

# Transformation of pine monoculture to biodiverse food forest

Baseline study of soil quality, carbon stock and biodiversity in a pine production forest on sandy soil in Overloon, the Netherlands.



By Maximiliaan Koebrugge

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Universiteit Utrecht & Copernicus Institute

Supervised by Dr. Pita Verweij  
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## **Abstract**

The ‘great divide’ between nature and culture has put immense pressure on the stable state of the Earth System. To keep the Earth in a stable state, a system change is necessary. The transition from conventional agriculture or silviculture to perennial agroforestry food systems promises prosperity, however, the legitimacy of these claims needs to be assessed and quantified. To assess such a transition, this study has provided a broad baseline assessment of the soil quality, aboveground woody carbon stock and biodiversity of a pine production forest that will be transformed into a food forest system. Additionally, the outcomes of two soil regeneration methods have been compared experimentally and the possible effect of the system transition has been explored.

The forest in Overloon is characterised by acidified, nutrient poor, sandy soils, with a low CEC that is mainly taken up by ‘useless’ cations ( $H^+$  and  $Al^+$ ). The average aboveground woody carbon stock of the forest in Overloon is 72.2 Mg C/ ha, just below the Dutch average. The plant diversity of the forest is low: a total of only 31 plant species has been found, including planted trees and mosses. The addition of rock flour to a forest clearing has a significant effect on the amount of herbaceous saplings that emerged and surprisingly none of these species had been found in the forest before.

To turn this forest into a productive food forest system, the soil conditions will need to improve. Rich leaf litter is expected to turn on the nutrient pump which will turn the system from its downward spiral into an upward spiral of increasing fertility and decreasing acidification. Possibly the nutrient pump will need a helping hand of minerals (rock flour) to get started. The restoration of a healthy soil ecosystem will benefit many species, from fungi and plants up to the big carnivores; thus the entire system will become more biodiverse, productive, and resilient.

**Keywords:** *Baseline study, soil quality, aboveground woody carbon stock, biodiversity, rock flour, soil regeneration, food forest, pine production forest, silviculture.*

## **Lekensamenvatting // Dutch lay summary**

De stabiliteit van onze aarde staat onder grote druk door de manier waarop wij mensen voedsel verbouwen. Onze huidige landbouw bestaat uit grootschalige, intensieve monoculturen, die jaarlijks geploegd, bemest en behandeld met pesticiden worden. Hierdoor nemen de bodemkwaliteit en productiviteit af, terwijl dit ook toe kan nemen. We hebben natuur en cultuur ver uit elkaar geplaatst, terwijl wij als mensen onlosmakelijk afhankelijk zijn van de natuur voor onze dagelijkse behoeftes, denk bijvoorbeeld aan schoon drinkwater, zuurstof, of voedsel.

Voedselbossen kunnen een bijdrage leveren aan het oplossen van het klimaatprobleem. Een voedselbos is een door mensen ontworpen productief ecosysteem, naar het voorbeeld van een natuurlijk bos, met een hoge diversiteit aan meerjarige planten, waarvan delen (vruchten, zaden, bladeren of stengels) eetbaar zijn. Dit onderzoek probeert in beeld te krijgen of voedselbossen hun beloftes kunnen waarmaken.

Dit onderzoek richt zich op een naaldbomen productiebos dat omgevormd wordt naar een voedselbos. We hebben een nulmeting uitgevoerd naar de huidige stand van de bodemkwaliteit, koolstofopslag en biodiversiteit, zodat kan worden vastgesteld wat het effect is van de transitie naar een voedselbos. Daarnaast hebben we gekeken naar de invloed van steenmeel en kalk op gecreëerde open plekken. Tot slot hebben we het verwachte effect van de transitie naar een voedselbos uiteengezet op basis van onze bevindingen en literatuur.

Het productiebos in Overloon heeft een verzuurde, zanderige bodem met schaarse voedingsstoffen. De bodem kan zijn nutriënten niet vasthouden, waardoor het systeem sterk verzuurd en verarmd is. Het bos heeft een koolstofopslag van 72 ton per hectare in bovengrondse biomassa, iets onder het Nederlands gemiddelde. De plantendiversiteit is erg laag, we hebben slechts 31 plantensoorten aangetroffen, inclusief mos en boomsoorten. Een interessante bevinding is dat de toevoeging van steenmeel aan de gecreëerde bosopeningen zorgt voor een significante toename aan ontkiemende kruiden. Deze kruidensoorten zijn nergens anders in het bos aangetroffen.

Om dit bos om te vormen naar een voedselproducerend systeem zal de bodemkwaliteit verbeterd moeten worden. Loofbomen produceren een rijkere strooissellaag, waardoor de nutriëntenpomp weer in gang kan worden gezet. Dit zal het systeem laten kantelen naar gestaag toenemende bodemvruchtbaarheid en afnemende verzuring. Mogelijk heeft de nutriëntenpomp een toevoeging van steenmeel nodig om op gang te komen. Het herstel van een gezond bodem ecosysteem zal een positieve invloed hebben op al het leven, van schimmels en planten tot grote carnivoren, en het hele systeem zal in biodiversiteit, productiviteit en veerkrachtigheid toenemen.

## Preface

Starting this project, Mats Kessel and I set out ambitiously to map every organism living in the deteriorated pine production forest at Overloon. We expected this unassuming forest to be unable to support a high diversity of life, however, when we took a closer look, we were quite surprised. Even though this *production* forest is not in its most *productive* state, it is teeming with life, if you know where to look.

First and foremost I would like to thank Mats Kessel, for the many heated discussions about the forest, its life within and methods regarding this major research project. You were excellent company during the many nights of camping and the long days in the lab, thank you.

Pita Verweij, thank you for the academic guidance and support on conducting this major research project. Jaco Appelman, thank you for your role as second supervisor and your creative input.

Jan-Willem van Bentum, Paul Slaats and Pieter van Bentum, thank you for the opportunity to conduct this research in your forest, the helpful lessons and discussions about food forests and the practical help with the research.

Big thanks to Naturalis and the ARISE project for granting us the necessary lab space, equipment and knowledge to identify the insect diversity of the forest. Specifically, big thanks to Tim Rietbergen, Hannco Bakker, Dionne van der Lugt and the ARISE DNA-team, without you we would not have been able to identify as many insect species as we have.

The expert ecologists, each in their own field, that helped us with identification, thank you Heinjo During, Leni Duistermaat and Roy Kleukers.

To the Vlinderstichting and specifically Jose Kok, thank you for the help with butterfly methodology and lending us the skinner trap.

Thank you Wouter van Eck for the insightful tour in food forest ‘Ketelbroek’ and to Stijn Heijs, chairman of ‘stichting Voedselbos’, for the helpful discussions about food forestry.

For the practical help with equipment, thank you: Pieter for the light bucket trap, Koen Wonders for the bat detector, Otto van de Beek for the drying stove and Berry Zwartjes for the camera trap.

Last but not least I would like to thank the volunteers that helped out with fieldwork along the way. Thank you Lisa Kluis, Fieke Koebrugge and Iza Nieuwenhuijs.

Altogether, this major research project was a great experience that gave me the chance to set up and perform my own research. I have learned how to properly execute a scientific study – by making many small mistakes – and gained sound knowledge on food forests and species identification. It was an interesting and mostly fun process and I can greatly recommend it to future students.

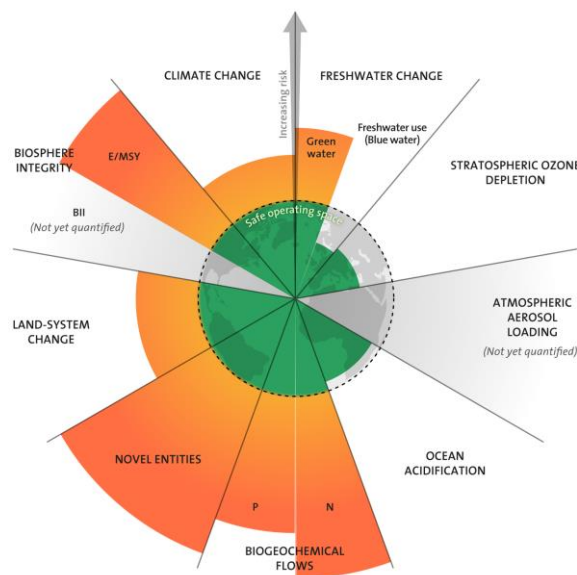
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## Introduction

The Anthropocene is the epoch in which the activities of a single species, us *Homo sapiens*, have altered global geophysical processes, threatening the resilience of the Earth System and its own very existence (Steffen et al., 2011). The safe operating space to keep the Earth System in its stable, accommodating environment of the Holocene has been determined using nine planetary boundaries (Rockström et al., 2009). Currently, we have transgressed the safe operating space for six of the nine planetary boundaries, thereby risking to cross thresholds that will cause non-linear, abrupt environmental change on a planetary scale (Rockström et al., 2009). The planetary boundaries at risk are: novel entities (environmental pollutants, including plastics), loss of biodiversity, biochemical flows (N&P loading), green freshwater change (water availability for plants), land-system change (for example deforestation), and climate change (Persson et al., 2022; Steffen et al., 2015; Wang-Erlandsson et al., 2022).



*Figure 1 The Conceptual framework of the planetary boundaries. The extent of the wedges depict an increasing risk to non-linear, abrupt environmental change. Licenced under CC BY-NC-ND 3.0 Credit: "Azote for Stockholm Resilience Centre, based on analysis in Wang-Erlandsson et al 2022".*

The environmental predicament that we are facing has predominantly been created by conventional agricultural systems: large-scale, intensive monocultures (Doran & Jones, 1996; Rohila Ansul et al., 2017). The system runs on annual crops that require extensive tilling, fertilization and pesticide use, thereby cyclically degrading the soil quality and reducing the relative yields (Benyus, 1997; Doran & Jones, 1996). Tilling practices expose the topsoil to the elements, leading to water and wind erosion. Recent estimates suggest that water erosion on croplands in Europe causes average soil loss rates of  $2.46 \text{ t ha}^{-1} \text{ yr}^{-1}$ , resulting in a total soil loss of 970 Mt in Europe each year (Panagos et al., 2015). Therefore, to keep the Earth System in a stable state, a system change is imperative.

The prevalent western definition of ‘nature’ – the whole of material reality, considered as independent of human activity and history – places a great divide between nature and culture (Ducarme & Couvet, 2020). This framework is relatively recent and conflicts with most other (non-European) visions of nature, however, it is currently used in public policies, conservation

science and environmental ethics. The ‘great divide’ between nature and culture is fundamental to our environmental predicament. Humans are an integral part of nature, we depend on the natural world for our survival and cannot place ourselves – including agriculture – outside of nature. It is preposterous to divide up the land in spaces restricted to either only nature or only agriculture, as proposed in the philosophy of “*land sparing*” (Fischer et al., 2014; Green et al., 2005). We must encompass natural processes wherever possible by following Benyus’ (1997) simple principle: “Life creates conditions conducive to life”.

Growing awareness of this ‘great divide’ and the issues caused by conventional agriculture, has led to a recent surge in interest in agroforestry (Dupraz et al., 2018). Agroforestry is defined as a “dynamic, ecologically based, natural resource management system that diversifies and sustains production in order to increase social, economic and environmental benefits for land users at all scales” (FAO, 2022). Agroforestry follows the life principle, as it uses biological processes to grow, fertilize, control pests and diseases and by doing so, the system mimics aspects of a ‘natural’ ecosystem. Agroforestry can take different shapes and forms, from a simple combination of crops and trees or trees and grazing to its ultimate form: an agrosylvopastoral system, a combination of trees, animals and crops. One of the most important benefits of perennial agroforestry is protection of the soil. An 8 year experiment has recently shown that a reduction in tilling significantly enhances soil organic carbon and nitrogen stocks, even in monoculture maize fields, while maintaining or even slightly increasing the yield (Fiorini et al., 2020). Moreover, advances have been made in the establishment of high-yield perennial varieties of major grain crops such as wheat and rice (Crews et al., 2014; Glover, 2022). These studies paint a promising picture for a future made up of perennial agroforestry food systems.

Food forests in the Netherlands are a form of perennial agroforestry systems and are defined as:

*‘Human-designed productive ecosystem, consisting of multiple layers and a rich soil system that mimics a natural forest, with a great diversity of perennial, woody species, of which parts (fruits, nuts, seeds, leaves, branches) serve as human food.’* (translated from Green Deal Voedselbossen, 2017).

Many studies and even a national monitoring program are set up to quantify the avowed benefits of food forests compared to conservative agriculture (Green Deal Voedselbossen, 2022). However, there is no research examining the effects of transforming a conventional silviculture system into a food forest system, even though 15% of the Dutch forests still consists of unmixed pine production forest (Schelhaas et al., 2022). Transformation to a food forest could increase biodiversity, productivity, carbon storage and economic return, however this has not yet been assessed. Therefore, a monitoring project is set up in a private silviculture plantation forest in Overloon, Noord-Brabant.

The forest is intended solely for wood production, periodically logged and, therefore, made up of different sections of even aged stands consisting of a single pine species. The owner plans to revitalize the forest by transforming the plantation into a food forest, considering the end goals of (1) an increase in carbon storage, (2) an increase in biodiversity and (3) an increase of



economic return (van Bentum, 2022). Due to its small size the forest will not bring about any structural change, however it can serve an educational purpose and inspire other forest owners.

This major research project is set up to provide a broad baseline study examining the current state of the soil quality, aboveground woody carbon stocks and biodiversity, in order to properly evaluate the effect of the planned changes to the forest in the future. In addition, I examine how this forest compares to other Dutch forests and what could be the effect of the transition to a food forest system. Next to that, an experiment is set up to assess the potential of two soil regeneration methods; the addition of chalk and rock flour.

For aspects of soil, carbon and biodiversity, this report first presents a separate baseline assessment, followed by a discussion exploring relationships and future potential. The study is guided by the following research questions.

### **Research Questions**

- 1) What is the state of
  - a. the soil, according to chemical, physical and biological variables;
  - b. aboveground woody carbon stocks;
  - c. plant and insect (*Lepidoptera* and *Odonata*) diversity?
- 2) Do these variables differ across the different forest types?
- 3) Considering these variables, what improvements are expected when turning this production forest into a food forest?
- 4) What are the short term effects of adding chalk and rock flour to a forest clearing and could this help to kickstart the nutrient cycle?

# Material & Methods

## Location

This study was conducted in a production forest near Overloon, Noord-Brabant, the Netherlands. The forest is made up of 26 hectares and mainly contains production species such as *Pinus sylvestris* (Scots Pine), *Pinus nigra* (Black pine) and *Pseudotsuga menziesii* (Douglas fir). The forest is a planted, managed system, consisting of even aged stands dominated by a single species in different densities. We mapped the area in detail using arcGIS and described each forest type (see figure 4, Results section). For the remainder of this report, the forest type codes (abbreviation of the scientific name of the dominant tree species) will be used when referring to a forest type.

The measurements to determine the soil quality, carbon storage and biodiversity were conducted in each of the major forest types: PsyOO, PsyJD, Pni, Pst and Pme. When conducting the different measurements, Pst and Pme were still, wrongfully, seen as one forest type, therefore the number of replications is not the same for all of the forest types.

## 1. Soil

### Soil quality

We collected an average soil sample for the major forest types (PsyOO, PsyJD, Pni and Pst/Pme), by taking a 0-25 cm core at 17 randomly chosen locations spread out across each major forest type. Additionally, a soil sample from the experimental plots was taken in each forest type. Plots located in the same forest area are considered homogenous (i.e. plot 1 and 2, and 3 and 4). Samples were collected by taking a 0-25 cm core from each of the quarters of the plot.

Several analyses of the soil samples were performed. Chemical analysis on the availability of nutrients (N, S, P, K, Ca, Mg and Na), physical analysis of the pH, organic matter, soil composition, CEC-analysis and water retention capacity and, lastly, biological analysis of the microbial biomass and activity were performed. These analyses were conducted by Eurofins, the methods used are included in appendix A.1.

### Soil water

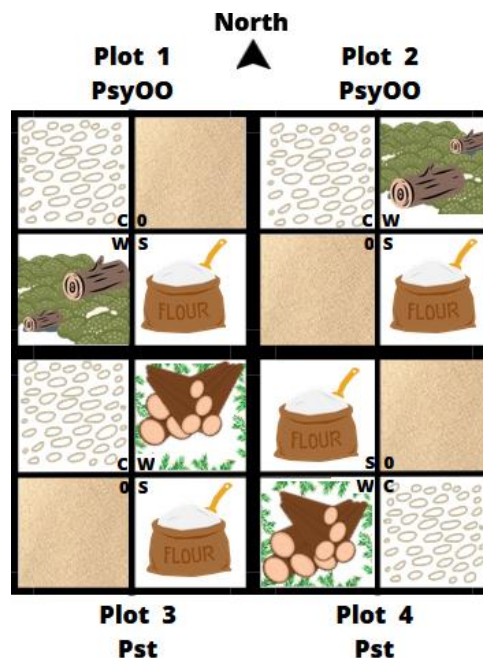
The soil water tables in the forest have been monitored from August 1973 until September 2018 by Brabantwater, a water extraction company. The data is publicly available and was retrieved from the website <https://www.dinoloket.nl/ondergrondgegevens>.

Next to that, we collected soil samples from different depths and examined the water content using the gravimetric method (Reynolds, 1970). Samples were collected on 01-09-22 after a period without rain. We used a soil corer to take sand from the depths 0-10 cm, 20-30 cm, 40-

50 cm, 100-110 cm, 150-160 cm and 200-210 cm. Samples were collected from the two largest forest types; PsyOO and PsyJD. The samples all weighed between 180 and 210 grams. The weight, with a precision of 0.1 mg, was noted and the samples were put in an incubator at 40 °C for 92 hours. After drying, the samples were weighed again and the relative evaporation weight was calculated.

## Experimental Plots

To examine different soil regeneration possibilities we created four square 10 x 10 m experimental plots, two in PsyOO and two in Pst. We chopped all trees on the plots on the 5<sup>th</sup> of March 2022 (Appendix B). We divided all plots into quarters with four different treatments (figure 2 below). The treatments were implemented on 29-06-22. Herbaceous seedlings were observed and counted per plot on 24-01-23.



*Figure 2 Treatments of the experimental plots. Four square 10x10m forest clearings were created, on forest type PsyOO and Pst, to test different soil regeneration methods. Each of the plots was divided in four to test effects of the different methods on plant growth: (W) Wood, (O) null, (C) chalk and (S) stone flour.*

(W) Wood: All of the chopped trees were left to decompose, without other interventions.

(O) Null: All organic material (trees, branches and leaf litter layer) was removed.

(C) Chalk: All organic material (trees, branches and leaf litter layer) was removed and 3.75 kg of DCM chalk (15% MgO) was added.

(S) Stone flour: All organic material (trees, branches and leaf litter layer) was removed and 7.5 kg of Stone flour was added. (see appendix A.2 for calculations)

## 2. Carbon stock

Above ground woody carbon stock of the forest was assessed by taking field measurements in the period May to August 2022. The carbon stock of was measured by taking the diameter at breast height (DBH; 130 cm) of all the trees in a square plot of 10 x 10 m. The tree height was determined using a Nikon forestry pro Laser Range Finder Hypsometer.

### Plot selection

Randomized plots were taken, spread out evenly across the different forest types. Due to their relative sizes, five plots were surveyed in the forest types PsyOO, PsyJD and Pni, and only one plot in the forest types Pst and Lde.

### Data collection

For each tree in a plot the species and circumference at breast height (CBH) was noted. The CBH was measured using a soft measuring tape. All woody species that were at least 130 cm in height were included in the measurements. If the CBH was thickened by a branch or cancer, the thinnest point below was used. If a tree separated into multiple stems below 130 cm, each of the stems was measured. The tree species was identified using the Heukels' Flora (van der Meijden, 2005), together with the identification app ObsIdentify. If identification was uncertain we consulted experts to identify the species.

### Carbon stock calculation

For the carbon stock calculation we used generalized species-specific allometric equations that have been developed in a comprehensive review that combined a total of 973 allometric equations (Forrester et al., 2017). The equation has species-specific parameters and is of the form:

$$\ln(Y) = \ln(\beta_0) + \beta \ln(D)$$

In which Y is the weight of the tree in kg and D the diameter in cm. The DBH is calculated from the CBH using the geometry formula  $c = 2\pi r$ . The  $\beta_0$  and  $\beta$  are species-specific parameters determined by Forrester et al.

These calculations yielded a biomass in kg per tree, which was transformed into a carbon mass by multiplying with the carbon content. The carbon content was set at 0.50, generally accepted and used in the Netherlands, as well as in other EU countries, to assess carbon stocks pools (Neumann et al., 2016).

The total area per forest type was calculated using ArcGIS and used to extrapolate the sampled carbon stocks to the total forest carbon stock.

### 3. Biodiversity

We used a variety of methods to examine the plant, butterfly (*Lepidoptera*) and dragonfly (*Odonata*) diversity that will each be described below. To conduct all measurements, we spent much time exploring each and every corner of the forest. Whenever we saw an animal, plant or fungus that we could identify, we would note it down. Next to that, we hung a camera trap at a small man-made pond to get to know its visitors.

#### Plant diversity

The plant diversity was measured by inventorying all plant species in a square 5 x 5 m plot. We examined a total of 5 plots in the forest types PsyOO, PsyJD and Pni, 4 plots in Pme and 1 plot in Pst. In each plot the cover or count of every plant species present was measured using a modified Braun-Blanquet scale (Colen, 2020; Hennekens, 2009). The scheme of the modified Braun-Blanquet scale that was used is shown in table 1 below.

Plant species were identified using the identification app ObsIdentify, in combination with the Heukels Flora (van der Meijden, 2005). When identification was uncertain, we consulted experts. The moss species were identified in consultation with Heinjo During.

All of the plots were sampled twice, in the beginning of April and in July, to account for seasonal differences. If the plant cover estimate from the second visit differed less than 10% from the first visit, the first observation value was used. Seedling *Pinus* trees could not be identified to species, therefore they were combined with the dominant *Pinus* species in and around the plot.

#### Data analysis

To determine the biodiversity of the different forest types the Shannon-Wiener index was calculated for every plot. The Shannon-Wiener index is commonly used and incorporates both the species richness and the evenness of species distribution (Spellerberg & Fedor, 2003). To calculate this biodiversity index, the cover values from the Braun-Blanquet method were converted to a relative cover midpoint (table 1). These values were used to calculate the Shannon-Wiener index using the vegan package in the statistical program R studio, software version 1.1.463 (Oksanen et al., 2019).

**Table 1 Modified Braun-Blanquet method to measure plant species abundance.** The cover midpoint gives the percentage value for each abundance category so that the Shannon-Wiener index could be calculated. These values are derived from the average percentage cover.

Code	Description	Cover	Abundance	Cover Midpoint	Relative Cover Midpoint
r	Very few	≤ 1%	1 individual	0.1%	0.001
+	Few	≤ 1%	2-5 individuals	0.5%	0.005
1	Numerous	≤ 5%	6-50 individuals	2%	0.020
2m	Very numerous	≤ 5%	>50 individuals	4.0%	0.040
2a	Random	5-12.5%	Not specified	8.0%	0.080
2b	Random	12.5-25%	Not specified	18.0%	0.180
3	Random	25-50%	Not specified	38.0%	0.380
4	Random	50-75%	Not specified	68.0%	0.680
5	Random	75-100%	Not specified	88.0%	0.880

### **Butterflies and Dragonflies (*Lepidoptera & Odonata*)**

The butterfly diversity is determined using the protocols from the *Vlinderstichting* so that the data could also be used for the national monitoring program. We created a ‘butterfly transect’, a route consisting of twelve 50 m sections, totalling 600 m, only crossing the PsyOO forest type (Figure 3). We sampled along the butterfly transects ten times in the months May to September. All butterflies and dragonflies spotted within a distance of 2.5 metres to either side, 5 metres in front and 5 metres above the ground were noted. Butterflies and dragonflies were captured and identified using ObsIdentify and field literature (Bos et al., 2016; Wynhoff et al., 2016).

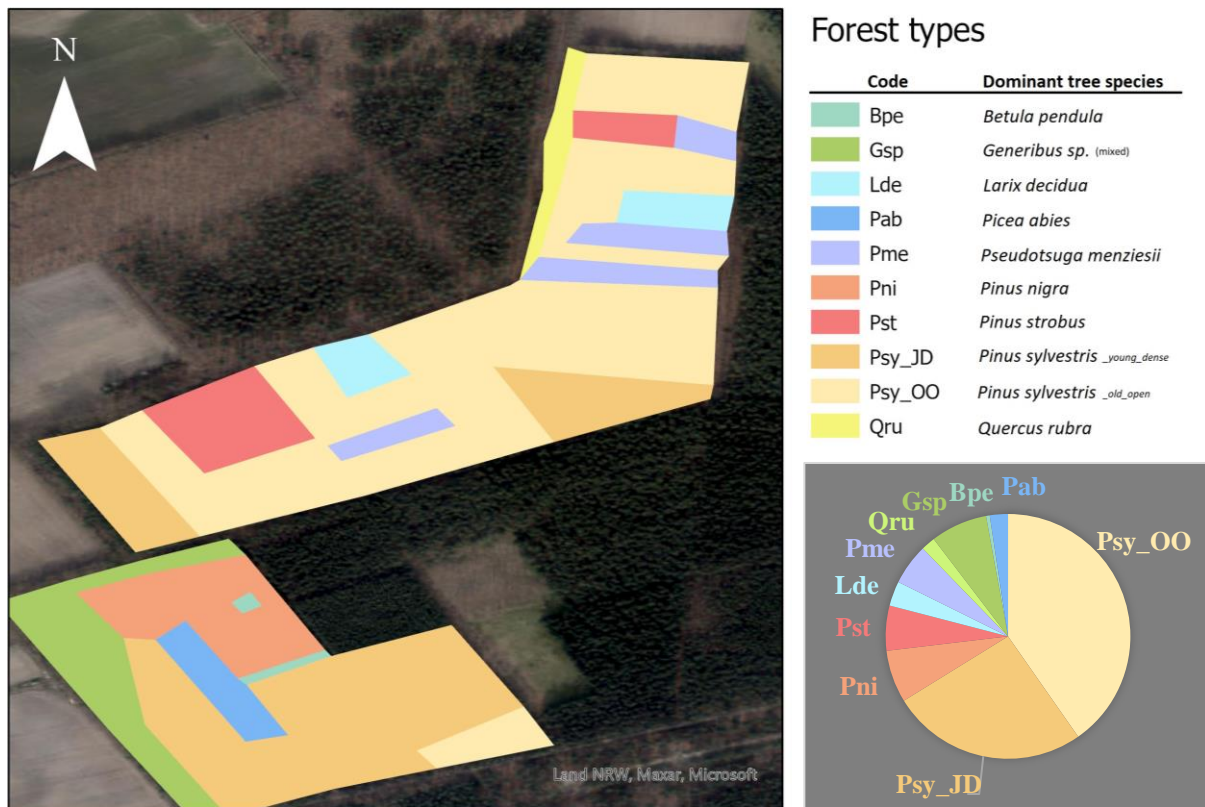


**Figure 3 Butterfly transect.** To measure butterfly diversity and abundance this butterfly transect, consisting of twelve 50 m sections, was created according to the 'Vlinderstichting' protocols.

To measure night butterfly diversity a light bucket was set on a fixed location, as described by the *Vlinderstichting* protocols. The light bucket consists of a rod covered with a LED UV-light (12 volt SMD 2835 UV LED Strip light of 395-405nm) suspended above the bucket between transparent plexiglass plates (see appendix A.3). A funnel is located underneath the plexiglass plates which leads to the inside of the bucket. The bucket was placed at sunset, and retrieved briefly after. All captured butterflies were identified using ObsIdentify and checked by admins from 'waarneming.nl'. A mosquito net was hung above the light bucket to prevent uncounted escapes.

## Results

The first phase of this study was to map the forest and describe the different forest types in detail. Each forest type reflects a homogenous stand of a certain age and density, dominated by a single species.



**Figure 4 Forest type map.** The forest consists of areas with homogeneous stands of different species and densities, these forest types have been mapped using ArcGIS. The map is displayed on a background layer provided by ArcGIS accredited to Beeldmateriaal, Maxar, Microsoft. Code names for the forest types use dominant tree species and scientific names are depicted. The pie chart portrays the relative size of each forest type.

Forest types were determined by the dominant tree species, density of the stand and ecological structure. We identified 10 different forest types, with their relative sizes depicted in the pie chart. Most of the forest is dominated by stands of *Pinus sylvestris* (66%). Abbreviations of the forest types are based on the scientific names of the dominant tree species. For the remainder of this report, the forest type codes will be used when referring to a forest type.



# 1. Soil

The soil quality assessment is divided into three parts in which the physical, chemical and biological properties of the soil are presented. Next to that, an overview of the water availability in the forest is provided. Lastly, the short term effect of chalk and stone flour on a forest clearing is assessed.

## *Physical soil quality*

Physical soil properties are presented in table 2 below. The soil is composed mainly of sand (90%) with a small proportion of clay and silt, in a slightly different composition in each forest type. The amount of organic matter is low (2-4%) and about half of it is carbon (C/OM-ratio). The low proportion of organic matter and clay bring about a low Cation-Exchange Capacity (CEC), therefore the capacities of the soil to bind and retain nutrients are low. The pH is between 3.3 and 3.5, so highly acidic soils prevail in all forest types. Due to the acidification of the forest soil, the CEC-complex is mainly taken up by H<sup>+</sup> and Al<sup>+</sup>.

Bulk density is slightly higher in the PsyOO forest type, suggesting the soil is slightly more compacted there than in the other forest types, however the difference is marginal. The amount of carbon stored in the topsoil is rather low, making up only 1-2% of the total weight.

*Table 2 Physical soil properties. Pooled soil sample, taken at 0-25 cm depth at 17 randomly chosen locations spread out across each forest type. Analysed in the lab by Eurofins using methods in Appendix A.1.*

	unit	Pst/Pme	PsyJD	PsyOO	Pni	Plots 1&2 (PsyOO)	Plots 3&4 (PstJD)
<b>Bulkdensity</b>	kg/m <sup>3</sup>	1365	1389	1419	1320	1429	-
<b>Acidity</b>	pH	3.3	3.4	3.4	3.4	3.5	3.3
<b>C-organic</b>	%	1.7	1.4	1.2	2.1	1	2.2
<b>Organic matter</b>	%	3.1	2.6	2	4.1	1.8	4
<b>C/OM-ratio</b>		0.55	0.54	0.6	0.51	0.56	0.55
<b>Carbonated lime</b>	%	0.7	0.6	0.4	0.2	0.2	0.9
<b>Clay</b>	%	<1	1	1.2	2	<1	2
<b>Silt</b>	%	5	3	2	10	8	5
<b>Sand</b>	%	91	93	92	84	90	88
<b>Cation exchange capacity (CEC)</b>	mmol/kg	15	14	17	17	<11	27
<b>CEC-saturation</b>	%	78	74	72	76	-	60
<b>Ca-saturation</b>	%	50	60	58	56	-	41

<b>Mg-saturation</b>	%	15	<0.1	<0.1	8.2	-	9.6
<b>K-saturation</b>	%	10	11	14	9.4	-	5.9
<b>Na-saturation</b>	%	3.3	2.9	<0.1	2.4	-	3
<b>H-saturation</b>	%	37	31	26	26	-	20
<b>Al-saturation</b>	%	40	40	40	40	-	40

### *Chemical soil quality*

Chemical analysis of the soil is depicted in table 3 and shows the availability of the essential nutrients for plant growth. Two values are given for each of the essential nutrients, plant available and soil stock. Plant available indicates the nutrients that are either dissolved in water or bound to the CEC-complex and therefore easily ingested by plants. Soil stock stands for the nutrients that are bound within minerals and remain stored in the soil in crystalline form.

Due to the low Cation Exchange Capacity, the soils do not hold much of the necessary nutrients, putting a serious restraint on plant growth. A chain is as strong as the weakest link, a principle that applies here very well. Plant growth is impaired if one of the essential nutrients is in low supply. For the forest in Overloon the limiting factors are primarily magnesium and phosphorus and to a lesser extent sodium.

*Table 3 Chemical soil quality. Pooled soil sample, taken from 0-25cm depth at 17 randomly chosen locations spread out across each forest type. Analysed by Eurofins using methods in Appendix A.1. Stock is the total nutrient content (bound in minerals), while available means nutrients are either dissolved in water or bound to the CEC-complex. All of the values are given in kg/ha. \* Ca-stock for plots 1&2 was too low to be measured (CEC insufficient)*

	<b>Pst/Pme</b>	<b>PsyJD</b>	<b>PsyOO</b>	<b>Pni</b>	<b>Plots 1&amp;2 (PsyOO)</b>	<b>Plots 3&amp;4 (PstJD)</b>
<b>N-stock</b>	2660	1950	2310	3660	1970	2520
<b>C/N ratio</b>	22	25	19	19	18	29
<b>N-releasing power</b>	20	10	25	35	20	0
<b>S-stock</b>	750	625	710	560	680	795
<b>S-available</b>	8	8	7	8	11	19
<b>C/S-ratio</b>	78	78	62	125	53	90
<b>S-releasing power</b>	10	8	11	3	12	9
<b>P-stock</b>	45	45	45	45	45	45
<b>P-available</b>	<1.1	<1.1	<1.1	<1.0	<1.1	4.6
<b>K-stock</b>	200	205	335	205	180	205
<b>K-available</b>	30	35	35	25	30	45

<b>Ca-stock</b>	515	585	695	630	0*	745
<b>Ca-available</b>	25	30	55	25	30	25
<b>Mg-stock</b>	95	45	45	55	50	105
<b>Mg-available</b>	<15	<15	20	15	<20	15
<b>Na-stock</b>	40	30	35	30	35	60
<b>Na-available</b>	20	25	20	25	<20	20

Nutrient availability and stock are mostly similar between the different forest types. Yet, forest type Pni seems to have a larger N stock (3660 kg/ha) than the other forest types (1950 – 2660 kg/ha). The calcium stock was too low to be adequately measured in the cleared experimental plots 1 and 2 (according to Eurofins), and therefore has value 0. The kalium stock is on average higher in the PsyOO forest type (305 kg/ha) than the other forest types (200-205 kg/ha).

### ***Biological soil quality***

The results of measurements of overall microbial biomass and activity are presented in table 4. The microbial biomass seems to differ substantially between the different forest types, however, no conclusions can be drawn because of the discrepancy between the results of the average soil samples (PsyOO and Pst/Pme) and those of the experimental plots. The microbial biomass and activity is very location bound and may differ a lot within each forest type.

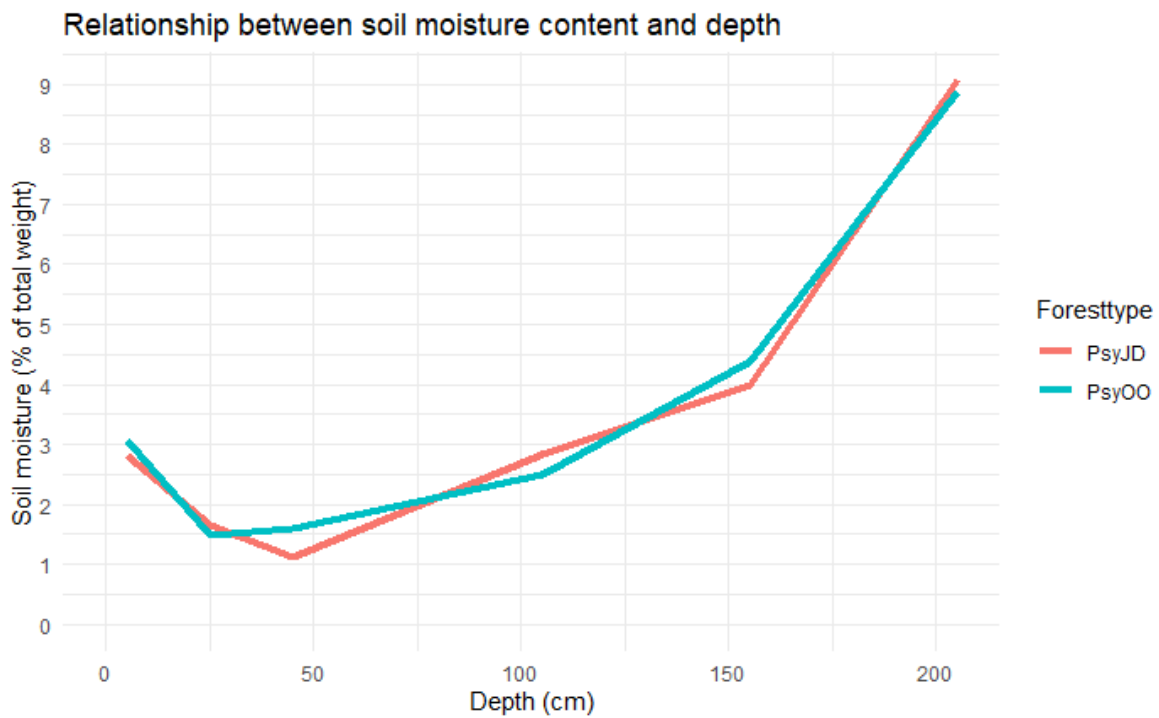
*Table 4 Biological soil quality. Pooled soil sample, taken from 0-25 cm depth at 17 randomly chosen locations spread out across each forest type. Analysed by Eurofins using methods in Appendix A.1.*

	Unit	Pst/Pme	PsyJD	PsyOO	Pni	Plots 1&2 (PsyOO)	Plots 3&4 (PstJD)
<b>Microbial biomass</b>	mg C/kg	179	276	193	279	276	312
<b>Microbial activity</b>	mg N/kg	7	3	10	12	6	17
<b>Fungi/bacteria-ratio</b>		0.9	1	0.6	0.4	0.5	1.2

## Soil water tables

Groundwater tables in the forest in Overloon have been rising over the past decades and currently fluctuate between 1 to 3 metres below the surface, during winter and summer respectively (for graph, see appendix B.1).

Soil moisture content has been measured at different depths after a period of drought and is depicted in figure 5. In the graph can be seen that water content is lowest at intermediate depth and increases with increasing depth, to a maximum of 9% water content at 2 meters depth. The water content is slightly higher in the top layer.

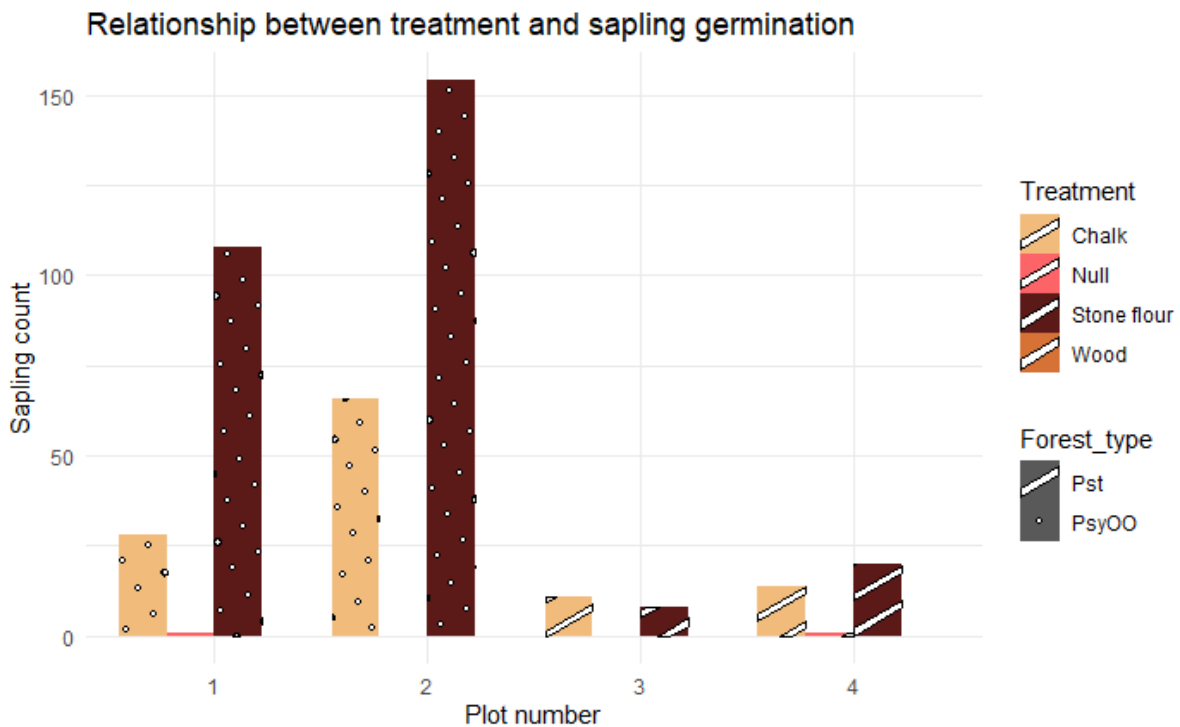


**Figure 5 Relationship between soil moisture content and depth.** Soil moisture content was measured using the gravimetric method at 6 different depths in forest types PsyOO and PsyJD at the height of summer (01-09-22). Displayed on the x-axis is the depth at which the sample was taken and on the y-axis the water content.

## Experimental plots

We found that in all forest clearings small herbaceous plant saplings have appeared in the quarters treated with chalk and rock flour, while almost no saplings appeared in either of the control treatments. (see figure 6).

Significantly more saplings emerged in the stone flour treatment, than in the null or wood treatment ( $p = 0.035, 0.034$ , respectively). The difference between chalk and stone flour, or chalk and the control treatments is not significant, but a clear trend can be observed. Moreover, the PsyOO plots have significantly more saplings than the Pst forest type ( $p = 0.036$ ).



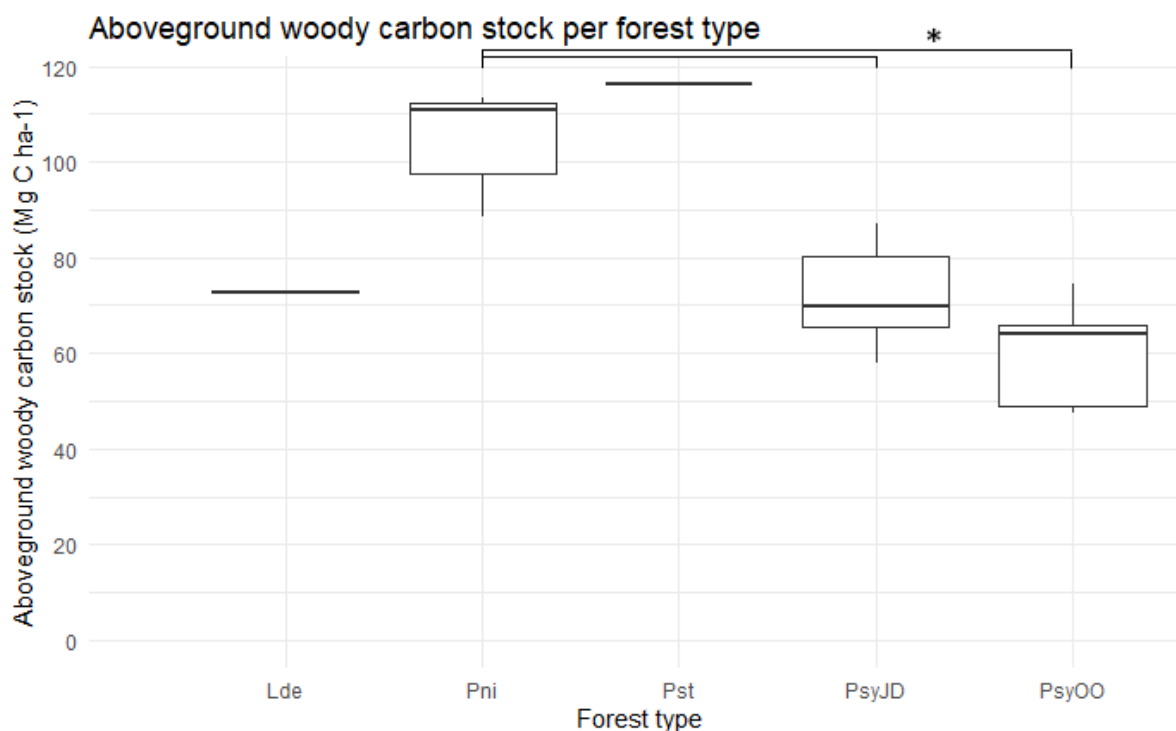
**Figure 6** *Herbaceous plant saplings per treatment per plot.* The herbaceous plant saplings emerging on the experimental plots have been counted on 24-01-23. The sapling counts are displayed on the y-axis, for each of the plot and treatment on the x-axis. Sapling count is significantly higher in PsyOO than Pst ( $p=0.036$ ) and in the stone flour treatment than the null or wood treatment ( $p = 0.035, 0.034$ , respectively).

Interesting about the plant saplings is that they are all species that have not been recorded in the forest before (pictures in appendix B.2). Their seeds might have been lying dormant in the seedbank for many years, or have arrived with the wind or other vectors after the leaf litter layer had been removed.

## 2. Carbon stock

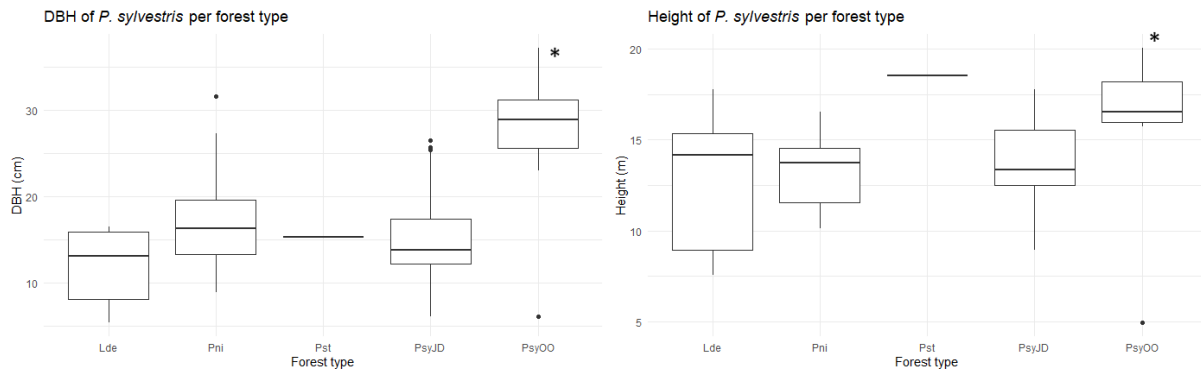
The carbon stored in aboveground woody biomass in the forest of Overloon was calculated for each forest type (see table 8, appendix B3). The average carbon stock is 72.2 Mg C per ha. Multiplying the carbon stocks by the forest type area, yielded a total carbon stock of 1724 Mg C in the entire forest.

The mean carbon stocks differ between the forest types (figure 7). An Anova indicated that the *Pinus nigra* forest type stores significantly more carbon than PsyJD and PsyOO ( $p = 0.0054847$  and  $0.0003940$ , respectively). Not enough duplicates were performed in forest type Lde and Pst to allow for statistical analysis.



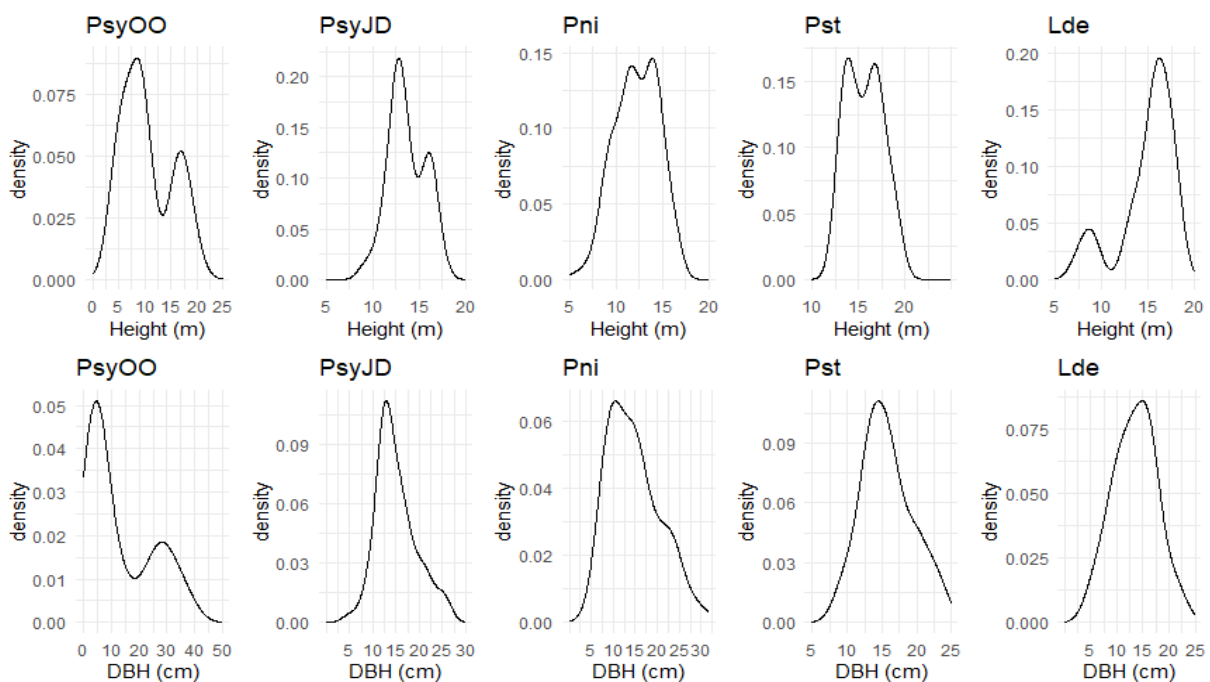
**Figure 7 Aboveground woody carbon stock per forest type.** Aboveground woody carbon mass based on the DBH of all trees in five 10x10m plots and general species specific allometric equations. Carbon mass is displayed on the y-axis in Mg C/ha for each of the forest types on the x-axis. Pni has significantly more carbon stored than PsyJD and PsyOO ( $p=0.0054847$  and  $0.0003940$ , respectively).

The stand structure – i.e. density, height, DBH and tree species – differs between the forest types (see table 7, appendix B.3). The majority of the forest belongs to the type PsyOO (*Pinus sylvestris*, Old Open). As the name suggests it is relatively open and made up of old, approximately 70 years, stands of Scots pine with an undergrowth of numerous smaller deciduous species. *Pinus sylvestris* can be found growing throughout the forest in all forest types; as expected it has a significantly larger height and DBH in PsyOO than in the other forest types (see figure 8).



**Figure 8 DBH and height of *Pinus sylvestris* for each forest type.** On the y-axis is the average DBH (left) and height (right) of *Pinus sylvestris* for each forest type. DBH and height are significantly higher in PsyOO than the other forest types (\*).

The other forest types are a lot denser and comprise of mainly the dominant production species with less undergrowth. The density plots of the height and DBH for the different forest types are depicted in figure 9. The distribution in the density plot illustrates the stand structure. PsyOO has two clear peaks, the first for its undergrowth and the second for the larger, dominant pine trees. PsyJD also shows a small second peak for height, indicating that some trees either grew tall quicker or were already there when the rest was planted. Pni and Pst show less deviation from a more normal distribution of tree height. Lde has a small peak for tree height in the undergrowth, these are small *Pinus sylvestris* that germinated in this forest type.



**Figure 9 Density plot of height and DBH for each forest type.** On the y-axis is the density and on the x-axis the height (top) and DBH (bottom). The density plots indicate the stand structure of the different forest types.

### 3. Biodiversity

The species diversity of the forest was measured in detail for vegetation, butterflies and dragonflies and is described in this chapter for each of these groups. Next to that, the vertebrate species list including reptiles, amphibians, birds and mammals that have been encountered in the forest is included in appendix B.6.

#### Plant diversity

The plant diversity of the forest in Overloon is low and is characterized by one dominant planted tree species, interspersed with some deciduous trees and shrubs and an undergrowth of grass, ferns and some mosses. There are strong differences in species richness between the different forest types (see table 10, appendix B.4). The richest plant communities are found in the PsyOO and Pni forest types. The Pst forest type has the lowest richness of all, not even moss species tolerate the conditions imposed by the dense rows and thick leaf litter layer of *Pinus strobus*.

Next to the plant diversity plots, we also identified all plants that were encountered during other visits or surveys. This yielded a list of herb species that are present in the open forest types and on the forest edges, but are not as common as the species listed in the species richness table (see table 11, appendix B.4). These species are of particular interest for the future changes to the forest as many produce edible fruits.

The Shannon index has been calculated for each of the surveyed plots to compare the floral diversity between the forest types. The mean diversity of each forest type is depicted in table 5 for both of the surveying rounds. PsyOO has the highest diversity index, followed by Pni and Pme. Forest types PsyJD and especially Pst have a very low Shannon index, as they comprise of very little species. The diversity indices are in line with the species richness of each forest type and it can be concluded that the plant diversity is highest in the open forest.

*Table 5 Average Shannon diversity for each forest type. Shannon diversity has been calculated for the different forest types in two survey rounds, round 1 in the beginning of June, round 2 half of July.*

Forest type	Shannon diversity round 1	Shannon diversity round 2
PsyOO	1.0521933	1.0624930
PsyJD	0.4937526	0.5599122
Pni	0.9088315	0.7549701
Pme	0.8191000	0.9697804
Pst	0.5004024	0.1914441



## Butterflies and Odonates

The diversity and abundance of diurnal butterflies is low, they have only been encountered sporadically. A total of 9 butterfly species is observed with a maximum total count of 3 (see table 12, appendix B.5). The nocturnal butterflies have been counted using specific protocols, the counts of the startled moths that were encountered on the butterfly transect have been included in table 13. This data cannot be interpreted due to its irregularity which gives an unfair representation of the actual diversity and abundance.

Four species of dragonfly have been recorded in the forest and a seasonality pattern can be observed for some species (Figure 10). The species *O. cancellatum* is present during the first four visits and becomes absent after that, while the species *S. striolatum* becomes more abundant towards the end of the season.

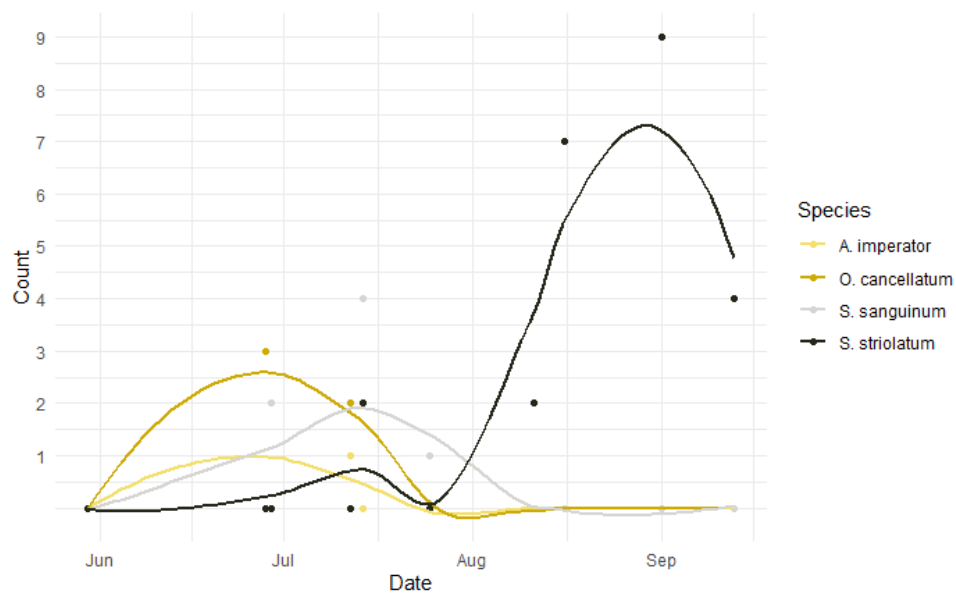


Figure 10 **Dragonfly counts over the season.** On the y-axis is the abundance and on the x-axis time, different species are indicated with colours.

The nocturnal butterfly diversity could only be surveyed from the end of August to the beginning of September. Nocturnal butterflies were sampled in four visits, during which a total of 42 moth species has been recorded with some species being very abundant (see table 14, appendix B.5). The Scopariinae, a family including nine species of the genera *Eudonia* and *Scoparia*, was very abundant and was counted over 400 times. In the beginning of September, the moth diversity and abundance dropped abruptly (Figure 18, appendix B.5).

To conclude, the butterflies and dragonflies have a low diversity and abundance in the forest in Overloon. Butterflies are only spotted occasionally, while the dragonflies are more frequent visitors and could often be found on the same locations of the transect. Next to that, a clear seasonal trend was observed for the different dragonfly species. The nocturnal butterflies are far more diverse and abundant, in particular the *Scopariinae* species are very abundant.

# Discussion

## 1. Soil

The forest in Overloon is characterised by acidified, nutrient poor, sandy soils. The dominant species of the forest is planted Scots pine that has relatively poor organic matter – mainly consisting out of carbon – which is hard to break down (Millar, 2012). The needles dropped by the trees accumulate on the forest floor and do not contribute to enrichment of the soil organic matter content, and thus, the soil has a low Cation Exchange Capacity. Essential nutrients that are fundamental for plant growth can only bind to organic matter, so a low organic matter content leads to a meagre potential for the soil to bind essential nutrients. Due to the acidic conditions the CEC-complex is mainly taken up by  $H^+$  and  $Al^+$ , thereby taking up the little space available to bind nutrients. Thus, not only is the CEC very low, it is comprised mainly of ‘useless’ cations. The soil is in a downward spiral of increasing acidification and decreasing fertility, as precious nutrients wash away.

The ground water tables are not that deep and trees and shrubs should be able to tap moisture from it with their taproots, even during very dry conditions. Saplings and juvenile plants, however, will have a harder time drawing in enough water, especially in summer. We showed that the water content only reaches 9% at 2 m depth in summer, therefore young trees and shrubs will not make it through times of drought without support from neighbouring trees or associated mycorrhizal fungi. Water stress causes trees to close their stomata, thereby halting photosynthesis and decreasing the total primary productivity and ecosystem respiration. In other words, the whole system pauses, in the middle of the growing season. However, it has been found that rainfall can rapidly lead to recovery of photosynthesis, even in young trees (Granier et al., 2007; Gallé et al., 2007). In severe drought experiments, 8 year old oak trees were exposed to 5 weeks of water stress (plastic foil over pots), during which photoprotective mechanisms acted to preserve the photosynthetic functionality, leading to a rapid recovery of photosynthetic activity after rewatering. Thus, there is hope for our young trees, even in times of extreme drought.

The current soil conditions are harsh and exert extreme pressure on the flora, shaping the forest undergrowth to its current shape. It will take a lot of time for the forest to build up an enriched soil system, where nutrients and water are retained. The forest is capable of restoring itself with natural regeneration of deciduous trees that will help build up rich humus, however it will take multiple generations before this is achieved. It is interesting to find out if the system can be given a helping hand to speed up to process.

Our experimental plots show that there is a significant short term effect of rock flour supplement on the growth of herbaceous plant saplings. The addition of chalk also shows a clear trend, but this was not significant, probably due to too few replications. The emergence of herbaceous saplings came as quite a surprise, as none of the species has been encountered in the forest before. Therefore, the seeds must either have been dormant in the seedbank for at least 20 years, or have arrived by wind or other vectors from outside the forest.

Significantly more saplings emerged on the PsyOO plots than on Pst, while the soil quality is similar; this is probably promoted by light availability or seed dispersal. The dense rows of *Pinus strobus* shed more needles, and block more light than the open *Pinus sylvestris* forest. The dense rows could also make it harder for seeds dispersed by wind to reach the clearings, possibly explaining how some of the herbaceous saplings got there at all.

Twice the amount of saplings appeared on the plots treated with rock flour than chalk in PsyOO. In the chemical soil analysis it was found that the limiting factors are primarily magnesium, phosphor and to a lesser extent sodium. Rock flour replenishes all of these stocks, while chalk only restocks magnesium. Even though chalk is often thought to have more effect on the short term, we found that rock flour has more potential. We expect that rock flour could potentially help jump start the nutrient pump in our forest and could speed up the process of soil restoration.

Adding stone flour to acidified forests is a method already employed on large scale by forest managers in the region (Bosgroep Zuid, 2023). They are trying to mitigate the effect of acidification and nitrogen eutrophication, by supplying essential nutrients in the form of pulverized rocks. The forest managers use helicopters to spread around 10 000 kg per hectare (1 kg per m<sup>2</sup>), three times more than we supplied in the experimental treatment.

## 2. Carbon storage

Forests are fundamental to mitigate climate change effects because of their ability to sequester carbon (Bowditch et al., 2020). Forests act as a carbon sink, as carbon dioxide is sequestered in living biomass and subsequently transferred into the soil or stored in a variety of wood products (Nabuurs et al., 2018). Dutch forests have all been planted to reclaim drift sands at the end of the 19<sup>th</sup> century and are extensively managed and monitored. National Forest Inventories are conducted every five years to monitor the structure, biomass and health of the Dutch forests (Schelhaas et al., 2022b). The most recent 7<sup>th</sup> Dutch National Forest Inventory found that as Dutch forests are aging, the share of large trees is increasing. Next to that, the composition is shifting from unmixed pine forests to more broad-leaved mixed forests, however, *Pinus sylvestris* is still the dominant species in 28% of the forested areas (Schelhaas et al., 2022b). The current average carbon storage in woody aboveground biomass is 88.7 Mg C/ha, while 15 years ago that was 72.6 Mg C/ha. Thus, Dutch forests have been acting as a net carbon sink the past decade sequestering 1.25 Mg C/ha yearly, sequestering an equivalent of 4.6 Mg of CO<sub>2</sub> per year from the atmosphere.

The average aboveground woody carbon stock of the forest in Overloon is 72.2 Mg C/ ha, thus it has less carbon stored than the average Dutch forest. This difference can be explained by the soil and species composition. Broad-leaved species skew the image of the Dutch average due to the larger wood density, as they make up 68% of the biomass, but only 54% of the volume. Moreover, as previously described, the forest in Overloon is growing on very poor soils, which would result in lower growth rates and a lower biomass. Therefore, the aboveground woody carbon stock of the forest in Overloon is representative for a monoculture pine forest growing on nutrient poor sandy soils.

Significant differences have been found in carbon stocks between the different forest types. The *Pinus nigra* forest stores significantly more carbon than the *Pinus sylvestris* forests, which can be explained by the stand density and to a lesser extent, the allometric equations. *Pinus nigra* comprises of dense stands with relatively large diameters resulting in a higher biomass per ha. Moreover, the parameters in the allometric equation are slightly higher than for *Pinus sylvestris*, so *Pinus nigra* has a slightly higher biomass for individuals with the same girth.

Due to time limitations not all the forest types were measured, we chose to sample the forest types covering the largest areas first, so that most of the total area (82%) is described. The Pst and Lde forest types are too small to allow for five replications, therefore only one plot was surveyed.

The allometric equations chosen to calculate tree biomass are generalized equations pooled from all available and adequate tree biomass studies (Forrester et al., 2017). These do not include tree height, but only use diameter at breast height as a variable to estimate the biomass. Due to the strong relationship between tree height and diameter, also found in this study, we believe these calculations should still give an accurate representation of the biomass (see figure 16 appendix B.3).

In this study, we focussed on the carbon stored in aboveground woody biomass, because of the affordable and accessible methods. The carbon stored in belowground biomass, dead organic matter, including leaf litter and soil organic matter was not considered in this study, even though organic carbon stocks in the soil can exceed those in plant biomass (Mayer et al., 2020). Moreover, it has been shown that pine forests can store as much carbon in the soil as in aboveground woody biomass (Osei et al., 2022). From the soil analysis can be concluded that the organic carbon stored in the top layer of soil is currently low (1-2%). To gain a more complete picture of the carbon budget of this forest, the other components should be investigated in more detail.

### **Future carbon potential**

The national trend of a shifting species composition can also be seen in the forest in Overloon. Natural regeneration is already playing a key role in the transformation process from even-aged, pure pine stands towards a more mixed forest, as *Betula pendula*, *Prunus serotina* and *Frangula alnus* are pioneering between the large pines in the open forest. The planned modifications to the forest will accelerate this process and given enough time the forest could have the capacity to store more carbon in aboveground woody biomass, as it will comprise of deciduous species that have more branches and denser wood. However, it remains unclear whether more biodiverse forests on these sandy soils store more carbon – in both biomass and soil – than monoculture pine forests.

Pine forests store more carbon in the topsoil and forest floor, due to slow decomposition rates occasioned by the high lignin/ N ratio and low pH (Vesterdal et al., 2013). On the other hand, broad-leaved species show enhanced carbon sequestration in the mineral soil, which however may be hindered on poor sites that do not allow macrofaunal activity (Mayer et al., 2020). Soil organic carbon stocks are very stable, it has been shown that even after 20 years of litter removal the soil organic carbon stocks remain unchanged compared to the control (Pierson et

al., 2021). Moreover, it has been shown that mixed species forestry, with beech, oak and pine, has at least equivalent carbon storage to the best performing monocultures, while providing more ecosystem services and better ecological resilience (Osei et al., 2022).

The forest at Overloon currently has relatively dense stands of different pine species that have a below average aboveground woody biomass. Most of the trees will in time be replaced by fruit and nut trees and other supporting broad-leaved species, that will have less dense stands but have the potential to store more aboveground carbon due to the larger wood density. Soil organic carbon content in the topsoil is currently very low, between 1-2%, in line with previous findings from primary succession Scots pine forests at the 'Veluwe' (Nadporozhskaya et al., 2006). The deciduous tree species produce qualitatively better leaf litter that should be more easily decomposed by macrofauna, soil fungi and bacteria, which should increase the soil organic carbon. Therefore, we hypothesize that carbon stocks of the forest in Overloon should increase with the transformation from monoculture pine plantation to a biodiverse food forest system.

### 3. Biodiversity

The sandy soils of Noord Brabant once supported rich communities, however, this former glory can only be observed in the fossil pollen record. Ancient forests on sand in the Netherlands (6000 – 3500 v. Chr) comprised of trees such as elm, ash, birch, hazel, European cherry, hornbeam, maple, holly, or yew combined with a typical forest herb layer comprised of species such as wood anemone, may lily, or Solomon's seal (Nyssen & Jans, 2016). Currently, the only remaining forest on the European continent that provides an insight into the structure and diversity of these ancient undisturbed forests is the Bialowieza forest in Poland. The Bialowieza forest covers an area of 1500 km<sup>2</sup> and provides a home to a total of 1280 plant species (Jaroszewicz et al., 2019).

The plant diversity of the forest in Overloon is severely diminished, only 19 common species including mosses and plantation trees have been encountered within the total surveyed area of 500 m<sup>2</sup>. When including the more rare herbs and shrubs that were found in clearings and on forest edges we come to a total of 34 plant species in the total forest area of 0.25 km<sup>2</sup>. Sunny sites in the Bialowieza forest with a crown layer of *Quercus robur* – that have a similar radiation as the sparse canopy of the pines (60%) – have an average herb layer diversity of 60-80 species per 100 m<sup>2</sup> (Jankowska-Błaszczuk et al., 1998).

Floral biodiversity is highest in the PsyOO forest type, due to the availability of light and reduced competition for scarce resources. In the open forest the stands are less dense, block less light and accumulate less indigestible leaf litter. Biodiversity is lowest in the Pst forest type, where the stands are most dense and the leaf litter is thickest, allowing no plants to grow other than the plantation species.

The Shannon index reflects both evenness and richness. The minimum value of the index is 0, when there is only one species present and thus no diversity. There is no maximum value of the index. When all species have the same evenness, i.e. the same number of individuals, the

index equals  $\log(k)$ , in which  $k$  is the number of species (Spellerberg & Fedor, 2003). The species richness of all surveyed plots combined is 19, therefore the maximum Shannon diversity for the forest in Overloon is  $\log(19) = 1.28$ . The diversity of the PsyOO forest type from the second survey (1.06) comes close to this maximum.

The barren state of the soil quality, together with nitrogen eutrophication (primarily caused by intensive agriculture) has a malnutrition build-up effect higher in the food chain. When plants have relatively more nitrogen available, they produce relatively more amino acids with a lot of nitrogen components than other essential amino acids – those that herbivores cannot produce themselves (Siepel et al., 2009). The leaching of nutrients in the soil, reduced intake by plants and possible shortages of essential amino acids in plants can lead to malnutrition in herbivores. Caterpillars, for instance, use the absolute amount of nitrogen as a marker for their feeding, therefore they have an increased risk to build up a shortage of essential amino acids. Most of these essential nutrients barely accumulate in the food chain, leading to more acute problems for top predators such as sparrowhawks. In nutrient poor forests on the Veluwe, the number of sparrowhawk breeding pairs has decreased drastically, due to reduced breeding success caused by shortages in essential amino acids (Burg, 2006). To compensate for their malnutrition, female sparrowhawks decreased their chest muscle circumference with up to 50% during breeding season. Thus, the degraded state of the soil is not only a problem for the plant productivity and diversity, but also has an effect higher in the food chain.

The diurnal butterfly diversity of the forest is low, butterflies are only encountered sporadically. This can easily be explained by the availability of food, as there are little flowering plants that can serve as a food source for the butterflies. Dragonflies are a bit more abundant and could often be found hunting at the same locations in the forest. Only four species have been observed and they showed a clear seasonal trend in abundance.

The nocturnal butterfly diversity and abundance is higher, which is a usual finding for forests (Vlinderstichting, 2023). The *Scopariinae* species are particularly abundant, which is probably induced by the abundance of their host plants: mosses and sometimes grasses (Léger et al., 2019). We found a total of 42 moth species, only a small fraction of the 865 macro- and 1480 micro moth species that can be found in the Netherlands (Vlinderstichting, 2023). There are probably more moth species present in the forest that we did not observe, but the overall diversity still seems to be low.

We could only sample the moth diversity towards the end of the season, due to technical complications with the skinner trap. The skinner trap has a light bulb that needs DC electricity, we tried different set-ups with car batteries, but were eventually forced to use an alternative. It was only by the end of August that we were able to borrow a light bucket with a led strip that can be powered simply by use of a power bank. We would recommend anyone in remote locations to use this setup as it is simple and reliable.

We set out to measure the insect diversity by using pit-, malaise and pan traps, however due to time constraints we were only able to sort-through about 20% of the pit traps. We were ambitious, as we wanted to identify all specimens to species level, even the mites smaller than 1 mm. We were given the opportunity at Naturalis to use DNA-techniques to ‘quickly’ identify

each of our morphospecies, however the results are still not in because the labs are understaffed our project has a low priority. Therefore most of the insect diversity is not included in this study, but will be published in a different format when the results become available.

Microbial aspects of the forest have not been examined in detail, but a general value for microbial biomass and activity has been acquired from the soil sample for each forest type. The discrepancy between the average samples for each forest type and the experimental plots imply that the microbial biomass and activity is very location specific. All that can be concluded from the microbial results is that the biomass (179 – 312 mg C/kg) and activity (3-17 mg N/kg) are relatively low when compared to findings from other European forests (average of 3330 mg C/kg, and 189 mg N/kg; Coûteaux et al., 2003). This could be related to high nitrogen deposits, as atmospheric N-loading has been correlated to decreased microbial, especially fungal, activity and biomass (Zechmeister-Boltenstern et al., 2011). To find out more about the microbial aspects of the forest, more research has to be conducted.

### **Future changes**

The owner of the forest aims to revitalize the forest and wants to (1) store more carbon, (2) increase the biodiversity, and (3) increase the economic return, by transforming this pine production forest into a food forest. The initial changes have been made by thinning the pines in one third of the forest and planting thousands of young linden, alder, and sweet chestnut trees. Together with the birch, black cherry, alder buckthorn, and european oaks – that can already be found in between the pines – these trees will produce rich leaf litter. The rich leaf litter is expected to turn on the nutrient pump, turning the system around from its downward spiral into an upward spiral of increasing fertility and decreasing acidification (Nyssen & Jans, 2016). Possibly the nutrient pump will need a helping hand of minerals (stone flour) to get started, but after that it will enrich itself.

When soil conditions improve, the forest will be able to support plants that demand a richer soil quality, such as fruit and nut trees, and a herb and shrub layer of flowering plants. As observed on the experimental plots: as soon as conditions improve, opportunistic herbaceous species will settle. The increased floral diversity will be able support a much larger diversity up the food chain. More flowering plants will attract pollinators, so the butterfly and moth diversity and abundance, for example, is expected to increase. This goes for all animals: herbivores, nectivores, insectivores, and carnivores are all expected to benefit from the planned changes to this forest. The only species that will decrease in abundance, are the ones specialised in pine. They will decrease in abundance, but will not disappear entirely, as not all pines will be removed from the forest.

## **Conclusion**

This baseline study assesses the current state of the soil quality, carbon storage and biodiversity of the planted forest in Overloon. The transition to a food forest promises substantial improvements to soil health, carbon stock and biodiversity, however, to assess the legitimacy of these claims, the forest must be monitored and compared to this baseline.

Currently, the soil quality is severely impaired and in a negative spiral of increasing acidification and decreasing fertility. The aboveground carbon stocks are below Dutch average and the plant and animal diversity is very low. If the functioning of the nutrient pump is restored, the system will turn around into a positive spiral. The aboveground carbon stocks will, probably, exceed the current level. Most of the potential for an increase in carbon storage is below ground, however, the baseline soil carbon stocks must be assessed in greater detail. The restoration of a healthy soil ecosystem will benefit many species: from fungi and plants up to the big carnivores, the entire system is expected to become more diverse, productive and resilient.



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## Appendix A - Methods

### A.1 - Soil analysis methods Eurofins

Analysis of soil quality parameters was performed in the lab by Eurofins, see methods used in table below.

*Table 6 Methods used by Eurofins for chemical, physical and biological soil analysis. Properties have been analysed using Near-InfraRed Spectroscopy (NIRS), calcium chloride solution (CCL3), ammonium lactate acetic acid solution (PAL1) and using glass electrode in 1:5 solution (PHC3).*

Measurement	Unit	Method
N-stock	kg N/ha	NIRS
S-available	kg S/ha	CCL3 (NEN 17924-2)
S-stock	kg S/ha	NIRS
P-available	kg P/ha	CCL3 (NEN 15923-1)
P-stock	kg P/ha	PAL1 (NEN 5793)
K-available	kg K/ha	CCL3 (NEN 17924-2)
K-stock	kg K/ha	NIRS
Ca-available	kg Ca/ha	NIRS
Ca-stock	kg Ca/ha	NIRS
Mg-available	kg Mg/ha	CCL3 (NEN 17924-2)
Mg-stock	kg Mg/ha	NIRS
Na-available	kg Na/ha	CCL3 (NEN 17924-2)
Na-stock	kg Na/ha	NIRS
Acidity	pH	PHC3 (Cf NEN ISO 10390)
C-organic	%	NIRS
Organic matter	%	NIRS
Clay	%	NIRS
Silt	%	NIRS
Sand	%	NIRS
Cation exchange capacity (CEC)	mmol+/kg	NIRS
Microbial biomass	mg C/kg	NIRS
Microbial activity	mg N/kg	NIRS
Fungal biomass	mg C/kg	NIRS
Bacterial biomass	mg C/kg	NIRS

## A.2 – Experimental plots

Four square 10 x 10 m forest clearings have been created to test different soil regeneration methods in an experimental setting. Trees were cut on the 5<sup>th</sup> of March, leaf litter and organic matter was removed and treatments were implemented on 29<sup>th</sup> of June.



*Figure 11 Experimental plots PsyOO. From top top bottom: PsyOO before clearing, after clearing, after treatment implementation during sapling count.*



*Figure 12 Experimental plots Pst. From top to bottom: Pst before clearing, after clearing, after treatment implementation.*



## Soil treatment calculations

Two soil regeneration methods were tested on the experimental plots: the addition of chalk and stone flour. They have been implemented in different concentrations, based on literature/manufacturers information. The DCM ‘groenkalk’ chalk contains 15% MgO. Stone flour consist of a variety of minerals, see table X below.

Chalk: DCM ‘groenkalk’ was used, manufacturer recommends a dose on acidic soils of 1.5 kg / 10 m<sup>2</sup>. We treated one 5x5 m quarter of the plot (25 m<sup>2</sup>), so a total of 3.75 kg of chalk was added (2.5\*1.5). As we did not have a weighing scale in the field, we measured the volume of the complete chalk stock, combined with its weight to calculate the volume we needed to add (=1.5 L). This was added using a volumetric 1L cup.

Stone Flour: Actimin-BT was used, producer recommends a dose of 3 kg / 10m<sup>2</sup> on acidified soils. We treated one 5x5m quarter of the plot (25m<sup>2</sup>), so a total of 7.5 kg of stone flour was added (2.5\*3). The stone flour is not so dense and we approximated that 7.5 kg would take up a volume of 10L.

*Table 7 Actimin-BT stone flour ('lavameel') composition, supplied by producer in Dutch. The first table gives the composition of the main elements, the second gives the concentrations of trace elements and the third table gives the mineral composition.*

### Hoofdelementen:

Element	Verbinding	Gehalte (%)
Silicium	SiO <sub>2</sub>	40 - 50
IJzer	Fe <sub>2</sub> O <sub>3</sub>	9,7 - 10,8
Calcium	CaO	7,5 - 8,4
Magnesium	MgO	5,4 - 7,8
Natrium	Na <sub>2</sub> O	3,0 - 3,4
Kalium	K <sub>2</sub> O	1,44 - 1,90
Fosfaat	P <sub>2</sub> O <sub>5</sub>	0,44 - 0,69
Zwavel	SO <sub>3</sub>	0,02

### Sporenelementen:

Element	Gehalte (mg/kg)	Element	Gehalte (mg/kg)
Cerium	66	<b>Molybdeen</b>	<b>2</b>
Cesium	0,2	Neodymium	33
Dysprosium	5	Praseodymium	7,8
Europium	2,2	Rubidium	32
Erbium	2,3	<b>Selenium</b>	<b>0,03</b>
Gadolinium	6,3	Scandium	18
Gallium	21	Tantaal	2,7
Germanium	1,3	Terbium	0,9
Hafnium	4	Thulium	0,3
Holmium	0,9	Tin	2
<b>Kobalt</b>	<b>38</b>	Ytterbium	1,8
<b>Koper</b>	<b>41</b>	Yttrium	23,5
Lanthaan	33,5	<b>Zink</b>	<b>97</b>
Lutetium	0,26	Zirkonium	169
<b>Mangaan</b>	<b>1300</b>		

### Mineralogie:

*XRD-analyse*

Naam	Verbinding	%
Plagioklaas	(Ca,Na)AlSi <sub>3</sub> O <sub>8</sub>	47
Clinopyroxenen	(Mg,Ca,Na,Fe)Si <sub>2</sub> O <sub>6</sub>	19
Anorthoklaas	(Na,K)AlSi <sub>3</sub> O <sub>8</sub>	18
Forsteriet	Mg <sub>2</sub> SiO <sub>4</sub>	7
Overige aluminosilicaten		6
Hydroxides		3

### A.3 – Methodology images

#### Night Butterfly Traps



Figure 13 **Skinner trap**. Left: Set-up just before dusk. Right: retrieval with net just after dawn.

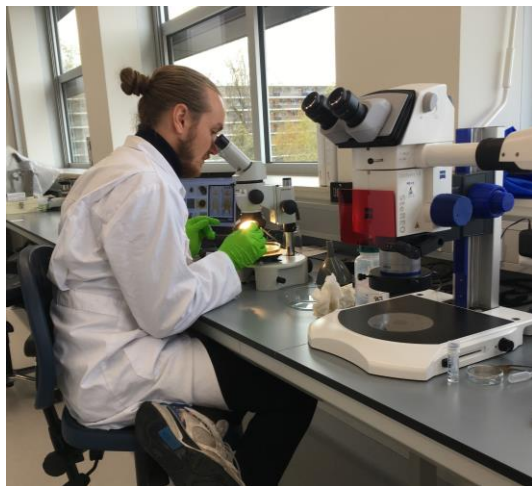


Figure 14 **Light Bucket**. Left: Bucket full of moths at dawn. Right: UV-light on at night

**Insect traps (unfortunately not included in this study)**



*Figure 15 Insect traps in field. Left: malaise trap. Right: pan trap*



*Figure 16 Analysis of invertebrate traps. Left: Sorting to morphospecies. Right: photographing each morphospecies.*

## Appendix B – Results

### B.1 – Groundwater

Ground water levels have been measured by Brabantwater, a water extraction company, from August 1973 until September 2018. The data is publicly available and can be retrieved, in Dutch, from the website <https://www.dinoloket.nl/ondergrondgegevens>.

In the figure below can be seen that the ground level is at 18.96 m relative to the Amsterdam Ordnance Datum (NAP in Dutch). Water levels are also depicted relative to NAP (y-axis) and fluctuate, since 2010, between 1600 and 1800 cm. Thus, relative to the ground level, the soil water table fluctuates between 3 to 1 meter. Yearly fluctuations can be observed from the table with highest water tables in winter, and lowest in summer. Moreover, it can be seen that water tables have been rising over the past decades, this is because Brabantwater stopped extracting water around 2000.

#### Grondwaterstanden

Identificatie: B46D0729  
Identificatie buis: B46D0729-001  
Coördinaten: 193448, 401196 (RD)  
Maaiveld: 18.96 m t.o.v. NAP

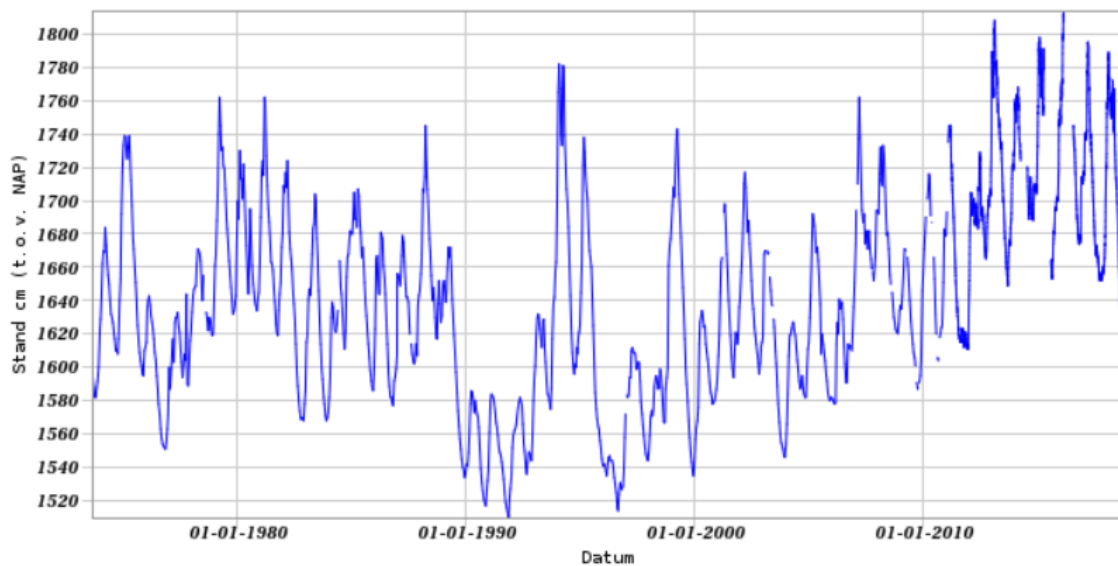


Figure 17 *Soil water tables*, supplied by Brabantwater. Soil water tables have been measured in the forest in Overloon from August 1973 until September 2018, and are depicted relative to the Amsterdam Ordnance Datum (NAP).

## B.2 – Experimental plots

Emerged seedlings were counted on 24-01-23. A total of at least 17 different morphospecies were identified, which is likely to be an underestimation. Species could not be identified with certainty, as they were still small seedlings.



*Figure 18 Saplings on experimental plots. Four morphospecies of the saplings that have emerged on the experimental plots, observed and counted on 24-01-23.*

### B.3 – Carbon

Structure of the forest was measured for the major forest types and is given in table 8. The aboveground woody carbon stock of each forest type has been multiplied with the forest type area to calculate the average carbon stock per ha of the complete forest, see table 9.

*Table 8 Structure per forest type. The species, height (m) and DBH (cm) of all the trees in a 10x 10 m plot was measured to calculate the carbon stored in aboveground woody biomass (Mg C/ ha) of each forest type. The amount of trees and carbon is depicted as the mean  $\pm$  standard deviation for each forest type, for height and DBH the minimum and maximum value are given.*

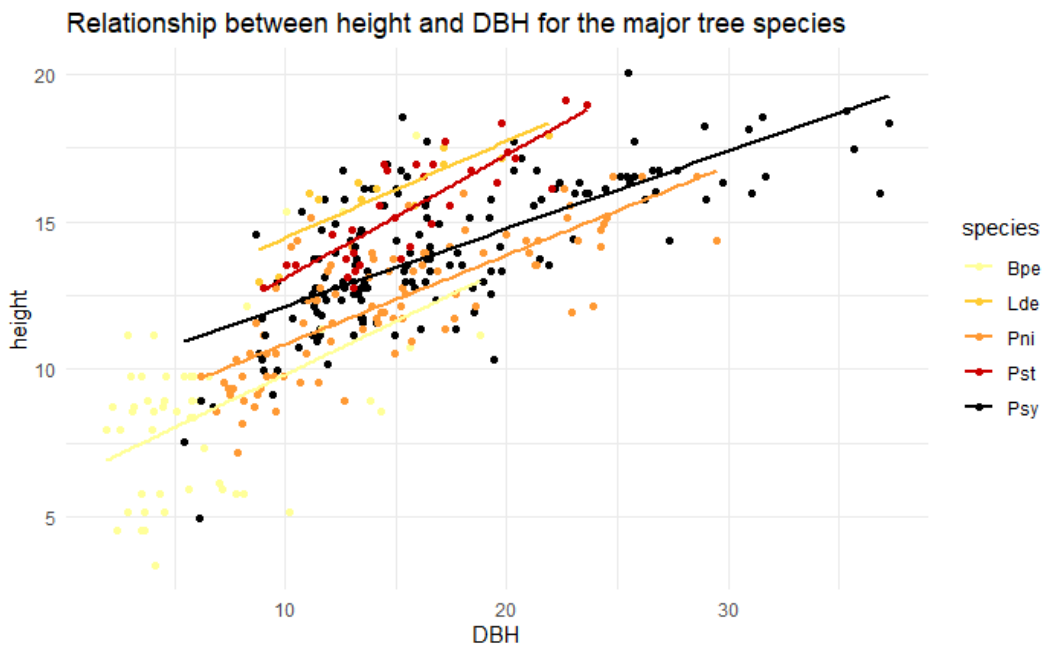
Forest type	Density (amount of trees per 100 m <sup>2</sup> )	Height (m) min. and max.	DBH (cm) min and max	Carbon (Mg C/ha)
PsyOO	8.6 $\pm$ 1.5	3.4 – 20.1	1.9 – 37.2	60.2 $\pm$ 11.7
PsyJD	20.6 $\pm$ 3.9	9.0 – 17.8	4.5 – 26.5	72.1 $\pm$ 11.6
Pni	23.8 $\pm$ 5.8	5.8 – 16.6	4.3 – 31.7	104.5 $\pm$ 11.0
Pst	32.0	12.8 – 19.2	9.0 – 23.6	116.2
Lde	21.0	7.6 – 18.0	5.4 – 21.9	72.8

The structure and carbon storage of the major forest types was measured in detail, by doing so, 82.3% of the forest was mapped in detail. The remaining small forest types were assumed to have an average carbon storage, so that the average carbon storage for the complete forest could be calculated. Some areas, such as the Birch dominated will have less carbon stored, others, such as Norway spruce or Douglas fir might have above average stocks.

*Table 9 Absolute carbon stock of the forest types and entire forest area. The average carbon stocks of each forest type were multiplied by the size, yielding an absolute carbon stock (Mg C). With the assumption that the remaining unsampled fraction is similar to the average, the absolute stock, and corresponding carbon storage of the entire forest was calculated.*

Forest type	Size (m <sup>2</sup> )	Fraction of total forest (%)	Carbon storage (Mg C/ ha)	Absolute stock (Mg C)
PsyOO	96201	40.28416	60.16748	578.8172
PsyJD	61877	25.91099	72.14635	446.42
Pni	16531	6.922355	104.52724	172.794
Pst	14182	5.938712	116.18506	164.7737
Lde	7793	3.263318	72.79127	56.72624
<b>Total</b>	196584	82.319539		1419.531
<b>Entire forest area</b>	238806	100	<b>72.20990</b>	1724.415635

The carbon stocks were calculated using allometric equations that only use the tree DBH and not the height. The relationship between height and DBH is shown in figure X. The regression lines are all significant and have an adjusted R-squared of 0.71, thus the relationship between DBH and height is fairly strong. The fluctuations in tree height (and thus biomass) are expected to be cancelled out by the high number of reference trees used to generate the generalized equations and should therefore not cause serious methodological errors.



*Figure 19 Relationship between tree height and DBH. Tree height and DBH have been plotted and their relation is predicted using a linear model for each of the frequently occurring tree species.*

## B.4 – Plant diversity

Table 10 **Plant species diversity.** This table contains a list of all the plant species encountered during the 5x5 m plot surveys, per surveyed forest type, categorised according to vegetation structure.

Type	PsyOO	PsyJD	Pst	Pme	Pni
Dominant tree	<i>Pinus sylvestris</i>	<i>Pinus sylvestris</i>	<i>Pinus strobus</i>	<i>Pseudotsuga mensiezii</i>	<i>Pinus nigra</i>
Undergrowth of trees and shrubs	<i>Ilex aquifolium</i>	<i>Ilex aquifolium</i>	<i>Ilex aquifolium</i>	<i>Ilex aquifolium</i>	<i>Ilex aquifolium</i>
	<i>Pinus sylvestris</i>	<i>Pinus sylvestris</i>	( <i>Pinus sylvestris</i> )	<i>Pinus sylvestris</i>	<i>Pinus sylvestris</i>
	<i>Betula pendula</i>	<i>Betula pendula</i>		<i>Betula pendula</i>	<i>Betula pendula</i>
	<i>Quercus robur</i>	<i>Quercus robur</i>		<i>Quercus robur</i>	<i>Quercus robur</i>
	<i>Quercus rubra</i>				<i>Quercus rubra</i>
	<i>Sorbus aucuparia</i>	<i>Sorbus aucuparia</i>		<i>Sorbus aucuparia</i>	<i>Sorbus aucuparia</i>
	<i>Prunus serotina</i>			<i>Prunus serotina</i>	<i>Prunus serotina</i>
	<i>Frangula alnus</i>			<i>Frangula alnus</i>	
Undergrowth of grasses ferns and herbs	<i>Avenella flexuosa</i>	<i>Avenella flexuosa</i>			<i>Avenella flexuosa</i>
	<i>Molinia caerulea</i>	<i>Molinia caerulea</i>			<i>Molinia caerulea</i>
	<i>Dryopteris carthusiana</i>				<i>Pteridium aquilinum</i>
	<i>Stellaria media</i>				
Mosses	<i>Hypnum cupressiforme</i>	<i>Hypnum cupressiforme</i>		<i>Hypnum cupressiforme</i>	<i>Hypnum cupressiforme</i>
	<i>Dicranum scoparium</i>	<i>Dicranum scoparium</i>		<i>Dicranum scoparium</i>	<i>Dicranum scoparium</i>
	<i>Polytrichum commune</i>	<i>Polytrichum commune</i>		<i>Polytrichum commune</i>	<i>Polytrichum commune</i>
	<i>Camylopus introflexus</i>			<i>Camylopus introflexus</i>	<i>Camylopus introflexus</i>
	<i>Pseudoscleropodium purum</i>			<i>Pseudoscleropodium purum</i>	<i>Pseudoscleropodium purum</i>
	<i>Eurhynchium praelongum</i>				

Other plant species have been encountered in the open forest and on the forest edges. These species have not been found during the 5x5m square plots surveys but are part of the complete floral diversity. This also applies to the plantation trees of the smaller forest types, such as Larch and Norway spruce.



Table 11 **Complete plant species list.** This list also includes the plants that have not been encountered on the surveyed plots. These grow on the forest edges and elsewhere in the open forest.

Scientific name	Dutch name	English name
<b>Conifer trees</b>		
<i>Pinus sylvestris</i>	Grove Den	Scotts pine
<i>Pinus strobus</i>	Weymouthden	Weymouth pine / White pine
<i>Pinus nigra subsp. laricio</i>	Corsicaanse den	Black pine
<i>Picea abies</i>	Fijnspar	Norway spruce
<i>Pseudotsuga menziesii</i>	Douglasspar	Douglas fir
<i>Larix decidua</i>	Europese lork	European larch
<b>Deciduous trees</b>		
<i>Quercus robur</i>	Zomereik	Common oak
<i>Quercus rubra</i>	Amerikaanse eik	Northern red oak
<i>Betula pendula</i>	Ruwe berk	Silver Birch
<i>Sorbus aucuparia</i>	Wilde lijsterbes	Rowan / mountain-ash
<i>Amelanchier lamarckii</i>	Amerikaanse krentenboompje	Juneberry
<i>Prunus serotina</i>	Amerikaanse vogelkers	Black cherry
<b>Shrubs, grasses and herbs</b>		
<i>Frangula alnus</i>	Sporkehout	Alder buckthorn
<i>Sambucus nigra</i>	Gewone vlier	Elderberry
<i>Rubus fruticosus</i>	Gewone Braam	Blackberry
<i>Vaccinium corymbosum</i>	Blauwbes	Northern highbush blueberry
<i>Calluna vulgaris</i>	Struikhei	Common heather
<i>Avenella flexuosa</i>	Bochtige smele	Wavy hair-grass
<i>Molinia caerulea</i>	Pijpestrootje	Purple moor-grass
<i>Ceratocarpus claviculata</i>	Rankende helmbloem	Climbing corydalis
<i>Alliaria petiolata</i>	look zonder look	Garlic mustard
<i>Digitalis purpurea</i>	Vingerhoedskruid	foxglove

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<i>Cardamine flexuosa</i>	Bosveldkers	wavy bittercress
<i>Stellaria media</i>	Vogelmuur	Chickweed
<i>Rumex acetosella</i>	Schapezuring	Sheep's sorrel
<b><i>Ferns and mosses</i></b>		
<i>Dryopteris carthusiana</i>	Smalle stekelvaren	Wood fern
<i>Dryopteris dilatata</i>	Brede stekelvaren	Broad buckler fern
<i>Pteridium aquilinum</i>	Adelaarsvaren	Bracken fern
<i>Hypnum cupressiforme</i>	Gesnaveld klauwtjesmos	Cypress-leaved plaitmoss
<i>Dicranum scoparium</i>	Gewoon gaffeltandmos	Broom forkmoss
<i>Polytrichum commune</i>	Gewoon haarmos	Common haircap
<i>Camylopus introflexus</i>	Grijs kronkelsteeltje	Heath star moss
<i>Pseudoscleropodium purum</i>	Groot laddermos	Neat feather-moss
<i>Eurhynchium praelongum</i>	Fijn laddermos	Common feather-moss

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## B.5 – Butterfly diversity

Butterfly diversity and abundance was measured during 10 butterfly transects during the period May to September 2022. Table 12 gives the total count of 9 butterfly species.

*Table 12 Butterfly species count. Diurnal butterfly species and counts, as observed during the butterfly surveys from May to September 2022 using the methodology of ‘de Vlinderstichting’.*

Species	Dutch common name	Total Count
<i>Aglais io</i>	Dagpauwoog	2
<i>Gonepteryx rhamni</i>	Citroenvlinder	1
<i>Jodis lactearia</i>	Melkwitte zomervlinder	1
<i>Lasiommata megera</i>	Argusvlinder	2
<i>Maniola jurtina</i>	Bruin zandoogje	1
<i>Ochlodes sylvanus</i>	Groot dikkopje	3
<i>Pieris brassicae</i>	Groot koolwitje	2
<i>Pieris rapae</i>	Klein koolwitje	2
<i>Vanessa atalanta</i>	Atalanta	1

Startled/ diurnally active moths that were encountered during the butterfly transects have also been counted, the total count is given in table 13. Most of these species are nocturnally active, so the diurnal surveys give an irregular representation of the actual moth abundances and diversity. However, for some species seasonality patterns were observed (Figure 17). *B. piniaria* was more abundant in May and disappeared in July while *C. margaritella* and *C. pusaria* had their peak abundance in the beginning of July. The *Scopariinae* species were present throughout the season (except for one survey) and had a peak abundance in the beginning of August.

*Table 13 Active or startled moths. Moth species and the total numbers that were observed during the butterfly surveys.*

Species	Dutch common name	Total Count
<i>Bupalus piniaria</i>	Dennenspanner	43
<i>Cyclophora albipunctata</i>	Berkenoogspanner	5
<i>Catoptria margaritella</i>	Gelijnde vlakjesmot	91
<i>Cabera pusaria</i>	Witte grijsbandspanner	26
<i>Ectropis crepuscularia</i>	Gewone spikkelspanner	2
<i>Scopariinae sp.</i>	Granietmot (sp.)	126

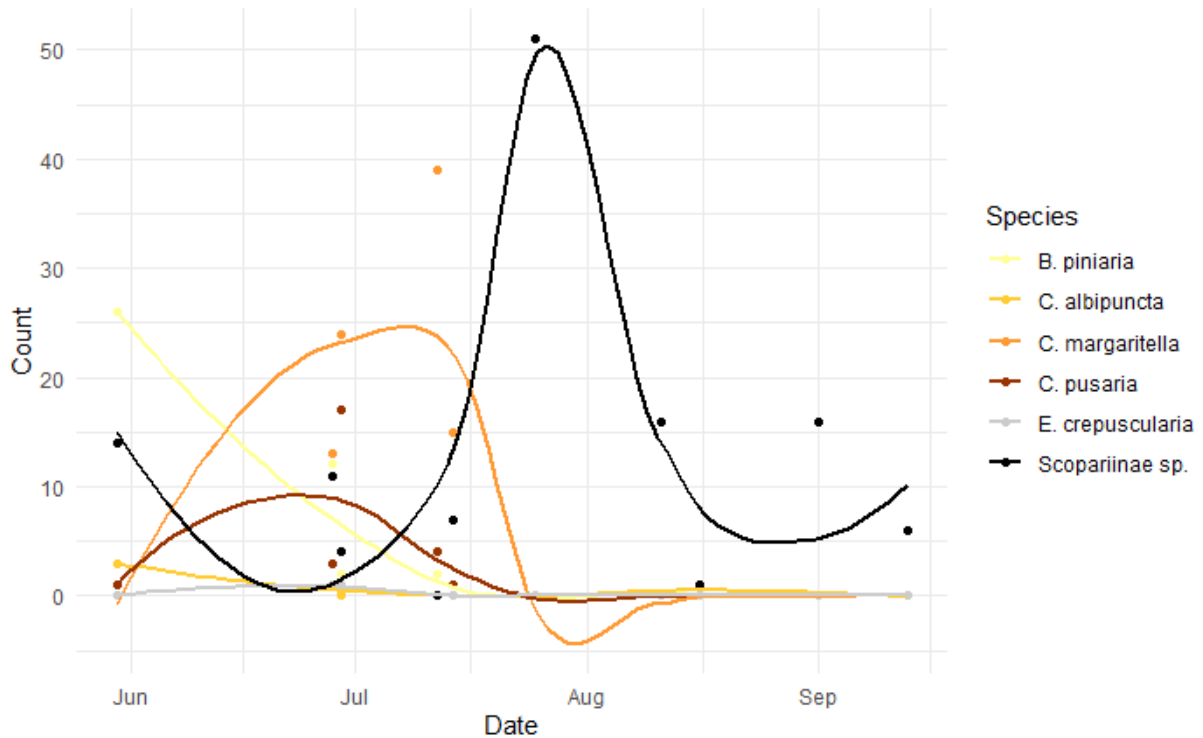


Figure 20 **Moth count over the season.** The number of moths, observed during the butterfly transects, over time. Seasonality patterns occur for some of the species.

Table 14 **Moth species list.** Moth species and their total counts, observed using a light bucket.

Scientific name	Dutch name	Total Count
<i>Campaea margaritaria</i>	Appeltak	6
<i>Pheosia gnoma</i>	Berkenbrandvlerkvinder	5
<i>Drepana falcataria</i>	Berkeneenstaart	2
<i>Cyclophora albipunctata</i>	Berkenoogspanner	1
<i>Agonopterix arenella</i>	Bleke kaartmot	1
<i>Evergestis pallidata</i>	Bonte valkmot	1
<i>Noctua fimbriata</i>	Breedbandhuismoeder	1
<i>Harpella forcicella</i>	Bruine molmboorder	3
<i>Xestia baja</i>	Bruine zwartstipuil	15
<i>Cydalima perspectalis</i>	Buxusmot	1
<i>Sphinx pinastri</i>	Dennenpijlstaart	1
<i>Macaria alternata</i>	Donker klaverblaadje	1
<i>Ancylis apicella</i>	Fijngestreepte haakbladroller	1
<i>Macaria liturata</i>	Gerimpelde spanner	1
<i>Cyclophora punctaria</i>	Gestippelde oogspanner	1
<i>Anania coronata</i>	Gewone coronamot	1

<i>Luperina testacea</i>	Gewone grasuil	7
<i>Scopariinae sp.</i>	Granietmot	400
<i>Crambidae spec</i>	Grasmot onbekend	5
<i>Eugnorisma glareosa</i>	Grijze herfstuil	1
<i>Noctua pronuba</i>	Huismoeder	1
<i>Cosmia trapezina</i>	Hyena	1
<i>Chiasmia clathrata</i>	Klaverspanner	1
<i>Mamestra brassicae</i>	Kooluil	1
<i>Ptilodon capucina</i>	kroonvogeltje	2
<i>Cataclysta lemnata</i>	kroosvlindertje	3
<i>Limnephilus marmoratus</i>	L. marmoratus	3
<i>Agriphila inquinatella</i>	Moerasgrasmot	1
<i>Eilema depressa</i>	Naaldboombeertje	3
<i>Unknown</i>	Onbekend (kleine beige vlinder oranje driehoek)	1
<i>Noctua janthe / janthina</i>	open/klein breedbandhuismoeder	2
<i>Hylaea fasciaria</i>	Rode dennenspanner	7
<i>Paramesia gnomana</i>	scherpbandbladroller	1
<i>Endotricha flammealis</i>	Strooiselmot	29
<i>Agriphila tristella</i>	variabele grasmot	1
<i>Carcina quercana</i>	Vuurmot	1
<i>Mythimna albipuncta</i>	Witstipgrasuil	1
<i>Xestia c-nigrum</i>	Zwarte-c-uil	13

In the beginning of September the moth abundance abruptly dropped to very low levels (see figure below). The moth diversity also dropped severely from August to September (from 23 and 27, to 7 and 2 species, respectively).

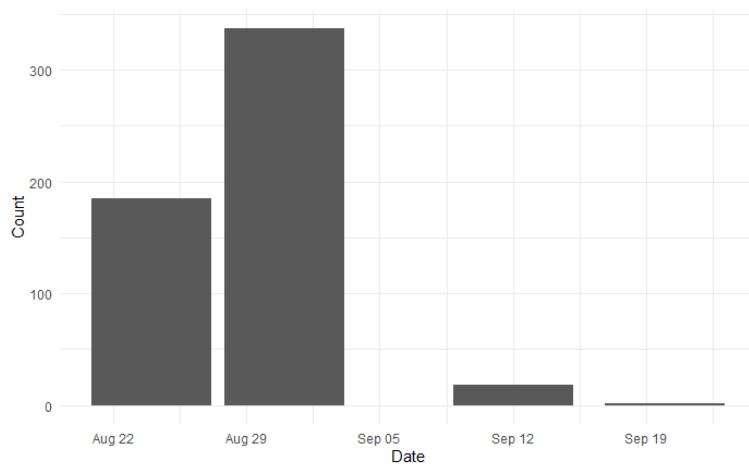


Figure 21 *Total moth count per survey.*

## B.6 – Vertebrate diversity

Table 15 **Mammal species list.** Mammal species that were observed in the forest by eye, camera trap, bat detector or, unfortunately (for the mice), pit trap.

<b>Scientific name</b>	<b>common name</b>	<b>Dutch common name</b>
<i>Pipistrellus pipistrellus</i>	Common pipistrelle	Gewone dwergvleermuis
<i>Myotis nattereri</i>	Natterer's bat	Franjestaart
<i>Nyctalus noctula</i>	Common noctule	Rosse vleermuis
<i>Capreolus capreolus</i>	Roe deer	Ree
<i>Meles meles</i>	Badger	Das
<i>Vulpes vulpes</i>	Fox	Vos
<i>Lepus europaeus</i>	Hare	Haas
<i>Myodes glareolus</i>	Bank vole	Rosse woelmuis
<i>Apodemus sylvaticus</i>	Wood mouse	Bosmuis
<i>Sorex minutus</i>	Eurasian pygmy shrew	Dwergspitsmuis

Table 16 **Reptile and amphibian species list.** Reptile and amphibian species of the forest as observed by eye.

<b>Scientific name</b>	<b>common name</b>	<b>Dutch common name</b>
<i>Podarcis muralis</i>	Common wall lizard	Muurhagedis
<i>Zootoca vivipara</i>	Viviparous lizard	Levendbarende hagedis
<i>Bufo bufo</i>	Common toad	Gewone pad
<i>Rana temporaria</i>	Common frog	Bruine Kikker
<i>Pelophylax sp.</i>	Water frog	Groene kikker
<i>Ichthyosaura alpestris</i>	Alpine newt	Alpenwatersalamander
<i>Lissotriton vulgaris</i>	Smooth newt	Kleine watersalamander

Table 17 **Bird species list.** Bird species observed in the forest by eye, sound or camera trap.

<b>Scientific name</b>	<b>Common name</b>	<b>Dutch name</b>
<i>Buteo buteo</i>	Common Buzzard	Buizerd
<i>Falco subbuteo</i>	Eurasian Hobby	Boomvalk
<i>Accipiter gentilis</i>	Northern Goshawk	Havik
<i>Accipiter nisus</i>	Eurasian sparrowhawk	Sperwer
<i>Strix aluco</i>	Tawny Owl	Bosuil
<i>Corvus corone</i>	Carrion Crow	Zwarte kraai
<i>Garrulus glandarius</i>	Eurasian Jay	Gaai
<i>Pica pica</i>	Eurasian Magpie	Ekster
<i>Dendrocopos major</i>	Great spotted woodpecker	Grote bonte specht
<i>Dryocopus martius</i>	Black Woodpecker	Zwarte specht
<i>Columba palumbus</i>	Common wood pigeon	Houtduif
<i>Turdus merula</i>	Common Blackbird	Merel
<i>Fringilla coelebs</i>	Common Chaffinch	Vink
<i>Turdus philomelos</i>	Song thrush	Zanglijster
<i>Sylvia curruca</i>	Lesser Whitethroat	Braamsluiper
<i>Certhia brachydactyla</i>	Short-toed Treecreeper	Boomkruiper
<i>Parus major</i>	Great tit	Koolmees
<i>Cyanistes caeruleus</i>	Eurasian blue tit	Pimpelmees
<i>Aegithalos caudatus</i>	Long-tailed tit	Staartmees
<i>Poecile palustris</i>	Marsh tit	Glanskop
<i>Phylloscopus collybita</i>	Common Chiffchaff	Tjiftjaf
<i>Erithacus rubecula</i>	European Robin	Roodborst
<i>Anthus trivialis</i>	Tree pipit	Boompieper

**B.7 – Images illustrating biodiversity**



Figure 22 **Viviparous lizard** (*Zootoca vivipara*), probably a 'pregnant' female.



Figure 23 **Common darter** (*Sympetrum striolatum*), female specimen.



Figure 24 **Common heather** (*Calluna vulgaris*) with Mats Kessel, during fieldwork.



Figure 25 **Common buzzard** (*Buteo buteo*).



Figure 26 **Badger** (*Meles meles*)



Figure 27 **Roe deer** (*Capreolus capreolus*)





*Figure 28 Sunset at the forest edge.*