Community Ecology, 2013, 14, 67-76.

Arbuscular mycorrhizal colonisation of roots of grass species differing in invasiveness

Endresz, G.[12]*, Somodi, I.[3], Kalapos, T.[1]

1 Department of Plant Systematics, Ecology and Theoretical Biology, Institute of Biology, Eötvös Loránd University, Pázmány Péter stny 1C, 1117 Budapest, Hungary
2 Present address: Szent László Secondary School, Kőrösi Csoma S. út 28-34., 1102 Budapest, Hungary
3 Centre for Ecological Research, Hungarian Academy of Sciences, Institute of Ecology and Botany, Alkotmány u. 2-4., 2163 Vácrátót, Hungary
* *corresponding author* – endreszgabor@gmail.com

Abstract

Recent research indicates that the soil microbial community, particularly arbuscular mycorrhizal fungi (AMF), can influence plant invasion in several ways. We tested if 1) invasive species are colonised by AMF to a lower degree than resident native species, and 2) AMF colonisation of native plants is lower in a community inhabited by an invasive species than in an uninvaded resident community. The two tests were run in semiarid temperate grasslands on grass (Poaceae) species, and the frequency and intensity of mycorrhizal colonisation, and the proportion of arbuscules and vesicles in plant roots have been measured. In the first test, grasses representing three classes of invasiveness were included: invasive species, resident species becoming abundant upon disturbance, and non-invasive native species. Each class contained one C3 and one C4 species. The AMF colonisation of the invasive *Calamagrostis epigejos* and *Cynodon dactylon* was consistently lower than that of the non-invasive native *Chrysopogon gryllus* and *Bromus inermis*, and contained fewer arbuscules than the post-disturbance dominant resident grasses *Bothriochloa ischaemum* and *Brachypodium pinnatum*. The C3 and C4 grasses behaved alike despite their displaced phenologies in these habitats. The second test compared AMF colonisation for sand grassland dominant grasses *Festuca vaginata* and *Stipa borysthenica* in stands invaded by either *C. epigejos* or *C. dactylon,* and in the uninvaded natural community. Resident grasses showed lower degree of AMF colonisation in the invaded stand compared to the uninvaded natural community with *F. vaginata* responding so to both invaders, while *S. borysthenica* responding to *C. dactylon* only. These results indicate that invasive grasses supposedly less reliant on AMF symbionts have the capacity of altering the soil mycorrhizal community in such a way that resident native species can establish a considerably reduced extent of the beneficial AMF associations, hence their growth, reproduction and ultimately abundance may decline. Accumulating evidence suggests that such indirect influences of invasive alien plants on resident native species mediated by AMF or other members of the soil biota is probably more the rule than the exception.

Keywords: arbuscular mycorrhizal fungi (AMF), *Calamagrostis epigejos*, *Cynodon dactylon*, grasses, invasive plants, semiarid temperate grassland.

Nomenclature: Tutin et al. (1964-1993).

Abbreviations: AMF – Arbuscular Mycorrhizal Fungi; a% – percentage arbuscule occurence of the AMF colonised root section; A% – percentage arbuscule occurence of the whole root; F% – frequency of root segments colonised by AMF; M% – intensity of mycorrhizal colonisation; v% – percentage vesicle occurence of the AMF colonised root section; V% – percentage vesicle occurence of the whole root;

Introduction

Several, mutually non-exclusive hypotheses have been proposed to explain the success of invasive plants in their new range (reviewed by Hierro et al. 2005, Mitchell et al. 2006): the enemy release hypothesis (Keane and Crawley 2002), the novel weapons hypothesis (Callaway and Ridenour 2004, Callaway et al. 2008, Wolfe et al. 2008), the evolution of increased competitive ability (EICA, Blossey and Nötzold 1995, Bossdorf et al. 2005), the theory of fluctuating resource availability (Davis et al. 2000), the framework of vacant niches (Moles et al. 2008), and the facilitation of further invaders as described in the invasional meltdown hypothesis (Simberloff and Von Holle 1999). More recently, attention has turned towards the soil biota as a possible mediator of plant-plant interactions in plant invasions (Klironomos 2002, Fitter 2005). Arbuscural mycorrhizal fungi (AMF) are particularly important in this respect due to their widespread occurrence and important role in communities and ecosystems (Brundrett 1991).

The majority of herbaceous plant species has AMF symbionts (Wang and Qiu 2006), yet data are insufficient whether invasive species are associated with AMF (Pringle et al. 2009). Although invasive plants may benefit from mutualistic relationships such as AMF symbioses (Richardson et al. 2000, Shah 2009b), it has been hypothesized that 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). The carbon cost of sharing assimilates with mycorrhizal fungi can be quite high (Douds et al. 1988). 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. Assimilates saved in this way can be spent on growth, hence resulting in greater competitive ability. Indeed, AMF associations were found less frequent among invasive species than native species (Vogelsang et al. 2004, Pringle et al. 2009). Furthermore, rapid evolution of the plant's decreased reliance on AMF mutualists during invasion has been documented (Seifert et al. 2009). Notwithstanding, opposite findings have also been reported. The invasive species possessed more intense AMF infection than species in the resident community (Marler et al. 1999, Greipsson and DiTommaso 2006), and there are several invasive plants heavily colonised by AMF in their new habitats (e.g. *Ambrosia artemisiifolia*, Kovács and Bagi 2001, Kovács and Szigetvári 2002, Fumanal et al. 2006).

Several mechanisms involving AMF have been proposed and experimentally tested that despite the low host-specifity of arbuscular mycorrhizal associations, AMF can take part in plant invasions (mutualist facilitation, degraded mutualism; Mitchell et al. 2006, Reinhart and Callaway 2006, Shah et al. 2009b, Vogelsang and Bever 2009). In its new habitat, the invasive plant can alter the soil microbial community (Kourtev et al. 2002, Batten et al. 2006, Greipsson and DiTommaso 2006). This can be achieved directly through exuded allelochemicals decimating indigenous AMFs (Stinson et al. 2006, Zhang et al. 2007). For example, such a mechanism has been reported for the European *Alliaria petiolata* in North American forests (Stinson et al. 2006, Hale et al. 2011, but see Barto et al. 2010). Alternatively, the soil microbial community may be changed indirectly via the mass effect of the invasive plant being non-mycorrhizal or having different AMF symbionts, hence local AMFs gradually attenuate (Niu et al. 2007, van der Putten et al. 2007, Vogelsang and Bever 2009, Meinhardt and Gehring 2012, Sanon et al. 2012). Different AMF species may have different colonisation phenologies (see e.g. Merryweather and Fitter 1998, Sánchez-Castro et al. 2012), thus an invasive plant associated with AMF having seasonal dynamics different from that of the AMF species of resident plant species can narrow the diversity of AMF partners available for the resident community. While Davison et al. (2012) found no seasonal variation in the AMF community structure in forest soil, other studies (Lingfei et al. 2005, Lugo et al. 2003) detected marked seasonality in grassland soil. Certain invasive plants are able to connect to the local AMF network and through that can draw assimilates from resident species. This was reported for the noxious exotic weed *Centaurea maculosa* interacting with the native *Festuca idahoensis* in Montana, USA (Marler et al. 1999), although the mechanism behind this phenomenon is not yet clear. Zabinski et al. (2002) suggested increased phosphorous uptake by exotic species through common mycorrhizal network and Carey et al. (2004) demonstrated net carbon transfer from *F. idahoensis* to the invasive plant. Interestingly, AMF appeared not to play obvious role in the invasion of the same *Centaurea* species on early successional sand dunes at the Great Lakes, USA (Emery and Rudgers 2012). Yet in other cases, the AMF community played a role in repelling plant invasion (Stampe and Daehler 2003, Barto et al. 2010).

These examples clearly show that the role of AMF in plant invasion is complex and context dependent. The aim of our study is to complement existing evidence and thus contribute to a deeper understanding of the problem. We included only grass (Poaceae) species in our study to minimise taxonomic variation, and because AMF symbiosis is common in this family (see e.g. Wang and Qiu 2006). Furthermore, grasses are dominant in grasslands, thus their contribution to ecosystem functions is decisive. We are aware of only few studies dealing with the role of AMF symbionts in invasion by grasses (Goodwin 1992, Hawkes et al. 2006, van der Putten et al. 2007). Specifically, we tested the following two hypotheses.

H1) Roots of invasive species show different rate of AMF colonisation than the roots of resident native species.

H2) Roots of resident native species show different rate of AMF colonisation in habitats infected by invasive species compared to habitats free from the invasive plant.

To examine these hypotheses, we conducted two separate field tests in semiarid temperate grasslands in Hungary. These grasslands are key components of the natural forest-steppe vegetation of East-Central Europe, cover substantial areas and have been greatly modified by human land uses over centuries. The spread of invasive species is one of the most serious threats to these grasslands today (Molnár et al. 2012). Data on AMF associations of plants in this vegetation started to appear in the last decade only (Kovács and Bagi 2001, Kovács and Szigetvári 2002, Endresz et al. 2005), and particularly little is known about alien plants in this respect. The grasses chosen for this study are common characteristic components of these grasslands and play different role in vegetation dynamics. *Calamagrostis epigejos* and *Cynodon dactylon* typically gain dominance in degraded habitats where otherwise their presence is negligible (invasive species), *Bothriochloa ischaemum* and *Brachypodium pinnatum* often become abundant after certain types of disturbances in grasslands they commonly inhabit (post-disturbance dominant residents), while *Bromus inermis* and *Chrysopogon gryllus* are characteristic elements of undisturbed vegetation (non-invasive species). Literature data on the mychorrizal status of these plants were available prior to our study for *B. ischaemum* (Shah et al. 2009a, Wilson et al. 2012), *B. pinnatum* (van der Heijden et al. 1998, Endresz et al. 2005), *B. inermis* (Turnau et al. 2008, Shah et al. 2009a), *C. epigeijos* (Rydlová and Vosátka 2001, van der Staaij et al. 2001) and *C. dactylon* (Cabello 1997, Lingfei et al. 2005). However, we are not aware of published data on the mycorrhizal status of *C. gryllus*.

Material and Methods

Study sites

The sampled grasslands lie in the Danube-Tisza Interfluve on the Great Hungarian Plain. The climate is semiarid temperate with wet spring and dry summer. Annual mean temperature is 10 °C, yearly precipitation is 550 mm in average. The bedrock is eolian or alluvial sediment (loess or sand, respectively). Hypothesis 1 was tested in a loess grassland developed on chernozem soil near the village Isaszeg, while hypothesis 2 was examined in a sand grassland at Fülöpháza. These localities were chosen because they are subjects of a wide range of ecological studies. As our H1 and H2 are independent, testing them at ecologically similar, yet different sites is methodologically tenable. Study sites were not replicated in this study.

Test 1

To test whether roots of invasive species differ in their colonisation rate by AMF than the roots of resident native species we examined the mycorrhizal colonisation of six grass species. The species studied (Table 1) fall into three categories of invasiveness: invasive grasses, resident species reaching local dominance upon disturbance and non-invasive native species. Of the two invasive grasses examined, *Cynodon dactylon,* a presumed archaeophyte in the Hungarian flora (Terpó et al. 1999), is one of the world's worst weeds (Holm et al. 1977), while *Calamagrostis epigejos* is classified as dangerous native species with tendencies to become invasive (Mihály and Demeter 2003). In each category of invasiveness, two plant species, one C3 and one C4, were selected. To exclude the possible effect of life form, only perennial grasses were studied. In June 2005, root samples were collected on the Great Hungarian Plain in a loess grassland near Isaszeg except for *C. dactylon*, where sampling took place in a sand grassland at Fülöpháza, the site of our second test. (*C. dactylon* was absent at the site where the other five species were sampled.) For each species 5 specimens, chosen randomly, were collected.

Test 2

To test whether native species exhibit a different degree of colonisation by AMF in invaded habitats we collected root samples from two native grass species (*Festuca vaginata* and *Stipa borysthenica*), both from a grassland stand without any sign of invasion and from stands where either *Calamagrostis epigejos* or *Cynodon dactylon* was predominant. From the three stands (no invasion, invasion by *C. epigejos,* invasion by *C. dactylon*) 5 specimens of both native species were collected. Root samples from 5 specimens of *C. epigejos* and *C. dactylon* were also collected. Both *F. vaginata* and *S. borysthenica* are characteristic dominant species of sand grasslands on the Great Hungarian Plain (Molnár et al. 2012). *Calamagrostis epigejos* has a tendency to invade native grassland habitats in Hungary (Somodi et al. 2008), while *C. dactylon* is considered an exotic and invasive species in Europe (Vilà and Weiner 2004). Root samples were collected on the Great

Hungarian Plain in a sand grassland sward near the village Fülöpháza in June 2006. For each species root samples were collected from randomly chosen specimens.

Measures of mycorrhizal colonisation

Root samples were preserved in 50% ethanol at 5 ºC until examination. Roots were cleaned in 10% KOH, and stained with aniline blue following the procedure of Grace and Stribley (1991). For each species, five specimens were assessed. From each specimen 30 root segments (of approx. 1-2 cm long) were examined and mycorrhizal structures (hyphae, arbuscules, vesicles) were determined in stained roots according to the method of Trouvelot et al. (1986). In both of our tests, the following variables were used to assess the degree of AM colonisation (see also in Endresz et al. 2005): frequency of root segments (approx. 1-2 cm) in the root system of a single plant where AMF was found (F%), intensity of mycorrhizal infection (estimated percentage of AMF in each root segment, M%), percentage arbuscule occurence of the AMF colonised root section (a%), percentage arbuscule occurence of the whole root (A%), percentage vesicle occurence of the AMF colonised root section (v%), percentage vesicle occurence of the whole root (V%).

Statistical analyses

Mycorrhizal colonisation measures 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. Since ANOVA is very robust to non-normality if sample sizes and variances are similar, and lack of normality is only a serious problem when coupled with variance heterogeneity (Quinn and Keough 2002), we did not test for normality, but tested variance homogeneity. We chose Levene's test with median centering, which is relatively insensitive to non-normality (Faraway 2002). If the homogeneity of variances assumption was violated, ANOVA with a sandwich covariance matrix estimator (referred to as ANOVA-S further) was used. With the sandwich covariance matrix estimator robustness can be achieved with respect to various types of model misspecification including heteroscedasticity (Zeileis 2006). Thus, using this estimator allows us to perform ANOVA on data where Levene's test indicated heteroscedasticity. Levene's test showed significant difference of variances only in one comparison and marginally significant difference ($0.05<p<0.1$) in four cases. Although the significance level for the tests was $p<0.05$ in general, we made an exception in this case and used modified ANOVA with sandwich estimators instead of simple ANOVA. This was to eliminate the possibility of using ANOVA when the homogeneity of variances assumption may have been violated. These comparisons are marked with "ANOVA-S" in figures. For the tests, the Graphpad Prism v.5.04 and the R statistical environment (R Development

Core Team 2009) were used. In the latter, the car package (Fox and Weisberg 2011) was used for Levene's test and the sandwich package (Zeileis 2004) for ANOVA S.

Results

Test 1

The frequency of roots colonised by AMF were approximately 100% for each species (Fig 1A). The intensity of AMF infection (M%) was between 31 and 91 %, and showed substantial variation with the two invasive species (*C. dactylon* and *C. epigejos*) reaching only half to two thirds of the value of the non-invasive species (*C. gryllus* and *B. inermis*, Fig 1B). The resident post-disturbance dominants were either intermediate (*B. ischaemum*) or comparable to non-invasives (*B. inermis*). Both measures of root arbuscule occurrence (a% and A%) followed the same pattern: non-invasive grasses and resident post-disturbance dominants reached several times higher arbuscule frequencies (33-68 %) than the invasive species (1-12 %, Fig 1C, D). Vesicle occurrence (v% and V%) was considerably lower (3-23 %) and the invasive species always reached lower value than either the non-invasive or the resident post-disturbance dominant species within its photosynthetic type (C3 or C4) subgroup (Fig 1E, F).

Test 2

Each collected species was colonised by AMF. The frequency of colonisation was nearly 100% for *C. epigeios*, *F. vaginata* and *S. borysthenica*, but significantly lower for *C. dactylon* (Fig 2A). The intensity of colonisation (M%) was remarkably lower for *C. dactylon* and *S. borysthenica* (23% and 38%, respectively) than for *C. epigejos* and *F. vaginata* (73% and 70%, respectively, Fig 2B). The same pattern in a more extreme manner appeared for both measures of arbuscule occurence (a% and A%, Fig 2C, D): mean a% was around 60% and mean A% was around 40% for *C. epigejos* and *F. vaginata* similarly, whereas mean a% remained below 17% and A% below 4% for *C. dactylon* and *S. borysthenica*. Vesicle occurrence in the AMF colonised root (v%) did not differ between species, while that in the whole root (V%) was lower for *C. dactylon* than for *F. vaginata* (Fig 2E, F).

Festuca vaginata displayed similarly high frequency of mycorrhizal colonisation (F%=90-100) in the uninvaded stand, in the stand invaded by *C. epigejos*, and in the stand invaded by *C. dactylon* (Fig 3A). In contrast, the mean intensity of colonisation (M%) for this species was lower (≤50%) in either of the two invaded stands than in the uninvaded resident community where it was 70% (Fig 3B). A similar trend was observed for arbuscule occurrence as well (a%, A%, Fig 3 C,D).

Vesicle occurence (v%, V%) in the roots of *F. vaginata* followed a comparable tendency, yet no significant difference was detected most probably because this trait showed the greatest variation among all mycorrhizal parameters examined (Fig 3 E,F).

For *S. borysthenica*, the frequency of mycorrhizal colonisation (F%) was lower in the *C. dactylon* invaded habitat than in the uninvaded resident community (97% and 69%, respectively), while the *C. epigejos* invaded stand did not differ from either of the two other swards (Fig 3A). Likewise, the intensity of AMF infection (M%) of *S. borysthenica* was remarkably lower (6%) when grown together with *C. dactylon* than in the uninvaded or the *C. epigejos* invaded stands (38% and 29%, respectively, Fig 3B). Mean arbuscule occurrence (a%, A%) was very low (<8%) for *S. borysthenica* and no difference appeared between the habitats compared (Fig 3C, D). Vesicle occurrence (v%, V%) showed the same trend as F% and M%; it was highest in the uninvaded community and lowest where *C. dactylon* was abundant (Fig 3E, F).

Discussion

In the first comparison we examined if the roots of invasive species are less intensively colonised by AMF than the roots of resident native species. The AMF colonisation of roots of the two invasive grasses were consistently lower than that of the resident grass species. This confirms our hypothesis (H1) and is in line with the first hypothesis of Pringle et al. (2009), namely that an invasive plant is likely to be nonmycorrhizal or a facultative symbiont. The remarkably low (≤10%) frequency of arbuscules found in the invasive grasses is particularly noteworthy. However, the nearly 100% frequency of AMF colonised roots (F%) irrespective of invasiveness category indicates that even invasive grasses are able to establish AMF associations in their new habitats, possibly due to the low host-specificity of AMF. The very low intensity of colonisation can mean that nutrient exchange between the alien grass and the resident AMF community is not substantial, although it was not measured in this study. . In this respect, C3 and C4 grasses behaved alike, despite substantial differences in their phenology and habitat preferences in the Hungarian flora (Kalapos 1991). Compared to native resident grass species, the invasive grass *Cenchrus biflorus* was found non-mycorrhizal in a Southern African savanna (van der Putten et al. 2007). In an earlier extensive survey of a shortgrass sand grassland community in Hungary, Kovács and Szigetvári (2002) found native grasses (e.g. *Festuca vaginata*, *Stipa capillata*) to possess intense AMF associations, but no or very weak mycorrhizal symbiosis for invasive or exotic grasses (e.g. *Cenchrus incertus*, *Tragus racemosus*), with the exception of the archaeophyte *Cynodon dactylon*, which possessed more intensive AMF infection. In the same grassland type we found *C. dactylon* to be colonised by AMF to a similar extent in 2005, in accordance with the results of Kovács and Szigetvári (2002), but to a smaller degree in 2006. The degree of colonisation of the other species we studied in 2006 was similar, albeit with slight differences, to the results of Kovács and Szigetvári (2002). A number of factors can account for the small differences. Although our assessment method did not differ considerably from that of Kovács and Szigetvári (2002), the procedure is based on estimation, hence it can not be as precise as a measurement-based technique. Furthermore, the extent of colonisation by AMF may vary according to season (Lugo et al. 2003, Busby et al. 2012) and probably between years as we have observed it for *C. dactylon*.

There was considerable difference in colonisation intensity and arbuscule occurence for *C. epigejos* between the two tests of our study. In Test 1, *C. epigejos* samples were taken from a closed loess grassland, whereas in Test 2 from an open sand grassland. The soil of closed loess grasslands is usually rich in humus and consequently plant nutrients, while the soil of open sand grasslands is seriously deficient in these (Kovács-Láng 1970, Molnár et al. 2012). The higher degree of AMF colonisation for *C. epigejos* in the open sand grassland than in the closed loess grassland might be due to the greater reliance of plants on AMF symbionts for nutrient uptake on nutrient poor than on nutrient rich soil. Yet, further studies are needed to clarify the background of differences in AMF colonisation for *C. epigejos* in different environments.

In our second test we examined if roots of resident native species show different rate of AMF colonisation in habitats infected by invasive species compared to habitats free from the invasive plant. We compared the mycorrhizal colonisation of two native grass species (*Festuca vaginata* and *Stipa borysthenica*) in uninvaded semi-natural habitats and in habitats invaded either by *Calamagrostis epigejos* or *Cynodon dactylon.* Although both invasive species form arbuscular mycorrhizal associations in the sand grasslands of the Great Hungarian Plain as it was shown in this and in previous studies (Kovács and Szigetvári 2002), invasive species can alter the AMF composition of the soil (Mummey et al. 2005, Wolfe and Klironomos 2005, Hawkes et al. 2006), and thus, influence plant-fungus interaction of native plants. There is ample evidence that AM associations are more host-specific than previously thought, at least in the response of plants to different types of AM fungi (van der Heijden et al. 1998, Klironomos 2000, Bever 2002a, Bray and Kitajima 2003, Klironomos 2003, Fitter 2005). Several studies demonstrated that AMF can enhance invasion success of plant species (eg. Bever 2002b, Roberts and Anderson 2001, Mummey et al. 2005). Our results support our hypothesis (H2) that invasive species can decrease the mycorrhizal colonisation of native species. 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.*

epigejos dominated sites. Arbuscule occurence showed the same trend in the case of *F. vaginata*, whereas for *S. borysthenica* vesicle occurence was markedly lower in *C. dactylon* stands than in uninvaded habitats. Others also reported decreased mycorrhizal colonisation of plants near invaders (Stinson et al. 2006, Zhang et al. 2007, Meinhardt et al. 2012). A possible mechanism responsible for our findings is explained by the degraded mutualist hypothesis, for which Bever et al. (2010) suggested a conceptual model, namely that the dominant species inhibits the mutualists of the resident community. Our study, however, does not give an underlying mechanism for the phenomenon. It was proposed by Vogelsang et al. (2005) that less mycorrhiza dependent weedy species allocate fewer of their resources to maintain mycorrhizal fungi and therefore the dominance of naturalised alien plant species could degrade resident AM fungal communities in time even without allelopathic exudates.

Further experiments are needed to understand whether it is a direct effect of the invasive species studied here (e.g. allelopathy), or the introduced plants indirectly decreased overall mycorrhiza density as was shown by Vogelsang and Bever (2009), or they altered the AMF community.

Conclusions

In conclusion, our case study demonstrated higher degrees of AMF colonisation for native resident grasses than for invasive grasses in a semiarid temperate grassland community. 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. These results point to the involvement of soil AMF community in the process of plant invasion. 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.

Acknowledgements

Authors thank Gábor M. Kovács for instructive comments on an earlier draft of the manuscript. Imelda Somodi was supported by the Hungarian Scientific Research Fund (OTKA) grant no. PD-83522.

References

Barto, K., C. Friese and D. Cipollini. 2010. Arbuscular mycorrhizal fungi protect a native plant from allelopathic effects of an invader. *Journal of Chemical Ecology* 36: 351-360.

Batten, K.M., K.M. Scow, K.F. Davies and S.P. Harrison. 2006. Two invasive plants alter soil microbial community composition in serpentine grasslands. *Biological Invasions* 8: 217-230.

Bever, J.D. 2002a. Host-specificity of AM fungal population growth rates can generate feedback on plant growth. *Plant and Soil* 244: 281–290.

Bever, J.D. 2002b. Negative feedback within a mutualism: host-specific growth of mycorrhizal fungi reduces plant benefit. *Proceedings of the Royal Society, London, Series B* 269: 2595–2601.

Bever, J.D., I.A. Dickie, E. Facelli, J.M. Facelli, J. Klironomos, M. Moora, M.C. Rillig, W.D. Stock, M. Tibbett, and M. Zobel. 2010. Rooting theories of plant community ecology in microbial interactions. *Trends in Ecology & Evolution* 25(8): 468-478.

Blossey, B. and R. Nötzold. 1995. Evolution of increased competitive ability in invasive nonindigenous plants: a hypothesis. *Journal of Ecology* 83: 887-889.

Bossdorf, O., H. Auge, L. Lafuma, W.E. Rogers, E. Siemann and D. Prati. 2005. Phenotypic and genetic differentiation between native and introduced plant populations. *Oecologia* 144: 1–11.

Bray, S. and K. Kitajima. 2003. Mycorrhizae differentially alter growth, physiology, and competitive ability of an invasive shrub. *Ecological Applications* 13: 565–574.

Brundrett, M. 1991. Mycorrhizas in natural ecosystems. *Advances in Ecological Research* 21: 171-313.

Busby, R.R, M. W. Paschke, M.E. Stromberger and D. L. Gebhart. 2012. Seasonal variation in arbuscular mycorrhizal fungi root colonization of cheatgrass (*Bromus tectorum*), an invasive winter annual. *Journal of Ecosystem and Ecography* S8: 001.

Cabello, M.M.. 1997. Hydrocarbon pollution: its effect on native arbuscular mycorrhizal fungi (AMF). *FEMS Microbiology Ecology* 22: 233-236

Callaway, R.M., D. Cippolini, K. Barto, G.C. Thelen, S.G. Hallett, D. Prati, K. Stinson and J.N. Klironomos. 2008. Novel weapons: invasive plant supresses fungal mutualists in America but not in its native Europe. *Ecology* 89: 1043-1055.

Callaway, R.M. and W. Ridenour. 2004. Novel weapons: invasive success and the evolution of increased competitive ability. *Frontiers in Ecology and Environment* 2: 436-443.

Carey, J.R., M.J. Marler and R.M. Callaway. 2004. Mycorrhizae transfer carbon from a native grass to an invasive weed: evidence from stable isotopes and physiology. *Plant Ecology* 172: 133-141.

Davis, M.A., J.P. Grime and K. Thompson. 2000. Fluctuating resources in plant communities: a general theory of invasibility. *Journal of Ecology* 88: 528-534.

Davison, J., M. Öpik, M. Zobel, M. Vasar, M. Metsis, and M. Moora. 2012. Communities of arbuscular mycorrhizal fungi detected in forest soil are spatially heterogeneous but do not vary throughout the growing season. *PLoS ONE* 7(8): e41938.

Douds, D.D., C.R. Johnson and K.E. Koch. 1988. Carbon cost of the fungal symbiont relative to net leaf P accumulation in a split-root VA mycorrhizal symbiosis. *Plant Physiology* 86: 491–496.

Emery, S.M. and J.A. Rudgers. 2012. Impact of competition and mycorrhizal fungi on growth of Centaurea stoebe, an invasive plant of sand dunes. *American Midland Naturalist* 167: 213-222.

Endresz, G., Á. Zöld-Balogh, and T. Kalapos. 2005. Local distribution pattern of Brachypodium pinnatum (Poaceae) - Field experiments in xeric loess grassland in N. Hungary. *Phyton-Annales rei Botanicae* 45:(2): 249-265.

Faraway, J.J. 2002. *Linear Models With R. Texts in Statistical Science.* Chapman & Hall/CRC, New York.

Fitter, A. 2005. Darkness visible: reflections on underground ecology. *Journal of Ecology* 93: 231–243.

Fox, J. and S. Weisberg. 2011. *An {R} Companion to Applied Regression*. Second Edition. Thousand Oaks CA: Sage. URL: http://socserv.socsci.mcmaster.ca/jfox/Books/Companion

Fumanal, B., C. Plenchette, B. Chauvel, and F. Bretagnolle. 2006. Which role can arbuscular mycorrhizal fungi play in the facilitation of *Ambrosia artemisiifolia* L. invasion in France? *Mycorrhiza*, 17: 25–35.

Goodwin, J. 1992. The role of mycorrhizal fungi in competitive interactions among native bunchgrasses and alien weeds: A review and synthesis. *Northwest Science* 66: 251-260.

Grace, C. and D.P. Stribley. 1991. A safer procedure for routine staining of vesicular arbuscular mycorrhizal fungi. *Mycological Research* 95: 1160–1162.

Graphpad Prism v.5.04 2007 GraphPad Software, San Diego California USA, www.graphpad.com

Greipsson, S. and A. DiTommaso. 2006. Invasive non-native plants alter the occurrence of arbuscular mycorrhizal fungi and benefit from this association. *Ecological Restoration* 24: 236-241.

Hale, A., S.J. Tonsor and S. Kalisz. 2011. Testing the mutualism disruption hypothesis: physiological mechanisms for invasion of intact perennial plant communities. Ecosphere 2: 110.

Hawkes, C.W., J. Belnap, C. D'Antonio and M.K. Firestone. 2006. Arbuscular mycorrhizal assemblages in native plant roots change in the presence of invasive exotic grasses. *Plant and Soil* 281: 369-380.

Hierro, J.L., J.L. Maron and R.M. Callaway. 2005. A biogeographical approach to plant invasions: the importance of studying exotics in their introduced and native range. *Journal of Ecology* 93: 5-15.

Holm, L.G., D.L. Plucknett, J.V. Pancho and J.P. Herberger. 1977. The world's worst weeds. Distribution and biology. -Honolulu, Hawaii: Univ. Press of Hawaii.

Kalapos, T. 1991. C3 and C4 grasses of Hungary: environmental requirements, phenology and role in the vegetation. *Abstracta Botanica* 15: 83-88.

Keane, R.M. and M.J. Crawley. 2002. Exotic plant invasions and the enemy release hypothesis. *Trends in Ecology and Evolution* 17: 164-170.

Klironomos, J. 2000. Host-specificity and functional diversity among arbuscular mycorrhizal fungi. In: Bell, C.R., M. Brylinski and P. Johnson-Green. (eds.) *Microbial biosystems: new frontiers*. *Proceedings of the 8th International Symposium of Microbial Ecology*, Halifax, NS, Canada. Atlantic Canada Society for Microbial Ecology. pp. 845–851.

Klironomos, J.N. 2002. Feedback with soil biota contributes to plant rarity and invasiveness in communities. *Nature* 417: 67-70.

Klironomos, J. 2003. Variation in plant response to native and exotic arbuscular mycorrhizal fungi. *Ecology* 84: 2292–2301.

Kourtev, P., J. Ehrenfeld and M. Häggblom. 2002. Exotic plant species alter the microbial community structure and function in the soil. *Ecology* 83: 3152–3166.

Kovács, M.G. and I. Bagi. 2001. Mycorrhizal status of plants in a mixed deciduous forest from the Great Hungarian Plain with special emphasis on the potential mycorrhizal partners of *Terfezia terfezioides* (Matt.) Trappe (Pezizales). *Phyton* 161-168.

Kovács, M.G. and Cs. Szigetvári. 2002. Mycorrhizae and other root-associated fungal structures of the plants of a sandy grassland on the Great Hungarian Plain. *Phyton* 42: 211-223.

Kovács-Láng, E. 1970. Fractional humus investigation of soils under sward communities (Festucetum vaginatae danubiale, Festucetum wagneri) growing on sandy sites. *Annales Universitatis Scientiarum Budapestiensis, Sectio Biologica* 12: 163-170.

Lingfei, L., Y. Anna and Z. Zhiwei. 2005. Seasonality of arbuscular mycorrhizal symbiosis and dark septate endophytes in a grassland site in southwest China. *FEMS Microbiology Ecology* 54: 367-373.

Lugo, M.A, M.E. González Maza and M.N. Cabello. 2003. Arbuscular mycorrhizal fungi in a mountain grassland II: Seasonal variation of colonization studied, along with its relation to grazing and metabolic host type. *Mycologia* 95: 407–415.

Marler, M.J., C.A. Zabinski and R.M. Callaway. 1999. Mycorrhizae indirectly enhance competitive effects of an invasive forb on a native bunchgrass. *Ecology* 80: 1180-1186.

Meinhardt, K.A. and C.A. Gehring. 2012. Disrupting mycorrhizal mutualisms: a potential mechanism by which exotic tamarisk outcompetes native cottonwoods. *Ecological Applications* 22: 532-549.

Merryweather, J. and A.H. Fitter. 1998. The arbuscular mycorrhizal fungi of *Hyacinthoides non-scripta* II. Seasonal and spatial patterns of fungal populations. *New Phytologist* 138: 131-142.

Mitchell, C.E., A.A. Agrawal, J.D. Bever, G.S. Gilbert, R.A. Hufbauer, J.N. Klironomos, J.L. Maron, W.F. Morris, I.M. Parker, A.G. Power, E.W. Seabloom, M.E. Torchin and D.P. Vázquez 2006. Biotic interactions and plant invasions. *Ecology letters* 9: 726–740.

Mihály, B. and A. Demeter. 2003. *Invasive alien species in Hungary. National Ecological Network* Vol. 6. Authority for Nature Conservation, Ministry of Environment and Water, Budapest.

Moles, A., M. Gruber and S. Bonser. 2008. A new framework for predicting invasive plant species. *Journal of Ecology* 96: 13-17.

Molnár, Zs., M. Bíró, S. Bartha and G. Fekete. 2012. Past trends, present state and future prospects of Hungarian forest-steppes. In: Werger, M.J.A. and M.A.van Staalduinen (eds.) *Eurasian steppes. Ecological problems and livelihoods in a changing world. Plant and vegetation, vol. 6.* Springer, Dordrecht. pp. 209-252.

Mummey, D.L., M.C. Rillig and W.E. Holben. 2005. Neighboring plant influences on arbuscular mycorrhizal fungal community composition as assessed by T-RFLP analysis. *Plant and Soil* 271: 83-90.

Niu, H.-b. , W.-x. Liu, F.-h. Wan and B. Liu. 2007. An invasive aster (*Ageratina adenophora*) invades and dominates forest understories in China: altered soil microbial communities facilitate the invader and inhibit natives. *Plant and Soil* 294: 73-85.

Pringle, A., J.D. Bever, M. Gardes, J.L. Parrent, M.C. Rillig and J.N. Klironomos. 2009. Mycorrhizal symbioses and plant invasions. *Annual Review of Ecology and Systematics* 40: 699-715.

Quinn, G.P. and J.M. Keough. 2002. *Experimental design and data analysis for biologists.* Cambridge University Press, New York

R Development Core Team 2009. R: *A language and environment for statistical computing.* R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Reinhart, K.O. and R.M. Callaway. 2006. Soil biota and invasive plants. *New Phytologist* 170: 445-457.

Richardson, D.M., N. Allsopp, C.M. D'Antonio, S.J. Milton and M. Rejmánek. 2000. Plant invasions - the role of mutualism. *Biological Review* 75: 65-93.

Roberts, K.J. and R.C. Anderson. 2001. Effect of garlic mustard [*Alliaria petiolata* (Beib. Cavara & Grande)] extracts on plants and arbuscular mycorrhizal (AM) fungi. *The American Midland Naturalist* 146: 146–152.

Rydlová, J. and M. Vosátka. 2001. Associations of dominant plant species with arbuscular mycorrhizal fungi during vegetation development on coal mine spoil banks. *Folia Geobotanica* 36: 85-97.

Sanon, A., Beguiristain, T., Cébron, A., J. Berthelin, S.N. Sylla and R. Duponnois. 2012. Differences in nutrient availability and mycorrhizal infectivity in soils invaded by an exotic plant negatively influence the development of indigenous Acacia species. *Journal of Environmental Management* 95: S275-S279.

Sánchez-Castro, I., N. Ferrol, P. Cornejo and J.-M. Barea. 2012. Temporal dynamics of arbuscular mycorrhizal fungi colonizing roots of representative shrub species in a semi-arid

Mediterranean ecosystem. *Mycorrhiza* 22: 449-460.

Seifert, E.K., J.D. Bever and J.L. Maron. 2009. Evidence for the evolution of reduced mycorrhizal dependence during plant invasion. *Ecology* 90: 1055-1062.

Shah, M.A., Z.A. Reshi and D. Khasa. 2009a. Arbuscular mycorrhizal status of some Kashmir Himalayan alien invasive plants. *Mycorrhiza* 20: 67-72.

Shah, M.A., Z.A. Reshi and D.P. Khasa. 2009b. Arbuscular mycorrhizas: drivers or passengers of alien plant invasion. *Botanical Review* 75: 397-417.

Simberloff, D. and B.V. Holle. 1999. Positive interactions of nonindigenous species: invasional meltdown? *Biological Conservation* 1: 21–32.

Somodi, I., K. Virágh and J. Podani. 2008. The effect of the expansion of the clonal grass Calamagrostis epigejos on the species turnover of a semi-arid grassland. *Applied Vegetation Science* 11: 187-192.

Stampe, E.D. and C. Daehler. 2003. Mycorrhizal species identity affects plant community structure and invasion: a microcosm study. *Oikos* 100: 362-372.

Stinson, K.A., S.A. Campbell, J.R. Powell, B.E. Wolfe, R.M. Callaway, G.C. Thelen, S.G. Hallett, D. Prati and J.N. Klironomos. 2006. Invasive plant suppresses the growth of native tree seedlings by disrupting belowground mutualisms. *PLoS Biology* 4: e140.

Terpó, A., M. Zajac and A. Zajac. 1999. Provisional list of Hungarian archaeophytes. *Thaiszia - Journal of Botany* 9: 41-47

Trouvelot, A., J.L. Kough and V. Gianinazzi-Pearson. 1986. Mesure du taux de mycorhization VA d’un systéme radiculare. Recherche de méthodes d’estimation ayant une signification fonctionelle. In: Mycorrhizae: physiology and genetics-Les mycorhizes: physiologie et génétique. Proceedings of the 1st ESM/1er SEM, Dijon, 1-5 July 1985.-INRA, Paris, pp. 217-221.

Turnau, K., T. Anielska, P. Ryszka, S. Gawronski, B. Ostachowicz and A. Jurkiewicz. 2008. Establishment of arbuscular mycorrhizal plants originating from xerothermic grasslands on heavy metal rich industrial wastes–new solution for waste revegetation. *Plant and Soil* 305: 267-280.

Tutin T.G., V.H. Heywood, N.A. Burges, D.M. Moore, D.H. Valentine, S.M. Walters, and D.A. Webb (eds.). 1964-1993. Flora Europaea, Vols. 1-5. Cambridge University Press, Cambridge, UK.

van der Heijden, M., T. Boller and A. Wiemken. 1998. Different arbuscular mycorrhizal fungal species are potential determinants of plant community structure. *Ecology* 79: 2082–2091.

van der Putten, W.H., G.A. Kowalchuk, E.P. Brinkman, G.T.A. Doodeman, R.M. van der Kaaij, A.F.D. Kamp, F.B.J. Menting and E.M. Veenendaal. 2007. Soil feedback of exotic savanna grass relates to pathogen absence and mycorrhizal selectivity. *Ecology* 88: 978-988.

van der Staaij, J., A. Rozema, A. van Beem and E. Aerts. 2001. Increased solar UV-B radiation may reduce infection by arbuscular mycorrhizal fungi (AMF) in dune grassland plants: evidence from five years of field exposure. *Plant Ecology* 154: 171-177.

Vilà M. and J. Weiner. 2004. Are invasive plant species better competitors than native plant species? - evidence from pair-wise experiments. *Oikos* 105: 229- 238.

Vogelsang K.M., J.D. Bever, M.S.P. Griswold and P.A. Schultz. 2004. The use of mycorrhizal fungi in erosion control applications. Sacramento, CA, USA: California Department of Transportation.

Vogelsang, K.M. and J.D. Bever. 2009. Mycorrhizal densities decline in association with nonnative plants and contribute to plant invasion. *Ecology* 90: 399-407.

Wang, B. and Y.-L. Qiu. 2006. Phylogenetic distribution and evolution of mycorrhizas in land plants. *Mycorrhiza* 16: 299-363.

Wilson, G.W., K.R. Hickman and M.M. Williamson. 2012. Invasive warm-season grasses reduce mycorrhizal root colonization and biomass production of native prairie grasses. *Mycorrhiza* 22: 327-336.

Wolfe, B.E. and J.N. Klironomos. 2005. Breaking new ground: soil communities and exotic plant invasion. *BioScience* 55: 477-487.

Wolfe, B. E., V.L. Rodgers, K.A. Stinson and A. Pringle. 2008. The invasive plant *Alliaria petiolata* (garlic mustard) inhibits ectomycorrhizal fungi in its introduced range. *Journal of Ecology* 16: 777–783.

Zabinski, C.A., L. Quinn and R.M. Callaway. 2002. Phosphorus uptake, not carbon transfer, explains arbuscular mycorrhizal enhancement of *Centaurea maculosa* in the presence of native grassland species. *Functional Ecology* 16: 758–765.

Zeileis, A. 2004. Econometric Computing with HC and HAC Covariance Matrix Estimators. *Journal of Statistical Software* 11: 1-17.

Zeileis, A. 2006. Object-oriented computation of sandwich estimators. *Journal of Statistical Software* 16: 1-16.

Zhang, Q., L.J. Yao, L.Y. Yang, J.J. Tang and X. Chen. 2007. Potential allelopathic effects of an invasive species *Solidago canadensis* on the mycorrhizae of native plant species. *Allelopathy Journal* 20: 71-78.

Figure captions

Figure 1. Measures of arbuscular mycorrhizal colonisation for roots of grass species in Test 1. **A)** frequency of root segments colonised (F%), **B)** intensity of mycorrhizal colonisation (M%), **C)** percentage arbuscule occurrence of the colonised root section (a%), **D)** percentage arbuscule occurrence of the whole root (A%), **E)** percentage vesicle occurrence of the AMF colonised root section (v%), **F)** percentage vesicle occurrence of the whole root (V%). Mean values, error bars indicate ±1SE. Different lowercase or uppercase letters above columns indicate significant differences between species. ANOVA S denotes that analysis of variance with a sandwich covariance matrix estimator instead of ordinary ANOVA was used.

Figure 2. Measures of arbuscular mycorrhizal colonisation for roots of grass species in the stand dominated by the species itself in Test 2. **A)** frequency of root segments colonised (F%), **B)** intensity of mycorrhizal colonisation (M%), **C)** percentage arbuscule occurrence of the colonised root section (a%), **D)** percentage arbuscule occurrence of the whole root (A%), **E)** percentage vesicle occurrence of the AMF colonised root section (v%), **F)** percentage vesicle occurrence of the whole root (V%). Mean values, error bars indicate ±1SE. Different lowercase letters above columns indicate significant differences between species. ANOVA S denotes that analysis of variance with a sandwich covariance matrix estimator instead of ordinary ANOVA was used.

Figure 3. Measures of arbuscular mycorrhizal colonisation for roots of *Festuca vaginata* and *Stipa borysthenica* in stands differing in invasion status (Test 2). **A)** frequency of root segments colonised (F%), **B)** intensity of mycorrhizal colonisation (M%), **C)** percentage arbuscule occurrence of the colonised root section (a%), **D)** percentage arbuscule occurrence of the whole root (A%), **E)** percentage vesicle occurrence of the AMF colonised root section (v%), **F)** percentage vesicle occurrence of the whole root (V%). Mean values, error bars indicate ±1SE. Different lowercase letters above columns indicate significant differences between species. ANOVA S denotes that analysis of variance with a sandwich covariance matrix estimator instead of ordinary ANOVA was used.

Table 1. Species selected for Test 1.

Photosynthetic pathway	**Invasive**	**Resident post-disturbance dominant**	**Non-invasive native**
C3	*Calamagrostis epigejos*	*Brachypodium pinnatum*	*Chrysopogon gryllus*
C4	*Cynodon dactylon*	*Bothriochloa ischaemum*	*Bromus inermis*

Figure 1.

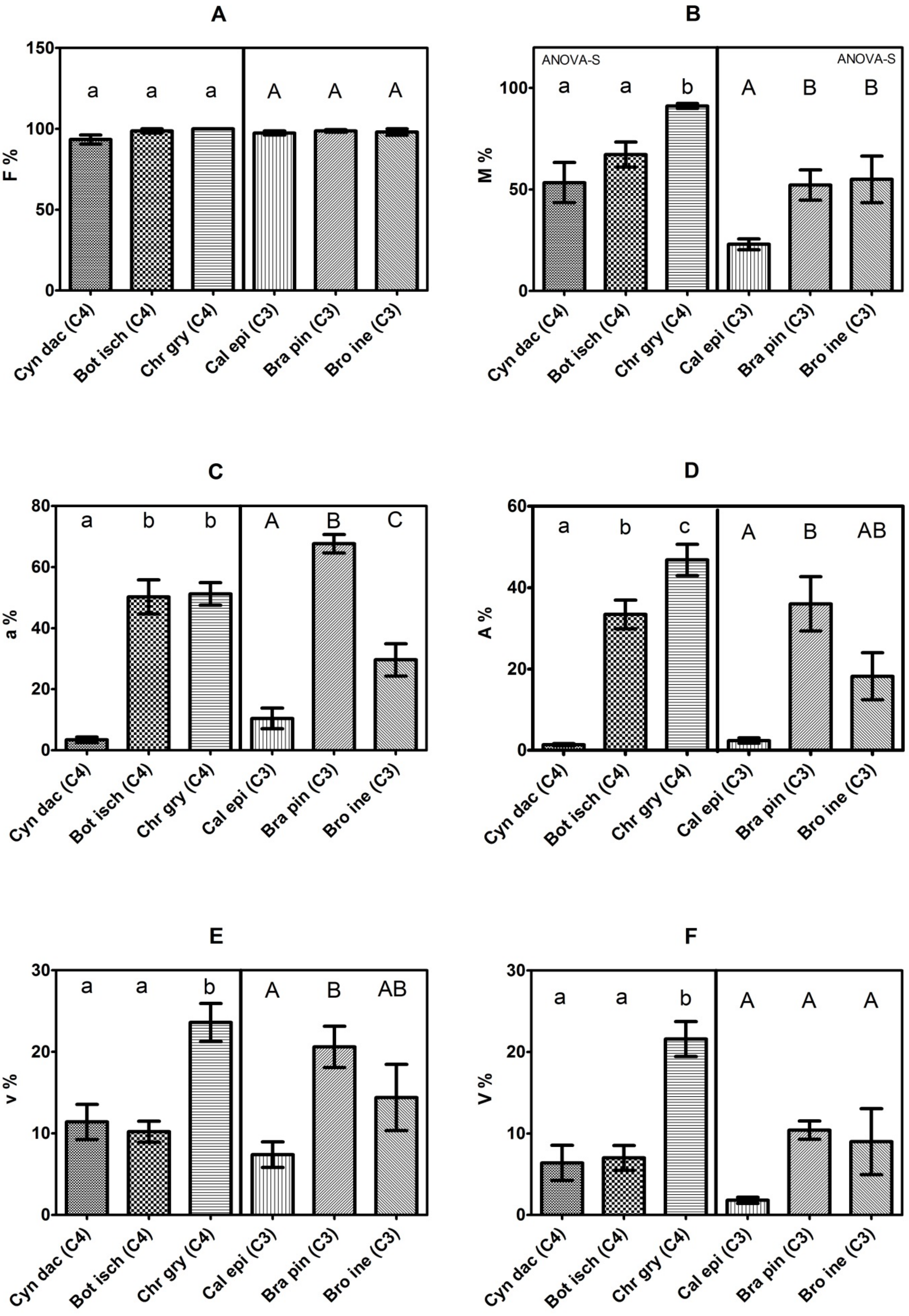

A
F %
0
50
100
150
a a a A A A
B
M %
0
50
100
ANOVA-S
ANOVA-S
a a b A B B
C
a %
0
20
40
60
80
a b b A B C
D
A %
0
20
40
60
a b c A B AB
E
v %
0
10
20
30
a a b A B AB
F
V %
0
10
20
30
a a b A A A
Cyn dac (C4)
Bot isch (C4)
Chr gry (C4)
Cal epi (C3)
Bra pin (C3)
Bro ine (C3)

Figure 2.

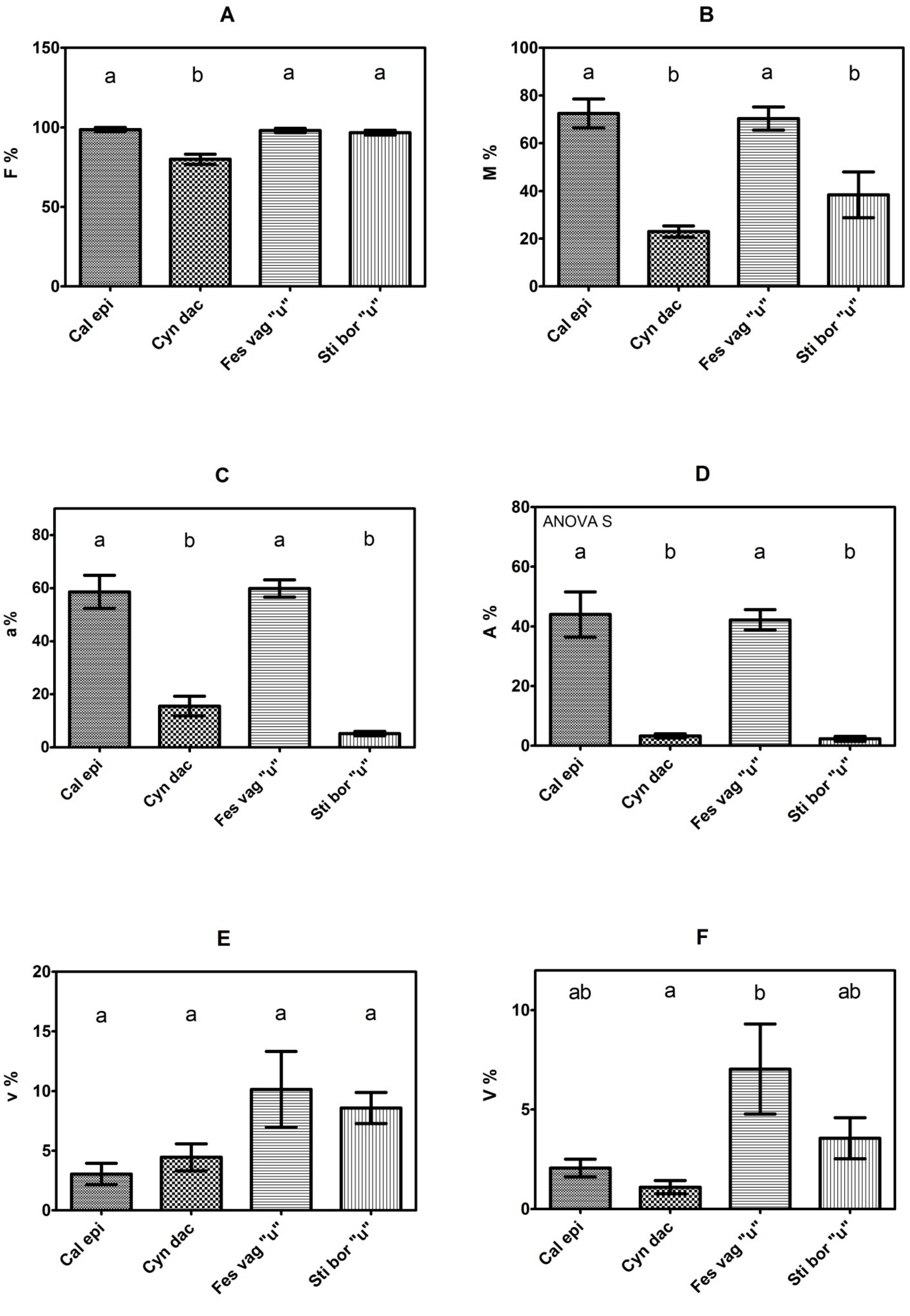
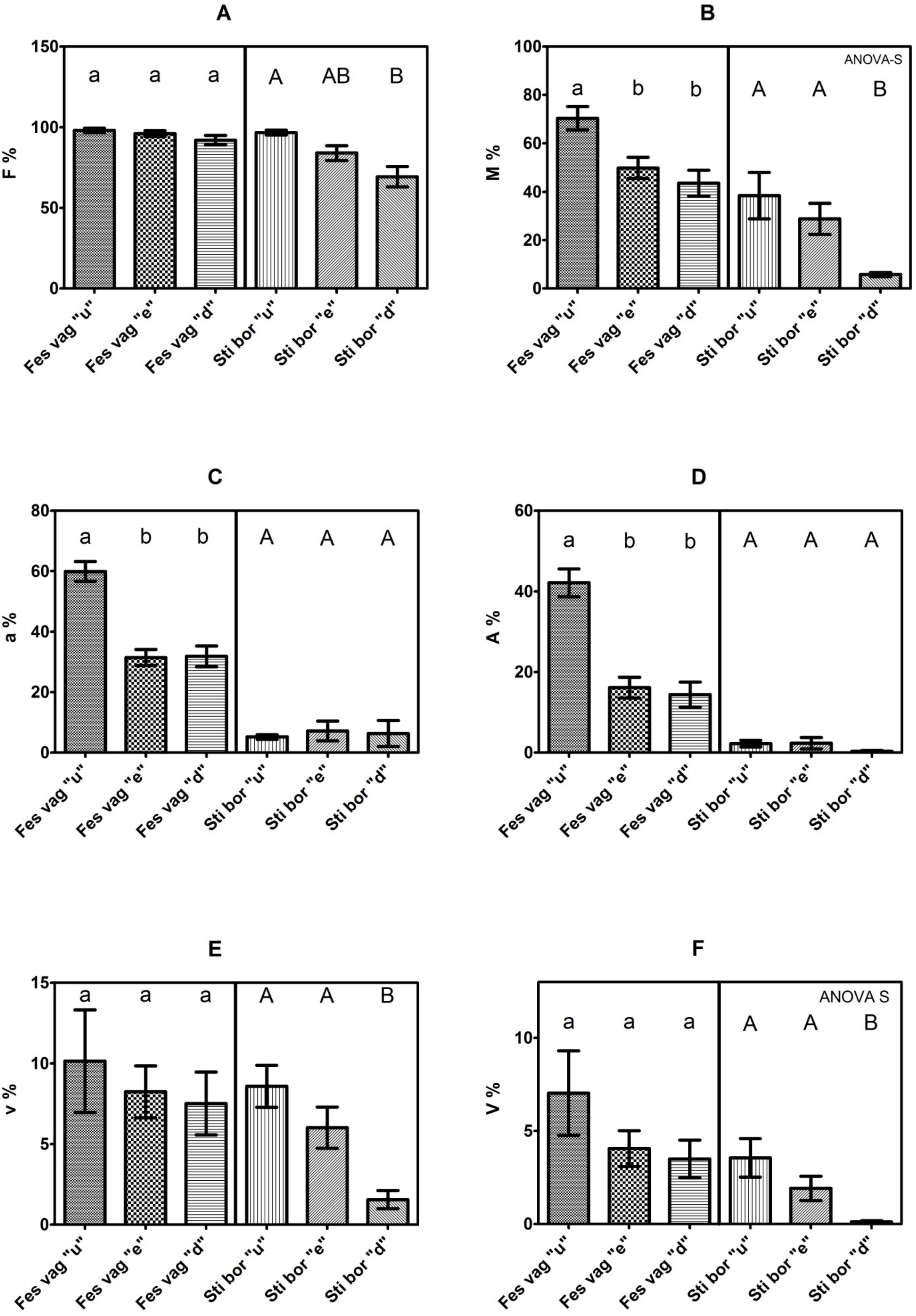

A
F %
150
100
50
0
a
b
a
a
Cal epi
Cyn dac
Fes vag "u"
Sti bor "u"
B
M %
100
80
60
40
20
0
a
b
a
b
Cal epi
Cyn dac
Fes vag "u"
Sti bor "u"
C
a %
80
60
40
20
0
a
b
a
b
Cal epi
Cyn dac
Fes vag "u"
Sti bor "u"
D
A %
ANOVA S
80
60
40
20
0
a
b
a
b
Cal epi
Cyn dac
Fes vag "u"
Sti bor "u"
E
v %
20
15
10
5
0
a
a
a
a
Cal epi
Cyn dac
Fes vag "u"
Sti bor "u"
F
V %
10
5
0
ab
a
b
ab
Cal epi
Cyn dac
Fes vag "u"
Sti bor "u"

Figure 3.


A
F %
150
100
50
0
a a a A AB B
Fes vag "u"
Fes vag "e"
Fes vag "d"
Sti bor "u"
Sti bor "e"
Sti bor "d"
B
M %
100
80
60
40
20
0
ANOVA-S
a b b A A B
Fes vag "u"
Fes vag "e"
Fes vag "d"
Sti bor "u"
Sti bor "e"
Sti bor "d"
C
a %
80
60
40
20
0
a b b A A A
Fes vag "u"
Fes vag "e"
Fes vag "d"
Sti bor "u"
Sti bor "e"
Sti bor "d"
D
A %
60
40
20
0
a b b A A A
Fes vag "u"
Fes vag "e"
Fes vag "d"
Sti bor "u"
Sti bor "e"
Sti bor "d"
E
v %
15
10
5
0
a a a A A B
Fes vag "u"
Fes vag "e"
Fes vag "d"
Sti bor "u"
Sti bor "e"
Sti bor "d"
F
V %
10
5
0
ANOVA S
a a a A A B
Fes vag "u"
Fes vag "e"
Fes vag "d"
Sti bor "u"
Sti bor "e"
Sti bor "d"