Spatio-temporal Patterns of Pollinator Persistence in Temperate Coniferous Forests: Implications for Forest Management and Pollinator Conservation

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Abstract

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Managed forests may serve as havens for native pollinators. However, very little is known about the abundance, diversity, and distribution of forest pollinators at the landscape-scale necessary to design effective long term conservation strategies. This research aims to shed light on the spatiotemporal dynamics of pollinator communities in response to various managed forest features and seasonal shifts in resource availability, elucidate the pathways pollinators use to move through the forest, and predict long term outcomes of alternative management scenarios. Data were collected using a network of blue vane traps dispersed throughout Gifford Pinchot National Forest – a 1.3-million-acre managed forest in Washington State – sampled bimonthly over the active seasons of most pollinators in temperate ecosystems for three years. To gain finer scale insight into the movement of pollinators through forest environments, I deployed a new method of in situ pollen tracking, applying quantum dot nanotechnology to trace the insect mediated dispersal of individual pollen grains over an extensive and successionally varied forest landscape. Analysis revealed distinct communities emerging at varying spatial and temporal scales, indicating the sensitivity of pollinators to landscape, stand, and within-stand level conditions facilitated by managed forests. Pollinators' foraging pathways were also influenced by forest landscape structure and exemplified the effective use of quantum dots as pollen analogues and the importance of floral resources to support pollinators in otherwise sparsely populated areas. To understand the practical implications of this research, I used a combination of occupancy and detection models and spatial simulation to characterize the habitat niches of wild bees – an important group of forest pollinators – and predict their presence across virtual landscapes representing alternative harvesting regimes. These simulations revealed that, at the temporal resolution of observed harvest rotations (i.e., 80-100 years), landscapes with large (350 acre) and small (50 acre) harvest patches both displayed a synchronized shift to mid succession forest and wild-bee communities exhibited a similar large-scale homogenization and loss of diversity over the harvest regime. Whereas moderate sized harvests (100 acres) maintained successional heterogeneity and wild-bee communities similarly sustained diversity. Collectively, this research supports the potential for managed forests as pollinator conservation havens if managed conscientiously and provides evidence for simple yet effective management practices – such as daylighting and seeding roadsides and maintaining moderate harvest sizes – to preserve the pollination services and persistence of wild pollinators over future forest landscapes.

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Chapter 1: An Introduction to the Importance and the Unknowns of Forest Pollinators

Interacting processes of pollination and photosynthesis have been sustaining the resilience of natural ecosystems for millions of years (Kooi and Ollerton, 2020). Through their coevolved behavioral and morphological traits, plants and pollinators impart diversity to natural systems that guards against synchronized collapse due to large-scale disturbances (Kudo and Cooper, 2019; Wei et al., 2021; Ehbrecht et al., 2021). Cross-pollination enhances ecosystem stability and the genetic resilience of plants, which extends to the rest of the ecosystem through the fertilization of a diverse phenotypic range of seeds. As seeds grow, they send roots into the soil that lock in nutrients, prevent erosion and filter groundwater. As plants they continue to grow, absorbing CO_2 and releasing oxygen, providing nutrient rich and varied vegetation for primary consumers, and pollen for pollinators to collect and repeat this process. By contrast, self-pollinating or clonal systems promote the same range of diversity in each generation without the plasticity of pollinator-mediated systems to adapt to change.

Native bees are responsible for pollinating approximately 75% of all flowering plants on Earth and 35% of our agricultural crops depend on insect-mediated pollination (Committee on the Status of Pollinators in North America, 2007; USGS, 2015). The diversified pollen loads of wild bees visiting agricultural areas not only enhance the genetic resilience of crops to disturbance through cross pollination but also contribute to higher quality produce and crop yield in proximity to wild pollinator habitat (Hoehn et al., 2008; Rogers, Tarpy and Burrack, 2014). As our industrialized society has come to rely on mass food production – primarily grains like corn, soy, and wheat – we have altered the natural composition of the landscape. Massive monocrop expanses lack diversity and local resilience to disturbance from pests or pathogens, resulting in unmitigated, large-scale die-offs. For example, the Asian Citrus Psyllid was introduced to Florida in the early 2000s and has since spread to Southern California, causing an estimated $224 million in damage annually to the citrus industry (Grafton-Cardwell, 2010; USDA, 2013).

To combat pest invasions, farmers apply systemic insecticides that spread beyond the intended crop area, bioaccumulating in the environment, and causing harm to other organisms (Hopwood et al., 2016; Siviter, Johnson and Muth, 2021). Specifically, neonicotinoids that infiltrate the air, soil, groundwater and living tissues of plants, are lethal to wild pollinators, impairing their nervous-system functioning, reproduction and decision making. They are now one of the leading causes of national pollinator decline (Committee on the Status of Pollinators in North America, 2007; Winfree et al., 2009; Hopwood et al., 2016; Fortuin, 2020; Siviter, Johnson and Muth, 2021). The fragments of natural habitat left in agricultural landscapes are becoming increasingly important as they are chipped away.

Another threat to native and wild bees are European-imported honeybees, *Apis mellifera* (Cunningham et al., 2022). From Texas to Washington, semi-trucks of honeybees intended to supplement diminishing native pollinator services are transported across the country to artificially assist in large-scale crop pollination of almonds, oranges, peaches, blueberries, apples, and many other crops (Bond et al., 2021). *A. mellifera* are extreme generalists that can wander into the wild from wherever their hives are deposited and outcompete local, wild, pollinators for nesting and foraging resources (Hung et al., 2019; Cunningham et al., 2022). Due to their biology, nesting behavior (in large eusocial hives) and overcrowding in agricultural areas, they are extremely susceptible to the infectious spread of varroa mites (the vectors of deformed wing virus) that often lead to widespread colony collapse (Dainat et al., 2012; Webster and Johnson, 2022). Native bees, however, are resilient to varroa mite infestations, which elevates their importance to the viability of pollinator-dependent crops (Webster and Johnson, 2022).

Climate change is another factor exacerbating pollinator declines (Scaven and Rafferty, 2013; Gérard et al., 2020). As plants and pollinators have evolved, they are tightly tuned to one another's phenology patterns. Pollinators emerge as buds are bursting to forage and provision their nests while rendering their pollination services to receiving flowers before returning to their solitary burrows to await the next vibrant spring. As irregular climate patterns initiate earlier bloom dates, many pollinators – queued to emerge by soil warming that occurs more gradually than warming air temperatures – may miss the peak abundance of their paired floral partners (Scaven and Rafferty, 2013; Kudo and Cooper, 2019). Particularly for oligolectic (i.e., more specialized) plant-pollinator partners, phenological mismatches can result in pollination limitation, wherein plants receive fewer visitations leading to lower rates of fertilization, and pollinators failing to secure sufficient pollen stores for their next generation or to overwinter (Gérard et al., 2020; Zoller, Bennett and Knight, 2023). Lower germination and survival rates are expressed the following season and by the next generation, perpetuating a cycle of degradation in natural pollination services and native pollinator populations. If this feedback cycle continues, we may see a shift from specialist to generalist pollinators over the next few decades depending on climate change, rates of land cover change, continued pesticide application and other environmental and anthropogenic factors influencing native pollinators (Ma et al., 2022; Zoller, Bennett and Knight, 2023).

As the importance of wild pollinator diversity to the sustained functioning of ecosystems is recognized, attention has been shifting to understanding the persistence of wild pollinators in the habitats where they still naturally persist (Romey et al., 2007; Bailey et al., 2014; Hanula, Ulyshen and Horn, 2016; Proesmans et al., 2019; Odanaka and Rehan, 2020). Among these places are forests. In Washington state, where my research is located, fifty percent of the state (42.6 million acres) is forestland (Arnold, 2017). Although nearly a third of these forestlands are under management, they still provide great swaths of natural habitat for wild pollinators in their meadows, wind throws, burns, clearings, riparian areas and roadside embankments (Arnold, 2017; Hanula, Ulyshen and Horn, 2016). Temperate coniferous forests are typically cast as habitat for megafauna – black bears, foxes, and badgers – and their tall, needled trees that rely on wind pollination. However, looking at a finer scale, forests are also home to an abundance of pollinators and have extensive potential for pollinator conservation (Romey et al., 2007; Bailey et al., 2014; Hanula, Ulyshen and Horn, 2016; Proesmans et al., 2019; Odanaka and Rehan, 2020) (Figure 1).

Figure 1: A longhorn bee, *Melissodes sp.*, rests on a floral disc of *Tanacetum vulgare* in a sunlit forest clearing.

Managed forests present highly heterogeneous landscapes conducive to pollinator diversity (Klein et al., 2018; Hass et al., 2018; Lázaro and Alomar, 2019). Pollution and pesticide application, including neonicotinoids, are comparably lower than in agricultural landscapes or urbanized areas (Park et al., 2015; Hopwood et al., 2016). Forest interiors remain relatively buffered from effects of light and noise pollution and incursions of invasive ornamental plants, pathogens and pests, including honeybees (Park et al., 2015;

Hopwood et al., 2016). They cover large areas of land, increasing the positive impact that conservation measures could have than in other less spatially extensive land-cover types. Existing forest-management practices also lend themselves to adopting pollinator conservation techniques for quick and actionable management.

As wild pollinator populations continue to dwindle, the depth of their importance to ecosystem functioning becomes more evident. Understanding their persistence patterns in diverse forest areas is critical to develop effective, long-term, conservation strategies.

The largest needs to fill in our knowledge of forest pollinators, and most pressing areas to address in terms of conservation, are:

1. Documentation of pollinator species, their abundances, and the spatial and temporal relationships, or communities, that they form throughout managed forests;
2. Empirical knowledge of pollinator movement and behavioral factors that can allow us to understand how forest structures affect patterns of pollinator distribution; and
3. Applied models of forest management to predict outcomes of native pollinators to various management techniques or strategies.

First, to determine pollinator presence across a forest in the context of spatially varied and seasonally dynamic behavior, we need to establish a sampling framework and baseline of knowledge that captures forest pollinator persistence in large heterogeneous managed forests. The vast landscape variation of managed forests is evident, quite literally from the top down, by satellite imagery that depicts an expansive patchwork of stands in various stages of succession and regeneration, each representing unique conditions for pollinators. Following principles of competitive exclusion and coevolutionary mutualisms and species-specific studies, pollinators within this spatially complex landscape maintain similarly diverse and multi-layered ecological relationships (Sargent and Ackerly, 2008; Cardinal and Danforth, 2013; Heller et al., 2019; Wei et al., 2021). Given highly varied microclimates and interactions, attempting to identify every species of pollinator present and examine each of their subsistence patterns in relation to various forest features and each other would be impossible with available resources. With this in mind, my first chapter establishes a landscape-scale analysis of pollinator communities at the genus level and evaluates abundance, richness, and diversity in response to the most salient spatial, seasonal, and structural features that comprise a managed forest.

Another critical aspect of pollination ecology is understanding how pollinators move through forests when confronted by different forest structures, such as forest stands in different successional stages, successional gradients and roadsides. This knowledge is important to predict how pollinator individuals, genera and communities may respond to alternative management strategies that alter stand arrangements, successional composition, including degrees of fragmentation and road densities. Currently, we lack reliable and efficient methods of tracking pollinators to observe these responses. Additionally, we need knowledge of not only pollinators' movement patterns, but also their floral interactions to understand how forest landscapes are serving them. For example, more movement may not always equate to better pollinator habitat if they are merely searching for suitable resources without gathering or pollinating. Alternatively, very little movement could indicate poor habitat if the population is isolated or dependent on a single foraging source.

Past methods of tracking pollinator movement have relied on fluorescent powders as pollen analogs and pollinator tags, mark and recapture techniques, electronic tracking devices, camera traps and software algorithms to record pollinator movement and pollination events (Lihoreau et al., 2012; Klaus et al., 2015; Sardiñas and Kremen, 2017; Ratnayake, Dyer and Dorin, 2021). However, each approach has shortcomings. Powders are difficult to apply precisely – often coating the entire insect or flower rather than analogous pollen-collection areas and can be transferred from pollinator to flower, or flower to flower, by other means than pollination (unintentional brushing of broadly applied powders while searching, resting, or sheltering; wind, rain or other disturbances). Thus, these methods often do not accurately reflect or result in flower fertilization or insect nutrient gain. Recapture rates in marking studies are usually very low, electronic trackers are only useful within the radius of their receivers, and cameras are limited by field of view and detection ability (Lihoreau et al., 2012). Therefore, to address the second gap in our knowledge of forest pollinators, I apply a new method of pollen tracking using quantum dot nanotechnology to trace the insect-mediated dispersal of individual pollen grains across stand successional gradients and road networks present in a large, operational, managed forest.

Quantum dots are nanoparticles, 2-10 nm in diameter, composed of semiconductor crystals that have undergone extreme compression such that energy levels within a particle are forced into a single valence band (Gammon, 2000). When exposed to ultraviolet (UV) light, excited electrons jump directly from the particle's core to the outer band and then fluoresce as they fall back to their ground states, releasing energy in the form of light. The color of light corresponds to the distance from core to shell, such that larger particles which incur a longer distance, or wavelength, appear on the "bluer" end of our visible spectrum while smaller particles produce a "redder" fluorescence. Most particles are equipped with a shell or coating that determines what surfaces the particle will bind to. Quantum dots used for this study have a lipophilic shell that encourages them to attach to the lipid-rich surfaces of individual pollen grains, which can then be followed like iridescent breadcrumbs through a dark forest to track the pathways of forest-dwelling pollinators with UV light.

The idea of quantum dots as pollen analogs was introduced by Dr. Minnaar in 2019 and has since been employed in the field of pollination ecology to determine species of pollen grains and observe rates of pollen transfer between conspecific flowers (Anderson and Minnaar, 2020; Konzmann et al., 2020). This, however, is the first application of quantum dots in a natural setting for tracking pollinator movement via plant-pollinator interactions. In addition to successfully providing insight to pollinator movement in response to forest structural features, developing this method for field sampling provides a demonstration for future researchers looking to employ this method.

Lastly, I used a quantitative occupancy modeling approach to assess the effects of alternative forest harvesting regimes on forest pollinators, specifically native bees. Quantitative measures whose importance is often overlooked in research design are the elements of resolution and scale. Across what area, or period of time, are we effectively "summing" the presence of taxa, natural resource availability, or some other factor? What does this quantification reveal or obscure? The effects of scale are present in each of my chapters as it is a constant factor in our study and understanding of the persistence of pollinators through space and time. At the finest resolution, it might be possible to observe pollinators at spatiotemporal resolutions of seconds or millimeters. Understanding patterns at landscape scales, though, requires that we sum these instances over space and time to understand how likely something is to occur over a specified period or area and with some level of certainty given our resolution and scale of study. The physical parameters of where something occurs is referred to as its habitat or spatial niche (Heggenes, Bagliniere and Cunjak, 1999; Schirmer et al., 2020).

To describe the habitat niche of each native bee genus at the landscape scale over which my data were collected, and the scale at which forest management decisions are made, I recorded the frequency at which each genus appeared in relation to successional and structural forest features over the three-year duration of this study. Describing each pollinator's niche in terms – and at the scale – of these features allowed me to project the likelihood of habitat niche occupancy of each genus across landscapes representing different forest harvesting scenarios. The potential outcomes of various logging scenarios on the prevalence of wild bees could then be compared, as well as the estimated yields of forest harvest from each management regime.

Each chapter provides additional insight to the spatiotemporal community dynamics and persistent patterns of wild pollinators, immediate actions that could be integrated to forest management practices to improve forest pollinator conservation, and explicitly outlines areas of additional research to continue filling the gaps in our knowledge of forest pollination ecology.

Chapter 2: Spatiotemporal Shifts of Pollinator Community Compositions Across Forest Landscapes

INTRODUCTION

Over a century of intensive forest management in Washington State has induced a landscape mosaic of succession and regeneration. Stands in stages of early seral to old growth forest, interspersed with meadows, streams, and roadways, provide the type of heterogeneous landscapes that pollinators favor (Harris and Johnson, 2004; Menz et al., 2011; Boscolo et al., 2017). However, the spatial and temporal diversity, abundance, and distribution of pollinators within these forests remains relatively unknown (Rivers et al., 2018 Hanula, Ulyshen and Horn, 2016; Hall and Reboud, 2019). As wild pollinators continue to decline in other ecosystems, the potential of managed forests as reservoirs of diversity has been identified by environmental and conservation scientists as an important and promising possibility (Romey et al., 2007; Bailey et al., 2014; Hanula, Ulyshen and Horn, 2016; Proesmans et al., 2019; Odanaka and Rehan, 2020).

In forest systems, pollinators' roles extend beyond servicing flowers. Once pollination successfully occurs, a fertilized seed can germinate, sending roots into the soil that lock in nutrients, prevent erosion, and filter groundwater into streams and wetlands for uptake by other forest flora and fauna. Upon germination, a seedling begins to photosynthesize, absorbing CO_2 and releasing oxygen, purifying the air as it grows. Primary consumers depend on the rich regrowth of vegetation as organisms at higher trophic levels depend on them. Additionally, pollinators' provisioning of cross-pollination (i.e., transfer of genetic material between two flowers), in contrast to self-pollination or cloning, strengthens the genetic resiliency of local plant communities (Huang, Tu and D'Odorico, 2021). The need for conservation techniques that support pollinator abundance and diversity in their design is important for pollinator communities as well as forest ecosystems.

To preserve these services and evaluate the potential of forests as diversity reservoirs, we need to understand which pollinators are present and associated with various successional stages, elevations, and edge effects within managed forests. Additionally, we need to understand how these communities shift in response to the spatial arrangements of these features and seasonal availability of resources over time. Such a landscape-level analysis of temperate forests is currently lacking (Hanula, Ulyshen and Horn, 2016; Rivers et al., 2018). Therefore, the purpose of this work is to (1) identify pollinator communities present in a large managed temperate coniferous forest; (2) understand how the composition of these communities shifts spatially and temporally over pollinators' active season in response to the arrangement of forest features, including stand- and site-level succession stages, elevation, and road proximity; (3) and identify areas of research that could be critical to preserving pollinator diversity in managed forests.

In temperate coniferous forests, which are dominated by wind-pollinated tree species of fir, pine, larch, cedar, hemlock, and spruce, pollinators are often only readily observed where the canopy opens, allowing light to penetrate the understory and initiate a photosynthesizing frenzy of floral resources. Pollinators depend on this abundance and diversity of foraging material to maintain similarly flourishing, complex communities (Harris and Johnson, 2004; Menz et al., 2011; Boscolo et al., 2017).

Large canopy openings that provide light to the understory may occur naturally due to wildfires, windfalls, landslides, floods, or tree pathogens. They can also result from timber harvesting. Studies have consistently found pollinators in greatest abundance and diversity at recently logged stands and forest edges where light and foraging resources are plentiful (Proctor et al., 2012; Roberts, King and Milam, 2017; Mullaly et al., 2019; Odonaka and Rehan, 2020). However, pollinators also require other resources to complete their life cycles, such as nesting habitat – in the cracks and crevices of dry snags, loose soil or abandoned burrows – and shelter from extreme weather events (Christensen, 2015; Han et al., 2021). The availability of these and other resources is largely determined by the successional structure of the forest and the abiotic conditions facilitated by different features. For example, recently logged stands with exposed soil and snapped stems supply ideal nesting substrate for miner and mason bees; open understories and shade-casting canopies of late-succession stands provide relief from summer heat; and abundant floral resources along roadsides act as habitat corridors (Hopwood, 2013; Xerces, 2023 Ren et al., 2023).

The spatial arrangement of these features is also important as all essential resources must be within a pollinator's foraging radius. While larger-bodied pollinators may forage as far as 2 km from their nests, smaller individuals are often bound by a much shorter radius (Gathmann and Tscharntke, 2002; Zurbuchen et al., 2010; Hofmann, Fleischmann and Renner, 2020).

Therefore, not only the availability of resources but also their spatial arrangement on the landscape is important to determine the distribution, abundance, and diversity of forest pollinators.

In seeking to document and explain the spatial patterns of pollinators in heterogeneous managed forest landscapes, I hypothesize that:

(1) Pollinator community composition varies between and within forest landscapes in response to stands of varying succession stages, elevation and the presence of logging roads, and their spatial arrangement, and

(2) Forests with greater successional heterogeneity and denser road networks may support more complex pollinator communities by providing a greater diversity of habitat and other resources.

Temperate coniferous forests also exhibit distinct seasonal structure. Cold winters (December – February) with heavy precipitation delivering snow at higher elevations are followed by lush springs (March – June). Summers (July – September) are hot and end gradually as floral resources fade into Fall (October – November). Pollinator and plant phenologies are closely tied to these seasonal changes, exhibiting synchronized emergence and budburst of mutual pollinator-plant partners (Duchenne et al., 2020). Competition between pollinators for floral resources and between plants for pollination services is reduced as phenology patterns of pollinator-plant communities are staggered over the season (Olesen and Jordano, 2002; Proesmans et al., 2019), resulting in spatially overlapping yet temporally distinct communities at the landscape scale. Given seasonal fluctuations in availability and suitability of resources offered by various forest features, I further hypothesize that:

(3) Community associations observed at finer levels of spatial and temporal resolution (e.g., within seasons and within landscapes) will provide a greater degree of differentiation between pollinator community compositions and association with forest structures than those based on coarser resolution data.

Investigation of this hypothesis also serves to test the importance of scale in studying the spatial and temporal dynamics of pollinator communities and any subsequent conservation actions, while providing information on the finer-scale variation in pollinator communities within landscapes and across the active season.

METHODS

Study Area: Gifford Pinchot National Forest is located between the eastern precipice of Mt. St. Helen's caldera and the western foothills of the Cascades Mountain range, in the Cascades Ecoregion, WA (Pater et al., 1998). The region is characterized by heavy rain and snowfall in winters with temperatures lingering between -5° and 3°C, thawing into a prolonged, wet spring that transitions into a dry summer with average highs from 24° to 32°C and a few days peaking over 38°C. From the lowest point of the forest at its northern entrance, the forest gains 3,600 m in elevation to the summit of Mt. Adams. Timber harvesting activities occur at elevations up to 1,500 m.

Gifford Pinchot National Forest is one of the oldest national forests in North America, originally included in the Mount Rainier Forest Reserve in 1897 (USFS, 2018). The forest has undergone intensive logging since the 1840's when the California Gold Rush increased demand for lumber on the West Coast (Wilma, 2003; Center for the Study of the Pacific Northwest, 2011). Thousands of acres were cleared over the next hundred years, the impacts of which remain visible in the first satellite images of the Forest taken in 1972 by NASA's LANDSAT 1 satellite (USGS, 2022). Implementation of the Forest Practice Act in 1946 requiring replanting of trees, and many amendments since – including restricted clearcut size in 1992 and designation of critical habitat areas by the Endangered Species Act – are also evident on the landscape as more recent harvests are fewer, smaller, and use less destructive methods (e.g., selective cutting vs. clearcutting) (Department of Natural Resources, 2013). The Forest is now predominantly composed of Douglas-fir stands in varying stages of succession, managed by the U.S. Forest Service and Port Blakely logging company (USFS, 2023). Ephemeral springs and tributaries of the Cispus River run through the forest, as well as a network of logging roads in various stages of abandonment and use creating a patchwork of open and closed canopy stands, laced with sunlit corridors.

Site Selection: To capture how pollinator communities vary over this successionally and structurally diverse landscape, I sampled pollinators at three stages of forest succession (early, mid, and late), along transition zones between pairs of succession stages (early-mid, mid-late and late-early), at different levels of proximity to roadsides (0 – 50 m), and at low (<450 m) and high (>1100 m) elevations. Early succession stands were defined as 1- 6 y.o. (i.e., since logging); mid succession as 15 – 30 y.o.; and late succession stands >60 y.o. (Powell, 2012; Franklin and Hemstrom, 1981). Transition zones between stands are important to distinguish and sample separately as they offer abiotically distinct areas compared to the adjacent stands. Pollinators have also exhibited different responses to stand edges than interiors (Ren et al., 2023).

Based on spatial data records of timber harvests kept by the Forest Service, along with a time series of high-resolution imagery maintained in GoogleEarth Pro, I located two sites each of adjacently-paired stands of different successional stages: early adjacent to mid (abbrev. EM), mid adjacent to late (ML), and late adjacent to early (LE). All sites were composed of two stands 74 – 110 ha in size (mean = 94.5 ha), roughly rectangular in shape, with one side bordered by a road (310-520 m long). Where the two stands met, near the centerline of the site, I considered as the “transition zone” between succession stages for 25 meters on either side of their meeting line. These transition zones I identified separately as an early to mid-transition zone (EMtz), a mid to late transition zone (MLtz), or a late to early transition zone (LEtz). For each type of site (EM, ML and LE), I identified one site each at low and high elevations, for a total of six sites (Figure 1). Sites were located a minimum of 2 km apart to ensure independence but were within a 2-

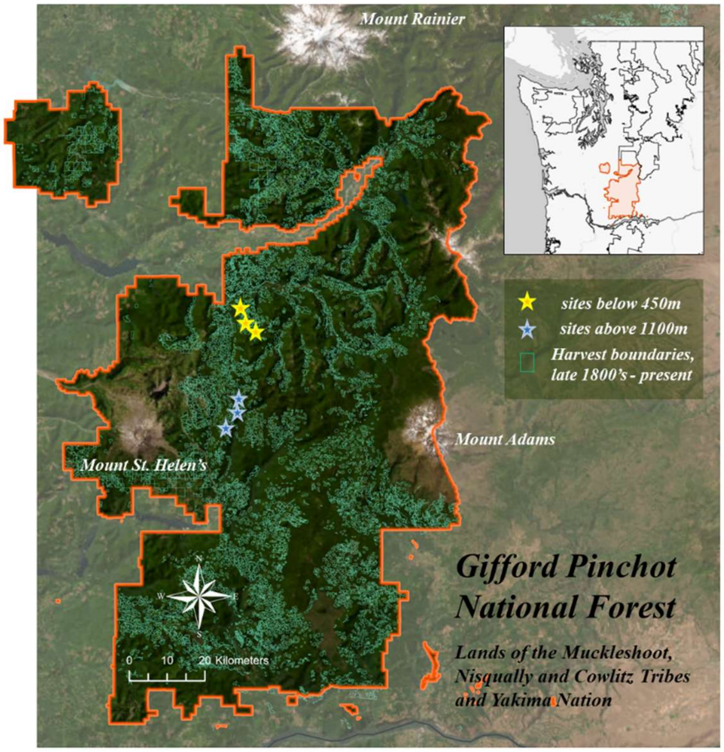


Figure 1: Study sites in Gifford Pinchot National Forest

hr drive of each other to maintain even sampling conditions. All sites were accessible by crushed aggregate or partially paved roads. Sampling took place from June to August, which reflects most pollinators' active seasons in temperate ecosystems (Hanula, Horn and O'Brien, 2015; Rivers et al., 2018). I conducted two three-day sampling events each month, allowing ten days to two weeks between each sampling event, from June – August, for the duration of three years (2020 – 2022).

Figure 2: A blue vane trap, hung along the roadside of an EM transition zone at a low elevation site.

Sampling Design and Trapping: Within each site, blue vane traps (BVTs) were deployed to sample pollinator populations (Figure 2). BVTs have been widely used to sample pollinator populations in heterogeneous environments (Galbraith, Vierling and Bosque-Pérez, 2015; Rhoades et al., 2018; Davis et al., 2020; Cunningham and Crist, 2020). The basin is made of fluorescent plastic that reflects ultraviolet (UV) light within the visible spectrum of most pollinating insects, mimicking the UV patterning displayed by flowers to entice visitors (Garcia et al., 2022; BanfieldBio, 2022; Koski et al., 2022). Traps were hung on green gardening stakes at the height of blooming floral resources and filled with a half-gallon of water and a few drops of scentless dish soap to reduce surface tension. BVTs are known to capture flying, large – bodied pollinators; however, they also capture many small-bodied, ground nesting and stem-climbing pollinators as well (Stephens and Rao, 2005). Once an insect flies or falls into the trap, they quickly slip below the water surface and are drowned. Specimens can remain in the traps for up to a week, depending on weather conditions, before showing signs of deterioration.

Traps were arrayed in parallel transects 30 – 50 m in length to capture the relationship of pollinator community composition to site type, succession stage, transition zones between stands, and roadside proximity (Figure 3). Five transects (A through E) were placed perpendicular to the roadside and at least 50 m apart to avoid oversampling the population in any one area. To describe responses of pollinator proximity to roadsides, I grouped insects collected in traps A0, B5, C0, D5 and E0. To describe responses of pollinator communities to their respective stand succession stage, I grouped traps A15, A50 and B5 with B30, and traps D5, D30 and E15 with E50. Lastly, I grouped traps C0, C15 and C50 to describe community composition in the

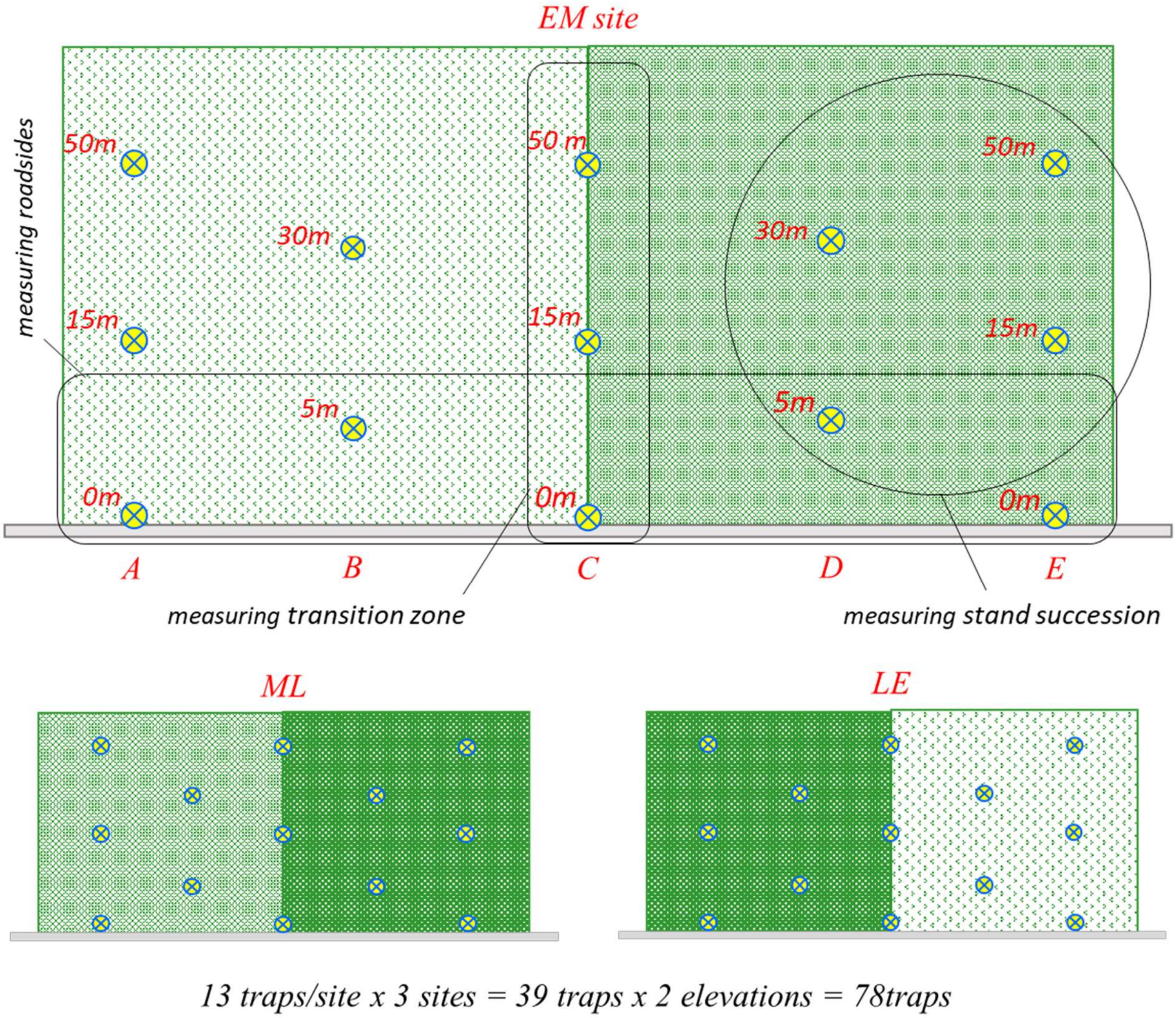


Figure 3: Transect sampling design and trap designation for measuring the response of pollinators to roadsides, stand successional interiors and transition zones between stands. This design was carried out at low and high elevations for every sampling event.

transition zone between stands.

In addition to logistical considerations (i.e., number of traps and dense understory), I limited all transects to fifty meters based on preliminary sampling in early 2020 indicating a steep decline in insect activity after 20m from the edge of roadsides within forested stands.

All traps were set at low elevation sites at the beginning of each sampling event, left for three days, emptied, and then reset at the high elevation sites and left for another three days. The traps were deployed before temperatures rose above 55°F on the first day and collected as temperatures dropped in the evening of the third day, totaling ≈ 35 hours of active collection time per trap per sampling period. While there is a wide range in the duration of time that traps have been dispatched among previous studies (8 hours to 3 months), I decided to leave these for three days as the soapy water used to set the traps was becoming murky and began to evaporate by the third day (possibly deterring other insects by scent) (Gibbs et al., 2017). Again, this also allowed for a minimum of ten days to two weeks between sampling periods for the population to recover.

Sampling was restricted to sunny days with < 20% cloud cover, peak temperatures between 65 – 90°F, low humidity, and no precipitation. I collected traps in the same order they were set to account for discrepancies in active time due to driving between sites. To reduce disturbance to bumble bee queens foraging or nesting in May and late September, I began sampling the first week of June, if weather conditions were acceptable, and continued through the first week of September. To apply even sampling, I attempted to sample during the first and third week of each month. However, due to COVID 19 restrictions in 2020, I didn't receive permission to start fieldwork until the second week of June. The two times I sampled in June of 2020 were therefore shifted towards the latter half of the month. In 2020 and 2021, wildfires within thirty miles of my low elevation sites prohibited sampling in September due to access and air quality that likely influenced pollinator behavior. Because I was unable to sample in September for two years of this three-year study, I decided to limit the analysis to exclude September data. Extreme heat events in July and August of 2021 and 2022 also forced me to move sampling events towards the beginning or end of each month. Nevertheless, a minimum of ten days was retained between sampling events to allow the community to recover and undergo change since the previous sampling.

To account for these discrepancies, rather than considering collections within each month separately, I combined them during analysis to provide a description of June, July, and August collectively rather than early June, mid-June, early July, etc. The use of months as a time period is somewhat arbitrary, although used in the literature to describe species' active periods, and could have been considered as beginning, middle, and end of the active season.

Sample Collection and Processing: Each BVT was strained into a fine mesh net, insects were separated from debris and preserved with 75% alcohol in individual Whirlpak© bags labeled with the trap ID, site, date, and elevation. Contents of each trap were processed following protocols of vanEngelsdorp Bee Lab (2014) and Droege et al. (2017). Captured insects were identified to genus using taxonomic keys, cross-referenced with information about pollinators' preferred floral and nesting resources, emergence times and known geographic ranges (Stephens, 1954; Gordon and Vandenberg, 1991; Goulet, 1993; Zeist, 2008; Discover Life, 2000; Whitehead, 2009; Gibbs et al., 2013; Miranda et al., 2013; Clark, Vlach, and LaBonte, 2016; Schapker, 2017; Koch, Strange and Williams, 2012; Engel, Rasmussen and Gonzalez, 2020; Packer, 2022).

Patterns of variability in response to landscape-scale differences in forest structure attributed at the genus level are likely characteristic of species-level ecological traits as well (Hadly, Spaeth and Li, 2009; Wiens et al., 2010). Thus, while characterization to species level would undoubtedly lend more detailed insight, time and resource constraints limited identification to the genus level. Non-pollinating insects were removed from the study pool.

Statistical Analysis: To identify patterns of co-occurrence between pollinator genera and landscape features over space and time, I used a non-metric multidimensional scaling (NMDS) analysis. NMDS is an ordination technique that considers the pairwise difference between observations in multiple dimensions, or in relation to multiple factors, as distances which can then be mapped to visualize dissimilarity in two dimensions (Oksanen et al., 2015; Bakker, 2023). NMDS is advantageous as an analytical ecological tool as it is very flexible and requires no assumptions about the data distribution. It allows the user to specify, rather than being assigned, a distance calculation method, like Euclidean distance in Principle Component Analysis (PCA). It also accepts mixed categorical and continuous variables and is robust to missing paired distances (Ramette, 2007). To identify how spatiotemporal collections of pollinators change over the active season at sites, stands, transition zones, and along roadsides, I conducted separate community ordinations (i.e., representations of potential groupings) for low- and high-elevation sites, early- mid- and late- successional stages, and by month. Ranked orders of Bray-Curtis dissimilarity were used to calculate distance matrices for each ordination. Then, data were log normalized and filtered to remove genera that appeared in less than 3% of sites that might skew results due to rarity. The analysis was completed in the vegan R package (Oksanen et al., 2015).

For each forest feature (elevation, month, succession stage, transition zones, and roadsides), I conducted an “umbrella” ordination, i.e., one that includes all levels of all factors (such as elevation encompassing both high and low areas, or seasonality inclusive of all months) within the chosen factor, and then constrained by sublevels within factors (e.g., early, mid and late stages within “succession”). Observing patterns that emerged with different groupings illustrates the effects of the chosen scale of study, or resolution, on ecological interpretation. Constraining each feature by sub-levels of the variables provided insight to the finer spatial and temporal dynamics of pollinator communities likely driving coarser resolution patterns. Examining month-to-month changes in pollinator communities, associated with each succession stage and further constrained by elevation, shed light on interactions at a finer spatiotemporal resolution (i.e., *succession x month x elevation*).

For each NMDS depicting the co-occurrence of genera at a given space and/or time I also performed an Analysis of Similarity (ANOSIM) test to determine if the groupings of pollinators were significantly different from one another (vegan R package by Oksanen et al., 2015; Bakker, 2023). The ANOSIM statistic R indicates whether similarities within groups are higher than those observed between groups such that when $R=1$, separation of communities is at its maximum and when $R=0$, no separation is found. Values of $R > 0.75$ indicate separate communities, $R > 0.5$ suggests communities are separate with some overlap and $R < 0.25$ suggests communities are similar, approaching indistinguishable (Somerfield, Clarke and Gorley, 2021).

Additionally, I conducted a genus-level Indicator assessment to identify which genera were most responsible for driving dissimilarity between assemblages and could be important indicators for conservation purposes (‘indispecies’ R package; Caceres, Jansen and Dell, 2023).

These genera are not always the most abundant in a community but those that distinguish communities from each other, making them instructive indicators. For example, *Bombus* appear in most ordinations but were only indicator genera in a few instances because, as they are present in most communities, they do not strongly distinguish between them (Caceres, Jansen and Dell, 2023).

In addition to characterizing community assemblages by genera composition, I calculated a measure of community diversity across elevations, succession stages, transitions, and road proximity levels to compare diversity across levels of forest features and over time. I used the Shannon-Wiener Index of diversity, due to its common usage in similar studies and simple conversion to effective numbers (Daly, Baetens and Baets, 2018). Pollinator diversity provides a partial measure of ecosystem health (Daly, Baetens and Baets, 2018). Analysis of variance (ANOVA) tests were performed to compare the effects of succession stages, transition zones, and road proximity on diversity over the active season and to contextualize community diversity in response to the examined forest features and feature combinations including intra-season variability (RStudio 3.6.2; R Core Team, 2021).

RESULTS

A total of 9,627 pollinators were identified to five orders, thirty-four families, and fifty-nine genera. Overall, pollinator community composition and diversity by genus varied by month, forest structural features and elevation (see the example in Figure 4 and Table 1, and full set of results in Appendix A). Conducting the ordinations on successive subsets of the data, based on these variables, revealed dependence of pollinator communities on these components and their interactions within managed forests.

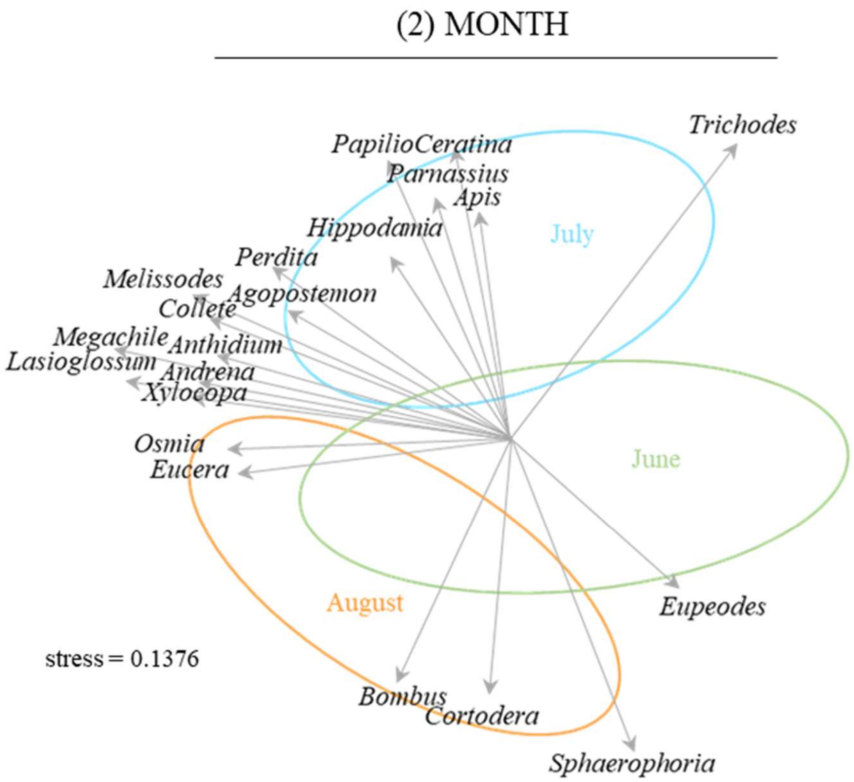
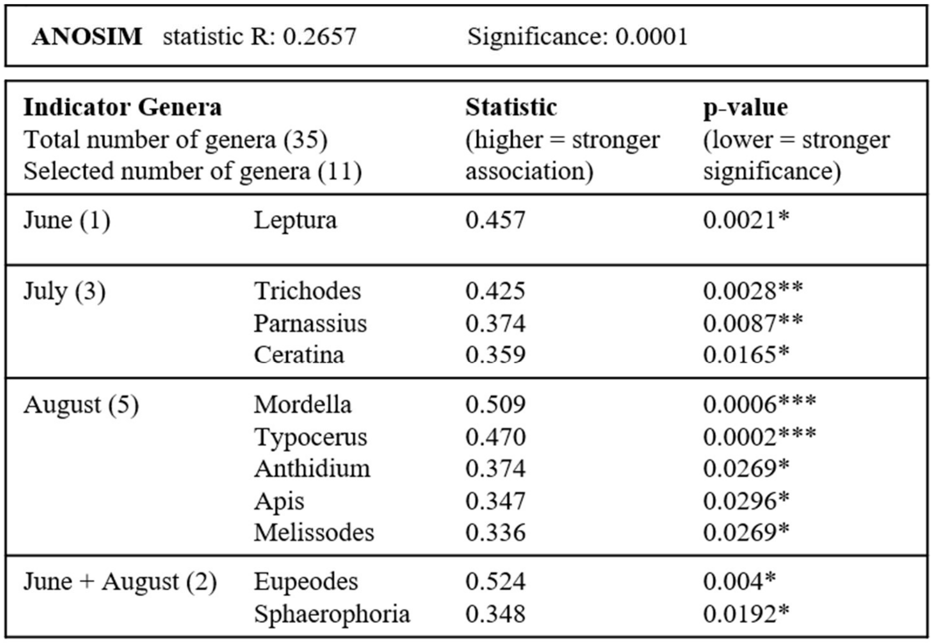


Figure 4: Example graphical result from NMDs ordination of pollinator communities by month (complete results presented in Appendix A).



ANOSIM statistic R: 0.2657 Significance: 0.0001

Indicator Genera Total number of genera (35) Selected number of genera (11)		**Statistic** (higher = stronger association)	**p-value** (lower = stronger significance)
June (1)	Leptura	0.457	0.0021*
July (3)	Trichodes	0.425	0.0028**
	Parnassius	0.374	0.0087**
	Ceratina	0.359	0.0165*
August (5)	Mordella	0.509	0.0006***
	Typocerus	0.470	0.0002***
	Anthidium	0.374	0.0269*
	Apis	0.347	0.0296*
	Melissodes	0.336	0.0269*
June + August (2)	Eupeodes	0.524	0.004*
	Sphaerophoria	0.348	0.0192*

Table 1: Example tabular result from ANOSIM test for Months (complete results presented in Appendix A).

Interpreting NMDS plots, ANOSIM statistics and Indicator Analysis: Within the ordination space, genera tend to be most abundant within or near the ellipse they are located closest to and therefore closeness of genera within the plots also indicates their similarity in spatial or temporal distribution on the ground. Length of the arrow signifies the genus' importance to maintaining balance in the ordination, often due to its greater abundance at a location or time. The stress score indicates how well the NMDS has been able to optimize dissimilarity between factors such that tension, or stress, is evenly dispersed and no one factor is influencing the location of any genera more than the other. Stress scores less than 0.2 are generally considered reliable reflections of genera distribution while scores above 0.2 approach an arbitrary arrangement of genera within the ordination.

At the first level of ordination (each variable, unconstrained by any others), high and low elevation traps did not display significantly different pollinator communities (Appendix 1A, R=0.019; p=0.26), although many more genera appeared associated with low elevation, likely due to greater overall abundance. At this coarse level of categorization (i.e., two elevations), the greatest proportions of pollinators composing high elevation communities were Coleoptera and Diptera, and those below 350m were largely Hymenoptera and Coleoptera.

Each month, or period of the active season, supported significantly different pollinator communities (Appendix A2, R=0.57; p=0.0068). Communities had a greater abundance of pollinators and were more genus rich from July to August, which was also the time period exhibiting stronger associations with indicator genera, suggesting pollinator populations progressively gain size and individuality over the active season.

Forest succession stages harbored significantly different pollinator communities (Appendix A3, R=0.45; p=0.0072). Pollinators were vastly more abundant in early succession stands with only one genus, *Typocerus*, found statistically associated to both early and mid-succession stands. Early succession stands supported larger and more diverse pollinator communities across all elevations and months the ordination encompassed.

Communities within transition zones between early, mid and late stands were significantly different from each other (Appendix A3, R=0.41; p=0.0081). However, EMtzs and LEtzs had many indicator genera in common, suggesting they are more similar than MLtzs.

Roadsides bordering or bisecting stands of different successional stages, supported statistically separate communities (Appendix A13, R = 0.40; p=0.0082). Pollinators were most abundant along early-succession roadsides, which also had more associated indicator genera than other roadside communities. The genera found in early-succession roadsides were similar to those found in early interior communities (Appendix A3).

When pollinator communities observed monthly were constrained by elevation (i.e., below 450m and above 1100m), unique seasonal structures emerged (Appendix A5). Many more pollinator genera were found at low elevations, particularly in June, while pollinator genera at high elevations did not peak until July. The number of keystone genera followed a similar pattern: more at lower than higher elevations. This does not necessarily mean there were more individuals of each indicator genus in each month, but that they were more important for distinguishing one month's community from others.

Pollinator communities associated with early-, mid-, and late-succession stages did not differ significantly when divided by low and high elevations. Early succession stands attracted much more abundant and genus-rich communities than either mid or late regardless of elevation (Appendix A6). Analyzing succession stages by month, however, provided insight into successional and seasonal variation among pollinator communities at the stand level (Appendix A7). Early succession stands that appeared important for genera like *Bombus* and *Osmia* in June, experienced a peak diversity of pollinator genera in July, then fewer but more strongly associated indicator genera in August. Mid- and late-succession stands both lacked sufficient specimens (i.e., observed abundance was too low) in June to form statistically significant communities, with the relatively low abundance of pollinators peaking in July and declining again into August. Early-succession stands at low and high elevations formed distinct communities through June, July and August (Appendix A8; R = 0.61; p = 0.0022 [low elevation]; R = 0.47; p = 0.0065 [high elevation]). Low stress values and high R values indicate very reliable interpretations. Early-succession, low-elevation stands containing early-emerging Hymenopterans, Diptera, and

Coleoptera in June, expanded to include a significant proportion of Lepidopterans in July. These pollinator-rich communities persisted into August.

By contrast, high-elevation pollinators in early-succession stands were much less abundant. From June to August, high-elevation communities became increasingly Dipteran-dominated, as compared to the multi-ordered communities at low elevations. Mid-succession stands were comparatively lacking pollinator diversity, with only seven and five genera found at low and high elevations, respectively. While the NMDS plot depicted distinct communities, the R statistic only weakly supported separation between communities. The indicator analysis did not identify any genera statistically more associated with one month than the next, due to the low pollinator abundances. Late succession stands were similarly lacking pollinator diversity at high and low elevations over the entire active season.

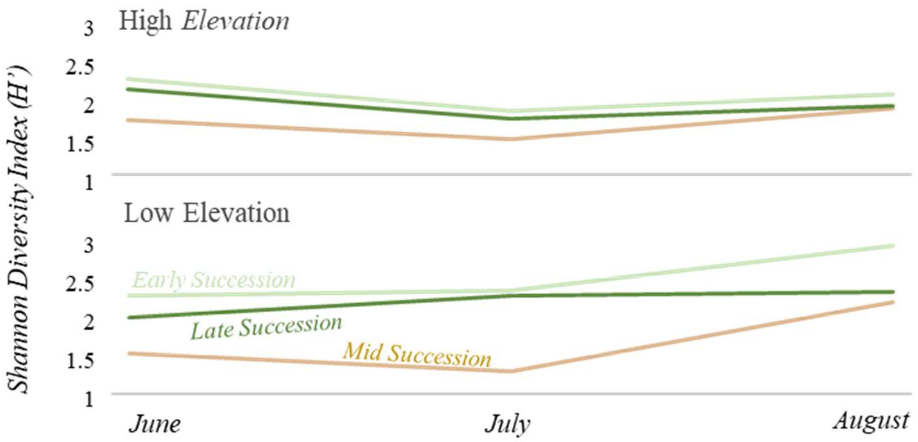
Transition zones between stands each supported distinct communities (Appendix A4; R = 0.41; p=0.0080) that shifted seasonally. LE and EM transitions supported much more abundant and rich communities than ML, while sharing many indicator genera. This pattern persisted at low and high elevations, although indicator genera for each transition zone changed from low to high elevation, indicating distinct communities dominated by Hymenoptera and Coleoptera, respectively. Communities also varied by month from smaller-bodied Hymenopteran-dominated communities in June growing to include a high proportion of Lepidoptera in July and greater presence of pollinating beetles in August, displaying statistically distinct and seasonally varied communities.

Pollinator communities found along early, mid, and late roadsides also displayed seasonal turnover (*Appendix A16*) with genera present throughout the season acting as important indicators for some months and not others. Across all elevations and months, pollinators were more abundant along early-succession roadsides than mid- and late-succession roadsides, though the latter supported a comparatively greater abundance of pollinators than their interiors. However, there were many more pollinators at low than at high elevations and their utilization of roadsides appeared to peak in August compared to other months.

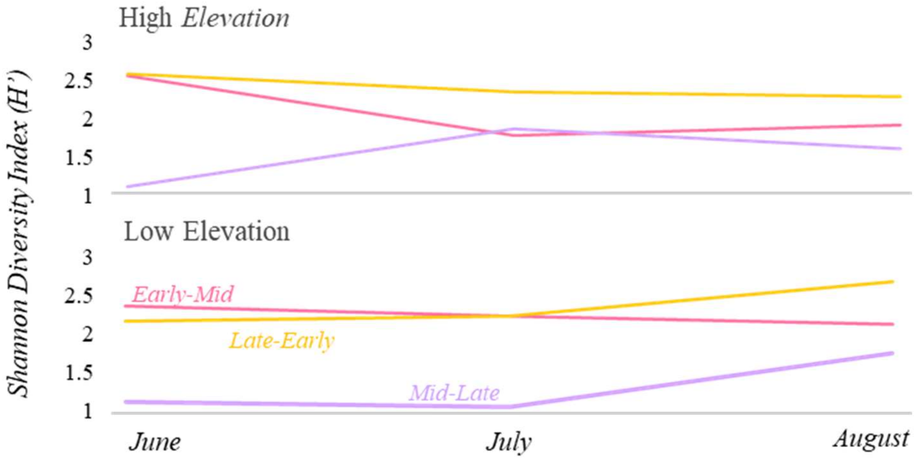
At low elevations, early- and late-succession stands gained diversity from June to August, although late stands plateaued from July through August (Figure 5a). Mid-succession stand diversity that dipped in July at low elevation, increased sharply in August ($p_{midJuly\_vs\_midAugust} = 0.019$). Early stands consistently supported greater diversity than mid stands ($p_{early\_vs\_mid} = 0.0091$). Late succession stands fluctuated between significantly similar community diversity as early stands in July ($p_{earlyJuly\_vs\_lateJuly} = 0.87$) and different from mid ($p_{midJuly\_vs\_lateJuly} = 0.0072$) to more similar diversity as mid-succession stands in August ($p_{midAugust\_vs\_lateAugut} = 0.66$) and slightly different from early ($p_{earlyAugust\_vs\_lateAugust} = 0.0077$). At high elevation sites, early, mid, and late succession stands did not display significantly different diversity trends over the season ($p_{early\_vs\_mid\_vs\_late} = 0.66$).

Transition zones (i.e., EMtz, MLtz and LEtz) at low elevations followed similar trends in significance as early, mid and late stands at low elevations (Figure 5b). However, while LEtzs remained significantly different from MLtzs over the duration of the season ($p_{LEtz\_vs\_MLtz} = 0.0095$), EMtzs were statistically different from MLtzs, and similar to EMtzs from June to July ($p_{EMtzJune\_vs\_MLtzJune} = 0.0034$; $p_{EMtzJuly\_vs\_MLtzJuly} = 0.692$) but were not statistically different from either in August.

At high elevations, EMtzs and LEtzs supported higher diversity in June than MLtzs ($p_{EMtzJune\_vs\_MLtzJune} = 0.0043$; $p_{LEtzJune\_vs\_MLtzJune} = 0.0043$), but all transitions zones became statistically indiscernible in July and August. MLtzs increased in diversity from June to July ($p_{MLtzJune\_vs\_MLtzJuly} = 0.023$) while EMtz and LEtzs decreased, though not statistically so, from June to August.



(a) Successional Stage



(b) Transition Zone

Figure 5**:** (a) Shannon Weiner Diversity Index (H) of early, mid and late succession stands at high and low elevation (b) diversity of EMtz, MLtzs and LEtzs over the active season.

Site type also influenced diversity. Ranked in order of decreasing diversity, LE sites had the highest diversity, followed by EM and then ML sites (Table 2).

Diversity in proximity to roadsides also showed distinct seasonal and successional patterns. Roadside diversity was significantly higher along early-succession stands than mid- and late-succession stands ($p_{earlyRds\_vs\_midRds} = 0.0016$; $p_{earlyRds\_vs\_lateRds} = 0.0020$), both of which displayed diminishing diversity with increased distance from roadside (Figure 6). Within early-succession stands, high diversity immediately along the roadside (from 0 – 5m) remained relatively constant throughout the stand. Meanwhile, in both late- and mid-succession stands diversity sharply declined after 5 m ($p_{mid0\text{-}5m\_vs\_mid15m} = 0.0033$; $p_{late0\text{-}5m\_vs\_late15m} = 0.0029$). Seasonality did not cause these patterns to deviate significantly at any successional stage or elevation (*Appendix A15* and *16*).

Site	Stand	Diversity (H’)	
		Total	Stand
EM	E	2.7109	2.6936
	M		2.1922
ML	M	2.1785	1.9432
	L		2.3145
LE	L	2.7360	2.3938
	E		2.7137

Table 2: Shannon-Weiner diversity calculated by site and stand types.

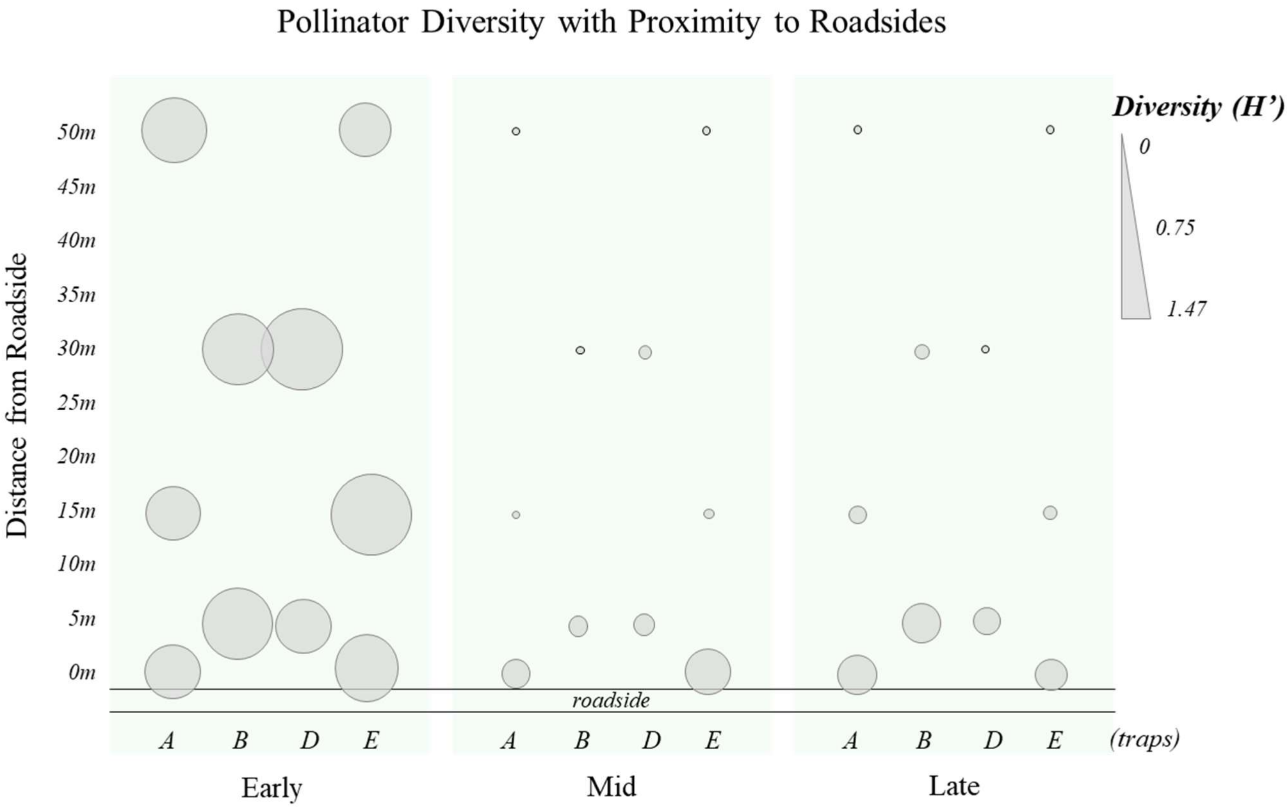


Figure 6: Trends of pollinator diversity in response to roadside proximity in all early, mid and late succession stands.

DISCUSSION

Composition of pollinator communities varied greatly in response to structural and successional features present in managed temperate coniferous forest of GPNF and these relationships shifted over the active season, providing insight to the complex spatiotemporal dynamics of forest pollinator communities.

First, pollinator communities at low and high elevations exhibited distinct compositional differences at the seasonal, site and stand levels, indicating the presence of spatially and temporally staggered communities. At low elevations, pollinators were much more abundant and emerged earlier than at high elevations, likely due to earlier ground warming and budburst (Kudo and Cooper, 2019; Duchenne et al., 2020). Indeed, small-bodied genera that are sensitive to discrete environmental changes, such as *Lasioglossum* and *Sphaerophoria*, appeared in June at low elevations, but did not appear until July at high elevations when lingering snow finally melted (Heinrich, 1974). This finding supports results from the handful of studies examining changes in forest pollinator communities over an elevational gradient (Warren, Harper and Booth, 1988; Lefebvre et al., 2018; Pepin et al., 2022). Variation in wind, precipitation, aspect and soil type controlled largely by local topology and regional weather patterns may also affect time and location of emergence (Lefebvre et al., 2018; Kudo and Cooper, 2019; Pepin et al., 2022).

As pollinators are sensitive to their surroundings, unique community dynamics arose at low and high elevations from the various environmental conditions facilitated by successional and structural forest features. For example, Lepidopterans appeared throughout the active season at low elevations but only briefly at high elevations, during the warmest months. There was also a shift from Hymenopteran to Dipteran dominance between low and high elevations that intensified over the active season, which has been observed in other alpine systems as well (Kearns, 1992; Lefebvre et al., 2018). Flies have a higher tolerance for colder temperatures, allowing them to persist later into the active season and at greater altitudes, while other pollinators have already begun retreating below ground (Kearns, 1992; Jordan, Hopwood and Morris, 2020).

Accompanying this observation, I found total pollinator diversity increased over the active season at low elevations while it declined, overall, at high elevations. This could be partially explained by the decline of floral resources above an altitude threshold, as well as shift to Dipteran dominated communities higher and later in the season. Higher elevation forests also have a much shorter hospitable gap between snowmelt and end-of-summer allowing fewer pollinator species to thrive.

Second, effects of elevation on total pollinator abundance and diversity were regulated by succession stages. Early-succession stands supported the most pollinators at high and low elevations, harboring species-rich communities of Coleoptera, Diptera, Hemiptera, Hymenoptera, and Lepidoptera. Pollinator diversity in early-succession stands increased steadily over the active season at low elevations, while generally decreasing at high elevations. Dips in pollinator diversity within mid-successional stands in July at high and low elevations could be explained by peak wildflower blooming in open sunlit areas, drawing pollinators from sparse, shaded, mid-succession interiors. Late-succession stands displayed differing seasonal trends at high and low elevations with diversity dropping in July at high elevations while peaking at low elevations, suggesting the role of late-succession stands for pollinators varies by elevation over the active season. For example, the drop of diversity in late-succession stands in mid-summer at high elevation may be due to pollinators spending most of their time in open areas where blooming has finally begun in earnest, while at low elevations some wildflowers have already peaked. Pollinators may be expanding foraging into forests to escape high summer temperatures.

Variations in community compositions at the stand level occurring at different elevation and months indicate that to detect separate communities and understand community turnover, we must study interactions of pollinators and their landscapes at least by month and in context of individual succession stages. Otherwise, changes in communities' spatial distributions may be obscured at the aggregated levels of season and landscapes. Variable responses across elevation and succession stages suggest that management changes aimed at affecting pollinator populations, e.g., by increasing the proportion of early succession stands, need to consider elevational effects on pollinator diversity at the landscape scale.

Third, transition zones between successional stages exhibit distinctive pollinator communities from that of either adjacent stand. Edge effects are documented phenomena affecting ecological processes in highly fragmented forests, grasslands, island systems, and croplands, and contributing to overall pollinator richness and abundance (Libran-Embid et al., 2021; Ren et al., 2023; Dodonov and Cazetta, 2023). To my knowledge, there have been no studies conducted within managed forests to examine the effects of edges between stands of differing successional stages on the transitioning pollinator communities. I found EMtz and LEtzs supported the most abundant and rich communities, sharing many strongly associated genera – *Bombus, Andrena, Ceratina, Xestoleptura*, and *Osmia*. At the beginning of the active season, both EMtz and LEtzs supported more diverse pollinator communities than those in either adjacent stand (Figure 5b and Figure 6) suggesting they may play an important role for early emerging pollinators. This is possibly due to the proximity of forest nesting sites to early seral flora.

At the site level, the effect of early-succession stands can be seen in the community compositions of the transition zones with neighboring stands. The community diversity of LEtzs and EMtzs both exhibited greater similarity to early-succession stands than late- or mid-succession stands, respectively. MLtzs followed more closely to mid- than late-succession stand trends (almost opposite to those in EMtzs), beginning with far less diverse communities and genera strongly associated to MLtzs in June not appearing until July at high elevation. A few genera, like *Perdita* and *Buprestidae*, were consistently found in transition zones which also supported genera from both adjacent stands. Overall, these results indicate that transition zones, particularly those adjacent to early-succession stands, may be some of the most diverse areas in managed forests. This finding is supported by work from Ma et al. (2022) who found the interior structural complexity of forests is complemented by forest floral diversity, which translates through the co-evolution of plants and pollinators to pollinator diversity (Kudo and Cooper, 2019; Ehbrecht et al., 2021).

Transition zones provide condensed examples of structural complexity, or contrast, in forests. For example, open canopy, early-seral stands abutting the dense, shaded understories of mid-succession stands provide stark contrast to older stands with understories of larger and well-spaced trees. Moving from late to early stands, the contrast may be less striking for pollinators than early-mid as light availability and open space – both important for pollinators – are more similar than encountering the shaded, thicketed edge of a mid-succession stand. Late- and mid-succession stands provide progressively less dramatic contrast as the latter ages and canopies meld. In terms of light availability, the contrast of early-to-mid- and early-to-late-succession stands was undoubtedly most drastic, the effects of which are reflected by the increase in pollinator diversity. Donato, Campbell and Franklin (2012) posited early- and late-succession forests may be the two most complex stages of forest succession, which could also explain the greater diversity of pollinators at LEtzs than EMtzs.

Structural complexity and community diversity of transition zones corresponded from most complex and diverse to least over all elevations and sites from beginning to end of the active season. The relationship of increasingly rich pollinator communities with greater structural complexity supports my hypothesis that more complex forest landscapes support more complex and diverse pollinator communities that will shift over the season with availability of resources at each forest feature.

Fourth, spatial adjacency of stands at the site scale was related to pollinator diversity within stands at the landscape level. Across sites, same succession stands showed varying levels of pollinator diversity dependent on the succession stage of the adjacent stand: early-succession stands adjacent to late stands were more diverse than when adjacent to mid-succession stands, and late-succession stands adjacent to early-succession were more diverse than when adjacent to mid-succession stands (Table 3). This illustrates that not only do pollinator communities fluctuate by succession stage at the stand level, but additionally in response to the spatial context of successional stages they exist within at the landscape level. This work complements the work of others on the effects of landscape pattern, including those that have found landscape fragmentation increases pollinator diversity (Libran-Embid et al., 2021; Ren et al., 2023; Dodonov amd Cazetta, 2023). Given the large-scale patterns of successional heterogeneity facilitated by managed forests, and evolution of pollinators relative to landscape complexity, I expect diversity would increase until a certain threshold of new harvests (i.e., early-succession stands). Thereafter, fragmentation may effectively merge the landscape into a potentially new entity, such as predominantly early or mid-succession landscape that may remove essential late forest resources and/or support entirely different pollinator communities. Knowing where this homogenous successional threshold exists would certainly be beneficial to inform forest management practices and avoid unforeseen and potentially sudden, irreversible, shifts in pollinator communities.

Fifth, roads that channel light and floral resources through densely canopied forest supported different communities than stand interiors or transition zones. Roadsides have been shown to provide valuable pollinator habitat in other ecosystems, maintaining richness and abundance along highways and increasing local network connectivity (Hopwood, 2013; Ren et al., 2023). This study adds the response of pollinators along roadsides in managed forests, which occupy 41% of global forested land, to our growing database of pollinator knowledge (World Resources Institute, 2023). Unlike most other forest features, I observed that pollinator communities along roadsides were relatively unaffected by elevation or season. They provided consistent habitat for pollinators in otherwise sparsely populated mid- and late-succession stands.

Communities of pollinators found along these roadsides contained higher proportions of small bodied Coleoptera (i.e., *Trichodes* and *Mordella*) than found within stands, as well as bee-mimicking Diptera (i.e., *Sphaerophoria* and *Eupeodes*), and some larger-bodied *Lepturinii* and *Apidae*. Roadsides have been documented as a source of invasive plants to remote ecosystems (Mortensen et al., 2009; Riitters et al., 2018). While only one species within the fifty-nine genera of pollinators I identified are considered invasive to GPNF (*Apis mellifera*), the patterns of roadside community abundance and assemblages were different than those found elsewhere in the forest. This suggests that while not necessarily facilitating invasion, roadsides may be fostering unfamiliar assemblages of pollinators via propagation of nonnative strips of floral resources through mid and late succession stands in TCF systems.

Natural forest features that mimic the linear and narrow, light-gap structure of roads include creeks and streams, isolated tree falls, or ridgelines. These features are still much less frequent and extensive than road networks, though, and often spatially restricted by elevation, slope, or other topographical features that roads crisscross. At the landscape level, roads may improve resilience of pollinator communities by adding structural complexity and community diversity. However, we need more information about the ecological interactions of the additional pollinator assemblages introduced (Ren et al., 2023).

Finally, this study illustrates the importance of considering multiple contextual factors that relate to the structure of forest pollinator communities. Interacting contextual variables for ordinations in both space (site, stands, transition zones, and road proximity) and time (full active season, and monthly) revealed which interactions were best suited to observation of changing communities within this forest system (through R, significance and stress values). Stress values, which indicate how well the forest features characterized pollinator communities, lessened as more spatial and temporal contextual factors were introduced. Fine-tuning the multivariate ordination by space and time revealed that the best-suited contextual factors to detect communities with the most confidence existed when communities were ordinated by *elevation x succession x month*. Ordinations of data that ignored these variables, i.e., included observations across elevation and over the full season, increased stress within the ordination plot and lessened significance. In a few instances (*Appendix A, 8a-b* and *8e-f*) there was insufficient data to identify statistically different communities. This occurred at high and low elevations in mid and late succession stands where observed abundance was too low to draw significant inferences about community compositions.

Areas of future research that could be critical to preserving pollinator diversity in managed forests include understanding forest heterogeneity in terms of additional features and variables (such as forest fragment size, aspect, harvesting techniques, intermediate elevations, and week-to-week temporal variation). How pollinator communities form in response to these multivariate environments informs our understanding and capacity to incorporate all necessary variables into decision-making or conservation practices regarding wild pollinators. The role of roads in managed forests should also be further investigated to determine their effect on local communities. Could the density or diversity of floral resources along roadsides draw pollinators away from the sparse flora within mid- and late-succession forest interiors, leading to pollination limitation?

Spatiotemporal patterns of occurrence of individual genera were also found that could provide groundwork for more in-depth studies: tiny shiny fairy bees, *Perdita sp.*, were identified as indicator pollinators within the transition zone of late and early stands more than any other parts of the forest. Are there unique conditions or resources present this pollinator requires? As we know very little about fairy bees except that they are extremely small and difficult to observe, this would be valuable information (Packer, 2022). Jewel beetles, *Buprestidae sp*., were only found within a 30 m range of mid- or late-succession stand edges, presumably spending their larval stages feeding on the associated trees. However, to my knowledge, no studies have estimated how far emerging, pollen-feeding, adults forage from forest edges, which could be useful for restoring specific wildflower seeding areas near forested areas post-harvest, accounting for other pollinators' foraging ranges as well.

Another pattern that emerged at multiple ordinations was the clustering of *Papilio* and *Parnassius* at low elevation, associated with early-succession stands and EMtzs and LEtzs, especially in July (*Appendix A4, 6a, 7a, 8a* and *10*). From field observations, this may be due to extreme heat driving pollinators typically observed in open areas to seek shade beneath mid- and late-succession canopies. Particularly large-bodied pollinators, like *Papilio* with a 3-4" wingspan, may overheat more quickly due to natural insulation to maintain muscular heat for flight and heat generated while flying (Heinrich, 1974; Brock, Brock and Kaufman 2003).

Additional studies could examine the role of forest structure on insect thermoregulation. For example, is there a relationship between the difference in degrees from sunny to shaded areas and the distance pollinators will travel into a forest? What other resources might pollinators be acquiring from older forests? While we know that nesting habitat is one forest use and shelter from weather events is another purpose, are there other relationships?

The indicator analysis I included is intended to establish a preliminary database of important pollinators present throughout managed forests. Much more work could be done to assess its practical use in monitoring community change and strength of relationships between indicator genera in communities. For example, how reliably does the presence of mid-July indicator genera, *Leptura* and *Bombus* (which are much more abundant and easily observed) indicate presence of other associated genera, like (the much harder to find) *Perdita* bee?

CONCLUSION

Managed forests will likely become increasingly important for the future of wild pollinators and the systems and cycles in which they play a vital role. Variations in the distributions of pollinators within stands, between stands, and across sites with different stand-adjacency characteristics lends layers of complexity to forests that strengthen resiliency to disturbances. Spatial and temporal factors strongly influenced pollinator communities, indicating their susceptibility to disturbance as well as potential for effective management. This study also highlights the importance of functional scale when studying pollinator systems. The complex nature of forest structure and communities became more visible with each level of ordination. More heterogeneous landscapes should be observed with finer spatiotemporal scales to detect how management practices implemented at the landscape level might affect communities from within, across and bordering forest stands.

Chapter 3: Tracking Pollen Flow Through the Forest Using Quantum Dot Nanotechnology

INTRODUCTION:

Due to their miniscule size, pollinators, and pollen have proven extremely difficult to track in natural settings. As pollinator populations continue to decline across nearly all landscapes (Dicks et al., 2021), understanding the spatiotemporal dynamics, pathways, and networks that knit together their communities and the botanical fabric of the environment around them is becoming increasingly important (Ollerton, 2017). Particularly in understudied systems that support an unknown amount of biological diversity, quickly assessing pollinators' patterns of abundance, diversity, and movement is critical to curating conservation strategies that support and protect them and the myriad of ecosystem services they provide (Ollerton, 2017; Krishnan et al., 2020; Porto et al., 2020). Developing pollinator-conscious landscapes, though, requires knowledge of not only pollinator movement, but additionally their floral interactions (Gong and Huang, 2007). As plants and pollinators have coevolved, the presence and health of one is inextricably linked to the other. When pollinators forage for nutrient-rich pollen and sugary nectars, they transfer grains of pollen between the anthers (male part) of one flower to the stigma (female part) of another, fertilizing seeds that will germinate, grow, and eventually bloom for next season's pollinators to emerge and repeat this process (Hu et al., 2008; Ollerton, 2017).

Tracking these plant-pollinator interactions has relied on fluorescent powders as pollen analogs and pollinator tags, mark and recapture techniques, electronic tracking devices, camera traps and software algorithms to record pollinator movement and pollination events (Lihoreau et al., 2012; Klaus et al., 2015; Sardiñas and Kremen, 2017; Ratnayake, Dyer and Dorin, 2021; Bjerge et al., 2021). However, each approach has shortcomings. Powders are difficult to apply precisely, often coating the entire insect or flower rather than analogous pollen collection areas and can be transferred from pollinator to flower, or flower to flower, by other means than pollination (e.g., wind, rain, unintentional brushing of broadly applied powders while searching, resting, sheltering, or other disturbances). Thus, they often do not accurately reflect or result in flower fertilization or insect nutrient gain. Recapture rates in marking studies are usually very low. Electronic trackers are only useful within the radius of their receivers, and cameras are limited by field of view and detection ability. Therefore, the ways in which these methods record pollinator movement and floral interactions are not always reliable and cannot consistently isolate when a pollination event occurred, or the efficacy of the event (i.e., amount and compatibility of pollen transferred and received). Observing pollen movement with this level of specificity is critical to develop our understanding of plant-pollinator community interactions.

To address this scientific gap and gain a better understanding of how pollinators use their environments, I implemented a novel method of pollen tracking using quantum dot nanotechnology to trace the insect-mediated dispersal of individual pollen grains across a managed forest in Washington state. Managed forests have received remarkably limited pollinator research (Rivers et al., 2018; Krishnan et al., 2020), although scientists have speculated that they may provide reservoirs of pollinator diversity (Hanula, Ulyshen and Horn, 2016; Proesmans et al., 2019; Rivers et al., 2018; Odanaka and Rehan, 2020). Additionally, they occupy the second greatest area of habitable land, after agriculture, which elevates the relevance of this research under current global pollinator declines (Dicks et al., 2021; Ritchie and Roser, 2021). Yet, there remains a paucity of knowledge of pollinators in forest landscapes and how to effectively study them (Rivers et al., 2018; Odanaka and Rehan, 2020).

Quantum dots (QDs) are nanoparticles, 2-10nm in diameter, composed of semiconductor crystals that have undergone extreme compression giving them unique optical and electrical properties (Critchley, 2019). Compression to this size creates discrete energy levels within the particle as electrons are forced into a single valence band (Gammon, 2000). When exposed to ultraviolet (UV) light, excited electrons jump from the core of the particle to the outer band and fluoresce as they fall back to their ground states, releasing energy in the form of light that can be detected through a specialized wavelength-filtering microscope attachment (Gammon, 2000; Yang et al., 2020). Most QDs are composed of inorganic elements with a shell or coating intended to keep the contents of the particle contained, as they can be harmful to organisms, as well as control what the dots attach to (Anderson and Minnaar, 2020; Yang et al., 2020). Dots used for this study are zinc-based, which makes them safe to apply in biological environments, and equipped with a lipophilic coating that encourages them to bind to the fatty surfaces of individual pollen grains (Valizadeh et al., 2012; Minnaar and Anderson, 2019; STREM Chemicals, 2020). By facilitating tracking of individual pollen grains, this method ensures that the transfer of pollen has actually occurred, unlike other methods that may record pollinator movement but cannot reliably document when a pollination event has occurred (Minnaar and Anderson, 2019). The persistent photoluminescent properties of QDs also enable more extensive spatiotemporal studies of pollen dispersal in natural environments than the degradation periods of powders, paints and longevity of electronic methods previously allow.

By designing a sampling approach that captures the dynamics of small-scale processes occurring over much larger areas, I was able to generate cross-scale (i.e., from the flower- to landscape-scale) insights that assist us in better understanding the impacts of forest management practices on forest pollinators. Based on knowledge of pollinator behavior and ecology, and the abiotic conditions facilitated by various structural features in managed forests, I expected distribution of pollination events to be related to landscape-level patterns of pollinator foraging and nesting resources. Furthermore, while some successional stages, stand arrangements and habitat edges may appear to support fewer or greater pollination events, spatially heterogeneous forest landscapes should facilitate greater pollen flow overall than structurally simpler forest landscapes. Additionally, the varying structures within the forest itself will influence density, distance, and direction of pollen flow at finer scales. As quantum dots are a new technology in pollination ecology, this study serves as a demonstration of the potential of QDs as a pollen analog in natural environments, and I suggest future directions in its practical application and avenues of research for forest pollinators.

METHODS:

Sites: The Gifford Pinchot National Forest (GPNF) is a 530,000-ha forest that extends from the foothills of Mt. Rainier and eastern slopes of the Mt. St. Helen's blast zone to the crest of the Cascades Mountain range and south to the banks of the Columbia River. Douglas fir dominates the forest, interspersed with Western hemlock, Western red cedar, and Ponderosa pine (USFS, 2023). GPNF has been under intensive management since the late

Figure 1: Example of an EM site in GPNF

1800s resulting in a patchwork of successional stages (USFS, 2018; Records of the Forest Service, 2022). Early (E), mid (M) and late (L) succession stands, defined by age since harvest as 2-3 y.o., 10-15 y.o., and >40 y.o., respectively, were classified by age from logging records and confirmed by satellite imagery (Claverie et al., 2018; Records of the Forest Service, 2022). Stands ranged from 15-40 ha in size and were all located along logging or secondary forest service roads below 610 m in elevation.

Three types of sites were chosen to exemplify three common, distinct patterns in successional adjacency: E stands adjacent to M stands (abbrev. EM; Figure 1), M stands adjacent to L stands (ML), and L adjacent to E (LE). Three of each type of site were sampled, for a total of nine sites (Figure 2). Similar sampling conditions were maintained with low wind speeds, moderate temperature 75-82°F, and no precipitation or pesticide application that would influence wind-dispersed pollen or insect pollinator behavior.

Lab Preparation: In lab, 500mg of $CuInS_2/ZnS$ QDs were diluted to a concentration of 5mg/mL toluene (supplying 100mL of QD solution) based on the amount needed to adequately coat an individual anther of flowers from four families (*Haemodoraceae, Iridaceae, Asteraceae, Oxalidaceae*), determined in a study by Minnaar and Anderson (2019) (approx. 0.5mL). Toluene is a non-polar solvent that prevents QDs from clustering and then quickly evaporates upon contact with air so QDs are left to bind to the lipid-rich surfaces of individual grains of pollen. Flowers present at my sites (*Achillea millefolium, Cirsium vulgare, Daucus carota, Digitalis purpurea, Epilobium angustifolium, Geranium robertianum, Hypochaeris radicata, Linaria vulgaris, Oxalis acetosella, Prunella vulgaris, Trifolium arvense, Trifolium pratense, Vaccinium ovatum*) include a similar range of anther size, and so 0.5mL was retained as the dosage for all anthers. To maintain consistent concentration, the QD solution was stored in 2mL vials (four 0.5mL doses) to prevent evaporation between applications.

In Field: To test the use of QDs as a pollen analogue and observe the influence of stand succession and adjacency on pollen flow, QDs were strategically placed along roadsides at the interface of two stands of different successional stages (EM, ML, and LE) and then tracked in five directions from the point of application (Figure 2).

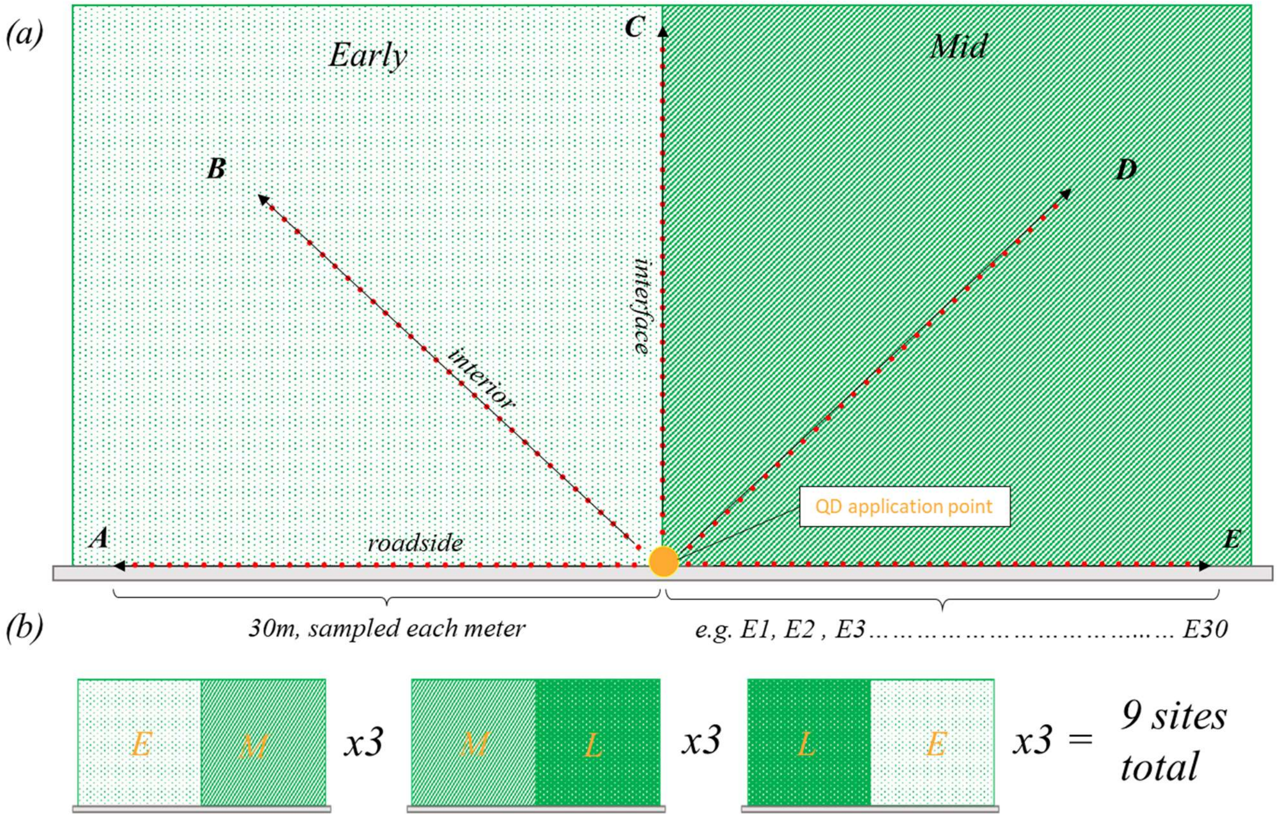


Figure 2: (a) Transects *A – E* characterize five distinct landscape gradients within each site, sampled every meter (b) Sites are of three types, defined by adjacency in successional stages.

Using a micropipette, four doses of QDs could be distributed in less than a minute (Figure 3a). At the application point of each site, three anthers on six different flowers were dosed with QD solution (for a total of eighteen 0.5mL doses per application point). I used at

Figure 3: (a) Micropipette and a 2mL vial of QD solution (b) Harvested flowers pressed on slides to view under a UV light microscope in lab.

least one flower of every species present; no site had more than six floral species at the application point. Sites were left for an average of 32 hours (ranging from 30-33hrs due to driving time between some sites) to allow pollinators to forage.

Figure 4: QDs fluorescing under UV light on a fireweed anther, a common floral resource in GPNF.

After the allotted time elapsed, five 30m-transects were established extending along roadsides (transects *A* and *E*), through the interior of each stand (transects *B* and *D*) and along the stand-succession interface (transect *C*) at each site (Figure 2). At one-meter intervals along each transect, anthers of all flowers within an arm's-reach radius were harvested, pressed onto glass slides with a coverslip and secured with clear tape, labeled, and stored in a portable freezer to prevent pollen grains shrinking and possibly detaching (Figure 3b). Sampling was conducted on three consecutive days, with one of each site type (EM, ML, LE) sampled every day. QDs applied on the first day were left to naturally disperse from approximately 8 am until the next morning, when anthers were harvested while the second batch was set just before at 8 am and allowed to disperse, followed by the third set of sites. After the first batch of sampling (including one of each site type) required preparing 722 slides, I adapted the QD-detection method to forego the process of preparing a slide(s) for each sample at every meter. Instead, I attached the microscope, equipped with a custom QD-viewing attachment[1], to a portable battery and added a black-out box so I could observe fluorescence in the field (Figure 4). Presence or absence of QDs on each flower and the total number of flowers were noted at every meter along each transect for all sites.

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[1] This attachment was modified from Minnaar's (2019) prototype by Frank Trush and I from materials we had available. High powered LEDs from a military grade flashlight were installed inside an aperture attachment made with parts printed from recycled filament on a homemade 3D printer and surrounded by a duct-tape fashioned black out box. The aperture opening was fitted with a UV filtering lens and attached by a hose clamp to a dissecting microscope to view fluorescence of QDs at the individual pollen grain resolution. Total cost, not including construction of the 3D printer, was $250. Commercially, fluorescence microscopes can cost thousands of dollars.

<u>*Statistical Analysis:*</u> To assess distribution patterns of pollination events at the landscape level, I fitted a generalized linear mixed model (GLMM) to estimate which forest features best explained QD distribution across all sites using quantum dot density (*qd_density*), measured at each sampling point, as the response variable. The model was fitted using maximum likelihood (loglink function, REML = FALSE), and I used likelihood ratio test (Chi^2) to compare Akaike Information Criterion (AIC) goodness of fit between included, excluded, and models at varying scales against the Null model (Table 1). Transect type (*A, B, C, D, E*) and floral availability (categorized by Jenks Natural Breaks as None [0 flowers], Low [1 – 10 flowers], Medium [11 – 30], High [31 – 80] and Very high [81 – 210]) were strongly correlated (Pearson's p-value < 2.2e-16) and therefore only floral availability, which best explained variation, was included as a fixed effect along with site type.

Variables	**AIC**	**P - value**	**Chi^2**
Null Model	**-1341.91**		NaN
Full Model	**-1274.51**		
Floral Availability + Site + Transect + (1|dist_rd) + (1|dist_intr) + (1|dist_intf)			2e-16 ***
Floral Availability (FA)		2e-16 ***	
Site (S)		0.0145*	
Transect (T)		0.0209*	
(1|dist_rd) + (1|dist_intr) + (1|dist_intf) = (random effects)			
Partial Models			
FA + S + random effects	-1318.29		2e-16 ***
Floral Availability		2e-16 ***	
Site		0.0115*	
T + S + random effects	-579.22		0.0423*
Transect		3.095e-10***	
Site		0.6268	
FA + S + (1|T)	-1320.63		0.0613
Floral Availability		2e-16 ***	
Site		0.0253*	
FA + S + (1|T) + random effects	-1133.94		1.000

Floral Availability	2e-16 ***
Site	0.0415*

Table 1: Null model and GLMM selection by comparison of AIC scores and Chi^2 goodness of fit. The optimal model used for this study is highlighted.

To assess the effect of forest spatial structure within-sites, each sampling point's location in relation to other features was specified by calculating distance from roadside transects *A* or *E* (*dist_rd)*, distance from interior transects *B* or *D* (*dist_intr)*, and distance from the interface transect *C* (*dist_intf).* The distances are all zero for the points on the transect being measured (i.e., on transects *A or E* the *dist_rd* will be equal to 0 for all points as they sit directly on the roadside), and additional distance variables were calculated as distance to the nearest corresponding point on the neighboring transects. For example, the fifth sampling point on transect *B* is located 5m from the application point, *dist_intr* = 0m from interior*, dist_rd*= 3.54m perpendicular to the fifth sampling point on the nearest roadside and *dist_intf* = 3.54m to the fifth corresponding point on the nearest interface. These distances were included as random effects in the model because proximity to roads and stand succession can influence pollinator behavior and may influence QD dispersal (Hopwood, 2013). To estimate the significance of random effects at a p > 0.05 level, I calculated a 95% confidence interval for each random effect, given the standard deviation and variance values from the GLMM, to see if it crossed zero (Table 2).

GLMM: Floral Availability + Site + (1|dist_rd) + (1|dist_intr) + (1|dist_intf)

Fixed Effects	**Estimate**	**Standard Error**	**t-value**	**p-value**	**Pr(>|z|)**
Intercept *(EM x High Floral Availability)*	0.2800	0.0223	12.537	2e-16***	2e-16***
Floral Availability					2e-16***
Low	0.0799	0.0211	3.790	0.0023**	
Medium	0.0112	0.0206	0.543	0.598	
Very High	-0.0417	0.0211	-1.974	0.061	
None	-0.2717	0.0152	-17.394	2e-16***	

Site Type					0.0115*
ML	-0.0271	0.0093	-2.905	0.008**	
LE	-0.0188	0.0093	-2.011	0.035*	
Random Effects	**Std. dev.**	**variance**	**95% conf. interval**		**significance**
dist_roadside	3.96e-02	1.57e-03	[1.57e-02, 1.26e-02]		p < 0.05*
dist_interface	1.62e-06	2.62e-12	[-5.80e-07, 5.80e-08]		p < 0.05
dist_interior	7.81e-02	6.11e-03	[-2.18e-02, 3.41e-02]		p < 0.05*

Table 2: Significance of attributes within each fixed effect on the QD variation found by the selected GLMM.

To investigate variation in distribution and dispersal patterns of QDs at the transect level (in comparison to the landscape-level assessed by the GLMM), two variables were developed to describe dispersal. Pollen flow weight (*Pfw*) measures the proportion of QD-labelled (pollinated) flowers out of the total flowers present averaged along a transect. Pollen flow reach *(Pfr*) measures the distance from the application point to the farthest QD-labelled flower detected on a transect. To test if the same drivers of variation within sites (transect level) remained significant as at the landscape level (site level), I calculated t-tests comparing *Pfw* and *Pfr* at each transect type and across sites (Table 3). (P-values were adjusted for multiple testing with Bonferroni corrections.) To indicate which direction QD-labelled pollen grains may disperse from a singular application point in relation to the presence and spatial arrangement of surrounding forest features, I plotted the results of *Pfw* and *Pfr* to illustrate the landscape scale implications of the results on pollen flow direction (*Pfd*). Analysis was completed in R Studio (R Core Team, 2021).

Feature	**Succession**	**Comparisons**	***Pfw* *t-value***	***Pfw* *p-value***	***Pfr* *t-value***	***Pfr* *p-value***
Roadside	Early	EM ~ LE	1.670	0.170	0.3941	0.714
	Mid	ML ~ EM	0.094	0.670	1.575	0.190
	Late	ML ~ LE	3.551	0.024*	0.2592	0.808

		Succession across sites				
		E ~ M	3.260	0.009**	5.251	0.004***
		M ~ L	0.1743	0.086	2.270	0.047*
		L ~ E	2.7562	0.020*	2.796	0.019*
Interior	Early	EM ~ LE	1.828	0.142	5.422	0.006**
	Mid	ML ~ EM	0.517	0.632	0.0056	0.264
	Late	ML ~ LE	0.350	0.744	0.0493	0.963
		Succession across sites				
		E ~ M	2.795	0.019*	2.857	0.017*
		M ~ L	2.952	0.015*	0.0427	0.967
		L ~ E	0.5912	0.568	2.508	0.031*
Interface		EM ~ LE	2.212	0.038*	0.596	0.583
		ML ~ EM	0.822	0.457	2.154	0.098
		ML ~ LE	0.3698	0.007**	3.419	0.027*

Table 3: Significance of forest features and succession stages in terns if Pf_w and Pf_r at the stand and site-levels.

RESULTS:

The GLMM results revealed that, across all sites, site type ($p < 0.01$) and floral availability ($p < 0.001$) explained the greatest variation in QD distribution (Table 2). Excluding floral availability significantly lowered models' predictive power (Table 1). Specifically, transects with Low and No floral availability had significantly lower QD density than sites with High floral availability. Site type also had a significant influence on distribution, with ML and LE sites both having significantly lower QD density than EM sites. Analysis of random effects revealed that distance from roadsides and interiors significantly affected QD variation as well (*dist_intf* had no effect).

While the GLMM captured QD distribution across all sites, pairwise comparisons at the transect level revealed dispersal patterns of pollen flow (Pf_w, Pf_r, Pf_d) supporting landscape level distributions (Figure 5 and Table 3). Within sites, Pf_w was highest, on average, in roadside transects adjacent to early successional stands (with a proportion of 0.43 QD-labelled/total flowers), LE stand interfaces (0.33), early stand interiors (0.30), and late successional interiors (0.29).

Early successional roadsides supported significantly heavier pollen flow than mid- and late-successional roadsides ($p < 0.005$), which were very similar (0.25 and 0.24, respectively). However, there were significantly more QD-labelled flowers on late-successional roadsides adjacent to early-successional stands than those adjacent to mid-successional stands (0.33 and 0.15, $p < 0.05$). Pf_w along EM and ML interfaces were not statistically different (EM=0.25 and ML= 0.18, p=0.1). Late- and early-successional stand interiors had significantly greater proportions of QD-labelled flowers (0.29 and 0.25,

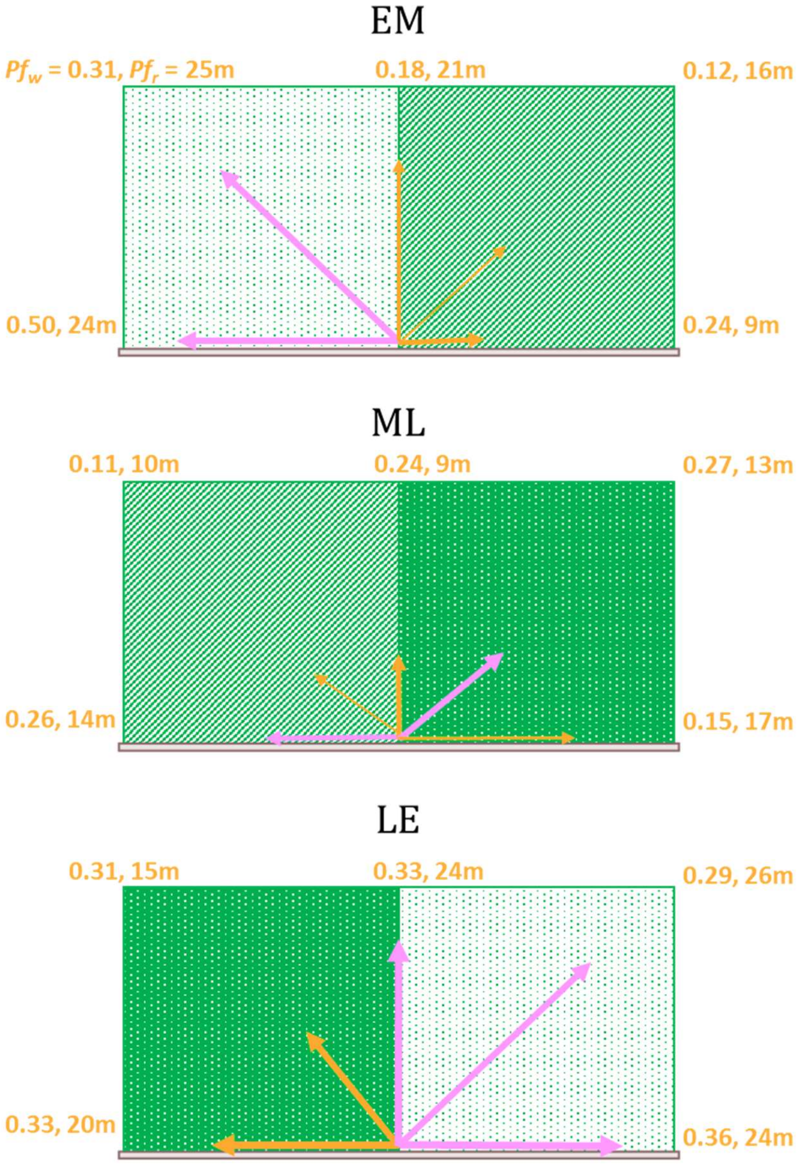


Figure 3: Pf_w is represented by the width of the arrow and Pf_r is represented by the length of the arrow. Pf_d is illustrated by the purple arrows, indicating where pollen was significantly greater in terms of Pf_w and Pf_r under different spatial arrangements of stand successional stages present at each site.

respectively) than mid-successional interiors (0.12; $p=0.015$ and $p=0.019$, respectively) and did not show variability in response to the successional stages of adjacent stands, as roadsides did.

Pf_r was highest, on average, along the LE boundary (24m) and early roadsides (23.67m), followed by early stand interiors (21.5m) (Figure 5). The LE and EM boundaries displayed significantly higher distances travelled of QDs from the application point (24m and 21m) than the ML boundary, which had the shortest distances of all transects (8.6m). QDs were found farther along early-succession roadsides (23.67m) than along late- and mid-succession roadsides (17.2m and 11.6m). They were transported greater distances into early-succession stands (21.5m) than mid- or late-successional stands (13m and 12.83m; $p < 0.05$), and significantly farther within early stands adjacent to mid than late stands (25m and 18m; $p > 0.001$).

Pf_d depended on the spatial adjacency of stands present at each site (Figure 5, Table 3). For example, if an early succession stand is located adjacent to a mid-successional stand, these results indicate QDs are more likely to travel away from an EM interface into an early stand interior or along an early-roadside than along the EM interface, into the mid stand interior, or along the mid-roadside.

DISCUSSION AND CONCLUSIONS:

Where past methods have been limited in their ability to accurately record pollination events, often equating pollinator presence to pollination, QDs address this issue by binding directly to individual pollen grains and acting as exact analogs (Minnaar and Anderson, 2019). Their persistent photoluminescence and suitability for nondestructive, in situ detection enables observation of pollen flow under entirely natural conditions. I was able to demonstrate the successful and practical use of QDs as a pollen analog in an uncontrolled natural environment to identify dispersal patterns of pollen grains in relation to various forest structures applied within a managed forest landscape. This improves upon past studies of pollen movement conducted in the lab or other controlled settings, which are unable to simulate all of the various natural factors that influence pollinator movement and pollen dispersal. Building on Minnaar's quantum box design (2019), this study also exemplified the cost-effectiveness of nanotechnology for pollination research. The most resource intensive step in this analysis was in the counting of QD-labelled anthers under a microscope, which requires minimal training.

The empirical results of this analysis suggest that the distribution and movement of pollen in managed forests is strongly influenced by the spatial adjacency and arrangement of successional stages of forest stands and road networks. At the landscape level, the concentration of pollination events within early successional stands and along roadsides is consistent with the behavior of pollinators observed in other studies (Hopwood, 2013; Rivers et al., 2018; Jordan, Hopwood and Morris, 2020). Recently harvested stands, with an open-canopy abundance of sunshine, foster early seral flora and a plethora of nesting sites within the cracks and crevices of splintering slag, abandoned burrows of other insects, birds, and small mammals and overturned ground, offering ideal habitat for many pollinators (e.g., mason, miner, carpenter, and cellophane bees) (The Xerces Society, 2023; Jordan, Hopwood and Morris, 2020). Roadsides are well documented habitat for pollinators, lined with flowers and embankments of warm, dry, soils studded with snapped stems and hollow twigs, ideal for foraging and nesting (Mader et al., 2011; Hopwood, 2013; Xerces Society, 2023).

That the abundance of floral resources present at these sites and features was the strongest predictor of pollination events is not surprising, given the mutual dependence of flowers and pollinators. However, this is the first study, to my knowledge, that has been able to identify the interacting influences of floral resources and forest succession stages on pollinators. Assuming pollination events are indicative of more pollinators, these findings support the findings of other studies documenting photophilic behavior by pollinators and demonstrates how this affects the interaction of pollinators and forest landscapes with important implications for future management.

The bivariate analysis of pollination patterns along transects, revealed new undocumented relationships of pollen flow to forest successional stages, roadsides, interiors and interfaces of adjacent stands. Within early successional stands at EM and LE sites, Pf_w and Pf_r were higher along roadsides and interiors at both site types. At the LE interface, though, pollen flow was substantially higher even though floral availability was similar to EM sites. This suggests factors other than floral availability can override pollen dispersal at the landscape level.

While this pattern of pollen flow between early- and late-successional stands, or forest pollen flow patterns in general, has not been empirically observed, existing literature on forest pollinators' foraging and nesting behaviors provide possible explanations. *Bombus sp*. prefer establishing their nests ≥100m from foraging areas in dry, sheltered soils, empty burrows or tree cavities that late successional stands supply in proximity to early stands with ample forage (Dramstad, Fry and Schaffer, 2003). *Halictidae sp*. seek rotten wood and moist areas along trickling streams found in older forest (Jordan, Hopwood and Morris, 2020). Many species of pollinating beetles (e.g., *Cerambycidae sp.* and *Buprestidae sp.*) spend their larval stages feeding on tree phloem before molting into adults that feed on floral tissues and pollen, inadvertently fertilizing flowers as they do (Jordan, Hopwood and Morris, 2020; USFS, 2020). Pollinating flies – including syrphids, bee flies, and other bee mimics – search for moist soils, stagnant water, rotting wood or other decomposing materials for their larvae to hatch and molt into flower-feeding adults (Finn, 2003; Inouye et al., 2015). Butterflies and skippers, easily observed fluttering between forage patches in large open areas, deposit their eggs on the undersides of deciduous tree leaves often in riparian buffer zones of coniferous forests (Jordan, Hopwood and Morris, 2020). After hatching, caterpillars hide in the leaf litter and some, like *Neophasia menapia*, or the Pine White butterfly (abundant in GPNF), subsist exclusively on the needles of coniferous trees (Landgren and Porter, 2023).

In addition to abundant pollination events along LE interfaces, another unstudied phenomenon suggested by these results is the damping effect of mid-successional forest on pollen flow. Within mid-successional stands, where competitive exclusion has yet to initiate natural thinning, canopies remain close-knit, blocking sunlight to a forest floor that is dominated by spore- and wind-pollinated plants that do not require rates of photosynthesis as high as insect-serviced angiosperms. Naturally, with lower floral availability and pollination demand, Pf_w within mid-succession stands, mid-roadsides, and associated EM and ML interfaces were also significantly lower, demonstrating low pollen flow at the stand level. Effects of mid-succession stands on pollen flow, however, extended beyond the individual stand to neighboring stands.

In ML and EM sites, Pf_w was significantly greater along late-successional roadsides adjacent to early-successional stands, and Pf_r was higher along early-successional interior transects of early-successional stands adjacent to mid- than late-successional forest, although floral availability was not significantly different. This indicates that floral availability is not as strong a predictor of QD dispersal or driver of pollen flow at the very fine scale (i.e., sampling points) as at the transect scale. Both responses likely reflect the lack of forage material in mid-successional forests that force pollinators to extend their search radiuses in the opposite direction to achieve adequate provisioning. Although not statistically significant, roadsides along early-successional stands with abundant floral resources also showed greater Pf_w and Pf_r when adjacent to mid- than late-successional stands, consistent with the redirecting effect of mid-successional stands on pollen flow. In terms of management, this suggests forest landscapes dominated by mid-succession forest may experience muted pollination services along roadsides and interfaces of early stands.

While the QD-tracking approach shows promise for field studies of pollen flow at the landscape scale, the process of preparing the analysis revealed opportunities for technical and methodological improvements. In the lab, separation of the QD solution into smaller doses could be improved by handling them only in a cold environment to reduce evaporation of toluene upon contact with air while transferring between vials that could affect equal concentrations. Different concentrations could introduce random, non-biased, error due to an unknown (but random and likely minute) amount of evaporation in the solutions applied at each site that could reduce the precision of statistical results. The more precise this process is made, the more sensitive the method will be at detecting patterns of pollen flow. To examine the concentration of each dose, doses could be compared via spectroscopy with a fluorescence spectrometer to ensure equal parts QDs/toluene after separation (lower fluorescence = lower concentration (Heyes, 2019)).

In the field, my original detection method was extremely labor intensive, requiring 1-10 slides prepared for every meter sampled, depending on the number of flowers present, so that I could examine them for QDs later in lab. After the first round of sampling, I adapted this method to check for presence of QDs on anthers in the field without preparing slides. While this adapted approach meant that flower anthers were not retained for future analysis, such as pollen genetics sequencing or evaluating QD density per anther rather than simply “presence or absence,” it greatly enhanced speed of collection so that transects could be sampled more evenly in terms of the amount of time pollinators had to forage and disperse pollen. Observing fluorescence in the field also reduced the number of pollen grains (i.e., potential QD detections) that could have become dislodged from anthers and lost while harvesting, preserving and transporting samples on slides for later observation.

In terms of sampling design, a better understanding of the total distance pollinators travel in managed forest landscapes (i.e., how far into a late successional stand will pollen travel from a roadside?) would likely be gained by applying QDs to the pollen carrying areas of a pollinator and then checking surrounding flowers for quantum dots to detect their pathways to or from foraging to nesting sites. From a singular point, QDs are inevitably depleted as they are transported and deposited without replacement by foraging insects. Insects likely deposit all their acquired QDs before they complete their journey any one direction. Therefore, the approach to applying QDs at a single application point may produce and underestimate the total distance travelled from the application point. Different colors of QDs could also be used to examine dispersal from the center of stands or test distance and degree to which pollinator foraging ranges between distant patches, time periods, elevations, or even between serviced floral guilds overlap.

Management Implications: In the context of a working managed forest, patterns of pollination events associated with successional stage and floral availability at the landscape level, and distinct responses of pollen flow to stands spatial adjacency at the site level indicate that forest management techniques could be readily developed to further, intentionally, support pollinators. Management approaches to this end include: developing harvest rotations that allow stands to reach full maturity and provide important nesting habitat in proximity to early stands; staggering the sizing and spacing of harvests to increase landscape heterogeneity; conducting maintenance along roadsides that exemplified high densities of pollination events before or after known pollinator active seasons and nesting periods; seeding sparse floral areas; and, perhaps, mechanically thinning mid-successional stands to increase understory regeneration and floral availability. Conservation actions like wildflower strips have been beneficially implemented in other ecosystems, including roadsides, and have proven extremely positive for local pollinator populations with minimal labor and resources required (Hopwood, 2013). The management framework for monitoring, and mitigating harm to, fauna and flora already in place in most national and large commercial forests also lends itself well to adopting pollinator conservation strategies.

Ecosystems require not only an abundance of pollinators, however, but also diversity in their plant and pollinator communities to remain ecologically stable. While the QD approach allowed me to examine distribution of pollination events, it did not allow me to identify species of pollen or pollinators. Applying knowledge of pollen flow to implement conservation strategies may require additional information at the species level. Without more detailed information about which pollinators are pollinating which plants, we may unintentionally eliminate specialist species that depend on less populated niches, like mid-succession stands, which are just as critical to maintaining diversity at a larger scale. As mentioned earlier, a foraging specialist niche, like late-successional forest interiors, may also be important nesting and rearing habitat for other pollinating insects during larval stages of their life cycles.

This study also illustrated the importance of investigating pollinator landscapes at multiple scales to capture the spatially varied behavior of pollinators and design conservation strategies that reflect this understanding. As research on forest pollinators continues to emerge, we should seek to maintain spatially, structurally, and successionally heterogenous working forest landscapes to promote pollen flow while preserving a range of habitats to sustain plant-pollinator diversity. However, this will require planning on the timescale of multiple forest successions and many more human generations. While this research helps inform management strategies to address the goals of supporting and maintaining pollinator diversity, these will goals will necessarily be balanced against other goals in forest management (e.g., wildlife conservation, watershed protection, and fire resilience). How to balance these multiple goals is beyond the scope of this dissertation.

The potential of QD nanotechnology in pollination ecology that this study demonstrates is promising and timely. As climate change continues to offset plant and pollinator phenologies (Gilman et al., 2012), tracking these interactions will become of increasing interest and importance for predicting future mismatches (particularly in agricultural landscapes) and to alleviate effects of shifting budburst and emergence times on native communities and ecosystem services.

One of many, future paths of investigation is delineating pollinator and plant communities by applying different colors of QDs to specific floral species, allowing forage time to elapse, and then recording other flower species colors are found upon and netting pollinators to dissect their pollen packages under UV light to identify their primary pollen sources. Seeding of common beneficial wildflowers, tailored to any area, could then be used to supplement where pollinator communities are declining, or habitat has been degraded. Other immediate research opportunities include assessing effects of seasonality and stand size on pollen dispersal, identifying pollinator-plant communities as described earlier, gridded application to observe true total distances of dispersal, investigating effects of other forest features like aspect, harvesting techniques (thinning, strip cutting, clearcutting, etc.), pesticide application (potentially even tagging and tracking pesticide particles similar to pollen), elevation, fragment size, or implemented in bird and bat pollinator studies and many more unexamined possibilities. The potential of QD nanotechnology to investigate landscape-level processes at the pollen-grain resolution is revelatory in the fields of spatial and pollination ecology and likely to become imminently important to protecting global pollination systems.

Chapter 4: The Presence of Wild Bees in Response to Alternative Forest Harvesting Regimes

INTRODUCTION:

Preservation and protection of wild pollinators is increasingly important as their populations continue to dwindle due to unsustainable land management practices, including large-scale monocropping and use of systemic insecticides (Kudo and Cooper, 2019; Dicks et al., 2021). Native bees are highly sensitive to habitat loss and critical to protect as they are the most indispensable group of pollinators and their diversity is intrinsic to sustaining the biological and ecological resilience of ecosystems at both local and global scales (Brosi, 2009; Wei et al., 2021; Xerces Society, 2023). Globally, native bees are estimated to pollinate nearly seventy-five percent of all flowering plants and significantly increase crop yields (Committee on the Status of Pollinators in North America, 2007; Hoehn et al., 2008; Rogers, Tarpy and Burrack, 2014). Once pollinator services and diversity are lost, it is uncertain whether a system can naturally regain them. Developing management strategies that protect wild bees in the places where they still exist in wild populations is, therefore, of the utmost importance. Among those places are forests.

While over two thirds of the forests in the U.S. are designated as "timberland," managed for the purpose of lumber production, they still provide large swaths of natural habitat for native bees (Klein et al., 2018; Mullaly et al., 2019; Hoover and Riddle, 2021). A plenitude of wildflowers burgeoning in early seral stands and dry soil embankments along logging roads provide foraging and nesting habitat, and forests' rural locations buffer the effects of urban light pollution, agricultural pesticides, and incursions of invasive ornamental plants (Park et al., 2015; Hanula, Ulyshen and Horn, 2016). Incorporating pollinator conservation strategies into forest management practices is a sagacious next step.

Production in managed forests is often assessed and managed at the level of landscapes (i.e., in terms of how many board feet produced per year from some geographic area) (Arnold, 2017). However, decisions of where and when to harvest are made at the stand level on a schedule of production and regeneration. As such, managed forests have come to present a unique, artificially generated patchwork of naturally regenerating succession stages, blanketing large expanses of forested land, concurrently governed by patterns of recovery driven by natural disturbances, like wildfire, defoliator damage, and wind throws (Shorohva et al., 2023). Within this checkered landscape, there are many factors influencing pollinator distribution (e.g., vegetation, precipitation, rain shadow, etc.), but the most salient at the forest landscape level are stand successional stages and the spatial arrangement of stands (e.g., large, small, clustered, dispersed).

Studies have examined wild bees' responses to specific features like canopy openings, suburbanization, nearby agriculture, and even their vertical canopy distribution (Ulyshen, Soon and Hanula, 2010; Carper et al., 2014; Roberts, King and Milam, 2017; Rahimi, Barghjelveh and Dong, 2022). The situational and sometimes species-specific scope of these studies, while adding valuable knowledge on forest bees, can be difficult to interpret and apply in practice at the scale of managed forest landscapes, and therefore does not provide guidance on specific, tangible conservation actions. The ability to estimate native bee occupancy at a resolution fitted to the landscape and in direct response to management scenarios would be incredibly useful for providing actionable conservation practices. The objective of this study, therefore, is to estimate the spatially occupied niches of wild bees at the scale of forest landscapes to evaluate their prevalence in response to alternative harvesting regimes and consider best practices.

Due to their size and solitary behavior, detecting pollinators in the wild is especially challenging and makes estimating their natural persistence patterns and the effects of various anthropogenic impacts difficult. Sampling techniques attempt to account for the difficulty of detection by repeated or staggered sampling events and multiple trapping methods, but it is still impossible to know how many species or individuals of each species went undetected (Welsh, Lindenmayer and Donnelly, 2013; Nogeire et al., 2013; MacKenzie et al., 2017). Unlike larger organisms, where we may know their established ranges and approximately how many individuals coexist in a habitat or territory, we do not have an estimated carrying capacity of pollinators in forest systems or even comprehensive lists of pollinators that may be present in most places. Furthermore, pollinators that are the rarest are often also the most difficult to detect, and their rarity can become amplified if modeling strategies lean on abundance as a predictor or do not account for uncertainty associated with detection (Gould et al., 2019).

To develop an understanding of wild bees' responses to landscape-scale management scenarios, I estimated the spatial habitat niches and distributions of native bees in managed forests using occupancy and detection models that incorporate the estimates for undetected and rare occurrences of wild bees, as well as the most abundant and common. Occupancy models allow for estimation of pollinator presence while taking into account the uncertainty and variability in ease of detection between genera (Mosher et al., 2019; Strimas-Mackey et al., 2020). This is achieved by relying on repeated surveys, wherein each visit represents a potential for detection rather than an absolute measurement of present or absent, which lends itself to estimating the probability of occupancy over time and across space (Welsh, Lindenmayer and Donnelly, 2013). As pollinators are highly sensitive to environmental factors, repeat visits under varying conditions are necessary to encounter as many of the pollinators present in an area as possible. Even so, some may be missed due to trapping method, observer error or chance, especially when considering pollinator distributions over highly heterogeneous forest landscapes.

No study to date has estimated the spatial habitat niches of wild bees across managed temperate forest landscapes. This is likely due in part to the intensive field work required to gather sufficient data for a landscape-scale study, and the diminutive size of pollinators versus their extensive ranges and sensitivities to environmental factors, which necessitates a multiscale analysis. To fill the need for studies that span the scales of pollinators' persistence and temperate forest landscapes, I examined the presence of wild pollinators in terms of managed forest features to understand how pollinator occupancy is affected by landscape level management decisions. Estimating pollinator occupancy in response to forest management allows us to investigate explicit outcomes of alternative management scenarios at the landscape level based on observed habitat niches of individual pollinator genera. To this end, I aimed to understand:

1. How do the spatial probabilities of the presence of native bee genera vary in response to site-level variables in managed forests?

Following principles of competitive exclusion and niche partitioning (Sargent and Ackerly, 2008; Wei et al., 2021), I expected occupancy probabilities of bee genera to be spatially dispersed according to the floral resources and nesting conditions facilitated by managed forests (Pyke, 1982; Wei et al., 2021). At the landscape scale, stand-level forest features can encompass a range of pollinator habitats (e.g., within a tenth-year stand, habitat varies greatly from edge to center). Spatial variation of these features can affect pollinator habitats at a finer proximal scale of interaction. Therefore, while pollinators co-occur within stands, their presences are likely partitioned at much finer scales, evident through comparative effects of landscape-level change.

2. How do occupancy probabilities compare under different forest harvesting regimes? And, how might each genus be expected to respond to alternative harvesting scenarios?

As harvest regimes alter landscape heterogeneity, degree of fragmentation, stand arrangement and composition, the probability of occupancy by genus will likely shift to reflect changes in the distributions of resource provisioned by different harvesting regimes (Winfree et al., 2009; Ferreira et al., 2015). Genera composed of predominantly polylectic (i.e., generalist) species may be relatively unaffected by alternative harvest regimes while those comprised of more oligolectic (i.e., specialist) species will be more sensitive to differences in regimes (Brosi, 2009; Botsch et al., 2017). How each genus responds to alternative management scenarios can help inform future conservation strategies for native bee populations.

3. Which management regimes support the greatest richness of wild bees over a full rotation of regeneration and harvest?

Following the principle of coevolutionary mutualism of plants and pollinators, more complex floral communities often support greater richness of wild bees (Cardinal and Danforth, 2013; Heller et al., 2019; Wei et al., 2021). As plant diversity and forest structural complexity go hand in hand, similar relationships might hold between pollinators and landscapes (Ehbrecht et al., 2021; Ma et al., 2022). Forest landscape complexity is often measured by such characteristics as patch size distribution or edge-to-interior ratios, i.e., rugosity. Therefore, smaller stand sizes that increase landscape complexity, supporting diversified communities of floral resources, may also support richer communities of wild pollinators. However, pollinator species have evolved to use the varying resources present within forest landscapes. Therefore, a threshold of fragmentation may be reached wherein pollinators that depend on unfragmented forest may decline and lead to a loss of total richness (Botsch et al., 2017; Librán-Embid et al., 2021; Dodonov and Cazetta, 2023). I expect managed forest landscapes that balance recent, recovered and regenerating stages of stand successions across the landscape and its longevity will likely support the greatest richness of wild bees over multiple harvest rotations.

4. How can management be improved to sustain pollinator occupancy and richness across an operational forest landscape?

Considering the plasticity of pollinator behavior and successional heterogeneity inherently provided by managed forests, managing for pollinators may not require drastic changes to current timber harvesting practices. At the landscape level, though, low-intensity actions could have much more drastic effects at smaller scales, the percolating effects of which should be monitored.

METHODS

Wild Bee Dataset: From the large, landscape-scale study of insect pollinators outlined in Chapter 2, I identified twenty-one genera of wild bees. The study was conducted in Gifford Pinchot National Forest (GPNF), a 1.3million acre temperate coniferous forest in Washington state, managed by the U.S. Forest Service and Port Blakely Logging company (National Forest Foundation, 2023). These forests have been considered as potential reservoirs of wild pollinator diversity, enhancing interest in understanding the various habitat niches pollinators occupy and their responses to management (Rivers et al., 2018; Hanula, Ulyshen and Horn, 2016; Roberts, King and Milam, 2017; Romey et al., 2007). Pollinators were collected using a network of 39 blue vane traps (BVTs) dispersed across an array of abiotic and biotic environmental conditions facilitated by various successional and structural features in managed forests, including stand successional stage, adjacency of successional stages, transition zones between stands, and proximity to roadsides. Traps were collected twice per month from June-August for three years (2020 – 2022). From this extensive dataset, all genera of wild bees were extracted for a finer-scale analysis of their niche occupancy and spatial distribution.

Single-season Single-genus Occupancy Models: To determine the spatial niche of each genus, described at the forest stand level, I used single-season, single-genus occupancy models (Welsh, Lindenmayer and Donnelly, 2013). Occupancy models estimate the probability of a given organism or taxonomic group's occurrence (sometimes referred to as "prevalence") at a location or under certain conditions based on its recorded presence/absence on past occasions (MacKenzie et al., 2017; Cove, 2011). Repeat visits allow it to also provide an estimate of detection, which quantifies uncertainty regarding actually observing or otherwise recording an organism if it is present, to evaluate the reliability of estimated probabilities of occupancy (MacKenzie and Bailey, 2004).

Detection estimates of data based on abundance with sparse counts, however, can be biased estimates towards extremes of detectability outcomes (1=100% probability of detection or 0=never present) (Welsh, Lindenmayer and Donnelly, 2013). As my data carry many instances of nondetection in mid and late stands, I excluded detection as a post-analysis metric. Quantification of uncertainty is inherently captured in the framework of occupancy modeling by relying on repeated visits with detection/nondetection data that account for false absences so the theoretical importance of detection in estimating occupancy is still captured (Strimas-Mackey et al., 2020; Mosher et al., 2019). Occupancy models are also particularly advantageous for studies encompassing large areas with constrained sampling, such as this. They also account for missed or misidentification of organisms, to which insect studies are particularly prone, given most insects' miniscule size and often microscopic discrepancies between species (MacIvor and Packer, 2016; Bailey, MacKenzie and Baileys, 2004; Gould et al., 2019).

Site-level covariates included stand succession (early 0-15 years; mid, 15-40 yrs; late >60 yrs), stand adjacency (early adjacent to mid, abbreviated EM; mid adjacent to late, ML; and late adjacent to early, LE), transitions zones between stands (EM-transition zone, abbreviated EMtz; ML transition zone, MLtz; and LE transition zone, LEtz) and distance from road (0-5m, 5-15m, 15-30m, and 50-100m) that correspond to the criteria used to collect data and describe managed forests in Chapter 2. 'Month' was retained as a sampling covariate to account for natural fluctuations in pollinators' phenology patterns. Distance from road was standardized (z-transformed) prior to input, following best practices for continuous variables with different units in occupancy models, and all other variables were assessed categorically (Cove, 2011 and 2020).

Binary presence (1) and absence (0) data for genera were compiled from the data collected in Chapter 2 by aggregating all observations at each trap-sampled location. This provided an exceptionally rich dataset of eighteen “present” or “absent” observations at 39 uniquely characterized forest locations. This was advantageous for three reasons. First, occupancy modeling assumes a “closed” system, meaning that the organisms are not expected to enter or leave over the duration of the study (MacKenzie et al., 2017). Combining all observations over months and years was consistent with this assumption without restricting pollinators’ presence due to temporal variation. Second, the repeat visits over the active season provides a richer dataset of pollinator occupancy than seasonally specific measurements would provide, with each site being visited eighteen times over the duration of the study (Mosher et al., 2019). Third, compiling pollinator sampling events over the season allowed me to retain a temporal scale of analysis consistent with the resolution of decision-making in forest management, which obscures the seasonal phenology of pollinators.

For each genus of wild bee, the best-fit model of predicted occupancy (ψ) and probability of detection (p) considering all sampled locations were tested additively and then deductively to arrive at a model with the least number of coefficients and lowest Akaike Information Criterion (AIC) score. From the selected AIC, the weighted AIC (AIC_w) was calculated to examine models’ predictive power (Wagenmakers and Farrell, 2004; Burnham and Anderson, 2004). The same fitting process was repeated for each genus at the sampled location level to estimate likelihood of occupancy at each of the 39 uniquely identified locations in the forest. Occupancy modeling was conducted using the software program PRESENCE (MacKenzie et al., 2017; Mosher et al., 2019).

<u>Forest Harvesting Scenarios:</u> Next, I used the estimated occupancy of each genus at every sampled location, to predict their probability of presence across landscapes under three forest harvesting scenarios. All scenarios were applied across a 250,000-acre virtual landscape, approximately a quarter of the area managed for logging in GPNF (National Forest Foundation, 2023). The three scenarios varied in the average size of stands used for harvest: ***Scenario 1*** represented large-stand harvests with harvested stands of 350 acres; ***Scenario 2*** employed moderately sized harvest stands of 100 acres, corresponding to the average stand size observed in GPNF; and ***Scenario 3*** used small-stand harvests of 50 acres. Stand sizes of each scenario were

determined from timber records kept by the U.S. Forest Service in GPNF since the late 1800s, binned into three categories using Jenks natural breaks classification and averaged by class (US Forest Service, 2023). Scenarios were simulated in a virtual landscape generator, *Landscape Generator,* with a cell size of 50 acres (~450 m on a side) for ***Scenarios 1*** and ***2***. For ***Scenario 3***, cell sizes of 12.5 acres (~225 m on a side) were used to allow for different configurations of 50-acre stands (Slager and de Vries, 2013).

Landscape simulations for each scenario were run over a 100-year period, approximately the time required for a harvested stand to fully regenerate from early seral to late succession stage (Smallidge, 2016; Franklin and Hemstrom, 1981; Powell, 2012). Landscapes were created with stands in 20-year increments. The starting states of each scenario contained equal areas of early, mid and late succession forest (defined as < 20 y.o. stands, 20-80 y.o. stands, and >80 y.o. stands, respectively) with the only discerning attribute being minimum and maximum stand size. The starting arrangement of stands for each scenario were tested in *Landscape Generator*, and selected for those that yielded the most harvested area over a century of management. Twenty-year timesteps were implemented to simulate and analyze stages of regeneration within a full harvest rotation that allowed stands logged at the first timestep to fully mature and be reconsidered for harvest by the last step.

At each step, the simulator attempted to harvest 55,000 acres of forest given the stand size specifications and limitation of late succession forest eligible to harvest. Following the natural cadence of forest succession, early stands fully transitioned to mid succession after one 20-year timestep, and then remained in mid succession state for the next three timesteps – undergoing reestablishment, competitive exclusion and understory opening – before being considered as late succession forest on the fifth time step (Ministry of Forests Research Program, 1998; Smallidge, 2016). Stands retained late succession status for at least one timestep before being reconsidered for harvest. Each landscape raster was exported to ArcGIS Pro v.3.1 (Esri, 2023).

To incorporate distance from roads, I created a map of forest roads for the first timestep of each scenario by delineating the shortest distance line between recently harvested stands, following existing stand edges. I added spurs for new harvests as needed at each subsequent timestep. Using these roads as input, I then created Euclidean distance rasters to identify the shortest distance between each cell of each landscape and the nearest road. Rasters were generated at 5 m^2 resolution and reclassified at radiating distances from roadsides by 0-5 m, 5-15 m, 15-30 m, 30-50 m and > 50 m to match the distance intervals sampled in the original dataset.

Assigning Bee Occupancy: Landscapes were rasterized to a cell size of 5 m^2 to align with the sampling design used in Chapter 2 and joined with corresponding road proximity rasters. Cells were initially assigned an identification value to label how their characteristics aligned with the areas sampled by the insect traps described in Chapter 2. For example, all cells in early-succession forest adjacent to a mid-succession stand and 0-5m from a roadside were assigned an initial value of *EM_early_0m* ("*site*"_"*succession*" _"*roadproximity*"). The contents of each trap used to sample this area was subsequently used to estimate each genus' occupancy and attach a probability of occurrence to each sampled location (or cell type). Then the predicted occupancy of each genus calculated from the trap situated within, for example, *early-mid adjacent, early succession stands and 0-5m from a roadside* was joined to these cells. This process was repeated for all other locations/cell types.

When stands were neighbored by different succession stages on either side, the "middle" stand was split between effects of adjacent stands. Transition zones were set to span a 50 m buffer between stands (extending 25 m, into each adjacent stand). For example, where an early- and mid-succession stand met along a roadside, five cells (25 m) to the left and right of this vertex and 1 cell (5 m) towards the interior were assigned a value of *EM_tz_0m*; cells 5-15 m from the road were assigned value *EM_tz_5m*, etc.

Where stand interiors extended beyond 100m, the value of the last cell on the transect was extended to the center of the stand. This assumes that occupancy probability remains the same from 50m to center of stands. This assumption is consistent with findings from pollinator studies that extend into mid or late succession temperate forests and indicate a steep, overall, decline in pollinators beyond 30m as edge effects weaken (Ewers, Bartlam and Diham, 2013; Jackson, Turner, and Pearson, 2014; McKechnie, Thomsen and Sargent, 2017; Mullaly et al., 2019; Odanaka and Rehan, 2020). Indeed, of the locations sampled at 50m in mid or late succession stands, in this study, only 4.7% contained records of a bee present and, of these, none exceeded more than two occurrences out of eighteen visits. In early-succession stands, edge effect was present along roadsides but appeared, from analysis in Chapter 2, to stabilize in terms of pollinator abundance and diversity by 15 m within the stand.

Once each cell was assigned a full identity (site type, succession stage or transition zone, and distance from road), the probability of occupancy per genus per cell type was projected over each landscape to create a prediction surface of prevalence for each genus (MacKenzie et al., 2017). Genera exhibiting < 50% likelihood of occupancy at a certain cell type were considered lost from that location, although they could still occur elsewhere on the landscape and later in the scenario (i.e., they were not deemed extinct) (Liu et al., 2005). Cells considered "occupied" were summed and multiplied by 5 m^2 to estimate the occupancy per genera per landscape per scenario as well as wild bee richness.

RESULTS

Habitat niches of native bees varied at the coarse level of forest stands by site-level covariates over the landscape (Table 1). Covariates that provided the most explanatory power at the landscape level, revealed finer levels of niche delineation when considered at the site level.

At the landscape level, predicted occupancies (ψ_{bee}) that describe the likelihood of each genus' presence considering all locations, were naturally lower than when calculated for specific sampled locations (if a bee was present). The vast majority of area within mid and late succession stands and along their transition zones and roadsides did not support a significantly high probability (or any) of occupancy. Of the 819 predicted likelihoods of occupancy (39 locations per each of 21 genus) 67.5% had a value of zero and the rest fell between 0.056 and 0.966 probability of occupancy.

Common name *Scientific name*	Best-fit model ψ_{bee}(site covariate), p_{bee}(sampling covariate)	AIC$_w$	K	ψ_{bee}(SE) (predicted occupancy)	p_{bee}(SE) (probability of detection)

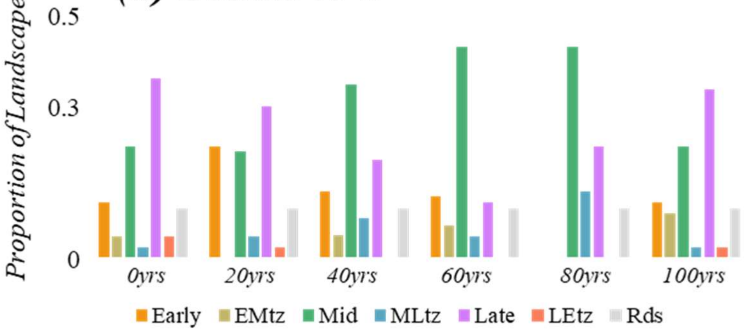
Striped Sweat bee *Halictidae Agapostemon sp.*	$\Psi_{agapostemon}$(site + succession), $p_{agapostemon}$(month)	0.417	8	0.041(0.021)	0.241(0.023)
Miner Bees - Andrena *Andrenidae Andrena sp.*	$\Psi_{andrena}$(succession), $p_{andrena}$(month)	0.533	5	0.257(0.048)	0.433(0.026)
Woolcarder bee *Megachilidae Anthidium sp.*	$\Psi_{anthidium}$(site + succession), $p_{anthidium}$(.)	0.550	8	0.124(0.036)	0.504(0.038)
Digger bee *Apidae Anthophora sp.*	$\Psi_{anthophora}$(site + succession + roadside), $p_{anthophora}$(.)	0.480	8	0.074(0.028)	0.336(0.051)
Bumble bee *Apidae Bombus sp.*	Ψ_{bombus}(succession + road proximity), p_{bombus}(.)	0.661	10	0.395(0.054)	0.604(0.022)
Carpenter bee, small *Apidae Ceratina sp.*	$\Psi_{ceratina}$(succession + road proximity), $p_{ceratina}$(.)	0.609	8	0.384(0.056)	0.584(0.041)
Cellophane bee *Colletidae Colletes sp.*	$\Psi_{colletes}$(succession + road proximity), $p_{colletes}$(month)	0.702	13	0.231(0.048)	0.327(0.027)
Longhorn bee - Eucera *Apidae Eucera sp.*	Ψ_{eucera}(succession + road proximity), p_{eucera}(month)	0.627	10	0.200(0.040)	0.443(0.041)
Furrow bee *Halictidae Halictus sp.*	$\Psi_{halictus}$(site + succession + road proximity), $p_{halictus}$(month)	0.596	13	0.021(0.013)	0.294(0.025)
Longhorn bee - Hoplitis *Megachilidae Hoplitis sp.*	$\Psi_{hoplitis}$(succession + road proximity), $p_{hoplitis}$(month)	0.519	13	0.059(0.026)	0.080(0.0020)
Masked bee *Colletidae Hylaeus sp.*	$\Psi_{hylaeus}$(site + succession + road proximity), $p_{hylaeus}$(month)	0.324	11	0.074(0.030)	0.024(0.0012)
Sweat bee *Halictidae Lasioglossum sp.*	$\Psi_{lassioglossum}$(site + succession + road proximity), $p_{lasioglossum}$(.)	0.788	13	0.307(0.049)	0.386(0.0278)
Leafcutter bee *Megachlidae Megachile sp.*	$\Psi_{megachile}$(succession + road proximity), $p_{megachile}$(month)	0.489	8	0.278(0.047)	0.598(0.033)

Longhorn bee – Melissodes *Apidae Melissodes sp.*	$\Psi_{mellisodes}$(site + succession), $p_{mellisodes}$(month)	0.650	6	0.247(0.049)	0.404(0.059)
Nomad bee *Apidae Nomada sp.*	Ψ_{nomada}(succession + road proximity), p_{nomada}(.)	0.507	10	0.056(0.023)	0.223(0.082)
Mason bee *Megachlidae Osmia sp.*	Ψ_{osmia}(succession + road proximity), p_{osmia}(month)	0.527	8	0.261(0.047)	0.327(0.027)
Fairy bee *Andrenidae Perdita sp.*	$\Psi_{perdita}$(site + succession), $p_{perdita}$(.)	0.460	6	0.030(0.018)	0.362(0.025)
Miner Bee - Pseudoparnurgus *Andrenidae Pseudoparnurgus sp.*	$\Psi_{perdita}$(site + succession), $p_{perdita}$(.)	0.608	8	0.169(0.041)	0.292(0.060)
Blood bee *Halictidae Sphecodes sp.*	$\Psi_{sphecodes}$(succession + road proximity), $p_{sphecodes}$(month)	0.373	10	0.058(0.025)	0.239(0.032)
Cuckoo bee *Apidae Triepolus sp.*	$\Psi_{triepolus}$(site + succession), $p_{triepolus}$(.)	0.382	6	0.027(0.014)	0.227(0.058)
Large Carpenter bee *Apidae Xylocopa sp.*	$\Psi_{xylocopa}$(site + succession + road proximity), $p_{xylocopa}$(month)	0.570	11	0.114(0.036)	0.218(0.054)
Landscape Null model	Ψ_{all_bees}(.), p_{all_bees}(.)	0	0	0.7436(0.0494)	0.732(0.019)

Table 1: Habitat niches of wild bees characterized by forest management practices identified in a landscape-scale study of GPNF. Covariates contributing to the best-fit model for each genus of wild bee at the landscape-level are given in the second column by ψbee(site covariate), $p$bee(sampling covariate), accompanied by the weighted AIC (AIC_w) score for each model, the number of parameters (K), and each genus' predicted occupancy and probability of detection across all sampled locations. .

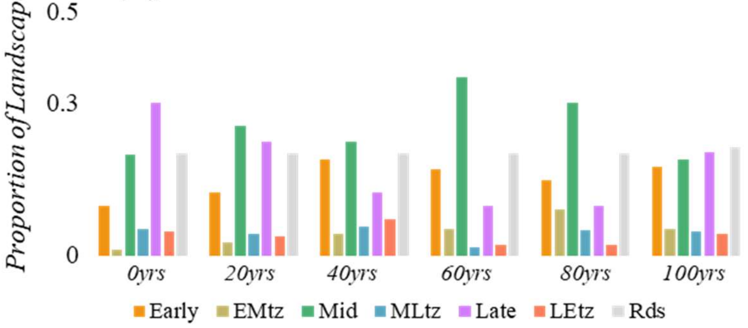
The structural composition of landscapes in each scenario varied, corresponding to greater or lesser area of acres harvested over each scenario (Figure 1). Overall, while ***Scenario 1*** and ***3*** exhibited greater areas of acres harvested at some timesteps, ***Scenario 2*** yielded the greatest acreage harvested over the complete harvest regime

(a) Scenario 1



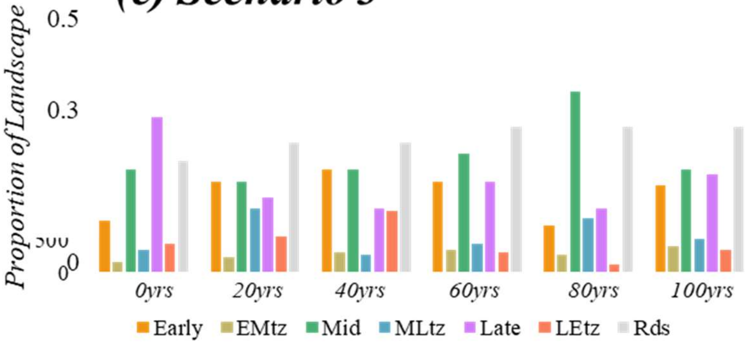
Acres Harvested: 170,500 acres
Max. Road Length: 290 km
Bee Richness: 14 – 21 genera
(min-max)

(b) Scenario 2



Acres Harvested : 240,500 acres
Max Road Length: 842 km
Bee Richness: 21 genera

(c) Scenario 3

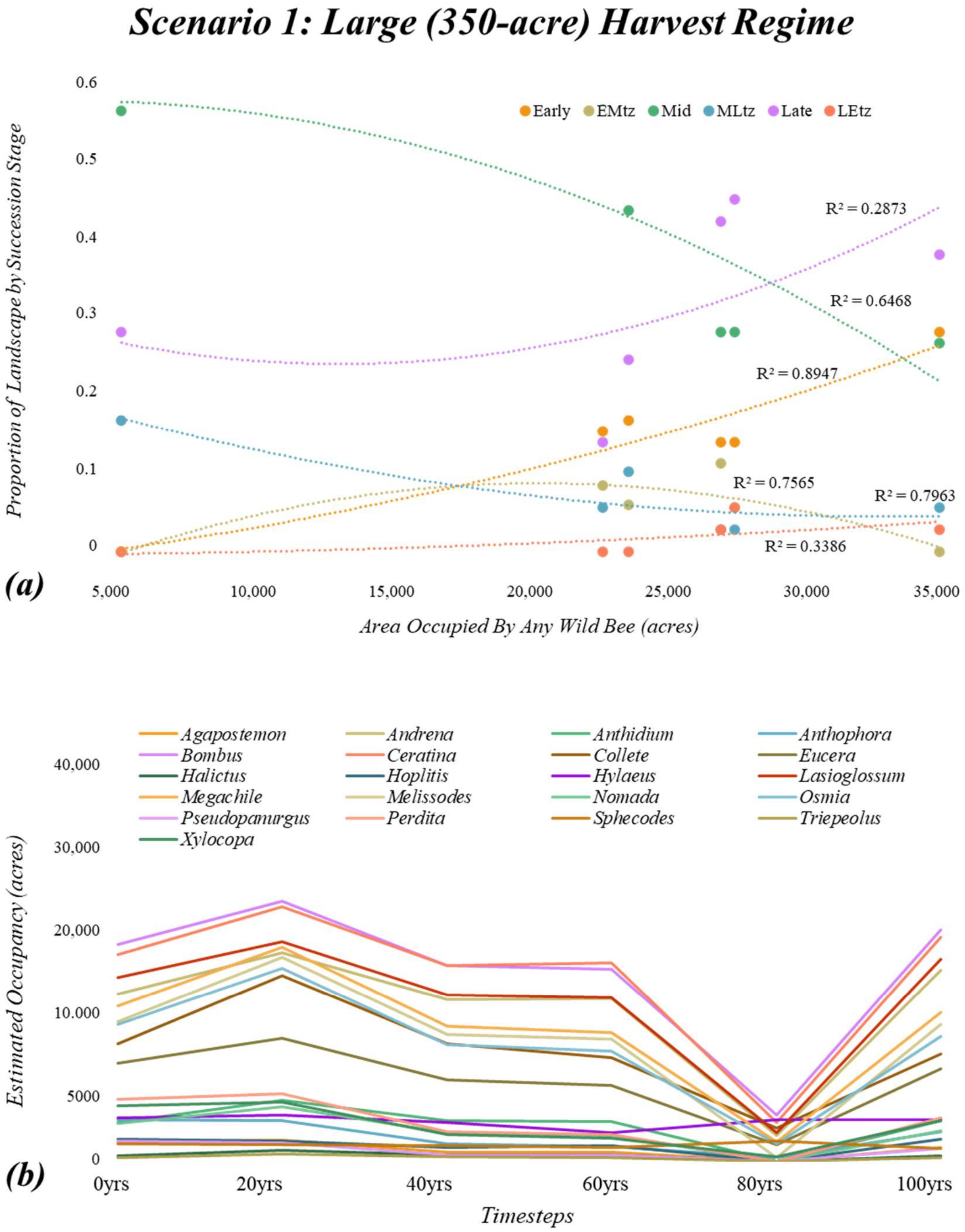


Acres Harvested : 226,000 acres
Max. Road Length: 990 km
Bee Richness: 21 genera

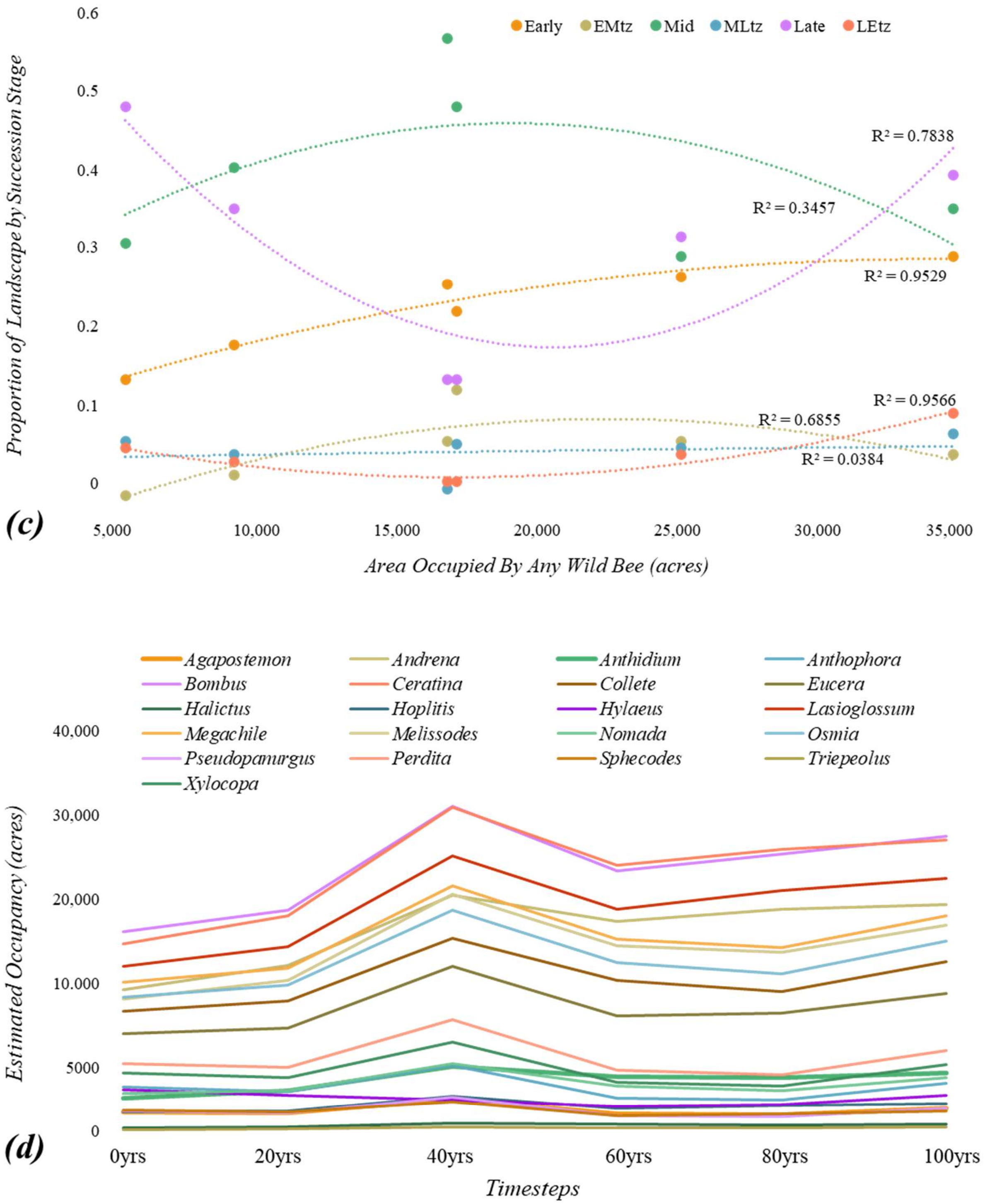
Figure 1: Landscape compositions of each timestep and scenario

The estimated area of the landscape occupied by each genus shifted in response to changes in landscape compositions introduced by each harvesting regime ((Figure 2).

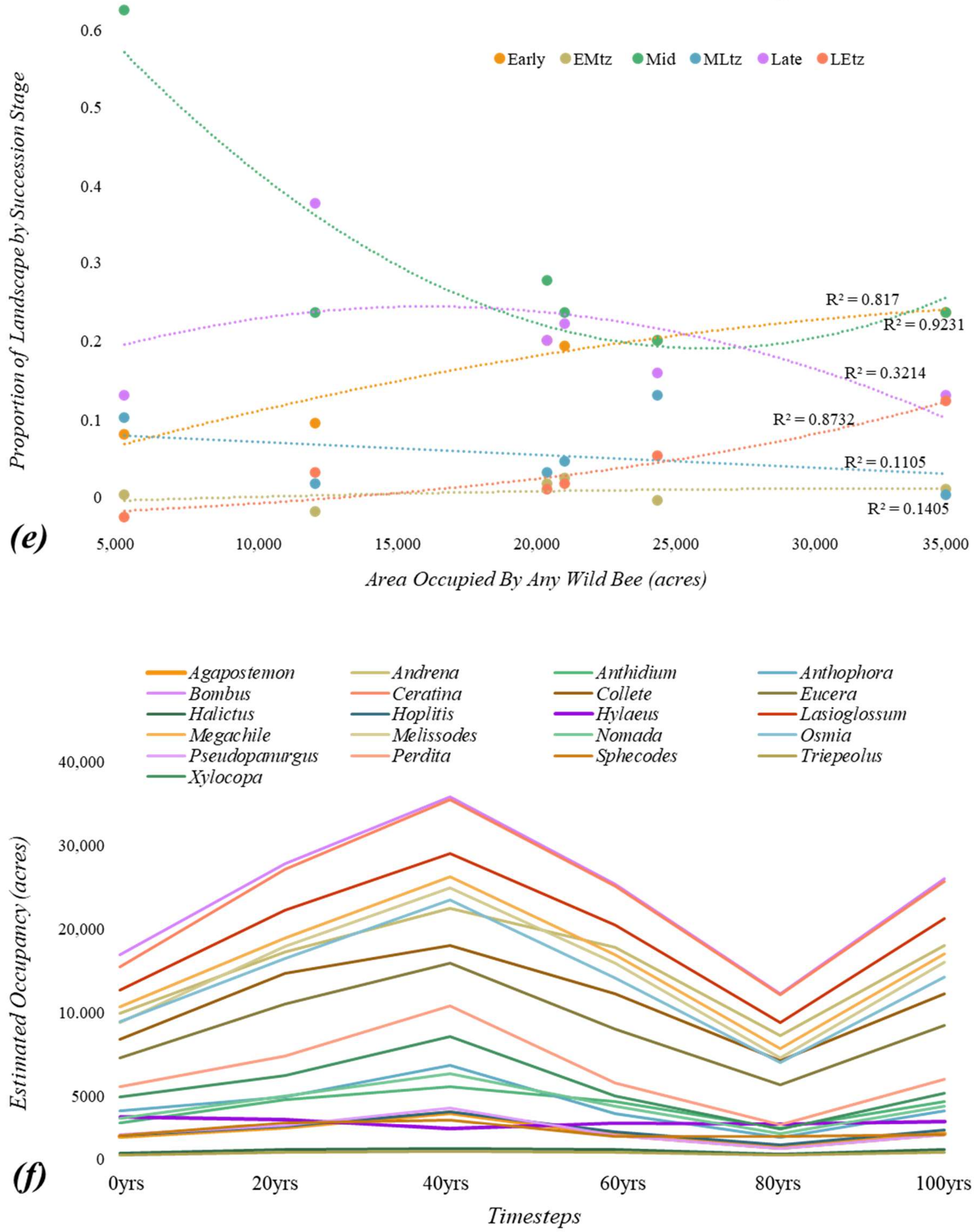
Figure 2: (a, c, e) Scatterplot with best-fit lines of landscape composition (% of landscape in each succession stage) versus total area occupied by any genera of wild bee for each of three harvest scenarios; (b, d, f) Estimated landscape occupancy (acres) of wild bee by genus for each of three harvest scenarios.



Scenario 2: Moderate (100-acre) Harvest Regime



Scenario 3: Small (50-acre) Harvest Regime



In ***Scenario 1***, the total area occupied by any genera of wild bee was positively related to the proportion of early succession forest present across the landscape ($R^2 = 0.89$) and negatively related to the proportion of mid-succession forest ($R^2 = 0.65$). Transition zones between these stands were positively correlated with greater bee presence ($R^2 = 0.76$) while the transition from mid- to late-succession stands signified a decline in occupancy ($R^2 = 0.80$). The proportion of mid- and late-succession stands did not show significant association with bee occupancy positively or negatively due to low abundance of bees observed in these stands. LE transition zones displayed a slight increase in occupancy on the landscape though not as strongly as other locations. Most genera followed similar patterns of presence, and estimated acreage, tied to the proportions of early and mid-succession stands. This is particularly evident at the 80yr timestep when the landscape lost presence of early succession forest and became dominated by mid succession (Figure 1(a)) and nearly all genera exhibit a synchronized shift to lower occupancy as well. Some – like *Agapostem*on *sp., Anthidium sp., Halictus sp., Hoplitis sp., Nomad sp., Pseudopargnasus sp.* and *Perdita sp*., that were only found (with > 50% occupancy) in areas association with early succession forest – disappeared entirely (Figure 2(b)). Other genera, which are much rarer and less abundant – like *Triepoeolus sp.* – were relatively unaffected. Interestingly, a few – i.e., *Hylaeus sp*. and *Sphecodes sp.* – exhibited a slight increase in occupancy.

In ***Scenario 2***, genera sustained much more stable levels of occupancy across the scenario (Figure 2(c)). Harvest patterns were also less drastic in terms of large shifts in successional composition (Figure 1(b)). For example, unlike in ***Scenario 1***, harvesting occurred at every timestep – maintaining the presence of early seral habitat throughout the scenario. At the 40-year timestep, all genera (excepting *Halictus sp*. and *Triepolus sp*.) showed an increase in landscape occupancy. This is in tandem with a comparative rise in early- and late-succession forest and consequently LE transition zone habitat area (Figure 1(b)). Although a similar harvesting bottleneck and build-up of maturing mid-succession stands was observed around the 60- and 80-year timesteps (as in ***Scenario 1)***, bee occupancy remained relatively stable, and all genera were present at all time steps. Pollinator occupancy exhibited extremely strong correlation with the proportion of early succession ($R^2 = 0.95$) and LE transition zones ($R^2 = 0.96$), as well as late succession ($R^2 = 0.78$) and EM transition zone ($R^2 = 0.69$). The amount of roadside habitat nearly tripled from ***Scenario 1*** to ***2*** (290 – 842 km), which may also play a factor in the increased stability of native bee occupancies (Figure 3).

At the landscape level, roadside habitat (calculated as any area extending ten meters (two cells) from a road, on either side) was not highly correlated with occupancy, although it was an important covariate for many genera (Figure 3). Broken down by habitat type though (e.g., mid or late successional roadsides) the amount of early succession ($R^2 = 0.70$) and LE transition zone roadsides ($R^2 = 0.78$) were strongly correlated with pollinator presence (Figure 3).

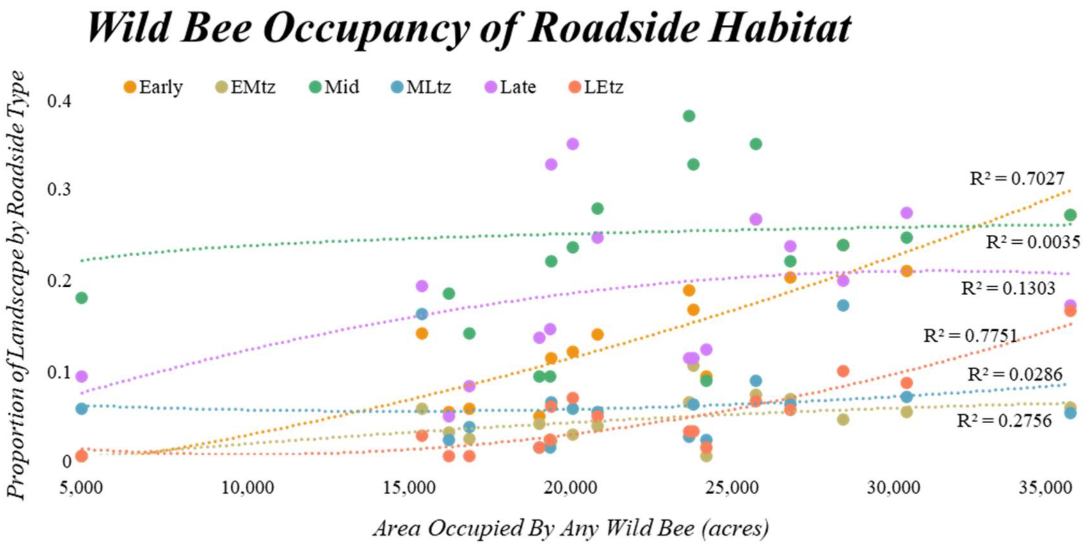


Figure 3: Roadside habitat and occupancy of wild bees across all scenarios

In ***Scenario 3***, smaller stand sizes (i.e., increased heterogeneity) resulted in a denser road network needed to service these stands (from 842 km, ***Scenario 2***, to 990 km). The estimated occupancy of each genus on the landscape was initially higher than other scenarios, reaching the greatest total occupancy of any landscape at the third timestep (Figure 2(f)). Similar to ***Scenario 1***, though, occupancy steeply declined approaching the 80-year timestep when presence of early succession diminished and mid-succession forest became the dominant succession stage (Figure 1(c)). No genus, however, was lost from the landscape as in the large-harvest scenario. Early succession and LE transition zones were positively associated with occupancy ($R^2 = 0.92$ and 0.87, respectively) although late-succession stands were not as strongly associated ($R^2 = 0.32$). The proportion of forest in mid-succession had a sweepingly negative effect on the estimated presence of wild bees ($R^2 = - 0.92$).

Within each scenario, acres harvested and presence of wild bees were strongly correlated ($0.82 < R^2 < 0.95$). ***Scenarios 2*** and ***3***, also exhibited greater bee richness with all twenty-one observed genera appearing at every timestep and individually occupying greater areas of the landscape than ***Scenario 1***.

DISCUSSION

As wild bees and the critical ecosystem services they provide continue to dwindle, understanding variation of their prevalence and the habitat niches they occupy across managed forests is increasingly important for large scale management and conservation (Klein et al., 2018). While theories of niche partitioning and evolutionary mutualism collectively imply that genera occupy specific and differentiated niches within forest systems, this is the first study to investigate what these niches may be at the applicable scale of forest management and the potential outcomes of alternative harvesting scenarios.

The presence and proportion of area occupied by genera fluctuated under different harvest regimes in response to the availability of habitat types supplied by various successional stages and arrangements. Given the variation in the resources these various areas provide (i.e., nectar, pollen, dry soil, hollow stems, etc.), wild bee spatial niches can vary according to their nesting, foraging, and subsistence behaviors. As the landscape shifted from a successionally heterogenous environment to low acreage of early successional forest and majority mid-succession forest in the first scenario (Figure 1a, Figure 2(a-b)), most genera drastically declined, or entirely disappeared. From large (350-acre) to moderate (100-acre) harvesting scenarios, most genera exhibited a gradual upward trend in occupancy, with a noticeable but far less exaggerated lag in occupancy towards the latter half of the scenario as the mid-succession regeneration periods of staggered harvests overlapped. Wild bee diversity (all 21 genera identified in the area) was sustained over the scenario, suggesting forest bees are well-suited to landscapes with higher and maintained successional heterogeneity.

The exceptions to the general pattern of declining presence with increasing mid-succession forests were *Hylaeus sp*. and *Sphecodes sp*., which occupied mid succession, mid-late transition zone edges and interiors ($\Psi_{Hylaeus}$(ML_mid_0m), $\Psi_{Hylaeus}$(ML_tz_0m), $\Psi_{Hylaeus}$(ML_late_30m) = 0.50, 0.66, and 0.51, respectively; $\Psi_{Sphecodes}$(ML_mid_0m), $\Psi_{Sphecodes}$(ML_mid_0m) = 0.51 and 0.51, respectively). This indicates that the resources these genera rely on, such as flowers along roadsides that are typically less abundant in mid-succession stands, are still available and potentially even more accessible because of reduction in competition. Pollinators may also be seeking other nutrition and energy sources from the forest (Ulyshen, Soon and Hanula, 2010). *Hylaeus sp.* are not equipped with the pollen-carrying scopae, present on other bee species, that are used to transport pollen back to nests; inferring pollen, and therein floral resources, may not be as important a part of *Hylaeus sp.'* ecology as other bees. *Sphecodes sp.* are, in fact, non-pollinating, kleptoparasitic bees that lay their eggs in the provisioned nests of other bees, which could explain their heightened presence in mid and late succession forest where nesting sites of bumblebee, carpenter, cellophane and other families provide opportunistic laying locations. Foraging behavior of bumblebees (which were present throughout the first scenario, though at times in low numbers) indicate that they prefer to nest up to 1.5km from their primary foraging areas, potentially in forested stands (Osborne et al. 2008; Withrow-Robinson et al., 2020; Mola et al., 2021). There are likely many other under-studied resources drawing bees to forests, such as sap or fungi (Ulyshen, Soon and Hanula, 2010; Christensen, 2015; Han et al., 2021), as the ecology of forest bees is largely unexplored in comparison to those in other ecosystems.

The only genera that deviated from this general pattern, initially increasing occupancy until a threshold of successional heterogeneity was reached, were *Andrena sp.* and *Nomada sp.*. After reaching an estimated occupancy of ≈ 35,000 acres and ≈ 10,000 acres, respectively, both genera plateaued. This suggests that while not detrimental enough to cause a decline at a moderate (100-acre stands) level, successional heterogeneity may not necessarily benefit some bees and, rather, continuous forest might play an important role in their distribution or ability to move through the forest (Rathcke and Jules, 1993; Gutiérrez-Chacón, Valderrama and Klein, 2020).

A greater number of stands (although the same total acreage of early succession stands at the starting states of all scenarios) requires additional access roads. Roadways, and roadside habitat, are an important covariate in the habitat niches of many genera at the landscape level (Table 1). Specifically, roads adjacent to early succession stands and transition zones into late stands supported high occupancy (Figure 3). The tripling of roadside area from ***Scenario 1*** to ***2*** may explain the sustained presence of pollinators during timesteps with lower early and predominantly mid succession stands. While most genera benefited from roadsides, a few (e.g., *Megachile sp.* and *Osmia sp.*) displayed higher probability of presence within early stands ($\Psi_{Megachile}$(LE_early_15-50m) = 0.58-0.77 > $\Psi_{Megachile}$(LE_early_0m) = 0.28; Ψ_{Osmia}(LE_early_15-50m) = 0.55-0.68 > Ψ_{Osmia}(LE_early_0m) = 0.33), suggesting roadsides may have a detrimental impact on their presence. Overall, however, the affinity of bees to forest edges in closed canopy environments and abundance along roadsides in general is well studied and may lead us to expect that the smaller stand size in ***Scenario 3***, resulting in more edge habitat and miles of roadsides, would host the highest occupancy and diversity of bees (Hopwood, 2013; Mullaly et al. 2019; Odanaka and Rehan, 2020).

Patterns of occupancy were consistent with this logic for the first half of the third scenario until availability of land for additional 50-acre harvests was limited while previously cut stands completed maturing to harvestable age. Lesser availability of early seral habitat was reflected by a decline in nearly all genera, which benefited from early succession stands. This pattern reiterates the dynamics in ***Scenario 1*** by reaching a level of disturbance wherein the landscape effectively re-homogenizes to a new succession state. No genus was lost from the landscape, although, over the full scenario the total area occupied by pollinators was less and the forest yielded fewer acres of harvested land than the more modest harvesting regime presented by ***Scenario 2***. A multitude of smaller stands necessitate additional road infrastructure as well, which may immediately benefit some pollinators but incurs added construction costs and could be harmful to other forest organisms.

Across all scenarios, some wild bees occupied the same spatial niche. This finding was not surprising as the scale of actionable forest management that this study seeks to characterize native bee niches is at a much coarser resolution than the evolutionarily honed behavior, morphology and ecologies of individual bee genera. However, these observations still provide important insight into niche occupancy and persistence patterns of wild bees in and at the applicable scale of managed forests systems. Landscapes with greater proportions of early succession stands consistently supported higher occupancies of all bees, except those that maintained low occupancy (i.e. *Halictus sp.* and *Hylaeus sp*.). *Bombus sp*. and *Ceratina sp*., and *Lasioglossum sp*. and *Andrena sp*. consistently exhibited high occupancy in all landscapes, regardless of harvesting regime, suggesting they may be extreme generalists.

Other co-occurring pairs included *Megachile sp.* and *Osmia sp*., *Perdita sp*. and *Xylocopa sp.* and *Agapostemon sp.* and *Pseudopargnasus sp.* whose probable patterns of estimated occupancy ran parallel in response to harvesting regimes. Persistent co-occupancy through different scenarios suggests that while these pollinators may favor similar environments in terms of temperature, humidity, vegetation types, etc. and conditions facilitated by various succession stages (i.e., canopy openness, groundcover and floral availability) they are not in competition but depend on spatially or temporally partitioned resources within these areas, like types of nesting sites or preferred flower morphologies. These relationships may change over time and under different landscape configurations such that pollinators that co-occurred in one landscape may not in another.

Genera that occupied lesser areas, like *Halictus sp.* and *Triepolus sp*., were relatively unaffected by any large-scale changes in the landscape, suggesting their habitat niches are derived at a finer spatial scale. *Halictus sp.*, a small, metallic, polylectic bee (for which considerable research has been conducted to understand their evolutionary shift to eusocial behavior) nest in clustered aggregations, which suggests, in context with their low spatial occupancy across all landscapes, that their nesting areas are limited in managed forests, likely highly defined and proximal (Soucy, 2002). *Triepeolus sp*., like *Hylaeus sp*., are kleptoparasitic bees that prey on nests of longhorned bees, like *Melissodes sp*., *Eucera sp*. and *Colletes sp*. also found in this forest, which may explain why their occupancy is not directly affected by landscape change as they can take advantage of the presence of several other genera (Rightmyer, 2008).

Spatial overlap of the occurrence of genera throughout alternative scenarios suggests – at the landscape scale – that genera have evolved to use similar yet not identical resources, thereby reducing competition and simultaneously increasing resource availability (i.e., niche partitioning). Changes in population associated with different landscape compositions and co-occupancy of genera in some locations, suggests niche augmentation occurs at the landscape level and is fine-tuned at the sub-stand level, illustrating the magnifying effect that management decisions can have on wild bee populations.

Comparing harvest regimes in terms of both wild bee occupancy and acres harvested revealed parallel trends in both manners of forest productivity. Although each landscape began with the same proportions of early-, mid-, and late-succession forest, controlled size and rules of harvesting led to different outcomes of forest yield and landscape occupancy by various genera. Due to the size of stand harvests, large-harvest landscapes in ***Scenario 1*** eventually became locked in a state of mid-successional recovery that spatially restricted new harvests, and creation of early seral habitat, from occurring until stands matured to a harvestable age (Figure 1). During this time, pollinator occupancy drastically declined (Figure 2).

While moderate and small harvest stands also experienced a bottleneck of productivity during the extended mid-succession regeneration period, smaller harvest footprints allowed for alternative configurations of harvesting that maintained presence of early successional stands on the landscape, although, at times, at lower proportions. Evidently, the lesser yet sustained presence of early seral habitat was enough for moderate- and small-harvest landscapes to support greater richness of wild bees as well as timber productivity, overall. These results are quite encouraging as they exemplify that we can manage forests for both timber production and pollinator conservation, if done with restraint and foresight.

Supplementary parameterization of this model could include incorporating, and likely collecting, empirical data on the presence of wild bees in variable forest stand sizes. Data used to parametrize this model were collected from stands averaging 95 ha in size. Some genera may be more prevalent in smaller or larger stands (Mullaly et al. 2019). If probabilities of occupancy are greater for some genera in stands smaller than 95 ha, then this model may be underrepresenting their occupancy in the third scenario; the reverse of which is true if some genera are found more present in larger stands.

Although prior studies and trends observed here suggest the majority of pollinator activity takes place along the sunlit edges of forest stands and early-succession interiors, extending sampling to the core of stands, would allow for a more qualitative comparison of alternative harvest scenarios on pollinator occupancy (Mullaly et al. 2019; Odanaka and Rehan, 2020). Extending occupancy estimates of 50 m from edge to stand interiors would provide additional insight to estimates of total bee occupancy and diversity per landscape and over each scenario. In large stands, the potential effects of uncertainties introduced through this estimation process are greater as there is more core area. Although, given the overwhelming abundance of pollinators in early stands, unless there is an unexpected and extreme increase in abundance or diversity of pollinators beyond 50 m in mid or late stands, the overall trends we are observing are not likely to change. For this analysis, only cells with a probability of > 50% presence were considered "occupied" (sensu Liu et al. 2005). Lower likelihoods could be included to inform results that encompass a wider breadth of potential occupancy. This may be desirable for conservation planning to ensure all potential and important areas for low-detection genera are included.

The scenarios I built were modelled after the National Forest Service harvest rotations, where the data used to parameterize this model were collected, and where I believe practices for supporting forest pollinators may be implemented most effectively. The amount of time between harvests on these forests, however, is not typical of many private forest owners or private corporate timberland owners who may harvest again in as soon as 50-60 years (Arnold, 2017). If this schedule were simulated over the same timeframe (100 years), two harvests could be fit into the one. More frequent harvesting may increase immediate pollinator abundance through greater generation of early seral habitat. However, as discussed and supported by findings in Chapter 2, there may be resources from older forests that are essential to some bee genera, which such frequent harvest rotations would fail to provide over time. For example, late-succession stands provide nesting sites and shelter that mid-succession forests typically do not. Comparing rotation lengths would be another useful application of this model to ensure management does not prioritize productivity over forest health in which pollinators play an important part.

This analysis of the implications of forest management for pollinator communities is not meant to provide single-objective recommendations for forest management. All forest management decisions are made in the context of broader sets of objectives considering the well-being of, or effects to, other forest flora and fauna and ecosystem services and functioning.

Rather, these recommendations provide scientifically based opportunities for implementation that managers can consider in context of their forests. How they are implemented is dependent on preexisting forest conditions, including presence of other threatened or endangered organisms, ecoregion, ownership and how much is known about the present pollinator communities. Conservation actions that may support pollinators, such as understory thinning of mid-forests, could also be beneficial for wildfire prevention or clearing nonnative vegetation. The specifics of how they can be incorporated into multi-objective decision making is outside the scope of this study, although provide another exciting and immediate avenue of research.

With the additional information that this research adds to the growing body of forest pollinator research, agent-based models, multi-species occupancy models and other process-based models that require estimates of total abundance or diversity, which were not available previously, could be developed to examine genera, or species, level spatial and temporal occupancy taking into account inter species, or intra community, dynamics (Tingley, Nadeau and Sandor, 2020). Additional environmental covariates could undoubtedly improve the best-fit model for each genus, as these parameters encompass a wide range of native bee ecologies from tiny, metallic, *Agapostemon sp*. to big and fuzzy *Bombus sp.*.

CONCLUSION

Overall, the occupancy of all genera of wild bees varied in response to each harvesting regime, indicating they are sensitive to landscape level management. Greater proportions of early succession drove higher mean occupancy of almost all genera and total landscape level occupancy. However, genera responded differently to levels of spatial and successional heterogeneity, or harvest size, used to generate early seral forest and the amount of roadside habitat created in response to harvests. While landscapes composed of predominately early- and late-succession forest with high proportions of roadside habitat may immediately seem to support the best landscapes for pollinators, early stands inevitably mature to mid-succession. Mid-succession stage is the longest maturation stage of forests and least beneficial to pollinators. Creating large areas of early succession forest, as exemplified in ***Scenario 3***, therefore, may not be advisable. Managers could consider ways to prolong early-succession stage of forests or experiment with harvesting some mid-succession stands for forest products that do not require large trunk diameters, while still retaining some mid-succession stands to fully mature to late-succession, needed for nesting habitat. However, the effects of alternative harvesting strategies on other organisms and forest processes would need to be considered. These results, in general, suggest that successional heterogeneity of forest landscapes, maintained through large-scale, long-term, forest management that sustains over multiple rotations, can protect the productivity of future forests and habitat niches necessary to support the persistence of wild bees.

In Conclusion

Over the course of my research, I spent around 80 nights and 120 days in the forest. I observed so many fascinating pollinator behaviors and interactions that I wished to pursue further but following statistically significant narratives turned my research down different paths. The value placed on statistical significance can sometimes feel inhibiting to me as it limits exploration of interactions and observations that fall beyond the determined bounds. I understand the necessity of statistical significance, though, as science is *a systematic reconciliation that builds and organizes general truths in the form of testable explanations concerned with the natural world* (Merriam-Webster, 2023). Significance solidifies knowledge into these buildable, organizable, and testable pieces that explanations and predictions are born from. My research subscribes to this definition and provides significant steps toward better understanding persistence and preservation of forest pollinators.

In response to the disciplinary gaps that each of my chapters set out to address, my research contributes:

1. Knowledge of the spatial and temporal dynamics of pollinator community compositions at varying scales in managed forest landscapes, including seasonal fluctuations of diversity and optimal spatiotemporal levels of study at the landscape scale.
2. A new method of tracking pollination events, which also elucidated the effects of forest structure on pollinator movement patterns.
3. Effects of forest fragment size over three harvesting regimes that revealed the importance of sustained successional heterogeneity across managed forest landscapes for wild bees.

To address the first disciplinary gap, my research contributes a field-tested and conceptual framework to delineate the spatial and temporal dynamics of pollinator communities associated with various forest features at the genus level and varying landscape levels that illustrate the effects of scale in observation and interpretation. Establishing landscape-scale trends provides guidance for future studies to build upon at the species level and finer spatial resolution where we otherwise may not have known to focus. For example, while transition zones between EM stands declined in diversity from June to August at low and high elevations, in July MLtzs displayed a juxtaposed peak in diversity at high elevation and drop in diversity at low elevation. Future studies may want to investigate if present pollinators are shifting with temperature rise from low elevation MLtzs in July to LE or EMtzs at higher elevation in August, or if completely new communities are emerging. This analysis also provided indicator genera for each community that could be important focal pollinators for additional studies. Emergence of distinct communities at each landscape level and sub level of forest feature and seasonal ordinations, illustrates the spatially and ecologically complex nature of pollinator communities across forest landscapes. This complexity lends resiliency to pollinator communities, the services they deliver and the landscape that they inhabit.

Landscape structure also affects how pollinators disperse through the forest. Using quantum dot nanotechnology, I was able to track the directionality and concentration of pollination events, representing movement decisions that pollinators made in response to various structural features of the forest. In concurrence with the abundant early seral communities identified in Chapter 2, early succession stands also contained higher densities of pollination events throughout as well as along roadsides and the edges of adjacent stands. Mid-succession stands, again supporting results of Chapter 2, had a muffling effect on pollination events and therein likely pollinator presence. While there were fewer pollination events within mid-stands and roadsides, pollinators actually traveled greater distances in the opposite direction from mid-stands into adjacent early or late stands or along early and late roadsides. While landscape structure clearly has a strong effect on dispersal, results of this analysis found that floral availability was another driving factor in pollinators' decision making. Collectively, these two chapters present strong implications to manage forests for successional heterogeneity to support diverse pollinator communities and to maintain an abundance of wildflowers whether through strategic harvesting to increase light availability and accessibility of early seral stands or seeding along roadsides to ensure ample floral resources. In addition to direct management actions, these two chapters exhibit the influence of landscape-level forest structure on the movement of pollinators and composition of pollinator communities.

To understand the potential outcomes of alternative forest management scenarios on pollinators, specifically wild bees, my third chapter examines three harvest regimes over a hundred years of management and the estimated occupancy of wild bees across various landscapes. These results were perhaps the most exciting from a management and conservation standpoint as they strongly suggest that forests can be managed harmoniously for native pollinators as well as for timber production.

At the end of each chapter, I included my observations which lacked enough evidence to be statistically significant, yet I believe are more than random, as recommendations for future research. Forests harbor great potential as conservation havens for wild pollinators if managed properly, taking into account the co-complexity of landscapes and communities, effects of structure on dispersal, and developing harvest regimes that maintain successional heterogeneity through long term forest planning to support regeneration of forest landscapes and the persistence of forest pollinators.

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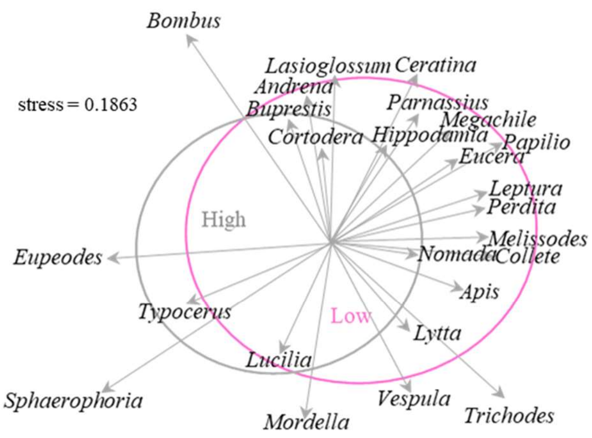
Appendix A

NMDs plots are based on 999 iterations of ranked order Bray Curtis dissimilarity matrices for each ordination, k=2 dimensions. Stress values > 0.2 indicate the ordination is a relatively unreliable representation of genera distribution, stress < 0.1 indicates a fair representations with little risk of misinterpretation, and stress <0.05 is an excellent representations. Which genera appear on the plot are determined by abundance. Meanwhile, the Indicator analysis presents which genera are driving dissimilarity between groups that are not necessarily the most abundant. ANOSIM statistic R value expresses whether communities interpreted by the NMDs are statistically separate (R < 0.2 indicates communities are nondistinctive, R <0.5 suggests separate yet overlapping, R >0.75 indicates communities are distinct with no overlap. The ANOSIM significance value helps to interpret strength of the R value. Conflicting R and significance (abbrev. "signif") values imply lack of data or high absence of pollinators in the given constrained ordination of features to draw conclusive inference. Plots that proved problematic for this reason are highlighted in red.

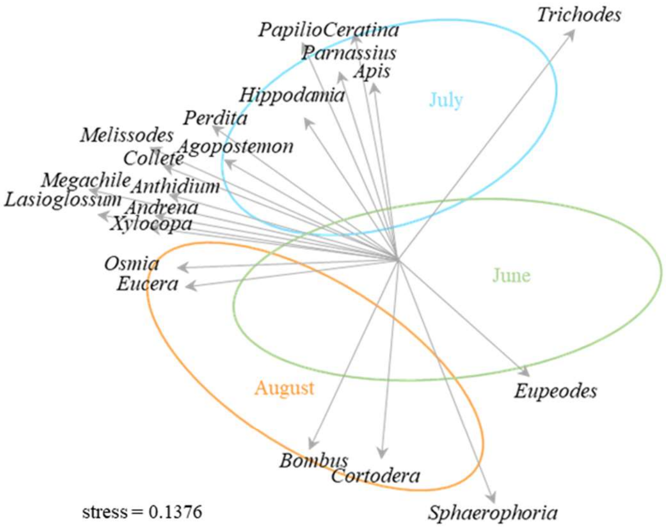
(1) ELEVATION



ANOSIM statistic R: 0.01933 Significance: 0.1885

Indicator Genera Total number of genera (35) Selected number of genera (9)		**Statistic**	**p-value**
High Elevation (1)	Sphaerophoria	0.278	0.0436*
Low Elevation (8)	Papilio	0.342	0.0008 ***
	Apis	0.339	0.0015 **
	Parnassius	0.307	0.0015 **
	Melanotus	0.285	0.0415 *
	Collete	0.282	0.0098 **
	Perdita	0.281	0.0471 *
	Melissodes	0.267	0.0088 **
	Megachile	0.253	0.0480 *

(2) MONTH



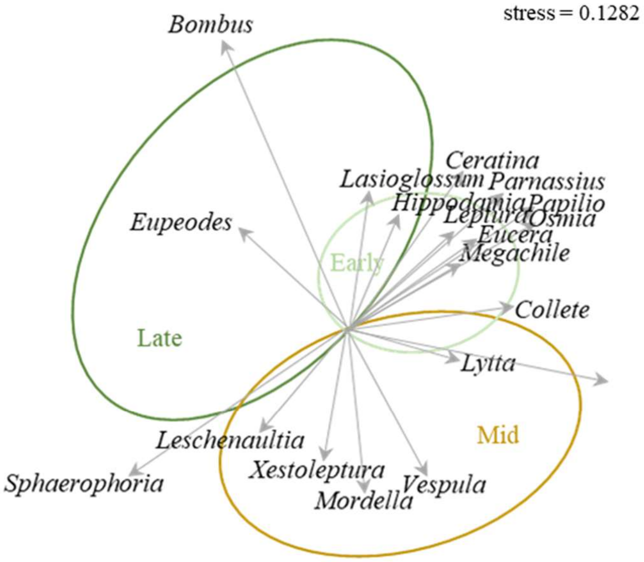
ANOSIM statistic R: 0.4657 Significance: 0.0001

Indicator Genera Total number of genera (35) Selected number of genera (11)		**Statistic**	**p-value**
June (1)	Leptura	0.457	0.0021*
July (3)	Trichodes	0.425	0.0028**
	Parnassius	0.374	0.0087**
	Ceratina	0.359	0.0165*
August (5)	Mordella	0.509	0.0006***
	Typocerus	0.470	0.0002***
	Anthidium	0.374	0.0269*
	Apis	0.347	0.0296*
	Melissodes	0.336	0.0269*
June + August (2)	Eupeodes	0.524	0.004*
	Sphaerophoria	0.348	0.0192*

1) Separate communities did not form at high and low ordinations of elevation (R = 0.01933, signif = 0.1885). NMDs stress value is approaching arbitrary (stress ≥ 0.2, infers an unreliable representation).

2) Separate pollinator communities formed in June, July and August (R = 0.4657, signif = 0.0001). ANOSIM R value approaching 0.5 indicates communities are statistically distinct yet temporally overlapping.

(3) SUCCESSION

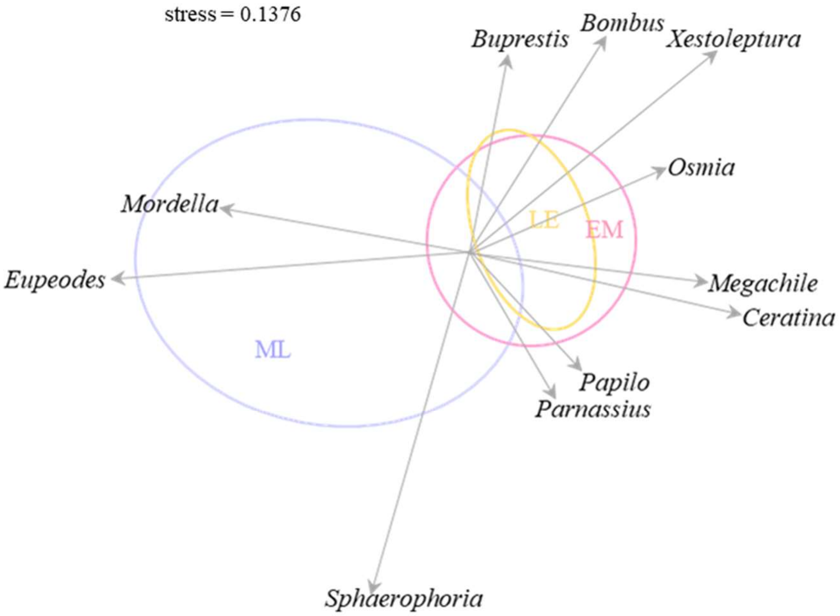


ANOSIM statistic R: 0.4525 Significance: 0.0001

Indicator Genera
Total number of genera (34)
Selected number of genera (26)

		Statistic	p-value
Early (25)	Bombus	0.745	0.0001 ***
	Lasioglossum	0.541	0.0001 ***
	Andrena	0.537	0.0001 ***
	Ceratina	0.510	0.0001 ***
	Megachile	0.508	0.0001 ***
	Leptura	0.502	0.0001 ***
	Osmia	0.457	0.0001 ***
	Eucera	0.440	0.0001 ***
	Papilio	0.379	0.0001 ***
	Mordella	0.368	0.0002 ***
	Trichodes	0.366	0.0001 ***
	Parnassius	0.297	0.0004 ***
	Cortodera	0.289	0.0014 **
	Apis	0.284	0.0019 **
	Nomada	0.266	0.0043 **
	Melissodes	0.261	0.0020 **
	Olibrus	0.258	0.0102 *
	Perdita	0.255	0.0043 **
	Collete	0.250	0.0017 **
	Xylocopa	0.247	0.0117*
	Sphex	0.237	0.0133 *
	Ochlodes	0.237	0.0167 *
	Anthidium	0.221	0.0284 *
	Eupeodes	0.206	0.0421 *
	Vespula	0.195	0.0460*
Early + Mid (1)	Typocerus	0.232	0.0159*

(4) TRANSITION ZONES



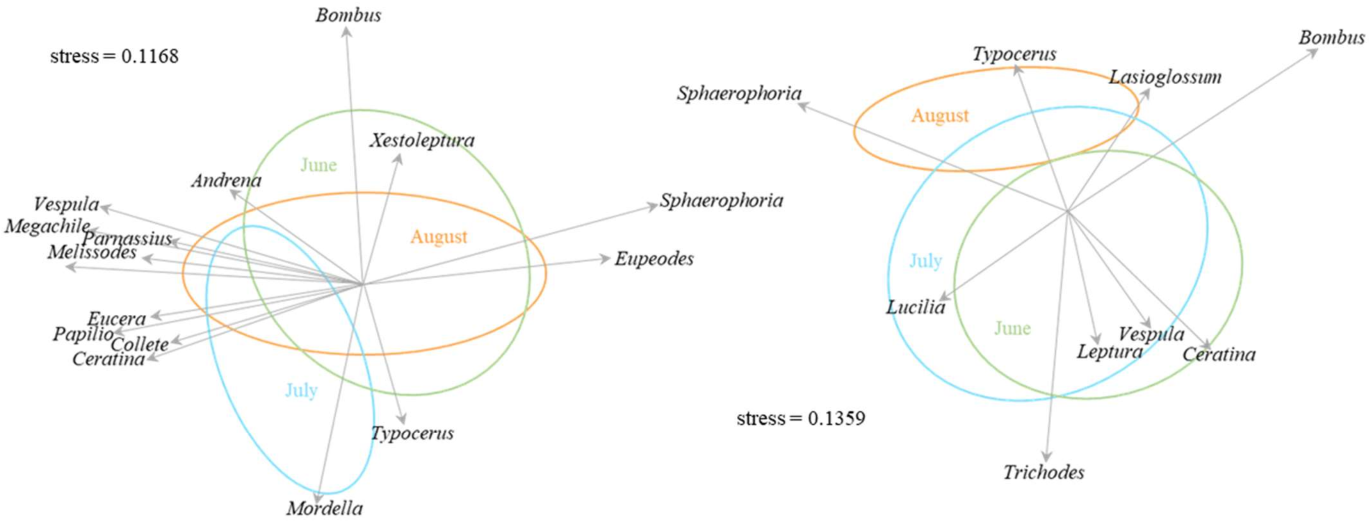
ANOSIM statistic R: 0.4051 Significance: 0.0001

Indicator Genera
Total number of genera (23)
Selected number of genera (8)

		Statistic	p-value
EM zone (1)	Vespula	0.426	0.009**
LE zone (2)	Megachile	0.514	0.0010***
	Lasioglossum	0.457	0.0024**
EM + LE (5)	Bombus	0.694	0.0001***
	Andrena	0.499	0.0009***
	Ceratina	0.437	0.0048**
	Xestoleptura	0.424	0.0068**
	Osmia	0.382	0.0162*

3) Separate communities formed at early, mid and late succession stages (R=0.4525, signif = 0.0001). Many more genera were strongly associated to early successional stages than mid or late. R indicates overlap between successional communities.
4) Separate communities formed at EM, ML and LE transition zones (R = 0.4051, signif = 0.0001). R indicates overlapping communities. NMDs plot and Indicator test suggest overlap may be occurring primarily in EM and LE zones.

(5) MONTH x ELEVATION


(a) LOW ELEVATION

(b) HIGH ELEVATION

ANOSIM statistic R: 0.3388 Significance: 0.0001

Indicator Genera Total number of genera (26) Selected number of genera (18)		**Statistic**	**p-value**
June (1)	Leptura	0.242	0.0398*
July (2)	Parnassius	0.510	0.0001***
	Papilio	0.399	0.0003***
August (7)	Mordella	0.445	0.0001***
	Melissodes	0.405	0.0001***
	Apis	0.400	0.0003***
	Anthidium	0.384	0.0003***
	Xestoleptura	0.363	0.0007***
	Eucera	0.341	0.0023**
	Megachile	0.262	0.0220*
August + June (4)	Eupeodes	0.332	0.0031**
	Sphaerophoroa	0.309	0.0083**
	Andrena	0.297	0.0072**
	Bombus	0.277	0.0160*
August + July (2)	Lasioglossum	0.354	0.0012**
	Ceratina	0.290	0.0086**
July + June (2)	Cortodera	0.249	0.0316*
	Osmia	0.237	0.0436*

ANOSIM statistic R: 0.3277 Significance: 0.0001

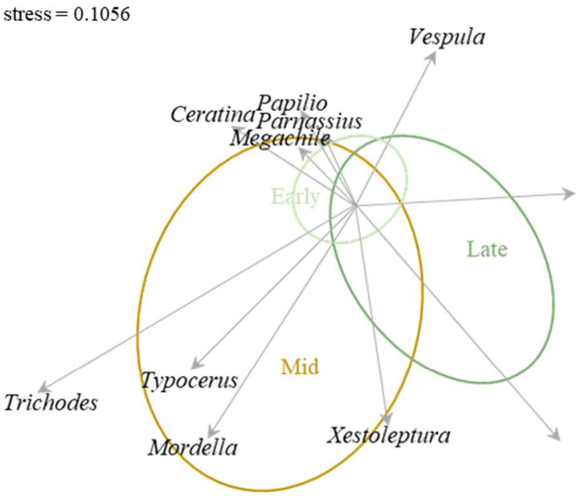
Indicator Genera Total number of genera (18) Selected number of genera (9)		**Statistic**	**p-value**
June (4)	Leptura	0.433	0.0002***
	Andrena	0.394	0.0006***
	Melanotus	0.319	0.0011**
	Osmia	0.277	0.0175*
July (1)	Ceratina	0.498	0.0001***
August (1)	Typocerus	0.577	0.0001***
August + June (2)	Eupeodes	0.335	0.0025*
	Cortodera	0.254	0.0280*
August + July (1)	Mordella	0.341	0.0025*

5a) Separate communities appeared in June, July and August at low elevation. R value (0.3388, signif = 0.0001) suggests, however, that some precaution should be taken in interpretation as communities are clearly overlapping in the NMDs plot and described by the Indicator analysis as well.

5b) Separate communities appeared in June, July and August at high elevation. R value (0.3277, signif = 0.0001) similarly suggests precaution should be taken in interpretation.

(6) SUCCESSION x ELEVATION

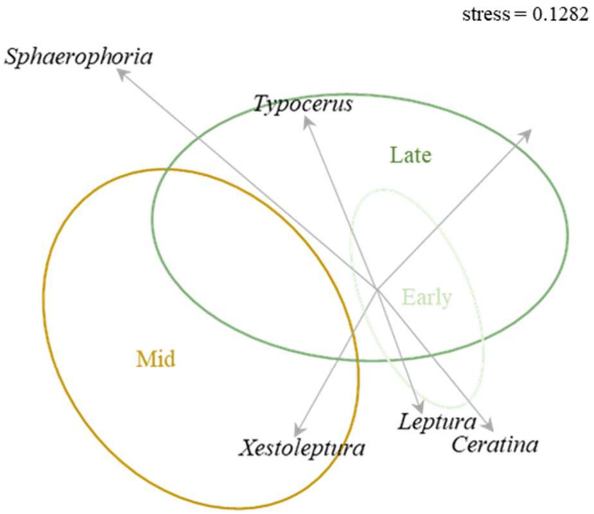
(a) LOW ELEVATION



ANOSIM statistic R: 0.4864	Significance: 0.0001

Indicator Genera Total number of genera (32) Selected number of genera (26)		**Statistic**	**p-value**
Early Succession (25)	Bombus	0.754	0.0001***
	Lasioglossum	0.662	0.0001***
	Megachile	0.610	0.0001***
	Andrena	0.608	0.0001***
	Leptura	0.580	0.0001***
	Ceratina	0.576	0.0001***
	Eucera	0.568	0.0001***
	Papilio	0.555	0.0001***
	Osmia	0.492	0.0001***
	Parnassius	0.422	0.0002***
	Trichodes	0.422	0.0003***
	Apis	0.412	0.0015**
	Mordella	0.406	0.0009***
	Cortodera	0.381	0.0031**
	Nomada	0.379	0.0031**
	Sphex	0.373	0.0024**
	Melissodes	0.372	0.0015**
	Olibrus	0.367	0.0073**
	Collete	0.337	0.0014**
	Ochlodes	0.337	00127*
	Perdita	0.331	0.0124*
	Xylocopa	0.317	0.0236*
	Anthidium	0.313	0.0254*
	Eupeodes	0.289	0.0389*
	Lytta	0.276	0.0483*
Early + Late (1)	Sphaerophoria	0.283	0..0469*

(b) HIGH ELEVATION



ANOSIM statistic R: 0.4525	Significance: 0.0001

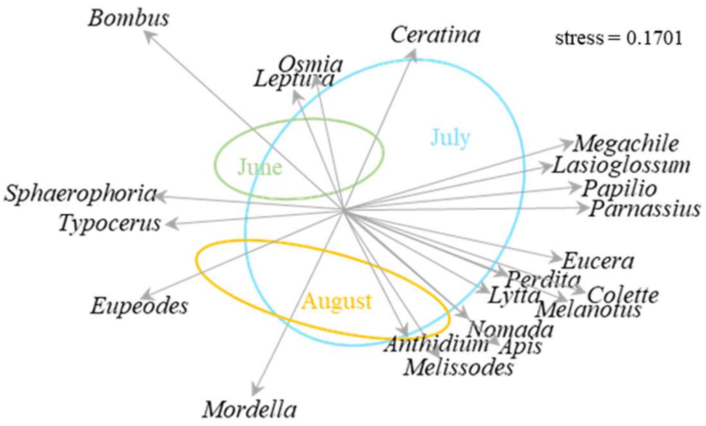
Indicator Genera Total number of genera (19) Selected number of genera (11)		**Statistic**	**p-value**
Early Succession (11)	Bombus	0.747	0.0001***
	Andrena	0.483	0.0001***
	Ceratina	0.443	0.0003***
	Osmia	0.421	0.0003***
	Megachile	0.416	0.0015**
	Lasioglossum	0.398	0.0022**
	Leptura	0.389	0.0023**
	Typocerus	0.334	0.0157*
	Mordella	0.329	0.0127*
	Trichodes	0.314	0.0216*
	Eucera	0.287	0.0387*
Early + Mid (1)	Typocerus	0.232	0.0159*

6a) Separate communities formed in early, mid and late succession stages at low elevation (R = 0.4864, signif = 0.0001). Similar to the ordination of succession, over all elevations (Appendix A 3), many more genera were strongly associated to early succession stands than mid or late. R value indicates overlap, as well.

6b) Separate communities formed in early, mid and late succession stages at high elevation (R = 0.4525, signif = 0.0001). This ordination was also similar to overall succession (Appendix A 3). R value indicates overlap.

(7) SUCCESSION x MONTH

(a) EARLY SUCCESSION



ANOSIM statistic R: 0.3528 Significance: 0.0001

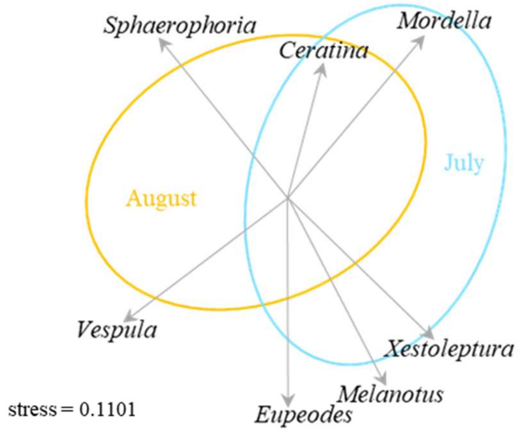
Indicator Genera Total number of genera (34) Selected number of genera (25)		**Statistic**	**p-value**
August (9)	Mordella	0.581	0.001***
	Typocerus	0.561	0.0001***
	Sphex	0.488	0.0004***
	Melissodes	0.456	0.0003***
	Apis	0.445	0.0015**
	Anthidium	0.396	0.0049**
	Eucera	0.357	0.0112*
	Nomada	0.324	0.0185*
	Lytta	0.285	0.0402*
July (1)	Ceratina	0.503	0.0003***
	Papilio	0.466	0.0006***
	Parnassius	0.431	0.0013**
June (2)	Leptura	0.454	0.0014**
	Osmia	0.343	0.0177*
August + June (3)	Andrena	0.481	0.0007***
	Eupeodes	0.454	0.0010***
	Sphaerophoria	0.386	0.0037**

7a) Separate communities formed in June, July and August within early succession stands, and displayed overlap between months (R = 0.3528, signif = 0.0001).

7b) Statistically distinct communities did not form each month at mid succession stands. Although the Indicator test found *Mordella* strongly associated to July and August, there was not enough data in June to form distinct communities at the community level.

7c) Statistically distinct communities did not form each month at mid succession stands, similarly due to absence of pollinators in June to draw statistical comparison of communities.

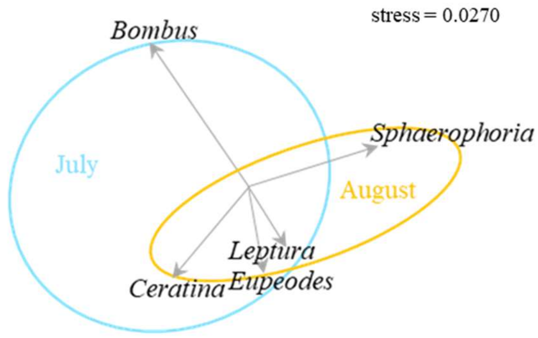
(b) MID SUCCESSION



ANOSIM statistic R: NA Significance: 0.0001

Indicator Genera Total number of genera (8) Selected number of genera (1)		**Statistic**	**p-value**
August + July (9)	Mordella	0.42	0.0258*

(c) LATE SUCCESSON



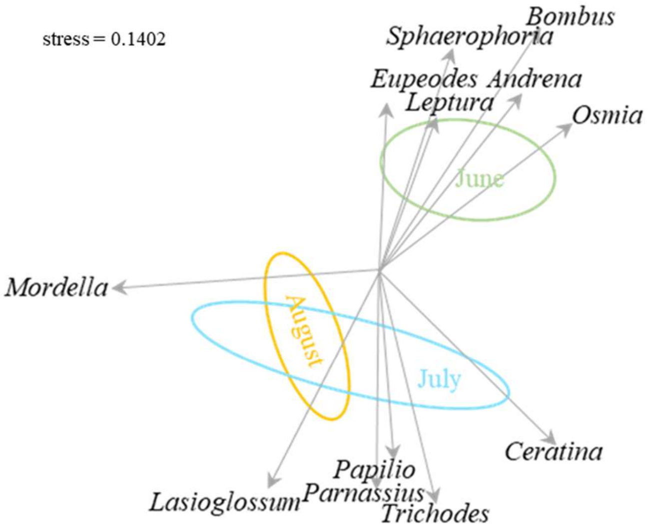
ANOSIM statistic R: NA Significance: NA

Indicator Genera Total number of genera (10) Selected number of genera (2)		**Statistic**	**p-value**
August (1)	Lasioglossum	0.376	0.0242*
July (1)	Ceratina	0.400	0.0104*

(8) SUCCESSION x MONTH x ELEVATION

LOW ELEVATION

(a) EARLY SUCCESSION



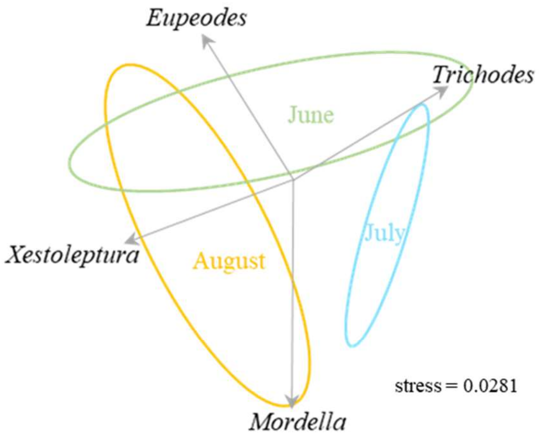
ANOSIM statistic R: 0.6063 Significance: 0.0001

Indicator Genera Total number of genera (30) Selected number of genera (19)		**Statistic**	**p-value**
August (9)	Mordella	0.766	0.0002***
	Apis	0.692	0.0003***
	Melissodes	0.684	0.0001***
	Eucera	0.594	0.0028**
	Anthidium	0.583	0.0022**
	Megachile	0.566	0.0015**
	Sphex	0.547	0.0043**
	Nomada	0.492	0.0091**
	Lytta	0.420	0.0268*
July (4)	Papilio	0.733	0.0001***
	Parnassius	0.623	0.0007***
	Trichodes	0.514	0.0068**
	Cortodera	0.437	0.0389*
August + June (4)	Bombus	0.821	0.0003***
	Andrena	0.483	0.0023**
	Eupeodes	0.434	0.0067**
	Sphaerophoria	0.431	0.0461*
August + July (2)	Lasioglossum	0.530	0.0091**
	Ceratina	0.445	0.0306*

8a) Separate communities formed in June, July and August within early succession stands at low elevation (R = 0.6063, signif = 0.0001).

8b) Distinct communities were not defined for each month within mid succession stands at low elevation (R = 0.1719, signif = 0.0356), although the stress value indicates an well-suited representation(0.0281).

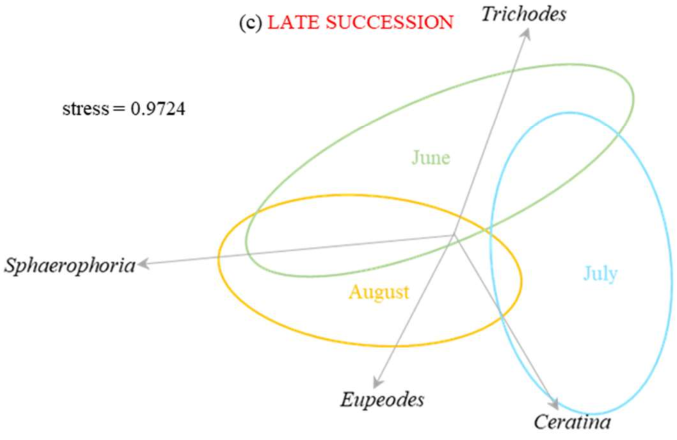
(b) MID SUCCESSION



ANOSIM statistic R: 0.1719 Significance: 0.0356

Indicator Genera Total number of genera (7) Selected number of genera (0)		**Statistic**	**p-value**
NA	NA	NA	NA

(c) LATE SUCCESSION



ANOSIM statistic R: 0.9579 Significance: 0.1034

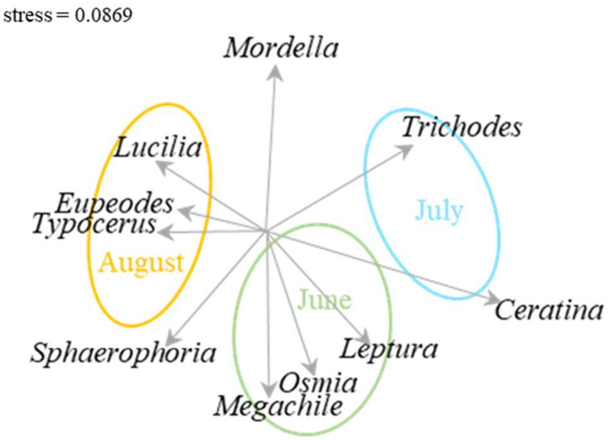
Indicator Genera Total number of genera (5) Selected number of genera (0)		**Statistic**	**p-value**
August + July (1)	Sphaerophoria	0.488	0.0491*

8c) Distinct communities were not defined each month within late stands at low elevation (stress = 0.9724, signif = 0.1034), although R value is extremely high (0.9579). These conflicting statistics are likely an artefact of low pollinator abundance within low stands at low elevation.

(8) SUCCESSION x MONTH x ELEVATION

HIGH ELEVATION

(d) EARLY SUCCESSION



ANOSIM statistic R: 0.4737	Significance: 0.0001

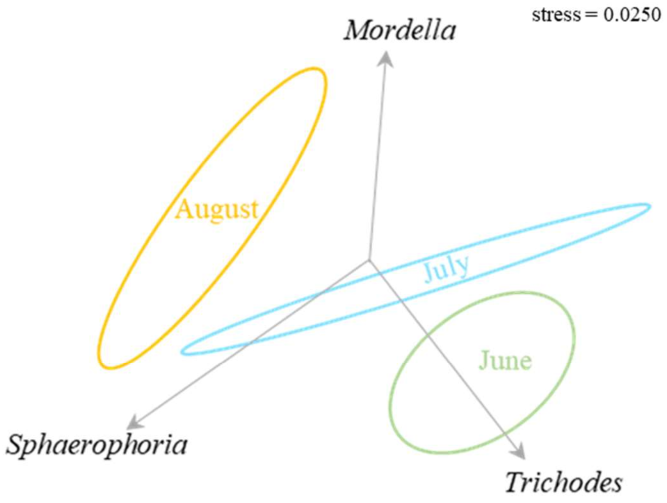
Indicator Genera Total number of genera (18) Selected number of genera (7)		**Statistic**	**p-value**
June (3)	Leptura	0.624	0.0012**
	Andrens	0.619	0.0009***
	Osmia	0.441	0.0276*
July (1)	Ceratina	0.673	0.0004***
August (3)	Typocerus	0.821	0.0001***
	Lucilia	0.483	0.0178*
	Sphaerophoria	0.434	0.0425*

8d) Separate communities formed at June, July and Agusut within early succession stands at high elevation (R = 0.4737, signif = 0.0001). R indicates some temporal overlap of communities.

8e) Distinct communities were not defined at June, July or August within mid succession stands at high elevation (R = 0.0537, signif = 0.0860), although the stress value indicates an extremely well-suited representation (0.0250).

8f) Distinct communities were not defined for each month within late stands at high elevation (R = 0.02183, signif = 0.5748), although the stress value indicates a realistic representation (0.0534). This may mean that the ordination is realistic but lacks a larger sample size to support it statistically.

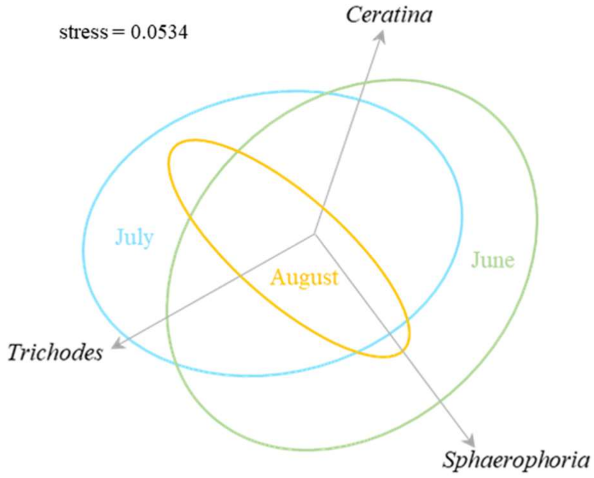
(e) MID SUCCESSION



ANOSIM statistic R: 0.0537	Significance: 0.0860

Indicator Genera Total number of genera (5) Selected number of genera (0)		**Statistic**	**p-value**
NA	NA	NA	NA

(f) LATE SUCCESSION

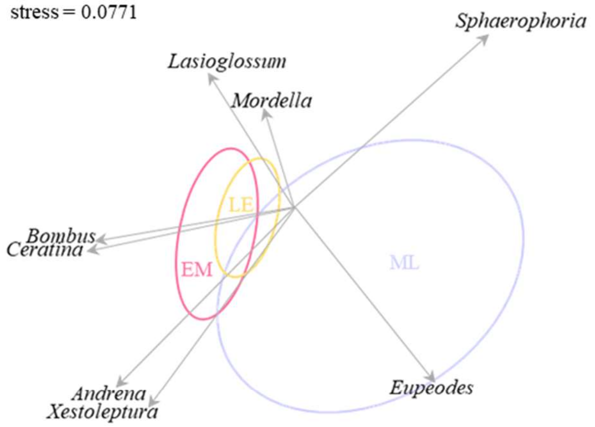


ANOSIM statistic R: 0.02183	Significance: 0.5748

Indicator Genera Total number of genera (7) Selected number of genera (0)		**Statistic**	**p-value**
NA	NA	NA	NA

(9) TRANSITION ZONES x ELEVATION

(a) LOW ELEVATION



ANOSIM	statistic R: 0.4936	Significance: 0.0001

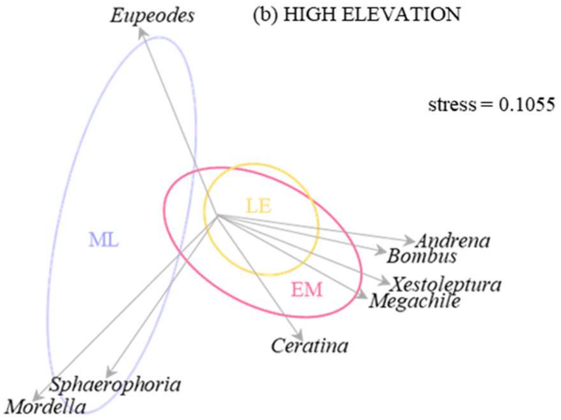
Indicator Genera Total number of genera (22) Selected number of genera (7)		Statistic	p-value
LE zone (3)	Bombus	0.666	0.0014**
	Megachile	0.5688	0.0126*
	Eupeodes	0.558	0.0149*
EM + LE (4)	Ceratina	0.537	0.0170*
	Andrena	0.514	0.0286*
	Lasioglossum	0.491	0.0447*
	Xestoleptura	0.467	0.0446*

9a) Separate pollinator communities emerged in each transition zone at high elevation (R = 0.4936, signif = 0.0001). R approaching 0.5 indicates communities are distinct yet overlapping. NMDs plot and Indicator suggest overlap may be occurring primarily in EM and LE transition zones.

9b) Separate pollinator communities emerged in each transition zone at high elevation (R = 0.4290, signif = 0.0001). NMDs plot and Indicator suggest overlap may be occurring primarily in EM and LE zones.

10) Distinct pollinator communities emerged in transition zones, collectively, each month (R = 0.4550, signif = 0.0001). R value indicates temporal overlap.

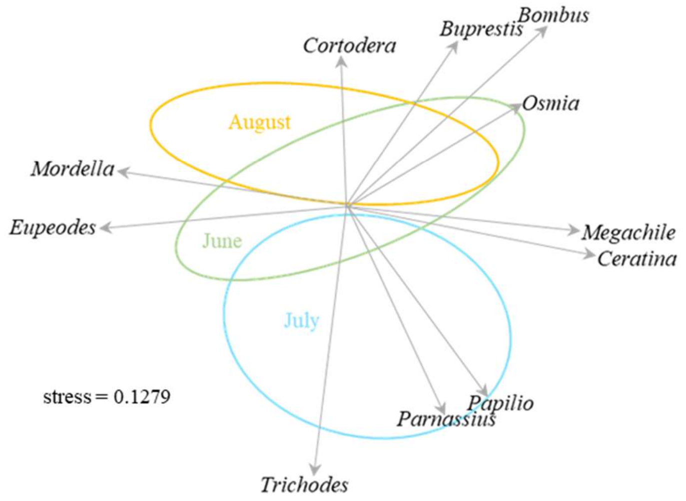
(b) HIGH ELEVATION



ANOSIM	statistic R: 0.4290	Significance: 0.0001

Indicator Genera Total number of genera (16) Selected number of genera (5)		Statistic	p-value
LE zone (3)	Bombus	0.552	0.0188*
	Lasioglossum	0.531	0.0332*
	Sphaerophoria	0.526	0.0346*
EM + LE (2)	Bombus	0.782	0.0002***
	Andrena	0.487	0.0477*

(10) TRANSITION ZONES x MONTH

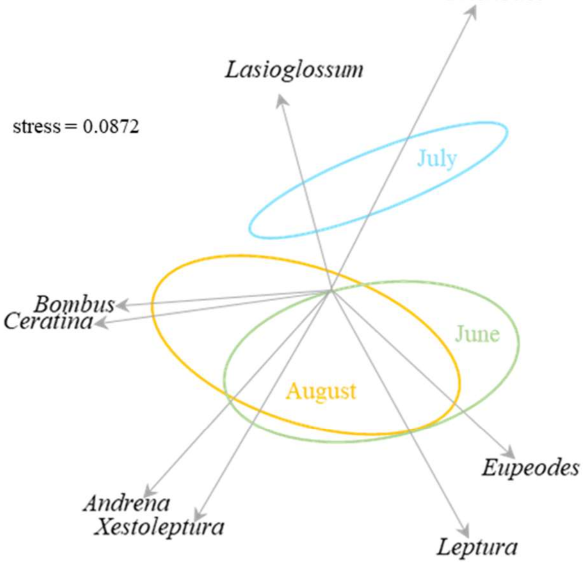


ANOSIM	statistic R: 0.455	Significance: 0.0001

Indicator Genera Total number of genera (24) Selected number of genera (12)		Statistic	p-value
August (5)	Eucera	0.502	0.0010***
	Typocerus	0.446	0.0040**
	Mordella	0.382	0.0138*
	Anthidium	0.370	0.0311*
	Melissodes	0.347	0.0387*
July (3)	Trichodes	0.522	0.0007***
	Parnassius	0.434	0.0058**
	Ceratina	0.406	0.0095**
June (3)	Leptura	0.564	0.0002***
	Andrena	0.485	0.0009***
	Osmia	0.369	0.0248*
August + July (1)	Lasioglossum	0.421	0.0072**

(11) TRANSITION ZONES x MONTH x ELEVATION

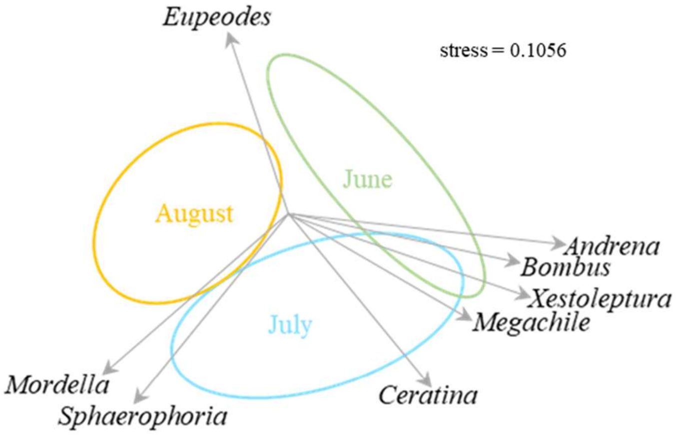
(a) LOW ELEVATION



ANOSIM statistic R: 0.4062	Significance: 0.0001

Indicator Genera Total number of genera (23) Selected number of genera (8)		Statistic	p-value
August (4)	Eucera	0.607	0.0085**
	Anthidium	0.524	0.0206*
	Xestoleptura	0.505	0.0267*
	Melissodes	0.502	0.0282*
July (2)	Parnassius	0.646	0.0030**
	Trichodes	0.643	0.0022**
June (1)	Leptura	0.51	0.0253*
August + July (1)	Lasioglossum	0.519	0.0204*

(b) HIGH ELEVATION



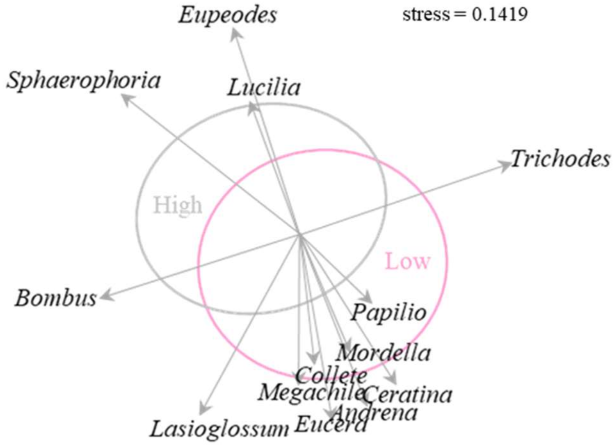
ANOSIM statistic R: 0.5712	Significance: 0.0002

Indicator Genera Total number of genera (15) Selected number of genera (5)		Statistic	p-value
August (1)	Typocerus	0.615	0.0196*
July (1)	Ceratina	0.610	0.0061**
June (2)	Leptura	0.627	0.0079**
	Andrena	0.555	0.0164*
July + June (1)	Trichodes	0.498	0.039*

11a) Separate communities formed at June, July and August within transition zones at low elevation (R = 0.4062, signif = 0.0001), although they displayed overlap. Stress value indicates a realistic representation of communities.

11b) Separate communities formed at June, July and August within transition zones at high elevation (R = 0.5712, signif = 0.0002). Overlap between communities is less so than at low elevation. Stress value also indicates a realistic representation of communities.

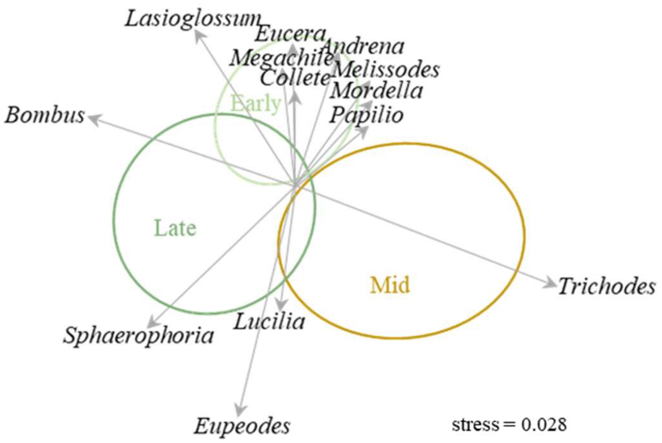
(12) ROADSIDES x ELEVATION



ANOSIM statistic R: 0.2153 Significance: 0.0001

Indicator Genera Total number of genera (26) Selected number of genera (7)		**Statistic**	**p-value**
High Elevation (1)	Sphaerophoria	0.221	0.0463*
Low Elevation (6)	Papilio	0.320	0.0057**
	Apis	0.272	0.0244*
	Ceratina	0.247	0.0311*
	Parnassius	0.233	0.0320*
	Collete	0.231	0.0237*
	Megachile	0.224	0.0399*

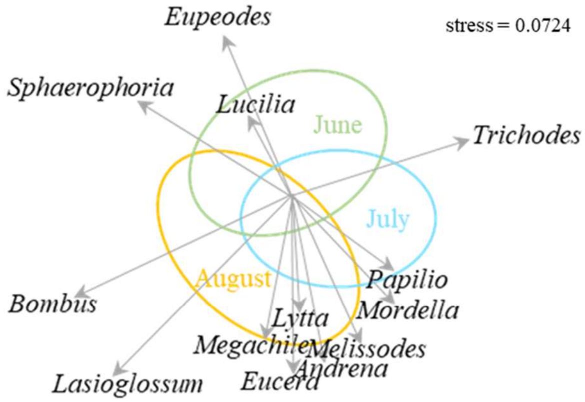
(13) ROADSIDES x SUCCESSION



ANOSIM statistic R: 0.4039 Significance: 0.0001

Indicator Genera Total number of genera (26) Selected number of genera (20)		**Statistic**	**p-value**
Mid + Late (1)	Sphaerophoria	0.221	0.0463*
Early (19)	Bombus	0.703	0.0001***
	Leptura	0.521	0.0001***
	Lasioglossum	0.510	0.0002***
	Andrena	0.491	0.0001***
	Mordella	0.484	0.0001***
	Trichodes	0.470	0.0002***
	Eucera	0.445	0.0005***
	Papilio	0.419	0.0010***
	Osmia	0.399	0.0011**
	Ceratina	0.391	0.0010***
	Megachile	0.382	0.0003***
	Olibrus	0.374	0.0103*
	Vespula	0.354	0.0048**
	Cortodera	0.343	0.0070**
	Parnassius	0.301	0.0250*
	Melissodes	0.297	0.0316*
	Apis	0.288	0.0360*
	Lytta	0.286	0.0317*
	Collete	0.285	0.0219*
	Xestoleptura	0.270	0.0443*

(14) ROADSIDES x MONTH



ANOSIM statistic R: 0.1536 Significance: 0.0001

Indicator Genera Total number of genera (22) Selected number of genera (8)		**Statistic**	**p-value**
June (1)	Sphaerophoria	0.221	0.0463*
August (5)	Mordella	0.488	0.0002***
	Typocerus	0.396	0.0016**
	Sphex	0.299	0.0251*
	Andreba	0.268	0.0460*
	Megachile	0.258	0.0488*
July (1)	Parnassius	0.349	0.0003***
August + June (2)	Eupeodes	0.336	0.0106*
	Melanotus	0.275	0.0458*

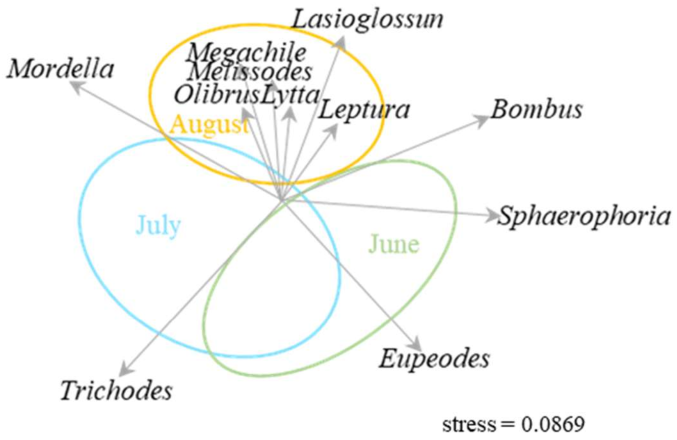
12) Distinct communities were not found along roadsides at high in comparison to low elevation (R = 0.2153, signif = 0.0001).

13) Separate communities formed along early, mid and late succession roadsides (R = 0.4039, signif = 0.0001). Communities displayed overlap with the vast majority of all pollinators present (26) strongly associated to early succession roadsides (19).

14) Communities along roadsides did not differ significantly by month (R = 0.1536, signif = 0.0001).

(15) ROADSIDES x MONTH x ELEVATION

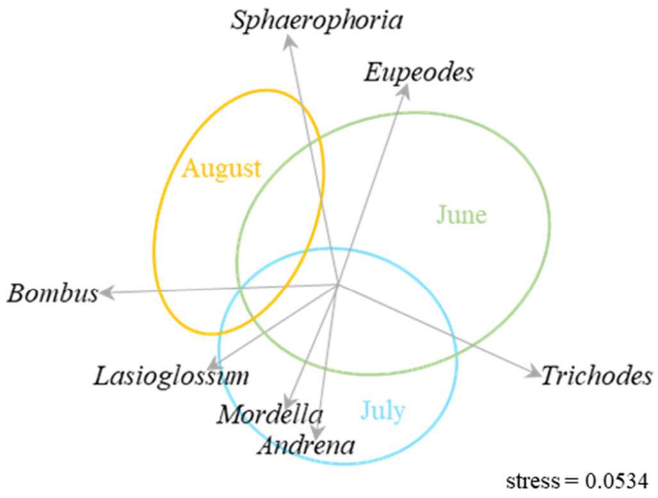
(a) LOW ELEVATION



ANOSIM statistic R: 0.3975 Significance: 0.0005

Indicator Genera Total number of genera (25) Selected number of genera (10)		**Statistic**	**p-value**
August (7)	Mordella	0.564	0.0010***
	Apis	0.540	0.0026**
	Lytta	0.422	0.0148*
	Andrena	0.421	0.0221*
	Melissodes	0.402	0.0491*
	Sphex	0.393	0.0313*
	Megachile	0.384	0.0288*
July (2)	Parnassius	0.482	0.0003***
	Trichodes	0.381	0.0413*
August + June (1)	Sphaerophoria	0.398	0.0299*

(b) HIGH ELEVATION



ANOSIM statistic R: 0.3388 Significance: 0.0002

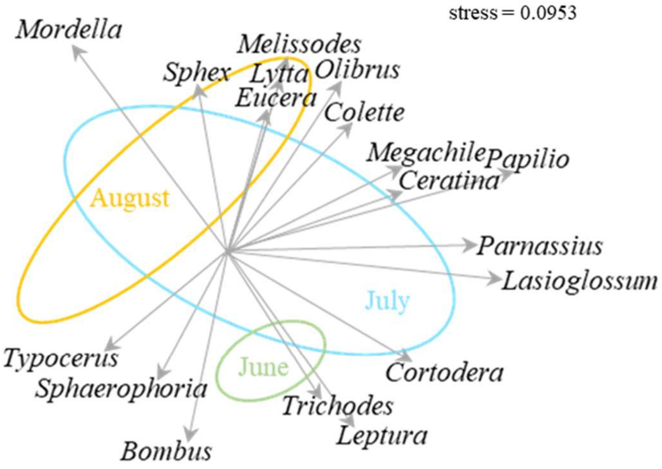
Indicator Genera Total number of genera (17) Selected number of genera (6)		**Statistic**	**p-value**
August (2)	Typocerus	0.544	0.0032**
	Mordella	0.407	0.0318*
July (1)	Ceratina	0.403	0.0392*
June (2)	Leptura	0.478	0.0180*
	Melanotus	0.421	0.0432*
July + June (1)	Trichodes	0.415	0.0249*

15a) Separate communities appeared along roadsides in June, July and August at low elevation (R = 0.3975, signif = 0.0005). Stress value suggests a realistic representation of genera distrtribution in the ordination. R indicates overlap between communities.

15b) Separate communities appeared along roadsides at June, July and August at high elevation (R = 0.3388, signif = 0.0002). Stress value suggests a realistic representation of genera distrtribution in the ordination. R indicates some overlap between communities,

(16) ROADSIDE x SUCCESSION x MONTH

(a) EARLY SUCCESSION



ANOSIM statistic R: 0.3881	Significance: 0.0002

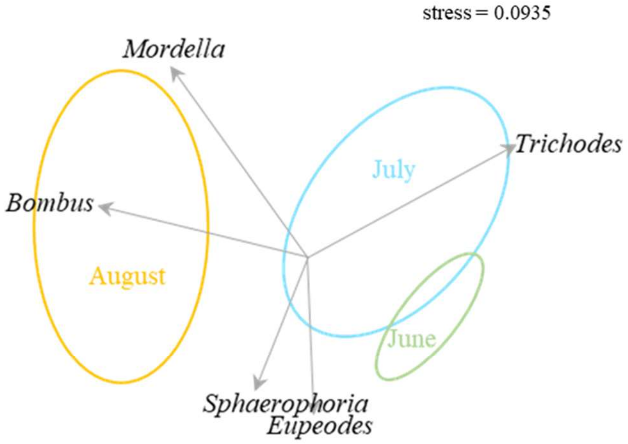
Indicator Genera Total number of genera (23) Selected number of genera (12)		**Statistic**	**p-value**
August (8)	Mordella	0.723	0.0004***
	Sphex	0.664	0.0008***
	Typocerus	0.621	0.0106*
	Lytta	0.529	0.0089**
	Andrena	0.514	0.0199*
	Melissodes	0.505	0.0395*
	Bombus	0.503	0.0300*
	Megachile	0.465	0.0319*
July (1)	Parnassuis	0.555	0.0138*
August + July (1)	Eucera	0.465	0.0438*
July + June (1)	Trichodes	0.481	0.0317*

16a) Separate communities appeared along early succession roadsides throughout the active season (R = 0.3881), although R indicates high overlap between communities.

16b) Separate communities formed along mid succession roadsides throughout the season (R = 0.4571, signif = 0.0002) with some overlap between months.

16c) Separate communities formed along late succession roadsides throughout the season as well (R = 0.4713, signif = 0.0155) with some overlap between months. ANOSIM signif value suggests some precaution should be taken considering statistical separation of communities.

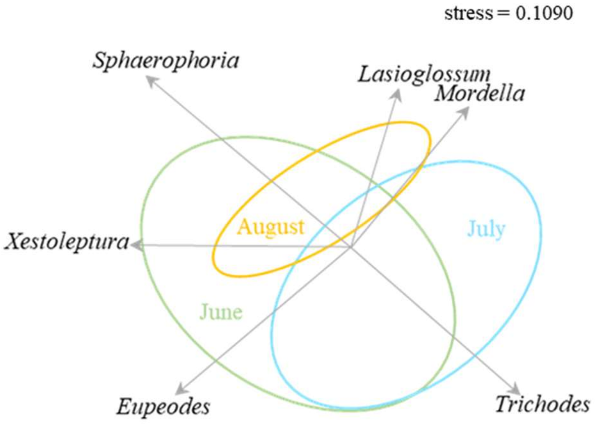
(b) MID SUCCESSION



ANOSIM statistic R: 0.4571	Significance: 0.0002

Indicator Genera Total number of genera (21) Selected number of genera (2)		**Statistic**	**p-value**
August (1)	Lasioglossum	0.542	0.019*
July (1)	Ceratina	0.521	0.0038*

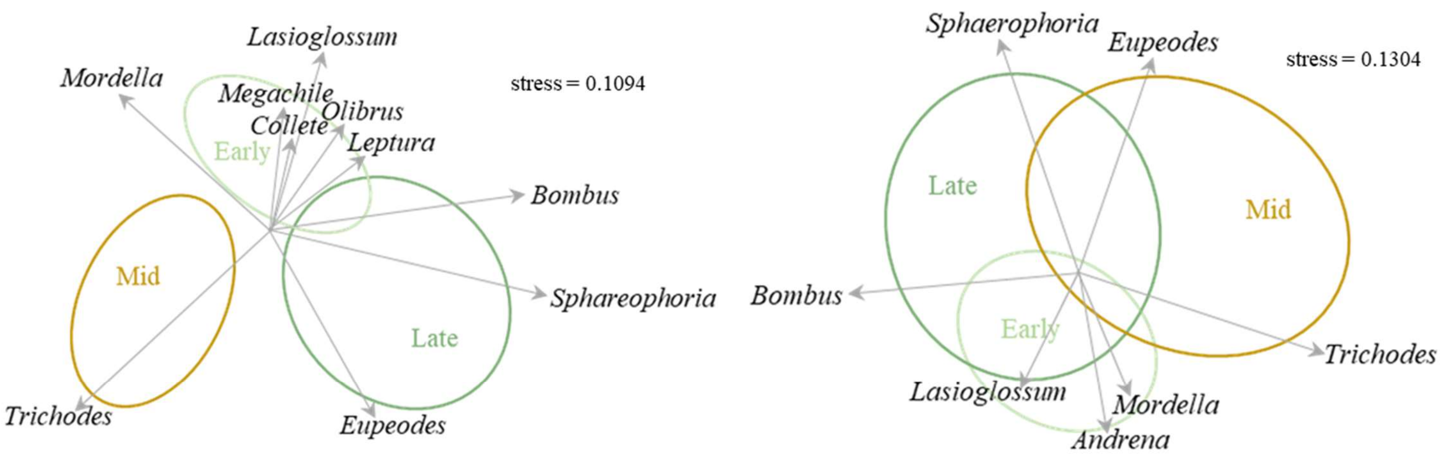
(c) LATE SUCCESSION



ANOSIM statistic R: 0.4713	Significance: 0.0155

Indicator Genera Total number of genera (9) Selected number of genera (3)		**Statistic**	**p-value**
August (2)	Mordella	0.610	0.0069**
	Bombus	0.520	0.0301*
July + June (1)	Trichodes	0.540	0.0114*

(18) ROADSIDE x SUCCESSION x ELEVATION


(a) LOW ELEVATION

(b) HIGH ELEVATION

ANOSIM statistic R: 0.5987 Significance: 0.0001

Indicator Genera
Total number of genera (25)
Selected number of genera (19)

		Statistic	**p-value**
Early (19)	Bombus	0.748	0.0001***
	Lasioglossum	0.703	0.0002***
	Leptura	0.657	0.0002***
	Papilio	0.610	0.0006***
	Eucera	0.598	0.0008***
	Andrena	0.577	0.000****
	Olibrus	0.537	0.0077**
	Ceratina	0.534	0.0014**
	Trichodes	0.525	0.0006***
	Megachile	0.522	0.0001***
	Cortodera	0.473	0.0078**
	Vespula	0.468	0.0051**
	Mordella	0.435	0.0085**
	Osmia	0.426	0.0173*
	Melissodes	0.421	0.0294*
	Parnassius	0.413	0.0261*
	Apis	0.412	0.0284*
	Lytta	0.404	0.0257*
	Collete	0.404	0.0161*

ANOSIM statistic R: 0.3188 Significance: 0.0001

Indicator Genera
Total number of genera (17)
Selected number of genera (4)

		Statistic	**p-value**
Early (4)	Bombus	0.691	0.0001***
	Mordella	0.517	0.0033**
	Andrena	0.483	0.0173*
	Trichodes	0.436	0.0147*

18a) Separate communities formed along early, mid and late succession roadsides at low elevation (R= 0.5987) with little overlap.

18b) Separate communities formed along early, mid and late succession roadsides at high elevation (R= 0.3188, signif = 0.001) but with much more overlap than at low elevation.