Seasonal variation in urban pollen resource use by north temperate European honeybees

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Abstract

Urban green infrastructure can provide important habitats for pollinators and support urban ecosystem services. Therefore, these areas must be managed to maximize biodiversity and density of pollinating insects. We used DNA metabarcoding to study honeybee pollen resource use over time and space in the city of Oslo, Norway, and to assess the role of green infrastructure as a resource for pollinators and the services they generate. Urban honeybees used diverse pollen resources throughout their active season. There was considerable seasonal turnover in pollen resource use that reflected flowering phenology. Non-native plants (including invasive species) were an important resource early in the season but were replaced by native plants later in the season. Hive location was not strongly correlated with resource use, likely indicating effective long-distance foraging in the fragmented urban landscape. However, flower visitation rates and floral resource density in public urban green spaces were coupled to pollen use. Honeybees collected pollen from a small number of preferred species but also visited other species, likely for nectar. To preserve pollinator services, urban planning should consider flower resource management, with particular focus on planting native species that can act as early season resources for bees. Public campaigns and other incentive mechanisms to promote the cultivation of native plants that are resources for pollinators and the protection of urban semi-natural habitats have the potential to enhance the value of green infrastructure to support urban pollinators and pollination services.

Keywords Urban ecology · Pollination · Seasonality · Pollen diversity · Flower resources · Urban green infrastructure · Citizen science

Introduction

Insect decline, particularly in pollinators, has been reported globally (Potts et al. 2010; Hallmann et al. 2017; Wagner et al. 2021) with detrimental effects to ecosystem processes and socio-economic impacts on food production and other insect-mediated ecosystem services (van der Sluijs 2020).

Habitat destruction and fragmentation driven by urbanization and intensified agroecosystems, as well as pollution, and climate change are identified as the main drivers explaining the decline in insect biomass and biodiversity (Sánchez-Bayo and Wyckhuys 2019). Urbanization is increasing, and by 2050 nearly 70% of all humans are expected to live in urban areas (Seto et al. 2012). Therefore, it is crucial to maintain ecosystem services within urban environments, including those supported by pollinating insects (Stange et al. 2018).

Pollination services are partly fulfilled by domesticated insects such as honeybees (*Apies mellifera*) (Garibaldi et al. 2013), and the number of hives has increased globally across the last five decades (Aizen and Harder 2009). Honeybees forage on a wide range of native plants, weeds, horticultural species, and invasive plants (Potter et al. 2019), and can be the most frequent flower visitors in both highly managed and natural areas (Hung et al. 2018). Owing to their generalist foraging strategy, honeybees' pollination represents

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an important ecosystem service for enhanced crop production and plant reproduction both within agroecosystems and urban areas. Honeybees also provide additional services including honey production and, particularly in urban systems, opportunities for nature-based leisure and educational activities (Stange et al. 2018).

With shifts in land use and increasing urbanization, there is increasing awareness of the importance of urban areas as pollinator habitats and how management can preserve pollination services and promote greater biodiversity. Although cities can harbour many pollinators, including rare taxa (Saure 1996; Baldock et al. 2015), floral resources are a limiting factor for urban pollinator populations (Aleixo et al. 2014). Particularly in the urban ecosystem, pollinators are impacted by fragmented vegetation cover that creates a patchy distribution of limited flower resources (Donaldson et al. 2002; Theodorou et al. 2020a). Urban honeybees use a wide range of flower resources with distinct seasonal patterns (Tanaka et al. 2020; Sponsler et al. 2020; Noël et al. 2021). Flower resource use patterns differ between urban honeybee hives and adjacent suburban and rural hives (Noël et al. 2021; Luce et al. 2019), highlighting the distinctness of the urban environment. However, the degree to which urban honeybees can track flower resources across green infrastructure mosaics is poorly understood.

Floral resource use by honeybees has been widely investigated (Potter et al. 2019; Wood et al. 2018) and a broad variation and seasonality in pollen foraging has been observed (Richardson et al. 2015; Hawkins et al. 2015; Danner et al. 2017; Lau et al. 2019; Sponsler et al. 2020). Plant phenology has been suggested as the main driver in pollinator forage choice (Biella et al. 2019), together with pollen taste (Muth et al. 2016). However, climate change driven changes in plant phenology, particularly in shifts in flowering onset (Piao et al. 2019; Fox and Jönsson 2019; Prevéy 2020), are predicted to result in mismatches between flower resources and pollinator foraging activities (Renner and Zohner 2018). North temperate ecosystems experience short growing seasons with rapid turnover in floral diversity in comparison to more equatorial climates, and disproportionately experience the effects of climate warming (Piao et al. 2019). This has the potential to disrupt the fine-tuned "supply and demand" system between flowers and pollinators. The assessment of temporal flower resource use can help identify seasonal resource gaps and thereby provides important knowledge to support management interventions aiming at designing climate-robust urban green infrastructure.

Pollinator resource use is traditionally assessed through microscopic morphological identification of pollen grains collected from the hive or honey. Advancements in sequencing technologies now allow investigation of pollen diversity using genetic techniques, and pollen metabarcoding has been applied to a variety of bee species (Sponsler et al. 2020)

addressing questions related to foraging patterns, flower visitation rates, insect movement, and plant-pollinator interactions (Bell et al. 2017; Pornon et al. 2016, 2017; Suchan et al. 2019). Here, we use Illumina metabarcoding of pollen collected by honeybees in the Oslo metropolitan area in Norway to assess the following: I) which plant species are used as pollen resources by honeybees in northern urban ecosystems, II) what is the temporal and spatial variation in pollen resource use, III) what is the relationship between flower resource availability and pollen use by honeybees in these systems, and IV) what is the relative importance of native versus exotic plants (both horticultural and invasive species)?

Materials & methods

Pollen sampling

Pollen was collected from beehives in Oslo, Norway, through a citizen science collaboration with the association of urban beekeepers in Oslo: ByBi. The association's members own and manage more than 100 honeybee hives spread across the metropolitan area. Pollen samples were collected from 23 hives at 2 week intervals between June and September 2017 (Table 1, Fig. S1). During each sampling event, beekeepers deployed a pollen trap at hive entrances and collected pollen over a 24-h period. Sample collection was coordinated via a social media platform to ensure that all samples were collected during a period without rainfall within the same 2–3 day window (typically a weekend). Samples were kept frozen (ca -18 °C) until delivery to the laboratory for processing. A total of 93 samples from 21 hives were analysed by DNA metabarcoding (Table 1). The bulk weight of pollen samples ranged from 1 to 14 g. Sampling intensity differed between hives due to variable pollen trapping success, and non-systematic sample return by participating beekeepers.

DNA isolation, amplification, and sequencing

DNA was isolated from pollen samples using a modified protocol for the Qiagen DNeasy Blood & Tissue Kit (Qiagen, Germany). Bulk pollen samples were homogenized with a small amount of Lysing Matrix D (MP Biomedicals) and a ceramic bead at 60 m/s for 30 s, and subsequently dried overnight in a drying cabinet at 55 °C. After subsampling the homogenized pollen into 2 mL tubes with lysing matrix D, lysis buffer was added according to the manufacturer's specifications and the samples were further homogenized at 60 m/s for 1 min. Then, 60 μL of proteinase K was added and the extraction mixture was incubated overnight prior to continuing the extraction protocol according to the manufacturer's instructions.

Table 1 Number of biweekly pollen samples collected per month across 21 urban beehives

Hive ID	Jun	Jul	Aug	Sep	Total
BH 1	1	1	-	-	2
BH 2	1	1	1	-	3
BH 3	1	-	-	-	1
BH 4	1	-	1	-	2
BH 5	2	3	2	-	7
BH 6	-	2	2	-	4
BH 7	1	4	2	-	7
BH 8	2	1	2	1	6
BH 9	1	-	-	-	1
BH 10	-	2	1	-	3
BH 13	2	-	-	-	2
BH 14	2	3	2	-	7
BH 15	2	3	2	-	7
BH 16	1	2	3	-	6
BH 17	3	-	-	-	3
BH 18	2	3	-	-	5
BH 19	2	2	2	-	6
BH 20	1	2	2	-	5
BH 21	2	1	1	-	4
BH 22	2	2	2	-	6
BH 23	2	2	2	-	6

The ITS2 region of plant DNA was amplified using the primers ITS-S2F (Chen et al. 2010) and ITS4 (White et al. 1990). PCR reactions were conducted in 25 μL volumes containing 1× KAPA HiFi HotStart ReadyMix (Fisher), 0.2 μM of each primer, and 12.5 ng of template DNA. Reaction conditions were as follows: an initial denaturation of 3 min at 94 °C followed by 25 cycles of 30 s at 95 °C, 30 s at 56 °C, and 30 s at 72 °C, and a final elongation step of 10 min at 72 °C. Amplicons were normalized to 25 ng using a SequalPrep normalization plate (Invitrogen). Adapters and indices for sequencing were added using the Nextera XT Index kit (Illumina). Reaction mixtures volumes were 50 μL and contained 1× KAPA HiFi HotStart ReadyMix (Fisher), 5 μL of each index primer, and 5μL of template DNA from the first PCR reaction. PCR reaction conditions were as follows: initial denaturation of 3 min at 95 °C followed by 8 cycles of 30 s at 95 °C, 30 s at 55 °C, and 30 s at 72 °C, and a final elongation step of 5 min at 72 °C. Libraries were then normalized using a SequalPrep normalization plate (Invitrogen) and pooled in equimolar amounts. The pooled libraries were sequenced on an Illumina MiSeq instrument using paired end 300 bp chemistry at the Genomics Core Facility at St. Olavs Hospital, Trondheim, Norway.

Bioinfomatic processing

Forward and reverse sequences were demultiplexed on the Illumina MiSeq instrument. Forward and reverse primers were removed using cutadapt v. 1.18 (Martin 2011), requiring a minimum match length of 17 bp, no indels, and < 0.15 expected errors over the primer length. Sequences were further quality filtered, error corrected, merged and chimera checked using the DADA2 R package (Callahan et al. 2016). All sequences < 50 bp, containing ambiguous bases or containing > 2 expected errors across the entire length were removed. Sequences were then truncated at the first instance of a base with Phred quality score < 15. Filtered sequences were merged with a minimum overlap of 30 bp and chimeric sequences were removed using the consensus method. Taxonomy was assigned to the resulting amplicon sequence variants (ASVs) using the SINTAX classifier (Edgar 2016) as implemented in vsearch v.2.14.1 (Rognes et al. 2016) and the PLANiTS database (Banchi et al. 2020). ASVs were assigned to the lowest possible taxonomic level receiving > 70% bootstrap support in the SINTAX analysis. Taxa were classified as either horticultural, native, or ambiguous based on their classification and status in the national flora (Lid & Lid 2005) and the Norwegian Biodiversity Information Centre (2018). Species were further classified as important pollen resources (utilized by > 40% of hives, mean proportional abundance > 10%), locally important (utilized by < 40% of hives, mean proportional abundance > 10%), globally abundant (utilized by > 40% of hives, mean proportional abundance < 10%), or incidental (utilized by < 40% of hives, mean proportional abundance < 10%, see Fig. 2).

Urban floral resources density and visitation rates

Floral inventories were conducted in 100 green spaces within the Oslo urban area between July 18 and August 4, 2017, generating a landscape level estimate of floral resource abundance for this time period. Sampling locations were extensions of previous work, with sites selected to capture a range of habitat suitability values as expressed in early versions of a model for urban pollinator habitat (Stange et al. 2017). The urban green spaces investigated were publicly accessible and included hedgerow, lawn, meadow, and waste areas containing primarily ruderal vegetation, as well as parks and cemeteries containing planted and tended vegetation (Table S1, Fig. S1). Green spaces composed of short cut lawns, forests lacking flowering ground cover, and private garden green spaces were excluded from the inventory. Each green space was visited once to determine floral resource density using 1 to 3 transects according to vegetation patch size and shape for a total of 20 transect meters. All open floral units were recorded within a 40 cm width along each transect. The definition of a unit (individual flower, entire

inflorescence) differed between species. For inflorescences with a high number of individual flowers (ex/ racemes, capitula), we used a multiplication factor to calculate the total number of flowers of that species in the transect. Multiplication factors were based on the average number of flowers per recorded unit, retrieved from the literature (Lid and Lid 2005; Mossberg et al. 1992; and the Nordic virtual flora at the Swedish Natural History Museum http://linnaeus.nrm.se/flora/). The mean floral resource density for the landscape was calculated per plant species across all the inventoried green spaces, as well as within a 1-, 3-, and 5-km radius of each individual hive. Landscape level honeybee visitation rates to different floral resources were calculated from 60 transect meters within each green space. One to four visits (mean 2.3) were made to each green space between July 18 and August 10, 2017, during which we walked each transect for approximately 5 min, allowing for additional time for specimen handling and species identification. We recorded the number of honeybees observed on flowers, including the plant species, within 2.5 m on both sides of the transect. Visitation rates were calculated as a mean across all transects in the landscape, as well as for all transects within a 1-, 3-, and 5-km radius of each individual hive.

Statistical analyses

All statistical analyses were conducted in the R statistical environment (R Core Team 2023). For analyses relying on number of species, the pollen data was rarefied to 5000 sequences per sample prior to tallying the number of species in order to account for variation in library sequencing depth (Fig. S2). For all analyses using proportional abundances, the proportional abundance of each species was calculated from the non-rarefied data. Variation in the plant species utilized by hives throughout the season was assessed using a GNMDS ordination with proportional abundance data and Bray-Curtis distance calculations, with the effects of hive and sampling date assessed using the *envfit* function in the R package vegan (Oksanen et al. 2019). After observing a strong effect of sampling date in the GNMDS ordination, a partial Mantel test using the Pearson correlation method and 999 permutations of the data was used to assess the correlation between the plant species utilized and the geographic location of hives, controlling for the effects of sampling date.

General linear mixed models as implemented in lme4 (Bates et al. 2015) were used to assess the relationship between sampling date and i) total number of species used, ii) number of horticultural species used, iii) number of native species used, and iv) the ratio of native to horticultural species used, with hive ID included as a random effect. For a subset of 14 hives that were sampled at 4 or more time points, general linear mixed models were used to assess the relationship between sampling date and i) proportional abundance of horticultural species used, ii) proportional

abundance of native species used, and iii) the ratio of sequences from native to horticultural species with hive ID included as a random effect. Turnover in pollen resource utilization throughout the season was examined by calculating the Bray-Curtis dissimilarity between successive sampling points for each individual hive throughout the season using the vegan package in R (Oksanen et al. 2019) and testing the effect of sampling date as a fixed effect in a linear model in the lme4 package in R (Bates et al. 2015).

Linear models were used to assess the relationship between flower availability and pollen resource utilization. Flower availability was calculated as the mean floral density for all transects occurring within 4 different projected resource use areas around each hive (1 km radius, 3 km radius, 5 km radius, total landscape). The mean proportional abundance of each plant species was further calculated across all hives sampled during the period beginning 7 days prior to the first transect sampling and 7 days after the last transect sampling (July 11 – August 11, typically 2 sampling events, range: 1–3). The relationship between the log transformed flower resource density and the log transformed pollen abundance was then tested for each projected resource use area (1 km radius, 3 km radius, 5 km radius, total landscape) using linear models. In order to avoid potential biases associated with insufficient sampling effort in flower resource inventories, and systematic detection bias related to primer bias in pollen inventories, the data was limited to only those plant species (55 species) detected at least once in both the flower resource inventories and the pollen inventories.

Results

Diversity of pollen resources

From the pollen collected at the 21 hives investigated, we detected 108 taxa that could be identified to the species level and an additional 150 taxa that could only be assigned to higher taxonomic levels (Table S2). Together, these represent 195 genera in 60 plant families with a mean of 15.65 genera (range: 5–49, sd: 7.23) and 10.47 families (range: 4–21, sd: 3.74) per hive at each sampling event. The taxa identified included 73 presumed native species, 123 presumed horticultural species, 21 non-native species, and 41 species that could not be placed in the preceding categories (predominantly due to ambiguous taxonomic assignments) (Table S2).

Temporal and spatial patterns of pollen use

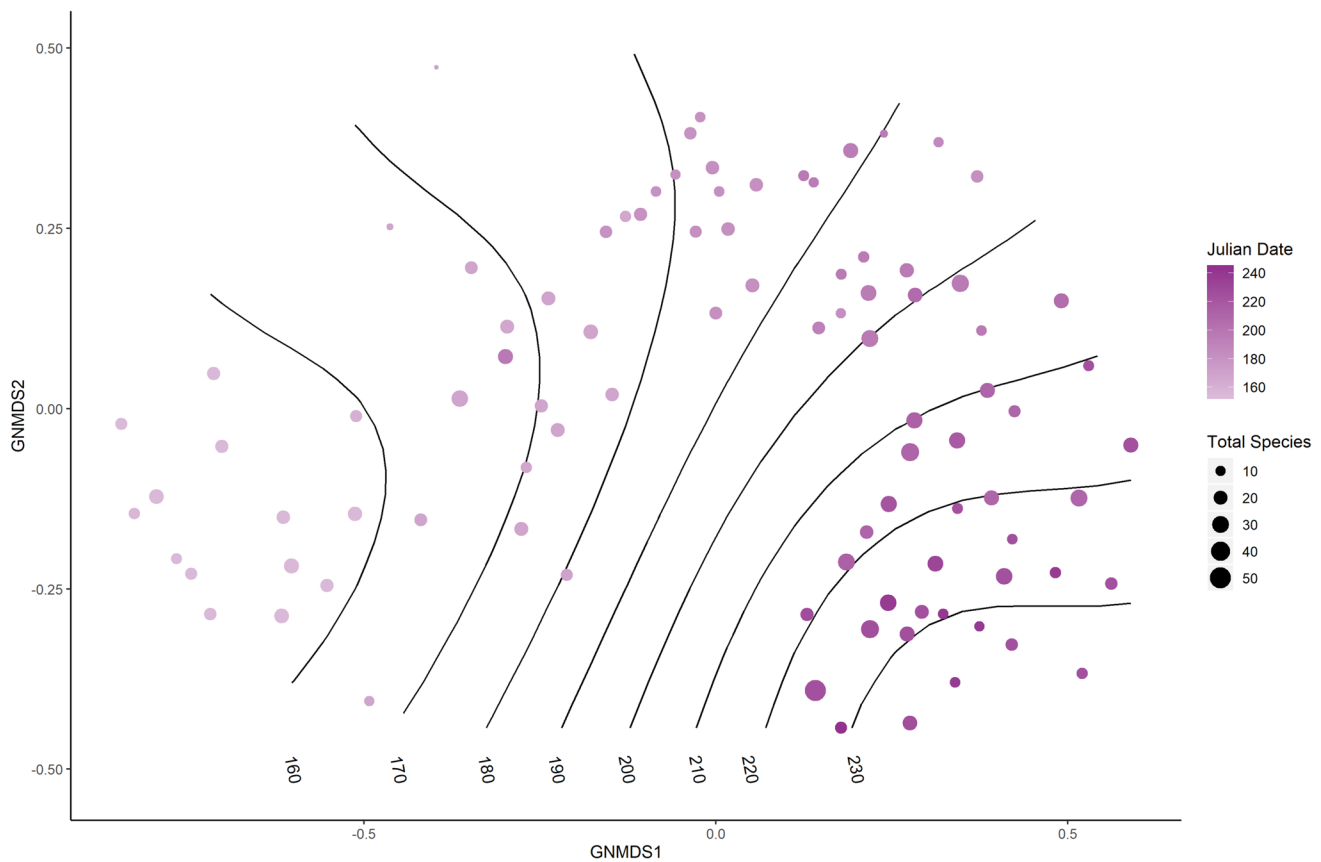Species composition of the pollen recovered from hives shifted seasonally (p < 0.001, Figs. 1, 2, and S3). *Hydrangea*

Fig. 1 GNMDS ordination diagram showing variation in pollen resource use between urban beehives over a season. Contour lines are fitted showing variation in Julian date and point size reflects the number of plant species detected in each pollen sample

petiolaris, Aegopodium podagraria, and *Rosa* sp. Were identified as important early season resources, each utilized by > 40% of hives and accounting for, on average, > 10% of the pollen collected by a given hive on a given day. Several species, including *Acer pseudoplatanus, Allium ursinum, Papaver* sp., and *Barbarea vulgaris* were identified as being locally important, accounting for > 10% of the pollen collected by one or a few hives on a given day. A number of pollen resources were collected by most hives, but in comparatively low amounts, including *Rubu*s sp., *Trifolium repens*, *Acer tartaricum*, and *Bunias orientalis* (Figs. 2A and S3). Later in the season, the most used pollen resources shifted to include *Filipendula ulmaria, Solidago* sp.*, Hydrangea paniculata, Calluna vulgaris,* and *Trifolium repens*. Locally important species included *Symphytum* sp. And *Tropaeolum* sp., while *Chamaenerion* sp.*, Melilotus* sp.*, Trifolium* sp.*, Rosa* sp.*, Cirsium arvense,* and *Dasiphora fruticosa* were all collected by many hives, but in comparatively low amounts (Figs. 2B and S3). Turnover of pollen resource use throughout the season, declined significantly in late summer (p < 0.001, Fig. 3).

Pollen composition among beehives on a given day was more similar than within a single hive throughout the season (Fig. S4). This pattern reflects the shifting dominance of particular flower resources throughout the season, and seasonal turnover in pollen resource use within individual hives. A partial mantel test for correlation between pollen resources used by the hives and their geospatial locations while controlling for the effects of sampling date was not significant (p = 0.427) (Fig. S4).

Pollen supply and use

There was a significant, positive relationship between per-species floral resource density and each hive's pollen resource utilization for all of the projected resource use areas investigated (1 km radius, 3 km radius, 5 km radius, entire landscape, p < < 0.001 for all) (Figs. 4 and S4). The fit of this correlation improved in the larger resource use areas, increasing from $R^2 = 0.03$ in a 1 km radius to $R^2 = 0.12$ when the entire urban landscape was considered (distances up to 17 km from the hive) (Fig. S5). A number of species were identified that had consistently high pollen utilization relative to their mean flower resource density, including *Filipendula ulmaria*, *Trifolium repens*, and *Calluna vulgaris* (Figs. 4 and 5A). Conversely, several species were identified

Fig. 2 Relative importance of pollen resources to urban honeybees during the **A** early season (prior to July 7) and **B** late season (after July 7). Species in the upper left quadrant are considered locally important pollen resources, right upper quadrant species are important pollen resources, lower right quadrant are globally abundant species, and lower left quadrant are incidental pollen resources
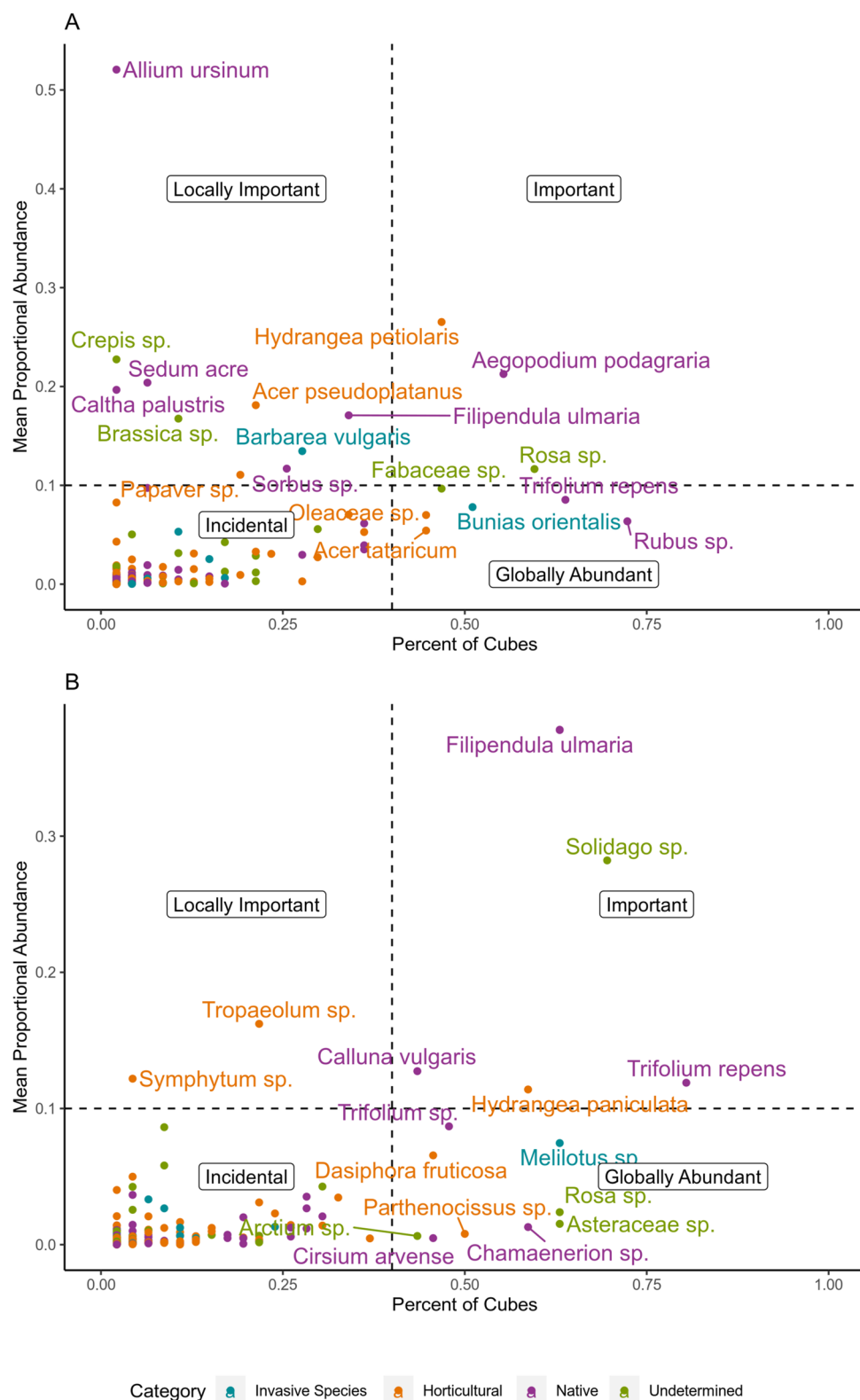
with comparatively low pollen utilization given their mean flower resource density, including *Cirsium arvense, Hypericum* sp., and *Ranunculus repens* (Figs. 4 and 5A). Per-species flower visitation rates were correlated with pollen resource

utilization (p < 0.001, Fig. 4B). While a number of species were identified that had both high visitation rates and pollen utilization, including *Solidago* sp. And *Filipendula ulmaria* (Fig. 5B), several species with high visitation rates
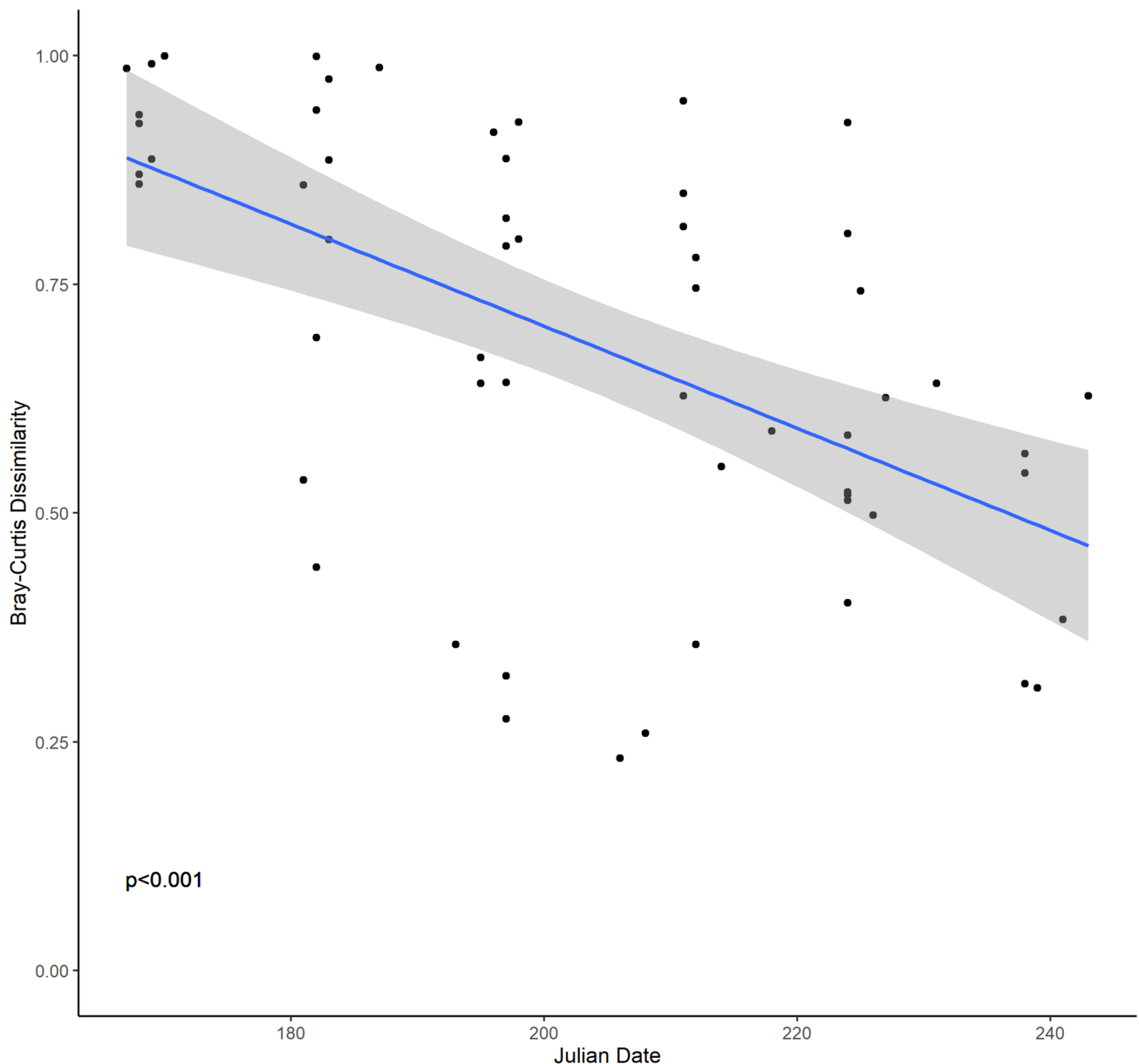
Fig. 3 Turnover in pollen resource utilization throughout the season for 13 urban bee hives. For each point, the Bray-Curtis dissimilarity has been calculated between the pollen collected at the focal sampling time and the previous sampling date

nevertheless had comparatively low pollen utilization rates, including *Cirsium* and *Arctium* species (Fig. 5B).

The total number of species used as pollen resources by an urban beehive at a given time significantly increased throughout the season ($p = 0.016$, Fig. 6A). The number of non-native horticultural species utilized was consistent throughout the season ($p = 0.199$, Fig. 6C) as was the ratio of horticultural to native species ($p = 0.387$, Fig. 6B). By contrast, the number of native species utilized increased significantly throughout the season ($p = 0.004$, Fig. 6D). The relative abundance of pollen collected from presumed horticultural plants did not vary significantly throughout the season ($p = 0.854$, Fig. 7A), nor did the relative abundance of pollen collected from native plants

($p = 0.126$, Fig. 7B). However, the ratio of native:horticultural pollen utilized throughout the season increased significantly ($p < 0.001$, Fig. 7C).

Discussion

Diversity of pollen resources used by urban honeybees

Over the course of the foraging season, pollen was identified from native, horticultural, and non-native trees, shrubs, and herbs, representing 195 genera from some 60 plant families.
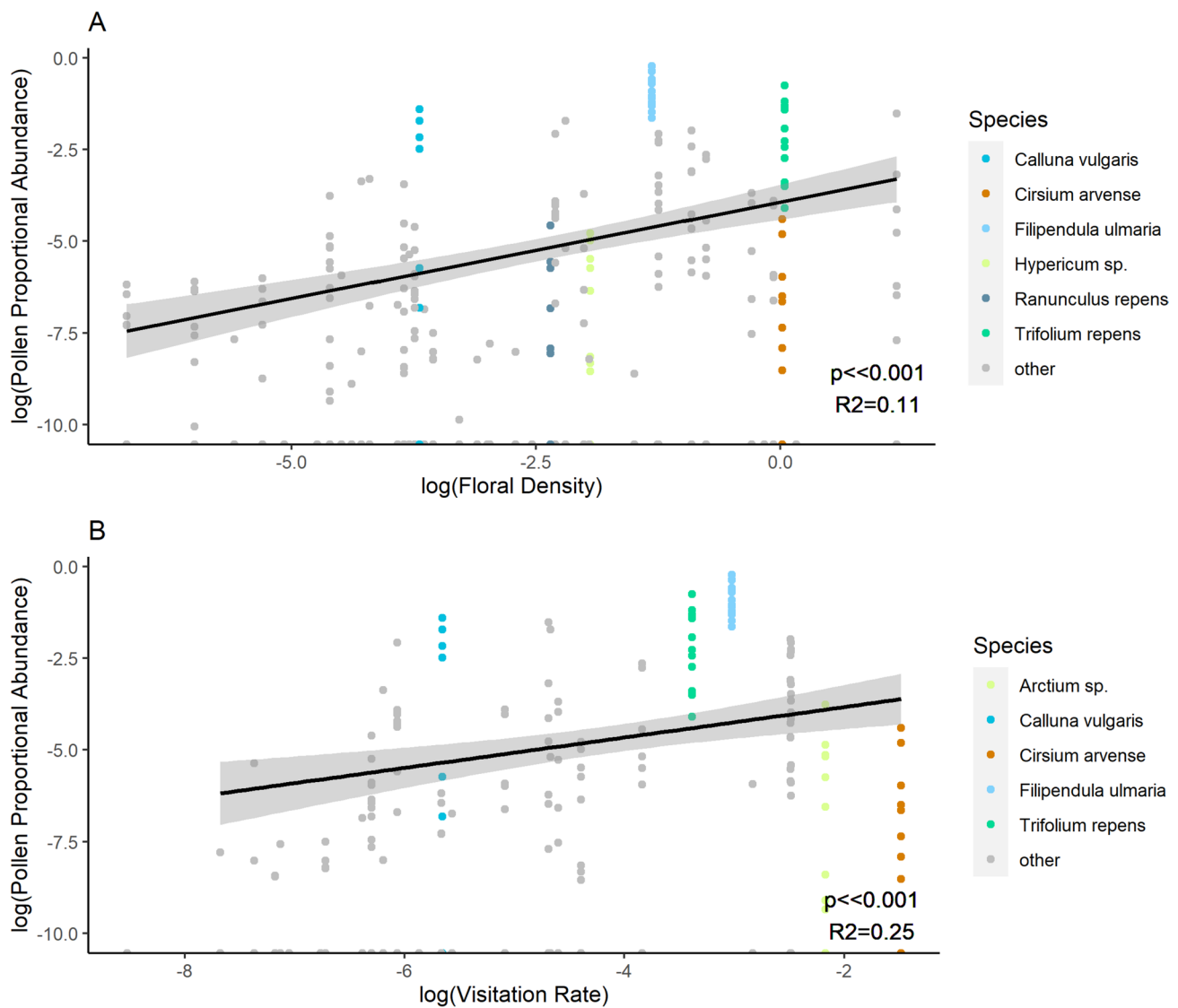
Fig. 4 The relationship between **A** per-hive pollen utilization and mean per-species flower resource density across the entire Oslo urban landscape, and **B** per-hive pollen utilization and mean per-species visitation rates across the entire Oslo urban landscape. The species visualized are limited to only those detected at least once in both flower inventories and pollen inventories. Selected species that consistently have comparatively low or high pollen utilization rates given the abundance of their flowers in the landscape or their mean visitation rate by honeybees are highlighted in colour

However, individual hive's pollen resource diversity at single time points was much lower (mean: 15.65 genera, 10.47 families) and falls on the low end of the range of previously reported resource diversities for *Apis mellifera* (12–39 families; Richardson et al. 2015; Lau et al. 2019; Hawkins et al. 2015; Tanaka et al. 2020; Sponsler et al. 2020; Noël et al. 2021; Richardson et al. 2015). These previous reports of pollen resource diversity are predominantly from more southern, warmer climates than the system studied here (i.e. coastal USA, Japan, United Kingdom). A lower diversity of pollen resources in north temperate urban ecosystems as compared to more southerly systems is not unexpected given

the decrease in plant diversity with latitude owing to harsher climatic conditions and a reduced growing season.

Pollen resource use at the city landscape level showed no clear geographic pattern at any given time point. Although we could detect locally important species (i.e., species that contributed with > 10% of the pollen in a particular beehive), a large proportion of the beehives used the same plants as pollen sources. This likely reflects a relatively homogeneous distribution of core pollen resources across green spaces in the urban environment, and/or relatively long distance foraging across the urban green infrastructure mosaic of heavily urbanized areas, gardens, and green
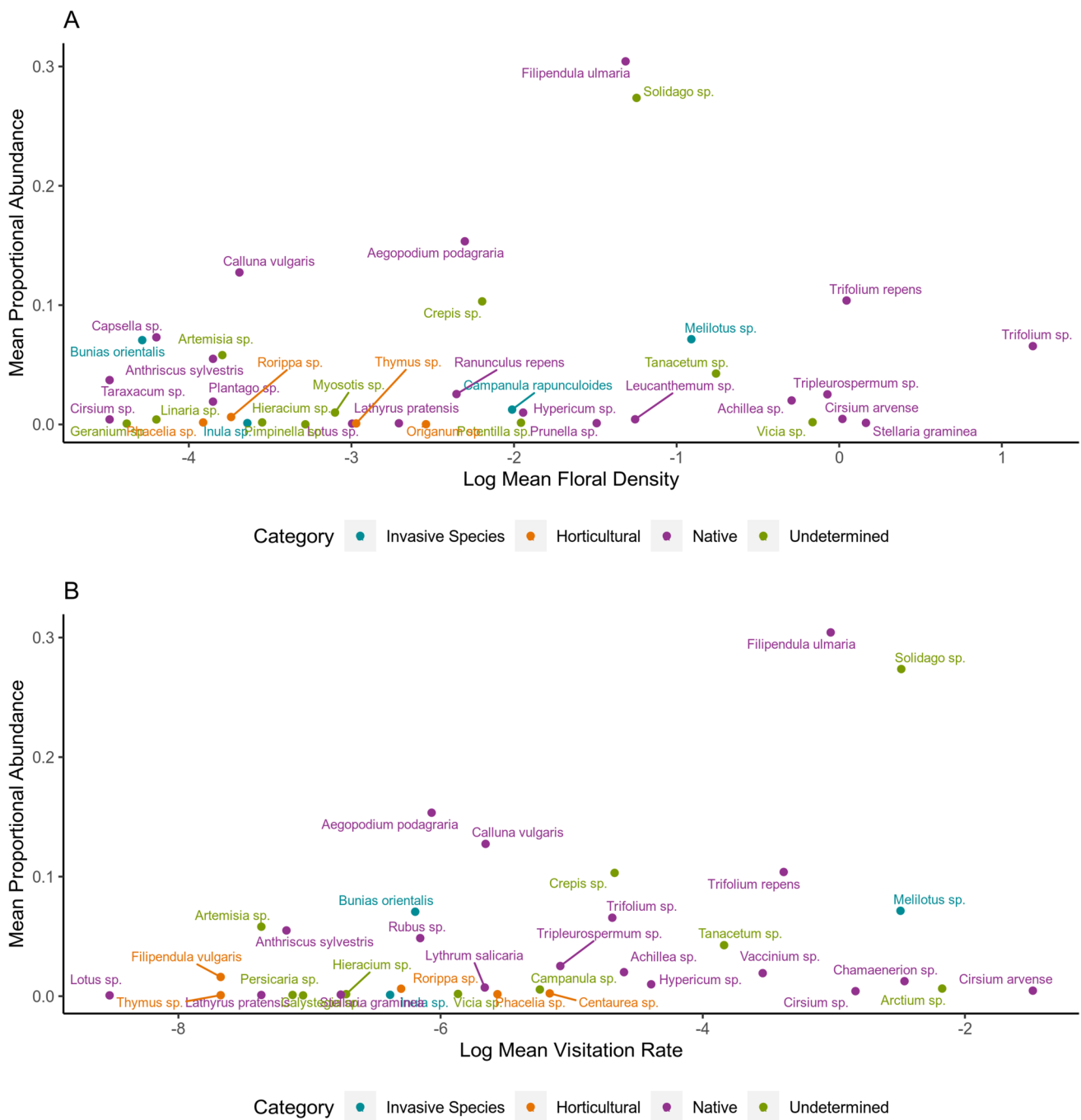
Fig. 5 Mean proportional abundance of collected pollen versus **A** mean flower resource density and **B** mean flower visitation rate. The species visualized are limited to only those for which both **A** metabar-coding and flower density or **B** metabarcoding and visitation rate data were available

spaces. Resource use in honeybees is connected to land use/land cover patterns i.e., degree of urbanization and type of green space such as percentage of gardens and other green spaces (Noël et al. 2021). Given the relatively fine-scale variability in these factors in an urban environment, we can

expect not to observe the classic distance-decay relationships in pollen resource use between urban beehives. Our data suggest that despite the fragmented nature of urban pollen resources, honeybees can track core resources across the urban matrix.
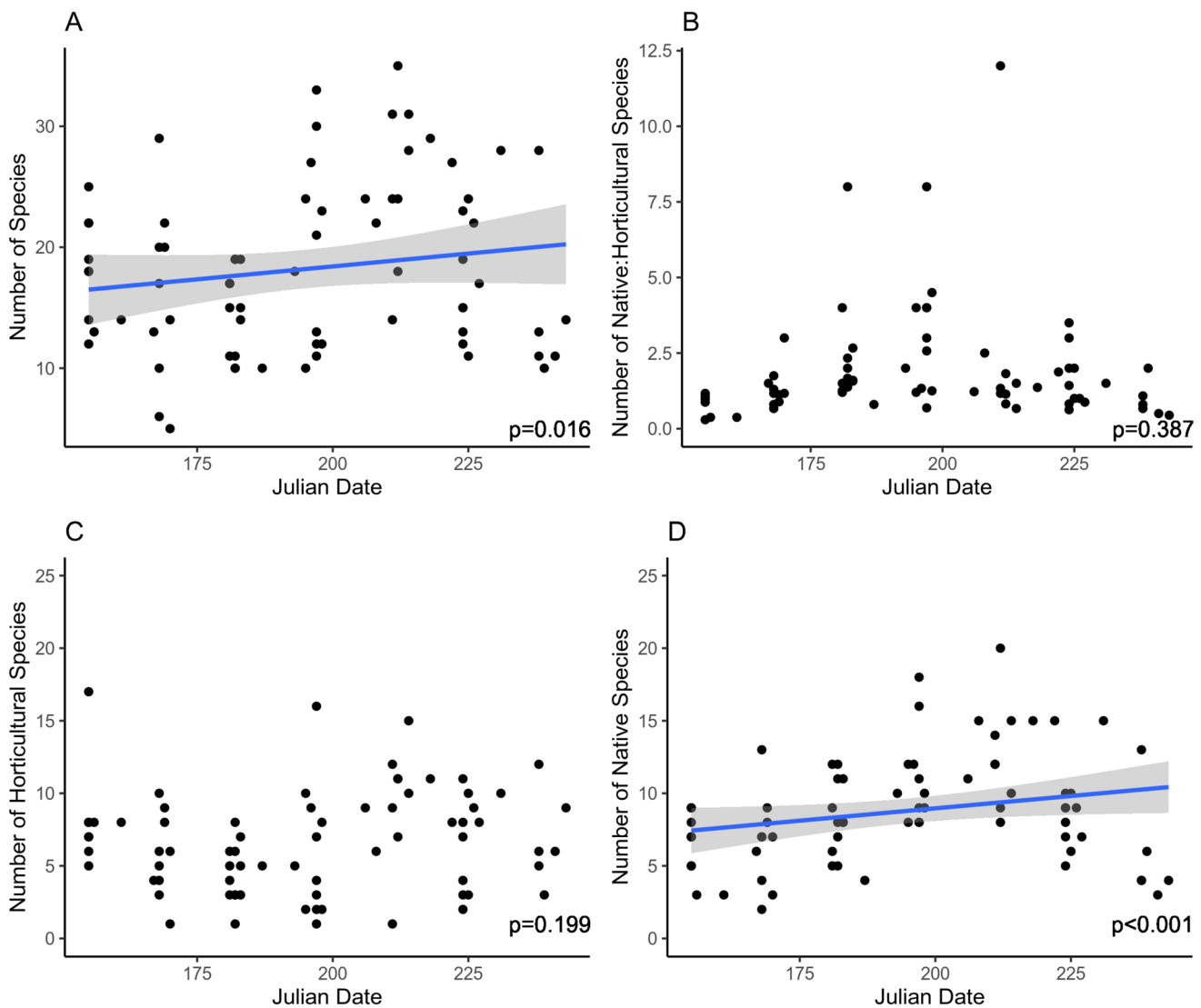
Fig. 6 Seasonal variation in the **A** total number of plant species, **B** ratio of native:horticultural plant species, **C** number of horticultural plant species and **D** number of native species. Regression lines are fitted where significant correlations are detected

Forage territories and preferential foraging among urban honeybees

Honeybees are restricted to spatio-temporally available resources, i.e., plants currently flowering within flight-radius of the hive. Honeybee foraging distances can be highly variable, ranging from only a few hundred metres to more than ten kilometres (Visscher and Seeley 1982; Beekman and Ratnieks 2000). In agricultural areas, honeybee foraging territories are frequently small, often not exceeding a 1 km radius from the hive (Beekman and Ratnieks 2000; Hagler et al. 2011; Sponsler et al. 2017). We observe a positive correlation between mean per-species pollen resource use in each hive and per-species estimates of floral resource density with an improved fit when larger forage territories are assumed (e.g. 5 km radius from the hive, or > 10 km radius from the hive). This would seem to suggest that honeybees engage in foraging over longer distances in urban environments than in natural or agricultural settings, ostensibly due to fragmented and reduced resource availability. However, these results must be interpreted with caution, as the sampling effort to characterize floral resource availability was greatest at larger spatial scales and smallest for the predicted resource use area of a 1 km radius around each hive. Furthermore, our floral resource inventories were limited to public green spaces and did not include private gardens, which clearly contain important floral resources (e.g. *Rosa* sp. And *Hydrangea* spp.) that were not detected in our floral inventories introducing potential bias to our
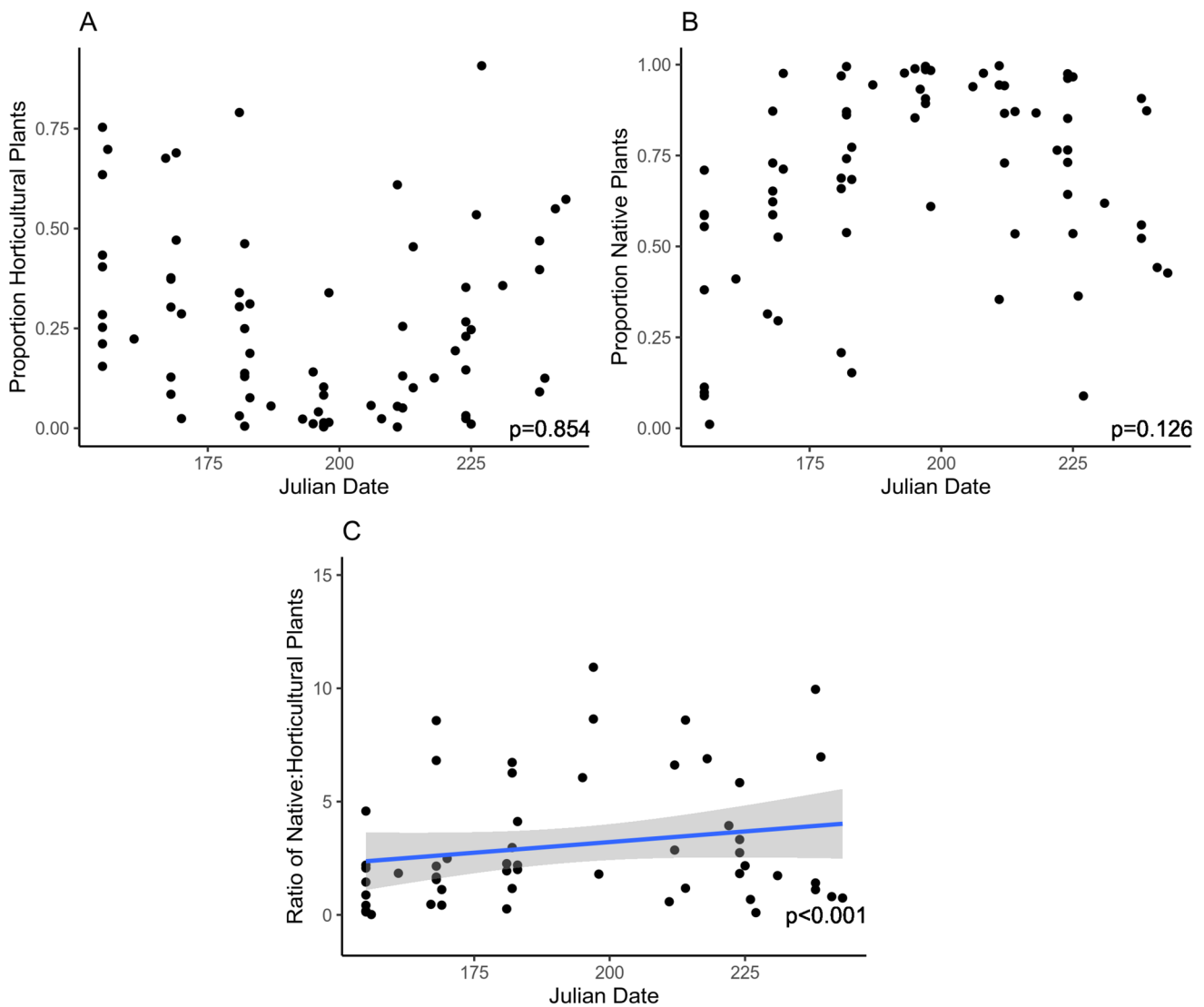
Fig. 7 Seasonal variation in the proportional abundance of **A** horticultural, **B** native and **C** the ratio of native:horticultural plant species. Regression lines are fitted where significant correlations are detected

data. In order to better characterize the forage behaviour and forage range size of honeybees in urban environments, it would be beneficial to estimate pollen resource utilization, flower visitation, and floral resource availability in both green spaces and private gardens and combine them with with waggle-dance decoding or mark-recapture experiments to gain quantitative information on foraging distances.

Despite observing an overall correlation between the proportion of pollen belonging to a particular plant species and both the density of its flowers in the landscape and its visitation rate by honeybees, we nevertheless find evidence for preferential foraging among urban honeybees. Honeybees from different hives consistently collected pollen from particular plant species and not from other plant species, irrespective of the comparative availability of their flowers in the surrounding landscape. Floral resource use patterning has been previously demonstrated in domesticated bees, and use of a limited range of species despite a high diversity of available flowers has been observed (de Vere et al. 2017). This implies that certain plant species are likely better pollen resources than others, and honeybees discriminate between them. For example, *Cirsium arvense* had high floral density and high visitation rates but was nevertheless detected in relatively low quantities in the pollen traps. This species is a high-quality nectar resource for bees, but a poor-quality pollen resource (Hicks et al. 2016). Ghosh et al. (2020) further demonstrate that honeybees use both nutritional value (protein content) and resource availability as criteria for selecting pollen resources, and as

such it is unsurprising that we find evidence of preferential foraging.

Seasonal variation in urban pollen resources

Honeybee pollen resource use is expected to vary across the hive's active period owing to differences in plant phenology i.e., flowering onset. Our results show considerable pollen resource turnover between two-week sampling intervals, which is consistent with numerous other studies (Bilisik et al. 2008; Hicks et al. 2016; Park and Nieh 2017; Lau et al. 2019; Tanaka et al. 2020; Sponsler et al. 2020; Noël et al. 2021) which observe seasonal patterns in pollen resource use across both Europe and North America. In the north-temperate urban ecosystem investigated here, we observe an increase in the diversity of pollen resources used throughout the hives' active season. This is in contrast to studies in more southerly, warmer urban environments (Lau et al. 2019; Noël et al. 2021; Sponsler et al. 2020) that document urban honeybees collecting a broad range of pollen resources early in the season with diversity decreasing over time. We suspect that flower phenology largely explains these contrasting patterns. Due to the short flowering season in Nordic areas, the plant community may flower incrementally rather than successively, with overlap in flowering periods giving an increase in resource availability throughout the season. This is supported by the decreasing turnover rate in pollen resources throughout the season despite a concomitant overall increase in the diversity of resources utilized. In particular, high turnover in the early season may be driven by the utilization of spring ephemeral herb species and other plants with short flowering periods. For example, in the early season, plants with short blossoming periods (< 1 month) including *Allium ursinum* and *Aegopodium podagraria* are identified as important pollen resources for hives, while plants with longer blossoming periods (> 1 month) such as *Rosa* sp., *Trifolium repens,* and *Filipendula ulmaria* are identified both in the early and late season.

Importance of cultivated and invasive species

In urban spaces, the plant community is not a result of ecological processes but rather is a constructed community based on urban planning, horticultural history and random events, the latter including remnants of the native flora surviving urbanization and introduction of non-native species. There is a strong correlation between urbanization and the occurrence of invasive species (Gaertner et al. 2017; Štajerová et al. 2017) and non-native plant species have been identified as important resources for pollinators, particularly honeybees, in urban ecosystems (Urbanowicz et al. 2020; Lowe et al. 2022). Our results pinpoint the importance of exotic species (including both horticultural and non-native

species) as a resource for honeybees across the season in urban settings. However, these species are more important during the early season in this north-temperate system. *Hydrangea petiolaris*, one of the most abundantly used early season resources, is a popular garden plant because it thrives in the Norwegian climate, endures shade and has profuse, early blossoms. *Aegopodium podagraria* a similarly important, introduced, early resource plant, is a shade tolerant garden weed forming extensive understory vegetation in roadside thickets and disturbed areas. Notably, two invasive species within Brassicaceae (*Barbarea vulgaris* and *Bunias orientalis*), both assessed to have severe negative ecological effects (Norwegian Biodiversity information Centre 2018), were identified as significant early season pollen resources for honeybees. This is consistent with other findings demonstrating that exotic plant species may be an important resource for bees, filling a forage gap during a seasonal dearth of flowering native species (Park and Nieh 2017; Wood et al. 2018; Sponsler et al. 2020). This is further supported by the example of the invasive *Melilotus* sp., which flowers in the mid-to-late season but is not preferentially collected by the bees.

Management of urban green spaces to preserve pollinator services must include consideration of floral resource availability for pollinators and assess the role of native, horticultural, and non-native plants in these highly anthropogenic habitats. Although horticultural and non-native plants are seemingly good resources for honeybees, the impact of cultivated and non-native flowers on native pollinator communities must also be considered(Szigeti et al. 2020). As urbanization often promotes replacement of native species, some studies have indicated a decrease in specialized pollinator taxa as a response (Bergerot et al. 2010; Theodorou et al. 2020b). To circumvent such effects planting native flowers that blossom throughout the season should be prioritized when planning green spaces within urban areas. Planting specific native taxa within green spaces could ensure local early flower resources also for wild pollinators within cities. For example, many species within the early flowering genus *Salix* are native within the northern hemisphere (Zhen-Fu 1987) and are known to be a preferential choice for many pollinators (Mosseler et al. 2020).

Conclusions

Honeybees in a north temperate urban ecosystem utilized a wide diversity of pollen resources throughout their active period. We observed preferential foraging on specific species that likely reflects resource quality, as well as shifting resource use patterns with decreased turnover in resource use in the late season likely coupled to flower phenology. Horticultural plants and other non-native plant species were found to be important pollen resources in the early season,

with native plants accounting for higher proportions of the pollen foraged in the late season. In order to preserve pollinator services and promote biodiversity, urban planning should take into account flower resource management, with particular focus on planting native species that can act as early season resources for honeybees.

Declarations

Ethics approval NA.

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