Eastern redcedar roots create legacy effects that suppresses growth of prairie species

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Research Article

Keywords: Plant-soil feedback, legacy effect, woody encroachment, allelopathy, range expansion

Posted Date: November 14th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-2219489/v1

Abstract

The expansion of woody species from their historical ranges into grasslands is a global problem. Understanding the mechanisms that enable species to successfully establish and then re-encroach following their removal is critical to effectively managing problem species. Legacy effects are a mechanism that could be critical to the re-establishment of woody encroachers following their removal. Legacy effects occur when a species alters the biotic and abiotic environment in a way that affects communities that establish subsequently. In this study, we assess whether *Juniperus virginiana*, a North American woody encroacher, generates legacy effects that affect communities that establish following removal of this species from an experimental grass community. We treated three soil groups with different percentages of *J. virginiana* roots. We found escalating suppression of overall community biomass and the biomass of each of the individual species with increasing percentages of root treatments. Our results suggest that *J. virginiana* exudes an allelochemical into soils that inhibits the growth of certain grasses and thus has legacy effects on future occupants. We suggest that the inhibition of the development of grasses in areas where *J. virginiana*has been removed is a mechanism that favors the re-establishment of *J. virginiana*. Our results indicate the legacy effects of *J. virginiana*must be considered when conducting removal and restoration of *J. virginiana* infested lands.

Highlighted Student Paper

We reveal an undocumented consequence of a widespread woody encroacher, an allelopathic legacy effect that persists in the soil and inhibits the growth of grasses that establish subsequently

Introduction

Species are moving into habitats outside their historical range in response to human-mediated translocation, habitat change, and alteration of climatic conditions (Hoegh-Guldberg et al. 2008; Poland et al. 2021). The introduction of new (or novel) species to a new range is deemed problematic when it results in negative changes to the ecosystem, habitat, or pre-existing resident species (e.g. Lambertini et al., 2011; Simberloff, 2011). These novel species can be of non-native or native origin and are frequently referred to as invasive species or range expanders, respectively (Tomiolo and Ward 2018). The introduction of a novel species into a community can lead to changes in competitive interactions, resource availability, and soil microbial communities (Kourtev et al. 2002; Callaway and Ridenour 2004; Perkins and Hatfield 2014). Due to the actual or potential negative effects on systems in their new range, novel species are frequently the target of management that seeks to extirpate them from an area (Pearson et al. 2016). However, there is some potential for the effects of novel species to persist following their extirpation and thus influence future residents, a phenomenon known as the legacy effect (Perry, 1987).

In ecology, legacy effects describe long-term indirect effects of species following their removal from an area. These effects can alter the successional trajectory of a system and are often attributed to human-mediated disturbance, herbivory, and invasive species (Cuddington 2011; Kostenko et al. 2012; Åkesson et al. 2021). Invasive species can alter the successional trajectory and community composition of future occupants through modifications to the soil environment and the resident soil-microbial community (Davis et al. 2005; Jordan et al. 2012). These legacy effects are often microbially-mediated, although abiotic alterations to the rhizosphere such as allelopathy can also be important (Bever 2003; Kardol et al. 2007; Del Fabbro and Prati 2015). Microbially-mediated plant-soil feedback is a commonly studied mechanism wherein the impact of novel plants on their near-soil environment alters the fitness of subsequent con- and heterospecifics (Levine et al. 2006; Suding et al. 2013). Plants accumulate specialist and generalist soil organisms in their rhizospheres that can be beneficial, neutral, or harmful to themselves and conspecifics (Bever et al. 1997). When subsequent species establish, the effects of pre-existing soil organisms can vary, based on the types of interactions that occur between them and the new plants. Soil organisms that are beneficial to existing occupant species and antagonistic to other species can promote the establishment of a monoculture (Smith and Reynolds 2012; Aldorfová et al. 2020). More typically, resident species accumulate soil microbes from antagonistic specialists over time which gives heterospecifics an advantage and promotes coexistence (Nijjer et al. 2007; Diez et al. 2010). This differential effect has been attributed as a major factor that promotes coexistence of species (Bonanomi et al. 2005; Bever et al. 2015). Abiotic conditions such as the physical and chemical characteristics of soils can reinforce or moderate the feedbacks generated by the microbial community and thus determine whether microbially mediated feedbacks have a meaningful impact on plant growth (Ehrenfeld et al. 2005; Bennett and Klironomos 2019).

Woody encroachment

Woody encroachment by native and non-native species is pervasive in North American grasslands (Barger et al. 2011; Morford et al. 2021). Human-mediated disturbances such as land-use changes, overgrazing, and greatly extended fire regimes have created a positive feedback that favors conversion from grasslands to woody systems (Heisler et al. 2003; Ratajczak et al. 2014; Miller et al. 2017). Eastern redcedar (*Juniperus virginiana*) is an aggressive range expander in natural and disturbed gaps in the eastern portion of its range but it is also known for its spread in the Great Plains that is rapidly converting grasslands and prairies to woodlands (Wang et al. 2017).

The legacy effects of eastern redcedar (hereafter, redcedar) encroachment on prairie plant communities is understudied. Recent research indicates burned redcedar stands may allow herbaceous biomass to recover to levels that are similar to unencroached areas (Limb et al. 2014). However, the result is often short-lived due to rapid re-establishment of redcedars (Bielski et al. 2021; Fogarty et al. 2021). Additionally, redcedar may contain allelopathic compounds that suppress the growth of certain grass species and thus favor re-establishment of redcedar following removal (Stipe and Bragg 1989; Bennion and Ward 2022).

We conducted a greenhouse study that assessed the abiotic legacies of eastern redcedar following removal. Our study design evaluated the biomass of four grasses (*Bromus inermis*, *Elymus canadensis*, *Bouteloua curtipendula*, and *Schizachyrium scoparium*) when grown together in potting mix or in live or sterilized field soils collected from eastern redcedar with varying levels of eastern redcedar root additions. Live field soils contained a living soil microbial community that was cultivated under a redcedar stand, whereas this community is removed in field soils that were sterilized. We examined the strength and direction of feedbacks derived from biotic (soil microbial community) and abiotic (eastern redcedar roots) sources on individual species and on the experimental plant community. If field soils from redcedar contain microbes that suppress the growth of certain grasses, we hypothesized that we would observe (1) a reduction in the cumulative and (2) individual biomass of grasses and between live and sterilized field soils in the absence of redcedar root additions. If the addition of redcedar roots introduced a source of allelochemicals, we hypothesized that we would observe a decrease (3) in the cumulative and (4) individual biomass of grasses with increasing levels of root addition of redcedars in each soil type. If there was a differential effect of redcedar roots on the growth of grass species, (5) we hypothesized that we would observe changes in the proportion of biomass of each species relative to the overall biomass of all other species.

Materials And Methods

Individuals of four common perennial grass species were grown together to evaluate if *Juniperus virginiana* roots could generate negative legacy effects on communities that establish following the removal of redcedar. Grasses were selected to be representative of a community that co-occurs, but also that captured the potential differences between cool- and warm-season grasses. We chose *Bromus inermis* (smooth brome) and *Elymus canadensis* (Canada wildrye) as representatives of common cool-season C_3 grasses. Cool-season grasses typically occur in the central mixed grass to northern shortgrass prairies of the Great Plains. Smooth brome and Canada wildrye are widespread, rhizomatous bunchgrasses of Eurasian and North American descent, respectively. We also included two warm-season C_4 grasses, *Bouteloua curtipendula* (side-oats grama) and *Schizachyrium scoparium* (little bluestem). Side-oats grama and little bluestem are both perennial clump-forming bunch grasses of North American origin typically occurring in warmer areas (Teeri and Stowe 1976).

In August 2021, field soils and redcedar roots were collected from a 60–80-year-old redcedar stand at the Edge of Appalachia Preserve in southern Ohio (38°42'N, 83°26'W). Soil samples were collected from under several large trees that were all within 100 m of each other. Large organic debris was swept away from the soil surface and samples were collected at a maximum depth of 10–15 cm. Soils were processed by running them through a 2 mm sieve and removing any rocks or organic matter. All soils were pooled and thoroughly mixed. A portion of the redcedar field soil was sterilized in an autoclave at 121 ºC for two one-hour cycles (e.g. Crawford & Knight, 2017). The sterilization of soils in an autoclave can release previously unavailable nutrients into soils. We sent samples of live and sterilized field soils to the Ohio State University Service Testing and Research laboratory in Wooster, OH to test for differences in

soil nutrient concentrations. Live and sterilized redcedar soil was mixed at a 50:50 ratio with Jolly Gardner Pro-line C/GP® germination mix that had been sterilized using the same procedure outlined above. We added 2.4 L of live or sterilized field soil mix or germination mix to 2.8 L pots.

Fine-root material collected from the field (> 0.5 cm in diameter) was separated from larger roots and retained for use in the experiment. To obtain the volume of redcedar roots needed for our experiment, additional root matter was collected from ~ 3-year-old redcedars that were growing in ProMix Ultimate potting mix in 5.6 L pots in our greenhouse. These redcedars were obtained as greenhouse-reared seedlings from Pinelands Nursery, New Jersey. All roots were washed to remove any remnant soil material and then lightly processed in an industrial grinder to break them into ~ 2.5 cm or smaller pieces. Roots were added to treatment pots of each soil type at the following volumes: 0 mL, 100 mL, 200 mL, or 400 mL. Roots were mixed into the soil to be evenly distributed in each pot. There were ten replicates of each soil and root treatment combination, resulting in 120 total pots in the experiment.

In late July 2021 seeds of *B. inermis*, *E. canadensis*, *B. curtipendula*, and *S. scoparium* seeds were germinated in preparation for the experiment. A monoculture of each species was grown in sand that had been sterilized following the procedure outlined above. One seedling of each species was transplanted into each pot in mid-August 2021. Any individuals that died during the first week were replaced. Grasses were grown in the greenhouse for 12 weeks. Pots were watered *ad libitum*. Due to the experiment taking place late in the year (August- November), we used supplemental lighting from 1000 W high pressure sodium bulbs nightly from 5:00–8:00 pm. The height of each grass was recorded weekly for the duration of the experiment. Grasses were harvested individually in November 2021. Aboveground biomass samples were dried in an oven at 65 ºC for over 48 h prior to being weighed.

Statistical Methods

To assess the effects of redcedar root additions on the cumulative and individual biomass of grasses we conducted a series of analyses that calculated Hedges' *g* effect sizes for contrasts between live and sterilized field soils and levels of root addition. Hedges' *g* is an unbiased measure of the difference between two groups and is calculated by subtracting the mean of one group (μ_b) from the mean of a second group (μ_a) and then dividing by the pooled variance (s_{pooled}) of the groups (Eq. 1) (Hedges 1981).

Equation 1:

$$g = (\mu_a - \mu_b)/s_{pooled}$$

We were interested in evaluating how redcedar root additions affected the biomass of grasses within the live and sterilized treatment groups. We calculated the effect of redcedar roots on cumulative shoot biomass by contrasting the treatment group with no root addition with the other three groups (100, 200, 400 mL of roots added). For each set of contrasts the summary statistics, Hedges' *g* effect size, and bias-corrected and accelerated bootstrapped 95% confidence interval of the effect size were calculated using the *dabestr* package in R version 4.1.1 (Ho et al. 2019). We then plotted the output using a Cummings

estimation plot (Ho et al. 2019). Following the same procedure, we evaluated whether there were differences in the total biomass of grasses grown in live and sterilized field soils by comparing paired contrasts of cumulative biomass at each level of root addition. In addition to the effects of redcedar root additions on the cumulative shoot biomass of grasses in the experiment, we were interested in the response of individual species to each treatment. For each grass species, we examined the effect of live and sterile soil treatments on shoot biomass at each level of redcedar root addition. We also evaluated the effect of root addition on shoot biomass for each grass species within the live and sterilized groups (Figure S2).

In addition to evaluating the raw shoot biomass we determined that a standardized feedback ratio could be useful for comparing the effects of varying levels of root additions on the biomass of individual species between the three different soil types. Within each species and soil type combination, we calculated the logarithm (log_{10}) of the ratio of the biomass of grass grown with 0 mL of added root additions ($Roots_0$) to the biomass of grass grown with 100, 200, or

400 mL ($Roots_x$ where x is 100, 200, or 400) of roots added (Eq. 2).

Equation 2:

$\text{Feedback ratio} = \log_{10}(\text{Roots}_0 / \text{Roots}_x)$

We chose a logarithmically standardized feedback ratio so the values could be compared between different soil types and between species (Crawford & Knight, 2017; see also Pernilla Brinkman et al., 2010; Petermann et al., 2008). The feedback ratio is centered on zero when there is no effect, is positive when grass biomass is larger without roots and is negative when the biomass of grasses grown with root additions is larger than those grown without. The data were split into potting, and live and sterile field soil types. Within each soil type we built a linear model of the feedback ratio as a function of the interaction between grass species and the level of root addition. Post-hoc testing of was completed using the *emmeans* (v1.7.1-1) package in R (Lenth et al., 2021). For each species, the estimated marginal means of the feedback ratio was contrasted between each the no root addition (0 mL) and each level of root addition (100, 200, 400 mL) treatments in potting, and live and sterilized field soils.

Finally, we determined whether the proportion of each species' biomass to the total biomass differed among treatments. The data was split to only include field soils. For each species we built a linear model of proportions as a function of the interaction between live or sterilized soil and the level of root addition. Post-hoc testing used the *emmeans* (v1.7.1-1) package in R (Lenth et al., 2021). For each species, the estimated marginal means of proportion of shoot biomass were contrasted between the no-root addition (0mL) and each level of root addition (100, 200, 400 mL) treatments in live and sterilized field soils. In addition, the proportion of shoot biomass between live and sterilized soil types were contrasted at each level of root addition (0, 100, 200, 400 mL).

Results

We evaluated the total shoot biomass of experimental community pots and found negative feedbacks derived from additions of redcedar roots (Fig. 1). In sterilized field soils, the 400 mL root-addition treatment resulted in a substantial reduction in biomass relative to the control group (Hedges' *g*: -2.26; 95% CI: -3.65 to -1.16). In live field soils, shoot biomass declined with each level of root addition, with the 400 mL treatment group having the largest effect (Hedges' *g*: -6.53; 95% CI: -7.93 to -5.29). Biomass in potting soil was reduced at the

200 mL (Hedges' *g*: -1.38; 95% CI: -2.4 to -0.47) and 400 mL (Hedges' *g*: -1.53; 95% CI: -2.57 to -0.66) root additions. For the pairwise comparison of shoot biomass between live and sterile field soils at the 0 mL root treatment levels, cumulative plant biomass in live soils was substantially smaller than in sterile field soils (Figure S1). However, we cannot assert the soil microbial community alone is responsible for observed differences when evaluating raw biomass. Soil testing revealed the sterilization procedure by autoclave released nutrients (notably, phosphorus) that were not available in live field soils (Table S1). For this reason, we did not consider differences between live and sterilized soil treatments derived from raw biomass as being caused solely by the soil microbial community.

We assessed the raw shoot biomass of each species grouped by soil type and root addition treatment and found mixed feedbacks (Fig. 2). In live field soils, *B. inermis* shoot biomass was reduced in the 200 mL (Hedges' *g*: -1.29; 95% CI -2.15 to -0.17) and 400 mL (Hedges' *g*: -1.73; 95% CI -2.74 to -0.68) root additions. There was no detected difference in biomass between root treatments in sterile field soils for *B. inermis*. There was a reduction in biomass of *B. inermis* at the 400 mL (Hedges' *g*: -1.11; 95% CI -1.93 to -0.18) root-treatment level in potting soil. In live field soils, *E. canadensis* shoot biomass was reduced in the 400 mL (Hedges' *g*: -2.58; 95% CI -3.73 to -1.57) root addition. In sterile soil, *E. canadensis* shoot biomass was reduced in the 200 mL (Hedges' *g*: -0.98; 95% CI -1.84 to -0.09) and 400 mL (Hedges' *g*: -1.43; 95% CI -2.46 to -0.49) root addition. Shoot biomass of *E. canadensis* was reduced when grown in potting soil at the 200 mL (Hedges' *g*: -0.9; 95% CI -1.61 to -0.07) and 400 mL (Hedges' *g*: -1.05; 95% CI -1.72 to -0.26) root additions. We detected positive feedback for *B. curtipendula* biomass grown in sterile field soil at the 200 mL (Hedges' *g*: 1.24; 95% CI: 0.49 to 1.77) and 400 mL (Hedges' *g*: 1.76; 95% CI 0.79 to 3.39) root additions. In contrast, *B. curtipendula* biomass grown in live field soil had a reduction in biomass at the 200 mL (Hedges' *g*: -1.20; 95% CI: -2.14 to -0.25) and 400 mL (Hedges' *g*: -1.70; 95% CI -2.78 to -0.69) root additions. There was no detected difference in the biomass of *B. curtipendula* among root treatments in potting soil. Our analysis found positive feedback for *S. scoparium* grown in sterile field soil with increased biomass at the 100 mL (Hedges' *g*: 4.14; 95% CI: 2.67 to 6.46), 200 mL (Hedges' *g*: 1.83; 95% CI: 0.79 to 2.50) and 400 mL (Hedges' *g*: 1.51; 95% CI: 0.78 to 2.16) levels of root addition. Conversely, the biomass of *S. scoparium* was reduced in live field soil at the 200 mL (Hedges' *g*: -1.74; 95% CI: -2.46 to -0.98) and 400 mL (Hedges' *g*: -1.96; 95% CI: -2.80 to -0.98) root additions. In potting soil, *S. scoparium* biomass was reduced at the 100 mL (Hedges' *g*: -1.04; 95% CI: -1.75 to -0.24), 200 mL (Hedges' *g*: -1.32; 95% CI: -1.98 to -0.68), and 400 mL (Hedges' *g*: -0.91; 95% CI: -1.61 to -0.14) levels of root addition.

We computed a feedback ratio to have a standardized metric that could be used to contrast the observed effect of root treatments across the three soil types (Fig. 3). In potting soil, *E. canadensis* had significant negative feedback with increased root addition when comparing the control and the 200 mL (t ratio = -2.0, p = 0.04) and 400 mL (t ratio = -2.24, p = 0.02) feedback ratios. Negative feedbacks were observed for *S. scoparium* grown in potting soil when contrasting the control and the 200 mL (t ratio = -2.15 p = 0.03) feedback ratios. In live field soils, *B. inermis* had strong negative feedbacks at all root-addition levels (t ratio < -3.0, p < 0.01). *Elymus canadensis* grown in live field soil had reduced growth when contrasting the feedback ratios of the control and 400 mL root-addition treatments (t ratio = -4.49, p < 0.001). Suppression of growth was observed for *B. curtipendula* when contrasting the feedback ratios of the control and the 200mL (t ratio = -3.01, p < 0.01) and 400 mL (t ratio = -3.82, p < 0.001) treatment groups in live soils. Similarly, *S. scoparium* had reduced growth in live soils for the contrasts of the control and the 200 mL (t ratio = -2.41, p = 0.02) and 400 mL (t ratio =-2.94, p < 0.01) root treatments. Sterile field soils had a mix of positive and negative feedbacks. Contrasts between control and the 200 mL (t ratio = -2.06, p = 0.04) and 400 mL (t ratio = -3.04, p < 0.003) root treatments showed negative feedback for *E. Canadensis* growth in sterile field soils. Conversely, a net gain in biomass was shown in the contrasts of feedback ratios at all levels of root treatment for *B. curtipendula* (t ratio > 2.3, p < 0.02) and *S. scoparium* (t ratio > 7.7, p < 0.001).

Within each soil type, we evaluated the overall proportion of biomass of each species relative to the total biomass in each pot at each level of root addition treatment. The proportion of *B. inermis* biomass did not change under root treatments in live or sterile field soil. However, when comparing live and field soils, the proportion of *B. inermis* biomass was reduced in the

0 mL (t ratio = -3.15, p = 0.047), 100 mL (t ratio = -5.44, p < 0.001), 200 mL (t ratio = -4.64, p < 0.001), and 400 mL (t ratio = -4.37, p = 0.001) levels of root treatment (Fig. 4a). No difference in proportion of biomass was detected for *E. canadensis* at each level of root treatment within or between live or sterile field soil types (Fig. 4b). Within live and sterile field soils, the proportion of *B. curtipendula* biomass did not differ at any level of root treatment. However, the proportion of *B. curtipendula* biomass increased in live compared to sterilized soil at the 0 mL (t ratio = 4.74, p < 0.001) and 100 mL (t ratio = 3.76, p < 0.01) root-treatment levels (Fig. 4c). No difference was observed in the proportion of *S. scoparium* within live or sterile soils at each level of root treatment. We found an increase in the proportion of *S. scoparium* biomass from the live soil group when compared to sterile soils at the 0 mL (t ratio = 5.68, p < 0.001), 100 mL (t ratio = 5.43, p < 0.001), and 400 mL (t ratio = 6.46, p < 0.001) levels of root treatment (Fig. 4d).

Discussion

The conversion of grasslands in the United States to eastern redcedar woodlands has slowed in areas with active management but is generally increasing across its range (Miller et al. 2017; Filippelli et al. 2020). We found redcedar root material in the soil reduced the overall productivity of an experimental grass community. In this experiment, we added root biomass to three different soil types and observed an overall reduction in biomass with increasing root addition. It should be noted that roots were not sterilized

prior to use in this experiment. They were washed thoroughly, but an extremely small amount of soil microbes likely remained and were transferred into experimental pots. Feedback experiments that use very small volumes of microbial inocula typically have a delayed effect on plant growth, due to the time required for soil microbial communities to build up (Kulmatiski and Kardol 2008). There is evidence the soil microbial community of redcedar soils can suppress the growth of grasses (Bennion and Ward 2022). In the case of redcedar, we suspect secondary metabolites are being released from roots into surrounding soil and persist long after the removal of stem material and plant death. Secondary metabolites exuded into soils from roots or litter can have allelopathic effects that range in effect from dampening positive microbial feedbacks to strongly suppressing the growth of certain species (Bennett and Klironomos 2019). Allelochemicals disrupt the function of plants by inhibiting their associated fungal communities or through direct phytotoxicity (Inderjit et al. 2011). The allelopathic effect of secondary metabolites may only exist when they are actively being exuded into the soils or can persist long after the source has been removed (Del Fabbro and Prati 2015). The inhibition of growth in prairie grass communities by redcedar soils and roots could make formerly occupied sites more susceptible to re-establishing redcedars.

When assessing differences in biomass (Figs. 1 and 2) or feedback ratio (Fig. 3), the suppressive effect of redcedar roots was observed for all species in the experimental plant community in untreated field soil. This is an important observation because this substrate is a reasonable representation of the type of soil environment that newly recruited grasses may encounter following the removal of redcedar. We did not detect a difference in the proportion of biomass represented by each species with increasing levels of roots, although the proportions differed between live and sterile soil types. This indicates that the effects of roots were not disproportionately affecting any single species more than another. Thus, there were no changes in the dominance structure of the community due to treatment.

We propose that roots remaining in the ground following removal of aboveground biomass could contribute to negative ecological feedbacks that promotes the return to a redcedar-dominated system. The phenomenon of organic materials such as roots or litter inducing hysteresis in systems for years following treatment has been well-documented (Grove et al. 2012; Reynolds et al. 2017). If the effect we observed experimentally exists in field conditions, then legacy effects of redcedar roots could be considered a mechanism that favors the

re-establishment of the species in treated areas. The strength of the suppressive effect increased at higher levels of root addition. In field conditions, redcedar roots are fibrous and widespread near the surface of soils (Burns 1990; Hiziroglu and Zhang 2010), and exist in the rooting zone of newly establishing grasses (Hamati 2022). Therefore, areas with a particularly high concentration of redcedar roots may inhibit grass growth enough to provide pockets of refugia suitable for redcedar establishment. To be clear, redcedar-laden soils will not eliminate grass establishment, but their suppressive strength may provide vegetation gaps to favor their return.

The outcomes of recent case studies in redcedar treatments align with our proposed mechanism of legacy effects derived from redcedar roots (e.g. Bielski et al. 2021; Fogarty et al. 2021). For example, Bielski et al. (2021) found the use of intense prescribed fire can result in high mortality of redcedars and the temporary restoration of a diverse and productive grassland community. These outcomes are short-lived, with redcedar re-establishing within one to two years following treatment (Fogarty et al. 2021). In the absence of seed limitation, we suggest the legacy effect we observed in this experiment could represent a key factor in the rate of

re-establishment. The observed window for redcedar re-establishing following treatment in Fogarty et al. (2021) is short enough (1–2 years) that even fine redcedar root materials should remain in the soil matrix. This means that the allelopathic effect of redcedar roots will likely persist long enough for seedlings to establish. It should be noted that there is no evidence of self-suppression for redcedars when growing in soil previously occupied by redcedar (Bennion, unpublished data).

Our findings indicate the impact of redcedar on the plant community persists following its removal. The legacy effect of redcedar is an example of a mechanism of encroachment that is not readily apparent but may have a significant impact on the success of the species. Further studies could evaluate the strength of this mechanism in field conditions through experimental removal of redcedars and monitoring the growth of establishing species.

Declarations

AUTHOR CONTRIBUTIONS

LDB conceived and designed the experiment, collected data, performed analysis, and led the writing of the manuscript. DW provided funding, advised on experimental design, and provided critical revisions to drafts of the manuscript.

ACKNOWLEDGMENTS

We thank Christian Combs for technical assistance, and Rachel Lasso for technical support. We thank the Herrick Foundation and the NSF-DEB 402109 for financial support.

CONFLICT OF INTEREST

None declared

DATA AVAILABILITY

The data collected in this experiment is archived in the Open Access Kent State (OAKS) repository (DOI TBD)

ETHICAL APPROVAL

This article does not contain any studies with human participants or animals performed by any

of the authors.

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Figures

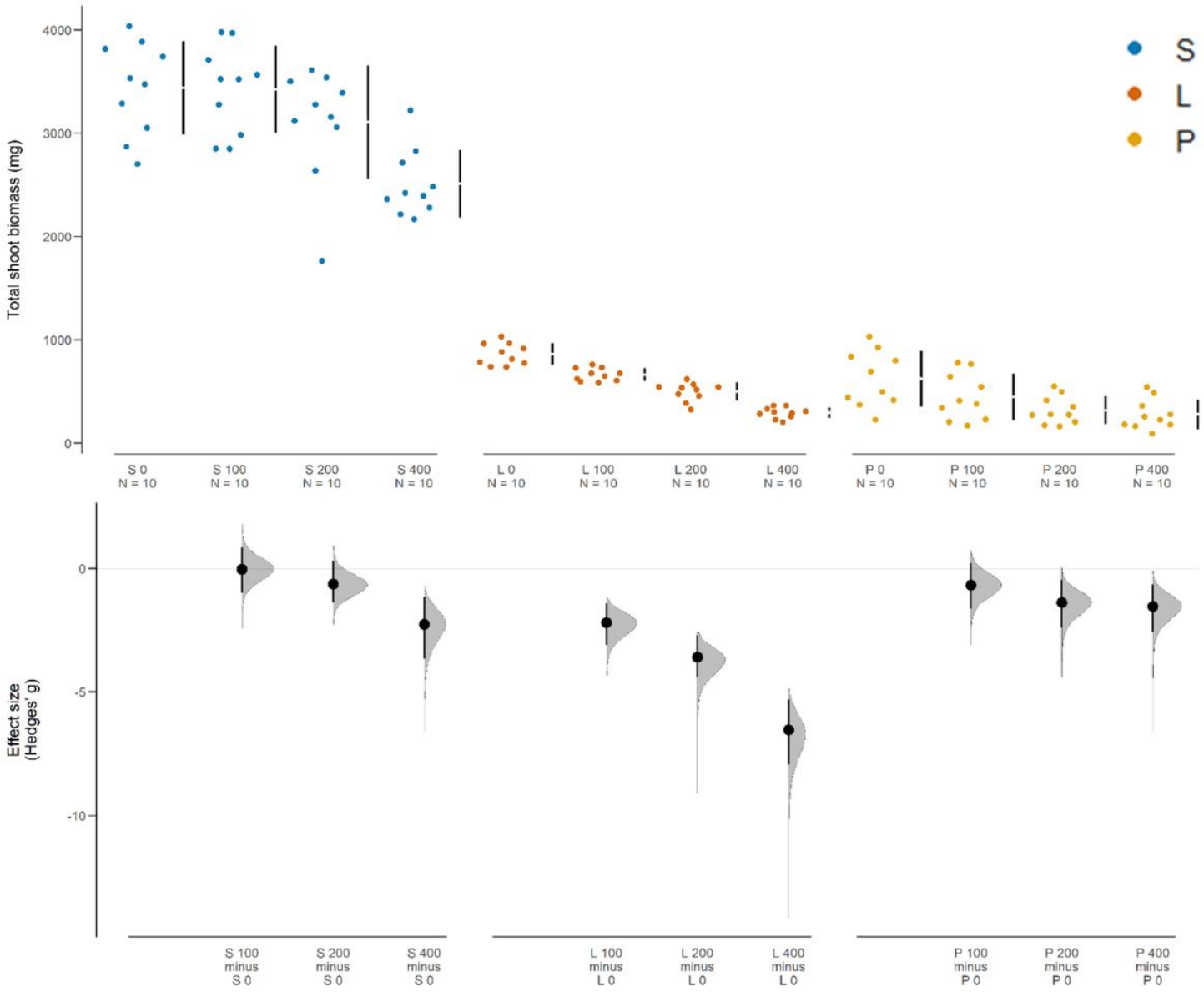

Figure 1

Cummings estimation plots (Ho et al. 2019) that compare the total shoot biomass of grasses grown in redcedar field or potting soils with varying levels of redcedar root addition (0, 100, 200, or 400 mL). Within sterile (S), live (L), or potting (P) soils, contrasts are between the 0 mL and the 100-, 200-, and 400-mL levels of root addition. The top panel shows the raw data (colored dots) and the mean and standard deviation for each group (vertical gapped lines). The bottom panel shows the Hedges' *g* effect size (black dot) and 95% confidence interval (vertical bar) for each pair of contrasts (listed on the x-axis). The grey filled-in curves represent the sampling distribution of the 95% confidence interval.

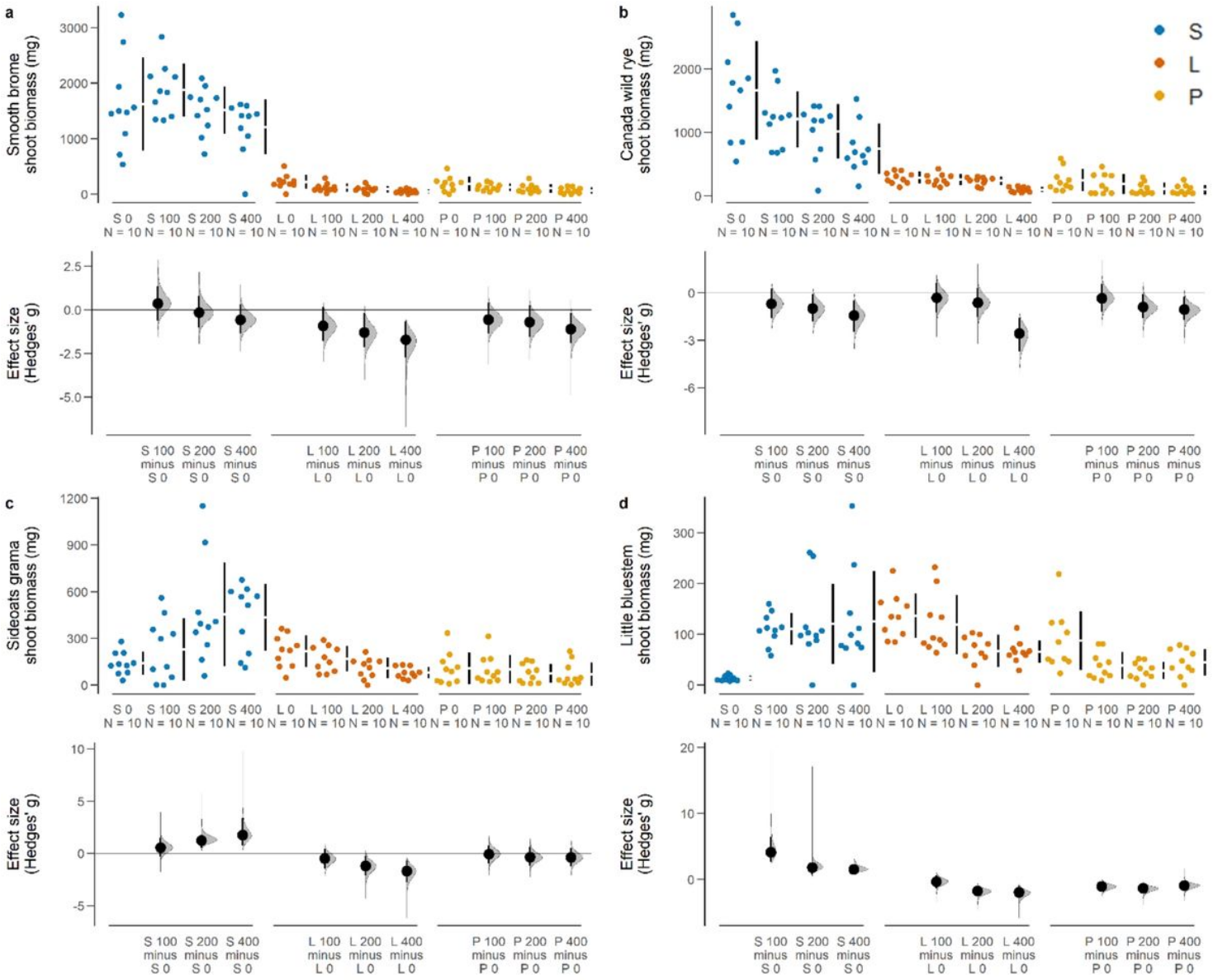

Figure 2

Cummings estimation plots that compare shoot biomass in control conditions (0 mL redcedar roots added) with each other level of redcedar root addition (100, 200, or 400 mL). Comparisons are grouped by soil type, either potting (P), sterile (S) or live (L), and species: (a) *Bromus inermis*, (b) *Elymus canadensis*, (c) *Bouteloua curtipendula*, or (d) *Schizachyrium scoparium*. The top panel in each plot shows the raw data (colored dots) and the mean and standard deviation for each group (vertical gapped lines). The bottom panel shows the Hedges' *g* effect size (black dot) and 95% confidence interval (vertical bar) for each pair of contrasts (listed on the x-axis). The grey filled in curves represent the sampling distribution of the 95% confidence interval.

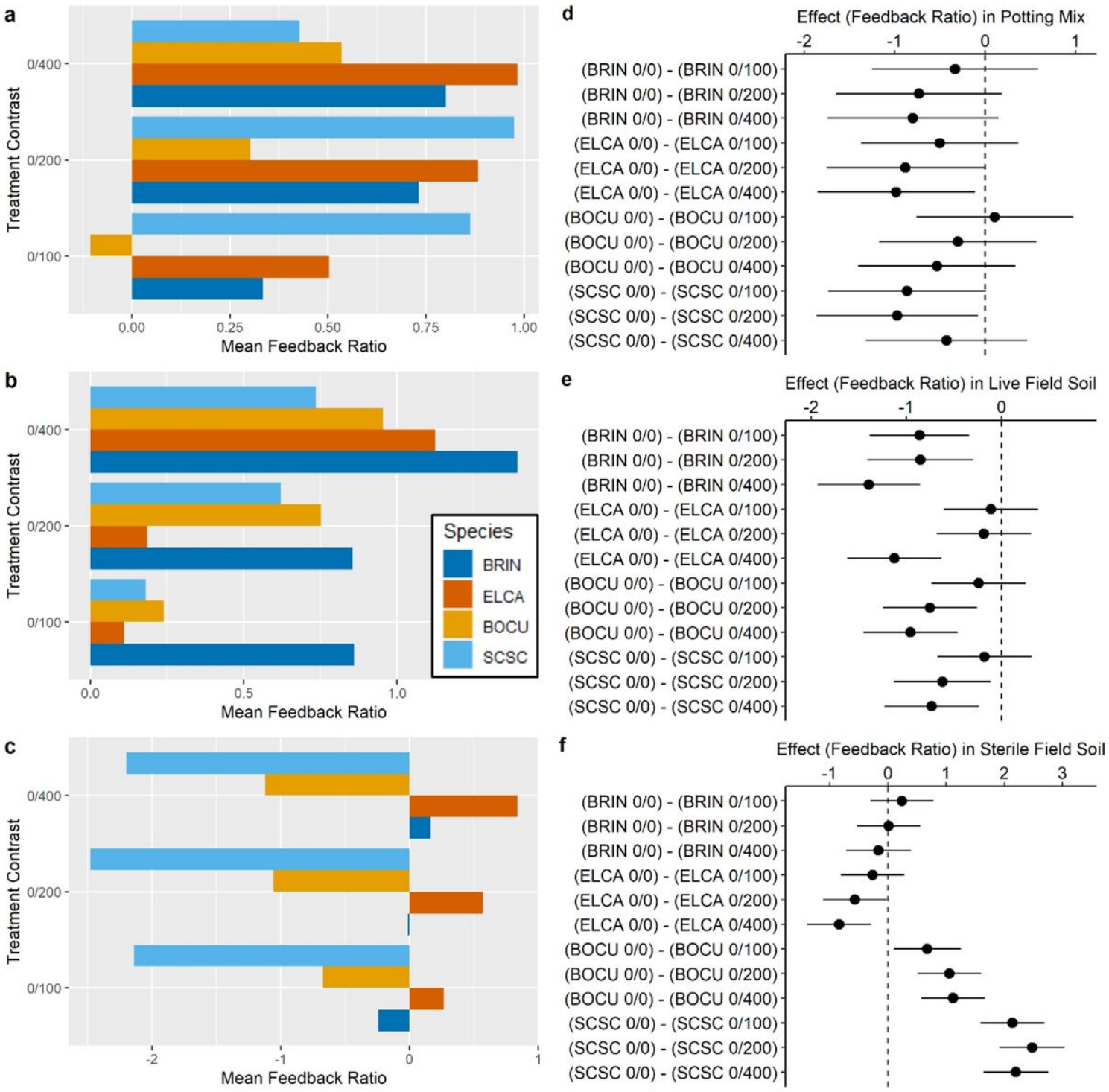

Figure 3

The mean feedback ratios for *Bromus inermis* (BRIN), *Elymus canadensis*(ELCA), *Bouteloua curtipendula* (BOCU), and *Schizachyrium scoparium* (SCSC) in (a) potting and (b) live or (c) sterile field soils. In each figure (a-c), the y-axis shows which contrast was calculated (0/100, 0/200, or 0/400) and the x-axis shows the mean feedback ratio. Positive values indicate a reduction in growth with root addition. The effects plots (d-f) show the difference in the estimated marginal mean feedback ratio between the control

(0/0) and the three levels of root addition (0/100, 0/200, or 0/400). In these plots (d-f), negative values indicate a reduction in biomass.

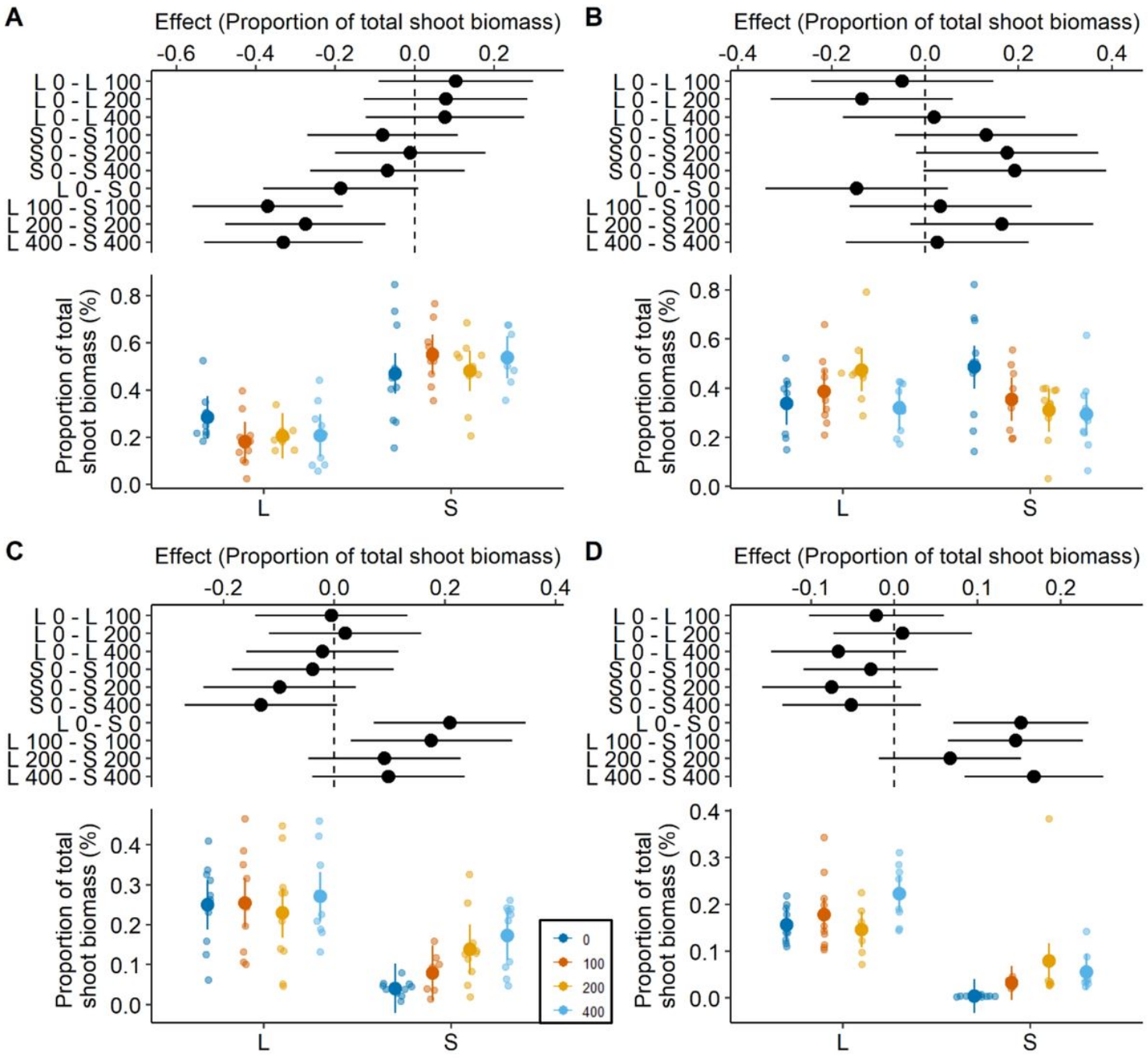

Figure 4

The proportion of total biomass for (A) *Bromus inermis*, (B) *Elymus canadensis*, (C) *Bouteloua curtipendula*, and (D) *Schizachyrium scoparium* in various treatments. The top panel in each figure shows the effects size contrasted between treatments within live (L) or sterile (S) soil types at each level of redcedar root addition (0, 100, 200, or 400 mL). The bottom panel show the raw data (dots) for the proportion of the shoot biomass of individual species relative to the total shoot biomass in each pot. The larger dot shows the mean and vertical bar shows the standard deviation

Supplementary Files

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