

What's on the menu? : Investigating diet shifts of the European garden spider along replicated urbanisation gradients.

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Preface:

This thesis is part of the SPIN City project: a research project that aims to study how species adapt to city life. It focuses on the trophic and thermal adaptations to urbanisation in the European garden spider (*Araneus diadematus*) and builds on an earlier developed citizen-science initiative (Spiderspotter). In this framework, I determined how urbanisation is causing shifts in the diets of European garden spiders via DNA metabarcoding and thereby showing the diversity of insects in cities.

What are we going to eat today? A question that is asked almost every day, even for spiders. The plate is replaced by a web and their menu depends on what they can catch. But what if you had to eat the same meal every day? Our European garden spiders are becoming more and more reliant on a narrow range of insects on their menu. With this research I want to recognise the important role that arthropods play in the food web and how urbanisation is contributing to their decline, which in turn is homogenising the diets of higher trophic levels.

This thesis would not have been possible without the help of Drs. K. De Wolf and Prof. Dr. D. Bonte. I am also very grateful for the opportunity to perform lab work and learn about the bioinformatics at the Department of Biology Genetics, Ecology and Evolution department of Aarhus University.

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Gent, 27th May 2024

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1 INTRODUCTION

1.1 Urbanisation

1.1.1 Urbanisation: definition and trends in Flanders:

The Earth is rapidly becoming an urban planet. Currently, over half of the world's population lives in cities, and this proportion is growing. It is not only that urban densification is happening fast, but that urban expansion does not seem to be stopping either. Our cities are becoming bigger and more densely populated at a rate that exceeds the speed limit. By definition, a rural area becomes urbanised as a result of economic development and industrialisation. In demographic terms, urbanisation refers to the redistribution of populations from rural to urban settlements over time (Kuddus et al., 2020).

In the European context, almost the entire area of Belgium is considered urban (Pisman & Vanacker, 2021). The effects of urban sprawl are the most pronounced in Flanders, the densely populated northern region of the country. Approximately 32.6% of its territory is occupied by settlements, and its landscapes are highly fragmented by ribbon and dispersed development (Vermeiren et al., 2018). Almost 28.7% of the total area of Flanders is considered urban when urbanisation is measured by the total amount of built-up area. This means that 28.7% of the 1,352,200 hectares are covered by buildings, which has various consequences for the living conditions in these areas (e.g., the urban heat island effect) (*Built-up Area*, n.d.). The expectations for 2050 are even more concerning, as they predict that the built environment will continue to expand, with 41.5% of Flanders being covered by buildings (*BRV_kleinwitboek_ENG.Pdf*, n.d.). Not only the number of buildings is increasing but the density of urbanised areas is also rising, which is leaving less and less space for green spaces.

If the future is urban, it is essential that we find solutions that lie within cities. As cities have an immense impact on biodiversity, energy, and the environmental balance of a country, it is clear that they must be at the center of attention.

1.1.2 Effect of urbanisation on the physical environment

The amount of land required to provide food, energy and materials for urban dwellers is expanding, with an ecological footprint that is often 200 times larger than the area of the city itself (Wigginton et al., 2016). The land changes needed to build cities and meet the demands of urban populations themselves drive other types of environmental change. Cities are known to alter the environment in many ways, from air, water, and noise pollution, as well as the production of artificial light. For instance, urban centres are the primary source of greenhouse gas emissions due to the concentration of traffic and industry, making them a point source of CO₂ and other greenhouse gases that affect Earth's climate, as well as trace gases such as NO, NO₂, NO₃, SO₃, HNO₃ and various organic acids (Wigginton et al., 2016). This has an impact on regional and global ecosystems, for example, through eutrophication in cities along rivers and coastlines.

One of the anthropogenic climate changes caused by urbanisation is the urban heat island effect (UHI). This is caused by an imbalance in the energy/material balance of cities compared to rural areas. Cities are characterised by differences in input and output flows, an increased number of constructions with higher heat absorption, increased heat production, increased run-off of precipitation and reduced evapotranspiration all of which contribute to the energy/material imbalance (Vujoovic et al., 2021). This

results in the formation of heat islands in urban areas, which are warmer than the surrounding rural and suburban areas (Fig. 1). The surfaces in the urban areas tend to warm more rapidly than those of the surrounding rural areas. The heat stored in urban building materials during the daytime is released, which makes city centres to become hotter. The elevated temperatures resulting from urban heat islands, especially during summer months, can have an impact on the environment and the quality of life of the communities (Grimm et al., 2008).

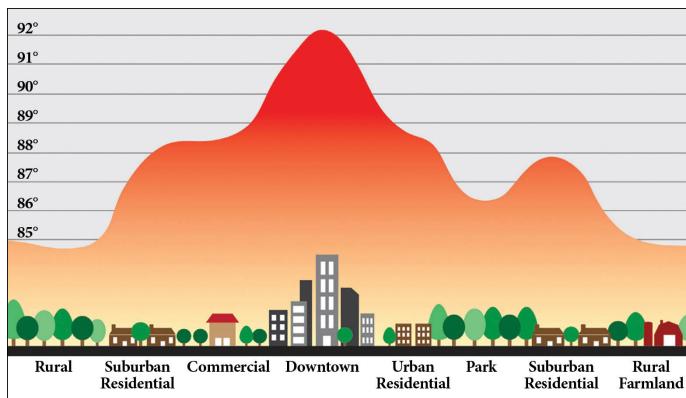


Fig. 1: Urban heat island effect (Grimm et al., 2008). Heath (y-axis) measured along the urbanisation gradient (x-axis). Lower temperatures are found towards more the rural sites compared to downtown, which is a highly urbanised habitat.

Another drastic ecological change is the expansion of impervious surfaces, such as paved roads and roofs. The imperviousness of paved materials prevents water from infiltrating into the soil, which in turn promotes the occurrence of floods and polluted runoff. This runoff harms aquatic ecosystems, which in turn reduces the diversity of insects as well. Along the urbanisation gradient urban cores (downtowns) are often characterised by low vegetation cover, elevated temperatures (UHI effect), elevated levels of chemical and light pollution, and poor water quality. Furthermore, they are isolated from one another and are dominated by non-native plant species (Grimm et al., 2008). As a consequence, environmental conditions in highly urbanised areas are expected to favour generalist species with broad diets, good dispersal capacities and high tolerance for heat and diverse pollutants (Burkman & Gardiner, 2014; Fenoglio et al., 2021; Langelotto & Hall, 2021).

1.1.3 Effects of urbanisation trend on biota

Insect populations are shrinking all over the world. Urbanisation is known to be a major factor in this global decline of insects and several other species around the world. In addition to the numerous forms of pollution (e.g., light and air) present in a city, construction also leads to habitat fragmentation, a well-known problem for biodiversity. Fragmentation reduces the connectivity between suitable habitat fragments, placing severe pressure on ideal habitats for arthropods living in cities (Dahirel et al., 2017; Lequerica Tamara et al., 2021). The impact of urbanisation is not a simple, straightforward effect; rather, it consists of multiple effects on multiple elements of the environment. This multiplicity of effects makes it difficult to predict how different species will respond to urbanisation. Consequently, selecting an appropriate range of spatial scales for the analysis of urbanisation and its impact on diversity patterns is of great relevance. A simplified urban ecology framework often fails to account for the multiple and contrasted urban habitat types contributing to the urban mosaic. The urban landscape is characterised by heterogeneity and fragmentation, comprising a complex variety of habitat types in three-dimensional mosaics (e.g., urban parks, forest remnants, dense residential areas, gardens, etc.) (McDonnell & Pickett, 1990). The incorporation of hierarchically nested sampling designs is a method that can be employed to address this complexity.

Cities homogenise the physical environment because they are built to meet the relatively narrow needs of just one species, namely our own (McKinney, 2006). The ability of species to withstand the urban environment, will determine more or less their presence in urban areas. As previously stated, these conditions are often very different from the surrounding rural areas, and a different set of species may be dominant or present in urban areas. In theory, the distinction is often made between ‘urban avoiders’, species that are very sensitive to human activity, and ‘urban exploiters’. The latter are those whose abundance increases with urbanisation, and they exploit the new and altered environment, based on the strategy of a species (Blair et al., 2001). Consequently, as urbanisation continues to expand across the globe, there is an increase in the biological homogenisation. This is due to the fact that the same species that are able to adapt to urban environments become increasingly widespread and locally abundant in cities across the planet (McKinney, 2006). It is important to note that the effects of urbanisation are taxon-specific. Every organism may experience the environment in a different way, and the same landscape can hence be perceived as heterogeneous by one species and as fragmented by another (Concepción et al., 2015).

1.2 Prey community assembly

1.2.1 The base of food webs

In food webs, species interact with other species living in the same area (Fig. 2). If only certain species survive, these interactions may be significantly altered, which affects the trophic relationships (Chapin et al., 2000). This also means that even if a species is able to withstand all the changes in physical conditions and habitat it will still not survive if its prey is not present there. The consequences of such changes in relationships will depend on the strength of the interaction and the sensitivity of the species (Ives & Carpenter, 2007). This is why generalist species, which do not depend on a single or a few relations, are often more resilient to environmental change than specialist species (Colles et al., 2009).

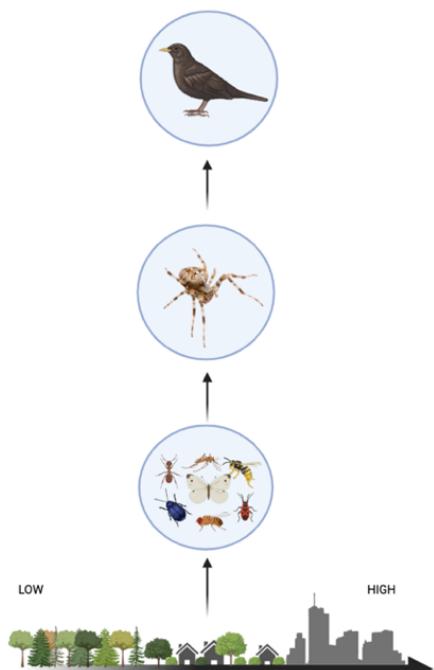


Fig. 2: Illustration of the cascading effects. At the base of the food web are the arthropods, followed by a higher level, the European garden spider, which in turn is the meal of the higher trophic level, e.g., insectivorous birds.

Arthropods represent a very diverse group of organisms that form the basis of food webs. Insects form the main animal building blocks of terrestrial ecosystems, as they are both immensely abundant and immensely diverse (Roslin, 2024). These flying insects provide essential ecological functions and human services, such as pollination, pest control and decomposition (Remmel et al., 2024). However, these functions and services are being threatened by anthropogenic declines in insect populations. Changes in arthropod richness and biomass can have cascading effects on higher trophic levels, including our study species the European garden spider, *Araneus diadematus* (Van Schrojenstein et al., 2021). These potential cascading effects have only been documented so far for insectivorous birds (Nyffeler et al., 2018). Previous research (Nyffeler & Bonte, 2020) has demonstrated a significant decline in the abundance of the orb-weaving spider *Araneus diadematus* over the last half-century, revealing a bottom-up trophic cascade in response to the

widespread insect losses over much of Europe in recent decades (Nyffeler & Bonte, 2020). This decline is paralleled by the dramatic declines in the abundance of other insectivorous animals, including insectivorous birds, bats, frogs, and lizards, which have been documented in recent decades (Nyffeler & Bonte, 2020). Currently, we are dealing with an ecological crisis in which trophic webs are being eroded and degraded as a result of human impacts on the environment over decades (Nyffeler & Bonte, 2020). This starvation will cause entire ecosystems to collapse (Lequerica et al., 2021). Despite the fact that terrestrial arthropods are involved in multiple functional processes, they remain understudied and generally overlooked in conservation strategies and large-scale monitoring (Leandro et al., 2024).

1.2.2 Biotic homogenisation

Common changes in the community composition due to urbanisation include biotic homogenisation (McKinney, 2006), species turnover (Fenoglio et al., 2021), and the dominance of urbanisation ‘winners’ (i.e., species with certain traits that can benefit from urbanisation) at the expense of sensitive species (Fenoglio et al., 2021). Cities can still maintain a relatively constant biodiversity, but it will be dominated by species that are generalists, mobile, small-bodied, or heat-tolerant (Hahs et al., 2023; Piano et al., 2016). Smaller arthropod species are likely less adversely affected than larger ones in perturbed and low-resource habitats. This is due to their lower energetic requirements, faster development, and higher probability of escaping from perturbations (Moretti et al., 2016; Peters, 1983). Species with higher drought tolerance may be favoured because they could cope better with the warmer environmental conditions that are typical of cities (Potapov et al., 2020; Weldon et al., 2016). Furthermore, species with a high dispersal capacity may be better able to colonise more fragmented and frequently disturbed habitats (Entling et al., 2011; Kneitel, 2018; Löveï & Sunderland, 2003).

The species community consists of an equal number of dominant and more tolerant species. This phenomenon is referred to as biotic homogenisation of species composition and leads to reduced genetic heterogeneity, taxonomic range and functional capacity between communities (Fenoglio et al., 2021). The homogenisation of biotic communities occurs across urban areas due to the similarities in environmental conditions (McKinney, 2006). Those species that are able to withstand similar conditions in different urban areas will be very similar. Consequently, non-native species are capable of enriching local biodiversity (Sax & Gaines, 2003), although this is offset by the subsequent extinction of unique local species that are lost to the global species pool (McKinney, 2006).

Species often differ in their contribution to a given ecosystem process, which means that the type of species involved is as important as the number of species in determining ecosystem functioning (Fenoglio et al., 2021). The diversity in species can have a significant impact on ecosystem functioning and the ecosystem services provided by the ecosystem. The results of our previous study (Urbanisatie en het verlies aan insectendiversiteit, Verstraelen N., 2022) and the study by Dahirel et al. (2017), demonstrate the influence of increasing urbanisation on the supply of flying insects (Fig. 3). Namely, a reduction in mean arthropod body size and lower diversity in highly urbanised areas due to the negative effects of urbanisation. In our study, the prey communities on sticky traps were identified and measured in order to gain an overview of the current insect communities along the urbanisation gradient. The total biomass of each unique location and diversity metrics (species richness, Shannon

and Simpson) were used to investigate the expected decline in body size and diversity with increasing urbanisation. The two diversity metrics, species richness and the Shannon diversity index, showed lower values in the urbanised sites at the local scale (200 x 200 m) within each of the landscape scales (3 x 3 km). The NMDS results showed evidence of biotic homogenisation in highly urbanised areas, with Diptera as the most abundant order.

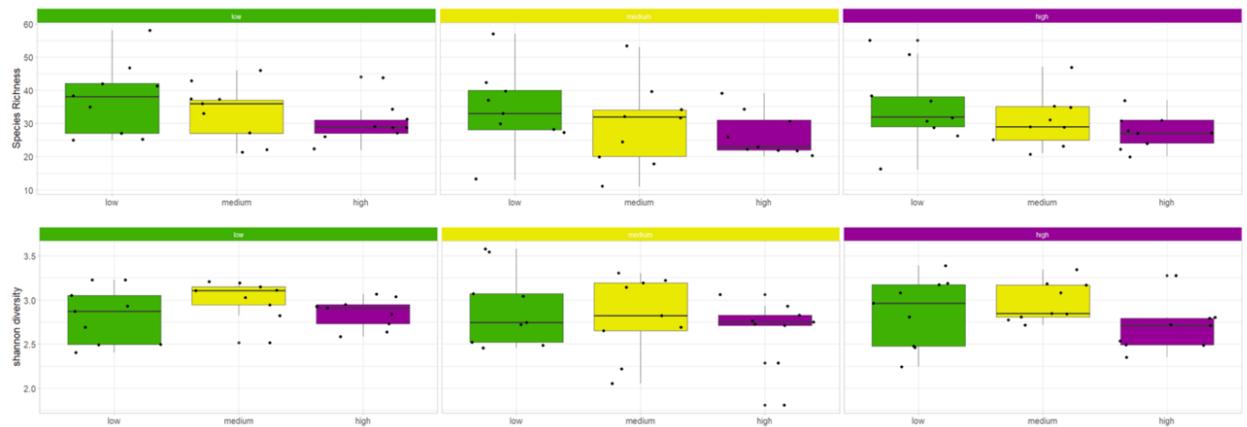


Fig. 3: Overview of the differences in species richness and Shannon's diversity indices collected on the sticky traps across urbanisation gradients. Each column represents the degree of urbanisation at the landscape scale (3x3 km) and within each of these columns, the three degrees of urbanisation on the local scale (200x200 m). Further explanation on how these degrees are assigned can be found in the Material and Methods section. Compared to urbanised sites (purple), rural sites (green) have higher median species richness at the local scale. This trend is also observed for the Shannon diversity index.

1.3 Spiders as model organism

In order to study the effect of urbanisation (e.g., pollution and fragmentation) on species, the order of the Araneae or spiders within the arthropods seems to be a particularly interesting group to use as study organisms. Several spider species can live in cities, due to their high ecological plasticity (Turnbull, 1973). They have an important sociological, agronomical, and economical role (Marc et al. 1999). Spiders fulfil this important role by adding an extensive part to pest control, by capturing and consuming great amounts of insects on a daily basis. Despite these crucial roles they perform in the ecosystem, only a few studies focus on spiders in urbanised areas. Furthermore, the spiders represent a significant source of food for a lot of birds, lizards, and mammals within the trophic food web (Collier et al., 2002).

More in general, the use of arthropods such as spiders to study urbanisation has several advantages, as demonstrated by McIntyre (2000). Firstly, they represent a diverse group that can be used to assess the overall biodiversity of an area. Secondly, they have a relatively short generation time, which allows them to respond quickly to anthropogenic activity. Thirdly, they are quite easy to sample. Finally, they represent a spectrum of trophic levels. Fluctuations in the availability and biomass of prey have an impact on the direct predators, such as spiders, which in turn affects the birds, lizards, and mammals that depend on them as a source of food. For example, birds rely strongly on insects as a food source for their chicks, and thus experience both directly as indirectly the consequences of a decline in prey availability and prey diversity.

1.3.1 *Araneus diadematus*

Our study organism, the European garden spider (*Araneus diadematus*), is an orb-weaver spider that constructs large orb webs. This species has a Holarctic distribution and is relatively common in both the Palaearctic and Nearctic regions (Bonte et al., 2008). Figure 4 illustrates the distribution of this species in Flanders over the past 15 years.

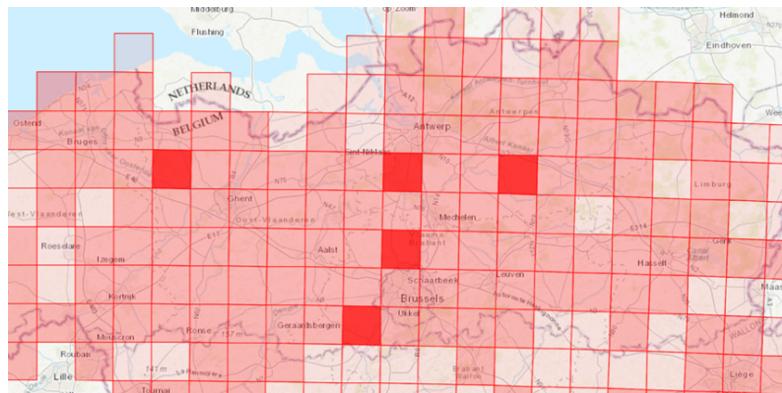


Fig. 4: A map illustrating the distribution of the European garden spider in Flanders over the past 15 years (source: www.waarnemingen.be). The saturation of the red squares indicates the abundance of the species at these sites. A higher saturation value indicates a higher abundance.

The spider can be found in a wide range of habitats, including different types of woodland, scrub and other vegetation, hedgerows and roadsides, buildings (sometimes inside) and gardens, as well as heathland, quarries, and cliffs (Lee & Thomas 2015). This allows us to study the effects of urbanisation along an urbanisation gradient, as our study organism occurs in both urban and rural habitats. The main determinant of these habitats is the availability of suitable attachment sites for the construction of the orb web (Wise, 1993). The webs are built between all types of vegetation at a height of approximately one metre (Herberstein, 1997) and have a diameter ranging from 15 to 25 cm with a mesh width of around 3 mm and a web area of 300 cm² (Nyffeler, 1982). The web surface and mesh size are reduced in areas of local-scale urbanisation, while the web area increases with landscape-scale urbanisation (Dahirel et al., 2018).

The spider hangs in the centre of the web, waiting for prey to be caught in the web (Fig. 5a). The webs are rebuilt and recycled on a daily basis, allowing the spiders to adapt to the current environmental conditions (Breed et al., 1964).

As with all orb weaving spiders, *A. diadematus* is a sit-and-wait predator. In order to use this ‘low’ energy hunting method, spiders need to build their typical costly aerial webs to trap flying insects (Fig. 5a). The diet of such sit-and-wait predators is mainly influenced by the composition of their prey community. The composition of the spider’s prey community depends on the species trapped in the spider’s sticky web and the community living near the spider’s web. In the temperate climatic zone, aerial web-spinning spiders primarily feed on dipterans (e.g., mosquitoes and flies), aphids and hymenopterans. These insects are among the most dramatically decreased in abundance and biomass in recent decades (Nyffeler & Bonte, 2020). The spiders strongly rely upon their flying prey and are therefore a highly vulnerable predator group in regions where there has been a significant decline of flying insects.

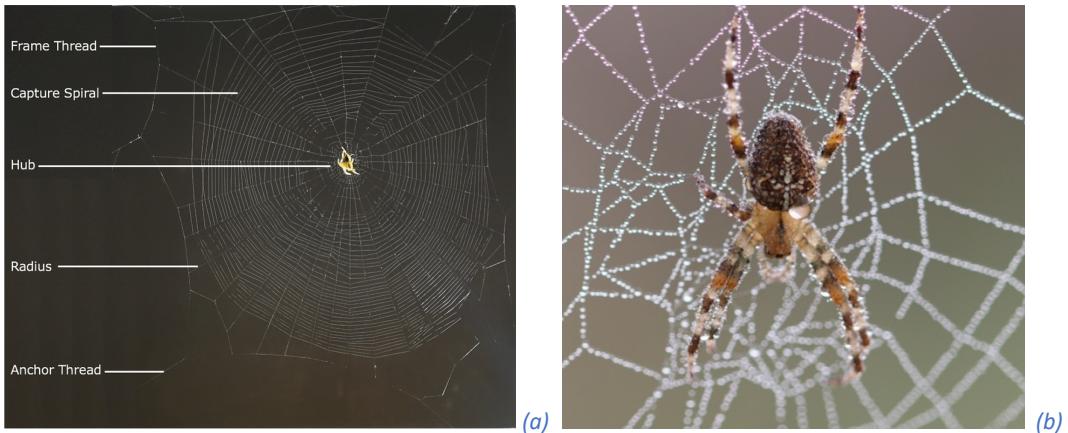


Fig. 5: (a) Web of the European garden with indications of main structures (Mulder et al., 2021). (b) Picture by Geert Vanhaverbeke of the European garden spider in its web

The size of *A. diadematus* varies between the sexes. Females are between 10 and 18 mm in length, with a large, triangular abdomen. Males are smaller, between 4 and 8 mm, with a much smaller, more elongated abdomen (Roberts, 1998). Their body coloration can vary from pale yellow to almost black. The species has a distinct cross-like pattern on its back (Fig. 5b), which makes them easily distinguishable from most other orb-web spiders (Foelix, 1982).

Adult females of *A. diadematus* are most active from early August to the end of November, although in some years individuals have been found from the end of June or even into December. Adult males, on the other hand, are only found during August and September, with occasional individuals found from mid-June or until the end of November (Roberts, 1998). At the end of the summer season, females engage in mating with males. After pairing, female spiders attempt to catch food in their web for the development of their eggs. Fertilised females deposit egg sacs containing several hundred eggs. The female remains in close proximity to the egg sac, protecting it until she dies in late autumn. In the following spring, around May, the eggs hatch, and after a period of staying together in a small ball, the young spiderlings disperse into the surrounding area (Burch et al., 1979).

1.4 Metabarcoding

In the past, researchers have typically relied on morphological identification of capture taxa, which can be time-consuming and requires taxonomic expertise (Leandro et al., 2024). The development of molecular tools, such as DNA metabarcoding, has opened up new ways of gathering information on patterns of diversity and studying changes in community composition (Creer et al., 2016; Taberlet et al., 2012; Valentini et al., 2009, Svenningsen et al., 2021). DNA metabarcoding enables the identification of multiple species from mixed samples based on high-throughput sequencing (HTS) of specific DNA markers (Liu et al., 2019). The DNA barcodes are used as a proxy for species detection and identification (Fig. 6). The 5' region of the mitochondrial DNA (mtDNA) gene cytochrome c oxidase I (COI) is the standard marker for DNA barcoding. It is a standardized 685 bp fragment proposed as a universal marker for species identification (Pentinsaari et al., 2016). Cytochrome C oxidase I (COI) sequences are used, as they are better represented in the reference sequencing databases compared to 16S rRNA. This approach allows for the distinguishing of animal taxa, given the relatively high mutation rate of the COI gene (Liu et al., 2019).

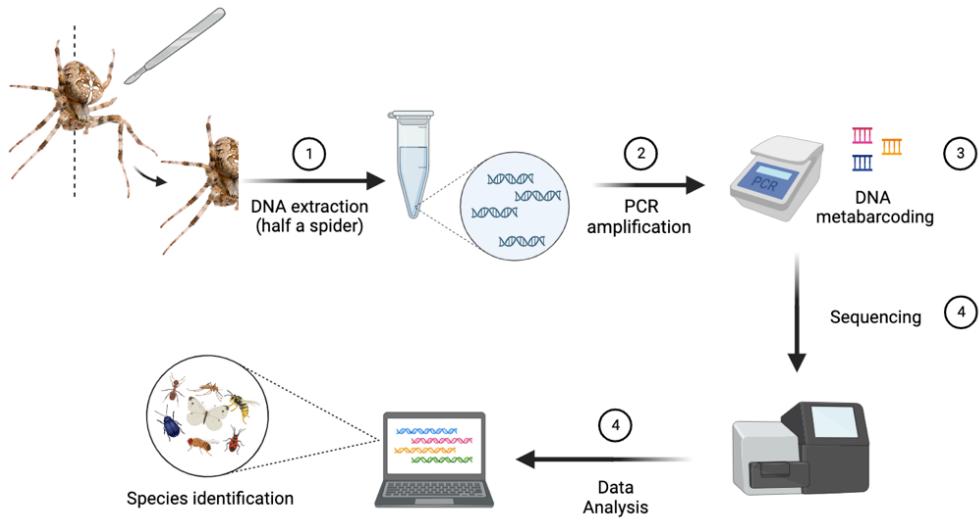


Fig. 6: Illustration of the different steps of DNA metabarcoding.

Primers TagF_ZBJ-ArtF1c and TagR_ZBJ-ArtF2c are a pair of arthropod primers used to amplify the mitochondrial COI gene (Zeale et al., 2010). One advantage of these primers is that they amplify different amplicon sizes and do not overlap in the COI region, thus eliminating the possibility of competition for the same primer binding sites (Batuellas et al., 2021). To prevent, or highly reduce, amplification of the spider COI sequence a blocking primer is used. This primer has a C3 spacer modification at the 3' end (Aradia-R-blk-C3: 5'-CCA AAT CCC CCA ATT AAA ATA GGT ATA-C3 spacer-3'). The blocking primer will bind to the DNA whose amplification is to be prevented, in this case, the DNA of the European garden spider, which enhances the detection of prey-specific DNA. The second PCR uses introduced linker-tagged indexed adapters (Illumina_MIDx_tagF, Illumina_MIDx_tagR) that were compatible with Illumina platforms and perfectly matched linker tags of the first. The dual tagging approach (Fig. 7) provides each sample with a unique label, allowing for the separation of samples after demultiplexing. Furthermore, dual tagging enables the detection of potential biases and errors resulting from cross-contamination and tag-jumping (Liu et al., 2019).

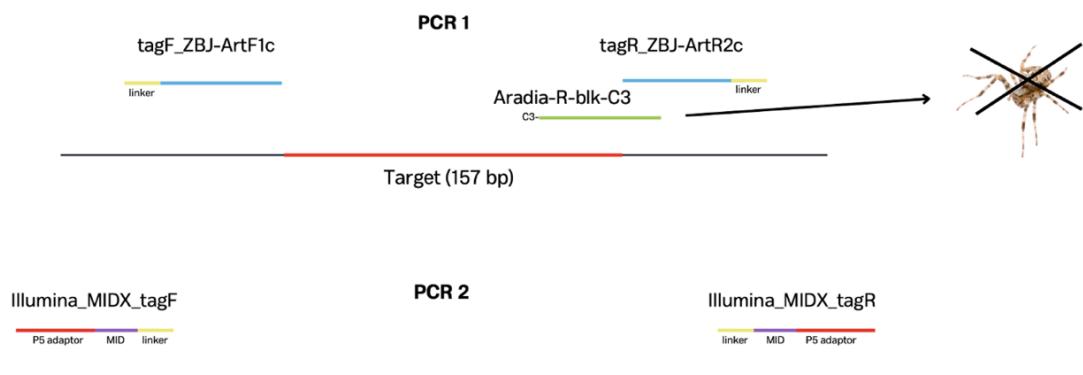


Fig. 7: Overview of the two PCR steps and the used primers and blocking primers in each of the steps.

After demultiplexing and removal of non-biological nucleotides (e.g., primers, adapters, linkers), the DADA2 pipeline generates an amplicon sequence variant table, a higher resolution analogue of the traditional OTU table (Callahan et al., 2016). It records the number of times each exact amplicon sequence variant was observed in each sample (Callahan et al., 2017). Taxonomic identities are assigned to the ASVs. Working with ASVs allows us to distinguish sequence variants that differ by as little as one nucleotide. It does not use a fixed sequence dissimilarity threshold, most commonly 3%, to cluster sequencing reads, which is done to construct molecular operational taxonomic units (OTUs) (Westcott & Schloss, 2015; Kopylova et al., 2016). This means that ASVs capture all biological variation present in the data (Callahan et al., 2017). Another advantage of using ASVs is that the ASVs derived from a given dataset can be reproduced in future datasets, allowing valid comparisons between datasets (Callahan et al., 2017). This is of particular interest if we want to replicate studies such as this one, which analyses the decline of insect communities along urbanisation gradients over time.

1.5 Response of spider to urbanisation

The study by Dahirel et al. (2018) has already demonstrated that smaller cross spiders are frequently observed in highly urbanised areas. The decrease in their body size is in line with the smaller biomass of prey available in cities and the heat island effect. Due to the strong built-up area (concrete and asphalt) and the disappearance of green vegetation, cities are on average warmer than rural areas (Horne et al., 2015; Kaiser et al., 2016). The higher temperatures, which are often notable in cities, cause increased metabolic rates that reduce the body size of ectothermic animals (Dahirel et al., 2017). As global warming intensifies, there is a shift expected towards communities composed of smaller individual. Body size is an important determinant of food webs and community dynamics (Merckx et al., 2018), and it has been demonstrated that ecosystem structure and function are affected when taxa in urban areas are represented by smaller species (Brose et al., 2017; Kalinkat et al., 2013).

Furthermore, it has been hypothesised that predators living in cities may require a higher intake of prey to maintain their necessary nutritional values (Hawley et al., 2014). The nutritional quality of prey varies and affects the survival, growth, and reproduction of spiders (Wilder et al., 2011; Wilder et al., 2013). Foraging on a diverse range of prey may allow generalist predators to maintain a balanced nutrient intake (Rendon et al., 2019). Previous research has demonstrated that spiders adapt their web-building behaviour in response to abiotic conditions and the presence and characteristics of potential prey (Bonte et al., 2008; Schneider & Vollrath, 1998; Vollrath et al., 1997). Related with this change in web-building, orb-weaving spiders have the potential to evolve through changing the intrinsic quality of biomaterials (e.g., silk) in response to the insect offer (Dahirel et al., 2018; Sensenig et al., 2010).

2 OBJECTIVES

2.1 Main objective

The overall objective of this research is to investigate the shifts in the diet of European garden spiders along replicated urbanisation gradients, both at the landscape and local scales. The diet of such sit-and-wait predators is mainly influenced by the composition of their prey community. The composition of the spider's prey community depends on the species trapped in the spider's sticky web and the community living near the spider's web. Therefore, *Araneus diadematus* is a suitable study species to investigate changes in prey composition due to urbanisation by metabarcoding of its diet.

The DNA metabarcoding analysis on the sampled European garden spiders combined with morphological identification data on prey community composition from insect sticky traps at the same collection sites will illustrate main differences in the flying insect community compositions along the urbanisation gradient. The main hypothesis is that spiders living in cities will consume less diverse flying prey compared to those living in rural areas due to the potential biotic homogenisation in cities. We try to resolve on which spatial scale urbanisation will affect prey species communities and if urbanisation alters species richness and diversity of the arthropod offer in the first place. The result will illustrate the decline in arthropod diversity across cities in Flanders and its effect on a higher trophic level.

2.2 Sub objective: Community composition

Previously, we performed sampling and studying on the arthropod prey communities of European garden spiders along the urbanisation gradients, which allowed us to quantify the flying prey supply in urban and rural sites in Flanders (Fig. 8). Both the diversity as well as the changes in their biomass along the gradient were studied to test whether there were significantly fewer large species found in urban areas, whether the species composition is less diverse in cities, and if the total prey biomass is lower. A reduction in mean arthropod body size and lower diversity in highly urbanised areas due to the negative effects of urbanisation were found together with an indication of biotic homogenisation in urbanised locations. The European garden spider is not a selective hunter. It consumes everything that gets trapped in its web and, as a result, represent the present species community. Therefore, we hypothesize that these differences will also be resembled in the analysis of the effective consumed prey.

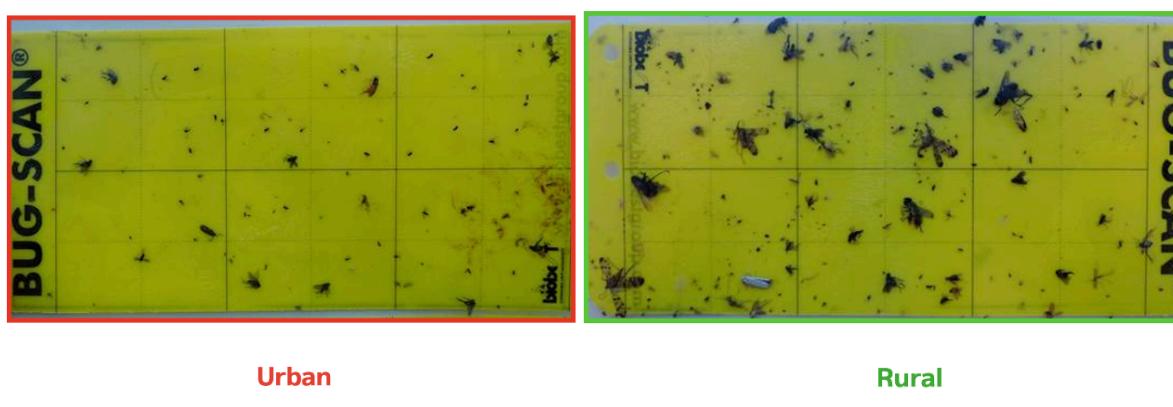


Fig. 8: The flying species communities caught on a sticky trap in an urban area compared to the community in a rural area. The community at rural sites is more diverse than that at urban sites. The total biomass of prey was found to be significantly lower in urban sites. A smaller number of large species were observed in urban areas.

2.3 Sub objective: Effective prey consumption

The effective prey consumption by the European garden spider will depend on the behaviour of the species, or in the case of orb-web spiders, on their web-building. Traditional trapping methods are constrained by limitations intrinsic to the community, including a tendency to favour certain groups and an oversampling of specific species (Leandro et al., 2024). With recent advances in metabarcoding, it is now possible to study trophic interactions on a larger scale, thereby avoiding the potential for overinterpretation. The use of DNA metabarcoding will facilitate the identification of additional species, particularly those that are challenging to distinguish morphologically (e.g., hoverflies). In other words, the use and improvement of metagenomics and barcode libraries will help to increase the knowledge and preservation of the ‘little things’ that have frequently been overlooked in conservation (Leandro et al., 2024).

To investigate the spider diet across urbanisation gradients, COI metabarcoding of the spider gut content will be analysed. While this method does not provide us with an absolute quantification, it will enable us to determine if certain orders/families are missing and if this is consistent across the urbanisation gradient. The results will provide further insight into the hypothesis that prey consumption in urban areas is less diverse and will help in understanding how declines in flying arthropod species richness will lead to shifts in the spider diet.

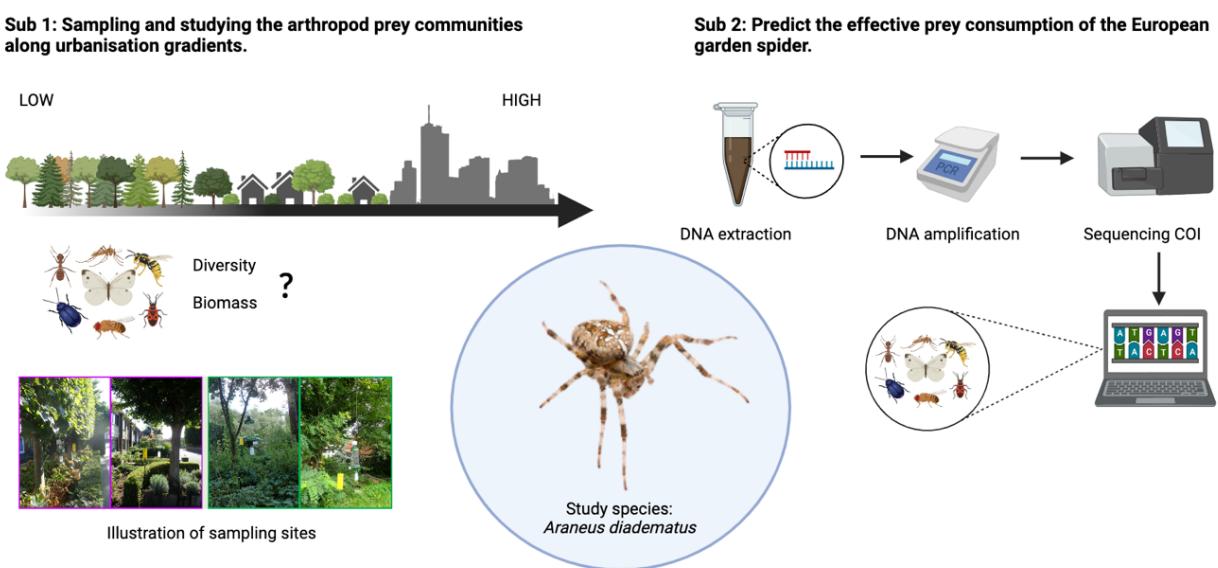


Fig. 9: Overview of the different objectives of this research. Sub. 1 focussed on the decline in diversity and biomass of arthropod prey communities along urbanisation gradients. Sub. 2 will aim to find the effective prey consumption of our European garden spider in rural versus urban locations and thereby validate the results of sub1.

3 MATERIAL & METHODS

3.1 Study sites & sampling design

The study area is situated in the Flemish region of Belgium, which is one of the most urbanised places in Europe. As in the previous study by Piano et al. (2017), sampling was carried out in a polygon of approximately 5000 km², including the city centres of Brussels, Antwerp, and Ghent. The sample sites in this region are densely populated, and the landscape consists of urban areas embedded within an agricultural or seminatural matrix (Piano et al., 2017). To gain insight into the effect of spatial scale, a hierarchically nested sampling design was used, whereby three local scale levels of urbanisation (non-urban, semi-urban and urban subplots) were repeatedly sampled across three landscape scale levels of urbanisation (non-urban, semi-urban and urban plots) (Piano et al., 2017). Including the effect of spatial scale in the analysis is important because it influences the effect of urbanisation and urban landscape diversity on arthropods (Egerer et al., 2017). The degree of urbanisation was assigned based on the percentage of built-up area (BU) (Merckx et al., 2018). This proxy for the amount of urbanisation takes into account only the area covered by buildings, excluding roads, pavements, parking places, etc. There are three scale levels as follows: low urbanised areas with less than 3% BU are shown in green, moderately urbanised areas with 5-10% BU are shown in yellow and locations with more than 15% BU area are assigned to the highly urbanised category, shown in red (Fig. 10). At the landscape scale, we sampled nine plots with low built-up area (3 x 3 km), nine plots with intermediate built-up area, and nine plots with a high built-up area. Within each plot, the impact of local-scale urbanisation was investigated by sampling three 200 x 200 m subplots, which were selected based on exactly the same built-up ratio levels as the landscape-scale. This resulted in a total of 81 subplots (Fig. 10). By utilising such a nested design, we will be able to extend the gradient and compare highly urbanised areas with more natural areas and all levels in between. The observed differences along the urbanisation gradient can inform us about species diversity in and around cities and provide an overview of potential biodiversity loss.

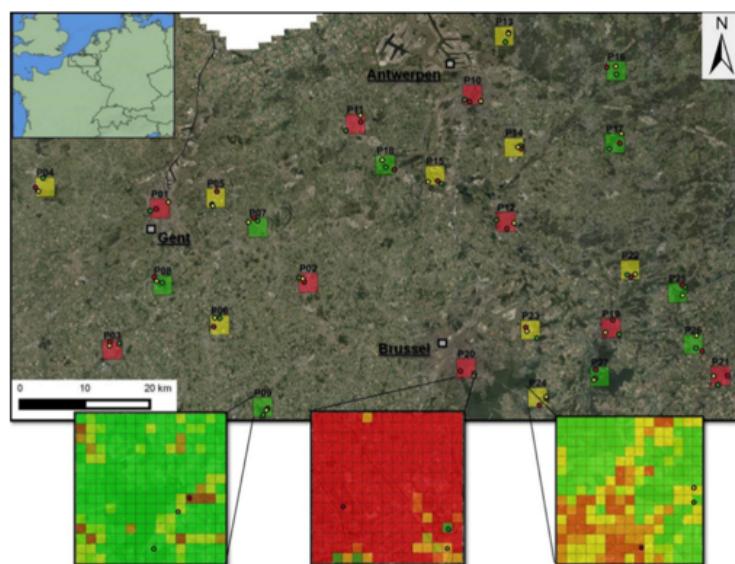


Fig. 10: Map of the study area around Antwerp, Ghent, and Leuven, showing the location of the 27 3 x 3 km plots. The different colours refer to the different classifications of urbanisation (green = low urbanisation; yellow = intermediate urbanisation; red = high urbanisation). Each plot is subdivided in 200 x 200 m subplots and assigned the same colour codes as for the plots. For each plot, three subplots belonging to the low, intermediate, and high urbanisation categories were selected as sampling sites. [Picture by Piano et al. 2017].

3.2 Data collection

3.2.1 Sampling of spiders

A total of 530 female European garden spiders were captured over a period of 23 days, from 21 September to 14 October 2021, along the urbanisation gradients. Sampling during the summer months was done in order to cover their activity periods as this is the time where the female spiders are engaged in the capture of food for the development of their eggs. The spiders were mature and had large webs during this time of the year, which made them easier to spot. A total of approximately five spiders were sampled at each unique location (subplotid). These five specimens will be used to represent the prey community present at each location. All 530 collected spider samples were placed in 2 mL labelled Eppendorf tubes labelled with the relevant information and stored at -80°C prior to DNA extraction.

Table 1: Overview of the different study sites of sampling. In this table the 27 sampling sites are listed with their location, region, and urbanisation degree on landscape scale (3 x 3 km). Each of these plotid's also has three local urbanisation levels (200 x 200 m), being high, intermediate, and low urbanised.

Plot ID	Study site	Region	Urbanisation on landscape scale	Landscape
P01	Gent	Gent	Strongly urbanised	R
P02	Aalst	Gent	Strongly urbanised	R
P03	Oudenaarde	Gent	Strongly urbanised	R
P04	Bellem	Gent	Intermediate level	Y
P05	Beervelde	Gent	Intermediate level	Y
P06	Hillegem	Gent	Intermediate level	Y
P07	Kalken	Gent	Rural	G
P08	Melsen	Gent	Rural	G
P09	Atembeke	Gent	Rural	G
P10	Antwerpen	Antwerpen	Strongly urbanised	R
P11	Sint-Niklaas	Antwerpen	Strongly urbanised	R
P12	Mechelen	Antwerpen	Strongly urbanised	R
P13	Brasschaat	Antwerpen	Intermediate level	Y
P14	Lint	Antwerpen	Intermediate level	Y
P15	Ruisbroek	Antwerpen	Intermediate level	Y
P16	Pulderbos	Antwerpen	Rural	G
P17	Herenthout	Antwerpen	Rural	G
P18	Bornem	Antwerpen	Rural	G
P19	Leuven	Leuven	Strongly urbanised	R
P20	Brussel	Leuven	Strongly urbanised	R
P21	Tienen	Leuven	Strongly urbanised	R
P22	Wezemaal	Leuven	Intermediate level	Y
P23	Kortenberg	Leuven	Intermediate level	Y
P24	Overijse	Leuven	Intermediate level	Y
P25	Houwvaart	Leuven	Rural	G
P26	Kerkom	Leuven	Rural	G
P27	Sint-Joris-Weert	Leuven	Rural	G

3.2.2 DNA extraction

DNA extraction was carried out with a custom magnetic bead protocol (Appendix A). The DNA of 530 spider samples was extracted using the MagAttract HMW DNA Kit (Qiagen 2020). The frozen spider was halved with one half can be placed in the solution to lyse the spider tissue, while the other half was stored in the freezer. The organism tissue was disrupted by crushing it in the solution. The tube was then incubated for two hours to prepare it for the DNA extraction step. High molecular weight

(HMW) DNA was extracted using the Qiagen MagAttract HMW DNA extraction kit. This protocol follows four steps: lyse, bind, wash and elute (Qiagen 2020). It is based on magnetic bead separation, which ensures that the isolated DNA is pure. The concentration of the purified DNA was quantified using a Nanodrop spectrophotometer. The final eluates were stored at a temperature below room temperature until PCR amplification, in order to reduce the rate of DNA degradation.

3.2.3 DNA Metabarcoding

In order to increase the amount of DNA of interest, and thereby enhance the probability of identifying as many taxa as possible, PCR is used to target specific short DNA regions for amplification (Pompanon et al., 2012; Taberlet et al., 2012). A total of 530 samples from 80 sites (Appendix Table B1.5) were used for DNA metabarcoding. Sequencing libraries containing the relevant sequences were generated by a two-step polymerase chain reaction (PCR), a dual indexing strategy for Illumina MiSeq sequencing. The MiSeq offers reasonable sequencing depth and low sequencing error rates at an affordable cost (Liu et al., 2019). All DNA samples were PCR-amplified using three different primers targeting the COI region of 157 bp DNA to study invertebrate communities present in the spider (Table 2). The primers used were tagF_ZBJ-ArtF1c as the forward primer, tagR_ZBJ-ArtR1c as the reverse primer and Aradia-R-blk-C3. The third primer, being a blocking primer, serves to prevent the amplification of spider DNA. This is a blocking probe instead of an exclusion PCR primer, in order to avoid that some taxa are excluded, mainly the ones phylogenetically close to the predator, during the amplification. The European garden spider is known to consume other spiders, and these close relatives are therefore important to include in our analysis.

Table 2: Target genomic region, the name of the primer and their sequence..

Locus/Target community	Primer name	Sequence
Arthropod primers pair	tagF_ZBJ-ArtF1c	ACGACGTTGTAAAAAGATATTGGAACWTTATTTTATTTTG
	tagR_ZBJ-ArtR1c	CATTAAGTCCCATTAWACTAATCAATTWCCAAATCCTCC
Blocking primer	Aradia-R-blk-C3	CCAAATCCCCAATTAAAATAGGTATA-C3 spacer

The COI region was amplified by polymerase chain reaction (PCR) using a two-step approach. The first round of PCR was performed in a total volume of 41 µl per sample, using 2 µl of template DNA. The PCR cycle programme included an initial denaturation at 95°C for 15 minutes, followed by 30 cycles of 94°C for 30 seconds, 46°C for 30 seconds, 72 °C for 60 seconds, and a final elongation at 72°C for 7 minutes. This was followed by a 40-cycle indexing PCR, known as the second PCR (PCR2), during which unique index combinations (tag1-40) and adapters were added (Appendix A). For PCR2, thermocycler conditions were 95°C for 15 minutes, 40 cycles of 94°C for 30 seconds, 60°C for 30 seconds, 72 °C for 60 seconds, and a final elongation at 72°C for 7 minutes. The amplicon size of the PCR products was checked by visualisation on a 2% agarose gel using GelRed™ staining. Subsequently, the amplicon products were purified using a purification method (Appendix A). The amplicon concentrations were quantified using a Qubit 4.0 fluorometer. Finally, the amplicons were pooled equimolarly for equal representation in the sequencing library, and sequencing was carried out using the Illumina MiSeq platform, at Aarhus University. Library building and sequencing on an Illumina NovaSeq 6000 was performed by Novogene using 250 PE sequencing for ITS and 150 bp paired-end (PE) sequencing for COI. For each library, an output of 10 Gb was requested. Figure 11 provides an overview of this DNA metabarcoding workflow.

It is important to note that each step in the metabarcoding workflow can introduce variability and bias into the output results. Depending on the sampling strategy, DNA source, DNA extraction kit, primers and bioinformatic pipeline, there will be differences in the output (Van Den Bulcke et al., 2023). Therefore, the full protocol used for this study is provided in the Appendix A Protocols.

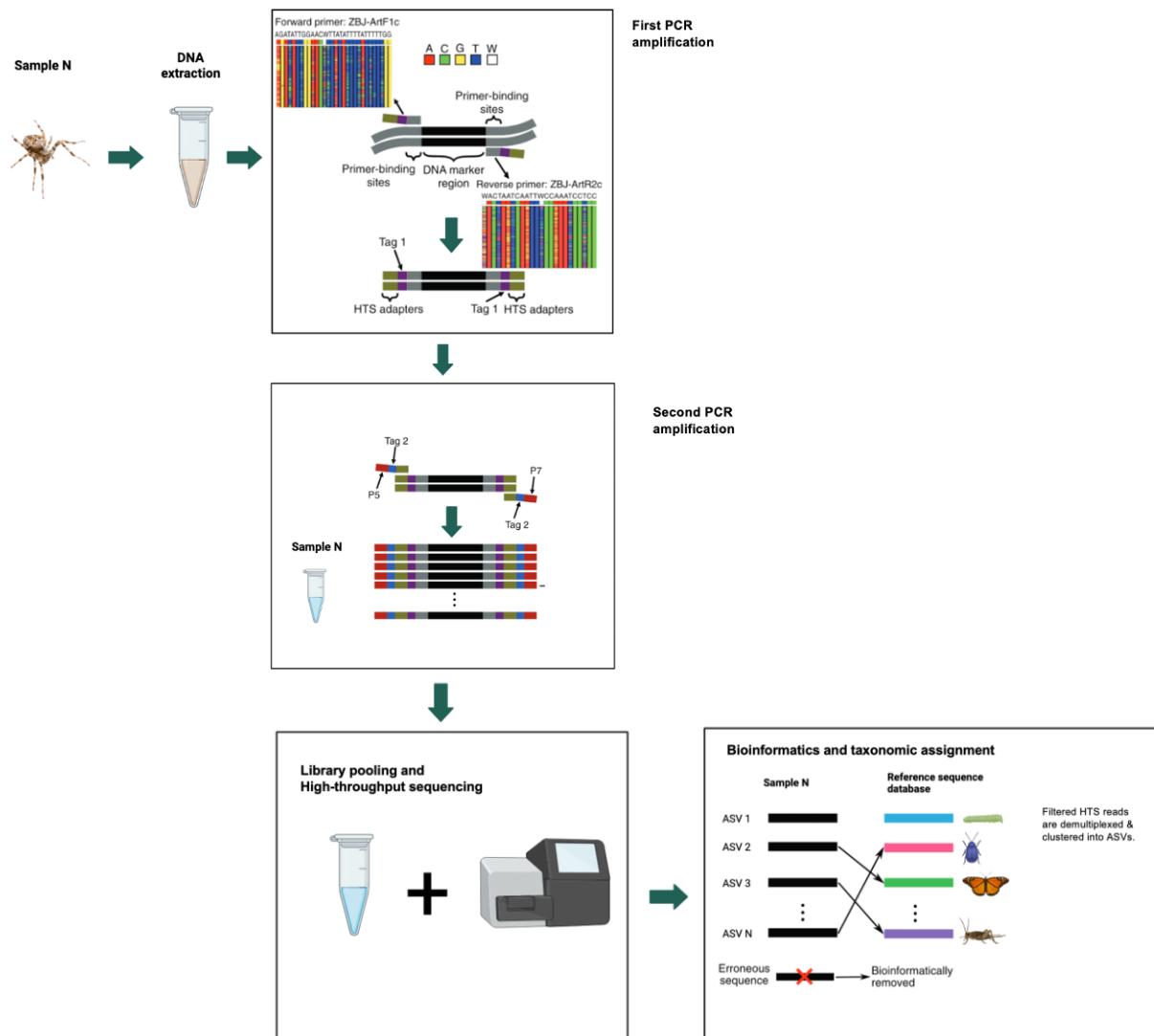


Fig. 11: Overview of the DNA metabarcoding protocol including the first polymerase chain reaction (PCR) amplification, the second PCR amplification, library pooling and high-throughput sequencing (HTS). This is followed by an illustration of the bioinformatics and taxonomic assignment. [This overview is created with BioRender based on the study by Liu et.al., (2019)].

3.2.4 Taxonomic identification & bioinformatic processing

The DNA reads obtained from the Illumina NovaSeq runs were analysed using the MetaBarFlow pipeline (Fig. 14, Sigsgaard et al., 2022). This pipeline converts high-throughput sequencing (HTS) data into an amplicon variant table and creates representative sequence files of each variant. In order to demultiplex the sequencing libraries based on the unique barcodes, the forward and reverse primers needed to be removed using Cutadapt (Martin, 2011). For each of the 14 libraries, the quality of the demultiplexed reads was evaluated using fastqc, in order to check the quality of the reads generated by the sequencer. Error filtering and removing chimeras was done with DADA2 (Callahan et al., 2016)

After initial filtering and the merging of paired reads, using the `mergePairs` function, the amplicon sequence variants (ASVs) of the COI sequences were blasted against a custom COI database. The database contains a combination of COI sequences from the GenBank nt database and the Barcode of Life Data Systems (BOLD) database (Ratnasingham & Hebert, 2007; www.boldsystems.org). Results were compared with a recent (January 2024) local download of the NCBI nt database.

Only blast hits with ≥97% identity and 100% query coverage (match across the entire query sequence) were included in the taxonomic assignment. Taxonomy was assigned using the `assignTaxonomy` function in the DADA2 package. Each ASV was assigned to the most recent common ancestor of the taxon (or taxa) yielding the hit with the highest sequence similarity, and any taxa showing an overlap in sequence similarities with those of the best-matching taxon (Thomassen et al., 2023). If the sequence similarity of an identification was at least 97%, the ASV received the assigned identity. When multiple species were equally probable identified for a specific ASV, the lowest shared taxonomic level would be used instead. Each of the ASVs were manually checked for occurrences in Belgium using the Global Biodiversity Information Facility (GBIF) (www.gbif.org) and waarnemingen.be. Taxids with no match in the NCBI taxonomy were classified manually or received an NA value. The metabarcoding taxa list was validated according to three criteria: (1) occurrence of the species in the GBIF database; (2) occurrence of the species in Belgium; (3) if the phylum is Arthropoda. This validation process helps to control for false positives by ensuring that only species confirmed to occur within the sampling area are analysed.

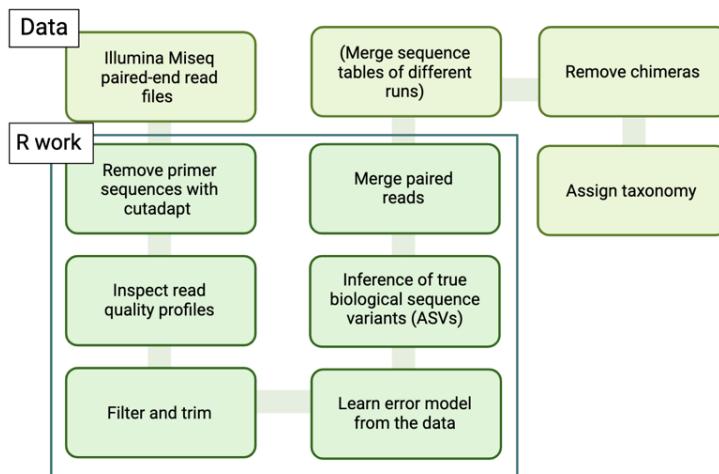


Fig. 12: Workflow of the MetaBarFlow pipeline and bioinformatic processing of the DNA reads obtained from the Illumina NovaSeq runs.

The amplicon sequence variants (ASV) tables and taxonomy files were imported into RStudio v.4.2.1 (R Core Team, 2022), where statistical analyses and data visualisations were performed using the PhyloSeq package (McMurdie et al., 2013). All sequences (ASVs) that were more prevalent in negative controls (DNA extraction blanks) than in the spider samples, were removed using the decontamination package (`decontam`; with a threshold=0.5). Furthermore, singletons, ASVs that only had one read in one sample, were removed.

3.2.5 Statistical analysis

All statistical analyses were conducted in RStudio (R 4.2). Each sequence variant included in the statistical analysis must have a match to the phylum Arthropoda and included comparisons to a greater than or equal to 97% reference match. Other species were expected to represent contamination. Only ASVs with a taxonomic assignment were included in the calculation of alpha and beta diversity. Diversity indices were calculated using the diversity function from the vegan package v2.5.7 and visualised in a bar plot. In this study, two different levels of diversity were used: alpha and beta diversity. Alpha diversity was assessed using several indices, including the Observed, Shannon, and Simpson indices. These indices focus on different aspects of biodiversity. The Observed value provides an indication of the number of prey items detected in each spider, thereby representing the species richness. The Shannon index reflects both the number of species (richness) and the evenness of the species' relative abundances. In contrast, the Simpson index assigns more weight to dominant species when measuring diversity.

A critical analysis was conducted to determine the most accurate option for filtering and rarefying the phyloseq object in order to find a robust option on which to base further analysis. In accordance with the methodology proposed by Coone et al. (2023), the application of rarefaction curves was performed on the phyloseq object (Appendix Fig. B3.1). The principle of this approach is that in order to reach the plateau phase, a minimum number of reads must be obtained. Samples that did not meet the required number were excluded from the analysis (Appendix Table B1.3). It is possible that these spiders had not yet consumed their prey, and thus their analysis will not represent the available prey community. Furthermore, a filtering threshold was applied, requiring a minimum relative abundance of at least 1e-5 for the occurrence of an ASV. Amplicon sequence variants that did not meet the threshold were excluded from the OTU table. The relative abundance values were calculated by dividing the observed number of sequences in an OTU by the total number of sequences in the sample (Schloß, 2023).

The diversity metrics used in this study are sensitive to differences in sequencing effort, therefore repeated rarefaction is necessary alongside the initial rarefying. This rarefaction method repeats the subsampling a large number of times (1000) and calculates the mean of the alpha or beta diversity index over those subsampling's (Schloß, 2023). When subsampling is repeated a large number of times the risks of ignoring or oversampling rare taxa are mitigated (Schloß, 2023). In accordance with the findings of Schloß et.al. (2023), this rarefaction step is repeated 1000 times prior to the calculation of the diversity indices using the rarefy function of the vegan R package. A minimum read depth of 100 is required to mitigate bias caused by varying sequencing depth between samples. This repeated rarefaction will enable us to ascertain what an alpha or beta diversity metric would have been for a collection of samples if they had been sequenced to the same depth (Schloß, 2023).

In addition to the importance of rarefaction for the alpha diversity metrics, the statistical power to detect differences between groups is maintained when rarefaction is performed. This crucial for the comparison of the different metrics along the urbanisation gradient. All of the samples are assigned an identification in the Group column according to their position on the urbanisation gradient (Fig. 13). The gradient contains nine categories, ranging from the most natural (LL) to the most urbanised (HH).

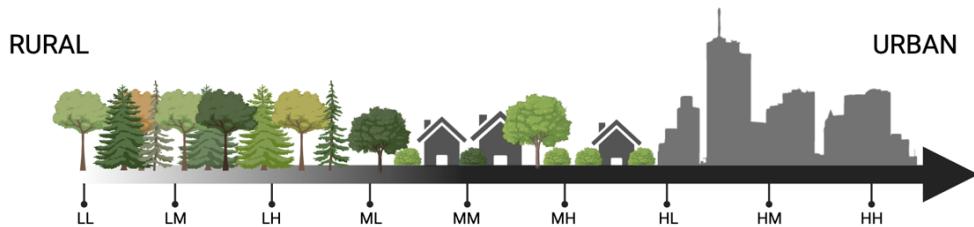


Fig. 13: The urbanisation scale divided into nine categories based on the degree of urbanisation observed at the landscape scale ($3 \times 3 \text{ km}$) and the local scale ($200 \times 200 \text{ m}$). The letters L, M, and H represent the three levels of urbanisation, which are defined as follows: L stands for low, M for medium, and H stands for high.

The shifts in the diet consumed by our European garden spider, which represents shifts in the available prey community, will be investigated using four different approaches (Fig. 14). Firstly, the analysis can be divided into two categories: examining each spider individual separately and examining each subplot (= sampling location) separately. By examining each subplot separately, it is possible to compare the prey consumed by a community of spiders at different locations. Within these two approaches there are two ways of analysing the received ASV data. The ASV-based approach posits that each amplicon sequence variant (ASV) present after all filtering steps is regarded as a unique barcode, representing a distinct species. The taxonomic level based approach aggregates all amplicon sequence variances (ASVs) that received the same taxonomic identification at a specified level (e.g., species, genus, family) into a single entity. This means that several unique barcodes with the same taxonomic identification are now regarded as a single entity.

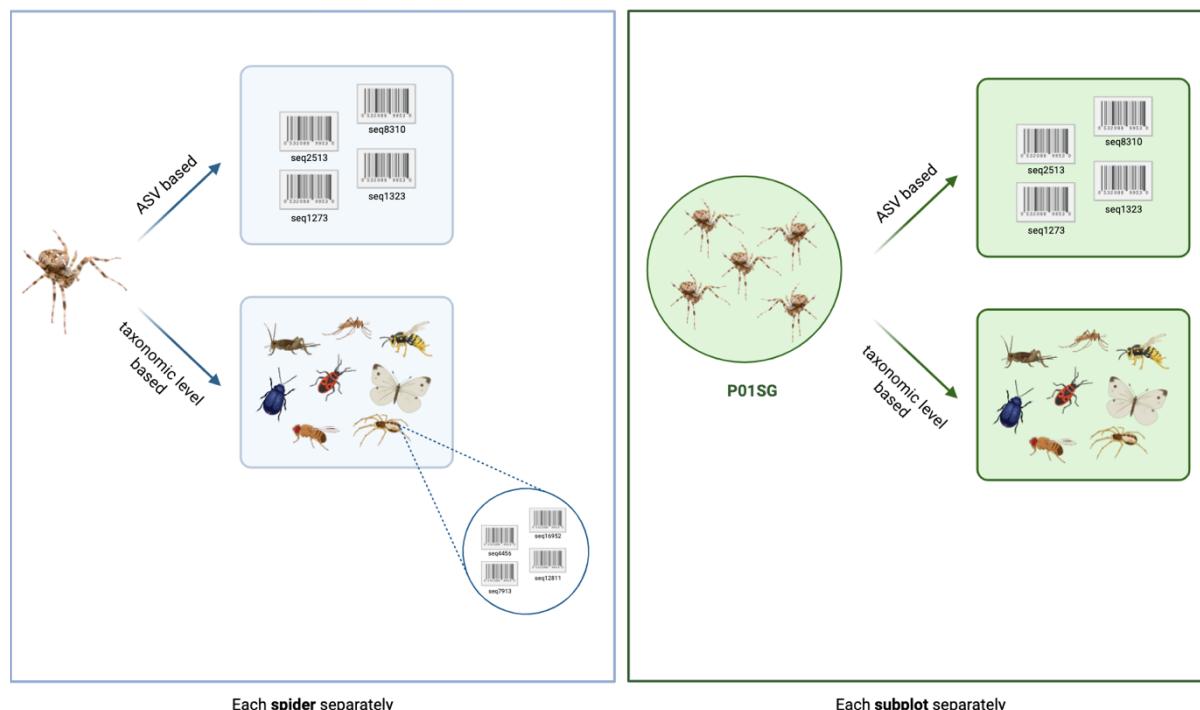


Fig. 14: Overview of the different approaches used in this study to analyse the received ASV data. The analysis can be divided into two categories: examining each spider individual separately and examining each subplot separately (two different squares). Within each category there are again two ways of analysing the received ASV data. The ASV-based approach posits that each amplicon sequence variant (ASV) present after all filtering steps is regarded as a unique barcode, representing a distinct species (ASV based). The taxonomic level based approach aggregates all amplicon sequence variances (ASVs) that received the same taxonomic identification at a specified level (e.g., species, genus, family) into a single entity (taxonomic level based). [Created with BioRender].

3.2.5.1 Effect of urbanisation on species richness

To determine the number of amplicon sequence variants (ASV richness) present in each spider, which can be interpreted as the number of prey items consumed by each spider, the total number of ASVs will be estimated. Secondly, the total number of ASVs for each spider community (subplot) at a distinct location within the study area is calculated. This enables the identification of the prey availability at each location. A generalised linear mixed model (glmer) was performed using the glmmTMB package (Brooks et al., 2017) on the total of ASVs for each subplotid, with U_local and U_landscape as fixed effects. The plotid was included as a random effect. This was done to test whether the number of ASVs is associated with the degree of urbanisation. It was assumed that the total ASV data followed a Poisson distribution. In order to account for the detected overdispersion, the nbinom2 function of the glmmTMB package was used as family distribution rather than the Poisson.

To test whether there were differences in species richness (taxonomic level based) for each spider sample (Observed) in response to local (U_local) and landscape (U_landscape) levels of urbanisation, a generalised linear mixed model was used. The model incorporates the primary effects of urbanisation at the landscape scale (U_landscape), urbanisation at the local scale (U_local), as fixed factors. The subplot identifier (subplotid) is included as a random factor. In this context, the term ‘subplotid’ refers to a unique identifier for each subplot, which accounts for the nested structure of the design. This allows for the spatial dependency of subplots within the same plot to be taken into account (Piano et al., 2020). The family function nbinom2 was used to account for overdispersion. Predicted values from the model based on specified terms U_landscape and U_local were generated using the ggpredict function from the ggeffects package (Lüdecke, 2018). The observed species richness values were also compared between the unique locations (subplotid) to test whether there were differences in species richness per subplotid in response to the levels of urbanisation. The generalised linear mixed model incorporates both urbanisation scales as fixed factors (U_landscape, U_local) and the plot identifier as a random factor (plotid).

Finally, in order to assess whether the observed ASV richness declined along the urbanisation gradient (Fig. 13), a Kruskal-Wallis test was conducted.

3.2.5.2 Effect of urbanisation on the Shannon & Simpson diversity

Linear mixed models were used to gain more insight into the spatial scale at which alpha diversity (Shannon & Simpson) is most affected by urbanisation. The models included the primary effects of at landscape scale urbanisation (U_landscape) and local scale urbanisation (U_local) as fixed factors. The subplot identifier (subplotid) is included in the model as a random factor when considering the diversity represented by each spider (Fig. 15). The plot identifier (plotid) is included as a random factor in the model when comparing diversity between different sites (Fig. 15). Gaussian is used as family distribution. Pairwise comparisons of levels within the two urbanisation scales (U_landscape, U_local) were performed using the emmeans package (Lenth et.al., 2018). The ANOVA with Type III, was used to calculate degrees of freedom and p-values of the linear mixed models. Models were plotted using ggplot2 (Wickham, 2016). Adjustments for multiple comparisons were corrected using Tukey.

Stacked bar plots were created to visualise the relative abundance of the represented species, genera or families in a given location. This, together with the Simpson index, can be used to visualise the trend towards biotic homogenisation in urbanised areas.

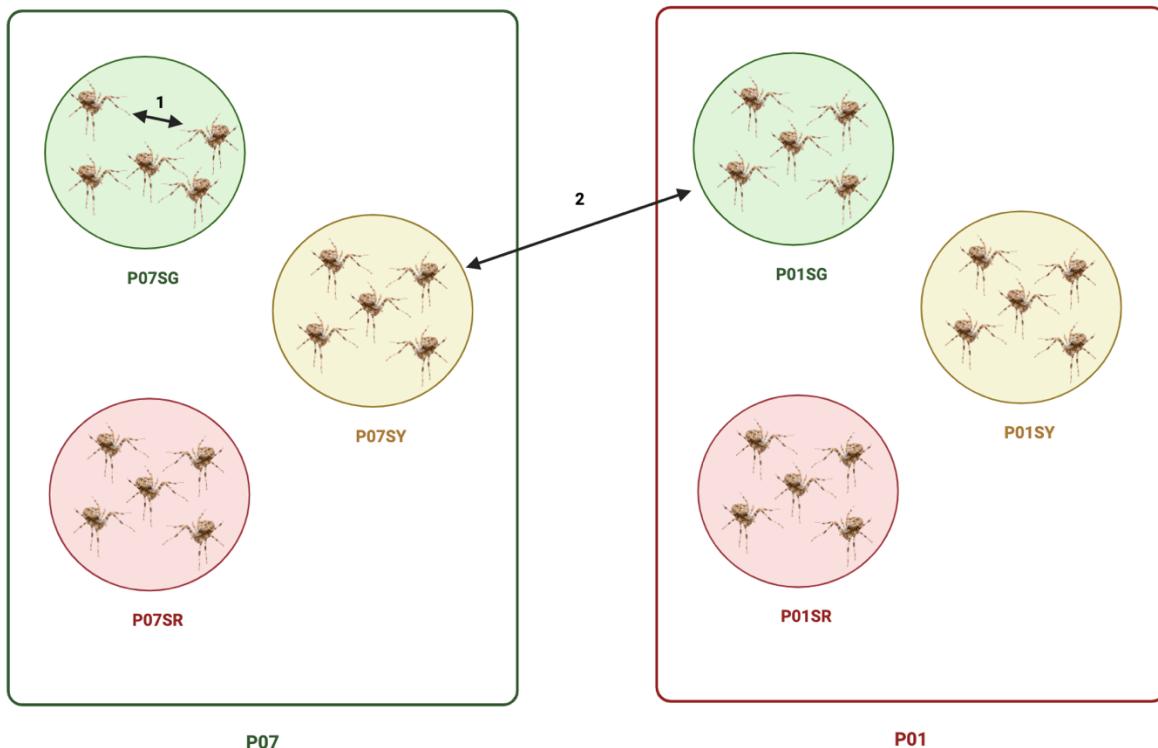


Fig. 15: Representation of the different ways (1 & 2) in which the Observed species richness, Shannon diversity and Simpson diversity are tested in this study. The circles represent the different subplots within each plot, i.e., the three different local scales within one landscape scale. In this example, a low urbanised site (plotid P07) is compared with a high urbanised site (plotid P01). (1) The first approach to examine diversity differences is to evaluate diversity metrics for each spider, thereby enabling comparisons between all individual spiders. (2) The second method involves comparing the diversity between unique locations (subplotids). Here, the diversity of all spiders in a particular subplot is combined, to represent the diversity at a unique location. Finally, diversity indices were compared along the urbanisation gradient. [Created with BioRender.]

3.2.5.3 Effect of urbanisation on species composition

Non-metric multidimensional (NMDS) ordination plots and principal coordinates analysis (PCoA) based on the Bray-Curtis dissimilarity index were constructed to visualise beta diversity (i.e., β -diversity) and study the diet variability (arthropod community composition) between high and low urbanised sites. The Bray-Curtis index, which ranges from 0 to 1, represents the proportion of the abundance that differs between communities. This index is more robust to sampling errors than other measures of β -diversity (e.g., Sorensen, Simpson) when abundance data are available (Schroeder & Jenkins, 2018). According to the level that required investigation, distance matrices were created. To do so, data was pooled per level: genus, family, species using the tax_glm function of the PhyloSeq package. The influence of urbanisation was tested using PERMANOVA (Anderson, 2017) through the function adonis2 from the vegan package (Oksanen et al., 2017). Additionally, a PCoA ordination with a rarefaction using 1000 iterations was made using the species-based distance matrix, to check if rarefying showed different patterns in beta diversity.

To determine whether cities promote biological homogenisation, the presence-absence of species was studied using the decostand function (ASV_matrix, pa) of vegan. This function converts all species abundance data into binary values: 1 if a species is present and 0 if a species is absent. The influence of urbanisation on the presence/absence of species was tested using PERMANOVA (adonis2, permutations = 999). Pairwise comparisons of levels within the two urbanisation scales (U_landscape, U_local) were performed using pairwise.adonis2 (method = 'jaccard', permutations = 10000). The Jaccard index is used to measure the dissimilarity.

An indicator species analysis was performed on both urbanisation scales to identify species that are strongly associated with urbanisation levels at each scale (U_local, U_landscape). The binary data from the presence-absence of species are used together with the multipatt function from the indicspecies package (De Cáceres & Legendre, 2009). This function uses a permutation test (nperm = 9999) to assess the significance of the species associations with specific urbanisation levels.

4 RESULTS

4.1 Sequencing output & ASV richness

The number of raw reads in the libraries received from Genome DK varied between ~4,697,100 and ~8,468,544 (Appendix Table B1.1), for the different samples. Following quality control procedures, which included the removal of PCR primers and merging of paired-end sequences our dataset consisted of 7646 unique amplicon sequence variants (ASVs) for 565 samples (ps_f). The dataset includes 530 spider samples and 35 blanco controls. Of the 530 spider samples, 37 contained only ASVs for which no taxonomic identification could be made (Table 3). These samples were removed, leaving 493 spider samples. The nine sequences that have been identified as contaminated (decontam) were removed (Appendix Table B1.2), leaving a total of 7637 ASVs. These nine sequences were more prevalent in negative controls than in the spider samples.

Table 3: The 37 spider samples without amplicon sequence variants (ASVs) that were removed from the phyloseq object. The three different urbanisation levels (LOW-MEDIUM-HIGH) on both scales are given in the U_landscape and U_local columns. Samples with high urbanisation levels on landscape (3 x 3 km) or local (200 x 200 m) scale are coloured in red.

Plotid	U_landscape	U_local	Plotid	U_landscape	U_local	Plotid	U_landscape	U_local
P08SG04	LOW	LOW	P15SG01	MEDIUM	LOW	P09SR10	LOW	HIGH
P08SG05	LOW	LOW	P15SG03	MEDIUM	LOW	P10SR08	HIGH	HIGH
P08SY06	LOW	MEDIUM	P15SR02	MEDIUM	HIGH	P04SR06	MEDIUM	HIGH
P09SR03	LOW	MEDIUM	P15SR04	MEDIUM	HIGH	P12SR11	HIGH	HIGH
P05SY16	MEDIUM	HIGH	P19SR02	HIGH	HIGH	P13SG09	MEDIUM	LOW
P22SR05	MEDIUM	MEDIUM	P19SR03	HIGH	HIGH	P14SY06	MEDIUM	MEDIUM
P22SR02	MEDIUM	HIGH	P01SG11	HIGH	LOW	P14SY08	MEDIUM	MEDIUM
P17SR16	LOW	HIGH	P01SG12	HIGH	LOW	P12SR05	HIGH	HIGH
P13SG03	MEDIUM	LOW	P01SY08	HIGH	MEDIUM	P22SG09	MEDIUM	LOW
P13SR03	MEDIUM	HIGH	P13SR01	MEDIUM	HIGH	P22SY01	MEDIUM	MEDIUM
P18SG02	LOW	LOW	P18SR03	LOW	HIGH	P27SR03	LOW	HIGH
P18SG05	LOW	LOW	P18SR06	LOW	HIGH			
P13SG01	MEDIUM	LOW	P07SY04	LOW	MEDIUM			

The number of ASVs received from Genome DK for each of the spider samples varied between ~1 and ~272, with a mean of approximately 32. Samples containing only one ASV (i.e., one prey identified) were predominantly observed in spiders sampled at an urban local or landscape site. This was the case for 18 out of 31 samples containing only one ASV. The highest number of ASVs (i.e., the highest number of prey consumed as ASVs) was observed in sample P12SY13, which is located at HM on the urbanisation gradient (Fig. 13, Appendix Table B1.5). Fourteen singletons, ASVs occurring only once in the entire dataset, and ASVs with zero occurrences were removed. The singletons are almost exclusively within the order Diptera and the family Tachinidae. The phyloseq object (ps_k) now contained 7576 taxa and 468 spider samples. After filtering of the phyloseq object (ps_k) for a relative abundance of at least 1e-5 (Appendix Table B1.3), the final phyloseq object (ps_k_filter) identified 3629 taxa in the OTU table for 468 samples. Diversity measurements were calculated based on this phyloseq object, where ASVs with really low abundances are filtered out.

We investigated whether the number of ASVs detected in a spider was the result of differences in sampling time, as this covered a period from 21 September to 14 October. There are less spiders with a very high ASVs count (Appendix Fig. B3.3), but there is no significant clear trend with sampling date is visible. Therefore, we did not include sampling date in our statistical analyses.

A comparison of the ASV richness for each of subplotid (i.e., the sum of ASVs of prey species consumed by the spider community at each unique location) showed that the total varied from 663 in P07SY, which decreased to only 5 ASVs in P17SY (Table 4). These locations both have a medium urbanisation degree over the two spatial scales (MM). In some cases, the low urbanised subplot (e.g., P17SG) within a plot (e.g., P17) contained a greater number of ASVs than the other subplots. However, this effect of urbanisation on ASV richness in each unique location was not significant ($\text{Chisq} = 3.5254$, $\text{df} = 2$, $p_{U_landscape} = 0.1717$; $\text{Chisq} = 0.5386$, $\text{df} = 2$, $p_{U_local} = 0.7639$). This indicates that the total number of ASVs did not significantly differ between the different locations (Appendix Fig. B2.3).

Table 4: Total number of amplicon sequence variants for each unique location (subplotid). Calculated based on the number of ASVs present in the community of spiders sampled on each location. This represents the available prey community at each location in terms of ASV counts. There were no ASVs found for subplotid P16SY and P09SY. The subplotid's are coloured based on their urbanisation degree at local scale (200 x 200 m). P20SR contains the subplotid's within P20SR and P20SE.

Subplotid	Total_asvs								
P01SG	177	P06SY	207	P11SR	75	P17SY	5	P22SR	20
P01SY	291	P06SR	100	P12SG	211	P17SR	79	P23SG	200
P01SR	234	P07SG	191	P12SY	202	P18SG	19	P23SY	472
P02SG	58	P07SY	663	P12SR	251	P18SY	105	P23SR	72
P02SY	63	P07SR	255	P13SG	48	P18SR	79	P24SG	106
P02SR	210	P08SG	34	P13SY	95	P19SG	171	P24SY	123
P03SG	81	P08SY	124	P13SR	110	P19SY	110	P24SR	270
P03SY	33	P08SR	226	P14SG	52	P19SR	37	P25SG	110
P03SR	235	P09SG	170	P14SY	107	P20SG	38	P25SY	46
P04SG	54	P09SY	X	P14SR	207	P20SY	109	P25SR	84
P04SY	91	P09SR	211	P15SG	111	P20SR	470	P26SG	123
P04SR	82	P10SG	63	P15SY	173	P21SG	254	P26SY	184
P05SG	62	P10SY	231	P15SR	83	P21SY	191	P26SR	147
P05SY	132	P10SR	183	P16SG	71	P21SR	159	P27SG	258
P05SR	91	P11SG	249	P16SR	110	P22SG	11	P27SY	47
P06SG	100	P11SY	105	P17SG	81	P22SY	105	P27SR	90

Comparisons of the ASV richness for each spider separately showed reducing trends in the effect of urbanisation on the number of observed ASVs. These were almost found to be significant at the landscape scale ($\text{Chisq} = 5.4306$, $\text{df} = 2$, $p_{U_landscape} = 0.06618$; $\text{Chisq} = 5.9318$, $\text{df} = 2$, $p_{U_local} = 0.05151$; Appendix Fig. B2.1). Lower numbers appear to be found at the landscape scale in locations with a medium urbanisation degree compared to those with a high degree (Fig. 15). The estimated difference in marginal means (EMMs) between medium and high sites on the landscape scale is -0.1844. This means that on average the ASV richness present in spiders at medium sites on the landscape scale seem to be -0.1844 units lower than urban sites.

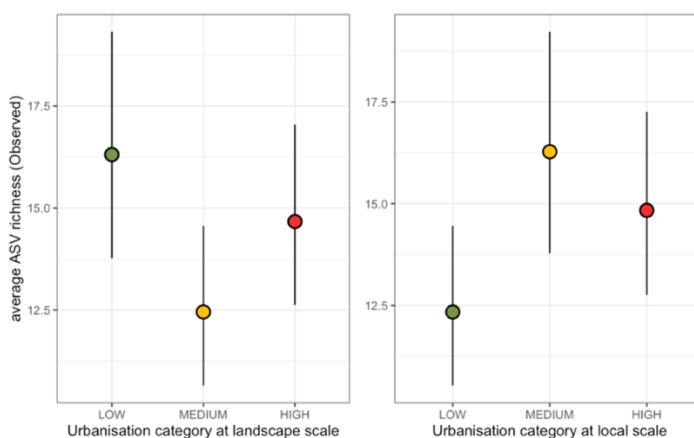


Fig. 15: The average ASV richness (y-axis) across different urbanisation categories at the landscape and local scale. Each point on the plot, coloured according to urbanisation category (green = low, yellow = medium, red = high), represents the average ASV richness, vertical lines around the point indicate the uncertainty or confidence intervals of these estimates.

Comparison of the ASV richness along the urbanisation gradient (Fig. 13) revealed that the highest prey numbers were observed in the HH category (995), representing highly urbanised locations, followed by the HL category (901), which represented high urbanisation on the landscape and low urbanisation on local scale. The location on the gradient with the lowest number of ASVs (563) was the ML category, which represents medium urbanisation at the landscape scale and low on the local scale (Table 5). The effect of placement on the urbanisation gradient on ASV richness demonstrated no significant effect ($p = 0.4335$). This indicates that the total number of amplicon sequence variants did not significantly differ between the nine urbanisation categories.

Table 5: Total number of amplicon sequence variants for each category on the urbanisation gradient. This represents the available prey community as number of observed ASVs, along the urbanisation gradient. Colours indicate the level of urbanisation at the landscape scale (3 x 3 km), green = low urbanised, yellow = medium, red = highly urbanised.

Category	Total_asvs	Category	Total_asvs	Category	Total_asvs
LOW – LOW	760	MEDIUM – LOW	563	HIGH – LOW	901
LOW – MEDIUM	680	MEDIUM – MEDIUM	881	HIGH – MEDIUM	838
LOW – HIGH	820	MEDIUM – HIGH	690	HIGH – HIGH	995

4.2 Overall spider diet

The OTU table (ps_k_filter) contained a total of 3629 ASVs of arthropods, after all cleaning and filtering steps. This resulted in the identification of 88 families, 192 genera, and at least 230 unique species, if NA and taxonomically unsolved sequences (f.e. o_Lepidoptera, g_Calliphoridae) were taken into account. Otherwise, this resulted in the identification of 79 families, 133 genera, and at least 97 unique species. Of the 3629 ASVs, 298 ASVs had not been assigned to an order level and 3263 ASVs had not been assigned to a species level. A total of 2583 ASVs were identified for the Diptera, representing 104 genera. In the case of Hymenoptera, 88 ASVs were identified, representing 14 genera. The order Lepidoptera was represented by 283 ASVs, corresponding to 31 genera, while the order Coleoptera was represented by 60 ASVs and 12 genera. The order Hemiptera was represented by 98 ASVs and 18 genera, while the order Orthoptera was represented by only 20 different ASVs and only one genus (o_Orthoptera). The orders Mecoptera and Neuroptera were only represented by respectively 9 and 7 ASVs (Table 6), containing 1 genus and 2 genera.

The European garden spider's diet is dominated by Diptera (2583), followed by Lepidoptera (283), Araneae (165), Hemiptera (98), Hymenoptera (88) and Coleoptera (60). The pie chart illustrates the high prevalence of Diptera in the overall diet of the spider, *Araneus diadematus*. The families that were most frequently consumed were Araneidae (155), Muscidae (126), Drosophilidae (110), Calliphoridae (105) and Ichneumonidae (77) (Table 7). With the exception of Araneidae (Araneae) and Ichneumonidae (Hymenoptera), these are all families within the order Diptera (Table 7). A total of 2131 ASVs remained unidentified at the family level, mainly within the order Diptera (1634) and Lepidoptera (161).

Table 6: The final number of ASVs for each phylum, class, and order in our Phyloseq object (*ps_k_filter*) after all of the filtering steps across all urbanisation categories. The pie chart provides a visual representation of the overall spider diet, indicating the proportion of each order that contributes to it. The colours indicate the order to which each item belongs.

Phylum	Nr. of ASVs
Arthropoda	3629
Class	Nr. of ASVs
Arachnida	165
Collembola	4
Insecta	3446
p_Arthropoda	14
Order	Nr. of ASVs
Araneae	165
c_Insecta	298
Coleoptera	60
Diptera	2583
Entomobryomorpha	4
Hemiptera	98
Hymenoptera	88
Lepidoptera	283
Mecoptera	9
Neuroptera	7
Orthoptera	20
p_Arthropoda	14

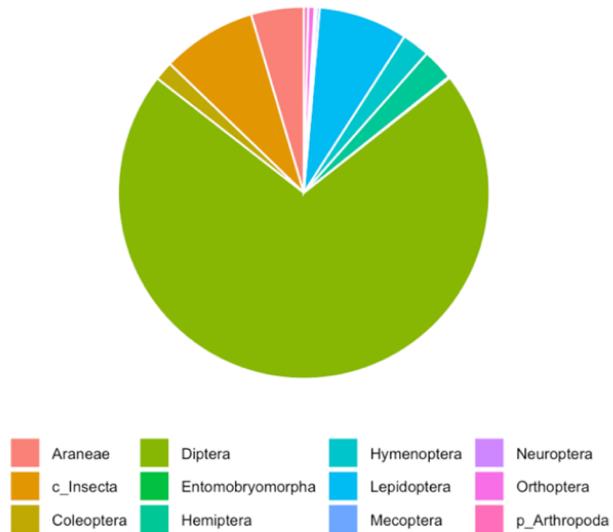


Table 7: The number of ASVs for each family in the Phyloseq object (*ps_k*) is presented in alphabetical order. The colour assigned to each family corresponds to which order they are part of. This colour coding is consistent with that used in the pie chart (Table 6). The five most abundant families are presented in the form of images. (a) Araneidae, (b) Muscidae, (c) Drosophilidae, (d) Calliphoridae, (e) Ichneumonidae. All images were sourced from www.waarnemingen.be.

Family	Nr. of ASVs	Family	Nr. of ASVs	Family	Nr. of ASVs
Adelgidae	1	Ichneumonidae	77	Sarcophagidae	32
Anisopodidae	33	Leiodidae	1	Scathophagidae	5
Anthocoridae	10	Limacodidae	1	Scatopsidae	3
Anthomyiidae	16	Linyphiidae	9	Sciariidae	55
Aphidiidae	3	Lonchopteridae	1	Sciomyzidae	24
Araneidae	155	Lygaeidae	3	Sepsidae	1
Asilidae	2	Miridae	44	Simuliidae	27
Calliphoridae	105	Muscidae	126	Sphaeroceridae	5
Carabidae	9	Mycetophilidae	3	Sphingidae	4
Cecidomyiidae	7	Nabidae	2	Staphylinidae	35
Cerambycidae	1	Nitidulidae	3	Stratiomyidae	4
Cercopidae	2	Noctuidae	40	Syrphidae	59
Chironomidae	50	Nolidae	4	Tabanidae	10
Chrysomelidae	8	Nymphalidae	19	Tachinidae	31
Chrysopidae	5	Oecophoridae	1	Tephritidae	30
Cicadellidae	1	Oestridae	1	Therevidae	1
Crambidae	14	Opomyzidae	13	Tipulidae	18
Culicidae	41	Orchesellidae	4	Tortricidae	3
Dolichopodidae	9	Pallopteridae	7	o_Araneae	1
Drepanidae	3	Panorpidae	9	o_Coleoptera	3
Drosophilidae	110	Pentatomidae	29	o_Diptera	1634
Empididae	2	Phoridae	42	o_Hemiptera	3
Ephydriidae	4	Pieridae	3	o_Hymenoptera	1
Erebidae	8	Pipunculidae	5	o_Lepidoptera	161
Fanniidae	17	Platystomatidae	5	o_Orthoptera	20
Gelechiidae	4	Polleniidae	12	p_Arthropoda	14
Geometridae	19	Pompilidae	2	c_Insecta	298
Heleomyzidae	3	Psychodidae	22	NA	1
Hemerobiidae	2	Pteromalidae	4		
Hybotidae	4	Rhinophoridae	3		



4.3 Diet richness, diversity, and composition

4.3.1 Diet richness

When comparing ASV richness between communities of spiders at different locations (Fig. 16, Appendix Fig. B2.3), no significant differences in ASV richness between subplots with varying levels of urbanisation were found ($p > 0.05$).

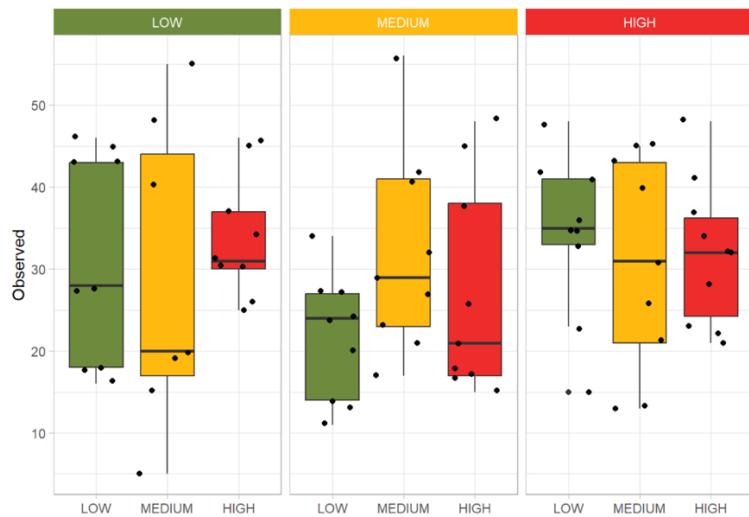


Fig. 16: The observed ASV richness (y-axis) for each of the different urbanisation categories (low-medium-high) on the local scale (200 x 200 m) within each category on the landscape plot (3 x 3 km). The local urbanisation degrees are listed on the x-axis. Each barplot received a colour based on their degree of urbanisation. There are no significant differences.

Once the taxonomic glomeration of the ASVs at the species level was completed, the species richness in each spider can be evaluated. The highest number of different prey species, 20, was observed in P06SY22, a spider sampled in a medium-urbanised site at both scales. In 47 spiders only one species was observed, 28 of these were sampled in an urbanised site at the landscape, local or both scales. This expected lower number of observed species richness to be found in city-living spiders compared to those living more rural was not significantly proven ($p > 0.05$; Appendix Fig. B2.2). However, there appears to be a trend at the local scale ($\text{Chisq} = 5.2643$, $df = 2$, $p_{U_local} = 0.07192$) of a higher average species richness in spiders living in more urban sites (Fig.17).

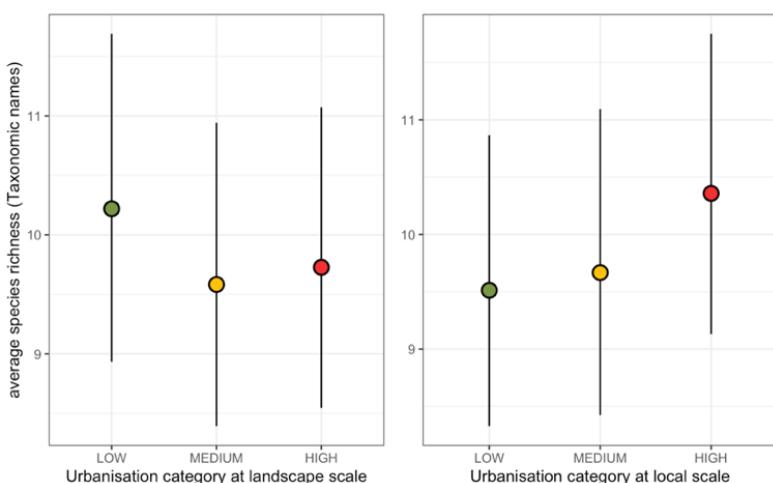


Fig. 17: The average species richness (y-axis) across different urbanisation categories at the landscape and local scale. Each point on the plot, coloured according to urbanisation category (green = low, yellow = medium, red = high), represents the average species richness, vertical lines around the point indicate the uncertainty or confidence intervals of these estimates. At the landscape scale the expected lower number of observed species richness in city-living spiders seems to be visible. However, this difference was not considered significant ($p = 0.22840$). The differences in species richness at the local scale are showing an almost significant trend ($p = 0.07192$) of a higher average species richness in spiders living at more urban sites.

The observed species richness for each community of spiders at a certain location showed no significant differences with varying levels of urbanisation at both scales (Appendix Fig. B2.4).

Comparison of the observed species richness along the urbanisation gradient (Fig. 13) revealed that the highest prey richness was observed in the HL category (113), representing the low urbanised locations within an urban landscape location. The category on the gradient with the lowest species richness was the ML category (77), which represents low urbanised locations within a medium landscape location. When comparing the categories at the local scale for urbanised and rural locations on the landscape scale (Table 8), the observed species richness seemed to be the highest in the more rural sites (HL = 113, LL = 94). However, the effect of placement on the urbanisation gradient on observed species richness demonstrated no significant effect ($p = 0.4335$). This indicates that species richness did not significantly differ between the nine urbanisation categories.

Table 8: The observed species richness (Observed) for each category on the urbanisation gradient. Colours indicate the level of urbanisation at the landscape scale (3x3 km), green = low urbanised, yellow = medium, red = highly urbanised.

Category	Observed	Category	Observed	Category	Observed
LOW – LOW	94	MEDIUM – LOW	77	HIGH – LOW	113
LOW – MEDIUM	80	MEDIUM – MEDIUM	104	HIGH – MEDIUM	100
LOW – HIGH	86	MEDIUM – HIGH	86	HIGH – HIGH	104

4.3.2 Shannon diversity

The results of the analysis of deviance tables from the linear mixed models, looking at each spider separately (ASV and taxonomic species based), indicated decreasing trends in Shannon diversity at the urbanisation scale (Appendix Fig. B2.5-B2.6). The effect of urbanisation on Shannon diversity for each spider appeared to be most visible at the taxonomic species level. The predicted emmeans appears to be higher for a spider living in a low-urbanised plot (U_landscape) compared to a spider living in a high-urbanised plot (Fig. 18). This contrast between LOW and HIGH was estimated to be 0.1398 (SE = 0.0825, df = 461, p = 0.2083). The difference between LOW and MEDIUM was estimated to be 0.1739 (SE = 0.0843, df = 461, p = 0.0988) (Appendix Fig. B2.6).

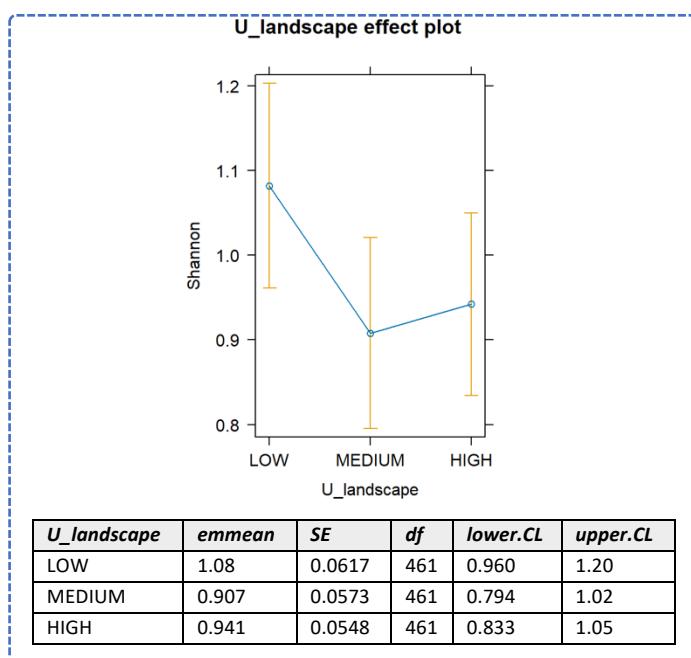


Fig. 18: Predicted emmeans values of the Shannon diversity index for each of the three urbanisation degrees on landscape scale (3 x 3 km), looking at each spider separately. Table gives the U_landscape emmeans predictors with their standard error (SE).

Looking at the Shannon diversity on the ASV level for each spider separately, there seems to be a trend at both scales (Appendix Fig. B2.5). Lower values of the Shannon diversity seem to be found in more rural-living (low) spiders at the local scale ($\text{Chisq} = 5.8959$, $\text{df} = 2$, $p_{U_local} = 0.05245$, Fig. 19).

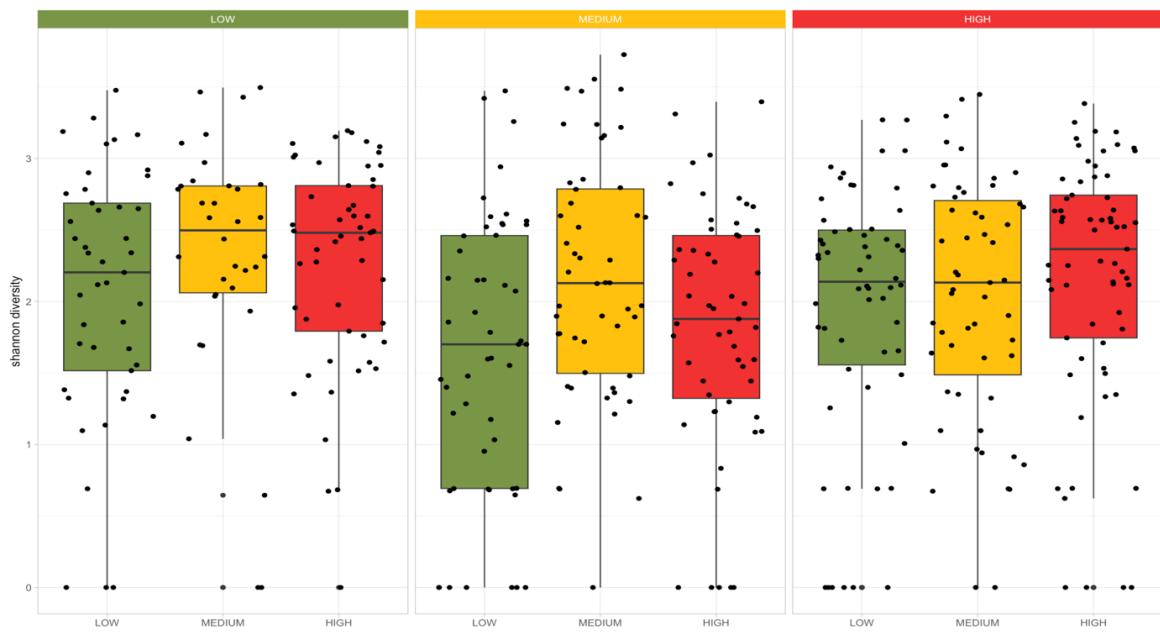


Fig. 19: Shannon diversity (ASV level) values looking at all spiders separately. The barplots within each of the landscape scales (columns), indicate the urbanisation degree at the local scale (200 x 200 m). Colours are received based on their degree (green = low, yellow = medium, red = high). All of the dots represent the Shannon diversity values of individual spiders. The x-axis represents the same urbanisation gradient as figure 13, going from LL to HH over 9 categories.

The Shannon diversity was also analysed for each subplotid, representing the Shannon diversity present in each spider community at a location. The landscape scale showed significant effects on the ASV based Shannon diversity ($\text{Chisq} = 9.1434$, $\text{df} = 2$, $p_{U_landscape} = 0.01034$; Appendix Fig. B2.7). However, it is important to mention that not all assumptions for this model were accomplished. When Shannon diversity was implemented in the model based on taxonomic species, the local scale appeared to have an impact ($\text{Chisq} = 4.7073$, $\text{df} = 2$, $p_{U_local} = 0.09502$, Appendix Fig. B2.8). The predicted emmeans for urbanised plots seemed to be higher compared to rural plots (Fig. 20).

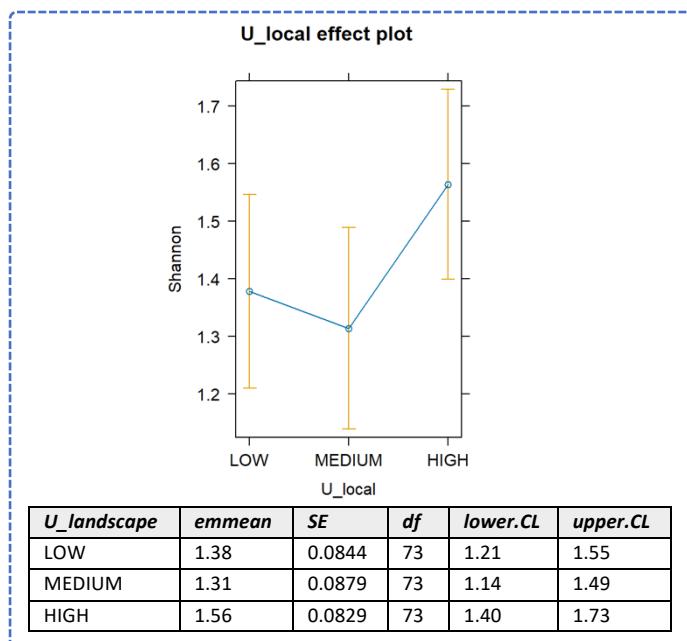


Fig. 20: Predicted emmeans values of the Shannon diversity index for each of the three urbanisation degrees on local scale (200 x 200 m), looking at spider communities. Table gives the *U*_local emmeans predictors with their standard error (SE).

4.3.3 Simpson diversity

The assumptions underlying the linear mixed models evaluating Simpson diversity, which considered each spider separately (ASV and taxonomic species based), were not consistently met. In some cases, notable discrepancies were observed. Therefore, the results of these tests are only given in the Appendix Fig. B2.9 and Fig. B2.10

The Simpson diversity was also analysed for each subplotid, representing the Simpson diversity present in each spider community at a location. The landscape scale showed significant effects on the ASV based Simpson diversity ($\text{Chisq} = 4.4310$, $\text{df} = 2$, $p_{U_landscape} = 0.02209$; Appendix Fig. B2.11). However, it is important to mention that not all assumptions for this model were accomplished.

Urbanisation on the local scale caused significant differences in the taxonomic species-based Simpson diversity ($\text{Chisq} = 7.472$, $\text{df} = 2$, $p_{U_local} = 0.03806$; Appendix Fig. B2.12). Comparison of the predicted emmeans, showed that the Simpson diversity index was significantly lower ($\text{Contrast}_{MH} = -0.10361$, $SE = 0.0433$, $\text{df} = 73$, $p = 0.0497$) in medium-urbanised sites compared to those with a high urbanisation level on the local scale (Fig. 21). The difference in Simpson diversity between low and high sites was almost significant ($\text{Contrast}_{LH} = -0.09729$, $SE = 0.0422$, $\text{df} = 73$, $p = 0.0610$).

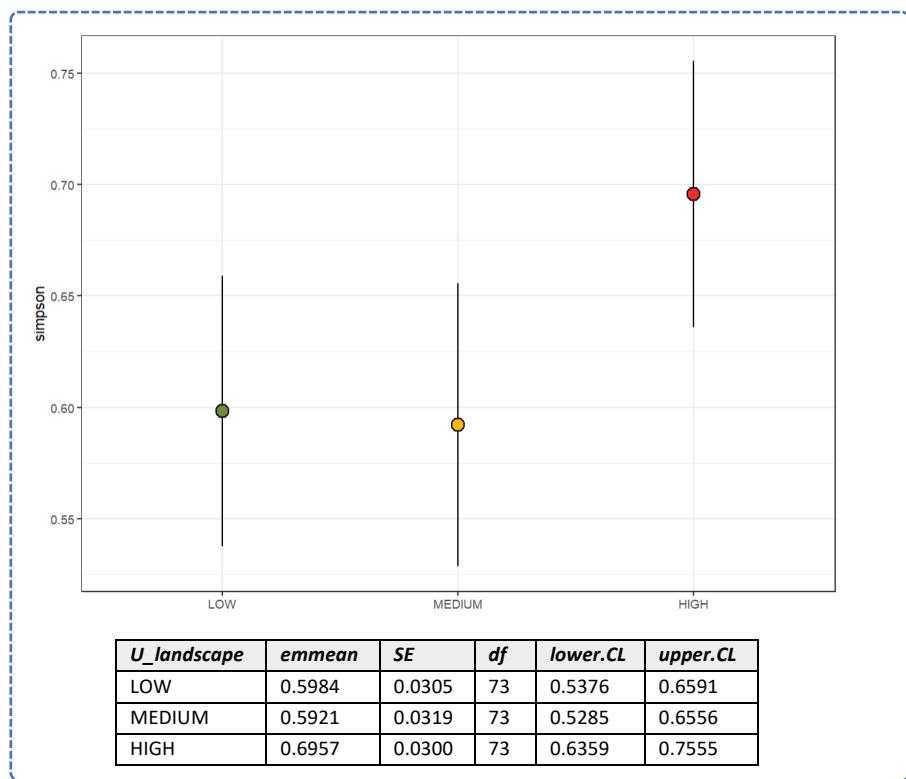


Fig. 21: Predicted emmeans values of the Simpson diversity index (y-axis) for each of the three urbanisation degrees on local scale (200 x 200 m), looking at spider communities. Each point on the plot, coloured according to urbanisation category (green = low, yellow = medium, red = high), represents the average Simpson index, vertical lines around the point indicate the uncertainty or confidence intervals of these estimates. Table gives the *U*_local emmeans predictors with their standard error (SE). Significant differences were found between MEDIUM – HIGH locations ($p = 0.0497$). The difference in Simpson index between LOW – HIGH was almost significant ($p = 0.0610$).

A lower Simpson index indicates higher diversity, signifying that the community contains many different species with relatively equal abundances. Based on the relative abundance data across the local scale urbanisation gradient, abundances seem to be more evenly divided for rural subplots within a plot compared to locally urbanised plots (Fig. 22). This is in line with lower Simpson indices for locally rural samples. The subplot P01SG shows abundances of the families, Miridae, Pentatomidae and Sciomyzidae, which are classified within the Hemiptera and Diptera. The relative abundance of each of these families within the diet consumed by the community present at this location is estimated to be approximately 25%. If we compare this with subplot P01SR, it seems that almost 50% of the diet, and thus the present prey community at this location, belongs to the Syrphidae. The subplot P01SY, which has a medium urbanisation degree at the local scale, was dominated by species that remained unidentified at the family level, representing approximately 75% of the total.

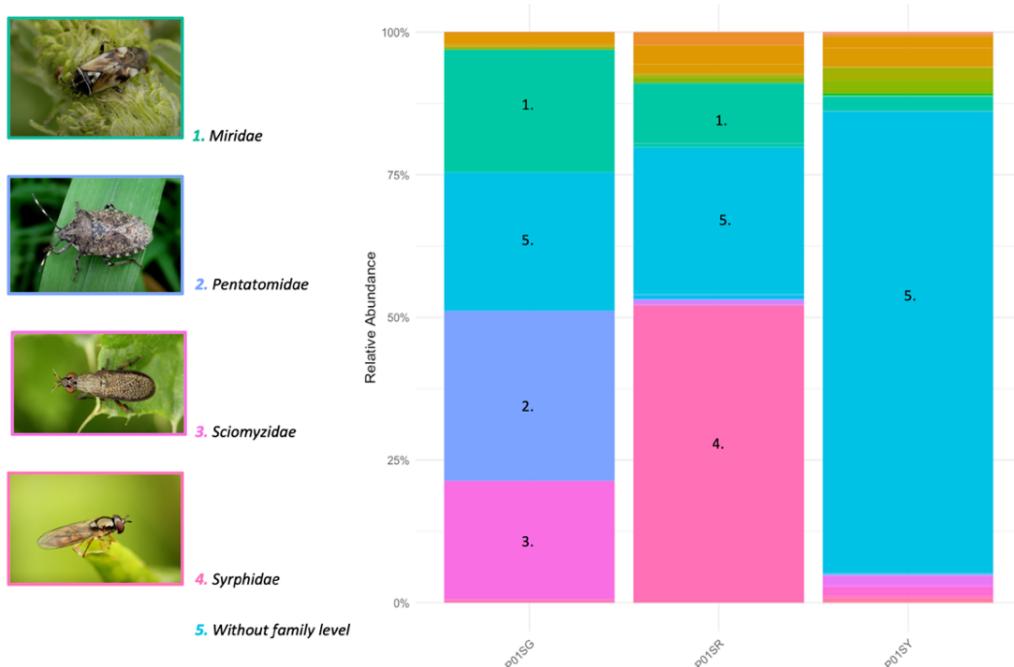


Fig. 22: The relative abundance of arthropod families within the different subplots in plotid P01 after taxonomic glomeration of the ASVs at family level. The subplotid's are listed at the x-axis. P01SG has a low urbanisation degree on local scale, P01SR has a high urbanisation degree, and P01SY has an intermediate urbanisation degree. The different colours in the barplots illustrate the relative abundance of the arthropod families. The most abundant five are visualised with a picture (www.waarnemingen.be) and family name at the left. Abundances seem to be more evenly divided for the rural subplot (P01SG) compared to a locally urbanised plot (P01SR).

Another example demonstrating that urban sites are more dominated by a few species is provided when taxonomic glomeration of the ASVs at genus level was performed. Subplot P01SG is divided in more equal relative abundances of genera, suggesting that the prey community at the more rural location contains a greater number of species, rather than just a few that are dominant (Fig. 23).

Figure 24 provides a final illustration of the observation that urban sites are more dominated by a few families. Here, the relative abundance of the top twenty most abundant arthropod families in our phyloseq object is calculated within the different subplots of plotid P27 (x-axis). P27 is situated in a low-urbanised location on the landscape scale and contains three subplotids, namely P27SG, P27SY and P27SR. Subplot P27SG is characterised by a low degree of urbanisation at the local scale, while P27SR exhibits a high degree of urbanisation and P27SY an intermediate degree. The relative abundances are more evenly distributed in P27SG compared to P27SR, which appears to be dominated

by o_Diptera. Subplot P27SY appears to be even more dominated by a single family. It should be noted, however, that the blue block in P27SY represents those ASVs that have only been identified at the order level of Diptera and Lepidoptera.



Fig. 23: The relative abundance of arthropod genera within the different subplot in plotid P01 after taxonomic glomeration of the ASVs at genus level. The subplot's are listed at the x-axis. The different colours in the barplots illustrate the relative abundance of the arthropod genera. Abundances seem to be more evenly divided for the rural subplot (P01SG) compared to a locally urbanised plot (P01SR).

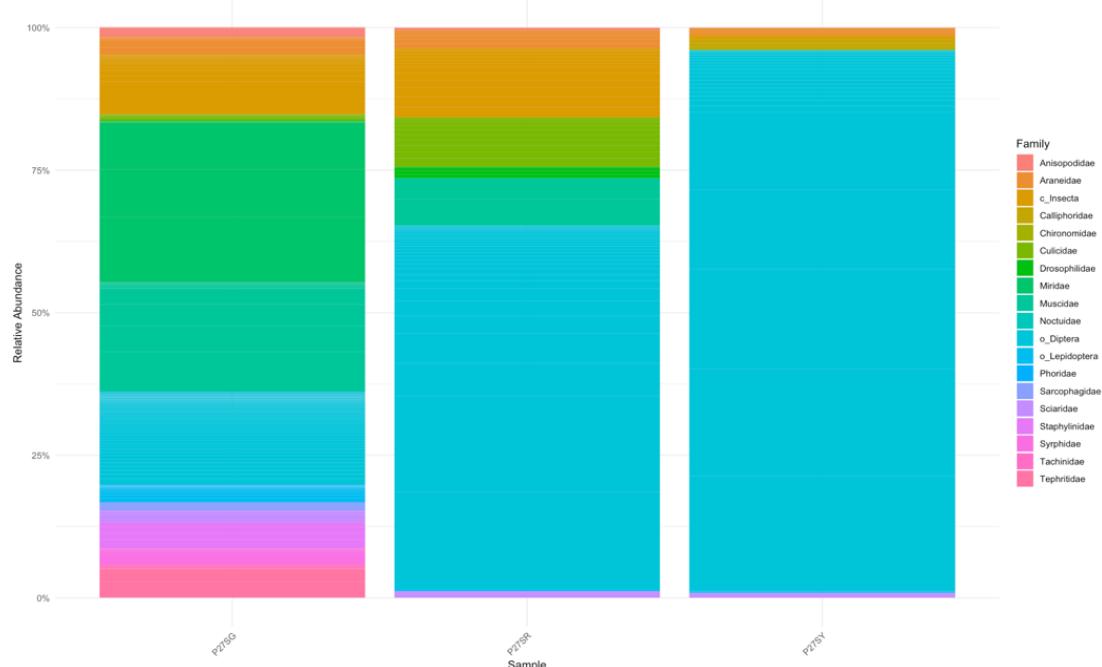


Fig. 24: The relative abundance (y-axis) of the top 20 arthropod families in the phyloseq object, within the different subplots of plotid P27 (x-axis). P27SG has a low urbanisation degree on local scale, P27SR has a high urbanisation degree, and P27SY has an intermediate urbanisation degree. The different colours in the barplots illustrate the relative abundance of the arthropod families. The relative abundances are more equally divided in P27SG compared to P27SR, which seems to be dominated by o_Diptera. Subplot P27SY appears to be even more dominated by one family.

4.3.4 Beta diversity

Visual inspection of the NMDS plot of the taxonomic family-based distance matrix reveals differences in prey community composition at the local scale (Fig. 25). The low urbanised subplots (dots) are more dispersed (larger ellipse), indicating greater variability. The ellipses of the medium and high urbanisation groups are smaller, indicating less dispersion and therefore more homogeneity. Communities in less urbanised sites appear to be more diverse than those in more urbanised sites. This is consistent with the expected homogenisation of the prey communities in more urban locations. The same pattern was observed at the genus-based distance matrix (Appendix Fig. B3.5). However, this clustering is less pronounced at the genus level, and even more so at the family level (Fig. 26).

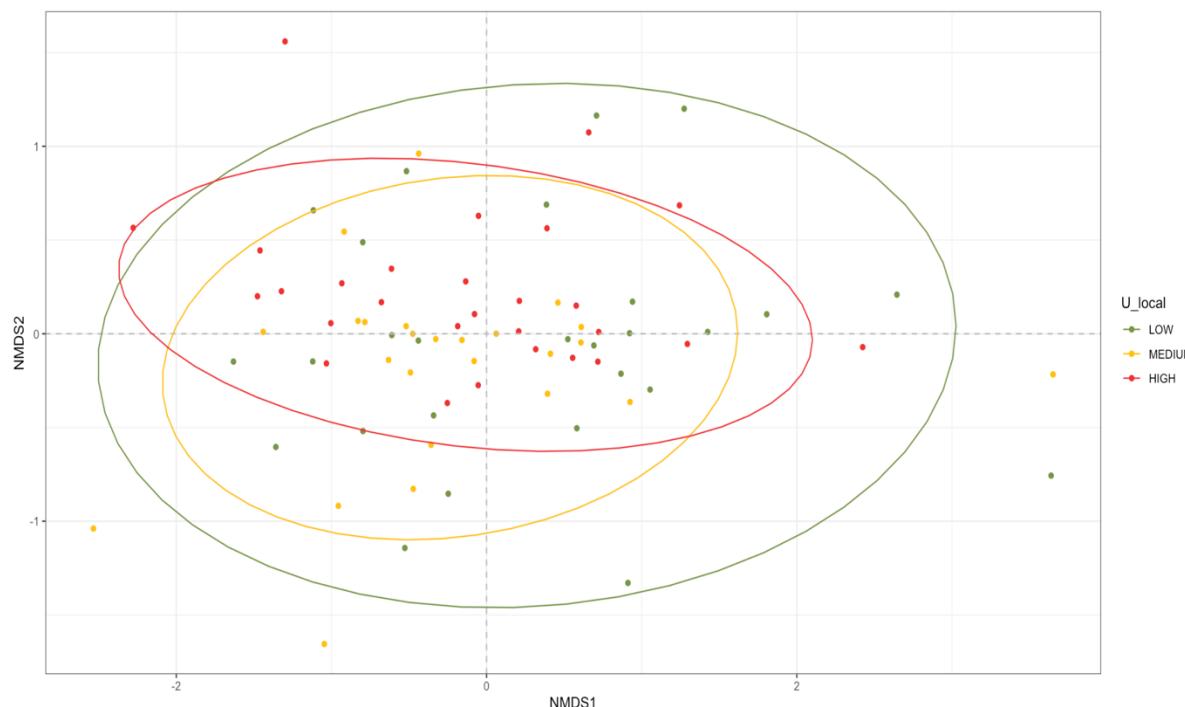


Fig. 25: Non-metric Multidimensional Scaling (NMDS) plot of the taxonomic family-based distance matrix. Points represent the 79 different locations (subplotids), coloured according to their degree of urbanisation at the local scale. The ellipses show the distribution of the different groups at the local scale, indicating overlap and dissimilarity between groups. The points of the low urbanised group are more spread out (larger ellipse), indicating more variability. The ellipses of the medium and high urbanisation groups are smaller, indicating less dispersion and therefore more homogeneity. This is consistent with the expected homogeneous prey supply in more urban locations.

This pattern was further confirmed by the PERMANOVA analysis (Appendix Fig. B2.13), which revealed significant differences in species composition at the landscape scale ($df = 2, F = 1.2090, p_{U_landscape} = 0.033$) and marginally significant differences at the local scale ($df = 2, F = 1.3914, p_{U_local} = 0.052$; Table 9). Permutation tests on the betadisper of both scales were not significant ($p > 0.05$, Appendix Fig. B2.13), indicating that the PERMANOVA results are reliable. This finding confirms that the landscape scale of urbanisation has a significant effect on the variation in species composition at the family level, and that the local scale has an almost significant effect.

Table 9: Results of the test for differences in species composition at the family level between urbanisation at the landscape (*U_landscape*) and local (*U_local*) level as inferred from PERMANOVA analysis. Df, degrees of freedom; SumOfSqs, sum of squares; MS, mean squares. Marginally significant or significant values are highlighted in bold. Significant values are indicated with *.

	Df	SumOfSqs	R2	F	Pr (>F)
<i>U_landscape</i>	2	0.8017	0.02990	1.2090	0.033 *
<i>U_local</i>	2	0.9227	0.03441	1.3914	0.052
<i>U_landscape: U_local</i>	4	1.5498	0.05780	1.1686	0.115
Residual	71	23.5413	0.87790		
Total	79	26.8156	1.00000		

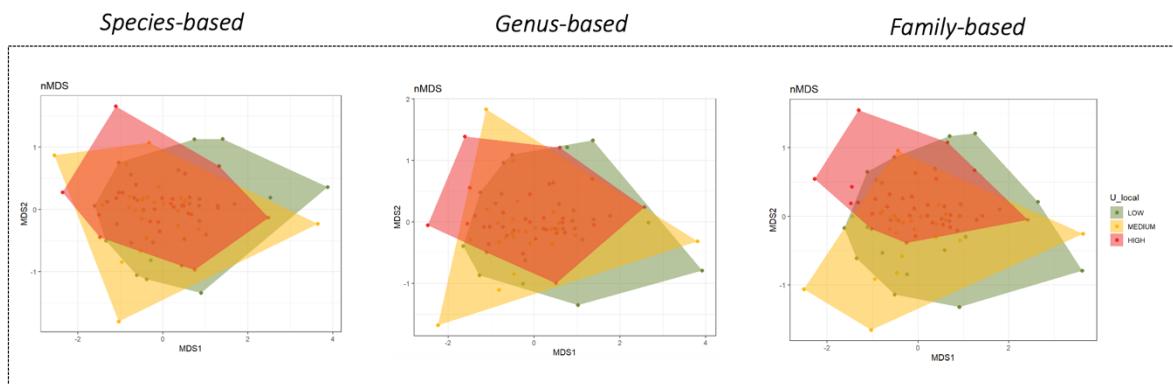


Fig. 26: Overview of the nMDS plots showing how prey communities cluster based on taxonomic levels – species, genus, and family. On the species-level distinct clustering according to urbanisation level is visible, while this clustering is less pronounced at the genus-level, and even more less at the family-level. Considering a finer taxonomic level (Species) provides greater resolution for detecting impacts of urbanisation on the prey community composition.

Visual inspection of the rarefied taxonomic species-based PCoA (principal coordinates analysis) plot shows differences in species composition of the different groups at the local scale (Fig. 27).

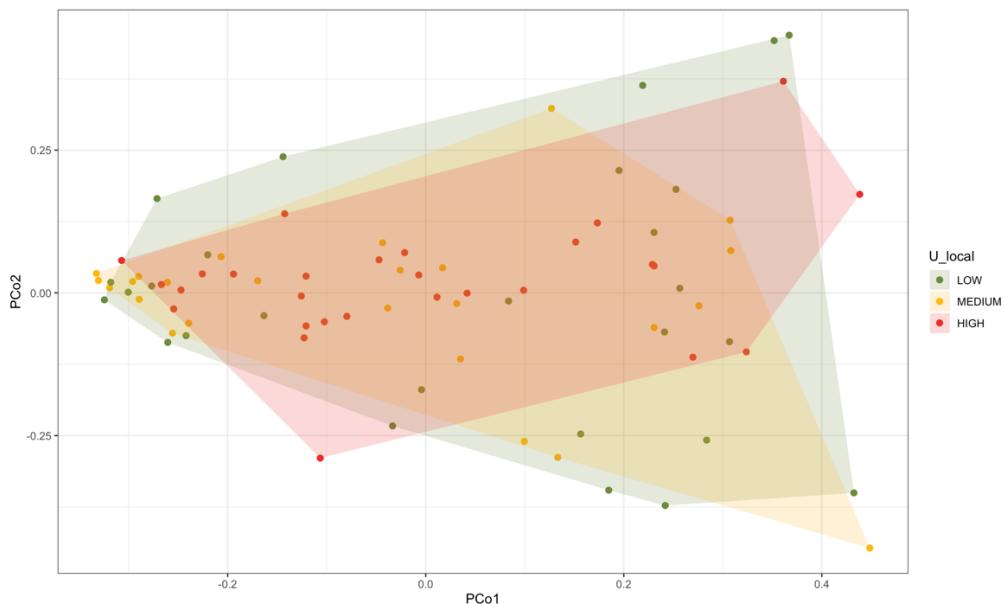


Fig. 27: The principal coordinates analysis plot of the rarefied, taxonomic species based distance matrix. Points represent the 79 different locations (subplotid), which are coloured according to their urbanisation degree at the local scale. The clustering of the data points indicates that there are distinct groups present. The points of the low urbanised group are more spread out.

This pattern was further confirmed by the PERMANOVA analysis (Appendix Fig.B2.14) of the rarefied distance matrix, which revealed marginally significant differences in species composition at the local scale ($df = 2$, $F = 1.3648$, $p_{U_local} = 0.052$) and landscape scale ($df = 2$, $F = 1.0522$, $p_{U_landscape} = 0.057$) (Table 10). This suggests that there may be a possible effect of the urbanisation scales on the distance matrix, although it is not significantly robust to be 100% confident. When analysing the average distance to the median (betadisper) at the local scale and performing a permutation test (Appendix Fig. B2.14), no significant differences were found between the dispersion of the groups. This result supports the assumption of a homogeneous distribution, which means that the PERMANOVA results for U_local are reliable and not biased by the distribution within groups. This assumption was not met at the landscape scale ($Pr(>F) = 0.036$). This means that only the local scale of urbanisation seems to have an almost significant effect on the variation in species composition.

Table 10: Results of the test for differences in species composition between urbanisation at the landscape (U_landscape) and local (U_local) level as inferred from PERMANOVA analysis. Df, degrees of freedom; SumOfSqs, sum of squares; MS, mean squares. Marginally significant values are highlighted in bold.

	Df	SumOfSqs	R2	F	Pr (>F)
U_landscape	2	0.7352	0.02627	1.0522	0.057
U_local	2	0.9536	0.03407	1.3648	0.052
U_landscape: U_local	4	1.4980	0.05352	1.0720	0.191
Residual	71	24.8040	0.88615		
Total	79	27.9908	1.00000		

Looking at the PERMANOVA results of the presence-absence model on the species-based distance matrix, both urbanisation scales have a significant effect ($df = 2$, $F = 1.4262$, $p_{U_landscape} = 0.034$; $df = 2$, $F = 1.4450$, $p_{U_local} = 0.036$; Fig. B2.15) on the presence or absence of a species. Both scales explain each 3.6% of the variation. Most of the variation (88%) is explained by other factors contributing to the variation (Table 11). Pairwise comparisons between the different levels of urbanisation at both scales suggest that the local levels of urbanisation may play a greater role in determining species composition than landscape factors. The local scale had a significant effect on the differences between HIGH and MEDIUM ($df = 2$, $F = 1.3551$, $p = 0.01120$).

Table 11: PERMANOVA results of the presence absence model on the species-based distance matrix. The model tested if differences were significantly influenced by landscape (U_landscape) and local (U_local) degree of urbanisation. Df, degrees of freedom; SumOfSqs, sum of squares; MS, mean squares. Marginally significant or significant values are highlighted in bold. Significant values are indicated with *.

	Df	SumOfSqs	R2	F	Pr (>F)
U_landscape	2	0.5962	0.03568	1.4262	0.034*
U_local	2	0.6041	0.03615	1.4450	0.036*
U_landscape: U_local	4	0.6696	0.04007	0.8008	0.909
Residual	71	14.8410	0.88810		
Total	79	16.1709	1.00000		

Finally, an indicator species analysis was performed on both urbanisation scales (Appendix Fig. B2.16). Almost all indicator species are visualised in Figure 28. At the landscape scale, seven indicator species were found to be significantly associated with particular landscape groups (Table 12a). Three species were associated with the high urbanised group: one within the family Platystomatidae (seq5268), one within the genus *Senotainia* (seq1927) and *Ephydria unangulata* (seq2103). The low urbanised group is

associated with seq2077 and seq1748, which are species within the family Phoridae (*Megaselia scalaris*, 95.261%) and a Diptera species. The highest levels of significance were found for seq5268 and seq2077. This is a signal fly (Platystomatidae) for the urban levels and a hump fly (*Megaselia scalaris*) for the rural levels. At the local scale, 10 indicator species were significantly associated with specific local groups (Table 12b). The high level (H) level indicator species are all within the order Diptera, which is a highly represented order (2583 ASVs). The low level (L) has indicator species within the Coleoptera and Mecoptera, which were observed to be less represented in the overall spider diet (60 & 9 ASVs). *Melanostoma*, an indicator species of the urbanised local scale, has a dispersal capacity of 1, indicating that it is one of the most dispersive species within its order. Two species of Culicidae, also known as mosquitoes, were found to be high and medium level indicator species. The third high and medium levels indicator species was *Tabanus*, a species within the horseflies. Looking at species that seem to characterise a specific category on the urbanisation gradient (Table 13c), *Lucilia sericata* also known as the common green bottle fly, was found to be an indicator species for the highly urbanised category (HH) and *Limata*, a horsefly, for the LM category (U_landscape = Low, U_local = Medium). *Lucilia sericata* has an average body size between 8-10 mm (Anderson & Kaufman, 2011). In comparison to *Limata*, the indicator species for more rural sites (LM) with an average body size between 10-20 mm (Mullens, 2019), *Lucilia* is smaller.

Table 12: Results of the indicator species analysis (a) at the landscape scale (b) at the local scale and (c) between categories on the urbanisation gradient. These are the species identified as significantly associated with a particular degree. The colours indicate the degree of urbanisation, no colour means present in multiple groups or a specific group on the urbanisation gradient. X indicates that this information was unknown. Size is given in mm and is the body size measured from head to abdomen, except for butterflies (Lepidoptera) where size is the length of the anterior wing. Dispersal indicates the dispersal capacity of that species as a relative value to other species of the same order. This means that a species with a dispersal of 1 is the most dispersive within its order, and zero the least dispersive.

<i>Seqid</i>	<i>Group</i>	<i>p-value</i>	<i>Order</i>	<i>Family</i>	<i>Genus (+ Species)</i>	<i>Size</i>	<i>Dispersal</i>
seq5268	H	0.0087	Diptera	Platystomatidae	X	X	X
seq1927	H	0.0346	Diptera	Sarcophagidae	Senotainia	X	X
seq2103	H	0.0413	Lepidoptera	Geometridae	Euphyia unangulata	13.5	X
seq2077	L	0.0094	Diptera	Phoridae	<i>Megaselia scalaris</i>	X	X
seq1748	L	0.0286	Diptera	X	X	X	X
seq3175	L + M	0.0408	Diptera	X	Leucophenga	X	X
seq261	L + H	0.046	Diptera	X	X	X	X

(a)

<i>Seqid</i>	<i>Group</i>	<i>p-value</i>	<i>Order</i>	<i>Family</i>	<i>Genus (+ Species)</i>	<i>Size</i>	<i>Dispersal</i>
seq1453	H	0.0007	Diptera	Muscidae	Helina	X	X
seq1273	H	0.0424	Diptera	X	X	X	X
seq711	H	0.0500	Diptera	Syrphidae	<i>Melanostoma</i>	8	1
seq2275	L	0.0183	Coleoptera	Carabidae	X	X	X
seq1001	L	0.0275	Mecoptera	Panorpidae	Panorpa	X	X
seq578	M	0.0084	Diptera	Tabanidae	<i>Limata</i>	X	X
seq1323	M	0.0275	Diptera	Drosophilidae	Scaptodrosophila	X	X
seq831	H + M	0.0221	Diptera	Culicidae	Culex	X	X
seq1342	H + M	0.0332	Diptera	Culicidae	X	X	X
seq215	H + M	0.0487	Diptera	Tabanidae	<i>Tabanus</i>	X	X

(b)

<i>Seqid</i>	<i>Group</i>	<i>p-value</i>	<i>Order</i>	<i>Family</i>	<i>Genus (+ Species)</i>	<i>Size</i>	<i>Dispersal</i>
seq347	HH	0.037	Diptera	Calliphoridae	<i>Lucilia sericata</i>	9	X
seq578	LM	0.0191	Diptera	Tabanidae	<i>Limata</i>	10-22	X

(c)

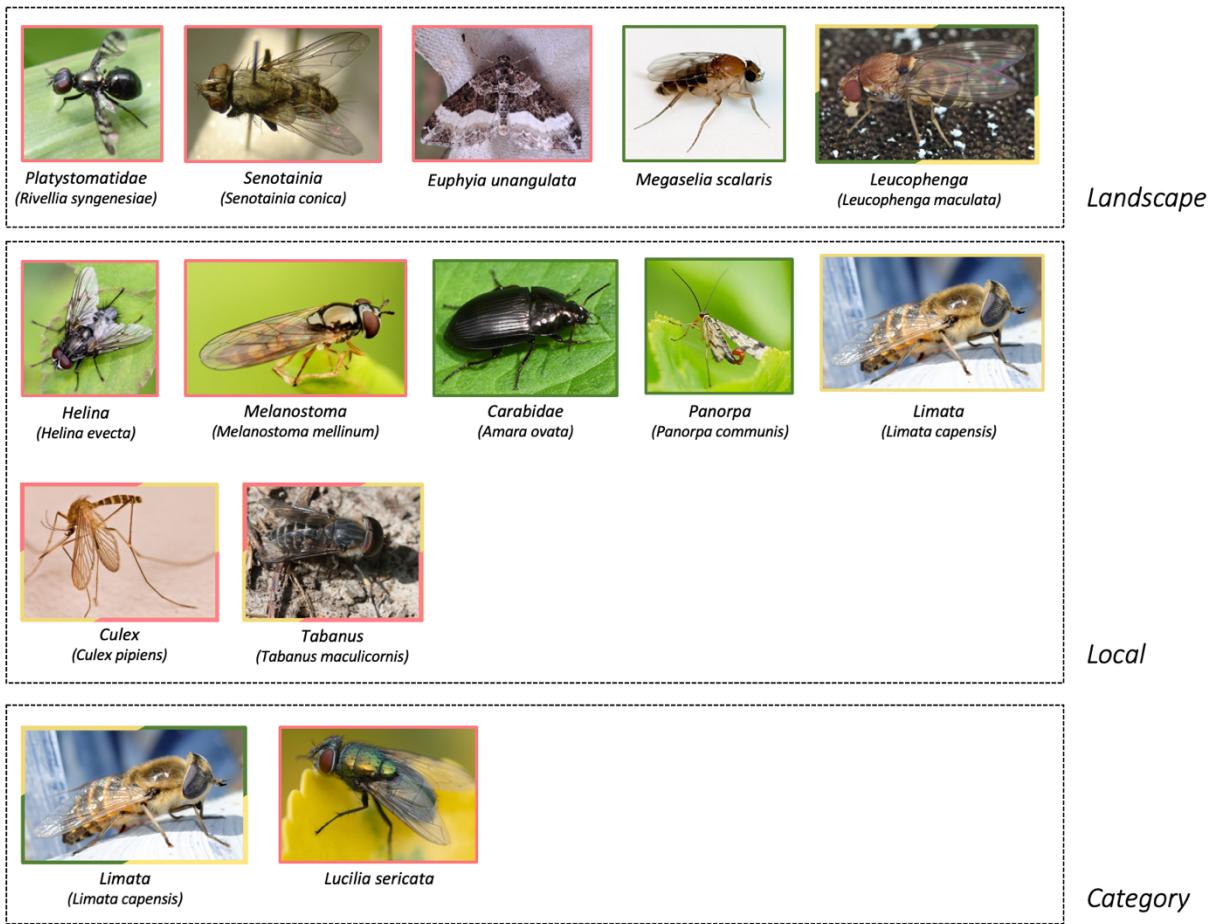


Fig 27. Pictures of the indicator species at the landscape scale, local scale and between categories on the urbanisation gradient. The name of each identified indicator species (Seqid) is similar to those in Table 12 (i.e., family, genus, or species name). Names in parentheses indicate which specific species is illustrated. Colours indicate at which urbanisation degree they occur (red = high, yellow = medium, green = low). [Images are all from www.waarnemingen.be or iNaturalist].

5 DISCUSSION

5.1 Sequencing output and its current limitations

A total of 468 spiders from 79 different locations, altogether covering the entire urban gradient (for 3 regions: Ghent, Antwerp, Leuven), were subjected to DNA metabarcoding. This was followed by species identification, which allowed for the discovery of a comparatively large number of species to be found across numerous taxonomic groups. The total number of unique species (ASVs) found after the application of all filtering steps in this study was 3629. This indicates that 3629 unique barcodes were identified across all spider samples. Despite the high number of ASVs identified, the number of ASVs that received no identification at the species level (3263) remained high. Nevertheless, this can be explained by the following limitations inherent to DNA metabarcoding techniques. The efficacy of the taxonomic identification steps of the metabarcoding process is only as good as the quality of the COI database in BOLD and GenBank. It is not possible to correctly identify all species or taxonomic groups based on the targeted 157 bp COI sequence. In order to identify and detect the rare species in the spider's gut, it is necessary for the prey species in question to be present in the database. However, this is not always the case. Many species still lack a reference barcode in the databases. Another limitation is that the application of thresholds during the filtering process may result in the inability to account for rare sequences as effectively as desired. Nevertheless, the number of taxa that cannot be resolved to the species level by metabarcoding will decline over time as barcode libraries continue to expand (Remmel et al., 2024). The current limitations can be addressed by implementing methodological improvements, such as the use multiple markers, as no single marker is capably of identifying all insects (Remmel et al., 2024).

5.2 Particular orders and families are dominating the overall spider diet.

The European garden spider's diet mainly consisted out of the orders Diptera (2583 ASVs), Lepidoptera (283), Araneae (165), Hemiptera (98), Hymenoptera (88) and Coleoptera (60). Additionally, there is a high abundance of certain families identified within these orders, including Araneidae (155), Muscidae (126), Drosophilidae (110), Calliphoridae (105) and Ichneumonidae (77). The high abundance of these families in the overall spider diet analysis may be due to the number of species within these families related to direct ecosystem (dis)services to humans (i.e., pollination, honey production, disease transmission or crop damage) (Remmel et al., 2024). In other words, they are more represented in the reference database used to identify unique barcodes until a certain taxonomic level. For example, the orders Orthoptera (grasshoppers), Mecoptera (scorpion flies) and Neuroptera (net-winged insects) for example only had 20, 9 and 7 ASVs in the overall spider diet analysis. These orders are not directly linked to ecosystem (dis)services provided to humans. Consequently, they may be underrepresented in the sequencing output. It is notable that none of the Orthoptera ASVs were assigned a family, genus, or species level. This may indicate a lack of reference sequences for these in the database, as no match could be found. These so-called barcode coverage gaps remain for non-charismatic species and for species not related to ecosystem (dis)services.

As observed in the study by Van Schrojenstein et al. (2021), Diptera appears to be the dominant component of the overall diet. The high abundances of several Diptera families, including Muscidae (houseflies), Drosophilidae (fruitflies) and Calliphoridae (blowflies), can contribute to the

homogenising effect of urbanisation on the spider's diet. Moreover, these Diptera families include numerous species that are considered pest species (Pedigo & Rice, 2014). This suggests that the environmental conditions in highly urbanised areas favour their abundance over others as confirmed by previous research (Burkman & Gardiner, 2014; Fenoglio et al., 2021; Langellotto & Hall, 2021).

Important to note is that the results of this diet analysis represent only a momentary recording of what the spider ate a few days prior. It is possible that the spider had not yet consumed its meal at the time of sampling, resulting in the absence ASVs not only caused by reduced prey availability. These samples were removed from further analysis. Additionally, it might be possible that spiders feed on adhered pollen or fungal spores when recycling their web (Eggs & Sanders, 2013). These resources provided by pollen may be an important dietary supplementation that are not taken into account in this study.

5.3 Urbanisation does not seem to affect the observed diet richness.

In contrast to the hypothesis that urbanised sites would exhibit lower richness based on the knowledge that declines in species richness occur along the urbanisation gradient (Piano et al., 2020), *Araneus diadematus* appears to have consumed an equal number of prey species regardless of the degree of urbanisation. Both the ASV richness and the taxonomic species richness did not significantly differ between locations on the urbanisation gradient. Comparing the species richness between spiders revealed a trend on the local scale, with city-living spiders consuming an average number of prey that was higher than that of spiders at rural sites.

Some flying taxa are thriving in urban areas, which can compensate for the losses of other more specialist species that were filtered out (Chatelain et al., 2023; Colles et al., 2009). This causes the number of observed species in cities to be approximately equal with more rural sites. *Araneus diadematus*, is adapting the characteristics of its webs in an adaptive way to maximize prey interception in environments with reduced prey availability, such as cities (Bonte et al., 2008; Dahirel et al., 2017). The adaptations in its web-building such as a smaller mesh width, to catch the urban occurring species, may reflect why the number of consumed species does not seem to be affected this much. This indicates how the availability of arthropods has a bottom-up effect, thus shaping foraging behaviour at the higher trophic level (Chatelain et al., 2023). Additionally, the sampling of spiders was performed towards the end of summer, which may also account for the higher abundances of prey observed in the urban diet. This is due to the fact that in more urban areas, due to warmer temperatures and/or higher food availability, the adult survival of some arthropod species is extended even after the end of the summer (Lowe et al., 2016).

5.4 Urbanisation negatively affects the spider's diet diversity, leading towards homogenisation of the menu in urbanised locations.

Despite the observed number of consumed species remaining unaffected by urbanisation, the diversity of the spider's diet was negatively affected. This confirms the hypothesis that the diet of the generalist predator *A. diadematus*, reflects similar diversity and composition patterns among flying insects, as observed in our previous research using morphological identification of prey arthropod species. The observed negative effects on the diversity of the diet as well as the presence or absence of a species in the diet were mostly dependent on the local land use type (rural, medium, urban for 200 x 200 m).

Results of comparing the Simpson diversity of subplots along the urbanisation gradient, showed significant differences between the medium and urban (HIGH) land use types and almost significant differences between the rural (LOW) and urban (HIGH) land use types. Less-urbanised locations appeared to have lower Simpson diversity. This indicates that the diet of spiders living in more rural areas contains a greater variety of species, with relatively equal abundances. An examination of the relative abundance of different prey species consumed provided further confirmation of this. The menus of urbanised spiders on the local scale tended to be more dominated by one or two families, in contrast to the menus of spiders at natural sites, where the options were more evenly distributed. This dominance of a few species in the urban diet, indicates the increasing occurrence of biotic homogenisation in cities.

It is important to know the ecology of the prey species that are typically associated with a particular landscape or local urbanisation group. Depending on what prey is available, the spider might need to change its behaviour to maintain its fitness (Venner & Casas, 2005). An indicator species analysis revealed seven indicator species at the landscape scale and 10 at the local scale. Particularly at the local scale, there was strong support for the hypothesis that the diet of urban spiders is less diverse. For example, all indicator species for urban sites were from the order Diptera, which was the most dominant order in the overall spider diet analysis, whereas those from more rural areas were species within the Coleoptera and Mecoptera, which were observed to be less represented in the overall spider diet (60 & 9 ASVs). This shows that there are differences in taxonomic diversity in the diet, with spiders living in rural areas consuming more rare prey compared to spiders living in urban areas consuming mainly the most abundant prey order. Other differences were found when comparing the ecology of some indicator species. One of the urbanised indicator species is *Melanostoma*, which received a dispersal capacity of 1, indicating that it is one of the most dispersive species within its order. This may indicate that highly mobile species are less affected by urbanisation (Entling et al., 2011; Kneitel, 2018; Löveï & Sunderland, 2003). There are also differences in the body size of the indicator species, which is important because spiders prefer larger prey on their menu, because these are more energy-efficient (Venner & Casas, 2005). The adult body length of species varies between 3-30 mm within the Mecoptera, between 0.35-200 mm within the Coleoptera and between 1-60 mm within the Diptera (Chown & Gaston, 2010). On average the body size of the indicator species in urbanised sites appears to be lower than that of more locally rural sites. This finding is in line with previous research by Merckx et al. (2018). Even when examining species that are presumed to characterise a specific category on the urbanisation gradient, the results demonstrated that the body size of prey in the highly urbanised sites (HH) was notably smaller. *Lucilia sericata* also known as the common green bottle fly, was found to be the indicator species with an average body size of 9 mm. In comparison to *Limata*, the indicator species for more rural sites (LM) with an average body size between 10-20 mm (Mullens, 2019), *Lucilia* is smaller.

The results on the analysis of indicator species and the NMDS (Fig.25) strongly indicated that the diet at rural sites was quite dissimilar from that observed at urban and medium sites, which exhibited a dominance of certain species with smaller body size or being highly mobile species. This homogenisation of the urban diet has drastic consequences for our orb-weaver *A. diadematus*, which is almost entirely dependent on flying insects as a food source. The loss of dietary diversity is expected to have a drastic negative impact on the survival and abundance of this spider species. Although *A. diadematus* is a generalist predator, it may prefer large prey over smaller ones in order to maintain its

fitness. The negative impact on fecundity of the spider is mainly caused by the lower abundance of larger prey, or so-called life-saving catches (Venner & Casas, 2005). Furthermore, foraging on diverse prey may allow generalist predators to balance their nutrient intake (Rendon et al., 2019). Previous research (Robinson & Robinson, 1970; Leborgne & Pasquet, 2005) has already shown that the reduced food intake of this spider in recent years is likely to have negatively impacted its fecundity and survival, which in turn may have led to the decline in abundance documented in the study of Nyffeler & Bonte, 2020. The decline in survival of the European garden spider thus illustrates the strong bottom-up trophic cascade in response to widespread homogenisation of flying insect prey.

6 CONCLUSION

This study highlights the significant impact of increasing urbanisation on the diet of *A. diadematus*, which represents the composition of arthropod communities at a unique location. Despite the fact that the diet richness of spiders living in more urban areas did not significantly decrease, their diversity did. The observed homogenisation of the diet of the European garden spider reveals a bottom-up trophic cascade effect in response to the widespread loss of flying insect prey. This dietary shift has drastic consequences for the fitness of the spider itself. Further research is needed to overcome some of the remaining limitations of DNA metabarcoding techniques, particularly the identification of rarer sequences in order to capture the full diversity of the diet. Nevertheless, the observed dietary shifts (less diverse and smaller prey at urban sites) were comparable to those observed in our previous study. Consequently, DNA metabarcoding may therefore provide a cost-effective approach to investigate the cascading effects urbanisation has on the diets of higher trophic levels. This study also highlights the necessity for further research into the potential impact of changes in the diet on the abundance of this spider, as they represent the next step in the food web to higher trophic levels, such as birds and lizards. It is crucial to determine whether the phenomenon of urban homogenisation and starvation could potentially lead to the collapse of entire food webs and ecosystems, as proposed by researchers.

7 SUMMARY

7.1 English summary

Insect populations are shrinking all over the world. Urbanisation is known to be a major factor in this global decline of insects and numerous other species worldwide. Although insects are frequently mentioned as the most functionally important organisms on Earth, an estimated 80% of insect species remain unknown to science. Arthropods represent a highly diverse group of organisms that serves as the base of food webs. Changes in arthropod richness and diversity can have cascading effects on higher trophic levels, including the study species *Araneus diadematus*, the European garden spider. The menu options of such sit-and-wait predators are mainly influenced by the composition of their prey communities. The composition of the spider's prey community depends on which species are caught in the spider's sticky web and on the community that live near where the spider has built its web.

Understanding how the distribution and abundance of insects are shifting in space, particularly in response to the rising level of urbanisation, is crucial. Urbanisation tends to favour adaptable generalist species over non-urban specialists, leading to biotic homogenisation. The overall objective of this study is to investigate the dietary shifts and patterns of European garden spiders along replicated urbanisation gradients in order to gain insight into the current insect communities along these gradients. Spiders living in cities are expected to consume a less diverse diet of flying prey than those living in more natural habitats. A sampling of the potential prey of European garden spiders along urbanisation gradients does not consider the specific characteristics of the web and the predator-prey interactions. In other words, this method will not accurately reflect the actual prey consumption. The application of DNA metabarcoding techniques enables the identification of the specific flying insects consumed by the spider within its home range. Such dietary studies are crucial in order to gain an understanding of how urbanisation affects higher trophic levels, as a bottom-up response to the lower availability and diversity of prey.

To achieve this, a hierarchically nested sampling design was used, whereby the proportion of built-up area (BU) was considered at two spatial scales: 3 x 3 km plots and 200 x 200 m subplots within each plot. This approach enabled us to investigate the spatial scale at which urbanisation had the greatest impact on diversity. Arthropod abundance, richness, and diversity (Shannon and Simpson index) were compared using generalised linear mixed models with urbanisation levels (both scales) as explanatory variable. Depending on the specific approach taken the correct random variable was implemented into the model: at each spider separately (random = subplotid) or at each unique location (random = plotid). Bray-Curtis dissimilarity and Jaccard distances were used to visualize dissimilarities in the diet composition and the presence/absence along the urbanisation gradient. An indicator species analysis was performed on both urbanisation scales to identify species that are strongly associated with urbanisation levels at each scale (U_local, U_landscape).

DNA metabarcoding of 468 spiders from 79 locations across Ghent, Antwerp and Leuven identified 3629 unique species (ASVs). Despite limitations inherent in current DNA metabarcoding techniques, including database incompleteness, our findings shed light on the predominant consumptions of European garden spiders. Diptera emerged as the dominant prey, particularly abundant in urban

environments, suggesting a high occurrence of pest species in the diet. Urbanisation did not reduce the number of prey for *Araneus diadematus*, but it did impact diet diversity, leading to homogenisation.

The homogenisation of urban diets, dominated by smaller, highly mobile prey species, poses challenges for spider survival and fitness. The loss of dietary diversity may impact fecundity and survival, potentially leading to a decline in *A. diadematus* abundance. These findings reveal a bottom-up trophic cascade effect in response to the widespread loss of flying insects. The observed changes were comparable to those observed in our previous study (smaller and less diverse prey) proving that DNA metabarcoding can be a cost-effective approach that requires less taxonomic expertise. Nevertheless, further research is necessary to overcome some remaining limitations, particularly the identification and representation of rarer sequences in the database in order to capture the full diversity on the menu.

This study also highlights the necessity for further research into the potential impact of shifts in the diet on the abundance of this spider, as they represent the next step in the food web to higher trophic levels, such as birds and lizards. It is crucial to determine whether the phenomenon of urban homogenisation and starvation could potentially lead to the collapse of entire food webs and ecosystems, as proposed by researchers.

7.2 Nederlandse samenvatting

De diversiteit aan insecten neemt wereldwijd af. Het is geweten dat verstedelijking een belangrijke factor is in deze wereldwijde achteruitgang van zowel insecten als talloze andere soorten. Hoewel insecten vaak vermeld worden als de meest functioneel belangrijke organismen op aarde, blijven er naar schatting nog 80% soorten onbekend voor de wetenschap. Insecten behoren tot een zeer diverse groep organismen die de basis vormt van het voedsel web. Veranderingen in hun rijkdom en diversiteit kan leiden tot cascade-effecten op hogere trofische niveaus, waaronder deze van onze studiesoort *Araneus diadematus*. De menuopties van dergelijke sit-and-wait predatoren worden voornamelijk beïnvloed door de samenstelling van hun prooi gemeenschap. De samenstelling van die prooi gemeenschap is afhankelijk van wat er rondom leeft en gevangen wordt in het kleverige web van de spin.

Het is belangrijk om te begrijpen hoe de verspreiding en rijkdom van insecten in de ruimte verschuift en vooral hoe dit samenhangt met de toenemende verstedelijking. Verstedelijking heeft de neiging om aan de stad aangepaste, typische generalistische soorten te bevoordelen ten opzichte van niet-stedelijke, gespecialiseerde soorten, wat op zijn beurt leidt tot biotische homogenisering. Het doel van deze studie is om de dieet verschuivingen van de kruisspin langs gerepliceerde verstedelijkingen gradiënten te onderzoeken om dan inzicht te krijgen in de huidige insecten gemeenschappen langs deze gradiënten. Van spinnen die in steden leven, wordt verwacht dat ze een minder gevarieerd menu aan vliegende prooien consumeren in vergelijking met spinnen die in een meer natuurlijke habitat leven. Het inzamelen van potentiële prooien van de kruisspin langs de verstedelijkingen gradiënten houdt geen rekening met de specifieke kenmerken van het web en de predator-prooi interacties. Met andere woorden, deze methode geeft niet de effectieve prooi inname weer. De toepassing van DNA-metabarcoding technieken maakt het mogelijk om de vliegende insecten die door de spin zijn

geconsumeerd te identificeren. Dergelijke dieet studies zijn cruciaal om inzicht te krijgen in hoe verstedelijking hogere trofische niveaus beïnvloedt, als bottom-up reactie op de lagere beschikbaarheid en diversiteit van de prooigemeenschap.

Een hiërarchische geneste steekproefopzet werd gebruikt, waarbij het aandeel bebouwd gebied (BU) werd beschouwd op twee ruimtelijke schalen: 3 x 3 km plots en 200 x 200 m subplotts binnen elke plot. Deze aanpak stelde ons in staat om de ruimtelijke schaal te onderzoeken waarop verstedelijking de grootste impact had op diversiteit. De abundantie, rijkdom en diversiteit (Shannon- en Simpson-index) van Arthropoda werden vergeleken met behulp van GLMM, met verstedelijkniveaus (beide schalen) als verklarende variabele. Afhankelijk van de specifieke benadering werd de juiste random variabele in het model geïmplementeerd: voor elke spin afzonderlijk (random = subplotid) of voor elke unieke locatie (random = plotid). Bray-Curtis disimmilariteit en Jaccard-afstanden werden gebruikt om verschillen in dieet samenstelling en de aanwezigheid/afwezigheid van soorten langs de verstedelijkingstraditie te visualiseren. Een indicator-soortenanalyse werd uitgevoerd op beide schalen om soorten te identificeren die sterk geassocieerd zijn met verstedelijkniveaus op elke schaal (U_landscape, U_local).

DNA-metabarcoding van 468 spinnen van 79 locaties in Gent, Antwerpen en Leuven identificeerde 3629 unieke soorten (ASVs). Ondanks de beperkingen die inherent zijn aan de huidige DNA-metabarcoding technieken, zoals de onvolledigheid van de database, werpen onze bevindingen licht op de overheersende consumptiepatronen van de kruisspin. Diptera bleken de dominante prooi te zijn, vooral in stedelijke omgevingen, wat kan duiden op een hoog voorkomen van plaagsoorten in het dieet. Verstedelijking verminderde het aantal prooien voor *A. diadematus* niet, maar had wel een invloed op de diversiteit van het dieet, wat leidde tot homogenisering.

De homogenisering van stedelijke diëten, die gedomineerd worden door kleinere, zeer mobiele prooisoorten, vormt een uitdaging voor de overleving en fitness van de spinnen. Het verlies aan diversiteit in hun voedsel kan invloed hebben op de vruchtbaarheid en overleving, wat mogelijk leidt tot een afname van de kruisspin in aantallen. Deze bevindingen onthullen een bottom-up trofische cascade-effect als reactie op het wijdverspreide verlies van vliegende insecten. De waargenomen veranderingen waren vergelijkbaar met deze in onze vorige studie (kleinere insecten en minder diversiteit), wat bewijst dat DNA-metabarcoding een kost effectieve aanpak kan zijn die minder taxonomische expertise eist. Niettemin is verder onderzoek nodig om enkele resterende beperkingen te overwinnen, met name de identificatie en vertegenwoordiging van zeldzamere sequenties in de database om de volledige diversiteit op het menu vast te leggen.

Deze studie benadrukt ook de noodzaak voor verder onderzoek naar de mogelijke impact van verschuivingen in het dieet op de abundantie van de kruisspin, aangezien zij de volgende stap in het voedsel web naar hogere trofische niveaus, zoals vogels en hagedissen, vormen. Op deze manier kunnen we dan vaststellen of het fenomeen van stedelijke homogenisering en uithongering mogelijk kan leiden tot de ineenstorting van hele voedselwebben en ecosystemen, zoals onderzoekers hebben voorgesteld.

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APPENDIX A Protocols

Protocol I: DNA extraction

The spider was dissected into two equal parts. One half of the specimen was used to extract the DNA of the spider, *Araneus diadematus*, using the MagAttract® HMW DNA Kit (Qiagen). The spiders were placed separately put in 1.5 mL Eppendorf tubes containing 440 µL Buffer ATL and 40 µL Proteinase K. The head and legs of the spider were first crushed in the tube, followed by the abdomen. Subsequently, the tubes were then incubated for two hours at 51.6°C at a speed of 300 revolutions per minute (rpm) in a thermal shaker until the sample was completely lysed. This was followed by a 5-minute centrifugation of the tubes. A volume of 200 µL of the lysate was then transferred to a new 2 mL tube. A total of 4 µL of RNase A was added to each sample, followed by 150 µL of Buffer AL, 280 µL of Buffer MB, and 40 µL of MagAttract Suspension G. Prior to the addition of any of these buffers or suspensions, the mixture was vortexed (1400 rpm) for 2 minutes at room temperature. The tubes were centrifuged in order to remove drops of liquid from the inside of the cap. The tubes were then incubated for two minutes at room temperature at 900 rpm. Afterwards, the samples are placed in the tube holder of the MagAttract Magnetic Rack for DNA isolation. The supernatant may be removed, with the pipette. It is important to avoid disturbing the magnetic bead pellet while during this process. Once all liquid has been removed, the washing steps commence. A total volume of 700 µL of MW1 Buffer was then added to the sample, which was then placed on a thermoshaker at 900 rpm for a period of two minutes. After bead separation, all liquid was removed. This step was repeated twice. A further 700 µL of PE Buffer was added to the sample, which was then incubated for two minutes at 900 rpm. The supernatant was then removed by placing the tube on the magnetic base, a process that was repeated twice. Finally, all traces of PE Buffer were removed with a small pipette tip. The final washing step involved rinsing the particles with 700 µL of distilled water, with the beads attached to the walls of the tube, and carefully removing the liquid, repeated two times. The tube holder was removed from the magnetic base and 115 µL of the Buffer AE was added. The tubes were Incubated the tubes one last time for five minutes at 1400 rpm. The method results in a high molecular weight genomic DNA concentration in the supernatant after centrifugation. The eluted DNA was transferred to two new 1.5 mL Eppendorf tubes (2 x 50 µL), one for metabarcoding and the other for a microbiome study.

Protocol II: Library preparation for metabarcoding of spider diet

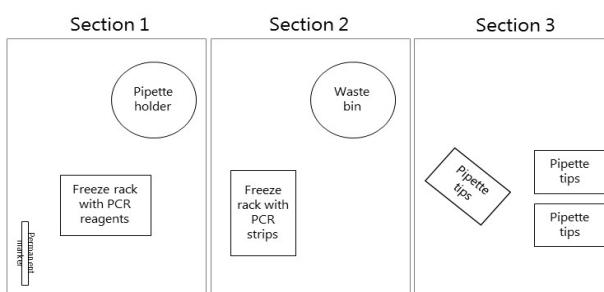


Fig. A1: Flow hood setup

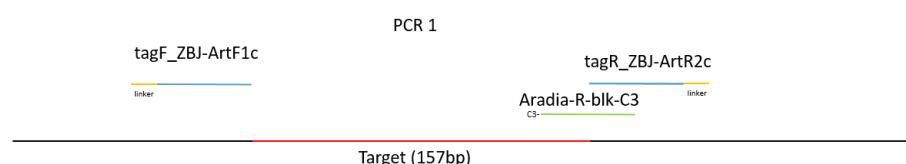


Fig. A2: Primers used for the first PCR reaction, targeting 157 bp of interest.

For the first PCR, the following three primers were used: tagF_ZBJ, tagR_ZBJ and Aradia-R-blk. These were first diluted (10x). The next steps were all carried out in the flow hood (Fig. A1). For tagF_ZBJ and tagR_ZBJ, the primers were vortexed and 10 µL of each were put into a separate tube and diluted with 90 µL H₂O. For Aradia-R-blk, it was 50 µL diluted with 450 µL H₂O. These primers are required for the master mix (Table A1) and are placed on ice. Calculations for this mix are based on the number of samples in a library, which is 39, but with some reserve 41. The vials are labelled from 1 to 39. After diluting the primers, the master mix can be prepared. Add 82 µL H₂O, 41 µL recombinant albumin to a 2 mL tube. Vortex and spin down the primers and add 41 µL of the forward primer (tagF_ZBJ), 41 µL of the reverse primer (tagR_ZBJ) and 205 µL of the blocking primer (Aradia-R-blk). After adding 410 µL of Hotstar, the master mix was vortexed and spun down. 20 µL of the master mix was added to each of the small tubes, the lids were sealed and placed on ice. Outside the flow hood, 2 µL of DNA was added to the tubes after vortexing, according to the label (e.g., 1 = P10 SY3) using a new pipette tip for each sample. The samples were transferred to the PCR room where each sample was vortexed before being placed in the PCR machine (Veriti 96 well Thermal Cycler Applied Biosystems). The programme for this PCR was set as follows: 95 °C 15 min, (94°C 30 sec, 46°C 30 sec, 72 °C 60 sec x 30 cycles), 72 °C 7,0 min, 4° C forever. When finished, all samples were placed in the freezer.

For the second PCR, The Illumina_MIDX tag forward and reverse tags are diluted (10x) together in a tube. These form the Illumina tags for the library (1-39) and must be melted before use (Fig. A3).

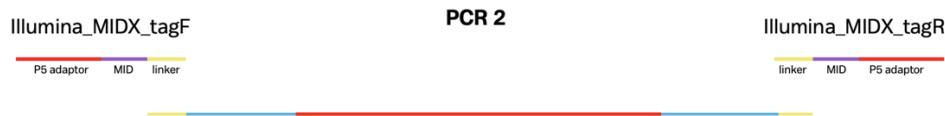


Fig. A3: Primers used for the second PCR reaction, using Illumina_MIDX tags.

First, a master mix (Table A1) was prepared containing 420 µL of Hotstar, 42 µL recombinant albumin and 294 µL H₂O. 18 µL of the master mix was added in each of the new labelled tubes (1-39). The box containing the Illumina tags was vortexed and spun down. Using a multi-tip pipette, 2 µL was added to the corresponding labelled tubes. The library from the first PCR was vortexed and spun down. 2 µL of the first PCR library is added to the correct library for the second PCR (corresponding labelled tubes). Each sample is spun down before being placed in the PCR machine (Veriti 96 well Thermal Cycler Applied Biosystems). The programme for this PCR was set as follows: initial denaturation at 95 °C 15 min, (denaturation at 94°C 30 sec, annealing at 60°C 30 sec, extension at 72 °C 60 sec x 40 cycles), final extension at 72 °C 7,0 min, 4° C forever. When finished, all samples were placed in the freezer.

Table A1: Primers used in this study, PCR settings, including reaction volumes, thermal settings, and number of cycles for the PCR reactions carried out for each primer set. The reaction volumes are written down for one sample, multiply by 40 to create a library containing 39 samples and one blanco.

Primers	Sequence	Target group	Amplified region	Reference	
Aradia-R-blk-C3	CCAAATCCCCAATTAAAATAGGTATA-C3 spacer	Araneae	COI-region spider	Van Schrojenstein Lantman et al. (2021)	
tagF_ZBJ-ArtF1c tagR_ZBJ-ArtR1c	ACGACGTTGAAAAAGATATTGGAACWTTAT ATTTTATTTTG	Arthropods	COI-region	Zeale et al. (2010)	
	CATTAAGTTCCCATTAWACTAATCAATTWCCA AATCCTCC				
PCR	Reaction volumes	Thermal settings		Cycles	
PCR1	10 µL HotStar	15 min at 95 °C (Initial denaturation)		1	

	1 µl tagF_ZBJ-ArtF1c 1 µl tagR_ZBJ-ArtR1c 1 µl Aradia-R-blk-C3 1 µl BSA 2 µl H2O 2 µl template DNA	30 s. at 94 °C (Denaturation) 30 s. at 46 °C (Annealing) 60 s. at 72 °C (Extension)	30
		7 min at 72 °C (Final extension)	1
PCR2	10 µl HotStar 1 µl Illumina_MIDx_tagF 1 µl Illumina_MIDx_tagR 1 µl BSA 7 µl H2O 2 µl PCR product	15 min at 95 °C (Initial denaturation)	1
		30 s. at 94 °C (Denaturation) 30 s. at 60 °C (Annealing) 60 s. at 72 °C (Extension)	40
		7 min at 72 °C (Final extension)	1

Table A2: Illumina_MIDx tags used for the second PCR reaction. The MID26 tag was not used in our study.

Forward	Reverse
MID1_tagF NNNAACAAACCGACGTTGTAAAA	MID1_tagR NNNAACAAACCATTAAGTCCCCATTA
MID2_tagF NNAACCGAACGACGTTGTAAAA	MID2_tagR NNAACCGACATTAAGTCCCCATTA
MID3_tagF NNNCCGGAAACGACGTTGTAAAA	MID3_tagR NNNCCGGAAACATTAAGTCCCCATTA
MID4_tagF NNAGTGTACGACGTTGTAAAA	MID4_tagR NNAGTGTACATTAAGTCCCCATTA
MID5_tagF NNNCCGCTGACGACGTTGTAAAA	MID5_tagR NNNCCGCTGCATTAAGTCCCCATTA
MID6_tagF NNAACGCGACGACGTTGTAAAA	MID6_tagR NNAACGCGCATTAAGTCCCCATTA
MID7_tagF NNNNGCTACAGCACGTTGTAAAA	MID7_tagR NNNNGCTACCATTAAGTCCCCATTA
MID8_tagF NNTTCTCGACGACGTTGTAAAA	MID8_tagR NNTTCTCGCATTAAGTCCCCATTA
MID9_tagF NNNTCACTCAGCACGTTGTAAAA	MID9_tagR NNNTCACTCCATTAAGTCCCCATTA
MID10_tagF NNGAACTAACGACGTTGTAAAA	MID10_tagR NNGAACTACATTAAGTCCCCATTA
MID11_tagF NNNCCGTCCACGACGTTGTAAAA	MID11_tagR NNNCCGTCCCATTAAGTCCCCATTA
MID12_tagF NNAAGACAACGACGTTGTAAAA	MID12_tagR NNAAGACACATTAAGTCCCCATTA
MID13_tagF NNNCGTGGCACGACGTTGTAAAA	MID13_tagR NNNCGTGGCCTTAAGTCCCCATTA
MID14_tagF NNGGTAAGACGACGTTGTAAAA	MID14_tagR NNGGTAAGCATTAAGTCCCCATTA
MID15_tagF NNNATAATTACGACGTTGTAAAA	MID15_tagR NNNATAATTATTAAGTCCCCATTA
MID16_tagF NNCGTACACGACGTTGTAAAA	MID16_tagR NNCGTACATTAAGTCCCCATTA
MID17_tagF NNNTTGAGTACGACGTTGTAAAA	MID17_tagR NNNTTGAGTCATTAAGTCCCCATTA
MID18_tagF NNAAGCAGACGACGTTGTAAAA	MID18_tagR NNAAGCAGCATTAAGTCCCCATTA
MID19_tagF NNNTTGCAAACGACGTTGTAAAA	MID19_tagR NNNTTGCAACATTAAGTCCCCATTA
MID20_tagF NNCACGTACATTAAGTCCCCATTA	MID20_tagR NNCACGTACATTAAGTCCCCATTA
MID21_tagF NNNTAACATACGACGTTGTAAAA	MID21_tagR NNNTAACATCATTAAGTCCCCATTA
MID22_tagF NNTGCGTGACGACGTTGTAAAA	MID22_tagR NNTGCGTGCTTAAGTCCCCATTA
MID23_tagF NNNNGTCGAACGACGTTGTAAAA	MID23_tagR NNNNGTCGACATTAAGTCCCCATTA
MID24_tagF NNCACTCTACGACGTTGTAAAA	MID24_tagR NNCACTCTCATTAAGTCCCCATTA
MID25_tagF NNNCTTGGTACGACGTTGTAAAA	MID25_tagR NNNCTTGGTACATTAAGTCCCCATTA
MID26_tagF	MID26_tagR
MID27_tagF NNNACTCAACGACGTTGTAAAA	MID27_tagR NNNACTCACATTAAGTCCCCATTA
MID28_tagF NNGCGAGAACGACGTTGTAAAA	MID28_tagR NNGCGAGACATTAAGTCCCCATTA
MID29_tagF NNNTGGAACACGACGTTGTAAAA	MID29_tagR NNNTGGAACATTAAGTCCCCATTA
MID30_tagF NNGTACACGACGTTGTAAAA	MID30_tagR NNGTACACCATTAAAGTCCCCATTA
MID31_tagF NNNAAGTGTACGACGTTGTAAAA	MID31_tagR NNNAAGTGTACATTAAGTCCCCATTA
MID32_tagF NNTCTGGACGACGTTGTAAAA	MID32_tagR NNTCTGGCATTAAGTCCCCATTA
MID33_tagF NNNAAGGTACGACGTTGTAAAA	MID33_tagR NNNAAGGTACATTAAGTCCCCATTA
MID34_tagF NNGGCGCAACGACGTTGTAAAA	MID34_tagR NNGGCGCACATTAAGTCCCCATTA
MID35_tagF NNNTCGACGACGACGTTGTAAAA	MID35_tagR NNNTCGACGCATTAAGTCCCCATTA
MID36_tagF NNCCTGTACCGACGTTGTAAAA	MID36_tagR NNCCTGTCCATTAAAGTCCCCATTA
MID37_tagF NNNAGAAGAACGACGTTGTAAAA	MID37_tagR NNNAGAAGACATTAAGTCCCCATTA
MID38_tagF NNAATAGGACGACGTTGTAAAA	MID38_tagR NNAATAGGCATTAAGTCCCCATTA
MID39_tagF NNNNGTTCTACGACGTTGTAAAA	MID39_tagR NNNNGTTCTCATTAAGTCCCCATTA
MID40_tagF NNTAATGAACGACGTTGTAAAA	MID40_tagR NNTAATGACATTAAGTCCCCATTA

Protocol III: Agarose gel

Amplification was confirmed and quantity assessed on a 2% agarose gel, stained with GelRed™. Add 3.0 g of agarose powder to 150 ml of diluted (1x) TAE buffer in a heat resistant bottle. Heat up this solution in the microwave for 1 minute at 800 watts with the cap lose on the lid. Remove the bottle from the microwave, close the cap slightly and mix the solution. Put the bottle back in the microwave at 800 watts for 1 minute until it starts to boil, then reduce it to 600 watts. Check that the agarose is completely dissolved. Move to the pull cabinet and take the rack to put the gel in. Use a pipette to add a little agarose solution to seal the 2 outsides. Cool down the rest of the bottle under the water. Take the GelRed™, add 7,5 µL to the agarose solution and mix it. Empty the whole bottle into the plate. Rinse the bottle 3 times with water, the first time throwing the waste in the bin. Allow the gel to cool for 30 minutes. Place the gel rack in the electrophoresis machine. Place parafilm over an empty tip box and make 6 rows of 8 wells, the last one containing 4 wells for the ladders. Add 3 µL of loading buffer and 5 µL of each sample, the PCR2 samples, to each well. 5 µL of 1:40 BP 100 marker is added to the ladders. Place the ladders in the first and last wells of the gel. Add 8 µL to each subsequent well. Close the lid of the electrophoresis unit and check that the polarity is correct. The machine to run the gel (PowerPAC Basic) is set at 125 volts for 60 minutes. After one hour the gel can be removed from the rack and placed in the gel imaging system, containing a UV light.

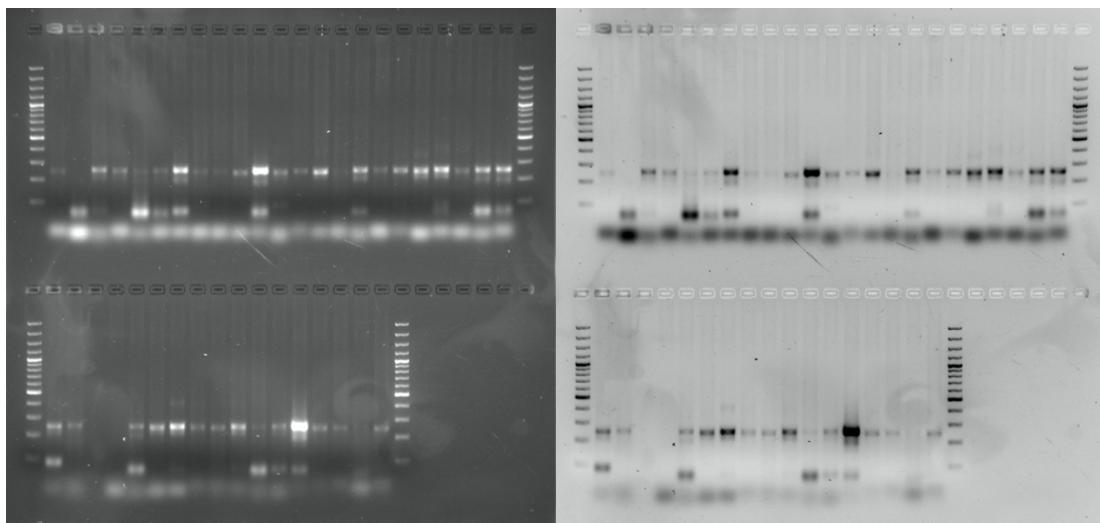


Fig. A4: Example of the visualisation of amplification on a 2% agarose gel. Library labelled Lib9, purified with Qiagen, MinElute, eluted 2 x 25 ul EB. Conc.: 6.4 ng/ul. The first and last wells of each row indicate the blank, used as reference.

Protocol IV: PCR purification and measuring DNA concentration of the library

Based on the intensity of the bands for each sample, a score of 1 to 4 was assigned, where 4 is almost not visible and 1 is very clear. The classification tells you how much of the sample (PCR2) needs to be add (e.g., 1 = 1 µL). After pooling of the samples, the DNA is purified using a MinElute kit (Qiagen). The quality and DNA concentration of the final pool was checked with the Qubit® 2.0 Fluorometer using the Invitrogen kit protocol.

Protocol V: Decontamination procedures in the laboratory

Decontamination procedures (e.g., UV-light is on for 1 hour each night) are in place, and all work is carried out using gloves and a lab coat. Thorough washing of all surfaces is done with DNA-away™ before and after working in the flow hoods, and pre- and post-PCR work is carried out in separate laboratories with a one-way system.

APPENDIX B Extra information data analysis

B1: Tables

Table B1.1: Sequencing output of the different libraries. Lane contains the Library ID_Flowcell ID_lane ID, for raw data file naming. Reads is the total amount of reads of raw data. For paired-end sequencing it equals the amount of read1 and read2. Data is the raw reads * sequence length. Effective equals to the number of clean reads divided by raw reads and multiplied with 100%. Error is the base error rate. Q20 and q30 are the base count of Phred value divided by the total base count. Gc is the G & C base count divided by the total base count.

Library	Lane	Reads	Data	Effective	Error	q20	q30	gc
Lib1_Ghent	ZKDN23H000095-1A_HMFM2DSX7_L3	63622356	9.5	97.88	0.02	98.69	95.38	35.50
Lib2_Ghent	ZKDN23H000096-1A_HMFM2DSX7_L3	84685442	12.7	98.93	0.02	98.60	95.15	35.21
LIB3	FKDN230510665-1A_HFLGWDSX7_L4	52715110	7.9	81.10	0.02	98.12	94.69	34.86
LIB4	FKDN230510666-1A_HFLGWDSX7_L4	55893636	8.4	91.28	0.02	98.45	95.20	35.28
LIB5	FKDN230510667-1A_HFLGWDSX7_L4	55160304	8.3	90.82	0.02	98.37	94.98	35.26
LIB6	FKDN230510668-1A_HFLGWDSX7_L4	46971006	7.0	94.48	0.02	98.33	94.97	34.96
LIB7	FKDN230510669-1A_HFLGWDSX7_L3	79661024	11.9	86.10	0.02	98.28	94.78	35.32
LIB8	FKDN230510670-1A_HFLTYDSX7_L4	47209414	7.1	92.27	0.03	98.11	94.01	35.17
LIB8a	FKDN230510671-1A_HFLTYDSX7_L4	50689818	7.6	95.64	0.02	98.20	94.17	35.05
LIB9	FKDN230510672-1A_HFLTYDSX7_L3	71004810	10.7	91.48	0.02	98.17	94.16	35.67
LIB10	FKDN230510673-1A_HFLTYDSX7_L3	50517164	7.6	98.63	0.02	98.23	94.07	34.83
LIB11	FKDN230510674-1A_HFLTYDSX7_L3	58523236	8.8	91.14	0.03	98.06	93.83	34.72
LIB12	FKDN230510675-1A_HFLTYDSX7_L3	70659816	10.6	95.21	0.03	98.00	93.93	34.01
LIB13	FKDN230510676-1A_HFLTYDSX7_L3	63278726	9.5	98.48	0.02	98.23	94.10	34.56
LIB14	FKDN230510677-1A_HFLTYDSX7_L3	74870434	11.2	88.17	0.02	98.36	94.40	35.19

Table B1.2: Sequences of contaminated ASVs. In addition, the percentage of taxonomic match is provided. Almost all sequences are within the genus Helina.

Seqid	Sequence	Taxonomic match
seq725	AGATATTGAACTTTATTTTATTTGGCGCTGAGCAGGAATAGTTGTACATCTTAAGTTACTAATTCGTGC TGAACTAGGGCAACCTGGCTACTTAATTGGTGTAGTCAAATTATAATGTTATTGTTACTGCCCATGCATTGTAAT AATTTCCTCATAGTTACCTATTCTAAATGGAGGATTGGTAATTGATTAGTA	90.094 %
seq393	AGATATTGAACTTTATTTTATTTGGAGCTTGATCAGGAATAATTGAACTTCATTAAGCATTAAATCGAAC TGAAATTAGGTCACCCTGGAGCTTAATTGGAGATGATCAAATCTATAATGTAATTGTAACAGCACATGCTTGTAA TAATTTTTTATAGTTACCTATTATAATTGGAGGATTGGTAATTGATTAGTA	99.526 %
seq435	TACTAATCAATTACCAAATCTCCAATTATAATAGGTATAACTATAAAAAAAATTACAAAAGCATGTGCTGTTAC AATTACATTATAGATTGATCATCTCCAATTAAAGCTCCAGGGTGACCTAATTCTGCTGAATTAAAATGCTTAATGA AGTCCAATTATCCTGATCAAGCTCCAAAAATAAAATATAAAGTTCCAATATCT	99.526 %
seq1059	TACTAATCAATTACCAAATCTCCAATTATGATAGGTATGACTATAAAAAAAATTATAATAAAAGCATGAGCTGTA CAATTACATTATAAAATTGATCATCTCCAATTATGCTCCAGGGATGACCTAATTCTGCTGAATTAAAATCTTAAAG AAGTCCCTACTATTCTGCTCAAGCCCCAAAAATAAAATATAAAGTTCCAATATCT	95.735 %
seq274	TACTAATCAATTCTCAAATCTCCAATTATAATAGGTATAACTATAAAAAAAATTACAAAAGCATGTGCTGTTAC AATTACATTATAGATTGATCATCTCCAATTAAAGCTCCAGGGTGACCTAATTCTGCTGAATTAAAATGCTTAATG AAGTCCAATTATCCTGATCAAGCTCCAAAAATAAAATATAAAGTTCCAATATCT	100 %
seq264	AGATATTGAACTTTATTTTATTTGGAGCTTGATCAGGAATAATTGAACTTCATTAAGCATTAAATCGAA CTGAATTAGGTCACTGGAGCTTAATTGGAGATGATCAAATCTATAATGTAATTGTAACAGCACATGCTTGT AATAATTTTTTATAGTTACCTATTATAATTGGAGGATTGGAAATTGATTAGTA	100 %
seq314	TACTAATCAATTACCAAATCTCCAATTAGGTATAACTATGAGAAAATTACAAAAGCATGAGCTGTA ACAATTACATTATAAAATTGTCATCTCCAATTATGCTCCAGGGTGACCTAATTCTGCTGAATTAAAATCTAA AGAAGTCCAATTATCCTGATCAGGAACCAAAATAAAATATAAAGTTCCAATATCT	99.526 %
seq702	TACTAATCAATTACCAAATCTCCAATTAGGTATAACTATGAGAAAATTACAAAAGCATGGGCAGTA ACAATAACATTATAAAATTGTCATCTCCAATTAGTAGCCAGGTTGCCCTAGTTCAGCACGAATTAGTAACTTA AAGATGTACCAACTATTCTGTCAGGCAGGCAAAATAAAATATAAAGTTCCAATATCT	90.094 %
seq328	AGATATTGAACTTTATTTTATGGAAATATGAGCAGGAATTAGGTACATCAAGATGAATTATTCGA ATTGAATTAGGAATACCTGGATCATTATTGGAGATGATCAAACATATAATGTTAGTTACAGCCCCATGCTTCA TCATAATTCTCATAGTTACCAATCATAATTGGAGGATTGGAAATTGATTAGTA	99.052 %

Table B1.3: The samples of spiders that were excluded from the `ps_k_filter` phyloseq object following the filtration step using the rarefaction curves. These are the samples that did not meet the required number of reads in order to reach the plateau phase (threshold = 0.0001). It is possible that these spiders had not yet consumed their prey.

Sample	Sample	Sample	Sample	Sample
P08SY07	P06SY21	P27SR02	P20SE02	P03SY08
P09SG03	P19SG13	P10SR05	P09SG12	P07SR12
P09SR04	P20SG12	P17SY01	P10SY08	
P22SY06	P25SG07	P13SR05	P03SY07	
P06SG07	P26SR08	P18SR05	P11SR15	

Table B1.4: Total number of ASVs (Observed) for each individual spider sampled for this study before performing rarefaction.

Sample	Observed	Sample	Observed	Sample	Observed	Sample	Observed	Sample	Observed
P01SY03	20	P03SG19	21	P01SY01	14	P01SY06	21	P11SY12	3
P01SY04	31	P03SR12	120	P01SY02	70	P13SR02	16	P11SY13	3
P01SY05	47	P06SR18	46	P10SR01	46	P13SR03	1	P11SY16	3
P02SG01	9	P07SG06	19	P10SR02	48	P13SR06	7	P11SY17	26
P02SG05	5	P07SG14	65	P10SR03	41	P17SG02	23	P12SG06	1
P02SR01	38	P07SR16	14	P10SR04	27	P17SG03	9	P12SG07	19
P02SR03	38	P07SY18	21	P12SG02	79	P17SG05	23	P12SG08	12
P02SR05	53	P07SY19	35	P12SG03	32	P17SR03	7	P12SG09	2
P02SY03	15	P12SR12	98	P12SG05	9	P17SR04	12	P12SG10	9
P08SG01	6	P12SY08	31	P13SG02	1	P20SE01	44	P12SR07	17
P08SG02	13	P14SR11	92	P13SG05	2	P20SE03	22	P12SR09	5
P08SG03	2	P16SR11	33	P13SG06	1	P20SE04	35	P12SY15	55
P08SR01	36	P16SR12	41	P13SY02	2	P20SE05	22	P12SY16	18
P08SR02	65	P18SY14	32	P13SY03	5	P20SG01	2	P12SY17	4
P08SR03	16	P19SG12	25	P13SY06	11	P20SG02	1	P12SY18	6
P08SR04	18	P19SG14	34	P13SY07	33	P20SG03	3	P13SG07	7
P08SR05	14	P19SG15	45	P17SY02	3	P20SG04	21	P13SG08	14
P08SY03	49	P19SG16	67	P17SY03	2	P20SG05	11	P13SG10	1
P08SY04	10	P20SR14	109	P18SG01	9	P07SY02	14	P13SG11	22
P08SY05	13	P20SR15	78	P18SG03	1	P07SY03	34	P13SR07	15
P09SG01	38	P23SY06	54	P18SG04	9	P07SY06	16	P13SR09	12
P09SG02	51	P23SY07	84	P18SY05	1	P07SY12	27	P13SR11	4
P09SG04	29	P23SY08	66	P02SY01	2	P07SY13	55	P13SR12	18
P09SG05	19	P23SY09	111	P02SY02	13	P08SG06	13	P13SR14	37
P09SR01	25	P23SY10	57	P02SY04	7	P08SR06	21	P13SY08	11
P09SR02	14	P24SY06	32	P12SG01	11	P08SR07	28	P13SY09	13
P09SR05	34	P26SG08	57	P15SG02	3	P08SR08	22	P10SY02	70
P10SG04	1	P26SR06	69	P15SG04	2	P08SR12	6	P13SY12	8
P03SR20	47	P26SR07	23	P15SG05	2	P08SY08	44	P13SY14	12
P04SGR01	1	P26SY10	40	P15SR01	1	P08SY11	17	P14SG06	2
P04SGR02	7	P27SG06	62	P15SR03	1	P09SG09	1	P14SG07	13
P05SRR20	27	P27SR01	37	P15SR05	2	P09SG10	32	P14SG08	11
P05SRR21	29	P27SY01	11	P15SY01	15	P09SR06	71	P14SR06	52
P05SY22	4	P03SG02	20	P15SY02	2	P09SR07	42	P14SR07	18
P06SR13	5	P03SG18	14	P15SY03	7	P09SR08	25	P14SR08	15
P06SY01	35	P03SR13	58	P15SY04	7	P10SG08	12	P14SR09	5
P10SG14	2	P05SRR22	28	P15SY05	20	P10SG09	6	P14SR10	25
P11SG14	32	P06SG09	12	P19SR04	6	P10SR06	11	P14SY07	23

P11SG17	75	P06SG17	27	P19SR05	1	P10SR07	6	P14SY09	31
P11SR11	1	P06SR07	27	P19SR06	1	P10SR09	2	P14SY10	53
P11SR18	35	P06SR17	7	P19SY01	35	P10SR11	2	P15SG06	11
P17SG13	16	P07SG15	23	P19SY02	4	P10SY06	1	P15SG07	4
P17SG15	5	P07SR15	30	P19SY03	2	P10SY07	4	P15SG08	33
P17SG16	5	P07SY15	85	P19SY04	6	P10SY09	17	P15SG09	39
P18SY13	25	P12SR14	55	P20SR01	12	P10SY11	14	P15SG10	17
P19SR12	29	P14SG11	24	P20SR04	30	P11SG07	16	P15SR13	22
P19SY12	30	P14SG12	2	P20SR05	32	P11SG08	14	P15SR14	6
P20SE12	30	P16SR14	13	P20SY01	15	P11SG09	8	P15SR15	5
P22SY03	52	P18SY12	1	P20SY03	19	P11SG10	2	P15SR16	18
P22SY04	15	P20SR11	56	P20SY04	6	P11SR10	2	P15SR17	12
P24SG02	28	P21SG02	29	P20SY05	69	P01SY10	31	P15SR19	9
P24SG04	11	P21SG07	14	P21SG01	75	P03SR06	5	P15SR20	7
P24SG05	16	P22SY05	29	P02SG02	28	P03SY09	7	P15SY08	38
P24SR02	58	P24SY01	15	P02SG03	1	P04SGR13	2	P15SY09	33
P24SR03	81	P24SY02	51	P02SG04	15	P04SGR14	9	P15SY10	48
P24SR04	82	P25SG03	30	P02SR02	52	P04SGR16	10	P15SY11	2
P24SR05	12	P25SG06	1	P02SR04	29	P04SGR17	10	P15SY12	1
P24SR06	37	P25SY01	30	P02SY05	26	P04SR05	3	P16SG07	20
P24SY04	21	P26SG06	14	P10SY01	31	P04SR07	21	P16SG08	21
P25SR01	5	P26SG07	32	P10SY04	46	P04SR08	34	P16SG10	8
P25SR08	34	P26SR09	25	P10SY05	33	P04SR11	5	P16SG14	4
P27SR05	25	P26SR10	30	P16SR01	1	P04SR15	1	P03SG16	1
P03SG17	25	P26SY06	59	P16SR03	12	P04SR16	8	P03SR15	5
P03SY11	26	P26SY07	35	P16SR04	6	P04SR19	3	P05SRR08	7
P04SGR18	15	P26SY08	25	P16SR05	4	P04SR20	7	P06SG13	1
P05SY17	20	P26SY09	25	P17SR02	27	P04SY03	5	P06SY03	11
P05SY18	36	P27SG02	46	P17SR07	31	P04SY04	18	P07SR11	33
P06SG15	60	5SG07REP	21	P18SR01	19	P04SY05	5	P07SY11	61
P06SR09	15	P05SY10	19	P18SR02	22	P04SY06	13	P07SY16	31
P06SY12a	89	P05SY19	52	P19SY05	33	P04SY07	20	P12SG11	1
P07SG13	70	P06SY22	72	P21SG03	59	P04SY12	18	P21SG20	18
P07SR13	29	P07SG16	6	P21SG04	31	P04SY14	12	P21SR06	37
P10SG10	32	P10SY12	15	P21SG05	23	P05SG02	8	P21SR08	16
P10SG13	10	P11SG18	102	P21SG06	5	P05SG03	15	P21SR12	44
P12SR16	4	P12SG13	36	P23SG01	28	P05SG04	6	P21SR13	53
P16SG12	5	P12SR17	35	P23SG02	11	P05SG05	12	P21SR17	9
P16SG13	4	P12SR18	4	P23SG03	24	P07SG12	8	P21SY06	68
P16SG16	9	P12SR19	33	P23SG04	1	P07SR01	15	P21SY09	1
P17SR15	2	P12SY11	16	P23SG05	73	P07SR02	43	P21SY10	25
P18SR07	38	P12SY13	72	P23SR01	17	P07SR04	57	P21SY11	31
P18SY11	46	OSR08REP	33	P23SR02	15	P07SR05	4	P21SY12	66
P22SR07	10	OSY06REP	28	P23SR03	11	P07SR07	24	P22SG02	3
P23SG06	63	P23SY13	56	P23SR04	1	P07SR08	4	P22SG05	5
P23SR06	23	P23SY16	44	P23SR05	5	P07SR09	2	P22SG06	2
P24SG01	15	P25SY02	16	P26SG01	20	P07SY01	73	P22SG08	1
P24SG03	36	P01SG01	56	P01SG07	41	P11SR12	1	P22SR04	4
P24SY05	4	P01SG03	8	P01SG08	20	P11SR13	8	P22SR06	6
P25SG01	44	P01SG04	27	P01SG10	10	P11SR16	14	P25SR02	22

P25SG05	35	P01SG06	15	P01SR06	32	P11SR19	14	P25SR07	15
P25SR06	8	P01SR01	19	P01SR07	47	P11SY03	21	P27SG04	55
P27SG03	54	P01SR02	50	P01SR08	20	P11SY05	5	P27SY02	11
P27SG05	41	P01SR03	15	P01SR09	13	P11SY06	19	P27SY06	8
P27SR04a	28	P01SR04	45	P01SR10	2	P11SY08	23		
P27SY03	17	P01SR05	29	P01SR11	19	P11SY11	2		

Table B1.5: For each location (Subplot): the number of spider samples sampled in the field (Sampled), the number of spider samples included in the further analysis after filtering (Remained), the number of observed amplicon sequence variants (Total_asvs) and the number of amplicon sequence variants after taxonomic glomeration at species level (Total_asvs_species) are included in the table for phyloseq object ps_k_filter. Colours indicate the urbanisation degree at the landscape scale: green = low urbanised, yellow = medium, red = highly urbanised (3 x 3 km).

Subplotid	Sampled	Remained	Total_asvs	Total_asvs_species	Subplotid	Sampled	Remained	Total_asvs	Total_asvs_species
P01SG	9	7	274	32	P14SY	5	3	137	26
P01SR	11	11	335	35	P15SG	10	8	131	25
P01SY	8	7	280	41	P15SR	12	10	88	17
P02SG	5	5	58	10	P15SY	10	10	216	30
P02SR	5	5	256	30	P16SG	7	7	78	20
P02SY	5	5	67	13	P16SR	7	7	129	28
P03SG	5	5	85	25	P17SG	6	6	83	17
P03SR	5	5	397	31	P17SR	6	5	81	16
P03SY	4	2	69	12	P17SY	3	2	5	3
P04SG	7	7	56	14	P18SG	5	3	19	6
P04SR	9	8	84	21	P18SR	6	3	83	17
P04SY	7	7	101	25	P18SY	5	5	137	24
P05SG	5	5	77	22	P19SG	5	4	245	35
P05SR	4	4	119	13	P19SR	6	4	48	12
P05SY	6	5	167	27	P19SY	6	6	124	24
P06SG	5	4	202	19	P20SE	6	5	171	24
P06SR	5	5	126	26	P20SG	6	5	54	10
P06SY	6	4	353	44	P20SR	7	7	613	40
P07SG	6	6	369	31	P20SY	5	5	158	28
P07SR	12	11	324	37	P21SG	8	8	397	46
P07SY	12	11	611	59	P21SR	5	5	213	24
P08SG	6	4	34	7	P21SY	5	5	277	30
P08SR	9	9	283	31	P22SG	5	4	11	8
P08SY	7	5	147	20	P22SR	5	3	20	13
P09SG	8	6	245	26	P22SY	5	3	124	20
P09SR	9	6	250	36	P23SG	6	6	254	26
P10SG	6	6	69	12	P23SR	6	6	79	15
P10SR	10	8	212	27	P23SY	7	7	696	48
P10SY	11	9	310	31	P24SG	5	5	119	16
P11SG	7	7	462	42	P24SR	5	5	518	43
P11SR	8	7	91	19	P24SY	5	5	165	25
P11SY	9	9	134	20	P25SG	5	4	114	25
P12SG	11	11	287	36	P25SR	3	5	88	25
P12SR	10	8	417	45	P25SY	2	2	65	15
P12SY	7	7	448	40	P26SG	4	4	189	28
P13SG	10	7	67	13	P26SR	5	4	223	23
P13SR	10	8	160	24	P26SY	5	5	253	31
P13SY	9	8	129	14	P27SG	4	5	399	45
P14SG	5	5	58	15	P27SR	5	3	100	19
P14SR	6	6	278	37	P27SY	2	4	53	11

B2: Statistical models

```

## Family: nbinom2 ( log )
## Formula: Observed ~ U_landscape + U_local + (1 | subplotid)
## Data: alpha_average_df_ps_k_filter
##
##      AIC      BIC   logLik deviance df.resid
##  3397.5  3426.6 -1691.8   3383.5     461
##
## Random effects:
##
## Conditional model:
## Groups Name Variance Std.Dev.
## subplotid (Intercept) 0.08021  0.2832
## Number of obs: 468, groups: subplotid, 80
##
## Dispersion parameter for nbinom2 family (): 2.38
##
## Conditional model:
##             Estimate Std. Error z value Pr(>|z|)
## (Intercept) 2.6380    0.1068 24.702 <2e-16 ***
## U_landscapeMEDIUM -0.2698   0.1175 -2.296  0.0217 *
## U_landscapeHIGH   -0.1060   0.1150 -0.922  0.3564
## U_localMEDIUM     0.2771   0.1167  2.374  0.0176 *
## U_localHIGH       0.1844   0.1115  1.654  0.0981 .
##
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```

```

## Analysis of Deviance Table (Type III Wald chisquare tests)
##
## Response: Observed
##          Chisq Df Pr(>Chisq)
## (Intercept) 610.2014 1 < 2e-16 ***
## U_landscape  5.4306 2 0.06618 .
## U_local      5.9318 2 0.05151 .
##
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```

(a)

(b)

Fig. B2.1: Statistical output of the observed **ASV richness for each spider** (i.e., the sum of ASVs of prey species eaten by each spider). Model: `modB <- glmmTMB(Observed ~ U_landscape+U_local+(1|subplotid), data=alpha_average_df_ps_k_filter, family=nbinom2)`. Family = `nbinom2` was used to correct for the detected overdispersion. (a) Summary output, (b) Anova results with Type III. The effect of the urbanisation scales on the ASV richness of each spider were found to be almost significant ($p_{U_landscape} = 0.06618$, $p_{U_local} = 0.05151$).

```

## Family: nbinom2 ( log )
## Formula: Observed ~ U_landscape + U_local + (1 | subplotid)
## Data: alpha_average_df_ps_k_filter_sp
##
##      AIC      BIC   logLik deviance df.resid
##  2297.7  2326.8 -1141.9   2283.7     461
##
## Random effects:
##
## Conditional model:
## Groups Name Variance Std.Dev.
## subplotid (Intercept) 0.06529  0.2555
## Number of obs: 468, groups: subplotid, 80
##
## Dispersion parameter for nbinom2 family (): 12.9
##
## Conditional model:
##             Estimate Std. Error z value Pr(>|z|)
## (Intercept) 1.61841  0.08719 18.562 <2e-16 ***
## U_landscapeMEDIUM -0.16385  0.09582 -1.710  0.0873 .
## U_landscapeHIGH   -0.09947  0.09362 -1.062  0.2880
## U_localMEDIUM     0.21171  0.09548  2.217  0.0266 *
## U_localHIGH       0.14975  0.09187  1.630  0.1031
##
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```

```

## Analysis of Deviance Table (Type III Wald chisquare tests)
##
## Response: Observed
##          Chisq Df Pr(>Chisq)
## (Intercept) 344.5485 1 < 2e-16 ***
## U_landscape  2.9534 2 0.22840
## U_local      5.2643 2 0.07192 .
##
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```

(a)

(b)

Fig. B2.2: Statistical output of the observed **species richness for each spider** (i.e., the sum of prey species eaten by each spider). Model: `modB <- glmmTMB(Observed ~ U_landscape+U_local+(1|subplotid), data=alpha_average_df_ps_k_filter_sp, family=nbinom2)`. Family = `nbinom2` was used to correct for the detected overdispersion. (a) Summary output, (b) Anova results with Type III. The effect of the local urbanisation scale on the species richness of each spider was found to be almost significant ($p_{U_local} = 0.07192$).

```

## Family: nbinom2 ( log )
## Formula: Observed ~ U_landscape + U_local + (1 | plotid)
## Data: alpha_average_df_merg_ps_k_filter
##
##      AIC      BIC  logLik deviance df.resid
##  630.5   647.2  -308.3    616.5     73
##
## Random effects:
##
## Conditional model:
## Groups Name      Variance Std.Dev.
## plotid (Intercept) 2.236e-10 1.495e-05
## Number of obs: 80, groups: plotid, 27
##
## Dispersion parameter for nbinom2 family (): 8.24
##
## Conditional model:
##             Estimate Std. Error z value Pr(>|z|)
## (Intercept)  3.40984   0.09803 34.78 <2e-16 ***
## U_landscapeMEDIUM -0.16580   0.11012 -1.51  0.132
## U_landscapeHIGH    0.01864   0.10762  0.17  0.862
## U_localMEDIUM     0.07087   0.10983  0.65  0.519
## U_localHIGH       0.06626   0.10617  0.62  0.533
##
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```

(a)

```

## Analysis of Deviance Table (Type III Wald chisquare tests)
##
## Response: Observed
##              Chisq Df Pr(>Chisq)
## (Intercept) 1209.8252 1 <2e-16 ***
## U_landscape  3.5245 2 0.1717
## U_local     0.5386 2 0.7639
##
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```

(b)

Fig. B2.3: Statistical output of the observed **ASV richness for each subplotid** (i.e., one value for each subplotid indicating the ASVs of prey species eaten by the community of spiders present at that unique location). Model: `modB <- glmmTMB(Observed ~ U_landscape+U_local+(1|plotid), data=alpha_average_df_merg_ps_k_filter, family=nbinom2)`. Family = `nbinom2` was used to correct for the detected overdispersion. (a) Summary output, (b) Anova results with Type III.

```

## Family: nbinom2 ( log )
## Formula: Observed ~ U_landscape + U_local + (1 | plotid)
## Data: alpha_average_df_merg_ps_k_filter_sp
##
##      AIC      BIC  logLik deviance df.resid
##  433.6   450.3  -209.8    419.6     73
##
## Random effects:
##
## Conditional model:
## Groups Name      Variance Std.Dev.
## plotid (Intercept) 1.782e-10 1.335e-05
## Number of obs: 80, groups: plotid, 27
##
## Dispersion parameter for nbinom2 family (): 51.7
##
## Conditional model:
##             Estimate Std. Error z value Pr(>|z|)
## (Intercept)  2.29053   0.08702 26.321 <2e-16 ***
## U_landscapeMEDIUM -0.06427   0.09636 -0.667  0.505
## U_landscapeHIGH   -0.04936   0.09512 -0.519  0.604
## U_localMEDIUM     0.01609   0.09775  0.165  0.869
## U_localHIGH       0.08516   0.09354  0.910  0.363
##
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```

(a)

```

## Analysis of Deviance Table (Type III Wald chisquare tests)
##
## Response: Observed
##              Chisq Df Pr(>Chisq)
## (Intercept) 692.8051 1 <2e-16 ***
## U_landscape  0.4875 2 0.7837
## U_local     0.9453 2 0.6233
##
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```

(b)

Fig. B2.4: Statistical output of the observed **species richness for each subplotid** (i.e., one value for each subplotid indicating the species richness in the community of spiders present at that unique location). Model: `modB <- glmmTMB(Observed ~ U_landscape+U_local+(1|plotid), data=alpha_average_df_merg_ps_k_filter_sp, family=nbinom2)`. Family = `nbinom2` was used to correct for the detected overdispersion. (a) Summary output, (b) Anova results with Type III.

```

## Family: gaussian ( identity )
## Formula: Shannon ~ U_landscape + U_local + (1 | subplotid)
## Data: alpha_average_df_ps_k_filter
##
##      AIC      BIC logLik deviance df.resid
##  1181.7  1210.7 -583.8  1167.7     461
##
## Random effects:
##
## Conditional model:
## Groups Name Variance Std.Dev.
## subplotid (Intercept) 0.1166  0.3414
## Residual          0.6278  0.7924
## Number of obs: 468, groups: subplotid, 80
##
## Dispersion estimate for gaussian family ( $\sigma^2$ ): 0.628
##
## Conditional model:
##                               Estimate Std. Error z value Pr(>|z|)
## (Intercept)                2.0265   0.1232 16.449 <2e-16 ***
## U_landscapeMEDIUM -0.3080   0.1361 -2.263  0.0236 *
## U_landscapeHIGH  -0.1549   0.1334 -1.162  0.2454
## U_localMEDIUM      0.3069   0.1348  2.276  0.0228 *
## U_localHIGH        0.2396   0.1289  1.858  0.0632 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```

(a)

```

## Analysis of Deviance Table (Type III Wald chisquare tests)
##
## Response: Shannon
##              Chisq Df Pr(>Chisq)
## (Intercept) 270.5576  1 < 2e-16 ***
## U_landscape  5.1315  2  0.07686 .
## U_local      5.8959  2  0.05245 .
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```

(b)

Fig. B2.5: Statistical output of the **Shannon diversity (ASV level)** for each spider. Model: `modC <- glmmTMB(Shannon ~ U_landscape+U_local+(1|subplotid), data=alpha_average_df_ps_k_filter, family=gaussian)`. (a) Summary output, (b) Anova test results with Type III. The effects of both urbanisation scales on the Shannon diversity (ASV level) of each spider were found to be almost significant ($p_{U_local} = 0.07686$, $p_{U_landscape} = 0.05245$).

```

## Family: gaussian ( identity )
## Formula: Shannon ~ U_landscape + U_local + (1 | subplotid)
## Data: alpha_average_df_ps_k_filter_sp
##
##      AIC      BIC logLik deviance df.resid
##  768.5    797.6  -377.3    754.5     461
##
## Random effects:
##
## Conditional model:
## Groups Name Variance Std.Dev.
## subplotid (Intercept) 0.04044  0.2011
## Residual          0.26379  0.5136
## Number of obs: 468, groups: subplotid, 80
##
## Dispersion estimate for gaussian family ( $\sigma^2$ ): 0.264
##
## Conditional model:
##                               Estimate Std. Error z value Pr(>|z|)
## (Intercept)                1.01556   0.07632 13.307 <2e-16 ***
## U_landscapeMEDIUM -0.17391   0.08430 -2.063  0.0391 *
## U_landscapeHIGH  -0.13977   0.08247 -1.695  0.0901 .
## U_localMEDIUM      0.08552   0.08338  1.026  0.3051
## U_localHIGH        0.11020   0.07969  1.383  0.1667
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```

(a)

```

## Analysis of Deviance Table (Type III Wald chisquare tests)
##
## Response: Shannon
##              Chisq Df Pr(>Chisq)
## (Intercept) 177.0729  1 < 2e-16 ***
## U_landscape  4.7095  2  0.09492 .
## U_local      2.0682  2  0.35554
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```

(b)


```

## contrast   estimate    SE df t.ratio p.value
## LOW - MEDIUM 0.1739 0.0843 461  2.063 0.0988
## LOW - HIGH   0.1398 0.0825 461  1.695 0.2083
## MEDIUM - HIGH -0.0341 0.0793 461  -0.431 0.9028
##
## Results are averaged over the levels of: U_local
## P value adjustment: tukey method for comparing a family of 3 estimates

```

	U_landscape	emmean	SE	df	lower.CL	upper.CL
## 1 LOW		1.08	0.0617	461	0.960	1.20
## 2 MEDIUM		0.907	0.0573	461	0.794	1.02
## 3 HIGH		0.941	0.0548	461	0.833	1.05

(c)

Fig. B2.6: Statistical output of the **Shannon diversity (taxonomic species level)** for each spider. Model: `modC <- glmmTMB(Shannon ~ U_landscape+U_local+(1|subplotid), data=alpha_average_df_ps_k_filter_sp, family=gaussian)`. (a) Summary output, (b) Anova test results with Type III, (c) Predicted emmeans and contrasts. The effect of the landscape urbanisation scale on the Shannon diversity of each spider was found to be almost significant ($p_{U_landscape} = 0.09492$).

```

## Family: gaussian ( identity )
## Formula: Shannon ~ U_landscape * U_local + (1 | plotid)
## Data: alpha_average_df_merg_ps_k_filter
##
##      AIC      BIC  logLik deviance df.resid
##  143.0    169.2   -60.5    121.0      69
##
## Random effects:
##
## Conditional model:
## Groups Name Variance Std.Dev.
## plotid (Intercept) 1.619e-10 1.272e-05
## Residual 2.658e-01 5.156e-01
## Number of obs: 80, groups: plotid, 27
##
## Dispersion estimate for gaussian family ( $\sigma^2$ ): 0.266
##
## Conditional model:
## Estimate Std. Error z value Pr(>|z|)
## (Intercept) 2.99836 0.17186 17.446 <2e-16 ***
## U_landscapeMEDIUM -0.59101 0.24305 -2.432 0.0150 *
## U_landscapeHIGH 0.08281 0.24305 0.341 0.7333
## U_localMEDIUM -0.34915 0.25983 -1.344 0.1790
## U_localHIGH 0.07645 0.24305 0.315 0.7531
## U_landscapeMEDIUM:U_localMEDIUM 0.91161 0.35579 2.562 0.0104 *
## U_landscapeHIGH:U_localMEDIUM 0.18362 0.35579 0.516 0.6058
## U_landscapeMEDIUM:U_localHIGH 0.22171 0.34372 0.645 0.5189
## U_landscapeHIGH:U_localHIGH -0.16798 0.33940 -0.495 0.6207
##
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```

(a)

```

## Analysis of Deviance Table (Type III Wald chisquare tests)
##
## Response: Shannon
##              Chisq Df Pr(>Chisq)
## (Intercept) 304.3734  1 < 2e-16 ***
## U_landscape 9.1434  2 0.01034 *
## U_local    2.9439  2 0.22948
## U_landscape:U_local 8.3226  4 0.08045 .
##
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```

(b)

Fig. B2.7: Statistical output of the *Shannon diversity (ASV level)* for each subplotid. Model: `modC_met_interactie <- glmmTMB(Shannon ~ U_landscape*U_local+(1/plotid), data=alpha_average_df_merg_ps_k_filter, family=gaussian)`. Not all assumptions were met (Fig.B3.6). (a) Summary output, (b) Anova test results with Type III. The effect of urbanisation at the landscape scale on the Shannon diversity (ASV level) was significant ($p_{U_landscape} = 0.01034$) when the interaction term between $U_landscape$ and U_local was implemented.

```

## Family: gaussian ( identity )
## Formula: Shannon ~ U_landscape + U_local + (1 | plotid)
## Data: alpha_average_df_merg_ps_k_filter_sp
##
##      AIC      BIC  logLik deviance df.resid
##  109.1    125.8   -47.5    95.1      73
##
## Random effects:
##
## Conditional model:
## Groups Name Variance Std.Dev.
## plotid (Intercept) 8.618e-10 2.936e-05
## Residual 1.922e-01 4.384e-01
## Number of obs: 80, groups: plotid, 27
##
## Dispersion estimate for gaussian family ( $\sigma^2$ ): 0.192
##
## Conditional model:
## Estimate Std. Error z value Pr(>|z|)
## (Intercept) 1.41138 0.11000 12.830 <2e-16 ***
## U_landscapeMEDIUM -0.05755 0.12181 -0.472 0.637
## U_landscapeHIGH -0.03981 0.12073 -0.330 0.742
## U_localMEDIUM -0.06421 0.12181 -0.527 0.598
## U_localHIGH 0.18551 0.11827 1.568 0.117
##
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```

(a)

```

## Analysis of Deviance Table (Type III Wald chisquare tests)
##
## Response: Shannon
##              Chisq Df Pr(>Chisq)
## (Intercept) 164.6205  1 < 2e-16 ***
## U_landscape 0.2325  2 0.89025
## U_local    4.7073  2 0.09502 .
##
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```

(b)


```

## contrast estimate SE df t.ratio p.value
## LOW - MEDIUM 0.0642 0.122 73 0.527 0.8583
## LOW - HIGH -0.1855 0.118 73 -1.568 0.2657
## MEDIUM - HIGH -0.2497 0.121 73 -2.068 0.1036
##
## Results are averaged over the levels of: U_landscape
## P value adjustment: tukey method for comparing a family of 3 estimates

```

	U_local	emmean	SE	df	lower.CL	upper.CL
	<fct>	<dbl>	<dbl>	<dbl>	<dbl>	<dbl>
## 1	LOW	1.38	0.0844	73	1.21	1.55
## 2	MEDIUM	1.31	0.0879	73	1.14	1.49
## 3	HIGH	1.56	0.0829	73	1.40	1.73

(c)

Fig. B2.8: Statistical output of the *Shannon diversity (taxonomic species level)* for each subplotid. Model: `modC <- glmmTMB(Shannon ~ U_landscape+U_local+(1/plotid), data=alpha_average_df_merg_ps_k_filter_sp, family=gaussian)`. (a) Summary output, (b) Anova test results with Type III, (c) Predicted emmeans and contrasts. The effect of the local urbanisation scale on the Shannon diversity was found to be almost significant ($p_{U_local} = 0.09502$).

```

## Family: gaussian ( identity )
## Formula: Simpson ~ U_landscape + U_local + (1 | subplotid)
## Data: alpha_average_df_ps_k_filter
##
##      AIC      BIC  logLik deviance df.resid
##     -21.8     7.2    17.9    -35.8     461
##
## Random effects:
## 
## Conditional model:
## Groups   Name        Variance Std.Dev.
## subplotid (Intercept) 0.00360  0.0600
## Residual           0.05118  0.2262
## Number of obs: 468, groups: subplotid, 80
##
## Dispersion estimate for gaussian family (sigma^2): 0.0512
##
## Conditional model:
##             Estimate Std. Error z value Pr(>|z|)
## (Intercept) 0.76443  0.02918 26.200 <2e-16 ***
## U_landscapeMEDIUM -0.07065  0.03212 -2.200  0.0278 *
## U_landscapeHIGH   -0.03679  0.03129 -1.176  0.2396
## U_localMEDIUM     0.07762  0.03167  2.451  0.0143 *
## U_localHIGH       0.06196  0.03018  2.053  0.0401 *
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```

```

## Analysis of Deviance Table (Type III Wald chisquare tests)
##
## Response: Simpson
##              Chisq Df Pr(>Chisq)
## (Intercept) 686.4252 1 < 2e-16 ***
## U_landscape  4.8420  2  0.08883 .
## U_local      6.9361  2  0.03118 *
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```

(b)

(a)

Fig. B2.9: Statistical output of the *Simpson diversity (ASV level)* for each spider. Model: `modD <- glmmTMB(Simpson ~ U_landscape+U_local+(1|subplotid), data=alpha_average_df_ps_k_filter, family=gaussian)`. (a) Summary output, (b) Anova test results with Type III. Strong deviations from the statistical assumptions were found (Fig.B3.7). The effect of local urbanisation on the Simpson diversity (ASV level) of each spider is significant ($p_{U_local} = 0.03118$).

```

## Family: gaussian ( identity )
## Formula: Simpson ~ U_landscape + U_local + (1 | subplotid)
## Data: alpha_average_df_ps_k_filter_sp
##
##      AIC      BIC  logLik deviance df.resid
##     36.6     65.7    -11.3    22.6     461
##
## Random effects:
## 
## Conditional model:
## Groups   Name        Variance Std.Dev.
## subplotid (Intercept) 0.005412 0.07357
## Residual           0.057067 0.23889
## Number of obs: 468, groups: subplotid, 80
##
## Dispersion estimate for gaussian family (sigma^2): 0.0571
##
## Conditional model:
##             Estimate Std. Error z value Pr(>|z|)
## (Intercept) 0.50606  0.03228 15.675 <2e-16 ***
## U_landscapeMEDIUM -0.07833  0.03562 -2.199  0.0279 *
## U_landscapeHIGH   -0.06350  0.03473 -1.828  0.0675 .
## U_localMEDIUM     0.02295  0.03511  0.653  0.5134
## U_localHIGH       0.04876  0.03352  1.454  0.1458
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```

```

## Analysis of Deviance Table (Type III Wald chisquare tests)
##
## Response: Simpson
##              Chisq Df Pr(>Chisq)
## (Intercept) 245.7026 1 < 2e-16 ***
## U_landscape  5.3773  2  0.06797 .
## U_local      2.1212  2  0.34624
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```

(b)

(a)

Fig. B2.10: Statistical output of the *Simpson diversity (taxonomic species level)* for each spider. Model: `modD <- glmmTMB(Simpson ~ U_landscape+U_local+(1|subplotid), data=alpha_average_df_ps_k_filter_sp, family=gaussian)`. (a) Summary output, (b) Anova test results with Type III. Deviations from the statistical assumptions were found (Fig.B3.8). The effect of the landscape urbanisation scale on the Simpson diversity of each spider was found to be almost significant ($p_{U_landscape} = 0.06797$).

```

## Family: gaussian ( identity )
## Formula: Simpson ~ U_landscape * U_local + (1 | subplotid)
## Data: alpha_average_df_merg_ps_k_filter
##
##      AIC      BIC logLik deviance df.resid
##      NA       NA     NA      NA      69
##
## Random effects:
## 
## Conditional model:
## Groups   Name        Variance Std.Dev.
## subplotid (Intercept) 0.001505 0.0388
## Residual    0.001505 0.0388
## Number of obs: 80, groups: subplotid, 80
##
## Dispersion estimate for gaussian family (sigma^2): 0.00151
##
## Conditional model:
##                               Estimate Std. Error z value Pr(>|z|)
## (Intercept)                0.921257  0.018288 50.37 < 2e-16 ***
## U_landscapeMEDIUM          -0.060307  0.025863 -2.33 0.01971 *
## U_landscapeHIGH             0.02981  0.025863 0.12 0.90824
## U_localMEDIUM              -0.045232  0.027649 -1.64 0.10185
## U_localHIGH                 0.010493  0.025863 0.41 0.68497
## U_landscapeMEDIUM:U_localMEDIUM 0.105224  0.037860 2.78 0.00545 **
## U_landscapeHIGH:U_localMEDIUM 0.039031  0.037860 1.03 0.30257
## U_landscapeMEDIUM:U_localHIGH 0.014801  0.036576 0.40 0.68572
## U_landscapeHIGH:U_localHIGH  -0.007984  0.036116 -0.22 0.82504
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```

```

## Analysis of Deviance Table (Type III Wald chisquare tests)
##
## Response: Simpson
##                                         Chisq Df Pr(>Chisq)
## (Intercept)                      2537.5742 1 < 2e-16 ***
## U_landscape                       7.6254 2 0.02209 *
## U_local                           4.4310 2 0.10910
## U_landscape:U_local               9.2430 4 0.05530 .
## 
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```

(b)

(a)

Fig. B2.11: Statistical output of the *Simpson diversity (ASV level)* for each subplotid. Model: `modD_met_interactie <- glmmTMB(Simpson ~ U_landscape*U_local+(1|subplotid), data=alpha_average_df_merg_ps_k_filter, family=gaussian)`. (a) Summary output, (b) Anova test results with Type III. Deviations from the statistical assumptions were found. The effect of urbanisation at the landscape scale on the Simpson diversity (ASV level) was significant ($p_{U_landscape} = 0.02209$) when the interaction term between *U_landscape* and *U_local* was implemented.

```

Family: gaussian ( identity )
Formula: Simpson ~ U_landscape + U_local + (1 | plotid)
Data: alpha_average_df_merg_ps_k_filter_sp
      AIC      BIC logLik deviance df.resid
-53.8    -37.2   33.9   -67.8      73
Random effects:
Conditional model:
Groups   Name        Variance Std.Dev.
plotid  (Intercept) 0.0007097 0.02664
Residual    0.0243888 0.15617
Number of obs: 80, groups: plotid, 27
Dispersion estimate for gaussian family (sigma^2): 0.0244
Conditional model:
Estimate Std. Error z value Pr(>|z|)
(Intercept) 0.601945 0.040271 14.947 <2e-16 ***
U_landscapeMEDIUM -0.008501 0.045304 -0.188 0.851
U_landscapeHIGH -0.002072 0.045054 -0.046 0.963
U_localMEDIUM -0.006325 0.043529 -0.145 0.884
U_localHIGH  0.097285 0.042160 2.308 0.021 *
---
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```

```

Analysis of Deviance Table (Type III Wald chisquare tests)
Response: Simpson
                                         Chisq Df Pr(>Chisq)
(Intercept)                      223.426 1 < 2e-16 ***
U_landscape                       0.039 2 0.98067
U_local                            7.472 2 0.02385 *
---
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```

(b)

(a)

```

contrast   estimate   SE df t.ratio p.value
LOW - MEDIUM  0.00633 0.0435 73  0.145  0.9884
LOW - HIGH    -0.09729 0.0422 73  -2.308  0.0610
MEDIUM - HIGH -0.10361 0.0433 73  -2.395  0.0497
Results are averaged over the levels of: U_landscape
P value adjustment: tukey method for comparing a family of 3 estimates

```

<i>U_landscape</i>	<i>emmmean</i>	<i>SE</i>	<i>df</i>	<i>lower.CL</i>	<i>upper.CL</i>
LOW	0.5984	0.0305	73	0.5376	0.6591
MEDIUM	0.5921	0.0319	73	0.5285	0.6556
HIGH	0.6957	0.0300	73	0.6359	0.7555

(c)

Fig. B2.12: Statistical output of the *Simpson diversity (taxonomic species level)* for each subplotid. Model: `modD <- glmmTMB(Simpson ~ U_landscape+U_local+(1|plotid), data=alpha_average_df_merg_ps_k_filter_sp, family=gaussian)`. (a) Summary output, (b) Anova test results with Type III, (c) Predicted emmeans and contrasts. The effect of the local urbanisation scale on the Simpson diversity was found to be significant ($p_{U_local} = 0.02385$).

```

Permutation test for adonis under reduced model
Terms added sequentially (first to last)
Blocks: ps_family@sam_data$plotid
Permutation: free
Number of permutations: 999

adonis2(formula = bray ~ ps_family@sam_data$U_landscape * ps_family@sam_data$U_local, permutations = perm_scheme)
   Df SumOfSq    R2
ps_family@sam_data$U_landscape           2   0.8017 0.02990
ps_family@sam_data$U_local              2   0.9227 0.03441
ps_family@sam_data$U_landscape:ps_family@sam_data$U_local  4   1.5498 0.05780
Residual                               71  23.5413 0.87790
Total                                    79  26.8156 1.00000
   F Pr(>F)
ps_family@sam_data$U_landscape           1.2090 0.033 *
ps_family@sam_data$U_local               1.3914 0.052 .
ps_family@sam_data$U_landscape:ps_family@sam_data$U_local 1.1686 0.115
Residual
Total
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```

(a)

```

Homogeneity of multivariate dispersions

Call: betadisper(d = bray, group = info$U_local)

No. of Positive Eigenvalues: 54
No. of Negative Eigenvalues: 25

Average distance to median:
  LOW MEDIUM HIGH
0.5912 0.5364 0.5497

Eigenvalues for PCoA axes:
(Showing 8 of 79 eigenvalues)
PCoA1 PCoA2 PCoA3 PCoA4 PCoA5 PCoA6 PCoA7 PCoA8
5.623 3.099 1.810 1.614 1.488 1.282 1.069 1.005

```

(b)

```

Permutation test for homogeneity of multivariate dispersions
Permutation: free
Number of permutations: 999

Response: Distances
   Df Sum Sq Mean Sq   F N.Perm Pr(>F)
Groups      2 0.04314 0.021567 1.8766   999  0.18
Residuals 77 0.88493 0.011493

Pairwise comparisons:
(Observe p-value below diagonal, permuted p-value above diagonal)
  LOW MEDIUM HIGH
LOW          0.079000 0.123
MEDIUM 0.076561          0.670
HIGH   0.094714 0.689872

```

(c)

```

Homogeneity of multivariate dispersions

Call: betadisper(d = bray, group = info$U_landscape)

No. of Positive Eigenvalues: 54
No. of Negative Eigenvalues: 25

Average distance to median:
  LOW MEDIUM HIGH
0.5562 0.5644 0.5639

Eigenvalues for PCoA axes:
(Showing 8 of 79 eigenvalues)
PCoA1 PCoA2 PCoA3 PCoA4 PCoA5 PCoA6 PCoA7 PCoA8
5.623 3.099 1.810 1.614 1.488 1.282 1.069 1.005

```

(d)

```

Permutation test for homogeneity of multivariate dispersions
Permutation: free
Number of permutations: 999

Response: Distances
   Df Sum Sq Mean Sq   F N.Perm Pr(>F)
Groups      2 0.00109 0.0005454 0.0486   999  0.951
Residuals 77 0.86329 0.0112115

Pairwise comparisons:
(Observe p-value below diagonal, permuted p-value above diagonal)
  LOW MEDIUM HIGH
LOW          0.77700 0.793
MEDIUM 0.76663          0.988
HIGH   0.80036 0.98742

```

(e)

Fig. B2.13: Statistical output of the *permanova analysis on the family-based NMDS*. Using Bray-Curtis distance: `bray <- phyloseq::distance(ps_family, method="bray", type='samples')`. Permutation scheme: `perm_scheme <- with(ps_family, how(nperm = 999, blocks = ps_family@sam_data$plotid))`. Model: `perm_adonis2 <- adonis2(bray ~ ps_family@sam_data$U_landscape*ps_family@sam_data$U_local, permutations=perm_scheme)`. (a) Permutation test output (`perm_adonis2`), (b) Betadisper for `U_local`, (c) Permutation test output for `U_local`, (d) Betadisper for `U_landscape`, (e) Permutation test output for `U_landscape`.

```

## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Blocks: merg_ps_k_filter_sp@sam_data$plotid
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = bray ~ merg_ps_k_filter_sp@sam_data$U_landscape * merg_ps_k_filter_sp@sam_data$U_local, permutations = perm_scheme)
##                                     Df
## merg_ps_k_filter_sp@sam_data$U_landscape          2
## merg_ps_k_filter_sp@sam_data$U_local              2
## merg_ps_k_filter_sp@sam_data$U_landscape:merg_ps_k_filter_sp@sam_data$U_local  4
## Residual                                         71
## Total                                           79
##                                     SumOfSqs
## merg_ps_k_filter_sp@sam_data$U_landscape          0.7352
## merg_ps_k_filter_sp@sam_data$U_local              0.9536
## merg_ps_k_filter_sp@sam_data$U_landscape:merg_ps_k_filter_sp@sam_data$U_local  1.4980
## Residual                                         24.8840
## Total                                           27.9908
##                                     R2
## merg_ps_k_filter_sp@sam_data$U_landscape          0.02627
## merg_ps_k_filter_sp@sam_data$U_local              0.03407
## merg_ps_k_filter_sp@sam_data$U_landscape:merg_ps_k_filter_sp@sam_data$U_local  0.05352
## Residual                                         0.88615
## Total                                           1.00000
##                                     F
## merg_ps_k_filter_sp@sam_data$U_landscape          1.0522
## merg_ps_k_filter_sp@sam_data$U_local              1.3648
## merg_ps_k_filter_sp@sam_data$U_landscape:merg_ps_k_filter_sp@sam_data$U_local  1.0720
## Residual
## Total
##                                     Pr(>F)
## merg_ps_k_filter_sp@sam_data$U_landscape          0.057
## merg_ps_k_filter_sp@sam_data$U_local              0.052
## merg_ps_k_filter_sp@sam_data$U_landscape:merg_ps_k_filter_sp@sam_data$U_local  0.191
## Residual
## Total
##                                     .
## merg_ps_k_filter_sp@sam_data$U_landscape          .
## merg_ps_k_filter_sp@sam_data$U_local              .
## merg_ps_k_filter_sp@sam_data$U_landscape:merg_ps_k_filter_sp@sam_data$U_local  .
## Residual
## Total
##                                     ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```

(a)

```

## Homogeneity of multivariate dispersions
##
## Call: betadisper(d = vegan_avgdist, group = metadata_vegan$U_local)
##
## No. of Positive Eigenvalues: 55
## No. of Negative Eigenvalues: 24
##
## Average distance to median:
##    LOW   MEDIUM   HIGH
## 0.5111 0.4761 0.4727
##
## Eigenvalues for PCoA axes:
## (Showing 8 of 79 eigenvalues)
## PCoA1 PCoA2 PCoA3 PCoA4 PCoA5 PCoA6 PCoA7 PCoA8
## 4.3119 2.2048 2.0484 1.5380 1.2743 1.1415 0.8449 0.7313

```

(b)

```

## Permutation test for homogeneity of multivariate dispersions
## Permutation: free
## Number of permutations: 999
##
## Response: Distances
##               Df  Sum Sq  Mean Sq      F N.Perm Pr(>F)
## Groups          2 0.02445 0.012225 0.5497   999  0.583
## Residuals       77 1.71250 0.022240
##
## Pairwise comparisons:
## (Observed p-value below diagonal, permuted p-value above diagonal)
##    LOW   MEDIUM   HIGH
## LOW           0.45300 0.315
## MEDIUM        0.42875 0.945
## HIGH          0.30374 0.93386

```

(c)

```

## Permutation test for homogeneity of multivariate dispersions
## Permutation: free
## Number of permutations: 999
##
## Response: Distances
##               Df  Sum Sq  Mean Sq      F N.Perm Pr(>F)
## Groups          2 0.13109 0.065546 3.2821   999  0.036 *
## Residuals       77 1.53777 0.019971
##
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Pairwise comparisons:
## (Observed p-value below diagonal, permuted p-value above diagonal)
##    LOW   MEDIUM   HIGH
## LOW           0.026000 0.027
## MEDIUM        0.031871 0.969
## HIGH          0.031598 0.957510

```

(d)

Fig. B2.14: Statistical output of the *permanova* analysis on the rarefied ordination (PCoA). Using Bray-Curtis distance: *bray* <- *phyloseq::distance*(*merg_ps_k_filter_sp*, method="bray", type='samples'). Permutation scheme: *perm_scheme* <- *with*(*merg_ps_k_filter_sp*, *how*(*nperm* = 999, *blocks* = *merg_ps_k_filter_sp*@*sam_data\$plotid*)). Model: *perm_adonis2_2* <- *adonis2*(*bray* ~ *merg_ps_k_filter_sp*@*sam_data\$U_landscape***merg_ps_k_filter_sp*@*sam_data\$U_local*, *permutations*=*perm_scheme*). (a) Permutation test output, (b) *Betadisper* for *U_local*: *bd_local* <- *betadisper*(*vegan_avgdist*, *metadata_vegan\$U_local*), (c) Permutation test for *U_local*, (d) Permutation test for *U_landscape*.

```

## Permutation test for adonis under reduced model
## Terms added sequentially (first to last)
## Permutation: free
## Number of permutations: 999
##
## adonis2(formula = binary_species_data ~ U_landscape * U_local, data = info, permutations = 999)
##          Df SumOfSqs      R2      F Pr(>F)
## U_landscape        2   0.5962 0.03568 1.4262  0.034 *
## U_local            2   0.6041 0.03615 1.4450  0.036 *
## U_landscape:U_local 4   0.6696 0.04007 0.8008  0.909
## Residual          71  14.8410 0.88810
## Total             79  16.7109 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```

(a)

```

## $parent_call
## [1] "dfAdonis[, 5:234] ~ U_landscape * U_local , strata = Null , permutations 999"
##
## $HIGHvsMEDIUM
##          Df SumOfSqs      R2      F Pr(>F)
## U_landscape        1   0.3749 0.02299 1.2570  0.08909 .
## U_local            2   0.8082 0.04957 1.3551  0.01120 *
## U_landscape:U_local 2   0.5100 0.03128 0.8550  0.87301
## Residual          49  14.6135 0.89617
## Total             54  16.3066 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## $HIGHvsLOW
##          Df SumOfSqs      R2      F Pr(>F)
## U_landscape        1   0.3919 0.02490 1.2963  0.07259 .
## U_local            2   0.5951 0.03781 0.9842  0.50635
## U_landscape:U_local 2   0.5439 0.03456 0.8996  0.76402
## Residual          47  14.2088 0.90274
## Total             52  15.7397 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## $MEDIUMvsLOW
##          Df SumOfSqs      R2      F Pr(>F)
## U_landscape        1   0.3696 0.02352 1.2067  0.13579
## U_local            2   0.7238 0.04605 1.1817  0.09999 .
## U_landscape:U_local 2   0.5347 0.03402 0.8730  0.83592
## Residual          46  14.0880 0.89641
## Total             51  15.7160 1.00000
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```

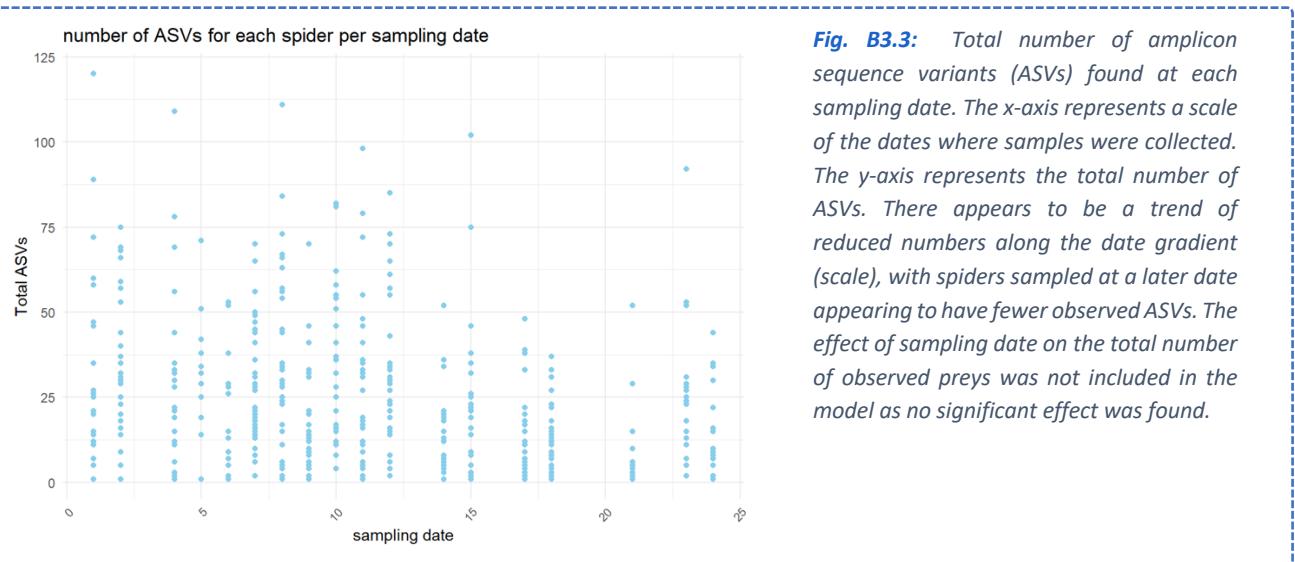
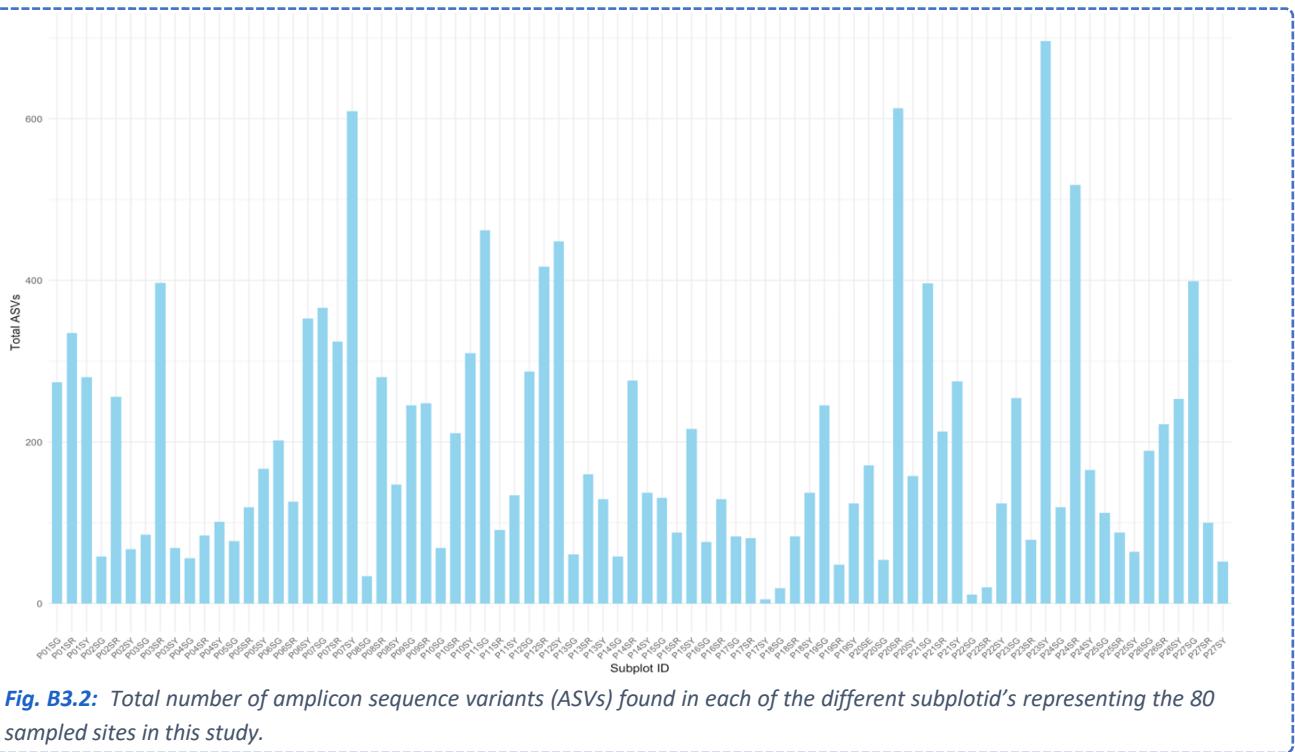
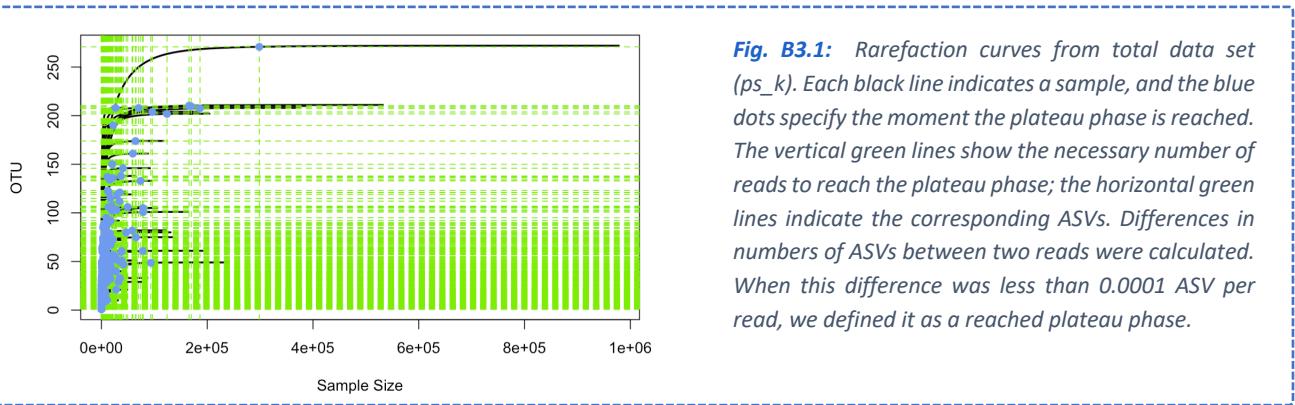
(b)

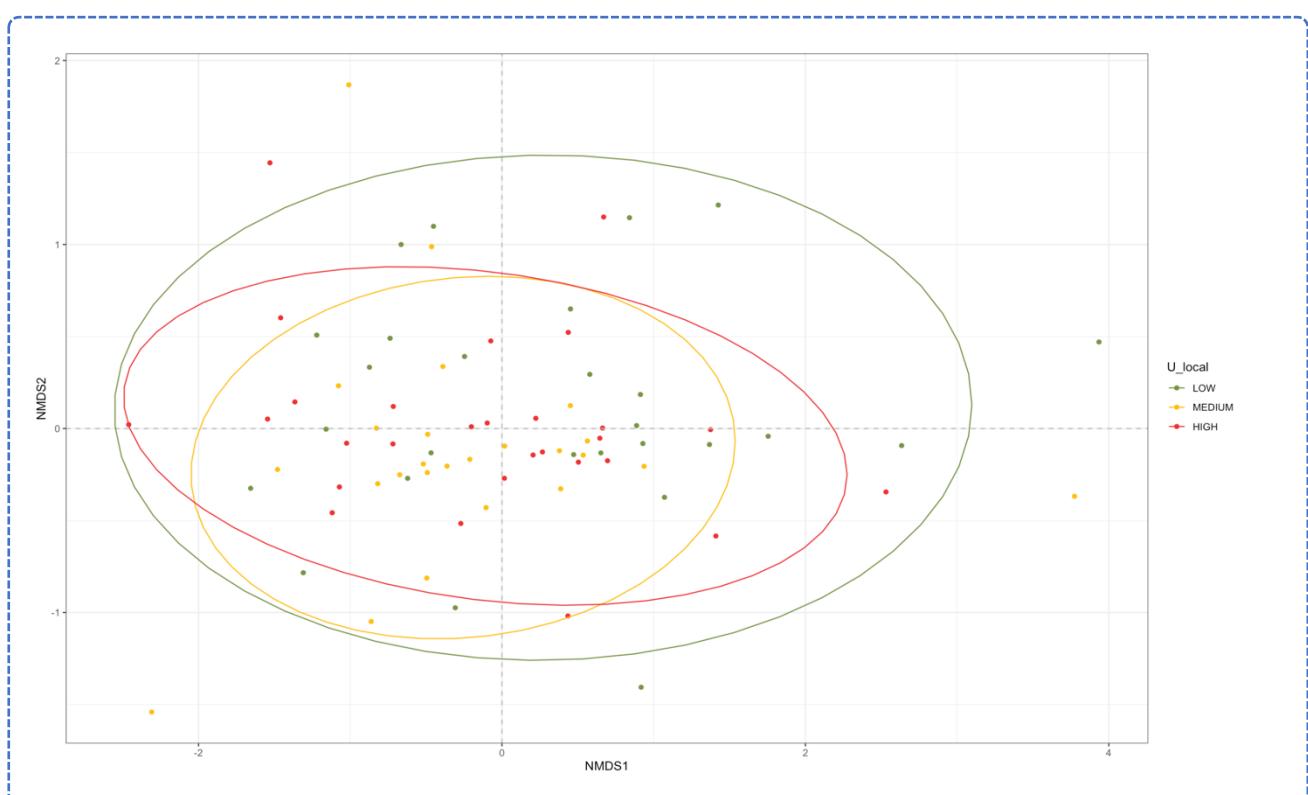
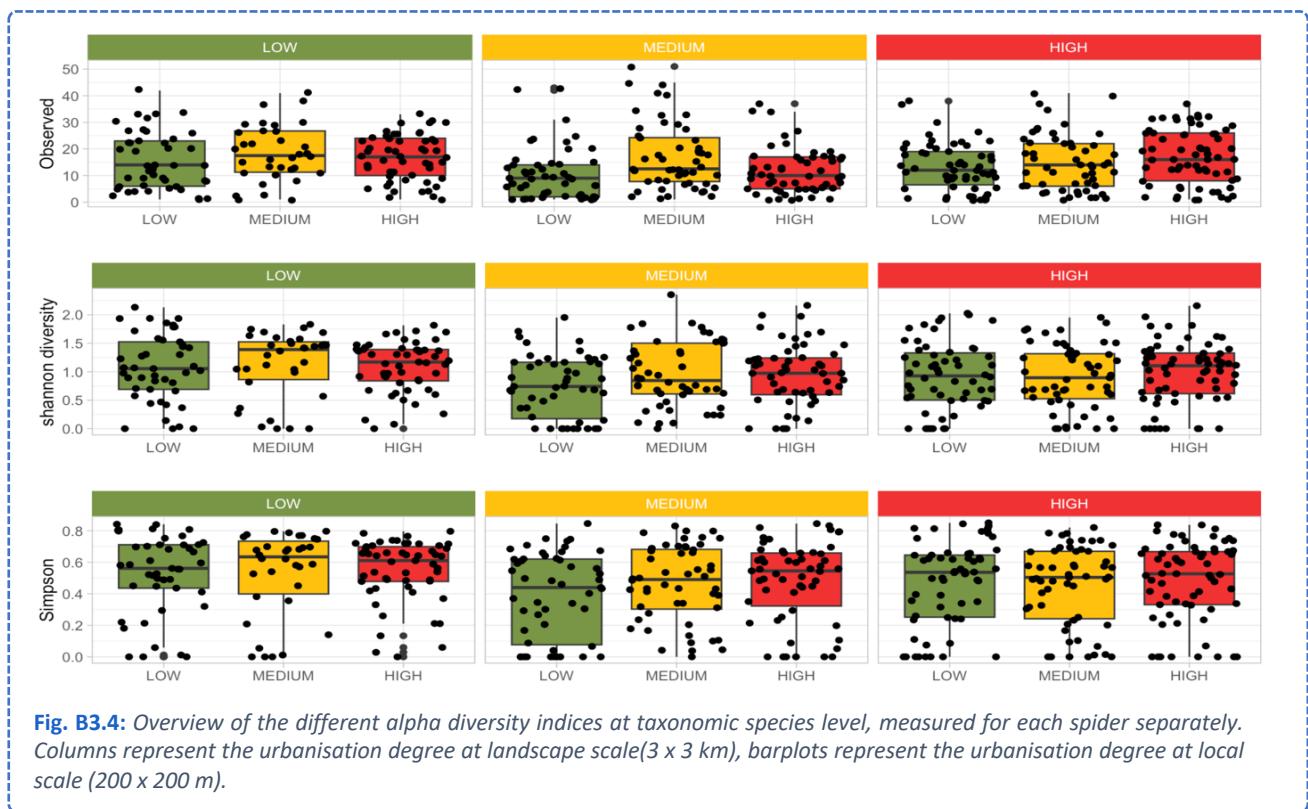
Fig. B2.15: Statistical output of the *permanova analysis on the presence-absence (PA)*. Using Jaccard distance: `binary_species_data <- decostand(ASV_matrix, "pa")`. Model: `permanova_result <- adonis2(binary_species_data ~ U_landscape * U_local, data = info, permutations = 999)`. (a) Permutation test output, (b) Output of pairwise testing

<pre> ## Multilevel pattern analysis ## ----- ## Association function: r.g ## Significance level (alpha): 0.05 ## ## Total number of species: 230 ## Selected number of species: 9 ## Number of species associated to 1 group: 6 ## Number of species associated to 2 groups: 3 ## ## List of species associated to each combination ## ## Group HIGH #sps. 5 ## stat p.value ## seq5268 0.356 0.0105 * ## seq347 0.296 0.0227 * ## seq1927 0.292 0.0332 * ## seq553 0.281 0.0381 * ## seq2103 0.280 0.0418 * ## ## Group LOW #sps. 1 ## stat p.value ## seq2077 0.337 0.0091 ** ## ## Group HIGH+LOW #sps. 1 ## stat p.value ## seq261 0.274 0.0481 * ## ## Group HIGH+MEDIUM #sps. 1 ## stat p.value ## seq514 0.321 0.0119 * ## ## Group LOW+MEDIUM #sps. 1 ## stat p.value ## seq3175 0.273 0.0434 * </pre>	<pre> ## Multilevel pattern analysis ## ----- ## Association function: r.g ## Significance level (alpha): 0.05 ## ## Total number of species: 230 ## Selected number of species: 10 ## Number of species associated to 1 group: 7 ## Number of species associated to 2 groups: 3 ## ## List of species associated to each combination ## ## Group HIGH #sps. 3 ## stat p.value ## seq1453 0.383 0.0018 ** ## seq1273 0.275 0.0456 * ## seq711 0.270 0.0444 * ## ## Group LOW #sps. 1 ## stat p.value ## seq2275 0.322 0.0191 * ## ## Group MEDIUM #sps. 3 ## stat p.value ## seq200 0.378 0.0018 ** ## seq578 0.319 0.0085 ** ## seq1657 0.275 0.0425 * ## ## Group HIGH+MEDIUM #sps. 3 ## stat p.value ## seq831 0.293 0.0233 * ## seq1342 0.289 0.0344 * ## seq215 0.261 0.0486 * </pre>	<pre> ## Multilevel pattern analysis ## ----- ## Association function: r.g ## Significance level (alpha): 0.05 ## ## Total number of species: 230 ## Selected number of species: 5 ## Number of species associated to 1 group: 2 ## Number of species associated to 2 groups: 0 ## Number of species associated to 3 groups: 3 ## Number of species associated to 4 groups: 0 ## Number of species associated to 5 groups: 0 ## Number of species associated to 6 groups: 0 ## Number of species associated to 7 groups: 0 ## Number of species associated to 8 groups: 0 ## ## List of species associated to each combination ## ## Group HIGHHIGH #sps. 1 ## stat p.value ## seq347 0.392 0.037 * ## ## Group LOWMEDIUM #sps. 1 ## stat p.value ## seq578 0.437 0.0191 * </pre>
(a)	(b)	(c)

Fig. B2.16: Statistical output of the *Indicator species analysis*. (a) for *U_landscape*, (b) for *U_local*, (c) for Group (categories on the urbanisation gradient).

B3: Figures





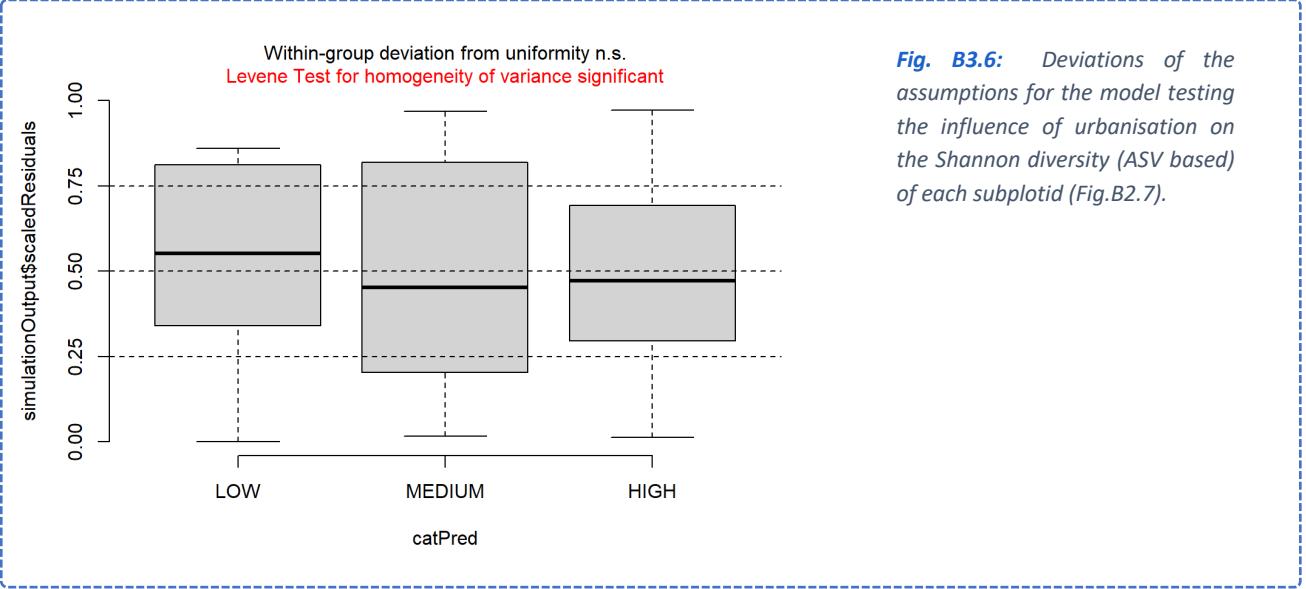


Fig. B3.6: Deviations of the assumptions for the model testing the influence of urbanisation on the Shannon diversity (ASV based) of each subplotid (Fig.B2.7).

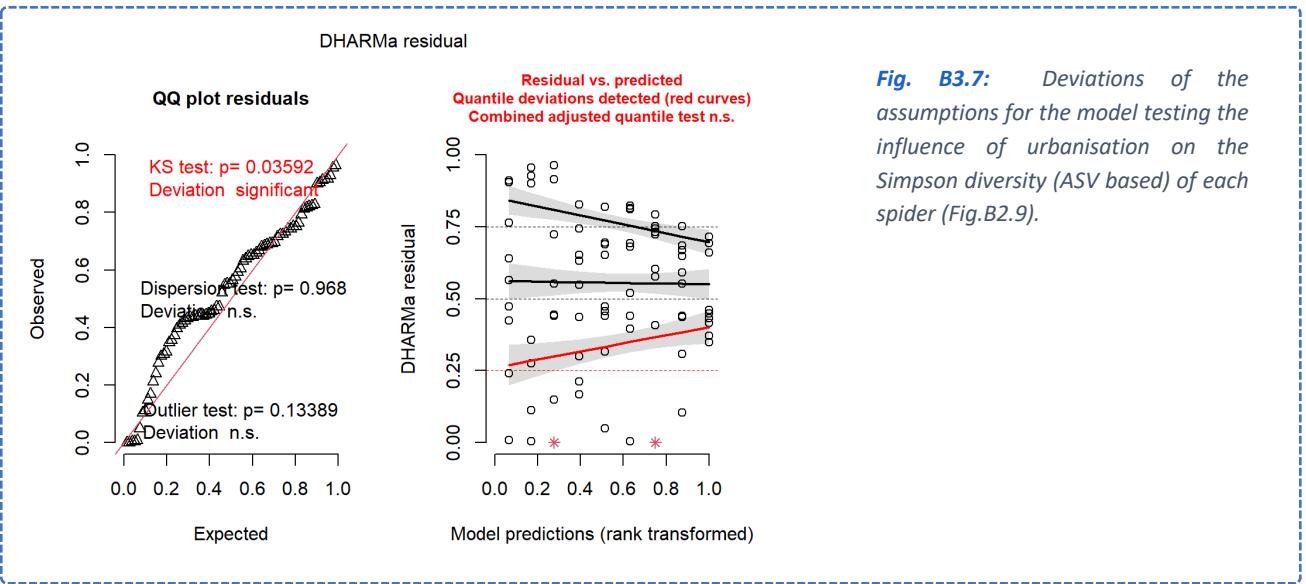


Fig. B3.7: Deviations of the assumptions for the model testing the influence of urbanisation on the Simpson diversity (ASV based) of each spider (Fig.B2.9).

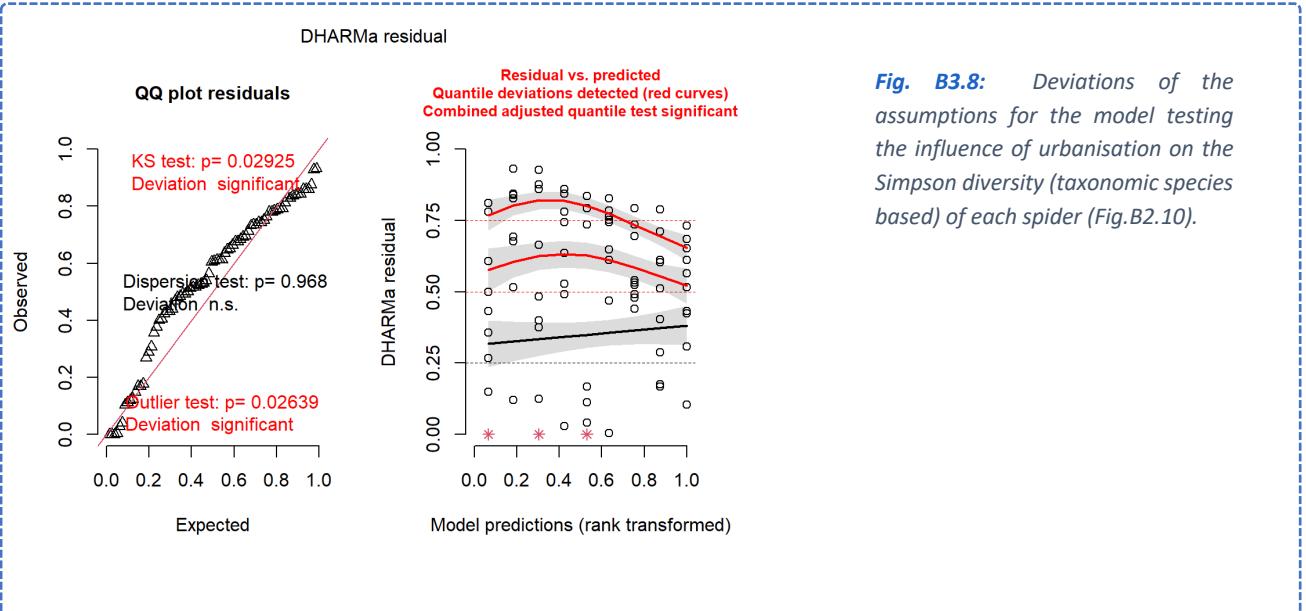


Fig. B3.8: Deviations of the assumptions for the model testing the influence of urbanisation on the Simpson diversity (taxonomic species based) of each spider (Fig.B2.10).