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REGULAR ARTICLE

Biocrust carbon isotope signature was depleted under a C_3 forb compared to interspace

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Abstract
Aims Plants and biological soil crusts (biocrusts) are the key producers in drylands, but biocrusts seldom show net CO_2 uptake. I hypothesized that biocrusts could augment CO_2 fixation by incorporating plant-derived carbon.
Methods I collected biocrusts located at the base of *Gutierrezia sarothrae* (C_3 forb), *Bouteloua gracilis* (C_4 grass), and from bare interspaces between plants, and from a mesocosm experiment with live *B. gracilis* or dead *B. gracilis* roots. To trace carbon sources, I determined ^{13}C values of the biocrust community, isolated cyanobacteria and lichen, and plant leaves because the photosynthetic pathway distinguishes the tissue ^{13}C values.
Results Biocrust communities and washed cyanobacteria and cyanolichen in *G. sarothrae* microsites were depleted by ~2‰ relative to other locations. Biocrust $\delta^{13}C$ did not differ between the interspace and live or dead *B. gracilis*.
Conclusions Potential mechanisms for the trend in biocrust $\delta^{13}C$ adjacent to C_3 plants include differences in microsite conditions, biocrust communities, use of respired CO_2 in the soil matrix for photosynthesis, or mixotrophic use of plant photosynthates. Further investigation of this observation may improve understanding of the degree to which the activities of dryland primary producers are coupled.

Keywords Biological soil crust · C_3 plant · C_4 plant · Delta ^{13}C · Carbon dynamics

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Introduction

Drylands cover ~45% of the earth's terrestrial surface (Prăvălie 2016) and are important drivers of interannual variability in global carbon dynamics (Ahlstrom et al. 2015). Primary producers in drylands include vascular plants and biological soil crusts ("biocrusts"). Biocrusts are present in arid and semi-arid communities globally (Belnap et al. 2001) and contain algae, cyanobacteria, mosses, lichens, bacteria, and fungi living at the soil surface. Desert and steppe biocrusts account for an estimated 0.59 Pg y^{-1} of annual global terrestrial CO_2 uptake (Elbert et al. 2012 from supplementary tables). Thus, understanding controls on carbon cycling through dryland biocrusts has potential to improve estimates of global terrestrial carbon flux.

Despite the presence of photosynthetic organisms, biocrusts typically exhibit a net release of CO_2 and only rarely demonstrate net uptake in field conditions (Wilske et al. 2008; de Guevara et al. 2014, Darrouzet-Nardi et al. 2015 include in situ field measurements that record fluxes from soil and roots in addition to biocrusts). In many drylands, the majority of moisture arrives during the hottest season (Zhou et al. 2008), with pulses of

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precipitation interspersed between long dry periods (Huxman et al. 2004). During these moist periods, autotrophic biocrust components must repair their tissues and initiate activity, so they initially respire before accumulating new carbon (Sponseller 2007). Thus, the total time available for biocrusts to fix carbon is often short because small rain events are followed by dry periods.

Some organisms are capable of supplementing their photosynthetic carbon capture by incorporating organic carbon compounds from external sources. For example, when grown in culture, some terrestrial cyanobacteria and algae use mixotrophic strategies (Yu et al. 2009; Gustavs et al. 2016). Biocrusts can take up organic and inorganic forms of carbon in situ (Green et al. 2008), but the importance of plant-derived carbon to biocrust function has not yet been investigated. Determining the controls on carbon uptake, exchange, and release in biocrusts has high potential to improve understanding of carbon cycling in drylands.

I made field observations to explore whether bulk tissue stable isotope patterns are consistent with biocrust use of plant-derived carbon. First, I determined the ^{13}C values of dryland primary producers: a dominant C_3 plant species, a dominant C_4 plant species, and biocrust communities in interspaces (*Objective 1*). Stable isotopes distinguish plant functional groups by their photosynthetic pathway and are expressed in parts per thousand (per mil, ‰): C_3 plants have $\delta^{13}C$ values near −28‰ and C_4 plants have values near −14‰ (O'Leary 1988). Because biocrusts are mixtures of functionally different microbes and macrobes, their $\delta^{13}C$ values can vary considerably (estimates range from −15 to −25‰, Aranibar et al. 2003, Cable and Huxman 2004, and Zelikova et al. 2012). Cyanobacteria and cyanolichens can have carbon-concentrating mechanisms (Badger and Price 1992), leading to relatively higher $\delta^{13}C$ values than C_3 plants; thus their $\delta^{13}C$ values are typically closer to those of C_4 plants (Raven et al. 2008).

Second, I compared $\delta^{13}C$ values of biocrust communities and isolated biocrust autotrophs from three microsites: at the base of a C_3 plant, at the base of a C_4 plant, or in interspaces between plants (*Objective 2*). The underlying assumption was that if biocrust autotrophs use plant C, then their isotopic signatures should differ among these locations because more plant carbon is available near plants than in interspaces. If biocrusts incorporate plant-derived carbon from C_3 or C_4 plants, then those biocrusts should be relatively depleted (in C_3 microsite) or relatively enriched (in C_4 microsite).

Third, to test whether photosynthetically active plants influenced biocrust isotopic signatures more strongly than dead plants, I opportunistically sampled from an established mesocosm experiment in which I paired biocrusts with living or dead (for 2 years) plants (*Objective 3*). Living plants produce root-respired CO_2 (generally depleted relative to leaf $\delta^{13}C$ values; Bowling et al. 2008) that may be available for biocrust fixation. Additionally, root exudates from recently fixed photosynthate can be processed and fractionated by the microbial community prior to reaching the biocrusts at the surface. Dead plant material receives no further input of photosynthate and undergoes no growth or maintenance respiration and so tissue can only be degraded through microbial or abiotic activity. The drivers of fractionation of soil organic material to CO_2 by microbes have not been fully resolved (Breecker et al. 2015), but if there are differences in the $\delta^{13}C$ values of the carbon released in different forms by plants when living vs. dead, and if the biocrusts use one or more of these forms of carbon derived from the plants, then the biocrusts carbon signature will vary if a living or dead plant is present. Differences in the biocrust isotopic signature adjacent to living versus dead plants suggest would indicate that a metabolically active plant affects the biocrust carbon signature.

Methods

Study sites I collected biocrusts from two sites in New Mexico. At the Sevilleta National Wildlife Refuge (SNWR) grassland (34.359, −106.736, WGS 84 Web Mercator, elevation 1600 m), I collected light cyanobacterial biocrusts dominated by the cyanobacteria *Microcoleus vaginatus* and *M. steenstrupii*; Garcia-Pichel et al. 2013). The grassland is dominated by the C_4 bunchgrasses blue grama (*Bouteloua gracilis* (Willd. ex Kunth) Lag. ex Griffiths) and black grama (*B. eriopoda* (Torr.) Torr.). Herbaceous C_3 plants include snakeweed (*Gutierrezia sarothrae* (Pursh) Britton & Rusby) as a dominant species. The climate is semi-arid with mean annual temperature of 13C and a summer monsoon season that delivers 60% of the ~250 mm mean annual precipitation (Moore 2016). Livestock grazing has been excluded since 1973.

At the La Puebla site (private property, 35.978, −105.995, elevation 1800 m), I collected dark, mature biocrusts. These biocrusts were composed of cyanobacteria (*Microcoleus* spp., *Scytonema* spp.,

Nostoc spp.), with scattered mosses (including *Bryum argenteum, Pterygoneurum ovatum, Syntrichia ruralis*) and lichens (*Collema* spp., *Placidium* spp., *Psora* spp.). This site is a one-seed juniper savanna (*Juniperus monosperma* (Engelm.) Sarg.) with few piñon pine (*Pinus edulis* Engelm.). The understory is dominated by the C_4 bunchgrass *B. gracilis* and the C_3 forb *G. sarothrae*. Mean annual temperature is 11C and a summer monsoon season delivers ~60% of 290 mm mean annual precipitation (Western Regional Climate Center 2015). Livestock grazing has been excluded since the 1950s.

Field mesocosm experiment At the La Puebla site, field mesocosms were set up in August 2013 by transplanting 80 individual *B. gracilis* with their associated soil and biocrusts, collected from nearby natural areas into separate 7.57 L pots. From half of the pots, the plant aboveground biomass and root crown were removed, killing the plant but leaving roots to decompose without disturbing the soil structure. The plants remained living in the other half of the pots. All pots were drip irrigated (RBY100MPTX filter, Rainbird, Azusa, CA, USA; 1.9 cm hose) with well water via maximum 12.11 L h^{-1} pressure compensating spray stakes (#22500–002030, Netafim USA, Fresno, CA, USA) and 91.4 cm dripper assembly (#40201–002020; Netafim USA, Fresno, CA, USA). For the small, frequent treatment, pots were watered once per week for 1 min using a timer (Orbit model 62,056, Orbit, Bountiful, Utah, USA). Although this design could provide up to 200 mL of water per pot, the water output emitted from each sprayer was actually 100 mL per event because the hose dried between applications. For the large, infrequent treatment, I hand-watered with 400 mL of well water once per month. Water additions occurred only during the summer months (May–September). There was no difference between watering treatments in plant size (20.8 g ± 1.9 s.e.; $F_{1,8} = 0.18$, $P = 0.68$) or biocrust $\delta^{13}C$ (−20.5‰ ± 0.3 s.e., $F_{1,8} = 0.06$, $P = 0.80$). Thus, I focused on comparisons of live plants versus dead roots across both water treatments, and I do not directly compare the mesocosm with field-collected samples due to the difference in water regime.

Sample collection Samples were collected in April 2015. Each biocrust sample was collected from the surface to the depth of biological aggregation of the soil (0.5–1.0 cm), excluding any visible live plant material or litter. Ten leaves were collected from five randomly chosen *B. gracilis* and *G. sarothrae* individuals from the Sevilleta site.

Microsite At each site, 10 samples each were collected at the base of the *G. sarothrae* (under the canopy in many cases, depending on size of the plant), at the base of *B. gracilis* (some leaves and stems shaded this area, but there was no "canopy" comparable to *G. sarothrae*), and from the center of interspaces between plants. I selected *G. sarothrae* and *B. gracilis* because they grow within meters of each other and are abundant in the same area. Biocrusts were collected from interspaces at least 25 cm but not greater than ~50 cm from any plant. From the mesocosm experiment, I collected biocrust samples from 10 pots with no living *B. gracilis* plants ("removed") and from 10 pots with living *B. gracilis* plants ("living").

Sample type I analyzed three sample types: plant leaves, biocrust communities, and washed cyanobacteria/lichen samples. Biocrust communities included substrate material bound by filaments. To obtain cyanobacteria or cyanobacteria with lichen tissue (when present), I wet community samples with deionized water and removed green filaments and lichens with forceps under a dissecting microscope at 20X magnification and washed them in water. Overall, percentage carbon by weight was ~3–5% higher in washed samples compared to community samples, and this amount of carbon was similar to the amount of organic material in the subsoil (supplementary material), suggesting this was an effective method for separating living tissue from dead organic material. Some substrate material was tightly bound to the filaments and could not be separated. I washed cyanobacteria and lichens from a subset of the *G. sarothrae*, *B. gracilis*, and interspace La Puebla field-collected samples and from *B. gracilis* living and removed mesocosm samples ($n = 5$).

Carbon isotope processing and analysis All leaf, community, and washed samples were dried at 60C for 3d. Biocrust community samples were passed through a 2 mm sieve, and both community samples and washed samples were ground using a mortar and pestle. 10 mg of communities and 1–4 mg of washed samples were placed into silver capsules (4 × 6 mm, Costech, Valencia, CA), and capsules were left open and acid fumigated with 12 M HCl for 30 h to remove carbonates (Harris et al. 2001). Samples were then air dried for 2 h. Silver

capsules were placed into tin capsules (4 × 6 mm, Costech, Valencia, CA) to improve combustion. Leaf samples were ground in liquid nitrogen with a mortar and pestle, and 4 mg were placed into tin capsules. All samples were run on an ECS 4010 Elemental Analyzer (Costech, Valencia, CA, USA) and a Delta V Isotope Ratio Mass Spectrometer (Thermo Scientific, Waltham, MA, USA) at the University of New Mexico Center for Stable Isotopes to obtain percentage carbon (supplementary material) and $\delta^{13}C$ values for each sample (relative to standard Vienna Pee Dee Belemnite). One *G. sarothrae* microsite and two *B. gracilis* microsite community samples from the Sevilleta site and one cyanobacteria/lichen from *B. gracilis* living mesocosm and one community sample from *B. gracilis* removed from the La Puebla site were excluded from $\delta^{13}C$ analyses because due to variability in carbon content between samples, the mass spectrometer did not produce reliable values when peaks were too large.

Analysis: Objective 1 I compared the $\delta^{13}C$ values among primary producers (*G. sarothrae*, *B. gracilis* plants, and the interspace biocrust community), sites (Sevilleta vs. La Puebla), and their interaction using a two-way ANOVA. Tukey honest significant difference (HSD) post-hoc tests were used to detect pairwise differences for factors with more than two levels. Additionally, I compared the plant $\delta^{13}C$ values collected from the Sevilleta site to the values from the TRY Plant Trait database (Kattge et al. 2011, dataset IDs 159, 163, 193) using a two-tailed *t*-test to assess whether the Sevilleta plants differed from the average for the species. All analyses were conducted in R (R version 3.1.3, 2015–03-09, R Core Team 2016).

Objective 2 To compare the $\delta^{13}C$ values of the biocrust community growing next to plants against those in the interspaces, I analyzed the effects of site (Sevilleta vs. La Puebla), microsite (*G. sarothrae*, *B. gracilis*, or interspace), and the interaction using ANOVA. To determine if the $\delta^{13}C$ values of living autotrophic components of the biocrust varied among microsites, I used only samples from the La Puebla site to compare the effects of microsite (*G. sarothrae*, *B. gracilis*, or interspace), sample type (biocrust community or cyanobacteria/lichen only), and their interaction using ANOVA.

To evaluate implications of one alternative hypothesis (see discussion), I used a two-source, single isotope mixing model (Tiunov 2007) to calculate the potential plant contribution if carbon in cyanobacteria/lichen were derived from leaf tissue. Mixing models can determine the relative contributions of isotopically different carbon sources to target organisms (Peterson and Fry 1987). I assumed that the interspace values represented fully autotrophic activity and thus if biocrusts under the plants used no plant photosynthate, the values would be the same as in the interspace. I assumed that biocrusts under the plants could use both plant photosynthate and autotrophic processes. I used leaf tissue values because 1) I did not have sufficient funding to additionally analyze root tissue and 2) the more depleted value provides a more conservative estimate of the amount of plant-derived carbon compared using values of roots/litter which are additionally enriched. I used the average and standard deviations from the TRY database value (−27.92 ± 1.72) because I did not have values from the La Puebla plants. I did not have a reliable estimate of fractionation of any transformation processes of organic material within the biocrust tissue, so I acknowledge that this may be an overestimation of the proportions of contributions and further refinement is needed. The mixing model was:

$$\delta^{13}C_{\text{cyanobacteria/lichen near GUSA2}} = (\alpha) \times \delta^{13}C_{\text{cyanobacteria/lichen in interspace}} + (1\text{-}\alpha) \times \delta^{13}C_{\text{GUSA2}} \quad (1)$$

where α was the proportion of the carbon fixed autotrophically and thus (1-α) is the proportion of the carbon derived from plant tissue. I used the siarmcmcdirichletv4 function in the siar package in R (Parnell et al. 2010) with 0 corrections.

Objective 3 To determine if killing the *B. gracilis* would affect the $\delta^{13}C$ value of the biocrust community or of cyanobacteria and lichen washed samples, I compared the effects of living vs. dead *B. gracilis*, biocrust sample type (community or washed samples), and their interaction using factorial ANOVA.

Results

Biocrust $\delta^{13}C$ values were intermediate between C_3 and C_4 plants

G. sarothrae and *B. gracilis* had distinctive $\delta^{13}C$ signatures reflecting their photosynthetic pathways. At the Sevilleta site, the $\delta^{13}C$ value of the C_3 plant *G. sarothrae* was ~13‰ lighter than that of the C_4 plant

B. gracilis (Fig. 1a, $F_{3,\ 26} = 120.2$, $P < 0.001$, all Tukey HSD post-hoc pairwise tests $P \leq 0.001$) as expected. The $\delta^{13}C$ values of *G. sarothrae* and *B. gracilis* from the Sevilleta site were not different from the TRY database values for these species (Fig. 1a, *G. sarothrae* TRY mean value = −27.92, $t_4 = 0.81$, $P = 0.46$; *B. gracilis* TRY mean value = −14.76, $t_4 = 1.56$, $P = 0.19$).

The $\delta^{13}C$ values of biocrust communities from both sites ranged from −23.0 to −16.6‰, likely reflecting the complex contributions of living and dead autotrophic and heterotrophic organisms present in biocrusts. Observed biocrust values were intermediate between C_3 and C_4 plants and significantly different from both plant sources (Fig. 1a, all Tukey HSD post-hoc pairwise tests $P \leq 0.05$). In addition, the biocrust community from the La Puebla site was depleted by 2.8‰ (Tukey HSD $P \leq 0.05$) compared to that from the Sevilleta site, perhaps reflective of different community compositions between sites.

Biocrust communities under *G. sarothrae* had depleted $\delta^{13}C$ values

The mean $\delta^{13}C$ value of the biocrust community near *G. sarothrae* was 2.1‰ lower than for biocrusts collected from interspaces (Fig. 1b, $F_{2,51} = 16.00$, $P < 0.001$; Tukey HSD $P \leq 0.001$) or under *B. gracilis* (Tukey HSD $P = 0.001$). These differences were consistent across both sites (microsite × site interaction: $F_{2,51} = 1.41$, $P = 0.253$; site: $F_{1,51} = 83.74$, $P < 0.001$). In contrast, there was no significant difference between the biocrust $\delta^{13}C$ values in the interspace versus under *B. gracilis* (Tukey HSD $P = 0.771$). Percentage carbon was similar in biocrust communities under *G. sarothrae* and in the interspace (supplementary material) despite differences in $\delta^{13}C$ values, so allocation patterns in cellular processes or community composition may differ.

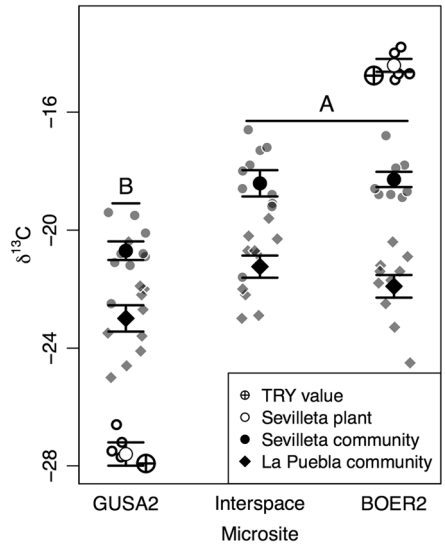

Fig. 1 Natural abundance $\delta^{13}C$ of *Gutierrezia sarothrae* (C_3 forb; "GUSA2") and *Bouteloua gracilis* (C_4 grass; "BOGR2") plant leaf tissue, the TRY database value for each species, and biocrust community collected from beneath GUSA2, BOGR2, and the interspace at two sites (Sevilleta and La Puebla) in New Mexico. Whiskers extend ±1 s.e. from the mean value. Letters show Tukey HSD post-hoc differences in biocrust community means for microsite across sites at $P \leq 0.05$

In samples from the La Puebla site, washed cyanobacteria and lichen collected under *G. sarothrae* had $\delta^{13}C$ values that were 1.4‰ lower than washed samples from the interspaces (Fig. 2a, $F_{2,39} = 8.51$, $P < 0.01$, Tukey HSD $P \leq 0.05$; sample type × microsite interaction, $F_{2,39} = 0.11$, $P = 0.89$). The $\delta^{13}C$ values of washed samples from interspaces did not differ from those collected under *B. gracilis* (Tukey HSD $P > 0.05$). The washed samples had 2.0‰ higher $\delta^{13}C$ values than the entire biocrust community ($F_{1,39} = 34.6$, $P < 0.01$).

The mixing model estimated that 16.4% (95%CI = 8.3–24.5%) of carbon in cyanobacteria and lichen washed samples could derive from C_3 plant photosynthate at the La Puebla site.

Biocrust $\delta^{13}C$ values were not affected by C_4 plant removal

In the mesocosm experiment, biocrust community $\delta^{13}C$ values did not significantly differ between pots with living *B. gracilis* and pots containing dead roots (Fig. 2b; microsite $F_{1,26} = 0.29$, $P = 0.59$; microsite × sample type $F_{1,26} = 2.59$, $P = 0.12$). In mesocosms, cyanobacteria and lichen washed samples had 1.5‰ higher $\delta^{13}C$ values than the biocrust community ($F_{1,26} = 10.5$, $P < 0.01$), similar to the difference observed for field washed samples.

I could not evaluate the mixing model under the hypothesis that biocrusts incorporated *B. gracilis* plant-derived carbon because there were no differences in the $\delta^{13}C$ values of cyanobacteria and lichens in the interspaces versus under the *B. gracilis*, or in the mesocosm pots with the living vs. removed *B. gracilis*.

Discussion

Biocrust cyanobacteria/lichens were depleted in $\delta^{13}C$ values when adjacent to *G. sarothrae,* a C_3 forb,

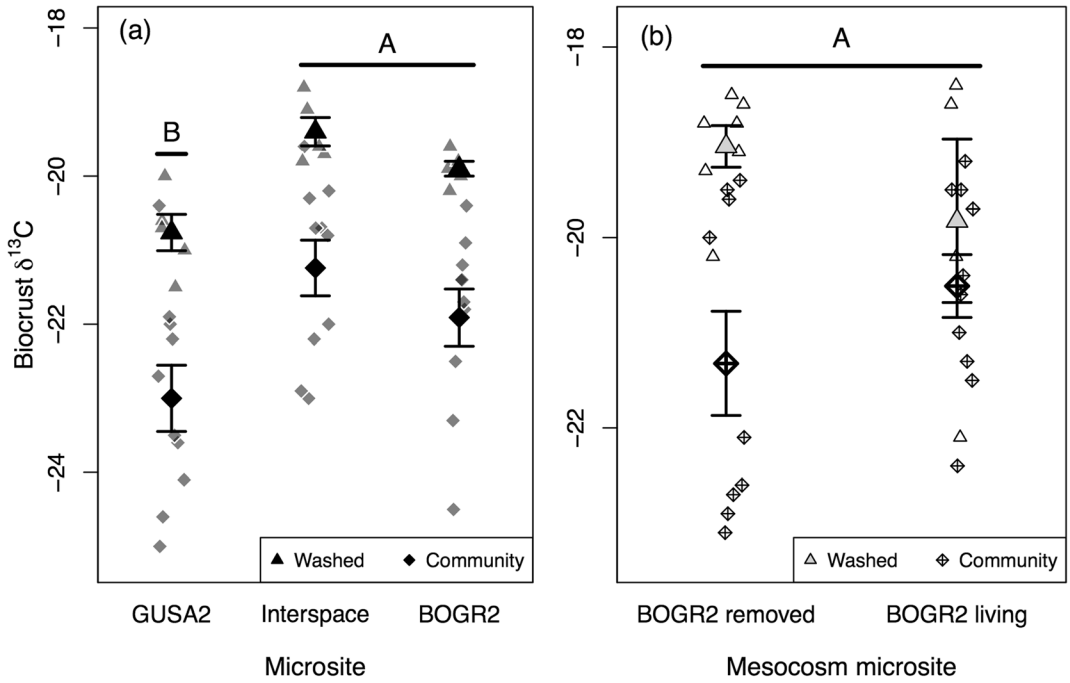

Fig. 2 Natural abundance $\delta^{13}C$ of biocrust community and washed cyanobacteria/lichen samples collected from **a** beneath naturally-occurring *Gutierrezia sarothrae* (C_3 forb; "GUSA2"), *Bouteloua gracilis* (C_4 grass; "BOGR2"), and interspace areas, and **b** in mesocosms that had either the *Bouteloua gracilis* removed or living. Whiskers extend ±1 s.e. from the mean value. Different letters within panels show Tukey HSD post-hoc differences in means for microsite across community/washed samples at $P \leq 0.05$

compared to unvegetated interspaces or adjacent to *B. gracilis*, a C_4 grass. Previous work has shown that shallow soils next to C_3 shrubs were depleted in ^{13}C compared to bulk soils in the surrounding C_4 grassland (Bai et al. 2012). Here, I have shown that $\delta^{13}C$ of the photosynthetic organisms at the soil surface, excluding soil heterotrophs and organic matter, was also spatially patterned rather than being independent of plant species identity. Further work is needed to determine if this trend holds for multiple species of C_3 and C_4 plants and across multiple sites and to resolve the mechanisms involved.

Biocrust $\delta^{13}C$ values were intermediate to C_3 and C_4 plant values but also varied considerably (>8.0‰) between sites and microsites. Higher isotopic discrimination (i.e., more negative $\delta^{13}C$ values in biocrust community) may occur in locations with higher rainfall (here, the La Puebla site), as has been observed in cyanolichens (Cuna et al. 2007) and in plants (Wang et al. 2016). Additionally, C_3 trees at the La Puebla site may contribute to root respiration and soil organic material, leading to more depleted $\delta^{13}C$ values in organic material and CO_2 in soils at this location. At a regional geographic scale, C_4 grasslands may retain a C_4 signal in the organic material and carbonates for long time periods (Breecker et al. 2009).

The washed samples of cyanobacteria and lichens had higher $\delta^{13}C$ values than samples of the biocrust consortium, and this trend could be attributed to the inclusion of soil organic material. Lignins from plant litter, for example, are relatively recalcitrant (Mun and Whitford 1998; although they are subject to photodegradation on the soil surface; Austin and Ballaré 2010), and lignins are depleted in ^{13}C compared to living plant tissue (Benner et al. 1987; Fernandez et al. 2003; Dumig et al. 2013). If there is high lignin content in the soil matrix surrounding biocrust organisms may thus explain the lower $\delta^{13}C$ values of community compared to washed cyanobacteria and lichens.

There was no evidence of any spatial patterning in the carbon signals of biocrusts adjacent to living or dead C_4 plants, and thus we have no evidence to support functional coupling in carbon cycling between biocrusts and the C_4 plant. The observed trends in biocrusts adjacent to C_3 plants can be explained by several alternative hypotheses which I did not attempt to resolve here, but pose possibilities for future research. Several

hypotheses assume no direct interaction with plants but simply differences in environmental conditions or biocrust community, while others assume that biocrusts benefit from growing near a living plant, suggesting that biocrust performance is linked to plant productivity.

Hypothesis 1: Microsite conditions differ between interspace and under C_3 plants

Plant cover may create different environmental conditions compared to unvegetated interspaces due to shading, nutrient accumulation (Schlesinger and Pilmanis 1998), or water dynamics (Berdugo et al. 2014), and these differences in abiotic conditions may lead to different fractionation during cyanobacterial/lichen photosynthesis in these microsites. For example, if soils remain moist longer under plant canopies, then biocrusts growing in microsites near plants may have depleted $\delta^{13}C$ values because of longer periods of favorable photosynthetic conditions. Cyanobacterial $\delta^{13}C$ values can vary plastically among seasons in lakes (Vuorio et al. 2006), with light conditions in pure culture (Wada et al. 2012), and with temperature in marine habitats (Hinga et al. 1994), while lichen $\delta^{13}C$ is affected by water availability (Cuna et al. 2007). The trend I observed is consistent with biocrusts photosynthesizing under more favorable conditions near *G. sarothrae*, However, the range in temperature, light, and moisture conditions of samples taken within ~25 cm of each other is likely small, and thus abiotic plasticity is unlikely to explain the magnitude of difference between biocrusts under *G. sarothrae* and interspaces.

To resolve this potential mechanism, researchers should collect microsite data on temperature, light, and soil moisture throughout the year, and should use pure cultures of dominant biocrust organisms grown under different environmental conditions to find the abiotic drivers of $\delta^{13}C$ in bulk tissues.

Hypothesis 2: Biocrust taxonomic composition differs by microsite

Communities of autotrophs may differ among plant and interspace microsites. Soil bacterial community composition can vary between interspaces and different dryland plant species (Kuske et al. 2002; Steven et al. 2014), and biocrust lichen and moss community composition can vary by micro-aspects (Bowker et al. 2006). If the composition of biocrusts differs between plant and interspace microsites *and* these different species or strains affect the fractionation of carbon during growth and maintenance, then the unique $\delta^{13}C$ values under plants versus in interspaces could simply reflect different biocrust community compositions. I did not identify each species or strain in each sample nor the proportion of biomass each contributed to the sample for $\delta^{13}C$, so I cannot determine if there were different communities present. However, at the Sevilleta site, ~60% of all cyanobacterial sequences in a composite sample from under *G. sarothrae* and composite sample from interspace microsites were the same species (*Microcoleus steenstrupii*; unpublished), suggesting that composition of biocrust samples for $\delta^{13}C$ analysis were similar in composition.

Even if compositions differ between microsite at the La Puebla site, if the fractionation of carbon during metabolism of different taxa is similar, then the composition would not explain differences in $\delta^{13}C$ by microsites. A previous study found that multiple genera of cyanobacteria grown in pure culture showed similar (<2‰) $\delta^{13}C$ values, while other species/strains differed by >>2‰ (Darby and Neher 2012). The high variability (range > 1‰) in isolate $\delta^{13}C$ within a given microsite could be due to differences in composition.

To resolve this potential mechanism, researchers should sequence each sample collected and create pure cultures that can be grown on carbon-free media to determine taxa-specific autotrophic fractionation.

Hypothesis 3: Biocrusts photosynthesize using CO_2 present in the soil matrix

Biocrusts are located at the intersection of the atmosphere and the soil and thus could access both atmospheric CO_2 and CO_2 rising to the surface from deeper in the soil. Respired CO_2 from roots is relatively depleted compared to the root tissue itself (by ~2‰ in C_3 plants and ~1‰ in C_4 plants; Werth and Kuzyakov 2010) and is much more negative than the atmospheric value of −8‰ (Keeling et al. 2010). I cannot resolve the source of CO_2 in my samples.

Within the soil, there are multiple pathways from plant photosynthate to CO_2. Roots respire CO_2 (Beck and Mayr 2012) and thus soil CO_2 may differ in isotopic concentration and/or composition between under plant vs. interspace microsites. Soil CO_2 concentration increases with vegetation cover (Amundson et al. 1989). For *G. sarothrae* and *B. gracilis,* most roots occur within 10 cm of the plant base (Milchunas et al. 1992);

therefore, root-respired CO_2 should have a higher concentration close to plants compared to interspaces. Isotopic composition varies by microsite also: $\delta^{13}C$ values for belowground respiration were close to C_3 leaf values during morning hours, and interspace values were less often depleted than those under plants (Bowling et al. 2011). Microbial respiration is another source of soil CO_2, and may also affect spatial pattern of concentration and isotopic composition of carbon. Microbial biomass is typically higher under plants than in interspaces (Kieft et al. 1998) and rhizosphere microbes can receive up to 30% of plant carbon, either direct transfers to organisms such as mycorrhizal fungi, or exudates and dead material (Brüggemann et al. 2011). Thus a large proportion of plant carbon is processed by microbes before being respired, and these processes occur close to the base of the plant. The net effect on isotopic fractionation can be altered by microbial species richness, with less discrimination against the heavy isotope in more complex, species rich communities (Yang et al. 2014), and thus the isotopic composition of soil CO_2 will vary with the composition of microbes in the rhizosphere vs. interspace microsite. Because the substrate material (root, litter, exudates) and microbial communities can differ with distance to the plant, I cannot resolve the effect of soil microbial respiratory CO_2 on biocrust carbon uptake. The trend I observed is consistent with biocrusts using isotopically depleted respired CO_2 for photosynthesis when growing close to the C_3 plant *G. sarothrae,* but I cannot resolve the pathway that generated the CO_2.

To resolve this potential mechanism, researchers could add labeled CO_2 gas either to the atmosphere above biocrusts or to the soil below biocrusts to discover the proportion of different carbon sources that the biocrusts use. To resolve sources within the soil, researchers could create mesocosms to compare root respiration under sterile conditions and root + microbe-respired CO_2 in inoculated mesocosms at different distances from the plant to determine concentration and $\delta^{13}C$ values of the gas.

Hypothesis 4: Biocrust cyanobacteria are mixotrophic

Biocrusts may use organic carbon sources from the soil which could differ among microsites in both their availability and their carbon signature. Aboveground litter (with $\delta^{13}C$ values similar to living material; Brüggemann et al. 2011) could fall onto the soil surface and degrade due to biotic and abiotic processes (e.g. photodegradation, Brandt et al. 2010), releasing carbon compounds which could be then taken up by biocrust organisms. The leaf litter of *G. sarothrae* decomposes more rapidly than litter of *B. gracilis* (Murphy et al. 1998), and *G. sarothrae* leaves had higher percentage carbon by weight than *B. gracilis* (supplementary material). Thus, carbon released from decomposing *G. sarothrae* might be taken up by biocrust organisms more readily than carbon from *B. gracilis*. Roots and root litter are relatively enriched in ^{13}C compared to photosynthetic tissue by ~1–2‰ in C_3 plants and that or less in C_4 plants (Hobbie and Werner 2004, Werth and Kuzyakov 2010, Brüggemann et al. 2011, supplementary material). Living roots also produce exudates (Jones et al. 2004) that vary in isotopic signature due to plant allocation and processing of those compounds (Brüggemann et al. 2011). However, root inputs may be relatively unimportant to the biocrusts under the *G. sarothrae* in this study because *G. sarothrae* has a low proportion of roots at shallow (0-10 cm) soil depths (Milchunas et al. 1992). Additionally, differential processing of soil organic carbon during microbial decomposition may result in plant-species and microbial community-specific changes in organic matter $\delta^{13}C$ (Wedin et al. 1995; Connin et al. 2001) which remains to be resolved.

To resolve this potential mechanism, researchers could label whole plants with enriched CO_2 and compare biocrust $\delta^{13}C$ values when grown with a living plant, aboveground litter, or belowground litter. Researchers could also look at compound-specific stable isotope values of plants and biocrust organisms to understand which compounds are derived from each autotroph.

Conclusion

Here, I report observational data that carbon use by biocrusts differs spatially among microsites. This new observation raises several testable, alternative hypotheses about the mechanisms that underlie spatial variability in the carbon isotopic signature of biocrust. The alternative hypotheses present non-exclusive mechanisms, and the ecological importance of any mechanism may be context-dependent, requiring further manipulative study. Additionally, further understanding of the impact on the performance of biocrusts is warranted.

Biocrusts are common across drylands globally; thus, understanding whether plant-derived carbon affects

biocrust functioning will help refine predictions on the roles of plant-microbe interactions in ecosystem services. In addition to primary production, biocrusts contribute to ecosystem structure and function by reducing soil erosion, fixing nitrogen, intercepting atmospheric deposition, and affecting infiltration of precipitation (Belnap et al. 2001; Belnap 2002; Elbert et al. 2012). Thus understanding the functional requirements in biocrusts will better enable maintenance of ecosystem services in drylands.

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