1 Introduction

The long term trajectory of vegetation succession after disturbance depends on the growth, successful reproduction, and competition for resources of species in the initial post-disturbance community, which is often of a different composition compared to undisturbed communities in the same region. Flowering species, of which 85% depend on animal pollination (Ollerton et al., 2011), compete for pollinators to increase their own reproductive fitness and in turn affect the fitness of the floral neighborhood, as neighboring species can compete for or facilitate pollination in each other (Bruckman & Campbell, 2016; Campbell & Motten, 1985, Morales & Traveset, 2008). Where recent fire disturbance has altered flowering community composition and pollinator availability, we might expect to see changes in the dynamics of pollinator interactions, such as changes to visitation rate and pollinator choice, which in turn could influence the long term recovery trajectory of the community.

Fire disturbance has both direct and indirect effects on pollinator activity. Immediate effects of fire include the destruction of nests and juveniles in less mobile stages, and also the clearing of above-ground vegetation matter that serves as nesting material (Potts et al., 2005). However, insect pollinators can recruit to flowering patches from a distance, with a positive relationship between body size and recruitment distance (Greenleaf et al., 2007) and recent burn history has been linked with increased pollinator diversity (Moretti et al., 2009; Potts et al., 2003). Landscape heterogeneity in the form of fire history patchiness has also been shown to help maintain pollinator diversity over time (Ponisio et al., 2016; Ulyshen et al., 2021). Studies in post-fire flowering in other regions have shown examples of increased flowering synchronicity and flowering time, which results in observations of longer periods of pollinator visitation (Mola & Williams, 2018).

Coastal sage scrub (CSS) is a plant community found in the coastal California ecoregion, with mature stands characterized by a dominant cover of low-growing native shrubs interspersed with a substory of herbaceous forbs and grasses. Native communities are adapted for persistence under historical fire regimes, with patterns of increased diversity shortly after fire slowly declining to the shrub dominated steady state at maturity, but shortened fire intervals are known to increase the proportion of invasive alien species (Keeley & Brennan). While vegetation succession has been well studied in southern California CSS, few have looked at post-fire flowering and pollination. Floral neighborhoods in CSS mostly consist of a mix of native shrubs and forbs, and non-native forbs. Many local forb species show high landscape heterogeneity and produce few flowers per individual, in contrast to the few dominant species which produce many flowers at a time: in mature stands, native shrubs such as *Salvia mellifera* and *Eriogonum fasciculatum* produce high vegetation cover as well as flowering, while intermediate stands are dominated by the forb/subshrub *Acmispon glaber*. Invasive mustards such as *Brassica nigra* and *Hirschfeldia incana* are also highly prevalent, particularly in recently disturbed areas. Fire following species also emerge in recently burned sites, further increasing immediate post-fire diversity and differentiating burned communities with mature communities. Past studies have examined the effects of these high density flowering invasives on neighboring native species and found facilitation of pollination with increased receipt of conspecific pollen and higher native seed set at low densities of invasives (Bruckman & Campbell, 2014), while higher diversity in the floral neighborhood increased visitation rates from native pollinators, which increased seed set for a focal species (Bruckman & Campbell, 2016).

We used collected data on flowering, pollinator activity, and pollen transfer from CSS in different post-fire stages in 2021 and 2022 to examine the following questions:

1. How does post-fire stage impact pollinator visitation and diversity, and can this be explained by flowering abundances and diversity present at these sites?
2. How does post-fire stage impact pollen transfer, including conspecific pollen and heterospecific pollen transfer?

2 Materials and Methods

2.1 Study Sites

We collected data on flowering and pollinator visitation from 22 coastal sage scrub transects spread across an area of ~20km2 in Orange County, California, USA from mid-March to mid-May in 2021 and 2022. Data collection took place during spring, corresponding with peak vegetation in coastal sage scrub (Cleland et al., 2016).

The transects are a subset of previous established sites for long-term vegetation monitoring by the Center for Environmental Biology at UC Irvine in partnership with The Nature Conservancy and the Natural Communities Coalition (Kimball et al. 2018). Three stages of recent fire history are included in the chosen transects: recently burned (B, 7 sites, burned in fall 2020), intermediate burned (M, 4 sites, burned in fall 2017), and unburned (U, 11 sites, over 10 years unburned). Each transect consisted of a 50m linear line marked by two permanent rebars at the start and end points, and flowering and pollinator data was collected from 1x1m quadrats distributed in 5m intervals alternating left and right of the transect line. To account for possible changes in flowering species and pollinator activity across the season, we visited transects three times each year in consecutive rounds, with the exception of two sites (one M and one U) which were added to the dataset in the second round of 2021, and one B site which was not visited in the second and third rounds of 2021.

2.2 Data Collection

2.2.1 Flowering

To account for flowering species abundance and diversity across the transect, all species with open flowers were recorded for each 1x1 quadrat, and flower abundances were counted by totaling the number of open flowers for each species in bloom or estimating high blooming species by counting the average number of open flowers on 10 randomly selected inflorescences and multiplying by the number of flowering inflorescences.

2.2.2 Pollinator visitation

To create estimates for pollinator activity and diversity, we collected visitation data through observations in the field. At each transect visit, up to 6 of the 1x1 quadrats were each monitored to get a spread of quadrats across the heterogeneous CSS transects. Quadrats were chosen to include the two quadrats with the highest flowering abundance and randomly selected for the rest. Quadrats where there were less than three individual blooms were not considered for observation due to the low chance of any pollinator activity.

20-minute observation blocks were taken for each selected quadrat to approximate the needed time for obtaining accurate estimates of flower visitation rates (Fijen & Klejin, 2017). A sole observer performed each observation and noted the start and end time. During the observation period, each pollinator entry into the quadrat, and each contact the individual pollinator makes with the reproductive structure of open flowers in the quadrat is recorded. Easily identified pollinators such as *Apis mellifera* and *Bombus* species were noted. Other species were noted for possible identifying features such as size, markings, and flying patterns. Due to our focus being the flower visitation of pollinators, it was not feasible to catch individuals for identification when they entered quadrats, due to interference in their activities. In 2021, when possible, individuals were caught, photographed, and released upon leaving the quadrat. In 2022 we also caught some individuals after visitations to also be identified in the lab. Ultimately, the pollinator groupings we used included *Apis mellifera, Bombus spp.,* and other bees in the 20, 10, and 5mm length categories. Other pollinators groups we observed included lepidopterans, syrphid flies, bombyliid flies, other flies, hummingbirds, and sawflies.

2.2.3 Stigma collection and pollen transfer

To look at pollen transfer, we collected flower stigmas from quadrats after completing our observations. Stigmas were collected from up to 10 individuals of each species in bloom in each quadrat and placed individually in labelled Eppendorf tubes. The stigmas were then stained with basic fuchsin gel (Kearns & Inouye, 1993) and examined under a light microscope to identify conspecific and heterospecific pollen presence. To quantify the amount of pollen transfer, we also counted conspecific and heterospecific pollen in the samples we collected in 2022.

2.3 Analysis

All statistical analyses were performed using R version 4.2.3.

2.3.1 Pollinator visitation

To calculate pollinator visitation rates for each 20 minute observation block as, we used 3(total number of visitations/total flower number) = visitations/flower/hour. To test for differences in pollinator visitation rates across different burn statuses and our two years of observation at the transect level, we averaged all quadrat pollinator visitation rates across each transect visit, and used linear mixed effect models (LME function, R nlme package 3.1) with burn status and year as fixed effects and transect identity included as a random effect, to account for our repeated visits across the season. We also used linear mixed models to compare the relationship between visitations and flowering number across the different burn statuses, including flowering and burn status as fixed effects and transect as random effect.

We were also interested to see whether pollinator visitation rates were significantly affected by differences in temperature at the time of observation, and ran correlation tests for the data in each year and burn status combination using the rmcorr package (Bakdash, 2017), to account for repeated measures of the same transect across the season.

2.3.2 Pollen transfer

As there was great variation in the presence of flowering species across different CSS, many of the rarer flowering species were not found across many transects or across different burn statuses. Because of this, we selected five of the most common flowering species in our dataset to serve as focal species for our comparisons on pollen transfer. These included three native species: *Salvia mellifera, Gilia angelensis, Euchrypta chrysanthemifolia,* and two invasives: *Hirshfeldia incana, Erodium cicutarium*. We compared (1) conspecific pollen deposition, (2) heterospecific pollen deposition, and (3) proportion of stigmas with heterospecific pollen presence across the different burn statuses in 2022. For each of these three dependent variables we first calculated the quadrat average for (1) and (2), and the proportion value for (3), and then averaged all quadrat values across the transect. We then analysed the averages used one-way ANOVA with burn status as the fixed factor. Post-hoc tests (TukeyHSD function, R stats package 3.6.2) were also performed on significant ANOVAs to look for differences between pairs of burn status means.

3 Results

In total, we performed 503 observation blocks totaling 168 observation hours. The majority of pollinator visitations came from *Apis mellifera*, comprising approximately 58% and 70% of total observed visitations in 2021 and 2022, respectively.

3.1 Pollinator visitations

Average transect pollinator visitation rates were not significantly different between different burn statuses or between our two years of observation (Slide 11 and 12). When looking at the visitation rates of *Apis mellifera* (Slide 14,15) and by non-*Apis* bees (slide 16,17) separately, the effects of burn status and year remain non-significant. The average proportion of all visits done by *Apis mellifera* (Slide 20,21) and non-*Apis* bees (Slide 22,23) was also not significantly affected by burn status or by year.

However, the relationship between quadrat visitation and flower number was significantly different across the three different burn statuses (Slide 25,26). Visitation of all species increased with flower number (p= 0.0012), and visitation differed across different burn statuses (p=0.0388). The significant interaction effect between flower number and burn status (p=0.00015) suggests differences in the relationship between visitation and flower number dependent on burn status, with the greatest rate if increase in visitations in order of burned, unburned and finally intermediate sites. Separating out visitations by *Apis mellifera* (Slide 29)and non-*Apis* bees (Slide 31), *Apis mellifera* visitations increased with flower number (p=0.000013), and increased the most relative to flower number in unburned sites (interaction, p=0.00123) though there was no significant difference in visitation numbers between the different burn statuses (p=0.063). In contrast, non-*Apis* bee visitations were not significantly affected by flower number (p=0.145), burn status (p=0.138), or interactions between flower number and burn status (p=0.288).

3.2 Pollen transfer

When we examined pollen transfer in the five selected common species (Slides 43,44,45), comparisons between burn statuses for conspecific pollen and heterospecific pollen deposition all showed no significant differences except for conspecific pollen transfer in *Salvia mellifera* (p=0.0396)*.* Post-hoc tests indicated that while intermediate sites looked like they had generally higher conspecific pollen deposition compared to both burned and unburned sites, the paired differences were not significant. The proportion of stigmas which had heterospecific pollen deposition for each species was also not significantly different across different burn statuses.