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The combined effects of earthworms and arbuscular mycorrhizal fungi on microbial biomass and enzyme activities in a calcareous soil spiked with cadmium

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Earthworms and arbuscular mycorrhizal fungi (AMF) are known to independently affect soil microbial and biochemical properties, in particular soil microbial biomass (SMB) and enzymes. However, less information is available about their interactive effects, particularly in soils contaminated with heavy metals such as cadmium (Cd). The amount of soil microbial biomass C (MBC), the rate of soil respiration (SRR) and the activities of urease and alkaline phosphatase (ALP) were measured in a calcareous soil artificially spiked with Cd (10 and 20 mg Cd kg^{-1}), inoculated with earthworm (*Lumbricus rubellus* L.), and AMF (*Glomus intraradices* and *Glomus mosseae* species) under maize (*Zea mays* L.) crop for 60 days. Results showed that the quantity of MBC, SRR and enzyme activities decreased with increasing Cd levels as a result of the elevated exchangeable Cd concentration. Earthworm addition increased soil exchangeable Cd levels, while AMF and their interaction with earthworms had no influence on this fraction of Cd. Earthworm activity resulted in no change in soil MBC, while inoculation with both AMF species significantly enhanced soil MBC contents. However, the presence of earthworms lowered soil MBC when inoculated with *G. mosseae* fungi, showing an interaction between the two organisms. Soil enzyme activities and SRR values tended to increase considerably with the inoculation of both earthworms and AMF. Nevertheless, earthworm activity did not affect ALP activity when inoculated with *G. mosseae* fungi, while the presence of earthworm enhanced urease activity only with *G. intraradices* species. The increases in enzyme activities and SRR were better ascribed to changes in soil organic carbon (OC), MBC and dissolved organic carbon (DOC) contents. In summary, results demonstrated that the influence of earthworms alone on Cd availability is more important than that of AMF in Cd-polluted soils; and that the interaction effects between these organisms on soil microorganism are much more important than on Cd availability. Thus, the presence of both earthworms and AMF could alleviate Cd effects on soil microbial life.

1. Introduction

Cadmium (Cd) is a trace and potentially toxic element entering the food chain through crop uptake and animal food intake (Kirkham, 2006; Smith, 2009). The accumulation of Cd in agricultural soils is derived primarily from industrial waste disposals, the application of phosphate fertilizers and biosolids added to soil as amendments and a source of plant nutrients and soil organic matter (SOM), especially sewage sludge (Smith, 2009). A high concentration of Cd in the soil is a serious threat to both the environmental quality and human health over the long-term (Kirkham, 2006). Cadmium is well-known to inflict a negative influence on soil biota (i.e., plants, microorganisms and fauna) in Cd-polluted environments. Generally, higher concentrations of Cd adversely affect plant growth and performance (Shahabivand et al., 2012), earthworm growth and activity (Domínguez-Crespo et al., 2012), and soil biological and biochemical properties such as soil microbial biomass C (MBC), enzyme activities and respiration (Landi et al., 2000; Vig et al., 2003). This may bring about different negative feedbacks, as there are significant multiple interactions among plants, soil microbial community and earthworms (Ma et al., 2006; Zarea et al., 2009; Li et al., 2012).

Clearly, the potential toxicity of Cd for soil biota often is better described by the fraction of bioavailable Cd than by the total Cd concentration (Vig et al., 2003; Kirkham, 2006). Several abiotic and biotic factors such as soil pH, SOM and dissolved organic carbon (DOC) contents, cation exchange capacity and soil organisms, and even plant species directly or indirectly affect Cd mobility and bioavailability (Adriano, 2001; Vig et al., 2003; Kirkham, 2006; Pelfrêne et al., 2012). Soil microorganisms and fauna are factors which may have an influence on the mobility and bioavailability

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of Cd in soil–plant systems (Edwards and Bohlen, 1996; Wen et al., 2004; Yu et al., 2005; Ma et al., 2006). Specifically, both earthworms and arbuscular mycorrhizal fungi (AMF) have been found to affect Cd chemistry with changes in its immobilization and mobilization or its speciation in Cd-polluted soils (Wen et al., 2004; Yu et al., 2005; Shen et al., 2006; Sizmur and Hodson, 2009; Janoušková and Pavlíková, 2010).

Earthworms and AMF are two taxonomically dissimilar soil organisms and play an important role in soil microbial activities and processes. They stimulate microbial activities and promote enzyme activities in soil with a positive consequence for the increases in plant growth through the enhancement of nutrient mineralization and availability (Raiesi and Ghollarata, 2006; Zarea et al., 2009; Li et al., 2012). These two soil organisms can also have an influence on Cd availability in the soil solution (Chen et al., 2004; Yu et al., 2005; Sizmur and Hodson, 2009; Natal-da-Luz et al., 2011). Arbuscular mycorrhizal fungi may often lower Cd mobility and toxicity by increasing soil pH (Chen et al., 2004; Shen et al., 2006), sequestering Cd inside extra-radical mycelium (Chen et al., 2004; Janoušková and Pavlíková, 2010) and binding Cd to glomalin (González-Chávez et al., 2004). Glomalin is an insoluble glycoprotein synthesized and released by AMF (Wright and Upadhyaya, 1998), and may bind heavy metals in the soil (González-Chávez et al., 2004). Previous studies have shown that glomalin production and the concentration of glomalin-related soil protein would increase under unfavorable growing conditions such as heavy metal (Cornejo et al., 2008) and salinity (Hammer and Rillig, 2011) stresses.

Earthworms may also have the potential to change the availability of Cd and other heavy metals in soil by changing the soil characteristics such as pH and DOC, and the stimulation of microbial activity (Ma et al., 2002; Wen et al., 2004; Yu et al., 2005; Sizmur and Hodson, 2009). However, the individual effects of earthworms and AMF on Cd availability are inconsistent in the literature (Yu et al., 2005; Shen et al., 2006; Suthar et al., 2008; Redon et al., 2009; Sizmur and Hodson, 2009; Natal-da-Luz et al., 2011). Apparently, this inconsistency could stem from differences in earthworm and fungal species, pollution mode and soil conditions, and cannot be generalized (Weissenhorn et al., 1995; Yu et al., 2005; Sizmur and Hodson, 2009). However, the net influence of earthworms and AMF co-inoculation on Cd availability is less studied (Liao et al., 2003; Yu et al., 2005). Additionally, these studies reported the interaction between earthworms and AMF in relation to Cd uptake and plant growth, and even far less is known about this interactive effect on soil microbial activities and biochemical processes for a better understanding of the ecology of Cd-polluted soils.

Soil microbial biomass (SMB) and enzymes are important indicators of soil biological activity and biochemical processes because they are involved in SOM decomposition, the dynamics of soil nutrient cycling and nutrient availability (Sparling, 1997; Dick, 1997). Soil microbial biomass levels and enzyme activities in soil have been reported to be greatly and differently affected by metals (Sparling, 1997; Dick, 1997; Landi et al., 2000). Soils polluted with Cd and other metals generally contain lower amounts of SMB and enzyme activities compared with unpolluted soils, due to high toxicity of metals for soil microorganisms (Zhang et al., 2010). On the other hand, the quantity of SMB and the activity of soil enzymes are affected by earthworm activity and AMF colonization (Raiesi and Ghollarata, 2006; Al-Maliki and Scullion, 2013). The effect of earthworms on SMB and soil enzymes is mainly through changes in soil pH, DOC content and microbial community structure as a result of their burrowing, feeding and casting activities (Edwards and Bohlen, 1996). Arbuscular mycorrhizal fungi colonization also affects SMB and soil enzymes owing to changes in root exudation and rhizodepositions following root colonization (Raiesi and Ghollarata, 2006; Zarea et al., 2009). Although the overall influences of earthworms and AMF either alone or in combination on the activity and functions of soil microflora have been studied extensively in uncontaminated soils, no studies were conducted to show their interactive effects in contaminated soils. The positive influence of these organisms on soil microbial processes might be particularly crucial in soils contaminated by Cd, since they may concurrently change Cd availability and mobility with a consequence for microbial population and processes (Liao et al., 2003; Yu et al., 2005). Therefore, the main aim of the current study was to study and report the response of soil MBC, the activity of enzymes involved in N and P cycles (i.e., urease and alkaline phosphatase) and soil respiration rate (SRR) to co-inoculation of earthworm (*Lumbricus rubellus*) and AMF (*Glomus intraradices* and *Glomus mosseae* species) in a calcareous soil artificially spiked with 10 and 20 mg Cd kg^{-1} soil under maize crop. We hypothesized that earthworms and AMF would individually or interactively affect Cd availability with subsequent changes in soil MBC contents, enzyme activities and SRR. If we accept or assume that earthworms usually increase while AMF decrease Cd mobility and availability individually, there is a possibility that AMF inoculation would counteract and mask the positive influence of earthworms on Cd toxicity, resulting in a negative interaction in Cd-polluted soils. It is therefore expected that one organism may cancel out the effect of the other organism under certain circumstances, as these two soil components may co-inhabit in most environments. In the presence of earthworms, Cd availability would decrease by AMF inoculation; and consequently SMB contents, soil enzymes and SRR could increase in Cd-polluted environments.

2. Materials and methods

2.1. Experimental set-up

The experiment was setup as $3 \times 2 \times 3$ factorial treatments of Cd level, earthworm and AMF inoculation organized in a completely randomized design with three replications. Treatments consisted of a full factorial combinations of three Cd levels (0, 10 and 20 mg kg^{-1}) applied as $CdCl_2$, two earthworm treatments (no earthworm, NE, and with *L. rubellus* species, WE) and three AMF treatments (*G. intraradices*, *G. mosseae*, and non-mycorrhizal control, NM) in a calcareous soil planted with maize (*Zea mays* L.) under greenhouse conditions. Experimental units consisted of plastic pots with the bottom covered with net to prevent earthworm escape. The average day and night temperatures were 30 ± 8 and $16 \pm 9\,^{\circ}C$, respectively and the average light intensity was 1060 mmol $m^{-2}\,s^{-1}$.

2.2. Soil, earthworm and AMF preparation

A typical sandy loam soil from the 0 to 30 cm layer was obtained in a cropland field without pollution history. The soil had not been under cultivation for 5 years. The soil was calcareous and classified as Typic Calcixerepts (Soil Survey Staff, 2010). The soil was transferred to the laboratory, air-dried, passed through a 2 mm sieve and autoclaved at 121 °C for 2 h. A subsample of the study soil was analyzed for general chemical and physical properties (Table 1). In total, 54 pots were prepared and filled with 8 kg (fresh weight) autoclaved soil. To establish and reactivate soil microorganisms, 50 mL soil suspension was added to each pot to inoculate the autoclaved soil with fresh microorganisms. To obtain the soil suspension, 500 g fresh soil was suspended in 1.5 L de-ionized water and filtered through a 25 μm for eliminating AMF spores (Schroeder and Janos, 2004). The soils were artificially contaminated with Cd (as cadmium chloride) at the following rates: 0 (control), 10 and 20 mg kg^{-1} on a dry weight basis. The control treatments were watered with distilled water and others with appropriate aliquots