USE OF ORGANIC AMENDMENT AND *ENDOMYCORRHIZAL FUNGI* FOR STEEL SLAG PHYTOSTABILISATION

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Introduction

In France, slag deposits resulting from metallurgical activities have been estimated to about 40 millions of tons, spread over several hundred sites. In most cases, these sites are left bare, without any protective measure; they are thus exposed to wind and rainfalls, causing them to erode and leach. In this context, phytostabilisation approaches (*i.e.* implementing a homogeneous plant cover without bringing an extra layer of clean soil)[1] could be quite efficient for sustainably managing large metallurgical slag dumps.[2]

Recent studies demonstrated that using "Composted Sewage Sludge" (CSS), as a cheap and easily available organic amendment, was an efficient treatment to promote plant development on slags, provided that an assemblage of adapted species (drought tolerant pseudo-metallophyte species) was used.[3] Nevertheless, decreasing Cr accumulation in plants used for phytostabilising slag dumpsites remains an important research issue. In this respect, a key factor to mitigate metal accumulation in plants could rely on stimulating the development of "*Mycorrhizal Fungi*" (AMF) symbioses. In addition to a better tolerance to soil nutrient deficiencies and drought stress, there are a growing number of evidences that AMF could also immobilise some metals in their hyphae (and especially Cr) thereby decreasing the accumulation of toxic metals in plants.[4]

In this work, we hypothesised that using *Rhizophagus irregularis* (Ri), a generalist, easily commercially available AMF which has been used for a long time for

agronomical practices, could be as efficient for phytostabilising metallurgical slags. In order to test this hypothesis, we performed a pot experiment, combining CSS amendment and AMF inoculation, to evaluate: 1) the effects of CSS and Ri inoculation on vegetation in terms of biomass production, Cr accumulation and P concentration in leaves and 2) the optimal amount of CSS in order to obtain the best rates of mycorrhisation.

Material and method

Slags used in this study were taken from a metallurgical landfill located at Châteauneuf (Loire, France) of about 4 ha. Pot experiments were performed to assess the combined effects of CSS level and Ri inoculation on plant growth, Cr accumulation and P concentration of two herbaceous species: *Festuca arundinacea* (*Poaceae*) (=*F.a.*) and *Melilotus officinalis* (*Fabaceae*) (=*M.o.*). Two CSS levels were compared: 60T·ha^{-1} (=CSS60) and 120T·ha^{-1} (=CSS120). AMF inoculation was done for each CSS level at the time of sowing. Thus, the overall experimental design comprised four treatments: CSS60-Ri0; CSS60-Ri; CSS120-Ri0 and CSS120-Ri, completely randomised with ten replicates by treatment. Root colonisation was quantified under optical microscope according to Trouvelot[5] using: frequency of mycorrhisation (F%), percentage of root cortex colonisation (M%) and overall abundance of arbuscules (A%). The Kruskal-Wallis tests indicate the significant differences between the treatments (Holm adjustment, $P < 5\%$).

Results and discussion

Shoot dry weights obtained for each species and treatment are shown in Figure 1. Plants grown in CSS60 had very low aerial biomass as compared with those from CSS120. These strong differences could be mostly due to an increasing of water holding capacity, nutrient supply as well as a more pronounced pH decrease in CSS120. Substrate pH around 11 without amendment limits nutrient availability and drives metals speciation and solubility. Plants inoculated with AMF had a slightly higher biomass, but this was not statistically significant.

The most problematic metal on the study site was Cr; its accumulation in leaves is presented in Figure 2a. Results showed that plants grown on CSS60 exhibited higher Cr contents; this could be related to the very small size of these plants. Indeed, for tree species as well as herbaceous species, metal concentrations are generally higher in the early vegetative growth stages. At the opposite, late growth stages present higher biomass production, leading to dilution of accumulated metals.

As one of the main essential plant nutrients, P concentrations in leaves are shown in Figure 2b. P foliar content showed contrasted differences between treatments. The

higher P contents were observed on plants growing on CSS120 in the presence of Ri (P < 5%). This is consistent with the general observation that mycorrhizal plants are able to solubilise higher P amounts from soil and improve its concentration in organs.

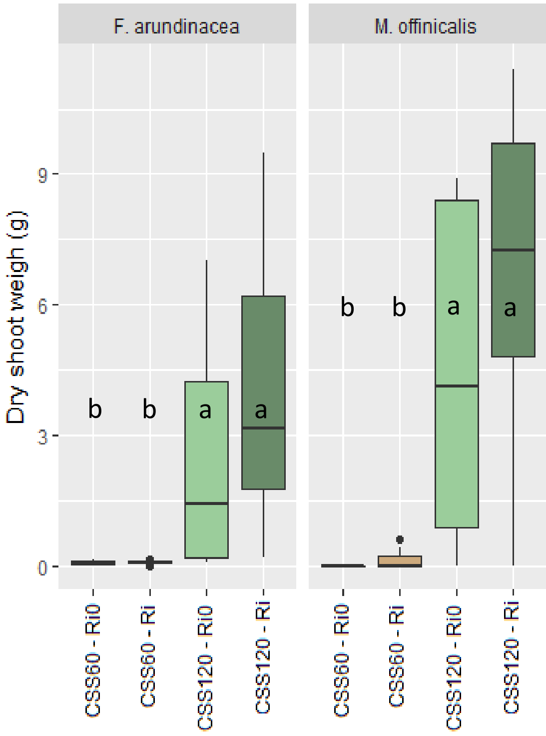

Figure 1: Shoot dry weight yields of *F. a. and M.o.* after 3 months growth; Different letters mean statistical significance (Kruskal-Wallis, P < 5%)

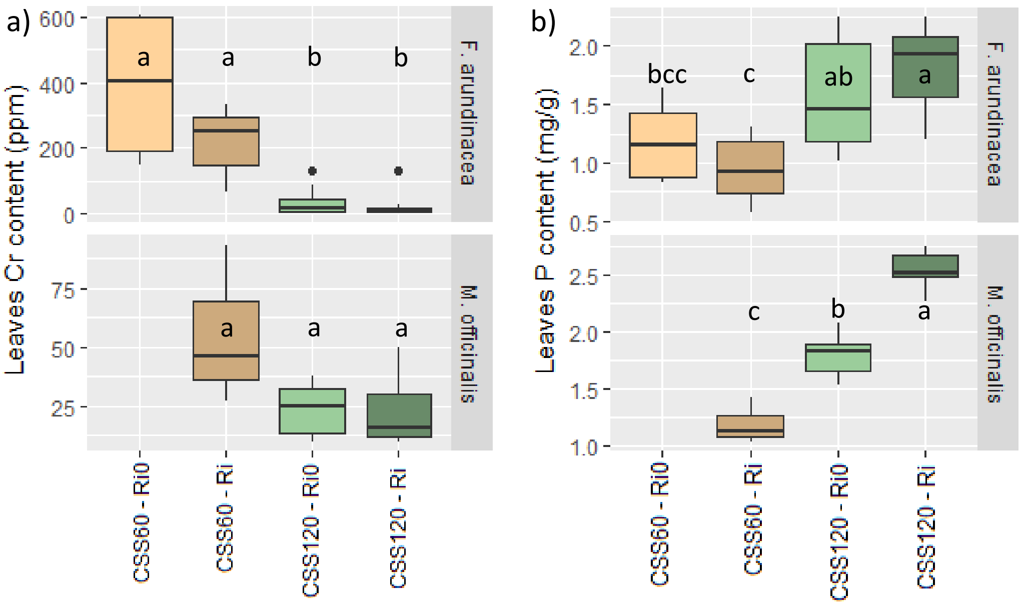

Figure 2: Cr (a) and P (b) concentration in leaves of *F. a.* and *M. o.* after 3 months growth; Different letters mean statistical significance (Kruskal-Wallis, P < 5%)

Indicators of root colonisation for both CSS levels are given in Table 1. The frequency of mycorrhisation was significantly higher on CSS120 than on CSS60 for both plant species. Likewise, *F. a.* showed the highest frequencies of colonisation when grown on CSS120 (P < 5%). Calculation of the M% and A% parameters did not evidence significant differences between species growing on CSS120 and confirmed that the extension of root colonisation was globally higher in that condition.

Table 1: Mycorrhisation status of *F. a.* and *M. o.* after 3 months growth; Different letters mean statistical significance (Kruskal-Wallis, P < 5%)

Plant species	Treatment	F%	M%	A%
F. arundinacea	CSS60	47 .50 ± 17 .85 (bc)	4 .90 ± 6 .88 (ab)	2 .40 ± 3 .90 (ab)
	CSS120	94 .00 ± 10 .20 (a)	14 .01 ± 10 .29 (a)	3 .36 ± 3 .87 (a)
M. officinalis	CSS60	27 .50 ± 10 .90 (c)	0 .48 ± 0 .43 (b)	0 .03 ± 0 .04 (b)
	CSS120	58 .89 ± 22 .83 (b)	17 .22 ± 15 .85 (a)	5 .52 ± 6 .38 (a)

Conclusion

In this study, we investigated how CSS amendment and AMF inoculation impact plant development and Cr bioavailability. In addition to increasing water holding capacity and substrate nutrient content, CSS drove the growth of plant species. In addition, CSS amended at $120T \cdot ha^{-1}$ led to a lower accumulation of Cr in plants leaves. AMF inoculation slightly increased aerial biomass and improved plant nutrition by stimulating phosphorus uptake.

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