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Report from the

6th Master Summer School
“Biodiversity Monitoring”,
Białowieża, Poland, 16–27 August 2024

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Cover photo

On the way back from the sampling sites after a long day in the field (Photo: Jürgen Dengler).

Disclaimer

The given authors are responsible for the content of the individual chapters.

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Preface

Iwona Dembicz & Jürgen Dengler

The Master Summer School "Biodiversity Monitoring" started in 2019 as a joint initiative between the Faculty of Biology of the University of Warsaw in Poland and the Institute of Natural Resource Sciences (IUNR) of the Zurich University of Applied Sciences (ZHAW) Wädenswil in Switzerland (Dengler 2020a, 2020b). It was planned to be conducted one year in Parc Ela, Switzerland, and every other year at the edge of the Białowieża National Park, Poland. However, the travel limitations of the Covid-19 years disturbed these plans to some extent and the event had to be conducted more often in Switzerland than originally planned. However, in 2022 we were able to conduct the Summer School for the first time in Białowieża, which was a great success (Dembicz & Dengler 2023a). This was also the year when the Kherson State University, Ukraine, joined as a third partner.

For the sixth conductance in August 2024, we returned to Białowieża. Under the guidance of seven teachers, 15 students learned for 10 days how to sample biodiversity in different taxonomic groups (vascular plants, orthopterans and small mammals), how to set up monitoring schemes and how to analyse these data with modern statistical methods in R. Both teachers and students were from all three countries, Switzerland, Poland and Ukraine. Therefore, the time in Białowieża was also a true intercultural experience, which was enhanced by the fact that we cooked ourselves and often had typical dishes from the three countries (Chusova et al. 2024).

However, there are also remarkable scientific outcomes, enabled through the careful sampling in 2024, combined with the data of the three previous conductance in Białowieża (2022). The three taxonomic groups were sampled on the permanently marked plots that were arranged always in triplets, each representing three landuse types (mown, recently abandoned, long-term abondoned) with otherwise similar environmental conditions (particularly concerning soil moisture) (Dembicz & Dengler 2023b). In interational teams, the students worked on a total of five research projects. To facilitate high-quality reports in the style of scientific papers, in autumn 2024, several months after the Summer School, we conducted a reciprocal peer review event on Zoom where each project team had to deliver one part of their "papers" to another team, which then reviewed its form and content. This element definitely contributed to the good quality of the student reports.

We hope you enjoy reading!

References

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Reports from the student projects

The participants of the Summer School have carried out five research projects in small teams of three students from at least two countries. These reports were prepared in the style of scientific papers. Please note that the projects are published as submitted by the students, except minor adjustments in the layout. The responsibility for the content solely rests with the authors. Overall, the quality is good, but individual reports might contain contain some errors or misinterpretations.

Project 1: Comparative analysis of species diversity of vascular plants in semi-natural grasslands using the method of permanent plots (Białowieża, Poland)

Nadin Kruschwitz, Navia Graf & Olena Miskova

Abstract

Changes in plant species composition and diversity were examined in semi-natural grasslands of the Białowieża region (Poland) between 2022 and 2024, comparing three management types: mowed meadows, unused grasslands, and forest areas. A total of 251 vascular plant species were recorded across both years, with species richness remaining relatively stable (215 species in 2022, 211 in 2024). Nevertheless, changes occurred, with 40 species found only in 2022 and 36 only in 2024. The calculated species turnover of 30% shows moderate changes in species composition. The analysis of pseudoturnover showed a total rate of 20.7%. Mowed meadows showed an increase in species diversity, while both unused and forested areas experienced a decline. The analysis of Ecological Indicator Values for Europe (EIVE) revealed significant differences across management types, particularly for "EIVE.T" and "EIVE.L". Species distribution diversity, measured through evenness and the Shannon Index, also showed a marked influence of management type, with mowed areas displaying higher diversity. Overall, this study emphasizes that management type has a substantial impact on species diversity and composition. Regular mowing can help maintain plant diversity, whereas unused or forested areas tend to experience species loss.

Keywords

Biodiversity, Ecological Indicator Values, Environmental Conditions, Field Sampling, Grassland Management, Species Richness, Species Turnover, Temporal Changes, Vascular Plants, Vegetation Succession.

Introduction

Grasslands are among the most biodiverse ecosystems globally, providing essential services such as carbon sequestration, soil conservation, and habitats for a wide range of species (Bengtsson et al., 2019; Buchmann et al., 2019). Semi-natural grasslands, in particular, are characterized by a long history of human management and support rich communities of vascular plants (Tälle, 2018). However, these ecosystems are increasingly threatened by changes in land use, abandonment, and intensification of agricultural practices, which disrupt their ecological balance and lead to shifts in species composition and biodiversity (Richter et al., 2024). Understanding how land use changes, such as the cessation of management, impact grassland biodiversity is crucial for conservation efforts and sustainable land management practices.

The abandonment of traditional grassland management practices, such as mowing and grazing, often leads to the process of succession, where woody species progressively invade and outcompete herbaceous plants. This process, known as woody encroachment, can significantly alter the structure and function of grassland ecosystems. Studies have shown that grasslands undergoing succession typically experience a reduction in species richness and shifts in ecological conditions, such as changes in light availability, soil moisture, and nutrient levels (Ballesteros et al., 2024; Molina et al., 2023; Wesche et al., 2012). Moreover, the cessation of mowing can accelerate the decline of light-demanding species, allowing shade-tolerant species and shrubs to dominate (Tälle, 2018). While the long-term effects of abandonment and succession have been well-documented, there is less understanding of the short-term changes that occur in plant communities during the initial years of management cessation.

Recent advancements in ecological research have introduced new methods for assessing environmental conditions and plant community responses. Among these is the Ecological Indicator Values for Europe (EIVE), a novel approach designed to provide standardized metrics for evaluating the environmental preferences of plant species. EIVE covers several key ecological variables, including light availability (EIVE.L), temperature (EIVE.T), moisture (EIVE.M), nitrogen availability (EIVE.N), and soil pH (EIVE.R) (Tichý et al., 2023). Despite being a recent development, EIVE offers a promising tool for assessing how changes in land use impact these environmental conditions and, consequently, plant communities.

In addition to changes in species composition and environmental conditions, the measurement of biodiversity metrics, such as species richness, evenness, and the Shannon Index, offers valuable insights into the health and stability of plant communities. Species richness refers to the number of species present within a given area, while evenness measures how evenly individuals are distributed across species. The Shannon Index combines both richness and evenness to provide a comprehensive assessment of community diversity (Dušek & Popelková, 2012).

One critical challenge in ecological monitoring is the issue of observer errors, including misidentification of species and inconsistent recording of species presence between years. These errors can inflate estimates of species turnover, leading to a phenomenon known as pseudoturnover, where apparent changes in species composition are driven by human error rather than true ecological shifts (Boch et al., 2022). It is important to account for these errors when analyzing temporal changes in plant communities, as they can obscure genuine patterns of species gain or loss.

This study aims to fill these gaps by conducting a comparative analysis of vascular plant diversity in semi-natural grasslands in the Białowieża Forest region, Poland, between 2022 and 2024. We focus on three different grassland types: regularly mown grassland, recently abandoned grassland, and long-term abandoned grassland undergoing woody encroachment. By examining changes in species richness, evenness, Shannon Index, and Ecological Indicator Values (EIVE) over two years, we seek to understand how grassland plant communities respond to short-term cessation of management and the onset of succession. We also account for observer errors by estimating pseudoturnover in species composition.

The specific objectives of this study are as follows:

1. To quantify the extent of species turnover between 2022 and 2024, accounting for observer errors and pseudoturnover.
2. To analyze differences in biodiversity metrics (species richness, evenness, Shannon Index) between 2022 and 2024 across three land use types.
3. To assess changes in Ecological Indicator Values for Europe (EIVE) in relation to shifts in environmental conditions over the two-year period.

We hypothesize that the cessation of mowing in the unused and woody encroachment plots will lead to a decrease in species richness and evenness, particularly in light-demanding species, and that EIVE scores will reflect these changes through shifts in light, moisture, and nitrogen availability.

Methods

Study site

The study was conducted in the Białowieża Forest region, located in northeastern Poland, an area known for its high biodiversity and semi-natural grasslands (Faliński, 1986). The permanent plots were established in semi-natural grasslands that are representative of different land-use histories and management intensities. The study area includes three types of grasslands:

- Mown grassland (M): Regularly managed by mowing once per year.
- Unused grassland (U): Recently abandoned and no longer subject to management for the past few years.
- Woody encroachment (W): Long-term abandoned grassland where natural succession has progressed, leading to the presence of shrubs and early-stage woodland development.

In these grasslands, the primary focus of this study was to assess vascular plant diversity and cover in relation to the changes observed between 2022 and 2024. The investigation covers **Triplet numbers 1-4 and 6-13**, with each triplet consisting of three permanent plots, one for each grassland type (M, U, W) (Figure 1).

Figure 1: Spatial distribution of vegetation sampling plots in Białowieża, Poland. Plots are labeled according to their management type (M = mowed meadow, U = unused, W = forest) and plot number. Plots marked with 'n' (Un, Wn) represent replacement plots established in 2024 due to management changes.

Field sampling

Field sampling was conducted in 2022 and again in 2024, with data collected by master students in collaboration with experts. Each of the permanent plots was structured as a triplet, representing the three different grassland types: Mown (M), Unused (U), and Woody (W). The sampling design followed a nested plot approach, with three grain sizes (Dengler, 2009):

- 10 m^2 plot (3.16 m x 3.16 m)
- 1 m^2 plot (1 m x 1 m)
- 0.1 m^2 plot (0.32 m x 0.32 m)

These plots were arranged in the southwestern corner of each permanent vegetation plot. The vegetation surveys recorded all vascular plant species present, with the cover of each species estimated as a percentage for each of the three vertical layers: herbs, shrubs, and trees. Since the cover was recorded additively for each layer, the total cover could exceed 100%. In addition to vascular plants, cover values for non-vascular species (e.g., cryptogams), litter, and bare soil surfaces were also estimated independently for each plot.

Mixed soil samples were collected from each plot by taking 3-5 subsamples from the uppermost 10 cm of soil, providing a representative sample for further analysis of soil characteristics. These samples were not included in this comparative analysis but are available for future studies on soil-plant interactions.

The primary aim of the field sampling was to monitor temporal changes in plant composition and cover between 2022 and 2024, with a specific focus on the overall difference between these two years, rather than on comparisons between the three grassland types.

Statistical analyses

The statistical analyses were designed to address the question: How does species composition develop over two years (2022 and 2024) across the three different land use types: Mown (M), Unused (U), and Woody encroachment (W)? To answer this, we performed the following key analyses:

All analyses (Appendix 2) were performed using R (version 4.2.1), with the complete R-script provided in Appendix B to ensure reproducibility of the results (R Foundation for Statistical Computing, 2022).

Packages Used:

- **dplyr** - for data manipulation and transformation. (Hadley Wickham et al., 2022)
- **ggplot2** - for data visualization and creating plots. (Hadley Wickham, 2016)
- **car** - for various regression diagnostics. (John Fox & Sanford Weisberg, 2019)
- **readxl** - for reading Excel files. (Hadley Wickham & Jennifer Bryan, 2022)
- **tidyverse** - a collection of packages for data science. (Hadley Wickham et al., 2019)
- **emmeans** - for estimated marginal means and pairwise comparisons. (Russell V. Lenth, 2024)
- **reshape2** - for reshaping data. (Hadley Wickham, 2007)

The raw data includes triplets numbered 1–4 and 6–13. Some of these plots (3U, 6U, 7U, 11U, 7W, and 10W) experienced a change in land-use type in 2024 and were thus repositioned slightly as new plots within the original land-use type, aiming to keep conditions as similar as possible to the previous year.

Based on this, two datasets were created for analysis:

1. «Dataset without altered plots»: This dataset excludes all plots affected by a land-use change. The actual triplet numbers used in this dataset are: «1, 2, 4, 6, 8, 9, 12, 13» (without 3Un, 3U, 6Un, 6U, 7Un, 7U, 11Un, 11U, 7Wn, 7W, 10Wn, 10W).
2. «Dataset with adjusted plots»: This dataset includes both the original, altered plots and the new, adjusted plots. The triplet numbers used here include «1–4, 6–13», with plots 3U, 6U, 7U, 11U, 7W, and 10W replaced by new, slightly adjusted observations.

These two datasets allow for comparisons between purely unaltered conditions and slightly adjusted conditions to capture differences more accurately.

Turnover and pseudoturnover

To evaluate the vegetation changes between 2022 and 2024, both ecological turnover and pseudo-turnover were analysed. Following (Boch et al., 2022) the analysis focused on distinguishing between actual ecological changes and changes due to methodological inconsistencies.

The analysis of vegetation changes included both quantitative turnover calculations and qualitative assessment of species dynamics. Species that appeared or disappeared between the survey years were specifically documented to understand patterns of species composition change. A detailed species list documenting these changes is provided in Appendix A.

Due to management measures such as mowing or clearing, some areas were classified differently between 2022 and 2024. Plots 3U, 6U, 7U, 11U, 7W and 10W were affected. For these areas, replacement plots (labelled with ‘n’: 3Un, 6Un, 7Un, 11Un, 7Wn and 10Wn) were set up in the close surroundings of the original plots. These new plots were treated as independent study plots and included in the analysis accordingly. All data presented are based on this adapted study design, unless otherwise indicated.

In the analysis, the actual ecological turnover and the pseudo-turnover caused by observation errors were analysed. The Turnover was calculated using the following formula:

$$Turnover = \frac{(\mathrm{n}_{22} + n_{23})}{\mathrm{N}}$$

n_{22} = number of species found only in 2022
n_{24} = number of species found only in 2024
N = total number of species recorded in both years

To determine the pseudoturnover, a manual verification of all species that only occurred in one of the two survey years was carried out. The following criteria were considered to identify possible cases of pseudoturnover:

- Temporal: Species pairs confused between 2022 and 2024
- Taxonomic: Species groups difficult to distinguish within the same year
- Methodological: Easily overlooked species due to size, flowering period, or visibility

The pseudo-turnover was then calculated as followed:

$$Pseudoturnover = \frac{(\mathrm{n}_{\mathrm{p}})}{N}$$

n_p = number of species identified as potential pseudoturnover cases
N = total number of species recorded in both years

Duplicate entries were identified for several species in the 2024 dataset: *Stellaria media* and *Luzula multiflora* (rows 2051 and 1469), *Hypochaeris radicata* (rows 1242 and 1805), *Plantago lanceolata* (rows 1246 and 1806), and *Tanacetum vulgare* (row 1812), they were changed in the datasheet. This correction was important to avoid overestimating species changes between the years.

Ecological Indicator Values for Europe (EIVE)

The analysis of Ecological Indicator Values for Europe (EIVE) was conducted to evaluate trends in ecological metrics between the years 2022 and 2024, focusing on potential shifts in environmental conditions affecting plant communities. This analysis centered on five standardized EIVE metrics: «EIVE.L» (light availability), «EIVE.T» (temperature conditions), «EIVE.M» (moisture levels), «EIVE.N» (nitrogen availability), and «EIVE.R» (soil pH). Each metric provides insights into environmental factors that influence plant growth, species composition, and overall ecosystem dynamics (Dengler et al., 2023).

To begin, an «analysis of variance (ANOVA)» was conducted for each EIVE metric to evaluate the effects of «Year» and «Type» (land-use type) on ecological indicators, as well as their interaction. Residuals for each model were checked for normality using the Shapiro-Wilk test, ensuring the validity of ANOVA assumptions. When the «Type» effect was found to be significant, post-hoc analyses were conducted to identify which specific land-use types differed significantly from each other.

For metrics where the «Type» effect approached significance or was significant, post-hoc tests were conducted to determine specific pairwise differences, with estimated marginal means (EMM) used to assess effect size and practical implications.

The analysis also included «visualization of data through boxplots» to represent the distribution of each EIVE metric across «Year» and «Type». The data were organized into two sets of boxplots: one showing the effect of «Year» alone and another showing the combined effects of «Year» and «Type» for the categories "Mown," "Unused," and "Woody." Each plot also included mean values and standard deviations displayed above the boxplots to highlight average scores for each «Year» and «Type» combination. Blue was used to represent data for 2022, and green for 2024.

Statistical analysis was conducted using several packages: «dplyr» for data manipulation, «ggplot2» for creating boxplots, «stats» for ANOVA and Shapiro-Wilk tests, and «emmeans» for estimated marginal means and post-hoc comparisons. This structured approach allowed for a comprehensive understanding of temporal trends and land-use impacts on EIVE metrics, highlighting how environmental and land-use factors shape ecological indicators across different conditions.

Biodiversity metrics

Biodiversity was assessed using three core metrics (Magurran, 2004):

- SP= Species richness: The number of species present in each plot, calculated for three plot sizes (0.1 m², 1 m², and 10 m²).
- EV = Evenness: A measure of the distribution of species abundance within the community, for the largest plot size (10 m²).

- SH = Shannon-Index: A commonly used biodiversity index that accounts for both species richness and evenness, for the largest plot size (10 m^2).

For these biodiversity metrics, a two-way ANOVA was conducted to evaluate the effects of both year (2022 vs. 2024) and land use type (Mown, Unused, Woody) on species richness, evenness, and the Shannon Index. The analysis included the calculation of mean values and variances for each metric by year and land use type. Additionally, the normality of residuals for each model was checked using the Shapiro-Wilk test to confirm the validity of the ANOVA assumptions.

Where significant effects were detected in the ANOVA, post-hoc tests were conducted to identify specific group differences. The post-hoc tests allowed for detailed pairwise comparisons between years and land use types to interpret interactions between these factors.

Results

Species composition and turnover/pseudoturnover

A total of 251 different plant species were recorded across both survey years. The total number of species remained relatively stable with 215 species in 2022 and 211 species in 2024. Despite this apparent stability, there were considerable fluctuations: 40 species were only found in 2022, while 36 species occurred exclusively in 2024. The calculated turnover value of 0.30 indicates a moderate change in species composition. Mowed meadows showed a high diversity with 165 total species. The number of species rose from 128 in 2022 to 138 in 2024, which indicates an increase in species diversity. The turnover value of 0.39 indicates a considerable fluctuation in the species composition, while unused areas also showed a high diversity with 160 total species. However, the number of species fell from 140 in 2022 to 122 in 2024. The turnover value of 0.36 shows a moderate change in the species composition. Forest areas recorded the highest total number of species with 172 species. However, a decline from 147 species in 2022 to 128 species in 2024 was also observed here. The turnover value of 0.40 indicates considerable fluctuation. As expected, newly unused areas and newly forested areas showed the most drastic changes. Both types had no species in 2022 as they had not yet been surveyed and were newly recorded in 2024 with 85 and 33 species respectively. The turnover value of 1.00 for both types confirms this fact.

The analysis of the pseudoturnover in the vegetation records revealed a total number of 52 species (20.7% of all species recorded), which can be attributed to methodological inaccuracies. These species can be divided into three main categories. The first category comprises the temporal component with 20 species (7.9%) that could be confused between the years 2022 and 2024. This includes three morphologically similar pairs of Carex species, two pairs of grasses of the genera Agrostis and Poa as well as other pairs of typical grassland species such as Juncus, Alopecurus and Festuca. The second category is the taxonomic component with 22 species (8.8%). These are mainly the genus Epilobium with 5 species that are difficult to distinguish, the genus Galeopsis with 4 morphologically similar species, four critical Carex species and several grasses of the genera Agrostis, Poa and Festuca.the third category is the methodical component with 10 species (4%). These species were easily overlooked due to their specific characteristics, including small-growing species such as Veronica serpyllifolia and Viola arvensis, species with a short flowering period such as Caltha palustris and inconspicuous species in dense vegetation such as Glechoma hederacea.The overall rate of pseudoturnover of 20.7% is in the middle range of the values described in the literature (10-30%) and reflects the methodological challenges of vegetation surveys.

We detected changes in the species composition for 2022 and 2024. This difference concerns both the general lists of flora species recorded on triplets and the species composition of the same plots. In 2022, 215 species were recorded in the studied areas, and 211 species in 2024. It was found that 40 species were not re-recorded in 2024, for example *Selinum carvifolia, Juncus articulatus, Alopecurus geniculatus, Carex disticha, C. echinata, Agrostis canina, Caltha palustris, Campanula patula*, etc. There are 36 new species found on plots in 2024: *Fraxinus pennsylvanica, Picris hieracioides, Vicia villosa, Carex cespitosa, C. spicata, C. vulpina, Glechoma hederacea, Poa compressa, P. nemoralis, Rumex crispus* and others (Appendix 1).

Changes in species richness in biotopes with different types of use are different. In 2024, the species richness is decreasing in unused and woody areas and increases in mowed plots.

The most common species is *Holcus lanatus* in both years, and we can see that its frequency increased in 2024. Other common species in 2022 were *Stellaria graminea,* Achillea *millefolium agg., Carex hirta, Veronica chamaedrys, Equisetum arvense,* and others. In 2024, the frequency of *Festuca rubra* aggr., *Juncus effusus, Lathyrus pratensis, Phleum pratense aggr., Rumex thyrsiflorus, Agrimonia procera* increases.

Ecological Indicator Values for Europe (EIVE) trends

The analysis of Ecological Indicator Values (EIVE) revealed significant changes across various metrics between the years 2022 and 2024, both in the analysis with land use changes and in the analysis without land use changes. Specifically, for «EIVE.T», the effect of «Year» was marginally significant, (F (1, 55) = 3.625), (p = 0.062), indicating a potential trend over time, while the «Type» had a significant effect, (F (2, 55) = 6.634), (p = 0.003). The interaction between «Year» and «Type» was not significant, (F (2, 55) = 0.277), (p = 0.759). The residuals for EIVE.T demonstrated a normal distribution, confirmed by the Shapiro-Wilk test (W = 0.977), (p = 0.216). The mean value for EIVE.T was 3.98 (SD = 0.146) for 2022 and 4.01 (SD = 0.181) for 2024 (Figure 2).

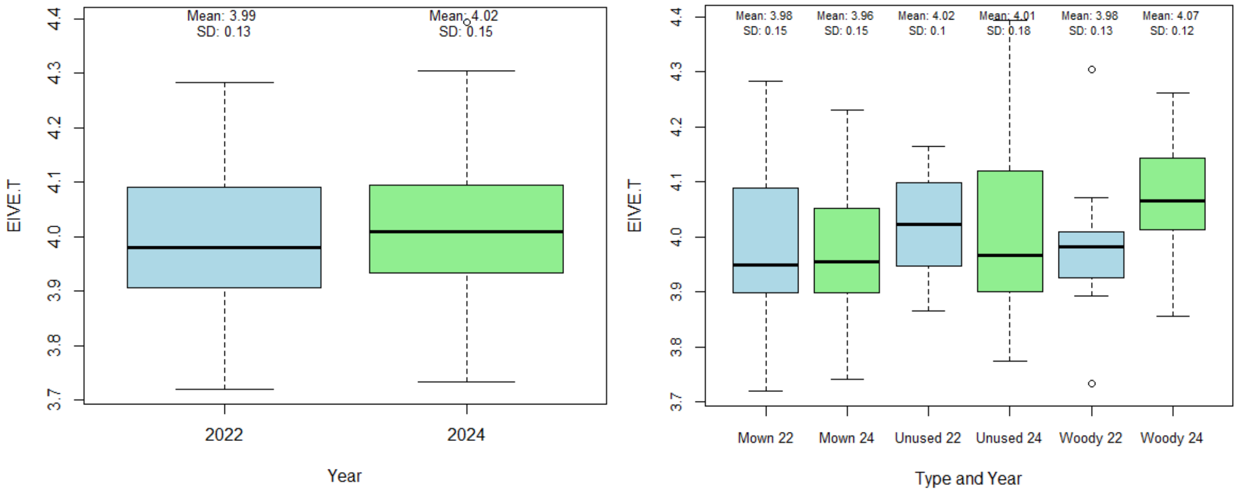

Figure 2. Boxplot of Ecological Indicator Values for Europe (EIVE.T). The right plot displays the effect of Year alone. The left plot illustrates the effect of Type and Year on EIVE.T, showing results for three types (Mown, Unused, Woody) in both 2022 (blue) and.

In the case of «EIVE.M», the «Year» effect was not significant, (F (1, 55) = 1.053), (p = 0.309), while «Type» approached significance with (F (2, 55) = 2.959), (p = 0.060). The interaction between «Year» and «Type» was also not significant, (F (2, 55) = 1.125), (p = 0.332). The residuals did not conform to a normal distribution, as indicated by the Shapiro-Wilk test (W = 0.944), (p = 0.003). The mean for EIVE.M was 5.03 (SD = 0.754) for 2022 and 5.04 (SD = 0.787) for 2024.

For «EIVE.L», the ANOVA indicated no significant effect of «Year», (F (1, 55) = 0.019), (p = 0.890), while «Type» had a highly significant effect, (F (2, 55) = 63.665), (p < 0.001). The interaction effect between «Year» and «Type» was not significant, (F (2, 55) = 0.813), (p = 0.449). The residuals for EIVE.L displayed a normal distribution (Shapiro-Wilk test: (W = 0.982), (p = 0.399)). The mean value for EIVE.L was 6.82 (SD = 0.206) for 2022 and 6.82 (SD = 0.197) for 2024.

For «EIVE.N», the effect of «Year» was not significant, (F (1, 55) = 0.017), (p = 0.896), while «Type» had a significant effect, (F (2, 55) = 6.797), (p = 0.002). The interaction between «Year» and «Type» was not significant, (F (2, 55) = 0.165), (p = 0.848). The residuals indicated a normal distribution (Shapiro-Wilk test: (W = 0.983), (p = 0.455)). The mean for EIVE.N was 4.77 (SD = 0.348) for 2022 and 4.82 (SD = 0.290) for 2024.

Lastly, for «EIVE.R», both the effects of «Year» (F (1, 55) = 0.124), (p = 0.727) and «Type» (F (2, 55) = 0.114), (p = 0.893) were not significant, as was the interaction effect (F (2, 55) = 0.056), (p = 0.946). The residuals exhibited a normal distribution (Shapiro-Wilk test: (W = 0.986), (p = 0.634)). The mean value for EIVE.R was 5.67 (SD = 0.246) for 2022 and 5.63 (SD = 0.239) for 2024.

When analyzing the dataset without land use changes (reduced data), similar ANOVA tests were performed to assess the effects of Year and Type on the dependent variables. For «EIVE.T», the «Year» effect was significant, (F (1, 43) = 5.825), (p = 0.020), and the «Type» effect was also significant, (F (2, 43) = 5.075), (p = 0.011), while the interaction between «Year» and «Type» was not significant, (F (2, 43) = 0.272), (p = 0.763). The residuals showed a normal distribution (Shapiro-Wilk test: (W = 0.983), (p = 0.563)). The mean for EIVE.T in this dataset was 3.98 (SD = 0.146) for 2022 and 4.01 (SD = 0.181) for 2024 (Figure 3).

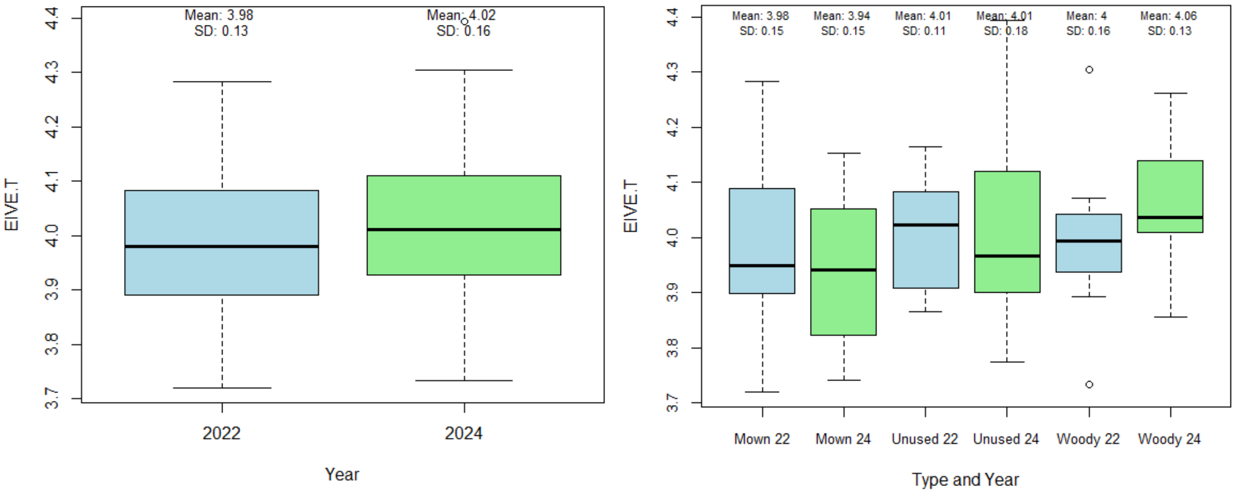

Figure 3. Boxplot of Ecological Indicator Values for Europe (EIVE.T) for the reduced dataset. The left plot illustrates the effect of Type and Year on EIVE.T, showing results for three types (Mown, Unused, Woody) in both 2022 (blue) and 2024 (green). The right pl.

For «EIVE.M», the «Year» effect was not significant, (F(1, 43) = 0.353), (p = 0.555), while «Type» was significant, (F(2, 43) = 4.670), (p = 0.015). The interaction was not significant, (F(2, 43) = 0.361), (p = 0.699).

The residuals again did not conform to a normal distribution (Shapiro-Wilk test: (W = 0.935), (p = 0.003)). The mean for EIVE.M was 5.03 (SD = 0.754) for 2022 and 5.04 (SD = 0.787) for 2024.

For «EIVE.L», the «Year» effect was not significant, (F (1, 43) = 0.012), (p = 0.912), while «Type» had a highly significant effect, (F (2, 43) = 46.144), (p < 0.001). The interaction effect was not significant, (F(2, 43) = 0.851), (p = 0.434). The residuals showed a normal distribution (Shapiro-Wilk test: (W = 0.986), (p = 0.741)). The mean value for EIVE.L was 6.82 (SD = 0.206) for 2022 and 6.82 (SD = 0.197) for 2024.

In the case of «EIVE.N», the «Year» effect was not significant, (F (1, 43) = 0.163), (p = 0.689), while «Type» was significant, (F (2, 43) = 5.047), (p = 0.011). The interaction was not significant, (F (2, 43) = 0.026), (p = 0.975). The residuals indicated a normal distribution (Shapiro-Wilk test: (W = 0.987), (p = 0.788)). The mean for EIVE.N was 4.77 (SD = 0.348) for 2022 and 4.82 (SD = 0.290) for 2024.

Finally, for «EIVE.R», the «Year» (F (1, 43) = 0.050), (p = 0.824) and «Type» (F (2, 43) = 0.373), (p = 0.691) effects were both non-significant, as was the interaction effect (F (2, 43) = 0.303), (p = 0.740). The residuals exhibited a normal distribution (Shapiro-Wilk test: (W = 0.986), (p = 0.744)). The mean value for EIVE.R was 5.67 (SD = 0.246) for 2022 and 5.63 (SD = 0.239) for 2024.

Biodiversity metrics

The analysis of biodiversity metrics covered species richness in three plot sizes (0.1 m², 1 m², and 10 m²), evenness, and the Shannon Index. Both datasets—one with land use changes and one excluding them—were analyzed for year and type effects.

For species richness in the smallest plot size (S.0.1), the two-way ANOVA indicated no significant year effect (p = 1.000 in the full dataset, p = 0.538 in the reduced dataset) or interaction effect (p = 0.712 and p = 0.723), but a significant effect for land use type (p < 0.001 and p < 0.001). In 2022, mean richness values were 12.9 (SD = 4.47) for Mown, 10.1 (SD = 3.42) for Unused, and 8.9 (SD = 3.94) for Woody, and in 2024, these values changed to 14.0 (SD = 5.80), 11.4 (SD = 4.96), and 8.4 (SD = 3.57), respectively. Normality of residuals was confirmed in both datasets (Shapiro-Wilk p-values > 0.05). Post-hoc tests revealed significant pairwise differences in the full dataset between Mown 2024 and Woody 2024 (p = 0.007), Mown 2024 and Mown 2022 (p = 0.046), and Mown 2024 and Unused 2022 (p = 0.015).

For species richness in medium-sized plots (S.1), the ANOVA showed no significant year effect (p = 0.927 and p = 0.867) or interaction effect (p = 0.232 and p = 0.391), but again a significant effect for type (p < 0.001 and p = 0.009). Mean richness values in 2022 were 18.7 (SD = 5.16) for Mown, 16.8 (SD = 5.04) for Unused, and 15.3 (SD = 4.92) for Woody, and in 2024, these values were 21.2 (SD = 8.14), 19.6 (SD = 7.06), and 15.9 (SD = 5.80), respectively. Residuals showed normality across both datasets. Post-hoc tests highlighted significant differences in the full dataset, with Mown 2024 differing from Mown 2022 (p = 0.041) and Unused 2022 (p = 0.067).

In the largest plot size (S.10), the year and type effects were not significant (p = 0.501 for year, p = 0.500 for type, and p = 0.092 for the interaction effect). Mean values for richness were consistent across years and types, and residuals were normally distributed. Post-hoc analysis did not reveal any significant pairwise differences.

For evenness (S.10.evenness), year had no significant effect (p = 0.370 and p = 0.246), but type had a significant effect (p = 0.003 and p = 0.005), with no interaction effect (p = 0.979 and p = 0.907). Mean evenness values in 2022 were 0.88 (SD = 0.04) for Mown, 0.83 (SD = 0.03) for Unused, and 0.81 (SD = 0.04) for Woody, with slight increases in 2024 to 0.90 (SD = 0.05), 0.84 (SD = 0.04), and 0.79 (SD = 0.05), respectively. Normality was confirmed, and post-hoc results showed significant differences between Mown and Woody types ($p = 0.046$).

Finally, for the Shannon Index (S.10.Shannon), no significant effect for year was found ($p = 0.376$ in the full dataset and $p = 0.512$ in the reduced dataset). Type was significant ($p < 0.001$ and $p = 0.005$), and interaction effects were non-significant ($p = 0.698$ and $p = 0.464$). Means for the Shannon Index in 2022 were 1.8 (SD = 0.127) for Mown, 1.5 (SD = 0.136) for Unused, and 1.2 (SD = 0.144) for Woody, with a slight increase in 2024. Normality of residuals was met, and post-hoc tests showed significant differences between Mown and Woody types ($p = 0.020$) and a near-significant trend between Mown 2024 and Woody 2022 ($p = 0.079$).

Overall, while no significant temporal effects on biodiversity metrics were observed, the significant influence of land use type on species richness, evenness, and the Shannon Index was evident across both datasets. These results emphasize that land use type, rather than temporal change, plays a crucial role in shaping biodiversity in this context.

Discussion

Species composition and turnover

The analysis of species composition changes between 2022 and 2024 revealed both methodological and ecological patterns across different management types. The overall species richness remained relatively stable (215 to 211 species), suggesting a general resilience of the vegetation communities. However, the detailed analysis revealed substantial dynamics in species composition, with a true ecological turnover of 24% after accounting for pseudoturnover.

The observed patterns varied markedly among management types. Mowed meadows showed an increase in species richness (128 to 138 species), indicating that regular mowing might promote species diversity by preventing competitive exclusion by dominant species, a pattern also observed by Dengler et al. (2014) in European grasslands. In contrast, unused areas experienced a decline in species numbers (140 to 122 species), possibly due to competitive exclusion and the accumulation of plant litter, which can inhibit seedling establishment (Ruprecht et al., 2010). This finding aligns with studies by Valkó et al. (2018) who documented similar species losses in abandoned grasslands.

The forest areas, despite showing the highest overall species richness (172 species), experienced the most pronounced decline (147 to 128 species). This reduction could reflect natural forest succession processes, where increasing canopy closure leads to the loss of light-demanding species, as documented by Verheyen et al. (2012) in temperate forests. The high turnover value (0.40) in forest areas reveals significant changes in plant species between 2022 and 2024. This observation is consistent with research by Holland et al. (1978), who showed that forest understory vegetation regularly undergoes natural changes. The changes likely result from plants competing for essential resources in the forest understory, such as light and nutrients.

The observed pseudoturnover rate of 20.7 % in our vegetation surveys aligns well with previously reported rates between 5 % and 25% (Boch et al., 2022). This analysis revealed three distinct components of pseudoturnover, each contributing to the overall methodological uncertainty in species identification and detection.

The temporal component (7.9 %) primarily reflects the challenges of species identification between survey years, particularly among morphologically similar species pairs. The confusion between Carex species pairs and Poaceae species (Agrostis, Poa) is a well-documented phenomenon in vegetation surveys, often attributed to seasonal variations in morphological characteristics and the complexity of vegetative identification (Morrison et al., 2020). This finding underscores the importance of consistent survey timing and the need for specialized expertise in identifying critical species groups.

The taxonomic component, representing the largest proportion (8.8 %), highlights the inherent difficulties in distinguishing between closely related species within certain genera. The high number of potentially confused species within Epilobium (5 species) and Galeopsis (4 species) genera reflects a common challenge in grassland surveys, where morphological similarities between relative can lead to identification uncertainties (Morrison, 2016).

The methodological component (4 %) represents the smallest but still significant contribution to pseudoturnover. The overlooked species, including small-growing plants and those with brief flowering periods, indicate the importance of survey timing and thoroughness. Like species such as Caltha palustris, with its short flowering period (Lauber et al., 2024).

Several limitations should be considered when interpreting these results. The two-year observation period may be too short to detect long-term vegetation trends, as highlighted by Kapfer et al. (2017) in their long-term vegetation studies. Additionally, annual variantions in weather conditions and phenology might influence species detection, and the establishment of replacement plots introduces some uncertainty in comparing temporal changes. Future research would benefit from longer observation periods to better understand succession patterns, as recommended by Chytrý et al. (2014) in their comprehensive review of vegetation monitoring methods.

Ecological Indicator Values for Europe (EIVE) trends

The analysis showed that there was a marginally significant effect of Year on EIVE.T, indicating a slight trend toward temperature change between 2022 and 2024. Although this could suggest potential shifts in environmental conditions, it is essential to recognize the limitations of a dataset that spans only two years. This limited timeframe is insufficient for making robust conclusions about long-term climate change. Instead, the observed changes may primarily reflect annual variability rather than a broader climate trend. As Tichý et al. (2023) highlighted, while EIVE scores are useful for tracking environmental changes, short-term variations should be interpreted with caution.

Short-term temperature fluctuations could be influenced by localized weather patterns or microclimatic changes, which are especially common in ecosystems such as semi-natural grasslands (Buzhdygan et al., 2020). Thus, these findings require careful interpretation. To truly assess climate change impacts, long-term monitoring is essential, ideally spanning multiple years or decades to distinguish natural interannual variability from sustained climatic trends.

In this context, the temperature shift observed in our study likely reflects standard year-to-year fluctuation rather than a definitive climate change signal. Despite this, such variability still plays a critical role in shaping plant communities. Even short-term temperature changes can affect species composition, phenology, and ecosystem functioning (Cleland et al., 2007). Continued monitoring over an extended period will be necessary to determine whether the trends identified here are part of a larger, more persistent pattern associated with climate change (Adler & Levine, 2007).

Biodiversity metrics

The analysis of biodiversity metrics, including species richness, evenness, and the Shannon Index, revealed no significant temporal changes between 2022 and 2024. These findings suggest that overall biodiversity in the studied semi-natural grasslands remained relatively stable over this short time frame. This is further supported by the non-significant results from ANOVA tests for species richness, evenness, and the Shannon Index. For instance, species richness did not differ significantly across years, regardless of plot size, indicating that short-term fluctuations in species composition were minimal. The mean values and standard deviations calculated for each metric also reinforce this stability over time.

The significant effect of land use type on biodiversity metrics, particularly on species richness and the Shannon Index, highlights the influence of land management practices on species diversity. Land use type consistently emerged as a key factor shaping biodiversity, aligning with expectations for semi-natural grasslands, where management intensity and habitat structure play crucial roles in determining species composition (Bengtsson et al., 2019).

Interestingly, while land use type had a clear impact, the lack of significant year-to-year differences across biodiversity metrics suggests that annual variability, such as weather conditions or short-term ecological changes, had less influence on biodiversity than management practices. This finding underscores the importance of long-term monitoring to detect more gradual changes that could relate to broader environmental shifts or climate change. For example, Wesche et al. (2012) documented substantial losses in species richness in Central European grasslands over a 50-year period, particularly in animal-pollinated plants, underscoring the importance of extended monitoring to reveal shifts in biodiversity that may not be apparent in short-term studies.

When replacement plots were excluded from the analysis, overall significance in some results diminished, likely due to the reduced sample size and potential biases introduced by plot modifications. However, the observed patterns suggest that land use remains the dominant factor affecting biodiversity, even when accounting for local plot disturbances or changes in land use type. Normality checks through Shapiro-Wilk tests were conducted on the residuals for each ANOVA, confirming normal distribution in most cases, supporting the reliability of the ANOVA results. The post-hoc analysis provided further detail on the effect of land use types, though differences between individual types were generally not statistically significant.

Methodological considerations and future directions

Our study provides valuable insights into the short-term dynamics of semi-natural grassland. The use of permanent plots and the consideration of observer error are strengths of our approach. However, the two-year time frame limits our ability to detect long-term trends. Future studies should aim for longer observation periods to capture gradual changes in plant communities and environmental conditions.

To summarise, our study provides a momentary record of the dynamics in the semi-natural grasslands of the Białowieża Forest region. The results emphasise the importance of land use practices for the composition and diversity of plant communities, while also highlighting the resilience of these ecosystems to short-term changes. Given the ongoing challenges of land-use change and climate change, continuous monitoring and adaptive management strategies are crucial for the conservation of these valuable ecosystems.

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Appendices

Appendix A: Species Turnover List

Species Turnover List. The list compares data for 2022 and 2024 for identical plots in triplets 1–4 and 6–13. Species that disappeared in 2024 are marked in red, and new species in 2024 are marked in yellow.

Species	**Frequency in 2022**		**Frequency in 2024**		**Total increase**	**Total decrease**	**Binom-test p-value**	**significance**
	Number of plots	**%**	**Number of plots**	**%**				
Acer negundo	1	2.8	1	2.8	0	0	1	
Acer platanoides	1	2.8	1	2.8	0	0		
Achillea millefolium aggr.	21	58.3	16	44.4	2	7	1	
Aegopodium podagraria	1	2.8	2	5.6	1	0	1	
Agrimonia eupatoria	2	5.6	2	5.6	1	1	1	
Agrimonia procera	16	44.4	15	41.7	2	3	1	

Species	**Frequency in 2022**		**Frequency in 2024**		**Total increase**	**Total decrease**	**Binom-test p-value**	**significance**
	Number of plots	**%**	**Number of plots**	**%**				
Agrostis canina	2	5.6	0	0.0	0	2	0.5000	
Agrostis capillaris	16	44.4	17	47.2	4	3	1	
Agrostis gigantea	6	16.7	11	30.6	7	2	1	
Agrostis stolonifera	1	2.8	2	5.6	2	1	1	
Ajuga reptans	1	2.8	1	2.8	0	0	1	
Alnus glutinosa	3	8.3	3	8.3	0	0		
Alopecurus geniculatus	3	8.3	0	0.0	0	3	0.1250	
Alopecurus pratensis	9	25.0	8	22.2	2	3	0.5000	
Angelica sylvestris	4	11.1	0	0.0	0	4	1	
Anthoxanthum odoratum	8	22.2	9	25.0	3	2	0.5000	
Anthriscus sylvestris	7	19.4	6	16.7	0	1	1	
Apera spica-venti	0	0.0	1	2.8	1	0	0.5000	
Arabis hirsuta	0	0.0	1	2.8	1	0	1	
Arenaria serpyllifolia	1	2.8	1	2.8	0	0	1	
Argentina anserina	8	22.2	8	22.2	1	1	1	
Arrhenatherum elatius	9	25.0	12	33.3	6	3	1	
Artemisia vulgaris	4	11.1	3	8.3	0	1	1	
Astragalus glycyphyllos	0	0.0	1	2.8	1	0	0.6250	
Avenula pubescens	5	13.9	5	13.9	3	3	1	
Betula pendula	9	25.0	9	25.0	4	4	0.3750	
Bistorta officinalis	1	2.8	1	2.8	1	1	1	
Briza media	4	11.1	3	8.3	1	2	1	
Bromus secalinus	1	2.8	0	0.0	0	1	1	
Calamagrostis canescens	2	5.6	4	11.1	3	1	0.5000	
Calamagrostis epigejos	2	5.6	3	8.3	1	0	0.5000	
Caltha palustris	2	5.6	0	0.0	0	2	0.5000	
Campanula patula	2	5.6	0	0.0	0	2	1	
Cardamine pratensis aggr.	5	13.9	1	2.8	0	4	1	
Carex ×elytroides	1	2.8	0	0.0	0	1	1	
Carex acuta	2	5.6	1	2.8	1	2	1	
Carex acutiformis	0	0.0	4	11.1	4	0	1	
Carex cespitosa	0	0.0	1	2.8	1	0	1	
Carex cuprina	2	5.6	0	0.0	0	2	1	
Carex disticha	3	8.3	0	0.0	0	3	1	
Carex echinata	3	8.3	0	0.0	0	3	1	
Carex elata	1	2.8	0	0.0	0	1		
Carex elongata	1	2.8	1	2.8	1	1	1	
Carex flava	5	13.9	1	2.8	0	4		
Carex hirta	20	55.6	18	50.0	1	3	0.5811	
Carex leporina	7	19.4	2	5.6	1	6	1	
Carex muricata aggr.	1	2.8	3	8.3	3	1	1	
Carex nigra	12	33.3	8	22.2	2	6	1	
Carex pallescens	6	16.7	0	0.0	0	6	0.5000	
Carex panicea	8	22.2	5	13.9	2	5	0.0313	*

Species	Frequency in 2022		Frequency in 2024		Total increase	Total decrease	Binom-test p-value	significance
	Number of plots	%	Number of plots	%				
Carex rostrata	2	5.6	4	11.1	3	1	0.4531	
Carex spicata	0	0.0	2	5.6	2	0	0.5000	
Carex sylvatica	0	0.0	2	5.6	2	0	0.5000	
Carex vesicaria	3	8.3	2	5.6	1	2	1	
Carex vulpina	0	0.0	1	2.8	1	0	1	
Carpinus betulus	6	16.7	4	11.1	0	2	0.2188	
Centaurea jacea	4	11.1	2	5.6	0	2	1	
Cerastium fontanum subsp. vulgare	10	27.8	5	13.9	1	5	0.6250	
Cerastium semidecandrum	1	2.8	2	5.6	1	0	1	
Chrysosplenium alternifolium	1	2.8	0	0.0	0	1	1	
Circaea lutetiana	1	2.8	1	2.8	0	0	1	
Cirsium arvense	7	19.4	6	16.7	1	2	1	
Cirsium palustre	4	11.1	6	16.7	5	3	0.5000	
Cirsium rivulare	16	44.4	11	30.6	1	6	0.5078	
Clinopodium vulgare	1	2.8	2	5.6	1	0	0.3750	
Comarum palustre	3	8.3	3	8.3	1	1	1	
Convolvulus arvensis	1	2.8	2	5.6	2	1	1	
Corylus avellana	1	2.8	1	2.8	0	0	0.1797	
Crataegus monogyna aggr.	3	8.3	3	8.3	0	0	0.2188	
Crepis biennis	1	2.8	0	0.0	0	1		
Crepis paludosa	1	2.8	0	0.0	0	1	1	
Dactylis glomerata	14	38.9	13	36.1	4	5	1	
Daucus carota	5	13.9	5	13.9	1	1	0.5000	
Deschampsia cespitosa	16	44.4	15	41.7	4	5		
Dryopteris filix-mas	1	2.8	2	5.6	1	0	0.1250	
Elytrigia repens	8	22.2	4	11.1	1	5	1	
Epilobium hirsutum	0	0.0	1	2.8	1	0	1	
Epilobium palustre	3	8.3	4	11.1	2	1	0.1250	
Epilobium parviflorum	1	2.8	0	0.0	0	1	0.0654	
Epilobium sp.	2	5.6	0	0.0	0	2	1	
Epipactis palustris	2	5.6	0	0.0	0	2		
Equisetum arvense	19	52.8	16	44.4	5	8	0.5000	
Equisetum fluviatile	3	8.3	4	11.1	1	0	1	
Equisetum palustre	9	25.0	6	16.7	2	5	0.4531	
Equisetum pratense	4	11.1	5	13.9	5	4	1	
Equisetum sylvaticum	3	8.3	1	2.8	0	2	1	
Erigeron annuus	2	5.6	6	16.7	4	0	1	
Erigeron canadensis	2	5.6	0	0.0	0	2	0.7266	
Eriophorum angustifolium	0	0.0	2	5.6	2	0	0.1250	
Euonymus verrucosus	1	2.8	0	0.0	0	1	1	
Fallopia convolvulus	2	5.6	1	2.8	0	1	0.4531	
Festuca rubra aggr.	18	50.0	19	52.8	6	5	0.2500	
Ficaria verna	1	2.8	1	2.8	1	1	0.3750	

Species	**Frequency in 2022**		**Frequency in 2024**		**Total increase**	**Total decrease**	**Binom-test p-value**	**significance**
	Number of plots	**%**	**Number of plots**	**%**				
Filipendula ulmaria	13	36.1	12	33.3	1	2	0.5000	
Fragaria vesca	3	8.3	3	8.3	2	2	0.0313	*
Frangula alnus	7	19.4	8	22.2	1	0	0.0313	*
Fraxinus excelsior	2	5.6	0	0.0	0	2	1	
Fraxinus pennsylvanica	0	0.0	2	5.6	2	0	1	
Galeopsis bifida	1	2.8	0	0.0	0	1	0.0156	*
Galeopsis pubescens	2	5.6	0	0.0	0	2	1	
Galeopsis speciosa	0	0.0	1	2.8	1	0	0.0078	**
Galium aparine	3	8.3	2	5.6	1	2		
Galium mollugo aggr.	7	19.4	7	19.4	2	2	1	
Galium palustre	10	27.8	8	22.2	1	3	1	
Galium rivale	2	5.6	3	8.3	2	1	0.2500	
Galium uliginosum	12	33.3	9	25.0	1	4	0.1250	
Galium verum	3	8.3	3	8.3	1	1	1	
Geranium robertianum	3	8.3	2	5.6	0	1	1	
Geum rivale	13	36.1	14	38.9	2	1	1	
Geum urbanum	3	8.3	1	2.8	0	2		
Glechoma hederacea	0	0.0	1	2.8	1	0		
Glyceria fluitans	0	0.0	1	2.8	1	0	0.2188	
Gnaphalium sylvaticum	2	5.6	2	5.6	1	1	1	
Helichrysum arenarium	1	2.8	1	2.8	0	0	0.5000	
Heracleum sphondylium	5	13.9	4	11.1	0	1	1	
Hieracium umbellatum	8	22.2	7	19.4	1	2	1	
Holcus lanatus	29	80.6	30	83.3	3	2	1	
Holcus mollis	1	2.8	1	2.8	1	1		
Hypericum ×desetangsii	1	2.8	0	0.0	0	1		
Hypericum maculatum	11	30.6	11	30.6	4	4	0.2500	
Hypericum perforatum	6	16.7	9	25.0	4	1	0.6250	
Hypochaeris radicata	4	11.1	5	13.9	2	1	1	
Impatiens noli-tangere	0	0.0	1	2.8	1	0	1	
Impatiens parviflora	1	2.8	1	2.8	0	0	1	
Inula britannica	0	0.0	1	2.8	1	0	1	
Jacobaea paludosa	1	2.8	2	5.6	1	0	0.5000	
Jacobaea vulgaris	3	8.3	1	2.8	0	2		
Juglans regia	1	2.8	1	2.8	0	0	0.6250	
Juncus articulatus	4	11.1	0	0.0	0	4	1	
Juncus compressus	1	2.8	0	0.0	0	1	1	
Juncus conglomeratus	6	16.7	1	2.8	1	6	1	
Juncus effusus	10	27.8	17	47.2	9	2	1	
Juncus inflexus	1	2.8	0	0.0	0	1	1	
Knautia arvensis	1	2.8	2	5.6	1	0	1	
Lamium galeobdolon	1	2.8	0	0.0	0	1	1	
Lathyrus pratensis	14	38.9	17	47.2	5	2	0.6250	
Leontodon hispidus	7	19.4	10	27.8	3	0	0.1250	

Species	**Frequency in 2022**		**Frequency in 2024**		**Total increase**	**Total decrease**	**Binom-test p-value**	**signifi cance**
	Number of plots	**%**	**Number of plots**	**%**				
Leucanthemum vulgare aggr.	5	13.9	8	22.2	4	1	0.1250	
Lolium perenne	0	0.0	2	5.6	2	0	1	
Lotus corniculatus	7	19.4	13	36.1	6	0	1	
Lotus pedunculatus	7	19.4	1	2.8	0	6	0.1797	
Luzula campestris aggr.	8	22.2	1	2.8	0	7	1	
Luzula multiflora	0	0.0	8	22.2	8	0	0.1797	
Lycopus europaeus	3	8.3	3	8.3	0	0	1	
Lysimachia nummularia	1	2.8	0	0.0	0	1	1	
Lysimachia thyrsiflora	1	2.8	4	11.1	3	0	1	
Lysimachia vulgaris	13	36.1	13	36.1	3	3	0.0078	**
Lythrum salicaria	12	33.3	11	30.6	0	1	1	
Maianthemum bifolium	1	2.8	2	5.6	1	0	1	
Malus sylvestris aggr.	1	2.8	1	2.8	0	0	0.7266	
Mentha arvensis	12	33.3	8	22.2	1	5	0.5000	
Myosotis arvensis	1	2.8	2	5.6	1	0	0.0313	*
Myosotis palustris aggr.	1	2.8	0	0.0	0	1	0.0490	*
Nardus stricta	1	2.8	2	5.6	1	0	1	
Orchidaceae sp.	1	2.8	0	0.0	0	1	1	
Parthenocissus quinquefolia	1	2.8	1	2.8	0	0	1	
Persicaria amphibia	1	2.8	1	2.8	0	0	1	
Peucedanum palustre	0	0.0	3	8.3	3	0	0.5000	
Phalaroides arundinacea	3	8.3	2	5.6	1	2	1	
Phleum pratense aggr.	15	41.7	15	41.7	5	5	1	
Phragmites australis	1	2.8	2	5.6	1	0	0.5000	
Picris hieracioides	0	0.0	2	5.6	2	0	1	
Pilosella caespitosa aggr.	0	0.0	1	2.8	1	0	1	
Pilosella cymosa aggr. s. l.	1	2.8	0	0.0	0	1	1	
Pilosella officinarum	2	5.6	3	8.3	1	0	1	
Pimpinella saxifraga aggr.	1	2.8	0	0.0	0	1	0.7266	
Pinus sylvestris	1	2.8	0	0.0	0	1	0.5000	
Plantago lanceolata	6	16.7	14	38.9	8	0	1	
Poa compressa	0	0.0	1	2.8	1	0	1	
Poa nemoralis	0	0.0	1	2.8	1	0		
Poa palustris	9	25.0	3	8.3	0	6	1	
Poa pratensis aggr.	19	52.8	10	27.8	4	13		
Poa trivialis	8	22.2	7	19.4	4	5	1	
Polygonum aviculare	0	0.0	1	2.8	1	0	1	
Populus tremula	2	5.6	3	8.3	2	1	1	
Potentilla argentea	1	2.8	0	0.0	0	1	0.3750	
Potentilla erecta	4	11.1	2	5.6	0	2	1	
Potentilla reptans	1	2.8	1	2.8	1	1	0.2891	
Prunella vulgaris	7	19.4	8	22.2	3	2		
Prunus avium	1	2.8	0	0.0	0	1	0.3877	
Prunus cerasifera	9	25.0	11	30.6	5	3	0.5000	

Species	**Frequency in 2022**		**Frequency in 2024**		**Total increase**	**Total decrease**	**Binom-test p-value**	**significance**
	Number of plots	**%**	**Number of plots**	**%**				
Prunus padus	1	2.8	3	8.3	2	0	1	
Prunus sp.	1	2.8	0	0.0	0	1	1	
Pyrola minor	1	2.8	1	2.8	0	0	1	
Pyrus communis	2	5.6	3	8.3	2	1	0.5000	
Pyrus communis aggr.	3	8.3	2	5.6	1	2	1	
Quercus robur	11	30.6	11	30.6	2	2		
Ranunculus acris	14	38.9	11	30.6	1	4	0.6250	
Ranunculus auricomus aggr.	8	22.2	4	11.1	2	6	1	
Ranunculus flammula	4	11.1	4	11.1	0	0	0.6875	
Ranunculus repens	17	47.2	13	36.1	4	8	0.2891	
Ribes nigrum	0	0.0	1	2.8	1	0	1	
Ribes rubrum aggr.	1	2.8	3	8.3	2	0	1	
Ribes uva-crispa	1	2.8	0	0.0	0	1	0.0010	***
Rubus caesius	1	2.8	1	2.8	0	0	0.2500	
Rubus idaeus	2	5.6	3	8.3	1	0	0.5000	
Rumex acetosa	13	36.1	2	5.6	0	11		
Rumex acetosella	0	0.0	2	5.6	2	0	1	
Rumex confertus	1	2.8	0	0.0	0	1	1	
Rumex crispus	0	0.0	1	2.8	1	0	1	
Rumex thyrsiflorus	3	8.3	16	44.4	13	0		
Salix aurita	1	2.8	2	5.6	1	0	0.0002	***
Salix caprea	3	8.3	2	5.6	2	3	1	
Salix cinerea	10	27.8	10	27.8	2	2	1	
Salix pentandra	2	5.6	3	8.3	2	1	1	
Schedonorus arundinaceus	0	0.0	1	2.8	1	0	1	
Schedonorus giganteus	0	0.0	1	2.8	1	0	1	
Schedonorus pratensis	6	16.7	8	22.2	5	3	1	
Scirpus sylvaticus	4	11.1	8	22.2	4	0	1	
Scutellaria galericulata	2	5.6	2	5.6	0	0	0.0625	
Selinum carvifolia	5	13.9	0	0.0	0	5	0.2500	
Setaria pumila	1	2.8	2	5.6	1	0	1	
Silene flos-cuculi	11	30.6	3	8.3	1	9	1	
Silene latifolia	1	2.8	1	2.8	0	0	1	
Solanum dulcamara	1	2.8	1	2.8	0	0	0.5000	
Solidago canadensis	3	8.3	3	8.3	0	0	1	
Solidago virgaurea	5	13.9	5	13.9	1	1	0.7266	
Stachys palustris	2	5.6	0	0.0	0	2	0.2188	
Stellaria graminea	23	63.9	16	44.4	1	8		
Stellaria media	0	0.0	3	8.3	3	0	0.2500	
Symphytum officinale aggr.	2	5.6	1	2.8	0	1	0.1250	
Tanacetum vulgare	3	8.3	3	8.3	0	0	1	
Taraxacum sect. Taraxacum	6	16.7	11	30.6	5	0	1	
Tilia cordata	2	5.6	1	2.8	0	1		
Torilis japonica	1	2.8	1	2.8	0	0	1	

Species	**Frequency in 2022**		**Frequency in 2024**		**Total increase**	**Total decrease**	**Binom-test p-value**	**significance**
	Number of plots	**%**	**Number of plots**	**%**				
Tragopogon pratensis	4	11.1	3	8.3	0	1	0.0625	
Trifolium arvense	1	2.8	1	2.8	0	0	1	
Trifolium campestre	0	0.0	1	2.8	1	0	1	
Trifolium hybridum	5	13.9	5	13.9	3	3	0.0215	*
Trifolium pratense	4	11.1	9	25.0	7	2	1	
Trifolium repens	11	30.6	7	19.4	1	5	0.5000	
Trisetum flavescens	2	5.6	0	0.0	0	2	1	
Urtica dioica	5	13.9	6	16.7	1	0		
Valeriana officinalis aggr.	4	11.1	2	5.6	1	3	0.5000	
Veronica arvensis	0	0.0	1	2.8	1	0		
Veronica chamaedrys	20	55.6	18	50.0	3	5	1	
Veronica longifolia	1	2.8	1	2.8	1	1		
Veronica serpyllifolia	1	2.8	0	0.0	0	1	1	
Vicia cracca	10	27.8	10	27.8	2	2	0.5000	
Vicia hirsuta	5	13.9	3	8.3	2	4	0.1250	
Vicia sativa subsp. nigra	4	11.1	1	2.8	0	3	0.2500	
Vicia sepium	1	2.8	6	16.7	5	0	0.1250	
Vicia tetrasperma	2	5.6	3	8.3	2	1	0.0391	*
Vicia villosa	0	0.0	2	5.6	2	0	1	
Viola canina	3	8.3	3	8.3	2	2	0.2500	
Viola palustris	7	19.4	6	16.7	3	4	1	
Viola reichenbachiana	1	2.8	0	0.0	0	1		
Viola riviniana	1	2.8	0	0.0	0	1	0.0625	

Project 2: Influence of land use and environmental conditions on vascular plant diversity in secondary grasslands of Bialowieza, Poland

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Abstract

This study investigates the influence of different land uses and environmental conditions on the diversity of vascular plants in secondary grasslands in Bialowieza, Poland. Specifically, it examines how parameters such as moisture and nutrient levels contribute to diversity indicators in these grasslands. Field surveys were carried out in 17 triplet plots, each characterising the mown, unmown and woody land uses. Each triplet consisted of three 10m² plots, each corresponding to one of the land use types (mown, unmown, woody encroachment). Within each 10m² plot, two smaller nested plots (1m² and 0.1m²) were sampled following the EDGG methodology. Our results show that land use has little effect on the biodiversity indicators, such as alpha diversity, Shannon index and evenness of secondary grasslands in Białowieża, but significantly influences beta diversity, with higher values in woody plots and lower values in mowed areas. Our findings do not support the intermediate disturbance hypothesis. Moisture and nitrogen levels were the primary drivers of biodiversity, with a unimodal relationship, highlighting the importance of environmental conditions over land management in secondary grasslands. These findings underscore the importance of environmental conditions, particularly moisture and nutrients, in shaping biodiversity in secondary grasslands, more so than land management practices alone.

Keywords

Grasslands, biodiversity, vascular plants, land use change, Bialowieza, species richness

Introduction

The biodiversity of ecosystems is a central focus of ecological research, as it directly influences ecosystem functions and services, which are crucial for both human well-being and environmental health (Cardinale et al., 2012; Hooper et al., 2012). Vascular plants play a key role in maintaining these functions by contributing to primary productivity, nutrient cycling, and habitat structure (Diaz et al., 2006; Tilman et al., 1997). Grasslands are spontaneously occurring herbaceous vegetation types, primarily dominated by grasses (Poaceae) or other graminoids (Cyperaceae, Juncaceae), with a relatively high herb-layer cover (typically over 10%). Woody species, such as dwarf shrubs, shrubs, and trees, if present, have much lower coverage compared to the herbs (Dengler et al., 2020). These habitats are among the most biodiverse ecosystems, hosting a wide range of vascular plant species, especially at small spatial scales (Wilson et al., 2012). However, the intensification of land use, environmental degradation and the abandonment of traditional land management practices are significant threats to grassland biodiversity across Europe (Dengler et al., 2014; Henle et al., 2008; Poschlod & WallisDeVries, 2002). The majority of grasslands in Eastern Europe are classified as secondary or semi-natural grasslands. Semi-natural grasslands are a crucial component of Europe's cultural landscape, shaped over centuries or even millennia of low-intensity land use, beginning with the Neolithic period. These grasslands are primarily utilized for livestock grazing (pastures) or for producing hay (meadows). Secondary grasslands are anthropogenic grasslands, which develop on formerly forested or cultivated land. They have become increasingly important for the conservation of vascular plant diversity, as they are greatly threatened by area and species loss. (Dengler et al., 2014, 2020; Török et al., 2020). Understanding the factors that influence plant diversity in these secondary grasslands is essential for developing effective conservation strategies, particularly in biodiversity hotspots like Bialowieza, Poland, and other areas of Eastern Europe (Mikusiński et al., 2018; Török et al., 2020).

Land use practices, such as grazing, mowing, and agricultural abandonment, along with environmental conditions like soil fertility, moisture, and climate, are recognized as key determinants of plant species composition and diversity in grasslands (Dengler et al., 2014; Helm et al., 2006; Pykälä, 2005). Numerous studies have shown that traditional land management practices, such as low-intensity grazing and periodic mowing, are critical for maintaining species richness in grasslands, as these practices prevent the dominance of a few competitive species and promote the coexistence of a wide range of plants (Dengler et al., 2020; Fischer et al., 2008). Plant species richness in herbaceous communities seem to peak at intermediate levels of management intensity (such as grazing, mowing, burning, or trampling), as a Study demonstrated through a trampling gradient in a European grassland (Grime, 1973). The abandonment of these practices often leads to a reduction in species diversity due to shrub encroachment and nutrient accumulation, which favour a smaller number of competitive species (Cramer et al., 2008; Dengler et al., 2014; Helm et al., 2006). In Poland, several phases of fallow land expansion have correlated with political and social factors, particularly since World War II and the political transformation between the late 1980's and early 1990's. As a result, many areas that were once grasslands have now become young forests. As this trend continues, there are also areas that have only recently been abandoned (Bomanowska & Kiedrzyński, 2011). Environmental factors such as soil pH, moisture, and light availability also play crucial roles in shaping plant community structures, with studies suggesting that land-use practices may affect these effects (Dengler et al., 2014). While these influences are generally well understood in primary grasslands (Dengler et al., 2014; Wilson et al., 2012), the dynamics of plant diversity in secondary grasslands, as they also occur in the areas of Bialowieza, are less explored (Dengler et al., 2014).

Despite substantial research on the effects of land use and environmental factors on grassland ecosystems, several knowledge gaps remain, particularly regarding the interactions between land use history and current environmental conditions in shaping vascular plant diversity in secondary grasslands (Dengler et al., 2020; Habel et al., 2013; Tälle et al., 2016). For example, the specific effects of different land management practices, such as rotational grazing or differences in mowing frequency, on plant diversity in secondary grasslands have not been fully clarified (Dengler et al., 2014; Tälle et al., 2016).

A critical unresolved question is whether secondary grasslands can fully replicate the biodiversity of primary grasslands under different management regimes (Tälle et al., 2016). Addressing these gaps is essential for informing conservation efforts and land use policies aimed at preserving plant diversity in grassland ecosystems (Dengler et al., 2020; Henle et al., 2008).

This study aims to investigate the influence of land use practices and environmental conditions on vascular plant diversity at three spatial scales in secondary grasslands of the Bialowieza region, Poland. The question we are addressing is whether different land use influences plant species richness, and whether this impact is dependent on spatial scale or a moisture gradient.

Methods

Study site

The study area was located around the village of Białowieża in the Podlaskie Voivodeship, Eastern Poland. The village of Białowieża is located within the boundaries of Białowieża National Park and is surrounded by its forest, though it is not officially part of the park itself. The park is renowned as the last remaining temperate lowland primeval forest in Europe and has been included on the UNESCO World Heritage List since 1979 (UNESCO World Heritage Center, n.d.). The climate in Białowieża National Park is characterised by average temperatures ranging from -6°C in winter to 23°C in summer. With an average altitude of 165 metres above sea level and a comparatively low annual precipitation of 392.7 mm, the region has a typical continental climate (*weatherspark.com*, n.d.).

Field sampling

Data was collected during the Summer School 2024, from August 16th to 27th. A total of 17 triplet-plots were surveyed, 13 of which were originally established in 2022, while four new triplet plots were first recorded during the Summer School 2024. The selection of the 17 triplet plots was done visually and subjectively from homogeneous grassland stands along the moisture gradient. Each triplet consists of three plots with different land uses: M = mowed grassland (mowed once or twice a year), U = unmowed grassland (abandoned about 5 years ago), and W = woody encroachment (abandoned about 30 years ago). The triplets consisted of three 10 m² (3.16 m x 3.16 m) plots, each marked with a magnet in the northeastern corner. GPS coordinates of the magnet were recorded to facilitate future surveys. The three plots, each representing a different land use, were located in close proximity to one another. The "EDGG Biodiversity Plots" method was applied for the establishment and sampling of the plots (Dengler et al., 2016). Sampling was conducted using a nested design with grain sizes of 0.1 m² and 1 m², located in the diagonally opposite southeastern corner from the magnet within each plot.

Map background – orthophotomap of Poland (source: mapy.geoportal.gov.pl)

Figure 1. The distribution of permanent triplets in the grasslands with three different land uses in Białowieża, Poland.

We recorded the presence and absence of vascular plants for all plot sizes, along with their cover, following the EDGG sampling methodology (Dengler et al., 2016). We recorded the cover percentage of various vegetation layers. This included the cover of trees (woody vegetation >5 m), shrubs (woody vegetation 0.5–5 m), and the herb layer (woody vegetation <0.5 m and herbaceous plants). The cover of each layer was measured as a percentage. In addition, the soil depth was also recorded according to the EDGG methodolgy (Dengler et al., 2016). In each 10 m^2 plot, soil samples were taken from five different spots within the top 10 cm of the soil to measure ist pH and electrical conductivity. The measurements were taken using a multi-parameter probe (HANNA Instruments HI991300, Woonsocket, Rhode Island, USA) in a mixture consisting of 10 g of the air-dried collected soil and 25 ml of distilled water. The moisture and nutrient levels for each plot were estimated using the EIVE system (Dengler et al., 2023). In addition to these parameters, the EIVE system also provided mean ecological indicator values for other environmental factors such as light, soil reaction, and temperature, enabling a comprehensive assessment of the ecological conditions within each plot. The Euro+Med PlantBase was used to standardise the nomenclature of the recorded species taxa, ensuring consistency and alignment with current taxonomic classifications.

Statistical analyses

All calculations were performed using the R programming language in version 4.4.0. Species richness (alpha diversity) was calculated as the number of species present for each plot and each grain size (0.1 m^2, 1 m^2 and 10 m^2). Beta diversity was determined as slope of alpha diversity in logarithmic scale, dependent on plot size. Shannon index was calculated only for the greatest grain size (10 m²) using *diversity* function from package *vegan* in version 2.6-8. Evenness was calculated as the Shannon index divided by logarithm of alpha diversity for the greatest grain size. ANOVA analysis was done with *aov* function from *stats* package. The diversity indicators were treated as dependent variables while management type, moisture levels and nitrogen levels were analysed as independent variables. The plot number was included as an error in the analysis. For the ANCOVA the same function was used, but the moisture and nitrogen values were treated as covariate in this analysis. The generalised linear mixed-effects models analysis was performed with the function *glmer* and *lmer* from the *lme4* library in version 1.1-35.5. The analyses were performed both with nitrogen and moisture as independent variables as well as with interaction between them and the land use type.

Results

Biodiversity Indicators

We assessed four different biodiversity indicators: alpha diversity, which was calculated for all three grain sizes, beta diversity, Shannon index and Evenness for the greatest grain size. The mean values for each management type can be seen in Table 1.

Table 1. Mean values of four different biodiversity indicators calculated for each type of management, for the 10m^2 plots. In brackets the value of standard error is given.

Management	Alpha diversity	Beta diversity	Shannon Index	Evenness
Mowed	25.71 (+/- 2)	0.17 (+/- 0.01)	2.14 (+/- 0.09)	0.67 (+/- 0.02)
Unmowed	27.12 (+/- 2.79)	0.2 (+/- 0.02)	2.04 (+/- 0.11)	0.64 (+/- 0.03)
Woody	26.53 (+/- 2.22)	0.29 (+/- 0.02)	1.86 (+/- 0.13)	0.58 (+/- 0.03)

Relationship between land use, moisture levels and biodiversity

In order to conduct ANOVA analysis, the moisture levels calculated using Ecological Indicator Values for Europe needed to be categorised. One category was given for each plot within one triplet. The EIVE values and corresponding moisture category for each triplet is shown in the Figure 2.

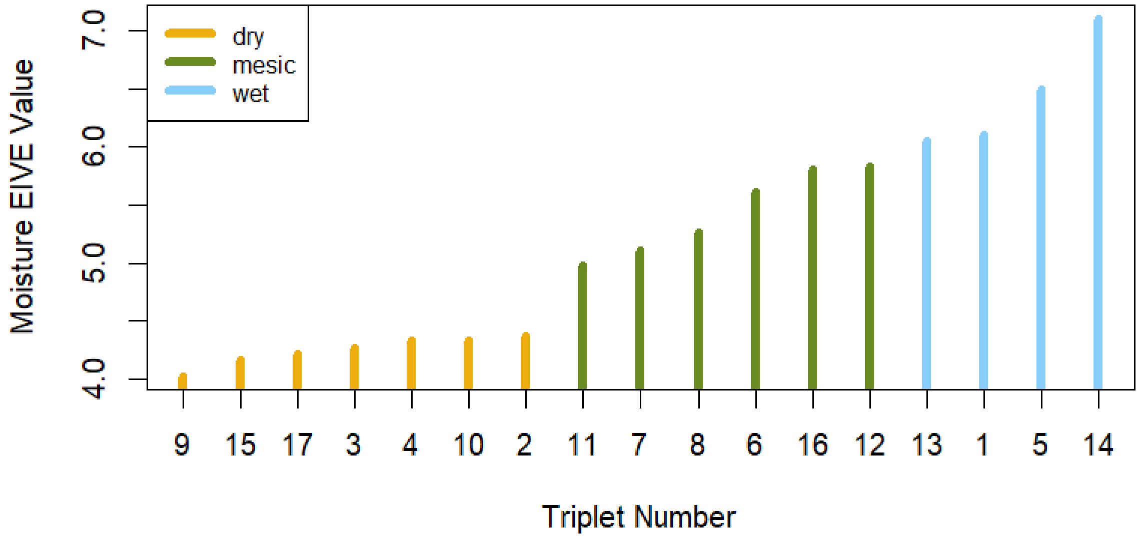

Figure 2. Moisture EIVE values for each triplet with assigned moisture category marked as different colours.

Both ANOVA and ANCOVA analysis suggested that management type had no effect on alpha diversity, but moisture was statistically significant in predicting the response variable. Same result was obtained for the generalised linear mixed-effects model - only moisture had any effect. Alpha diversity reached highest values for intermediate moisture values (mesic category), as is shown in the Figure 3.

For beta diversity however, only management type had a statistically significant effect in both ANOVA and ANCOVA analysis. In a generalised linear mixed-effects model an interaction was observed between moisture and woody and unowed management type. The differences between management types were statistically significant.

Only moisture levels affected the Shannon index for ANOVA and ANCOVA analysis, with the same results for the generalised linear mixed-effects model.

Not management type, nor moisture had a statistically significant effect on evenness in ANOVA and ANCOVA analysis. For generalised linear mixed-effect model only woody and mowed management types had statistically significant differences.

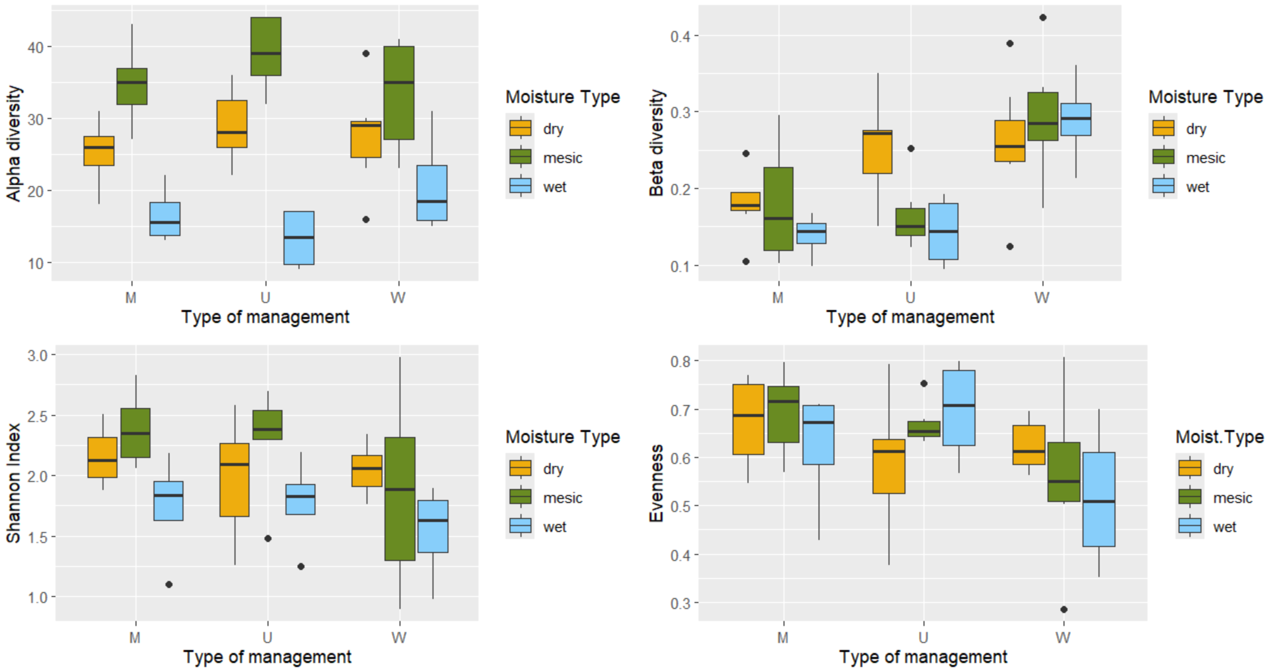

Figure 3. Values of four biodiversity indicators (alpha diversity, beta diversity, Shannon index and evenness) for each type of management (denoted as W - woody encroachment, M - mowed grassland, U - unmowed grassland) and moisture level.

Relationship between land use, nitrogen levels and biodiversity

As for the moisture values, for nitrogen levels a category needed to be assigned in order to conduct ANOVA analysis. The same category was given to each plot within a triplet. Figure 4 shows how the EIVE values for each triplet were assigned a nitrogen category.

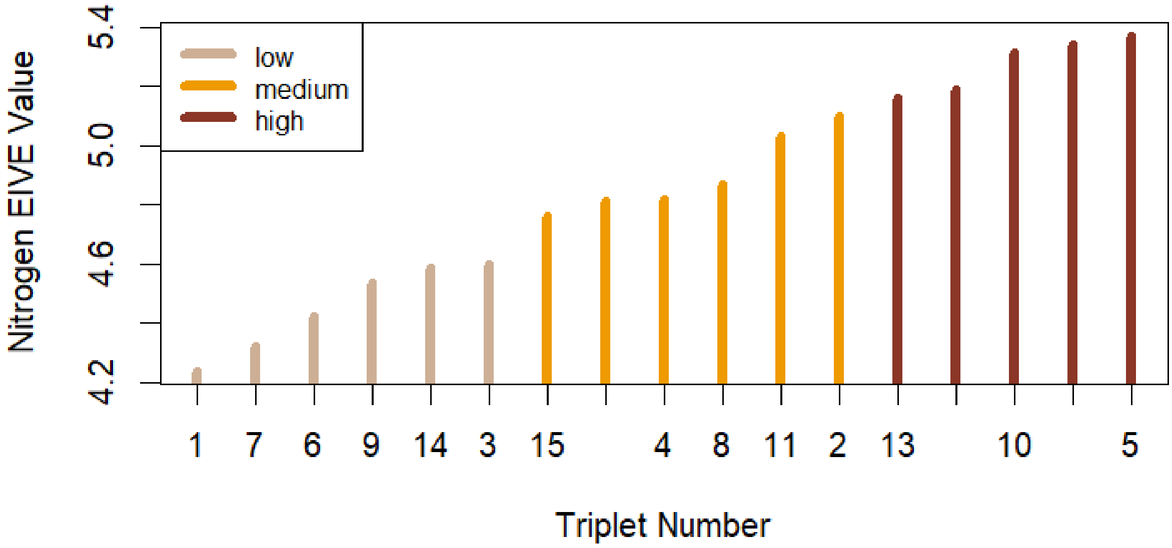

Figure 4. Nitrogen EIVE values for each triplet with assigned nitrogen level category marked as different colours.

Alpha diversity did depend on nitrogen levels for ANOVA and on nitrogen EIVE value for ANCOVA, but management type had no statistically significant effect on it. For the generalized linear mixed-effects model only nitrogen levels had an effect on the dependent variable.

As with moisture levels, beta diversity did not depend on nitrogen levels, but it depended on management type in both ANOVA and ANCOVA analysis. In the generalized linear mixed-effects model long-term abandonment (woody type) and unmowed management had statistically significant differences.

Shannon index was dependent on nitrogen levels, but not on management type for ANOVA and ANCOVA analysis. Generalised linear mixed effects model showed that nitrogen levels had an effect on Shannon Index.

Evenness did not depend on nitrogen levels nor on management type in ANOVA and ANCOVA analysis and in generalised linear mixed-effects models.

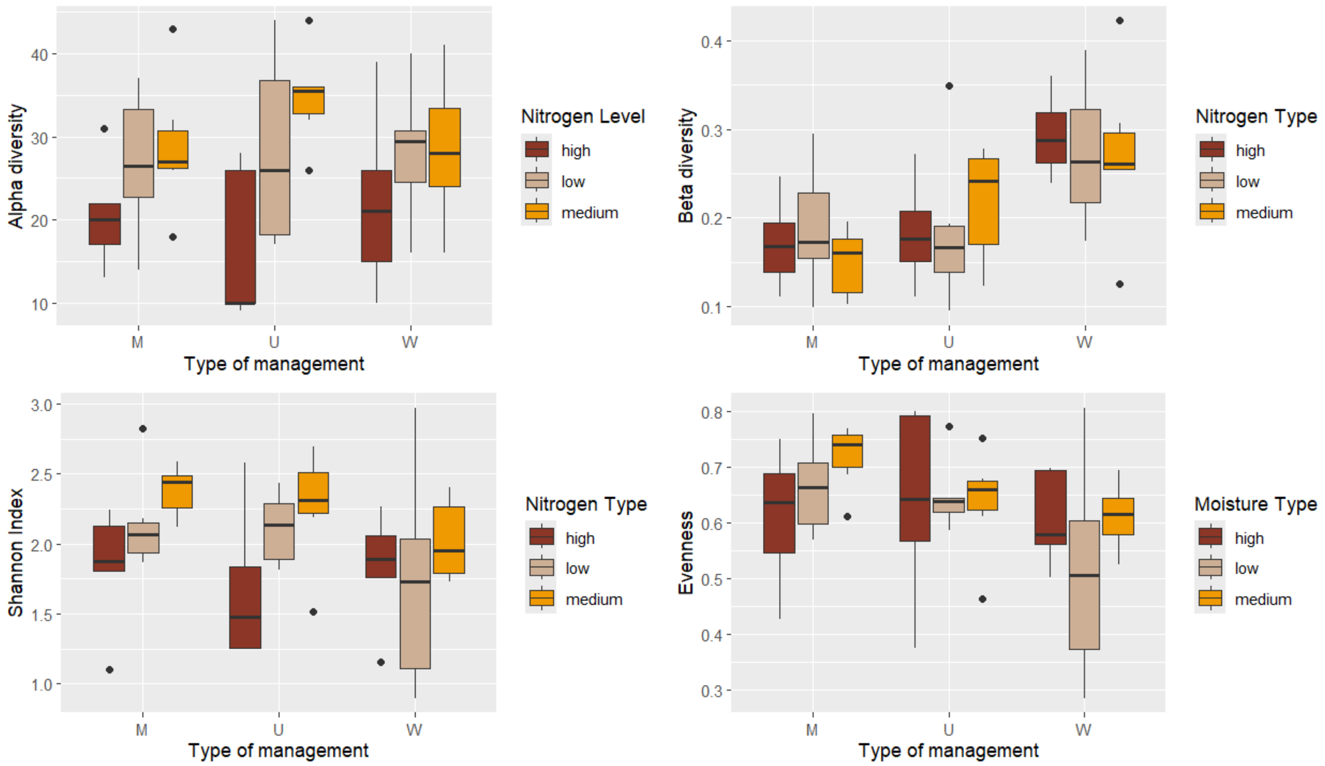

Figure 5. Values of four biodiversity indicators (Alpha diversity, beta diversity, Shannon index and Evenness) for each type of management (denoted as W - woody encroachment, M - mowed grassland, U - unmowed grassland) and nitrogen level.

Discussion

Results presented in our study support the relation between land use and beta diversity for secondary grasslands in Białowieża, however, no relation was observed for other biodiversity indicators – alpha diversity, Shannon index or evenness. Our findings are in line with results obtained by Dembicz et al., 2023, whose study was conducted in the same area and for the same plots. In order to achieve greater statistical power, we included additional 12 plots in our study. The beta diversity was the highest for woody plots, intermediate for unmowed plots and the lowest for mowed plots. Because alpha diversity was not dependent on management type, we can only conclude that for mowed plots rare species were more frequent than for woody (abandoned). Similar results were obtained for Molinion meadows in Poland, where abandonment and management had no significant effect on the number of species, but a relation was noted for species richness per sampling unit (Swacha, et al. 2018).

Our findings do not support the intermediate disturbance hypothesis which states that low levels of disturbance lead to low diversity through competitive exclusion, while intensive disturbance decreases the number of species that are unable to compete under high stress conditions (Conell, et al. 1978). Mowing can be classified as a non-selective type of disturbance – therefore we would expect that it has an effect on biodiversity indicators, especially alpha diversity, however that was not the case. In the previous report for Białowieża secondary grasslands (Dembicz & Dengler et al. 2023) the authors suggest that increasing the number of sample sites might help mitigate the influence of high variability between the plots – therefore we included additional plots that represent high and low moisture levels, to achieve more even moisture gradient between the studied sites. However, the intermediate disturbance hypothesis has been criticised for marginal support by empirical studies (Hughes, et al. 2007; Fox 2013). It could be argued that natural processes and variables that affect biodiversity indicators are numerous, thus often obscuring the effect of intermediate disturbance on diversity (Fox 2013). To see if other environmental parameters had any correlation with studied biodiversity indicators, we analysed them as covariates in our study. Only the moisture level and nitrogen level had a statistically significant effect on biodiversity indicators.

For nitrogen, the low and high values decreased alpha diversity, while intermediate values promoted it. Our results are consistent with previous observations – the increased levels of nitrogen cause higher accumulation of biomass, reducing the amount of light that is able to reach ground level and thus decreasing the number of less-competitive species (Hautier et al. 2009). However, the microrelief did not depend on nitrogen levels in our study. Others have also found that the relationship between nutrient levels and plant species richness reaches highest values for intermediate nutrient levels (Tilman, 1982). Our findings agree with conclusions from meta-analysis that looked at 189 studies of herbaceous communities (Soons et al. 2017).

Moisture levels had a statistically significant effect on biodiversity indicators such as alpha diversity and Shannon index. The highest biodiversity was again observed for plots with intermediate moisture levels, while the lowest – for plots with high moisture. Based on a study in the Loess Plateau in China (Wang et al., 2021), the greatest species diversity in the semi-arid steppes was found at a soil moisture content of 6–8%. This range was identified as a crucial threshold, where biodiversity reached its peak. When soil moisture dropped below this level, a trade-off occurred, with increased biodiversity resulting in a reduction of soil moisture. However, at moisture levels above 8%, the relationship between soil moisture and species diversity became more synergistic, suggesting that this moisture range is optimal for maximising biodiversity in these areas. A study by Smerdova et al., (2013), looked at grassland species alongside moisture gradient for sites located at calcium-rich part of the Western Carpathian flysch zone. Their observations suggest an unimodal relationship between moisture in the top part of the soil and species richness, which agrees with our results, where mesic grasslands had the highest alpha diversity. Moreover, Smerdova et al. notes that in higher moisture habitats the number of specialised species of vascular plants rises.

However, it should be acknowledged that our nitrogen levels and moisture levels were not measured directly but assessed using Ecological Indicator Values for Europe. This approach fails to capture precise parameters that were present at a given site. Moreover, because EIVEs are calculated based on species present and then averaged across the site, it can be argued that a correlation can be present between number of species and EIVE for a given site. This approach could be sensitive when a studied plot has a low number of species - presence of a single species has greater impact on the EIVE value and can shift it closer to one of the extremes. Therefore, in order to achieve more reliable values direct measurements of the parameters should be made. However, Diekmann, 2003, reports that indicator values can reliably predict real moisture levels, while nitrogen levels of the indicator values best correlate with biomass. Same author concludes that when conditions are homogenous within the plots, the environmental gradient is sufficiently large and if there is no sudden environmental shift, then the mean indicator values can be reliable. Moreover, Dengler et al. 2023, in their paper introducing the Ecological Indicator Values for Europe the authors point out that the regional EIVs and real environmental variables are in close relationship. Since the goal of this study was not to directly measure the nitrogen nor moisture levels but rather look for a relationship between those parameters and diversity indicators, then EIVEs should be sufficient.

Another criticism could be raised in regard to the sampling method itself. The species determination and abundance assessment was done by many different groups of students and researchers, with varying amounts of experience. The observer prone errors can be categorised into three types: overlooking error, misidentification error and estimation error (Morrison, 2016). Klimeš et. al. (2001), reports that for sampling grasslands, the error among observers reached 10-20% which was primarily attributed to misidentification. Moreover, the highest discrepancy was seen for the smallest plots (33%). Therefore, in order to achieve more reliable results and better capture the real world phenomena a smaller sampling team of more experienced researchers could be employed.

Conclusions

Our results demonstrate that land use has a significant effect on the beta diversity of secondary mesic grasslands, while other biodiversity indicators, such as alpha diversity, Shannon index and evenness were not affected. For beta diversity higher values were correlated with woody plots, while lower with mowed. Therefore, we can conclude that mowing allows more rare species present in the plot to become more frequent, possibly by non-selective reduction of biomass which in turn reduces competition for light. The phytocommunity of mowed plots was more homogenised, while abandonment promoted heterogeneity.

Acknowledgments

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List of AI tools used

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- Translate text passages

OpenAI. (2024). ChatGPT (Version 3.5). https://chatgpt.com/

- Phrasing and spelling help

Project 3: The influence of land abandonment on vegetative traits in grasslands

Nina Direnko, Joshua Gerteiser & Uliana Zakhariia

Abstract

It is assumed that plant functional diversity plays an important role regarding ecosystem goods and services. However, there is still a lack of understanding how plant species exactly adapt their traits to changes in their environment, especially also to changes in land use. There is a mosaic of still cultivated grasslands and those that have been abandoned over time in our study area around the village of Białowieża Poland. We posed the question of how flexibly different grassland species can adapt their vegetative traits to those changes in land use and whether they differ in their ability to adapt. We therefore sampled two grass- and four forb-species, measured the specimen’s height, leaf area and leaf length-width ratio and compared the results between mowed, recently abandoned (unmowed) and long term abandoned (woody) sites. Our results suggest that there is a considerable difference between rosette-forming species and erosulate species in terms of their vegetative traits mentioned above. While the rosette-forming species grew highest and formed the largest leaves on unmown areas overall, for the erosulate species this was the case on woody areas. Our results indicate that all species recorded are able to react to changing environmental conditions – light availability being the decisive factor – but are limited in their ability to adapt due to their physiological characteristics at the same time.

Keywords

Erosulate species, functional traits, functional diversity, land use, leaf area, leaf length, leaf width, rosette-forming species, vegetative traits

Introduction

The Białowieża Forest, a UNESCO World Heritage Site (World Heritage Convention UNESCO, 2014), is one of the last and largest remaining parts of the primary forest that once covered much of Europe. With its natural habitats, which are home to a wide variety of species, it is considered as a natural laboratory for biology, ecology and forestry (Jaroszewicz et al. 2019). Around the village of Białowieża there is a mosaic of differently cultivated grasslands. While some meadows are still used for agriculture today, others have been abandoned over the years. It is evident that changes in land use practices significantly influence species composition, which has even been extensively investigated in our study area (Adamowski W.& Bomanowska A. 2009; 2011). However, recent studies indicate that human disturbance also significantly alters plant functional diversity compared to natural habitats. This alteration goes in the direction of functional homogenization (Rosa et al. 2024). For example, rosette-forming species seem to have an advantage over erosulate species in regularly mowed grasslands (Janečková et al., 2017). At the same time, it is assumed that functional diversity plays an important role regarding ecosystem goods and services (Chapin Iii et al. 2000; Díaz et al. 2013; Lavorel S. 2013).

Interspecific variation in vegetative and reproductive plant phenology is assumed to be heavily influenced by the differences in their functional traits (Sporbert et al. 2022). The ability of a species to adapt its traits to changing environmental conditions can therefore be decisive for its survival.

While the general idea behind plant’s functional traits is widely confirmed (Sing S. & Verma A. 2020), there is still a lack of in-depth understanding of the exact processes. Many models developed to date attempt to describe the reactions of plants to changing environmental conditions. There are indications of the great importance of leaf construction and growth potential, between which a trade-off must be made (Díaz et al. 2016). A lot of models combine vegetative and reproductive traits, such as the often cited LHS scheme, which explains relationships between specific leaf area SLA, height of the plant’s canopy and seed mass (Westoby M. 1998).

In our study we focus on vegetative traits only, working with six frequently occurring species in the region - *Dactylis glomerata, Holcus lanatus, Hypericum perforatum, Hypericum maculatum, Veronica chamaedrys*, and *Hieracium umbellatum*. We aimed to assess how their plant height, leaf area, leaf length and leaf width vary in three different types of land use: cultivated (mowed), recently abandoned (unmowed) and long-term abandoned (woody) areas to identify potential implications for plant competition and ecosystem functioning. We posed the question of how flexibly the species studied can adapt their vegetative traits to changes in land use and whether the species surveyed differ in their ability to adapt.

Methods

Study area

Between August 19 and 25 2024 we carried out our surveys in the vicinity of Białowieża in eastern Poland. We selected a total of four different sites with mesic soil conditions and pH values between 5 and 7. To ensure comparability between differently managed areas, the sites had to include mown (M) areas that are still managed, unmown (U) areas that have recently been abandoned, and woody (W) areas that were abandoned some time ago. While the mowed and unmowed areas consisted of a herb layer only, the woody areas were composed of additional shrub (0.5m - 5m) and tree (>5m) layers which were largely composed of *Betula pendula* and occasionally other species such as *Quercus robur* and *Prunus cerasifera*. To ensure that the ten targeted samples per species could be achieved, each area was screened as a whole. As *Dactylis glomerata*, only one of two recorded grasses, had a low abundance in the recorded plots, it was additionally sampled at a further site "11+" close to triplet "11". This resulted in a total of five triplets (Fig. 1).

Figure 1. Location of the analysed plots (outlined in red) in the vicinity of Białowieża.

Field sampling and measurements

For species selection, we followed the recommendations of (Cornelissen et al. 2003). We selected the most frequently occurring species. Based on previously recorded vegetation surveys, we were able to determine which species were to be found in most plots. Phanerophytes were excluded. In addition, we wanted to survey both grasses and forbs. We therefore collected *Dactylis glomerata, Holcus lanatus, Hypericum perforatum, Hypericum maculatum, Veronica chamaedrys* and *Hieracium umbellatum*. Where possible, we recorded ten individuals per species and plot. The plant height and the length, width and area of one leaf each were measured. For forbs, the absolute length was measured in the extended state, for grasses we measured the height above the ground of the vegetative plant parts (not extended state). For the leaf measurements, average-sized and healthy leaves were chosen - approximately in the middle of the stem in the case of forbs. While the leaf length and width were measured manually, we determined the leaf area digitally by scanning the leaves and measuring them using the program ImageJ (Rasband W. 2024).

Figure 2. Three leaf samples of *Holcus lanatus* as an example of our collected leaves which were scanned and measured digitally.

Statistical analyses

For the statistical analyses, we used the software R (v4.3.1, (R Core Team 2023)). To compare each functional trait between different land use types (M, U, W) we performed an ANOVA for each species and each functional trait (significance level: p value < 0.05). We used all the collected data from the plots mentioned above (measured in cm) and performed a log-transformation. We checked the model diagnostics visually using Residuals vs. Fitted Plots, Normal Q-Q Plots, Scale-Location Plots and Constant Leverage Plots (Residuals vs. Factor Levels) and compared the results with the untransformed data to ensure the improvement. In cases of significant results, we performed Tukey's post-hoc test (package: agricolae (De Mendiburu, F. 2023)). We visualised the results of Tukey's post-hoc test by creating homogeneous groups (A, B, C).

Results

Plant height

Significant differences in plant height were found between the land use types for all species except *Holcus lanatus*. The pattern differs for *Dactylis glomerata* and the forbs. *Dactylis glomerata* was significantly higher in unmowed areas than in mowed and woody areas. This tendency can also be observed for *Holcus lanatus*, but not significantly. Meanwhile, all the forbs were significantly smaller in mowed areas than in the other two land use types. *Veronica chamaedrys* and *Hypericum perforatum* were also significantly higher in woody plots than in unmowed plots. This tendency (not significantly) can also be observed for the other forbs.

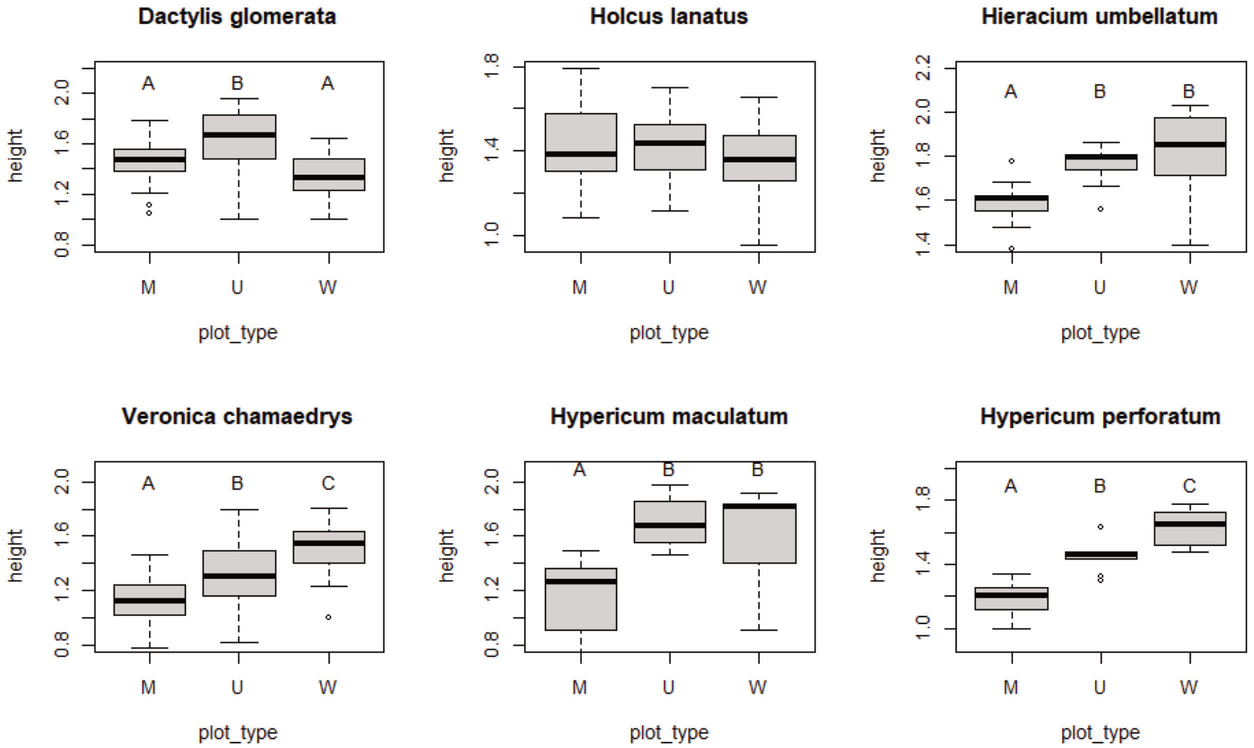

Figure 3. Plant height (logarithmic) of the different species differentiated according to land use (mowed, unmowed and woody) across all plots surveyed.

Table 1. Plant height (logarithmic) ANOVA (F = F statistic, df = degrees of freedom, p = p value) and Tukey's post-hoc test with M = mowed, U = unmowed and W = woody (p = value); green = significant results with p value <0.05.

Species	ANOVA	M - U	M - W	U - W
D. glomerata (n = 90)	F = 19.38, df = 2, p = $1.094e^{-7}$	p = 0.0006	p = 0.0644	p = 0.0000
H. lanatus (n = 120)	F = 2.54, df = 2, p = 0.08281	-	-	-
H. umebellatum (n = 54)	F = 21.43, df = 2, p = $1.761e^{-7}$	p = 0.0000	p = 0.0000	p = 0.2633
V. chamaedrys (n = 120)	F = 40.2, df = 2, p = $5.156e^{-14}$	p = 0.0000	p = 0.0000	p = $1e^{-4}$
H. maculatum (n = 77)	F = 37.96, df = 2, p = $4.518e^{-12}$	p = 0.0000	p = 0.0000	p = 0.4727
H. perforatum (n = 30)	F = 47.25, df = 2, p = $1.518e^{-9}$	p = 0.0000	p = 0.0000	p = 0.0013

Leaf area

Only *Dactylis glomerata*, *Holcus lanatus*, *Hieracium umbellatum* and *Veronica chamaedrys* showed significant differences in leaf area depending on the different land use types. The leaves of *Dactylis glomerata* were significantly larger in unmowed plots than in mowed plots and woody plots, while those of *Holcus lanatus* were only significantly larger in unmowed plots compared to mowed plots. The leaves of *Hieracium umbellatum* and *Veronica chamaedrys*, on the other hand, were significantly larger on woody plots.

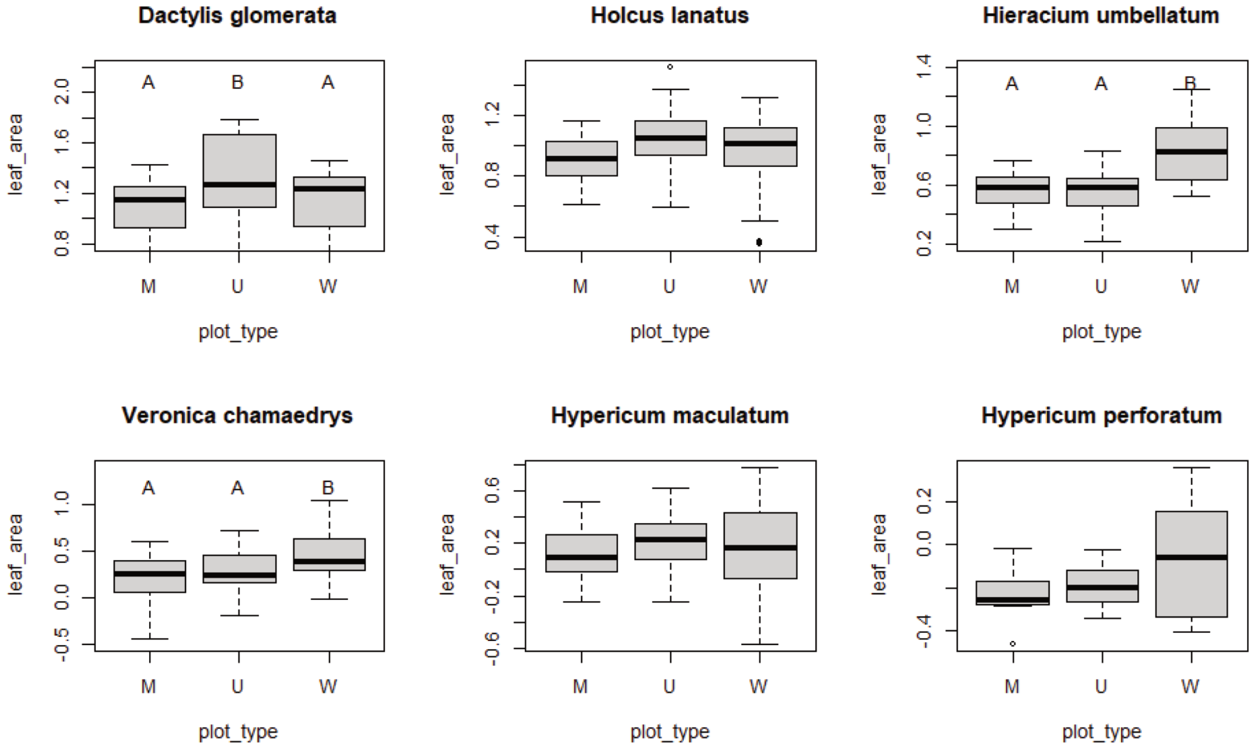

Figure 4. Leaf area (logarithmic) of the different species differentiated according to land use (mowed, unmowed and woody) across all plots surveyed.

Table 2. Leaf area (logarithmic) ANOVA (F = F statistic, df = degrees of freedom, p = p value) and Tukey's post-hoc test with M = mowed, U = unmowed and W = woody (p = value); green = significant results with p value <0.05.

Species	**ANOVA**	**M - U**	**M - W**	**U - W**
D. glomerata (n = 90)	F = 5.86, df = 2, p = 0.004041	p = 0.0060	p = 0.8996	p = 0.0210
H. lanatus (n = 120)	F = 4.353, df = 2, p = 0.01502	p = 0.0114	p = 0.4893	p = 0.1796
H. umebellatum (n = 60)	F = 17.31, df = 2, p = $1.332e^{-6}$	p = 0.9982	p = 0.0000	p = 0.0000
V. chamaedrys (n = 120)	F = 11.09, df = 2, p = $3.884e^{-5}$	p = 0.3195	p = 0.0000	p = 0.0057
H. maculatum (n = 84)	F = 0.7675, df = 2, p = 0.4675	-	-	-
H. perforatum (n = 30)	F = 1.832, df = 2, p = 0.1795	-	-	-

Leaf length / leaf width ratio

Only *Dactylis glomerata*, *Hieracium umbellatum* and *Hypericum perforatum* showed significant differences in leaf ratio depending on the different land use types. *Dactylis glomerata* and *Hieracium umbellatum* showed significantly higher leaf ratios in woody plots, which means that the leaves were more elongated in shape. In addition, the leaves of *Hieracium umbellatum* had a significantly higher ratio in the unmowed plots than in the mowed plots. This effect could not be detected for *Dactylis glomerata*. Meanwhile *Hypericum perforatum*'s leaf ratio was significantly lower in mowed plots compared to unmowed plots, but not compared to woody plots.

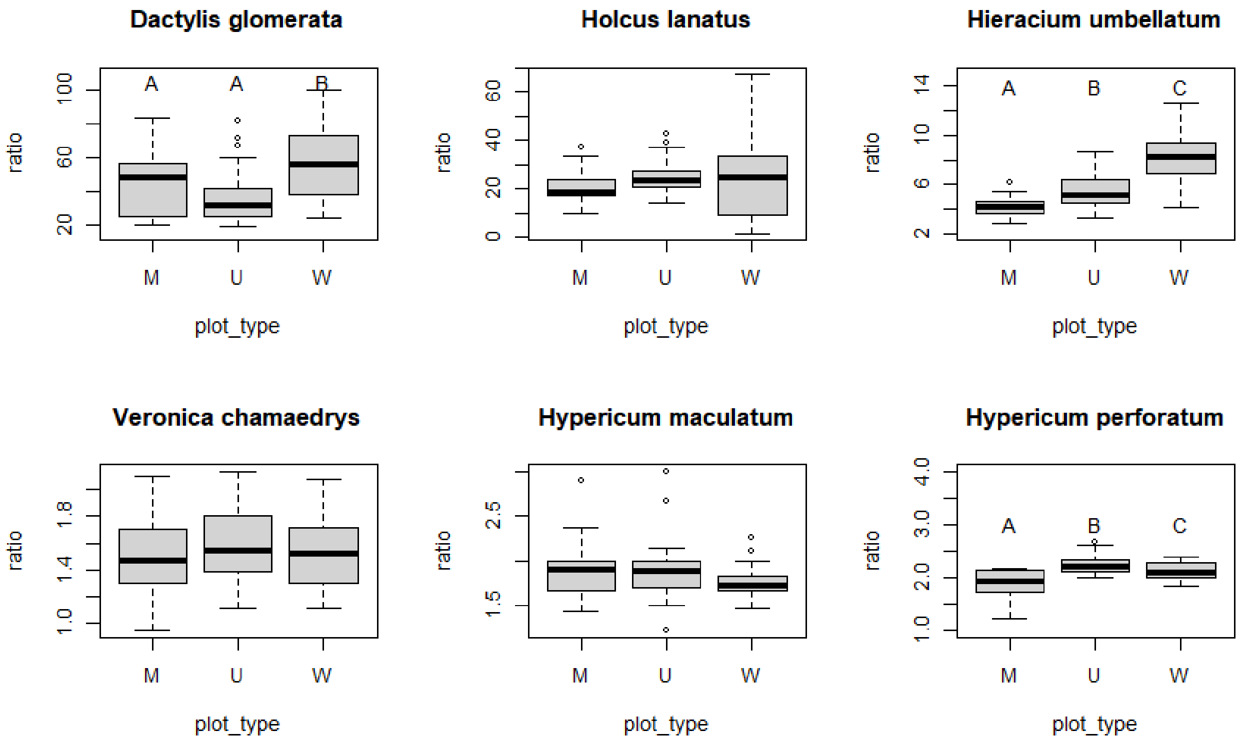

Figure 5. Leaf length / leaf width ratio (logarithmic) of the different species differentiated according to land use (mowed, unmowed and woody) across all plots surveyed.

Table 3. Leaf length / leaf width ratio (logarithmic) ANOVA (F = F statistic, df = degrees of freedom, p = p value) and Tukey's post-hoc test with M = mowed, U = unmowed and W = woody (p = value); green = significant results with p value <0.05.

Species	**ANOVA**	**M - U**	**M - W**	**U - W**
D. glomerata (n = 90)	F = 8.447, df = 2, p = 0.0004441	p = 0.2289	p = 0.446	p = 0.0003
H. lanatus (n = 120)	F = 1.771, df = 2, p = 0.1748	-	-	-
H. umebellatum (n = 60)	F = 33.64, df = 2, p = $2.247e^{-10}$	p = 0.0388	p = 0.0000	p = 0.0000
V. chamaedrys (n = 120)	F = 1.08, df = 2, p = 0.3428	-	-	-
H. maculatum (n = 84)	F = 1.96, df = 2, p = 0.1475	-	-	-
H. perforatum (n = 30)	F = 6.435, df = 2, p = 0.005185	p = 0.0042	p = 0.0717	p = 0.4498

Discussion

Our study shows that land use has a significant influence on plant height and leaf construction of the species surveyed. It was shown that *Dactylis glomerata* grew tallest in unmowed areas, an effect that was also observed to a non-significant extent for *Holcus lanatus*. Meanwhile, all forbs were higher in the unmowed areas than in the mowed areas as well, but even higher in the woody areas. This effect was observed for all species, but only to a significant extent for some of them. It was predictable that all species grew higher in unmowed areas than in mowed areas, as their growth was not disturbed by mowing. Those findings are consistent with previous studies (Lindborg R. & Eriksson O. 2005). The fact that the forbs grew even higher in woody areas unlike the grasses is probably due to their physiology and our measuring method. While *Dactylis glomerata* and *Holcus lanatus* form their leaves in basal rosettes, the forbs recorded are species of the erosulate growth type. They are able to form a longer stem so that the leaves can be formed at a higher level when light availability is low.

Dactylis glomerata and *Holcus lanatus* also produced the largest leaves in unmowed areas, although the difference between woody areas and unmowed areas was not significant for *Holcus lanatus*. Meanwhile, the leaves of *Hiercium umbellatum* and *Veronica chamaedrys* were largest in woody areas (also for both *Hypericum sp*., but not significantly). A possible explanation here would also be the physiological difference between the two rosette-forming and the remaining erosulate species. While the erosulate species can do without leaves near the ground and invest more energy in the remaining leaves in the upper part, the rosette-forming species are denied this possibility.

For *Dactylis glomerata*, leaves with significantly higher length/width ratio (elongated leaves) could be detected in woody areas (also for *Holcus lanatus*, but not significantly), presumably because the plant height is linked to the length of the leaves due to rosette formation. The only other species for which this effect was detected as well was *Hieracium umbellatum*. Compared to the other forbs, *Hieracium umbellatum* has rather lanceolate leaves. Another possible explanation would therefore be that species with more lanceolate leaves invest increasingly in leaf length with increasing leaf growth.

In grassland areas, the availability of light is probably the decisive environmental factor to which plants must adapt to through changes in land use (Opdekamp et al., 2012). Unsurprisingly, our results indicate that all species can react - albeit in different ways - to those changing environmental conditions by altering their traits. However, they appear to be physiologically limited. There seems to be a considerable difference between rosette-forming species and erosulate species. While the erosulate species develop a longer stem under reduced light availability and can therefore invest a larger part of their energy specifically in the higher leaves, rosette-forming species must invest in longer leaves to gain height, although these are presumably less efficient near the ground. However, as our study was limited to a few species, further studies would be necessary to substantiate this claim. Furthermore, as we did not record the species' abundance, no conclusions can be drawn on the importance of this adaptability for the competitiveness of the species.

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Project 4: Drivers of *Orthoptera* assemblages: the role of soil moisture and herb diversity across varied grassland habitats

Sharon Lustenberger, Miriam Steinhauer & Patryk Werner

Abstract

Białowieża, located in the Podlaskie Voivodeship of Poland, has experienced significant landscape alterations due to changes in agricultural practices and human intervention. This study aims to analyse the impact of different grassland management practices on Orthoptera species abundance and richness in the grasslands surrounding Białowieża National Park. Field sampling was conducted in fifteen triplets of 10 x 10 m plots representing three management types: intermittently mowed, recently abandoned (unmowed), and long-term abandoned (woody). A total of 826 Orthoptera individuals belonging to 21 species were collected using a combination of sweep netting and container methods. Statistical analysis involved generalized linear mixed models (GLMMs) to assess the effects of management practices and environmental variables on Orthoptera populations. Results revealed that Orthoptera abundance was highest in mowed plots, followed by unmowed and woody plots. Species richness was similar in mowed and unmowed plots yet differed significantly in woody plots. No significant effect of soil moisture on species richness or abundance was found. On the other hand, a positive relationship between plant diversity and Orthoptera species richness and abundance was observed. Therefore, regularly mowed plots support higher Orthoptera abundance, while plant diversity enhances species richness and abundance. These findings suggest that complex plant communities offer microhabitats and resources that support Orthoptera population diversity.

Keywords

Orthoptera, meadows, Białowieża, grasslands, soil moisture relation, herb diversity

Introduction

Grasslands, forming 20 - 40 % of the world's land area, are among the most agriculturally useful habitats (Nunez, 2023). Grasses are the naturally dominant vegetation in this type of biome, and they occur naturally where there is not enough precipitation for the growth of a forest but enough for there not to be a desert. Depending on factors such as temperature, humidity, soil moisture and fertility, grasslands can be divided into mesic, dry, wet, mountain grasslands, and agricultural meadows and pastures (Nunez, 2023). Grasslands are one of the most diverse ecosystems in Europe, they can host over eighty plant species per square metre, as well as a high diversity of small insects, birds, rodents, large herbivores and other animals (BISE, 2021). Poland is home to primarily semi-natural grasslands and managed meadows. Conserving semi-natural lowland and mesic meadows largely depends on treatments such as mowing and grazing livestock (Meserszmit et al., 2022). Nowadays, the condition of the grasslands is deteriorating mainly due to agricultural intensification, as well as complete land-use abandonment (Fumy et al., 2021; Meserszmit et al., 2022). These land-use changes shift plant communities of natural and semi-natural grasslands (Geppert et al., 2021). The reduced species richness of plants affects many trophic levels and leads to the decline of microbial biomass, fungal abundance, and species richness of arthropods, especially that of herbivorous insects (Chisté et al., 2016).

Orthoptera are essential to the functioning of grassland ecosystems due to their dual role as herbivores and prey (Fumy et al., 2021). They can also reflect microclimatic conditions and the naturalness of ecosystems (Stefanidis et al., 2023). The character of land use strongly influences the microclimate and the vertical and horizontal structure of vegetation, affecting orthopteran assemblages (Geppert et al., 2021; Gutiérrez et al., 2020; Kenyeres et al., 2020; Popa, 2020; Stefanidis et al., 2023). Orthopterans, with their complex habitat requirements, respond quickly to environmental changes, disturbance and habitat restoration (Fumy et al., 2021; Kenyeres et al., 2020). Both terricolous and phytophilous species profit from uneven vegetation height and density, which provide diverse habitat possibilities (Stefanidis et al., 2023). Bare ground is also vital for Orthoptera, serving as basking and courtship ground (Stefanidis et al., 2023). Furthermore, abiotic factors, such as temperature, humidity and soil moisture also determine the habitat quality and can limit Orthoptera generations and their abundance (Popa, 2020). Soil moisture significantly impacts reproductive success, food availability, microclimate conditions and predator avoidance (Brandon & Oswald, 2009; Fartmann et al., 2012; Ingrisch & Köhler, 1998; Kenyeres et al., 2020). For these reasons, Orthoptera have been widely used as indicator species to investigate the effects of land-use changes (Bazelet & Samways, 2011; Fartmann et al., 2012; Fumy et al., 2021; Löffler et al., 2019). Research in temperate grasslands reveals that intensive grazing reduced orthopteran species richness and Shannon diversity, and mowing strongly reduced grasshopper densities (Chisté et al., 2016). Intensively used meadows, which are mowed up to three times during Orthoptera season (May-September), lead to a substantial loss of biomass and population (Stefanidis et al., 2023). Therefore, the impact of land use on Orthoptera diversity and abundance is driven by the intensity of its management.

In this study, we analysed the effects of land-use intensity on orthopteran richness and diversity, focusing on different maintenance types: mowed, unmowed, and woody (abandoned). In addition to assessing the response of orthopteran assemblages to land use practices, we also examined their response to variations in soil moisture and herbal diversity. Our investigation sought to address the following research questions:

- How does land-use intensity influence the Orthoptera species richness and abundance?
- How does soil moisture influence the species richness and abundance of Orthoptera in mowed, unmowed, and woody habitats?
- Is Orthoptera species richness and abundance correlated with the species richness and abundance of herbs within their habitat?

Methods

Study site

The research area was situated in the vicinity of Białowieża, located in the Podlaskie Voivodeship, Powiat Hajnowski, Poland. Białowieża serves as the entrance to the Białowieża National Park, which is part of the Białowieża Primeval Forest, recognized as the last remaining temperate lowland primeval forest in Europe. However, Białowieża itself is surrounded by meadows and grasslands. Human interventions prevented the natural succession of forests, maintaining the open habitats as fields. However, around 1980's, there was a decline in agricultural activity around the village of Białowieża. The proportion of fields in the area dropped from 56% in 1950 to just 5% by 2005. Some of the fields were developed into the meadows and grasslands seen today, because it was profitable. As a result, the Białowieża landscape has transformed into a mosaic of mowed and abandoned meadows at various stages of secondary succession, alongside tree plantations and fields (Adamowski & Bomanowska, 2011). Over time, these areas became an integral part of the region's landscape.

Field sampling

Sampling was performed in permanent plot triplets, located around (52° 42′ 14″ N, 23° 51′ 9″ E) Białowieża on grassland with different types of maintenance: intermittently being *mowed (m)*, recently abandoned (*unmowed = u*) and long-term abandoned, overgrown woody shrubs that are shifting into forest habitat (*woody = w*). A total of fifteen triplets were sampled for Orthoptera in the study area. They consisted of 45 plots (10 x 10 m), of which 30 were permanent and established by the Summer School in 2022 and 15 were established by the Summer School in 2024. There were 2 triplets (Nr. 5, 15) not sampled this year due to limited time and planning problems (Figure 1).

Recording abundance of Orthoptera was performed differently than in 2022 sampling edition due to some important factors. Thanks to the previous experience and data that teachers provided we were able to tailor the most suitable method of catching. In the previous sampling, it was evaluated that the use of a sweep net requires a certain level of experience, which the students lacked, thus they decided to use plastic containers for catching. They laid out a 50 m tape measure on the sides of the plot to mark its boundaries. Then they positioned themselves on the same side of the plot to be sampled and slowly moved to the opposite side catching disturbed individuals. This method, while effective, is not particularly precise and may potentially miss smaller or dark-coloured Orthopterans. Therefore, our procedure was as follows: We also laid out a 50 m tape measure on the sides of the plot to mark its boundaries. SE corners of 100 m^2 plots were shared with the NW corner of the vegetation sampling (in order not to disturb orthopterans by vascular plant recording) as described in permanent plots sampling in 2022 edition. Then, one person positioned themselves with a sweep net at the SE corner to begin sampling, followed by others who could collect Orthoptera missed by the person sweep netting, using containers. This approach allowed for the capture of small and inconspicuous individuals, while scouting behind the sweep netter worked well with the varying vegetation heights in our plots. After all fieldworkers reached the opposite side of the plot, we dispersed to randomly walk the vegetation a second time to locate escaped individuals. The search and trapping process lasted approximately 15 minutes in most cases. However, in plots with a particularly high abundance of individuals, sampling time was extended to up to 30 minutes.

Figure 1. Orthoptera sampling sites around Białowieża. Each number indicates a plot triplet with three plots, each treated with a different management type (mowed, unmowed, woody).

Following the trapping phase, the subsequent procedures were conducted as in 2022. Specimens were categorized by morphotype, identified, and counted in the field with the assistance of supervisors and identification keys. Additional data, including sex (male, female) and life stage (adult, larvae), were recorded. Specimens that could not be definitively identified in the field using a hand lens were transported to the facility in Białowieża for further examination under a binocular microscope. Upon identification, all specimens were released at approximately the same location where they had been captured.

Statistical analyses

The collected data was first organized into an Excel file. Each variable was placed in its own column consecutively and included the plot ID, plot number, treatment type (mown, unmowed, or woody), species, species richness, species abundance, total individual abundance, and other relevant parameters. Further data preparation and statistical analysis was carried out in RStudio (RStudio Team, 2022) with R version 4.3.3 (R Core Team, 2024). For each plot, the Shannon diversity index, Evenness index and the Simpson index was calculated. Prior to modelling the data was visually investigated and general patterns were analysed by descriptive statistics. For all the analyses no model included more than one continuous or categorical predictor, such that variable scaling and correlations between continuous variables was not considered.

For each analysis part a generalized linear mixed model (GLMM) was fitted using the *glmmTMB* package. The associated family and link function for the GLMM models varied depending on the structure of the response variable, the *poisson* family with logit link function was used for count data, where a *negative binomial* family was used alternatively if dispersion was significant, while the *gaussian* family with identity function was used for ~normally distributed data. With an experimental setup including plot triplets, each with a different management type, spatial autocorrelation may have occurred and was accounted for by testing the management type, the plot triplet and both nested as random factors before conducting the model selection. For this step, full models with different combinations of the random factors were fitted and compared by AICc (Akaike information criterion for small sample sizes) using the restricted maximum likelihood (REML) estimation of variance components, which maximizes the likelihood of the data only for the random effect, being more suitable for the comparison of random effects than the maximum likelihood (ML) approach (default in *glmmTMB*) (Zuur et al., 2009). From this point on, fixed effects were estimated using the maximum likelihood (ML) approach, since post-hoc tests often could not handle REML. The best model was selected by AICc, where two candidate models were regarded equal with dAICc < 4. For the model validation the following functions from the *DHARMa* package were used: testDispersion-function, simulateResiduals-function and plot-function to test deviation for critical dispersion, autocorrelation, outliers and possible violation of the model assumptions. Post-hoc tests were conducted using the *marginaleffects* package and results were described using the *ggplot2* package for visualisations. The level of significance was defined as $\alpha < 0.05$.

Differences between the data of the two years 2022 and 2024 were tested at the beginning of each analysis, to decide whether data of both years could be included. This was done fitting GLMMs (family again depending on the type of response variable) with year as a predictor and triplet and/or management as random factors. Data of the year 2022 was only included in the analysis if data of the two years did not differ.

Results

General results

A total of 826 orthoptera individuals were caught in 2024, which belonged to 21 different species (Appendix 1) from four families (*Acrididae*, *Gryllidae*, *Tetrigidae*, *Tettigoniidae*), where most species (12) were found belonging to the Acrididae family. The mean species richness per plot was 3.90, with 12 species being the highest species richness per plot. *Chorthippus dorsatus* marked the most abundant species, it was found in 26 plots with a total of 274 individuals being caught.

The contrast analysis revealed no difference of the species abundance nor the species richness between the years 2022 and 2024 (details not shown). The mean species abundance was 7.82 ± 1.84 in 2022 and 4.96 ± 1.17 in 2024, whereas the mean species richness was 2.91 ± 1.73 in 2022 and 2.48 ± 1.48 in 2024. Also, there was no difference found between the years 2022 and 2024 concerning the orthoptera diversity and the herbal diversity (range: 0.90 - 3.28) measured by Shannon index (details not shown). The mean orthoptera diversity (Shannon index) was 0.85 ± 0.30 in 2022 and 0.93 ± 0.30 in 2024, whereas the mean herbal diversity (Shannon index) was 2.10 ± 0.075 in 2022 and 2.19 ± 0.073 in 2024.

Analysis of orthoptera species richness and abundance across different management types

The best model to investigate the influence of management on orthoptera species abundance included management as a predictor as well as the experimental triplet as a random factor. Orthoptera species abundance varied among the different management types, with the species abundance being highest in a regularly mowed land type (Table 1). The species abundance found on abandoned fields was mediocre, followed by woody plots, with the lowest species abundance (Figure 2).

To investigate the influence of management on orthoptera species richness, the best model included management as a predictor and the experimental triplet as a random factor. The orthoptera species richness did not differ between the management types mowed and unmowed, however there was a clear difference between field plots (mowed, unmowed) and the woody plots (Table 2, Figure 3).

Table 1: Contrast analysis of the orthoptera species abundance between the different management types. Pairwise contrasts of m = mowed, u = unmowed and w = woody are listed, along with the slope (estimate), standard deviation (SE), Wald z-statistic (z-value), P-value and the 95% lower and upper Wald confidence intervals (CL and CU).

Contrast	*Estimate*	*SE*	*z-value*	*p-value*	*CL*	*CU*
mean(u) - mean(m)	-2.25	0.79	-2.85	0.004	-3.80	-0.70
mean(w) - mean(m)	-6.17	0.83	-7.44	< 0.001	-7.80	-4.55
mean(w) - mean(u)	-3.92	0.60	-6.54	< 0.001	-5.10	-2.75

Table 2: Contrast analysis of the orthoptera species richness between the different management types. Pairwise contrasts of m = mowed, u = unmowed and w = woody are listed, along with the slope (estimate), standard deviation (SE), Wald z-statistic (z-value), P-value and the 95% lower and upper Wald confidence intervals (CL and CU).

Contrast	*Estimate*	*SE*	*z-value*	*p-value*	*CL*	*CU*
mean(u) - mean(m)	-0.48	0.66	-0.72	0.470	-1.78	0.82
mean(w) - mean(m)	-5.10	0.52	-9.73	< 0.001	-6.12	-4.07
mean(w) - mean(u)	-4.62	0.50	-9.21	< 0.001	-5.60	-3.64

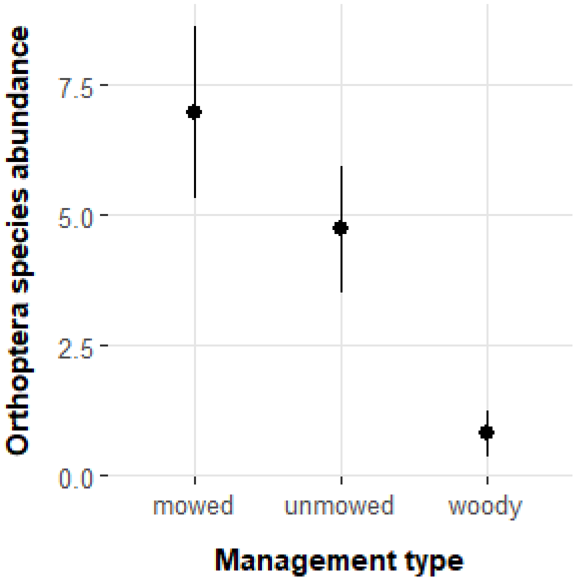

Figure 2. Orthoptera species abundance divided by the type of management. Standard errors indicated by vertical lines.

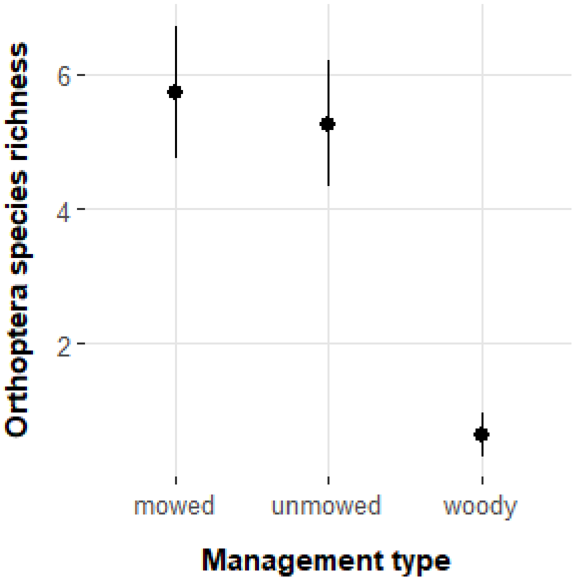

Figure 3. Orthoptera species richness divided by the type of management. Standard errors indicated by vertical lines.

Analysis of orthoptera species richness at different levels of soil moisture

To describe the possible effects of soil moisture (range: 2.78 - 4.73) on orthoptera species richness (2024 data only), the best model included both the soil moisture and the management type as predictors, as well as the experimental triplet as a random factor. Also, an equally performing model included the same random factor and the interaction of soil moisture and management type.

The model without an interaction revealed no effects of soil moisture on the orthoptera species richness, however, as reported in the previous result chapter, management influences the species richness (details shown in: “Management type on orthoptera species richness and abundance”). No effects were found investigating the interaction model (details not shown).

Analysis of species abundance of C*horthippus dorsatus* at different levels of soil moisture

The best model to investigate the effects of soil moisture on C*horthippus dorsatus* abundance included soil moisture as the only predictor and the experimental triplet as a random factor, the link-function family used here was negative binomial due to dispersion, the model performed equal to the null model. No relationship was found between the abundance of C*horthippus dorsatus* and soil moisture (details not shown).

Analysis of orthoptera species richness and abundance in relation to herb diversity

To describe the potential relationship between the herbal diversity and the orthoptera diversity, the best model included the Shannon index of the herbal diversity and the management type as a predictor, whereas the experimental triplet was included as random factor. We found a positive relationship between the herb diversity and the orthoptera species richness and abundance (Table 3, Figure 4). Furthermore, the orthoptera diversity and abundance (Shannon index) differed between the management types mowed and woody as well as unmowed and woody (Table 4, Figure 5). The model including the interaction of the herbal diversity, and the management type was not significantly worse (dAICc = 3.4) than the best model, though no interaction was found (details not shown).

Table 3: Modelled description of the effect of herbal diversity (Shannon index) on the orthoptera diversity (Shannon index). Given are the slope (estimate), standard deviation (SE), Wald z-statistic (z-value), P-value and the 95% lower and upper Wald confidence intervals (CL and CU).

Estimate	*SE*	*z-value*	*p-value*	*CL*	*CU*
0.29	0.10	2.86	0.004	0.09	0.48

Table 4: Contrast analysis of the orthoptera diversity and abundance (Shannon index) between the different management types. Pairwise contrasts of m = mowed, u = unmowed and w = woody are listed, along with the slope (estimate), standard deviation (SE), Wald z-statistic (z-value), P-value and the 95% lower and upper Wald confidence intervals (CL and CU).

Contrast	*Estimate*	*SE*	*z-value*	*p-value*	*CL*	*CU*
mean(u) - mean(m)	0.05	0.11	0.49	0.622	-0.16	0.26
mean(w) - mean(m)	-0.94	0.11	-8.27	< 0.001	-1.17	-0.72
mean(w) - mean(u)	-1.00	0.11	-9.14	< 0.001	-1.21	-0.78

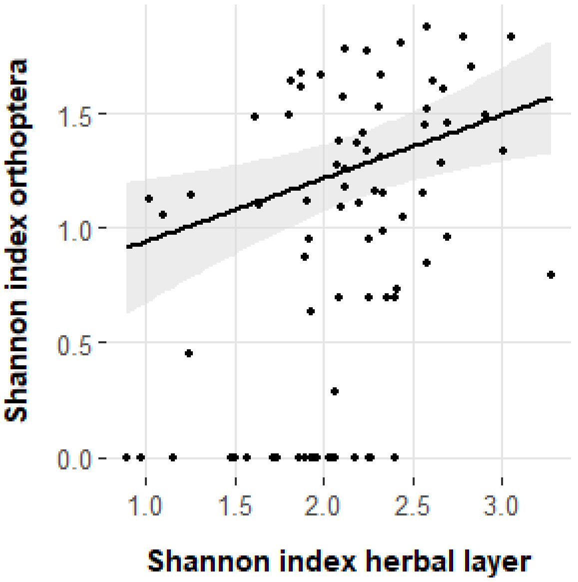

Figure 4. Regression line of predicted orthoptera diversity (Shannon index) against herbal diversity (Shannon index), with shaded area indicating confidence intervals and the observed data points in the background.

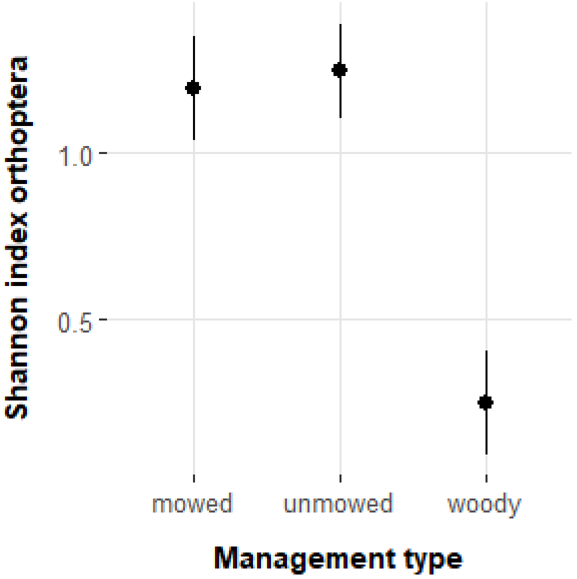

Figure 5. Orthoptera diversity and abundance (Shannon index) divided by the type of management. Standard errors indicated by vertical lines.

Discussion

During our fieldwork, we captured a total of 826 individuals representing 21 species. This reflects a decrease of 156 individuals and an increase of 6 species compared to the findings reported in 2022 Białowieża Summer School. The observed differences could be easily explained. We implemented a methodology designed to enhance precision and better represent the actual species composition and we expanded the number of plots sampled as mentioned above, so they were probably more heterogeneous.

However, this approach likely resulted in a slightly slower capture rate, which may have accounted for the reduced number of individuals collected. Regardless, these differences weren't enough to create any statistical significance for species richness and species abundance so combined with the text above this just probably shows yearly fluctuations in species populations.

Management type on orthoptera species richness and abundance

The significant effect of management type on Orthoptera abundance aligns with previous studies emphasizing the role of habitat structure in determining insect communities (Gardiner et al., 2002) Not surprisingly, regularly mowed meadows exhibited the highest Orthoptera abundance, followed by abandoned plots, with woody plots supporting the lowest abundance. Mowing practices enhance habitat suitability for Orthoptera by maintaining open grassland environments that facilitate mobility and feeding. The consistent removal of biomass in mowed fields may reduce shading, which enables basking and competition, creating favourable microhabitats with greater accessibility to sunlight and warmer soil temperatures, which are critical for Orthoptera development and activity (Specht et al., 2008). This highlights the importance of maintaining open landscapes through active management to support Orthoptera populations, which are crucial for grassland food webs and ecosystem functions, such as nutrient cycling and primary production (Belovsky et al., 2000).

Soil moisture on orthoptera species richness

Nevertheless, the reasoning above does not explain the lack of statistical differences between mowed and unmowed species richness. While higher soil moisture was generally found in unmowed areas, it did not directly enhance Orthoptera richness. This result contrasts with well-established knowledge that soil moisture benefits vegetation and, by extension, herbivorous insects (Perner et al., 2005, Haddad et al., 2009). This lack of differentiation in richness may be due to species turnover in response to niche shifts. As noted, some Orthoptera species are restricted by their need for warm, drier conditions, leading to species replacement rather than increased richness as soil moisture changes. For example, nearly all bush cricket species (*Tettigoniidae*) were found exclusively in wetter, unmowed plots, where conditions align with their ecological requirements.

This pattern suggests that an additional, likely reproductive, factor may be influencing species composition across moisture gradients without affecting overall richness. Specifically, oviposition patterns may play a critical role. In drier, mesic meadows, such as those primarily represented by mowed plots in our study, most species e.g. *Chorthippus brunneus* tend to deposit eggs in the soil, which must be sufficiently moist to support hatching but not overly saturated (Choudhuri, 1958). In contrast, species common in wetter grasslands, such as *Conocephalus fuscus*, typically lay eggs in vegetation (Gardiner, 2020). Consequently, even if bush cricket species could theoretically thrive in drier, mowed areas, mowing activities likely destroy their vegetation-bound eggs, which could result in similar species richness levels between mowed and unmowed plots but visible differences in species composition.

This reproductive pattern suggests that while certain Orthoptera species are capable of existing in both dry and wet habitats, their reproductive success — and thus their persistence in managed versus unmanaged meadows — varies significantly, reflecting adaptations to specific environmental conditions.

In connection to that pattern, we suggest further analysis of soil moisture effects on species abundance. We investigated effects of soil moisture on C*horthippus dorsatus* abundance, which is a grasshopper species who usually lays eggs in the soil (Cárdenas et al., 2017). However, our described approach did not show any significant relation. C*horthippus dorsatus* was the most abundant species as well as the most opportunistic, sometimes even found in woody plots. With that said, it is possible that C*horthippus dorsatus* does not follow the patterns described above. Lastly, soil moisture was the only predictor included in the model together with the experimental triplet as a random factor, however the small number of sampled plots could have decreased the statistical power of the model. For future studies it would be interesting to sample larger and more heterogeneous plots.

Orthoptera species richness and abundance in relation to herbal diversity

We found a positive correlation between plant diversity and both Orthoptera diversity and abundance, underscoring the idea that diverse plant communities support diverse insect populations. Greater plant diversity often results in a wider range of microhabitats, food sources, and shelter, which facilitates species coexistence among Orthopterans. While the Shannon index for the herbal layer, which is somewhat linked to management type, effectively reflected meadow conditions, assessing Orthoptera diversity using this index was more challenging due to species replacement discussed above. It would be interesting to link some functional diversity indices of Orthoptera with management type with a broader scale experiment in the future.

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We would like to thank the Zurich University of Applied Sciences and the University of Warsaw for this amazing opportunity of participating in the Summer School Biodiversity Monitoring 2024 in the Białowieża village in Poland. Also, we are grateful for the organisation, effort and support of all our teachers and supervisors: Jürgen Dengler, Iwona Dembicz, Paweł Radzikowski, Ludmiła Szewczak, Olha Chusova, Aleksandra Wróbel and Marcin Mazurkiewicz. The summer school was fun, and we gained a lot of experience!

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Appendix

Appendix 1 All orthopteran species found during the field work of this project in 2024, including the family and species abundance.

species	***family***	***species abundance***
Chorthippus albomarginatus	Acrididae	6
Chorthippus biguttulus	Acrididae	37
Chorthippus brunneus	Acrididae	34
Chorthippus dorsatus	Acrididae	250
Chorthippus mollis	Acrididae	2
Chorthippus vagans	Acrididae	3
Chrysochraon dispar	Acrididae	9
Conocephalus dorsalis	Acrididae	26
Conocephalus fuscus	Acrididae	57
Gryllus campestris	Gryllidae	27
Omocestus viridulus	Acrididae	32
Phaneroptera falcata	Tettigoniidae	19
Pholidoptera griseoaptera	Tettigoniidae	3
Pseudochorthippus montanus	Acrididae	58
Pseudochorthippus parallelus	Acrididae	113
Roeseliana roeselii	Tettigoniidae	20
Stethophyma grossum	Acrididae	88
Tetrix subulata	Tetrigidae	26
Tetrix tenuicornis	Tetrigidae	4
Tetrix undulata	Tetrigidae	4
Tettigonia cantans	Tetrigidae	3
Tetrix sp.	Tetrigidae	6

Project 5: Changes in small mammal abundance and diversity from 2022 to 2024 in Białowieża: impact of mown, unused, and woody land uses

Jakub Czasak, Fabienne Grawehr & Nadja Pfister

Abstract

Rodents play an important role in the ecosystem, such as in seed dispersal, and serve as a major food source for many predators, making them essential for biodiversity. This study investigated the effects of land use on small mammal communities in Białowieża, focusing on mowed, unused, and wooded areas. Fieldwork included trapping sessions across nine sites, with analysis of abundance, species composition, and diversity across different land use types. We additionally compared rodent abundances and diversity between 2022 and 2024. We revealed that unused and wooded areas supported greater species diversity and abundances than mown areas, which are more disturbed. However, the rodent diversity remained constant between 2022 and 2024 however noticeable increase in abundance of forest habitat specialist was noted suggesting post-mast year surge in population numbers. Long-term trends and improved land management strategies for biodiversity conservation should be considered in future studies.

Keywords

Abundance, biodiversity, Białowieża, grassland, habitat use, land management, land use, rodents, small mammals, species diversity

Introduction

With over 2,300 species, Rodentia is among the most successful and diverse groups in the mammalian order. Rodents make up almost 40 % of all known mammals and new ones are still being discovered (Gazzard et al., 2023). Their success is primarily due to their ecological and biological characteristics, such as their high reproductive potential. Rodents are extremely adaptable in their choice of food plants and can thrive in a wide range of habitats across the globe, from pristine natural environments to areas heavily influenced by human activities. Species like the brown rat (*Rattus norvegicus*) and house mouse (*Mus musculus*) exemplify this adaptability, benefiting significantly from anthropogenic modifications. They also have a high tolerance for habitat fragmentation (Associazione Teriologica Italiana Onlus, 2020).

Rodents play a crucial role in the preservation of biodiversity. They aid in seed dispersal within their habitat, helping sustain local plant populations by foraging and caching behaviours(Gómez et al., 2019). In addition to dispersing seeds, rodents can support plant growth by creating safe locations where seeds are more likely to survive unpredictable events (Godó et al., 2022). Additionally, they can aid in germination of aborted fruits by gnawing through their extremely hard pericarp thus enabling emergence of seedlings (Fedriani et al., 2020). Previous studies on zoochory focused mainly on large mammals and birds (Traveset et al., 2007) but following global decline and extinction of seed-dispersing large herbivores, rodents have taken over many of the seed dispersal functions (Ripple et al., 2015). Additionally, their abundance makes them a primary source of food for many predators, such as birds of prey, snakes, and felines, making them essential for the survival of many other vertebrates (Associazione Teriologica Italiana Onlus, 2020).

The factors influencing the rodent species composition and densities are multifaceted and interconnected. One of the primary drivers of rodent species diversity in grasslands is the vegetation community structure and plant diversity. Grassland plant communities that are subjected to natural and human-induced disturbances, such as grazing or mowing, can undergo rapid changes in their composition, creating resource opportunities for the establishment of new plant species, including invasive ones. The compatibility of these new plant species with the existing regime of competitors and predators can directly impact the suitability of the habitat for different rodent species, leading to shifts in species composition and densities (Benedek & Sîrbu, 2018). The availability and quality of food resources are another crucial determinants of rodent abundance and diversity. Within Białowieża region multi-annual patterns of rodent abundances were identified. They consist of 4-7 years of moderate densities followed by a year of very high abundance due to the mast years, a phenomenon characterised by the synchronised and abundant production of fruits and nuts (Jędrzejewska & Jędrzejewski, 2010). Rodents, with their rapid reproductive cycles and adaptability, have the potential to respond quickly to the influx of food resources during mast years. This obviously leads to a rapid crash in rodent abundance when the mast year ends and there are no resources to feed the enlarged population (Morales-Díaz et al., 2019). Climate and weather patterns are another significant factor shaping rodent communities in grasslands. Variations in temperature, precipitation, and other climatic variables can affect the availability of resources, the prevalence of diseases, and the suitability of the habitat for different rodent species, ultimately influencing their composition and densities (Imholt et al., 2014). Moreover, recent studies have highlighted the impact of lunar cycles on rodent behaviour. During full moon phases, small mammals significantly reduce their activity, primarily due to the increased risk of predation under bright moonlight. This behaviour is particularly pronounced in open or less densely vegetated areas, where predation risk is higher (Chakraborty, 2020; Taylor et al., 2023). In addition to these environmental factors, management practices, such as grazing intensity and frequency, can also play a pivotal role in determining rodent species composition and densities. Overgrazing can lead to a reduction in plant diversity and structural complexity, creating conditions that favour opportunistic and generalist rodent species, while under grazing can allow for the proliferation of more specialised rodent species (Godó et al., 2022). The landscape heterogeneity favours higher biodiversity. In areas with low intensity farming practices, characterised by small patches of crops connected by ditches, field margins and small forests, communities of specialists and generalists can coexist (Benedek & Sîrbu, 2018). Regrettably, such landscapes are increasingly rare and give way to extensive monocultures.

Ultimately, the factors that govern rodent species composition and densities in grassland ecosystems are multifaceted and interconnected. Small terrestrial mammals (Soricomorpha and Rodentia) could potentially be strongly affected by changes in land use and subsequent shifts in cover and vegetation height, vegetation diversity, and abiotic changes such as moisture and sun exposure. Grasslands in Central Europe are increasingly threatened by land management changes, particularly land abandonment following the collapse of traditional farming practices (Dengler et al., 2014; Török et al., 2020). Abandonment and mowing practices alter the species composition and biodiversity of these ecosystems, impacting the plant and rodent communities (Bomanowska & Kiedrzyński, 2011; Wan et al., 2016). While the effects of mowing on small mammal communities are under-researched, especially in Europe, studies from North America show that mowing reduces shelter and food availability, leading to increased predation risk and altered behaviour among species like voles and mice (Jacob & Brown, 2000; Slade & Crain, 2006).

Understanding land use impacts on rodent populations is crucial, not just for biodiversity conservation, but also for managing ecosystems where rodents are key players in the food chain and plant regeneration cycles. This study offers valuable insights for environmental ecologists, researchers in the Białowieża region, and land managers by examining the effects of land use and human activities on small mammals, particularly rodents. The findings contribute to understanding how land management practices impact biodiversity in grasslands, providing a foundation for future research on sustainable land use and its implications for small mammal populations.

This study examines the effects of land use and human activities on small mammals, particularly rodents, in the Białowieża region, comparing data from 2022 and 2024. The area in which the study took place has experienced a significant decline in agricultural use since the 1980s. The arable area decreased from 56 % in 1950 to only 5 % in 2005, transforming the Białowieża meadow into a mosaic of mown and abandoned meadows in various stages of secondary succession, tree plantations and few remaining fields (Bomanowska & Kiedrzyński, 2011). By examining the abundance, composition, and diversity of rodents across different land use types (mowed, unused, and woody meadow), this research seeks to understand how these communities evolve in response to environmental changes by management practices.

Our research aimed to study the effect of land use on small mammal communities. As a result, we tested the following hypotheses:

- Hypothesis 1: Rodent species are expected to be most abundant and diverse in the unused plots compared to mowed and woody plots.
- Hypothesis 2: The abundance and diversity of rodent species will not change significantly compared to 2022 studies.

Additionally, we seek to analyse habitat preferences of studied species by identifying habitat generalists and specialists.

Methods

Study area

The survey of small mammals took place near the village of Białowieża in the Podlaskie Voivodeship, eastern Poland (Fig. 1). The study site lies on a flat plain, with elevations ranging from 132 to 196 m a.s.l. The Narewka River flows through this area from southeast to northwest, the Białowieża Forest surrounds the region.

Around 200 years ago, this area was deforested and converted into meadows that were mowed once a year in summer. In recent decades, many of the meadows have been gradually abandoned, with mowing being completely stopped and natural succession allowed to take its course. Currently, there is a mosaic of grassland areas with varying ages of managed fallow (Kołos & Banaszuk, 2013).

Figure 1. Location of Białowieża in the eastern part of Poland. Source: https://creativecommons.org/licenses/by-sa/3.0/de/legalcode.

Sampling design

In and around the area of Białowieża, nine study sites were defined that correspond to different moisture levels (wet – mesic – semi-dry). In each site three spatially close plots were selected and represented the three different land use types: currently mown grassland (M), unused grassland abandoned 5-10 years ago, dominated by tall-grass or tall-forb community (U), and wooded area abandoned around 30 years ago now covered by woody vegetation mainly: *Betula sp., Salix sp.* and *Populus sp.* (W). Those triplets were as similar as possible in all other respects. These study sites were specifically selected to investigate the effects of different land uses on small mammal populations. On all plots, six pairs of wooden live traps were placed in a line roughly 20 metres apart. When it was not feasible to arrange all traps in a straight line, they were instead positioned in a square pattern. This setup resulted in a total of 36 traps per study site. This method of sampling yielded overall 972 trap nights, significantly more than during the 2022 study (540 trap nights). Trap nights are defined as a sum of occasions where traps were set, and the capture of studied animals was possible. This discrepancy in the number of trap nights between 2022 and 2024 comes from the lower number of traps used in 2022 (18 per study site) and because of the loss of one study site (no. 5) due to conversion in its land use (Dembicz & Dengler, 2023).

Trapping methods

The survey consisted of two sessions. First between 17 and 19, and the second between 21 and 24 August 2024. During the first session studies were carried out on study sites: 1, 3, 4, 12. In the second session on study sites: 7, 8, 9, 10, 11 (fig. 2, tab.1). Oats and carrots were used as bait. Insectivores were not specifically baited. Traps were checked twice a day, except for one occasion (17 August) when the traps were closed during the day due to the hot temperatures. They were reopened in the evening. Captured animals were marked by trimming a small patch of fur to track recaptures and monitor movement between plots. For each captured individual, the following data was recorded: species, sex, age category (juvenile, subadult, or adult), and weight. Remarkable features such as sexual activity or pregnancy were noted. For some individuals of specific species and/or sex could not be determined due to young age.

Identifying habitat preferences

Habitat preferences of small mammals were analysed by comparing numbers of trapped animals within different habitats. The results were visualised by utilizing ternary plot which represents a proportion among three components - in this case, mown, woody, and unused land use types. This method allowed for broad classification of studied small mammals into habitat generalists and specialists.

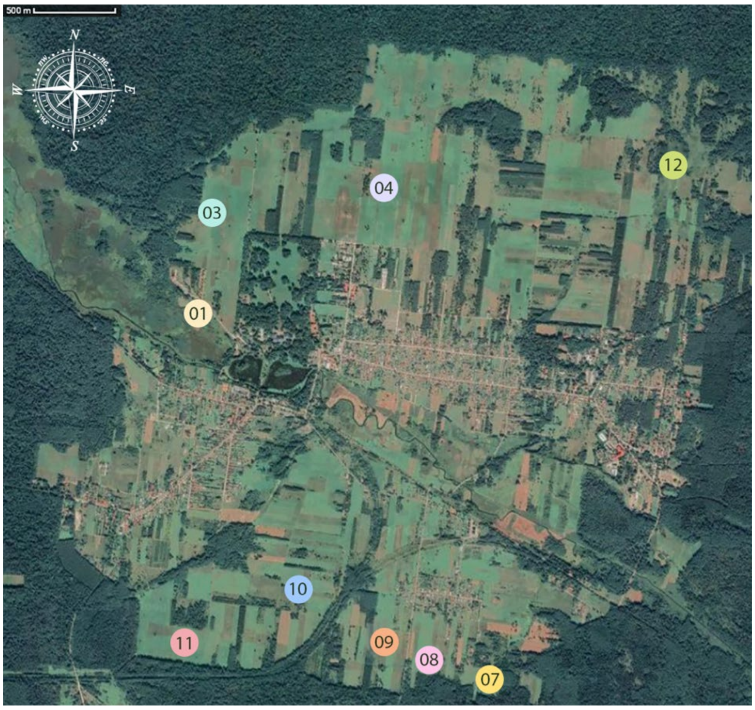


Figure 2. An overview of the plot locations around Białowieża. The locations are marked with colours in the map and in the table 1. Exact coordinates of the locations for each of the three land use types: woody, unused, and mown, showed below.

Table 1. Exact coordinates of the surveyed areas, colour coordinated with the map above.

Plot ID	Latitude (°N)	Longitude (°E)
01M	52.70430	23.83922
01U	52.70392	23.84069
01W	52.70366	23.84049
03M	52.70937	23.83974
03U	52.70934	23.83820
03W	52.70998	23.83804
04M	52.71225	23.85684
04U	52.71212	23.85538
04W	52.71193	23.85476
07M	52.68405	23.86620
07U	52.68462	23.87550
07W	52.68410	23.86542
08M	52.68525	23.86024
08U	52.68500	23.86148
08W	52.68453	23.86069
09M	52.68670	23.85694
09U	52.68650	23.85772
09W	52.68741	23.85740
10M	52.68839	23.85975
10U	52.68871	23.84885
10W	52.68811	23.84904
11M	52.68613	23.83880
11U	52.68689	23.83784
11W	52.68563	23.84078
12M	52.71281	23.88238
12U	52.71207	23.88235
12W	52.71260	23.88174

Statistical analyses

The data was cleaned and prepared in R (version 4.4.1) after removing recaptured individuals and non-rodent species. Missing values were replaced with NA, and the dataset was standardised for the year 2024.

Sex (‘M’, ‘F’) was recorded as ‘male’ and ‘female’, age categories (‘Juv’, ‘Sub’, ‘A’) were changed to ‘Juvenile’, ‘Subadult’, and ‘Adult’, and habitat categories (‘W’, ‘U’, ‘M’) were adjusted to ‘woody’, ‘unused’, and ‘mown’.

Diversity metrics, such as the Shannon Diversity Index, Shannon Evenness, species diversity, and total abundance, were calculated using grouped data by species and land use type. These metrics were then used for subsequent analysis.

To investigate the impact of land use on rodent populations, Generalised Linear Models (GLMs) and Generalised Linear Mixed Models (GLMMs) were applied. In these models:

- the response variable for the Quasi-Poisson GLM was the number of individuals (count data) per plot;
- the response variable for the logistic GLM was the presence or absence of individual species per habitat type (binary data: presence = 1, absence = 0);
- the predictor variable for all models was land use type (mown, unused, woody);
- in the logistic GLMM, plot was used as a random factor to account for variability between triplet plots, ensuring that differences between plots were controlled for but not directly analysed.

Residual analysis was performed using the DHARMa package to check the quality of the models and ensure that statistical assumptions were met. The differences in Shannon diversity index, Shannon evenness, species diversity, and total abundance between land-use types were analysed using a Quasi-Poisson GLM, with a significance level of 0.1 to determine statistically significant differences. In addition, species composition differences across land-use types were assessed using Detrended Correspondence Analysis (DCA), which was performed with functions from the vegan package. Results were visualised using boxplots, illustrating diversity metrics across habitat types.

Results

A total of 91 small mammals representing eight different species were captured during the study (table 2). Of these, 18 individuals were recaptures and were excluded from statistical analysis, along with five insectivores that were caught incidentally. As shown in table 3 those results don't differ significantly from the study conducted in 2022. No migration between plots was observed. As shown in table 2, the highest abundance was recorded in unused plots (48 individuals), followed by woody plots (37 individuals). Mown plots had the lowest abundance (6 individuals).

Table 2. Overview of the species caught during the fieldwork in 2024.

Species	woody	unused	mown	male	female	sex N/A	adult	subadult	juvenile	age N/A	total
Apodemus agrarius	6	10	2	13	4	1	8	6	2	2	18
Apodemus flavicollis	14	4	0	8	8	2	13	2	2	1	18
Microtus arvalis	1	18	4	9	14	0	20	3	0	0	23
Microtus oeconomus	2	4	0	2	4	0	6	0	0	0	6
Microtus sp.	0	6	0	0	5	1	0	0	6	0	6
Myodes glareolus	14	1	0	8	7	0	11	3	0	1	15
Sorex araneus	0	4	0	0	3	1	4	0	0	0	4
Sorex minutus	0	1	0	0	1	0	0	0	0	1	1
total	**37**	**48**	**6**	**40**	**46**	**5**	**62**	**14**	**10**	**5**	**91**

Table 3. Summary of animals caught, specimens analysed, recaptures, plots, species recorded, and trap nights for 2022 and 2024.

Year	2022	2024
Animals caught	121	109
Specimens used in statistical analysis	82	86
Animals recaptured	35	18
Number of plots	10	9
Species recorded	9	7
Trap nights	540	972

Abundance and diversity in different land-use types

Rodent abundance and diversity were highest in unused and woody plots, with ANOVA showing significant differences between land-use types ($p = 0.029$). Although the Shannon diversity index did not reach statistical significance ($p = 0.056$), it displayed a trend toward lower diversity in mown areas compared to higher diversity in woody and unused plots. This suggests that less disturbed environments support richer rodent communities. An analysis of the interaction effects between land use, year, and species composition was conducted (Fig. 3a & 3b). The interactions between land use and year ($p = 0.862$), land use and species ($p = 0.985$), and the three-way interaction between land use, year, and species ($p = 0.906$) were not significant. The most abundant species: *Microtus arvalis* was additionally examined. Results from a paired t-test indicated a decrease in *Microtus arvalis* abundance from 2022 to 2024, but this change was not statistically significant ($p = 0.303$). Clear shift in abundance was identified within woody land use type. The number of trapped animals nearly doubled compared to 2022 (increase from 20 to 37). Changes in *Apodemus flavicollis* and *Myodes glareolus* numbers were mainly responsible for this. Noticeably two species were not found in 2024 namely: *Apodemus sylvaticus* and *Microtus agrestis.*

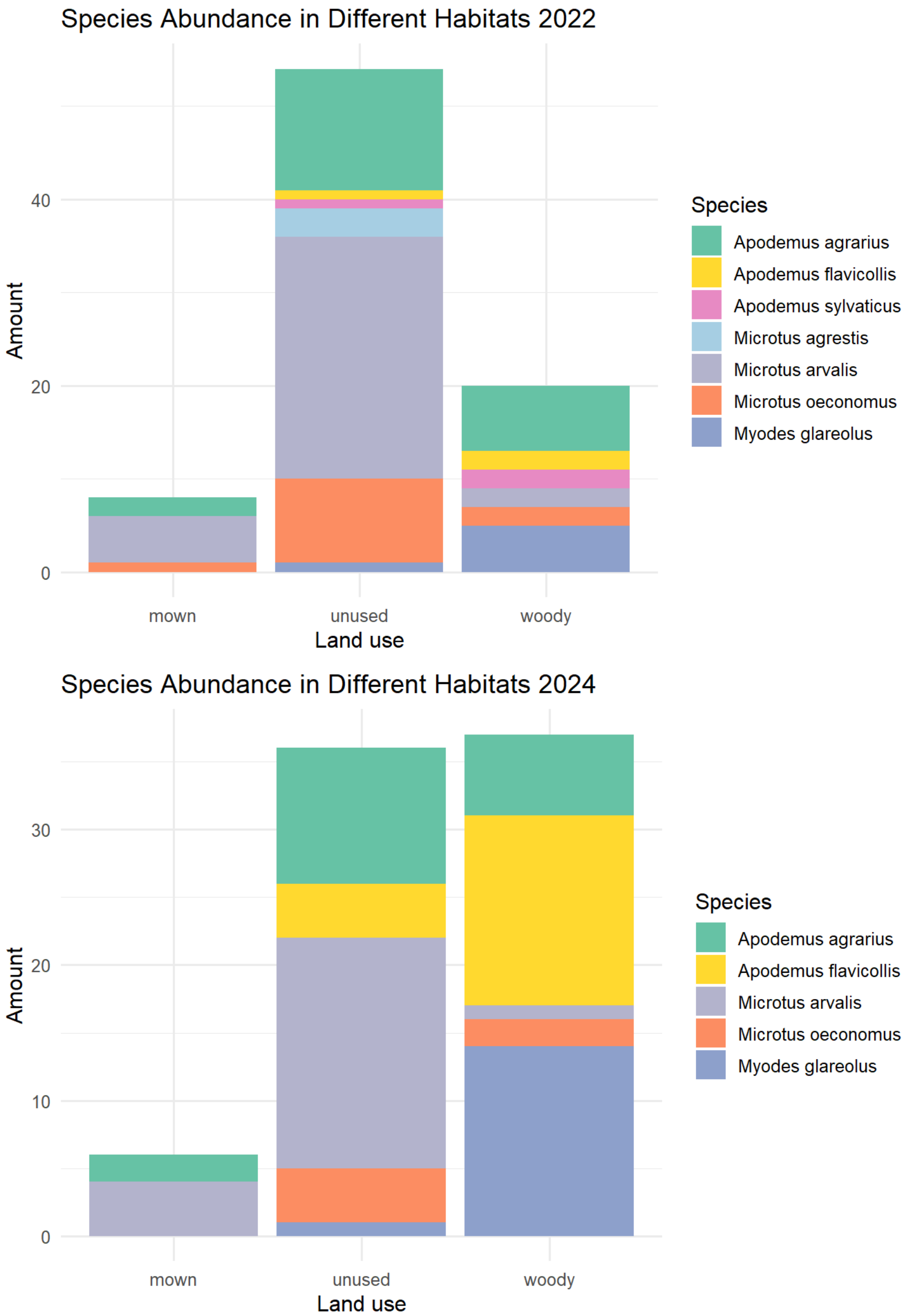


Figure 3. The bar graphs show the total number of individuals caught per land-use type (mown, unused, woody). These figures illustrate the significant differences in abundance between land-use types, highlighting that unused plots had the highest abundance in 2022 (upper) and 2024 (lower).

Identification of habitat preferences

The Figures 4a) and 4b) indicate habitat preference of different species. *Myodes glareolus* and *Apodemus flavicollis* clearly are more prevalent in woody land use types. This seems to be less of a case in 2022. Still abundance of those two species increased significantly in woody habitat. This alongside the prevalence of those species in woody land use seems to indicate them as clear forest habitat specialists. *Apodemus sylvaticus* despite being similar in its distribution to two species mentioned above could not be categorized due to small sample size. In turn *Microtus arvalis* seemingly prefers more open spaces and is found both in mown and unused land use types, while being much more abundant in the latter. This pattern remains consistent in 2022 and 2024. This and small amounts of this species trapped in woody land use types suggest clear meadow specialization. In 2022 three members of *Microtus agrestis* were trapped, exclusively on the unused land. On surface level it would suggest specialization toward this type of land use, however due to low sample size it can't be confirmed. *Apodemus agrarius* is present across all land use types and is abundant both in woody and unused plots, suggesting that it is a habitat generalist. Similarly, *Microtus oeconomus* could be considered generalist but it is less abundant in woody habitat making this species less of generalist.

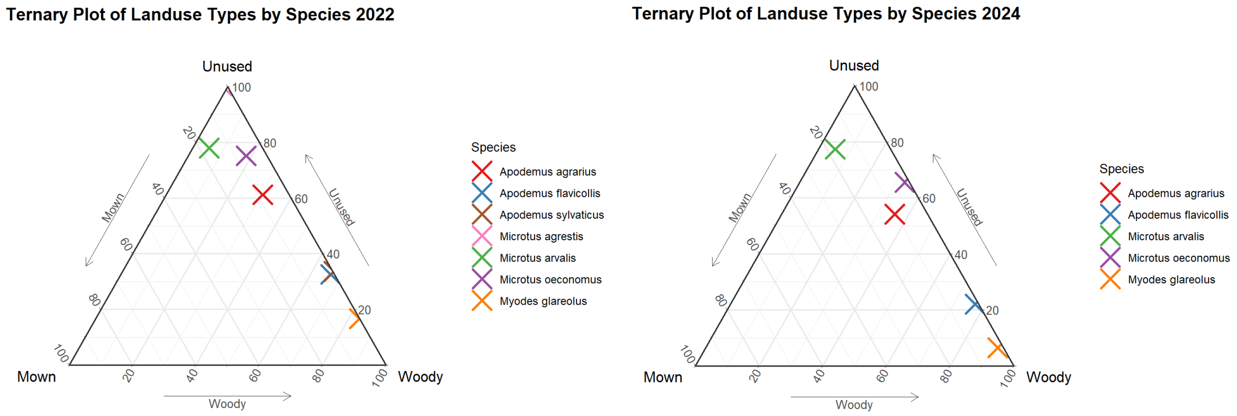


Figure 4. The relationships between land use, and species composition. The three corners of the triangle represent 100% species prevalence in each land type.

In a detailed analysis (tab. 4) of the interaction effects between land use, year, and species composition, GLMs were used to investigate the interactions between the variables. The number of individuals caught served as the dependent variable, while land use, year and species were considered as independent (explanatory) variables. To assess the statistical significance of the interactions, the chi-square test was used to determine whether the effects of the independent variables on the dependent variable were significant.

Table 4 Results (Quasipoisson GLM, Binomial GLM, and Binomial GLMM) showing the influence of land use (Unused, Woody) on the abundance of Rodentia species in Białowieża. The Binomial GLMM includes Triplet ID as a random factor to account for variability. Lower p-values indicate stronger evidence of land use effects on species abundance.

Species	**Quasipoisson GLM**		**Binomial GLM**		**Binomial GLMM (Random factor: Triplet ID)**	
Land use	Unused	Woody	Unused	Woody	Unused	Woody
Apodemus agrarius	$7,58^{-194}$	$3,82^{-191}$	0,99	1	$1,59^{-3}$	0,037
Apodemus flavicollis	$1,42^{-234}$	NA	0,99	NA	$1,45^{-8}$	NA
Microtus arvalis	$5,23^{-212}$	$3,46^{-200}$	0,99	1	$1,77^{-9}$	0,179
Microtus oeconomus	$7,82^{-51}$	NA	0,99	NA	0,21	NA
Myodes glareolus	$3,80^{-141}$	NA	0,99	NA	$8,47^{-3}$	NA

Quasipoisson GLM generally shows very strong effects of land use on species abundance across all species and land use types. Binomial GLM often does not show significant effects, suggesting that the binary modelling approach without random factors does not capture the effects of land use as well for this data. Binomial GLMM reveals significant effects for most species in unused land types, indicating that when site variability (triplet ID) is considered, land use tends to impact species abundance in unused land use types more strongly than in woody land use types.

Changes in abundance and diversity over time

The Detrended Correspondence Analysis (DCA) (fig. 5) revealed clear patterns in species composition relative to land-use types. The ordination diagram showed that species composition varied most significantly between mown and woody plots, while unused plots overlapped with both habitats. In 2022 the unused land use types have a broader spread indicating higher variability in species composition and has shifted in 2024, which indicates a reduction in variability in this area. It suggests that the species composition might have become more consistent, or that fewer species are dominant here compared to 2022. The woody land use form a distinct group in 2022 that overlaps somewhat with unused areas but is more compact. It has shifted slightly to the left on the DCA1 axis, indicating a shift in species composition. This might reflect an increase in species more associated with woody environments or a reduction in species found in the unused areas. Mown land use types overlap significantly with both the unused and woody areas, suggesting that mown areas had a similar species composition to the other land-use types. By 2024, the overlap with other land types significantly diminished making it no longer comparable.

- 2022 (Fig. 5 left): The DCA ordination graph for 2022 shows more overlap between the three habitat types, although a separation between the mown and wooded areas is also evident here. The unused areas showed more overlap with the other habitats this year, suggesting that they are in a transitional stage that has elements of both mowed and forested areas.
- 2024 (Fig. 5 right): The DCA analysis for the year 2024 shows a clear separation in species composition between the mown and forested areas. The unused areas overlap in their species composition with both the mown and forested areas.

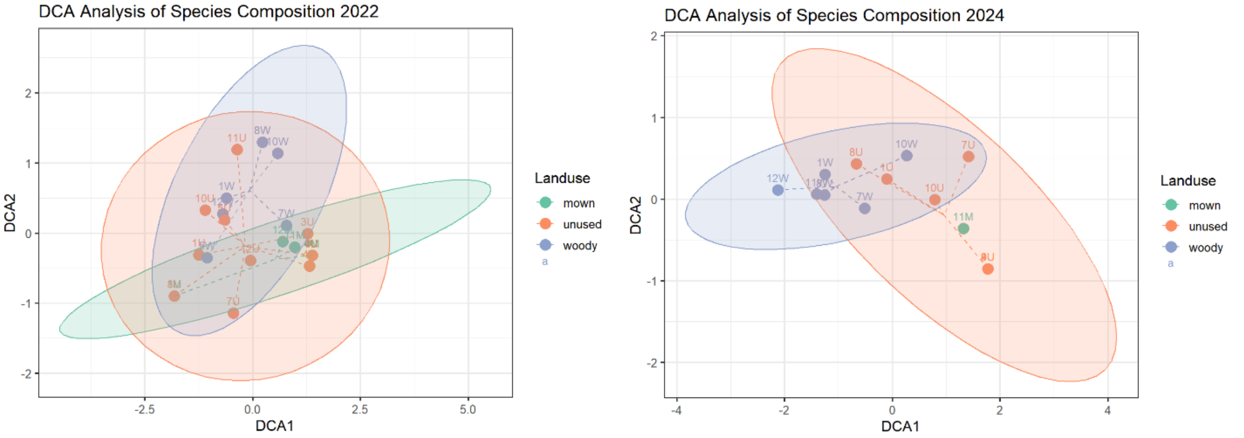


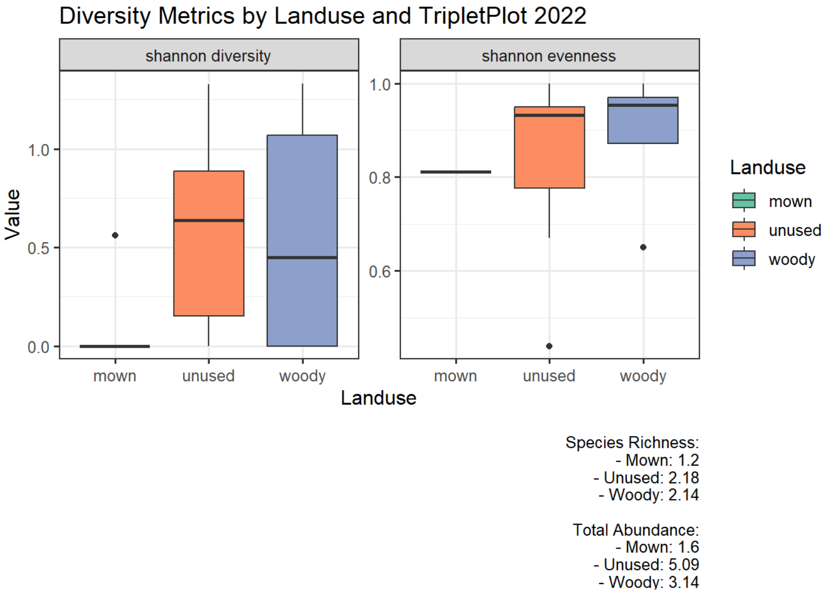
Figure 5. A DCA ordination plot displaying species composition across the three land-use types (mown = green, unmown = orange, woody = blue). This figure highlights how species composition differs significantly between mown and woody habitats.

Regarding the hypothesis 2, significant temporal differences between 2022 and 2024 were found ($p < 0.001$). However, species diversity only decreased slightly between 2022 and 2024 across all land-use types, and this change was not statistically significant according to the Wilcoxon test (*p* = 0.174). The stability of species composition across the land-use types also showed no significant differences, as indicated by a Chi-square test (*p* = 0.977). Thus, there was no significant shift in overall abundance and diversity compared to the 2022 study (fig. 5a & 5b).

Shannon diversity indexes showed that woody land use types have the highest median diversity, though there is considerable variation, indicating that diversity levels in woody areas can be quite variable. The diversity in those areas decreased compared to 2022 with less variation as well. The woody land use types also have highest evenness indicating a more balanced distribution of species. It decreased slightly in 2024 but remained the highest with little more variability.

Unused land use types have lower diversity, with a narrower range, though there is some variation. They maintain relatively similar diversity to 2022 but appear more consistent with a smaller interquartile range. Evenness haven't changed significantly compared to 2022.

The mown area shows the lowest Shannon diversity with a median value close to 0 although it increased slightly compared to 2022. Evenness also increased slightly but remained low.



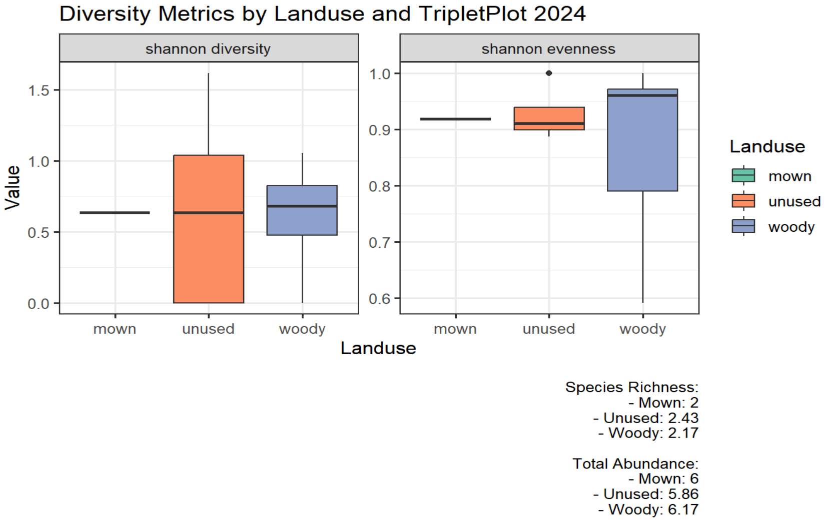


Figure 6. Boxplots showing species diversity and abundance between 2022 and 2024 across different land-use types.

As shown in the graph (Fig. 7), 2024 generally had lower mean numbers of animals per 100 trap nights compared to 2022 across most sites. Unused plots (orange) consistently had the highest animal captures, especially in sites like 3, 4, and 9 in 2022, while in 2024, these numbers generally decreased. Woody areas (blue) were abundant in rodents, particularly in the plots 1, 8, and 12. In the plots 3, 4, 9 and 12, only one specific land-use type contributed to trapping success, either unused or woody. Mown areas (green) consistently showed the lowest capture rates across all sites, with only plot 11 showing noticeable activity on this land-use type.

Catch rates were calculated as the mean number of animals caught per 100 trap nights (dependent variable), and these data were broken down by the different land use types (independent variable) and the years 2022 and 2024.

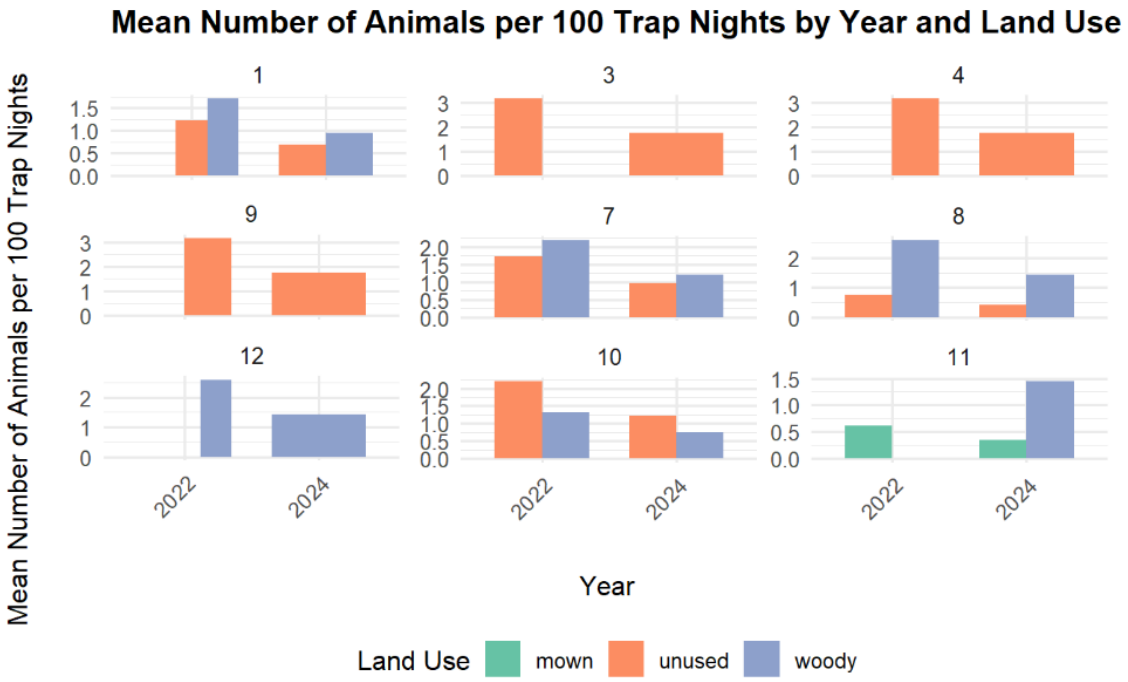


Figure 7. The graph depicts the mean number of animals per 100 trap nights for each study site in 2022 and 2024, categorized by land-use types (mown, unused, woody). It highlights the overall decrease in animal captures in 2024, with unused and woody areas showing the highest capture rates, while mown areas consistently had the lowest.

Discussion

The results of our study show the complexity of the relationships between land use, species composition and small mammal abundance in the Białowieża region. Our analysis confirmed significant differences in the abundance of rodent communities between 2022 and 2024, indicating the influence of land use practices such as mowing and natural succession. These results reflect how highly disturbed habitats such as mowed areas have lower structural diversity and food availability, which negatively affects species diversity. We showed that mowed areas represent a highly disturbed environment, which is reflected in lower species diversity and species richness. Wooded areas, on the other hand, offer more stable conditions that favour higher diversity and a specific species composition. Unused plots of land appear to be an intermediate habitat that exhibits characteristics of both disturbed and more stable habitats.

Ecological significance

Our hypothesis that unused areas would have the highest species diversity and abundance was confirmed. The highest rodent species diversity was observed in unused areas, supporting the prediction that these areas provide more stable living conditions due to their structural complexity and richer food resources. These results are consistent with previous studies showing that mowing reduces the structural complexity of vegetation and limits the availability of cover and food, which negatively affects rodent communities (Slade & Crain, 2006).

Forested patches also provided more stable conditions and greater species diversity compared to mowed patches, suggesting that less disturbed habitats support more specialized rodent species. In this study we established significant shift in abundances of two forest specialists: *Myodes glareolus* and *Apodemus flavicollis.* Additionally, slightly lesser diversity on woody land use type suggests occurrence of conditions favourable to forest specialists. Most likely explanation is sudden proliferation of forest fruits following mast year. This dynamic is well established and is a great source of abundance variation in Białowieża region.

The results of the ANOVA and DCA analyses highlight the crucial role that land-use practices play in shaping rodent communities. In particular, mowing, which leads to habitat disturbance, reduces structural complexity and food availability, which has a negative impact on species diversity. Wooded habitats, on the other hand, offer more stable conditions that promote greater species diversity and a more specific composition of rodent communities. The unutilised areas act as transitional habitat that may become forested habitat over time, unless altered by human intervention.

Identifying specialists and generalists

Obtained data showed clear habitat preferences for different rodent species which were largely consistent with established knowledge (Schlinkert et al., 2016). It is advisable to establish and monitor habitat preferences of different rodent species, since it provides the framework for interpretation of shifts in behaviour. Such adaptations may be in response to changes in environmental factors such as availability of food or changes in land use. Additionally, there is a risk that generalist species may become more abundant due to their better adaptation to different habitats. Ratios of generalist and specialist species may provide interesting metric for studying changing environmental conditions and associated population shifts.

Interpretation and hypothesis testing

These results partially confirm the second hypothesis that there are no significant changes in abundance and diversity between the years 2022 and 2024. Despite the temporal fluctuations in the total number of individuals caught, species diversity showed no significant changes in either year, which is supported by the Wilcoxon test. The species composition also remained largely stable. To summarise, although abundance fluctuated significantly between 2022 and 2024, the diversity and composition of rodent communities remained stable. This stability indicates that the land use types analysed (mowed, unused, wooded) provide stable conditions for the rodent communities, even if abundance varies in some years.

The results of this study highlight the complex relationships between land use, species composition and small mammal abundance in the Białowieża region. Significant differences in the abundance of rodent communities were found between 2022 and 2024, indicating that land use practices such as mowing and natural succession have a significant impact on these populations. Nevertheless, our results show that species diversity has remained relatively stable, which is supported by the lack of statistical significance of the decline in Shannon diversity between 2022 and 2024.

Influence of land use

The highest rodent richness was found in unused areas, supporting the assumption that these areas provide more stable living conditions for rodent communities due to their structural complexity and higher food resources. Wooded areas also showed higher species diversity and more stable populations than mowed areas, which are characterised by regular disturbance. These results are consistent with previous studies showing that mowing reduces the structural complexity of vegetation and limits the availability of cover and food, which negatively affects rodent diversity and abundance. Studies have shown that mowing increases the risk of predation, as rodents have less cover from predators in mowed areas. The more structurally complex wooded and unmanaged areas provide more stable conditions that favour higher species diversity and more specialised rodent communities (Benedek & Sîrbu, 2018).

Stability of species composition

The stability of species composition between 2022 and 2024 was supported by non-significant differences, indicating that no dramatic shifts in the ecological structure of rodent communities occurred. This suggests that rodents in the habitats studied are relatively flexible in their response to changes in land use, particularly in the more complex forested and unmanaged areas. Studies show that conserving habitats with higher vegetation complexity promotes more stable populations and higher biodiversity, as these habitats provide more shelter and food .While generalists, such as *Microtus arvalis*, were found in all land use types, specialised species showed stronger preferences for certain habitats. This is typical of generalists, which are more resilient to environmental change due to their broader feeding niches and ability to survive in different environments (Bomanowska & Kiedrzyński, 2011; Gazzard et al., 2023).

Temporal changes and the influence of the lunar cycle

The abundance data showed that the average number of captured rodents decreased in 2024 compared to 2022 in most of the plots. Populations of many rodent species are known to exhibit cyclical fluctuations in abundance, which can occur in periodic cycles of few years (refs). These cycles are often driven by factors such as food resources and predation (Morales-Díaz et al., 2019). But also, full moon phases could also play a role. It is well documented that small mammals reduce activity during full moon phases to minimise predation risk, especially in open or less structured habitats (Chakraborty, 2020; Taylor et al., 2023). As some of the trapping nights in the studies coincided with full moon phases, this may explain why fewer rodents were caught. In 2024, the full moon occurred on August 19, while in 2022 it was on August 12, outside the trapping period which was 15-25 August (Wetteronline, 2022, 2023). In the Białowieża region, such natural population cycles could explain the differences in abundance between the two study years.

Significance for management

Our results in this study have implications for land use strategies in the Białowieża region. The clear dominance of unmanaged and wooded areas in supporting rodent communities suggests that reducing mowing and encouraging natural succession could lead to greater stability and diversity of rodent populations. In the long term, this could also promote the biodiversity of other animal groups, as rodents play a key role in the food chain and serve as a prey for a variety of predators (Associazione Teriologica Italiana Onlus, 2020). These results highlight the importance of considering environmental factors such as lunar cycles and natural population cycles when designing survey and management strategies, in order to obtain a complete picture of the dynamic interplay between habitats and rodent communities.

To further validate the results, future studies could be conducted over a longer period, as the comparison between 2022 and 2024 only covers a short period and the data collection was not entirely identical (increase in sample size and different plots). Repeated surveys in the coming years could help to identify long-term trends more clearly and gain deeper insights into the development of rodent populations. This can show which type of land use is the most sustainable for small mammals in the long term and how the biodiversity of these animals can be kept as high as possible. Further studies in this area could increase the sample size and better capture potentially hidden effects.

Conclusions

Our research shows that land use has a significant influence on the abundance and composition of rodent communities in Białowieża. Unused and forested areas show higher species diversity and more stable populations, while mowed areas show lower diversity and less structural complexity. A significant change in abundance of forest specialist species was found indicating favorable shift in condition on this land use, most likely post-mast year increase in available food. Rodent abundance was significantly higher in unused plots compared to mowed plots, indicating that reduced disturbance supports larger populations. Despite cyclical population changes, species diversity remained relatively stable between 2022 and 2024. Future studies should consider longer time periods to capture long-term trends and improve land management strategies to promote biodiversity in these ecosystems.

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Appendix

Appendix 1. Calculation of the plot nights 2024

	Northern plots	**Southern plots**
No. of trapping days	3	3
No. of plots	4	5
No. of traps each plot	36	36
Total no. of trap days	432	540
Total from both plots		972

Appendix I: Species list

Compiled by Marcin Mazurkiewicz and Jürgen Dengler

Both students and teachers recorded species of any taxonomic group with the iNaturalist App in a specific project set up for the Summer School. Only taxa determined at least at species level were included in the following list of terminal taxa (i.e. genera are listed only if there was no species of this genus definitely determined). Records from iNaturalist were briefly checked and corrected by experts where necessary (e.g. addition of "aggr." where iNaturalist erroneously claimed the determination of the microspecies). We then merged these data with the records from the five projects and the photographers. In total we recorded 456 taxa during the 11 days (Table 1). They are presentend in alphabetical order within the respective phlya in Table 2.

Table 1. Statistics of the species and other terminal taxa recorded with iNaturalist and other sources as well as the resulting totals.

Regnum	Phylum	iNaturalist	Additions	Total
Protozoa				
	Myxomycota	1	0	1
Plants				
	Bryophyta	18	0	18
	Tracheophyta	262	72	334
Fungi				
	Ascomycota	8	0	8
	Basidiomycota	19	0	19
Animals				
	Mollusca	8	0	8
	Arthropoda	51	1	52
	Chordata	12	4	16
Total		**379**	**77**	**456**

Table 2. Complete list of all species and other terminal taxa determined during the 11 days in Białowieża.

Myxomycota

Ceratiomyxa sp.

Bryophyta

Alleniella complanata
Anomodon viticulosus
Atrichum undulatum
Barbula unguiculata
Bryum argenteum
Bryum dichotomum
Calliergonella cuspidata
Climacium dendroides
Dicranum montanum
Dicranum scoparium
Hypnum cupressiforme
Orthotrichum sp.
Plagiomnium affine
Plagiomnium sp.
Plagiomnium undulatum
Polytrichum formosum
Schistidium sp.
Thuidium tamariscinum

Tracheophyta

Acer negundo
Acer platanoides
Acer pseudoplatanus
Achillea millefolium aggr.

Actinidia sp.
Aegopodium podagraria
Agrimonia eupatoria
Agrimonia procera
Agrostis capillaris
Agrostis gigantea
Agrostis stolonifera
Ajuga reptans
Alchemilla sp.
Alliaria petiolata
Alnus glutinosa
Alnus incana
Alopecurus pratensis
Anchusa arvensis
Anchusa officinalis
Anthoxanthum odoratum
Anthriscus sylvestris
Apera spica-venti
Arabidopsis arenosa
Arabis hirsuta
Arenaria serpyllifolia
Argentina anserina
Armoracia rusticana
Arrhenatherum elatius
Artemisia absinthium
Artemisia vulgaris
Asarum europaeum
Astragalus glycyphyllos
Athyrium filix-femina
Avenula pubescens
Ballota nigra
Bergenia sp.
Betula pendula
Bidens frondosa
Bistorta officinalis
Brachypodium sylvaticum
Briza media
Bromus hordeaceus
Bromus inermis
Calamagrostis canescens
Calamagrostis epigejos
Campanula patula
Campanula trachelium
Capsella bursa-pastoris
Cardamine pratensis aggr.
Carex acuta
Carex acutiformis
Carex cespitosa
Carex elongata
Carex flava
Carex hirta
Carex leporina
Carex muricata aggr.
Carex nigra
Carex panicea
Carex paniculata
Carex pilosa
Carex remota
Carex rostrata
Carex sylvatica
Carex vesicaria
Carex vulpina
Carpinus betulus
Centaurea cyanus
Centaurea jacea
Cerastium fontanum subsp. vulgare
Cerastium semidecandrum
Chelidonium majus
Chenopodium sp.
Cichorium intybus
Circaea lutetiana
Cirsium arvense
Cirsium oleraceum
Cirsium palustre
Cirsium rivulare
Cirsium vulgare
Clinopodium vulgare
Comarum palustre
Convallaria majalis
Convolvulus arvensis
Corylus avellana
Crataegus monogyna aggr.
Dactylis glomerata
Daphne mezereum
Daucus carota
Deschampsia cespitosa
Digitaria sanguinalis
Dipsacus fullonum
Dryopteris carthusiana
Dryopteris filix-mas
Echinochloa crus-galli
Echium vulgare
Elytrigia repens
Epilobium hirsutum
Epilobium montanum
Epilobium palustre

Equisetum arvense
Equisetum fluviatile
Equisetum palustre
Equisetum pratense
Equisetum sylvaticum
Eragrostis minor
Erigeron annuus
Erigeron canadensis
Eriophorum angustifolium
Eriophorum sp.
Erodium cicutarium
Euonymus europaeus
Euphorbia esula
Euphorbia helioscopia
Fallopia convolvulus
Festuca rubra aggr.
Festuca trachyphylla
Ficaria verna
Filipendula ulmaria
Fragaria vesca
Frangula alnus
Fraxinus excelsior
Fraxinus pennsylvanica
Galeopsis bifida
Galeopsis speciosa
Galeopsis tetrahit
Galium aparine
Galium mollugo aggr.
Galium odoratum
Galium palustre
Galium rivale
Galium uliginosum
Galium verum
Gentiana pneumonanthe
Geranium molle
Geranium pusillum
Geranium pyrenaicum
Geranium robertianum
Geum rivale
Geum urbanum
Glechoma hederacea
Glyceria fluitans
Gnaphalium sylvaticum
Helianthus tuberosus
Helichrysum arenarium
Hepatica nobilis
Heracleum sphondylium aggr.
Herniaria glabra
Hieracium umbellatum
Holcus lanatus
Holcus mollis
Hordelymus europaeus
Hottonia palustris
Humulus lupulus
Hylotelephium maximum
Hypericum maculatum
Hypericum perforatum
Hypochaeris radicata
Impatiens noli-tangere
Impatiens parviflora
Inula britannica
Jacobaea paludosa
Jacobaea vulgaris
Jasione montana
Juglans regia
Juncus conglomeratus
Juncus effusus
Knautia arvensis
Lamium galeobdolon
Lathyrus pratensis
Lathyrus vernus
Leontodon hispidus
Leucanthemum vulgare aggr.
Lolium arundinaceum
Lolium giganteum
Lolium perenne
Lotus corniculatus
Lotus pedunculatus
Luzula campestris aggr.
Lycopus europaeus
Lysimachia thyrsiflora
Lysimachia vulgaris
Lythrum salicaria
Maianthemum bifolium
Malus sylvestris aggr.
Malva sp.
Medicago lupulina
Melilotus albus
Mentha arvensis
Mercurialis perennis
Milium effusum
Moehringia trinervia
Molinia caerulea
Mycelis muralis
Myosotis arvensis
Nardus stricta

Neottia nidus-avis
Oenothera sp.
Omalotheca sylvatica
Ononis arvensis
Ononis spinosa
Oxalis acetosella
Paris quadrifolia
Parthenocissus quinquefolia
Pastinaca sativa
Pentanema britannica
Persicaria amphibia
Persicaria lapathifolia
Peucedanum palustre
Phalaris arundinacea
Phalaroides arundinacea
Phleum pratense aggr.
Phragmites australis
Phytolacca acinosa
Picris hieracioides
Pilosella caespitosa aggr.
Pilosella officinarum
Pimpinella saxifraga aggr.
Plantago lanceolata
Plantago major
Poa annua
Poa compressa
Poa nemoralis
Poa palustris
Poa pratensis aggr.
Poa trivialis
Polygonatum multiflorum
Polygonum aviculare aggr.
Populus tremula
Potentilla argentea
Potentilla erecta
Potentilla reptans
Prunella vulgaris
Prunus cerasifera
Prunus padus
Prunus serotina
Pteridium aquilinum
Pulmonaria obscura
Pyrola minor
Pyrus communis aggr.
Quercus robur
Rabelera holostea
Ranunculus acris
Ranunculus auricomus aggr.
Ranunculus flammula
Ranunculus lanuginosus
Ranunculus repens
Rhus typhina
Ribes nigrum
Ribes rubrum aggr.
Rorippa amphibia
Rubus caesius
Rubus idaeus
Rudbeckia hirta
Rumex acetosa
Rumex acetosella
Rumex crispus
Rumex obtusifolius
Rumex thyrsiflorus
Sagina procumbens
Salix aurita
Salix caprea
Salix cinerea
Salix pentandra
Sanicula europaea
Saponaria officinalis
Schedonorus arundinaceus
Schedonorus giganteus
Schedonorus pratensis
Scirpus sylvaticus
Scleranthus annuus
Scorzoneroides autumnalis
Scrophularia nodosa
Scutellaria galericulata
Setaria pumila
Silene flos-cuculi
Silene latifolia
Silene vulgaris
Solanum dulcamara
Solidago canadensis
Solidago virgaurea
Sonchus arvensis
Sonchus oleraceus
Sparganium erectum
Spergula arvensis
Spergularia rubra
Spiraea salicifolia
Stachys palustris
Stachys sylvatica
Stellaria graminea
Stellaria media
Stellaria nemorum

- Succisa pratensis
- Succisella inflexa
- Symphytum officinale aggr.
- Tanacetum vulgare
- Taraxacum sect. Taraxacum
- Tilia cordata
- Torilis japonica
- Tragopogon pratensis
- Trifolium arvense
- Trifolium campestre
- Trifolium hybridum
- Trifolium medium
- Trifolium montanum
- Trifolium pratense
- Trifolium repens
- Typha angustifolia
- Ulmus glabra
- Urtica dioica
- Urtica galeopsifolia
- Vaccinium myrtillus
- Valeriana officinalis aggr.
- Verbascum nigrum
- Verbascum sp.
- Veronica arvensis
- Veronica chamaedrys
- Veronica longifolia
- Veronica officinalis
- Veronica scutellata
- Veronica serpyllifolia
- Viburnum opulus
- Vicia cracca
- Vicia hirsuta
- Vicia sativa subsp. nigra
- Vicia sepium
- Vicia tetrasperma
- Vicia villosa
- Viola canina
- Viola mirabilis
- Viola palustris
- Viola reichenbachiana
- Viscum album
- Vitis vinifera

Ascomycota

- Cladonia sp.
- Claviceps bavariensis
- Evernia prunastri
- Parmelia sulcata
- Peltigera sp.
- Rhytisma acerinum
- Xanthoria parietina
- Xylaria polymorpha

Basidiomycota

- Amanita muscaria
- Coprinellus sp.
- Echinoderma sp.
- Fomitopsis pinicola
- Fomitopsis sp.
- Hypholoma sp.
- Laccaria amethystina
- Lycoperdon sp.
- Marasmius oreades
- Mycena sp.
- Phallus impudicus
- Pholiota sp.
- Picipes badius
- Rhodotus palmatus
- Rickenella sp.
- Russula sp.
- Trametes sp.
- Typhula sp.
- Xylobolus sp.

Mollusca

- Arion sp.
- Arion vulgaris
- Cepaea nemoralis
- Fruticicola fruticum
- Helix pomatia
- Krynickillus sp.
- Limax maximus
- Succinea putris

Arthropoda

- Aelia sp.
- Apis mellifera
- Apterygida albipennis
- Araneus quadratus
- Arge pullata
- Argiope bruennichi
- Carabus granulatus
- Carpocoris sp.
- Charagmus gressorius
- Chiasmia clathrata
- Chorthippus albomarginatus

Chorthippus biguttulus
Chorthippus brunneus
Chorthippus dorsatus
Chorthippus mollis
Chorthippus vagans
Chrysochraon dispar
Conocephalus dorsalis
Conocephalus fuscus
Deilephila elpenor
Ectobius lapponicus
Eratigena atrica
Eurygaster testudinaria
Euthrix potatoria
Forficula auricularia
Gryllus campestris
Haematopota sp.
Ixodes sp.
Lacinius dentiger
Lycaena dispar
Mantis religiosa
Misumena vatia
Oecanthus pellucens
Omocestus viridulus
Panorpa vulgaris
Phaneroptera falcata
Phlogophora meticulosa
Pholidoptera griseoaptera
Pseudochorthippus montanus
Pseudochorthippus parallelus
Roeseliana roeselii
Ruspolia nitidula
Singa sp.
Stethophyma grossum
Tetrix subulata
Tetrix tenuicornis
Tetrix undulata
Tettigonia cantans
Timandra comae
Vespa crabro
Xestia sp.
Zelotes sp.

Chordata

Apodemus agrarius
Apodemus flavicollis
Bos bonasus
Bufo bufo
Clethrionomys glareolus
Curruca communis
Hyla orientalis
Lacerta agilis
Microtus arvalis
Microtus oeconomus
Myodes glareolus
Natrix natrix
Pelophylax sp.
Rana temporaria
Vulpes vulpes
Zootoca vivipara

Appendix II: Photo plates

Compiled by Patryk Werner with photos by Jürgen Dengler (if not otherwise indicated)

Geobotanical station in Białowieża (photo by station website) and our way of transportation around Białowieża.

First part of the Summer School: joint excursions and sampling.

Second part of the Summer School: sampling for projects (orthopterans, vegetation and small mammals [live capture traps and *Clethrionomys glareolus* by Nadin Kruschwitz]).

Determining *Orthoptera* and analysing gathered data at the station (*Tettigonia cantans* on the third photo).

Landscapes of Białowieża at the first sign of dawn (by Nadin Kruschwitz).

Landscapes of Białowieża: unused mesic and wet meadows (by Joshua Gerteiser)

Trip to Białowieża's National Park.

Huge oaks and fallen trees are common sight in Białowieża's National Park.

Bison (*Bos bonasus*) “hunting” around Białowieża (1st photo by Joshua Gerteiser, 2nd by Nadin Kruschwitz).

Organisms around Białowieża. From left to right and top to bottom: *Hyla orientalis*, *Xylaria polymorpha* (by Nadia Pfister), *Mantis religiosa*, *Lythrum salicaria* and *Vulpes vulpes* (by Nadin Kruschwitz).