

**BUMBLEBEE RESPONSE TO GLYPHOSATE APPLICATION WITHIN
MANAGED FORESTS OF NEW BRUNSWICK, CANADA**

by

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ABSTRACT

Pesticides are used to target undesired pests, but their use can also affect non-target organisms. Non-target effects can be both direct (such as a toxic response) or mediated effects, where the response from the focal community is observed because of changes within another community. Herbicide use in managed forests occur in late summer/ early fall when some pollinators are still flying and foraging. This puts them at risk of direct exposure to spray either during the application or after through contact with residues in pollen and nectar resources. Pollinators also rely on the plant community and alterations to the plant community structure from herbicide applications could influence pollinator responses. To test for this, I assessed bumblebee community patterns in forest blocks sprayed with a glyphosate-based herbicide and unsprayed (control) blocks. Overall, there were more individual bumblebees caught in sprayed blocks, but higher richness in unsprayed blocks. Bumblebee response to herbicide use was not significant between application and the bumblebee community (direct) but was observed when mediated through changes within the floral community. Herbicide application significantly increased cover of flowering plants and this resulted in higher abundance and diversity of bumblebees. Overall, the use of minor canopy disturbances (such as herbicide application) following the creation of early successional stands (from clearcutting) benefit plant species that bumblebees use, and this can result in increases in abundance and diversity.

DEDICATION

I dedicate this thesis to my family, as without them this would only be a dream.

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I would like to acknowledge that my field work and studies were carried out on the traditional unceded territory of the Wolastoqiyik (Maliseet) and Mi'kmaq Peoples. My journey through this degree has brought me to some beautiful locations where I could bask in the sun while listening to the birds' chirp high in the trees as the rivers flowed past my feet. These moments are the basis of many Indigenous teachings. Take time to enjoy and appreciate what is around you, but also remember we are here to protect and live in unison with Mother Earth. I would like to honor that teaching as I move through my career and promise to pause and be grateful, but also lend my hand and knowledge when needed.

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Introduction

Global pesticide applications were expected to exceed 3.5 million tonnes in 2020 (Sharma *et al.*, 2019). Pesticides are essential for the maintenance of global trade markets and without them there would be significant reduction in crop yields from managed lands, such as agricultural lands or lands managed for wood and fibre products (Zhang, 2018; Sharma *et al.*, 2019). Their effectiveness and efficacy makes their use critical to some land operations, but their use carries some risk to human and environmental health (Zhang, 2018; Hedlund *et al.*, 2020; Canada, 2021).

Pesticides are applied to target a particular 'pest', such as insects (insecticides), fungus (fungicides) and vegetation (herbicides) (Mahmood *et al.*, 2016). Some pesticides are designed to be efficient and effective for a particular target organism(s) (Zacharia, 2011). However, when broad spectrum, non-selective pesticides are used there is a risk that non-target organisms will be affected (Zacharia, 2011; Mahmood *et al.*, 2016; Rolando *et al.*, 2017). The effects observed on non-target organisms can influence ecosystem health and functioning by altering relationships between biotic and abiotic components within the ecosystem (Zacharia, 2011; Stanley *et al.*, 2015; Mahmood *et al.*, 2016; Sharma *et al.*, 2019). Predicting non-target effects at the local scale can be difficult due to interactions with abiotic and biotic factors, which vary across the landscape, which can create multi-directional outcomes that can contradict one another (Laigle *et al.*, 2021). Additionally, non-target effects on organisms within a system can be positive or negative. The directionality of non-target effects creates challenges because they require land managers to consider multiple outcomes from land

management operations and how these operations could affect the ecosystem (abiotic and biotic interactions) as a whole (Laigle *et al.*, 2021). For example, the application of an herbicide to control a weed that hinders crop production could help produce large crop yields. However, if the application of the herbicide affects beneficial insects (e.g. pollinators), then the crop yield could be impacted from the loss of pollination services. In this example, the land manager evaluates the benefit of herbicide use against environmental risk in an attempt to meet multiple goals (crop yield and pollination services) simultaneously.

Pesticide use is one of several factors that has led to a global decline in pollinator abundance and diversity (Vanbergen & Initiative, 2013; Goulson *et al.*, 2015; De Palma *et al.*, 2016). In addition, local pollinator population declines have also been attributed to a variety of factors, such as habitat loss, fragmentation, land-use intensification, encroachment of alien plant and insect species, and pathogens (Vanbergen & Initiative, 2013; De Palma *et al.*, 2016; Hanula *et al.*, 2016). Globally, insect pollinators are responsible for the pollination of nearly 90% of flowering plant species (Ollerton *et al.*, 2011). Insect pollination services are a highly valued ecosystem service (De Palma *et al.*, 2016; Hanula *et al.*, 2016) and an important component in the production of nutrient rich foods, such as fruits, vegetables and nuts (Aizen *et al.*, 2009). The cumulative and interactive effects from the variety of factors affecting pollinators is serious as they are an extremely important ecological group (De Palma *et al.*, 2016; Hanula *et al.*, 2016). Therefore, it is critical that land managers address potential impacts land operations could have on pollinators and help mitigate these impacts through sustainable management plans.

Currently, pollinator management and consideration in management plans is dominated by urban landscapes and agroecosystems as these areas were once thought to be the most important areas for pollinators and their conservation (Hanula *et al.*, 2016; Mola *et al.*, 2021). However, as land degradation and land use intensification in these areas increases and we learn more about pollinators and their habitats, conservation efforts have shifted to include previously less valued habitat to meet conservation goals (Hanula *et al.*, 2016; Proesmans *et al.*, 2018; Mola *et al.*, 2021). One of these areas is within managed forests, where researchers have shown that early successional forest stands could provide sufficient habitat to some pollinator species (Taki *et al.*, 2013; Hanula *et al.*, 2016; Mola *et al.*, 2021). Previously, forested areas were thought to have little value to pollinators, but recent advancements in pollinator conservation have shown that pollinators are using these areas more than previously thought (Taki *et al.*, 2013; Hanula *et al.*, 2016; Proesmans *et al.*, 2018; Mola *et al.*, 2021). Forest management operations create a matrix of landscape types that could benefit pollinators through increasing pollinator habitat characteristics (Hanula *et al.*, 2016; Mola *et al.*, 2021). Harvesting alters local light and temperature and create favorable abiotic conditions for many pollinators (Hanula *et al.*, 2016; Mola *et al.*, 2021). Following the removal of mature tree species, the colonization of disturbance generalists and early successional species increase pollinator friendly plant species that can be used for food resources (Taki *et al.*, 2013; Hanula *et al.*, 2016; Mola *et al.*, 2021). In addition, forests have a greater diversity of flowering phenologies than grassland counterparts, which is critical in supporting pollinators throughout their entire lifecycles (Taki *et al.*, 2013; Mola *et al.*, 2021; Smith *et al.*, 2021). Little is still known about the state of pollinators

within forests (Hanula *et al.*, 2016; Mola *et al.*, 2021), particularly the Acadian Forest in Eastern North America which represents a diverse and unique forest type from the mixing of northern boreal and southern temperate forest species (Brooks & Nocera, 2020). With little known about pollinators within the Acadian Forest, less is known about how forest operations could be affecting the quality and quantity of pollinator habitat in this region.

In New Brunswick, forested areas account for approximately 6.1 million hectares, or 83% of New Brunswick's land mass (SGS Belgium S.A., 2018). A tenet of modern sustainable forest management is to ensure that in addition to fibre and timber supply, ecological and biological objectives are met, with the goal of maintaining forest health long term (Thiffault & Roy, 2011; Natural Resources Canada, 2020; Thiffault, 2021). Forest management plans are tasked with generating product (wood and fibre), and maintaining forest function, such as hydrological maintenance, carbon sequestration, and wildlife habitat (O'Hara, 2006; Nyland, 2016; Natural Resources Canada, 2020).

Forest management plays a critical role in the maintenance and functioning of wildlife habitat as it directly influences the quality and quantity of habitat across a landscape (O'Hara, 2006). Forest habitat goes beyond just the protection of old growth or mature forests, as preference for stand age varies among wildlife species and some prefer stands that have been disturbed (O'Hara, 2006). The succession between a disturbance (natural or anthropogenic) and mature forests provides varying types of habitat for all kinds of wildlife (O'Hara, 2006). Wildlife habitat in managed forest landscapes relies on forest management plans to create, maintain and improve habitat through precise long term planning objectives (O'Hara, 2006; Nyland, 2016). These

long-term plans describe the amount, location and type of harvest as well as regeneration plans (Nyland, 2016). To achieve the goals outlined in a forest management plan, silviculture techniques are applied to guide a stand to a specific forest stand class (Nyland, 2016). A variety of techniques can be applied post-harvest to increase the growth and success of coniferous (conifer) tree species. Techniques can include mechanically preparing the soil, planting species suited for local conditions, and manual, mechanical, or chemical tending to remove competitive hardwood vegetation (Nyland, 2016).

In New Brunswick, approximately one quarter of harvested land is replanted with coniferous tree species to promote their establishment and growth (Nyland, 2016; SGS Belgium S.A., 2018; Natural Resources Canada, 2020). However, conifer growth is slower in comparison to some early successional species, many of which are angiosperms, and if left unmanaged, successional influences would result in reduced yields and longer periods between harvests (Rolando *et al.*, 2017). To increase survival and success of planted conifers, early intervention silviculture techniques can be applied to planted sites to impede the growth of competitive angiosperms species (Nyland, 2016; Rolando *et al.*, 2017). The most commonly used early intervention technique in Canadian forestry is the application of a herbicide (Rolando *et al.*, 2017).

Of the herbicides available on the market for use in Canada, glyphosate-based herbicides are the most commonly used and account for 95% of herbicide use in Canadian forestry (Rolando *et al.*, 2017), and are also the most commonly used in the world (Benbrook, 2016). The high use of glyphosate-based herbicides can be attributed to their low cost, efficacy, and relative safety in the environment (Sullivan & Sullivan,

2003; Duke & Powles, 2008; Helander *et al.*, 2012; PMRA, 2017; Rolando *et al.*, 2017).

Several international reviews have concluded that glyphosate, as typically used in forestry, is not expected to pose a unacceptable risk to non-target species (PMRA, 2017; US EPA, 2020). However silviculture techniques are used to influence stand dynamics and the regeneration trajectory (Nyland, 2016), which, by definition, will have an effect on the ecosystem (Kremen & Merenlender, 2018) and could lead to indirect effects on organisms of concern.

Glyphosate works by inhibiting the production of proteins needed for growth by stopping the enzymic shikimic acid pathway that is only found in plants and some microorganisms (Duke & Powles, 2008; Richmond, 2018). The non-selective nature of glyphosate-based herbicides make it an ideal candidate for non-target effects because applications do not target one species or group of species and can have significant influences on plant community structure (Freedman, 1990; Rolando *et al.*, 2017). Insect pollinators are of particular interest due to their ecological importance and connection to areas where herbicides are applied (Battisti *et al.*, 2021). Their charismatic nature and relevance to the climate crisis have made bees, both managed and wild, the focus in the discussion around the loss of pollination services from anthropogenic actions (Goulson *et al.*, 2015; Cullen *et al.*, 2019).

Research on the potential effects of glyphosate-based herbicide use on bees is limited in comparison to other pesticides (Straw & Brown, 2021). From the available literature, LD₅₀ and LC₅₀ endpoints have been established for honeybees (Lewis *et al.*, 2016) and current work aims to expand critical endpoints for other bee species (Franklin & Raine, 2019). In addition, studies have reported both lethal (Abraham *et al.*, 2018;

Battisti *et al.*, 2021) and sublethal (Balbuena *et al.*, 2015; Motta *et al.*, 2018; Blot *et al.*, 2019; Odemer *et al.*, 2020; Battisti *et al.*, 2021) effects which result from olfactory disruptions, cognitive impairment and increased vulnerability to pathogens (Battisti *et al.*, 2021). Much of the literature on the effect of glyphosate-based herbicides on bees is dominated by laboratory studies which use *Apis mellifera* (honeybees) as the focal taxon (Battisti *et al.*, 2021). Some field studies have been conducted but again, these studies focus primarily on honeybees within agroecosystems (Battisti *et al.*, 2021), resulting in less knowledge of glyphosate-based herbicide effects in other management regimes, such as managed forests and on wild bees (Miller & Miller, 2004; Hanula *et al.*, 2016; Cullen *et al.*, 2019).

The conclusions on the effects of glyphosate-based herbicides on bees have not been unanimous (Battisti *et al.*, 2021). Many regulatory agencies conclude that there is little risk associated with the use of glyphosate-based herbicides on honey bees under typical applications in agriculture and forestry (EFSA, 2015; PMRA, 2015; US EPA, 2020); however, individual studies have found significant lethal and sublethal effects on bees from both worst case (manufacturer recommended dosing) and ecologically relevant exposures (Abraham *et al.*, 2018; Motta *et al.*, 2018; Seide *et al.*, 2018; Odemer *et al.*, 2020; Battisti *et al.*, 2021). Exposure to glyphosate-based herbicides was found to increase mortality in all life stages, but worst case dosing experiments had higher mortality rates than ecological relevant dosing studies (Battisti *et al.*, 2021). Sublethal effects were found to decrease homing ability, disrupt olfactory senses and decrease hive success through loss of worker bees (mortality or exclusion) or impairments to larval development affecting adulthood performance (Battisti *et al.*, 2021).

The use of glyphosate-based herbicides in managed forests generally occurs during the early successional stages of stand regeneration. The habitat offered by early successional stands is more preferred by bee species that inhabit forested areas (Taki *et al.*, 2013; Hanula *et al.*, 2016; Proesmans *et al.*, 2018). Therefore, bees could be exposed at higher rates to glyphosate-based herbicides in these areas. The direct mechanisms of exposure that could be observed can be broken down into three major routes: 1) contact with spray during application or with residues following an application 2) consumption of pollen and nectar resources containing residues and 3) contact with residues in nesting substrates (soil or vegetation). The frequency and magnitude of exposure for the routes mentioned above is critical in determining the risk of exposure on wild bees (Sgolastra *et al.*, 2019; Willis Chan *et al.*, 2019).

Direct exposure to glyphosate during herbicide application could occur through contact with the spray cloud (Stanley & Preetha, 2016). Bees within the spray cloud zone could make direct contact with the herbicide spray, which could result in a high level of exposure at the time of contact. The duration of this exposure route would not persist long as the spray cloud moves about the harvest block as the application is completed. The frequency and magnitude of exposure through the consumption of pollen and nectar containing glyphosate is difficult to quantify (Stanley & Preetha, 2016). The consumption of glyphosate through pollen and nectar can have long lasting effects on honey bees and their developing brood (Abraham *et al.*, 2018; Motta *et al.*, 2018; Battisti *et al.*, 2021) but, would depend on the amount of residues consumed over time and their sensitivity to the pesticide (Arena & Sgolastra, 2014; Battisti *et al.*, 2021b). Exposure to glyphosate residues following spray and in nesting substrates is

difficult to predict. The exposure concentration would depend on the dose as well as degradation profile (Franklin & Raine, 2019; Battisti *et al.*, 2021). This would likely have a greater effect on bees than high doses over a short period of time (Abraham *et al.*, 2018). The time of year when herbicides are applied to managed forests (late August into October) is when bees are entering or preparing for dormancy (Michener, 2000; Williams *et al.*, 2014; Mola *et al.*, 2021). During dormancy, glyphosate residues could have serious negative effects on developing brood or over-wintering adults as the length of exposure would be prolonged (Stanley & Preetha, 2016; Seide *et al.*, 2018). The direct effects of glyphosate on bees is an active area of study, where studies have suggested that ecologically relevant dosing of glyphosate-based herbicides do not pose a significant risk to wild bees (Balbuena *et al.*, 2015; PMRA, 2017; Motta *et al.*, 2018; Seide *et al.*, 2018; Odemer *et al.*, 2020). However, glyphosate is used to alter the vegetation community and those alterations may lead to indirect effects on other components of the ecosystem (Edge *et al.*, 2020), such as the bee community.

Herbicides are applied to areas where control of competitive vegetation is needed to improve local site conditions for crops, easements, or aesthetics (Duke & Powles, 2008; Richmond, 2018). In doing so, reductions in plant species abundance occur, which alters the local vegetative landscape (Sullivan & Sullivan, 2003; Rolando *et al.*, 2017; Richmond, 2018). As bees rely on vegetation for food and nesting resources, alterations to this component of the landscape can have significant effects (Taki *et al.*, 2013; Mola *et al.*, 2021). In forested landscapes, the application of a herbicide are generally used to suppress overhead canopy species to help improve growing conditions for planted conifers (Nyland, 2016). The changes in canopy density shift local abiotic factors, such

as the amount of light reaching the forest floor which results in higher local temperatures (Freedman, 1990; Sullivan & Sullivan, 2003; Helander *et al.*, 2012). The shift in abiotic factors can directly affect bees by creating favored site conditions (enough light, and warmer temperatures) as well as indirectly by improving site conditions for vegetation used by pollinators (Sullivan & Sullivan, 2003; Helander *et al.*, 2012). Increases in low herbaceous groundcover and floral output can be observed in areas where overhead canopy density has been suppressed (Sullivan & Sullivan, 2003), which would be beneficial to bees as it would increase food resources.

As glyphosate is applied to areas and habitats used by pollinators for foraging and nesting it is possible that bees may be indirectly affected by targeted changes in the ecosystem (Laigle *et al.*, 2021; Mola *et al.*, 2021). The goal of herbicide application is to alter the vegetation community structure through reductions in competitive plant species (Sullivan & Sullivan, 2003; Nyland, 2016). Alterations in the vegetation community could have an impact on the pollinator community through changes in foraging resource types and/ or availability of food resources. Following a herbicide application the greatest mortality is expected to occur in plants that occupy the canopy layer (e.g. hardwood shrub species) and this mortality will create a canopy gap (Sullivan & Sullivan, 2003; Nyland, 2016; Rolando *et al.*, 2017). The creation of a gap will alter local abiotic conditions (Freedman, 1990; Taki *et al.*, 2013; Nyland, 2016). Changes in abiotic factors, such as increase in light availability, increased temperatures and increased water and nutrient availability favor the establishment of early successional/ generalist plant species (Bell & Newmaster, 2002; Sullivan & Sullivan, 2003; O'Hara, 2006; Wagner *et al.*, 2011). Generally, species richness and diversity of the plant

community remain the same or increase slightly from 1 year post clearcut to post herbicide application (Sullivan & Sullivan, 2003). However, there are decreases in the abundances of species following herbicide application (Sullivan & Sullivan, 2003). In addition, changes to local abiotic factors from a disturbance (harvest or herbicide application) can help facilitate ideal growing conditions for low herbaceous groundcover (Boateng *et al.*, 2000). Reductions in the woody shrub layer have a positive effect on low herbaceous groundcover, and more importantly the floral community (Bell & Newmaster, 2002; Sullivan & Sullivan, 2003). These effects also persist for some time, where documented vegetation community structure differences between treated and control blocks have been observed up to 12 years post-treatment (Freedman *et al.*, 1993). The effects on abiotic conditions, vegetation composition, and floral abundance could lead to changes in the pollinator community due to changes in food resources and other habitat aspects (Bell & Newmaster, 2002; Mola *et al.*, 2021).

Objectives of Study

Anthropogenic alterations in ecosystems can have far reaching impacts that could persist over long periods of time or severely impact ecosystems services and biodiversity (Díaz *et al.*, 2019). Ecosystems are extremely complex, making it difficult to fully understand the extent of which changes in one community can affect another (Díaz *et al.*, 2019; FAO and UNEP, 2020). As glyphosate use continues to increase globally (Duke & Powles, 2008; Richmond, 2018), it is critical that research addresses the risk of exposure to both target and non-target organisms. To address the potential impacts of glyphosate-based herbicide use on bumblebees within managed forests of

New Brunswick, I structured a causal model that describes potential effects on the bumblebee community that could occur from forest management and changes in the vegetation community (Figure 1). In addressing these potential effects, I predict that:

1. The application of a glyphosate-based herbicide in managed forests will result in changes to the vegetation community, specifically reductions in the canopy layer are expected to increase floral resources through decreased competition. I predict that the change in the vegetation community from increases in floral resources will have a positive effect on the bumblebee community by increasing abundance, richness and diversity.
2. The effects from herbicide exposure (during spray, contact with or consumption of glyphosate residues in/on floral resources, or residues in nesting substrates) could negatively affect the bumblebee community by increasing mortality rates in areas treated with a glyphosate-based herbicide. I predict that herbicide application will result in a negative effect on bumblebee abundance from increased mortality rates from herbicide exposure.

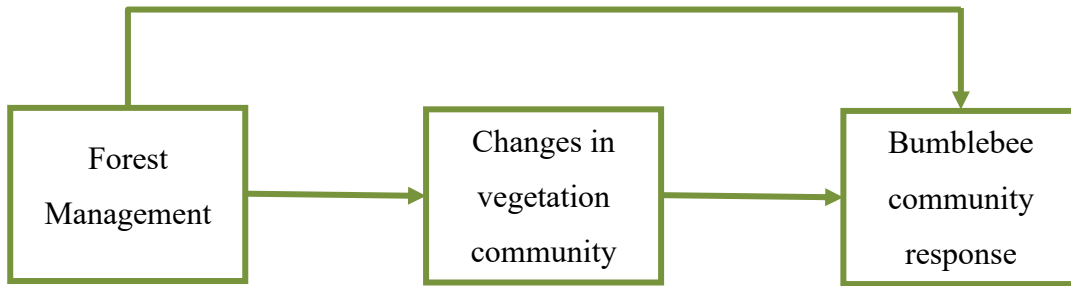


Figure 1. Causal model for the predicted flow of effects of forest management activities (herbicide application and harvesting) on the bumblebee community within managed forest of New Brunswick. The model addresses the potential effect of forest management on the bumblebee community and the mediated effect from changes within the vegetation community.

Methods

To evaluate the impact of glyphosate on non-target forest plant and pollinator communities I conducted a large-scale study in operational harvest blocks (clearcuts) in New Brunswick, Canada (46.5653° N, 66.4619° W). A total of 31 harvest blocks ranging in size between 4 – 54 ha in were selected within a 150 km radius of Fredericton, New Brunswick (45.9636° N, 66.6431° W). Selected clearcuts were sprayed with a glyphosate-based herbicide (sprayed, n = 18) or not sprayed after the current harvest (unsprayed, n = 13) which acted as a control in the experiment (Table 1; Figure 2).

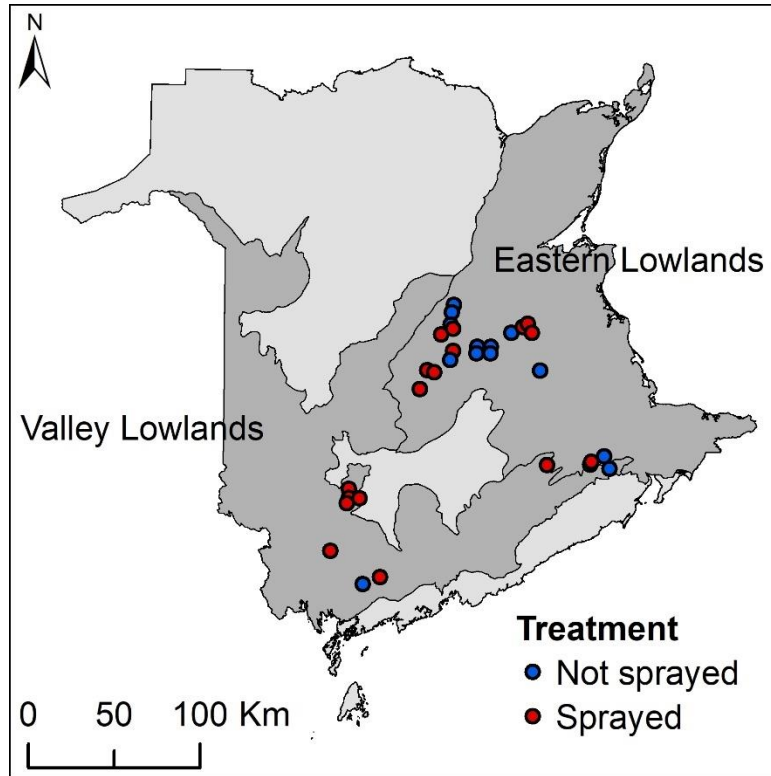


Figure 2. Harvest blocks selected for study within the Valley Lowlands and Eastern Lowlands ecoregions of New Brunswick, Canada. Of the 31 clearcut sites selected, seven are within the Valley Lowlands ecoregion and 24 sites are within the Eastern Lowlands ecoregion.

All sites were clearcut between 2005 to 2016 and planted with a single species of conifer or a mix of conifer species 1 to 3 years after harvest. Clearcuts were treated with an aerial application of a glyphosate-based herbicide 1 to 4 years after planting. Of the 18 clearcuts that received a glyphosate-based herbicide application, seven harvest blocks received two applications ranging from 2 to 4 years after the initial application. The second herbicide applications were made when the first application did not result in the desired level of vegetation control. Glyphosate products VisionMAX™ (540 g a.e./L), FORZA™ (360 g a.e./L), VP480™ (480 g a.e./L) and Weed-Master™ (356 g a.e./L) were applied to sprayed blocks in the months of August to September. Weed-Master™

was the most common product applied, where 15 of the 18 spray blocks used Weed-Master™ in one of the applications (initial or second, but never both). Application rates varied between 3.1 to 5.0 L/ha, where 4.7 L/ha was the most common rate applied.

Table 1. Tally of unsprayed and sprayed blocks based on year of harvest (clearcut year). Sites that received an application of a glyphosate-based herbicide are broken down into spray applications (sprayed once or twice) and last application year is indicated in brackets.

Clearcut Year	Unsprayed	Sprayed (Year)		Total per Year
		Sprayed once	Sprayed twice	
2005	1			1
2007	1	1(2010)		2
2008			1(2016)	1
2009			1(2015)	1
2010	1			1
2011	1			1
2012	2		1(2016), 1(2018)	4
2013	2	1(2016)		3
2014	2	1(2017)	2(2018)	5
2015	2	5(2017)	1(2019)	8
2016	1	2(2018), 1(2019)		4
Total per treatment	13	11	7	31

Study location

Of the 31 harvest blocks selected 7 (6 sprayed and 1 unsprayed) sites were in the Valley Lowland ecoregion and 24 (12 sprayed and 12 unsprayed) sites were in the Eastern Lowlands ecoregion. The climate within in the Valley Lowlands and Eastern Lowlands ecoregions is similar, however there are differences in the plant communities found within each ecoregion (Zelazny, 2007).

The Eastern Lowlands forest canopy is dominated by more boreal-like species, such as red spruce (*Picea rubens* Sargent, 1898), black spruce (*Picea mariana* [Britton, Sterns & Poggenburg. 1888]), balsam fir (*Abies balsamea* [Miller, 1768]), eastern

hemlock (*Tsuga canadensis* [Carrière, 1855]), white pine (*Pinus strobus* [Linnaeus, 1753]), and jack pine (*Pinus banksiana* [Lambert, 1803]) (Zelazny, 2007), with an understory plant community consisting of lambkill (*Kalmia angustifolia* [Linnaeus, 1753]), mountain holly (*Ilex mucronata* [Powell, Savolainen & Andrews, 2000]), speckled alder (*Alnus incana* [Moench, 1794]), partridgeberry (*Mitchella repens* [Linnaeus, 1753]), three-leaf goldthread (*Coptis trifolia* [Salisbury, 1807]), bunchberry (*Cornus canadensis* [Linnaeus, 1753]), bristly club-moss (*Spinulum annotinum* [Haines, 2003]) and sphagnum (*Sphagnum* spp. [Schimper, 1858]) (Zelazny, 2007).

The Valley Lowlands canopy is dominated by more tolerant mixedwood forests of red pine (*Pinus resinosa* [Aiton, 1789]), white pine, red oak (*Quercus rubra* [Linnaeus, 1753]), aspen (*Populus* spp. [Linnaeus, 1753]), yellow birch (*Betula alleghaniensis* [Britton, 1904]), American beech (*Fagus grandifolia* [Ehrhart, 1788]) and white ash (*Fraxinus americana* [Linnaeus, 1753]) (Zelazny, 2007), with an understory plant community being characteristic of a mixedwood forest, such as violets (*Viola* spp. [Linnaeus, 1753]), hay-scented fern (*Dennstaedtia punctilobula* [Smith, 1875]), sensitive fern (*Onoclea sensibilis* [Linnaeus, 1753]), and Christmas fern (*Polystichum acrostichoides* [Schott, 1834]) (Zelazny, 2007).

Data collection occurred from June to September 2020 and consisted of pollinator community sampling, a rapid flowering plant survey and vegetation surveying. All data collection occurred within a 1-hectare study plot. The study plot was established ~150 metre from the access point and was positioned approximately in the middle of each harvest block to reduce edge effects.

Vegetation surveying

In each study plot ($n = 31$), three 30 m long by 1 m wide belt transects was established. The first transect was laid haphazardly at the centre point of the study plot and the remaining two transects were laid haphazardly at the edges of the delineated study plot. Each 30 m belt transect was divided into 5 m long by 1 m wide segments to reduce estimation error when completing visual cover estimation of present vegetation. Vegetation species within each segment was recorded and their percent cover estimated for a total area of 90 m² surveyed in each harvest block. Vegetation surveys were completed between July 13th, 2020 to August 7th, 2020. Visual cover estimation of present species was calculated using the Braun-Blanquet method which assigns cover estimate ranges a value from + to 5 to gather accurate data in a timely fashion (Table 2; Braun-Blanquet 1932; Wikum and Shanholtzer 1978). A representative photo was taken when vegetation species could not be identified in the field and taken back to the lab to be identified.

Recorded Braun-Blanquet scale values were converted to midpoint of cover estimates for each present species to facilitate further analysis (Table 2). Site vegetation data from each segment ($n = 6$) within each transect ($n = 3$) were summed for each present plant species and then averaged by the number of segments to get percent cover of each recorded species. Identified plant species were then assigned to their seed-bearing type (angiosperm or gymnosperm). The total percentage cover of angiosperms in each harvest block was calculated by summing the averaged percent cover of all angiosperm plant species at a site. Treatment percent cover for angiosperms and plants

in flower was calculated by summing each variable by treatment and finding the average.

Table 2. Braun-Blanquet cover-abundance scale including conversion to midpoint of cover range.

Braun-Blanquet scale	Range of % cover	Midpoint of cover range (%)
+	< 5; few individuals	0.1
1	< 5; many individuals	2.5
2	5 – 25	15.0
3	25 – 50	37.5
4	50 – 75	62.5
5	75 – 100	87.5

Floral resources and bumblebee sampling

In the study plot, three blue vane traps were set approximately 50 m apart from one another. Traps were set at or above mean vegetation height so that the view of the blue vane portion of the trap was not obstructed by surrounding vegetation (Droege *et al.*, 2016). Each trap contained ~100 mL of 100% Propylene Glycol (Robinson Supply, Calgary, Alberta) as a preservative. Propylene Glycol is often used as a preservative in insect traps left in the field for more than a couple of days as it has a low rate of evaporation (Droege *et al.*, 2016). Blue vane traps were placed between August 10th, 2020 to August 27th, 2020 and were opened for one week. Upon collection of specimens from the blue vane traps, samples were placed in specimen containers with propylene glycol from the field for transportation. In the lab, samples were sorted to remove by-catch (any non-bee pollinator). Bees were placed in 70% ethanol and stored until bee specimens could be mounted. Bees were mounted following the National Protocol Framework for the Inventory and Monitoring of Bees (Droege *et al.*, 2016) and placed in Schmitt boxes. Identification of bee species was undertaken on the genus *Bombus*

(bumblebees) and will be the focus for the remainder of this thesis. Identifications were completed using the *Bumble Bees of North America: An Identification Guide* (Williams *et al.*, 2014) and supplemented with the Discover Life bee species guide and world checklist (Ascher & Pickering, 2020).

Bumblebee community metrics was calculated for each harvest block and consisted of abundance, species richness, species evenness (Equation 1; (Pielou, 1966), Shannon Weaver diversity index (H' ; (Shannon & Weaver, 1963) and Simpson's diversity index (D_1 ; Equation 3; (Simpson, 1949). Community metrics were calculated using the *vegan* package in R (Oksanen *et al.*, 2020).

Equation 1. Pielou's evenness

$$J = \frac{H'}{\log(S)}$$

Where H' is the value derived from the Shannon Weaver diversity index and S is the number of species (Oksanen *et al.*, 2020).

Equation 2. Shannon Weaver diversity index

$$H' = - \sum_{i=1}^S p_i \log_b p_i$$

Equation 3. Simpson diversity index

$$D_1 = 1 - \sum_{i=1}^S p_i^2$$

Where p_i is the proportion of species (i), and S is the number of species (Oksanen, 2020).

The Shannon Weaver diversity index (H') accounts for both evenness and richness,

where the latter has a stronger influence on the diversity measure (Bell & Newmaster, 2002; Daly *et al.*, 2018). Simpson diversity index accounts for the proportion of species and looks at the chance that two individuals are from two different species (Daly *et al.*, 2018). Both diversity indices were calculated because of their relevance in literature and to the dataset. Shannon Weaver diversity is a common measure of diversity in many ecological studies (Daly *et al.*, 2018), warranting consideration in this study. However, the dataset showed high abundance values for two bumblebee species, warranting the inclusion of Simpson diversity to address unequal relative abundances of bumblebee species in the dataset (Daly *et al.*, 2018).

During placement of blue vane traps, a rapid assessment of available floral resources was conducted within the study plot. Rapid assessments utilized the Braun-Blanquet method for percent estimation to facilitate comparisons with other vegetation data collected. When conducting the rapid assessment for floral resources, the observer would walk around the 1 ha study plot and record the percent cover of all species that were in flower. The Braun-Blanquet scale values were converted to their midpoint value (Table 2) and a combined floral coverage value was calculated by summing the total percentage of flowering plants for each site. Combined floral coverage values can exceed 100% cover as the midpoint values were summed. While possible, this outcome was not common ($n = 2$) in the dataset.

Statistical analyses

All statistical analyses were completed in R version 4.0.3 (R Core Team, 2020). Differences between community metrics (abundance, evenness, diversity and

rarefaction) observed between sprayed and unsprayed blocks were calculated using Wilcoxon rank sum test (Wilcoxon, 1945). The package *coin* (v1.4-2, Hothorn *et al.*, 2006) was used to compute the exact p-values and W statistic as there were ties (same value) among the data. Effect size was calculated using r (Equation 4):

Equation 4. r effect size

$$r = Z/\sqrt{N}$$

Where Z is the test statistic generated from the Wilcoxon rank sum test and N is the total number of observations within the dataset (Cooper & Hedges, 1993).

The causal model describing the effects of vegetation management on the bumblebee community was further described to include measurable variables (Figure). This was done by incorporating measured variables that relate to vegetation management, changes in the vegetation community and bumblebee community response. Vegetation management was broken down to include:

- a. Herbicide – a categorical variable that indicates if the harvest block received a glyphosate-based herbicide or not
- b. Time since clearcut – a interval variable that describes the amount of time that has passed since the block was clearcut

Changes in the vegetation community were described by:

- a. Angiosperm cover – a numerical variable that describes the percent cover of all angiosperms found within the transects

- b. Floral cover – a numerical variable that describes the percent cover of plant species that are in flower during blue vane trap placement

Wild bee community response was described by:

- a. Abundance – total abundance for all bumblebee species caught
- b. Richness – total number of bumblebee species caught
- c. Evenness – distribution of abundance among bumblebee species caught
- d. Diversity – described by both Shannon Weaver and Simpson’s diversity indices

To test if the covariance in the dataset was consistent with the causal pathways described, structural equation modelling (SEM) was used. SEM calculations were generated using the *lavaan* package in R (v0.6-7; Rosseel 2012). The variability in the types of measurements collected required the data to be mean centred and scaled to the variance to reduce large variations in the observed variances allowing for meaningful comparisons between observed effects. Causal models were fitted using a global estimation approach to include the direct effect of herbicide application on bumblebee community metrics described above, as well as mediated (indirect) effects through changes in vegetation from herbicide application and time since clearcut (Figure 3). Goodness-of-fit for each model were evaluated using Chi squared testing (χ^2), root mean square error of approximation (RMSEA) and comparative fit index (CFI). In all models the χ^2 was non-significant ($p > 0.05$) and other fit statistics were acceptable (Table 5). Standardized beta (β) values were used to compare effect sizes among variables and significant results ($p < 0.05$) are included in the results. Statistics for all variable relationships are given in the appendices (Appendix 1 - 5).

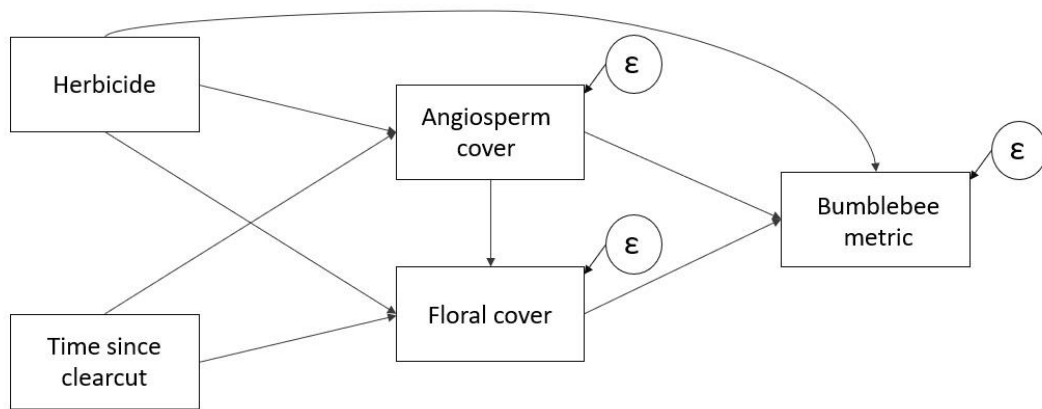


Figure 3. Causal pathway model for predicting covariance between the effect forest management operations (herbicide and time since clearcut) have on bumblebee community metrics (direct effect) and the effect changes in the vegetation community (angiosperm cover and floral cover) from forest management operations could have on the bumblebee community (indirect effect). Arrows predicts the expected direction of the effect. Epsilon represent the error term for each variable.

Results

Vegetation community change

A total of 88 plant species were recorded between sprayed and unsprayed blocks, of which 6 species (*Cirsium vulgare* [Petraik, 1912], *Tussilago farfara* [Linnaeus, 1753], *Galeopsis bifida* [Boenninghausen, 1824], *Veronica officinalis* [Linnaeus, 1753], *Fallopia convolvulus* [Löve, 1970], *Rumex acetosella* [Linnaeus, 1753]) were classified as introduced species according to the Vascular Plants of Canada database (Brouillet *et al.*, 2010). The cover of plants in flower was significantly higher in spray blocks, but angiosperm cover was similar between sprayed and unsprayed blocks (Table 3).

Table 3. Comparison of vegetation community variables between sprayed and unsprayed blocks. Percentages represent average cover for each variable within each treatment. Treatment comparisons are given using Wilcoxon sum rank and significant results ($p < 0.05$) are in bold. Effect size is represented by r .

	Sprayed	Unsprayed	W	Z	p	95% CI	r
Angiosperm cover	27.70%	27.48%	128	0.44	0.68	[-8.52 15.38]	0.07
Floral cover	56.91%	8.03%	24	-3.74	< 0.001	[-62.5 -27.5]	0.67

Bumblebee community metrics

A total of 933 bumblebee individuals were collected, representing eight species (Figure 4). Of these species two, *Bombus flavifrons* (Fabricius, 1793) and *B. impatiens* (Cresson, 1863) were only caught in unsprayed blocks. *Bombus ternarius* (Say, 1837) was the most common species caught ($n = 598$), followed by *B. borealis* (Kirby, 1837; $n = 260$). A total of 743 bumblebee individuals were caught within spray blocks and 199 individuals were caught in unsprayed blocks (Figure 5).

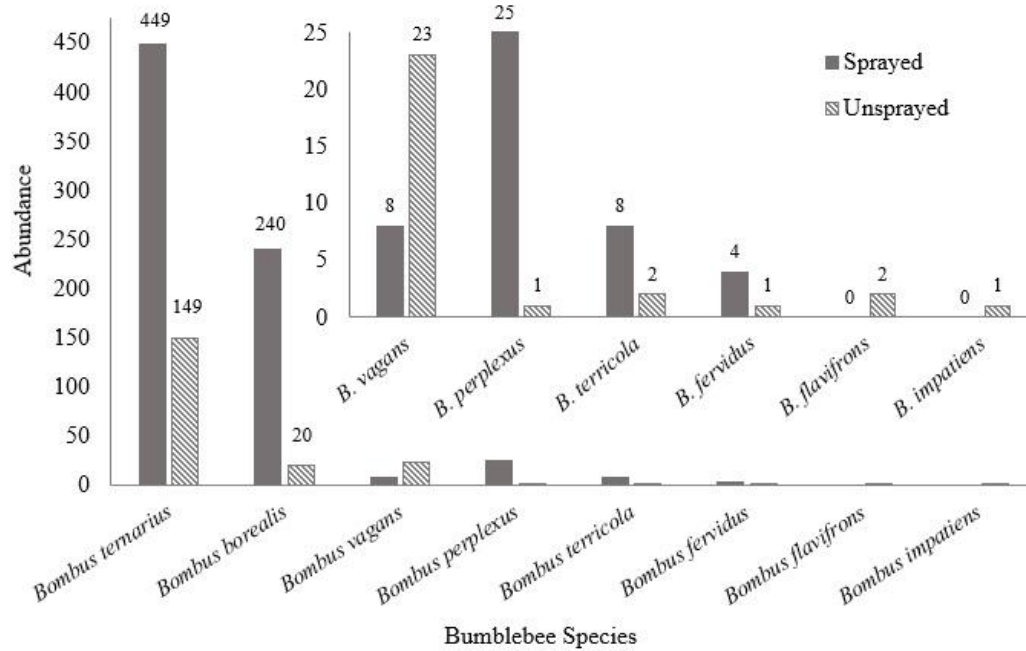


Figure 4. Total abundance of *Bombus* spp. caught in sprayed and unsprayed harvest blocks. Species are labeled in descending order based on total individuals caught for each species. Insert graph is used to visualize less abundant species. Individual counts are above each bar.

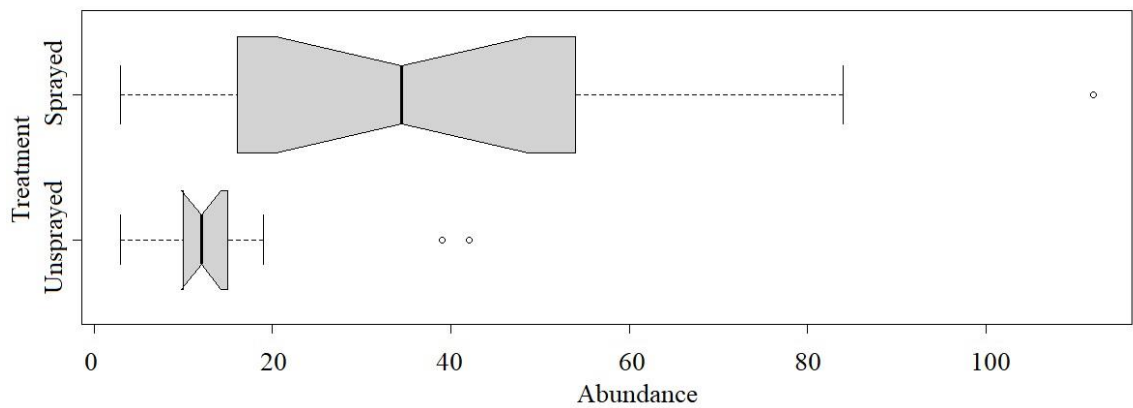


Figure 5. Distribution of abundance among caught *Bombus* spp. within sprayed and unsprayed blocks.

The relationship between treatment (sprayed or unsprayed) and the five community metrics measures produced medium (≥ 0.3) to large (≥ 0.5) effect sizes but

only total abundance and Shannon Weaver diversity were statistically significant ($p < 0.05$, Table 4). Bumblebee abundance and Shannon Weaver diversity was significantly higher in sprayed blocks than in unsprayed blocks.

Table 4. Bumblebee abundance, richness, evenness, Shannon's diversity index, Simpson's diversity index and rarefaction scores for sprayed and unsprayed blocks. Treatment comparisons are given using Wilcoxon sum rank and significant results ($p < .05$) are in bold. Effect size is represented by r .

	Sprayed	Unsprayed	W	Z	p	95% CI	r
Abundance	734	199	45.5	-2.86	0.03	[-39 -6]	0.51
Richness	6	8	69.0	-1.99	0.053	[-2 0]	0.36
Evenness	0.29	0.20	74.0	-1.73	0.09	[-0.19 0.15]	0.31
Shannon Weaver Diversity Index (H')	0.91	0.87	66.0	-2.05	0.04	[-0.65 0]	0.37
Simpson's Diversity Index (D ₁)	0.52	0.42	69.0	-1.93	0.06	[-0.37 0]	0.35

Effects from forest herbicide use

The five SEM models that address effects on bumblebee abundance, richness, evenness, and the two diversity indices provided non-significant χ^2 values ($p > 0.05$; Table 5). Therefore, the proposed causal models are able to predict covariance in the dataset, as exogenous (herbicide application and time since harvest) and mediator endogenous (angiosperm coverage and floral resources) variables are influencing the bumblebee community simultaneously. Generally, the direct effect of herbicide application did not significantly affect bumblebee abundance, richness, evenness or the two diversity measures ($p > 0.05$). Rather, I found that effects on the bumblebee community were significant when mediated through forest management effects on the vegetation community.

Table 5. Model fit statistics for each bumblebee community response variable. Fit statistics include Chi-squared (χ^2), root mean square error of approximation (RMSEA) and the comparative fit index (CFI). Fit statistics indicate non-significance in all models, meaning the causal models are able to predict covariance relationships in the dataset.

Response Variable	<i>df</i>	χ^2 (p)	RMSEA (p)	CFI
Abundance	1	1.28 (0.26)	0.10 (0.28)	0.99
Richness	1	0.25 (0.62)	0.00 (0.63)	1.00
Evenness	1	2.20 (0.14)	0.20 (0.15)	0.96
Shannon Diversity	1	1.60 (0.21)	0.14 (0.22)	0.98
Simpson Diversity	1	2.23 (0.14)	0.20 (0.15)	0.96

Effect of forest management on the vegetation community

I found herbicide application and time since harvest had contrasting effects on the vegetation community, but not all effects were significant at explaining covariance among exogenous and endogenous variables (Figure 6). Herbicide application had a strong positive effect ($p < 0.01$) on floral cover, and time since harvest had a weaker negative effect ($p = 0.016$) on floral cover. Neither herbicide application ($p = 0.7$) or time since harvest ($p = 0.5$) had a significant effect on angiosperm coverage.

Interestingly, angiosperm coverage within the transects did not influence the measure of floral cover in the study plot ($p = 0.4$).

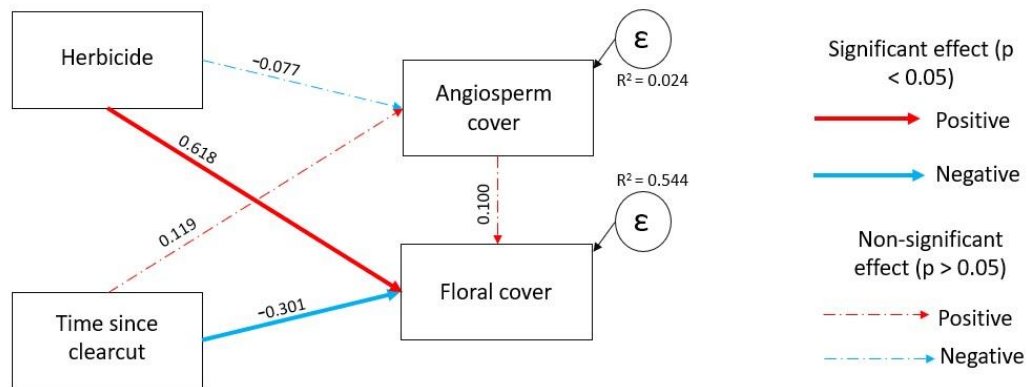


Figure 6. Summary of results for the structural equations model (SEM) for the effects of forest management on the vegetation community. The model is observing the direct effects of forest management activities (treatment and time) on angiosperm coverage and availability of floral resources. Arrows indicate the direction of the effect, and arrow colour indicates the type of effect: red is a positive effect and blue is negative. Line style represents significance of effect: dashed lines are non-significant ($p > 0.05$) and solid lines are significant ($p < 0.05$). Epsilon represents the error term for each tested variable and the amount of error is represented by R^2 values. The standardized coefficients are given for direct effects and are shown on each arrow.

Effects of forest management activities on the bumblebee community

Floral cover was generally a stronger predictor of the bumblebee community than angiosperm cover. Increases in floral cover had positive effects on bumblebee abundance ($p = 0.004$), richness ($p = 0.017$), evenness ($p = 0.016$, Figure 7, Appendix 1-3) and the two diversity indices (H' $p = 0.009$, D_1 $p = 0.007$, Figure 8, Appendix 5 & 6). In contrast, angiosperm cover had a weaker positive effect on bumblebee richness ($p = 0.021$, Figure 7, Appendix 3) and the two diversity measures (H' $p = 0.012$, $D_1 = 0.022$, Figure 8, Appendix 5 & 6) but did not predict abundance or evenness (Figure 7, Appendix 1 & 2).

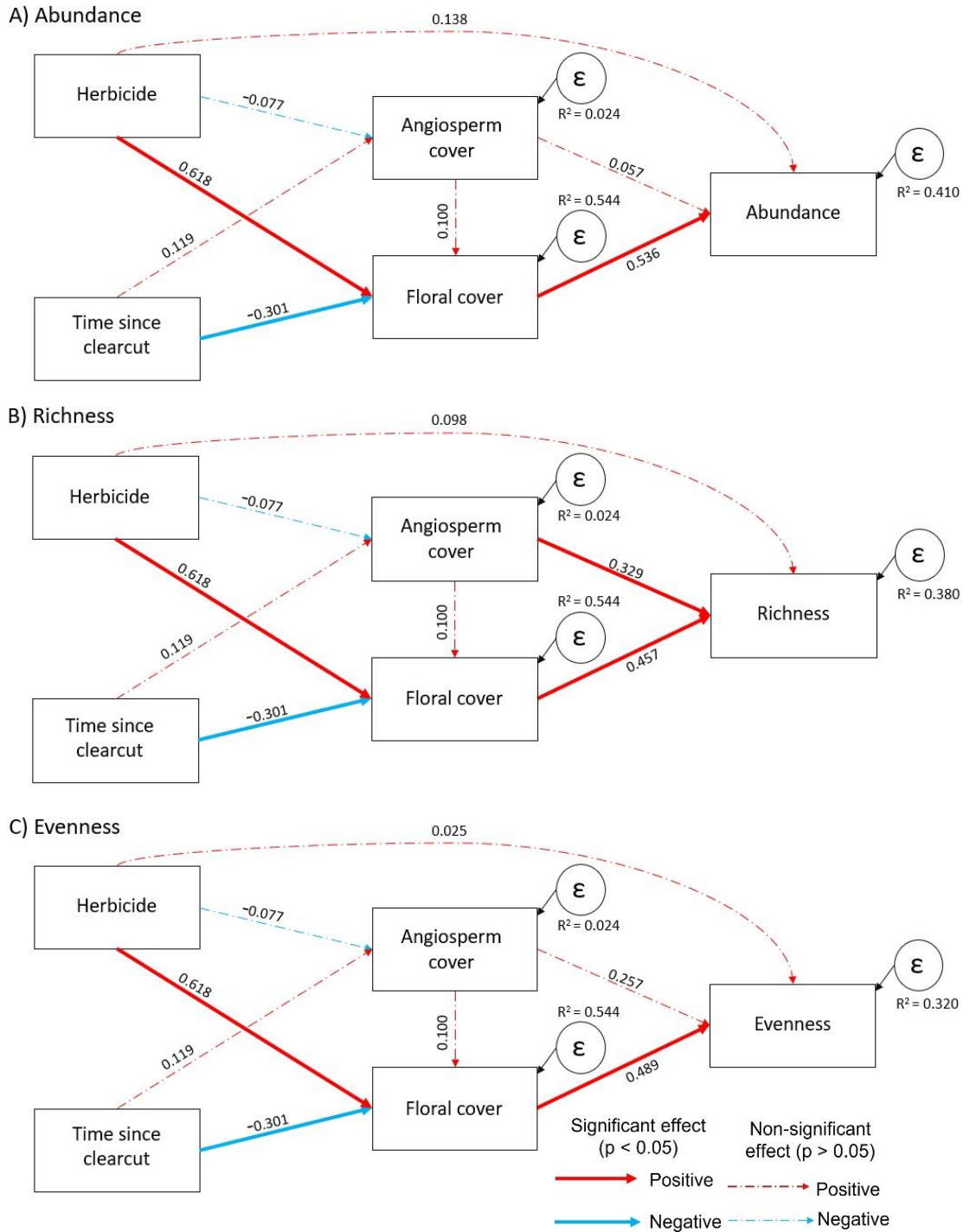


Figure 7. Summary of results for the structural equations models (SEM) for the effect of forest management on bumblebee community metrics. Models highlight the direct effects of forest management activities (treatment and time) and vegetation community variables (angiosperm coverage and floral resources) on (A) bumblebee abundance, (B)

bumblebee richness, and (C) bumblebee evenness. Arrows indicate the direction of the effect, and arrow colour indicates the type of effect: red is a positive effect and blue is negative. Line style represents significance of effect: dashed lines are non-significant ($p > 0.05$) and solid lines are significant ($p < 0.05$). Epsilon represents the error term for each tested variable and the amount of error is represented by R^2 values. The standardized coefficients are given for direct effects and are shown on each arrow.

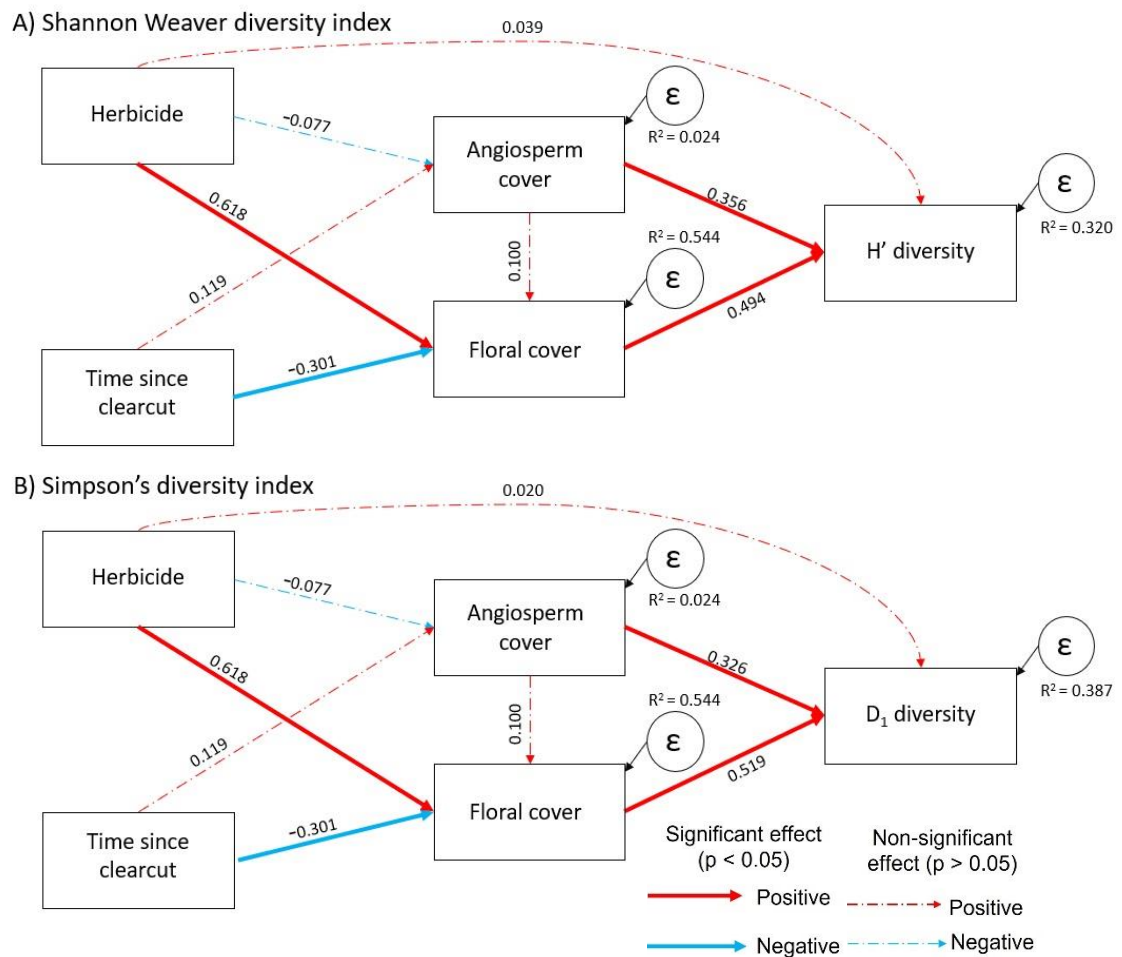


Figure 8. Summary of results for the structural equations model (SEM) for the effect of forest management on bumblebee diversity. Models highlight the direct effects of forest management activities (treatment and time) and vegetation community variables (angiosperm coverage and floral resources) on (A) Shannon Weaver (H') and (B) Simpson's (D_1) diversity. Arrows indicate the direction of the effect, and arrow colour indicates the type of effect: red is a positive effect and blue is negative. Line style represents significance of effect: dashed lines are non-significant ($p > 0.05$) and solid lines are significant ($p < 0.05$). Epsilon represents the error term for each tested variable

and the amount of error is represented by R^2 values. The standardized coefficients are given for direct effects and are shown on each arrow.

In all five models, the total indirect effect was significant ($\beta > 1$, $p < 0.02$), but the direct effect of treatment on bumblebee abundance, richness, evenness and diversity was not significant (Appendix 1-5). However, the overall effect (direct + indirect pathways) was significant in all models and produced a strong positive effect indicating that significant and non-significant pathways were important in predicting the overall effect on the bumblebee community. The positive effect herbicide had on floral cover always produced a positive indirect effect on the bumblebee community, but time since clearcut's direct negative effect on floral cover did not indirectly affect the bumblebee community. Interestingly, mediation through angiosperm cover alone was not a significant pathway, but when the effect was mediated through both angiosperm cover and floral cover a significant positive effect was observed on the bumblebee community.

Discussion

Overall, there were no observed direct effects on the bumblebee community from herbicide application. In fact, in all five models the relationship between herbicide and bumblebee abundance, richness, evenness, Shannon Weaver diversity and Simpson diversity had a non-significant positive effect (Figure 7; Figure 8). Data for this project was collected greater than 1 year post application which could be influencing the non-significant effect, but even though the relationship is non-significant it is interesting that the predicted effect is positive. Furthermore, a significant positive effect was observed when mediated through changes in the floral community following spray. Blocks that

were treated with a glyphosate-based herbicide application had significantly more plants in flower than those that were not sprayed (Table 3). Spray blocks had higher bumblebee abundance, evenness and diversity, but richness was higher in unsprayed blocks. The mostly positive trends with herbicide application and the bumblebee community promote the inclusion of pollinator objectives within forest management plans.

Most studies that have addressed the effects of glyphosate on bees have found negative effects (Seide *et al.*, 2018; Vázquez *et al.*, 2018, 2020; Tomé *et al.*, 2020; Battisti *et al.*, 2021; Straw *et al.*, 2021). Ecologically relevant exposure to glyphosate has increased mortality in adult and larval stages (Seide *et al.*, 2018; Vázquez *et al.*, 2020; Battisti *et al.*, 2021) or had sublethal effects where cognitive ability, pathogen susceptibility and olfactory learning have been negatively affected (Zhu *et al.*, 2015; Seide *et al.*, 2018b; Vázquez *et al.*, 2018). If these effects were the case in this study, I would have expected to see a negative relationship between herbicide and bumblebee response. However, the relationship between herbicide and the bumblebee community was non-significant. The lack of significance between herbicide and the bumblebee community coupled with the higher abundance, evenness and diversity observed in spray blocks must be the result of mediated effects. Pesticide risk can be buffered by landscape effects (Williams *et al.*, 2012; Park *et al.*, 2015; Leclerc *et al.*, 2021), where landscape structure and/or changes in the system can influence the interaction between bees and pesticides (Park *et al.*, 2015). The changes within the vegetation community follow spray must be driving the positive response observed within the bumblebee community. The positive effect of herbicide on floral cover (Figure 6) and floral covers significant relationship with the bumblebee community (Figure 7; Figure 8) is buffering

the expected negative effect from herbicide to bumblebees. Furthermore, the result of increases in floral cover following spray positively influences bumblebee responses because it increases food resources in these areas.

The positive effect from floral cover to the bumblebee community is not surprising, but why the mediated path exists is. By design, forest management operations will have an effect on the vegetation community (Nyland, 2016; Thiffault, 2021) and the magnitude of effect will vary depending on the management activity (Figure 5). Major disturbances, such as a harvest, are going to drastically change the vegetative structure from mature growth to early successional species (Nyland, 2016) and this will create habitat that bumblebees prefer to use (Hanula *et al.*, 2016b; Mola *et al.*, 2021; Smith *et al.*, 2021b). The transition from mature growth to early successional stand is going to attract bumblebees into the area because the open warm habitat is preferred over closed cold habitat offered in denser canopy stands (Taki *et al.*, 2013; Hanula *et al.*, 2016; Mola *et al.*, 2021; Smith *et al.*, 2021). As the age of the stand increases the canopy will close over and preferred bumblebee habitat characteristics will be lost (Taki *et al.*, 2013). However, vegetation management strategies, such as herbicide could influence vegetation structure enough in stands to extend the period of early successional habitat (Boateng *et al.*, 2000; Wagner *et al.*, 2011). In addition, controlling for competitive canopy species has shown to increase low herbaceous ground cover which is where many flowering producing species in the forest reside (Boateng *et al.*, 2000; Wagner *et al.*, 2011). Therefore, herbicide application is able to influence success of food resources in managed stands and through reducing overhead competition is able to promote

suitable bumblebee habitat (Wagner *et al.*, 2011; Williams *et al.*, 2012; Smith *et al.*, 2021; Xiao *et al.*, unpub.).

Floral cover produced a strong positive effect on all bumblebee community metrics of abundance, richness, evenness and diversity (Figure 7; Figure 8). Interestingly, angiosperm cover only predicted richness and the two diversity indices (Figure 7; Figure 8). The two vegetation variables measure food resources at a site, but their temporal relationships differ. Floral cover was estimated the day blue vane traps were set and angiosperm cover was calculated from the vegetation survey cover results and did not account for bloom status. The temporal variation in the variables partitions the results observed between the vegetation community and five bumblebee community responses. Floral cover which describes the cover of resources at a particular time will influence the attractiveness of a site at a specific point in time, where higher amounts of floral cover will likely attract more bees to come forage in that area (Cartar, 2004; Williams *et al.*, 2012; Goulson *et al.*, 2015). In comparison, angiosperm cover which describes the potential cover of food resources at a site will influence to some degree the suitability of the site (Cartar, 2004; Williams *et al.*, 2012). The difference between current attractiveness (floral cover) and overall attractiveness (angiosperm cover) influences how the bumblebee community metrics are going to respond. Higher amounts of plants in flower at a given time are going to influence the number of bumblebees in the area (abundance), but also how many individuals for each species can be there (evenness) because the more resources available the more individuals an area can support (Cartar, 2004; Goulson *et al.*, 2015). Suitability of an area is going to determine what kinds of species are there (richness), but also how species can be supported

(diversity) (Williams *et al.*, 2012). Therefore, current attractiveness (floral cover) at a site will influence all bumblebee community metrics because it attracts bees into an area and the availability of food resources (cover estimate) will influence how many of each species (evenness) can be supported there at that time, but also what species are there (richness and diversity) (Williams *et al.*, 2012). Overall attractiveness (angiosperm cover) at a site will have less of an effect on how many individuals were caught because it addresses quality of habitat, such that it will describe what kind of species are there but does not influence their foraging habitats at the time of trapping (Cartar, 2004; Williams *et al.*, 2012).

Herbicide and time since harvest did not significantly influence angiosperm cover and angiosperm cover did not have a significant relationship with floral cover (Figure 6). However, when assessed together the relationships between forest management and the vegetation community variables produced significant positive effects on bumblebee abundance, richness, evenness, and the two diversity measures (Appendix 1-5). With the lack of significance between direct relationships from forest management to angiosperm cover (Figure 6) and angiosperm cover's limited influence on the bumblebee community (Figure 7; Figure 8), it begs the question of what could be driving the mediated effect from angiosperm cover and floral cover to the bumblebee community from forest management. One possible explanation could be the diversity of flowering phenologies offered within forested areas (Proesmans *et al.*, 2018; Mola *et al.*, 2021). Forested sites generally support a wider range of flowering phenologies, where bloom periods span a greater period of time than those in open grassland habitat, but forests have lower overall plant diversity (Taki *et al.*, 2013; Mola *et al.*, 2021). The

tradeoff between lower plant diversity for longer bloom periods could be important for bumblebees as this genus has a long foraging season that requires floral resources in early spring into late fall to support current and future colonies (Mola *et al.*, 2021). Because of their long foraging season, bumblebees are usually classified as floral generalists, meaning they use a variety of flowering species throughout the active season (Michener, 2000; Williams *et al.*, 2014). Forested areas could be beneficial in supporting this long foraging season as angiosperms in these areas have some of the earliest and latest blooms in comparisons to other pollinator habitat (Proesmans *et al.*, 2018; Mola *et al.*, 2021). Future work should aim to address differences in flowering phenologies between various degrees of management (i.e. sprayed or not sprayed) to tease apart relationships between bees and angiosperms within managed forests.

The study has an unbalanced design, where 7 sites (six sprayed and one unsprayed) represented the Valley Lowlands ecoregion. Ecoregion was not included in the models as there was not biological justification to include it as an exogenous variable. Bee community data can be scaled up to describe community distinctions between ecoregions to facilitate comparisons and differences (Strange & Tripodi, 2019). However, the result is not because of the ecoregion itself, but rather the habitat types that are offered within the ecoregion (Strange & Tripodi, 2019). Thus, for inclusion in the model a habitat characteristic variable would better relate to the bumblebee community than ecoregion. Additionally, the measured vegetation variables would not have significant influences from ecoregion, as ecoregion describes the types of plant species in the region but would not significantly influence cover of the present species (Zelazny, 2007; Jones *et al.*, 2009). Adding of another variable to the model would also reduce

power as additional relationships would have been tested from a limited sample size ($n = 31$). However, to ensure the observed trends were not resulting from an ecoregion effect, all five models were run with data from the Eastern Lowlands ecoregion generating a balanced design that included 24 sites (sprayed = 12, unsprayed = 12). The results followed the same trends that were observed from the complete dataset ($n = 31$), but with the reduced sample size there was not enough power and significant relationships were lost in most cases.

Conclusions

Overall, forest herbicide use did not affect the bumblebee community but changes in the vegetation community from vegetation management had an indirect positive effect on the bumblebee community. The lack of direct effects from herbicide application are likely due to the timing of the study, where data collection occurred greater than 1 year post herbicide application. Direct effects are most likely to occur immediately after herbicide application when it is present in the environment at concentrations which could have an effect on non-targets species (Stanley & Preetha, 2016; Battisti *et al.*, 2021; Weidenmüller *et al.*, 2022). Future research should consider this when addressing the impact of forest herbicide use on non-target organisms to address immediate and long term direct and indirect effects.

Through the creation of early successional stands and applications of various vegetation management strategies, managed forests could help boost pollinator habitat within currently under represented areas of pollinator conservation (Taki *et al.*, 2013; Mola *et al.*, 2021). Vegetation management, such as herbicide, to control for competitive

species is also benefiting plant species used by bumblebees which positively effects bumblebee abundance, richness, evenness and diversity from increases in food resources. The findings from this study will help to bridge the gap in knowledge of pollinators within the Acadian Forest and more importantly, how forest management operations are influencing pollinators within these areas.

Bibliography

- Abraham, J., Benhotons, G.S., Krampah, I., Tagba, J., Amisshah, C. & Abraham, J.D. (2018) Commercially formulated glyphosate can kill non-target pollinator bees under laboratory conditions. *Entomologia Experimentalis et Applicata*, 166, 695–702.
- Aizen, M.A., Garibaldi, L.A., Cunningham, S.A. & Klein, A.M. (2009) How much does agriculture depend on pollinators? Lessons from long-term trends in crop production. *Annals of Botany*, 103, 1579–1588.
- Arena, M. & Sgolastra, F. (2014) A meta-analysis comparing the sensitivity of bees to pesticides. *Ecotoxicology (London, England)*, 23, 324–334.
- Ascher, J.S. & Pickering, J. (2020) Discover Life bee species guide and world checklist (Hymenoptera: Apoidea: Anthophila). [WWW Document]. URL http://www.discoverlife.org/mp/20q?guide=Apoidea_species [accessed on .
- Balbuena, M.S., Tison, L., Hahn, M.-L., Greggers, U., Menzel, R. & Farina, W.M. (2015) Effects of sublethal doses of glyphosate on honeybee navigation. *Journal of Experimental Biology*, 218, 2799–2805.
- Battisti, L., Potrich, M., Sampaio, A.R., Castilhos Ghisi, N. de, Costa-Maia, F.M., Abati, R., *et al.* (2021a) Is glyphosate toxic to bees? A meta-analytical review. *Science of The Total Environment*, 767, 145397.
- Bell, F.W. & Newmaster, S.G. (2002) The effects of silvicultural disturbances on the diversity of seed-producing plants in the boreal mixedwood forest. *Canadian Journal of Forest Research*, 32, 1180–1191.
- Benbrook, C.M. (2016) Trends in glyphosate herbicide use in the United States and globally. *Environmental Sciences Europe*, 28, 3.
- Blot, N., Veillat, L., Rouzé, R. & Delatte, H. (2019) Glyphosate, but not its metabolite AMPA, alters the honeybee gut microbiota. *PLOS ONE*, 14, e0215466.
- Boateng, J.O., Haeussler, S. & Bedford, L. (2000) Boreal Plant Community Diversity 10 Years After Glyphosate Treatment. *Western Journal of Applied Forestry*, 15, 15–26.
- Braun-blanquet, J. (1932) Plant sociology. The study of plant communities. First ed. *Plant sociology. The study of plant communities. First ed.*
- Brooks, D.R. & Nocera, J.J. (2020) Bumble bee (*Bombus* spp.) diversity differs between forested wetlands and clearcuts in the Acadian forest. *Canadian Journal of Forest Research*, 50, 1399–1404.
- Brouillet, L., Coursol, F., Meades, S.J., Favreau, M., Anions, M., Bélisle, P., *et al.* (2010) Database of Vascular Plants of Canada (VASCAN) [WWW Document]. URL <https://data.canadensys.net/vascan/search?lang=en> [accessed on 2010].
- Canada, H. (2021) PMRA Guidance Document, A Framework for Risk Assessment and Risk Management of Pest Control Products [WWW Document]. URL <https://www.canada.ca/en/health-canada/services/consumer-product-safety/reports-publications/pesticides-pest-management/policies-guidelines/risk-management-pest-control-products.html> [accessed on 3 February 2021].

- Cartar, R.V. (2004) Resource Tracking by Bumble Bees: Responses to Plant-Level Differences in Quality. *Ecology*, 85, 2764–2771.
- Cooper, H. & Hedges, L.V. (1993) *The Handbook of Research Synthesis*. Russell Sage Foundation.
- Cullen, M.G., Thompson, L.J., Carolan, J.C., Stout, J.C. & Stanley, D.A. (2019) Fungicides, herbicides and bees: A systematic review of existing research and methods. *PLOS ONE*, 14, e0225743.
- Daly, A.J., Baetens, J.M. & De Baets, B. (2018) Ecological Diversity: Measuring the Unmeasurable. *Mathematics*, 6, 119.
- De Palma, A., Abrahamczyk, S., Aizen, M.A., Albrecht, M., Basset, Y., Bates, A., *et al.* (2016) Predicting bee community responses to land-use changes: Effects of geographic and taxonomic biases. *Scientific Reports*, 6, 31153.
- Díaz, S.M., Settele, J., Brondízio, E., Ngo, H., Guèze, M., Agard, J., *et al.* (2019) *The global assessment report on biodiversity and ecosystem services: Summary for policy makers*. Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services.
- Droege, S., Engler, J.D., Sellers, E. & O'Brien, L.E. (2016) *National Protocol Framework for the Inventory and Monitoring of Bees*. Fort Collins, Colorado.
- Duke, S.O. & Powles, S.B. (2008) Glyphosate: a once-in-a-century herbicide. *Pest Management Science*, 64, 319–325.
- Edge, C.B., Baker, L.F., Lanctôt, C.M., Melvin, S.D., Gahl, M.K., Kurban, M., *et al.* (2020) Compensatory indirect effects of an herbicide on wetland communities. *Science of The Total Environment*, 718, 137254.
- EFSA. (2015) Conclusion on the peer review of the pesticide risk assessment of the active substance glyphosate. *EFSA Journal*, 13, 4302.
- FAO and UNEP. (2020) *The State of the World's Forests 2020: Forests, biodiversity and people*. The State of the World's Forests (SOFO). FAO and UNEP, Rome, Italy.
- Franklin, E.L. & Raine, N.E. (2019) Moving beyond honeybee-centric pesticide risk assessments to protect all pollinators. *Nature Ecology & Evolution*, 3, 1373–1375.
- Freedman, B. (1990) Controversy over the use of herbicides in forestry, with particular reference to glyphosate usage. *Environmental Carcinogenesis Reviews*, 8, 277–286.
- Freedman, B., Morash, R. & MacKinnon, D. (1993) Short-term changes in vegetation after the silvicultural spraying of glyphosate herbicide onto regenerating clearcuts in Nova Scotia, Canada. *Canadian Journal of Forest Research*, 23, 2300–2311.
- Goulson, D., Nicholls, E., Botías, C. & Rotheray, E.L. (2015) Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. *Science*, 347, 1255957.
- Hanula, J.L., Ulyshen, M.D. & Horn, S. (2016) Conserving Pollinators in North American Forests: A Review. *Natural Areas Journal*, 36, 427–439.

- Hedlund, J., Longo, S.B. & York, R. (2020) Agriculture, Pesticide Use, and Economic Development: A Global Examination (1990–2014). *Rural Sociology*, 85, 519–544.
- Helander, M., Saloniemi, I. & Saikkonen, K. (2012) Glyphosate in northern ecosystems. *Trends in Plant Science*, 17, 569–574.
- Hothorn, T., Hornik, K., Wiel, M.A. van de & Zeileis, A. (2006) A Lego System for Conditional Inference. *The American Statistician*, 60, 257–263.
- Jones, P.D., Hanberry, B. & Demarais, S. (2009) Stand-Level Wildlife Habitat Features and Biodiversity in Southern Pine Forests: A Review. *Journal of Forestry*, 107, 398–404.
- Kremen, C. & Merenlender, A.M. (2018) Landscapes that work for biodiversity and people. *Science*, 362.
- Laigle, I., Moretti, M., Rousseau, L., Gravel, D., Venier, L., Handa, I.T., *et al.* (2021) Direct and Indirect Effects of Forest Anthropogenic Disturbance on Above and Below Ground Communities and Litter Decomposition. *Ecosystems*, 24, 1716–1737.
- Leclerc, M.-A.F., Daniels, L.D. & Carroll, A.L. (2021) Managing Wildlife Habitat: Complex Interactions With Biotic and Abiotic Disturbances. *Frontiers in Ecology and Evolution*, 9.
- Lewis, K.A., Tzilivakis, J., Warner, D.J. & Green, A. (2016) An international database for pesticide risk assessments and management. *Human and Ecological Risk Assessment: An International Journal*, 22, 1050–1064.
- Mahmood, I., Imadi, S.R., Shazadi, K., Gul, A. & Hakeem, K.R. (2016) Effects of Pesticides on Environment. In *Plant, Soil and Microbes: Volume 1: Implications in Crop Science* (ed. by Hakeem, K.R., Akhtar, M.S. & Abdullah, S.N.A.). Springer International Publishing, Cham, pp. 253–269.
- Michener, C.D. (2000) *The Bees of the World*. JHU Press.
- Miller, K.V. & Miller, J.H. (2004) Forestry herbicide influences on biodiversity and wildlife habitat in southern forests. *Wildlife Society Bulletin*, 32, 1049–1060.
- Mola, J.M., Hemberger, J., Kochanski, J., Richardson, L.L. & Pearse, I.S. (2021) The Importance of Forests in Bumble Bee Biology and Conservation. *BioScience*, 71, 1234–1248.
- Motta, E.V.S., Raymann, K. & Moran, N.A. (2018) Glyphosate perturbs the gut microbiota of honey bees. *Proceedings of the National Academy of Sciences*, 115, 10305–10310.
- Natural Resources Canada. (2020) The State of Canada’s Forests - Annual Report 2020, 96.
- Nyland, R.D. (2016) *Silviculture: Concepts and Applications, Third Edition*. Waveland Press.
- Odemer, R., Alkassab, A.T., Bischoff, G., Frommberger, M., Wernecke, A., Wirtz, I.P., *et al.* (2020) Chronic High Glyphosate Exposure Delays Individual Worker Bee (*Apis mellifera* L.) Development under Field Conditions. *Insects (2075-4450)*, 11, 664–664.
- O’Hara, K. (2006) Multiaged forest stands for protection forests: Concepts and applications. *Forest Snow and Landscape Research*, 80, 45–55.

- Oksanen, J. (2020) Vegan: ecological diversity, 12.
- Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., *et al.* (2020) *vegan: Community Ecology Package*.
- Ollerton, J., Winfree, R. & Tarrant, S. (2011) How many flowering plants are pollinated by animals? *Oikos*, 120, 321–326.
- Park, M.G., Blitzer, E.J., Gibbs, J., Losey, J.E. & Danforth, B.N. (2015) Negative effects of pesticides on wild bee communities can be buffered by landscape context. *Proceedings of the Royal Society B: Biological Sciences*, 282, 20150299.
- Pielou, E.C. (1966) The measurement of diversity in different types of biological collections. *Journal of Theoretical Biology*, 13, 131–144.
- PMRA. (2015) Proposed Re-evaluation Decision PRVD2015-01, Glyphosate [WWW Document]. *aem*. URL <https://www.canada.ca/en/health-canada/services/consumer-product-safety/pesticides-pest-management/public/consultations/proposed-re-evaluation-decisions/2015/glyphosate/document.html> [accessed on 13 April 2015].
- PMRA. (2017) Re-evaluation Decision RVD2017-01, Glyphosate [WWW Document]. *aem*. URL <https://www.canada.ca/en/health-canada/services/consumer-product-safety/reports-publications/pesticides-pest-management/decisions-updates/registration-decision/2017/glyphosate-rvd-2017-01.html> [accessed on 28 July 2017].
- Proesmans, W., Bonte, D., Smagghe, G., Meeus, I. & Verheyen, K. (2018) Importance of forest fragments as pollinator habitat varies with season and guild. *Basic and Applied Ecology*.
- R Core Team. (2020) *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria.
- Richmond, M.E. (2018) Glyphosate: A review of its global use, environmental impact, and potential health effects on humans and other species. *Journal of Environmental Studies and Sciences*, 8, 416–434.
- Rolando, C.A., Baillie, B.R., Thompson, D.G. & Little, K.M. (2017) The Risks Associated with Glyphosate-Based Herbicide Use in Planted Forests. *Forests*, 8, 208.
- Rosseel, Y. (2012) lavaan: An R Package for Structural Equation Modeling. *Journal of Statistical Software*, 48, 1–36.
- Seide, V.E., Bernardes, R.C., Pereira, E.J.G. & Lima, M.A.P. (2018) Glyphosate is lethal and Cry toxins alter the development of the stingless bee *Melipona quadrifasciata*. *Environmental Pollution*, 243, 1854–1860.
- Sgolastra, F., Hinarejos, S., Pitts-Singer, T.L., Boyle, N.K., Joseph, T., Luckmann, J., *et al.* (2019) Pesticide Exposure Assessment Paradigm for Solitary Bees. *Environmental Entomology*, 48, 22–35.
- SGS Belgium S.A. (2018) *Forest sustainability in the province of New Brunswick, Canada* (No. 180373).
- Shannon, C.E. & Weaver, W. (1963) *The mathematical theory of communication*. University of Illinois Press, Urbana.

- Sharma, A., Kumar, V., Shahzad, B., Tanveer, M., Sidhu, G.P.S., Handa, N., *et al.* (2019) Worldwide pesticide usage and its impacts on ecosystem. *SN Applied Sciences*, 1, 1446.
- Simpson, E.H. (1949) Measurement of Diversity. *Nature*, 163, 688–688.
- Smith, C., Harrison, T., Gardner, J. & Winfree, R. (2021a) Forest-associated bee species persist amid forest loss and regrowth in eastern North America. *Biological Conservation*, 260, 109202.
- Smith, C., Harrison, T., Gardner, J. & Winfree, R. (2021b) Forest-associated bee species persist amid forest loss and regrowth in eastern North America. *Biological Conservation*, 260, 109202.
- Stanley, D.A., Garratt, M.P.D., Wickens, J.B., Wickens, V.J., Potts, S.G. & Raine, N.E. (2015) Neonicotinoid pesticide exposure impairs crop pollination services provided by bumblebees. *Nature*, 528, 548–550.
- Stanley, J. & Preetha, G. (2016) Pesticide Toxicity to Pollinators: Exposure, Toxicity and Risk Assessment Methodologies. In *Pesticide Toxicity to Non-target Organisms: Exposure, Toxicity and Risk Assessment Methodologies* (ed. by Stanley, J. & Preetha, G.). Springer Netherlands, Dordrecht, pp. 153–228.
- Strange, J.P. & Tripodi, A.D. (2019) Characterizing bumble bee (*Bombus*) communities in the United States and assessing a conservation monitoring method. *Ecology and Evolution*, 9, 1061–1069.
- Straw, E.A. & Brown, M.J.F. (2021) No evidence of effects or interaction between the widely used herbicide, glyphosate, and a common parasite in bumble bees. *PeerJ*, 9, e12486.
- Straw, E.A., Carpentier, E.N. & Brown, M.J.F. (2021) Roundup causes high levels of mortality following contact exposure in bumble bees. *Journal of Applied Ecology*, 58, 1167–1176.
- Sullivan, T.P. & Sullivan, D.S. (2003) Vegetation management and ecosystem disturbance: impact of glyphosate herbicide on plant and animal diversity in terrestrial systems. *Environmental Reviews*, 11, 37.
- Taki, H., Okochi, I., Okabe, K., Inoue, T., Goto, H., Matsumura, T., *et al.* (2013) Succession Influences Wild Bees in a Temperate Forest Landscape: The Value of Early Successional Stages in Naturally Regenerated and Planted Forests. *PLOS ONE*, 8, e56678.
- Thiffault, N. (2021) *Forest vegetation management: Key functions, alternatives to chemical herbicides and challenges. Information report FI-X-023.*
- Thiffault, N. & Roy, V. (2011) Living without herbicides in Québec (Canada): historical context, current strategy, research and challenges in forest vegetation management. *European Journal of Forest Research*, 130, 117–133.
- Tomé, H.V.V., Schmehl, D.R., Wedde, A.E., Godoy, R.S.M., Ravaiano, S.V., Guedes, R.N.C., *et al.* (2020) Frequently encountered pesticides can cause multiple disorders in developing worker honey bees. *Environmental Pollution*, 256, 113420.
- US EPA. (2020) Glyphosate: Interim Registration Review Decision Case Number 0178, 36.
- Vanbergen, A.J. & Initiative, the I.P. (2013) Threats to an ecosystem service: pressures on pollinators. *Frontiers in Ecology and the Environment*, 11, 251–259.

- Vázquez, D.E., Iliina, N., Pagano, E.A., Zavala, J.A. & Farina, W.M. (2018) Glyphosate affects the larval development of honey bees depending on the susceptibility of colonies. *PLOS ONE*, 13, e0205074.
- Vázquez, D.E., Latorre-Estivalis, J.M., Ons, S. & Farina, W.M. (2020) Chronic exposure to glyphosate induces transcriptional changes in honey bee larva: A toxicogenomic study. *Environmental Pollution*, 261, 114148.
- Wagner, S., Fischer, H. & Huth, F. (2011) Canopy effects on vegetation caused by harvesting and regeneration treatments. *European Journal of Forest Research*, 130, 17–40.
- Weidenmüller, A., Meltzer, A., Neupert, S., Schwarz, A. & Kleineidam, C. (2022) Glyphosate impairs collective thermoregulation in bumblebees. *Science*, 376, 1122–1126.
- Wikum, D.A. & Shanholtzer, G.F. (1978) Application of the Braun-Blanquet cover-abundance scale for vegetation analysis in land development studies. *Environmental Management*, 2, 323–329.
- Wilcoxon, F. (1945) Individual Comparisons by Ranking Methods. *Biometrics Bulletin*, 1, 80.
- Williams, N.M., Regetz, J. & Kremen, C. (2012) Landscape-scale resources promote colony growth but not reproductive performance of bumble bees. *Ecology*, 93, 1049–1058.
- Williams, P.H., Thorp, R.W., Richardson, L.L. & Colla, S.R. (2014) *Bumble Bees of North America: An Identification Guide*. Princeton University Press.
- Willis Chan, D.S., Prosser, R.S., Rodríguez-Gil, J.L. & Raine, N.E. (2019) Assessment of risk to hoary squash bees (*Peponapis pruinosa*) and other ground-nesting bees from systemic insecticides in agricultural soil. *Scientific Reports*, 9, 11870.
- Xiao, J., Yakimowski, S., Brown, M., Hartz, S., Parachnowitsch, A. & Edge, C. (unpub.) Changes to the vegetation community 20 years after herbicide use in the Acadian Forest.
- Zacharia, T.J. (2011) Ecological Effects of Pesticides. In *Pesticides in the Modern World - Risks and Benefits*, Dr. Margarita Stoytcheva (Ed.). InTech, p. 560.
- Zelazny, V.F. (2007) *Our Landscape Heritage: The Story of Ecological Land Classification in New Brunswick*. 2nd edn. New Brunswick Department of Natural Resources, Fredericton.
- Zhang, W. (2018) Global pesticide use: Profile, trend, cost / benefit and more, 8, 1–27.
- Zhu, Y.C., Adamczyk, J., Rinderer, T., Yao, J., Danka, R., Luttrell, R., *et al.* (2015) Spray Toxicity and Risk Potential of 42 Commonly Used Formulations of Row Crop Pesticides to Adult Honey Bees (Hymenoptera: Apidae). *Journal of Economic Entomology*, 108, 2640–2647.

Appendices

Appendix 1. SEM regression statistics for vegetation management effect of bumblebee abundance. Significant relationships are in bold ($p < 0.05$).

	Estimate	Standard Error	Z	p	Standardized Latent Variable Estimate	Standardized coefficient (β)
Regressions:						
Abundance ~						
Treatment	0.138	0.189	0.728	0.467	0.138	0.138
Angiosperm Coverage	0.057	0.140	0.404	0.682	0.057	0.057
Floral Resources	0.536	0.188	2.849	0.004	0.536	0.536
Angiosperm Coverage ~						
Treatment	-0.077	-0.425	-0.425	0.671	-0.077	-0.077
Time	0.119	0.653	0.653	0.514	0.119	0.119
Floral Resources ~						
Treatment	0.618	0.125	4.951	<0.001	0.618	0.618
Time	-0.301	0.125	-2.404	0.016	-0.301	-0.301
Angiosperm Coverage	0.100	0.123	0.817	0.414	0.100	0.100
Variances:						
Abundance	0.574	0.146	3.937	<0.001	0.574	0.593
Angiosperm Coverage	0.944	0.240	3.937	<0.001	0.944	0.976
Floral Resources	0.442	0.112	3.937	<0.001	0.442	0.456
R-squared:						
Abundance	0.407					
Angiosperm Coverage	0.024					
Floral Resources	0.544					
Defined parameters:						
Tr. > An. > A.	-0.004	0.015	-0.295	0.768	-0.004	-0.004
Ti. > An. > A.	0.007	0.020	0.347	0.729	0.007	0.007
Tr. > Fl. > A.	0.331	0.134	2.469	0.014	0.331	0.331
Ti. > Fl. > A.	-0.161	0.088	-1.837	0.066	-0.161	-0.161
Tr. > An. > Fl. > A.	0.528	0.189	2.791	0.005	0.528	0.528
Ti. > An. > Fl. > A.	0.548	0.189	2.890	0.004	0.548	0.548
Total indirect	1.247	0.450	2.774	0.006	1.247	1.247
Tr. > A.	0.138	0.189	0.728	0.467	0.138	0.138
Total effect	1.385	0.356	3.891	<0.001	1.385	1.385

Tr. = Treatment, Ti. = Time, An. = Angiosperm coverage, Fl. = Floral resources, A. = Abundance

Appendix 2. SEM regression statistics for vegetation management effect of bumblebee richness. Significant relationships are in bold ($p < 0.05$).

	Estimate	Standard Error	Z	p	Standardized Latent Variable Estimate	Standardized coefficient (β)
Regressions:						
Richness ~						
Treatment	0.098	0.193	0.501	0.610	0.098	0.098
Angiosperm Coverage	0.329	0.143	2.304	0.021	0.329	0.329
Floral Resources	0.457	0.192	2.380	0.017	0.457	0.457
Angiosperm Coverage ~						
Treatment	-0.077	-0.425	-0.425	0.671	-0.077	-0.077
Time	0.119	0.653	0.653	0.514	0.119	0.119
Floral Resources ~						
Treatment	0.618	0.125	4.951	<0.001	0.618	0.618
Time	-0.301	0.125	-2.404	0.016	-0.301	-0.301
Angiosperm Coverage	0.100	0.123	0.817	0.414	0.100	0.100
Variances:						
Richness	0.600	0.152	3.937	<0.001	0.600	0.620
Angiosperm Coverage	0.944	0.240	3.937	<0.001	0.944	0.976
Floral Resources	0.442	0.112	3.937	<0.001	0.442	0.456
R-squared:						
Richness	0.387					
Angiosperm Coverage	0.024					
Floral Resources	0.544					
Defined parameters:						
Tr. > An. > R.	-0.025	0.061	-0.418	0.676	-0.025	-0.025
Ti > An. > R.	0.039	0.062	0.628	0.530	0.039	0.039
Tr. > Fl. > R.	0.283	0.132	2.145	0.032	0.283	0.283
Ti > Fl. > R.	-0.138	0.081	-1.691	0.091	-0.138	-0.138
Tr. > An. > Fl. > R.	0.450	0.193	2.326	0.020	0.450	0.450
Ti > An. > Fl. > R.	0.469	0.194	2.424	0.015	0.469	0.469
Total indirect	1.078	0.470	2.294	0.022	1.078	1.078
Tr. > R.	0.098	0.193	0.510	0.610	0.098	0.098
Total effect	1.176	0.377	3.123	0.002	1.176	1.176

Tr. = Treatment, Ti. = Time, An. = Angiosperm coverage, Fl. = Floral resources, R. = Richness

Appendix 3. SEM regression statistics for vegetation management effect of bumblebee evenness. Significant relationships are in bold ($p < 0.05$).

	Estimate	Standard Error	Z	p	Standardized Latent Variable Estimate	Standardized coefficient (β)
Regressions:						
Evenness ~						
Treatment	0.025	0.202	0.102	0.900	0.025	0.025
Angiosperm Coverage	0.257	0.150	2.298	0.086	0.257	0.257
Floral Resources	0.489	0.201	2.718	0.015	0.489	0.489
Angiosperm Coverage ~						
Treatment	-0.077	-0.425	-0.425	0.671	-0.077	-0.077
Time	0.119	0.653	0.653	0.514	0.119	0.119
Floral Resources ~						
Treatment	0.618	0.125	4.951	<0.001	0.618	0.618
Time	-0.301	0.125	-2.404	0.016	-0.301	-0.301
Angiosperm Coverage	0.100	0.123	0.817	0.414	0.100	0.100
Variances:						
Evenness	0.658	0.167	3.937	<0.001	0.658	0.680
Angiosperm Coverage	0.944	0.240	3.937	<0.001	0.944	0.976
Floral Resources	0.442	0.112	3.937	<0.001	0.442	0.456
R-squared:						
Evenness	0.320					
Angiosperm Coverage	0.024					
Floral Resources	0.544					
Defined parameters:						
Tr. > An. > E.	-0.020	0.048	-0.412	0.680	-0.020	-0.020
Ti > An. > E.	0.031	0.050	0.610	0.542	0.031	0.031
Tr. > Fl. > E.	0.302	0.139	2.181	0.029	0.302	0.302
Ti > Fl. > E.	-0.147	0.086	-1.709	0.088	-0.147	-0.147
Tr. > An. > Fl. > E.	0.481	0.202	2.378	0.017	0.481	0.481
Ti > An. > Fl. > E.	0.501	0.203	2.471	0.013	0.501	0.501
Total indirect effect	1.147	0.486	2.360	0.018	1.147	1.147
Tr. > E.	0.025	0.202	0.125	0.900	0.025	0.025
Total effect	1.173	0.387	3.030	0.002	1.173	1.173

Tr. = Treatment, Ti. = Time, An. = Angiosperm coverage, Fl. = Floral resources, E. = Evenness

Appendix 4. SEM regression statistics for vegetation management effect of bumblebee Shannon Weaver diversity. Significant relationships are in bold ($p < 0.05$).

	Estimate	Standard Error	Z	p	Standardized Latent Variable Estimate	Standardized coefficient (β)
Regressions:						
Shannon's Diversity ~ Treatment	0.020	0.191	0.207	0.836	0.039	0.039
Angiosperm Coverage	0.356	0.141	2.519	0.012	0.356	0.356
Floral Resources	0.494	0.190	2.600	0.009	0.494	0.494
Angiosperm Coverage ~ Treatment	-0.077	-0.425	-0.425	0.671	-0.077	-0.077
Time	0.119	0.653	0.653	0.514	0.119	0.119
Floral Resources ~ Treatment	0.618	0.125	4.951	<0.001	0.618	0.618
Time	-0.301	0.125	-2.404	0.016	-0.301	-0.301
Angiosperm Coverage	0.100	0.123	0.817	0.414	0.100	0.100
Variances:						
Shannon's Diversity	0.587	0.149	3.937	<0.001	0.587	0.606
Angiosperm Coverage	0.944	0.240	3.937	<0.001	0.944	0.976
Floral Resources	0.442	0.112	3.937	<0.001	0.442	0.456
R-squared:						
Shannon's Diversity	0.394					
Angiosperm Coverage	0.024					
Floral Resources	0.544					
Defined parameters:						
Tr. > An. > H'	-0.027	0.066	-0.419	0.675	-0.027	-0.027
Ti > An. > H'	0.042	0.067	0.632	0.527	0.042	0.042
Tr. > Fl. > H'	0.305	0.133	2.302	0.021	0.305	0.305
Ti > Fl. > H'	-0.149	0.084	-1.765	0.078	-0.149	-0.149
Tr. > An. > Fl. > H'	0.486	0.191	2.545	0.011	0.486	0.486
Ti > An. > Fl. > H'	0.506	0.191	2.643	0.008	0.506	0.506
Total indirect effect	1.164	0.468	2.484	0.013	1.164	1.164
Tr. > H'	0.039	0.191	0.207	0.836	0.039	0.039
Total effect	1.203	0.377	3.189	0.001	1.203	1.203

Tr. = Treatment, Ti. = Time, An. = Angiosperm coverage, Fl. = Floral resources, H' = Shannon Weaver diversity

Appendix 5. SEM Regression statistics looking at the effect of vegetation management on bumblebee Simpson diversity. Significant relationships are in bold (p< 0.05)

	Estimate	Standard Error	Z	p	Standardized Latent Variable Estimate	Standardized coefficient (β)
Regressions:						
Simpson's Diversity ~						
Treatment	0.020	0.192	0.102	0.918	0.020	0.020
Angiosperm Coverage	0.326	0.142	2.298	0.022	0.326	0.326
Floral Resources	0.519	0.191	2.718	0.007	0.519	0.519
Angiosperm Coverage ~						
Treatment	-0.077	-0.425	-0.425	0.671	-0.077	-0.077
Time	0.119	0.653	0.653	0.514	0.119	0.119
Floral Resources ~						
Treatment	0.618	0.125	4.951	<0.001	0.618	0.618
Time	-0.301	0.125	-2.404	0.016	-0.301	-0.301
Angiosperm Coverage	0.100	0.123	0.817	0.414	0.100	0.100
Variances:						
Simpson's Diversity	0.593	0.151	3.937	<0.001	0.593	0.613
Angiosperm Coverage	0.944	0.240	3.937	<0.001	0.944	0.976
Floral Resources	0.442	0.112	3.937	<0.001	0.442	0.456
R-squared:						
Simpson's Diversity	0.387					
Angiosperm Coverage	0.024					
Floral Resources	0.544					
Defined parameters:						
Tr. > An. > D1	-0.025	0.060	-0.418	0.676	-0.025	-0.025
Ti > An. > D1	0.039	0.062	0.628	0.530	0.039	0.039
Tr. > Fl. > D1	0.321	0.135	2.383	0.017	0.321	0.321
Ti > Fl. > D1	-0.156	0.087	-1.801	0.072	-0.156	-0.156
Tr. > An. > Fl. > D1	0.512	0.192	2.662	0.008	0.512	0.512
Ti > An. > Fl. > D1	0.531	0.192	2.760	0.006	0.531	0.531
Total indirect effect	1.221	0.469	2.600	0.009	1.221	1.221
Tr. > D1	0.020	0.192	0.102	0.918	0.020	0.020
Total effect	1.240	0.377	3.287	0.001	1.240	1.240

Tr. = Treatment, Ti. = Time, An. = Angiosperm coverage, Fl. = Floral resources, D1 = Simpson diversity

Curriculum Vitae

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Universities attended (with dates and degrees obtained):

University of New Brunswick, 2019, Bachelor of Science in Environment and Natural Resource Management

Publications:

Edge, C. B., **Brown, M. I.**, Hertz, S., Thompson, D., Ritter, L., & Ramadoss, M. (2021). The persistence of glyphosate in vegetation one year after application. *Forests*, 12(5), 601–601. <https://doi.org/10.3390/f12050601>

Xiao, J., Yakimowski, S., **Brown, M.**, Hertz, S., Parachnowitsch, A. & Edge, C. (unpub.) Changes to the vegetation community 20 years after herbicide use in the Acadian Forest.

Conference Presentations:

Atlantic Forestry Centre Winter Speaker Series, 2021. “Response of pollinators to glyphosate application in managed forests of New Brunswick”

34th Annual Meeting of the Scandinavian Association for Pollinator Ecology, 2020. “Effect of Glyphosate-based herbicide use on flowering plant and pollinator communities in managed forests”

SERG International Workshop, 2020. “Response of pollinator communities to herbicide application”