Using the AudioMoth - a novel passive acoustic monitoring technology - to monitor bat diversity in a rewilded landscape

Master thesis • Koen Pronk • October 2022



Using the AudioMoth - a novel passive acoustic monitoring technology - to monitor bat diversity in a rewilded landscape

Koen Pronk

Supervisor: Assistant supervisor:	Sheila Holmes, SLU, Joris Cromsigt, Swedish University of Agricultural Sciences
Examiner:	
Credits:	30
Level: Course title:	A2E Master Thesis in Environmental Science, A2E – Wildlife, Fish and Environmental Studies
Course code:	EX0988
Programme/education:	Master thesis – Freestanding Course
Course coordinating dept:	Department of Wildlife, Fish and Environmental Studies
Place of publication:	Amsterdam
Year of publication: Cover picture: Part number	2022 © Inaturalist.org 2022:30
Keywords:	Passive Acoustic Monitoring, Bioindicator, Chiroptera, AudioMoth, Trophic rewilding



Abstract

Ecosystem restoration through the reintroduction of grazers is gaining interest across Europe. Large herbivores are associated with landscape heterogeneity, restoration of trophic cascades and biodiversity. Projects implementing this form of trophic rewilding benefit from adequate monitoring techniques to assess the progress of ecosystem restoration. Bats have been put forward as suitable bioindicators, providing generalizable insight into responses of a multitude of taxonomic orders further down the food chain. At the same time, novel acoustic methods such as the AudioMoth allow for upscaling studies concerning the behavior of sound producing animals, which may be used to monitor progress of rewilding projects. In this study, I explored the relative detection probabilities of the AudioMoth in cluttered, edge and open habitat types, where clutter refers to areas with more trees and understory vegetation. Clutter reduced detection probability with the AudioMoth with sounds played over a speaker at 10kHz and at three ascending volumes. The AudioMoths were also used to assess the impact of three habitat types (forest, forest edge and open grassland) on the presence of functionally different bat genera, by placing the devices in each habitat to record bats for 3-4 days over the course of three months. The forest-adapted genus Plecotus was not more likely to be detected in any of the habitats. I did find evidence that the edge-adapted genus Myotis was more likely to be detected in open compared to forest habitat, and open-adapted genera Nyctalus and Seronitus were more likely to be detected in forest edges and open grasslands compared to forest habitats. Lastly, I compared bat genus richness between a rewilded area and its non-rewilded nature surroundings in Dutch national landscape 'Het Groene Woud'. There was no effect of rewilding on genus richness. Further efforts to monitor rewilding projects should consider the methods used in this study, as AudioMoth provides a low-cost and energy-efficient option, and bats an indication of wider taxonomic patterns. My data and findings may be used as a baseline study for bat and rewilding research in the local context of Het Groene Woud.

Table of Contents

LIST OF TABLES	6
LIST OF FIGURES	7
1. INTRODUCTION	9
1.1 IMPLICATIONS FOR SUSTAINABLE DEVELOPMENT	10
2. THEORETICAL BACKGROUND	12
 2.1 PARADIGM SHIFT IN CONSERVATION 2.2 SHIFTING MOSAICS 2.3 BAT DIVERSITY IN HETEROGENEOUS LANDSCAPES 2.4 NOVEL PASSIVE ACOUSTIC MONITORING TECHNOLOGY: AUDIOMOTH 2.5 HYPOTHESES 	12 13 14 16 17
3. RESEARCH DESIGN	18
 3.1 RESEARCH AREA 3.2 SITE SELECTION 3.3 DATA COLLECTION 3.3.1 AudioMoth Configuration 	18 20 22 22
3.3.2 Active test	22
3.4 DATA PROCESSING 3.4.1 Automated and manual identification	23 23
3.4.2 Minimalization of errors in manual ID	24
3.5 STATISTICAL ANALYSES	24
4. RESULTS	26
4.1 GENERAL FINDINGS 4.2 ACTIVE TEST 4.3 IMPACT OF HABITAT AND REWILDING ON FUNCTIONAL GROUPS 4.4 OUTCOME GLMM GENUS RICHNESS	26 27 29 33
5. DISCUSSION	35
 5.1 AUDIOMOTH – DETECTION PROBABILITY 5.2 UBIQUITOUS PRESENCE OF <i>P. PIPISTRELLUS</i> 5.3 INDISTINCT HABITAT SELECTION 5.3.1 Forest functional group 	35 36 36 36
5.3.2 Edge functional group	36
5.3.3 Open functional group	37
5.4 PREFERENCE FOR HABITAT HETEROGENEITY	37

 5.5 GENUS RICHNESS: HABITAT TYPE 5.6 GENUS RICHNESS: REWILDED VS. NON-REWILDED 5.7 BIODIVERSITY INDICATOR: BASELINE STUDY 5.8 LIMITATIONS AND PRACTICAL IMPLICATIONS FOR FUTURE RESEARCH 	38 39 40 40
6. CONCLUSION	.42
REFERENCES	.43
POPULAR SCIENCE SUMMARY	. 52
ACKNOWLEDGEMENTS	. 52
APPENDIX I: CODING	. 54
APPENDIX II: TEMPLATE CALLS	. 57
SUPPLEMENTARY MATERIALS	. 67

List of tables

 Table 1. Bat species of HGW.

Table 2. Findings per treatment combination after manual review inside the deer enclosure. NoID is the number of times I was not able to ID a sound file, Total bats is the total number of identified bats, UBIRD is the number of times I ascribed a sound that was automatically identified as a bat species to a different sound, such as a bird call.

Table 3. Findings per treatment combination after manual review outside the deer enclosure. NoID is the number of times I was not able to ID a sound file, Total bats is the total number of identified bats, UBIRD is the number of times I ascribed a sound that was automatically identified as a bat species to a different sound, such as a bird call.

Table 4. Results of six Kuskal-Wallis tests with habitat type and rewilded as the independent variables respectively and detection distance as the dependent variable with sounds played at soft, intermediate and loud volume.

Table 5. Post-hoc test of Kruskal-Wallis test showing the pairwise comparisons between habitat types and soft, intermediate and loud volume.

Table 6. Results of GLMM investigating the effect of habitat type and rewilding on detection of Forest-adapted genus (*Plecotus*).

Table 7. Results of Tukey test (pairwise comparison) for the GLMM investigating the effect of habitat type and rewilding on detection of Forest-adapted genus (*Plecotus*).

Table 8. Results of GLMM investigating the interaction effect of habitat type and rewilding on detection of Edge-adapted genus (*Myotis*).

Table 9. Post-hoc tests (Tukey tests) of GLMM comparing the effects of habitat types inside and outside (1 and 0 respectively) the rewilded area on detection of functional group 'Edge'.

Table 10. Results of GLMM investigating the effect of interaction between habitat type and rewilding on detection of Open-adapted genera (*Nyctalus* and *Plecotus*).

Table 11. Results of Tukey test (pairwise comparison) for the GLMM investigating the effect of habitat type and rewilding on detection of Open-adapted genera (*Nyctalus* and *Plecotus*).

Table 12. Results of the final GLMM investigating the effect of habitat type and rewilding on genus richness.

Table 13. Post-hoc test (Tukey test) of GLMM, showing the comparisons between the effects of habitat types and inside and outside the deer enclosure on genus richness.

List of figures

Figure 1. Depiction of a typical spatial mosaic landscape and the cyclic succession of vegetation types in temperate woodlands with large grazers. Grazing pressure maintains a grassy state through trampling and consumption of saplings and seedlings. In locally enriched or disturbed patches, non-palatable forbs and shrubs may form (A/B), which in turn may facilitate tree growth through associational resistance (C). The shading of a mature tree and grazers' preference of vicinity to such a tree limits vegetation growth underneath its canopy (D) until vegetation is largely absent (E). When the tree dies the patch eventually returns to a grassy state (F). See text for more detailed description. Adopted from Olff *et al.* (1999). \mathbb{C} *Icons in figure: Jino, Gregory Montigny, Andre Buand, Laymik, Sewon Park, Andi, Assaf Katz, Softscape and Travis Bird from the Noun Project.*

Figure 2. Blakey *et al.* (2019) showed that bat adaptations to forest structure are determinants of presence of bat species. Typical adaptations (scale from small bodies and high frequency calls in cluttered environments to large bodies and low frequency calls in open environments) are depicted. *Figure adopted from Blakey et al.* (2019). © Icons in figure: Jino, Gregory Montigny, Andre Buand, Laymik, Sewon Park, Andi, Assaf Katz, Softscape and Travis Bird from the Noun Project.

Figure 3. If a bat emits sound within the detection radius surrounding an AudioMoth, the call will be recorded (**A**). However, the detection radius may be affected by factors such as call volume, distance to the device and the presence of clutter near the device (**B**). *Figure adopted from Browning et al. (2017).*

Figure 4. Map of research area: rewilded area in the center, surrounded by non-rewilded area. Colors depict grasslands (open habitat) or forests (cluttered habitat) inside and outside the deer enclosure. The transition zones (edge habitat) in-between forests and grasslands are located on the borders between forests and grasslands. The black dots represent the sites where the AudioMoths were deployed in blocks (grey blocks in figure). *Data retrieved from Van der Velde (2021)*.

Figure 5. Examples of sites and AudioMoth placement. Pictures on the left-hand side show how I placed the AudioMoths in each environment, pictures on the right-hand side show the direction in which the device was aimed. From first to last: 1. Forest within deer enclosure, 2. Edge within deer enclosure, 3. Open within deer enclosure, 4. Forest outside deer enclosure, 5. Edge outside deer enclosure, 6. Open outside deer enclosure.

Figure 6. Schematic depiction of data collection scheme. The colored circles represent AudioMoths placed in the three habitats (square boxes). The first nine are shown as an example, I continued this scheme until data was collected at all 90 sites (18 sampling periods in total).

Figure 7. To minimize the chance of two recorders simultaneously recording the same sound, the devices were deployed at a minimum distance of 100 meters from each other.

Figure 8. Mean distances AudioMoths were able to record 10kHz sound played at different volumes. On the y-axis, the distance to the AudioMoth in meters is shown. On the x-axis, the three volumes are depicted (played at 10kHz), showing the comparison between habitat types.

Figure 9. Index of relative distance (mean of soft, intermediate and loud sounds) AudioMoths were able to record 10kHz sound played from different directions. The sample size was too small for standard error calculation (see section 3.3.2), therefore error bars were omitted. On the y-axis, the distance to the AudioMoth in meters is shown. On the x-axis, the direction of sounds played over the speaker to the AudioMoth are depicted, showing the comparison between the six different treatment combinations.

Figure 10. Figure showing the percentage of total samples of each treatment combination in which *P. pipistrellus* was detected.

Figure 11. Depiction of output of GLMM investigating the effect of habitat type and rewilding on detection of Forest-adapted genus (*Plecotus*).

Figure 12. Depiction of output of GLMM investigating the effect of habitat type and rewilding on detection of Open-adapted genera (*Nyctalus* and *Plecotus*).

Figure 13. Depiction of output of GLMM investigating genus richness in three different habitats and within and outside the rewilded area.

Figure 14. Mean of total genera found in 90 samples across all treatment combinations (15 samples per treatment combination) within and outside the rewilded area.

1. Introduction

Many natural habitats around the world have decreased in size over the course of the past century, mostly through fragmentation and agricultural land conversion (Jongman, 2002; Newbold *et al.*, 2015). As a result, global studies on the state of biodiversity show that for many species, population sizes are declining and risk of extinction is increasing (Clavel *et al.*, 2011; Tittensor *et al.*, 2014; Lanz *et al.*, 2018). In particular, large-bodied animals can be affected by land-use change (Dirzo *et al.*, 2014). These apex consumers are particularly important for ecosystem functioning as many of them are ecosystem engineers; through their consumption they influence vegetation structure and ecosystem dynamics (Svenning *et al.*, 2016). The impact of large-bodied carnivores and herbivores is thereby propagated downward through food webs. These dynamic, top-down trophic interactions are known as trophic cascades (Paine, 1980). Decreasing population sizes of large-bodied animals, or ultimately their extinction, might therefore alter plant and animal species composition. This process is known as trophic downgrading (Estes *et al.*, 2011).

An important example of the impact of large herbivores on their environment is grazing. In temperate terrestrial ecosystems where large grazers are absent, there is often quick succession from grassland to closed canopy forest (Olff *et al.*, 1999). In contrast, the reintroduction of large grazers promotes the co-existence of successional stages in the landscape – for example, their grazing limits the growth of later successional species in heavily grazed areas, while trampling and dung deposition may promote growth of later successional species elsewhere (detailed explanation in section 2.2). In other words, large grazers function as ecosystem engineers and may create or maintain a spatial mosaic containing grassland, shrub thickets and forest (Olff *et al.*, 1999). Over the past two decades, reintroducing large grazers has become a central part of the restoration of landscape dynamics and ecosystems across Europe and other parts of the world (Jepson *et al.*, 2016; Svenning *et al.*, 2016; Svenning *et al.*, 2019). This reintroduction of the functional role of large grazers is an important example of so-called trophic rewilding, which focuses on ecosystem restoration by reintroducing large animals and their trophic impacts (Svenning *et al.*, 2016).

The increasing interest in trophic rewilding projects can be seen as a response to the ongoing anthropogenic impact on natural ecosystems (Soulé & Noss, 1998). The aim of these projects is to restore trophic cascades and tackle trophic downgrading through, for example, the impact of grazers on their ecosystems (Svenning *et al.*, 2016; Cromsigt *et al.*, 2018; Jepson *et al.*, 2018). In this way, trophic rewilding projects also aim to restore biodiversity. For example, the diversity of vegetation types associated with the presence of large grazers is linked to increased animal diversity (Olff *et al.*, 1999). In order to assess the success of rewilding projects, it is fundamental to monitor the state of the ecosystem and the effect rewilding initiatives have on biodiversity (Stem *et al.*, 2005; Mata *et al.*, 2021). However, the technical and funding resources required to monitor progress in rewilding projects are often limiting (Mata *et al.*, 2021). One cost-effective option to assess biodiversity within rewilded areas is to use certain taxa as biodiversity indicators (Torres *et al.*, 2018).

A taxonomic order that shows strong responses to the effect of restoration efforts is the order *Chiroptera* (bats). Bats represent a species-rich order with great variation in their habitat, roost and prey preferences, and their diversity is therefore suggested to increase with landscape heterogeneity (Blakey, 2019). The structure of vegetation in an ecosystem has shown to be a determinant of insectivorous bat assemblages (Blakey *et al.*, 2017), where genera differ in terms of their preference for hunting in open habitats, cluttered habitats (with a complex

vegetation structure, such as forests), or in transition zones from grassland to forest (Blakey *et al.*, 2019). As discussed above, trophic rewilding with large grazers enhances vegetation heterogeneity and creates mosaics of open landscapes and cluttered habitat (Olff *et al.*, 1999; McMillan *et al.*, 2019). Therefore, a rewilded landscape through the reintroduction of large grazers may facilitate bat species richness.

Insectivorous bats are particularly informative for rewilding research due to their sensitivity to anthropogenic impacts on ecosystems (Jones *et al.*, 2009). They occupy high trophic levels, provide several ecosystem services, and show taxonomic stability. They are also present in many ecosystems across the globe, allowing for comparative studies across spatial scales (Jones *et al.*, 2009). For these reasons, bats have great potential to serve as indicators for the impact of rewilding projects on biodiversity. Assessment of bat diversity is complicated by their nocturnal lifestyle and avoidance of humans (Russo & Voigt, 2016). However, developments in passive acoustic monitoring (PAM) technology mean that studying bat species is becoming increasingly affordable and efficient (Browning *et al.*, 2017; Piel *et al.*, 2021). PAM devices are small and can be deployed in the research area for longer periods of time, meaning the method is non-invasive and enables researchers to monitor animals during the night (Medeiros *et al.*, 2017; Hill *et al.*, 2019; Piel *et al.*, 2021).

I used the AudioMoth (Hill *et al.*, 2019), a recently developed PAM device, to test if and how rewilding with large grazers affects bat genus richness in 'Het Groene Woud' (HWG), a nature area in the Dutch province of Noord-Brabant. This area was selected because in 2017, red deer (*Cervus elaphus*) were reintroduced into HGW, in addition to the already (seasonally) present cattle, with the intent to create a natural mosaic landscape with grassland, forest and edge habitats (ARK Natuurontwikkeling, n.d.). I deployed the AudioMoth in these three habitat types within and outside the deer enclosure, in order to contrast the presence of functional bat genera in forest, transition zones and grasslands in a rewilded and a non-rewilded area. As I used a newly developed technology, I tested the effectiveness of the AudioMoth prior to and during the fieldwork in the three habitats by conducting active tests. I played sounds at different volumes over a speaker in order to establish the maximum distance to which the device is able to record bat calls. The research thereby contributes to improved practical assessment of the AudioMoth by establishing the probability of detection of bats in various habitats. This research setup leads to the following research questions:

RQ1: How do forest, edge and grassland habitats affect probability of detection with AudioMoth?

RQ2: How does the presence of functionally different bat genera (inferred by detection) vary across forest, edge and grassland habitats in- and outside a rewilded area?

RQ3: How does bat genus richness vary across forest, edge and grassland habitats in- and outside a rewilded area?

1.1 Implications for sustainable development

I aimed to contribute to the broader field of sustainable development by further testing of biodiversity monitoring techniques. Passive acoustic methods are widespread monitoring techniques and adequate testing of novel technology leads to more time-, energy- and cost-efficient research design. This is directly linked to biodiversity research within the context of the Sustainable Development Goals of the United Nations (Browning *et al.*, 2017; Stowell &

Sueur, 2020). In addition, I contributed to monitoring a rewilding project, which are associated with various ecosystem services related to biodiversity (Cerqueira *et al.*, 2015). Natural regeneration may allow soil recovery, increased carbon sequestration, increased nutrient availability, regulation of hydrological cycles and better infiltration of water into groundwater reserves (Cerqueira *et al.*, 2015; Jepson *et al.*, 2016). Better understanding of the potential of ecosystem restoration through trophic rewilding is essential to restore and maintain these vital ecosystem services.

2. Theoretical background

2.1 Paradigm shift in conservation

The rewilding concept has gained much attention globally over the past decades. The classical example of trophic rewilding is the reintroduction of wolves into Yellowstone National Park, USA. After the extirpation of wolves in the region, their absence had significant impact on the ecosystem (Lorimer et al., 2015). For example, riverine cottonwoods and aspen woodlands were declining after the disappearance of wolves due to increased grazing pressure from elk (Ripple & Beschta, 2012). Coyote populations increased, resulting in increased predation on small herbivores such as rodents (Ripple & Beschta, 2012). Attempting to restore the natural habitat, 66 wolves were reintroduced into the national park in 1995 and 1996 (Ripple & Beschta, 2012). After reintroduction, elk populations declined, likely due to reestablished hunting pressure by wolves (Fortin et al., 2005). Decreased grazing pressure subsequently contributed to improved woody recruitment, and thereby the restructuring of the natural vegetation in Yellowstone (Ripple & Beschta, 2012). For example, riverside willows grew taller and in higher quantities, restoring aquatic ecosystems. Bison populations increased, likely due to less interspecific competition as elk became less abundant. Similarly, results suggest that red foxes and badgers benefitted from wolf predation on coyote populations (Ripple & Beschta, 2012).

Parallel to the Yellowstone reintroduction project, rewilding took a prominent position in the Dutch nature management debate and in government policies in the 1990s (LNV, 1990). The conviction that lost natural processes can be restored, often in accordance with historical analogies, forms the basis for this new paradigm (Vera, 2009). In the Netherlands, the most well-known example of large-scale rewilding is the Oostvaardersplassen: a 6000ha nature reserve established in 1968 when a polder in the province of Flevoland was reclaimed from Lake IJssel (Vera, 2009). This project was inspired by a shift in ecological thinking from the assumption that forests are the natural ecosystem archetype in Europe (i.e. without human influence, the entire mainland would be covered by trees), to the idea that large herbivores have shaped the landscape throughout history, resulting in mosaic landscapes of grassland, shrubland and forest (Remmert, 1991; Olff *et al.*, 1999; section 2.2). Influenced by these new insights, contemporary livestock, Heck cattle, Konik ponies and Red deer were introduced in the Oostvaardersplassen as substitutes for their extinct ancestors (i.e. Aurochs and Tarpan), with the aim to create a resilient and diverse ecosystem through natural grazing (Vera, 2009).

This paradigm shift has impacted nature management throughout the Netherlands and abroad (Svenning *et al.*, 2016). Grazing has obtained a central role in nature management in various countries around the world (e.g. Svenning *et al.*, 2016; Jepson *et al.*, 2018). In order to restore steppe vegetation in Siberia, bison and other herbivores were reintroduced to mimic the function of mammoths in shaping the landscape. This rewilding project is known as the 'Pleistocene Park' (Svenning *et al.*, 2016). After countries like Russia, the USA and the Netherlands, the call for rewilding is now growing in Eastern and Southern Europe (Jepson *et al.*, 2016), South America (Galetti *et al.*, 2017; Root-Bernstein *et al.*, 2017) and Southeast Asia (Gray *et al.*, 2019).

2.2 Shifting mosaics

An important element for the role of large grazers in rewilding is the theory of shifting mosaics. Intending to better understand vegetation dynamics and guide reintroduction projects with large herbivores, Olff et al. (1999) outlined the mechanisms by which grazers create and maintain diverse vegetation structures in temperate woodlands. They showed that tree growth is limited in grasslands as tree saplings are outcompeted by the fast-growing grasses. In addition, as attractive grasses are grazed, saplings of trees or seedlings of thorny shrubs growing in the grass-dominated patch are consumed as well. This process is known as associational palatability. However, when a grassland patch is disturbed - for example by trampling - or enriched - for example by fertilization with dung – the dominant grasses can be locally outcompeted by herbaceous species adapted to be herbivore resistant (e.g. through thorns, spines, thistles or toxicity). The large herbivores may subsequently avoid these areas, which gives thorny shrub saplings the opportunity to grow. Grazers may convert these patches back into grasslands before the shrubs are established (Olff et al., 1999). However, in some cases, particularly when grazing pressure is low enough that not every location within an area is grazed every year, shrubs do establish and spread out over a larger area, giving opportunities for woody recruitment to occur and, eventually, for trees or patches of forest to form. This process is called associational resistance (Olff et al., 1999; figure 1.).



Figure 1. Depiction of a typical spatial mosaic landscape and the cyclic succession of vegetation types in temperate woodlands with large grazers. Grazing pressure maintains a grassy state through trampling and consumption of saplings and seedlings. In locally enriched or disturbed patches, non-palatable forbs and shrubs may form (A/B), which in turn may facilitate tree growth through associational resistance (C). The shading of a mature tree and grazers' preference of vicinity to such a tree limits vegetation growth underneath its canopy (D) until vegetation is largely absent (E). When the tree dies the patch eventually returns to a grassy state (F). See text for more detailed description. Adopted from Olff *et al.* (1999). © *Icons in figure: Jino, Gregory Montigny, Andre Buand, Laymik, Sewon Park, Andi, Assaf Katz, Softscape and Travis Bird from the Noun Project.*

The mechanisms of associational palatability and associational resistance have been observed to function in a shifting or cyclic manner (Olff et al., 1999). Through local disturbance or enrichment, non-palatable plant species establish in patches within a grassland. These nonpalatable forbs may facilitate the growth of non-palatable shrubs, which in turn provide refuges for trees to grow (associational resistance). Once a tree is grown, the shading of that tree limits growth of the unpalatable species underneath it. In addition, grazers trample seedlings and saplings as they prefer proximity to the tree for shelter and scouring. These mechanisms imply that a tree or a group of trees that is established within a grassland in the shifting system remain without a lot of undergrowth (Vera, 1997). When the tree dies, the protection for woody recruitment is no longer present and there is more sunlight available to facilitate growth of grasses. The patch may subsequently return to grassland. Alternatively, if the branches of the dead tree provide enough shelter against large herbivores for a long enough period of time, a new tree may grow (figure 1.). The dynamic nature of this successional cycle and its dependence on multiple factors (e.g. soil heterogeneity, random tree mortality or clonal shrub growth) lead to a shifting mosaic landscape with varying vegetation structural types consisting of forest, grassland and edge habitats (Olff et al., 1999; Schulze, 2018).

2.3 Bat diversity in heterogeneous landscapes

Another fundamental concept in ecology is the relationship between landscape heterogeneity – for example as created and maintained by grazers in shifting mosaics - and biodiversity (Reynolds *et al.*, 2018). There is evidence that a landscape with diverse vegetation structures increases plant and animal diversity (Benton *et al.*, 2003; Tews *et al.*, 2004; Navarro *et al.*, 2015), although this effect may differ across spatial scales and taxonomic orders (Katayama *et al.*, 2014). Bats, the most diverse order of mammals, and the most numerous next to rodents (Neuweiler, 1990), exhibit a high variety of adaptations to vegetation, in which prey are concealed from predators. Other bat species prefer open landscapes or edge environments, where prey detection is less complex and less maneuverability is required (Blakey *et al.*, 2017). These different habitat preferences are reflected by differences in morphology and calls among bat species. For example, species with smaller bodies and shorter, wider wings (low aspect ratio), are well-adapted to cluttered habitats as they can maneuver in small spaces (Broders et al., 2004).

The suitability of insectivorous bat diversity as a biodiversity indicator is outlined by Jones *et al.* (2009). Biodiversity indicators function as an index of, for example, presence/absence or abundance for other species, and thereby function as surrogates for taxa within ecosystems when monitoring all taxa is out of scope (Moreno *et al.*, 2007). Bioindicators should therefore be organisms that provide generalizable insight into the responses of a multitude of taxonomic orders. They should, in addition, reflect biodiversity components like species diversity and species richness (Jones *et al.*, 2009; Russo *et al.*, 2021). Species that represent higher trophic levels are especially interesting in that respect, because their abundance and diversity is an outcome of the degree of food availability lower down in the food chain. The presence and diversity of functional bat species may therefore be indicative of species richness of the insect prey population and plants and pollinators (Jones *et al.*, 2009). As reintroduction projects are associated with increased vegetation heterogeneity (McMilan *et al.*, 2019), bat diversity may respond to such projects through this trophic interaction. Furthermore, bats are informative because they are sensitive to habitat conversion. They respond to changes in water quality, noise, light, agricultural activities and landscape connectivity (Jones *et al.*, 2013).

The echolocation calls used by open-, edge- and forest-adapted bat species show traitenvironment relationships (figure 2). These auditory adaptations can be explained through the acoustic constraints of the foraging habitat. Echolocation in open habitats requires sound to travel long distances. The attenuation of sound in the atmosphere due to energy absorption exponentially increases with higher frequencies. Open-space foragers therefore use relatively low frequency calls of long duration (≥ 10 ms) in order to distinguish prey in an otherwise empty auditory space (Neuweiler, 1990). Bats foraging close to bushes or inside forests are confronted with a completely different situation. They receive echoes from a multitude of objects and, potentially, prey. They have to distinguish the echoes that resemble prey from a set of time-smeared echoes as objects might be at different distances. A high frequency call with a large bandwidth allows clutter-adapted bats a fine spatial grain and better directionality (Neuweiler, 1990). These calls are of shorter duration. However, airborne low frequency (<30 kHz) calls of open-adapted bat species carry up to 50m, whereas high frequency (>100kHz) calls mean that for clutter-adapted species the world is soundless after a few meters (Neuweiler, 1990).



Decreasing call bandwidth and frequency, increasing call duration and body size

Decreasing vegetation density: transition from cluttered to open habitat

Figure 2. Blakey *et al.* (2019) showed that bat adaptations to forest structure are determinants of presence of bat species. Typical adaptations (scale from small bodies and high frequency calls in cluttered environments to large bodies and low frequency calls in open environments) are depicted. *Figure adopted from Blakey et al.* (2019). © *lcons in figure: Jino, Gregory Montigny, Andre Buand, Laymik, Sewon Park, Andi, Assaf Katz, Softscape and Travis Bird from the Noun Project.*

2.4 Novel passive acoustic monitoring technology: AudioMoth

For many animal species from ocean to tropical rainforest, sound recordings are an effective way to decipher numbers, communication and behavior (Stowell & Sueur, 2020). Eco-acoustic research using sound recorders has been conducted for decades, and has increased in popularity over recent years (Browning et al., 2017). However, eco-acoustic devices are often costly and energy intensive, especially the full-spectrum detectors that are needed for bat monitoring (Browning et al., 2017). Developed by Andrew Hill and Peter Prince (2018, 2019), the AudioMoth is designed to be cost- and energy-efficient. Battery life is preserved, for example, with an on-board algorithm that enables the device to only record sounds in a preset frequency band (Browning et al., 2017). Keeping costs low was another important criterium, with the main aim to make eco-acoustic research more available and widespread (Hill et al., 2018; 2019). The AudioMoth is a full-spectrum acoustic technology with a high enough sample rate to record bat calls exceeding 100 kHz (Browning et al., 2017). In particular, recording ultrasonic sounds is usually a high-cost method, but the AudioMoth provides this ability without the associated high costs (Browning et al., 2017). An additional benefit of the low cost of the AudioMoth is a reduced risk and consequences of theft of deployed AudioMoths, further increasing accessibility of PAM projects for large-scale biodiversity monitoring, for example through citizen science (Browning et al., 2017).

Although its potential is clear, in-the-field testing of the AudioMoth is still necessary in a variety of ecological contexts (Browning *et al.*, 2017). For this study, I assessed the probability of detection at various different distances from the deployed device. Detection distances may be impacted by multiple factors, such as humidity, anthropogenic sounds in the surroundings of the device, or vegetation (Browning *et al.*, 2017; figure 3). Because I deployed AudioMoths in three habitats with different vegetation, I performed an active test at the deployment sites in order to establish the probability of detection are hence informative of AudioMoth's potential in cluttered, edge and open habitats. More importantly, it is essential to correct for potential biases that are caused by an effect of habitat on detection of the AudioMoth.



Figure 3. If a bat emits sound within the detection radius surrounding an AudioMoth, the call will be recorded (**A**). However, the detection radius may be affected by factors such as call volume, distance to the device and the presence of clutter near the device (**B**). *Figure adopted from Browning et al. (2017).*

2.5 Hypotheses

I expected the probability of detection, based on the distance of detection, to be higher in open habitats. In addition, I expected to record clutter-, edge- and open-adapted bats in the habitats corresponding to their acoustic and morphological adaptations. My hypotheses are described in more detail below:

- 1. Probability of detection: I expected the probability of detection of bats with AudioMoth to be higher in open habitats compared to cluttered habitats. I expected clutter, or dense vegetation in patches of forest, to negatively affect the distance at which the AudioMoth can record species in its surroundings. Edge habitats, or transition zones from forest to grassland, are expected to have a partly compromised detection due to the presence of vegetation and vicinity to forest patches, but are expected to have a higher probability of detection compared to cluttered habitats.
- 2a. Cluttered habitat (forests): in patches of forests (figure 4), I expected to detect more bat genera with morphological and acoustic adaptations to forest habitats (i.e. to detect members of the forest functional group in table 1). Genera with traits such as a relatively small body, shorter, wider wings (low aspect ratio) and high frequency calls of short duration and with a large bandwidth, were expected to be present in cluttered habitats.
- 2b. Edge habitat (forest edges): in transition zones from forest to grassland, I expected to record both open- and clutter-adapted bat species. In addition, I expected it to be more likely to detect members of the edge functional group. Species with intermediate morphological and acoustic adaptations compared to theretofore described habitats in edge habitats.
- *2c. Open habitat (grasslands):* in grasslands, I expected that higher detection of the grassland functional group. Species with traits such as a relatively large body, longer, narrower wings (high aspect ratio) and low frequency calls of long duration and with a small bandwidth, were expected to be present in open habitats.
- 3. Genus richness: Lastly, I expected there to be higher genus richness within the deer enclosure. The presence of large grazers is expected to facilitate a higher diversity in vegetation and vegetation structure, which in turn results in more food availability for insects, increasing their abundance and diversity, which results in increased prey for more functional groups of bats. As edge habitats provide a suitable environment for edge-, but also for clutter- and open-adapted species, I expected the highest genus richness to be detected in this habitat.

3. Research design

3.1 Research area

Het Groene Woud (HGW) is a National Landscape of roughly 35000 hectares in the triangle between the cities of Tilburg, Eindhoven and Den Bosch (Steur, 2014; Tutelaers, 2017). The area encompasses small towns, small-scale agricultural landscapes and 7500 hectares of nature reserves. HGW is further characterized by a mosaic landscape, consisting of a heterogeneous mix of grassland, heathland, loam forest and swamps (De Beijer *et al.*, 2012). The nature reserves are managed by a set of organizations such as Brabants Landschap, Staatsbosbeheer and Natuurmonumenten. HGW is managed with consideration for historical value, ecological value and landscape connectivity, and recreation for nature tourists from the three cities surrounding the area and from the rest of the Netherlands (Brabants Landschap, 2020).

Roe deer and Aberdeen Angus cattle (for approximately two decades, personal communication, Sjors de Kort, 2022) already roamed HGW when ungulate diversity was supplemented by reintroducing Red deer in a fenced protected area in 2017. Red deer are native to Noord-Brabant, but had been absent for over 150 years (Tielemans, 2017; Allen, 2020). As of 2022, 80 Red deer (20 stags, 23 does and 17 calves counted in April, with 20 new born calfs added to the population in May, personal communication, Sjors de Kort, 2022) are present in the enclosure. The Angus cattle are released into the enclosure yearly around May 1st, depending on the carrying capacity of the grasslands, and removed in November (personal communication, Sjors de Kort, 2022).

The protected area lies in the center of HGW and encompasses 1.790 hectares (Brabants Landschap, 2020; figure 4). There is a high variety of small-scale land types such as grasslands, loam forests and mesotrophic meadows, arranged in a mosaic composition. The patches of land types range from < 1 hectare to > 35 hectares over the whole research area. The area is characterized by a temperate climate, with average 24-hour temperatures of 3.0 to 4.5 °C in winter, 6.0-14.0 °C in spring and 16.0-18.5 °C in summer (KNMI, 2020).

There are potentially eleven species and five genera of echolocating bats in HGW. These species are either known to be present in HGW, or are likely to be present as they are present in surrounding nature areas (Twisk, 2013; Waarneming.nl, n.d.). These include the brown longeared bat (*Plecotus auritus*), Grey long-eared bat (*Plecotus austriacus*), Daubenton's bat (*Myotis daubentonii*), Natterer's bat (*Myotis nattereri*), Whiskered bat (*Myotis mystacinus*), Greater mouse-eared bat (*Myotis myotis*), Common pipistrelle (*Pipistrellus pipistrellus*), Nathusius's pipistrelle (*Pipistrellus nathusii*), Common noctule (*Nyctalus noctula*), Lesser Noctule (*Nyctalus leisleri*) and Serotine bat (*Eptesicus serotinus*). These species are members of five genera in total, of which the morphological and acoustic adaptations as described in Blakey *et al.* (2019) are primarily associated with cluttered (i.e. forest), edge and open habitats. *Plecotus spp* are associated with forest habitats, *Myotis spp* and *Pipistrellus spp* with edge habitats, and *Nyctalus spp* and *Eptesicus spp* with open habitats (more detailed description in table 1).

Genus	Species	Morphological adaptations ²		Vocal adaptations ¹		Habitat preference ⁴
					Average call	
		Mass (g)	Wing type	Frequency (kHz)	duration (ms)	
	Brown long-eared bat					Forest (Schnitzler
Plecotus	(Plecotus Auritus)	4.5-12.0	Wide	27-56	2.5	& Kalko, 2001)
	Grey long-eared bat					Forest (Schnitzler
	(Plecotus austriacus)	7.0-14.0	Wide	18-45	5.8	& Kalko, 2001)
	Daubenton's bat ³					
Myotis	(Myotis daubentonii)	8.0-17.0	Wide	32-85	3.3	Edge
	Natterer's bat					
	(Myotis nattereri)	5.0-12.0	Wide	23-115	3.8	Forest
	Whiskered bat					
	(Myotis mystacinus)	4.0-8.0	Wide	40-65	4.7	Edge
	Greater mouse-eared bat					
	(Myotis myotis)	28.0-40.0	Wide	30-70	-	Edge
	Common Pipistrelle					
Pipistrellus	(Pipistrellus pipistrellus)	3.5-8.0	Narrow	45-76	5.6	Edge
	Nathusius's Pipistrelle					
	(Pipistrellus nathusii)	6.0-15.5	Wide	36-62	6.9	Edge
	Noctule bat					
Nyctalus	(Nyctalus noctula)	25.0-35.0	Long, narrow	17-25	11.5	Open
	Lesser noctule					
	(Nyctalus leisleri)	13.0-20.0	Long	25-54	8.5	Open
	Serotine bat					
Eptesicus	(Eptesicus serotinus)	15.0-35.0	Long	25-55	8.8	Open

 Table 1. Bat species of HGW.

¹Vocal adaptations adopted from Parson & Jones (2000) and Obrist et al. (2004).

²Morphological adaptations adopted from Parson & Jones (2000) and Obrist et al. (2004).

³Based on description provided in Bogdanowicz (1994).

⁴Based on foraging guilds as described in Müller et al. (2013).



Figure 4. Map of research area: rewilded area in the center, surrounded by non-rewilded area. Colors depict grasslands (open habitat) or forests (cluttered habitat) inside and outside the deer enclosure. The transition zones (edge habitat) in-between forests and grasslands are located on the borders between forests and grasslands. The black dots represent the sites where the AudioMoths were deployed in blocks (grey blocks in figure). *Data retrieved from Van der Velde (2021).*

3.2 Site selection

I recorded bat calls with AudioMoths (Hill *et al.*, 2019). Five AudioMoths (versions 1.1.0 and 1.2.0) were deployed in HGW from March 17th, 2022 to July 14th, 2022. Due to health issues, I was not able to conduct fieldwork between May 26th and June 22nd. The devices were deployed inside and outside the deer enclosure in patches of forest, transition zones from forest to grassland, and in open grassland. The comparative design between the rewilded area (inside the deer enclosure, labeled 'Deer') and the surrounding area (outside the deer enclosure 'labeled NoDeer') meant that there were six treatment combinations (figure 5). As I had five AudioMoths available, the devices were relocated in a rotational scheme (figure 6). Over the course of the total data collection period each treatment combination was recorded 15 times.

I identified all possible blocks of open, edge and cluttered habitat sites within the research area through on-site observations and Google Earth. A limit of 1-kilometer distance from the deer enclosure was used as a criterium in the selection of sites outside the deer enclosure to limit travel time and to minimize environmental differences other than the presence/absence of deer. These areas outside the deer enclosure were all property of Brabants Landschap, and consisted of nature areas with organically managed grassland grazed by livestock. Management of the rewilded area was very similar to the management of the surrounding nature areas, with the exception that less mowing was needed inside the deer enclosure as grassland is grazed by deer and cattle (personal communication, Sjors de Kort, 2022). 22 sites were identified inside the deer enclosure and 19 outside the deer enclosure. Out of the total sites, 15 sites were randomly selected for each treatment. This site selection resulted in 15 blocks of three habitats (cluttered, edge and open) within and 15 blocks outside the deer enclosure for a total of 90 samples (15 blocks * 3 habitats * 2 treatments).

The devices were placed on tree trunks at a height of approximately 3 meters (figure 5). In open grassland the devices were placed on lone standing trees, with a minimum distance of 50 meters to other trees. In cases where this was not possible in all directions, the AudioMoth was placed facing the grassland with at least 50 meters distance to other trees in that direction. In edge and cluttered habitats, devices were deployed at a distance of at least 1.5 meters away from other trees or shrubs to decrease acoustic interference (Newson *et al.*, 2015). The devices faced the habitat type of interest and were deployed in waterproof cases (AudioMoth IPX7).

To ensure independence of recordings, the distance between each device had to be at least 100 meters in the block setup (figure 7), as echolocation calls of open-adapted species carry up to 50 meters. Hence, as bat echolocation calls had a smaller range than the AudioMoth detection range, the issue of overlap in the recordings was determined by the calls of bats rather than the recording range of the AudioMoths. Larger distances between devices would eliminate overlap completely, but in some cases the small scale of land type patches made larger distances between devices impossible.



Figure 5. Examples of sites and AudioMoth placement. Pictures on the left-hand side show how I placed the AudioMoths in each environment, pictures on the right-hand side show the direction in which the device was aimed. From first to last: 1. Forest within deer enclosure, 2. Edge within deer enclosure, 3. Open within deer enclosure, 4. Forest outside deer enclosure, 5. Edge outside deer enclosure, 6. Open outside deer enclosure.



3.3 Data collection3.3.1 AudioMoth Configuration

Prior to the fieldwork, I conducted a few tests in a woodland area near my residence. By playing sounds at various distances to the AudioMoth, and at different volumes and frequencies, in cluttered, edge and open habitat, I established the most suitable configuration for the AudioMoth. This configuration is in line with the findings of the Somersat Bat Group, who investigated the best AudioMoth configuration for recording bat species (Somersat Bat Group, n.d.).

- 1. 192 kHz sample rate
- 2. Medium gain
- 3. 55s recording 5s sleep intervals
- 4. High band filter >20 kHz

Based on bat research guidelines in the Netherlands (Netwerk Groene Bureaus, 2021), the recording schedule of the AudioMoths was set to one hour before sunset until two hours after sunset, and two hours before sunrise until one hour after sunrise. During this period, bats are most likely to be active and vocalizing. Because of a substantial distance (>100km) between my residence and the research area, and the limited availability of transportation, the recording schedule lasted for three or four days alternately.

3.3.2 Active test

In order to establish the relative detection probability across habitat types (research question 1), an active test was performed at each site. I performed an active surround test in three directions (in front, to one side and behind), where I played tones at three different sound levels (soft; ~74dB, intermediate; ~82dB and loud; ~90dB) and at different distances (10, 20, 40, 80 and 160 meters). The speaker that was available (JBL Charge 3) was not able to produce

frequencies that are most common among bats. Instead, I used the highest frequency the speaker could produce sounds on, which was 10kHz, to approach bat frequencies with the available equipment. The test was carried out during daytime, to minimize impact on active bats.

Due to time constraints during the fieldwork days, it was not feasible to conduct the full active surround test for each block. For twelve out of fifteen blocks in each treatment combination, I conducted the same test, but only in front of the device.

3.4 Data processing

3.4.1 Automated and manual identification

For each treatment combination (DeerCluttered, DeerEdge, DeerOpen, NoDeerCluttered, NoDeerEdge, NoDeerOpen) the presence of bat species was determined using the bat analysis function in Kaleidoscope Pro (version 5.4.7). Kaleidoscope provides a function called 'auto ID for bats', which matches sound recordings with the pre-programmed calls of temperate bat species. Each sound in the recordings is labelled by the program as 1) a specific bat species, 2) an unidentified bat species, 3) as noise. However, as the accuracy of the software is not 100% (65-70% for versions 3.1.0, 4.3.0 and 5.1.0 as tested by Goodwin & Gillam in 2021), all of the files labelled a certain bat species and the files labelled 'NoID' (meaning the program identified the call to belong to a bat, but it could not determine which species it was) were manually reviewed - I confirmed or discarded the identification provided by Kaleidoscope, and I reviewed the files where Kaleidoscope was unable to identify the species to check if there were false negatives. I aimed to increase the accuracy of the identification, as tests suggest Kaleidoscope should be used as a means to organize the recordings for manual review, rather than as a final identification (Goodwin & Gillam, 2021). In the bat analysis provided by Kaleidoscope, 'Bats of Europe 5.4.0' was selected under the 'Auto ID for Bats' tab, including all European bat species in the analysis. If the automated selection identified species other than the species I expected to be present beforehand (overview in table 1), the identification was treated with extra caution during the manual review.

The manual review was based on template spectrograms of the species included in the analysis. I compared bat passes, defined as 'one or more bat echolocation calls during a sound recording' (Kerbiriou *et al.*, 2019, p. 2), that were identified as a specific species by Kaleidoscope to verified template calls and guidelines provided by Marckmann & Pfeiffer (2020) and Bas *et al.* (2011). If I decided that Kaleidoscope's ID was correct, I labelled the file with the name of that species. If it was clear the call belonged to a different species, I assigned the call to that species. If the call was not a clear match with one species, for example because it could be assigned to multiple species, I labelled that call as 'NoID'. I aimed to be conservative in this selection.

Two main criteria were considered prior to matching bat passes to bat species: 1) within the 55 second recordings, there had to be a sequence of at least two echolocation pulses of one species before it was considered a bat pass and assigned to that species (following Hawksworth & Bull, 2007). 2) Within each one-minute time block, there could be maximum one bat pass per species. Miller (2001) showed that a 1-minute time block provides an appropriate temporal scale; greater time scales would not give a resolution fine enough to detect minor variation in bat passes. These criteria were adopted in order to minimize the effect of multiple bats passing at the same time, or conversely one bat passing multiple times, as it is currently not possible to

recognize individuals with acoustic methods such as the AudioMoth. The bat passes that were identified were subsequently treated as belonging to one individual (Meaney & Gerson, 2018).

3.4.2 Minimalization of errors in manual ID

A pronounced limitation of the manual identification is the fact that I am an inexperienced acoustic ecologist, with minimal prior knowledge of acoustic research and no experience with bat call identification. A few measures were considered during the identification process to minimize the effect of my lack of expertise on the results. 1) As put forward in the research question, I identified at genus level instead of species. This decision limits misidentification of species that are closely related to each other (D'acunto *et al.*, 2018). Kaleidoscope's auto ID for bats function identified species rather than genera, so my manual selection translated that identification into genus. For example, if Kaleidoscope was not able to ID a call on the species level, but it was clear that it belonged to one particular genus, the sound was assigned to the most common species of that genus. 2) In order to evenly distribute misidentification over all treatment combinations, I first analyzed five samples of each treatment combination. The idea behind this structure was that each treatment combination was equally affected by my own learning curve. 3) As explained in section 3.4.1, I relied on external information such as reference calls of bat species known to be present in HGW (appendix II) and bat identification guides.

3.5 Statistical analyses

The statistical analysis of the data was conducted in R (version 4.1.1). To test hypotheses 2a, 2b, and 2c, three generalized linear mixed models (GLMM) were constructed using the 'glmer' function from the package 'lme4' (also see appendix I for overview of the models). In the first model, detection of members of the forest functional group was used as a binomial dependent variable. Independent factors included habitat type, rewilded or not, and the interaction between these two variables. Models two and three maintained the same independent variables with detection of members of the edge and open functional groups as their respective dependent variables.

To assess the impact of the interaction between habitat type and rewilding status on genus richness, I conducted a GLMM with poisson family. In this model, the dependent variable was the total number of genera detected by each deployed AudioMoth. The independent variables were once again habitat type and rewilding status.

In order to test the differences within each block in the research setup, aiming to isolate the impact of habitat type as opposed to some other factor, all GLMMs included the blocks as a random factor. In addition, a post-hoc Tukey test was conducted after each model was run. This was necessary to distinguish between levels of the categorical variable, habitat type.

For the active test conducted in order to assess the probability of detection, I ran six Kruskal-Wallis tests to determine which factors influenced the maximum detection distance of the sounds played over a speaker in front of the AudioMoths. The first three tests aimed to assess the effect of clutter in the sound environment (habitat type), as well as the effect of volume (soft, intermediate, loud) on the maximum distance to which the AudioMoth could recognize sound. The second three tests aimed to assess the effect of whether a recorder was within (rewilded = 1) or outside deer enclosure (rewilded = 0). For the Kruskal-Wallis test involving

habitat type (a categorical variable with three groups) a pairwise Wilcoxon test was conducted to determine where the difference in detection occurred across habitat types.

4. Results

4.1 General findings

In total, 23,470 sound files were automatically identified as containing bat sounds. Out of those, 11,417 were ascribed to a particular species, while the rest were labeled 'NoID'. After the manual review, I had assigned 8434 sound files to a particular species. These files consisted of automated ID's by Kaleidoscope that I confirmed, and files that were automatically labelled 'NoID' that I assigned to a certain bat species. In total, 17.35% of the automated selection was altered. During the manual review I corrected 3405 false positives (where Kaleidoscope Pro identified a certain species, but I discarded that identification or changed the ID to another species), and 668 false negatives (where I ascribed a file automatically labelled 'NoID' to a certain species). Table 2 & 3 give an overview of the findings per treatment combination. In all habitats, *Pipistrellus pipistrellus* was the most common bat species by far, hence the genus *Pipistrellus* is most common too. During the manual review it became clear that a considerable number of sound files (1082) was automatically assigned to a certain species by Kaleidoscope, while those files in fact contained an alternative sound such as a bird call or sound of the wind. I labeled these files as unknown bird or 'UBIRD', meaning a sound other than a bat call (i.e. also including wind).

Table 2. Findings per treatment combination after manual review inside the deer enclosure. NoID is the number of times I was not able to ID a sound file, Total bats is the total number of identified bats, UBIRD is the number of times I ascribed a sound that was automatically identified as a bat species to a different sound, such as a bird call.

	Deer Forest	Deer Edge	Deer Open	Total
NoID	2704	2044	1499	6247
Pipistrellus	661	1525	1261	3447
Plecotus	33	10	23	66
Myotis	2	19	41	62
Nyctalus	6	83	142	231
Eptesicus	6	10	22	38
Total bats	708	1647	1489	3844
UBIRD	530	348	204	1082
Total	3942	4039	3192	11,173

Table 3. Findings per treatment combination after manual review outside the deer enclosure. NoID is the number of times I was not able to ID a sound file, Total bats is the total number of identified bats, UBIRD is the number of times I ascribed a sound that was automatically identified as a bat species to a different sound, such as a bird call.

	No Deer Clut	No Deer Edge	No Deer Open	Total
NoID	2766	2434	2001	7201
Pipistrellus	1138	1756	1331	4225
Plecotus	10	6	20	36
Myotis	6	23	9	38
Nyctalus	10	129	123	262
Eptesicus	5	4	20	29
Total bats	1169	1918	1503	4590
UBIRD	196	212	98	506
Total	4131	4564	3602	12297

4.2 Active test

The active test in front of the device with three different sound volumes resulted in 12-14 samples for each treatment combination (figure 8). The direction test was conducted 3-4 times for each treatment combination.

The results of the Kruskal-Wallis tests and the post-hoc Tukey tests are presented in tables 4 & 5. Generally, sounds were detected at further maximum distances in more open habitat types. The pairwise comparison shows that there was very strong evidence for a difference in detection distance between Forest and Open habitats, for sounds played at soft and intermediate volumes (p = 0.000021 and p = 0.00002, respectively). There was strong evidence for a difference in detection distance between Edge and Open habitats with soft volume (p = 0.0042), Forest and Edge habitats with intermediate volume (p = 0.0019) and Forest and Open habitats with loud volume (p = 0.0026). There was moderate evidence for a difference in detection distance between Edge and Open habitats with loud sound (p = 0.0262). Weak evidence (i.e. p < 0.1) was found for a difference in detection distance between Forest and Edge with soft sounds (p = 0.0786) and between Edge and Open with intermediate sounds (p = 0.0952). No evidence was found for a difference between Forest and Edge with loud sounds played over the speaker (p = 0.2472), or for a difference in detection distance between inside the deer enclosure (soft: p = 0.634, intermediate: p = 01865, loud: p = 0.4803).

Volume	Habitat/rewilded	chi-squared	p-value
Soft	HabitatType	21.4270	0.00002224***
Intermediate	HabitatType	23.7140	0.000007089***
Loud	HabitatType	12.6490	0.001792**
Soft	Rewilded	0.22664	0.634
Intermediate	Rewilded	1.7447	0.1865
Loud	Rewilded	0.49819	0.4803

Table 4. Results of six Kuskal-Wallis tests with habitat type and rewilded as the independent variables respectively and detection distance as the dependent variable with sounds played at soft, intermediate and loud volume.

**Strong evidence (p < 0.01)

***Very strong evidence (p < 0.001)

Table 5. Post-hoc test of Kruskal-Wallis test showing the pairwise comparisons between habitat types and soft, intermediate and loud volume.

Volume	Comparison	p-value
Soft	Forest-Edge	0.0786
Soft	Forest-Open	0.000021***
Soft	Edge-Open	0.0042**
Inter	Forest-Edge	0.0019**
Inter	Forest-Open	0.00002***
Inter	Edge-Open	0.0952
Loud	Forest-Edge	0.2472
Loud	Forest-Open	0.0026**
Loud	Edge-Open	0.0262*

*Moderate evidence (p < 0.05)

**Strong evidence (p < 0.01)

***Very strong evidence (p < 0.001)



Figure 8. Mean distances AudioMoths were able to record 10kHz sound played at different volumes. On the yaxis, the distance to the AudioMoth in meters is shown. On the x-axis, the three volumes are depicted (played at 10kHz), showing the comparison between habitat types.



Figure 9. Index of relative distance (mean of soft, intermediate and loud sounds) AudioMoths were able to record 10kHz sound played from different directions. The sample size was too small for standard error calculation (see section 3.3.2), therefore error bars were omitted. On the y-axis, the distance to the AudioMoth in meters is shown. On the x-axis, the direction of sounds played over the speaker to the AudioMoth are depicted, showing the comparison between the six different treatment combinations.

4.3 Impact of habitat and rewilding on functional groups

For the Edge functional group, the model would not converge due to ubiquitous presence of *Pipistrellus pipistrellus*. The converge error in the model was caused by the fact that there was no variation in the data for some of the treatment combinations (figure 10). This species was the most commonly detected species in the total number of bat passes and the most dominantly present species across all treatment combinations (supplementary material: figures S1 and S2). Its dominance resulted in a lack of variability in the outcome of the model. I subsequently ran the model for just the second genus in the Edge functional group: *Myotis*.

The GLMMs did not provide any evidence for an interaction effect of habitat and rewilding on the presence the Forest and Open functional groups within the treatment combinations (functional group Forest - Open*Rewilded: p = 0.263 & Edge*Rewilded: p = 0.545, functional group Open - Open*Rewilded: p = 0.5025 & Edge*Rewilded: p = 0.7356). Hence, I re-ran the models without interaction terms for the Forest and Open genera for more straight-forward interpretation and higher statistical power. For the Edge functional group, weak evidence was found for an interaction between Open habitats and rewilding (p = 0.0594), and no evidence was found for an interaction effect, I decided to still run the model with the interaction for *Myotis*, in order to assess the extent to which the interaction was visible in the post-hoc test.

The Forest-adapted genus *Plecotus* was more likely to be detected in Open (p = 0.0339) compared to Forest habitats (table 6). However, the Tukey test only showed weak evidence of this difference (p = 0.0856, table 7). The Tukey test for the model for the Edge-adapted genus *Myotis* showed weak evidence of higher detection in Open habitats within the rewilded area compared to Forest habitats within the rewilded area (p = 0.0731, table 9). Both the model (table 10) and the Tukey test (table 11) showed strong evidence that Open-adapted species were more likely to be detected in Edge (model: p = 0.00184, Tukey test: p = 0.00523) and Open (model: p = 0.00108, Tukey test: p = 0.00303) habitats compared to Forest. I did not find evidence supporting a difference in detection between rewilded and non-rewilded sites for any of the functional groups (Forest: p = 0.5436, Edge: p = 0.3623, Open: p = 0.9879).



Figure 10. Figure showing the percentage of total samples of each treatment combination in which *P. pipistrellus* was detected.

Table 6	. Results o	of GLMM	investigati	ng the effe	ct of habita	t type and	rewilding	on detection	n of Foi	rest-adap	oted
genus (Plecotus)										

	Estimate	Std. Error	z-value	p-value
(Intercept)	-1.168e+00	5.709e-01	-2.046	0.0407*
HabitatEdge	1.313e+00	6.189e-01	2.121	1.0000
HabitatOpen	-3.988e-06	6.048e-01	0.000	0.0339*
Rewilded	3.509e-01	5.777e-01	0.607	0.5436

*Moderate evidence (p < 0.05)



Figure 11. Depiction of output of GLMM investigating the effect of habitat type and rewilding on detection of Forest-adapted genus (*Plecotus*).

*Moderate evidence (p < 0.05)

Table 7. Results of Tukey test (pairwise comparison) for the GLMM investigating the effect of habitat type an
rewilding on detection of Forest-adapted genus (<i>Plecotus</i>).

0	1 0 1	/
Comparison	Estimate	p-value
Edge-Forest	-3.988e-06	1.0000
Open-Forest	1.313e+00	0.0855
Open-Edge	1.313e+00	0.0856
Rewilded-Non-rewilded	0.3509	0.544

Table 8. Results of GLMM investigating the interaction effect of habitat type and rewilding on detection of Edgeadapted genus (*Myotis*).

	Estimate	Std. Error	z-value	p-value
(Intercept)	-1.8103	0.6121	-2.957	0.0864
HabitatEdge	1.3824	0.6344	2.179	0.4139
HabitatOpen	1.5325	0.6403	2.394	0.6731
Rewilded	0.5565	0.5356	1.039	0.3623
EdgeRewilded	1.5861	1.3025	1.218	0.0594
OpenRewilded	2.5688	1.3625	1.885	0.2233

Table 9. Post-hoc tests (Tukey tests) of GLMM comparing the effects of habitat types inside and outside (1 and 0 respectively) the rewilded area on detection of functional group 'Edge'.

Habitat + treatment	Comparison	Estimate	p-value
Forest, non-rewilded	Edge, non-rewilded	-0.6886	0.9638
Open, non-rewilded	Edge, non-rewilded	-0.3295	0.9986
Edge, rewilded	Edge, non-rewilded	0.6246	0.978
Forest, rewilded	Edge, non-rewilded	-1.6501	0.6051
Open, rewilded	Edge, non-rewilded	1.2777	0.7069
Open, non-rewilded	Forest, non-rewilded	0.359	0.9983
Edge, rewilded	Forest, non-rewilded	1.3131	0.6946
Forest, rewilded	Forest, non-rewilded	-0.9615	0.9427
Open, rewilded	Forest, non-rewilded	1.9663	0.306
Edge, rewilded	Open, non-rewilded	0.9541	0.8841
Forest, rewilded	Open, non-rewilded	-1.3206	0.8007
Open, rewilded	Open, non-rewilded	1.6073	0.4961
Forest, rewilded	Edge, rewilded	-2.2747	0.2242
Open, rewilded	Edge, rewilded	0.6532	0.9678
Open, rewilded	Forest, rewilded	2.9278	0.0731

	Estimate	Std. Error	z-value	p-value
(Intercept)	-1.1299	0.6519	-1.733	0.08304
HabitatEdge	2.4707	0.7931	3.115	0.00184**
HabitatOpen	2.6953	0.8245	3.269	0.00108**
Rewilded	-0.0107	0.7069	-0.015	0.98792

Table 10. Results of GLMM investigating the effect of interaction between habitat type and rewilding on detection of Open-adapted genera (*Nyctalus* and *Plecotus*).

**Strong evidence (p < 0.01)



**Strong evidence (p < 0.01)

Table 11. Results of Tukey test (pairwise comparison) for the GLMM investigating the effect of habitat type and rewilding on detection of Open-adapted genera (*Nyctalus* and *Plecotus*).

Comparison	Estimate	p-value
Edge-Forest	2.4707	0.00525**
Open-Forest	2.6952	0.00306**
Open-Edge	0.2245	0.93992
Rewilded-Non-rewilded	-0.0107	0.988

**Strong evidence (p < 0.01)

4.4 Outcome GLMM genus richness

The GLMM for genus richness did not provide evidence for an interaction effect (Rewilded*Edge: p = 0.5236 & Rewilded*Open: p = 0.2501). Hence, the interaction effect was left out of the model for more straight-forward interpretation and higher statistical power. I also did not find evidence that there was an overall effect of rewilded compared to non-rewilded sites in terms of genus richness detected by AudioMoths (p = 0.2038). The model did show strong evidence of a difference between Edge habitat and Forest habitat (p = 0.009131), and very strong evidence of a difference between Open and Forest habitat (p = 0.000402). I found moderate evidence with the Tukey test that Edge habitats hold higher genus richness compared to Forest habitats (p = 0.02467). The post-hoc test showed that there was strong evidence that Open habitats hold higher genus richness compared to Forest habitats (p = 0.00114). I have included figure 14 to show that, although I did not find evidence of a difference between rewilded and non-rewilded, there was a substantial difference in genera detected between Open within the rewilded area compared to Open outside the rewilded area.

	Estimate	Std. Error	z-value	p-value
(Intercept)	0.4218	0.1591	2.651	0.008034 **
HabitatEdge	0.4700	0.1803	2.607	0.009131 **
HabitatOpen	0.6206	0.1754	3.539	0.000402 ***
Rewilded	0.1708	0.1344	1.271	0.203804

Table 12. Results of the final GLMM investigating the effect of habitat type and rewilding on genus richness.

genus

three

in

**Strong evidence (p < 0.01)

***Very strong evidence (p < 0.001)



***Very strong evidence (p < 0.001)

Table 13. Post-hoc test (Tukey test) of GLMM, showing the comparisons between the effects of habitat types and inside and outside the deer enclosure on genus richness.

Comparison	Estimate	p-value
Edge-Forest	0.4700	0.02467*
Open-Forest	0.6206	0.00114**
Open-Edge	0.1506	0.58358
Rewilded-Non-rewilded	0.1708	0.204

*Moderate evidence (p < 0.05)

**Strong evidence (p < 0.01)





- 5. Discussion
- 5.1 AudioMoth detection probability

The active test showed that sounds played over a speaker can be detected from greater maximum distances by the AudioMoth in an open environment compared to a densely vegetated environment. Hypothesis 1, where I expected open habitats to have the greatest probability of detection, forest to have the lowest and edge to be in between, is thereby supported. This result is in line with studies investigating the attenuation of sound in various habitats. Attenuation rates of emitted sounds are lower in grassland and edge habitats compared to forest habitats (Morton, 1975; Swearingen, 2013), and are positively associated with tree density and maturity, particularly for high frequency sounds (Fricke, 1984). Darras *et al.* (2006) similarly show that clutter affects sound signal propagation.

This implies that bat calls produced in grasslands have a higher probability of being detected by the deployed AudioMoth compared to edge or forest habitats. Hence, it can be assumed that the true number of bats calling within vicinity of the AudioMoths in forests is actually higher relative to edge and open habitats than detected in this study. Bats calling from a greater distance than the maximum detection distance would not have been recorded in forests, while they would have been recorded in open grassland (Browning *et al.*, 2017). In general, this is the case for soft, intermediate and loud volumes, although I did not find evidence for a difference between forest and Edge habitats with soft and loud volumes, and for a difference between edge and open habitats at intermediate volumes. When the auditory adaptations as described in section 2.3 (i.e. clutter-adapted bat calls carry less far compared to edge- and open-adapted bat calls) are also considered, it is not surprising that the fewest bat passes were recorded in the forest habitat. The underrepresentation of bat presence in forests should be considered when interpreting the results.

Furthermore, the analysis of the sound files resulted in a higher number of unidentified bat passes in forest compared to edge and open. This discrepancy might imply that not only is the detection distance affected by clutter, but the quality of recordings also decreases. This finding can likely be explained by the high level of interfering sounds in forest compared to edge and open (i.e. fluttering leaves, singing birds & wind blowing along tree branches). This explanation seems to be backed by the number of filed labelled 'UBIRD' (unrecognized sounds that were wrongly identified as bat calls by the automated bat identification in Kaleidoscope Pro), although I would suggest caution with drawing conclusions from these files, as the source of the sound was often unclear.

The directional component of detection probability needs further research. As the results presented here are comprised of a small sample size. the impact of direction (front, side and behind) on the probability of detection presented in figure 9 should be interpreted as a preliminary indication rather than strong evidence. However, they do indicate that detection probability substantially differs with sounds played in front of, to the side of and behind the device. The AudioMoth will capture sounds in front of the speaker at farther distances than to the side and back. Future studies using the AudioMoth should therefore consider the direction the device faces when it is deployed.

5.2 Ubiquitous presence of P. pipistrellus

The data showed ubiquitous presence of the *Pipistrellus* genus, and one species within that genus in particular: *Pipistrellus pipistrellus*. This species is, by far, the most common of all species in HGW and was present in nearly all samples that contained bat calls. In forest edges and open areas within the rewilded area, *P. pipistrellus* was present in every sample, which obstructed statistical analysis due to lack of variation leading to the model not converging. This ubiquity can potentially be explained by the variety of roosting and foraging strategies *P. pipistrellus* uses. Although the morphological and acoustic adaptations indicate this species is specialized to edge environments, their habitat is not limited to forest edges (Nicholls & Racey, 2006).

P. pipistrellus forage over large distances (e.g. Davidson-Watts & Jones (2006) showed a colony home range of 1526ha), using forest edges for foraging and navigation. The landscape within their home range, is characterized by habitat heterogeneity (Nicholls & Racey, 2006). Nicholls & Racey (2006) confirmed that *P. pipistrellus* prefers to forage on leeward sides of hedgerows, treelines or human buildings where insect prey accumulates. They hence deem it plausible that *P. pipistrellus* is not specialized to one specific habitat, but rather specialized to habitat structure. *P. pipistrellus* benefits from the presence of landscape heterogeneity; a combination of forest patches, hedgerows, treelines and clearings in a woodland environment, or even human-created landscape elements (Davidson-Watts & Jones, 2006; Rijksdienst voor Ondernemend Nederland, 2014). As *P. pipistrellus* uses a broad range of habitats, the ascription to the edge functional group based on their acoustic and morphological adaptations might be an oversimplification of the species' habitat selection as it employs a more generalist strategy, and shows dependence on a heterogeneous habitat structure.

5.3 Indistinct habitat selection *5.3.1 Forest functional group*

Although acoustic and morphological adaptations may indicate specialization to specific habitats, the results show that, similar to *P. pipistrellus*, habitat selection by the three groups of bat genera included in this study is quite indistinct. Firstly, no evidence was found that the forest functional group's (*Plecotus spp*) presence could be predicted by habitat type in HGW, rejecting hypothesis 2a. In fact, I found weak evidence that this genus is more likely to be detected in open habitat compared to forest habitat. Other research suggests that *Plecotus spp* prefer areas with habitat heterogeneity, and show substantial variation of habitat selection when comparing across species within the genus (Ashrafi *et al.*, 2013). Therefore, *Plecotus spp* may select habitats within HGW that are not clearly linked to their acoustic and morphological clutter-adaptations.

5.3.2 Edge functional group

A similar case can be observed for the edge functional group. *Myotis spp* were more likely to be detected in edge habitats than in forest habitats, partly supporting hypothesis 2b. However, I also found moderate evidence (p = 0.0437) that *Myotis spp* are more likely to be found in open habitats compared to forest. Similar to the earlier described genera *Plecotus* and *Pipistrellus*, a preference for habitat heterogeneity is a plausible explanation for this finding. Out of all four *Myotis spp*, *M. daubentonii* was detected most frequently in the research area. This species prefers sheltered foraging sites (Boonman, 2000; Fawcett & Ratcliffe, 2015), and is known in particular for foraging above open water bodies (in fact the Dutch name for *M*.

daubetonii directly translates to 'waterbat'; Twisk, 2013). Although the sheltered ponds preferred by *M. daubentonii* are often located in close proximity to forest edges (Todd & waters, 2017), this species is also known to prefer larger water bodies, such as rivers and lakes, than other bat species who may only forage over smaller ponds (Downs & Racey, 2006). This may result in greater divergence from forest edges when ranging over the water body, and relatively high detection in both edge and open habitats.

Another contextual component that may have resulted in a higher detection rate of *Myotis* within open grasslands is the small scale of some of the grassland patches included in this study (Kusch *et al.*, 2004). *M. daubentonii* prefers water without overhanging vegetation, but along forest edges (Todd & Waters, 2017). When foraging, the species benefits from a partly open, but background cluttered sound space, which places it in between species preferring uncluttered spaces and species preferring densely cluttered spaces (Fawcett & Ratcliffe, 2015). Considering these components of foraging site selection of *M. daubentonii*, a small-scale grassland may provide enough background clutter, obscuring the exposure of distinct patterns of use.

5.3.3 Open functional group

Lastly, I found strong evidence (p = 0.0030) that genera belonging to the open functional group were more likely to be present in open habitats over forest habitats, partly supporting hypothesis 2c. However, I also found strong evidence (p = 0.0052) that these genera are more likely to be detected in edge habitats over forest habitats. In contrast to clutter- and edgeadapted species, open-adapted species do use a highly specialist strategy and are unlikely to use cluttered airspace (Müller *et al.*, 2013). However, the observed indistinct detection of openadapted genera in both open and edge habitats is not remarkable, because 'open' essentially refers to a space free from acoustic interference during echolocation (Neuweiler *et al.*, 1990), and is therefore not descriptive of a specific habitat type. For example, there is little acoustic interference when open-adapted species glide over grasslands, and they are known to use grasslands as foraging habitats (Neuweiler, 1990). However, other acoustically empty spaces, such as the space above the forest canopy, are also frequently used as foraging habitats by species belonging to these genera (Müller *et al.*, 2013).

This behavior of combining grassland and above-canopy foraging might explain the high detection rate of open-adapted species in edge habitats, although more detailed research – for example by examining the stratum from which calls were emitted - is needed. It is likely that calls emitted above the canopy of forests had a lower probability of detection due to the umbrella effect of leaves (Müller *et al.*, 2013), compared to above-canopy strata close to forest edges in this research setup. In addition, the strength of signals emitted in the above-canopy stratum reduces over distance due to atmospheric attenuation and spreading loss (Lawrence & Simmons, 1982; Plank *et al.*, 2012). These mechanisms would decrease the likelihood of detecting above-canopy foraging of open genera in forest habitats more so than edge habitats, due to a continuous canopy and higher tree growth (Müller *et al.*, 2013).

5.4 Preference for habitat heterogeneity

The pattern observed here is that none of the genera included in this study, although physically specialized to a certain environment, make exclusive use of the particular habitat associated with those adaptations. Several factors may lead to a preference of bats for heterogeneous habitats. For example, previous studies showed that bat activity is higher along forest edges or

clearings within woodlands, compared to the forest interior (Kusch *et al.*, 2004; Morris *et al.*, 2010). An explanation for this is the availability of suitable foraging sites. These open areas within woodlands, which may consist of grassland patches or ponds and lakes, provide shelter against predators as well as the elements (Ekman & De Jong, 1996; Verboom & Spoelstra, 1999). At the same time, a high proportion of clearings and water bodies in a woodland area positively relates to diversity of insect prey (Kalda *et al.*, 2015). Morris *et al.* (2010) indicate that for insectivorous bat species, a heterogeneous woodland structure provides hunting opportunities in the form of windbreaks, where large densities of insects are often present. Anderson *et al.* (2020) showed that greater structural heterogeneity increases insect diversity and abundance. Which indicates a particular need for academics and nature managers to pay attention to heterogeneity in vegetation structure.

In addition, heterogeneous habitat structure may provide suitable landscape elements used for navigation when commuting from roost to foraging sites (Verboom, 1998). As bats use their sonar to navigate on these commuting routes, acoustic contact with vertical objects has to be maintained (Verboom, 1998). Forest edges provide enough echoes from the background of the tree lines for navigation (Schnitzler & Denzinger, 2003). Verboom (1998) emphasized that the distance of individual bat species to clutter determines the type of their echolocation call (high frequency, broad bandwidth calls when in or nearby clutter, low frequency, narrow bandwidth calls when in and open environment). Although these species generally emit calls that can be linked to a certain habitat, the ability of individual bat species to slightly alter these components of echolocation dependent on the habitat they are in at that moment, indicates that they are adapted to habitat heterogeneity to some extent (Kalko & Schnitzler, 1993).

Another indication that bat species may not exclusively use the habitat that suits their acoustic and morphological specialization, is their selection of roosts. Cavities in trees within the forest interior are used by various edge- and open-adapted bat species as roosting sites (for example by *M. daubentonii* and *N. noctula*, as described in Boonman, 2000). In general, older trees with low branches are preferred roosting sites for bats, and in order to promote bat diversity special attention should hence be paid to preserving these sites in proximity to or within open sites. Bats have been shown to prefer their roosting sites to be in close vicinity to their foraging sites to reduce commuting flight costs (Boonman, 2000). Hence, a higher degree of landscape heterogeneity, and the subsequent higher availability of forest edge, results in higher facilitation of suitable trees for roosting (Boonman, 2000).

5.5 Genus richness: habitat type

I found evidence that open and edge habitats facilitated higher genus richness compared to forest habitat, partly rejecting hypothesis 3. As discussed in section 5.1, lower detection probability likely decreased detection within the forest interior in this study, which may have impacted the results for genus richness when comparing the three habitat types. A larger sample size, particularly for sounds played over the speaker to the side and behind, is needed to quantify a correction and estimate the true relative detection rate compared to forest edges and grasslands.

Several other factors may explain the observed difference. The first factor may be the abundance and diversity of insect prey within the three habitat types (Rautenbach *et al.*, 1996; Kolkert *et al.*, 2020). The presence of different functional bat genera is an outcome of food availability further down the food web, as bats rely on the presence of insect prey, who in turn rely on plants and pollinators for their food availability (Kusch *et al.*, 2003; Jones *et al.*, 2009).

Habitats with high plant species richness are associated with a more divers and stable insect population, facilitating bat genus richness (Anderson *et al.*, 2020). An explanation for higher genus richness in edge habitats may therefore be the higher relative insect abundance that is associated with edge habitats (Limpens & Kapteyn, 1991).

In contrast, some studies showed that there is low bat activity even in forest patches with high abundance of insect prey (Grindal & Brigham, 1999). Brigham *et al.* (1997) suggested that this contradiction is caused by clutter density. At two sites known to be used by bats, they artificially increased clutter density, while maintaining the same density of insect prey. Through this exclusive manipulation of spatial complexity, activity of even small bat species associated with cluttered environments was negatively affected. Hence, forests may only provide suitable foraging habitats for specialized bat species, and only to a certain degree of clutter density.

5.6 Genus richness: rewilded vs. non-rewilded

I did not find evidence of a difference in genus richness between the rewilded area and the nonrewilded area. Based on correspondence with Het Brabants Landschap and my own observations during the fieldwork, it became clear that the structural difference of the landscape within and outside the deer enclosure may not currently be substantially different. The observed effects of cattle and deer grazing within the enclosure by nature managers are specifically 1) pruning of woody recruitment such as hazel, willow and bird cherry and browsing of bramble, and generally 2) higher variation in transition zones from grassland to forest, increased structure in grasslands, and more openings in dense bramble bushes (personal communication, Sjors de Kort, 2022). Attempts were made to quantify these observations by prior studies, which showed there is no significant change in woody vegetation structure in the deer enclosure in HGW, but there are indications of Red deer affecting particular tree species in different ways by their grazing (Allen, 2019; Van der Velde, 2021). However, substantial changes in habitat structure that may impact bat behavior are not yet visible in the area.

In particular, the apparent diversity in transition zones from grassland to forest within the deer enclosure may potentially provide opportunities for bats in the future. High plant species richness in transition zones within the deer enclosure is expected to provide notable opportunities for edge-adapted insects (personal communication, Sjors de Kort, 2022). The effect of this qualitative observation is not confirmed by my results, but does deserve attention in future studies.

There is also not a substantial difference in management strategies when comparing the deer enclosure with its surroundings. I restricted my fieldwork to property of Het Brabants Landschap, which consisted of nature areas and livestock farms. There were two main differences between the rewilded area and the non-rewilded area. 1) Mowing is substituted in the rewilded area by cattle and deer grazing, so mowing pressure is lower (approximately 1/3 of the grasslands; ~45/130 ha within the deer enclosure is still mowed yearly, personal communication, Sjors de Kort, 2022). The grasslands outside the deer enclosure are all mowed. And, 2) the vast majority of livestock farmers (14 out of 15 contracted farmers, personal communication, Sjors de Kort, 2022) outside the deer enclosure use dewormers to protect their cattle from internal parasites. These chemicals are released into the grassland ecosystem as dung, and are known to have an impact on dung fauna (Lahr *et al.*, 2007). Further research is needed to specifically assess the impact of dewormers on dung-dwelling insects, which may affect bat species as beetles in particular are a known food source (Ancillotto *et al.*, 2017).

It would be interesting to further investigate the presence of bats in the intensively managed farmlands that are also present in the area, but that were not included in this study. The type of agricultural management of pasture (e.g. tillage, temporary vs. permanent pastures) and cattle (e.g. rotational grazing vs. set stocked) can have a substantial impact on vegetation and soil structure, which in turn can impact invertebrate prey populations and ultimately the presence of bats (Anderson *et al.*, 2020). Put *et al.* (2018) also underscore the impact of agrochemical use on bats by showing that bat abundance and diversity as well as abundance of insect prey all positively respond to organic farming. Grasslands owned by Het Brabants Landschap that were assessed in this study were all managed organically (personal communication, Sjors de Kort, 2022).

5.7 Biodiversity indicator: baseline study

One of the main arguments to choose bats as the subject of research for this study was their potential as a bioindicator (section 2.3). Using AudioMoth, I found differences in detection of the different functional groups and genera across habitat types, that suggest that bats vary in their use of different landscape features. Hence, landscape dynamics associated with trophic rewilding will likely influence bat species richness long-term. However, care must be taken to account for differences in detection probability across habitat type.

It remains of interest to investigate bat genus or species richness as a surrogate for wider biodiversity in HGW, as Red deer were reintroduced only recently to complement cattle grazing. The impact on landscape dynamics may only start influencing bat behavior over a longer period of time, as the mosaic landscape dynamic (section 2.2) becomes more prevalent and vegetation structure and heterogeneity increases. Continued efforts to monitor vegetation growth and bat presence may provide better insight in the relation between the two and the impact of rewilding on that relation. It would also be valuable to link bat research to research on associated ecosystem elements such as insect abundance or plant species richness, in order to substantiate the utility of bats as a bioindicator.

Hence, I would argue that the data of this study can function as a baseline for further research in HGW. A yearly repetition of this study in the same area will be more informative of longterm patterns that might have been instigated by the rewilding project. Russo *et al.* (2021) point out that the observed responses of bats to specific environments should also be related to similar studies in different geographic contexts. In this way, the potential geographic bias that may have influenced the results of my research can be tested. In addition, future studies should aim to overcome the main limitations of my research.

5.8 Limitations and practical implications for future research

Although I have shown that there is a difference in detection probability within cluttered-, edge- and open-environments and with sounds played at soft, intermediate and loud volumes, additional information on the detection probability is needed to account for detection probability in future bat research with AudioMoth. A larger sample size in all directions would enable researchers to correct for differences. Also, it was beyond the scope of this study to use acoustic equipment that could replicate bat frequencies. An ultrasonic speaker enables researchers to use playbacks of actual bat sounds and determine the differences in probability of detection for each species across habitat types.

Secondly, although the research setup consisted of 30 blocks and 90 individual sites, there was still a relatively small sample size due to the high number of categories (Forest, rewilded; Edge, rewilded; Open, rewilded; Forest, non-rewilded; Edge, non-rewilded; Open, non-rewilded). A larger sample size would increase the statistical power of my analyses. I would recommend to enhance the spatial and temporal scales in future studies, where this study may be used as a baseline study for longevity.

As previously discussed, the small scale of the landscape may have impacted the results. The grasslands in HGW, particularly in the rewilded area, were sometimes smaller than one hectare, and I was limited to placing the AudioMoths on lone standing trees in open fields. As a result, although I was always able to prevent overlap between the devices within the same block, it was not always possible to ensure a large enough (100 < meters) distance between the device and other habitat types. This may have resulted in a degree of false positives when bats foraging in one habitat were detected in another. In order to overcome this limitation, I recommend using a suspension system or poles placed in dug out holes in the open areas to place the devices on.

6. Conclusion

I aimed to investigate 1) the relative probability of detection with the AudioMoth in forest, edge and open habitat, 2) the impact of those habitats and the rewilding project within a deer enclosure in HGW on the detection of functionally different bat genera, 3) whether the difference in vegetation structure within those treatment combinations is associated with bat genus richness. The results provide insights in the detection probability with the AudioMoth in different habitats and in the likelihood of detecting functionally different bat genera in the treatment combinations within the context of HGW. Hence, this study can be used to further understand and improve the ability and use of a novel passive acoustic monitoring technology, as well as provide further understanding of the behavior of bats in HGW, which could subsequently be applied to management strategies. Future studies should consider the effect of clutter on detection probability and conduct further research on the directional component of detection with AudioMoths. The forest-adapted genus Plecous was not more likely to be detected in any of the habitats. I did find evidence that the edge-adapted genus Myotis was more likely to be detected in open compared to forest habitat, and open-adapted genera Nyctalus and Seronitus were more likely to be detected in forest edges and open grasslands compared to forest habitats. Lastly, I compared bat genus richness between a rewilded area and its non-rewilded nature surroundings in Dutch national landscape 'Het Groene Woud'. There was no effect of rewilding on genus richness. Further efforts to monitor rewilding projects should consider the methods used in this study, as AudioMoth provides a low-cost and energyefficient option, and bats an indication of wider taxonomic patterns. My data and findings may be used as a baseline study for bat and rewilding research in the local context of Het Groene Woud.

References

- Ancillotto, L., Ariano, A., Nardone, V., Budinski, I., Rydell, J., & Russo, D. (2017). Effects of free-ranging cattle and landscape complexity on bat foraging: implications for bat conservation and livestock management. *Agriculture, Ecosystems & Environment, 241*, 54-61.
- Anderson, M., Norton, L., & Mathews, F. (2020). Grassland management affects vegetation structure, bats and their beetle prey. *Diversity*, *12*(10), 406.
- Allen, G. C. R. (2020). Trophic rewilding with red deer in Het Groene Woud, the Netherlands (Master's thesis).
- ARK Natuurontwikkeling, (n.d.). Edelhert: Co-evolutie van planten en dieren. Available from: <u>https://www.ark.eu/natuurontwikkeling/natuurlijke-</u>processen/begrazing/edelhert (accessed January, 2022).
- Ashrafi, S., Rutishauser, M., Ecker, K., Obrist, M. K., Arlettaz, R., & Bontadina, F. (2013). Habitat selection of three cryptic Plecotus bat species in the European Alps reveals contrasting implications for conservation. *Biodiversity and conservation*, 22(12), 2751-2766.
- Bas, Y., Cornut, J. & Colombo, R. (2011). *Détermination visuelle des Myotis sur*sonograme. Translated by Chris Corben, Aug 2016.
- Benton, T. G., Vickery, J. A., & Wilson, J. D. (2003). Farmland biodiversity: is habitat heterogeneity the key?. Trends in ecology & evolution, 18(4), 182-188.
- Blakey, R. V., Law, B. S., Kingsford, R. T., & Stoklosa, J. (2017). Terrestrial laser scanning reveals below-canopy bat trait relationships with forest structure. Remote Sensing of Environment, 198, 40-51.
- Blakey, R. V., Webb, E. B., Kesler, D. C., Siegel, R. B., Corcoran, D., & Johnson, M. (2019). Bats in a changing landscape: Linking occupancy and traits of a diverse montane bat community to fire regime. Ecology and evolution, 9(9), 5324-5337.
- Bogdanowicz, W. (1994). Myotis daubentoniid. Mammalian Species, 475: 1-9.
- Boonman, M. (2000). Roost selection by noctules (Nyctalus noctula) and Daubenton's bats (Myotis daubentonii). *Journal of Zoology*, 251(3), 385-389.

Brabants Landschap. (2020). Jaarverslag. Stichting Het Noordbrabants Landschap.

- Brigham, R. M., Grindal, S. D., Firman, M. C., & Morissette, J. L. (1997). The influence of structural clutter on activity patterns of insectivorous bats. *Canadian journal of* zoology, 75(1), 131-136.
- Broders, H. G., Findlay, C. S., & Zheng, L. (2004). Effects of clutter on echolocation call structure of Myotis septentrionalis and M. lucifugus. Journal of Mammalogy, 85(2), 273-281.

- Browning, E., Gibb, R., Glover-Kapfer, P., & Jones, K. E. (2017). Passive acoustic monitoring in ecology and conservation.
- Cerqueira, Y., Navarro, L. M., Maes, J., Marta-Pedroso, C., Pradinho Honrado, J., & Pereira, H. M. (2015). Ecosystem services: the opportunities of rewilding in Europe. In *Rewilding European Landscapes* (pp. 47-64). Springer, Cham.
- Clavel, J., Julliard, R., & Devictor, V. (2011). Worldwide decline of specialist species: toward a global functional homogenization?. Frontiers in Ecology and the Environment, 9(4), 222-228.
- Cromsigt, J. P., Kemp, Y. J., Rodriguez, E., & Kivit, H. (2018). Rewilding Europe's large grazer community: how functionally diverse are the diets of European bison, cattle, and horses?. Restoration Ecology, 26(5), 891-899.
- D'Acunto, L. E., Pauli, B. P., Moy, M., Johnson, K., Abu-Omar, J., & Zollner, P. A. (2018). Timingand technique impact the effectiveness of road-based, mobile acoustic surveys of bats. *Ecology and evolution*, 8(6), 3152-3160.
- De Beijer, Van Geelen & Zollinger. (2012). Gebiedsgerichte uitwerking leefgebiedsplan het Groene Woud: Maatregelen plan tbv soortenbescherming. Ministerie van Economische Zaken, Landbouw en Innovatie & RAVON.
- Dirzo, R., Young, H. S., Galetti, M., Ceballos, G., Isaac, N. J., & Collen, B. (2014). Defaunation in the Anthropocene. science, 345(6195), 401-406.
- Downs, N. C., & Racey, P. A. (2006). The use by bats of habitat features in mixed farmland in Scotland. *Acta chiropterologica*, 8(1), 169-185.
- Ekman, M., & De Jong, J. (1996). Local patterns of distribution and resource utilization of four bat species (Myotis brandti, Eptesicus nilssoni, Plecotus auritus and Pipistrellus pipistrellus) in patchy and continuous environments. *Journal of Zoology*, 238(3), 571-580.
- Estes, J. A., Terborgh, J., Brashares, J. S., Power, M. E., Berger, J., Bond, W. J., ... & Wardle, D. A. (2011). Trophic downgrading of planet Earth. science, 333(6040), 301-306.
- Fawcett, K., & Ratcliffe, J. M. (2015). Clutter and conspecifics: a comparison of their influence on echolocation and flight behaviour in Daubenton's bat, Myotis daubentonii. Journal of Comparative Physiology A, 201(3), 295-304.
- Fortin, D., Beyer, H. L., Boyce, M. S., Smith, D. W., Duchesne, T., & Mao, J. S. (2005). Wolves influence elk movements: behavior shapes a trophic cascade in Yellowstone National Park. Ecology, 86(5), 1320-1330.
- Fricke, F. (1984). Sound attenuation in forests. *Journal of Sound and Vibration*, 92(1), 149-158.

- Galetti, M., Root-Bernstein, M., & Svenning, J. C. (2017). Challenges and opportunities for rewilding South American landscapes. Perspectives in ecology and conservation, 15(4), 245-247.
- Goodwin, K. R., & Gillam, E. H. (2021). Testing Accuracy and Agreement among Multiple Versions of Automated Bat Call Classification Software. *Wildlife Society Bulletin*.
- Gray, T. N. E., Eames, J. C., Lyon, J. R. A., & Meyerhoff, M. (2019). Rewilding in Southeast Asia: an assessment of conservation. Cambodian Journal of Natural History, 98.
- Grindal, S. D., & Brigham, R. M. (1999). Impacts of forest harvesting on habitat use by foraging insectivorous bats at different spatial scales. *Ecoscience*, *6*(1), 25-34.
- Hawksworth, D. L., & Bull, A. T. (Eds.). (2007). Vertebrate conservation and biodiversity. Springer.
- Hill, A. P., Prince, P., Piña Covarrubias, E., Doncaster, C. P., Snaddon, J. L., & Rogers, A. (2018). AudioMoth: Evaluation of a smart open acoustic device for monitoring biodiversity and the environment. *Methods in Ecology and Evolution*, 9(5), 1199-1211.
- Hill, A. P., Prince, P., Snaddon, J. L., Doncaster, C. P., & Rogers, A. (2019). AudioMoth: A low-cost acoustic device for monitoring biodiversity and the environment. HardwareX, 6, e00073.
- iNaturalist.org. (n.d.). Bat spectrograms. Available from: <u>https://www.inaturalist.org/projects/bat-spectrograms</u> (accessed September, 2022).
- Jepson, P. (2016). A rewilding agenda for Europe: creating a network of experimental reserves. *Ecography*, 39(2).
- Jepson, P., Schepers, F., & Helmer, W. (2018). Governing with nature: a European perspective on putting rewilding principles into practice. Philosophical Transactions of the Royal Society B: Biological Sciences, 373(1761), 20170434.
- Jongman, R. H. (2002). Homogenisation and fragmentation of the European landscape: ecological consequences and solutions. Landscape and urban planning, 58(2-4), 211-221.
- Jones, G., Jacobs, D. S., Kunz, T. H., Willig, M. R., & Racey, P. A. (2009). Carpe noctem: the importance of bats as bioindicators. Endangered species research, 8(1-2), 93-115.
- Jones, K.E., Russ, J., Bashta, A.-T., Bilhari, Z., Catto, C., Csosz, I.,Gorbachev, A., Gyorfi, P., Hughes, A., Ivashkiv, I., Koryagina, N., Ku-rali, A., Langton, S., Maltby, A., Margiean, G., Pandourski, I., Par-sons, S., Prokofev, I., Szodoray-Paradi, A., Szodoray-Paradi, F., Tilova, E., Walters, C., Weatherill, A. & Zavarzin, O. (2013). Indicator Bats Program: a system for the global acoustic monitoring of bats. Biodiversity Monitoring and Conservation: Bridging the Gaps Between GlobalCommitment and Local Action (eds B. Collen, N. Pettorelli, S. Durant, L. Krueger & J. Baillie) Wiley-Blackwell, London.

- Katayama, N., Amano, T., Naoe, S., Yamakita, T., Komatsu, I., Takagawa, S. I., ... & Miyashita, T. (2014). Landscape heterogeneity–biodiversity relationship: effect of range size. PloS one, 9(3), e93359.
- Kerbiriou, C., Bas, Y., Le Viol, I., Lorrillière, R., Mougnot, J., & Julien, J. F. (2019). Bat pass duration measurement: an indirect measure of distance of detection. *Diversity*, 11(3), 47.
- KNMI. (2020). Klimaatviewer, langjarig gemiddelde 1991-2020. Koninklijk Nederlands Metereologisch Instituut. Available from: <u>https://www.knmi.nl/klimaat-viewer/kaarten/temperatuur/gemiddelde-temperatuur/juli/Periode_1991-2020</u> (accessed January, 2022)
- Knops, J. M., Tilman, D., Haddad, N. M., Naeem, S., Mitchell, C. E., Haarstad, J., ... & Groth, J. (1999). Effects of plant species richness on invasion dynamics, disease outbreaks, insect abundances and diversity. *Ecology Letters*, 2(5), 286-293.
- Kolkert, H., Smith, R., Rader, R., & Reid, N. (2020). Insectivorous bats foraging in cotton crop interiors is driven by moon illumination and insect abundance, but diversity benefits from woody vegetation cover. *Agriculture, Ecosystems & Environment, 302*, 107068.
- Kusch, J., Weber, C., Idelberger, S., & Koob, T. (2004). Foraging habitat preferences of bats in relation to food supply and spatial vegetation structures in a western European low mountain range forest. *FOLIA ZOOLOGICA-PRAHA-*, *53*(2), 113-128.
- Lagerveld, S., Poerink, B. J., & de Vries, P. (2015). Monitoring Bat activity at the Dutch EEZ in 2014 (No. C094/15). IMARES.
- Lahr, J., Van Kats, R. J. M., & Crum, S. J. H. (2007). Ontwormingsmiddelen in de natuur. *Vakblad Natuur Bos Landschap*, 4(2), 22-23.
- Lanz, B., Dietz, S., & Swanson, T. (2018). The expansion of modern agriculture and global biodiversity decline: an integrated assessment. Ecological Economics, 144, 260-277.
- Lawrence, B. D., & Simmons, J. A. (1982). Measurements of atmospheric attenuation at ultrasonic frequencies and the significance for echolocation by bats. *The Journal of the Acoustical Society of America*, 71(3), 585-590.
- Kapteyn, K., & Limpens, H. (1991). Determineren met een bat-detector. Zoogdier, 2(2), 14-19.
- LNV. (1990). Natuurbeleidsplan. Ministerie van Landbouw, Natuurbeheer en Visserij. Den Haag.
- Lorimer, J., Sandom, C., Jepson, P., Doughty, C., Barua, M., & Kirby, K. J. (2015). Rewilding: science, practice, and politics. Annual Review of Environment and Resources, 40, 39-62.

- Marckmann, U. & Pfeiffer, B. (2020). Analysis of Bat Call Recordings and Criteria for the Evaluation of Acoustic Identification of Species. Part 1 – Genera Nyctalus, Eptesicus, Vespertilio, Pipistrellus (nyctaloid and pipistrelloid Species), Barbastelle, Long-eared Bats and Horseshoe Bats in Bavaria. Bayerisches Landesamt für Umwelt.
- Mata, J. C., Buitenwerf, R., & Svenning, J. C. (2021). Enhancing monitoring of rewilding progress through wildlife tracking and remote sensing. PloS one, 16(7), e0253148.
- McMillan, N. A., Kunkel, K. E., Hagan, D. L., & Jachowski, D. S. (2019). Plant community responses to bison reintroduction on the Northern Great Plains, United States: a test of the keystone species concept. *Restoration Ecology*, *27*(2), 379-388.
- Meaney, C., & Gerson, J. (2018). Bat Acoustical Surveys at the National Renewable Energy Laboratory, National Wind Technology Center. *Contract*.
- Medeiros, C. I., Both, C., Grant, T., & Hartz, S. M. (2017). Invasion of the acoustic niche: variable responses by native species to invasive American bullfrog calls. Biological Invasions, 19(2), 675-690.
- Moreno, C. E., Sánchez-Rojas, G., Pineda, E., & Escobar, F. (2007). Shortcuts for biodiversity evaluation: a review of terminology and recommendations for the use of target groups, bioindicators and surrogates. International Journal of Environment and Health, 1(1), 71-86.
- Morris, A. D., Miller, D. A., & KALCOUNIS-RUEPPELL, M. C. (2010). Use of forest edges by bats in a managed pine forest landscape. *The Journal of Wildlife Management*, 74(1), 26-34.
- Müller, J., Brandl, R., Buchner, J., Pretzsch, H., Seifert, S., Strätz, C., ... & Fenton, B. (2013). From ground to above canopy—Bat activity in mature forests is driven by vegetation density and height. *Forest Ecology and Management*, 306, 179-184.
- Navarro, L. M., Proença, V., Kaplan, J. O., & Pereira, H. M. (2015). Maintaining disturbance-dependent habitats. In *Rewilding European Landscapes* (pp. 143-167). Springer, Cham.
- Neuweiler, G. (1990). Auditory adaptations for prey capture in echolocating bats. Physiological reviews, 70(3), 615-641.
- Newbold, T., Hudson, L. N., Hill, S. L., Contu, S., Lysenko, I., Senior, R. A., ... & Purvis, A. (2015). Global effects of land use on local terrestrial biodiversity. Nature, 520(7545), 45-50.
- Newson, S.E., Evans, H.E. & Gillings, S. (2015). A novel citizen science approach for largescale standardised monitoring of bat activity and distribution, evaluated in eastern England. Biol. Conserv., 191, 38–49.

Nicholls, B., & A. Racey, P. (2006). Habitat selection as a mechanism of resource

partitioning in two cryptic bat species Pipistrellus pipistrellus and Pipistrellus pygmaeus. *Ecography*, 29(5), 697-708.

- Obrist, M. K., Boesch, R., & Flückiger, P. F. (2004). Variability in echolocation call design of 26 Swiss bat species: consequences, limits and options for automated field identification with a synergetic pattern recognition approach.
- Olff, H., Vera, F. W., Bokdam, J., Bakker, E. S., Gleichman, J. M., De Maeyer, K., & Smit, R. (1999). Shifting mosaics in grazed woodlands driven by the alternation of plant facilitation and competition. Plant biology, 1(2), 127-137.
- Paine, R. T. (1980). Food webs: linkage, interaction strength and community infrastructure. Journal of animal ecology, 49(3), 667-685.
- Parsons, S., & Jones, G. (2000). Acoustic identification of twelve species of echolocating bat by discriminant function analysis and artificial neural networks. Journal of experimental biology, 203(17), 2641-2656.
- Petz, K., & van Oudenhoven, A. P. (2012). Modelling land management effect on ecosystem functions and services: a study in the Netherlands. International Journal of Biodiversity Science, Ecosystem Services & Management, 8(1-2), 135-155.
- Piel, A. K., Crunchant, A., Knot, I. E., Chalmers, C., Fergus, P., Mulero-Pázmány, M., & Wich, S. (2021). Noninvasive Technologies for Primate Conservation in the 21st Century. International Journal of Primatology, 1-35.
- Plank, M., Fiedler, K., & Reiter, G. (2012). Use of forest strata by bats in temperate forests. *Journal of Zoology*, 286(2), 154-162.
- Put, J. E., Fahrig, L., & Mitchell, G. W. (2019). Bats respond negatively to increases in the amount and homogenization of agricultural land cover. *Landscape Ecology*, 34(8), 1889-1903.
- Remmert, H. (1991). The mosaic-cycle concept of ecosystems—an overview. The mosaic-cycle concept of ecosystems, 1-21.
- Reynolds, C., Fletcher, R. J., Carneiro, C. M., Jennings, N., Ke, A., LaScaleia, M. C., ... & McCleery, R. A. (2018). Inconsistent effects of landscape heterogeneity and land-use on animal diversity in an agricultural mosaic: a multi-scale and multi-taxon investigation. Landscape Ecology, 33(2), 241-255.
- Rijksdienst voor ondernemend Nederland. (March 2014). Soortenstandaard Gewone dwergvleermuis Pipistrellus pipistrellus. Available from: <u>https://www.rvo.nl/sites/default/files/2015/01/Soortenstandaard%20Gewone%20dwer</u> <u>gvleermuis.pdf</u> (accessed September, 2022).
- Ripple, W. J., & Beschta, R. L. (2012). Trophic cascades in Yellowstone: the first 15 years after wolf reintroduction. *Biological Conservation*, 145(1), 205-213.

Rodríguez-San Pedro, A., Allendes, J. L., Beltrán, C. A., Chaperon, P. N., Saldarriaga-

Córdoba, M. M., Silva, A. X., & Grez, A. A. (2020). Quantifying ecological and economic value of pest control services provided by bats in a vineyard landscape of central Chile. *Agriculture, Ecosystems & Environment, 302*, 107063.

- Russo, D., & Voigt, C. C. (2016). The use of automated identification of bat echolocation calls in acoustic monitoring: A cautionary note for a sound analysis. Ecological Indicators, 66, 598-602.
- Russo, D., Salinas-Ramos, V. B., Cistrone, L., Smeraldo, S., Bosso, L., & Ancillotto, L. (2021). Do We Need to Use Bats as Bioindicators?. *Biology*, *10*(8), 693.
- Saldaña-Vázquez, R. A., & Munguía-Rosas, M. A. (2013). Lunar phobia in bats and its ecological correlates: A meta-analysis. *Mammalian Biology*, 78(3), 216-219.
- Schnitzler, H. U., & Kalko, E. K. (2001). Echolocation by insect-eating bats: we define four distinct functional groups of bats and find differences in signal structure that correlate with the typical echolocation tasks faced by each group. *Bioscience*, *51*(7), 557-569.
- Schnitzler, H. U., Moss, C. F., & Denzinger, A. (2003). From spatial orientation to food acquisition in echolocating bats. *Trends in Ecology & Evolution*, 18(8), 386-394.
- Schulze, K. A., Rosenthal, G., & Peringer, A. (2018). Intermediate foraging large herbivores maintain semi-open habitats in wilderness landscape simulations. Ecological Modelling, 379(March), 10–21. https://doi.org/10.1016/j.ecolmodel.2018.04.002
- Somersat Bat Group. (n.d.). AudioMoth which sample rate is best for bat detection?. Available from: <u>https://somersetbat.group/advice/which-bat-</u><u>detector/audiomoth/testing/sample-rate/</u> (accessed September, 2022).
- Soulé, M., & Noss, R. (1998). Rewilding and biodiversity: complementary goals for continental conservation. Wild Earth, 8, 18-28.
- Stahlschmidt, P., & Brühl, C. A. (2012). Bats as bioindicators-the need of a standardized method for acoustic bat activity surveys. *Methods in Ecology and Evolution*, *3*(3), 503-508.
- Stone, E. L., Harris, S., & Jones, G. (2015). Impacts of artificial lighting on bats: a review of challenges and solutions. *Mammalian Biology*, 80(3), 213-219.
- Swearingen, M. E., White, M. J., Guertin, P. J., Albert, D. G., & Tunick, A. (2013). Influence of a forest edge on acoustical propagation: Experimental results. *The Journal of the Acoustical Society of America*, 133(5), 2566-2575.
- Svenning, J. C., Pedersen, P. B., Donlan, C. J., Ejrnæs, R., Faurby, S., Galetti, M., ... & Vera, F. W. (2016). Science for a wilder Anthropocene: Synthesis and future directions for trophic rewilding research. Proceedings of the National Academy of Sciences, 113(4), 898-906.

Svenning, J. C., Munk, M., & Schweiger, A. N. D. R. E. A. S. (2019). Trophic rewilding:

ecological restoration of top-down trophic interactions to promote self-regulating biodiverse ecosystems. *Rewilding*, 73-89.

- Stem, C., Margoluis, R., Salafsky, N., & Brown, M. (2005). Monitoring and evaluation in conservation: a review of trends and approaches. Conservation biology, 19(2), 295-309.
- Steur, J. (2014). Het Groene Woud, vlindertuin van Noord-Brabant. Vlinders, 29(3), 10-13.
- Stowell, D., & Sueur, J. (2020). Ecoacoustics: acoustic sensing for biodiversity monitoring at scale. *Remote Sensing in Ecology and Conservation*, 6(3), 217-219.
- Tews, J., Brose, U., Grimm, V., Tielbörger, K., Wichmann, M. C., Schwager, M., & Jeltsch, F. (2004). Animal species diversity driven by habitat heterogeneity/diversity: the importance of keystone structures. Journal of biogeography, 31(1), 79-92.
- Tittensor, D. P., Walpole, M., Hill, S. L., Boyce, D. G., Britten, G. L., Burgess, N. D., ... & Ye, Y. (2014). A mid-term analysis of progress toward international biodiversity targets. Science, 346(6206), 241-244.
- Todd, V. L. G., & Waters, D. A. (2017). Small scale habitat preferences of Myotis daubentonii, Pipistrellus pipistrellus, and potential aerial prey in an upland river valley. *Acta Chiropterologica*, *19*(2), 255-272.

Torres, A., Fernández, N., Zu Ermgassen, S., Helmer, W., Revilla, E., Saavedra, D., ... & Pereira,

H. M. (2018). Measuring rewilding progress. Philosophical Transactions of the Royal Society B: Biological Sciences, 373(1761), 20170433.

- Tutelaers, P. (2017). Spinnen (Arachnida: Araneae) uit de leembossen van Het Groene Woud. entomologische berichten, 77(6), 274-282.
- Twisk, P. (2013). Harige viervoeters en gevleugelde muizen. From: Poelmans, W., Van der Straaten, J., Veling, K. (2013). Leembossen in Het Groene Woud, schatkamer van biodiversiteit. Picture Publishers, Woudrichem, 193-209.
- Van der Velde, W. (2021). Rewilding with red deer in Het Groene Woud: impact on woody recruitment and vegetation structure (Master's Thesis).
- Van Klink, Roel, and Michiel F. WallisDeVries. "Risks and opportunities of trophic rewilding for arthropod communities." Philosophical Transactions of the Royal Society B: Biological Sciences 373.1761 (2018): 20170441.
- Vera, F. W. (1997). *Metaforen voor de wildernis: eik, hazelaar, rund en paard*. Wageningen University and Research.
- Vera, F. W. (2009). Large-scale nature development--The Oostvaardersplassen. British Wildlife, 20(5), 28.
- Verboom, B. (1998). The use of edge habitats by commuting and foraging bats. Wageningen

University and Research.

- Verboom, B., & Spoelstra, K. (1999). Effects of food abundance and wind on the use of tree lines by an insectivorous bat, Pipistrellus pipistrellus. *Canadian Journal of Zoology*, 77(9), 1393-1401.
- *Waarneming.nl.* (n.d.). Waarneming.nl. Consulted August 29, 2022, from: https://waarneming.nl/

Popular science summary

Bats have been suggested to be suitable indicators of the general state of ecosystems. Hence, monitoring bat diversity may provide an adequate technique to assess progress in reintroduction projects. Reintroduction projects with large grazers, which are gaining popularity across Europe, are associated with wider impacts on the ecosystem. Large grazers affect the landscape and interactions within the food web through their grazing, trampling and dung deposition. These reintroduction projects are commonly referred to as rewilding projects. In order to investigate the effect of large grazers on their environment, progress of these rewilding projects needs to be monitored. Bats may function as so-called 'bioindicators', which provide generalizable insight into responses of multiple other plant and animal species further down the food chain. At the same time, a newly developed audio-recorder that allows for recording bat calls, such as the AudioMoth, provides cheap, non-invasive and energy-efficient monitoring of bats. In my study, I explored the distance to which this device could record bat calls in forests, forest edges and open habitat types, in order to further develop the use of the AudioMoth in scientific research.

Forest habitat reduced detection probability with the AudioMoth with sounds played over a speaker at 10kHz and at three ascending volumes. The devices were also used to assess the impact of three habitat types (forest, forest edge and open grassland) on the presence of bat genera that had adaptations specific to one of those habitats. I placing the devices in each habitat to record bats for 3-4 days over the course of three months. The genus *Plecotus*, which was adapted to forests, was not more likely to be detected in any of the habitats. I did find evidence that the genus *Myotis*, which was adapted to forest edges, was more likely to be detected in open compared to forest habitat, and open-adapted genera *Nyctalus* and *Seronitus* were more likely to be detected in forest edges and open grasslands compared to forest habitats. Lastly, I compared bat genus richness between a rewilded area, where Red deer were reintroduced in 2017, and its non-rewilded nature surroundings in Dutch national landscape 'Het Groene Woud'. There was no effect of rewilding on genus richness. My data and findings may be used as a baseline study for bat and rewilding research in the local context of Het Groene Woud.

Acknowledgements

The first thank you goes out to my supervisor Sheila Holmes, who guided me through the research project and was always available to answer questions or provide feedback. In our extensive online meetings and email correspondence she demonstrated expert knowledge, academic precision and empathy for my learning process, all of which I recognize and greatly appreciate. I can honestly say that I could not have wished for better supervision.

I also owe a big thank you to my second supervisor, Joris Cromsigt. Drawing from his experience and great expertise, he provided a critical perspective, exposing issues that Sheila and I might have missed otherwise. Next to his crucial guidance in the thesis process, I want to thank Joris for his lecture sparking my interest in rewilding two years ago.

Thirdly, thanks to Sjors de Kort from Het Brabants Landschap, who provided contextual information regarding the research area, and was always ready to answer additional questions regarding the fieldwork. His enthusiasm for nature management was infectious, and his practical perspective reminded me of the utility of my study.

I also thank Ine Dorresteijn, the second reader of this thesis.

Last but not least I want to thank my parents, Corinne van der Velden and Bert Pronk, for letting me use their car to drive 200km twice a week to conduct fieldwork. I also thank them, and all others who supported me in various ways during these years, for making sure my academic time was enriching, educational and enjoyable.

Appendix I: Coding

Active test: Kruskal-Wallis

#Initialization
my_data <- KWtest
library(dplyr)
str(my_data)
KWtest\$DetectionDistanceSoft <- as.numeric(KWtest\$DetectionDistanceSoft)
KWtest\$DetectionDistanceIntermediate <as.numeric(KWtest\$DetectionDistanceLoud <- as.numeric(KWtest\$DetectionDistanceLoud)</pre>

#Kruskal-Wallis HabitatType

kruskal.test(DetectionDistanceSoft ~ HabitatType, data = my_data)
kruskal.test(DetectionDistanceIntermediate ~ HabitatType, data = my_data)
kruskal.test(DetectionDistanceLoud ~ HabitatType, data = my_data)

#Kruskal-Wallis Treatment

kruskal.test(DetectionDistanceSoft ~ Treatment, data = my_data)
kruskal.test(DetectionDistanceIntermediate ~ Treatment, data = my_data)
kruskal.test(DetectionDistanceLoud ~ Treatment, data = my_data)

#Pairwise Wilcox HabitatType

#Pairwise Wilcox Treatment

Functional group detection: GLMM

#Initialization

library(lme4) library(GLMMadaptive) library(multcomp) str(FINALANALYSIS) #Factorize Rewilded FINALANALYSIS\$REWILDED <- as.factor(FINALANALYSIS\$REWILDED)

#rescale variables
control=glmerControl(optimizer="bobyqa",optCtrl=list(maxfun=2e5))

#GLMM of forest, edge and open

Forest1 <- glmer(FGFOREST~HABITAT+REWILDED+(1|BLOCK), data=FINALANALYSIS, family =
binomial(link="logit"))
summary(Forest1)</pre>

FINALANALYSIS\$HABITATREWILDED <- interaction(FINALANALYSIS\$HABITAT, FINALANALYSIS\$REWILDED) Edge1 <- glmer(FGEDGE~HABITATREWILDED+(1|BLOCK), data=FINALANALYSIS, family = binomial(link="logit")) summary(Edge1)

Open1 <- glmer(FGOPEN~HABITAT+REWILDED+(1|BLOCK), data=FINALANALYSIS, family = binomial(link="logit")) summary(Open1)

#Tukey tests

comp.FINALANALYSISFOREST1 <- glht(Forest1, linfct=mcp(HABITAT="Tukey"))
summary(comp.FINALANALYSISFOREST1)
comp.FINALANALYSISFOREST2 <- glht(Forest1, linfct=mcp(REWILDED="Tukey"))
summary(comp.FINALANALYSISFOREST2)</pre>

comp.FINALANALYSISEDGE <- glht(Edge1, linfct=mcp(HABITATREWILDED="Tukey"))
summary(comp.FINALANALYSISEDGE)</pre>

comp.FINALANALYSISOPEN1 <- glht(Open1, linfct=mcp(HABITAT="Tukey"))
summary(comp.FINALANALYSISOPEN1)
comp.FINALANALYSISOPEN2 <- glht(Open1, linfct=mcp(REWILDED="Tukey"))
summary(comp.FINALANALYSISOPEN2)</pre>

Genus richness: GLMM

#Insert packages and data

library(Ime4) library(GLMMadaptive) library(multcomp) str(FINALANALYSIS)

#Factorize Rewilded FINALANALYSIS\$REWILDED <- as.factor(FINALANALYSIS\$REWILDED)

#rescale variables

control=glmerControl(optimizer="bobyqa",optCtrl=list(maxfun=2e5))

#GLMM GenusRichness

GenusRichness1 <- glmer(TOTALGENERA~HABITAT+REWILDED+(1|BLOCK), data=FINALANALYSIS, family = poisson) summary(GenusRichness1)

#Tukey test

comp.FINALANALYSIS1 <- glht(GenusRichness1, linfct=mcp(HABITAT="Tukey"))
summary(comp.FINALANALYSIS1)</pre>

comp.FINALANALYSIS2 <- glht(GenusRichness1, linfct=mcp(REWILDED="Tukey"))
summary(comp.FINALANALYSIS2)</pre>

Appendix II: Template calls

Brown & grey long-eared bat (Plecotus Auritus & Plecotus austriacus)



Figure A1. Typical sequence of brown and grey long-eared bats. Figure adopted from Marckmann & Pfeiffer (2020, p. 67).



Figure A2. Spectrograms of various short calls (< 4 ms) from brown and grey long-eared bats. Figure adopted from Marckmann & Pfeiffer (2020, p. 67).



Figure A3. Spectrograms of various medium to long calls (4-6 ms) from brown and grey long-eared bats. Figure adopted from Marckmann & Pfeiffer (2020, p. 67).



Figure A4. Spectrograms of various long calls (6-8 ms) from brown and grey long-eared bats. Figure adopted from Marckmann & Pfeiffer (2020, p. 67).

Daubenton's bat (Myotis daubentonii)



Figure A5. Depiction and spectrogram of Daubenton's bat calls. The figure shows the common sigmoidal shape and relatively low starting frequency (<100kHz). Figure adopted from Bas *et al.* (2011, p. 3).



Figure A6. Examples of template calls from Daubenton's bat obtained during the data analysis for this study.

Natterer's bat (Myotis nattereri)



Figure A7. Depiction and spectrogram of Natterer's bat calls. The figure shows the common wide bandwidth and end frequency into the audible (<20kHz). Figure adopted from Bas *et al.* (2011, p. 5).





Figure A8. Depiction of Whiskered bat calls. The figure shows decreasing end frequency with increasing call duration, which distinguishes *M. mysticanus* from *M. daubentonii*. Figure adopted from Bas *et al.* (2011, p. 6).





Figure A9. Depiction and spectrogram of Natterer's bat calls. The figure shows and end frequency between 12 and 30 kHz. This species can emit long calls (>10 ms). Figure adopted from Bas *et al.* (2011, p. 5).

Common Pipistrelle (Pipistrellus pipistrellus)



Figure A10. Typical sequence of common Pipistrelle. Figure adopted from Marckmann & Pfeiffer (2020, p. 59).



Figure A11. Spectrograms of quasi constant frequency calls from common Pipistrelle. Figure adopted from Marckmann & Pfeiffer (2020, p. 60).



Figure A12. Spectrograms of various frequency modulated ending in quasi constant frequency calls short calls from common Pipistrelle. Figure adopted from Marckmann & Pfeiffer (2020, p. 60).



Figure A13. Spectrograms of various frequency modulated calls from common Pipistrelle. Figure adopted from Marckmann & Pfeiffer (2020, p. 61).

90KHZ	90KHZ
80kHz	80kHz
70kHz	70kHz
60kHz	60kHz
50kHz	50kHz
40kHz	40kHz
30kHz	30kHz
20kHz	20kHz
10kHz	10kHz
0kHz	0kHz
0ms	0mc
	UIIS

Figure A14. Examples of template calls from common Pipistrelle obtained during the data analysis for this study.





Figure A15. Typical sequence of Nathusius's Pipistrelle. Figure adopted from Marckmann & Pfeiffer (2020, p. 53).



Figure A16. Spectrograms of quasi constant frequency calls from Nathusius's Pipistrelle. Figure adopted from Marckmann & Pfeiffer (2020, p. 54).



Figure A17. Spectrograms of frequency modulated calls ending in quasi constant frequency from Nathusius's Pipistrelle. Figure adopted from Marckmann & Pfeiffer (2020, p. 54).



Figure A18. Spectrograms of frequency modulated calls from Nathusius's Pipistrelle. Figure adopted from Marckmann & Pfeiffer (2020, p. 55).

9UKHZ		
80kHz	_	
70kHz		
60kHz		
50kHz		
40kHz		
30kHz	A REAL PROPERTY.	
20kHz		
10kHz		
0kHz		
0ms	20ms	40ms

Figure A19. Example of template call from Nathusius's Pipistrelle obtained during the data analysis for this study.

Noctule bat (Nyctalus noctula)



Figure A20. Typical sequence of the Noctule, depicting the 'plip-plop' calls that characterize this species. Figure adopted from Marckmann & Pfeiffer (2020, p. 30).



Figure A21. Spectrograms of quasi constant frequency calls from the Noctule. Figure adopted from Marckmann & Pfeiffer (2020, p. 31).



Figure A22. Spectrograms of frequency modulated calls ending in quasi constant frequency from the Noctule. Figure adopted from Marckmann & Pfeiffer (2020, p. 31).



Figure A23. Spectrograms of frequency modulated calls from the Noctule. Figure adopted from Marckmann & Pfeiffer (2020, p. 32).

Lesser noctule (Nyctalus leisleri)



Figure A24. Typical sequence of Lesser Noctule. Figure adopted from Marckmann & Pfeiffer (2020, p. 34).



Figure A25. Spectrograms of quasi constant frequency calls from the Lesser Noctule. Figure adopted from Marckmann & Pfeiffer (2020, p. 35).



Figure A26. Spectrograms of frequency modulated calls ending in quasi constant frequency from the Lesser Noctule. Figure adopted from Marckmann & Pfeiffer (2020, p. 35).



Figure A27. Spectrograms of frequency modulated calls from the Lesser Noctule. Figure adopted from Marckmann & Pfeiffer (2020, p. 35).

Serotine bat (Eptesicus serotinus)



Figure A28. Typical sequence of Serotine bat with frequency modulated ending in quasi constant frequency calls. Figure adopted from Marckmann & Pfeiffer (2020, p. 41).



Figure A29. Spectrograms of quasi constant frequency calls from the Serotine bat. Figure adopted from Marckmann & Pfeiffer (2020, p. 42).



Figure A30. Spectrograms of frequency modulated calls ending in quasi constant frequency from Serotine bat. The middle three calls show an uppercut in the frequency, which characterizes the Serotine. Figure adopted from Marckmann & Pfeiffer (2020, p. 42).



Figure A31. Spectrograms of frequency modulated calls from the Serotine bat. Figure adopted from Marckmann & Pfeiffer (2020, p. 43).



Supplementary materials





Figure S2. Figure showing the percentage of total samples in which each genus was detected.



Figure S3. Examples of Forest species *Pipistrellus pipistrellus* (left) and *Plecotus auritus* (right). ©*Images: Wikimedia Commons*



Figure S4. Examples of Edge species *Myotis Daubentonii* (left) and *Myotis myotis* (right). ©Images: Wikimedia Commons



Figure S5. Examples of Open species *Nyctalus noctula* (left) and *Eptesicus seronitus* (right). ©*Images: Wikimedia Commons*