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Herbivore Influence on Post-Fire California Sage Scrub Plant and Soil Microbial Assemblages

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Abstract: California sage scrub (CSS) is an endangered, shrub-dominated, southern California ecosystem type threatened by increasing fire frequencies and conversion to non-native grasslands. As CSS harbors a rich endemic flora and fauna and is critical for maintaining key ecosystem services; significant attention has been focused on understanding how many key factors (N-deposition, drought, and competition with non-natives) influence post-fire CSS recovery. Unfortunately, few studies have examined the role of herbivores. For five years, we investigated the influence of herbivores on post-fire CSS succession by annually surveying plant assemblages in control (herbivores allowed access) and experimental (herbivore excluded) plots at the Bernard Field Station following a fire in May 2017. We found that herbivores reduce the cover of both invasive grasses and native shrubs, and negatively impact *Artemisia californica* survivorship. Consequently, post-fire plant restoration efforts should not discount the effects of herbivores. While native forb cover was elevated in plots that permitted herbivores, plots near the fire edge experienced increased herbivory pressure and lower cover of most plants. Despite modifications in plant assemblages, we did not observe differences in soil microbe abundances, fungi assemblage structure, or nutrient concentrations. We suggest that restoration practitioners consider caging 'shrub regeneration' areas to facilitate post-fire CSS recovery.

Keywords: *Bromus* grass; carbon; fungi; microbe; nitrogen; plant succession; restoration

1. Introduction

Fire is an increasingly common disturbance in southern California [1]. Unfortunately, frequent fires, in combination with drought and high nitrogen (N) deposition rates, facilitate invasion of non-native annuals and reductions in native plant abundance and diversity [2–4]. Because invasive annuals are easy to ignite and are often the first species to become established following a fire, ignition probabilities and fire frequencies often increase following a fire, creating invasive plant-fire cycles [5,6]. As most fires in southern California are caused by human ignition, invasive plant-fire cycles are particularly common near areas with high human population densities [7,8]. Invasive plant-fire cycles often result in type-conversion of native habitats to areas dominated by non-native grasses and forbs [3,9]. However, if native shrubs are able to recover before non-native species become abundant, shrub cover can reduce non-native annual abundances [10–13]. Consequently, the first few years following a fire disturbance are critical in the post-fire establishment of southern California native plant communities [14,15].

Native to low elevation areas of southern California, the California sage scrub (hereafter CSS) ecosystem, dominated by drought-deciduous shrubs, is one of the most threatened ecosystem types in North America [16,17]. Estimates suggest that less than 10% of the original CSS distribution remains, and that approximately 50% of CSS lost in natural areas (e.g., areas not converted because of agricultural expansion and urbanization) since

1930 was primarily driven by fire disturbances and resulting type-conversion to systems dominated by non-native annuals [3]. Part of the California Floristic Province, a world biodiversity hotspot, the rich and endemic CSS flora and fauna make it an ecosystem of special conservation importance, and its preservation is critical to the persistence of CSS-dependent species [18–23]. In addition, protecting CSS is vital to preserving important ecosystem services. For example, type conversion of CSS to either non-native grass or non-native forb dominated systems impacts soil microbial abundances and assemblage structure, which in turn can influence C and N storage and soil respiration rates [24–26]. Changes in soil microbial assemblages can also differentially influence plant germination and growth in the CSS ecosystem, which can alter the plant assemblages supported in these areas and complicate CSS restoration efforts [27–29]. Therefore, understanding factors that hinder or facilitate CSS successional trajectories following a fire disturbance are critically important.

While numerous studies have explored key factors that influence CSS recovery following a fire [2,15,30–34], few have explored how herbivores influence succession (see research of the effects of herbivores in chaparral [35–37]). The limited attention to the effects of herbivores following fire may have resulted from early work in CSS, conducted primary in the cool-moist spring and early summer months, which found that herbivores preferentially consumed herbaceous vegetation facilitating shrub seedling survivorship [10,11,38]. However, recent studies conducted through the hot-dry late-summer and autumn months highlight that herbivores reduce CSS shrub seedling survivorship and growth [39,40]. Therefore, while reductions of non-native annuals may help CSS shrub germination and survivorship in the cool-moist Mediterranean season [10,11,38], shrub recovery will only occur if herbivory pressure is low enough in the hot-dry season. The extent to which herbivory or competition may influence shrub survivorship and CSS successional trajectories will also likely be mediated by rainfall and N-deposition levels. Low rainfall years reduce shrub recruitment and survivorship, particularly in areas with elevated N-deposition [4,10,15,41]. Though recent research suggests that herbivory may be a significant factor structuring CSS plant assemblages [39,40], research was conducted in unburned CSS, so the extent to which herbivores influence CSS shrub recovery following a fire remains difficult to predict.

Predicting how herbivores influence soil microbial assemblages and key ecosystem properties following a fire is also difficult. While, Cox et al. [42] found that the direct effects of fire have little effect on soil bacterial assemblages in southern California, changes in plant assemblage structure has a significant influence on soil microbial abundances and assemblage structure, which, in turn, can influence soil nutrient concentrations [24–26,42,43]. For example, soil C and N concentrations are elevated in CSS relative to type converted non-native grasslands [24,25,43]. However, soils in recovering CSS, composed of both shrubs and non-native annuals, may not differ from CSS in C and N storage [43]. Therefore, supporting CSS recovery will likely result in elevated C and N concentrations, both in soil and above ground biomass [43]. Consequently, high herbivore pressure on CSS shrubs could lead to the lost opportunity to capture and store carbon in above- and below-ground components [43]. Similarly, decomposition in non-native grasslands is elevated compared to CSS shrublands, elevating greenhouse gasses emissions to the atmosphere [44]. In contrast, herbivores could "fertilize" soil with scat, increasing soil C and other important nutrients that can enhance photosynthetic rates [45]. The extent to which direct effects (consumption of plants) and indirect effects (changes in ecosystem processes and fertilization of soil) influence soil C and N concentrations during CSS succession post-fire is largely unknown.

In this study, we conducted an experiment to explore how herbivores influence plant and soil fungal assemblages and soil C and N concentrations over the first five years following a fire. We hypothesized that herbivores: (1) reduce non-native grass and forb cover and native CSS shrub cover, slowing succession and CSS recovery, (2) reduce the abundance of *Artemisia californica*, the dominate CSS shrub species and a plant species preferred by herbivores [39], (3) reduce bacterial and fungal abundances and modify soil microbial assemblages, and (4) reduce soil C and N concentrations, though nutrients from

herbivore scat may ameliorate those effects. By understanding how herbivores influence CSS successional patterns and soil conditions following a fire, we provide key insights for land managers and conservation practitioners to preserve this increasingly endangered ecosystem type.

2. Materials and Methods

2.1. Study Site

We conducted this study at the Robert J. Bernard Field Station (BFS). The BFS is located at the foot of the San Gabriel Mountains on the eastern edge of Los Angeles County in Claremont CA, USA (34°6′ N, 117°42′ W; 348 m elevation). The climate is Mediterranean with cool-moist winters and warm-dry summers. Winter rains typically start in October and end in April or May, with the onset of the hot-dry season beginning in late June. Annual rainfall averages 41.5 cm [15]. During our study, annual rain fall varied from 2.11 to 73.76 cm (Appendix A).

The BFS harbors five distinct habitat types and is surrounded by urban habitats [46]. Approximately 25 ha is composed of intact CSS, characterized by native drought-deciduous and drought-tolerant shrubs, though this area also supports a variety of native forbs and non-native grass and forb species at low densities. While the three most common shrubs are *Artemisia californica, Eriodictyon trichocalyx,* and *Eriogonum fasciculatum*, CSS at the BFS contains a high diversity of native plants including evergreen shrubs common to CSS (*Salvia apiana* and *Ericimeria pinifolia*), as well as evergreen shrubs (e.g., *Rhus integrifolia* and *Malosma laurina*) more typical of CSS along the coast. As the BFS is located near the foothills of the San Gabriel Mountains, increased orographic precipitation likely supports the diverse CSS plant community at the BFS [47].

In May 2017, a human-ignited fire burned approximately 1.96 ha, mostly burning previously intact CSS areas, as well as areas near the BFS lake. There had not been a fire in our study area in at least the last 40 years, though there was another fire at the BFS in 2013 [46]. This study was conducted in the CSS burned areas (Figure 1).
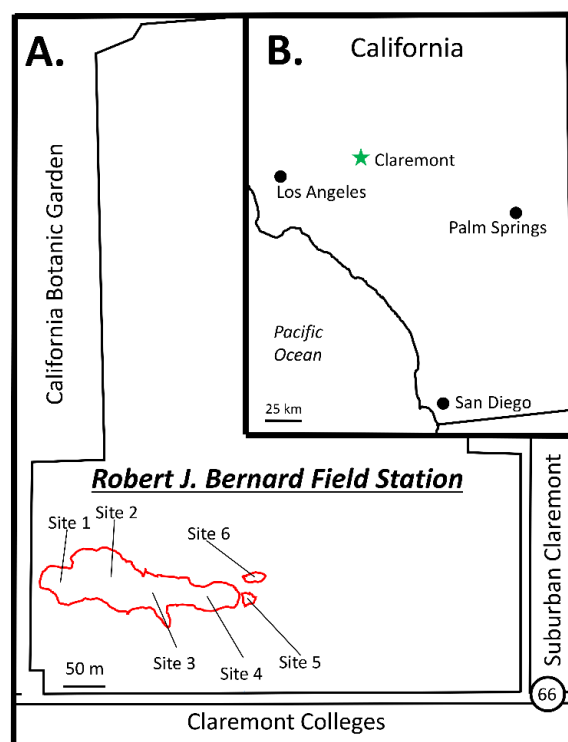

Figure 1. Map of the 2017 fire perimeter (red) and the location of the six study sites within the Robert J. Bernard Field Station (**A**), located in eastern Los Angeles County (**B**). Site 5 and 6 are in small spot fire areas.

2.2. Experimental Design, Sample Collection, and Sample Processing

We identified six sites in the 2017 fire scar (Figure 1), with four sites located within the primary fire perimeter and two located in small areas where spot fires burned portions of intact CSS (Figure 1). At each site, we established two 5 × 5 m plots, separated by a 2 m buffer. Each plot was randomly assigned to one of two treatments: an experimental herbivore excluded treatment or a control treatment that allowed herbivores access (Figure 2). Experimental plots were surrounded by chicken wire attached to t-posts. The chicken wire extended ca. 0.75 m above the surface of the soil and was buried 0.25 m below the soil surface. Treatments were paired to control for site differences, as CSS plant compositions differed across the study area before the fire.
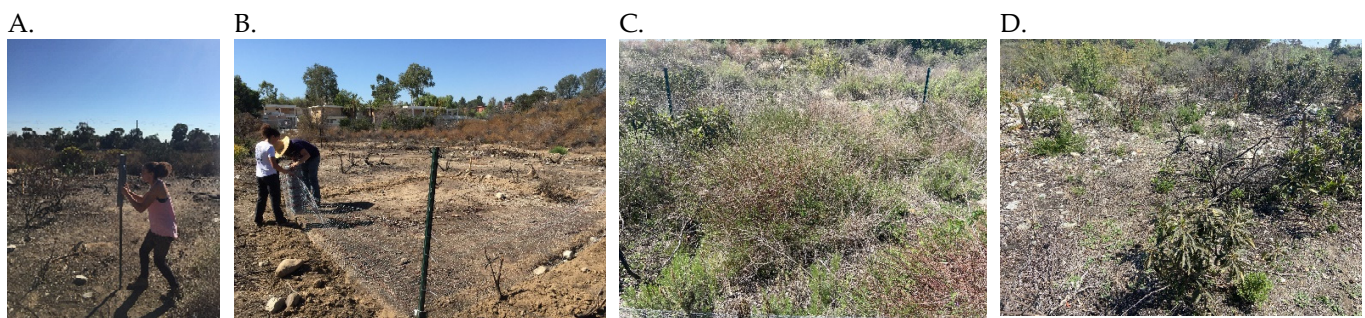
A.

B.

C.

D.

Figure 2. Pictures of our sites: (**A**) Caitlin Halligan putting in a t-post for the experimental plot in fall 2017; (**B**) Caitlin Halligan and Wallace Meyer fencing in an experimental plot to prevent herbivores access in 2017; (**C**) site six experimental plot that excluded herbivores in 2021; (**D**) the adjacent site 6 control plot that allowed herbivores access in 2021.

At the BFS, as well as in many CSS fragments surrounded by urban development, deer are excluded. Consequently, the herbivore community in CSS fragments is primarily composed of small herbivores, including: the desert cottontail, *Sylvilagus audubonii*; the California ground squirrel, *Otospermophilus beecheyi*; the deer mouse, *Peromyscus maniculatus*; the San Diego desert woodrat, *Neotoma lepida intermedia*; the big-eared woodrat, *Neotoma macrotis*; and the Pacific kangaroo rat, *Dipodomys agilis* [39].

We measured vegetative cover annually in all plots for five years beginning in spring 2018 and ending in spring 2022 using a point intercept method. We used the point intercept method as it is more repeatable and precise than visual cover estimates [48]. In each plot, we established 4 transects spaced at 1 m intervals from south to north. Along each transect, we estimated percent cover for each plant species by taking point-samples at 0.5 m intervals (36 measurement per plot). For each point-sample, we placed a 19 mm diameter pole perpendicular to the ground and recorded all the plant species that touched that pole. All plants were identified to the lowest taxonomic level possible. For most perennial species, we could identify individuals to species, but for most annuals we could only identify plants to genus. While flowers and seeds are often required to identify annual forbs and grasses to species, these characteristics were often absent during our sampling. Consequently, we could effectively identify most species only to genus (e.g., *Bromus* spp.). Unidentified species, typically small sprouts with few identifying features, represented 0 to 2 percent cover in our plots and were not included in analyses. Percent cover was calculated by dividing the number of points a plant species was found at by 36.

Following point-intercept sampling, we surveyed the entire plot and counted all *Artemisia californica* individuals in the plot. We were unsuccessful at tagging individuals from 2018 to 2019 and were not able to track individual survivorship among years. In 2018, we did record how many *A. californica* plants were resprouts. They were first identified as being larger and more robust than most other seedlings. Following identification, we gently removed soil around the base of the stem and confirmed that they were attached to well developed root system.

In October 2021, prior to last spring plant sampling, we collected three soil samples from each plot. All samples were collected from the soil A horizon (first 10 cm; [24]), making sure to remove the O horizon before taking the samples. First, we collected one 30 mL sample from the middle of each plot for DNA extraction and analyses of soil fungal assemblages. Second, we collected one 500 mL composite sample collected by combining five 100 mL samples, four samples that were collected approximately 1 m at a 45 degree angle from each corner and one taken from the center of the plot, to determine total and active fungi and bacteria. These samples were sent immediately after collection to Earthfort Laboratories™ (Corvallis, Oregon). Direct enumeration through microscopy was used to quantify total and active bacteria and fungi [49–52]. Finally, we collected four 20 mL samples of loose soil for C/N analyses. These samples were collected 1 m from each corner of the plot. Samples were stored in a −20 °C freezer before processing. To analyze percent C and N, we used an Elementar vario Micro cube elemental analyzer (Elementar, Mt. Laurel, NJ, USA).

DNA Extraction, Sequencing, and Sequence Analysis

DNA was extracted from soil using the DNeasy Power Soil Pro Kit by QIAGEN (DNeasy PowerSoil Pro Kit n.d.) according to kit procedure without optimal incubations. We used approximately 0.25 g of soil from each sample and sent extracted DNA to Molecular Research LP (https://www.mrdnalab.com/) for sequencing using ITS1-F (CTTGGTCATTTAGAGGAAGTAAGAAC) and ITS2 (GCTGCGTTCTTCATCGATGC) to amplify the fungal ITS1 region.

PCR was performed using the HotStarTaq Plus Master Mix Kit (Qiagen, USA) with the following cycle: 3 min at 94 °C, then 30 cycles of 30 s at 94 °C, 40 s at 53 °C, and finally 1 min at 72 °C, followed by a final 5 min elongation step at 72 °C. PCR products were used to create an Illumina DNA library, then sequenced using Illumina MiSeq v3 2 × 300 bp sequencing according to manufacturer guidelines (Illumina, San Diego, CA, USA). All sequences used in this paper are available at GenBank under Bioproject PRJNA893696.

Sequence analyses were performed with QIIME 2 [53]. Sequences of all samples were imported into QIIME 2, and the ITS2 region was extracted from each read using the Q2_ITSxpress plugin [54]. These filtered reads were processed using DADA2 [55] (using the q2-dada2 qiime plugin) to generate a table of unique amplicon sequence variants (ASV) and their counts per sample. Taxonomy for each ASV was determined using the q2-feature-classifier plugin [56] consensus-vsearch taxonomy classifier against the Unite database version 8.3 for eukaryotic ASVs [57]. ASVs classified as fungal were kept for further analyses. Samples were rarified to 12,000 reads based on the number of reads of the sample with fewer reads, and beta diversity measures were calculated using Qiime's diversity plugin.

2.3. Statistical Analyses

To explore if herbivores influenced successional trajectories and plant assemblage structure, we ran a three factor PERMONOVA with year, site, and treatment as factors. The similarity matrix was created using the Bray-Curtis coefficient and plant cover data collected in each plot every year. We did exclude cover data for yerba santa (*E. trichocalyx*) as all individuals were resprouts, meaning abundances in plots were correlated with pre-fire abundances, and we saw no evidence that herbivores were impacting resprouts. To visualize successional trajectories, we created an MDS plot that plotted relationships among plots for each year surveyed. The goal was to assess if control and experimental plots moved in similar or divergent trajectories. Divergent trajectories suggest that herbivores significantly impact plant assemblage structure during succession. Finally, we ran a two-factor PERMANOVA with site and treatment as factors using just the 2022 (year 5) data to examine if plant assemblages differ among treatments after 5-years. When treatment effects were significant, we ran SIMPER tests to see which species were driving differences. All analyses were run using PRIMER-E with the PERMANOVA+ add on [58].

Because we observed differences between plant successional trajectories for control sites located within spot fires and those in control sites in the primary fire perimeter which were similar to all experimental sites, we ran one additional *post-hoc* SIMPER test to explore how plant assemblages in control plots differed between sites in spot fires and in the primary fire perimeter.

To examine if herbivores impact abundance and survivorship of *A. californica* sage seedlings, the most abundant CSS species at the BFS and most other CSS areas, we plotted the abundance of *A. californica* in the plots during the second survey year (2019) and during our fifth survey year (2022). We did this as there is a peak and high variance in *A. californica* seedling emergence the first two years after a fire [15]. We expected higher abundances and higher survivorship, which we define as the number of *A. californica* individuals in 2022 divided by the number of individuals in 2019 in each plot. As dynamics differ among plots and we did not record individuals in some plots either in 2019 or 2020, we did not run a statistical analysis.

To explore if herbivores influenced soil biotic and abiotic conditions, we ran a variety of two-factor PERMONOVA tests using site and treatment as factors. First, we tested if herbivores influenced soil fungal or bacterial abundances. For this analysis we used the Euclidian distance coefficient to construct similarity matrices. Second, we examined if herbivores impacted soil fungal compositions, using the Bray-Curtis coefficient and the relative abundance of fungal ASVs. Finally, we examined if herbivores influenced soil C and N concentrations, using the Euclidean distance coefficient.

3. Results

Plant assemblages significantly differed across years ($F_4 = 3.50$, $p = 0.0001$) and sites ($F_5 = 4.33$, $p = 0.0001$), and between herbivore treatments ($F_1 = 3.92$, $p = 0.0035$). While we expected a significant year x site interaction ($F_{20} = 2.80$, $p = 0.0001$), it was interesting not to find a year x treatment interaction ($F_4 = 0.84$, $p = 0.66$) and to simultaneously find a significant site x treatment interaction ($F_5 = 2.81$, $p = 0.0003$). These interaction results may be explained by examining the successional trajectories in the different plots (Figure 3). Trajectories for herbivore allowed and excluded plots differed in sites found in spot fires (i.e., areas with intact sage scrub close by and sites 5 and 6, Figure 2), but trajectories between treatments in the primary fire perimeter were similar.
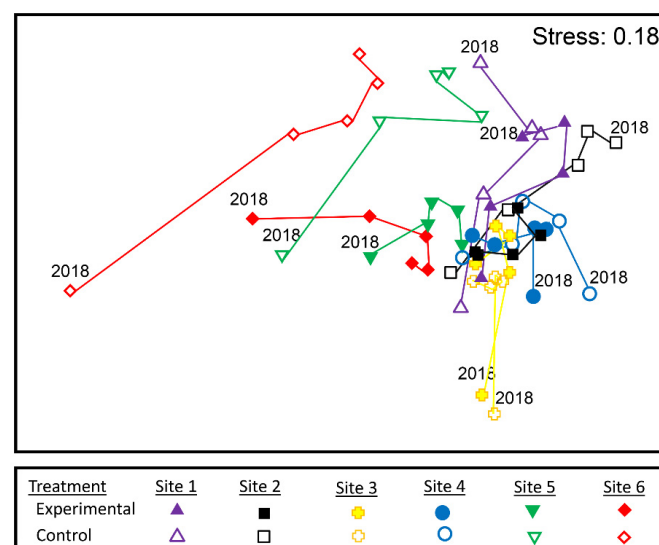

Figure 3. MDS ordination for all plots across the five sampling years (2018–2022) according to the composition and relative cover of plants in each plot. Experimental plots excluded herbivores while control plots allowed herbivores access. Lines connect samples from each plot across sequential years (2018 denotes the first year). Sites 5 and 6 were in small spot fires with intact CSS close to plots.

The SIMPER test comparing which species drove differences between all control and experimental plots revealed that herbivores reduced native shrub and non-native grass and forb cover, while native forbs had elevated cover in the control plots (Table 1). Shrub species that typify later CSS successional stages had low cover across most sites and plots (*A. californica*, averaging 1%; *E. fasciculatum*, <1%; *Salvia melifera*, <1%). The post hoc SIMPER test revealed that comparing control (herbivore allowed plots) at sites in spot fires to those in the primary fire perimeter had significantly different assemblages (85.22% average dissimilarity among spot fire and primary fire perimeter plots) (Table 2). In general, abundances of most species were reduced, except for the native forb *Camissoniopsis* spp and the native shrub *A. californica*. Increases in *A. californica* cover in control plots can be explained by elevated recruitment and evidence of resprouting in the spot fires.

Table 1. Results from SIMPER analyses listing the most important species that contribute to differences in plant assemblages between experimental (herbivore excluded) and control (herbivore allowed) plots. Subscripts indicate a plant's designation as either a native shrub (NS), native annual forb (NF), native perennial forb (NPF), non-native grass (NNG), or non-native annual forb (NNF).

Plant Taxa	Average Percent Cover		Average Dissimilarity ± 1 SD
	Experimental	Control	
Acmispon glaber [NS]	50	22	27.54 ± 1.35
Eriodium spp. [NNF]	19	19	15.58 ± 0.99
Bromus spp. [NNG]	20	5	11.47 ± 1.04
Schimus barbatus [NNG]	11	7	8.64 ± 0.84
Cryptantha spp. [NF]	7	10	7.26 ± 0.88
Mustards *,[NNF]	5	6	5.48 ± 0.72
Phacelia spp. [NF]	3	5	4.21 ± 0.65
Solaum spp. [NPF]	4	2	4.04 ± 0.44
Apiastrum angustifolium [NF]	1	4	2.99 ± 0.63
Camissoniopsis spp. [NF]	0	3	1.72 ± 0.40
Centaurea melitensis [NNF]	2	0	1.51 ± 0.50

* Mustards at the BFS represent species in a variety of genera, but *Hirschfeldia incana* is most common in our plots.

Table 2. Results from SIMPER analyses listing the most important species that contribute to differences in plant assemblages between experimental (herbivore excluded) and control (herbivore allowed) plots. Each species is followed by a subscript indicating its designation as either a native shrub (NS), native annual forb (NF), native perennial forb (NPF), non-native grass (NNG), or non-native annual forb (NNF).

Plant Taxa	Average Percent Cover		Average Dissimilarity ± 1 SD
	Sites 1–4	Sites 5 & 6	
Acmispon glaber [NS]	49	0	37.78 ± 2.37
Cryptantha spp. [NF]	17	0	12.15 ± 1.54
Eriodium spp. [NNF]	19	3	12.02 ± 1.41
Schimus barbatus [NNG]	10	4	6.58 ± 1.45
Camissoniopsis spp. [NF]	0	8	5.85 ± 0.91
Phacelia spp. [NF]	9	0	5.56 ± 0.54
Bromus spp. [NNG]	8	7	5.25 ± 1.05
Mustards *,[NNF]	5	4	3.85 ± 1.80
Artemisia californica [NS]	0	4	2.91 ± 0.91

* Mustards at the BFS represent species in a variety of genera, but *Hirschfeldia incana* is most common in our plots.

Despite low cover, *A. californica* survivorship was elevated in experimental plots that excluded herbivores, leading to enhanced abundances in five of the six sites after year 5. The control at site 5 had higher seedling recruitment following the fire compared to the

experimental at site 5 (Figure 4). In addition, the control at site 5 had two resprouts, the only two *A. californica* resprouts recorded.
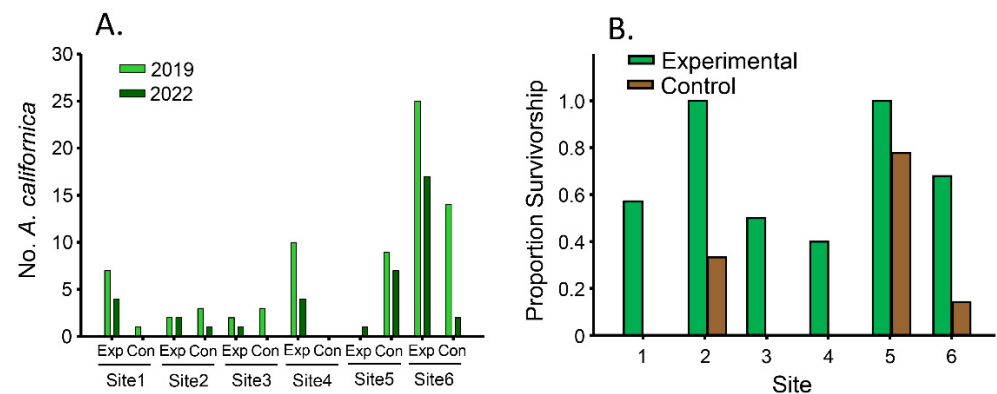

Figure 4. Number of *Artemisia californica* plants in each plot in 2019 and 2022 (**A**), and *A. californica* survivorship in each plot ((**B**) Survivorship = number of plants in 2022/number of plants in 2019).

We did not observe differences in soil biotic or abiotic characteristics between herbivore treatments that allowed and excluded herbivores. For example, bacterial ($F_1 = 0.46$, $p = 0.52$) and fungal ($F_1 = 0.44$, $p = 0.63$) abundances did not differ between control and experimental plots. Similarly, soil fungal assemblages did not differ ($F_1 = 0.84$, $p = 0.58$; Figure 5) when herbivores were excluded or allowed access. In addition, soil C ($F_1 = 1.14$, $p = 0.47$) and N ($F_1 = 0.44$, $p = 0.63$) concentrations did not differ between herbivore treatments. We reviewed the bacterial and fungal abundance data and C and N concentration data and confirmed that there were no differences that could be explained by partitioning data to compare sites in spot fires to those in the primary fire scar. Differences among sites were observed for fungal assemblage ($F_5 = 1.42$, $p = 0.035$) carbon concentrations ($F_5 = 8.58$, $p = 0.004$) and nitrogen concentrations ($F_5 = 7.26$, $p = 0.002$), though sites did not differ in microbial abundances (bacterial abundance $F_5 = 1.14$, $p = 0.427$; fungal abundance $F_5 = 4.02$, $p = 0.051$).
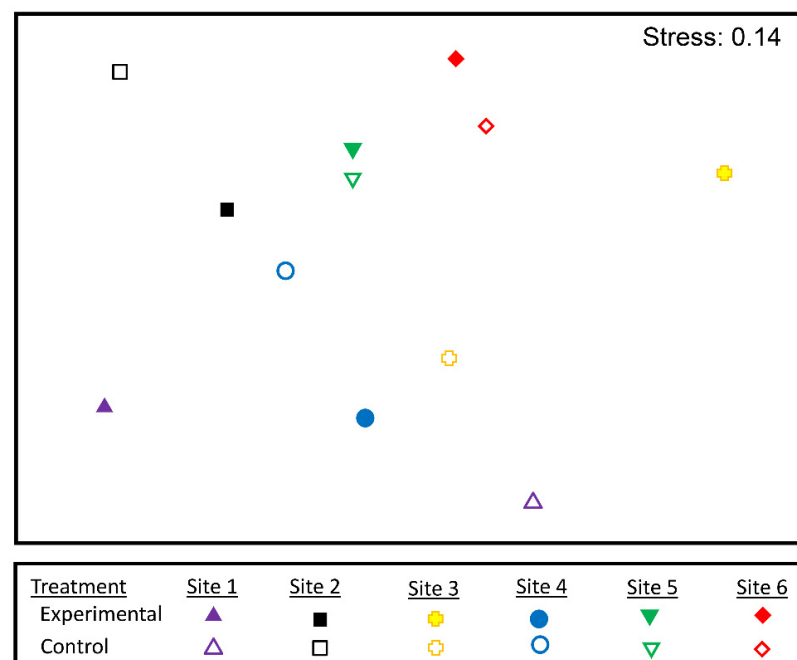

Figure 5. MDS ordination for all plots according to the composition and abundance of soil fungi in each plot. Similarity was calculated using the Bray-Curtis coefficient.

4. Discussion

Our results highlight that herbivore effects on post-fire CSS plant assemblages are complex and are contingent on location within the fire scar. While we found that herbivores influence post-fire CSS plant assemblages, their effects were elevated at spot fire sites. For example, while most plant species, including *Acmispon glaber*, a fire-following shrub, and non-native grasses had reduced cover in plots that allowed herbivores, their cover was significantly reduced in plots exposed to herbivores at spot fire sites compared to similar plots within the main fire perimeter. In addition, CSS successional trajectories in control plots at sites located within spot fires were different to those in all the other plots. These findings are not surprising as elevated herbivory near CSS shrubs has been a subject of interest for a half century [10,11,38]. Combined, results support the idea that areas near the fire edge with adjacent shrub cover have elevated herbivore impacts.

At the beginning of the experiment, we were unsure how herbivores would impact *A. californica* seedling recruitment and survivorship, and thus CSS recovery. While recent research has shown that herbivores reduce shrub seeding survivorship in intact CSS [39,40], we were unsure if similar patterns would be found in burned habitats. In particular, we wanted to explore the relative importance of competition with non-native annual grasses, which are often consumed by the herbivore community, as opposed to the direct effects of herbivores in limiting *A. californica* recruitment [15,59,60]. Our findings support the idea that herbivores reduce *A. californica* seedling survivorship slowing CSS recovery. Other factors (e.g., abiotic stress and competition) are clearly impacting *A. calfornica* populations as survivorship varied among plots, including those that excluded herbivores. However, *A. californica* survivorship at all sites was reduced in plots that allowed herbivores relative to plots that excluded them. Elevated *A. californica* survivorship in plots that excluded herbivores were observed despite elevated cover of non-native grasses in these plots. Results demonstrate that herbivores are an important factor influencing *A. calfornica* post-fire recovery and highlight that the direct impacts of herbivores should not be discounted.

While survivorship is reduced in the presence of herbivores, exposure to herbivory may not predict *A. californica* abundance. For example, in plot 5, we recorded higher *A. californica* abundances in the control plot that allowed herbivores than the experimental plot that excluded herbivores at the end of five years. The elevated abundances are correlated with elevated recruitment, suggesting that this plot had a more robust seed bank. In addition, the only two *A. californica* resprouts recorded in this study were in this plot. Crown resprouting can play a key role in CSS recovery [33,61,62] as resprouts can grow and set seeds quickly [33,34]. As reproduction from resprouting shrubs after the fire can lead to enhanced germination in subsequent years [33,34], presence of *A. californica* resprouts may enhance *A. californica* populations even in the presence of herbivores. Consequently, presence of resprouts, particularly in inland areas where resprouting rates are often low [15,63], may indicate an area with high recovery potential.

Combined, these results provide key insights into how herbivores impact post-fire plant assemblages and early CSS succession. We found that herbivores reduce cover of native shrubs and non-native grasses but have limited effects on native forbs. For example, we found that herbivores reduced the cover of the fire-following shrub *Acmispon glaber*, particularly in spot fire sites. Reductions of *A. glaber* may have negative long-term effects, as this shrub can increase soil nitrogen and other nutrients and act as a nurse plant, potentially improving recruitment and growth of climax CSS shrub species [64,65]. In contrast, reductions in cover of non-native grasses, particularly *Bromus* spp., may help ameliorate competition between non-native grasses and native CSS shrubs, enhancing CSS shrub recovery. However, we found that herbivores reduce *A. californica* seedling survivorship despite reductions in non-native grass cover, highlighting the importance of direct effects of herbivores. In contrast to native CSS shrubs, native forbs seem to do well in the presence of herbivory, as we observed increased cover of *Cryptantha* spp. and *Camissoniopsis* spp. suggesting that herbivory may benefit these species, though abundances of native forbs were significantly reduced in spot fire plots where herbivory is elevated.

Despite these insights, after 5 years our study sites remain in the early stages of CSS succession with plant assemblages being dominated by fire-following shrubs (*A. glaber*), non-native annuals, and native and non-native forbs. Therefore, we are uncertain if CSS, with an intact and abundant native shrub community, will return. Consequently, tracking plant succession for more than 5 years is required to confirm if CSS plant assemblages can recover. In addition, exploring if succession near fire edge differs from succession in the fire perimeter will help elucidate how herbivory pressure impacts CSS recovery.

Contrary to our hypotheses, we found that herbivores have little influence on below ground properties following fire in CSS. Though we did not test it, we expected that the fire would have few direct effects on soil microbial assemblages or nutrient concentrations (see [43]). However, changes in plant assemblages in southern California can impact soil microbe abundance, microbe assemblage structure, and nutrient concentrations with CSS having higher C and N concentrations relative to non-native grasslands [24–26,42,43]. Therefore, we hypothesized that herbivores, through their effects on plant abundances and assemblages, would reduce bacterial and fungal abundances and soil C and N concentrations. We also hypothesized that these below-ground and above-ground changes would result in different soil microbial assemblages in herbivore allowed and herbivore excluded plots. Instead, we found that microbe abundance, soil C and N concentrations, and soil assemblage structure did not differ between plots that allowed or excluded herbivores after 5 years. Large differences among sites make it difficult to explore if patterns in microbial assemblage structure differ in spot fires where the effects of herbivores were elevated. Wheeler et al. [43] found that soils in recovering CSS, composed of both shrubs and non-native annuals, did not differ in C and N storage from intact CSS. Consequently, understanding how herbivores will impact key ecosystem processes/services will ultimately require understanding if CSS recovers or if the area becomes type-converted to a non-native grassland [24,25].

5. Conclusions

CSS recovery following a fire disturbance is a critical conservation goal in southern California. Our results highlight that despite reducing cover of invasive grasses, herbivores slow CSS succession by reducing native shrub cover and reducing *A. californica* survivorship. While ameliorating the effects of drought and competition with non-native grasses are also critical to improving CSS post-fire recovery [41,59,60], the direct effects of herbivores on CSS shrub assemblages should not be discounted. These results suggest that effective restoration should incorporate efforts to reduce herbivory effects on *A. californica* and other shrub seedlings, as these efforts could facilitate recovery of native shrubs, especially obligate seeders and facultative seeders in areas where resprouting is low. Native forbs do well in the presence of herbivores, increasing their cover. Therefore, we suggest that restoration practitioners consider caging 'shrub regeneration' areas to facilitate CSS scrub recovery following a fire. Similar actions are not be required for native forbs, except near the fire edge where caging may be critical for CSS recovery. For example, we recommend that restoration studies explore the efficacy of caging individual seedlings [39,40] or larger areas like we did here, and potentially targeting non-native grass removal efforts in these treatments [15], as this could increase shrub survivorship and enhance the likelihood of CSS recovery.

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Appendix A

Table A1. Total annual rainfall, calculated using data from the Western Regional Climate Center's weather station in Claremont, California. Rain years were considered to be from July 1 of the year noted to June 30th of the following year. Average annual rainfall is 44.3 cm/year (1894–2016; [66]).

Rain Year	Average Preciptiatation (cm)
2017–18	22.73
2018–19	73.76
2019–20	18.54
2020–21	2.11
2021–22	36.55

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