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Earthworm biomass and population structure are negatively associated with changes in organic residue nitrogen concentration during vermicomposting

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ABSTRACT

Vermicomposting is an efficient and environmentally friendly technology to dispose of agricultural organic residues. The efficiency of organic residue decomposition during vermicomposting is directly affected by the biomass and population structure of earthworms. In this study, we investigated how the earthworm biomass and population structure responded to changes in the physicochemical properties of six types of organic residue (cattle dung, herbal waste, rice straw, soybean straw, garden waste, and tea residues) during vermicomposting. Each type of organic residues was placed in a pot with earthworms *Eisenia fetida*, and the physicochemical properties of the organic residues and earthworm growth dynamics were recorded at 0, 30, 60, and 90 d of vermicomposting. The biomass and population structure of earthworms were stable or increased in rice straw, garden waste, and cattle dung within 60 d of vermicomposting, whereas in tea residues and herb waste, very little earthworm activity (3 adults and 2 cocoons) was recorded on day 30. Among the physicochemical parameters, the substrate C/N ratio was negatively correlated with earthworm growth dynamics. Decomposing organic residues showed higher NH_4^+-N and NO_3^--N concentrations but a lower total organic carbon content, which negatively affected earthworm growth and reproduction. We recommend that chemical properties of vermicomposting systems should be monitored regularly. At the threshold levels of decomposing organic residue NH_4^+-N and NO_3^--N concentrations, earthworms should be removed and the vermicompost can be harvested. Small- and large-scale farmers thus need to monitor the physicochemical properties of vermicompost to sustain active earthworm populations.

Key Words: ammonium nitrogen, decomposing, earthworm biological traits, *Eisenia fetida*, nitrate nitrogen, organic residue dispose, physicochemical property

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INTRODUCTION

More than 40 million tons of agricultural organic residues are produced each year globally (Kumar *et al*., 2013), and a major part of these residues can be recycled as organic fertilizer after a suitable treatment to eliminate odors, reduce pathogens and weed seeds, and decrease the moisture content (Londhe and Bhosale, 2015). Vermicomposting is an effective way to achieve these goals and to make nutrients available to plants through synergistic decomposition of organic residues by earthworms and microorganisms. The earthworm *Eisenia fetida* is often used in vermicomposting owing to its ability to decompose agricultural organic residues, its adaptability to different environmental conditions, and its rapid reproduction (Molina *et al*., 2013; Sharma and Garg, 2018). The advantage of vermicomposting is that earthworms increase the mineralization and humification rates of organic residues, thereby reducing composting time and generating a product with a higher plant-available nutrient content and more stable than that produced by thermal composting (Albanell *et al*., 1988; Bhat *et al*., 2017). Vermicompost improves soil fertility and promotes plant growth, and it can be an efficient substitute for chemical fertilizers (Arai *et al*., 2013; Nattudurai *et al*., 2014; Bhat *et al*., 2018).

Efficiency of vermicomposting depends on earthworm biomass and population structure (*i.e*., proportions of adults and juveniles) (Rajapaksha *et al*., 2013). The biological basis of population structure is reproduction, and biomass is related to growth rate, both of which are influenced by environmental conditions such as light, temperature, and humidity (Auerswald *et al*., 1996; Sarwar *et al*., 2006). Earthworms exhibit negative phototaxis and avoid strong light. Lowe and Butt (2005) reported adverse effects on the growth and reproduction of earthworms exposed to natural light, thus darkness is generally considered important for optimal growth and reproduction. Moreover, temperature affects earthworm metabolic activities responsible for growth and reproduction (Uvarov *et al*., 2011). A study by Moreau-Valancogne *et al*. (2013) found that both the abundance and

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hatching rate of cocoons increased and then decreased with increasing temperature, and optimum temperatures for development and growth were 21.2 and 17.6 °C, respectively. Earthworm respiration depends on body surface moisture; too much or too little water is not conducive for absorption of oxygen through the epidermis (Dominguez *et al*., 2001). Water also affects the abundance and hatching rate of cocoons, which ultimately affects the earthworm population (Wani *et al*., 2013). Tripathi and Bhardwaj (2004) found that *E. fetida* growth was optimal at 25 °C in an organic substrate with 70% humidity and pH 6.5.

Earthworm-mediated decomposition releases plant-available forms of nitrogen (N), phosphorus, and potassium from organic residues during vermicomposting. In the course of vermicomposting, earthworms ingest organic material, grind it with gizzard, and pass it through gut (Garvìn *et al*., 2000). This result in an increase in the active sites for the microbial activity in the substrate. As the microbial activity increases, a portion of the carbon (C) content is lost in form of CO_2 and, subsequently, the mineral content in the substrate increases (Kim *et al*., 2010). Mineralization of N occurs, resulting in increased concentrations of nitrate and ammonium. However, changes in chemical properties of decomposing organic residues affect the biomass and population structure of earthworms (Hong *et al*., 2011). The chemical composition of leaves, particularly their C/N ratio, affects the abundance and population dynamics of earthworms (Lavelle *et al*., 2006). Organic residues with a C/N ratio between 20 and 30 are considered suitable for earthworm growth (Fusilero *et al*., 2013). This is because the activity and community structure of decomposer microbes respond to the C/N ratio of organic residues (Sarathchandra *et al*., 2006; Pang *et al*., 2009). In addition to the C/N ratio, earthworms are sensitive to the pH and salt content, which can be assessed by measuring electrical conductivity (EC), of organic residues. Excessively acid or alkaline condition alters earthworm activity (Klok *et al*., 2007) and interferes with their oxygen metabolism (Saroja, 1964), while salt concentrations exceeding an EC level of 1.03 dS m^{-1} reduce earthworm abundance and weight (Owojori *et al*., 2009; Tao *et al*., 2012).

The chemical composition of agricultural organic residues determines their suitability for vermicomposting. Assessing organic residues is generally based on their initial physicochemical properties, prior to introducing earthworms; however, the physicochemical properties of organic residues change with time and should therefore be assessed at different time points. Here, we measured the changes in physicochemical properties of six types of agricultural organic residues and assessed the corresponding earthworm activity in a vermicomposting unit for a period of 90 d. We chose the earthworm species *E. foetida* owing to its strong adaptability to different feeds and environmental conditions and its high reproduction rate. The objective of this study was to evaluate the effects of six types of agricultural organic residues on earthworm biological traits after 0, 30, 60, and 90 d of vermicomposting. Our results may be useful for small- and large-scale farmers for sustaining earthworm population when vermicomposting a variety of agricultural organic residues.

MATERIALS AND METHODS

Agricultural organic residues and earthworms

The experiment was conducted in Guangzhou, China and six types of agricultural organic residues were used. Cattle dung was collected from a cattle ranch in Guangzhou. Herbal waste (roots, stems, leaves, and seeds of plants such as *Panax pseudoginseng* Wall and *Glycyrrhiza uralensis* Fisch) was obtained from a pharmaceutical company in Huizhou, China. Rice straw and soybean straw were acquired from research facilities of South China Agricultural University, Guangzhou. Garden waste included branches and leaves pruned from green trees in the university campus. Tea residues, *i.e.*, residues after production of bottled green tea, were obtained from a beverage manufacturer in Guangzhou. All organic residues were air-dried and ground to $<$ 2 mm using a pulverizer, as residues of this size are more easily decomposed by earthworms than larger particles (Zaller and Saxler, 2007). About 10 kg of each type of organic residues was moistened (65%–70% humidity) and naturally fermented for 30 d in a plastic box (80 cm $\times$ 40 cm $\times$ 20 cm). This pre-decomposition process allows thermophilic bacteria to degrade labile substrates, but releases heat and gases that can harm earthworms (Wani *et al*., 2013). *Eisenia fetida* earthworms were purchased from a farm in Guangzhou and allowed to acclimate for 7 d in a compost mixture containing equal amounts (dry weight) of the six types of pre-decomposed organic residues. Then, healthy adults (*i.e.*, with a fully formed clitellum) were selected for vermicomposting experiments.

Experimental design

Plastic pots (15 cm in diameter, 20 cm in height) were filled with 400 g (dry weight) of one of the six types of organic residues (after pre-decomposition for 30 d). Next, 30 healthy adult earthworms were rinsed, weighed, and placed into each pot. The bottom and top of the pot were covered with 80-mesh nylon screen. Nine replicates (pots) were used for each type of organic residues (treatment). Mean weight of individual earthworms was 0.35 $\pm$ 0.03 g and total weight of earthworms was approximately 10 g, which was the same in each treatment. The experiment lasted for 90 d. All organic residues were maintained at 60% moisture content, on average, during the 90-d experimental period by daily sprinkling the pots using distilled water. Temperature in the laboratory was maintained at 25 ± 1 °C throughout the experiment. Physicochemical properties of the organic residues and earthworm populations were recorded at 0, 30, 60, and 90 d. This involved removing three randomly chosen treatment pots from the experiment after 30, 60, and 90 d. All earthworms were removed from the pots, rinsed using distilled water, gently dried on filter paper, and then weighed. Adults, juveniles, and cocoons were counted. Organic residues removed from the pot were air-dried for physicochemical analysis; the material consisted of a mixture of pre-decomposed residues plus earthworm-digested vermicompost.

Physicochemical analyses of organic residues

The pH and EC were determined in a suspension of organic residues and double-distilled water (1:10, weight:volume) that was agitated mechanically for 30 min and then filtered through quantitative filter paper (Yadav and Garg, 2009). Total organic C (TOC) was measured by the potassium dichromate oxidation method. Organic residues were digested using concentrated H_2SO_4 and 30% H_2O_2. Total N (TN) in the acid digest was determined using the micro-Kjeldahl analysis, total phosphorus (TP) using the vanadium-molybdenum colorimetric method, and total potassium (TK) using a flame photometer (Loh *et al.*, 2005). Organic residue was extracted with 0.01 mol L^{-1} $CaCl_2$ (1:20, weight:volume), and NO_3^--N and NH_4^+-N were measured using a flow injection analyzer (FIACompact, MLE, Germany) (Tylova-Munzarova *et al.*, 2005).

Statistical analyses

Effects of physicochemical properties of organic residues on earthworm biomass and population structure were tested with correlation and stepwise regression analyses. On day 0, physicochemical properties of organic residue did not have any effect on earthworms added that day; therefore, data from day 0 were not included in the correlation and stepwise regression analyses. In the correlation and stepwise regression analyses, data from the six types of organic residues on days 30, 60, and 90 were used, with 3 replicates per treatment and time point (n = 54). Statistical tests were performed using SAS 8.0 software (SAS Institute, Inc., Cary, USA).

RESULTS

Biomass and population structure of Eisenia fetida

Abundance of adults declined during the 90-d experimental period; however, the rate of decline differed significantly among the organic residuals (Fig. 1). On day 30, there were fewer than 3 adults in the pots with herb waste and tea

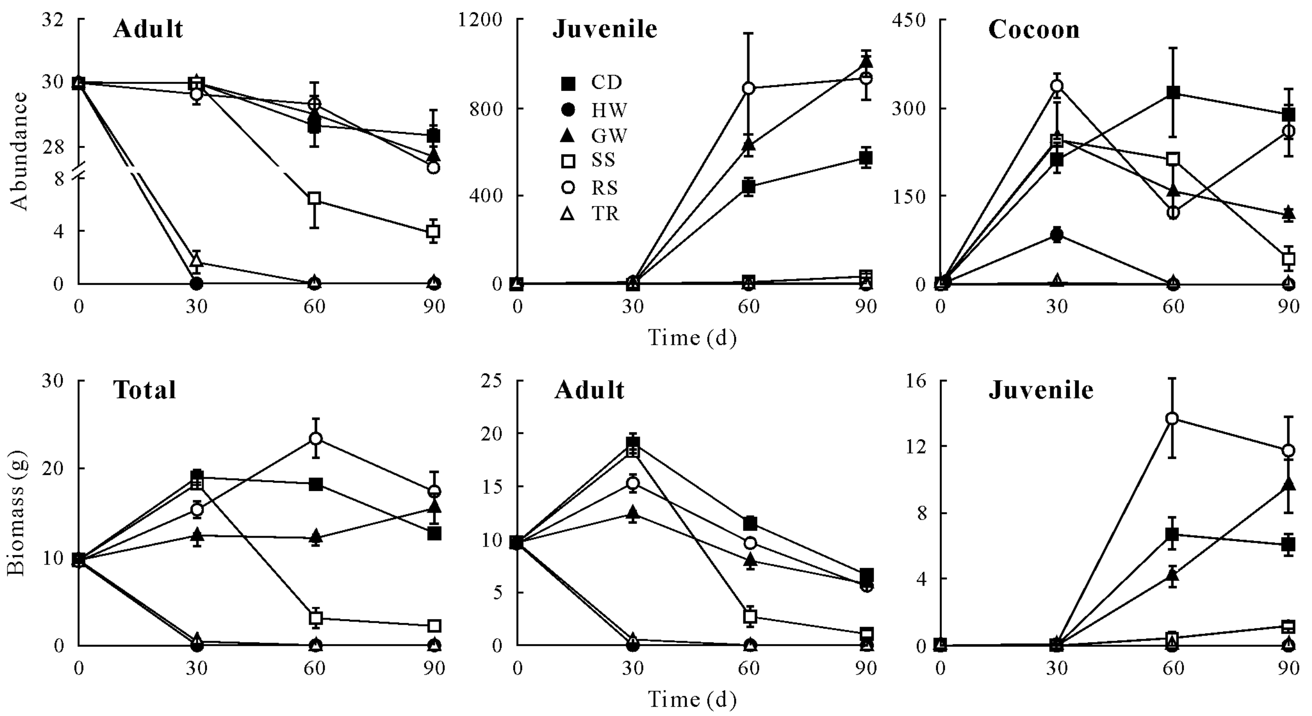

Fig. 1 Population structure and biomass of *Eisenia fetida* at different time points of vermicomposting as affected by six types of agricultural organic residues. Vertical bars represent standard errors of the means (n = 3). CD = cattle dung; HW = herb waste; GW = garden waste; SS = soybean straw; RS = rice straw; TR = tea residues.

residues, whereas the abundance of adults in the pots with other organic residuals did not change. On day 60, few adults were found in the pots with soybean straw, and the abundance of adults in the other pots declined during 60–90 d. On day 30, juveniles appeared, and the abundance of juveniles in the pots with rice straw, garden waste, and cattle dung increased to 405–1 220 per pot on day 90. However, there were almost no juveniles in the pots with herb waste, soybean straw, and tea residue. Furthermore, no cocoons were observed in the pots with herb waste and tea residues, whereas in the pots with soybean straw, up to 80 cocoons were found on day 90. Total biomass and adult biomass were affected by the type of organic residue (Fig. 1). In the pots with herb waste and tea residues, total biomass and adult biomass decreased with time, whereas in the pots with rice straw, garden waste, soybean straw, and cattle dung, total biomass increased and then decreased. Juvenile biomass increased with time in the pots with rice straw, garden waste, and cattle dung, but typically low in the pots with other organic residues (Fig. 1).

Changes in physicochemical properties of organic residues during vermicomposting

Organic residues decomposed over 90 d, resulting in a decrease in TOC content with time in all types of agricultural organic residue (Fig. 2). Concentrations of TN, TP, and TK showed an increasing trend (Fig. 2). As TOC decreased and TN was relatively stable, the C/N ratio of organic residues produced a decreasing trend with time (Fig. 2). On day 90, the C/N ratio of soybean straw declined from 36.53 to 19.12 and that of tea residues from 8.43 to 7.50; however, NO_3^--N and NH_4^+-N concentrations increased by 1.3- to 12-fold (Fig. 2). The NO_3^--N concentration increased by 119%–1 163% in all organic residues. The NH_4^+-N concentrations was the

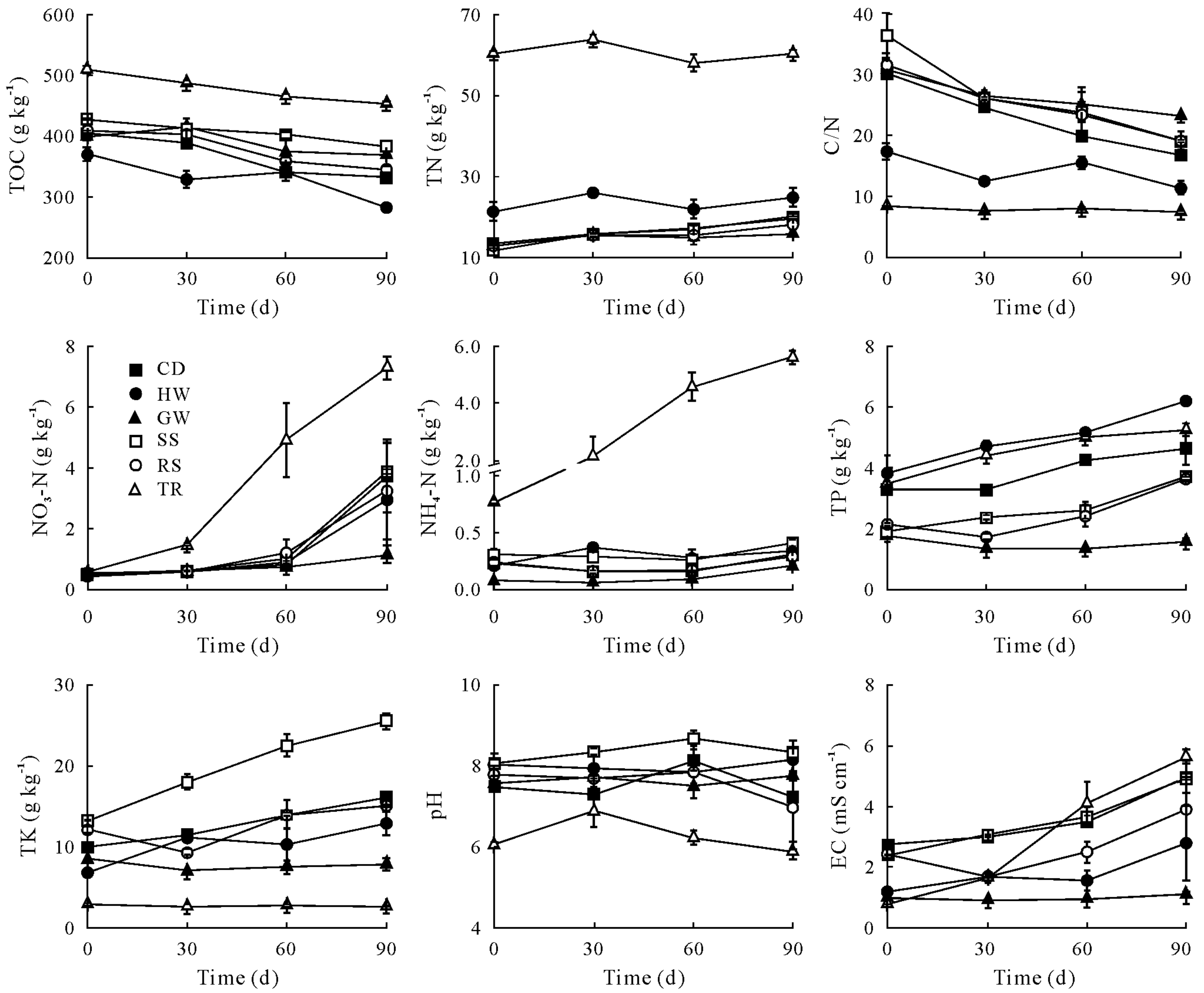

Fig. 2 Changes in physicochemical properties, including total organic C (TOC), total N (TN), C/N ratio, NO_3^--N, NH_4^+-N, total P (TP), total K (TK), pH, and electrical conductivity (EC), of six types of agricultural organic residues over time during vermicomposting. Vertical bars represent standard errors of the means ($n = 3$). CD = cattle dung; HW = herb waste; GW = garden waste; SS = soybean straw; RS = rice straw; TR = tea residues.

highest (an increase by 733%) in tea residues on day 90, with lower increase rates in the other organic residues (Fig. 2). The pH remained stable over time, whereas EC showed an increasing trend (Fig. 2).

Correlations between earthworm biological traits and physicochemical properties of organic residues

Abundance and biomass of adults was negatively correlated with the concentrations of TN ($R_{\text{abundance}} = -0.65$**; $R_{\text{biomass}} = -0.56$**), NO_3^--N ($R_{\text{abundance}} = -0.42$**; $R_{\text{biomass}} = -0.49$**), NH_4^+-N ($R_{\text{abundance}} = -0.53$**; $R_{\text{biomass}} = -0.44$**), and TP ($R_{\text{abundance}} = -0.71$**; $R_{\text{biomass}} = -0.62$**) of organic residues and was positively correlated with C/N ratio ($R_{\text{abundance}} = 0.79$**; $R_{\text{biomass}} = 0.77$**) (Table I). Abundance and biomass of juveniles was negatively correlated with TOC ($R_{\text{abundance}} = -0.34$*; $R_{\text{biomass}} = -0.34$*), TN ($R_{\text{abundance}} = -0.35$**; $R_{\text{biomass}} = -0.34$*), and NH_4^+-N ($R_{\text{abundance}} = -0.28$*; $R_{\text{biomass}} = -0.27$*) concentrations and was positively correlated with C/N ratio ($R_{\text{abundance}} = 0.30$*; $R_{\text{biomass}} = 0.27$*) (Table I). Abundance of cocoons was negatively correlated with TN ($R = -0.60$**), NO_3^--N ($R = -0.37$**), NH_4^+-N ($R = -0.50$**), and TP ($R = -0.51$**) concentrations and was positively correlated with C/N ratio ($R = 0.67$**) and TK concentration ($R = 0.31$*).

Stepwise regression analysis of earthworm biological traits and physicochemical properties of organic residues

Stepwise regression analysis revealed that abundance and biomass of adults were mainly influenced by C/N ratio, TN, NH_4^+-N, and NO_3^--N of organic residues. Total biomass of earthworm showed correlations with C/N ratio, TOC, TN, and pH (Table II). Abundance and biomass of juveniles were affected by TOC, TN, TP, and pH, and abundance of cocoons were most strongly affected by the C/N ratio (Table II).

DISCUSSION

Changes in physicochemical properties of organic residues during vermicomposting affected earthworm biomass and population structure. Abundance of adults decreased with time in all treatments; however, the rate of decline in earthworm abundance varied among the organic residuals. The differences were due to the nature of the respective residues and the degree of change in the material properties. The abundance of adults in tea residues and herb waste decreased to 0 on days 60 and 90, suggesting that changes in certain components of these two types of residues can affect survival of earthworms. In cow dung, garden waste, soybean straw, and rice straw, adult earthworms showed normal survival at early stage; however, abundance of adults declined to varying degrees with time, indicating that changes in some of the physicochemical properties of organic residues affected adult survival. Results of the correlation and stepwise regression analyses revealed that C/N, NH_4^+-N, and NO_3^--N were the predominant factors affecting abundance of adults.

Nitrogen concentration can affect reproduction of earthworms. Almost no juveniles were produced in the herb waste, soybean straw, and tea residue treatments. Furthermore, no cocoons were observed in herb waste and tea residues, whereas a large number of cocoons were found in soybean

TABLE I

Correlation coefficients between earthworm biological traits and physicochemical properties[a)] of organic residues ($n = 54$)

Biological trait	TOC	TN	C/N	NO_3^--N	NH_4^+-N	TK	TP	pH	EC
Abundance of adults	NS[b)]	−0.65**	0.79**	−0.42**	−0.53**	NS	−0.71**	NS	NS
Abundance of juveniles	−0.34*	−0.35**	0.30*	NS	−0.28*	NS	−0.33*	NS	NS
Abundance of cocoons	NS	−0.60**	0.67**	−0.37**	−0.50**	0.31*	−0.51**	NS	NS
Total biomass	NS	−0.62**	0.75**	−0.41**	−0.49**	NS	−0.62**	NS	NS
Adult biomass	NS	−0.56**	0.77**	−0.49**	−0.44**	NS	−0.62**	NS	NS
Juvenile biomass	−0.34*	−0.34*	0.27*	NS	−0.27*	NS	NS	NS	NS

*, **Significant at $P < 0.05$ and $P < 0.01$, respectively.
[a)]TOC = total organic C; TN = total N; TK = total K; TP = total P; EC = electrical conductivity.
[b)]Not significant.

TABLE II

Stepwise regression results of earthworm biological traits and physicochemical properties[a)] of organic residues

Biological trait	Regression equation	F value	P value
Abundance of adults	$Y = 1.7\text{C/N} - 3.3\text{NH}_4^+\text{-N} - 10.8\text{pH} + 68.79$	58.20	< 0.000 1
Abundance of juveniles	$Y = -16.4\text{TN} - 249.5\text{pH} + 2\,560.99$	8.50	0.000 6
Abundance of cocoons	$Y = 14.2\text{C/N} + 20.0\text{EC} - 176.2$	25.53	< 0.000 1
Total biomass	$Y = -0.2\text{TOC} + 0.8\text{TN} + 2.5\text{C/N} - 3.7\text{pH} + 31.3$	28.68	< 0.000 1
Adult biomass	$Y = 0.2\text{TN} + 1.8\text{C/N} - 0.9\text{NO}_3^-\text{-N} + 3.1\text{TP} - 3.5\text{pH} - 13.8$	30.04	< 0.000 1
Juvenile biomass	$Y = -0.1\text{TOC} - 1.5\text{TP} - 2.4\text{pH} + 47.9$	7.73	0.000 2

[a)]TN = total N; EC = electrical conductivity; TOC = total organic C; TP = total P.

straw. These findings suggest that certain properties of herb waste and tea residues inhibit cocoon production, whereas soybean straw can promote cocoon production but appears to hamper cocoon hatching. Stepwise regression analysis revealed that TOC showed a strong effect on abundance of juveniles, whereas abundance of cocoons was mainly affected by TN, C/N ratio, and NH_4^+-N. Considering the composition analysis results for soybean straw, the findings indicate that NH_4^+-N may affect hatching of cocoons.

Nitrogen concentration can affect biomass and survival of earthworms. Experiments by Curry *et al*. (2008) showed that earthworm populations are enhanced by legumes with a high N content. Yang and Chen (2009) found that earthworms prefer palatable sources of food and therefore select soils under plant species with high N concentrations. García and Fragoso (2003) reported that high N levels are necessary for sexual maturation of earthworm *Pontoscolex corethrurus*; however, Piotrowska *et al*. (2013) found that the relationship between earthworm abundance and type of plant species is significantly affected by the amount of added N. Larger amounts of N input reduced earthworm abundance and biomass when maintained with white clover *Trifolium repens*. In the present study, low N concentrations were found to be beneficial to the growth and reproduction of earthworms, whereas increases in N concentrations (*i.e*., TN, NH_4^+-N, and NO_3^--N) reduced earthworm abundance and biomass. Some properties of soybean straw may affect survival of adults and inhibit cocoon incubation, as the pots with soybean straw produced low earthworm biomass and abundance; however, average individual adult and juvenile weights were higher in the pots with soybean straw than those with other organic residuals. These findings suggest that soybean straw is conducive to increasing earthworm biomass. Considering the results of correlation and stepwise regression analyses, it can be speculated that a suitable C/N ratio promotes earthworm biomass, whereas NH_4^+-N appears to have an adverse effect on earthworm biomass. Previous studies on vermicomposting of biosolids showed that a suitable C/N ratio is required for optimal earthworm digestion and that earthworm biomass increased with increasing C/N ratios within a range of 10 to 25, with a C/N ratio of 25 being most favorable for growth (Ndegwa and Thompson, 2000). Some studies showed that a low C/N ratio in dairy manure is important for supporting a large earthworm population (Hurisso *et al*., 2011; Frouz *et al*., 2013), which partially explains the results of the present study.

CONCLUSIONS

Decomposition of agricultural organic residues was accompanied by a decline in TOC content and increases in NH_4^+-N and NO_3^--N concentrations, which negatively affected growth and reproduction of *E. fetida*, ultimately reducing vermicomposting efficiency and earthworm survival. We recommend that NH_4^+-N and NO_3^--N concentrations in the vermicomposting system should be monitored regularly. Once high levels of NH_4^+-N and NO_3^--N have accumulated, earthworms should be harvested and the vermicompost is ready to be used as fertilizer. For sustaining the earthworm population for over 30 d, adding TOC to a continuously feeding vermicomposting system with cow dung or rice straw is recommended to prevent NH_4^+-N and NO_3^--N accumulation. Since dynamic changes in agricultural organic residues directly affect earthworms and vermicomposting efficiency, we conclude that not all organic residues are suitable for vermicomposting or a prolonged pre-decomposition period may be required before earthworms can be added.

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