Earthworm Community In Relation To Soil Properties And Metals In Hebei Province, North China

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Abstract

In this study, we examined the influence of soil properties (pH, total potassium (TK), available potassium (AK), total nitrogen (TN), total phosphorus (TP), available potassium (AP), cation exchange capacity (CEC), and soil organic carbon (SOC)), and metals (Cd, Pb, Cu, and Zn) on the density, diversity, and species composition of earthworms in the Hebei Province, North China. In total, 535 earthworms were collected from 20 sites in the study area, and belonged to three families, six genera, and ten species. *Amynthas hupeiensis* (39.4%) and *Drawida gisti* (37.8%) were the dominant species. The correlations between soil variables and earthworm composition determined using redundancy analysis indicated that SOC, TK, and AK enhanced earthworm density (total, adult, and juvenile) and species (*A. hupeiensis* and *D. gisti*) abundances. Earthworm composition remained unaffected by the metals (Cd and Pb) in the uncontaminated sites; in contrast, species were absent in areas with high metal concentrations (S19 and S20). Soil TN content was negatively and positively related to Shannon and Peilou indexes ($p<0.05$), respectively, indicating that TN may be pivotal in influencing earthworm diversity and species evenness. Overall, the soil properties such as K, SOC, and TN were the key variables affecting earthworm density, diversity, and species dominance.

1. Introduction

As a widely recommended model organism, earthworms promote formation of soil and the organic matter decomposition in terrestrial ecosystems (Butenschoen et al., 2009; Romanes, 2009; Datta et al., 2016). Extensive ecotoxicological data on earthworm's response to metal pollution has been collected to diagnose and evaluate the ecological risks. Soil metal contamination has become a severe environmental issue that adversely affects the life-history of earthworms, leading to genetic erosion or population extinction (Rinklebe et al., 2019; He et al., 2019; Sun et al., 2019; Rybak et al., 2020; Yang et al., 2020; Zhong et al., 2020).

Earthworms can be effective bioindicators in ecological and toxicological risk assessments (Valchovski, 2010; Bottinelli et al., 2020). Numerous studies have focused on the effects of short-term metal toxicity on earthworms in freshly spiked soil (OECD, 1984; Martikainen, 1996; Lowe and Butt, 2007). Furthermore, information collected from laboratory studies may be impractical (OECD 222, 2004; McBride et al., 2009). Alterations in earthworm community structure and abundance can accurately reflect the metal toxicity in fields (Wang et al., 2018; Huang et al., 2020), where metal contamination often comprises a mixture of contaminants (Pascaud et al., 2014). Compared to short-term exposure, chronic metal exposure in fields may be detrimental to earthworms; the long-term conditions may exhibit unexpected slow toxicity, even at low metal concentrations in soils (Tang et al., 2017).

The soil physical, chemical, and biological factors significantly affect the performance of earthworms inhabiting its environments. Metal transfer in soil depends on compartmentalization and distribution of contaminants, which are affected by properties in soil (such as pH value, organic carbon, and soil type) (Chenot et al., 2013; Shahid et al., 2013). Soil properties can be considered as predictors that affect the patterns of metal distribution and earthworm species (Xu et al., 2013; Xie et al., 2018). The earthworm communities in contaminated fields become unstable or simplified, wherein the density or abundance of sensitive species may either decrease or even disappear under contaminant stress (Lévêque et al., 2015; Wang et al., 2018). Therefore, in our study, the associations between the dynamics of earthworm communities (diversity and density) and the variations in soil properties and chronic metal contamination that impact their distribution patterns were investigated. The approach from the perspective of correlation between soil parameters and earthworm communities in this study provides important information for ecological risk assessment.

This study was conducted in the agricultural land and near an abandoned industry that locating in the vicinity of Baoding City, Hebei province of North China, and the objectives were to (i) investigate the variations in the composition, density (total, adult and juvenile worms), dominant species, and diversity (using Shannon index, Simpson index, and Pielou index) of earthworms in the concerned study areas; (ii) evaluate the soil factors (including pH, total nitrogen (TN), organic carbon (SOC), available phosphorous (AP), total phosphorus (TP), total potassium (TK), available potassium (AK), cation exchange capacity (CEC), and metal (Cd, Pb, Cu, and Zn) concentrations) influencing the earthworm community, which would provide a theoretical basis for ecological risk assessment in this area.

2. Method And Materials

2.1. Site descriptions

This study was conducted in the vicinity of Baoding City, Hebei Province, North China (Fig. 1). The specific coordinates of the elevations and locations are listed in Table S1. The elevations of the sites ranged from 1.0 to 15.8 m. Sites, which had similar agricultural land use with loam based soil, situated near an abandoned factory at distance of approximately 0.5-1.0 km were selected. The general area has a temperate continental monsoon climate. The annual average temperatures ranged from 4.3℃ to 26.4℃. The average annual precipitation was 499 mm, and the average precipitation in October was 21.7 mm. In total, 100 samples of soil were collected from the 20 study sites (n = 5) (18 sites contained earthworm species) that displayed variations in soil factors (soil properties and the metal concentrations). Soil and earthworm samples were processed in five replicates, covering a radius of 20 m for each category, and were sampled during early October 2019.

2.2. Soil properties

Soil samples were randomly collected from the upper soil horizon (0–20 cm) from each plot using a plastic shovel, labeled, placed into polyethylene bags, and subsequently transported to the laboratory to determine the soil properties and metal concentrations (Lévêque et al., 2013, 2015). Soil samples were air-dried and sieved to determine the soil properties (including the pH, SOC, TN, AP, TP, TK, AK, and CEC) and metal concentrations (Cd, Pb, Cu, and Zn).

Furthermore, pH of the soil suspensions (1:2.5 (w/v) soil/water); SOC content was determined using the $K_2Cr_2O_7$ titration method (Walkley and Black, 1934). The CEC values were determined using an NH_4OAc solution (Metson, 1956). TN content was measured using the Kjeldahl apparatus (Jamali et al., 2006). AP and AK contents were determined by extracting with $NaHCO_3$ (0.5 M). The AK content was extracted by NH_4OAC and determined using the flame photometric method. Furthermore, TP and TK contents were determined using the NaO-Mo-Sb anticolorimetric method and the flame photometric method (NaOH), respectively. Heavy metal (Cd, Pb, Cu, and Zn) concentrations in soil were digested with aqua regia (HNO_3:HCl (1:3 (v/v)), and determined using atomic absorption spectrophotometer (Atomic absorption AA6300c, Japan) .

2.3. Earthworm sampling and analysis

Earthworms were collected from the plots (50 cm × 50 cm × 25 cm) at each site. Living earthworms were separated and depurated for 24 h on moist filter paper, and subsequently stored in ethanol (70%) for morphological identification and analysis (Lévêque et al., 2015). After 48 h, the specimens were preserved in 95% ethanol. The earthworm samples were observed under a dissecting microscope and were identified based on anatomical and morphological characteristics (Xu and Xiao, 2010). The earthworm density at each site was expressed as the mean density (inds m^{-2})(n=5), and the presence of adult and juvenile earthworms (with clitellum and non-clitellum) were recorded at each site.

2.4 Statistical analysis

Statistical analyses were performed using SPSS statistical software (IBM, version 20.0). Data were plotted using R software (Version 4.3). The significant variations among the earthworm community descriptors (density, diversity, and species) and soil factors (soil properties and metal concentrations) were analyzed using Redundancy analysis (RDA). RDA approach allows the use of various methods to model the structure in a data matrix, which is performed using the R software with “vegan” and “ade4” packages. The projection and angle between the soil and earthworm variable arrows reveal the correlation coefficients (Mariet et al., 2020). Pearson’s correlation was calculated to determine the relationships between soil (pH, CEC, SOC, TK, TP, TN, AK, AP content, and concentrations of Cd, Pb, Cu, and Zn) and earthworm variables (diversity, density, and species) ($p < 0.05$).

Shannon (1948) and Simpson (1949) indexes were calculated based on the density and diversity of earthworm species at each site.

Shannon index: $H = -\sum P_i \ln P_i$

Simpson index: $D = 1-\sum (P_i)^2$

where P_i is the percentage of earthworm individuals represented by species i on the total number of individuals.

High Shannon index values and low Simpson index values indicate a high diversity of earthworms.

The representation of earthworm individuals by taxon (evenness) was evaluated using the Pielou (1969) index:

Pielou index: $H/\ln(\text{total number of taxa})$

High Pielou index values indicate high evenness in population sizes of different taxa, in contrast, low values for the Pielou index indicate dominance of one or more species in the community.

3. Results

3.1. Soil properties and metal concentrations

The results of the soil properties (including the pH, TK, AK, TP, AP, TN, SOC, and CEC) and concentrations of Cd, Pb, Cu, and Zn in soil determined at the sampling sites are shown in Tables S2 and S3. In the study sites (Table S2), the pH values, TK, AK, TP, AP, TN, SOC, and CEC contents ranged from 7.9 to 8.3, from 11.18 to 25.18 g kg^{-1}, from 82.24 to 342.1 mg kg^{-1}, from 1.04 to 2.04 g kg^{-1}, from 15.01 to 87.16 mg kg^{-1}, from 1.08 to 2.54 g kg^{-1}, from 6.62 to 11.88 g kg^{-1}, and from 6.92 to 11.51 cmol kg^{-1}, respectively. In the study sites, Cd, Pb, Cu, and Zn concentrations ranged from 0.17 to 5.94, from 24.23 to 283.2, from 16.39 to 462.3, and from 44.45 to 263.2 mg kg^{-1}, respectively (Table S3). The descriptive data showed that Cd, Cu, and Pb concentrations exceeded the threshold legal values at sites S19 and S20 (as stipulated in the Chinese Environmental Quality Standard for Soils, prescribed by the Ministry of Environmental Protection of China (GB 15618-2018); Table S3].

3.2. Earthworm community

Earthworm species collected at the study sites are listed in Table 1. In total, 535 individuals were collected from all study sites (earthworm species were absent in sites S19 and S20), and comprised three families, six genera, and ten species across the sites. Five species belonged to the family Megascolecidae, with one species from the genus *Amynthas* (*Amynthas hupeiensis*) and other four from the genus *Metaphire* (including *Metaphire guillemi, Metaphire asiatica, Metaphire kiangsuensis,* and *Metaphire tschiliensis*). Two species belonging to the family Moniligastridae were *Drawida gisti* and *Drawida japonica.* The rare species *Eisenia foetida, Aporrectodea trapezoids*, and *Bimastus parvus* belong to the family Lumbricidae.

In the study sites, the ecological category with endogeic species was the most diverse and abundant group; five species were endogeic, four were epigeic, and only one was an anecic species (Table 1). The most abundant species was *A. hupeiensis* (Michaelsen, 1895) (accounting for 39.4% of the total number of individuals), followed by *D. gisti* (Michaelsen, 1931) (37.8%), and *D. japonica* (16.3%) (Michaelsen, 1892). The rare species in the study sites were *M. guillemi* (1.87%) (Michaelsen, 1895), *M. tschiliensis* (Michaelsen, 1928) (0.17%)*, M. asiatica* (0.56%) (Michaelsen, 1900), *M. kiangsuensis* (Chen, 1930) (1.5%), and *A. trapezoids* (Duges, 1828) (0.73%). Furthermore, Shannon (H), Simpson (D), and Pielou (P) indexes were calculated to determine the alterations of earthworm diversity and species evenness, as shown in Table 2. The results showed that the values of H, D, and P indexes ranged from 0.45 to 1.34, from 0.28 to 0.68, and from 0.55 to 1.0, respectively.

3.3 Relationships between soil variables and earthworms

Relationship between earthworm community (including the total, adult, and juvenile individuals, species, diversity and evenness indexes), and soil properties (including the pH, TN, SOC, TP, TK, AK, AP, and CEC) and concentration of metals (Cd, Pb, Cu, and Zn) were analyzed using RDA (Figs. 2 and 3) to determine the potential impact of the soil variables on earthworm composition. The correlations between different soil properties, metals in soil, and earthworm parameters from the RDA are shown in Tables 3 and 4.

In Fig. 2, the RDA results showed the ranking of soil variables and earthworm parameters, with the first principal component axes explaining 96.4% of the total variance, suggesting that sites S5 and S4 differed from the other study sites. Pearson's correlations between soil and earthworms showed that soil properties including TK, AK, and SOC contents significantly correlated with earthworm density (total, adult, and juvenile worm individuals) (p<0.05, Table 3), respectively, in study sites (except for the sites of S19 and S20 with no earthworms). The Cd and Pb concentrations influenced the earthworm density (total and adult worms) at the study sites (p<0.05). Juvenile worms were negatively correlated with pH values (p<0.05). In this study, the earthworm diversity and evenness variations by Shannon, Simpson, and Pielou indexes did not correlate with the soil variables (p>0.05, Table 3), except for the noted decreased and increased values of Shannon and Peilou index, respectively, with increasing soil TN content (p<0.05).

In Fig. 3, the RDA results showed the ranking of the soil variables and different earthworm species at each site; the first principal component axes explained 91.6% of the total variance. Pearson's correlation coefficients between earthworm species and soil variables are shown in Table 4. RDA ranking results showed that soil variables, including the AK, SOC, and Cd and Pb concentrations affected the density of the dominant species *A. hupeiensis* and *D. gisti* (p<0.05, Table 4). In addition, the AP and TN contents were positively correlated with *D. japonica* (p<0.05), respectively. In contrast, AP levels in the soil were negatively correlated with the density of *M. asiatica, M. kiangsuensi*, and *D. gisti* (p<0.05), respectively.

4. Discussion

4.1 Earthworm composition

The study site majorly contained *A. hupeiensis* (anecic, 39.4%) and *D. gisti* (endogeic, accounting for 37.8%) (Table 1), which are widely distributed across China (Zhang and Sun, 2014). High earthworm densities were observed in sites S4 (containing 9.6 and 29.6 inds per m^{-2} of *A. hupeiensis* and *D. gisti*, respectively) and S5 (containing 56 and 92 inds per m^{-2} of *A. hupeiensis* and *D. gisti*, respectively) (Table 1). The high TK (20.4 and 20.5 g kg^{-1}), AK (293.4 and 251 mg kg^{-1}), and SOC contents (11.88 and 11.7 g kg^{-1}) in sites S4 and S5 (Table S2), respectively, provides a considerably favorable habitat for earthworms in agricultural soils. However, earthworm species were absent in sites S19 and S20, which may be attributed to the elevated metal concentrations (2.04 and 5.94 mg Cd kg^{-1}, 88.7 and 283.2 mg Pb kg^{-1}, and 118.3 and 462.3 mg Cu kg^{-1}, respectively) that exceeded the threshold (GB 15618-2018); additionally, higher concentrations of TK (25.18 and 24.56 g kg^{-1}), AK (216.9 and 342.1 mg kg^{-1}) and SOC (11.78 and 11.64 g kg^{-1}) were observed. This indicates an unstable earthworm composition in highly contaminated soils. Consistent with our previous studies, the earthworm density changes in response to the soil variables, and the absence of sensitive species in highly contaminated soils resulted in reduced species richness and diversity (Wang et al., 2018).

Different niche partitions of earthworms exhibit different responses to environmental stress (Zhang et al., 2018). In the study sites, the ecological categories of endogeic, anecic, and epigeic species accounted for 42.5%, 40%, and 17.5% of the total individuals, respectively. The endogeic species are possibly the least affected by soil factors as compared with that of epigeic and anecic earthworm populations (Mariotte et al., 2016). Anecic and epigeic earthworms are competitive; they use the same food resources, which influences their distribution patterns (Palm et al., 2013). Furthermore, anecic and epigeic display relatively lower abundance and diversity than their endogeic counterparts in agricultural fields, wherein soil plowing damage the earthworm burrows, disturbing their distributions (Solomou et al., 2013; Chen et al., 2020).

4.2 Relationships between soil variables and earthworm community

The possible correlations between the soil physicochemical variables and earthworm composition were obtained by calculating RDA and Pearson's correlations (p<0.05). Descriptive approaches provide information on the relationships among earthworm community parameters (total, adult or juvenile densities, diversity, and species evenness) and soil properties and metals (Cd, Pb, Cu and Zn) in the study sites (Figs. 2 and 3, and Tables 3 and 4).

Among the factors examined, the earthworm population size (total, adult, and juvenile individuals) was positively correlated with the SOC levels (ranged from 6.62 to 11.88 g kg^{-1}, p<0.05, Fig. 2), indicating that the increase in SOC level favored earthworm density and abundance (van Vliet et al., 2007). Meanwhile, RDA ranking results indicated that earthworm density (total, adult, and juvenile individuals) increased with the increase in TK or AK content (p<0.05), suggesting that K was a crucial factor that enhanced the growth of earthworms; in contrast, earthworm activities also exhibit synergistic effects on soil fertility (such as affecting nitrogen mineralization, and increasing the availability of phosphorous and potassium), promotes the survival of soil organisms even in presence of metal contamination (Maity et al., 2008; Quenea et al., 2009; Dulaurent et al., 2020).

Moreover, negatively correlations between juvenile worms and pH values (p<0.05, Table 3) suggesting that juveniles may display pH sensitivity, which directly or indirectly influences the metal pools and earthworm community. Our study sites were located in the areas of North China with alkaline loam soil and pH ranging from 7.9 to 8.3 units, decreased the bioavailability of metals to earthworms, and possibly inhibiting the growth or reproduction of earthworms (Umiker et al., 2009; Huang et al., 2020). A previous study observed that the earthworm communities (total density and abundance) were influenced by the soil properties (such as organic matter, pH, nitrogen, phosphorus, and potassium) in natural and agricultural ecosystems (Ernst and Emmerling, 2009; Solomou et al., 2013; Carnovale et al., 2015; Singh et al., 2020). This highlights the importance of considering the altered SOC and K levels to evaluate the impacts of metals on earthworm communities dwelling in uncontaminated or contaminated sites.

As shown in the RDA ranking (Fig. 2), soil TN content was negatively correlated with Shannon index, but positively correlated with Peilou index (p<0.05, Table 3), suggesting that TN reduced earthworm diversity and increased species evenness in uncontaminated sites. This indicated that Shannon and Peilou were the dominant indexes, as the formula provides increased weight diversity and evenness than that of the Simpson's index. This disparity in earthworm diversity has been reported in previous studies (Bardgett et al., 1999; Curry et al., 2008; Piotrowska et al., 2013), where the soil nitrogen (N) inputs supported the high abundance and biomass of earthworms. However, elevated mineral N levels can reduce earthworm population and richness, wherein the higher N application accelerates soil decomposition processes (Tian et al., 1993; Yang and Chen, 2009).

In the sites S1 to S18, RDA ranking results showed that the earthworm parameters (including the total, adult, and juvenile individuals) correlated with Cd (2.4-fold variances) and Pb concentrations (2.21-fold variances) (p<0.05, Fig. 2 and Table 3). This could be attributed to the relatively low concentrations of Cd and Pb (0.17–0.41 mg Cd kg^{-1}; 24.23–53.44 mg Pb kg^{-1}) that were within the threshold (GB 15618-2018), and the earthworm composition remained unaffected by Cd or Pb in the uncontaminated sites. It can also be associated with the strong positively correlations among soil properties (SOC, TK, and AK) and metal (Cd and Pb) concentrations (p<0.05, Table S4), while the high SOC, TK, and AK contents enhanced the survival of earthworms. Kamitani and Kaneko (2007) reported that compared to the metal contamination levels, soil properties (pH and clay fraction) considerably influenced the earthworm community. Conversely, in sites S19 and S20, the earthworm species were absent in the heavily metal-contaminated soils (Table S3), even at high levels of SOC, TK, and AK, thereby adversely affecting the earthworm community structure (Spurgeon and Hopkin, 1999; Wang et al., 2018).

According to the RDA ranking results (Fig. 3), soil variables influenced the distribution patterns of earthworm species. Significant correlations were observed between the soil variables and the density of *A. hupeiensis* and *D. gisti* (p<0.05, Table 4), indicating that the two species preferentially inhabited high-SOC and -AK regions of the uncontaminated sites, but were absent in sites contaminated with heavy metals (Cd or Pb).

Although the density of *A. hupeiensis* and *D. gisti* correlated with the soil metal concentrations (p<0.05, Table 4), RDA indicated that K and SOC levels, which were significant among the analyzed soil properties, may determine the earthworm community and species (p<0.05, Fig. 3 and Table S4). Meanwhile, *D. japonica* (epigeic, accounting for 16.3% of total individuals) increased with the increase in AP and TN contents (p<0.05, Fig. 3 and Table 4), suggesting its distribution at sites with increasing AP and TN, which considerably vary with the other ecological category species. Rare species such as *A. trapezoids*, *B. parvus*, and *M. tschiliensis* remained unaffected by the determined soil properties. Therefore, in the present study, at low metal concentrations in the soil, and the soil factors should be considered to estimate the impact of soil contamination on earthworms (Kamitani and Kaneko, 2007), and heavy metal contamination significantly affects the earthworm species composition.

5. Conclusions

In this study, we investigated the distribution pattern and diversity (Shannon, Simpson, and Pielou indexes) of earthworm species in the Hebei Province, North China. The correlations between soil properties, metal concentrations, and earthworm communities at the field scale were obtained using the RDA results. Changes in soil physicochemical properties altered earthworm composition and species distribution. Our data indicated that soil properties, including AK, TK, and SOC enhanced earthworm abundance or density. Furthermore, nitrogen was considered to be a crucial factor influencing earthworm species evenness and diversity in uncontaminated sites. The dominant species *A. hupeiensis and D. gisti*, were widely distributed in the study sites and preferentially inhabited relatively high SOC and AK sites. Earthworm composition was highly-sensitive to and absent in heavy metal-contaminated soils. This study provides information on the dynamics of earthworm communities that correlate with the different nutrients (such as potassium and nitrogen), and metals (Cd and Pb) in soil, which provides a framework for research in ecological studies focusing on conservation agriculture of disturbed soil systems.

Declarations

This manuscript has not been published or presented elsewhere in part or in entirety and is not under consideration by another journal.

Ethics approval and consent to participate

Not applicable.

Consent for publication

The authors declare that we consent for publication in this journal.

Availability of data and materials

The source of data and materials were mentioned in the manuscript, in support of the results. **Competing interests**

The authors declare that they have no competing interests that could have appeared to influence the work reported in this paper.

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Authors' contributions

Dianwu Wang: Conceptualization, Validation, Supervision, Project administration, Writing- Reviewing and Editing; **Yue Yin** and **Kun Wang**: Conceptualization, Investigation, Resources, Data curation, Formal analysis, Visualization, Writing- Original Draft□Writing- Reviewing and Editing; **Miaomiao Chen**: Investigation, Formal analysis, Writing-Reviewing and Editing; **Xiaoquan Mu**: Investigation, Writing- Reviewing and Editing; **Bo Li**: Investigation, Writing-Reviewing and Editing; **Yang Yang**: Investigation, Writing- Reviewing and Editing; All authors read and approved the final manuscript.

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Tables

Table 1. Earthworm species and density in the study sites (inds m^{-2}, n = 5).

Family	Species	Ecotypes	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S1
Megascolecidae	*Amynthas hupeiensis* (Michaelsen, 1895)	Anecic	0.8	4	6.4	9.6	56	13.6	8	4	4	7.2	7.2	12	4	8	1.6
	Metaphire guillemi (Michaelsen, 1895)	Endogeic		5.6	1.6								0.8				
	Metaphire asiatica (Michaelsen, 1900)	Endogeic			2.4												
	Metaphire kiangsuensis (Chen, 1930)	Endogeic			4		2.4										
	Metaphire tschiliensis (Michaelsen, 1928)	Endogeic						0.8									
Moniligastridae	*Drawida gisti* (Michaelsen, 1931)	Endogeic	9.6	12.8	13.6	29.6	92									0.8	0.8
	Drawida japonica (Michaelsen, 1892)	Epigeic						5.6	8.8	12.8	5.6	8	4	1.6	2.4	5.6	5.6
	Eisenia foetida (Savigny,1826)	Epigeic	1.6	2.4													
Lumbricidae	*Aporrectodea trapezoides* (Duges, 1828)	Epigeic															
	Bimastus parvus (Eisen,1874)	Epigeic										0.8				0.8	

Note: Density (inds m^{-2}) represented the average for individuals per site, respectively.

Table 2. The values of Shannon index, Simpson index, and Pielou index for the earthworm community in each site.

Index	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18
H	0.63	1.2	1.34	0.56	0.72	0.75	0.69	0.55	0.68	0.86	0.85	0.45	0.66	1.02	0.8	1.16	1.02	0.6
D	0.34	0.65	0.68	0.37	0.46	0.46	0.5	0.36	0.49	0.55	0.52	0.28	0.47	0.58	0.46	0.56	0.5	0.51
P	0.57	0.86	0.83	0.8	0.66	0.68	1.0	0.79	0.98	0.78	0.78	0.65	0.95	0.73	0.73	0.72	0.63	0.55

Abbreviation: H, D, and P, represented the values of Shannon index, Simpson index, and Pielou index in each site, respectively.

Table 3. Pearson's correlation coefficients between soil properties and metal concentrations with earthworm density and diversity in the study sites (n = 18).

Coefficients	pH	TK	AK	TP	AP	TN	SOC	CEC	Cd	Pb	Cu	Zn
TO	-0.37	0.536*	0.662**	-0.323	-0.405	-0.259	0.67**	-0.267	0.883**	0.801**	-0.371	-0.095
AD	-0.312	0.475*	0.579*	-0.39	-0.401	-0.306	0.607**	-0.231	0.854**	0.756**	-0.333	-0.059
JU	-0.484*	0.556*	0.737**	0.359	-0.137	0.242	0.602**	-0.31	0.435	0.521*	-0.354	-0.268
H	0.294	-0.188	-0.343	-0.074	-0.342	-0.522*	-0.161	-0.032	-0.13	-0.34	0.19	-0.249
D	0.341	-0.236	-0.415	-0.139	-0.217	-0.363	-0.216	0.131	-0.255	-0.325	0.304	-0.224
P	-0.178	-0.008	-0.008	0.28	0.296	0.501*	0.072	0.426	-0.221	0.047	0.168	0.14

Abbreviation: TO, AD, and JU, represented the total, adult, and juvenile number of earthworms in each site; H, D, and P, represented the values of Shannon index, Simpson index, and Pielou index in each site, respectively. TK, total potassium; AK, available potassium; TP, total phosphorus; AP, available potassium; TN, total nitrogen; SOC, soil organic carbon; CEC, cation exchange capacity.

* and ** indicated the significantly differences at $p < 0.05$ and $p < 0.01$, using a Duncan's test.
n, number of soil sampling sites.

Table 4. Pearson's correlation coefficients between soil properties (pH, TK, AK, TP, AP, TN, SOC, and CEC) and metal concentrations (Cd, Cu, Zn, and Pb) with the earthworm species in study sites (n = 18).

Correlation coefficients	pH	TK	AK	TP	AP	TN	SOC	CEC	Cd	Pb	Cu	Zn
Amynthas hupeiensis	-0.38	0.447	0.516*	-0.466	-0.274	-0.112	0.492*	-0.193	0.787**	0.724**	-0.351	0.051
Metaphire guillemi	0.373	-0.066	-0.047	0.225	-0.206	-0.53*	0.147	-0.132	-0.022	-0.181	0.073	-0.29
Metaphire asiatica	0.106	-0.126	-0.039	0.124	-0.515*	-0.381	0.059	0.073	0.109	-0.241	-0.005	-0.166
Metaphire kiangsuensis	-0.097	0.145	0.241	-0.150	-0.621**	-0.444	0.333	-0.014	0.525*	0.191	-0.186	-0.123
Metaphire tschiliensis	0.344	-0.247	-0.101	-0.182	0.012	0.142	-0.26	-0.105	-0.164	-0.097	0.121	-0.07
Drawida gisti	-0.355	0.596**	0.744**	-0.251	-0.517*	-0.37	0.742**	-0.341	0.921**	0.831**	-0.401	-0.215
Drawida japonica	-0.071	-0.246	-0.289	0.073	0.594**	0.561*	-0.294	0.348	-0.434	-0.098	0.218	0.213
Eisenia foetida	0.526**	-0.056	0.104	0.218	-0.152	-0.531*	0.233	-0.411	0.097	-0.039	0.206	-0.251
Aporrectodea trapezoids	0.038	0.231	-0.185	-0.270	0.03	0.067	-0.216	-0.125	-0.05	-0.327	-0.243	0.073
Bimastus parvus	0.046	-0.114	-0.317	-0.104	0.203	0.004	-0.262	-0.123	-0.231	-0.217	0.209	0.077

* and ** indicated the significantly differences at $p < 0.05$ and $p < 0.01$, using a Duncan's test.

n, number of soil sampling sites.

Figures

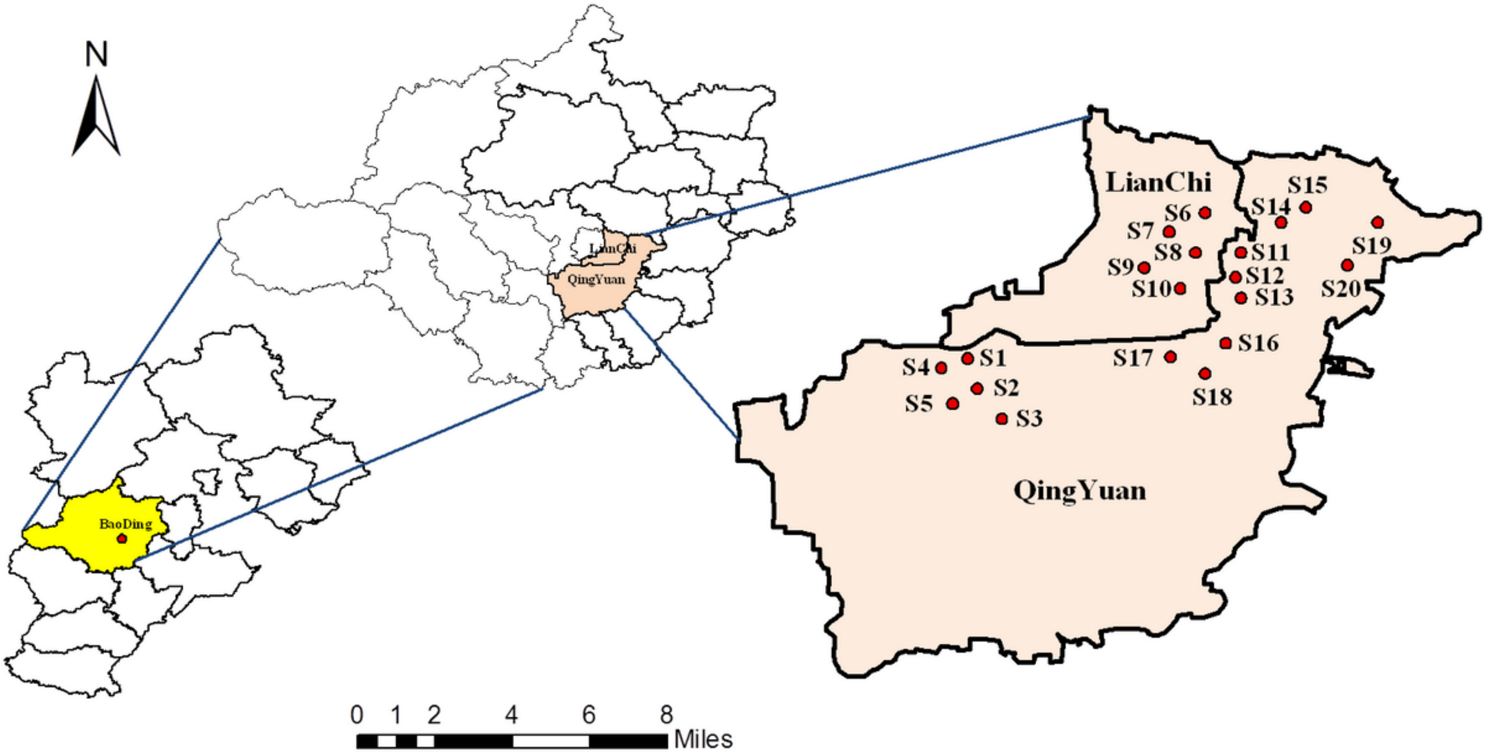

Figure 1

The location of the sampling sites in vicinity of Baoding City, Hebei Province of North China.

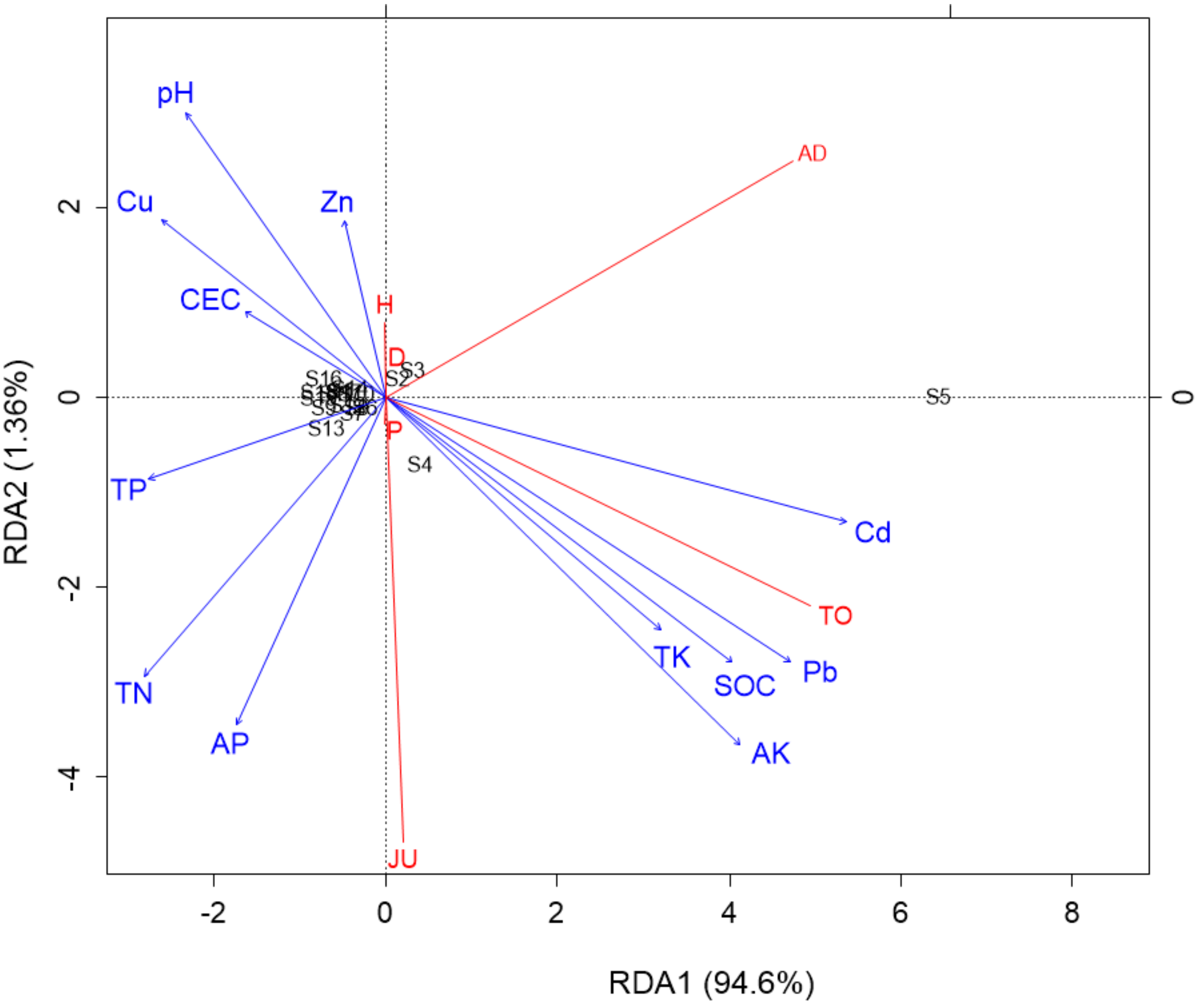

Figure 2

Redundancy analysis (RDA) of the correlations of soil properties and metals and earthworms in soil sites (n= 18). The percentages of variance explained by each soil factor [including soil properties (pH, TK, AK, TP, AP, TN, SOC, and CEC) and the metals (Cd, Pb, Cu, and Zn)] and earthworm density and diversity (including the TO, AD, JU, and index of H, P, D, and S). Abbreviation: TK, total potassium; AK, available potassium; TP, total phosphorus; AP, available potassium; TN, total nitrogen; SOC, soil organic carbon; CEC, cation exchange capacity. TO, AD, and JU, represented the total, adults, and juveniles number of earthworms in each site; H, D, and P, represented the values of Shannon index (H'), Simpson index (D'), and Pielou index (P') in each site, respectively.

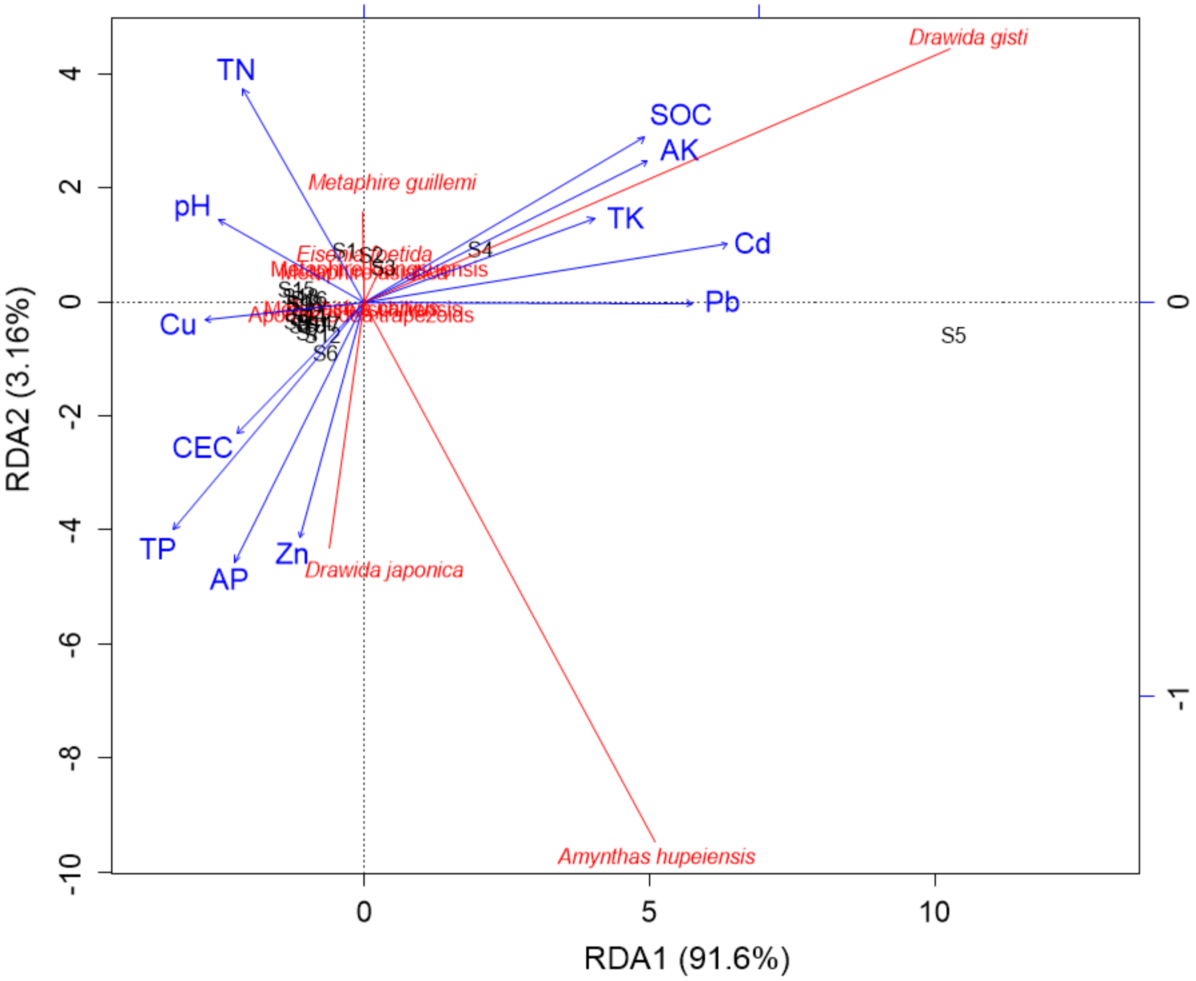

Figure 3

Redundancy analysis (RDA) of the correlations of soil properties and metals and earthworms in soil sites (n= 18). The percentages of variance explained by soil properties (pH, TK, AK, TP, AP, TN, SOC, and CEC) and the metals (Cd, Pb, Cu, and Zn) and the earthworm species in each site.

Supplementary Files

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