Figure 1: A) Lichen-dominated biocrust from San Clemente Islands (SCI). B) Dried soil bryophyte from SCI. C) Moss biocrust underneath grasses on SCI. D) Patchy distribution of a diversity of lichen biocrusts on SCI. E) Active cyanobacteria after a rainstorm in the Mojave Desert.

More Questions than Answers: *The Recovery of Biological Soil Crust Communities After Prescribed Burns*

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Background

Biological soil crusts (biocrusts) are complex communities of macroscopic and microscopic organisms found on every continent and in a variety of ecosystems, including grasslands. Generally, biocrusts can be categorized into three broad functional groups that are easily identifiable without a microscope: cyanobacteria, lichen, or bryophyte. Each of these functional groups provides different functions for the ecosystem (Belnap, Büdel, and Lange 2003). Cyanobacterial crusts are dominated by microbes such as Nostoc and Microcoleus (Büdel et al. 2016). These microbes form colonies on the soil surface that are visible with the naked eye, particularly when they are wet (Figure 1 E). They also excrete a sugar syrup, called exopolysaccharides, from their cells and the sugar binds soil particles together, forming a crust (Büdel et al. 2016). Lichen biocrusts are often more highly developed than cyanobacteria biocrusts and provide even more soil stability (Castillo-Monroy et al. 2015, Figure 1 A, D). The organisms in both cyanobacterial and lichen biocrusts fix atmospheric nitrogen and provide more available nutrients in the soil (Belnap 2002). Bryophyte biocrusts are composed of liverworts and mosses and are often difficult to see when they are dry (Figure 1 B, C). Bryophyte crusts hold more water than the other crust types and have been shown to increase the soil water content (Michel et al. 2013).

Previous studies have assessed how biocrusts respond to disturbance, although the responses vary depending on the biocrust composition and the severity of disturbance (Belnap and Eldridge 2001). For example, when biocrusts are trampled, they can take anywhere from one month to one century to recover to the pre-disturbance state (Zhao et al. 2016). Researchers have looked at biocrust disturbance by measuring the changes in biocrust cover, changes in the macroscopic composition, and changes in the microbial community. The primary disturbance of interest to us is fire. As fire is increasing in frequency and severity across the globe, it is important to think about how components of the ecosystem respond. Previous work in the Great Basin found some fire-resistant cyanobacteria in biocrusts (Bowker et al. 2004), although fire did reduce the overall diversity of biocrust lichens (Root et al. 2017). Compared to other ecological communities, the response of biocrusts has been understudied and primarily focused on deserts and cold shrublands with little emphasis on grasslands. In fact, biocrusts in grasslands have been largely overlooked.

Why does this matter?

Biocrusts provide a variety of different ecosystem functions. Previous work indicated that a decline in biocrust cover increased soil erosion and led to depleted nutrients in the soil (Belnap 2002; Eldridge and Leys 2003; Morillas and Gallardo 2015). Changes to or loss of biocrust cover can thus result in a loss of ecosystem function at larger scales (Barger et al. 2006; Chamizo et al. 2012). Therefore, it is critical that we not only understand how biocrusts respond to fire but also understand how to best protect them and the services they provide.

This has a variety of implications for grassland management. First, prescribed burns are often used in grasslands as a management practice. It is important to know how those prescribed burns are impacting biocrusts and weigh the potential loss of biocrust function with the potential benefits of the fire. Secondly, the presence of biocrusts after a fire may help with ecosystem recovery.

From 2018–2020, we attempted to understand how these complex biocrust communities respond to prescribed fire in a California coastal grassland.

Where did we do it?

The project took place on San Clemente Island (SCI), the southernmost island in the California Channel Islands. It is owned by the US Navy and home to a variety of ecological research projects through the United State Geological Survey (USGS) and the Soil and

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Ecology Restoration Group (SERG) at San Diego State University. The plateaus of the island are dominated by a coastal perennial grassland while the canyons and mesic areas are characterized as coastal sage scrub. The interspaces between the grasses are filled with a diverse cover of biocrusts including cyanobacteria, lichens, and bryophytes (Figure 2). We used two sites that were burned using prescribed fire in 2012 and 2017. The sites are named Perennial Grassland East (PGE) and Perennial Grassland West (PGW). The goal of the prescribed burns was to promote the growth of the native bunchgrass, *Stipa pulchra*. Both prescribed burns occurred in the same ten $10m^2$ plots at all three sites with adjacent unburned plots. This created an opportunity for us to measure the effects of fire on the biocrusts of the island.

What we did

In the springs of 2018, 2019, and 2020, we surveyed the percent cover of biocrusts within each burned plot using $1m^2$ Daubenmire frames. We repeated this measurement four times in each plot for a total of forty burn measurements and forty control measurements in each site. Unfortunately, we did not measure the percent cover of biocrusts before the fire, although the control plots are adjacent to the burned plots and may be similar to the pre-fire community. We characterized the functional group of the dominant biocrust cover as cyanobacteria-, lichen-, or bryophyte-dominated. We hypothesized that in the year after the fire, cyanobacteria-dominated biocrusts would be the most common and would gradually increase in lichen and bryophyte cover. We expected greater biocrust cover in the control plots and more highly developed biocrusts in the control plots.

To understand how the microbial community changed with fire, we used shotgun metagenomics (Quince et al. 2017). This technique allows the sequencing of all the DNA present in a small biocrust sample. DNA was extracted from biocrusts collected in 2018. We sequenced four samples from each site-treatment combination for a total of 16 metagenomes. More extensive DNA sequencing will occur in the future. The sequences were uploaded to the MG-RAST database where we were able to extract taxonomic and functional profiles for each sample.

What we found

Contrary to our hypothesis, there was greater biocrust cover in the burned plots compared to the control plots across all three sampling years and there were significant differences between treatment and site. In general, the eastern sites (PGE) had more biocrust cover in the burn and control compared to the western sites (PGW). As the time since the fire progressed, the total biocrust cover in the burned plots decreased. But in the three years since the fire, it has not declined to the same coverage as the control plots (Figure 3).

Although cyanobacteria dominated crusts occupied more space throughout the years, there was a difference in the cover of the different biocrust types as the ecosystem recovered from the fire. In 2018, one year after the fire, PGE and PGW both had significant

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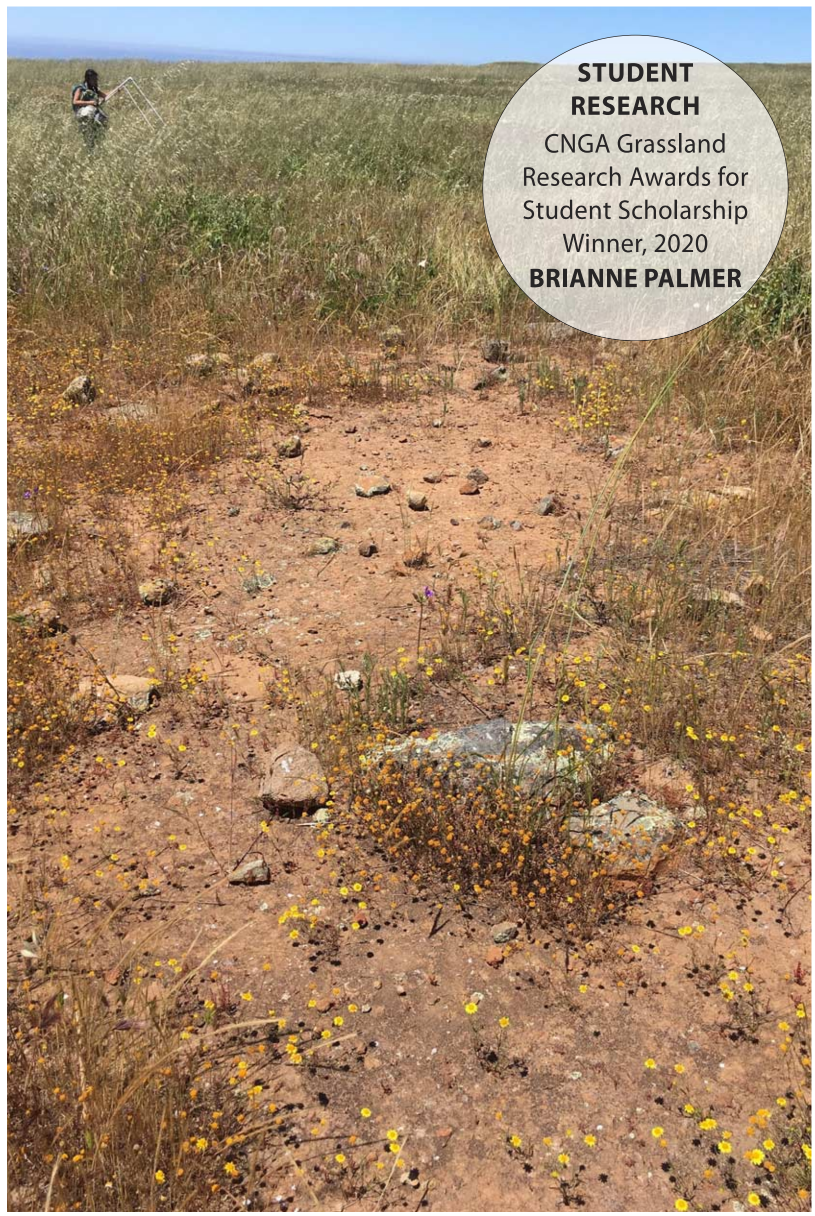

Figure 2: Typical plot on San Clemente Island. The vegetation is patchy with continuous swaths of grasses and forbs broken up by large patches of biocrust.

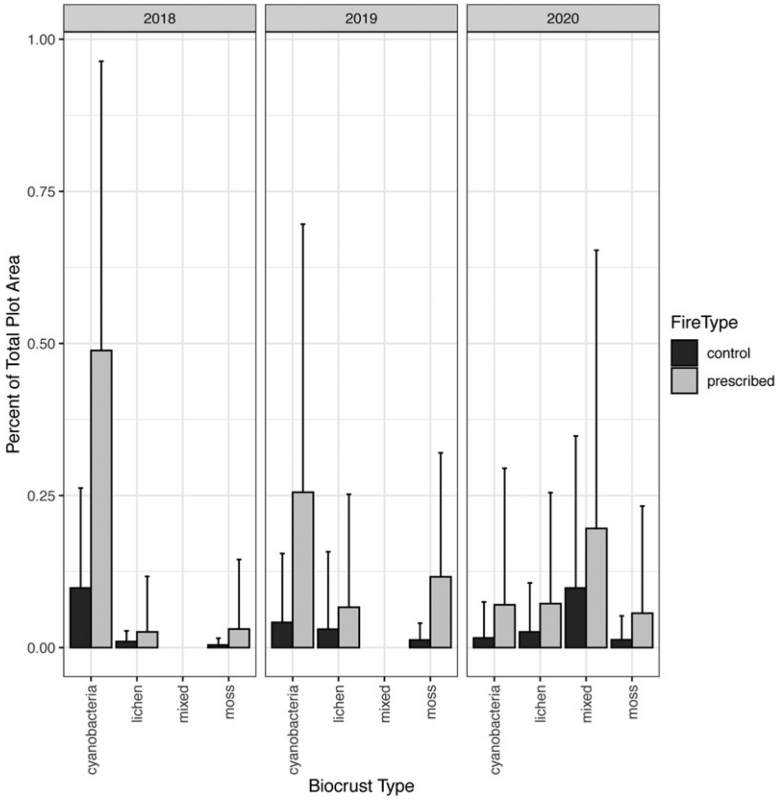

Figure 3: Proportion of the total plot area covered by each biocrust type for 2018, 2019, and 2020. Mixed biocrusts were recorded when the biocrust types were overlapping and we were unable to distinguish between the types.

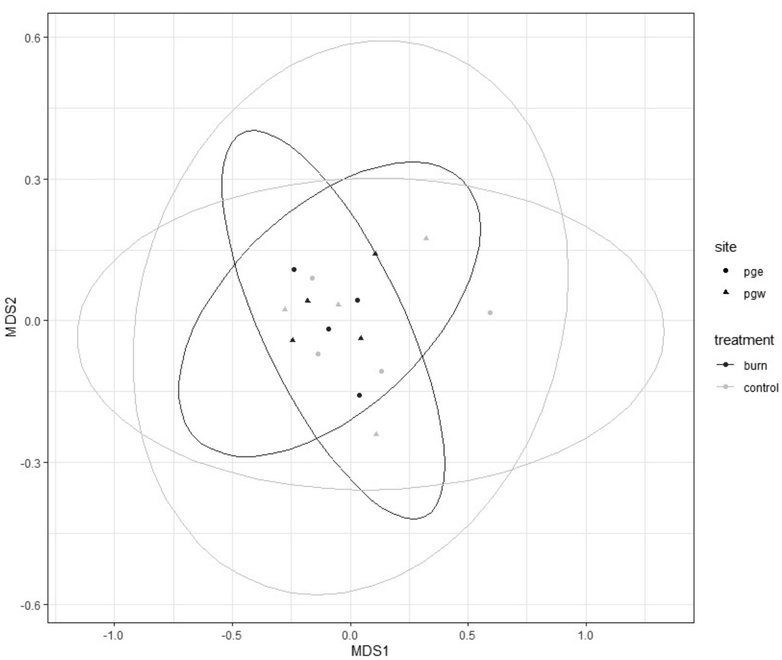

Figure 4: NMDS plot of the microbial community at each site (shapes) and treatment (colors). The closer the points are together and the more overlap there is between the ellipses, the more similar communities are to each other. There is no difference in community composition between treatment (P=0.4) or site (P=0.9).

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cyanobacterial cover in the burned treatments. Cyanobacteria were still the most dominant biocrust type in the control plots but had less overall cover. In 2019, cyanobacterial biocrusts were still common at both sites, although there was an overall decline in biocrust cover and an increase in the percentage of lichen and moss biocrusts in the burned plots. Then in 2020, biocrust cover continued to decline but again, the proportion of lichen and moss biocrusts increased (Figure 3).

Based on the stark differences between the biocrust cover between the burned and control plots, we expected to see these differences reflected in the microbial community. We used the sequences at the genus level to look for differences in richness and diversity and found no difference between treatment or site. Then using Bray-Curtis distances, we analyzed the communities for each site and treatment and again, found no difference in the community composition between treatment or site (Figure 4).

What could this mean?

Contrary to other studies, in this grassland, there was greater biocrust cover in the burned plots one to three years after a prescribed burn. There are a variety of explanations to describe this result. The first is the product of the experimental design. Each plot was only burned in the 10m^2 area leaving the grassland around the plot unburned. This undisturbed area may be an inoculum source of biocrust microbes that allowed for swift colonization of the plots immediately after the fire. It is unknown how biocrusts are colonized from adjacent areas, but it is a growing area of study. A possible hypothesis is that there are biocrust forming microbes, most likely cyanobacteria, that are aerosolized and blown into the burned area where they settle and can swiftly form a biocrust in the absence of competition from plants. Alternatively, there may be an inoculum source of biocrust forming microbes living deeper in the soil layers that can survive the fire. When conditions are right, these microbes may move to the surface and form the biocrust (Garcia-Pichel and Pringault 2001). The fires in grassland ecosystems are generally low severity and move quickly through the duff. San Clemente, in particular, has relatively sparse vegetation compared to other grasslands leading to less fuel and possibly cooler fires. This may be enough to allow the biocrust to remain intact and recover quickly after the fire.

There is likely greater biocrust in the burned plots because they colonized the soil surface before the vascular plants. Several

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cases document the prevalence of cyanobacterial crusts during secondary succession (Lan et al. 2014; Arróniz-Crespo et al. 2014; Pessi et al. 2019). In some cases, the biocrusts may provide a habitat that promotes plant growth and thus leads to a decline in the biocrust itself. Biocrusts can promote plant growth in a variety of ways including increasing the amount of available nitrogen in the soil, increasing water infiltration, and reducing erosion— all ecosystem functions that are useful after a fire (Eldridge, Zaady, and Shachak 2000; Belnap 2002; Breen and Lévesque 2006; Bowker et al. 2008; Godínez-Alvarez, Morín, and Rivera-Aguilar 2012). This may also explain the gradual decline in biocrust cover over time. As more plants establish, they outcompete the biocrust for space and light. This may be particularly true in the control plots where we see the little biocrust cover. These plots are dominated by exotic annual grasses such as *Avena barbata* and *Bromus madritensis* which create dense monocultures and thick thatch layers that may either inhibit biocrust growth or add an additional barrier for researchers surveying the percent cover of the biocrusts (Figure 5).

However, this study is not without its flaws. The microbial analysis was highly selective towards prokaryotic organisms and may have missed much of the eukaryotic diversity, particularly mosses and the fungi associated with lichens. Additionally, in the field, on dry days it was difficult to distinguish the different types of biocrust types. Therefore, the total biocrust cover is a better variable than the proportion of lichen, moss, and cyanobacteria. Most likely, most of the biocrust cover should have been characterized as mixed.

What are the implications for management?

There are a few takeaways from this experiment. The first is that there is still an incomplete understanding of how grassland biocrusts recover after a prescribed fire. There is a need to more fully understand the inoculum sources and how biocrusts are colonized. However, the swift recovery of biocrusts after fire would be beneficial for fire management, particularly due to their ability to reduce erosion, modulate water content, and influence nutrient regimes. Based on this research, prescribed fire in a coastal grassland should not reduce the cover of biocrust nor change the microbial community and may therefore leave some of the biocrust functions intact. Managers can take advantage of these biocrust functions when restoring a post-fire landscape. Either by utilizing the biocrust present or by transplanting other biocrust inoculum, biocrusts will readily colonize a landscape and provide more nutrients and soil stability. Rather than managing fire to promote and conserve biocrusts, biocrusts themselves may be used to passively improve the post-fire landscape overall.

California grasslands generally have high plant cover and low plant stature which presents a unique challenge for biocrusts. Without space to grow, the biocrust forming cyanobacteria may lurk in the soil, awaiting a patch of light to begin photosynthesizing and form a crust. When restoring grasslands, land managers should evaluate the type of grassland structure they are aiming for. Do they want endless fields of continuous plants or the patchy distribution of biocrusts? The decision will probably be based on evaluating the trade-offs of each community, restoration goals, and the disturbance history of the site. A continuous grassland may provide more forage and more carbon storage. Perhaps, a continuous grassland matches the reference community used by restorationists and provides ecosystem functions. But consider what this grassland may have looked like in an early successional state. As we learned from this study, there was greater cover of early successional biocrusts in plots that were burned. Perhaps restoring a grassland to a state where biocrusts can do what biocrusts do would be beneficial for the ecosystem and require less hands-on management. These are simply speculations and they require research. However, we urge land managers to consider, even briefly, the function of biocrust on their land and the potential benefits of a healthy biocrust community after a disturbance.

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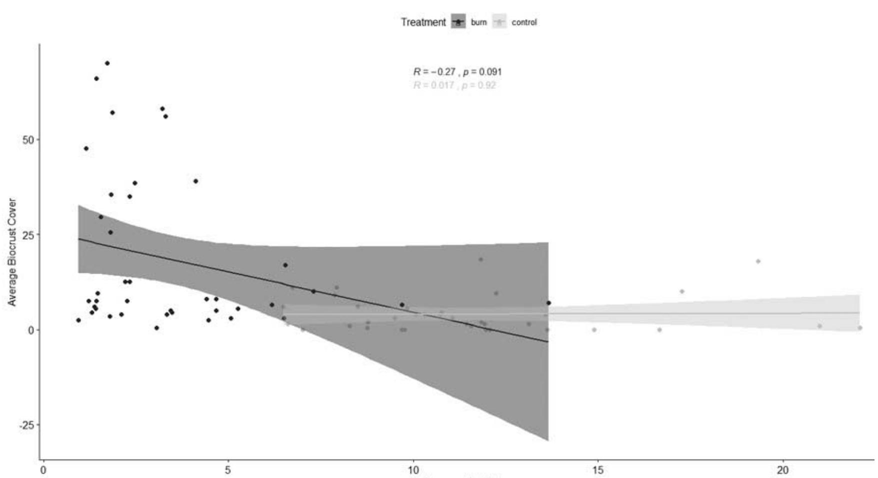

Figure 5: There is a significant negative correlation (Spearman's correlation coefficient) between biocrust cover and plant cover in the burned plots. There is no correlation between the cover of these two communities in the control plots.