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Sewage sludge application strongly modifies earthworm impact on microbial and biochemical attributes in a semi-arid calcareous soil from Iran

Hanye Jafari Vafa*, Fayez Raiesi, Alireza Hosseinpur

Department of Soil Science and Engineering, Faculty of Agriculture, Shahrekord University, P.O. Box 115, Shahrekord, Iran

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ABSTRACT

Application of municipal sewage sludge (MSS) may modify the influence of earthworm activity on soil microbial and biochemical properties through an increase of substrate availability. However, the effect of earthworm activity on microbial community is poorly known in calcareous soils amended with MSS. Such knowledge would lead to better understanding of how earthworms and microorganisms interact following MSS application in agroecosystems. This study aimed to investigate how MSS application can modify the impact of earthworms on microbial performance and biochemical attributes of a calcareous soil amended with MSS. Experimental treatments were MSS application (without and with 1.5% MSS) and earthworm addition (no earthworm, epigeic *Eisenia fetida*, endogeic *Aporrectodea caliginosa* and a combination of the two earthworm species) setup as a 2×4 full factorial experiment using a completely randomized design with three replications for each treatment combination under greenhouse conditions over 90 days. Addition of MSS had a positive effect on the measured biochemical and microbiological indicators of the study soil. Municipal sewage sludge application resulted in a significant ($p < 0.001$) increase in the amounts of microbial biomass carbon (MBC) and basal respiration rate (RR); but without an effect on the level of dissolved organic carbon (DOC). While the presence of earthworms decreased the quantity of DOC and MBC ($p < 0.001$); other soil attributes such as basal respiration rate, the metabolic quotient (qCO_2) and the ratio of fungal to bacterial (F/B) respiration were all increased significantly ($p < 0.001$) with earthworm addition. Nevertheless, the magnitude of the earthworm effect was largely dependent upon the MSS treatment. Application of MSS decreased the stimulatory effect of earthworms on soil microbial indicators and this effect was less pronounced in the presence of *E. fetida* than *A. caliginosa* species. This is probably due to the preference of epigeic *E. fetida* to feed on MSS as an essential food resource rather than only feeding and grazing on microbial populations. In contrast, the endogeic *A. caliginosa* species was not dependent upon MSS as food resources. Moreover, the interaction effect between the two earthworm species on soil microbial and biochemical properties was mostly additive in nature (without interaction) in both MSS-amended and unamended soils. Results indicated that earthworm effect and its interaction with MSS were important factors for soil microbial biomass, community composition and performance and that earthworm effects should decrease in MSS-treated soils.

1. Introduction

Earthworms are the most important soil saprophagous fauna and much of the faunal biomass is attributed to the presence of these organisms in the soil (Edwards and Bohlen, 1996; Edwards and Arancon, 2004; Blouin et al., 2013). Earthworms are commonly recognized as ecosystem engineers due to their crucial role in soil physical, chemical and biological processes, and ultimately their great contribution to ecosystem functioning and services (Edwards and Arancon, 2004; Blouin et al., 2013). By burrowing, feeding and casting activities, this group of soil organisms generally promotes the incorporation of plant residues in soil layers, the biochemical breakdown of plant residues, soil humus development and decomposition, the conversion of soil nutrients from an unavailable into an available form for plants and microorganisms, the creation of macro-biopores and the uniform distribution of organic matter throughout the soil profile (Edwards and Bohlen, 1996; Edwards and Arancon, 2004; Bohlen et al., 2004; Blouin et al.,

* Corresponding author. Fax: +98 38 34424401.
E-mail address: hjafarivafa@gmail.com (H.J. Vafa).

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2013). As a consequence of improved soil conditions and the enhancement of nutrient mineralization, a positive effect of earthworms on plant growth and productivity has been shown for different crops (Paz-Ferreiro et al., 2014). Hence, the abundance, diversity and activity level of earthworms may provide a useful indicator of soil quality in agroecosystems (Linden et al., 2004). Most specifically, the burrowing and casting activities of earthworms and consumption of microorganisms by earthworms can also affect the activity, biomass and diversity of soil microorganisms as well as soil enzyme activities (Edwards and Bohlen, 1996; Zhang et al., 2000; Kızılkaya and Hepsen, 2004; Blouin et al., 2013; Paz-Ferreiro et al., 2014).

Earthworms were often found to stimulate organic matter decomposition, microbial activity and nutrient availability, but the magnitude of their effect largely depends upon soil characteristics, food availability and quality, the location of the organic matter, the species and functional group, and their interactions with other soil organisms (Flegel and Schrader, 2000; Zhang et al., 2000; Caravaca and Roldán, 2003). For instance, *Eisenia fetida* activity decreased microbial biomass C and increased microbial metabolic quotient in a sandy soil but with no effect in the clay loam and clay soils (Caravaca and Roldán, 2003). The positive effect of earthworm *Aporrectodea caliginosa* on soil O_2 uptake, microbial biomass and enzyme activities was greater in the presence than absence of ryegrass residues as food resources for earthworm ingestion (Ross and Cairns, 1982). Flegel and Schrader (2000) reported that soil enzyme activities in the earthworm (*Dendrobaena octaedra*) casts were highly influenced by the different food sources and showed the activities of dehydrogenase and phosphomonesterase in the earthworm casts were greatest when the earthworms were feeding on dandelion and lupin residues compared with rye, alder, beech and larch residues. This effect was mainly ascribed to differences in the food quality (*i.e.*, C:N ratio) and consumption rate by the earthworms (Flegel and Schrader, 2000). Sewage sludge as a soil organic amendment is a valuable source of nutrients essential for plants, and an appropriate source of energy, organic carbon and nutrients for both earthworms and heterotrophic microbial communities (Singh and Agrawal, 2008; Smith, 2009). Soil application of MSS has been an important and attractive practice for (1) improving soil physical, chemical and biological properties as well as plant nutrient level and (2) reducing the disposal problems associated with increased production of urban wastes or biosolids (Singh and Agrawal, 2008; Smith, 2009). Sewage sludge is known to increase the growth, biomass, reproduction and population density of earthworms (Barrera et al., 2001; Emmerling and Paulsch, 2001; Rorat et al., 2013) and was found to be the most favorable organic solid for food consumption by some earthworms species (Emmerling and Paulsch, 2001). However, the effect of MSS disposal to soil on earthworm growth, reproduction and activity, and the subsequent microbial community depends on the level of toxic substances in MSS, in particular toxic metals (Barrera et al., 2001; Domínguez-Crespo et al., 2012). Earthworms consume and feed on the organic fragments added by MSS, which are suitable for microorganisms after passing through the earthworm gut. This might lead to the stimulation or occasionally inhibition of microbial and enzyme activities in the soil, depending on the level of toxic metals (e.g., Cd, Cu, Pb, Ni, Zn) in MSS (Kızılkaya and Hepsen, 2004).

Nevertheless, the effect of earthworm activity on microbial activity and biomass in soils amended with MSS, in particular in arid environments where earthworm growth and activity is constrained by low organic matter resources, is poorly known (McDaniel et al., 2013). Such information would lead to better understanding of how earthworms and microorganisms interact following MSS application, which is important for managing earthworms and land disposal of MSS. In this study, we focused on how two earthworm species (epigeic *E. fetida* and endogeic *A. caliginosa*) alone or in combination affect microbial performance following MSS application, and whether MSS could modify the effect of earthworms on microbial indicators of soil quality in a typical calcareous soil. It is hypothesized that earthworms would increase soil microbial activity and biomass through the enhancement of their growth and activity with MSS application.

2. Materials and methods

2.1. Soil and MSS sampling and preparation

A calcareous soil from 0 to 30 cm layer was obtained from a farmland field under fallow without cultivation history for ten years. The study soil was classified as mesic Fluventic Haploxerepts with clay loam texture (19% sand, 51% silt and 30% clay). The soil was air-dried and passed through a 2-mm sieve for the experiment. Sewage sludge as the soil organic amendment was collected from Wastewater Treatment Plant (WTP) in Shahrekord, which treats a large quantity of MSS each day from the municipality. Sewage sludge was air-dried and grounded to pass through a 1-mm sieve for a uniform mixture with soil matrix. The chemical properties of both soil and MSS analyzed were electrical conductivity, EC (Rhodes, 1996), pH (Thomas, 1996), total nitrogen, TN (Bremner, 1996), organic carbon, OC (Nelson and Sommers, 1996), available phosphorus, AP (Olsen and Sommers, 1982), and available copper (Cu), lead (Pb) and zinc (Zn) extracted with DTPA-TEA (Lindsay and Norvell, 1978) using an atomic absorption spectrophotometer (AAS Model GBC 913 plus). The soil water holding capacity (WHC) was determined based on the method described by Rey et al. (2005). In brief, 100 g of disturbed soil was placed in a metal cylinder with the bottom covered with plastic net to prevent soil losses and soaked in tap water for 24 h to saturate the soil. Soils were covered with a plastic sheet to prevent evaporation losses and left for 72 h to drain before weighing. The soil was then oven-dried at 105 °C to constant mass and WHC (%) was considered as the gravimetric moisture content retained in the soil. Total metal contents in soil and MSS subsamples (extracted with 4 M HNO_3 at 80 °C overnight) were determined according to the method described by Sposito et al. (1982). Sewage sludge was characterized by pH 6.01, 359 g OC kg^{-1} and 55.9 g N kg^{-1} based on oven-dry weight. The total concentration of metals in MSS was 73.2 mg kg^{-1} for Cu, 46.1 mg kg^{-1} for Pb and 1163 mg kg^{-1} for Zn. The characteristics of the study soil and MSS are listed in Table 1.

2.2. Earthworm preparation

Epigeic earthworms *E. fetida* and endogeic earthworms *A. caliginosa*, the most common species present in the studied area, were manually obtained from WTP in Shahrekord and a garden soil, respectively, and transferred to the laboratory and allowed reproduction separately for four months in wooden boxes filled with soil and sufficient plant residues. *E. fetida* is an epigeic species which is mostly present in the top layer of the soil feeding mainly on organic matter and decaying plant residues with less burrowing into or ingesting much soil, while *A. caliginosa* is an endogeic earthworm which can burrow greatly below soil surface (the upper 10–15 cm) and obtain their food by ingesting a mixture of soil and organic matter, and is thought to be an important species in terms of organic matter incorporation and decomposition (Edwards and Bohlen, 1996; Edwards and Arancon, 2004). Before their transfer to the pots, earthworms were washed with distilled water to remove surface soil particles and kept in sterilized vessels for 24 h in tap water to allow the complete evacuation of their gut contents.