Thesis of Doctoral Dissertation

The long-term effect of restoration practices and landscape composition on the restoration success of Pannonian grasslands

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Budapest,
2021

1. INTRODUCTION

In order to counteract land degradation and the resulting loss of biodiversity and ecosystem services, ecological restoration of degraded lands is considered crucial. To achieve this goal, ecological restoration should be scaled up that includes extending restoration to any kind of unused land, restoring larger landscapes, considering landscape features for prioritizing areas for restoration globally, and considering landscape factors and longer time scales when assessing restoration efforts (Strassburg et al. 2020).

Approximately 70% of the world's grasslands have been cleared or transformed to agricultural lands over the last two centuries or lost due to afforestation and, still, little attention has been paid to conservation and restoration of these habitats globally (Temperton et al. 2019). Grassy biomes and savannahs cover around a third of the land surface (Dudley et al. 2020), and host high species diversity (Habel et al. 2013). They also provide many other ecosystem services, e.g. water supply and flow regulation, carbon storage, erosion control, climate mitigation, pollination and, in Europe, they are also important for the cultural services they provide due to the long tradition of extensive management (Bengtsson et al. 2019). Currently, the main causes of grassland degradation besides land use change, i.e. conversion to arable lands, afforestation, land abandonment and incorrect management are urbanization, biological invasion and climate change (Habel et al. 2013).

In Hungary, grassland restoration has been classified among the top 50 conservation research priorities by Mihók et al. (2015). The need to restore more areas meets the opportunity, since the rate of land abandonment was accelerated in the last three decades (Valkó et al. 2016). There are several ongoing restoration projects and research that help elaborate best practices for grassland restoration. Restoration efforts aim to overcome the major limitation of spontaneous recovery: dispersal limitation that can be overcome by the introduction of target species (Kövendi-Jakó et al. 2019), abiotic factors, like the excess of nutrients (mostly nitrogen) in the soil after the abandonment of agricultural cultivation that can be reduced e.g. by carbon amendment (Halassy et al. 2021) and biotic factors, i.e. competitive dominance that is often managed by mowing that increase species diversity through the creation of establishment gaps (Valkó et al. 2012). The knowledge gained in previous projects might be scaled up in space and time to help restoration prioritization and to meet global restoration targets.

2. AIMS

The main aims of my study were (1) to evaluate and synthesize the long-term outcome of previous restoration interventions carried out by the Restoration Ecology Group of the Institute of Ecology and Botany, Centre for Ecological Research in the Kiskunság Biosphere Reserve, Central Hungary, and (2) to complete the evaluation with the impact of landscape characteristics in order to select best practices that can support scaling up the restoration of Pannonian sand grasslands in the Hungarian lowland, especially in the Kiskunság Biosphere Reserve.

2.1. The long-term effect of restoration practices on the restoration success of Pannonian grasslands

In the first part of my thesis, I have evaluated the long-term effect of different restoration practices in three separate field experiments. The first restoration experiment aimed to assist the recovery of Pannonian sand grasslands after the elimination of black locust (*Robinia pseudoacacia)* stands with the help of mowing. I studied the impact of initial mowing on vegetation recovery in the long term, up to 22 years. My questions were: 1. How does the vegetation develop with time due to initial mowing according to trajectory analysis? 2. How does initial mowing impact the relative cover of target and neophyte species in the long-term and in comparison to reference grasslands?

The second experiment aimed to assist the recovery of sand grasslands at abandoned agricultural fields by soil nitrogen immobilization through carbon amendment. I studied the long-term impact of initial carbon amendment on vegetation recovery, 20 years after the first application. My questions were: 1. How does the vegetation develop with time due to initial carbon amendment according to trajectory analysis? 2. How does initial carbon amendment impact the relative cover of target and neophyte species in the long-term and in comparison to reference grasslands?

The third experiment also aimed to assist the recovery of sand grasslands at abandoned agricultural fields, but treatments included mowing, carbon amendment and seeding. I studied the impact of initial treatments on vegetation recovery in the long-term, 16 years after the first applications. My questions were: 1. How does the vegetation develop with time due to initial seeding, mowing and carbon amendment according to trajectory analysis? 2. How do initial seeding, mowing and carbon amendment impact the relative cover of target and neophyte species in the long-term compared to untreated control?

2.2. The impact of landscape composition on the restoration success of Pannonian grasslands considering all experiments and treatments

In the second part, I have evaluated the impact of landscape composition on the success of restoration practices related to the three experiments. For this purpose, I have compared the impact of initial treatment (seeding, mowing and carbon amendment), the landscape composition (abundance of target and neophyte species in the landscape and distance from propagule sources) and the elapsed time since the restoration has started. The specific questions addressed were: 1. What is the importance of treatment (seeding, carbon amendment, mowing), the landscape composition and elapsed time on the restoration success in terms of target species and neophyte species? 2. Which of the studied treatments was the most effective in restoring Pannonian sand grasslands? 3. What is the impact of the external abundance of target and neophyte species and the distance from nearby propagule sources on restoration success? 4. What is the impact of the time elapsed since restoration started on restoration success?

3. METHODS

Mowing experiment at clear-cut black locust plantations. Three *Robinia pseudoacacia* stands were chosen that reflected a gradient of propagule availability of target species within the adjacent landscape (Bugac, Fülöpháza and Izsák). The *R. pseudo-acacia* plantations were clear cut and herbicide treated (Garlon® 4E) in 1994-1995. After removing the tree cover, a block of twelve adjacent plots of 10 mx 10 m was allocated for the experiment at each site, with six control (unmowed) and six treatment (mowed) plots randomly selected. Mowing was applied as a restoration practice twice a year (early June and early September) for five years between 1995-1999 in Bugac and seven years between 1995-2001 in Fülöpháza and Izsák. Reference areas were also selected in the neighboring open sand grasslands at each site to characterize the target community.

Carbon amendment experiment at abandoned arable fields. Three abandoned agricultural sites were chosen for the carbon amendment experiment. The three sites were approximately 200 m apart within the same abandoned farmland at Fülöpháza (Fabók-tanya). The three fields were arranged along an elevation and productivity gradient, varying from lower altitude and more productive to higher elevation and lower productivity. The sites were abandoned between 1991 and 1995. The experimental design was similar to the first experiment, with a block of twelve plots of 10 m x 10 m, where six of these were randomly set as control (no carbon applied) and six plots treated with carbon at each site. We applied carbon

amendment in the form of sucrose and sawdust, with different rates for the three sites for six years between 1998 and 2003. Two reference areas of open sand grasslands were also selected for comparison.

Seeding, mowing and carbon amendment experiment at abandoned arable fields. Three abandoned agricultural fields were originally involved in the third experiment, but only two of them could be followed on the long-term. The two fields differed in the time of abandonment (abandoned approx. in 1999 and in 1987). The same experimental design was applied in both sites. Ploughing and harrowing was applied as a preparatory treatment in 2002 to reduce the effect of standing vegetation. A block with 64 plots of 1 m^2 with 1 m paths between the plots was marked. The experimental design consisted of eight types of treatments randomly assigned to eight plots within each row, and the eight rows served as replicates. The treatments were control, mowing, carbon amendment, seeding alone or in combinations. Seeding was carried out by hand in 2002 and contained a mixture of five species (3.8 g/m^2) including: two dominant grass species, *F. vaginata* and *S. borysthenica*, a subordinate grass, *Koeleria glauca*, plus two forb species *Dianthus serotinus* and *Euphorbia seguieriana*. Mowing plus hay removal was applied twice in 2003 and once a year in September from 2004 until 2008. Carbon amendment was applied in the form of sucrose addition at a rate of 45 g/m^2 four times per year from 2003 to 2008. Only the main treatments (no combinations) were included in the present thesis.

Assessment of long-term vegetation development. The vegetation monitoring protocol was similar for all experiments, but the size and number of plots varied slightly (2 m x 2 m quadrates inside of the 10 m x 10 m plots for the first two experiments and only the treated plots, 1 m x 1 m for the third experiment). We estimated the cover of each vascular plant species in the permanent plots twice (in June and August) each year from the start till the end of experimental manipulations, and later re-sampled the sites at less frequent intervals. Of the two estimations within year, we used the maximum estimated cover value for each species per plot per year for further analysis.

Assessment of landscape composition. I have included all three experiments presented above in the landscape composition analysis. I considered each site as a separate block independent of the experiment. Reference plots were not considered. I used the long-term datasets of the three experiments to calculate the effect sizes based on the relative cover of target species and the relative cover of neophyte species as indicators of restoration success. Landscape variables, 1) the weighted abundance of target species and 2) the weighted abundance of neophytes, 3) the distance from primary grasslands and 4) plantations (as a source

of target and neophyte species, respectively) were assessed in a 500-m landscape buffer around the blocks based on field sampling.

Data analysis. For the long-term analyses, I have used PCoA to describe the trajectories of vegetation development by calculating the centroids of control, treatment and reference plots for each year and site along the first two axes based on the cover of species using the Euclidean distance. In addition, I have tested the changes in relative cover of target or neophyte species with time and treatment with the help of separate linear mixed effects models for each experiment and indicator followed by post hoc test.

In the landscape scale analysis, I have used two separate linear mixed effects models to investigate the effect of treatments, landscape variables and the time elapsed on restoration success. In the first model, I used the effect size of target species as response variable, and treatments, the weighted abundance of target species and the distance from semi-natural grasslands, plus time as fixed factors. In the second model, I used the effect size of neophyte species as response variable, and treatments, the weighted abundance of neophyte species and the distance from plantations, plus time as fixed factors. Significant fixed factors (treatments) were analyzed by post hoc test, and in case of significant or near significant ($p<0.05$) landscape variables, Pearson correlation was calculated between the response variable and the predictors.

4. NEW SCIENTIFIC RESULTS

4.1. The long-term effect of restoration practices on the restoration success of Pannonian Grasslands

4.1.1. Long-term effect of mowing at clear-cut black locust plantations

- According to the trajectory analysis, initial mowing successfully accelerates the recovery of sand grassland vegetation after clear-cutting *R. pseudoacacia*, where the dispersal of target species is not inhibited by surrounding forest plantation.
- In such cases, initial mowing helps the establishment and spread of target species compared to control.
- Mowed plots are also more prone to secondary invasion of non-native species, therefore can remain different from the reference grasslands even after 22 years without additional treatments.

4.1.2. Long-term effect of N immobilization through carbon amendment at abandoned arable fields

- Carbon amendment has only minor impacts on the vegetation development at abandoned fields.
- Carbon amendment has a delayed and temporary, but positive impact on the cover of target species.
- Carbon amendment does not affect the cover of neophyte species, that can stall the recovery of sand grassland vegetation.

4.1.3. Long-term effect of seeding, mowing and carbon amendment at abandoned arable fields

- Initial seeding proved to be the best method to accelerate the recovery of sand grassland vegetation.
- Seeding results in a higher relative cover of target species compared to control, mowing and carbon amendment. Seeded species are able to establish, persist and also spread into neighboring areas obscuring the differences between treatments with time, but assisting the restoration of old fields on the long-term.
- Seeding also suppresses the cover of neophyte species.

4.2. The impact of landscape composition on the restoration success of Pannonian grasslands considering all experiments and treatments

- From the studied factors (treatment, landscape composition and time) treatment presents the highest impact on the restoration success.
- All treatments positively affect target species but seeding has the most positive impact. Seeding suppresses, while mowing slightly favors invasion.
- A larger abundance of neophyte species in the landscape and the proximity to plantations increase the cover of neophytes in restoration areas.
- Treatment and landscape scale factors obscure the impact of time, when considering all experiments together.

5. CONCLUSION

- Restorative treatments can have the highest influence on the success of sand grassland restoration, overwriting the impact of landscape factors and time.
- From the three treatments applied, early seeding with a low diversity seed mixture of target species proved to be the best method in restoring sand grasslands in the long run, indicating that dispersal limitation is the most important constraint in grassland recovery and restoration.
- Seeded species were able to spread from the small introduction plots and colonize old fields, supporting the idea that there is no need to introduce seeds through the whole area, instead, the creation of smaller establishment windows from where the species can spread to the whole site can be a cheaper solution for large scale restoration.
- Early seeding, besides favoring the establishment of target species, can halt invasion spread in restoration areas, but further research is needed for understanding which native species should be introduced and when to prevent invasion.
- Mowing can be used to control woody encroachment – involving the re-sprouting of *R. pseudoacacia* – and to open up space for colonization, but the new spaces can be occupied also by other invasive species. Based on these findings, we suggest that mowing should be applied with low intensity and in combination with other treatments, e.g. seeding of target species and/or control of invasive species.
- Carbon amendment can temporarily lower soil available nitrogen, creating a window of opportunity for the development of target vegetation if applied in combination with seeding right after cropland abandonment.
- Landscape factors should be considered in restoration prioritization to increase efficiency and to support scaling up the restoration of degraded dry grasslands.
- A higher abundance of neophyte species in the surrounding landscape and a shorter distance from plantations negatively affect the success of restoration on the long term, indicating that further management is needed to assist the recovery of such areas.
- Long-term monitoring is essential in restoration ecology, as treatment effects need several years to result in a visible impact on vegetation development and initial trends are not always confirmed on the long-term.
- Abandoned croplands should be considered as priority areas to scale up ecological restoration, since they offer an opportunity to create new semi-natural habitats.

6. RELATED PUBLICATIONS

6.1. Accepted papers

Llumiquinga Y.B., **Reis B. P.**, Sáradi N., Török K., Szitár K. & Halassy M. (2021). Long-term results of initial seeding, mowing and carbon amendment on the restoration of Pannonian sand grassland on old-fields. (under publication in *Tuexenia*).

Halassy M., Kövendi-Jakó A., **Reis B. P.,** Szitár K., Seyidova Z. & Török, K. (2021). N immobilization treatment revisited: a retarded and temporary effect unfolded in old field restoration. *Applied Vegetation Science*, e12555.

Halassy M., Kövendi-jakó A., **Reis B. P**., Sáradi N., Szitár K. & Török K. (2020). Nyílt homokpusztagyep helyreállítási lehetőségei akác ültetvények helyén: a kaszálás hosszú távú hatása. *Természetvédelmi Közlemények* 26: 28–38.

Reis B.P., Kövendi-Jakó A., Szitár K., Török K. & Halassy M. (2020). Long-term effect of mowing on the restoration of Pannonian sand grassland to replace invasive black locust plantation. *Restoration Ecology*.

6.2. Manuscripts under review

Reis B. P., Szitár K., Kövendi-Jakó A., Török K., Sáradi N., Csákvári E. & Halassy M. (2021). The long-term effect of restoration interventions, landscape composition, and time on the restoration success of Pannonic sand grasslands. (manuscript sent in *Land degradation & Development*).

Reis B. P., Kövendi-Jakó A., Csákvári E., Szitár K., Török K., Sáradi N., Llumiquinga Y.B. & Halassy M. (2021). Seeding of target species is more effective than mowing or carbon amendment to restore sandy grasslands in old-fields based on long-term evaluation. (manuscript sent in *Ecological Engineering*).

6.3. Oral and poster presentations in conferences

Reis B. P., Kövendi-Jakó A., Szitár K., Török K. & Halassy M. (2020). Restoration of sand grasslands to replace invasive *Robinia pseudoacacia* plantation: effects of long-term mowing. In: Vili N., Báldi A., Kovács-Hostyánszky A., Mázsa K. (szerk.) Abstracts, *6th Student Conference on Conservation Science*, 25 – 29 August 2020, Centre for Ecological Research, Tihany, Hungary, pp. 24. (Oral)

Sáradi N., **B. P. Reis**, Szitár K., Csákvári E., Török K. & Halassy M. (2020). The appearance and spread of invasive species during the restoration of Pannonic sand steppes. In: Vili N., Báldi A., Kovács-Hostyánszky A., Mázsa K. (szerk.) Abstracts, *6th Student Conference on Conservation Science*, 25 – 29 August 2020, Centre for Ecological Research, Tihany, Hungary, pp. 26. (Oral)

Reis B.P., Kövendi-Jakó A., Szitár K., Török K. & Halassy M. (2019). Long-term effect of mowing on the restoration of Pannonian sand grassland to replace invasive black locust plantation. *Öbi Szeminárium*, 7 November 2019. (Oral)

Reis B.P., Kövendi-Jakó A., Szitár K., Török K. & Halassy M. (2019). Long-term effect of management to replace invasive tree plantations with Pannonian sand grassland. *European Ecological Federation Congress*, Lisbon, Portugal, 29-02 August 2019 - Book of abstracts. p. 421. (Oral)

Reis B.P., Kövendi-Jakó A., Szitár K., Török K. & Halassy M. (2018). Long-term effect of management on the restoration success of Pannonian sand steppe on previous *Robinia*

pseudo-acacia plantation. *8th Meeting of PhD students in Plant Ecology and Botany*, Telč, Czech Republic, 26-28 October 2018. (Oral)

Reis B.P., Kövendi-Jakó A., Szitár K., Török K. & Halassy M. (2018). Long-term effect of mowing on the restoration success of Pannonian sand steppe at clear-cut *Robinia pseudo-acacia* plantation. *Student Conference in Conservation Science (SCCS) Europe***,** Tihany (Lake Balaton), Hungary, 4 - 8 September 2018. (Oral)

Halassy M., **Reis B.P.,** Kövendi-Jakó A. & Török K. (2018). Long-term effect of climate on the restoration success of Pannonian sand grassland. *Society of Ecological Restoration Europe Conference* (SERE), Reykjavik, Iceland, 9-13 September 2018 - Book of abstracts p.30. (Oral)

Reis B.P., Kövendi-Jakó A., Szitár K., Török K. & Halassy M. (2019). Long-term effect of mowing on the restoration success of Pannonic sand steppe at clear-cut *Robinia pseudo-acacia* plantation. *International Association for Vegetation Science (IAVS) Symposium*, Bremen, Germany, 14-19 June 2019. (Poster)

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