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Application of leaves to induce earthworms to reduce phenolic compounds released by decomposing plants

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ABSTRACT

The negative impacts (allelopathic effects) of phenolic compounds (PCs) in soil on plant growth and microbial communities have recently received considerable attention. Similarly, there have been several recent studies on the effects of microbes on the degradation of PCs. Because of the profound effects of their feeding and burrowing activity, earthworms should have significant effects on PCs degradation; however, few studies have examined this potential effect, and particularly, factors that may affect the course of degradation. We tested different earthworm species and density, different conditions (sterilization or not) of mixture of soil and plant residual, and different source of PCs to evaluate the capacity of earthworms to accelerate PCs degradation. In addition, earthworm behavior experiments were set up to test whether adding plant leaves can stimulate earthworm feeding activity. The results showed that native earthworms exhibited a higher capacity than compost earthworms for degrading PCs; furthermore, when the number of *Metaphire guillemi* reached 300 individuals m^{-2} in our experimental units, the PCs decreased most quickly, and the residual PCs concentration was 105 μg (*p*-coumaric acid)g^{-1} (dry soil) less than that of control group. The source of PCs also affected their degradation rate, as PCs derived from leaves seemed to degrade more quickly. The results of our experiment suggested that earthworms avoid feeding on phenolic acids, but can be induced to do so by adding leaves to the substrate. These results indicate that earthworm activity can accelerate the degradation of total PCs, and that this may be further facilitated by incorporation of organic matter, which may be used to alleviate allelopathic effects of PCs in soil.

1. Introduction

Phenolic compounds (PCs) are the most important and common plant allelochemicals in soil ecosystems. They are chemical compounds consisting of a hydroxyl group bound directly to an aromatic hydrocarbon group [1], which are the most widespread secondary plant metabolites known [2,3]. PCs are released into the environment through foliar leachate, root exudation, residue decomposition [4,5], among which aboveground and belowground litter are the dominant pathways [3,6]. Some PCs are phytotoxic, and can inhibit plant growth through interaction with the mitochondrial membrane and impairment of dark respiration and ATP synthesis [4,5]. Similarly, phytotoxicity can disrupt the microbial community balance by modifying the population and community structure in the rhizosphere [7,8]. According to Blum and Gerig (2005), phenolic acid treatment of seedlings inhibits transpiration, water utilization, leaf area, and the absolute and relative rates of leaf expansion [9].

Several management approaches have been taken to alleviate allelopathic stress caused by PCs on plants. For example, activated charcoal has been used to adsorb accumulated phytotoxic chemicals with the result of improved growth and yield in strawberry [10], several leafy vegetables, and some ornamentals [11,12]. Similarly, several studies have focused on the application of microorganisms that can biodegrade phenolic acids and colonize the

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rhizosphere of plants to alleviate the stress of autotoxic allelochemicals exuded by crop roots or left by plant residues [13–16]. Although several measures to degrade PCs have been tested and shown to be successful, a major component of natural and agricultural ecosystems, earthworms [17], has received little attention.

Earthworms form a major part of the soil decomposer macrofauna, and play an important role in temperate ecosystems [18] and some tropical ecosystems. They can alter soil structure, accelerate the nutrient cycling processes in soil, and significantly impact microbial communities [19–23]. In addition, as an ecosystem engineer, earthworms can promote the degradation of many compounds in soil. Several studies have examined the direct or indirect relationship between earthworms and some toxic substances. For example, studies have examined the effect of earthworms on altering mineralization and biodegrading atrazine [24,25], as well as earthworm mediated enhancement of the removal of dichloro-diphenyl-tricgloroethane or polycyclic aromatic hydrocarbons [22,26]. Thus, earthworms may have the potential to degrade single cyclic molecules such as phenolic acid or other simple PCs with simpler structures than the complex substances mentioned above. Although Butenschoen et al. (2009) studied the fate of catechol affected by earthworms, they did not examine the influence of earthworms on total PCs, nor did they focus on the basic conditions for earthworms to degrade PCs [18]. Furthermore, they only used endogeic earthworms in their experiments, and thus is it unclear whether the different feeding and burrowing activities of epigeic or anecic earthworms might be more (or less) efficient at degradation of PCs. So, it is important to evaluate whether epigeic earthworms and anecic earthworms can accelerate the decomposing of PCs.

The objectives of this paper are: first, to evaluate the ability of earthworms to accelerate the degradation of PCs released by decomposing plants residue (strawberry was used as our model plant); second, to investigate influence factors for earthworms to biodegrade phenolics; and finally, to examine the trophic behavior of earthworms in relation to phenolic acid.

2. Materials and methods

2.1. Soil and earthworms

Soil was obtained from a continuously cropped experimental field at China Agricultural University in Beijing, where the strawberry cultivar ‘Benihoppe’ (*Fragaria ananassa Duch*) had been planted for 3 years. The soil was air-dried, sieved (2 mm), and thoroughly mixed with smashed roots and strawberry leaves, creating an experimental mixture of soil (96% mass ratio), root (2% mass ratio) and leaf (2% mass ratio) (MSRL) with the following properties: pH (H_2O:soil, 2.5:1) of 7.82, 2.25% soil organic matter, 0.12% total nitrogen, 50.38 mg kg^{-1} available phosphorus (Olsen-P), 255.23 mg kg^{-1} available potassium (NH_4OAc-K).

Earthworms *Metaphire guillemi* [27] (anecic) native to the experimental fields of China Agricultural University were collected at the same location where the soil was obtained. Compost earthworms *Eisenia fetida* (epigeic) were purchased from Beijing Lvhuan Kemao Company. Both species of earthworm were placed in MSRL for 1 week to acclimate to the matrix and replace their gut contents with experimental soil. Adult earthworms with fresh weight of 2.4–2.5 g for *M. guillemi*, and 0.4–0.5 g for *E. fetida* were chosen to be used as test animals. Prior to fresh weight determinations, the earthworms were washed and blotted with filter paper [23].

2.2. Experimental procedure

Plastic pots (top diameter 19 cm, bottom diameter 18 cm and 15 cm depth) with a small hole in the bottom were filled with 3 kg MSRL and 500 mL deionized water. A plastic net (±1 mm) was placed in the bottom of every pot to prevent earthworms from escaping. All pots were placed in an artificial climate incubator with temperature maintained between 15 and 20 °C. All pots were irrigated every 5 days with 100 mL deionized water.

Four experiments were conducted to test the influence of earthworm species, density of *M. guillemi*, sterilization and the source of PCs on degradation rate of PCs (Table 1). In the first experiment, there were 3 treatments: one with 6 individuals of *M. guillemi* (M), 30 individuals of *E. fetida* (E) (this resulted in approximately equal biomass for each species) and a control without earthworms (CK). In the second experiment, there were six densities of *M. guillemi* added to the pots: treatments consisted of 0, 3, 6, 9, 12, and 15 individuals per pot (marked as Z, T, SI, N, TW and F respectively). In the third experiment, there were four treatments: sterilized (autoclaved at 101 kpa, 121 °C, 30 min, twice) MSRL with *M. guillemi* (SM), sterilized MSRL without earthworms (S), ordinary MSRL with *M. guillemi* (OM), and ordinary MSRL without earthworms (O). In order to verify whether most of the microorganisms in sterilized MSRL have been eliminated, we used dilution-plate method on LB (lysogeny broth) (10 g MSRL and 100 mL sterile normal saline to obtain extraction) to test the effect of sterilization (Fig. 1). The last experiment included six treatments: mixture of soil and ground leaves with *M. guillemi* (ML), *E. fetida* (EL) and without earthworm (L), and mixture of soil and ground roots with earthworm *M. guillemi* (MR), *E. fetida* (ER), and without earthworm (R). Each treatment in each experiment mentioned above had 4 replicates. All of the experiments lasted for 30 days.

The avoidance/selection behavior of earthworms was studied in

Table 1
Earthworms added to pots (Initial) and recovered after 30-day experiments (Final).

Experiment	Treatment	Earthworm abundance (Individuals per pot)		Earthworm survival rates (%)
		Initial	Final	
E1	M	6	6	100
	E	30	31.00 ± 1.41	103.3 ± 4.7
	CK	0	0	–
E2	Z	0	0	–
	T	3	3	100
	SI	6	6	100
	N	9	9	100
	TW	12	12	100
	F	15	14.50 ± 0.58	96.7 ± 3.8
E3	SM	6	6	100
	S	0	0	–
	OM	6	6	100
	O	0	0	–
E4	ML	6	6	100
	EL	30	31.00 ± 0.82	103.3 ± 2.7
	L	0	0	–
	MR	6	6	100
	ER	30	31.75 ± 1.71	105.8 ± 5.7
	R	0	0	–

Values are mean (standard deviation is zero) or mean ± standard deviation of 4 replicates. Abbreviations:
E1: M: treatment with *M. guillemi*; E: treatment with *E. fetida* (E); CK: Control group.
E2: Z: treatment without earthworm; T: treatment with three individuals of earthworm; SI: treatment with six individuals of earthworm; N: treatment with nine individuals of earthworm; TW: treatment with twelve individuals of earthworm; F: treatment with Fifteen individuals of earthworm.
E3: SM: sterilized matrix added with *M. guillemi*; S: only sterilized matrix; OM: ordinary matrix added with *M. guillemi*; O: only ordinary matrix.
E4: ML: matrix composed of leaves and soil with *M. guillemi*; EL: matrix composed of leaves and soil with *E. fetida*; L: matrix composed of leaves and soil; MR: matrix composed of roots and soil with *M. guillemi*; EL: matrix composed of roots and soil with *E. fetida*; R: matrix composed of roots and soil.