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THE EFFECTS OF LEAF LITTER ON GERMINATION IN THE SERPENTINE ENDEMIC *BOECHERA CONSTANCEI*

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ABSTRACT

Leaf litter accumulation may negatively impact seedling germination by altering key ecosystem properties, such as light availability and soil moisture. The impacts of litter depth may be particularly strong for species in dry environments with low vegetative cover and high light availability. The rare species *Boechera constancei* (Rollins) Al-Shehbaz (Brassicaceae) is endemic to the serpentine outcrops of Plumas and Sierra Counties, where litter accumulation is highly variable and *B. constancei* inhabits a range of litter depths (0–40 mm). In this study, we tested whether increased litter depth impedes germination, and whether populations vary in tolerance to litter depth. Specifically, we quantified the impacts of treatments representing the observed range of litter depth occupied by *B. constancei* on the germination of seed collected from nine populations. Differences in germination were analyzed using a two-way ANOVA. While both source population and litter treatment influenced germination, the interaction term was not significant. Post-hoc Tukey's honest significance tests revealed that germination under both median and high litter treatments was significantly lower than under no litter. The median and high litter treatments did not differ significantly. Differences in germination were observed during dark, cold stratification, suggesting that light availability was not a factor. These results indicate that early life stages of this species may benefit from litter reduction treatments, as both median and high litter treatments reduced germination.

Key Words: *Arabis constancei*, *Boechera constancei,* endemic, germination, leaf litter, management, rare, serpentine.

Plant life histories are shaped by abiotic and biotic influences from the environment. Litter (dead plant material of a small size that is loose on the ground) can facilitate or impede plant growth, by altering temperature, moisture, and light availability (Facelli and Pickett 1991, Baskin and Baskin 1998), and can affect life history stages differently (Muturi et al. 2017). The amount of litter accumulation in an ecosystem determines key properties and functions such as light availability, microbial community composition, and nutrient cycling rates (Sayer 2006). The chemical composition of water and soil is also influenced by litter type and depth. Litter accumulation is typically low on poor nutrient soils, such as serpentine, as plant productivity is lower than on more fertile soils (McNaughton 1968, Gulmon 1992). Therefore an increase in historical litter accumulation may influence the ecology of plants that inhabit these low productivity soils.

Serpentine soils are derived from ultramafic serpentinite and peridotite parent rock material, and comprise over 6000 km^2 of the state of California (Safford et al. 2005). These soil types are low in essential plant nutrients such as calcium, potassium, phosphorus, and nitrogen, and are rich in heavy metals such as magnesium and iron. In addition, toxic trace elements such as nickel, cobalt, and chromium are often present in these soils (Safford et al. 2005). This combination results in harsh conditions for plant growth, and few plants are able to tolerate these soil types. These inhospitable soil conditions result in a high degree of plant species endemism (Kruckeberg 1954). Harsh environments like serpentine soils typically have sparse vegetative cover, and a low amount of litter accumulation on the soil surface (Anacker et al. 2012).

In this study we examine the effects of litter on germination in populations of the rare serpentine endemic plant species *Boechera constancei* (Rollins) Al-Shehbaz (Brassicaceae) (Fig. 1). *Boechera constancei* (formerly *Arabis constancei* Rollins) is a strict serpentine endemic, meaning that is found growing on serpentine soils over 95% of the time (Safford et al. 2005). This rare perennial mustard species is ranked as 1B.1 on the California Rare Plant Rank list, as it is considered "Seriously endangered in California" (CNPS, 2018). *Boechera constancei* populations are restricted to serpentine rocky outcrops in northeastern California (Plumas, Sierra, Lassen, and Nevada Counties). Fire suppression practices in the Sierra Nevada mountain range have caused forests in these areas to accumulate more litter than was historically present (Stephens et al. 2012). Populations of *B. constancei* occur predominately on open

FIG. 1. Photos of *Boechera constancei*. (a) Seedlings in the no litter (control) treatments (photo by Alexander C. Yang), (b) rosette in median litter conditions in Plumas National Forest, and (c) flowering individuals in Plumas National Forest. Photos b and c by Erica J. Case.

rocky outcrops, but can also be found in the nearby mixed conifer forest understory (Case and Harrison 2017). Litter accumulation is highly variable on the serpentine outcrops of Plumas National Forest, and *B. constancei* has been observed to inhabit areas with litter depths ranging from 0–40 mm (Case and Harrison 2017).

Populations of *Boechera* exist in relatively undisturbed environments that have existed for thousands of years (Brunelle et al. 2005). Due to this history, ecologically important genetic polymorphisms have been found to occur across populations of *Boechera* species (Rushworth et al. 2011). In addition, the genus *Boechera* is predominantly self-pollinating, and most species are diploid and reproduce sexually (Schranz et al. 2005, Rushworth et al. 2011). Therefore, we expect to see a high degree of population differentiation with respect to functional traits in this species, including germination. We predict that we might also see differential responses of populations to abiotic or biotic conditions, such as the presence or depth of litter.

Litter alters the physical and chemical environment on the soil surface, and can impact the demography of plant populations (Facelli and Pickett 1991). Litter presence can inhibit or facilitate seedling emergence, and can have different influences across life history stages in individual plants (Li et al. 2016). Genotype by environment interactions can be strong in some species, and ecotype differentiation in germination among populations has been shown in common garden experiments (Rathcke and Lacey 1985, Facelli and Pickett 1991). Because there is high variability in the litter layer depth between sites where *B. constancei* occurs, and what is known about *Boechera* genetics, it is possible that populations of

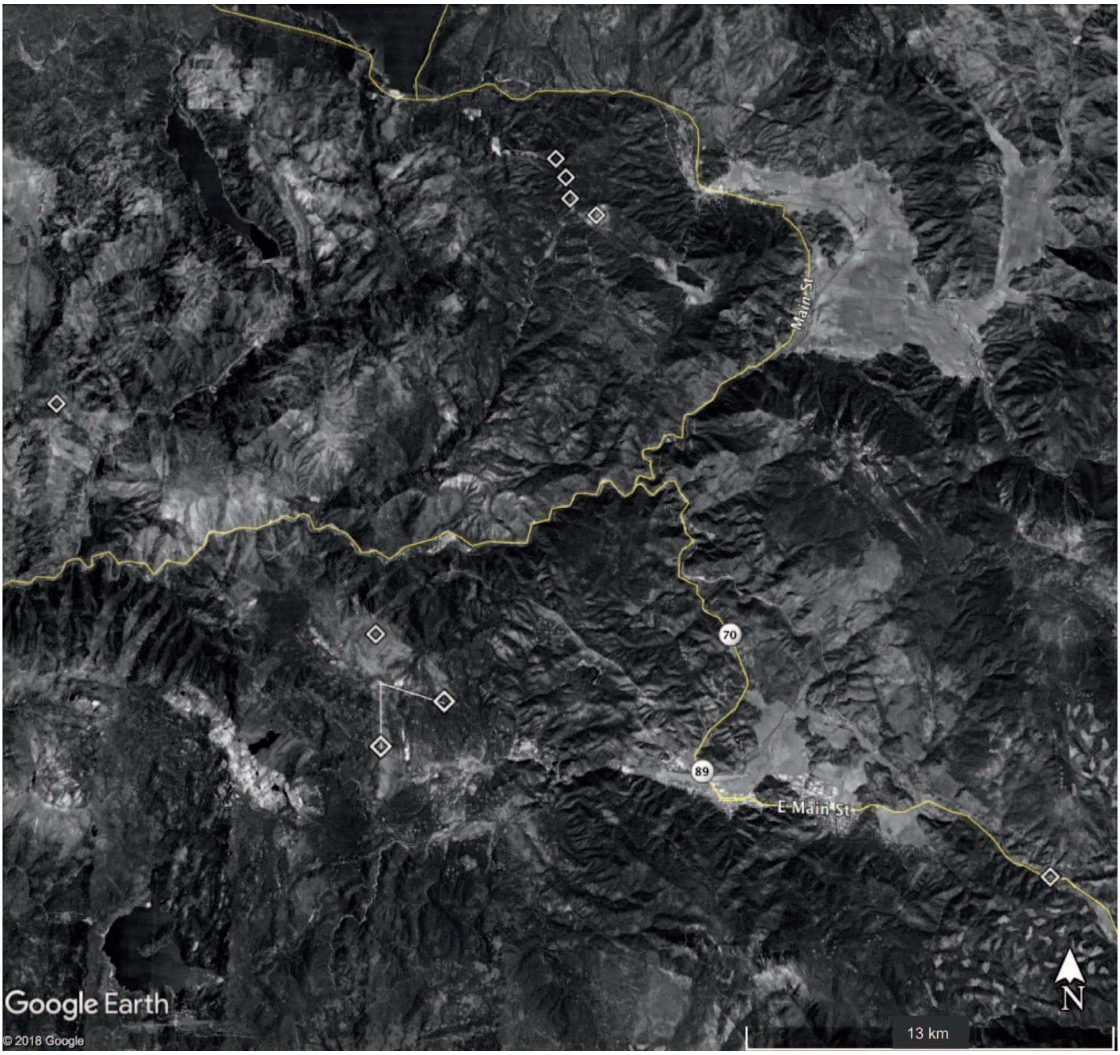

FIG. 2. Map of *Boechera constancei* populations in Plumas Co. White diamonds indicate population locations (n = 9) (Imagery: Google Earth 2017).

this species may be differently adapted in their tolerance to litter depth.

This study examines whether increased litter depth impedes germination of *B. constancei*, and whether populations of this species vary in their tolerance to litter depth. Specifically, we test the following hypotheses: H1: Increased litter will negatively impact the proportion of germination in *B. constancei*. H2: Populations of *B. constancei* from areas with higher litter accumulation will show greater germination in high litter treatments, due to being locally adapted to these conditions.

METHODS

Nine populations of *Boechera constancei* from Plumas National Forest were selected for this study. Populations were defined as groups of *B. constancei* individuals that were at least 150 m apart, and up to 40 km from each other (Fig. 2). Sixty populations were visited in total, and seed collection was limited to sites with more than 50 fruiting individuals. Populations used for this study were selected to span the geographical range of species as well as the range of observed litter depths. Seeds were collected from between five and nine individuals per population. No more than 10% of available seed was collected from the population in order to ensure minimal impact to plant populations, and in compliance with our collection permits.

Seed mixes of 240 seeds were then created for each population, and split into 12 replicates of 20 seedlings each. Each replicate was then weighed and assigned to one of three litter depth treatments. Seeds were cleaned in a 20% bleach and 80% deionized (DI) water solution for 20 minutes, and

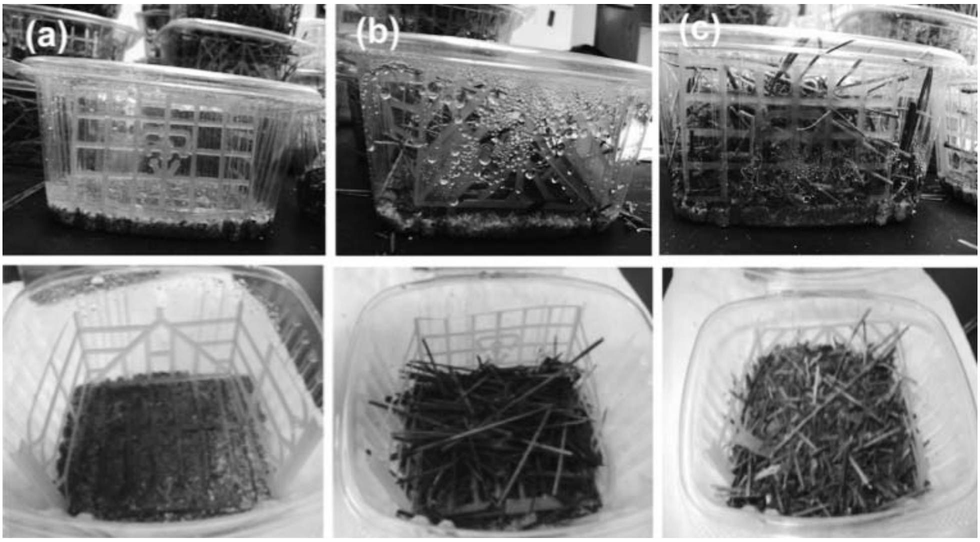

FIG. 3. Litter weight treatments. Shown are two stacked images of each of the following treatments: (a) no litter control, (b) median litter treatment (8 g), and (c) high litter treatment (27 g).

then rinsed with 100% DI water twice before experimental treatments were administered.

Litter treatments were based on field observations of the median (12 mm), high (38 mm) and low (0 mm) litter depths in the areas where *B. constancei* populations occur in Plumas National Forest. To more precisely control the amount of litter in each treatment, litter depths were converted to litter weights by multiplying by average litter density (Case, unpublished data). Specific weights associated with the litter treatments were 27 g for high (38 mm) litter depth observed, 8 g for the average (12 mm) litter depth observed, and 0 g for no litter (control) (Fig. 3). Leaf litter was collected from areas where *B. constancei* populations were found in Plumas National Forest, and consisted mainly of needles and scales fallen from Jeffery pine (*Pinus jeffreyi* Grev. & Balf.*)*, Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco var. *menziesii*), incense cedar (*Calocedrus decurrens* (Torr.) Florin), sugar pine (*P. lambertiana* Douglas), and Ponderosa pine (*P. ponderosa* Douglas ex. Lawson & C.Lawson). There were four replicates of each litter treatment per population. Treatments were organized in a randomized block design, with 108 total samples, (9 populations x 3 litter treatments x 4 replicates each treatment = 108). This study was conducted at the University of California, Davis.

Previous studies established that *Arabis* spp. (now *Boechera*) seeds require cold stratification at 5°C, for simulation of winter conditions, in order to germinate effectively (Baskin and Baskin 2002; Bloom et al. 1990). We determined via a pilot study on *B. constancei*, that at least 52 days of cold stratification at 5°C produced the highest amount of germination (Olliff-Yang, unpublished data). The replicates were initially put into plastic containers on wetted germination paper and placed into cold stratification (5°C) in an unlit germination chamber, to simulate winter conditions. The seeds remained in this dark cold stratification for nine weeks (from 8 December 2011–15 February 2012)). On 15 February 2012, the seeds were moved into containers with wet sand. Seeds were placed on top of the sand. Litter treatments were administered at this time. Dried litter was weighed and added to plastic mesh baskets (made from half pint strawberry containers) and placed on top of the seeds (Fig. 3). The mesh baskets were used to remove the litter for watering and to count germination during the study. Control treatments included these baskets as well. Seeds were then put back into dark cold stratification for 23 more days, and then moved into a greenhouse for two weeks. Throughout the experiment, treatments were checked for fungal growth, and when fungal hyphae were observed, seeds were moved into a new treatment container with fresh sand and litter.

Germinated individuals were counted on Day 0, Day 16, and Day 23 of the experiment during dark and wet cold stratification, on Day 27 (after four days in the greenhouse, exposed to light) and on Day 35 (after 12 days in the greenhouse, exposed to light). A seed was considered to have germinated if the radicle had emerged. The development of cotyledons on germinants was also noted. Differences in proportions of germinated seed by litter treatment and population were then analyzed using a two-way ANOVA, after testing to ensure variables met normality assumptions. Post-hoc Tukey's Honest Significant Difference (HSD) tests were done to determine where differences occurred. All analyses were performed in JMP (JMP, Version 9, SAS Institute Inc., Cary, NC).

TABLE 1. EFFECTS OF LEAF LITTER AND SOURCE POPULATION ON GERMINATION. Analysis of variance table of germination by population, litter weight (in grams), and the interaction between population and litter weight. Asterisks indicate significance level: * = $P \leq 0.05$, ** = $P \leq 0.01$, and *** = $P \leq 0.001$. 'NS' (not significant) is indicated where $P > 0.05$.

Effect	df	Sum of squares	Mean square	F-ratio	P-value
Population	8	3.092	0.3865	10.874	***
Litter weight (g)	2	0.658	0.6575	18.500	***
Pop x Litter	8	0.356	0.0445	1.253	NS
Residual Error	90	3.199	0.0355		

RESULTS

Litter weight had a significant negative impact on *Boechera constancei* germination ($P < 0.001$, Table 1, Figs. 4 and 5). The germination proportion per replicate of *B. constancei* seeds, as well as the total number of germinants, was significantly reduced in both litter-addition treatments compared to the control treatment (Table 1, Figs. 4 and 5). Post-hoc Tukey's HSD tests revealed that germination under both 8 g and 27 g of litter were significantly lower than under no litter (Fig. 5, $P < 0.001$). However, both the median and high litter weight had similar effects, and there was no significant difference between the median and high litter treatments ($P = 0.97$, Fig. 5).

Source population had no significant effect on the germination litter relationship. The population that *B. constancei* seeds were collected from did significantly impact germination success, ($P < 0.001$, Table 1 and Fig. 6). However, while both population ($P < 0.001$, Table 1) and litter ($P < 0.001$, Table 1) treatments influenced germination, the interaction term was not significant ($P = 0.278$, Table 1). This reveals that germination success in populations of *B. constancei* does not differ in tolerance to litter weight.

Litter treatments reduced the germination of *B. constancei* in dark cold stratification, suggesting that light availability was not a factor in germination inhibition. Germinationand number of total germinants was reduced under litter while *B. constancei*

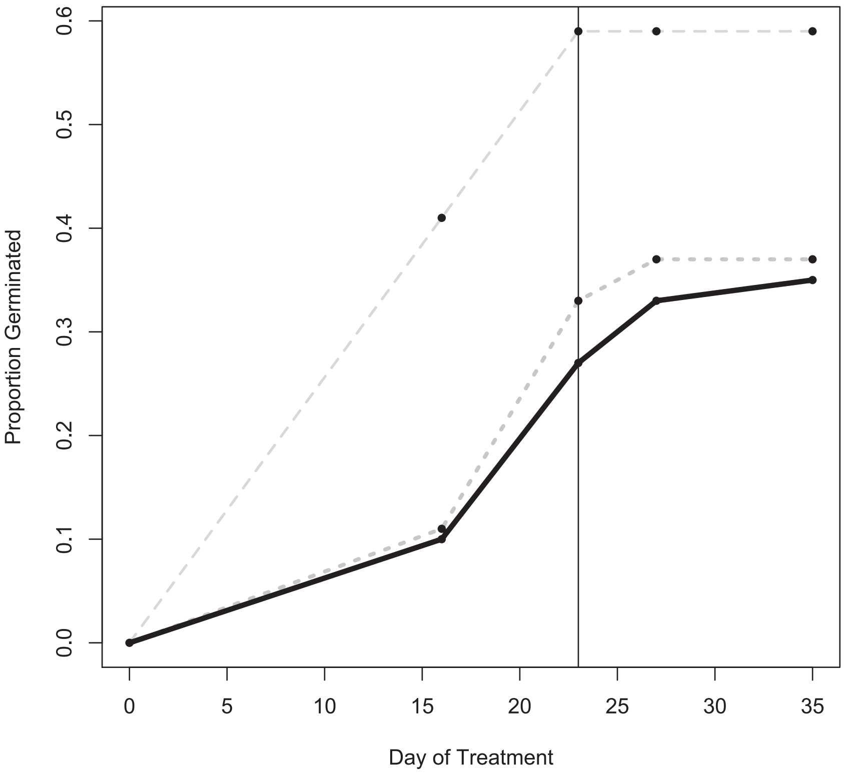

FIG. 4. Mean germination in each treatment over time. Mean proportion of germinated seeds per replicate during the experiment in 0 grams of litter (long dashed light grey line), 8 grams of litter (dotted dark grey line), and 27 grams of litter (solid black line). All replicates were in cold stratification from Day 0 though Day 23 (indicated with a vertical line) of the experiment.

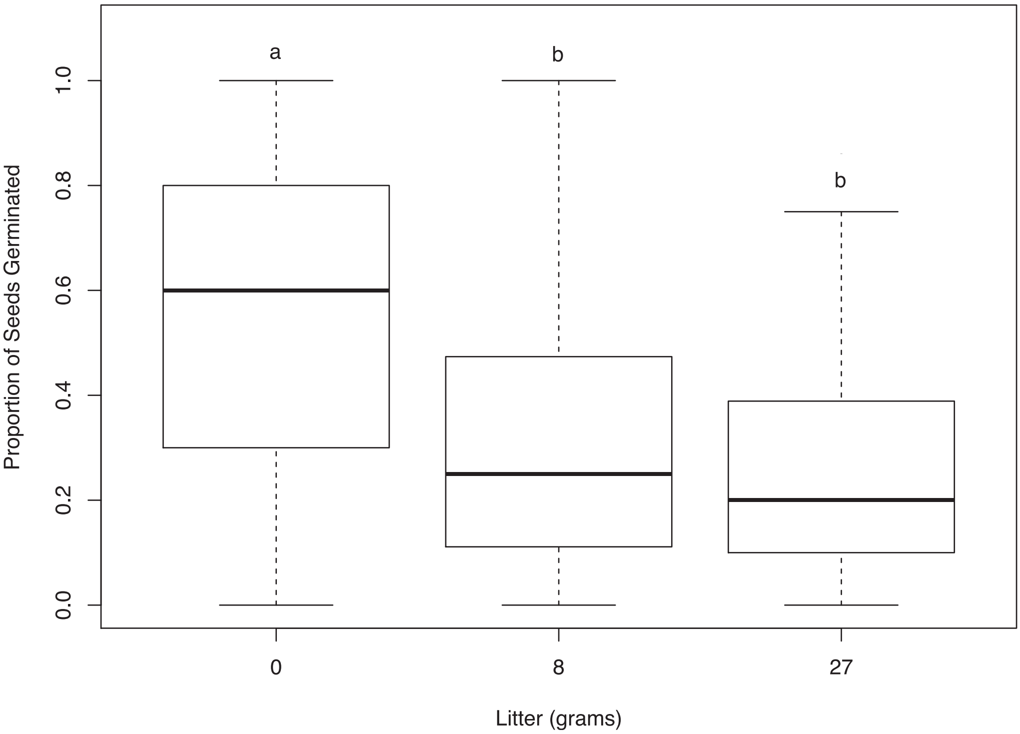

FIG. 5. Proportion of germination by treatment. Proportion of total seedlings germinated per replicate under each treatment, 0 grams of litter (no litter control), 8 grams of litter (median), and 27 grams of litter (high). Midline indicates the median germination, the box indicates the middle 50% of the data, and whiskers show the upper and lower quartiles of the data. Different letters above whiskers indicate significant differences in mean germination proportion between treatments after Bonferroni correction.

seeds were still in cold dark conditions (Days 0–23, Fig. 4). Once moved to lit conditions, there was higher germination under litter treatments than control treatment (Days 24–30, Fig 4). Germination under all litter treatments ceased after 30 days of the experiment (Fig. 4).

DISCUSSION

The germination of *Boechera constancei* was strongly inhibited by the presence of leaf litter, and populations were all similarly impacted (Pop x Litter interaction NS, Table 1). Both the median and maximum litter treatments had lower germination than the control treatment, indicating that *B. constancei* populations could experience reduced germination due to litter accumulation. This finding suggests that litter reduction treatments (such as prescribed burning or manual litter removal) may benefit this species. However, this does not necessarily scale up to the population level, as a recent study reports that *B. constancei* population demography from these and other nearby sites were unaffected by prescribed and natural fires (Case and Harrison 2017). This may indicate demographic differences in the effects of litter on different life stages.

Litter affected *Boechera constancei* germination in dark conditions, revealing that light availability was not the main cause of reduced germination. This suggests that there may be a chemical component to germination inhibition from litter presence. Germination may have been inhibited by tannins or acid from the litter, both of which have been found to delay and inhibit germination (Rice 1979; Kuiters 1989). Once moved to lit conditions, there was higher germination under litter treatments than the control treatment (Fig. 4), but this is most likely due to a chemical component in the litter delaying germination of seeds. Light availability was not a treatment, so we cannot say whether the effects of litter would be different in fully dark or fully light conditions throughout the experiment. We suggest a germination study with dark and light condition treatments to tease apart this question.

Other possible explanations for inhibition of germination include moisture or thermal differences, or differences in fungal growth between the litter and non-litter treatments. Slight temperature or moisture differences may have made a difference between litter and non-litter treatments, as leaf litter buffers temperature (Evans and Young 1970; Facelli and Pickett 1991) and can slow evaporation (Williams et

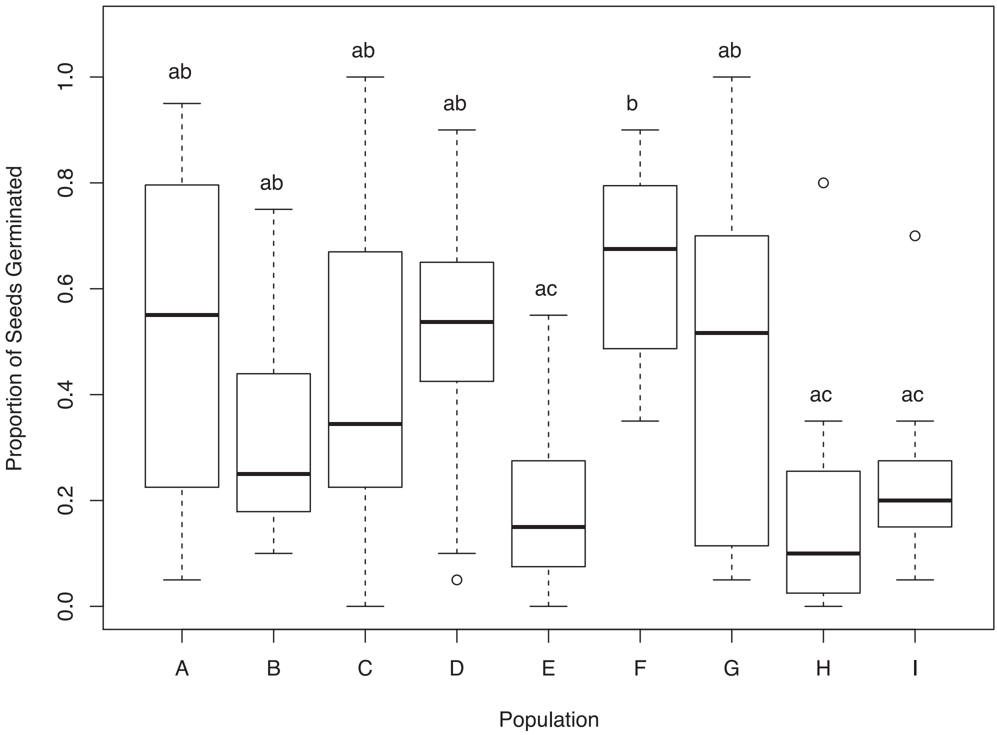

FIG. 6. Germination by population. Mean proportion of seeds germinated compared across populations. Letters correspond to latitude and are arranged in alphabetical order from A, the most southern population (39.91231 N) to I, the most northern population (40.15036 N). Midline indicates the median germination, the box indicates the middle 50% of the data, and whiskers show the upper and lower quartiles of the data. Distinct lettering above the whiskers indicates significant differences in mean germination between populations after Bonferroni correction.

al. 1990). In addition, while we took precautions to reduce fungal growth on the seeds (washing the seeds, drying leaf litter, and moving seeds when fungus was present), litter-addition increased the prevalence of fungal growth in this experiment (Olliff-Yang, unpublished data), which may have contributed to the decrease in germination under the litter treatments. It is unclear whether the same fungal growth under litter occurs in the field. We suggest additional studies manipulating specific abiotic (light, temperature, chemical exudates) and biotic (fungal growth) variables associated with litter accumulation to identify the mechanism(s) by which litter inhibits germination in this species.

There were significant differences among populations in proportion germination (Table 1, Fig. 6), which is consistent with other studies on germination (Baskin and Baskin 1998). We expected to see some difference among populations of *B. constancei*, as this genus is estimated to have lived in relatively undisturbed environments for thousands of years (Brunelle et al. 2005), and because *Boechera* is predominantly self-pollinating (Schranz et al. 2005, Rushworth et al. 2011). However, source population had no significant effect on the germination litter relationship (Pop x Litter interaction NS, Table 1). Therefore, germination differences detected between populations may be due to local adaptation to other biotic or abiotic factors in the environment. Variation in germination response between populations can occur due to different temperature optima (McArthur et al. 1987). For example, populations of Australian *Cardamine* species vary in their temperature requirement for germination across an elevational gradient (Thurling 1966). Differences in germination at low temperatures can also vary across a species range, as seen in rabbitbrush (*Chrysothamnus nauseosus* (Pursh) G.L.Nesom & G.I.Baird) where germination at low temperatures decreases in populations from cooler higher elevation areas, likely due to differential risks associated with germination in cold temperatures (McArthur et al. 1987). Seeds from different populations have also been observed to vary in sensitivity to soil moisture (*Abies cephalonica* Loudon, Fady 1992), pH and calcium (*Euphorbia thymifolia* Pursh, Ramakrishnan 1965), and salinity (*Dactyloctenium aegyptium* (L.) K.Richt., Okusanya and Sonaike 1991). Differences may also simply be due to variation in seed dormancy proportions between populations (Andersson and Milberg 1998).

Germination is just one stage in the plant life cycle, and therefore inhibition of germination does not directly indicate population decline. When germination is reduced, other vital life history stages (survival, growth, reproduction) may increase, compensating for the decline, and yielding little or no difference to the overall population growth rate. For example, litter depth may inhibit germination, while at the same time enhance seedling growth and survival, as found in dry woodland species in Africa (Muturi et al. 2017). Opposing influence on different vital rates, also known as demographic compensation (Villellas et al. 2015), may be the reason that litter is shown here to impact germination, while prescribed fire was not shown to affect overall population growth rates in full demographic models (Case and Harrison 2017). However, litter depth did not significantly affect transition rates between plant size classes or variation in reproduction in full demographic models (Case and Harrison 2017).

Litter weight had a negative effect on *B. constancei* germination regardless of population (Table 1, Fig. 5). Litter reduction, such as prescribed fires, may therefore improve the germination success of this species. While overall population demographic rates are not affected by fire (Case and Harrison 2017), the establishment of new populations may be affected, as our results suggest that germination is inhibited by litter depth. Additionally, litter depth should be considered in conservation planning, as germination will be essential for success if management actions include planting or moving seed. Findings from this study have been presented to Plumas National Forest, and results were used in the creation of demographic models for this species (Case and Harrison 2017).

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