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Interaction between earthworms and arbuscular mycorrhizal fungi on the degradation of oxytetracycline in soils

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ABSTRACT

The interactive impact of earthworms (*Eisenia fetida*) and arbuscular mycorrhizal fungi (*Rhizophagus intraradices*, AM fungi) on the degradation of oxytetracycline (OTC) in soils was studied under greenhouse conditions. Treatments included maize plants inoculated vs. not inoculated with AM fungi and treated with or without earthworms at low (1 mg kg^{-1} soil DM) or high (100 mg kg^{-1} soil DM) OTC rates. The root colonization rate, the hyphal density of mycorrhizae, the residual OTC concentration in soils, catalase, dehydrogenase, urease, soil microbial biomass C, Shannon–Wiener index (H) for microbial communities from T-RFLP profiles were measured at harvest. The results indicated that earthworms and AM fungi would individually or interactively enhance OTC decomposition and significantly decreased the residual OTC concentration at both high and low OTC rates. Both earthworms and AM fungi could promote the degradation of OTC by increasing soil microbial biomass C at both high and low OTC rates. The effect of soil enzyme activity and soil microbial diversity on OTC decomposition was different between high and low OTC rates. *Hyphomicrobium* and *Bacillus cereus* were dominant bacteria, and *Thielavia* and *Chaetomium* were dominant phyla of fungi at all occasions. Earthworm activity stimulated the growth of *Hyphomicrobium* and *Thielavia*, while AM fungi may stimulate *B. cereus*, *Thielavia* and *Chaetomium*, *resulting in* greater OTC decomposition. The interaction between earthworms and AM fungi in affecting the degradation of OTC may be attributed to different mechanisms, depending on soil microbial biomass, function (enzyme activity) and communities (the abundance of *Hyphomicrobium*, *B. cereus*, *Thielavia* and *Chaetomium*) in the soil.

1. Introduction

Veterinary antibiotics are widely used for the treatment and prevention of disease and the promotion of animal growth, particularly in livestock (Sarmah et al., 2006). As not all antibiotics are metabolized completely in the digestive system of the animals (De Liguoro et al., 2003; Kumar et al., 2005), up to 90% of these antibiotics are released into the environment unaltered, either directly in faeces or urine or indirectly through the application of manure as a fertilizer (Halling-Sørensen et al., 1998; ElSayed and Prasher, 2014). A large range of veterinary medicines, such as macrolides, sulphonamides, tetracyclines and antifungals, have increasingly been monitored in slurry, soils, surface waters and ground water (Fatta-Kassinos et al., 2011). Therefore, there is a growing concern regarding the potential adverse effects of veterinary antibiotics in soils. In agro-ecosystems, agricultural application of untreated or even treated antibiotic-containing animal wastes might influence plant growth (Chitescu et al., 2013) and indigenous soil microbial activity indices, such as respiration (Vaclavik et al., 2004; Kotzerke et al., 2008), nitrification (Gomez et al., 1996; Kotzerke et al., 2011), iron reduction (Thiele-Bruhn, 2005; Toth et al., 2011). Thus, veterinary antibiotics might influence or even ruin the function and productivity of the soil ecosystem.

Oxytetracycline (OTC) is one of the most widely used veterinary antibiotics, which exhibits broad-spectrum antimicrobial activity against a variety of bacterial infections in livestock production (Gao et al., 2013). OTC is poorly metabolized in the digestive tract of animals and most is excreted through faeces and urine as unmetabolized parental compounds (Sarmah et al., 2006). OTC may

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remain in the soil for at least 40 days if the concentration of OTC is greater than 25 μg ml^{-1} (Hou et al., 2009), which suggested that OTC could accumulate in soils and pose a potential risk to the soil ecosystem. Some studies disclosed the adverse effects of OTC on soil enzymes (Gao et al., 2013), the soil microbial community (Liu et al., 2009), and the root and shoot elongation of wheat (Jin et al., 2009). Conversely, OTC could also be absorbed by soybean and rice (Boonsaner and Hawker, 2010, 2012), which could expose humans to veterinary medicines through the food chain (Boxall et al., 2006). Therefore, OTC in the form of cations, zwitterions, or net negatively charged ions in soils complicates the prediction of its sorption characteristics and potential bioavailability and toxicity (Kong et al., 2012).

The remediation of soil organic contaminants has focused traditionally on chemical treatments or physical removal, but bioremediation has recently been promoted, as it has a far less destructive effect on the environment (Juwarkar et al., 2010). Earthworms function as ecosystem engineers, i.e., they directly or indirectly modify the chemical, physical and biochemical properties of the soil, thus affecting the availability of resources to other organisms at different spatial and temporal scales (Brussaard et al., 2007; Blouin et al., 2013). Earthworms have been used recently to indicate the removal of contaminants from soil (Rodriguez-Campos et al., 2014) or degrade unrecyclable compounds in soils (Gupta and Garg, 2009). Earthworm species at high densities, such as *Eisenia fetida* and *L. terrestris*, effectively accelerate the removal of high concentrations of herbicides when the feed for earthworms is sufficient (Tejada and Masciandaro, 2011). Earthworms can accelerate the removal of atrazine from 41% to 93% after 9 days (Kersanté et al., 2006), and can also increase the removal of PCBs and PAHs in the soil for 40% (Hernández-Castellanos et al., 2013; Rodriguez-Campos et al., 2014). To date, the positive effect of earthworms on the removal of organic contaminants, such as herbicides, PAHs and PCBs, has been reported in several laboratory studies. However, few studies have been conducted to indicate that earthworms could increase the removal of veterinary antibiotics, especially OTC, from soil.

Arbuscular mycorrhizal (AM) fungi, which form a permanent, obligate symbiotic relationship with the roots of over 80%–90% of all plant species, can significantly affect the growth and physiology of host plants by extending the exploited soil volume (Smith and Read, 2008). Most previous studies have found that AM fungi have positive effects on the decomposition of organic contaminants and improve plant growth by alleviating the toxicity of pollutants (Huang et al., 2009). The atrazine concentration was increased in the mycorrhizal inoculated roots by 132.4%–260.5% compared to the non-inoculated roots (Huang et al., 2007). Previous studies have also found that AM fungi have positive effects on the decomposition of other organic contaminants, such as PAHs (Joner and Leyval, 2003; Wu et al., 2008a) and DDT (Wu et al., 2008b), in soils. AM fungi may therefore play a critical role in the degradation of organic contaminants in soils. However, very little information is available concerning the impact of AM fungi on the degradation of veterinary antibiotics in the soil (Hillis et al., 2008).

Earthworms and AM Fungi interactions are known to influence soil fertility and plant growth by changing the physical environment and the soil nutrient cycles, including microbial and enzymatic activities, that enhance nutrient mineralization and availability in the soil (Wardle et al., 2004; Zarea et al., 2009; Li et al., 2012b,c). However, the mechanisms involved the independent and combined impact of earthworms and AM fungi on the degradation of veterinary antibiotics remain unclear. It is likely that the management of AM fungi and earthworms may increase veterinary antibiotic degradation in the soils. On the other hand, earthworms facilitate and increase the contact between a contaminant and soil microorganisms (Hickman and Reid, 2008), and AM hyphae influence the surrounding soil, which could result in the development of distinct microbial communities in the rhizosphere and bulk soil (Cheng and Baumgartner, 2006; Purin and Rillig, 2008). It is reasonable to expect that soil microbial communities modified by earthworms and AM fungi play a key role in the degradation of veterinary antibiotics. Based on the rationale above, we hypothesized that earthworm (*E. fetida*) and AM fungi (*Rhizophagus intraradices*) could separately and interactively promote the degradation of OTC through impacts on the soil microbial community and diversity.

2. Materials and methods

2.1. Antibiotics and soil

The experiment was conducted using sterilized 180 mm × 220 mm plastic containers as the culture vessel. A completely randomized block design with a 2 × 2 factorial arrangement of treatments was utilized. The factors were AM fungi (with or without) and earthworms (added or not added). There were two groups of containers with different concentrations of OTC (1 mg kg^{-1} soil DM, 100 mg kg^{-1} soil DM). The concentration of 1 mg kg^{-1} was selected to encompass environmentally relevant concentrations based on a review of the literature (Toth et al., 2011). The concentration of 100 mg kg^{-1} was the scenario simulation, and the concentration level which may threaten soil life and health. The antibiotics used in the study were OTC (Terramycin®, 200 mg ml^{-1} base as OTC dihydrate). The soil, a silt loam, was obtained from the 0- to 20-cm layer of long-term no-farming land in the Shangzhuang experimental station, and had received no manure or fertilizer with veterinary antibiotics for at least 10 years. The bulk soil sample was air-dried, sieved to pass 2.53 mm, and sterilized with 25 kGy 60 Co γ-radiation at the Beijing Radiation Application Research Centre. The soil had a total nitrogen concentration of 0.10%, an organic matter concentration of 1.25%, a pH of 7.40 (H_2O, soil 2.5:1, v/v), a cation exchange capacity of 96 mg kg^{-1}, an Olsen-P of 13.65 mg kg^{-1} and an NH_4Cl-exchangeable K of 143.0 mg kg^{-1}.

2.2. Earthworm, mycorrhiza and maize

The earthworms (*E. fetida*) were kept in sterilized glass vessels for 24 h to minimize the impact of naturally occurring mycorrhizal propagules before being inoculated into the test soil (Li et al., 2012b). The AM fungi inoculum was *R. intraradices* (BEG JX04B), propagated on maize and white clover host plants in a growth chamber at 30 °C/18 °C with a 16 h/8 h light/dark regime and 50–75% relative humidity. Maize seeds were *Zheng Dan 958*, which were surface sterilized with a 10% (v/v) solution of H_2O_2 for 10 min, rinsed thoroughly with deionized water, then placed on autoclaved filter papers soaked with sterile distilled water and incubated at 25 °C for 24 h (Li et al., 2012b).

2.3. Experimental design and inoculation

Plastic containers were filled with 1.5 kg sterilized soil that was mixed with 8.75 g sterilized oven-dry wheat straw (equivalent of 8 t/ha biomass). Live or inactivated mycorrhizae innoculum was placed approximately 2 cm under the soil surface at 50 g kg^{-1}. Ten ml of a soil filtrate (0.45 μm pore size) and sterilized mycorrhiza were added to the pots with sterilized mycorrhiza to provide nutrients similar to those supplied from the live inoculum (Calvet et al., 1993). Two seeds of *Zheng Dan 958* were planted into each container. All treatments received basal fertilization of 150 mg kg^{-1} N (NH_4NO_3), 50 mg kg^{-1} P (KH_2PO_4), 150 mg kg^{-1} K