Effect of earthworms (*Eisenia fetida*) on the fractionation and bioavailability of rare earth elements in nine Chinese soils

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Abstract

The effect of earthworm (*Eisenia fetida*) activity on soil pH, dissolved organic carbon (DOC), fraction distribution pattern and bioavailability of rare earth elements (REEs) Y, La, Ce, Pr and Nd in nine Chinese soils were investigated using pot experiments. A three-step extraction procedure recommended by the European Community (Standards, Measurements and Testing Programme) was used to fractionate REEs in soils into water soluble, exchangeable and carbonate bound (B1), Fe- and Mn-oxides bound (B2) and organic matter and sulfide bound (B3). Inoculated with earthworms, the soil pH, DOC and water-soluble rare earth elements fraction increased. A significant correlation was obtained between the increased DOC and the increased water-soluble REEs. REEs in fraction B1 increased after earthworm inoculation, while those in fraction B3 decreased. No significant differences were observed for REEs in fraction B2. The biomass and the concentrations of REEs in wheat shoots and roots increased after the treatment with earthworms. The results demonstrated that earthworm activity increased the mobility and bioavailability of REEs in soils.

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1. Introduction

In recent years, more and more rare earth elements (REEs) enter the environment through various pathways because of the rapid increase of the exploitation of REE resources and its applications in modern industry, agriculture and everyday life. Many efforts have

been made to understand the chemical behavior and bioavailability of REEs in the environment (Wytenbach et al., 1998; Li et al., 1998; Zhang and Shan, 2001; Lu et al., 2003). It is well established that the physico-chemical properties of soils are the main factors controlling the mobility, transformation, and bioavailability of REEs in soils (Shan et al., 2004). The chemical fractionations afford valuable information in evaluating the bioavailability of REEs in soils (Cao et al., 2000; Wen et al., 2001).

Earthworms are ubiquitous species living in soils. Earthworm activity plays an important role in changing

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soil properties (Lee, 1985; Pallant and Hilster, 1996; Boyle et al., 1997; Capowiez et al., 2000; Ponder et al., 2000). By making burrows, earthworms greatly influence soil structure and therefore modify soil transport characteristics (Jégou et al., 2000). By ingesting organic debris, they enhance soil nutrients (for example C, N and P) availability beneficial to the plant growth. By excreting mucus and urine, they change the distribution and activity of microbes and soil biota (Binet et al., 1998; Toyota and Kimura, 2000; Salmon, 2001). Moreover, it has been reported that after treatment with earthworms, the fraction distribution of heavy metals is changed significantly, thus, affecting their bioavailability (Devliegher and Verstraete, 1996; Cheng and Wong, 2002; Ma et al., 2002). This effect was attributed (Wen et al., 2004) to the enhancement effect of dissolved organic matter. However, the effect of earthworms on the fractionation and bioavailability of REEs is still unclear.

The aim of the present study was to investigate the effect of earthworm activity on the fractionation and bioavailability of REEs. The soil pH, DOC, REE fraction distributions and the concentrations of REEs in wheat roots and shoots with and without earthworms were compared.

2. Materials and methods

2.1. Soil sample collection and preparation

Nine Chinese cultivated soils were collected from Jiangxi Province, southern China (Yingtian soil); Beijing, northern China (Beijing soil); Hubei Province, central China (Wuhan soil), Shandong Province, eastern China (Rongcheng soil), Heilongjiang Province, northeastern China (Heilongjiang soils), Guizhou Province, southeast China (Anshun soils), Shanghai, east China (Shanghai soil), Fujian Province, southeast China (Chaozhou soil), and Shanxi Province, northern China (Changzhi soil). Soil samples were taken from the cultivated surface layer (0–20 cm). The soils were air-dried, ground and screened through a 1-mm nylon fiber sieve to remove stones, plant roots, and other large particles.

Soil pH was measured in de-ionized water-soil suspension using a ratio of 1:1 (w:v), organic C was determined by the Walkley-Black procedure (Nelson and Sommers, 1982), and cation exchange capacity (CEC) was measured by the method of Rhoades (1982). Amorphous iron (Fe) and aluminum (Al) oxides were determined by ammonium oxalate extraction method (Blakemore et al., 1987), and crystalline Fe and Al oxides by the oxalate-ascorbic acid extraction method of Shuman (1982). Manganese oxide content was determined by extraction with 0.1 mol l⁻¹ of hydroxyl-ammonium hydrochloride (Shuman, 1982).

2.2. Pot experiments

One kg of soil was placed in a plastic pot before planting. 12 earthworms (*Eisenia fetida*), purchased from China Agricultural University, 0.60 ± 0.03 g fresh weights each, 3.5–4.5 cm in length, were inoculated in each pot under greenhouse conditions. The soils were adjusted to approximately 60% water-holding capacity, and water loss was compensated daily by addition of deionized water. Wheat seeds (*Triticum aestivum* L.) were thoroughly rinsed with water, and germinated on a filter paper moistened with de-ionized water, ten uniformly germinated seeds with radical emerged were sown in all soil types. A total of 20 seeds were sown per pot and subsequently reduced to 15 plants. Winter wheat in pots without earthworms served as controls. All treatments were replicated three times.

Wheat shoots and roots were harvested 6 weeks after germination. The plant shoots were cut off with a plastic knife near the soil surface and washed with dilute detergent solution followed by several rinses with de-ionized water. The roots were separated from soil by washing with de-ionized water carefully. The plant materials were dried at 70 °C for 48 h and the dried plant tissue was weighed and finely ground before metal determination.

The soil collected from each pot after harvest was air-dried, mixed, sieved (1-mm), and then analyzed for DOC and fractionations of REEs by the three-step BCR method (Quevauviller et al., 1993).

2.3. Water-soluble REEs and DOC

Water-soluble REEs and DOC were obtained by shaking 1.0 g of dried soil with 5.0 ml de-ionized distilled water in 50 ml polypropylene centrifuge tubes for 24 h. After centrifuging at 4000g for 30 min, the supernatant was filtered with 0.45 µm membrane. The concentrations of REEs and the DOC in the supernatants were determined by inductively coupled plasma mass spectrometry (ICP-MS) and TOC instrument (Shimadzu 5000), respectively.

2.4. Fractionation of REEs

Three-stage sequential fractionation procedure proposed by the European Community (Standards, Measurements and Testing Programme, BCR) (Quevauviller et al., 1993) was carried out. The REE fractions were specified as fraction B1: water soluble, exchangeable and carbonate bound; fraction B2: Fe–Mn oxide bound; and fraction B3: organic matter and sulfide bound. Dried soil (0.50 g) was used for fractionation. The procedure was replicated three times for each soil sample. Following each extraction, the mixture was centrifuged at 4000g for 30 min to separate the supernatant from the residues. The residues were washed with

de-ionized water, and washings were combined with the supernatant fraction. The residues were then subject to the next step of BCR extraction method.

2.5. Determination of REEs

The concentrations of REEs in wheat shoots and roots, soil and each soil fraction were determined by ICP-MS (PQ3, Fisons Instruments, UK). ^{115}In was added as an internal standard to monitor matrix effects and signal drifting. General instrumental operating conditions and sample digestion methods have been described previously (Wen et al., 1999). The analyses were carried out in triplicate.

2.6. Reagents and statistics

All reagents used were of analytical reagent grade or better.

Statistical analyses were conducted with the software SPSS 11.5 for Windows (SPSS Inc. Chicago, USA). All values including soil pH, DOC, total concentrations of REEs in soils and in soil fractions, in wheat roots and shoots, biomass of dry wheat, are reported as mean $\pm$ standard errors. Paired-samples *t*-test analysis was performed to establish significant differences between treatments with and without earthworms.

3. Results

3.1. Soil and earthworms

The soils represented a wide range of physicochemical properties (Table 1). The REE concentrations of soils are shown in Table 2.

No mortality was observed 6 weeks after inoculation. The average fresh weight of each earthworm inoculated was 0.60 g. After wheat shoots and roots were harvested 6 weeks after planting, still no mortality was observed,

Table 2

Total concentrations of REEs in soils ($\mu\text{g g}^{-1}$)^a

Soils	Y	La	Ce	Pr	Nd
Beijing	12.4	29.8	62.7	7.21	27.5
Yingtian	1.53	4.60	7.50	0.78	4.20
Helongjiang	9.11	19.6	35.1	4.36	15.7
Rongcheng	19.9	27.2	33.2	2.93	8.26
Wuhan	21.7	35.7	30.1	3.61	10.3
Anshun	20.2	48.8	44.1	6.41	17.7
Shanghai	25.7	34.9	34.2	4.25	11.7
Chaozhou	28.1	71.9	35.7	5.68	20.9
Changzhi	17.9	31.8	35.2	4.83	14.6

^a Relative standard deviations are less than 10% for six measurements.

and the average fresh weight of each earthworm was 0.61 g.

3.2. Effect of earthworms on the soil pH and DOC

The pH values of all soils increased from 0.2 to 1.1 units due to earthworm activity (Fig. 1). All differences were significant ($p < 0.05$, $n = 6$) except for one soil ($p < 0.1$, $n = 6$). The pH of Beijing soil was affected the most significantly since it increased from 6.9 to 8.0, while pH of Heilongjiang soil was only slightly elevated from 7.3 to 7.5.

Earthworm activity significantly ($p < 0.1$) increased DOC for all nine soils (Fig. 2). The DOC increase factors ranged from 1.23 (Chaozhou soil) to 2.50 (Changzhi soil) after the treatment with earthworms.

3.3. Effect of earthworm activity on water-soluble REEs

The concentrations of REEs in the water-soluble fraction were compared between soils with and without earthworms (Fig. 3). Soils treated by earthworms significantly increase ($p < 0.05$) the REEs water-soluble fraction. The increase of water-soluble REEs fraction was

Table 1
Selected properties of soils

Soil	Type	Sand (%)	Silt (%)	Clay (%)	Fe (%)		Al (%)		Mn (mg kg ⁻¹)	CEC (cmol kg ⁻¹)	pH	Organic C (%)
					Crystal	Amorphous	Crystal	Amorphous				
Beijing	Mollisols	37.6	51.6	10.8	0.88	0.32	0.36	0.11	112	15.7	6.90	0.78
Yingtian	Ultisol	19.5	38.7	42.4	3.52	0.72	1.39	0.28	27.6	14.2	4.56	0.89
Helongjiang	Mollisols	9.8	62.8	27.4	1.50	0.40	1.60	0.34	326	26.0	7.35	3.71
Rongcheng	Mollisols	89.7	3.91	6.40	11.7	0.33	0.97	0.011	475	9.82	6.08	0.39
Wuhan	Alfisol	52.9	21.4	25.7	23.5	0.73	1.05	0.012	409	22.8	6.73	1.72
Anshun	Cambisols	37.2	32.8	30.1	0.62	0.42	0.14	0.13	103	51.3	6.29	6.76
Shanghai	Luvisols	21.4	65.5	13.2	1.23	0.29	0.43	0.35	26.1	36.6	5.53	3.02
Chaozhou	Ferralsols	29.6	33.7	36.7	1.99	4.43	0.60	0.046	107	18.1	5.96	1.81
Changzhi	Luvisols	24.6	52.3	18.1	0.55	0.34	0.77	0.71	50.8	21.9	7.29	4.12

Fig. 1. Effects of earthworm activity on soil pH. White column: earthworms; black column: no earthworms; bars represent standard errors.

Fig. 2. Effects of earthworm activity on soil DOC. White column: earthworms; dark column: no earthworms; bars represent standard errors.

the greatest in Changzhi soil (Fig. 3i) and the lowest in the Heilongjiang soil (Fig. 3c).

3.4. Effect of earthworms on the REE fraction distribution

The soils with and without earthworms were subjected to BCR sequential extraction, and the distributions patterns of REEs among B1, B2 and B3 fractions were compared in order to investigate their binding site shift. For the nine soils studied, REEs increased in B1 ($p < 0.1$) by factor of 1.3–2.9, 1.2–5.2, 1.2–3.7, 1.3–3.0 and 1.1–2.2 for Y, La, Ce, Pr and Nd, respectively, while those in B3 decreased ($p < 0.1$) by 21–44%, 15–44%, 12–42%, 14–40%, and 17–51%, respectively, after earthworm treatment. No significant changes in B2 fraction of REEs were found ($p > 0.1$). Since the presence of earthworms had a similar effect on the distribution of Y, La, Ce, Pr and Nd among three fractions B1, B2 and B3, only La results were displayed graphically in Fig. 4.

3.5. Relationship between DOC and water-soluble REEs

The comparison between the DOC and REE contents of the water-soluble fraction can provide

an evidence for the effect of DOC on the solubility of REEs. We calculated the ratios of $\text{DOC}_{\text{earthworms}}$ to $\text{DOC}_{\text{no earthworms}}$ and the ratios of the Water-soluble $\text{REEs}_{\text{earthworms}}$ to water-soluble $\text{REEs}_{\text{no earthworms}}$. There were strongly positive correlations ($p < 0.01$) between the $\text{DOC}_{\text{earthworms}}/\text{DOC}_{\text{no earthworms}}$ values and the water-soluble $\text{REEs}_{\text{earthworms}}/\text{water-soluble REEs}_{\text{no earthworms}}$ values. The correlation coefficients of Y, La, Ce, Pr, and Nd were 0.98, 0.92, 0.92, 0.93, and 0.91, respectively.

3.6. Effects of earthworms on wheat biomass and REE accumulation in wheat roots and shoots

Earthworm treatments significantly increased the yield of wheat shoots and roots by 1.2–1.6 and 1.5–1.9 times, respectively, in the nine soils ($p < 0.1$). The mean and range of REEs in wheat roots and shoots with and without earthworms are given in Table 3. The REE concentrations of wheat roots in nine soils increased by 27–53%, 25–61%, 16–33%, 18–45% and 30–76% for Y, La, Ce, Pr and Nd in the presence of earthworms, respectively ($p < 0.1$), while REEs in wheat shoots increased by 15–39%, 13–34%, 12–26%, 16–31% and 19–50% for Y, La, Ce, Pr and Nd, respectively ($p < 0.1$).

Fig. 3. Effect of earthworm activity on the concentrations of water-soluble REEs. White column: earthworms; dark column: no earthworms; bars represent standard errors: (a) Beijing soil, (b) Yingtan soil, (c) Heilongjiang soil, (d) Rongcheng soil, (e) Wuhan soil, (f) Anshun soil, (g) Shanghai soil, (h) Chaozhou soil and (i) Changzhi soil.

4. Discussion

It is well established that soil pH is a key factor affecting the adsorption-desorption behaviors and hence bioavailability of REEs (Cao et al., 2001; Li et al., 2001; Wen et al., 2002). Therefore, it is important to determine the pH change due to the earthworm activity. The increasing soil pH was attributed to the increase N associated excreta by earthworm such as alkaline urine (Parkin and Berry, 1999; Salmon, 2001). Another explanation for the increasing pH could be earthworm calciferous glands (Lee, 1985). Regardless of the precise mechanism, it seems likely that the earthworms play an active role in pH regulation of the soil.

Generally, increased soil pH leads to the enhancement of the affinity of soils for REEs due to pH-dependent surface-charge density on colloids, resulting in the less concentration of REEs in soil solution (Cao et al., 2001; Shan et al., 2002). However, this phenomenon was not observed in our experiments. Instead, the treatment with earthworms elevated the water-soluble REEs (Fig. 3). In view of the increase of soil pH and the elevation of water-soluble REEs, one may deduce that there must be some REE-chelating metallophores produced by earthworm.

It is well documented that earthworm activity improves soil properties through physical, chemical, bio-

logical and biochemical mechanisms, such as enhances the fragmentation and humification of coarse particulate organic matter into finer fractions and influences the distribution of organic matter in soil (Martin, 1991). Parkin and Berry (1999) found that the DOC in burrow soil was much higher than that in nonburrow soil. Jones (1997) observed that there was a strong relationship between the large amount of DOC and the desorption amount of REEs. He suggested that DOC may be able to bind REEs in solution, thereby increasing REE desorption to the water phase. Moreover, DOC could enhance the bioaccumulation of REEs in wheat roots and shoots significantly (Gu et al., 2001). The positive relationship between water-soluble REEs and DOC revealed the basic mechanisms of how earthworms affected the behaviors of REEs in soils.

In addition, the fragmentation was also associated with microbes, whose population and activity were increased by earthworms (Toyota and Kimura, 2000; Binet et al., 1998). Results obtained by our previous experiments showed that in the presence of earthworms, the population of bacteria, actinomycetes and fungus in Beijing soil were much higher than those in the absence of earthworms (Wen et al., 2004). These microbes can exude some low-molecular-weight organic acids, such as acetic, citric and oxalic acids (Jones, 1998), which could enhance the supply of

Fig. 4. Effect of earthworm activity on the fraction distribution pattern of La in B1, B2 and B3 in nine soils. White bar: earthworms; dark bar: no earthworms; bars represent standard errors: (1) Beijing, (2) Yingtan, (3) Heilongjiang, (4) Rongcheng, (5) Wuhan, (6) Anshun, (7) Shanghai, (8) Chaozhou and (9) Changzhi soil.

Table 3

Mean and range of REEs in wheat grown in all nine soils with and without earthworm treatments

Element	Concentration of REEs in roots and shoots ($\mu\text{g g}^{-1}$)							
	In roots				In shoots			
	With earthworms		Without earthworms		With earthworms		Without earthworms	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Y	3.61	0.98–6.12	2.55	0.77–4.01	0.22	0.07–0.39	0.17	0.05–0.30
La	5.48	0.85–9.25	3.40	0.67–8.26	0.35	0.22–0.56	0.21	0.20–0.49
Ce	13.6	5.01–18.1	10.2	3.98–16.1	0.58	0.30–1.27	0.42	0.26–1.09
Pr	1.61	1.01–2.99	1.11	0.93–1.51	0.08	0.05–1.01	0.05	0.03–0.80
Nd	3.54	1.69–7.34	2.01	0.98–5.68	0.21	0.15–0.46	0.13	0.10–0.38

REEs to roots (Shan et al., 2002), and enhance the uptake of REEs by plants (Han et al., 2005). Such,

exudation of microbe is an important source of metallophores.

Information of the REE binding site shift can help to understand the REE behavior as affected by earthworms. The increase in REE concentrations in B1 and the decrease in B3 fractions suggested that the distribution pattern of REEs among different fractions was changed due to earthworm activity. One of the DOC sources is the decomposition of soil organic matter by earthworms and microbes. Since B1 represented the most bioavailable form of REEs to plants (Li et al., 1998; Wang et al., 2001), we hypothesized that earthworm treatments increased the bioavailability of REEs in soils.

Earthworm activity improves nutrient mineralization, which also benefits to plants. The results obtained by Pallant and Hilster (1996) showed that the presence of earthworms significantly increased the yields of plants, which is in agreement with our results. Furthermore, the concentrations of La, Ce, Pr and Nd in both wheat roots and shoots with earthworm treatments were significantly higher than those without earthworm treatments, which demonstrated that earthworms could enhance the bioavailability of REEs.

Although in this study, we proved that the earthworm activity could increase the disassociation and bioavailability of REEs by enhancing the DOC, it should be mentioned that the pH, DOC, and water-soluble REEs were determined after soil mixing and extraction. No efforts were made to distinguish the earthworm cast and drilosphere soil from bulk soil. There is also no information if the increased DOC is the result of the earthworm activity or soil microorganism activation. In addition, our study was based on the inoculation of 12 earthworms for 12 weeks in 1 kg soil and the soil moisture was maintained at 60% water-holding capacity. Higher earthworm density may lead to higher metabolic rates of the microflora community and higher production of dissolved organic matter. Besides, it is reported that microbial activity, nutrient and C mineralization were increased in earthworm casts over the period of a few days, but after drying out and remaining stable for a few months or years, the same casts had lower microbial activity and C mineralization rate (Brown et al., 2000). Therefore, the long-term consequences should be considered too.

5. Conclusion

It was demonstrated that the soil pH, DOC and water-soluble REEs are increased by earthworm activities. The decrease of B3 form, the increase of B1 form, and a simple relationship between DOC and water-soluble REEs indicated that earthworm activity could increase the bioavailability of REEs by decomposing organic matter into smaller components. These results revealed that earthworm activity is an important factor

that affected the fractions distribution pattern, mobility and bioavailability of REEs in soil.

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