Foraging preferences of honeybees (Apis melliferaL.) analysed by pollen metabarcoding along an urban-rural gradient, across seasons

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In collaboration with Chiba University (Japan)

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Abstract

In a context of global biodiversity lost, recent studies support that well-managed cities could improve the preservation of Hymenoptera and thus provide hot spots for pollination services. Therefore, cities are in the spotlight of studies in order to determine if they have sufficient resources to host both domesticated and wild bees. One solution would be to investigate their floral preferences to promote the biodiversity through integrated urban greening projects.

Part of this continuum, the present work analyses the pollen collected from 18 different apiaries distributed in the Kanto region of Japon. The sampling was realised from March to September 2019. Prior to the analysis, the pollen was identified by pollen metabarcoding. Next, the objectives were to assess if the species richness and diversity were influenced influenced by by landscape and/or sampling period. In addition, a trait-based analysis was conducted to determine if the plant nature (Woody or Herbaceous) and the native status (Native, Alian or Cultivar) were influenced differ among the landscapes and over the course of the seasons.

To do this, several test were applied including a K-means clustering associated with a PCA to determine the landscape classes, a NMDS ordination followed by permutation-based multivariate analysis of variance (PERMANOVA) and a post-hoc multilevel pairwise analysis to evaluate the differences in the pollen composition. Then the diversity was assessed by a 2ways mixed ANOVA and the Hill indices along rarefaction and extrapolation curves. Finally, the trait-based analysis was based on a G–test of independence for contingency followed by a post-hoc pairwise comparisons.

It has been evidenced that the landscape explains minor variations in the plant composition foraged by honeybees. In contrast, the species richness, pollen diversity and plant composition showed a strong dependence to the seasons. Regarding the taxonomic composition, the Fabaceae, Rosaceae, Brassicaceae, Plantaginaceae and Onagraceae represent the families with the most frequent observations in all samples combined.

The present study contributes to a broader understanding of the ecology and floral preferences foraged by honeybees on which the urban planning can rely in order to promote the biodiversity in the cities.

Résumé

Dans un contexte de perte de biodiversité, des études récentes soutiennent que des villes bien gérées pourraient améliorer la préservation des hyménoptères et ainsi fournir des pôles de pollinisation. C'est pourquoi les villes font l'objet d'études visant à déterminer si elles disposent de ressources suffisantes pour accueillir à la fois des abeilles domestiques et des abeilles sauvages. Une solution serait d'étudier leurs préférences florales afin de promouvoir la biodiversité par le biais de projets intégrés d'écologisation urbaine.

Dans le cadre de ce continuum, le présent travail analyse le pollen collecté dans 18 ruchers différents répartis dans la région de Kanto au Japon. L'échantillonnage a été réalisé de mars à septembre 2019. Avant l'analyse, le pollen a été identifié par le métabarcodage du pollen. Ensuite, les objectifs étaient d'évaluer si la richesse et la diversité des espèces étaient influencées par le paysage et/ou la période d'échantillonnage. En outre, une analyse basée sur les caractéristiques a été menée pour déterminer si la nature de la plante (ligneuse ou herbacée) et le statut d'indigène (indigène, alien ou cultivar) étaient influencés différemment selon les paysages et au cours des saisons.

Pour ce faire, plusieurs tests ont été appliqués, notamment un regroupement de K-means associé à une ACP pour déterminer les classes de paysage, une ordonnée NMDS suivie d'une analyse multivariée de la variance basée sur la permutation (PERMANOVA) et une analyse par paires multiniveaux post-hoc pour évaluer les différences dans la composition du pollen. Ensuite, la diversité a été évaluée par une ANOVA mixte à deux voies et les indices de Hill le long des courbes de raréfaction et d'extrapolation. Enfin, l'analyse des traits a été basée sur un test G d'indépendance pour la contingence, suivi de comparaisons par paires post-hoc.

Il a été démontré que le paysage explique des variations mineures dans la composition des plantes butinées par les abeilles. En revanche, la richesse des espèces, la diversité du pollen et la composition des plantes montrent une forte dépendance aux saisons. En ce qui concerne la composition taxonomique, les Fabaceae, Rosaceae, Brassicaceae, Plantaginaceae et Onagraceae représentent les familles dont les observations sont les plus fréquentes dans tous les échantillons combinés.

La présente étude contribue à une meilleure compréhension de l'écologie et des préférences florales des abeilles domestiques sur lesquelles l'urbanisme peut s'appuyer pour promouvoir la biodiversité dans les villes.

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1 Introduction

1.1 Pollinator decline and its impacts

Between 1 and 10% of biodiversity is lost every decade (Abrol (2012)), resulting mainly from ecological niche loss, landscape degradation, invasion of non-native pests, pollution, over-harvesting, diseases and climate change (Wilcove et al. (1998); Potts et al. (2010); Hoffmann et al. (2010); Dirzo et al. (2014); Goulson et al. (2015)). In addition to directly impacting ecosystems, it affects severely vital ecosystem services, such as the pollination (Kluser and Peduzzi (2007)). Despite shifts of pollinator populations remain poorly documented, especially for wild species, experts agree on the reliability of a collapse (Vanbergen et al. (2013); Lever et al. (2014); Goulson et al. (2015)). Indirect assignments of pollinator loss are issued from communities studies along gradients of agricultural intensification and habitat fragmentation as temporal predictors of change (Larsen et al. (2005); Winfree et al. (2008)). Local-scale researches report a global decline in pollinator richness and abundance. Since most of the world's natural landscapes have been altered by human disturbances, it is likely that this finding can be broadened in many regions of the world (Potts et al. (2010)).

The scientific consensus agrees that the pollinator decline results from multiple interacting drivers, including: agricultural intensification with the continuous use of agrochemicals (e.g.: neonicotinoids) (Barnett et al. (2007); Desneux et al. (2007); van der Sluijs et al. (2015)); the extensive land use as well as the fragmentation and degradation of habitats through agriculture and urbanisation (Rathcke and Jules (1993); Steffan-Dewenter et al. (2005); Kremen et al. (2007); Garibaldi et al. (2011)); increasing parasitism (e.g viruses,mites) (Le Conte et al. (2010); Cameron et al. (2011); Evison et al. (2012)); invasion of alien plants or animals with their collateral consequences (Goulson (2003); Singh et al. (2010); Goulson and Sparrow (2009); Pyšek et al. (2012)); and impacts of global and local climate changes (Memmott et al. (2007); Schweiger et al. (2008); Forister et al. (2010)). This loss will have serious, direct impacts on both wild and crop flowers.

Biotic pollination is an essential regulating, supporting and cultural ecosystem service through interactions between the fauna and the flora (Kevan and Menzel (2012); Chagnon et al. (2015)). It is performed by a wide diversity of animals, mainly insects (honeybees, bumblebees, solitary bees, butterflies, wasps, ...) but also some vertebrates such as bats, squirrels, hummingbirds, some primates and humans with hand pollination (Allen-Wardell et al. (1998); Klein et al. (2007)). 87 of humanity's main food crops depend on the animal pollination (Klein et al. (2007)), including vegetables, fruits, nuts, edible oils and proteinaceous crops, spices and condiments (Maxim and van der Sluijs (2013)). Although these crops account for only 35% of global production (Klein et al. (2007)), their production value per ton has been estimated to be five times higher than for the crops dependent on abiotic pollination (Gallai et al. (2009)).
The wild bees play a significant role in the pollination of a wide range of crops (Morandin and Winston (2005); Winfree et al. (2008)). Moreover, in many countries, the domesticated bees are

used in the agriculture to supplement the local pollinators (Olmstead and Wooten (1987); Robinson (1989)). Therefore, the bees are considered as the largest and most economically valuable group of pollinators in most parts of the world (Kluser et al. (2010)). Gallai et al. (2009) estimates the total economic value of insect pollination to 153 billion euros. Furthermore, in addition to this aspect, there is also a whole ecological issue. The loss of pollinators would lead to a decline in the associated biodiversity by losing the mutualism pollinator-plant interaction with potential trophic cascades. As a final note, van der Sluijs and Vaage (2016) stated that "pollinator decline is an issue that is characterised by complexity, deep uncertainty, high stakes and urgency".

1.2 Honeybees (*Apis* spp.)

1.2.1. Description

Throughout the world two social domesticated species (*Apis* spp.) are commonly used : *Apis mellifera* L. (European honey bee) and *Apis cerana* L. (Asiatic honey bee). Thanks to their morphological adaptations and behaviours, they become the key pollinator of agriculture and horticulture (Abrol (2012)). The honeybees are easily manageable, in contrast with solitary bees, bumblebees or other pollinators (Aizen et al. (2008); VanEngelsdorp and Meixner (2010)). The beekeeper can influence the behaviours of the colony by modifying the reward system of the plant and/or nectar and pollen storage in the unsealed brood (Free (1965c,b,a, 1967); Cale (1968); Barker (1971); Al-Tikrity et al. (1972)). The hives can be relocated depending on the pollination needs (Jay (1986); Mardan (1995)).

In comparison with other pollinators, honeybees are more persistent throughout the seasons and have a greater foraging force given the high number of individuals in a colony. Therefore, they can accumulate more working hours in total (Abrol (2012); Mahmood et al. (2017)). In fact, the number of potential foragers is estimated to 25-30% of the colony, so around 10 000 foragers in a strong colony. However, not all forage constantly (van der Steen (2015)). Furthermore, the colony benefits from an effective communication system for locating and collecting food. Another advantage is the size of their body and proboscis length which allow them to forage a wide range of floral hosts (McGregor (1976); Free (1993); Sihag and Mishra (1995)).

1.2.2. Foraging behaviour

The colony has a complex communication system allowing its workers to locate and collect food. It works as a dynamic system collecting information from an environment and adapting its behaviour consequently (Stephens and Krebs (1986); Tereshko and Loengarov (2005)). Once back at the hive, the scouts recruit foragers by communicating, through the waggle dance, the distance and the direction of resources regarding to the sun (von Frisch (1965)). After foraging a specific plant specie, the *Apis* forager continues as long as this resource is not depleted. This action is defined as the floral fidelity or constancy which is essential for the cross-pollination success (Wells and Wells (1983); Waser (1986)). The resources (nectar, pollen, water) being not constant and reliable along the year, the colony must balance its food supply between present

consumption and reserve for the future. To this end, negative feedbacks are extremely essential for the colony's foraging process. Moreover, to counter the resource availability, colonies maintains a strong deployable workforce to fully benefit of the abundance period. The workers can extend their search radius significantly in order to find suitable resources (Seeley (1995)). According to several researches (Visscher and Seeley (1982); Roubik (1989); Wenner et al. (1991); Seeley (1995)), characteristic distances can be assumed and are listed in the table 1. However, it is clear that these distances are proper to the experimental conditions, but they give a good first indication. Steffan-Dewenter and Kuhn (2003) observed that the foraging distance is influenced significantly by the complexity of landscapes expressed by the proportion of arable land and semi-natural habitats and mean patch area.

Table 1 – Characteristic distance to forage site [km] from Visscher and Seeley (1982), Roubik (1989), Wenner et al. (1991) and Seeley (1995)

.

modal distance	0.7
median distance	1.6
mean distance	2.2
maximum distance	10.9
95^{th} percentile distance	6.0

Beekman et al. (2004) discovered that the mean of foraging distances differ between small (6000 bees) and large (20 000 bees) colonies but also according to the months considered. In fact, small and large colonies foraged a comparable distance in July when resources was abundant, while in August large colonies flied much further as the floral offer became scarcer. Pollen and nectar production is intense for most of the floral plants during only a portion of day (Southwick (1983). Therefore, the recruitment priorities of the colony vary considerably between the morning and the afternoon. Domroese and Johnson (2017) and Grabowski and Wilde (2000) have reported that efficiency of worker's flight is higher during the morning and reach its maximum at midday before starting to decrease. Thus, the scouting distance of colonies from a same region is influenced by the colony strength, the availability of food supply, the weather, the month and period of the day.

A colony will cease collecting nectar once its combs are filled of honey, while only a low reserve of pollen is gathered (Jeffree and Allen (1957); Fewell and Winston (1992)). In addition of being a source of lipids, vitamins and minerals, pollen provides the primary protein supply for honeybee (Haydak (1970)). Approximately between 15 to 30 kg of pollen is collected annually from which nearly all is consumed (Avni et al. (2009, 2014)). The colony uses proteins for brood production principally during the summer (Seeley (1995)). The related quantities of larval brood and stored pollen is highly correlated with the colony's pollen needs (Dreller and Tarpy (2000); Weidenmüller and Tautz (2002)). Therefore, the sustainability of the colony is highly dependent on the pollen

gathered (Smart et al. (2016)). Furthermore, not only the quantity is important but also the diversity and quality of the pollen is necessary for the well being of bees (Käpylä (1974); Wilde et al. (2003); Alaux et al. (2010); Di Pasquale et al. (2013); Danner et al. (2017)). The nutritional quality of pollen has been correlated to honey bee physiology, worker longevity and parasite tolerance (Di Pasquale et al. (2013); Wang et al. (2014)).

1.2.3. Coexistence with native pollinators

The mass introduction of domesticated honeybees may impact negatively the interactions between plants and native species causing disruptions in ecosystem functioning (Thomson (2006)). The competition and pathogen spread appear as the most disruptive mechanisms (Graystock et al. (2016); Geslin et al. (2017)).

The exploitative competition with native pollinators for limited resources may lead to a reduction of the fitness of at least one of the interacting species (Roubik (1978); Sugden and Pyke (1991); Stout and Morales (2009); Henry and Rodet (2018)). To ensure the best yield, beekeepers boosted their hives by providing sugar during the low nectar flows, which gives no chance to wild pollinators. As an example, Thomson (2004) noticed that colonies of *Bombus occidentalis* suffered from increased lack of nectar while they competed with *Apis* spp. To offset this deficiency, they redeploy more workforce from the pollen to the nectar collection, leading to a decrease in progeny production due to lack of pollen. Thus, Apis spp. may threaten the existence of important pollinators with potential cascading effects on the related endemic plant communities (Gross and MacKay (1998); Gatoria et al. (2000); Paini (2004)). Similarly, *Apis mellifera* replaced *Apis cerana japonica* in China and Japan and *Apis cerana indica* in Indian subcontinent (Sakagami (1959); Sakai (1992); Abrol (2012)).

Introduced alien pollinators can host parasites potentially harmful to endemic populations of honeybees or wild bees (Allen et al. (1990); Saville (2000); Goulson (2003); Woolhouse et al. (2005)). This is especially the case for viruses, such as the deformed wing virus DWV (Ribière et al. (2008)), which can infect different host species (Eyer et al. (2009)). Thus, it is more likely they can be transmitted to wild bees and vice versa.

Furthermore, some studies reviewed how honeybees may disrupt mutualism between native plants and pollinators (Kearns et al. (1998); Dohzono and Yokoyama (2010); Watts et al. (2012); Traveset and Richardson (2014)). The pollination to be efficient needs a concordance between the flower and the pollinator morphology in order to transfer the pollen (Burd (1994)). Collecting the floral rewards without proper transfer of pollen is synonym of floral parasitism (McDade and Kinsman (1980)). Observations support that introduced honeybee can lower the reproductive success of native plants by stealing nectar or pollen (Kenta et al. (2007); Hargreaves et al. (2009)) or by physically damaging the flower (Dohzono and Yokoyama (2010)). From Australian and American data set, Butz Huryn (1997) concluded that honeybees perform an efficient pollination for most of native plants, although they operate as a floral parasites for few species.

Finally, some researches pointed out that the introduction of non-native honeybees may promote the spread of invasive or exotic species (e.g. Stimec and Scott-Dupree, CD McAndrews (1997); Stout et al. (2002); Montalva et al. (2011)). On the other hand, opposite observations have also been reported. For example, Sanguinetti and Singer (2014) found in Argentina that *Bombus terrestris* (L.) ensures a better reproductive success to an indigenous orchid by increasing the number of visits compared with what the native *Bombus dahlbomii* (Guérin-Méneville) does. Therefore, this point requires thorough investigations.

1.3 Urban beekeeping

Urbanization is one of the major causes of habitat segmentation and related biodiversity losses (Concepción et al. (2015); Geslin et al. (2016)). However, rural areas no longer represent optimal habitats for pollinators due to landscape homogenisation, loss of habitats and excessive use of pesticides which reduce floral resources diversity (Ollerton et al. (2014); Banaszak-Cibicka et al. (2016); Kaluza et al. (2016)). In this regard, observations from recent studies (Samuelson et al. (2018); Theodorou et al. (2020)) support that Hymnoptera, in particular bees, can thrive more in urban than in rural areas.

In addition to the benefits of the pollination, beekeeping offers an activity that can generate profits from the sale of the byproducts (honey, wax, ...) at a local scale. Moreover, it can also bring a social aspect by involving the population into environmental education, but also with the need of integrate stakeholders into projects. This cooperation between the different actors allows the emergence of new interactions and the promotion of more sustainable activities with respect to the environment.

Lately, some cities want to put in place some actions in order to preserve and promote the biodiversity. *Apis mellifera* embodies the biodiversity for the general public due to campaigns and public policies that tend to focus on the introduction of its colonies. Consequently, urban beekeeping has become more and more popular, leading to a surge of *Apis mellifera* colony density in the cities (Geslin et al. (2013); FAO (2018)). However, the floral resources availability needs to be sufficient enough to host both domesticated honeybees and the local wild pollinators. The percentage of impervious surfaces plays a major role in the pollinating biodiversity (Geslin et al. (2016)). Therefore, on one side, populations of managed honeybees must be regulated so that wild pollinator populations are not adversely affected (Geslin et al. (2017); Mallinger et al. (2017)). On the other side, green areas must be managed and well distributed to meet the demands of the pollinator community (Blackmore and Goulson (2014)). Garbuzov et al. (2015) identified, in a city of UK, that among the ornamental flowers only 33% were attractive to insect pollinators. Many flower beds managed in the cities are not sources of pollen. Thus, in order to promote biodiversity in cities, it is essential that the greening projects examine the appropriate floral composition by favouring diverse honey-bearing species.

To summary, well-managed cities could improve the preservation of hymenoptera and thus provide hot spots for pollination services. To do so, decision-makers should focus on regulating the introduction of honeybees (selection of native bee species, colony density, control of pathogens and parasites) and on the availability of resources (proportion of impermeable surface area, melliferous plant species, landscape diversity) (Mallinger et al. (2017); Geslin et al. (2017)).

1.4 Pollen analysis

The mutualistic interactions between pollinators and plants are essential for preserving the proper functioning of ecosystems. To assess these interactions several methods are used : observation of the frequency of pollinator visit; mark recapture, agent-based foraging models, digital tracking systems, chemical signatures, genetic sequencing and light microscopy (Cornman et al. (2015)). The methods that analyses the pollen collected from pollinators are more relevant to study the interactions on a global scale. Traditionally, the pollen present in the honey is analysed through light microscopy following dichotomous keys (Cornman et al. (2015)). It is a laborious and time-consuming process, called melissopalynology (De França Alves and De Assis Ribeiro DosSantos (2014)), requiring an expert eye in pollen identification and resulting in a low taxonomic resolution, usually to family rank (Kaškoniene and Venskutonis (2010); Keller et al. (2014); Bell et al. (2016); Danner et al. (2017). However, it remains a useful diagnostic tool when combined with other techniques (Hawkins et al. (2015)).

Another method relies on the chemical composition of the pollen in aroma compounds, free amino acids or minerals and trace elements (Conti et al. (2016)). Pollen quality, in regards to amino acid profile and total protein content, varies significantly depending on the floral origin (Auclair and Jamieson (1948); Roulston et al. (2000); Cotte et al. (2004)). Nevertheless, it involved advanced and expensive equipment (Hermosín et al. (2003); Fernández-Torres et al. (2005)) and still result in little information on the plant species of honey samples (Laha et al. (2017)).

Over the last two decades, the sequencing of taxonomic "barcode" genetic loci has become a robust approach to taxonomic identification. DNA barcoding described the sequencing of a standardized barcode marker that reveals intra-species identity and interspecies variability (Hebert et al. (2003); Borisenko et al. (2009). Thanks to innovative high-throughput sequencing, the metabarcoding analysis is able to classify pollen collected from bees thanks to chloroplast (rbcL,matK, and trnH-psbA) and nuclear ribosomal (ITS region) barcoding markers (Keller et al. (2014); Kraaijeveld et al. (2014); Richardson et al. (2015b); Bell et al. (2016)). In theory, a single operation can quickly define taxonomic profiles for dozens of pollen samples at the milligram scale (Cornman et al. (2015)). DNA metabarcoding provides faster and higher taxonomic resolution of pollen in comparison to traditional approaches (Bell et al. (2017)).

Nevertheless, while qualitative assessment of species composition in the pollen load mix is largely correct, its robustness of quantitative analysis remains controversial (Lamb et al. (2019); Piñol et al. (2019). In fact, recent studies have tried to quantify the different components of the pollen load mix carried by pollinators. Most of them have reported a poor correlation between the number of sequence reads and visually identified pollen grains (Kraaijeveld et al. (2014); Keller et al. (2014); Richardson et al. (2015b)). Bell et al. (2018) stated that the metabarcoding method is globally reliable in the assessment of pollen presence/absence but, given current knowledge, the number of reads should not be used to predict relative abundance of specie's pollen. In contrast, up to-date, four studies have succeeded in quantifying pollen for certain plant species using the "trnL" marker (Kraaijeveld et al. (2014); Pornon et al. (2016); Richardson et al. (2018); Baksay et al. (2020)). Moreover, lately, Baksay et al. (2020) concluded that the robustness of the correlation between the number of sequence reads and visually identified pollen grains depends on pollen counting methodology, the marker choice, plant samples and the number of PCR cycles. Despite, this technique still need investigations and improvements, it it is clear that it will allow strong progress in studying plant-pollinator interactions and the floral preferences of pollinating insects.

1.5 Objectives

Better understandings of ecology in the cities are essential for nature resource management, preservation and tackling environmental issues in urban areas. The urban matrix is highly fragmented with usually small, remote and intensely maintained green spaces (Bastin and Thomas (1999)). Consequently, the foraging behaviour of pollinators must adapt in order to ensure that sufficient net energy gain is maintained (Fontaine et al. (2006)). The foraging distance with the quantity and diversity of pollen may be influenced. However, studies on this last point remain sparse. Danner et al. (2017) found no influence of the agricultural landscape diversity in the pollen pollen amount and diversity. As the honeybees rely on a broad variety of flowers, it is suggested that they could counterbalance lower landscape diversity where resources are poor by enlarging their foraging area (Steffan-Dewenter and Kuhn (2003); Danner et al. (2016)).

However, pollen availability is not only dependent on landscape diversity but is also sensitive to seasonal shifts. As colonies' pollen demands also differ seasonally, the foraged flower types could vary over time. Danner et al. (2017) discovered a strong seasonal effect on species richness. In fact, April and the first half of May had significantly lower number of species than the end of May, June, July. Furthermore, they observed differences in the amount of collected pollen among the seasons, with April and May being the most abundant. This could be explained by higher protein requirements for the brood production in the colony development during the spring. While June reported low supply, matching with the shortage period, also documented by Requier et al. (2015). More recently, Sponsler et al. (2020) reported also seasonal variations in the traits composition of the flower. The spring was dominated by trees and shrubs, the summer presented more herbaceous species, while woody vines characterized fall samples.

Part of this continuum, the present study analyses the pollen foraged by honeybees from different locations along a urban-rural gradient across the season in order to answer the following questions:

— Are the species richness and diversity determined through the pollen analysis influenced by the landscape type and/or the month period ?
— How the floral composition and its traits differ between the different landscapes and over the course of the seasons ?

In the end, the results may assist in the selection of appropriate plant composition in urban planning to promote honeybees and the biodiversity. The understanding of which flowers are favoured by honeybees and their distribution and availability in cities can support stakeholder decisions in promoting green spaces. This research is willing to provide further knowledge for urban planning to ensure the preservation and development of biodiversity in cities.

2 Material and methods

2.1 Study area and experimental set-up

The study carried out 18 different locations distributed in the Kantō region of Japan (Figure 1). As it can be observed in the table 2, the meteorological conditions and altitude are homogeneous between the different sites. Therefore, these variables can be interpreted as constant among the site as they should not explain a significant proportion of the variability in the observations. 18 observations hives were used, one per site, owned by different beekeepers collaborating with the project.

Theoretically, pollen samples were supposed to be collected once a month with a constant interval from March to September. However, the sampling being sensitive to the weather conditions and the personal schedule of beekeepers, the sampling date, collection frequency and operational time varied from site to site. For the subsequent analysis, the sampling dates have been discreetly grouped by month period. The appendix A shows the details of the sampling among the sites. In order to determine if the data can be treated independently of the sampling length, the Spearman's Rank-Order correlation was tested between the number of species per sample and the sampling length in hours. Pollen traps were placed at the entrance of the hive for several hours in order to collect the pollen balls from the bee's legs (Figure 2 and 3). Although multiple designs of pollen trap have been developed, the basis remains identical i.e. a bee size mesh to remove the pollen from the bees and a tray to collect the pollen (Mahmood et al. (2017)). Then, the contents of the pollen traps were discharged into 50 ml conical tubes, referenced with the date and the location. The samples from the different beekeepers were received every month and stored at -20°C. Finally, all the samples were sent to the private company Bioengineering Lab. Co., Ltd. (https://www.gikenbio.com/, consulted on 20/07/2020) which realises the DNA pollen metabarcoding analysis.

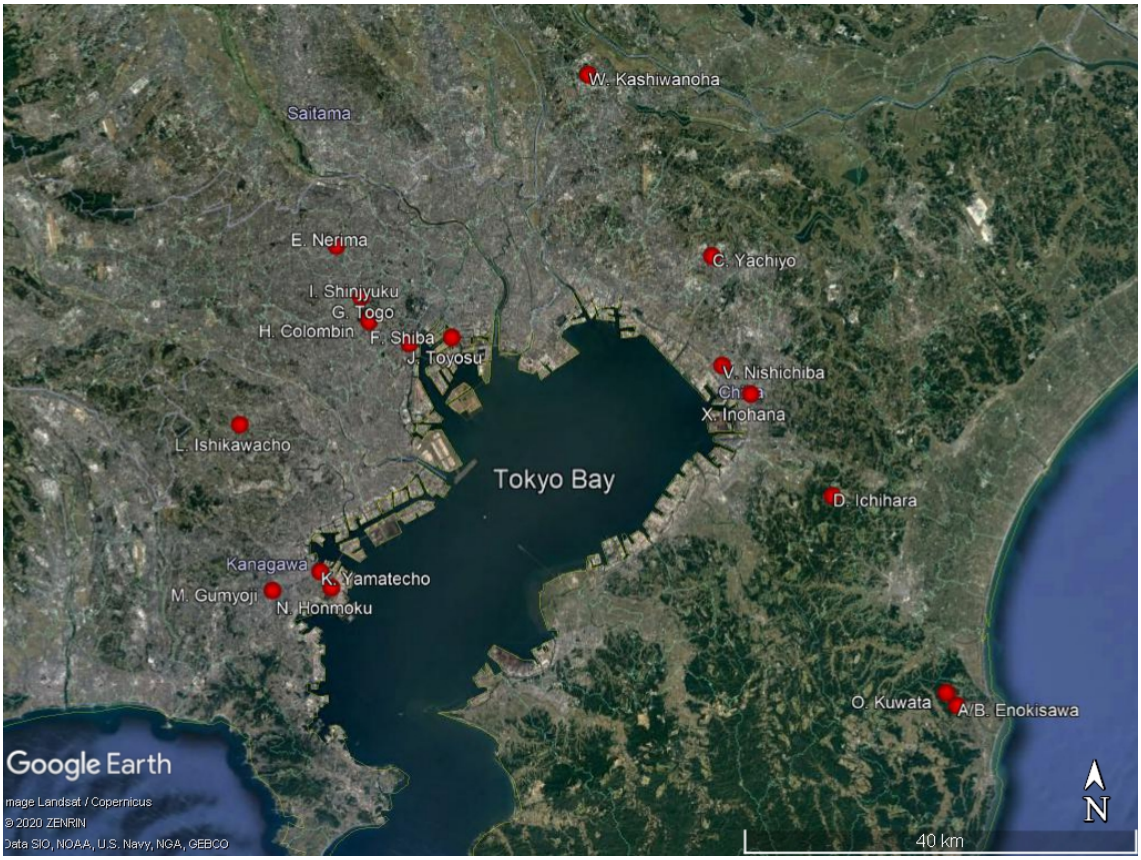


Figure 1 – Study sites distributed along the Tokyo bay in the Kantō region, Japan (maps issued from ©Google satellite images). Red dots represents the location of the beehives

Figure 2 – Pollen trap at the entrance of a hive *(location: Nishi-Chiba campus Chiba University, 25 March 2020)*

Figure 3 – Pollen trap close-up with pollen balls collected in the trail *(location: Nishi-Chiba campus Chiba University, 17 June 2020)*

Table 2 – Characteristics of the different locations

ID	Location	Prefecture	Elevation [m]	Mean temperature [°C]	Precipitation [mm]
A-B	Enokisawa	Chiba	14	15.6	1428
O	Kuwata	Chiba	23	15.6	1428
C	Yachiyo	Chiba	31	14.9	1394
D	Ichihara	Chiba	36	15.5	1550
E	Nerima	Tokyo	38	15.1	1448
F	Shiba	Tokyo	6	15.4	1442
G	Togo	Tokyo	29	15.4	1442
H	Colombin	Tokyo	24	15.4	1442
I	Shinjyuku	Tokyo	32	15.4	1442
J	Toyosu	Tokyo	6	15.4	1442
K	Yamatecho	Kanagawa	23	15.6	1554
L	Ishikawacho	Kanagawa	29	14.7	1488
M	Gumyoji	Kanagawa	13	15.6	1554
N	Honmoku	Kanagawa	12	15.6	1554
V	Nishichiba	Chiba	17	15.3	1435
W	Kashiwanoha	Chiba	19	14.7	1358
X	Inohana	Chiba	17	15.3	1435

[1] issued from ©Google Earth Pro

[2] from ©Climate-Data.org (consulted 16/07/2020)

2.2 DNA isolation, sequencing and bioinformatics

The use of DNA metabarcoding to characterize interactions between plant and pollinators alleviates some of the limitations associated with traditional methods such as the need of an expert eye, time consuming and the cost (Pornon et al. (2016)). Moreover, it allows to identify bigger pollen samples and provides the researchers a greater taxonomic resolution. Therefore, DNA metabarcoding is considered as a better approach to analyse the relationship between bees and the surrounding environment. For these reasons, the present study decided to use this method even though technical advances are still needed to make it more accurate, consistent and quantitative (Bell et al. (2016))

DNA extraction

At first, the pollen were lyophilized using a Lyophilizer Freeze dryer VD-250R (TAITEC, Koshigaya, Saitama, Japan). After being ground at 1500 rpm for 2 min using a ShakeMaster NEO homogenizer (bms, Shinjyuku, Tokyo, Japan), DNA extraction was realised with the kit MPure Bacterial DNA Extraction Kit(MP Biomedicals, Irvine, CA, USA). Then a purification with a MPure-12 Automated Nucleic Acid Purification System (MP Biomedicals, Irvine, CA, USA) was applied. Concentration of extracted DNA solutions were measured using Synergy H1 (BioTek, Winooski, VT) and QuantiFluor dsDNA System (Promega, Madison, WI, USA).

Library preparation and sequencing

Libraries were produced from a 2-step tailed Polymerase Chain Reaction (PCR) method. The first PCR analysis was conducted with ITS primers coupled with MiSeq-specific adapters and Illumina index sequences, and the second was conducted with index primers. Even if plastid intron such as trnL demonstrated a higher reliability in the quantitative analysis of mixed pollen samples when compared to internal transcribed spacer (ITS) of the nuclear ribosomal locus (Richardson et al. (2015a); Baksay et al. (2020), ITS markers are strongly divergent and allow a relatively high taxonomic resolution (Chen et al. (2010); Wang et al. (2015)).
PCR reactions were carried out in a reaction volume of 10μl containing 10XEx Buffer 1.0 μl, nucleoside triphosphate dNTPs (each 2.5mM) 0.8 μl, 0.5μl for both Forward and Reverse primer at a concentration of 10μM, Template DNA※1(max0.5ng/μl) 2.0μl, DNA polymerase ExTaq (TaKaRa, Otsu, Shiga, Japan)(5U/μl) 0.1μl and Double-distilled water DDW 5.1μl. PCR programs were proceeded as followed : 2 min denaturation at 95°C; followed by 30 then 10 cycles (30s denaturation at 95 °C, 30s annealing at 57 °C, 30s elongation at 72 °C) and a final elongation at 72°C for 5 min. At the end of each PCR, the products were purified using AMPure XP(Beckman Coulter, Brea, CA, USA) (the amount of AMPure added equal PCR solution volume x 0.7). Library concentrations were determined with a Synergy H1 microplate reader (BioTek, Winooski, VT) and a QuantiFluor dsDNA System (Promega), and library quality was evaluated by using a Fragment Analyzer (Advanced Analytical Technologies, Ankeny, IA, USA) with a dsDNA 915 Reagent Kit (Agilent, Santa Clara, CA, USA). The generated library was then sequenced using the MiSeq Illumina technology (Illumina, San Diego, CA, USA) through 2 × 300 paired-end run.

Data analysis

"FASTX Barcode Splitter" [1] from Fastx toolkit, a short-reads pre-processing tools, was used to extract only the Miseq reads with sequences readings matching exactly the primers used. Next, the reads were clipped and filtered using the Sickle tool [2] with a quality value of 20; then, trimmed reads and paired-end reads with fewer than 150 bases were discarded. The remaining reads were merged by using the FLASH (version 1.2.11) paired-end merge script (Magoč and Salzberg (2011)) under the following conditions: fragment length after merge, 420 bases; read fragment length, 280 bases; and minimum overlap length, 10 bases.The paired readings were then aligned with the reference sequences using the open-source bioinformatics pipeline Qiime 2.0. (Bolyen et al. (2019)). A Qiime workflow script “pick_de_novo.py” (Caporaso et al. (2010)), with the default parameter values, was used for OTU creation and taxonomic assignments. RDP classifier was used for taxonomic assignments with the output sequence.

Following alignments, further filtering of the raw data were applied in R (R Core Team (2020)). First, all Operational taxonomic units (OTUs) assignation below the identity thresholds of 97% were discarded (Danner et al. (2017); Smart et al. (2017)). Next, in the same way as Sponsler et al.

1. A. Gordon et al. (2008). FASTX-Toolkit: FASTQ/A Barcode splitter (Version 0.0.13) Available at http://hannonlab.cshl.edu/fastx_toolkit/download.html

2. Joshi NA, Fass JN. (2011). Sickle: A sliding-window, adaptive, quality-based trimming tool for FastQ files (Version 1.33) [Software]. Available at https://github.com/najoshi/sickle.

(2020) did, the number of reads were sorted by genus and by sample (i.e. site and date) and was then expressed for each genus as the ratio between the read counts and the sum of number of reads per sample. Genera accounting less than 0.05% of the total number of readings for a single sample were excluded to prevent low false positives. Moreover, a sample was removed if it accounts less than 1000 reads to limit inferences from insufficient sequencing depth.

2.3 Landscape characterization

Landscape structure was investigated within a 6 km radius around the hive's location (table 1) using remote sensing. This study used 3-m pixel resolution multi-spectral images (RGB, NIR) from ©Planet Labs Inc. With approximately 130 satellites, PlanetScope constellation takes daily images of the entire land surface of the Earth. In order to fully exploit the whole potential of the data, the cloud cover condition was set to maximum 5%. However, as Japan is most of the time cloudy, the possibilities of adequate images were limited. In the end, the images from the different locations are issued from either April, May or June. It is believed that this heterogeneity would not have significant impact in the further analysis.

As report by Miller et al. (2019), Planet data are relevant for computing and mapping high resolution terrestrial above-ground vegetation at the landscape scale. For each Planet image, the normalized difference vegetation index (NDVI, eq. 1) was computed using the Red and Near-InfraRed bands. The chlorophyll pigment of a plant in good conditions absorbs the majority of the visible red light and reflects most of the near-infrared light. Therefore, a dense vegetation with high photosynthetic activity has higher reflectance in the NIR and low one in the red band.

$$NDVI = \frac{NIR - RED}{NIR + RED} \tag{1}$$

Where:

NIR : spectral reflectance measured in the near infrared waveband
RED : spectral reflectance in the red waveband

Based on this principle, band rationing allows to distinguish vegetation cover from other type of land cover (Xue and Su (2017)). Classes were created with the function reclassify from the R package 'Raster' (Hijmans et al. (2020)) by defining NDVI threshold value to 0.2 in order to distinguish the non vegetation (NDVI: -1-0.199) from the vegetation (NDVI: 0.2-1) (Taufik et al. (2016); Hashim et al. (2019)). Then, a majority filter, with 6x6 filter kernel size, from the R package 'Whitebox' (Lindsay (2016)) was applied in order to smooth the result and accumulate regions of high uncertainty.

Separate landscape classifications were performed using as inputs demographic data [3] and landscape metrics from the 'lconnect' and 'landscapemetrics' R packages (Mestre and Silva (2019); Hesselbarth et al. (2019)). The landscape matrix along the urban-rural gradient should differ in terms of landscape diversity, connectivity and aggregation between the patches (Bastin and Thomas (1999); Hadley and Betts (2012)). Based on these assumptions, the retained landscape metrics were: Integral index of connectivity (Saura and Pascual-Hortal (2007)); effective mesh size (Spanowicz and Jaeger (2019)); Shannons's evenness index (Danner et al. (2017)); vegetation cover proportion; patch density (Threlfall et al. (2015)); and median of vegetation class's NDVI.

$$IIC = \frac{\sum_{i=1}^{n} \sum_{j=1}^{n} \frac{a_i . a_j}{1+nl_{ij}}}{A_L^2} \tag{2}$$

Where [4]:

n : total number of patches
a_i : area of each habitat patch
nl_{ij} : number of links in the shortest distance between patches i and j
A_L : total landscape area

The Integral index of connectivity is derived from habitat availability and a binary binary link model, in opposition to a probabilistic. On one hand, for non-connected patches the sum's numerator is null as $nl_{ij} = \infty$. On the other hand, for i = j then $nl_{ij} = 0$ as no link required to reach itself. IIC increase with the connectivity between the patches within a landscape [0:1]. Therefore, IIC=1 corresponds to the case where only one habitat occupied the whole landscape (Pascual-Hortal and Saura (2006)).

$$MESH = \frac{1}{A_L} \sum_{i=1}^{n} a_i^2 \tag{3}$$

The effective mesh size assesses the probability that two randomly selected points in a region are linked and thus part of the same patch (Jaeger (2000)). The lower is the effective mesh size, the more fragmented the landscape is. In other words, the higher it is, the bigger the area covered by this class is.

3. ©人口・面積・人口密度, 昼夜間人口比率ランキング　全都道府県市区町村　平成22年国勢調査 , http://demography.blog.fc2.com/blog-entry-6677.html (consulted on 28/07/2020)
4. Note that only variables not yet mentioned previously are precised for every equation

$$SHEI = \frac{-\sum_{i=1}^{n}(P_i . \ln P_i)}{\ln n} \tag{4}$$

Where:

P_i : proportion of class i

Shannon evenness index gives information on the diversity of a landscape. It corresponds to the ratio of the actual Shannon's diversity index for the patch type i to its maximum value. SHDI equal to zero when there is only patch in the landscape (no diversity) and tends to 0 as the patches' classes are becoming more even, dominated by one class. In opposition, classes with same proportional abundances shows a SHEI equal to 1.

$$Pd = \frac{n}{A_L} \tag{5}$$

The patch density Pd describes the fragmentation of the landscape. Maximum when each cell of the raster is a different patch.

$$P_{A_1} = \frac{A_L - \sum_{i=0}^{k} pixel_i}{A_L} \tag{6}$$

Where:

$pixel_0$: pixel having a NDVI <0.2

The vegetation cover P_{A_1} corresponds to the proportion of area showing a NDVI superior than 0.2 relative the total area of the study site.

$$A_1 = \frac{1 - A_0}{A_L} \tag{7}$$

Where:

A_0 : non vegetation area characterized by a NDVI<0.2

The unsupervised clustering method of K-means was applied in order to identify which sites are similar, and potentially categorize into k groups to establish an urban-rural gradient. Before initiating the analysis, the data was standardized using the R function "*scale*", to make variables comparable and so, the clustering algorithm independent of any variable unit. The number of k groups required to be defined as a first step was determined by the elbow method (Kodinariya and Makwana (2013)). The method consists in plotting the total within-cluster sum of square in function of each k group. The location of the bend in the plot can be interpreted as the adequate

number of clusters. The clustering K-means analysis was proceeded using the "*kmeans*" function and the R package 'factoextra' for the graphical representations (Kassambara and Fabian (2020)). First, the algorithm selects k objects from the observations as the initial centroids. Then, the remaining observations are assigned to their closest center on the basis the Euclidean distance. The "*kmeans*" function offers an nstart option that performs several initial configuration attempts and only retains the best one. As recommended (Strickland (2014)), nstart equal to 25 was added to the "*kmeans*" function. After this assignment step, the algorithm updates each groups centers. Iteratively, the total within sum of square, which measures the compactness of the clustering, is minimized until the convergence is achieved or the maximum number of iterations is reached. At the end, all the study sites are classified into the k distinct groups.

2.4 Taxonomic analyses

Given the poor correlation between the biomass collected during the sampling and the sequencing read proportions (Lamb et al. (2019)), the analyses undertaken are based exclusively on incidence based approaches. In other words, the results issued from the data constructed from barcoding are based on presence/absence binary arrays. This process prevents misinterpretation of DNA metabarcoding data.

The taxonomic composition of samples was studied across sites, sampling periods and landscape classes using the Jaccard dissimilarity metric from the R package 'vegan' (Oksanen et al. (2019)). This asymmetric distance coefficient addresses the problem of double zero, which is essential when studying data on community composition along a gradient. Differences in the plant composition between sampling periods and landscape classes were investigated by permutation-based multivariate analysis of variance using the function "*Adonis*" (vegan R package, Oksanen et al. (2019)). Adonis segments a distance matrix between categorical or continuous variables, and evaluates the robustness and significance level of the predictors (Anderson (2001). The significance was measured using 999 permutations. If the PERMANOVA results in significant level, a post-hoc multilevel pairwise analysis with Bonferroni correction was performed using the "*pairwise.adonis()*" function from 'pairwiseAdonis' R package (Martinez Arbizu (2020)). The dissimilarities in plant communities structures were displayed using non-metric multidimensional scaling (NMDS) with 999 permutations. This iterative algorithm approach reshapes multivariate data into important axes to simplify interpretation of patterns and variations between groups. It aims in illustrating, as faithfully as possible, pairwise dissimilarity between components in a low-dimension space. Points that are clustered can be interpreted as more likely similar than those further apart. The stress value defines degree of correspondence between the distances among points into the reduced dimension compared to the complete multidimensional space. Better is the representation as the stress is minimized. Compared to other ordination methods, NMDS can be advantageous when data sets present several different gradients of variance (Minchin (1987)). In fact, in a two dimension ordination plot, with the NDMS method, all data set variance is used to allocate the objects in the low dimensional space, whereas for PCA/CA/PCoA the first two dimensions only exploit partially this variance (Legendre and Legendre (2012); Paliy and Shankar (2016)).

Following, species richness of the samples, i.e. number of distinct species, was analyzed as function of the months and the landscape classes. Differences were assessed by a two-way mixed ANOVA on species richness (response), across independent landscape classes (between subject factors) and along time (within subjects factor). Prior to the test, the data was mathematically root squared transformed to respect the normality (shapiro test) assumption for each combinations of factor levels. Then, the assumptions on homogeneity of variance (Levene's test), homogeneity of covariances (Box's M-test) and the sphericity (Mauchly's test) of the landscape classes were verified at each level of time variable. Finally, a Tukey's post hoc test was applied to analyzed the pairwise differences. The graphics were generated using 'ggplot2' (Wickham (2016)).

The package 'iNEXT' (Chao et al. (2014); Hsieh et al. (2020)) was used to analyze the diversity from the three Hill's numbers of order q = 0, 1, 2 corresponding respectively to species richness (eq.8.a), Shannon diversity (the exponential of Shannon entropy, eq.8.b) and Simpson diversity (the inverse of Simpson index, eq.8.a). Hill's numbers derived from presence/absence data measure the effective number of uniformly frequent (in all samples) in the system (Alberdi and Gilbert (2019)). The order of diversity q adapts the sensivity to frequent and rare OTUs. The higher the order is, the greater are the weights attributed to dominant OTU (Chao et al. (2014)). Therefore, for q=0, rare OTUs are over-represented, q=1 weighs without discriminating rare or most frequent OTUs, while the most frequent OTUs are over-weighed for q=2 (Keylock (2005); Jost (2006)). In the case of incidence data, sample size refers to number of sampling units (here pollen traps).

$$ {}^{q}\Delta = (\sum_{i=1}^{S}[\frac{\pi_i}{\sum_{j=1}^{S}\pi_j}]^{q})^{1/(1-q)} \qquad q \geq 0, q \neq 1 \tag{8a} $$

$$ {}^{1}\Delta = \lim_{q \to 1}{}^{q}\Delta = exp(-\sum_{i=1}^{S}\frac{\pi_i}{\sum_{j=1}^{S}\pi_j}.log\frac{\pi_i}{\sum_{j=1}^{S}\pi_j}) \tag{8b} $$

Where:

$ {}^{q}\Delta $: effective number of equally frequent species in the assemblage
S : number of species
q : the sensitivity to the relative frequencies
π_i : incidence probabilitie of the specie i

To each computed index, a 95% confidence intervals was associated, as well as rarefaction and extrapolation (R/E) curves. Concerning the species richness (q=0), the argument endpoint designating the maximum sample size of the R/E computation was set to not exceed the double of the original sample size. In fact, above this setting, the prediction bias can be significant leading potentially to unreliable results. In contrast, if the data are not scattered, this precaution is not necessary for the two other measures (Hsieh et al. (2016)). In order to realise a sample-size-based

rarefaction and extrapolation, the base sample size was determined following the methodology described by Chao et al. (2014). Moreover, completeness curves from coverage-based rarefaction/extrapolation were generated in order to illustrate the sampling effort required to obtain a given level of sample completeness (Good (1953)). The base size is fixed by taking the minimum size when comparing the maximum reference sample size and the minimum of the double reference sample sizes. The reference sample size is defined by the number of samples in the objects to be compared (here landscape and season). The same procedure is applied to determine the base coverage with coverage instead of sample size. The two parameters allow to perform more robust inferences on the assemblage comparison within the sampling range below them (Chao et al. (2014); Hsieh and Chao (2017)).

2.5 Indicator species and trait-based analysis

Similarity percentage (SIMPER) analysis was conducted with the '*simper*' function from R package 'vegan' (Oksanen et al. (2019)) in order to identify how the taxonomic composition differs from the environmental conditions (landscape type) and the environmental changes (time). This step allowed to determine which sampled species contribute significantly to the dissimilarities among the months and/or landscapes, but also which species are cosmopolitan and are foraged over seasons. The contribution of each species to the dissimilarity between two groups is determined from the Bray-Curtis dissimilarity. When implemented with a binary matrix, this index is none other than Sørensen index.

Finally, to analyze the foraging preferences of honeybees concerning plant's traits, each specie identified in the samples were characterized by its nature, i.e. herbaceous (no woody stems above ground) or woody species(tree, shrub, liana), and its native status, i.e. native, alian, cultivar species. The database has been built up obtaining the information from different database : Ylist (Yonekura and Kajita (2007); and ©Species2000 (Roskov et al. (2019)). To determine if proportion between the different traits varies with season and landscape type, the G–test of independence for contingency table was performed using the R package 'RVAideMemoire' (Hervé (2020)). It is based on Log likelihood ratio and tests if the relative proportions of one categorical variable (here, plant nature or nativity status) are independent of the second categorical variable (season or landscape). Therefore, a reject of the null hypothesis allows to conclude that there is a statistical significant relation between the two variables. Next, a post-hoc pairwise comparisons between pairs of proportions with Bonferroni corrections of the p-values was conducted (MacDonald and Gardner (2000)), using the '*pairwise.G.test*' function from R package 'RVAideMemoire'.

3 Results

3.1 Landscape classification

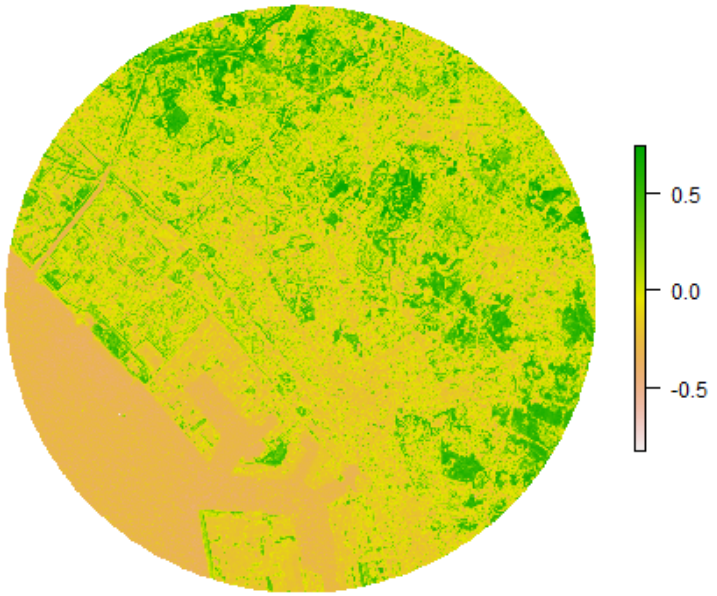
The method to differentiate the vegetation from the impervious surfaces using the NDVI yields to convincing results after cross-visualisation checking, even in complex environment such as the urban matrix. The Figure 4 illustrates an example of the result for one of the study site.

According to the elbow method (Figure 5), the optimal number of k distinct groups is four. Therefore, the K-means clustering method classified the different sites into four clusters based on demographic and landscape metrics (Figure 6). The four different classes represent different landscape environments: rural; suburban; urban; and urban center. As the two first dimensions describe a high percentage of variance, the interpretations can be reliable. In support to table 3, the biplot issued from Principal component analysis (Figure 7) allows to visualize how variables explain the differences between the landscape classes. The urbanized locations are driven by a much higher demographic density compared to the other landscape. Morever, following the decrease in the proportion of vegetation along the rural-urban gradient, it can be assumed that the higher patch density in the cities is induced by the presence of many smaller plots, such as gardens. patches, such as gardens. In contrast, the rural sites testifies of a higher connectivity between the patches. Finally, the suburban landscape really acts as a transitional bridge between rural and metropolitan areas. The detailed results of the principal component analysis are presented in the appendix B.

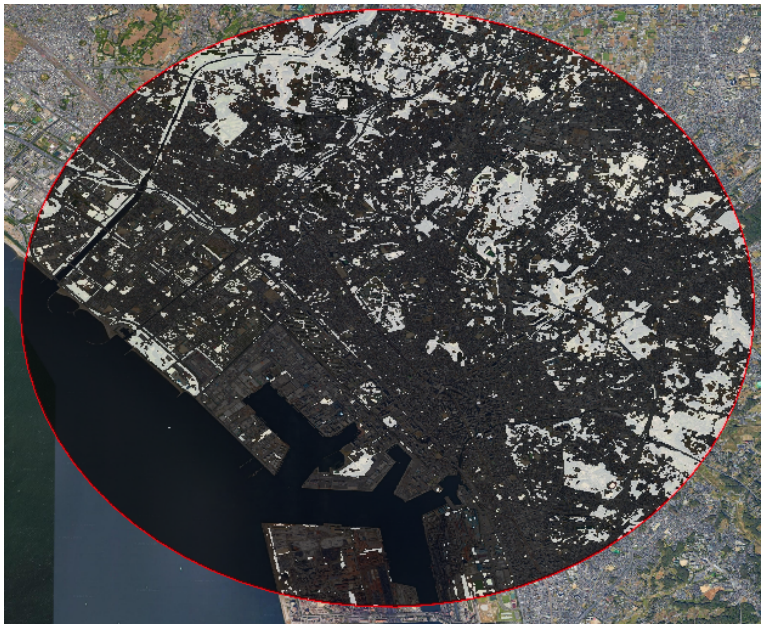
Table 3 – Mean and standard deviation of variables among the landscape classes. The standard deviation is situated between brackets below the mean.

Cluster	**dpop [in./km²]**	**IIC [-]**	**MESH [ha]**	**NDVI median [-]**	**SHEI [-]**	**veg cover (%)**	**Pd [nbr/km^2]**
rural	398 (291)	0.3533 (0.0366)	2414.8 (904.9)	0.49 (0.05)	0.93 (0.05)	0.67 (0.05)	0.000044 (0.00004)
suburban	6475 (4839)	0.0572 (0.0342)	186.3 (122.9)	0.48 (0.05)	0.93 (0.1)	0.56 (0.08)	0.000117 (0.00003)
urban	10741 (2104)	0.0023 (0.0021)	2.7 (1.3)	0.32 (0.04)	0.51 (0.12)	0.27 (0.08)	0.000276 (0.00003)
urban center	16532 (1499)	0.0009 (0.0006)	1.4 (1.1)	0.32 (0.03)	0.38 (0.14)	0.19 (0.07)	0.000437 (0.00010)

(a) Satellite base map using QuickMapServices (NextGIS (2019),©Google images) with 6 km radius around the hive.



(b) Computed NDVI map, June 2019.



(c) Vegetation highlighting (NDVI= $\langle 0.2 : 1 \rangle$) in white.

Figure 4 – Successive remote sensing outputs for the case of Nishichiba

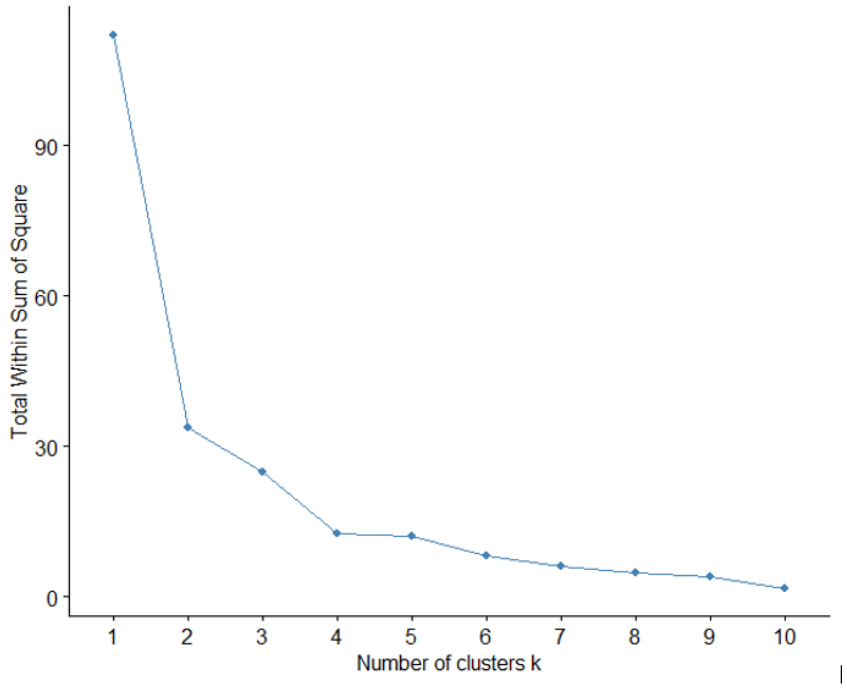


Figure 5 – Elbow method plotting the total within sum of square explained in function of the number of k clusters. The elbow of the curve suggests the number of groups to retain for kmeans clustering analysis.

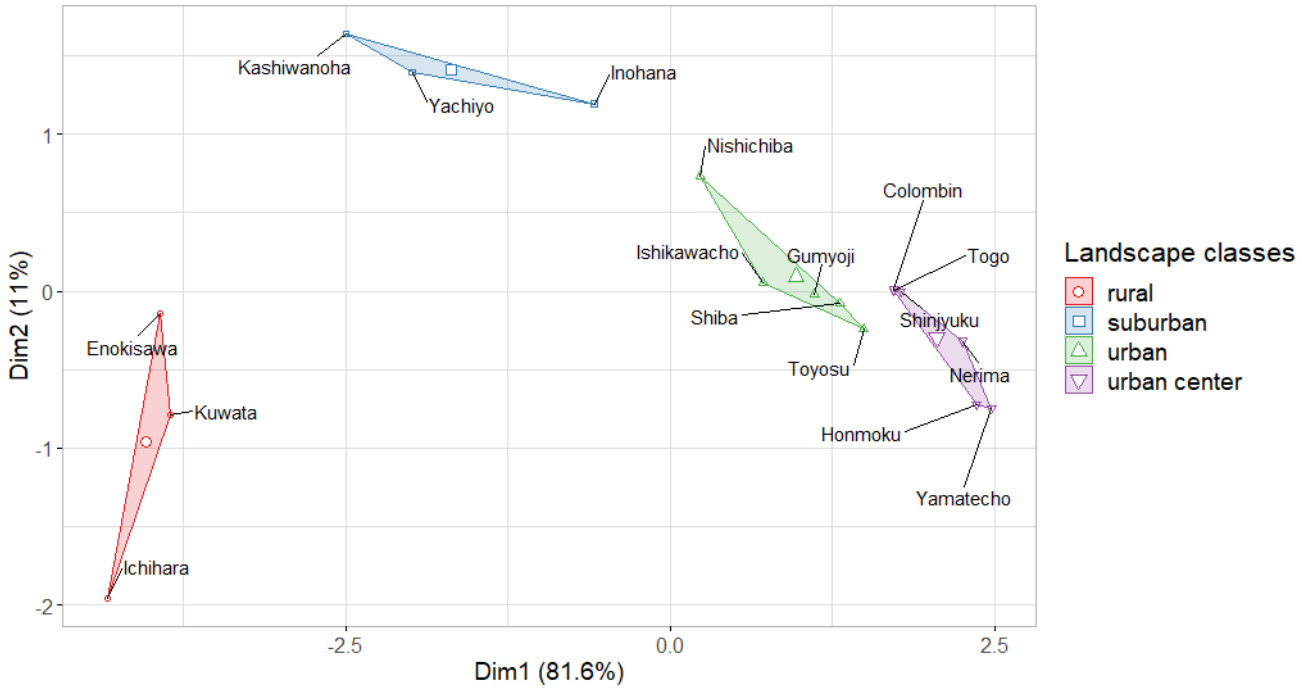


Figure 6 – Cluster plot of the study sites from k-means clustering analysis. The axes represent the first two principal components of the PCA analysis.

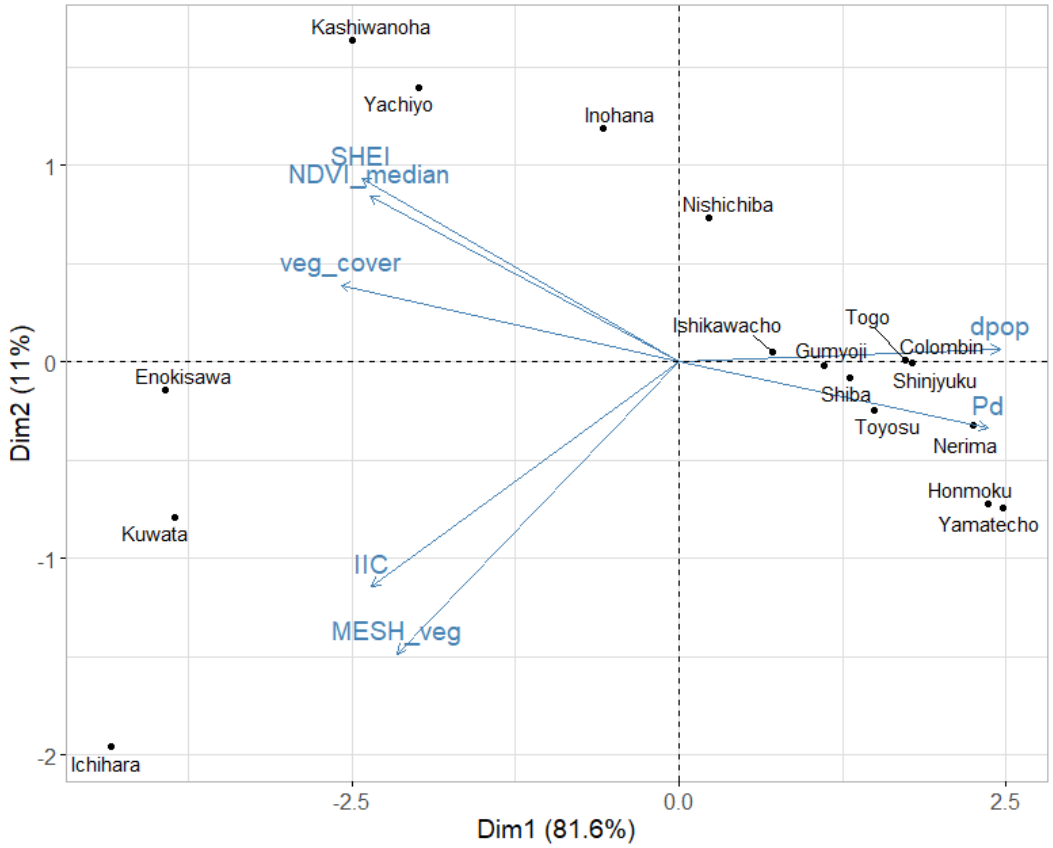


Figure 7 – Principal component analysis (PCA) biplot of individuals and variables. Pd: Patch density [patches/km^2], dpop: demographic density (number of inhabitant/km^2), MESH_veg: effective mesh size of vegetation [-], IIC: Integral index of connectivity [-], veg_cover: vegetation cover (%), NDVI_median: median of the NDVI of the cells superior to 0.2 [-], SHEI: Shannons's evenness index [-]

3.2 Richness and diversity of pollen samples

From a total of 8,179,602 raw reads initially, the data set includes 6,806,967 reads after quality filtering (identity$\geq$ 97%) with an average sampling rate of 47548 reads ($SD = 27464$) for the 143 samples. The further filtering reduced the number of reads to 6,799,314, distributed among 307 taxa from 74 families and 187 genera. 301 are determined to the specie level and the 6 to the genus. The species richness, defined as the number of distinct taxa in the samples, extends from 3 to 42 per sample with an average of 12 ($SD = 6.2$). No interaction is found between the landscape type and the time on the squared root species richness ($F = 0.95, p = 0.52$). While the landscape type implies no statistical significant differences ($F = 2.00, p = 0.12$), the pollen diversity (squared root species richness) varies highly significantly across the months ($F = 5.22, p < 0.001$) with the early spring (March and April) testifying a significantly higher diversity (Tukey test, $p < 0.05$) than the late growth season (July, August, September) (Figure 8). Prior to the analysis, the Spearman's rank-order correlation showed a very weak relation [5] ($r_s[143] = -0.17, p < 0.05$) between the number of species and the sampling length. Despite a significant p-value, because of the large

5. Correlation strength evaluation following the guides of Fowler et al. (1998)

size of sample, the weak correlation is statistically justified and representative of the population (Schober et al. (2018)).

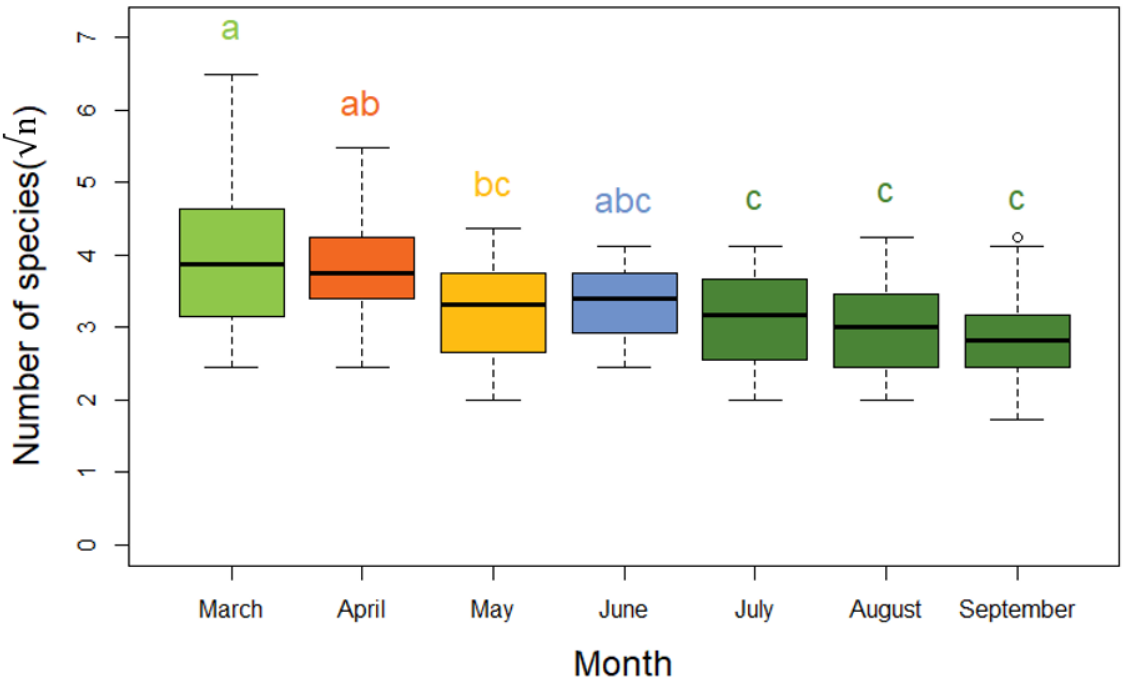


Figure 8 – Boxplot of squared root species richness by sampling period. Letters indicate significant differences according to Tukey's test.

NMDS shows variabilites in the pollen composition accros the month periods (Figure 9). The greatest discontinuity is found between the Spring season (March, April, May) and the Autumn (september). The floral composition foraged by the honeybee during May and August serve as a transition to the next season. The permutation tests reveal that the month period ($F = 6.87, R^2 = 0.23, p < 0.001$), the site ($F = 1.27, R^2 = 0.1, p < 0.01$) and the landscape class ($F = 2.01, R^2 = 0.03, p < 0.001$) are significant explanatory variables of the pollen composition in the samples, but the sampling period explains a larger proportion of the variance. From post-hoc pairwise adonis (letters from Figure 9), the urbanized sites (urban and urban center) appears to host a similar plant community. Moreover, taken as a whole, the plant composition foraged by honeybees varies considerably over the months until early autumn, when the floral composition appears similar to the one of late summer.

The results from sample-size-based rarefaction and extrapolation, and sample completeness curves are shown in the Figures 10 for the landscapes and in Figures 11 for the seasons.
Independently of the factor, the null order sampling curves increase sharply with the sampling size, while the ones from higher orders tend to flatten out. This illustrates the increasing influence of dominant species in the measure. The curves for the highest order start stabilizing at lower sampling size and present smaller confidence intervals. In fact, the most frequent species having more weigh, they can be observed with lower sampling effort.
Prior to the comparison of diversity among the factors, the base sample size and the base coverage were determined and equal to 48 and 87.2% for the landscape, and 110 and 94.6% for the season.

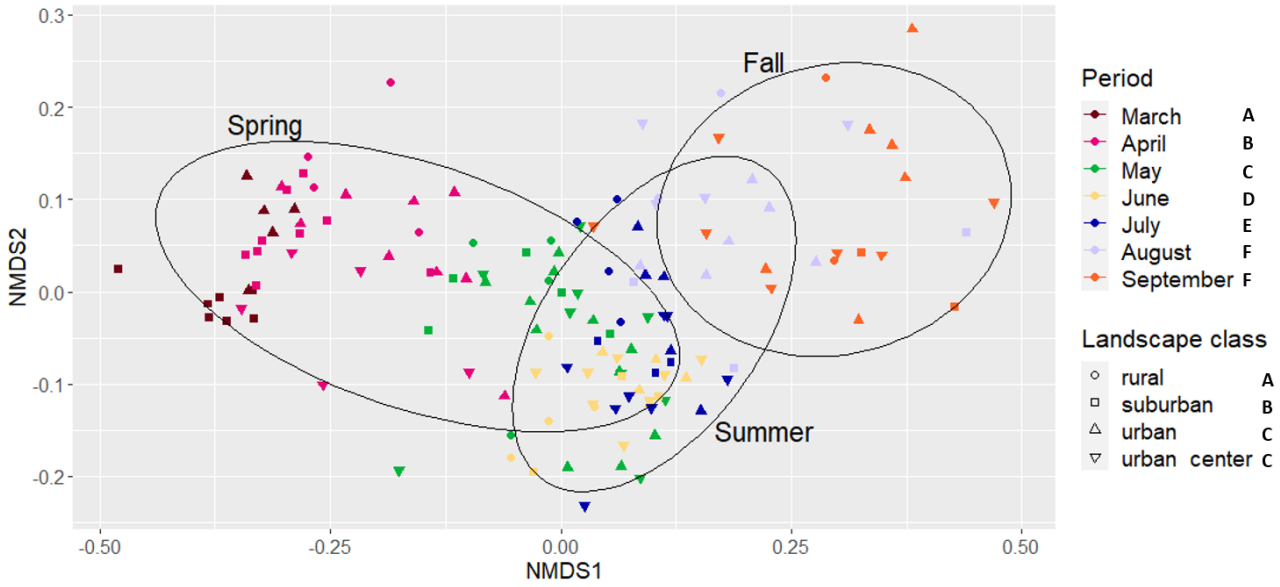
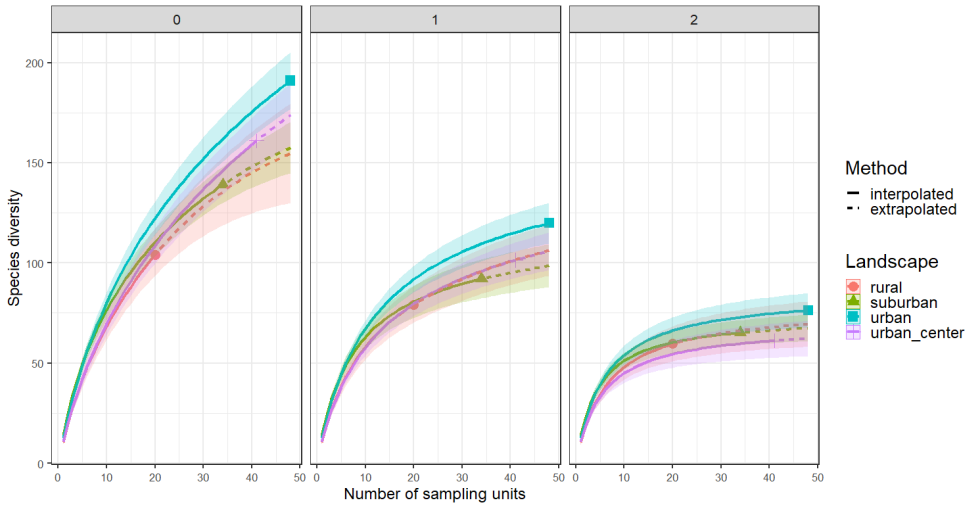


Figure 9 – Non-metric multidimensional scaling (NMDS) of plant communities from the pollen incidence data. Pollen samples' landscape type are symbolized by different shapes. Sampling periods are illustrated by color, and seasons are represented by 80% prediction confidence ellipses. Stress value = 0.086, non-metric fit,R^2=0.99 and linear fit, R^2=0.96 determined from Shepard diagram (appendix C). Letters indicate significant differences according to the post-hoc pairwise comparisons, with Bonferroni correction, of the plant communities among the landscape gradient and the sampling period.

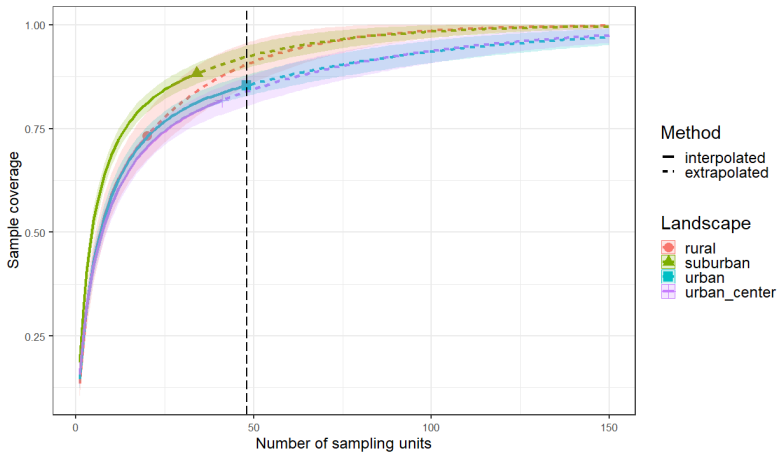
Regarding to the landscape factor (fig.10.a), the urban class appears to have the highest diversity independently of the order. The difference is only justified statistically compared to suburban class when the sample size exceeds 30 (for the order q=0 and q=1). Furthermore, the 95% confidence intervals tend to not overlap for q=0,on one side, for the urban and rural classes, and on the other side for q=2, between the urban and urban center. Moreover, for the rural, suburban and urban center, in contrast to q=0, the order of the curves is inverted. Without being statistically significant, this implies that urban center's plant community presents more rare OTUs compared to rural and suburban areas. On the other hand, it also indicates that proportion of frequently common OTUs is higher in the rural than in the suburban and urban center landscapes.
The sample coverages for the four landscape (rural, suburban, urban and urban center) was evaluated respectively to 73.3%, 88.3%, 85.4% and 81.8% and increased to 90.5%, 92.3%, 85.4% and 84.0% with the extrapolation. The Figure 10.c displays coverage-based rarefaction and extrapolation curves up to the base coverage of 87.2%. Similar trends as for the sample size-based curves (fig. 10.a) can be observed for the order 1 and 2. However, for the species richness (q=0), the urbanized landscapes differ significantly from rural and suburban landscapes at a higher level of coverage (from 80% of sample coverage to the base coverage).

Turning to the season factor, being composed of only one month (September), the fall season has far less sample units compared to the two others seasons. Being too restrictive, it has not been considered in the determination of the base size and coverage size. Hence, the estimation with extrapolation of the species richness (q=0) for the fall season cannot be reliable and is excluded from the interpretations of this graph. The spring season presents a significantly higher diversity independently of the sampling size, coverage and the order of Hill number. In contrast, for order superior to zero, the fall and the summer do not differ in species diversity at any sample size.
When the reference sample size is extrapolated to the base size, the sample coverage is increase from 92.5%, 89.,% and 71.3% to 95%, 94.6% and 99% for the spring, summer and fall. Consequently, the comparison of covered-based sampling curves allows to contrast three equally complete samples. The patterns observed for the Figure 11.c are consistent with the ones based on sample-size-based curves.

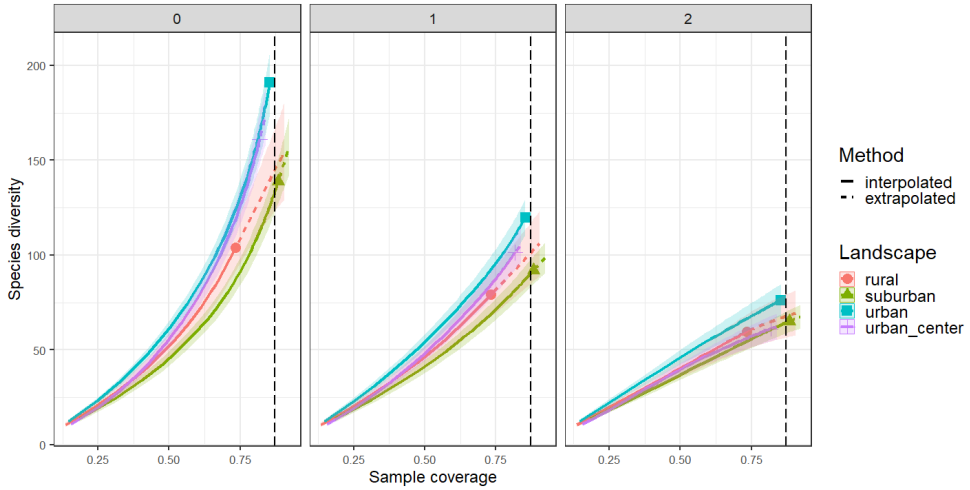
The coverage-based comparison ensures that the differentiation level between multiple communities is due to the intrinsic differences between these communities and not to the sampling effort. It allows a better guidance on the sampling efforts required to support interpretations on differences between communities rather than samples (Chao and Jost (2012)). Here, the conclusion is different depending on factors studied. For the analysis of seasons, the sampling efforts is sufficient to reach a complete sample, while for the landscape, it would require more samples, especially for the rural landscape which limits the extrapolation and thus interpretations.



(a) Sample-size-based rarefaction and extrapolation curves

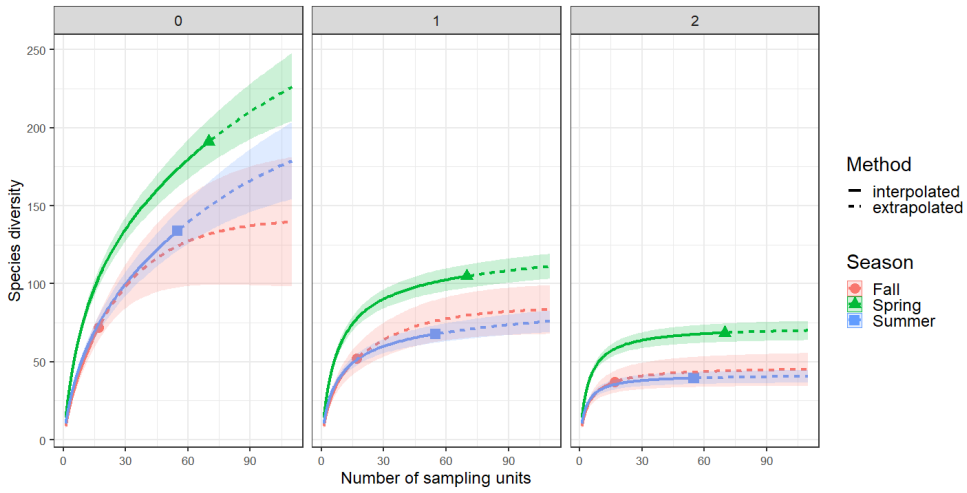


(b) Sample completeness curve

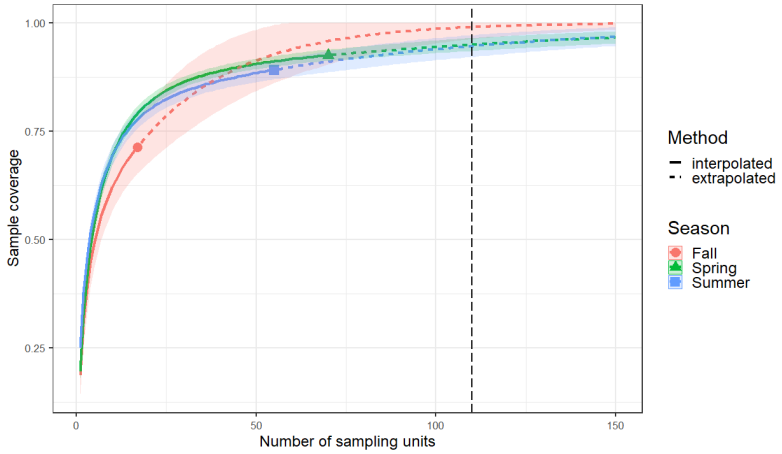


(c) Coverage-based rarefaction and extrapolation curves

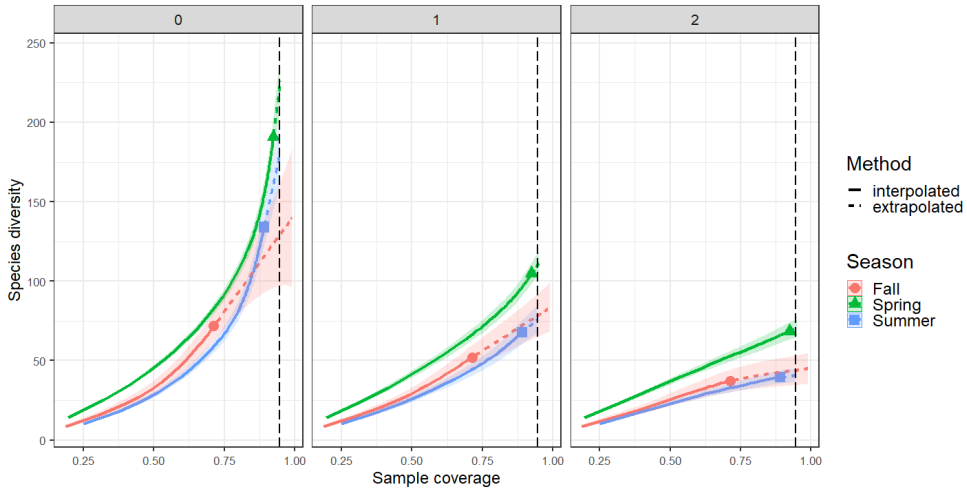
Figure 10 – Comparison of three types of sampling curves showing plant species diversity depending on the landscape for Hill numbers of order q=0 (Specie richness), q=1 (Shannon diversity), q=2 (Simpson diversity).
Reference samples, 95% confidence intervals, rarefaction and extrapolation are indicated respectively by solid shapes, shaded areas, solid lines and dotted lines. The vertical dotted lines represent the base size equal to 48 on (b) and the base coverage , 87.2%, on (c).



(a) Sample-size-based rarefaction and extrapolation curves



(b) Sample completeness curve



(c) Coverage-based rarefaction and extrapolation curves

Figure 11 – Comparison of three types of sampling curves illustrating the plant species diversity depending on the season for Hill numbers of order q=0 (Specie richness), q=1 (Shannon diversity), q=2 (Simpson diversity).
Reference samples, 95% confidence intervals, rarefaction and extrapolation are indicated respectively by solid shapes, shaded areas, solid lines and dotted lines. The vertical dotted lines represent the base size equal to 110 on (b) and the base coverage , 94.6%, on (c).

3.3 Trait-based and dominant taxa

The results from the G–test of independence reveal that over the months, the traits composition of plants foraged by the honeybees varied significantly, both in nature ($G = 99.0, p < 0.001$) and native status ($G = 69.1, p < 0.001$). In contrast, regarding the landscape, only the nature of the plants shows a dependence ($G = 10.7, p < 0.05$). The results of the post-hoc comparisons are depicted on the Figure 15 by letter combinations which reflect significant differences ($p < 0.05$) between the pairs of proportions.

Effect of landscape

According to the Figure 12, the proportion of herbaceous species among the samples appears to be significantly higher for the rural landscape compared to the others. Apart from this observation, plant composition traits are independent of landscape type. The Figure 13 shows that among the 307 different species identified in the study, 144 species are only found in specific landscape. In term of ratio, it corresponds respectively to 27%, 22%, 24% and 26% of the total species identified in the rural, suburban, urban and urban center landscapes. In contrast, 35 species are observed in every landscape. They correspond to 45% of the species found in all the samples combined. These species are divided between 18 families and 25 genera.

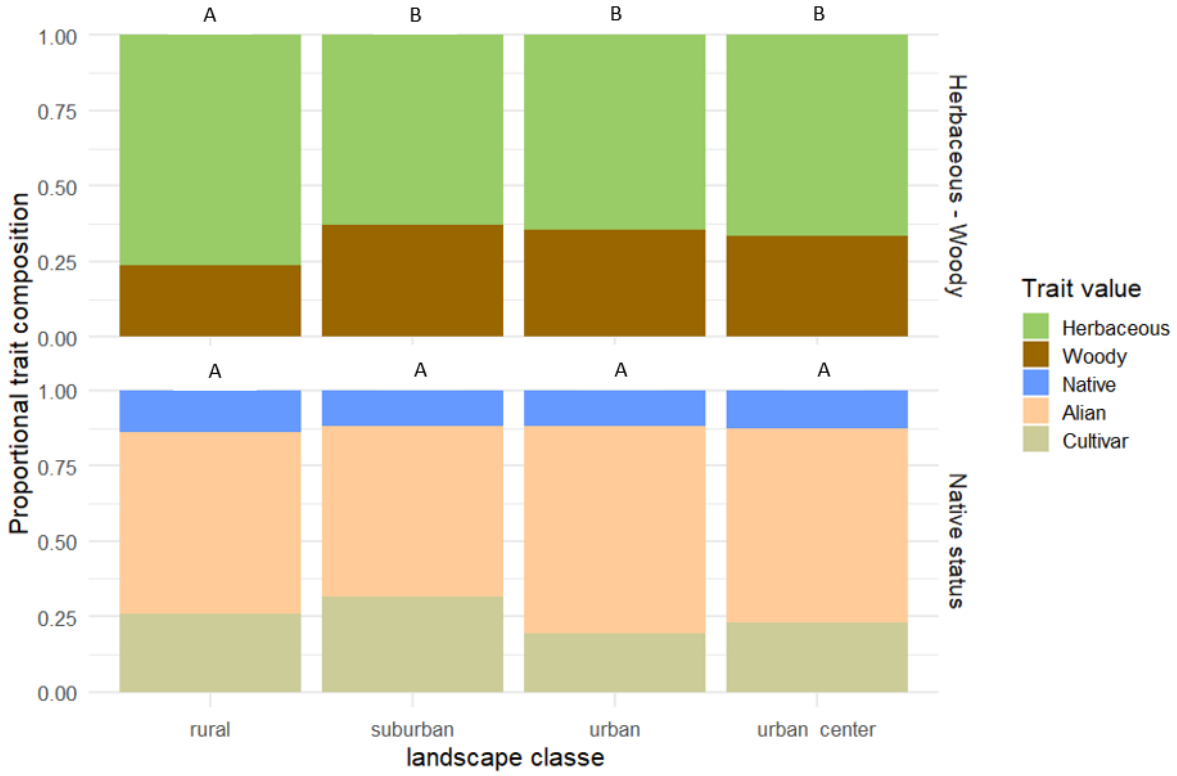


Figure 12 – Comparison of proportional occurrence of the different plant traits along landscapes. Letters on top of the bar indicate significant differences between pairs of proportions according to the result of post-hoc pairwise comparison with Bonferroni correction.

Independently of the landscape, Fabaceae, Rosaceae, Brassicaceae, Asteraceae, Plantaginaceae and Onagraceae are respectively the most frequent taxonomic families encountered in all samples. However, as support by the Figure 14, the order varies in function of the landscape. The suburban area stands out from the other classes as it presents higher frequency for Brassicaceae, Ranunculaceae and Rosaceae, to the detriment of the Fabaceae family. In contrast, the proportions of the Fabaceae are equal for the urban and urban center landscapes This finding supports the result from the NMDS ordination of diverse plant community among the landscape type (rural, suburban and urbanized landscape). Moreover, to be noted, the presence of Poaceae among the families most represented in the samples. This family is almost exclusively formed by anemophilous species. More surprisingly, it is the most frequent in the urbanized landscape, while this family is usually associated in majority to the countryside.

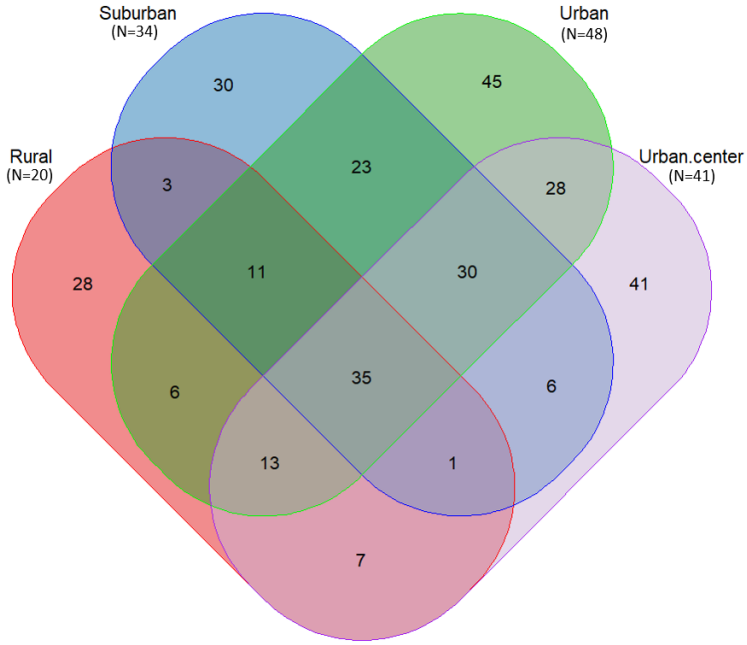


Figure 13 – Venn Diagram illustrating the overlap of plant species within different landscapes. The "N" between brackets corresponds to the number of samples per landscape.

Effect of sampling period

The proportions of woody species decrease significantly over the season ($G = 87.5, p < 0.001$). The Figure 15 highlights the monthly variation with the highest proportion in April (46%) and the lowest in September (10%). It can be observed that there is a strong drop between June and July (-12%) and between August and September (-10%). On the other hand, the proportion in native status($G = 32.9, p < 0.001$), the spring stands out compared to the other two seasons with a higher presence of cultivar in the samples. Throughout the sampling period, native species are the least common, in opposition to the exotic species.

March and April are dominated by *Prunus* and *Brassica* spp (Fig. 16), from which half of the observations being cultivar species. A strong presence of the genera *Acer* (maples) and *Helleborus* is

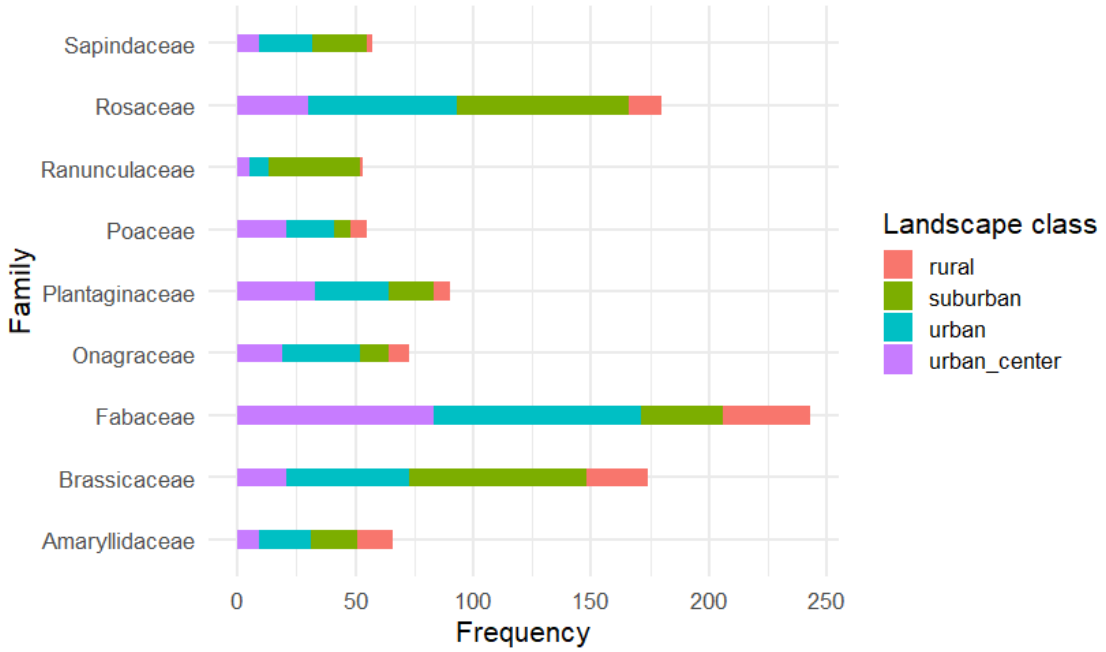


Figure 14 – Bar plot showing the 9 most frequent family observed in the samples in contrast with the landscape classes.

detected, which are known to bloom early in the season. These genera disappear from samples, later in the season. In contrast, four of the eleven most frequent genera emerge from late spring in May. Between April and June, a noticeable phenological turnover in the pollen composition (Fig.14) can be evidenced, with May serving as a transition bridge. This has already been highlighted by the discontinuities in the NMDS ordination (Fig.9). Following this shift, the genus *Trifolium* spp. is highly dominant in June and July samples. In addition, are also found in large proportions, the herbaceous genera *Plantago* and *Oenothera* spp, and the woody genera *Mallotus, Hydrangea* spp. . In August, the species from the genus *Oenothera* arer the most represented with *Trifolium* despite a reduction in its occurrence. August and September denote a shift in pollen composition trends with a reduction of high proportional occurrence genera. In other word, plants detected in August and September are more dispersed between genera. Only genus *Allium* shows an increase from August to September. Finally, *Trifolium* spp., *Rosa* spp and *Allium* spp. are the only genera that are observed across the whole study period.

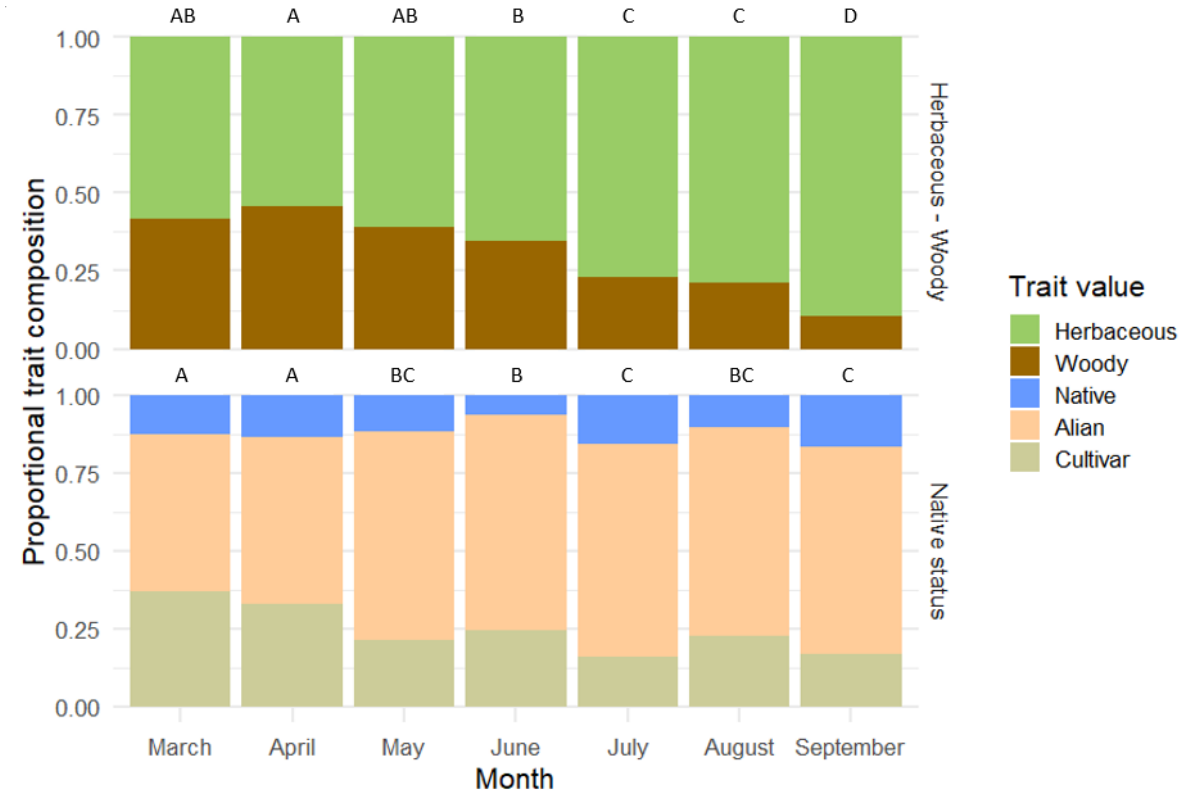


Figure 15 – Proportional occurrence of the different plant traits across the sampling period. Letters on top of the bar indicate significant differences between pairs of proportions according to the result of post-hoc pairwise comparison with Bonferroni correction.

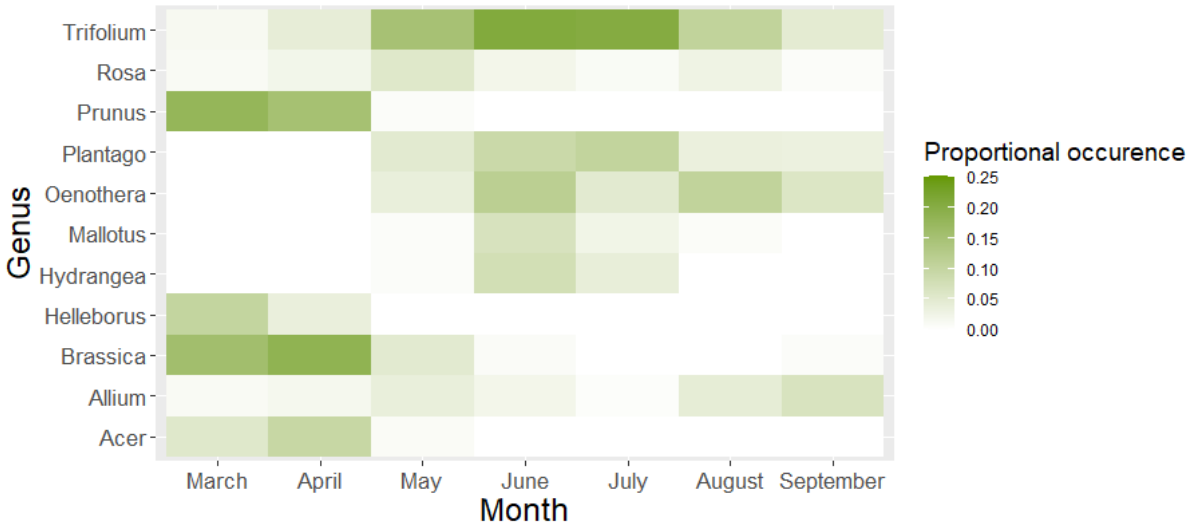


Figure 16 – Heatmap representing the 11 most frequent genera (62 species) in all samples according to their monthly proportional occurrence. The proportional occurrence corresponds to the ratio of the number of observations for a genus per month to the number of samples for the specific month. The 11 genera were chosen according to the condition that their observation frequency is superior to 10% of the total occurrence of at least one season. In total, they account for 47% of all observations of the study period. Note that the genera are ordered according to their prevalence, with the most frequent on the top.

4 Discussion

The study analyzed the pollen collected from 18 different sites for 7 months, from March to September, in 2019. Using DNA metabarcoding to identify the pollen composition of the samples, the methods yields to the identification of 307 taxas of which 301 to the specie level. As advised by Bell et al.(2018), the generated data set is considered as incidence data (presence/absence).

The pollen composition is assessed over the seasons and along an urban-rural gradient. For the latter, the use of landscape metrics allowed to include influences of ecological processes at a landscape level (foraging, plant dispersal) by assessing the diversity, connectivity and aggregation of the patches (Baguette et al. (2012); Doherty and Driscoll (2018)). Four different landscapes were identified : rural; suburban; urban; and urban center (Fig.6, table 3).

Landscape diversity does not show significant difference in the Species Richness (Fig. 10.a (q) and Two way ANOVA). However, the rural sample shows limitation in its interpretation as it accounts a lower sample size not comparable to the others, that could mislead the extrapolation (see section 3.2). Therefore, its analysis must be treated with caution. For the others, with regard to Coverage-based rarefaction and extrapolation curves (Fig.10.c, q=0 and q=1), the suburban sites appear to have a significantly lower diversity compared to the urbanized areas (urban and urban center). In fact, except for the urbanized areas that are similar, the plant communities among the landscape differ independently of the season (Fig.9). This in opposition to related studies that report no effect of the landscape diversity on the pollen composition (Steffan-Dewenter and Kuhn (2003); Danner et al. (2017). The hypothesis is that as many ornamental flowers used in the city are unattractive for the pollinators(Garbuzov et al. (2015)), in order to meet nutritional demands with diverse pollen diet, the honeybees need to increase their foraging range to find available resources increasing the probability to meet a higher diversity of plants (Avni et al. (2014); Danner et al. (2016)). Moreover, the urban matrix having smaller spread patches, the resources are less dense, so the foragers to search further or for substitutes. Hendriksma and Shafir (2016) report that in case of nutritional deficiency, honeybees do not only integrate new sources but also focus in searching complementary nutritional ones.

Despite this difference in community composition, the trait-based analysis reveals no significant differences in the characteristics of sampled plants (Fig.12). This reflects a great diversity of plant resources and highlight foraging patterns regardless the landscape. In the samples, 35 species are found in all landscape which corresponds for each class to 45% of the observations from all the samples combines. Fabaceae, Rosaceae and Brassicaceae are the most occurring taxonomic families in the samples (Fig.14), which is in agreement with previous findings (Park and Nieh (2017); Doherty and Driscoll (2018). Furthermore, the presence of the Poaceae family among the most frequent families in the samples support the findings of Saunders (2018) to take in consideration the anemophilous plants as resources for pollinators. The presence in higher proportion for the urbanized landscape support the hypothesis of a nutrional deficiency that foragers try to compensate.

In contrast to the minor variations due to the landscape diversity, a strong seasonal effect has been evidenced on the species richness (Fig.8, Fig.11.a), the pollen diversity and the plant composition (Fig.11.a,.c and Fig.9). The samples from the Spring have significantly higher diversity compared to the other two seasons (Fig.11), same observations have been made by Lau et al. (2019). Woody species appear to occur more in the Spring (Fig.15), as they constitutes an important source of pollen for the early season (Brodschneider et al. (2019); Sponsler et al. (2020)). The genera *Prunus* spp. and *Acer* spp. are the most represented (Fig.16). It is also during this period that the cultivar species occur the most with the genera *Prunus* spp. and *Brassicaceae* spp. Next, the proportion of woody species is gradually replaced by herbaceous plants when in September, they only represents 10% of the occurrence (Brodschneider et al. (2019); Sponsler et al. (2020)). Concerning the native status, the cultivar frequency tends to decrease in time to the benefits of alian species which dominates the observations. These observations are at odds with the ones from Williams et al. (2011) and Urbanowicz et al. (2020) who report both no overall preference for native or alian species. The dominance of non native species in the samples could be explained by a higher presence of non native species in the vegetation communities as the region is composed of highly modified landscape favouring the development of non native plants at the expense of the indigenous. However, this statement should be alleviated and requires further research. A plants inventory representative enough of populations surrounding the hives would allow to remove some uncertainties.

The plant composition of May and August denote a qreongn to the next season (Fig.9, Fig.16). The summer shows a presence much more important of dominant taxa in the samples. This pattern corresponds to the findings of Liolios et al. (2015) evidencing the behaviour of honeybees to focus on certain abundant plant with a long flowering period (floral consistency). Therefore, the low diversity during the Summer matches with the peak of occurence of certain families (Fig.16) such as Fabaceae with the genus *Trifolium* spp and Plantaginaceae, *Plantago* spp. This finding is in line with other researches (Donaldson-Matasci and Dornhaus (2012); Brodschneider et al. (2019)). Finally, August reflects a break in the preponderance of the dominant taxa in the pollen composition, that translate the transition to seasonal dearth with the resources that are decreasing (Fig.9,Fig.15). To mitigate this transition, the honeybees increase their foraging radius requiring to extra effort from the foragers sometimes for worthless rewards. Therefore, in an urban greening context, it would be relevant to put in place measures to alleviate this seasonal dearth by ensuring enough resources close to the apiaries.

Limitations

The urban-rural gradient used in this research was determined from an unconventional approach. Despite, the method led to convincing results, certain caveats can be emitted. In fact, the image resolution used was 3 meters which could lead to some limitations and approximations, especially in complex landscape matrices such as urban area. Therefore, a higher satellite resolution or images captured by drones would be more appropriate. Then, the choice of variables can be questioned. Despite, these limitations, this approach gave relevant results, open to further investigations.

Concerning the DNA metabarcoding, the method yields the identification of a large number of taxa, 307 from which 306 at the specie level. This results is higher than what I have been found the literature. However, this technique is still under development and needs further investigations and improvements, especially in the question of the relation between the number of reads and the pollen abundance, which remains to date not reliable (Baksay et al. (2020)). Too many steps are still arbitrary, such as the choice of the identity threshold, set in this study to 97%. Consequently, with the upcoming progress, it will require to be standardized in order to enable the comparison of data between different studies.

Finally, some implementation would allow for further interpretations of the samples. A monitoring of strength of each colony (brood combs, stored food,...) would help to compare the conditions of the colonies. Moreover, during the pollen collection, the weighting of the samples would give some insights about the abundance.

5 Conclusion

The present study aims to promote biodiversity and urban greening in cities by adopting a more reasoned and conscious urban planning. Pollen was used as the source of the observations in this study as it is vital to the growth and well-being of the colony (Herbert (1992)), but also because it highlights the plant-pollinator interactions.

In this context, pollen was collected from 18 different locations during the period from March to September 2019. The pollen was analyzed by DNA metabarcoding yielding in the identification of 307 different taxa. Next, the study conducted an analysis of the pollen sampled depending on two factors : across the seasons (Spring, Summer, Fall); and along an urban-rural gradient (rural, suburban, urban and urban center). The goals of the study were to determine if the landscape and/or the season explain some variations in the species richness and diversity. In addition, the floral composition was characterize by specific traits: nature (Woody or Herbaceous); and native status (Native, Alien and Cultivar).

It has been evidenced that the landscape explains minor variations in the plant composition foraged by honeybees. In fact, only a higher diversity in the pollen composition was observed for the urbanized locations (combination of urban and urban center) compared to suburban areas compared. The hypothesis is in favour of an unattractive plant composition in the urban areas that forces the bees to forage further to increase the plant diversity in order to find suitable pollen. Moreover, smaller patches in the urbanized landscape yield in fewer resources requiring the foragers to visit more patches. However, despite this difference any difference in the trait-based analysis was identified. It reflects large plant communities dominated by the occurrence of alian species independently of the landscape classes.

In contrast, the species richness, pollen diversity and plant composition showed a strong dependence to the seasons. Woody species appearing more often the sample of the early Spring provide an important source of pollen to the honeybees in the early growth season. Over the months, the proportion of woody species is decreasing in favour of herbaceous plants. Regarding the native status, the alian species dominates the observation. The cultivar species occurrence tends to decrease constantly over the seasons, while native species are sparsely foraged.

Regarding the taxonomic composition, the Fabaceae, Rosaceae, Brassicaceae, Plantaginaceae and Onagraceae represent the families that are the most frequently observed in the sample. The genus the most represented is by far *Trifolium* spp., reaching its peak in Summer, period of low diversity due to the pervasive presence of dominant taxa. In opposition, the end of the Summer shows a reduction in the presence of dominant taxa in the sample, which corresponds to the seasonal dearth. To conclude, the present study contributes to a deeper understanding of the ecology and floral composition foraged by honeybees on which the urban planning can rely in order to promote the biodiversity in the cities.

References

Abrol, D. P. (2012). Pollination Biology: Biodiversity conservation and agricultural production. *Pollination Biology: Biodiversity Conservation and Agricultural Production*, 9789400719:1–792.

Aizen, M. A., Garibaldi, L. A., Cunningham, S. A., and Klein, A. M. (2008). Long-Term Global Trends in Crop Yield and Production Reveal No Current Pollination Shortage but Increasing Pollinator Dependency. *Current Biology*, 18(20):1572–1575.

Al-Tikrity, W. S., Benton, A. W., Hillman, R. C., and Clarke, W. W. (1972). The relationship between the amount of unsealed brood in honeybee colonies and their pollen collection. *Journal of Apicultural Research*, 11(1):9–12.

Alaux, C., Ducloz, F., Crauser, D., and Le Conte, Y. (2010). Diet effects on honeybee immunocompetence. *Biology Letters*, 6(4):562–565.

Alberdi, A. and Gilbert, M. T. P. (2019). A guide to the application of Hill numbers to DNA-based diversity analyses. *Molecular Ecology Resources*, 19(4):804–817.

Allen, M. F., Ball, B. V., and Underwood, B. A. (1990). An isolate of Melissococcus pluton from Apis laboriosa. *Journal of Invertebrate Pathology*, 55(3):439–440.

Allen-Wardell, G., Bernhardt, P., Bitner, R., Burquez, A., Buchmann, S., Cane, J., Cox, P. A., Dalton, V., Feinsinger, P., Ingram, M., Inouye, D., Jones, C. E., Kennedy, K., Kevan, P., Koopowitz, H., Medellin, R., Medellin-Morales, S., and Nabhan, G. P. (1998). The potential consequences of pollinator declines on the conservation of biodiversity and stability of food crop yields. *Conservation Biology*, 12(1):8–17.

Anderson, M. J. (2001). A new method for non-parametric multivariate analysis of variance. *Austral Ecology*, 26(1):32–46.

Auclair, J. L. and Jamieson, C. A. (1948). A qualitative analysis of amino acids in pollen collected by bees. *Science*, 108(2805):357–358.

Avni, D., Dag, A., and Shafir, S. (2009). Pollen sources for honeybees in Israel: Source, periods of shortage, and influence on population growth. *Israel Journal of Plant Sciences*, 57(3):263–275.

Avni, D., Hendriksma, H. P., Dag, A., Uni, Z., and Shafir, S. (2014). Nutritional aspects of honey bee-collected pollen and constraints on colony development in the eastern Mediterranean. *Journal of Insect Physiology*, 69(C):65–73.

Baguette, M., Blanchet, S., Legrand, D., Stevens, V. M., and Turlure, C. (2012). Individual dispersal, landscape connectivity and ecological networks. *Biological Reviews*, 88(2):310–326.

Baksay, S., Pornon, A., Burrus, M., Mariette, J., Andalo, C., and Escaravage, N. (2020). Experimental quantification of pollen with DNA metabarcoding using ITS1 and trnL. *Scientific Reports*, 10(1):4202.

Banaszak-Cibicka, W., Ratyńska, H., and Dylewski, Ł. (2016). Features of urban green space favourable for large and diverse bee populations (Hymenoptera: Apoidea: Apiformes). *Urban Forestry and Urban Greening*, 20:448–452.

Barker, R. J. (1971). The Influence of Food Inside the Hive on Pollen Collection by a Honeybee Colony. *Journal of Apicultural Research*, 10(1):23–26.

Barnett, E. A., Charlton, A. J., and Fletcher, M. R. (2007). Incidents of bee poisoning with pesticides in the United Kingdom, 1994–2003. *Pest Management Science*, 63(11):1051–1057.

Bastin, L. and Thomas, C. D. (1999). The distribution of plant species in urban vegetation fragments. *Landscape Ecology*, 14(November):493–507.

Beekman, M., Sumpter, D. J. T., Seraphides, N., and Ratnieks, F. L. W. (2004). Comparing foraging behaviour of small and large honey-bee colonies by decoding waggle dances made by foragers. *Functional Ecology*, 18(6):829–835.

Bell, K. L., Burgess, K. S., Botsch, J. C., Dobbs, E. K., Read, T. D., and Brosi, B. J. (2018). Quantitative and qualitative assessment of pollen DNA metabarcoding using constructed species mixtures. *Molecular Ecology*, 28(2):431–455.

Bell, K. L., De Vere, N., Keller, A., Richardson, R. T., Gous, A., Burgess, K. S., and Brosi, B. J. (2016). Pollen DNA barcoding: Current applications and future prospects. *Genome*, 59(9):629–640.

Bell, K. L., Fowler, J., Burgess, K. S., Dobbs, E. K., Gruenewald, D., Lawley, B., Morozumi, C., and Brosi, B. J. (2017). Applying Pollen DNA Metabarcoding to the Study of Plant–Pollinator Interactions. *Applications in Plant Sciences*, 5(6):1600124.

Blackmore, L. M. and Goulson, D. (2014). Evaluating the effectiveness of wildflower seed mixes for boosting floral diversity and bumblebee and hoverfly abundance in urban areas. *Insect Conservation and Diversity*, 7(5):480–484.

Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., Alexander, H., Alm, E. J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J. E., Bittinger, K., Brejnrod, A., Brislawn, C. J., Brown, C. T., Callahan, B. J., Caraballo-Rodríguez, A. M., Chase, J., Cope, E. K., Da Silva, R., Diener, C., Dorrestein, P. C., Douglas, G. M., Durall, D. M., Duvallet, C., Edwardson, C. F., Ernst, M., Estaki, M., Fouquier, J., Gauglitz, J. M., Gibbons, S. M., Gibson, D. L., Gonzalez, A., Gorlick, K., Guo, J., Hillmann, B., Holmes, S., Holste, H., Huttenhower, C., Huttley, G. A., Janssen, S., Jarmusch, A. K., Jiang, L., Kaehler, B. D., Kang, K. B., Keefe, C. R., Keim, P., Kelley, S. T., Knights, D., Koester, I., Kosciolek, T., Kreps, J., Langille, M. G. I., Lee, J., Ley, R., Liu, Y.-X., Loftfield, E., Lozupone, C., Maher, M., Marotz, C., Martin, B. D., McDonald, D., McIver, L. J., Melnik, A. V., Metcalf, J. L., Morgan, S. C., Morton, J. T., Naimey, A. T., Navas-Molina, J. A., Nothias, L. F., Orchanian, S. B., Pearson, T., Peoples, S. L., Petras, D., Preuss, M. L., Pruesse, E., Rasmussen, L. B., Rivers, A., Robeson, M. S., Rosenthal, P., Segata, N., Shaffer, M., Shiffer, A., Sinha, R., Song, S. J., Spear,

J. R., Swafford, A. D., Thompson, L. R., Torres, P. J., Trinh, P., Tripathi, A., Turnbaugh, P. J., Ul-Hasan, S., van der Hooft, J. J. J., Vargas, F., Vázquez-Baeza, Y., Vogtmann, E., von Hippel, M., Walters, W., Wan, Y., Wang, M., Warren, J., Weber, K. C., Williamson, C. H. D., Willis, A. D., Xu, Z. Z., Zaneveld, J. R., Zhang, Y., Zhu, Q., Knight, R., and Caporaso, J. G. (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology*, 37(8):852–857.

Borisenko, A. V., Sones, J. E., and Hebert, P. D. (2009). The front-end logistics of DNA barcoding: Challenges and prospects. *Molecular Ecology Resources*, 9(SUPPL. 1):27–34.

Brodschneider, R., Gratzer, K., Kalcher-Sommersguter, E., Heigl, H., Auer, W., Moosbeckhofer, R., and Crailsheim, K. (2019). A citizen science supported study on seasonal diversity and monoflorality of pollen collected by honey bees in Austria. *Scientific Reports*, 9(1):16633.

Burd, M. (1994). Bateman's principle and plant reproduction: The role of pollen limitation in fruit and seed set. *The Botanical Review*, 60(1):83–139.

Butz Huryn, V. M. (1997). No Title. *The Quarterly Review of Biology*, 72(3):275–298.

Cale, G. (1968). Pollen gathering relationship to honey collection and egg laying in honeybees. 108:8–9. *Am. Bee. J.*, 108:8–9.

Cameron, S. A., Lozier, J. D., Strange, J. P., Koch, J. B., Cordes, N., Solter, L. F., and Griswold, T. L. (2011). Patterns of widespread decline in North American bumble bees. *Proceedings of the National Academy of Sciences of the United States of America*, 108(2):662–667.

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., Fierer, N., Peña, A. G., Goodrich, J. K., Gordon, J. I., Huttley, G. A., Kelley, S. T., Knights, D., Koenig, J. E., Ley, R. E., Lozupone, C. A., McDonald, D., Muegge, B. D., Pirrung, M., Reeder, J., Sevinsky, J. R., Turnbaugh, P. J., Walters, W. A., Widmann, J., Yatsunenko, T., Zaneveld, J., and Knight, R. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nature Methods*, 7(5):335–336.

Chagnon, M., Kreutzweiser, D., Mitchell, E. A., Morrissey, C. A., Noome, D. A., and Van Der Sluijs, J. P. (2015). Risks of large-scale use of systemic insecticides to ecosystem functioning and services. *Environmental Science and Pollution Research*, 22(1):119–134.

Chao, A., Gotelli, N. J., Hsieh, T. C., Sander, E. L., Ma, K. H., Colwell, R. K., and Ellison, A. M. (2014). Rarefaction and extrapolation with Hill numbers: A framework for sampling and estimation in species diversity studies. *Ecological Monographs*, 84(1):45–67.

Chao, A. and Jost, L. (2012). Coverage-based rarefaction and extrapolation: Standardizing samples by completeness rather than size. *Ecology*, 93(12):2533–2547.

Chen, S., Yao, H., Han, J., Liu, C., Song, J., Shi, L., Zhu, Y., Ma, X., Gao, T., Pang, X., Luo, K., Li, Y., Li, X., Jia, X., Lin, Y., and Leon, C. (2010). Validation of the ITS2 region as a novel DNA barcode for identifying medicinal plant species. *PLoS ONE*, 5(1):e8613.

Concepción, E. D., Moretti, M., Altermatt, F., Nobis, M. P., and Obrist, M. K. (2015). Impacts of urbanisation on biodiversity: The role of species mobility, degree of specialisation and spatial scale. *Oikos*, 124(12):1571–1582.

Conti, I., Medrzycki, P., Grillenzoni, F. V., Corvucci, F., Tosi, S., Malagnini, V., Spinella, M., and Mariotti, M. G. (2016). Floral diversity of pollen collected by honey bees (Apis mellifera L.) - validation of the chromatic assessment method. *Journal of Apicultural Science*, 60(2):209–220.

Cornman, R. S., Otto, C. R., Iwanowicz, D., and Pettis, J. S. (2015). Taxonomic characterization of honey bee (Apis mellifera) pollen foraging based on non-overlapping paired-end sequencing of nuclear ribosomal loci. *PLoS ONE*, 10(12):e0145365.

Cotte, J. F., Casabianca, H., Giroud, B., Albert, M., Lheritier, J., and Grenier-Loustalot, M. F. (2004). Characterization of honey amino acid profiles using high-pressure liquid chromatography to control authenticity. *Analytical and Bioanalytical Chemistry*, 378(5):1342–1350.

Danner, N., Keller, A., Ha, S., and Steffan-dewenter, I. (2017). Honey bee foraging ecology : Season but not landscape diversity shapes the amount and diversity of collected pollen. *Plos One*, 12(8):9–12.

Danner, N., Molitor, A. M., Schiele, S., Härtel, S., and Steffan-Dewenter, I. (2016). Season and landscape composition affect pollen foraging distances and habitat use of Honey bees. *Ecological Applications*, 26(6):1920–1929.

De França Alves, R. and De Assis Ribeiro DosSantos, F. (2014). Plant sources for bee pollen load production in Sergipe, northeast Brazil. *Palynology*, 38(1):90–100.

Desneux, N., Decourtye, A., and Delpuech, J.-M. (2007). The sublethal effects of pesticides on beneficial Arthropods. *Annual Review of Entomology*, 52(1):81–106.

Di Pasquale, G., Salignon, M., Le Conte, Y., Belzunces, L. P., Decourtye, A., Kretzschmar, A., Suchail, S., Brunet, J. L., and Alaux, C. (2013). Influence of pollen nutrition on honey bee health: Do pollen quality and diversity matter? *PLoS ONE*, 8(8):e72016.

Dirzo, R., Young, H. S., Galetti, M., Ceballos, G., Isaac, N. J. B., and Collen, B. (2014). Defaunation in the Anthropocene. *Science*, 345(6195):401–406.

Doherty, T. S. and Driscoll, D. A. (2018). Coupling movement and landscape ecology for animal conservation in production landscapes. *Proceedings of the Royal Society B: Biological Sciences*, 285(1870):20172272.

Dohzono, I. and Yokoyama, J. (2010). Impacts of alien bees on native plant-pollinator relationships: A review with special emphasis on plant reproduction. *Applied Entomology and Zoology*, 45(1):37–47.

Domroese, M. C. and Johnson, E. A. (2017). Why watch bees? Motivations of citizen science volunteers in the Great Pollinator Project. *Biological Conservation*, 208:40–47.

Donaldson-Matasci, M. C. and Dornhaus, A. (2012). How habitat affects the benefits of communication in collectively foraging honey bees. *Behavioral Ecology and Sociobiology*, 66(4):583–592.

Dreller, C. and Tarpy, D. R. (2000). Perception of the pollen need by foragers in a honeybee colony. *Animal Behaviour*, 59(1):91–96.

Evison, S. E., Roberts, K. E., Laurenson, L., Pietravalle, S., Hui, J., Biesmeijer, J. C., Smith, J. E., Budge, G., and Hughes, W. O. (2012). Pervasiveness of parasites in pollinators. *PLoS ONE*, 7(1):e30641.

Eyer, M., Chen, Y. P., Schäfer, M. O., Pettis, J., and Neumann, P. (2009). Small hive beetle, Aethina tumida , as a potential biological vector of honeybee viruses . *Apidologie*, 40(4):419–428.

FAO (2018). Why bees matter. *FAO*, page 16.

Fernández-Torres, R., Pérez-Bernal, J. L., Bello-López, M. Á., Callejón-Mochón, M., Jiménez-Sánchez, J. C., and Guiraúm-Pérez, A. (2005). Mineral content and botanical origin of Spanish honeys. *Talanta*, 65(3):686–691.

Fewell, J. H. and Winston, M. L. (1992). Colony state and regulation of pollen foraging in the honey bee, Apis mellifera L. *Behavioral Ecology and Sociobiology*, 30(6):387–393.

Fontaine, C., Dajoz, I., Meriguet, J., and Loreau, M. (2006). Functional diversity of plant–pollinator interaction webs enhances the persistence of plant communities. *PLoS Biology*, 4(1):e1.

Forister, M. L., McCall, A. C., Sanders, N. J., Fordyce, J. A., Thorne, J. H., O'Brien, J., Waetjen, D. P., and Shapiro, A. M. (2010). Compounded effects of climate change and habitat alteration shift patterns of butterfly diversity. *Proceedings of the National Academy of Sciences*, 107(5):2088–2092.

Fowler, J., Cohen, L., and Jarvis, P. (1998). *Practical statistics for field biology*. Wiley, Chichester(New York), 2nd ed. edition.

Free, J. (1967). Factors determining the collection of pollen by honeybee foragers. *Animal Behaviour*, 15(1):134–144.

Free, J. B. (1965a). The Ability of Bumblebees and Honeybees to Pollinate Red Clover. *The Journal of Applied Ecology*, 2(2):289.

Free, J. B. (1965b). The behaviour of honeybee foragers when their colonies are fed sugar syrup. *Journal of Apicultural Research*, 4(2):85–88.

Free, J. B. (1965c). The effect on pollen collection of feeding honey-bee colonies with sugar syrup. *The Journal of Agricultural Science*, 64(2):167–168.

Free, J. B. (1993). *Insect pollination of crops*. Academic Press, London, 2nd ed edition.

Gallai, N., Salles, J. M., Settele, J., and Vaissière, B. E. (2009). Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. *Ecological Economics*, 68(3):810–821.

Garbuzov, M., Samuelson, E. E., and Ratnieks, F. L. (2015). Survey of insect visitation of ornamental flowers in Southover Grange garden, Lewes, UK. *Insect Science*, 22(5):700–705.

Garibaldi, L. A., Steffan-Dewenter, I., Kremen, C., Morales, J. M., Bommarco, R., Cunningham, S. A., Carvalheiro, L. G., Chacoff, N. P., Dudenhöffer, J. H., Greenleaf, S. S., Holzschuh, A., Isaacs, R., Krewenka, K., Mandelik, Y., Mayfield, M. M., Morandin, L. A., Potts, S. G., Ricketts, T. H., Szentgyörgyi, H., Viana, B. F., Westphal, C., Winfree, R., and Klein, A. M. (2011). Stability of pollination services decreases with isolation from natural areas despite honey bee visits. *Ecology Letters*, 14(10):1062–1072.

Gatoria, G., Chhuneja, P., and Singh, D. (2000). Import of pollinator, package bees, queen bees and associated problems. *Indian Bee J*, 62:1–9.

Geslin, B., Gauzens, B., Baude, M., Dajoz, I., Fontaine, C., Henry, M., Ropars, L., Rollin, O., Thébault, E., and Vereecken, N. J. (2017). Massively introduced managed species and their consequences for plant–pollinator interactions. In *Advances in Ecological Research*, volume 57, chapter 4, pages 147–199. Elsevier Ltd.

Geslin, B., Gauzens, B., Thébault, E., and Dajoz, I. (2013). Plant Pollinator Networks along a Gradient of Urbanisation. *PLoS ONE*, 8(5):e63421.

Geslin, B., Le Féon, V., Folschweiller, M., Flacher, F., Carmignac, D., Motard, E., Perret, S., and Dajoz, I. (2016). The proportion of impervious surfaces at the landscape scale structures wild bee assemblages in a densely populated region. *Ecology and Evolution*, 6(18):6599–6615.

Good, I. J. (1953). The Population Frequencies of Species and the Estimation of Population Parameters. *Biometrika*, 40(3/4):237–264.

Goulson, D. (2003). Effects of Introduced Bees on Native Ecosystems. *Annual Review of Ecology, Evolution, and Systematics*, 34(1):1–26.

Goulson, D., Nicholls, E., Botías, C., and Rotheray, E. L. (2015). Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. *Science*, 347(6229):1255957–1255957.

Goulson, D. and Sparrow, K. R. (2009). Evidence for competition between honeybees and bumblebees; effects on bumblebee worker size. *Journal of Insect Conservation*, 13(2):177–181.

Grabowski, P. and Wilde, J. (2000). Composition and weight of pollen loads gathered by high and low pollen-colecting honeybee colonies. *Apidologie*, 31(October):607–609.

Graystock, P., Blane, E. J., McFrederick, Q. S., Goulson, D., and Hughes, W. O. (2016). Do managed bees drive parasite spread and emergence in wild bees? *International Journal for Parasitology: Parasites and Wildlife*, 5(1):64–75.

Gross, C. L. and MacKay, D. (1998). Honeybees reduce fitness in the pioneer shrub Melastoma affine (Melastomataceae). *Biological Conservation*, 86(2):169–178.

Hadley, A. S. and Betts, M. G. (2012). The effects of landscape fragmentation on pollination dynamics: absence of evidence not evidence of absence. *Biological Reviews*, 87(3):526–544.

Hargreaves, A. L., Harder, L. D., and Johnson, S. D. (2009). Consumptive emasculation: The ecological and evolutionary consequences of pollen theft. *Biological Reviews*, 84(2):259–276.

Hashim, H., Abd Latif, Z., and Adnan, N. A. (2019). Urban vegetation classification with NDVI threshold value method with very high resolution (VHR) Pleiades imagery. *International Archives of the Photogrammetry, Remote Sensing and Spatial Information Sciences - ISPRS Archives*, 42(4/W16):237–240.

Hawkins, J., De Vere, N., Griffith, A., Ford, C. R., Allainguillaume, J., Hegarty, M. J., Baillie, L., and Adams-Groom, B. (2015). Using DNA metabarcoding to identify the floral composition of honey: A new tool for investigating honey bee foraging preferences. *PLoS ONE*, 10(8):e0134735.

Haydak, M. H. (1970). Honey Bee Nutrition. *Annual Review of Entomology*, 15(1):143–156.

Hebert, P. D., Cywinska, A., Ball, S. L., and DeWaard, J. R. (2003). Biological identifications through DNA barcodes. *Proceedings of the Royal Society B: Biological Sciences*, 270(1512):313–321.

Hendriksma, H. P. and Shafir, S. (2016). Honey bee foragers balance colony nutritional deficiencies. *Behavioral Ecology and Sociobiology*, 70(4):509–517.

Henry, M. and Rodet, G. (2018). Controlling the impact of the managed honeybee on wild bees in protected areas. *Scientific Reports*, 8(1):9308.

Herbert, E. W. (1992). Honey bee nutrition. In Graham, J., editor, *The hive and the honey bee*, pages 197–233. Dadant & Sons, Hamilton (USA), revised edition.

Hermosín, I., Chicón, R. M., and Cabezudo, M. D. (2003). Free amino acid composition and botanical origin of honey. *Food Chemistry*, 83(2):263–268.

Hervé, M. (2020). RVAideMemoire: Testing and Plotting Procedures for Biostatistics.

Hesselbarth, M. H., Sciaini, M., With, K. A., Wiegand, K., and Nowosad, J. (2019). Landscapemetrics: an open-source R tool to calculate landscape metrics. *Ecography*, 42:1648–1657.

Hijmans, R. J., van Etten, J., Sumner, M., Cheng, J., Baston, D., Bevan, A., Bivand, R., Busetto, L., Canty, M., Forrest, D., Ghosh, A., Golicher, D., Gray, J., Greenberg, J. A., Hiemstra, P., Hingee, K., Institute for Mathematics Applied Geosciences, Karney, C., Mattiuzzi, M., Mosher, S., Nowosad, J., Pebesma, E., Perpinan Lamigueiro, O., Racine, E. B., Rowlingson, B., Shortridge, A., Venables, B., and Wueest, R. (2020). raster: Geographic Data Analysis and Modeling.

Hoffmann, M., Hilton-Taylor, C., Angulo, A., Böhm, M., Brooks, T. M., Butchart, S. H., Carpenter, K. E., Chanson, J., Collen, B., Cox, N. A., Darwall, W. R., Dulvy, N. K., Harrison, L. R., Katariya, V., Pollock, C. M., Quader, S., Richman, N. I., Rodrigues, A. S., Tognelli, M. F., Vié, J. C., Aguiar, J. M., Allen, D. J., Allen, G. R., Amori, G., Ananjeva, N. B., Andreone, F., Andrew, P., Ortiz, A. L. A., Baillie, J. E., Baldi, R., Bell, B. D., Biju, S. D., Bird, J. P., Black-Decima, P., Blanc, J. J., Bolaños, F., Bolivar-G., W., Burfield, I. J., Burton, J. A., Capper, D. R., Castro, F., Catullo, G., Cavanagh, R. D., Channing, A., Chao, N. L., Chenery, A. M., Chiozza, F., Clausnitzer, V., Collar, N. J., Collett, L. C., Collette, B. B., Cortez Fernandez, C. F., Craig, M. T., Crosby, M. J., Cumberlidge, N., Cuttelod, A., Derocher, A. E., Diesmos, A. C., Donaldson, J. S., Duckworth, J. W., Dutson, G., Dutta, S. K., Emslie, R. H., Farjon, A., Fowler, S., Freyhof, J., Garshelis, D. L., Gerlach, J., Gower, D. J., Grant, T. D., Hammerson, G. A., Harris, R. B., Heaney, L. R., Hedges, S. B., Hero, J. M., Hughes, B., Hussain, S. A., Icochea M., J., Inger, R. F., Ishii, N., Iskandar, D. T., Jenkins, R. K., Kaneko, Y., Kottelat, M., Kovacs, K. M., Kuzmin, S. L., La Marca, E., Lamoreux, J. F., Lau, M. W., Lavilla, E. O., Leus, K., Lewison, R. L., Lichtenstein, G., Livingstone, S. R., Lukoschek, V., Mallon, D. P., McGowan, P. J., McIvor, A., Moehlman, P. D., Molur, S., Alonso, A. M., Musick, J. A., Nowell, K., Nussbaum, R. A., Olech, W., Orlov, N. L., Papenfuss, T. J., Parra-Olea, G., Perrin, W. F., Polidoro, B. A., Pourkazemi, M., Racey, P. A., Ragle, J. S., Ram, M., Rathbun, G., Reynolds, R. P., Rhodin, A. G., Richards, S. J., Rodríguez, L. O., Ron, S. R., Rondinini, C., Rylands, A. B., De Mitcheson, Y. S., Sanciangco, J. C., Sanders, K. L., Santos-Barrera, G., Schipper, J., Self-Sullivan, C., Shi, Y., Shoemaker, A., Short, F. T., Sillero-Zubiri, C., Silvano, D. L., Smith, K. G., Smith, A. T., Snoeks, J., Stattersfield, A. J., Symes, A. J., Taber, A. B., Talukdar, B. K., Temple, H. J., Timmins, R., Tobias, J. A., Tsytsulina, K., Tweddle, D., Ubeda, C., Valenti, S. V., Van Dijk, P. P., Veiga, L. M., Veloso, A., Wege, D. C., Wilkinson, M., Williamson, E. A., Xie, F., Young, B. E., Akçakaya, H. R., Bennun, L., Blackburn, T. M., Boitani, L., Dublin, H. T., Da Fonseca, G. A., Gascon, C., Lacher, T. E., Mace, G. M., Mainka, S. A., McNeely, J. A., Mittermeier, R. A., Reid, G. M. G., Rodriguez, J. P., Rosenberg, A. A., Samways, M. J., Smart, J., Stein, B. A., and Stuart, S. N. (2010). The impact of conservation on the status of the world's vertebrates. *Science*, 330(6010):1503–1509.

Hsieh, T. C. and Chao, A. (2017). Rarefaction and extrapolation: Making fair comparison of abundance-sensitive phylogenetic diversity among multiple assemblages. *Systematic Biology*, 66(1):100–111.

Hsieh, T. C., Ma, K. H., and Chao, A. (2016). iNEXT: an R package for rarefaction and extrapolation of species diversity (Hill numbers). *Methods in Ecology and Evolution*, 7(12):1451–1456.

Hsieh, T. C., Ma, K. H., and Chao, A. (2020). iNEXT: Interpolation and extrapolation for species diversity.

Jaeger, J. (2000). Landscape division, splitting index, and effective mesh size: new measures of landscape fragmentation. *Landscape Ecology*, 15(February):115–130.

Jay, S. C. (1986). Spatial management of honeybees on crops. *Bee World*, 67(3):98–113.

Jeffree, E. P. and Allen, D. M. (1957). The Annual Cycle of Pollen Storage by Honey Bees. *Journal of Economic Entomology*, 50(2):211–212.

Jost, L. (2006). Entropy and diversity. *Oikos*, 113(2):363–375.

Kaluza, B. F., Wallace, H., Heard, T. A., Klein, A. M., and Leonhardt, S. D. (2016). Urban gardens promote bee foraging over natural habitats and plantations. *Ecology and Evolution*, 6(5):1304–1316.

Käpylä, M. (1974). Diurnal flight activity in a mixed population of Aculeata (Hym.). *Annales Entomologicae Fennicae*, 40(2):61–69.

Kaškoniene, V. and Venskutonis, P. R. (2010). Floral Markers in Honey of Various Botanical and Geographic Origins: A Review. *Comprehensive Reviews in Food Science and Food Safety*, 9(6):620–634.

Kassambara, A. and Fabian, M. (2020). factoextra: Extract and Visualize the Results of Multivariate Data Analyses.

Kearns, C. A., Inouye, D. W., and Waser, N. M. (1998). Endangered mutualisms: The conservation of plant-pollinator interactions. *Annual Review of Ecology and Systematics*, 29(1):83–112.

Keller, A., Danner, N., Grimmer, G., Ankenbrand, M., von der Ohe, K., von der Ohe, W., Rost, S., Härtel, S., and Steffan-Dewenter, I. (2014). Evaluating multiplexed next-generation sequencing as a method in palynology for mixed pollen samples. *Plant Biology*, 17(2):558–566.

Kenta, T., Inari, N., Nagamitsu, T., Goka, K., and Hiura, T. (2007). Commercialized European bumblebee can cause pollination disturbance: An experiment on seven native plant species in Japan. *Biological Conservation*, 134(3):298–309.

Kevan, P. G. and Menzel, R. (2012). The plight of pollination and the interface of neurobiology, ecology and food security. *Environmentalist*, 32(3):300–310.

Keylock, C. J. (2005). Simpson diversity and the Shannon-Wiener index as special cases of a generalized entropy. *Oikos*, 109(1):203–207.

Klein, A.-M., Vaissière, B. E., Cane, J. H., Steffan-Dewenter, I., Cunningham, S. A., Kremen, C., and Tscharntke, T. (2007). Importance of pollinators in changing landscapes for world crops. *Proceedings of the Royal Society B: Biological Sciences*, 274(1608):303–313.

Kluser, S., Neumann, P., Chauzat, M.-P., and S. Pettis, J. (2010). Global Honey Bee colony disorders and other threats to insect pollinators. Technical report, UNEP.

Kluser, S. and Peduzzi, P. (2007). 'Global Pollinator Decline : A Literature Review', UNEP/GRID Europe. © UNEP 2007. (September):4.

Kodinariya, T. M. and Makwana, P. R. (2013). Review on determining number of Cluster in K-Means Clustering. *International Journal of Advance Research in Computer Science and Management Studies*, 1(6):90–95.

Kraaijeveld, K., de Weger, L. A., Ventayol García, M., Buermans, H., Frank, J., Hiemstra, P. S., and den Dunnen, J. T. (2014). Efficient and sensitive identification and quantification of airborne pollen using next-generation DNA sequencing. *Molecular Ecology Resources*, 15(1):8–16.

Kremen, C., Williams, N. M., Aizen, M. A., Gemmill-Herren, B., LeBuhn, G., Minckley, R., Packer, L., Potts, S. G., Roulston, T., Steffan-Dewenter, I., Vázquez, D. P., Winfree, R., Adams, L., Crone, E. E., Greenleaf, S. S., Keitt, T. H., Klein, A.-M., Regetz, J., and Ricketts, T. H. (2007). Pollination and other ecosystem services produced by mobile organisms: a conceptual framework for the effects of land-use change. *Ecology Letters*, 10(4):299–314.

Laha, R. C., De Mandal, S., Ralte, L., Ralte, L., Kumar, N. S., Gurusubramanian, G., Satishkumar, R., Mugasimangalam, R., and Kuravadi, N. A. (2017). Meta-barcoding in combination with palynological inference is a potent diagnostic marker for honey floral composition. *AMB Express*, 7(1):132.

Lamb, P. D., Hunter, E., Pinnegar, J. K., Creer, S., Davies, R. G., and Taylor, M. I. (2019). How quantitative is metabarcoding: A meta-analytical approach. *Molecular Ecology*, 28(2):420–430.

Larsen, T. H., Williams, N. M., and Kremen, C. (2005). Extinction order and altered community structure rapidly disrupt ecosystem functioning. *Ecology Letters*, 8(5):538–547.

Lau, P., Bryant, V., Ellis, J. D., Huang, Z. Y., Sullivan, J., Schmehl, D. R., Cabrera, A. R., and Rangel, J. (2019). Seasonal variation of pollen collected by honey bees (Apis mellifera) in developed areas across four regions in the United States. *PLoS ONE*, 14(6):e0217294.

Le Conte, Y., Ellis, M., and Ritter, W. (2010). Varroa mites and honey bee health: Can Varroa explain part of the colony losses? *Apidologie*, 41(3):353–363.

Legendre, P. and Legendre, L. (2012). Ordination in reduced space. In *Numerical Ecology*, chapter 9, pages 425–520. Elsevier, Amsterdam (Netherlands), 3 edition.

Lever, J. J., van Nes, E. H., Scheffer, M., and Bascompte, J. (2014). The sudden collapse of pollinator communities. *Ecology Letters*, 17(3):350–359.

Lindsay, J. B. (2016). Whitebox GAT: A case study in geomorphometric analysis. *Computers and Geosciences*, 95:75–84.

Liolios, V., Tananaki, C., Dimou, M., Kanelis, D., Goras, G., Karazafiris, E., and Thrasyvoulou, A. (2015). Clasificación del polen de las plantas melíferas en función de su aportación de proteínas para las abejas de la miel. *Journal of Apicultural Research*, 54(5):582–592.

MacDonald, P. L. and Gardner, R. C. (2000). Type I error rate comparisons of post hoc procedures for I × J chi-square tables. *Educational and Psychological Measurement*, 60(5):735–754.

Magoč, T. and Salzberg, S. L. (2011). FLASH: Fast length adjustment of short reads to improve genome assemblies. *Bioinformatics*, 27(21):2957–2963.

Mahmood, R., Asad, S., Sarwar, G., Ahmad, W., Qadir, Z. A., Balouch, A., and Rafque, M. K. (2017). Pollen collection and pollen foraging behaviour of honeybees (apis mellifera) during different time intervals from brassica campestris L. *Pakistan Journal of Zoology*, 49(2):733–736.

Mallinger, R. E., Gaines-Day, H. R., and Gratton, C. (2017). Do managed bees have negative effects on wild bees?: A systematic review of the literature. *PLoS ONE*, 12(12):e0189268.

Mardan, M. (1995). Varied Pollinators for Southeast Asian Crops. In Roubik, D. W., editor, *Pollination of cultivated plants in the tropics*, chapter 2.5.1, pages 142–148. FAO, Rome, fao 1995 edition.

Martinez Arbizu, P. (2020). pairwiseAdonis: Pairwise multilevel comparison using adonis.

Maxim, L. and van der Sluijs, J. P. (2013). Seed-dressing systemic insecticides and honeybees. In *Late lessons from early warnings: science, precaution, innovation'*, chapter 16, pages 401–438. Copenhagen, no1/2013 edition.

McDade, L. A. and Kinsman, S. (1980). The impact of floral parasitism in two neotropical Hummingbird-pollinated plant species. *Evolution*, 34(5):944–958.

McGregor, S. E. (1976). Insect pollination of cultivated crop plants. *Agriculture handbook*, 496(July):411.

Memmott, J., Craze, P. G., Waser, N. M., and Price, M. V. (2007). Global warming and the disruption of plant-pollinator interactions. *Ecology Letters*, 10(8):710–717.

Mestre, F. and Silva, B. (2019). lconnect: Simple Tools to Compute Landscape Connectivity Metrics.

Miller, G. J., Morris, J. T., and Wang, C. (2019). Estimating aboveground biomass and its spatial distribution in coastal wetlands utilizing planet multispectral imagery. *Remote Sensing*, 11(17):2020.

Minchin, P. R. (1987). An evaluation of the relative robustness of techniques for ecological ordination. In *Theory and models in vegetation science*, pages 89–107. Springer Netherlands, Dordrecht.

Montalva, J., Dudley, L., Arroyo, M. K., Retamales, H., and Abrahamovich, A. H. (2011). Geographic distribution and associated flora of native and introduced bumble bees (Bombus spp.) in Chile. *Journal of Apicultural Research*, 50(1):11–21.

Morandin, L. A. and Winston, M. L. (2005). Wild bee abundance and seed production in conventional, organic, and genetically modified canola. *Ecological Applications*, 15(3):871–881.

NextGIS (2019). QuickMapServices: Collection of easy to add basemaps.

Oksanen, J., Blanchet, F. Guillaume Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., Szoecs, E., and Wagner, H. (2019). vegan: Community Ecology Package.

Ollerton, J., Erenler, H., Edwards, M., and Crockett, R. (2014). Extinctions of aculeate pollinators in Britain and the role of large-scale agricultural changes. *Science*, 346(6215):1360–1362.

Olmstead, A. L. and Wooten, D. B. (1987). Bee Pollination and Productivity Growth: The Case of Alfalfa. *American Journal of Agricultural Economics*, 69(1):56–63.

Paini, D. R. (2004). Impact of the introduced honey bee (Apis mellifera) (Hymenoptera: Apidae) on native bees: A review. *Austral Ecology*, 29(4):399–407.

Paliy, O. and Shankar, V. (2016). Application of multivariate statistical techniques in microbial ecology. *Molecular Ecology*, 25(5):1032–1057.

Park, B. and Nieh, J. C. (2017). Seasonal trends in honey bee pollen foraging revealed through DNA barcoding of bee-collected pollen. *Insectes Sociaux*, 64(3):425–437.

Pascual-Hortal, L. and Saura, S. (2006). Comparison and development of new graph-based landscape connectivity indices: Towards the priorization of habitat patches and corridors for conservation. *Landscape Ecology*, 21(7):959–967.

Piñol, J., Senar, M. A., and Symondson, W. O. (2019). The choice of universal primers and the characteristics of the species mixture determine when DNA metabarcoding can be quantitative. *Molecular Ecology*, 28(2):407–419.

Pornon, A., Escaravage, N., Burrus, M., Holota, H., Khimoun, A., Mariette, J., Pellizzari, C., Iribar, A., Etienne, R., Taberlet, P., Vidal, M., Winterton, P., Zinger, L., and Andalo, C. (2016). Using metabarcoding to reveal and quantify plant-pollinator interactions. *Scientific Reports*, 6(1):27282.

Potts, S. G., Biesmeijer, J. C., Kremen, C., Neumann, P., Schweiger, O., and Kunin, W. E. (2010). Global pollinator declines: Trends, impacts and drivers. *Trends in Ecology and Evolution*, 25(6):345–353.

Pyšek, P., Jarošík, V., Hulme, P. E., Pergl, J., Hejda, M., Schaffner, U., and Vilà, M. (2012). A global assessment of invasive plant impacts on resident species, communities and ecosystems: the interaction of impact measures, invading species' traits and environment. *Global Change Biology*, 18(5):1725–1737.

R Core Team (2020). R: A Language and Environment for Statistical Computing.

Rathcke, B. J. and Jules, E. S. (1993). Habitat Fragmentation and Plant Pollinator Interactions. Current Science 65: 273-277F2529. *Current Science*, 65(August):—-.

Requier, F., Odoux, J. F., Tamic, T., Moreau, N., Henry, M., Decourtye, A., and Bretagnolle, V. (2015). Honey bee diet in intensive farmland habitats reveals an unexpectedly high flower richness and a major role of weeds. *Ecological Applications*, 25(4):881–890.

Ribière, M., Ball, B., and Aubert, M. F. A. (2008). Natural History and Geographical Distribution of Honey Bee Viruses. In Ribière, M., Ball, B., Aubert, M., Eds, Aubert, M., Ball, B., Fries, I., Moritz, R. F. A., Milani, N., and Bernardinelli, I., editors, *Virology and the Honey Bee*, number EUR 21937, chapter 1, pages 15–84. European Commission, eur 21937 edition.

Richardson, R. T., Curtis, H. R., Matcham, E. G., Lin, C. H., Suresh, S., Sponsler, D. B., Hearon, L. E., and Johnson, R. M. (2018). Quantitative multi-locus metabarcoding and waggle dance interpretation reveal honey bee spring foraging patterns in Midwest agroecosystems. *Molecular Ecology*, 28(3):686–697.

Richardson, R. T., Lin, C., Quijia, J. O., Riusech, N. S., Goodell, K., and Johnson, R. M. (2015a). Rank-based characterization of pollen assemblages collected by honey bees using a multi-locus metabarcoding approach. *Applications in Plant Sciences*, 3(11):1500043.

Richardson, R. T., Lin, C.-H., Sponsler, D. B., Quijia, J. O., Goodell, K., and Johnson, R. M. (2015b). Application of ITS2 Metabarcoding to Determine the Provenance of Pollen Collected by Honey Bees in an Agroecosystem. *Applications in Plant Sciences*, 3(1):1–6.

Robinson, M. L. (1989). Corporate family searching on dialog. Techniques for the business searcher. *On-line review*, 13(6):477–484.

Roskov, Y., Ower, G., Orrell, T., Nicolson, D., Bailly, N., Kirk, P., Bourgoin, T., DeWalt, R., Decock, W., van Nieukerken, E., Zarucchi, J., and Penev, L. (2019). Species 2000 & ITIS Catalogue of Life, 2019 Annual Checklist.

Roubik, D. W. (1978). Competitive interactions between neotropical pollinators and africanized honey bees. *Science*, 201(4360):1030–1032.

Roubik, D. W. (1989). *Ecology and Natural History of Tropical Bees*. Cambridge University Press.

Roulston, T. H., Cane, J. H., and Buchmann, S. L. (2000). What governs protein content of pollen: Pollinator preferences, pollen-pistil interactions, or phylogeny? *Ecological Monographs*, 70(4):617.

Sakagami, S. F. (1959). Some Interspecific Relations Between Japanese and European Honeybees. *The Journal of Animal Ecology*, 28(1):51–68.

Sakai, T. . (1992). Apis cerana beekeeping in Japan. In Verma, L. R., editor, *Honeybees in mountain agriculture*. Oxford & IBH Pub. Co, New Delhi.

Samuelson, A. E., Gill, R. J., Brown, M. J., and Leadbeater, E. (2018). Lower bumblebee colony reproductive success in agricultural compared with urban environments. *Proceedings of the Royal Society B: Biological Sciences*, 285(1881):20180807.

Sanguinetti, A. and Singer, R. B. (2014). Invasive bees promote high reproductive success in Andean orchids. *Biological Conservation*, 175:10–20.

Saunders, M. E. (2018). Insect pollinators collect pollen from wind-pollinated plants: implications for pollination ecology and sustainable agriculture. *Insect Conservation and Diversity*, 11(1):13–31.

Saura, S. and Pascual-Hortal, L. (2007). A new habitat availability index to integrate connectivity in landscape conservation planning: Comparison with existing indices and application to a case study. *Landscape and Urban Planning*, 83(2-3):91–103.

Saville, N. M. (2000). Farmer-participatory extension in Jumla, Western Nepal. In Matsuka, M., Verma, L., Wongsiri, S., Shrestha, K., and Partap, U., editors, *Asian bees and beekeeping: progress of research and development*, chapter 61, pages 230–236. Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi, illustrate edition.

Schober, P., Boer, C., and Schwarte, L. A. (2018). Correlation Coefficients: Appropriate Use and Interpretation. *Anesthesia & Analgesia*, 126(5):1763–1768.

Schweiger, O., Settele, J., Kudrna, O., Klotz, S., and Kühn, I. (2008). Climate change can cause spatial mismatch of trophically interacting species. *Ecology*, 89(12):3472–3479.

Seeley, T. D. (1995). *The wisdom of the hive*. Harvard University Press, illustrate edition.

Sihag, R. and Mishra, R. (1995). Crop pollination and Apis cerana. In Kevan, P. G., editor, *The Asiatic hive bee: apiculture, biology, and role in sustainable development in tropical and subtropical Asia.*, pages 135–142. Enviroquest Ltd., Cambridge, Ontario, illustrate edition.

Singh, R., Levitt, A. L., Rajotte, E. G., Holmes, E. C., Ostiguy, N., VanEngelsdorp, D., Lipkin, W. I., DePamphilis, C. W., Toth, A. L., and Cox-Foster, D. L. (2010). RNA Viruses in Hymenopteran Pollinators: Evidence of Inter-Taxa Virus Transmission via Pollen and Potential Impact on Non-Apis Hymenopteran Species. *PLoS ONE*, 5(12):e14357.

Smart, M., Pettis, J., Rice, N., Browning, Z., and Spivak, M. (2016). Linking measures of colony and individual honey bee health to survival among apiaries exposed to varying agricultural land use. *PLoS ONE*, 11(3):e0152685.

Smart, M. D., Cornman, R. S., Iwanowicz, D. D., McDermott-Kubeczko, M., Pettis, J. S., Spivak, M. S., and Otto, C. R. (2017). A comparison of honey bee-collected pollen from working agricultural lands using light microscopy and its metabarcoding. *Environmental Entomology*, 46(1):38–49.

Southwick, E. E. (1983). Nectar biology and nectar feeders of common milkweed, Asclepias syriaca L. *Bulletin - Torrey Botanical Club*, 110(3):324–334.

Spanowicz, A. G. and Jaeger, J. A. (2019). Measuring landscape connectivity: On the importance of within-patch connectivity. *Landscape Ecology*, 34(10):2261–2278.

Sponsler, D. B., Shump, D., Richardson, R. T., and Grozinger, C. M. (2020). Characterizing the floral resources of a North American metropolis using a honey bee foraging assay. *Ecosphere*, 11(4).

Steffan-Dewenter, I. and Kuhn, A. (2003). Honeybee foraging in differentially structured landscapes. *Proceedings of the Royal Society B: Biological Sciences*, 270(1515):569–575.

Steffan-Dewenter, I., Potts, S. G., and Packer, L. (2005). Pollinator diversity and crop pollination services are at risk. *Trends in Ecology & Evolution*, 20(12):651–652.

Stephens, D. W. and Krebs, J. R. (1986). *Foraging Theory*. Princeton University Press, New Jersey, illustrate edition.

Stimec, J. and Scott-Dupree, CD McAndrews, J. (1997). Honey Bee, Apis mellifera, pollen foraging in southern Ontario. *Canadian Field-Naturalist*, 111(3):454–456.

Stout, J. C., Kells, A. R., and Goulson, D. (2002). Pollination of the invasive exotic shrub Lupinus arboreus (Fabaceae) by introduced bees in Tasmania. *Biological Conservation*, 106(3):425–434.

Stout, J. C. and Morales, C. L. (2009). Ecological impacts of invasive alien species on bees. *Apidologie*, 40(3):388–409.

Strickland, J. S. (2014). *Predictive Analytics using R*. Lulu Inc.

Sugden, E. A. and Pyke, G. H. (1991). Effects of honey bees on colonies of Exoneura asimillima, an Australian native bee. *Austral Ecology*, 16(2):171–181.

Taufik, A., Syed Ahmad, S. S., and Azmi, E. F. (2016). Classification of landsat 8 satellite data using unsupervised methods. *Journal of Telecommunication, Electronic and Computer Engineering*, 8(4):37–40.

Tereshko, V. and Loengarov, A. (2005). Collective decision-making in Honey Bee foraging dynamics. *Computing and Information Systems Journal*, 9.

Theodorou, P., Radzevičiūtė, R., Lentendu, G., Kahnt, B., Husemann, M., Bleidorn, C., Settele, J., Schweiger, O., Grosse, I., Wubet, T., Murray, T. E., and Paxton, R. J. (2020). Urban areas as hotspots for bees and pollination but not a panacea for all insects. *Nature Communications*, 11(1):576.

Thomson, D. (2004). Competitive interactions between the invasive European honey bee and native bumble bees. *Ecology*, 85(2):458–470.

Thomson, D. M. (2006). Detecting the effects of introduced species: A case study of competition between Apis and Bombus. *Oikos*, 114(3):407–418.

Threlfall, C. G., Walker, K., Williams, N. S., Hahs, A. K., Mata, L., Stork, N., and Livesley, S. J. (2015). The conservation value of urban green space habitats for Australian native bee communities. *Biological Conservation*, 187:240–248.

Traveset, A. and Richardson, D. M. (2014). Mutualistic Interactions and Biological Invasions. *Annual Review of Ecology, Evolution, and Systematics*, 45(1):89–113.

Urbanowicz, C., Muñiz, P. A., and McArt, S. H. (2020). Honey bees and wild pollinators differ in their preference for and use of introduced floral resources. *Ecology and Evolution*, 10(13):6741–6751.

van der Sluijs, J. P., Amaral-Rogers, V., Belzunces, L. P., Bijleveld van Lexmond, M. F. I. J., Bonmatin, J.-M., Chagnon, M., Downs, C. A., Furlan, L., Gibbons, D. W., Giorio, C., Girolami, V., Goulson, D., Kreutzweiser, D. P., Krupke, C., Liess, M., Long, E., McField, M., Mineau, P., Mitchell, E. A. D., Morrissey, C. A., Noome, D. A., Pisa, L., Settele, J., Simon-Delso, N., Stark, J. D., Tapparo, A., Van Dyck, H., van Praagh, J., Whitehorn, P. R., and Wiemers, M. (2015). Conclusions of the Worldwide Integrated Assessment on the risks of neonicotinoids and fipronil to biodiversity and ecosystem functioning. *Environmental Science and Pollution Research*, 22(1):148–154.

van der Sluijs, J. P. and Vaage, N. S. (2016). Pollinators and Global Food Security: the Need for Holistic Global Stewardship. *Food Ethics*, 1(1):75–91.

van der Steen, J. (2015). The foraging honey bee. *BBKA News - The British Bee Journal*, 2015(February):43–46.

Vanbergen, A. J., Garratt, M. P., Vanbergen, A. J., Baude, M., Biesmeijer, J. C., Britton, N. F., Brown, M. J., Brown, M., Bryden, J., Budge, G. E., Bull, J. C., Carvell, C., Challinor, A. J., Connolly, C. N., Evans, D. J., Feil, E. J., Garratt, M. P., Greco, M. K., Heard, M. S., Jansen, V. A., Keeling, M. J., Kunin, W. E., Marris, G. C., Memmott, J., Murray, J. T., Nicolson, S. W., Osborne, J. L., Paxton, R. J., Pirk, C. W., Polce, C., Potts, S. G., Priest, N. K., Raine, N. E., Roberts, S., Ryabov, E. V., Shafir, S., Shirley, M. D., Simpson, S. J., Stevenson, P. C., Stone, G. N., Termansen, M., and Wright, G. A. (2013). Threats to an ecosystem service: Pressures on pollinators. *Frontiers in Ecology and the Environment*, 11(5):251–259.

VanEngelsdorp, D. and Meixner, M. D. (2010). A historical review of managed honey bee populations in Europe and the United States and the factors that may affect them. *Journal of Invertebrate Pathology*, 103:S80–S95.

Visscher, P. K. and Seeley, T. D. (1982). Foraging strategy of honeybee colonies in a temperate deciduous forest. *Ecology*, 63(6):1790–1801.

von Frisch, K. (1965). *Tanzsprache und Orientierung der Bienen*. Springer Berlin Heidelberg, Berlin, Heidelberg.

Wang, H., Zhang, S. W., Zeng, Z. J., and Yan, W. Y. (2014). Nutrition affects longevity and gene expression in honey bee (Apis mellifera) workers. *Apidologie*, 45(5):618–625.

Wang, X. C., Liu, C., Huang, L., Bengtsson-Palme, J., Chen, H., Zhang, J. H., Cai, D., and Li, J. Q. (2015). ITS1: A DNA barcode better than ITS2 in eukaryotes? *Molecular Ecology Resources*, 15(3):573–586.

Waser, N. M. (1986). Flower Constancy: Definition, Cause, and Measurement. *The American Naturalist*, 127(5):593–603.

Watts, S., Ovalle, D. H., Herrera, M. M., and Ollerton, J. (2012). Pollinator effectiveness of native and non-native flower visitors to an apparently generalist Andean shrub, Duranta mandonii (Verbenaceae). *Plant Species Biology*, 27(2):147–158.

Weidenmüller, A. and Tautz, J. (2002). In-hive behavior of pollen foragers (Apis mellifera) in honey bee colonies under conditions of high and low pollen need. *Ethology*, 108(3):205–221.

Wells, H. and Wells, P. H. (1983). Honey Bee Foraging Ecology: Optimal Diet, Minimal Uncertainty or Individual Constancy? *The Journal of Animal Ecology*, 52(3):829.

Wenner, A. M., Meade, D. E., and Jon Friesen, L. (1991). Recruitment, Search Behavior, and Flight Ranges of Honey Bees. *American Zoologist*, 31(6):768–782.

Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York.

Wilcove, D. S., Rothstein, D., Dubow, J., Phillips, A., and Losos, E. (1998). Quantifying Threats to Imperiled Species in the United States. *BioScience*, 48(8):607–615.

Wilde, J., Bratkowski, J., and Siuda, M. (2003). Pollen collection by 3 subspecies of honeybee Apis mellifera L . *Acta Biologica Universitatis Daugavpiliensis*, 3(2):101–106.

Williams, N. M., Cariveau, D., Winfree, R., and Kremen, C. (2011). Bees in disturbed habitats use, but do not prefer, alien plants. *Basic and Applied Ecology*, 12(4):332–341.

Winfree, R., Williams, N. M., Gaines, H., Ascher, J. S., and Kremen, C. (2008). Wild bee pollinators provide the majority of crop visitation across land-use gradients in New Jersey and Pennsylvania, USA. *Journal of Applied Ecology*, 45(3):793–802.

Woolhouse, M. E., Haydon, D. T., and Antia, R. (2005). Emerging pathogens: The epidemiology and evolution of species jumps. *Trends in Ecology and Evolution*, 20(5):238–244.

Xue, J. and Su, B. (2017). Significant remote sensing vegetation indices: A review of developments and applications. *Journal of Sensors*, 2017(May):1–17.

Yonekura, K. and Kajita, T. (2007). Japanese botanical name-scientific name index YList.