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RESEARCH ARTICLE

Seasonal progression and differences in major floral resource use by bees and hoverflies in a diverse horticultural and agricultural landscape revealed by DNA metabarcoding

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Abstract

1. Gardens are important habitats for pollinators, providing floral resources and nesting sites. There are high levels of public support for growing 'pollinator-friendly' plants but while plant recommendation lists are available, they are usually inconsistent, poorly supported by scientific research and target a narrow group of pollinators. In order to supply the most appropriate resources, there is a clear need to understand foraging preferences, for a range of pollinators, across the season within horticultural landscapes.
2. Using an innovative DNA metabarcoding approach, we investigated foraging preferences of four groups of pollinators in a large and diverse, horticultural and agricultural landscape, across the flowering season and over 2 years, significantly improving on the spatial and temporal scale that can be achieved using observational studies.
3. Bumblebees, honeybees, non-corbiculate bees and hoverflies visited 191 plant taxa. Overall floral resources were shared between the different types of pollinators, but significant differences were seen between the plants used most abundantly by bees (Hymenoptera) and hoverflies (Diptera).
4. Floral resource use by pollinators is strongly associated with seasonal changes in flowering plants, with pollinators relying on dominant plants found within each season, with preferences consistent across both years.
5. The plants identified were categorised according to their native status to investigate the value of native and non-native plants. The majority of floral resources used were of native and near-native origin, but the proportion of horticultural and naturalised plants increased during late summer and autumn.
6. *Synthesis and applications.* Plant recommendation lists for pollinators should distinguish between bees and hoverflies and provide evidence-based floral recommendations throughout the year that include native as well as non-native plants

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for use in the United Kingdom and Northern Europe. Specific management recommendations include reducing mowing to encourage plants such as dandelion *Taraxacum officinale* and buttercups *Ranunculus* spp., and reducing scrub management to encourage bramble *Rubus fruticosus*.

KEYWORDS

bees, DNA metabarcoding, foraging, gardens, hoverflies, pollen eDNA, pollinator conservation, pollinator ecology

1 | INTRODUCTION

The decline in pollinating insects is well documented globally, leading to potentially severe impacts on floristic biodiversity and human health due to the loss of pollination ecosystem services (Klein et al., 2007; Lundgren et al., 2016; Smith et al., 2015). Pollinator declines have occurred due to a combination of habitat loss, climate change, pests and diseases and the use of pesticides (Potts et al., 2010). As the availability of floral resources limits pollinators (Goulson et al., 2015), understanding foraging preferences is a key knowledge need for their effective conservation.

Gardens are important, heterogeneous habitats, covering significant areas in urban landscapes (Loram et al., 2007). Gardens can provide pollinators with pollen, nectar and nesting sites (Osborne et al., 2008), supporting pollinators in agricultural (Timberlake et al., 2020) and urban (Potter et al., 2019) settings while increasing habitat connectivity within the landscape (Goddard et al., 2009).

The limited number of studies in the United Kingdom (Wignall et al., 2019) and elsewhere in Northern Europe (Schonfelder & Bogner, 2017) on the public perception of pollinators suggests that attitudes towards their conservation is very positive. However, while there is a wealth of information available on the best plants for pollinators, only a small number of recommendation lists are based on empirical evidence (Garbuzov & Ratnieks, 2014), with most plants sold in UK garden centres relatively unattractive to flower-visiting insects (Garbuzov et al., 2017). Moreover, these lists broadly target pollinators, leading to generalisation across a wide range of functional groups and species.

Consequently, there is a clear need to provide scientific evidence for effective floral use in gardens to support pollinators. Although foraging can vary between pollinator groups (Bänsch et al., 2020), most studies in gardens focus on a single group (de Vere et al., 2017). Honeybees and bumblebees are the most frequently studied, however, non-corbiculate bees and hoverflies have important roles in pollination and ecosystem function (Klein et al., 2007). Additionally, seasonality and annual variation can influence forage choice (Petanidou et al., 2014), highlighting the need to provide information on floral use throughout the year.

There are conflicting perspectives as to whether native or non-native plants are preferred by pollinators, but it is imperative to

understand this for effective conservation. When surveying pollinator visits to a variety of plants, Salisbury et al. (2015) found a greater abundance of pollinators on native and near-native taxa than those defined as exotic. Additionally, introduced plant species have been shown to attract fewer species of flower visitors than natives and those closely related to natives (Memmott & Waser, 2002).

DNA metabarcoding has been used to identify pollen within honey (de Vere et al., 2017; Jones, Brennan, et al., 2021), from the bodies of insects (Lucas et al., 2018a; Richardson et al., 2021), and from brood provision in nests (Vaudo et al., 2020). The advantages of pollen metabarcoding approaches include increased taxonomic resolution (Brennan et al., 2019) and the elimination of the taxonomic expertise required for pollen microscopy (Hawkins et al., 2015). DNA metabarcoding overcomes the limitations of observational methods by revealing interactions previously unseen due to spatial and temporal limitations (Arstingstall et al., 2021), however, it must be accompanied by a comprehensive reference library to ensure accurate identification. In the United Kingdom, the Barcode UK project provides 98% coverage of all native flowering plants and conifers using three plant DNA barcode markers, *rbcl*, *matK* and *ITS2*, allowing reliable identification at the species and genus level for the majority of plants (de Vere et al., 2012; Jones, Twyford, et al., 2021).

This study identifies plants used by pollinating insects in an extensive, well characterised, and complex horticultural and agricultural landscape, using a multi-locus (*rbcl* and *ITS2*) pollen DNA metabarcoding approach. We specifically answer the following questions:

1. How does foraging differ between pollinator groups (bumblebees, honeybees, non-corbiculate bees and hoverflies)?
2. Do ecological functional categories within these groups (related to tongue length in bumblebees, body size in non-corbiculate bees and larval requirements in hoverflies) affect the plant taxa used?
3. How does foraging change over the flowering season and year?
4. Do pollinators prefer native or non-native plants?

The results are used to present novel plant recommendations for gardeners, landowners and conservation organisations based on time resolved, empirical data, to support pollinator populations and ensure effective conservation.

2 | MATERIALS AND METHODS

2.1 | Insect sampling

Bees and hoverflies were sampled monthly from March to October during 2018 and 2019 at the National Botanic Garden of Wales, UK (51°50'33.4"N 4°08'49.2"W). The site is a diverse landscape (230 ha) set within a predominately semi-improved (based on the extent of agricultural improvement) landscape and consists of formal garden and organic farmland, designated as a National Nature Reserve, Waun Las NNR (Figure 1). The Botanic Garden contains over 5,000 plant taxa from throughout the world, including many horticultural plants grown throughout Western Europe. Eight areas were selected for pollinator sampling covering broadleaved woodland and hedgerows, horticultural and grassland habitat. The most abundant plants across both years per transect area per season are provided in Appendix S1.

Within each sampling area, a 210 m × 2 m transect was established and divided into 3 × 70 m sections, walked independently of each other. Transect walks were preferentially undertaken between 11:00 and 15:00 when the temperature was over 10°C. When this was not possible, transects were walked on dry days with little wind. All bees and hoverflies seen on the transect were caught individually and stored at -20°C prior to pollen removal. Further information on field sampling is provided in Appendix S2. Permission for field work and ethical approval was granted by the National Botanic Garden of Wales.

2.2 | Pollen removal

Pollen was washed from insects following a modified version of the protocol described by Lucas et al. (2018b). Insects were first transferred to a sterile 1.5 ml collection tube using sterile forceps and cleaned with 70% ethanol between each insect. The tube used to catch insects

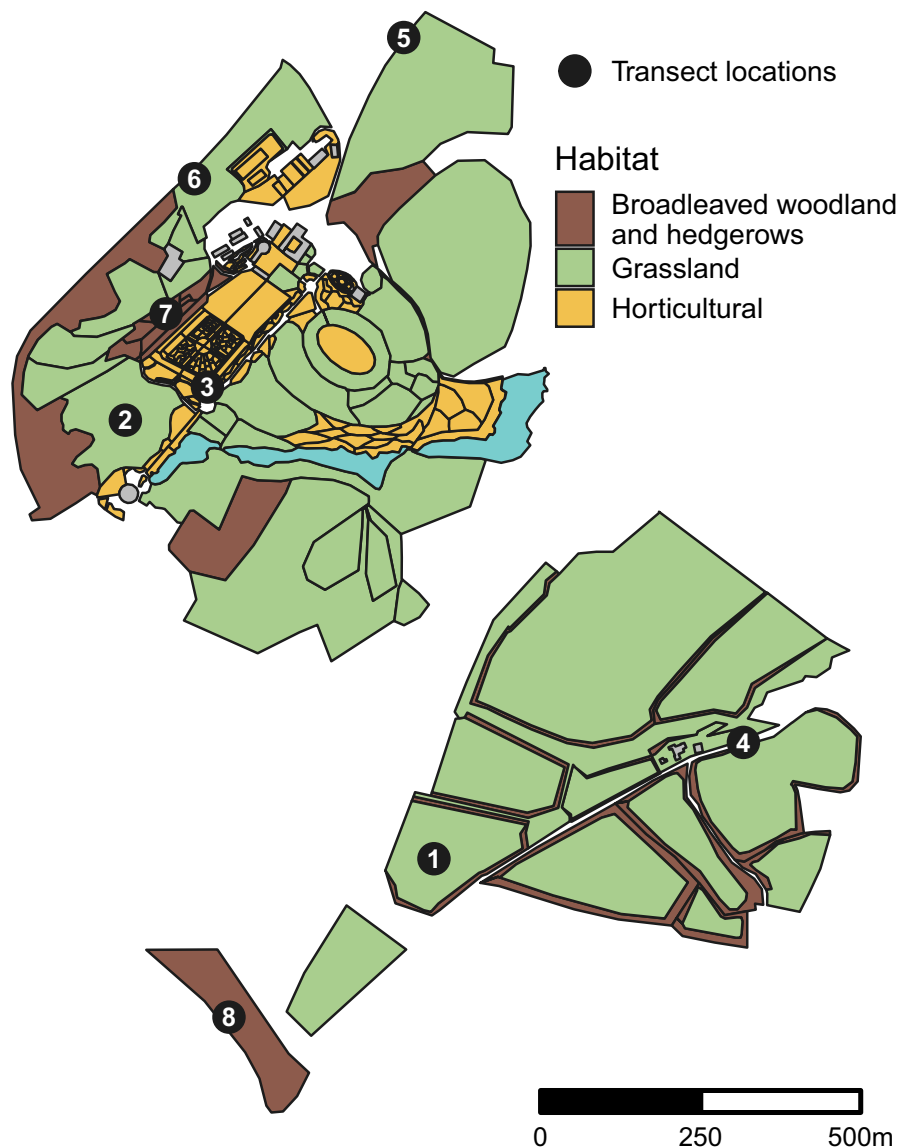


FIGURE 1 Habitat map of the National Botanic Garden of Wales and Waun Las National Nature Reserve showing location of transects where pollinators were collected. The grassland in the study site is mainly composed of semi-improved grassland and lowland hay meadows. Maps were created in QGIS v. 3.6.1 and R v. 4.0.2 from OS data © Crown Copyright (2021) licensed under the Open Government Licence

was washed with 1 ml of 1% sodium dodecyl sulphate (SDS) and 2% polyvinylpyrrolidone (PVP) solution, ensuring any pollen residue on the sides was collected and transferred to the tube containing the insect. Samples were shaken using a TissueLyser II (Qiagen) for 1 min at 8.5 Hz, stood at room temperature for 5 min, then shaken again for 20 s at 8.5 Hz. Each insect was removed using sterile forceps and placed into a 1.5 ml microcentrifuge tube containing 70% ethanol, prior to species identification (see Taxonomic assignment of insects, Appendix S2). The tube containing the detergent and pollen pellet was centrifuged at 16,200 g for 5 min and the supernatant removed. The pollen pellet was resuspended in 400 µl buffer, made up of 400 µl AP1 from the DNeasy 96 Plant Kit (Qiagen) and 80 µl (1 mg/ml) of Proteinase K (Qiagen).

2.3 | DNA extraction

A modified version of the DNeasy 96 Plant Kit was used for DNA extraction. Samples were incubated in a water bath at 65°C for 1 hr and 1 µl RNase (Qiagen) added before disruption using a TissueLyser II for 4 min at 30 Hz with 3 mm tungsten carbide beads. The remaining steps were carried out according to the manufacturer's protocol, excluding the use of the QIAshredder and the second wash stage. A negative control was included within each extraction.

2.4 | Amplification and sequencing

Two barcode regions, *rbcl* and ITS2 were amplified via a two-step PCR protocol (Table S1, Appendix S3). The initial PCR used a final volume of 20 µl: 2 µl template DNA, 10 µl of 2× Phusion Hot Start II High-Fidelity Mastermix (New England Biolabs UK), 0.4 µl (2.5 µM) forward and reverse primers, and 7.2 µl of PCR grade water. Each PCR was repeated twice more and pooled before purification using the Illumina 16S metabarcoding protocol, with a 1:0.6 ratio of product to Agencourt AMPure XP beads (Beckman Coulter). The purified product was amplified further to anneal custom unique and matched *i5* and *i7* indices to each sample (UltraM, Integrated DNA Technologies). This second stage PCR used a final volume of 25 µl: 5 µl of purified first-round PCR product, 12.5 µl of 2× Phusion Hot Start II High-Fidelity Mastermix (New England Biolabs UK), 1 µl of *i5* and *i7* Index Primer and 6.5 µl of PCR grade water. All thermal cycling conditions are available in Appendix S2. Tag addition was confirmed with visualisation on a 1% agarose gel. A second Illumina clean-up stage was followed with a 1:0.8 ratio of product to beads. Products were quantified using a Qubit 4.0 (Thermo Fisher Scientific) and pooled at equal concentrations. The negative extraction and PCR controls from each plate were sequenced with the pollen samples on an Illumina MiSeq (2 × 300 bp) at Liverpool University's Centre for Genomic Research (Liverpool, UK). Laboratory contamination controls can be found in Appendix S2.

2.5 | Sequence analysis

Sequence reads were processed following Ford and Jones (2020). Initially, raw sequences were trimmed to remove low-quality

regions, paired and merged. Only sequences greater than 450 bp (*rbcl*) and 350 bp (ITS2) were used in downstream analysis. Identical reads were dereplicated within each sample and clustered at 100% identity across all samples with singletons (sequence reads occurring once across all samples) removed. Sequences were compared to a custom reference library containing 5887 plant species (Jones, Brennan, et al., 2021), comprising native plants of the United Kingdom (Stace, 2019), naturalised and alien species (Preston et al., 2002) and horticultural species from the IRIS BG database at the National Botanic Garden of Wales.

2.6 | Assigning taxonomic classifications

Sequences were compared against the reference library using blastn, summarising the top 20 BLAST hits and combining all sequences with identical BLAST results across all 20 hits. Sequences with bit scores below the first percentile were excluded. Sequences were assigned so that if the top bitscore matched a plant species, the sequence was assigned to that species. If the top bitscore matched different species within the same genus, the sequence was assigned to that genus. If the top bitscore belonged to multiple genera of the same family, then a family designation was made for that sequence. Sequences returning top bitscores of multiple families within different orders were removed, assuming that these were poor quality sequences. The botanical veracity of the plants identified across all insect samples was assessed by considering whether those plants were present within the study site and wider landscape. Taxonomic assignment of sequences was compared between markers on a sample-by-sample basis for further verification.

Once the identifications were complete, a consensus identification was reached to combine the taxa identified by both markers at differing taxonomic resolution using a rule-based, objective, and conservative decision process (see Using *rbcl* and ITS2 markers, Appendix S2). The number of *rbcl* and ITS2 sequences for each consensus taxon within a sample were then summed to combine the results of each marker. Sequences assigned to taxa identified using one marker alone were retained. Plants identified to genus and species were assigned to a status category following Stace (2019). The category 'native and near native' comprised native species and also genera that include native species and horticultural varieties which are functionally similar. Naturalised plants were those which have been introduced and become widespread and self-perpetuating in the wild. All remaining non-native plants were classified as horticultural.

2.7 | Statistical analysis

The DNA metabarcoding data were treated as semi-quantitative with relative read abundance used for all analyses (Deagle et al., 2019), either using the proportion of taxa as a percentage or, for the models, the number of sequences, controlling for sequencing depth by setting the total number of sequences per sample as an offset, comparable to proportion (Jones, Brennan, et al., 2021; Appendix S2).

Using the package *MVABUND* (Wang et al., 2012), a multivariate generalised linear model with a negative binomial distribution was used to understand how pollen load composition changed through time. The data best fit a negative binomial distribution due to the strong mean–variance relationship (Figure S1, Appendix S3), likely from distributions of rare taxa where mean abundance is low, a common observation in multivariate abundance data.

To understand the effect of time and pollinator type on plant composition, the effect of season (coded as 1–3, starting with spring), year and pollinator group/order was included as predictor variables, with the number of sequence reads for each plant taxon set as the multivariate response variable. The number of reads per sample was included as an offset to control for differences in sampling depth (Deagle et al., 2019; Jones, Brennan, et al., 2021). Seasonal changes in the composition of pollen loads were visualised using non-metric multidimensional scaling (NMDS) ordination of Bray–Curtis dissimilarity indices (based on the proportion of reads returned for each plant taxa), using the *VEGAN* package (Dixon, 2003). A Chi-square contingency test was used to investigate differences in major taxa (constituting over 5% of sequences) between pollinator orders (based on the relative read abundance overall), with Holm correction for multiple testing. Each pollinator group was split into categories based on a unique ecological functional trait (see Functional diversity analysis, Appendix S2) and Chi-square contingency tests were used to investigate differences in taxa constituting over 1% of sequences between functional categories within broader groups.

To investigate the change in use of native plants over time, the plant taxa were grouped by their status categories. A multivariate generalised linear model was run, with season and year included as predictor variables and the response variable being the number of reads, retaining the use of the offset. All statistical analyses were carried out in R v 4.0.2 using the consensus identification. Analysis of *rbcl* and ITS2 was also carried out separately to support the use of combining markers (Appendix S4).

3 | RESULTS

3.1 | Overview

Throughout the study, 382 insects were caught with successful sequencing of pollen from 369 individuals (Table 1). No insects were caught in October despite surveys being carried out. Pollinators were grouped into hoverflies (Syrphidae, $n = 195$), bumblebees (*Bombus* spp., $n = 108$), honeybees (*Apis mellifera*, $n = 44$) and all other non-corbiculate bees ($n = 22$; Table S2, Appendix S3). A total of 40,800,709 reads were returned with 22,510,682 remaining after stringent quality control (11,305,697 *rbcl* and 11,204,985 ITS2). Using the *rbcl* and ITS2 regions combined, 191 plant taxa were identified with the majority of taxa identified at genus level (Appendix S1). Six taxa were found on over 50% of insects sampled (Figure 2): bramble (*Rubus* spp.), thistles, knapweeds and cat's ear (*Cirsium/Centaurea/Hypochaeris* spp.), buttercups and lesser celandine

TABLE 1 Summary of the number of insects sampled along with the mean and standard deviation (SD) of plant taxa identified from the pollen on their bodies

Order	Group	Successfully sequenced	Sequencing success rate (%)	Number of pollinator species (successful samples)	Mean number of plant taxa identified per individual	Mean number of plant taxa identified per individual (>1% sequence reads)	Plant taxa unique to group (%)
Diptera	Hoverfly (Syrphidae)	195	95.1	41	14 (SD = 7.76)	4 (SD = 2.45)	11.7
Hymenoptera	Bumblebee (<i>Bombus</i> spp.)	108	100	6	21 (SD = 11.31)	4 (SD = 2.72)	5.8
	Honeybee (<i>Apis mellifera</i>)	44	97.8	1	20 (SD = 9.20)	3 (SD = 2.77)	4.3
	Non-corbiculate bee (<i>Andrena</i> , <i>Lasioglossum</i> , <i>Halictus</i> , <i>Nomada</i>)	22	91.7	9	13 (SD = 8.30)	4 (SD = 2.04)	0.0



FIGURE 2 Plant taxa found in over 50% of pollen samples retrieved from pollinators. Those plants identified to species or genus level only are illustrated, with species given as an example of taxa represented. Images (a, d, e, h, i) by Natasha de Vere All rights reserved, (b) by Matt Lavin CC BY-SA 2.0, (c, f, g) by Bruce Langridge All rights reserved. All images have been cropped and adjusted

(*Ranunculus/Ficaria* spp.), angelica and hogweed (*Angelica/Heracleum* spp.), daisy family (Asteraceae) and meadowsweet (*Filipendula ulmaria*). An average of 17 (SD = 9.76) plant taxa were found on each individual insect with an average of 4 (SD = 2.55) taxa contributing >1% of reads (Table 1).

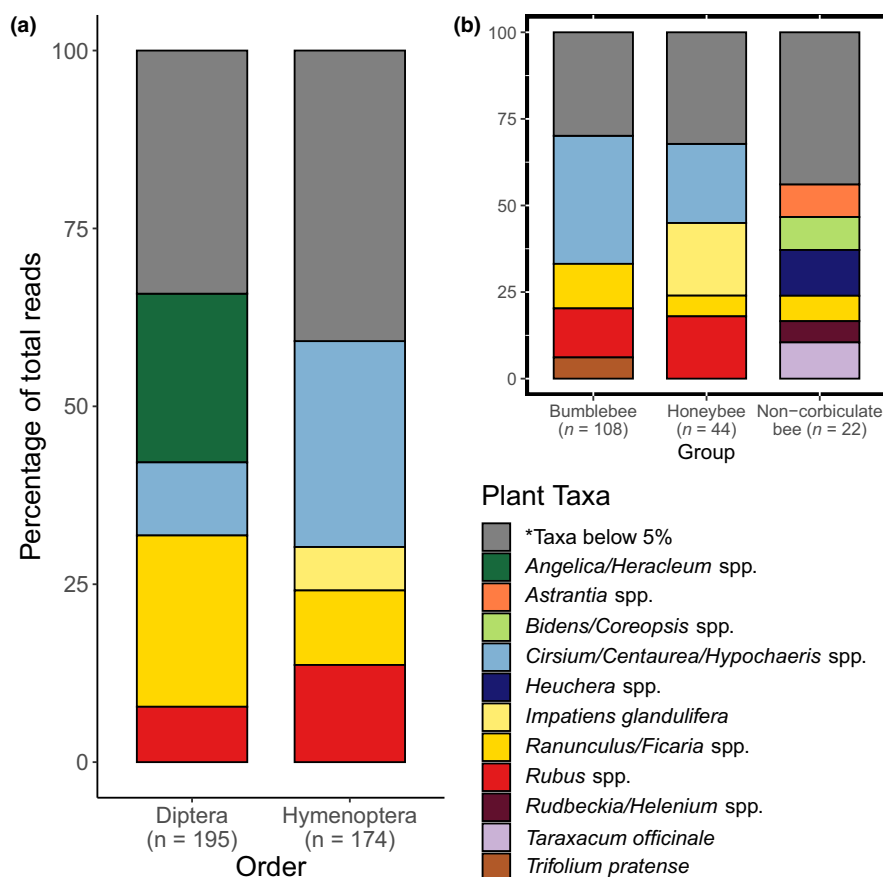
3.2 | Variation in foraging between pollinators

Overall, we found little variation in foraging habits between the four pollinator groups. Neither pollinator group nor pollinator order predicted pollen composition when all plant taxa were included in the model (pollinator group: $LR_{1,363} = 1753.8$, $p = 0.999$, order: $LR_{1,365} = 953.9$, $p = 1.000$). The ability of the model to predict pollen composition was greatest when characterising pollinators by their taxonomic order (Diptera, Hymenoptera) rather than group (bumblebees, honeybees, non-corbiculate bees and hoverflies; Table S3, Appendix S3). There was, however, a significant difference in the composition of plant taxa constituting over 5% of sequences carried by Diptera and Hymenoptera ($\chi^2 = 46.26$, $df = 5$, $p < 0.001$; Figure 3). A large proportion of pollen sequences from hoverflies (Diptera) belonged

to *Angelica/Heracleum* spp., but these were not found to be as valuable for bees (Hymenoptera). *Cirsium/Centaurea/Hypochaeris* spp. contributed a large proportion of sequences for bees but made up a lower proportion of sequences for hoverflies, while hoverflies used *Ranunculus/Ficaria* spp. more abundantly than bees.

Within pollinator groups, differences in foraging were found between ecological functional categories (Figure 4). A significant difference was found in the composition of plant taxa represented by over 1% of sequences from short- and long-tongued bumblebees ($\chi^2 = 50.179$, $df = 20$, $p < 0.001$). A large proportion of pollen was attributed to *Ranunculus/Ficaria* spp. across short-tongued species (*Bombus hypnorum*, *B. lapidarius*, *B. pratorum*, *B. lucorum/terrestris* agg.) while long-tongued species (*B. hortorum*, *B. pascuorum*), utilised more *Trifolium pratense* and *Rubus* spp. (Figure 4). Honeybees' foraging habits were broadly similar to bumblebees but utilised a greater proportion of *Impatiens glandulifera* than any other group (Figure 4). Within the non-corbiculate bees, the total proportion of pollen collected was significantly different between body size groups ($\chi^2 = 433.01$, $df = 52$, $p < 0.001$), with extra small bees carrying mostly *Heuchera* spp., small-sized carrying mostly *Rudbeckia/Helenium* spp. and medium-sized carrying mostly *Taraxacum*

FIGURE 3 (a) Plant taxa represented by >5% of total sequence reads for each pollinator order, where Diptera includes hoverflies only, and Hymenoptera comprises bumblebees, honeybees and non-corbiculate bees. The proportion of taxa illustrated was significantly different between orders ($\chi^2 = 46.26$, $df = 5$, $p < 0.001$). (b) Plant taxa represented by >5% of total sequence reads for each pollinator group within Hymenoptera



officinale. Pollen composition from hoverfly species differed between various larval requirements ($\chi^2 = 235.4$, $df = 48$, $p < 0.001$), with carnivorous and detritivorous species utilising a greater diversity of plant taxa than herbivorous species (Figure 4).

3.3 | Annual and seasonal variation in pollinator foraging

Season was a good predictor of pollen composition ($LR_{2,367} = 2632.8$, $p < 0.001$), regardless of year of sampling ($LR_{2,366} = 816.2$, $p = 0.828$; Figures S2–S4, Appendix S3). There were 147 taxa found in 2018 and 170 in 2019, and of these 71 were identified in both years. NMDS ordination scaling shows that pollen samples collected in the same season are most similar to each other (Figure 5). Seasonal progression is visible for each pollinator group when assessing the most abundantly foraged plants throughout the year (Table 2) using the consensus data and *rbcL* and ITS2 separately (Figures S5–S7, Appendix S3).

3.4 | Relationship to native status

The largest proportion of DNA reads returned from pollinators were attributed to native and near-native plants (Figure 6). Native and near-native plants were predominately used in the spring and the use of naturalised and horticultural plants increased during the summer

and autumn ($LR_{1,367} = 58.10$, $p = 0.001$) (Figure 6), regardless of year of sampling ($LR_{1,366} = 3.14$, $p = 0.369$). In transects with more horticultural plants, we see that pollinators use a diverse array of plants with no dominant taxa identified (Figure S9, Appendix S3), compared to predominately native areas (Figures S8, S10–S12, Appendix S3).

4 | DISCUSSION

Using DNA metabarcoding, we reveal the most frequently visited plants by key pollinator groups, across a broad taxonomic range covering bumblebees, honeybees, non-corbiculate bees and hoverflies. We show that while common resources are shared across all groups, differences are seen in the major taxa visited by hoverflies (Diptera) and bees (Hymenoptera) and between ecological functional categories within. This choice in foraging is strongly influenced by season, with clear changes in floral use through the year. Pollinators were shown to predominately utilise native and near-native plants, with increased use of horticultural and naturalised plants towards the end of the season.

4.1 | Pollinators share resources with differences between insect orders in major taxa

Floral resources were shared overall among pollinator groups (hoverflies, bumblebees, honeybees and non-corbiculate bees),

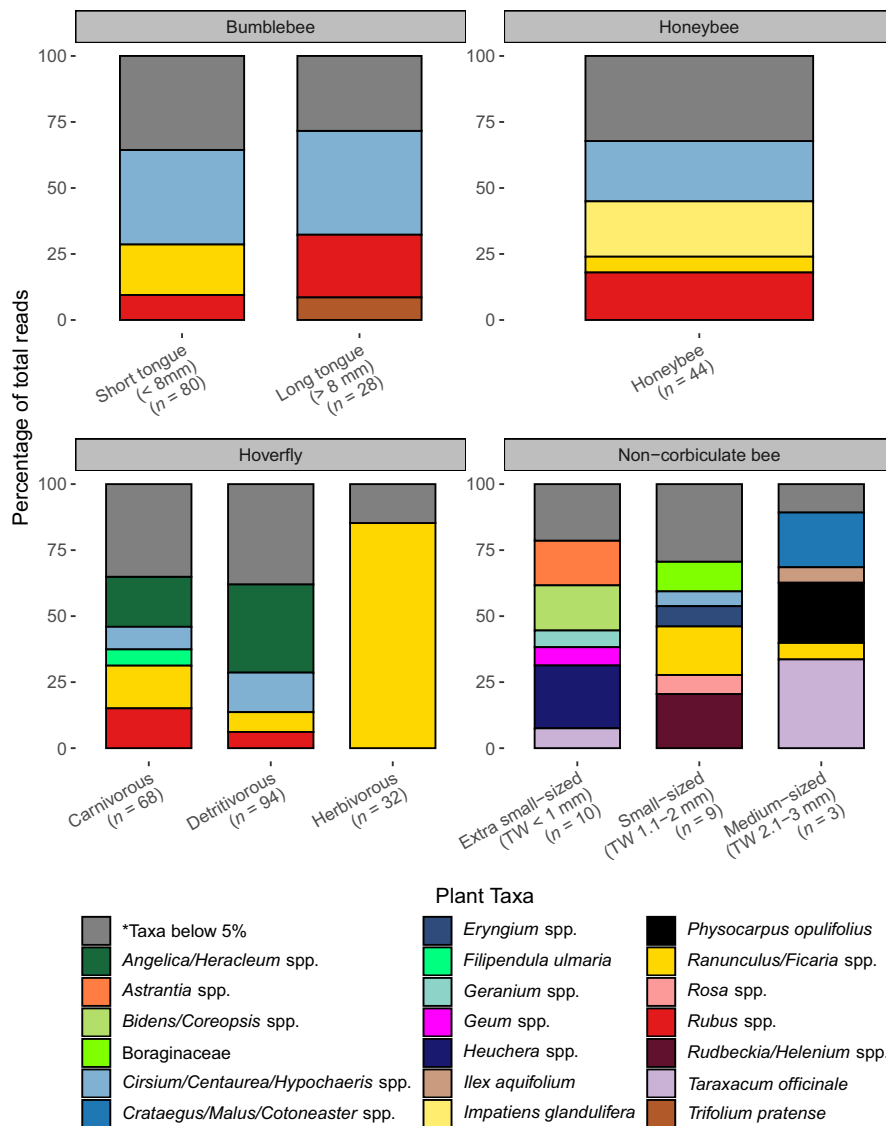


FIGURE 4 Plant taxa represented by over 5% of sequence reads for ecological functional categories of pollinators. The proportion of taxa constituting >1% of reads for each functional group was significantly different within bumblebees ($\chi^2 = 50.179$, $df = 20$, $p < 0.001$), hoverflies ($\chi^2 = 235.4$, $df = 48$, $p < 0.001$), and non-corbiculate bees ($\chi^2 = 433.01$, $df = 52$, $p < 0.001$)

but clear differences were seen between the taxa used most abundantly by Diptera (hoverflies) and Hymenoptera (bees). In comparison to hoverflies, bees utilised thistles more and umbelliferous plants less. A possible explanation for the preference differences between the major plants of Diptera and Hymenoptera is that the accessibility of nectar may be limited by the morphology of the plants, influencing which plants are visited by pollinators. The hoverflies recorded here generally have shorter tongues than bees (King, 2012), and may have difficulty fully removing nectar from the long corollas found in the genera *Cirsium*, *Centaurea* and *Hypochaeris*. While hoverflies are evidently able to utilise this resource, the issue of accessibility may be a reason for hoverflies prioritising the shorter, open flowers of *Angelica/Heraclium* spp.

We demonstrate that within broad pollinator groups, resources may be partitioned further based on ecological functional traits shared by species. When studying the diversity and abundance of pollen collected by insects, Cullen et al. (2021) found that traits had a greater impact than local floral diversity

itself, highlighting the importance of understanding this relationship. Tongue length is widely known to affect forage choice in bumblebees and is thought to influence species' vulnerability to extinction as long-tongued species tend to specialise more on species with long corollae (Goulson et al., 2005). While we did find differences in forage relating to bee size in non-corbiculate bees, the small sample size and long sampling period mean these results must be interpreted with caution and further work is required. Non-corbiculate bees comprise a cosmopolitan suite of ecologically distinct taxa in the United Kingdom. However, this study was limited to bees within Halictidae and *Andrena*, along with the kleptoparasitic *Nomada* which are all relatively small (thoracic width < 3 mm) making comparisons within this group difficult. As body size limits the foraging distance of bees (Greenleaf et al., 2007), the floral resources used by these species may have been predicted by the species immediately available to them in the area sampled. The relationship with floral resources is more complex in hoverflies, as larval requirements influence the habitats which species occupy (Schirmel

FIGURE 5 Non-metric multidimensional scaling (NMDS) plot of pollen samples in relation to season of collection and insect order

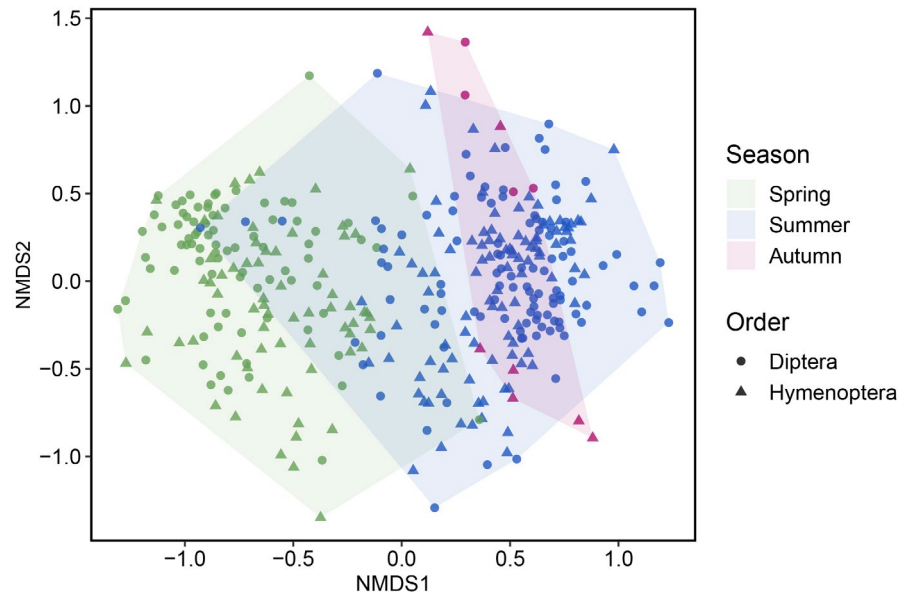






TABLE 2 Major plant taxa constituting over 10% of sequence reads in each season, using the consensus taxa which combines *rbcl* and ITS2. Reads for 2018 and 2019 were combined as year was not found to have a significant effect on pollen composition

	Spring	Summer	Autumn
Bumblebee 	<i>Ranunculus/Ficaria</i> spp. <i>Rubus</i> spp.	<i>Cirsium/Centaurea/Hypochaeris</i> spp. <i>Rubus</i> spp.	<i>Aster</i> spp. <i>Clematis</i> spp. <i>Rubus</i> spp.
Honeybee 	<i>Ranunculus/Ficaria</i> spp. <i>Taraxacum officinale</i>	<i>Cirsium/Centaurea/Hypochaeris</i> spp. <i>Impatiens glandulifera</i> <i>Rubus</i> spp.	<i>Actaea</i> spp. <i>Heuchera</i> spp. <i>Impatiens glandulifera</i>
Non-corbiculate bee 	<i>Geum</i> spp. <i>Ranunculus/Ficaria</i> spp. <i>Taraxacum officinale</i>	<i>Bidens/Coreopsis</i> spp. <i>Heuchera</i> spp. <i>Rudbeckia/Helenium</i> spp.	<i>Astrantia</i> spp.
Hoverfly 	<i>Ranunculus/Ficaria</i> spp.	<i>Angelica/Heracleum</i> spp. <i>Cirsium/Centaurea/Hypochaeris</i> spp. <i>Rubus</i> spp.	<i>Angelica/Heracleum</i> spp. <i>Bidens/Coreopsis</i> spp. <i>Rudbeckia/Helenium</i> spp.

et al., 2018) although the link between these requirements and floral resources used is little studied. While we identified differences in floral resource use between these functional guilds, we also highlight that hoverflies use plants for mate seeking, therefore additional work is required to fully understand which plants are being used for food, breeding sites or oviposition in phytophagous species (Rotheray & Gilbert, 2011).

4.2 | Plant use changes throughout the season

Season of collection was found to be the biggest predictor of plant use, with pollinators relying on key plants within each season (Appendix S5). The phenological patterns of plants result in shifting of floral availability, temporally limiting the foraging habits of insects. These shifts in available resources require pollinators to alter their use of resources throughout the season to survive, with those with long flight periods utilising a greater diversity of plant taxa than those with short flight periods (Ogilvie & Forrest, 2017).

4.3 | Dependence of pollinators on native and near-native plants

Pollinators use native and near-native plants more often than non-native plants, however, the non-native plants play a key role at the end of the flowering season. These findings are supported by Salisbury et al. (2015) who showed that native and near-native plants attracted a greater number of pollinators than non-native plants in a garden, however, the non-native plants extended the flowering period. The greater use of naturalised plants in summer and autumn can be attributed to the high use of *Impatiens glandulifera* by honeybees, highlighting the importance of this species for nectar provision. However, such an observation comes with a broader conservation caveat since *I. glandulifera* is a highly invasive, non-native plant and so it must not be grown due to its ability to displace other plant species (Chittka & Schürkens, 2001). While a lower proportion of non-native plants were used compared to native and near-native plants, they may contribute by increasing the diversity of pollinator diets. For example, *Taraxacum officinale* is used abundantly in the spring, however, it must be supplemented with additional resources as it

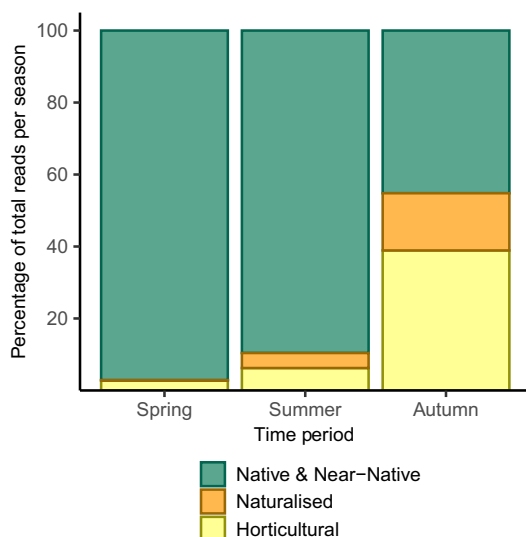


FIGURE 6 Proportion of sequence reads assigned to the native status of identified plants for all pollinators combined, by season. Plant taxa that were unable to be assigned a native status due to level of taxonomic rank were excluded. The use of plants within each category was affected by season of collection ($LR_{2,367} = 64.97$, $p < 0.001$) but not by year of collection ($LR_{2,367} = 59.48$, $p < 0.001$). Spring ($n = 148$), summer ($n = 210$) and autumn ($n = 11$)

lacks essential amino acids needed for pollinator health (Génissel et al., 2002).

4.4 | Using DNA metabarcoding to study plant-pollinator interactions

The multi-locus metabarcoding approach used here allows the relationship between plants and pollinators to be studied on a fine scale, improving both the number of plant taxa that can be detected and the level of discrimination achievable with the use of one marker alone (Jones, Twyford, et al., 2021) or alternative methods (Brennan et al., 2019). We highlight the ability of DNA metabarcoding to not only provide a greater depth of information, but also to support knowledge provided by traditional techniques, for example here the frequent use of taxa with large open inflorescences by hoverflies (Branquart & Hemptinne, 2000). Due to potential biases in sampling, along with extraction, amplification and sequencing of DNA (Bell et al., 2016), the data should be treated as semi-quantitative, with the abundance of DNA reads treated as estimates of relative abundance (see Analysing DNA metabarcoding data using semi-quantitative approaches, Appendix S2). Frequent taxa may be over represented, and rare taxa more difficult to detect, however, this is also the case using pollen microscopy (Hawkins et al., 2015). Recent developments suggest that in some cases metabarcoding data may be quantitative (Richardson et al., 2021), particularly regarding the most abundant taxa in a sample (Bänsch et al., 2020), however, further work is needed to fully understand this relationship (Piñol et al., 2019). Furthermore, species-level discrimination

in plants using DNA metabarcoding is challenging due to no single marker meeting the requirements for an ideal barcode (CBOL Plant Working Group, 2009). While genus-level designations have limitations in understanding fine-scale plant-pollinator interactions, these provide a conservative approach to identification, using the most universal and discriminative plant DNA markers available, to provide accurate taxonomic information across a wide study scale (Jones, Twyford, et al., 2021). Our conclusions therefore focus on the plants most abundantly used by pollinators, and how we can provide these in gardens and wider landscapes.

4.5 | Synthesis and applications

As public awareness and enthusiasm for pollinator conservation increases, improving plant recommendation lists for gardeners and encouraging suitable management practices has the potential to support pollinator populations at risk. This study provides an evidence base for recommendations that will support pollinators. We recommend that plants for pollinator lists should:

- distinguish between bees and hoverflies as a minimum
- provide recommendations throughout the seasons
- include native as well as non-native plants

Native and near-native plants can be provided in gardens by planting or through changing garden management regimes. For example, reducing mowing to encourage plants such as dandelion *Taraxacum officinale* and buttercups *Ranunculus* spp., and reducing scrub management to encourage bramble *Rubus fruticosus*. While the availability of floral resources may limit pollinators, we also highlight the importance of providing suitable nesting habitat within gardens. In particular, providing pre-existing hollow cavities will support aerial nesters, while having a variety in sward length within grassland will benefit ground-nesting bees. Egg laying in hoverflies can be encouraged by providing a diversity of floral resources, aquatic habitats and decaying wood to support the diversity of larval requirements.

The results of this study allow us to provide an evidence-based plant recommendation list to support a range of pollinators throughout the season including native and horticultural plants across a range of growth forms (Table S4, Appendix S3). We improve on previous lists by providing foraging information from the perspective of the insect, increasing both the temporal and spatial scope possible compared to using observations of plants (Arstingstall et al., 2021). This recommendation list is based on taxa found within the United Kingdom, with relevance to Northern Europe and can be used by gardeners, land managers, plant producers and policy makers to inform decisions on planting within gardens and urban greenspace to ensure pollinators are appropriately supported.

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CONFLICT OF INTEREST

None of the authors have a conflict of interest.

AUTHORS' CONTRIBUTIONS

The study was conceived by A.L., N.d.V. and S.C. Data collection and laboratory work was carried out by A.L.; The data were compiled by A.L. and analysed by A.L. and L.J. with suggestions from N.d.V., S.C. and G.B.; The manuscript was written by A.L. with contributions from all the authors. All authors gave final approval for publication.

DATA AVAILABILITY STATEMENT

Raw sequence data are available on the Sequence Read Archive at PRJNA763761. Data available via the Dryad Digital Repository <https://doi.org/10.5061/dryad.rjdfn2z9s> (Lowe et al., 2022). All code is available at <https://github.com/colford/nbgw-plant-illumina-pipeline>.

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