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Master thesis

INSECT BIODIVERSITY AND BIOMASS IN A FORESTED REGION IN BELGIUM

A case study in Bosland, Limburg

Master in Applied Ecology

2021

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I started gaining an interest in insects through my membership of the youth movement JNM (Youth movement for nature and environment). Looking at the amazing variety of insects and learning about them, makes you see nature differently. The fact that most people would not notice a row of ants crossing a path, or a beetle on the search for food, makes them even more interesting to me.

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“Nature is not a place to visit. It is home.”

Gary Snyder

Contents

1. Introduction

The importance of insects has become widely known. Next to pollination, insects provide a lot of other benefits to humans. Medical uses, like minimizing the clinical symptoms of Multiple sclerosis (MS) by Apitherapy (Helal, Hegazi and Al-menabbawy, 2014) or surgical maggots that clean out wounds (Nebraska-Lincoln, no date) are just the tip of the iceberg of what can be done with insects. Biological control is a great way to reduce the use of pesticides when fighting pests in crops. We can think of the lady beetles eating aphids or parasitic wasps controlling beetles in cabbages (Cornell University, no date; Jordan et al., 2020). Even waste recycling is done by insects, think of dung beetles taking apart faeces of cattle(Yamada, Imura and Shi, 2007). Natural ecosystems in which insects thrive provide a variety of services and we humans depend on a lot of these for our survival (Schowalter, Noriega and Tscharntke, 2018).

The abovementioned uses of insects are all reasons for conservation of their diversity and abundance. However, the loss of insects has been a common topic the last few years, even being called an “Ecological Armageddon” (Leather, 2018). Recent studies showing a decline in biomass are concerning and show that insect decline is happening here and now (Sorg et al., 2013, Hallmann et al., 2017, Wagner et al., 2021). Not only pollution and chemical substances used in agriculture, but habitat loss, fragmentation and degradation have a big negative impact on insect diversity and abundance. Next to these, global climate change and the spread of invasive species are causing a mass insect extinction (Cardoso et al., 2020).

Insects form a crucial part of every food web in nature. Insects are prey for a variety of other animals (Schowalter, Noriega and Tscharntke, 2018), so the decline in insects has an effect on other levels of the food web. In North America the number of insectivorous birds, bird species for which insects are a significant proportion of the diet, have declined significantly over the last 50 years. This happens while other species, which do not depend on insects, are gaining in abundance (Rosenberg et al., 2019). The same decline in insectivorous bird abundance was found in mainland Europe (Bowler et al., 2019). Although breeding success depends on multiple factors like nest site availability, weather and predation, the fact remains that food abundance will have a big impact on the population size of these bird species (Sherry et al., 2015). It is therefore important to preserve the insect diversity and biomass.

In this study we investigate if a decline in insect biomass is observable in a forested region in Belgium. In addition, we take a look at the present day biodiversity and biomass that is found in the Bosland region in Belgium. Due to the recent return of the European wolf (Canis lupus) and the raven (Corvus corax), Bosland has gained a lot of attention in the media (Nield, 2018; Agentschap voor Natuur en Bos, no date). The region also harbours a variety of endangered bird species, of which a lot rely on insects to survive and reproduce.

One bird species that has received a lot of attention in the last years is the nightjar (Caprimulgus europaeus), a crepuscular insectivorous bird. One of the main habitats for this bird in Belgium is our study area Bosland, in the Province of Limburg in Flanders. The bird species feels at home in the open semi-natural habitats that Bosland offers. Sparse trees within a heather field or sandy dunes are the ideal nesting habitat (Cramps, Simmons and Perrins, 1985; Sierro et al., 2001). However, the extent of these ideal habitats have declined significantly. Heathlands have, due to anthropogenic activities, decreased by 95% in size in the last 150 years (Webb, 1998). The heathlands in present day Belgium are fragmented pieces between woodlands, agricultural fields and urban areas (Evens et al., 2017). Keeping and managing the breeding areas of the nightjar ensures that suitable nesting habitat remains. However, studies in our study area have found that the nightjar forages in different habitats, travelling long distances for ideal foraging habitat, with an average maximum of over 2600 m (Evens et al., 2017). Habitats used for foraging vary, with nightjars being observed in meadows, grasslands and oak scrubland (Alexander and Cresswell, 1990; Sierro et al., 2001; Sharps et al., 2015). In our study area, the key foraging habitats have been found to be extensively-cultivated grasslands and recreational areas (Evens et al., 2017). Population sizes of this bird species have been on a rise, mainly due to the good management of the breeding habitats (Morris, Burges and Fuller, 1994; Paelinckx, Van Landuyt and de bruyn, 2008). But the limited population sizes have placed the nightjars on the Belgian red list as 'vulnerable' for more than 14 years (Langston et al., 2007).

The relatively new insight that the nightjars travel great distances for suitable foraging habitat (Evens et al., 2017), has raised questions about the reason why these birds travel to these extensively-cultivated grasslands for foraging. The biomass of moths, which make up the biggest part of the nightjar's diet (65%), has been found to be higher in these foraging habitats compared to the breeding habitats of the nightjar (Evens et al., 2018). High biomass of moths was also found in forest habitat, which is not used for foraging. The fact that this habitat is not used for foraging is expected to be due to low visibility. Because the nightjar hunts at dusk, high densities of trees lead to low visibility for foraging (Sierro et al., 2001). In this study we want to look at the insect biomass from other taxonomic groups than Lepidoptera (butterflies). The aim is to get a better picture of what habitats harbour the highest biomass in Bosland.

To calculate the biomass of these insects, a preliminary study was done to evaluate three different methods of biomass calculations. Calculating insect biomass is becoming more and more important in studying the health and processes in ecosystems. Biomass can be used to provide a more accurate picture of the changes in community structures as compared to using biodiversity. (Saint-germain et al., 2007).

Length-weight regressions provide a specific method of estimating insect biomass. By calculating the specific length and weight of a species, a function can be created and used in further research. General length-weight regressions like used in Rogers, Hinds and Buschbom, 1976, covering multiple species or families of insects, are an easy way to estimate insect biomass.

Next to using a power function, wet insect biomass is a common method. This method consists of measuring the mass of insects captured in the trap, all species confounded, sorted to family level or after identification and straining the preservation fluid by the use of a sieve. Sorg et al., 2013 used this method over a period of 24 years, being able to compare the insect biomass between these years. The main advantage is that the method is relatively simple and not very time consuming. More importantly, the samples are not destroyed and can be used for further research.

The last method used in this study is oven dry biomass. Drying the samples in an oven is quite easy, only needing an industrial oven. The advantage of this method is that it is stable and little space is left for errors. The method standardizes the measure and gets rid of the interstitial preservation fluid or fluid that was used to capture the animals. Drying samples however means that specific species weights are not calculated and only comparison between samples is possible, making it less usable in studies trying to answer species specific questions. The method can be used to compare insect biomass between years and study sites, as only the dry weight is measured with little error.

In the past (2012, 2013, 2014) light traps were installed in four different habitats in Bosland. Building further on the interesting preliminary results, we aim to further finetune the experimental set-up in time and space, to be able to make sounder conclusions regarding the difference in insect composition and density across the different habitats. Beside the contribution that this study will provide in solving the foraging behaviour of the nightjar, this study (due to the availability of multiple year data) will allow us to study the trend in insect biomass for these habitats and compare it to the global declining insect trends.

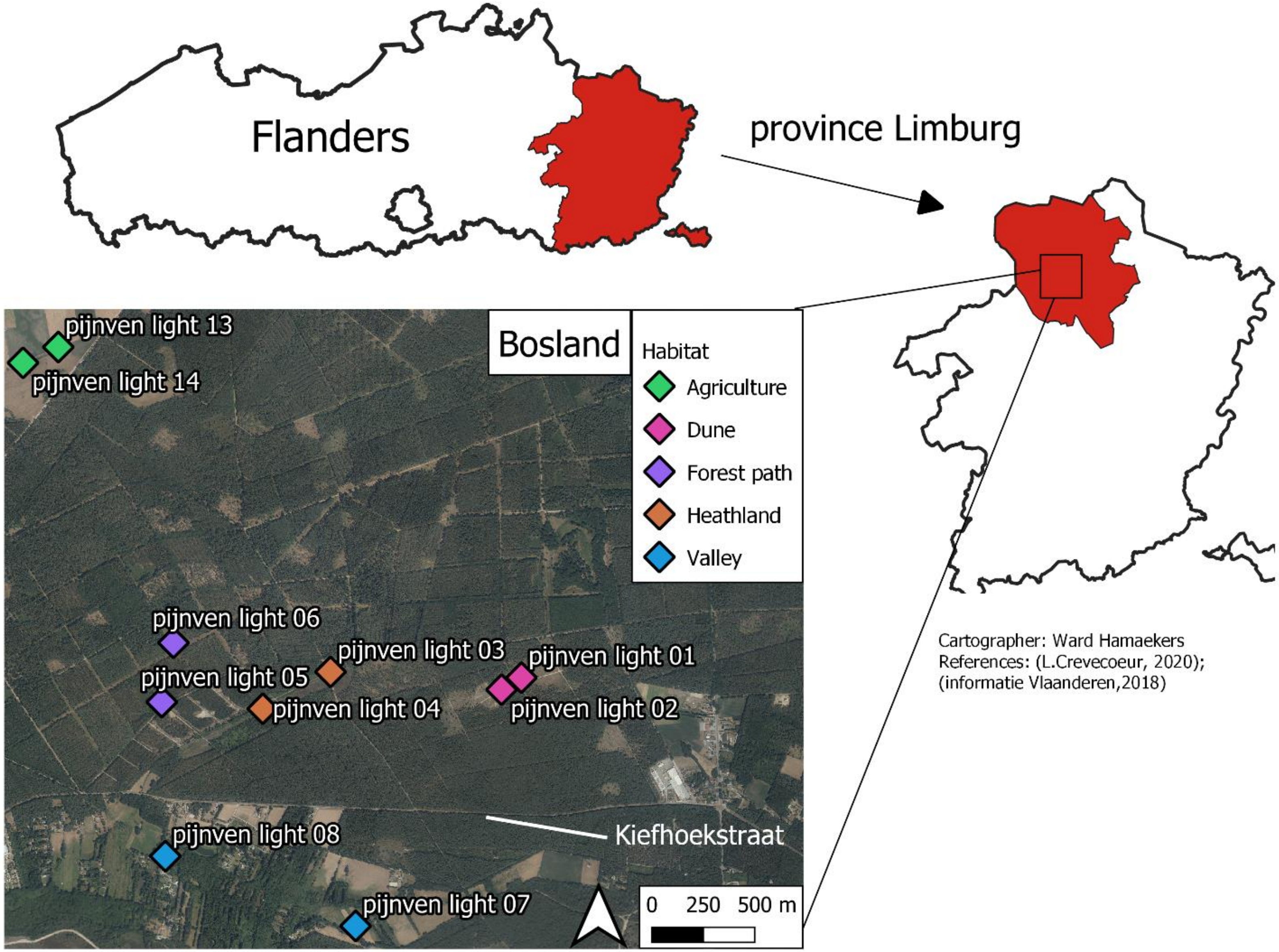
2. Materials and methods

2.1 Study area

Bosland is a forest-rich area in the northwest of the Limburg province in Belgium (Figure 1). It is a partnership that was formed in 2008 between the municipalities of Lommel, Hechtel- Eksel, Pelt and Peer, together with the Agentschap voor Natuur en Bos (ANB-. The area is a mosaic of different forest and nature reserves ,9500 ha, together with agricultural areas ,5000 ha, and recreational area (Coördinatiecel Bosland, 2012). One of the reasons this partnership was formed, next to recreational use, was to protect the unused and unmanaged heathland habitats that were being planted with Scots pine (Pinus sylvestris) and Corsican pine (Pinus nigra var Corsicana)(Evens, 2011). Due to its location, the region acts as a connection between different heathland habitats in Belgium and the Netherlands, situated to the north of Bosland (Evens, 2011).

Bosland can be divided into sub-areas. Most of our traps are located in the nature reserve Pijnven, which is located north of the kiefhoekstraat, in the middle of Bosland (Figure 1). The nature reserve Pijnven is subdivided into two areas. An area open to the public for recreational use (70ha), located in the west, and a closed nature reserve (864 ha) (Gorissen, 2006). The area is characterized by the big amount of Scots pine (Pinus sylvestris) and Corsican pine (Pinus nigra var Corsicana), which make up around 91% of the trees in the area (Waterinckx and Roelandt, 2001). Big amounts of heathland are found in the open areas of the nature reserve, alternated with some open dune habitats (Gorissen, 2006).

Two sampling locations are located in ‘De Vriesput’, to the northwest of the nature reserve Pijnven. This region is characterized by intense agriculture, together with some extensively managed grasslands and wooded hedges.



Cartographer: Ward Hamaekers
References: (L.Crevecoeur, 2020);
(informatie Vlaanderen,2018)

Figure 1: Our study area situated in the Province of Limburg in Flanders (Belgium), more specifically located in Bosland. Location of the light traps in the 2020 sampling period, two sampling locations in five different habitats are sampled.

2.2 Insect trapping protocol

To determine the insect diversity and biomass, 10 light traps are placed in different habitats in Bosland. During previous study visits, ten light traps were placed in 5 different habitats used by the nightjar. These were classified as: dune restoration, overgrown forest paths with grass, forest, heathland restoration and valley habitat (Evens *et al.*, 2013). Eight of these light trap locations are resampled in this research. This enables us to look at differences through time. Forest habitat, due to low sampling amount, is excluded from resampling in 2020. This study aims to expand previous research in time and space, meaning there will be an additional sampling location close to farmland to the North of the other locations. Different to Evens *et al.*, 2018, the height of the traps is standardized at 2 meters to get statistically comparable results.

Naming of the sampling locations was done by including the specific name of the region (Pijnven), the sampling method (light) and a number. Abbreviations are possible, only including the name of the region (PV) together with an identification number.

Pijnven light 07 (PV07) is located on a border between a species rich permanent cultural grassland and a grassland type characterized by marsh-marigold (*Caltha palustris)*, with a biological appreciation code (BWK-code) hc- (Ecopedia, no date). Slow formation of bog type habitat and reed vegetation are also present in this grassland. Pijnven light 08 (PV08) was located in a similar habitat as trap number 07, though of a less biologically valuable type. This can be due to some species not being present in the habitat. The BWK (hp + kbb) shows that there is a row of *Betula*-species at the border of this species rich grassland.

Pijnven light 03 (PV03) and 04 (PV04) are located in Natura2000 protected habitat types. Natura2000 is a European network, aiming to protect valuable habitats (Vlaamse overheid, no date)**.** PV03 and PV04 are located in a dry heathland on young sand deposits (Natura2000 code 2310). This habitat type is the main breeding ground for the nightjar *(Caprimulgus europaeus)* in which the presence of some small trees and some higher trees is beneficial for the nightjar. The bird uses these trees to sing from (Bowden and Green, 1991; Scott *et al.*, 1998).

The habitat found around Pijnven light 11 (PV11) and 12 (PV12) is characterized by *Corynephorus* and *Agrostis* species. This Natura2000 (code 2310_bu) habitat is a sparse, grassy vegetation with a species rich layer of lichen. The soil is very nutrient-poor and dry (T'jollyn *et al.*, 2009).

Pijnven light 05 and 06 are located in open forest paths with heather vegetation types. Trap 05 has a bigger abundance of *Corynephorus* species and has more sandy and open vegetation, while trap 06 is characterized by its dry heather vegetation (code 4030).

The two traps located at the border of agricultural field (trap 13 and 14) are located within a row of rough pioneer vegetation, BWK code ku+. The grassland located opposite of the cultivated field is used to create a species rich environment for insects, complementing the goals that were set for the forest reserve Vriesput (Opstaele and Berten, 2013).

Table 1: An overview of the sampling locations. All traps were hung at a height of 2 meters.

Number	Surrounding	Habitat
PV01	Dune habitat, close to forest with Corsican pine *(Pinus nigra* subsp. l*aricio)*	Dune habitat
PV02	Dune habitat, close to forest with Corsican pine *(Pinus nigra* subsp. l*aricio)*	Dune habitat
PV03	Forest edge, heather	Dry heather field
PV04	Dry heathland, recently cleared	Degraded heather field with eagle fern *(Pteridium aquilinum)*
PV05	Forest path within Scots pine *(Pinus sylvestris)*	Forest
PV06	Forest path within Scots pine *(Pinus sylvestris)*	Forest
PV07	Wet grassland within valley of a brook, low row of trees and reeds	Wet grassland
PV08	Wet grassland with *(Juncus effusus),* close to a brook	Overgrown grassland with blackberry *(Rubus fruticosus)*
PV13	Arable field with low row of trees and species rich grassland	Field road
PV14	Arable field with low row of trees and species rich grassland	Field road

2.3 Sampling

The insect traps were placed following Evens et al., 2018, each having a 15watt UVA-lamp to attract and collect insects from dusk to dawn. Arthropods were collected in an ethanol mixture (70% ethanol, 30% water and a drop of detergent). The action radius of a light-activated trap is around five meters for moths (Truxa and Fiedler, 2012). We activated the traps 3 times a week from June 13th to the end of August. In September, traps were activated only once a week. Traps are activated during the last hours of light and deactivated the next morning.

Samples were strained from the ethanol mixture and sorted into groups to estimate biomass. Coleoptera (beetles), Lepidoptera (butterflies), Nepomorpha (true water bugs), Formicidae (ants), Arachnidae (spiders) were sorted. For the Coleoptera, beetles from the family Carabidae were sorted separately to simplify the species determination. Further storage was done in ethanol 99%.

2.4 Analysis of insect biodiversity

Biodiversity analysis is done using R package ‘vegan’ version 2.5-7 (Oksanen et al., 2020). Shannon-Wiener, Simpson’s, Margalef, Pielou evenness indices and species richness are calculated for the insect species data across all sampling years.

2.5 Analysis of insect biomass

2.5.1 Calculating insect wet biomass

Species identification was only possible by optimally preserving the samples and weighing them in a alcohol-wet state. The samples were first taxonomically sorted (see section 2.3). Calculating wet insect biomass was then done by subtracting fluid content by using a standardized measurement protocol (Schwan, Sorg and Stenmans, 1993). The different samples were placed in a sieve with a small mesh size (0.5 mm), to avoid losing any insects in the straining process. The sieve was placed at a 30 degree angle to accelerate the first runoff of alcohol and in this way the whole measuring process. When the interval between drops reached 10 seconds, the straining process was done and the samples were measured using a laboratory scale to the nearest 0.001grams (Schwan, Sorg and Stenmans, 1993).

2.5.2 Calculating insect dry biomass

From the different sorted groups, only the Nepomorpha and Carabidae will be used in the calculation of dry insect biomass. The amount of Arachnidae captured was too low to justify this. During species identification, the Formicidae were not counted. Including these data makes calculation of dry insect biomass difficult, as we have no idea how many specimens were present in the sample. Furthermore, species identification on male alates was not done, due to difficult identification. Calculating biomass using a length-weight regression is not possible if the species and number of specimens is not known.

Oven dry biomass

Usual dry insect biomass calculations use a method of drying the samples in an oven. However, this method destroys all specimens and makes them unusable for further research or reference material. Due to the big amount of insects captured for this research, the decision was made to preserve as many insect specimens as possible. Some specimens will be kept in private collections and used as reference material, remaining specimens will be donated to the Royal Belgian Institute of Natural Sciences (KBIN) in Brussels to be used in further research. Due to the destruction of specimens when drying in an oven, only 30 samples of Nepomporpha and 30 samples of Carabidae are dried in an oven at 60 °C for 48 hours. Measuring was done using a laboratory scale to the nearest 0.001grams.

Length-weight regression

Next to drying the specimens, length-weight regressions make for good estimations of insect biomass if species data are available. Calculations were done by using a linear regression by (Rogers, Hinds and Buschbom, 1976). In this study a generalized regression was developed using 500 individuals, covering eight insect orders. The specimens were measured, oven-dried at 65°C and weighed to get an estimation of biomass (Rogers, Hinds and Buschbom, 1976).

$$\mathrm{W} = 0.0305\ \mathrm{L}^{2.62}$$

where W is weight in mg and L is length in mm.

This general regression, made for the insects in North America, is not as accurate as restricted regressions made for specific taxa. This is due to variety in insect morphology between different orders used to generate this length-weight regression (Ganihar, 1997; D. Johnson and Strong, 2000; Gowing and Recher, 1984). Gowing and Recher, 1984 found no significant difference between the newly calculated data and the regression found by Rogers, Hinds and Buschbom, 1976. They conclude that it is acceptable to use length-weight regressions for the estimation of invertebrate biomass, irrespective of continent of origin.

A later study found that latitude and physical habitat (aquatic or terrestrial) are positively correlated with allometric coefficients used in length-weight regressions. The similarities found in Gowing and Recher, 1984 could be explained by this. D. Johnson and Strong, 2000 also found that arthropods in Jamaica were relatively thin and light compared to drier regions. Due to the absence of specific length-weight regressions for insects in Europe, the regression as proposed by Rogers, Hinds and Buschbom, 1976 was used as a proxy for dry biomass in this study.

The species length was extracted from the literature. Stoffelen *et al.*, 2013 was used to get the average length of the *Nepomorpha* found in the study by calculating the mean length. Fourteen species of *Sigara* genus were grouped during species identification due to the difficulty of identifying them to species level. The mean length of this group was calculated by taking the mean length of each species in the group. Thereafter, these fourteen results were used to calculate the mean length of the group. For the *Carabidae*, mean length was calculated from Muilwijk *et al.*, 2015. As all insects were caught in light traps, we can assume that all specimens were macropterous (having long wings).

2.5.3 Analysis of insect biomass

R version 3.6.1 (R Core Team, 2019) was used to perform the statistical analysis of the data.

We used a generalized linear mixed-effects model (GLMM) approach to model the relationship between 1) wet biomass and oven-dried biomass 2) wet biomass and biomass calculated from a length-weight power function 3) oven-dried biomass and biomass calculated from a length-weight power function. These models are fit for both the Nepomorha and the Carabidae datasets, giving us a total of 6 models. The package glmmTMB (Brooks, Kristensen and J., 2017) is used in the analysis.

We fitted one model for the Carabidae, and one model for the Nepomorpha, because these are the most abundant groups captured during the sampling period. Log-transformation is done in both the predictor and the response to enhance the fit of the models and normalize the distribution. The fit of the models was checked using the package DHARMa (Hartig, 2021).

3. Results

3.1 Insect diversity

3.1.1 Ants (Formicidae)

In 2020, 12 species of ants were caught in the light traps. These were all female or male alates that were caught during or after nuptial flight, except some workers of Lasius platythorax that found their way into the trap from the ground. The preliminary Red List of ants from Dekoninck, Vankerkhoven and Maelfait, 2003:

- Vulnerable: Lasius meridionalis
- Highly endangered: Strongylognathus testaceus
- Introduced: Hypoponera puntatissima

Hypoponera punctatissima is an ant species that is introduced by humans, known for being found in anthropogenic environment (Dekoninck, Vankerkhoven and Maelfait, 2003). The species used to be only found inside buildings or in heat-producing organic material. Lately, the species is also found in natural habitats. This shift is interpreted as being a consequence of global warming (Seifert, 2018). The species has been found in two different traps. In 2012, the species was also found in two different traps on the 19th of august.

Figure 2: Hypoponera punctatissima (Nobile, 2007)

strongylognathus testaceus is an obligatory parasite on ants of the genus Tetramorium. Due to the species living inside of Tetramorium-nests, the species is difficult to find and only males or queens will be found during nuptial flight (Dekoninck, Vankerkhoven and Maelfait, 2003). The species was found throughout June and July on five different sites. It was only found once before with light trapping in 2012 in a heathland habitat.

Lasius meridionalis is a species known for living in warm heathland and dry grassland habitats on sandy soils (Seifert, 1996). The species is a temporal parasite on Lasius psammophilus, Lasius alienus and Lasius niger (Boer, Noordijk and van Loon, 2018). The species was found in small numbers in four different locations (PV03, PV11, PV12 and PV14). Great densities of this species in sandy habitat with Corynephorus species may be an indication of a valuable habitat. These grass species, as well as Lasius meridionalis are present in PV11 and PV12 (paragraph 2.2) (Boer, 2001).

3.1.2 Spiders (Arachnidae)

Although the number of spiders captured with light traps is only due to ballooning, a method of aerial movement during which spiders get carried by the wind (Cho et al., 2018), or to accidentally falling into the traps, it is still interesting to look at the species found.

In 2020, only four spiders were caught, all from different species. One of the spiders is mentioned in the Red List of the spiders of Flanders (Baert, Alderweireldt and Janssen, 1998).

- Endangered: Cheiracanthium virescens

Roberts, 1998 classifies all species found as species from dry, sandy habitats, which corresponds to the heatland of the study area Bosland.

Figure 3: Cheiracanthium virescens(Alberts, 2010)

3.1.3 True water bugs (infraorder: Nepomorpha)

21 species of water bugs were found in the 2020 sampling period. One specimen turned out to be classified as regionally extinct, Sigara longipalis. The species however was already present in the data from 2012-2014, being captured 16 times across different years and habitats. The species is known to live in big bright and open bodies of water with little growth of floating plants and a sandy soil (Stoffelen et al., 2013).

One new find is Corixa dentipes, only being found once in sampling location 05 on the 21st of July. The species is classified as vulnerable and known to live in open, shallow, nutrient-poor and acidic waters, such as bogs or fens (Stoffelen et al., 2013).

Glaenocorixa propingua, an endangered species found in bogs and fens, and Micronecta scholtzi were only found in 2012-2014 (Stoffelen et al., 2013).

Before looking at the species diversity indices, notice has to be given to the complex Sigara. 14 species of water bugs are merged together due to these species being difficult to identify to species level. This means that capture rates are high for this complex of species and that individual species will not have an effect on biodiversity indices, meaning that conclusions taken from the analysis could change if species-level identification was possible.

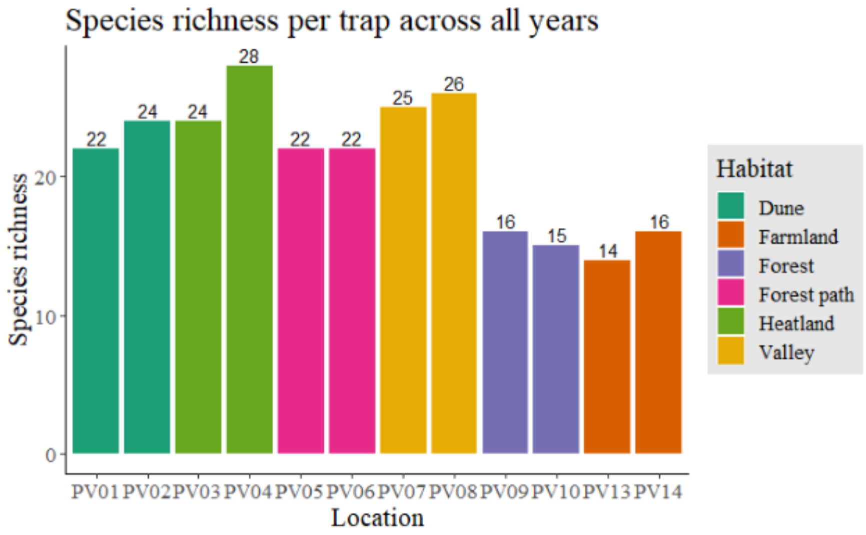
Looking at the diversity indices (page below and appendix 1), PV09 and PV10 score highest on the Shannon-Wiener index, with Simpson’s index showing similar results. The sampling intensity in these locations is lowest (N = 223 for PV09 and N= 251 for PV10) due to only being sampled at low intensity in 2013 and 2014. This can also be seen in the rarefaction curves for these data (Appendix 2), which show that the number of species does not increase linearly with the number of individuals. These locations score highest on the Pielou evenness index, which means that species are distributed evenly in numbers through the habitat.

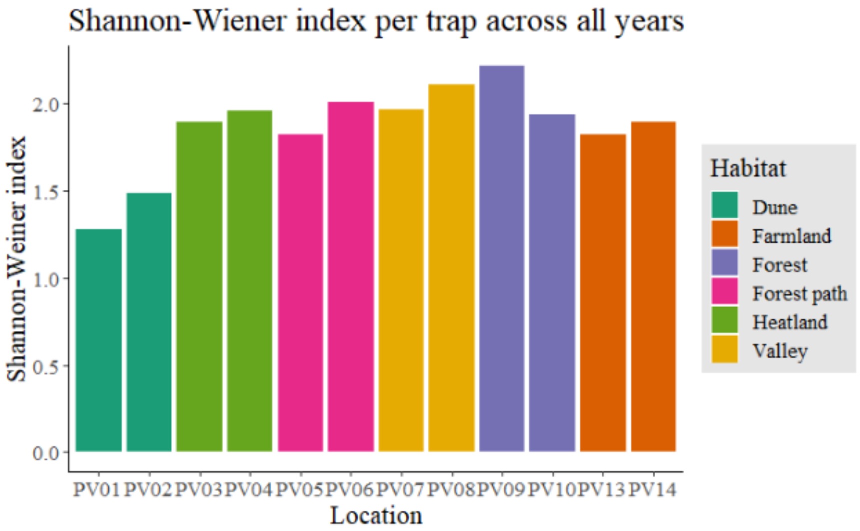
Species richness in itself however is lowest in the forest habitat (PV09 and PV10), though with similar results in the farmland habitat (PV13 and PV14). The largest number of species can be found in the traps located in the valley or in heathland habitat.

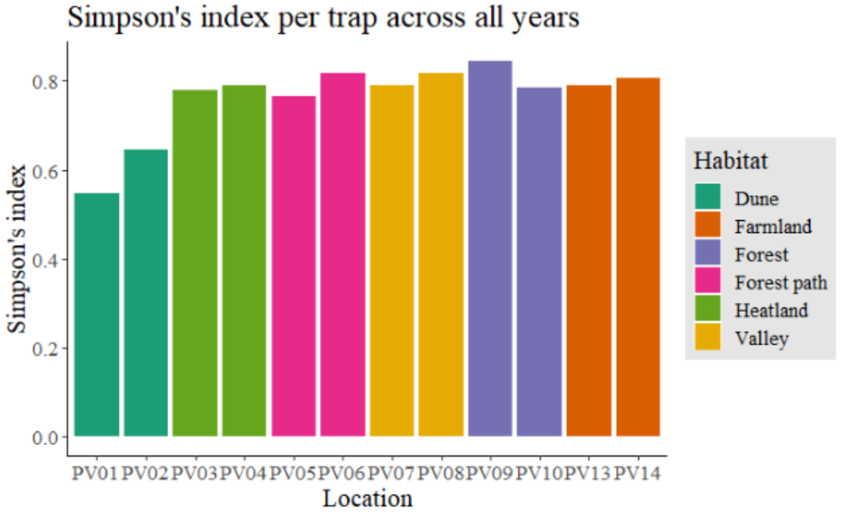
The most abundant species caught are Callicorixa praeusta (958 specimens), Sigara lateralis (1598 specimens) and Sigara striata (923 specimens). All are widely spread species in Belgium and are known from different habitat characteristics. Sigara lateralis is known for living in water with a higher Ph level and avoids living in bogs, while Callicorixa praeusta is known to live in waters with a lower Ph. This species can, when present in huge quantities, be an indicator for organic pollution (Stoffelen et al., 2013).

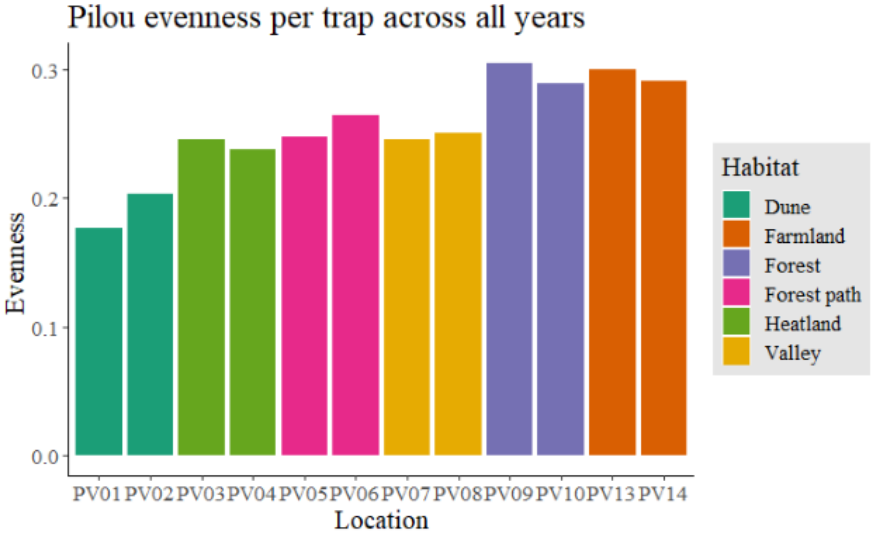
Figure 4: Callicorixa praeusta (Vergoossen, 2020).

Species diversity indices for the Nepomorpha









3.1.4 Ground beetles (Carabidae)

Nine new species of *Carabid*-beetles were caught in the 2020 sampling period (Table 2). Three of these species are mentioned in the red list for the *Carabidae* in Belgium (Desender *et al.*, 2008). *Bradycellus caucasicus* is an endangered species found in heather vegetation and dune vegetation (Muilwijk *et al.*, 2015). Only 1 specimen was caught in location PV06, forest path, on the 25th of July. *Bembidion octomaculatum* is known for living on shaded banks and wet forest habitats. 1 specimen was caught in PV05 on the 11th of august. *polistichus connexus* is an interesting find. A total of 114 specimens were caught across multiple habitats. The species is known to live in hayfields and swamps (Muilwijk *et al.*, 2015).

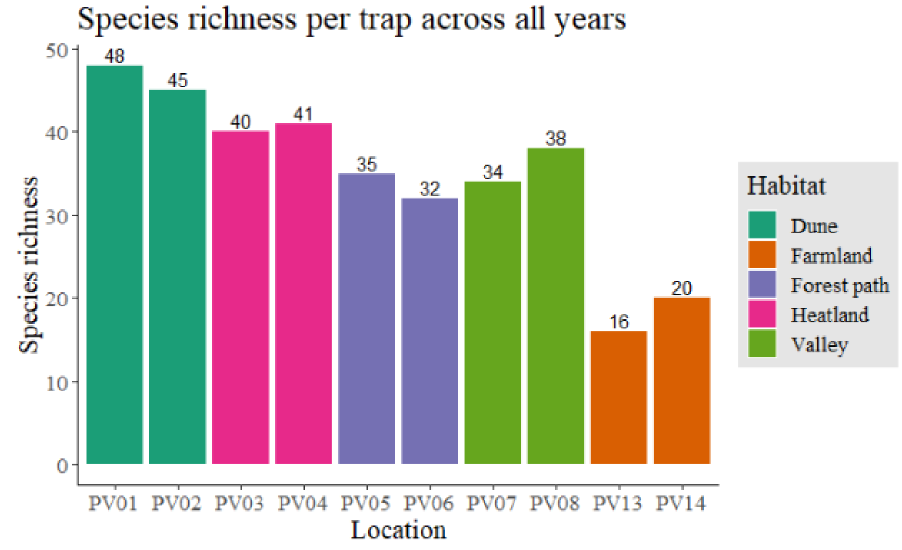
Table 2: Newly captured species in the 2020 sampling period with their red list status (Desender et al., 2008).

Species	Red list status
Bembidion assimile	Currently not endangered
Stenolophus teutonus	Currently not endangered
Bradycellus caucasicus	*Endangered*
Bembidion octomaculatum	Endangered
Bembidion lunulatum	*Currently not endangered*
Badister lacertosus	Currently not endangered
polistichus connexus	*Probably Endangered*
Bembidion assimile	Currently not endangered
Stenolophus teutonus	*Currently not endangered*

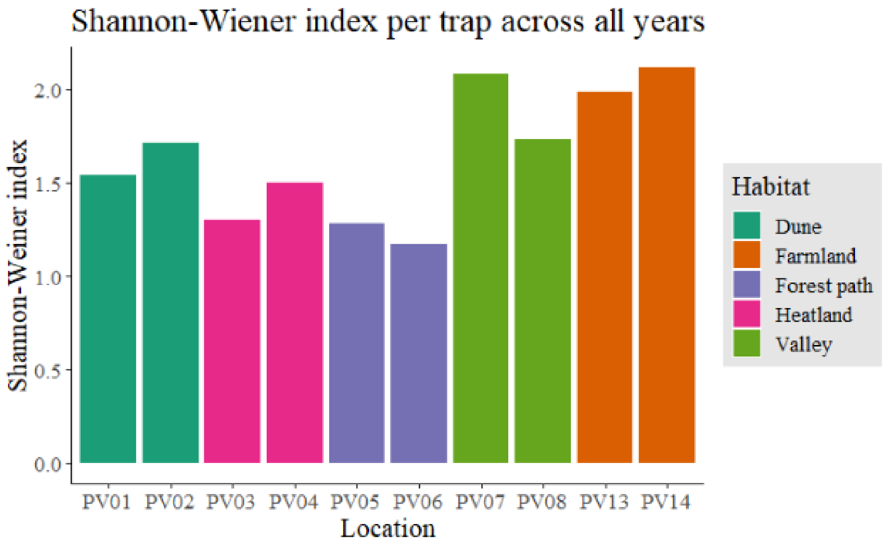
The most abundant species in 2020 are Bradycellus harpalinus (4723), Bradycellus verbasci (4515) and Harpalus griseus (1369), which are species of heathland and dune habitats (Muilwijk et al., 2015).

The number of Carabid-beetles captured differs a lot throughout the years. With a sample size of 32.245 specimens in 2012, sample sizes of 509 and 627 (2013 and 2014 respectively), are very low. 13.052 specimens were caught in the 2020 sampling period. The highest number of different species was caught in dune habitats (PV01 and PV02), 48 and 45 species respectively. Farmland scores lowest with only 16 (PV13) and 20 (PV14) species (page below and appendix 1). The Farmland habitat however scores highest when looking at the Shannon-Wiener and Simpson's indices, together with the trapping locations in the valley, but scores higher on the Pielou evenness scale. The rarefaction curves for these data (Appendix 2) show that the asymptote has not been reached for PV13 and PV14 due to the low number of individuals caught. This could change the diversity indices. The diversity of Carabidae is lowest in the forest path habitats (PV05 and PV06). The rarefaction curves show that we can be more certain about these data, having a big enough sample size and the curve flattening.

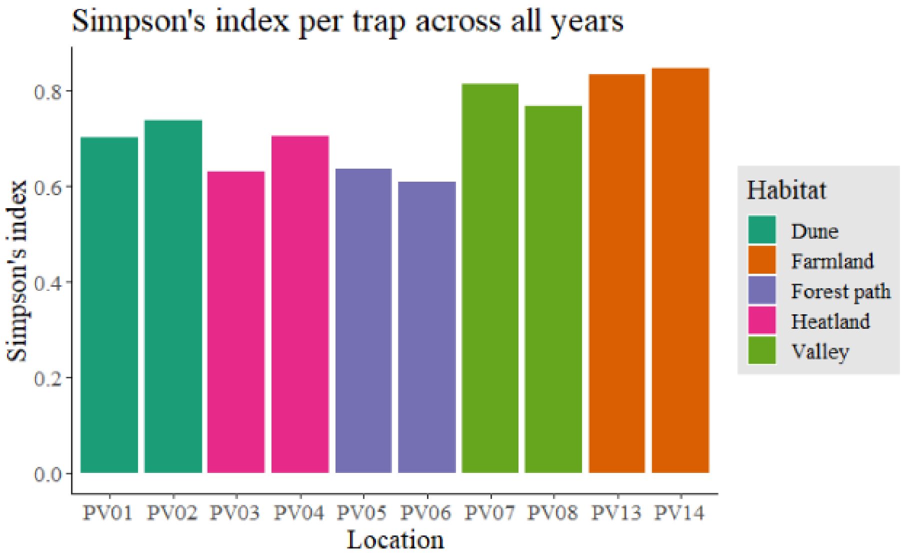
Species diversity indices for the Carabidae



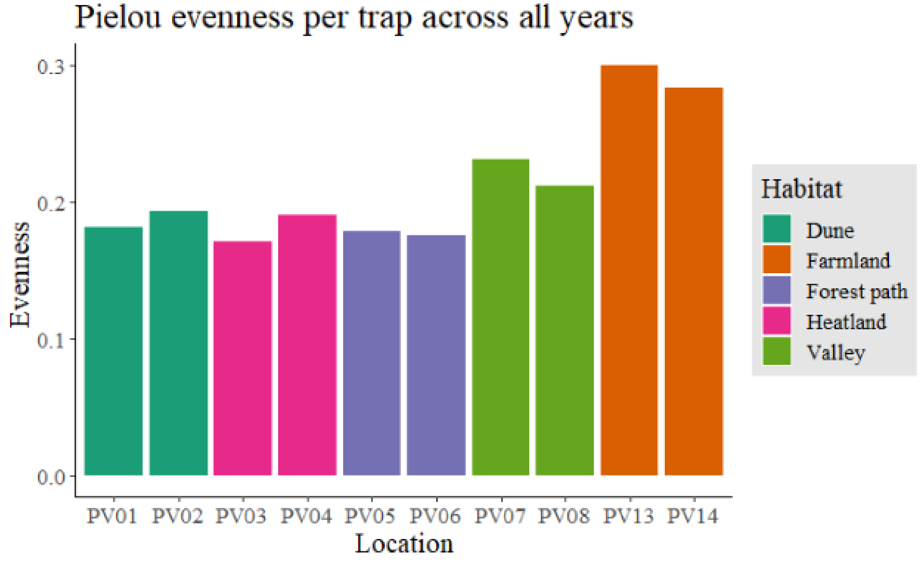
Species richness per trap across all years



Shannon-Wiener index per trap across all years



Simpson's index per trap across all years



Pielou evenness per trap across all years

3.2 Biomass methodology comparison

DHARMa’s simulation of the residuals shows a good distribution and no deviation from normality, but shows a deviation from uniformity in Residuals vs. predicted values for some of the models. This worse fit has to do with the way the data were gathered and the uncertainty it brings. The models are accepted and the fit will be further discussed later in this report. We excluded 4 observations (Carabidae) and 3 observations (Nepomorpha) for which, during species identification, sorting was found to be done incorrect.

Table 3: Models to evaluate different methods for biomass calculation.

Data	Formula	Family	β	95% CI
Carabidae	log(BiomassPow) ~ log(BiomassWet)	Gaussian	1.03	[0.98, 1.08]
Carabidae	Log(BiomassDry) ~ log(BiomassWet)	Gaussian	1.06	[0.98, 1.14]
Carabidae	log(BiomassPow) ~ log(BiomassDry)	Gaussian	0.96	[0.87, 1.04]
Nepomorpha	log(BiomassDry) ~ log(BiomassWet)	Gaussian	1.10	[0.91, 1.28]
Nepomorpha	log(BiomassPow) ~ Log(BiomassWet)	Gaussian	1.01	[0.96, 1.06]
Nepomorpha	log(BiomassPow) ~ log(BiomassDry)	Gaussian	0.87	[0.79, 0.95]

Model fit was best when comparing wet insect biomass (BiomassWet) with oven dry biomass (BiomassDry) in both datasets. The models including biomass calculated using the length-weight power function (BiomassPow) all show a deviation from uniformity. Due to the better fit, comparing BiomassWet and BiomassDry is preferred.

As expected, the different methods of calculating insect biomass yield similar results. BiomassPow shows a slightly bigger β coefficient compared to the BiomassDry (Table 3). The fact that we find this when comparing BiomassPow to BiomassDry could be due to the uncertainty when calculating BiomassPow.

The prediction plots show small confidence intervals in all models, as can be seen in Figure 5 or Appendix 3 for the other models. BiomassWet has a slightly higher β coefficient, which could mean a slight overestimation of BiomassWet when compared to BiomassDry.

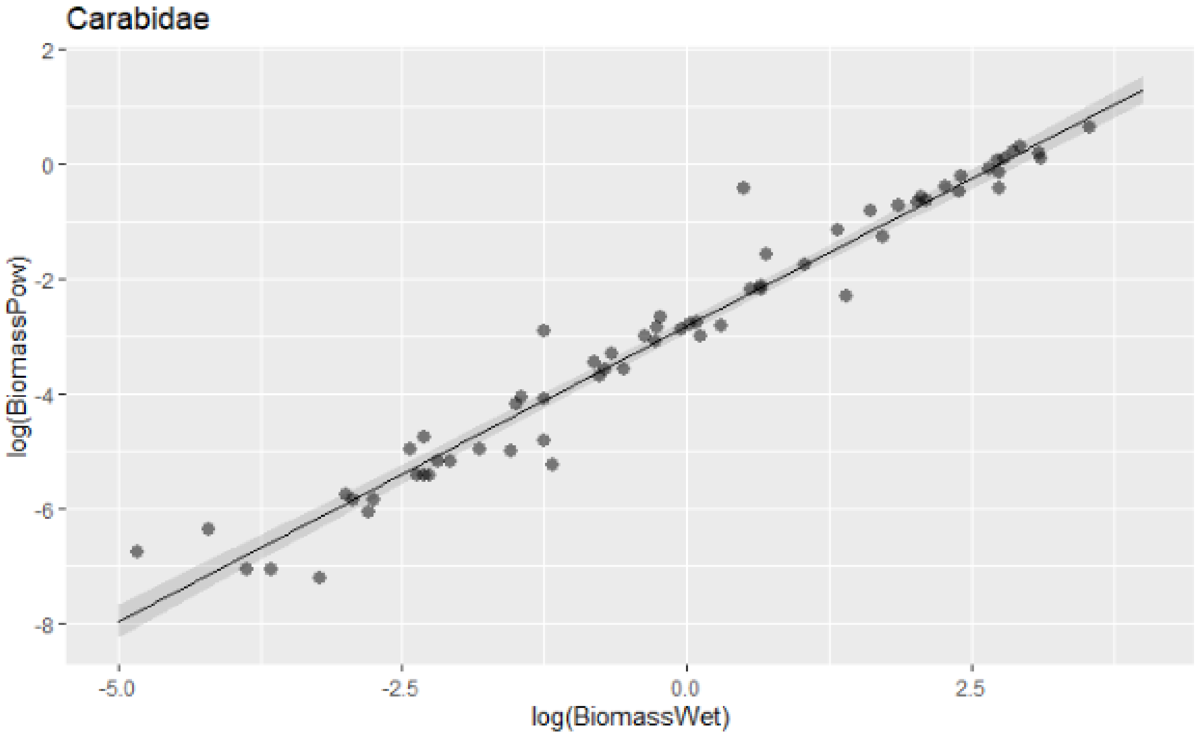


Figure 5: Prediction plot showing one of the models. (beta = 1.03, 95% CI [0.98, 1.08], p < .001; Std. beta = 0.98, 95% CI [0.93, 1.03]).

3.3 Insect biomass analysis

Due to the absence of insect biomass data from previous research, the American biomass power function by Rogers, Hinds and Buschbom, 1976 was used to estimate the insect biomass for all years in this analysis. Mean insect biomass differs a lot between different habitats and years when looking at both datasets.

Average weight of Nepomorpha was highest in PV01 and PV02, the dune habitats. This is followed by the biomass in heathland habitats. We can observe a decline in mean insect biomass across years for almost all habitat types, with a strong decline in the dune and heathland habitats (Figure 6). This is confirmed by two simple one- way ANOVA tests. These show a statistically significant correlation between Nepomorpha insect biomass and year (F(1, 480) = 6.31 , p = 0.012, α = 0.05, 90% CI [1.53e-03, 0.03]), as well as a significant difference between insect biomass and the different habitats (F(5, 476) = 2.91 , p=0.013, α = 0.05, 90% CI [3.42e-03, 0.05]).

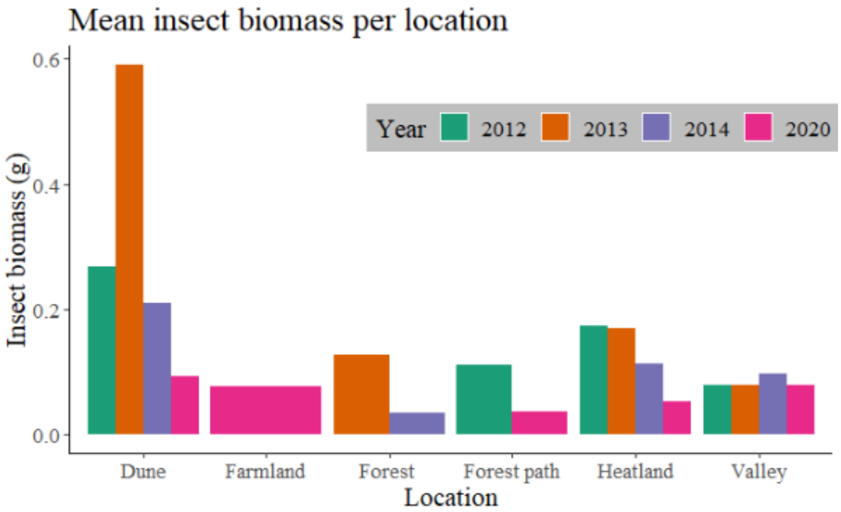


Figure 6: Mean Nepomorpha biomass per trapping habitat.

Carabid-beetle biomass shows similar results as found with the Nepomorpha dataset, showing a strong relation between Carabidae insect biomass and year (F(1, 171) = 9.87, p = 0.002, α = 0.05, 90% CI [0.01, 0.12]) and a smaller relation with the different habitats (F(3, 169) = 0.91, p = 0.438, α = 0.05, 90% CI [0.00, 0.05]). Mean insect biomass across the years differs greatly, showing a big decline from 2012 to 2013. Average biomass found in 2020 was slightly higher, showing a slight increase when compared to 2013 and 2014, but retaining the big difference with 2012

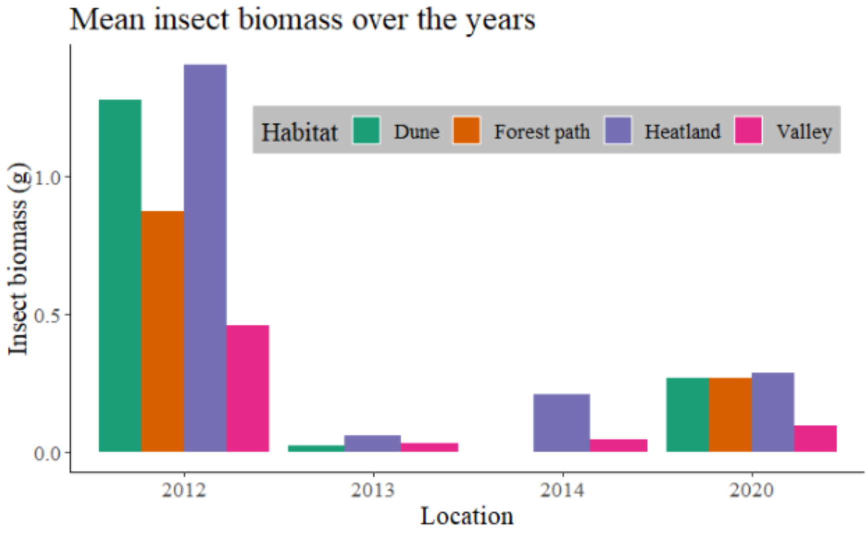


Figure 7: Mean Carabid beetle biomass over all years, showing a big decline after the 2012 capturing period.

4. Discussion

4.1 Biodiversity analysis

4.1.1 *Nepomorpha* biodiversity

Insect biodiversity indices give us more insight in how the habitat is doing. A more divers habitat has a better chance of surviving and keeping itself in balance.

It is interesting that the highest biodiversity for Nepomorpha can be found in the forested areas (PV09 and PV10). Due to these locations not being sampled in 2020 and having not sampled the 2012 -2014 data myself, we can only speculate as to why this is the case. Direct species richness is lowest in the forest habitat. Diversity indices, like the Shannon-Wiener and in lesser amounts Simpson’s index are dependent on the sample size. This could have an effect when comparing low sample size locations to locations with a much higher sample size. It is argued that this effect is bigger when looking at samples with a higher diversity and higher number of rare species, especially for the Shannon-Wiener diversity index (Soetaert and Heip, 1990). This can lead to the fact that we are not able to infer whether the differences are due to differences in diversity, richness or sampling intensity (Statsdirect, no date). The rarefaction curve (Appendix 2) shows that these locations have a very low sample size, compared to the other sampling locations, telling us to be careful with the interpretation of biodiversity indices. A bigger sample size, despite scoring highest on the Pielou evenness index, could score very differently.

The fact that some species were grouped together due to difficult identification (Sigara complex), could have an effect on biodiversity indices. If these species were all identifiable, species diversity could possibly change between habitats.

We have to keep in mind that the only specimens actually caught are all macopterous (having fully developed wings with the ability to fly). Nepomorpha are known to underdevelop these wings, mainly in the summer generation. This is done to quickly develop to the adult phase and fully take advantage of suitable ecological circumstances (Stoffelen et al., 2013). The high number of Nepomorpha captured in the dune habitats could indicate unsuitable circumstances in the habitat of these Nepormorpha. The continuous increase of average temperatures and the almost yearly record breaking temperatures could have a tremendous effect on water reserves and habitat for these water bugs (Willemsen, 2021); Deboosere, no date). The dune habitat is very open with little plant cover and could be used as a highway to new and more suitable breeding habitat. The same could be said about the heathland habitat.

4.1.2 Ground beetle biodiversity

Carabid-beetles diversity was shown to be the highest in farmland habitat (PV13 and PV14). The fact that these locations were only sampled in 2020 means that the sample size is small compared to the other locations. Rarefaction curves support the fact that many new species could possibly be found in this habitat, by not coming close to an asymptote. Carabid-beetles are found in a wide variety of habitats, with different species being linked to specific habitats (Muilwijk et al., 2015). The trapping locations PV13 and PV14 were located at the edge of a cornfield and a species rich grassland, the latter forming a perfect habitat for these beetles. Wang and Liu, 2009 found a high correlation between land use and species occurrence when looking at Carabid- beetles in Northern China. The less disturbed habitats showed a higher diversity than habitats with higher human disturbance (farmland). However, the fact that the traps were located at the edge of an agricultural field may lead to a lesser effect of this human disturbance. The grassland habitat may act as a refuge for not only Carabid-beetles, but also for their prey. This refuge can buffer the negative effect that an intensely managed crop field can have on insect biodiversity (Lee, Menalled and Landis, 2001). Further research will show how diverse this grassland is and if there are any effects of the intensive agriculture next to it.

The sampling locations located in the dune habitat (PV01 and PV02) score lowest on almost all of the biodiversity indices, despite being the locations with the biggest sample and having the highest number of different species. This could be due to rarer species of beetles being captured. As shown, the Pielou index is lowest in these habitats, meaning there is a big difference in the abundance of each species in the habitat. Having a lot of ‘rare’ species could also have an effect on the Shannon-Wiener index (Soetaert and Heip, 1990).

4.2 Biomass methodology comparison

Calculating insect biomass is becoming more and more important in studying the health and processes in ecosystems. Many insect families are being used as bioindicators, indicating disturbances in the environment and reflecting the responses of other animals. The two insect groups focused on in this study, Carabidae and Nepormorpha are both being used to indicate the health of the habitats they live in by looking at their diversity and specific species habitat preferences and sensitivity to changes (Rainio and Niemela, 2003; De Pauw and Vannevel, 1993). Although the biodiversity of these insect groups can tell us a lot about the health of the ecosystem, it is argued that, when looking at trophic interactions, biomass could provide a more accurate picture of the changes in community structures (Saint-germain et al., 2007).

Conducting good research into this requires using the right methods to achieve the goal of the study and has to be reproducible and replicable (Editage Insights, 2019). In this research we look at three methods of estimating insect biomass to investigate their comparability and find out if it is possible to derive different biomass estimations by only calculating one estimation and creating a coefficient of proportionality between these methods.

Comparing the different methods of estimating insect biomass has shown that wet biomass and dry biomass are the methods that are comparable. The models including these methods show the best fit, while having small confidence intervals and showing a straight linear relation.

The positive side of methods using dry insect weight is that these are easily replicable and there is a small chance of human error. Working with wet insect biomass means that small differences are easily created. Using a different sieve, waiting a few more seconds for water to drop or having bigger or smaller samples, could all have a big impact on the amount of water remaining in the sample when weighing. This can create an over- or underestimation when comparing insect biomass between habitats, time or studies. This over- or underestimation could not be proven during in this research. Further research will need additional samples.

Drying insects is a better estimation for insect biomass, as it is less susceptible to small changes in methodology and insects do not need to be determined to species level. Further research has to be done to make the models concerning oven dry biomass more reliable, with only a small number of samples dried for the Carabidae (N =30) as well as for the Nepomorpha (N =30).

The good results when comparing dry to wet insect biomass signify that we could, if carefully used, use the model as a coefficient of proportionality. Research can benefit from this when one of the methods is not available or cannot be calculated. In research where insect identification is not needed, wet biomass is easy to calculate from samples. Being able to derive dry insect biomass from wet biomass is useful when comparing between different studies, making it possible to get a better understanding of the decline in insect biomass.

In this study we found bad fits for the models including biomass calculated from the American power function. Using length-weight regression methods, it is important to account for the uncertainty. By only providing an average weight per insect length, big errors can be expected when working with species that are longer and thinner than average. In this study, we did not account for this uncertainty.

The bad fits found in this study can be explained by looking at the method used in this research. An average length per species was calculated using sources that estimate maximum and minimum length of a species. Using an American power function to estimate insect biomass in Belgium is another cause of uncertainty. As mentioned in section 2.5, this method requires more region specific functions to be used. A small change in the morphology of insects i.e. American insects being slightly longer or slimmer than the insects in Europe, could have an effect on the parameters used to calculate the estimated insect biomass in this study. Another point has to be made about the fact that we used two species groups in the analyses, causing us to be more specific than the model was made for. In conclusion, the worse fit with the biomass estimation resulting from a length-weight power function has to do with the way the data were gathered. Keeping this in mind, the power function is still a useful and easy method of estimating insect biomass and comparing biomass between habitats, when carefully used. Interesting would be if more specific length-weight regressions would exist for Europe. Creating a regression for specific taxa, like the Coleoptera or just Carabidae in mainland Europe, would make the estimation of biomass more reliable. However, to use this method, species information has to be present, making it very time consuming in studies that do not need species information. In addition, all specimens caught will be destroyed in the process, making these specimens unsuitable for further research.

4.3 Biomass analysis

The mean insect biomass per trap and location shows interesting results when looking at both species groups we worked with. The significant decline in mean insect biomass is disturbing. The fact that we can find a significant decline in insects when working with a small number of sampling locations and only having repeating data for four years, shows that insect decline is happening everywhere. Due to the unavailability of local weather data for 2012, further modelling of these results was not possible and inferring anything about the amount of insect decline would be unfounded. The changes found here, although important to note and disturbing, could be due to changes in weather, land use or other habitat characteristics. To get bigger and statistically more robust results of insect decline, like in Hallmann et al., 2017, further research will be necessary. Screening average temperature, precipitation and hours of sunlight per month do not show big differences between 2012, 2013, 2014 and 2020. Average temperature across the whole year increased from 10.6 °C to 12.2 °C (from 2012 to 2020), sunlight increased from 1528 to 1838 hours and precipitation decreased from 976.5 mm to only 731.9 mm (MeteoBelgië, no date). A steady increase in average temperature, and sunlight, with a decrease in precipitation across the whole year, might have an effect on insect development, reproductive success and activity (Jaworski and Hilszczański, 2013). One other subject that this research does not consider is the amount of moonlight present during the sampling periods. Moonlight does have a strong negative effect on sampling effectiveness in light bait trapping on noctuid moths (Yela and Holyoak, 1997). It is acceptable to infer that the same is true for other insects that are attracted by light bait trapping methods.

The fact that mean Carabid-beetle biomass shows a strong decline from 2012 to 2013 is strange. 2012 showed a very big average biomass compared to the other sampling years. Some explanation can be found in examining the most abundant species, Bradycellus harpalinus, Bradycellus verbasci and Harpalus griseus. These are species of heathland and dune habitats (Muilwijk et al., 2015). Potential management or disturbance in or around the sampling locations, could have created a more suitable habitat in 2012. However, this does not explain the strong decline from the 2012 to 2013 sampling period.

Numerous recent articles show a strong decline in insect abundance and biodiversity (Sorg et al., 2013, Hallmann et al., 2017, Wagner et al., 2021. The latter calls the decline in insects

“Death by a thousand cuts”. Warren et al., 2021 found that the abundance of butterflies in Belgium declined by 30% between 1992 and 2007. Although we cannot arrive at concrete results from our data, we cannot ignore the fact that the biomass did decline significantly.

We already mentioned that beetles are a big part in the diet of various other species, like the nightjar (Caprimulgus europaeus), for which about 10% of the diet consists of these insects (Evens et al., 2020). A significant decline could have a big impact on the food availability of these birds, making them travel longer distances to suitable hunting ground, forcing them to use a lot of energy in the process. The nightjars’ average maximal foraging range, from their breeding ground, was found to be 2603 m in Bosland, with some individuals travelling over 5 km (Evens et al., 2017). Declining densities of insects could change the habitat use of different bird species, making some habitats more suitable as foraging areas, or could affect the foraging efficiency and making a foraging session less rewarding. This could have an effect on nest survival or changes in nesting ecology. Barrientos, Bueno-Enciso and Sanz, 2016 showed that tit species react differently to changes in food availability, with hatching asynchrony being one of the mechanisms to cope with this.

5. Conclusion and remarks

After comparing different methods to calculate insect biomass, we can conclude that the setup of the study determines which biomass calculation method is feasible, depending on how the insects were collected and what has been done to them. If insect identification to the species level is needed for the scope of the study, then calculating the biomass using an accurate length-weight power function would save a lot of time and resources. However, accurate length-weight regressions on specific taxa are not available for European insects. More research has to be done, on big sample sizes, to calculate an accurate and usable length-weight regression. If the scope of the study is to accurately measure the difference in insect biomass, the oven dry biomass is the most accurate and is easy to perform. The downside is that insect specimens will be destroyed in the process. This study found a good model between dry and wet insect biomass. This model could be used as a coefficient of proportionality to derive one biomass estimation from the other.

It is disturbing that, even with the limited sample sizes, inaccurate calculation of insect biomass, limited amount of replications over time and the absence of weather data to explain more of the variation, we can still see a significant decline in insect biomass in the Bosland region of Belgium. Although we have to be careful with the interpretation, these results should show us that something is going on with our insects. More consistent data from longer time series could show the real rate of insect decline. Further research, modelling the effect of weather on insect biomass, could give more insight in the exact biomass fluctuations. An other interesting factor to include is moonlight, as it is shown to have an effect on the light trap captures. Working together with moonlight is cloud cover. Having a higher cloud cover negates the negative effect of moonlight on captures when using light traps (Yela and Holyoak, 1997). Insects are an important part of every ecosystem. Being the most important food source for insectivorous birds like the nightjar, means that a decline in insect abundance can have a tremendous effect on other animals that live in the same habitat. Protecting the abundance of insects ensures that protection efforts, like for the nightjar in Bosland, are essential.

The samples close to agricultural land showed, against expectations, to be one of the most biodiverse habitats for ground beetles, together with the traps in the wet valley. Although sampling in the former was only executed in 2020 and rarefaction curves show the possibility this could change with further research. The same goes for the Nepomorpha biodiversity. Further research is needed to examine how biodiversity will change with bigger sample sizes. Nevertheless, trapping locations in the forest show the highest amount of biodiversity at this point. However, we can conclude that heathland scores high in both instances when looking at species diversity.

It could be interesting to look at species turnover for both the Carabidae as the Nepomorpha. This is the number of species that are replaced in a certain habitat per time. Having some species leave the habitat because of unfavourable conditions or human disturbance, can lead to a shift in insect assemblages (MacArthus and Wilson, 1967). The warming of the climate could also start a big shift in the composition of insect communities (Nooten, Andrew and Hughes, 2014). Biodiversity indices alone do not cover this part of ecology. Discovering that a shift may happen over time, can say a lot about the state of a certain habitat. Due to time shortage, species turnover was not calculated in this research, but further research on this topic might be useful and interesting.

This research focussed only on the Nepomorpha and Carabidae groups. Including other species groups could result in more robust and accurate results when looking at biomass as well as biodiversity, giving the chance for further modelling.

Insects form a big part of ecosystems and we should make the protection of their abundance and biodiversity a priority. Although the causes could vary from habitat fragmentation, pesticides or normal fluctuations to changes in climate, these changes could have a domino effect, having a big impact on ecosystems in Belgium. There is an urgent need to unravel the reasons behind this decline and understand the effects of this decline on ecosystems and ecosystem services.

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I started gaining an interest in insects through my membership of the youth movement JNM (Youth movement for nature and environment). Looking at the amazing variety of insects and learning about them, makes you see nature differently. The fact that most people would not notice a row of ants crossing a path, or a beetle on the search for food, makes them even more interesting to me.

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7. Figures

7.1 Tables

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8. Abstract

Insects, being the first in the food chain, form a perfect animal group to estimate the vitality of an ecosystem. Their diversity and abundance could have tremendous impact on plants and other animals living in the ecosystem. Good research on insects requires using the best available methods. In this study I used a generalized linear mixed-effects models (GLMM) approach to model and evaluate three different methods of calculating insect biomass: dry insect biomass, wet insect biomass and insect biomass obtained from a length-weight power function. Results show that the use of oven dried insect biomass is most accurate with a low opportunity for human error and less uncertainty than the other methods. Most apparent differences are the way of gathering these data. Using length-weight regressions, although easiest to calculate, requires insect identification to the species level, making it time consuming in comparison to research that does not require specific species data. This research may help further research in gathering valuable data for analysis of insect biomass, by making a usable model to compare wet and dry insect biomass.

This study aimed to evaluate the present biomass and biodiversity in different habitats within Bosland, a forested area in the north of the Limburg province, Belgium, focusing on both *Nepomorpha* (true water bugs) and *Carabidae* (ground beetles). Insect biomass declined for both groups from 2012 till 2020. Although uncertain, these results are concerning when looking at the broader picture of climate change and the effects these declines might have on other animals in the ecosystem.

Nepomorpha diversity was found to be similar between different habitats, but high abundances of animals were captured in dune habitats, suggesting that this habitat may be used as a ‘highway’ to travel to a more suitable habitat. *Carabidae* biodiversity was highest in the valley and farmland habitats, although highest species richness was found in dune habitat. Rarefaction curves show that further research is needed, as asymptotes are not reached.

Further research is warranted to get a better estimation of the actual insect biomass decline over the years. I suggest including and modelling with weather data and adding the amount of moonlight. Looking at insect turnover in the different habitats could give more insight in the changes in insect diversity through time. Next to these, development of length-weight regressions for European insects could enhance and simplify the estimation of insect biomass in studies looking at both insect diversity and biomass.

9. Appendix

9.1 Appendix 1

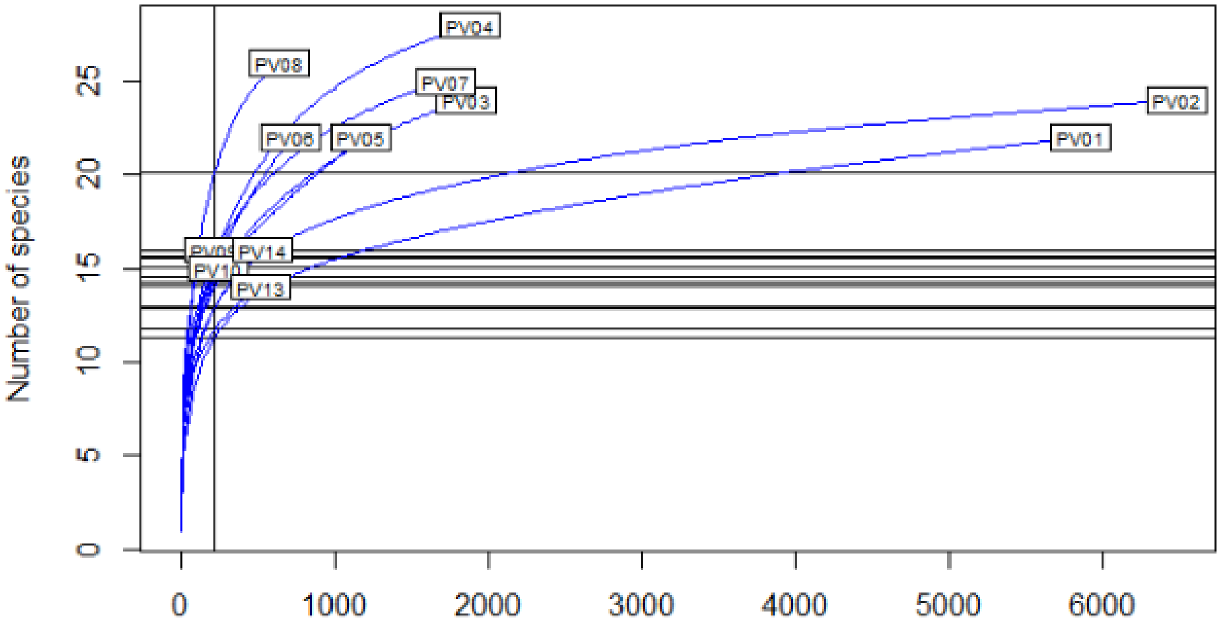
Results showing the insect biodiversity across all years of the *Nepomorpha* (top) and *Carabidae* (bottom) species groups. Diversity indices were not calculated for the PV09 and PV10 locations with the *Carabidae* species data due to insufficient data between 2012-2014 and not being resampled in 2020.

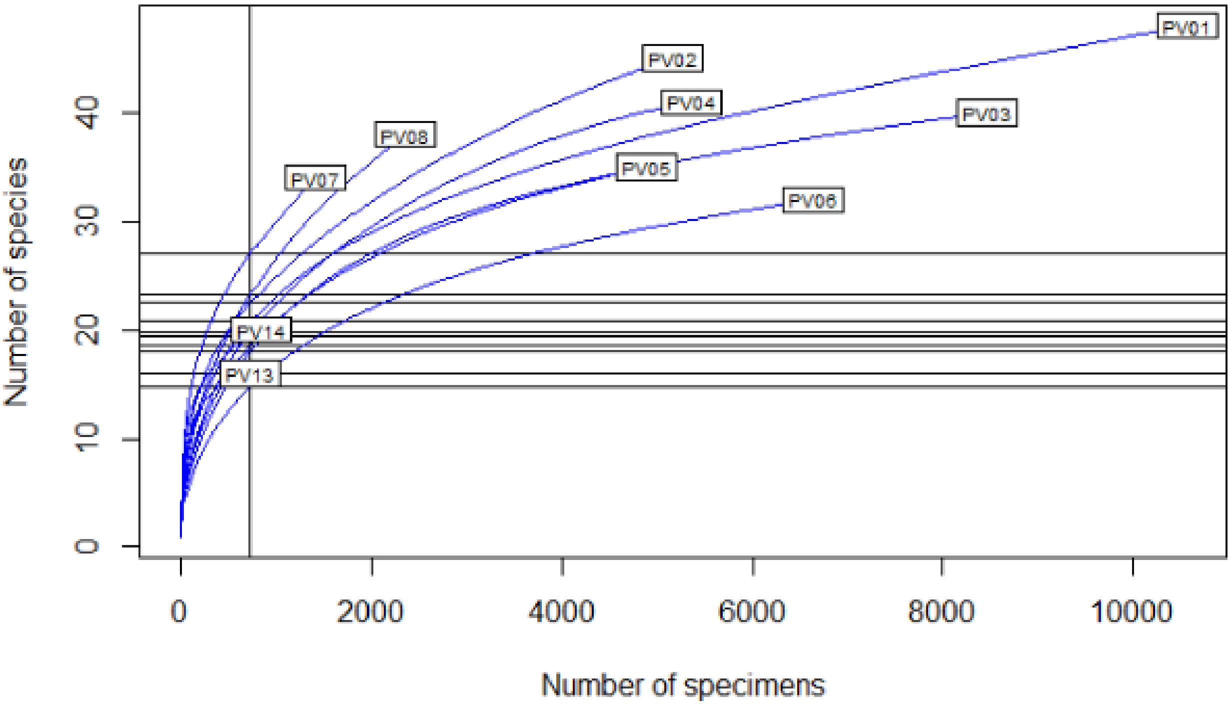
Location	Richness	Margalef	Abundance	Shannon-Wiener	Simpson	Evenness
PV01	22	2.42	5858	1.27	0.54	0.17
PV02	24	2.62	6481	1.48	0.64	0.20
PV03	24	3.05	1861	1.89	0.77	0.24
PV04	28	3.57	1887	1.95	0.79	0.23
PV05	22	2.96	1177	1.82	0.76	0.24
PV06	22	3.19	716	2.00	0.81	0.26
PV07	25	3.22	1723	1.96	0.79	0.24
PV08	26	3.86	640	2.10	0.81	0.25
PV09	16	2.77	223	2.21	0.84	0.30
PV10	15	2.53	251	1.93	0.78	0.28
PV13	14	2.08	517	1.82	0.79	0.29
PV14	16	2.38	539	1.89	0.80	0.29

Location	**Richness**	**Margalef**	**Abundance**	**Shannon-Wiener**	**Simpson**	**Evenness**
PV01	48	5.07	10554	1.54	0.70	0.18
PV02	45	5.14	5170	1.71	0.73	0.19
PV03	40	4.31	8460	1.30	0.63	0.17
PV04	41	4.65	5363	1.49	0.70	0.18
PV05	35	4.00	4895	1.28	0.63	0.17
PV06	32	3.52	6636	1.17	0.60	0.17
PV07	34	4.54	1425	2.08	0.81	0.23
PV08	38	4.76	2360	1.73	0.76	0.21
PV13	16	2.27	736	1.99	0.83	0.29
PV14	20	2.81	850	2.11	0.84	0.28

9.2 Appendix 2

Rarefaction curves for the Nepomorpha (top) and Carabidae (bottom) biodiversity datasets. The labels for the lines correspond to the trapping locations. A rarefaction curve for PV09 and PV10 locations with the Carabidae species data was not included due to insufficient data between 2012-2014 and not being resampled in 2020.





9.3 Appendix 3

Prediction plots showing the different models made for comparing different biomass calculations. Models using BiomassDry (oven dry biomass) have a limited sample size, only having 30 samples for Carabidae and 30 samples for Nepomorpha.

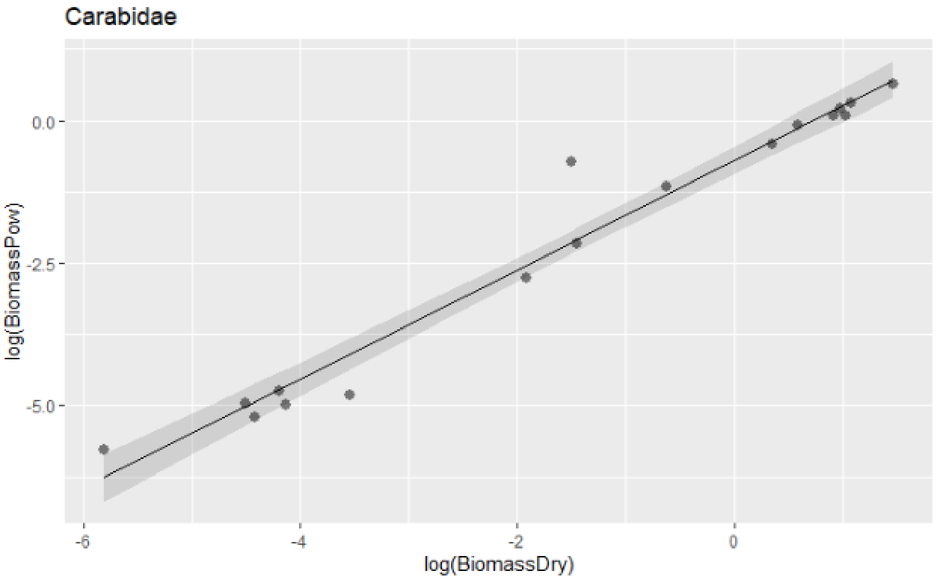

Carabidae

Figure 8: (beta = 0.96, 95% CI [0.87, 1.04], p < .001; Std. beta = 0.98, 95% CI [0.90, 1.07]).

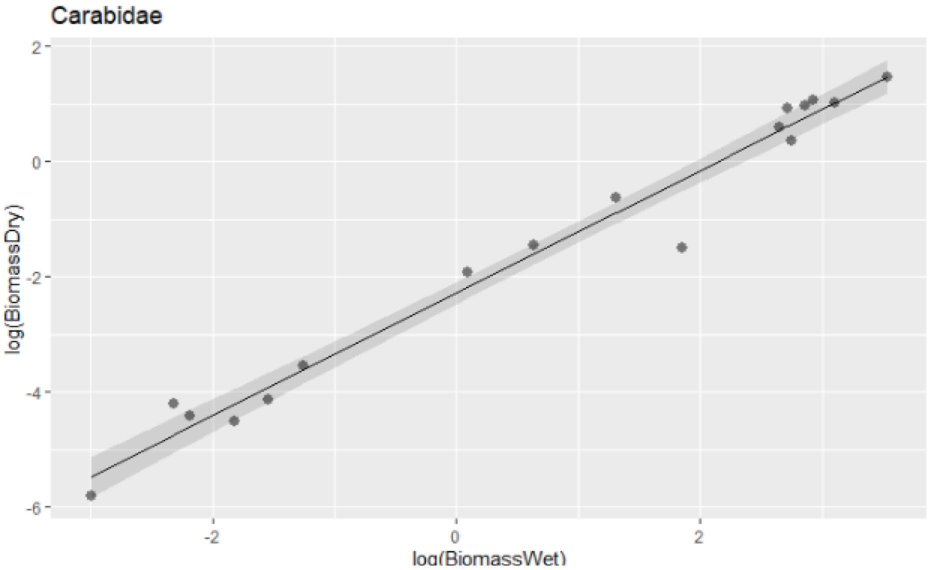

Carabidae

Figure 9: (beta = 1.06, 95% CI [0.98, 1.14], p < .001; Std. beta = 0.99, 95% CI [0.91, 1.06])

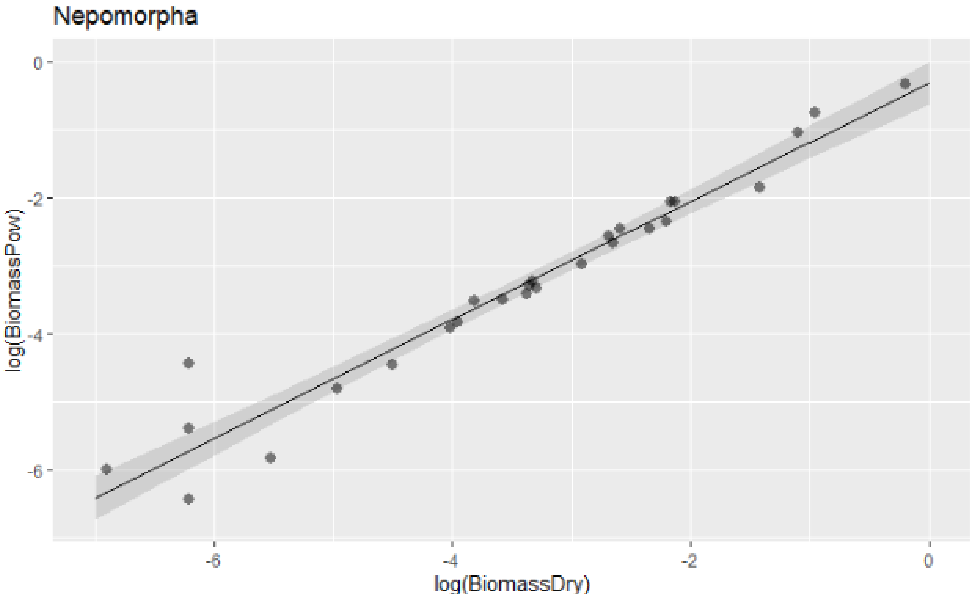


Figure 10: (beta = 0.87, 95% CI [0.79, 0.95], p < .001; Std. beta = 0.97, 95% CI [0.88, 1.06])

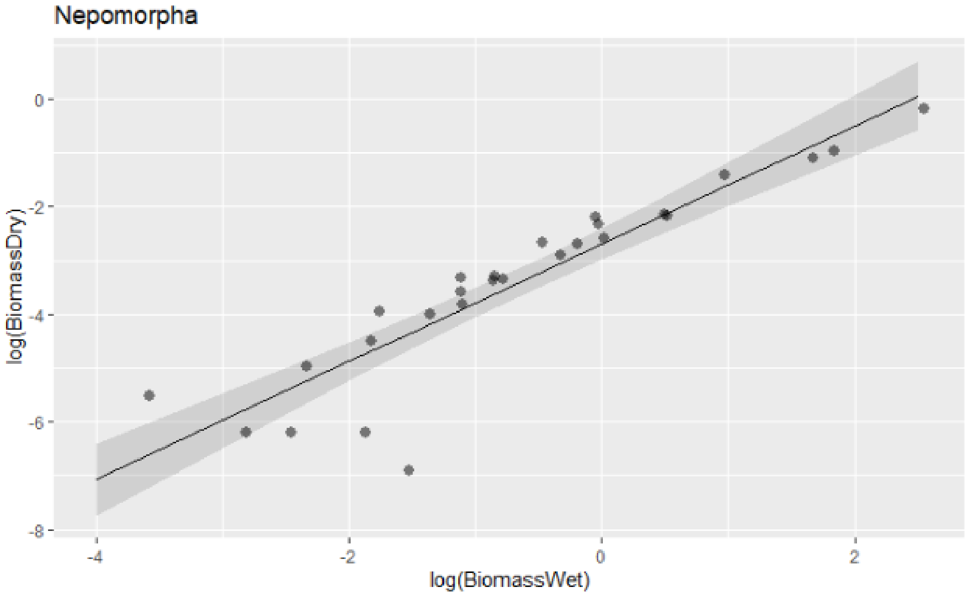


Figure 11: (beta = 1.10, 95% CI [0.91, 1.28], p < .001; Std. beta = 0.91, 95% CI [0.76, 1.07])

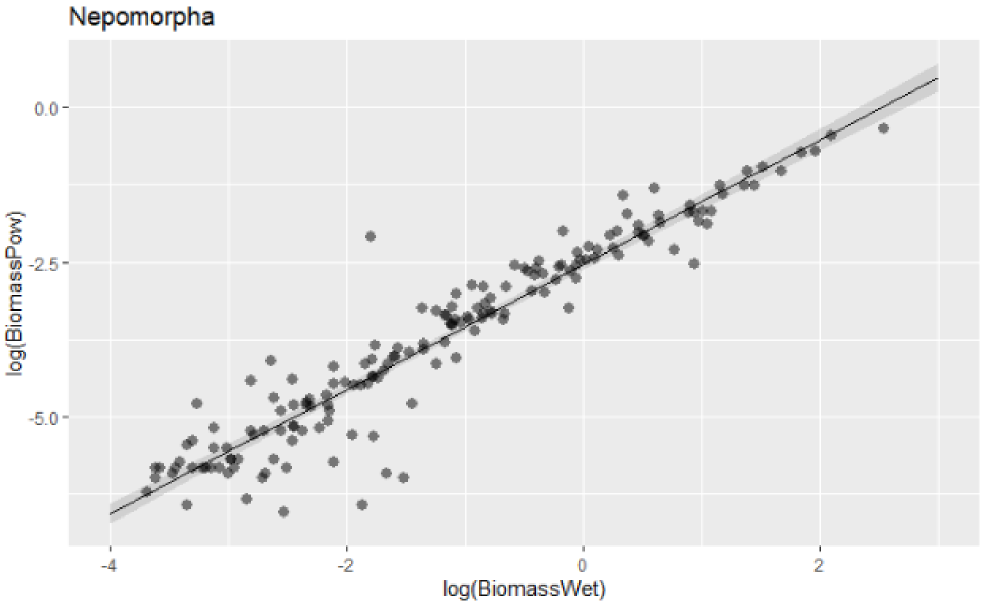


Figure 12:(beta = 1.01, 95% CI [0.96, 1.06], p < .001; Std. beta = 0.95, 95% CI [0.90, 1.00])