# Plant-soil feedback from eastern redcedar (Juniperus virginiana) inhibits the growth of grasses in encroaching range

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**Abstract**

1. The encroachment of woody plants into grasslands is an ongoing global problem that is largely attributed to anthropogenic factors such as climate change and land management practices. Determining the mechanisms that drive successful encroachment is a critical step towards planning restoration and long-term management strategies. Feedbacks between soil and aboveground communities can have a large influence on the fitness of plants and must be considered as potentially important drivers for woody encroachment. 2. We conducted a plant-soil feedback experiment in a greenhouse between eastern redcedar Juniperus virginiana and four common North American prairie grass species. We assessed how soils that had been occupied by redcedar, a pervasive woody encroacher in the Great Plains of North America, affected the growth of big bluestem, little bluestem smooth brome, and western wheatgrass over time. We evaluated the effect of redcedar on grass performance by comparing the height and biomass of individuals of each grass species that were grown in live or sterilized conspecific or redcedar soil. 3. We found that redcedar created a negative plant-soil feedback that limited the growth of two species. These effects were found in both live and sterilized redcedar soils, indicating redcedar may exude an allelochemical into the soil that limits grass growth. 4. Synthesis. By evaluating the strength and direction of plant-soil feedbacks in the encroaching range, we can further our understanding of how woody pants successfully establish in new plant communities. Our results demonstrate that plant-soil feedback created by redcedar inhibits the growth of certain grass species. By creating a plant-plant interaction that negatively affects competitors, redcedars increase the probability of seedling survival until they can grow to overtop their neighbors. These results indicate plant-soil feedback is a mechanism of native woody plant encroachment that could be important in many systems yet is understudied.

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large influence on the fitness of plants and must be considered as potentially important drivers for woody encroachment.

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**Keywords:**

Plant-soil feedback, woody encroachment, eastern redcedar, allelopathy, prairie, soil community, *Juniperus virginiana* , range expansion

## 1 | Introduction

Plants make species-specific changes to the biotic and abiotic conditions of their near-soil environment which can affect the fitness of future occupants (Bever *et al.* 1997; Bezemer *et al.*2006; Gundale and Kardol 2021). This phenomenon, deemed plant-soil feedback, can have a large influence on competitive interactions, community composition and function (van der Putten *et al.* 2013; Lekberg *et al.* 2018; Crawford *et al.* 2019). The strength and direction of a feedback is the product of several interacting mechanisms including soil-nutrient availability, the presence of pathogenic natural enemies and beneficial mutualists, and the effects of secondary chemicals (i.e. allelochemicals) that are exuded from plants (Bennett and Klironomos 2019).

Woody plant encroachment into grasslands is a global phenomenon that alters ecosystem function (Eldridge *et al.* 2011; Naito and Cairns 2011). The conversion of grasslands to woodlands can decrease biodiversity, change ecosystem structure and function, reduce productivity for livestock, alter water resource availability, and change the carbon balance (Barger *et al.* 2011; Ratajczak *et al.* 2012; Anadón *et al.* 2014; Acharya *et al.* 2018). Managing for encroaching species is difficult because the influence of factors differs between study species and systems (Tomiolo and Ward 2018). Fire suppression and livestock grazing are land-management practices that are frequently cited as the primary drivers of woody plant encroachment (Briggs *et al.* 2005; Van Auken 2009). The global trend of climate change, specifically increased temperature, nutrient deposition, and elevated $CO_2$ levels, may also explain continental-scale patterns of woody species expansion (Devine*et al.* 2017). An additional factor that may promote encroachment is plant-soil feedback, which is a mechanism that can promote the establishment of woody species and reinforce the dominance of a woody state (Peters *et al.* 2020).

In North America, woody encroachment is occurring in the deserts and rangelands of the west, the savannas of the south, and the grasslands of the Great Plains region (Van Auken 2000; Ratajczak *et al.* 2012). Tree cover in rangelands of the western United States has increased by as much as 50% in the last 30 years, resulting in ˜$5 billion in lost revenue (Morford *et al.* 2021). Encroachment in the Great Plains region of the United States is particularly concerning, with invading woody shrubs (e.g. *Cornus drummondii* ) and trees (e.g. *Juniperus virginiana* ) replacing grassland plant communities at a rate of up to 1.7 % per year (Barger *et al.* 2011).

Understanding how successful woody encroachers establish and spread is critical to being able to manage them effectively and efficiently. It is of particular importance to understand mechanisms that provide an advantage to species in their expanded range and to quantify the strength of that advantage. This paper explores plant-soil feedback as a potential mechanism that has facilitated the movement of eastern redcedar (*J. virginiana* ) from its historical range into the prairies of the Great Plains and into disturbed areas within their current ranges. Eastern redcedar (hereafter redcedar) is the most common, widely distributed conifer that is native to eastern North America (Fowells, 1965; Ward, 2020).

Redcedar tolerates a wide variety of climatic conditions including temperature extremes and drought. Redcedar is considered a long-lived, early seral species that can be dominant in a forest or woodland habitat until later seral species establish (Lawson, 1990; Briggs et al., 2002). Historically, populations persisted where there was reduced threat of fire, such as on rocky outcrops or barrens (Guyette *et al.* 2002; Briggs *et al.* 2002). Several mechanisms have been proposed that explain why redcedar is a successful encroacher. In tallgrass prairies there is strong evidence for the interaction of extended fire regimes and livestock grazing intensity being determinants of redcedar expansion (Briggs *et al.* 2005). The transition from grassland to woodlands in the Great Plains is largely attributed to land-management practices that have greatly extended fire-return intervals beyond their pre-European settlement levels (Briggs *et al.* 2005; Bielski*et al.* 2021; Fogarty *et al.* 2021). There is also some evidence that the $C_3$ photosynthetic pathway may provide an advantage to redcedar trees under elevated $CO_2$conditions over many of the warm-season $C_4$ grasses that co-occur in its range (Iverson *et al.* 2008; Huntley and Baxter 2013).

### 1.1 | Plant-soil feedbacks and woody plant encroachment

Plant-soil feedback could favor an encroaching species if it benefits the encroacher (intraspecific positive feedback) or inhibits competitors (interspecific negative feedback) or both (Bever *et al.* 1997; Aldorfová *et al.* 2020). A typical experimental approach to determine if the soil microbial community is driving plant-soil feedbacks is to compare plant growth in soils with live microbial communities with soils that have had their microbial communities sterilized with heat or fungicides (Kulmatiski and Kardol 2008). Greenhouse feedback-experiments typically have a training phase, where soil is conditioned by the growth of a species of interest and a phytometer phase, where plants are grown in the training soil to evaluate whether a feedback affects their growth. A positive feedback occurs when the fitness of subsequent conspecific or heterospecific plants benefit from growing in soil altered (conditioned) by a given species. Conversely, a negative feedback describes a reduction in fitness when growing in conditioned soil (Kulmatiski *et al.*2008a). Plant-soil feedback is a well-documented mechanism that can favor the fitness of range-expanding and invasive species in plant communities (Kulmatiski and Kardol 2008; Aldorfová *et al.* 2020).

We conducted a fully-crossed greenhouse experiment between redcedar and four common North American prairie grasses (*Andropogon gerardi* ,*Schizachyrium scoparium* , *Bromus inermis* , *Pascopyrum smithii* ) to evaluate if redcedar creates plant-soil feedback with any of those species and to determine the strength and direction of that feedback. If plant-soil feedbacks are a mechanism that help redcedars encroach into prairies, we hypothesize that we would observe the following outcomes: (a) redcedar would have neutral or positive conspecific feedbacks; (b) grass growth in redcedar soils would be reduced when compared to growth in intraspecific soils; (c) grass growth in live redcedar soil would be reduced when compared to sterile redcedar soil.

## 2 | Materials and Methods

### 2.1 | Study Species

We selected four common perennial grass species to be phytometers of soil conditioned by eastern redcedar. We selected two $C_3$and two $C_4$ grasses for this experiment because both photosynthetic pathways are common in North America and frequently co-occur, although they partition dominance along a gradient of temperature at the continental scale (Teeri and Stowe 1976; Still*et al.* 2003). *Andropogon gerardi* (big bluestem) and*Schizachyrium scoparium* (little bluestem) are common, native warm-season $C_4$ bunchgrasses with ranges that typically overlap in tall- or mixed-grass prairies (Weaver 1954; Wang *et al.* 2013). *Pasco-*

*pyrum smithii* (western wheatgrass) is a common, native cool-season $C_3$ rhizomatous grass that occurs in mixed-grass prairies (Dong *et al.* 2014). *Bromus inermis*(smooth brome) is a common, Eurasian cool-season $C_3$rhizomatous grass that has rapidly spread across North American grasslands since its introduction in the late 1800s (Vogel 2004).*B. inermis* occurs in all contiguous states of the United States. All four grass species can co-occur with each other and with redcedar in portions of their range (Weaver 1942; Burns 1990).

### 2.2 | Phase I: Training Phase

In the training phase of the experiment individuals are grown in potting mix to condition (or train) soils for use in the feedback phase. In February 2020 four shallow trays were filled with sterilized sand. Sand was steam sterilized in a pressurized autoclave at 121 ºC for ˜60 min, cooled and then sterilized for an additional cycle (e.g. Crawford & Knight, 2017). Each tray was sown with a monoculture of *A. gerardi* , *S. scoparium, B. inermis* , or*P. smithii* . All seeds were purchased from OPN Seed, Ohio, USA. In early March 2020, 30 seedlings (mean grass height ˜ 5 cm) of each species were transplanted into 5.6 L pots of common potting mix (120 pots total). Plants were grown in a greenhouse and received auxiliary lighting in the evening hours to promote growth. Ten randomly selected pots of each grass species were harvested in mid-June following ˜16 weeks of growth. In addition, we randomly selected ten pots from a pool of ˜18-month-old redcedars that had been growing in 5.6 L pots in the same greenhouse for the previous ten-months. Grass and tree samples were clipped at the root collar and aboveground biomass was dried in a 65 ºC oven and weighed. Training soils were separated from root materials manually by running material through a 2 mm sieve. Half of the soil (> 2 L) collected from each sample was set-aside for sterilization in an autoclave. Each pot was processed individually, and all materials used in processing were sterilized with an alcohol solution in-between each sample. This procedure was established to prevent the transfer of soil particles and microbes between samples.

### 2.3 | Phase II: Phytometer Phase

In June 2020, we germinated seeds of the same four grass species following the procedure outlined above. Eastern redcedar were purchased from Pinewoods nursery, New Jersey. Individual grass and redcedar seedlings were transferred into 2.8 L pots that contained *home* or*away* soils that were either *live* or *sterilized* . They were planted in pots using the following method: We added 1.3 L of sterilized sand, then 0.4 L of conditioned training soil from one of the five above-mentioned species, followed by a 0.3 L cap of sterilized sand (Appendix Figure 1). We used a full-factorial design with ten replicates of each phytometer- and conditioned-soil combination, resulting in a total of 500 experimental pots (Figure 1). Grasses were grown in controlled greenhouse conditions for 96 days. Eastern redcedar pots were allowed to grow for 13 months due to their slower growth rate. The maximum height of each plant was measured twice a week for the duration of the experiment. At the end of the experiment, each sample was cut at the root collar and dried in an oven prior to weighing above- and belowground biomass.

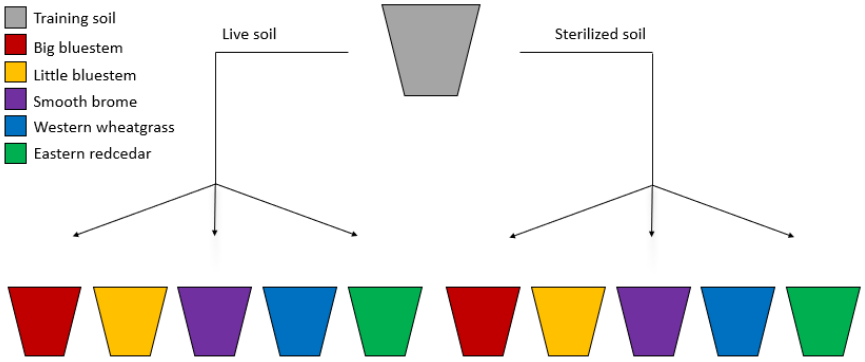


**Figure 1:** Illustration showing how soil from each training pot was distributed to ten new pots for the phytometer phase. There were 50 total training sample pots, ten from each study species. The grey pot

represents one of the 50 training pots. The remaining pots are colored according to the phytometer that was grown in the soil conditioned by a given species in the training phase.

### 2.4 | Statistical Analysis

Height data were recorded at regular intervals over the course of the experiment to aid in determining when plant-soil feedbacks occurred and to assess their strength and direction. The rate of plant growth is variable over time, which means non-linear models will generally perform better than linear models at capturing how height changes over time. We chose to use generalized additive models (GAMs) to evaluate grass growth over time. GAMs are similar to generalized linear models except that they replace linear covariates with local smoothing functions that enable modeling of non-linear processes (Hastie and Tibshirani 1986). To help us understand the overall effect and timing of plant-soil feedbacks on the four phytometers, we built GAMs of the height data of each treatment group over time using the *mgcv* package (v1.8-34; Wood, 2011) in R. The following is a simplification of the generalized additive model (GAM) formula that was used for each group of phytometers (Yee and Mitchell 1991).

$$\mathbb{E}\log \mathbf{y} = x_1 : x_2 + (\sum \{(\mathbf{t_i}) + \ \{(\mathbf{t_i})x_1 : x_2) + (1|x_3)$$

The formula relates the expected value ($\mathbb{E}$) $\log_{10}$-transformed height ($\log \mathbf{y}$) as a function of the interaction between the factors conditioning species ($x_1$) and sterilization status ($x_2$), the sum ([?]) of smoothing ({) variables time ($\mathbf{t_i}$) and time given each level of the interaction of the two factors ($\{(\mathbf{t_i})x_1 : x_2$), and a random intercept ($1|x_3$)) using the unique ID for each pot in the phytometer phase of the experiment. The random intercept was selected to account for repeated measures on each phytometer (Pedersen et al., 2019). The models used the Gaussian family and identity link function. Model selection was done by comparing the AIC for candidate models. We found this model formulation to explain the most variance while retaining only the variables that contribute to explanatory power of the model. We plotted the output of these generalized additive models (GAMs) using the *tidymv* R package to visualize and facilitate comparison of plant height over time under different treatments (Coretta, 2022).*Post hoc* comparisons were done using the *emmeans* package (v1.7.1-1; Russell, 2021). For each phytometer species, the mean estimated height was contrasted between each treatment group. Significance was determined using a Tukey *post hoc* comparison adjustment for a family of ten estimates.

**Table 1.** The model type and $R^2$ value for each biomass type (Shoot, Root, or Total) and phytometer *Andropogon gerardi* (ANGE), *Schizachyrium scoparium* (SCSC), *Bromus inermis* (BRIN), and *Pascopyrum smithii* (PASM). Model types are mixed effects (M) or linear (L) and either contain an interaction term (I) between conditioning soil type and sterilization status or do not include the interaction term (no I). Asterisks (*) denote models that have significant main effects. Adjusted $R^2$ (adj) quantifies the explained variance of fixed effects in linear models. Conditional $R^2$ (cond) quantifies the variance described by fixed and random effects in mixed models. See*Methods* section for detailed model description.

| Phytometer | Biomass | Model Type | R-squared (type) |
|---|---|---|---|
| ANGE | Shoot | M, I, * | 0.81 (cond) |
| ANGE | Root | M, I, * | 0.73 (cond) |
| ANGE | Total | M, I, * | 0.76 (cond) |
| SCSC | Shoot | L, I | 0.01 (adj) |
| SCSC | Root | L, I | 0.01 (adj) |
| SCSC | Total | L, I | 0.02 (adj) |
| BRIN | Shoot | L, I, * | 0.63 (adj) |
| BRIN | Root | M, I, * | 0.51 (cond) |
| BRIN | Total | L, I, * | 0.54 (adj) |
| PASM | Shoot | L, I, * | 0.53 (adj) |
| PASM | Root | M, no I, * | 0.66 (cond) |

| Phytometer | Biomass | Model Type | R-squared (type) |
|---|---|---|---|
| PASM | Total | M, no I, * | 0.64 (cond) |

We assessed how the aboveground, belowground, and overall biomass differed between treatments, splitting the dataset into observations from each phytometer species. We ran a mixed-effects model (GLMM) relating biomass (transformed to the $\log_{10}$ scale) as a function of the conditioning species, the sterilization status of the soil, and the interaction between the two. The pot ID number of the conditioned training soil was used as a random intercept with a fixed mean. Conditioned soils came from individual pots in the training stage that may differ in their abiotic and biotic features, so we chose to use mixed-effects models to account for the variance in the strength of feedback due to these differences. If the random effect was not significant (i.e. individual pots from the training stage did not differ in their effect on the feedback), we ran the same formula as a generalized linear model (GLM). For GLMMs or GLMs of aboveground, belowground, and overall biomass data, the most parsimonious model was selected through comparison of AIC between full and reduced models. The type of model, whether an interaction term was used, and the $R^2$ value for each model is indicated (Table 1). To determine if any of the simple main effects were significant, we ran the same formula as an ANOVA using the linear model to calculate degrees of freedom and sum of squares error. We were particularly interested in comparing the effects of live and sterilized eastern redcedar soil to live and sterile home soils for each phytometer species. To elucidate this relationship for each phytometer species, we performed *post hoc* pairwise comparisons to obtain the estimated marginal means (also called least-squares means) using the *emmeans* package (Russell 2021).

We visualized differences in phytometer biomass between live and sterile home and redcedar soils using effects plots that were derived from the linear model fit for each set of contrasts (Ho *et al.* 2019; Wilschut and van Kleunen 2021). These plots illustrate simple mean differences between contrasts of interest with 95% confidence intervals using the sample data. The second part of these plots shows the modeled means and 95% confidence intervals paired with raw data points (Figure 2).

## 3 | Results

In general, soils conditioned by *Juniperus virginiana* (redcedar) suppressed the $C_3$ grasses *Pascopyrum smithii* and*Bromus inermis* relative to growth in their home soils (Table 2 and Appendix Table 1). The $C_4$ grasses *Andropogon gerardi* and *Schizachyrium scoparium* showed mixed feedbacks in soil conditioned by redcedar when compared to the height and biomass of plants grown in their home soils (Table 2 and Appendix Table 1).

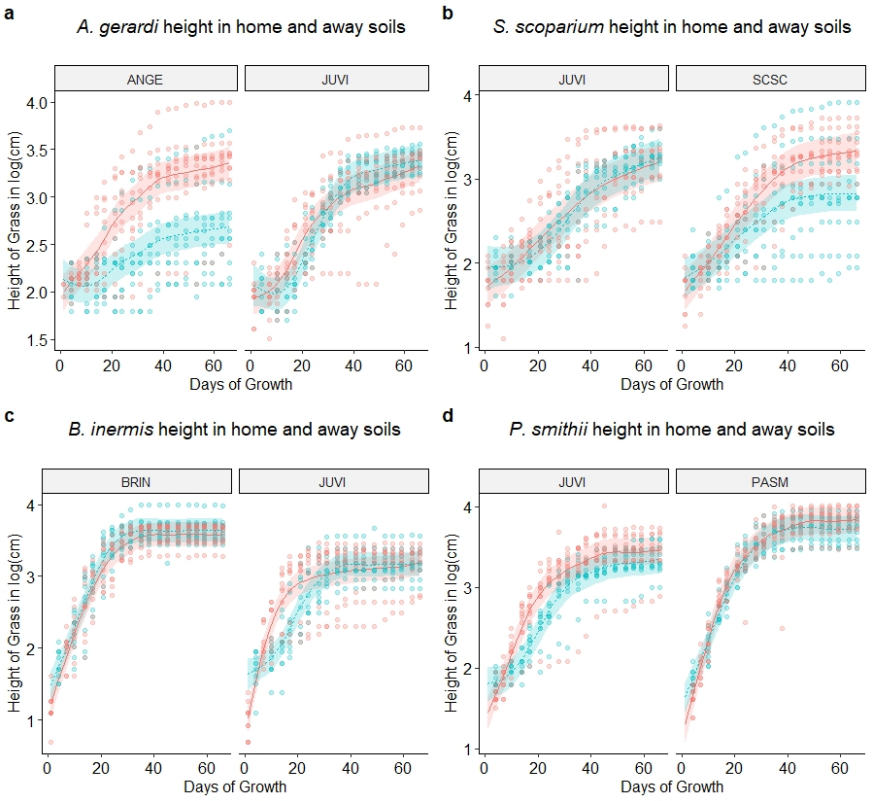


**Figure 2.** Plot showing modeled grass heights (mean line and 95% confidence intervals) and raw data (points) for the phytometers (A)*Andropogon gerardi* , (B) *Schizachyrium scoparium* , (C)*Bromus inermis* , and (D) *Pascopyrum smithii* grown in their home or away (*Juniperus virginiana* ) soils. The shaded areas illustrate 95% confidence intervals. Modeled means and confidence intervals are derived from the output of generalized additive models of $\log_{10}$(height) as a function of the interaction between the factors *soil sterilization status* and *conditioning soil type* and the smoothing variables *days of growth* , *days of growth given the interaction of treatment factors* , and the random intercept of *pot ID* for each plant. Grasses grown in live or sterile soils are indicated by red or blue coloration, respectively. Species names within each subfigure are abbreviated as follows: *A. gerardi* (ANGE), *S. scoparium* (SCSC), *B. inermis* (BRIN),*P. smithii* (PASM), and *J. virginiana* (JUVI).

**Table 2.**

The mean estimate, variance, and confidence intervals of effects on shoot biomass for contrasting interactions of each home and away (redcedar (JUVI)) and soil sterilization status. Phytometers and conditioned soil types are abbreviated as follows: *Andropogon gerardi* (ANGE), *Bromus inermis* (BRIN), *Pascopyrum smithii*(PASM), and *Schizachyrium scoparium* (SCSC). Soils are either live (L) or sterile (S).

| Phytometer | Contrasts | estimate | SE | df | lower CL | upper CL | t ratio | p |
|---|---|---|---|---|---|---|---|---|
| ANGE | ANGE L - JUVI L | -1.662 | 0.324 | 25.382 | -2.554 | -0.771 | -5.126 | <0.001 |
| | ANGE L - ANGE S | -1.279 | 0.193 | 18 | -1.823 | -0.734 | -6.634 | <0.001 |
| | ANGE L - JUVI S | -1.509 | 0.324 | 25.382 | -2.4 | -0.618 | -4.652 | <0.001 |
| | JUVI L - ANGE S | 0.384 | 0.324 | 25.382 | -0.508 | 1.275 | 1.183 | 0.643 |
| | ANGE S - JUVI S | -0.23 | 0.324 | 25.382 | -1.121 | 0.661 | -0.709 | 0.892 |
| | JUVI L - JUVI S | 0.154 | 0.193 | 18 | -0.391 | 0.698 | 0.797 | 0.855 |

| Phytometer | Contrasts | estimate | SE | df | lower CL | upper CL | t ratio | p |
|---|---|---|---|---|---|---|---|---|
| SCSC | JUVI L - SCSC L | 0.405 | 0.773 | 36 | -1.677 | 2.487 | 0.524 | 0.953 |
| | JUVI L - JUVI S | 1.049 | 0.773 | 36 | -1.033 | 3.132 | 1.357 | 0.534 |
| | JUVI L - SCSC S | -0.332 | 0.773 | 36 | -2.415 | 1.75 | -0.43 | 0.973 |
| | SCSC L - JUVI S | 0.644 | 0.773 | 36 | -1.438 | 2.726 | 0.833 | 0.838 |
| | SCSC L - SCSC S | -0.737 | 0.773 | 36 | -2.82 | 1.345 | -0.954 | 0.776 |
| | JUVI S - SCSC S | -1.382 | 0.773 | 36 | -3.464 | 0.701 | -1.787 | 0.296 |
| BRIN | BRIN L - JUVI L | 0.987 | 0.155 | 36 | 0.57 | 1.405 | 6.373 | <0.001 |
| | BRIN L - BRIN S | -0.096 | 0.155 | 36 | -0.513 | 0.322 | -0.618 | 0.926 |
| | BRIN L - JUVI S | 0.708 | 0.155 | 36 | 0.29 | 1.125 | 4.567 | <0.001 |
| | JUVI L - BRIN S | -1.083 | 0.155 | 36 | -1.5 | -0.666 | -6.991 | <0.001 |
| | JUVI L - JUVI S | -0.28 | 0.155 | 36 | -0.697 | 0.137 | -1.806 | 0.287 |
| | BRIN S - JUVI S | 0.803 | 0.155 | 36 | 0.386 | 1.221 | 5.185 | <0.001 |
| PASM | JUVI L - PASM L | -0.925 | 0.198 | 36 | -1.46 | -0.391 | -4.663 | <0.001 |
| | JUVI L - JUVI S | -0.301 | 0.198 | 36 | -0.835 | 0.233 | -1.517 | 0.438 |
| | JUVI L - PASM S | -1.206 | 0.198 | 36 | -1.741 | -0.672 | -6.079 | <0.001 |
| | PASM L - JUVI S | 0.624 | 0.198 | 36 | 0.09 | 1.159 | 3.145 | 0.017 |
| | PASM L - PASM S | -0.281 | 0.198 | 36 | -0.816 | 0.253 | -1.416 | 0.498 |
| | JUVI S - PASM S | -0.905 | 0.198 | 36 | -1.44 | -0.371 | -4.562 | <0.001 |

### 3.1 | Plant height

Comparisons between the estimated mean height of each phytometer species grown in home and redcedar soils revealed many significant differences (Appendix Table 1). *A. gerardi* height in live home soils showed a strong negative feedback when compared to height in sterile home soils (t = 17.2, p<0.001). Height of *A. gerardi* in sterile home soils was greater than in sterile redcedar soils (t = 3.3, p = 0.029), but greater than height in home live soils (t = 15.0, p <0.001) (Figure 2a). Height of *A. gerardi* in live home soils was significantly shorter than in live redcedar soils (t = -16.3, p < 0.001). Similarly, *S. scoparium* height in home sterile soils was much greater than in home live soil (t = 10.3, p<0.001), indicating a strong negative feedback. *S. scoparium* height in live (t = 7.6, p < 0.001) and sterile (t = -7.7, p < 0.001) redcedar soils were shorter than in home sterile soils. There was no detectable difference in *S. scoparium*height when comparing growth in home live soils and sterile or live redcedar soils (Figure 2b). There was no detectable difference in*B. inermis* height in live home soils and sterile home soils (t =

-2.7, p = 0.194). The height of *B. inermis* was suppressed in sterile redcedar soils relative to live (t = -15.2, p < 0.001) and sterile (t = 13.0, p <0.001) home soils. (Figure 2c). The height of *B. inermis* was also suppressed in live redcedar soils relative to live (t = 13.5, p < 0.001) and sterile (t = 11.2, p <0.001) home soils (Figure 2c). The height of *P. smithii* showed no detectable difference between live home soils and sterile home soils (t = 0.5, p = 1.0). The height of *P. smithii*growth was suppressed in sterile redcedar soils relative to sterile (t = -10.4, p < 0.001) and live (t = -13.0, p <0.001) home soils. The height of *P. smithii* growth was also suppressed in live redcedar soils relative to sterile (t = 13.9, p < 0.001) and live (t = -16.6, p <0.001) home soils. Live redcedar soils suppressed the height of *P. smithii* relative to growth in sterile redcedar soils (t = 4.5, p < 0.001) (Figure 2d).

### 3.2 | Plant biomass

There were many significant differences on the final shoot biomass of each species in the effects of the interaction between home or away (redcedar) soil types and the main effects of whether the soil was live or sterilized (Table 2). Root biomass and total biomass results generally aligned with those of shoot biomass (see Appendix Figures 2, 3 and Tables 2, 3).

Plant-soil feedbacks where soil conditioned by redcedar suppressed shoot biomass were not detected for either $C_4$ grass species in the study. *A. gerardi* shoot biomass in live home soils showed a strong negative

feedback (estimate = -1.3, $p < 0.001$) when compared to the biomass of samples grown in sterile home soils. Shoot biomass of *A. gerardi* grown in live home soils was less than its biomass when grown in redcedar soils that were live (estimate = -1.6, $p < 0.001$) or sterile (estimate = -1.5, $p < 0.001$). No significant effects or interactions were found when modeling shoot biomass as a function of growth in home or away soils and soil sterilization status.

The $C_3$ grasses in this experiment showed strong negative feedbacks when grown in redcedar soil (Figure 3). However, the shoot biomass of *B. inermis* did not show any significant feedback when growth between live and sterile home soils (estimate = -0.10, $p = 0.93$) was contrasted. Shoot biomass of *B. inermis* was reduced when grown in live (estimate = 0.99, $p < 0.001$) or sterile (estimate = 0.71, $p < 0.001$) redcedar-soils in comparison to shoot biomass in live home soils. Similarly, shoot biomass of *B. inermis* was reduced when grown in live (estimate = -1.1, $p < 0.001$) or sterile (estimate = 0.80, $p < 0.001$) redcedar soils in comparison to shoot biomass in sterile home soils. Shoot biomass of*B. inermis* did not differ when grown in live or sterile redcedar-conditioned soils (estimate = -0.28, $p = 0.29$). The shoot biomass of *P. smithii* grown in home live or sterile soils did not differ (estimate = -0.28, $p = 0.50$). Shoot biomass of *P. smithii*grown in sterile away soils was reduced significantly when compared to live (estimate = 0.62, $p = 0.017$) or sterile (estimate = -0.91, $p < 0.001$) home soils. Shoot biomass of *P. smithii* was reduced when grown in live redcedar soils when compared to live (estimate = -0.93, $p < 0.001$) or sterile (estimate = -1.2, $p < 0.001$) home soils. Shoot biomass of *P. smithii* did not differ when grown in live or sterile redcedar-conditioned soils (estimate = -0.30, $p = 0.44$).

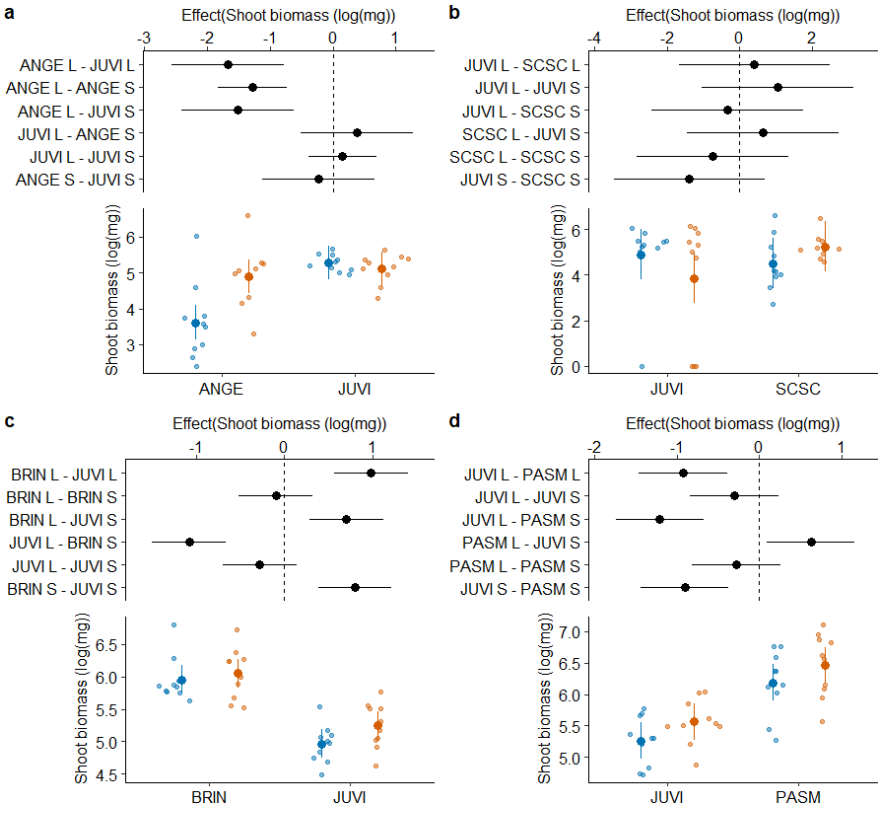


**Figure 3.** These plots illustrate the effect of home- and away-conditioned soils and whether the soil is sterilized (S) or live (L) on the shoot biomass of (a) *Andropogon gerardi* (ANGE), (b)*Schizachyrium scoparium* (SCSC), (c) *Bromus inermis* (BRIN), and (d) *Pascopyrum smithii* (PASM). Top of each figure: Effects plot

showing the difference in means between home and away soils and sterilization status of those soils. The horizontal black bars show 95% confidence intervals of the effects. The horizontal dashed line shows where there is no difference between groups, a 95% confidence interval that crosses this dashed line indicates no significant difference in the effects of contrasting pairs of treatment groups. The x-axis scale is $\log_{10}$(biomass, mg). The Y-axis lists the contrasts between each pairing of treatment types. Bottom of each figure: This portion of each plot shows the modeled response to each treatment pair, where the large solid dot is the mean and the vertical bars are the modeled 95% confidence intervals. Semi-transparent dots illustrate the raw data for each treatment combination. Blue indicates live (L) soils and orange indicates soils that were sterilized (S).

## 4 | Discussion

The growth of woody species is limited by above- and belowground competition during early stages of establishment in grasslands (Bush and van Auken 1990; Ward 2020). Identifying mechanisms that could promote survivorship and growth of woody species during their seedling stage is critical to understanding how they encroach into grasslands (Van Auken 2000). We observed a negative plant-soil feedback that suppressed the height and biomass of grasses grown in soil conditioned by redcedar for two of the four species used in this experiment. This suggests plant-soil feedback may facilitate the establishment of redcedar in its encroaching range depending on the local plant community at the site of establishment, much has it has done for other species combinations (Aldorfová *et al.* 2020).

In our experiment, grass growth in live and sterilized away (redcedar) soil was reduced when compared to growth in live and sterilized home soils for the $C_3$ grasses *B. inermis* and *P. smithii* . Plants frequently experience strong negative feedback when growing in live home soils due to accumulation of specialized predators (Bever 1994; Petermann *et al.* 2008; Lekberg *et al.* 2018). Therefore, the observed suppression of grass growth in redcedar-conditioned soils relative to home soils is noteworthy and may represent a key factor in redcedar expansion into grasslands. Negative feedbacks from dissimilar heterospecific species on target species can be derived from either an antimicrobial effect of soil biota in the conditioned soil (Haichar *et al.* 2014) or from the production of allelochemicals that negatively affect the growth of the target plant directly or by inhibiting the establishment of beneficial soil microbial communities (Mommer *et al.* 2008; Bennett and Klironomos 2019). In this experiment, we observed the inhibition of phytometer growth in sterilized away soils, which may be indicative that redcedar exudes an allelochemical into its near-soil environment. We are uncertain why $C_3$ species showed negative feedbacks and not $C_4$ species. A possible explanation is that the $C_3$ redcedar has novel weapons against these two species (Callaway and Ridenour 2004; Orians and Ward 2010). The Eurasian origins of *B. inermis* that now occupies the entire contiguous United States and the recent switch to dominance of *P. smithii* in parts of the Great Plains during the Dust Bowl could indicate that these species have had relatively limited exposure to any secondary chemicals produced by redcedar (Weaver 1942; Knapp *et al.* 2020). Another possibility is that because redcedar is a $C_3$ plant, it produces a stronger negative feedback with other $C_3$plants. Further study of more $C_3$ grass species will be needed to determine if this is a causal relationship or a coincidence.

The modification of the soil environment by allelopathic woody plants is an important process that can create a positive feedback for their encroachment (Eldridge *et al.* 2011; Caracciolo *et al.*2016). Researchers have explored the possibility of allelopathy in several North American *Juniperus* species with mixed results (Schott and Pieper 1985; Norman and Anderson 2003). Past investigations of redcedar allelopathy have focused on germination rates of prairie plants. For example, Corbett and Lashley (2017) found redcedar litter additions did not negatively affect germination of test species. However, Stipe and Bragg (1989) noted suppression of germination for a different pool of test species grown in soil collected from a redcedar stand. Our findings take this research one step further by demonstrating the suppression of plant performance following successful germination. Taken together, the ability of redcedar to reduce the germination rate of grasses and suppress their growth following establishment may be a key factor in its successful encroachment of prairies.

Our experimental results show a negative feedback for grasses grown in soil conditioned by redcedar, but

interpretation of these results must also consider the myriad factors that influence plant-plant interactions in the field. Our study examined growth of individuals in a greenhouse, using potting mix and sand as soil substrates, and comparing live inoculations of conditioned soil with those that had been sterilized under heat and pressure. The strength of plant-soil feedbacks measured in artificial conditions have been found to be inflated relative to those observed in field conditions (Kulmatiski and Kardol 2008). Confounding factors that could change the relative strength of feedback in field conditions include the near-neighborhood community composition and competitive interactions. For example, we observed strong suppression of individuals of *B. inermis* and *P. smithii*grown in live and sterilized redcedar soils. In field conditions, individuals of *B. inermis* and *P. smithii* could be expected to grow in patches where they have many conspecific neighbors (Fink and Wilson 2011; Ott and Hartnett 2015). In the prairies of the Great Plains, *B. inermis* has been shown to have positive conspecific plant-soil feedback that can exclude heterospecific plants (Vinton and Goergen 2006). Additionally, when *B. inermis* occurs at high density, it has been shown to be a strong competitor with redcedar seedlings (Hamati *et al.* 2021). In mixed-grass prairies, *P. smithii* invests heavily in spreading its resources through rhizomes that aid in ensuring plant survival in changing conditions (Ott and Hartnett 2015). Taken in this context, it is unlikely that the allelopathic effect of redcedar seedlings could fully displace *B. inermis* or *P. smithii* in a dense monoculture. However, if the suppressive effect of redcedar is sufficiently large to allow redcedar individuals to establish and survive long enough to overtop their competitors, then plant-soil feedbacks could be an important factor in the spread of the redcedars. Inherently, this effect will only apply to near neighbors that overlap in the rooting zone of redcedars (i.e. over a short distance). Further studies are needed to determine the strength of this effect in field conditions, the size of the area of impact around trees, the longevity of the effect in the soil, and how the strength of suppression changes with tree size or age.

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## AUTHOR CONTRIBUTIONS

Leland Bennion conceptualized experiment, collected data, performed analysis, and led the writing of the manuscript. David Ward provided funding, advised on experimental design, and provided critical revisions to drafts of the manuscript.

## DATA AVAILABILITY

Our data will be archived in the Dryad repository.

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## Appendix

### Table A1.

This table shows contrasts between modeled height of the four study grasses grown in home and away (redcedar) soils. The grasses are abbreviated using the following notation: *Andropogon gerardi*(ANGE), *Schizachyrium scoparium* (SCSC), *Bromus inermis*(BRIN), and *Pascopyrum smithii* (PASM). These contrasts are derived from generalized additive models of $\log_{10}$(height) as a function of the interaction between the factors *soil sterilization status* (Live = L and Sterile = S) and *parent soil type* and the smoothing variables *days of growth* , *days of growth while accounting for interactions of treatment factors* , and the random intercept of *pot ID* for each plant. The significance of contrasts was calculated by comparing the ratio between the estimated marginal means of different groups of interest. If the ratio is <1, this indicates the first term of the contrast is less than the second term. Conversely, if the ratio is >1, this indicates that the first term of the contrast is greater than the second term. The significance of these differences was calculated using a Tukey adjustment of $\alpha$ for a family of ten estimates.

| Phytometer | Contrast | ratio | SE | df | lower CL | upper CL | null | t ratio | p |
|---|---|---|---|---|---|---|---|---|---|

| Phytometer | Contrast | ratio | SE | df | lower CL | upper CL | null | t ratio | p |
|---|---|---|---|---|---|---|---|---|---|
| ANGE | ANGE S / JUVI S | 1.12 | 0.04 | 1698.20 | 1.01 | 1.25 | 1 | 3.3 | 0.029 |
| ANGE | ANGE S / ANGE L | 1.88 | 0.07 | 1698.20 | 1.68 | 2.11 | 1 | 17.2 | <0.001 |
| ANGE | ANGE S / JUVI L | 1.02 | 0.04 | 1698.20 | 0.91 | 1.15 | 1 | 0.6 | 1.000 |
| ANGE | JUVI S / ANGE L | 1.68 | 0.06 | 1698.20 | 1.50 | 1.87 | 1 | 15.0 | <0.001 |
| ANGE | JUVI S / JUVI L | 0.91 | 0.03 | 1698.20 | 0.82 | 1.02 | 1 | -2.6 | 0.207 |
| ANGE | ANGE L / JUVI L | 0.54 | 0.02 | 1698.20 | 0.48 | 0.61 | 1 | -16.3 | <0.001 |
| SCSC | JUVI S / SCSC S | 0.74 | 0.03 | 1547.97 | 0.66 | 0.84 | 1 | -7.7 | <0.001 |
| SCSC | JUVI S / JUVI L | 1.04 | 0.05 | 1547.97 | 0.90 | 1.19 | 1 | 0.8 | 0.998 |
| SCSC | JUVI S / SCSC L | 1.06 | 0.04 | 1547.97 | 0.95 | 1.18 | 1 | 1.8 | 0.761 |
| SCSC | SCSC S / JUVI L | 1.40 | 0.06 | 1547.97 | 1.22 | 1.61 | 1 | 7.6 | <0.001 |
| SCSC | SCSC S / SCSC L | 1.43 | 0.05 | 1547.97 | 1.28 | 1.60 | 1 | 10.3 | <0.001 |
| SCSC | JUVI L / SCSC L | 1.02 | 0.04 | 1547.97 | 0.90 | 1.17 | 1 | 0.6 | 1.000 |
| BRIN | BRIN S / JUVI S | 1.65 | 0.06 | 1692.73 | 1.46 | 1.87 | 1 | 13.0 | <0.001 |
| BRIN | BRIN S / BRIN L | 0.91 | 0.03 | 1692.73 | 0.82 | 1.02 | 1 | -2.7 | 0.194 |
| BRIN | BRIN S / JUVI L | 1.52 | 0.06 | 1692.73 | 1.35 | 1.71 | 1 | 11.2 | <0.001 |
| BRIN | JUVI S / BRIN L | 0.55 | 0.02 | 1692.73 | 0.49 | 0.63 | 1 | -15.2 | <0.001 |
| BRIN | JUVI S / JUVI L | 0.92 | 0.04 | 1692.73 | 0.81 | 1.05 | 1 | -2.0 | 0.578 |
| BRIN | BRIN L / JUVI L | 1.66 | 0.06 | 1692.73 | 1.48 | 1.87 | 1 | 13.5 | <0.001 |
| PASM | JUVI S / PASM S | 0.67 | 0.03 | 1704.72 | 0.60 | 0.76 | 1 | -10.4 | <0.001 |
| PASM | JUVI S / JUVI L | 1.19 | 0.05 | 1704.72 | 1.05 | 1.34 | 1 | 4.5 | <0.001 |
| PASM | JUVI S / PASM L | 0.68 | 0.02 | 1704.72 | 0.62 | 0.75 | 1 | -13.0 | <0.001 |
| PASM | PASM S / JUVI L | 1.76 | 0.07 | 1704.72 | 1.55 | 2.01 | 1 | 13.9 | <0.001 |
| PASM | PASM S / PASM L | 1.02 | 0.03 | 1704.72 | 0.92 | 1.13 | 1 | 0.5 | 1.000 |
| PASM | JUVI L / PASM L | 0.58 | 0.02 | 1704.72 | 0.52 | 0.64 | 1 | -16.6 | <0.001 |

**Table A2.**

The mean estimate, variance, and confidence intervals of effects on root biomass for contrasting interactions of each home and away (Redcedar) and soil sterilization status. Phytometers and parent soil types are abbreviated as follows: *Andropogon gerardi* (ANGE),*Schizachyrium scoparium* (SCSC), *Bromus inermis* (BRIN), and*Pascopyrum smithii* (PASM). Soils are either live (L) or sterile (S).

| **Phytometer** | **Contrasts** | **estimate** | **SE** | **df** | **lower CL** | **upper CL** | **t ratio** | **p** |
|---|---|---|---|---|---|---|---|---|
| ANGE | ANGE L - JUVI L | -1.813 | 0.323 | 29.568 | -2.691 | -0.935 | -5.617 | <0.001 |
| | ANGE L - ANGE S | -1.464 | 0.236 | 18 | -2.131 | -0.798 | -6.211 | <0.001 |
| | ANGE L - JUVI S | -1.561 | 0.323 | 29.568 | -2.44 | -0.683 | -4.837 | <0.001 |
| | JUVI L - ANGE S | 0.349 | 0.323 | 29.568 | -0.53 | 1.227 | 1.08 | 0.704 |
| | JUVI L - JUVI S | 0.252 | 0.236 | 18 | -0.415 | 0.918 | 1.067 | 0.713 |
| | ANGE S - JUVI S | -0.097 | 0.323 | 29.568 | -0.975 | 0.781 | -0.3 | 0.99 |
| SCSC | JUVI L - SCSC L | 0.381 | 0.802 | 36 | -1.778 | 2.541 | 0.476 | 0.964 |
| | JUVI L - JUVI S | 0.968 | 0.802 | 36 | -1.191 | 3.127 | 1.207 | 0.626 |
| | JUVI L - SCSC S | -0.498 | 0.802 | 36 | -2.658 | 1.661 | -0.621 | 0.925 |
| | SCSC L - JUVI S | 0.587 | 0.802 | 36 | -1.573 | 2.746 | 0.732 | 0.884 |
| | SCSC L - SCSC S | -0.88 | 0.802 | 36 | -3.039 | 1.28 | -1.097 | 0.694 |
| | JUVI S - SCSC S | -1.466 | 0.802 | 36 | -3.626 | 0.693 | -1.829 | 0.277 |
| BRIN | BRIN L - JUVI L | 1.162 | 0.236 | 35.948 | 0.525 | 1.799 | 4.914 | <0.001 |

| Phytometer | Contrasts | estimate | SE | df | lower CL | upper CL | t ratio | p |
|---|---|---|---|---|---|---|---|---|
| | BRIN L - BRIN S | -0.169 | 0.232 | 18 | -0.824 | 0.487 | -0.728 | 0.885 |
| | BRIN L - JUVI S | 0.357 | 0.236 | 35.948 | -0.279 | 0.994 | 1.512 | 0.441 |
| | JUVI L - BRIN S | -1.331 | 0.236 | 35.948 | -1.968 | -0.694 | -5.628 | <0.001 |
| | JUVI L - JUVI S | -0.805 | 0.232 | 18 | -1.46 | -0.149 | -3.469 | 0.013 |
| | BRIN S - JUVI S | 0.526 | 0.236 | 35.948 | -0.111 | 1.163 | 2.226 | 0.136 |
| PASM | JUVI L - PASM L | -1.185 | 0.17 | 18 | -1.665 | -0.706 | -6.984 | <0.001 |
| | JUVI L - JUVI S | -0.538 | 0.154 | 19 | -0.97 | -0.106 | -3.503 | 0.012 |
| | JUVI L - PASM S | -1.723 | 0.229 | 36.42 | -2.339 | -1.107 | -7.529 | <0.001 |
| | PASM L - JUVI S | 0.647 | 0.229 | 36.42 | 0.031 | 1.263 | 2.827 | 0.036 |
| | PASM L - PASM S | -0.538 | 0.154 | 19 | -0.97 | -0.106 | -3.503 | 0.012 |
| | JUVI S - PASM S | -1.185 | 0.17 | 18 | -1.665 | -0.706 | -6.984 | <0.001 |

**Table A3.**

The mean estimate, variance, and confidence intervals of effects on total biomass for contrasting interactions of each home and away (redcedar (JUVI)) and soil sterilization status. Phytometers and conditioned soil types are abbreviated as follows: *Andropogon gerardi* (ANGE), *Bromus inermis* (BRIN), *Pascopyrum smithii*(PASM), and *Schizachyrium scoparium* (SCSC). Soils are either live (L) or sterile (S). Non-integers for d.f. derive from mixed models. See details in Figure S3.

| Phytometer | Contrast | estimate | SE | df | lower CL | upper CL | t.ratio | p |
|---|---|---|---|---|---|---|---|---|
| ANGE | ANGE L - JUVI L | -1.764 | 0.318 | 28.088 | -2.631 | -0.896 | -5.551 | <0.001 |
| | ANGE L - ANGE S | -1.408 | 0.218 | 18 | -2.023 | -0.793 | -6.468 | <0.001 |
| | ANGE L - JUVI S | -1.537 | 0.318 | 28.088 | -2.404 | -0.67 | -4.837 | <0.001 |
| | JUVI L - ANGE S | 0.356 | 0.318 | 28.088 | -0.511 | 1.223 | 1.12 | 0.68 |
| | JUVI L - JUVI S | 0.227 | 0.218 | 18 | -0.388 | 0.842 | 1.042 | 0.728 |
| | ANGE S - JUVI S | -0.129 | 0.318 | 28.088 | -0.996 | 0.738 | -0.406 | 0.977 |
| BRIN | BRIN L - JUVI L | 1.14 | 0.201 | 36 | 0.599 | 1.68 | 5.678 | <0.001 |
| | BRIN L - BRIN S | -0.13 | 0.201 | 36 | -0.671 | 0.411 | -0.647 | 0.916 |
| | BRIN L - JUVI S | 0.451 | 0.201 | 36 | -0.089 | 0.992 | 2.248 | 0.13 |
| | JUVI L - BRIN S | -1.27 | 0.201 | 36 | -1.81 | -0.729 | -6.325 | <0.001 |
| | JUVI L - JUVI S | -0.688 | 0.201 | 36 | -1.229 | -0.148 | -3.429 | 0.008 |
| | BRIN S - JUVI S | 0.581 | 0.201 | 36 | 0.041 | 1.122 | 2.896 | 0.031 |
| PASM | JUVI L - PASM L | -1.079 | 0.153 | 18 | -1.511 | -0.647 | -7.062 | <0.001 |
| | JUVI L - JUVI S | -0.435 | 0.143 | 19 | -0.838 | -0.032 | -3.032 | 0.032 |
| | JUVI L - PASM S | -1.514 | 0.21 | 36.703 | -2.078 | -0.95 | -7.223 | <0.001 |
| | PASM L - JUVI S | 0.644 | 0.21 | 36.703 | 0.08 | 1.208 | 3.073 | 0.02 |
| | PASM L - PASM S | -0.435 | 0.143 | 19 | -0.838 | -0.032 | -3.032 | 0.032 |
| | JUVI S - PASM S | -1.079 | 0.153 | 18 | -1.511 | -0.647 | -7.062 | <0.001 |
| SCSC | JUVI L - SCSC L | 0.325 | 0.868 | 36 | -2.012 | 2.662 | 0.375 | 0.982 |
| | JUVI L - JUVI S | 1.141 | 0.868 | 36 | -1.196 | 3.479 | 1.315 | 0.56 |
| | JUVI L - SCSC S | -0.488 | 0.868 | 36 | -2.826 | 1.849 | -0.563 | 0.942 |
| | SCSC L - JUVI S | 0.816 | 0.868 | 36 | -1.521 | 3.154 | 0.94 | 0.783 |
| | SCSC L - SCSC S | -0.814 | 0.868 | 36 | -3.151 | 1.524 | -0.937 | 0.785 |
| | JUVI S - SCSC S | -1.63 | 0.868 | 36 | -3.967 | 0.708 | -1.878 | 0.255 |

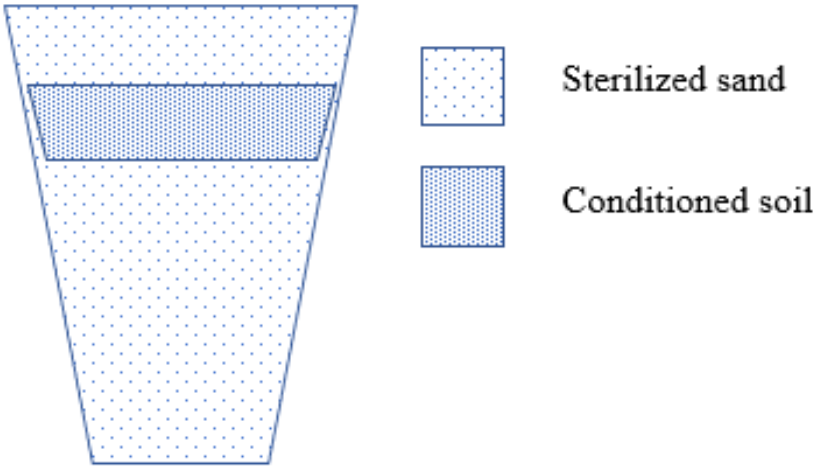


**Figure A1:** This is a basic illustration of how substrates were combined in 2.8 L pots. Sand was sterilized in an autoclave and cooled prior to being added to each pot. Conditioned soils from the training phase were added and then capped with additional sand. One individual phytometer was transplanted into each prepared pot.

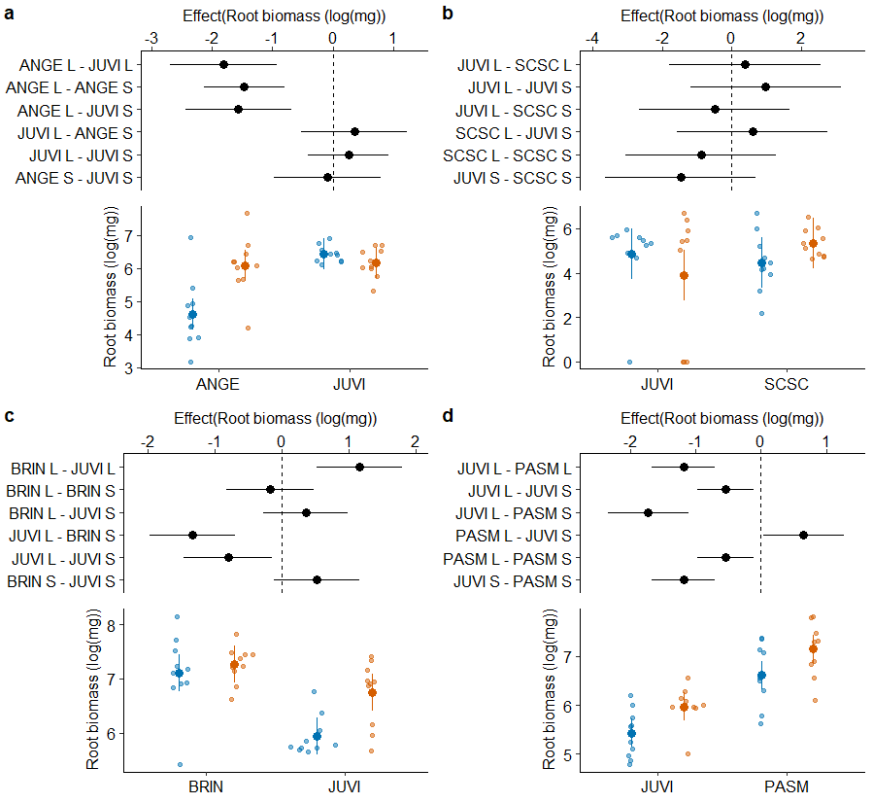


**Figure A2.** These plots illustrate the effects of home- and away-conditioned soils and whether the soil is sterilized (S) or live (L) on the root biomass of (a) *Andropogon gerardi* (ANGE), (b)*Schizachyrium scoparium* (SCSC), (c) *Bromus inermis* (BRIN) and (d) *Pascopyrum smithii* (PASM). Top of each figure: Effects plot showing the difference in means between home and away soils and sterilization status of those soils. The horizontal black bars show 95% confidence intervals of the effects. The horizontal dashed line shows where there is no difference between groups, a 95% confidence interval that crosses this dashed line indicates no significant difference in the effects of contrasting pairs of treatment groups. The x-axis scale is $\log_{10}$(biomass, mg). The Y-axis lists the contrasts between each pairing of treatment types. Bottom of each figure: This portion of each plot shows the modeled response to each treatment pair, where the large solid dot is the mean and the vertical bars are the modeled 95% confidence intervals. Dots illustrate the raw data for each treatment combination. Blue coloration indicates live (L) soils and orange coloration indicates soils that were sterilized (S).

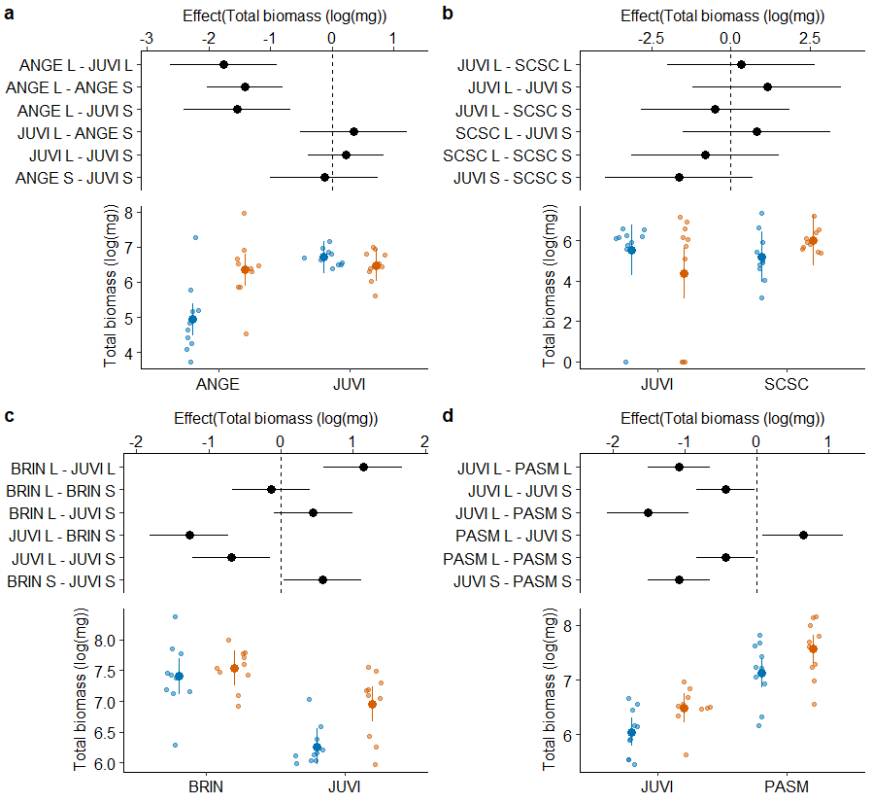


**Figure A3.** These plots illustrate the effect of home- and away-conditioned soils and whether the soil is sterilized (S) or live (L) on the total biomass of (a) *Andropogon gerardi* (ANGE), (b)*Schizachyrium scoparium* (SCSC), (c) *Bromus inermis* (BRIN), and (d) *Pascopyrum smithii* (PASM). Top of each figure: Effects plot showing the difference in means between home and away soils and sterilization status of those soils. The horizontal black bars show 95% confidence intervals of the effects. The horizontal dashed line shows where there is no difference between groups, a 95% confidence interval that crosses this dashed line indicates no significant difference in the effects of contrasting pairs of treatment groups. The x-axis scale is $\log_{10}$(biomass, mg). The Y-axis lists the contrasts between each pairing of treatment types. Bottom of each figure: This portion of each plot shows the modeled response to each treatment pair, where the large solid dot is the mean and the vertical bars are the modeled 95% confidence intervals. Dots illustrate the raw data for each treatment combination. Blue indicates live (L) soils and orange indicates soils that were sterilized (S).