A little-known world - assessing a non-bee crop flower visiting community using metabarcoding

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Abstract

Pollinator diversity is critical for optimal ecosystem service and function. While bees are frequently the most efficient pollinators, they represent only a small fraction of pollinator diversity. Non-bee pollinators have received little recognition for their role in commercial agricultural pollination despite representing 95% of flower visitor diversity. Many non-bee pollinators are more resilient to land-use intensification and climate change due to their nomadic life-history and tolerance of inclement weather. Our research characterizes non-bee pollinator communities, their foraging preferences, and floral fidelity in strawberry crops. We caught 608 non-bee flower visitors, across three field sites, during three months of the flowering period (May–August) of day-neutral strawberries in southern Ontario. DNA metabarcoding provided species-level identifications of the non-bee flower visiting community. Diptera (64%) and Hymenoptera (22%) (primarily bee species) were the most abundant flower visitors; Coleoptera and Hemiptera were also collected from flowers. Metabarcoding of pollen identified pollen from 110 genera representing 48 different families. Species with a high floral fidelity (flower constancy) for visiting strawberries were likely to be more effective pollinators (vectors of conspecific pollen between reproductively receptive strawberry plants). Additionally, small amounts of pollen from other plant genera suggested that insects are active and mobile, rather than staying stationary on a single flower.

Introduction

The importance of pollination for terrestrial ecosystem function and services is well known. It is estimated that 78% of temperate flowering plant species rely to some extent on the activity of animal pollinators for reproduction (Ollerton et al. 2011). Many of these are critical for maintaining ecosystem functions providing important services for humans (e.g., increased water and air quality, prevention of soil erosion, timber, fruit and nut production; Kearns et al. 1998, Ashman et al. 2004, Cardinale et al. 2012). In addition, insect pollinators are critically important to global food crops, contributing to 65% of the produced crop volume (Klein et al. 2007). All these ecosystem services are often solely credited to bees (Woodcock 2012, Dicks et al. 2013) and although they are obligate nectar and pollen foragers, this assumption leaves out many other important flower visiting insects (Müller et al. 2006). In fact, there are more than 330,000 insect species such as flies, beetles, wasps, moths and butterflies which may provide pollination services that are often unaccounted for (Larson et al. 2001, Wardhaugh 2015, Rader et al. 2016, Ollerton 2017). Such diverse flower visitor assemblages have been shown to lead to evenness of pollination services; resulting in higher fruit set and fruit weight with fewer blemishes due to insufficient pollination (Nye and Anderson 1974, Lopez-Medina et al. 2006, Hodgkiss et al. 2018).

This evenness of pollination service is the result of each species having their own foraging preferences and tolerances, which leads to more frequent and thorough visitation of flowers from different pollinators, thus increasing the likelihood of conspecific pollen transfer (Fontaine et al. 2005, Hoehn et al. 2008, Blüthgen and Klein 2011, Garibaldi et al. 2013, 2014, Rogers et al. 2014). For example, some insect communities are abundant early in the growing season, maintaining large populations for pollination services for only a week

or two, while others peak later in the season, whereas others have a lower population density that is sustained throughout the season (Bartomeus et al. 2013). Thus, there is always a reasonably large pollinator community with potentially complementary and overlapping population peaks (Garratt et al. 2018). Additionally, species have different foraging strategies. For instance, honey bees tend to forage along the tops of plants and travel in a linear fashion, while solitary bees and flies tend to forage on lower branches in a random pattern (Brittain et al. 2013, Brunet et al. 2019). The combination of these two foraging behaviours ensures that the entire plant receives pollination and therefore sets fruit evenly. Finally, different environmental conditions can affect which insects are foraging. High winds and cloudy days tend to keep honey bees inside their hives, while flies, bumblebees and some solitary bees will continue to forage in the rain and the cold (Morgan and Heinrich 1987, Brittain et al. 2013).

One of the leading justifications usually given for the exclusion of non-bee species in pollination studies is that bees are often the most efficient pollinators (Buchman and Nabhan 1996, Kennedy et al. 2013). Bees have a nectar and pollen-dependent diet; as such, their behaviour and foraging techniques often result in high pollen release and frequent flower visiting (Sheffield 2014, Russo et al. 2017, Campbell et al. 2018). When considering non-bee pollinators, their average pollination efficiency per flower visit may be comparatively low, but their ubiquity can lead to high visitation frequency, resulting in equal, or greater, pollen deposition than bees (Larson et al. 2001, Skevington and Dang 2002, Rader et al. 2009, Orford et al. 2015, Rader et al. 2016). This is especially true when considering Diptera, which are particularly speciose and abundant (Skevington and Dang 2002).

To ensure accurate estimation of relative contribution from all crop-pollinating taxa, it is crucial to include non-bee pollinators in crop-pollination surveys, pollination estimates, and pollinator-management practices to avoid taxonomic bias. However, it can be difficult to assign reliable species-level identification of non-bee pollinators and the plant pollen they carry. Given the taxonomic breadth of flower visitor communities, the number of taxonomic experts required for accurate species-level identification is typically unobtainable. Equally, the identification of plant pollen has traditionally been achieved using light microscopy, comparing samples to an extensive palynological collection. However, this method often restricts taxonomic resolution to the genus or family level and requires extensive expertise (Rahl 2008, Keller et al. 2015). DNA-based identification methods, such as DNA barcoding (Hebert et al. 2003) and metabarcoding (Cristescu 2014), can generate sufficient taxonomic resolution to overcome those barriers. The mitochondrial cytochrome *c* oxidase I (COI) gene is the community-wide accepted standard DNA barcoding region for animals (Hebert et al. 2003) while metabarcoding of the plastid barcode standard gene region of *rbcL* (large subunit of RuBisCo) (Hollingsworth et al. 2009) and the nuclear region ITS2 (internal transcribed spacer) has been explored for its accuracy in the qualification and quantification of pollen samples (e.g., Bell et al. 2019, Potter et al. 2019, de Melo Moura et al. 2022).

For this study we aimed to identify a community of non-bee flower visitors considered probable pollinators living on three fields of strawberry (*Fragaria* spp.) crops in Southern Ontario, Canada. We performed species-level identifications of the non-bee flower visiting insects by using DNA barcoding. In addition, pollen loads collected from the bodies of those flower visitors were quantified by particle characterization and identified utilizing *rbcL* metabarcoding. Subsequently, we assessed how non-bee flower visiting communities changed across the season and in response to variation in local environmental conditions, including temperature, humidity, solar radiation, and wind speed.

Materials and Methods

Field Sampling

Three large-scale crop production strawberry fields in Southern Ontario were selected. Since crop field size was the presiding selection criteria, fields had differing crop varieties, surrounding habitat, and pollinator-friendly additions, or lack thereof. Fields were within 120 km of each other and within a latitudinal gradient of 0.15 degrees. The narrow latitudinal gradient was chosen to increase the similarity in flowering time and temperature fluctuations. All fields had a seven-day spraying rotation; however, pesticides used are largely

unknown and therefore were not considered. Each field was sampled weekly between May 1st and August 31st of 2018 when the crop reached at least 20% bloom. Bloom percentage was assessed by walking up a row from the field edge and counting the number of flowers on each side, to a depth of 50 flowers (100 flowers total); this was repeated in the field centre, and numbers were averaged. Sampling took place from 09:30 to 16:00 and consisted of five, hour-long periods followed by a 30-minute break. Each sampling event was divided into a 30-minute active period and a 30-minute observation period with five sampling events in a day.

During active sampling periods non-bee flower-visitors (excluding Lepidoptera and *Drosophila* spp.) were collected directly into sterile vials and set aside for lab work. Lepidoptera were excluded from these collection samples, because the scales from their wings would disrupt the quantification of pollen found on individuals.*Drosophila* spp. were excluded because they were far too numerous to capture without affecting the capture rate of other specimens. Samples were placed in a small cooler containing freezer packs at the end of each sampling to minimize grooming behaviour and regurgitation. The remaining 30 minutes of each sampling hour were for observational sampling, where all flower-visiting insects were identified to the lowest confident taxonomic unit. This approach provided a non-lethal sampling method to determine the insect community (including bees) and reduce collector bias. A small number of bees were sporadically collected, to provide comparative data on pollen sources and their abundance. Because this sampling was not standardized, the abundance of these collections cannot be considered representative of true populations. Only observational data are representative of bee abundance at each site. The order of active and passive sampling portions of each hour periods were randomly selected. Sampling periods rotated between edge habitat (the edge of the crop, to 50 m into the interior) and interior habitat (at least 60 m into the crop), while randomizing whether the first period was interior or exterior. Measurements were collected for wind speed, rainfall, humidity, and temperature using AcuRite weather station and solar radiation using a TES 1333R Solar Power Meter, every half-hour. These environmental measurements were taken every 90 minutes during sampling (five per sampling day) and averaged for the analysis.

DNA Barcoding of Insects

Trace files and sequences were uploaded to the Barcode of Life Data system (BOLD) and automatically assessed for quality based on predefined parameters (Ratnasingham and Hebert 2007). All sequences and associated metadata can be found in the public dataset DS-NBPP (GenBank Accessions OQ622469 - OQ623072). A Barcode Index Number (BIN, proxies for species distinguished sequences without an assigned taxonomic name), was assigned using the RESL algorithm on BOLD (Ratnasingham and Hebert 2013).

Pollen Removal and Quantification

Pollen was removed from the exterior of insect bodies following the protocol by Lucas et al. (2018). Each specimen was washed in 500 µL of wash solution containing 2% PVP and 1% SDS (buffer solution) using a 1.5 mL Eppendorf tube. For larger specimens (>8 mm) additional wash solution was added until they were submerged. The specimens and controls were agitated by hand for 1 minute and then centrifuged at 15800 g for 20 seconds. Afterwards they were incubated for 5 minutes and shaken for an additional 20 seconds to resuspend pollen. The insects were then removed from the tube and stored in 95% ethanol. The remaining washing solution and suspended pollen were centrifuged at 15800 g for 5 minutes. The supernatant was discarded, and samples stored at -20°C.

The pollen pellet was resuspended in 250 µL of 95% ethanol by vortexing for 4 minutes. Samples that were difficult to homogenize were heated at 56°C for 5 minutes and vortexed for an additional 4 minutes. An aliquot of 50 µL was taken and dried in a sterile incubation oven for quantification and the remainder was used for metabarcoding. Pollen counts were determined for each sample using a Multisizer 3 Coulter Counter (Beckman Instruments, Fullerton, CA, USA). A blank of 10 mL of Isoton II diluent was measured in a 30 mL cuvette and used to calibrate the machine. The pollen sample was suspended in 300 µL of diluent by vortexing for 10-20 seconds. This pollen suspended diluent was added to the measuring cuvette. Additional diluent was added to reach 11 mL of liquid. The cuvette was gently vortexed for 3-5 seconds to homogenize

the sample. The Coulter counter was then used to quantify the number of particles in the size range 10-120 μm for three 1 mL samples.

Pollen Quantification

Pollen loads were assessed by comparing non-bee insect visitors to the genus of bee with the largest pollen loads, *Halictus* , using a generalized linear model (GLM) using quasi-poisson distribution. A GLM was used in place of a linear model to account for the non-normal distribution of data. As the data were also overdispersed, a quasi-poisson distribution was chosen (Ver Hoef and Boveng 2007). The pollen counts for each respective genus were used as response variable. Each non-bee genus was treated as a factor and included as explanatory variables. Visualizations of pollen loads, and insect abundance were presented using TreeMaps generated in R (version 2.5-5). To assess total available pollen contribution, data from observations were combined with pollen counts from collected specimens, total pollen = observed abundance x average pollen count. The percentage of total pollen was calculated by taking the average pollen load for each insect taxon and dividing it by the absolute sum of total pollen counts.

Molecular Identification of Pollen

Pollen DNA was extracted using a modified glass fibre protocol (Ivanova et al. 2008). The remaining 200 μL of ethanol suspended pollen samples were dried under a sterile hood and resuspended in 300 μL of insect lysis buffer. Samples were transferred into 96 well plates with microbeads (MP Biomed, lysis matrix E, OH, USA). Samples were randomly assigned a location in the plate matrices. In order to detect contamination, 116 negative controls were added into the matrices, randomly assigned with at least one negative control per column in the 96 well plate matrix. Pollen grains were pulverized by shaking samples at 28 Hz for two minutes. Samples were incubated at 56°C for 2 hours, followed by 1 hour at 65°C. Following incubation, 6M GuSCN buffer was added to lysate in a (2:1 to lysate, 400 μL to 200 μL), mixed briefly by vortexing, centrifuged at 1000 g for 20 seconds. The lysate was transferred to a glass fibre filter plate (PALL Corp) and centrifuged at 5000 g for 5 mins, followed by the addition of 300 μL of binding mix and centrifuged at 5000 g for 2 mins. The glass fibre plate was then washed twice with 600 μL of wash buffer and spun down at 5000 g for 5 mins. The plate was spun for an additional 5 mins at 5000 g and incubated at 56°C for 30 mins to dry the plate. DNA was eluted into a PCR plate with 25 μL of elution buffer and incubated at 56°C for 1 minute and then centrifuged at 5000 g for 5 mins.

To assess plant diversity, we amplified a 184 bp fragment of *rbcL*(large subunit of RuBisCo) using rbcL1 and rbcLB (Palmieri et al., 2009) (Table 1). A total of 284 samples w selected for sequencing. The Qiagen multiplex plus master mix (QIAGEN, Hilden, Germany) was used for PCR. Amplification was performed under the following thermal conditions: 5 mins at 95°C; 35 cycles of 30 s at 95°C, 30 s at 50°C, and 1 min at 72°C; 5 min at 72°C; then held at 4°C. The 25 μL PCR reaction mix included 12.5 μL of Master Mix, 1.25 μL of each 10X PCR forward and reverse *rbcL* primer and 10 μl of DNA template (Palmieri et al. 2009, Little 2014). PCR amplicons were visualized on a 1.0% agarose gel using GelRed® Nucleic Acid Gel Stain (Biotium, Hayward, CA, USA). Samples were indexed with a secondary PCR using fusion primers and run under the same thermal conditions (Elbrecht and Steinke 2018). Fusion primers were used to attach unique molecular identifiers (UMIs) along with TruSeq sequencing adaptors for Illumina MiSeq sequencing (Supplemental Table S2). The PCR mix included 12.5 μL of Master Mix, 9 μL of molecular grade water, 1.25 μL of each 10X PCR forward and reverse primer with custom tags (Elbrecht and Steinke 2018) and 1 μL of DNA template. The samples were cleaned and normalized using the SequalPrep Normalization Plate Kit (Invitrogen, Thermo Fisher Scientific Inc., MA, USA) following manufacturer's instructions. Libraries were pooled and subsequently went through clean up using the SPRIselect Kit (Beckman Coulter) and the Left Side Size Selection procedure with a sample-to-volume ratio of 0.75. Final quantification was done using a Qubit Fluorometer with the Qubit dsDNA HS Assay Kit according to manufacturer's instructions. Sequencing was done using an Illumina MiSeq with the 600 cycle Reagent Kit v3 (2 × 300) at the Advanced Analysis Centre at the University of Guelph.

Sequence Analysis

Pollen libraries were analyzed using JAMP (https://github.com/VascoElbrecht/JAMP). In summary, the pipeline demultiplexed the sequences using the assigned custom tags, trimmed the primers using cutadapt (v. 2.4; Martin 2011), filtered by length (184 +/- 10 bp) and expected error (1), and denoised using Usearch (Edgar 2010). The resulting exact sequence variants (ESV) were queried against a custom *rbcL* library (Braukmann et al. 2017, Kuzmina et al. 2017) using MegaBlast (Tan et al. 2006) in Geneious (ver 9.1.1; Kearse et al. 2012). The extracted hits were then queried against the ESV using the classify sequences command in Geneious with a minimum 99% identity match and 0.5% to the next best hit. A 99% threshold was chosen to allow more sequences to be included, as *rbcL* markers are distinct at the family-level. Singletons and ESVs below 0.01% were excluded as these are likely not represent true diversity but rather sequencing or PCR errors.

Data Analysis

Treemaps to display diversity and abundance were generated using the R package Treemap v. 2.4-3 (Tennekes, 2022). Plant-flower visitor networks were build using the bipartite package in R (Dormann et al. (2008). A Redundancy analysis (RDA) was used to assess how the non-bee community changed due to environmental variance, such as the parameters measured in the experimental methods: wind speed (km/h), solar radiation (W/m^2), humidity (%), temperature (°C) and edge effect (binary: interior or exterior). These variables only explained 14% of the variance, therefore time and date were also added to the model as explanatory variables. A Hellinger transformation was applied to remove the arch effect by normalizing the data through reducing the effect of zeros (Legendre and Gallagher 2001). The significance of the model and the axes generated were tested using an ANOVA like permutation test for Constrained Correspondence Analysis build into the function 'anova.cca' in vegan (Oksanen et al. 2019). All analyses were completed using R (version 2.5-5).

Results

Diversity and Pollen Loads

In total 3352 insects were observed; of those 972 were *Apis mellifera* (29%), 496 were other bees (15%), and 1884 were non-bee flower visitors (56%) (Supplementary Table 1).

In addition, a total of 604 insects were collected and successfully sequenced (GenBank Accessions OQ622469 – OQ623072). 515 of those were non-bee visitors belonging to 4 orders, 29 families, 53 genera, 62 species (Table 2; Supplementary Table 2). Sequence read lengths ranged from 359 to 658 bp, with an average of 644 bp. For pollen load comparison 89 bee specimens were caught and successfully sequenced, 26 were assigned species level identification representing three families containing 13 species (Table 2). For two genera (*Nomada* and*Sphecodes*) DNA barcodes did not provide consistent identifications due to a poorly parameterized reference library on BOLD. Consequently, both were excluded from pollen counts.

When considering only the data from collected specimens, the non-bee families which contributed the most pollen (average pollen count x abundance) were flies of the families Syrphidae, Polleniidae and Anthomyiidae (Figure 1a). Overall, the species that carried the most pollen (in the order of 10,000-70,000 pollen grains on average per individual) were *Eristalis tenax* > *Eristalis similis* > *Halictus confusus* >*Lasioglossum pectorale* > *Ceratina mikmaqi* > *Neocnemodon coxalis* > *Callirhytis tumifica* > *Bombus impatiens* (Figure 1b; Table 2). Of the non-bee flower visitors, 30 of the 53 genera caught, had pollen loads that were not significantly different in size from the genus of bee (*Halictus*) with the highest pollen count (Table 3).*Eristalis* was the only genus that carried more pollen than*Halictus* (Figure 1b; Table 3). Variation in pollen load size was substantial, even within a species group. For example, *Eristalis tenax* individual loads ranged from 1,617-316,300 pollen grains (Table 2).

Pollen Metabarcoding and Pollinator Networks

The pollen loads of 267 insects were successfully analyzed for plant family or genus composition. The total read count after filtering was 15,205,661 ranging from 22 (*Sylvanelater cylindriformis*) to 2.7 M (*Toxomerus marginatus*) (Supplementary Table 3). After removal of OTUs (operational taxonomic units) from negative controls, we found 129 plant genera with at least 98% hit match to reference library (Supplementary Table

4). As a more conservative estimate, 49 plant families were found across all insects (Supplementary Table 3, Figure 2). The relative abundance of sequence reads was used as a proxy of relative abundance of pollen load composition for the remaining analysis (Richardson et al. 2015, Kraaijeveld et al. 2015, Pornon et al. 2017).

All species, apart from one undetermined cecidomyiid had strawberry pollen on their bodies. Species with only strawberry pollen on their bodies were the dipterans *Lucilia sericata, Liohippelates bishoppi, Discomyza incurva* , the coleopterans *Collops quadrimaculatus, Macrodactylus subspinosus, Sylvanelater cylindriformis* , and the hemipteran *Lygaeus kalmii* (Supplementary Table 3). The most generalist families were Syrphidae (for which pollen data from 32 plant families, and 73 genera were recorded), Pollenidae (29 plant families, 55 genera), and Anthomyiidae (22 plant families, 43 genera). At first glance syrphids appeared quite generalist in their choice of flower visitations. However, this picture was driven by two generalist species (*Toxomerus marginatus* and *Sphaerophoria scripta*) and the remaining species were more selective, carrying pollen from only 2-6 plant species (Figure 3).

Environmental Variance of Community Structure

Redundancy analysis (RDA) modelling was applied to Hellinger transformed observational data with respect to five environmental explanatory variables (wind speed, solar radiation, temperature, humidity, and field edge effects). While the model was statistically significant ($F = 3.94$, $p < 0.001$), it only explained 14% of the variance. All environmental variables are well represented by the axes but did not match the spread of communities in the model. The effect of sites on community composition appeared to be low since there was no distinct clustering with this variable. However, the model including temporal variables explained 65.2 % (R^2 adj = 53.9%) of the variance (Figure 4a) was significant ($F = 5.79$, $p < 0.001$); and so were first 7 axes (ANOVA, $p < 0.001$). Most of the temporal variance was explained by sampling date (ANOVA, $p < 0.001$). Models that included date improved the explained variance by more than 45%. Time of day was also significant (ANOVA, $p < 0.03$), with ellipses depicting a small gradient across the communities (Figure 4a). General trends in insect community composition across the season showed consistent presence of solitary bees and Hemiptera, while the abundance of Syrphids and other Diptera, as well as observed *Apis mellifera*showed more seasonal variation (Figure 4b). While Formicidae (ants) were generally rare or absent, there was a large surge in their abundance on flowers for one week (May 18^{th}). Coleoptera and Lepidoptera consistently showed low levels of occurrence on strawberry flowers (Figure 4b).

Discussion

For a long time, the pollination capacity of non-bee insects visiting flowers has remained largely unexplored (Solomon and Kendall 1970, Boyle and Philogène 1983, Currah and Ockendon 1983, Kumar et al. 1985). Only recently developed molecular methods, such as metabarcoding, seem to have the potential to change this (Keller et al. 2015, Lucas et al. 2018) as confirmed by the much greater resolution and diversity of pollen present on the bodies of flower visiting insects collected from strawberry flowers in our study.

We observed a high diversity of non-bee visitors on day-neutral strawberry flowers at three sites in Southern Ontario and used metabarcoding to identify the pollen they carried. More than half of the non-bee genera were found to transport similar amounts of pollen to native bees from the genus *Halictus* (Figure 1). In fact, two members of the syrphid genus *Eristalis* had the largest overall pollen loads per individual (Figure 1, Table 3).

When assessing pollen loads at a coarse level, Syrphidae had the most available pollen, contributing more than four-times as much pollen as the species of Halictidae (Figure 1, Table 2). This was primarily due to the high pollen loads found on *Eristalis tenax* and*Eristalis similis* (Figure 1b; Table 2), as well as the high abundance of *Toxomerus marginatus* (n = 114), which carried much less pollen per individual, but considering total pollen available in the field, all three species contributed meaningfully (Figure 1b; Table 2). Our observations during sampling indicated that syrphids were not stationary on flowers, they took flight at the slightest disturbance and alighted on neighbouring flowers. This observation is consistent with previous findings in studies on effective syrphid pollination, showing large pollen loads and rapid flower-

flower movement (Bohart and Nye 1970, Solomon and Kendall 1970, Kendall and Solomon 1973, Nye and Anderson 1974, Kumar et al. 1985, Hodgkiss et al. 2018). Syrphid abundance has also been correlated with an increase in pollination and fruit set with a decrease in malformation of strawberry fruits (Stewart et al. 2017).

The fly families Polleniidae and Anthomyiidae were also found with larger amounts of pollen (Figure 2A). Polleniid flies are already known to be efficient pollinators of strawberry, imparting services equivalent to honey bees and have been used for stocking greenhouses (Free 1966, Carden and Emmett 1973, Clements 1982). Anthomyiids, also known as root-maggot flies, are a crop pest to strawberries, and thus their role as potential pollinators needs to be weighed against the consequence of their pest status. Interestingly, two of the three recorded ant species (*Prenolepis imparis* and *Formica subsericea*) are confirmed pollinators (Ashman and King 2005) but the proportion of strawberry pollen on them varied substantially (30% to 100% respectively) (Appendix 1). The third ant species, *Tetramorium caespitum*(Formicidae), mostly carried strawberry pollen (92%; Appendix 1). The exclusion of Lepidoptera is unlikely to affect the assessment of non-bee flower visitors as their abundances were low (Figure 4B). The exclusion of *Drosophila* was necessary given the resources and collection methods. However due to their high abundance, even with small pollen loads, it is possible that they collectively provide substantial pollination services that we were unable to track. However, while observing them in the field they often did not move from flower to flower, but rather stayed clustered together and stationary on a single flower.

Most of the pollen found on non-bee flower visitors collected on strawberry flowers was indeed strawberry pollen, with an average of 69% across all measured pollen loads (Figure 3A). The species with the largest pollen loads carried over 70% strawberry pollen:*Eristalis tenax* (N= 350,017, 85% strawberry), *Eristalis similus* (N = 216,117, 70% strawberry), *Toxomerus marginatus* (N = 141,783, 76% strawberry), and *Pollenia rudis* (N = 105,320, 87% strawberry). Thus, these flower visiting species are likely contributing significantly to pollination and should be investigated further to rule out the possibility that they are stationary on a particular flower and therefore cannot be classified a pollinator.

Interestingly, the most generalist families (counted by plant genera) were also those that carried the largest amount of pollen (Figure 2, Supplementary Table 3), Syrphidae (58 plant genera), Polleniidae (53 plant genera), and Anthomyiidae (35 plant genera). Anthomyiid flies have been recorded as a largely generalist family of flower visitors (Larson et al. 2001). Within the Syrphidae, we found two generalist and one specialist species within *Toxomerus* (Figure 3). The genus*Toxomerus* included both a generalist (*T. marginatus*) and a specialist (*T. geminatus*) which could be the result of speciation due to differing food exploitation strategies (Schluter et al. 1985). However, all plant (pollen) diversity estimates should be treated cautiously when considering which plants these insects visit, as many of the genera identified with metabarcoding were grasses (Poaceae) with 15 genera identified, and other wind-pollinated plants (Rabinowitz et al. 1981). The presence of wind-pollinated plants in the samples could be incidental, found on these insect bodies as the result of contact with windborne pollen when flying, rather than a confirmed visit to the plant itself (although this also cannot be excluded as a possibility).

The RDA analysis demonstrated that a number of environmental variables were poor predictors of insect community visitation to strawberry flowers (Figure 4A). A strong explanatory variable in the model was sampling date and to a lesser degree time of day (during sampling days). This suggests that the flower visitor community was quite different on each day of sampling. As such, this model could be detecting phenological patterns of the non-bee visitors; insects that emerge and are abundant for a short time and not recorded outside of their biological timeline. This is supported by observations (see Figure 4B), where large peaks of activity can be found for some taxa, particularly Syrphidae and Formicidae. Many insects are restricted to narrow ranges of temperature for flight, as endothermy is a rare trait in insects, requiring a rise in ambient temperature or basking in sunlight to warm their flight muscles (Inouye et al. 2015). Most syrphid species, however, do have endothermic capabilities which allows them to forage in cloudy and cool weather (Morgan and Heinrich 1987). Other dipteran families also forage when bees and butterflies do not (Hooper 1932, Inouye et al. 2015). Indeed, during field sampling, syrphids and other flies were foraging on

cool, overcast days and even in light rain. Low abundance of solitary bees, particularly *Dialictus* , were out visiting strawberry flowers during these less-than-ideal weather conditions but they were stationary, and not apparently actively engaged in pollinating during this time. This range in degree of specialization(s) could reduce the effect of the environmental variables in the model.

Conclusion

Our study found a high diversity of non-bee flower visitors, and the primary non-bee pollinators were flies. Even though bee-mediated pollination is important, our results suggest that non-bee species significantly contribute to total pollination services for strawberry crops in Ontario. On average, syrphids carried more pollen than native bees, contextualizing their role as putative pollinators. The collective contribution of three fly families, Syrphidae, Polleniidae and Anthomyiidae, represented most of the active pollen within the investigated fields. Although these families also tended to be the most generalist foragers, their pollen loads contained large proportions of strawberry pollen. Generalist pollinators are highly valuable in agriculture; they contribute to the diversity of pollinators visiting crop flowers and therefore increase pollination success, and they are more robust in the face of ongoing landscape intensification (Ghazoul 2005, Blüthgen and Klein 2011, Garibaldi et al. 2014). Furthermore, generalists may be more resilient to adverse weather conditions (Heinrich and Mcclain 1986, Inouye et al. 2015). However, further research is needed to understand the quality of pollination services of non-bee pollinators.

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Author contributions

E.R., N.E.R. and D.S. conceived the original idea for the study. E.R. collected the field data. E.R. and T.B. conducted lab work. E.R., T.B. and D.S. contributed to data curation, formal analysis, and data visualization. N.E.R. and D.S. contributed to funding acquisition. E.R. (lead), T. B., N.E.R. and D.S. contributed to writing-original draft. E.R. (lead), T. B., E.R. and D.S. contributed to writing-review and editing.

Conflict of interest statement

There is no conflict of interest.

Data availability statement

All DNA barcode data associated metadata for insect species sequenced can be found in the public BOLD dataset DS-NBPP (GenBank Accessions OQ622469 - OQ623072). All raw HTS datasets for pollen sequencing are deposited in the Sequence Read Archive (SRA www.ncbi.nlm.nih.gov/sra/) under the BioProject accession number PRJNAXXXXXX. Other ancillary information is provided within this paper and associated supplementary data.

References

Ashman, T.-L., and E. A. King. 2005. Are flower-visiting ants mutualists or antagonists? A study in a gynodioecious wild strawberry. American Journal of Botany 92:891–895.

Ashman, T.-L., T. M. Knight, J. A. Steets, P. Amarasekare, M. Burd, D. R. Campbell, M. R. Dudash, M. O. Johnston, S. J. Mazer, R. J. Mitchell, M. T. Morgan, and W. G. Wilson. 2004. Pollen limitation of plant

reproduction: ecological and evolutionary causes and consequences. Ecology 85:2408–2421.

Bartomeus, I., M. G. Park, J. Gibbs, B. N. Danforth, A. N. Lakso, and R. Winfree. 2013. Biodiversity ensures plant-pollinator phenological synchrony against climate change. Ecology Letters 16:1331–1338.

Bell, K. L., K. S. Burgess, J. C. Botsch, E. K. Dobbs, T. D. Read, and B. J. Brosi. 2019. Quantitative and qualitative assessment of pollen DNA metabarcoding using constructed species mixtures. Molecular Ecology 28:431–455.

Blüthgen, N., and A.-M. Klein. 2011. Functional complementarity and specialisation: the role of biodiversity in plant–pollinator interactions. Basic and Applied Ecology 12:282–291.

Bohart, G. E., and W. P. Nye. 1970. Onion pollination as affected by different levels of pollinator activity. Utah Agricultural Research Station Bulletin 482:3-45.

Boyle, R. M. D., and B. J. R. Philogène. 1983. The native pollinators of an apple orchard: variations and significance. Journal of Horticultural Science 58:355–363.

Braukmann, T. W. A., M. L. Kuzmina, J. Sills, E. V. Zakharov, and P. D. N. Hebert. 2017. Testing the efficacy of DNA barcodes for identifying the vascular plants of Canada. PLoS ONE 12: e0169515.

Brittain, C., C. Kremen, and A.-M. Klein. 2013. Biodiversity buffers pollination from changes in environmental conditions. Global Change Biology 19:540-547.

Brunet, J., Y. Zhao, and M. K. Clayton. 2019. Linking the foraging behavior of three bee species to pollen dispersal and gene flow. PLoS ONE 14: e0212561.

Buchmann, S., and G. P. Nabhan. 1996. The Forgotten Pollinators. Washington D.C., Island Press/ Shearwater Books.

Campbell, J. W., J. C. Daniels, and J. D. Ellis. 2018. Fruit set and single visit stigma pollen deposition by managed bumble bees and wild bees in *Citrullus lanatus* (Cucurbitales: Cucurbitaceae). Journal of Economic Entomology 111:989-992.

Carden, P. W., and D. J. Emmett. 1973. Artificially introduced blowflies show promise in strawberry pollination trials. Grower 79:1017-1019.

Cardinale, B. J., J. E. Duffy, A. Gonzalez, D. U. Hooper, C. Perrings, P. Venail, A. Narwani, G. M. Mace, D. Tilman, D. A. Wardle, A. P. Kinzig, G. C. Daily, M. Loreau, J. B. Grace, A. Larigauderie, D. S. Srivastava, and S. Naeem. 2012. Biodiversity loss and its impact on humanity. Nature 486:59–67.

Clements, R. 1982. Blowflies worth considering for tunnel pollination. Grower 97:24-26.

Cristescu, M. E. 2014. From barcoding single individuals to metabarcoding biological communities: towards an integrative approach to the study of global biodiversity. Trends in Ecology & Evolution 29:566–571.

Currah, L., and D. J. Ockendon. 1983. Onion pollination by blowflies and honeybees in large cages. Annals of Applied Biology 103:497–506.

de Melo Moura, C. C., Setyaningsih, C. A., Li, K.,Merk, M. S., Schulze, S., Raffiudin, R., Grass, I., Behling, H., Tscharntke, T., Westphal, C., Gailing, O. 2022. Biomonitoring via DNA metabarcoding and light microscopy of bee pollen in rainforest transformation landscapes of Sumatra. BMC Ecology and Evolution 22:51.

Dicks, L. V., A. Abrahams, J. Atkinson, J. Biesmeijer, N. Bourn, C. Brown, M. J. F. Brown, C. Carvell, C. Connolly, J. E. Cresswell, P. Croft, B. Darvill, P. De Zylva, P. Effingham, M. Fountain, A. Goggin, D. Harding, T. Harding, C. Hartfield, M. S. Heard, R. Heathcote, D. Heaver, J. Holland, M. Howe, B. Hughes, T. Huxley, W. E. Kunin, J. Little, C. Mason, J. Memmott, J. Osborne, T. Pankhurst, R. J. Paxton, M. J. O. Pocock, S. G. Potts, E. F. Power, N. E. Raine, E. Ranelagh, S. Roberts, R. Saunders, K. Smith, R. M. Smith, P. Sutton, L. A. N. Tilley, A. Tinsley, A. Tonhasca, A. J. Vanbergen, S. Webster, A. Wilson,

and W. J. Sutherland. 2013. Identifying key knowledge needs for evidence-based conservation of wild insect pollinators: a collaborative cross-sectoral exercise. Insect Conservation and Diversity 6:435–446.

Dormann, C.F., Gruber, B., Fruend, J. (2008). Introducing the bipartite Package: Analysing Ecological Networks. R News 8(2), 8-11.

Edgar, R. C. 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26:2460–2461.

Elbrecht, V., and D. Steinke. 2018. Scaling up DNA metabarcoding for freshwater macrozoobenthos monitoring. Freshwater Biology 64:380–387.

Folmer, O., Black, M., Hoeh, W., Lutz, R., and R, Vrijenhoek. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology, 3, 294–299

Fontaine, C., I. Dajoz, J. Meriguet, and M. Loreau. 2005. Functional diversity of plant–pollinator interaction webs enhances the persistence of plant communities. PLoS Biology 4:e1.

Free, J. B. 1966. The pollination of the beans *Phaseolus multiflorus* and *Phaseolus vulgaris* by honeybees. Journal of Apicultural Research 5:87–91.

Garibaldi, L. A., I. Steffan-Dewenter, R. Winfree, M. A. Aizen, R. Bommarco, S. A. Cunningham, C. Kremen, L. G. Carvalheiro, L. D. Harder, O. Afik, I. Bartomeus, F. Benjamin, V. Boreux, D. Cariveau, N. P. Chacoff, J. H. Dudenhöffer, B. M. Freitas, J. Ghazoul, S. Greenleaf, J. Hipólito, A. Holzschuh, B. Howlett, R. Isaacs, S. K. Javorek, C. M. Kennedy, K. M. Krewenka, S. Krishnan, Y. Mandelik, M. M. Mayfield, I. Motzke, T. Munyuli, B. A. Nault, M. Otieno, J. Petersen, G. Pisanty, S. G. Potts, R. Rader, T. H. Ricketts, M. Rundlöf, C. L. Seymour, C. Schüepp, H. Szentgyörgyi, H. Taki, T. Tscharntke, C. H. Vergara, B. F. Viana, T. C. Wanger, C. Westphal, N. Williams, and A. M. Klein. 2013. Wild pollinators enhance fruit set of crops regardless of honey bee abundance. Science 339:1608–1611.

Garibaldi, L. A., L. G. Carvalheiro, S. D. Leonhardt, M. A. Aizen, B. R. Blaauw, R. Isaacs, M. Kuhlmann, D. Kleijn, A. M. Klein, C. Kremen, L. Morandin, J. Scheper, and R. Winfree. 2014. From research to action: enhancing crop yield through wild pollinators. Frontiers in Ecology and the Environment 12:439–447.

Garratt, M. P. D., R. Brown, C. Hartfield, A. Hart, and S. G. Potts. 2018. Integrated crop pollination to buffer spatial and temporal variability in pollinator activity. Basic and Applied Ecology 32:77–85.

Ghazoul, J. 2005. Buzziness as usual? Questioning the global pollination crisis. Trends in Ecology & Evolution 20:367–373.

Hebert, P. D. N., A. Cywinska, S. L. Ball, and J. R. deWaard. 2003a. Biological identifications through DNA barcodes. Proceedings of the Royal Society B: Biological Sciences 270:313–321.

Hebert, P.D.N., Ratnasingham, S., de Waard, J.R., 2003b. Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. Proeedings of the Royal Society, London B Biological Sciences 270, S96–S99.

Heinrich, B., and E. Mcclain. 1986. "Laziness" and hypothermia as a foraging strategy in flower scarabs (Coleoptera: Scarabaeidae). Physiological Zoology 59:273–282.

Hernández-Triana, L. M., S. W. Prosser, M. A. Rodríguez-Perez, L. G. Chaverri, P. D. N. Hebert, and T. Ryan Gregory. 2014. Recovery of DNA barcodes from blackfly museum specimens (Diptera: Simuliidae) using primer sets that target a variety of sequence lengths. Molecular Ecology Resources 14:508–518.

Hodgkiss, D., M. J. F. Brown, and M. T. Fountain. 2018. Syrphine hoverflies are effective pollinators of commercial strawberry. Journal of Pollination Ecology 22:55–66.

Hoehn, P., T. Tscharntke, J. M. Tylianakis, and I. Steffan-Dewenter. 2008. Functional group diversity of bee pollinators increases crop yield. Proceedings of the Royal Society B: Biological Sciences 275:2283–2291.

Hollingsworth, P. M., L. L. Forrest, J. L. Spouge, M. Hajibabaei, S. Ratnasingham, M. van der Bank, M. W. Chase, R. S. Cowan, D. L. Erickson, A. J. Fazekas, S. W. Graham, K. E. James, K.-J. Kim, W. J. Kress, H. Schneider, J. van AlphenStahl, S. C. H. Barrett, C. van den Berg, D. Bogarin, K. S. Burgess, K. M. Cameron, M. Carine, J. Chacón, A. Clark, J. J. Clarkson, F. Conrad, D. S. Devey, C. S. Ford, T. A. J. Hedderson, M. L. Hollingsworth, B. C. Husband, L. J. Kelly, P. R. Kesanakurti, J. S. Kim, Y.-D. Kim, R. Lahaye, H.-L. Lee, D. G. Long, S. Madriñán, O. Maurin, I. Meusnier, S. G. Newmaster, C.-W. Park, D. M. Percy, G. Petersen, J. E. Richardson, G. A. Salazar, V. Savolainen, O. Seberg, M. J. Wilkinson, D.-K. Yi, and D. P. Little. 2009. A DNA barcode for land plants. Proceedings of the National Academy of Sciences of the United States of America 106:12794–12797.

Hooper, C. H. 1932. The insect visitors of fruit blossoms. Journal of the Royal Society of Arts 81:86–105.

Inouye, D. W., B. M. H. Larson, A. Ssymank, and P. G. Kevan. 2015. Flies and flowers III: ecology of foraging and pollination. Journal of Pollination Ecology 16:115–133.

Ivanova, N. V., J. R. Dewaard, and P. D. N. Hebert. 2006. An inexpensive, automation-friendly protocol for recovering high-quality DNA. Molecular Ecology Notes 6:998–1002.

Ivanova, N. V., A. J. Fazekas, and P. D. N. Hebert. 2008. Semi-automated, membrane-based protocol for DNA isolation from plants. Plant Molecular Biology Reporter 26, 186-198.

Kearns, C. A., D. W. Inouye, and N. M. Waser. 1998. Endangered mutualisms: the conservation of plant-pollinator interactions. Annual Review of Ecology and Systematics 29:83–112.

Kearse, M., R. Moir, A. Wilson, S. Stones-Havas, M. Cheung, S. Sturrock, S. Buxton, A. Cooper, S. Markowitz, C. Duran, T. Thierer, B. Ashton, P. Meintjes, and A. Drummond. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28:1647–1649.

Keller, A., N. Danner, G. Grimmer, M. Ankenbrand, K. von der Ohe, W. von der Ohe, S. Rost, S. Härtel, and I. Steffan-Dewenter. 2015. Evaluating multiplexed next-generation sequencing as a method in palynology for mixed pollen samples. Plant Biology 17:558–566.

Kendall, D. A., and M. E. Solomon. 1973. Quantities of pollen on the bodies of insects visiting apple blossom. Journal of Applied Ecology 10:627.

Kennedy, C. M., E. Lonsdorf, M. C. Neel, N. M. Williams, T. H. Ricketts, R. Winfree, R. Bommarco, C. Brittain, A. L. Burley, D. Cariveau, L. G. Carvalheiro, N. P. Chacoff , S. A. Cunningham, B. N. Danforth, J. H. Dudenhoffer, E. Elle, H. R. Gaines, L. A. Garibaldi, C. Gratton, A. Holzschuh, R. Isaacs, S. K. Javorek, S. Jha, A.-M. Klein, K. Krewenka, Y. Mandelik, M. M. Mayfield, L. Morandin, L. A. Neame, M. Otieno, M. Park, S. G. Potts, M. Rundlof, A. Saez, I. Steffan-Dewenter, H. Taki, B. F. Viana, C. Westphal, J. K. Wilson, S. Greenleaf, and C. Kremen. 2013. A global quantitative synthesis of local and landscape effects on wild bee pollinators in agroecosystems. Ecology Letters 16:584–599.

Klatt, B., F. Klaus, C. Westphal, and T. Tscharntke. 2014. Enhancing crop shelf life with pollination. Agriculture & Food Security 3:14.

Klein, A.-M., B. E. Vaissière, J. H. Cane, I. Steffan-Dewenter, S. A. Cunningham, C. Kremen, and T. Tscharntke. 2007. Importance of pollinators in changing landscapes for world crops. Proceedings of the Royal Society B: Biological Sciences 274:303–313.

Klein, A.-M. 2011. Plant–pollinator interactions in changing environments. Basic and Applied Ecology 12:279–281.

Kraaijeveld, K., L. A. de Weger, M. Ventayol García, H. Buermans, J. Frank, P. S. Hiemstra, and J. T. den Dunnen. 2015. Efficient and sensitive identification and quantification of airborne pollen using next-generation DNA sequencing. Molecular Ecology Resources 15:8–16.

Kumar, J., R. C. Mishra, and J. K. Gupta. 1985. The effect of mode of pollination on *Allium* species with observation on insects as pollinators. Journal of Apicultural Research 24:62–66.

Kuzmina, M. L., W. A. Braukmann, A. J. Fazekas, S. W. Graham, S. L. Dewaard, A. Rodrigues, B. A. Bennett, T. A. Dickinson, J. M. Saarela, M. Catling, S. G. Newmaster, D. M. Percy, E. Fenneman, A. Lauron-Moreau, B. Ford, L. Gillespie, S., J. Whitton, L. Jennings, D. Metsger, C. P. Warne, A. Brown, E. Sears, J. R. Dewaard, E. V Zakharov, and P. D. N. Hebert. 2017. Using herbarium-derived DNAs to assemble a large-scale DNA barcode library for the vascular plants of Canada. Applications in Plant Sciences 5:1700079.

Larson, B., P. G. Kevan, and D. W. Inouye. 2001. Flies and flowers: taxonomic diversity of anthophiles and pollinators. The Canadian Entomologist 133:439–465.

Legendre, P., and E. D. Gallagher. 2001. Ecologically meaningful transformations for ordination of species data. Oecologia 129:271–280.

Little, D. P. 2014. A DNA mini-barcode for land plants. Molecular Ecology Resources 14(3): 437-446.

Lopez-Medina, J., A. Palacio-Villegas, and E. Vazquez-Ortiz. 2006. Misshaped fruit in strawberry, an agronomic evaluation. V International Strawberry Symposium 708:77–78.

Lucas, A., O. Bodger, B. J. Brosi, C. R. Ford, D. W. Forman, C. Greig, M. Hegarty, P. J. Neyland, and N. de Vere. 2018. Generalisation and specialisation in hoverfly (Syrphidae) grassland pollen transport networks revealed by DNA metabarcoding. Journal of Animal Ecology 87:1008–1021.

Martin, M. 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet.journal 17:10.

Morgan, K. R., and B. Heinrich. 1987. Temperature regulation in bee- and wasp-mimicking syrphid flies. Journal of Experimental Biology 133:59-71.

Müller, A., S. Diener, S. Schnyder, K. Stutz, C. Sedivy, and S. Dorn. 2006. Quantitative pollen requirements of solitary bees: implications for bee conservation and the evolution of bee–flower relationships. Biological Conservation 130:604–615.

Nye, W., and J. Anderson. 1974. Insect pollinators frequenting strawberry blossoms and the effect of honey bees on yield and fruit quality. Journal of the American Society for Horticulture Science 99:40–44.

Oksanen, J., F. G. Blanchet, R. Kindt, P. Legendre, R. B. O'Hara, G. L. Simpson, P. Solymos, M. H. H. Stevens, and H. Wagner. 2019. Vegan: community ecology package.

Ollerton, J. 2017. Pollinator diversity: distribution, ecological function, and conservation. Annual Review of Ecology, Evolution, and Systematics 48:353–376.

Ollerton, J., R. Winfree, and S. Tarrant. 2011. How many flowering plants are pollinated by animals? Oikos 120:321-326.

Orford, K. A., I. P. Vaughan, and J. Memmott. 2015. The forgotten flies: the importance of non-syrphid Diptera as pollinators. Proceedings of the Royal Society B: Biological Sciences 282:20142934.

Palmieri, L., E. Bozza, L. Giongo, L. Palmieri, E. Bozza, and L. Giongo. 2009. Soft fruit traceability in food matrices using real-time PCR. Nutrients 1:316–328.

Pornon, A., C. Andalo, M. Burrus, and N. Escaravage. 2017. DNA metabarcoding data unveils invisible pollination networks. Scientific Reports 7:16828.

Potter, C., de Vere, N., Jones, L. E., Ford, C. R., Hegarty, M. J., Hodder, K. H., Diaz, A., Franklin E. L. 2019. Pollen metabarcoding reveals broad and species-specific resource use by urban bees. PeerJ 7:e5999.

Rabinowitz, D., J. K. Rapp, V. L. Sork, B. J. Rathcke, G. A. Reese, and J. C. Weaver. 1981. Phenological properties of wind- and insect-pollinated prairie plants. Ecology 62:49–56.

Rader, R., I. Bartomeus, L.A. Garibaldi, M.P.D. Garratt, B.G. Howlett, R. Winfree, S.A. Cunningham, M.M. Mayfield, A.D. Arthur, G.K.S. Andersson, R. Bommarco, C. Brittain, L.G. Carvalheiro, N.P. Chacoff, M.H. Entling, B. Foully, B.M. Freitas, B. Gemmill-Herren, J. Ghazoul, S.R. Griffin, C.L. Gross, L. Herbertsson, F. Herzog, J. Hipólito, S. Jaggar, F. Jauker, A-M. Klein, D. Kleijn, S. Krishnan, C.Q. Lemos, S.A.M. Lindström, Y. Mandelik, V.M. Monteiro, W. Nelson, L. Nilsson, D.E. Pattemore, N. de O. Pereira, G. Pisanty, S.G. Potts, M. Reemer, M. Rundlöf, C.S. Sheffield, J. Scheper, C. Schüepp, H.G. Smith, D.A. Stanley, J.C. Stout, H. Szentgyörgyi, H. Taki, C.H. Vergara, B.F. Viana, and M. Woyciechowski. 2016. Non-bee insects are important contributors to global crop pollination. Proceedings of the National Academy of Sciences of the United States of America 113:146-151.

Rader, R., B. G. Howlett, S. A. Cunningham, D. A. Westcott, L. E. Newstrom-Lloyd, M. K. Walker, D. A. J. Teulon, and W. Edwards. 2009. Alternative pollinator taxa are equally efficient but not as effective as the honeybee in a mass flowering crop. Journal of Applied Ecology 46:1080–1087.

Rahl, M. 2008. Microscopic identification and purity determination of pollen grains. Methods in Molecular Medicine 138:263–269.

Ratnasingham, S., and P. D. N. Hebert. 2007. BOLD: the barcode of life data system (http://www.barcodinglife.org). Molecular Ecology Notes 7:355–364.

Ratnasingham, S., and P. D. N. Hebert. 2013. A DNA-based registry for all animal species: the barcode index number (BIN) system. PLoS ONE 8:e66213.

Richardson, R., C.-H. Lin, J. Quijia, N. Riusech, K. Goodell, and R. Johnson. 2015. Rank-based characterization of pollen assemblages collected by honey bees using a multi-locus metabarcoding approach. Applications in Plant Sciences 3:1500043.

Rogers, S. R., D. R. Tarpy, and H. J. Burrack. 2014. Bee species diversity enhances productivity and stability in a perennial crop. PLoS ONE 9:e97307.

Russo, L. A., M. G. Park, E. J. Blitzer, and B. N. Danforth. 2017. Flower handling behavior and abundance determine the relative contribution of pollinators to seed set in apple orchards. Agriculture, Ecosystems & Environment 246:102-108

Schluter, D., T. Price, and P. Grant. 1985. Ecological character displacement in Darwin's finches. Science 227:1056–1059.

Sheffield, C. S. 2014. Pollination, seed set and fruit quality in apple: studies with *Osmia lignaria* (Hymenoptera: Megachilidae) in the Annapolis Valley, Nova Scotia, Canada. Journal of Pollination Ecology 12:120-128.

Skevington, J. H., and P. T. Dang. 2002. Exploring the diversity of flies (Diptera). Biodiversity 3:3–27.

Solomon, M., and D. Kendall. 1970. Pollination by the syrphid fly,*Eristalis tenax* . 101. [Annual Report]

Stewart, R. I. A., G. K. S. Andersson, C. Brönmark, B. K. Klatt, L.-A. Hansson, V. Zülsdorff, and H. G. Smith. 2017. Ecosystem services across the aquatic–terrestrial boundary: linking ponds to pollination. Basic and Applied Ecology 18:13–20.

Tan, G.-M., L. Xu, D.-B. Bu, S.-Z. Feng, and N.-H. Sun. 2006. Improvement of performance of MegaBlast algorithm for DNA sequence alignment. Journal of Computer Science and Technology 21:973–978.

Tennekes, M. 2022. Treemap: Treemap Visualization. R package.*https://CRAN.R-project.org/package=treemap*

Ver Hoef, J. M., and P. L. Boveng. 2007. Quasi-poisson vs. negative binomial regression: how should we model overdispersed count data? Ecology 88:2766–2772.

Wardhaugh, C. W. 2015. How many species of arthropods visit flowers? Arthropod-Plant Interactions 9:547–565.

Woodcock, T. S. 2012. Pollination in the agricultural landscape best management practices for crop pollination. Canadian Pollination Initiative (NSERC-CANPOLIN), University of Guelph report. 113 pp.

Captions

Figure 1 : Treemap of total pollen load on non-bee strawberry flower visitors by family **(A).** Size of the rectangle represents total pollen collectively carried by family members. Treemap of average pollen carried by species visiting strawberry flowers **(B).** Size of the rectangle represents mean quantity (corresponds to the number given in each box) of pollen carried per individual for that species.

Figure 2 : Plant-flower visitor network matrix **(A)** at the family level and corresponding bipartite network **(B)** .*Fragaria* is included at the genus level for distinction of strawberry pollen. The relative number of reads for each plant family (or genus) per insect family is represented by a gradient from dark blue (1) to white (0).

Figure 3 : Plant-flower visitor network matrix **(A)** for members of the family Syrphidae and corresponding bipartite network**(B)** . *Fragaria* is included at the genus level for distinction of strawberry pollen. The relative number of reads for each plant family (or genus) per flower fly species is represented by a gradient from dark blue (1) to white (0).

Figure 4 : Triplot of redundancy analysis with species scaling**(A)** includes explanatory environmental variables, time was also included as a continuous variable (blue arrows), temperature, humidity, solar radiation and wind, and temporal variables (blue x's), date and time (ellipses), and the response variables (black circles) are the insect floral visiting community and their composition (red crosses). Data are Hellinger transformed. Box and whisker plot representation of observed abundance for 8 taxa across 25 dates **(B)** . In each box the horizontal bar is the median, whilst the lower and upper edges represent the 25% and 75% quartiles respectively. Whiskers indicate the maximum and minimum values that are not outliers, and outliers are represented by filled circles.

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