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How do earthworms affect decomposition of residues with different quality apart from fragmentation and incorporation?

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ABSTRACT

Earthworms especially contribute to decomposition by fragmenting, incorporating, and mixing residues into the soil. However, residues are already mechanically fragmented and incorporated into the soil by combine harvesters in most arable cropping systems of China, which may diminish the effects of earthworms. How earthworms affect the decomposition of residues with different quality, apart from fragmentation and incorporation, is still not clear. Thus, the aim of the present 60-day soil microcosm incubation experiment was to assess how earthworms affect the decomposition of residues of different quality, aside from fragmentation and incorporation. Four residues with different quality (high quality: rapeseed cake; medium quality: corn leaf; low quality: rice straw and corn stalk) were fragmented to 1-mm pieces using a laboratory blender and then thoroughly mixed with soil. The decomposition rates of different residues with or without earthworms (*Metaphire guillelmi*) were determined by measuring the CO_2 emission. We hypothesized that earthworms accelerate high-quality residue decomposition more than low-quality residues. Results showed that earthworms increased cumulative CO_2 emission for each residue type separately. Carbon derived from the residues was calculated and the decomposition kinetics was fitted with the first-order exponential model. During the initial 20 days of the incubation, the decomposition rate constant (k) of low-quality residues was significantly decreased by earthworm addition and significantly increased during the later period (20–60 days) of the incubation. However, no significant difference was detected between earthworm presence and absence treatments on the k value with high- and medium-quality residue incorporation. In general, earthworm addition increased inorganic nitrogen contents in most residue treatments during the entire incubation period, except for the rapeseed cake-treated soil. Our results indicated that earthworms have no effect on the decomposition of high-quality residues, but have a positive effect on the later stages of low-quality residue decomposition.

1. Introduction

Crop residues are a potent source of nutrients and organic matter during decomposition and can thus maintain soil fertility. Therefore, returning crop residues during harvest by combine harvester has become a common soil management practice in most arable cropping systems of China. The decomposition of crop residues is the result of complex microbial processes controlled by numerous factors, including the chemical composition of the residue and the decomposer community (Li et al., 2011; Pena-Pena and Irmler, 2016).

The chemical composition of the residues exerts an important influence on decomposition processes. The nitrogen (N) concentration of residues is the dominant parameter determining residue quality due to the influence of N availability on microbial metabolism (Parton et al., 2007). Consequently, the carbon-nitrogen ratio (C/N) is often used as an indicator of either net N mineralization or immobilization during residue decomposition (Begum et al., 2014). Additionally, lignin and polyphenols have been identified as important quality parameters, as lignin is one of the most recalcitrant carbon (C) compounds and polyphenols can bind with proteins to immobilize N (McDaniel et al., 2014; Nikolaidou et al., 2010). Therefore, the conceptual classification system for organic residue management divides residues into quality classes based on N, lignin, and polyphenol contents (Table 1) (Palm et al., 2001). In general, high-quality residues can decompose faster in comparison with low-quality residues (Li et al., 2011; Tian et al., 2007; Trinsoutrot et al., 2000). While Li et al. (2011) indicated that the

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Table 1
The contents of C, N, lignin, polyphenols and quality class of crop residues.

Residues	TC (%)	TN (%)	C/N	Lignin (%)	Polyphenols (%)	Quality class[a]
Rapeseed cake (RC)	43.82 ± 0.41	6.02 ± 0.09	7.28	14.91 ± 0.08	1.22 ± 0.02	High quality
Corn leaf (CL)	41.41 ± 0.52	2.30 ± 0.05	18.00	14.62 ± 0.05	2.53 ± 0.03	Medium quality
Rice straw (RS)	33.21 ± 0.45	0.80 ± 0.04	41.51	23.31 ± 0.11	3.31 ± 0.05	Low quality
Corn stalk (CS)	41.70 ± 0.32	0.80 ± 0.02	52.13	24.04 ± 0.12	2.73 ± 0.03	Low quality

Residue C and N contents were determined by an elemental analyzer (Vario El cube, Elementar, Germany). Residue lignin and polyphenol contents were determined using a modified Klason lignin method and Folin-Ciocalteu method, respectively.

[a] Residue quality classified according to Palm et al. (2001). High-quality residues (class I) have high N, low lignin and low polyphenol contents (> 2.5% N; < 15% lignin; < 4% polyphenols). Medium quality residues have high N, high lignin and high polyphenol contents (> 2.5% N; > 15% lignin; > 4% polyphenols) as class II, or low N and low lignin contents (< 2.5% N; < 15% lignin) as class III. Low-quality residues (class IV) have low N and high lignin contents (< 2.5% N; > 15% lignin).

decomposition rate of residues was positively correlated with the initial residue N content in the initial stage of incubation, but negatively correlated with litter N and P concentrations in the late stage.

Decomposer community is another crucial factor that influences the residue decomposition process. Earthworms are considered an effective part of the decomposer community, and play a key role in plant material decomposition and increase the rate of turnover of organic matter (Ernst et al., 2009; Gomez-Brandon et al., 2010; Lubbers et al., 2017). Through comminution of residues and their vertical redistribution in the soil profile, earthworm activity leads to a greater surface area availability for microbial colonization and further decomposition (Ernst et al., 2009; Seeber et al., 2008). Moreover, through ingestion, earthworms modify and stimulate microorganisms when they pass through the earthworm gut, and this results in differences in the microbial community in the cast, which could enable better exploitation of resources either because of the appearance of microbial species in the fresh substrate or the pool of readily assimilable compounds in the casts (Chen et al., 2015; Knapp et al., 2009). Nevertheless, residue breakdown by earthworms is also strongly related to the chemical properties of the residue. Previous studies have reported that residue decay by earthworms was highest in N-rich maize litter (C/N 34.8) and lowest in *Miscanthus* litter (C/N 134.4) (Ernst et al., 2009). This was also confirmed by Martin Flegel and Schrader (2000) and Araujo et al. (2004), as they stated that the palatability of residue for earthworms increased with decreasing C/N of the residue (Curry and Schmidt, 2006). However, these results were conducted via litterbag experiments or the loss of litter from the soil surface in natural ecosystem, which may not reflect the actual situation in the farmland.

In general, earthworms contribute to decomposition by fragmenting, incorporating and mixing residues into the soil in natural habitats, which strongly relates to the chemical properties of the residue. However, residues are mechanically chopped to around 5–10 cm stalks, and then incorporated into the soil to a 10–15 cm depth by combine harvesters on fields, which may diminish the effects of earthworms. How earthworms affect the decomposition of residues with different quality, apart from fragmentation and incorporation, is still not clear. Therefore, we homogeneously mixed residues of < 1 mm with soil in the present microcosm experiment. This particle size enabled a homogenous mixing of residues with soil and a valid comparison between treatments based on crop residue quality. Our objective was to assess the impact, apart from fragmentation and incorporation, of earthworms (*Metaphire guillelmi*) on the decomposition and mineralization of a variety of residues (rapeseed cake, corn stalk, corn leaf and rice straw) with different chemical composition and quality. We hypothesize that earthworms may accelerate high-quality residue decomposition more than low-quality residues.

2. Material and methods

2.1. Soil, earthworm and residue preparation

Soil and earthworms were collected in the summer of 2016 from the same vegetable field in Wuhan, Hubei Province, China. The anecic earthworm *M. guillelmi* is a dominant species in this field (~42 individuals m^{-2}). The surface layer (0–30 cm) of soil was collected and sieved (5 mm) to remove debris, and sterile distilled water was added to achieve a moisture content of 52% water-filled pore space (consistent with the field when we collected soil), and this was pre-incubated for 7 days. At the beginning of the experiment, soil total nitrogen (TN) content was 1.25 g kg^{-1}, total organic carbon (TOC) content was 13.95 g kg^{-1}, dissolved organic carbon (DOC) content was 35.89 mg kg^{-1}, and soil inorganic N (NH_4^+-N and NO_3^--N) content was 27.49 mg kg^{-1}. Adult earthworms (*M. guillelmi*) of similar size and weight were collected, purged following the filter paper method, and then rinsed with sterile distilled water before using.

Rice, maize, and rape are the main crops in Hubei Province. Four residues from these crops (rapeseed cake, corn leaf, rice straw and corn stalk) with different qualities (Table 1) were collected from the field, dried at 60 °C for 2 days, and then chopped with a laboratory blender in order to obtain residues of < 1 mm. The rapeseed cake used in this study was a byproduct of oil manufacture; it we selected because of its low C/N ratio and considered as a plant residue because it originates from rapeseed. In addition, some farmers in the vicinity of the oil factory return the rapeseed cake to the soil.

2.2. Incubation experiment

An experiment with three replicates was carried out using microcosm system incubation; it consisted of the following eight treatments: 1) rapeseed cake (RC), 2) rapeseed cake + earthworms (ERC), 3) corn stalk (CS), 4) corn stalk + earthworms (ECS), 5) corn leaf (CL), 6) corn leaf + earthworms (ECL), 7) rice straw (RS), and 8) rice straw + earthworms (ERS). In total, 482 g of fresh soil (equal to 400 g dry soil) were placed in an 800-ml glass bottle. Each residue (4 g) was incorporated and thoroughly mixed with soil. Depending on the treatments, three adult earthworms were added to the microcosm (~260 individuals m^{-2}). We used a high earthworm density in this experiment because (1) earthworm densities of up to 277 individuals m^{-2} have been reported in the red soil of China (Hu and Wu, 1993), and (2) the experiment was designed to study mechanistic soil processes, so this density minimized the risk of finding no effects. Treatments without residue incorporation were set up as the corresponding control (ECK and CK).

Each microcosm was covered with a black polyethylene cloth and a piece of plastic mesh (1.5 mm) held tightly with a rubber band to prevent earthworms from escaping but to allow aeration. All microcosms were incubated in the dark at air temperatures of 25 ± 2 °C for 60 days, as the average air temperatures of Wuhan in summer are

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