Reducing Overstory in Pacific Northwest Forests Enhances Forage for Bumble Bees

Without Increasing Microparasite Prevalence

by

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THESIS ABSTRACT

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Title: Reducing Overstory in Pacific Northwest Forests Enhances Forage for Bumble Bees
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Bumble bees (*Bombus* spp.) are the most effective pollinator group in temperate and boreal regions, but habitat loss and disease are contributing to steep declines of several forest-associated species. Forest bumble bees benefit when forest management decreases overstory cover and enhances understory forage plant species, but effects can be short-lived. Forest restoration techniques that prioritize canopy openness may prolong forage availability through later successional stages. Management that increases floral resources may aggregate bumble bee populations and increase disease risk. We examined effects of forest management strategies on understory plant and bumble bee communities and evaluated whether plant and bee community characteristics were correlated with bumble bee disease dynamics. We surveyed the abundance and diversity of flowering plants and bumble bees within stands of varied canopy cover in the Coast Range (n = 98 stands) in Oregon, USA and screened bumble bees for six microparasite taxa (n = 191 bees). We found that canopy openness was positively correlated with flowering plant abundance and diversity and flowering plant communities were positively correlated with bumble bee richness and diversity. Our parasite prevalence rates were comparable to those of other North American bumble bee populations and were not correlated with characteristics of flowering plant or bee communities. Our data suggest that thinning in dense forests can enhance bumble bee habitat without increasing disease prevalence, informing efforts to conserve, restore, and expand forest habitat for imperiled bumble bee species.

This thesis includes unpublished co-authored material.

AUTHOR CONTRIBUTIONS

ACKNOWLEDGMENTS

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CHAPTER I

REDUCING OVERSTORY IN PACIFIC NORTHWEST FORESTS ENHANCES FORAGE

FOR BUMBLE BEES WITHOUT INCREASING MICROPARASITE PREVALENCE

Introduction

Global ecosystem health and agricultural production rely on wild pollinators, especially

social and solitary bees (Kearns et al., 1998; Willmer et al., 2017; Winfree et al., 2007). Wild bee

populations are in decline worldwide (Steffan-Dewenter et al., 2005), a trend best documented in

bumble bees (*Bombus* spp.) (Potts et al., 2010). Bumble bees are crucial pollinators in temperate

regions but have experienced dramatic population declines and range contractions in recent

decades (Colla et al., 2012; Soroye et al., 2020), prompting calls for habitat conservation, state

and federal endangered species designation, and targeted research (Cameron, 2011; Graves et al.,

2020). Primary contributors to bumble bee decline include land conversion, broad-scale pesticide

use, and disease (Cameron, 2011). Several declining bumble bees are temperate and boreal

species associated with forested landscapes (e.g., *Bombus occidentalis, B. caliginosus,* and *B.

sitkensis*) (Jackson et al., 2022; Williams et al., 2009). Forest management that results in

abundant and diverse floral resources may support populations of these and other bee species.

Across forest types, bee occurrence is often associated with sparse overstories, where

availability of warmth and light facilitate the growth of flowering understory vegetation (Hanula

et al., 2016; Korpela et al., 2015; Odanaka and Rehan, 2020). In temperate coniferous forests,

tree mortality caused by fire, windfall, and insect kill create structurally complex canopies with

small-scale openings (Frankline et al., 2002; Swanson et al., 2011). Heterogeneous canopies may

have sufficient flowering vegetation to support bees even in mature stands (e.g., Proesmans et

al., 2019), though bee occurrence data in this forest type is lacking in the Pacific Northwest. Anthropogenic canopy reduction can also increase forest bee populations: studies in stands managed for timber production show that tree harvest creates rapid recruitment of herbaceous vegetation (Yang et al., 2005; Harris and Betts, 2021). These stands support abundant and diverse wild bee communities for approximately 6-12 years, but practices such as dense tree replanting result in rapid canopy reclosure (Rivers et al., 2018; Rivers and Betts, 2021; Zitomer et al., 2023). After closure, planted stands have dense canopies (Wimberly 2002) that limit understory plant recruitment and diversity (Phalan et al., 2019) and support few to no wild bees (Rodríguez and Kouki, 2017; Rivers et al., 2018; Zitomer et al., 2023). Twenty percent of present-day Pacific Northwest forests are harvested and replanted stands under 100 years old (Oswalt et al., 2019). In 1994, the Northwest Forest Plan shifted federal forestry management objectives regarding these replanted stands away from timber harvest (Franklin and Johnson, 2012). As a result, many are now managed for return to late-successional conditions (Franklin and Johnson, 2012; Chamberlain et al., 2021). Strong positive bee responses following timber harvest support the potential of forest restoration to support forest bees in this region.

Forest restoration practices intended to increase structural complexity may also increase floral resources for wild bees. A primary restoration technique used is thinning (selective removal of trees across a stand to reduce overall density) (Spies et al., 2018), often conducted at variable densities to increase heterogeneity. Thinning mature coniferous forests can increase understory plant abundance and thinning at variable densities benefits plant species richness (Ares et al., 2010). Some herbaceous species that need fully open gaps may not benefit from thinning (Lindh and Muir, 2004), but can be supported if thinning is combined with group selection forestry (removal of trees to create small-scale openings (<5 acres); hereafter referred

to as "gap creation") (BLM, 2020). Gap creation is limited to <10% of the stand (BLM, 2020) and creates forage-rich patches with higher wild bee abundance and diversity (Roberts et al., 2017; Mullally et al., 2019), while thinning may increase connectivity between these open patches by providing supplemental forage in the matrix (Librán-Embid et al., 2021). Increasing overall openness may especially benefit bumble bees, who move freely through an open coniferous forest matrix (Mola et al., 2020) and have wider foraging ranges than other bee taxa (Librán-Embid et al., 2021). Floral resources in forests may also attract bumble bee species that require protected areas for nesting (Lee et al., 2021; Mola et al., 2021a). Thinning can enhance wild bee diversity and abundance in southeastern coniferous and boreal deciduous forests (Pengelly and Cartar, 2010; Odanaka et al., 2020) and floral availability in Northwest coniferous forests (Ares et al., 2010; Neill and Puettmann, 2013). While gap creation benefits wild bees in other forest types, impacts vary across pollinator functional guilds (Proctor et al., 2012; Roberts et al., 2017; Mullally et al., 2019) and effects on bumble bees are not well understood.

Forest management strategies intended to enhance floral diversity and richness may indirectly influence bumble bee health. Understanding the effects of forest characteristics on wild bee disease transmission is necessary to inform conservation decisions for forest-specialized bumble bees. Parasite and pathogen infection is one of the strongest predictors of bee decline (Szabo et al., 2012), especially prevalence of the genera *Nosema* and *Crithidia* (Meeus et al., 2011; Cameron, 2011; Ivers and Jha, 2023). Infection can reduce colony reproductive success, male and worker lifespans, and queen overwintering survival (Meeus et al., 2011). Some widespread and potentially pathogenic bumble bee parasites include the microsporidian genus *Nosema (Vairimorpha)*, the trypanosomatid genus *Crithidia,* the fungal genus *Ascosphaera,* and the neogregarine genus *Apicystis* (Meeus et al., 2011). Bee parasites may be endemic or

introduced via imported colonies of nonnative bees, as has been observed with nonnative bumble

bees (Hicks et al., 2018), honey bees (*Apis mellifera*) (Pereira et al., 2019), and mason bees

(*Osmia* spp.) (Müller et al., 2019). Transmission occurs among nestmates, among colonies when

foraging workers visit contaminated flowers, and between generations when infected gynes raise

offspring (Tripodi et al., 2014; McArt et al., 2017). Body size is positively related to

transmission rates, which show high inter- and intraspecific variation in bumble bees (Figueroa

et al., 2020; Van Wyk et al., 2021). Forests may be associated with large-bodied bumble bee

species (Wray et al., 2014), underlining the need to investigate forest bumble bees separately.

In coniferous forests, flower availability drives high bee abundance in post-harvest stands

(Pengelly and Cartar, 2010; Rivers and Betts, 2021). However, hyper-abundant and time-limited

food resources may aggregate bees, amplifying disease transmission (Becker et al., 2018). Bees

have higher microparasite infection rates in agricultural landscapes with dense, mass-blooming

monocultures than in natural ecosystems (Piot et al., 2019; Cohen et al., 2021). Managed forests

may present a similar pulse of concentrated floral resources, as early successional stands are

often predominated by flowering forbs (Lindh, 2008; Pengelly and Cartar, 2010). Conversely,

abundant forage resources may attract diverse pollinators that vary in infection and transmission

rates, diluting overall parasite prevalence (Graystock et al., 2015; Fearon and Tibbetts, 2021).

High floral abundance and diversity may also reduce an individual bee's likelihood of

encountering another infected individual (Figueroa et al., 2020; Cohen et al., 2021) and increase

immune system strength through improved nutrition, preventatively reducing disease prevalence

(McNeil et al., 2020; Fearon and Tibbetts, 2021). In conifer forests, plant species diversity peaks

in early seral areas (Widenfalk and Weslien, 2009; Swanson et al., 2011; Rivers and Betts,

2021). Accordingly, forests with high floral resource availability may dilute disease rates through

increased biodiversity, amplify disease rates due to host aggregation, or both, with the strength of either effect determined by plant and bumble bee community characteristics. In this study system, imported mason bees (*Osmia lignaria*) displayed both parasite amplification and dilution (Ponisio et al., in press), but disease dynamics in wild bee populations have yet to be assessed.

To determine the potential cascading effects of forest management on flowering plants, bumble bees, and parasite prevalence, we identified survey areas in a Pacific Northwest region across a gradient of forest openness, from no canopy to total overstory cover. We selected sites in private forests managed primarily for timber production and in federally owned forests managed for restoration to late-successional conditions. Sites included planted stands before and after canopy closure, older planted stands thinned for restoration, and older stands with no thinning treatment. We surveyed all stands for the abundance and diversity of flowering plants and bumble bees. We examined the effects of canopy openness on flowering plant abundance and diversity and the relationship between the flowering plant community and the bumble bee community. We then screened bumble bees from all canopy types for seven common parasite taxa and reported parasite prevalence in forest-associated bumble bees. To evaluate evidence of amplification and dilution and to assess indirect effects of canopy cover, we tested the effects of plant and bumble bee community characteristics on the prevalence of bumble bee disease.

Methods

Study system

We conducted three years (2020-2022) of surveys in the central Oregon Coast Range in North America, a low-elevation (0 - 1,250 m) coastal mountain range characterized by temperate conifer rainforest (Yang et al., 2005) (see Fig. 1). Prior to widespread forestry practices, the area of old growth forest was approximately 40% (Wimberly 2002). At the time of sampling the

Oregon Coast Range was 80% forested, approximately 1-2% of which was structurally complex old growth (Ohmann et al., 2007) and approximately 45% of which was planted stands of Douglas-fir (*Pseudotsuga menziesii*) (Zybach, 2004). Planted stands were harvested at 30-50 years of age, replanted at high densities (>300 trees per hectare) (Puettmann et al., 2016), and commonly treated with broadleaf herbicide one to three times in the first three years after replanting to suppress competing vegetation (ODF, 2022). Post-harvest stands had native floral resources such as Pacific blackberry (*Rubus ursinus*), fireweed (*Chamaenerion angustifolium*), and lupine (*Lupinus* spp.), and nonnative forbs such as foxglove (*Digitalis purpurea*), creeping thistle (*Cirsium arvense*), and hairy cat's-ear (*Hypericum radicata*). Approximately 10% of the Coast Range was under federal ownership (United States Forest Service, Siuslaw National Forest), characterized by mixed-age multispecies conifer forest (Franklin and Forman, 1987; Yang et al., 2005) composed of western hemlock (*Tsuga heterophylla*), western redcedar (*Thuja plicata*), and Sitka spruce (*Picea sitchensis*). Perennial understory shrubs such as vine maple (*Acer circinatum*), huckleberry (*Vaccinium* spp.), and Western azalea (*Rhododendron occidentalis)* constituted most of the floral resources. Within Siuslaw National Forest ownership, we targeted a selection of stands being restored to late-successional conditions. Restored stands (hereafter referred to as "thinned stands") were all second-growth planted Douglas-fir stands 30-50 years of age, harvested between 2019-2021 using variable-density thinning and group-cut harvest techniques (USDA, 2007). Thinned stands were replanted with a mix of native conifer tree species (Douglas-fir, western hemlock, western redcedar, and Sitka spruce) (USDA, 2007). In some thinned stands, existing log landings (widened road areas created to stage logging equipment and process timber) were excavated and the soil layers inverted to bury the existing non-native seedbank and support understory shrub and forb recruitment (USDA, 2007).

Survey stand selection

Within this region, we combined spatial datasets from the US Fish & Wildlife service, Symbiota Collections of Arthropods Network, and Oregon Bee Atlas to identify where one or more West Coast bumble bee species of concern (*Bombus occidentalis, B. suckleyi, B. morrisoni, B. caliginosus, B. franklini, B. crotchii, B. fervidus, B. vandykei,* and *B. huntii*) had been detected since 2010. Using an existing Environmental Responses to Watershed Composition framework, we delimited all fourth-order watersheds within the Oregon Coast Range, quantified average forest age of each watershed, and randomly selected watersheds of equal proportions with average forest ages <60 years and >120 years. All watersheds selected were within 20 km of bumble bee species of concern detection areas. From the delimited watersheds, we selected 8 that were accessible via agreements with landowners. We also chose 8 watersheds within Siuslaw National Forest ownership. Our watersheds had an average area of 24±10 km². To identify stands within each stratum, we used a metric of forest age assessed from a combination of the 2017 Gradient Nearest Neighbor (GNN) raster (Ohmann and Gregory, 2002), LiDAR (DOGAMI), and landowner stand age records to identify young early seral forest following timber harvest (<6 years) and mature forest (>70 years). We added a third category of forests thinned for restoration using spatial data from Siuslaw National Forest. We stratified our sampling within these categories. Using the Create Random Points tool (ArcGIS Pro, 2021), we generated a set number of random points within each watershed. Around each point, we used ownership boundaries or satellite imagery to manually draw stand boundaries, defining "stand" as a forested region uniform enough in tree age and composition to visually distinguish it from the surrounding forested area. For our study, we restricted stands such that stands could be adjacent to but not bisected by roads. We included skid roads (temporary trails used by logging equipment to

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transport logs to a roadside area for processing) within stand boundaries because we assumed herbaceous revegetation in skid roads occurred at similar rates to the rest of the stand interior. In watersheds that did not contain thinning treatments, we randomly selected 3 stands from the young age class (<6 years) and 3 from the mature age class (>70 years). In watersheds that contained thinning treatments, we performed the same age categorization but selected 1 young stand, 1 mature stand, and 3-5 thinned stands. We sampled all available thinned stands.

We surveyed 98 stands, including 27 young, 35 old, and 36 thinned stands across three years. Selected stands included 61 stands in public ownership (US Forest Service, Siuslaw National Forest) and 37 stands in private ownership (6 landowners). Stand elevation ranged from 69-950 meters above sea level. Within each stand we randomly placed 6 points at least 50 m from the stand edge and at least 100 m apart (using Create Random Points, ArcGIS) for later survey site selection.

Field methods

We surveyed flowering plant and bee communities throughout the flowering season. Start dates varied due to a combination of the COVID-19 pandemic and spring weather conditions. We surveyed from July - September 2020, April - September 2021, and May - September 2022. Within each stand, we chose hand-net survey transects by randomly selecting 3 interior points (randomly generated during stand selection) and 1 point along a road. At each interior site we delimited two paired 32 m transects, randomizing the direction of each pair. At each roadside site we delimited a single 32 m transect that ran alongside the road edge. In total, we surveyed 224 m of transect in each stand sampling visit.

We collected tree size and canopy information once per season to ground-truth GNN age estimates used during stand selection and to quantify canopy openness in variably thinned stands.

At the start of each interior transect, we used a spherical densiometer to quantify canopy cover (Fiala et al., 2006) and a Biltmore stick to measure dominant tree class DBH (diameter at breast height) (Fiala et al., 2006).

We collected floral data during each visit. Along each transect, we surveyed flowering plants in a 1 m quadrat every 4 m for a total of 8 survey plots per transect (a total of 56 survey plots per stand). Within each quadrat we recorded all blooming plant species, number of individual plants of each species, and average number of blooms per individual. We defined blooming vegetation as any flower with internal structures open to pollination, excluding the pollen-bearing structures of graminoids and conifers. For plant species with small, clustered flowers (e.g., oceanspray [*Holodiscus discolor*]), we counted blooms in bunches by standardizing the number of individual flowers within an inflorescence and counting by inflorescence.

To sample bees, we conducted 10-minute active netting surveys along each transect. We recorded wind speed (km/h) and temperature (degrees Celsius) using a Kestrel Instruments wind and weather meter (Shawnee on Delaware, Pennsylvania, USA) at the start of each survey. We conducted surveys when it was not steadily raining, temperature was at least 15.5°C, and wind speeds were under 2.7m/s (or 9.6 km/h). Field technicians conducted surveys by walking slowly and capturing any insects that touched the reproductive parts of flowers. Once an insect was captured, we recorded the plant species from which we collected the insect. We did not survey flowers in the canopy (i.e., bigleaf maple [*Acer macrophyllum*]). To standardize netting effort, field technicians started a timer at the beginning of the survey, paused it while euthanizing a pollinator and recording the associated plant species, and restarted it once they returned to active netting. To prevent parasite contamination among individuals, we collected pollinators into

sterile vials and wore latex gloves during surveys. We sprayed gloves and nets with 10% bleach, allowed them to dry, and sprayed them with 70% ethanol before sampling and after each capture. We did not euthanize bumble bee queens to avoid fatal take of reproductive gynes of sensitive species. We euthanized all other pollinating insects with dry ice, kept them on dry ice in the field, and stored them at -80° C until screened. We returned to stands for repeat sampling 1-3 times per season as weather conditions allowed.

Taxonomy, species characterization, and phylogenetic distance determination

All collected bumble bee specimens were identified to species by a professional taxonomist (L. R. Best, Oregon State University). Foraging distance (km) for each *Bombus* species was calculated by measuring the mean of intertegular distance across ten randomly selected specimens and allometrically calculating approximate foraging distance (Greenleaf et al., 2007). We accessed foraging distance estimates from a publicly available dataset of Coast Range bee traits compiled by Zitomer et al. (2023). We sourced foraging distance for species not included in the Coast Range dataset (*B. melanopygus* and *B. vandykei*) from publicly available Northern California bee traits (Sardiñas et al., 2016). We constructed a bee community phylogeny to account for non-independence among bee species due to their relatedness. To determine phylogenetic distance among species, we accessed publicly available sequences from Genbank, performed multiple sequence alignment using the function AlignSeqs from the DECIPHER package (Wright, 2020), and constructed a phylogenetic tree using the function as.DNAbin from the phangorn package in program R (Schliep, 2011).

Parasite screening

To determine internal parasite presence, we selected up to 10 bumble bees from each unique combination of species, stand, and sampling round for screening. When fewer than 10

bumble bees were collected from a sampling event, we screened all collected bumble bees. We only screened female bumble bees, as male bumble bees may be parasitized at different rates than workers (Malfi and Roulston, 2014) and our collection of male bees (n = 37) was too low to establish differences in infection rate. Bee specimens were removed from -80° C storage and kept on dry ice until immediately before dissection. We used sterilized tweezers to remove the gut from each bee and extracted the DNA from each gut sample using the Qiagen DNeasy kit following the provided protocol included in the kit, with the following altered lysis step: we added 180 µL Qiagen buffer ATL and one sterile 5 mm stainless steel bead to each gut sample and lysed samples in a Qiagen Tissue Lyser II for 4 minutes. We used a multiplex protocol with parasite-specific primers to screen for presence of the following taxa: the species *Nosema bombi*, *Nosema ceranae, Crithidia bombi,* and *Crithidia expoeki*, and the genera *Apicystis, Ascosphaera,* and *Crithidia* (excluding *C. bombi* and *C. expoeki*) (Graystock et al., 2020). Primer references and conditions are provided in Table 1. We included a negative and positive control for each sample. To confirm that samples contained bee DNA, we amplified a gene sequence (EF– 1α) associated with bees (Hines et al., 2006) and confirmed amplification with electrophoresis on a 1% agarose gel. We confirmed positive parasite detection by submitting a subset of samples for Sanger sequencing. We did not screen bumble bees for parasites in 2020.

Table 1. PCR primers and conditions used for the detection of parasites.

Parasite	Sp.	dntp	Taq	10x	F	R	DNA	Total	Denat.I		Denat.		Anneal		Extend		X	Elong.		Size	Reference
		µL	µL	µL	µL	µL	µL	µL	Min	Temp	Min	Temp	Min	Temp	Min	Temp		Min	Temp	bp	
Apicystis spp.		0.2	0.05	1.00	0.02	0.02	1	10	2	94	:30	94	:30	60.7	:45	72	35	3	72	220	(Meeus *et al.*, 2010)
Ascosphaera spp.		0.2	0.05	1.00	0.02	0.02	1	10	10	94	:45	94	:45	62	1	72	35	5	72	550	J(James & Skinner, 2005)
Crithidia multiplex		0.8	0.15	2.8			1.25	20	3	94	:45	94	:45	57	1	72	42	7	72		(Graystock *et al.*, 2020)
	C. spp				0.8	0.8														470	
	C. bombi				0.8	0.8														279	
	C. expoeki				0.8	0.8														163	
Nosema multiplex		0.8	0.15	2.8			1.25	20	3	94	:45	94	:45	57	1	72	42	7	72		(Graystock *et al.*, 2020)
	N. ceranae				0.8	0.8														288	
	N. bombi				0.8	0.8														185	

Statistical analysis

We fitted Structural Equation Models (SEMs) in a Bayesian framework to test the relationships among stand characteristics, the flowering plant community, the bee community, and parasite prevalence. We fit models using the brms package in R (Bürkner 2017).

Canopy openness and floral abundance and diversity. To test effects of canopy cover on the flowering plant community, we included canopy openness (ranging from 0% (closed-canopy) to 100% (open-canopy)) as an explanatory variable and floral abundance (log transformed) and floral diversity (Shannon's diversity index) as response variables. We also included day of year (Julian date) to account for plant phenologies and survey year (2020, 2021, 2022) to account for variation across years. Because we surveyed a stand multiple times, we included stand as a random effect. Due to the high proportion of stands with no floral diversity, we assumed hurdle log normal error for floral diversity. We assumed Gaussian error for the log floral abundance.

Flowering plant community effects on bee abundance and richness. To test the effect of flowering plant community on *Bombus* species, we first rarefied *Bombus* species richness to achieve better estimates across variable stands. We then modeled rarefied *Bombus* richness as a response variable and floral diversity as an explanatory variable. To account for differences in weather across surveys, we included temperature at the start of a survey as an explanatory variable. We include a random effect of stand. Due to the high proportion of stands with zero bees collected, we assumed a hurdle log normal model. To test the effect of the plant community on *Bombus* abundance, we modeled *Bombus* species abundance as a response to vegetation abundance and again included temperature at the start of the survey and a random effect of stand. We assumed hurdle Poisson error to account for the high number of zeros in the data (Feng 2021).

Bumble bee and plant community effects on parasitism. Before modeling parasite presence, we excluded *Apicystis* spp., *Ascosphaera* spp., and *Nosema* spp. due to low rates of detection (see Fig. 2) and instead focused on prevalence of all *Crithidia* species, assigning presence or absence (1 if a bee had any *Crithidia* species, 0 if it had none or any other parasite). In this model layer, we added the explanatory variable of estimated species-specific foraging distance to account for increased risk of exposure to wider-ranging bees. We included effects of flowering plant diversity and rarefied *Bombus* spp. richness to test for disease dilution. We included flowering plant abundance and *Bombus* spp. abundance to test for disease amplification. We added a random effect of stand due to resampling of stands. We included phylogenetic covariance of bee species to account for relatedness among species. Since the presence or absence of *Crithidia* spp. was dichotomous, we assumed Bernoulli error (Shapiro and Zahedi, 2018).

Results

Across our three-year sampling period we performed 245 unique round/stand/year visits across the 98 selected stands, totaling 163 hours of active netting effort. We caught 420 bumble bees (34 in 2020, 62 in 2021, 323 in 2022), composed of 7 species (Fig. 1b, 1c). The most collected *Bombus* species, *Bombus vosnesenskii*, composed 54% (367 bees) of the total.

Figure 1 (next page). Study region and bumble bee collection summary.
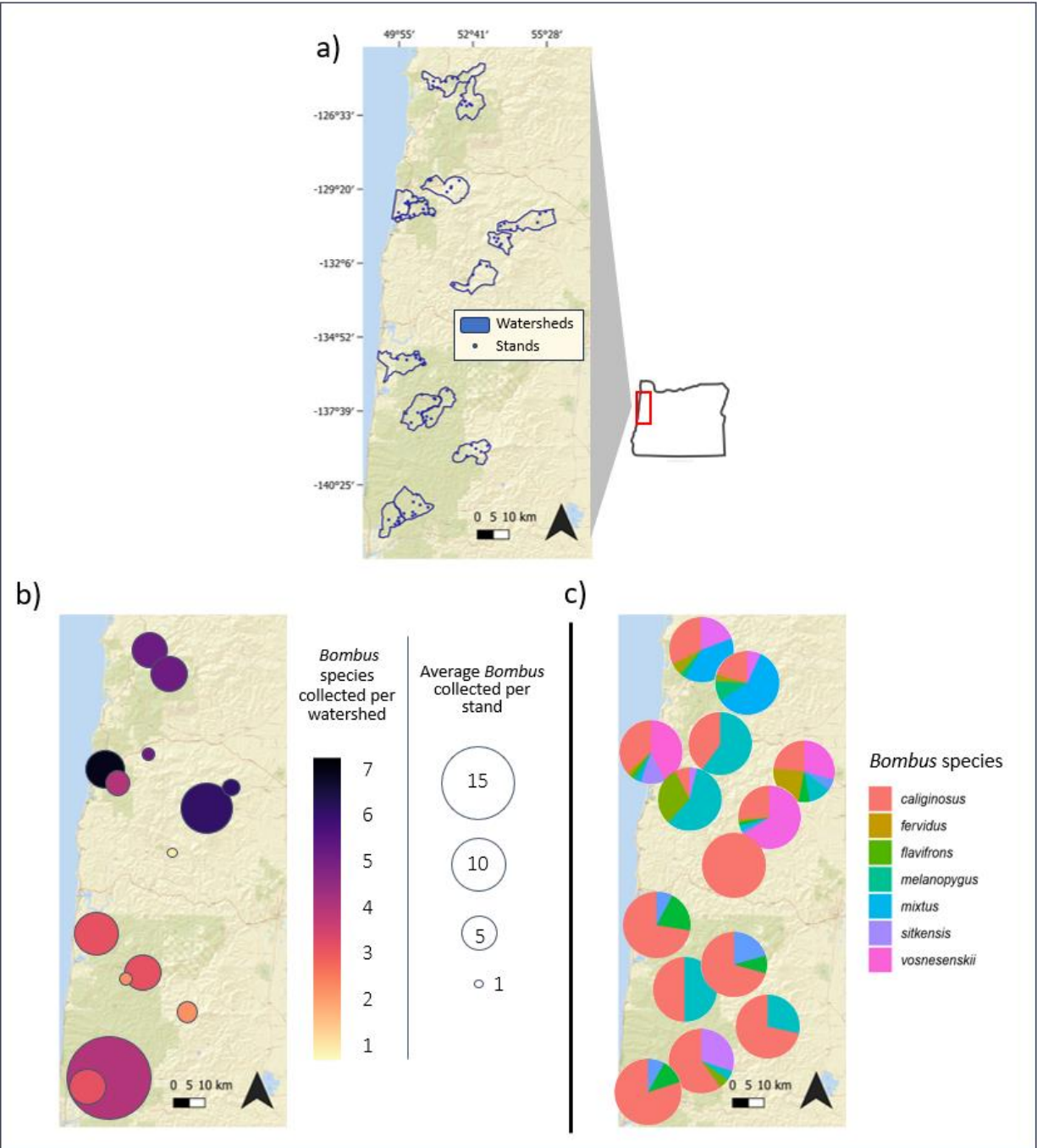
Figure 1. Study region and bumble bee collection summary. a) A map of the study area with watersheds and n = 96 sampled stands; location of study within Oregon is shown in the inset. b) The study area with the number of *Bombus* species collected per watershed indicated by the hue of the circle and the average number of bumble bees collected per stand indicated by the size of the circle. c) The species composition of collected bumble bees by watershed.
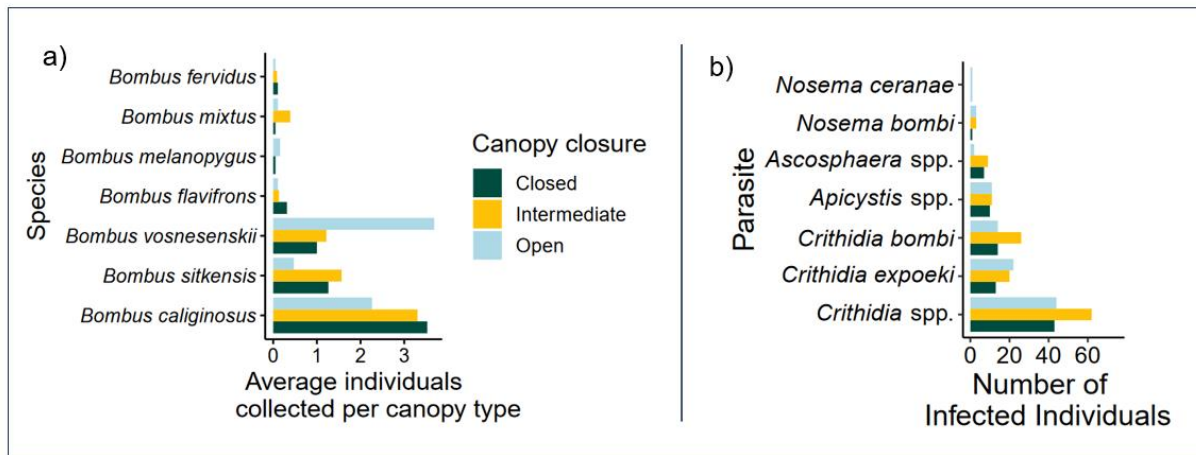
Figure 2. Bumble bee and parasite occurrence summarized by stand type. (a) The average number of collected individuals of each *Bombus* spp. separated by stand type. (b) The total number of individual parasite detections separated by stand type. We categorized stands as closed (0-25% open), intermediate (25-75% open), and open (75-100% open).

All statistical analyses use a range of canopy openness from 0-100, but we used ground-truth data to categorize stands as closed (0-25% open), intermediate (25-75% open), and open (75-100% open) for summary visualization (Figs. 2-4). We captured bumble bees most frequently on foxglove and hairy cat's-ear in open-canopy stands, on foxglove and salal (*Gaulteria shallon*) in intermediate-canopy stands, and silverleaf phacelia (*Phacelia hastata*) and big deervetch (*Lotus crassifolius*) in closed-canopy stands, (Fig. 3).
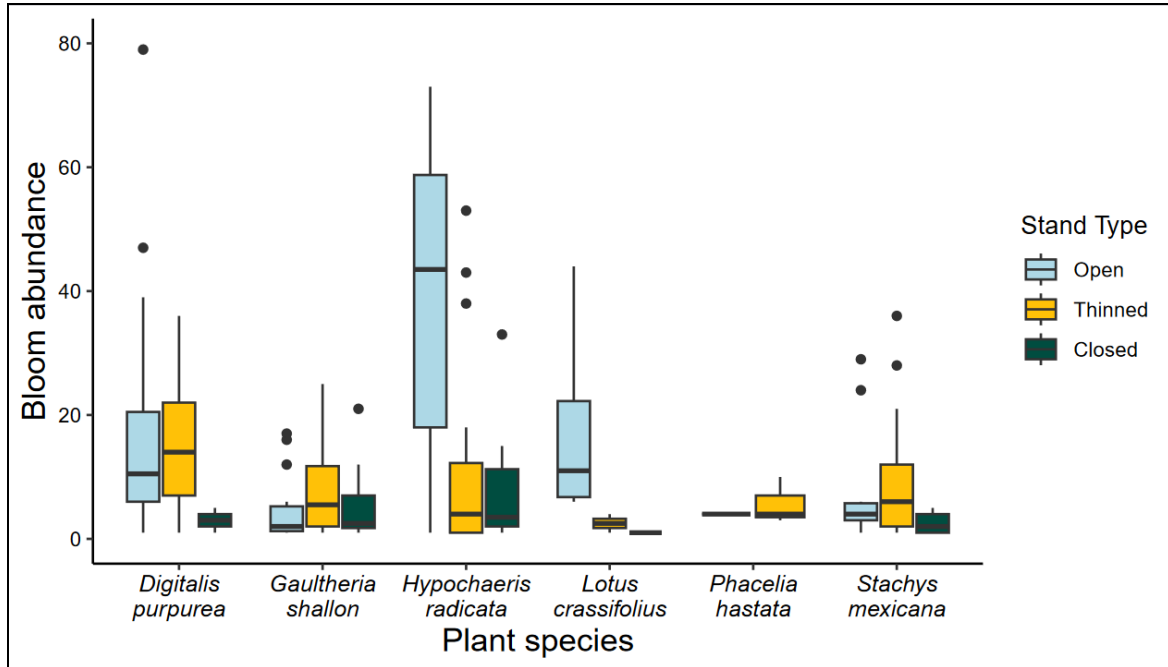
Figure 3. Bloom abundance of the six flowering plant species that bumble bees were most commonly collected on. Abundance of each species is separated by stand type. We categorized stands as closed (0-25% open), intermediate (25-75% open), and open (75-100% open).

Examples of plant-pollinator interaction networks for each stand type are shown in Figure 4. Most bumble bee species were caught approximately equally across canopy types except for *Bombus vosnesenskii* (Figs. 2, 4). The number of collected bumble bees was too low to statistically establish preferences of individual bumble bee species for canopy type or forage plant species.

Figure 4 (next page). Examples of bumble bee and plant interaction networks.

Figure 4. **Examples of bumble bee and plant interaction networks.** Three plant-pollinator networks are pictures, with one shown for each sampled canopy type. The number of sampled stands in each network is indicated beneath the network. Each network shows the collected bumble bee species linked to the plant species they were collected on. Number of individual observations is shown next to the plant or bee species name. Networks from different watersheds are shown in order to display similar sample sizes.
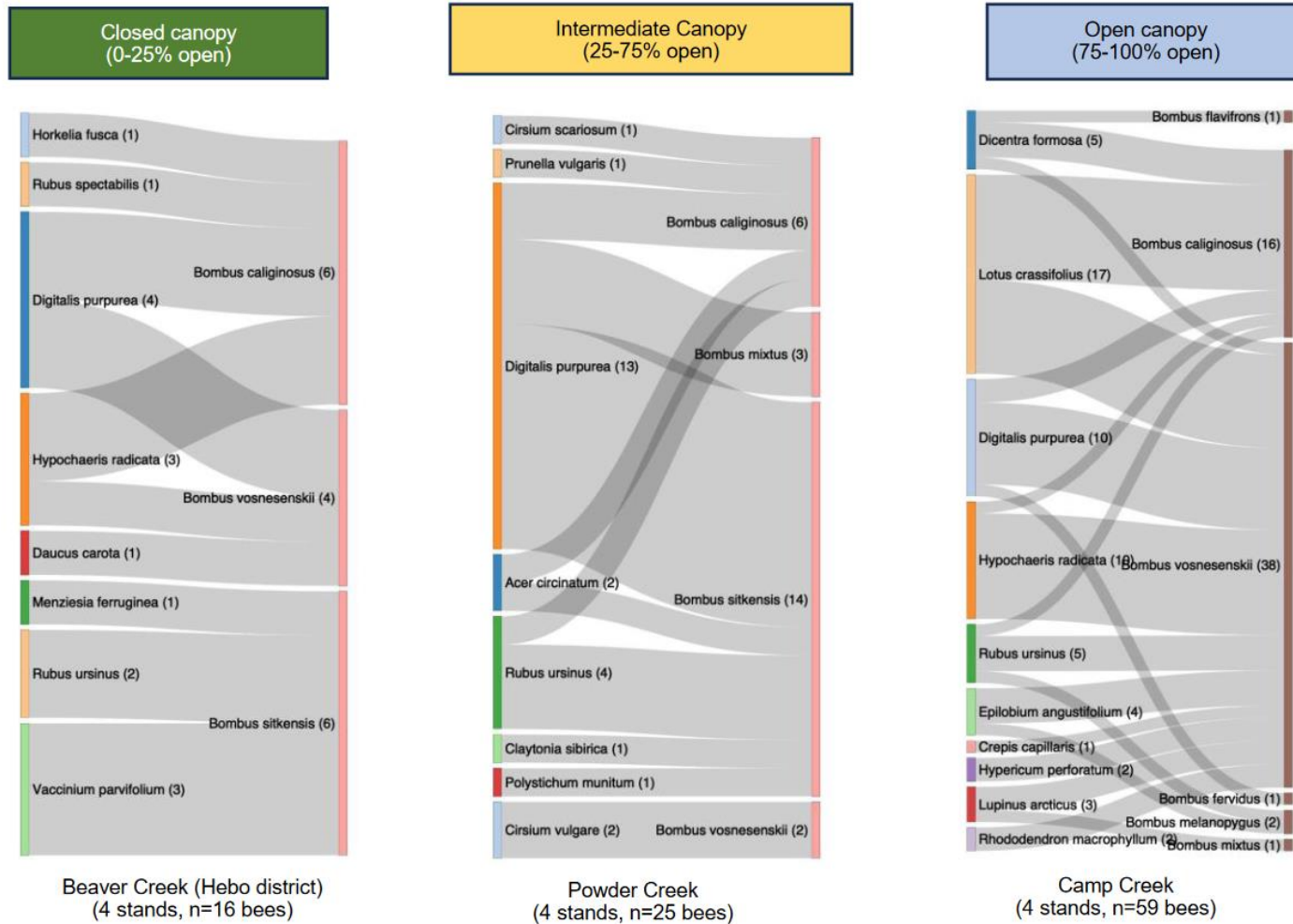
Effect of canopy cover on plant communities. Thinned stands had variable canopy openness ranging from 12-77% despite having similar-sized trees (Fig. 5). We found a positive effect of canopy openness on flowering plant diversity and abundance (Tab. 2, Fig. 6).
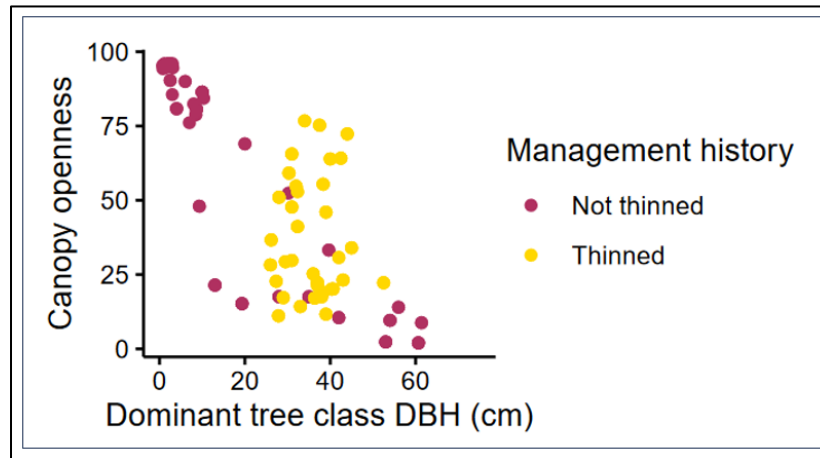
Figure 5. Canopy cover and tree diameter at breast height (DBH) of the dominant tree class of each stand. Thinned stands are depicted in yellow and non-thinned in purple.

Effect of forest plant communities on bee communities. We found weak support for a relationship between flowering plant diversity and *Bombus* richness (Tab. 2, Fig. 7a) and support for a positive relationship between flowering plant abundance and *Bombus* abundance (Tab. 2, Fig. 7b). Starting temperature had a positive relationship with *Bombus* diversity and rarefied species richness (Tab. 2, Fig. 8).

Table 2. Model results. The parameter estimates, lower (L-95 CI) and upper (U-95 CI) highest density 95% credible intervals, R-hats, bulk effective sample size (ESS), tail ESS, and proportion of the posterior samples > and < 0. Variable names for strong and moderate support are bolded; ***, **, and * indicate strong statistical support (≤0.02), moderate support (≤0.05), or weak support (≤0.10), respectively. All R-hats were equal to 1, indicating chain convergence. Years are compared to the base year 2020.

| Response	Explanatory	Estimate	Std. Error	L-95 CI	U-95 CI	Rhat	Bulk ESS	Tail ESS	P>0	P<0	P > 0	P < 0
Floral diversity	Intercept	0.18	0.23	-0.27	0.64	1	19948.18	13115.74	0.79	0.21		
	Year (2021)	-0.23	0.28	-0.78	0.32	1	18157.67	12664.91	0.21	0.79		
	Year (2022)	0.14	0.22	-0.29	0.56	1	18772.15	12696.81	0.73	0.27		
	Day of year	0.02	0.07	-0.11	0.15	1	27789.27	13013.87	0.61	0.39		
	Day of year^2	-0.26	0.07	-0.4	-0.11	1	25274.57	10890.97	0	1		***
	Mean canopy	0.16	0.09	-0.02	0.35	1	16813.1	11493.94	0.96	0.04	**	
	Mean canopy^2	-0.06	0.11	-0.27	0.16	1	19393.79	11834.1	0.3	0.7		
Floral abundance	**Intercept**	7.89	0.58	6.77	9.04	1	15770.09	11696.47	1	0	***	
	Year (2021)	-2.28	0.71	-3.71	-0.9	1	8666.74	10597.13	0	1		***
	Year (2022)	-0.79	0.54	-1.88	0.24	1	16104.66	12328.82	0.07	0.93		*
	Day of year	-0.62	0.17	-0.94	-0.29	1	15958.76	12383.52	0	1		***
	Day of year^2	-0.56	0.17	-0.89	-0.22	1	24174.55	11208.18	0	1		***
	Mean canopy	0.39	0.24	-0.1	0.85	1	15118.95	12345.66	0.95	0.05	**	
	Mean canopy^2	0	0.28	-0.55	0.54	1	16465.51	11848.87	0.51	0.49		
Bombus abundance	Intercept	-0.03	0.39	-0.84	0.7	1	17824.21	12622.29	0.48	0.52		
	Floral abundance	0.14	0.05	0.04	0.25	1	19703.37	12987.38	1	0	***	
	Temperature	0.15	0.08	0	0.31	1	23508.08	12904.66	0.97	0.03	**	
Rarefied *Bombus* species richness	**Intercept**	0.42	0.14	0.15	0.7	1	26751.72	12163.56	1	0	***	
	Floral diversity	0.17	0.11	-0.05	0.38	1	26090.82	11743.17	0.94	0.06	*	
	Temperature	0.12	0.07	-0.01	0.26	1	28906.85	11923.18	0.96	0.04	**	
Crithidia presence	**Intercept**	3.17	1.7	0.01	6.7	1	13064.34	11598.34	0.98	0.02	***	
	Bombus abundance	0.01	0.06	-0.12	0.13	1	14761.74	10883.19	0.57	0.43		
	Floral diversity	-0.23	0.47	-1.14	0.69	1	21361.91	13184.15	0.31	0.69		
	Floral abundance	-0.26	0.2	-0.67	0.11	1	15996.55	10979.85	0.08	0.92		*
	Forage distance (km)	-0.09	0.49	-1.07	0.86	1	15584.87	11321.66	0.43	0.57		
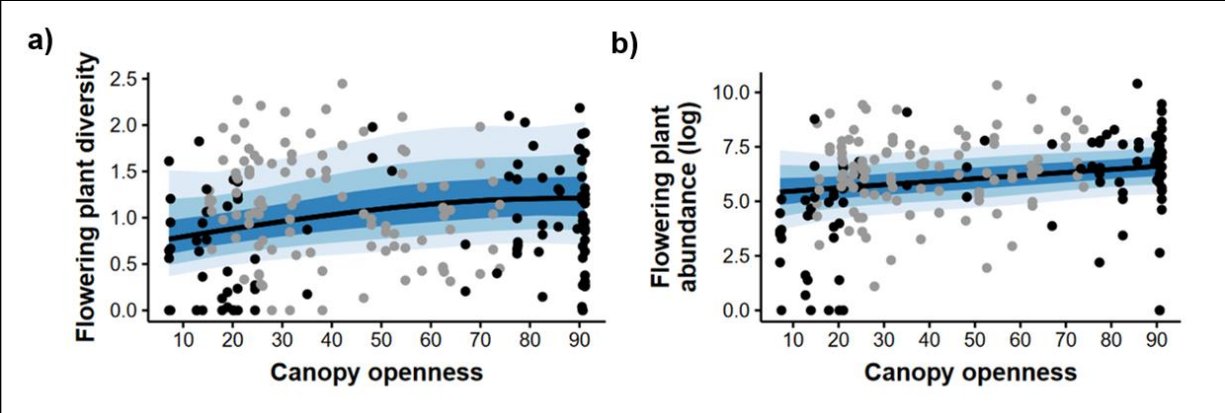
Figure 6. The relationships between canopy openness and a) flowering plant diversity and b) log of flowering plant abundance. The solid black line indicates the mean of the posterior and the colored fill zones from dark to light are the 50%, 80%, and 95% credible intervals around our estimate. The shades of blue indicate that 90-100% of the posterior was above or below zero. Thinned stands are displayed as gray points and unthinned stands are displayed as black points.
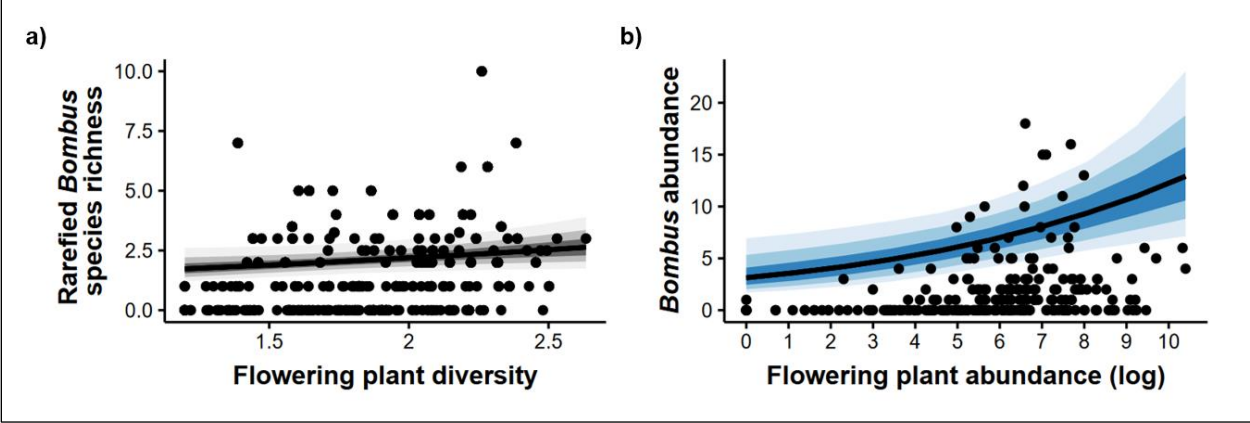
Figure 7. The relationships between a) rarefied *Bombus* species richness and flowering plant diversity and b) *Bombus* abundance and log of flowering plant abundance. The solid black line indicates the mean of the posterior and the colored fill zones from dark to light are the 50%, 80%, and 95% credible intervals around the estimate. If 90-100% of the posterior was above or below zero, the curves are shades of blue; if 0- 90% of the posterior was above or below zero, the curves are gray.
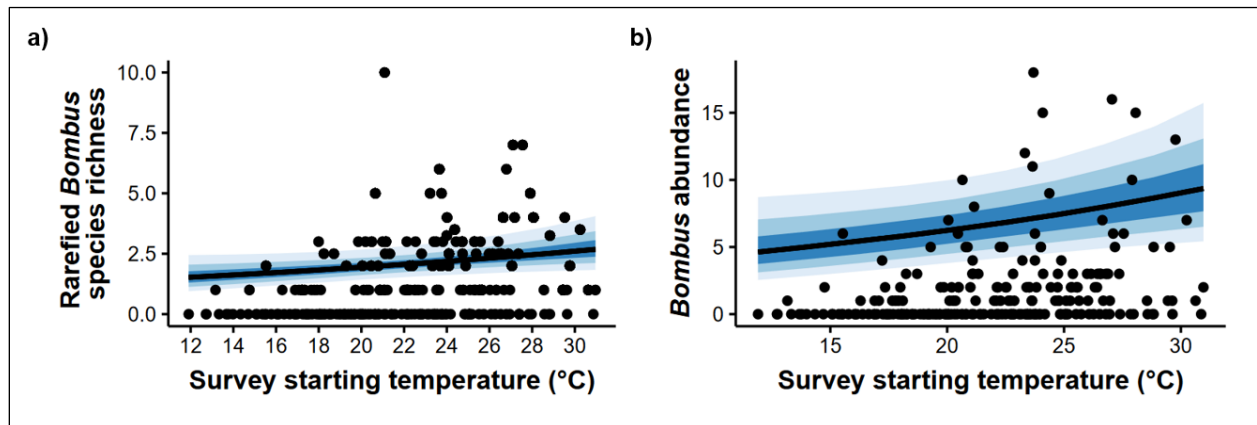
Figure 8. The relationships between a) rarefied *Bombus* species richness and the hand-netting survey starting temperature and b) *Bombus* abundance and the survey starting temperature. The solid black line indicates the mean of the posterior, and the colored fill zones from dark to light are the 50%, 80%, and 95% credible intervals around the estimate. The shades of blue indicate that 90-100% of the posterior was above or below zero.

Parasite prevalence in forest bumble bees. We screened 191 *Bombus* spp. individuals (31 from 2021, 160 from 2022). Of the screened bees, 78% (149 bees) had unknown *Crithidia* spp., 28.3% (55 bees) had *Crithidia expoeki*, 27.8% (54 bees) had *Crithidia bombi*, 16.7% (32 bees) had *Apicystis* spp., 9.4% (18 bees) had *Ascosphaera spp.*, 3.6% (7 bees) had *Nosema bombi,* and 0.5% (1 bee) had *Nosema ceranae* (Fig. 2b). Of the screened Bombus, 1% (2 bees) had 4 parasites, 16% (30 bees) had 3 parasites, 45% (86 bees) had 2 parasites, 17% (32 bees) had one parasite, and 21% (41 bees) had none of the parasites for which we screened.

Effect of plant communities on parasite prevalence. We found no effect of floral abundance or diversity on prevalence of *Crithidia* spp. in bumble bees (Tab. 2).

Evidence of amplification and dilution effects in bumble bees. We did not find evidence
to support a relationship between *Bombus* abundance and *Crithidia* spp. prevalence or between
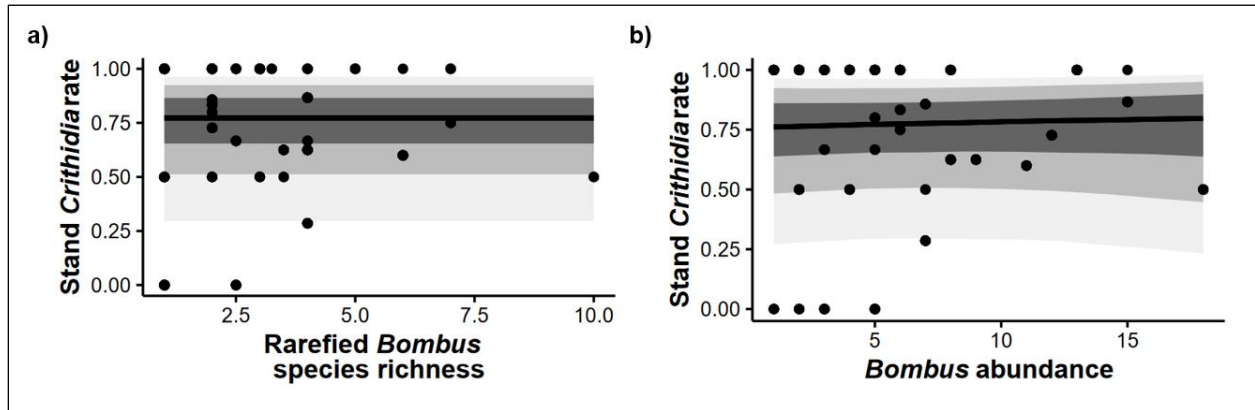rarefied *Bombus* species richness and *Crithidia* spp. prevalence (Tab. 2, Fig. 9).

**Figure 9. The relationships between a) rate of *Crithidia* spp. prevalence in each stand and
Bombus abundance and b) rate of *Crithidia* spp. prevalence in each stand and rarefied
Bombus species richness.** The solid black line indicates the mean of the posterior, and the
colored fill zones from dark to light are the 50%, 80%, and 95% credible intervals around the
estimate. The shades of gray indicate that 0- 90% of the posterior was above or below zero.

Effect of average foraging distance on parasite prevalence. We did not find support for
a relationship between foraging distance and *Crithidia* spp. prevalence (Tab. 2).

Discussion

Similar to previous studies in the region, we found that flowering plant communities
responded positively to canopy openness created by timber harvest (Rivers et al., 2018; Rivers
and Betts, 2021; Zitomer et al., 2023). Open-canopy stands had an average of 176.8 (±34.6)
more blooming flowers and 0.12 (±0.74) more flowering plant species than closed-canopy stands
per season. Since stand age was correlated with canopy cover, flowering plant abundance and
diversity was highest in young stands and lowest in mature stands (Fig. 6). Bumble bee
abundance tracked floral abundance, reflecting previous studies (Rivers et al., 2018; Rivers and

Betts, 2021; Zitomer et al., 2023), so canopy openness indirectly affected bumble bees via its effect on the floral community. Closed-canopy stands had an average of 241.1 (±397.6) blooming flowers and 1.05 bumble bees (±0.78) per season. Open-canopy stands had an average of 417.9 (±801.8) blooming flowers and 1.93 bumble bees (±1.36) per season. As expected, increased warmth and lateness of the foraging season partially explained bee presence across stand types, with bumble bee abundance and species richness responding positively to the starting temperature of the survey, day of year, and day of year squared.

We expected that thinned stands would host smaller and less diverse flowering plant and bee populations than those in open-canopy planted stands (Rivers et al., 2018; Zitomer et al., 2023). In our system, intentional irregularity in thinning protocols between stands resulted in highly variable canopy openness (range = 25-72%, median = 44.7% ± 14.5). The effect of thinning on the floral community was variable depending on the stand's remaining canopy (Fig. 6, see variability in colored points), consistent with the positive relationship between canopy openness and floral diversity and abundance. Given the positive relationship between floral abundance and bumble bee abundance (Fig. 7), the indirect effect of thinning on bee communities was also variable. In our study area, U.S. Forest Service implementation of these practices varied on a site-by-site basis (D. Williams, U.S. Forest Service, personal comm.) and may have contributed to the highly variable canopy in thinned stands. Although thinning supported less understory vegetation than open-canopy stands, such stands may have floral resources for longer durations (Brockerhoff et al., 2008; Swanson et al., 2011). While tree thinning without sufficient irregularity may allow existing trees and shrubs to exploit the new openings and re-close the canopy, highly variable thinning and practices such as gap creation can increase longevity of understory resources (Neill and Puettmann, 2013). Replanting of native tree

species was also conducted in thinned stands. Tree seedlings were planted in 2019-2021 and were too small to affect determinations of canopy cover at the time of our surveys, but the presence of a subcanopy layer can drastically decrease understory vegetation (Barbier et al., 2008) and effects of this age class should be reassessed in the coming decades. Our surveys occurred only 1-3 years after thinning treatments were conducted; future studies would benefit from monitoring the success of these and other techniques in maintaining canopy gaps and early seral resources for bees across longer time scales.

We found high *C. bombi* (27.8%)*, C. expoeki* (28.3%), and other *Crithidia* species (78%) prevalence, comparable to surveys of wild bumble bee parasites conducted regionally (Kissinger et al., 2011) and elsewhere in North America (Gillespie, 2010; Cordes et al., 2012). Interestingly, these surveys found *C. bombi* at higher rates in bumble bee species with stable populations and at lower rates in rare and declining species (Gillespie, 2010; Kissinger et al., 2011; Cordes et al., 2012). *C. bombi* and *C. expoeki* also show increased prevalence in northern latitudes (Ivers and Jha, 2023). The virulence of *Crithidia* spp. is poorly understood, and under low-stress conditions infections may be largely benign (Brown et al., 2003). However, virulence increases during stressful life cycle stages such as colony founding, when queens are highly resource-limited (Brown et al., 2003). Bumble bee queens in forests may be at additional risk due to decreasing availability and increasing phenological mismatch of early-season floral resources (Williams et al., 2009; Mola et al., 2021b). Lethal and sublethal effects may also increase in severity throughout the year if colonies suffer from food stress (Brown et al., 2003). Increasing rates of *C. bombi* infection are consistent with a decline in pollen-collecting efficiency in individual workers (Gegear et al., 2005; Otterstatter and Thomson, 2007), which may have outsize consequences in forested landscapes where floral resources are already relatively limited.

Our parasite screening revealed low *Nosema* spp. prevalence (4.2%). These low rates are likely not explained by intraseasonal variation of *Nosema* spp. (Graystock et al., 2020), as we sampled the extent of the foraging season, but may be partially explained by the low density of bumble bees, which has been shown to decrease prevalence (Ivers and Jha, 2023). We detected *N. ceranae* in only a single bee (*B. fervidus*). *N. ceranae* primarily infects honey bees, though it has been found in bumble bees (Pereira et al., 2019). The relative scarcity of honey bees in coniferous forests may decrease the overall rate of *N. ceranae* prevalence in wild bees. *N. ceranae* is also highly sensitive to cold and may be excluded by winter temperatures in northern regions (Gisder et al., 2010). We also found low rates of *Nosema bombi* (3.7%). Host specificity in *N. bombi* is related to host life history traits, with higher prevalence and more severe consequences of infection in bumble bee species with larger colonies and longer seasonal foraging stages (Rutrecht and Brown, 2009); the comparatively limited colony sizes and foraging seasons of higher-latitude bumble bees (Cueva del Castillo et al., 2015) may limit prevalence of *Nosema* species in our study region. North American surveys of multiple *Bombus* species have found comparably low overall presence of *N. bombi* but high rates in declining species, such as *B. occidentalis*, which displays high *N. bombi* infection rates (37% - 44%) across the continent (Cordes et al., 2012; Koch and Strange, 2012; Tripodi et al., 2014). *B. occidentalis* is extirpated from much of the Oregon Coast Range (Sheffield et al., 2016), but no studies were conducted during the species' decline to assess historical parasite prevalence in the region. Given the low rates of *Nosema* prevalence in these forests, *Nosema* prevalence would be unlikely to impede attempts to reintroduce *B. occidentalis* in temperate conifer forests (e.g., Wildlife Protection Canada, 2020).

We detected low rates of *Ascosphaera* spp. (3.6 %) and *Apicystis* spp. (9.4%).

Ascosphaera spp. are widespread across pollinator taxa, with bumble bee species showing high

prevalence but no pathogenic effects, suggesting they serve as non-host vectors (Evison et al.,

2012). Our *Apicystis* spp. detections represent *Apicystis bombi*, the only species in the genus

known to infect bumble bees. *A. bombi* is found at comparably low prevalence in bumble bees

both regionally and globally (Lipa and Triggiani, 1996; Kissinger et al., 2011; Maxfield-Taylor

et al., 2015), but is correlated with high mortality of spring queens (Rutrecht and Brown, 2008).

A. bombi infection in bumble bees is poorly understood and warrants future investigation (Meeus

et al., 2011). Both *Ascosphaera* spp. and *Apicystis* spp. can also infect honey bees (Meeus et al.,

2011) and native solitary bees (Youssef et al., 1985; Plischuk et al., 2011; Graystock et al., 2020;

Cohen et al., 2021) at high rates, presenting the risk of cross-infection between bumble bees and

the greater pollinator community.

Contrary to other studies on parasite prevalence in wild bee communities (Piot et al.,

2019; Cohen et al., 2021), we did not detect any effect of the bumble bee or plant community

amplifying or diluting *Crithidia* spp. prevalence. Bumble bee density may be too low in forests

for community-level epidemiological effects, potentially due to population isolation by the

closed-canopy matrix and the relatively small sizes of early seral bee communities. Dilution may

be difficult to detect due to low bumble bee species richness. Bumble bees may also not be

sufficiently diverse in transmission potential to cause a dilution effect: studies that observe

dilution effects in wild bees incorporate multiple bee genera (Fearon and Tibbets, 2021; Ponisio

et al., in press). Also contrary to previous studies, we did not detect an effect of bee body size on

parasite prevalence (Cohen et al., 2021; Van Wyk et al., 2021). Infection rates were highly

variable in stands ($R^2 = 0.14\%$), indicating that factors beyond those we examined shape parasite

communities. Our supporting values (R^2) were similar to those found in a local community-level study on parasite prevalence (Ponisio et al., in press), providing further evidence that other variables affecting infection warrant further exploration. Unfortunately, many of these variables are difficult to measure in the field, including the nesting density of conspecifics and congenerics (Halliday et al., 2020; Ivers and Jha, 2023), colony size (Rutrecht and Brown, 2009), quality of nutrition (McNeil et al., 2020), host and parasite genotypes (Barribeau et al., 2014), and bee bacterial microbiomes (Engel et al., 2016). Though many epidemiological questions remain, the prevalence statistics we report suggest that bumble bees in managed coniferous forests do not have high transmission rates compared to other populations and will not experience increased disease risk resulting from strategies that enhance open canopies.

Our sampling efforts returned considerably lower numbers of bees than previous studies in the region, which used a combination of hand netting, pan traps, and blue vane surveys to assess bee populations (Hanula et al., 2016; Rivers et al., 2018; Rivers and Betts, 2021; Zitomer et al., 2023). Passive traps collect higher numbers of bees per unit of sampling effort but preclude later parasite screening. Forest bumble bee studies based on hand netting have shown similar capture rates to our surveys (Pengelly and Cartar, 2010; Rivers et al., 2018). In closed canopies, hand-net insect capture may be biased due to difficulty of netting in dense vegetation and reaching mid-canopy floral resources. Literature from other North American forest types supports that bumble bees are generally less abundant in closed canopies and, if present, are more likely to use closed-canopy forests for non-foraging activities such as nesting and hibernation (Lee et al., 2021; Mola et al., 2021a).

Though we detect a weak effect of vegetation diversity, much of the variation in *Bombus* species richness is still unexplained, as evidenced by the model's low R^2 (0.09%). In general, the

number of bumble bees at any stand was low (maximum = 31, mean = 2.2) and highly variable

(SD = 4.1). Of the 7 bumble bee species we collected, only one (*Bombus vosnesenskii*) was

caught more often in open canopies than other stand types (Fig. 2a); while our sample size is too

low to draw conclusions about species distribution, this pattern suggests incorporating species-

specific habitat preferences may help explain the presence of bee species based on canopy types.

Further understanding of species-level canopy type preference can focus conservation efforts:

while areas with the most open canopy may have the highest overall flowering plant and bumble

bee abundance, they may support generalists instead of more forest-adapted species of

conservation concern. Other stand characteristics, such as the availability of suitable nesting

sites, may also help predict bumble bee richness. Additionally, stochastic processes such as

chance dispersal to a stand may shape communities. We have a limited understanding of bee

movement through forests, but evidence exists that structural characteristics such as road

corridors may benefit dispersal in managed forests (Wojcik and Buchmann, 2012). A better

understanding of how bees access, colonize, and persist in forest openings may improve our

ability to predict bumble bee richness.

By finding that canopy reduction increases forage abundance and indirectly benefits the

bumble bee community without exacerbating disease, we fill existing gaps in forest pollinator

literature and support a growing body of work in Northwest forests showing that forest

stewardship decisions can create and revitalize habitat for bees and other threatened wildlife.

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