

Germination and seedling establishment of two annual grasses on lichen-dominated biological soil crusts

Lynell Deines · Roger Rosentreter ·
David J. Eldridge · Marcelo D. Serpe

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Abstract Biological soil crusts dominated by lichens are common components of shrub-steppe ecosystems in northwestern US. We conducted growth chamber experiments to investigate the effects of these crusts on seed germination and initial seedling establishment of two annual grasses; the highly invasive exotic *Bromus tectorum* L. and the native *Vulpia microstachys* Nutt. We recorded germination time courses on bare soil and two types of biological soil crusts; one composed predominantly of the lichen *Diploschistes muscorum* (Scop.) R. Sant. (lichen crust) and the other comprised of an assortment of lichens and mosses (mixed crust). Final germination on the lichen crust for both grass species was about a third of that on the bare soil surface. Mean germination time (MGT) was 3–4 days longer on the lichen crust compared with the bare soil. In

contrast, there was no difference in germination percentage or MGT between the mixed crust and bare soil, and results were similar for both grass species. For both species, root penetration of germinating seeds on the lichen crust was lower than on the bare soil or mixed crust surfaces. The combined effects of the lichen crust on germination and root penetration resulted in an overall reduction in seedling establishment of 78% for *V. microstachys* and 85% for *B. tectorum* relative to the bare soil treatment. Our results clearly demonstrate that lichen-dominated biological soil crust can inhibit germination and root penetration, but the extent of these effects depends on the composition of the crust.

Keywords *Bromus tectorum* · *Diploschistes muscorum* · Patchy vegetation · Root penetration · *Vulpia microstachys*

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L. Deines · M. D. Serpe (✉)
Department of Biology, Boise State University, 1910
University Drive, Boise, ID 83725, USA
e-mail: mserpe@boisestate.edu

R. Rosentreter
USDI Bureau of Land Management, 1387 S. Vinnell
Way, Boise, ID 83709, USA

D. J. Eldridge
School of Biological, Earth, and Environmental Sciences,
University of New South Wales, Sydney, NSW 2052,
Australia

Introduction

A combination of empirical evidence and theoretical predictions suggests that in arid and semiarid ecosystems, essential resources such as water, seeds, nutrients, and organic matter have a patchy distribution (Noy-Meir 1972; Tongway 1995). Resource-rich patches such as grass tussocks, shrub hummocks and groves of trees appear to act as sinks for essential resources derived from the intervening runoff patches or resource-shedding sections of the landscape

(Aguilar and Sala 1999; Peters et al. 2006). Regulation of the movement of resources from runoff patch to fertile patch is controlled by vegetation and geomorphology (Peters et al. 2006).

Spatial heterogeneity in arid and semiarid landscapes is partly attributed to the presence of biological soil crusts (Belnap et al. 2001; West 1990). These crusts are complex assemblages of mosses, lichens, liverworts, bacteria, fungi, and algae that form an intimate association with the soil surface and dominate the less vegetated interspaces between vascular plants (Eldridge and Greene 1994; Harper and Marble 1988). The crust organisms and their byproducts form a surface of soil particles bound together by organic material, often covering up to 70% of the surface area of drylands that are not cultivated nor dominated by sandy soils (Belnap 2003, 2006).

Biological soil crusts may contribute to the development of patchy vegetation patterns through an effect on seed germination and establishment. Biological soil crusts can influence the germination and establishment of vascular plants, but the particular effect varies among vascular plant species and the biological soil crust composition (Lesica and Shelly 1992; Prasse and Bornkamm 2000; Rivera-Aguilar et al. 2005; Serpe et al. 2006; Zaady et al. 1997). For example, St. Clair et al. (1984) observed significantly higher germination of three grass species on algal crusts compared with mixed crusts of lichens, mosses and algae. Similarly, Zamfir (2000) showed that seedling emergence was much lower on lichen mats compared with moss mats for three out of four species studied.

The effect of biological soil crusts on vascular plants may also be more indirect. Biological soil crusts affect water flow across the landscape and into the soil (Eldridge et al. 2002; West 1990; Williams et al. 1995). Hydrophobic crusts redistribute runoff water, while crusts with rough surfaces tend to retain more water compared with crust-free soil (Belnap 2006; Warren 2001). Crusts are also a source of organic carbon, and free-living cyanobacteria and some lichens fix atmospheric nitrogen (Evans and Belnap 1999; Forman and Dowden 1977; West 1990). Furthermore, biological soil crust provide habitat for soil microarthropods, which are important for decomposition and mineralization processes (West 1990). Through their various effects on soil microtopography and water and nutrient flow, crusts

may affect the distribution of resources in arid lands, which could contribute to the development of patchy vegetation patterns.

In western US, biological soil crusts are significant components of plant interspaces (Hilty et al. 2004; Rosentreter and Belnap 2001). Excessive livestock trampling and the invasion of exotic annual plants over the past century have, however, reduced crust cover and transformed relatively stable, diverse sagebrush steppe communities into more homogenous grasslands dominated by exotic annuals (Brooks et al. 2004; Mack 1981). In particular, grasslands dominated by cheatgrass (*Bromus tectorum* L.) now cover over 30 million ha of public lands (Knapp 1996). Cheatgrass litter creates a rather continuous and homogeneous layer of fine fuels, sufficient to drastically reduce fire-return intervals to as little as 5 years and eliminate the fire-sensitive native shrubs (Brooks et al. 2004; D'Antonio and Vitousek 1992; Whisenant 1990).

Cheatgrass tolerates disturbances, including livestock grazing and fire, better than many native plants, and competes successfully for resources with other vascular plants (Harris 1977; Melgoza et al. 1990). Cheatgrass is a prolific seeder, can germinate in most seasons, and the seed bank can remain viable for 5 years (Rice and Dyer 2001). Cheatgrass, however, may be less effective at invading areas with an intact biological soil crust (Kaltenecker et al. 1999). This notion is supported by field observations and growth chamber experiments that indicate that the presence of certain types of biological soil crusts decreases cheatgrass germination compared to bare soil (Larsen 1995; Serpe et al. 2006). In a recent study (Serpe et al. 2006), we have observed that a biological soil crust dominated by the short moss *Bryum argenteum* Hedw. significantly inhibited germination of cheatgrass and three perennial grasses. In contrast, a tall moss crust dominated by *Syntrichia ruralis* (Hedw.) Web. & Mohr had little effect on germination.

In the northern portion of the Great Basin, various types of biological soil crusts are common including moss-dominated crusts, lichen-dominated crusts, and mixed crusts where no particular organism prevails over the surface cover (Rosentreter and Belnap 2001). These biological soil crusts occur in arid and semiarid shrublands and can cover up to 80% of the soil surface. Biological soil crusts in the Great Basin play an essential role in increasing the organic content of the soil and reducing erosion by wind

and water (Belnap 2003). Other effects of biological soil crusts on processes such as water retention and nitrogen fixation vary depending on the composition of the crusts (Belnap 2003; Evans and Johansen 1999).

Moss-dominated, lichen-dominated, and mixed crusts differ in their chemical and structural characteristics and thus may have a different effect on seed germination (Belnap 2006; Zamfir 2000). To explore this possibility we compared in the present study, germination, root penetration, and shoot emergence of two grass species on bare soil and two crust types, one dominated by the lichen *Diploschistes muscorum* (Scop.) R. Sant. and the other consisting of a mixture of thin lichens, short mosses, and cyanobacteria. The grass species tested were the native *Vulpia microstachys* Nutt. and the exotic *B. tectorum*. Our goal was to determine whether lichen-dominated and mixed crusts could act as natural barriers to the germination of annual grasses. Furthermore, we investigated the specific effect of the crusts on root penetration. Although various studies suggest that biological soil crusts are not a barrier to root penetration (Belnap et al. 2001; Belnap and Gardner 1993), this situation may be different for lichen-dominated crusts. Physicochemical characteristics of the lichens such as the presence of hydrophobic and/or hard layers, or the secretion of allelopathic compounds, may affect root growth and ultimately penetration (Dietz and Hartung 1998; Gardner and Mueller 1981; Kidron et al. 1999; Lakatos et al. 2006). Overall, a better understanding of the effects of biological soil crusts on germination and seedling establishment may also help to identify factors that determine vegetation patterns and patchiness in arid lands.

Materials and methods

Soil crust and seeds

We collected two types of naturally occurring biological soil crusts in the fall of 2004 from shrubland communities in southwestern Idaho, USA. Lichens dominated both crust types, but they differed in their composition and cover. The ‘mixed crust’ type, containing the lichens *Aspicilia*, *Caloplaca*, *Candelariella*, *Collema*, and *Placidium*, the cyanobacterium *Microcoleus*, and a low growing

form of the moss *Syntrichia caninervis* Mitt., was collected from a silty-loam soil in a salt-desert shrub community near Grandview (42°54′N, 116°02′W). The ‘lichen crust’ type contained only *D. muscorum* (Scop.) R. Sant., and was collected on a silty-loam soil in a sagebrush (*Artemisia tridentata* Nutt. spp. *wyomingensis* Beetle and Young) steppe community near Boise (43°32′N, 116°08′W).

To collect the crust samples, we removed the top 10–15 cm of the soil when the soil was slightly moist and less vulnerable to breakup. Samples were placed into large plastic trays (47 × 47 cm²). We collected the control soil treatment, without biological soil crust, at the same Boise location where we collected the lichen crust samples. The control, bare soil was autoclaved to remove any effect of biological elements on the surface. On the lichen and mixed crusts, organisms were identified to the genus level. To estimate the surface area occupied by different organisms, we recorded the organisms present at each of 50 random points per tray on five trays of each crust type.

We collected *B. tectorum* seeds at Kuna Butte (43°26′N, 116°26′W) during the summer of 2002. Seeds were exposed to after-ripening conditions in order to break dormancy. *V. microstachys* seeds were commercially obtained cultivars supplied by the Bureau of Land Management in Boise, Idaho. We surface sterilized the seeds by soaking them in 70% ethanol for 1 min and then in 0.5% sodium hypochlorite for 20 min. The seeds were then rinsed four times with sterile water, allowed to dry, and stored at 4°C. To verify that the seeds were viable and non-dormant, we tested the percent germination of the grass species. For each species studied, we placed three replicates of 25 seeds in 9 cm Petri dishes containing filter papers moistened with distilled water. Seeds were incubated in a growth chamber programmed for a 12 h photoperiod with day/night conditions of 15/10 ± 1°C air temperature.

Germination and initial seedling establishment experiment

Experimental trays (10 × 10 × 3.5 cm³) were prepared with either mixed crust, lichen crust, or bare soil. Holes were drilled into the bottoms of the trays to allow drainage. Any large spaces remaining between the edges of the lichen or between the lichen and the

tray were filled with sterilized sand to create a more even surface. Fifty seeds of *B. tectorum* or *V. microstachys* were sown in each tray. Seeds were randomly placed on the surface rather than being buried to mimic the initial position of the seeds after wind dispersal. On the lichen crust, we did not place the seeds on the sand between the lichens, but only on the lichen surface. This approach was followed because most of the large cracks between lichens were created during the collection of the crusts and thus were not representative of the initial crust condition.

Each trial in the germination and initial establishment experiment included three seedbed surfaces (soil, mixed crust, and lichen crust) and two grass species (*B. tectorum* and *V. microstachys*). Each treatment combination (grass species $\times$ seedbed surface) was replicated seven times in the same growth chamber. Trays were placed randomly in the growth chamber and rotated daily. We repeated this procedure three times resulting in 21 trays for each seedbed surfaces time grass species combination and 126 experimental units (trays). We programmed the growth chamber for a 12 h photoperiod with day/night conditions of $15/10 \pm 1^\circ\text{C}$ air temperature and $55/75 \pm 7\%$ relative humidity. Fluorescent lamps supplied $75 \mu\text{mol m}^{-2} \text{s}^{-1}$ of PAR. Every second day we watered the trays with about 1 mm of water, which was applied as a fine mist.

Germination was recorded at radicle emergence (≈ 2 mm) daily, and trials were terminated after 14 days. At the end of each trial, we measured the following variables: (1) the number of seeds that germinated, (2) the number of seeds whose roots penetrated the seedbed surface, (3) the number of seeds that formed a shoot (coleoptile emergence), (4) the weight and length of the aboveground biomass produced (shoot production), and (5) the total number of seeds found in each tray. Root penetration was determined by pulling the seedlings from the seedbed surfaces. If seedling removal was difficult or resulted in damage to the root, we recorded this as evidence that the root had penetrated the surface. Percent root penetration is reported as the proportion of seedlings that entered the soil or crust over the number of seeds that germinated.

Daily measurements of germination were used to determine the mean germination time (MGT) for both grasses on each of the three surfaces. MGT, an

indication of the speed of germination, was calculated as $\text{MGT} = \sum_1^i n_i \cdot t_i / N$; where n_i is the number of seeds that germinated within consecutive intervals of time, t_i is the time between the beginning of the test and the end of a particular interval of measurement, and N is the total number of seeds that germinated (Hartmann and Kester 1983).

Effect of the lichen crust on seed viability

We conducted a separate experiment to analyze the effect of the lichen crust on seed viability. Trays were prepared and incubated as described above and after 14 days on the crust, the seeds that did not germinate were tested for viability by two methods. Half of the seeds were incubated for 12 h in solutions containing 0.1% tetrazolium chloride (Hartmann and Kester 1983). The development of red color throughout the embryo was taken as an indication that the seeds were viable. The rest of the seeds were transferred from the lichen crust to Petri dishes containing wet filter paper; seed germination was determined after 7 days. For each species, three replicates with at least 25 seeds each were used to determine viability and germination.

Root penetration of seeds germinated on filter paper

To further investigate the effect of the lichen crust on root penetration, we conducted a separate experiment with seed that were first germinated on wet filter paper. After the radicle had emerged 2–5 mm, we transferred the seeds to bare soil or lichen crust. Fifty germinated seeds of *V. microstachys* or *B. tectorum* were used per tray and each treatment combination was replicated three times. The trays were watered every other day with 1 mm of water and were incubated in a growth chamber under the conditions described earlier. About 8 days after transferring the seeds to bare soil or lichen crust, we determined root penetration and shoot formation.

Morphological measurements

Measurements of seed and root dimensions were made on digital images acquired through a stereo-zoom microscope. We measured root diameters at

40 × magnification and seed length and width at 12 × magnification using Qcapture Pro software (Qimaging Corporation, Austin, TX, USA). Values are reported as the mean ± standard deviations (SD) of measurements made of at least 30 seeds or roots.

Statistical analyses

We analyzed the effect of seedbed surface treatments on germination and initial establishment using the MIXED procedure model in SAS 9.1 (SAS Institute Inc., Cary, NC, USA) for a randomized complete block design with replications within blocks. The three trials conducted represented the blocks. Random factors in the analysis were block, block × grass species, block × seedbed surface, and block × grass species × seedbed surface. For the seedbed surfaces, different variances were modeled into the MIXED procedure to allow for unequal variances in the lichen crust treatment (Littell et al. 1996). We treated seedbed surface, grass species, and seedbed surface × grass species as fixed factors. Significant differences between treatments were determined using Tukey–Kramer least square means test at $P < 0.05$. This statistical method was used to analyze the effect of both seedbed surface and grass species on percent germination, MGT, root penetration, shoot emergence, shoot length, and shoot weight.

The results of the experiments on seed viability and root penetration of seed germinated on filter paper were analyzed by ANOVA. The significance of the differences between treatments was determined using Tukey–Kramer least square means test at $P < 0.05$ (JMP 5.1, SAS Institute).

Results

Biological soil crust composition

The lichen crust was solely dominated by the crustose lichen *D. muscorum*. The mixed crust contained 52% lichens ($17.3 \pm 1.8\%$ *Aspicilia*, $16.8 \pm 1.5\%$ *Collema*, $12.1 \pm 3.2\%$ *Candelariella*, $5.1 \pm 1.4\%$ *Placidium*, $0.8 \pm 0.9\%$ *Caloplaca*), 17% moss (mainly *S. caninervis*), 8% cyanobacteria (mainly *Microcoleus*) and 23% bare soil.

Seed germination

For both *B. tectorum* and *V. microstachys*, germination on the bare soil and mixed crust surfaces was higher than on the lichen crust. Germination of *B. tectorum* on bare soil was somewhat higher than on the mixed crust, but these differences were not statistically significant. Similar results were obtained in the three trials (Fig. 1).

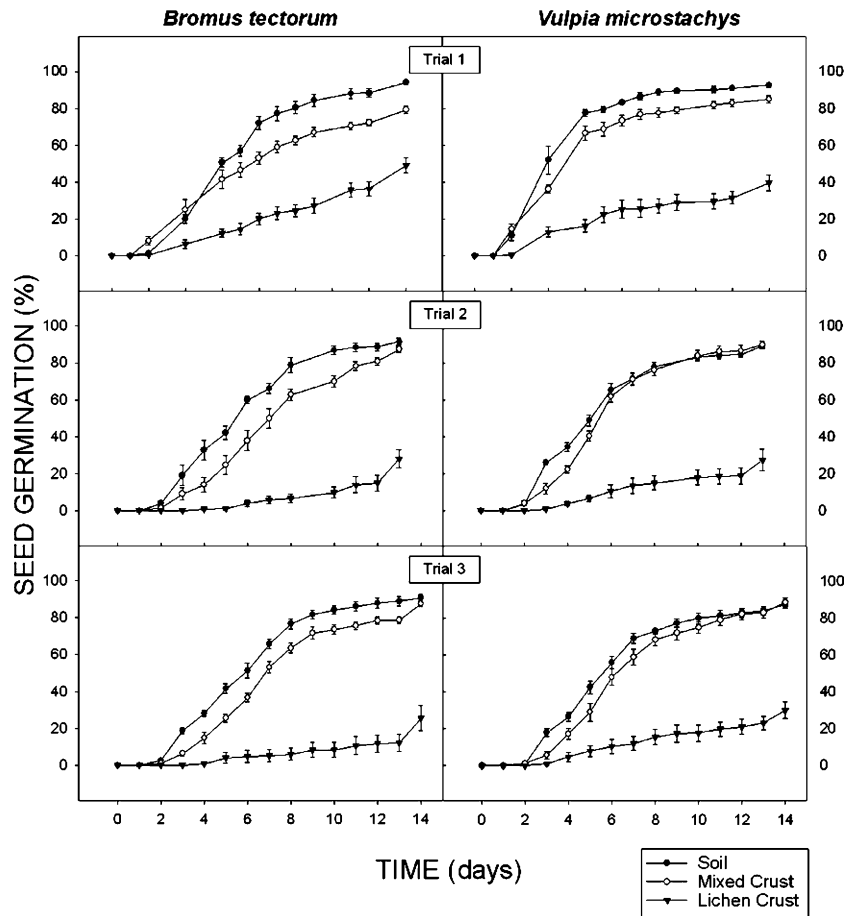
Both grass species had similar patterns in MGT, which for both grass species took longer on the lichen crust than on the mixed crust or bare soil (Fig. 2a). MGT differed significantly among seedbed surfaces ($P = 0.0013$) and grass species ($P < 0.0001$), but there was no significant interaction between the two factors ($P = 0.06$, Table 1). On the two crust types, MGT for *V. microstachys* was significantly shorter than that of *B. tectorum* (Fig. 2a). For *V. microstachys*, MGT was between 5 and 6 days on the soil and mixed crust, but over 8 days on the lichen crust. Similarly, the MGT for *B. tectorum* was between 6 and 7 days on the bare soil and mixed crust, but nearly 11 days on the lichen crust (Fig. 2a).

There was a significant seedbed surface effect on percent germination ($P = 0.0088$), but no significant effect of grass species ($P = 0.83$) or grass species × seedbed interaction ($P = 0.31$; Table 1). The percent germination after 14 days was significantly higher for *V. microstachys* and *B. tectorum* on the soil surface compared to the lichen crust, while no detectable difference in germination was found between the soil and mixed crust (Fig. 2b). For *V. microstachys* there was only a 2% difference in percent germination between the soil and mixed crust, but 58% lower germination on the lichen crust compared to the bare soil. *B. tectorum* showed a similar pattern (Fig. 2b). Germination was relatively high, at least 85% on the soil and mixed crust. In contrast, on the lichen crust, both species showed significantly less germination.

Root penetration

Root penetration was significantly different among the surfaces ($P = 0.0038$), and there was a significant effect of grass species × seedbed interaction on root penetration ($P = 0.006$, Table 1). The percent root penetration for both grass species was ~99% on the bare soil (Fig. 2c). For *B. tectorum* there was

Fig. 1 Effect of lichen-dominated biological soil crusts on germination of *Bromus tectorum* and *Vulpia microstachys*. Three types of seedbed surfaces were used; the soil surface was devoid of any biological soil crusts, the mixed crust surface was comprised of a variety of lichens, mosses, and cyanobacteria, and the lichen crust contained only *Diploschistes muscorum* lichens. Each point represents the mean seed germination for that day ($\pm$ SE) of seven trays



significantly less root penetration on the mixed crust surface compared with the soil surface. In contrast, for *V. microstachys*, there were no significant differences between the soil and mixed crust surfaces. Root penetration was significantly lower on the lichen crust for both species, with significantly lower root penetration for *B. tectorum* compared with *V. microstachys* (Fig. 2c).

Overall seedling establishment

Overall seedling establishment combines the effects of the seedbed surface on both germination and root penetration. For overall seedling establishment, there was grass species $\times$ seedbed interaction ($P = 0.0003$, Table 1). For both species, seedling establishment was less on the mixed crust surfaces than on the bare soil surface (Fig. 2d). However, these differences

were only significant for *B. tectorum*. For *V. microstachys*, overall seedling establishment after 14 days was $88.7 \pm 1.5\%$ on the soil surface and $82.4 \pm 2.3\%$ on the mixed crust. Similarly, for *B. tectorum* overall establishment was $91.3 \pm 1.5\%$ and $74.0 \pm 2.3\%$ for the bare soil and mixed crust surfaces, respectively. On the lichen crust surface, both grass species had much lower seedling establishment, only $11.1 \pm 0.95\%$ for *V. microstachys* and $6.6 \pm 0.95\%$ for *B. tectorum*.

In order to determine the contribution of root penetration to reducing establishment, we compared the percent germination to the overall seedling establishment for each species (Fig. 2b, d). For *V. microstachys*, overall establishment was 78% higher on bare soil than on lichen crust. Of this difference, 57% was attributed to the effect of the lichen crust on germination and 21% to the reduction in root

Fig. 2 Mean germination time (a), germination percentages (b), root penetration percentages (c), and overall seedling establishment (d) of *Vulpia microstachys* and *Bromus tectorum* on the bare soil, mixed crust, and lichen crust surfaces. Percent root penetration represents the proportion of seedlings that entered the soil or crust over the number of seeds that germinated. Each bar represents the mean ($\pm$ SE) of 20–21 trays. Overall seedling establishment was calculated as the percentage of seeds that had root penetration into the seedbed surface out of the total seeds placed on the trays. Fifty seeds were used in each tray. Bars not labeled with the same letter are significantly different ($P < 0.05$) based on Tukey–Kramer least square means test

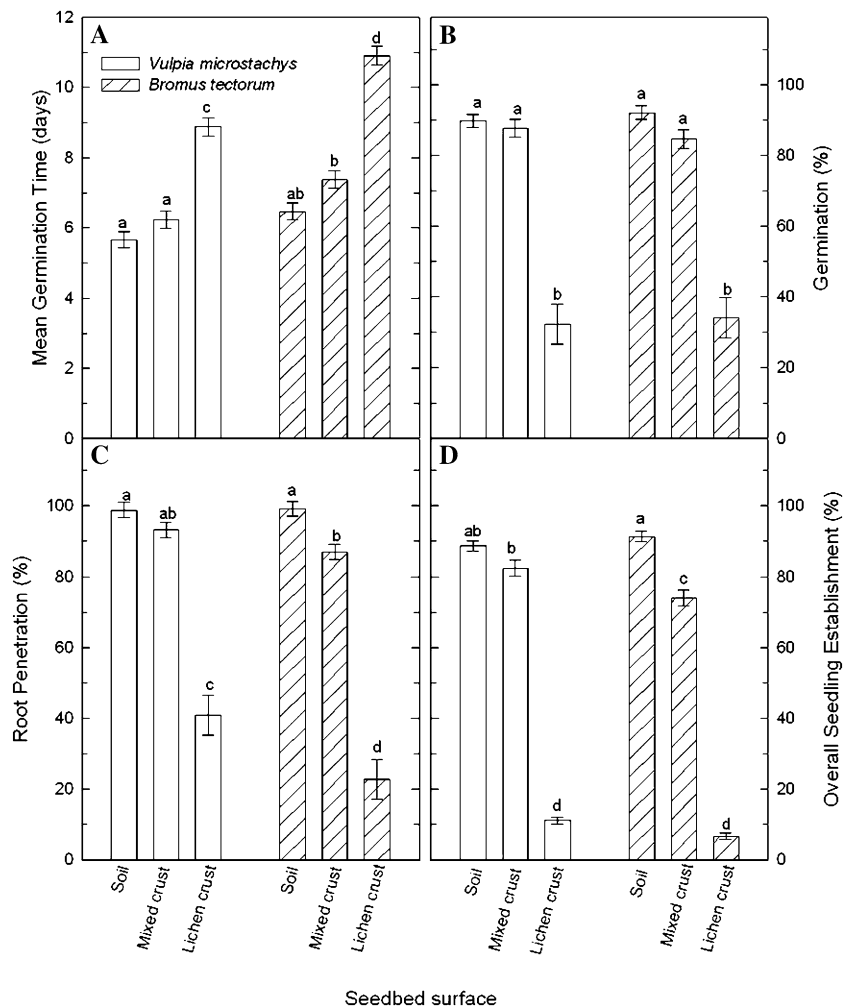


Table 1 Test for significance of fixed factors (grass species, seedbed surface, and grass species $\times$ seedbed surface) on mean germination time (MGT), percent germination (PG), percent root penetration (PRP), and overall seedling establishment (OSE) after 14 days

Factor	DF Num	DF Den	MGT/PG/PRP/OSE	Mean germination time		Percent germination		Percent root penetration		Overall seedling establishment	
				F-value	P-value	F-value	P-value	F-value	P-value	F-value	P-value
Seedbed surface	2		3.12/2.81/2.6/2.76	106.19	0.0013	39.59	0.0088	93.68	0.0038	454.66	0.0003
Grass species	1		112/112/112/112	38.74	<0.0001	0.05	0.8315	11.66	0.0009	10.23	0.0018
Grass species $\times$ seedbed surface	2		112/112/112/112	2.91	0.0583	1.18	0.3107	5.36	0.006	8.91	0.0003

penetration. Similarly, for *B. tectorum* overall establishment was 85% higher on bare soil than on lichen crust. About 58% of this difference was attributed to

the effect of the lichen crust on seed germination and 27% to the reduction in root penetration. Thus, the effect of the lichen crust on reducing seed

germination is quantitatively more important than the effect on root penetration, but both effects contributed to the reduction in seedling establishment (Fig. 2b, d).

Shoot emergence and growth

To further characterize the effects of the crusts on seedling development, we also compared shoot emergence, shoot length, and shoot weight among the treatments. Shoot emergence, measured as the number of seeds that developed coleoptiles as a percentage of those that germinated, was highest for the seeds on the soil and mixed crust surfaces (Table 2). Both species showed less shoot emergence on the lichen crust with significantly lower shoot emergence for *B. tectorum* than for *V. microstachys*.

For those seeds that formed shoots, there were significant differences in the length per shoot produced, and the shoot weight (Table 2). The average shoot length was about the same for the soil and mixed crust treatments, and significantly less for the lichen crust (Table 2). Both species showed this same pattern and the trend was similar for average shoot weight. Shoot weight was significantly higher on the soil and mixed crust than on the lichen crust (Table 2).

Effect of the lichen crust on seed viability

To further investigate the effect of the lichen crust on the seeds, we collected seeds that did not germinate on this crust and analyzed their viability. We did not observe differences in viability between control seeds (tested immediately after removal from storage) and those placed on lichens for 14 days (Table 3). When

tested for germination, the seeds that were transferred from the lichen crust to wet filter paper showed somewhat lower rates of germination than control seeds. However, the differences were not statistically significant (Table 3).

Root penetration of seeds germinated on filter paper

For seeds that were first germinated on wet filter paper and then transferred to bare soil or lichen crust, root penetration was significantly higher on the bare soil. After 8 days, root penetration for *V. microstachys* seedlings was 98.6 (SD $\pm$ 1.2) and 4.5 ($\pm$ 2.0)% on the bare soil and lichen crust, respectively. Similarly, root penetration for *B. tectorum* seedlings was 93.8 ($\pm$ 3.6) and 1.9 ($\pm$ 3.3)% on the bare soil and lichen crust, respectively. The values obtained for shoot emergence were very similar to those observed for root penetration (data not shown). Based on these results, the lichen crust can drastically inhibit root penetration.

We also explored the possibility that negative effects of the lichen crust on germination and/or root penetration were attributed to compounds secreted by *D. muscorum*. To test this notion, we collected substances from the lichen by blotting their moist surface with dry filter paper and by inverting the lichen and letting it leak chemicals overnight to a small volume of water ($\sim$ 4 cm² of thallus surface area per ml of water). The material collected in this manner had a pH of about 6. Seeds placed on filter paper moistened with the solution obtained from the lichen chemical exudates germinated and produced normal seedlings (data not shown). Based on these results, lichen chemicals do not appear to be responsible for the decrease in germination and root

Table 2 Shoot emergence (%), average shoot length (cm), and average shoot weight (mg fresh weight) of *Vulpia microstachys* and *Bromus tectorum* on bare soil, mixed crust, and lichen crust

Attribute	<i>Vulpia microstachys</i>			<i>Bromus tectorum</i>		
	Soil	Mixed crust	Lichen crust	Soil	Mixed crust	Lichen crust
Shoot emergence	88.7 $\pm$ 1.5 ^a	82.4 $\pm$ 2.3 ^a	11.1 $\pm$ 0.9 ^b	91.3 $\pm$ 1.5 ^a	74.0 $\pm$ 2.3 ^a	6.6 $\pm$ 0.9 ^c
Shoot length	6.5 $\pm$ 0.3 ^a	6.5 $\pm$ 0.3 ^a	4.4 $\pm$ 0.3 ^b	4.3 $\pm$ 0.3 ^b	4.4 $\pm$ 0.3 ^b	2.7 $\pm$ 0.3 ^d
Shoot weight	0.9 $\pm$ 0.08 ^a	0.9 $\pm$ 0.1 ^a	0.4 $\pm$ 0.03 ^c	1.0 $\pm$ 0.08 ^a	0.9 $\pm$ 0.1 ^a	0.3 $\pm$ 0.04 ^c

Values represent the mean ($\pm$ SE) of 20–21 trays. Fifty seeds were used in each tray. Percent shoot emergence represents the proportion of seedlings that develop coleoptiles over the number of seeds that germinated. Within an attribute, values not labeled with the same letter are significantly different ($P < 0.05$) based on Tukey–Kramer least square means test

Table 3 Percent viability and germination of *Vulpia microstachys* and *Bromus tectorum* seeds that did not germinate on the lichen crust during a period of 14 days

	<i>Vulpia microstachys</i>		<i>Bromus tectorum</i>	
	Control	After placement on lichen crust	Control	After placement on lichen crust
Viability	96.4 ± 1.7 ^a	94.7 ± 1.7 ^a	97.3 ± 1.7 ^a	95.7 ± 1.7 ^a
Germination	80.7 ± 6.5 ^a	69.0 ± 6.5 ^a	80.6 ± 6.5 ^a	58.1 ± 6.5 ^a

Control seeds represent seeds that were tested for viability or germination immediately after removal from storage. Values represent the mean (±SE) of three replicas with at least 25 seeds per replica. Viability was measured by the tetrazolium test. Germination was determined after the seeds were incubated for 7 days on wet filter paper. Values labeled with the same letter are not significantly different ($P < 0.05$) based on Tukey–Kramer least square means test

penetration observed on the lichen crust. The possibility cannot be discarded, however, that non-diffusible molecules bound to the lichen surface had a negative effect, particularly on the root tips as they begin to penetrate the *D. muscorum* thalli.

Discussion

Our study showed that two distinct biological soil crusts had significantly different effects on the germination of two grass species now common in the western US. While the lichen crust inhibited germination, the mixed crust, made up of lichens, mosses, and cyanobacteria had no effect on germination compared with a bare soil surface. The effects of the lichen crust on decreasing and delaying germination demonstrate that this crust can act as a natural barrier to the establishment of *B. tectorum*, a major invader of extensive areas of rangeland in the western US.

The biological soil crusts tested had similar effects on *V. microstachys* and *B. tectorum*, which may be attributed to similarities in the physiological and anatomical characteristics of the seeds. *V. microstachys* and *B. tectorum* are cool season grasses that lack self-burial mechanisms and can germinate on the soil surface in early fall. In addition, the caryopses of *V. microstachys* and *B. tectorum* have comparable lengths (4.8 ± 0.4 mm for *V. microstachys*; 7.0 ± 2.28 mm for *Bromus*-excluding awns) and widths. Seed size may be a factor affecting germination on biological soil crusts (Li et al. 2005; Sedia and Ehrenfeld 2003; Zamfir 2000). The dimensions of the seeds combined with the morphological characteristics of the crust may partially determine

whether the crust has microsites suitable for germination (Li et al. 2005).

In this and a previous study (Serpe et al. 2006), we observed that biological soil crusts had similar effects on the exotic and native grasses tested. This contrasts with results reported by other researchers (Eldridge and Simpson 2002; Kaltenecker et al. 1999; Larsen 1995). In a field study, Larsen (1995) observed that a mixed crust of mosses and lichens inhibited the germination of *B. tectorum* but did not affect the germination of the native perennial grasses *Hesperostipa comata* (Trin. & Rupr.) Barkworth and *Achnatherum thurberiana* (Piper) Barkworth. Unlike the species used in our studies, *H. comata* and *A. thurberiana* have hygroscopic awns that facilitate horizontal seed displacement and/or penetration through the crust. The presence of hygroscopic awns in these species may account for the lack of an inhibitory effect of the crust on germination.

Differences in germination between exotic and native species have also been observed on biological soil crusts from a semiarid woodland in Australia (Eldridge and Simpson 2002). Intact biological soil crusts suppressed the germination of two weedy forbs (*Marrubium vulgare* L. and *Brassica tournefortii* Gouan), but enhanced the germination of the native perennial grass *Austrodanthonia caespitosa* (Gaudich.) H. P. Linder compared with a soil from which the crust had been removed by rabbit digging (Eldridge and Simpson 2002). Clearly, the above results indicate that germination of some native species is unaffected or promoted by the crust. Thus, even among native species biological soil crusts can have differential effects on seed germination (Hawkes 2004; Serpe et al. 2006). These differential effects may play a role in determining the local

distribution of plant species (Golberg and Barton 1992; Kirpatrick et al. 2006; Sedia and Ehrenfeld 2003).

To our knowledge, this is the first study to show conclusively that biological soil crusts can inhibit root penetration. Although both grass species established roots on all surfaces, the degree to which they penetrated the surface varied among the different seedbeds (cf. Fig. 2c). For both species, root penetration was significantly lower on the lichen crust, and for *B. tectorum*, was less on the mixed crust compared with the bare soil by about 11%. The 60% or higher difference in root penetration between the bare soil and lichen crust strongly suggests that the lichen surface effectively restricted root penetration. Furthermore, the inhibitory effect of the lichen crust on root penetration was largely independent on the effect on MGT. When we placed germinated seeds on bare soil or lichen crust, the roots had the same time to penetrate these surfaces. However, high rates of root penetration only occurred on bare soil.

Some discrepancies in root penetration were also observed between species. For the seeds that germinated on the lichen crust, root penetration was significantly higher for *V. microstachys* than for *B. tectorum* (cf. Fig. 2c). Root morphological characteristics can affect root penetration (Gilker et al. 2002; Iijima et al. 2004; Kislev et al. 1979; Materechera et al. 1992). We measured the diameter of *V. microstachys* and *B. tectorum* roots at about 1 mm from the root tip and they were virtually identical, 262 (± 10) and 274 (± 25) μm , respectively. Based on these measurements, root diameter does not appear to be a factor that could lead to differences in root penetration. An alternative explanation for the difference in root penetration between grass species on the lichen crust is the disparity in MGTs. In average *V. microstachys* germinated 3 days earlier than *B. tectorum*, resulting in less opportunities for the latter to penetrate the lichen crust. When the difference in germination time was experimentally removed by placing seeds on the crust after germination, there were not differences in root penetration between the two grass species. Thus, differences in MGT cannot explain the dissimilarities in root penetration among surfaces, but appear to account for the difference in root penetration between the grass species on the lichen crust.

An important consequence of limited root penetration on the lichen crust may be an increase in seedling mortality due inadequate water absorption and desiccation. This scenario is particularly likely to develop in natural settings, where the seedlings are in general exposed to drier conditions than the ones used in this study. Thus, even if viable seeds eventually germinate on the crust, their prospects for successful establishment and survival would be largely restricted by the inability of the seedlings to penetrate the crustose lichens.

The distinct morphological characteristics of the surfaces tested suggest that differences in seed to seedbed contact and seed water absorption can be responsible for the lower germination on the lichen crust (Sedia and Ehrenfeld 2003; Zamfir 2000). The lichen crust represents a more homogenous and flat surface than the mixed crust. The mixed crust had many micro-depressions, which would act as sinks for water and seeds (Boeken and Shachak 1994; Eckert et al. 1986; Eldridge and Greene 1994; Li et al. 2005). Moreover, the seeds tended to become enclosed in mixed crust depressions presumably resulting in higher seed to seedbed contact than on the flatter surface of the lichen crust. Depressions also developed in the soil control surface. Initially this surface was flat, but even though we watered it with a very fine mist, this was sufficient to cause some rainsplash displacing soil particles and creating micro-depressions. These particles partially covered the seeds, increasing the degree of seed to soil contact, which may have facilitated seed water absorption and germination.

Apart from their morphological differences, the crusts tested differed in other structural characteristics. The lichen crust is thicker and denser than the mixed crust. The compact lichen crust may mechanically restrict root growth, an effect that could account for the low root penetration observed on the lichen surface. In addition, the mixed crust included organisms such as cyanobacteria, gelatinous lichens, and mosses that can absorb several times their volume in water (Campbell 1979; Galun et al. 1982; Proctor et al. 1998; Verrecchia et al. 1995). In contrast, crustose lichens such as *D. muscorum* tend to absorb lesser amounts of water (Belnap 2006; Blum 1973). Thus, the organisms in the mixed crust perhaps maintained higher water content than *D. muscorum*. The maintenance of moist conditions on

the mixed crust surface would favor germination and subsequent root growth.

Independent of the mechanisms involved, the inhibitory effects of the lichen crust on germination and root penetration indicate that this crust can be a barrier to the establishment of both native and exotic grasses. The extent of this effect would depend on the soil surface area occupy by the crust. *Diploschistes* spp. are common soil inhabiting lichens in arid and semiarid regions throughout the world. In areas where livestock have been excluded it is not uncommon for individual lichen thalli to exceed 150 mm in diameter (Eldridge 1998). However, *Diploschistes* spp. do not generally dominate an area exclusively, but occur extensively with a suite of other crustose species. On lichen-dominated soils in arid areas, their combined coverage may exceed 40% of the soil surface (Eldridge and Ferris 1999; Martinez et al. 2006). In these areas, negative effects of crustose lichens on seedling establishment would tend to decrease vegetation cover and contribute to the development of a patchy vegetation distribution. Patchiness would result in a less continuous dry biomass during the summer, particularly in areas threatened by cheatgrass invasion, thus reducing the frequency and extent of wild fires (Brooks et al. 2004; D'Antonio and Vitousek 1992; Kaltenecker et al. 1999).

In summary, our findings illustrate the complex nature of seedling establishment on biological soil crusts. Distinct crusts had different effects on seed germination, root penetration, and shoot emergence. A combination of physical and chemical factors such as placement of seeds on microsites of varying microtopography, dissimilarities in surface hydrophobicity and water holding capacity between the seedbeds studied could have resulted in the differences we observed between surface types. The exact mechanisms by which the lichen crust reduced seed germination and root penetration requires further investigation. Similarly, more work is needed to ascertain the ecological significance of the results presented.

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