The Tenants of Apple Orchards: Evaluating the Effects of Additional Nesting Habitat on Bee Populations

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Abstract

Identifying the resources that limit bee populations is essential both for bee conservation and pollination management in agroecosystems. Land-use change typically leads to decreased habitat availability for wild pollinators including loss of nesting habitat, which is an essential but often-overlooked resource for wild bees. Cavity-nesting bees, such as many *Osmia* spp. (Hymenoptera: Megachilidae), occupy holes in wood or reeds to build their nests; due to their nesting habits, they are frequently scarce in agricultural settings, although, under the right circumstances, these bees can be ideal pollinators of apple and other orchard crops. Artificial nesting structures ("bee hotels", "trap nests", or "nest boxes") are used to study cavity-nesting bees and have been posited as solutions for promoting bee conservation. To evaluate the effects of additional nesting habitat on the local abundance of *Osmia* spp., and on bees more generally, artificial nesting structures for cavity-nesting bees were installed at 24 sites in apple orchards around Ottawa in 2021 and 2022. Each site had two treatments: one with nest boxes, and one without (control). Transect walks were conducted to measure overall bee contact (including contact by *Osmia* spp.) with apple blossoms and, after the end of apple bloom, with flowers in the undergrowth and/or in shrubs. Numbers of apple buds and developing fruit were also recorded. *Osmia* spp. and overall bee numbers were both significantly higher in the treatment with nest boxes (44% and 15% higher, respectively, in 2021, and 113% and 47% higher, respectively, in 2022); however, there was no difference in fruit set (apple count/bud count) between the two treatments. Thus, nest boxes seem to locally increase *Osmia* spp. numbers as well as total bee numbers, but they have no apparent effect on apple yield, likely because apple production was not pollinator limited in the years of this study. These findings suggest that bee populations in apple orchards are limited by nesting resources, which has important implications for orchard management practices and bee conservation policy.

Key Words: Apple orchard, Cavity-nesting, Fruit set, Mason bees, Nesting habitat, Pollination, Resource limitation, Solitary bees, Trap-nest

Résumé

L'identification des ressources qui limitent les populations d'abeilles est essentielle pour la conservation des abeilles et la gestion de la pollinisation dans les agroécosystèmes. Les changements d’utilisation des terres entraînent généralement une diminution de la disponibilité d'habitat pour les pollinisateurs sauvages, y compris la perte d'habitat de nidification, une ressource essentielle mais souvent négligée pour les abeilles sauvages. Les abeilles nichant dans les cavités, comme *Osmia* spp. (Hymenoptera : Megachilidae), occupent des trous dans le bois ou les roseaux pour construire leurs nids ; en raison de leurs habitudes de nidification, elles sont souvent rares dans les milieux agricoles, bien que, dans les bonnes circonstances, ces abeilles puissent être des pollinisateurs idéaux pour les pommiers et autres récoltes des vergers. Les structures de nidification artificielles ("hôtels à abeilles", "nids pièges" ou "boîtes à nids") sont utilisées pour étudier les abeilles nichant dans des cavités et ont été proposées comme des solutions pour promouvoir la conservation des abeilles. Pour évaluer les effets d'installer des ressources de nidification supplémentaires sur l'abondance locale d'*Osmia* spp., et sur les abeilles en général, des structures de nidification artificielles pour les abeilles nichant dans des cavités ont été installées à 24 sites dans des vergers de pommiers autour d'Ottawa en 2021 et 2022. Chaque site avait deux traitements : un avec des boîtes de nidification et un sans boîtes de nidification (témoin). Des marches transversales ont été effectuées pour mesurer le contact global des abeilles (y compris le contact d'*Osmia* spp.) avec les fleurs de pommiers et, après la fin de la floraison des pommiers, avec les fleurs des sous-bois et/ou des arbustes. Le nombre de bourgeons de pommiers et de fruits en développement ont également été enregistrés. Le nombre d’*Osmia* spp. et le nombre global d'abeilles était tous deux significativement plus élevé dans le traitement avec les nids (44 % et 15 % plus élevé, respectivement, en 2021, et 113 % et 47 % plus élevé, respectivement, en 2022) ; cependant, il n'y avait aucune différence dans la mise à fruits (nombre de pommes/nombre de bourgeons) entre les deux traitements. Ainsi, les nids semblent augmenter localement le nombre d’*Osmia* ainsi que le nombre total d'abeilles, mais ils n'ont aucun effet apparent sur le rendement en pommes, probablement parce que la production de pommes n'était pas limitée par les pollinisateurs au cours des années de cette étude. Ces résultats suggèrent que les populations d'abeilles dans les vergers de pommiers sont limitées par les ressources de nidification, ce qui a des implications importantes pour les pratiques de gestion des vergers et la politique de conservation des abeilles.

Mots clés : Abeilles maçonnes, Abeilles solitaires, Habitat de nidification, Limitation des ressources, Mise à fruit, Nidification dans des cavités, Nid-piège, Pollinisation, Verger de pommiers

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Chapter 1: Introduction

1.1 Crop Pollination and Wild Bees

With a 30% growth in agricultural land cover and a surge in demand, global agriculture has become twice as dependent on pollinators as it was half a century ago (Potts *et al.*, 2016). Wild bees play a prominent role in global crop production (Reilly *et al.*, 2020), supplementing or even exceeding the contributions of the domesticated honeybee, *Apis mellifera* (Koh *et al.*, 2018; Pérez-Méndez *et al.*, 2019; Boyle *et al.*, 2020; McKerchar *et al.*, 2020). Honeybees are widely used in various crop systems for pollination (IPBES, 2016). However, relying solely on honeybees for pollination is increasingly risky and wild bees are progressively being perceived as potential replacements (Greenleaf and Kremen, 2006; Brittain *et al.*, 2013; IPBES, 2016). In the United States, wild bees are as commonly found on crop flowers as honeybees (Reilly *et al.*, 2020) and they can be more effective pollinators of some fruit crops (Garibaldi *et al.*, 2013; MacInnis and Forrest, 2019; Pérez-Méndez *et al.*, 2019). Even though technological advancements over the past 70 years have led to overall improved yields, pollinator-dependent crops have experienced less growth in yield than other crops, suggesting that pollinator-dependent crops could still benefit from additional pollination services (Potts *et al.*, 2016; McKerchar *et al.*, 2020).

Although pollinators support crops, they are also heavily impacted by agricultural practices (Potts *et al.*, 2016). The indirect effects (sensu Roulston and Goodell, 2011) of the conversion of natural habitat for human use (hereafter land-use change), among other factors, have led to pollinator declines, chiefly in agroecosystems (Potts *et al.*, 2016; Zattara and Aizen, 2021). Land-use change often leads to decreased abundance and diversity of floral resources, as well as, for many species, reductions in nesting resources. The latter are an often overlooked yet essential component of wild bee habitat (Roulston and Goodell, 2011; Belsky and Joshi, 2019). Because high floral visitor densities are the greatest contributor to yields of pollinator-dependent crops, declines in pollinator abundance and diversity have been associated with reduced crop yield in agricultural landscapes (Potts *et al.*, 2016), showcasing the delicate balance between agricultural productivity and pollinator conservation.

1.2 Resource Limitation and Cavity-Nesting Bees

Identifying the resources that limit bee populations is essential for maintaining pollination services to crops and wildflowers as well as conserving bee biodiversity and abundance. Floral resource availability (particularly in agroecosystems) has long been the primary focus in studies of resource limitation in bees (Ghazoul, 2004; Ebeling et al., 2008; Fornoff *et al.*, 2017; Bloom, Northfield and Crowder, 2019; Theodorou et al., 2020). For example, studies of apple orchards, have shown that the presence of flowering crop succeeding apple bloom (Martins *et al.*, 2018), floral strips (Heller *et al.*, 2019), semi-natural habitat (Bihaly *et al.*, 2020) and even floral resources in the orchard ground cover (Rosa García *et al.*, 2014), increases wild bee abundance and diversity.

This focus on floral resources has left the role of nesting resources in regulating bee populations largely unexplored (reviewed by Roulston and Goodell, 2011). Most current evidence on the effects of nesting resources on bee populations is indirect, due to difficulties in establishing appropriate controls in field experiments (Steffan-Dewenter and Schiele, 2008; Bihaly *et al.*, 2020). Several studies have shown that the abundance and richness of bees is correlated, to some degree, with the availability of nesting resources (i.e., bare ground, dead wood, soil burrows, and cavities) in the local environment (Caudill *et al.*, 2017; Rodríguez and Kouki, 2017; Westerfelt, Weslien and Widenfalk, 2018; Bartholomée *et al.*, 2020). However, the existence of nesting resource limitation has not been in tested in a controlled and replicated experiment (reviewed by Roulston and Goodell, 2011), even though there is reason to suspect that bee populations could be nest-site limited in certain situations. In particular, cavity-nesting bees tend to be rare in agroecosystems compared to their ground-nesting counterparts, raising the question whether availability of nesting cavities in such habitats limits their abundance (Williams, 2011; Forrest *et al.*, 2015; Bartholomée *et al.*, 2020; Bihaly *et al.*, 2020).

Artificial nesting structures for bees that nest in above-ground cavities, also known as trap nests or bee hotels (Geslin *et al.*, 2020), have long been used to study wild cavity-nesting bees (e.g., Krombein, 1967; Steffan-Dewenter and Schiele, 2004; Seidelmann *et al.*, 2016; Groulx and Forrest, 2018), and also, more recently, as a potential tool for pollinator conservation in urban and agricultural settings (MacIvor and Packer, 2015; Boyle and Pitts-Singer, 2017). Some studies have shown that bees occupy artificial nesting structures and that the number of bees occupying these structures may increase over a period of a few years as the structures attract new colonists as well as the progeny of previous years' occupants (Kraemer, Favi and Niedziela, 2014; Dainese *et al.*, 2018). Other studies have contributed to our understanding of the effectiveness of nesting structures by evaluating attractiveness of different nest-site characteristics (such as colour, size, placement, shape) (Artz *et al.*, 2014; Fortel *et al.*, 2016; Boyle and Pitts-Singer, 2017; MacIvor, 2017). Yet no study has examined whether the provision of nesting structures actually increases local bee populations in comparison to control sites, establishing that bees are nest-site limited (Roulston and Goodell, 2011; Dainese *et al.*, 2018; Westerfelt, Weslien and Widenfalk, 2018). While we do have promising evidence that bees occupy these structures, we do not know whether nesting structures increase local bee populations (Fortel *et al.*, 2016; Dainese *et al.*, 2018).

1.3 Study Systems: Osmia spp. and Apple

Apple (*Malus domestica*; varieties: Honeycrisp, Gala, Spartan, Golden Delicious, Cortland, McIntosh, etc.) is a common crop in temperate regions of the world including eastern Canada (Ontario Apple Growers, 2019). Apple is typically self-incompatible and can be pollen limited, as it relies heavily on animal pollination for adequate fruiting (Reilly *et al.*, 2020; Osterman *et al.*, 2021). In spring, in the northern hemisphere, most visitors to orchard flowers (including apple) are honeybees, bumble bee queens (*Bombus* spp.), and wild solitary bees such as *Andrena* spp. ("mining bees") and *Osmia* spp. ("mason bees") (Márquez, Bosch and Vicens, 1994; Sekita, 2001; Delaplane and Mayer, 2004; Martins, Gonzalez and Lechowicz, 2014; Carvell *et al.*, 2015; Park *et al.*, 2016; Pérez-Méndez *et al.*, 2019). In North America, *Osmia lignaria* (the "blue orchard bee") in particular, distinguishes itself from honeybees by its ability to fly at cooler temperatures (Isaacs *et al.*, 2017; Boyle and Pitts-Singer, 2019) and deposit higher pollen loads on a wide-range of flowers (Földesi *et al.*, 2016). Notably, some *Osmia* spp. can have synergistic effects on orchard fruit yield by altering the foraging behaviour of honeybees, thereby increasing pollen transfer, fruit set and ultimately, crop yield (Brittain *et al.*, 2013; Martins, Gonzalez and Lechowicz, 2014). Many *Osmia* spp., including *O. lignaria*, are cavity-nesters; thus, they may be less abundant on apple orchards than they would be if orchards provided nesting habitat (i.e., reeds, twigs, dead wood, etc.).

1.4 Objectives

The goal of this study is to understand the degree to which populations of *Osmia* spp., a genus that includes several apple pollinators, are limited by nesting resources in orchards. I use a controlled, replicated field experiment to test **(a)** whether *Osmia* spp. in apple orchards are limited by nesting habitat, such that the addition of nesting structures increases *Osmia* spp. abundance in apple orchards. However, *Osmia* spp. are not the only pollinators relevant to orchard environments; if cavity-nesting bee populations increase due to the addition of nesting structures, other bee populations could decrease in response. Alternatively, the presence of greater numbers of cavity-nesting bees might cause other bees to alter their activity patterns in ways that increase flower visitation and pollination. To determine how the addition of nesting structures impacts bee communities more broadly, I also examine **(b)** whether the presence of additional nesting structures affects overall bee visitation to flowers in apple orchards. Finally, to determine if the addition of nesting structures produces biologically relevant effects on apple pollination, I test **(c)** whether nesting structures affect apple yield and quality.

Chapter 2: Methodology

2.1 __Osmia__ spp. Biology

Osmia breed individually like other solitary bees but often nest near other individuals (Fortel *et al.*, 2016). They nest in cavities in which they construct series of brood cells each of which contains one egg, along with a mass of pollen and nectar; multiple nests can be built in succession (Kemp and Bosch, 2005). Nests not only provide a site for larval development but also protect adults and larvae from predators, parasites, disease and environmental extremes (Roulston and Goodell, 2011). In this study region, *Osmia* are univoltine.

2.2 Study Sites

I selected 24 field sites within 16 apple orchards (with one to three sites per orchard) around Ottawa, Ontario, Canada (*Appendix IV: Figure S1*). All orchards planted several varieties, that vary slightly in flowering and fruiting time, including early-season apples like Paul Red and Ginger Gold as well as late-season apples like Russet and Cortland. I used a paired design, with each site including a treatment and a control plot, to account for variation among orchards (*Appendix IV: Figure S2*). Sites were established to obtain the greatest number of sites within a single orchard based on orchard dimensions (see below). Orchards were located 4 to 166 km from each other. *Osmia lignaria* and other bees like *Apis mellifera* and *Bombus* spp. have the capacity to travel distances up to 1,200 m, but these distances are unlikely when abundant floral resources are present within 100 to 200 m of their nests (Osborne *et al.*, 2008; Guédot, Bosch and Kemp, 2009). Smaller-bodied bees like most *Osmia* spp., such as *Osmia pumila* (which were expected to be the most common *Osmia* species in orchards, based on earlier surveys within the region (Forrest, unpublished), typically forage within 100 m (Greenleaf *et al.*, 2007; Zurbuchen *et al.*, 2010). Because of this, each site was separated by a minimum of 200 m from all other sites, while paired plots within sites were separated by a minimum of 100 m (μ = 150 ± 5 m, range: 100 m to 200 m). Each plot (treatment or control) was 0.3 ha in area (50 m x 60 m or 30 m x 100 m, depending on the shape of the orchard). The locations of treatment plots (with nest boxes) and control plots (without nest boxes) were assigned randomly and there were no notable or consistent landscape feature differences between treatment and control plots (*Appendix I: Quantifying Floral Resources*).

2.3 Nesting Structures

Treatment plots were supplemented with 10 nest boxes each with 10 to 14 nesting holes, placed at equally spaced distances (*Appendix IV: Figure S2*). while control plots received no nesting habitat supplementation. In 2022, the same nest boxes that were installed in 2021 were re-installed in 2022 at the same sites.

Nest boxes were made of blocks of wood (spruce, pine, or fir) with drilled, 15 cm deep holes for the nesting bees to occupy. Since my goal was to attract a variety of *Osmia* species, cavities ranged in diameter from 6 to 10 mm. All nest holes in the nest boxes were lined with paper straws of the same diameter as the cavities. All nest boxes were placed at around 1 m above ground and tied to apple tree trunks (to avoid damaging trees). This ensured that nests were not blocked by understory vegetation and were easily accessible for examination.

At the end of summer 2021, all nest boxes from the 24 sites were placed in plastic boxes with holes covered by window mesh to allow air to flow (10 nest boxes from a site in each box) and stored outdoors from September 2021 to April 2022. The lids of the plastic boxes were cleared of rain and snow, as needed. In April 2022, these nests from 2021 were placed in open, waxed-cardboard milk cartons attached to apple trees in the centres of the treatment plots from which they were collected. This allowed surviving progeny to emerge and potentially colonize the nest boxes in 2022.

2.4 Quantifying Bee Abundance

Bee visitation to flowers was used as a metric of bee abundance to compare the two treatments. In 2021, each site was sampled on one occasion during apple bloom (May 12–18) as well as twice more after apple bloom (May 21–June 10 and June 6–23) to provide data on bee numbers over the growing season. Sampling took place on sunny days with wind speeds below 3.3 m/s and above a recorded temperature of 12 ºC (Bosch and Kemp, 2002; Delaplane and Mayer, 2004) between 7:00 and 19:30.

On each sampling occasion, I conducted three replicate transect walks (described below). This amounts to a total of 9 transect walks per plot (18 per site). Because analysis of the 2021 data revealed that the strongest treatment effects occurred during the period of apple bloom (*3.3 Osmia and Bee Abundance*), I restricted bee sampling in 2022 to that period. Therefore, in 2022, each site was sampled only during apple bloom from May 16 to May 22. This amounts to a total of 3 transect walks per plot (6 per site) in the second year of the study (*Appendix III: Table S1*).

For transect walks in both years, a field technician and I noted all observable bee visits to apple blossoms (or other flowers in the undergrowth or in shrubs after apple bloom in 2021) during 15 min sampling occasions along a 100 m transect with a width of approximately 1 m (*Appendix IV: Figure S2*) in each plot (a linear transect for plots of 30 m x 100 m; a U-shaped transect for plots of 50 m x 60 m). Observers alternated transect walks between control and treatment plots to eliminate observer bias. After apple bloom in 2021, if there were no flowers growing in the undergrowth or in shrubs along the designated 100 m transect, the 100 m transect was shifted so that it passed through areas with a dense growth of flowers within the plot. This sampling effort is comparable to that of other studies (Martins, Gonzalez and Lechowicz, 2014; Russo *et al.*, 2015).

Bees were identified visually in the field since *Osmia* spp. are readily distinguished from other pollinators. *Osmia* spp. were identified to genus, and all other bees were identified to morphospecies (*Appendix I: Quantifying Bee Abundance*) and subsequently grouped into a single category ("other bees") for analysis. A bee visit was counted if the insect contacted the sexual organs of flowers. If evident, the sex of the bee was also recorded.

2.5 Collecting Data from Nesting Structures

In parallel to bee visitation observations at each site, nest box occupancy was monitored during apple bloom and twice more after apple bloom in 2021 to determine when bees were nesting in relation to apple bloom (*Appendix I: Nesting Structure Occupancy*). Nest box occupancy data are not available in 2022.

To monitor nest boxes, each straw within a nest box was temporarily extracted. Brood cells within a straw (nest hole) were counted to record the total number of cells as well as the number of new cells that were made since the last monitoring event. Taxa were identified based on the material used in nest construction (macerated leaves or mud for *Osmia* spp. and most *Megachile* spp., mud for wasps, and resin for *Megachile* [*Chelostomoides*]). *Megachile* nests (other than *Chelostomoides*) could be distinguished from *Osmia* nests by the presence of leaf pieces lining the interior of the straw (see *Appendix I: Nesting Structure Occupancy*). Wasps and *Osmia* nests were distinguished by snipping a small window in the straw to view the nest provisions. After apple bloom, pollen was sampled from *Osmia* nests to identify the floral taxa used for provisioning (*Appendix I: Pollen Usage*).

In November 2021, to determine *Osmia* survival rates, a Pinnacle X-Ray Solution microCT system was used to picture and examine the bee nest holes without dissecting them (*Appendix IV: Figure S3*). The system consists of a Hamamatsu 150kV Microfocus L12164-07 source, which was run on the small focal spot setting (7 μm), coupled with a Varian PaxScan 1313DX flat panel detector. The optimal parameters for this application were determined to be 40 kV, 225 mA, 7.5 frames per second, 10 frames average (cf. Mader, Spivak and Evans, 2010). X-ray images were examined as DICOM files in ImageJ © (Schneider, Rasband and Eliceiri, 2012). Survival was determined by counting the number of apparently viable *Osmia* individuals within each nest hole visible in the X-ray image (*Appendix I: Nesting Structure Occupancy*). Male and female *Osmia* were differentiated based on size (females are about twice as large as males) (see *Appendix IV: Figure S3*).

2.6 Quantifying Apple Yield and Quality

During initial surveys of the orchard, three trees, equally spaced within the 100 m transect of each plot (control and treatment), were chosen for estimating fruit set. On each of the trees, one north-facing and one south-facing branch were tagged. During apple bloom, the number of buds or open flowers (henceforth “buds”) present in a 1 m section of each branch (measured 1 m from the tip of the branch) was counted. A few weeks after apple bloom, the number of developing apples was counted on the same branch sections; this represents the number of fruit “pre-thinning” (May 21–June 10, 2021, and June 6–20, 2022). Thinning of fruit trees (either chemically or physically) is a common practice in fruit orchards, including apple orchards (Ministry of Agriculture and Affairs, 2020; Pardo and Borges, 2020). Therefore, before harvest, I also counted the number of fruit post-thinning (August 3–18, 2021). Fruit set was measured as the ratio of ripe fruits (pre-thinning in 2021 and 2022 and post-thinning in 2021) to buds (using numbers of fruits and buds summed across both branches for each tree) on each of the 144 trees. Apple quality is typically measured by weighing apples or counting seed set in apples around harvest time (Brookfield *et al.*, 1996; Sonoda *et al.*, 2022). However, to avoid interfering with orchard production, I instead measured the circumference of the apples. A measure of mean apple circumference for three apples (one at the tip of the flagged section of the branch, one in the centre, and one at the other end of the flagged section) on the north-facing branch and three apples on the south-facing branch was calculated for each tree (n = 144 trees). This serves as a second measure of pollination success and provides a basis for recommendations to apple producers (Földesi *et al.*, 2016).

2.7 Statistical Analyses

All models were run in R version 4.1.2 (R Core Team, 2021) with the lme4 package (Bates *et al.*, 2021). All model assumptions were met.

For the 2021 data, I used a generalized linear mixed model (GLMM) with a Poisson distribution to test whether **(a)** the abundance of *Osmia* spp. (i.e., the number of observed flower visits per plot per sampling occasion) differed significantly between plots with and without nesting structures. Observations from three transect walks per sampling occasion were summed so that there was a single independent observation for each plot on each sampling occasion (n = 48 plots x 3 sampling occasions = 144). Thus, the response variable was the number of observed *Osmia* visits per sampling occasion, and the fixed predictors were treatment (addition or no addition of nesting structures) and sampling occasion (a categorical variable with three levels: apple bloom, pre-apple thinning, and post-apple thinning). Site nested in orchard was included as a random-intercepts term. A similar model was run to test **(b)** the effect of additional nesting structures on overall bee numbers in 2021.

The data obtained in 2022 (from apple bloom period only) was combined with the 2021 apple bloom data to run two additional models. Data from the three transect walks per plot were summed so that there was a single datapoint for each sampling occasion in each year (n = 48 plots x 1 sampling occasion x 2 years = 96). For these models, the response variables were the number of observed *Osmia* visits to flowers or the total number of bees observed; the primary fixed predictor was treatment; year was coded as a categorical variable with two levels (2021 and 2022); and site nested in orchard was included as a random-intercepts effect.

I used three GLMMs with binomial distributions to test the effect of treatment on **(c)** apple yield in 2021 (pre- and post-thinning fruit-set) and in 2022 (pre-thinning fruit-set only). For the post-thinning 2021 and the pre-thinning 2022 models, the random effect (site nested in orchard) explained none of the variance, resulting in singular fit errors; however, it was retained in the model to reflect the experimental design and account for non-independence of observations made at the same site or orchard. . I also ran a linear mixed model (LMM) to test the effect of treatment on mean apple circumference. Site nested in orchard was included as a random-intercepts term in the model

Chapter 3: Results

3.1 Nest Box Occupancy

In 2021, out of a total of 2,742 nesting holes, 1,582 were filled by cavity-nesting insects; this included *Osmia* spp. (245 nests; ~9% of all available holes), wasps (1,108; ~40%), *Megachile* (*Chelostomoides*) (104; ~4%), and other *Megachile* spp. (125; ~5%). The remaining 1,160 nesting holes remained unoccupied (*Appendix IV: Figure S4*). 6,804 brood cells were completed by all cavity-nesting insects; this includes 1,559 *Osmia* brood cells, 4,266 wasp cells, 369 *Megachile* (*Chelostomoides*) cells, and 609 other *Megachile* cells (*Appendix IV: Table S2* and *Appendix IV: Figure S5*). Occupancy data from 2022 have not yet been collected.

3.2 Osmia Survival and Pollen Usage

53% of all *Osmia* brood cells constructed in 2021 survived to adulthood, and 67% of these brood cells were female; thus, overall, 36% of *Osmia* brood cells resulted in surviving female adults. Given that, in this study, the average filled nest contained 4.59 ± 3.59 brood cells, we would expect each nest to yield 1 to 2 surviving adult female bees (*Appendix IV: Figure S6*). Survival data of other taxa were not recorded.

Collectively, *Osmia* individuals provisioned their nests with 27% *Malus* spp. pollen (most likely apple), 25% Fabaceae pollen, 15% *Cornus* spp. pollen, and the remaining 33% a combination of miscellaneous pollen: Asteracaea, Lamiacaea, *Lonicera* spp., *Fragaria* spp., *Rubus* spp., etc. During apple bloom and pre-apple thinning, the most productive taxon was *Osmia* spp. which produced 198 new cells across all sites (1.83% of all new cells produced) and 618 new cells (9% of all new cells produced), respectively. During this period, most of the pollen used to provision nests was from apple as well as some Fabaceae. Post-apple thinning, wasps were becoming more productive but did not surpass *Osmia* as the most productive taxon, producing 281 new brood cells (4% of all new cells produced) while *Osmia* spp. produced 543 new cells (8% of all new cells produced; *Appendix IV: Figure S7*).

3.3 Osmia and Bee Abundance

In 2021, during apple bloom, out of a total of 3,717 individuals, the most common taxa identified visiting apple blossoms were *Apis mellifera* (2,123; 57%), followed by *Bombus* spp. (347; 9%), *Andrena* spp. (304; 8%), *Lasioglossum* spp. (160; 4%), and *Osmia* spp. (47; 1%). In the same year, pre- and post-apple thinning, out of a total of 4,432 individuals, the most common taxa observed visiting flowers in the undergrowth and in shrubs were *Apis mellifera* (3,019; 68%), *Bombus* spp. (1,604; 36%), *Andrena* spp. (1,083; 24%), *Lasioglossum* spp. (893; 20%), and *Osmia* spp. (181; 5%). In 2022, the most common taxa, out of a total of 4,226 individuals seen visiting apple blossoms, were *Andrena* spp. (1,398; 33%), followed by *Apis mellifera* (1,224; 29%), *Bombus* spp. (571; 13%), and *Osmia* spp. (168; 4%). We did not collect voucher specimens; however, based on past work with cavity nesting *Osmia* spp. in this study area (Forrest, unpublished), we suspect that most individuals were *Osmia pumila*; *O. lignaria* may also have been present.

In 2021, *Osmia* abundance (measured as visitation to apple blossoms, or to other flowers after apple bloom) was 44% greater on average in treatments plots with nest boxes (μ ± s.e. = 1.50 ± 0.294 bees per plot) than in control plots (μ = 1.0 ± 0.2; ß = 0.44, p = 0.0047; *Figure 1*). *Osmia* numbers also differed significantly among sampling occasions (χ^2 = 8.3, p = 0.015), as did the effect of treatment (interaction: treatment x sampling occasion; χ^2 = 8.4, p = 0.015). *Osmia* abundance was highest and the treatment effect was stronger during apple bloom (*Figure 1*).

Based on the combined apple bloom visitation data from 2021 and 2022, *Osmia* abundance was again significantly greater in treatments plots with nest boxes (μ = 3.4 ± 1.9 bees per plot) than in control plots (μ = 1.0 ± 0.5; ß = 1.2, p <0.00001; *Figure 1*). This corresponds to a 113% difference between the two plot types (*Appendix III: Table S3*).

In 2021, across all sampling rounds, total bee abundance (i.e., including *Osmia* spp.) was also significantly greater in treatment plots (μ = 3.8 ± 0.28 bees per plot) than in control plots (μ = 3.3 ± 0.2; ß = 0.15, p <0.00001). This corresponds to a 15% difference between plot types (*Figure 1*). Similar trends were observed with the combined 2021 and 2022 bee visitation data from the period of apple bloom: bee visitation to apple blossoms was 47% greater in treatment plots (μ = 101 ± 13, bees per plot) compared to control plots (μ = 63 ± 7; ß = 0.46, p <0.00001; *Figure 2*; *Appendix III: Table S3*).

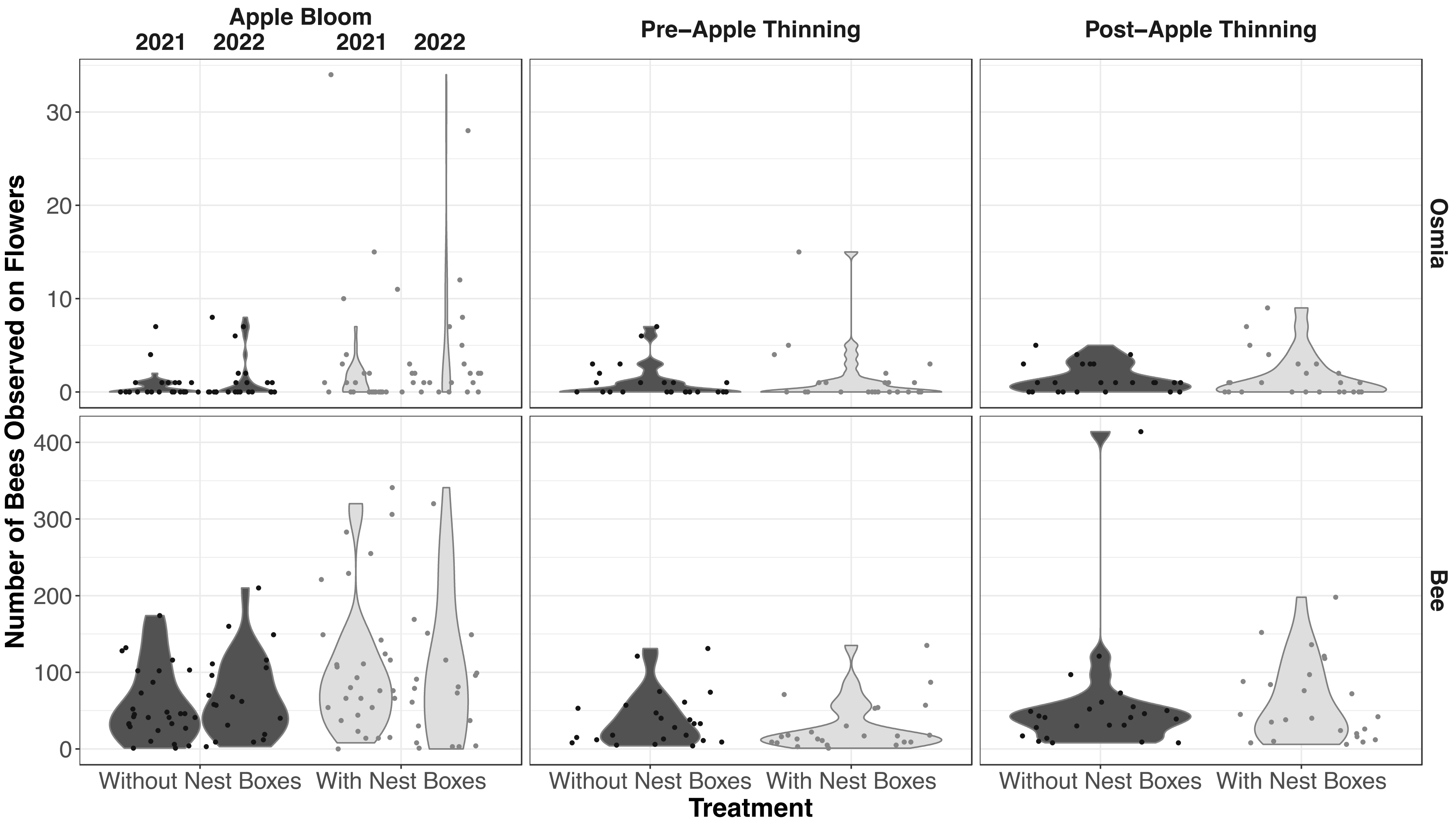

Figure 2 Total numbers of *Osmia* spp. and total numbers of bees on flowers (on apple blossoms during apple bloom and on flowers in the groundcover and/or shrubs after apple bloom) per site (n = 24) for treatment plots (with nest boxes) and for control plots (without nest boxes), during three sampling occasion (during apple bloom [in 2021 and 2022]; and after apple bloom but before apple thinning [2021 only]; and after apple thinning [2021 only]). Each data point represents the total number of *Osmia* or bee individuals visiting the reproductive parts of flowers during all 15 min transect walks combined

(15 min x 3 = 45 min for each plot on each sampling occasion). Violin plots represent the distribution (density) and peaks of the total number of visits to flowers per site.

3.4 Apple Yield and Quality

In 2021, apple fruit set pre-thinning was not significantly greater in treatment plots (μ = 0.47 ± 0.030, with nest boxes) compared to control plots (μ = 0.50 ± 0.030, without nest boxes; ß = 0.09, p = 0.80, *Figure 2*). Post-thinning results were similar, showing no difference in post-thinning apple fruit set between the treatment plots (μ = 0.093 ± 0.015) and the control plots (μ = 0.083 ± 0.016; ß = -0.01, p = 0.99; *Figure 2*). Similarly, there was no difference in apple circumference between the treatment plots (μ = 9.3 ± 0.9 cm) and the control plots (μ = 8.5 ± 1.0 cm; ß = 0.72, p = 0.52; *Figure 2*). In 2022, apple fruit set pre-thinning was 29% greater on average in treatment plots (μ = 0.252 ± 0.0271) compared to control plots (μ = 0.187 ± 0.02); however, this difference was not significant (ß = -0.054, p = 0.93; *Figure 2; Appendix III: Table S4*).

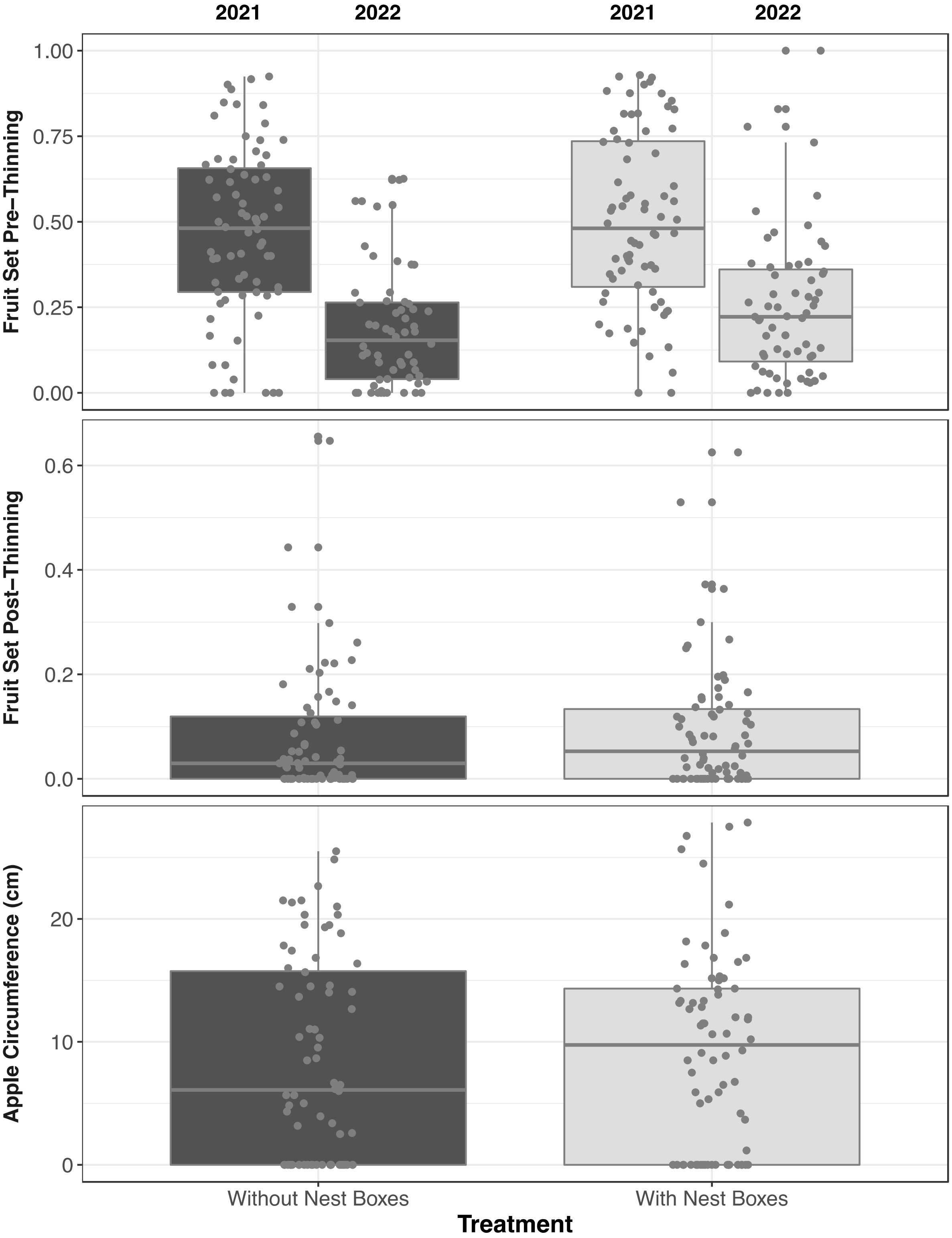

Figure 2 Apple fruit set and quality in the two experimental treatments. The top two panels, show apple fruit set pre-thinning and post-thinning (number of developing fruit/number of buds) per tree for treatment plots (with nest boxes; n = 24 in 2021, n = 21 in 2022) and for control plots (without nest boxes; n = 24 in 2021, n = 21 in 2022). Each

data point represents the total number of fruit divided by the total number of buds from the sampled branches of each tree (i.e., [fruit count north + fruit count south]/[bud count north + bud count south]). The bottom panel shows apple quality measured (in 2021 only) as the mean circumference (cm) of six apples per tree (3 trees per plot) for treatment plots (with nest boxes; n = 24) and for control plots (without nest boxes; n = 24). Each data point represents the mean circumference of six apples from a tree (3 from the north-facing branch and 3 from the south-facing branch). Box plots showcase the first quartile, median, and third quartile of the total fruit set and apple circumference per site.

Chapter 4: Discussion

The availability of nesting resources is important to cavity-nesting bees such as *Osmia* spp. since they provide a site for larval development and protect adults and larvae from predators, parasites, and environmental extremes (Roulston and Goodell, 2011; Kline and Joshi, 2020). To my knowledge, this study is the first to test the effect of experimentally supplementing nesting structures on bee populations. I found that the addition of nesting structures in apple orchards significantly increases **(a)** the numbers of *Osmia* spp. and **(b)** overall bee numbers on orchards flowers near nesting structures.

4.1 <u>Osmia</u> Abundance on Orchard Flowers

This study adds to a growing body of literature, using a variety of approaches, that suggest that adding nesting habitat promotes bee abundance (Steffan-Dewenter and Schiele, 2008; Dainese *et al.*, 2018; Martins *et al.*, 2018; Bartholomée *et al.*, 2020). However, whether these nesting structures are beneficial from a conservation perspective is uncertain. Nesting structures, rather than attracting native bees, could host non-native bee species, which could be more productive in foreign lands and less susceptible to parasitization than their native counterparts and through their nest construction, may even inhibit native bee emergence (MacIvor and Packer, 2015; Geslin *et al.*, 2020; Straffon-Díaz *et al.*, 2021). The prevalence of parasitization and amount of nesting occupancy could be due to differences in artificial nesting structure construction, distribution, and maintenance (Artz *et al.*, 2013, 2014; Seidelmann *et al.*, 2016; Koh *et al.*, 2018; McCallum, McLean and Cutler, 2018).

In the first year of my study (2021), the positive effect of nesting structures on *Osmia* numbers was most likely due to immigration rather than in situ reproduction. This is because *Osmia* populations in southern Canada only complete a single generation per year and thus, the subsequent generation (their progeny) could not have contributed to the increased numbers observed in 2021. It follows then that in the second year of my study (2022), the positive effect of nesting structures could have been due to both immigration of new *Osmia* individuals and emergence of progeny from the previous generation of nesting structure occupants. The fact that the effect measured in 2022 (112% difference between treatment and control plots) was greater than that observed in 2021 (44% difference) is consistent with this hypothesis (*3.3 Osmia and Bee Abundance*). These results suggest that *Osmia* populations are nest-site limited in apple orchards, such that installation of artificial nesting structures can attract nesting bees to an area and potentially maintain or increase their numbers over time.

While a large portion of the observed increase in *Osmia* numbers was likely due to immigration in both years, their origin is unknown. In this case, *Osmia* populations must have come either from outside the orchard or from elsewhere within the orchard. I did not observe greater occupancy of nest boxes located closer to the orchard edge, regardless of the adjoining habitat (*Appendix II: Effects of Distance to Orchard Edge on Nest Box Occupancy*). This suggests–but does not prove–that bees were immigrating from elsewhere within the orchard.

If bees were immigrating from elsewhere in the orchard, they may have been coming from areas not included in the experiment (such as the external margins of the treatment plots) or alternatively, the control plots. If bees immigrated from control to treatment plots, this could have inflated the effect sizes I observed, since the increases in population size observed in treatment plots would have been driven in part by concurrent decreases in population size in control plots. While control plots were installed such that they were sufficiently distant from treatment plots to account for *Osmia* foraging distances (typically less than 100 m; *2.2 Study Sites*), individuals might fly a larger distances from their emergence site when selecting a nesting site (but see Steffan-Dewenter and Schiele, 2004; Roulston and Goodell, 2011; Talašová *et al.*, 2018; Bosch *et al.*, 2021). Given the relatively small orchard area constituted by the control plots (0.3 ha), relative to non-experimental area (> 1 ha), it is unlikely that bees came primarily from control plots. Most likely, *Osmia* immigrated from areas surrounding treatment plots in addition to adjacent natural habitat. If the bees were immigrating solely from orchard areas surrounding treatment plots, it is possible that the bees were merely redistributing themselves around the orchard in response to the availability of the introduced nesting structures. In this scenario, it is unlikely that addition of nesting structures throughout the entire orchard would produce an orchard-wide increase in *Osmia* numbers similar in magnitude to that observed here. Conversely, if bees were immigrating primarily from adjacent natural habitat, then increased *Osmia* numbers would reflect an additional input of pollinators to the orchard. Most likely, it is a combination of immigration from adjacent natural habitat, from margins of treatment plots, and to a lesser degree, from control plots, that led to the observed increase in *Osmia* numbers on flowers.

Because their flight season corresponded with the timing of apple bloom, *Osmia* spp. were the most productive nest box occupants during the apple flowering period. While we observed great productivity in *Osmia* spp. nests, it is also important to consider what occurs to *Osmia* individuals after they occupy nesting structures. *Osmia* spp. survival seems low (53%), and only 36% of brood cells yielded surviving female offspring

(*3.1 Nest Box Occupancy*; cf. Jayasingh and Freeman, 1980). Mortality was in part due to parasitoids such as *Melittobia* spp. (Hymenoptera: Eulophidae) that were found when inspecting nesting structures in the field and in the lab (cf. Delaplane and Mayer, 2004; Kovács-Hostyánszki *et al.*, 2021). It is unknown whether bees would have experienced greater levels of overwinter mortality if they had been left in the field rather than placed in plastic boxes (*2.3 Nesting Structures*). Nevertheless, with each *Osmia* nest yielding, on average, 1–2 surviving females, and with individual females potentially building multiple nests in succession (cf. Wong and Forrest, 2021), the population appears to be potentially growing, at least in the short term. However, as nesting structure occupancy increases, parasites may also increase, especially if nesting holes and structures are at high density (see Torchio, 1979; Steffan-Dewenter and Tscharntke, 2001; Groulx and Forrest, 2018). So, over several years, populations could be decimated by parasitoids or other parasites potentially leading to the creation of population cycles where the hosts (nesting structure occupants) would potentially rebound after the parasites eventually dwindle.

4.2 Bee Abundance on Orchard Flowers

In both years, nesting structures not only increased the numbers of *Osmia* recorded on flowers but also the total numbers of bees observed on flowers. These increases in total bee numbers cannot be solely explained by the increases in *Osmia* since the latter only make up a small percentage (1% to 5% during apple bloom) of total bee observations (*3.3 Osmia and Bee Abundance*). Instead, the increase in overall bee numbers could be due to species interactions that affect foraging. In particular, interactions between honeybees and native bees can influence the rate at which individuals move between flowers (Greenleaf, Kremen and Matson, 2006; Geslin *et al.*, 2017; Bartholomée *et al.*, 2020). These synergies between floral visitors have been shown to improve crop pollination in almond orchards and sunflower (Greenleaf, Kremen and Matson, 2006; Brittain *et al.*, 2013).

While it is possible that synergistic species interactions contributed to the increase in bee abundance that I observed, the reverse can also occur. Interspecific competition among bees can lead to the extirpation of native bees and decreases in native bee reproduction (Morales *et al.*, 2013; Aizen *et al.*, 2014). Some studies have shown that the presence of honeybee hives rather than promoting bee visitation to crop, can decrease wild bee abundance, species richness, and fruit yield (Mallinger, Gaines-Day and Gratton, 2017; Angelella, McCullough and O'Rourke, 2021). Because of this, it is uncertain whether synergistic interactions that affect bee foraging would have a positive effect on flower visitation year-to-year or in different environments.

Other variables also influence bee numbers on flowers. In my study, honeybees were the most more common flower visitors during apple bloom in 2021 (57% of all visits), but their relative abundance was substantially lower in 2022 (only 29% of all visits). The lower number of honeybees observed in 2022 could be due to unprecedented honeybee colony losses reported by 10 out of 16 apple growers within the area. However, it seems that honeybee visits were compensated by *Andrena* spp. in 2022, another common apple orchard visitor (Delaplane and Mayer, 2004).

4.3 Apple Yield and Quality

Globally, apple (*Malus domestica*) is an economically important crop with an annual value of $45 billion USD in 2016 (Pardo and Borges, 2020) and a l production of 86 million tonnes (FAOSTAT, 2018). In Ontario, the most recent projections suggested that Ontario apple orchard acreage would increase by at least 650 acres (263 ha) per year from 2016 to 2018 (Ontario Apple Growers, 2016). Because apple orchards are a pollinator-dependent crop, and pollinators such as honeybees are in high demand, identifying methods to support alternative pollinators within such ecosystems is crucial.

Even though apple is pollinator-dependent and apple production can be pollen limited (Delaplane and Mayer, 2004; Pardo and Borges, 2020), there was little evidence from this study that the presence of additional nesting structures in apple orchards increases apple yield and quality, even if these structures increase the numbers of bees observed on flowers. The lack of effect of the experimental treatment on fruit set may have been due to orchard production not being pollinator limited in the study area during the years of the study. Indeed, a post-hoc test found no correlation between overall bee visitation to apple orchard flowers and pre-thinning fruit set (in 2021, Pearson's $r = -0.078$, $p = 0.598$, $n = 48$ [24 sites x 2 plots]; and in 2022, Pearson's $r = 0.0702$, $p = 0.658$, $n = 42$ [21 sites x 2 plots]). In the absence of pollen limitation, there is no reason to expect that increasing pollinator numbers will increase apple yield. However, studies have found a link between pollinator abundance or richness and yield or quality of a variety of crops (e.g., Magalhães and Freitas, 2013; Isaacs *et al.*, 2017). In particular, apples grown near *Osmia* spp. nests tend to have a larger number of seeds, greater symmetry, and higher fruit set (Delaplane and Mayer, 2004; Pérez-Méndez *et al.*, 2019; Pardo and Borges, 2020). It is possible that local weather conditions (extreme heat in March 2021 followed by frost in April 2021 and brief apple bloom in May 2022) had a larger impact on apple yield than did pollinator visitation. In other words, apple production may have been resource limited rather than pollinator limited during the years of this study. Resource limitation would not be expected to occur in all years. Having a greater abundance and diversity of pollinators visiting apple blossoms can provide insurance for years when abiotic conditions are good and plants may become pollinator limited (Winfree *et al.*, 2008; Albrecht *et al.*, 2012; Bartomeus *et al.*, 2014; Blitzer *et al.*, 2016).

4.4 Future Applications

Based on my results, it is possible that promoting the availability of nesting resources in various systems, but particularly the productive environments of agroecosystems, could have positive effects on local bee populations. Not only can added nesting habitat promote bee immigration to orchard environments; it may also sustain bee population through multiple generations. Practices such as planting floral strips and (according to evidence from my study) installing nesting structures for promoting pollinator populations can both play a hand in increasing pollination services in agroecosystems (Klein *et al.*, 2007; O'Brien and Arathi, 2021; Raderschall *et al.*, 2021).

Future orchard management practices should not only consider resources present within an orchard such as flowers and nesting habitat but also the surrounding landscape and its resources. Typically, semi-natural habitat such as grassland, forest patches, and floral strips (or hedgerows) can provide sufficient floral and nesting resources for pollinators (Bartholomée *et al.*, 2020; Halinski *et al.*, 2020). For instance, if an orchard is within a matrix of semi-natural habitat, it is possible that the supplementation of nesting resources would not be needed (Bihaly *et al.*, 2020). Proximity to mass-flowering crops (i.e., oilseed rape) (Jauker *et al.*, 2012) and other flowering crop fields (i.e., strawberry, blueberry, raspberry) (Martins *et al.*, 2018) also has the potential to support orchard pollinator populations. Conversely, the homogenization of landscapes and land-use intensification can severely deplete resources and lead to local pollinator declines (Bartholomée *et al.*, 2020). Landscape heterogeneity and the passive provisioning of floral and nesting resources through semi-natural habitat and floral strips insures that orchard environments are not bereft of resources outside of the crop's flowering period and, thus, that installed nesting structures are not acting as ecological traps or population sinks, attracting pollinators during crop bloom only to trap and starve them when bloom is over (Timus *et al.*, 2017).

While bees have been shown to be key contributors to apple pollination, cavity-nesting wasps play important roles in agroecosystems by feeding on common pests and pollinating flowers. Having a variety of insects with a diversity of functional ecologies within agroecosystems can improve pollination and ecosystem services (Martins, Gonzalez and Lechowicz, 2014; Blitzer *et al.*, 2016; Botzas-Coluni *et al.*, 2021; Sonoda *et al.*, 2022). Given the strong affinity of solitary wasps for nest boxes (40% nest hole occupancy in my nest boxes), there is a good potential for promoting wasp populations (along with that of other cavity-nesting pollinators) through the installation of nesting structures (*Appendix III: Table S2*).

Further study should examine how the interaction between floral resources and nesting resources can be used to improve orchard management practices (cf. Westerfelt, Weslien and Widenfalk, 2018), and whether the addition of nesting structures (along with the addition of floral resources) can be replicated–and prove beneficial–in other ecosystems (i.e., urban, suburban, etc.), climates, and agricultural intensities. Moreover, studies to experimentally test the effects of addition of nesting resources for other bee nesting guilds like bumblebees (Lye *et al.*, 2011) and ground-nesting bees (Antoine and

Forrest, 2021) could be valuable for clarifying how different pollinators are impacted by the addition of different nesting resources. With unstable honeybee stocks, relying solely on honeybees for pollination is increasingly precarious (Becher et al., 2013; Henry et al., 2012; Potts et al., 2010; Wakgari & Yigezu, 2021), making investment in supporting wild bees (which can compensate for the absence of honeybees) a sensible strategy (Martins, Gonzalez and Lechowicz, 2014; Osterman *et al.*, 2021).

A long-term experimental study of the effect of nesting habitat addition on bee population sizes could be useful for determining whether the beneficial effects observed in this study are maintained over time. In addition, it is important to consider the potentially harmful effect (due to increased competition) of increasing cavity-nesting bee numbers to on other bee populations. Competitive behaviour is observed between managed honey bees and wild bees in agroecosystems (Hudewenz and Klein, 2013; Henry and Rodet, 2018; Angelella, McCullough and O’Rourke, 2021) and could also occur between nesting structure occupants and non-occupants.

There are also some logistics to consider in the installation of nesting structures in agroecosystems. In my experiment, nesting structures were removed from the field in the winter and stored outdoors, in sealed plastic boxes, safe from rain and other hazards. Such an approach would be difficult to replicate on a larger scale, especially by orchard managers that may not have the time or resources to care for nesting structures over a long period. Moreover, nesting structures need to be maintained and replaced as needed. An alternative (low-maintenance) solution to installing nesting structures could consist of drilling holes in fences and trees (other than fruit trees) within and around the orchard;

however, it is unclear whether such alternatives would be equally effective in attracting and supporting cavity-nesting bees.

In conclusion, my study shows that supplemental nesting resources, in the form of artificial nesting structures, can increase overall bee abundance (measured as visitation to apple blossoms) and most notably, *Osmia* spp. abundance. It is unclear if the observed increases in abundance will lead to sustained population growth and if this sustained population growth is beneficial. Future, long-term studies to test the effect of additional nesting structures on bee populations can help with answering these questions.

Data Availability Statement

The data and code that support the findings of this study are *privately* available on GitHub at https://github.com/BatouleHyjazie/The-Tenants-of-Apple-Orchards.git. The data and code will be publicly available after publication.

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Appendix I: Supplementary Methodology

Quantifying Bee Abundance

Bees were visually assigned the following morphospecies: *Apis mellifera* (honeybees)., *Bombus* spp., *Halictus* spp., *Hoplitis spp.*, *Ceratina* spp., *Lasioglossum* spp., *Megachile* spp., bright green bees (*Agapostemon* spp. and *Augochlorini* spp.), *Nomada* spp., *Sphecodes* spp., *Hylaeus* spp., *Colletes* spp., and *Osmia* spp. (as in Martins, Gonzalez and Lechowicz, 2014; Trip *et al.*, 2020). Morphospecies were identified by observing a bee's size, its stripes or colour, and location of pollen storage (abdomen or corbiculae). If a bee could not be identified to morphospecies during transect walks, descriptive characteristics were used to name the individual bee (i.e., black bee, striped bee, and green bee).

Orchard Characteristics

Orchards varied in management practices (e.g., pesticide application, mowing schedule, thinning practices, honeybee rental, etc.), weather and climate (topography and latitude), and landscape features such as alternative crops (e.g., strawberry, blueberry, squash, etc.). Weather conditions were recorded by taking temperature and wind speed measurements with a Kestrel © thermometer/anemometer. I recorded tree height, number of branches more than 50% of tree height, and diameter at breast height but these data were not used in the main analysis of the study.

Nesting Structure Occupancy

To monitor nesting structure occupancy, each nest hole was identified with an ID associated with the orchard, site, and trap nest number. The nest hole number was assigned based on its location (from right to left, top to bottom) in the nest box. If a nest hole straw was colonized by a bee, a fine-tipped sharpie was used to mark a 0.5 cm line at the entrance of the straw to mark the nests orientation. Another line was drawn to mark the most recent pollen provision and the sampling date was written next to this line. The number of new cells (egg with pollen provision) constructed since the last sampling date was counted and this was added to the total number of cells constructed. Half-completed cells were recorded as 0.5. If a nesting hole straw was sealed, then the type of seal (i.e., leaf, mud, resin) was recorded.

Quantifying Floral Resources

In 2021, to estimate the abundance of floral resources within the apple orchard, the quantity of flowers or inflorescences (in short, floral units) of all non-graminoid species within 100 m of the plots (treatment and control) was estimated. Floral abundance (across all plant taxa combined) was quantified as a percentage coverage within 1 m x 1 m quadrats (5 in total, 4 in each corner of the plot and 1 in the centre), estimated to the nearest 5% (i.e., 5%, 10%...85%, 90%, etc.). A paired t-test was run to test if floral resources in control plots and treatment plots differed significantly. No difference was found (t = 0.503, p-value = 0.616).

Pollen Usage

After apple bloom, pollen was sampled from *Osmia* nests to identify the floral taxa visited by nesting *Osmia* (Boyle *et al.*, 2020). This was done by snipping with microscissors a 2 mm opening in the paper straw containing the nest at the location of the first constructed brood cell (at the site of the pollen provision). A small sample of pollen was removed using the forceps and rubbed on a fuchsin jelly cube (~2 mm^3). All openings were sealed with tape. The pollen-covered fuchsin jelly cube was placed on the microscope slide, a cover slip was placed over the fuchsin jelly, and the fuchsin jelly placed on the microscope slide was melted over an open flame (Kearns and Inouye, 1983). A second sample was taken from the most recently constructed brood cell of each nest.

Pollen samples from brood cells were collected to characterize the degree to which apple blossoms are utilized as a food source in comparison to other available food sources in early spring such as *Taraxacum officinale*. To create a pollen reference library, pollen samples were taken of flowers within the study sites if the taxa were not already present within our lab's pollen reference library. Each microscope slide was analyzed under a compound microscope (40X) and individual pollen grains were identified to genus using this pollen reference collection.

There were two separate pollen mounts on each microscope slide, representing the first and last brood cells of each nest. For each pollen mount, a small line was drawn with a sharpie approximately at the centre of the pollen mount to separate the pollen mount in half and delineate two separate transects. Within each transect, starting from the top corner of the drawn line, the first 100 pollen grains encountered were counted and identified.

Appendix II: Effects of Distance from Orchard Edge on Nest Box Occupancy

Introduction

Proximity to the orchard edge (if it is undisturbed) might provide a greater access to food resources and lead to a higher number of nests. Higher occupancy at orchard edges could also potentially indicate that bees are immigrating from outside the orchard. In addition to the objectives outlined in the main manuscript, it would also be interesting to evaluate the possible effects of proximity to orchard edge on nesting structure occupancy as determined by the number of occupied nest holes per nesting structure.

Methods

To measure the effect of distance from orchard edge on nesting occupancy, in each site, distance of each nest from orchard edge was measured in metres. The type of edge (road, field, or forest) was also recorded. This provides information on the nesting habits of wild bees and particularly, the influence of various apple orchard habitats on nesting structure occupancy. A GLMM with a Poisson distribution was run to test the effect of distance to edge on total number of occupied nest holes. The response variable was the number of occupied nest holes, the fixed predictor was distance to edge and site was included as a random-intercepts term.

Results and Discussion

There was no effect of orchard edge on nesting structure occupancy (total number of occupied nest holes) (z = -0.38, p = 0.70) (*Appendix III: Figure S8*). In addition, there was no interaction between edge type and distance from orchard edge (χ^2 = 3.02, p = 0.22). Thus, there is no indication that bees are predominantly coming from beyond the orchard. Isolation from semi-natural habitat (such as grassland) has been shown to have a negative impact on the abundance and richness of pollinators (Bartholomée *et al.*, 2020). However, another study found that proximity to forest edges was correlated with pollinator diversity rather than abundance (McKechnie, Thomsen and Sargent, 2017). This might explain why no correlation existed between distance to orchard edge and nest hole abundance in my study.

Appendix III: Supplementary Tables

Supplementary Table 1

Summary of bee sampling rounds (sampling effort) in 2021 and 2022. In 2021, there were 9 transect walks per plot (48 plots in total) and 18 per site (24 sites in total) for a total of 492 transect walks. In 2022, there were 3 transect walks per plot (48 plots in total) and 6 per site (24 sites in total) for a total of 144 transect walks (see *2.7 Statistical Analyses* for how these data were analyzed).

Year	Period in Relation to Apple Production	Dates	# Transect Walks per Plot	# Transect Walks per Site
2021	During Apple Bloom	May 12 – May 18	3	6
	Pre-Apple Thinning	May 21 – June 10	3	6
	Post-Apple Thinning	June 6 – June 23	3	6
2022	During Apple Bloom	May 16 – May 22	3	6

Supplementary Table 2

Summary of nest box occupancy in 2021. There were 10 boxes per treatment plot (10 boxes per site) for a total of 240 boxes (24 sites in total). Each box contained between 10 and 14 nesting holes. Overall, there was a total of 2,742 nest holes (μ = 11.43 ± 1.80 per box). In each nesting hole, each taxon can occupy a certain number of holes (summarized in column 1) and an insect can produce a certain number of brood cells (summarized in column 2). If a cell is incomplete, it can be fractional (ex; 25% complete = 0.25 cells).

Taxa Occupant	# of Total Holes	# of Total Cells	# of Surviving *Osmia*	# of Surviving Female *Osmia*
Chelostomoides	104	369.25		
Megachile	125	609.75		
Osmia	245	1,559.75	832	561
Unoccupied	1,160	0		
Wasp	1,108	4,266		
Total	2,742	6,804.75		

Supplementary Table 3

Summary of GLMMs (Poisson distribution) run to test the effect of treatment (addition of nest boxes) on *Osmia* spp. abundance and total bee abundance. The last row from the column "Year" refers to the fact that the data from apple bloom in 2021 and data from apple bloom 2022 were combined to run the model. In 2021 and in 2022, the three transect walks were summed, so that each plot was represented by a single datapoint (see *Appendix III: Table S1*). Statistics refer to the significance of the treatment effect.

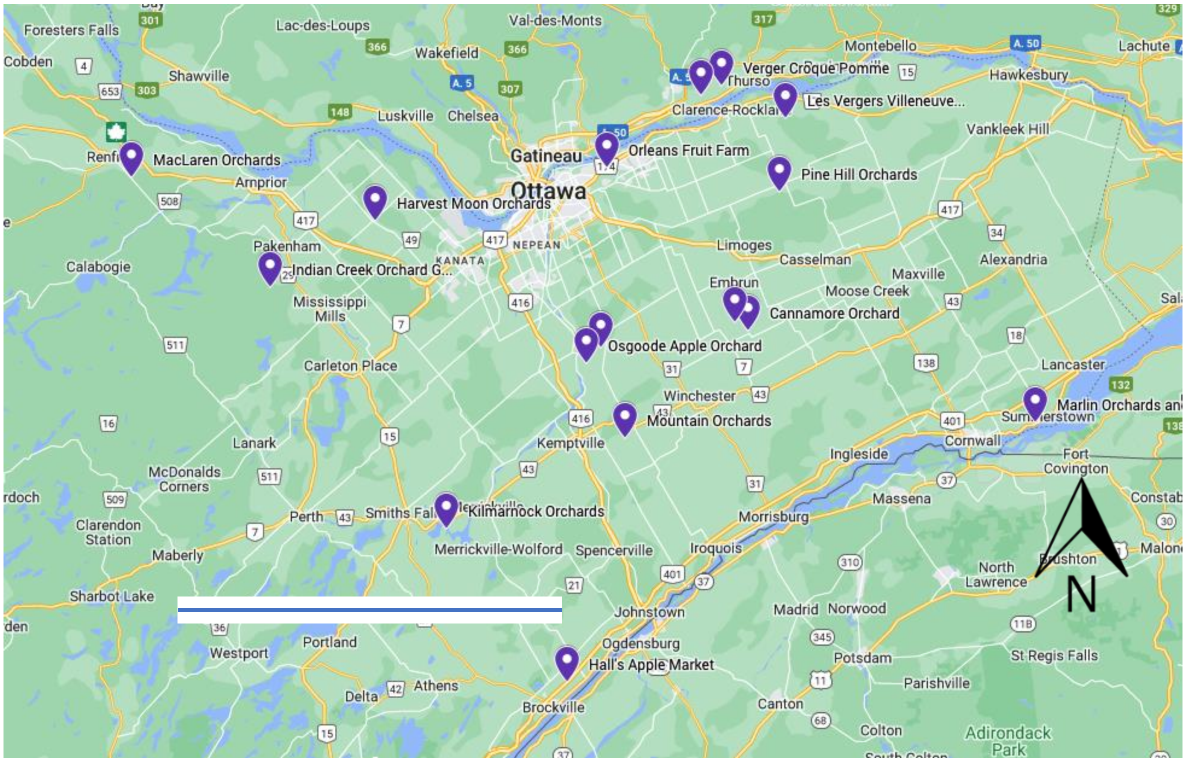
Year	Response Variable	Explanatory Variables	n	Estimate (ß)	Std. Error	P-value
2021	*Osmia spp.* Abundance	Treatment + Round + (1|Orchard/Site)	144	0.441	0.156	0.005
	Bee Abundance		144	0.157	0.024	<0.00001
2021 + 2022	*Osmia spp.* Abundance	Treatment + Year + (1|Orchard/Site)	96	1.24	0.165	<0.00001
	Bee Abundance		96	0.460	0.024	<0.00001

Supplementary Table 4

Summary of GLMMs (for apple fruit set) and LMM (for apple quality) run to test the effect of treatment (addition of nest boxes) on apple yield pre-thinning and post-thinning and apple quality pre-harvest. In 2022, 3 sites were not included in apple yield measurements which amounts to 6 trees which were not included in the original n = 144 sample size (n = 138). Statistics refer to the significance of the treatment effect.

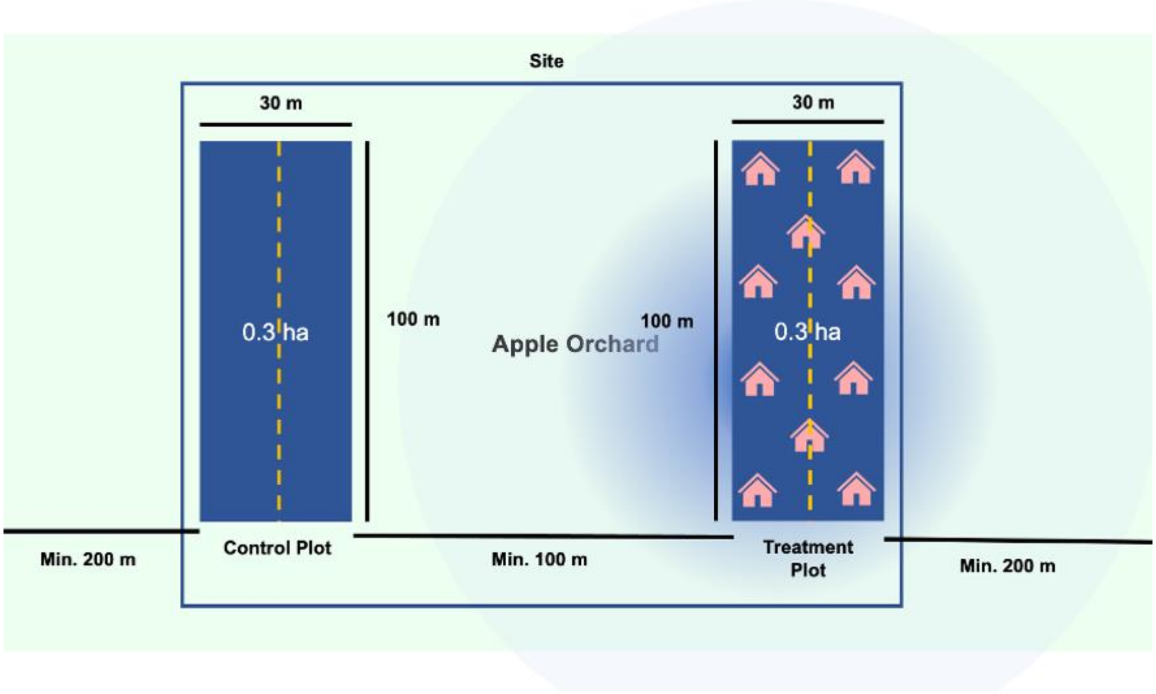
Year	Response Variable	Distribution	Explanatory Variables	n	Estimate (ß)	Std. Error	P-value
2021	Apple Fruit Set Pre-Thinning	Binomial	Treatment + (1|Orchard/Site)	144	0.094	0.368	0.798
	Apple Fruit Set Post-Thinning	Binomial			-0.014	1.01	0.989
	Apple Quality	Normal	Treatment + (1|Orchard/Site)		0.721	1.13	0.523
2022	Apple Fruit Set Pre-Thinning	Binomial	Treatment + (1|Orchard/Site)	138	-0.054	0.608	0.929

Appendix IV: Supplementary Figures



Supplementary Figure 1
Map of 16 apple orchards around Ottawa that were used to establish the 24 sites each with control and treatment plots. The blue line represents a 50 km distance.

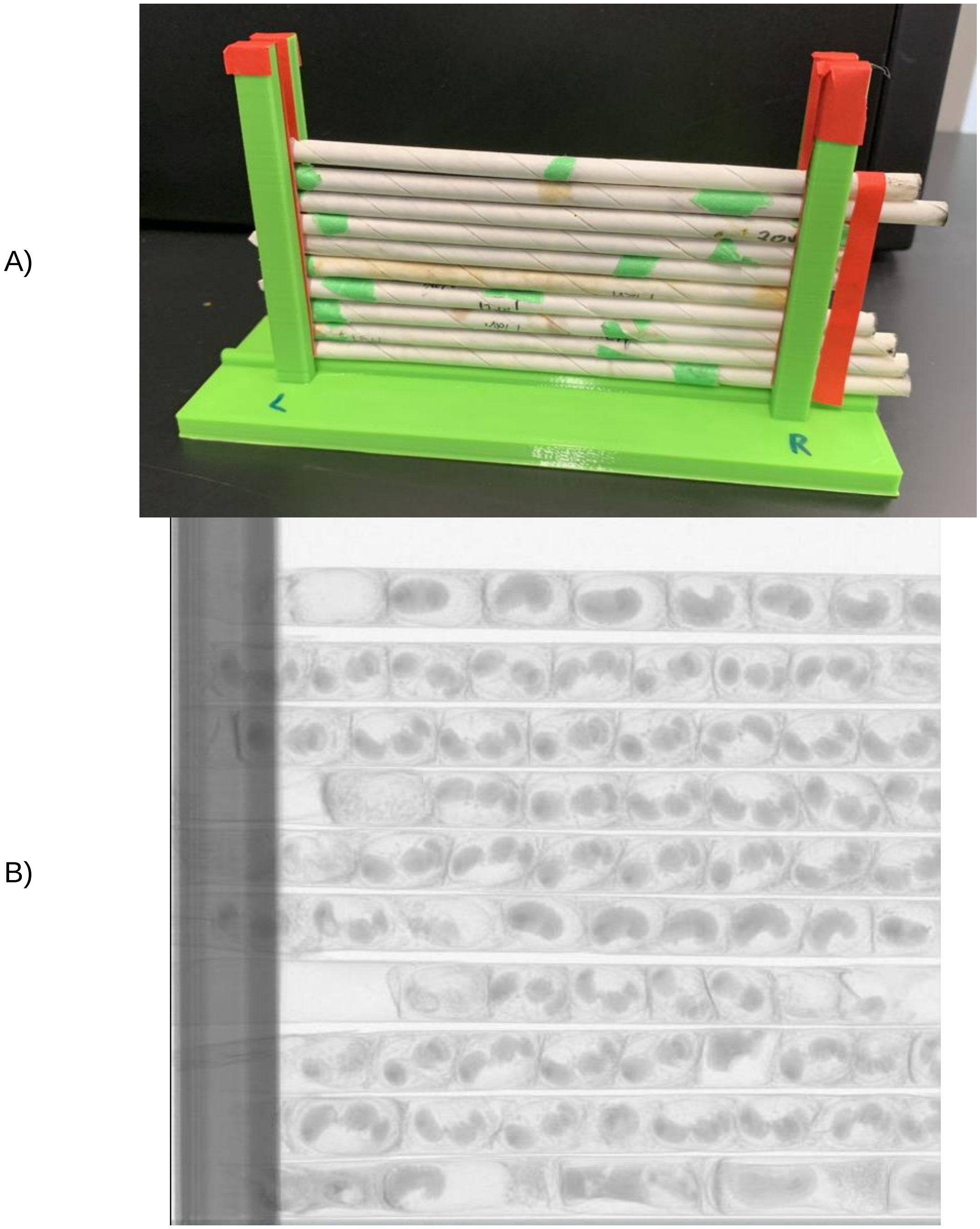
A)



B)

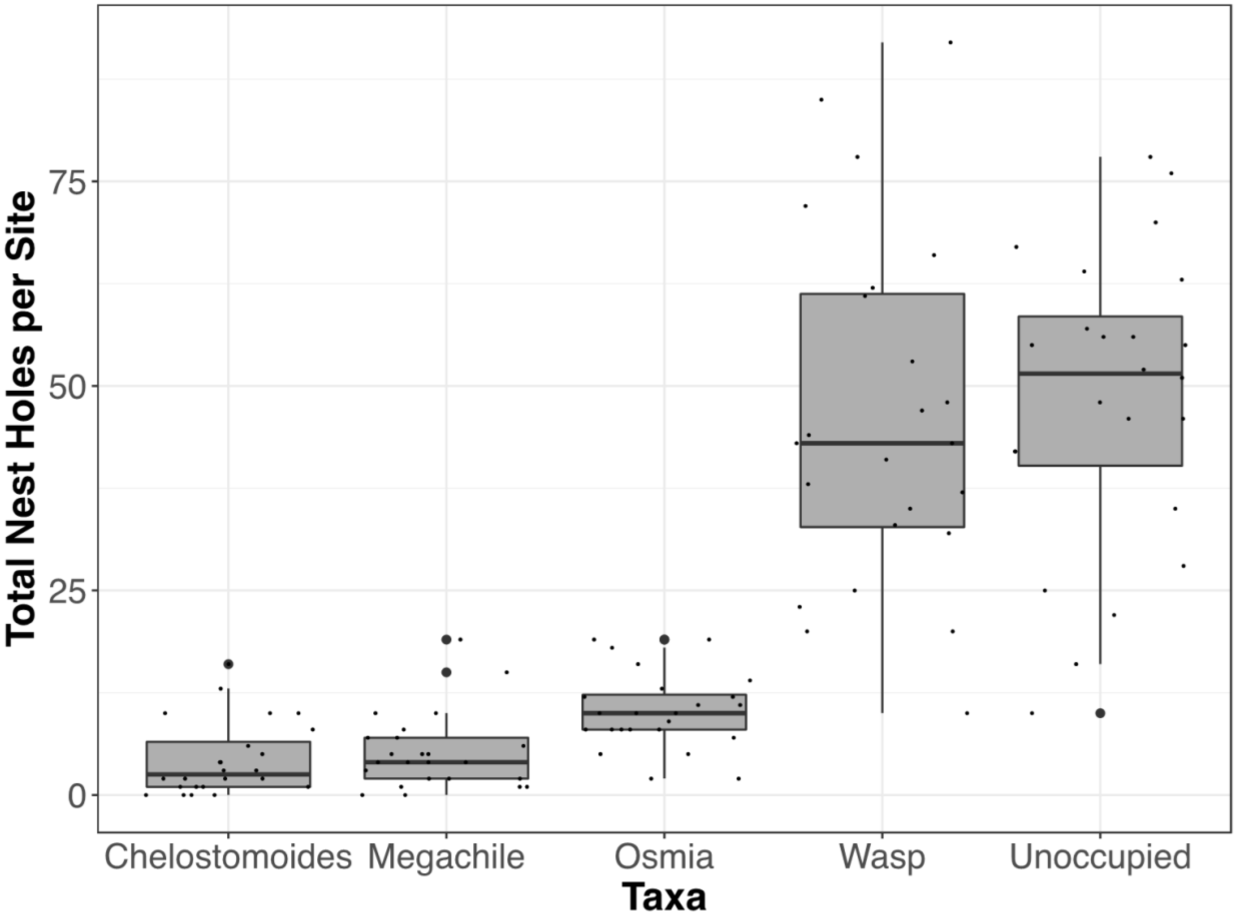
Supplementary Figure 2

A) Schematic diagram of experimental design where the green background represents the apple orchard, the blue rectangles represent the treatment and control plots, and the orange houses represent the regularly distributed nesting structures. Transects are represented by the yellow dashed line but may also be U-shaped in plots of 50 m x 60 m. The opaque circle serves to visualize the assumed typical 100 m foraging range of *Osmia* spp. Specific plot dimensions varied among sites, but the area of the plots remained the same. The 100 m distance separating the two plots represents a minimum distance. The arrows on the left and right of the plots represent the 200 m minimum distance between sites. **B)** Photo taken by Batoule Hyjazie of trap nests tied to apple tree in apple orchard. The bottom-most left-hand nesting hole is filled with macerated leaves while the hole immediately above it is occupied by a female *Osmia* provisioning her nest.



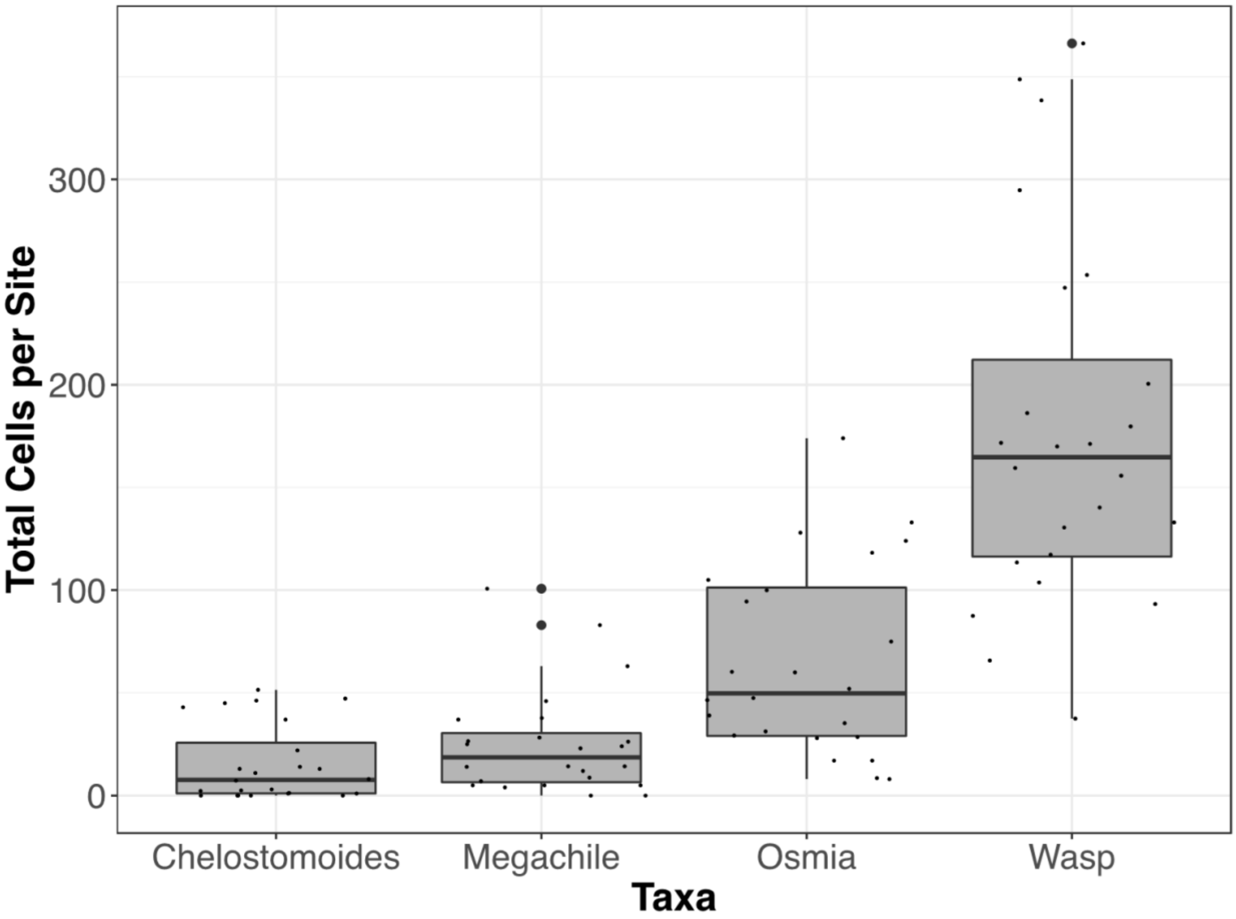
Supplementary Figure 3

A) 3D printed X-ray stand (designed by Dr. Samuel Morfin) for holding the straws in place during X-ray image processing. **B)** Example of X-ray image taken of 10 the back half of straws (*Osmia* spp. nest holes, ID from top to bottom: CO1-3-1, CO1-3-3, CO1-3-5, CO1-3-13, CO1-5-3, CO1-5-7, CO1-7-4, CO1-7-6 and CO1-8-2) (for X-ray parameters, see *2.5 Collecting Data from Nesting Structures*) (image taken by Dr. Samuel Morfin). An individual was coded as surviving if it had an opaque and dense (non-translucent) body with three clear sections (head, thorax, and abdomen). An individual was coded as male if it was approximately half the size of a female. In this image, there are more females than males because female eggs are typically laid at the back of the nest (earlier in the foraging season).

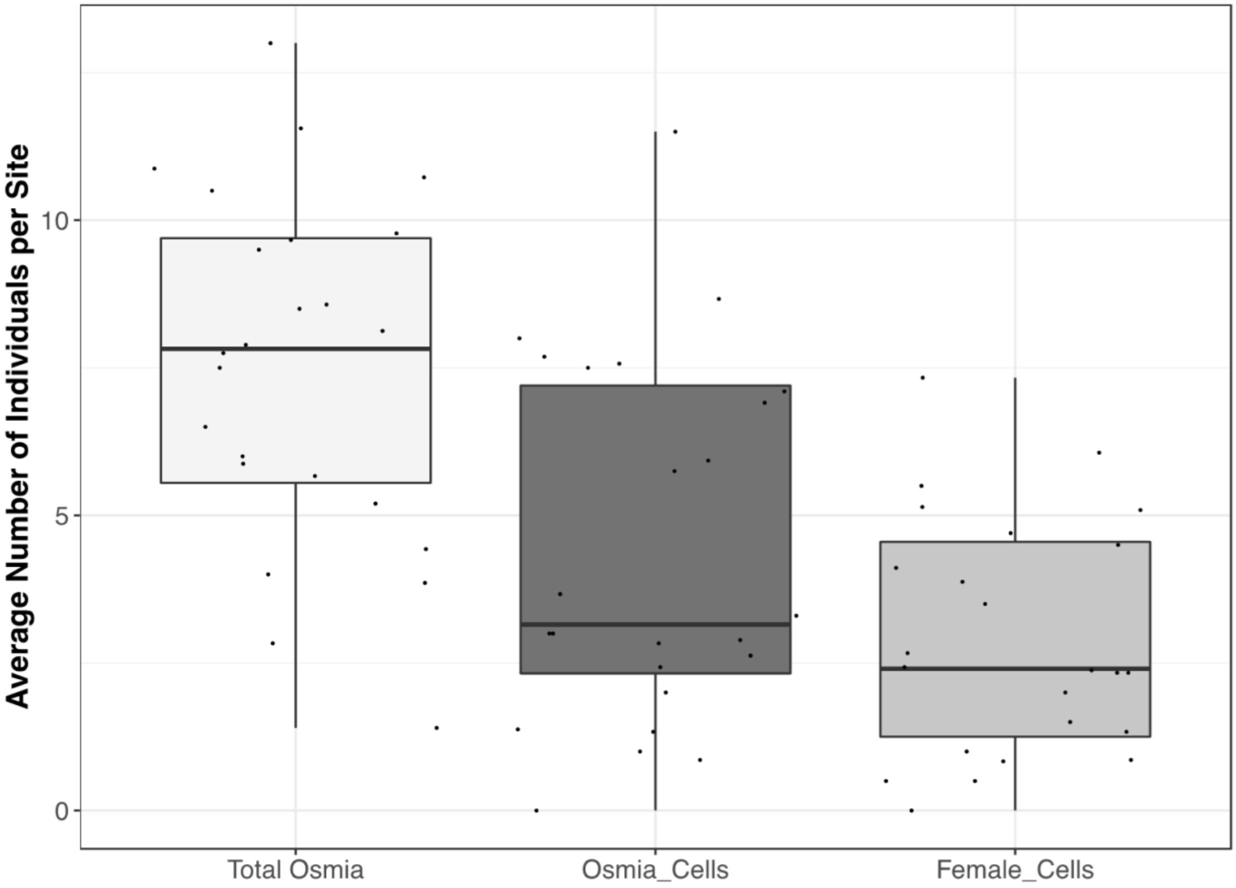


Supplementary Figure 4

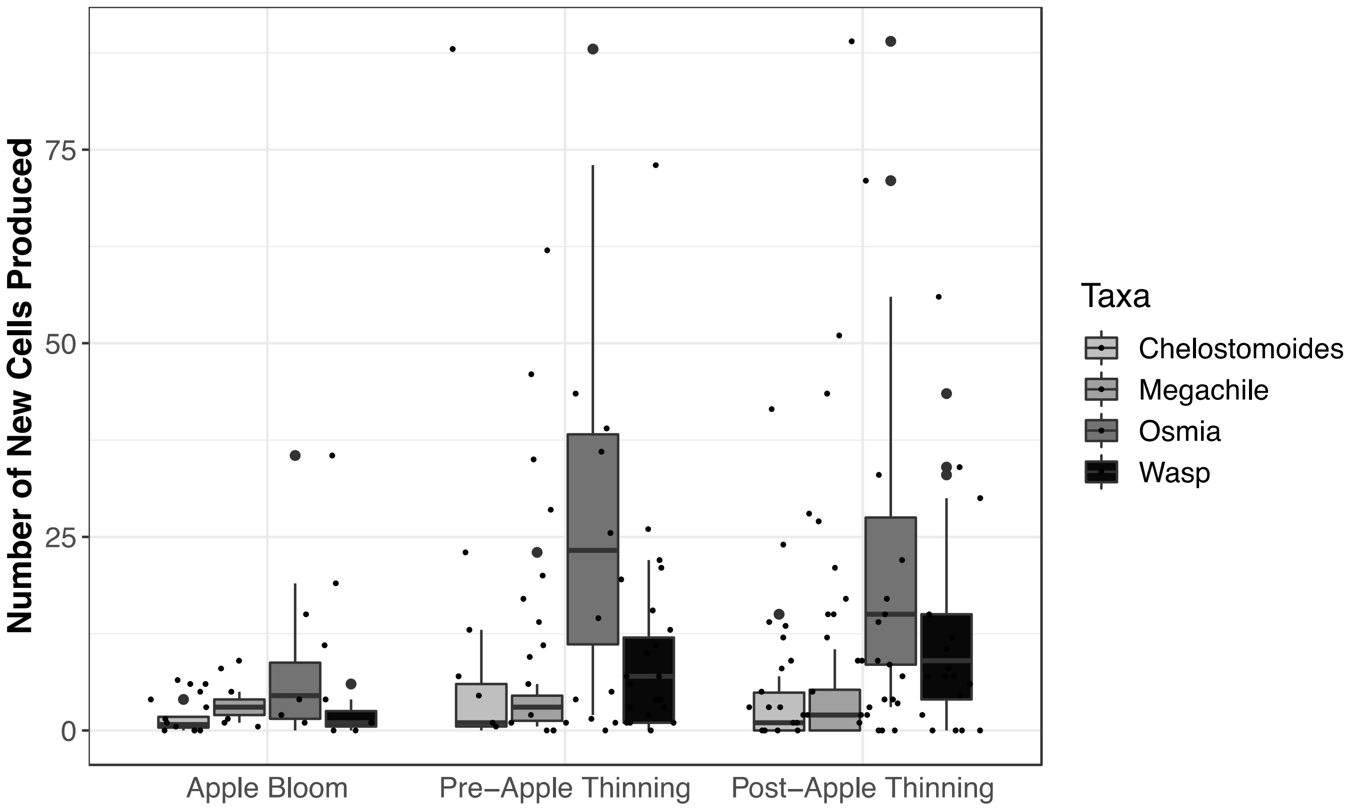
Box plots summarizing total counts of occupied nest holes (straws) by taxon out of a total of 2,742 nest holes. There is one category for unoccupied nest holes that have remained empty throughout the foraging season. Each data point represents the total number of nest holes at each site (n = 24).



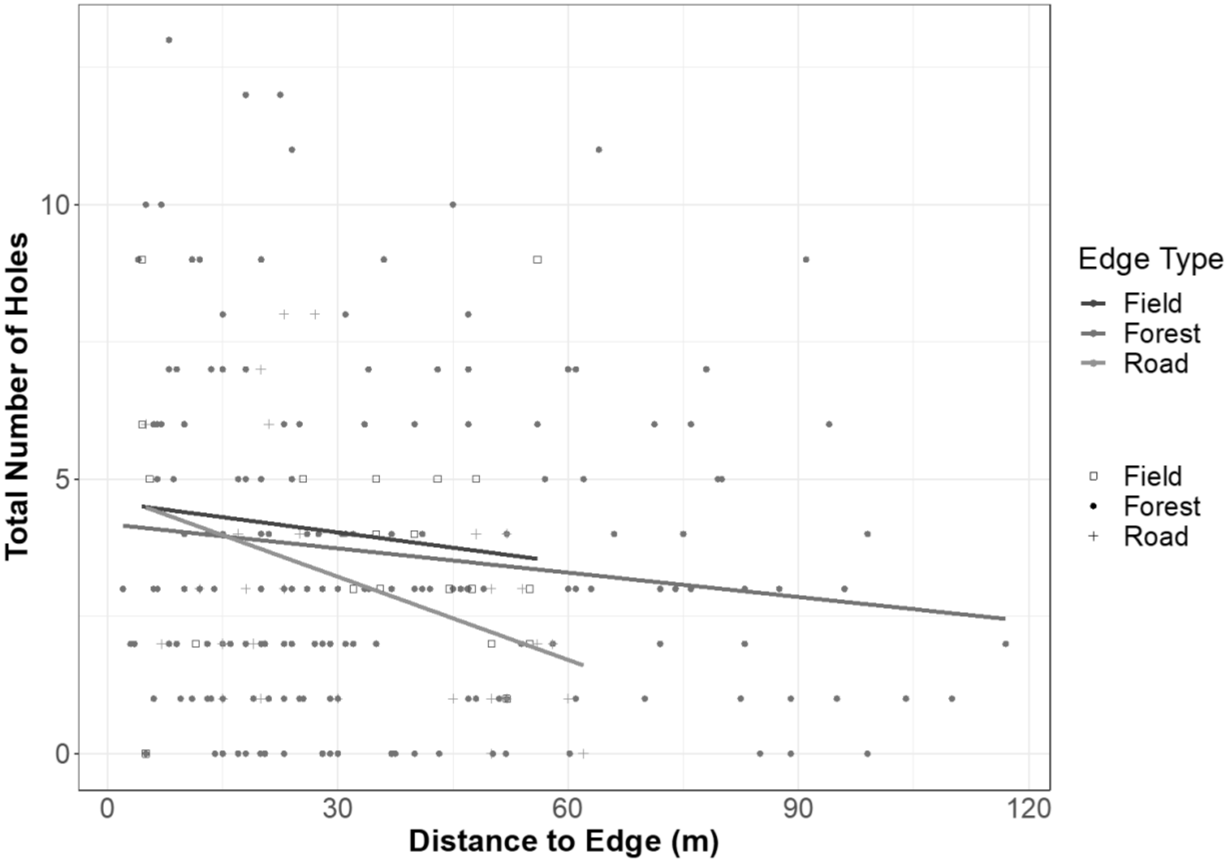
Supplementary Figure 5
Box plots summarizing total counts of produced cells (i.e., eggs laid in straws) by taxon. Each data point represents the total number of cells at each site (n = 24).



Supplementary Figure 6
Box plots summarizing counts of *Osmia* spp. individuals, of surviving *Osmia* spp. individuals, and of surviving *Osmia* spp. females. Each data point represents an average per nest at each site (n = 24).



Supplementary Figure 7
Box plots summarizing counts of new brood cells produced by cavity-nesting insects summarized across all nests for a given taxon at a site since the previous site visit. Each data point represents an average at each site (n = 24).



Supplementary Figure 8
Total number of occupied holes per distance to edge (m) of each nest box (n = 240). There are three edge types: field (full of wildflowers and undeveloped agricultural land), forest (covered in trees and shrubs), and road (a dirt or paved road).