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Short communication

Effects of biological soil crust and water availability on seedlings of three perennial Patagonian species

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ABSTRACT

Biological soil crusts are communities composed of mosses, lichens and cyanobacteria. They cover a large portion of the arid and semiarid regions of the world and play key roles in nutrient cycles and soil erosion diminution. Biological soil crust might affect germination, establishment and growth of vascular plants. In this study we examined the differential response to soil cover and water availability of grasses and shrubs. Our hypothesis was that seed germination and seedling survival are greater in soils with biological soil crust cover because crust have positive effects on vascular plant establishment. We evaluated the effect of biological soil crust cover on seed emergence, survival and seedling growth of *Nassella tenuis*, *Senecio subulatus* and *Larrea divaricata*. The experiment had four treatments combining biological soil crust (C) or bare soil (BS) with drought (D) or soil at field capacity (FC). Seeds of *L. divaricata* did not germinate and *S. subulatus* only germinated in BS-FC. Emergence and biomass of seedlings of *N. tenuis* were higher with C and at FC. There were no differences in seedling survival. Soil cover with biological soil crust and water availability influenced germination and seedling growth and their effects are different according to the species or plant functional groups. Germination of seeds varies with the composition and structure of the biological soil crust and the vascular plants, and the response may change with different species or plant functional groups.

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Biological soil crusts cover a large portion of the arid and semiarid regions of the world. These communities are composed of mosses, lichens and cyanobacteria (Belnap et al., 2001) and they play key roles in nutrient cycles and in the diminution of erosion (Maestre et al., 2011). The community structure of biological soil crust also influences soil microfaunal activity and, depending on the dominant group, they may change the water distribution because lichens are negatively associated with infiltration, whereas mosses are positively correlated with infiltration (Eldridge et al., 2010). Some species of mosses can rehydrate and store considerable volumes of water (Eldridge et al., 2010). They show high tolerance to extreme temperatures and light, which allows them to survive under conditions that limit growth in vascular plants (Belnap, 2003).

The changes produced by crusts on the soil surface might affect

the germination, establishment and growth of vascular plants (Belnap et al., 2001; Gomez et al., 2012). The influence of biological soil crust on germination may be positive, negative or neutral (Belnap et al., 2001; During and Van Tooren, 1990) mainly due to changes in temperature and moisture.

Patagonian Monte is a shrubland with vegetation distributed in patches dominated by shrubs with grasses, subshrubs, herbs and biological soil crust growing under their canopy, and immersed in a matrix of bare soil (Bertiller and Ares, 2011). The biological soil crust are principally dominated by moss, and cover most inter-shrub spaces in areas of low grazing intensity where trampling by livestock is reduced (Funk et al., 2014). On the other hand, under grazing conditions, biological soil crust grows in association with dominant shrub species, such as the observed with *Larrea* spp. (Gómez et al., 2012).

In our study we examined the differential response to soil cover and water availability of grasses and shrubs. Our hypothesis was that seed germination and seedling survival are greater in soils with biological soil crust cover because crust have positive effects on vascular plant establishment. To test this hypothesis, we performed

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a greenhouse experiment for nine weeks, and we evaluated the effect of biological soil crust cover and the response of seed emergence, survival and seedling growth of three native perennial species under drought and watering to field capacity regimes.

The experiment was conducted with four treatments: pots with biological soil crust at field capacity (C-FC); pots with biological soil crust and drought (C-D); pots with bare soil at field capacity (BS-FC); and pots with bare soil and drought (BS-D). Soil and biological soil crust were collected from natural sites in the Patagonian Monte, located in Patagones department, Buenos Aires province (40°39'S, 62°54'W), during the winter of 2013. Biological soil crust samples were collected using a cylindrical soil sampler of 6 cm diameter and 5 cm depth. Samples were placed in 300 cm³ circular pots of 6 cm diameter, filled with soil from the area and they were placed in the greenhouse. The biological soil crust were distributed as an intact layer, when it was possible, over the entire surface of the pots, covering >90% of its surface. Biological soil crust are composed of two species of mosses: *Syntrichia princeps* (De Not.) Mitt. and *Ceratodon purpureus* (Hedw.) Brid. In addition, seeds of *Nassella tenuis* (Phil.) Barkworth, *Senecio subulatus* D. Don ex Hook. et Arn. and *Larrea divaricata* Cav. were collected in the same area from at least 20 different mother plants during spring-summer 2012–2013 and stored in paper bags in a dark and dry place until the start of the experiment. *L. divaricata* seeds were scarified to break primary seed dormancy. We chose *N. tenuis* as a representative grass species because it is widely spread in the Monte system, has a high germination power and is important as a forage species. We chose two shrub species of different communities of Monte. *L. divaricata* is a dominant species of the typical community composition and *S. subulatus* is a dominant species in sandy soil communities (Velasco and Siffredi, 2010), where biological soil crust is not characteristic.

The experiment was completely randomized for evaluating the effect of biological soil crust coverage ($k = 2$, with and without biological soil crust) and soil moisture ($k = 2$, field capacity and drought). For the treatment with crust (C), the soil in the pots was covered with biological soil crust, while pots without crust (BS) were only filled with soil. Pots were weighed and watered to field capacity or to half field capacity (drought treatment, Moretto and Distel, 1997) every 48 h. Field capacity was determined by watering the pots until the soil was saturated and allowing them to drain for 48 h. After this, the weight of each pot was recorded. In order to establish the amount of water used in the treatments at field capacity and under drought, a subset of pots was oven dried (105 °C) and the moisture content (grams of water per pot) was determined

by the gravimetric method. In this way, a fixed weight was assigned to each pot depending on its water content, according to each treatment. Each treatment combination had 10 replicates, totaling 40 experimental units for each species. Ten seeds of one species were sown in each pot. The pots were kept in a greenhouse under a natural light cycle with media of 14 h of light and 10 h of darkness. Average temperature for the period of the experiment was 36 °C, and the mean minimum and maximum temperature were 10 and 46 °C. The temperature registered in the greenhouse was similar to the temperature of the soil surface in the Patagonian Monte (Busso and Bonvissuto, 2009). Prior to the experiment, we tested the germination percentage by placing 20 seeds of each species in Petri dishes (6 cm diameter) containing filter paper saturated with water ($n = 4$). Seeds were incubated in a germination stove at 20 °C with a photoperiod of 8/16 h for 40 days. The seed germination percentage under these conditions was 48.75% for *S. subulatus*, 15% for *L. divaricata* and 53.75% for *N. tenuis*. In addition, eight pots without any seed were used as controls and were maintained at field capacity in order to verify the absence of seeds of the three species in the soil or biological soil crust.

All seedlings were kept in the pots for nine weeks and then they were harvested. Seedling mortality was observed during this period. For each harvested plant, we measured the seedling biomass.

The effects of biological soil crust coverage and soil moisture on germination were tested in a two-way ANOVA. Statistic interaction between moisture and soil cover was not found in any of the variables analysed in this study. The assumption of normal distribution was tested with Shapiro–Wilks test. Total seedling biomass data were not normally distributed and they were natural log ($x + 1$) transformed. When normalization failed, as for seedling survival, aerial and root biomass, Kruskal–Wallis tests were employed. Means were compared using Tukey HSD tests. All statistics were performed with Infostat software (Di Rienzo et al., 2014).

Seeds of *L. divaricata* did not germinate under any treatment conditions. In the case of *S. subulatus*, this species only germinated in BS-FC. So, we did not compare the results of these two species, and only the results of *N. tenuis* are presented and discussed.

Emergence of seedlings of *N. tenuis* was higher in treatments with biological soil crust than in pots with bare soil ($P = 0.008$) (Fig. 1). It was also higher in pots at field capacity ($P < 0.0001$), without showing any differences between those having the surface covered with biological soil crust or bare soil (Fig. 1). In the bare soil treatments, emergence was $29.00 \pm 6.02\%$, whereas in the treatments with biological soil crust it was $46.50 \pm 5.59\%$. Similarly, in

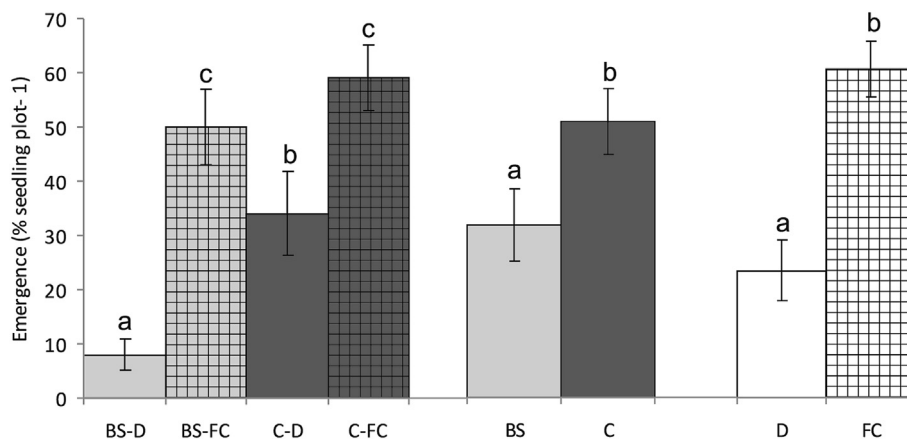


Fig. 1. Emergence percentage of *Nassella tenuis* (seedlings per plot) measured during the experiment in different treatments. References: C, biological soil crust; BS, bare soil; FC, field capacity; D, drought. Means \pm S.E. Different letters indicate significant differences between treatments at $P < 0.05$.

Table 1
Mean values of all parameters measured during the experiment in different treatments. References: C, biological soil crust; BS, bare soil; FC, field capacity; D, drought. Means \pm S.E. Different letters indicate significant differences between treatments at $P < 0.05$.

Variables	BS-D	BS-FC	C-D	C-FC
Emergence (%)	8 \pm 2.91 ^a	50 \pm 6.83 ^c	34 \pm 7.77 ^b	59 \pm 6.05 ^c
Survival (%)	83.33 \pm 40.82	89.82 \pm 16.50	96.56 \pm 6.85	93.50 \pm 14.15
Total biomass (g/seedling)	0.02 \pm 0.01 ^a	0.13 \pm 0.04 ^{bc}	0.11 \pm 0.02 ^b	0.17 \pm 0.02 ^c
Aerial biomass (g/seedling)	0.01 \pm 0.01 ^a	0.05 \pm 0.01 ^b	0.07 \pm 0.01 ^{bc}	0.09 \pm 0.01 ^c
Radical biomass (g/seedling)	0.01 \pm 0.01 ^a	0.08 \pm 0.02 ^{bc}	0.05 \pm 0.01 ^{ab}	0.08 \pm 0.01 ^c

the pots that were at field capacity, 54.50 \pm 4.56% of the seedlings emerged; whereas in pots under drought conditions only 21.00 \pm 5.02% of the seedlings emerged.

The control pots did not show any seedlings of these species during the experimental period.

There were no significant differences in seedling survival between any treatments during the experiment (Table 1).

Total seedling biomass was higher in treatments at field capacity than in those under drought conditions ($P = 0.0061$) and in treatments with a cover of biological soil crust than in bare soil ($P = 0.03$) (Fig. 2). Moreover, it was significantly different in the combination of treatments, because C-FC was higher than C-D and BS-D (Fig. 2, Table 1). Radical biomass showed the same results as total biomass whereas in aerial biomass there were differences

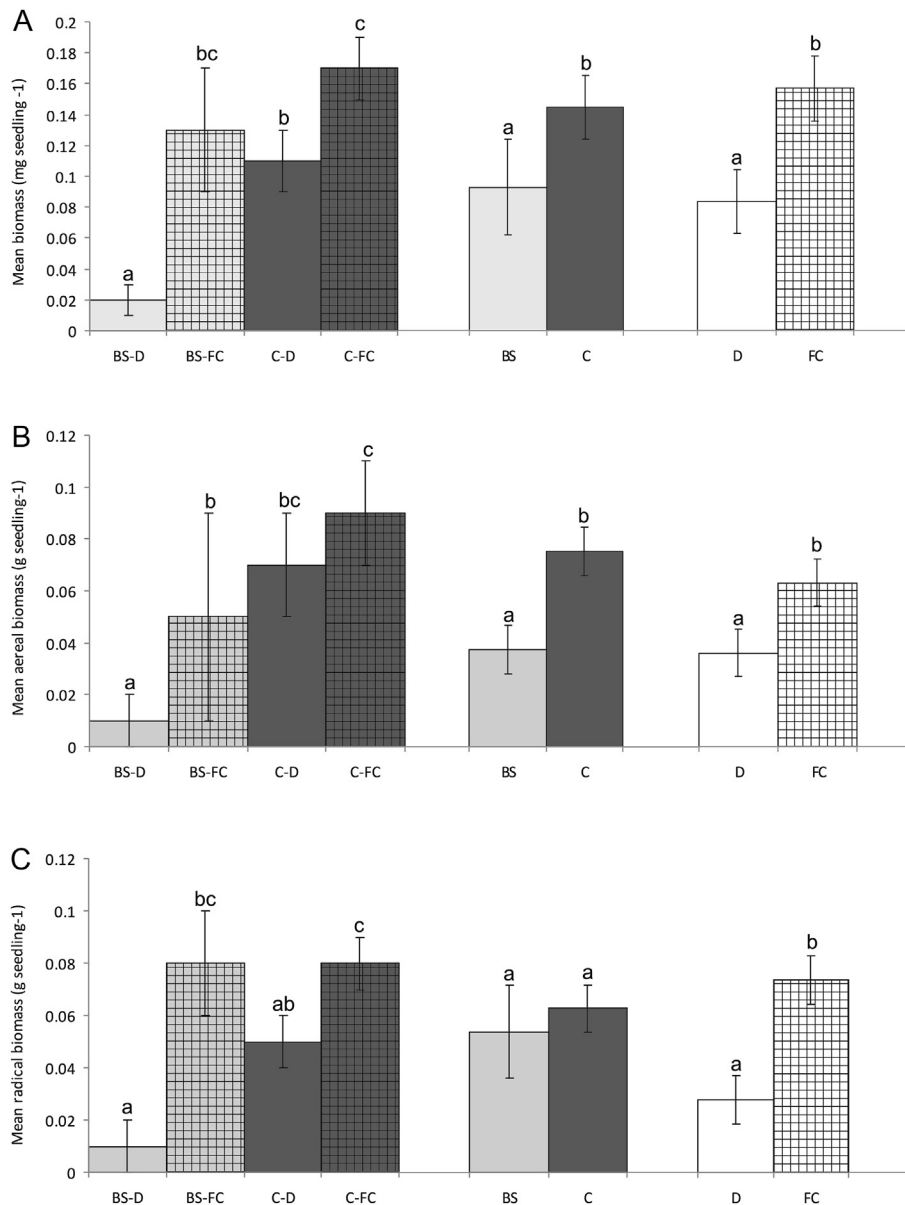


Fig. 2. Mean biomass of seedlings of *Nassella tenuis* (g, per seedling) measured during the experiment in different treatments. A) Total biomass. B) Aerial biomass. C) Radical biomass. References: C, biological soil crust; BS, bare soil; FC, field capacity; D, drought. Means \pm S.E. Different letters indicate significant differences between treatments at $P < 0.05$.

between treatments at field capacity, where C-FC was higher than BS-FC (Fig. 2, Table 1).

Our results support our hypothesis that soil cover with biological soil crust and water availability influenced both the germination and seedling growth and their effects are different according to the species. In opposition to these conclusions, neither soil cover nor water availability would have affected seedling survival.

The reason for the absence of emergence of *S. subulatus* in the covered soil could be because biological soil crust is not characteristic of the sandy soil in the Monte province.

In contrast with the results of Bisigato and Bertiller (1999) we did not observe any establishment of *L. divaricata*. However, already Barbour (1968) mentioned that it is a very difficult species to establish under laboratory or greenhouse conditions, even in a natural habitat. Here, we only observed radical emergence, but neither cotyledon emergence nor seedling establishment.

We observed a positive effect of soil covered by biological soil crust and of water availability in opposition to the results of Bisigato and Bertiller (1999), who did not observe any differences between the effects of microsites (beneath vegetation patches and inter-patches) or the soil water content on the emergence and survival of *N. tenuis*. In a previous work with another perennial grass, *Poa ligularis*, Funk et al. (2014) found that biological soil crust have a negative effect on the emergence of this species, and Langhans et al. (2009) mentioned that the establishment of perennial species is usually inhibited by crust cover, and that this negative effect seems to be stronger for grass species (Langhans et al., 2009; Serpe et al., 2006). In our study there was no difference in emergence between soil with and without biological crust at field capacity, but under dry conditions, the presence of a biological soil crust improved the emergence of *N. tenuis*. We did not measure soil moisture, but Funk et al. (2014) mentioned for the same system (same soil type and same species of the biological soil crust) that there were no differences in the water loss between soil with and without biological crust. We considered that changes in bulk density and nutrient cycles did not occur, because the soil used to fill the pots was collected at the same site and the time of possible influence of the biological soil crust on soil quality was too short. Escolar et al. (2015) found that biological soil crust diminish the effect of the high temperature on the soil. Hence, we conclude that the key factor for the emergence of *N. tenuis* could be the protection of the soil against high temperature, besides other variables that could depend directly or indirectly of crust cover. In the case of the greenhouse in this study, with high temperatures, we think that the emergence would be greater in soil covered by biological crust because of that protection.

Some morphological aspects, such as the shape of seeds and the characteristics of the biological soil crust may determine if the crust enhance or inhibit germination (Li et al., 2008). Seeds of *N. tenuis* have a large size and hygroscopic awns as anchorage appendages, and they are dispersed after reaching the soil surface (Pazos and Bertiller, 2007). Since biological soil crust act as seed traps (Bertiller and Ares, 2011), in nature, seeds of *N. tenuis* are trapped in biological soil crust, especially when it is principally composed of mosses.

In relation to seedling growth, both water availability and biological soil crust cover have positive effects on biomass, as the aerial, radical and total biomass were higher at field capacity and with biological soil crust. These results are consistent with those found by Langhans et al. (2009) and Funk et al. (2014), where the continuous availability of water enhanced the survival and biomass of seedlings of the native species studied. Belnap et al. (2001) postulated that the effect of biological soil crust on growth was due to the greater retention of humidity, which was higher than for bare soil. In our experiment we did not study whether the pots with

biological soil crust retained a higher proportion of water as compared with the pots with bare soil, therefore we could not verify that conclusion. But the fact that biological soil crust cover had the same effect than field capacity water supply points that higher water availability in soil crusts dominated by mosses could be behind the results found for biomass.

This study showed that the response of the germination of seeds to water availability and soil cover is different in different species. So, we provide evidence to previous conclusion (Maestre et al., 2011) that the effect of biological soil crust on the germination of seeds varies with the composition and structure of the biological soil crust and the vascular plants. So, it is difficult to generalize the results, since the response may change with different species or plant functional groups, or when the biological soil crust are dominated by organisms other than mosses, such as lichens or cyanobacterias (Zaady et al., 1997).

Author contributions

GP has performed the experiment, data analysis, drafted most of the paper and achieved the financial support. CVL also conducted the experiment and the edition of the paper. FAF has conceived the design of the experiment and also performed the edition of the paper. All the authors have contributed with suggestions and ideas for summarizing and organizing all the information on the material, conscientiously revised the overall article, and approved the submitted version.

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