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SETBACKS AND SURPRISES

Unexpected side effects in biocrust after treating non-native plants using carbon addition

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Carbon addition has been proposed as an alternative to herbicide and manual removal methods to treat non-native plants and reduce non-target effects of treatments (e.g. impacts on native plants; surface disturbance). On Mojave Desert pavement and biocrust substrates after experimental soil disturbance and carbon addition (1,263 g C/m^2 as sucrose), we observed declines in lichens and moss cover in sucrose-treated plots. To further explore this unforeseen potential side effect of using carbon addition as a non-native plant treatment, we conducted biocrust surveys 5 and 7 years after treatments, sampled surface soils to observe if treatments additionally affected soil filamentous cyanobacteria, and conducted laboratory trials testing the effects of different levels of sucrose on cyanobacteria and desert mosses. Sucrose addition to biocrust plots reduced lichen and moss cover by 33–78% and species richness by 40–80%. Sucrose reduced biocrust cover in biocrust plots to levels similarly detected in pavement plots (<1%). While cyanobacteria in the field did not appear to be affected by sucrose, laboratory tests showed negative effects of sucrose on both cyanobacteria and mosses. Cyanobacteria declined by 41% 1 month after exposure to 5.4 g C/m^2 equivalent solutions. We detected injury to photosynthesis in mosses after 96 hour exposure to 79–316 g C/m^2 equivalent solutions. Caution is warranted when using carbon addition, at least in the form and concentration of sucrose, as a treatment for reducing non-native plants on sites where conserving biocrust is a goal.

Key words: biocrust, carbon addition, invasion, Mojave Desert, non-native plant, soil amendment, sucrose

Implications for Practice

- Carbon addition in the form of sucrose as a soil amendment to treat invasive non-native annual plants could negatively affect surface biocrust.
- Further research is needed to assess factors such as carbon source and application rate for carbon addition as a non-native plant treatment in ecosystems containing biocrusts to avoid severely damaging them.

Introduction

Recognition of non-target effects of invasive plant treatments and reducing non-target effects are major challenges for restoration practitioners who frequently treat non-native plants as part of initial restoration and subsequent maintenance management. Published literature in the last two decades has highlighted potential for non-target effects such as to native plants, wildlife including pollinators, soil properties and microbial communities, and ecosystem functions, such as nutrient cycling (Pyšek et al. 2012; Skurski et al. 2014). The growing recognition of non-target effects of commonly used treatments has stimulated research on trade-offs among candidate treatments and research into alternatives which may mitigate trade-offs or have fewer trade-offs.

Herbicide treatments are commonly applied to reduce negative effects of non-native plants (e.g. competition with native plants; increased fire frequency) and have been successfully used to remove target non-natives. However, invasion by non-native plants and activities implemented to treat invasions can have persistent legacy effects (Skurski et al. 2014). For example, broad-spectrum herbicides can negatively affect native forbs (Pearson et al. 2016), which in turn can negatively affect plant seed production, seed banks, and seed performance (Olszyk et al. 2004; Wagner & Nelson 2014) and, thus, ecosystem recovery. Herbicide treatments can also trigger secondary invasion by other non-native plants (Skurski et al. 2014; Pearson et al. 2016), necessitating further management.

Carbon addition was proposed as an alternative to herbicides and manual removal methods to treat non-native plants and stimulate natural resiliency of an invaded ecosystem (Morgan 1994). Carbon addition stimulates soil microbial communities to hypothetically alter the soil environment and shift competition in favor of native species (Blumenthal et al. 2003). By immobilizing plant-available nitrogen in soil (Morgan 1994), native plants adapted to low-resource environments

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receive competitive advantages over non-native species often adapted to high-resource environments (Blumenthal et al. 2003; Pysek & Richardson 2008). In drylands, carbon addition showed some promise as a potential alternative to herbicide and labor-intensive manual methods for reducing non-native plants (e.g. Steers et al. 2011).

After we implemented an experiment involving experimental soil surface disturbance and applying carbon in the form of sucrose to further explore effects on non-native annual plants in drylands, we noticed that cover of lichen-moss biocrust was nearly or completely eliminated from plots treated with sucrose 5 years earlier (Fig. S1). Biocrusts provide important ecosystem functions in dryland systems, such as aggregating soil particles and contributing to surface soil nutrient cycling (Chamizo et al. 2016; Colesie et al. 2016). Removal or loss of biocrusts can alter ecosystem structure and processes, such as reduced soil stability triggering soil erosion (Zaady et al. 2016). Although there are several studies and reviews examining carbon addition as a method for controlling or treating non-native plants, we were unable to identify literature which presented possible explanations for our observations with biocrust or within a context of carbon addition for treating non-native plants.

The apparent non-target effects to biocrust from carbon addition that we observed seemingly represented an unacceptable trade-off from non-native plant treatments. This surprise and setback triggered us to further explore relationships between biocrust and sucrose treatment and the implications to biocrust organisms of using sucrose to treat non-native plants. We conducted biocrust community surveys 5 and 7 years after disturbance and sucrose treatments and performed laboratory experiments to better understand how sucrose addition affected biocrust organisms. We asked (1) did sucrose treatments reduce biocrust cover, (2) did trends 5 years after treatment persist 7 years after treatment, and (3) how did sucrose treatments specifically affect biocrust components? Results have implications for further understanding potential unintended consequences of restoration actions and how undesirable side effects to soil health may be minimized when non-native plants are treated.

Methods

Site Description

The field study was located in the Mojave Desert, U.S.A. at Lake Mead National Recreation Area (National Park Service), 40 km from Las Vegas, NV (36°14′49″N, 114°31′50″W) at an elevation of 633 m. Soils are Haplocalcids, Petrocalcids, and Haplogypsids (Lato 2006). The 0.4 ha site contains two intermixed substrate patch types: desert pavement and non-pavement, low rock and gravel cover (Fig. S2). Desert pavement is a millennia-old surface with interlocking coarse gravel to cobble with little bare surface cover overlaying a relatively gravel- or cobble-free horizon (Verheye 1986). Pavement patches at the study site contain low perennial plant cover. Biocrust, which includes lichens, mostly *Collema coccophorum* and *Placidium lacinulatum*, occurs between gravel and cobble. Non-pavement patches contain low-density shrub-dominated patches and a lichen-moss biocrust under drip canopies of shrubs and in interspaces between shrubs (Fig. S2). Dominant plant species in both substrate types include creosote bush (*Larrea tridentata*) and white bursage (*Ambrosia dumosa*). Biocrust species observed generally in non-pavement patches include the lichens *C. coccophorum*, *P. lacinulatum*, *Placidium squamulosum*, *Peltula patellata*, and the mosses *Aloina bifrons*, *Bryum argenteum*, and *Syntrichia caninervis*. Although cyanobacteria were assumed present in soils because of previous work near the current site in similar substrates (Chiquoine et al. 2016), cyanobacteria are not visually obvious without use of a hand lens. Cyanobacteria can be a significant microscopic component of biocrust in hot deserts (Chamizo et al. 2016).

During the 2008–2016 study period, mean daily average maximum/minimum temperatures were 14/4°C for winter from December through February and 41/28°C for summer from July through September (23 km from study site; Valley of Fire, NV; NCEI, NOAA). Average annual rainfall during the study period (2008–2016) was 156 mm and varied between 79 mm (2009) and 315 mm (2010). In most years more rainfall occurred during winter (December–February; 24% higher in winter), while the remainder occurred as summer monsoons (July–September).

Field Treatments

The original experimental treatments, which included experimental surface disturbance and sucrose addition applied to plots situated within the two substrate patch types, desert pavement and non-pavement (hereafter biocrust patches), were applied in February 2009. Twenty 1-m^2 plots were established in each substrate type (Fig. S3). To avoid perennial plant canopy within plots and ensure each plot was completely within the assigned substrate type, plot dimensions varied to maintain the 1-m^2 area (1 m × 1 m, 1.50 m × 0.67 m, or 1.25 m × 0.8 m). The disturbance only, sucrose addition only, and factorial combination of disturbance and sucrose addition treatments each had four replications per substrate type, totaling 12 plots per substrate type. To act as controls, the remaining eight plots per substrate type did not receive treatments. Disturbance treatment was applied, then sucrose treatment the same day. Disturbance involved raking the top 2 cm of the soil surface with a rake, tearing surface material and dislodging surface rocks (Fig. S4). Three liters of a 2.9M sucrose solution (3,000 g sucrose) were applied per plot with a backpack sprayer delivering 1,263 g C/m^2. This was similar to amounts of carbon addition used in past studies that reduced non-native plants (e.g. Reever Morghan & Seastedt 1999; Alpert & Maron 2000). For plots that did not receive sucrose treatment, 3 L of water was applied using a backpack sprayer (Fig. S4).

Data Collection

Field Assessments

During a site visit 5 years (April 2014) after treatments, we observed what appeared to be lower biocrust cover in sucrose-treated plots (Fig. S1). Upon brief inspection, we observed fewer lichen and moss species in plots which received

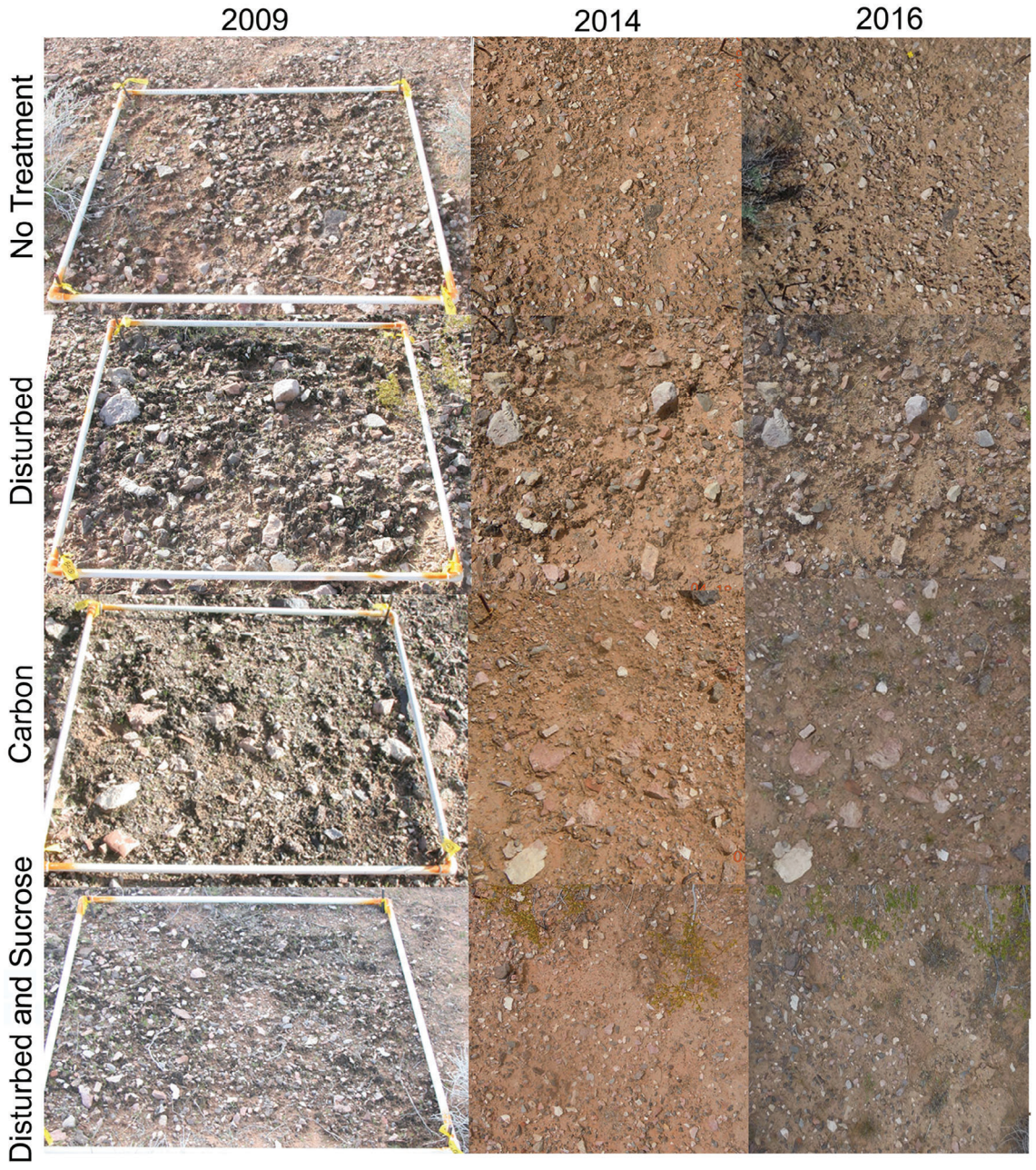

Figure 1. Examples of 1 m^2 biocrust plots before (2009) and 5 (2014) and 7 years (2016) after surface disturbance and sucrose addition (1,263 g C/m^2) treatments in the Mojave Desert, U.S.A.

sucrose. To assess whether sucrose in fact affected biocrust in plots, we conducted surveys 5 (April 2014) and 7 years (April 2016) after treatments (Fig. 1). For surveys, we estimated percent cover of individual lichen and moss species by estimating overall biocrust cover, then estimating the proportion of cover contributed by each species. Cyanobacteria were not included in the estimated cover, as this component was not visually obvious and difficult to visually estimate. Seven years after treatments, we collected four randomly located surface samples (each 1 cm × 1 cm × 1 cm) from each biocrust plot and composited the four samples on a plot basis to examine the filamentous cyanobacteria community microscopically.

Each composited sample was pulverized and homogenized for 2 minutes using a mortar and pestle. Three 1.00-g subsamples were extracted per sample. Each subsample was separately serially diluted using polished water to produce a 10^{-1} suspension, then a 10^{-2} suspension. Suspensions were vortexed between dilutions for 2 minutes to break apart additional aggregated soil particles. The 10^{-2} suspension was vortexed for 2 minutes before adding a 0.01 mL drop of the 10^{-2} suspension

to a glass slide with a 20 mm × 20 mm grid. The slide was examined using a compound microscope at 400× to 1,000× magnification. Filamentous cyanobacteria (filaments ≥2 cells sheathed) were counted within the whole grid. Although this method does not directly estimate biomass or density, this method is a relative measure among samples which received the same timed preparation methods. We calculated density or estimated mean number of filaments detected in 1 g of soil per biocrust plot.

Laboratory Assessments

To better understand how sucrose affected biocrust constituents, we conducted laboratory trials specifically on cyanobacteria and mosses. To examine sucrose effects on cyanobacteria, we conducted two trials of sucrose addition. For this first trial, we tested field equivalents of 53.6 g C/m^2 (1.0 g sucrose/dish), 134.0 g C/m^2 (2.5 g sucrose/dish), 268.1 g C/m^2 (5.0 g sucrose/dish), or 536.1 g C/m^2 (10.0 g sucrose/dish) and a no-sucrose control ($N = 5$) on field-collected biocrust ($N = 5$). For the second trial, we tested lower levels of sucrose addition at field equivalents of 5.4 g C/m^2 (0.1 g sucrose/dish), 13.4 g C/m^2 (0.3 g sucrose/dish), or 26.8 g C/m^2 (0.5 g sucrose/dish) and a no-sucrose control ($N = 4$). Lichen-moss material was obtained 50 m away from treatment plots in the same substrate type. Thirty 1-cm deep, 3-cm diameter cores targeting intact lichen-moss biocrust were pulverized and homogenized to use as inoculant. Dry sucrose was added to 10-cm diameter sterile petri dishes containing 40.00 g of autoclaved dry fine sand; autoclaving was necessary to reduce contamination of non-local microbial constituents that could interfere with native microbial constituent responses. Each dish was inoculated with 10.00 g of the well-homogenized inoculant. Dishes were watered with sterile water until saturated, sealed with parafilm, and placed in a Percival model GL-136L Intellus Environmental Controller (Percival Scientific, Inc., Perry, IA, U.S.A.) set on a diurnal setting with dark/light temperatures at 15/20°C and 100 µmol m^{-2} s^{-1} light irradiance. We included four no-inoculation, no-sucrose dishes containing 40.00 g of autoclaved sand to observe if any contamination persisted in sand after autoclaving or occurred in dishes during the experiment. After 1 month, dishes were slow dried for 48–52 hours in a drying oven at 30°C. Dish material was pulverized and homogenized. Three 1.00-g subsamples per dish were used to make separate 10^{-2} suspensions as described above to examine cyanobacteria microscopically. We calculated the mean filamentous cyanobacteria per 1 g soil. No contamination was observed in the non-inoculated dishes.

We used chlorophyll fluorescence as a response variable to test effects of sucrose solutions on laboratory-cultured moss *Bryum argenteum* and *Funaria hygrometrica*. Because *Bryum* is strongly stress tolerant (Wood 2007), while *Funaria* does not exhibit high stress tolerance from desiccation (Werner et al. 1991), we hypothesized that *Funaria* would display a greater stress response than *Bryum*. Both species have been previously detected in the region (Brinda et al. 2007), although only *Bryum* was observed at our site. *Bryum* was collected from the San Joaquin River Gorge, Fresno County, CA, U.S.A. (L.R. Stark 2008). *Funaria* was collected from a site near Pecos, New Mexico, San Miguel County, U.S.A. (J.L. Greenwood 2016). Mosses were grown in sterile fine sand in petri dishes for a minimum of 18 months in a Percival model E30B (Boone, IA, U.S.A.) on a diurnal setting with dark/light temperatures of 8/20°C, 55 µmol m^{-2} s^{-1} light irradiance, and 65% relative humidity. Mosses occasionally received a 30%-Hoagland's nutrient solution.

For each species separately, using 1-mL cell culture plates, we placed 10 mature and fully water-hydrated moss shoots per cell containing 0.8 mL of each of three levels of sucrose solution or sterile water as a control ($N = 4$). Sucrose concentrations as field carbon addition equivalents included (sucrose per milliliter solution): 78.9 g C/m^2 (0.06 g sucrose/mL), 157.9 g C/m^2 (0.01 g sucrose/mL), or 315.8 g C/m^2 (0.25 g sucrose/mL). The highest sucrose concentration was an equivalent of one-quarter the concentration of sucrose applied to field plots. The solution was diluted by 50% two times to produce the medium and lowest concentrations, respectively. Maximum concentration and dilutions were selected purposefully to induce a gradient response to sucrose levels. Shoots were incubated for 24 hours in a Percival model GL-136L on a diurnal setting with dark/light temperatures at 4/16°C and 100 µmol m^{-2} s^{-1} light irradiance.

Shoots per cell were removed from solutions and dark-adapted in leaf clips for 30 minutes in the laboratory (20°C) while still exposed to solutions using filter paper. The number of shoots necessary to produce an adequate fluorescence signal was determined beforehand using healthy, well-hydrated, and non-stressed shoots from the same laboratory-cultivated material used for this experiment. Dark- and light-adapted fluorescence and non-photochemical quenching (NPQ) were monitored using a pulse-modulated chlorophyll fluorometer (FMS2, Hansatech Instruments Ltd., Norfolk, United Kingdom) using a saturation pulse method (Genty et al. 1989; Bilger et al. 1995). A decrease of the first two metrics and an increase in NPQ would indicate reduced photosynthetic efficiency (stress) leading to dissipation of unused photosynthetic energy either via NPQ or thermal radiation. We conducted additional fluorescence measurements at 96 and 144 hours for *Funaria* and at 96 and 168 hours for *Bryum*, replacing shoots into clean solutions respective of their treatments between measurements and dark-adapting samples for 30 minutes before additional measurements. For *Funaria* at the end of the experiment, we collected leaf tissue to examine sucrose effects on cells using a compound microscope.

Data Analysis

We asked the following questions to guide our statistical analyses:

(1) How did field treatments (disturbance and sucrose addition) affect macrospecies lichen and moss cover and richness between surface types (biocrust, pavement) 5 and 7 years after treatment?

Table 1. Mean cover ± standard error of means for biocrust species detected in biocrust and desert pavement plots and percent of plots in which a species was detected 5 and 7 years after sucrose treatments (1,263 g C/m^2) in the Mojave Desert. Treatments included experimental disturbance and sucrose addition. Disturbance did not significantly affect lichen and moss cover or richness.

	Biocrust Plots				*Desert Pavement Plots*			
	5 yr		*7 yr*		*5 yr*		*7 yr*	
	Cover (%)	*Plots detected (%)*	*Cover (%)*	*Plots detected (%)*	*Cover (%)*	*Plots detected (%)*	*Cover (%)*	*Plots detected (%)*
No sucrose ($N = 12$)								
Collema coccophorum	26.5 ± 4.5	100	11.9 ± 1.6	100	0.2 ± 0.0	16	0.4 ± 0.1	58
Peltula patellata	5.5 ± 0.8	92	6.4 ± 1.4	100	0.1 ± 0.0	8	0.4 ± 0.1	16
Placidium lacinulatum	18.5 ± 1.6	100	14.2 ± 2.3	100	0.7 ± 0.0	8	0.2 ± 0.1	16
Syntrichia caninervis	0.9 ± 0.4	58	4.0 ± 0.8	75	0.0 ± 0.0	0	0.0 ± 0.0	0
Sucrose ($N = 8$)								
Collema coccophorum	0.3 ± 0.1	50	0.2 ± 0.2	100	0.5 ± 0.2	50	<0.1	12.5
Peltula patellata	0.0 ± 0.0	0	0.0 ± 0.0	12.5	0.0 ± 0.0	0	0.0 ± 0.0	0
Placidium lacinulatum	0.5 ± 0.0	25	0.4 ± 0.2	25	0.0 ± 0.0	0	0.0 ± 0.0	0
Syntrichia caninervis	0.0 ± 0.0	0	0.0 ± 0.0	0	0.0 ± 0.0	0	0.0 ± 0.0	0

(2) Within surface types, did responses observed within 5 years of treatments at the field site persist to 7 years after treatments, or were there additional responses to treatments?
(3) In biocrust field plots, how did field treatments affect estimated filamentous cyanobacteria 7 years post-treatment, and did laboratory trials result in similar responses?
(4) How did different concentrations of sucrose solutions affect photosynthetic efficiency in mosses?

We used generalized linear mixed models to analyze data in SAS v 9.4 (SAS Institute 2013). To address if sucrose affected macrospecies cover and richness in field plots within years (2014, 2016), we tested effects of disturbance (yes, no) and sucrose addition (yes, no) in two substrate types (biocrust, pavement) and all two- and three-way interactions between substrate type, disturbance treatment, and sucrose treatment for each year, with substrate type and the manipulative treatments (disturbance, sucrose addition) as fixed effects. To address if responses detected during our 5-year survey persisted into our 7-year survey separately per substrate type, we conducted a repeated-measures analysis by analyzing effects of the manipulative treatments (disturbance, sucrose addition) between years and all two-way and three-way interactions between year and manipulative treatments, with manipulative treatments and year as fixed effects. To assess the response by cyanobacteria collected from biocrust field plots 7 years after field treatments, we used a model similar to our first model but with substrate type removed. To assess laboratory results for cyanobacteria and for mosses, we conducted one-way analyses of variance. For all analyses, where transformations did not improve normality of data, we examined probability plots and goodness-of-fit statistics to identify distributions and assigned those distributions in our statistical analyses. Tukey-adjusted post hoc tests were used to further explore any significant effect or interaction.

Results

Field Study

In both study years, biocrust plots had greater macrospecies cover compared to pavement plots, and more species were detected more frequently among biocrust plots compared to pavement plots (Table 1). However, although more macrospecies occurred at the study site (see site description), only three species of lichen (*C. coccophorum*, *P. lacinulatum*, and *P. patellata*) and one species of moss (*S. caninervis*) were detected among plots during both survey years (Table 1).

At 5 and 7 years after treatments, we detected a significant ($p \leq 0.05$) substrate type × sucrose addition interaction for macrospecies cover and richness (Fig. 2 and Table S1). Compared to non-sucrose-treated biocrust plots, sucrose-treated biocrust plots had significantly reduced cover and richness comparable to levels detected in pavement plots both survey years. Sucrose treatment did not statistically reduce cover either year or richness 5 years after treatments in pavement plots. However, we detected a mean loss in cover and a significant loss in richness 7 years after treatment (Table 1).

We detected changes in macrospecies cover and richness between our 5- and 7-year surveys for both substrate types (Table S2). Specifically for pavement plots, we detected year × sucrose addition interactions for cover and richness (Fig. 3). Cover and richness did not differ between sucrose and non-sucrose plots during the 5-year survey. Trends in response detected during the 5-year survey did not persist into the 7-year survey. During the 7-year survey, we detected a decline in both cover and richness in sucrose-treated plots. For cover, this interaction was only moderately significant ($p \leq 0.10$). The reversal of the response trend of richness on pavement between the 5- and 7-year surveys can be explained by an increase in detection of species among non-sucrose plots during the 7-year compared to the 5-year survey. Concomitantly, a decrease in detection or a loss of species among sucrose addition plots occurred during the 7-year compared to the 5-year survey (Table 1). For

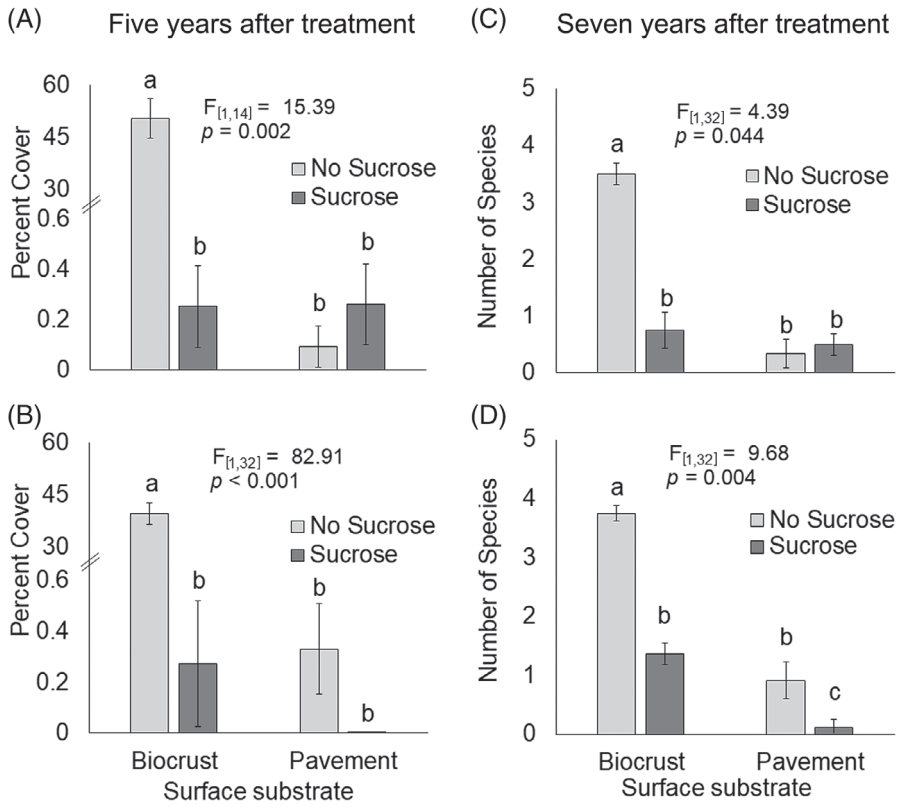

Figure 2. Significant interactions of sucrose addition (1,263 g C/m^2) × substrate type (biocrust, desert pavement) on lichen and moss cover (top) and richness (1 m^2) (bottom) 5 (left) and 7 (right) years after disturbance and sucrose treatments. Disturbance was not significant, and responses are averaged across disturbance treatments. Letters indicate significant ($p \leq 0.05$) groups. Error bars are ±1 SE.

macrospecies cover in biocrust plots, year was not a significant factor, only sucrose addition which significantly reduced cover (Fig. 1, Fig. 4, and Table S2). For richness in biocrust plots, year and sucrose addition had significant main effects (Fig. 4). We detected an increase in species among biocrust plots 7 years after treatments, but we observed continued declines in species detected in sucrose-treated plots during the 7-year survey.

In general, we observed more macrospecies more frequently among biocrust and pavement plots, particularly among non-sucrose plots, at 7 years as compared to 5 years after treatments (Table 1). Sucrose-treated pavement plots were the only exception. We observed fewer species and species less frequently among sucrose-treated plots both years we surveyed. For example, we did not detect *S. caninervis* in sucrose-treated biocrust plots and we only detected one lichen species, *C. coccophorum*, in sucrose-treated pavement plots both years. For the cyanobacteria component from biocrust field plots, neither disturbance nor sucrose addition significantly affected abundance in biocrust plots (Fig. 5).

Laboratory Experiments

Field and laboratory examinations of the effect of sucrose on cyanobacteria differed. In the laboratory, we observed a significant negative sucrose effect on cyanobacteria after 1 month. In the initial trial with doses closer to field levels, we did not observe easily countable cyanobacteria but instead what appeared to be distressed cyanobacteria (e.g. lysed or shriveled cells, sheaths without cyanobacteria cells). Cyanobacteria were only observed in control samples and appeared to be healthy (data not shown). In the second trial, cyanobacteria significantly and progressively declined with increasing sucrose concentrations (Fig. 5).

Both moss species were negatively affected by sucrose at higher concentrations and after longer exposure times (Figs. 6 & 7). Dark- and light-adapted fluorescence response to sucrose solutions resulted in similar trends and similar significant post hoc results of treatments for each moss species. Only dark-adapted fluorescence is presented for each moss species. At 24 hours, sucrose solutions had little effect on fluorescence. However, after 96 hours of exposure, fluorescence decreased while NPQ increased, particularly at the highest sucrose concentration. Continued exposure to sucrose caused further declines in fluorescence and increases in NPQ, especially in the higher concentration. *Funaria* displayed a greater stress response than *Bryum*. Microscopic examination of *Funaria* shoot tissue incubated in the highest sucrose concentration showed chloroplasts clustered in the center of cells compared to along the margins of cells in less stressed tissues (Fig. 8). We could not identify if the plasma membrane had lysed or plasmolysis had occurred.

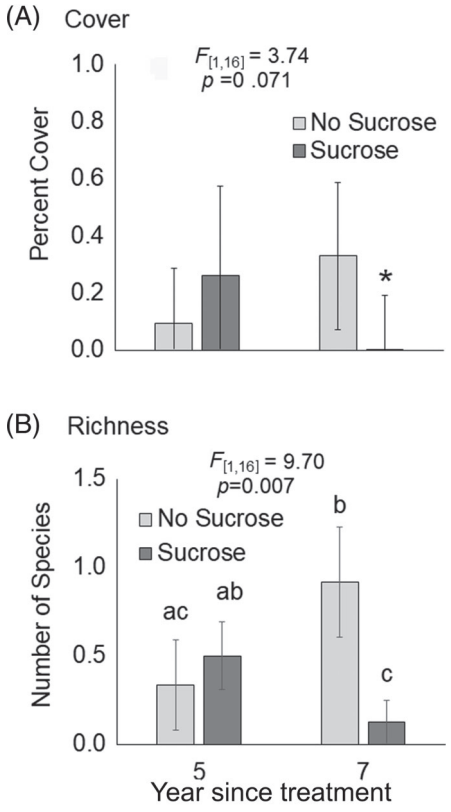

Figure 3. Significant effects between survey years on lichen and moss cover and richness (1 m^2) in desert pavement plots. Treatments included disturbance and sucrose addition (1,263 g C/m^2). Disturbance treatment was not significant, and responses are averaged across disturbance treatments. Letters indicate significant ($p \leq 0.05$) groups. The asterisk indicates significance ($p \leq 0.10$). Error bars are ±1 SE.

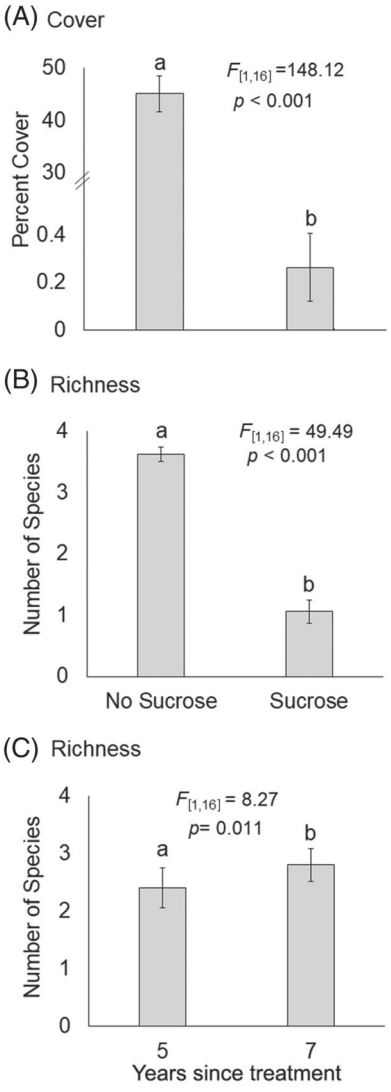

Figure 4. Significant effects between sucrose treatments or between survey years on lichen and moss cover and richness (1 m^2) in biocrust plots. Treatments included disturbance and sucrose addition (1,263 g C/m^2). Disturbance treatment was not significant, and responses are averaged across disturbance treatments. Letters indicate significant ($p \leq 0.05$) groups. Error bars are ±1 SE.

Discussion

After treating non-native plants using carbon addition, we observed a side effect on biocrust organisms. Results of the field study suggest that sucrose addition had minimal apparent influence on cyanobacteria in biocrust but sharply reduced lichen and moss cover and altered species composition. These effects persisted for at least 7 years. To more fully explore this undesirable setback and surprise from treating non-native plants, follow-up laboratory experiments reinforced the field study and revealed that sucrose at different concentrations negatively affected cyanobacteria and mosses. The mechanisms by which sucrose might be affecting these biocrust organisms are unclear but could potentially relate to several hypothesized factors.

Hypothetical Mechanisms for the Negative Effects of Sucrose Addition on Biocrust

Although we do not currently have direct evidence to explain our results, existing literature and our laboratory studies assist to formulate hypotheses, which are not necessarily mutually exclusive:

(1) Sucrose solutions are hypertonic compared to cellular cytoplasm. Water moved out of cells to a region of higher solute concentration causing cells to desiccate more rapidly. In turn, sucrose reentered solution during hydration events and reduced or hindered cellular absorption of water.
(2) Biocrust organisms experienced extreme desiccation events under the effects of sucrose solutions. Because sucrose solutions drew water out of cells, organisms experience extreme desiccation greater than their desiccation tolerances.
(3) Repeated insufficient rehydration (sub-turgor conditions) during hydration events caused by sucrose damaged cellular functioning (e.g. reduced photosynthetic efficiency), creating a carbon deficit.

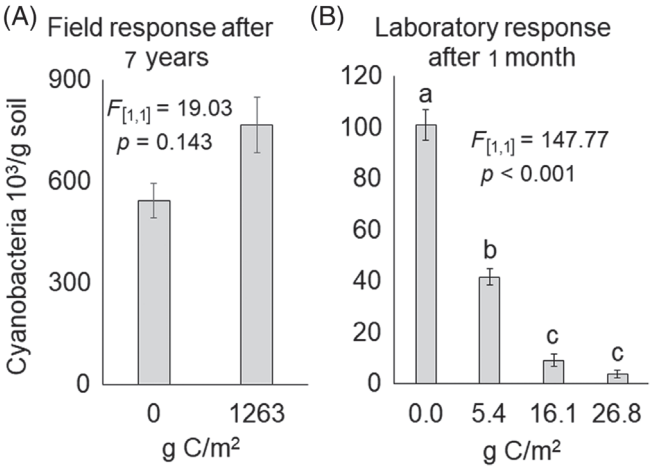

Figure 5. Estimated filamentous cyanobacteria per 1 g soil (A) from field plots 7 years after sucrose addition and (B) in a laboratory study 1 month after four levels of sucrose treatment. Sucrose addition is expressed in carbon addition equivalents per 1-m^2 application area. Post hoc tests are shown for significant results. Letters indicate significant ($p \leq 0.05$) groups. Error bars are ±1 SE.

Sucrose Decreased Rate of Drying or Hindered Water Absorption

Many lichens, mosses, and cyanobacteria are desiccation tolerant (Potts 1999; Proctor et al. 2007; Kranner et al. 2008). Desiccation tolerance is the ability to rehydrate and return to normal biological activity after a desiccation event (i.e. available water has been lost to surrounding dry atmosphere; Lüttge et al. 2011). Preparation for a desiccated state and successful recovery from a desiccation event (e.g. achieve net positive photosynthesis) requires time, although time varies among different organisms (Lüttge et al. 2011). In some organisms, less preparation for a desiccated state is required; such is the case for constitutively desiccation-tolerant organisms. For other species, desiccation tolerance can be induced by environmental conditions (Greenwood & Stark 2014; Stark et al. 2014). The desiccation-tolerant moss *Syntrichia ruralis* can withstand rapid desiccation (30 minutes) because it is constitutively desiccation tolerant (Oliver & Bewley 1984). The moss *Physcomitrella patens* requires significantly more time (days) to induce a desiccation-tolerant phenotype (Greenwood & Stark 2014). Species may also exhibit a range of resiliency to desiccation generally and to specific events.

Biocrust species in hot deserts would be expected to express phenotypes which tolerate relatively rapid drying. However, extremely rapid drying or an extended period in a stressed sub-turgor state before desiccation may impede cellular protective measures or recovery (Pressel & Duckett 2010). Sucrose solutions used in this study may have been hypertonic compared to cellular cytoplasm, triggering more rapid drying than an organism can recover from during a subsequent hydration event. Osmotically induced dehydration using a sucrose solution has been demonstrated previously in lichens (Jensen et al. 1999; Hájek et al. 2006). In turn, sucrose present in soils reenters solution during a hydration (or precipitation) event and may reduce or hinder cellular absorption of water, reducing the reactivation of photosynthesis and metabolic response to damage.

Sucrose Solutions Caused Desiccating Events Greater Than Organisms' Desiccation Tolerances. Biocrust organisms are poikilohydric, meaning they lack an ability (structural or functional) to maintain or regulate water content and are more susceptible to changing water conditions in their environments compared to vascular plants. Although biocrust organisms have structures that absorb water and increase time to desiccation (e.g. gelatinous cyanobacteria sheaths, Lange 1976; thick hyphal cell walls, Kranner et al. 2008), as poikilohydric organisms, they gain and lose water relatively rapidly and internal cellular water content equilibrates to the external environment. When desiccated, these organisms hold onto strongly bound water, while no unbound water remains in cells.

Salts and sugars function as osmolytes when dissolved in fluids, influencing osmolarity and the direction in which water moves across membranes. In the presence of a hypertonic sucrose solution, organisms may experience extreme desiccation events beyond their usual tolerances. Jensen et al. (1999) observed sucrose caused declines in water potential and chlorophyll fluorescence in lichens. Hájek et al. (2006) observed changes in distribution and size of a lichen photobiont and inhibition of photosynthetic processes using a 2.5M sucrose solution, a concentration less than the solution applied to our field plots (2.9M sucrose). Because of the lack of ability of biocrust organisms to regulate water content, sucrose may have caused extreme water loss and breakdown of cellular structures. This possibly is what we observed in *Funaria* cells and is similar to what occurs in food preservation (e.g. meat jerky or fruit preserves).

Repeated Insufficient Hydration Period Causes a Cellular Carbon Deficit. Several studies have demonstrated that short hydration events will stress desiccation-tolerant, poikilohydric species. For example, Coe et al. (2012) demonstrated this condition in the moss *S. caninervis*, a desiccation-tolerant moss. Smaller precipitation events and increasing time of a desiccation period before a precipitation event resulted in carbon deficits. Repeated short rainfall events that do not allow mosses such as *Syntrichia* to fully hydrate can result in degradation of pigments, or chlorosis (Barker et al. 2005), and loss of aboveground biomass (Stark et al. 2011). In our field study, corresponding carbon deficits accumulating over time might have led to losses in moss or other biocrust species' biomass.

Reconciling Field and Laboratory Effects of Sucrose Addition on Cyanobacteria. The differences in response to sucrose by cyanobacteria at field sites compared to laboratory trials suggest environmental conditions were likely a factor. Laboratory samples were continuously hydrated and thus likely continuously under osmotic stress. Meanwhile, cyanobacteria under field conditions were likely only biologically active after a precipitation event. Our observations that estimated cyanobacteria in

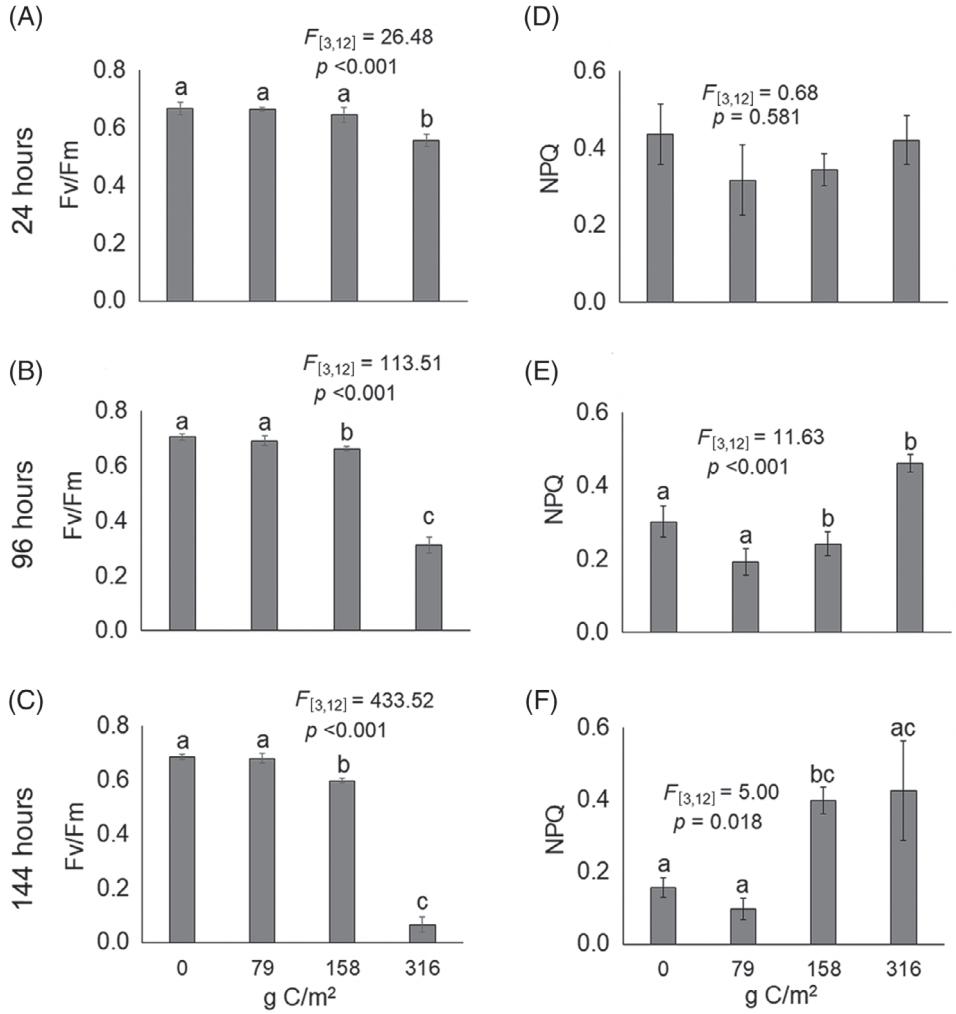

Figure 6. Dark-adapted chlorophyll fluorescence (Fv/Fm) and non-photochemical quenching (NPQ) for the moss *Funaria hygrometrica* after 24, 96, and 144 hours exposure to three levels of sucrose solutions and compared to a control. Sucrose addition is expressed in carbon addition equivalents per 1-m^2 application area. Post hoc tests are only shown for significant results. Letters indicate significant ($p < 0.05$) groups. Error bars are ±1 SE.

untreated and sucrose-treated plots were similar 7 years after treatment could have several explanations: (1) cyanobacteria were not affected by sucrose addition, or cyanobacteria were more resistant than other biocrust constituents we examined; (2) sucrose applied to field plots dissipated over time, lessening the effect or allowing for cyanobacteria recovery, possibly suggesting cyanobacteria are more resilient than other biocrust constituents we examined; or (3) cyanobacteria were initially stimulated by carbon addition similar to other microbial responses (e.g. Hamada 1993), and what we detected was actually a slower response compared to other biocrust constituents we examined and a slow decline. Additional sampling over time and assessing soil carbon content could elucidate responses of biocrust components to sucrose in a field setting.

Unanticipated Trade-Offs of Carbon Addition

The assumption that carbon addition stimulates the soil microbial community does not appear to extend to all biocrust constituents. Carbon addition has previously been demonstrated to provide short-term benefits to microbial populations, particularly when carbon is added as a labile and rapidly available source like sucrose (Szili-Kovács et al. 2007; Steers et al. 2011). Exposure over longer terms to carbon sources can have relatively positive effects (Tilston et al. 2009), no effect, or negative effects on certain microbial constituents (Lange 1976; Steers et al. 2011). Some biocrust constituents may have benefited from carbon addition; for example, specific groups of cyanobacteria (Hamada 1993).

Biocrust response to experimental disturbance and carbon addition was not a primary focus during the initial non-native plant treatment experiment. Hypothetically, we might have missed declines in biocrust during the first years after sucrose addition, or initial declines 1 or 2 years after treatments were not yet evident. Declines in *Collema*, a cosmopolitan species constituting most of the biocrust cover at the study site, were more obvious than declines in other constituents.

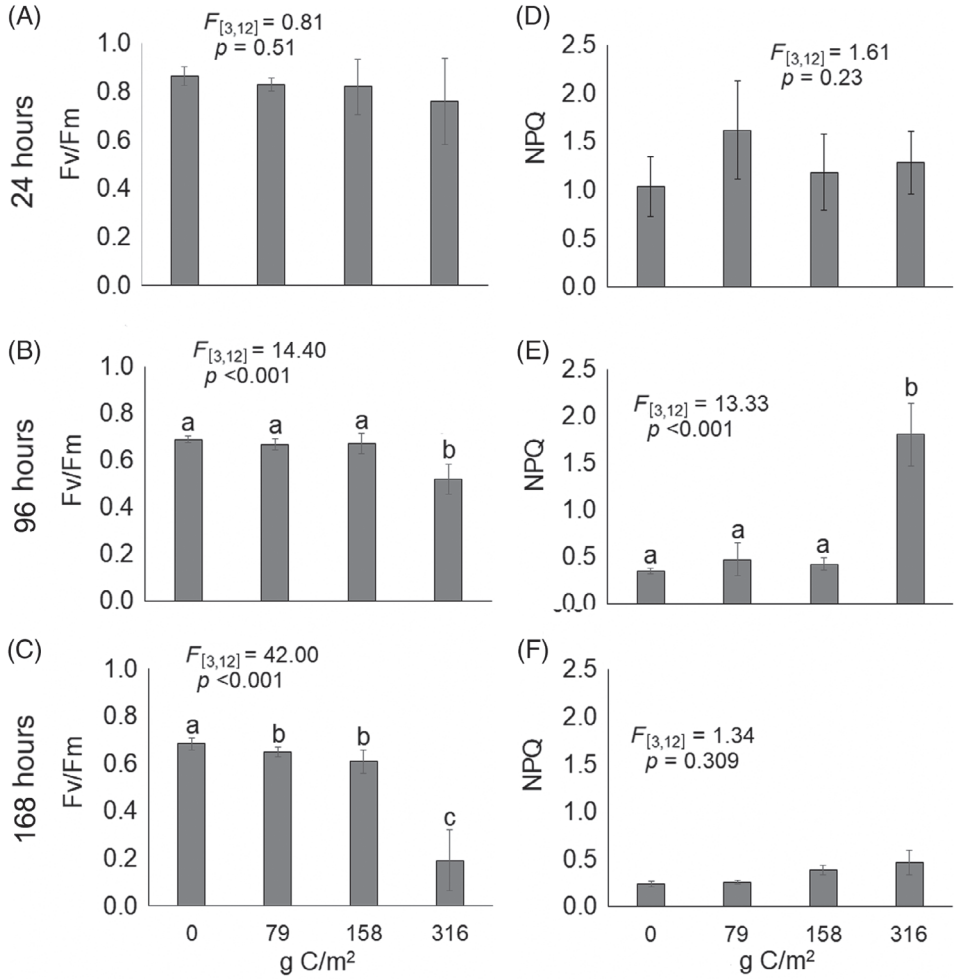

Figure 7. Dark-adapted chlorophyll fluorescence (Fv/Fm) and non-photochemical quenching (NPQ) for the moss *Bryum argenteum* after 24, 96, and 168 hours exposure to three levels of sucrose solutions and compared to a control. Sucrose addition is expressed in carbon addition equivalents per 1-m^2 application area. Post hoc tests are shown for significant results. Letters indicate significant ($p \leq 0.05$) groups. Error bars are ±1 SE.

Addressing Trade-Offs in Non-native Plant Management and Soil Conservation

Restoration prescriptions often are not able to account for all ecosystem components during monitoring, and responses to treatments might not initially be obvious or obvious without continued monitoring. We observed symptoms of homogenization with loss of species and simplification of the surface biocrust community, but only after what appeared to be a significant loss of surface biocrust and unmistakably square-shaped patches of bare soil (Fig. S1). Homogenization occurs when an environmental change promotes loss of genetic, taxonomic, or functional distinctiveness and multifunctionality over time (Olden et al. 2004). Losses of species diversity or of specialized species cause declines in functional diversity (Olden & Rooney 2006; Clavel et al. 2011). A main implication of carbon addition in the form of sucrose on relatively intact soil surfaces with biocrust is a potential significant change in the biocrust community, which may not be initially recognizable with limited monitoring because of seasonal variability in biocrust communities.

Biocrusts are hypothesized to vary with environmental conditions, although there are few long-term studies on biocrusts and which have captured seasonal or yearly variability. In our study system, lichens and mosses occurred patchily in interspaces between perennial vegetation, with the remaining surface either bare soil or patches of cyanobacteria-dominated biological and physical crust. We detected variability of biocrust at our study site among years. However, the effect of sucrose was more significant than temporal variation, particularly on biocrust cover. Yearly or more frequent surveys would be necessary to understand responses of the biocrust community to differences in seasonal or yearly precipitation and interactions with the long-term consequences of sucrose addition.

The attributes that make biocrust organisms unique and resilient in extreme ecosystems may be antagonistic to the effects of some forms of carbon. For example, desiccation tolerance, poikilohydry, and the ability to withstand and recover from desiccation could be negatively affected by sucrose addition. Destruction of biocrust by carbon addition is likely

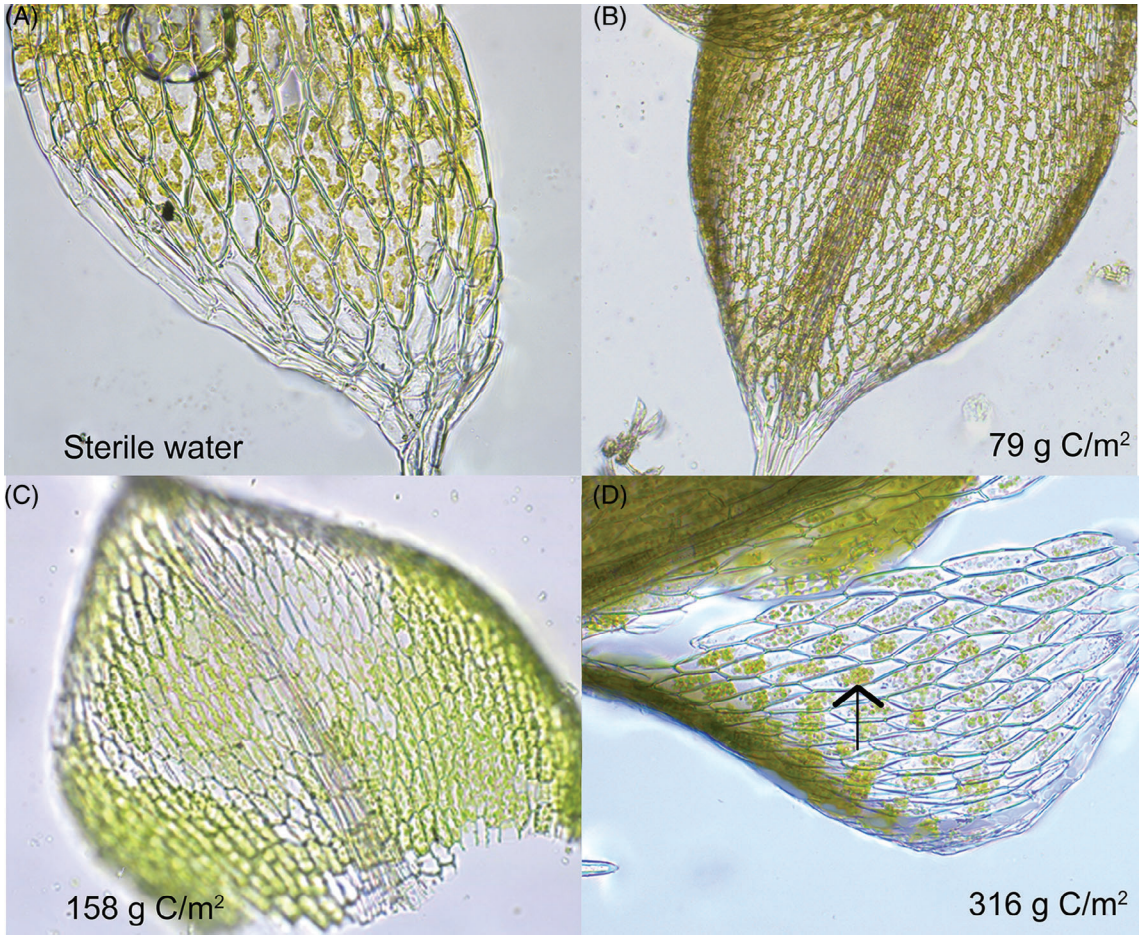

Figure 8. Microscopic images (400× magnification) of *Funaria hygrometrica* tissue 144 hours after submersion in sucrose solutions (A–D). The arrow indicates clustering of chloroplasts at the center of cells in the highest sucrose addition treatment.

an unacceptable trade-off of this form of non-native plant management due to the important functions biocrust provides in drylands. Biocrust and cyanobacteria communities affect soil physicochemical properties, including soil aggregate stability, water retention, and organic carbon and nitrogen content (Chamizo et al. 2012). Removal of lichens and mosses can reduce soil stability, making soils more vulnerable to wind and water erosion (Belnap & Gillette 1998; Belnap 2006). Increased vulnerability to erosion can promote persistent disturbance. These losses of functions provided by biocrust could, ironically, be counterproductive to the intended goal of carbon addition treatments by actually increasing disturbance and abundance of non-native plants over the long term.

Developing Carbon Addition Treatments in Drylands

Similar to the varying plant responses to different forms of carbon and in different environments, biocrust and subsurface microbial communities may also have different responses depending on the form of carbon, concentration, and environment. Most carbon addition studies occur in temperate regions where biocrusts are not prominent features of landscapes (Perry et al. 2010, except see Allen et al. 2011). Most carbon addition studies have used sucrose (reviewed in Alpert 2010; Perry et al. 2010), although some have used glucose (Blumenthal 2009) or plant materials (e.g. sawdust, plant litter; Zink & Allen 1998; Corbin & D'Antonio 2004; Tilston et al. 2009). Although larger woody materials may be problematic for biocrusts (e.g. disturbance to incorporate material into surfaces or problems associated with burial; Jia et al. 2008, 2012; Rao et al. 2012), labile forms of carbon, such as simple sugars or polyols that are similar to naturally occurring photosynthetic and metabolic pathway constituents (Hill & Smith 1972; Honegger 1991), may in fact benefit some biocrust species or at least not affect them as negatively. Glucose is a primary product and an important metabolic regulator in photosynthetic organisms. Low levels of glucose have benefited microbes in laboratory studies (e.g. Rippka 1972; Lange 1976; Kuzyakov et al. 2000). Mannitol is one of the most abundant polyols in nature and a widely occurring polyol in fungi, algae, and lichens (Stoop et al. 1996). Testing different concentrations and forms of carbon would be necessary to determine the best form of carbon delivery to maximize effects on vascular non-native plants while minimizing non-target effects on biocrusts.

Balancing the desired effects of carbon addition on non-native plants without negatively impacting biocrusts requires additional studies. If carbon addition is perceived as a non-native invasive plant control measure for drylands, and if biocrust or other microbial surface communities are present, further testing of the effects, types, and concentrations of carbon are necessary to evaluate effects on vascular plants and surface biocrust communities.

Acknowledgments

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Supporting Information

The following information may be found in the online version of this article:

Table S1. Effect of experimental disturbance and sucrose addition on the biocrust community 5 and 7 years after treatments on two surface types, desert pavement and biocrust.
Table S2. Effect of disturbance and sucrose on the biocrust community over time on two surface types, desert pavement and biocrust, on the biocrust community.
Figure S1. Image showing biocrust negatively impacted by sucrose addition.
Figure S2. Images of patches of desert pavement and biocrust.
Figure S3. Examples of pavement and biocrust plots.
Figure S4. Demonstration of treatment during installation in February 2009.

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Supplementary materials

Unexpected side effects in biocrust after treating non-native plants using carbon addition

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Includes:

Table S1. Effect of experimental disturbance and sucrose addition on the biocrust cover and richness five and seven years after treatments on two surface types, desert pavement and biocrust. Treatments were applied to 1-m^2 plots. Disturbance involved raking and tearing surface material. Sucrose addition added 1263 g C/m^2. P values ≤0.10 are in italics and P values ≤0.50 are in bold.

Variable	df	Surface		Disturbance		Sucrose		Sucrose × Disturbance		Surface × Sucrose		Disturbance × Sucrose		Surface × Disturbance × Sucrose	
5 yr		F	P	F	P	F	P	F	P	F	P	F	P	F	P
Cover	1,14	5.72	**0.031**	2.09	0.170	14.73	**0.002**	1.86	0.195	15.39	**0.002**	0.01	0.934	0.00	1.000
Richness	1,32	11.31	**0.002**	0.04	0.841	1.68	0.204	0.36	0.554	4.39	**0.044**	2.16	0.152	3.48	*0.071*
7 yr															
Cover	1,32	85.24	**<0.001**	0.00	0.946	86.10	**<0.001**	0.00	0.993	82.91	**<0.001**	0.00	0.966	0.01	0.918
Richness	1,32	68.84	**<0.001**	0.27	0.608	45.45	**<0.001**	0.00	1.000	9.68	**0.004**	0.27	0.608	1.08	0.308

Table S2. Results of repeated measures analysis of variance of the effect of disturbance and sucrose addition on the biocrust community in two surface types, desert pavement and biocrust. Treatments were applied to 1-m^2 plots. Disturbance involved raking and tearing surface material. Sucrose addition added 1263 g C/m^2. Plots were assessed 5 and 7 yr after treatment. P values ≤0.10 are in italics and P values ≤0.50 are in bold.

Variable	Year			Disturbance			Sucrose			Year × Disturbance			Year × Sucrose			Sucrose × Disturbance			Year × Sucrose × Disturbance		
Pavement																					
	df	F	P	df	F	P	df	F	P	df	F	P	df	F	P	df	F	P	df	F	P
Cover	1,16	0.00	0.947	1,16	0.55	0.470	1,16	0.57	0.460	1,16	0.01	0.939	1,16	3.74	*0.071*	1,16	0.66	0.429	1,16	0.00	0.992
Richness	1,16	0.39	0.542	1,16	0.07	0.797	1,16	1.28	0.274	1,16	0.04	0.838	1,16	9.70	**0.007**	1,16	1.71	0.210	1,16	0.39	0.542
Biocrust																					
	df	F	P	df	F	P	df	F	P	df	F	P	df	F	P	df	F	P	df	F	P
Cover	1,12	1.01	0.334	1,16	0.02	0.901	1,16	148.12	**<0.001**	1,12	0.82	0.382	1,12	0.06	0.810	1,16	0.00	0.973	1,12	1.11	0.312
Richness	1,16	8.27	**0.011**	1,16	0.05	0.823	1,16	49.49	**<0.001**	1,16	1.04	0.323	1,16	3.24	*0.091*	1,16	0.56	0.467	1,16	0.10	0.759

Figure S1. Top: An example of a biocrust patch plot (1 m × 1 m) in which biocrust appeared to be negatively affected by the sucrose addition (1263 g C/m^2) in a Mojave Desert ecosystem. Photograph taken April 2014. Below: Left, a close up of a plot in April 2016 that received sucrose addition in 2009. Right, a close up in April 2016 of a plot that did not receive sucrose addiction treatment. Length of white bars represents 10 cm. Photographs by L.P. Chiquoine.

Figure S2. Images of the study site in April 2014. The study was conducted in the Mojave Desert, USA at Lake Mead National Recreation Area (National Park Service), 40 km from Las Vegas, Nevada at an elevation of 633 m (36°14'49"N, 114°31'50"W). The 0.4-ha site contained patchy desert pavement (left) and shrub-dominated communities with surface lichen-moss biocrust occupying interspaces and under the dripline of shrubs (right). In pavements, lichens were observed between some gravel and cobble. Photographs by S.R. Abella.

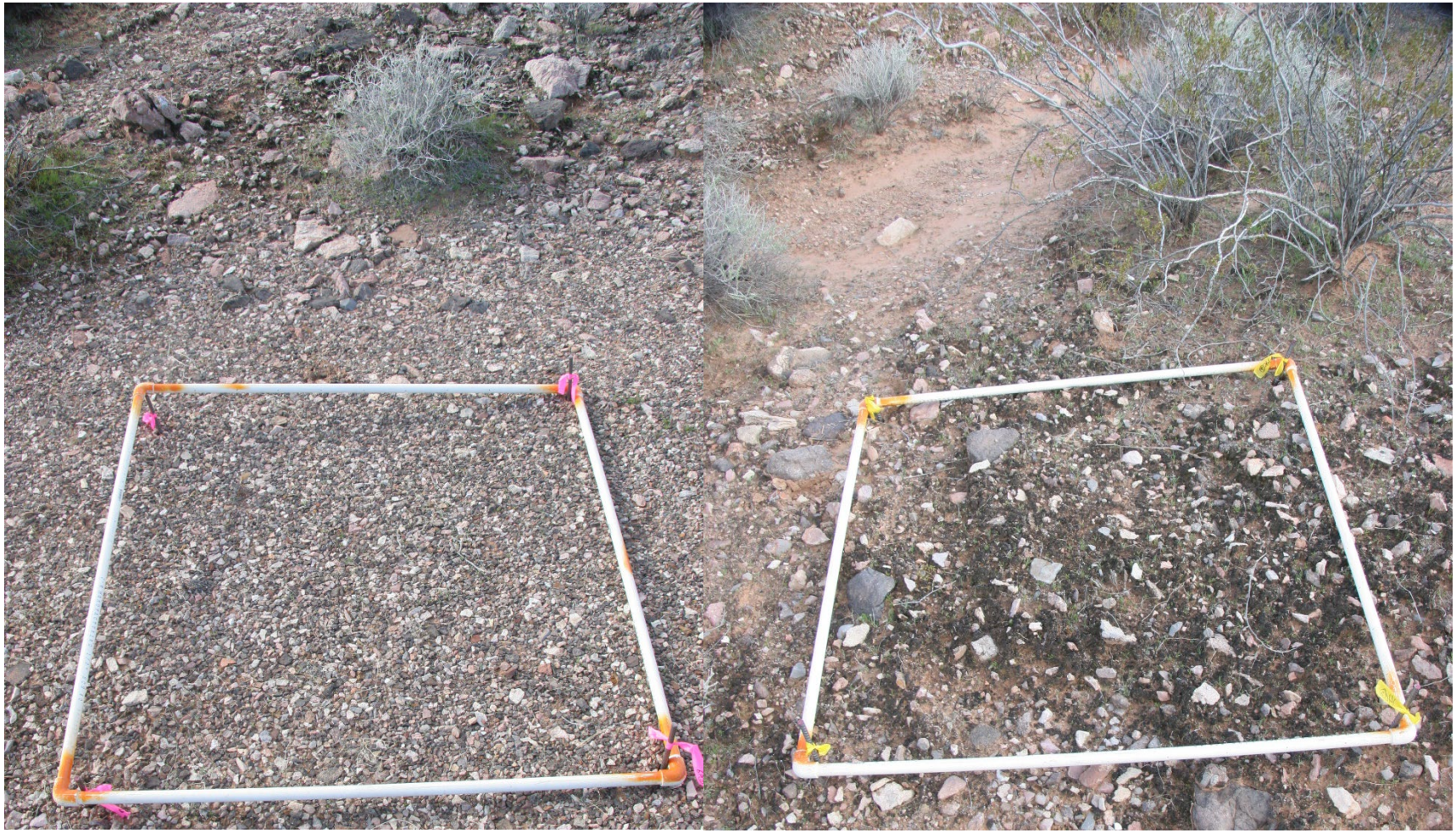

Figure S3. Examples of a desert pavement (left) and biocrust plot (right) in February 2009. Photographs by A. DeCorte.

Figure S4. Example of treatments installed in February 2009. Disturbance treatments (left) were conducted by raking the surface to rip surface material and dislodge surface rocks. Carbon addition was applied as a 2.9 *M* sucrose solution delivering 1263 g C/m^2 using a backpack sprayer. Photographs by A. DeCorte.