Final report – PD 128302

Biodiversity, soil seed banks and grassland restoration

1 Aims of the project

In grassland restoration seed sowing is a widely established practice, but still there are several open questions related to the composition, density and sowing time of the seed mixtures. Grassland restoration projects have gained a new momentum recently, but it is important to fine-tune the existing methods to maximize the restoration success. Seed bank can have a role in spontaneous regenerative capacity of the grasslands and it can be used for predicting the dynamics of invasive and weed species; thus, the knowledge of species composition of the seed bank is important in grassland restoration projects. During the project we evaluated the success of seed sowing in grassland restoration, analyzed the role of the density, diversity and sowing time of the seed mixtures and we also studied the role of the seed bank in the vegetation dynamics of the restored grasslands. Four papers in WoS journals have been published from the results of the project along with 6 papers partly connected to our research.

2 Key results of the project

2.1 Timing of seed sowing: grasses and diverse seed mixtures

In a sowing experiment we examined the success of grassland restoration using different seed sowing measures. The arrangement of the experiment was the following: altogether in 36 sampling blocks nine different seed mixture combinations (treatments) were sown. On four study site we sown low diversity seed mixture that consist of only grass species. Plots received grass seeds (G; *Festuca pseudovina*), diverse seed mixture (D) or the combination of both (G+D). In all treatments, grasses were sown in 2014 (Year 0); while sowing the diverse mixture had four levels: sowing in Year 0, 1, 2 or 3, i.e., sowing together with, or 1, 2 or 3 years after the sowing of grass seeds. Each treatment had four replications. We performed vegetation sampling in late spring (in May) and in autumn (in October) in each year during the OTKA project. Within the blocks, there were four 1-m^2-sized permanents plots where we recorded percentage cover of vascular plants. With repeated measures GLMM models we evaluated the effect of grass-matrix age (i.e., age of F. pseudovina sown plots before D seed mixture was sown (0-3) or fallow age (i.e, age of empty plots before D seed mixture was sown (0-3)). The dependent variables were total vegetation cover, weed cover, cover and species richness of sown species. We found that weed control was the most effective in treatments where the grass and diverse seed mixtures were sown together or maximum one year later. Where only grass seeds were sown, we observed a fast weed suppression already in a first year, but the developed vegetation remained species-poor during the whole experiment. We found that sowing diverse mixture 2 or 3 years after both grass sowing was much less effective compared to treatments

where the D mixture was sown together or 1 year later than the grass seeds. Also, we found that sowing of diverse seed mixture is more effective when there is no or only 1-year-long fallow period; after longer fallow periods, the establishment success of sown species was lower.

2.2 Seed bank of restored grasslands

We sampled the soil seed bank composition in restored grasslands under two settings: sites sown with grass seeds in Hungary, and sites restored by diverse seed mixture sowing in the White Carpathians, Czech Republic. In Hungary, we followed the vegetation development and seed bank dynamics of 14 sites sown with two types of grass seed mixture 3 and 8 years after restoration, to study both early- and mid-successional stages. We also studied the effect of continued management vs. abandonment on the vegetation and seed bank dynamics. We found that despite the successful recovery of the aboveground vegetation in the managed grasslands, the seed bank remained dominated by weed species even 8 years after restoration. We also found that the continuation of the management is crucial for maintaining the results of the restoration projects, as in the abandoned plots the weeds could effectively establish from their dense seed bank. In the Czech Republic we made vegetation sampling in permanent plots in restored grasslands along a chronosequence to compare the dynamics of vegetation and seed bank. We also sampled the seed bank of arable fields (representing starting conditions of restoration) and reference grasslands (target state of restoration). We germinated the seed bank samples in a greenhouse for two years to have a complete list of the seed bank species pool. The germination project was ended in 2021, and now we are in the phase data recording and processing. Based on the preliminary results, we found that the similarity of vegetation and seed bank was generally low, and the similarity decreased during the succession.

3 Publications

3.1 Core papers related to the project

Importance of the long-term management. We found that abandonment of management had considerable effect both on the vegetation and seed bank of restored grasslands, which suggests that it is important to plan the long-term management of the restored sites. Cessation of mowing resulted in a decline of the cover of the sown perennial grass species and increased the cover of weeds. The seed bank was characterized by annual weeds and just only some target species even eight years after restoration. This implies that the build-up of the seed bank of target species is a low process in the studied ecosystem and the seed bank has only a limited potential to maintain the species richness of the restored alkaline and loess grasslands (Valkó et al. 2021 *Restoration Ecology*).

Establishment gaps in species-poor grassland. In this study we use establishment gaps to increase the species richness of grasslands that were restored by sowing low diversity seed mixtures. Establishment gaps were effective in decreasing microsite limitation (removal of the aboveground biomass) and propagule limitation (sowing diverse seed mixtures). We studied

the effect of gaps size and found that the most feasible was the 4 m^2 gap for the establishment of target species and also for the colonization of the target species in the surrounding species-poor grasslands (Kiss et al. 2021 *Restoration Ecology*).

Germination experiment of invasive species. In restoration ecology it is important to know the germination capacity of invasive species under different environmental conditions. We examined the effect of soil burial depth and litter accumulation on the germination of different invasive species. In general we assumed that the seed size was an important driver shaping the specific responses to soil burial or litter cover. However we found large species-specific differences that highlight the importance of using specific information on species' response to burial depth and litter accumulation in planning management or restoration in areas threatened by plant invasions (Sonkoly et al. 2020 *Journal of Vegetation Science*).

Biomass production in restored grasslands: effects of sowing density and diversity. In this experiment we sown seed mixtures with different grass and forb ratio. We examined the relationship of biomass production and diversity and we studied the effect of perennial grass biomass and species diversity, evenness and functional diversity. We found that the species number and functional richness decreased with the increasing abundance of perennial grasses, but species evenness showed a humped-back relationship (Sonkoly et al. 2019 *Scientific Reports*).

3.2 Other papers partly related to the project

During the project period, we published other papers that are partly related to the OTKA project. These papers are all important for certain aspects of grassland restoration, invasive species control or post-restoration management.

- We studied effect of grazing on the dispersal of sown species from establishment gaps and tested which plant traits contribute to the successful establishment of sown species to the surrounding grasslands (Kiss et al. 2021 *Journal of Vegetation Science*).
- We compared the effects of grazing of traditional breeds and crossbred beef cattle in lowland marshes and wet alkaline grasslands (Kovácsné Koncz et al. 2020 *Applied Vegetation Science*).
- We examined human-mediated seed dispersal on cloths, that can be an important pathway of the dispersal of invasive plant species (Valkó et al. 2020 *Neobiota*).
- We tested the dietary choices of cattle in species-rich meadow steppes (Balogh et al. 2021 *Tuexenia* in press).
- We studied the effect of different mowing regimes vs abandonment on the biomass of fen meadows (Kiss et al. 2018 *Gyepgazdálkodási Közlemények*).
- We published a book chapter where we gave practical recommendations about grassland restoration for the wide public (Valkó et al. 2019).

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