Quantifying the effects of wildfire reintroduction on native pollinators in ponderosa pine forests of the Pacific Northwest

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Abstract

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Pollinators are an essential component of ecosystem function, and declining bee populations are a global conservation concern. Despite this importance, there is a lack of understanding regarding the distribution and demography of native bee species across western North American landscapes. Furthermore, the effects of disturbance on these essential fauna are understudied. Fire is a major driver of biodiversity and structural integrity in fire prone ecosystems. Over the last century, historical wildfire regimes have shifted due to climate change, exclusion of Indigenous fire stewards, and land management philosophies. As a result of these pressures, forests have experienced increases in fuel buildup that threatens dry forest resilience across western North America. In these post-fire landscapes, pollination is critical for vegetation recovery and ecosystem health. Pollinators are critical for successful angiosperm reproduction, food security, and ecosystem resilience. Because pollination services can vary by species and across spatial and temporal scales, land managers and ecologists seek to understand the spatial and temporal effects of fire reintroduction on native bee communities. An increased understanding of the effects of fire on pollinators can inform conservation management and ecosystem restoration.

In this dissertation, I used field based and molecular methods to evaluate the effects of fire reintroduction on bee community composition across a temporal range of 1-9 years post-fire, and in an unburned control, in the Okanogan-Wenatchee National Forest in Washington, USA. In Chapter 1, I quantified native bee richness at nine forested plots using blue vane and pan traps, and determined that native bee genus richness was driven by the interaction between burn severity and burn age. Study areas that had been recently burned at higher severities had higher bee genus richness values. Furthermore, the proportion of above ground nesting bees in landscapes 1-8 years post-fire were greatest at sites that burned with moderate fire severity and had more available nesting habitat. In Chapter 2, I highlighted shifts in foraging resources for pollinator communities in post-fire landscapes based on DNA metabarcoding of pollen collected in blue vane and pan traps. The findings suggest that herbaceous perennials such as *Achillea millefolium*, *Lupinus* spp., and *Phacelia hastata* are important floral resources for post-fire pollinator communities. Burn age and burn severity were important drivers of plant-pollinator network richness, and plant species richness increased with burn age and burn severity. This work is the first to use pollen suspended in trap solutions to quantify floral resources for insect pollinators and highlights the utility of trap byproducts to provide insightful ecological information. In Chapter 3, I evaluated changes in pollinator demography to provide insights for bee community health following disturbance by wildfire. By deploying nesting materials across three burned landscapes and one unburned control, I quantified native wood-cavity-nesting bee reproductive success for two exemplar pollinator species: *Osmia lignaria propinqua* and *Osmia kincaidii*. The results suggest that burn age is the main driver of wood-cavity-nesting bee oviposition. Furthermore, DNA metabarcoding of pollen from nest boxes revealed herbaceous perennials such as *Phacelia hastata* and *Epilobium brachycarpum* to be important floral resources for wood-cavity-nesting pollinators. Finally, in Chapter 4, I documented new records of *Melissodes nigracauda* LaBerge, *Dufourea dilatipes* Bohart, *Atoposmia abjecta abjecta* Cresson, *Coelioxys*

funerarius Smith, *Dianthidium cressonii* Dalla Torre, *Dianthidium singulare* Cresson, *Osmia cyaneonitens* Cockerell, and *Stelis heronae* Sheffield. These eight new records supplement the ~565 bee species previously documented in Washington state. Collectively, I assessed the effects of wildfire in ponderosa pine forests on native pollinator community composition, wood-cavity-nesting bee reproductive success, and plant-pollinator networks. These findings highlight the importance of fire reintroduction for native pollinator conservation in dry forest landscapes.

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Published Materials

A portion of the materials presented in this dissertation have been published through the peer review process. This article is reproduced in full here as Chapter 4 and is reprinted from Check List.

Maust, A., Gardner, J., Wright, K. W., Griswold, T., & Tobin, P. C. (2025). First records of eight native bee species (Hymenoptera, Anthophila) in Washington, USA. *Check List*, *21*(2), 374-399.

Introduction

Pollination is an important ecosystem function to monitor following a disturbance due to its ecological and economic value. Pollination services are critical for sustaining food systems and are valued at >$150 billion dollars annually (Porto et al. 2020). Pollinators also increase biodiversity, assisting in the reproduction of ~75% of angiosperms (Mader et al. 2011), making them essential for plant community recovery post fire (Potts et al. 2006). In addition to pollination services, pollinators contribute to ecosystem function through herbivory and are a food source for other trophic levels (Waltz and Covington 2004).

Among pollinators, bees (Hymenoptera: Anthophila) depend exclusively on pollen and nectar during their life cycle, and prefer plants that provide both, which consequently makes them highly efficient pollinators (Brown and Paxton 2009). Bees also adhere to flower constancy when foraging, visiting the same type of flower repeatedly. This constancy yields a higher probability of receiving pollen from the same species, which is critical for successful pollination. Furthermore, bees have evolved morphological features, such as scopa (pollen carrying hairs) and corbicula (pollen baskets), that facilitate efficient pollen collection and transportation (Mader et al. 2011). The culmination of these factors makes bees the most important pollinating taxon to monitor.

Several bee species, particularly the European honey bee, *Apis mellifera* L. (Hymenoptera: Anthophila), are well studied due to their importance in agriculture (Beekman and Ratnieks 2000, Human et al. 2013). However, native bees are often more reliable and efficient pollinators for many agricultural crops and could reduce agricultural dependance on the non-native honey bees (Winfree et al. 2007). Wild bee species provide reliable pollination services for agricultural production (Kremen et al. 2002) in addition to pollination of natural biodiversity (Potts et al. 2006). Research on native bees is increasingly critical considering widely documented native bee declines facilitated by large and increasingly severe disturbances, which are predicted to increase as the climate continues to warm (Buchmann and Nabhan 1996, Allen-Wardell et al. 1998, Cane and Tepedino 2001).

One of the main disturbances that affects native bee communities in forest ecosystems is wildfire. Fire is a major driver of biodiversity and structural integrity at every ecological scale in fire prone ecosystems (Agee 1993). Historically, native fire regimes fostered heterogeneous landscapes that supported an abundance of flora and fauna. Until the mid 1800s, fire was driven by cultural burning and lightning ignited wildfires, resulting in frequent fire across most western North American dry interior forests. Specifically, dendrochronological evidence coupled with charcoal records suggests that historical fire intervals in forest ecosystems in eastern Washington were 1-25 years (Everett et al. 2000, Hessburg and Agee 2003, Hessburg et al. 2005). Following settler colonialism, fire regimes markedly changed with steep reductions in fire throughout much of the 20th century and increases in fire size and severity over the past several decades (Reilly et al. 2017, Parks and Abatzoglou 2020). Following a century of fire suppression and exclusion coupled with agriculture, logging, and urbanization, many dry forest landscapes have shifted outside their historical ranges of variability, leading to shifts in fire frequency and severity (Hessburg and Agee 2003, Hagmann et al. 2021, Cansler et al. 2022).

Fires are predicted to continue to shift landscapes outside their historical range of variability, thus threatening the resilience of fire-prone forest ecosystems (Xu et al. 2020). Landscapes in the western United States remain in a ‘fire deficit’ despite increases in both annual area burned and fire severity (Marlon et al. 2012, Baron et al. 2022). The current rate of biomass burning is not unusual compared to long term historical burn rates (Whitlock 1992) but is out of sync with current climate conditions (Marlon et al. 2012). In addition, high severity fires in places that historically experienced low or mixed severity fire regimes, such as eastern Washington, increase the risk of losing native biota by destroying foliage, seed stocks, and roots. To ensure the retention and resilience of dry forest biodiversity, an increased understanding of the effects of fire on dry forest plant-pollinator communities is critical (Potts et al. 2006; Burkle et al. 2019).

When wildfire is restorative, most fire effects on pollinator communities are positive (Peralta et al. 2017, Galbraith et. al. 2019). Post-fire landscapes are often rich in components that create high quality bee habitat, including floral resources, structural complexity, exposed soil, and nesting habitat (Nyoka 2010). However, climate change can compound the effects of fire, thus threatening dry and mixed conifer forests and the wildlife they support. Climate change has concentrated precipitation in winter, making water less available and increasing the flammability of fire prone ecosystems (Swain et al. 2018). Wildfire occurrence within western North America is highly sensitive to the timing of spring snowmelt and changes in moisture (Westerling 2016). This sensitivity could lead to an increase in fire frequency as climate change shortens the temporal extent of the North Cascades snowpack, thus altering the hydrologic dynamics of eastern Washington. The direct effects of these shifting climatic processes on pollinator communities are unknown.

The overarching goal of this dissertation is to provide novel data that can be used to inform management practices for pollinator conservation. Management strategies that support and enhance pollinator communities also promote food security for both nearby commercial orchards and Indigenous foraged foods within national forests (i.e., the Okanogan-Wenatchee National Forest). The Syilx (Okanagan), Yakama, and Confederated Tribes of the Colville Reservation have retained foraging rights to national forestlands and rely on native pollinators to sustain culturally important foods including huckleberry (e.g., *Vaccinium* spp.) and many herbaceous plants common to dry forest and *Artemesia* steppe landscapes.

In this dissertation, I evaluated how recent wildfires in Chelan County, Washington, USA, have affected native plant-pollinator communities both compositionally and demographically. After collecting data over four years, I used generalized linear mixed models and molecular methods to determine shifts in bee community metrics from one to nine years following fire, and in an unburned control. A central question guided this dissertation: *How might land managers effectively reintroduce wildfire and simultaneously support native pollinator biodiversity in interior dry forests of the Pacific Northwest?* Each chapter addresses a component of this question: in Chapter 1 I quantified seasonal changes in native bee genus richness at four field study areas 1-9 years post-fire, and at an unburned control; in Chapter 2 I used DNA metabarcoding on bee-collected pollen to reveal high-priority floral resources for pollinators in post-fire conditions; in Chapter 3, I assessed bee demographic traits of wood-cavity-nesting bee species to understand how disturbance influences bee reproductive success; finally, in Chapter 4 I identified native bee faunas of Chelan County, and highlighted eight new records for the state of Washington. While previous studies have determined trends in pollinator community shifts following disturbance in other systems, effective conservation necessitates targeted research on the specific bee species that are present in this region, and the unique response of native plant-pollinator networks to fire.

Chapter 1. Native bee richness increases with burn severity in ponderosa pine forests

1.1 Abstract

Fire is a major driver of biodiversity and structural integrity in fire prone ecosystems. Increases in forest fuel buildup coupled with pressure from climate change has threatened dry forest resilience across western North America. In these post-fire landscapes, pollination is critical for vegetation recovery. Because pollination services can vary by species and across spatial and temporal scales, understanding the effect of fire on pollinator populations informs conservation management and ecosystem restoration. We quantified native bee richness in the Okanogan-Wenatchee National Forest in Washington, USA at nine forested plots that burned in 2021, 2018, and 2015, or were unburned since 1968. Data was collected in 2021, 2022, and 2023 biweekly from April to August using blue vane and pan traps. Our findings suggest that native bee genus richness was driven by the interaction between burn severity and burn age. Study areas that had been recently burned at higher severities had higher bee genus richness values. Furthermore, the proportion of above ground nesting bees in landscapes 1-8 years post-fire were greatest at sites that burned with moderate fire severity and had more available nesting habitat. Our findings suggest that the reintroduction of fire with mixed severity fire effects in ponderosa pine landscapes promotes native bee biodiversity.

1.2 Introduction

Landscapes in western North America are increasingly vulnerable to seasonal and episodic drought, warming events, and wildfires (Hagmann et al. 2021). Compared to historical fire regimes, wildfires have increased in severity, affecting both the landscape (Agee 1993) and the wildlife that inhabits it (Ager et al. 2007, Galbraith et al. 2019, Sanderfoot et al. 2022). Wildfires are expected to continue increasing in frequency and severity as dry seasons lengthen and become more intense under climate change (Stephens et al. 2020, Hessburg et al. 2021). These rapid changes in ecosystems necessitate targeted research and conservation on the biota in fire prone areas. Pollinators are receiving particular attention due to the economically valuable pollination services they provide to food systems (Porto et al. 2020). Wild bee species in fire-prone forest ecosystems are historically understudied relative to pollinators in agricultural systems (Delaplane and Mayer 2000, Lentini et al. 2012, Garibaldi et al. 2014). Given documented global declines in native bee abundance, studies are increasingly focusing on native bee populations and strategies for their conservation (Kearns et al. 1998, Burkle et al. 2013, Zattara and Aizen 2021).

Fire effects on pollinator communities vary across ecosystems (Allen et al. 2002, Nyoka 2010, Geroff et al. 2014, Loffland et al. 2017, Tonietto et al. 2017, Simanonok and Burkle 2019, Leone et al. 2022). The direct negative effects of fire on bees may include the immediate loss of habitat and disruption of behavior. However, most fire effects are positive over longer time scales (Waltz and Covington 2004, Potts et al. 2006, Cane and Neff 2011, Peralta et al. 2017, Rodriguez and Kouki 2017, Rivers et al. 2018, Galbraith et al. 2019, Teixido et al. 2024). Specifically, post-burn forest landscapes are often rich in floral resources, which can likely be attributed to increases in light availability. Fire may improve nesting habitat for ground nesting species by increasing available bare earth and leaf litter. Above-ground nesting pollinator species also benefit due to an increase in available nesting resources including cavities, hollow reeds, host plants, beetle galleries, and dead wood (Westerfelt et al. 2015, Gelles et al. 2022).

Historically, ponderosa pine (*Pinus ponderosa*) forests in eastern Washington, USA were maintained by frequent fires that were often of low or moderate fire severity (Agee 1993, Hessburg and Agee 2003, Cansler et al. 2022). As with many fire-adapted ecosystems across the globe, historical fires included cultural burns (Trauernicht et al. 2015, Lake and Christianson 2019) and lightning ignitions (Whitlock 1992, Marlon et al. 2012). However, historic fire regimes in western North America have been disrupted by a century of fire suppression following Euro-American settler colonialism, which was exacerbated by the forced removal of Indigenous fire stewards (Lake and Christianson 2019, Long et al. 2020). Through a prolonged absence of fire, mixed conifers and shrubs encroached on open forests with a concomitant increase in fuel accumulations, from organic soils to layered forest canopies (Hagmann et al. 2021).

Furthermore, landscapes with increased pyrodiversity and heterogeneity support a greater diversity of pollinators and may protect against drought induced scarcity of floral resources (Ponisio et al. 2016, Parreño et al. 2024). Landscape heterogeneity creates patch mosaics that reduce fire risk and contribute to fire- and climate-resilient landscapes (Hessburg et al. 2016, Stevens et al. 2019, Cansler et al. 2022). Thus, measuring the direct effects of mixed severity fire on pollinator communities can clarify best practices for simultaneously managing wildfire risk and supporting pollinator biodiversity across western North American forests.

There are 573 documented native bee species in Washington state, with an additional 102 undocumented species that are likely to occur (Bartholomew et al. 2024, Maust et al. 2025). To effectively conserve native bee biodiversity, targeted research on native bee distributions and associated host plants is needed. A positive relationship between local wildfire severity and wild bee diversity was reported in a fire-adapted mixed conifer forest in southwestern Oregon, USA (Galbraith et al. 2019). This finding was especially true for ground nesting bee species that were capable of surviving soil heating by wildfire (Cane and Neff 2011). Furthermore, wood-cavity nesting success in lodgepole pine forests was greatest in years immediately following fire, and declined with increasing time-since-burn across a temporal gradient of 3-25 years (Simanonok and Burkle 2019). Hypothesized direct negative effects of fire on above ground nesting bee species include displacement and mortality, but the immediate effects of fire on resident native bee community in recently burned landscapes (1-3 years) are unknown.

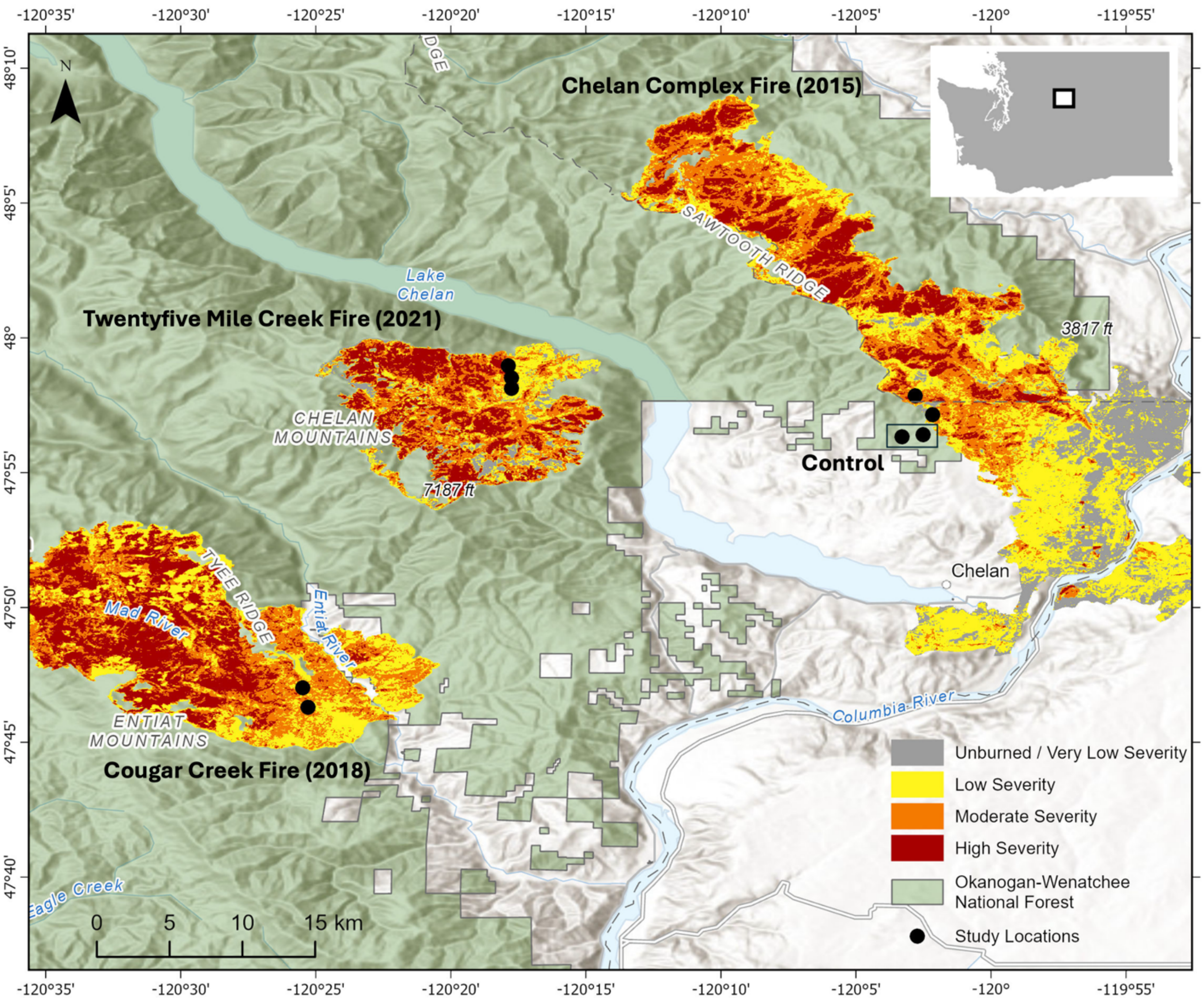
In this study, we measured the effect of wildfire burn severity and burn age (from 1 to 8 years post fire) on pollinator community structure over three years (2021-2023) in eastern Washington, USA. We hypothesized that inter-community variation would be best predicted by time since burn and burn severity, and that native bee genus richness would be greater in more recently burned study areas, reflecting the short-term positive effects of fire on bee communities. Specifically, we hypothesized that (1) burn age and burn severity are the main drivers of inter-community variation (variation in β-diversity). We also conjectured that (2) specific bee species are associated with unique burn ages and severities, highlighting key associations for conservation. Lastly, we quantified the relative abundance of above ground nesting species across post-fire landscapes. We hypothesized that (3) due to displacement and mortality of above ground nesting bees during active wildfires, there is likely an immediate decrease in the proportion of above ground nesting bees on the landscape. Increases in the proportion of above ground nesting bees 2-3 years following fire are most likely driven by bee recolonization rates and the abundance of nesting habitat.

1.3 Materials and Methods

Study Areas

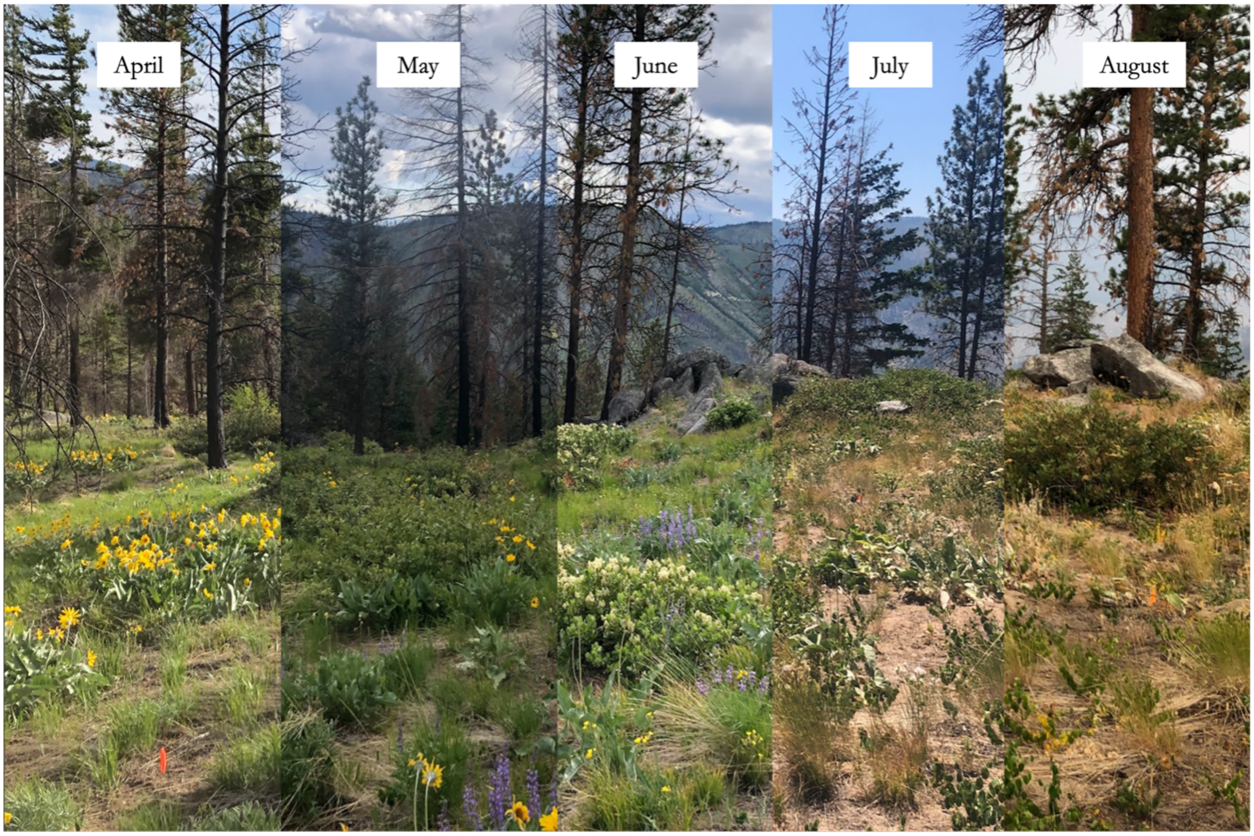
We established study areas within three fires that burned in eastern Washington, USA in 2015, 2018, and 2021 (Figure 1). The Twentyfive Mile Creek fire burned 8,990 ha approximately 40 km west of Chelan, Washington in August of 2021. In July of 2018, lightning sparked the Cougar Creek fire, located 32 km northwest of Entiat, Washington, and burned 16,760 ha. The Chelan

Figure 1.1. Locations of nine study plots within four study areas in the Okanogan-Wenatchee National Forest in Washington, USA. All study areas were established in Chelan County within the Chelan and Entiat US Forest Service Ranger Districts. Specimens were collected from three burned areas, and one "control" that was unburned since 1968. Fire severity is displayed as Composite Burn Index (CBI), and all study areas were burned by mixed-severity fire effects. Each 10×10-m pixel was categorized as unchanged (0-0.1), low (0.101-1.24), moderate (1.241-2.24), or high (2.241-3) severity.



Complex fire burned 22,055 ha along the south shore of Lake Chelan in August of 2015. All three fires burned with mixed severity fire effects. In addition to these three fires, we established a fourth study area in which fire has been suppressed since 1968 as an "unburned control" for comparative purposes. All study areas were in Chelan County, Washington, and on land within the Okanogan-Wenatchee National Forest. In all selected study areas, the dominant vegetation included ponderosa pine, Douglas-fir (*Pseudotsuga menziesii*), bearberry (*Arctostaphylos uva-ursi*), wax currant (*Ribes cereum*), snowbrush ceanothus (*Ceanothus velutinus*), and arrowleaf balsamroot (*Balsamorhiza sagittata*) (Figure 2).

Figure 1.2. Temporal variation in the plant community within the Cougar Creek fire. This study area had a burn age of three years when this series of photographs was taken in 2021. Across all sites, floral resources and water availability decrease throughout the season. In August 2021, air quality also decreased due to smoke from the nearby Twentyfive Mile Creek Fire, which we began sampling in April 2022. Dominant flowering plants included *Balsamorhiza sagittata*, *Lupinus* spp., and *Ceanothus velutinus*.



Field Methods

Within each study area, sites were selected based on accessibility, the presence of understory angiosperm species, and south-facing aspects to ensure consistency among fire and habitat types. The selected fires were similar in size, and had mixed soil burn severity. Additional selection criteria included public ownership within the burn and adequate distance from amenities that would indicate high levels of human visitation and potentially disrupt data collection. For each post-burn study site and the unburned control, with the exception of sites within the Twentyfive Mile Creek fire, we installed and sampled two replicated plots in 2021, 2022, and 2023 every two weeks between April and August. In 2022, following the Twentyfive Mile Creek fire, we established three replicated plots for sampling in 2022 and 2023, also every two weeks between April and August. At all study areas, plots were ≥ 600m apart to limit the effect of spatial autocorrelation in data analyses and to account for the average foraging range of wild bee species (Gathmann and Tscharntke 2002). The timing of the first and final sampling intervals for all years was influenced by study area accessibility (e.g., snow melt for the first interval, wildfire warnings for the final interval); thus, we sampled a total of seven, eight, and nine times in 2021, 2022, and 2023, respectively.

In each plot, we deployed two sets of pan traps and two blue vane traps to ensure the capture of a diverse pollinator community (Campbell and Hanula 2007, Gezon et al. 2015, Packer and Darla-West 2021). Traps were placed approximately 0.25–0.5-m above the soil surface to mirror average floral height and deployed for 24–36 hours at each sampling interval. Each pan trap contained two white, two fluorescent blue, and two fluorescent yellow cups, each with a capacity of ~96 mL. Pan and blue vane traps were filled with a solution of water and soap to ensure effective capture, avoid data loss due to predation, and minimize harm to wildlife. Initial blue vane trap deployment was delayed by ~2 weeks to avoid collection of *Bombus* queens (Kimoto et al. 2012).

Processing of Field-Collected Data

Upon collection of field data, samples were labeled with the collection date, study site, and plot number. Pan and blue vane trap specimens were stored separately at 0°C. Trap contents were first separated by taxonomic order and stored in vials containing 70% ethanol until identification. From trapping devices, native bee pollinators within Hymenoptera (Anthophila) were identified to species with taxonomic keys as outlined in Maust et al. (2025). When species level identifications could not be determined, a morphospecies group was assigned. A total 2,954 bees were analyzed in this study. Due to initial species identification errors, which were corrected on voucher specimens but could not be corrected in the data due to an inability to reassociate locality information, most specimens from 2021 and 2022 were identified to the genus level. Individuals that could not be reassociated with site at the genus level were excluded from subsequent analyses. These errors were corrected in 2023, for which most specimens were identified at the species level, and all were associated with a locality. Because data collected in 2023 had the highest robustness, quality, and quantity, it was analyzed separately to confirm patterns detected in the three-year dataset (2021-2023).

Beetles (Coleoptera), butterflies (Lepidoptera), and wasps (Hymenoptera) were identified to family. Coleopterans were identified using keys from Borror et. al (1989), Evans and Hogue (2006), and Marshall (2018). Lepidopterans were sorted using the taxonomic key by Miller and Hammond (2003). Wasps (Hymenoptera) were identified using Marshall 2023. Additionally, two pollinating families of Diptera (Bombyliidae and Syrphidae) were identified using Cole (1969).

Additional Field Variables

Burn severity and landscape characteristics are important factors to consider when conducting post-fire insect studies (Mason et. al 2021). We quantified burn severity using predicted Composite Burn Index (CBI) values generated in a 10×10-m scale with Google Earth Engine using methodology developed by Parks et al. (2019) (Figure 1). The CBI is a standardized method for assessing burn severity in forests that combines ecologically relevant variables across five horizontal strata into one numeric site index (Key and Benson 2006), allowing for a quantifiable and comparable measure of burn severity across study areas. Values of CBI range from 0-3, where higher values indicate increased burn severity and higher overstory tree mortality (Key and Benson 2006). Because mixed severity fires contain a range of spatially heterogenous fire effects (i.e. areas of minimal fire effects and areas of greater overstory tree mortality), we used the mean and standard deviation of CBI values within a 350-m radius of each plot center to represent the range of fire effects at each study area. Due to uncertainties in predicting on-the-ground fire effects, continuous values for CBI, which range from 0-3, were used as a measure for burn severity in statistical analyses. In addition, CBI values were binned into categories: unchanged (0-0.1); low (0.1-1.24); moderate (1.25-2.24), and high (2.25-3.0) (Miller and Thode 2007) for graphical purposes and indicator species analyses. All data processing was performed in ArcGIS Pro 3.3.1 (Environmental Systems Research Institute, Inc., Redlands, California).

Bee community metrics are known to decrease along an increasing elevational gradient (Smith et al. 2015). To account for this pattern, we used elevation values measured at a 30×30-m scale from the USGS Digital Elevation Model. We extracted values within a 350-m radius from plot center to estimate the mean plot elevation. However, because elevation was relatively consistent, with a range of 831–1156 meters above sea level across all plots, it was omitted from statistical analyses.

We measured the basal area (m^2 ha^{-1}) at each plot as an indicator of canopy openness. Basal area was measured for the three original study areas in 2021. The Twentyfive Mile Creek study area was quantified in 2022. All stems ≥ 5 cm diameter at standard height (~1.4 m from the ground) within a 50-m radius from plot center were measured and identified to species. In the same 50-m radius, we quantified potential nesting habitat as the number of snags and coarse woody debris present in each plot.

Temperature varies from year-to-year, likely affecting bee seasonality. Across the three-year study, we collected fewer bees when temperatures were <15°C, which corresponds to known thermal thresholds for bee activity (Oyen et al. 2016, Heinrich 1979). Specifically, average daily temperatures in 2022 were generally below 15°C until late June. Consequentially, fewer bees were captured between late April and late June of 2022 than in other sampling years (Supplemental Figure 1.1). We accounted for temperature effects on bee community dynamics by recording hourly temperatures in each year using one HOBO® Pro v2 device (Onset Computer Corp., Bourne, MA) per study site. Devices were deployed during the first sampling interval and removed at the last sampling interval. For each site and across all years, we averaged hourly temperatures to calculate the overall site mean and site standard deviation. We then calculated the standard normal deviate according to,

$$\frac{(t_i - T_i)}{SD_i}, \qquad \text{(Eq. 1)}$$

where t_i is the mean temperature at a site (i) for a given year, T_i is the three-year average temperature at a site (i) across all three years of data collection, and SD_i is the standard deviation of the three-year temperature data at a site (i). We used the standard normal deviate as a measure of annual variation in temperature for each site.

Statistical Analyses

Richness is a commonly used metric for quantifying community composition (Wilson 1990, Currie 1991, O'Brien 1998). To calculate genus richness, specimens were grouped by burn age at both the site and plot scale and summarized by the unique number of genera present. Non-native European honeybees (*Apis mellifera* Linnaeus) were omitted. Sampling effort is known to affect richness values (Chazdon et al. 1998, Endres et al. 2021). Genus richness was thus rarefied to the minimum common number of samples to address sampling imbalances across collection years using rarefy() in the R package vegan (Oksanen et al. 2025). For the 2023 dataset, when the Twentyfive Mile Creek, Cougar Creek, and Chelan Complex fires were 2, 5, and 8 years post fire, respectively, we considered unrarefied genus and species richness. All analyses were conducted in R version 4.4.2 (R Core Team, 2025). Figures were developed using the function ggplot() in the R package ggplot2 (Wickham et al. 2024).

Inter-community variation across burn age and burn severity

A generalized linear mixed model (GLMM; Type III Analysis of Variance using Satterthwaite's Method) was developed for the full dataset (2021-2023) using the rarefied plot scale genus richness values as the response variable. We included burn severity, burn age, and their interaction as fixed effects. The control study area was omitted from analyses because it had a burn severity of zero and no relevant burn age. Sampling year and the standard normal deviate of temperature (Eq. 1) were included as random effects. Rarefied data were checked for normality, and a GLMM was conducted using lme4 (Bates et al. 2025) and lmerTest (Kuznetsova et al. 2020).

Bee dissimilarity in relation to fire severity and burn age was calculated using the Bray-Curtis index. These sampling units were clustered and visualized graphically using nonmetric multidimensional scaling (NMDS; Minchin 1987). Ordination plots were generated using the metaMDS function in the R package vegan (Oksanen et al. 2025). The stress of the ordination was assessed against a threshold < 0.2.

An additional set of GLMMs were run for data collected in 2023 to compare the patterns detected in the three-year model to those of the higher quality one-year dataset. Both unrarefied genus richness and species richness at the plot scale were used as the response variables. Burn severity, burn age, and their interaction were considered as fixed effects. Both response variables were modeled with a generalized Poisson distribution to address under-dispersion (Consul and Famoye 2007) and analyzed in glmmTMB (Brooks et al. 2017, McGillycuddy et al. 2025).

Indicator Species Analysis

An Indicator Species Analysis (ISA) can be used to identify species that are strongly associated with a particular group with statistical significance assessed through permutation tests (Dufrêne & Legendre 1997). An ISA was conducted to measure associations among bee species and burn age. A community matrix was constructed from the 2023 dataset, when study areas were 2, 5, and 8 years post fire, or unburned since 1968, to determine if specific bee species were significantly associated ($p \leq 0.01$) with post-fire burn ages. A second ISA was conducted to determine if specific bee species were significantly associated with a burn severity category. Plots from the unburned study area were designated as a control category. Both tests were conducted with the classical parameters (duleg = TRUE) using the package indicspecies (De Cáceres et al. 2024).

Variation in above ground nesting bees

Bee nesting preferences are diverse and can vary among and within species (Danforth et al. 2019). To capture the diversity of native bee nesting behavior, nesting preferences were recorded in thirteen categories summarized in Supplemental Figure 1.2A and Supplemental Table 1.3. Select genera (namely *Lasioglossum*) have assumed or unknown nesting preferences that have not been observed or recorded and were categorized to reflect this uncertainty (i.e. assumed soil, assumed wood or cavity, unknown). To assess the effects of wildfire on bees by nesting guild, bee nesting preferences were subsequently binned as either nesting below ground (assumed soil, prefer soil, soil) or above ground (assumed wood, surface, stem, twig, wood). Bee nesting preferences within the Twentyfive Mile Creek fire were considered separately to determine the previously unrecorded effects of wildfire on above ground-nesting bee species 1-2 years after the disturbance (Supplemental Figure 1.2B). The proportion of all bees classified as above ground nesting species was calculated at the plot scale for each year of data collection. A value of 0.001 was added to all proportions to account for 0 data and normalized using ln(proportion/(1-proportion). Normalized proportions of above ground nesting species across all study areas and collection years were considered as the response variable. Sampling year was included as a random effect to account for annual variation among sites. We used a GLMM in the package lme4 (Bates et al. 2025) and included the main effects of available nesting habitat, basal area, CBI, and the interaction between CBI and nesting habitat. Evaluations for all GLMMs were conducted to ensure model assumptions were met using the DHARMa package (Hartig 2024).

1.4 Results

Across all sampling years, a total of 23,896 insects were collected. Of these, 5,676 were bees (~23.8% of all insects), of which 2,954 bees were analyzed in this study. The remaining 2,722 could not be reassociated with site at the genus level and were excluded. A complete record of all collected bees, including first records for Washington state and Chelan County, are documented in Maust et al. (2025). In total, our dataset for this study consisted of 169 bee species/morphospecies in 29 genera (Supplemental Table 1.1) and represent five of the six North American bee families;

Andrenidae (n=115), Apidae (n=394), Colletidae (n=16), Halictidae (n=1,886), and Megachilidae (n=543).

Other insect groups collected in blue vane and pan traps are summarized in Supplemental Table 1.2. A total of 21,266 Coleopterans from 12 families were collected. Notably, 84.5% of the insects collected were soft-winged flower beetles in the family Melyridae (n = 20,185). Additionally, 648 wasps from more than eight families were collected, the majority of which were pollinating, thread-waisted wasps in Sphecidae (n=302). Bee flies (Bombyliidae) and hoverflies (Syrphidae) comprised ~7.3% percent (n=223) of the total Dipterans (n=1,629). Finally, 92 butterflies and moths (Lepidoptera) from more than four families were identified.

Among all sampled sites, bee genus richness was highest two years post fire (24 genera) and lowest four years post fire (10 genera). Across all years, the average plot-scale genus richness value was ~15 genera. Site level genus richness 5- and 8-years post-fire (19) was comparable to the unburned control (22; Figure 3). Most bee genera (Supplemental Figure 1.3) and bee species (Supplemental Figure 1.4) were collected between late May and early July across all burn ages.

Figure 1.3. Bee genus richness across burn ages. Plot level boxplots of genus richness across burn ages. Genus richness at the site level is shown as a gray line. Among all plots, bee genus richness was highest at two years post fire (24 genera) and lowest at four years post fire (10 genera). Across all years, the average plot level genus richness value was ~15 genera.

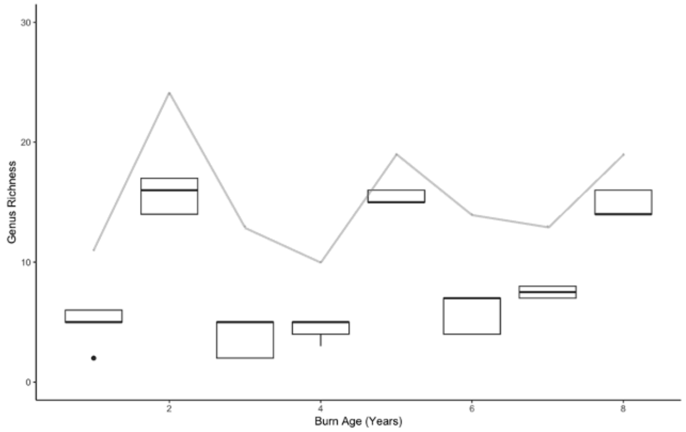
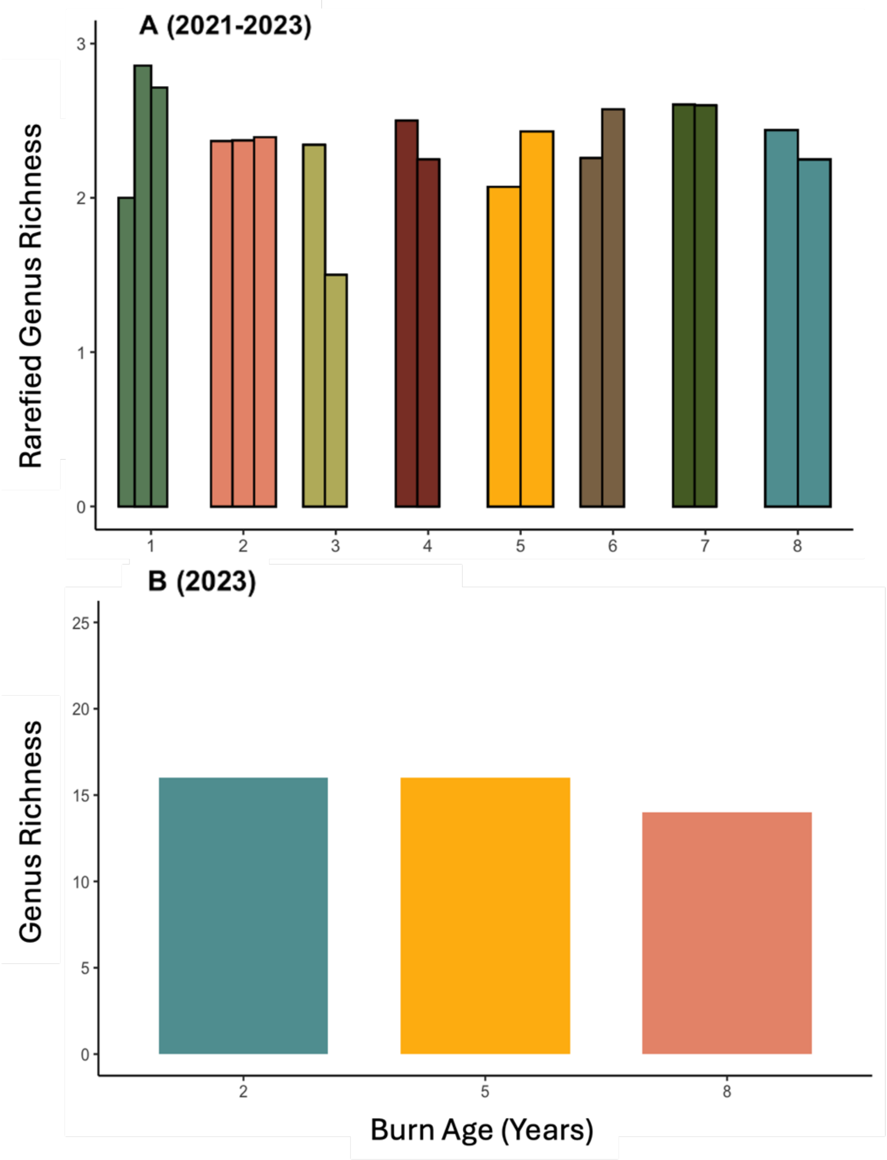


Figure 1.4. Bee genus richness decreases with increasing burn age. A slightly negative relationship can be observed among bee genus richness and burn age at the plot level in both the three-year (2021-2023; A) and one-year (2023; B) datasets.

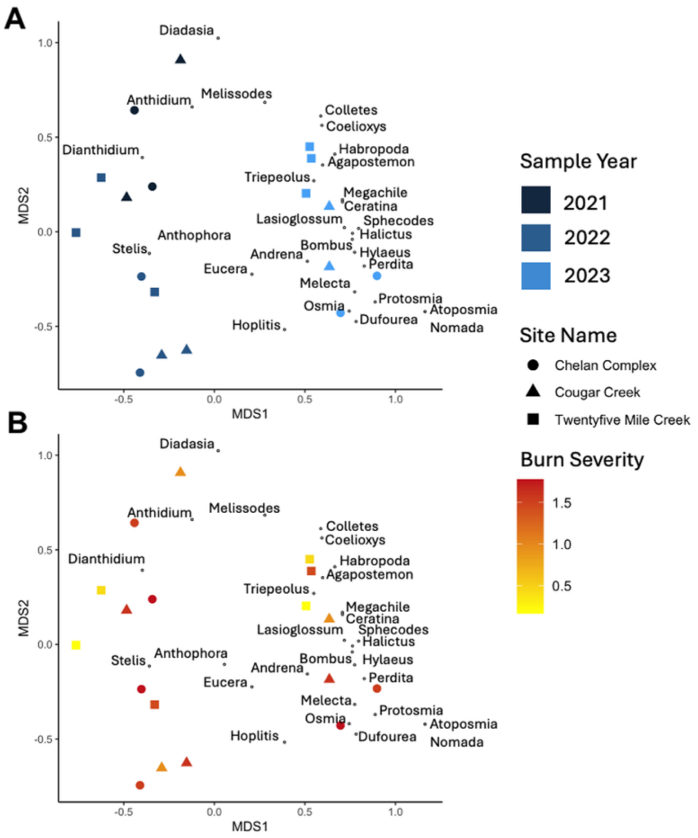


Inter-community variation across burn age and burn severity

We used a GLMM with the main effects of burn severity, burn age, and their interaction as fixed effects on bee genus richness sampled in 2021-2023 with year and the standard normal deviate of temperature included as random effects. At the plot scale, burn age ($p = 0.012$, $F = 15.7$, Figure 4A), burn severity ($p = 0.\ 003$, $F = 8.9$, Figure 1.5B), and their interaction ($p < 0.001$, $F = 183.9$)

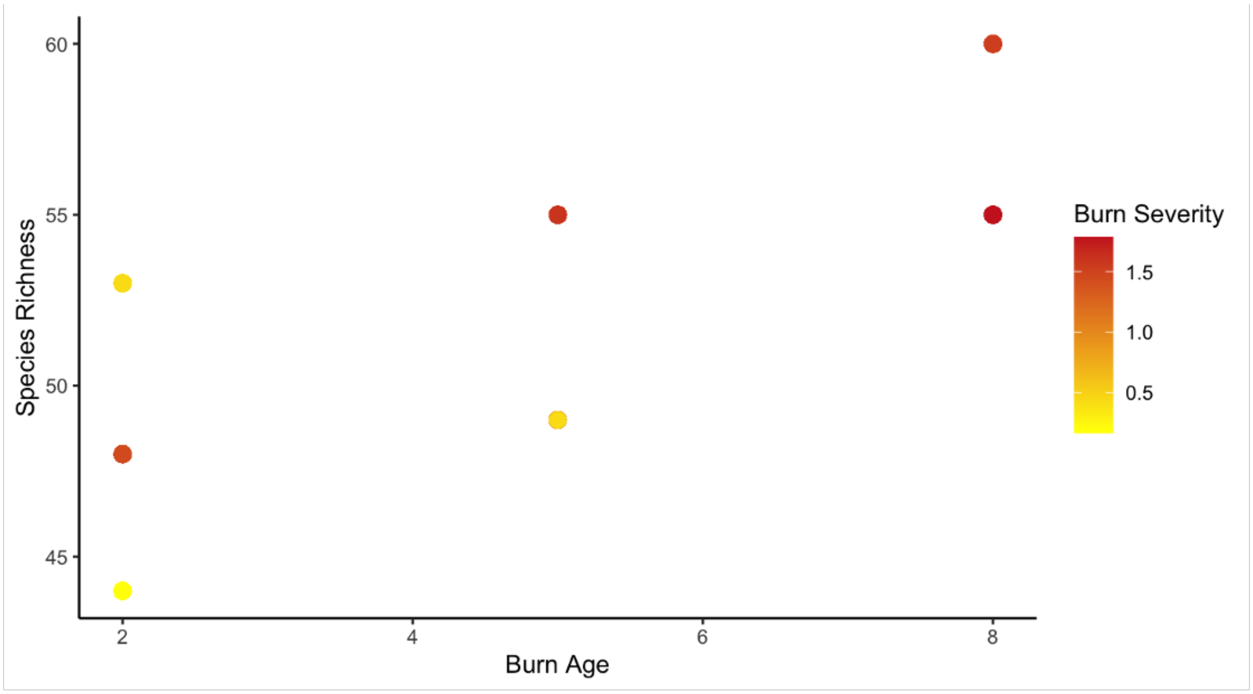
were significant drivers of genus richness. Local, plot scale genus richness was highest one-year post-fire (Figure 1.4A). NMDS was used to graphically demonstrate the dissimilarity between variables in three dimensions ($R^2 = 0.988$, stress value = 0.11). Data visualization with NMDS ordination suggests that the positive effects of increasing fire severity on bee genus richness are subtle, with small but significant increases in genus richness observed (Figure 1.5B). Plots that burned with low severity fire effects tended to be associated with fewer genera. Moreover, the significant interaction effect between burn age and burn severity detected in the GLMM was likely due to the higher number of bee genera identified in 2023 relative to 2021 and 2022, underscoring the importance of rarefaction in the three-year dataset to minimize the effects of imbalanced sampling (Figure 1.5A).

Figure 1.5. NMDS of the bee community matrix. Nonmetric multidimensional scaling (NMDS) of bee genera (small open circles) by sample year (A) and burn severity (B). Site name reflects burn age (Chelan Complex, 4-6 years; Cougar Creek, 2-5 years; Twentyfive Mile Creek, 1-2 years.



Generalized linear mixed models of the 2023 data revealed similar patterns to values across all three years (Figure 1.5A). Specifically, plot level genus richness increased with increasing burn severity ($p < 0.001$, z value = 6.6, Figure 1.5B), and decreased with burn age ($p = 0.03$, z value = -2.1, Figure 4B). Furthermore, the interaction of burn age and burn severity significantly affected plot level genus richness ($p = 0.002$, z value = -3.0). Contrarily, plot level species richness increased with burn age ($p = 0.0212$, z value = -2.3), burn severity ($p = 0.0465$, z value = -2.0), and the interaction between burn severity and burn age ($p < 0.001$, z value = 10.1, Figure 1.6).

Figure 1.6. Bee species richness increases with burn age and burn severity. Species richness was calculated for data collected in 2023 when the Twentyfive Mile Creek, Cougar Creek, and Chelan Complex fires were 2, 5, and 8 years post fire, respectively. Bee species richness was positively associated with burn age and severity.



Indicator Species Analysis

Approximately 22.2% (32/144) of the bee species collected were significantly associated with burn age based upon permutation tests (Table 1.1). Higher burn ages generally had fewer associated species. Additionally, 13.9% (20/144) of bee species were significantly associated with a specific burn severity category (Table 1.2). Plots that were burned at higher severities had more associated above ground nesting species, including bee species in *Osmia* (Cane et al. 2007) and *Lasioglossum nigroviride* Graenicher*,* which was the only known above-ground nesting *Lasioglossum* species collected in this study (Gibbs et al. 2017).

Table 1.1. Bee species collected in 2023 that were significantly associated ($p \leq 0.01$) with a burn age, which collectively comprised ~22.2% of the total number of bee species collected.

Burn Age Site	Associated Species	Test Statistic	P value
2 Twentyfive Mile Creek	*Bombus vancouverensis*	0.102	0.025
	Bombus vosnesenskii	0.127	0.005
	Melissodes agilis	0.068	0.015
	Melissodes microstictus	0.088	0.005
	Agapostemon subtilior	0.380	0.005
	Lasioglossum anhypops	0.135	0.005
	Lasioglossum knereri	0.111	0.005
	Lasioglossum nevadense	0.231	0.005
	Lasioglossum reasbeckae	0.097	0.010
	Dianthidium subparvum	0.112	0.005
	Megachile montivaga	0.068	0.035
5 Cougar Creek	*Bombus vandykei*	0.094	0.015
	Halictus farinosus	0.397	0.005
	Lasioglossum cf. *cooleyi*	0.216	0.005
	Lasioglossum helianthi	0.068	0.035
	Lasioglossum mellipes	0.090	0.010
	Osmia californica	0.175	0.005
8 Chelan Complex	*Andrena melanochroa*	0.062	0.050
	Bombus centralis	0.186	0.005
	Melecta pacifica	0.078	0.045
	Halictus confusus	0.081	0.010
	Lasioglossum glabriventre	0.154	0.005
	Lasioglossum sandhousiellum	0.091	0.015
	Osmia albolateralis	0.124	0.005
	Osmia exigua	0.076	0.005
	Osmia montana montana	0.389	0.005
unburned Unburned Control	*Perdita* aff. *tortifoliae*	0.074	0.025
	Perdita wyomingensis sculleni	0.083	0.005
	Bombus fervidus	0.157	0.005
	Halictus tripartitus	0.519	0.005
	Hoplitis albifrons	0.107	0.035
	Megachile brevis	0.083	0.015

Table 1.2. Bee species collected in 2023 that were significantly associated ($p \leq 0.01$) with a burn severity, which collectively comprised ~12.9% of the total number of bee species collected. Burn severity was binned into categories according to Miller and Thode (2007).

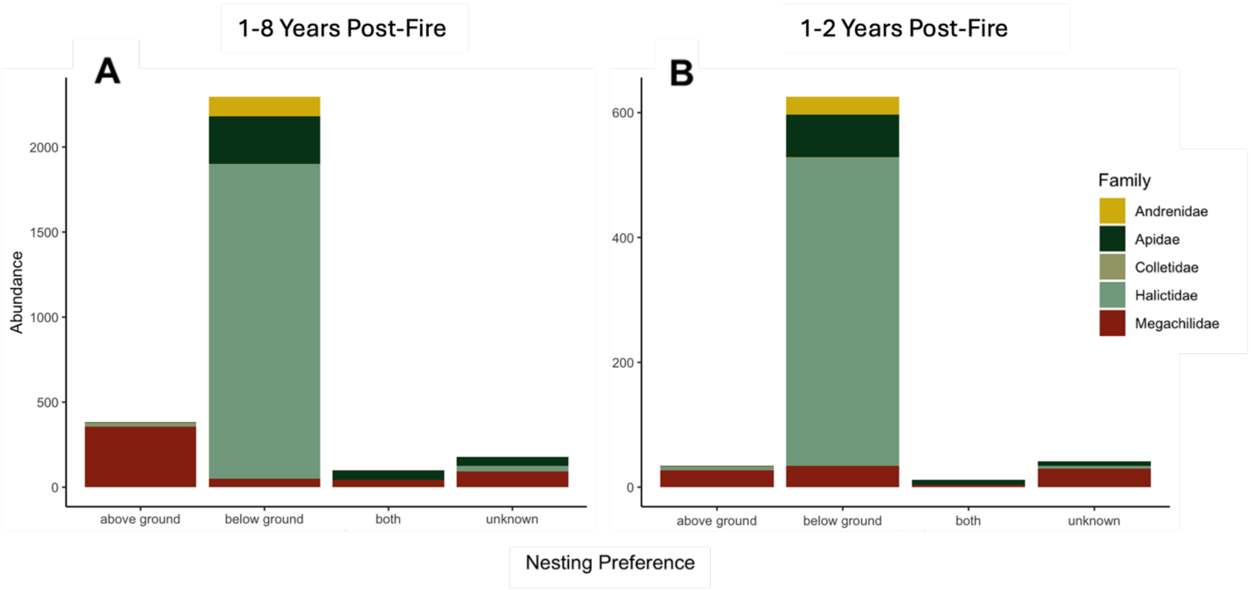
Burn Severity Category	Associated Species	Statistic	p value
Low (n=7) CBI=0.1-1.24	*Bombus vosnesenskii*	0.115	0.005
	Agapostemon subtilior	0.329	0.005
	Halictus farinosus	0.286	0.005
	Lasioglossum mellipes	0.101	0.005
	Lasioglossum nevadense	0.265	0.005
	Lasioglossum reasbeckae	0.086	0.010
	Megachile perihirta	0.089	0.030
Moderate (n=6) CBI=1.25-2.24	*Lasioglossum glabriventre*	0.156	0.005
	Lasioglossum nigroviride	0.064	0.035
	Lasioglossum prasinogaster	0.071	0.030
	Osmia albolateralis	0.114	0.005
	Osmia californica	0.213	0.005
	Osmia montana montana	0.322	0.005
Control (n=7) CBI = 0	*Perdita* aff. *tortifoliae*	0.081	0.010
	Perdita wyomingensis sculleni	0.083	0.005
	Bombus centralis	0.141	0.010
	Bombus fervidus	0.172	0.005
	Halictus tripartitus	0.554	0.005
	Hoplitis albifrons	0.124	0.010
	Megachile brevis	0.083	0.010

Variation in above ground nesting bees

Of the bees collected (n=2,954) across all years and sites, 2,294 nest below ground, 383 nest above ground, 98 nest variably, and 179 had a nesting preference that was unknown (Figure 1.7A). We also assessed data from the Twentyfive Mile Creek separately to determine the immediate fire effects in sites with a burn age of 1-2 years. In this subset, above ground nesting behavior occurred at a significantly lower frequency when compared to burn ages from 1-8 years ($\chi^2 = 39.627$, $p = <0.001$, Figure 1.7B).

Approximately 63.8% of the bees collected across all years were in the genera *Halictus* and *Lasioglossum* (Hymenoptera: Halictidae). *Halictus* is known to be exclusively ground nesting (Carril and Wilson 2023), while the more speciose and understudied genus *Lasioglossum* is assumed to be

Figure 1.7. Binned bee nesting preference by family. Abundance of bee species by simplified nesting preferences summarized across burn ages 1-8, and the control site that is unburned since 1968 (A), and in young burns 1-2 years post-fire (B). Although most bees were below ground nesting species, we did observe an immediate decrease in above ground nesting species 1-2 year post fire.



ground nesting, likely with exceptions (e.g. the aforementioned *Lasioglossum nigroviride*, Gibbs et al. 2017). The mean proportion of above-ground nesting bees found through time was highest between 6 and 8 years post fire (0.35), and generally lower (~0.16) between 2 and 5 years post fire. We also observed more variation in the proportion of above-ground nesting bees, based on the interquartile range, 1-2 years post-fire (i.e., Twentyfive Mile Creek fire; Figure 8A). Overall, the there was a high proportion of above-ground nesting species found one year post fire, which decreased until 3-4 years post fire, and then increased to its highest levels 8 years post-fire (Figure 1.8B).

When using a GLMM to test the main effects of nesting habitat, stand basal area, burn severity, and the interaction between nesting habitat and burn severity, on the proportion of above ground nesting bees, we observed that increases in nesting habitat (p = <0.001, F statistic = 25.854) and burn severity (p = <0.001, F statistic = 136.507) increased the proportion of above ground nesting bees. Furthermore, plots with higher burn severities had more nesting habitat, and supported increased proportions of above ground nesting bee species (*p* = <0.001, F statistic = 36.7051; Figure 1.9). Not surprisingly, the mean stand basal area in unburned areas was approximately two times the stand basal area of burned plots. Within the burned plots, stand basal area was between 0.065 and 6.645-m^2 ha^{-1}, but did not significantly influence the proportion of above ground nesting bees in the landscape.

Figure 1.8. Temporal variation in bee nesting proportions. Proportion of above ground nesting bee species summarized by study areas (A). Twentyfive Mile Creek, Cougar Creek, and Chelan Complex had a burn age of 1-2, 3-5, and 6-8 years, respectively. The Control had not burned since 1968. The proportion of above ground nesting bee species summarized by burn age (B) in which there was a decrease in the proportion one year following fire, and did not increase until after ~4 years.

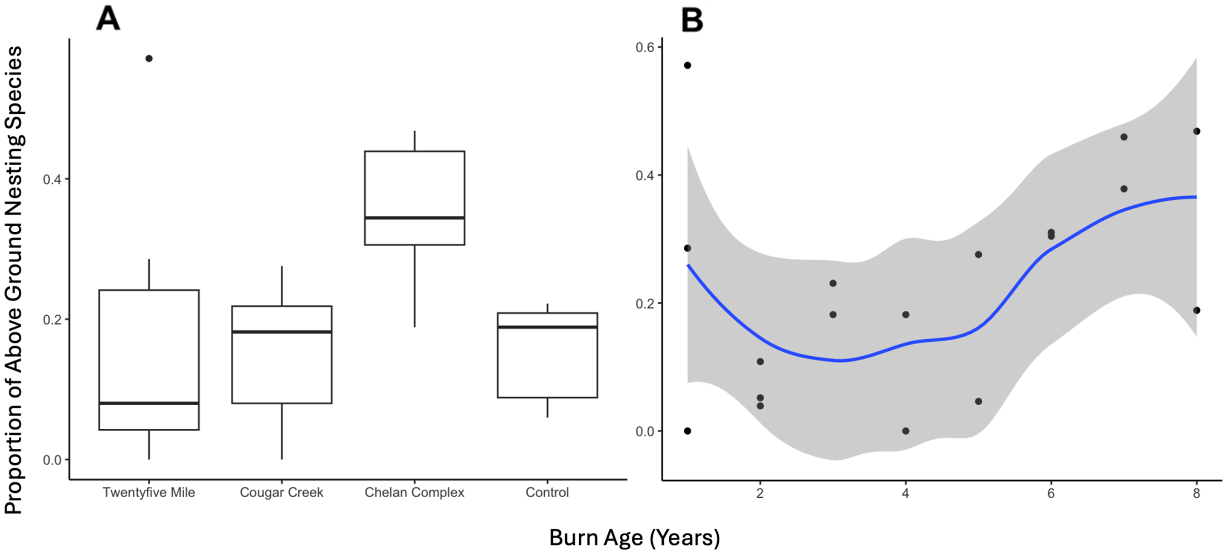
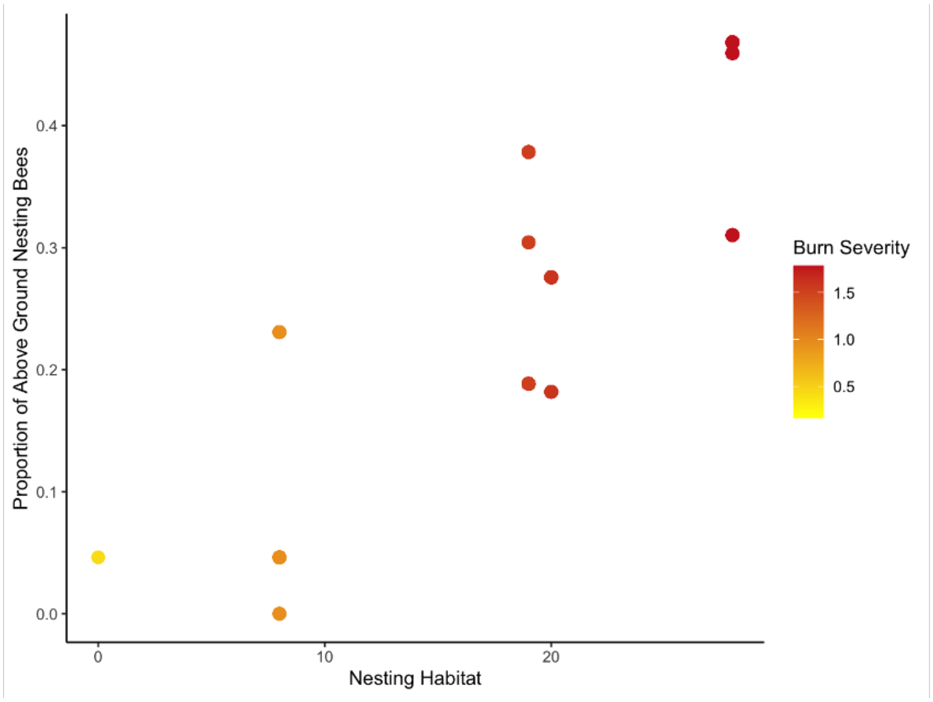


Figure 1.9. Bee nesting habitat and burn severity. Effects of nesting habitat availability and burn severity (CBI) on the proportion of above ground nesting bees.



1.5 Discussion

This study is the first to identify drivers of bee community variation in ponderosa pine forests following mixed severity wildfire, highlighting the importance of fire reintroduction for bee health protection in dry-forest ecosystems. Increased burn severity and low burn age maximizes bee genus richness in post-fire ponderosa pine systems. However, both burn age and burn severity were positively associated with bee species richness. Understanding the nuances of these drivers is critical for successful conservation of forest pollinators.

Bee community variation across burn age and burn severity

Our data support the hypothesis that burn age and burn severity are important drivers of inter-community bee variation. Analyses indicated that bee genus richness is highest in recently burned areas and decreases with time since burn (Figure 1.4A and 1.4B). Specifically, bee genus richness was highest in a landscape with a burn age of two years (Figure 1.3) and positively associated with burn severity (Figure 1.5B). Across the three-year study, we generally collected fewer bees when temperatures were <15°C (Supplemental Figure 1.1). Temperature was thus controlled for in the models as a random effect, and the three-year dataset was rarefied to account for climactic variation and sampling effort. Analysis with generalized linear mixed models revealed that bee species richness increased with both burn age and burn severity (Figure 1.6). These findings suggest that recently burned landscapes may support more bee genera, but fewer bee species, reflecting the immediate effects of increased floral abundance, light availability, and nesting habitat coupled with delays in bee recolonization in post-fire landscapes.

Indicator bee species

Understanding indicator species associations with post-fire landscape characteristics provides novel insights for bee conservation. As hypothesized, we observed that specific bee species were associated with specific burn severity categories (e.g., unchanged, low, moderate) and specific burn ages (e.g., 2, 5, or 8 years, or unburned). Study areas with more recent burn ages had more associated bee species, highlighting the importance of fire as a habitat-generating disturbance in ponderosa pine forests (Table 1.1). Furthermore, while the number of bee species significantly associated with a specific burn severity were generally uniform across burn severity category (Table 1.2), we did observe differences in nesting preferences. Most of the species associated with low severity and unburned landscapes were below-ground nesting bees in Andrenidae, Apidae, and Halictidae (Hymenoptera: Anthophila). By contrast, a greater proportion of bees collected in moderate burn study areas are above-ground nesting species including *Lasioglossum nigroviride* and three species in the genus *Osmia* (Megachilidae). We surmise that low severity fires might reduce the abundance of above-ground species due to direct mortality without necessarily providing the increase in above-ground nesting resources, such as hollow reeds, stems, and beetle galleries, that might occur in areas that experienced moderate severity fire effects. Increases in above-ground nesting resources would hypothetically support an increase in bee diversity, reinforcing the importance of fire as a disturbance agent in ponderosa pine forests.

Above-ground nesting habitat

Nesting above ground is a less common trait amongst forest pollinators when compared to soil nesting (Figure 1.7). We hypothesized that recently burned study areas may have the lowest proportion of above-ground nesting species due to the immediate displacement and mortality from fire. Indeed, a 1- and 2-year post fire landscape had a greater proportion of below-ground nesting bees (Figure 1.7B) compared to an average across all burn ages from 1-8 years (Figure 1.7A). This observation is consistent with a previous study that reported most below-ground nesting bee species were capable of surviving soil heating by fire, while megachilids (*Osmia, Megachile*), which generally nest in shallow soils or in above-ground nests, were more vulnerable (Cane and Neff 2011).

In ponderosa pine forests that have burned at low-moderate severity, the 1-2 years following mixed severity fire resulted in a decrease in above-ground nesting bee presence, with a gradual increase in abundance until 6-8 years post-fire (Figure 1.8B). Our findings suggest that the time needed for above-ground nesting bee recolonization in ponderosa pine systems is at least 4 years. Study areas that were four years post-fire had comparable proportions of above-ground nesting bee species to the control study area that had not burned since 1968 (Figure 1.8A). Interestingly, the Chelan Complex Fire, with a burn age of 6-8 years, had the highest average proportion of above-ground nesting species. This could be due to the high severity fire effects at this study area. Indeed, the Chelan Complex study area had the highest average burn severity, and consequently the highest average amount of above ground nesting habitat, which was furthermore linked to the highest proportion of above-ground nesting bees (Figure 1.9).

Similarly, the greatest variation in the proportion of above-ground nesting bees was among plots in the Twentyfive Mile Creek burn scar, which had a burn age of 1-2 years (Figure 1.8A). This can be attributed to the breadth of fire severities experienced by plots within this study area. For example, one plot within this study area had the lowest burn severity of any plot in the entire study (CBI = 0.1637), from which no above-ground nesting bees were observed. Another plot experienced some of highest severity fire effects (CBI = 1.4499), from which the highest proportion (0.43) of above-ground nesting bees, across all sites and years, were observed. Thus, the combination of low burn age and high burn severity supported the largest proportion of above ground nesting species at this plot (Figure 1.8B). Our findings support the previously recorded positive relationship between fire severity and above ground nesting bee reproduction in mixed-conifer forests (Galbraith et al. 2021).

Stand basal area (a proxy for stand density) did not affect the proportion of above ground nesting bees present on the landscape, likely because stand basal area was consistently low across all plots. Instead, available nesting habitat, burn severity, and their interaction all significantly influenced the proportion of above ground nesting bees across burned areas ages 1–8 years old (Figure 1.9). This observation suggests that, to promote above ground nesting bees during post-fire restoration and salvage logging, the incorporation of standing snags and coarse woody debris would increase the available nesting habitat for bees and other biodiversity (Riffell et al. 2011).

Along a gradient from 3 to 25 years post-burn, nesting success of wood-cavity-nesting bees in a lodgepole pine forest was highest immediately following high severity fire and decreased with time (Simanonok and Burkle 2019). Lodgepole pine systems are characterized by high-severity fire events and fire return intervals of 10-80 years (US Department of Agriculture, Forest Service, and Missoula Fire Sciences Laboratory 2012). By contrast, ponderosa pine systems generally have lower stand basal areas, less fuel, and experience mixed severity fire with a fire return interval of 5-25 years (Agee 1993). While the temporal patterns between the two systems differ, the overall trend of increased above ground nesting bee abundance with higher fire severities and lower burn ages is consistent. This variation in fire effects among fire regimes and forest types highlights the need for targeted sampling of bee communities across fire-prone ecosystems.

Caveats

We acknowledge that the additional 2,722 bees collected in 2021–2022 and excluded from analysis due to data loss have potential to alter the findings of this study. However, because bees were excluded randomly the majority of genera were still represented. Furthermore, the use of rarefaction amongst the data when using the three-year dataset accounted for sampling imbalances. Additionally, the 2023 data had no loss and largely supported our findings in the three-year dataset when analyzed separately, which suggests interesting patterns worthy of investigating in other burned forest landscapes.

Implications for conservation

Fire effects vary across forest type, burn severity, and burn age, with implications for the native bee community. Our findings highlight the need for targeted investigation of native bees across fire-prone forest systems. Furthermore, because pollinators are an essential component of ecosystem function, and declining bee populations are a global concern, understanding the effects of disturbance on bees is critical for successful conservation. Our findings suggest that the number of bee genera, bee species, and the available bee nesting habitat increases with burn severity in ponderosa pine forests. Furthermore, while low burn ages support more bee genera and higher burn ages support more bee species, not all species and genera conformed to this pattern. For example, some species are only found in recently burned sites, as exemplified by the eleven bee species significantly associated with a young burn age of 2 years (Table 1.1). Therefore, a patch mosaic of burn ages across will maximize bee community richness. Thus, the reintroduction of fire to ponderosa pine landscapes may simultaneously reduce wildfire risk while benefitting native bee populations.

Additional Information

Funding sources

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Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and should not be construed to represent any official USDA or U.S. Government determination or policy.

Data statement

All data that support the findings of this study are available in the main text and Appendix A. The data that support the findings of this study are openly available in UW Research Works at https://digital.lib.washington.edu/researchworks/. The complete collection data is deposited in ecdysis (https://ecdysis.org/).

Appendix A

Supplemental Table 1.1. Bee species analyzed in this study and their total abundance from data collection in 2021-2023. All physical specimens have been vouchered in the Washington State University M.T. James Entomological Collection (WSUC) in Pullman, Washington, USA. References for the generalized nesting preference of each bee species are indicated. When nesting preference or species-specific information is unknown, the nesting preference was listed as NA. Cleptoparasitic cuckoo bees were assigned the nesting preference of their known hosts.

Family	**Species**	**Total Abundance**	**Nesting Preference**	**Nesting Reference**
Andrenidae (n=115)	*Andrena angustitarsata*	1	below ground	Lebuhn 2013; Carril and Wilson 2023
	Andrena astragali	1		
	Andrena candida	1		
	Andrena candidiformis	2		
	Andrena lawrencei	3		
	Andrena melanochroa	3		
	Andrena microchlora	2		
	Andrena nigrocaerulea	4		
	Andrena nivalis	2		
	Andrena pallidifovea	1		
	Andrena prunorum	44		
	Andrena quintiliformis	3		
	Andrena salicifloris	2		
	Andrena sp. 1	1		
	Andrena sp. 2	1		
	Andrena spp.	6		
	Panurginus atriceps	1	below ground	
	Panurginus nigrellus	2		
	Panurginus spp.	15		
	Perdita aff. tortifoliae	9	below ground	
	Perdita aff. wyomingensis	2		
	Perdita nevadensis nevadensis	2		
	Perdita spp.	1		
	Perdita wyomingensis sculleni	6		
Apidae (n=394)	*Anthophora bomboides*	2	below ground	
	Anthophora porterae	11	below ground	
	Anthophora terminalis	1	above ground	
	Anthophora urbana	4	below ground	
	Bombus appositus	26	below ground	Williams et al. 2014

	Bombus centralis	92	below ground	Williams et al. 2014; Carril and Wilson 2023
	Bombus fervidus	50	both	
	Bombus insularis	3	both	Williams et al. 2014
	Bombus nevadensis	3	below ground	
	Bombus rufocinctus	2	both	Williams et al. 2014; Carril and Wilson 2023
	Bombus spp.	54	unknown	NA
	Bombus sylvicola	1	below ground	Williams et al. 2014
	Bombus vancouverensis	24	below ground	Carril and Wilson 2023
	Bombus vandykei	16	below ground	Williams et al. 2014
	Bombus vosnesenskii	31	below ground	Williams et al. 2014; Carril and Wilson 2023
	Ceratina nanula	2	twig	Carril and Wilson 2023
	Ceratina sequioae	3	above ground	
	Diadasia diminuta	1	below ground	Lebuhn 2013; Carril and Wilson 2023
	Diadasia nitidifrons	2		
	Eucera delphinii	7	below ground	
	Eucera edwardsii	28		
	Eucera fulvitarsis	1		
	Eucera spp.	2		
	Habropoda cineraria	1	below ground	Carril and Wilson 2023
	Habropoda morrisoni	1	below ground	
	Melecta pacifica	9	below ground	
	Melissodes agilis	3	below ground	Lebuhn 2013; Carril and Wilson 2023
	Melissodes lupinus	1		
	Melissodes microstictus	7		
	Melissodes nigracauda	3		
	Nomada spp.	2	below ground	
	Triepeolus sp. 1	1	below ground	Carril and Wilson 2023
Colletidae (n=16)	*Colletes fulgidus*	2	below ground	
	Hylaeus affinis	3	unknown, prefer above ground	Lebuhn 2013; Carril and Wilson 2023
	Hylaeus ?conspicuus	4		
	Hylaeus spp.	2		
	Hylaeus wootoni	5		
Halictidae (n=1,886)	*Agapostemon femoratus*	5	below ground	
	Agapostemon subtilior	255		

	Agapostemon texanus	95		
	Agapostemon virescens	1		
	Dufourea dilatipes	5	below ground	Lebuhn 2013
	Halictus confusus	6	below ground	Lebuhn 2013; Carril and Wilson 2023
	Halictus farinosus	400		
	Halictus ligatus	2		
	Halictus rubicundus	5		
	Halictus spp.	4		
	Halictus tripartitus	457		
	Halictus virgatellus	1		
	Lasioglossum albohirtum	3	below ground	Carril and Wilson 2023*
	Lasioglossum anhypops	28	below ground	
	Lasioglossum aspilurus	3	both, prefer below ground	
	Lasioglossum athabascense	7	below ground	
	Lasioglossum buccale	10	below ground	
	Lasioglossum cf. cooleyi	53	below ground	
	Lasioglossum cf. ruficorne	3	below ground	
	Lasioglossum egregium	6	below ground	
	Lasioglossum foxii grp.	2	below ground	
	Lasioglossum fratellum grp.	2	unknown	NA
	Lasioglossum glabriventre	59	below ground	Carril and Wilson 2023*
	Lasioglossum helianthi	3	below ground	
	Lasioglossum incompletum	2	below ground	
	Lasioglossum inconditum	1	below ground	
	Lasioglossum knereri	20	below ground	
	Lasioglossum macroprosopum	22	below ground	
	Lasioglossum marinense	1	below ground	
	Lasioglossum mellipes	15	below ground	
	Lasioglossum nevadense	235	below ground	
	Lasioglossum nigroviride	6	above ground*	Gibbs et al. 2017
	Lasioglossum ovaliceps	1	below ground	Carril and Wilson 2023*
	Lasioglossum pacificum	2	below ground	
	Lasioglossum prasinogaster	7	below ground	
	Lasioglossum pruinosum	2	below ground	
	Lasioglossum punctatoventre	27	below ground	
	Lasioglossum reasbeckae	6	below ground	
	Lasioglossum ruidosense	5	below ground	

	Lasioglossum sandhousiellum	9	below ground	
	Lasioglossum sedi	10	below ground	
	Lasioglossum sisymbrii	23	below ground	
	Lasioglossum (*Sphecodogastra*) sp. 1	5	below ground	
	Lasioglossum (*Sphecodogastra*) sp. 2	11	below ground	
	Lasioglossum (*Sphecodogastra*) sp. 5	8	below ground	
	Lasioglossum (*Sphecodogastra*) sp. 6	2	below ground	
	Lasioglossum spp.	31	unknown	NA
	Lasioglossum trizonatum	13	below ground	Carril and Wilson 2023*
	Sphecodes sp. 1	1	unknown, likely below ground	Carril and Wilson 2023
	Sphecodes sp. 2	3		
	Sphecodes sp. 3	1		
	Sphecodes spp.	2		
Megachilidae (n=543)	*Anthidium banningense*	5	unknown	Lebuhn 2013; Carril and Wilson 2023
	Anthidium formosum	3	unknown	
	Anthidium mormonum	2	above ground	
	Anthidium utahense	2	both	Jaycox 1966; Lebuhn 2013; Carril and Wilson 2023
	Atoposmia abjecta abjecta	1	unknown	NA
	Coelioxys funeraria	1	above ground	Baker 1975
	Dianthidium cressonii	35	both	Carril and Wilson 2023
	Dianthidium heterulkei	3	unknown	NA
	Dianthidium pudicum	4	above ground	Lebuhn 2013; Carril and Wilson 2023
	Dianthidium singulare	1	above ground	Grigarick and Stange 1968
	Dianthidium subparvum	18	unknown	Lebuhn 2013; Carril and Wilson 2023
	Hoplitis albifrons	59	above ground	Lebuhn 2013; Carril and Wilson 2023
	Hoplitis fulgida fulgida	3	above ground	
	Hoplitis hypocrita	1	unknown	
	Hoplitis producta	1	above ground	
	Hoplitis sambuci	2	unknown	
	Megachile apicalis	1	above ground	Trostle and Torchio 1994; Barthell et al. 1998
	Megachile brevis	5	both	

	Megachile ?coquilletti	1	unknown, likely above ground	Carril and Wilson 2023
	Megachile montivaga	3	both, prefers below ground	
	Megachile ?onobrychidis	1	probably below ground	Discover Life 2025
	Megachile pascoensis	6	unknown	Lebuhn 2013; Carril and Wilson 2023
	Megachile perihirta	43	below ground	
	Megachile pugnata	3	above ground	
	Megachile spp.	9	unknown	NA
	Megachile subnigra	2	probably below ground	Lebuhn 2013; Carril and Wilson 2023; Discover Life 2025
	Megachile wheeleri	1	below ground	Gordon 2000; Carril and Wilson 2023
	Osmia albolateralis	27	above ground	Cane et al. 2007
	Osmia atrocyanea	54	above ground	
	Osmia brevis	1	unknown	NA
	Osmia bruneri	1	above ground	Cane et al. 2007
	Osmia bucephala	1	above ground	
	Osmia californica	59	above ground	
	Osmia calla	3	unknown	NA
	Osmia cara	5	unknown	
	Osmia (*Cephalosmia*) spp.	1	unknown	
	Osmia cobaltina	4	above ground	Cane et al. 2007
	Osmia coloradensis	1	above ground	
	Osmia cyanella	1	above ground	
	Osmia cyaneonitens	1	unknown	NA
	Osmia densa	6	above ground	Cane et al. 2007
	Osmia exigua	9	above ground	
	Osmia juxta	7	unknown	NA
	Osmia kincaidii	1	above ground	Cane et al. 2007; personal observation
	Osmia marginipennis	3	above ground	Cane et al. 2007
	Osmia (*Melanosmia*) spp.	5	unknown	NA
	Osmia montana montana	111	above ground	Cane et al. 2007
	Osmia nemoris	1	both	
	Osmia ?nifoata	1	below ground	

	Osmia ?odontogaster	1	unknown	NA
	Osmia simillima	1	above ground	Cane et al. 2007
	Osmia spp.	8	unknown	NA
	Osmia trevoris	6	unknown	
	Protosmia rubifloris	3	above ground	Carril and Wilson 2023
	Stelis heronae	2	unknown	NA
	Stelis montana	3		

* Most bees in the genus *Lassioglossum* are assumed to be ground-nesting bees. However the nesting behavior of *Lasioglossum* is understudied, and there are likely exceptions (see *L. nigroviride*).

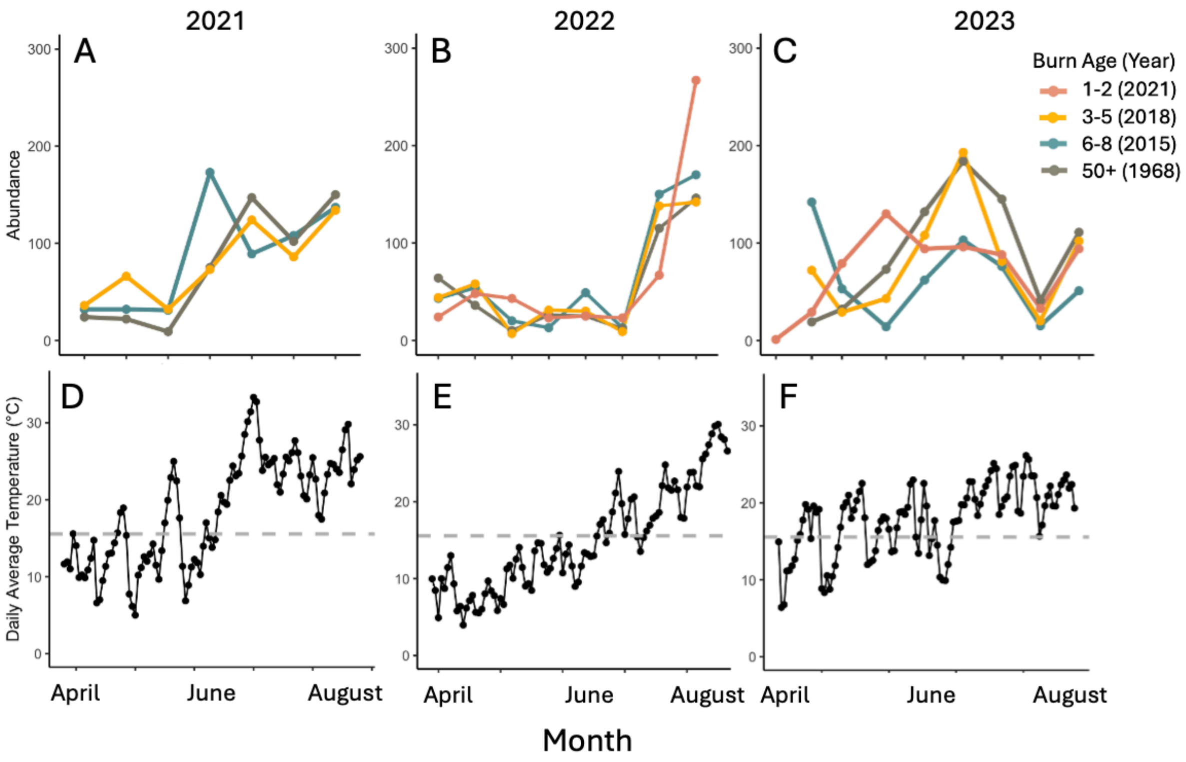
Supplemental Table 1.2. Abundance of Hymenoptera (non-Anthophila), Coleoptera, Diptera, and Lepidoptera collected in blue vane and pan traps. All specimens have been vouchered at the University of Washington, Seattle, WA. Sites were sampled a total of seven, eight, and nine times in 2021, 2022, and 2023, respectively. Abundance values are not standardized for sampling effort.

Order	Family	Number of Individuals Collected			Total
		2021	2022	2023	
Hymenoptera (n = 648 individuals)	Chrysididae	1	15	31	47
	Crabronidae	6	5	19	30
	Formicidae	0	0	2	2
	Ichneumonidae	0	0	1	1
	Pompilidae	0	0	4	4
	Sphecidae	25	114	163	302
	Tiphiidae	15	0	2	17
	Vespidae	35	151	58	244
	Unidentified	1	0	0	1
Coleoptera (n=21,541 individuals)	Buprestidae	139	206	196	541
	Cerambycidae	31	36	28	95
	Chrysomelidae	28	56	101	185
	Cleridae	28	31	123	182
	Coccinellidae	8	11	10	29
	Curculionidae	7	7	13	27
	Elateridae	2	2	6	10
	Meloidae	2	5	5	12
	Melyridae	3,381	3,344	13,460	20,185
	Mordellidae	58	48	126	232
	Scarabaeidae	1	10	4	15
	Stenotrachelidae	6	4	4	14
Diptera (n=1629 individuals)	Bombyliidae	9	16	16	41
	Syrphidae	55	32	95	182
	Other Dipterans	293	492	621	1406
Lepidoptera (n=92 individuals)	Hesperiidae	7	1	10	18
	Lycinidae	10	11	17	38
	Nymphalidae	0	1	0	1
	Papilionidae	0	6	6	12
	Other Lepidoptera	2	3	18	23

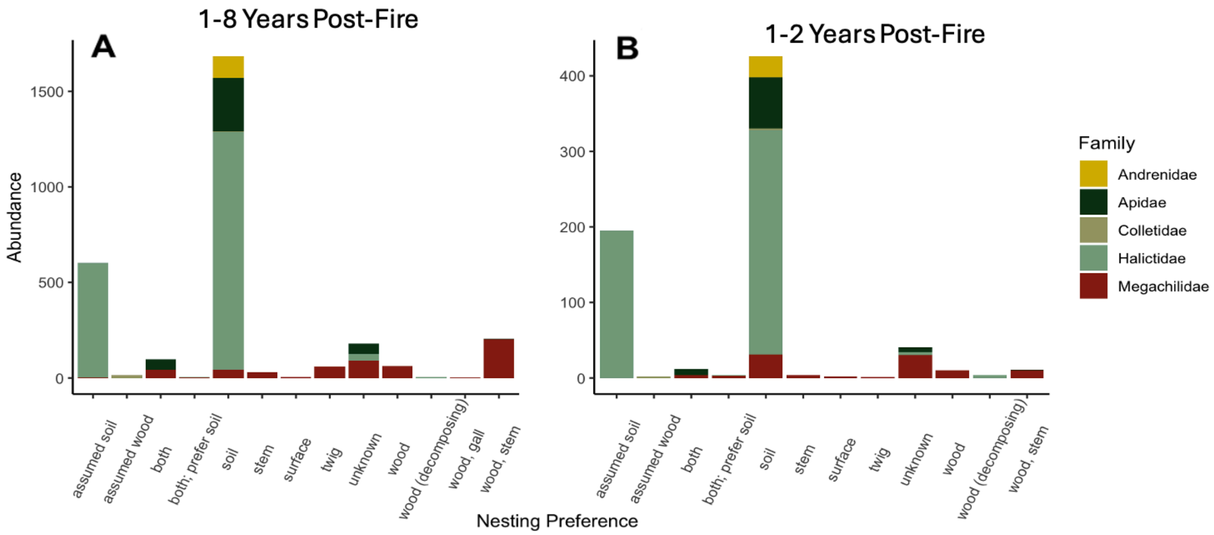
Supplemental Table 1.3. Bee abundance summarized by nesting preference.

Nesting Preference	Categorized Nesting Preference	Abundance
Assumed soil	Below ground	604
Assumed wood or cavity	Above ground	14
Both below and above ground	Both	98
Both; prefer soil	Below ground	6
Soil	Below ground	1684
Stem	Above ground	31
Surface	Above ground	4
Twig	Above ground	59
Unknown	Unknown	179
Wood	Above ground	61
Rotten wood	Above ground	6
Wood, gall	Above ground	1
Wood, stem	Above ground	207

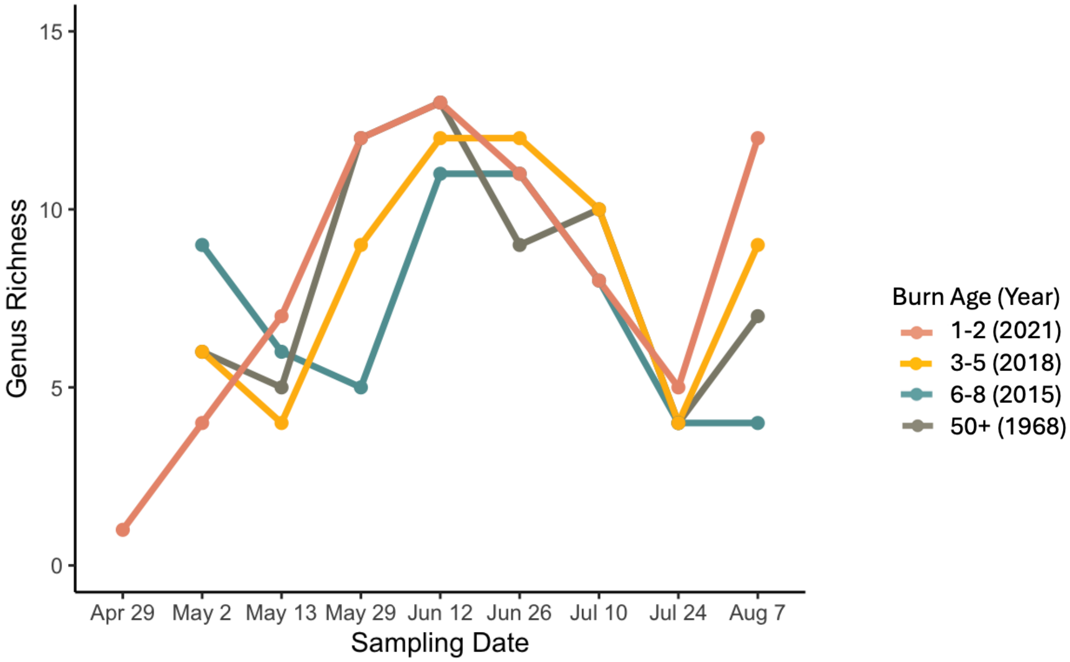
Supplemental Figure 1.1. Variation in annual bee abundance (A-C) is correlated with average daily temperature (D-F). Temperature (D-F) was calculated as an average across all study areas using localized temperature probes. The gray dashed line indicates a temperature threshold (~15 °C; Heinrich 1979, Oyen et al 2016) above which most bees are active. Average daily temperatures in 2022 were generally below the threshold until late June. Consequentially, fewer bees were captured between late April and late June of 2022 than in other sampling years. Annual variation in temperature was quantified as the annual deviation from the site specific three-year average.



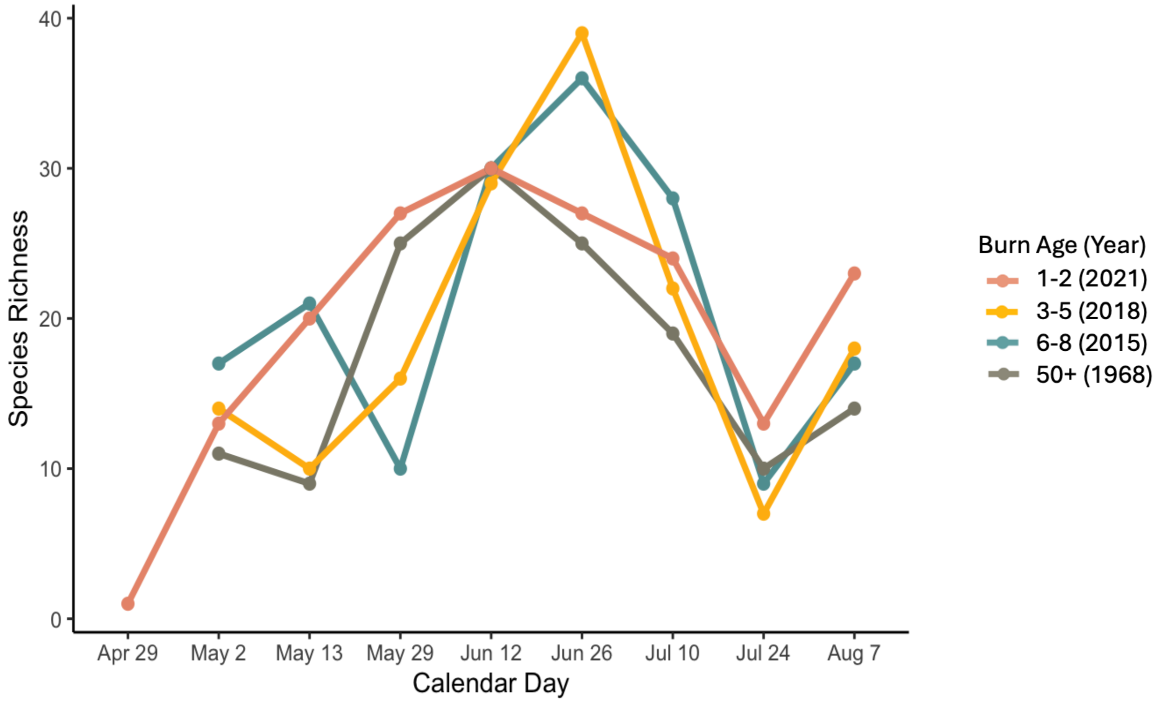
Supplemental Figure 1.2. Bee abundance and nesting preference. Across all temporal scales, most bees are below ground nesting. The number of above ground nesting bees was higher for all study areas (1-8 years post-fire; A) than for sites with burn ages of 1-2 years (B).



Supplemental Figure 1.3. Genus richness across all study areas in 2023. Study areas are indicated by color: Twentyfive Mile Creek (pink), Cougar Creek (yellow), Chelan Complex (blue), and Control (gray) which were 2, 5, 8, and >53 years post-fire, respectively. Genus richness was greatest between late May and early July, which aligns with known temporal bee distributions.



Supplemental Figure 1.4. Species richness across all study areas in 2023. Study areas are indicated by color: Twentyfive Mile Creek (pink), Cougar Creek (yellow), Chelan Complex (blue), and Control (gray) which were 2, 5, 8, and >53 years post-fire, respectively. Species richness was greatest between late May and early July, which aligns with known known temporal bee distributions.



Chapter 2. DNA metabarcoding reveals herbaceous perennials are key floral resources for native pollinators in post-fire landscapes

2.1 Abstract

Wildfires are a natural disturbance in dry ponderosa pine forest ecosystems and are increasing in frequency and severity across western North America. The effects of wildfire on biotic and abiotic conditions have important implications for higher trophic levels, including native bees. We sequenced pollen from pan and blue vane traps deployed bimonthly in 2022 and 2023 to quantify shifts in foraging resources for pollinator communities in landscapes age 1-8 years post-fire, and in an unburned area. DNA metabarcoding of pollen suspended in blue vane and pan traps revealed herbaceous perennials such as *Achillea millefolium*, *Lupinus* spp., and *Phacelia hastata* to be important floral resources for post-fire pollinator communities. Burn age and burn severity were important drivers of plant-pollinator network richness, and plant species richness increased with burn age and burn severity. Bee species richness varied among burn severities, supporting management for patch mosaics and landscape heterogeneity as a fundamental principal of fire reintroduction. Our findings underscore the importance of bioinformatics threshold selection in plant list generation, and highlights the role of burn age, burn severity, and plant richness in effectively conserving pollinator species across fire-effected landscapes. This study is the first to use pollen suspended in trap solutions to quantify floral resources for insect pollinators and highlights the utility of trap byproducts to provide insightful ecological information. Understanding specific plant-pollinator relationships is critical for successful conservation of angiosperm flora and bee faunas.

2.2 Introduction

Among pollinators, bees (Hymenoptera: Anthophila) depend exclusively on pollen and nectar during their life cycle, preferring plants that provide both (Brown and Paxton 2009). They also adhere to flower constancy when foraging, yielding a higher probability of receiving pollen from the same species and ensuring successful pollination. Furthermore, bees have evolved morphological features, such as scopa and corbicula, that assist in the efficient collection and transport of pollen (Mader et al. 2011). The culmination of these factors makes bees the most important insect taxon in providing pollination services, and by extension an important taxon to consider when quantifying plant-pollinator relationships. Understanding these relationships is especially critical given global bee population declines due to urbanization, agriculture, and climate change (Buchmann and Nabhan 1996, Allen-Wardell et al. 1998, Cane and Tepedino 2001).

Pollen is an essential resource for insect pollinators (Brown and Paxton 2009, Goulson et al. 2015), and is selected based on nutritional content, protein quality, color, and odor (Kunze and Gumbert 2001, Kitaoka and Nieh 2009). These preferences vary among bee species, and both pollen quality and quantity have direct effects on bee fitness and resilience to disturbance (Di Pasquale et al. 2013, Dharampal et al. 2019, Westreich et al. 2023a).

Though the immediate effects of fire on pollinator communities are complex, bees generally benefit from fire (Waltz and Covington 2004, Potts et al. 2006, Cane and Neff 2011, Rivers et al. 2018), likely due to a concomitant increase in light availability and floral resources (Nyoka 2010). However, the relationship between bees and angiosperms is too complex for broad generalizations. While most North American bees are floral generalists, many have floral preferences. For example, several bee species are oligolectic and prefer pollen from a single angiosperm family or genus (Robertson 1926). Additionally, while monolectic bees are uncommon, they represent an important group to monitor for bee biodiversity conservation. Due to the complexity of plant-pollinator networks, even small disturbances can have strong effects on these unique, localized relationships.

Furthermore, as fires become more frequent and intense (Marlon et al. 2012), the risk of forest conversion increases, causing compositional changes in existing post-fire vegetative communities (Reilly et al. 2017). Forest conversion is a result of microclimatic shifts following high severity fire that favor xeric species (Stevens et al. 2019). The result may be the loss of moist-mesic biota to thermophilic vegetation. For example, snowbrush ceanothus (*Ceanothus velutinus)* is an abundant drought-tolerant, fire-adapted woody shrub in western North American dry forest landscapes. Thermophilic shrubs, such as snowbrush ceanothus, are better adapted to warmer, drier conditions, but the quality of the pollen they provide to pollinators compared to moist-mesic understory forbs is unknown. Previously, woody shrubs were identified as important for generalist pollinators in urbanized landscapes (Casanelles-Abella et al. 2021), likely because they produce multiple blossoms when flowering. The same may be true for post-fire ponderosa pine forest communities. However, because fine scale information on plant-pollinator relationships is not known for many fire adapted landscapes, the effects of forest conversion on pollinator communities are difficult to predict.

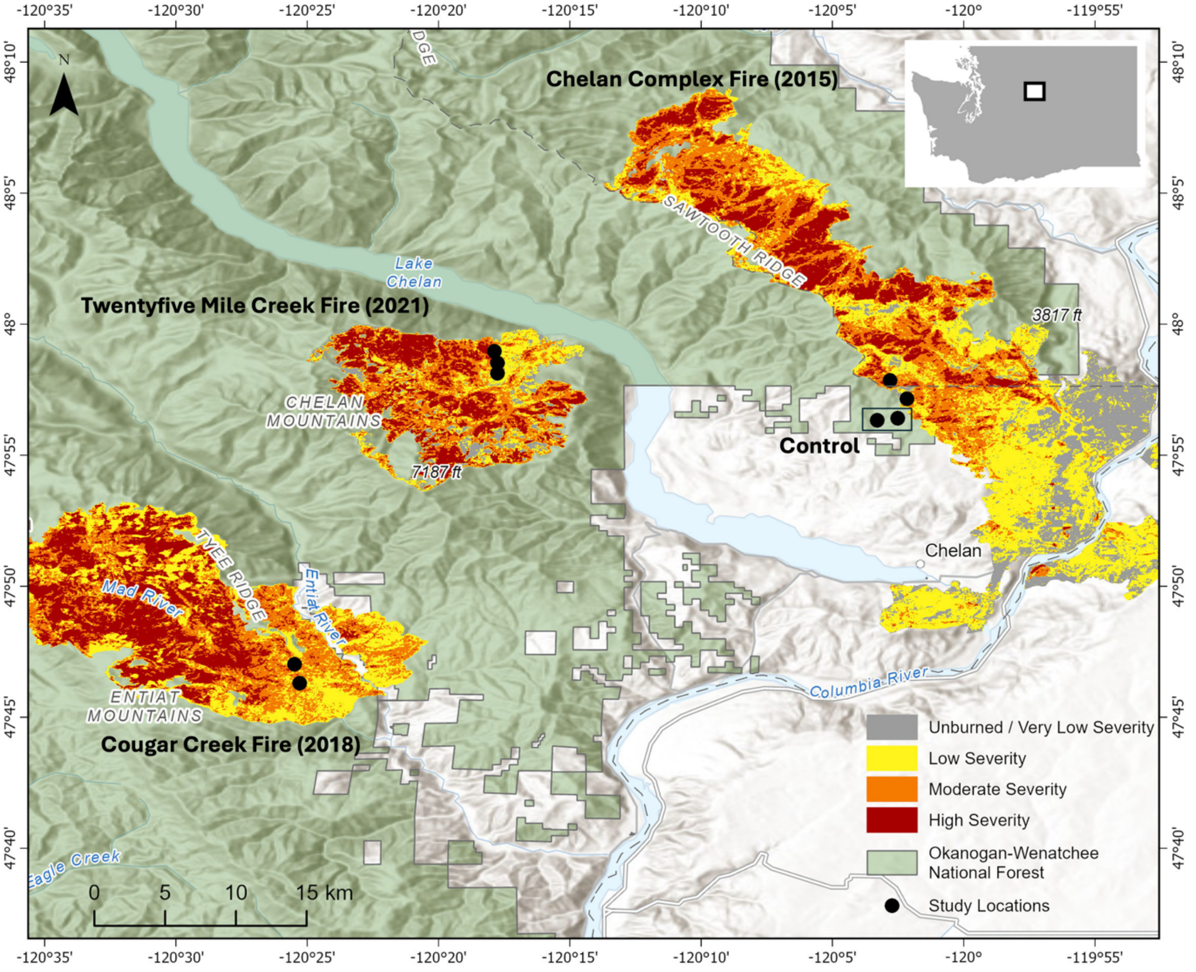
Determining plant-pollinator relationships can also be challenging due to the time and specialized taxonomic training required. Classical methods of identifying plant-pollinator relationships include direct observations from the field (e.g., netting and identifying bee species actively foraging on flowers and identifying plants *in situ*), and microscopic identification of pollen collected directly from bees (e.g., from bee corbicula and scopa). While these approaches are effective, they may not capture all plants visited by pollinators due to the relatively short temporal window of feasible collection relative to the total time pollinators are actively engaged in pollen collection over the larger landscape (Milla et al. 2022). Furthermore, classic palynology requires specialized training to microscopically identify individual pollen grains, which can be time consuming and subjective, occasionally resulting in misidentification.

DNA metabarcoding has facilitated the use of genetic material in ecological studies. Metabarcoding techniques use high throughput simultaneous sequencing methods to identify a sample containing DNA from multiple species (Cristescu 2014, Hawkins et al. 2015, Keller et al. 2015). Next-generation sequencing (NGS) is a specific molecular method used to accomplish metabarcoding (Keller et al. 2015). DNA metabarcoding can be used on pollen (Hawkins et al. 2015) to accurately record more plant-pollinator interactions than through field-based surveys alone (Milla et al. 2022) and has been used to highlight the fungal and bacterial symbionts of bees (Westreich et al. 2023b) and foraging patterns (Bosch et al. 2009). DNA metabarcoding has also been used to quantify temporal variation in plant community composition (Wilmshurst et al. 2014).

While NGS is effective at identifying the presence of plant species in a mixed pollen sample, it does not yet provide an accurate representation of the relative abundances within the sample (Bell et al. 2018). Furthermore, the NGS process can be subject to taxonomic biases due to incomplete libraries (Bell et al. 2016, Bell et al. 2018). Despite these limitations, pollen DNA metabarcoding is still an effective tool for identify floral resources visited by bees, from which plant-pollinator network information can be ascertained.

In this project, we developed a comprehensive list of post-fire plants that provide foraging resources for native pollinator communities. We predicted that locally abundant, thermophilic woody shrubs (e.g., *Purshia tridentata, Ceanothus velutinus*) would be the main pollen source for bees due to their ability to produce multiple blossoms and thrive in a hot, dry climate. We sought to determine if plant species identified by DNA metabarcoding were consistent with plant species observed bi-weekly in the field using 10×1-m transects. We also measured the effect of burn age and burn severity on floral resource richness and hypothesized that plant-pollinator community relationships would be affected by these factors. Our final hypothesis posits that specific plant species would be associated with unique burn ages and severities, highlighting key relationships for plant-pollinator network conservation.

Figure 2.1. Locations of nine study plots within four study areas in the Okanogan-Wenatchee National Forest in Washington, USA. All study areas were established in Chelan County within the Chelan and Entiat USDA Forest Service Ranger Districts. Specimens were collected from three burned areas and one "control" that was unburned since 1968. Fire severity is displayed as Composite Burn Index (CBI), and all study areas were burned by mixed-severity fire effects. Each 10×10-m pixel has been categorized as unchanged (CBI=0-0.1), low (CBI= 0.101-1.24), moderate (CBI= 1.241-2.24), or high (CBI= 2.241-3) severity.

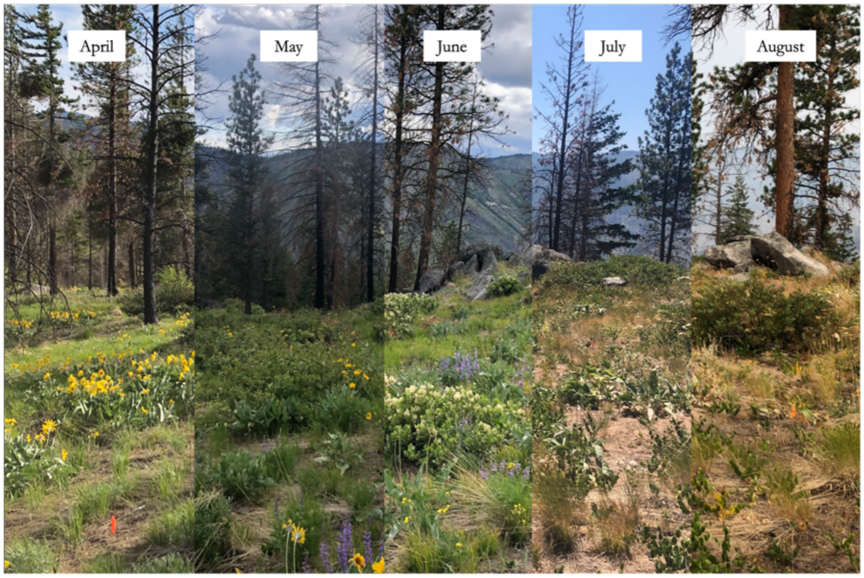


2.3 Materials and Methods

Study Areas

We established study sites within three fires that burned in eastern Washington in 2015, 2018, and 2021 (Figure 2.1). All study areas were established in Chelan County, Washington, USA and on land within the Okanogan-Wenatchee National Forest with permission from the USDA Forest Service. Located on the eastern slopes of the Cascade Mountain Range, Chelan County is a landscape dominated by dry *Pinus ponderosa* Douglas ex. C. Lawson (Pinaceae) forest and *Artemisia* L. (Asteraceae) steppe (Franklin and Dryness 1973; Figure 2.2). The Twentyfive Mile Creek fire burned 8,990 ha approximately 40 km west of Chelan, Washington in August of 2021. In July of 2018, lightning sparked the Cougar Creek fire, located 32 km northwest of Entiat, Washington, and burned 16,760 ha. The Chelan Complex fire burned 22,055 ha along the south shore of Lake Chelan in August of 2015. We also established a fourth study area that had been unburned since 1968 as a control for comparative purposes.

Figure 2.2. Temporal variation in the plant community within the Cougar Creek fire. This study area had a burn age of three years when this series of photographs was taken in 2021. Across all sites, floral resources and water availability decrease throughout the season. In August 2021, air quality also decreased due to smoke from the nearby Twentyfive Mile Creek Fire, which we began sampling in April 2022.



All three fires burned with mixed severity fire effects. Fire severity was determined using predicted Composite Burn Index (CBI) values generated in a 10×10-m scale with Google Earth Engine using methodology developed by Parks et al. (2019) (Figure 2.1). The CBI is a standardized method for assessing burn severity in forests that combines ecologically relevant variables across five horizontal strata into one numeric site index (Key and Benson 2006), allowing for a quantifiable and comparable measure of burn severity across study areas.

Field data collection

At each study site, we installed and sampled two to three replicated plots in 2022 and 2023. All plots were ≥600m apart to limit the effect of spatial autocorrelation in data analyses and to account for the average foraging range of wild bee species (Gathmann and Tscharntke 2002). In each plot, we deployed two sets of pan traps and two blue vane traps to ensure the capture of a diverse pollinator community (Campbell and Hanula 2007, Packer and Darla-West 2021). Each pan trap contained two white, two fluorescent blue, and two fluorescent yellow cups, each with a capacity of ~96 mL. Pan and blue vane traps were deployed every two weeks between April and August for a total of eight and nine sampling trips in 2022 and 2023, respectively.

Foraging bees captured by blue vane and pan traps often carry pollen loads on scopa or corbiculae, which is transferred into the soapy water within the trap upon capture. Little is known about pollen collection from blue vane and pan traps. We attempted to ascertain whether byproduct pollen could be effectively collected from trap water solutions for sequencing. Thus, when collecting samples from blue vane and pan traps, all trap contents (bees, water, pollen) were transferred to sealed containers for transport to the laboratory. Pan and blue vane traps were rinsed with molecular grade water in the field to ensure complete pollen residue collection.

During bi-monthly sampling efforts, we also measured floral composition using two randomly established, static 10×10-m quadrats per study plot. Within each quadrat, two 1×10-m transects were randomly selected during each sampling trip. The percent ground cover was estimated by identifying all plants to species and measuring the blossom area of each individual plant in cm^2.

Sample preparation

Field samples were pooled by trap type at the plot level. All visible pollen provisions on the scopa and corbiculae of bees were removed with a sterilized probe and forceps and stored in 15 mL microcentrifuge tubes. All bees were lightly shaken for 20 s in a 60% ethanol solution to ensure complete pollen collection, then set aside for identification. Each bee-derived pollen specimen was labeled as bee pollen (BP) with a unique identification number.

Trap pollen (TP) was collected from the soapy water used in blue vane and pan traps by filtering the solution through 3µm pore filter paper. Because the minimum size of a pollen grain is 5µm (Buchmann and O'Rourke 1991), this methodology ensured sufficient pollen capture. Dry filters were scraped with a sterilized scalpel to free embedded pollen particles. Pollen was washed from the filter into a sterile vial using 60% ethanol. The site, plot, date, year, and trap type were recorded for all pollen samples, which were then stored at 3°C. Once collection was complete, trap samples were centrifuged at 13,300 rpm for 3 minutes to allow pollen grains to form a pellet. The un-pelleted supernatant was pipetted off and discarded. When minimal ethanol remained, the sample tubes were opened and allowed to evaporate within a chemical fume hood. Empty tubes were added to act as negative controls and were included in the subsequent genetic analysis to ensure no airborne contamination occurred. All field materials and lab tools were submerged in a 15% bleach solution for four hours and air dried before redeployment in the field to avoid contamination.

Native bee pollinators within Hymenoptera (Anthophila) were labeled with the collection date, study site, and plot number. Bees were identified to species with taxonomic keys outlined in Maust et al. (2025). When species level identifications could not be determined, a morphospecies group was assigned.

DNA extraction and PCR amplification

DNA was extracted from pollen samples using the DNeasy Plant Mini Kit (Cat. No. 69106, Qiagen, Germany) following the standard kit protocol with the exception of the tissue disruption step, which was omitted (Qiagen 2016). Extracted DNA samples were stored at -80°C. The ITS2 genetic marker was selected as a PCR target to identify plant species from mixed pollen samples. The ITS2 region has been previously used as a DNA metabarcoding marker (Keller et al. 2015, Casanelles-Abella et al. 2021, Dürrbaum et al. 2022) and performs well for plants (Chen et al. 2010, Buchheim et al. 2011). Primers ITS-S2F (5'- ATGCGATACTTGGTGTGAAT-3') (Chen et al. 2010) and ITS4R (5'-TCCTCCGCTTATTGATATGC-3') (White et al. 1990) were chosen to amplify a ~340 base pair (bp) fragment of DNA from the ITS2 region. The primers were modified with an addition of Illumina's sequencing adapter nucleotide chains at the 3' ends, notated in bold: ITS-S2F (5'-**TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**ATGCGATACTTGGTGTGAAT-3'), ITS4R (5'**GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**TCCTCCGCTTATTGATATGC-3').

Initial amplicon polymerase chain reactions (PCRs) were 25μL in volume, consisting of 12.5μL of Multiplex Master Mix (Qiagen), 1.25μL of each primer (10μM starting concentration), and 10μL of DNA extract. Amplicon PCR conditions were the following: 1) an initial 95°C hot-start denaturing step for 15 minutes; 2) 35 cycles of 95°C for 30s, 55°C for 30s, and 72°C for 60 seconds; and 3) a final elongation step of 72°C for 10 minutes. Following amplification, samples were purified using SPRI beads, then quantified using a Qubit fluorometer with a dsDNA HS kit (Life Technologies, Carlsbad, CA, USA). Samples above average concentrations were diluted to be within a normalized range, determined independently for each PCR plate. Cleaned, normalized PCR products then underwent index PCR, where unique dual indexes (IDT for Illumina) were attached to the 5' and 3' ends of the amplicons within each reaction. Index PCRs took place in 10µL reactions, with 5µL of Multiplex Master Mix (Qiagen), 1µL of each i5 and i7 index, 2µL of PCR product, and 1µL of RNAse-free water. The Index PCR thermal cycling conditions were as follows: 1) an initial 95°C hot-start denaturing step for 15 minutes; 2) 8 cycles of 95°C for 30s, 55°C for 30s, and 72°C for 30 seconds; and 3) a final elongation step of 72°C for 5 minutes. Following index PCR, samples were pooled by PCR plate, SPRI bead-cleaned, quantified, normalized, then pooled into one final library. The pooled library was subsequently sequenced at Northwest Genomics Center (Seattle, Washington, USA) with the Illumina MiSeq System (Illumina V3 600 Flow Cell). Illumina software was used to demultiplex barcodes and sort reads to their corresponding samples.

Statistical Analysis

Bioinformatics and taxonomic assignment

Amplicon sequence variant (ASV) generation was accomplished using the Divisive Amplicon Denoising Algorithm 2 (DADA2) bioinformatics pipeline (Callahan et al. 2016). DADA2 is open-source R package that uses a model-based approach to correct Illumina amplicon errors and analyze sequencing data. It transforms the paired-end fastq files returned from the Northwest Genomics Center into merged, denoised, chimera-free sample-associated sequences.

Primer sequences were trimmed from raw reads using the command line tool cutadapt. The default parameters of DADA2 were used for quality filtering (Callahan et al. 2016). To account for differences in data quality across collection types, trap pollen and bee pollen were analyzed in separate pipelines with identical parameters. Samples for which all sequences were filtered out during the trimming and denoising process (e.g., fume hood controls, PCR negatives) were removed from analysis. No substantial contamination was detected from extraction and PCR negatives, nor from fume hood controls.

Trimming thresholds strongly effect conclusions drawn from pollen metabarcoding studies (Arstingstall et al. 2023). Strict thresholds reduce the likelihood of false positives. However, more liberal thresholds, resulting in more plant detections, may highlight rare floral resources critical for oligolectic and specialist pollinators. To account for this, each bioinformatics model was run twice. Each pipeline was conducted with both strict (maxEE=2) and liberal (maxEE=8; Rolling et al. 2022) maximum allowed errors indicated in the filterandtrim() function to generate a strict and a liberal species list, respectively. The threshold selection procedure for the liberal threshold is illustrated in Supplemental Figure 2.1.

Database selection informs taxonomic assignment, and specialized local databases reduce the potential of regional mismatches (Arstingstall et al. 2021). Taxonomy was assigned to the ASVs using a Chelan County nucleotide database aggregated by the Washington Native Plant Society (2023) and generated by GenBank using the blastn() function in DADA2 (Callahan et al. 2016). Our county-specific database was supplemented with species data obtained from iNaturalist (2025) to account for potential agricultural and horticultural floral resources. ASVs with matches meeting strict blastn() criteria were identified and applied using the assignTaxonomy() function in DADA2.

To limit errors across taxonomic assignments, the minimum bootstrap confidence interval needed to assign taxonomy at any level was set to 80% using the minBoot parameter.

Taxonomic assignments were used to construct one strict and one liberal plant list for each collection type. Bee (BP) and trap (TP) pollen samples were then merged into one dataset according to their corresponding maxEE() thresholds. Sample occurrence data for both the strict and liberal threshold lists was visualized using ggplot with the R package treemapify (Wilkins 2023). All analyses were conducted in R version 4.4.2 (R Core Team 2025). All figures were developed using the function ggplot() in the R package ggplot2 (Wickham et al. 2024).

Comparison of DNA and field data

The relationship between sequenced wild bee-collected pollen with a strict filtering threshold and field-based measures of floral availability were examined using a correspondence analysis. Proportions of DNA and transect identifications were developed by dividing the total unique plant species detected by each method by the total plant species detected across both methods. This was conducted at both the species and genus level. The proportion of plants detected in both methods across species and genera was also calculated. Proportions were grouped by sampling date and summarized in a contingency table. Then, two correspondence analyses were conducted to determine if plant lists at the species and genus level differed by collection method using the ca package in R (Greenacre et al. 2022).

Drivers of plant and bee species richness

Species richness is a commonly used metric for quantifying ecological community composition (O'Brien 1998). Pollen species richness at the plot scale was calculated by the unique number of species and morphospecies present, and was used in model analyses. We also calculated pollen species richness at the site level for graphical purposes. The control study area was omitted because it had a burn severity of zero and no relevant burn age. We then used a generalized linear mixed model (GLMM; Type III Analysis of Variance using Satterthwaite's Method) to test the main effects of burn severity, burn age, and their interaction on plot level plant pollen species richness with year as a random effect. Data were checked for normality, and a GLMM was conducted using lme4 (Bates et al. 2025) and lmerTest (Kuznetsova et al. 2020).

To calculate bee richness, specimens were grouped by burn age at both the site and plot scale and summarized by the unique number of genera present. Non-native European honeybees (*Apis mellifera* Linnaeus) were omitted. Sampling effort is known to affect richness values (Chazdon et al. 1998, Endres et al. 2021). Genus richness was thus rarefied to the minimum common number of samples to address sampling imbalances across collection years using rarefy() in the R package vegan (Oksanen et al. 2025). For the 2023 dataset, when the Twentyfive Mile Creek, Cougar Creek, and Chelan Complex fires were 2, 5, and 8 years post fire, respectively, we considered both genus and species richness.

We also used a GLMM to measure the effect of burn severity, burn age, their interaction, and pollen species richness on rarefied bee genus richness. Sampling year was included as a random effect, and the response variables were tested for normality and found to be normally distributed. Analyses were conducted in lme4 (Bates et al. 2025) and lmerTest (Kuznetsova et al. 2020).

Due to the known importance of species level identifications in mellitology, the relationship between pollen and bee richness at the species level was assessed using data from 2023 when data collection was most robust. We tested the main fixed effects of burn severity and burn age and plot level pollen species richness on plot level bee species richness. Bee species richness was modeled with a generalized Poisson distribution to address under-dispersion (Consul and Famoye 2007) and analyzed in glmmTMB (Brooks et al. 2017, McGillycuddy et al. 2025). Evaluations for all GLMMs were conducted to ensure model assumptions were met using the DHARMa package (Hartig 2024).

Indicator species analysis

We used an Indicator Species Analysis (ISA) to identify pollen species that were associated with a specific burn age, as determined by statistical significance ($p \leq 0.05$) assessed through permutation tests (Dufrêne and Legendre 1997). We constructed a community matrix from data collected in 2022 and 2023 when study areas were 1, 2, 4, 5, 7, and 8 years post fire, or unburned since 1968. We also used an ISA to identify plant species that were associated with a fire severity category. Fire severity, based on CBI, was binned as either a low or moderate burn severity (Miller and Thode 2007). Plots from the unburned study area were designated as a control category. Both ISAs were conducted with the classical parameters (duleg = TRUE) using the package indicspecies (De Cáceres et al. 2024).

2.4 Results

Comparing the strict and liberal DADA2 bioinformatics thresholds

The liberal threshold identified an additional 2 families, 6 genera, and 15 insect-pollinated plant species compared to the strict threshold. Although we present details from the ASVs generated by the DADA2 pipeline from both thresholds, only the plant species dataset generated by the strict threshold was used in subsequent analyses.

Strict threshold (maxEE=2)

After filtering, trimming, and denoising the sequences, a total of 194,247 reads were generated from the bee pollen and 924,832 reads were generated for the trap pollen data. From these reads, we used the DADA2 pipeline to identify 2,171 unique ASVs that were assigned to 186 unique species/morphospecies categories. These species spanned 45 families and 128 genera.

Several of the identified plant species (45 species/morphospecies) were suspected to be the result of wind contamination and were removed from further analyses. Because traps were open to the environment when deployed in the field, it follows that wind pollinated plant pollen would be deposited into the soap water solution. Plants suspected of being the result of wind contamination include Alaskan yellow cedar (*Callitropsis nootkatensis,* 49 samples), variegated horsetail (*Equisetum variegatum*, 25 samples), and mountain alder (*Alnus incana*, 17 samples); a complete list is provided in Supplemental Table 2.1.

After suspected wind contamination was removed, a total of 1,798 observations remained. These observations were assigned taxonomic identifications in 30 families and 95 genera for 138 species of insect-pollinated plants (Figure 2.3). The family Asteraceae had the most occurrences across all samples (631), contributing 25.3% (24/95) of the total genera and 26.1% (36/138) of the total species/morphospecies (Figure 2.4). A complete list of insect pollinated plant species identified using the strict filterandtrim() threshold is available in Table 2.1. The twenty most frequently detected insect-pollinated plants are shown in Figure 2.5A, and a complete list of plants that occurred in more than ten samples is shown in Figure 2.6A.

Figure 2.3. Bee collected pollen richness and diversity for all samples. A total of 30 plant families, in 95 genera and 138 species, were identified with pollen deposited in blue vane and pan traps by insect pollinators.

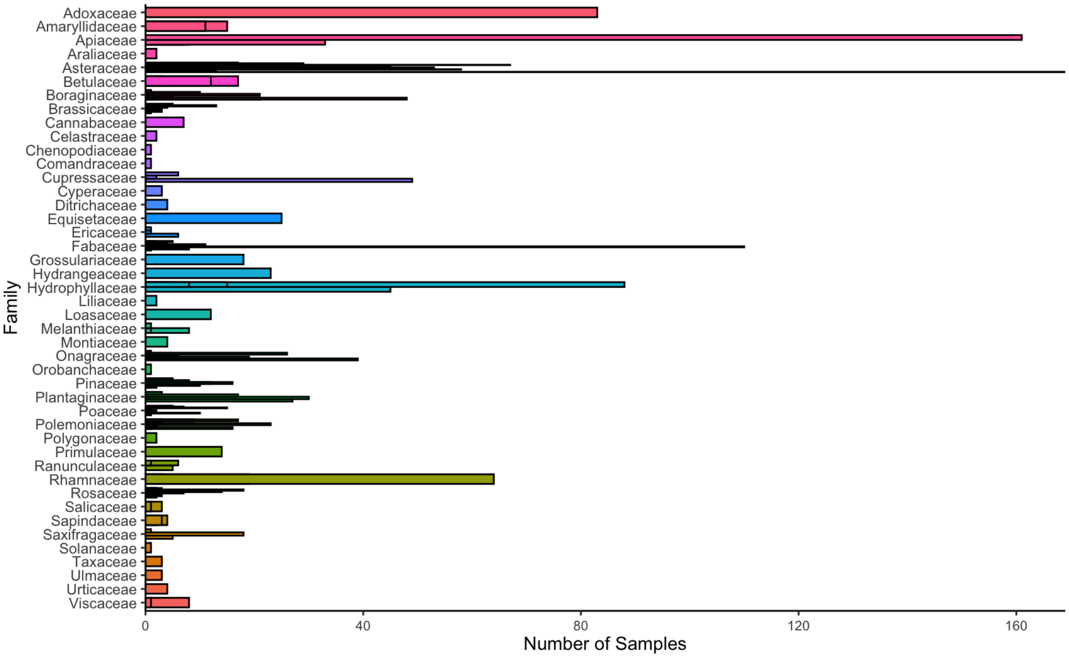


Figure 2.4. Across all 1,798 observations, Asteraceae pollen was the most common and specious (631 occurrences). It contributed 25.3% of the total genera (24/95) and 26.1% (36/138) of the total species/morphospecies. Yarrow (*Achillea millefolium*) was the most prevalent and appeared in 169 samples.

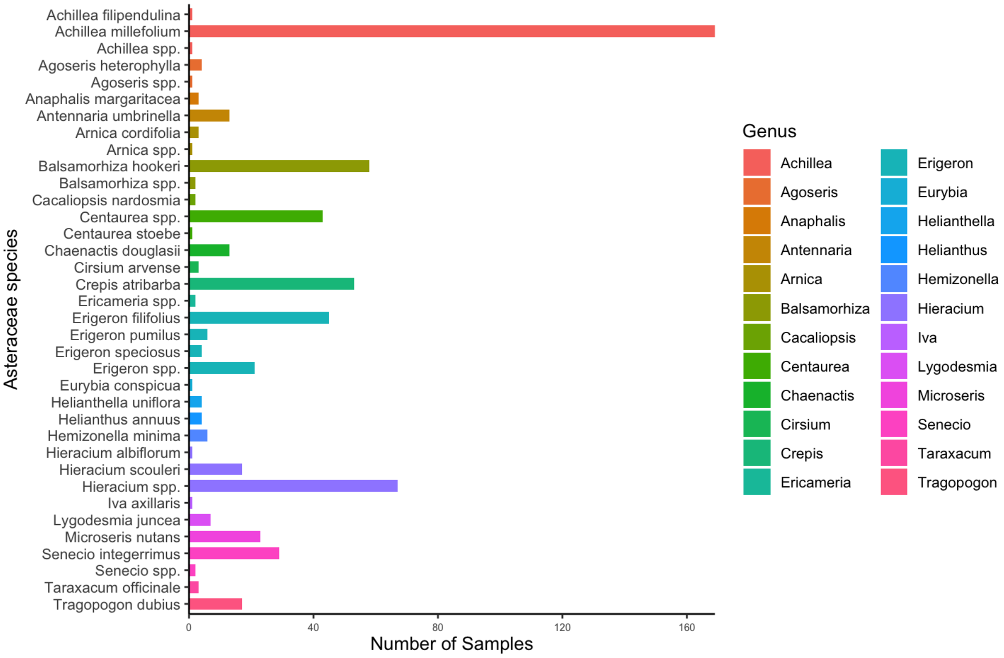
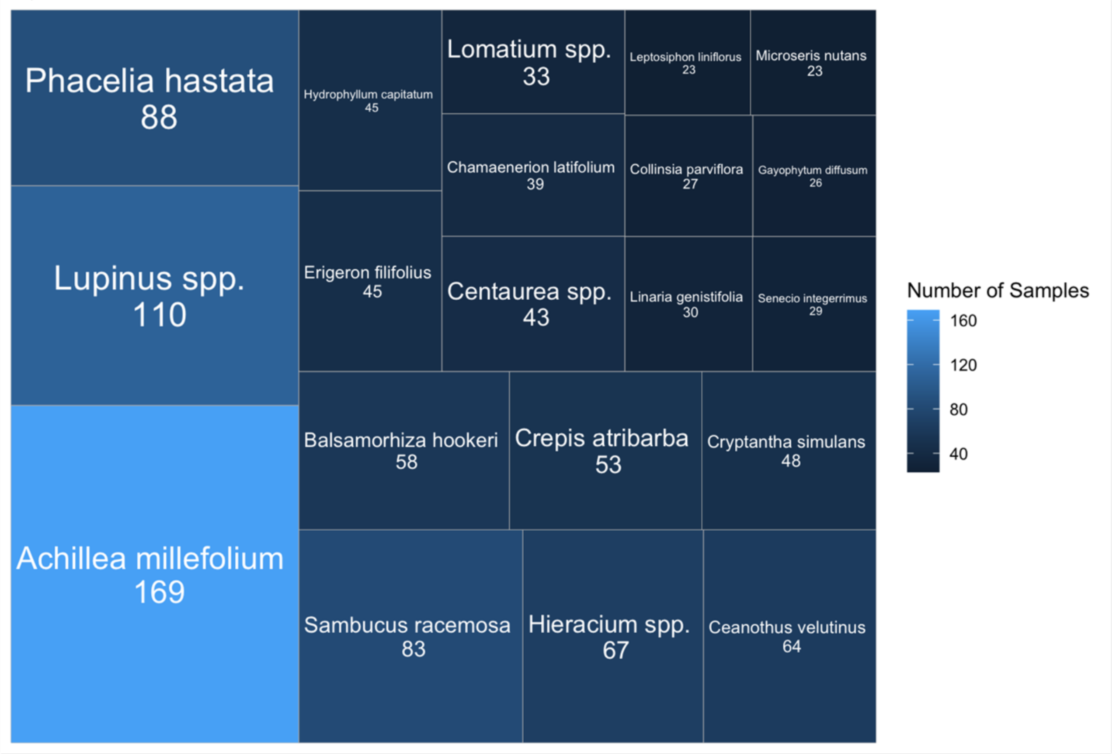


Figure 2.5. Treemaps of the 20 most frequently detected plant species after applying a strict (A) and liberal (B) bioinformatics filter and trim threshold. The size of the box reflects the number of samples in which each species was detected across two sampling years (2022-2023). Lighter colors indicate more species were detected.

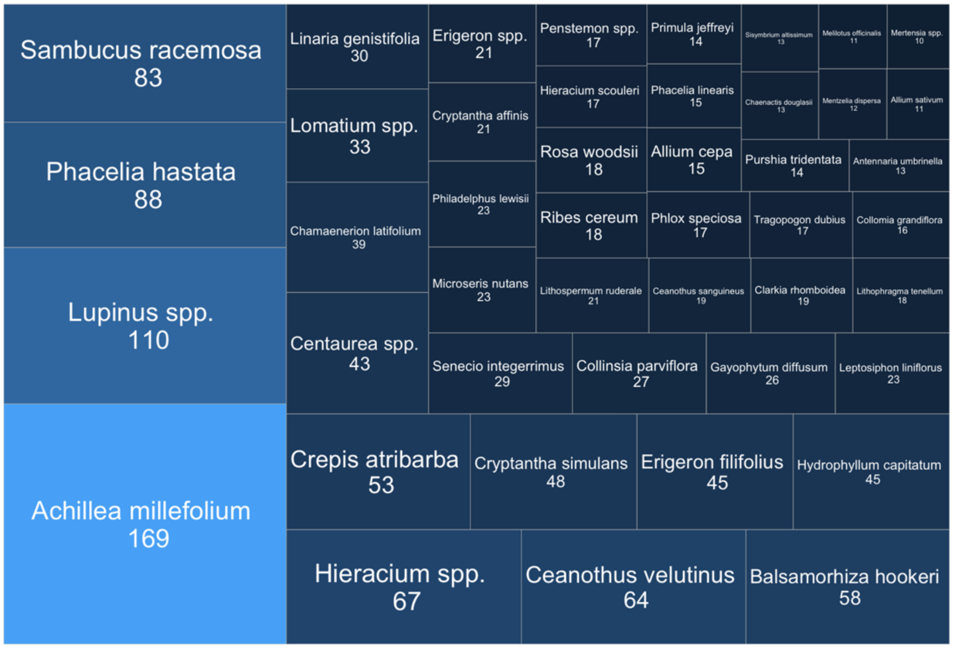
A) Strict threshold



B) Liberal threshold

Figure 2.6. Treemaps of the plant species detected in more than ten samples based on DNA metabarcoding of pollen using both a strict (A) and liberal (B) bioinformatics filtering and trimming threshold. The overall size of the box in each treemap reflects the number of samples in which each species that was detected across two sampling years (2022-2023). Lighter colors indicate more species were detected.

A) Strict threshold



B) Liberal threshold

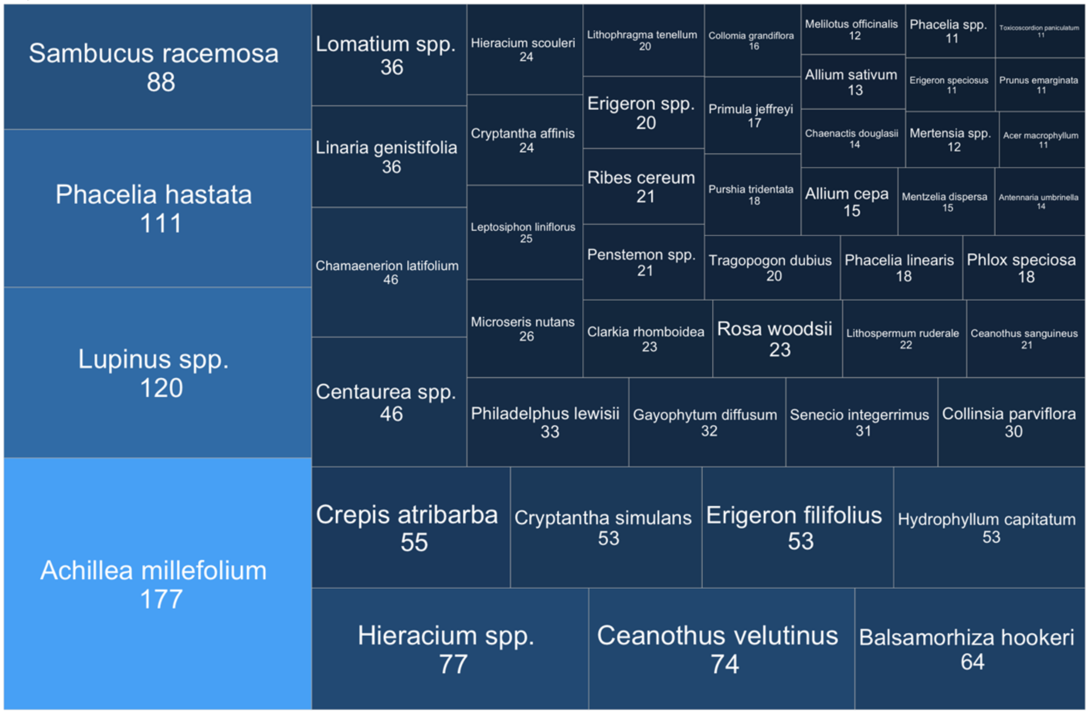


Table 2.1. Insect pollinated plant species identified by the DADA2 assignTaxonomy() function with a strict trimming threshold. A total of 1,798 observations were identified across all samples, which include 30 families, 95 genera, and 138 species. Species that did not appear in the liberal threshold list are in bold. Additional data regarding common names, pollination, and origin was derived from the Burke Herbarium (2025).

Family	Latin Name	Common Name	Pollinator	Origin	Sample Frequency
Adoxaceae	*Sambucus racemosa*	red elderberry	insect	native	83
Amaryllidaceae	*Allium cepa*	onion	insect	agricultural	15
	Allium sativum	garlic	insect	agricultural	11
Apiaceae	*Lomatium ambiguum*	swale desert parsley	insect (Andrena)	native	8
	Lomatium dissectum	fernleaf biscuitroot	insect and asexual	native	7
	Lomatium spp.	biscuitroot	insect and asexual	native	33
Araliaceae	*Oplopanax horridus*	devil's club	insect	native	2
Asteraceae	***Achillea filipendulina***	fernleaf yarrow	insect	introduced	1
	Achillea millefolium	yarrow	insect	native	169
	Achillea spp.	yarrow	insect	unknown	1
	Agoseris heterophylla	annual agoseris	insect	native	4
	Agoseris spp.	agoseris	insect	native	1
	Anaphalis margaritacea	pearly everlasting	insect	native	3
	Antennaria umbrinella	pussytoes	insect	native	13
	Arnica cordifolia	heart-leaf arnica	insect	native	3
	Arnica spp.	arnica	insect	native	1
	Balsamorhiza hookeri	hookers balsamroot	insect	native	58
	Balsamorhiza spp.	balsamroot	insect	native	2
	Cacaliopsis nardosmia	silvercrown luina	insect	native	2
	Centaurea spp.	knapweeds	insect	native	43
	Centaurea stoebe	spotted knapweed	insect	introduced	1
	Chaenactis douglasii	Douglas' dustymaiden	insect	native	13
	Cirsium arvense	creeping thistle	insect	introduced	3
	Crepis atribarba	slender hawksbeard	insect	native	53
	Ericameria spp.	rabbitbrush	insect	native	2
	Erigeron filifolius	threadleaf fleabane	insect	native	45
	Erigeron pumilus	shaggy fleabane	insect	native	6

Asteraceae (cont.)	*Erigeron speciosus*	showy fleabane	insect	native	4
	Erigeron spp.	fleabane	insect	native	21
	Eurybia conspicua	western showy aster	insect	native	1
	Helianthella uniflora	Rocky Mountain helianthella	insect	native	4
	Helianthus annuus	common sunflower	insect	native / horticultural	4
	Hemizonella minima	smallhead tarweed	insect	native	6
	Hieracium albiflorum	white hawkwed	insect	native	1
	Hieracium scouleri	hound-tongue hawkweed	insect	native	17
	Hieracium spp.	hawkweeds	insect and asexual	native	67
	Iva axillaris	deeproot	insect	native	1
	Lygodesmia juncea	rush skeletonweed	insect	native	7
	Microseris nutans	nodding microsteris	insect and asexual	native	23
	Senecio integerrimus	lambstongue ragwort	insect	native	29
	Senecio spp.	ragwort/groundsel	insect	unknown	2
	Taraxacum officinale	common dendelion	insect	introduced	3
	Tragopogon dubius	yellow salsify	insect	native	17
Boraginaceae	*Cryptantha affinis*	side-grooved cryptantha	insect	native	21
	Cryptantha simulans	pine woods cryptantha	insect	native	48
	Cryptantha torreyana	Torrey's cryptantha	insect	native	2
	Hackelia micrantha	meadow forget-me-not	insect	native	3
	Hackelia spp.	stickseed	insect	native	5
	Lithospermum ruderale	western stoneseed	insect	native	21
	Mertensia spp.	bluebells	insect	native	10
	Myosotis laxa	bay forget-me-not	insect	native	1
Brassicaceae	*Alyssum alyssoides*	small alyssum	insect	introduced	1
	Brassica juncea	brown mustard	insect	introduced	3
	Descurainia incana	mountain tansymustard	insect	native	1
	Descurainia sophia	flixweed	insect	introduced	1
	Descurainia spp.	tanseymustard	insect	unknown	2
	Erysimum capitatum	sand dune wallflower	insect	native	3
	Sandbergia whitedii	fissurewwort	insect	native	4

Brassicaceae (cont.)	*Sisymbrium altissimum*	tall tumble-mustard	insect and asexual	introduced	13
	Thelypodium laciniatum	cut-leaf thelypody	insect	native	5
Celastraceae	*Paxistima myrsinites*	Oregon boxleaf	insect	native	2
Comandraceae	*Comandra umbellata*	bastard toadflax	insect	native	1
Ericaceae	*Arctostaphylos nevadensis*	pinemat manzanita	insect	native	6
	Orthilia secunda	sidebells	insect and asexual	native	1
	Phyllodoce spp.	mountain heath	insect	native	1
Fabaceae	*Alhagi maurorum*	camelthorn	insect	introduced	1
	Astragalus miser	timber milkvetch	insect	native	8
	Lathyrus spp.	pea	insect	unknown	7
	Lupinus polyphyllus	bigleaf lupine	insect	native	5
	Lupinus spp.	lupine	insect	native	110
	Medicago sativa	alfalfa	insect	introduced	5
	Melilotus officinalis	sweet yellow clover	insect	native	11
	Phaseolus vulgaris	common bean	insect and asexual	agricultural	4
	Trifolium repens	white clover	insect	introduced	4
	Vicia americana	American vetch	insect	native	2
	Vicia spp.	vetch	insect	unknown	2
	Vicia villosa	hairy vetch	insect	introduced	5
Grossulariaceae	*Ribes cereum*	wax currant	insect	native	18
Hydrangeaceae	*Philadelphus lewisii*	Lewis' Mock Orange	insect	native	23
	Hydrophyllum capitatum	ballhead waterlead	insect	native	45
	Phacelia hastata	silverleaf phacelia	insect	native	88
	Phacelia heterophylla	varileaf phacelia	insect	native	5
	Phacelia linearis	threadleaf phacelia	insect	native	15
	Phacelia spp.	phacelia	insect	native	8
Liliaceae	*Tulipa gesneriana*	garden tulip	insect	introduced	2
Loasaceae	*Mentzelia dispersa*	bushy blazingstar	insect	native	12
Melanthiaceae	*Toxicoscordion paniculatum*	foothill death camas	insect (Andrena)	native	8
	Toxicoscordion venenosum	meadow death camas	insect	native	1
	Veratrum californicum	California false hellebore	insect	native	1
Montiaceae	*Claytonia rubra*	red miners lettuce	insect and asexual	native	4

Onagraceae	*Chamaenerion latifolium*	drawf fireweed	insect	native	39
	Clarkia rhomboidea	diamond clarkia	insect	native	19
	Epilobium brachycarpum	autumn willow herb	insect	native	6
	Gayophytum diffusum	spreading groundsmoke	insect	native	26
	Oenothera pallida	pale evening primrose	insect	native	1
Orobanchaceae	*Pedicularis groenlandica*	bull elephants head	insect, hummingbird	native	1
Plantaginaceae	*Collinsia parviflora*	small-flowered collinsia	insect	native	27
	Linaria genistifolia	broom-leaved toadflax	insect	native	30
	Penstemon davidsonii	Davidsons penstemon	insect	native	2
	Penstemon spp.	penstemon	insect, hummingbird	native	17
Polemoniaceae	*Collomia grandiflora*	large-flowered mountain trumpet	insect	native	16
	Collomia tenella	diffuse collomia	insect	native	1
	Ipomopsis aggregata	scarlet gilia	hummingbird	native	2
	Leptosiphon liniflorus	narrowflower flaxflower	insect	native	23
	Microsteris gracilis	slender phlox	insect and asexual	native	3
	Phlox hoodii	Hood's phlox	insect	native	9
	Phlox speciosa	showy phlox	insect	native	17
	Phlox spp.	phlox	insect	native	3
Polygonaceae	*Eriogonum* spp.	buckwheat	insect	native	2
Primulaceae	*Primula jeffreyi*	jeffrey's shootingstar	insect (Bombus)	native	14
Ranunculaceae	*Clematis ligusticifolia*	western clematis	insect	native	5
	Ranunculus repens	creeping buttercup	insect	introduced	6
Rhamnaceae	*Ceanothus sanguineus*	redstem ceanothus	insect	native	19
	Ceanothus spp.	ceanothus	insect	native	3
	Ceanothus velutinus	snowbrush ceanothus	insect	native	64
Rosaceae	*Amelanchier alnifolia*	saskatoon serviceberry	insect	native	2
	***Drymocallis* spp.**	cinquefoil	insect	native	3
	Fragaria spp.	strawberry	insect	native	2
	Fragaria vesca	woodland strawberry	insect	native	1

Rosaceae (cont.)	*Fragaria virginiana*	mountain strawberry	insect	native	1
	Prunus emarginata	bitter cherry	insect	native	7
	Purshia tridentata	antelope bitterbrush	insect	native	14
	Rosa woodsii	wood's rose	insect	native	18
	Rubus parviflorus	thimbleberry	insect	native	2
	Rubus spp.	blackberry	insect	unknown	3
	Rubus ulmifolius	elm-leaf blackberry	insect	introduced	2
Salicaceae	*Salix spp.*	willow	insect	unknown	4
Sapindaceae	*Acer circinatum*	vine maple	insect and wind	native	2
	Acer macrophyllum	big-leaf maple	insect and wind	native	4
	Acer saccharinum	silver maple	insect and wind	introduced	2
	Acer spp.	Maple	insect and wind	unknown	3
Saxifragaceae	*Heuchera spp.*	alumroot	insect (Diptera)	native	5
	Lithophragma glabrum	bulbous woodland star	insect	native	5
	Lithophragma parviflorum	small-flower woodland star	insect	native	1
	Lithophragma spp.	woodland star	insect	native	3
	Lithophragma tenellum	woodland star	insect	native	18
	Micranthes ferruginea	rusty saxifrage	insect	native	1
Solanaceae	*Solanum spp.*	nightshade	insect and wind	unknown	1

Table 2.2. Insect pollinated plant species identified using the liberal filter and trim bioinformatics threshold in DADA2. Species that did not appear in the strict plant list are in bold. Of the total 2,077 observations, 34 families, 102 genera, and 154 insect pollinated species were identified. Additional data regarding plant common name, pollination type, and origin was derived from the Burke Herbarium (2025).

Family	Latin Name	Common Name	Pollinator	Origin	Sample Frequency
Adoxaceae	*Sambucus racemosa*	red elderberry	insect	native	88
Amaryllidaceae	*Allium cepa*	onion	insect	agricultural	15
	Allium sativum	garlic	insect	agricultural	13
	***Allium* spp.**	onion	insect	agricultural	1
Apiaceae	*Lomatium ambiguum*	swale desert parsley	insect (Andrena)	native	9
	Lomatium dissectum	fernleaf biscuitroot	insect and asexual	native	8
	Lomatium spp.	biscuitroot	insect and asexual	native	36
Araliaceae	*Oplopanax horridus*	devil's club	insect	native	2
Asteraceae	*Achillea millefolium*	yarrow	insect	native	177
	Achillea spp.	yarrow	insect	unknown	6
	Agoseris heterophylla	annual agoseris	insect	native	4
	Agoseris spp.	agoseris	insect	native	1
	Anaphalis margaritacea	pearly everlasting	insect	native	4
	Antennaria umbrinella	pussytoes	insect	native	14
	Arnica cordifolia	heart-leaf arnica	insect	native	4
	Arnica spp.	arnica	insect	native	2
	Balsamorhiza hookeri	hookers balsamroot	insect	native	64
	Balsamorhiza spp.	balsamroot	insect	native	3
	Cacaliopsis nardosmia	silvercrown luina	insect	native	2
	Centaurea spp.	knapweeds	insect	native	46
	Centaurea stoebe	spotted knapweed	insect	introduced	2
	Chaenactis douglasii	Douglas' dustymaiden	insect	native	14
	Cirsium arvense	creeping thistle	insect	introduced	3
	Crepis atribarba	slender hawksbeard	insect	native	55
	Ericameria spp.	rabbitbrush	insect	native	2
	Erigeron filifolius	threadleaf fleabane	insect	native	53
	Erigeron pumilus	shaggy fleabane	insect	native	7
	Erigeron speciosus	showy fleabane	insect	native	11
	Erigeron spp.	fleabane	insect	native	20
	Eurybia conspicua	western showy aster	insect	native	2
	Helianthella uniflora	Rocky Mountain helianthella	insect	native	4

Asteraceae (cont.)	*Helianthus annuus*	common sunflower	insect	native / horticultural	9
	Hemizonella minima	smallhead tarweed	insect	native	6
	Hieracium albiflorum	white hawkwed	insect	native	1
	Hieracium scouleri	hound-tongue hawkweed	insect	native	24
	Hieracium spp.	hawkweeds	insect and asexual	native	77
	Hieracium triste	alpine hawkweed	insect	native	1
	Lactuca serriola	prickly lettuce	insect	introduced	1
	Lygodesmia juncea	rush skeletonweed	insect	native	7
	Microseris nutans	nodding microsteris	insect and asexual	native	26
	Senecio integerrimus	lambstongue ragwort	insect	native	31
	Senecio spp.	ragwort/groundsel	insect	unknown	2
	Senecio triangularis	arrowleaf groundsel	insect	native	1
	Taraxacum officinale	common dendelion	insect	introduced	4
	Tragopogon dubius	yellow salsify	insect	native	20
	***Tragopogon* spp.**	goatsbeard/salsify	insect	unknown	1
Boraginaceae	*Cryptantha affinis*	side-grooved cryptantha	insect	native	24
	Cryptantha simulans	pine woods cryptantha	insect	native	53
	Cryptantha torreyana	Torrey's cryptantha	insect	native	3
	Hackelia micrantha	meadow forget-me-not	insect	native	6
	Hackelia spp.	stickseed	insect	native	5
	Hydrophyllum capitatum	ballhead waterlead	insect	native	53
	Lithospermum ruderale	western stoneseed	insect	native	22
	Mertensia spp.	bluebells	insect	native	12
	Myosotis laxa	bay forget-me-not	insect	native	1
	Phacelia hastata	silverleaf phacelia	insect	native	111
	Phacelia heterophylla	varileaf phacelia	insect	native	5
	Phacelia linearis	threadleaf phacelia	insect	native	18
	Phacelia spp.	phacelia	insect	native	11
Brassicaceae	*Alyssum alyssoides*	small alyssum	insect	introduced	1
	Brassica juncea	brown mustard	insect	introduced	6
	Cardamine pensylvanica	Pennsylvania bittercress	insect	native	1
	Descurainia incana	mountain tansymustard	insect	native	1
	Descurainia sophia	flixweed	insect	introduced	1
Brassicaceae (cont.)	*Descurainia* spp.	tanseymustard	insect	unknown	2
	Erysimum capitatum	sand dune wallflower	insect	native	3

	Sandbergia whitedii	fissurewwort	insect	native	4
	Sisymbrium altissimum	tall tumble-mustard	insect and asexual	introduced	4
	Thelypodium laciniatum	cut-leaf thelypody	insect	native	5
	Thlaspi arvense	field pennycress	insect	introduced	1
Caryophyllaceae	***Cerastium beeringianum***	alpine chickweed	insect	native	1
Celastraceae	*Paxistima myrsinites*	Oregon boxleaf	insect	native	2
Comandraceae	*Comandra umbellata*	bastard toadflax	insect	native	1
Ericaceae	*Arctostaphylos nevadensis*	pinemat manzanita	insect	native	6
	Orthilia secunda	sidebells	insect and asexual	native	2
	Phyllodoce spp.	mountain heath	insect	native	1
	Vaccinium membranaceum	tall huckleberry	insect	native	4
Fabaceae	*Astragalus miser*	timber milkvetch	insect	native	9
	Lathyrus spp.	pea	insect	unknown	7
	Lupinus polyphyllus	bigleaf lupine	insect	native	4
	Lupinus spp.	lupine	insect	native	120
	Medicago sativa	alfalfa	insect	introduced	6
	Melilotus officinalis	sweet yellow clover	insect	native	12
	Phaseolus vulgaris	common bean	insect and asexual	agricultural	4
	Trifolium pratense	red clover	insect	introduced	1
	Trifolium repens	white clover	insect	introduced	5
	Vicia americana	American vetch	insect	native	2
	Vicia spp.	vetch	insect	unknown	2
	Vicia villosa	hairy vetch	insect	introduced	5
Grossulariaceae	*Ribes cereum*	wax currant	insect	native	21
Hydrangeaceae	*Philadelphus lewisii*	Lewis' Mock Orange	insect	native	33
Leguminosae	*Alhagi maurorum*	camelthorn	insect	introduced	1
Liliaceae	*Tulipa gesneriana*	garden tulip	insect	introduced	2
Loasaceae	*Mentzelia dispersa*	bushy blazingstar	insect	native	15
Melanthiaceae	*Toxicoscordion paniculatum*	foothill death camas	insect (Andrena)	native	11
	Toxicoscordion venenosum	meadow death camas	insect	native	1
	Veratrum californicum	California false hellebore	insect	native	1
Montiaceae	***Claytonia lanceolata***	lanceleaf springbeauty	insect	native	1
	Claytonia rubra	red miners lettuce	insect and asexual	native	4
Onagraceae	*Chamaenerion latifolium*	drawf fireweed	insect	native	46
Onagraceae (cont.)	***Chamaenerion*** **spp.**	fireweed	insect	native	1
	Clarkia rhomboidea	diamond clarkia	insect	native	23
	Epilobium brachycarpum	autumn willow herb	insect	native	9

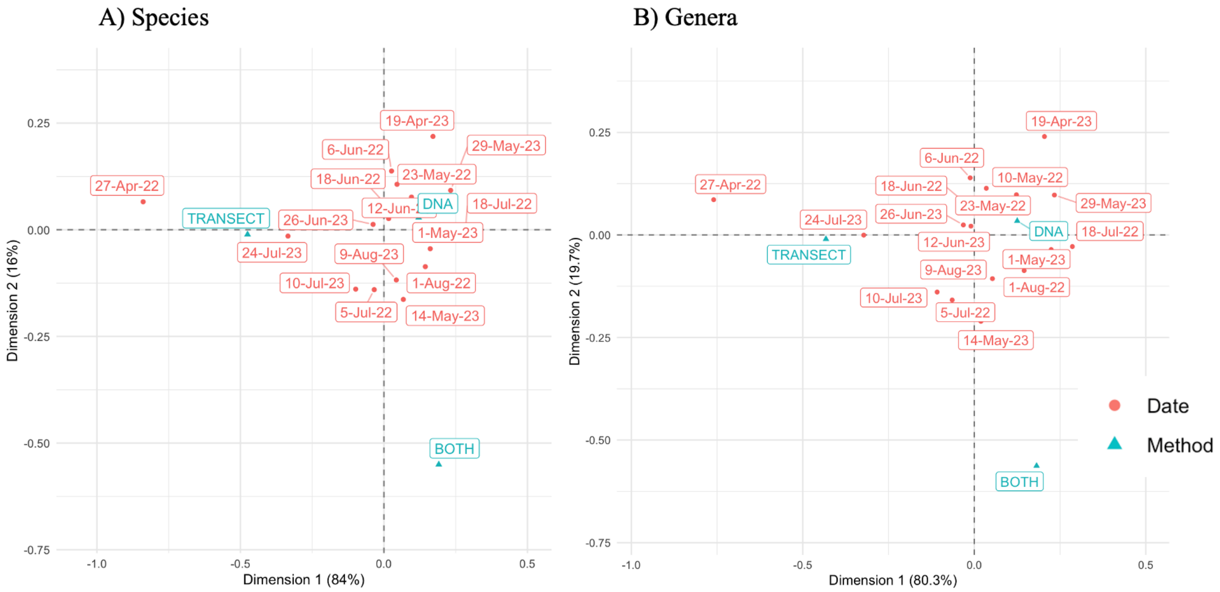
	Gayophytum diffusum	spreading groundsmoke	insect	native	32
	Oenothera pallida	pale evening primrose	insect	native	1
Orobanchaceae	*Pedicularis groenlandica*	bull elephants head	insect, hummingbird	native	1
Plantaginaceae	*Linaria genistifolia*	broom-leaved toadflax	insect	native	36
	Penstemon spp.	penstemon	insect, hummingbird	native	21
Polemoniaceae	*Collomia grandiflora*	large-flowered mountain trumpet	insect	native	16
	Collomia tenella	diffuse collomia	insect	native	1
	Ipomopsis aggregata	scarlet gilia	hummingbird	native	2
	Leptosiphon liniflorus	narrowflower flaxflower	insect	native	25
	Microsteris gracilis	slender phlox	insect and asexual	native	3
	Phlox hoodii	Hood's phlox	insect	native	8
	Phlox speciosa	showy phlox	insect	native	18
	Phlox spp.	phlox	insect	native	4
Polygonaceae	***Eriogonum heracleoides***	parsnip-flowered buckwheat	insect	native	4
	Eriogonum spp.	buckwheat	insect	native	3
Primulaceae	*Primula jeffreyi*	jeffrey's shootingstar	insect (Bombus)	native	17
Ranunculaceae	*Clematis ligusticifolia*	western clematis	insect	native	6
	Ranunculus aquatilis	white western buttercup	insect	native	1
	Ranunculus repens	creeping buttercup	insect	introduced	7
Rhamnaceae	*Ceanothus sanguineus*	redstem ceanothus	insect	native	21
	Ceanothus spp.	ceanothus	insect	native	3
	Ceanothus velutinus	snowbrush ceanothus	insect	native	74
Rosaceae	*Amelanchier alnifolia*	saskatoon serviceberry	insect	native	2
	Drymocallis arguta	cordilleran drymocallis	insect	native	4
	Fragaria spp.	strawberry	insect	native	1
	Fragaria vesca	woodland strawberry	insect	native	2
	Fragaria virginiana	mountain strawberry	insect	native	2
Rosaceae (cont.)	***Holodiscus discolor***	creambush ocean-spray	insect	native	2
	Prunus emarginata	bitter cherry	insect	native	11
	Purshia tridentata	antelope bitterbrush	insect	native	18
	Rosa woodsii	wood's rose	insect	native	23
	Rubus parviflorus	thimbleberry	insect	native	3
	Rubus spp.	blackberry	insect	unknown	5

	Rubus ulmifolius	elm-leaf blackberry	insect	introduced	2
Rubiaceae	***Galium aparine***	common bedstraw	insect	native	1
Salicaceae	***Salix exigua***	coyote willow	insect	native	1
	Salix spp.	willow	insect	unknown	8
Sapindaceae	*Acer circinatum*	vine maple	insect and wind	native	2
	Acer macrophyllum	big-leaf maple	insect and wind	native	11
	Acer platanoides	Norway maple	insect and wind	introduced	2
	Acer saccharinum	silver maple	insect and wind	introduced	2
	Acer spp.	Maple	insect and wind	unknown	3
Saxifragaceae	*Heuchera* spp.	alumroot	insect (Diptera)	native	6
	Lithophragma glabrum	bulbous woodland star	insect	native	4
	Lithophragma parviflorum	small-flower woodland star	insect	native	1
	Lithophragma spp.	woodland star	insect	native	5
	Lithophragma tenellum	woodland star	insect	native	20
	Micranthes ferruginea	rusty saxifrage	insect	native	1
Scrophulariaceae	*Collinsia parviflora*	small-flowered collinsia	insect	native	30
	Verbascum thapsus	flannel mullein	insect (Bombus)	introduced	1
Solanaceae	*Solanum* spp.	nightshade	insect and wind	unknown	1

Liberal threshold (maxEE=8)

When the filterandtrim() threshold was relaxed to a maxEE value of 8, a total of 997,590 bee pollen and 5,978,556 trap pollen reads were generated. These were binned into 3,502 ASVs that were assigned to 207 species/morphospecies categories. ASVs that could not be assigned to a family were omitted. The resulting species included 48 families and 139 genera, which were then refined to only insect-pollinated plants. In the remaining 2,077 ASVs, 34 families, 102 genera, and 154 insect-pollinated species were identified. A complete list of insect-pollinated plant species identified using the liberal threshold is available in Table 2.2. The twenty most frequently detected insect-pollinated plants by the liberal threshold are shown in Figure 2.5B. The total number of insect-pollinated species that occurred in more than ten samples is shown in Figure 2.6B.

Figure 2.7. Correspondence analyses of the variation in plant species (A) and genera (B) detection between field sampling methods (i.e. transects and DNA metabarcoding), and sampling date. Across both analyses, most of the variation was explained by sampling date. More species were identified with DNA than with field based transects.

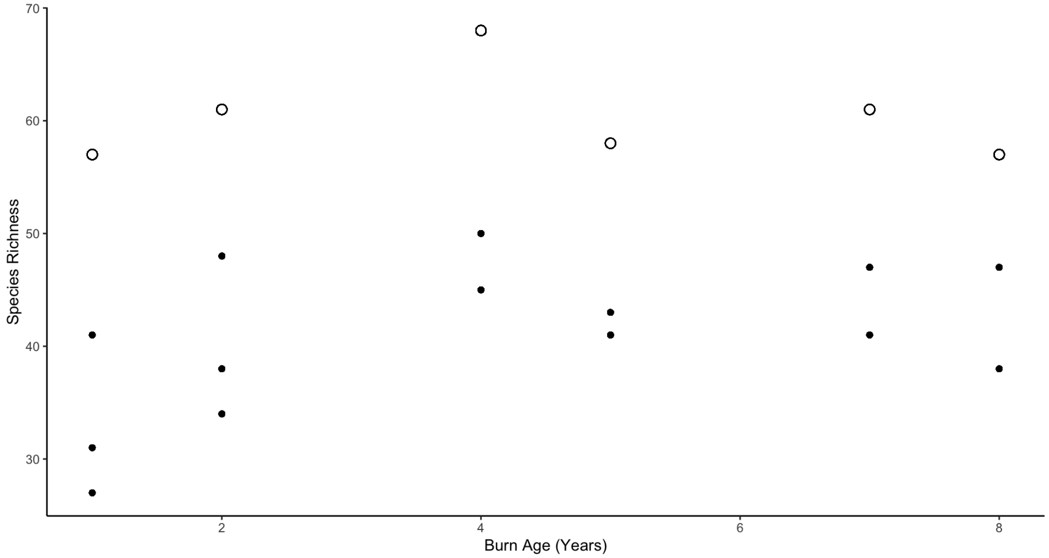

A) Species

B) Genera

Comparison of DNA and field data

Field transect surveys identified 55 plant species and 48 genera. Of these, 21 species and 35 genera were identified by both the field transect surveys and the DNA metabarcoding results. Thus, 15.2% and 36.8% of the total species and genera, respectively, that were detected with genetic analysis were also identified during field data collection (Supplemental Figure 2.2). Correspondence analyses revealed that, at the species level, 84% of the variation was explained by sampling date (eigenvalue = 0.059203), and 15% was explained by sampling method (eigenvalue = 0.011268; Figure 2.7A). Most of the samples were identified with DNA, with a shift toward increased detection from transects during late summer sampling intervals. Among plant genera, 80.3% of the variation was explained by sampling date (eigenvalue = 0.055509), and 19.7% by sampling method (eigenvalue = 0.013604; Figure 2.7B). Similarly, most samples were identified with DNA. However, plant occurrence coordinates were more centrally positioned between transects and DNA, reflecting the increased level of overlap between the two methodologies at the genera level (Figure 2.7B).

Figure 2.8. Variation in site (open circles) and plot level (closed circles) plant species richness across burn ages. Among all sampled sites and plots, plant species richness was highest at sites that were four years post fire (68 species) and lowest at sites that were one- and eight- years post fire (57 species).



Drivers of plant and bee species richness

Across all sites, plant species richness was highest four years post fire (68 species) and lowest one- and eight-years post fire (57 species). Across the 2022 and 2023 sampling years, the average plot-level genus richness value was ~42 species (Figure 2.8). Plot level plant species richness was significantly affected by burn age ($p < 0.001$, $F = 363.62$) and the interaction between burn age and severity ($p < 0.001$, $F = 158.19$; Figure 2.9). Local, plot scale species richness was highest four years post-fire at a site that burned at moderate severity. Rarefied bee genus richness across all plots was significantly affected by burn age ($p < 0.001$, $F = 78.85$), the interaction of burn age and severity ($p < 0.001$, $F = 60.56$), and plant species richness ($p = 0.0015$, $F = 10.15$; Figure 2.10). Plant species richness is positively correlated with bee genus richness, and richness values were higher at plots with older burn ages. Analysis of data from 2023 revealed similar patterns to those observed in the two-year dataset. Specifically, unrarefied bee species richness at the plot level significantly increased with increased burn severity ($p < 0.001$, z = 50.8) and plant species richness ($p < 0.001$, z = 73.5; Figure 2.11).

Figure 2.9. Interacting effects of burn age and burn severity (CBI) on localized (plot level) plant pollen species richness, showing highest plant species richness at higher severity fires with a burn age of 4 years.

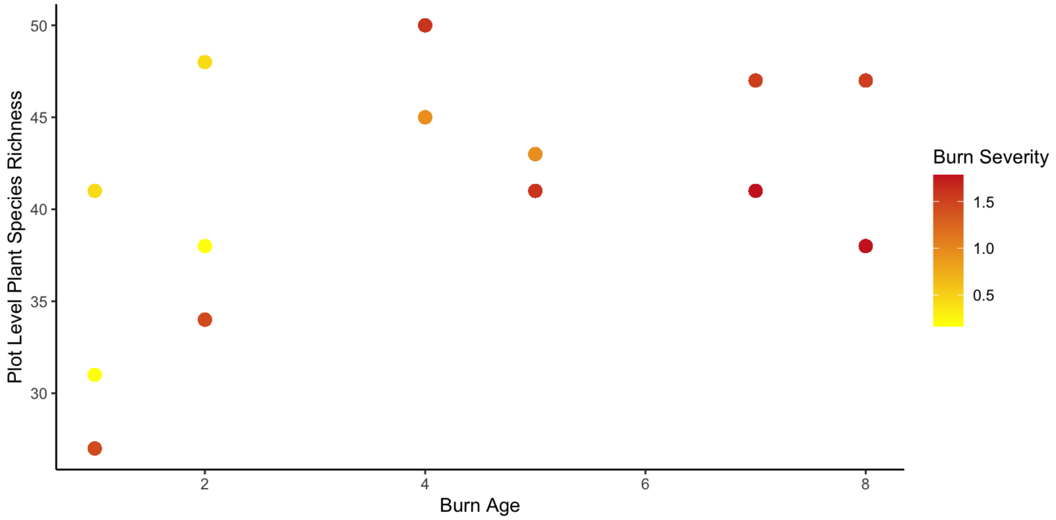
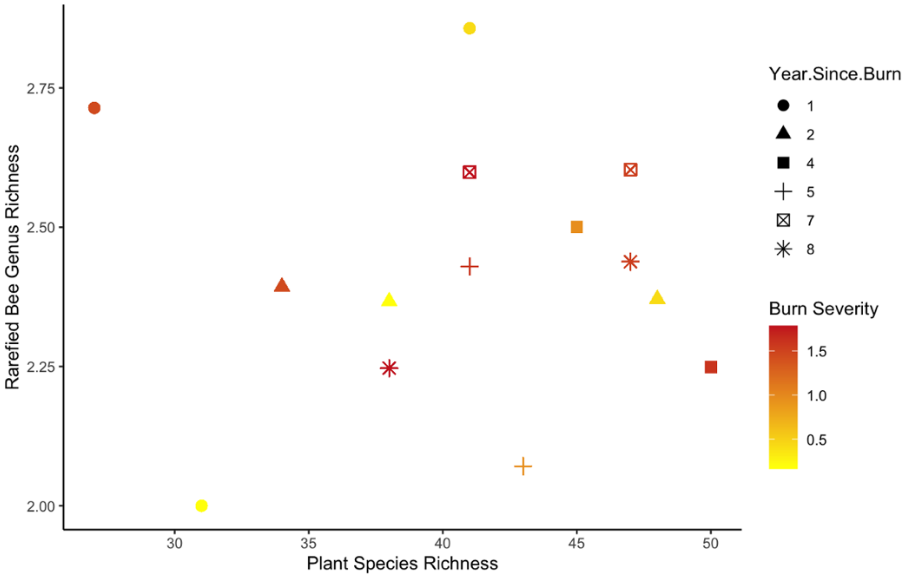


Figure 2.10. Effects of plant pollen species richness, burn severity (CBI), and burn age on rarefied bee genus richness (2022-2023). Bee genus richness tended to increase with plant genus richness. Higher burn ages generally yielded higher bee and plant richness values.



Indicator species analysis

Approximately 17.4% (24/138) of the insect pollinated plant species identified were significantly associated with a specific burn age (Table 2.3). Higher burn ages generally had more associated species, and a burn age of 7 years had the highest number of associated species (5). Additionally, 13% (18/138) of plant species were significantly associated with a specific burn severity category (Table 2.4), with plots that were burned at higher severity having fewer associated plant species.

Table 2.3. Approximately 17.4% (24/138) of the insect pollinated plant species identified from data collection in 2022 and 2023 were significantly associated ($p \leq 0.05$) with a specific burn age.

Burn Age (years)	**Associated Species**	**Test Statistic**	**p value**
1	*Phacelia linearis*	0.205	0.005
	Chamaenerion latifolium	0.134	0.010
	Sisymbrium altissimum	0.108	0.040
	Gayophytum diffusum	0.106	0.035
2	*Melilotus officinalis*	0.184	0.005
	Phacelia hastata	0.147	0.035
	Sandbergia whitedii	0.111	0.010
4	*Collomia grandiflora*	0.123	0.01
	Ceanothus sanguineus	0.103	0.05
5	*Philadelphus lewisii*	0.106	0.050
7	*Toxicoscordion paniculatum*	0.117	0.015
	Cryptantha torreyana	0.111	0.010
	Phlox hoodii	0.111	0.030
	Erigeron pumilus	0.101	0.025
	Antennaria umbrinella	0.101	0.025
8	*Crepis atribarba*	0.137	0.025
	Lithophragma glabrum	0.131	0.010
unburned control	*Senecio integerrimus*	0.120	0.015
	Linaria genistifolia	0.118	0.020
	Primula jeffreyi	0.136	0.020
	Prunus emarginata	0.136	0.005
	Lomatium dissectum	0.134	0.005
	Helianthella uniflora	0.121	0.005
	Acer macrophyllum	0.093	0.015

Figure 2.11. Effects of plant pollen species richness, burn severity (CBI), and burn age on bee species richness in 2023. Bee species richness increased with greater plant species richness and burn severity. Furthermore, plots with higher burn ages tended to have more plant and bee species richness values.

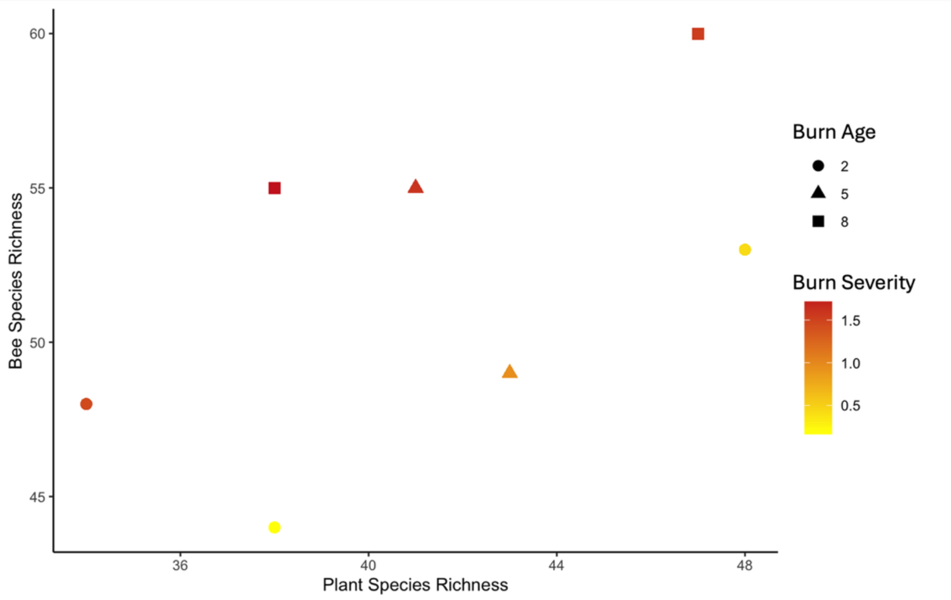


Table 2.4. Approximately 13% (18/138) of plant pollen collected was significantly associated ($p \leq 0.05$) with a specific burn severity category. Burn severity was binned into categories defined by Miller and Thode (2007).

Burn Severity Category	**Associated Species**	**Statistic**	**p value**
Low CBI=0.1-1.24	*Phacelia hastata*	0.180	0.020
	Phacelia linearis	0.109	0.030
	Sisymbrium altissimum	0.103	0.015
	Melilotus officinalis	0.092	0.035
	Erigeron speciosus	0.084	0.010
Moderate CBI=1.25-2.24	*Chamaenerion latifolium*	0.132	0.04
	Gayophytum diffusum	0.116	0.04
unchanged control CBI = 0	*Lithospermum ruderale*	0.157	0.005
	Senecio integerrimus	0.147	0.005
	Linaria genistifolia	0.146	0.005
	Primula jeffreyi	0.128	0.005
	Lomatium dissectum	0.121	0.005
	Chaenactis douglasii	0.110	0.010
	Lygodesmia juncea	0.107	0.010
	Prunus emarginata	0.105	0.005
	Helianthella uniflora	0.092	0.015
	Clematis ligusticifolia	0.083	0.035
	Arnica cordifolia	0.079	0.025

2.5 Discussion

Understanding plant-pollinator relationships is critical for successful conservation of angiosperm flora and bee faunas. We generated a list of key floral resources frequently visited by pollinators. Our results suggest that burn age, burn severity, and plant richness are the main drivers of bee richness, and should be considered by managers wishing to conserve pollinators in in fire-affected landscapes. This study is the first to use pollen deposited in pan and blue vane traps to quantify floral resources for insect pollinators and highlights the utility of trap byproducts to provide insightful ecological information.

Comparing the strict and liberal DADA2 bioinformatics thresholds

Filtering and trimming ASVs with a liberal threshold identified 15 more plant species than the strict threshold. Interestingly, there were four species/morphospecies that appeared exclusively in the strict species list: *Achillea filipendulina* and *Iva axillaris* (Asteraceae), *Penstemon davidsonii* (Plantaginaceae), and *Drymocallis* spp. (Rosaceae; Table 1). This finding indicates that, while adjustments to the filter and trimming thresholds by increasing the maxEE parameter typically results in more species identifications, some species level identifications are lost.

Liberal thresholds have the capacity to detect more rare interactions (Arstingstall et al. 2023). Indeed, of the additional plant species detected with the liberal threshold, none appeared in more than four samples. The most frequently occurring species across all samples that were added with the liberal threshold were *Vaccinium membranaceum* (Ericaceae), *Eriogonum heracleoides* (Polygonaceae), and *Drymocallis arguta* (Rosaceae), each of which appeared in four samples. It is likely that the *Drymocallis* spp. identified with the strict threshold was *Drymocallis arguta,* as identified by the liberal threshold, indicating that a finer resolution to species level identifications is possible with increased filter and trim threshold flexibility.

Tall huckleberry (*V. membranaceum*) was only detected with the liberal threshold. *Vaccinium membranaceum* is an important food source for both Indigenous peoples (Richards and Alexander 2006) and recreational gatherers (Carroll et al. 2003). The dense shrubby habitat is also used for nesting by bird species (Martin et al. 1951), and the berries are an important food source for black and grizzly bears (McLellan and Hovey 1995, Welch et al.1997, McLellan 2015). Furthermore, elk (Edge et al. 1988), deer (Klebenow 1965), moose (Pierce 1984), and other birds and small mammals eat the vegetative and woody parts of the plant. Bees in the genera *Andrena* (Andrenidae), *Apis*, *Bombus*, *Ceratina*, *Eucera*, *Habropoda*, *Nomada* (Apidae), *Lasioglossum*, *Sphecodes* (Halictidae), *Megachile*, and *Osmia* (Megachilidae) are known to visit *Vaccinium* spp. blossoms (Cane and Payne 1993, Oregon State University 2025). Thus, the detection of *V. membranaceum* has important implications ecologically, culturally, and economically, particularly considering predicted decreases in habitat suitability due to climatic shifts along much of its native range (Prevéy et al. 2020).

While liberal filter and trim thresholds revealed more rare interactions than strict thresholds, there were some key plants present in the study landscape that were not detected in either plant list. Specifically, three female and two male specimens of the rare pollinator *Dufourea dilatipes* (Halictidae) were collected in blue vane and pan traps in 2022 and 2023 (Maust et al. 2025). This bee species is oligolectic on Mariposa lilies (*Calochortus* spp.), of which two species, *Calochortus lyalli* and *Calochortus macrocarpus*, were recorded with field based transects. However, no *Calochortus* pollen was detected by either bioinformatics threshold. While it is possible that the three females *D. dilatipes* collected had no pollen in their scopa when they were captured, a more likely conclusion is that pollen occurred in too small a quantity to be effectively detected using DNA metabarcoding. This highlights the need for targeted, field-based surveys to ascertain rare plant-pollinator relationships and ensure effective documentation of rare species with oligolectic relationships.

As predicted, some thermophilic woody shrubs were an important resource for pollinators in post-fire landscapes. Specifically, snowbrush ceanothus (*Ceanothus velutinus*) occurred in more than 60 of the 299 samples. However, antelope bitterbrush (*Purshia tridentata*) only appeared in 14 samples. Interestingly, herbaceous perennials like *Lupinus* (110 samples) and *Phacelia hastata* (88 samples) were more frequently detected. These findings indicate the secondary importance of thermophilic woody shrubs to drought tolerant herbaceous perennials for native insect pollinators in post-fire landscapes.

Indeed, the most frequently occurring herbaceous perennial was yarrow (*Achillea millefolium*), indicating its value as a key floral resource for pollinating insects. There are many varieties of both native and non-native yarrow in the Pacific Northwest, USA and it is occasionally considered a pest due to its ability to widely reproduce via both rhizomes and seeds (Reed 1970, Robocker 1977). However, yarrow can withstand long periods of drought with little to no precipitation and is generally thermophilic (Clausen et al. 1941). It also has a long bloom duration and can be detected from February to October (Burke Herbarium 2025), thus providing floral resources for pollinating insects over an extensive temporal and climatic range (Warwick and Black 1981). As wildfires in eastern Washington, USA become more frequent and intense (Marlon et al. 2012), xeric herbaceous perennials such as yarrow may become increasingly important resource for native pollinators.

Genetic analysis of pollen from pan and blue vane traps has the potential to amplify wind pollen as well as bee-collected pollen due to the open nature of the traps during deployment in the field. Future work using this methodology should be sure to cross-reference findings with known pollination mechanisms of local plant species to ensure wind-pollinated plants are removed from analysis. However, while wind-pollinated plants have not evolved to rely on insect pollination for successful reproduction, there are records of bee visitation and pollen collection from many wind-pollinated plants (Saunders 2017) such that the use of wind-dispersed pollen by bees cannot be fully discounted. However, we contend that the wind-pollinated plants we identified (Supplemental Table 2.1) were not likely be main food supplements for native pollinator species and therefore were not counted in measures of pollen richness to ascertain high-priority plants.

Additional limitations of this methodology include the lack of clarity regarding specific plant-pollinator relationships. Pan and blue vane traps caught insects among many insect orders including Coleoptera, Diptera, Hemiptera, Lepidoptera, and Raphidioptera. Thus, pollen collected in traps cannot be attributed to bees exclusively, nor to any specific bee species or genera. While bees are likely responsible for depositing most of the pollen in traps due to their external pollen-carrying morphological features (i.e. corbicula, scopa), other insects will occasionally carry pollen from flower to flower on their bodies, resulting in effective pollination. Despite this, we maintain that this is an effective way to gather additional information from popular trapping methodologies with a product that would otherwise be considered bycatch.

The plants species identified with DNA metabarcoding in this study may be used to inform the composition of seed mixes used in post-fire restoration to support native pollinators and angiosperms (Figure 2.6). Specifically, the twenty most frequently detected insect pollinated species could be considered high priority plants for plant-pollinator network conservation in dry forest landscapes (Figure 2.5).

Comparison of DNA and field data

DNA metabarcoding is known to accurately record more plant-pollinator interactions than field-based surveys alone (Milla et al. 2022). It is therefore unsurprising that the genetic analyses conducted in this study detected more plants than bi-monthly 1×10-m field transect surveys. Most of the variation in plant genus and species richness was explained, unsurprisingly, by sampling date, given variation in plant species phenology. In a correspondence analysis, we visually detected overlap between transect and DNA detection methods, particularly at the genus level (Figure 2.7; Supplemental Figure 2.2). At the species level, early season plant species were better detected with DNA, while late season sampling periods tended to be well represented by both transect and DNA analyses. This is likely a result of declining species richness across the landscape during the warmest, driest parts of the summer when only thermophilic species (i.e. *Achillea millefolium*) remain in bloom (Figure 2.2).

While there appears to be little overlap between the species detected in transects and with DNA metabarcoding (Supplemental Figure 2.2), it is possible that matches among the two datasets are underrepresented due to plant hybridization. For example, arrowleaf balsamroot (*Balsamhoriza sagittata*) was abundant in the landscape and was identified with field based transects. However, the species Hooker's balsamroot (*Balsamhoriza hookeri*) was assigned to the ASVs generated by genetic analysis. *Balsamhoriza hookeri* and *B. sagittata* are known to hybridize, and both species are reported to occur in Chelan County (Burke Herbarium 2025). While the ITS2 region of the *Balsamhoriza* on the landscape may match *B. hookeri* genetically, the species more closely resembles *B. sagittata* morphologically. Other such species-level disagreements may be present among the two datasets, thus underrepresenting the overlap between transect and DNA-derived species lists. This discrepancy highlights the need for complete regional libraries that reflect the diversity of plants on a landscape for effective taxonomic assignment at the species level.

Drivers of plant and bee species richness

In burned landscapes, plant species richness was highest four years post fire, after which it generally remained constant across burn ages (Figure 2.8). Our data supports the hypothesis that burn age and burn severity are important drivers of plant-pollinator network richness in post fire landscapes. Analyses at the local plot scale indicated that plant species richness increased with burn age and the interaction of burn age and severity (Figure 2.9), likely as a result of slower recolonization rates for mesic plant species following higher severity fire. Additionally, plant species richness, burn age, and the interaction of burn age and burn severity were positively associated with bee genus richness (Figure 2.10). Similarly, when the robust data from 2023 was assessed, bee species richness was positively associated with burn severity and plant species richness (Figure 2.11). Positive associations between plant and bee richness are well documented (Grundel et al. 2010, Rhoades et al. 2018). Overall, plots with moderate to high burn severities had higher plant species richness values. A suite of burn ages and severities support different plant-pollinator networks, suggesting that a mosaic of burn ages across a landscape maximizes angiosperm and pollinator diversity.

Indicator plant species

Understanding indicator species associations with post-fire landscape characteristics provides novel insights for plant-pollinator network conservation. As hypothesized, we did observe specific plant species associations with burn ages (e.g., 1, 2, 4, 5, 7, or 8 years, or unburned; Table 2.3) and burn severity categories (e.g., unchanged, low, moderate; Table 2.4). The number of plant associations were generally uniform across burn age and ranged from 1 (burn age of 5) to 5 (burn age of 7) species. A total of 7 plant species were associated with unburned sites, including Bigleaf maple (*Acer macrophyllum*; Sapindaceae), which is generally associated with mesic western landscapes in Washington, USA. *Acer macrophyllum* takes many years to establish, reflecting the vegetative effects of fire exclusion in unburned control sites.

Five plant species were significantly associated with low burn severity, and only two were associated with moderate severity. Of these two, *Chamaenerion latifolium* (dwarf fireweed; Onagraceae) is a pioneer genus known for its rapid colonization of post fire landscapes (Gustafsson et al. 2021). Its pyrophytic nature is reflected by its association to sites with higher severity fire effects. Eleven plant species were significantly associated with unchanged landscapes (CBI = 0). Six of these eleven plant species also appear in the unburned category for the burn age ISA (i.e. *Lomatium dissectum* (Apiaceae), *Helianthella uniflora* and *Senecio integerrimus* (Asteraceae), *Linaria genistifolia* (Plantaginaceae), *Primula jeffreyi* (Primulaceae), *Prunus emarginata* (Rosaceae)), indicating agreement between the two analyses.

2.6 Conclusion

DNA metabarcoding of pollen from traps revealed herbaceous perennials such as *Achillea millefolium*, *Lupinus* spp., and *Phacelia hastata* to be important floral resources for post-fire pollinator communities. Understanding the foraging needs of pollinators in post-fire landscapes informs plant-pollinator network conservation, particularly considering predicted increases in fire frequency and severity due to the ongoing fire deficit in western North America. Future work should assess the nutritional utility of herbaceous perennial pollen, and model projected range shifts under various climate change scenarios.

Burn age and burn severity were the main drivers of plant and pollinator richness in post fire landscapes, and plant species richness increased with burn age and burn severity. Unsurprisingly, plant richness was found to be positively associated with bee richness. Bee species richness varied among burn severities, supporting management for patch mosaics and landscape heterogeneity as a fundamental principal of the reintroduction of fire to xeric landscapes. The findings of this study may inform seed mix compositions for use in post-fire restoration efforts to promote resilient plant-pollinator networks in disturbed landscapes.

Additional Information

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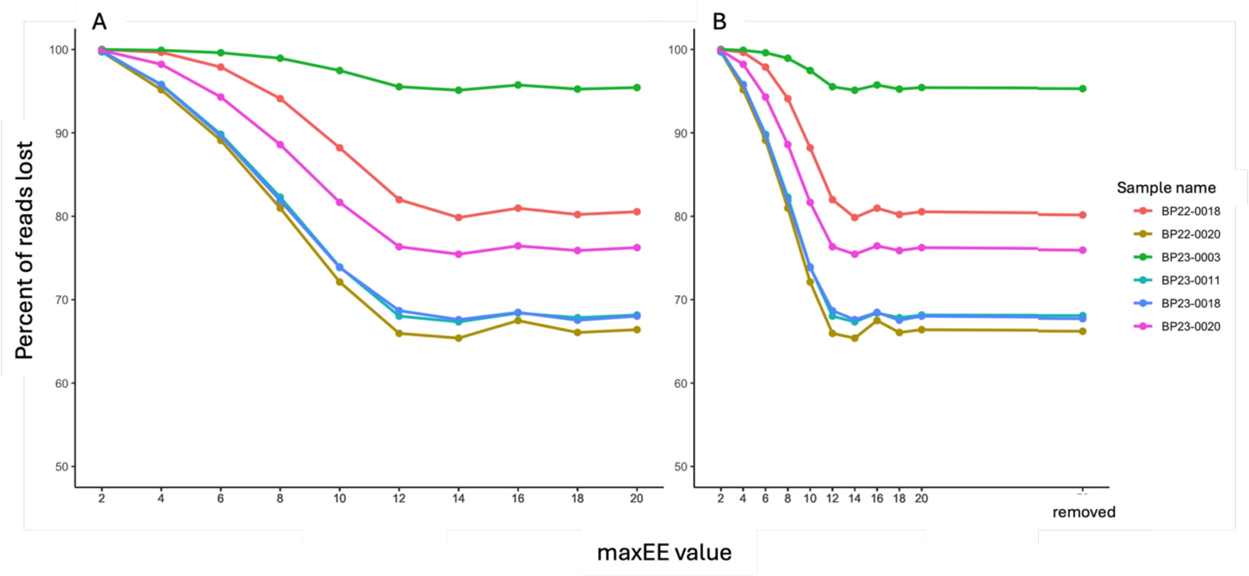
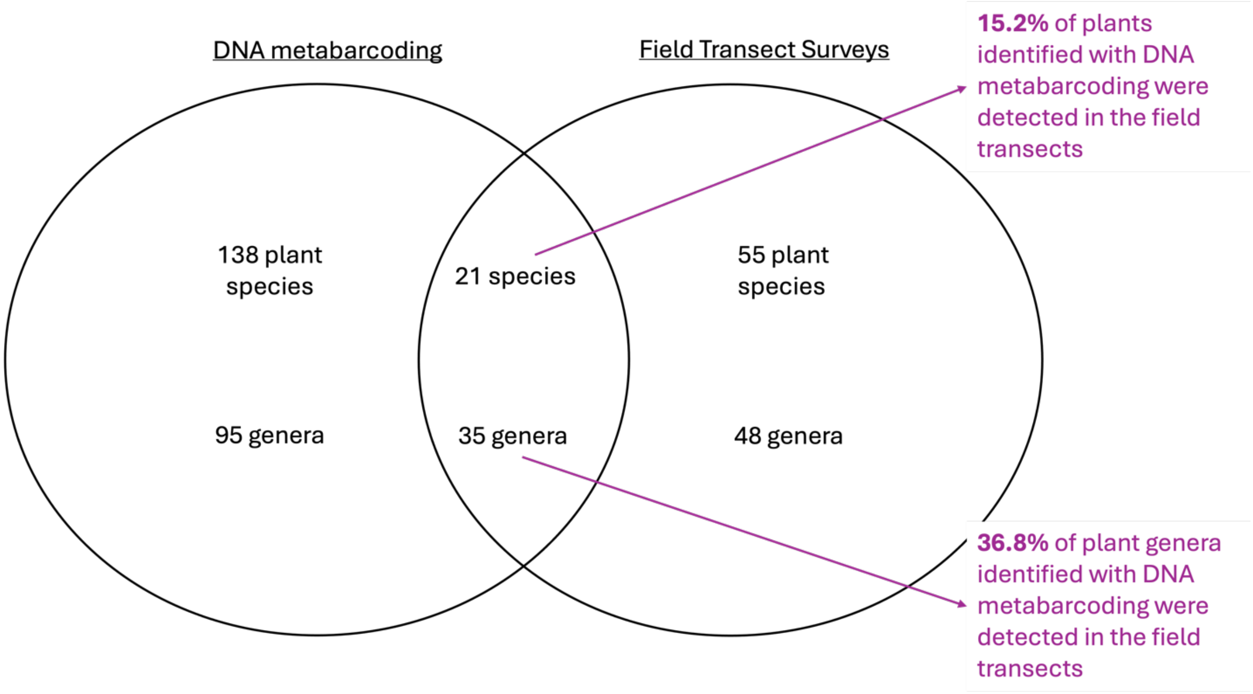
2.7 Appendix B

Supplemental Figure 2.1. Justification for the filterandtrim() maxEE threshold selection. Threshold selection was iteratively determined by analyzing the percent of reads that were lost from six individual samples (indicated by color) along a range of maxEE values in DADA2. The maxEE() parameter is the expected errors calculated by summing up the probability of an error based on the quality scores at all remaining bases in a read (Callahan 2016). MaxEE() parameter values of 2-20 were input in increments of 2 to iteratively assess the effects on the percent of reads removed from the dataset (A). When the parameter was omitted, the percent lost was roughly equivalent to a maxEE value of 12 (B). Because a maxEE value of 8 was the approximate median, we adopted it as our liberal threshold. Furthermore, a previous study tested multiple iterations of filterandtrim() with maxEE values between 1 and 10 and found that maxEE value of 8 yielded the best results (Rolling et al. 2022).



Supplemental Figure 2.2. Comparison of DNA metabarcoding and field data from transect surveys. More plant species and genera were detected with DNA metabarcoding than with randomly placed 1×10-m transects in the field, reinforcing known limits of field transect surveys in ascertaining complete plant-pollinator networks and accurate species richness values (Milla et al. 2022). Mis-identification of plants, particularly at the species level, was more likely to occur in the field than with DNA metabarcoding due to hybridization within plant genera, temporal and spatial limitations, and taxonomic inexperience.



15.2% of plants identified with DNA metabarcoding were detected in the field transects

36.8% of plant genera identified with DNA metabarcoding were detected in the field transects

Supplemental Table 2.1. A complete list of wind pollinated plants that were considered to be airborne contamination, as derived from the strict (maxEE=2) threshold. While these plants have evolved to be predominantly wind pollinated, we have indicated previous reports of bee visitation and pollen collection for some plant species.

Latin Name	Common Name	Distribution in Washington	# Samples	Insect Pollination
Abies lasiocarpa	subalpine fir	native	1	*Apis mellifera* on *Abies* (O'Neal & Waller 1984)
Abies spp.	fir	native	2	
Agrostis capillaris	colonial bentgrass	introduced	3	
Agrostis gigantea	black bentgrass	introduced	1	
Alnus incana	mountain alder	native	17	*Bombus* and *Andrena* on *Alnus incana* (Moisan-Deserres et al. 2014)
Alnus rhombifolia	white alder	native	3	*Apis mellifera* on *Alnus* (Girard et al. 2012)
Alnus spp.	Alder	native	12	
Alopecurus aequalis	little foxtail	native	1	
Arceuthobium campylopodum	western dwarf mistletoe	native	8	
Arceuthobium douglasii	Douglas-fir dwarf mistletoe	native	1	
Artemisia furcata	forked wormwood	native	1	
Artemisia spp.	mugwort, sagebrush, wormwood	unknown	1	
Artemisia tilesii	cascade wormwood	native	1	
Avena sativa	cultivated oats	agricultural	1	
Bromus hordeaceus	soft chess	introduced	2	
Bromus tectorum	cheatgrass	introduced	10	
Calamagrostis canadensis	bluejoint reedgrass	native	1	
Callitropsis nootkatensis	alaska yellow cedar	native	49	
Carex spp.	sedge	unknown	3	
Ceratodon purpureus	fire moss	native	4	
Chenopodium album	pigweed	unknown	1	
Dactylis glomerata	orchard grass	introduced	1	
Equisetum variegatum	variegated horsetail	native	25	
Humulus lupulus	hops	agricultural	7	*Celastrina humulus* on *Humulus lupulus* var. *neomexicanus* (Mooney et al. 2023)
Juniperus communis	common juniper	native	2	
Lolium perenne	perennial ryegrass	introduced	2	

Pascopyrum smithii	western wheatgrass	native	1	
Phalaris arundinacea	reed canarygrass	introduced	1	
Picea engelmannii	englmann spruce	native	10	*Apis mellifera* on *Picea* (Girard et al. 2012)
Pinus contorta	lodgepole pine	native	12	*Apis mellifera* on *Pinus* (Girard et al. 2012); *Bombus* and *Andrena* on *Pinus* (Moisan-Deserres et al. 2014); *Osmia rufa* on *Pinus* (Teper & Bilinski 2009)
Pinus ponderosa	ponderosa pine	native	16	
Pinus spp.	pine	native	8	
Plantago lanceolata	English plantain	introduced	2	
Plantago major	common plaintain	introduced	3	
Poa pratensis	Kentucky bluegrass	both native and introduced	1	
Poa spp.	bluegrass	unknown	15	
Prunus spp.	fruit tree	unknown	1	*Osmia lignaria* on *Prunus* (Bosch et al. 2006); (Delaplane and Mayer 2000)
Pseudotsuga menziesii	Douglas fir	native	8	*Apis mellifera* on *Pseudotsuga* (O'Neal & Waller 1984)
Secale cereale	cultivated rye	introduced	7	
Taxus brevifolia	pacific yew	native	3	
Thuja plicata	western red cedar	native	6	*Apis mellifera* on *Thuja occidentalis* (Avitabile 1982)
Triticum aestivum	bread wheat	agricultural	5	
Tsuga mertensiana	mountain hemlock	native	5	
Ulmus pumila	siberian elm	introduced	3	*Osmia rufa* on *Ulmus* (Teper & Bilinski 2009); *Apis mellifera* on *Ulmus* (Baum et al. 2004)
Urtica dioica	stinging nettle	introduced	4	James et al. 2015

Table 2.1 References

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Chapter 3. Frequent fire increases nesting success and oviposition in two solitary bee species

3.1 Abstract

Understanding plant-pollinator relationships is critical for successful conservation of angiosperm flora and bee faunas. Specifically, quantifying changes in pollinator demography can provide insights for bee community health. We deployed nesting materials across three burned landscapes and one unburned control to quantify plant-pollinator relationships using DNA metabarcoding. We also quantified native wood-cavity-nesting bee reproductive success using nest boxes and assessed *Osmia lignaria propinqua* and *Osmia kincaidii* as exemplar species. DNA metabarcoding of pollen from nest boxes revealed herbaceous perennials such as *Phacelia hastata* and *Epilobium brachycarpum* to be important floral resources for wood-cavity-nesting pollinators in post fire landscapes. Our results suggest that burn age is the main predictor of wood-cavity-nesting bee oviposition, with higher ovipositional rates in burns ages 1-5. This study highlights the importance of fire reintroduction for wood-cavity-nesting bee reproductive success and native pollinator conservation in dry forest landscapes.

3.2 Introduction

Wildfire is a driver of forest structure and function in western North American dry interior forests. Fires are projected to become more frequent and intense in coming decades (Stephens et al. 2020, Hessburg et al. 2021) and, despite the occurrence of large fires, western North America remains in a fire deficit (Marlon et al. 2012). Climate driven disturbances of increased frequency and severity affect both biotic and abiotic conditions, with cascading effects across ecosystems and trophic levels. Specifically, fire shapes the food and habitat resources for many forest animals.

One particularly important taxon to monitor amongst these shifts is pollinating insects, specifically bees, whose declines have been globally documented (Kearns et al. 1998). While the effects of wildfire on ground nesting bees have been reported to be minimal (Cane and Neff 2011), wood-cavity-nesting bee species may be more strongly affected by fire as a result of damage to nesting materials and increased mortality (Williams et al. 2010, Simanonok and Burkle 2019). Above-ground-nesting bees (namely *Osmia* and *Megachile*, Megachilidae) also serve as reliable and efficient pollinators in agriculture (Winfree et al. 2007). Therefore, it is important to understand the effects of disturbance on above-ground nesting bee biology given their ecological and economic importance.

The reintroduction of wildfire to fire-excluded landscapes is often positive for forest health and pollinator habitats (Cane and Neff 2011, Peralta et al. 2017, Rodriguez and Kouki 2017, Galbraith et al. 2019, Teixido et al. 2024). Wood-cavity-nesting pollinators rely on existing galleries from wood boring beetles (Westerfelt et al. 2015, Sydenham et al. 2016) and benefit from increases in coarse woody debris post fire (Gelles et al. 2022). However, post fire vegetative recovery takes time, and is a process that occurs slower in xeric ponderosa pine systems with increasing burn severity (Chen et al. 2010, Haffey et al. 2018, Korb et al. 2019). Consequently, wood-cavity-nesting pollinator species in ponderosa pine forests that rely on vegetative and floral resources likely face challenges recolonizing following disturbance. Despite this, post-fire landscapes typically support more cavity nesting bees compared to unburned areas. Furthermore, the proportion of wood-cavity-nesting bees increases with increasing burn severity and available nesting habitat (Chapter 1).

Many studies have quantified the community composition of native pollinators following disturbance (Chapter 1, Rodríguez and Kouki 2017, Rivers et al. 2018, Galbraith et al. 2019, Gelles et al. 2022). Furthermore, studies on demography in post-fire landscapes highlight patterns in bee foraging behavior (Peralta et al. 2017), nesting success (Simanonok and Burkle 2019), and sex ratios (Galbraith et al. 2021). Quantification of fire effects on other demographic traits, such as reproductive success of wood-cavity-nesting bee species, is lacking, yet has critical implications for persistence following fire and pollinator conservation.

Wood-cavity-nesting bee species represent an exemplar study system for demographic research because their nesting behavior can be quantified under field conditions using nest boxes. Hollow tubes within nest boxes are filled by wood-cavity-nesting bees from back to front, and each egg is laid with a pollen provision separated by soil or other debris (Danforth et al. 2019). Unlike many insect species that may oviposit once and in one egg mass (Cury et al. 2019), cavity nesting bees oviposit one egg per pollen provision. Thus, nest boxes provide information on the cavity nesting species present on the landscape (Peralta et al. 2017, Simanonok and Burkle 2019), and the specific pollen resources used by those species (Abel and Wilson 1998, Cane et al. 2011, MacIvor 2017). Nest boxes also provide a method to measure oviposition and developmental success (Sexton et al. 2021, Westreich et al. 2023a), and the effects of natural enemies and fungal pathogens on bee progeny (Kamke et al. 2008, Mayr et al. 2020). Lastly, because cavity nesting solitary bees generally forage <600 m from their nests (Gathmann and Tscharntke 2002), nest boxes can provide localized information about habitat resources and quality.

DNA metabarcoding uses high throughput simultaneous sequencing methods to identify a sample containing genetic material from multiple species (Cristescu 2014, Hawkins et al. 2015) and records more plant-pollinator interactions than field-based surveys alone (Milla et al. 2022). Pollen metabarcoding also has the potential to highlight the fungal and bacterial symbionts of bees

(Westreich et al. 2023b) as well as foraging patterns (Bosch et al. 2009) and can quantify temporal variation in plant community composition (Wilmshurst et al. 2014).

In this study, we quantified oviposition and successful larval development in both released and wild wood-cavity-nesting bees across post-burn landscapes 1-8 years since burn, and at one unburned control. We also used DNA metabarcoding on bee-collected pollen to identify plants visited by wood-cavity-nesting bees in post-fire landscapes. We hypothesized that pollinator reproductive success would be positively correlated with increases in pollen richness, which is greatest approximately four years post-fire (Chapter 2). We also hypothesized that, at the species level, cavity nesting bee oviposition and larval developmental success would be highest 3-4 years following fire and be positively associated with burn severity and burn age. Lastly, we hypothesized that increases in available nesting habitat would positively affect nesting success in post-fire landscapes.

3.3 Materials and Methods

Study system and field methods

We established study areas within three fires that burned in Chelan County, Washington, USA in 2015, 2018, and 2021. In addition, we established a fourth study area in which fire has been suppressed since 1968 as an unburned control for comparative purposes (Figure 3.1, Table 3.1). All study areas were in the Okanogan-Wenatchee National Forest, where the dominant trees are ponderosa pine (*Pinus ponderosa*) and Douglas-fir (*Pseudotsuga menziesii*). Dominant understory plants include bearberry (*Arctostaphylos uva-ursi*), wax currant (*Ribes cereum*), snowbrush ceanothus (*Ceanothus velutinus*), and arrowleaf balsamroot (*Balsamorhiza sagittata*).

Figure 3.1. Locations of eight study plots within four study sites in the Okanogan-Wenatchee National Forest in Washington, USA. All study areas were established in Chelan County within the Chelan and Entiat USDA Forest Service Ranger Districts. Data was collected from three burned areas and one "control" that was unburned since 1968. Fire severity is displayed as Composite Burn Index (CBI), and all study areas were burned by mixed-severity fire effects. Each 10×10-m pixel has been categorized as unchanged (CBI=0-0.1), low (CBI= 0.101-1.24), moderate (CBI= 1.241-2.24), or high (CBI= 2.241-3) severity.

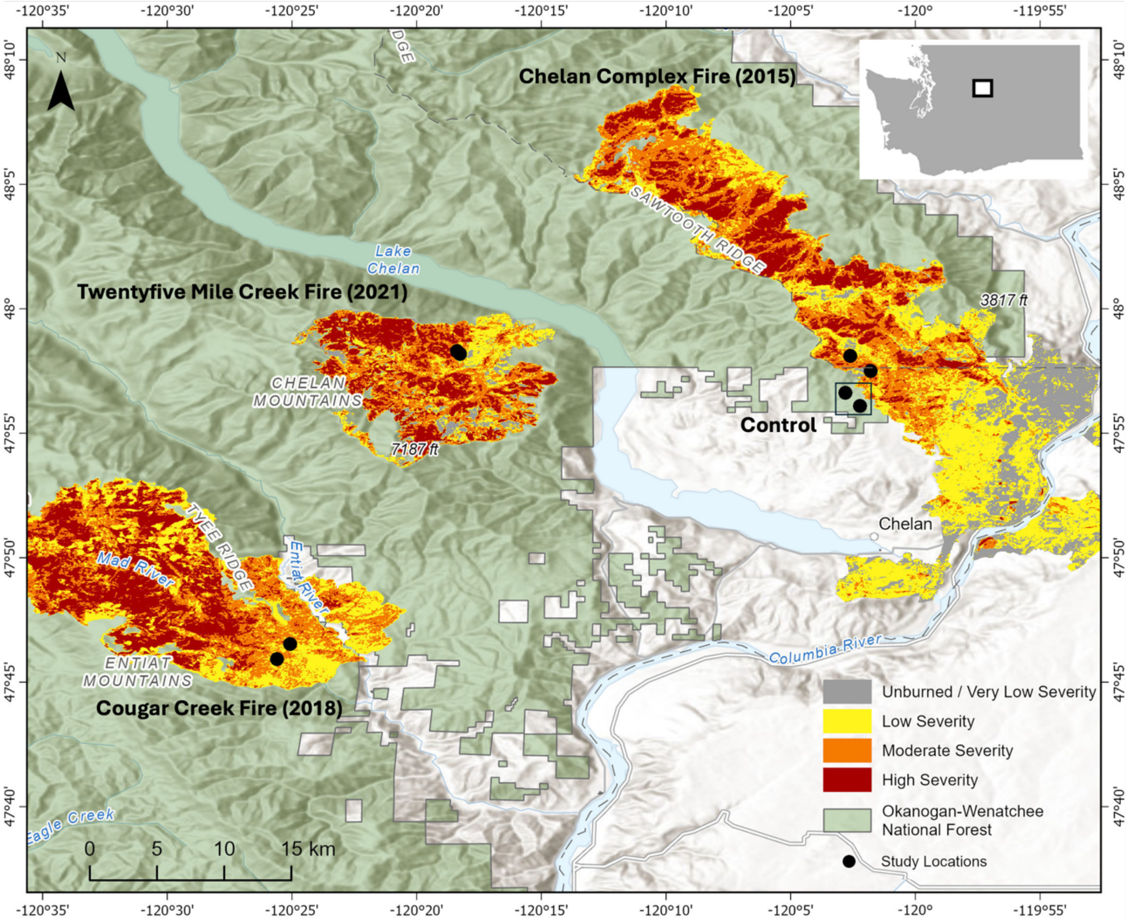
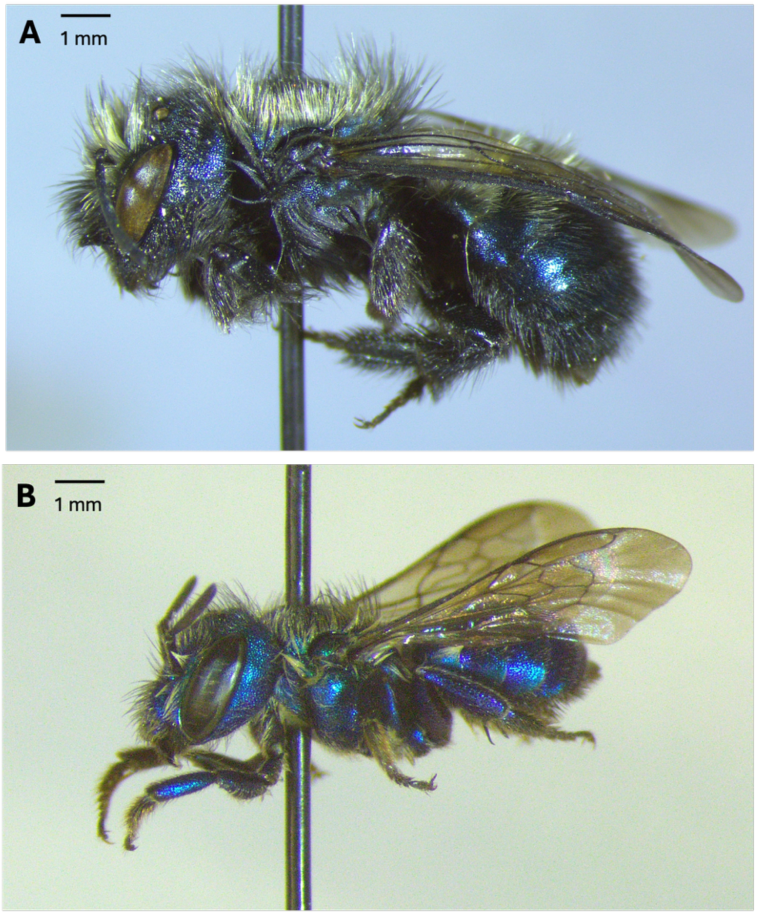


Table 3.1. Summary of site characteristics. Each of the four sites had two plots, where wood-cavity-nesting bees were sampled with nest boxes from April until August in 2021-2024.

Fire name	**Year of burn**	**Burn age**	**Mean burn severity ± SD**	**Mean nesting habitat ± SD** (abundance of coarse woody debris and snags)
Twentyfive Mile Creek	2021	1-3	1.39 ± 0.208	8.5 ± 8.5
Cougar Creek	2018	3-6	1.24 ± 0.0288	20 ± 4
Chelan Complex	2015	6-9	1.33 ± 0.363	14 ± 8
Unburned Control	(1968)	(53-56)	NA	1

In 2021 and 2022 we sourced *Osmia lignaria propinqua* Cresson (Figure 3.2A) pupae from a commercial producer in western Washington (Watts Solitary bees, Bothell, WA) for field releases. *Osmia lignaria propinqua* is a wood-cavity-nesting bee native to western North America (Rust 1974) and commercially reared. Pupae were deployed in late April when average daily temperatures were consistently >10°C. Approximately 200 pupae were retained under laboratory conditions to verify adult emergence, and to ascertain a baseline sex ratio. Across both years, adults emerged from >81% pupae with a 7:1 female:male sex ratio.

Figure 3.2. The two most abundant pollinators to colonize the deployed nesting materials were the Blue Orchard Mason Bee, *Osmia lignaria propinqua* (A) and Kincaid's Mason Bee, *Osmia kincaidii* (B).



Nest boxes were installed in two replicated plots per study area and were ≥ 600 m apart from each other to account for known cavity nesting bee foraging ranges and to limit the effect of spatial autocorrelation in data (Gathmann and Tscharntke 2002). Four 2 m T-posts were positioned at each plot in a south-facing orientation. Two nest boxes, each made up of a 7.62 cm diameter PVC pipe approximately 15 cm long with the nest closed at one end with a PVC cap, were attached to each T-post. One pupal release capsule was attached to each nest box (Supplemental Figure 3.1). Each pupal release capsule, consisting of a 2.54 cm diameter PVC pipe approximately 13 cm long, contained ~100 *O. lignaria propinqua* pupae for a total of 4,800 pupae released across all three study sites in 2021 and 6,400 pupae released across four study sites in 2022.

In 2021, each nest box contained twenty 8mm paper nesting tubes, which is the preferred diameter for *O. lignaria propinqua*. Beginning in 2022, we used nest boxes that contained paper nesting tubes that were 4, 6, 8, or 10mm in diameter to capture a variety of wild wood-cavity-nesting bees. Prior to deployment, each paper tube was pre-cut (excluding 4 mm tubes) lengthwise to ensure bee provisions, larvae, and pupae could be subsequently counted and extracted in the lab without damage. Approximately 80 nesting tubes of each size were deployed per plot. Nest boxes were inspected monthly from late April until their collection in early August. Filled nesting tubes were retrieved and replaced to ensure adequate nesting resources for pollinators with differing phenologies.

Processing field-collected data

Collected nesting tubes were labeled with the collection date, study site, and plot number and left to develop to cocoons under laboratory ambient conditions until November. Tubes were then opened using sterile tools and nitrile gloves to minimize contamination. Nesting was quantified according to the number of tubes with nesting success, nesting attempts (i.e., denoted by an unfinished pollen provision, or remnants of partial mud partitions within the tube), or no nesting activity. Within the filled nesting tubes, the number of pollen provisions was quantified as a proxy for oviposition. The number of eggs that successfully hatched and developed into larvae was also assessed. The proportion of eggs that successfully completed development to a cocoon was used as a measurement of developmental success.

In 2022, 2023, and 2024, pollen provisions from nest boxes that were not consumed by larvae (i.e., such as the case of failed egg hatch or early instar death) were removed from nesting tubes using sterile forceps and stored in 15 mL microcentrifuge tubes for genetic analysis. The associated site, plot, year, and nest tube diameter were recorded, and each sample was given a unique identifier. Pollen samples were stored in a -80°C freezer prior to DNA extraction.

Pupal cocoons were then removed from their nesting tubes and separated from natural enemies and fungal pathogens with a light sterile water wash. Cleaned cocoons were placed in labeled petri dishes and maintained in Percival Scientific biological incubators (Perry, Iowa, United States) to undergo an artificially condensed winter period. This process ensured that overwintering individuals were exposed to diapause termination cues, such as exposure to a chilling period followed by warming temperatures (Stephen et al. 1969). Incubator temperatures were decreased from 20°C to 0°C by decreasing the temperature by 5°C each week and then increased by 5°C each week and maintained at 20°C until adult emergence. Adults were removed from their respective dishes, pinned, and labeled for identification. Each emerged insect was identified to the species level using the following taxonomic keys: Michener 1939 (*Ashmeadiella*), Sheffield et al. 2011 (*Megachile*), Gardner and Gibbs 2022 (*Lasioglossum*)and Sandhouse 1939 (*Osmia*). Due to a multitude of factors that can contribute to overwintering mortality, emerged adults were only used for identification and the establishment of plant-pollinator relationships.

Additional field variables

We quantified burn severity using predicted Composite Burn Index (CBI) values generated in a 10×10-m scale with Google Earth Engine using methodology developed by Parks et al. (2019) (Figure 3.1). Values of CBI range from 0-3, where higher values indicate increased burn severity and higher overstory tree mortality (Key and Benson 2006). Because mixed severity fires contain a range of spatially heterogenous fire effects (i.e. areas of minimal fire effects and areas of greater overstory tree mortality), we used the mean and standard deviation of CBI values within a 350-m radius of each plot center to represent the range of fire effects at each study area. Burn severity data processing was performed in ArcGIS Pro 3.3.1 (Environmental Systems Research Institute, Inc., Redlands, California). Additionally, potential nesting habitat was quantified in a 50-m radius from each plot center by tallying snags and coarse woody debris. Both snags and coarse woody debris are critical habitat resources for wood-cavity-nesting bees (Potts et al. 2005, Grundel et al. 2010, Gelles et al. 2022).

DNA extraction and PCR amplification

To account for variation among provisional pollen within each nesting tube, pollen samples were homogenized by vigorously vortexing and inverting the sample tubes for at least 1 minute. A single 25 mg subsample was collected from each homogenized sample for DNA extraction. DNA was extracted from pollen samples using the DNeasy Plant Mini Kit (Cat. No. 69106, Qiagen, Germany) following the standard kit protocol with the exception of the tissue disruption step, which was omitted (Qiagen 2016). Primers ITS-S2F (5'- ATGCGATACTTGGTGTGAAT-3') (Chen et al. 2010) and ITS4R (5'TCCTCCGCTTATTGATATGC-3') (White et al. 1990) were chosen to amplify a ~340 base pair (bp) fragment of DNA from the ITS2 region. The primers were modified with an addition of Illumina's sequencing adapter nucleotide chains at the 3' ends, notated in bold: ITS-S2F (5'-**TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**ATGCGATACTTGGTGTGAAT-3'), ITS4R (5'**GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**TCCTCCGCTTATT GATATGC-3').

Initial amplicon polymerase chain reactions (PCRs) were 25μL in volume, consisting of 12.5μL of Multiplex Master Mix (Qiagen), 1.25μL of each primer (10μM starting concentration), and 10μL of DNA extract. Amplicon PCR conditions were the following: 1) an initial 95°C hot-start denaturing step for 15 minutes; 2) 35 cycles of 95°C for 30s, 55°C for 30s, and 72°C for 60 seconds; and 3) a final elongation step of 72°C for 10 minutes. Following amplification, samples were purified using SPRI beads, then quantified using a Qubit fluorometer with a dsDNA HS kit (Life Technologies, Carlsbad, CA, USA). Samples concentrations were diluted to 10 ng/μL of DNA to be within a normalized range, all samples under 2 ng/μL were considered failed. Cleaned, normalized PCR products then underwent index PCR, where unique dual indexes (IDT for Illumina) were attached to the 5' and 3' ends of the amplicons within each reaction. Index PCRs took place in 10μL reactions, with 5μL of Multiplex Master Mix (Qiagen), 1μL of each i5 and i7 index, 2μL of PCR product, and 1μL of RNAse-free water. Index PCR thermal cycling conditions were as follows: 1) an initial 95°C hot-start denaturing step for 15 minutes; 2) 8 cycles of 95°C for 30s, 55°C for 30s, and 72°C for 30 seconds; and 3) a final elongation step of 72°C for 5 minutes. Following index PCR, samples were pooled by PCR plate, SPRI bead-cleaned, quantified, normalized, then pooled into one final library. The pooled library was subsequently sequenced at Northwest Genomics Center (Seattle, Washington, USA) with the Illumina MiSeq System (Illumina V3 600 Flow Cell). Illumina software was used to demultiplex barcodes and sort reads to their corresponding samples.

Statistical analysis

Bioinformatics and taxonomic assignment

Amplicon sequence variant (ASV) generation was accomplished using the Divisive Amplicon Denoising Algorithm 2 (DADA2) bioinformatics pipeline with the default parameters (Callahan et al. 2016). Primer sequences were trimmed from raw reads using the command line tool cutadapt. Samples for which all sequences were removed during the trimming and denoising process (e.g., negative controls) were removed from analysis. No substantial contamination was detected from extraction and PCR negative controls.

Because database selection is known to inform taxonomic assignment, and specialized local databases perform better (Arstingstall et al. 2021), taxonomy was assigned to the ASVs using a Chelan County species list aggregated by the Washington Native Plant Society (2023). Sequences for this nucleotide database were generated with GenBank using the blastn() function in DADA2 (Callahan et al. 2016). Our county-specific database was supplemented with species data obtained from iNaturalist (2025) to account for potential agricultural and horticultural floral resources. ASVs with matches meeting strict blastn() criteria were identified and applied using the assignTaxonomy() function in DADA2. To limit errors across taxonomic assignments, the minimum bootstrap confidence interval needed to assign taxonomy at any level was set to 80% using the minBoot parameter.

Sample occurrence data was visualized in ggplot with the R package treemapify (Wilkins 2023). Plant pollinator network visualizations were generated using the plotweb function in the R package bipartite (Dormann et al. 2009). All other figures were developed using the function ggplot() in ggplot2 (Wickham et al. 2024). All analyses were conducted in R version 4.4.2 (R Core Team, 2025).

Wood-cavity-nesting bee reproductive success

We considered oviposition, larval development, and nesting success of wood-cavity-nesting bees as response variables. Oviposition was recorded as the number of eggs laid in a single nesting tube by a female bee. Larval development was quantified as the proportion of eggs that successfully completed development to a cocoon. Nesting success was defined as the proportion of all deployed tubes where at least one egg was oviposited. We considered these variables for released *Osmia lignaria propinqua* and for the most abundant wild wood-cavity-nesting bee. A value of 0.001 was added to all proportions of larval development to account for 0 data with the exception of proportions = 1, which were modified to 0.999. Proportions were normalized according to ln(proportion/(1-proportion).

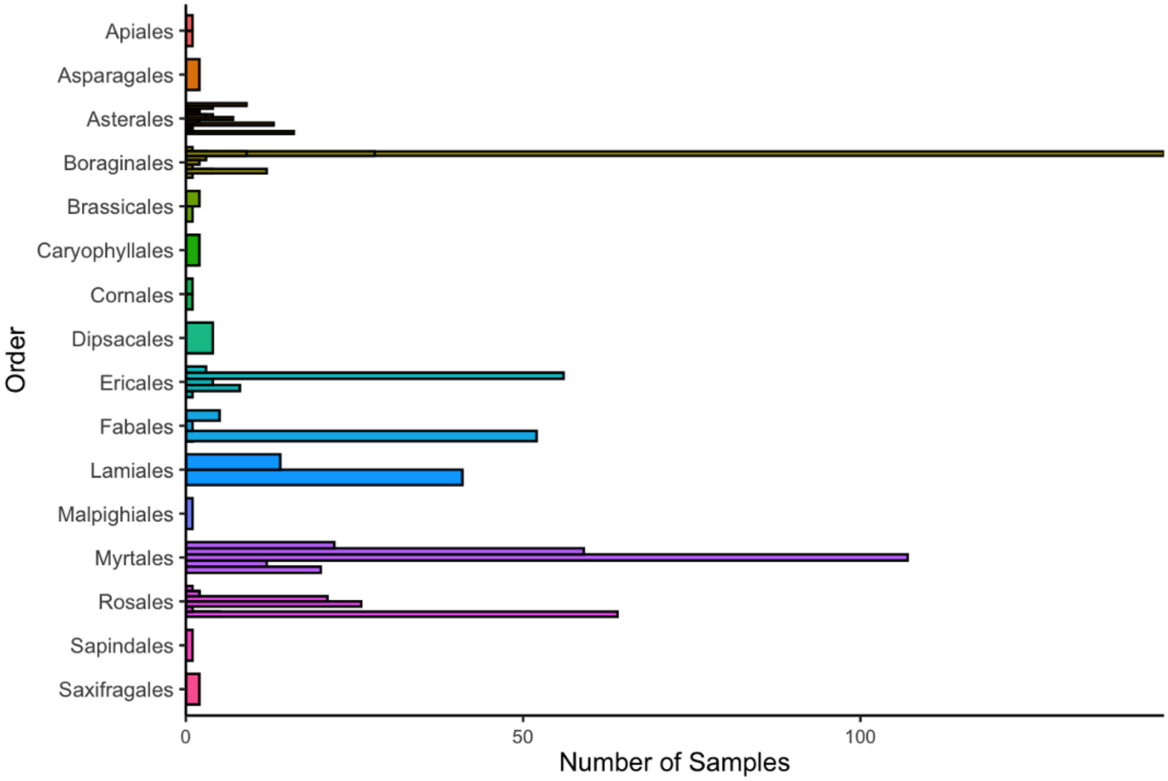
We used generalized linear mixed models (GLMM; Type III Analysis of Variance using Satterthwaite's Method) in the package lme4 (Bates et al. 2025) and lmerTest (Kuznetsova et al. 2020) to test the main effects of burn age, burn severity, and available nesting habitat on each response variable. Sampling year was included as a random effect. All response variables were tested for normality and modeled with Gaussian distributions. Evaluations for all GLMMs were conducted to ensure model assumptions were met using the DHARMa package (Hartig 2024).

3.4 Results

Genetic analysis of pollen provisions

After filtering, trimming, and denoising the sequences, a total of 323,471 reads were generated from the wood-cavity-nesting bee collected pollen. From these reads, 512 unique amplicon sequence variants (ASVs) were detected. Taxonomic assignments were conducted in DADA2, and wind pollinated plants were removed. A total of 60 insect-pollinated plant species/morphospecies in 16 orders, 20 families, and 45 genera were identified among the ASVs (Figure 3.3, Table 3.2).

Figure 3.3. Bar chart of pollen foraged by wild wood-cavity-nesting bees and detected using DNA metabarcoding by order (color). Each bar is a genus, which has been subdivided by the number of species in that genus.



Asterales was the most diverse order, and comprised 11/45 genera and 14/60 species, but had low overall abundance and only appeared in 57 out of 789 samples. By contrast, Boraginales were the most abundant order and appeared in 209 samples. However, Boraginales were less diverse than Asterales and were comprised of only 6/45 genera and 12/60 species. (Figure 3.3). *Phacelia hastata* (Boraginales, Hydrophyllaceae) and *Epilobium brachycarpum* (Myrtales, Onagraceae) were the most frequently detected plants across all samples. The fifteen most frequently detected insect-pollinated plants across all samples are shown in Figure 3.4A, and a complete list of plants that occurred in more than ten samples is shown in Figure 3.4B.

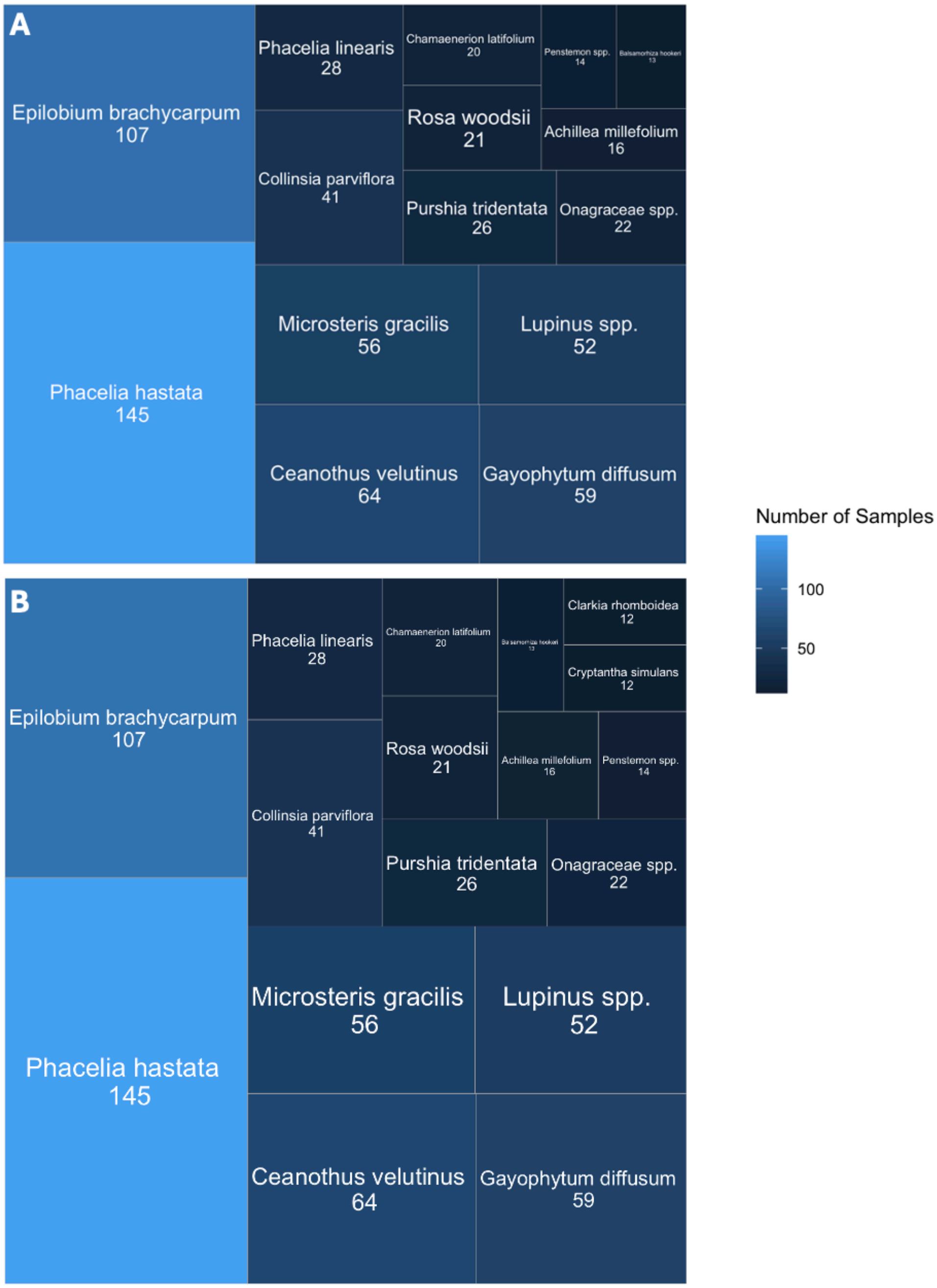
Table 3.2. Insect pollinated plant species identified from pollen provisions collected by wood-cavity-nesting bees. Of the total 789 observations, 16 orders, 20 families, 46 genera, and 60 insect pollinated species were identified. Plant common names and origins were derived from the Burke Herbarium Image Collection (2025).

Family	Latin Name	Common Name	Origin	Sample Frequency
Adoxaceae	*Sambucus racemosa*	red elderberry	native	4
Amaryllidaceae	*Allium sativum*	garlic	agricultural	2
Apiaceae	*Lomatium ambiguum*	Wyeth biscuit-root	native	1
	Osmorhiza occidentalis	Sierran sweet-cicely	native	1
Asteraceae	*Achillea millefolium*	yarrow	native	16
	Agoseris grandiflora	large-flowered agoseris	native	1
	Agoseris heterophylla	annual agoseris	native	1
	Arnica cordifolia	heart-leaf arnica	native	1
	Balsamorhiza hookeri	hairy balsamroot	native	13
	Balsamorhiza spp.	balsamroot	native	1
	Chaenactis douglasii	hoary chaenactis	native	2
	Crepis atribarba	slender hawksbeard	native	7
	Erigeron filifolius	Peck's threadleaf fleabane	native	4
	Erigeron pumilus	shaggy fleabane	native	3
	Hieracium spp.	hawkweed	unknown	2
	Microseris nutans	nodding microseris	native	2
	Taraxacum officinale	common dandelion	introduced	4
Boraginaceae	*Amsinckia lycopsoides*	bugloss fiddleneck	native	1
	Cryptantha affinis	common cryptantha	native	4

	Cryptantha simulans	pine woods cryptantha	native	12
	Hackelia spp.	stickseed	native	1
	Lithospermum ruderale	western stoneseed	native	3
Brassicaceae	*Lepidium draba*	heart-podded hoarycress	introduced	1
Ericaceae	*Arctostaphylos nevadensis*	pinemat manzanita	native	1
Fabaceae	*Lupinus polyphyllus*	bigleaf lupine	native	1
	Lupinus sericeus	silky lupine	native	1
	Lupinus spp.	lupine	native	52
	Medicago sativa	alfalfa	agricultural	1
	Melilotus officinalis	yellow sweet-clover	introduced	5
Grossulariaceae	*Ribes cereum*	wax currant	native	2
Hydrangeaceae	*Philadelphus lewisii*	Lewis' mock orange	native	1
Hydrophyllaceae	*Hydrophyllum capitatum*	ballhead waterleaf	native	2
Hydrophyllaceae	*Phacelia hastata*	silverleaf phacelia	native	145
	Phacelia heterophylla	varileaf phacelia	native	3
	Phacelia linearis	thread-leaf phacelia	native	28
	Phacelia sericea	silky phacelia	native	1
	Phacelia spp.	phacelia	native	9
Loasaceae	*Mentzelia dispersa*	bushy blazing-star	native	1
Montiaceae	*Claytonia rubra*	cushion miner's lettuce	native	2
Onagraceae	*Chamaenerion latifolium*	alpine fireweed	native	20
	Clarkia rhomboidea	common clarkia	native	12
	Epilobium brachycarpum	autumn willow-herb	native	107
	Gayophytum diffusum	spreading groundsmoke	native	59
Plantaginaceae	*Collinsia parviflora*	small-flowered blue-eyed Mary	native	41
	Penstemon spp.	penstemon	native	14
Polemoniaceae	*Collomia grandiflora*	large-flowered collomia	native	8
	Leptosiphon liniflorus	thread-stem linanthus	native	4
	Microsteris gracilis	slender phlox	native	56
Rhamnaceae	*Ceanothus sanguineus*	redstem ceanothus	native	5
	Ceanothus spp.	ceanothus	native	1
	Ceanothus velutinus	snowbrush ceanothus	native	64
Rosaceae	*Drymocallis arguta*	cordilleran drymocallis	native	1
	Drymocallis spp.	cinquefoil	native	1
	Purshia tridentata	antelope-brush	native	26
	Rosa woodsii	pearhip rose	native	21
	Rubus idaeus	red raspberry	native	1
	Rubus parviflorus	thimbleberry	native	2

	Rubus ulmifolius	elm-leaf blackberry	introduced	2
Salicaceae	*Salix* spp.	willow	unknown	1
Sapindaceae	*Acer* spp.	maple	unknown	1

Figure 3.4. Treemap of the 15 most frequently detected plant species (A) and the plant species detected in more than ten samples (B) for the wood-cavity-nesting bee community. The relative size of the box reflects the number of samples in which each species was detected across four sampling years (2021-2024). Lighter colors indicate more species were detected.



Several of the identified plant species (7 species/morphospecies) were suspected to be the result of wind contamination and were removed from further analyses. These include variegated horsetail (*Equisetum variegatum*, 115 samples) and fire moss (*Ceratodon purpureus*, 29 samples). All other wind dispersed pollen (e.g. *Callitropsis nootkatensis*, *Phaseolus vulgaris*, and *Agrostis capillaris*) was detected in less than 5 samples.

Plant-pollinator networks

A total of 130 individuals successfully emerged as adults following artificial winter conditions, from which five wood-cavity-nesting bee species were identified and associated with plant species (Supplemental Figure 3.2). The most frequently detected wood-cavity-nesting pollinators were *O. lignaria propinqua* (Figure 3.2A), which was released in 2021 and 2022, and Kincaid's Mason Bee, *Osmia kincaidii* Cockerell (Figure 3.2B), which were detected every year (2022-2024) in which community nesting materials were deployed. Both *Osmia* species are generalists with differing floral preferences (Supplemental Figure 3.3). *Osmia lignaria propinqua* visited a total of 45 species in 36 genera and 18 families. Among the 248 total observations that could be identified as *O. lignaria propinqua* samples, the plant species most frequently visited were *Phacelia hastata* (61 samples, Hydrophyllaceae) and an unidentified species of lupine (34 samples, Fabaceae; Figure 3.5A). By contrast, *O. kincaidii* was associated with 310 samples, and visited 25 species in 20 genera and 10 families. The most frequently visited plants were *Epilobium brachycarpum* (87 samples, Onagraceae), *Phacelia hastata* (47 samples, Hydrophyllaceae), *Microsteris gracilis* (44 samples, Polemoniaceae), and *Gayophytum diffusum* (42 samples, Onagraceae; Figure 3.5B).

Indicator species

Approximately 17% (10/60) of the insect pollinated plant species identified from wood-cavity-nesting bee provisional pollen were significantly associated ($p \leq 0.05$) with a specific burn age (Table 3.3). Significantly associated species were evenly distributed across burn ages. Sites with a burn age of 5 years were the only group to have more than one significantly associated species (*Epilobium brachycarpum* and *Phacelia heterophylla*). Additionally, sites with higher burn ages were more likely to be associated with woody shrubs such as snowbrush ceanothus (*Ceanothus velutinus,* burn age of 9) and wild rose (*Rosa woodsii,* unburned control).

Figure 3.5. Treemap of the plant pollen collected by *Osmia lignaria propinqua* (A) and *Osmia kincaidii* (B) that was found in more than ten samples. While both species foraged on *Phacelia hastata*, floral resources generally differed between the two mason bee species.

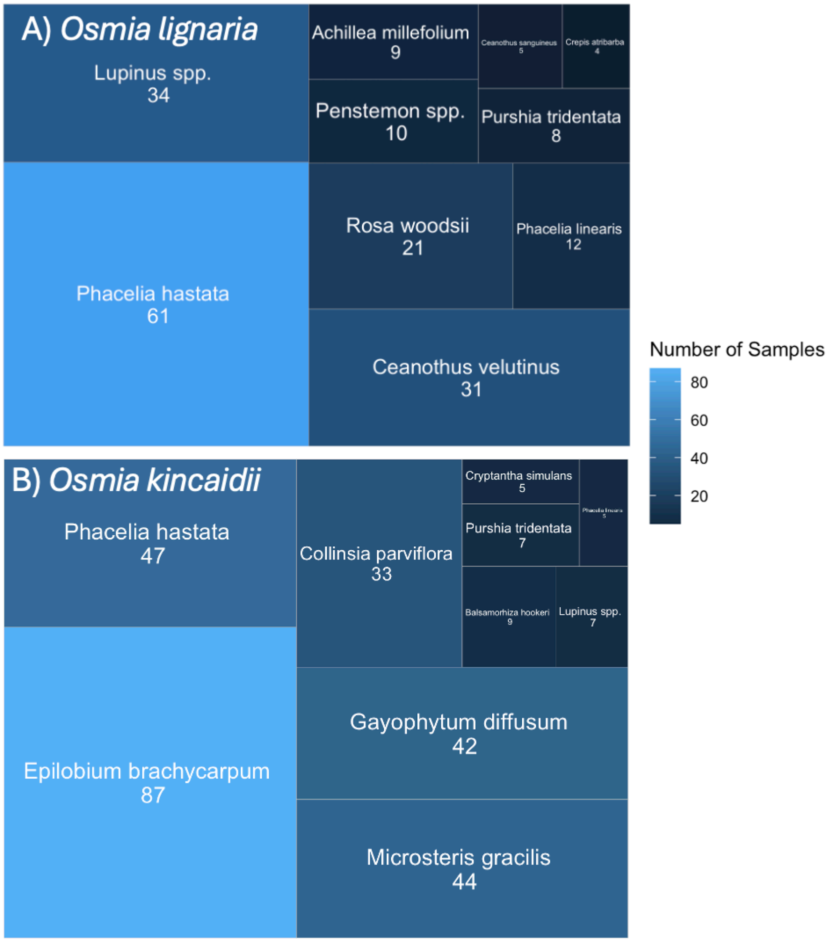


Table 3.3. List of the insect pollinated plant species identified from wood-cavity-nesting bee provisional pollen that were significantly associated ($p \leq 0.05$) with a specific burn age.

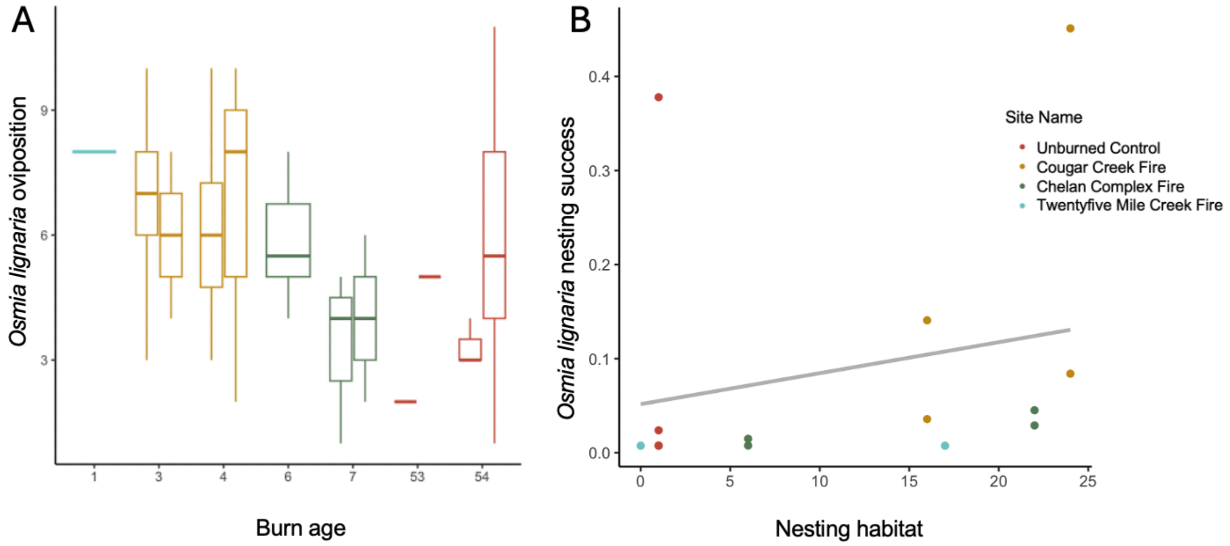
Burn Age (years)	Associated Species	Test Statistic	p value
1	*Phacelia linearis*	0.34	0.015
2	*Clarkia rhomboidea*	0.337	0.025
3	*Chamaenerion latifolium*	0.491	0.005
4	*Phacelia hastata*	0.364	0.02
5	*Epilobium brachycarpum*	0.396	0.005
	Phacelia heterophylla	0.265	0.040
7	*Penstemon* spp.	0.477	0.005
9	*Ceanothus velutinus*	0.511	0.005
unburned control	*Rosa woodsii*	0.397	0.01
	Microsteris gracilis	0.35	0.015

Table 3.4. List of plant pollen collected by wood-cavity-nesting bee species significantly associated ($p \leq 0.05$) with a specific burn severity category. Burn severity was binned into categories defined by Miller and Thode (2007).

Burn Severity Category	Associated Species	Statistic	p value
Low CBI=0.1-1.24	*Penstemon* spp.	0.228	0.005
	Achillea millefolium	0.217	0.005
	Purshia tridentata	0.204	0.025
	Rubus parviflorus	0.128	0.020
Moderate CBI=1.25-2.24	*Phacelia hastata*	0.376	0.005
	Epilobium brachycarpum	0.284	0.050
	Gayophytum diffusum	0.244	0.025
	Chamaenerion latifolium	0.222	0.005
unchanged control CBI = 0	*Microsteris gracilis*	0.387	0.005
	Collinsia parviflora	0.261	0.005
	Rosa woodsii	0.232	0.005
	Balsamorhiza hookeri	0.139	0.045
	Taraxacum officinale	0.124	0.040
	Leptosiphon liniflorus	0.124	0.020

Additionally, approximately 23% (14/60) of plant pollen collected by wood-cavity-nesting bee species was significantly associated ($p \leq 0.05$) with a specific burn severity category (Table 3.4). Significantly associated species were evenly distributed across burn severities, which were binned into categories defined by Miller and Thode (2007). Plots that were burned at low severities or were unburned had significantly associated shrub species (*Purshia tridentata, Rubus parviflorus*, *Rosa woodsii*), while sites that experienced moderate burn severities did not.

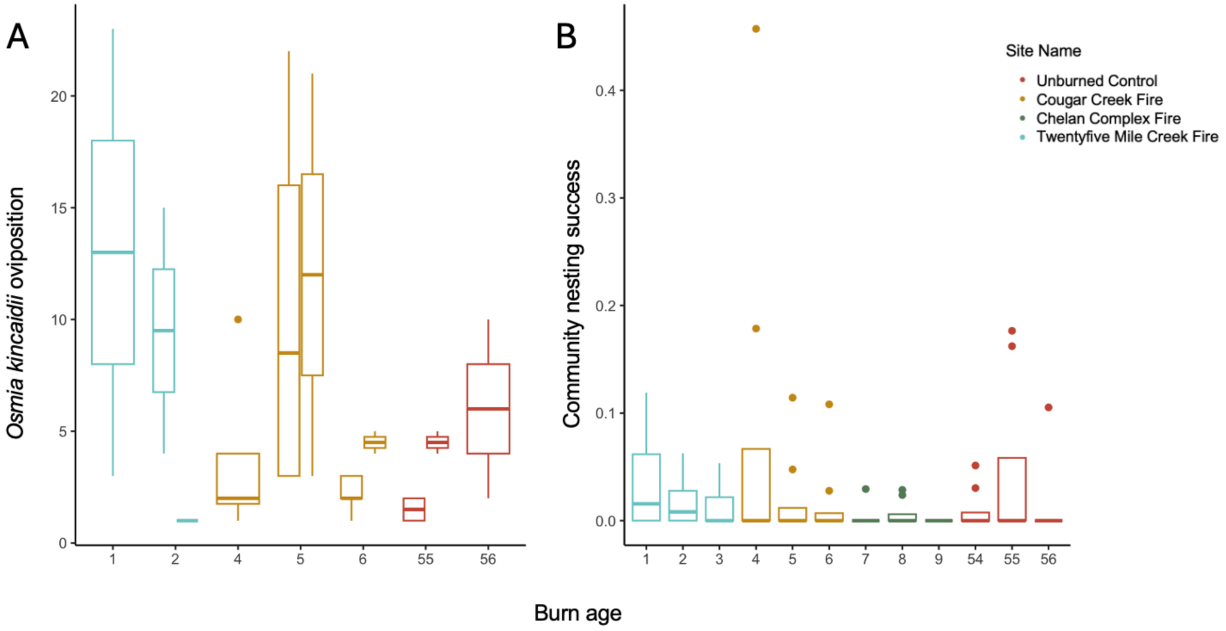
Figure 3.6. *Osmia lignaria propinqua* oviposition generally decreased with burn age (A). Nesting success increased with available nesting habitat (B).



Wood-cavity-nesting bee reproductive success

Using the GLMM approach, we observed that *O. lignaria propinqua* oviposition significantly decreased with increasing burn age ($p < 0.001$, $t = -3.776$, Figure 3.6A). In contrast, neither burn age, burn severity, or nesting habitat were significant predictors of *O. lignaria propinqua* larval development. *Osmia lignaria propinqua* nesting success significantly increased with available nesting habitat ($p = 0.011$, $t = 3.806$, Figure 3.6B). We observed similar patterns for *O. kincaidii*. Specifically, *O. kincaidii* oviposition significantly decreased with increasing burn age ($p < 0.025$, $t = -2.787$, Figure 3.7A) but also nesting habitat ($p = 0.042$, $t = -2.252$). We also did not detect any significant effects of burn age, burn severity, or nesting habitat on *O. kincaidii* larval development. Overall nesting success across all wild wood-cavity-nesting bee species decreased with increasing burn age (p = 0.003, t = -3.095, Figure 3.7B) but was not affected by available nesting habitat or burn severity.

Figure 3.7. *Osmia kincaidii* oviposition was significantly affected by year since burn (A) and nesting habitat. Nesting success of the wild wood-cavity-nesting bee community slightly decreased with time since burn (B).



3.5 Discussion

In general, nesting in wood cavities is a relatively uncommon behavior among native pollinators, but it likely represents the guild most directly impacted by wildfire (Cane and Neff 2011). Understanding plant-pollinator relationships for this guild is critical for successful conservation of angiosperm flora and bee faunas. We generated a list of floral resources frequently visited by wood-cavity-nesting pollinators in post-fire landscapes. We also quantified native wood-cavity-nesting bee reproductive success using nest boxes and assessed *O. lignaria propinqua* and *O. kincaidii* as exemplar wood-cavity-nesting species. Our results suggest that burn age is the main driver of wood-cavity-nesting bee oviposition, and that larval development is not affected by wildfire. This study highlights the importance of fire reintroduction for native pollinator conservation in dry forest landscapes.

Plant-pollinator networks

Of the five wood-cavity-nesting bee species identified, *O. lignaria propinqua* and *O. kincaidii* were the most frequently detected (Supplemental Figure 3.2). *Osmia lignaria propinqua*, released in 2021 and 2022, is identified by the swollen projection extending from the malar space and its dark metallic blue color (Sandhouse 1939, Messinger Carril and Wilson 2023; Figure 3.2A). Additionally, *O. kincaidii* colonized the 4mm nesting materials every year materials were deployed (2022-2024). Female *O. kincaidii* can be distinguished from similar species by their small size (~7mm), bright metallic blue-green color, and untruncated clypeus (Sandhouse 1939; Figure 3.2B). Its range extends north to British Columbia and east to Colorado, USA (Ikerd 2019). *Osmia kincaidii* may be a better exemplar pollinator when considering wood-cavity-nesting species in post-fire forests in Chelan County because it is native to the landscape and not commercially produced; thus, its detection on the landscape would indicate a resident population. Although *O. lignaria propinqua* is also native to the western United States, it is commercially produced and released for its pollination services such that its detection may not necessarily indicate a resident population. Interestingly, in this study, only one *O. lignaria propinqua* female was detected in the years when it was not released, suggesting that it is not an abundant pollinator in our study region.

The list of plants visited by wood-cavity-nesting bees comprised 16 orders and 20 plant families (Figure 3.3, Table 3.2). Wood-cavity-nesting bees in this study were generalist foragers, and species in the plant families Asteraceae (Asterales) and Hydrophyllaceae (Boraginales) were detected in the most samples. Specifically, *Phacelia hastata* (Hydrophyllaceae) was the most frequently detected plant across all samples and represents an important floral resource for wood-cavity-nesting bees in this region (Figure 3.4). *Epilobium brachycarpum* (Myrtales, Onagraceae) was also frequently collected, particularly by *Osmia kincaidii* (Figure 3.5B).

Foraging preferences influence bee development and vary in nutritional composition (Vaudo et al. 2016, 2020). To meet their nutritional needs, generalist solitary bees visit a variety of floral resources (Jha and Kremen 2013). Both exemplar *Osmia* species are generalists with differing floral preferences (Supplemental Figure 3.3). *Osmia lignaria propinqua* collected pollen from more total plant species than *O. kincaidii.* Additionally, *O. lignaria propinqua* most frequently visited *Phacelia hastata* and an unidentified species of lupine (*Lupinus* spp.). *Osmia kincaidii* also visited *P. hastata* but more frequently foraged on *Epilobium brachycarpum*. Additionally, many *O. kincaidii* pollen provisions contained *Microsteris gracilis* and *Gayophytum diffusum,* indicating that it may have a more diverse diet compared to *O. lignaria propinqua*.

Silverleaf phacelia (*Phacelia hastata*) is native to western North America and is widespread in Washington state. It flowers from May to August, providing a valuable floral resource over a long temporal extent. Furthermore, *P. hastata* is adapted to a wide range of elevations, and prefers sandy, dry soils and extensive sunlight (Burke Herbarium 2025), all of which are characteristics of the Okanogan-Wenatchee National Forest. The second most abundant plant, tall annual willowherb (*Epilobium brachycarpum*) is also native to western North America. By contrast, it is a late season floral resource that typically blooms in July and August. It thrives in dry, open meadows and grasslands to lightly wooded areas (Burke Herbarium 2025).

Seven species of plant pollen were presumed to be a result of wind contamination and were subsequently removed from analyses. The most frequently detected wind pollinated plants were variegated horsetail (*Equisetum variegatum*) and fire moss (*Ceratodon purpureus*). All other wind dispersed pollen occurred in less than 5 samples. Flowers may be contaminated by wind pollen that drifts onto the available pollen and is unintentionally picked up by bees. While wind-pollinated plants have not evolved to rely on insect pollination for successful reproduction, there are records of bee visitation and pollen collection from many wind-pollinated plants (Saunders 2017) such that the intentional collection of wind-dispersed pollen by bees cannot be fully discounted. However, we contend that the wind pollinated plants we identified were not likely be main food supplements for native pollinator species, particularly because no records of any species in the family Megachilidae pollinating *Equisetum* or *Ceratodon* spp. were found.

The plants species identified with DNA metabarcoding in this study may be used to inform the composition of seed mixes used in post-fire restoration to support plant-pollinator networks (Supplemental Figure 3.2 and 3.3). While wood-cavity-nesting is a less common trait in bees, they represent an important guild to monitor to effectively conserve both bee faunas and associated flora. The fifteen most frequently detected insect pollinated plant species (Figure 3.4) could be considered for managers working to re-seed in post fire landscapes, or conserve wood-cavity-nesting bees in the genus *Osmia*.

Indicator species

Burn age

Understanding associations among specific bee species and post-fire landscape characteristics facilitates plant-pollinator network conservation. Nine plant species and one morphospecies (*Penstemon* spp.) were significantly associated with a specific burn age via permutation test (e.g., 1, 2, 3, 4, 5, 7, or 9 years, or unburned; Table 3.3). Of these plants, eight were among the fifteen most visited floral resources by native wood-cavity-nesting bees (Figure 3.4A), indicating they are important floral resources to identify for conservation. The number of significant plant associations was uniform across burn age and ranged from 1 to 2 plant species. This suggests that most of the species present in this study can be found at more than one burn age. However, targeted conservation of oligolectic and specialist pollinators who frequent these ten species for pollen and nectar should consider fire as a restoration and conservation strategy to ensure the longevity of these plants on the landscape.

Plant associated with burn ages 1-7 were all herbaceous perennials, which are critical resources for pollinators in post fire landscapes (Chapter 2). Specifically, *P. hastata* was significant associated with a burn age of four years post fire. The aforementioned importance of *P. hastata* for the wood-cavity-nesting pollinator community emphasizes that frequent fire has significant associations with critical foraging resources for wood-cavity-nesting bees. Furthermore, *O. lignaria propinqua* had high levels of successful oviposition in landscapes 4 years post-fire. Both *P. hastata* and *E. brachycarpum* are often visited by *O. kincaidii* (Figure 3.5B, Supplemental Figure 3.2 and 3.3) and were significantly associated with burn ages of 4 and 5 years, respectively. *Osmia kincaidii* oviposition and community nesting success were both high in landscapes that were 4 to 5 years post fire, suggesting an ecological relationship among burn age, the presence of *P. hastata* and *E. brachycarpum*, and reproductive success of this wood-cavity-nesting bee species (Figure 3.7).

A greater number of woody shrubs were significantly associated with later burn ages, including *Ceanothus velutinus* and *Rosa woodsii*. *Ceanothus velutinus* and *R. woodsii* appeared in 64 and 21 of the 789 total samples collected from woody cavity nesting bee nests, respectively. While these were not the most frequently detected plants, they do represent an important foraging resource for wood-cavity-nesting bees. Furthermore, as fires become more frequent and intense (Marlon et al. 2012), the risk of forest conversion increases, causing compositional changes in existing post-fire vegetative communities (Reilly et al. 2017). The result may be the loss of moist-mesic biota to thermophilic vegetation. Snowbrush ceanothus (*C. velutinus)* is an abundant drought-tolerant, fire-adapted woody shrub in western North American dry forest landscapes that generally thrives in post-fire conditions. Thermophilic shrubs, such as snowbrush ceanothus, are better adapted to warmer, drier conditions, they may become a dominant feature of interior dry forests. This is particularly true considering limits to ponderosa pine regeneration with increasing distance to a seed source and decreasing elevation following high severity fire (Haffey et al. 2018).

Burn severity

Some plant species were also significantly associated with specific burn severity categories (e.g., unchanged, low, moderate; Table 3.4). Four plant species were associated with each burn severity category, and six with the unburned control. Of the four plants associated with moderate severity burns, *Chamaenerion latifolium* (dwarf fireweed; Onagraceae) is a pioneer genus known for its rapid colonization of post fire landscapes (Gustafsson et al. 2021, Montana Field Guides 2025). Its pyrophytic nature is reflected by its association to sites with higher severity fire effects. While burn severity was not a significant driver of wood-cavity-nesting bee reproductive success, it does have significant associations with key plant species. The aforementioned *E. brachycarpum* was significantly associated with moderate burn severities. Land managers that seek to maximize the presence of high priority floral resources for wood-cavity-nesting bee species should consider the effects of severity on plant communities, though its effect on bee reproductive success was not significant.

Wood-cavity-nesting bee reproductive success

In burned landscapes, both *O. lignaria propinqua* and *O. kincaidii* oviposition decreased with increasing burn age. The immediate effects of fire are typically increases in floral resources and light availability, which are favorable characteristics in high quality bee habitat. An increase in floral resources could also result in shorter foraging distances and energy expenditure, resulting in increased oviposition. *Osmia kincaidii* oviposition was also affected by available nesting habitat. As available habitat increased, *O. kincaidii* oviposition, as measured through deployed nest boxes decreased. However, we contend that this finding could be explained by the nesting preferences of *O. kincaidii* for natural landscape features over deployed nest boxes; thus, as available natural nesting habitat increased on the landscape, their use of deployed nesting materials decreased. Overall, our findings support the hypothesis that the reintroduction of fire to dry forest landscapes benefits wood-cavity-nesting bees.

For both exemplar *Osmia* species, burn age, burn severity, and available nesting habitat had no significant effects on larval development. However, it is important to note that solitary bee species require adequate pollen and nectar resources for their offspring, and mothers may oviposit only when pollen and nectar resources are sufficient for a developing larva. As a consequence, *Osmia* spp. likely would respond by ovipositing less when resources were scarce without a consequence to successful larval development.

Responses in nesting success to fire differed among the exemplar wood-cavity-nesting pollinator species. *Osmia lignaria propinqua* nesting success increased with increasing nesting habitat (Figure 3.6B) and was unaffected by burn age and burn severity. However, *O. lignaria propinqua* was released on the landscape, so any intergenerational effects of fire on reproductive success were negated by these releases. By contrast, *O. kincaidii* nesting success decreased with increasing burn age

(Figure 3.7B) and was unaffected by burn severity or available nesting habitat. Patterns in *O. kincaidii* nesting success are likely more reflective of the effects of burn metrics on wood-cavity-nesting species due to it undeniable presence as a resident species that would have been affected by the localized burn history.

3.6 Conclusion

DNA metabarcoding of pollen from nest boxes revealed herbaceous perennials such as *Phacelia hastata* and *Epilobium brachycarpum* to be important floral resources for wood-cavity-nesting pollinators in post fire landscapes. Both species were significantly associated with burn ages of 4 to 5 years post fire, indicating that frequent fire intervals may support plant-pollinator networks.

Burn age was observed to be a main driver of wood-cavity-nesting bee oviposition in post fire landscapes. Oviposition and nesting success varied among the exemplar wood-cavity-nesting species, supporting management for patch mosaics and landscape heterogeneity as a fundamental principal of the reintroduction of fire to xeric landscapes. Future work should assess the nutritional utility of pollen provisions for native solitary bees that may contribute to successful larval development and oviposition.

The findings of this study may inform seed mix compositions for use in post-fire restoration efforts to promote resilient plant-pollinator networks in disturbed landscapes. It also provides novel insights into the effects of disturbance on bee reproductive success, and highlight the functionality of *O. lignaria propinqua* and *O. kincaidii* as exemplar wood-cavity-nesting species to monitor in recently burned landscapes.

Additional Information

Funding

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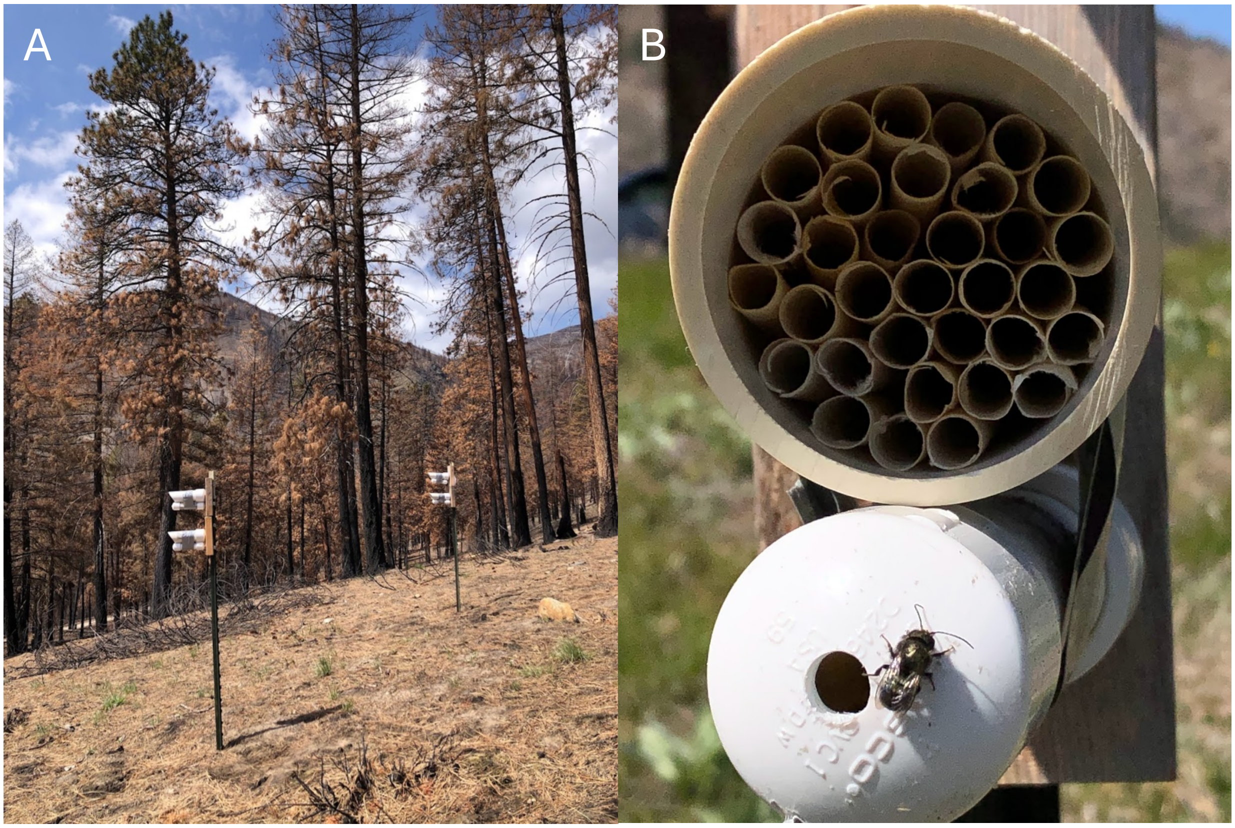
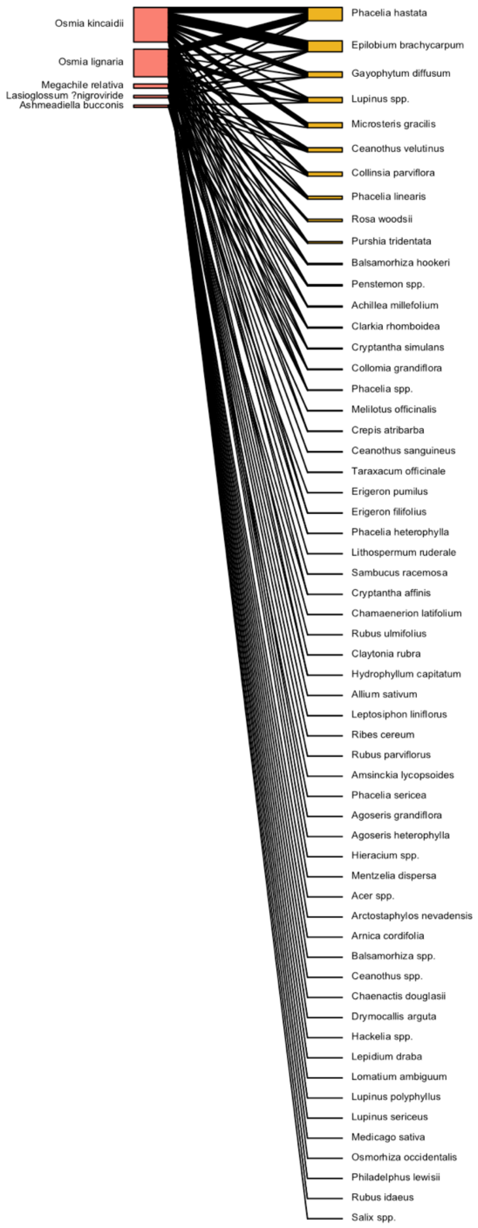
3.7 Appendix C

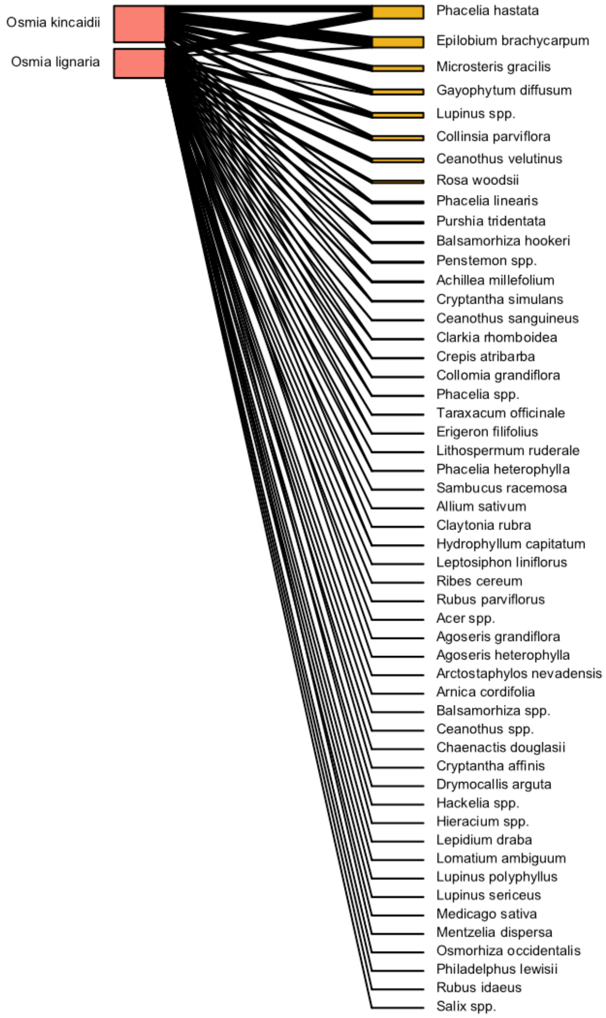
Supplemental Figure 3.1. Nest boxes, each made from a 7.62 cm diameter PVC pipe approximately 15 cm long and beveled for a rain shield were attached to each of the four T-posts per site through a PVC cap (A). One pupal release capsule was attached to each nest box. Each pupal release capsule, consisting of a 2.54 cm diameter PVC pipe approximately 13 cm long, contained ~100 *O. lignaria* pupae for a total of 4,800 pupae released across all three study sites in 2021 and 6,400 pupae released across four study sites in 2022 (B).



Supplemental Figure 3.2. Plant-pollinator network for five wood-cavity-nesting pollinators sampled using nest boxes in post-fire landscapes. Pollen from *Phacelia hastata* was present in the most provisions. All wood-cavity-nesting bee species were generalists, and visited a variety of plant genera and species.



Supplemental Figure 3.3. Plant-pollinator network for *Osmia kincaidii* and *O. lignaria propinqua* using nest boxes in post-fire landscapes. Pollen from *Phacelia hastata* and *Epilobium brachycarpum* were present in the most provisions. Both wood-cavity-nesting bee species were generalists, and visited a variety of plant genera and species.



Chapter 4. First records of eight native bee species (Hymenoptera, Anthophila) in Washington, USA

4.1 Abstract

Pollinators are an essential component of ecosystem function, and declining bee populations are a global conservation concern. Despite this importance, there is a lack of understanding regarding the distribution of native bee species across western North American landscapes. This study documents new records of *Melissodes nigracauda* LaBerge, *Dufourea dilatipes* Bohart, *Atoposmia abjecta abjecta* Cresson, *Coelioxys funerarius* Smith, *Dianthidium cressonii* Dalla Torre, *Dianthidium singulare* Cresson, *Osmia cyaneonitens* Cockerell, and *Stelis heronae* Sheffield. These eight new records supplement the ~565 bee species previously documented in Washington state.

4.2 Introduction

Bees are globally important and contribute to essential ecosystem services. However, distributional knowledge for many bee species is limited. Systematically documenting regional bee faunas is vital for monitoring ecosystem health and provides a foundation for detecting declines in biodiversity (Mathiasson and Rehan 2019; Decker et al. 2020).

Washington is an ecologically diverse state characterized by wet western coastal forests and a dry eastern interior, generally separated by the Cascade Mountain Range (Franklin and Dryness 1973). Located on the eastern slopes of the Cascades, Chelan County is a landscape dominated by dry *Pinus ponderosa* Douglas ex. C. Lawson (Pinaceae) forest and *Artemisia* L. (Asteraceae) steppe (Franklin and Dryness 1973). While xeric landscapes typically contain high bee biodiversity

(Michener 1979; Cane 2011), bees in Chelan County are understudied (Bartholomew et al. 2024). One of the main disturbances that affects native bee communities in this ecosystem is wildfire. When fire is restorative, the effects on pollinator communities are generally positive (Peralta et al. 2017; Galbraith et. al. 2019; Gelles et al. 2022). However, disturbances like wildfire are predicted to become more frequent and intense as the climate continues to warm (Dale et al. 2001; Marlon et al. 2012; Seidl et al. 2017). Changing climate has also affected the distributional ranges of several bee species (Kuhlmann et al. 2012; Nooten and Rehan 2020), making fine-scale documentation of bee faunas essential to ensure the conservation and preservation of native biodiversity.

We report the first records of eight bee species for the state of Washington, USA: *Melissodes nigracauda* LaBerge, 1961 (Hymenoptera, Apidae), *Dufourea dilatipes* Bohart, 1948 (Hymenoptera, Halictidae), *Atoposmia abjecta abjecta* Cresson, 1878, *Coelioxys funerarius* Smith, 1854, *Dianthidium cressonii* Dalla Torre, 1896, *Dianthidium singulare* Cresson, 1879, *Osmia cyaneonitens* Cockerell, 1906, and *Stelis heronae* Sheffield, 2024 (Hymenoptera, Megachilidae).

4.3 Methods

Study sites (*n* = 8) in Chelan County, Washington were selected within the Okanogan–Wenatchee National Forest, with permission from the Entiat and Chelan United States Forest Service Ranger Districts. Six sites were established in areas that were burned by wildfire within the last decade (Figure 4.1). In all selected study areas, dominant vegetation included Ponderosa Pine (*Pinus ponderosa* Douglas ex. C. Lawson, Pinaceae), Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco, Pinaceae), Bearberry (*Arctostaphylos uva-ursi* L. Spreng, Ericaceae), Wax Currant (*Ribes cereum* Douglas, Grossulariaceae), Snowbrush Ceanothus (*Ceanothus velutinus* Douglas, Rhamnaceae), and Arrowleaf

Balsamroot (*Balsamorhiza sagittata* Pursh, Asteraceae). We established and sampled two replicated plots across four randomly selected sites from 2021 to 2023.

In each plot, two pan traps and two blue vane traps were deployed. Traps were placed approximately 0.25–0.5 m above the ground to mirror average floral height. Pan and blue vane traps were deployed for 24–36 hours every two weeks, from late April to early August, to account for seasonal variation among species. Each pan trap contained two white, two fluorescent blue, and two fluorescent yellow cups, each with a capacity of ~96 mL. Pan and blue vane traps were filled with soapy water to ensure effective capture. Blue vane deployment was delayed by ~2 weeks to avoid destruction of early season *Bombus* Latreille, 1802 queens (Kimoto et al. 2012).

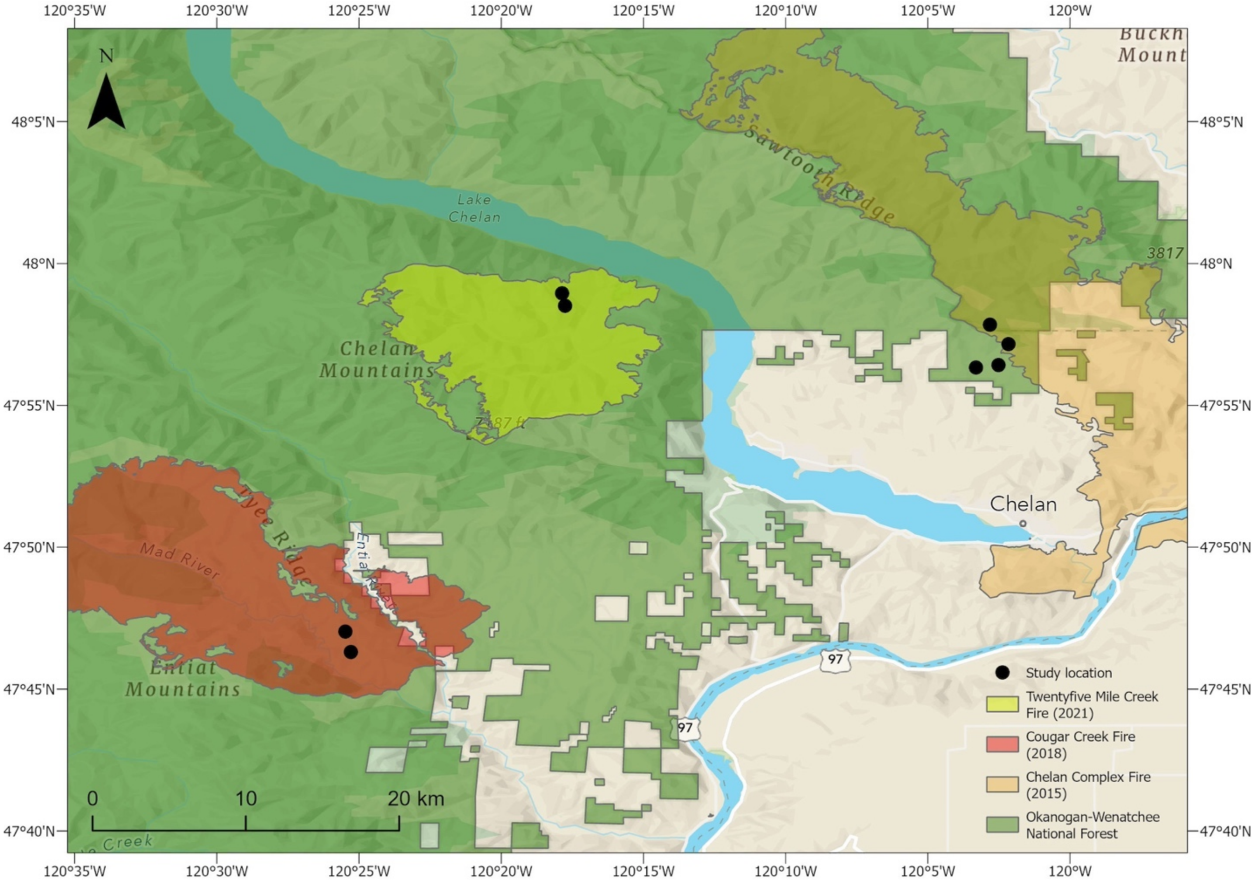


Figure 4.1. Study locations where first record species were collected in Washington, USA.

Upon collection of field data, samples were labeled with the collection date, study site, and plot. Field samples were transported to the lab and stored at 0 °C. Trap contents were then separated by taxonomic order and stored in vials containing 70% ethanol prior to identification. The specimens were rehydrated, dried, pinned, and labeled. Native bee pollinators (Hymenoptera, Anthophila) were identified to the species level using taxonomic keys (Supplemental Table 4.1). The specific keys used to identify first records are as follows: *Melissodes* (LaBerge 1961); *Dufourea* (Dumesh and Sheffield 2012); *Coelioxys* (Baker 1975); *Dianthidium cressonii* (Schwarz 1926); *Dianthidium singulare* (Grigarick and Stange 1968); *Osmia* (Sandhouse 1939); and *Stelis* (Sheffield 2024). Voucher specimens were deposited in the M. T. James Entomological Collection at Washington State University in Pullman, Washington, USA (WSUC) and the United States Department of Agriculture, Agriculture Research Service, Pollinating Insects Collection (formerly Bee Biology and Systematics Laboratory) at Utah State University in Logan, Utah, USA (BBSL). All maps were generated using ArcGIS Pro v. 3.3.1 (Esri Inc., Redlands, CA, USA), with species distributional data aggregated by the Global Biodiversity Information Facility.

4.4 Results

The result of these sampling efforts is the discovery of eight new records for Washington, USA described below. A complete list of new state records and their voucher locations are summarized in Table 4.1. Additionally, 100 new records for Chelan County, Washington, USA are reported in Table 4.2. A complete list of species collected and their abundance by year is provided in Supplemental Table 4.2.

Table 4.1. First records of eight native bee species (Hymenoptera, Anthophila) collected in the Okanogan–Wenatchee National Forest in Chelan County, Washington, USA by determiners Autumn Maust (AM), Joel Gardner (JG), Karen W. Wright (KW), and Terry Griswold (TG). Due to a database error the specific locations within the study region cannot be determined for some specimens, and are listed as NA. Specimens were deposited in the M. T. James Entomological Collection at Washington State University in Pullman, Washington, USA (WSUC) and the United States Department of Agriculture, Agriculture Research Service, Pollinating Insects Collection (formerly Bee Biology and Systematics Laboratory) at Utah State University in Logan, Utah, USA (BBSL). Table continues on next page.

Family	Voucher ID No.	Species	Latitude (°N)	Longitude (°W)	Date of collection	Depository (determiner)
Apidae	AM21-0440	*Melissodes nigracauda*	NA	NA	2021[*]	WSUC (KW)
	AM21-1494	*Melissodes nigracauda*	NA	NA	2021[†]	WSUC (KW)
	AM21-1497	*Melissodes nigracauda*	NA	NA	2021[†]	WSUC (KW)
	AM21-1560	*Melissodes nigracauda*	47.7718	120.4216	3-Aug 2021	WSUC (KW)
	AM21-1561	*Melissodes nigracauda*	47.7718	120.4216	3-Aug 2021	WSUC (KW)
	AM22-1126	*Melissodes nigracauda*	NA	NA	2022[‡]	WSUC (KW)
	AM23-2436	*Melissodes nigracauda*	47.7718	120.4216	7-Aug 2023	WSUC (KW)
Halictidae	AM22-0786	*Dufourea dilatipes*	47.9526	120.0360	2022[§]	WSUC (KW)
	AM23-0471	*Dufourea dilatipes*	47.9388	120.0550	29-May 2023	WSUC (KW)
	AM23-0552	*Dufourea dilatipes*	47.9641	120.0469	29-May 2023	WSUC (KW)
	AM23-0596	*Dufourea dilatipes*	47.7837	120.4246	29-May 2023	WSUC (KW)
	AM23-1015	*Dufourea dilatipes*	47.7837	120.4246	12-Jun 2023	WSUC (KW)
Megachilidae	AM21-0062	*Atoposmia abjecta abjecta*	NA	NA	2021[¶]	WSUC (TG)
	AM21-0063	*Atoposmia abjecta abjecta*	NA	NA	2021[¶]	WSUC (TG)
	AM21-1507	*Atoposmia abjecta abjecta*	NA	NA	2021[#]	WSUC (TG)
	AM23-1376	*Atoposmia abjecta abjecta*	47.9526	120.0360	26-Jun 2023	WSUC (TG)
	AM23-1109	*Coelioxys funerarius*	47.9826	120.2978	12-Jun 2023	WSUC (TG)
	W21-0015	*Dianthidium cressonii*	47.9403	120.0418	6-Jul 2021	WSUC (KW)
	W21-0016	*Dianthidium cressonii*	47.9403	120.0418	6-Jul 2021	WSUC (KW)
	W21-0018	*Dianthidium cressonii*	47.9403	120.0418	6-Jul 2021	WSUC (KW)
	W21-0019	*Dianthidium cressonii*	47.7837	120.4246	6-Jul 2021	WSUC (KW)
	W21-0020	*Dianthidium cressonii*	47.7837	120.4246	6-Jul 2021	WSUC (KW)
	W21-0021	*Dianthidium cressonii*	47.7837	120.4246	6-Jul 2021	WSUC (KW)
	W21-0050	*Dianthidium cressonii*	47.9526	120.0360	22-Jun 2021	WSUC (KW)
	W21-0051	*Dianthidium cressonii*	47.9526	120.0360	22-Jun 2021	WSUC (KW)
	W21-0052	*Dianthidium cressonii*	47.9388	120.0550	22-Jun 2021	WSUC (KW)
	W21-0053	*Dianthidium cressonii*	47.9403	120.0418	22-Jun 2021	WSUC (KW)
	W21-0056	*Dianthidium cressonii*	47.9526	120.0360	6-Jul 2021	WSUC (KW)
	W21-0057	*Dianthidium cressonii*	47.9526	120.0360	6-Jul 2021	WSUC (KW)
	W21-0060	*Dianthidium cressonii*	47.7718	120.4216	6-Jul 2021	WSUC (KW)
	W21-0061	*Dianthidium cressonii*	47.9641	120.0469	22-Jun 2021	WSUC (KW)
	W21-0063	*Dianthidium cressonii*	47.9641	120.0469	22-Jun 2021	WSUC (KW)

	W21-0064	*Dianthidium cressonii*	47.9641	120.0469	22-Jun 2021	WSUC (KW)
	W21-0067	*Dianthidium cressonii*	47.9388	120.0550	6-Jul 2021	WSUC (KW)
	W21-0070	*Dianthidium cressonii*	47.9388	120.0550	8-Jun 2021	WSUC (KW)
	W21-0072	*Dianthidium cressonii*	47.9403	120.0418	8-Jun 2021	WSUC (KW)
	W21-0073	*Dianthidium cressonii*	47.7837	120.4246	22-Jun 2021	WSUC (KW)
	W21-0074	*Dianthidium cressonii*	47.7837	120.4246	22-Jun 2021	WSUC (KW)
	W22-0109	*Dianthidium cressonii*	47.9403	120.0418	1-Aug 2022	WSUC (KW)
	W22-0111	*Dianthidium cressonii*	47.9388	120.0550	1-Aug 2022	WSUC (KW)
	W22-0112	*Dianthidium cressonii*	47.9388	120.0550	1-Aug 2022	WSUC (KW)
	W22-0116	*Dianthidium cressonii*	47.9641	120.0469	1-Aug 2022	WSUC (KW)
	W22-0130	*Dianthidium cressonii*	47.9641	120.0469	18-Jul 2022	WSUC (KW)
	W22-0131	*Dianthidium cressonii*	47.9641	120.0469	18-Jul 2022	WSUC (KW)
	W22-0133	*Dianthidium cressonii*	47.9526	120.0360	1-Aug 2022	WSUC (KW)
	W22-0134	*Dianthidium cressonii*	47.9641	120.0469	1-Aug 2022	WSUC (KW)
	W22-0135	*Dianthidium cressonii*	47.9826	120.2978	1-Aug 2022	WSUC (KW)
	W23-0081	*Dianthidium cressonii*	47.9403	120.0418	10-Jul 2023	WSUC (KW)
	W23-0091	*Dianthidium cressonii*	47.9526	120.0360	12-Jun 2023	WSUC (KW)
	W23-0153	*Dianthidium cressonii*	47.9752	120.2959	26-Jun 2023	WSUC (KW)
	W23-0156	*Dianthidium cressonii*	47.9826	120.2978	26-Jun 2023	WSUC (KW)
	W23-0171	*Dianthidium cressonii*	47.7837	120.4246	26-Jun 2023	WSUC (KW)
	W22-0142	*Dianthidium singulare*	47.9403	120.0418	1-Aug 2022	WSUC (AM)
	AM23-0542	*Osmia cyaneonitens*	47.9526	120.0360	29-May 2023	WSUC (JG)
	AM22-0597	*Stelis heronae*	NA	NA	2022[**]	BBSL (TG)
	AM22-1314	*Stelis heronae*	NA	NA	2022[††]	WSUC (TG)
	AM23-1730	*Stelis heronae*	47.9388	120.0550	10-Jul 2023	BBSL (TG)
	AM23-1732	*Stelis heronae*	47.9388	120.0550	10-Jul 2023	WSUC (TG)

[*] Jun 8–Jul 6 2021; [†] Jun 8–Aug 3 2021; [‡] Jul 5–Aug 1 2022; [§] May 10–Jul 5 2022; [¶] Apr 28–Jul 6 2021; [#] Jun 22–Aug 3 2021; [**] Jul 18–Aug 1 2022; [††] Jul 5–Aug 1 2022

Table 4.2. New records of 100 species in Chelan County, Washington, USA and their corresponding abundance by year. New records for the state of Washington are bold. Species that have not been documented in Washington since before the year 2000 are annotated (Bartholomew et al. 2024). Species-level identifications with ambiguity are omitted.

Family	Species	No collected 2021	No. collected 2022	No. collected 2023
Andrenidae	*Andrena angustitarsata*	2	1	0
	Andrena astragali	0	0	1
	Andrena buckelli	1	0	0
	Andrena candida	0	0	1
	Andrena candidiformis	0	3	1
	Andrena cleodora	0	1	0
	Andrena lawrencei	0	6	1
	Andrena lupinorum	3	0	0
	Andrena microchlora	1	0	0
	Andrena nivalis	1	5	1
	Andrena quintiliformis	0	5	0
	Andrena salicifloris	1	1	0
	Andrena schuhi	1	0	0
	Andrena trevoris	0	1	0
	Andrena vicinoides	0	1	0
	Panurginus atriceps	0	6	1
	Panurginus nigrellus	1	7	1
	Perdita wyomingensis sculleni	9	0	5
Apidae	*Anthophora terminalis*	0	1	1
	Ceratina nanula	5	1	2
	Ceratina sequioae[*]	2	1	3
	Eucera delphinii	1	7	1
	Habropoda morrisoni[†]	0	0	1
	Melecta pacifica	4	1	9
	Melissodes communis	0	10	0
	Melissodes grindeliae/robustior	0	1	0
	Melissodes lupinus	0	0	1
	Melissodes nigracauda	5	1	1
Colletidae	*Colletes consors*	0	1	0
	Colletes fulgidus	0	1	2
	Hylaeus affinis[‡]	0	0	3
Halictidae	***Dufourea dilatipes***	0	1	4
	Halictus tripartitus	328	167	457

	Lasioglossum albohirtum	1	2	3
	Lasioglossum aspilurum[§]	1	1	2
	Lasioglossum athabascense	8	10	7
	Lasioglossum buccale[¶]	8	2	10
	Lasioglossum glabriventre	13	25	59
	Lasioglossum helianthi	2	0	3
	Lasioglossum inconditum[#]	0	11	1
	Lasioglossum knereri	2	3	20
	Lasioglossum macroprosopum	15	9	22
	Lasioglossum marinense	1	0	1
	Lasioglossum mellipes	13	16	15
	Lasioglossum pacificum	0	0	2
	Lasioglossum prasinogaster	6	6	7
	Lasioglossum pruinosum	1	0	2
	Lasioglossum punctatoventre	15	16	27
	Lasioglossum reasbeckae	1	1	6
	Lasioglossum ruidosense	14	7	5
	Lasioglossum sandhousiellum	19	4	9
	Lasioglossum trizonatum	14	22	11
Megachilidae	*Anthidium banningense*	2	0	3
	Anthidium formosum	1	0	2
	Anthidium mormonum	2	0	0
	Anthidium utahense	0	1	1
	Ashmeadiella californica	1	0	0
	Ashmeadiella cubiceps cubiceps	0	1	0
	Atoposmia abjecta abjecta	3	0	1
	Atoposmia elongata	1	0	0
	Coelioxys funerarius	0	0	1
	Coelioxys octodentata	0	1	0
	Dianthidium cressonii	21	9	5
	Dianthidium heterulkei	1	1	1
	Dianthidium pudicum	3	1	0
	Dianthidium singulare	0	1	0
	Dianthidium ulkei	1	0	0
	Heriades carinatus	0	1	0
	Hoplitis fulgida fulgida	3	3	1
	Hoplitis hypocrita	0	0	1
	Hoplitis producta	2	0	0
	Hoplitis sambuci	0	2	2
	Megachile apicalis	0	0	1

	Megachile brevis	0	1	5
	Megachile gemula	0	1	0
	Megachile lapponica	0	1	0
	Megachile montivaga	4	6	3
	Megachile onobrychidis	2	0	0
	Megachile pascoensis	11	7	6
	Megachile subnigra	1	0	2
	Megachile wheeleri	0	0	1
	Osmia brevis	1	1	0
	Osmia bucephala	0	2	1
	Osmia cahuilla	0	0	1
	Osmia calla	1	1	3
	Osmia cara[**]	1	4	5
	Osmia cyaneonitens	0	0	1
	Osmia exigua	1	5	3
	Osmia kincaidii	0	0	1
	Osmia montana montana	5	15	108
	Osmia nanula	0	1	0
	Osmia nemoris	0	1	0
	Osmia nifoata	1	0	0
	Osmia proxima	1	0	0
	Osmia sedula	1	0	0
	Osmia simillima	0	0	1
	Osmia texana	0	1	0
	Osmia trevoris	2	1	5
	Stelis heronae	0	2	2
	Stelis subcaerulea	0	1	0

[*] *Ceratina sequioae*; not documented since 1919.

[†] *Habropoda morrisoni*; not documented since 1995.

[‡] *Hylaeus affinis*; not documented since 1970.

[§] *Lasioglossum aspilurum*; not documented since 1973.

[¶] *Lasioglossum buccale*; not documented since 1970.

[#] *Lasioglossum inconditum*; not documented since 1985.

[**] *Osmia cara*; not documented since 1935.

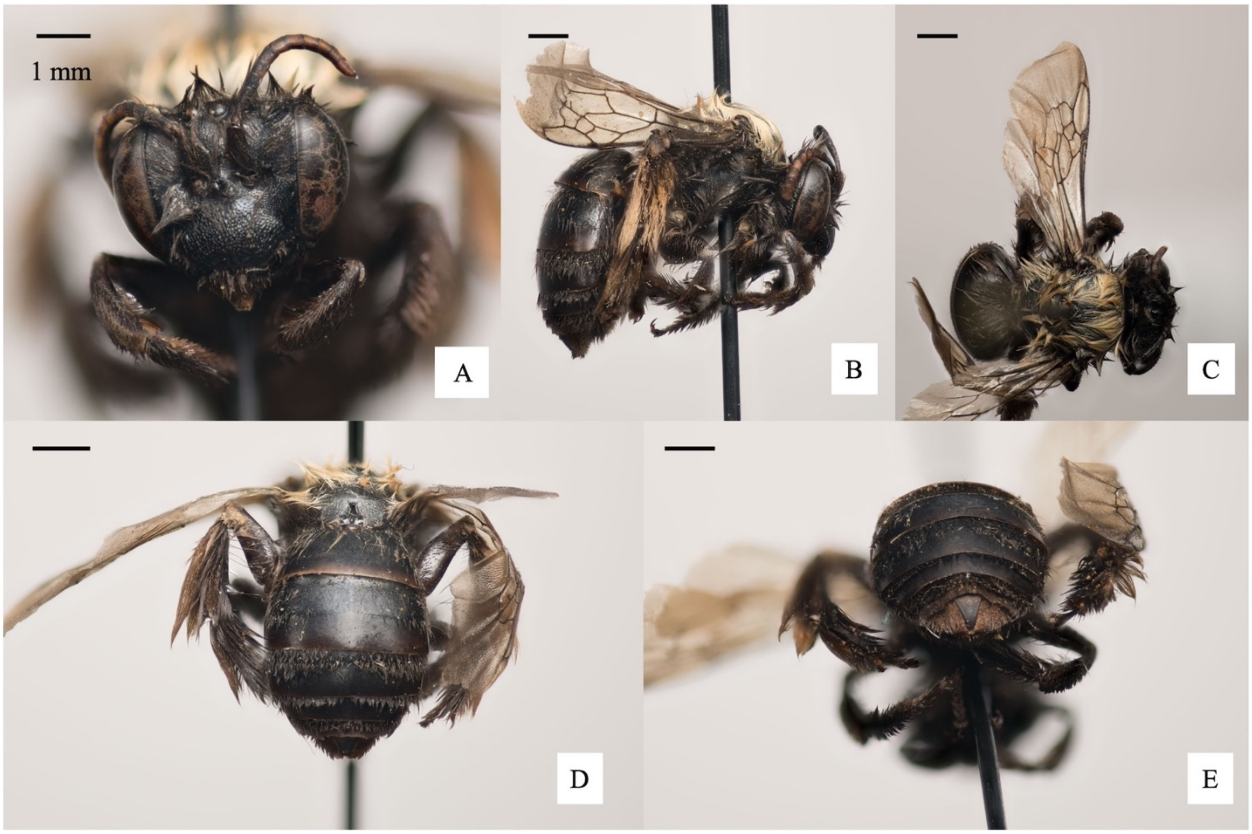


Figure 4.2. Female *Melissodes nigracauda.* **A.** Face, frontal view. **B.** Body, lateral view. Vestiture of the head, abdomen, and sides of the thorax dark brown to black. Scopa brown to black. **C.** Thorax, dorsal view. Dorsum of thorax ochraceous to slightly rufescent. **D.** Abdomen, dorsal view. Apical edges of tergites impunctate. **E.** Abdomen, dorsal view. Pygidial plate triangular. Scale is approximate.

Melissodes nigracauda **LaBerge, 1961**

Figure 4.2

New records. (*n* = 3♀). UNITED STATES OF AMERICA – **Washington** • Okanogan Wenatchee National Forest, Entiat Ranger District; Chelan County, Ardenvoir; 47.7718°N, 120.4216°W; 890 m a.s.l.; 2–4 Aug 2021; A. Maust leg.; blue vanes; det. KW Wright 2024; 2♀, AM21-1560 and AM21-1561• ibid.; 7–9 Aug 2023; 1♀, AM23-2436.

Four additional specimens were captured, but due to a database error their specific locations within the Okanogan–Wenatchee National Forest in Chelan County, Washington cannot be determined. Three female specimens were collected in 2021 (AM21-0440, AM21-1494, and AM21-1497), and one male specimen was collected in 2022 (AM22-1126).

Identification. Females of *M. nigracauda* have black pile on the head, episterna, and metasoma, and brown to black scopa. Males have a long first flagellar segment and flattened segments 5–11 (LaBerge 1961). *Melissodes nigracauda* paratypes were borrowed from The University of Kansas Entomology Collection and directly compared to the Washington state specimens to confirm identification.

Distribution. The previously known distributional range of *M. nigracauda* is California (LaBerge 1961, Figure 4.3). The nearest known location of *M. nigracauda* is ~1,105 km south of the Washington collection site in Contra Costa County, California, USA (37.87901°N, 121.9203°W, det. LaBerge 1959, Dombroskie 2024). The spatial distance between *M. nigracauda* populations in central Washington and California suggests that the two populations are genetically distinct, or that the intervening region is under sampled. Further investigations using DNA barcoding from specimens in each location coupled with increased sampling would ascertain the extent of genetic differences between these two populations.

Figure 4.3. Known distribution of *Melissodes nigracauda* in North America (represented by gray circles) with new records in Washington state (black square). Known occurrence records were derived from Ikerd (2019) and Dombroskie (2024).

Habitat. LaBerge hypothesized that "this species is restricted to plants of the genus *Stephanomeria* Nutt. (Asteraceae) for sources of pollen. However, the data are as yet too sparse to come to a firm conclusion regarding oligolecty" (1961:330). *Stephanomeria* spp. have been recorded in Chelan County where the specimen was collected (Consortium of Pacific Northwest Herbaria 2024). LaBerge also noted other plants from which *M. nigracauda* has been collected, including species of *Erigeron* L. and *Senecio* L., both of which were present in the study area (personal observation).

Remarks. All specimens were collected in August, which aligns with the known phenology of *Melissodes* Latreille, 1829 as primarily late summer bees (Messinger Carril and Wilson 2023). The two female specimens collected in 2021 were discovered in a landscape that was three years post-fire. The remaining female was collected from the same location two years later. The Cougar Creek Fire (2018) was located 32 km northwest of Entiat, Washington and consumed 16,760 ha., burning at moderate severity. This ground-nesting species either survived the soil heating by wildfire or recolonized within three years after the burn.

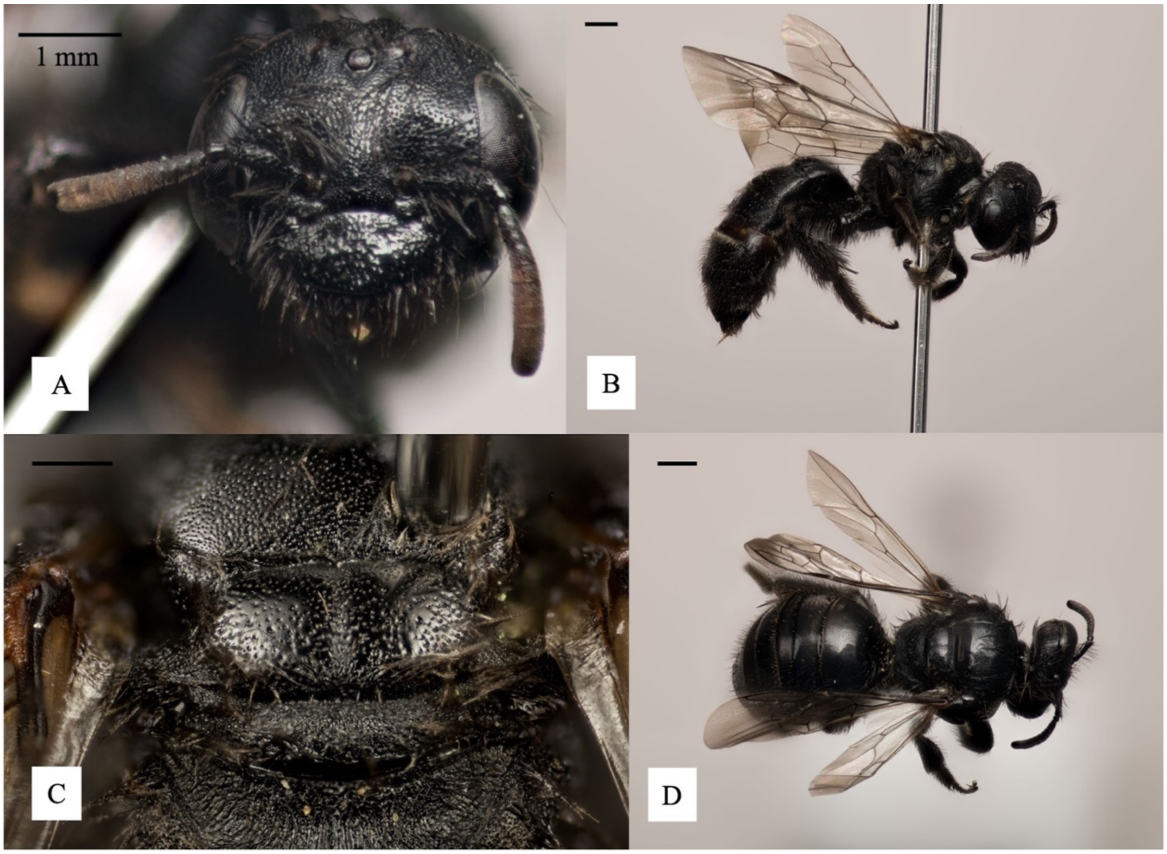


Figure 4.4. Female *Dufourea dilatipes*. **A.** Face, frontal view. Clypeus long. **B.** Lateral view, brown body pubescence **C.** Dorsal view, propodeal triangle dull. Mesoscutellum densely punctate. **D.** Dorsal view. Scale is approximate.

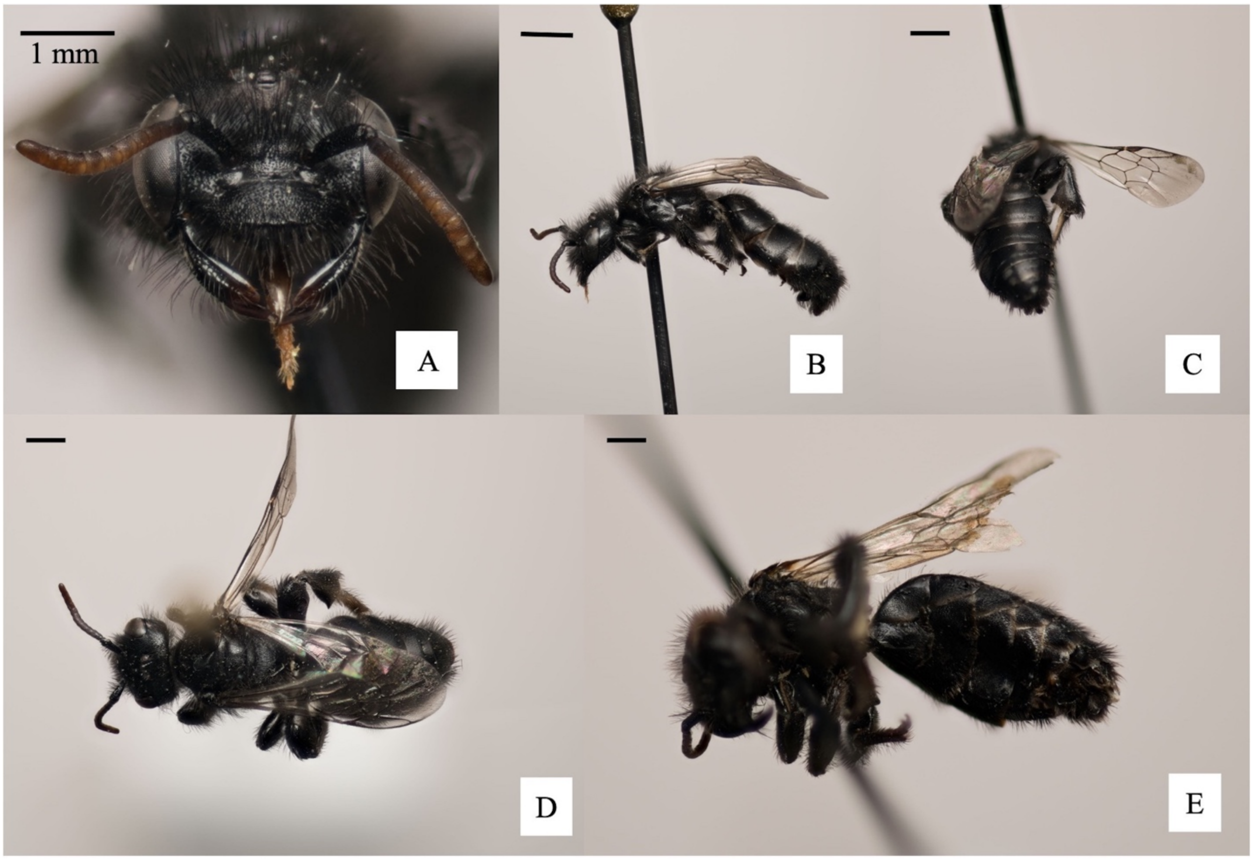


Figure 4.5. Male *Dufourea dilatipes*. **A.** Face, frontal view. Clypeus long. **B.** Lateral view, brown body pubescence. **C.** Hind leg with robust hind femur and triangular hind tibia **D.** Dorsal view, propodeal triangle dull. Mesoscutellum densely punctate. **E**. Ventral view, S4 with medial process on apical margin, S6 with medial process gradually curved. Scale is approximate.

Dufourea dilatipes **Bohart, 1948**

Figures 4.4, 4.5

New records (*n* = 3♀, 2♂). UNITED STATES OF AMERICA – **Washington** • Okanogan Wenatchee National Forest, Chelan Ranger District; Chelan County, Chelan; 47.9525°N, 120.0360°W; 1060 m a.s.l.; May 10–Jul 5 2022; A. Maust leg.; det. KW Wright 2024; 1♀, AM22-0786 • ibid.; 47.9388°N, 120.0550°W; 850 m a.s.l.; 29–30 May 2023; 1♀, AM23-0471 • ibid.; 47.9641°N, 120.0470°W; 1160 m a.s.l.; pan traps; 1♂ AM23-0552 • ibid., Entiat Ranger District; Ardenvoir; 47.7837°N, 120.4246°W; 1010 m a.s.l.; 1♂ AM23-0596• ibid.; 47.7837°N, 120.4246°W; 1010 m a.s.l.; 12–13 Jun 2023; 1♀ AM23-1015.

Identification. *Dufourea dilatipes* are distinguished from other species in *Dufourea* Lepeletier, 1841 by the dark brown to black pubescence, including scopa. *Dufourea maura* is similarly dark, but females of *D. dilatipes* have a smoother, duller propodeum and a densely punctate scutellum, while male *D. dilatipes* have a greatly expanded, triangular metatibia (Dumesh and Sheffield 2012).

Figure 4.6. Known distribution of *Dufourea dilatipes* in North America (represented by gray circles) with new records in Washington state (black squares). Known occurrence records were derived from Sheffield and Heron (2018), Ikerd (2019), and Sheffield (2019).

Distribution. The nearest known record of *D. dilatipes* is from southern British Columbia, Canada (49.025°N, 119.567°W, det. Sheffield, Sheffield, and Heron 2018), which is ~145 km from the collection location. *Dufourea dilatipes* has also been recorded in Shasta County, California, USA (41.0037°N, 121.9050°W, leg. Brown 1965, det. T. Griswold 2006, Ikerd 2019) and Montana, USA (48.4870°N, 113.3668°W, leg. Michener 1988, Ikerd 2019), which are ~777 km south and ~510 km east of the Washington study area, respectively (Figure 4.6).

Habitat. *Dufourea dilatipes* is oligolectic on Mariposa Lilies in the genus *Calochortus* Pursh (Liliaceae). Our findings concur, as *Calochortus lyallii* Baker, 1874 and *Calochortus macrocarpus* Douglas, 1828 are present on the landscape where *D. dilatipes* was collected (Consortium of Pacific Northwest Herbaria 2024; personal observation). *Dufourea dilatipes* is a ground nesting species that was detected only at study sites >5 years post-fire. This may indicate a sensitivity to soil heating or consumption by wildfire for both *D. dilatipes* and *Calochortus* species. The loss of *Calochortus* spp. on landscape due to wildfire would result in unsuitable habitat for *D. dilatipes*. However, one species in the genus, *Calochortus plummerae* Greene*,* has been documented one-year post-fire (Horton and Kraebel 1955; Williams et al. 2021), which suggests *Calochortus plummerae* seeds may be fire-adapted. As an alternate hypothesis, Cane and Neff (2011) observed that bees with nests >10cm beneath the soil horizon were insulated from soil heating. Little is known about the nesting habits of *D. dilatipes*; however, because this species was exclusively detected >5 years post-fire, it may have a shallow nest depth.

Remarks. Due to a limited number of recorded observations, one could infer that *D. dilatipes* is a rare pollinator. Furthermore, the oligolectic behavior of this species emphasizes its importance for the conservation of native *Calochortus* species in dry forest landscapes.

Figure 4.7. Female *Atoposmia abjecta abjecta*. **A.** Face, frontal view. **B.** Mesoscutum, coarsely punctured. The central part with punctures separated by >2 puncture widths extending out to the parapsidal lines. **C.** Lateral view, scopa brownish black. **D.** Abdomen, lateral view. Scale is approximate.

***Atoposmia abjecta abjecta* Cresson, 1878**

Figure 4.7

New record. UNITED STATES OF AMERICA – **Washington** • Okanogan Wenatchee National Forest, Chelan Ranger District; Chelan County, Chelan; 47.9526°N, 120.0360°W; 1060 m a.s.l.; 26–27 Jun 2023; A. Maust leg.; blue vanes; det. T. Griswold 2024; 1♀, AM23-1376.

Three additional female *A. abjecta abjecta* specimens were captured in 2021, but due to a database error their specific locations within the Okanogan–Wenatchee National Forest in Chelan County, Washington cannot be determined. Two female specimens were collected between 28 April and 6 July (AM21-0062 and AM21-0063) and one female specimen was collected between 22 June and 3 August (AM21-1507).

Identification. Females of *Atoposmia abjecta abjecta* are diagnosed by the combination of dark scopa and coarse punctures on the mesoscutum. These punctures are often separated by two or more puncture widths and, in females, extend from the midpoint to the parapsidal lines. While *A. anthodyta* (Michener, 1943) and *A. oregona* (Michener, 1943) have similarly punctured mesoscuta, the punctures are closer and the mandibles, narrower (Hurd and Michener 1955).

Figure 4.8. Known distribution of *Atoposmia abjecta abjecta* in North America (represented by gray circles) with new records in Washington state (black square). Known occurrence records were derived from Ikerd (2019), Best et al. (2022), and Bentley and Falin (2025).

Taxonomic Remarks. *Atoposmia* was previously considered a subgenus of the genus *Anthocopa* by Hurd and Michener (1955).

Distribution. *Anthocopa* (*Atoposmia) abjecta* is reported as occurring in California, Colorado, Oregon, Utah, and Wyoming. The subspecies *A.*

abjecta abjecta is present in California, Oregon, and east to the Rocky Mountains (Hurd and Michener 1955). Five specimens collected in Hood River County (45°N, 122°W, Best et al. 2022) are ~275 km southeast of the sampling area (Figure 4.8). A specimen has also been recorded in southern British Columbia (Sheffield and Heron 2018) which is at least 116 km from the collection location. This new record fills a gap in the known distributional range of *Atoposmia abjecta abjecta*.

Habitat. *Atoposmia abjecta* has been reported to occur in British Columbia's Montane Cordillera ecosystems, a category which includes conifer forests (Sheffield and Heron 2018). This aligns with vegetative observations from the study area, where Ponderosa Pine (*Pinus ponderosa)* was the dominant tree species.

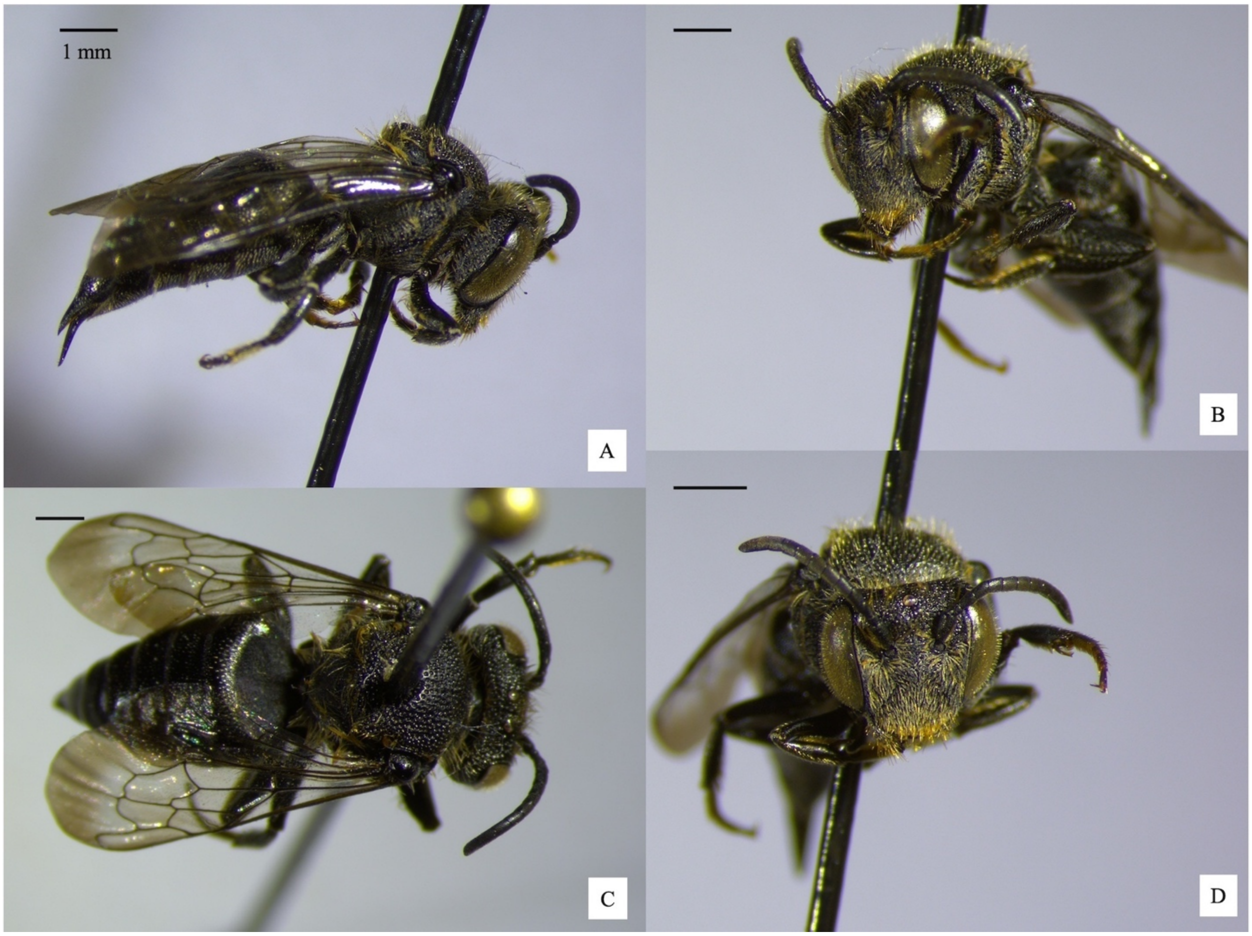


Figure 4.9. Female *Coelioxys funerarius*. **A.** Lateral view, clypeus convex, slightly outcurved margin. **B.** Face, frontal and lateral view. Axilla visible. **C.** Dorsal view, fasciae on the scutum absent. **D.** Face, frontal view. Scale is approximate.

Coelioxys funerarius **Smith, 1854**

Figure 4.9

New record. UNITED STATES OF AMERICA – **Washington** • Okanogan Wenatchee National Forest, Chelan Ranger District; Chelan County, Chelan; 47.9826°N, 120.2978°W; 980 m a.s.l.; 12–13 Jun 2023; A. Maust leg.; pan traps; det. T. Griswold 2024; 1♀, AM23-1109.

Identification. *Coelioxys funerarius* is the only species in the subgenus *Schizocoelioxys* Mitchell, 1973 that occurs in North America. Female specimens are distinguished by the moderately punctured scutum, which lacks fasciae, and the hump on the inner surface of the mandible (Baker 1975). *Coelioxys funerarius* resembles *C. moesta* Cresson, 1864 but is usually larger. Furthermore, the clypeus of female *C. funerarius* is convex with a slightly outcurved margin, while the female of *C. moesta* has a flat clypeus with a triangularly produced margin (Baker 1975).

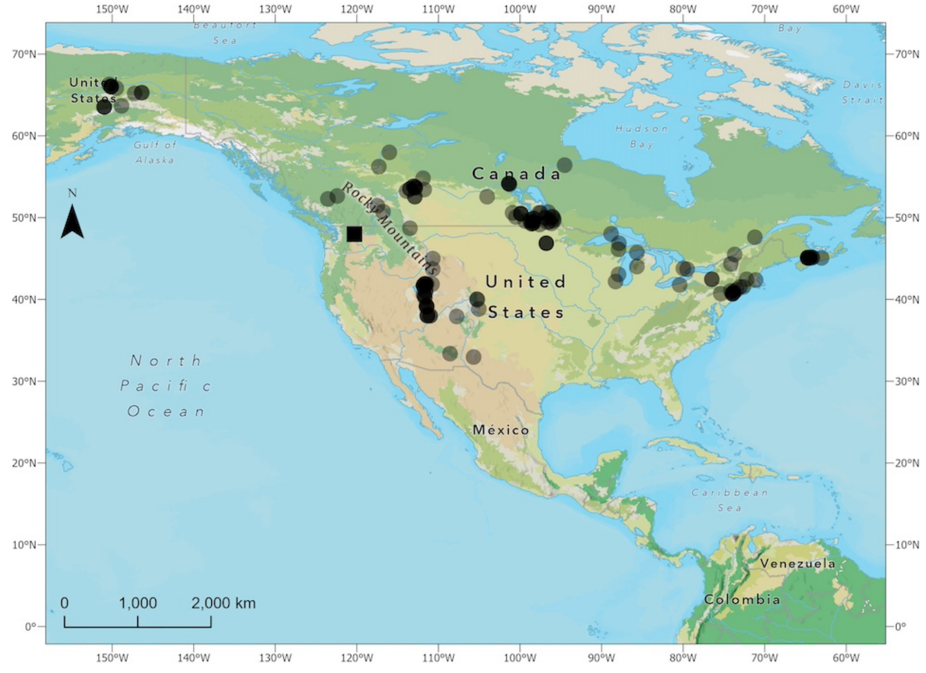


Figure 4.10. Known distribution of *Coelioxys funerarius* in North America (represented by gray circles) with new records in Washington state (black square). Known occurrence records were derived from Ikerd (2019), Gibbs (2024), Sikes (2024), Bentley and Falin (2025), and York University

Distribution. This is the first record of *C. funerarius* with known physical vouchers in the state of Washington. Two records of *C. funerarius* were published on a distribution map by Baker (1975). However, its presence could not be confirmed because the provenance of the voucher specimens associated with those records, if they exist, was not given and they could not be located. The nearest known record of *C. funerarius* were collected in British Columbia (Buckell 1950), approximately 400 km northeast of the study location. The known distribution of *Coelioxys funerarius* is shown in Figure 4.10.

Remarks. *Coelioxys funerarius* is a known cleptoparasite of five species of *Megachile* spanning three subgenera in North America: *M. rotundata* Fabricius, 1787, *M. inermis* Provancher, 1888, *M. relativa* Cresson, 1878, *M. frigida* Smith, 1853, and *M. latimanus* Say, 1823 (Baker 1975). Of these recorded hosts, *Megachile frigida*, *Megachile relativa,* and *Megachile rotundata* (Bartholomew et al. 2024) are present in Washington, though were not collected in this study. Due to the wide breadth of hosts, coupled with the extensive distributional range of the species, *C. funerarius* is considered a generalist parasite (Baker 1975). Therefore, it could likely attack any of the *Megachile* species found in this study as a host (Supplemental Table 4.2).

Coelioxys funerarius was collected within the burn perimeter of the Twentyfive Mile Creek Fire, which burned 8,990 ha approximately 40 km west of Chelan, Washington, in August of 2021. The site at which *C. funerarius* was collected burned at moderate severity, which is higher than the historically low severity fire experienced by this landscape (Agee 1993). All hosts are known cavity-nesting species (Messinger Carril and Wilson 2023), suggesting an ability of both the hosts and parasite to recolonize a landscape from adjacent unburned landscapes within two years following moderate severity wildfire. *Coelioxys funerarius* was captured in a pan trap between the 12th and 13th of June 2023, which aligns with the known phenology of the genus to fly from late May to mid-September (Messinger Carril and Wilson 2023) with most specimens collected from mid-June through August (Baker 1975).

Figure 4.11. Female *Dianthidium cressonii*. **A.** Face, frontal view. **B.** Abdomen, dorsal view of T6. **C.** Abdomen, dorsal view of terga I–V. **D.** Lateral view. Black with yellow pubescence. Strong yellow maculations. Large, approximately 11 mm. **E.** Dorsal view, segment T2 three-spotted. Scale is approximate.

Dianthidium cressonii **Dalla Torre, 1896**

Figures 4.11, 4.12

New records (*n* = 23♀, 12♂). UNITED STATES OF AMERICA – **Washington** • Okanogan Wenatchee National Forest, Chelan Ranger District; Chelan County, Chelan; 47.9388°N, 120.0550°W; 850 m a.s.l.; 21–23 Jun 2021; A. Maust leg.; blue vanes; det. KW Wright 2024; 1♂, W21-0052 • ibid.; 47.9526°N, 120.0360°W; 1060 m a.s.l.; 21–23 Jun 2021; 1♀, 1♂, W21-0051, W21-0050 • ibid.; Ardenvoir; 47.7837°N, 120.4246°W; 1010 m a.s.l.; 1♀, 1♂, W21-0073, W21-0074 • ibid.; Chelan; 47.9826°N, 120.2978°W; 980 m a.s.l.; 1–3 August 2022; 1♀, 1♂, W22-0135. The remaining 29 specimens are summarized in Table 4.1.

Figure 4.12. Male *Dianthidium cressonii*. **A.** Face, frontal view. **B.** Terminalia, dorsal view. **C.** Abdomen, dorsal view. Edges of tergites inflated. **D.** Lateral view, left. Spines on coxae unusually long. **E.** Dorsal view. Scale is approximate.

Identification. Both sexes of *Dianthidium cressonii* are readily identified by the laterally inflated metasomal terga; the male additionally has the median process of T7 straight and parallel to the lateral lobes. *Dianthidium singulare* also shares these characters, but the lateral inflations of the metasomal terga are not as strongly developed in *D. cressonii* (about twice as large in *D. singulare*), and the apical margin of the female T6 is slightly more rounded (Grigarick and Stange 1968) (Figure 4.11B).

Distribution. According to Grigarick and Stange (1968: 54), "*D. cressonii* occurs in Colorado and Nevada and may eventually be found in eastern California". This hypothesis has not yet come to

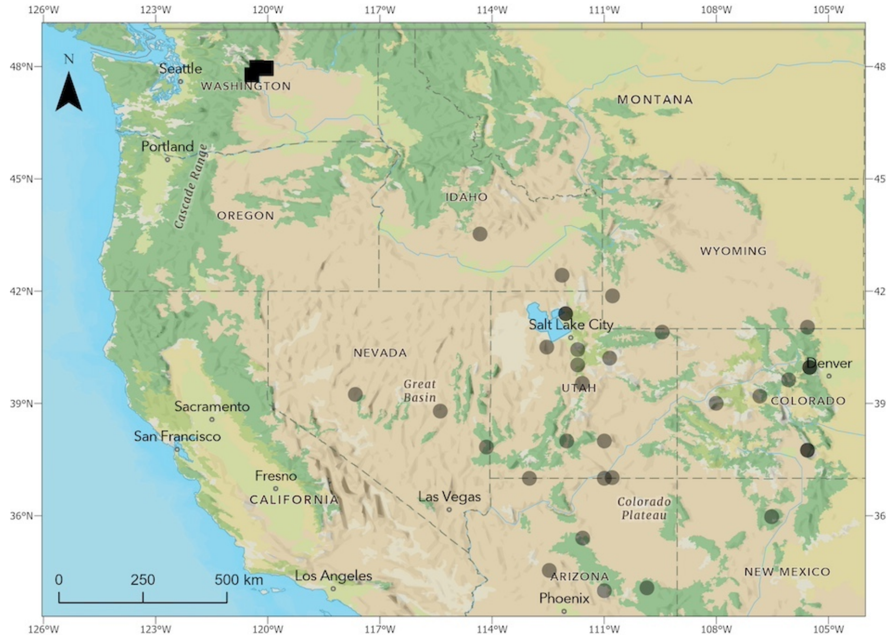


Figure 4.13. Known distribution of *Dianthidium cressonii* in North America (represented by gray circles) with new records in Washington state (black squares). Known occurrence records were derived from Ikerd (2019) and Johnson (2020).

fruition, as there are no documented records of *D. cressonii* in the state of California (Figure 4.13). Schwarz (1926) cited *D. cressonii* as occurring only in Colorado and Utah. The nearest georeferenced record is from Blaine, Idaho (43.525°N, 114.321°W, Johnson 2020), ~664 km southeast of the study area. The farthest record is from Sandoval County, NM (35.9747°N, 106.5228°W, leg. 2009, det. K.T. Huntzinger 2011, Ikerd 2019), ~1,735 km southeast of the study area.

Habitat. *Dianthidium* Cockerell, 1900 is known to construct nests of gravel or pebbles on branches and rock surfaces using mud and resin, or more rarely, build nests in the ground (Messinger Carril and Wilson 2023). Though the specific nesting preferences of *Dianthidium cressonii* are unknown, the closely related *Dianthidium singulare* nests on rock surfaces (Grigarick and Stange 1968). The presence of *D. cressonii* one year post fire, coupled with known similarities among nesting preferences of related species, suggests that *D. cressonii* is likely a rock surface nesting species with a rapid recolonization rate to post-fire areas.

Remarks. *Dianthidium cressonii* was the most abundant new record identified in this study, with 35 specimens recorded. It was collected each year of sampling (2021–2023) across all four sites with both blue vane and pan traps between early June and early August. *Dianthidium cressonii* was found both in landscapes that had recently burned (1–3 years post fire) and landscapes that had not burned in more than 50 years (Figure 4.1), suggesting a lack of dependency on disturbance for survival. Notably, *D. cressonii* was found approximately one year post fire (August 2022) within the burn perimeter of the aforementioned Twentyfive Mile Creek Fire. Arid deserts associated with *D. cressonii* span the western United States and have climatic and vegetative similarities. Thus, it is probable that *D. cressonii* is distributed throughout temperate desert ecosystems across the Northern Basin and Range ecoregion and into the Columbia Plateau. The comparatively common occurrence of newly documented *D. cressonii* on the landscape highlights the need for species level identification and long-term monitoring of bee populations across Washington state to ensure native bee biodiversity can be effectively protected.

Figure 4.14. Female *Dianthidium singulare*. **A.** Face, frontal view. **B.** Abdomen, dorsal view. T6 shape rectangular. **C.** Abdomen, dorsal view. Maculations bright yellow. **D.** Lateral view. Lateral margins of terga decreasingly inflated. **E.** Thorax, dorsal view. Scale is approximate.

***Dianthidium singulare* Cresson, 1879**

Figure 4.14

New record. UNITED STATES OF AMERICA – **Washington** • Okanogan Wenatchee National Forest, Chelan Ranger District; Chelan County, Chelan; 47.9403°N, 120.0418°W; 960 m a.s.l.; 1–3 Aug 2022; A. Maust leg.; pan traps; det. A. Maust 2024; 1♀, W22-0142.

Identification See the identification of *D. cressonii* above.

Distribution. *Dianthidium singulare* has been frequently reported in the mountainous regions of eastern and southern California and is collected less frequently in Nevada (Grigarick and Stange 1968). There are three records of *D. singulare* from Osoyoos, British Columbia, which is ~130 km north of the collection location (49.0069°N, 119.488°W, Sheffield 2019). The known distribution of *Dianthidium singulare* in North America is shown in Figure 4.15.

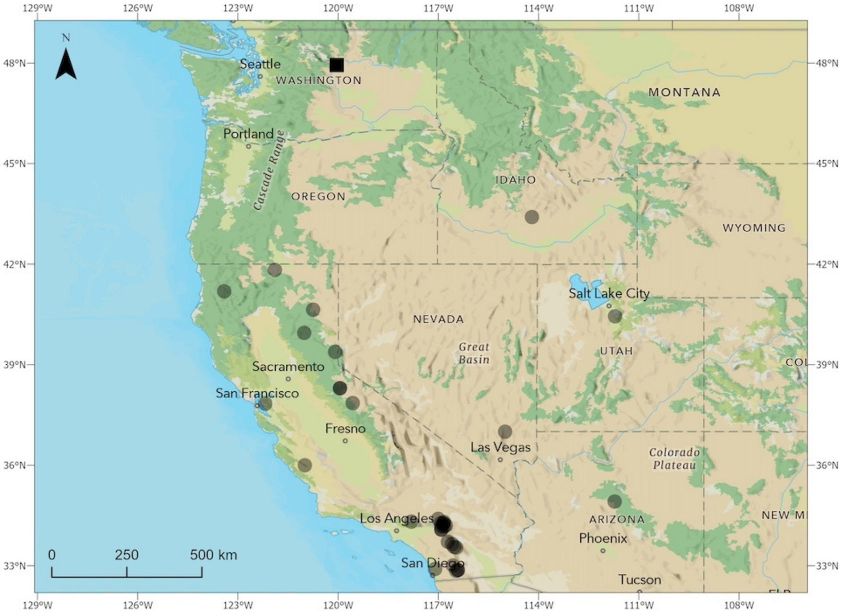


Figure 4.15. Known distribution of *Dianthidium singulare* in North America (represented by gray circles) with new records in Washington state (black square). Known occurrence records were derived from Sheffield (2019) and Ikerd (2019).

Habitat. *Dianthidium singulare* constructs individual cells from resin and pebbles to nest on the surface of rocks (Grigarick and Stange 1968). The limited foraging records associated with *D. singulare* suggest a floral preference for Asteraceae, which is abundant on the study landscape (personal observation). Specifically, Arrowleaf Balsamroot (*Balsamorhiza sagittata*) is the main floral resource early in the season (personal observation). More recently, Sheffield and Heron (2018) reported *D. singulare* as associated with the Western Interior Basin ecozone. This ecozone includes the lower Okanagan Valley dominated by Antelope Bitterbrush (*Purshia tridentata* Pursh, Rosaceae) in British Columbia. *Purshia tridentata* was present across the study area (personal observation; Consortium of Pacific Northwest Herbaria 2024) and thus reflects the landscape traits associated with this species.

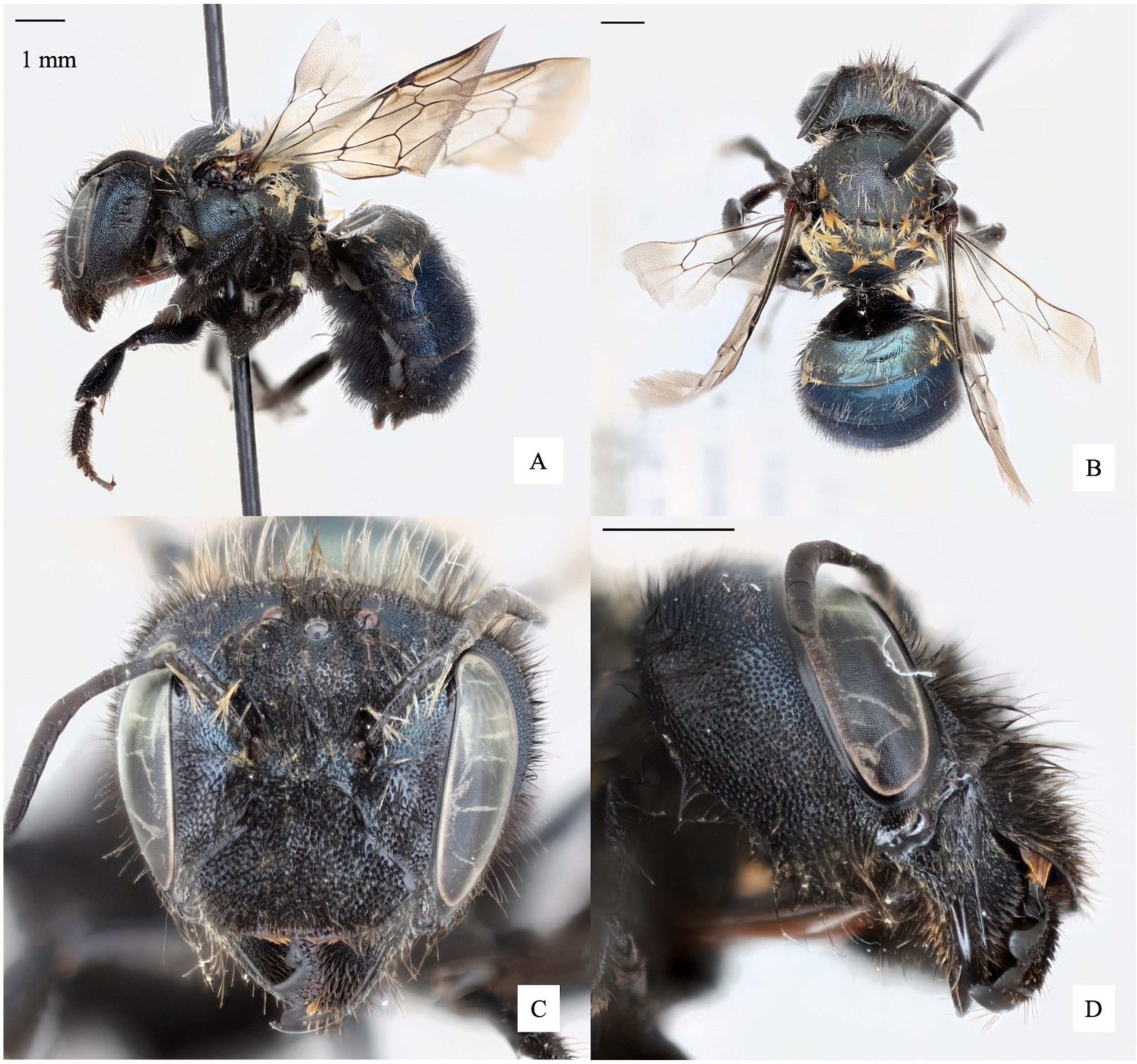


Figure 4.16. Female *Osmia cyaneonitens*. **A.** Lateral view. **B.** Dorsal view. **C.** Face, frontal view. Coarse punctures and proclinate bristles on the face, mandibles wide apically. **D.** Face, lateral view. High projection of the hypostomal carina.

Osmia cyaneonitens **Cockerell, 1906**

Figure 4.16

New record. UNITED STATES OF AMERICA – **Washington** • Okanogan Wenatchee National Forest, Chelan Ranger District; Chelan County, Chelan; 47.9526°N, 120.0360°W; 1060 m a.s.l.; 29–30 May 2023; A. Maust leg.; blue vanes; det. J. Gardner 2024; 1♀, AM23-0542.

Identification. Female *O. cyaneonitens* are distinguished by the presence of coarse, proclinate bristles on the frons (similar to those in *O. brevis* and *O. pentstemonis*), apically broadened mandibles (similar to the "*Acanthosmioides*" group of species), and high hypostomal carina abruptly reduced at the lateral angle (Sandhouse 1939).

Distribution. The distributional range of *O. cyaneonitens* spans North America and stretches from California east to Kanas, USA and north to Manitoba, Canada (Figure 4.17). The closest georeferenced specimen was collected in southern British Columbia, which is at least 116 km from the collection location (Elwell et al. 2016). Across British Columbia, *O. cyaneonitens* has been reported to reside in the Western Interior Basin ecozone, which is comparable to the plant communities found in the Okanogan Wenatchee National Forest (Sheffield and Heron 2018; Ikerd 2019). The next nearest georeferenced specimens are from Sanders County, Montana, USA which is at least 325 km east of the collection site (Ikerd and Engler 2023).

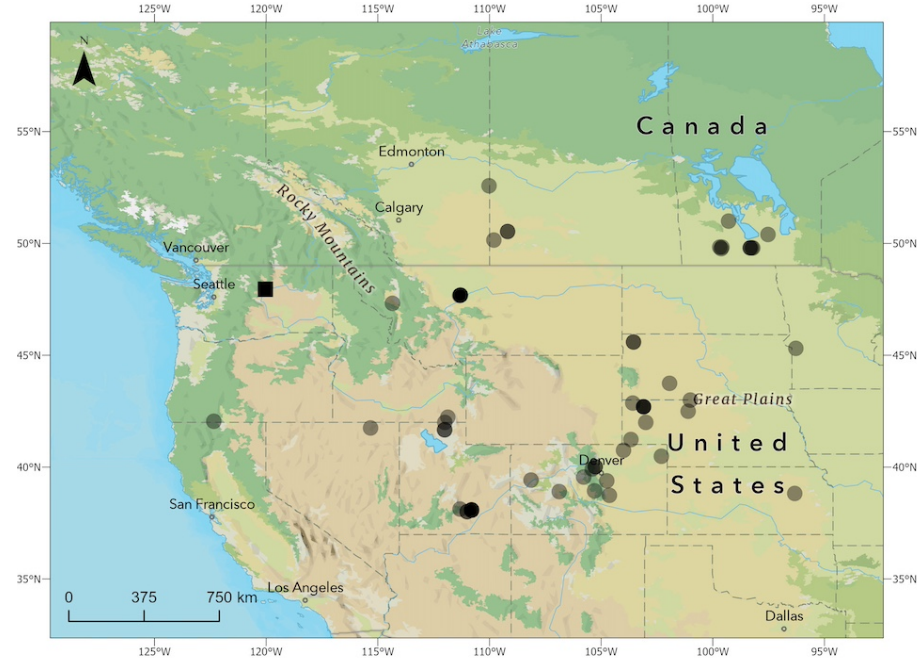


Figure 4.17. Known distribution of *Osmia cyaneonitens* in North America (represented by gray circles) with new records in Washington state (black square). Known occurrence records were derived from Ikerd (2019), Ikerd and Engler (2023), and Gibbs (2024).

Remarks. Idaho Fish and Game regards *O. cyaneonitens* as native to Idaho, though only in the southeastern corner of the state (2024). Its conservation rank according to this source is G3G4, which means it is rare or uncommon, but not imperiled. Discover Life (2024) cites the floral hosts for this species as Fabaceae and Scrophulariaceae, which are present on the collection landscape (Consortium of Pacific Northwest Herbaria 2024; personal observation). Specifically, *O. cyaneonitens* is a known pollinator of *Penstemon* Schmidel species, including Blowout Penstemon (*Penstemon haydenii* S. Watson, Plantaginaceae; Tepedino et al. 2006; Tepedino et al 2006), which is considered Endangered by the United States Fish and Wildlife Service (1987). The *O. cyaneonitens* specimen collected in this study was found in a landscape that was 8 years post-fire. The nesting habitat for this species is unknown.

Figure 4.18. Female *Stelis heronae*. **A.** Dorsal view. **B.** Lateral view, left. **C.** Face, frontal view. Clypeus entirely black. **D.** Abdomen, dorsal view. Pale maculations on terga I–IV. **E.** Ocelli, frons, vertex. Dorsal view. Ocelli normal sized. Upper frons and vertex densely punctate. Pronotal lobe and tegula black. Scale is approximate.

Stelis heronae **Sheffield, 2024**

Figures 4.18, 4.19

New records (*n* = 2♂). UNITED STATES OF AMERICA – **Washington** • Okanogan Wenatchee National Forest, Chelan Ranger District; Chelan County, Chelan; 47.9388°N, 120.0550°W; 850 m a.s.l.; 10–11 Jul 2023; A. Maust leg.; pan traps; det. T. Griswold 2024; AM23-1730 and AM23-1732.

Two additional female *S. heronae* specimens were collected in 2022, but due to a database error their specific locations within the Okanogan–Wenatchee National Forest in Chelan County, Washington cannot be determined. One specimen was collected between 18 July and 1 August (AM22-0597), the other was collected between 5 July and 1 August (AM22-1314).

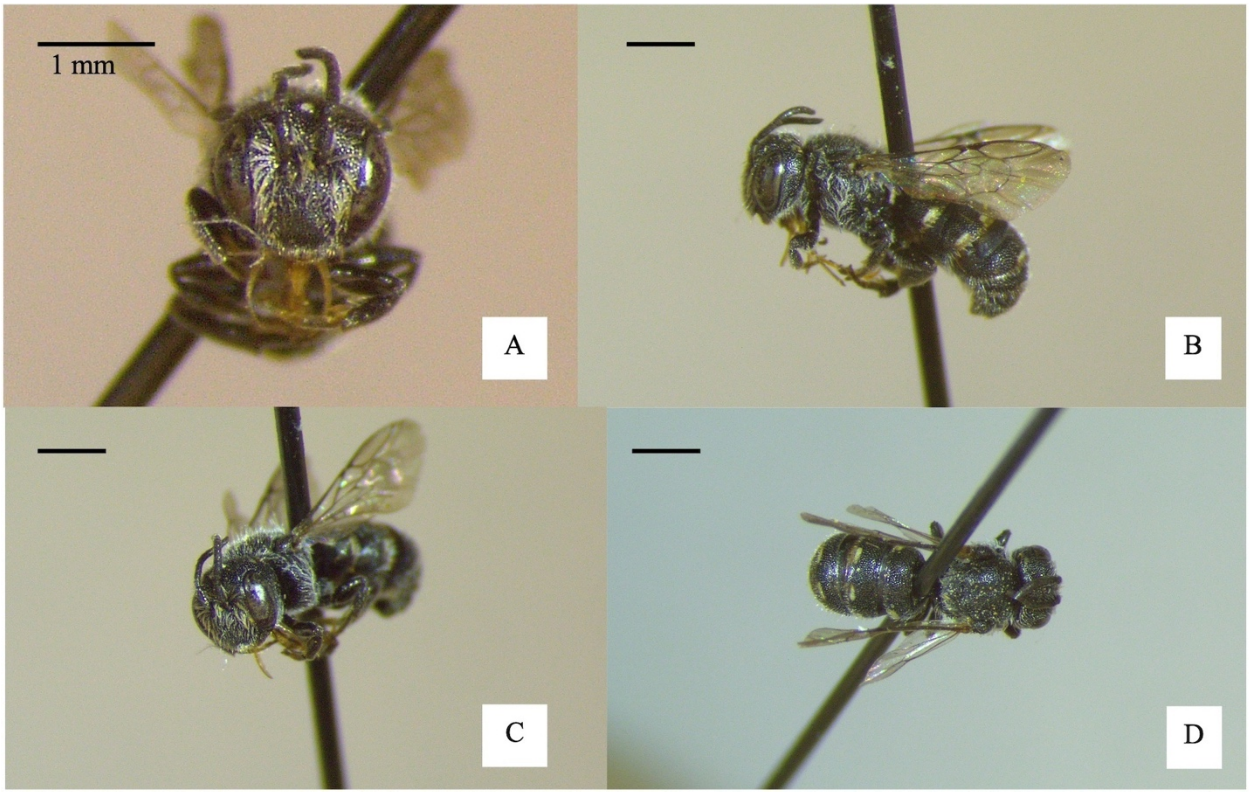


Figure 4.19. Male *Stelis heronae*. **A.** Face, frontal view. Clypeus black. **B.** Lateral view, left. Maculations visible. **C.** Face and lateral view, left. **D.** Dorsal view, scutum and abdominal maculations visible. Scale is approximate.

Identification. Females of *S. heronae* are distinguished from other western species in *Stelis* Panzer, 1806 by the pale maculations on terga 1–4 or 5. *Stelis anasazi* closely resemble *S. heronae*, but *S. anasazi* only has pale maculations on terga 1–3 (Sheffield 2024).

Taxonomic Remarks. *Stelis heronae* was separated from other members of the *Stelidium* group by Sheffield (2024).

Distribution. Three *S. heronae* types were collected in southern British Columbia from Lillooet (50°54'46.1"N, 122°14'17.7"W, leg. Bennett, Copley, and Copley 2012), Kelowna (49.8111°N, 119.4402°W, leg. Dawson and Heron 2016), and Vaseux Lake (49.2614°N, 119.508°W, leg. Packer 2009) which are at least 338 km north, 222 km northeast and 162 km northeast of the study area, respectively (Figure 4.20). *Stelis heronae* is distributed across the western interior basin ecozone of southern British Columbia for which similarities to the study area have been noted. Specimens for this study were collected in July of 2022 and 2023, which aligns with the documented phenology of the species (Sheffield 2024).

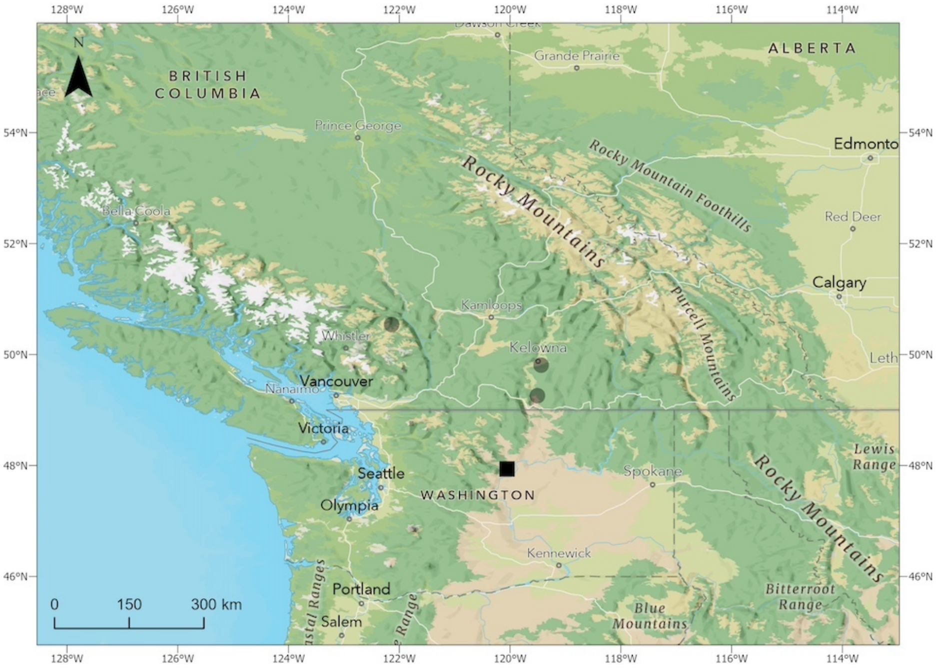


Figure 4.20. Known distribution of *Stelis heronae* in North America (represented by gray circles) with new records in Washington state (black square). Known occurrence records were derived from Sheffield (2024).

4.5 Discussion

Washington state contains a vast array of ecosystems that vary topographically, climatically, and vegetatively (Franklin and Dryness 1973). Each system has a unique associated bee community (Cane 2011; Looney and Eigenbrode 2012; Orr et al. 2021). There is a deficit of species level bee data in some regions of Washington, USA, as exemplified by the 100 new records for Chelan County resulting from this study. Therefore, it is likely that continued fine-scale bee diversity research and repeated monitoring in undersampled regions will reveal new records. Furthermore, climate shifts may result in less suitable habitat for key forest species across the state (Littell et al. 2010). Thus, this study provides a reference of bee distributional ranges in anticipation of further environmental changes, such as those resulting from increased habitat loss and climate change. We specifically examined the effects of fire on bee communities and used known nesting information to infer recolonization and survival following disturbance. Understanding the geographic distribution of native forest bee species facilitates targeted conservation and restoration efforts for both native bees and associated foraging and nesting plants. This is particularly critical for rare, native pollinators with highly specialized relationships to floral hosts.

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Additional information

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Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and should not be construed to represent any official USDA or U.S. Government determination or policy.

Data availability

All data that supports the findings of this study are available in the main text and Appendix D. The complete raw data is deposited in ecdysis and is available at https://ecdysis.org/.

4.6 Appendix D

Supplemental Table 4.1. Taxonomic keys used for species level identification of Hymenoptera, Anthophila species in Washington, USA. Species level identifications were determined by Autumn Maust (AM), Joel Gardner (JG), Karen W. Wright (KW), and Terry Griswold (TG). Genera for which the determiner listed is N/A did not have available keys and therefore were not identified to the species level. Identification to genera was completed using the CANPOLIN Bee Genera key (AM).

Family	**Genus**	**Taxonomic Key**	**No. of individuals**	**Determiner (year)**
Andrenidae	*Andrena*	Ribble 1968; Bouseman and Laberge 1973, 1977, 1980, 1985, 1989; LaBerge 1978; Michener 2007	153	JG (2023)
	Panurginus	Michener 1935	52	JG (2023)
	Perdita	Timberlake 1954-1980	34	KW/TG (2024)
Apidae	*Anthophora*	Ascher and Pickering 2016; Discover Life 2024	31	AM/KW (2023)
	Apis	CANPOLIN Bee Genera	27	AM (2023)
	Bombus	Williams et al. 2014	572	AM (2023)
	Ceratina	Daly 1973	14	KW (2024)
	Diadasia	Sipes 2001	5	KW (2024)
	Eucera	Timberlake 1969	56	KW (2024)
	Habropoda	Discover Life 2024	2	AM/KW (2024)
	Melecta	Discover Life 2024	16	AM (2023)
	Melissodes	LaBerge 1956, 1961*	33	KW (2024)
	Nomada	Key not available	2	N/A
Colletidae	*Colletes*	Stephen 1954	4	KW (2024)
	Hylaeus	Snelling 1966; Snelling 1970	39	KW/JG (2024)
Halictidae	*Agapostemon*	Roberts 1973; Portman et al. 2024	542	AM/KW/JG (2024)
	Dufourea	Bohart 1948; Dumesh and Sheffield 2012*	5	KW (2024)
	Halictus	Roberts 1973	1,734	AM (2023)
	Lasioglossum	McGinley 1986; Gardner and Gibbs 2022	1,475	JG/AM (2023)
	Sphecodes	Key not available	8	N/A
Megachilidae	*Anthidium*	Gonzalez and Griswold 2013; Burrows et al. 2021	13	AM (2024)
	Ashmeadiella	Michener 1939	3	AM/TG (2024)
	Atoposmia	Hurd and Michener 1955*	4	TG (2024)

	Coelioxys	Baker 1975*	2	TG (2024)
	Dianthidium	Schwarz 1926*; Grigarick and Stange 1968*	65	AM/KW (2024)
	Heriades	Hurd and Michener 1955	1	TG (2024)
	Hoplitis	Discover Life 2024	161	AM/JG/TG (2024)
	Megachile	Sheffield et al. 2011	130	AM/TG (2024)
	Osmia	Sandhouse 1939*; Rightmyer et al. 2010	480	JG/AM/TG (2024)
	Protosmia	CANPOLIN Bee Genera	5	AM (2023)
	Stelis	Michener 2007; Sheffield 2024*	8	TG (2024)
	Triepeolus	Key not available for males	1	N/A

*Key used to identify new Washington, USA records.

Supplemental Table 4.2. Sampling of Okanogan–Wenatchee National Forest bee fauna from 2021–2023 yielded 5,676 bee specimens representing 32 genera and 201 species. Species that have not been documented in Washington, USA since before the year 2000 have been annotated (Bartholomew et al. 2024). Of the specimens collected, 1,885 individuals (33.21%) are new records for Chelan County and 58 individuals (1.02%) are new records for the state of Washington.

Family	Species	No. collected 2021	No. collected 2022	No. collected 2023	Total
Andrenidae	*Andrena angustitarsata*	2	1	0	3
	Andrena astragali	0	0	1	1
	Andrena buckelli	1	0	0	1
	Andrena candida	0	0	1	1
	Andrena candidiformis	0	3	1	4
	Andrena cleodora	0	1	0	1
	Andrena lawrencei	0	6	1	7
	Andrena lupinorum	3	0	0	3
	Andrena melanochroa	3	2	2	7
	Andrena microchlora	1	0	0	1
	Andrena nigrocaerulea	0	3	2	5
	Andrena nivalis	1	5	1	7
	Andrena pallidifovea	1	5	1	7
	Andrena pertristis	2	0	0	2
	Andrena prunorum	23	34	34	91
	Andrena quintiliformis	0	5	0	5
	Andrena salicifloris	1	1	0	2
	Andrena schuhi	1	0	0	1
	Andrena (Trachandrena) sp. 1	0	1	0	1
	Andrena (Trachandrena) sp. 2	0	0	1	1
	Andrena trevoris	0	1	0	1
	Andrena vicinoides	0	1	0	1
	Panurginus atriceps	0	6	1	7
	Panurginus nigrellus	1	7	1	9
	Panurginus spp.	25	3	8	36
	Perdita nevadensis nevadensis[*]	1	0	2	3
	Perdita aff. *tortifoliae*	6	0	8	14
	Perdita wyomingensis sculleni	9	0	5	14
	Perdita aff. *wyomingensis*	1	0	2	3
Apidae	*Anthophora bomboides*	3	1	0	4
	Anthophora porterae	5	4	6	15
	Anthophora terminalis	0	1	1	2

	Anthophora urbana	4	4	2	10
	Apis mellifera	5	10	12	27
	Bombus appositus	19	28	20	67
	Bombus centralis	84	26	72	182
	Bombus fervidus	36	42	45	123
	Bombus insularis	3	3	0	6
	Bombus melanopygus	0	2	0	2
	Bombus mixtus	2	0	0	2
	Bombus nevadensis	0	0	3	3
	Bombus rufocinctus	2	2	2	6
	Bombus sp. 1	1	0	0	1
	Bombus sp. 2	1	0	0	1
	Bombus sylvicola	1	0	1	2
	Bombus vancouverensis	28	21	24	73
	Bombus vandykei	13	23	14	50
	Bombus vosnesenskii	8	18	28	54
	Ceratina nanula	5	1	2	8
	Ceratina sequioae[†]	2	1	3	6
	Diadasia diminuta	1	0	0	1
	Diadasia nitidifrons[‡]	2	1	1	4
	Eucera actuosa	0	1	0	1
	Eucera delphinii	1	7	1	9
	Eucera edwardsii	8	28	9	45
	Eucera fulvitarsis	0	0	1	1
	Habropoda cineraria	0	0	1	1
	Habropoda morrisoni[§]	0	0	1	1
	Melecta pacifica	4	1	9	14
	Melecta seperata	1	1	0	2
	Melissodes agilis	0	0	3	3
	Melissodes communis	0	10	0	10
	Melissodes grindeliae/robustior	0	1	0	1
	Melissodes lupinus	0	0	1	1
	Melissodes microstictus	5	1	5	11
	Melissodes nigracauda	5	1	1	7
	Nomada spp.	0	0	2	2
Colletidae	*Colletes consors*	0	1	0	1
	Colletes fulgidus	0	1	2	3
	Hylaeus affinis[¶]	0	0	3	3
	Hylaeus ?conspicuus	14	0	3	17
	Hylaeus ?polifolii	0	1	0	1

	Hylaeus ?rudbeckiae	0	2	0	2
	Hylaeus spp.	3	1	1	5
	Hylaeus wootoni	5	2	4	11
Halictidae	*Agapostemon femoratus*	0	0	5	5
	Agapostemon subtilior	163	52	321	536
	Agapostemon virescens	0	0	1	1
	Dufourea dilatipes	0	1	4	5
	Halictus confusus	1	2	5	8
	Halictus farinosus	154	218	381	753
	Halictus ligatus	0	1	2	3
	Halictus rubicundus	7	5	5	17
	Halictus tripartitus	328	167	457	952
	Halictus virgatellus	0	0	1	1
	Lasioglossum albohirtum	1	2	3	6
	Lasioglossum anhypops	4	17	27	48
	Lasioglossum aspilurum[#]	1	1	2	4
	Lasioglossum athabascense	8	10	7	25
	Lasioglossum buccale[**]	8	2	10	20
	Lasioglossum cf. *cooleyi*	32	121	47	200
	Lasioglossum egregium	5	18	5	28
	Lasioglossum foxii grp.	1	0	2	3
	Lasioglossum fratellum grp.	2	0	2	4
	Lasioglossum glabriventre	13	25	59	97
	Lasioglossum helianthi	2	0	3	5
	Lasioglossum incompletum	2	0	2	4
	Lasioglossum inconditum[††]	0	11	1	12
	Lasioglossum knereri	2	3	20	25
	Lasioglossum macroprosopum	15	9	22	46
	Lasioglossum marinense	1	0	1	2
	Lasioglossum mellipes	13	16	15	44
	Lasioglossum nevadense	115	124	231	470
	Lasioglossum nigroviride	0	0	6	6
	Lasioglossum ovaliceps	1	2	1	4
	Lasioglossum pacificum	0	0	2	2
	Lasioglossum prasinogaster	6	6	7	19
	Lasioglossum pruinosum	1	0	2	3
	Lasioglossum punctatoventre	15	16	27	58
	Lasioglossum reasbeckae	1	1	6	8
	Lasioglossum cf. *ruficorne*	4	1	3	8
	Lasioglossum ruidosense	14	7	5	26

	Lasioglossum sandhousiellum	19	4	9	32
	Lasioglossum sedi	2	2	10	14
	Lasioglossum sisymbrii	18	22	22	62
	Lasioglossum (*Sphecodogastra*) sp. 1	2	7	5	14
	Lasioglossum (*Sphecodogastra*) sp. 2	2	16	11	29
	Lasioglossum (*Sphecodogastra*) sp. 3	1	10	0	11
	Lasioglossum (*Sphecodogastra*) sp. 4	0	5	0	5
	Lasioglossum (*Sphecodogastra*) sp. 5	14	16	8	38
	Lasioglossum (*Sphecodogastra*) sp. 6	33	2	2	37
	Lasioglossum spp.	1	8	0	9
	Lasioglossum trizonatum	14	22	11	47
	Sphecodes sp. 1	1	0	0	1
	Sphecodes sp. 2	1	0	3	4
	Sphecodes sp. 3	0	1	1	2
	Sphecodes spp.	0	0	1	1
Megachilidae	*Anthidium banningense*	2	0	3	5
	Anthidium formosum	1	0	2	3
	Anthidium mormonum	2	0	0	2
	Anthidium utahense	0	1	1	2
	Anthidium sp. 1	1	0	0	1
	Ashmeadiella californica	1	0	0	1
	Ashmeadiella cubiceps cubiceps	0	1	0	1
	Atoposmia abjecta abjecta	3	0	1	4
	Atoposmia elongata	1	0	0	1
	Coelioxys funerarius	0	0	1	1
	Coelioxys octodentata	0	1	0	1
	Dianthidium cressonii	21	9	5	35
	Dianthidium heterulkei	1	1	1	3
	Dianthidium pudicum	3	1	0	4
	Dianthidium singulare	0	1	0	1
	Dianthidium subparvum	8	5	8	21
	Dianthidium ulkei	1	0	0	1
	Heriades carinatus	0	1	0	1
	Hoplitis albifrons	64	42	41	147
	Hoplitis fulgida fulgida	3	3	1	7
	Hoplitis hypocrita	0	0	1	1
	Hoplitis producta	2	0	0	2
	Hoplitis sambuci	0	2	2	4
	Megachile angelarum	0	1	0	1
	Megachile apicalis	0	0	1	1

	Megachile brevis	0	1	5	6
	Megachile ?coquilletti	0	0	1	1
	Megachile gemula	0	1	0	1
	Megachile lapponica	0	1	0	1
	Megachile melanophaea	3	1	0	4
	Megachile montivaga	4	6	3	13
	Megachile onobrychidis	2	0	0	2
	Megachile pascoensis	11	7	6	24
	Megachile perihirta	21	27	20	68
	Megachile pugnata	0	1	3	4
	Megachile subnigra	1	0	2	3
	Megachile wheeleri	0	0	1	1
	Osmia albolateralis	5	16	25	46
	Osmia atrocyanea	11	40	53	104
	Osmia brevis	1	1	0	2
	Osmia bruneri	0	1	1	2
	Osmia bucephala	0	2	1	3
	Osmia cahuilla	0	0	1	1
	Osmia californica	13	37	57	107
	Osmia calla	1	1	3	5
	Osmia cara[‡‡]	1	4	6	11
	Osmia ?cara	0	1	1	2
	Osmia cobaltina[§§]	1	3	2	6
	Osmia coloradensis	1	1	0	2
	Osmia cyanella	0	1	1	2
	Osmia cyaneonitens	0	0	1	1
	Osmia densa	0	4	11	15
	Osmia ?densa	0	1	0	1
	Osmia exigua	1	5	3	9
	Osmia juxta	2	2	4	8
	Osmia kincaidii	0	0	1	1
	Osmia marginipennis	0	1	3	4
	Osmia montana montana	5	15	108	128
	Osmia nanula	0	1	0	1
	Osmia nemoris	0	1	0	1
	Osmia nifoata	1	0	0	1
	Osmia ?nifoata	0	0	1	1
	Osmia proxima	1	0	0	1
	Osmia sedula	1	0	0	1
	Osmia simillima	0	0	1	1

	Osmia spp.	0	1	1	2
	Osmia texana	0	1	0	1
	Osmia trevoris	2	1	5	8
	Osmia ?vandykei	0	1	0	1
	Protosmia rubifloris	2	0	3	5
	Stelis heronae	0	2	2	4
	Stelis montana	1	1	1	3
	Stelis subcaerulea	0	1	0	1
	Triepeolus sp. 1	0	0	1	1

[*] *Perdita nevadensis*; not documented since 1949.

[†] *Ceratina sequioae*; not documented since 1919.

[‡] *Diadasia nitidifrons*; not documented since 1919.

[§] *Habropoda morrisoni*; not documented since 1995.

[¶] *Hylaeus affinis*; not documented since 1957.

[#] *Lasioglossum aspilurum*; not documented since 1973.

[**] *Lasioglossum buccale*; not documented since 1970.

[††] *Lasioglossum inconditum*; not documented since 1985.

[‡‡] *Osmia cara*; not documented since 1935.

[§§] *Osmia cobaltina*; not documented since 1977.

Conclusion

Ponderosa pine forests are historically adapted to low severity, short interval fire regimes. Although contemporary shifts in fire frequency and severity have altered disturbance dynamics, the reintroduction of wildfire to fire adapted forests has positive effects on native pollinator communities.

Key findings

This work documents novel records of bee species for both Washington state and Chelan County. Variation in bee community composition across post-fire landscapes was primarily influenced by burn severity and time since fire. Recently burned study areas that experienced moderate severity fire supported greater floral and bee species richness compared to sites that burned at low severity or were unburned. These results suggest that mixed severity wildfire is beneficial for native pollinators, likely due to concomitant increases in floral resources and nesting habitat. Furthermore, herbaceous perennials such as *Achillea millefolium* and *Phacelia hastata* were important floral resources for pollinator communities in post-fire environments. Demographic assessments further revealed increased oviposition by wood-cavity-nesting bees in recently burned sites, with reproductive activity declining over time post burn. Collectively, these findings underscore the ecological importance of wildfire reintroduction for sustaining native pollinator populations in dry forest ecosystems.

Broader implications

Native bees are understudied in forested ecosystems across North America. Increased documentation of bee distributional ranges is essential for the accurate identification of at-risk species and the development of targeted conservation strategies. Despite globally reported pollinator declines, native pollinators are underrepresented on national and international endangered species lists. Comprehensive documentation of native bee distributions constitutes a critical first step toward monitoring population trends, quantifying biodiversity loss, and implementing effective, evidence-based conservation measures.

The significant association of unique bee species across a gradient of burn ages and severities underscores the ecological value of pyrodiversity and supports the incorporation of spatiotemporal heterogeneity into landscape-scale management plans for comprehensive bee conservation. Burn age emerged as a key determinant of bee community composition, reproductive success, and floral resource abundance, and should be explicitly considered in management plans that seeks to promote pollinator biodiversity. Additionally, the retention of unburned refugia in fire-treated forests may serve as critical source habitats for above-ground nesting bees in post-fire landscapes.

Drought tolerant, thermophilic plant species such as *Achillea millefolium* were frequently visited by native bees and are likely to be increasingly important under projected climate warming scenarios. To support the resilience of plant-pollinator networks, seed mixes used in post-fire restoration and salvage logging should incorporate drought tolerant herbaceous perennials with strong associations with native pollinators.

Directions for future research

Future research should prioritize the systematic, species-level documentation of bee occurrence data to establish comprehensive baseline inventories of native bee species, which are essential for informing conservation assessments. Additionally, investigations into the nutritional composition of floral resources utilized by native bees in post-fire landscapes are needed to evaluate pollen quality and determine whether the dietary requirements of different bee taxa are being met. Seed mixes spread in post-fire restoration efforts should be formulated to promote network resilience and ensure diverse bee nutritional needs are satisfied for all developmental stages. Lastly, predictive models that forecast shifts in the spatial and temporal distributions of native bees under various climate change scenarios should be generated, with particular attention to phenological mismatches between pollinators and their floral hosts.

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