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REGULAR ARTICLE

Seed water status and root tip characteristics of two annual grasses on lichen-dominated biological soil crusts

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Abstract Biological soil crusts can affect seed germination and seedling establishment. We have investigated the effect of biological soil crusts on seed water status as a potential mechanism affecting seed germination. The seed water potential of two annual grasses, one exotic *Bromus tectorum* L. and another native *Vulpia microstachys* Nutt., were analyzed after placing the seeds on bare soil, on a crust that contains various lichens and mosses (mixed crust), or on a crust dominated by the crustose lichen *Diploschistes muscorum* (Scop.) R. Sant. (*Diploschistes* crust). Seed water potential and germination were similar on the bare soil and the mixed crust, except for the initial germination of *V. microstachys*, which was higher on the mixed crust than on the bare soil. For the two grasses studied, seed water potential was significantly higher on the bare soil and mixed crust than on the *Diploschistes* crust. These differences in water potential correlated with differences in germination, which was much lower on the lichen crust. Experiments were conducted under two watering regimens. Increasing the frequency of watering amplified the differences in seed water potential and germination between the *Diploschistes* crust and the other two surfaces. For a particular watering regimen, the bare soil, mixed crust, and *Diploschistes* crust received the same amount of water, but they reached significantly different water potentials. Throughout the experiments, the water potential of the soil and mixed crust remained above −0.6 MPa, while there was a marked decline in the water potential of the *Diploschistes* surface to about −4 MPa. To ascertain that water was the major factor limiting germination on the *Diploschistes* crust, we conducted germination tests in an environment with 100% relative humidity. Under these conditions, germination on the *Diploschistes* crust was similar to that on the bare soil. However, the seeds that germinated on the *Diploschistes* crust did not penetrate this surface and approximately 60% of their root tips became necrotic. Our results indicate that the presence of *D. muscorum* can inhibit seedling establishment by two mechanisms: a reduction in seed water absorption and an increase in root tip mortality.

Keywords *Bromus tectorum* · Crustose lichens · *Diploschistes muscorum* · Seed germination · *Vulpia microstachys* · Water potential

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Introduction

Biological soil crusts are soil surface communities formed by various organisms including mosses, lichens, algae, cyanobacteria, and heterotrophic microorganisms (Rosentreter and Belnap 2001). These communities are common in arid and semiarid lands, where they tend to occupy the open interspaces between vascular plants. Biological soil crusts play important ecological roles; they contribute to carbon assimilation and nitrogen fixation, and secrete polysaccharides and metal chelators that help in maintaining soil fertility (Evans and Johansen 1999; Belnap 2003). Furthermore, the presence of biological soil crusts modifies soil surfaces by altering surface roughness and physicochemical characteristics of the soil (Harper and Marble 1988; Belnap 2006). Through these changes in the characteristics of the soil surface, biological soil crusts reduce soil erosion, affect water infiltration, and contribute to the development of patchy vegetation patterns (Belnap 2006). In many arid ecosystems, a patchy vegetation distribution reduces the risk of fire and is critical for the survival of native wildlife (Aguilar and Sala 1999; Rosentreter and Belnap 2001).

By altering the characteristics of the soil surface, biological soil crusts may also affect seed germination and establishment. Both positive and inhibitory effects of biological soil crusts on germination have been reported (Zamfir 2000; Hawkes 2004; Morgan 2006). The various effects of biological soil crusts on seed germination are likely to reflect differences in crust composition, environmental conditions, and/or the type of seed placed on the crust (Hawkes and Menges 2003; Otsus and Zoel 2004; Li et al. 2005; Morgan 2006). Differences in crust composition can result in crusts with very different morphological, physical, and chemical characteristics; thus resulting in different seedbed environments (Eldridge and Rosentreter 1999; Belnap et al. 2001). Furthermore, seeds have different germination requirements and anatomical characteristics and may respond different to the conditions created by the crust (Zaady et al. 1997; Baskin and Baskin 1998; Li et al. 2005; Morgan 2006).

While several studies indicate that biological soil crusts have an influence on seed germination, the mechanisms that mediate such effects are not clearly understood (Belnap 2006). Even under the same weather conditions, the temperature and moisture of biological soil crusts often differ from those of adjacent bare soil (Verrecchia et al. 1995; Belnap et al. 2001; Warren 2001). Any effect of biological soil crusts on moisture and temperature are likely to affect seed germination because these environmental conditions have a primary influence on seed imbibition and metabolism (Bradford 1995; Baskin and Baskin 1998). Furthermore, in arid and semiarid lands, water is the main factor limiting plant establishment and growth. Through an effect on the water status of the soil surface, biological soil crusts may play an important role in determining germination success and seedling survival in xeric environments (Belnap 2003).

In a previous study, we have investigated the effect of two types of lichen-dominated biological soil crusts on the germination and establishment of two annual grasses, the native grass *Vulpia microstachys* Nutt. and the exotic and highly invasive grass *Bromus tectorum* L. (Deines et al. 2007). *Vulpia microstachys and B. tectorum* have similarities in the physiological and anatomical characteristics of their seeds. They 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. We studied the germination of these grasses on two types of biological soil crusts that naturally occur in steppe communities of the Great Basin of North America. One of the crusts studied contains various lichens, mosses, and cyanobacteria (mixed crust). Lichens are the main components of the mixed crust and constitute more than 50% of the crust surface. The other crust studied is solely dominated by the lichen *Diploschistes muscorum* (Scop) R. Sant. (*Diploschistes* crust). *Diploschistes muscorum* has a hard and rather continuous thallus, which is somewhat unusual among soil lichens (McCune and Rosentreter 2007). The mixed crust is of widespread occurrence in arid and semiarid shrublands, where it can cover up to 80% of the soil surface (Rosentreter and Belnap 2001). The *Diploschistes* crust is much less common. The scarcity of *D. muscorum* in many areas of the Great Basin partly reflects that livestock trampling has destroyed this lichen and that *D. muscorum* is a late successional species with very slow recovery following disturbances (McCune and Rosentreter 2007). Notwithstanding these events and

characteristics, *D. muscorum* can be found in arid and semiarid regions throughout western North America where it often occurs with a suite of other crustose species (St. Clair 1999, McCune and Rosentreter 2007).

Independent of the area occupied by lichens, knowledge about the effect of these organisms on germination may provide information regarding mechanisms that increase lichen survival on the soil surface. Lichens in general have very low growth rates, of only a few mm per year (Hale 1959). The mechanisms that allow *D. muscorum* and other lichens to persist on the soil and contend with organisms with much faster growth rates are not clearly understood. Analysis of the effect of soil lichens on germination might help to identify processes that give lichens some competitive advantage over seed plants.

Our previous study indicated that the mixed and *Diploschistes* crusts had different effects on seedling establishment, even though both crusts are dominated by lichens. Seedling establishment on the mixed crust was similar to that on bare soil. In contrast, the *Diploschistes* crust significantly inhibited establishment of the two grasses tested (Deines et al. 2007). The main effect responsible for about two thirds of the overall decrease in seedling establishment was a reduction in seed germination, which was much lower on the *Diploschistes* crust than on bare soil. The other factor that contributed to the reduction in seedling establishment was root penetration. Following germination, only a small proportion of the emerged roots penetrated the *Diploschistes* crust, while almost all roots penetrated the bare soil surface. Although our results showed that the *Diploschistes* crust inhibited seed germination and root penetration, the mechanisms responsible for these effects remained unknown.

In the present study, we have investigated the effect of the mixed crust and *Diploschistes* crust on seed water status as a potential mechanism affecting germination. In addition, we have analyzed whether the effect of these crusts on germination varies with different moisture conditions. This phenomenon has been observed with seeds germinating on bryophyte mats, but much less is known about seeds germinating on lichens (Zamfir 2000; Hawkes and Menges 2003; Otsus and Zoel 2004; Morgan 2006). Furthermore, one of the moisture conditions tested involved placing the seeds on the *Diploschistes* crust and incubating them in an environment with virtually 100% relative humidity. The purpose of this experiment was to remove water as a limiting factor for germination and determine whether other physicochemical characteristics of the *Diploschistes* crust have an inhibitory effect on germination or seedling establishment.

Materials and methods

Soil crust and seeds

We collected two types of naturally occurring biological soil crusts in the spring of 2006 from sagebrush steppe communities in southwestern Idaho. Lichens dominated both crust types, but they differed in the lichen species present and the overall coverage of lichens. The mixed crust contained approximately 50% lichens (*Aspicilia*, *Collema*, *Candelariella*, *Placidium*, *Caloplaca*), 25% moss (mainly *Syntrichia caninervis* Mitt.), 8% cyanobacteria (mainly *Microcoleus*) and 17% bare soil. We collected this crust near Grandview, Idaho (42° 53.82′ N, 116° 1.80′ W). The *Diploschistes* crust contained only *Diploschistes muscorum* and was collected on a silty-loam soil in a sagebrush (*Artemisia tridentata* Nutt. spp. *wyomingensis* Bettle & 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. We collected the control soil treatment, without biological soil crust, at the Boise location where we collected the *Diploschistes* crust samples.

Bromus tectorum seeds were collected at Kuna Butte, Idaho (latitude 43° 26.40′ N, longitude 116° 25.87′ W) during the summer of 2005. Seeds were exposed to dry-warm conditions in order to break dormancy. *Vulpia microstachys* seeds were provided by the Bureau of Land Management in Boise. The seeds were surface sterilized, allowed to dry, and stored in a cool room at 4°C until used (Deines et al. 2007).

Seed water status and germination

Experimental trays (10×10×3.5 cm) were prepared with bare soil, mixed crust, or *Diploschistes* crust. All trays were watered to soil saturation and allowed to

drain. We then placed a hundred seeds of *B. tectorum* or *V. microstachys* on each tray. Seeds were randomly placed on the surface of the soil or biological soil crusts. Collection of the *Diploschistes* crust resulted in the formation of large cracks between lichens. Care was taken to keep the seeds off these cracks because they were not representative of the initial crust condition. After seed placement, all trays were sprinkled with approximately 1 mm of water, which was supplied as a fine mist. Subsequently, we placed the trays in a growth chamber that provided a 12-h photoperiod with day/night conditions of 15/10±1°C air temperature and 60/85±7% relative humidity. Fluorescent lamps supplied 75 μmol m^{-2} s^{-2} of PAR at the tray level.

The experiment included a total of 12 treatment combinations, 3 seedbed surfaces (bare soil, mixed crust, and *Diploschistes* crust), 2 species (*V. microstachys* and *B. tectorum*), and 2 watering regimes (dry and wet treatment). Trays in the dry treatment did not receive any additional water, while trays in the wet treatment received 1 mm of water every 48 h. These watering regimens were selected based on the water holding capacity of the trays and the amount of precipitation that occurs in Boise during September and October when both *V. microstachys* and *B. tectorum* germinate. The average precipitation in Boise for September and October is about 3.85±3.7 mm per week (Boise WSFO airport). Within this range, we selected two amounts of watering. The 1 mm used in the dry treatment is below average, but sufficient to induce some germination on the bare soil. In the wet treatment, the seeds received 4 mm during the eight days of the experiments, which is similar to the average precipitation. We applied only 1 mm per watering event because this amount was sufficient to bring the trays to field capacity. We prepared three trays per each treatment combination. One of the trays was used to determine germination, seed water potential, and seed water content 4 days after seeding and the other two to determine the same parameters 8 days after seeding. We used more trays for the measurements after 8 days to have enough un-germinated seeds for the determination of seed water potential. The whole experiment was repeated four times over consecutive periods.

After 4 and 8 days, we removed one or two trays per treatment combination and placed them inside a chamber maintained at high relative humidity in order to minimize changes in seed water content. For each tray, we first counted the seeds that had germinated; germination was recorded at radicle emergence. Subsequently, we collected the un-germinated seeds and used them to determine seed water potential and seed water content. The experiments were conducted for only 8 days. After this period, most of the seeds had germinated on the bare soil and mixed crust and there were not enough seeds to make accurate measurements of seed water potential and water content.

Seed water potential was measured with a dew-point water potential meter (WP4-T Decagon Devices, Inc., Pullman, WA). The WP4-T was set to operate at 25°C in a continuous mode and the calibration was tested before each use as recommended by the manufacturer. We used 50 to 100 seeds for each determination of water potential. Water potential measurements were recorded after readings outputs had stabilized, which varied between 30 minutes and 2 h. For each treatment and sampling period, we made four measurements of seed water potential.

As a complementary measurement of seed water status, we also analyzed the seed water content by weight. Seed water content was measured in the same seeds that were used for the determination of water potential. The percent water content was estimated as $[(\text{seed fresh weight} - \text{seed dry weight})/\text{seed dry weight}] \times 100$. Fresh weight represents the seed weight after the completion of the water potential measurement and dry weight represents the seed weight after drying at 130°C for 24 h. Fifty to 100 seeds were used for each determination of percent water content and four measurements of water content were made for each treatment and sampling period.

The values of germination, water potential, and water content were analyzed statistically using the MIXED procedure in SAS 9.1 (SAS Institute Inc., Cary, NC) for a completely randomized block design. The four repeats of the whole experiment represented the blocks. The fixed factors in the analysis were seedbed surface, species, water regimen, and the interactions between these three factors. For some analyses, we modeled different variances in the MIXED procedure to allow for unequal variances between treatments (Littell et al. 1996). Significant differences between treatments were determined using Tukey–Kramer least square means test at $P \angle 0.05$. All estimates of treatment variability are reported as standard errors.

Water potential of the bare soil, mixed crust, and *Diploschistes* crust

Differences in seed water status among treatments can be due to differences in the water potential of the seedbed surfaces, which in our study were the bare soil, mixed crust, and *Diploschistes* crust. To investigate this possibility, we measured the water potential of the three surfaces over the course of 8 days. Experimental trays were prepared as previously described. All trays were initially watered to saturation, allowed to drain, and placed in the growth chamber under the conditions described earlier. Subsequently, trays in the dry treatment did not receive any additional watering, while trays in the wet treatment received 1 mm of water every 48 h. At daily intervals, we removed three trays per each seedbed surface and watering regimen and collected samples from the upper 5 mm of each tray with a razor blade. The water potential of each sample was determined with the WP4-T potentiometer as described above. The water potential values 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).

To gain an insight into the hydraulic properties of the three seedbed surfaces, we also estimated their water holding capacities. For this purpose, we used five trays per each seedbed surface and collected about 5 g of sample from the upper 5 mm of each tray. The water holding capacity was estimated as [(weight at field capacity − dry weight)/volume]. Dry weight represents the weight after drying at 130°C for 24 h.

Germination and root penetration under 100% relative humidity

We conducted a separate experiment to determine the germination of *B. tectorum* and *V. microstachys* seeds on the *Diploschistes* crust under very moist conditions. For each species, we prepared three trays with *Diploschistes* crust and three with bare soil (control treatment). The trays were watered to soil saturation and on each tray, we placed 100 seeds, which received 1 mm of water after seeding and thereafter 1 mm of water every 48 h. Wet filter papers were added to the sides of the trays and the trays were covered with plastic lids. The goal was to maintain moist conditions at the crust and bare soil surfaces by creating an environment with 100% relative humidity. Germination and root penetration was recorded after 8 days. Root penetration was determined by pulling the seedlings from the soil or lichen surface. 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 *Diploschistes* crust over the number of seeds that germinated.

For seedlings growing on the *Diploschistes* crust, we also examined their root tips for symptoms of injury. Seedlings were observed at 12X magnification. On seedlings with brown root tips, we also analyzed cell viability and callose deposition. Loss of cell viability was determined by propidium iodide exclusion; cells with damaged membranes allow this dye to enter the cells where it binds to the nuclei generating bright red fluorescence (Jones and Senet 1985). We incubated the roots for 15 min in a 1.5 μM solution of propidium iodide in phosphate buffer saline and rinsed them with water prior to observation using a fluorescence microscope. For localization of callose, root tips were fixed in 4% (w/v) paraformaldehyde in 50 mM Pipes (pH 6.9) containing 5 mM $MgSO_4$ and 5 mM EGTA and embedded in 4:1 (v/v) butyl methacrylate to methyl methacrylate (Polysciences, Inc., Warrington, PA, USA) as described by Baskin et al. (1992). Samples were sectioned at a thickness of 3 μm and affixed to slides coated with 3-aminopropyltriethoxysilane. Callose was detected using a mouse monoclonal antibody that recognizes (1→3)-β-D-glucans including callose (Meikle et al. 1991, Biosupplies Australia, Parkville, Australia) and an Alexa Fluor 488 rabbit anti-mouse secondary antibody. Representative images were selected from observations of at least five seedlings.

Effect of *Diploschistes* extracts on seed germination and root growth of *Bromus tectorum*

We used two approaches to collect chemicals from *D. muscorum*. One approach was to invert the lichen and letting it leak chemicals overnight to a small volume of water (~4 cm^2 of thallus surface area per ml of water). We used this water extract to soak filter papers where we placed the seeds. The second approach

involved homogenizing dry samples of *D. muscorum* thalli in liquid nitrogen and extracting 5 g of the homogenate with 50 ml of 80% acetone. The samples were extracted overnight at 4°C. Afterward, we filtered the suspension through Whatman #1 paper and used the filtrate to analyze the effect of this acetone extract on germination and root growth. Three layers of filter paper were placed on plastic trays (10×10×4 cm) and the papers were soaked with either 8 ml of 80% acetone (control treatment) or 8 ml of *Diploschistes* acetone extract. The trays were then left for 48 h in a fume hood to vaporize the acetone. Subsequently, the filter papers were rewetted with 8 ml of distilled water or 8 ml of 20 mM 2-(*N*-morpholine)-ethane sulfonic acid (MES) buffer (pH 6.5) and 50 seeds were placed in each tray. We covered the trays with lids and incubated them in a growth chamber under the conditions described earlier. After eight days, we determined the percent germination, the average root length, and the percent of roots with brown tips.

Results

Seed water status and germination

Four days after seeding, we observed significant differences in seed water potential among the seedbed surfaces (Table 1). For both grass species, the water potential of seeds on the *Diploschistes* crust was significantly lower than that of seeds on the soil or mixed crust surfaces (Fig. 1a). These differences were detected in both the samples that were watered every other day (wet treatment) and those that were watered only once, immediately after seeding (dry treatment). The statistical analysis also indicated that the watering regimen had a significant effect on water potential ($P<.0001$). This reflects that the water potential of seeds in the wet treatment was overall somewhat higher than that of seeds in the dry treatment. However, there were significant interactions between the factors tested (Table 1). Comparison between the wet and dry treatment for the same species-seedbed surface combination (i.e. *V. microstachys*-soil-wet versus *V. microstachys*-soil-dry) revealed that differences in water potential were only statistically significant for *V. microstachys* seeds on the *Diploschistes* crust (Fig. 1a).

The analysis of seed water content by weight yielded similar results to those obtained by measurements of seeds water potential. Prior to seeding, the seeds had water contents between 8 and 10%. During 4 days on bare soil or biological soil crusts, the seeds gained water albeit in different amounts. For both grass species, the water content of seeds on the *Diploschistes* crust was significantly lower than that of seeds on the other seedbed surfaces (Fig. 1b). In addition, on the soil and mixed crust the water content of *B. tectorum* seeds in the wet treatment was higher than those in the dry treatment.

Differences in water potential and water content between treatments roughly correlated with differ-

Table 1 Test for significance of fixed factors (grass species, seedbed surface, watering regimen and the interaction of these factors) on water potential, water content, and germination of *Vulpia microstachys* and *Bromus tectorum* seeds after 4 and 8 days from seeding

Factors	DF Num	DF Den	4 days			8 days		
			Pr > *F*			Pr > *F*		
			Water potential	Water Content	Germination	Water potential	Water Content	Germination
Grass species	1	33	<0.0001	<0.0001	0.0004	<0.0001	<0.0001	<0.0001
Seedbed surface	2	33	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Watering regimen	1	33	<0.0001	<0.0001	0.0207	<0.0001	<0.0001	<0.0001
Species × Surface	2	33	0.0341	0.0007	0.0004	0.1065	0.0903	<0.0001
Species × Watering	1	33	0.0575	0.0345	0.2369	0.1838	0.4224	0.6444
Surface × Watering	2	33	0.0765	0.0823	0.2181	0.0345	0.0004	<0.0001
Species × Surface × Watering	2	33	0.03	0.016	0.515	0.1265	0.3374	0.6166

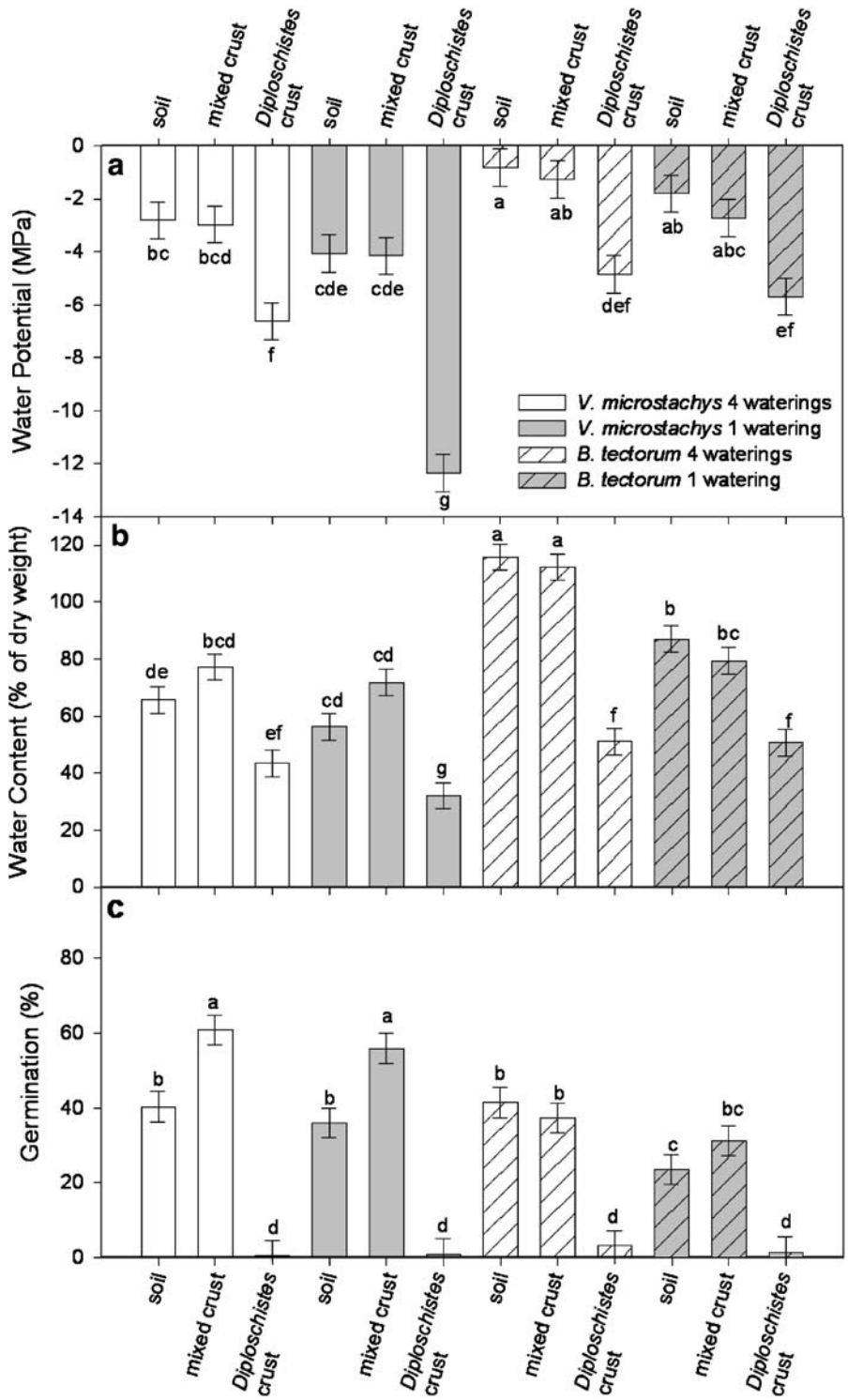

Fig. 1 Water potential (**a**), water content (**b**), and germination (**c**) of *V. microstachys* and *B. tectorum* after 4 d from seeding on soil, mixed crust, and *Diploschistes* crust. Each bar represents the mean (±SE) of four trays. Only un-germinated seeds were used to determine water potential and water content. Approximately 50 seeds were used for each determination of water content and water potential. Bars not labeled with the same letter are significantly different ($P<0.05$) based on Tukey–Kramer least square means test

ences in germination. For both grass species, germination was more than ten times higher on the soil and mixed crust than on the *Diploschistes* crust, where germination was less than 5% (Fig. 1c). For *V. microstachys*, the germination was also higher on the mixed crust than on the soil, even though no clear differences in water potential and water content were observed between the seeds on these surfaces.

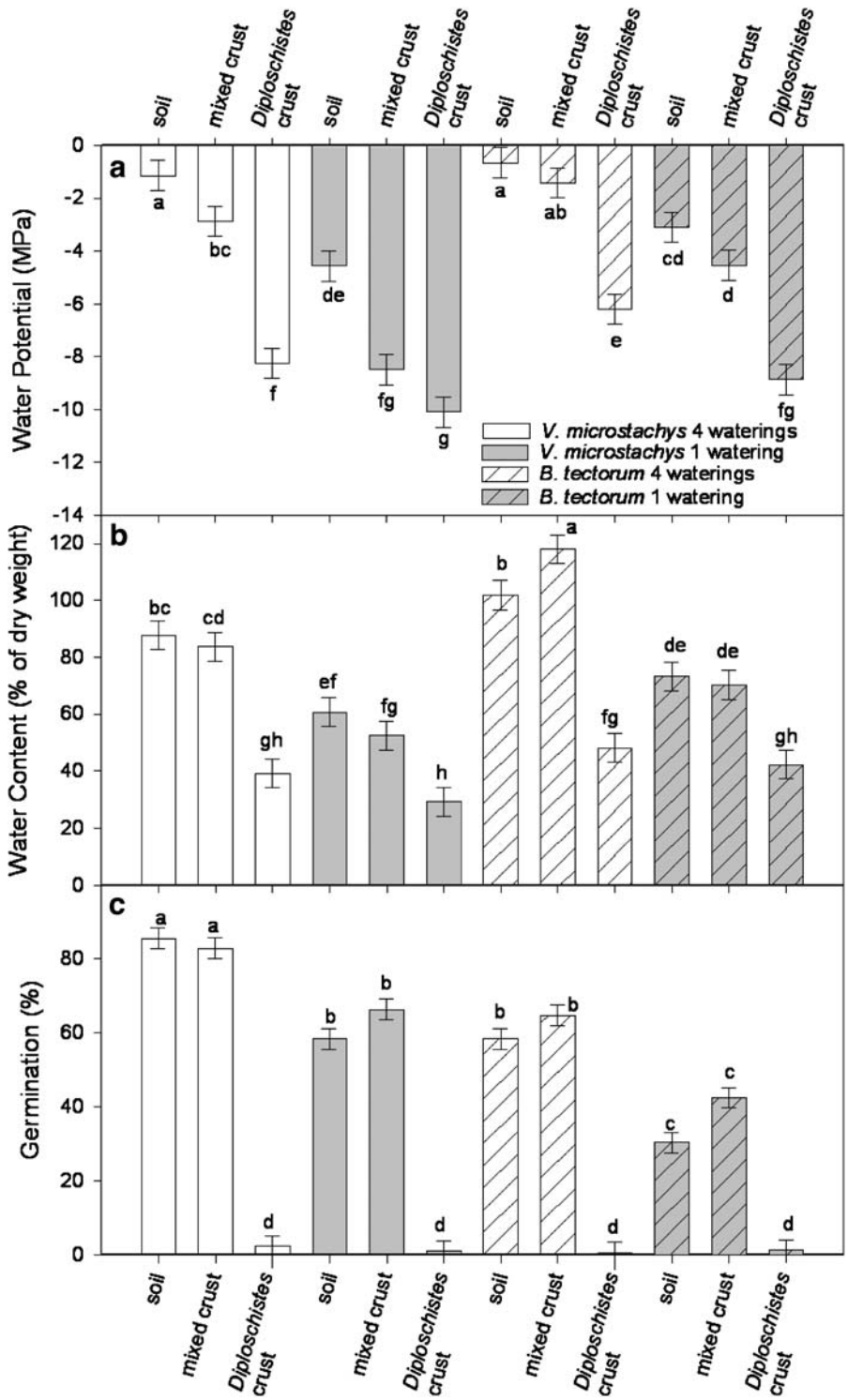

Fig. 2 Water potential (**a**), water content (**b**), and germination (**c**) of *V. microstachys* and *B. tectorum* after 8 d from seeding on soil, mixed crust, and *Diploschistes* crust. Each bar represents the mean (±SE) of at least four trays. Only un-germinated seeds were used to determine water potential and water content. Approximately 50 seeds were used for each determination of water content and water potential. Bars not labeled with the same letter are significantly different ($P<0.05$) based on Tukey–Kramer least square means test

Eight days after seeding, when most of the germination had occurred on the moist soil treatment, all the factors tested (grass species, seedbed surface, and water regimen) had a significant effect on seed water potential, water content, and germination (Table 1). In the wet treatment, the water potential of seeds on the *Diploschistes* crust was significantly lower than that of seeds on the other surfaces (Fig. 2a). We observed

similar results under dry conditions, except for *V. microstachys* seeds on the mixed crust that had a water potential similar to that of seeds on the *Diploschistes* crust (Fig. 2a). Differences in seed water potential were also apparent between the wet and dry treatment. For each of the seedbed surfaces tested, the water potential of seeds in the wet treatment was higher than that of seeds in the dry treatment (Fig. 2a).

Determination of the seed water content confirmed that seeds on the soil and mixed crust had gained more water than those on the *Diploschistes* crust (Fig. 2b). Also, for seeds on the soil and mixed crust the seed water content was significantly higher in the wet than in the dry treatment. In contrast, for seeds on the *Diploschistes* crust there were no significant differences in water content between the wet and dry treatment. Moreover, seeds on the *Diploschistes* crust in the wet treatment had lower water content than seeds on the soil or mixed crust in the dry treatment.

For both species, there were not significant differences in germination between the soil and mixed crust (Fig. 2c). On these surfaces, the germination was higher for the wet than for the dry treatment and higher for *V. microstachys* than for *B. tectorum*. The differences in germination between species were similar to those that we observed when we tested germination on moist filter paper (data not shown). On the *Diploschistes* crust, there were no differences in germination between wet and dry conditions or between species. Overall, germination on the *Diploschistes* crust was drastically lower than on the other surfaces.

The statistical analysis of the data also indicated that on day 8 the interaction between seedbed surface and watering regimen had a significant effect on all the dependent variables measured (Table 1). This reflects that the wet treatment increased seed water potential, water content, and germination on the soil and mixed crust, but had much less effect on improving seed water status and germination on the *Diploschistes* crust surface.

Water potential of the bare soil, mixed crust, and *Diploschistes* crust

During most of the experiment, the water potentials of the bare soil and mixed crust remained above −0.3 MPa (Fig. 3). The water potentials under the wet treatment were not significantly different from those in the dry treatment until day 8. By day 8, the water potentials of the soil and mixed crust in the dry treatment were lower than that of the soil-wet treatment. The *Diploschistes* crust showed more variation in water potential than the other surfaces. Four hours after the initial watering, the *Diploschistes* crust had water potentials between −0.3 and −0.7 MPa, which were not statistically different from those in the soil and mixed crust treatments. In contrast, on days 1 through 8 the water potentials of the *Diploschistes* crust were in most cases significantly lower than those of the soil or mixed crust. This trend was observed for both the wet and dry treatments. Thus, repeated watering every 48 h did not have a lasting effect on increasing the *Diploschistes* water potential. Overall, *Diploschistes* water potentials measured between 1 and 8 days from the last watering were low ranging in value between −2 and −9 MPa.

Germination and root penetration under 100% relative humidity

When experiments were conducted in an environment with nearly 100% relative humidity, we did not detect significant differences in germination between seeds on the *Diploschistes* crust and those on bare soil. Eight days after seeding, the percent germination of *V. microstachys* was 86.4 (±4.2) and 87.7 (±3.8) on the *Diploschistes* crust and bare soil, respectively. Similarly, the percent germination of *B. tectorum* was 70.1 (±4.3) and 66.1 (±8.9) on the *Diploschistes* crust and bare soil, respectively. Even though the wet environment resulted in an increase in germination, this was not accompanied by a parallel increase in seedlings establishment. On the bare soil, all the seeds that germinated showed root penetration and seedling establishment. In contrast, root penetration through the *Diploschistes* crust was negligible, 1.8 (±1.5) and 1.4 (±1.1) % for *V. microstachys* and *B. tectorum*, respectively.

On the *Diploschistes* crust, many roots showed symptoms of injury. In particular, a large proportion of the roots developed brown tips (Fig. 4a and b). The percent of roots with brown tips was 60.1 (±8.4) and 64.5 (±8.7) for *V. microstachys* and *B. tectorum*, respectively. The roots with brown tips also showed swollen regions, changes in the morphology of epider-

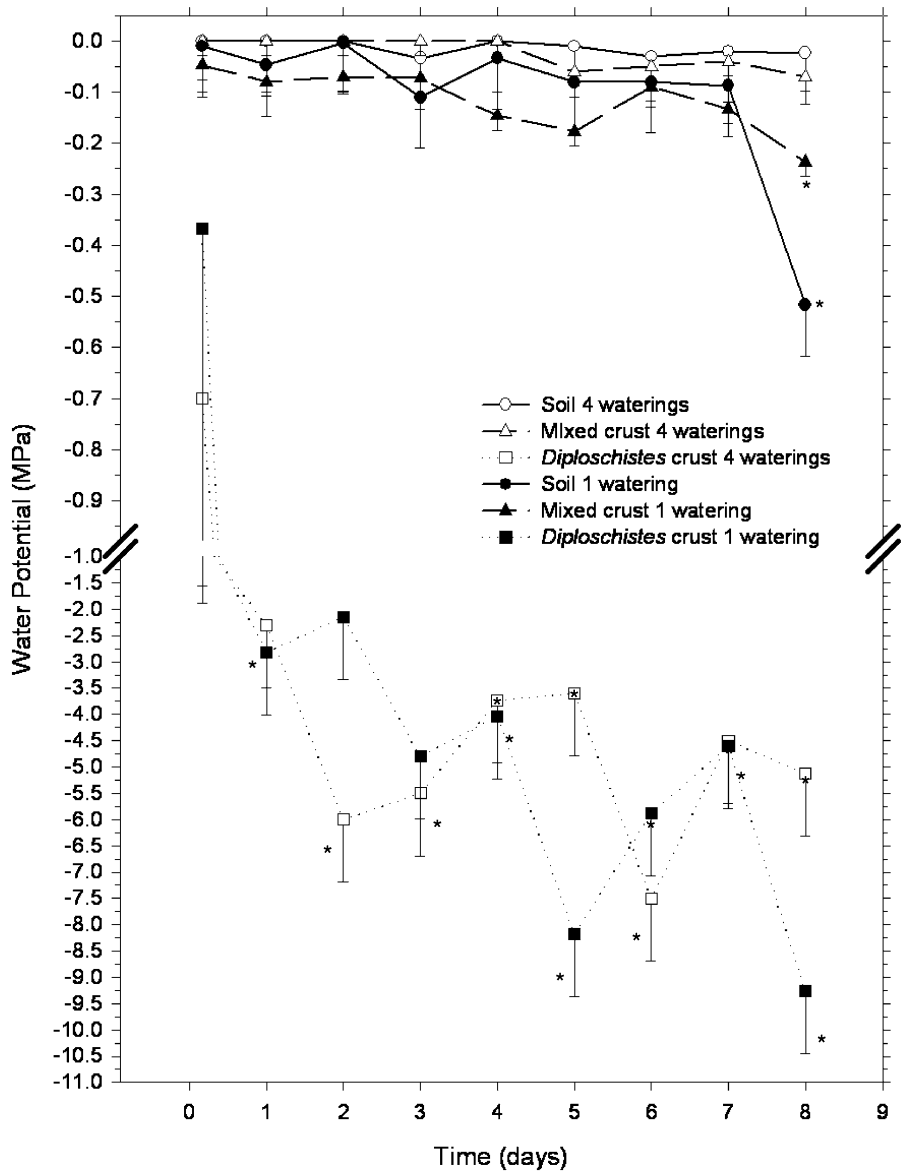

Fig. 3 Water potentials of the bare soil surface, mixed crust, and *Diploschistes* crust during the time course of the experiment. Means (±SE) of at least 3 trays. Means with an asterisk are significantly different from the bare soil treatment ($P<0.05$) based on Tukey–Kramer least square means test

mal cells, and accumulation of callose (Fig. 4d and f). Ultimately, the root tips became necrotic as indicated by the high incorporation of propidium iodide into most cells (Fig. 4h). In contrast, control roots only showed positive staining with propidium iodide in border cells of the root cap and a few epidermal cells (Fig. 4g).

Effect of *Diploschistes* extracts on seed germination and root growth of *Bromus tectorum*

Seeds placed on water extracts of *D. muscorum* had a percent germination virtually identical to that of seeds placed on water, 67.6 (±5.4) and 68.9 (±4.2), respectively. Root growth was also similar. Eight days after seeding, the average root length was 9.1 (±1.8) mm for the seedlings on the lichen extract and 10.7 (±1.2) mm for those on water. Furthermore, we did not detect differences on the percent of brown root tips, which was about 2% for both treatments.

In contrast to the water extract, the acetone extract of *D. muscorum* decreased germination, significantly inhibited root growth, and triggered browning of the root tips (Table 2). The acetone extract acidified the water added to the filter paper to pH 4.0. When the pH of the acetone extract was maintained at about 6.5

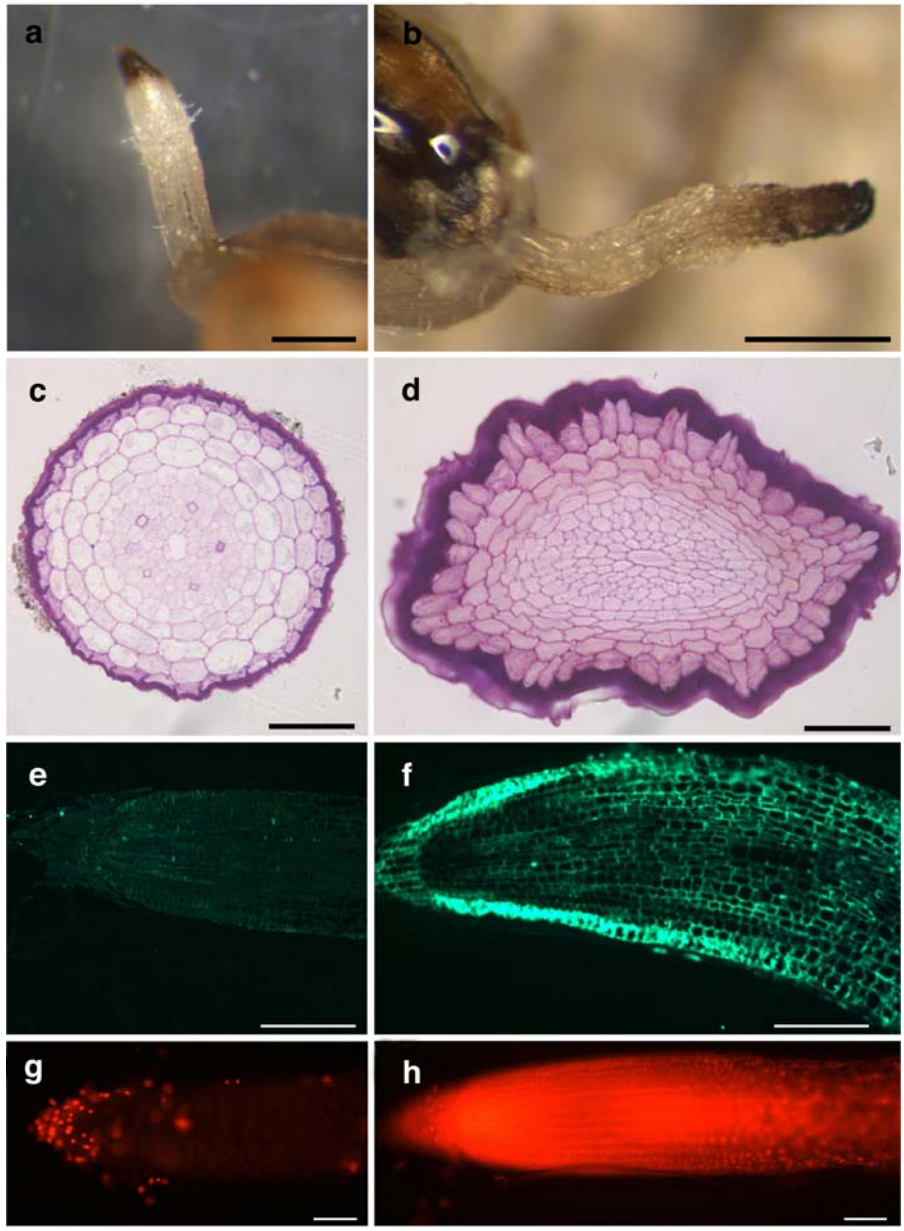

Fig. 4 Effect of the *Diploschistes* crust on young roots of *Bromus tectorum*. *Bromus tectorum* roots with damaged root tips (**a**, **b**). (**c**, **e**, and **g**) Roots from control plants. (**d**, **f**, and **h**) Roots from seeds that germinated on the *Diploschistes* crust. (**c** and **d**) Cross sections stained for polysaccharides by the periodic acid-Schiff's reaction. (**e** and **f**) Immunolocalization of callose on longitudinal sections. (**g** and **h**) Whole mounts of root tips incubated with propidium iodide to detect loss of cell viability. Bars: in **a**, **b**, 500 μm; in **c**, **d**, **e**, **f**, 50 μm; in **g** and **h**, 100 μm

Table 2 Effect of acetone extracts of *Diploschistes muscorum* on germination, root growth, and root tip browning of *Bromus tectorum*

	Germination (%)	Average Root Length (mm)	Brown Root Tips (%)
Water	72.3 ± 3.9^{a}	7.4 ± 0.4^{bc}	0.6 ± 0.6^{b}
Acetone extract + water	42.9 ± 3.9^{b}	1.8 ± 0.4^{d}	63.1 ± 7.5^{a}
20 mM MES buffer (pH 6.5)	70.0 ± 3.9^{a}	11.9 ± 0.4^{a}	0.9 ± 0.9^{b}
Acetone extract + 20 mM MES buffer (pH 6.5)	54.2 ± 3.9^{ab}	9.2 ± 0.4^{b}	3.0 ± 0.4^{b}
20 mM K-phosphate buffer (pH 4.0)	56.1 ± 3.9^{ab}	5.5 ± 0.4^{c}	0.9 ± 0.9^{b}

Within a column, values not labeled with the same letter are significantly different ($P<0.05$) based on Tukey–Kramer least square means test.

Means (±SE) of 3 trays, 50 seeds were used in each tray.

by addition of MES, the average root length and the percent of brown tips were similar to the control treatments (Table 2). A phosphate buffer at pH 4.0 did not mimic, however, the effects of the acetone extract on the roots. The percent of brown root tips was only 0.9 in the phosphate buffer and 63% on the unbuffered acetone extract. The combined results suggest that the acetone extract has compounds that inhibit root growth but only at low pH. Based on measurements made using pH paper, the moist surface of the *Diploschistes* crust had a pH of about 6.0. Similarly, water that has been in contact overnight with the *Diploschistes* surface had a pH of 6.0. At this pH, the acetone extract did not have an inhibitory effect on the roots. Consequently, it is unlikely that compounds present in the lichen were responsible for the browning of root tips observed on the intact crust.

Discussion

Based on our results, the *Diploschistes* crust inhibited germination of *B. tectorum* and *V. microstachys* through an effect on seed water status. The water potentials of seeds on the *Diploschistes* crust were between 1 and 6 MPa lower than those of seeds on the bare soil or mixed crust. These differences in water potential corresponded with differences in germination, which was significantly lower on the *Diploschistes* crust than on the other two surfaces tested. The germination response in an environment with 100% relative humidity also indicated that water was the main factor limiting germination on the *Diploschistes* crust. In the high humidity environment, water was presumably no longer restricting imbibition and some free water was present on the crust due to condensation during the dark periods. Under these conditions, seed germination on the *Diploschistes* crust was similar to that on the bare soil surface.

The notion that the presence of biological soil crusts can decrease water availability during seed germination is in agreement with other studies (Otsus and Zoel 2004; Morgan 2006). For example, Otsus and Zoel (2004) reported that to reach similar percent germination, *Festuca* seeds required more water when they were on bryophyte mats than on bare soil. Moreover, in a previous study, we have shown that the water potential and germination of *B. tectorum* seeds were significantly lower on the short moss *Bryum argenteum* Hedw. than on bare soil (Serpe et al. 2006). Thus, the effect of biological soil crusts on reducing seed water potential and germination is not limited to the *Diploschistes* crust used in this study.

Although the water status of the seeds in general corresponded with differences in germination, there were some exceptions. Four days after seeding, *V. microstachys* seeds on bare soil had similar water potential to those on the mixed crust, but germination was significantly higher on the latter one (cf. Fig. 1). Also, on the soil and mixed crust, germination rates were up to 50% in treatments where the seed water potentials were below −2 to −3 MPa; which are too low to sustain any germination (Bauer et al. 1998). These apparent incongruities may reflect that our measurements most likely underestimated the overall water potential of the seeds. Within each tray, variations in seed water potential probably developed due to surface roughness. The mixed crust in particular has many micro-depressions that act as sinks for water. Seeds in these depressions may imbibe water and germinate sooner than those on bare soil or small mounds on the mixed crust. Because we only measured the water status of seeds that had not germinated, we may have preferentially measured the driest seeds in the trays.

Two factors that can affect seed water status are the water potential of the medium surrounding the seeds and the degree of contact between the seeds and the seedbed (Collis-George and Hector 1966; Rogers and Dubetz 1980; Bradford 1995). The water potential of the *Diploschistes* crust was significantly lower than that of the other surfaces, which at least partially accounts for the low water status of seeds on the *Diploschistes* surface. In this regard, the *Diploschistes* crust differed from the moss crust dominated by *B. argenteum*, which also inhibited germination of *B. tectorum* (Serpe et al. 2006). Seed water potential was lower on both crusts, but the water potential of the moss crust was similar to that of the bare soil. This suggests that the lichen, and moss crust reduced seed imbibition by somewhat different mechanisms.

Differences in seed water status were also observed between the dry and wet treatments. In contrast, for the three seedbed surfaces there were no clear differences in water potential between the dry and wet treatment. The higher water potential for seeds in the wet treatment may be attributed to direct water absorption by the seeds during the watering events.

In addition, rain splash caused by watering may have increased seed displacement to micro-depressions or detachment of soil particles that subsequently covered the seeds (Eckert et al. 1986; Chambers 2000). Both of these events would tend to maintain moister conditions around the seeds resulting in higher seed water potential (Chambers 2000; Morgan 2006).

While some displacement of soil particles occurred in our experiments, this phenomenon may increase under field conditions, particularly during the summer when the seeds are in a dormant state and are exposed to the action of wind for prolonged periods. Furthermore, movement of soil particles is more likely to occur on bare soil than on biological soil crusts (Belnap et al. 2001). A higher rate of seed burial in the bare soil than in the mixed crust, for example, could result in higher germination in the former. Similarly, seed displacement may be different in a natural setting. In our experiments, we placed the seeds at random over the crust where most of the seeds remained. Under field conditions, the seeds may be preferentially displaced outside the crust or between lichens. Under this scenario, the effect of the *Diploschistes* crust on inhibiting germination in a particular area would be reduced because a larger proportion of the seeds would be outside rather than on the crust. Experiments aimed at comparing the fate of seeds on the soil and biological soil crusts would help to ascertain whether unequal burying of seeds and seed displacement play a role in determining germination success under field conditions (Chambers 2000).

Although we watered the three seedbed surfaces with the same amounts of water, only the bare soil and mixed crust maintained high water potentials. At field capacity, the water holding capacity of the soil, mixed crust, and *Diploschistes* crust was 0.31 (±0.02), 0.7 (±0.1), and 0.23 (±0.07) g of water per cm^3 of seedbed. The lower water holding capacity of the *Diploschistes* crust may partly account for the rapid decrease in water potential of this surface. The water holding capacity of the *Diploschistes* crust was, however, not significantly lower than that of the bare soil. Thus, other factors appear to determine the water status of these surfaces. One possibility is that the bare soil maintains a better hydraulic contact with layers beneath the surface than the *Diploschistes* crust. *Diploschistes muscorum* is a stratified lichen. Lichens with this type of anatomy tend to have, even when fully hydrated, many air filled spaces in their inner thalli due to presence of hydrophobic molecules that prevent waterlogging (Lange et al. 1997; Trembley et al. 2002; Lakatos et al. 2006). The presence of such spaces would decrease water replenishment via capillary action.

Independent of the mechanisms involved, an important result of this study was the observation that *D. muscorum* had significantly lower water potentials than the soil and mixed crust. It is somewhat counterintuitive that an organism adapted to live in arid environments cannot maintain high water potentials even under frequent watering. Nevertheless, this characteristic may be of adaptive value to *D. muscorum*. The average water potential for the *Diploschistes* crust over 8 days was −4.3 and −4.7 MPa for the wet and dry treatment, respectively. These water potentials are above the moisture compensation point of *D. muscorum*, which occurs at about −20 MPa (Lange 2001). Furthermore, Lange et al. (2001) reported that at −5 MPa some lichens have photosynthetic rates that are only 5% below the maximum. Thus, under our experimental conditions *D. muscorum* had water potentials that allow photosynthetic carbon gain, but that are too low to sustain plant growth. The low water potentials measured in *D. muscorum* are likely to prevent germination of most seeds and moss spores, and reduce the growth of fungal hyphae (Money 1997; Manandhar 1998; Wiklund and Rydin 2004). This would limit the establishment of plants and fungi on the *D. muscorum* surface, thereby favoring lichen persistence in areas occupied by this organism.

In conclusion, our study showed that the biological soil crust dominated by the lichen *D. muscorum* had a negative effect on seed water status, which correlated with a decrease in germination of the two annual grasses tested. Even when water was not limiting germination, *D. muscorum* inhibited seedling establishment. The root tips did not penetrate the *Diploschistes* crust and became necrotic. The factors that triggered root tip browning require further investigation but may include physical stresses associated with growth on a hard surface or drying of the roots tips despite the high humidity environment (Potters et al. 2007). The ability of *D. muscorum* to reduce seed germination and root penetration may be of adaptive value to this lichen. Suppression of plant growth would tend to reduce the mechanical damage associated with root penetration, decrease vegetation cover over the crust, and lessen

the spread of bryophytes and microorganisms on the lichen surface (Wiklund and Rydin 2004; Belnap et al. 2006). It would be interesting to determine whether other crustose lichens have similar effects on seedling establishment. Crustose lichens are common components of biological soil crusts in the Great Basin (Rosentreter and Belnap 2001). An inhibition of seedling establishment by these organisms may not only provide an adaptive advantage to them, but also tend to reduce the spread of invasive species such as *B. tectorum* and contribute to maintaining a patchy vegetation distribution (Deines et al. 2007). Patchiness reduces the incidence of wildfires and promotes the establishment of diverse sagebrush communities (Brooks et al. 2004; Bowker 2007).

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