

# Biodiversity effects of broadleaved tree shelterbelts around pine plantations

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## Preface

I would like to express my gratitude to Hervé Jactel and Elena Valdés-Correcher at the National Research Institute for Agriculture, Food and the Environment (INRAE) for their valuable collaboration. Their expertise and willingness to share necessary information have been instrumental in completing this master's thesis. In addition, their guidance and support have greatly enriched my research journey. I believe that their guidance and mentorship will greatly strengthen the quality and depth of my research. The skills and knowledge acquired were instrumental in the data analysis phase and in interpreting the results accurately.

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Special thanks goes to Sanne Verdonck, whose guidance and expertise in utilizing the R software have been so valuable. The time she dedicated to me away from her PhD, insightful suggestions and patient assistance have significantly enhanced my proficiency in R programming. I am deeply thankful for her mentorship and the meaningful meetings we have had throughout the process. In the end, being a part of your PhD research project at KU Leuven presented a valuable educational opportunity that enhanced my knowledge and experience to adopt these practices in a professional setting, which further enriched my understanding in the field. Your advice during this training will significantly contribute to the overall success of my master's thesis.

I am deeply indebted to my supervisor, Professor Bart Muys, for granting me this remarkable opportunity to embark on this research project. Under his mentorship, I have acquired invaluable knowledge, skills, and experiences that will undoubtedly shape my future career. I am immensely grateful for his unwavering support, constructive feedback, and the privilege of being a part of the esteemed KU Leuven Division Forest, Nature and Landscape (DFNL). My involvement in this thesis project proved to be a valuable and enlightening experience, expanding my knowledge and insights in the realm of qualitative research and analysis, which presented me with a new venture.

To my beloved friends (you know who you are), family and almost-to-be mother, my sister, I am eternally grateful for your unwavering love, support, and encouragement throughout this research journey. I am very excited to be the best aunt for Joa. Despite the physical distance separating us, your constant belief in me and unyielding support have been a driving force behind my motivation. Your presence and encouragement have uplifted me, even in moments of self-doubt.

Lastly, I am truly humbled and honoured to have had the privilege of collaborating with such exceptional individuals and institutions. Their contributions and support have been invaluable in shaping the outcomes of this research attempt. I extend my deepest appreciation to all those who have played a part in this journey, and I am confident that their influence will continue to resonate in my future venture.

## Contribution statement

Collaborating with my co-supervisor Hervé Jactel and supervisor Professor Bart Muys, we have developed the primary thesis objectives and the larger context of the research. Regarding the experimental design and data collection, Hervé already developed an outlined procedure, based on his experience with previous research he had done on the methods that would be used in the experiment. This was important seeing that the materials were needed beforehand to prepare for fieldwork. During the development of the standardized protocols, I had to review extensive literature to articulate the methods and models needed to do completed in this thesis project. For this, Elena Valdés-Correcher guided me through the process of identifying the data and how to analyse the data properly. Afterwards, two separate drafts of the protocols were made beforehand and were sent to my supervisors for review purposes were they gave additional suggestions on how to improve my approach before going out in the field. Further, the materials needed were gathered and assembled by me, with additional help from PhD students at the Geoinstitute for the first part of the experiment. As for fieldwork, KU Leuven field technician, Eric van Beek and Professor Muys assisted me in deploying and collecting the models over the two consecutive years. Inspections of the models were completed by me as well as the creation of the data frame.

Regarding the data analysis, I received assistance from a PhD student, Sanne Verdonck. She helped me to understand and grasp the fundamentals working with R software, as it was my first time using it. However, for the results I wrote the R scripts and created the graphs myself. As for the rest of the text in the document, the structuring and writing of each section was completed by me. Professor Muys gave additional suggestions in the structuring department as well as incorrect grammar usage. Hervé also gave some good guidelines and suggestions for my results, where I adapted to it accordingly.

Last but not least, I have to give credit to ChatGPT. This generative artificial intelligence helped me throughout the analysis process to build codes, perform various statistical tests, visualising my boxplots and occasionally used it to resolve errors when they occurred. However, I did also accomplish all of these goals by searching for the problems or returning to some excellent coding education websites. Nevertheless, ChatGPT was super helpful in a sense that it gets the job done faster thus me managing my time accordingly.

## Summary

Estimating forest biodiversity has become one of the major tools for management in landscapes. A standardized and practical methodology was developed for the monitoring of some important aspects of biodiversity in forests on a stand-scale level. The landscape structures have undergone numerous unfavourable changes as a result of prolonged human activity. The most prominent adverse impact has been the simplification of the landscape structure due to the decrease of stable ecosystems with the incorporation of mixed tree species in forests, and their replacement with monoculture reforestation. In fact, mixed-species forests are suggested as a viable method of sustainable forestry management that allows for increased wood production while simultaneously promoting ecological and social advantages that would be otherwise diminished by conventional monoculture-based approaches. One significant ecological advantage of mixed forestry is the potential to decrease plant damage caused by specialized insect pests, however, increase predator species. This is coupled with the fact that mixed species also improve soil health, increase biodiversity, and support additional habitats. This is based on the notion that diverse habitats created by these structures are likely to restrict the fluctuations in the population dynamics of pest insects. However, fragmentation can have negative consequences by disrupting stand connectivity thus contributing to the decrease in biodiversity, making it less likely for forest stands to be resistant against natural disasters. Moreover, factors such as diseases and pests also pose a threat to natural forests globally. Overall, linear structures, like shelterbelts, could play a crucial role in the prolonged conservation of endemic forest species within fragmented habitats by fulfilling multiple essential functions. To test this, two hypotheses was created to explain the biodiversity effect impacted by shelterbelts. First, building the knowledge from the bottom-up effect, whereas pine trees planted in a diverse stand possess enhanced defence mechanisms against defoliators, making them more effective in repelling defoliators. Second, suggesting that tree species diversity contributes to the creation of diverse habitats, which in turn facilitates a greater presence of natural enemies that control pest populations. Unfortunately, the underlying mechanisms that drive the bottom-up and top-down effects are not tested In this study.

Further, this master's thesis aimed to evaluate the connectivity by looking at the edge-to-center gradient within the stand and then comparing these positions to the two different stands, one containing a shelterbelt and one without. Species have specific fulfilment of niches, hence, in order to test this, forest stand edges positioned in different directions, North or South, were investigated to prove if the different stands influence the occurrence of species. The assessment will involve the use of plasticine prey as a quantitative model measurement and active pheromone trapping as a means of indirect assessment.

During a summer season trial in a temperate woodland in Belgium, different marks and frequencies left by attacks on artificial caterpillars were observed, as a proxy for the rate of predation and investigated the relationship between predation and forest pests. Invertebrates and avian beak marks were the most common predators. Predation rates were not as high in the late dry summer season, suggesting that this could be a functional rather than a numerical response of predators (predator populations increasing after with peak in prey abundance), or adaptively timed phenology (breeding time to follow with the seasonal increase in prey abundance), and that predation would essentially decrease the abundance of pest Lepidopteran communities. Results showed that there was a higher predation rate occurring

in the center for the models but not at the edges. Upon further analysis, it was found that the fluctuation in numbers among moth defoliators exhibited a more pronounced pattern in mixed forests compared to monocultures, in regards to the position within the stand. Furthermore, it was observed that forest heterogeneity did not have a noticeable impact on predators, as they demonstrated an equal ability to locate their hosts in both simple and complex habitats.

In addition, this study highlights the relevance of understanding predator and pest species effects in broadleaved shelterbelts that can help inform sustainable forest management practices and provide possible monitoring recommendations, such as the design and placement of shelterbelts, and the selection of tree species for planting. By incorporating this information into forest management plans, it is possible to enhance the ecological sustainability of forest monocultures. Evaluating the predation variation is, therefore, crucial to understand the mechanisms underlying the complex forest ecosystems. The research also needed to include information about the management of these communities and species.

## List of abbreviations and list of symbols

ANOVA - Analysis of Variance

ES - Ecosystem Services

EU - European Union

GLM – Generalized Linear Model

GLMM - Generalized Linear Mixed Model

g – gram

ha – Hectares

IPM - Integrated Pest Management

SDG - Sustainable Development Goal

SES - Social-ecological systems

UN - United Nations

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# 1. Introduction

## 1.1. Climate Challenges and its Impact on Forests: An Overview

Often perceived as a human-induced activity, global change is commonly perceived as changes in the Earth system involving irregular fluctuations in climate (van den Berge, 2021). Numerous climate research provided clear compelling evidence observing a consistent warming trend, with the global average temperature that increased by 0.8 °C since 1900 (Lindner et al., 2010). With this being said, record breaking temperatures have been recorded over the last twelve years globally (Lindner et al., 2010). Climate change can be defined as the rapid change in environmental conditions associating with occurrences of frequent and extreme abiotic disturbances like heatwaves, droughts, and heavy rainfall, affecting both food security and terrestrial forest ecosystems negatively (Sebald et al., 2021). Whilst forests are considered as important carbon sinks, areas that absorb more carbon than released, the rate to which the frequency and intensity of droughts is happening, especially affects forest ecosystems by influencing their structure and composition (van den Berge, 2021). In fact, studies indicate that the release of atmospheric carbon dioxide gas is expected to triple by 2030, thus further resulting in frequent wildfire outbreaks as well as altered water cycles (Maddelain et al., 1990). Due to forests' vulnerability, their physiological tolerances can be surpassed leading to their ability to adapt to rapid environmental shifts quite limited, impacting tree growth and survival. While forest ecosystems have demonstrated ecological resilience and adaptability to the shifting conditions, continuous changes might exceed the initial adaptive potential of forest species or ecosystems (Sturrock et al., 2011). Following this, tree mortality rates are expected to rise, further influencing species composition, structure and function (D'Amato et al., 2013; Lindner et al., 2010). Forests play a vital role serving as sanctuaries for terrestrial biodiversity, and with strong evidence indicating that climate alterations will continue to affect species and possibly lead to the extinction of numerous species across various taxonomic groups (Sturrock et al., 2011). In turn, increasing the number of endangered species resulting in a biodiversity crisis, recognized as the sixth major mass extinction (Freer-Smith et al., 2019; van den Berge, 2021).

As recent global warming caused many changes in forests, some species benefit from this. Climate change facilitates the extended range of geographical distribution of both indigenous and non-native pest species (Jactel et al., 2012). As the susceptibility of trees are lowered, the most likely biological invasions will occur. Pathogen agents and forest insect pests cause disease outbreaks resulting in tree mortality especially in forested landscapes (Jactel et al., 2021). On the contrary, trees located at the edge of forest stands are most prone to attacks by pests further exposing them to possible pathogen infections (Jactel et al., 2021). Forest edges are prevalent features in numerous European regions, resulting from historical processes of forest fragmentation influenced by agricultural and urbanization activities (Terraube et al., 2016). Forest fragmentation primarily attributes to the increased length of forest edges and establish pronounced differences between edges and the center of forest stands (Guyot et al., 2019). While the significance of forest edges in supporting diverse forms of biodiversity has been recognized in the past, there has been limited extended research done into the specific mechanisms that contribute to variations in the extent of edge effects and the surrounding landscape (Terraube et al., 2016). According to Dulaurent et al. (2012), several studies proved that edges do contain higher pest infestation than the trees located in the center of a stand. The phenomenon known as the 'edge effect' can be further supported by the fact

that there are higher quality of sun-exposed leaves further increasing the feeding mechanism of insects due to their characteristics of being often warmer and drier associated with higher photosynthetic radiation capability than in the center (Anderson et al., 2019). Thus, creating the presence of preferable microclimatic conditions favouring poikilothermic organisms and as a result, produce more generations annually through having higher survival rate thus increasing their expansion range, and reproduction rate (Jactel et al., 2019; Roy, 2006; Valdés-Correcher et al., 2019; Anderson et al., 2019; Dulaurent et al., 2012). This seems to be the case for the defoliator lepidopteran species like *Lymantria monacha*, *Panolis flammea*, and *Dendrolimus pini* benefitting from these changing environmental conditions and are considered to increase as serious pests to pine trees (Melin et al., 2020; Johansson et al., 2002). In addition, significant financial losses and expenditures for forest protection, outbreaks can have a significant influence on the forests' capacity to function as a proper carbon sink and net ecosystem production (Beule et al., 2017). With the increasing dispersal pattern of forest insect pests boosts the bottom-up system (Anderson et al., 2019; Tvardikova & Novotny, 2012). Similarly, top-down control of herbivory will eventually be enhanced through an increase in insects (Tvardikova & Novotny, 2012; Sinclair & Krebs, 2002). Take for example, when birds or other predator population decrease, herbivorous insectivore populations significantly increase impacting herbaceous fauna health negatively ultimately, displaying trophic cascades (Tvardikova & Novotny, 2012).

## 1.2. Problem Statement

Although the idea of sustainability is widely accepted in the forestry industry, it is not always put into effect (Liu et al., 2018). Initially, forest plantations were managed as part of an important component within the timber industry, contributing to the economy of numerous countries. With the concept of afforestation being a commonly known practise, implemented globally, these plantations are still managed as monoculture species stands (Liu et al., 2018). This is problematic seeing that monoculture stands have similar genetic makeup, with their close proximity making them susceptible to pests and may lead to the decline in local bird species, thus, lowering their conservation value (Jactel et al., 2021). Adopting practices like mixed-species plantations will lead to an increased number of ecological niches by providing species with habitats and increasing resistance to pests (Liu et al., 2018). This particular resistance is referred to as associational resistance and is often attributed to the concept of resource concentration (Castagneyrol et al., 2014). Following the resource concentration hypothesis, insect herbivores would therefore be less likely to locate and persist in patches where their host plants are present. This can be attributed to either a straightforward 'host dilution effect' or the physical concealment of the target plant by neighbouring heterospecific plants, which in this case broadleaved tree shelterbelts (Castagneyrol et al., 2014).

Unfortunately, with the growing importance of plantation forestry, these structures have often been perceived negatively, particularly regarding their impact on biodiversity richness (Powers, 1999; Williams et al., 2013). Planted forests are characterized by the formation of extensive edge lengths due to fragmentation however, the quality of these edges in terms of biodiversity conservation can be inconsistent. Within planted forests, a majority of edges tend to be sharp and abrupt, and a substantial portion of these edges are exposed, particularly those bordering open habitats such as clearcuts or young stands (van Halder et al., 2011). It is argued that planted forests were structured in a way that is biologically and physiologically simplified apprehending a lowered resilience to natural disturbance agents and typically referred to as 'biological deserts' (Powers, 1999). Consequently, it follows that the productivity of plantations

is considered unpredictable and, in all likelihood, unsustainable (Powers, 1999). Considerable factors may come into play when implementing such practise. This may involve accidental poor selection of a single introduced, non-native tree species which can link to many issues regarding to policy rights, social issues, land tenure, declining biodiversity, and negative environmental effects, especially in terms of increased water use (Freer-Smith et al., 2019). Not only does the alteration of tree species create environmental problems, but it also hinders habitat formation necessary for species survival (Bernaschini et al., 2019). Birds in particular, that in general are well adapted to climax-forest communities, are especially affected by land-use change. Their ability to only occupy narrow niche breadths make them especially vulnerable (Iezzi et al., 2018). Further, plantation forestry is seen by ecological managers and conservation planners as a dynamic process characterized by the gradual reduction of habitats into disconnected patches that experience increased isolation also known as a process referred to as fragmentation (Echeverría et al., 2007; Terraube et al., 2016). Further, these patches experience increased microclimatic changes in the edges, other known as the abiotic edge effect (Echeverría et al., 2007). With numerous studies providing evidence indicating forest edges exhibiting distinct characteristics compared to the forest center, drastic changes abiotic changes would be expected. Due to prolonged exposure to sunlight, these areas experience increased soil temperatures resulting in lowered moisture levels therefore putting trees at risk for drought (Bernaschini et al., 2019). As a result, these changes can influence the community composition, ecological process such as predator-prey relationships and increased susceptibility to disturbances (Bernaschini et al., 2019; Echeverría et al., 2007).

### 1.3. The practice of afforestation: A Solution

At the end of the twentieth century, planted forests have experienced a significant expansion from 168 to 278 million ha and as a result, the total area covered by both natural and planted forests now accounts for approximately 7% of the world's total land area (Freer-Smith et al. 2019). Countries in Europe especially experienced an increasing trend of forested areas, expanding approximately at a rate of 0.8 million ha annually since 1990. This positive trajectory is anticipated to persist in the foreseeable future (Valdés-Correcher et al., 2019). The reason for this drastic expansion can be attributed to several factors, such as the widespread abandonment of agricultural lands, reduced grazing pressure, and increased reforestation programs (Fischer et al., 2010). In 2015, several global initiatives and agreements like the Forest Europe process, and the Paris Climate Agreement, underscored the critical importance of conserving natural forests and recognizing the valuable benefits offered by forest plantations (Fischer et al., 2010). The advantages provided included social, environmental, and economic aspects, further contributing the mitigation of climate change, preserving soil health, and generating employment opportunities (Fischer et al., 2010). It also presents tremendous opportunities for conservation, despite the fact these areas were essentially developed as a land-saving strategy, focusing on intensive wood production with yields marking up to 33% of the world's roundwood supply (Freer-Smith et al., 2019).

Although, the conversion of natural areas into forest plantations lead to changes in biodiversity, in some instances, similar biodiversity levels can be found when comparing the two areas with each other, further suggesting a positive impact on biodiversity resulting from the establishment of forestry plantations (Sousa-Silva et al., 2018). Looking at the landscape scale, natural disturbances in plantations along with changes to spatiotemporal heterogeneity through creating an even-aged structural matrix, may assist in the restoration of forest biodiversity (Sousa-Silva et al., 2018). This creates and serve as an alternative environment



for native species offering a viable yet preferable habitat as canopies promote growth for plants residing in the understory thus providing shelter and additional food resources (Iezzi et al., 2018). However, the rate to which certain disturbances occur, pose a threat as resource managers currently face the most pressing challenge posed by climate change, uncertainty. Predictions surrounding future conditions of forests urge immediate strategy development thus aiming to enhance adaptive capacity and possibly reducing ecosystem vulnerability (D'Amato et al., 2013). These strategies involve but are not limited to modifying the structure and composition of forests therefore fostering the incorporation of tree species diversity in plantations further promoting ecosystem productivity. In the end, this will have the potential to mitigate the intense impacts of climate change (D'Amato et al., 2013). Equally considered important is the fact that mixed forests further enhance biodiversity, evidence showing a rise in bird and beetle population richness and abundance in the forest (Felton et al., 2010). Adopting mixed-species afforestation practice will result in these forests being well adapted to disturbance regimes (Sousa-Silva et al., 2018). In turn, playing a crucial role in enhancing the resilience of forest ecosystems against potential pest outbreaks (Sousa-Silva et al., 2018; Guyot et al., 2019).

Due to its effectiveness, the approach of employing land-use mosaics, which combine forest plantations with shelterbelts, is implemented in numerous regions (de Bonilla et al., 2012). This method serves to enhance ecological integrity, enhance their capacity to support biodiversity while addressing climate and environmental challenges. However, for the designing process of mixed species shelterbelt plantations in order to optimize the benefits, additional research is necessary to understand spatial ecology (Freer-Smith et al., 2019; de Bonilla et al., 2012). Shelterbelt forest plantations are often a significant component of landscape-scale restoration and can bring degraded land back into production and improve the provision of ecosystem services (Freer-Smith et al., 2019). In the end, mixed-species forests offer numerous benefits to many end users, while simultaneously mitigating the production risks of biotic and abiotic factors, resulting in an interest to adopt policy initiatives in many countries promoting the transformation of certain coniferous monocultures into mixed-species stands (Felton et al., 2010).

### 1.3.1. What are shelterbelts?

Depending on their region or intended use, shelterbelts are known by others as windbreaks, hedgerows, or firebreaks (Mize et al., 2008). Broadleaved tree shelterbelts create a range of benefits. Normally planted in a forest monoculture setting, 5 to 10 meters from the edge of forest stands, shelterbelts are able to preserve a wide range of biodiversity through increasing the quality of habitats and ensure the stability of outlying areas (Dulaurent et al., 2012). Shelterbelts are generally maintained to offer control and/or stop the overgrowth of trees into nearby fields or other forest stands (Dulaurent et al., 2012). Their ability to favour structural complexity enables them to protect tree host species prone to pest, while providing essential ES, thus making them a runner-up to use as a barrier for natural pest control (Dulaurent et al., 2012; Felton et al., 2010). By minimizing wind erosion, reducing fire risk and enhancing water usage, shelterbelts conserve many young trees throughout the summer and winter seasons. This, in turn, decrease the overall biotic and abiotic susceptibility towards prolonged environmental stress (Griess & Knoke, 2011). Considered to function as corridors, these structures ensure connectivity amongst the forest landscape enhancing the movement of species further improving seed dispersal and mitigating the effect of habitat fragmentation on semi-natural areas (Dover & Sparks et al., 2000). Shelterbelts are especially utilized by

predators to travel between forest stands, therefore facilitating access to additional resources and establish new population (Dover & Sparks et al., 2000). This is beneficial due to the fact that the integrity of species metapopulations are maintained, aiding in genetic exchange and variation between local species.

However, it is important to note that while shelterbelt networks may play a crucial role in facilitating predator movement between preferred habitats, they also provide an opportunity for pest species to invade and exploit within the corridors (Dover & Sparks et al., 2000). Due to its linear exposed structure, a complementary habitat is created that fulfil a range of niches harboured by insect herbivores, especially pests, thus, representing a difficult incorporation strategy to individual landowners, forest managers, and society (van Den Berge, 2021; Mize et al., 2008). According to Roy (2006), there has been some uncertainty concerning the ability of these marginal habitats to perform specific ecological functions, particularly in the sense for poor dispersers, but van den Berge (2021) and Felton et al. (2010), both stated that many migration species still rely on shelterbelts as temporary stop-overs. Even for poor dispersers, the introduction of shelterbelts exerts small-scale changes through creating vital micro-habitats in turn, enhancing species diversity within large-scale pine plantations (Terraube et al., 2016). The positive correlation between the increased diversity of insect herbivores and mixed tree species would lead to an increase of more predator species, therefore providing a method of natural pest control (Jactel et al., 2021). Managing shelterbelts is not a new technique nor unfamiliar, in fact, centuries ago, shelterbelts have been utilized to manage the natural environment in forested settings, and more recently, the border between rural-urban areas. These systems have been considered to create several positive economic, social, and environmental benefits for society (Mize et al., 2008).



*Figure 1: Illustration of two stands with and without broad-leaved shelterbelts. The stand on the right contains a broadleaved shelterbelt whereas the stand on the left does not have a shelterbelt.*

### 1.3.2. Ecology of predator-prey interaction

Ecology is a term centred to focus on the pattern of organism distribution and population size through studying their various interactions within ecosystems (McGill et al., 2006). One of the most fundamental interactions in ecology is biological control, through predation of naturally occurring prey species (Howe et al., 2009). The dynamics of predator-prey interactions stand as a pivotal cornerstone within ecological systems being most profound and recognizable when studying predators such as bird species. These predators offer key ecosystem services while enhancing the stability and structure of surrounding ecosystems in forested landscapes through controlling insect herbivores or pests (Muiruri et al., 2016, Gunnarsson et al., 2018; Lövei & Ferrante, 2017). This way predators can indirectly regulate the quantity of plant biomass consumed in these natural landscapes by implementing top-down trophic cascades (Valdés-Correcher et al., 2022). Ecosystem processes like carbon sequestration, biomass productivity, and nutrient cycling can all be significantly impacted by insect herbivores if not managed properly (Muiruri et al., 2016). Up to date, bird predation can be considered as the primary mortality factor of externally feeding caterpillars of the Lepidoptera order (Jonason et al., 2014). The intensity of predation is influenced by the type of forest and the level of disturbance, which can affect the abundance of predators or their ability to locate prey. Specifically, in fragmented forests with sudden edge effects emerging from disturbances, a correlation can be seen with an increased abundance of pests (Tvardikova & Novotny, 2012). Nevertheless, there remains a significant conservation challenge in comprehending the impact of habitat edges on mixed forests and the ways in which specific traits influence predator communities (Terraube et al., 2016). This is considered crucial, as it has the potential to trigger detrimental effects on the functioning of predation that contribute to important ecosystem services in forests, including pest control (Terraube et al., 2016). The distribution of resources and the interactions among various species are the primary mechanisms that govern the responses of individual species to habitat edges (Halder et al., 2022). Hence why the use of artificial caterpillars as "sentinels" has gained popularity among ecologists in recent years, as one of the various methods employed to investigate predation (Valdés-Correcher et al., 2022). This method offers a number of benefits, to which it being cheaper, easy to produce, replicate, and being able to easily manipulate prey distribution and density, but most importantly, it is possible to identify an array of predators (Lövei & Ferrante, 2017). However, determining a particular mark, whether it comes from a bird or mammal can be often challenging and can remain indistinguishable in cases. This uncertainty poses a barrier to gaining a comprehensive understanding of predation patterns and infers estimations of predation intensity experienced by local prey communities unreliable (Valdés-Correcher et al., 2022).

### 1.3.3. Recreational Potential of Mixed Species

Not only does mixed forest species provide ecological benefits, but also have the potential to offer a range of social benefits, providing services that includes a variety of recreational activities enforcing SES (Eriksson, 2012). SES can be defined as the relationship between the biophysical environment, in this case forests and the human society (Eriksson, 2012). In some regions, activities like berry picking holds significant cultural importance in forests while identifying other species like birds pose as an environmental education purpose in forests (Lindhagen, 2012). Mixed forests are particularly favourable for such activities due to the positive influence of tree diversity on the abundance and diversity of both fauna and flora (Eriksson, 2012). Another popular activity is cycling and nature-watching of various local birds

and small mammals. To this extent, studies also shown that the presence of small roe deer and wild boar ought to increase the tree diversity (Lindhagen, 2012).

#### 1.4. Research Aim and Objectives

Based on the context and challenges faced by forest plantations identified in the literature, this study aimed specifically to compare the performance of different forest stands either containing a shelterbelt or not. Through this, the information will then be considered as a method implemented for sustainable plantation forestry to promote species diversity and abundance. More specifically, this study utilizes plasticine caterpillars as an easily replicable and cost-effective tool to monitor ecological patterns and study predator-prey relationship of various predators. In addition, the use of pheromone traps are also considered as a convenient method to study pest species to observe and evaluate the abundance and diversity of pests. Stating this, a hypothesis can be made that forests plantations incorporating stands with broadleaved tree shelterbelts will indicate a higher biodiversity value compared to the stands without shelterbelts.

The presence of shelterbelts has a relatively positive impact on the diversity of local fauna. These shelterbelts are also expected to provide habitats for migrating birds and support other insects. Considering the edge of any forest stand, microclimatic conditions come into play when evaluating different species in mixed species stands, increasing the viability of potential pest infestation. Studies conducted in the Northern Hemisphere, have observed that south-facing forest edges experienced more sunlight therefore increasing temperatures (Bernaschini et al., 2019).

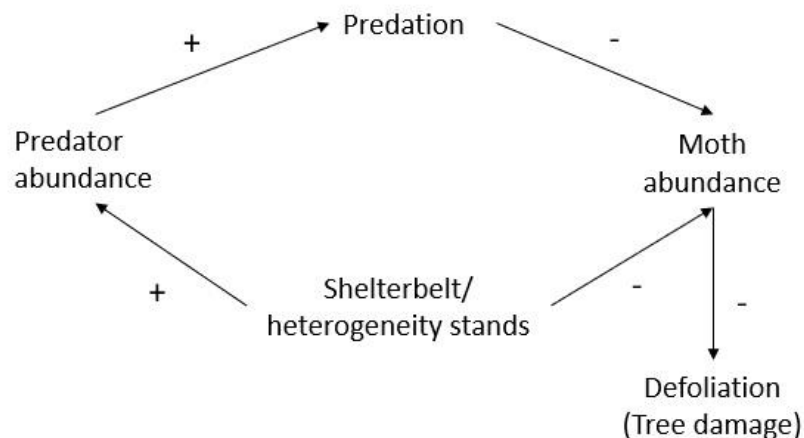


Figure 2: The following is an illustration of the hypothesized effects between the different systems in heterogeneous forest landscape. The arrows indicate the direct interaction, direction of the predicted and observed interactions between the system's components. The + denotes a favourable effect, - indicates a negative impact (Adapted from Bereczki et al., 2014).

Given the hypothesis mentioned, four core objectives have been formulated:

**Objective #1:** By looking at natural occurring predators, increased predation pressure is located at the edge of a stand containing a shelterbelt further revealing that shelterbelts can be seen as an important biodiversity support.

**Objective #2:** Determining if broadleaved tree shelterbelts might serve as a natural barrier, reducing potential pine moth pest infestation towards the center of forest stands.

Objective #3: Assessing stand edges, especially containing broadleaved tree shelterbelts that is located in the South indicate lowered pest infestation, looking at the predation pressure of natural occurring pest control species as well as the amount of moths captured.

Objective #4: Assess the ecological connectivity within the two types of stands, looking at the interaction effect between the different plots and if the interaction facilitates movement of both pest and predator species.

Through addressing the research objectives, this study further contribute to the literature on the inclusion of mixed tree species in monoculture forest stands and further promote conservation thereof. The findings can aid forest managers to carry out the necessary resource management plans that promotes long-term viability and resilience of forest stands. The acquired knowledge can be utilized to further improve management practises that would improve environmental conditions and increase conservation of biodiversity.

## 2. Materials and Methods

### 2.1. Site Characteristics

#### 2.1.1. Selection of study site

Forests cover approximately 11% of the total land area in Flanders, Belgium with nearly half of the forested areas comprising pine plantations, while only one-third of the forested areas are occupied by mixed broadleaved tree stands consisting of native species like oak (*Quercus* spp.), beech (*Fagus sylvatica* L.) (Vandekerkhove, 2013). Therefore, the forested regions of Ravels and Pijnven were specifically chosen for evaluation for this project as it contained mature pine forests with broadleaved tree shelterbelts. For this reason, it will help gain valuable insights from these regions to apply and adapt the findings in other pine plantation areas worldwide.

The “Gewestbos van Ravels” is situated in the province of Antwerp, north of Turnhout and features a diverse mix of coniferous (pine) and deciduous (oak) trees, as well as bogs and heathlands. On the other hand, “Pijnven Bosland”, located in the province of Limburg, is predominantly composed of planted coniferous mixture stands alongside grasslands and heathlands. Upon evaluating and comparing the stands in both forests, it became apparent that Ravels had fewer desired broadleaved tree shelterbelt stands to the stands compared in Pijnven. Pijnve offered a greater abundance of broadleaved shelterbelt trees that were more accessible in terms of height, for installation purposes.

#### 2.1.2. Climate

Pijnven offers temperate and often pleasant weather conditions during the summer months of June to August, with average temperatures ranging from 20°C up to 35°C. On the other hand, February is considered as the coldest month, with an average maximum temperature of 5°C (Campioli et al., 2012). This region is situated along the northern gradient of Belgium as it receives less sunlight than the south, due to cloudiness and recently a study confirmed that there is a considerable amount of decline in annual sunshine with decreased hours from 1723 to 1541. Coupled with this, a corresponding increase in annual precipitation from 660 mm to 1015 mm was also found (Neiryneck et al., 1998).

#### 2.1.3. Topography

Situated at the periphery of the high campine plateau, the Pijnven forest encompasses a mean elevation range of 50 to 58 meters characterized by tertiary sands and gravel-rich sands deposited by the Meuse River (Neiryneck et al., 1998). This area is also structured relatively flat, with the altitudes increasing towards the south whereas the west and northern areas are lowlands.

#### 2.1.4. Soil

The forest soil in this area consists of nutrient-poor sandy-loam particles, known as Carbic podzols (Vangansbeke et al., 2015; Neiryneck et al., 1998) The area went through immense plowing, converting from heathland to pine afforestation, in order to break the iron-accumulation horizon that was prevalent in the early nineteenth century (Verheyen et al., 2013). Subsequently, Scots pine (*Pinus sylvestris* L.) and Corsican pine (*Pinus nigra* L.) were cultivated for three consecutive years to enhance nitrogen fixation in the soil. In addition, supplementary fertilization with phosphorus and lime was applied (Jonckheere et al., 2005).

#### 2.1.5. Vegetation

Situated near Hechtel-Eksel, the state forest Pijnven covers approximately 10,000 ha of the landscape (Vandekerkhove, 2013). As mentioned before, heathlands in the area were afforested with Scots pine (*Pinus sylvestris* L.), however, in most of these stands, significant development has occurred with the introduction of deciduous tree species. The forested area consist mostly of pine trees, while European beech (*Fagus sylvatica* L.) and oaks (*Quercus* spp.) account for approximately one third of the plantation (Vandekerkhove, 2013). The stands containing shelterbelts consist of various tree species with the ingrowth primarily dominated by exotic tree species namely the American red oak (*Quercus rubra* L.) and black cherry (*Prunus serotina*) (Maddelein et al., 1990).

#### 2.1.6. Management

During the early twentieth century, the forest practice were primarily centered on regulating the rate and manner of wood exploitation adopting methods such as artificial afforestation thus, resulting in monocultures solely based on timber purposes (Farrell et al. 2000). Fortunately, this practice later evolved towards a more sustainable and balanced approach, managing various forest goods and services while placing considerable emphasis on conserving and sustainably utilizing forest products (Lindenmayer et al. 1999). To date, this type of practice is widely endorsed in Flanders and in numerous regions across Western Europe, further promoting the shift from monoculture species production to a more diverse forest landscape with multifunctional, mixed tree species forest stands (Maes et al. 2011).

### 2.2. Experimental design

During the design phase of the project, scheduling and extensive literature review was considered as a very important steppingstone. For the first part of this experiment, 52 plots were selected in the stands with their edges varying from north to south. For this experiment, stands were selected based on the tree species, estimated age and density. Furthermore, the environmental conditions for each chosen tree were overall similar thus providing more or less the same habitat conditions and were not located near areas affected by water. This is considered important as species can utilize water resources better than others resulting in the creation of different micro-habitats (Valdés-Correcher et al., 2022). This method of selection creates a diversity gradient within the stands so that habitat connectivity can be assessed. The total above-ground biomass of stands in the area consisted of species varying between 72 and 96 year of first-generation tree species (Maddelein et al., 1990). Broad roads give origin to the geometric pattern that divides the forest into rectangular stands of 4 to 5 ha each. With this being said, some stands located along these roads are therefore surrounded by shelterbelts consisting of broadleaved tree species. Although the spatial distribution of stands with and without shelterbelts is not perfectly random, it was deemed acceptable for this study. 12 stands with broadleaved tree shelterbelts and 8 stands without broadleaved tree shelterbelts were eventually chosen. To further elaborate on the 12:8 ratio, due to spatial clustering in this area, it was originally thought that there would not be enough sufficient suitable stands without shelterbelts to find that would allow for comparison. This was how it would be determined to sample the centres of the stands with shelterbelts as a proxy for the situation in a stand without shelterbelts. The stands containing shelterbelts had a total of three trees sampled with the broadleaved tree of interest, ideally being an oak species, *Quercus rubra* or *Quercus robur*, represent as a sample plot for the shelterbelt. While plots at the edge and center, containing pine species *Pinus Strobus*, Douglas fir or Scots Pine as illustrated in Fig.3. The stands

containing no shelterbelt had a total of two trees sampled with the plots consisting of only of pine trees located at the edge and center. Following the same procedure, the second part of this experiment sampled two plots per stand both in stands consisting of a shelterbelt and not, thus excluding the shelterbelt as a plot, making the total number of sampled plots 40.

As illustrated in Fig. 5, each stand containing shelterbelts had an estimated measure of 5 meters in width from the edge with each stand being approximately 200m x 200m each. Lastly, using a Garmin GPSMAP® 64s handheld navigator assisted in plotting the waypoints for accurate mapping. The waypoints will then be used as reference points in the field and further mapped using QGIS Standalone Installer Version 3.22 Long term release.



*Figure 3a): Example of a stand with shelterbelt that contained the broadleaved (diamond-shaped symbol) and pine tree (circle symbol); b) illustrating a second-generation tree marked with a x symbol.*



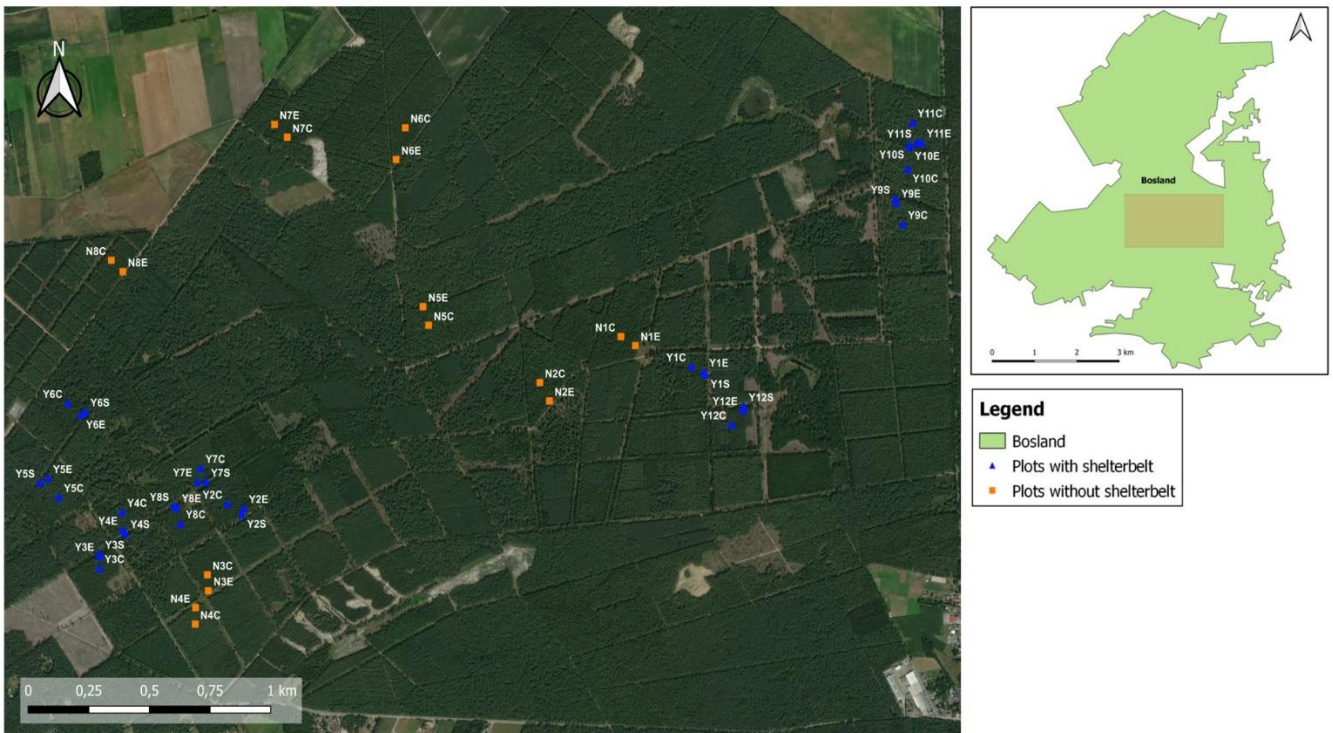


Figure 4: Map of the study area located in Bosland, a nature reserve located in the municipality of Hechtel-Eksel, Flanders, Belgium.

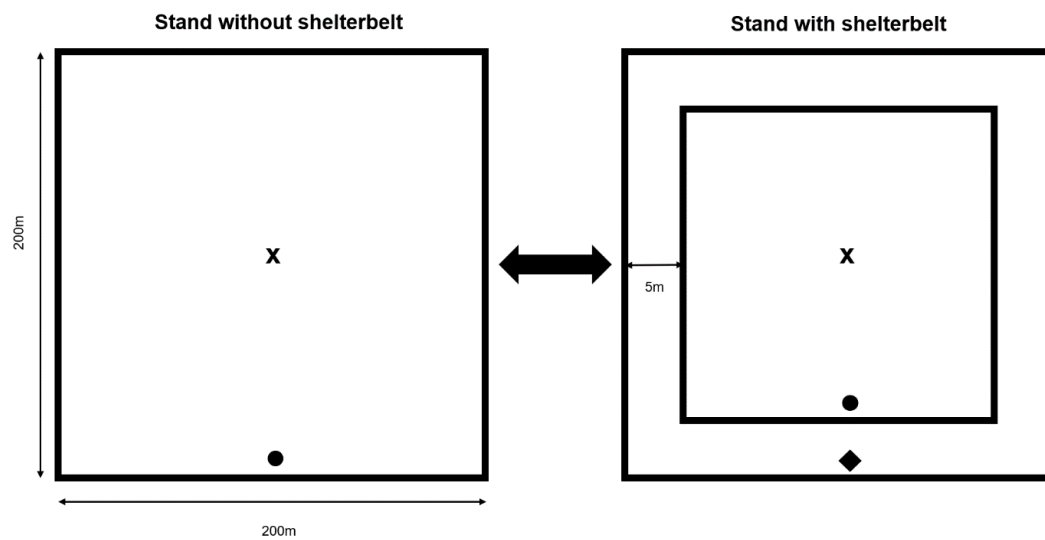


Figure 5: Illustration of experimental design of pine stands with broadleaved shelterbelts and stands without shelterbelts. The crosses indicate plots located in the centre of the stand while the circles are plots located at the edge of the stand. The diamond represents the plot located in the shelterbelt.

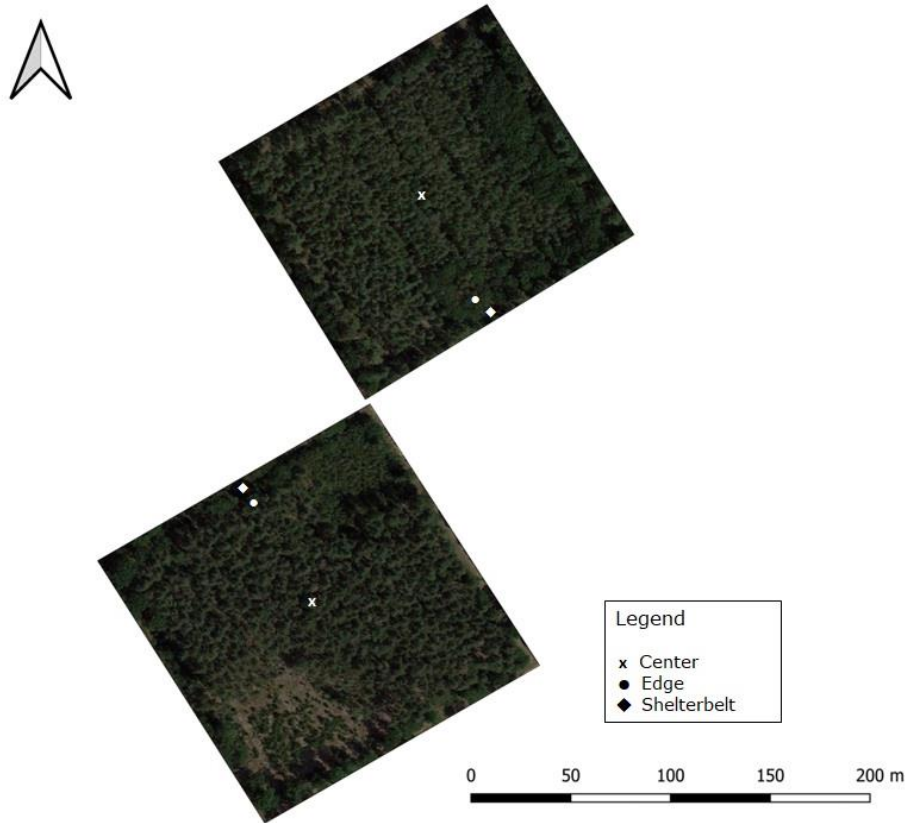


Figure 6: Example of two plots with shelterbelt with each of their respective edges facing in different directions. The stand edge situated at the top is facing south while the stand edge underneath is facing north.

## 2.3 Caterpillar experiment

### 2.3.1. Plasticine caterpillars as sentinel prey

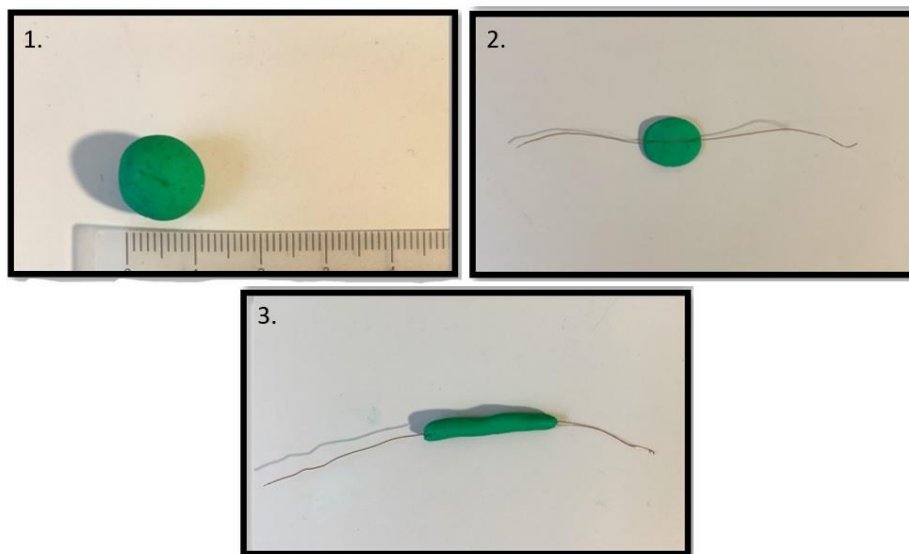
It should be noted that the process of creating caterpillars relies on the malleability and flexibility of the plasticine or other known as modelling clay, that needed to be shaped by hand into a cylindrical form that closely resembles a live caterpillar, capturing the exact body size, colour, and morphology. Numerous experiments that investigate predator-prey interactions utilize caterpillar models regarding the fact that the process to make them is easy and less time-consuming. In addition, models can be crafted in large numbers for more accurate results. The malleability of plasticine makes it possible to see traces left behind by a potential predator, unlike other materials such as clay or wax. Further, this type of plasticine can be used in various types of environments as it can be non-toxic, consisting without any traces of polymer, which can potentially harm the environment as well as the organisms that digest it. What makes the plasticine most preferable, would be its' durability in the environment when exposed over long periods of time in harsh weather conditions.

On the other hand, plasticine is available in a wide variety of colours, offering scientists the opportunity to simulate and study how predators react to pest caterpillars resembling striking colours. Bright colours are typically seen as a defence mechanism to warn off potential predators, however, for this study, only green plasticine is used. According to Valdés-Correcher et al. (2022), green caterpillars are often used in studies and are seen as the most effective technique to study predator-prey relationships. A study done by Roeder et al. (2022), compared different colours of plasticine with each other in the field and how predators react to

them. It was found that green caterpillars often performed the best when compared to other colours possibly giving the intention that green caterpillars are more palatable and characterized as undefended prey (Howe et al., 2009).

### 2.3.2. Creation of caterpillars

Following the protocol attached in Annex 2, the necessary material listed was acquired and utilized to create the caterpillars. These included STAEDTLER 8421 Noris Club Plasticine Clay 1000 g Green to create the 'prey caterpillars' by hand, Crochet Wire - 0.30 mm Diameter, 50 m, Jewellery Wire Colour Copper in order to fix these caterpillars onto the desired surface of the tree branch or stem. Using the protocol in Annex 2, Fig. 7 illustrates the initial steps towards creating a plasticine caterpillar. The first step starts with preparing a ball of plasticine clay, 1 cm in diameter, weighing approximately 1 gram. Next, the ball is flattened fixed with a thin wire measured at 12 cm in length. Roll the clay to at least 3 cm in length, with the wire still intact until it takes the shape of an elongated worm. Although the models can have some shape variation, uniformity is often the best practice for accurate results. Before installation, each caterpillar should be inspected for any deformities and indentations. If any indentations commonly made by human nails would occur on the surface of the caterpillars, make sure that it has been smoothed out. In order to avoid accidental markings, is recommended that the completed caterpillars should be fixed onto styrofoam blocks.



*Figure 7: Step by step illustration on how to create a plasticine caterpillar.*

### 2.3.3. Installing the caterpillars

Following the steps indicated by Annex 2, Fig. 8 applies the method of installation that was carefully carried out. Upon choosing the desired tree, it would be beneficial to flag it with a brightly coloured ribbon for easy identification in the field. Flag each tree at the base of each tree instead of placing it near the caterpillar placement. This will avoid bias data and conflict with predators that associate bright colours as a present caterpillar. Following Fig. 8, the placement of each caterpillar should be aligned along with the branch, mimicking the actual position of live prey making sure that each branch is cleared (10-15 cm) of leaves to avoid "brushing" and should not be too thick. A suitable branch of less than 1 cm diameter would be optimal. If the branch would be too thick, the wire would be too short and cannot fasten the

caterpillar. One end of the wire should be carefully wrapped around the branch with the plasticine model placed between the thumb and index finger. Carefully wrap the other side of the caterpillar around the branch. Take into account when handling the caterpillar models, as too much handling can inflict marks deemed as false positives in the experiment. In addition, spatial configurations and densities of the caterpillars should accurately represent the patterns found in naturally existing prey populations. Three caterpillars were placed based on four cardinal directions namely, north, south, east, and west according to the predator's discretion, concluding with 12 caterpillars in each chosen tree.

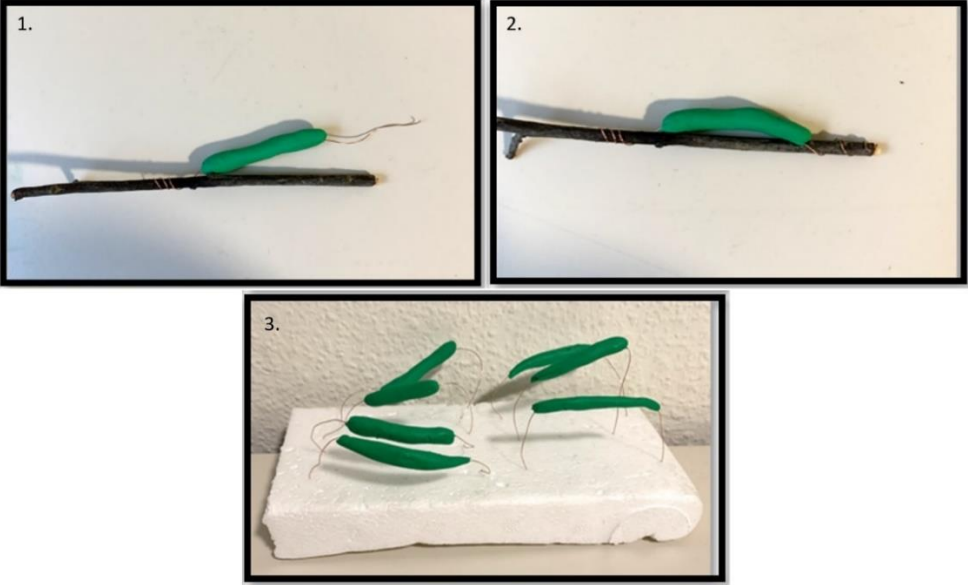


Figure 8: Step by step illustration of installing a caterpillar. 5.3. Shows caterpillars fixed on a styrofoam block for easy transportation and handling.



Figure 9: Illustrated is a plot containing the installed caterpillars indicated by the red arrow.

#### 2.3.4. Retrieval process

Seasonality plays a significant role when analyzing predator-prey interactions as the breeding and feeding seasons of various predators can influence the data. Avian predators such as the Eurasian Great Tit (*Parus major*) breed approximately four months throughout the year, March to June, with nest feeding occurring in the same time period as well (Pimentel & Nilsson, 2007). Concluding that the installation would be commenced in late May, and collection happening in late June.

Initially planned as a two-week experiment trial to which the caterpillars would stay in the field, upon inspection it was found that the models did not show any empirical evidence whether predation had happened. Taking this into account, it was decided that another two-week 'incubation' period would be beneficial for optimal results. After four weeks, the caterpillar models were removed from the field and analysed. During the process of removal caution should be taken as additional marks can lead to false positives. It is often best to collect the caterpillars still intact with the whole stem ensuring minimal handling, if caterpillars would be examined ex situ, best to fix them on marked styrofoam blocks stabilized in a cardboard box (Fig. 10).



Figure 10: Representation of identifying and stabilizing caterpillars onto marked Styrofoam in a cardboard box.

The inspection happened in situ. Predation levels can be estimated using counts of predation and parasitism on artificial caterpillars. As expected, the presence of predation marks left by birds was the common occurrence observed. These marks are distinguishable by their distinctive "V" shape as indicated in Fig. 11, while the presence of slugs can be identified by their distinct repeating chewing marks. Surprisingly, marks by rodents were found, as these marks occur infrequently. Lastly, airholes can be seen made onto the caterpillar by potential parasites.

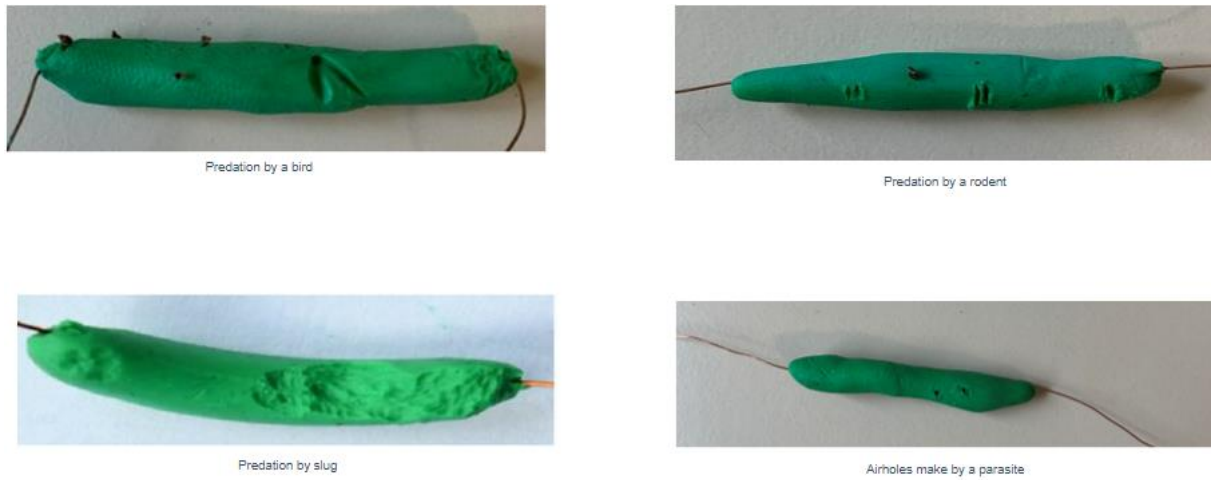


Figure 11: Different types of predations found on the plasticine caterpillars.



Figure 12 a): Caterpillar fixed on a branch, b) Collection and inspection process of caterpillars.

## 2.4. Pheromone trapping experiment

### 2.4.1. Study organisms

The species chosen are regarded as one of the most destructive pests in European coniferous forests, posing significant threats to the forest ecosystem (Morewood et al., 2000). Furthermore, the damage inflicted on the trees caused by the defoliation (loss of leaves) of these species can impact tree growth further affecting decreased timber production (Morewood et al., 2000). Severe and repeated defoliation can have even more severe consequences, potentially resulting in the death of the tree, especially in young trees and trees growing on unfavorable soil conditions. Additionally, trees weakened by defoliation become more vulnerable to secondary pests, like bark beetles (Jacquet et al., 2012). Verified by their occurrence made through various observations by citizens, a local website called waarneming.be confirmed their presence in the study area.

*Lymantria monacha*, known as the nun moth (Fig. 13a), has a distinct medium-sized white and black pattern and is considered a significant defoliator of coniferous forests across the

European forests (Wang et al., 2019). This species is quite polyphagous seeing that it feeds on a wide range of species, from *Quercus* to *Pinus* while its' larvae feed on the needles of pine trees and adults feeding on conifer cones and leaves. Outbreaks happen sporadically, meaning that their outbreaks are likely triggered by a series of consecutive hot and dry summers and frequently manifest across expansive regions (Vanhanen et al., 2007).

The *Dendrolimus pini*, other known as the pine-tree lappet or sawfly moth (Fig. 13b), is considered one of the most prominent harmful insect species that significantly affects forests especially predominantly observed in Scots pine (*Pinus sylvestris*) thriving in impoverished and sandy soil conditions. Covering areas from Western Europe to North Africa, species outbreaks has been recorded frequently. In Belgium, the *D. pini* is the only species to overwinter as larvae (Skrzecz et al., 2020).

The moth species known as the pine beauty (Fig 13c), scientifically referred to as *Panolis flammea*, is a significant pest that poses a threat to various tree species belonging to the Pinaceae family in Europe. The caterpillar larvae of this moth have been observed feeding on a range of trees, including Douglas fir, pine, specifically Scots pine, and spruce (Gilligan & Passoa, 2014). The early instar larvae have a feeding behaviour where they consume the needles of newly grown foliage, while the on-stage instar larvae feed on older foliage. Outbreaks of *P. flammea* have been reported in pine plantations across the United Kingdom and Europe, leading to substantial damage covering extensive areas of land and causing significant mortality among the affected trees (Gilligan & Passoa, 2014).

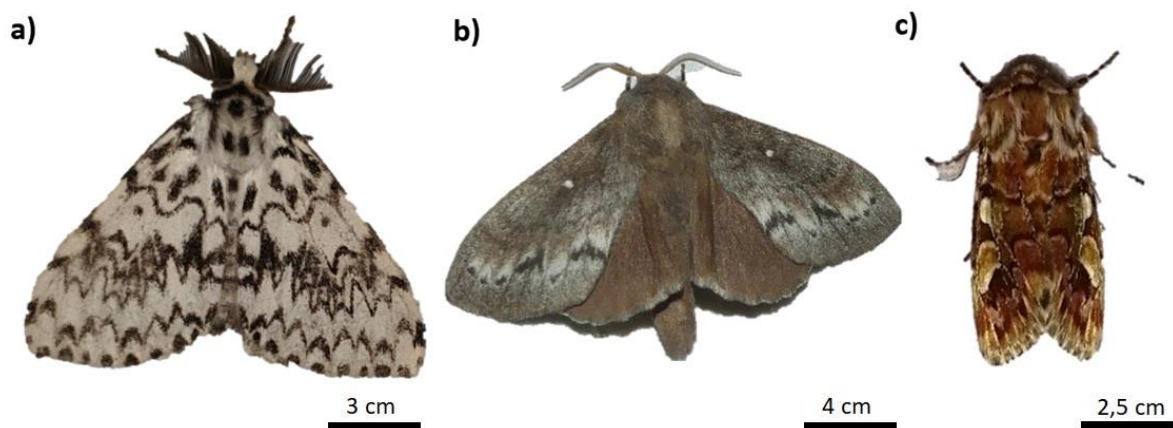


Figure 13: Illustrations of the moth species captured. a) *Lymantria monacha*, b) *Dendrolimus pini*, c) *Panolis flammea*.

#### 2.4.2. Trap setup

The same stands were used in this experiment; however, the experimental setup did not include hanging the traps along the shelterbelt edge but did take into account the stands that contained shelterbelts and those without.

Pheromone trapping is commonly used to compare and provide a quantitative estimate of population density and abundance of defoliators in different forest stands (Hielscher & Engemann, 2012). The act of trapping males also serves as a method to decrease the quantity

of eggs laid by females, as their ability to mate is hindered (Jactel et al., 2014). An essential aspect of utilizing pheromone trap catches for predicting outbreaks and understanding population dynamics of defoliating insects, is the correlation in the population between the number of males captured in traps and the abundance of females (Jactel et al., 2019). The use of pheromone-based monitoring mimics the pheromone released by females during breeding season therefore providing an alternative approach to estimate the number of pest moth species within a short period of time (Guerrero et al., 2014). Commonly known as a standard bucket trap in conjunction of a pheromone lure, this method can assist in monitoring especially male defoliator populations of different species while remaining constant and sufficient regardless of the number of insects caught (Guerrero et al., 2014). Synthetic pheromones are known to lure males from at least 200m distance (Melin et al., 2020). Another reason for the use of bucket traps is the fact that its effectiveness in capturing a high quantity of moths without causing damage to their distinguishing features is preferred by many. Once in the trap, the moths cannot escape making them easy to count and detect.

Fourty synthetic funnel traps each containing three lures each containing the specific pheromones of the moth species namely Panowit to attract *P. flammaea*, Lymowit for *L. monacha* and Dendrowit S. for *D. pini*.



Figure 14: Overview of the materials used during installation of the traps.

These traps were deployed in the study area in early June until the beginning of July (3 weeks) 2023. This period is essential because this is the estimated time frame to which male moths take flight to actively breed with females (Melin et al., 2020). Additionally, the duration to which pheromones remained effective is an estimated 6 to 8 weeks (Melin et al., 2020). It should be considered that the quantity of pests captured may be influenced by factors such as trap design, placement, and density. Hence, the exclusion of the shelterbelt due to its close proximity to the edge. Each individual lid consisted of three different pheromones as well as a label to identify the trap to each stand. Filter paper was used to equip the traps with an insecticide, this method will assist to kill the captured moths instantly so that the wings, necessary for identification, would not get damaged. Before assembling the traps, a one and a half meter string is cut in order to tie the string to the lid so that the trap can be hanged. Each trap was installed at the edge of the forest stand and in the center, each located at least 50 meters apart from each other to avoid interference. The traps were set to hang about 1-2

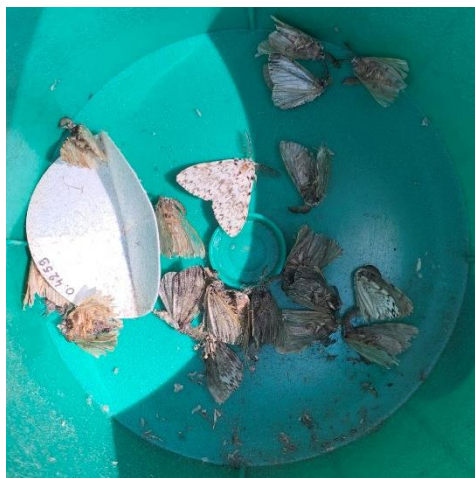


meters high above the ground from a tree branch, ensuring optimal trapping of the species of interest. If no branches are available, it is firmly tied around the tree trunk.



*Figure 15a) Installation method of the traps, b) Example of one of the traps installed in the field.*

For the retrieval process, the buckets were emptied all at once each in their respective marked plastic bag. These bags contained each a paper towel to draw any moisture from the moths, preventing them from rotting. To preserve the moths, they can be frozen and thawed when analysis is underway.



*Figure 16: Assemblages of moths in the bucket*

## 2.5. Statistical Analysis

### 2.5.1. Predation pressure: Caterpillar experiment

All 52 sampling sites were pooled where a log link function probability was used in R software studio 4.2.2. The correct assumptions of parametric statistics for the caterpillar predation density, a normality assumption was done with a Q-Q normal probability plot and the independence assumption creating a plot of the residuals on the observations used. Beforehand, the response variable (y variable = the number of markings made by the predators) was logarithmically transformed adding a very small constant value to the data ( $\log x + 0.1$ ). The purpose was to analyze the relationship of average predated caterpillars of each

plot to the fixed effects, that is, the x variables, the direction of each plot, the type of plot, and the position. A random effect was used to account for the variability between different levels of a grouping variable (in this case the "PlotID"). The model was created placed by measures of GLMER built using the GLMMTMB package for the distribution of the gamma family regression for the continuous non-normal data, the total average markings accounted for. Upon examining the GLMER, identification of data overdispersion needed to be done and spatial autocorrelation within the residuals. Luckily, the analysis revealed no significant evidence of data overdispersion. Further, analysing the effects of the model by looking at the caterpillar attacks and if the plot had a shelterbelt through a Tukey's posteriori test for pairwise contrast presented a feasible method to interpret the results. Lastly, the Kruskal-Wallis test was carried out to investigate the impact of the position and direction of the stands on the average of the total marking amount.

Using the Spearman correlation test, the proportion to which the caterpillar was attacked, and the frequency of attack offers insights into the intricate dynamics of predation pressure and the underlying abundance of predatory organisms. Giving an overview of the year-round dynamics of predator-insect interactions. It offers insight into the collective impact of predators that consistently target insects.

In order to assess the impact of shelterbelts and the position of plots within the stand, it is deemed more feasible to focus solely on testing the bird predation rate due to their high predation numbers compared to other predators. A Welch Two Sample pairwise t-test was performed on the main predator, birds solely focusing on the presence and absence of shelterbelts in the stands regarding the plots' position.

#### 2.5.2. Moth pheromone trapping

All 40 sampling sites were pooled and where no log link function probability was used in the R software studio 4.2.2. The correct assumptions of parametric statistics for the moth distribution density was analysed, a normality assumption was done with a Q-Q normal probability plot and the independence assumption creating a plot of the residuals on the observations used. The purpose was to analyze if the moth distribution was influenced of each plot by the fixed effects, that is the x variables, the direction of each plot, the type of plot as well as the position. A random effect was used to account for the variability between different levels of a grouping variable (in this case the "PlotID"). The model was created placed by measures of GLMER built using the GLMMTMB package for the distribution of the gamma family regression for the continuous non-normal data, the weight of the total moth captured. It was also opted in utilizing the Poisson family for the discrete non-normal data, the number of moths captured Upon examining the GLMER, identification of data overdispersion needed to be done and spatial autocorrelation within the residuals. Luckily, the analysis revealed no significant evidence of data overdispersion. In terms of the moth distribution analysis, the same was method applied to analyse the influence of plot location (South vs. North), presence of shelterbelt (Yes vs. No), and position within the plot (Edge vs. Center) For each plot, Shannon-Wiener diversity indices and species richness were computed. Prior to analysis, the abundance of the moths and Shannon-Wiener diversity values underwent a log transformation, which was deemed suitable based on model diagnostics. This transformation was applied to ensure the appropriateness of the data for subsequent analyses.

All 40 sampling sites were nested together in the experimental sites of interest where no log link function probability was used in the R software studio 4.2.2. The correct assumptions of

parametric statistics for the moth distribution density were analysed, a normality assumption was done with a Q-Q normal probability plot and the independence assumption creating a plot of the residuals on the observations used. Using the Poisson distribution analysis, the focus was shifted to the main species, *L. monacha*, as captures of the other species are relatively low and may not provide enough data for meaningful analysis to observe the influence of the stand characteristic to the effects of its distribution. Accounting for the autocorrelation and create a more accurate representation of the *L. monacha* captures, it is therefore more statistically accurate to merge the catches from the traps collected from the variable 'Position within the stand', namely edge and center. By merging the catches, the spatial dependency between traps is considered, and the analysis becomes robust. The inclusion of the variable 'The amount of markings per caterpillar' in the model serves as a proxy in controlling the potential effect of predation on *L. monacha* captures that may have influenced the behaviour and distribution of *L. monacha* and possibly the other species as well.

The Pearson correlation analysis was conducted on log10-transformed data on the species, which is appropriate due to the multiplicative relationship. The study involves traps placed in both edge and center of the stands. However, it is observed that the catches in these traps are auto-correlated, meaning that there is some underlying relationship between the catches in nearby traps.

### 3. Results

#### 3.1. Caterpillars attacked and recovered back

In the end, 432 caterpillars was placed within stands containing shelterbelts, 192 caterpillars placed in stands without shelterbelts making the grand total of caterpillars in the field 624. Of the 624 caterpillars that were exposed, over 52 sampling sites, it was found that only 138 caterpillars were attacked of which 101 (73,19%) were due to birds, 25 (18,11%) by rodents, 56 (40,58%) by parasites and 77 (55,80%) by slugs. The rate of predation was identified by the indentation marks left on the caterpillars. Overall, a total of 820 attacks were recorded (pooled over space), for which 404 attacks were made by birds, 57 by rodents, 115 by parasitoids, and 158 by slug mandibles. 86 of the markings were classified as false positives made either by human indentation by nails, pine needles, or tree branches that it was fixed on. Of those unrecovered, the missing caterpillars were 25 (4%) but were not replaced. We excluded the missing caterpillars and the false positives from the analysis because it was impossible to determine whether they had been attacked by particular predators, either formed by human error or, but might that also clay had been stripped from the wire.

Looking at Table 1, the variable for the 'Direction of plot [South]', has a positive estimate value when referenced to the North, further, the indication of the 0.12 estimate states that if the stand is oriented in the south, the expected predation frequency will be slightly higher compared to the stands situated in the north. However, there is no statistical difference as shown by the wide 95% Confidence Interval (CI) that does include 1 in its range, indicating non-statistical significance for the high p-value of 0.76 ( $p > 0.05$ ). Similarly, the coefficient estimates for the variable 'Type of stand with shelterbelt [Yes]' referenced to No, also show a positive value indicating that the stands containing shelterbelts will have an overall slightly increased predation rate than stands without shelterbelts but show no statistical significance. The same can be seen for the 'The position within the stand' variables when compared to the center. Further, this model resulted in the adjusted R-squared value being 0.02 indicating that the variables influencing the predation in the model explain only 0.2% of the variance in the predation and might be that not enough data can be efficient to explain predation in this forest. Therefore, there can be no positive edge effect stated due to the small assemblage of predation trend in the dataset. Given that the model is not over dispersed, the ratio of which a Pearson chi-squared to residual degrees of freedom is calculated, 0.86 indicates that the model's goodness-of-fit is acceptable, and the observed results are not that different from one another under the assumed gamma distribution. Further, since  $\tau_{00}$  Plot is zero, it suggests that the variable 'Plot' fixed effect does not have a significant effect on the frequency of predation even with 17 distinct groups of plots identified.

In summary, it can be statistically inferred that there are no positive correlations nor significant differences between the chosen variables that could influence the overall predation rate, not rejecting the null hypothesis and the mean of the predation pressure overall on the caterpillars are not equal and conclude that the intercept is different from zero. This means that the difference we observed could have happened by chance and is not necessarily due to the presence or absence of shelterbelts. Based on the chi-square tests, none of the variables (direction of the stand, type of stand with a shelterbelt, and position within the plot) have a significant independent effect on the outcome variable.

Table 1: The coefficients of a generalized linear mixed model analysis utilizing the Gamma family for the overall predation. The model comprises of an independent variable to three explanatory variables, with their respective estimates, standard errors and p-values. In this model, the random effect is the PlotID as it accounts for the variability between different levels of the fixed effects (independent variables).

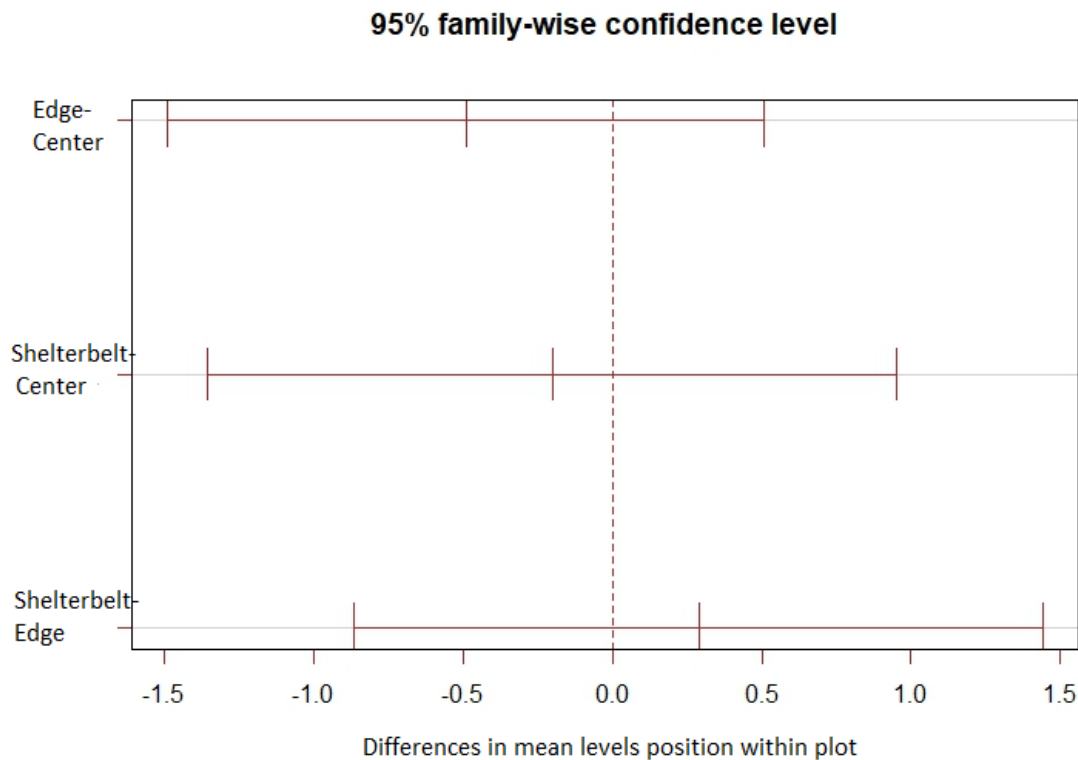
<b>Frequency of predation</b>					
<b>Predictors</b>	<b>Estimates</b>	<b>std. Error</b>	<b>CI</b>	<b>t value</b>	<b>p</b>
<b>(Intercept)</b>	0.51	0.44	0.68 – 4.06	1.14	0.26
<b>Type of stand with shelterbelt [Yes]</b>	0.01	0.47	0.40 – 2.59	0.03	0.98
<b>Direction of plot [South]</b>	0.12	0.40	0.51 – 2.53	0.31	0.76
<b>Position within the stand [Edge]</b>	0.23	0.48	0.48 – 3.33	0.48	0.64
<b>Position within the stand [Shelterbelt]</b>	0.07	0.57	0.37 – 3.14	0.14	0.89
<b>Random Effects</b>					
$\sigma^2$	0.75				
$T_{00}$ Plot	0.00				
$N$ Plot	17				
<b>Observations</b>	52				
<b>Marginal R<sup>2</sup> / Conditional R<sup>2</sup></b>	0.019 / NA				

All three of the confidence intervals for the mean value between the different groups contain the value zero, which indicates that there is a statistically not significant difference in the mean between all three groups (Fig. 14). This stays consistent with the fact that two of the p-values for the indicator values from the hypothesis are above 0.05 and the edge position is equal to 0.05. Hence, concluding that the mean values of the predation that happened on the edge is significantly higher than the mean values of the center and shelterbelt.

Further, a slightly significant positive Shelterbelt-Edge effect suggests that both positions experience competitive predation effects in the mixture stands relative to the monoculture stands. The proximity of the two positions might be an explanation. This positive effect is however partly offset by a negative Shelterbelt-Center effect, due to centers experiencing predation of the in mixed species stands and possibly due to management benefitting the stands containing shelterbelts.

As seen in Figure 9, all three of the confidence intervals for the mean value between the different groups contain the value zero, which indicates that there is a statistically not significant difference in the mean between all three groups. This stays consistent with the fact that two of the p-values for the indicator values from the hypothesis are above 0.05 and the edge position is equal to 0.05. Hence, concluding that the mean values of the predation that happened on the edge is significantly higher than the mean values of the center and shelterbelt.

Further, a slightly significant positive Shelterbelt-Edge effect suggests that both positions experience competitive predation effects in the mixture stands relative to the monoculture stands. The proximity of the two positions might be an explanation. This positive effect is however partly offset by a negative Shelterbelt-Center effect, due to centers experiencing predation of the in mixed species stands and possibly due to management benefitting the stands containing shelterbelts.



*Figure 17: Tukey ad hoc test of overall differences of position in the plot*

Spearman's rank correlation coefficient ( $\rho$ ) was found to be 0.82, indicating a strong and positive monotonic relationship between the variables 'Proportion of predation' and 'Frequency of predation'. The p-value associated with the correlation was extremely low (1.61), suggesting that the observed correlation is highly unlikely to have occurred by chance, consequently, rejecting the null hypothesis. This states that there is no correlation, and the data provide evidence to support the presence of a significant, positive correlation between the frequency of markings per caterpillar and the extent of caterpillar attacks. This implies that as the frequency of markings on caterpillars increases, there is a corresponding rise in the number of attacks by other organisms, underscoring a potential ecological linkage of the abundance of predators, frequency of predation and proportion to which predation happens.

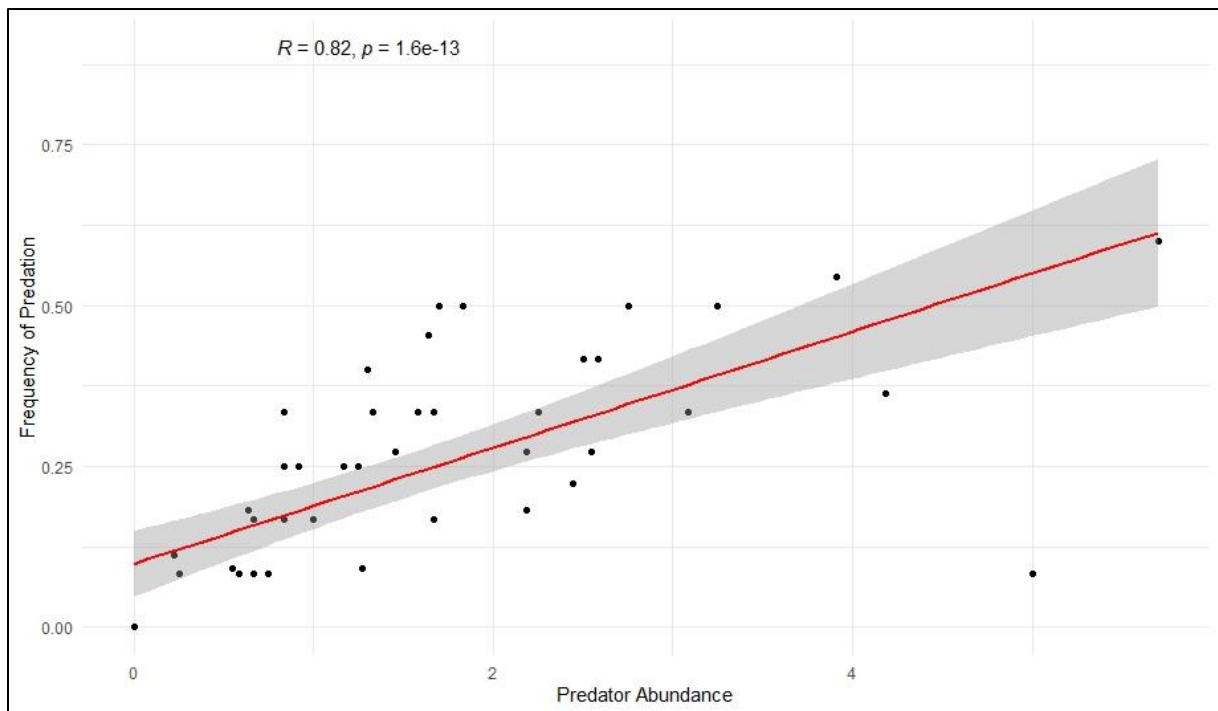


Figure 18: Spearman regression analysis based on the proportion of each caterpillar based on predator abundance attacked and the frequency of attack on the same caterpillars.

The stands that contained shelterbelts had a predation rate that fluctuated between 0 and 5 with a mean of 1.48 (SD = 1.17). There was no significant difference in the number of predation marks and the position in the stand (Kruskal-Wallis chi-squared = 3.60, df = 2, p-value = 0.16), with the direction of the stand also not indicating significance (Kruskal-Wallis chi-squared = 0.002, df = 1, p-value = 0.96). More specifically, the stand that did not contain shelterbelts fluctuated between 0 and 4 with a mean of 1.48 (SD = 1.60). With no sign of significance as the p-value is greater than 0.05.

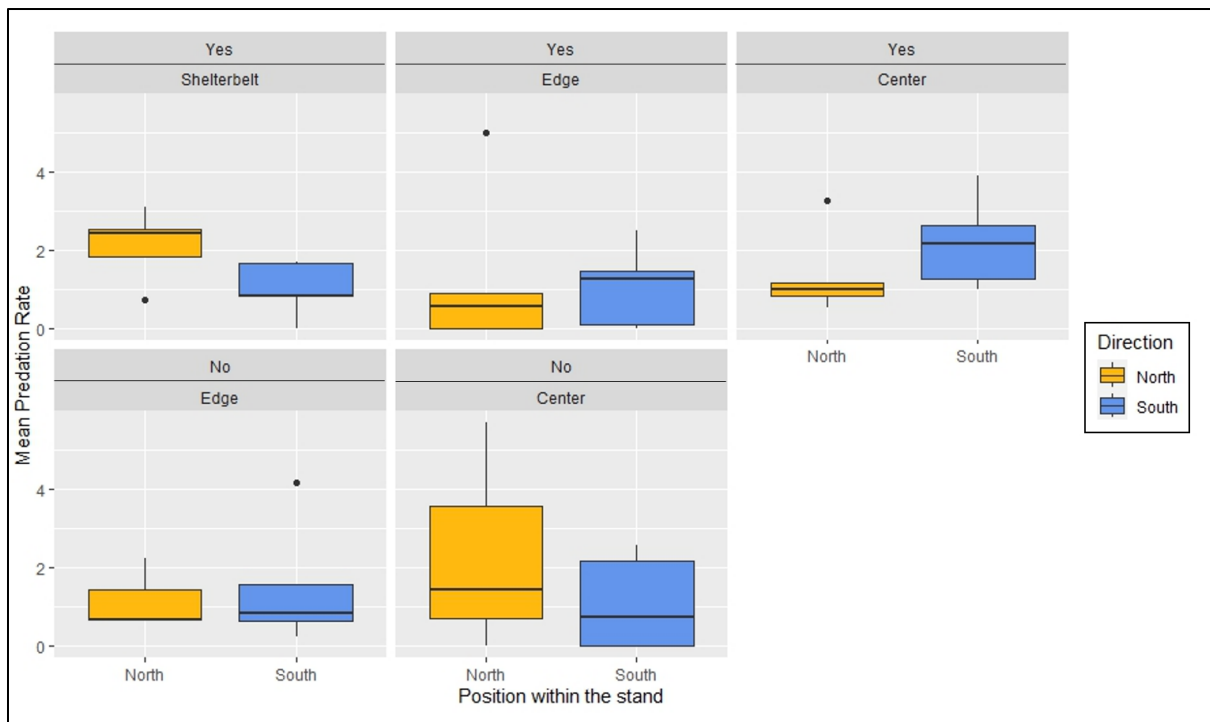


Figure 19: Boxplots showcasing each separate stand containing a shelterbelt and without on the position and direction to which the plot is located.

Four categories of predation classes were identified namely, birds, rodents, parasite, and slug. The predators identified that were responsible for the highest number of attacks on artificial caterpillars were the birds ( $0.72 \pm 0.94\%$ ) and slugs ( $0.26 \pm 0.33\%$ ). Other identified predators were parasites ( $0.19 \pm 0.22\%$ ) and rodents ( $0.10 \pm 0.15\%$ ). We did not encounter marks from beetles nor ants. When comparing the AIC values of the overall predation model ( $\Delta AIC = 61.66$ ) with the models focusing on individual predators and their interaction, it becomes evident that the bird model performed the best among the others. The violin plot visually tells us that the highest probability of attack by a predator would be either by slugs and/or birds, with the lowest probability being mammals.



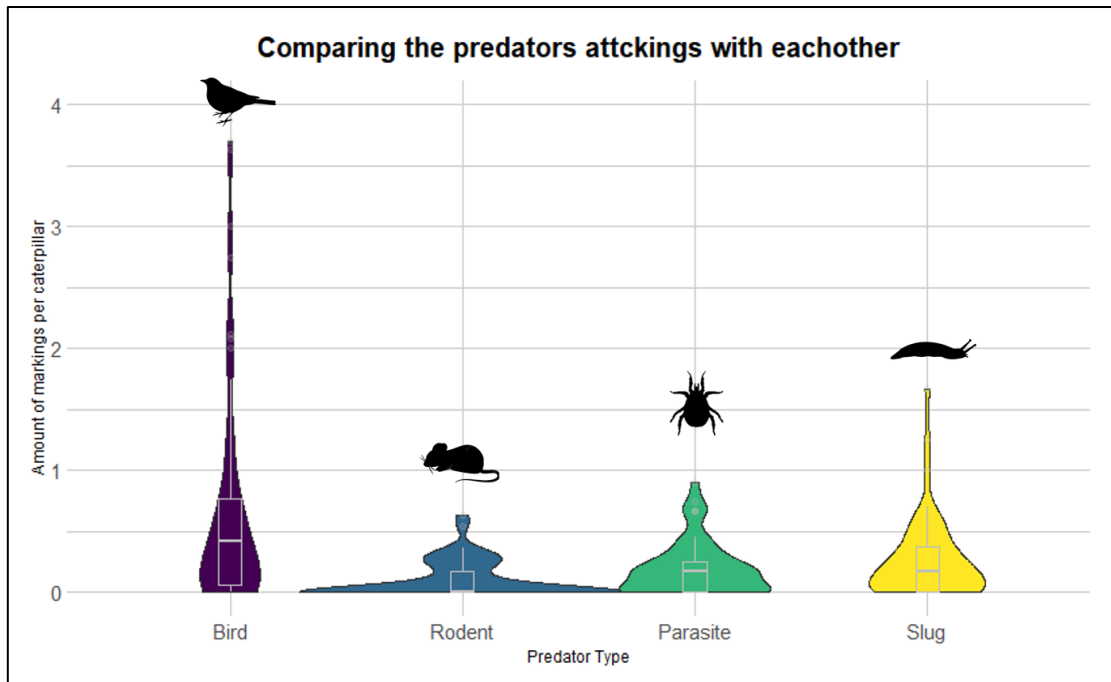


Figure 20: Mean ( $\pm$  SD;  $n = 21$ ) frequency of the predation rate by birds, rodents, parasites, and slugs in the stands with and without shelterbelt.

The mean total for each predator species were higher at the stands that had no shelterbelt than in the stands with shelterbelt. (GLMM,  $\text{ivar} \times$  type of stand with shelterbelt:  $z = -0.005$ ;  $P = 1$ ). As depicted the results from the GLMMs indicated no effect of tree diversity on the proportion of attacked models by either birds (Yes:  $0.15 \pm 0.21$ ; No:  $0.27 \pm 0.15$ ), slugs (Yes:  $0.14 \pm 0.11$ ; No:  $0.53 \pm 1.17$ ), parasites (Yes:  $0.49 \pm 2.22$ ; No:  $0.49 \pm 1.55$ ) or rodents (Yes:  $2.22 \pm 0.42$ ; No:  $1.39 \pm 0.24$ ).

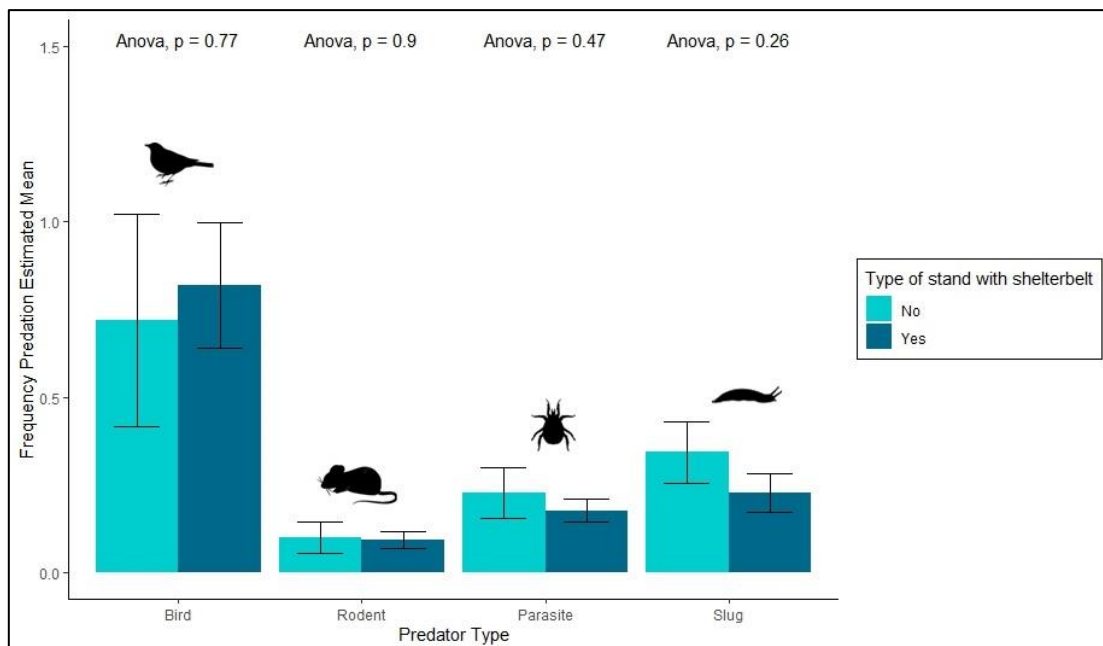


Figure 21: Attacks on caterpillars by looking at the effects of tree diversity (Yes vs No) by each predator type. Standard error bars depict  $\pm 95\%$  interval.

Predator assemblages were considerably different between the two directions. In the north, the bird attack rate was 58.17% which accounted for over of attacks by all predator groups and the remaining attacks consisted of damages by rodents (5%), parasites (10%) and slugs (30%). Further the ANOVA chi-test proved that the variables Direction of the stand:  $\chi^2 = 0.02$ ,  $P < 0.88$ , Type of stand with shelterbelt:  $\chi^2 = 0.16$ ,  $P < 0.7$ , Position within the stand:  $\chi^2 = 4.46$ ,  $P < 0.12$ , did not have a significant effect.

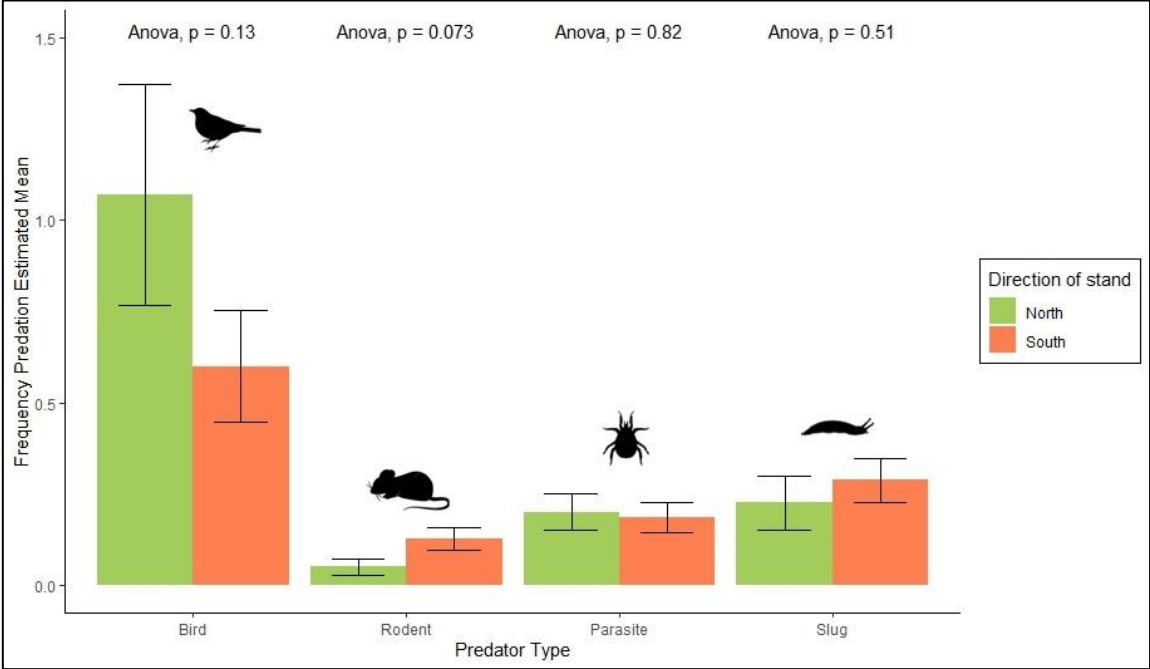


Figure 22: Attacks on caterpillars by direction (North vs South) and predator type. Error bars depict ± 95% CI interval.

Predator species respond to a complex forest habitat which includes the leaf area and branch structure with the overall composition and by looking at Figure 21, the edge containing the broadleaved tree shelterbelt had a higher predation pressure than the pine trees.

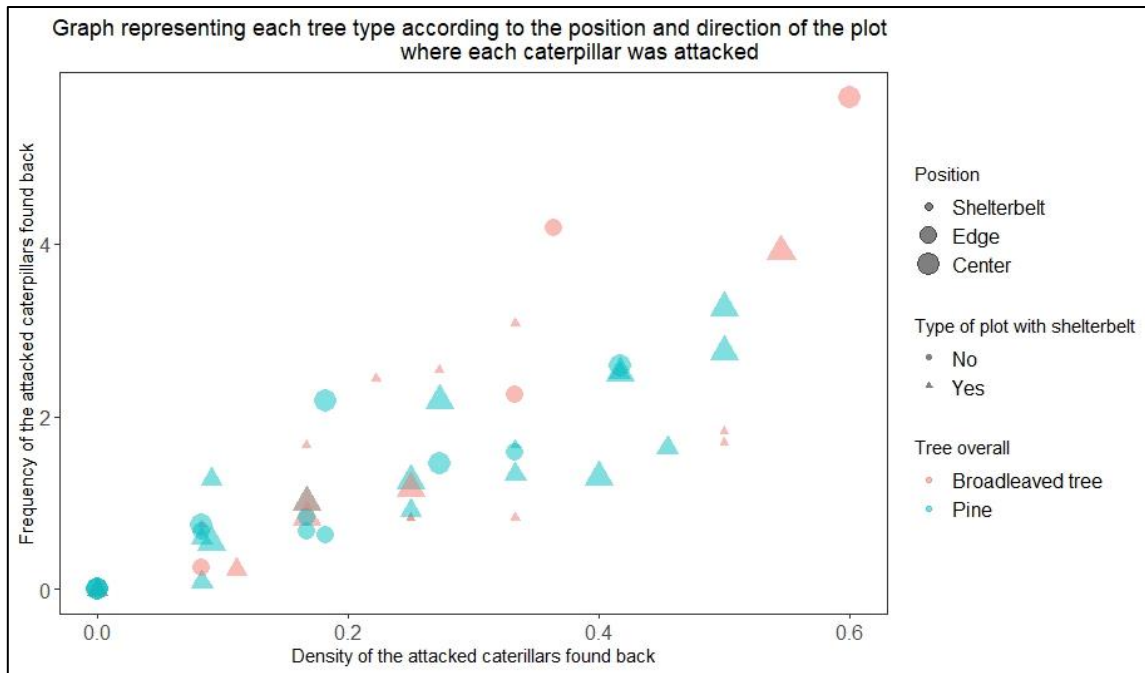


Figure 23: Predation can be affected by the distribution and abundance of prey in different species of trees depending on their leaf traits.

### 3.1.1. Attack rate by Birds

The pairwise t-test (Table 2), indicate that there is no statistically significant difference in the frequency of bird predation between the 'Center' and 'Edge' positions within the stand that contained present with a shelterbelt and without. The mean difference between the two positions is relatively small not far from zero making it not statistically different from one value to another. Therefore, based on this analysis, it cannot be concluded that the position within the stand had a significant effect on the frequency rate of bird predation. Further, the 95% interval includes values close to one and zero making it less likely for the data to be significant.

Table 2: The following analysis explored the relationship between the variables that would influence the bird predation rate referencing stands that had a presence or absence of a shelterbelt (Yes, No), with the plot's position within the stand (Center, Edge) utilizing the pairwise t-test.

Frequency of bird predation				
Variable	t-value	df	95% CI (d)	p
Center	0.49	12.36	[-0.90, 1.43]	0.63
Edge	-0.08	16.535	[-1.29, 1.19]	0.94

Further summarizing the interaction effect between the presence and absence of shelterbelts between the center and edge alone, the average bird predation shown no interaction regarding the position within the stand. Taking the center position regarding the absence to the presence of shelterbelt the pairwise t-test analysis shown no significant indication (df = 11, p = 1.18). Similar findings were also found at the edge of the stand (df = 1, p = 0.75).

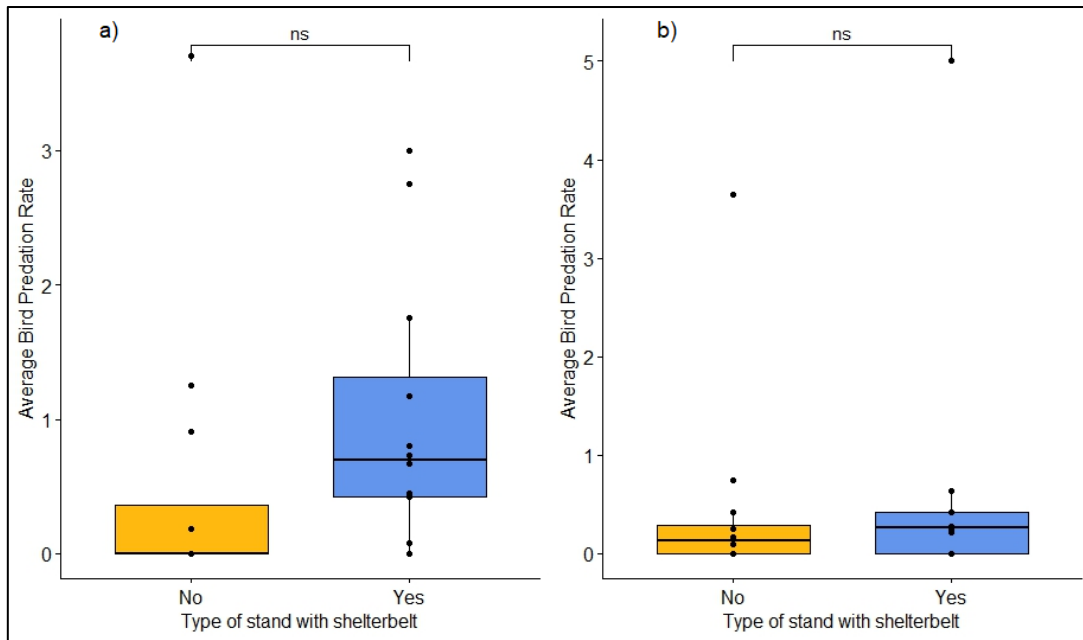


Figure 24 a) Type of stand with shelterbelt interaction for the center; b) type of stands with shelterbelt referenced to their respective edges calculated on average bird predation.

Only comparing stands containing a shelterbelt, the interaction effects between the different positions within the plot was analysed. Upon analysis, the center to the edge, center to shelterbelt and edge to shelterbelt ( $P > 0.05$ ), positions indicated that they had an effect on the bird predation rate in stands containing a shelterbelt. A paired t-test analysis was done resulting in the p-adjusted values for the groups center to shelterbelt and edge to shelterbelt being 1. The only group that had a value less than one was the center to the edge with the p value greater than the threshold 0.85.

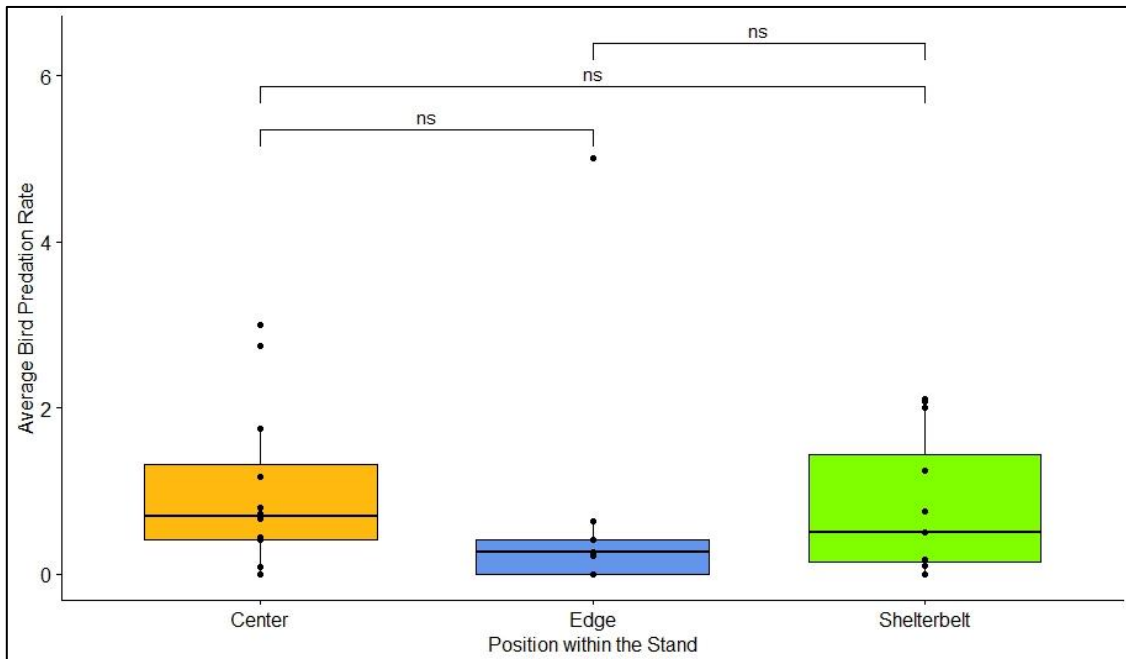


Figure 25: Interaction between the different positions within the stand based on the presence of shelterbelts to the average bird predation rate.

When doing a two-way ANOVA model, it can be best explained through the variation attack by birds the best included all the independent variables ('Direction of the stand', 'Position within the stand' and 'Type of stand'). All dependent variables did not significantly affect the attack rate by birds (Direction of the stand:  $\chi^2 = 0.9575$ ,  $P < 0.3957$ , Type of stand with shelterbelt:  $\chi^2 = 0.0387$ ,  $P < 0.8644$ , Position within the stand:  $\chi^2 = 4.4027$ ,  $P < 0.1904$ ). Upon closer inspection figure 23 shows a small difference when looking at the shelterbelt. What was evident that there was a higher mean predation for each unit to which predation would happen in the shelterbelt located in the North would increase.

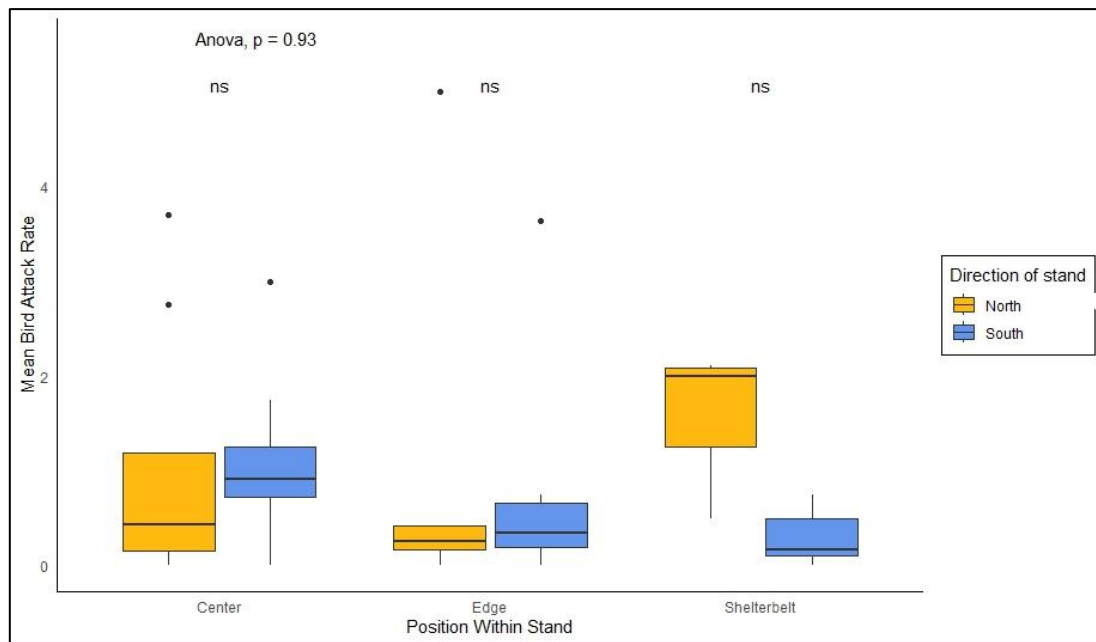


Figure 26: The effects of the dependent factors on the mean ( $\pm$  SE) attack rates by birds. The effect of position (Center, Edge, Shelterbelt) is shown.

### 3.1.2. Attack rate by Rodents

The interaction between all the dependent variables resulted in the direction of the stand being significant (ANOVA: Direction of the stand:  $\chi^2 = 0.85036$ ,  $P < 0.05066$ , Type of stand with shelterbelt:  $\chi^2 = 0.00077$ ,  $P < 0.95318$ , Position within the stand:  $\chi^2 = 0.69575$ ,  $P < 0.20961$ ). The attack rate was the highest in the center located in the South and declined in the North.

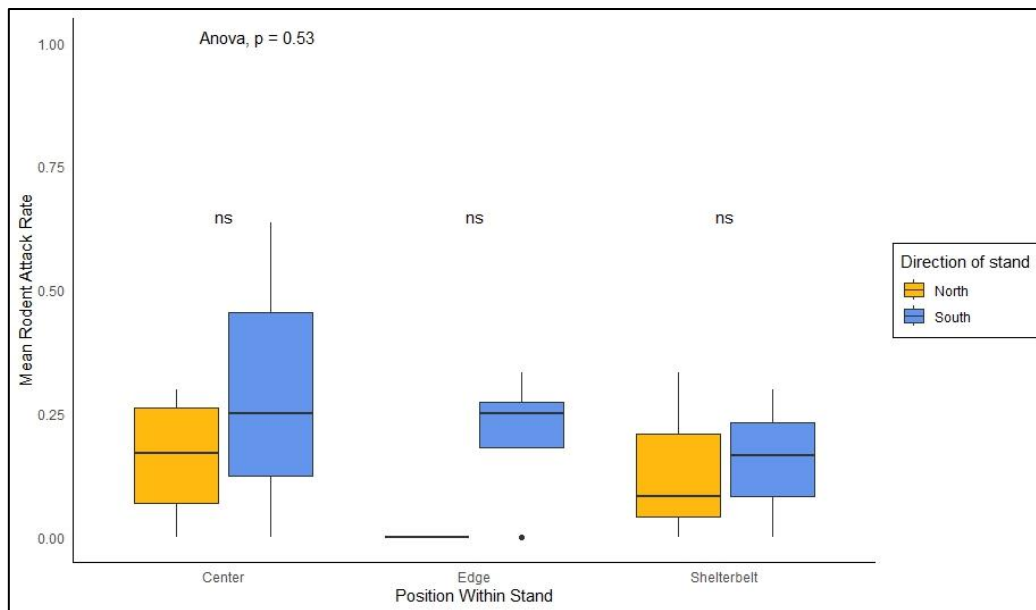


Figure 27: The effects of the dependent factors on mean ( $\pm$  SE) attack rates by rodents. The effect of position (Center, Edge, Shelterbelt) are shown.

### 3.1.3. Attack rate by Parasites

The interaction between all the dependent variables resulted in the direction of the stand being significant (Direction of the stand:  $\chi^2 = 0.01341$ ,  $P < 0.8138$ , Type of stand with shelterbelt:  $\chi^2 = 0.13908$ ,  $P < 0.4483$ , Position within the stand:  $\chi^2 = 0.98097$ ,  $P < 0.1316$ ).

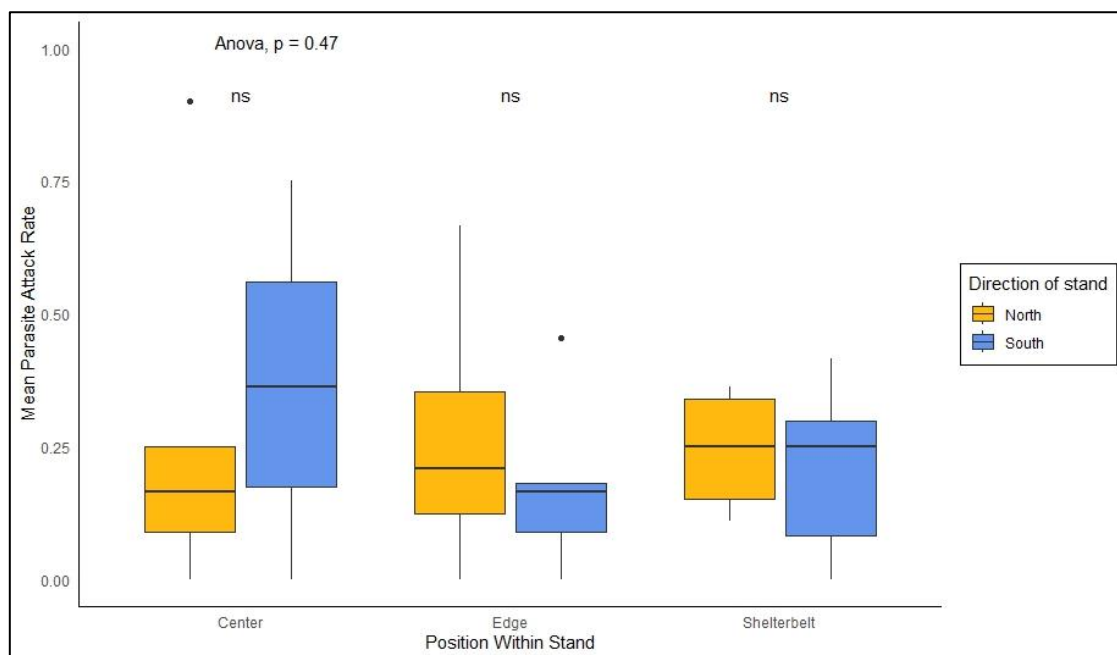


Figure 28: The effects of the dependent factors on the mean ( $\pm$  SE) attack rates by parasites. The effect of position (Center, Edge, Shelterbelt) is shown.

### 3.1.4. Attack rate by Slugs

The interaction between all the dependent variables resulted in the direction of the stand being significant (ANOVA: Direction of the stand:  $\chi^2 = 0.18762$ ,  $P < 0.6649$ , Type of stand with shelterbelt:  $\chi^2 = 0.50125$ ,  $P < 0.4790$ , Position within the stand:  $\chi^2 = 0.09346$ ,  $P < 0.9543$ ).

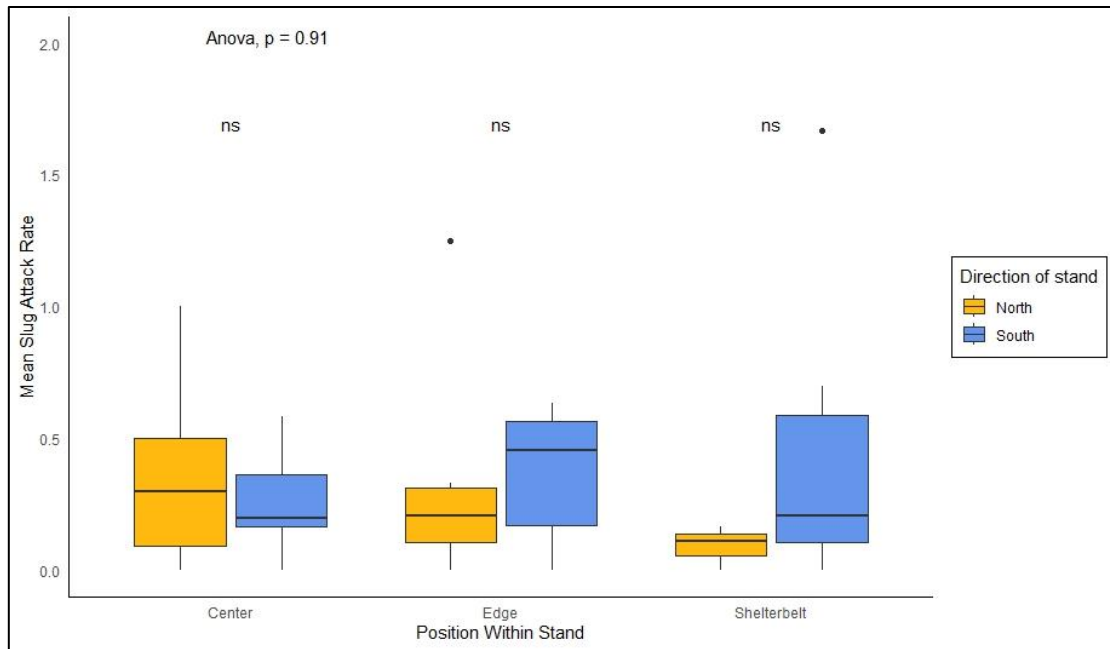


Figure 29: The effects of the dependent factors on the mean ( $\pm$  SE) attack rates by slugs. The effect of position (Center, Edge, Shelterbelt) is shown.

### 3.2. Moth distribution

The total of 3 species of moths were caught and 421 individuals in 39 pheromone traps observed throughout the sample period. In the center of the stand, 216 individuals observed and 205 individuals in the edges of the stands with all three species found in each position of the stand. Of the 421 individuals caught, 406 (96,44%) were *Lymantria monacha*, 5 (1,19%) were *Panolis flammea* and 10 (2,38%) were *Dendrolius pini*. *P. flammea* and *D. pini* were absent in most of the edges and centers if the stand and were considered as rare with less than 15 individuals recorded. Initially, there were 40 traps deployed, however, upon the moth retrieval in the field, one of the traps was identified to be faulty due to the wire breaking attaching the trap to the tree, resulting in zero captures, leading to the conclusion of excluding the plot from the analysis. The mean  $\pm$  SE number of captures per trap was  $10,79 \pm 1,15$ . With the mean being 10,79, it revealed a skewed distribution of the number of captures. In addition to the total moths captures, each plot was weighted in grams respectfully. Accounting for the weight of all moths captured in the 39 traps, the weight measured at 12,88g. The mean  $\pm$  SE weight of captures per trap was  $0,33 \pm 0,04$ . Similar to the total number of moths caught, it was revealed that a skewed distribution of the weight per number of traps would be present.

Traps located at the edges but facing south had a total of 114 ( $9,5 \pm 2,03$ ) individuals weighing 3,8g ( $0,32 \pm 0,07$ ) whereas traps located in the north had a total of 91 ( $20,22 \pm 1,95$ ) individuals.

Table 3: The analysis explored the relationship between the total amount of moths captured and three independent variables, such as the direction of the plot, the presence of a shelterbelt, and the position within the stand utilizing various predictors in a Poisson distribution with a random effect the PlotID as it accounts for the variability between different levels of the fixed effects (independent variables).

<b>Total amount of moths captured</b>						
<b>Predictors</b>	<i>Estimates</i>	<i>Incidence Rate Ratios</i>	<i>std. Error</i>	<i>CI</i>	<i>t- value</i>	<i>p</i>
<b>(Intercept)</b>	2.21	9.12	2.43	5.40 – 15.39	8.28	<b>&lt;0.001</b>
<b>Type of stand with shelterbelt [Yes]</b>	0.07	1.08	0.34	0.58 – 1.98	0.24	0.81
<b>Direction of plot [South]</b>	0.05	1.05	0.21	0.70 – 1.56	0.22	0.82
<b>Position within the stand [Edge]</b>	-0.13	0.87	0.09	0.72 – 1.06	-1.37	0.17
<b>Random Effects</b>						
$\sigma^2$	0.10					
$\tau_{00 \text{ Plot}}$	0.35					
<b>ICC</b>	0.77					
$N_{\text{Plot}}$	17					
<b>Observations</b>	39					
<b>Marginal R<sup>2</sup> / Conditional R<sup>2</sup></b>	0.014/0.774					

When looking at table 3 the intercept suggests that there could be a significant relationship between the dependent and independent variables with high incidence rate ratios having a strong effect on the number of moths captured. The p-value supports this by suggesting that this effect is highly unlikely to be due to random chance. Next the variable ‘Direction of plot’, (South compared to North) does not have a statistically significant effect on the incidence rate (mean number) of moths captured. The estimate of 0.05 suggests a slight increase in the incidence rate in the "South" direction, but this difference is not statistically significant, as evidenced by the wide confidence interval and the p-value greater than 0.05. The same can be said for the variable “Type of stand with shelterbelt” (Yes compared to No). The variable "Position within the stand" (edge referenced to the center) does not have a statistically significant effect on the incidence rate (mean number) of the total moths captured. The estimate of -0.13 suggests a slight decrease in the incidence rate at the "Edge" compared to the "Center," but this difference is not statistically significant, hence not completely different from one another, and evidenced by the narrow confidence interval, that includes 1, and the p-value greater than 0.05. Therefore, based on this table, there is no strong evidence to support a significant difference in moth capture rates between the different variables used within the stands. Upon further inspection solely based on the ANOVA procedure, it was



revealed that the direction of the stand only becomes a significant predictor when considered in isolation. This implies that while the combined effects of all predictor variables in the mixed model may not have yielded a significant result for the direction of the stand, it is still a relevant factor that influences the total moth count when analyzed separately.

The ICC value of 0.77 suggests that there is quite a large portion of the total variance in the number of moths captured attributed to the variability between different plots identified. The small value of marginal  $R^2$  (0.014) indicates that the independent variables in the model have a limited impact on explaining the impact of the presence of moth species in the stands. However, when considering both the fixed (independent variables) and random effects (Plot) together, the model has high goodness of fit, with no overdispersion, as indicated by the large value of Conditional  $R^2$  (0.774).

The variability of the weight of the total moths in stands with shelterbelts proved to be higher compared to the stands without shelterbelt (Fig. 26).

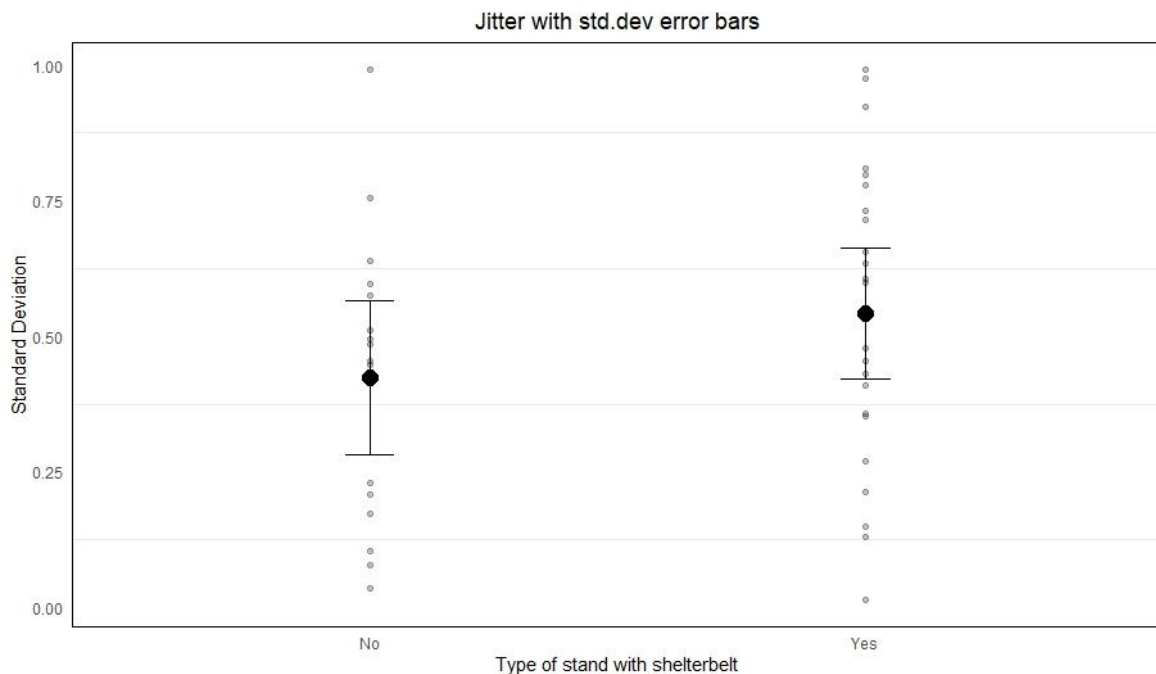


Figure 30: The mean standard deviation of moth weights within the overall groups captured for stands with shelterbelts and stands without shelterbelts (No, Yes); the error bars represent the confidence interval of the mean.

Further, for each trap located at the center of the stand, 216 ( $11,37 \pm 1,85$ ) individuals were caught weight 6,28g ( $0,33 \pm 0,06$ ). Edges having 205 ( $19,52 \pm 1,43$ ) individuals' weight in a 6,60g ( $0,63 \pm 0,05$ ). The two-way ANOVA revealed that none of the preferred habitats revealed significance ( $n = 39$ ,  $F = 0.23$ ,  $P < 0.63$ ). Taken separately, both the center and edge samples were performed under the one-way ANOVA, proving to have similar results with no effective effect on each other (center;  $n = 19$ ,  $F = 0.08$ ,  $P < 0.78$ ; edge;  $n = 20$ ,  $F = 0.08$ ,  $P < 0.78$ ).

Lastly, traps situated in stands with shelterbelts had 257 ( $11,17 \pm 1,58$ ) individuals in total weighing 7,90g ( $0,34 \pm 0,05$ ) with traps located in stands without shelterbelts having 164 ( $10,25 \pm 1,69$ ) individuals weighing 4,98g ( $0,31 \pm 0,05$ ).

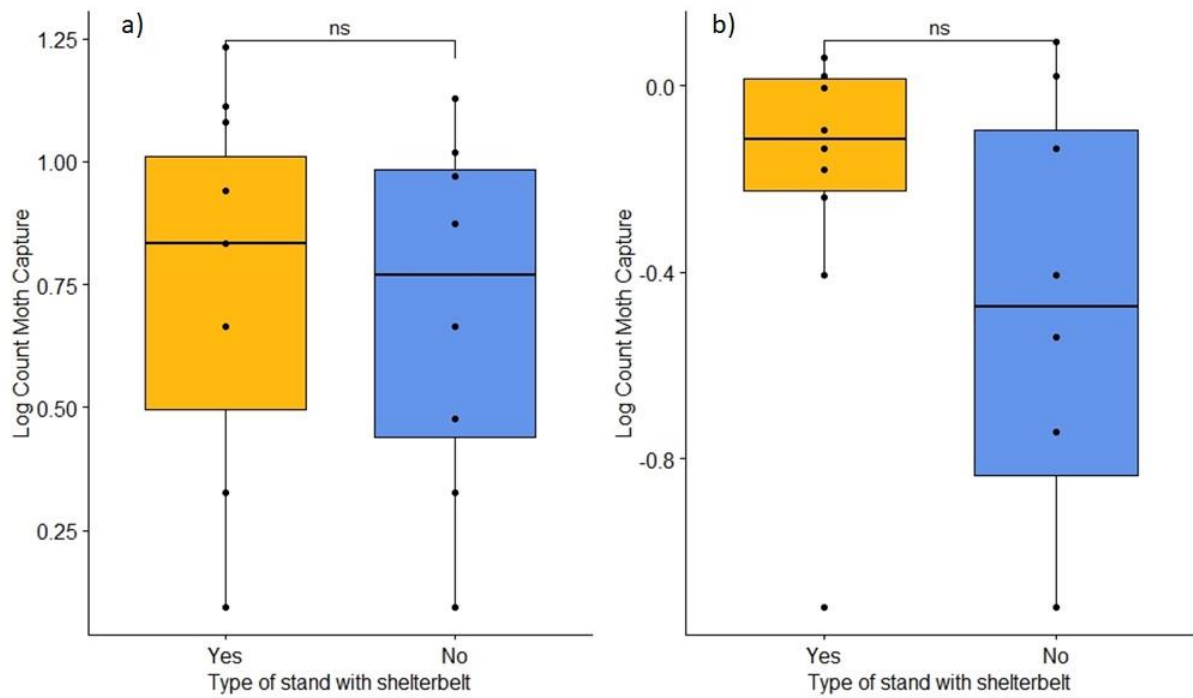


Figure 31 a): Mean values ( $\pm$ SE) for the total abundance of the moths captured as a function from the center. Significance values are shown on the top of the boxplots for the results of the two-way ANOVA for the effect of the position in the stand on the variable type of stand with shelterbelt (\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ ). b) Type of stand with compared with the edge.

### 3.2.1. *L. monacha* captures

As seen in Table 4, none of the variables have a significant effect ( $p > 0.05$ ) on the moth distribution, stating that *L. monacha* is totally independent of the stand type, position to which the edge is facing and predation. This indicates variability in the response variable between different plots ( $\tau$ 00 Plot and ICC), indicating that the "Plot" variable has a significant effect. The high ICC suggests that a large portion of the variability in the response can be attributed to differences between plots.

Table 4: The analysis explored the relationship between the total amount of *L.monacha* captured and three independent variables, such as the direction of the plot, the presence of a shelterbelt, and the amount of markings per caterpillar utilizing various predictors in a Poisson distribution with a random effect the PlotID as it accounts for the variability between different levels of the fixed effects (independent variables).

<b>Total amount of <i>L. monacha</i> captured in the stand</b>					
<b>Predictors</b>	<b>Incidence Rate Ratios</b>	<b>std. Error</b>	<b>CI</b>	<b>Statistic</b>	<b>p</b>
<b>(Intercept)</b>	17.73	4.96	10.25 – 30.66	10.28	<b>&lt;0.001</b>
<b>Direction of stand [South]</b>	1.06	0.22	0.70 – 1.60	0.27	0.784
<b>Type of stand with shelterbelt [Yes]</b>	1.03	0.31	0.57 – 1.85	0.09	0.927
<b>The amount of markings per caterpillar</b>	0.97	0.05	0.87 – 1.07	-0.60	0.550
<b>Random Effects</b>					
<b><math>\sigma^2</math></b>	0.06				
<b>T<sub>00</sub> Plot</b>	0.32				
<b>ICC</b>	0.85				
<b>N<sub>Plot</sub></b>	17				
<b>Observations</b>	20				
<b>Marginal R<sup>2</sup> / Conditional R<sup>2</sup></b>	0.016 / 0.849				

As seen in figure 25, the species accumulation curve provides an estimate of the number of species present in the study area, revealing a distinct break or transition in species composition. The curve reaches an asymptote at approximately 30 species, indicating that the number of unique species observed in the area stabilizes at this point.

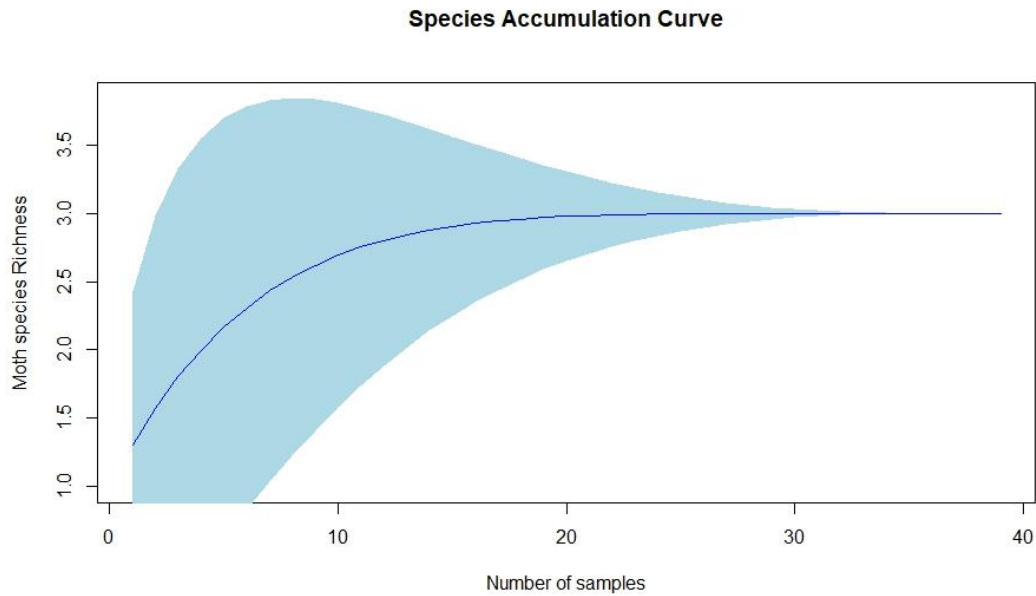


Figure 32: Species accumulation curve based on samples based on 50 permutations for total moth species (solid blue line). Light blue shaded areas mark 95% CI. In total, 3 species were collected belonging to *Lymantria monacha*, *Panolis flammea* and *Dendrolimus pini*.

A Pearson correlation test was done to look if the bird predation rate could have had an influence on the abundance of *L. monacha* the stands. When analysing the stands together there was no significant effect of bird predation to the abundance of moths in the stands ( $t = -0.7$ ,  $df = 18$ ,  $p = 0.49$ ). In addition, when looking at the stand heterogeneity of bird predation to moth abundance, there was no significant difference. Looking at the type of stand to the predator rate ( $t = -0.13$ ,  $df = 18$ ,  $p = 0.9$ ), to the moth abundance ( $t = 0.34$ ,  $df = 18$ ,  $p = 0.73$ ), the results indicated similar findings regardless of the heterogeneity. However, when looking at the type of stands with shelterbelts alone either indicating "Yes" or "No", it was found that stands containing shelterbelt have some degree of significance ( $p = 0.065$ ) when birds are present to the abundance of moth species compared to the stands without shelterbelts.

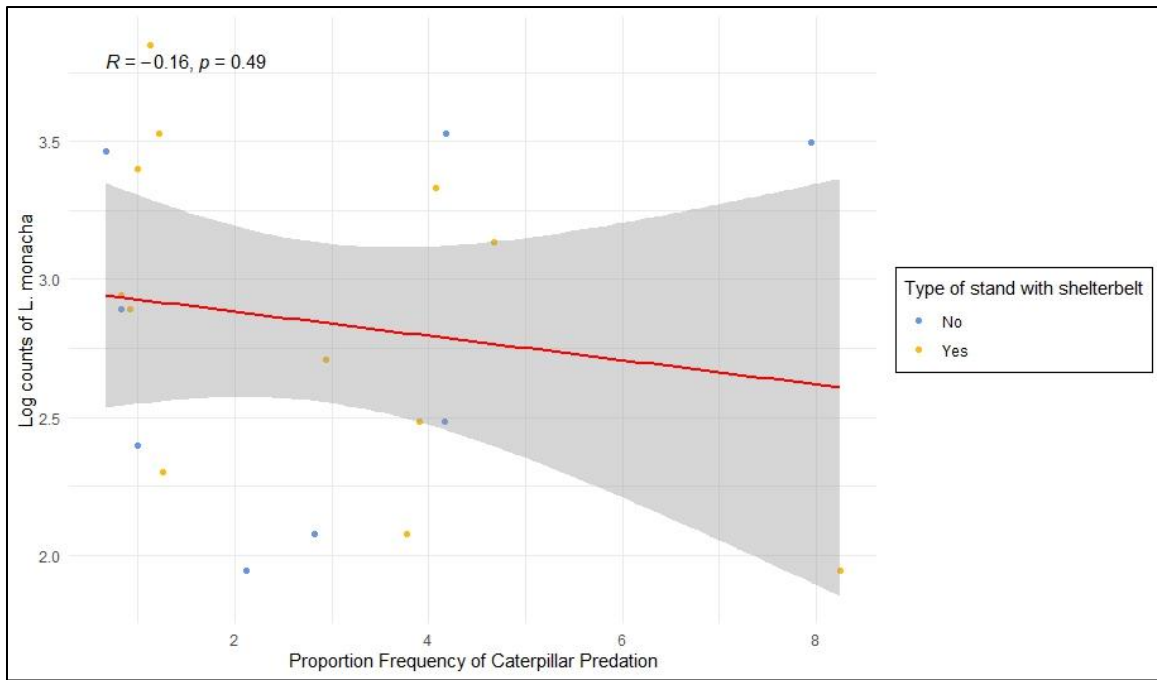


Figure 33: Pearson's correlation test between the amount of Log count of *L. monacha* caught in the stands with and without shelterbelt to the overall predation rate within each stand respectively.

The pairwise t-test statistical evaluation to compare between plots featuring shelterbelts and those lacking them, with respect to the log count of the total moths caught variable, did not uncover a statistically significance. The p-value of 0.1 is above the 0.05 value signifying that the disparities observed between the two groups are not adequately robust to substantiate a significant statistical association.

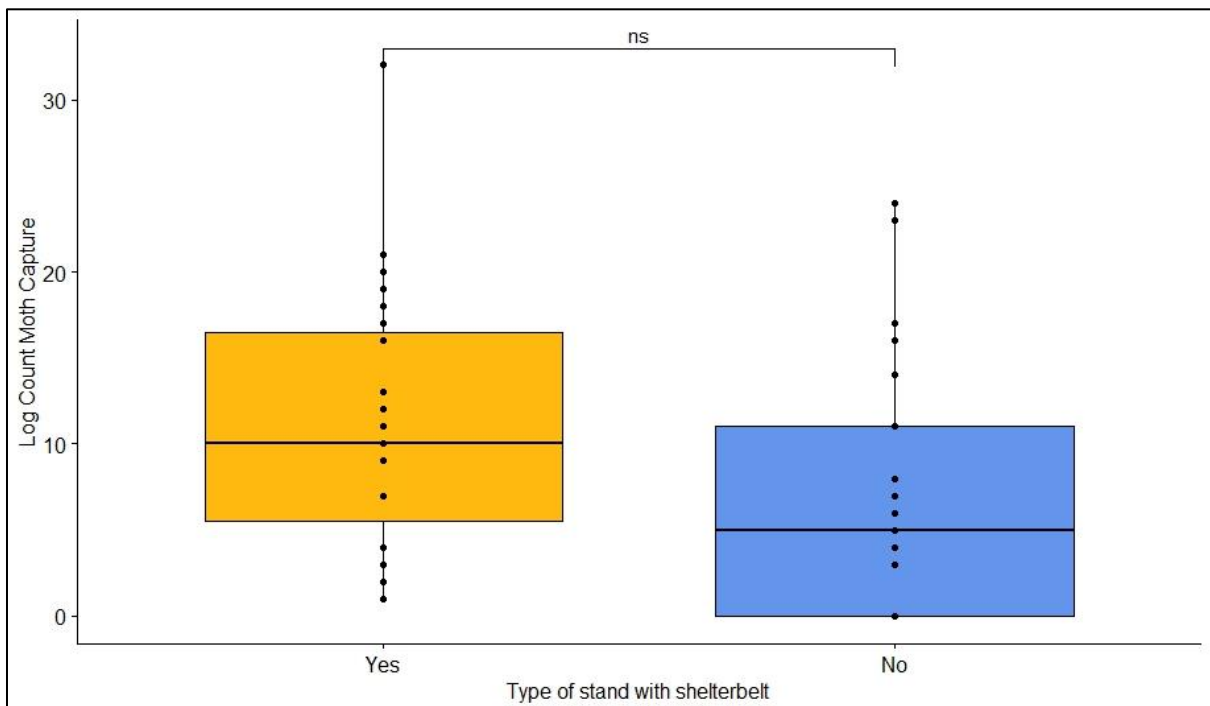


Figure 34: Pairwise t-test interaction test correlated to the type of stand with shelterbelt to the amount of moth capture.

The dominant species, *Lymantria monacha* showed a habitat preference of occurring in all stands regardless of their position, while *Dendrolimus pini* and *Panolis flammea* seemed to prefer the habitats with an overall closed canopy, the center. *Lymantria monacha* (Position within the stand:  $\chi^2 = 31.9$ ,  $P = 0.422$ , Type of stand with shelterbelt:  $\chi^2 = 2.5$ ,  $P = 0.821$ ) showing no significant effect of the influence of both the variables on the abundance of the moth species. Same applies to *Dendrolimus pini* (Position within the stand:  $\chi^2 = 0.360$ ,  $P = 0.68$ , Type of stand with shelterbelt:  $\chi^2 = 0.398$ ,  $P = 0.295$ ) and *Panolis flammea* (Position within the stand:  $\chi^2 = 0.113$ ,  $P = 0.72$ , Type of stand with shelterbelt:  $\chi^2 = 0.189$ ,  $P = 0.158$ ) showing no significant effects.

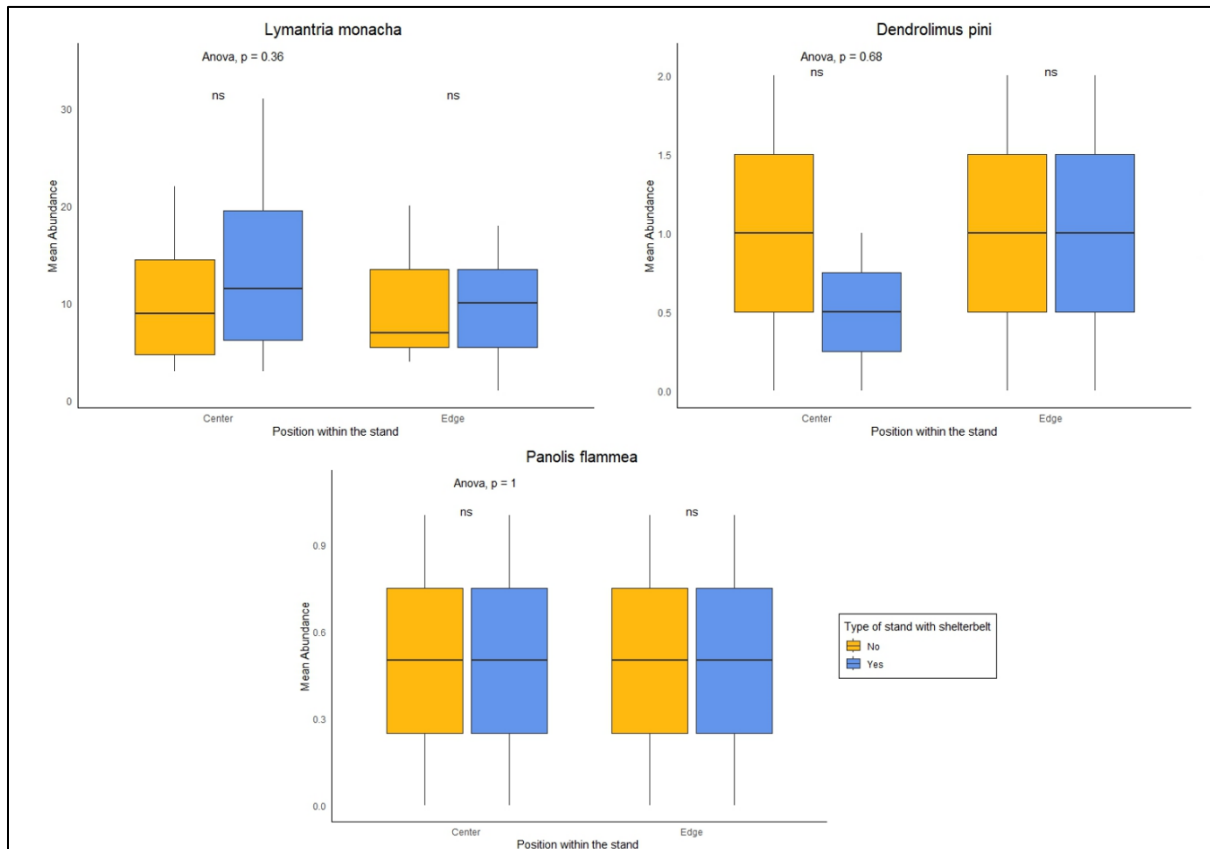


Figure 35: Mean values ( $\pm SE$ ) for the total abundance of each of the moth species captured as a function from the edge and center. Significance values are shown on the top of the boxplots for the results of the two-way ANOVA for the effect of the position in the stand on the variable type of stand with shelterbelt (\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ ).

The p-value is 0.29, which is greater than the significance level of 0.05 for the center. This indicates that there is no significant effect of "Position within the stand" on the response variable, however, it is seen that the mean abundance to which the moth species occur, is located in the center of the stand located north. Similarly, The p-value for the edge is 0.68, which is also greater than the significance level of 0.05. Therefore, there is no significant effect of "Direction of plot" on the response variable. Leading to the conclusion, that the hypothesis would be rejected. It is seen in the graph that it correlated with the north direction as the abundance of moths are higher in the stand located in the north.

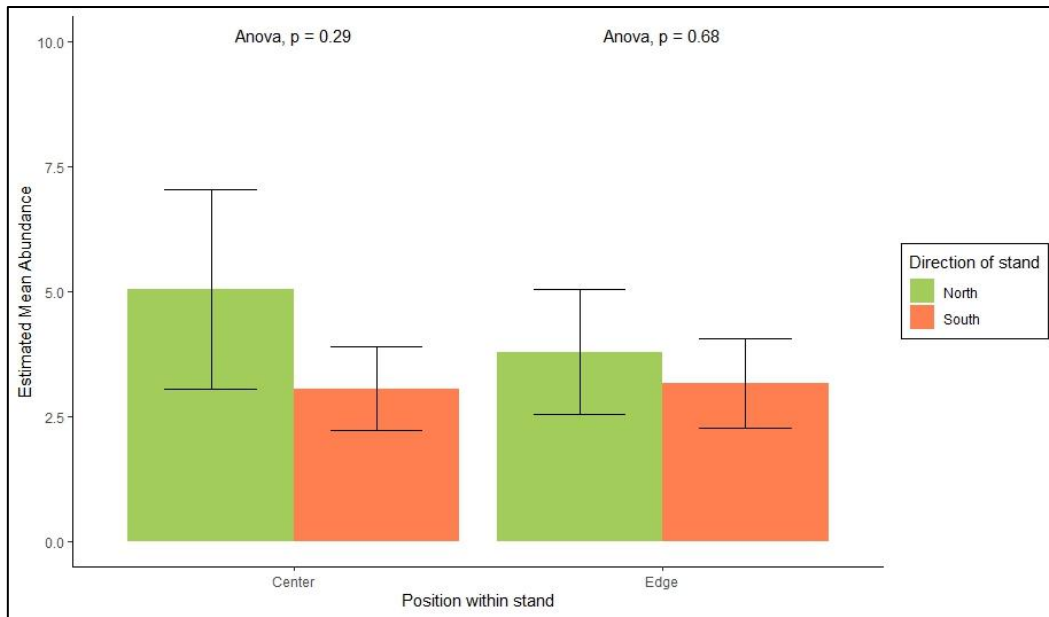


Figure 36: Mean values ( $\pm$ SE) for the total abundance of the total moth species captured as a function from the direction of the stand. Significance values are shown on the top of the boxplots for the results of the one-way ANOVA for the effect of the direction of the plot on the variable species type (\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ ).

Based on the ANOVA analysis of *L. monacha* there is evidence of a potential difference between the species and the variable "Direction of plot", but it does not reach statistical significance at the conventional alpha level of 0.05 ( $P > 0.05$ ). As expected, *D.pini* and *P.flammea*, shows no significant evidence that suggest that there are differences between the species that occurred in the plantation based on the variable "Direction of plot" ( $P > 0.05$ ). The variability observed can be attributed to random variation or other factors not considered in this analysis. Therefore, failing to reject the null hypothesis.

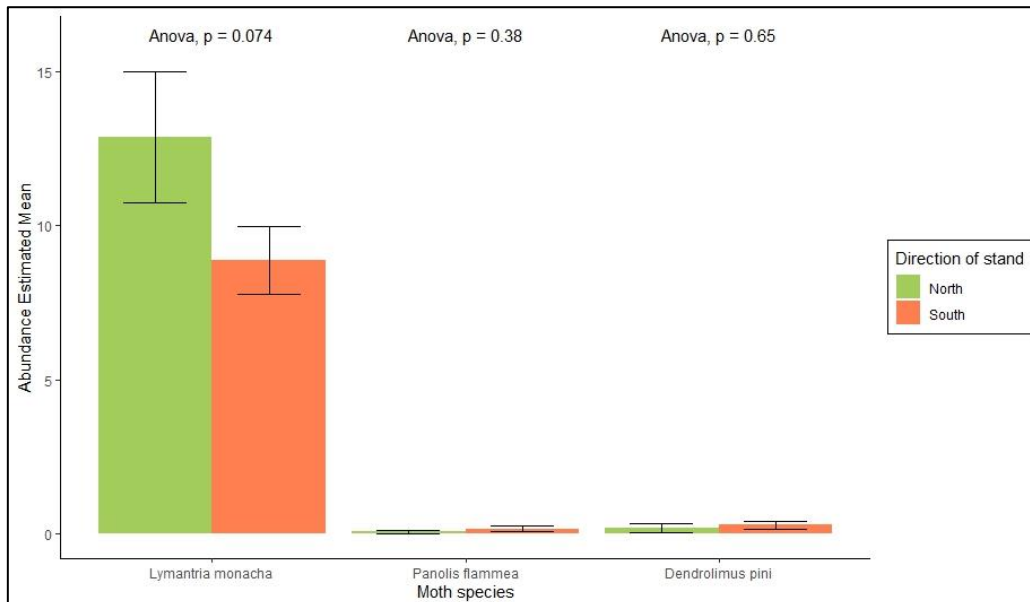


Figure 37: Mean values ( $\pm$ SE) for the total abundance of each of the moth species captured as a function from the direction of the stand. Significance values are shown on the top of the boxplots for the results of the one-way ANOVA for the effect of the direction of the plot on the variable species type (\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ ).

## 4. Discussion

### 4.1 Caterpillar predation

The selection of foraging habitat amongst predators is a dependent relationship between the availability and accessibility of food, influenced by the vegetation structure at forest stands (Barbaro et al., 2011). For this reason, the question emerges if the preference of forage abilities at shelterbelt edges occur due to increased prey abundance or rather to enhanced accessibility when compared to stands without shelterbelts? Specifically, the recordings of four different predator species were assessed looking at the absence and presence of broadleaved tree shelterbelt stands with their respected edges and interiors. In addition, both stands were also evaluated based on their edge orientation either facing from the south or to the north. Data showed that there was no significant effect that stands containing shelterbelts influenced the predation rate inflicted on the plasticine caterpillars, even though there were signs of predation in these stands, the hypothesis cannot be proved true. While the experiment involving caterpillar models may not directly yield estimates of the natural to which predation occur, it is plausible that the relative frequency of predation incidents could exhibit comparability across different habitats. Hence using these models to serve as a relative measure to represent the diversity and intensity of attacks (Tvarikova & Novotny, 2012).

Playing a vital role in forest ecosystems, insectivorous birds are considered to be good bio-indicators as they indicate the level of biodiversity present within forest stands (Tvarikova and Novotny, 2012). The majority of damages inflicted on the caterpillars were caused by avian predators, further suggesting that birds can be considered as the most important and frequent predators across forest stands (Bereczki et al., 2014). Essentially it should be emphasized that the presence of bird markings does not necessarily indicate that birds are the main predators in forested areas but also be considered together with the co-occurrence of other opportunistic predators residing in the same areas such as arthropods and ants (Cupitra-Rodríguez et al., 2023). Nonetheless, it's crucial to bear in mind that the method involving plasticine caterpillars may not be as appropriate for obtaining absolute measurements of predation by distinct predators (Mrazova & Sam, 2018). Even though this study did not find evidence of ants and arthropod predators Gardarin et al. (2023), characterized arthropods exhibiting a small fraction of predation and conversely, found that slow opportunistic consumers like slugs were considered more significant predators. Slugs were seen as the second most frequent predator to attack the caterpillars. Supported by Nimalrathna et al. (2023), the increased attack rate by slugs, could potentially stem from a combination of behavioural, olfactory, and visual cues emitted by live prey, however on the models it was merely stemmed off of olfactory cues as some of the minerals or salts can be attractive and cannot be considered as intentional predator attacks (Mrazova & Sam, 2018; Nimalrathna et al., 2023). The diversity of avian species can be increased in a landscape with compositional complexity (Berg, 1997). Thus, the higher the heterogeneity, the higher the species density in the edge. Therefore, the planting of shelterbelts creates habitat heterogeneity at the edge, allowing the co-existence of diverse predator species (Berg, 1997). Essentially, the absence of shelterbelts also facilitated the occurrence of predators like rodents and occasionally parasites potentially providing a niche preference suitable for survival (Berg, 1997). As explained in a study done by Ferrante et al. (2017), the few mammal markings found were not considered as an important source of predatory attack in temperate areas mainly due to the fact that this type of predation could have happened by chance. Although identified as less significant predators, small rodents still play a significant role in the ecosystem functioning (Tvarikova & Novotny, 2012).



The overall average of bird predation was low, in fact, the degree to which predation happened between the different plots and stands, showcased inconsistent results. The synoptic population model of Southwood and Comins (1976), may explain the effect that caused the lowered predation pressure on the plasticine caterpillars. The model suggests that the restriction effect that most of the generalist predators possess, such as birds, cannot be expressed at high prey densities. During these situations, a possibility of diseases can lead to a population decline. (Southwood & Comins, 1976). Thus, the role to which birds play to maintain caterpillar abundance is low, not suppressing high caterpillar populations, thereby extending the time between pest outbreaks (Berecki et al. 2014). Other factors are also considered, due to the fact that birds selectively prey on caterpillars based on visual cues influencing the attack rather out of cautiousness than intentional (Ferrante et al., 2017). Birds develop traits that recognise objects that do not offer any nutritional benefit nor resemble real prey possibly due to the absence of any movement (Tvarikova & Novotny, 2012; Berecki et al. 2014). On the other hand, the possibility to which the caterpillars were installed in the field contributed to the lack of bird predation cannot be completely ruled out. Indicating that the consequence of the prey arrangement may be at fault, thus leading to no significant effect of higher predation in the stands (Ferrante et al., 2017). Given that a majority of birds in forested habitats typically forage for their prey on branches and leaves oriented at specific heights, they could develop the ability to recognize and steer clear of objects that are either exposed over a longer period of time or found at low hanging branches (Berecki et al., 2014). Part of the caterpillar installations happened on younger trees, thus providing less structural diversity and limited feeding ground than larger, older trees, possibly making the data biased (Berecki et al. 2014), failing to prove if birds serve as a natural pest management alternative. However, a positive predation correlation was found, ensuring that predators did benefit to some extent in terms of the different tree sizes and ages (Berecki et al. 2014).

According to Whelan (2001), the foraging behaviour of predators can also be determined by the distance of leaves on branches, neighbouring leaves from one another as well as the shape of leaves on different species of trees. Most apparent factor by broadleaves, is shading that might have had an influence on the caterpillars from being predated as well as the sparse distribution of leaves in oak trees poses challenges for predators in effectively accessing their prey (Whelan, 2001). In fact, one might argue that the shade provided by oak trees could offer some protection against the impacts of climate change. However, the interplay between climate, habitat characteristics, and trends in predator abundance still requires thorough investigation and validation (Blumgart et al., 2022). These factors can be characterized as robust defence mechanisms against herbivores to survive and therefore, might've indicated the lowered desired predation in the shelterbelts (Whelan, 2001). Although shelterbelts may have not shown significance in predation, the shelterbelts provided a corridor for species to move from outside the stand towards the edge (Roy, 2006).

Separating edge effects from area effects requires extensive environmental data and is considered a concern for landscape ecologists when examining how fragmentation impacts prey-predator relationships with other components of forest biodiversity (Barbaro et al., 2012). The little to no statistical significance displayed likely attributed to the fragmented or the so-called 'island' stand sizes and the varying levels of human interference within the fragments (de Castro & van den Berg, 2013). However, as the edges and the habitat stand sizes interact, the frequency and intensity of ecological processes in these areas often increase due to organisms' ability to adapt (Barbaro et al., 2012). Overall, broadleaved tree shelterbelts planted

in combination with pine stands are often preferred by foraging birds due to the presence of substantial nesting cavities within oaks, thus further supporting the evidence of higher predation in the shelterbelt edges (Barbaro et al., 2011). These forest stands in turn, create a larger niche preference most often preferred by birds due to the ability to fulfill both breeding and foraging necessities as shown in fig. 26 (Barbaro et al., 2011; Berecki et al. 2014).

While the findings indicate to some extent the impact of stand type on the predation rate the same cannot be said for the interaction gradient of shelterbelt-to-edge-to-center of bird predation as the study was unable to establish any discernible effect in terms of in heterogenous stand connectivity. In addition, similarities was found when looking at the connectivity between the edges and centers between the two stands. Edge to center facilitates the dispersal activity of numerous species, especially for species with limited dispersal abilities (Roy, 2006). Any indication of significance would've implied that these stands supported species that would not typically occur in the area as the stand wouldn't match the species' niche requirements (Roy, 2006). This could be due to the fact that some of the habitat quality might have already been lost as a result of fragmentation implying that community structure of natural occurring prey is already fluctuating both within and between stands (Gray et al., 2022; de Castro & van den Berg, 2013). These similarities matched with de Castro and van den Berg (2013) and found that a relatively uniform structure but also the close proximity of the stands may have had influence, resulting in minimal to no contrast.

Upon visualizing the data the center containing a shelterbelt proved to be a preferred hunting ground for birds as a higher predatory preference can be seen throughout when compared to the edge. This partly supports the hypothesis as shelterbelts harbour higher biodiversity to which the centers of these stands, create a specific niche to which certain species can accommodate, in addition, appearing to necessitate distinct center habitat conditions (de Castro & van den Berg, 2013). Species that have specific adaptations for utilizing resources exclusively within the center of stands, thus tend to avoid edges where the habitat quality, predation risk, and/or micro-climate conditions would be less favourable (van Halder et al., 2011). These adverse effects have been proven to be consistent across various taxonomic groups, including avian species (van Halder et al., 2011). This finding was in contrast to Barbaro et al. (2012) and Ouin et al. (2015), observing higher bird species assemblages in the edge that resulted in increased predatory rates as the dispersal of birds was mainly concentrated at the edges. In a landscape predominantly characterized by pine plantations, it would be assumed that the that broadleaved shelterbelts would have served as appropriate habitats or supplementary feeding sources for predators (Castagneyrol et al., 2014). However, mechanisms such as increased solar radiation are more profound in edges than in the center therefore, creating a microclimate to such extent that would be beneficial for opportunistic organisms such as pest species (Pulgarin Diaz et al., 2022). Pest species such as lepidopteran larvae tend to prefer increased temperatures to enhance their survival rates, thus increasing the availability of food resources in the edges (Pulgarin Diaz et al., 2022; Barbaro et al., 2012; Berecki et al., 2014). The same accounts when comparing the predation rates referencing to the direction of the stand, to which north-facing stands implying an increased abundance of predator species. However, Lövei and Ferrante (2017), implies that forests across different geographical latitudes did not reveal a statistically significant rise when moving from north to south similar to this study. Nevertheless, the possibility of north-facing stands exposed to sunlight, could mean that there is a presence of sun-dependent plant species that benefit from this further creating microhabitats (Lövei & Ferrante, 2017). As the microclimate changes

within habitats, prey species are expected to experience the northward shift as warmer areas in combination with increased light availability leading to changes in productivity both in larval development and leaf formation (Delzon et al., 2013). As a result, broadleaved edges therefore create suitable nesting, feeding and breeding opportunities for potential avian predators (Barbaro et al., 2012). This assumption, nevertheless, has not always been proven in practice, by the fact that bird insectivory can be higher in forest edges despite higher defoliation or herbivory on trees found in the center as seen in the moth trapping experiment (Barbaro et al., 2012). Certain native species may display a seasonal shift in their edge occurrence towards the center, potentially linking to the fact that the accessibility of prey is influenced by the structure of the forest understory in the center (Barbaro et al., 2012). For instance, specialized species are sensitive to seasonal changes and prefer to occur within the forest center, therefore relying on habitat connectivity within the stand forest to maintain viable breeding populations (de Castro & van den Berg, 2013). Further, this behaviour is also likely influenced by the tendency of crucial resources like leaf-litter arthropods as prey occurring in the centers (Barbaro et al., 2012).

This study did not focus on the influence of microhabitats in the edges explicitly, the data does support the likelihood of enhanced predation in mixed forest plantations leading to the conclusion of positive effects of shelterbelt presence on the mixed stand forest ecosystem, possibly extending beyond the boundaries of the studied stands (Bereczki et al., 2014). Even though it remains uncertain whether there is a significance between predation rates and stand heterogeneity, the provided data showed clear indication of predation within the center of stands containing shelterbelts. Thus, leading to the conclusion that the presence of broadleaved trees may increase species richness and that the role of predators plays an essential part in plantation forests, providing vital ecosystem services and pest management less. Overall, while the use of the plasticine caterpillar method is a novel approach, the findings emphasize the importance of further research that compares actual predation rates to those observed using caterpillars as a proxy for predation in forest mixed stands. Hence, suggesting that a long-term study would be required to establish whether the attack rates of predation is influenced by the presence or absence of broad-leaved tree shelterbelts.

#### 4.2 Pheromone attraction of the moth species

The results obtained from this study showed the rate at which attacks inflicted by defoliators would happen in plantation forests. Specifically, the capture of the three moth species were assessed looking at the absence and presence of broadleaved tree shelterbelt stands with their respected edges and interiors. In addition, both stands were also evaluated based on their edge orientation either facing from the south or to the north. The experiment indicated that the stands containing broadleaved tree shelterbelts captured the most defoliator species, specifically *L. monacha*. The presence of this species further indicates that stands containing mixed tree species experience a higher attacking rate resulting in more damage to trees. Considering that mixed stands have associational resistance, the stands sampled weren't able to support the associational resistance theory, stating that stand diversity had no effect on lowered occurrence of moths (Jactel et al., 2021; Alalouni et al., 2014).

Trees grown in mixed stands prove to be less vulnerable to pests and diseases than in pure monoculture stands (Damestoy et al., 2020; Jactel et al., 2021). However, in some instances, associational susceptibility can be observed in mixed stands showing equal or higher susceptibility to insect herbivores compared to pure stands. The presence of certain tree

species in mixed stands enhances the vulnerability of neighbouring trees that, in turn, favours certain herbivorous insects (Damestoy et al., 2020). Plus, stands that serve as an ecotone, a region between forest stands and surrounding habitats, are probably more accessible for insects, ensuring movement from one forest patch to another at each generation (de Castro & van den Berg, 2013). While tree diversity in planted forests has shown the ability towards lowering pest impact in stands, the studied relationship between diversity and resistance in semi-natural mature forests is still not yet fully comprehended (Guyot et al., 2016; Dulaurent et al., 2012). A study done by Castagneyrol (2014), showed that the impact of tree diversity can be influenced by both the phylogenetic diversity of within mixed stands and the specialization of defoliators. It was found that tree mixtures with higher phylogenetic diversity exhibit higher resistance against generalist defoliators, resulting in lower levels of infestation by the pests studied (Castagneyrol, 2014). However, this experiment was not specifically designed to examine the effects of phylogenetic diversity on the occurrence of pest species in mixed stands. Therefore, the hypothesis must be rejected stating that the presence of broadleaved tree shelterbelts in combination with edges could serve as a barrier, further reducing infestation by preventing defoliators damaging pine trees.

The results provided no statistical evidence regarding herbivore-plant interaction to trap placement, whether placed at the forest edge or center, further giving no indication of interaction between the varying plots within each stand. The data showed that the total catches in traps placed in the edge and center of each stand were auto-correlated, meaning that there was some underlying relationship between the catches in nearby traps and that the experiment contained small sample sizes, resulting in reduced statistical influence for the study (Bergès et al., 2013; Jactel et al., 2021). Further, the observed pattern can be attributed to two possible explanations, which are not mutually exclusive (Précigout & Robert, 2022). Firstly, it is possible that the traps attract defoliators equally in stands with and without shelterbelt. Secondly, defoliators are equally abundant in both types of stands (Précigout & Robert, 2022). On the contrary, the findings indicate that there is little to no difference between the edge and center between the two different stands.

There was an observed pattern that stands containing shelterbelts had a higher occurrence of pest species than stands without shelterbelt. As the development and growth of moths are influenced by the quality of its food source, elements such as leaf carbohydrates, nitrogen content and other compounds provided by broadleaved trees such as oaks are most preferred (McCollin, 1998). However, the assumption that edges exhibiting higher heterogeneity compared to the center is not supported by the results. Responses at the edge can vary significantly based on the preference of a particular species, thus heavily influenced by the specific characteristics encountered (van Halder et al., 2011). Higher numbers of moth species can be typically found in edges due to interception as they stop on the first host tree they come across in breeding season (Pulgarin Diaz et al., 2022; Dulaurent et al., 2012). However, explained by Castagneyrol et al. (2014), the majority of larval nests are located at the edges of stands, and the abundance of these nests could be approximately 25% lower behind shelterbelts than in the absence of a shelterbelt. According to Dulaurent et al. (2012), when edge pines are disguised by high shelterbelts, olfactory cues that female moths utilise in selecting the most obvious trees are compromised. Further, this may indicate that these species might either avoid, or inhibit no edge preference in these stands (van Halder et al., 2011). Yet, it may also be possible that the way the pheromones were exposed in the field contributed to the minor occurrence of the other species, such as *D. pini* and *P. flammaea* and

*L. monacha* being the most abundant pest. In addition, *L. monacha* is considered highly polyphagous, feeding on various trees, thus capable of expanding its' feeding range, supporting the fact that more moth species were found in heterogenous stands (Jactel et al., 2021). Further, *L. monacha* also has the ability to adapt to fluctuating temperatures, experienced in heterogenous stands therefore, giving reason for the high number of captures whereas *D. pini* and *P. flammaea* would be more susceptible to long-term climate changes as they cannot adapt as fast (Haynes et al., 2014; Jactel et al., 2021).

As seen throughout the experiment, as trees situated in the center of mixed stands showed a higher likelihood of pest infestation, confirming similar results found by Guyot et al. (2019). As stated in their study, *L. monacha* often forage within the centers of stands in combination with lowered levels of management, therefore, exhibiting a more widespread utilization of habitats (Guyot et al., 2019). Some research suggests that the highest diversity of moths occur in large, unfragmented forests characterized by minimal edge effects, while others state the opposite (McCollin, 1998). This sort of connectivity enables moth species to spread and persist. However, habitat fragmentation breaks this connectivity by separating habitats in smaller patches thus resulting in lowered fitness of trees, increased edge effects for pest species to invade (McCollin, 1998). Dulaurent et al. (2012), stated that defoliators prefer monoculture stand edges more due to its favourable environmental conditions provided by increased exposure to sunlight, thus increasing in temperatures, further affecting the surrounding microclimate. However, this was not found as possible biotic environmental factors like wind, altering the active dispersal of pheromones, may have also had an impact on the edge species capture (Williams & Jonusas, 2018). A study done by Slade et al. (2013), observed that the abundance of moth species exhibited a notable pattern in relation to distance from the edge to the interior in monoculture stands, showing an increase in numbers as the edge distance increased from the center. An edge effect can be particularly pronounced within a 100 meter range highlighting the importance of considering the proximity of connectivity (Slade et al., 2013). While the stands without shelterbelt had a distance 100 meter distance between the edges and centers, the same cannot be applied to the stands containing a shelterbelt, as this distance was decreased. Nevertheless, stands containing shelterbelts had a higher occurrence of pest moth species. These observed patterns may have further been influenced by methodological issues like pheromone placement.

The orientation of forest edges can significantly impact the intensity of edge effects. As the climate change issue is expected to exacerbate this issue even further, with temperatures expected to increase, eggs laid by female moths could double, further resulting in earlier hatching, and enhanced survival rates of pupae (Pulgarin Diaz et al., 2022). A recent study done in Finland showcased that moth pest species utilize their ability to rapidly respond to rising temperatures by expanding their geographical range (Melin et al., 2020). Corresponding to the results of Melin et al. (2020), an observed northward movement of moth species is seen indicating that certain life-history traits of species like *P. flammaea*, showed a positive correlation with temperature. However, population levels may continue to fluctuate locally due to alterations in habitat suitability (Pulgarin Diaz et al., 2022). Planted forests cannot create the same quality range of ecological richness compared to natural forest areas suggesting that changes to stand structure due to forest management and increased herbivore browsing could be contributing to this decline (Blumgart et al., 2022). Further, in the northern hemisphere, south-facing edges tend to exhibit more pronounced edge effects compared to north-facing edges due to higher exposure to sunlight altering underlining environmental conditions

(Bernaschini et al., 2019). Characterized by this, south-facing edges are known to have a higher abundance of light-demanding and competitive plant species thus creating a distinct edge effect (Boeraeve, 2019). Observed was the non-significance of catches in the south compared to the north-facing edge, making the results inconsistent with the hypothesis. Due to the fact that the pest infestation was higher in the north than in the south, it would be therefore, likely be attributed to a smaller conspicuous edge effect in the south-facing stand edges (Boeraeve, 2019). The low abundance in the southern regions is further supported by the same observed pattern in broadleaf stands during the caterpillar experiment. These findings suggest that defoliators are currently experiencing unfavorable conditions in the south-facing edges compared to the north-facing edges. In contrast to the findings, Régolini et al. (2014), found that the edges experiencing more shade, specifically those facing north, exhibited lower rates of infestation compared to edges facing the south that experienced intermediate levels of infestation. The edge effect experienced in the south-facing edges, creates an optimal habitat for poikilothermic organisms such as nocturnal lepidopterans that prefer to mate and lay their eggs on sun-exposed trees for their warmth (Dulaurent et al., 2012; Parlak et al., 2019).

The observed inverse trend regarding the presence of birds to *L. monacha* abundance implies that there is an observed increase in predation located at the center due to the occurrence of *L. monacha* at higher densities in stands containing shelterbelts. Therefore, the higher the predation, the lowered occurrence of the pest species. As explained by Guyot et al. (2019), the results may have been influenced by edge effects interacting with various environmental conditions within the forest center, thereby influencing ecological processes within the forest stands, altering the quantity, quality, and connectivity of habitats and in turn, species. Proposing that bird populations respond numerically to variations in prey density, as indicated by the primary influence of bird abundance on predation rate. Although this intensified accumulation of pest species in the center of the stands could potentially impede further growth in defoliator abundance, therefore, not supporting the hypothesis that shelterbelts provide effectively reduced infestation in terms of predator species supporting biological control (Bereczki et al., 2014). With moths serving as an important food source for important predators, both during their larval and adult stages, the low observed numbers caught may pose a threat to species that depend on them as prey, such as insectivorous birds. For instance, studies have reported an observed insect decline leading to the decline of insectivorous bird populations, particularly among European species (Blumgart et al., 2022). Even though some countries experienced an increase in planted forest areas, there is still a continuous loss of mature, natural forests due to urban development and production (Blumgart et al., 2022).

### 4.3. Important considerations for future research

#### 4.3.1. Remarks and Sampling strategies

Quantifying the extent of predation pressure and identifying predators pose as a challenge in ecological research (Zvereva & Kozlov, 2022). Therefore, the use of plasticine caterpillars can be one indirect approach to observe predation. However, a main concern that arise when conducting such study is whether the observed predation rates accurately reflect the actual intensity of predation (Zvereva & Kozlov, 2022). Considering this, it is important to note whether there is any bias in estimating predation intensity, and if so, whether this bias varies among different predator groups present in a specific location at a given time (Zvereva & Kozlov, 2022). Plasticine caterpillars do not mimic most of the natural features when compared to a real caterpillar, in terms of movement and chemical release. This means that anti-predator

traits would not come into play during an attack that would make most avian predators avoid pecking on the models. Most avian predator attacks rely on visual cues, reacting on small movements rather than on chemical cues (Zvereva & Kozlov, 2022). Fortunately, for this study, results did show evidence of various predators in the environment, therefore providing a positive bias, as the surrounding habitat is already conducive to support their presence.

A general recommendation for improving this type of study would be to perform a quantitative assessment and have a comprehensive understanding beforehand, as this would ensure that the evaluation process of using plasticine models be easily identified by their strengths and drawbacks (Birkhofer et al., 2017). Further, both fieldwork and sample collection occurred during the spring-summer period of May to June 2022, this would be considered as an optimal experimental period seeing that the activity of bird predators increases due to nesting, breeding and, optimally hunting for resources. However, numerous other studies have indicated that the timeframe to which such an experiment is most effective would be the months of March to May (Bereczki et al., 2014). Regardless of the data analysed, the experiment ran only for one trial over a period of three to four weeks, leading to substantially different results when compared to other studies that ran multiple trials over subsequent years. Therefore, it is suggested that the incorporation of at least two trials would enhance the proper interpretation of prey-predator interactions in heterogenous species forest stands. Likewise, increasing ecological data collection in terms of further analysis on additional variables would also improve the statistical models for further evaluation for the influence of stand heterogeneity on predation and pest monitoring. Additional explanatory variables such as stands age and tree size heterogeneity, were reported by Bereczki et al. (2014), to prove a positive correlation with the abundance of species.

Another factor should be considered as a comprehensive understanding is needed when studying the associational effects between the forest edge and center, therefore, it would be essential to identify the species of interest and examine their biological traits, focusing especially on their dispersal abilities and diet (Williams et al., 2013). This will allow valuable insights into the mechanisms responsible for the plot differences measured in each stand. Synthetic pheromone lures are known to be effective in attracting male moths hence, playing a fundamental role in establishing an effective monitoring system. The use of synthetic sex pheromones is increasing globally as a method to monitor systems for numerous insect pests, with a particular focus on Lepidopteran pests (Williams et al., 2013). Thus, it is essential to ensure that the mixture of the pheromone components are correct. Any small changes to the composition of the lure could alter the effectiveness of the pheromone (Williams et al., 2013). The limited number of captures observed in the funnel traps placed approximately 20 to 50 meters apart, leading to the possible attribution of close proximity of the traps. Traps placed close to each other could lead to interference and competition between the pheromone lures potentially affecting the trapping efficiency (Régolini et al., 2014). De Smedt et al. (2019), stated that the distance to which the traps had been placed between the forest center and edge could influence the edge effects on the abundance of moth species. Leading to the recommendation of placing traps further from one another, improving the success of the data in the future. When looking at the experimental design of this study, the data that was gathered occurred in one day. By gathering repetitive, additional data on a smaller scale, the model's accuracy of the moth distribution can be improved, enabling a more precise calculation of the distribution between stands with and without shelterbelt required for species to complete its life cycle. It should be mentioned that it will also be more accurate to separate the pheromones

respectively placing each in their own trap. In the study done by Jactel et al. (2006), the data found that the traps baited with pheromones that were cleaned regularly, maintained its efficiency, rather than traps left uncleaned for a period of time, in turn reducing the risk of odors released by captured insects possibly contaminating the traps. The analysis presented in this study focuses solely on describing abundance patterns, lacking the ability to provide a comprehensive biological explanation. Therefore, further research is needed, that would include long-term data collection and experimental studies, to elucidate and describe the biological mechanisms that connect organism abundance with annual thermal sums and other weather variables (Pulgarin Diaz et al., 2022).

Lastly, establish a clear link between the moth population density and the actual levels of defoliation observed in the stands. This method would help to focus on understanding the relationship between moth abundance, predation pressure, shelterbelt position, and additional environmental variables such as temperature (Leroy et al., 2021). This information could be proven essential to identify areas that are most vulnerable to moth damage and possibly indicate a high predation rate. Gathering such data will contribute to improved forest management practices and a more comprehensive understanding of the ecological dynamics within forest ecosystems (Leroy et al., 2021).

#### 4.3.2. Sustainable Land-use Management: Important role of shelterbelts

While the current study may not have yielded conclusive implications for forest restoration and management concerning the integration of broadleaved tree shelterbelts in forest plantations, it's important to acknowledge that similar research offers valuable insights of forest heterogeneity towards restoring forest ecosystems and the promotion of biodiversity. Forest plantations face multiple threats regarding the impact of climate change increasing in both frequency and intensity (Freer-Smith et al., 2019). Therefore, forest managers and landscape ecologists have been enhancing their understanding of the relationship between specific tree species and their surrounding environment, as this knowledge enhances biodiversity conservation and ecosystem resilience (Freer-Smith et al., 2019; van den Berge, 2021). From this perspective, it would be reasonable to consider that the selection of tree species is undeniably the most critical factor. These species for example include but are not limited to birch and pedunculate oak (Lust et al., 1998). The value in selecting native tree species for shelterbelts is considered important, as native tree species are able to maintain the balance of resource utilization, attributing to genetic diversity and, the method is cost-efficient (Lust et al., 1998; Valdés-Correcher et al., 2019). Maintaining such genetic diversity within the forest plantation, offers a crucial advantage in terms of long-term economic viability, particularly in the face of potential challenges such as global climate change and pest outbreaks (Lust et al., 1998). Therefore, effectively sustaining native species within forest plantations, adopting a multi-purpose approach that aligns with sustainable forest management principles are needed. Further, the design of such areas also holds immense importance as they will serve as corridors for various species facilitating the movement of diverse species across landscapes (Brockerhoff et al., 2008). Accounting for factors such as stand age and species composition, further ensures the creation of habitat heterogeneity that accommodates the niche requirements of different organisms further utilizing the ability of shelterbelts to serve as a connection to fragmented habitats, providing a refuge and resources especially for migratory birds (Brockerhoff et al., 2008; Freer-Smith et al., 2019; Terraube et al., 2016). Interestingly enough, one of the factors attributed to the long-term population decline of Afro-Palaearctic migrant birds is the loss of suitable stopover edge sites due to land-use changes in European



landscapes (Terraube et al., 2016). Nevertheless, shelterbelt edges support the increased response of predator-prey interaction as seen in previous studies (Terraube et al., 2016).

In light of the this, several key recommendations emerge. First and foremost, the compelling case made to prioritize the inclusion of stand broadleaved edges within early warning detection systems (Fält-Nardmann et al., 2018). It is suggested by Fält-Nardmann et al. (2018), that shelterbelts provide a risk reduction system, making them disturbance-resistant and ecologically stable against pest infestations. The expansion of the defoliators prompt the need for an effective monitoring system to effectively determine and monitor their expansion range and population growth. With this being said, the results of this study indicate that through identifying the important variables that might have an impact on moth captures in pheromone traps would be crucial to identify beforehand, for the purpose of developing a successful and sustainable monitoring program (Williams et al., 2013).

Despite this, with the shelterbelts providing a dilution of insect pest species filtrating into the stand, further reducing the likelihood of pest insects finding a suitable host and support the presence of predators and their accessibility to resources is significantly greater at the edges than in the interior of the forest (Jactel et al., 2021; Dulaurent et al., 2012). Therefore, a comprehensive assessment in evaluating the influence of climate and land use changes at landscape scale can further advocate utilizing remote sensing as a cost-effective and a continuous practical strategy to cover extensive areas (Régolini et al., 2014). By utilising these techniques, managers can transcend from the traditional strategic limitations and gain a more comprehensive understanding of the complex interactions in the forest understorey, especially at the edges thus contributing to adaptive and responsive management approach (Terraube et al., 2016).

If managed accordingly, forest plantations can act as supporting systems that may establish successful conservation efforts through the provision of additional habitats, enhancing the connectivity and resilience, in reference to complementing and supplementing existing ones (Halder et al., 2011; Brockerhoff et al., 2008). Standard management strategies and the traditional land-use planning approach lack the necessary tools to address the complex and interconnected nature of the sustainability challenges confronting society in the present era (Vangansbeke et al., 2014). As efforts to combat climate change in combination with the transition towards a bio-based economy, the demand for wood products serving as a base for energy production is expected to increase as well (Eggers et al., 2019). Over the last decade, the need for research and alternative methods that explicitly embrace system thinking that involved the long-term viability of forests has steadily increased to regulate ES (Eggers et al., 2019). These strategies include the implementation of forest plantations to relieve commercial timber demands. Nevertheless, with the global expansion of plantation forests sparking extensive debates, regarding their purpose for biomass production and biodiversity conservation, alternative management strategies need to be considered for the optimization of carbon sequestration and ecosystem functioning (Bremer & Farley, 2010; Eggers et al., 2019). Although the forest cover is expanding, there is still a significant risk regarding the functioning of forest ecosystems mainly due to the continued deterioration of quality indicators such as soil, hence the need for biodiversity monitoring (Ahumada et al., 2013). Monitoring biodiversity enables informed decision-making aimed at improving the resilience of ecosystems through effective management and conservation strategies. In the same sense that monitoring pest species can indicate a warning system for forest rangers (Ahumada et al., 2013; Fält-

Nardmann et al., 2018). In forest areas where stands are susceptible to defoliators but currently not experiencing outbreaks, as for the study area, it is advised to establish a centralized and efficient monitoring network for defoliators. This monitoring system should be designed to require minimal effort while providing continuous surveillance of pest population dynamics. Its primary focus should be on detecting increases in population densities, expansions in distribution and outbreak ranges, and the earliest signs of defoliation (Fält-Nardmann et al., 2018).

While the majority of existing research of forest biodiversity has centered on enhancing forest resistance, there is an emerging perspective that suggests altering forest management practices to enhance resilience could hold significant appeal for local stakeholders and managers (Vangansbeke et al., 2014). Through this shift, an interactive democracy can be formed where legislation on forest management plans can be revised and improved (Vangansbeke et al., 2014). Incorporating stakeholders, which includes groups or individuals who are impacted by forest management, in development strategies is becomes relevant as the shift in focus becomes particularly relevant given the substantial uncertainty associated with global change thus promoting comprehensive understanding and enhancing transdisciplinary knowledge (Eggers et al., 2019). Rather than focusing on resistance, the concept of fostering resilience through adaptive management strategies could provide a more flexible and effective approach to navigating the challenges posed by a rapidly changing environment (Vangansbeke et al., 2014). This assist in working towards establishing the viability of ES in the long-term strategic plans within these plantations maximizing both the provisioning and regulating services (Bailey, 2007; Lust et al., 1998).

## 5. Conclusion

Monoculture pine stands is often characterised with lowered biodiversity and has gathered rather significant criticism in comparison to mixed tree species stands. In practise, this study investigated the extent to which the benefit of forest heterogeneity might have in monoculture pine plantations, by comparing the biodiversity effect in stands containing broadleaved tree shelterbelts to stands without broadleaved tree shelterbelts.

The first part of the research focused on the crucial role of insectivorous bird predators in diverse tree plantations, serving as natural enemies for insect herbivores. The enemy hypothesis suggests that there should be an existing correlation between bird predation rate and increased tree species richness, even if it occurs at a small spatial scale. On the contrary, the findings failed in demonstrating the rate to which predation would take place correlates with habitat heterogeneity, thus drawing conclusions from similar experiments that showed significant evidence suggesting the incorporation of shelterbelts to be crucial habitats in forests. In addition, results shown that forest centers are habitats with valuable properties from forest edges regardless of their direction. The consistent higher predation in the center of the stands throughout the study suggest that predators may use edges located in the North as corridors. There is a need for a better understanding of the factors influencing species' presence as future research should focus on including environmental variables, such as temperature, percentage of forest cover and possibly leaf area index in time. The presence of predators contributes to the provisioning of valuable ecosystem services in both the presence or absence of shelterbelts in stands. Lastly, the experiment proved that if the population of a new caterpillar pest would dramatically increase, a combination of birds, rodents, slugs and parasites demonstrate their ability to remove pest species.

As for the second part of the study, the research investigated the ability of broadleaved tree shelterbelts in enhancing associational resistance in pine stands. Establishing an edge comprised of broadleaved tree species could potentially offer a more efficient way to manage pest populations. It appeared that the increased effect of *Lymantria monacha* residing in the study area, was affected by the stand heterogeneity as numbers increased towards the center. Thus, explaining the fact that there is reduced biological control of pest species provided by the diversity seen in heterogenous stands. Although not a new phenomenon, the occurrence of the pest species observed can possibly be explained by climate change, specifically the increasing temperatures and decreased precipitation patterns creating favourable conditions for them to mate and lay their eggs. Next, would be the explanation of edge effect created by forest fragmentation. Numerous artificial roads run through the stands, reducing connectivity between neighbouring stands, and leading to the formation of edge effects. Moreover, regular monitoring of pest numbers during the summer season should be implemented. To summarize, it is crucial to maintain a continuous pheromone trap monitoring system. This is particularly important for IPM programs in coniferous forests, representing as an effective and reliable pheromone-based monitoring system that plays a key role in managing defoliating insect pests.

Overall, evidence show that the diversity of stands cannot simply be equated with effective predation nor suppression on pest species. As a result, assessments of predator abundance are seldom integrated into decision-making processes for the management of pest Lepidoptera. However, coupled with findings from other experiments, this can offer valuable insights that can inform the adoption of certain management practices. A strategic approach

that involves planting a diverse mixture of tree species together, rather than in isolated patches containing individual species, could yield more effective results in terms of insectivorous bird-mediated pest management within production forests. By incorporating native tree shelterbelts around pine plantations, forest managers can aim to achieve not only high productivity but also a range of ecological benefits such as maintaining biodiversity, promoting sustainable approaches and management of pest species. By conducting fieldwork in the forest that investigated similar environmental variables compared with previous experiments, it was possible to assess the effectiveness of shelterbelts in the context of a monoculture plantation.

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## Report Internship

**Organization:** KU Leuven Division Forest, Nature and Landscape (DFNL) & Instituut voor Natuur en Bosonderzoek (INBO).

**Professor:** Professor. Bart Muys & Myriam Durmortier

### **Presentation of own position and internship activities**

When describing my internship, I refer to the practical work of my thesis. Before starting the fieldwork, I had the privilege to receive valuable training in May 2022. This involved working alongside professionals at INRAE, Bordaux, France. Scientists who carry out, organize, and coordinate, in response to a governmental request, with scientific research such as forestry. During my time at INRAE, I received comprehensive training in various aspects related to my thesis project. Elena Valdés-Correcher hosted and guided me to analyse data collection and analysis and in addition assisted me towards creating and installing the plasticine caterpillars, crucial for assessing biodiversity impacts. Hervé Jacktel provided hands-on training on the deployment of pheromone funnel bucket traps, an essential technique for capturing and studying pest moth species.

Fieldwork preparations began in the summer examination period in late May to early June 2022. The aim was to execute the fieldwork as precisely as possible without seasonal challenges. As a first step of finalizing the preparation for the fieldwork, different materials were needed and had to be purchased. During the month of May, the plasticine caterpillars used in the fieldwork were handmade according to the protocol. In June, plasticine caterpillars were deployed in the field. The second part of the fieldwork was conducted in June to July 2023 evaluating important pest moth species occurring in the study area. This consisted of funnel traps that needed to be prepared in early June, pheromones that had to be purchased and be set up in the field. Afterwards, the caterpillar markings and moth species were counted and used for data analysis.

### **Contribution of the internship activities**

Given that the internship equates to the practical work of the thesis, it was mainly important for collecting and processing the data. With the protocol established by me, it made it easier to decide and carry out the variables chosen for this fieldwork (i.e. North to South, presence or absence of shelterbelt and different plot positions) happened in 2022 and again in 2023. I read a wide range of research to gain a solid understanding of the established methods to measure such variables in order to adequately prepare for the method that I would apply. In chapter two as well as Index 2 and 3 of this project, a thorough explanation of the methodology used to measure the various variables is given. The activities during the internship made it possible to conduct the research project, to analyze and discuss the results. In other words, the activities formed the basis for the research through evaluating if the incorporation of broadleaved tree shelterbelts would lead to a higher incidence of biodiversity and lowered pest invasion. The primary objective of this research project was to advance sustainable forest management practices that support biodiversity, ecosystem resilience, and ecological sustainability, while concurrently preserving and safeguarding ecosystem services. Specifically, the study investigated the optimal management intensity that optimizes both ecosystem service provision and yield. The findings from this research have the potential to significantly contribute

to the preservation and enhancement of forest biodiversity, ecological resilience, and the promotion of sustainable forest management practices, while also ensuring the continued provision of essential ecosystem services. Such information is necessary as declining biodiversity in forest ecosystems due to human activities poses significant challenges, impacting the natural control mechanisms. Therefore, understanding the influence of predators and pests within shelterbelt-containing stands becomes crucial for informed decision-making by forest managers. This knowledge not only promotes sustainable forest management practices but also facilitates the development of integrated pest control strategies.

In terms of the materials that were required, I had the responsibility to purchase certain items to carry out the measures, including plasticine clay, wire, insect repellent and plastic bags. Making 624 caterpillars in a few days were difficult and probably near to impossible when you also have to study for upcoming exams in June. Fortunately, I received assistance from a few local students in the lab to complete the duties on time. As a result, I simultaneously improved communication skills with people who have varied educational levels (i.e. PhD, postdocs and master students) which is crucial in a working setting. Further, I needed to organise the delivery of the pheromones with a local company that distributed them. One challenge I faced regarding the requirement of these materials was the fact that one of the pheromones were out of stock and the project was further delayed into mid-June. Due to this, I had to work both on the analysis and creating a new data frame at the same time to complete those measures. I contributed most of my internship preparing the dataset created for my thesis and delivered two cleaned datasets towards my data analysis. Sanne Verdonck provided me guidance on how to analyse the different variables in my dataset and which model would be appropriate for the analysis in R Studio.

### **Additional internship activities**

I dealt with two major projects as additional activities to my internship activities dealt with threats related to biodiversity decline. The first short-term internship I did was at the Department of Earth and Environmental Sciences at KU Leuven. It lasted about two weeks as part of the Bos, Natuur en Landschap division with Sanne Verdonck, a PhD student studying the 'Optimization of climate-smart forest management to alleviate drought stress in temperate forest systems' with Professor Muys as her supervisor. The internship activities were conducted in facility of the GEO – Institute on campus Heverlee. This project involved me in separating beetles from woodlice necessary for data analysis used in her project. Although this activity was not linked to my thesis topic nor was the data used in my thesis, the experience at KU Leuven has enriched both my academic persona being flexible and collaborate amongst students. No doubt that the project exposed to a new topic, sorting beetles from woodlice which in a similar way I would utilize the techniques learned applying it to my project but with moths. The project consisted of pitfall traps, that collected the invertebrates whose distribution is affected by the taxon's desiccation sensitivity. It captured my interest and curiosity to become part of my methodology which I have used in my fieldwork. In this sense, the experience at the KU Leuven allowed me to discover unexplored areas and get out of my comfort zone as a researcher. This internship took place in November 2022, with a flexible arrangement of two to three days per week due to other students also working on the same project as a student job.

The second long-term internship, about three months from December 2022 to February 2023 involved me participating in research involving the effects of pesticide use on the Belgian



agriculture sector, resulting in 15% reduction in pesticide usage by farms at INBO (Instituut voor Natuur en Bosonderzoek), Brussels. Alongside, senior researcher Myriam Dumortier, who was my supervisor, I also assisted with drafting legislation recommendations to European Union policymakers based on analysis of Belgium's pesticide policies, contributing to a 20% increase in support for stricter regulations. This practical experience allowed me to apply the theoretical knowledge gained during my studies to real-world scenarios, advancing in my problem-solving skills. The engagement with drafting legislation recommendations, and interaction with other colleagues provided me with valuable insights into the intricacies of environmental policy-making processes and the practical application of scientific findings in a policy context.

## **Reflection**

Upon arriving at INRAE, the collaborative and supportive environment, allowed me to gain practical skills and knowledge in a practical work setting and with this being said, applied what I learnt there directly to my research. It was an immense opportunity to observe and learn from experienced researchers, benefiting from their expertise and insights. This allowed me actively engage in fieldwork, employing new methodologies that were previously unfamiliar to me. This in turn, acquired me to gain invaluable skills that I can utilize in my future career as an environmental scientist and create professional relationships with possible future collaborators. For this reason, I can apply my skills to contribute to further research projects that will be aimed at mitigating environmental impacts, evaluating the effectiveness of sustainability initiatives, and informing evidence-based decision-making. While the trajectory of my career may or may not lead me back to INRAE, one thing is certain: the methodologies and inquiries I have learned during these internships will be with me. The hands-on experience has enhanced my understanding of the subject matter and has provided me with a solid foundation for conducting further research as an established scientist.

Throughout my bachelor's and master's studies, the datasets provided for various assignments were already completed, which resulted in less effort required for data preparation before analysis. However, when I had to work with my dataset, I realized that my skills in RStudio had to be significantly improved. I had to learn from scratch how to input data from my data set into R and how to analyse it. This process was slow, going through various YouTube videos and articles while mistakes occurred and the process of learning became more challenging for me to grasp the intricacies of the dataset, but luckily Sanne Verdonck assisted me throughout this process, and I completed my result interpretation. Through this engagement process, it offered me a more comprehensive understanding of analysing my own research findings, along with diverse and complementary viewpoints on data interpretation. Moreover, interpreting the analysis results served as an invaluable opportunity to further develop my professional skills. From this experience, I concluded that it is beneficial to be involved in both the sampling and analysis stages, as it provides a more comprehensive understanding of the data and minimizes potential issues.

An area where I could have enhanced my approach involves conducting an even more comprehensive review of relevant studies prior to the internship. This preparation would have allowed me to better grasp diverse strategies and protocols for assessing the various variables. The internship tasks may have been completed much more effectively in this way. However, I should acknowledge that my preparation time was limited, occurring during the demanding semesters as a master student, coinciding with an intensive exam period. Despite these

challenges, I am confident that I successfully accomplished the stipulated objectives of my internship. In the end, I discovered that you acquire a lot of knowledge while being under pressure and that protocols can be altered on the fly, which will undoubtedly also be the case for my upcoming career, during which I will pick up a lot of knowledge on the job and exhibit flexibility. In conclusion, my internship proved to be a valuable and fulfilling experience, characterized by supportive colleagues and engaging tasks. Not only did it provide me with the opportunity to further my knowledge in conducting extensive environmental research, which is essential for my thesis, but it also granted me valuable insights into the world of research as a career path. The internship significantly enhanced my professional skills within a scientific research setting, equipping me with practical expertise that will undoubtedly prove beneficial in future employment opportunities.

On a personal level, with all the internships that I completed for this project assisted me in terms of personal growth by challenging me to step out of my comfort zone and undertake tasks that demanded a high level of responsibility and giving me a sense of independence. Navigating complex issues or challenges that came forth, meeting certain deadlines, and adapting to a fast-paced research environment further developed my organizational and time-management skills.

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## Annex 1:

### Additional results

#### Caterpillar predation

*Table 5: The analysis explored the relationship between bird predation and three independent variables, such as the direction of the stand, the presence of a shelterbelt, and the position within the stand utilizing various predictors in a Gamma distribution with a random effect the PlotID as it accounts for the variability between different levels of the fixed effects (independent variables).*

<b>Frequency of bird predation</b>					
<b>Predictors</b>	<i>Estimates</i>	<i>std. Error</i>	<i>CI</i>	<i>t value</i>	<i>p</i>
<b>(Intercept)</b>	0.87	0.97	0.33 – 16.92	0.89	0.37
<b>Type of stand with shelterbelt [Yes]</b>	-0.13	1.04	0.11 – 7.13	-0.13	0.90
<b>Direction of plot [South]</b>	0.72	0.93	0.31 – 13.28	0.77	0.44
<b>Position within the stand [Edge]</b>	0.40	1.04	0.18 – 12.18	0.38	0.71
<b>Position within the stand [Shelterbelt]</b>	0.20	1.12	0.13 – 11.70	0.18	0.86
<b>Random Effects</b>					
$\sigma^2$	2.02				
$\tau_{00}$ Plot	0.00				
$N_{Plot}$	17				
<b>Observations</b>	52				
<b>Marginal R<sup>2</sup> / Conditional R<sup>2</sup></b>	0.074 / NA				

When examining Table 3, at the “Direction of plot [South]” referenced to North, it was found that stands oriented towards the south had a positive estimated coefficient, suggesting a slight increase in the incidence rate of bird predation. However, this effect was not statistically significant since the p-value is greater than 0.05. A larger standard error can also be seen throughout the model, providing evidence that there is a high uncertainty in the intercept value. The same can be said when considering the position within the stand, being at the edge and shelterbelt of the stand when referenced to the center, it was associated with a slight increase in the estimate rate of bird predation but cannot be proved true with a non-significant p-value. Regarding the presence of a shelterbelt, the estimated coefficient for stands with a shelterbelt was -0.13 indicating a 1.3 times lower incidence rate of bird predation compared to stands without a shelterbelt. Further, the residual variance ( $\sigma^2$ ) is 2.02 and  $\tau_{00}$  Plot is 0, indicating the amount of unexplained to no variation in the frequency of bird predation when the fixed and random effects are not accounted for. The marginal R-squared ( $R_2$ ) is 0.074 indicating that the fixed effects explain about 7.4% of the total variability in the frequency of bird predation. In summary, the statistical analysis did not provide strong evidence for significant associations

between these factors and bird predation incidence rate, hence not rejecting the null hypothesis for birds being the main predator.

In figure 15, it is shown that the increased caterpillars that had markings retrieved from the stand had a positive relationship with the frequency of the predation, meaning that the predation on the same caterpillar happened or in the same tree. However, this is proven to be not statistically significant as the relationship between 'The amount of each caterpillar attacked': 'Position within the plot' [Shelterbelt],  $p > 0.05$  as well as 'The amount of each caterpillar attacked': 'Position within the stand' [Edge],  $p > 0.05$ . With my  $R^2$  value being 0.6739, approximately 67.39% of the variability in the predation frequency can be explained by the amount of each caterpillar attacked and position within the stand in my linear regression model stating that the line does a decent job of explaining the pattern in the data.

Overall, based on the equation and  $R^2$  value, we can say that there is a moderate-to-strong positive relationship between the amount of markings on the caterpillars and the frequency of predation. As the amount of markings on the caterpillars increases, the frequency of predation tends to increase as well.

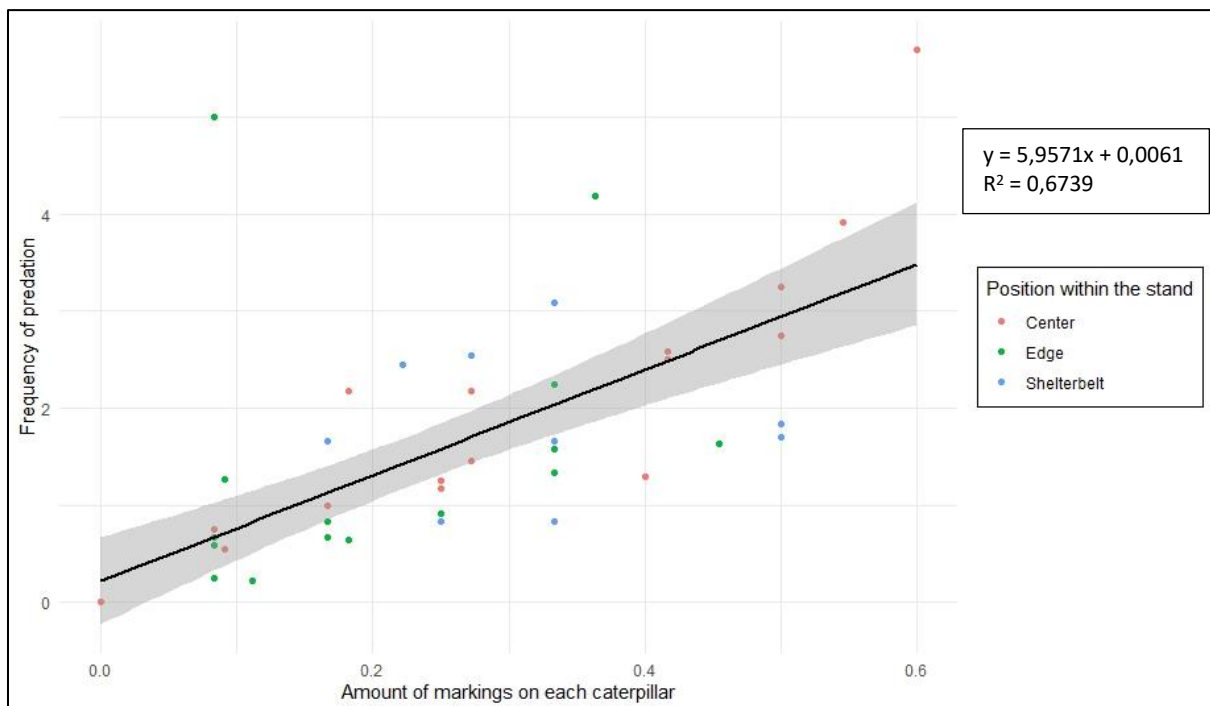


Figure 38: Relationship between the predation frequency on the amount of caterpillars that had markings in each position of the stand ( $R$  square = 0.6739).

Utilising the paired t-test, the gradient of interaction between the center to edge of the stand in terms of absence in shelterbelt was analysed. With this, it was evident that no effect was seen in terms of bird predation to position of the stand whatsoever, indicating no reliable pattern of predation regarding the position within the stand ( $df = 7$ ,  $p = 0.81$ ).

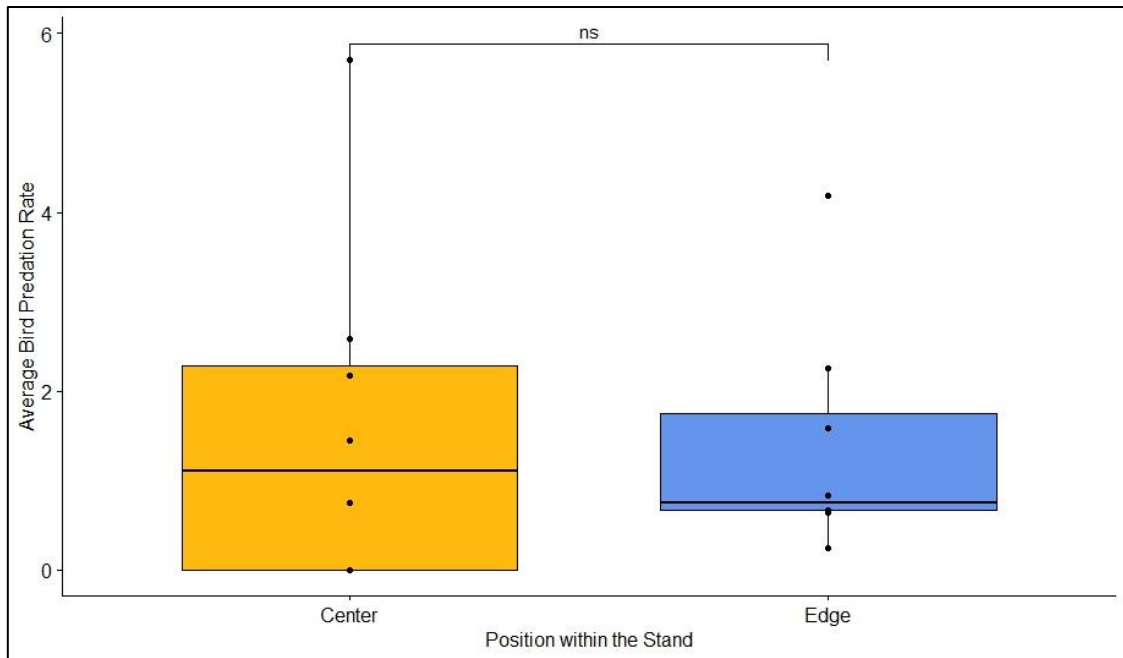


Figure 39: Interaction between the center and edge position regarding the average bird predation in the stands containing no shelterbelts.

#### Moth captures

Based on the data in Table 4, the weight and the total moths captured did not completely correlate with one another hence statistically analyzing the moth captured according to weight. Although the model was performed through the Gamma family, there is no strong evidence to support a significant difference in moth weight between the different variables used within the stands. The results for the weight of total moths captured align with the findings for the total moths captured model, indicating that the variables “Direction of plot”, “Type of stand with shelterbelt”, and “Position within the stand” do not have a significant effect on both the total amount and weight of moths captured.

Table 6: The analysis explored the relationship between the total amount of the moth weight captured and three independent variables, such as the direction of the stand, the presence of a shelterbelt, and the position within the stand utilizing various predictors in a Gamma distribution with a random effect the PlotID as it accounts for the variability between different levels of the fixed effects (independent variables).

Total weight of moths captured					
Predictors	Estimates	std. Error	CI	t-value	p
(Intercept)	3.70	1.25	3.15 – 511.44	2.95	<b>0.006</b>
Type of stand with shelterbelt [Yes]	-0.20	1.06	0.10 – 6.71	-0.20	0.85
Direction of stand [South]	0.28	0.94	0.20 – 8.89	0.30	0.77
Position within the stand [Edge]	0.04	0.60	0.33 – 3.34	0.08	0.94
<b>Random Effects</b>					

$\sigma^2$	0.38
T <sub>00</sub> Plot	1.40
ICC	0.79
N <sub>Plot</sub>	17
Observations	39
Marginal R <sup>2</sup> / Conditional R <sup>2</sup>	0.016 / 0.789

Although most of variance was explained by site direction, there was a non-significant effect of type of stand composition on male captures ( $\chi^2 = 20.99$ , df. = 22, P = 0.52). Specifically, the captured species, calculated with two-way ANOVA, were on average, 1,4 times more moths in stands with shelterbelts ( $23,73 \pm 2,38$ ) than in stands without shelterbelts ( $21,13 \pm 0,56$ ). In the interior of the stand, individuals were observed and in the edge of the stand's individuals. Species that were mostly absent in both the center and edge of the stands were identified as rare with less than 2 individuals per trap found.

The calculated Pearson correlation coefficient of -0.19 suggests a weak negative linear relationship between the variables 'The amount of markings per caterpillar' and the '*L. monacha* captures'. Implying that if the predation in the stand increases, the trend might lead a slight decrease in the *L. monacha* species. However, with the p-value, calculated as 0.43, exceeding the significance threshold of 0.05, the provided evidence in Fig. 30 does not substantiate the rejection of the null hypothesis. This suggests that the observed correlation between the variables is likely attributable to random variability rather than a meaningful relationship.

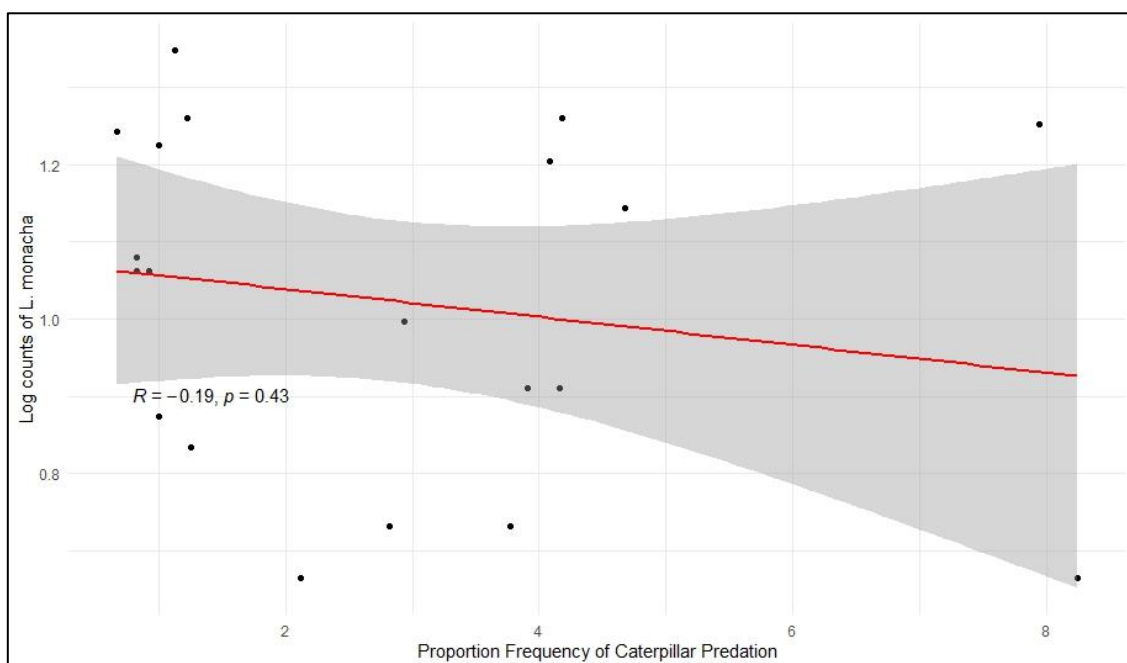


Figure 40: Pearsons correlation test between the amount of Log count of *L. monacha* caught in the stands to the overall predation rate within each stand respectfully.

## Annex 2:

### Protocol for the predation assessment on fake caterpillars:

This protocol describes in detail the different tasks that needed to be carried out during the project.

#### Key steps

- As soon as possible, identify several **pine trees and additional oak trees** of at least 6 cm in diameter, measured at 1,30m in height, containing low laying branches, preferably having leaves, to facilitate the tree species identification.
- Make 12 fake caterpillars per tree, using the material provided.
- 15-20 days after the caterpillar instalment, carefully remove the caterpillars and count the predator marks. Carefully fix all caterpillars onto small blocks of styrofoam in a box.

#### Materials:

- Modelling clay Staedler (Noris Club 8421, green)
- Thin metallic copper wire, 0.5 mm diameter (3m)
- A complete caterpillar to serve as a reference.
- Styrofoam and cardboard boxes

#### Choosing the right time

The climate varies a lot from Southern (Spain and Portugal) to Northern (Sweden and Finland) Europe. This has a strong impact on tree phenology of which include the timing of budburst and leaf fall. It may also have a strong impact on insect herbivores feeding on leaves and on naturally occurring enemies (predators). It is therefore important that the installation of the caterpillars happens at the same phenological stage. This should be between late April and late May, depending on your country.

#### Making the caterpillars

- Prepare a ball of modelling clay of 1 cm diameter, weighing approximately 1 gram and 12 cm of thin wire



- Gently press the clay between your fingers onto the middle of the wire



- Roll the modelling clay around the wire so as to obtain a 3-cm long "caterpillar"





### Installing caterpillars

Prior to installing the caterpillars, select **4 low hanging branches** easily reached from the ground, **one facing North, South, East and West**. Attach a short (less than 5 cm) coloured ribbon **at the base of the tree** to make it easier to retrieve.

Attach 4 caterpillars per branch, leaving approximately 15cm between caterpillars.

- Find a thin twig with a clear area of about 10-15 cm without leaves. Remove one or two leaves if there is a risk that they will brush the caterpillar.
- Align the caterpillar along the branch and wrap one of the free ends of the wire around the branch.
- Carefully grip the caterpillar between the thumb and index finger and wrap the other end of the wire around the branch.



- Tada! The caterpillar is ready. **Make sure that caterpillar surface is completely smooth** (there should be no marks left by fingernails).

### For each caterpillar:

Gently remove each caterpillar from the branch. You can fix each one onto the styrofoam blocks for easy transport **and making sure that the caterpillar is protected and that its surface will not be touched by other loose metallic wires**.

**Once all caterpillars have been removed and stored** (should be 12, but some may have disappeared).

Inspect each caterpillar with the magnifying glass. Note all the marks on a sheet of paper. If any mark is visible on the caterpillar surface, google each different mark by searching something like “plasticine caterpillar bird mark”.

### For data analysis:

Clearly write down the number of installed caterpillars, the number of undamaged or lost. Then record the **total number of damaged caterpillars** (regardless of predator type) and the number of caterpillars with predation marks left by each predator type. For example, if predation marks left by both birds and insects are visible on the same caterpillar, you will count this caterpillar as damaged once in total, and once by birds and once by insects. Indicate 0 (zero) instead of leaving the cell empty if no damage was observed.

### Assessing predation rate

The predation frequency will be assessed by **inspecting the** predation marks twice, ideally for the first **15 days**, then **30 days** after caterpillar installation.

You will need to use a **magnifying glass** as some damage may be poorly impressed into the modelling clay. The most common marks are left by bird beaks and insect mandibles. Less often, you may see teeth marks left by mammals (mice) or lizards.

**It is crucial that caterpillars are handled with care to avoid “false positive” (marks that are not left by predators but by leaves, branches or fingernails).**

### Predation marks left by birds:

The presence of predation marks left by birds is a common occurrence. These marks are distinguishable by their distinctive 'V' shape. This can vary in depth, with some being relatively superficial while others are noticeably deeper. The 'V' shape indicates the pecking behaviour of birds, as they grasp the caterpillar with their beaks and create an impression in the clay. These marks are frequently encountered, suggesting that avian predation is a significant factor affecting the caterpillars in the study area.



### Predation marks left by slugs

The presence of slugs can be identified by their distinct chewing marks, which can be observed repeatedly that they went back and repeated their chewing process. These marks are typically concentrated at the ends of the caterpillar, indicating the feeding behaviour of slugs. Sometimes this is an irregular pattern and can vary in depth and intensity. The surface of the caterpillar may exhibit visible signs of slug feeding, with shallow depressions or trails created by their movement.

### Predation left by other predators

In some instances, it is possible to come across caterpillars displaying marks that do not align with the previously described characteristics. These alternative marks may have been caused by mammals such as mice or lizards, although such occurrences are infrequent.

### False positive:

It is important to note that not all marks observed on the caterpillars necessarily indicate predation. Some marks may fall under the category of "false positives," indicating non-predatory interactions or incidental damage. The presence of several parallel thin and superficial scratches on the leaves of the tree suggests that friction between adjacent leaves has occurred. Additionally, grooves can be observed, which are marked by the wire or nails.

Furthermore, imprints of the branch are noticeable, indicating the locations where the installation took place.

## Annex 3:

### Protocol for the moth species assessment using pheromone traps:

This protocol describes in detail the different tasks that needed to be carried out during the project.

#### Key steps:

- As soon as possible, identify several **locally known moth species** by looking at websites such as waarneming.be.
- Assemble all 40 traps that should be deployed in the field
- 15-20 days after the pheromone funnel trap instalment, carefully remove the moths and count the species accordingly. Carefully pack all moths in a marked ziplock bag.

#### Materials:

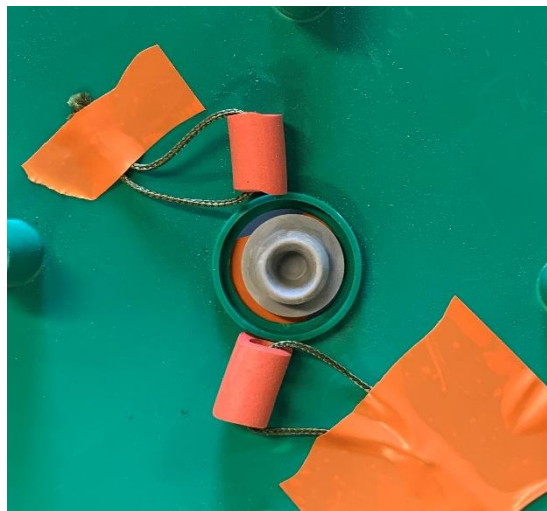
- Green INRAE bucket funnel traps equipped with a plastic cage in the lid
- Desired pheromones ordered from a local distributor
- Rope or wire to suspend the traps from the trees
- Ziplock bags
- Cardboard boxes

#### Choosing the right time

As such, the mating period of the female and male moths begins in the peak of summer (June, depending on the country of instalment) when they take flight. This has a strong impact on tree phenology when trees developed their matured leaves (ideal for shading) leading to a strong impact on insect herbivores feeding on leaves and on naturally occurring enemies (predators). It is therefore important that the installation of the traps happens at the same phenological stage.

#### Assembling

Take out a pheromone lure from its packaging and carefully position it inside the lure plastic cage. It is recommended to wear gloves while handling the lures. Close the cage by attaching the cap, and then insert the assembled cage through the circular opening located at the center of the trap lid. If the cap does not fit tightly into the lid opening, it can be secured with tape for added stability.



Connect a wire handle to the lid by passing it through the two loops provided. A one-meter wire or string can be utilized as an acceptable form of support. However, it is worth noting that string may not have the same durability as wire and may need to be replaced more frequently.



### Installing the traps

The plastic bucket trap is a durable and effective method for monitoring and detecting different moth species when combined with a lure. It is particularly favoured for its ability to capture a high volume of moths without causing damage to their distinguishing features. The trap consists of four components *i.e.* a lid, a lure basket with a cap, a funnel, and a bucket. It is offered in a variety of colour combinations to cater to specific needs.



It is also important to note that labelling the containers would be an essential part when installing the traps. This is to ensure complete accuracy when doing data analysis. Attach a waterproof label either on top of the buckets or on the side with a water-proof black marker on the bucket trap indicating which site it is.



Placement of traps should be done about 10 meters from each other. In this case, the shelterbelts will be excluded from the sampling method due to the close proximity. Note that the traps should also be placed 1 to 1.5 meters above ground to ensure easy retrieval and emptying of traps.

After the three week of installation, the bucket traps may be emptied and stored in marked zip lock plastic bags according to the site. Place an absorbent paper, such as a piece of a paper tissue, inside each plastic bag to reduce moisture and to pad the specimens for their protection. If longer-term storing is necessary, freezing works best, but refrigeration is acceptable as well.

#### Assessing the numbers of captures

The capture frequency will be assessed by **inspecting and emptying the** traps twice, ideally for the first **15 days**, then **30 days** after installation.

You will need to use a **magnifying glass** to assist in the distinction of each moth species from one another. Some moths may have damaged their wings in the process, however, if the moth did have no wings for identification, they would be excluded. Similarly, remove any moth that is vastly different from the target and all other arthropods (beetles, flies, spiders).

#### For data analysis:

Clearly write down the number of installed traps and the number of traps lost.

Then record the **total number of moths in each stand** and the number of species caught per trap. Indicate 0 (zero) instead of leaving the cell empty if no moth capture was observed.