Biocrusts benefit from plant removal

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PREMISE OF THE STUDY: Productivity in drylands may depend on the sensitivity of interactions between plants and biocrusts. Given future climate variability, it is essential to understand how interactions may be context-dependent with precipitation regime. Furthermore, little is known about the additional interactions of these producers with the belowground biota (e.g., roots, fungi, microarthropods). We evaluated the effect of removal (such as could occur following disturbance) and net interaction of plants and biocrusts and additionally manipulated the abiotic and biotic context.

METHODS: We established field mesocosms containing grass (*Bouteloua gracilis*) and surrounding biocrusts, then clipped the plant or heat-sterilized the biocrust to simulate the loss of dryland producers. To test for context-dependency on the precipitation pattern, we imposed a large, infrequent or small, frequent precipitation regime. A mesh barrier was used to impede belowground connections that may couple the dynamics of producers. Productivity was assessed by plant biomass and biocrust chlorophyll content.

KEY RESULTS: Biocrusts increased chlorophyll content more when plants were removed than when they were present in the first year, but only in the small, frequent precipitation regime. In contrast, plant growth slightly declined with biocrust removal. Plant biomass and biocrust chlorophyll content were negatively correlated in the second year, suggesting net competition. Belowground connectivity weakly promoted overall biocrust relative productivity, but was generally weakly detrimental to plant relative productivity.

CONCLUSIONS: Altered precipitation patterns can amplify positive effects of plant removal on biocrust producers. Furthermore, we discovered that belowground networks contributed to dryland productivity by promoting biocrust performance.

KEY WORDS   biocrust; *Bouteloua gracilis*; competition; cyanobacteria; belowground connections; fungi; plant-microbe interactions; precipitation regime; producer removal.

Identifying factors that alter the strength of interactions among different primary producers is important for predicting net ecosystem productivity under future conditions. Dryland producer communities include both vascular plants and biological soil crusts (biocrusts). Biocrusts are composed of autotrophs and heterotrophs, including cyanobacteria, mosses, lichens, algae, and fungi that live on the soil surface between plant canopies. The net interaction of plants and biocrusts growing together may vary along the competition-facilitation continuum. Plants can shade the soil surface, reducing sunlight for photosynthetic biocrusts. Biocrust microtopography can alter surface soil moisture, and biocrusts with pigments can reduce soil albedo (Rutherford et al., 2017). In addition, plants contribute the majority of organic matter to dryland soils through above- and belowground litter as well as exudates from living plants (Aguilar and Sala, 1999). Biocrusts fix nitrogen (Belnap, 2002) and

intercept most atmospherically deposited resources (e.g., nitrogen). Previous studies have found that biocrusts can enhance or impede plant germination and seedling survival and increase plant nutrient status (reviewed by Zhang et al., 2016). Other recent studies investigated germination and emergence under greenhouse conditions (Tavili et al., 2017) and cold ecosystems (Li et al., 2016), and compared early life history of native, invasive (Song et al., 2017), perennial (Peter et al., 2016) and annual (Ferrenberg et al., 2017a) plant species growing with biocrusts.

The effect of removal of mature plants or biocrusts on each other through disturbances such as grazing, fire, pollution, extreme weather, or mechanical disturbance (Belnap and Eldridge, 2001) is poorly resolved for most ecosystems. Removal can directly affect the resource availability though loss of biomass from the system or release of resources into the system as the organisms die. Additionally,

any other mechanisms underlying plant-biocrust interactions, such as modification of the physical environment or resource cycling, are disrupted by removal as well. There is potential for complex interactions, but little is known about net outcomes of mature plants and biocrusts when one or the other is removed because of disturbance under field conditions: Do these producers function independently because of their spatial separation, compete for limiting resources, or facilitate each other by ameliorating abiotic stress?

Interaction outcomes and responses to the removal of producers may be context-dependent on abiotic conditions (Chamberlain et al., 2014), and understanding the potential for context-dependency is critical to predicting dryland productivity under future climate scenarios. Precipitation regimes are predicted to vary in the future, including the total amount and the intra-annual variability around the mean (Garfin et al., 2014; Shields et al., 2016). Water availability is the key constraint on primary production in drylands (Schwinning and Sala, 2004; Shi et al., 2014), but the rainfall regime can have different effects on plants versus biocrusts. Biocrusts intercept rainfall events of all sizes, enabling their activity (Belnap et al., 2004). However, although small rains initiate biocrust metabolism, without sufficient soil moisture to replenish initial carbon losses via photosynthesis, autotrophic members of biocrusts can decline (Cable and Huxman, 2004; Ferrenberg et al., 2015). Only large rain events increase soil moisture in the rhizospheres of plants sufficiently to activate plant photosynthesis (Huxman et al., 2004). Thus, the ecological effect of the same total amount of water could vary dramatically depending on the frequency and duration of rain events (Knapp et al., 2008). If plant-biocrust response to removal or net interactions vary with precipitation regime, future climates could alter the net productivity in dryland ecosystems.

Plant and biocrusts may additionally interact through belowground networks of roots, fungal hyphae, or trophic webs. Little is known about how these belowground networks affect biocrusts independently (Zhang et al., 2016), but considerable work has shown that belowground biota affect plant performance through herbivory, parasitism, or mutualism (Bardgett and van der Putten, 2014). The potential exists for the belowground organisms to mediate interactions between plants and biocrusts by linking soil surface microsites with deeper rhizosphere microsites. In studies of these potential networks, plant rhizospheres and biocrusts shared approximately half of their fungal taxa (Porras-Alfaro et al., 2011) and one-third of prokaryotic taxa (Steven et al., 2014). Functionally, root-associated fungi can increase plant water and nutrient uptake (van der Heijden, 2015), and this improved plant growth may indirectly feed back to biocrusts via shading or increased litter quality or root exudates. Additionally, belowground biota may directly couple the resource dynamics of plants and biocrusts. This "fungal loop hypothesis" proposed that fungal hyphae connect plants and biocrusts and thereby transfer and retain resources in a biotic pool, increasing overall productivity (Collins et al., 2008). Initial support for the fungal loop hypothesis includes evidence that substrate movement through hyphae is faster than through dry soil (Frey et al., 2003; Ruth et al., 2011), and that isotopically labeled C and N can be translocated between plants and biocrusts (Green et al., 2008; Zhuang et al., 2015). However, experiments that directly test whether belowground connections affect the outcome of plant or biocrust removals or net plant-biocrust interactions, independently of their effects on each producer separately, are lacking. Furthermore, the influence of belowground connections on plant-biocrust interactions following losses of producers may depend on

precipitation regime. Filling this gap in knowledge is important to understand the aboveground-belowground interactions that affect productivity in drylands.

In this study, we addressed two questions: (1) What is the net interaction and effect of removal of mature plants and biocrusts, and how much does the precipitation regime alter these effects? Removal of one producer may release resources that the other producer can use. The net interaction between plants and biocrusts could range from net beneficial to net antagonistic, and thus each producer's response to removal could be positive or negative. The precipitation regime will likely affect the response to removal. Here, we tested whether a small, frequent precipitation regime promoted facilitative plant-biocrust interactions relative to a large, infrequent regime (with the same total volume), or instead intensified plant-biocrust antagonism because water availability was more limited. (2) Do belowground networks affect interactions between plants and biocrusts, and is the effect of belowground connections sensitive to the precipitation regime? We hypothesized that belowground connections would benefit both producers, and that the benefits of connections would be strongest under a large, infrequent precipitation regime when total productivity is higher. If belowground connections are more beneficial when both producers are present, this would support part of the fungal loop hypothesis of direct resource transfer that mediates plant-biocrust interactions. To investigate these questions, we established field mesocosms in which we manipulated the presence/removal of a dominant grass and cyanobacterial-cyanolichen biocrust, the precipitation regime, and the presence of belowground connections.

MATERIALS AND METHODS

Site

We established a mesocosm experiment in La Puebla, New Mexico on private property (lat/lon: 35.978, –105.995, 1800 m). This site is a juniper savanna with scattered piñon pine (*Pinus edulis*) and one-seed juniper (*Juniperus monosperma*) trees and is dominated by the bunchgrasses blue grama (*Bouteloua gracilis*) and Indian rice grass (*Achnatherum hymenoides*). The mean annual temperature is 11°C and the site receives an average of 290 mm of precipitation annually (Western Region Climate Center 2015), 190 mm of which falls in the warm months (May–October). Livestock grazing has been excluded since the 1950s.

Focal taxa

We focused on *B. gracilis*, a widespread, dominant C_4 bunchgrass, which hosts root-endophytic fungi including arbuscular mycorrhizal fungi (Glomeromycota) and dark septate endophytes (Ascomycota) (Herrera et al., 2011, Porras-Alfaro et al., 2008). Mature biocrusts contained cyanobacteria (*Microcoleus* sp., *Scytonema* sp., *Nostoc* sp.), mosses (including *Bryum argenteum*, *Syntrichia* sp. *Pterygoneurum* sp.), and lichens (*Collema* sp., *Placidium* sp.).

Mesocosms

We used a 3 × 2 × 2 experimental design to manipulate producer removal (three levels: both living, biocrust removed, or plant

removed), precipitation regime (small, frequent or large, infrequent), and belowground connections (intact or impeded), with $n = 20$ replicates per treatment combination (however, five plants died in the first year across treatments). We transplanted field-collected *B. gracilis* with intact rhizosphere soil and neighboring biocrusts into plastic pots (7.6 L, 22.9 cm diameter) in July–August 2013. We targeted plants that were 3–5 cm in root crown diameter (estimated shoot biomass of ~5 g) to increase transplant survival compared to smaller plants. The pots were sunk into the ground to keep root conditions similar to the field, but the plastic isolated the target plant and biocrusts from the influence of neighboring soils. The 240 pots were arranged ~50 cm apart in a randomized grid of 15 rows by 20 columns (additional pots were interspersed in the grid that were not discussed in this article). The plot was fenced to exclude large herbivores.

Producer removal

To assess the productivity of plants and biocrusts growing together or when one was removed, *B. gracilis* was either living or the root crown was removed (Plant + or Plant –). This mimicked the loss of a plant due to a grazer removing all aboveground biomass. The remaining roots were left to die to minimize soil disturbance. We estimate that plant roots in transplants weighed approximately 10 g (SE = 2.3) based on above:belowground relationships ($R^2 = 0.29$; data not shown). Roots ($n = 10$) averaged 43.8% C (SE = 0.31) and 0.6% N (SE = 0.009). Thus, the average release of resources would have been 4.3 g C (up to 7.6 g using top 95% *CI* values) and 0.06 g N (up to 0.1 g using top 95% *CI* values) into the pots.

Biocrusts were either living or heat-sterilized (autoclaved for 3 h and then replaced on the soil surface; Biocrust + or Biocrust –). We removed the biological activity without disrupting the microtopography similar to what happens during biocrust die-offs that occur during droughts (Belnap et al., 2004, Ferrenberg et al., 2015). We estimate that biocrusts weighed approximately 225 g (SE = 1.2 g) and contained 4.5% C (SE = 0.16) and 0.42% N (SE = 0.02; $n = 10$). Thus, the maximum release of resources would have been 10.1 g C (up to 10.9 g using top 95% *CI* values) and 0.94 g N (up to 1.04 g using top 95% *CI* values) in the pot. The combination of both producers removed (Plant –/Biocrust –) lacked biological relevance and was not included.

Precipitation regime

We compared the effects of the same quantity of well water delivered in different frequencies. The watering regime was either large, infrequent events (400 mL once per month, a 10 mm event; delivered by hand through a watering can with ~4 mm holes to prevent disrupting the soil surface) or small, frequent events (100 mL once per week, a 2.5 mm event; delivered with spray emitters), mimicking patterns typical for this region (Western Regional Climate Center, 2015). Our watering system (RBY100MPTX filter, Rainbird, Azusa, California, USA; 1.9 cm hose) provided well water to the pots via 12.11 L h^{-1} pressure compensating spray stakes (#22500-002030, Netafim USA, Fresno, California, USA) and 91.4 cm dripper assembly (#40201-002020; Netafim USA, Fresno, California, USA). For the small, frequent treatment, pots were watered once per week for 1 min (Orbit model 62056, Orbit, Bountiful, Utah, USA). The water output emitted from each sprayer was measured as 100 mL per event because the hose dried between applications. Well water

was enriched in salts compared to precipitation (Appendix S1, see the Supplemental Data with this article), but no salts visibly accumulated in the pots.

Precipitation was altered during the frost-free months surrounding months with most of the annual rainfall. We watered following the experiment set-up in August through October in 2013 (our additions were 20% of the ambient rainfall received during those months) and May through October in 2014–2015 (30% and 21% of ambient rainfall for those months in 2014 and 2015, respectively; Appendix S2). Water additions were intended to increase the probability that the producers would survive during the hot season, despite recent severe drought conditions in the region (Climate Assessment for the Southwest, 2013), but we expected that the small, frequent precipitation regime would be less favorable to producers because small rains evaporate quickly and do not penetrate to the rooting zone. Thus, the small, frequent regime would provide less time for plants and microbes to be active (given the same total water volume) compared to the large, infrequent regime. The precipitation regime treatment effectively changed soil moisture in the pots, with a single large, infrequent event increasing soil moisture ~80% more than a single small, frequent event (Appendix S3).

Belowground connections

To determine the effects of belowground connections on plant and biocrust productivity, hydrophilic mesh was used to reduce connections. Control pots had all connections intact with no mesh. Mesh with 0.45 μm pores (GE Healthcare Life Science, Pittsburg, Pennsylvania) impeded both roots and fungi (Teste et al., 2006) because hyphal diameters range from 2–20 μm (Dwivedi and Arora, 1978), and fine roots are >50 μm (Ares, 1976), but bacteria/archaea could pass through (Reed and Martiny, 2007). Arthropod movement was also impeded (Kampichler and Bruckner, 2009), potentially altering trophic webs. Mesh was placed horizontally under the biocrust with a hole cut in the center allowing the aboveground part of the bunchgrass to pass through. Although we could not completely eliminate the potential connections around the edges of the mesh, we impeded the majority of the surface area between the biocrusts and rooting zone soil. We did not observe any effect of the hydrophilic mesh on soil moisture (Appendix S3). The mesh treatment allowed us to investigate if the belowground connections affected plant performance independently of biocrust activity (Plant +/Biocrust –), if the connections affected biocrust performance independently of plant activity (Plant –/Biocrust +), or if the connections only affected producer performance when both producers were present (Plant +/Biocrust +).

In autumn 2014, we checked a subsample of ~15 pots with living plants for roots occurring above the 0.45 μm mesh and found none. By the time of harvest in October 2015, all plants had produced some roots above the mesh (mean = 27% ± 1.9 SE of total root biomass across all pots), indicating that by the second year, some connections formed between roots and biocrust; however, the mesh still impeded the majority of belowground connections.

Response variables

Plant and biocrust performance were assessed after setting up the experiment and starting the watering treatment in September 2013 and at the end of the growing season (October) in 2014 and 2015. To nondestructively monitor plant productivity through time, we

developed allometric equations for aboveground biomass using small nearby plants located outside of the experiment (to represent size when first transplanted) and our destructively harvested plants from the pots (see below) (shoot biomass (g) = 0.5858 × largest dimension of root crown (cm) + 0.2734 × perpendicular dimension of root crown (cm) + 0.0688 × largest dimension (cm) × perpendicular dimension (cm); R^2 = 0.89, $F_{3,180}$ = 473.4, $P < 0.001$). In 2015, we destructively harvested plants and present total biomass results (shoot + root) in Appendix S4; we did not have the power to resolve among multiple models with strong support.

We used chlorophyll *a* content as a measure of biocrust productivity because we were interested in the photosynthetic components of biocrusts. We do not have data for total microbial biomass because the heterotrophic organisms were not the focus of this study. We collected soil from the top 5 mm from two randomly selected areas (excluding areas occupied by the plant root crown) into 11 mm diameter microcentrifuge tubes. We air dried, sieved (2 mm), and incubated samples in 1 ml dimethyl sulfoxide (DMSO) for 3 d at room temperature, then recorded absorbance of the supernatant at 664 nm and 750 nm on a Synergy H1 Hybrid plate reader (Biotek, Winooski, Vermont, USA). Absorbance at 750 nm accounted for turbidity in the sample (Castle et al., 2011). We only extracted once (~75% yield predicted; Castle et al., 2011) so our absolute estimates of chlorophyll content are likely low, but we can compare the relative values. We then calculated chlorophyll *a* content per grams of soil using the equation for DMSO (not acidified) in Castle et al. (2011).

Analysis

All analyses were conducted in R (R version 3.3.1 [2016-06-21]). First, we directly assessed the outcome and magnitude of the plant and biocrust interaction by determining the productivity of each producer when the other was removed. There were treatment effects immediately after setting up the experiment, as well as significant correlations between initial performance and change in performance (Appendix S5). Therefore, we used the relative change in chlorophyll *a* or plant biomass as our response variable to separate short-term versus longer-term responses to manipulations. We calculated these intervals independently: the difference from 2013 to 2014 divided by the value in 2013, and the difference from 2014 to 2015 divided by the value in 2014.

We analyzed biocrust and plant responses separately using model selection (Anderson 2008) of general linear mixed models (Bates et al., 2015) with plant individual as the random effect to account for repeated measures. We compared small sample size-corrected Akaike information criterion (AICc) values. All models included the main effects of producer removal, precipitation regime, belowground connection treatment, and year (2013–2014 or 2014–2015 as a categorical variable). We evaluated two models that included no context-dependency of the effect of removal on precipitation or belowground connection: Model 1 included all the main, fixed effects. Model 2 additionally included the 2-way interaction of producer removal with year. Next, we evaluated two models that included context dependency of producer removal only on precipitation regime (Question 1): Model 3 included the 2-way interaction between removal and precipitation regime. Model 4 included all 2- and 3-way interactions among removal, precipitation regime and year. Finally, we evaluated two models that accounted for context dependency of the removal treatment on both the precipitation regime and belowground connections (Question 2): Model 5 included all 2- and 3-way interactions

among the removal, precipitation regime, and belowground connection treatments. Model 6 included all 2-, 3-, and 4-way interactions.

Second, we further evaluated the net interaction of biocrusts and plants in the subset of 80 pots containing both living biocrust and a living plant. This analysis accounted for differences in biomass rather than the effect of a discrete disturbance that removed biomass (loss of one producer) and allowed exploration of the potential influence of precipitation regime and belowground connections on net producer interactions when both producers were present and could potentially be connected to each other. There was no relationship between plant biomass (natural log transformed) and biocrust chlorophyll *a* content (natural log transformed) immediately after the experiment was set up in September 2013 (Pearson *r* = 0.02, t_{75} = 0.18, $P = 0.89$); thus, we evaluated the data for October 2014 and 2015 only. Biocrust chlorophyll *a* (ln transformed) was the response variable, and plant biomass (ln transformed), precipitation regime, belowground treatment, and year were fixed effects, with plant identity as a random effect. A negative slope between biocrust chlorophyll and plant biomass would indicate net competition, and a positive slope would indicate net facilitation. Our key questions were whether the strength or direction of this slope was altered by the precipitation regime (Question 1), or also by the presence of belowground connections (Question 2). Differences in the intercepts of the biocrust-plant relationship under altered precipitation or belowground connections would indicate an overall boost/decline in biocrust production that was independent of any competitive/facilitative dynamics with plant biomass. The six candidate models were structured similarly to the analyses described above, with the first two models evaluating the relationship of plants and biocrusts with no context dependency, with or without an interaction with year; the next two models evaluating the relationship as context dependent on precipitation regime; and the final two models evaluating the relationship as context dependent on both the precipitation regime and the belowground connection treatment.

RESULTS

Plant removals initially benefited biocrusts

The relative change in biocrust chlorophyll content by removal treatment was context dependent on precipitation regime, and the effect varied by year (Table 1: Models 4, 6, Figure 1). During the first year (2013–2014) in the small, frequent precipitation regime, the relative increase in chlorophyll content was 60–81% higher when plants were removed than when they were present. However, in the large, infrequent precipitation regime, there was little response to the plant removal treatment (5% higher when plants were removed than present). During the second year (2014–2015), there was little difference in biocrust chlorophyll content (<7% absolute magnitude in either Model 4 or Model 6, Figure 1) in response to plant removal or precipitation regime (Table 1: Models 4, 6).

Multiple models had equal support for explaining the relative change in plant biomass (Table 1, Figure 1, Appendix S6). Across models (Table 1: Models 1, 4, 6), plants grew relatively less (4–29%, but see the positive effect in Model 6, below) when biocrusts were removed than when they were present, indicating a weak net benefit of biocrust presence for plants. Overall, plants grew relatively more in the large, infrequent precipitation regime. However, the effect of biocrust removal was potentially context dependent on the

TABLE 1. *AICc* values and weights from candidate models for relative change in biocrust chlorophyll *a* and relative change in plant shoot biomass. Fixed main effects included producer removal, precipitation regime, belowground connection treatments, and year (2013–2014 or 2014–2015) and were included in all models. The best models (ΔAICc < 2) are shown in bold; models with moderate support (ΔAICc < 7) are shown in italics.

Model	Factors	Implications	Biocrust		Plant	
			AICc	Weight	*AICc*	Weight
1	Removal + Precipitation + Belowground + Year	The removal effect was **not context-dependent** on precipitation, belowground connection, or year.	1391.9	0.01	**767.9**	0.28
2	Removal × Year + Precipitation + Belowground	The removal effect differed between the two years, but it was **not context-dependent** on the precipitation regime or belowground connection treatment.	1392.5	0.01	*771.5*	0.04
3	Removal × Precipitation + Belowground + Year	The removal effect was **context-dependent on precipitation**, but not on belowground connections or year.	1391.7	0.01	*771.3*	0.05
4	Removal × Precipitation × Year + Belowground	The removal effect was **context-dependent on precipitation** and this dependency differed between years, but was not dependent on belowground connections.	**1383.2**	0.73	**768.0**	0.26
5	Removal × Precipitation × Belowground + Year	The removal effect was **context-dependent on both the precipitation regime** and **the belowground treatment** but not year.	1394.0	0.00	775.4	0.01
6	Removal × Precipitation × Belowground × Year	The removal effect was **context-dependent on both the precipitation regime and the belowground treatment**, and these interaction effects differed between years.	*1385.4*	0.24	**767.4**	0.36

precipitation regime (Table 1: Models 4, 6). In the small, frequent precipitation regime, plants grew relatively less (16–19%) when biocrusts were removed than when present (Figure 1). In the large, frequent precipitation regime, plants grew only 6–10% less when biocrusts were removed than when present (Figure 1). A positive effect of biocrust removal on plants was evident in only one model (Model 6) and so does not have strong support: During the first year of growth (2013–2014), when belowground connections were intact in the large, infrequent precipitation regime, plant growth was 40% higher when biocrusts were removed than when present. In the small, frequent precipitation regime, the increase in plant growth with biocrust removal was only 5% compared to biocrust presence, consistent with the overall result that the effect of biocrust removal on plant growth depended on the precipitation regime.

Belowground connections promoted biocrust growth

Across both time periods, the relative increase in biocrust chlorophyll content was 8% lower when connections were impeded than intact (Table 1: Model 6, Figure 1). Thus, regardless of whether a plant was present or removed, biocrusts performed slightly better when there was no impediment between them and the deeper soil.

In contrast to biocrusts, plants generally had higher relative growth (26%; Table 1: Models 1, 4) when belowground connections were impeded compared to intact. One model (Table 1: Model 6) suggested that the effects of belowground connections changed over time. In the first year across both precipitation regimes, plants in pots with impeded belowground connections grew 29% less when biocrusts were removed than when present. In contrast, with intact connections, there was weak to moderately higher (5–40%) growth when biocrusts were removed compared to present (Table 1: Model 6, Figure 1, Appendix S6). However, in the second year, the intact belowground connections amplified the detriment of biocrust removal (15–22% lower growth when biocrusts were removed than present) compared to when the connections were impeded (4–17% lower growth when biocrusts were removed than present), suggesting a potential role of belowground connections in mediating interactions between plants and biocrusts.

Plant biomass and biocrust chlorophyll content were negatively correlated

Given that the removal treatments indicated that biocrusts benefited from plant removal while plants had weakly negative or little response from biocrust removal, we further investigated the net relationship between these producers in the subset of mesocosms in which living biocrusts and plants grew together. In these treatments, there was no potential nutrient release or other by-product of removing the producer from the system. Year strongly affected the strength of the relationship between biocrust chlorophyll and plant biomass (Table 2: Model 2). In the first year, there was no relationship between plant and biocrust performance (95% *CI* of the slope spanned zero [–0.31 to 0.31]). However, during the second year, when plants were larger, there was a strongly negative relationship (95% *CI* for the slope excluded zero [–0.94 to –0.14]), suggesting net competition (Figure 2). The model without an interaction with year had moderate support, showing that over both years, there was an overall weak negative relationship between plant aboveground biomass and biocrust chlorophyll content (Table 2: Model 1; slope mean [95% *CI*] = 0.19 [–0.46 to 0.08]).

Models with context dependency of the plant – biocrust relationship on the precipitation regime had strong to moderate support (Table 2: Models 3, 4; Figure 2). Overall, there was a stronger negative relationship between plants and biocrusts in the large, infrequent precipitation regime (Model 3: slope mean [95% *CI*]= –0.36 [–0.67 to –0.05]; Model 4: 2014 slope mean [95% *CI*] = –0.31 [–0.74 to 0.10]; 2015 slope mean [95% *CI*] = –1.06 [–1.66 to –0.46]) than the small, frequent regime (Model 3: slope mean [95% *CI*] = –0.07 [–0.36 to 0.22]; 2014 slope mean [95% *CI*] = 0.31 [–0.13 to 0.74]; 2015 slope mean [95% *CI*] = –0.18 [–0.69 to 0.32]).

DISCUSSION

Our experiment revealed that plants and biocrusts responded differently to the removal of the other producer, with biocrusts benefiting from plant removal but plants generally reducing growth with biocrust removal. Biocrusts may have been shaded by the growing
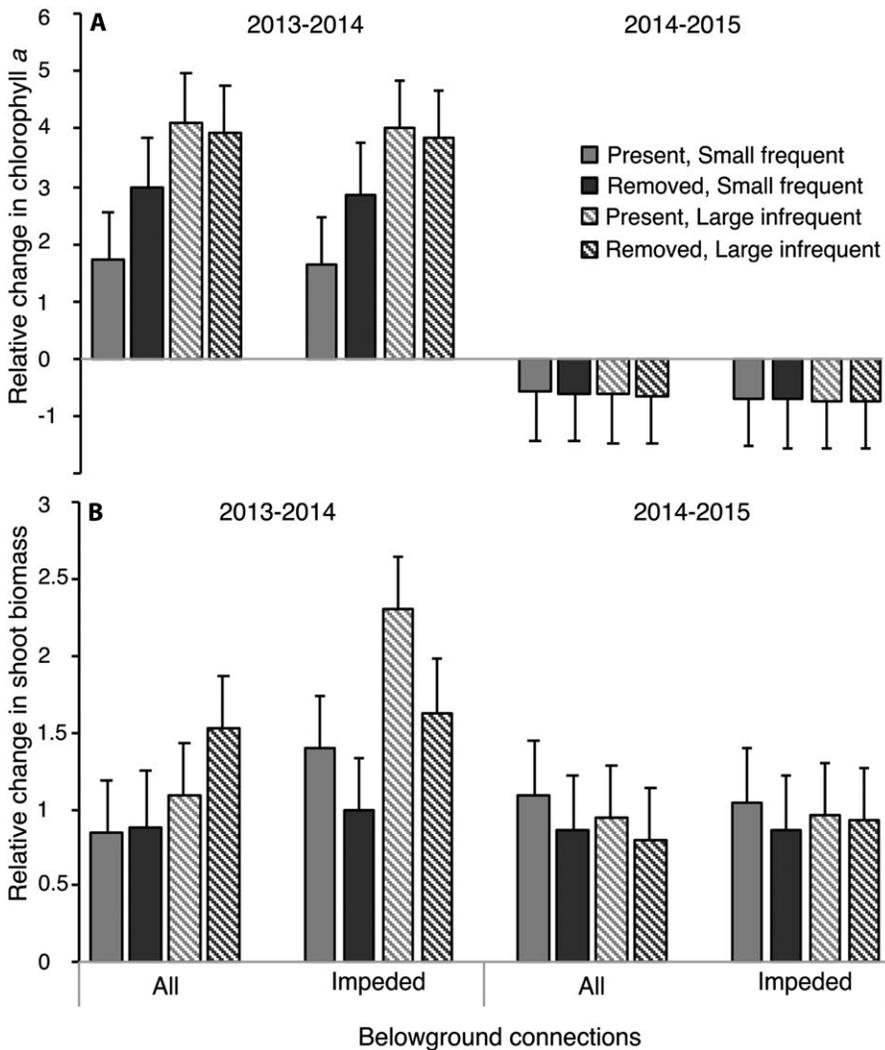
FIGURE 1. Means + 95% *CI* for (A) relative change in biocrust chlorophyll *a* content and (B) relative change in plant biomass for the belowground connection treatment (Intact or Impeded), removal treatment (Living = light gray; Removed = dark gray), precipitation regime (Small, frequent = unfilled, Large, infrequent = hashed), and years of study (2013–2014, 2014–2015).

plants or experienced some other effect due to changes in the aboveground conditions because there was little effect of the belowground connection on the plant removal effect. Plants may have benefited from improved nitrogen content from growing with living biocrust (Harper and Belnap, 2001), potentially mediated through belowground connections (Table 1: Model 6, Figure 1). Removals of plants had the strongest effects on biocrusts during the first year, consistent with the hypotheses that plant removal caused either a release of resources or a release from competitive interactions with biocrusts.

The beneficial effects of biocrust presence on plants that we observed in mature plants and biocrusts have also been seen at different life stages (Thiet et al., 2014; Zhang et al., 2015), but may vary with plant species identity, making it difficult to generalize across plant species. For example, Chung and Rudgers (2016) reported positive effects of biocrust additions on *Bouteloua eriopoda,* a congener of our focal species, but neutral to negative effects of the same additions on our focal grass *B. gracilis.* Further research could lead to a more holistic understanding of effects of biocrusts across plant life stages, plant species, and biocrust taxa that may improve long-term predictions on the consequences of plant or biocrust disturbances.

The relationship between plant aboveground biomass and biocrust chlorophyll content indicated that the net interaction of these producers was generally competitive because there was a negative relationship between producers' productivities when both were grown together, and that relationship was strongest in the second year of the experiment (Figure 2). The plants appear to be the stronger competitor because they grew larger over time, whereas the biocrust performance declined over time

TABLE 2. *AICc* values from candidate models of biocrust performance (chlorophyll *a*, µg g⁻¹ soil; ln-transformed) as a function of plant shoot biomass (g, ln-transformed) in 2014 and 2015. Candidate models compare the effects of the precipitation regime, belowground treatment, and year on this relationship. All main effects are included in all models. The best models (Δ*AICc* < 2) are shown in bold, and models with moderate support (Δ*AICc* < 7) are shown in italics.

Model	Factors	Implications	*AICc*	Weight
1	Biomass + Precipitation + Belowground + Year	The plant-biocrust relationship was **not context-dependent** on precipitation regime, belowground connection treatment, or year.	*187.6*	0.17
2	Biomass × Year + Precipitation + Belowground	The plant-biocrust relationship differed between the two years but was **not context-dependent** on precipitation regime or belowground treatment.	**185.6**	0.48
3	Biomass × Precipitation + Belowground + Year	The plant-biocrust relationship was **context-dependent on precipitation regime**, but not on belowground treatment or year.	*188.1*	0.13
4	Biomass × Precipitation × Year + Belowground	The plant-biocrust relationship was **context-dependent on precipitation regime** and year, but was not context-dependent on belowground connections.	**187.3**	0.20
5	Biomass × Precipitation × Belowground + Year	The plant-biocrust relationship was **context-dependent on both the belowground connections treatment and the precipitation regime** but not year.	*191.6*	0.02
6	Biomass × Precipitation × Belowground × Year	The plant-biocrust relationship was **context-dependent on the belowground connections treatment, the precipitation regime**, and the year.	200.4	0.00

(Figure 1). Such relationships between mature plants and biocrusts may depend on species identity or the functional group of the producers. In one study, biocrust cover had no relationship with the cover of native bunchgrasses but was negatively correlated with the cover of invasive grasses (Dettweiler-Robinson et al., 2013). Our results highlight the need for additional studies that examine relationships across different species and functional groups to develop better generalizations about the conditions under which plant-biocrust interactions are positive, neutral, or negative.

We found context-dependency of plant-biocrust interactions on the precipitation regime because biocrusts were especially benefited by plant removal whereas plants generally had an increased detriment of biocrust removal in the harsher small, frequent precipitation regime compared to the large, infrequent regime. Thus, our results suggest that in future regimes dominated by small, frequent precipitation events, biocrusts will be at a greater disadvantage than plants (Figure 1). In contrast, when both biocrusts and plants were present, biocrusts experienced stronger negative relationships with plant biomass in the large, infrequent regime than in the small, frequent regime. While all of these results point to a role for precipitation regime in altering the outcome and magnitude of plant-biocrust interactions, further experimentation will be required to determine how precipitation affects competitive dynamics in this system. At this stage, we can predict that in years with most of the rainfall occurring in many, small events, biocrust performance will be most sensitive to plant removals (disturbances, grazing), and that in years dominated by few, large rains, biocrusts may be most sensitive to large increases in plant biomass.

Belowground connections between deeper soils and surface soils were weakly beneficial to biocrusts but weakly harmful to plants. We found some support for context-dependency of biocrust removals on belowground connections for plant relative growth, with intact connections increasing the benefit of biocrust presence for plant growth, consistent with a belowground link that couples resource dynamics between plants and biocrusts (Collins et al., 2008). Currently, we cannot resolve the potential roles of roots, microbes, or arthropods in these belowground interactions. Thus, it remains unclear if the benefit of connections to biocrusts is a direct result of their interactions with belowground biota, or an indirect consequence of parasitism, disease, or herbivory harming the apparently competitively dominant plants. However, few roots were present in the biocrusts (0.56 mg ± 5.53 s.d. in 2.5 cm diameter soil core to a depth of 1 cm) in mesocosms without mesh; so, roots directly accessing resources at the surface is not likely to be a driver. Further experimentation with different mesh sizes could help resolve the influences of different soil taxa (e.g., Lemons et al., 2005).

Our results have implications for land management and restoration efforts. Although we detected evidence of net competitive interactions between *B. gracilis* and biocrusts (Figure 2), we do not suggest that managers should remove biocrusts for the sake
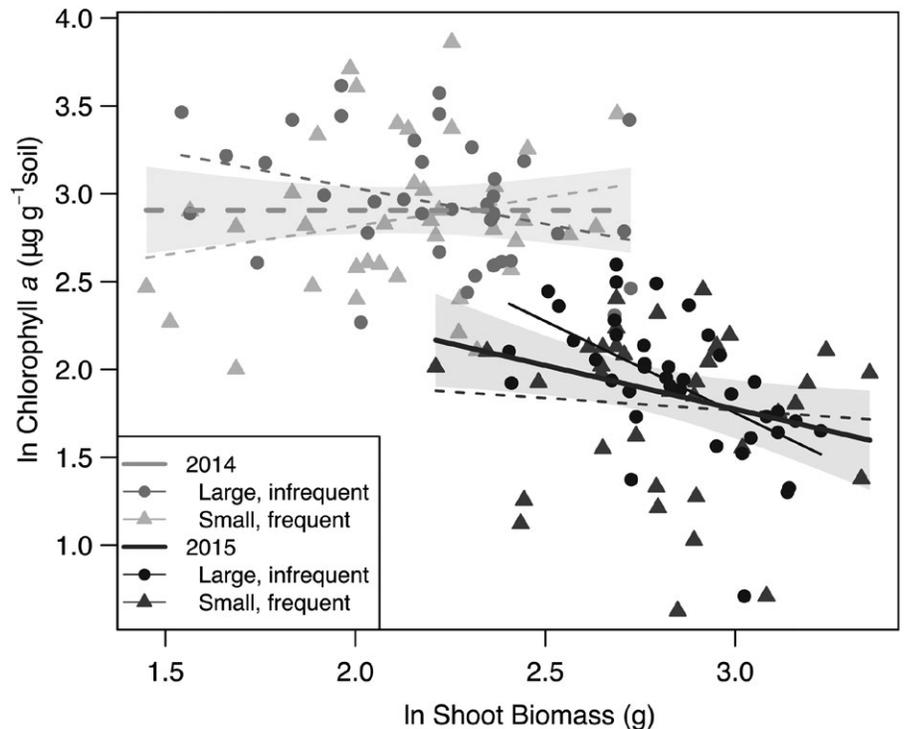

FIGURE 2. Relationship between plant aboveground biomass (g, ln transformed) and biocrust chlorophyll *a* (µg g⁻¹ soil content, ln transformed), in mesocosms with both producers present. Relationships are shown separately for each year (2014 = light grey; 2015 = dark grey) and for each precipitation regime (Small, frequent = triangles, Large, infrequent = circles). Thin lines show results from Table 2: Model 4 with context-dependency of the relationship on both year and precipitation regime; thick lines with band (slope 95% *CI*) show results from Table 2: Model 2 with differences only by year. Dashed lines indicate that the relationship is not different from zero; all 95% *CI*s presented in text.

of improving grass production, because biocrust removal was not beneficial to plants (Figure 1). In addition, biocrusts have other important roles in the ecosystem, such as reducing soil erosion and fixing N (Ferrenberg et al., 2017b). Thus, a loss of biocrusts at the landscape scale could have significantly negative effects on nitrogen dynamics and dust production that feedback to reduce grassland production. Our experiments were not designed to resolve dynamics at this large scale. We also showed that belowground connections benefited both groups of primary producers under some conditions. Thus, our results suggest that belowground processes and taxa should be considered in land management and restoration activities in drylands, particularly in ecosystems that are threatened by changing climate.

ACKNOWLEDGEMENTS

AUTHOR CONTRIBUTIONS

EDR contributed to the design and carried out field and lab work for the study, performed data analysis and interpretation, and wrote the manuscript. RS contributed to the design of the study, interpretation, and writing of the manuscript. JR contributed to design of study and data collection, data analysis, interpretation, and writing of the manuscript.

DATA ACCESSIBILITY

Data are archived in FigShare: 0.6084/m9.figshare.6081143.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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