GROWTH AND MYCORRHIZAL COLONIZATION OF FOUR GRASSES IN A MN-AMENDED LOW QUALITY SANDY SOIL

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***Abstract**: Colonisation of arbuscular mycorrhiza fungi (AMF) was studied in a pot experiment with four grasses (Bromus inermis, Lolium perenne, Festuca rubra and Poa pratensis) in low quality, calcareous, slightly humic (1.5 %) sandy soil. A regular organic treatment, the propylene glycol (PG) was used to enhance the microbial activity of the soil, diluted in water at 12.500 mg/L ratio. Soil was amended with Mn sludge (500 mg/kg) from Úrkút, Hungary before the sowing. Plants were grown 8 weeks and sampled several times during the growing season. Dry matter production, element uptake and AMF colonization and total catabolic enzyme activity of microbial biomass, as fluorescent-diacetate analysis (FDA) were studied. Available element content of potassium (K_2O) and phosphorous (P_2O_5) or the Fe and Mn microelements was assessed by ICP analysis from the propylene-glicol (PG)- and/or the Mn-mud-treated soil samples. Great differences were found among the grasses and mycorrhiza colonization. Total catabolic enzymatic activity (FDA) and colonization with arbuscular mycorrhizal fungi (AMF) responded positively to the organic treatment of fast-degradable propylene-glycol. A reduced biomass production was recorded at those hosts at both treatments during the plant growth. The fresh (and dry) shoot biomass production was decreasing with the plant age as a function also with the applied treatments. Especially high AMF colonization intensity (M%) and arbuscular richness (A%) could be found at the Poa pratensis. Organic PG amendment enhanced the mycorrhiza intensity and function at all grasses, as well as the total catabolic enzyme activities (FDA) of microbes. Both PG and Mn-treatments had increased the availability of potassium in soil on a same extent, but no changes appeared in soil P content was found. We consider that variability in interaction and plant-microbe respond can provide an appropriate tool for the plant-selection in PG- and Mn-affected soils.*

Key words: *Mn-Fe availability, propylene glycol, sandy soil, grasses, mycorrhiza, toxicity*

INTRODUCTION

Mycorrhizal fungi are known to develop a beneficial symbiosis with more than 80% of higher plants (SMITH AND READ, 2008). The symbiosis can be beneficial for both the microsymbiont fungi and also for the macrosymbiont hosts (BIRÓ et. al., 2005). Not only the plant growth and development, but also the stress-tolerance abilities and the plant fitness can be improved by the symbiosis, as it was measured by using chlorophyll-fluorescence techniques of OJIP test (KÖVES-PÉCHY et al., 1999). The inoculation of known mycorrhiza species is an accepted technology therefore in the horticultural practice (SCHMIDT et al. 2010) and also at the acclimatization of micropropagated fruit trees (BALLA et al., 1998). Beside this, the combination of several beneficial microbes, such as the nitrogen-fixing *Rhizobium* and *Azospirillum* bacteria (BIRÓ et al. 2000*)* in arable soils or the *Brevibacillus* strains in heavy-metal contaminated soils can be also a good practice (VIVAS et al. 2003a,b, 2006) for improving the plant growth. The adapted arbuscular mycorrhizal fungi (AMF) can determine the reduction of the toxic metals' uptake of their host-plant (BIRÓ et al. 2005, 2010). The indigenous fungi in a certain environment can become a great help for the plant growth not only at the early stage, but also at the later periods, when the circumstances become more serious (i.e. at summer drought or at salinity). The initiative for the symbiosis-development is increasing at environmental stress, and AM fungi will be functioning better by improving the arbusculum richness (FÜZY et al. 2007).

The mycorrhizal colonization and functioning in low quality sandy soils, however needs to be improved by the physical-, chemical- and/or (micro-) biological treatments. One of the necessities in the soil could be the requirements for the better nutrient availabilities. For this reason inorganic (N, P, K) fertilizers are often used in the arable practice. Knowledge about the use of organic additives is rather missing. The propylene glycol, as one of the highly degradable organic sugar-alcohol is known to enhance the specific microbial activities in the low quality soils (DOMONKOS, 2009). This happens also during the snow-melt (at about $4^{o}C$), in cold (arctic) climatic conditions. Among such stress-situations the rhizosphere properties, i.e. the interaction between the higher plants, the mycorrhiza fungi and also the so called „helper" bacteria has more advantage. Question arises therefore to study, how the highly degradable organic additives of the PG are improving the soil biological activities by the regular addition during the plant growth? Are there any differences among the typical grass-types on the colonization abilities with the microsymbiont arbuscular mycorrhizal fungi? How the developed symbiosis can be functioning, when applying high amounts of Mn-mud, from a Manganese-Ore?

The Úrkút Ore is operating from 1917 in Hungary. The Fe-Mn-mud, as a side-product of the industry is considered as potentially applicable row material in the agriculture (VIGH, 2005). The Mn-sludge has great amounts of microelements, beside the Fe- and Mn content (Table 1). Sludge was improving the structure of the sandy soils and better yield was found with several crops (POLGÁRI et al. (Eds.), 2000). Mn-sludge is containing high amount of Fe, which is known to improve the chlorophyll content and several enzymatic reactions. (HARGITAI, 1998). Among basic conditions chlorotic symptoms can be found due to the low availability of the Fe and Mn ions, which highlights the necessity of the application of Mn-sludge of industrial origins. Manganese is known as a key-element in the soil which affects the oxido-reductive conditions (HARGITAI, 1998), and also for the formation of complex humic materials and organic acids. Acidic sandy soils are high in manganese, but calcareous soils are poor. Monocotyledonous plants require more Mn, than dicotyledonous ones. During the vegetation periods the Mn-content is eliminated *(HTTP1)*. Differences among the grass species require, therefore more attention.

MATERIALS AND METHODS

Pot experiment was designed to study the effect of Mn-sludge addition on the growth and development of four representative grass species, such as the *Bromus inermis* (R), *Lolium perenne* (A), *Festuca rubra* (V), *Poa pratensis* (P), potentially applicable both in Hungary and in cold climate in available grass-mixtures (Soil-CAM project). Low quality, calcareous, slightly humic (1.5 %) sandy soil was used in the pots (300 mg dry soil in each). For improving the soil-microbial activity a concomitant addition of the propylene-glycol (PG) organic amendment (12.500 mg/L of water) was applied regularly twice a week, at watering the pots.

Mn mud was used for some of the pots as 500 kg/ha on a dry weight basis. Some of the element content of mud is shown in Table 1.

Pots were sampled several times depending on the studied parameters during the 8 weeks of growth period. Row and dry-matter production, ICP-element content in soil and in plant biomass was assessed after 8 weeks of plant-growth in a light-chamber. Total catabolic enzyme activity of microbial biomass, as fluorescent-diacetate analysis (FDA) was estimated as described by VILLÁNYI et al., 2006.

Table 1.
Some macro- and trace-element contents of Mn-mud from Úrkút (Hungary), measured by ICP analysis (n=3).

Total element contents by aqua-regia method (g/t)						
Si	**Al**	**K**	**Ca**	**Mg**	**Fe**	**Mn**
158790,4	67189,7	27343,7	14669,2	11059,5	157852,4	139258,1
Co	Ni	Cr	Cu	Zn	Ti	Ba
261,6	171,5	224,9	77,2	143,5	3871,7	1057,0

After an aniline staining of root samples, colonization with arbuscular mycorrhizal fungi (AMF) was assessed by the TROUVELOT et al. (1986) method using an Olympus type microscope. All data were subjected to variance analysis. Mean and significant differences are shown in Tables and Figures.

Both treatments could enhance the microbial activity during the growth of the grasses (except the first sampling period). The activity was concomitant increasing with the plant age. A reduced microbial activity was produced, however at the last sampling at all the treatments. Plant age and treatments are affecting mainly the activities of microbes in the soils. The same conclusion was reported by BAREA et al.(2002).

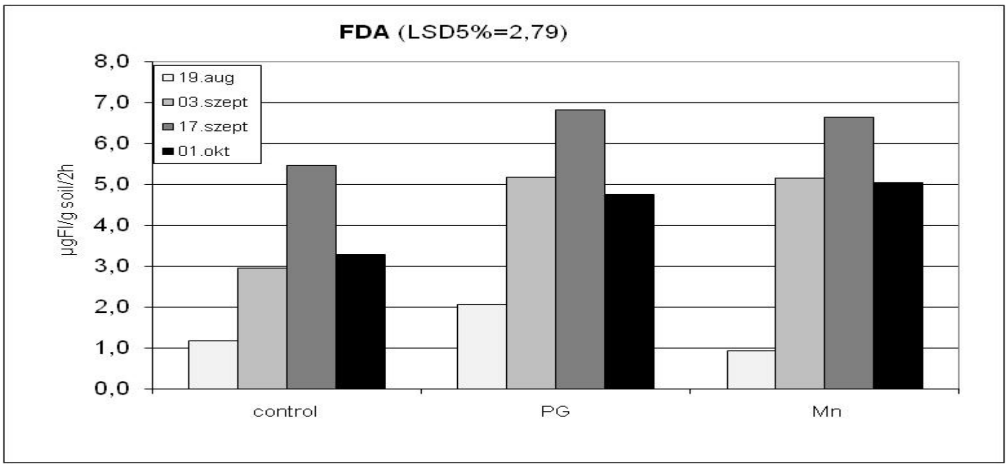

Figure 1. Total catabolic enzymatic activity (µg Fl/g soil/2 hours), measured by the fluorescent diacetate hydrolysis (FDA) of the slightly humic sandy soil, as a function of different treatments. PG=propylene glycol, Mn=Mn-mud treatments. Further details in text. (n=3)

Biomass production of 4 grasses with treatments

The biomass production of the four grasses we investigated is shown in Table 2. Both the shoot and the root or the total biomass production is listed as the total amount of the first (2010. 08.17) and the second (2010.10.01) sampling. We could realize a reduced biomass production at the second sampling at all the two treatments (data not shown). An increased root biomass production was found at *Lolium perenne* (A) and a reduced one at *Bromus inermis* (R). The other two grasses, the *Festuca rubra* (V) and *Poa pratensis* (P) were not responding positively for the treatments we applied.

A reduced biomass production was recorded at those hosts at both treatments during the plant growth. The fresh (and dry) shoot biomass production was decreasing with the plant age as a function also with the applied treatments. When considering the combined PG- and Mn treatments, chlorotic symptoms were found at the test plants.

Table 2.

Shoot, root and total biomass production of four grasses at propylene glycol (PG) and manganese-mud (Mn) treatment in a slightly humic sandy soil. Further details in text. (n=3)

Treatments	Shoot (DW)	Root (DW)	Total biomass production (DW)
Lolium perenne (A)			
0	0.35 +/- 0.03	0.10 +/- 0.08	0.45
PG	0.32 +/- 0.33	0.62 +/- 0.24	0.94
Mn	0.29 +/- 0.33	0.54 +/- 0.24	0.83
Bromus inermis (R)			
0	0.53 +/-0.05	0.19 +/- 0.11	0.72
PG	0.33 +/- 0.03	0.79 +/- 0.31	1.12
Mn	0.33 +/- 0.03	0.64 +/- 0.31	0.97
Festuca rubra (V)			
0	0.55 +/- 0.05	1.24 +/- 0.15	1.79
PG	0.64 +/- 0.04	0.96 +/- 0.24	1.60
Mn	0.82 +/- 0.04	1.00 +/- 0.24	1.82
Poa pratensis (P)			
0	0.46 +/- 0.02	0.35 +/- 0.06	0.81
PG	0.47 +/- 0.01	0.40 +/- 0.05	0.97
Mn	0.63 +/- 0.01	0.29 +/- 0.05	0.91

Table 3.

Available macroelement (P, K) and Fe, Mn microelement-contents of slightly humic sandy soil under 2 testplants, as a function of propylene-glycol (PG) and manganese-mud (Mn treatments). Further details in text.

Available nutrients in soil (mg/kg)				
Treatments	Fe	K_2O	Mn	P_2O_5
Control (without plants)	81,1	37,6	113	40,0
Poa pratensis (P)				
0	77,2	26,8	112	41,5
PG	155	32,4	156	34,9
Mn	127	33,8	234	40,9
Festuca rubra (V)				
0	76,4	23,0	116	38,9
PG	125	28,7	148	35,3
Mn	106	30,4	178	37,4

Element content in soil and in plants at different treatments

Available element content of potassium (K_2O) and phosphorous (P_2O_5) or the Fe and Mn microelements was assessed by ICP analysis from the propylene-glicol (PG)- and/or the

Mn-mud-treated soil samples. Both PG and Mn-treatments had increased the availability of potassium in soil on a same extent. On the other hand no changes in soil P content was found (Table 3).

Approximately the double amount of Mn-content was found in Mn-mud treated soils. This enhanced availability could increase of the Mn content in shoot biomass of the test plants. Between the two host plants great differences were found. According to Table 4, a greater amount (at about 10 times more) of Mn was found in *Poa pratensis* (P), while only 4-times more at the *Festuca rubra* (V) host plant. The used Mn-mud equally increased the Fe-content of 2 test plants.

Table 4.

Macroelement (P, K) and Fe, Mn microelement contents of two test plants grown in propylene-glycol (PG) and manganese-mud (Mn)-treated slightly humic sandy soil. Further details in text.

Element contents in plant shoot (mg/kg)				
	Fe	K_2O	Mn	P_2O_5
Poa pratensis (P)				
0	2773	21727	658	3653
PG	4607	9760	1141	2631
Mn	11574	6456	5920	1677
Festuca rubra (V)				
0	972	37243	801	4809
PG	3310	14805	1003	3639
Mn	10150	7388	4296	1838

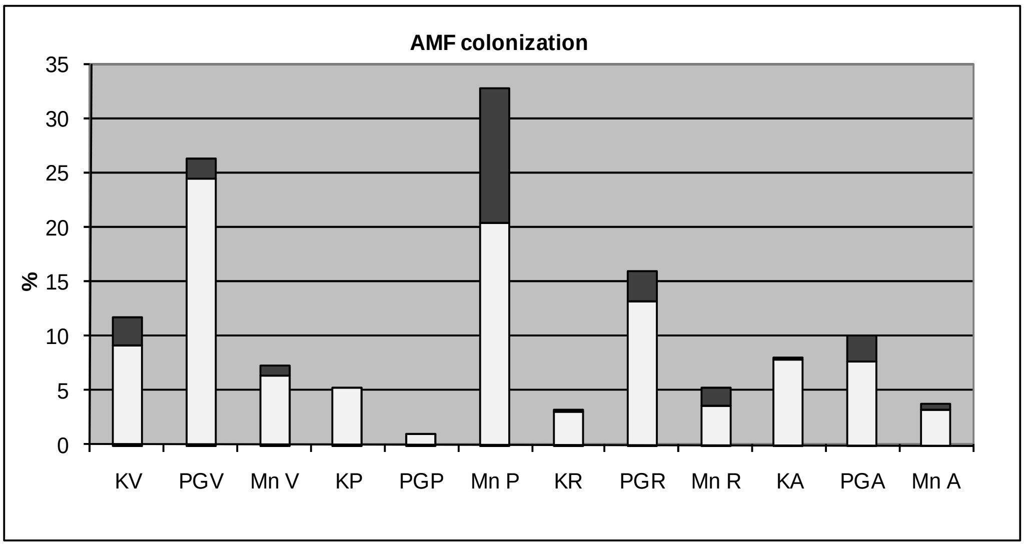

Figure 2. Colonization of arbuscular mycorrhizal fungi (AMF), as colonization intensity (M%, white parts) and arbuscular richness (A%, black parts) of four grasses, grown in propylene-glycol (PG), Mn-mud (Mn) treated or in control (K) soils.

Testplants are: V= *Festuca rubra*, P= *Poa pratensis*, R= *Bromus inernis*, A= *Lolium perenne*

Mycorrhiza colonization of 4 grasses with treatments

According to Figure 2, the colonization of arbuscular mycorrhizal fungi (AMF) is highly depending on the host plants we used. A relatively strong AMF symbiosis was found at *Festuca rubra* (V), while the greatest variability determined by the treatments was found at (*Poa pratensis,* P) and also the highest colonization values were found at this host.

Addition of propylene-glycol could generally increase the percentage of mycorrhizal colonization. Among the four hosts, only the *Poa pratensis* was not responding positively to the PG addition by enhancing the mycorhizal colonization. In case of a further treatment of Mn-mud a strengthened necessity of microsymbiont (AMF) help could be realised at test-plants, as it was shown by other authors (BAREA et al., 2002; VIVAS et al., 2003a,b; 2006). On the other hand, deleterious effect of high toxic manganese amounts could not be diminished by the mycorrhizal fungi, even though the better functioning (greater arbuscularity) of the fungi at *Poa pratensis*. We assume, however, that a more efficient plant-mycorrhizal fungi relationship can develop on the long-term level of the toxic elements. In those heavy-metal polluted soils the adapted mycorrhizal fungi are able to confer the tolerance to the higher plants (BIRÓ et al. 2005, 2010). Indigenous AM fungi were not adapted preliminary to the PG and Mn-treatments, therefore the high amount of Mn-mud proved to be deleterious both for the microsymbiont fungi and also for the macrosymbiont hosts.

CONCLUSIONS

Microbial parameters, measured as total catabolic enzymatic activity (FDA) and colonization with arbuscular mycorrhizal fungi (AMF) responded positively to the organic treatment of fast-degradable propylene-glycol. Regarding the Mn-mud treatments, the enhanced FDA activity has remained, but the mycorrhizal colonization was diminished, which shows a grater sensitivity of the AM fungi and the plant-mycorrhizal fungi symbiosis. Variability in interaction and plant-microbe respond can provide an appropriate tool for the plant-selection in PG- and Mn-affected soils.

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