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Abstract

Soil-exchangeable aluminum (Al) has toxic effects on living organisms in acidic soils. Earthworm presence and activity can alter soil pH, which has a significant influence on Al toxicity. However, the effects of earthworms on soil Al toxicity and fractions are still largely unknown. This laboratory study focused on the effects of three earthworm species (endogeics Pontoscolex corethrurus and Amynthas robustus, anecis Amynthas aspergillum) on soil acidification, Al fraction distribution, and base cation release. Three native earthworm species and a soil (latosolic red soil) collected from a botanical garden in South China were incubated under laboratory conditions. After 40 days of incubation, six Al fractions in soil, namely exchangeable (Al_{Ex}), weakly organically bound (Al_{Orw}), organically bound (Al_{Or}), amorphous (Al_{Amo}), Al occluded in crystalline iron oxides (Al_{Oxi}), and amorphous aluminosilicate and gibbsite (Al_{Aag}) fractions, were extracted using a sequential procedure. Soil pH; organic carbon; total nitrogen; total Al (Al_{Total}); exchangeable K, Na, Ca, Mg contents; and CEC were determined as well. Compared to control soil, pH values increased by 0.79, 0.41, and 0.57 units in casts in the presence of P. corethrurus, A. robustus, and A. aspergillum, and 0.70, 0.32, and 0.50 units in non-ingested soil, respectively. Compared to control soil, the 61.7%, 30.7%, and 36.1% of Al_{Ex} contents in casts and 68.5%, 25.9%, and 39.0% of Al_{Ex} in non-ingested soil significantly decreased with the addition of P. corethrurus, A. robustus, and A. aspergillum, respectively. Moreover, compared to control soil, the 78.7%, 37.7%, and 40.1% of exchangeable Ca^{2+} and 12.3%, 24.7%, and 26.8% of exchangeable Mg^{2+} contents in casts significantly increased with the presence of P. corethrurus, A. robustus, and A. aspergillum, respectively. Soil treated with P. corethrurus had higher soil pH values, exchangeable Ca^{2+} contents, and lower Al_{Ex} than those with A. robustus and A. aspergillum. Results of principal component analyses showed that P. corethrurus, A. robustus, and A. aspergillum casts and non-ingested soil differ for soil pH, Al fractions, and exchangeable base cations release. These results indicate that earthworms, especially P. corethrurus, can reduce soil Al toxicity, increase soil pH, and affect the release of exchangeable base cations.

Highlights

• P. corethrurus decreased 61.7% exchangeable Al content significantly in cast compared to control soil.

• P. corethrurus was more effective to reduce soil acidification than A. robustus and A. aspergillum.

• Casts had higher exchangeable Ca^{2+}, Mg^{2+}, K^{+}, CEC, TN, and SOC than non-ingested soil for the three earthworms.

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Keywords Earthworms · Aluminum fractions · Soil pH · Exchangeable base cations · Latosolic red soil

Introduction

Soil acidification is a global ecological and environmental issue that has however received little attention until now (Guo et al. 2010). It has naturally occurred across tropical and subtropical regions (Hodson and Donner 2013) and is strongly influenced by anthropogenic atmospheric acid depo-sition (Qiao et al. 2015; Zhao et al. 2009). It is well known that acid soils are commonly associated with Al toxicity, which is terribly deleterious to various soil organisms. Kunito et al. (2016) reported that high levels of exchangeable Al can be expected to increase stresses on organisms via Al toxicity; Zhang et al. (2013) found that the critical value of Al in soil for earthworm Eisenia fetida was 50 mg kg^{-1}; Gestel and Hoogerwerf (2001) reported that $AlCl_3$ was more toxic to earthworm Eisenia Andrei compared to Al_2O_3 and $Al_2(SO_4)_3$, with LC_{50} value of 316 mg kg^{-1} dry artificial soil at a pH_{KCl} of 3.5.

Al fractions determine its toxicity besides its total concen-tration (Kubová et al. 2005; Ščančar and Milačič 2006) and are also closely related to soil pH (Xu 2012). When soil pH is below 5.0, exchangeable Al mainly includes Al^{3+}, $AlOH^{2+}$, $Al(OH)_2{}^{+}$, $Al(OH)_3$, and $Al(OH)_4$ species Al^{3+} being the most toxic (Baquy et al. 2018). Most of Al in soil is bound to organic matter in the form of polymers and monomers that are of low toxicity (Buodot et al. 1994). In general, Al can be divided into several fractions in soils such as Al_{Ex} (ex-changeable), Al_{Orw} (weakly organically bound), Al_{Or} (organ-ically bound), Al_{Amo} (amorphous), Al_{Oxi} (occluded in crystal-line iron oxides), and Al_{Aag} (occluded in amorphous alumino-silicate and gibbsite) (Larssen et al. 1999). Hagvall et al. (2015) investigated the effects of organic matter on the frac-tions of Al in soils. Evans and Jacobs (2016) evaluated the seasonal changes in Al fractions in soil. However, no infor-mation exists regarding the impacts of soil fauna on Al speci-ation and toxicity recently in tropical soils.

Earthworms are an essential part of soil fauna in most soils (Blouin et al. 2013), and they play an important role in the transformations of soil chemical elements such as Cd, Cu, Pb, and Zn, and so on (Richardson et al. 2018; Zhang et al. 2016). Basker et al. (1994) demonstrated that soil ingestion by earth-worm A. caliginosa and L. rubellus increased exchangeable K content in Raumai soil. Previous studies have addressed the effects of earthworms on soil metal (loid) (Cd, Cu, Zn, and As) mobility, fractions, and availability through soil pH change (Sizmur and Hodson 2009) and the degradation and redistri-bution of organic matter (Tica et al. 2013; Sizmur et al. 2011; Zhang et al. 2009). However, far less attention has been paid to the impact that earthworms have on soil Al in terms of its toxicity and fractions. Endogeic species such as Pontoscolex corethrurus (Müller 1857) and Amynthas robustus (Perrier 1872) and anecics species Amynthas aspergillum (Perrier 1872) are the most common earthworms in south China (Huang et al. 2015; Lin et al. 2012). However, there is no data on how the ecology of these earthworm species affects soil Al fractions and toxicity in subtropical soils.

Therefore, in the present study, the objectives were to in-vestigate the effects of different earthworm species such as Pontoscolex corethrurus, Amynthas robustus, and Amynthas aspergillum on soil acidification, Al speciation and exchange-able base cation release and also evaluate the impacts of earth-worm presence and activity on reducing soil acidity and changing Al fractions.

Materials and methods

Soil and biological preparation

Natural uncontaminated soil was collected from surface horizon (0–10 cm) at a botanical garden in Guangdong province, South China (23°9′33″N and 113°21′22″E). It was air-dried and then sieved at 3 mm. pH value, fine texture, and contents of organic carbon and total nitrogen were determined in soil (Table 1).

All earthworms (Pontoscolex corethrurus, Amynthas robustus, and Amynthas aspergillum) were hand-sorted in the field from the same location. Mature individuals with de-veloped clitellum were selected to apply in the laboratory tests. To ensure the soil contained a large proportion of earth-worm casts, a relatively large number of earthworms were added to a comparatively small volume of soil and a short treatment time was selected (Zhang et al. 2000; Zhang et al. 2016). The average fresh weights of P. corethrurus were 0.29 g fresh weight (ranging from 0.27 to 0.31 g ind^{-1}), and 41 individuals were added to 0.5 kg soil. A. robustus were 2.91 g fresh weight on average (ranging from 2.10 to 3.17 g ind^{-1}), and 4 worms were added to 0.5 kg soil. A. aspergillum were 2.34 g fresh weight (ranging from 1.10 to 4.07 g ind^{-1}) on average, and 6 individuals were added to 0.5 kg soil.

Experimental design

Half a kilogram of soil was placed in 1.5 L plastic plots (9 cm × 12 cm × 13 cm) allocated to four different treatments:

1. CS: control soil without earthworms
2. PS: soil inoculated with 12.0 ± 0.0 g fresh weight of P. corethrurus
3. RS: soil inoculated with 12.5 ± 0.1 g fresh weight of A. robustus

Table.1 Main physicochemical characteristics and Al contents of soil samples (mean ± S.E., n = 3)

	pH	Organic C	Total N	C: N ratio	Clay content (< 0.002 mm)	Cation exchange capacity (CEC)	Exchangeable acid quantum	Exchangeable hydrogen	Exchangeable Al	Total Al
	(H_2O)	$g \cdot kg^{-1}$	$g \cdot kg^{-1}$	–	(%)	cmol (+)·kg^{-1}	cmol (+)·kg^{-1}	cmol (+)·kg^{-1}	cmol (+)·kg^{-1}	$g \cdot kg^{-1}$
Soil	4.25 ± 0.02	27.0 ± 0.7	1.85 ± 0.05	14.6 ± 0.2	22.0 ± 0.5	12.3 ± 1.8	5.52 ± 1.03	0.77 ± 0.12	4.75 ± 0.82	68.2 ± 5.5

4. AS: soil inoculated with 12.1 ± 0.0 g fresh weight of A. aspergillum

Each treatment with five replicates was incubated for 40 days in the laboratory at a temperature of 25 °C and moisture was adjusted by weight at field capacity every 2 days. Organic matter was not added during the whole culture period so as not to influence soil properties. In the medium term of the incubation, surface casts were collected. After 40 days of incubation, both internal and surface casts were collected according to the method of Zhang et al. (2016). After being dried in an air-circulating room, surface casts and internal casts were mixed together and used as the casts in this study. The casts and non-ingested soil were weighed to estimate total cast pro-duction (Wen et al. 2006).

Analytical determinations

Earthworm responses

Surviving earthworms were collected, counted, and weighed at the beginning, after 20 days and at the end of the culture period. Ingestion rate (I in g dry soil ingested daily per g fresh weight earthworm biomass) was calculated as follows:

$$I = \frac{(C/d)}{[(W_0 + W_{40})/2]} \times 100\%$$

where C is the total cast production (surface cast + internal cast) in g dry weight, d is culture duration in days, and W_0 and W_{40} is the biomass of earthworms at the beginning and end of the culture period (Zhang et al. 2016).

Soil and casts analyses

Soil pH value was determined in water suspension [1: 2.5 (w/v) soil/distilled water ratio] by a Sartorius PB-10 pH meter. Exchangeable acid quantum and hydrogen were measured according to KCl exchange-neutralization titration method (SSIR 2004). Cation exchange capacity (CEC) was determined by the method of ammonium acetate saturation (CH_3COONH_4, 1 mol L^{-1}, pH 7.0). Soil was digested by hot HF, HNO_3, $HClO_4$, and H_2O_2 mixture and analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES) (Varian 710-ES) in order to determine total Al concentration (Kubová et al. 2005). The optimized six-step sequential extraction scheme was applied according to the method of Larssen et al. (1999), and Al concentrations in the extracts were also determined by ICP-OES (Varian 710-ES) (Table 2). Soil-exchangeable Ca, Mg, K, and Na were extract-ed with 1 mol L^{-1} CH_3COONH_4 (pH 7.0) and then measured by atomic absorption spectrometry as previously described (Moro et al. 2014). All subsequent data were expressed on dry weight bases.

Analysis of earthworms

Earthworms were placed in Petri dishes with one filter paper and a few drops of distilled water to keep them moist. They were kept at 25 °C for 7 days, and filter papers were changed daily to allow complete evacuation of the gut contents. Earthworms were killed in liquid nitrogen and then oven-dried at 100 °C for 24 h. Dry earthworms were crushed (< 0.2 mm) and digested as follows: 0.2 g of sample was mixed with 8 ml concentrated HNO_3 and 2 ml concentrated $HClO_4$ for 12 h, heated progressively up to 250 °C with electric heating plate digestion for 2 h. After cooling at ambient tem-perature, the solution was filled up to 50 ml with ultra-pure distilled water (Dai et al. 2004). Metal contents in earthworms were determined by ICP-OES (Varian 710-ES). Biota-to-soil accumulation factors (BSAF) of the metals in the earthworms were calculated by the following formula: BSAF = metal con-tent in earthworm / total metal content in soil (Cortet et al. 1999).

Statistical analysis

All data were analyzed using the software SPSS version 23.0 (SPSS Inc., Chicago, IL) and R version 3.0.2 (ade 4 library) (R Development Core Team 2007; Thioulouse et al. 1997), re-spectively. Differences between the means were evaluated using a one-way ANOVA which was tested by Duncan's test. $p < 0.05$ was considered as statistically significant. Results were expressed as mean ± standard error. Principal component analysis (PCA) was performed using the software R (ade 4 library), coupled with a Monte Carlo permutation test that allowed to test for multivariate differences between treatments.

Table 2 Experimental conditions in the optimized six-step sequential extraction scheme

Step	Abbreviation	Fraction	Chemical reagents, analytical conditions, and references
1	Al_{Ex}	Exchangeable	0.1 mol· L^{-1} $BaCl_2$ (w/v = 1/10) for 2 h, extract twice, rinsing by redistilled H_2O
2	Al_{Orw}	Weakly organically bound[a]	0.5 mol L^{-1} $CuCl_2$ (w/v = 1/10) for 2 h, extract twice, rinsing by redistilled H_2O
3	Al_{Or}	Organically bound	0.1 mol L^{-1} $Na_4P_2O_7$ (pH 10) (w/v = 1/40) for 16 h, extract twice, rinsing by 1 mol L^{-1} Na_2SO_4
4	Al_{Amo}	Amorphous	NH_4 oxalate (0.2 mol L^{-1} at pH 3) (w/v = 1/40) for 4 h in dark, rinsing by redistilled H_2O
5	Al_{Oxi}	Occluded in crystalline iron oxides	Citrate dithionite for 15 min, 80 °C (w/v = 1/40), rinsing by 1 mol L^{-1} NaCl
6	Al_{Aag}	Amorphous aluminosilicate and gibbsite	Boiling 0.1 mol·L^{-1} NaOH for 2.5 min (w/v = 1/500).
–	Al_{Min}[b]	Occluded in layered aluminosilicate	Al_{total}−∑Al
–	Al_{Total}	Total Al	decomposed by hot HF, HNO_3, $HClO_4$ and H_2O_2 mixture (Kubová et al. 2005)

aExtraction with 0.5 mol L^{-1} $CuCl_2$ is considered organo-Al complexes of low to medium stability (Álvarez et al. 2012)

bAl is occluded in layered silicates in most of soils and cannot be extracted by above chemical reagents, $Al_{Min} = Al_{Total}$ - ∑Al, ∑Al = Al_{Ex} + Al_{Orw} + Al_{Or} + Al_{Amo} + Al_{Oxi} + Al_{Aag} (Shao et al. 1998)

Results

Earthworm response to soil

Earthworm survival percentage, biomass, and weight loss rate for the different treatments are shown in Table. 3. At the end of the experiment, P. corethrurus had the lowest survival percentage (79.1%) and biomass (8.89 g) but the highest weight loss rate (25.98%) com-pared to other earthworm species. In contrast to P. corethrurus, A. robustus had the highest survival per-centage (97.1%), biomass (12.5 g), and lowest weight l o s s r a t e (− 0 . 1 2 %) . T h e w e i g h t l o s s r a t e o f P. corethrurus was significantly higher than that of A. robustus ($p < 0.05$). However, there was no signifi-c a n t d i f f e r e n c e i n w e i g h t l o s s r a t e b e t w e e n P. corethrurus and A. aspergillum. The survival percent-age, biomass, and weight loss rate of A. aspergillum w e r e a l l b e t w e e n t h o s e o f P. c o r e t h r u r u s a n d A. robustus, respectively.

Surface cast production of P. corethrurus (67.9 g kg^{-1}) and A. aspergillum (53.4 g kg^{-1}) were significantly higher than that of A. robustus (67.0 g kg^{-1}) ($p < 0.05$, Fig. Error! Reference source not found.a). Inter cast productions of the three earthworms were significantly different and ranked as P. corethrurus > A. robustus > A. aspergillum, with the mean values of 296.0, 224.0, and 61.4 g·kg^{-1}, respectively ($p < 0.05$, Fig. 1b). The amounts of the inter cast of P. corethrurus and A. robustus were 4.4- and 4.2-fold higher than the surface cast ($p < 0.05$), respectively. Generally, P. corethrurus and A. robustus produced significantly greater amounts of total casts than A. aspergillum ($p < 0.05$, Fig. 1c). Soil ingestion rates were 1.99- and 0.94-fold higher, respec-tively, for P. corethrurus and A. robustus ($p < 0.05$) than for A. aspergillum (Fig. 1d). Bioaccumulation values of Al in earthworms assessed by BSAF were ranked as A. robustus > P. corethrurus > A. aspergillum, with the mean values of 0.087, 0.158, and 0.002, respectively ($p < 0.05$). BSAFs were 42.3- and 77.8-fold higher, respectively, for P. corethrurus and A. robustus ($p < 0.05$) than A. aspergillum (Fig. 1e).

Table 3 Variations of survival percentage, biomass, and weight loss rate of different earthworm species during the incubation period (mean ± S.E., n = 5)

Treatments	Incubation days						
	0 d		20 d		40 d		
	Survival rate (%)	Biomass (g)	Survival rate (%)	Biomass (g)	Survival rate (%)	Biomass (g)	Weight loss rate (%)
PS	100 ± 0a	12.0 ± 0.0a	87.5 ± 5.0b	11.5 ± 0.6a	79.1 ± 2.2b	8.89 ± 0.5b	26.0 ± 4.5A
RS	100 ± 0a	12.5 ± 0.1b	97.1 ± 2.9a	14.9 ± 0.5a	97.1 ± 2.9a	12.5 ± 0.3b	− 0.12 ± 2.8B
AS	100 ± 0a	12.1 ± 0.0ab	97.8 ± 2.2a	13.4 ± 0.7a	85.8 ± 4.2b	10.5 ± 0.8b	13.4 ± 6.3AB

The different lowercase letters relate to the different levels of Duncan in one-way ANOVA analysis at different incubation days (α = 5%). The different capital letters relate to the different levels of Duncan in One-way ANOVA analysis of treatments in weight loss rate as follows (α = 5%)

PS soil + P. corethrurus, RS soil + A. robustus, AS soil + A. aspergillum

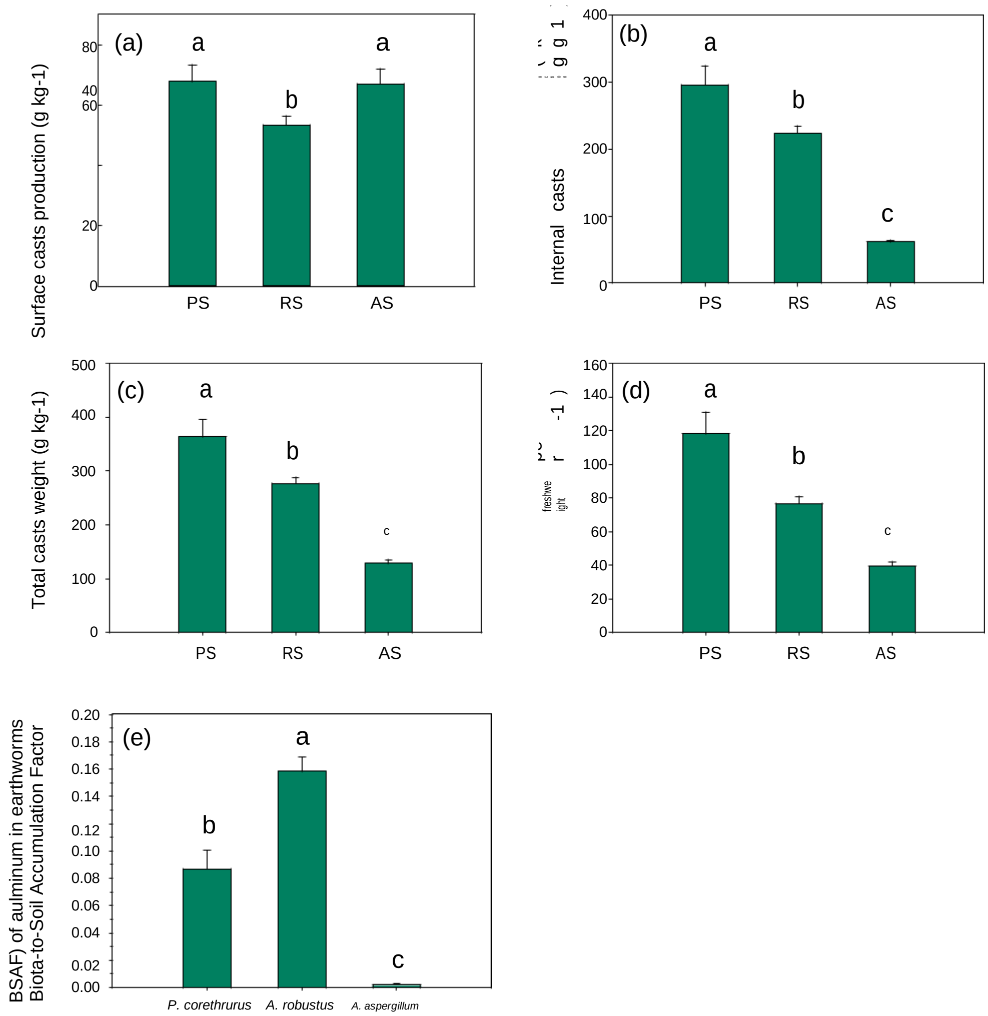


Fig. 1 Earthworm cast production, ingestion rate, and BSAF in different treatments. PS: soil + P. corethrurus; RS: soil + A. robustus; AS: soil + A. aspergillum. The different lowercase letters relate to the different levels of Duncan in one-way ANOVA analysis of treat-ments ($n = 5$, $\alpha = 5\%$)

Variations of soil pH in treatments

The addition of the three species of earthworm significantly increased pH values in casts and non-ingested soil ($p < 0.05$, Fig. 2). Compared to control soil, P. corethrurus, A. robustus, and A. aspergillum increased pH values by 0.79, 0.41, and 0.57 units respectively in casts. Similarly, pH values in non-ingested soil were 0.70, 0.32, and 0.50 higher, respectively, with P. corethrurus, A. robustus, and A. aspergillum than for the control soil. Moreover, all earthworm casts had significantly higher pH values than the corresponding non-ingested soil (P. corethrurus: $p < 0.05$; A. robustus: $p < 0.05$; A. aspergillum: $p < 0.01$, Fig. 2), respectively.

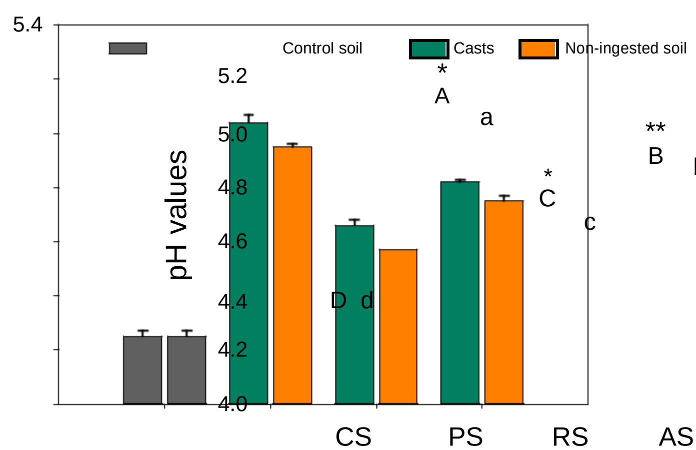


Fig. 2 Soil pH affected by earthworm in different treatments (CS: control soil; PS: soil + P. corethrurus; RS: soil + A. robustus; AS: soil + A. aspergillum). The different capital letters relate to the different levels of Duncan in one-way ANOVA analysis of treatments in casts group as follows ($\alpha = 5\%$). The different lowercase letters relate to the different levels of Duncan in one-way ANOVA analysis of treatments in non-ingested soil group as follows ($n = 5$, $\alpha = 5\%$). Significant differences between casts and the non-ingested soil group of T test are note by ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$; ns $p > 0.05$

Variation of Al fractions and distribution in treatments

Al fractions assessed by chemical extractions varied significantly among treatments in casts as well as non-ingested soil (Table 4). Compared to control, 61.7% (130 mg kg^{-1}), 30.7% (64.0 mg kg^{-1}), and 36.1%

(76.0 mg kg^{-1}) decreases were observed in Al_{Ex} contents in the casts of PS, RS, and AS treatments, respectively, and 68.5% (144 mg kg^{-1}), 25.9% (54.0 mg kg^{-1}), and 39.0% (82.0 mg kg^{-1}) decreases of that found in non-ingested soil as well. In the meantime, differences of Al_{Ex} contents between casts and non-ingested soil were earthworm species dependent and ranked as follow: Al_{Ex} contents in non-ingested soil with A. robustus > A. robustus casts > P. corethrurus casts > non-ingested soil with P. corethrurus ($p < 0.05$); however, no significant difference was found between A. aspergillum casts and non-ingested soil ($p > 0.05$). In the presence of earthworms, Al_{Orw} fractions in casts and non-ingested soil varied notably. Compared to control soil, Al_{Orw} contents in non-ingested soil significantly decreased by 21.4%, 7.64%, and 6.18% due to the activities of P. corethrurus, A. robustus, and A. aspergillum, respectively. Higher Al_{Orw} contents were found in casts compared to non-ingested soil (P. corethrurus: $p < 0.05$; A. robustus: $p < 0.01$; A. aspergillum: $p < 0.01$, Table 4). Earthworm addition could increase Al_{Or} contents not only in casts but also in non-ingested soil. In comparison to control, Al_{Or} contents in casts increased by 0.74-, 0.78-, and 0.48-fold, respectively, in casts of P. corethrurus and A. robustus and A. aspergillum ($p < 0.05$). Al_{Or} contents in non-ingested soil affected by A. robustus and A. aspergillum were significantly higher than control soil ($p < 0.05$). Compared with non-ingested soil, the ingestion by P. corethrurus and A. robustus significantly increased the Al_{Or} in casts (P. corethrurus: $p < 0.01$; A. robustus: $p < 0.05$, respectively, Table 4). Al_{Amo} fractions in casts and non-ingested soil were influenced by the activities of the different earthworm species. Compared to control soil, Al_{Amo} contents in casts generally increased significantly ($p < 0.05$). However Al_{Amo} contents in non-ingested soil significantly decreased by 30.2%, 15.2%, and 20.6%, for P. corethrurus and A. robustus and A. aspergillum ($p < 0.05$), respectively. In addition, Al_{Amo} contents in casts were significantly higher than for non-ingested soil (P. corethrurus: $p < 0.01$; A. robustus: $p < 0.05$; A. aspergillum: $p < 0.01$, Table 4). Thus, earthworm addition could decrease Al_{Oxi} contents. Al_{Oxi} contents were significantly lower in casts with addition of P. corethrurus and A. robustus than for control soil ($p < 0.05$). Al_{Oxi} contents were 44.0%, 7.46%, and 20.0% lower in non-ingested soil treated with P. corethrurus, A. robustus, and A. aspergillum, respectively, than control. It was also noted that Al_{Oxi} contents of the other two earthworms, and in general, Al_{Oxi} contents in casts, were higher than that in non-ingested soil ($p < 0.001$). The presence of earthworms decreased Al_{Aag} contents, especially in A. aspergillum and P. corethrurus casts and non-ingested soil. Earthworm activities increased Al_{Min} contents, especially in A. aspergillum casts and non-ingested soil and in P. corethrurus non-ingested soil ($p < 0.05$). There was no significant difference between Al_{Aag} contents of casts and non-ingested soil for all earthworms, so as for Al_{Min} (Table 4).

Average proportions of seven fractions in total contents varied notably among treatments (Fig. 3a, b). Al_{Ex} fractions in casts and non-ingested soil were 0.10–0.23%, which were lower than control (0.31%), especially for P. corethrurus, indicating that three earthworm activities could decrease the exchangeable Al contents. There were 1.52-–1.78-fold increases of Al_{Or} contents in three casts compared to control. The non-ingested soil of A. aspergillum had the highest Al_{Or} contents (785.21 mg kg^{-1}) of the three earthworms.

Table 4 Al fractions and contents in soil (casts and non-ingested soils) (mean ± S.E., n = 5)

Treatments		Aluminum fractions						
		Al_{Ex}	Al_{Orw}	Al_{Or}	Al_{Amo}	Al_{Oxi}	Al_{Aag}	Al_{Min}
		(Al mg·kg^{-1})			(Al g·kg^{-1})			
Casts	PS	80.5 ± 6.7C*	271 ± 16A*	627 ± 43A**	1.32 ± 0.07A**	0.69 ± 0.01AB***	10.9 ± 0.5B ns	54.4 ± 0.5AB ns
	RS	146 ± 3B	267 ± 12A**	634 ± 65A*	1.38 ± 0.08A*	0.67 ± 0.02B ns	11.2 ± 1.4AB ns	54.0 ± 1.4AB ns
	AS	134 ± 7B ns	290 ± 2A**	567 ± 73A	1.47 ± 0.04A**	0.61 ± 0.01C ns	9.56 ± 0.79B ns	55.6 ± 0.8A ns
Non-ingested soil	PS	66.2 ± 3.2d	198 ± 3c	275 ± 22bc	0.90 ± 0.03c	0.50 ± 0.02c	10.2 ± 0.3b	56.1 ± 0.3a
	RS	156 ± 3b*	232 ± 8b	350 ± 17b	1.12 ± 0.03b	0.67 ± 0.01a	12.6 ± 0.6a	53.2 ± 0.6b
	AS	128 ± 14c	236 ± 7b	785 ± 66a ns	1.07 ± 0.04b	0.60 ± 0.02b	9.46 ± 0.41b	56.0 ± 0.4a
Control	CS	210 ± 2Aa	251 ± 17Aa	228 ± 16Bc	1.29 ± 0.03Aa	0.72 ± 0.02Aa	14.0 ± 1.0Aa	51.6 ± 2.0Bb

The content of total Al in soil is 68.2 ± 5.5 g kg^{-1} . The different capital letters relate to the different levels of Duncan in one-way ANOVA analysis of casts of treatments in cast group as follows (α = 5%). The different lowercase letters relate to the different levels of Duncan in one-way ANOVA analysis of treatments in non-ingested soil group as follows (α = 5%)

CS control soil, PS soil + P. corethrurus, RS soil + A. robustus, AS soil + A. aspergillum, ns p > 0.05

Significant differences between the casts and the non-ingested soil group of T test are noted by ***p < 0.001; **p < 0.01; *p < 0.05

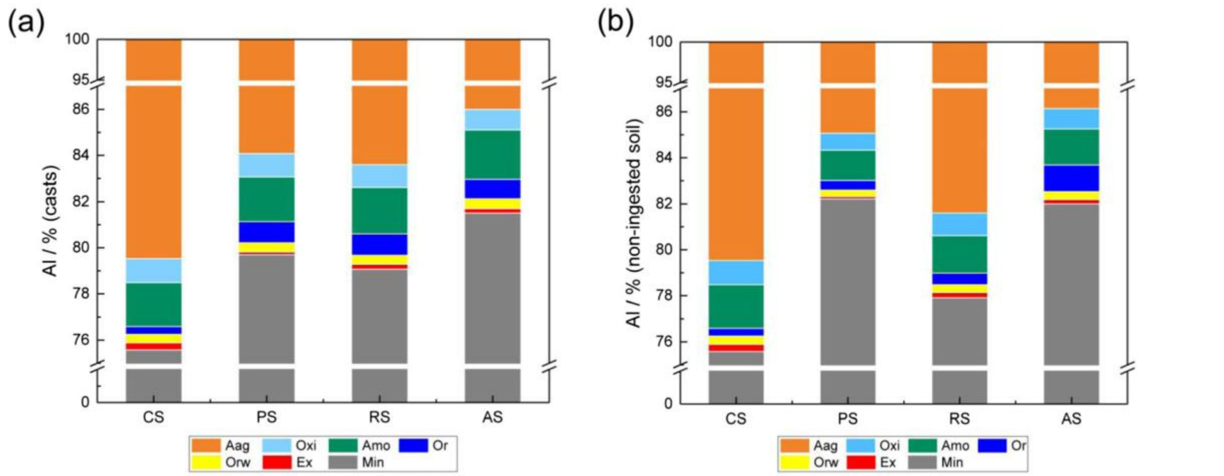


Fig. 3 The percentage of seven fractions of Al in casts (a) and non-ingested soil (b) of three earthworm species determined by sequential extraction (n = 5)

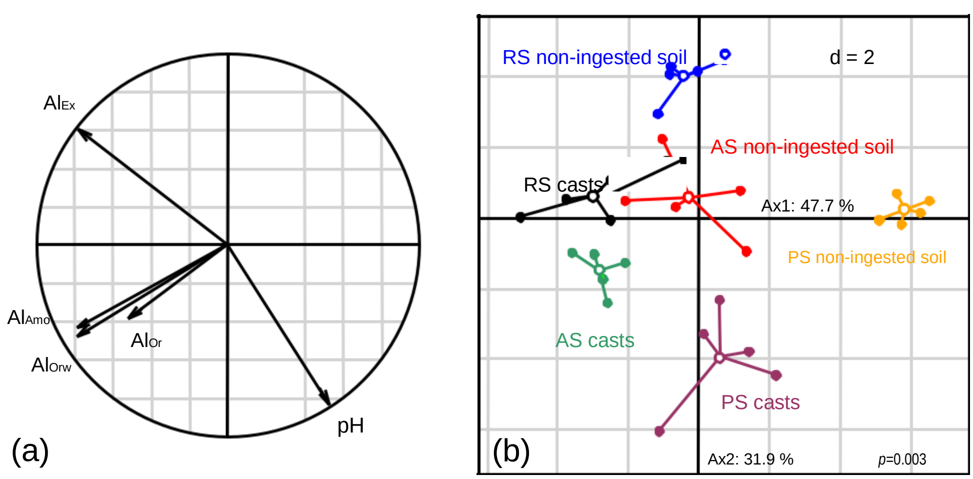
General effects of treatments on Al fractions in casts and non-ingested soils

PCA was performed to explain the relationship among aluminum fractions, and pH values of earthworm casts and non-ingested soil (Fig. 4). Soil Al fractions in casts and non-ingested soils of three earthworms allowed to identify differences of Al fractions among the large set of variables observed (Fig. 4a, b). Experiments with casts could be clearly separated from the non-ingested soils along axis 1 that explains 47.7% of total inertia: casts of P. corethrurus, A. robustus, and A. aspergillum were associated with higher contents of Al_{Orw}, Al_{Or}, and Al_{Amo} in comparison with the non-ingested soil. The second principal component (31.9% of total inertia) indicated differences of earthworm species on pH value and Al_{Ex} and showed that P. corethrurus was more helpful in reducing soil acidification. Results of correlation analysis showed that there was a remarkable correlation respectively between pH and Al_{Ex} in casts ($r = -0.909^{**}$) and non-ingested soil ($r = -0.937^{**}$).

Variations of exchangeable base cations, soil C, N, and C/N ratio

Earthworm addition generally increased the contents of exchangeable base cations. The ingestion of P. corethrurus and A. robustus significantly increased contents of exchangeable K (K_{Ex}), Ca (Ca_{Ex}), and Mg (Mg_{Ex}) in casts compared with control soil and non-ingested soil ($p < 0.01$, Table 5). The exchangeable Na (Na_{Ex}) contents were significantly higher in casts and non-ingested soil with addition of P. corethrurus than that in control soil ($p < 0.05$), and the trend was as follows: Na_{Ex} contents in non-ingested soil > in casts > control ($p < 0.05$). Moreover, Ca_{Ex} and Mg_{Ex} contents were significantly higher in casts with addition of A. aspergillum than that in non-ingested soil and control soil ($p < 0.01$, Table 5). Cation exchange capacity (CEC) was significantly higher in the casts of P. corethrurus and A. aspergillum than in control soil as well as non-ingested soils. The highest CEC was observed in the

Fig. 4 Correlation circles of several Al fractions and pH characteristics in treatments (a). Projection of experimental points according to treatments (b) casts vs. non-ingested soil. n = 5



n=5)

Treatments	K_{Ex}	Na	Ca	Mg	CEC	SOC	TotalN	C/N ratio
	mg·kg^{-1}	$^{-1}$	$^{-1}$	$^{-1}$	cmol (+)·kg^{-1}	g·kg^{-1}	g·kg^{-1}	–
[illegible]							2.±0.01A***41	11. ± 0.4C8
[illegible]							911.±0.06BC ns	814. ± 0.3A ns
[illegible]							042.±0.07B ns	513. ± 0.2B ns
[illegible]							601.±0.07b	213. ± 0.4 bns
[illegible]							1.±0.01a82	14. ± 0.4b0
[illegible]							791.±0.06a	413. ± 0.2b
[illegible]							851.±0.05Ca	614. ± 0.2Aa

analysisofcasts and control,
group and

non-ingested soil with addition of A. robustus among all treatments, while the lowest value was in the non-ingested soil with P. corethrurus.

After the ingestion of earthworms, higher soil organic carbon (SOC) contents and total nitrogen contents were observed in all earthworm casts than those in control soil. Significant differences among earthworm species were shown only in the latter, and the highest TN contents were found in P. corethrurus casts ($p < 0.05$). Concerning the non-ingested soil, lower SOC and TN contents were observed than those in control soil in the presence of earthworm, especially for P. corethrurus and A. aspergillum ($p < 0.05$, Table 5). Earthworm generally decreased C/N ratio in casts and non-ingested soil in comparison with control, and significant dif-ferences could be seen with the addition of P. corethrurus and A. aspergillum ($p < 0.05$).

General effects of earthworms on exchangeable base cations, soil C, N, and C/N ratio in casts and non-ingested soils

PCA was performed to identify general trends of soil Al fractions, exchangeable base cations, soil C and N characteristics of earthworm casts, and non-ingested soils (Fig. 5a, b). The axis 1 (32.1% of total inertia) isolated treatments according to their respective pH, Al_{Or}, Al_{Orw}, C/N ratio, Al_{Ex}, Na_{Ex}, Ca_{Ex}, Al_{Oxi}, and Al_{Aag}, especially for treatments incubated with P. corethrurus and A. aspergillum (Fig. 5). The casts of P. corethrurus, A. robustus, and A. aspergillum were clearly separated from their non-ingested soil of that along axis 2 that explained 26.2% of total inertia: the ingestion activities of all earthworms allows to increase the contents of Ca_{Ex}, K_{Ex}, Mg_{Ex}, CEC, TN, and SOC in casts in comparison with non-ingested soil ($p < 0.01$, Fig. 5). Moreover, Al_{Or} contents in casts showed a significant correlation with SOC contents in casts ($r = 0.693$**). Unlike casts, AlOrw and AlAmo had significant correlation with SOC in non-ingested soil ($r = 0.600$** and 0.782**), respectively. For casts, pH values showed significant correlation between contents of KEx ($r = 0.711$**), CaEx ($r = 0.812$**), and MgEx ($r = -0.537$**), respectively. In addition, pH values also showed significant correlation between NaEx ($r = 0.618$**), CaEx ($r = 0.686$**), and MgEx ($r = -0.822$**) for non-ingested soil, respectively.

Discussion

Earthworm growth and Al accumulation

It is well known that earthworm density and survival are typically low in acidic soils (Chan and Mead 2003). However, in our study, high survival rates and low biomass loss were observed for the three earthworm species. Some previous studies

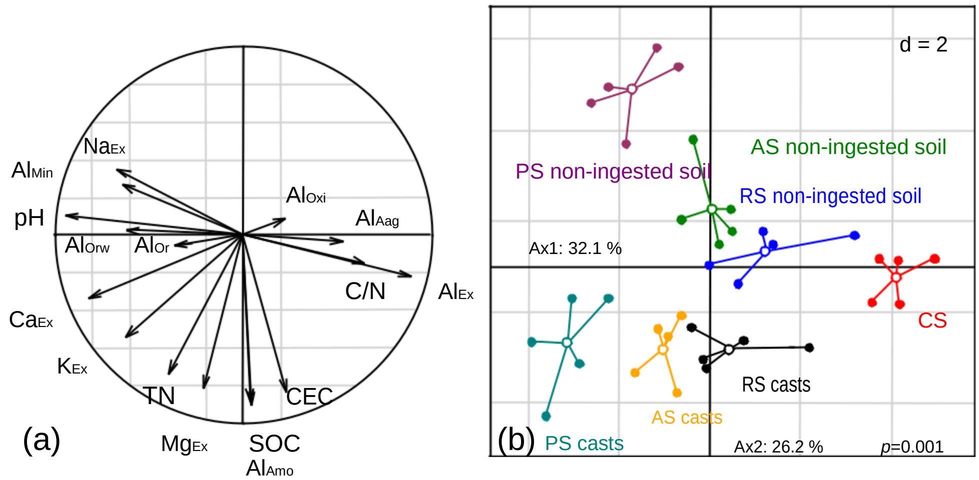


Fig. 5 Projection of soil Al fractions, exchangeable base cations, CEC, pH, SOC, TN, and C/N ratio in casts and non-ingested soils in different treat-ments by PCA. a Correlation cir-cle of variables. b Sore plots in treatment. PS: soil + P. corethrurus; RS: soil + A. robustus; AS: soil + A. aspergillum. n = 5

demonstrated that Amynthas sp. and P. corethrurus have out-standing ability to survive in acidic soils (pH 3.8) (Huang et al. 2015; Shao et al. 2017), and this adaptive advantage may explain the widespread occurrence of these species in south China (Lin et al. 2012).

The cast is the crucial biological aggregate produced by earthworms, which has a positive influence on soil properties (Bossuyt et al. 2005). It was observed that surface soil (0– 20 cm) can be all digested by earthworms in 50 years in a tropical forest (Benckiser 1997). In our laboratory culture ex-periments, endogeic species, especially P. corethrurus, have a higher total cast production than anecic species A. aspergillum (Fig. 1d). Soil ingestion rates of P. corethrurus was similar with that of epi-endogeic species (Amynthas morrisi) in sub-tropical areas (Zhang et al. 2016), about 10-fold lower than comparable measurements made for temperate areas endogeic species (Aporrectodea caliginosa) (Zhang et al. 2009) and approximately 80 times lower than that for tropical savanna endogeic species (Millsonia anomala) (Lavelle and Spain 2001). Thus, the cast production of P. corethrurus is higher than that of A. robustus and A. aspergillum, indicating its greater impact on soil properties which is beneficial for soil remediation.

Total Al contents accumulated in the tissues of P. corethrurus, A. robustus, and A. aspergillum were 5933.9, 10,803.9, and 137.1 mg kg^{-1} (the corresponding BSAF were 0.087, 0.158 and 0.002) in our work, respectively, which sug-gested that Al accumulation in earthworms is species depen-dent. Dai et al. (2004) found that two earthworm species (Aporrectodea caliginosa and Lumbricus rubellus) have dif-ferent patterns of metal (Zn, Cd, and Pb) bioaccumulation in the same contaminated soil. In detail, the behavioral and eco-logical characteristics of different earthworms, namely, the food selectivity and the ability to accumulate excess Al, play an important role in their BSAF (Dai et al. 2004). In addition, Zhang et al. (2013) found that the Al content in Eisenia fetida was about 1500 mg kg^{-1} the acidic soil inoculated with earthworm being collected from the same location as in our work (top 20 cm latosol), which shows that the earthworm species is the major factor that causes different BSAF in earth-worm. A. robustus is a typical endogeics species that derives its nutrition through consumption of large quantities of min-eral soil, which caused the highest BSAF. As anecic species, A. aspergillum, prefers to feed on the mixture of organic com-pound and soil (Lavelle and Spain 2001); therefore, it has lower accumulation of Al than A. robustus. The organic inges-tion amount of P. corethrurus is between A. robustus and A. aspergillum.

Impacts of earthworm on soil acidification and base cation release

In acidic forest soils of south China, the addition of earth-worms significantly increased soil pH from 0.41 to 0.79 units (Fig. 2). The highest increase of pH values in the presence of P. corethrurus confirmed its strong ability to reduce soil acid-ification in subtropical soil. A similar phenomena was showed in some previous studies (Udovic and Lestan 2007; Wen et al. 2006; Yu et al. 2005), which indicated that this may be due to calciferous glands (García-Montero et al. 2013; Karaca 2011) and the cutaneous mucus secretion, including urine, NH_4^+, and exchangeable Ca^{2+}, Mg^{2+}, and K^+ (Salmon 2001; Sizmur and Hodson 2009). In our work, casts of P. corethrurus and A. aspergillum had significantly higher cation exchange capacity (CEC) than their non-ingested soil ($p < 0.05$, Table 5). Especially significantly higher exchange-able K^+, Na^+, and Ca^{2+} contents of the P. corethrurus casts were observed compared to the control soil (Table 5). It is well known that pH has an extremely significantly positive corre-lation with exchangeable Ca^{2+} and Mg^{2+} (Wang et al. 2018). On one hand, the exchangeable base cation release in earth-worm casts in this study may result in the increase of soil pH values (Kunito et al. 2016). On the other hand, soil pH in-crease may be due to the secretion of calcium carbonate from earthworm calciferous glands (Huang et al. 2015), which may have an important role in regulating the acid-base balance (Briones et al. 2008). Exchangeable Ca^{2+} and Mg^{2+} contents and pH values of all earthworm casts were higher than that of the non-ingested soil ($p < 0.05$, Fig. 2; Table 5) in our study, which indicated that the intestine was the main pathway to release Ca^{2+} and Mg^{2+} and intestinal secretions were more effective on soil acidification remediation than skin mucus. Above all, in this work, it was not clear whether soil pH increase affected by earthworms resulted from the Ca^{2+} and Mg^{2+} replacing acid cations on exchange sites or others. A single study may not be sufficient to demonstrate these effects.

Impacts of earthworm on Al fractions

In general, according to PCA analysis (Figs. 4 and 5), earth-worm modified Al fractions in the soils and transferred Al from the exchangeable fraction to the residual fraction and the fraction bound to organic matter and thus reduced the bio-toxicity of Al in our study. This phenomenon may be due to the change of pH and the availability of cations (Sizmur and Hodson 2009) and their ingestion on soil organic matter (Zhang et al. 2016). Considering earthworms were col-lected in the same site as the soil, there would be no net loss of Al after worms were taken away although P. corethrurus, A. robustus, and A. aspergillum can accumulate Al in their bodies. Significant negative relationship between pH and Al_{Ex} in our study (Fig. 4) confirmed the positive effect of soil acidification on the increase of soil-exchangeable Al (Bailey et al. 2005). Exchangeable Al [Al^{3+}, $AlOH^{2+}$, $Al(OH)_2^{+}$, $Al(OH)_3$, and $Al(OH)_4$] contents represent an essential factor for soil potential acid-producing capacity in subtropical soil, which is deleterious to soil organisms (Kunito et al. 2016; Baquy et al. 2018). Lower Al_{Ex} contents in the presence of earthworm confirmed earthworm effects on decreasing soil Al toxicity in subtropical soil. Al_{Orw}, Al_{Or}, and Al_{Amo} are always regarded as potentially reactive Al pool in acidic soil (Jou and Kamprath 1979) and strongly influenced by soil organic mat-ter (Pierart et al. 2018; Shao et al. 1998). It is generally thought that most of the Al bounded to organic matter in the soil is of low toxicity (Buodot et al. 1994). Al extracted by acid ammo-nium oxalate (Al_{Amo}) provides an estimate of the non-crystalline Al (Álvarez et al. 2012). Al_{Oxi}, Al_{Aag}, and Al_{Min} are usually considered as the relatively stable species (Shao et al. 1998). In the presence of three earthworms, moreover in our study, the increase of the Al_{Orw}, Al_{Or}, and Al_{Amo} contents in all earthworm casts may be due to the higher SOM contents in casts resulting in a strong affinity between organic matters and Al (Li et al. 2006), as the positive relationship between Al_{Amo} and SOM contents is shown in Fig. 5. Therefore, just like other metal(loid)s, such as Cd, Cu, Zn, and As, earth-worms may regulate the content of Al_{Orw}, Al_{Or}, and Al_{Amo} in casts by affecting the degradation and redistribution of soil organic matter (Tica et al. 2013).

Higher Al_{Ex}, Al_{Orw}, Al_{Or}, and Al_{Amo} in earthworm casts than non-ingested soil may indicate that Al metabolization from the gut is the main route instead of skin mucus (Table 4). Moreover, Al fractions affected by earthworm may be species dependent. A. aspergillum, as anecic species, may secrete special mucus resulting in the increased contents of Al_{Or} in non-ingested soil compared to casts (Table 4). In addition, different ecological earthworm species differ in their feeding preference for soil organic matter (Lavelle and Spain 2001). In our study, P. corethrurus may prefer soil particles with higher organic matter content than A. robustus and se-cretes more exchangeable K^{+} and Ca^{2+} contents in guts (Fig. 5), which results in their stronger effect on the decrease of Al_{Ex} contents and the increase of stable fractions (Table 4; Figs. 3 and 4) than for other earthworm species. Further study should be conducted on earthworm physiological characteris-tics, such as calciferous glands, the composition of intestinal secretions and skin mucus, owning to their great effect on Al fractions.

Conclusions

Our study demonstrated that P. corethrurus, A. robustus, and A. aspergillum have different impacts on soil acidification, Al fractions, and base cation release in a south China subtropical soil. Firstly, three earthworms, especially P. corethrurus, sig-nificantly reduced soil acidification. Subsequently, the in-creases of pH values in casts and non-ingested soil may be due to the release of exchangeable cation from their intestinal secretions and mucus. The intestine may be the main pathway for the release of Ca^{2+} and Mg^{2+}. Finally, all three earthworms altered Al fractions and reduced Al toxicity, especially P. corethrurus which decreased 61.7% of exchangeable Al contents in cast compared to control soil while increasing the contents of Al_{Orw}, Al_{Or}, and Al_{Amo}.

Acknowledgements Thanks to Qijun Yang, Haoyu Wang, and Huan Qian for laboratory assistance.

Funding information This research was supported by the Natural Science Foundation of China (Grant No. 41201305 and 41601227) and National Key Research and Development Program of China (2016YFD0201301 and 2016YFD0201200).

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