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PhD Thesis Abstract

**THE SIGNIFICANCE OF ARBUSCULAR MYCORRHIZAL
ASSOCIATION IN THE ECOPHYSIOLOGY OF GRASS SPECIES
DIFFERING IN INVASIVENESS**

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I. Introduction and aims

Plant invasions are today among the greatest threats to biodiversity worldwide. Research programmes in ecology and conservation biology have an important role in investigating invasion events and exploring their causes in the hope of developing useful preventive measures against invaders. After establishment, invasive species can spread quickly in natural habitats, thus altering original ecological interactions, which – together with their better competitive ability – results in the gradual disappearance of resident species. Their ability to outcompete other species lies partly in their phenology and physiology. By modifying ecological interactions, invasive plants alter the biotic environment of native species in invaded habitats in a short period of time. This rapid change favours native species that have greater plasticity to environmental factors. From the invaders' perspective plasticity is also favoured, since not only they have to establish new biotic interactions with native species, but furthermore, they have to tolerate the abiotic environment of their new habitat as well. In conclusion, physiological and morphological plasticity can help plants to populate spatially and/or temporally heterogeneous environments (e.g. Fekete 1974, Bazzaz 1996, Mojzes *et al.* 2003).

In Hungary native or naturalised herbaceous species show invasive properties in the Poaceae family (they invade new habitats, decrease the abundance of resident species and modify the functioning of ecosystems), henceforth these species will be referred to as invasive (Valéry *et al.* 2008, 2009). *Cynodon dactylon* and *Calamagrostis epigeios* were thus considered invasive species in this study. *C. dactylon* is listed on second place among the world's most noxious weeds (Holm *et al.* 1977), while *C. epigeios* invades new habitats and facilitates their degradation. *Brachypodium pinnatum* and *Bothriochloa ischaemum* are resident species in the natural habitats studied here. These species were considered post-disturbance dominant “local invaders” due to their aggressive expansion upon disturbance.

Arbuscular mycorrhizal fungi may be intermediaries in plant invasions due to their wide range and key role in natural ecosystems. Exotic plants without obligate dependence on an AMF symbiont have greater chance to become invasive in the new community compared to those with strong AMF associations (Pringle *et al.* 2009). Facultative mycorrhizal plants can establish symbiosis with new AMF taxa, but cooperation may be less efficient with different fungi. The carbon cost of sharing assimilates with mycorrhizal fungi can be quite high. Thus, lacking tight interspecific associates not only allows easier spread and establishment of the species, but also spares the plant the considerable cost of the fungal symbiont, resulting in greater competitive ability. Indeed, AMF associations were found less frequent among invasive species than native species (Pringle *et al.* 2009).

Soil microbial community may be changed indirectly via the mass effect of the invasive plant being non-mycorrhizal or having different AMF symbionts favouring the invader over resident species (e.g. Callaway *et al.* 2008, Zhang *et al.* 2010). An intact and undisturbed soil microbial community increases the resistance of natural ecosystems against invasion. However, invasive species can disrupt and alter soil AMF community, making persistence or regeneration harder for these ecosystems. Human activities that result in vegetation and soil disturbance often make AMF communities degrade (e.g. Reeves *et al.* 1979).

Through three case studies I examined the effect of restrictive abiotic environment on the phenology, morphology, photosynthesis and mycorrhization of grass species differing in invasiveness both in the field and in growth room. Furthermore, I studied how mycorrhizal colonisation of resident species changes in habitats invaded by either a „local invader” or a naturalised invasive plant.

The following questions were addressed:

1. What are the effects of the warmer, drier microclimate with greater solar irradiation on the phenology, photosynthesis and mycorrhization of *Brachypodium pinnatum* transplanted to SW-facing slopes of a loess grassland?
2. Is the phenotypic plasticity of *B. pinnatum* enough to tolerate the microclimate of the SW slopes?
3. Does mycorrhizal colonisation of roots of non-invasive species differ from those of invasive species?
4. Is there a difference in the extent of mycorrhizal colonisation of non-invasive grass species in invaded and uninvaded habitats?
5. Does the lack of mycorrhizal association influence the response of invasive and non-invasive species to soil drying?

II. Material and methods

In the first case study I examined the effects of the warmer, drier microclimate on *B. pinnatum* plants transplanted from the NE-facing slopes of a loess grassland to the SW-facing slopes near Isaszeg, in a valley at the northernmost part of the Great Hungarian Plain. Soil monoliths with *B. pinnatum* plants were transplanted from the steppe type *B. pinnatum* grassland on the NE-facing slope to the xeric grassland on the SW-facing slope. I used *B. pinnatum* plants transplanted *in situ* on the NE-facing slope as control. I observed shoot morphology, leaf phenology of the plants during a vegetation period in 2002. Also, chlorophyll fluorescence induction measurements were performed using a pulse-modulated chlorophyll fluorometer in June and July 2002 and in July

2003. Mycorrhizal colonisation was measured in a similar loess steppe situated at the northern edge of the Mezőföld region, approx. 55 km away from the first site, where plants were transplanted in a similar fashion, from the NE-facing slope to the SW-facing slope of a valley. Root samples were collected three times in 2002 (May, August and October) from 5 plants (control and transplanted as well) each time of sampling and stained with aniline blue, in order to stain fungal structures inside the roots. Colonisation of roots by AM fungi, frequency of arbusules and vesicles were estimated by using the method described by Trouvelot *et al.* (1986). Repeated measures ANOVA (fixed effect was the slope aspect, while the repeated effect was the time of day – dawn and noon) was used to analyze the effect of different slope aspect on means of maximum photochemical efficiency (Fv/Fm). Two-way ANOVA with seasons and exposures as grouping variables was used to analyse significant differences among means of calculated mycorrhizal indices (with Sidak post hoc test). The other variables were compared with two sample t-test. When data points differed between the two datasets t-test with Welch correction was used. Significance level for the tests was set to $P < 0.05$ throughout.

In the second case study I examined whether roots of invasive species differ in their colonisation rate by AMF from the roots of resident native species and whether native species exhibit a different degree of colonisation by AMF in invaded habitats compared to uninvaded ones. To test the first hypothesis I examined the mycorrhizal colonisation of six grass species in a loess grassland near Budapest. For each species 5 specimens, chosen randomly, were collected. The species studied fall into three categories of invasiveness and in each category of invasiveness, two plant species, one C₃ and one C₄, were selected: invasive grasses (C₃: *C. epigeios*, C₄: *C. dactylon*), resident species reaching local dominance upon disturbance –so called „local invaders” (C₃: *B. pinnatum*, C₄: *Bothriochloa ischaemum*) and non-invasive native species (C₃: *Bromus inermis*, C₄: *Chrysopogon gryllus*). To test the second hypothesis I studied the effect of *C. dactylon* and *C. epigeios* gaining dominance in sand grasslands on the Great Hungarian Plain. Native, non-invasive species were *Festuca vaginata* and *Stipa borysthena*, both characteristic dominant species of sand grasslands. From the three stands (no invasion, invasion by *C. epigeios*, invasion by *C. dactylon*) 5 specimens of both native species were collected. Colonisation of roots by AM fungi, frequency of arbusules and vesicles were estimated by using the method described by Trouvelot *et al.* (1986). Mycorrhizal colonisation indices were compared between invasiveness classes by using one-way ANOVA with Tukey HSD post hoc test for comparison of means. The significance level was set to $P < 0.05$ throughout. If the homogeneity of variances assumption was violated, ANOVA with a sandwich covariance matrix estimator was used.

In the third case study in a growth room experiment I tested whether the presence of the original soil microbiota ameliorates the influence of soil drying on the growth and physiology of two non-invasive resident (*Danthonia alpina*, *Ch. gryllus*) and two invasive (*C. epigeios*, *C. dactylon*) grass species typical in semiarid temperate grasslands in Hungary. Seeds were germinated on sterilized sand in petri dishes under laboratory conditions. Seedlings of the same species were planted in pots (of 1.5 l volume) filled with the same amount of a 1:1 mixture of perlite and sieved soil collected in the plant's original habitat. A factorial experiment was set up to test the effects of AMF and water shortage on photosynthesis and growth of the plants differing in invasiveness. For the AMF treatment, half of the pots were filled with sieved field soil (thus containing the original soil microbiota, including AMF), while the other half with sterilized sieved field soil. Soils were sterilized (heat pasteurized) through several consecutive cycles of heating in a forced ventilation oven at 80 °C for six hours and subsequent moist incubation at room temperature for one day. With this procedure I aimed at depleting the AMF spore bank in the soil. Drought treatment started at 42-45 days after planting (DAP). Aboveground parts were harvested at 132-140 DAP, when plant biomass, growth rate, leaf relative water content, leaf density and root colonisation by AMF were measured. Leaf gas exchange and chlorophyll-a fluorescence were measured at 103-107 DAP. For each measure of plant performance, two-way ANOVA with drought and soil sterilization as fixed main effects was used. Means were compared by using the Sidak post hoc test. Two sample t-tests – with Welch's correction if sample variances differed – were used for the comparison of mycorrhizal colonisation of roots in well-watered versus drought treated field soil. The significance level was set to $P < 0.05$ throughout.

III. Results

*The effect of the abiotic environment on the phenology, photosynthesis and mycorrhization of *Brachypodium pinnatum**

1. In July, with the beginning of the hot and dry summer, mortality of shoots on the SW side increased considerably. Meanwhile, on the NE slope most of the shoots were still alive in September suggesting more severe stress on the SW side. Leaf longevity in spring did not differ significantly on the two slopes. However, estimated average leaf longevity was greater on the NE-facing slope.
2. Shoot height on the NE slope was greater than on the SW slope most of the year. In spring total leaf area per shoot was greater on the SW-facing slope, early summer it was similar in both exposures, while in mid- and late-summer leaf area per shoot on the NE slope surpassed that of the SW side shoots. In autumn total leaf area per shoot was similar again

in both exposures, because new shoots emerged on the SW-facing slope. In spring the proportion of green leaf area per shoot was similar in both exposures, then in July leaves started to show rapid senescence on the SW-facing slope.

3. Maximum photochemical efficiency of PSII in July 2002 was much smaller on the SW-facing slope compared to the NE-facing slope at noon, which – together with the small value measured at dawn – indicates the photoinhibition of photosystems. On the SW side depression at midday was greater than on the NE side. Non-photochemical quenching did not differ on the two slopes in June 2002 and July 2003, while in July 2002 (contrary to expectations) it decreased significantly on the SW slope compared to the NE side.
4. In summer strong decrease appeared in arbuscule content of the roots on both slopes with more conspicuous change on the SW slope. In autumn root arbuscule content was greater on the SW side. After summer drought new shoots emerged in autumn due to regular precipitation resulting in more favourable microclimate and arbuscule content increased on both exposures.

The effect of post-disturbance dominant „local invaders” and invasive grasses on the mycorrhizal colonisation of non-invasive species

1. The intensity of AMF infection showed substantial variation with the two invasive species (*C. dactylon* and *C. epigeios*) reaching only half to two thirds of the value of the non-invasive species (*Ch. gryllus* and *B. inermis*). The resident post-disturbance dominants were either intermediate (*B. ischaemum*) or comparable to non-invasives (*B. inermis*).
2. Root arbuscule occurrence followed the same pattern: non-invasive grasses and resident post-disturbance dominants reached several times higher arbuscule frequencies than the invasive species.
3. The mean intensity of colonisation (M%) for *F. vaginata* was lower ($\leq 50\%$) in either of the two invaded stands than in the uninvaded resident community. A similar trend was observed for arbuscule occurrence as well, it was lower in either of the two invaded stands than in the uninvaded community.
4. In *S. borysthenica* roots both frequency (F%) and intensity (M%) of mycorrhizal colonisation was lower in invaded stands than in the uninvaded one.
5. Vesicle occurrence in *S. borysthenica* was lower in the stand invaded by *C. dactylon* than either in the uninvaded community or where *C. epigeios* was dominant.

Plant responses to soil drying with and without mycorrhizal association of invasive, non-invasive and post-disturbance dominant „local invader” grass species

1. For non-invasive species, the reduction of leaf, stem, shoot and total plant masses in response to soil sterilisation was similar (*D. alpina*) or even greater (*Ch. gryllus*) than in response to the imposed drought. Root weight declined in non-invasive species due to soil drying, while for *Ch. gryllus* it decreased by 39% in response to soil sterilisation.
2. Plant biomass and its components in invasive species decreased in response to drought alone, while soil sterilisation alone either did not change biomass (*C. epigeios*) or even increased it (*C. dactylon*) compared to control. In the combined treatment – when plants were subjected to drought in sterilised soil – biomass accumulation was not smaller than that achieved in response to drought treatment in intact field soil.
3. The responses of the „local invader” *B. ischaemum* to the treatments were mixed. No response was observed for plants grown in intact soil when subjected to drought. Its root biomass increased considerably due to soil sterilisation, thus total biomass was also greater than in field soil. In the combined treatment its biomass allocation into the root system was less than in plants growing in intact soil and subjected to drought.
4. Compared to control, relative growth rate was reduced with drought, sterile soil, and drought + sterile soil treatments at an increasing extent in this order for the non-invasive *D. alpina* and *Ch. gryllus*. For the invasive species, sterile soil alone did not decrease relative growth rate (even increased it for *C. dactylon*), while the reduction brought about by drought was not greater for plants grown in sterile than in intact soil. The growth rate of *B. ischaemum* was similar to *C. dactylon*: it increased in sterilised soil compared to control, while drought affected it only in sterilised soil and not in intact soil.
5. Treatment responses in biomass proportions were less marked. When grown in sterilized soil – irrespective of water supply – *D. alpina* and *C. dactylon* displayed higher biomass allocation into the roots and less into the stem or leaves compared to that achieved in field soil. For *Ch. gryllus* shoot biomass allocation was lower in sterile soil than in the drought treatment in field soil. This was due to the slight increase in root biomass allocation and the slight decrease in leaf and stem biomass allocation. Drought increased root biomass allocation for *C. epigeios* in sterilised soil, while it decreased leaf biomass ratio both in intact and sterile soil.
6. Compared to control, the non-invasive *D. alpina* and *Ch. gryllus* underwent more marked reduction in total plant foliage area than the two invasive species when grown in sterile soil, and this was most marked when both soil sterilisation and drought was applied. For

the invasive *C. epigeios* and *C. dactylon* total leaf area was similar in drought treatments irrespective of soil quality (intact or sterilized).

7. For *B. ischaemum* root biomass ratio decreased, shoot biomass ratio increased in the combined treatment, while neither of the treatments alone elicited any response to biomass allocation compared to control. Leaf biomass ratio was similar to control in every treatment.
8. Drought treatment decreased leaf relative water content to a greater degree in sterilised than in intact soil for the non-invasive species, while caused no significant change under both watering treatments for invasive grasses. A decline in P_n and g_s appeared for *D. alpina* in the drought treatments in both intact and sterilised soil.
9. Actual and maximal photochemical efficiency of PSII declined for *D. alpina* both in the soil sterilisation and combined treatment. Photochemical quenching (qP) also declined for *D. alpina* in the soil sterilisation treatment, while for *Ch. gryllus* PSII antenna efficiency reduced in response to soil sterilisation or drought treatment alone. No treatment effect on leaf gas exchange and photochemistry appeared for either of the two invasive species.

IV. Conclusions

The field transplant experiment indicated that the physical environment may play an important role in the exclusion of *B. pinnatum* from South-facing slopes despite its gregarious appearance in adjacent hillsides exposed to the N or E in the loess forest steppe landscape mosaic of the Hungarian Great Plain. The hot and dry microclimate of South-facing slopes in this loess-covered landscape seems to impose intolerable stress on this broadleaved perennial grass at least in drier-than-average years when the experiment was conducted. Seasonal shoot development began earlier on the S-facing slope compared to the N-facing one probably due to the warmer microclimate of the former. However, the reduction of photosynthetic leaf area occurred earlier and in a greater extent on the slope with SW aspect, furthermore shoot height was also lower. Under „average” weather conditions leaf photochemistry was well-protected from radiation damage as chlorophyll fluorescence induction measurements revealed. The severe drought in July 2002 coupled with high radiation load on the S-facing slope, however, resulted in the photodamage of leaves indicated by decreased capacity for photochemical reactions and also for non-photochemical channelling of excess energy. In this study *B. pinnatum* showed a high frequency and extent of VA micorrhizal colonisation, 50-80% depending on exposure and season. The greater-than-average summer drought in 2002 reduced the amount of arbuscules and this was more marked on the S-facing slope than on the N-facing one. This reduction of arbuscule

frequency may affect not only P acquisition of the plant, but its water uptake in addition as arbuscular symbiosis may alleviate plant responses to moderate moisture deficit by increasing water uptake.

AMF colonisation of roots of the two invasive grasses was consistently lower than that of the resident grass species. This confirms the hypothesis that an invasive plant is likely to be nonmycorrhizal or a facultative symbiont. Additionally, native resident grasses displayed lower degree of AMF colonisation when grown in a grassland infected by an invasive grass species compared to the uninvaded seminatural grassland. The extent of mycorrhizal colonisation for both resident grass species was the greatest in their native site, the lowest in *C. dactylon* invaded stands and moderate in *C. epigeios* dominated sites. Arbuscule occurrence showed the same trend in the case of *F. vaginata*, whereas for *S. borysthenica* vesicle occurrence was markedly lower in *C. dactylon* stands than in uninvaded habitats.

The experiment in the growth room confirmed the hypothesis that invasive species do not benefit from AMF association in alleviation of water stress, while non-invasive resident species do. Under water deficit the non-invasive *D. alpina* and *Ch. gryllus* performed markedly better in almost all measures of growth (except for root and total plant biomass of *D. alpina*) when grown in intact soil containing native AMF community than in sterilized field soil. In contrast, the invasive *C. epigeios* and *C. dactylon* displayed no difference in growth rate and biomass accumulation between intact and sterilised soil when subjected to water stress. Furthermore, when plants were grown well watered but deprived of AMF symbionts, both *D. alpina* and *Ch. gryllus* achieved weaker growth than in AMF containing soil at adequate watering, while neither *C. epigeios* nor *C. dactylon* displayed any reduction.

These results point to the involvement of soil AMF community in the process of plant invasion. The role of AMF in plant invasion is complex and context dependent. The nature of the interaction and its effect on the ecosystem is determined by the plant and AMF community and on habitat properties on a case-by-case basis. However, it is reasonable to conclude that as changes in the soil AMF community caused by invasive species are profound and last even after the removal of the alien plant, restoration efforts should include the rehabilitation of the soil microbial community in order to achieve a fully functional community.

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