Effects of Non-Native *Eucalyptus globulus* on Biological Soil Crusts in Berkeley's Tilden Park

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ABSTRACT

Biological soil crusts are a community of species which are vital to arid and semi-arid ecosystems because they regulate water and nutrient cycling and maintain soil cohesion. Despite this, biocrusts are often understudied with little to no research being done on the impacts of soil acidification on their well-being. *Eucalyptus globulus* is one plant with allelochemicals that causes soil acidification and harms biocrusts. The invasive impacts of eucalyptus can be seen in the mixed forests of the San Francisco Bay Area. To obtain a better understanding of how these volatile chemicals impact biocrust growth and diversity, I analyzed biocrust samples from Tilden Park in Berkeley, California along a eucalyptus exposure gradient. I then exposed these samples to varying concentrations of allelochemical "teas" made by straining *E. globulus* leaves and pouring this liquid onto the samples to simulate leaching from rainfall. The tea concentrations spanned a pH range of approximately 7 to 1. The effect on cyanobacteria was not able to be measured. After exposure to tea treatments, overall surface biocrust biodiversity dropped from an average of 0.024 genera per individual to a mean of 0.014. Plant and fungal growth also decreased. Average moss length dropped by 30% while average lichen height dropped by 50%. Soil cohesion dropped by an average of 16%. Overall, lichen and fungi were the most impacted while mosses were impacted to a lesser degree which is to be expected given that mosses tend to be more resilient in acidic soils than other plants.

KEYWORDS

soil acidification, invasive species, soil leaching, soil litter, allelochemicals

INTRODUCTION

Biological soil crusts, more commonly referred to as biocrusts, are cryptobiotic communities composed primarily of cyanobacteria, fungi, lichen, and mosses. The term cryptobiotic refers to early evolutionary and successional stage species including but not limited to mosses and lichens. Biocrusts' cover spans around 12% of the world's terrestrial ecosystems, and they are particularly important in arid and semi-arid systems (Belnap et al. 2001, Maier 2018, Rodriguez-Caballero et al. 2018). Biocrusts act as keystone communities; they are versatile and play key roles needed to maintain ecosystems. Biocrusts are responsible for maintaining and carrying out multiple processes within arid ecosystems. Primarily, they are responsible for nutrient and water cycling and maintaining soil cohesion (Belnap 2001, Belnap et al. 2016). Their versatility is due to the different components found within these communities. For example, water cycling is partially due to the morphology of mosses (Belnap et al. 2001). Mosses have hand-like papillae which help "grab" and spread water across their surface and to nearby surfaces (Belnap et al. 2001). Additionally, increased soil cohesion can be attributed to the mycorrhizal components of the fungi found due to their root-like filaments which tightly pack soil (Belnap et al. 2001). Beyond ecosystem services, biocrusts also serve as biological indicators of overall ecosystem health. For example, mosses in these communities can act as an indicator for water availability due to their natural desiccation resistance; low water availability leads to an easy to identify "dormant" state within mosses serving as a visual bioindicator (Belnap et al. 2001). Despite their importance, biocrusts are threatened by natural sources of soil acidification.

A source of soil acidification is exposure to allelochemicals. Allelochemicals are volatile, biological chemicals known to produce harm to other species within their ecosystem. This harm comes in the form of understory suppression of plant growth via soil acidification. This suppression is an evolutionary trait adopted by plants to reduce competition for nutrients and is a common management concern of invasive species (Hiero and Callaway 2003). Allelochemicals are spread by leaching from plant matter into soil and directly onto neighboring plants (Molina et al. 1991). This process is facilitated via rainfall which directly washes these chemicals onto varied substrates (Molina et al. 1991). Other allelochemical spreading mechanisms also exist including plant matter decay (Molina et al. 1991). The effects of allelopathic flora on non-cryptobiotic flora

is well studied and understood (Hiero and Callaway 2003, Jose and Gillespie 1998, May and Ash 1990 Molina et al. 1991). However, this is not the case for biological soil crusts.

Studies focused on anthropogenic damage to biocrusts are limited in scope despite the importance of biocrusts to arid and semi-arid ecosystems. While some studies looking at the effects of anthropogenic damage exist (Ferrenberg et al. 2015, Reed et al. 2016, Rodriguez-Caballero et al. 2018), they are limited to trampling, climate change, and water availability. However, the effects of natural soil acidification on these cryptobiotic communities are not well understood. To obtain a better understanding, we can look at the impacts of the invasive *Eucalyptus globulus* found in Northern California. Studies have been conducted on this tree's effects on other flora with research concluding that *E. globulus* is able to suppress non-cryptobiotic flora found below these trees (May and Ash 1990, Molina et al. 1991), but research on the effects on biocrusts is limited.

Considering the gaps in studies regarding biocrusts, my study sought to determine how eucalyptus allelochemicals impact the diversity and growth of biocrusts. Specifically, I asked i) Does biocrust overall diversity change along gradients of eucalyptus tree density? ii) Does the species community composition of biocrusts change along gradients of allelochemical exposure and iii) Does laboratory exposure to prepared allelochemical teas impact biocrust growth? Answering these subquestions, informs our understanding of the effects of allelochemical exposure on biocrust biodiversity, individual species lengths, soil cohesion, and successional stages. I predicted that the introduction of allelochemicals would acidify soils leading to a decrease in diversity, a decrease in individual lengths, a loss of soil cohesion, and a reverse successional shift (May and Ash 1990).

METHODS

Study site

To determine how allelochemical exposure impacts biocrusts, I sampled biocrusts along a Eucalyptus gradient within Tilden Park. These gradients were found at the following coordinates: N 37°54'43.272" W 122°16'1.164", N 37°54'45.7812" W 122°15'51.7932", N 37°54'45.7812" W 122°15'51.7932", N 37°54'42.588" W 122°15'54.774", and N 37°54'44.372 W 122°16'1.280. Tilden Park is located between the Berkeley Hills and San Pablo Ridge in the San Francisco Bay

Area. Tilden Park is composed of mixed habitat types with varying concentrations of invasive eucalyptus trees. The mixed forest habitats allowed me to identify line tracts with a wide range of allelochemical exposures. *Eucalyptus globulus* was first introduced into California in the 1850s and expanded its range. The invasive species is known to leach its chemicals from its bark and leaves into the soil facilitated by rainfall. The volatile chemicals leached from plant matter are known to suppress understory plant growth.

Field allelochemical exposure gradients

To quantify allelochemical exposure, I identified line transects throughout Tilden Park. I defined my line tracts as allelochemical exposure gradients ranging from areas that have no exposure (areas surrounding native species) to areas with direct exposure (areas directly under *Eucalyptus globulus*) with areas of medium exposure (defined as areas between the direct and no exposure gradient points) in between. I selected these line tracts from an unmarked path near Wildcat Peak Trail (37.914720, -122.264783) (Figure 1). I chose this location because the area has little human activity to ensure biocrust communities would not be trampled. I established line transects at five sites and randomly sampled from three sites and measured the pH at each of them using a soil probe. I only used three randomly selected sites out of the five sites to avoid any bias; using random selection allowed me to not look for specific sites that may be more visually diverse and thus skew data away from what happens in nature.

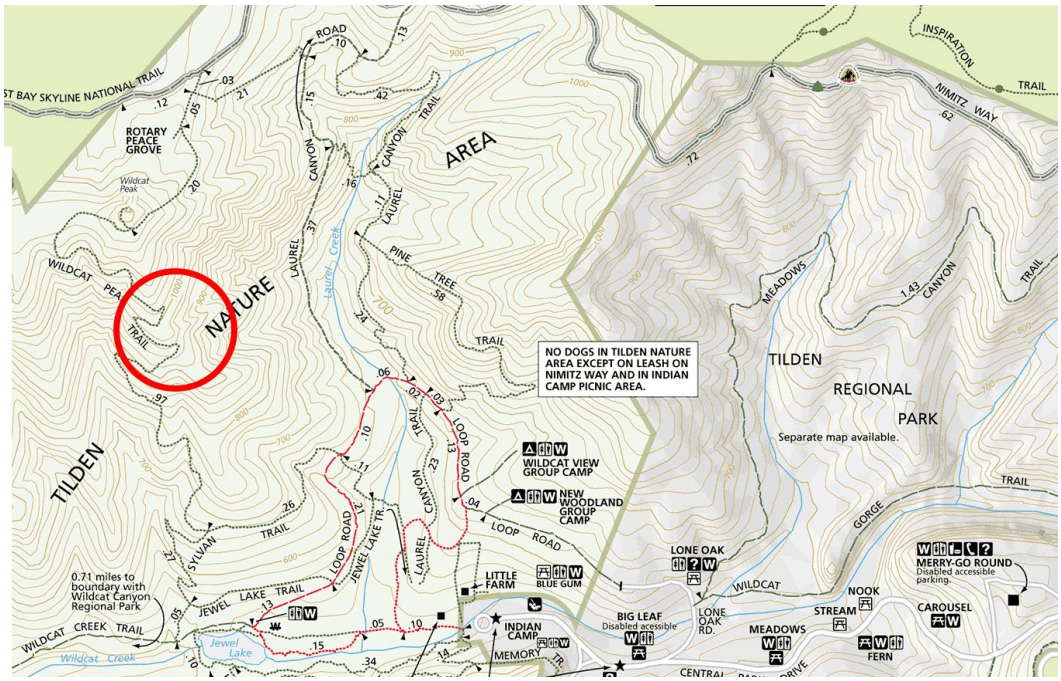


Figure 1. A Map of Tilden Park's Nature Area. I obtained this map from the East Bay Regional Park District website and cropped to only include the most relevant information. The area circled is an approximation of the study site.

Biocrust field sampling

To determine the biocrust diversity at each transect, I collected 95-square centimeter samples of biocrusts from the "no exposure" sites. I measured diversity at these sites because they had an average pH of 7. I did not select samples from other sites because the direct exposure regions and mid-exposure regions had more acidic pH readings due to their exposure to allelochemicals. I collected eight samples total each with a surface area of approximately 95-square centimeters while attempting to maintain as much mycorrhizal mass intact. To collect the biocrusts, I carved samples out of the ground after outlining them with 95-square centimeter petri dishes to get the correct sizes. In these samples, I counted the individual mosses and lichen which I later used to calculate biodiversity.

Biocrust growth assay

To develop an understanding of how allelochemical exposure and soil acidification affects biological soil crust overall community health, I exposed these samples to varying concentrations of allelochemicals from *E. globulus* to obtain a pH range of approximately 7 to 1. To do so, I extracted allelochemicals by first collecting leaves and bark samples from *E. globulus* which I then turned into a tea. I then poured varying concentrations of this solution into the samples to obtain the approximate pH range stated above. I measured pH using a standard garden soil pH probe. I also measured any changes in growth by measuring the heights of the biocrusts' mosses and lichens at the beginning and the end of the two-week study period.

Data analysis

To assess shifts in successional stages, I measured biodiversity across all samples. I measured biodiversity both at the beginning and at the end of the study period. To calculate biodiversity, I divided the total number of genera by the total number of individuals. I then divided these values by the surface area to obtain the approximate biodiversity per square centimeter for each sample. I also calculated biodiversity using R version 3.6.0 to avoid any potential mistakes (R Core Team, 2019). After this, I did a regression analysis measuring biodiversity against pH. To assess effects on growth, I calculated regression between changes in sample length against pH. I had measured height at the beginning and end of the study period, and I calculated the difference in height after the study period. To assess impacts on mycorrhizal fungi, I analyzed soil cohesion as a proxy source of data. Soil cohesion is a unitless measurement which follows a scale of 0 through 5 with 0 indicating a loose, sandy consistency and 5 indicating tightly packed soil. I also categorized each sample's successional stage on a scale of 1 through 4. Stage 1 indicated a cyanobacteria dominant stage; stage 2 indicated a mycorrhizal dominant stage; stage 3 indicated a moss dominant stage; stage 4 indicated a lichen dominant stage. I used .5 values (i.e., 1.5, 2.5, 3.5) to indicate that a sample seemed to be transitioning from one successional stage to the next. I labeled samples at the beginning and end of the study period.

RESULTS

Allelochemical exposure gradients and sampling

The areas with no exposure had an average pH of 6.8 (Figure 2). Areas with limited exposure had an average pH of 5.8 (Figure 2). The areas with direct exposure had an average pH of 4.3 (Figure 2).

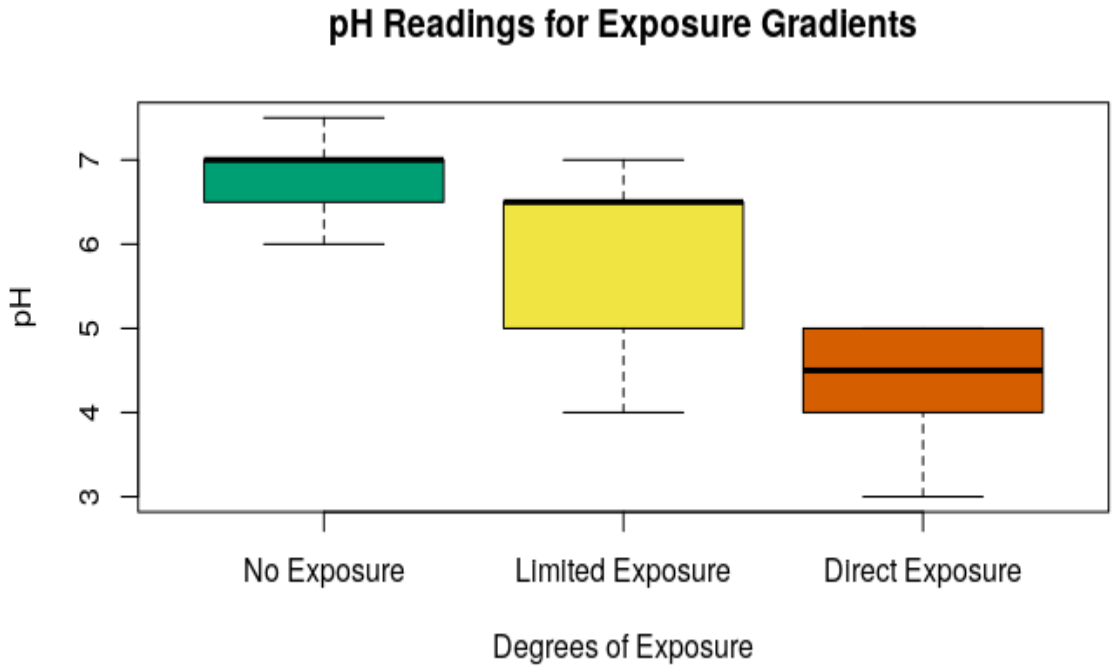


Figure 2. Box Plots Detailing pH Readings for Each Exposure Gradient Point. These box plots were created using R 3.6.0.

Data analysis

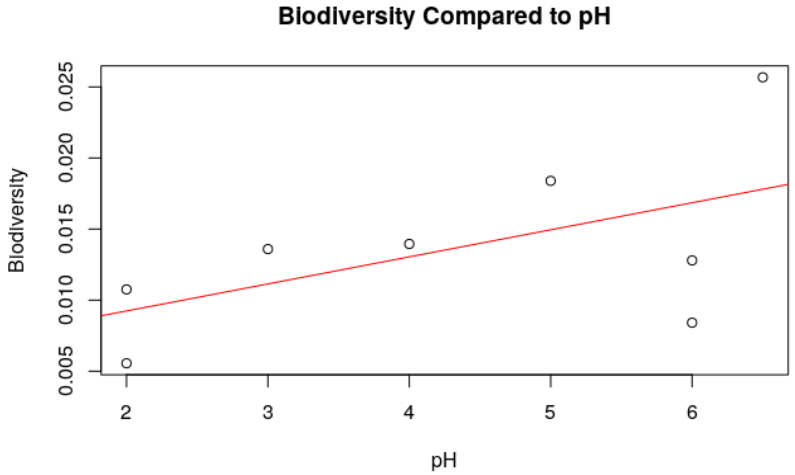
Biodiversity

In the samples I collected, I identified moss and lichen growing on the surface with mycorrhizal fungal mass and cyanobacteria growing alongside both. After exposing soil crusts to allelochemicals to produce an approximate pH range of 7 to 1, I found a decrease in biodiversity as soil pH levels moved away from 7. I calculated biodiversity by dividing the number of genera

by the number of individuals. Overall biodiversity decreased by 0.19% as soil pH decreased by a unit (Figure 3). A unit decrease in pH among soil samples decreased the moss surface coverage by 0.87% with no plateau noted at any specific pH reading (Figure 3). Similarly, a unit decrease in pH decreased the lichen surface coverage by 4.42% with no plateau noted (Figure 3). Biodiversity was highest at more neutral pH levels with soils samples with a pH level of 7 having the most diversity within the sample throughout the experimental period.

Table 1. Summary of surface biodiversity before and after allelochemical exposure.

Sample	Surface Biodiversity	Surface Biodiversity Per Square Centimeter	pH	Biodiversity After Exposure	Biodiversity Per Square Centimeter After Exposure
1	0.027027	0.000406	6.5	0.025676	0.000386
2	0.009346	0.000109	6	0.008411	9.83E-05
3	0.014706	0.000172	6	0.012794	0.00015
4	0.022989	0.000302	5	0.018391	0.000242
5	0.023256	0.000367	4	0.013953	0.00022
6	0.019417	0.000204	3	0.013592	0.000143
7	0.011111	0.000146	2	0.005556	7.31E-05
8	0.021505	0.000251	2	0.010753	0.000126



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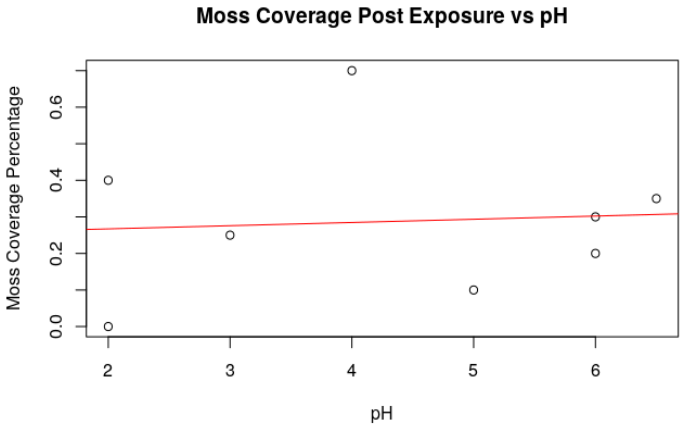

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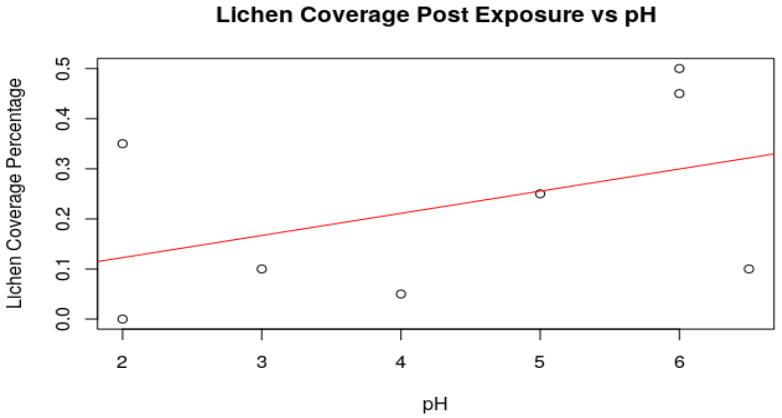

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Figure 3. Trends of Shifts in Biodiversity and Surface Coverage in response to pH shifts.

Table 2 lists the specific numbers for each genus on the surface and their percent coverage per 95-square centimeters of soil. I recorded coverage before and after exposure to *E. globulus'* allelochemicals as seen below.

Table 2. Moss and Lichen Superficial Coverage Before and After Allelochemical Exposure.

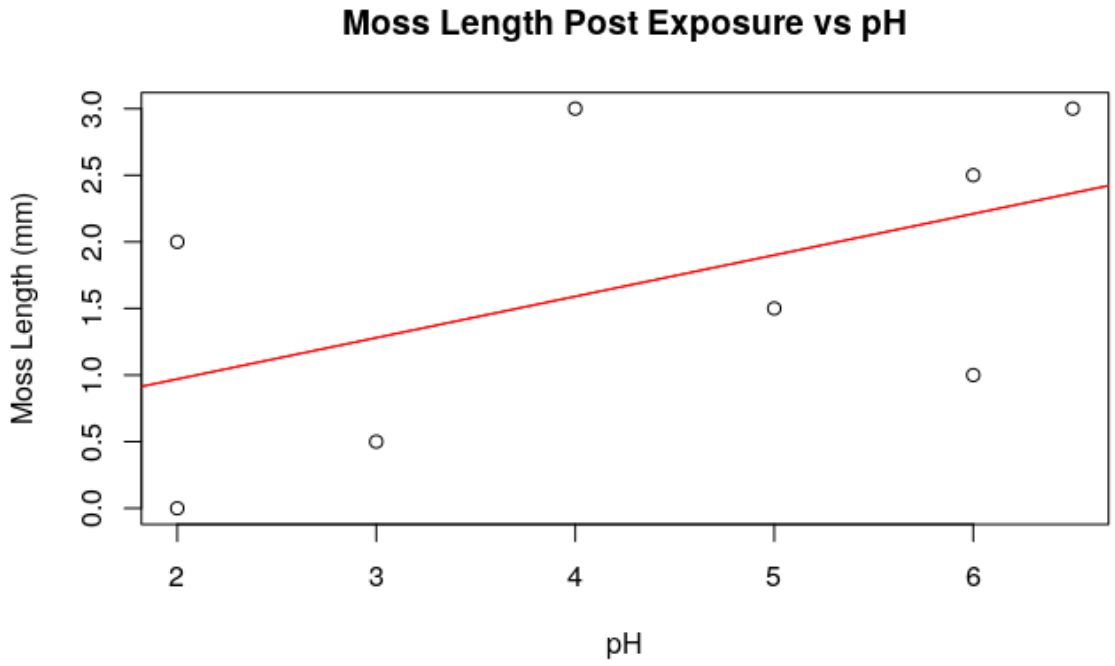
Sample	Moss Coverage	Lichen Coverage	pH	Post Moss Coverage	Post Lichen Coverage
1	0.35	0.1	6.5	0.35	0.1
2	0.3	0.6	6	0.3	0.5
3	0.2	0.45	6	0.2	0.45
4	0.15	0.4	5	0.1	0.25
5	0.85	0.1	4	0.7	0.05
6	0.4	0.2	3	0.25	0.1
7	0.1	0.6	2	0	0.35
8	0.75	0.05	2	0.4	0

Sample lengths

I noted a decrease in height for species within the samples which had a pH other than 7. I calculated a 31% decrease in moss length with each unit decrease in pH (Figure 4). Lichen had a less drastic decrease in height; I calculated a 13% decrease in lichen length with a unit decrease in pH (Figure 4).

Table 3. Moss and Lichen Length in Millimeters (mm) Before and After Allelochemical Exposure.

Sample	Moss Height	Lichen Height	pH	Post-Exposure Moss Height	Post-Exposure Lichen Height
1	3	0.5	6.5	3	0.5
2	2.5	1	6	2.5	1
3	2	1	6	1	1
4	2	0.5	5	1.5	0.25
5	5	1.5	4	3	1
6	2.5	1	3	0.5	0.5
7	1	1.5	2	0	0.25
8	2.5	0.5	2	2	0



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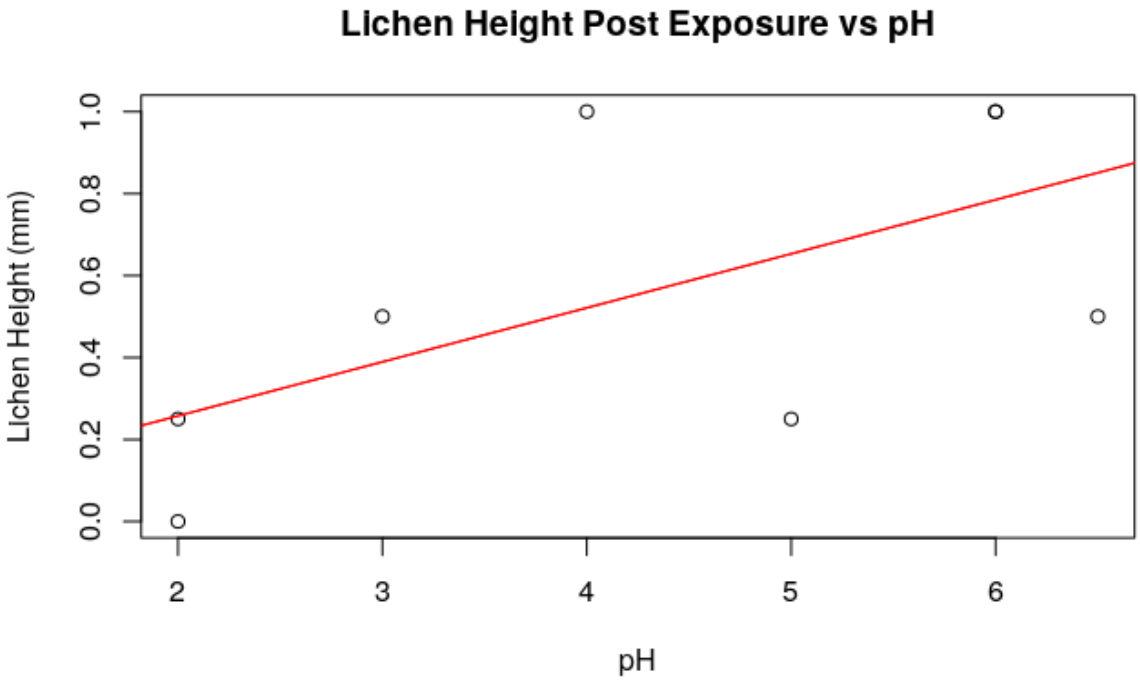

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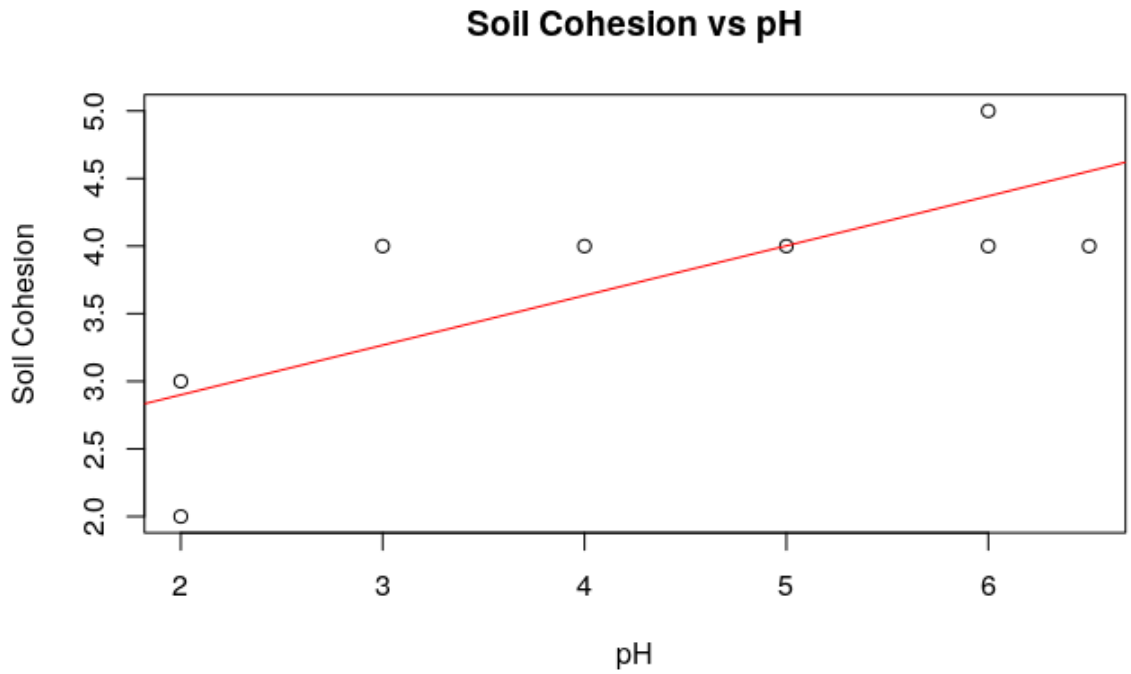
Figure 4. Trends of Shifts in Moss and Lichen Heights in response to pH shifts.

Soil cohesion and successional stages

I noted an overall decrease in soil cohesion and a negative shift in successional stages as pH approached 1. I calculated a 36% drop in soil cohesion with a one unit drop in pH, and I also calculated a 3% shift backwards in successional stage as pH shifted closer to a value of 1 (Figure 5).

Table 4. Soil Cohesion and Sample Successional Stage Before and After Allelochemical Exposure.

Sample	Cohesion	Successional Stage	pH	Cohesion Post-Exposure	Successional Stage Post-Exposure
1	4	3.5	6.5	4	3.5
2	5	4	6	5	3
3	4	4	6	4	3.5
4	5	3.5	5	4	3.5
5	5	3.5	4	4	3
6	4	3	3	4	2.5
7	5	4	2	2	4
8	4.5	3	2	3	3



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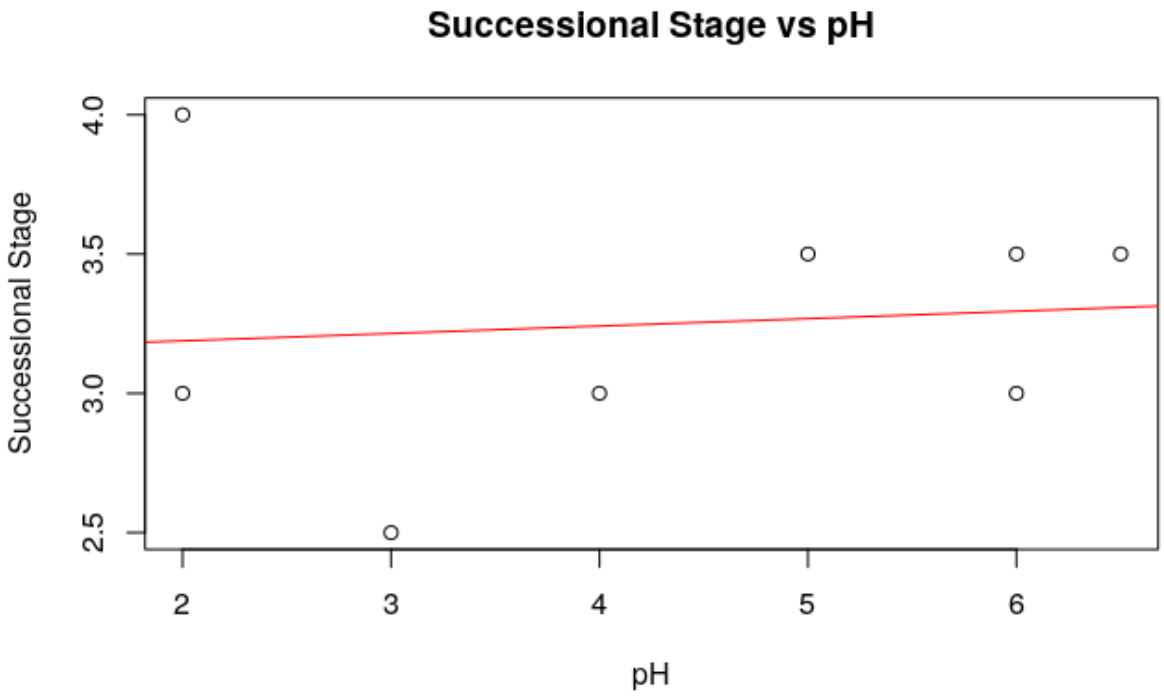

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Figure 5. Trends of Shifts in Soil Cohesion and Succession Stages in response to pH shifts.

DISCUSSION

My study provided evidence showing that *Eucalyptus globulus* did suppress biocrust diversity and growth. Eucalyptus allelochemicals decreased overall biodiversity and individual species coverage. Exposure to allelochemicals also reduced biocrust height as well as soil cohesion. Most samples also experienced a shift backwards in successional stage with only two exceptions. Moss was the least impacted species, but that is not to say that there were no significant impacts on this genus. Rather, the results indicate a natural tolerance found within the genus.

Biodiversity and community composition

I found that *Eucalyptus globulus* did have an acidifying effect on soil which negatively impacted the biodiversity and growth of biocrusts. I noticed an overall decrease in biodiversity as the concentration of allelochemicals increased. Moss was impacted less than lichen and mycorrhizal fungi. This aligns with my hypothesis that moss would be the most prevalent genus as pH decreased (Gauthier et al. 2018, May and Ash 1990). Similarly, I noticed a decrease in overall biomass length with moss being the most resistant to changes in exposure. This once again falls in line with my hypothesis that moss would be the most resilient as concentrations increased.

By applying various amounts of allelochemicals to different biocrust samples, I noted a decrease in biodiversity. Moss had the highest prevalence after the trial period which aligned with the pre-established notion that *E. globulus* will suppress all species that do not have a high range of tolerance (Gauthier et al. 2018). My findings should be used to further other shifts in soil pH levels to obtain a broader understanding of the impact of substrate on cryptobiotic diversity and overall ecosystem health especially in arid ecosystems.

Biocrust growth

I noted decrease in overall height as allelochemical exposure increased. Moss had the lowest mean percent change in height which aligns with my original predictions (Gauthier et al. 2018). While the regression slope for moss was larger than for lichen, this did not necessarily conflict with my original hypothesis given that lichen does not tend to have much vertical growth and thus cannot lose as much biomass height.

Soil cohesion and successional stages

I used soil cohesion as a proxy measure for mycorrhizal fungi. Thus, decreases in soil cohesion indicated a decrease in fungal mass within the samples. This was expected but not to the degree seen in the study. Lichen and mycorrhizal fungi share similar pH ranges which they can tolerate, so shifts in their prevalence should have been similar. This drop may indicate that using soil cohesion as a proxy measure for fungal mass is not effective and should be further tested.

Successional stages also decreased with increased exposure. However, two outliers in my data bring into question the use of successional stages when defining biocrusts. Samples 7 and 8 presented no shifts in succession despite their extreme exposures to allelochemicals. However, the impacts of soil acidification could easily be seen when looking at sample 7 and 8's soil cohesion which fell more in line with the treatments provided. This may be evidence against the use of successional stages alone for defining biocrusts, for this method disregards the shifts seen within sample 7 and 8. As a result, I propose the potential inclusion of "pseudo-stages" to define biocrust samples that look like they are at a particular successional stage but are not truly at said stage.

Limitations and future studies

Future studies in the field should look at how these communities react to natural sources of alkalization – that is, if any of these species were to react to alkalization at all. Future studies may benefit from exploring how soil chemistry (i.e., nutrient availability and chemical composition) could impact pH readings. I did not explore this factor since I isolated samples and would thus have fewer chemical fluctuations occurring as nutrients are cycled throughout the ecosystem and as soil chemistry potentially changes. Future studies should include field ecosystem dynamics to include interaction between flora and fauna and how these influence the results seen above. Overall, while my study had its limitations, my research can provide the opportunity for future studies in the field.

Broader impacts

As mentioned before, biocrusts are often overlooked due to them being perceived as "primitive" in an evolutionary sense despite their importance to many arid ecosystems. If we are to protect and conserve the ecosystems in which these cryptobiotic communities are found, it is imperative that we reduce the damage produced by humans. While climate change and water availability are issues being addressed worldwide, it is also important to aim to remove these allelopathic trees harming biocrusts and by extension the communities in which they are found. I hope my study can provide a source of data to further expand the repertoire of information currently being used to lobby for the removal of *E. globulus* within California and other areas where it may exist in an invasive manner. In doing so, not only can we protect these ecosystems, but we can also avoid the threat of landslides and dust storms which often occur due to a lack of proper soil cohesion. Through this, we can protect the intrinsic value of arid ecosystems as well as potentially reduce the chance of physical harm that may come to humans due to heavy wind erosion.

ACKNOWLEDGEMENTS

Brent Mishler and Javier Jauregui were excellent mentors throughout this entire process. Javier provided valuable insight and lit the fire which drove my passion for this thesis, and Brent alongside the rest of the Bryolab provided the additional guidance and knowledge needed to make this paper what it is today. Thank you to Patina Mendez and Leslie McGinnis for the insightful and deep feedback which helped improve the overall quality of the paper and for also providing important resources both for academic support as well as personal improvement. Thank you to Sunny Elliot and the rest of the Crusty Bugs workgroup for their insightful peer reviews as well as for simply listening to my biocrust spiel the hundreds of times I rehearsed it. I extend my gratitude as well to my mother, Horfelinda Martinez, my brother, Jose Gonzalez, and my partner, Salma Hernandez, for providing the support both in person as well as over the phone necessary to keep me going as well as the space to actively speak about this thesis in a less formal and more passion-driven sense. Finally, thank you to my therapists throughout the last three semesters that helped me keep it together and finish this thesis.

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