FINAL REPORT

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The invasion of non-native plant species has become a pressing ecological issue, impacting biodiversity and ecosystem functions globally. While the effects of single invasive species on native communities are often documented, less is known about the combined effects and interactions between multiple invasive species.

The aim of the project was to gain new knowledge about how multi-species invasions affect vegetation. More specifically, I targeted questions about the main and interaction effects of co-invading alien plants on the taxonomical and functional alpha and beta diversity, both on local and landscape scales, as well as the assembly rules of recipient communities. The study object is the grasslands and old-fields in the north-eastern edge of the Gödöllő Hills region, where *Asclepias syriaca*, *Solidago gigantea* and *S. canadensis* are widely spreading.

WP1 - Testing the main and interaction effects of the cover of Solidago spp. and Asclepias syriaca on community diversity

This work package examined the individual and combined effects of *Asclepias syriaca* and *Solidago* spp. on the taxonomic and functional diversity of dry, sandy old-fields on local scale. The objectives were to (1) assess the individual and combined impacts of *Asclepias* and *Solidago* on plant community diversity and structure and (2) test whether interactions between these invasive species modify their main effects on the invaded community.

Four old-field sites were selected based on their invasion by *Asclepias* and *Solidago*. The study included 80 2m x 2m plots across the four sites, sampled in 2022. Plots varied in *Asclepias* and

Solidago cover. In each plot, the cover of vascular plant species was visually estimated. Plant traits (e.g., height, specific leaf area, seed mass) were obtained from open databases to calculate functional diversity metrics.

I calculated several diversity indices, including species richness, community completeness, and functional diversity (Rao quadratic diversity for all traits and for each trait separately, community-weighted means [CWM] for each trait). These metrics were evaluated both with and without including the invasive species' traits to capture their influence on overall ecosystem function and on accompanying species, respectively. Generalized linear models (GLMs) and mixed models (GLMMs) were applied to examine relationships between invasive cover and diversity metrics, adjusting for site-specific variations. Attila Rigó contributed to this work with data management.

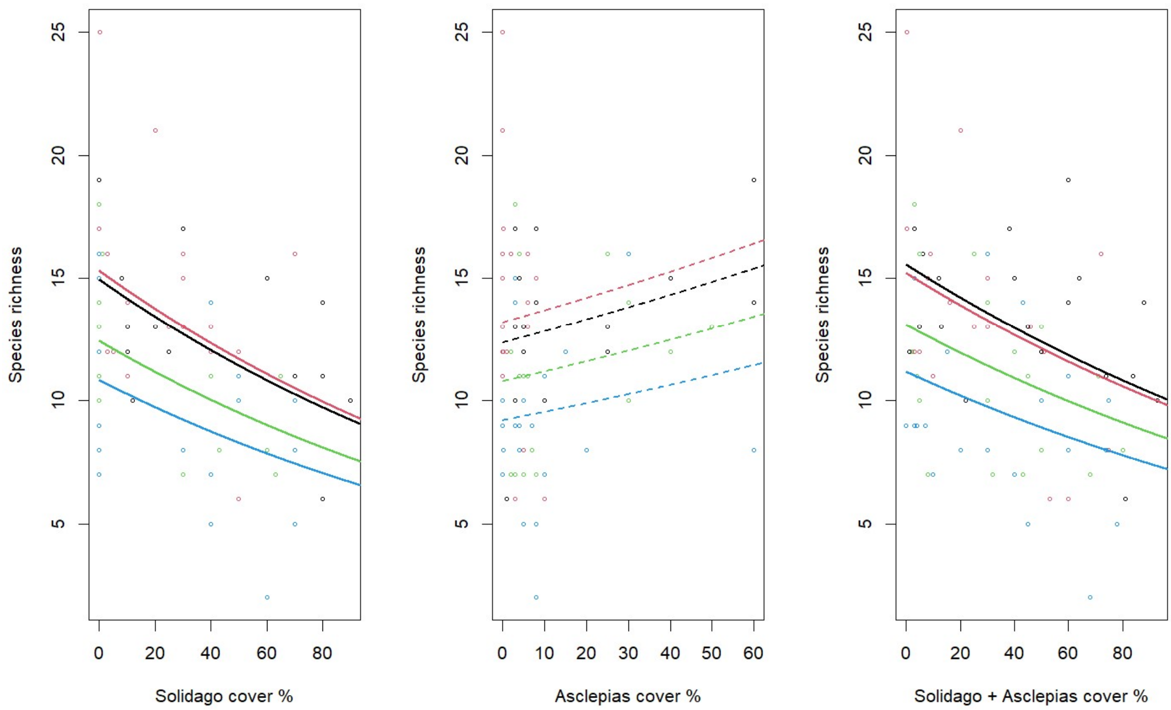

Figure 1. Relationship of *Solidago* cover, *Asclepias* cover, and their summed cover with species richness without considering invasive species. Solid line: significant relationship; dashed line: non-significant relationship; colours: sites.

Solidago cover significantly reduced species richness and community completeness, confirming that it strongly limits plant diversity in the invaded areas (Fig. 1). *Asclepias* cover had no significant impact on species richness or community completeness. This pattern aligns with earlier studies suggesting that *Solidago* is more disruptive to native communities than *Asclepias*, which may even facilitate certain native species under stress, such as drought conditions. *Asclepias* and *Solidago* cover was negatively correlated, and their combined effects did not show any significant interactions. This lack of interaction suggests that each species impacts the ecosystem independently rather than synergistically.

Including the traits of *Asclepias* and *Solidago* in the analysis revealed distinct shifts in CWMs or functional diversity. This was due to the fact that both *Asclepias* and *Solidago* possess some extreme trait values in comparison with other species of the sites. In accordance with the mass ratio hypotheses (Grime 1998), if a species with a particular trait value becomes dominant, the trait distribution of the community will follow this trend.

I found surprisingly few significant effects of invasive cover on trait-based response variables. Only the CWM of bud bank score increased with *Solidago* cover. This trait (or some of its correlates) seems to be key for resident species to coexist with *Solidago.*

The study's results have significant implications for managing multi-species invasions in sandy grasslands. Given *Solidago*'s dominant effect on reducing diversity, management strategies may need to prioritize controlling *Solidago* populations in sensitive areas. In contrast, *Asclepias* may not require as intensive management, as it appears less harmful to local diversity. The lack of interaction between the two species also suggests that they can be managed independently, without the need to account for combined effects on biodiversity.

After two conference poster publications, I have submitted a manuscript with these results to the special issue dealing with biological invasions in the Journal of Vegetation Science (Q1). The manuscript is available as a preprint here: https://doi.org/10.32942/X2VH0J

WP2 – Effect of invasive species dominance on community assembly rules

The aim of this work package was to examine how *Asclepias* and *Solidago* invasion influences the landscape-scale distribution of fine-scale vegetation diversity. The realization deviated from the plans at some points of the methodology. Instead of nested 'microplots' nested within 'macroplots', I only sampled a pair of 'microplots' (hereafter only called 'plots'), from which the 'macro-'-level analogue could be obtained by pooling the pair. With this, I avoided complications of having species absent in microplots but present in macroplots. I sampled 40 pairs of plots invaded by *Asclepias* (18 pairs) or *Solidago* (22 pairs) and uninvaded, in close vicinity (<10 metres), with the assumption that there is no difference between the members of the pair in environmental background and history. Despite the abundance of *Asclepias* and *Solidago* in the landscape, it was unexpectedly challanging to find suitable locations for the plots because in many cases, the populations of invasive plants followed fine-scale historical patterns, e.g. wild boar rooting or management borders, which would strongly limit comparability within the pair. I also tried to sample vegetation with as narrow time interval as possible to minimize the effect of temporal changes. The drought in 2022 also reduced the period suitable for sampling. Finally, the number of plots has been lower than planned, but still 2x as high as what Hejda et al. (2009) used in a similar study (10 pairs per species), thus I consider them sufficient for a conclusive study. With the inclusion of drier and more mesic sites, I covered a longer environmental gradient than in WP1.

I tested pairwise difference in species richness, effective species number with various *q* values, functional diversity and community-weighted mean trait values between invaded and uninvaded plots using the same traits as in WP1. In 2024, for some species missing from the trait databases (e.g. *Festuca pseudovaginata*, *Hierochloe repens*, *Pilosella echioides*), I obtained new measurements for leaf size, fresh mass and dry mass, from which specific leaf area and leaf dry matter content traits can be calculated. Anikó Csecserits contributed to trait measurements.

Originally, I planned to collect soil samples from each plot making it a significant item in the budget plan. However, since the submission of the application (early 2021), all types of planned expenses had been increasing so steeply that I had to re-arrange the budget. The scientific role of soil sampling was not to test hypotheses on soil properties but to verify that the plot pair is coming from a homogeneous environment. However, I realized that soil samples may not be fully reliable in this respect anyway, because invasive species can change the soil properties when becoming dominant. That is, there can be difference in soil properties even if the location of the plot pair had been originally homogeneous before the invasion (Zhang et al. 2009). Hence, I decided to remove soil sampling from the plans.

I fitted generalized linear mixed effect models on the diversity and CWM indices in which the predictors were the invadedness of the plot (invaded/uninvaded), the identity of the invasive species represented in the plot pair (*Asclepias*/*Solidago*), and the interaction term of these two. The plot pair was used as a random factor.

Surprisingly, I found no significant effect of invasion in any of the models at $p<0.05$ level. That is, neither invasive cover, nor the identity of the invasive species had any general effect on the species richness, trait diversity and trait composition of the sampled grasslands (e.g. Table 1). This result contradicts the popular perception of the effects of alien species, and much care should be taken to avoid misinterpretation. These negative findings seem to be another example of a recurrent pitfall in invasion biology: context-dependence (Pyšek et al. 2020, Fenesi et al. 2023). The most likely explanation is that the sampled grasslands are so heterogeneous that the fine-scale dominance of an alien species might not cause the same type of directional change for all of them.

Model parameters	*z*	*p*
(INTERCEPT)	43.11	<2e-16 ***
INVASIVE_COVER	-1.43	0.154
INVASIVE_SPECIES	0.65	0.513
INVASIVE_COVER * INVASIVE_SPECIES	0.92	0.357

Table 1. Results of generalized linear mixed-effect models fitted on species richness. None of the predictors had a significant effect

Due to the lack of clear-cut conclusions, I could only publish two conference posters together with the results of WP3.

WP3– Effect of invasive species dominance on beta-diversity

This work package complemented WP2 with a multivariate approach. The data were the same as in WP2 but the aims were to test the effect of *Asclepias*/*Solidago* dominance on community composition. I calculated dissimilarity matrices between all plots using dissimilarity indices sensitive to presence/absence and abundance data, with and without accounting for trait similarity between species. I tried the methodology based on the regression of dissimilarity matrices proposed in the research plan but it seemed too complicated and not straightforward.

Therefore, I fitted PERMANOVA models instead on the dissimilarity matrices with the invadedness, the invasive species, and their interaction as explanatory variables. I also ran non-metric multidimensional scaling ordination to visually inspect the multivariate structure of the data.

Model terms	*Degrees of freedom*	R^2	*F*	*p*
INVASIVE_COVER	1	0.01133	0.901	0.594
INVASIVE_SPECIES	1	0.0239	1.8999	0.010 **
INVASIVE_COVER * INVASIVE_SPECIES	1	0.00873	0.6944	0.909
RESIDUALS	76	0.95603		
TOTAL	79	1		

Table 2. Result of the PERMANOVA model fitted on the between-plot taxonomical dissimilarities without considering invasive species. Only the main effect of the invasive species was significant; however, the model explained only the 4.5% of the total variation.

I found only one significant effect ($p<0.05$) in three models: the main effect of invasive species was significant on community taxonomical dissimilarity with and without considering the invasive species with presence/absence data, and with considering the invasive species with abundance data (e.g. Table 2). In accordance with this, the ordination showed a slight shift in between plot pairs characterized by *Asclepias* and *Solidago* (Fig. 2).

These results suggest that invasion by *Asclepias* and *Solidago* does not affect the community composition in a consistent way. The significant effect of the invasive species can be attributed to the difference between the habitat preference of the two focal alien plants, *Asclepias* being more concentrated on more drier sites.

As with WP2, the lack of support to the effect of invasive species is counter-intuitive. Especially because I cannot provide satisfactory causal explanation to them, I published only two conference posters together with the results of WP2.

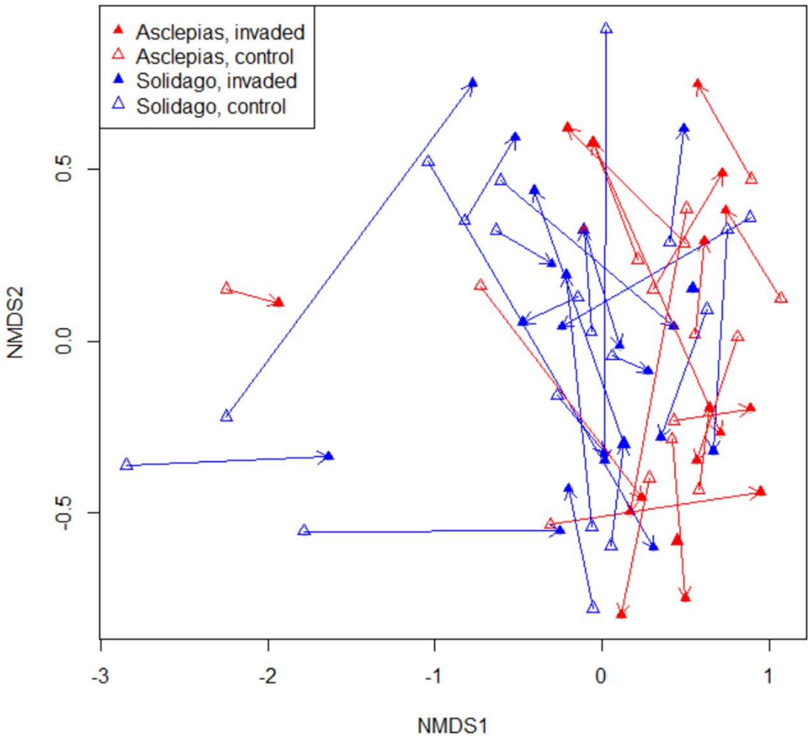

Figure 2. Scattergram of plot pairs in the space of non-metric multidimensional scaling based on 1-Jaccard dissimilarity. There is a hint of separation according to the invasive species characterising the plot pairs.

Follow-up 1 – Synthesis of functional dissimilarity indices

Partly due to the difficulties in finding 'positive' results, I was experimenting with a large variety of methods on the data for all WPs, especially for seeking patterns in trait composition and diversity. This re-ignited one of my abandoned works with Zoltán Botta-Dukát, a comparative review of trait-based dissimilarity indices. Earlier we worked on an empirical test of the indices but could not reach a proper final conclusion, and we could not publish it in a journal. Recently, we chose a more theoretical way, and provided a conceptual synthesis of trait-based dissimilarity indices through evaluating the methodological decisions that should be considered to choose among them. The first such decision should be made according to the type of trait data available, with three options: (1) typical values, (2) discrete sets, (3) hypervolumes (Fig. 3). The second is the concept of dissimilarity to follow: (1) distance, (2) disagreement.

Out of these 3×2 combinations we discuss the five realistic ones, and review the indices representing them. This work was published in the Ecography journal (Lengyel & Botta-Dukát 2023).

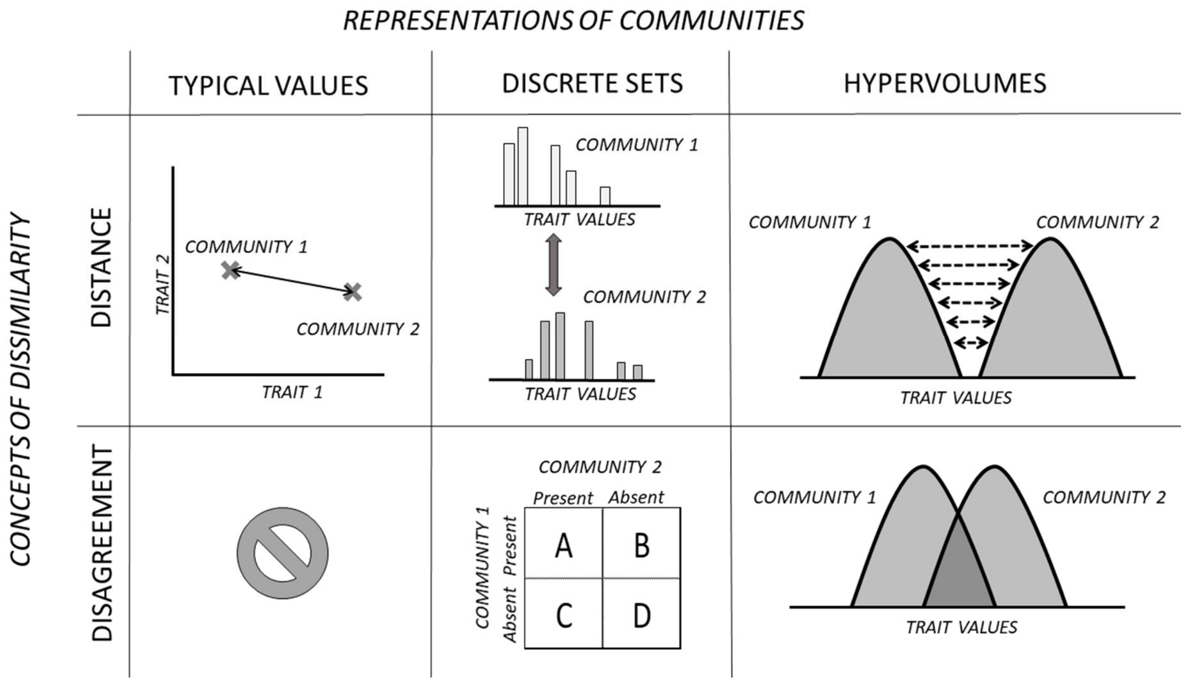

Figure 3. A scheme of the classification of functional dissimilarity measures

Follow-up 2 – Effect of invasive species on the phenology of resident plants

In seek for the mechanism behind the lack of support for invasive species affecting recipient plant communities, I considered the possibility that the effect might not be a change in presence or abundance of particular accompanying species but the phenology of these species. I recognized it as a research gap. I planned to investigate how the phenology of resident species changes if they are dominated by *Asclepias* or *Solidago*. Luckily, I was contacted by a student, Levente Horváth from the University of Veterinary Medicine, looking for supervisor. With my guidance to locate plot pairs in field and to sample vegetation, in 2024, Levente sampled 9 uninvaded/invaded pairs of plots with *Asclepias* and 12 pairs with *Solidago* from March to September each month, and recorded the abundance of flowering species. Then, we fitted generalized linear mixed effect models on the number of flowering individuals as response variable with sampling date, invadedness, and their interactions for each resident species and each invasive species. We compared models with AIC, and selected the best model for each pair of resident and invasive species.

For most species, under *Solidago* invasion, the model containing the sampling date and the invasive species without interaction was the best model. *Viola arvensis* was the only species showing positive response to *Solidago* invasion, all the others had similar or reduced number of flowering individuals in the invaded plots. Under *Asclepias* invasion, there were larger variation in the best models. In two cases, the model with interaction terms was the best: *Cerastium brachypetalum* and *Plantago arenaria* flowered earlier under *Asclepias* compared with uninvaded plots.

The results were published on a conference and is submitted for Conference of Scientific Student Associations ("TDK").

Conclusions

I supported earlier results, e.g. *Solidago* reducing species number of recipient communities, the dubious role of *Asclepias* in dry, sandy environments. The effects on alien plant dominance on local vegetation seems highly context-dependent making general conclusion difficult to achieve. The interaction effect between these two alien species does not seem significant. Based on preliminary results, the phenological change of the recipient community might be a promising field of future research.

References

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