Interaction between Arbuscular Mycorrhizal Fungi and rhizobia on the growth of subclover under Mn toxicity: The role of Extraradical Mycelium.

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ABSTRACT
When Arbuscular Mycorrhizal (AM) colonization started from an intact extraradical mycelium (ERM) its bioprotective effect on subclover was enhanced in comparison with other sources of inoculum. The presence in the soil of an intact ERM, developed previously on mycotrophic plants tolerant to Mn toxicity, resulted in the earlier colonization of subclover, reduced Mn concentration in the roots, improved development and activity of root nodules, and enhanced N acquisition.

INTRODUCTION
Arbuscular Mycorrhizal Fungi (AMF) can protect plants against several different abiotic stresses, including Al and Mn (Yano and Takaki, 2005; Nogueira *et. al*., 2007). However, a well-established AM infection is crucial for an adequate degree of protection (Garg and Chandel, 2010). When compared with other sources of AM propagules, colonization initiated from an intact ERM starts earlier and develops faster (Fairchild and Miller, 1988; Martins and Read, 1997). We hypothesized that the presence in the soil of an intact ERM developed on Mn tolerant plants, at the time of subclover seeding, could enhance the bioprotection of subclover, and its associated rhizobia, against Mn toxicity.

MATERIAL AND METHODS
A two-stage experiment was conducted in pots containing a sandy loam Cambisoil, where toxic levels of Mn were detected previously. In Stage 1, Mn tolerant weed species, *Silene gallica L.*, *Rumex bucephalophorus L., Lolium rigidum L. and Ornithopus compressus L.* (developer plants), with different levels of mycotrophy, grew for 6 weeks. At the end of Stage 1, half of the pots were disturbed (ERM of mycotrophic developers disrupted), while glyphosate was applied to the weed shoots in the other half, thereby keeping the ERM of mycotrophic developers intact. For Stage 2 of the experiment, 6 seedlings of clover were introduced into the pots inoculated with an appropriate and effective strain of rhizobia. Three of these plants were sampled after 3 weeks to determine the arbuscular colonization of the clover, while the three remaining plants were allowed to grow for a total of 6 weeks.

RESULTS AND DISCUSSION
By the end Stage 1, the arbuscular colonization rate (AC) of ERM developer plants indicates different levels of mycotrophy and, therefore, different amounts of ERM were present in the soil. Developer plants did not change the availability of Mn in the soil. The AM colonization of subclover after 3 weeks was significantly greater when an intact ERM was present at the time of planting, that is, after *Lolium* and *Ornithopus* in the undisturbed treatment (Table 1). The dry matter and N content of subclover after 6 weeks was also significantly increased after the mycotrophic, than non-mycotrophic plants. AC of the clover at 3 weeks (AC3) was positively and significantly related to shoot weight at 6 weeks but inversely proportional to the Mn concentration in the roots

(Figure 1A). The AC3 was significantly and positively related with N content (NS) of subclover shoots and nodule dry weight at 6 weeks (NDW) (Figure 1B), whereas Mn concentration in the roots was inversely related to NS and NDW (Figure 1C). An intact ERM, at the seeding of the subclover enhanced earlier AM colonization and with N-fixing rhizobia, was the basis of a more effective tripartite symbiosis. This resulted from better N acquisition associated with larger and more active root nodules, the consequence of the smaller concentration of Mn in the roots. We conclude that AM colonization starting from an intact ERM greatly enhances the bioprotection granted by AMF against Mn toxicity.

Table 1. Arbuscular colonization rate (AC) and Mn in soil solution of developers at the end of stage 1; and AC at 3 weeks, Shoot dry weigh (SDW), Nodule dry weight (NDW), Shoot N and Mn concentration, Root Mn concentration and Shoot N content of clover at 6 weeks of growth.

ERM Developer Plants			**Subclover**							
							Concentration			Content
	AC	Mn in soil solution (mg/L)	Disturbance	AC 3 wks	SDW 6 wks (g/pot)	NDW 6 wks (µg/nod)	Shoot N (g/Kg)	Shoot Mn (mg/Kg)	Root Mn (mg/Kg)	Shoot N (mg/pot)
Silene	0.01 C	0.76	Undisturbed	0.355 BC	1.372 CD	24 B B	4.9 A	141	312	66 B
			Disturbed	0.145 E	0.720 DE	42 B B	5.0 A	118	231	36 B
Rumex	0.01 C	0.22	Undisturbed	0.329 BD	1.246 CE	28 B B	4.9 A	132	171	61 B
			Disturbed	0.219 DE	0.836 BE	61 B B	4.8 A	135	247	41 B
Lolium	0.54 B	0.24	Undisturbed	0.615 A	3.670 B	418 A A	3.2 B	125	134	120 AB
			Disturbed	0.404 B	1.341 CE	105 B B	4.6 A	130	191	59 B
Ornithopus	0.73 A	0.83	Undisturbed	0.682 A	4.373 A	160 B B	3.6 B	129	108	155 A
			Disturbed	0.240 CE	0.707 DE	25 B B	4.6 A	140	261	33 B

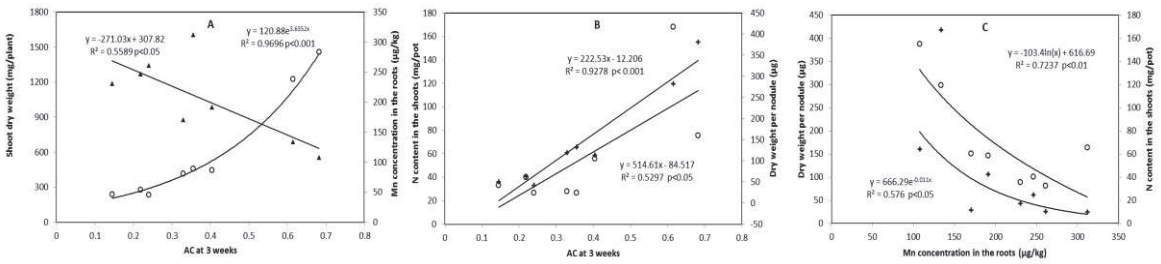

Figure 1. (A) Relationship between arbuscular colonization rate (AC) at 3 weeks and shoot dry weight (open circles) and Mn concentration in the roots (closed triangles) of subclover at 6 weeks; (B) Relationship between AC at 3 weeks and N content in the shoots of subclover at 6 weeks (crosses) and the dry weight of nodules at 6 weeks (open circles); (C) Relationship between Mn concentration in the roots and dry weight of nodules (crosses) and N content in the shoots (open circles) at 6 weeks of clover growth.

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