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Rhizosphere interactions between earthworms (*Eisenia fetida*) and arbuscular mycorrhizal fungus (*Funneliformis mosseae*) promote utilization efficiency of phytate phosphorus in maize

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ABSTRACT

Rhizosphere interactions between earthworms (*Eisenia fetida*) and arbuscular mycorrhizal fungus (AMF, *Funneliformis mosseae*), suggested to promote phytate mineralization and maize phosphorus (P) content were studied in the Organization for Economic Co-operation and Development (OECD) (non-phosphorus active soil). Maize was planted and amended with AMF and/or earthworms (E) as well as insoluble phytate-P ($C_6H_{18}O_{24}P_6$), in comparison to not amended maize (control). The maize shoot and root biomass, shoot and root P uptake, soil alkaline phosphatase activity, soil microbial biomass P, available P, and the Shannon–Wiener index (H) for the bacterial communities from T-RFLP profiles were measured at harvest. The results indicated that both AM and AM + E treatments significantly increased the utilization efficiency of phytate-P in maize. Our results suggest there are significantly positive interactions between earthworms and AMF in terms of promoting the utilization efficiency of phytate-P in maize and enhancing soil microbial biomass P and alkaline phosphatase activity. The positive interaction enhanced soil microbial biomass P and alkaline phosphatase activity by regulating the bacterial diversity in the soil. The bacterial diversity was significantly correlated with alkaline phosphatase activity, shoot and root P content, biomass and the utilization efficiency of phytate-P. Additionally, alkaline phosphatase activity was positively correlated with microbial biomass P. *Flavobacteriaceae* and *Xanthomonadales*, which were the dominant bacteria under all conditions. A significant correlation was observed between *Flavobacteriaceae* and alkaline phosphatase activity as well as shoot P content, and there was also a significant correlation between *Betaproteobacteria* and the root and shoot P content. Earthworm and AMF interaction could enhance the phytate-P mineralization in the belowground and improve linking between the aboveground and belowground for phytate-P utilization.

1. Introduction

Phosphorus (P) is an essential element for plant growth and productivity. Plants take up P from soil predominantly as free phosphate ions (HPO_4^{2-} and $H_2PO_4^-$, Bucher, 2007). However, P in soil exists predominantly in inorganic fractions that are either adsorbed into soil mineral surfaces or occasionally appear as available precipitates, which are also in an organic form that is either adsorbed, incorporated into biomass or associated with soil organic matter (Richardson and Simpson, 2011). Only a small component of total soil P is in a form directly available for plant or microbial uptake (Hinsinger, 2001). Because P is one of the most limiting macronutrients in soil, shortage of available P in soils is one of the key chemical factors limiting plant growth (Becquer et al., 2014). To increase agricultural production, large amounts of soluble-P fertilizers are widely applied. However, studies predict that phosphate rock will be depleted within a short to medium number of years (Cordell et al., 2009; Van Vuuren et al., 2010). Therefore, promoting the soil phosphorus cycle and enhancing P availability in soil has been a considerable research interest for many decades.

Generally, organic P (Po) in the soil accounts for approximately 50% of total soil P and up to 80% of the P for pasture soils (McLaughlin et al., 1990). Although a large proportion of the Po in soil remains poorly characterized, the inositol phosphates

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synthesized by microorganisms and plants, as well as the various metal-ion derivatives of these phosphates (e.g., soil phytate), are major components of Po in soil (Behera et al., 2014). The mobilization of soil phytate and rates of P diffusion within soil are the major limitations to generating an adequate supply of P for plant growth (Richardson and Simpson, 2011). Previous studies showed that plants grown in axenic conditions had very poor capacity to use phytate as a sole source of P because the plants had little or no capacity for releasing specialized enzymes (phytases) into the external medium (Hayes et al., 2000; Richardson et al., 2000, 2001; Becquer et al., 2014). Recent studies suggest that bacteria (e.g., *Pseudomonas alcaligenes* M2O) play a major role in the mineralization of nutrients, such as P, through trophic relationships because these bacteria can produce specific phosphatases, such as phytases, to degrade phytate and increase available P (Jorquera et al., 2008a,b; Maougal et al., 2014; Zhang et al., 2014). Bacteria are also more effective than other microorganisms or plants at immobilizing free phosphate ions. Consequently, a large fraction of P preleased from phytate is immobilized and locked into the bacterial biomass in soil. The soil microbial biomass P (MBP) is one of the major biological sinks for P. P held in microbial biomass acts as a labile pool that prevents fixation and becomes available for plant capture during microbial turnover (Becquer et al., 2014). Therefore, bacteria are integral to the soil P cycle and play an important role in mediating the availability of P to plants by regulating the phytases and soil microbial biomass P.

After mineralization of phytate and other Po compounds, the phosphate released must be absorbed by plants and mainly by AMF, which are more efficient than the roots (Becquer et al., 2014). AMF affects the growth and physiology of most plants because of AMF's extraradical mycelia, which increase the nutrient uptake capacity of roots by extending the amount of soil volume a plant can exploit (Tuffen et al., 2002; Li et al., 2013a). In the soil P cycle, AMF are considered to be P pumps that can improve the availability of soil P and increase the acquisition of P in different ecosystems and for different host plants (Eisenhaue et al., 2009; Wurst et al., 2004). AM fungi are able to release protons to mobilize insoluble soil phosphates and extend their extensive hyphae from a P depletion zone to explore soil for inorganic P sources (Smith and Smith, 2011). Additionally, AMF can also affect the growth of soil bacteria directly (Mansfeld-Giese et al., 2002) or indirectly via AM spore-associated bacteria (Johansson et al., 2004; Bharadwaj et al., 2012). The interactions between AM fungi and phosphate-solubilising bacteria that influence plant P uptake have been studied since the late 1990s (Zhang et al., 2014). Few studies have been conducted to identify the means for improving organically derived P through the interactions between AMF and soil biota in agricultural soils (Grace et al., 2006; Herridge et al., 2008; Paul and Lade, 2014).

Earthworms are considered to be "ecosystem engineers," and they can modify C and N cycles in the soil as well as improve soil fertility by affecting the nutrient dynamics at different spatial and temporal scales (Brussaard et al., 2007). Earthworms improve the turnover of organic P in the soil (Zhang et al., 2000) by changing the biogeochemical status of P (availability, organic P pool, enzymes activities) in their guts and in the drilosphere (Jiménez et al., 2003; Bünemann et al., 2010). Depending on the location of the worms in the soil profile (near or far away the drilosphere, for instance), earthworms will directly and/or indirectly influence P dynamics (Le Bayon and Binet, 2006 ; Le Bayon and Milleret, 2009). This influence includes chemical aspects of the soil (proportions of total, organic and inorganic P), kinetics (mobility, sorption processes), and the availability of P for plants and microorganisms (bacteria and fungi). The large amounts of mucus secreted in the earthworm gut, which release carboxyl groups from carbohydrate compounds that can block and compete for P absorption, increase soluble P (López et al., 1993). Moreover, Kuczak et al. (2006) have conducted a fractionation of the different chemical forms of P recovered in casts, and they found that passage through the earthworm gut and/or selective ingestion could influence both available P (extracted through a resin or with bicarbonate) and moderate available P. Earthworms can also increase microbial activity during their digestion processes (López et al., 1993), which can produce a high phosphatase activity (both acid and alkaline forms) in worm castings to promote P availability in the soil. However, less attention has been paid to the combined influence of earthworms and soil microorganisms on the availability of organic P (especially phytate).

Earthworms and AMF are at different trophic levels in the soil ecosystem, and both are involved in important soil processes, including microbial and enzymatic activities that enhance nutrient mineralization and availability as well as plant growth (Wardle et al., 2004; Raiesi and Ghollarata, 2006; Zarea et al., 2009; Li et al., 2012a,b). The nature of the interaction between AMF and earthworms may depend on the presence of limiting nutrients, such as N and P (Milleret et al., 2009). Because earthworms promote new hyphal growth and improve P transportation by AMF, which increases the P content in plant leaves and roots (Wurst et al., 2011; Li et al., 2013b), improved management of AMF and earthworms may increase soil P availability and uptake by plants. We hypothesized that earthworms and AMF could also interactively promote phytate mineralization in the soil and could help maize (*Zea mays* L.) to absorb the free phosphate ions that lead to increases in P content in maize shoot and root tissue (Fig. 1). The objective of this study was to determine whether the interactions between earthworms (*Eisenia fetida*) and AMF (*Funneliformis mosseae*) enhance the availability of phytate-P through regulation of the soil bacterial community, which affects the soil microbial biomass P and alkaline phosphatase activity.

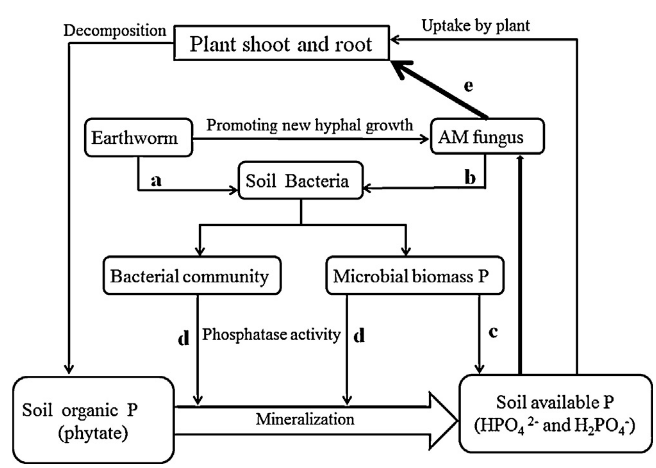

Fig. 1. Schematic representation of the importance of rhizosphere interaction between earthworms and AMF, which impact the use of soil phytate by regulating the bacterial community. (a) earthworms stimulate the activity of bacteria; (b) AM fungi affect the growth of soil bacteria directly; (c) P held in the microbial biomass acts as a labile pool, which prevents fixation and becomes available for plant capture during microbial turnover; (d) bacteria produce specific phosphatases, such as phytases, to degrade phytate and increase the available P pool; (e) hyphae from the fungus help plants to absorb the free phosphate ions, which leads to increased P content in the shoot and root tissue. Bacteria are integral to the soil P cycle and play an important role in mediating the availability of P to plants by regulating phytases (specific phosphatases that facilitate the mineralization of phytate) and the P in the microbial biomass of the soil.