aston 1972) to meet analysis of variance (ANOVA) assumptions. A correction of 0.000001 was added to the units with 0% mortality and subtracted from units with 100% mortality to prevent undefined logits. This correction was deemed sufficiently small as making it smaller did not change the outcome of the tests. Mortality in the Defoliation experiment was fit using a mixed model with water, defoliation intensity, defoliation frequency, and their interactions as fixed effects and block as a random factor. Mortality in the No Defoliation experiment was fit using a mixed model with water as a fixed factor and block and block × water as random factors.

Models were fit using residual maximum likelihood estimation and a Kenward-Roger degrees of freedom approximation (Kenward and Roger, 1997). A standard 95% level was used for significance, but we have informed this statistic with additional measures of effect size. When interactions were significant, simple effects were examined using the SLICE command in the LS MEANS statement. All variables were transformed to meet ANOVA assumptions (Table 1). In some cases, one to two data points had to be excluded from the Defoliation experiment analysis due to missing data, creating an unbalanced design (see Table 1).

Three different measures of effect size were used. The amount of variance explained by each model was estimated using ANOVA R^2 calculated,

$$R^2 = \frac{V_{null} - V_{full}}{V_{null}} \tag{1}$$

Where V_{null} is the residual covariance of the model when only random factors are included, and V_{full} is the residual covariance of the model when all factors are included (Selya et al., 2012).

Cohen's f^2 was used to estimate the magnitude of the variance explained by each term within the model as it is one of the few effect size statistics that can be used with mixed models with unbalanced designs. More commonly used statistics with ANOVA, such as η (eta), ω (omega), and their squares, only work when sums of squares estimation can be used (Fritz et al. 2012). Cohen's f^2 is calculated from ANOVA R^2,

$$f^2 = \frac{R^2{}_{model} - R^2{}_{model-A}}{1 - R^2{}_{model}} \tag{2}$$

Where $R^2{}_{model}$ is the variance explained by the full model calculated earlier, and $R^2{}_{model\text{-}A}$ is the variance explained when the term of interest and all its interactions have been removed from the model. Cohen's f^2 is considered small at 0.02, medium at 0.15, and large at 0.35 (Cohen 1992), but the value can go much higher. Negative values mean that the factor actually reduces the amount of the variance the model explains.

Hedges' *g* was used to estimate the magnitude of the difference between means when simple effects were examined within interactions (Fritz et al. 2012). Hedges' *g* can be interpreted as the number of standard deviations by which two means differ and are directional—values close to zero ($g < 0.4$) indicate that the means are similar, values >1.1

Table 1
Definitions of response variables used in this study.

Response variables	Definition	Original units	Transformation	*N*
Aboveground final	Average mass at harvest of all AGB within a sample unit	g	Square root	48/95
AGB	Average mass of AGB within a sample unit including defoliated vegetation and mass at harvest	g	Square root	48/94
Total BGB	Average mass at harvest of all BGB within a sample unit	g	Square root	48/95
Root-to-shoot ratio	AGB/BGB	Proportion	Log	48/94
Tillers	Average number of tillers within sample unit at harvest	Count	Log	48/95
Mortality	Number of dead divided by total number of individuals within a sample unit	Proportion	Logit	16/32

AGB, aboveground biomass; BGB, belowground biomass.
Numbers separated by a slash in the N column represent the experimental units included in the No Defoliation versus the Defoliation analyses. Occasionally, experimental units had to be excluded from analysis due to missing data. Defoliation and No Defoliation experiments could not be combined into a single analysis due to an additional treatment applied to the latter (HIGH vs. LOW frequency defoliation). Mortality was only examined for Sandberg bluegrass, as other species experienced no or almost no mortality.

indicate differences that are easily observable, and large values ($g > 2.7$) indicate strong effects (Ferguson, 2009). Hedges' g is calculated,

$$g = \frac{mean_1 - mean_2}{s^*} \tag{3}$$

Where s^* is a pooled estimate of the standard deviation of both sampled populations as calculated by (Morris and DeShon 2003).

$$s^* = \sqrt{\frac{((n_1 - 1) \times std_1^2) + \left((n_2 - 1) \times std_2^2\right)}{((n_1 + n_2) - 2)}} \tag{4}$$

Where n_1 is the number of samples in the first mean, std_1 is the standard deviation of those samples, n_2 is the numbers of samples in the second mean, and std_2 is the standard deviation of the second mean.

Although directly testing treatment differences between seedlings in the No Defoliation and Defoliation experiments was not possible due to the differing number of treatments applied, we compared the individual 95% confidence intervals around two means within a species under the same watering conditions. An example is the confidence interval for BGB in crested wheatgrass in the No Defoliation experiment, WET treatment (0.083–0.17 g) versus crested wheatgrass in the Defoliation experiment, all WET treatments: intensity 30%, frequency LOW (0.17–0.19 g); intensity 30%, frequency HIGH (0.088–0.15 g); intensity 70%, frequency LOW (0.11–0.16 g), and intensity 70%, frequency HIGH (0.086–0.10 g). In this case, all confidence intervals overlap; therefore, no differences likely exist between BGB in crested wheatgrass when defoliated as opposed to when undefoliated under well-watered conditions.

Results

Species

Under control, unstressed conditions (i.e., No Defoliation experiment, WET treatment) species differences in plant performance measures were minimal between bluebunch wheatgrass and crested wheatgrass. They both had similar AGB (bluebunch: 0.14 g; crested; 0.14 g; $g = |0.07|$), and bluebunch wheatgrass had BGB that was only slightly higher than crested wheatgrass (bluebunch: 0.16 g; crested: 0.12 g; $g = |0.58|$) and correspondingly similar root:shoot ratios (bluebunch ratio: 1.1, crested ratio: 0.9, $g = |0.71|$). However, bluebunch wheatgrass seedlings had approximately one more tiller on average (3.9 tillers) than crested wheatgrass seedlings (2.8 tillers) ($g = |1.63|$). Sandberg bluegrass had similar BGB to the other two species (Sandberg: 0.17 g; $g < |0.90|$) but lower AGB (Sandberg: 0.06 g; $g > |1.99|$), resulting in a root:shoot ratio that was nearly triple that of the larger grasses (ratio of 2.7, $g > |3.98|$). Sandberg bluegrass had an average of 7 tillers as compared to crested wheatgrass's 2.8 and bluebunch wheatgrass's 3.9 ($g > |3.47|$). All three species responded differently to the stress treatments, as discussed in the following sections.

No mortality occurred in crested wheatgrass in either the No Defoliation or Defoliation experiment. One bluebunch wheatgrass seedling died in the Defoliation experiment, and a total of 20 Sandberg bluegrass seedlings died in both experiments: 5 in the No Defoliation experiment and 15 in the Defoliation experiment.

Defoliated Seedlings versus Undefoliated Seedlings

In the vast majority of cases, there was no difference (confidence intervals overlapped) between performance measurements in the No Defoliation experiment and corresponding means in the Defoliation experiment for the same species and watering level. However, for BGB and root:shoot ratio in all species, the treatment combination DRY, intensity 70%, and frequency HIGH resulted in confidence intervals that were below the corresponding interval in the No Defoliation experiment (see Supplementary Tables 1 and 2). BGB and root:shoot ratio also had lower confidence intervals for Sandberg bluegrass in the WET, intensity 70%, and frequency HIGH treatment as compared with the No Defoliation WET treatment. The only case of a nonoverlapping confidence interval for tillering was Sandberg bluegrass where tilling was lower in one Defoliation treatment (WET, intensity 70%, frequency HIGH) than the corresponding No Defoliation WET treatment. Final aboveground biomass only differed between the Defoliation and No Defoliation experiments once. Crested wheatgrass final AGB (DRY, clipping level 30%, frequency HIGH) was lower than in the corresponding No Defoliation DRY treatment. There was one case of a positive nonoverlapping confidence interval. For bluebunch wheatgrass (WET, intensity 70%, frequency LOW), AGB was higher than the corresponding No Defoliation WET treatment.

Water

Water in the No Defoliation Experiment

In the absence of defoliation, seedlings in the DRY treatment performed worse than seedlings in the WET treatment (Fig. 1, Left; Table 2). For AGB, watering explained a large amount of the variance seen between seedlings, but the difference in means was not large ($g = -0.81$). In the case of BGB (see Fig. 1, Right) and tillering (Fig. 2, Right), species and water interacted to determine seedling response to treatments; water stress did not cause reductions in performance for crested wheatgrass and bluebunch wheatgrass, but it did for Sandberg bluegrass. Sandberg bluegrass seedlings demonstrated around a three standard deviation reduction in both performance measures under dry conditions (BGB $g = -2.9$; tillers $g = -3.1$). For BGB this interaction explained a medium amount of the variance between seedlings, and for tillering it explained a large amount of the variation. Watering had no significant effect on root:shoot ratios ($g = 0.1$) or mortality (Table 3): 4.2% and 1.7% of Sandberg bluegrass seedlings died in the DRY and WET treatments, respectively.

Water in the Defoliation Experiment

Water stress generally reduced performance in defoliated seedlings. For Sandberg bluegrass, the only species with substantial mortality,

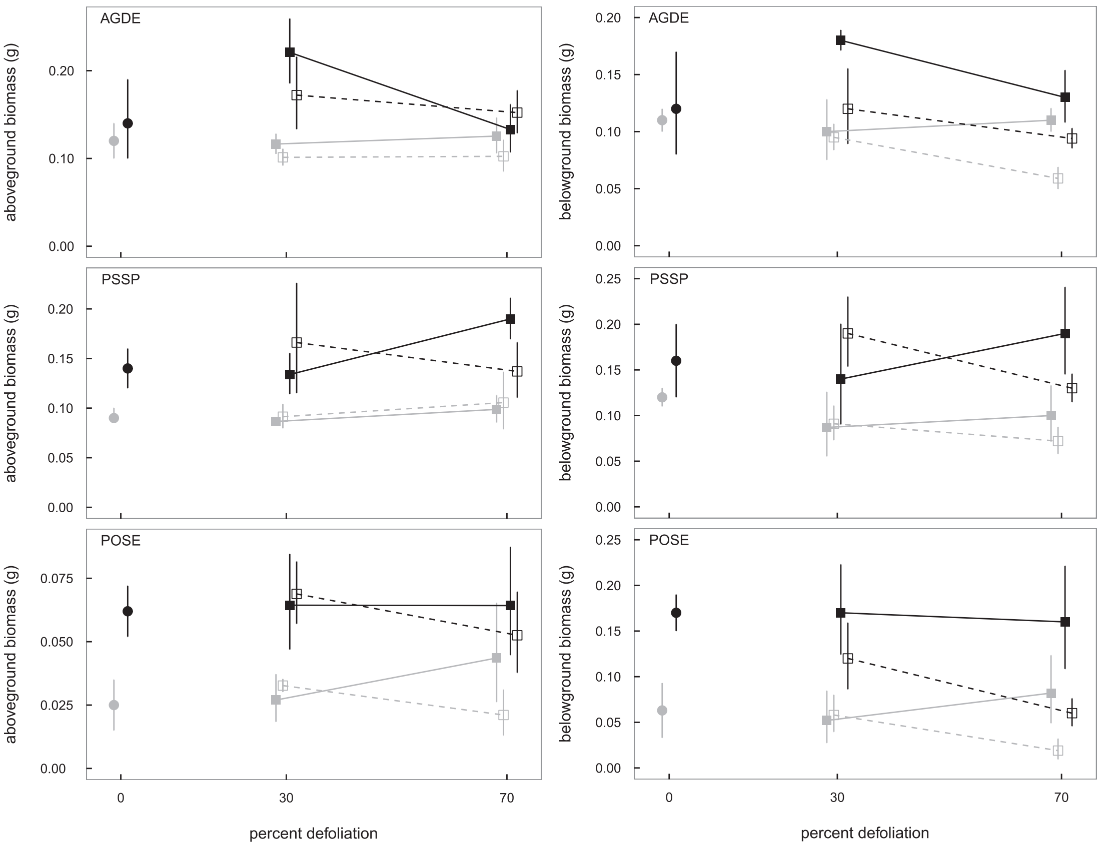

Figure 1. Left, Back transformed means for aboveground total biomass (AGB). **Right,** Back transformed means for belowground total biomass (BGB). Black points represent the wet treatment, and gray points represent the dry treatment. Circular points are undefoliated plots, square points are defoliated plots: solid, low-frequency defoliation; hollow, high-frequency defoliation. Error bars represent 95% confidence intervals. Note that No Defoliation and the Defoliation treatments had to be analyzed separately due to a differing number of treatments applied.

Table 2
Simplified analysis of variance (ANOVA) table of results for models fit to No Defoliation and Defoliation Experiments for 4 main response variables of interest.

	AGB		BGB		Root-to-shoot ratio		Tillers	
	F Value	Cohen's F^2 [1]	*F* Value	Cohen's F^2 [1]	*F* Value	Cohen's F^2 [1]	*F* Value	Cohen's F^2 [1]
No Defoliation								
Species (S)	53.23***	2.5	1.64	0.19	56.01***	3.2	57.66***	2.8
Water (W)	25.41***	0.66	17.62***	0.68	0.90	0.10	32.96***	1.1
S × W	1.69	-0.0025	5.84*	0.16	1.26	0.10	8.9***	0.36
R^2	0.75		0.41		0.76		0.77	
Defoliation								
Species (S)	227.21***	5.7	15.87***	0.60	84.42***	2.1	75.75***	2.3
Water (W)	140.25***	2.0	114.8***	1.5	4.90*	0.080	50.38***	0.82
Intensity (I)	0.66	0.43	7.95**	0.40	10.75**	0.30	0.64	0.32
Frequency (F)	3.49	0.26	34.23***	0.84	30.14***	0.67	0.19	0.46
S × W	0.25	0.14	4.63*	0.10	2.34	0.022	8.90***	0.26
S × I	4.46*	0.36	1.88	0.077	1.57	−0.030	0.68	0.31
S × F	0.88	0.22	4.94**	0.17	5.87**	0.12	5.24**	0.46
W × I	6.15*	0.22	0.77	−0.0040	2.72	−0.0066	0.01	0.017
W × F	0.02	0.13	0.64	0.0040	0.05	−0.012	0.04	0.27
I × F	3.46	0.26	22.05***	0.34	17.79***	0.19	5.10*	0.37
S × W × I	2.33	0.17	0.34	0.0065	0.60	−0.036	1.49	0.031
S × W × F	0.85	0.12	0.74	0.017	1.79	−0.0044	1.84	0.041
S × I × F	5.10**	0.13	2.81	0.075	0.64	−0.035	12.57***	0.33
W × I × F	0.13	0.12	0.48	0.017	1.47	−0.019	1.01	0.018
S × W × I × F	5.51**	0.13	1.88	0.025	0.06	−0.026	1.58	0.018
R^2	0.87		0.71		0.71		0.73	

Asterisks indicate significant effects ($^{*} < 0.05$, $^{**} < 0.01$, $^{***} < 0.001$). Full ANOVA tables, including degrees of freedom can be found in Supplementary Materials. See Table 1 for definitions of AGB, BGB, root-to-shoot ratios, and tillers.

[1] The measure of effect size Cohen's F^2 is considered small at 0.02, medium at 0.15, and large at 0.35 + (Cohen 1992). Negative values mean that the factor actually reduces the amount of the variance the model explains.

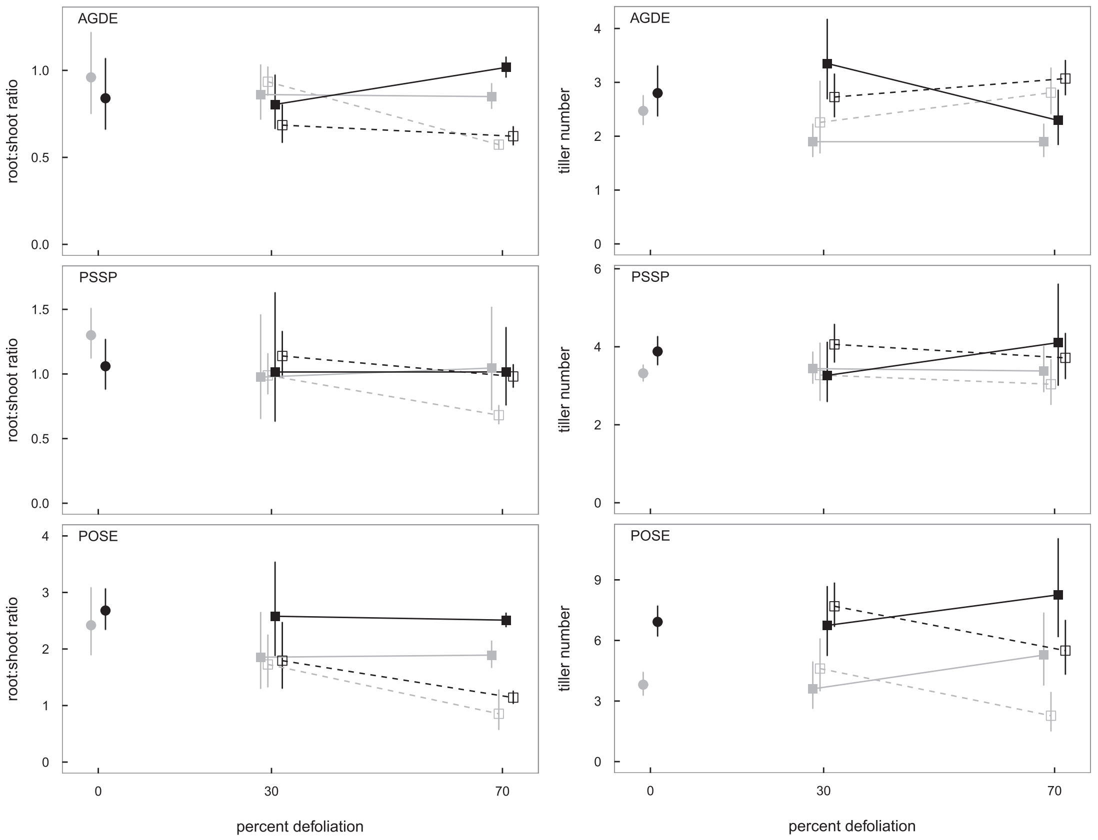

Figure 2. Left, Back transformed means for root:shoot ratios. **Right,** Back transformed means for tillers per plant. Black points represent the wet treatment, and gray points represent the dry treatment. Circular points are undefoliated plots, square points are defoliated plots: solid, low-frequency defoliation; hollow, high-frequency defoliation. Error bars represent 95% confidence intervals. Note that No Defoliation and Defoliation treatments had to be analyzed separately due to differing number of treatments applied.

watering explained a large amount of the variation in survival between seedlings; 29.3% of seedling died in the DRY treatment while only 2.1% died in the WET treatment (see Table 3). Water stress also reduced root:shoot ratios, but the amount of variance explained was small (see Table 2 and Fig. 2, Left). Seedlings in the DRY treatments, regardless of species, defoliation level, or frequency, had significantly lower root:shoot ratios than seedlings in the WET treatments, but the difference was considerably less than one standard deviation ($g = -0.2$). The remaining performance measures (AGB, BGB, and tillering) also responded to water stress but had significant higher-order interactions, so they will be discussed in more detail later.

Table 3
Mortality analysis for Sandberg bluegrass.

	F Value$_{(\text{df1, df2})}$	P value	Cohen's F^2 [1]
No Defoliation			
Water	2.45 $_{(1,\ 3)}$	0.21	0.00
Defoliation			
Water (W)	13.66 $_{(1,\ 21)}$	0.0013**	0.55
Intensity (I)	0.12 $_{(1,\ 21)}$	0.73	−0.11
Frequency (F)	5.85 $_{(1,\ 21)}$	0.025*	0.23
W × I	0.09 $_{(1,\ 21)}$	0.77	−0.045
W × F	3.17 $_{(1,\ 21)}$	0.090	0.088
I × F	0.09 $_{(1,\ 21)}$	0.77	−0.045
W × I × F	0.87 $_{(1,\ 21)}$	0.36	-0.045

$N = 16$ for No Defoliation, and $N = 32$ for Defoliation experiment. The No Defoliation model had an R^2 of 0.00003. The watering treatment explained essentially none of the variance that occurred in the model (covariance from block = 0, covariance from water · block = 3.59). R^2 for 30/70% clipping model is 0.38. Block covariance is 2.50. Asterisks indicate significant effects (* < 0.05, ** < 0.01, *** < 0.001).

[1] The measure of effect size Cohen's F^2 is considered small at 0.02, medium at 0.15, and large at 0.35 (Cohen 1992). Negative values mean that the factor actually reduces the amount of variance the model explains.

Water: AGB Interactions

All four treatments interacted to determine seedling response in terms of AGB, though the variation explained by the interaction was small (see Table 2). Seedlings in DRY treatments had lower AGB in almost all treatment combinations; these reductions were moderate to large, ranging from 1.9 to 5.2 standard deviations. However, at high defoliation intensity there were occasionally no significant differences between the DRY and WET treatments. Treatments without significant reductions were crested wheatgrass ($g = -0.3$) and Sandberg bluegrass ($g = -1.0$ [defoliation intensity 70%, frequency LOW] and bluebunch wheatgrass ($g = -1.1$) [defoliation intensity 70%, frequency HIGH], though effect sizes are still moderate in the latter two cases.

Water: BGB Interactions

Species and water interacted to determine seedling performance in terms of BGB when defoliated regardless of defoliation level or intensity (see Table 2 and Fig. 1 Right). When well-watered, all three species had similar BGB. However, when water stressed Sandberg bluegrass, BGB was reduced by nearly three standard deviations ($g = -2.9$). This was not the case for the other two bunchgrasses (bluebunch: $g = -0.9$; crested: $g = -0.2$).

Water: Tillering Interactions

Species and water also interacted to determine seedling tillering when defoliated (see Table 2 and Fig. 2, Right), regardless of defoliation level and intensity when water stressed Sandberg bluegrass and crested wheatgrass seedlings tillered less ($g = -1.8$ and -1.1, respectively).

Only a smaller, insignificant difference was seen in bluebunch wheatgrass tillering ($g = –0.9$) under water-stressed conditions.

Defoliation Intensity

Defoliation intensity on its own did not play a large role in determining seedling performance for AGB, BGB, root:shoot ratios, or tillering but did modify how seedlings responded to other treatments (see Table 2). Additionally, Sandberg bluegrass mortality was not affected by defoliation intensity (12.6% and 18.8% mortality at the 30% and 70% intensities, respectively) (see Table 3).

Defoliation Intensity: AGB Interactions

Seedling AGB was influenced by a fourth-order interaction among species, water, defoliation intensity, and defoliation frequency, which explained a small amount of the variance (see Table 2 and Fig. 1, Left). In most cases, no difference in AGB was found between seedlings subjected to 30% versus 70% defoliation. Defoliation intensity did not modify AGB for Sandberg bluegrass at all, but large, though opposing, responses to defoliation intensity were seen in bluebunch wheatgrass and crested wheatgrass under limited conditions. When well-watered at LOW defoliation frequency, crested wheatgrass seedlings defoliated at an intensity of 30% had higher AGB than those receiving 70% defoliation ($g = 2.7$). Bluebunch wheatgrass seedlings receiving 30% defoliation had lower AGB than those defoliated at the 70% level under the same conditions ($g = –2.7$).

Defoliation Intensity: BGB Interactions

Defoliation intensity and frequency interacted to determine seedling BGB, regardless of species and watering regime (see Table 2 and Fig. 1, Right), and explained a large amount of the variation between seedlings. At LOW frequency defoliation there was no difference between seedlings defoliated at a 30% level versus those defoliated at 70% ($g = –0.1$). However, at HIGH frequency defoliation, seedlings defoliated only at 30% maintained their BGB while those defoliated at 70% did not ($g = –1.0$), though the difference was only of 1 standard deviation.

Defoliation Intensity: Root:Shoot Ratio Interactions

A medium amount of the variation in root:shoot ratios between seedlings was explained by a defoliation intensity and frequency interaction (see Table 2 and Fig. 2, Left). Like BGB, seedling root:shoot ratios did not differ between defoliation intensities when defoliated at LOW frequency ($g = –0.2$), while only seedlings receiving 70% defoliation had reduced root:shoot ratios at HIGH frequency ($g = –1.1$). Also like BGB, the difference in means between the 30% and 70% defoliation treatments was not large.

Defoliation Intensity: Tiller Interactions

Seedling response to defoliation intensity was moderated by both defoliation frequency and species by not by watering treatment (see Table 2 and Fig. 2, Right). At low stress (30% intensity, LOW frequency defoliation) Sandberg bluegrass produced the most tillers (5.1 per seedling), crested wheatgrass produced the least (2.7 per seedling), and bluebunch wheatgrass was in between (3.3 per seedling). Under combined 70%, HIGH frequency defoliation Sandberg bluegrass decreased its tillering (3.9 per seedling; $g = –1.3$), while crested wheatgrass seedlings increased tillering (2.9 per seedling; $g = 0.9$) and bluebunch wheatgrass remained unchanged (3.3 per seedling; $g = –0.4$), resulting in all three species having the same number of tillers. Conversely, Sandberg bluegrass increased tillering at the 70% defoliation level over the 30% level ($g = 0.7$) at LOW defoliation frequency, but this effect was small. No other treatment combinations resulted in a change in tillering in response to defoliation intensity.

Defoliation Frequency

Like defoliation intensity, defoliation frequency affected seedling AGB, BGB, root:shoot ratios, and tillering by interacting with other treatments (see Table 2). Unlike defoliation intensity, defoliation frequency independently explained a medium amount of the variation seen in Sandberg bluegrass mortality (see Table 3). HIGH frequency defoliation resulted in higher mortality (6.3% vs. 25.1% mortality in the LOW and HIGH frequency treatments, respectively).

Defoliation Frequency: AGB Interactions

The effect of defoliation frequency on seedling AGB interacted with species, water, and defoliation intensity (see Table 2 and Fig. 1, Left) to explain a small amount of the variation seen in that performance measure. In most cases defoliation frequency did not affect seedling AGB; however, in three cases HIGH frequency defoliation reduced AGB by a moderate amount over LOW frequency defoliation: bluebunch wheatgrass ($g = –2.2$) (WET, 70% defoliation), crested wheatgrass ($g = –1.2$) (WET, 30% defoliation), and Sandberg bluegrass ($g = –1.5$) (DRY, 70% defoliation).

Defoliation Frequency: BGB Interactions

Defoliation frequency interacted separately with both defoliation intensity and species to determine seedling BGB (see Table 2 and Fig. 1, Right). Bluebunch wheatgrass was unresponsive to defoliation frequency ($g = –0.2$), while both crested wheatgrass ($g = –1.2$) and Sandberg bluegrass ($g = –0.9$) reduced BGB under HIGH frequency defoliation by a small to moderate amount. Across all species and watering regimes, seedlings defoliated at HIGH frequency had lower BGB than those defoliated at LOW frequency when subjected to 70% defoliation intensity ($g = –1.3$). However, there was no difference between HIGH and LOW frequency defoliation at an intensity of 30% ($g = –0.1$).

Defoliation Frequency: Root:Shoot Ratio Interactions

Mirroring the response seen in BGB, defoliation frequency interacted separately with species and intensity to determine seedling root:shoot ratio (see Table 2 and Fig. 2, Left). HIGH versus LOW frequency defoliation did not change Bluebunch wheatgrass root:shoot ratios ($g = –0.2$) but reduced root:shoot ratios in crested wheatgrass ($g = –1.3$) and Sandberg bluegrass ($g = –1.5$) by just over 1 standard deviation. HIGH frequency defoliation resulted in reduced root:shoot ratios compared with LOW frequency defoliation at 70% defoliation intensity ($g = –1.1$), but not at 30% ($g = –0.2$).

Defoliation Frequency: Tiller Interactions

Defoliation frequency affected tillering as part of an interaction with species and defoliation intensity, explaining a medium amount of the variation (see Table 2 and Fig. 2, Right). HIGH frequency defoliation reduced tillering over LOW frequency defoliation in Sandberg bluegrass at 70% defoliation ($g = –1.3$). The same treatment combination increased tillering in crested wheatgrass ($g = 2.1$). Defoliation frequency did not affect tillering in either grass at 30% defoliation ($gs < |0.6|$). Bluebunch wheatgrass did not respond to change in frequency of defoliation at any defoliation intensity ($gs < |0.4|$).

Discussion

In this study we investigated whether defoliation on seedlings could be a driver of restoration failure. We hypothesized that seedlings would be more sensitive to high-intensity defoliation, and high-frequency defoliation would exacerbate reductions in performance measures (AGB, BGB, tillering, and root:shoot ratios) and/or mortality. Additionally, we put forth two competing hypotheses regarding how grass seedlings may respond to defoliation in a high-stress (i.e., low water) environment: Water stress would either exacerbate reductions in performance resulting from the defoliation treatments or reduce differences in performance between defoliated treatments. Finally, we predicted that the introduced species of perennial grass would have a greater ability to tolerate defoliation and drought stress than the native species, potentially explaining why exotics establish better in most planting studies. We found that defoliation intensity on its own did not influence seedling performance in terms of survival, aboveground and belowground growth, root:shoot ratios, or tillering. However, combined high-frequency defoliation and 70% defoliation did reduce seedling performance, mostly in belowground measures. Seedlings of the non-native species, crested wheatgrass, performed considerably better than Sandberg bluegrass seedlings, but not bluebunch wheatgrass seedlings. Sandberg bluegrass was the only species to experience extensive mortality. Water stress and defoliation did not interact in a clear-cut pattern as expected, with treatment interactions differing across performance measures.

While previous research with adult plants has indicated that intensity of vegetation removal is rarely predictive of plant response to defoliation (reviewed in Ferraro and Oesterheld, 2002), there has been some indication that defoliation intensity may be more important in seedlings when plants are still dependent on their cotyledon tissues for nourishment (Hanley and May, 2006; Hanley and Fegan, 2007). However, our results support Barton and Hanley's (2013) assertion that species display similar responses to defoliation intensity across life history; defoliation intensity rarely affected our seedlings on its own and did not appear to cause mortality. In a concurrent study to ours, photosynthetic rates were measured over the lifetime of the bluebunch wheatgrass and crested wheatgrass seedlings in the present study, with these seedlings able to increase photosynthetic rates in response to defoliation, though this was dependent on when defoliation occurred in the course of development (Hamerlynck et al., 2016). The ability of our grass seedlings to modulate photosynthetic rates might explain why defoliation intensity did not strongly affect aboveground growth. However, increased vegetative growth from increased photosynthetic rates may come at a cost; frequent, high-intensity (70%) defoliation caused reductions in belowground growth across all species as compared with undefoliated plants and reductions in tiller number in Sandberg bluegrass.

Reduction in root growth can have a long-lasting effect on developing plants. Restricted BGB may affect provisioning for future growth, potentially leading to reduced reproductive output (Pyke, 1986; Hanley and May, 2006; Hanley and Fegan, 2007; Zhang et al., 2011) and fewer tillers being initiated for next year's growth (Busso and Richards, 1995), which may result in reduced plant size. Previous work has indicated that seedlings are more likely to die if individuals are smaller in size (Harris, 1967; Gross, 1981). Reduced root biomass also has an immediate effect potentially affecting seedling ability to survive drought and additional defoliation (Roundy et al., 1985; Atwater et al., 2015). That said, while we found reductions in belowground provisioning, these responses were fairly small, around one order of magnitude. It is uncertain if there is a large enough effect to carry over into adulthood (Buwai and Trlica, 1977) or affect survival into the coming year (Ries and Svejcar, 1991). In addition, native populations and specific germplasm lines of bluebunch wheatgrass have been found to vary considerably in root growth response to defoliation (Ray-Mukherjee et al., 2013). This suggests that quantifying responses to defoliation across a wider range of plant material sources is needed before we can fully understand the consequences of defoliation on successful seedling development in sagebrush steppe ecosystems.

Unlike defoliation intensity, frequent defoliation or grazing on its own has been shown to reduce the success of both seedlings (Roundy et al., 1985) and adult plants (reviewed in Ferraro and Oesterheld, 2002). Roundy et al. (1985) found that defoliation had little effect on crested wheatgrass seedling survival until defoliation frequency dropped to ≤ 4 wk when plants were well watered, with sharp declines occurring at defoliation rates of every ≤ 2 wk. However, repeated clipping intervals of ≤ 5 wk reduced root growth by more than half. Our results indicate that sensitivity to frequent defoliation may be species specific. As in Roundy et al. (1985), we found crested wheatgrass seedlings had reduced root growth under high-frequency defoliation (a 4-wk interval); however, no mortality was observed, perhaps because our observed reductions in BGB were much smaller (only 25 − 33%). Sandberg bluegrass seedlings only had reduced root growth when frequent defoliation occurred at high intensity (70% tissue removal) but had reduced survival whenever high-frequency defoliation occurred. High-frequency defoliation did not result in reduced survival or reduced BGB in bluebunch wheatgrass. These species-specific responses have interesting implications for the debate regarding whether to use native or exotic seed for restorations.

Non-native species like crested wheatgrass have frequently been found to establish better than native species such as Sandberg bluegrass and bluebunch wheatgrass (Ferrell et al., 1998; Asay et al., 2001; Sheley et al., 2001). There is some evidence that this is due to better grazing tolerance at younger plant stages (Asay et al., 2001). However, our results indicate that bluebunch wheatgrass may be more resistant to defoliation than crested wheatgrass during the seedling stage, at least based on belowground measures of plant performance. This resistance to defoliation at the seedling stage could be an adaptation to cope with small native grazers, which have been found to prey on grass seedlings at times (Pyke, 1986). Bluebunch wheatgrass seedlings being particularly successful at resisting defoliation suggests a possible mechanism to explain how the species came to be a dominant component of sagebrush steppe community (Miller et al., 1994), even though it is sensitive to grazing as a mature plant (Clark et al., 1998; Ralphs, 2009). On the other hand, increased tiller production is sometimes cited as the mechanism by which non-native grasses tolerate grazing (Zhang and Romo, 1995), a feature crested wheatgrass seedlings in our study displayed at high-intensity, high-frequency defoliation, while bluebunch wheatgrass had no such increase and Sandberg bluegrass individuals decreased tillering under the same conditions. Since tiller number has implications for long-term survival (Ries and Svejcar, 1991; Lollato, 2015), it is difficult to say if bluebunch or crested wheatgrass is more resistant to defoliation at the seedling stage. This is not the only case where a native grass has been found to perform as well or better than non-natives (Pyke, 1990; Busso and Richards, 1995; McArthur, 2004), but such studies still seem the exception rather than the rule (Ferrell et al., 1998; Asay et al., 2001; Sheley et al., 2001; James et al., 2011). On the basis of our study, Sandberg bluegrass appears to be more sensitive to defoliation than the other two species at the seedling phase. This finding is somewhat surprising as Sandberg bluegrass is known to increase in abundance under grazing pressure (Howard, 1997), perhaps because of its small stature, which may allow it to mostly avoid defoliation from herbivores in the wild. A better understanding of how to improve Sandberg bluegrasses success at the seedling phase is certainly needed as it is one of the few species that competes successfully with cheatgrass as seedlings and adults (Link et al., 1990; Espeland and Hammond, 2013).

With our watering treatment we hoped to assess whether additional stress exacerbated reductions in performance and survival seen in defoliated seedlings or mediated such reductions as both responses have theoretical basis. This information could potentially help land managers decide when defoliation could be a concern in planting decisions. We had expected that seedlings would respond by either showing reduced sensitivity to defoliation at high resource levels (i.e., well-watered), as had been found in monocots previously (Hawkes and Sullivan, 2001), or that water stress would dampen differences between defoliation treatments, as has been found in a number of other studies combining defoliation and water treatments (synthesized in Wise and Abrahamson, 2007). However, we found that water and defoliation interacted differently depending on the performance measure examined. Tillering, root:shoot ratios, and BGB were all reduced in the low-water treatment, but no interaction was found with the defoliation treatments—not an expected result. AGB was always lower in the low-water treatments, except at high frequency, 70% defoliation, when no difference was found between the watering treatments, consistent with Wise and Abrahamson (2007). Mortality, on the other hand, was reduced in well-watered treatments as opposed to dry treatments. Sandberg bluegrass displayed increased mortality when frequently defoliated at both high and low intensities when dry as opposed to wet; however, no change in mortality was noted between watering regimes when plants were not defoliated, which agrees with Hawkes and Sullivan (2001).

Seedlings did not fit nicely into our either/or paradigm for combining water and defoliation stress, but a theory proposed by Wise and Abrahamson (2007), the Limiting Resource Model (LRM), may explain why. The LRM states that plants may display increased, decreased, or no change in sensitivity to their primary stressor when another stress is applied depending on what resource is actually limiting plant growth and whether the secondary stressor actually modifies that condition. In the case of tillering, BGB, and root:shoot ratios, there were no watering treatment interactions with defoliation, but reductions were seen under water stress. This indicates that water was the limiting resource for these performance measures and remained so regardless of defoliation extent or intensity. For AGB, where high-intensity, high-frequency defoliation eliminated the difference between watering treatments, we can assume that water was the limiting resource, but that when repeatedly defoliated at such high intensities, something else became the limiting resource, perhaps the ability to capture carbon from the air (Stafford, 1989). Finally, mortality (as there were no differences in Sandberg bluegrass mortality in the undefoliated treatments water stress) did not appear to be the primary driver of mortality. However, when defoliated, well-watered seedlings displayed higher survival than those in water stress treatments, suggesting that defoliation caused water to become a limiting resource for seedling survival. All of this indicates that predicting the response to combined water stress and defoliation under field conditions will be difficult as different and opposing interactions may exist within the plant, though in terms of restoration success mortality may be the most important factor and the one of primary concern to land managers.

Implications

Our study indicates that grass seedlings are most sensitive to the combination of repeated and heavy defoliation but can generally compensate for damage short of that threshold. While seedling performance and potential long-term survival may be reduced in poor water years, herbivory is not likely to exacerbate that problem except in the case of Sandberg bluegrass. If seedling herbivory is expected to be a problem at a restoration site, it would be best to select species that are more resistant to damage, such as bluebunch wheatgrass and crested wheatgrass. While the present work was just a pot study, it indicates that herbivory could have consequences on restoration success, at least in some species. Further research is needed to determine how general these findings are in a field setting and how sensitive other commonly seeded natives and exotics are to these types of combined stressors, as well as how long-term population dynamics may be influenced.

Acknowledgments

The authors wish to thank Lori Ziegenhagen and Brett Bingham for their invaluable assistance in gathering data for this experiment.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.rama.2017.06.014.

References

Adeel, Z., Safriel, U., Niemeijer, D., White, R., 2005. Millennium Ecosystem Assessment, 2005. Ecosystems and Human Well-being: Desertification Synthesis. World Resources Institute, Washington, DC.

Arkle, R.S., Pilliod, D.S., Hanser, S.E., Brooks, M.L., Chambers, J.C., Grace, J.B., Knutson, K.C., Pyke, D.A., Welty, J.L., Wirth, T.A., 2014. Quantifying restoration effectiveness using multi-scale habitat models: implications for sage-grouse in the Great Basin. Ecosphere 5, 1–32.

Asay, K.H., Horton, W.H., Jensen, K.B., Palazzo, A.J., 2001. Merits of native and introduced Triticeae grasses on semiarid rangelands. Canadian Journal of Plant Science 81, 45–52.

Aston, W., 1972. The logit transformation, with special reference to its uses in bioassay. Hafner Publishing Co, New York, NY, USA, p. 96.

Atwater, D.Z., James, J.J., Leger, E.A., 2015. Seedling root traits strongly influence field survival and performance of a common bunchgrass. Basic Applied Ecology 16, 128–140.

Bailey, T.G., Davidson, N.J., Close, D.C., 2012. Understanding the regeneration niche: microsite attributes and recruitment of eucalypts in dry forests. Forest Ecology Management 269, 229–238.

Barberá, G.G., Navarro-Cano, J.A., Castillo, V.M., 2006. Seedling recruitment in a semi-arid steppe: the role of microsite and post-dispersal seed predation. Journal of Arid Environments 67, 701–714.

Barton, K.E., Hanley, M.E., 2013. Seedling-herbivore interactions: insights into plant defence and regeneration patterns. Annals of Botany 112, 643–650.

Bleak, A.T., Frischknecht, N.C., Plummer, A.P., Eckert, R.E.J., 1965. Problems in artificial and natural revegetation of the arid shadscale vegetation zone of Utah and Nevada. Journal of Range Management 18, 59–65.

Brewer, T.K., Mosley, J.C., Lucas, D.E., Schmidt, L.R., 2007. Bluebunch wheatgrass response to spring defoliation on foothill rangeland. Rangeland Ecology & Management 60, 498–507.

Busso, C.A., Richards, J.H., 1995. Drought and clipping effects on tiller demography andgrowth of two tussock grasses in Utah. Journal of Arid Environments 29, 239–251.

Buwai, M., Trlica, M.J., 1977. Defoliation effects on root weights and total nonstructural carbohydrates of blue grama and western wheatgrass. Crop Science 17, 15–17.

Clark, P.E., Krueger, W.C., Bryant, L.D., Thomas, D.R., 1998. Spring defoliation effects on bluebunch wheatgrass: II. Basal area. Journal of Range Management 51, 526–530.

Cohen, J., 1992. A power primer. Psychological Bulletin. 112 (1), 1–8.

Dalzell, C.R., 2004. Post-fire establishment of vegetation communities following reseeding on Southern Idaho's Snake River Plain. Boise State University, Boise, ID, USA.

Davies, K.W., Boyd, C.S., Beck, J.L., Bates, J.D., Svejcar, T.J., Gregg, M.A., 2011. Saving the sagebrush sea: an ecosystem conservation plan for big sagebrush plant communities. Biology Conservation 144, 2573–2584.

Defossé, G.E., Robberecht, R., Bertiller, M.B., 1997. Seedling dynamics of *Festuca* spp. in grassland of Patagonia, Argentina, as affected by competition, microsties, and grazing. Journal of Range Management 50, 73–79.

Engelbrecht, B.M.J., Kursar, T.A., Tyree, M.T., 2005. Drought effects on seedling survival in a tropical moist forest. Trees Structure Function 19, 312–321.

Espeland, E., Hammond, D., 2013. Maternal effects on growth and competitive ability in a commonly used restoration species. Native Plants Journal 14, 231–242.

Evetts, L.L., Burnside, C., 1973. Early root and shoot development of nine plant species. Weed Science 21, 289–291.

Fehmi, J.S., Niu, G., Scott, R.L., Mathias, A., 2014. Evaluating the effect of rainfall variability on vegetation establishment in a semidesert grassland. Environmental Monitoring Assessment 186, 395–406.

Ferguson, C.J., 2009. An effect size primer: a guide for clinicians and researchers. Professional Psychology Research Practice 40, 532–538.

Ferraro, D., Oesterheld, M., 2002. Effect of defoliation on grass growth. A quantitative review. Oikos 98, 125–133.

Ferrell, M.A., Whitson, T.D., Koch, D.W., Gade, A.E., 1998. Leafy sparge *(Euphorbia esula)* control with several grass species. Weed Technology 12, 374–380.

Fritz, C., Morris, P., Richler, J., 2012. Effect size estimates: current use, calculations, and interpretation. Journal of Experimental Psychology 141 (1), 2–18.

Gross, K.L., 1981. Predictions of fate from rosette size in four "biennial" plant species: *Verbascum thapsus*, *Oenothera biennis*, *Daucus carota*, and *Tragopogon dubius*. Oecologia 48, 209–213.

Hamerlynck, E.P., Smith, B.S., Sheley, R.L., Svejcar, T.J., 2016. Compensatory photosynthesis, water-use efficiency, and biomass allocation of defoliated exotic and native bunchgrass seedlings. Rangeland Ecology & Management 69, 206–214.

Hanley, A.M.E., Fenner, M., Edwards, P.J., Hanley, M.E., Fenner, M., Edwards, P.J., 1995. The effect of seedling age on the likelihood of herbivory by the slug *Deroceras reticulatum*. Functional Ecology 9, 754–759.

Hanley, M., Fenner, M., Whibley, H., Darvil, B., 2004. Early plant growth: identifying the end point of the seedling phase. New Phytologist 163 (1), 61–66.

Hanley, M.E., Fegan, E.L., 2007. Timing of cotyledon damage affects growth and flowering in mature plants. Plant, Cell Environment 30, 812–819.

Hanley, M.E., May, O.C., 2006. Cotyledon damage at the seedling stage affects growth and flowering potential in mature plants. New Phytology 169, 243–250.

Hardegree, S.P., Jones, T.A., Roundy, B.A., Shaw, N.L., 2016. Assessment of range planting as a conservation practice. In: Briske, D.D. (Ed.), Conservation benefits of rangeland practices assessment, recommendations, and knowledge gaps Lawrence KS, USA:.

Harris, G.A., 1967. Some competitive relationships between *Agropyron spicatum* and *Bromus tectorum*. Ecology Monographs 37, 89–111.

Hawkes, C.V., Sullivan, J., 2001. The impact of herbivory on plants in different resource conditions: a meta-analysis. Ecology 82, 2045–2058.

Hemstrom, M.A., Wisdom, M.J., Hann, W.J., Rowland, M.M., Wales, B.C., Gravenmier, R.A., 2002. Sagebrush-steppe vegetation dynamics and restoration potential in the interior Columbia Basin, U.S.A. Conservation Biology 16, 1243–1255.

Herrick, J.E., Schuman, G.E., Rango, A., 2006. Monitoring ecological processes for restoration projects. Journal Nature Conservation 14, 161–171.

Howard, J.L., 1997. *Poa secunda* [WWW Document]. Index Species Inf. Available at: http://www.fs.fed.us/database/feis/plants/tree/acesac/all.html, Accessed date: 1 December 2016.

Huber-sannwald, E., Pyke, D.A., 2005. Establishing native grasses in a big sagebrush – dominated site: an intermediate restoration step. Restoration Ecology 13, 292–301.

Isselstein, J., Tallowin, J.R.B., Smith, R.E.N., 2002. Factors affecting seed germination and seedling establishment of fen-meadow species. Restoration Ecology 10, 173–184.

James, J.J., Boyd, C.S., Svejcar, T., 2013. Seed and seedling ecology research to enhance restoration outcomes. Rangeland Ecology & Management 66, 115–116.

James, J.J., Svejcar, T.J., Rinella, M.J., 2011. Demographic processes limiting seedling recruitment in arid grassland restoration. Journal of Applied Ecology 48, 961–969.

Karcher, D.E., Richardson, M.D., Hignight, K., Rush, D., 2008. Drought tolerance of tall fescue populations selected for high root/shoot ratios and summer survival. Crop Science 48, 771–777.

Kenward, M.G., Roger, J.H., 1997. Small sample inference restrictred max likelihood. Biometrics 53, 983–997.

Kimball, S., Schiffman, P.M., 2003. Differing effects of cattle grazing on native and alien plants. Conservation Biology 17, 1681–1693.

Knick, S.T., Dobkin, D.S., Rotenberry, J.T., Schroeder, M.A., Vander Haegen, W.M., van Riper, I.I.I.C., 2003. Teetering on the edge or too late? Conservation and research issues for avigauna of sagebrush habitats. Condor 105, 611–634.

Larrison, E.J., Johnson, D.R., 1973. Density changes and habitat affinities of rodents of shadscale and sagebrush associations. Great Basin Nature 33, 255–264.

Larson, J.E., Sheley, R.L., Hardegree, S.P., Doescher, P.S., James, J.J., 2015. Seed and seedling traits affecting critical life stage transitions and recruitment outcomes in dryland grasses. Journal of Applied Ecology 52, 199–209.

Leishman, M.R., Westoby, M., 1994. The role of seed size in seedling establishment in dry soil conditions—experimental evidence from semi-arid species. Journal of Ecology 82, 249–258.

Link, S.O., Gee, G.W., Downs, J.L., Link, S., Gee, G.W., Downs, J.L., 1990. The effect of water stress on phenological and ecophysiological characteristics of cheatgrass and Sandberg's bluegrass. Journal of Range Management 43, 506–513.

Lloret, F., Casamovas, C., Penuelas, J., 1999. Seedling survival of Mediterranean shrubland species in relation to root: root ratio, seed size and water and nitrogen use. Functional Ecology 13, 210–216.

Lollato, R., 2015. Factors to consider in winter survival wheat. Kansas State University, Manhattan, KS, USA.

McAdoo, J.K., Barrington, M.R., Ports, M.A., 2006. Habitat afinities of rodents in Northeastern Nevada rangeland communities. Western North American Nature 66, 321–331.

McArthur, T.O., 2004. Emergency fire rehabilitation of BLM lands in the Great Basin: revegetation & monitoring. Oregon State University, Corvallis, OR, USA.

Miller, R.F., Knick, S.T., Pyke, D.A., Cara, W., Hanser, S.E., Wisdom, M.J., Hild, A.L., 2011. Characteristics of sagebrush habitats and limitations to long-term conservation. In: Knick, S.T., Connelly, J.W. (Eds.), Greater sage-grouse ecology and conservation of a landscape species and its habitats. University of California, Press Studies in Avian Biology, Berkeley, CA, USA, pp. 145–184.

Miller, R.F., Seufert, J., Haferkamp, M., 1994. The ecology and management of bluebunch wheatgrass *(Agropyron spicatum)*: a review. Agriculture Experimental Station—Oregon State University, Corvallis, OR, USA Station Bulletin 669.

Moles, A.T., Westoby, M., 2004. Seedling survival and seed size : a synthesis of the literature. Journal of Ecology 92, 372–383.

Monsen, S.B., Stevens, R., 2004. Seedbed preparation and seeding practices. In: Monsen, S.B., Stevens, R., Shaw, N.L. (Eds.), Restoring Western ranges and wildlands. USDA Forest Service Rocky Mountain Research Station, Fort Collins, CO, USA, pp. 121–154.

Morris, S., DeShon, R., 2003. Estimating common metric effect sizes from a variety of research designs. https://pdfs.semanticscholar.org/8d6e/eaf4888283375aea80153faf63a76c8d39b1.pdf?_ga=2.266322572.268606822.1501535967-1155778919.1501535967.

Moser, L.E., Smart, A.J., 1997. Relation of root and shoot morphology of grass seedlings. International Grassland Congress Proceedings XVIII. 5, pp. 49–50.

Noss, R.F., LaRoe, E.T., Scott, J.M., 1995. Endangered ecosystems of the United States: a preliminary assessment of loss and degradation. Department of the Interior, Washington, DC, USA.

Pyke, D.A., 2011. Restoring and rehabilitating sagebrush habitats. Studies in Avian Biology 38, 531–548.

Pyke, D.A., 1990. Comparative demography of co-occurring introduced and native tussock grasses: persistence and potential expansion. Ecology 82, 537–543.

Pyke, D.A., 1987. Demographic responses of *Bromus tectorum* and seedlings of *Agropyron spicatum* to grazing by small mammals: the influence of grazing frequency and plant age. Journal of Ecology 75, 825–835.

Pyke, D.A., 1986. Demographic responses of *Bromus tectorum* and seedlings of *Agropyron spicatum* to grazing by small mammals: occurrence and severity of grazing. Journal of Ecology 74, 739–754.

Pyke, D., Chambers, J., Pellant, M., Knick, S., Miller, R.F., Beck, J.L., Doescher, P., Schupp, E., Roundy, B., Brunson, M., McIver, J., 2015. Restoration handbook for sagebrush steppe ecosystems with emphasis on greater sage-grouse habitat—Part 1. Concepts for understanding and applying restoration, circular. 1 ed. US Geological Survey, Reston, VA, USA.

Ralphs, M.H., 2009. Response of broom snakeweeds *(Gutierrezia sarothrae)* and cool-season grasses to defoliation. Invasive Plant Science Management 2, 28–35.

Ray-Mukherjee, J., Jones, T.A., Monaco, T.A., 2013. Biomass and defoliation tolerance of 12 populations of *Pseudoroegneria spicata* at two densities. Rangeland Ecology & Management 66, 706–713.

Ries, R.E., Svejcar, T.J., 1991. The grass seedling: when is it established? Journal of Range Management 44, 574–576.

Roundy, B.A., Cluff, G.J., McAdoo, J.K., Evans, R.A., 1985. Effects of jackrabbit grazing, clipping, and drought on crested wheatgrass seedlings. Journal of Range Management 38, 551–555.

Salvo, M., 2008. The shrinking sagebrush sea: spatial analyses of threats to sagebrush-steppe and greater sage-grouse. WildEarth Guardians, Santa Fe, NM, USA.

Selya, A.S., Rose, J.S., Dierker, L.C., Hedeker, D., Mermelstein, R.J., Saverio, D., Costa, J., 2012. A practical guide to calculating Cohen's f2, a measure of local effect size , from PROC MIXED. Frontline Psychology 3, 1–6.

Sheley, R.L., Jacobs, J.S., Lucas, D.E., 2001. Revegetating spotted knapweed infested rangeland in a single entry. Journal of Range Management 54, 144–151.

Stafford, R.A., 1989. Allocation responses of *Abutilon theophrasti* to carbon and nutrient stress. American Midland Nature 121, 225–231.

Tsakaldimi, M., Ganatsas, P., Jacobs, D.F., 2013. Prediction of planted seedling survival of five Mediterranean species based on initial seedling morphology. New Forestry 44, 327–339.

US Department of Interior, 2004. Guidance for the management of sagebrush. Bureau of Land Management national sage-grouse habitat conservation strategy. BLM, Washington, DC, USA.

Verts, B.J., Carraway, L.N., 1998. Land mammals of Oregon. University of California Press, Berkeley, CA, USA.

White, R., Rohweder, M., Murray, S., 2000. Pilot analysis of global ecosystems: grassland ecosystems. Washington, DC, USA.

Wilson, M.V., Ingersoll, C.A., Clark, D.L., 2004. Why pest plant control and native plant establishment failed: a restoration autopsy. Nature Areas Journal 24, 23–31.

Wise, M.J., Abrahamson, W.G., 2007. Effects of resource availability on tolerance of herbivory: a review and assessment of three opposing models. American Nature 169, 443–454.

Young, J.A., Sparks, B.A., 2002. Cattle in the cold desert—expanded edition, ed 1. University of Nevada Press, Reno, NV, USA.

Zhang, J., Romo, J.T., 1995. Impacts of defoliation on tiller production and survival in northern wheatgrass. Journal of Range Management 48, 115–120.

Zhang, S., Zhao, C., Lamb, E.G., 2011. Cotyledon damage affects seed number through final plant size in the annual grassland species *Medicago lupulina*. Annals of Botany 107, 437–442.