# Effect of arbuscular mycorrhizal fungi and mycoremediated dry olive residue in lead uptake in wheat plants

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# Role of the extraradical mycelium of arbuscular mycorrhizal fungi combined with mycoremediated dry olive residue in Pb transport and plant protection

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**Abstract**

This study aims to evaluate the role of the extraradical mycelium of arbuscular mycorrhizal fungi in Pb transport. We also investigate how these fungi, alone or combined with the mycoremediated dry olive residue (MDOR), provide protection against Pb. We established a container system consisting of a central compartment and two lateral compartments separated by a hydrophobic membrane. The central compartment was filled with sterilized soil in which wheat plants, inoculated and non-inoculated with *Funneliformis mosseae*, were grown. The lateral compartments were filled with sterile, Pb-contaminated or MDOR-amended soil or combinations of both. In contrast to shoots and grains, wheat roots accumulated larger amounts of Pb with or without applications of MDOR. The extraradical mycelium (ERM) and the glomalin related protein content were significantly boosted by adding MDOR to Pb-contaminated soil samples. Wheat root biomass was decreased as the result of Pb contamination with no increases in plant phosphorous (P) uptake. However, MDOR, when added to Pb-contaminated soil samples, only boosted the accumulation of P in roots, with P content and biomass remaining unchanged in wheat shoots and grains. Our study highlights the role of the ERM in Pb transport its accumulation in wheat roots and how the protection effect exerted by AMF seemed to rely on MDOR application by increasing the P uptake rather than Pb.

**Key-words**: Dry olive residue, Container system, *Funelia floccose*, *Funneliformis mosseae*, Hydrophobic polytetrafluoroethylene membrane, Lead (Pb)

## 1. Introduction

Lead (Pb), which is widely distributed in soils, is complexed with organic matter, adsorbed by clays and oxides and precipitated as carbonates, hydroxides and phosphates (Epstein et al., 1999). However, industrial activities, such as mining, smelting, the burning of fossil fuels and the manufacture of pesticides and fertilizers, are the main cause of lead soil pollution (Sharma and Dubey, 2005; Tchounwou et al., 2012). Arbuscular mycorrhizal fungi (AMF), which form a symbiotic relationship with over 80% of terrestrial plants (Smith and Read, 2008), have been shown to take an active part in plant resistance to contamination by heavy metals such as Pb (Arriagada et al., 2005, 2007). The so-called growth dilution effect of AMF described by some authors is based on enhanced plant growth through higher phosphorous (P) uptake (Arias et al., 2010; Chen et al., 2001). However, other authors have reported that the alleviation of plant metal toxicity by AMF might be associated with the immobilization of large quantities of metals in the extraradical mycelium (ERM) and their subsequent translocation from plant roots to shoots (Nayuki et al., 2014; Rufyikiri et al., 2002; Weiersbye et al., 1999). This could be connected with the high cation exchange capacity (CEC) of the ERM which may favour metal adsorption on the surface of fungal hyphae (Chen et al., 2001; Joner et al., 2000). Nevertheless, even if the ERM is involved in their transport to mycorrhizal roots, the metals could be stored in fungal structures i.e. spores or easily-extractable glomalin related soil protein (EE-GRSP), thus preventing their transfer across symbiotic surfaces between AMF and root cells (Gao et al., 2019; Göhre and Paszkowski, 2006; Joner and Leyval, 1997; Nayuki et al., 2014; Salazar et al., 2018).

On the other hand, despite the reported ability of AMF to explore metal-degraded environments their diversity and abundance, with the exception of some more resistant strains, can be depleted by increasing metal content (Hildebrandt et al., 2007; Zarei et al., 2010). Organic amendments, such as mycoremediated dry olive residue (MDOR), have significantly increased the abundance of AMF populations and their capacity to adapt to different metals such as As, Cd, Pb and Zn, thus enhancing the nutrient status of wheat plants (García-Sánchez et al., 2017, 2019). MDOR is characterized by a high degree of organic matter humification and low levels of toxic phenol substances as a result of biological stabilization through the use of saprophytic fungi such as *Funelia floccose*, which produce a set of extracellular oxidoreductases (Reina et al., 2013; Sampedro et al., 2007, 2009; Siles et al., 2014). Although it has been reported the suitability of combining MDOR application and AMF inoculation for recovering metal-degraded soils (García-Sánchez et al., 2019), the specific mechanisms involving the ERM of the AMF in presence of MDOR on Pb transport and in hosting plant response (in terms of plant protection, P uptake and yield) to Pb has been scarcely studied. Therefore, the hypothesis of the present study was aimed to investigate whether: i) the ERM of *F. mosseae* was directly involved in the transport of Pb to wheat roots, or by contrary, the Pb was immobilized in the external mycelia, ii) the MDOR application had a beneficial impact on the development of ERM, leading to increased Pb transport, and iii) the exogenous carbon (C) substances and nutrients supplied by MDOR might improve P uptake and plant yields.

## 2. Material and methods

### *2.1. Materials*

*2.1.1. Soil site description*

The soil used in this experiment was obtained from the field "Cortijo Peinado", a farm in the province of Granada in southern Spain (37°13′N; 3° 45′W), at an altitude of 550 m. The soil was described as Haplic Regosol (Ortega et al., 1991), and its principal properties are represented in the Table 1. The climate in the region is Mediterranean with the mean annual precipitation about 357 mm with extended periods of drought. Mean annual temperature is 15.1 °C; the coldest month is January (mean 6.7 °C), and the warmest month is July (mean 24.8 °C) (http://www.aemet.es).

*2.1.2. Arbuscular mycorrhizal fungi (AMF) inocula*

The mycorrhizal inoculum used, *Funneliformis mosseae* BEG 12 (Banque Européene des Glomales), was collected from *Medicago sativa* L. trap pot culture, consisting of soil, spores, mycelia and colonized root fragments (10 sporocarps $g^{-1}$, 1–5 spores per sporocarp).

*2.1.3. Mycoremediated dry olive residue (DOR)*

The mycoremediation of dry olive residue (DOR) was carried out using the fungus *Funnelia floccose* (Spanish Type Culture Collection, CECT 20449T). The fungal strain was pre-cultured on 2% malt extract agar (MEA) for 2 weeks at 24 °C to maintain the fresh inoculum. DOR, provided by Sierra Sur S.L. (Granada, Spain), was sterilized by autoclave three times at 121 °C for 20 min, frozen and stored at −20 °C for later use. DOR was mycoremediated under solid state fermentation (SSF) conditions as described elsewhere (Reina et al., 2013). To do this, four fungal agar plates, homogenized in 80 ml of sterile water, were used to pre-culture *F. floccose* for one week in a medium containing 18 g of barley seeds and 30 ml of sterile water. Subsequently, the barley-seed media inoculated with *F. floccose* was mixed with sterilized DOR in a 1:1 ratio and moistened with sterile water. After four weeks of

127 incubation at 28 ºC, the MDOR was heat-inactivated by autoclaving; thereafter, samples
128 were sieved (5 mm mesh) and the remaining barley seeds were manually removed. The
129 samples were stored at 4 ºC for later use. Prior to the experiment, MDOR, whose
130 chemical composition is shown in Table 2, was characterized.

131 *2.2. Experimental design and description of treatments*

132 The experiment was carried out using the container system described by (Mäder
133 et al., 1993) with some modifications, which consisted of a central compartment (CC)
134 and two lateral compartments (LCs), 24 cm long, 12 cm high and 2 cm wide, made of
135 polychlrorure of vinyl (PVC) (Supplementary Fig. S1). The central container was
136 separated from the two lateral containers by a screen system composed of 32 μm mesh
137 nylon combined with polytetrafluoroethylene (PTFE) hydrophobic GORE-TEX®
138 membranes with nominal pore diameters of 5 μm (Fig. 1) which have been shown to
139 efficiently inhibit the mass flow and diffusion of mobile ions in a soil solution in an
140 abiotic system, they can be penetrated by the extraradical mycelium of AMF (Mäder et
141 al., 2000).

142 The experiment had a completely randomized factorial system with three factors of
143 variation consisting of two levels. The first factor comprised soil inoculated with and
144 without *F. mosseae*. The second one included contaminated soil with Pb or not, and the
145 third was the soil with or without MDOR application (Table 3). The CC of the container
146 system was filled with a mixture of soil and quartz sand (2:3, v/v) which was previously
147 steam-sterilized. 15-d-old wheat plants were planted in the CC of the container systems
148 and only one half received the *F. mosseae* inoculum (8 g) (Table 3). A similar amount
149 of inoculum filtrate (Whatman no. 1 filter paper) containing soil microflora free of AM
150 fungal propagules was added to the other half. The LCs of each container systems,
151 AMF-inoculated (+ AMF) and non AMF-inoculated (- AMF) in the CC, was filled with

sterile soil samples (Control), Pb-contaminated soil samples (+Pb), MDOR-amended soil samples (+ MDOR) or combined Pb-contaminated/MDOR-amended soil samples (+Pb + MDOR). Pb-polluted soil samples were artificially contaminated with an aqueous Pb $(NO_3)_2$ solution up to a final concentration of 3000 mg Pb $kg^{-1}$. MDOR was applied and manually mixed with the soil up to a final concentration of 50 g $kg^{-1}$, as previously suggested by Siles et al. (2014). Three replicates of each container system were stablished per each treatment. The experiment was conducted under greenhouse conditions (supplementary light at 25/19 °C and 50% relative humidity), and the wheat plants were watered weekly with 10 ml water without P (Hewitt, 1952) and grown for two months. At the end of the experiment, soil samples contained in each compartment (CC and LCs) were separately collected, and in the case of the LCs, the soil samples were manually mixed to obtain a composite soil. Subsequently, soil samples were homogenised, sieved (2 mm mesh) and kept at 4 ºC for further analysis. Wheat plants were also harvested and separated into roots, shoots and grains and then divided into two sub-samples. The first sub-samples were used to record plant biomass after dying the material at 105 ºC for 72 h. The second sub-sample was air-dried and analyzed for total Pb and P content.

*2.3. Analyses*

*2.3.1. Analytical methods*

For the content of Pb and P the aliquots (0.5 g) of soil samples or plant biomass were decomposed in a digestion vessel contained 8 ml of 65% $HNO_3$ and 2 ml of 30% $H_2O_2$. The mixture was heated in an Ethos 1 (MLS GmbH, Germany) microwave-assisted wet digestion system for 30 min at 220 ºC. After cooling, the digests were transferred to 20 ml glass tubes, which were filled to the mark with deionized water. Each extraction was carried out in triplicate. Extracts were centrifuged in a Hettich

177 Universal 30 RF (Germany) at 3000 rpm (i.e., 460 g) for 10 min at the end of each
178 extraction procedure and the supernatants were stored at 4 ºC prior to analysis.
179 Inductively coupled plasma-atomic emission spectrometry (ICP-OES, Agilent
180 720, Agilent Technologies Inc., USA), equipped with a two-channel peristaltic pump, a
181 Struman-Masters spray chamber, and a V-groove pneumatic nebulizer made of inert
182 material, was used to determine the Pb and P contents of soil and plant digests.
183 *2.3.2. Quantification of the root length colonization percentage and external mycelium*
184 The percentage of root length colonization was estimated using the methodology
185 described by Giovannetti and Mosse (1980) by randomly selecting fresh root systems
186 which were cleared and stained (Phillips and Hayman, 1970). 3 g of fresh soil were
187 sieved through 700 and 100 μm nylon mesh to retain the extraradical mycelium. The
188 fungal hyphae in the 100 μm sieve were transferred to a nylon membrane (32 μm) and
189 stained with fuchsine acid solution (0.05%); hyphae length was then quantified under a
190 stereoscopic microscope at 100× (Giovannetti and Mosse, 1980).
191 *2.3.3. Determination of easily extractable glomalin-related-soil protein (EE-GRSP)*
192 The EE-GRSPs were determined after removing 1 g of soil with 8 ml of citrate
193 buffer (20 mM, pH 7.0) followed by autoclaving at 121 °C for 30 min (Wright and
194 Upadhyaya, 1998). Samples were then centrifuged at 10,000g for 15 min and filtered
195 through a Whatman No. 1 filter. Protein content was determined using the Bradford
196 Bio-Rad Protein Assay (Bio-Rad Labs) with bovine serum albumin as standard (Wright
197 et al., 1996).
198 *2.4. Statistical analysis*
199 Data analyses were carried out using IBM SPSS Statistics version 26.0 software.
200 All data presented are the means of three replicates with standard deviation (mean ±
201 DE). One-way analysis of variance (ANOVA), followed by the post hoc Tukey HSD

test ($p<0.05$), were used to evaluate: i) the Pb transport and plant variables (P uptake and biomass) when AMF was present or absent and, ii) the ERC development (in terms of fungal hyphae density and EE-GRSPs) between the LCs and CC. Using one-way ANOVA, we statistically analysed the effect of Pb contamination, MDOR and the combination of MDOR and *F. mosseae* inoculation on: i) Pb accumulation in wheat plants, ii) fungal hyphae density, iii) EE-GRSPs, iv) the percentage of root colonization, v) P uptake and vi) plant biomass. Differences among treatments, which were analysed by post-hoc tests, are indicated by different letters.

## 3. Results and discussion

### *3.1. The role of the ERM in Pb transport*

The transport of Pb from the lateral compartments to the central compartment through the ERM was evaluated by measuring Pb content in wheat plants. In the absence of *F. mosseae* (-AMF), wheat roots unexpectedly accumulated a relatively small amount of Pb after Pb-polluted soil samples were added to the LC (Table 4). While this could indicate that Pb is diffused through the screen system, no statistical differences were found between the Control, + Pb, + MDOR and + Pb + MDOR treatments (Table 4). This could be related to the presence of trace Pb content in the soil used, with values observed to be below plant toxic limits (Table 1). Conversely, the presence of *F. mosseae* (+AMF) in the container system significantly increased Pb levels in wheat roots with respect to the container system without AMF (-AMF) when the soil samples were artificially contaminated (Table 4). After evaluating the translocation of Pb to the upper parts of wheat plants, interestingly, we detected no Pb in either wheat shoots or grains. This could point to the involvement of the *F. mosseae* ERM in the transport of Pb from the lateral compartment to wheat roots. This strategy might be considered as a plant protection mechanism which decreased the translocation

of Pb to the upper parts of the plant in the presence of MDOR, as other authors suggested (Arriagada et al., 2005).

The addition of MDOR (+ MDOR) to soil samples showed the same values for Pb content regardless of the presence or absence of *F. mosseae* (-AMF/ + AMF) (Table 4). By contrast, we observed a sharp increase in Pb content when Pb-contaminated soil samples were treated with MDOR (Pb+-MDOR) in the presence of *F. mosseae,* which differed significantly from + Pb soil samples (Table 4). This could indicate that the nutrients supplied by MDOR application could be involved in the stimulation of the ERM which in turn favoured the transport of Pb through the ERM with its subsequent accumulation in roots decreasing the translocation of Pb to the upper parts of the plant in the presence of MDOR. However, this hypothesis requires further investigation.

Regardless of the soil treatment used, the development of the external mycelium and EE-GSRPs was less marked in the LCs than in the CC (Fig. 1a-b), given the presence of the *F. mosseae* inoculum in these soil samples. The exposure of AMF to Pb did not significantly reduce the external mycelium, whose values were similar to those for control treatments (Fig. 1).

*F. mosseae* clearly possesses a mechanism of protection against Pb toxicity which presumably immobilizes Pb in fungal structures due to the great sorption and accumulation capacity of the ERM (García-Sánchez et al., 2016) (Fig. 1a). MDOR supplies soils with labile C compounds as a result of fungal transformation, which can be used as an energy source by microorganisms, as reported Siles et al. (2014). This is in line with our finding that MDOR (+MDOR), rather than the control treatment, greatly enhanced the external mycelium (Fig. 1a). The combined application of Pb and MDOR (+Pb + MDOR) also stimulated the development of ERM in Pb-contaminated soil samples (Fig. 1a). Thus, MDOR stimulates AMF development by increasing nutrient

252 solubilisation, which boosts soil aggregates, thus favouring AMF hyphal growth, as
253 described by García-Sánchez et al. (2019).

254 According to Purin and Rillig (2007), glomalin plays an important
255 environmental role in soil by boosting feedback between plant production, soil
256 aggregation and external AMF hyphal growth. We observed no significant differences
257 in glomalin content in the CC and LCs between the treatments tested except in the case
258 of + Pb and +Pb +MDOR (Fig. 1b). The presence of Pb in soil samples greatly
259 increased glomalin content in the CC as compared to control samples, while Pb levels in
260 the CC were similar following the control and +Pb treatments (Fig. 1b). This could
261 indicate that Pb is immobilized by surface complexation with cysteine-containing
262 ligands of glomalin as reported elsewhere (González-Chávez et al., 2004). Increased
263 glomalin content is usually observed following the addition of organic sources, such as
264 manure, crop stubble and compost (Curaqueo et al., 2014). This finding is in line with
265 our results which found that MDOR increased glomalin content in both the CC and
266 LCs, whose glomalin levels differed significantly from those observed in control
267 samples (Fig. 1b). Contrary to expectations, the addition of MDOR to Pb-contaminated
268 soil samples did not boost glomalin production significantly in LC with respect to +Pb
269 treatments. This contradicts other studies which report that the quantity of glomalin
270 extracted from soils is typically related to AMF hyphal density (González-Chávez et al.,
271 2009; Lovelock et al., 2004). However, we found that the increase in the external
272 mycelium exceeded that in glomalin content when MDOR was added to Pb-polluted
273 soils. Nevertheless, reduction in glomalin production could be due to the presence of
274 certain chelating humic substances supplied by MDOR rather than to the presence of
275 Pb.

276 *3.2. Role of AMF in plant protection*

We also evaluated the role of mycorrhiza in plant protection, which is well known to lead to an increase in biomass and P plant uptake. Some studies indicate that AMF have the ability to absorb P from adjacent soil which is rapidly translocated to the fungus-plant interface and subsequently absorbed by roots (Smith et al., 2003, 2004). This explains the well-known positive effects of AMF on plant P nutrition, especially under stressful conditions. Figure 2 shows the results of the analysis of P content and plant biomass in wheat roots, shoots and grains.

The *F. mosseae* inoculation resulted in an overall increase in P plant uptake in relation to the non-inoculated container system, which could be associated with the higher levels of wheat biomass (Fig. 2a-f). Interestingly, P uptake by wheat roots in the presence of AMF was not greatly affected by exposure to Pb, as similar P levels were found in both control and +Pb treatments (Fig. 2a). This could, in turn, be explained by the similar levels found for root biomass following these treatments (Fig. 2d). As suggested by others authors, our results could indicate that P uptake is superceded by Pb transport in soils that are highly contaminated by this metal (Xiong, 1997). ERM development was not greatly affected by exposure to Pb, which could be explained by its possible translocation to root cells rather than by its possible accumulation in vesicles and spores, as some authors have reported (Salazar et al., 2018). This hypothesis concurs with our finding that the presence of Pb did not have a significant effect on AMF-root colonization in soil samples (Table 4). Arriagada et al. (2005) have reported a similar finding with respect to Eucalyptus plants inoculated with *Rhizophagus irregularis* in soil highly contaminated by Pb. On the other hand, we observed no significant increase in wheat and grain P uptake in Pb-polluted soil samples (+Pb) as compared to control treatments (Fig. 2b), which is consistent with the unchanged levels of shoot and grain biomass (Fig. 2e).This could indicate that AMF

play a major stabilizing role in plant protection by reducing Pb translocation to shoots. Thus, Pb, which is transported to the cytosol across the cell wall and cell membrane via active metabolism, is accumulated inside the cell and/or via passive metabolism by which Pb adheres to fungal surface molecules (sorption) (Mishra and Malik, 2013), which could also explain the decrease in root biomass. However, the binding of Pb to fungal tissues associated with roots could be involved in creating a physical barrier against Pb translocation to the plant (García-Sánchez et al., 2016).

MDOR significantly improved P uptake by wheat roots and shoots (Fig. 2a, and b), which could be associated with increased biomass, but only in roots (Fig. 2d, e and f). The addition of MDOR to Pb-polluted soil samples (+Pb+MDOR) resulted in increased P uptake which proved to be higher than that observed after +Pb treatment (Fig. 2a). However, root biomass did not increase significantly, with +Pb and +Pb+MDOR treatments producing similar results. An increase in P content caused by MDOR might determine the transport of P and Pb through the ERM to wheat roots. However, Pb could be transferred from fungal hyphae to root cells which, in turn, would lead to a reduction in root biomass. This reasoning concurs with our finding that MDOR did not increase AMF root colonization (Table 5). Conversely, no differences in wheat shoot and grain P content were observed between +Pb and +Pb+MDOR treatments, which, in turn, did not modify shoot biomass levels. This could point to possible competition between Pb and P for the same P transporter in plant plasma membranes, as some authors such, as Smith et al. (2010), have reported with regard to other metals. However, AMF could induce a resistance mechanism based in the uptake of both, P and metals, through the same phosphate transporters or by contrary can discriminate between P and metals which in turn reduces the metal uptake via the ERM resulting in lower toxicity (Christophersen et al., 2012). In our case, MDOR could stimulate other

AMF plant resistance mechanisms involving reduced Pb uptake due to the suppression of high-affinity P uptake systems.

## 4. Conclusions

This study highlights the feasibility of using a container system with a GORE-TEX® hydrophobic membrane to evaluate the role of the *F. mosseae* ERM in Pb transport. Our results show that the ERM is responsible for Pb accumulation in wheat roots. *F. mosseae* also protected wheat plants by stabilizing Pb content in roots and by preventing its translocation to shoots and grains in the upper part of the plant. Pb may have been transferred by AMF to the cytosol through the cell wall and membrane via active metabolism and have been accumulated inside the cell and/or via passive metabolism, by which Pb adheres to fungal surface molecules (sorption) as a result of decreased root biomass. In addition, MDOR greatly improved P uptake which probably led to a reduction in Pb uptake. The development of the ERM was unaffected by exposure to Pb, while the nutrients supplied by MDOR significantly increased fungal hyphae density and glomalin production. Our study highlights the transport of Pb through the ERM, its accumulation in wheat roots and how the increase in the uptake of P rather than Pb by MDOR is a plant protection mechanism triggered by AMF.

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**Fig. 1**. External mycelium (a) and easily-extractable glomalin-related soluble protein (EE-GRSP) content (b) in the central compartment (CC) and lateral compartments (LCs) of the container system inoculated with *F. mosseae*. Data expressed as mean (bar) and standard deviation (line segment) (n = 3). Similar letters above bars denote non-significant statistical differences between treatments ($p<0.05$). Asterisks above bars denote statistically significant differences ($p<0.05$) between central and lateral compartments.

**Fig. 2.** P content (mg kg-1) and dry biomass (mg) in roots, shoots and grains of wheat plants grown in the container system. Black bar: treatment without *F. mosseae* inoculation (- AMF). Grey bar: treatment with *F. mosseae* inoculation (+ AMF). a: P content in roots; b: P content in shoots; c: P content in grains; d: Dry biomass in roots; e: dry biomass in shoots; f: dry biomass in grains. Error bars represent standard deviation from replicate experiments. Lowercase letters above bars denote non-statistically significant differences ($p<0.05$) between treatments. Asterisks above bars show statistically significant differences ($p<0.05$) between AMF inoculations.

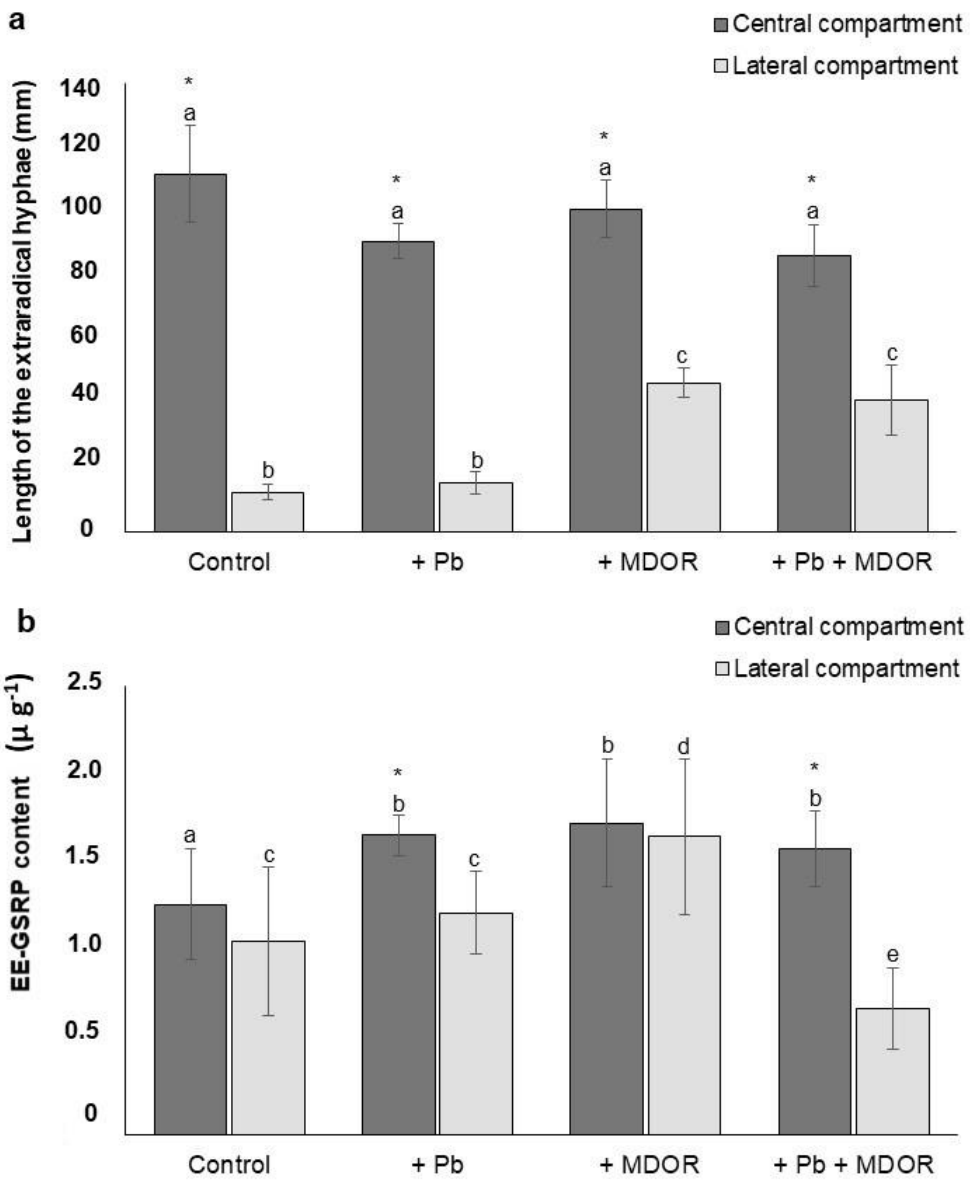
a
Central compartment
Lateral compartment
Length of the extraradical hyphae (mm)
140
120
100
80
60
40
20
0
Control
+ Pb
+ MDOR
+ Pb + MDOR
b
Central compartment
Lateral compartment
EE-GSRP content (µ g-1)
2.5
2.0
1.5
1.0
0.5
0
Control
+ Pb
+ MDOR
+ Pb + MDOR

**Fig 1**

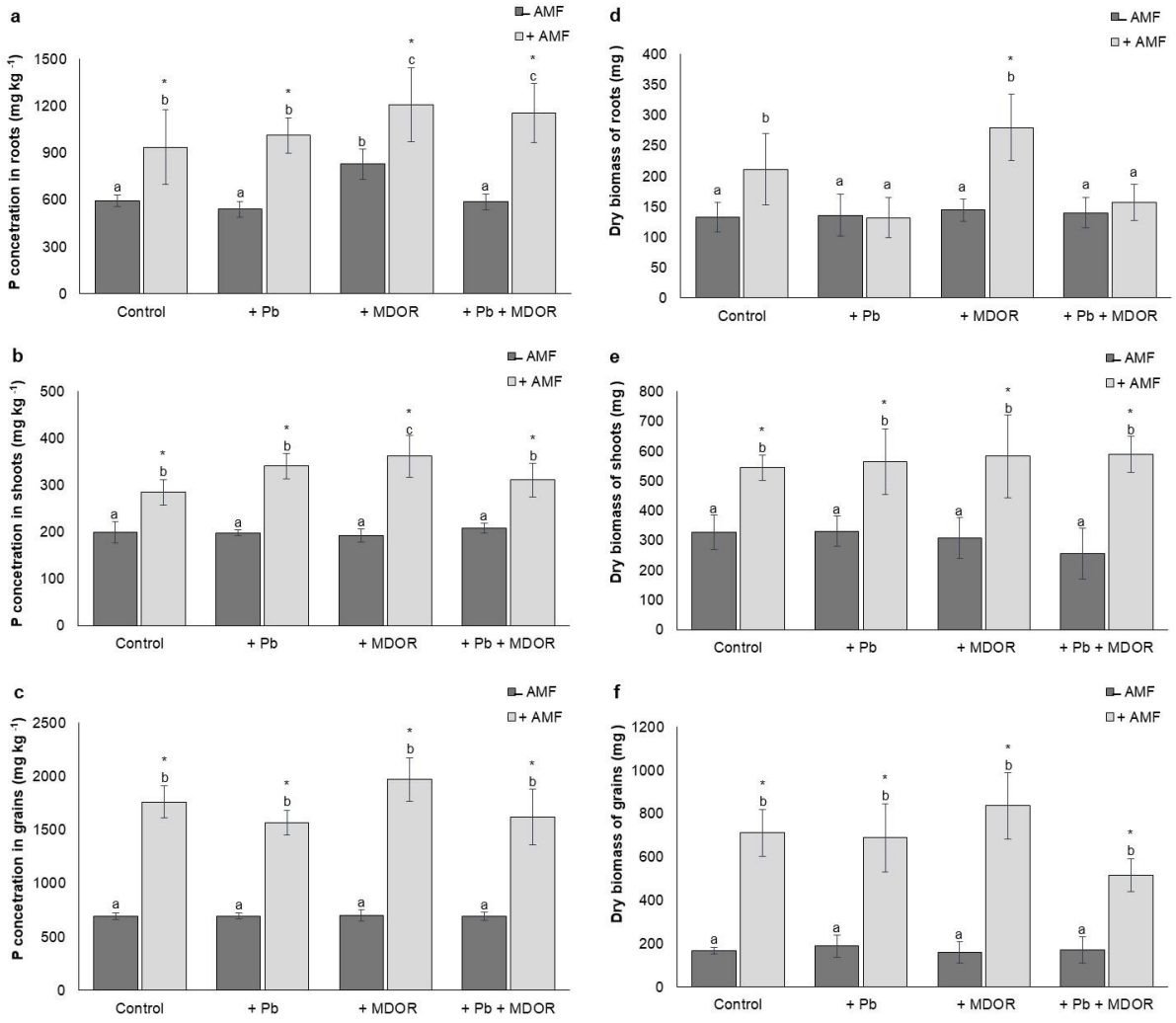
a
AMF
+ AMF
P concetration in roots (mg kg -1)
1500
1200
900
600
300
0
Control
+ Pb
+ MDOR
+ Pb + MDOR
d
Dry biomass of roots (mg )
400
350
300
250
200
150
100
50
0
b
P concetration in shoots (mg kg -1)
500
400
300
200
100
e
Dry biomass of shoots (mg )
800
700
600
c
P concetration in grains (mg kg -1)
2500
2000
1500
1000
f
Dry biomass of grains (mg )
1200
1000
800
600
400
200

**Fig. 2**

**Table 1** Chemical properties of soil (n = 3).

| Properties | Concentration |
|---|---|
| TOC* (g $kg^{-1}$) | 10.67 ± 0.52 |
| WSC* (g $kg^{-1}$) | 4.83 ± 0.02 |
| TN* (g $kg^{-1}$) | 1.52 ± 0.21 |
| Ca (g $kg^{-1}$) | 61.90 ± 3.65 |
| Mg (g $kg^{-1}$) | 17.66 ± 0.11 |
| K (g $kg^{-1}$) | 8.63 ± 0.21 |
| Na (g $kg^{-1}$) | 1.78 ± 0.05 |
| P (g $kg^{-1}$) | 0.59 ± 0.25 |
| Fe (g $kg^{-1}$) | 20.97 ± 0.13 |
| Mn (mg $kg^{-1}$) | 435.92 ± 19.36 |
| Zn (mg $kg^{-1}$) | 73.24 ± 4.32 |
| Cu (mg $kg^{-1}$) | 30.28 ± 0.15 |
| Pb (mg $kg^{-1}$) | 26.49 ± 1.12 |

*Total organic carbon (TOC).
*Water soluble organic carbon (WSOC).
*Total nitrogen (TN).
(Siles et al., 2014)

**Table 2** Main characteristics of MDOR.

| | **MDOR** |
|---|---|
| pH | 5.60 ± 0.02 |
| CEC (mmol $kg^{-1}$) | 512 ± 11 |
| C (g $kg^{-1}$) | 472 ± 2 |
| N (g $kg^{-1}$) | 22.8 ± 1.11 |
| C/N | 20.8 ± 0.9 |
| TOC (g $kg^{-1}$) | 152 ± 3 |
| P (g $kg^{-1}$) | 1.03± 0.4 |
| K (g $kg^{-1}$) | 6.95 ± 3.25 |
| Mg (g $kg^{-1}$) | 0.05 ± 0.001 |
| Ca (g $kg^{-1}$) | 1.55 ± 0.05 |
| Phenol content (µg $g^{-1}$) | 2.3±0.05 |

**Table 3** Experimental design.

| Treatments | Soil samples in each compartment | |
|---|---|---|
| | **CC** | **LC (both sides)** |
| **Control** | − AMF-inoculated soil | Sterile soil |
| | + AMF-inoculated soil | |
| **+ Pb** | − AMF-inoculated soil | Pb-polluted soil |
| | + AMF-inoculated soil | |
| **+ MDOR** | − AMF-inoculated soil | MDOR-amended soil |
| | + AMF-inoculated soil | |
| **+ Pb + MDOR** | − AMF-inoculated soil | Pb-polluted and MDOR amended |
| | + AMF-inoculated soil | soil samples |

**Table 4** The Pb content (mg $kg^{-1}$) accumulated in wheat roots, shoots and grains grown in the central compartment (CC) of the container system inoculated or not with *F. mosseae* in presence of: i) sterile soil (control) or ii) Pb-polluted soil (+Pb), or iii) MDOR-amended soil (+MDOR), or iv) Pb-polluted/MDOR amended soil (+Pb +MDOR) in the lateral compartment (LC) at the end of the experiment.

| Treatments | | Pb content (mg $kg^{-1}$) | | |
|---|---|---|---|---|
| | | **Root** | **Shoot** | **Grain** |
| **Control** | **− AMF** | 19.75 ± 1.7[a] | ND | ND |
| | **+ AMF** | 25.09 ± 4.5 [a] | ND | ND |
| **+ Pb** | **− AMF** | 15.00 ± 3.6 [a] | ND | ND |
| | **+ AMF** | 81.10 ± 6.9[b*] | ND | ND |
| **+ MDOR** | **− AMF** | 18.25 ± 5.6 [a] | ND | ND |
| | **+ AMF** | 22.65 ± 3.6 [a] | ND | ND |
| **+ Pb + MDOR** | **− AMF** | 20.25 ± 7.2 [a] | ND | ND |
| | **+ AMF** | 125.5 ± 20.5[c*] | ND | ND |

ND: Under detection limit

Data expressed as mean and standard deviation (n = 3).

Same letters indicated not statistical significance at $p<0.05$ between treatments.

*Asterisk shown statistical significance at $p<0.05$ in presence or absence of *F. mosseae*.

**Table 5** Percentage of AMF-root colonization.

| Treatments | % Mycorrhizal colonization |
|---|---|
| **Control** | 43.0 ± 8.3 |
| **+MDOR** | 44.8 ± 1.7 |
| **+Pb** | 39.0 ± 5.5 |
| **+Pb+MDOR** | 42.0 ± 5.2 |