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**Conservation importance of habitat heterogeneity
in Pannonian sandy forest-steppes**

Summary of Ph.D. dissertation

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1. Introduction

Forest-steppe ecosystems consist of differently sized forest and grassland patches of various structure and composition, and an intricate network of their contact zones (i.e., habitat edges). These mosaics can be found in North America, South America, and Eurasia. Representing a transitional zone between the closed forests and the treeless grasslands, Eurasian forest-steppes cover vast areas from the Pannonian region to the Russian and Chinese Far East, and are among the most complex non-tropical ecosystems.

Earlier ecological studies have focusing on habitat heterogeneity in sandy forest-steppes mainly examined species composition and simple species-based indices (e.g., species richness and Shannon diversity), ignoring phylogenetic and functional diversity. Therefore, it is unknown how these parameters relate to taxonomic diversity, and how they are distributed among the different habitats of the forest-steppe mosaic.

The assembly of plant communities is usually represented as a series of various filters that define which traits can enter the realized local plant community. The filter proposed by Grime's scheme acts on traits that are directly connected to competitive ability, stress tolerance, and disturbance tolerance. These traits display a plant's general strategy. Consequently, by studying the traits and strategies of the species composing a plant community, we can gather information about the primary drivers that determine community composition. However, knowledge of Grime's scheme-based filters (i.e., CSR strategy) is still unknown in sandy forest-steppes, which may limit understanding of habitat heterogeneity in this ecosystem.

Earlier investigations measured microclimate parameters for only a very short period (typically 24 hours on a selected summer day) in forest-steppes. These short-term measurements may not be able to capture the most critical microclimatic conditions. To gain more informative microclimate background data, repeated measurements are needed throughout the vegetation period, from spring to autumn. Furthermore, earlier studies did not take into account the full variety of forest-grassland mosaics: some works disregarded the edge habitat,

while others restricted their attention to small forest patches and a single type of grassland. Thus, measuring microclimate in a broader spectrum of near-natural forest-steppe habitats is necessary to fill the above knowledge gap.

In the Pannonian region, plantations of *Pinus nigra*, *P. sylvestris*, *Robinia pseudoacacia*, and different *Populus* species are widespread in forest-steppe landscapes. These plantations span over large areas and are in continuous contact with grasslands as well as natural and near-natural forests. However, it is largely unknown to what extent tree plantations of various species can substitute near-natural forests in terms of different aspects of diversity (taxonomic, functional, and phylogenetic).

Although the number of studies on edges and the two adjacent habitats in forest-grassland mosaics is increasing, the existing knowledge regarding the general composition, taxonomic, edge-related species, functional, and phylogenetic diversity patterns in Pannonian sandy forest-steppes is limited.

2. Aims and study questions

The general aim of this study was to provide information on the habitat heterogeneity of sandy forest-steppes to support the conservation of this ecosystem in the Pannonian region. Based on the general aim, the following studies were designed:

Study 1: The aim of this study was to examine how species composition as well as taxonomic, functional, and phylogenetic diversity vary in multiple habitat types (large forest patches, medium forest patches, small forest patches, north-facing forest edges, south-facing forest edges, closed perennial grasslands, open perennial grasslands, and open annual grasslands) in a sandy forest-steppe ecosystem. Our specific questions were the following: (1) Is there a compositional gradient from shady forests to semi-arid grasslands? (2) Do edges have their own species that are rare in habitat interiors (edge-species)? (3) Does taxonomic diversity peak at forest edges (i.e., at the middle of the gradient? (4) Do the patterns of functional diversity and phylogenetic diversity follow the pattern of taxonomic diversity?

Study 2: Taking into account the eight habitats mentioned above, the aim of this study was to reveal the dominant CSR strategies of the communities of sandy forest-steppes, and infer the assembly processes responsible for their species composition. The following primary questions are answered: Is community composition driven by competitive exclusion in the more productive environment of forest patches? (2) Is stress (i.e., environmental filtering) the most important driver under the harsher conditions of open grasslands? (3) Which habitats are most affected by disturbance?

Study 3: We aimed to describe the microclimate conditions of multiple habitat types (large forest patches, medium forest patches, small forest patches, north-facing forest edges, south-facing forest edges, open perennial grasslands, and open annual grasslands) throughout the vegetation period, in a sandy forest-steppe ecosystem. Our questions were: (1) How do air temperature and humidity differ among the studied habitats during the growing season, from April to October? (2) Which habitats are more stressed to vegetation growth in terms of vapor pressure deficit?

Study 4: Our aim was to compare the species composition, diversity, and ecological value of near-natural forests with those of various types of tree plantations (native deciduous, non-native evergreen, and non-native deciduous) in the Kiskunság. We addressed the following questions: (1) How distinct or overlapping is the species composition of the studied habitats? (2) How do taxonomic, functional, and phylogenetic diversity indices vary among the four habitat types? (3) What is the ecological value of the studied habitats in terms of protected, endemic, and red listed species and naturalness status?

Study 5: Our aim was to assess how the species composition and diversity of edge habitats are related to those of the forest and grassland interiors in two forest-steppe ecosystems in the Pannonian region: in the Kiskunság Sand Ridge (Hungary) and the Deliblato Sands (Serbia). We asked the following questions: (1) Does the species composition of the edges differ from the habitat interiors? (2) Do edge-related species (i.e. species that favor edge habitats and are rare or absent

in habitat interiors) exist? (3) Do taxonomic, functional, and phylogenetic diversity of the edges differ from those of the forest and grassland interiors?

3. Materials and Methods

3.1. Study 1: species composition, edge-related species, taxonomic, functional, and phylogenetic diversity in forest-grassland mosaics

Thirteen study sites of the Kiskunság Sand Ridge, central Hungary were selected. We differentiated eight habitats of forest-steppe: large forest patches (> 0.5 ha), medium forest patches (0.2-0.4 ha), small forest patches (< 0.1 ha), north-facing forest edges, south-facing forest edges, closed perennial grasslands, open perennial grasslands, and open annual grasslands. 25-m^2 plots (5 m × 5 m plots for forest interiors and grasslands and 2 m × 12.5 m plots for forest edges) were used.

Non-metric multidimensional scaling (NMDS) was performed using Bray-Curtis dissimilarity on the square-root transformed percentage cover values to reveal the species composition. To confirm compositional distinctness of the habitats, we used permutational multivariate analysis of variance (PERMANOVA). Species richness and Shannon diversity were calculated for each plot. The diagnostic species of each habitat were identified by calculating phi-coefficients as indicators of fidelity. To quantify overall functional diversity (FD), we calculated plot-level Rao's quadratic entropy (RaoQ) with the combination of nine traits: start of flowering, flowering duration, specific leaf area (SLA), mean plant height, thousand seed mass, life form, seed dispersal, pollination type, and reproduction type. Functional diversity was also calculated for each individual trait, with the exception of two individual traits (flowering start and flowering duration), which were combined to form a trait group (called flowering time), upon which its functional diversity was assessed. We also selected RaoQ to measure phylogenetic diversity (PD) based on phylogenetic tree. To eliminate the effect of species richness on RaoQ, and to determine whether the habitats are functionally and phylogenetically over- or underdispersed, the standardized effect size of RaoQ (SES.RaoQ) was measured as (observed RaoQ value – mean expected RaoQ values)/standard deviation of expected RaoQ values.

To compare species richness, Shannon diversity, and SES.RaoQ, linear mixed-effects models (GLMMs) were applied. We used analysis of variance (ANOVA) to test these models. Pairwise comparisons of factor levels were performed, and the Bonferroni method wase used for multiple comparisons.

3.2. Study 2: Plant strategies and community assembly rules in forest-grassland mosaics

All relevés in study 1 were for this study. We calculated the strategy (separate C, S, and R values) for each species based on values of the three leaf traits: leaf area (LA, mm^2), leaf dry matter content (LDMC, mg/g), and specific leaf area (SLA, mm^2/mg) by the "StrateFy" tool. Based on the C, S, and R components of the strategy for each species, we calculated the unweighted and weighted mean C, S, and R values for each plot, using presence/absence data for the former situation and square-root transformed cover scores for the latter one. To reveal how ecological strategies (C, S, and R) correlate with the gradient, we used linear regression between plot ordination scores (the first DCA scores) and each strategy (C, S, and R).

The statistical approach is similar to Study 1, i.e., linear mixed-effects models (GLMMs) were used to analyze each strategy (C, S, and R).

3.3. Study 3: microclimate among the habitats of a forest-steppe ecosystem

We selected the Fülöpháza site, located in the center of the Sand Ridge, Hungary. Microclimate measurements were carried out in large forest patches, medium forest patches, small forest patches, north-facing forest edges, south-facing forest edges, open perennial grasslands, and open annual grasslands. We used three replicates for each habitat type. The air temperature (ºC) and relative air humidity (%) were measured once every month using MCC USB-502 data loggers in all seven habitats from April to October 2022.

The following variables were computed from the obtained microclimate data: mean daily air temperature (MDAT), mean daytime air temperature (MDtAT), mean nighttime air temperature (MNtAT), mean daily relative air humidity

(MDAH), mean daytime relative air humidity (MDtAH), and mean nighttime relative air humidity (MNtAH). We calculated these variables for each replicate.

Vapor pressure deficit (VPD) was calculated from the air temperature (t, °C) and relative air humidity (H, %) as follow:

$$VPD = (100 - H) \times 6.112 \times e^{(17.67 \times t/(t + 234.5))}$$

We focused on the exceedance rate, which is the percentage of VPD values above an appropriate threshold (1.2 or 3.0 kPa) over a 24-h period. We constructed a VPD duration curve (DC) using 1440 VPD values that were averaged over three replicates collected over the period of 24 hours for each habitat per month. Based on this DC, we calculated the exceedance rate per replicate, which was then used for statistical analysis.

The variables related to mean daily air temperature, mean daytime air temperature, mean nighttime air temperature, mean daily relative air humidity, mean daytime relative air humidity, and mean nighttime relative air humidity, as well as exceedance rate were analyzed using general linear models. We then tested the general linear models using analysis of variance (ANOVA). Pairwise comparisons of the fixed factor levels were performed and the p-values were adjusted with the false discovery rate (FDR) method.

3.4. Study 4: species composition, edge-related species, taxonomic, functional, and phylogenetic diversity of near-natural forests and tree plantations

We chose nine study sites of the Kiskunság Sand Ridge, central Hungary. Four habitat types were sampled: near-natural poplar forests (forest patches located in forest-steppe mosaics), plantations of native *Populus alba*, plantations of non-native *Pinus nigra*, and plantations of non-native *Robinia pseudoacacia.* To sample the vegetation, we used 5 m × 5 m plots in the interior of the habitats.

Similar to study 1, we also performed NMDS, diagnostic species analysis, and RaoQ for overall functional diversity and phylogenetic diversity. Shannon diversity was calculated for each plot.

We counted the number of non-native and native species per plot. A Venn-diagram was created to show how many of the species with high conservation

importance (protected, endemic, and red-listed plant species) are restricted to some of the habitats and how many occur in two or more habitats. The unweighted mean naturalness value per plot was calculated using the species present in each plot.

The number of non-native and native species, Shannon diversity, RaoQ, and naturalness values were analysed using linear mixed-effects models. Analysis of variance was computed to test the linear mixed-effects models, and if the model had a significant proportion of variability, all pairwise comparisons of the fixed factor levels were performed, and the p-values were adjusted with the Bonferroni method.

3.5. Study 5: Forest edges revisited: species composition, edge-related species, taxonomic, functional, and phylogenetic diversity

Forest-steppes in the Kiskunság Sand Ridges and the Deliblato Sands were selected. Four main habitat types were differentiated at both sites: forest patches (>0.5 ha), north-facing forest edges, south-facing forest edges, and grasslands. The method to sample the vegetation using is the same as for Study 1.

The approach for data analysis was similar to study 1 (NMDS, diagnostic species, species richness, Shannon diversity, SES.RaoQ).

The differences in the species richness, Shannon diversity, functional and phylogenetic diversity of the four habitat types in the two study sites (8 groups = 4 habitats × 2 sites) were tested using the Kruskal-Wallis test. Pairwise comparisons of the habitat types of the two study sites were performed, and the p-values were adjusted using the false discovery rate method.

4. Key findings

4.1. Study 1: species composition, edge-related species, taxonomic, functional, and phylogenetic diversity in forest-grassland mosaics

Our study clearly desmontrated that there was a compositional gradient, ranging from large forest patches through smaller-sized forest patches and edges to closed and open grasslands. Our findings confirmed the existence of edge-

species: we were able to identify species that preferred either north-facing or south-facing edges while they were rare in habitat interiors. The highest species richness was found at north-facing edges, followed by south-facing edges, gradually decreasing both towards forest patches and grasslands. This study strongly supports the edge-effect hyphothesis. Shannon diversity was high at edges and at the semi-dry end of gradients (i.g., open annual and open perennial grasslands).

Functional diversity was significantly higher in woody habitats (forests and edges) than in grasslands. When studying the functional diversity of individual traits, the functional diversities of flowering time, seed dispersal, reproduction type, plant height, and thousand seed mass were higher in woody habitats than in grassland habitats. For life form and mean plant height, south-facing edges had the highest functional diversity, followed by north-facing edges and small forest patches. Closed perennial grasslands, open annual grasslands, and medium forest patches had the highest functional diversity for SLA, while the functional diversity of pollination type reached its maxima towards the endpoints of the gradient.

Phylogenetic diversity tended to show a peak near the middle of the gradient when all species were considered, but this pattern disappeared when non-angiosperms were excluded from the analysis. Phylogenetic diversity was significantly higher in woody than in grassland habitats, which may be related to the evolutionary age of the habitats. Importantly, our results underline that taxonomic diversity is not necessarily informative of functional and phylogenetic diversity. Thus, in addition to simple taxonomic indices, studies on diversity patterns should also take into account functional and phylogenetic.

The fact that taxonomic, functional, and phylogenetic diversity peaks do not coincide along the studied compositional gradient indicates that maintaining habitat heterogeneity in sandy forest-steppe ecosystems is crucial in practical nature conservation.

4.2. Study 2: Plant strategies and community assembly rules in forest-grassland mosaics

Based on CSR values, habitat types constituted three main groups: large and medium forest patches belonged to one group, small forest patches, north- and south-facing edges, and closed grasslands formed another group, and open perennial and open annual grasslands comprised the third group. Each habitat type was dominated by the stress-tolerator strategy, which reflects the relatively harsh environmental conditions prevailing in the study region. The importance of the stress-tolerator strategy decreased towards the less harsh end of the gradient (i.e., from grasslands to forests), while the competitor strategy showed a reverse pattern. The ruderal strategy was weakly correlated with the gradient, although its proportion increased towards the harsh end of the gradient.

With ongoing climate change, an increasing importance of environmental filtering is expected in the assembly of the vegetation types in the studied forest-grassland mosaics. We suggest that CSR strategies offer a useful tool for studying plant community assembly rules along environmental gradients.

4.3. Study 3: microclimate among the habitats of a forest-steppe ecosystem

In comparison to grasslands, forests are cooler during the daytime and warmer during the nighttime, and more humid during the daytime. In our study, especially for air temperature, south-facing edges tended to be more similar to grasslands, while north-facing edges tended to be more similar to forests. In terms of vapor pressure deficit, open grasslands were the most stressed for vegetation growth from May to October. During the summer season, forest patches had a small moderating effect at the limiting threshold of 1.2 kPa VPD, but a stronger moderating effect at the 3.0 kPa threshold. We found that even small forest patches (less than 0.1 ha in size) have a very important function in mitigating macroclimatic harshness.

Our study had two main suggestions: (1) the remaining near-natural forest stands in sandy forest-grassland ecosystems should be protected and (2) scattered trees or groups of trees of native species should be encouraged in extensive

treeless grasslands (where woody vegetation has been destroyed anthropogenically).

4.4. Study 4: species composition, edge-related species, taxonomic, functional, and phylogenetic diversity of near-natural forest and tree plantations

We found that there were significant differences in species composition among the four habitats (near-natural poplar forests, plantations of native *Populus alba*, plantations of non-native *Pinus nigra*, and plantations of non-native *Robinia pseudoacacia*). We found that all habitats had some diagnostic species that were significantly concentrated within them while being rare or absent in the other habitats, but near-natural forests had the highest number of diagnostic species, most of which were native shrubs. Our study revealed that near-natural forest patches were more valuable than plantations in terms of native and non-natives species richness, naturalness index, and the number of species with special conservation importance. Also, near-natural forests had high functional and phylogenetic diversity. Among the plantations, *Populus alba* plantations were ecologically more valuable than *Pinus* or *Robinia* plantations in most respects, and the latter proved the ecologically least valuable. Our results suggest that the remaining near-natural forests of the study region should be prioritized for conservation and restoration. If establishing plantations is unavoidable, the native *Populus alba* should be preferred over the exotic *Pinus* or *Robinia*.

4.5. Study 5: Forest edges revisited: species composition, edge-related species, taxonomic, functional, and phylogenetic diversity

Based on species composition and the existence of edge-related species, we confirmed that edges should be considered separate habitats in sandy forest-grassland mosaics. Although species richness and Shannon diversity were the highest at north-facing edges in the Kiskunság, they did not differ significantly between the grasslands and the edges in the Deliblato, indicating that the edge effect hypothesis is not a general rule and the topic needs further studies.

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The multi-trait functional diversity was significantly higher in woody habitats (forest patches and edges) than in grasslands in the Kiskunság, while it was similar among the four habitat types in the Deliblato. This clearly contradicts the stress-dominance hypothesis and emphasizes that functional diversity depends on several factors such as canopy openness and the traits of the dominant species. We conclude that it is too early to draw general conclusions on functional diversity patterns across edges.

We found that phylogenetic diversity was higher in woody habitats than in grasslands, which may be explained by the younger evolutionary age of grasslands. We found that taxonomic diversity is not always indicative of functional and phylogenetic diversity, highlighting the need of considering multiple aspects of diversity in future studies.

5. Implications

Species composition and CSR strategy gradient were arranged along the environmental harshness gradient. Taxonomic, phylogenetic, and functional diversities, as well as conservation value, had different patterns, peaking in different habitats of sandy forest-steppes in the Pannonian region. Consequently, practical conservation has to focus on multiple habitats simultaneously in sandy forest-steppes and similar mosaic-like ecosystems. Non-native tree plantations cannot substitute near-natural forests located in sandy forest-steppes regarding diversity and ecological values; therefore, protecting and reconstructing these forest patches are of high-priority.

We do not advocate further large-scale afforestation in sandy forest-steppes. However, whenever the establishment of plantations is unavoidable because of economic or legal reasons in the Kiskunság region, native tree species (e.g., *Populus alba*) should be preferred to non-native tree species. These plantations could serve as buffers around near-natural stands, and as green corridors among protected areas. Less intensive forestry, mimicking natural processes, could even increase the ecological value of these plantations while maintaining their commercial value. In addition, some *Populus alba* plantations could be set aside as it is reasonable to

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assume that, in the long run, their ecological value will increase. Also, selective thinning (rather than clear-cutting) would be beneficial in *Populus alba* plantations, as it would retain a continuous forest with low canopy cover (resembling the naturally low canopy cover of the near-natural forests). Importantly, our study indicated that forest edges were important in terms of diversity and conservation values; thus, including edges among native tree plantation patches (i.e., embedding open habitat patches into the planned plantation patch-mosaic) could increase habitat heterogeneity and support higher biodiversity.

LIST OF PUBLICATIONS RELATED TO THE THESIS

MTMT ID: 10080804

1. Erdős, L., **Ho, K. V.,** Bátori, Z., Kröel-Dulay, G., Ónodi, G., Tölgyesi, C., Török, P., & Lengyel, A., 2023. Taxonomic, functional and phylogenetic diversity peaks do not coincide along a compositional gradient in forest-grassland mosaics. Journal of Ecology, 111, 182–197. https://doi.org/10.1111/1365-2745.14025. **IF$_{2022}$ = 5.5 (D1)**

2. Erdős, L., **Ho, K. V.,** Bede-Fazekas, A., Kröel-Dulay, G., Tölgyesi, C., Bátori, Z., & Péter Török, P., 2024. Environmental filtering is the primary driver of community assembly in forest-grassland mosaics: a case study based on CSR strategies. Journal of Vegetation Science, 35. https://doi.org/10.1111/jvs.13228. **IF$_{2022}$ = 2.8 (Q1).**

3. **Ho, K. V.,** Süle, G., Kovács, B., & Erdős, L., 2024. Strong differences in microclimate among the habitats of a forest-steppe ecosystem. Időjárás, 128, in press. https://doi.org/10.28974/idojaras.2024.1. **IF$_{2022}$ = 0.9 (Q4)**

4. **Ho, K. V.,** Kröel-Dulay, G., Tölgyesi, C., Bátori, Z., Tanács, E., Kertész, M., Török, P., & Erdős, L., 2023. Non-native tree plantations are weak substitutes for near-natural forests regarding plant diversity and ecological value. Forest Ecology and Management 531, 120789. https://doi.org/10.1016/j.foreco.2023.120789. **IF$_{2022}$ = 3.7 (D1).**

5. **Ho, K. V.,** Ćuk, M., Šikuljak, T., Kröel-Dulay, G., Bátori, Z., Tölgyesi, C., Fűrész, A., Török, P., Hábenczyus, A. A., Hegyesi, A., Coşgun, Z. L., & Erdős, L., 2023. Forest edges revisited: species composition, edge-related species, taxonomic, functional, and phylogenetic diversity. Global Ecology and Conservation 46 (2023) e02625. https://doi.org/10.1016/j.gecco.2023.e02625. **IF$_{2022}$ = 4 (Q1).**

6. **Ho, K. V.,** Kröel-Dulay, G., Lengyel, A., Ónodi, G., Bátori, Z., Tölgyesi, C., Török, P., & Erdős, L., 2022. Functional structure reveals distinctions in plant community assembly of forest-grassland mosaics – a study case in hungary.

In: Molnár Dániel and Molnár Dóra (Eds), XXV. Tavaszi Szél Konferencia 2022, Absztraktkötet. May 6-8, Pécs, Hungary, p. 166-167.

7. Erdős, L., Török, P., Bátori, Z., Kröel-Dulay, G., **Ho, K. V**., Weldman, J. W., & Tölgyesi, C., 2022. Forest-grassland coexistence in Eurasian forest-steppes. In: Hrivnák R. & Slezák M. (eds), 30th Conference of the European Vegetation Survey: Plant communities in changing environment. Abstract book. May 9–13, 2022, Bratislava (Slovakia) Slovakia, p. 58.

8. **Ho, K.,** Kröel–Dulay, G., Lengyel, A., Ónodi, G., Bátori, Z., Tölgyesi, C., Török, P., & Erdős, L., 2023. Patterns of taxonomic, functional, and phylogenetic diversity in forest–grassland mosaics. In: Zasadil Petr, Ludvíková Vendula & Báldi András (Eds), 6th European Congress of Conservation Biology: Biodiversity crisis in a changing world. Book of abstracts, August 22–26, 2022, Prague, Czech Republic, p. 26.

9. **Ho, K. V.,** Kröel-Dulay, G., Tölgyesi, C., Bátori, Z., Tanács, E, Kertész. M., Török, P., & Erdős, L., 2023. Plantations cannot well substitute for near-natural forests in terms of diversity patterns and ecological value - a case study in Hungary. In: Hajdú Péter, (Eds), XXVI. Tavaszi Szél Konferencia 2023, Absztrakt kötet, May 5-7, 2023, Miskolc, Hungary, p. 101-102

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