Nematodes as indicators of soil recovery in tailings of a lead/zinc mine

Yuanhu Shao [a,b], Weixin Zhang [a,b], Juecui Shen [c], Lixia Zhou [a], Hanping Xia [a], Wensheng Shu [d], Howard Ferris [e], Shenglei Fu [a,*]

[a] Institute of Ecology, South China Botanical Garden, Chinese Academy of Sciences, Xingke Road 723, Guangzhou 510650, China
[b] Graduate University of the Chinese Academy of Sciences, Beijing 100049, China
[c] College of Materials Science and Chemical Engineering, Harbin Engineering University, Harbin 150001, China
[d] School of Life Sciences, Sun Yat-Sen University, Guangzhou 510275, China
[e] Department of Nematology, University of California, Davis, CA 95616, USA

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ABSTRACT

Trophic groups and functional guilds of soil nematodes were measured under four mine tailing subsystems in the Baoshan lead/zinc mine, Hunan Province, southern China to test the indicator value of nematodes for heavy metal pollution. No obvious correlation was found between heavy metal concentration and the total number of nematodes. However, the densities of c–p3, c–p4 and c–p5 nematodes were negatively correlated with Pb and Zn concentrations, suggesting that the abundance of nematode groups of high c–p values is useful indicators of heavy metal contamination. The "weighted faunal analysis" provided a better assessment of soil health condition than Maturity Index (MI) in situations where there were extremely low numbers of soil nematodes. Results showed that the effect of heavy metal contamination on soil nematodes might be strongly influenced by plants. Although the abundance of plant-feeding nematodes did not reflect the heavy metal conditions in the soil, it might be used as an index for assessing the soil remediation potential of pioneering plants. Patrinia villosa seems superior to Viola baoshanensis as a pioneer plant species for soil remediation based on analysis of rhizosphere nematode community.

1. Introduction

Nematodes are an important component of the soil ecosystem with profound effects on organic matter decomposition, nutrient transformation and energy transfer (Anderson et al., 1981; Freckman, 1988; Yeates and Bongers, 1999; Neher, 2001; Coleman et al., 2004). Many reports suggest that nematodes are useful bioindicators in soil and aquatic ecosystems. Their indicator value in relation to soil functioning or soil properties has been well illustrated (Goralczyk, 1998; Bongers and Ferris, 1999; Ekschmitt et al., 2001). Omnivore–predator nematodes are most sensitive to environmental disturbance (Bongers and Bongers, 1998; Georgieva et al., 2002), while bacterivorous and fungivorous nematodes respond at different rates to residue application under conventional tillage and no-till agroecosystems (Fu et al., 2000). Graphic representation of the relative biomass of bacterivorous, fungivorous and herbivorous nematodes has been used to explain structure of the soil food web in relation to resource inputs (Ferris and Bongers, 2006).

Soil nematodes are ubiquitous; they are well adapted to a wide range of environmental conditions; and they respond rapidly to disturbance. In addition, nematodes are transparent, their diagnostic internal features can be seen without dissection, and their life course is short. These features enhance their bioindicator potential for assessment of environmental health (Bongers and Ferris, 1999; Urzelai et al., 2000; Diemont et al., 2006; Ferris and Bongers, 2006). In recent years, the relationship between heavy metal contamination and soil nematodes has attracted increasing attention (Bardgett et al., 1994; Nagy, 1999; Korthals et al., 2000; Li et al., 2006).

Bongers (1990) developed the Maturity Index (MI) based on the weighted mean frequency of nematodes in five colonizer–persister (c–p) classes. The c–p scaling is based on functional responses of soil nematodes to resource and environmental change (Ettema and Bongers, 1993; Freckman and Ettema, 1993; Bongers, 1999; Bongers and Ferris, 1999). Ferris et al. (2001) developed a graphic faunal analysis system, which integrates the information of c–p scaling and trophic groups of nematodes and allows diagnosis of food web structure and soil health condition.

In this study, trophic groups and c–p scaling of soil nematodes were investigated under different vegetation types and in slag and sludge mine tailings. The objectives were: (1) to determine how soil nematodes respond to heavy metal contamination and (2) to test

* Corresponding author. Tel.: +86 20 37252592; fax: +86 20 37252615.
E-mail address: sfu@scbg.ac.cn (S. Fu).

how the diagnostic tools (i.e. Maturity Index, weighted faunal analysis) perform in assessing soil recovery from lead and zinc contamination in mine tailings.

2. Material and methods

2.1. Site description

The sampling was conducted in tailings of the Baoshan lead/zinc mine which is located in Guiyang County (E112°35′, N25°42′), Hunan Province, China, at an elevation of 400–650 m. High yielding Pb and Zn deposits have been extracted since the Tang dynasty (A.D. 618–907) but mining ceased in 1996 because of low production (Liu et al., 2004). Based on vegetation coverage and soil substrate, the mine tailing area can be roughly categorized into the following four subsystems: I: soil covered with *Viola baoshanensis* (≈30%) and *Patrinia villosa* (≈60%) plants by 10 years after mining ceased; II: soil covered (≈60%) with Polygonaceae plants within 5 years after mining ceased; III: mine tailing slag without vegetation; IV: mine tailing sludge without vegetation. For this study, we randomly selected three replicate sampling plots for each subsystem. The area of each sampling plot was approximately 400–600 m^2 (Fig. 1).

2.2. Soil analyses

Soil pH was determined in 1:2.5 (w/v) soil solutions, and soil moisture was measured by oven drying for 72 h at 105 °C. Soil organic C was determined by combustion of dry soil in a muffle furnace at 490 °C for 8 h. Total nitrogen was measured colorimetrically using the indophenol blue method (Keeney and Nelson, 1982). Total soil Pb and Zn concentrations were determined by inductively coupled plasma atomic emission spectrometry (ICP-AES) after acid digestion (McGrath and Cunliffe, 1985). Microbial Biomass C (C_{mic}) was determined with the fumigation extraction procedure (Vance et al., 1987).

2.3. Nematode sampling and analyses

Five composite soil samples were taken in each sampling plot at each subsystem site. The composite samples were comprised of three soil cores taken from the upper 0–5 cm soil. Because *V. baoshanensis* and *P. villosa* were the two dominant plant species in subsystem I, rhizosphere soils of the two plants were sampled separately in order to compare the soil nematode community structure between rhizosphere and non-rhizosphere soil. The rhizosphere soils of *V. baoshanensis* and *P. villosa* were designated as Ir1 and Ir2, respectively. Sampling was conducted on January 5, 2006. The mean annual temperature in this region is 17.2 °C, the mean rainfall is 1525 mm, and the mean evaporation is 701.2 mm.

For each composite soil sample, nematodes were extracted from 50 g of moist soil using the Baermann funnel method (Barker, 1985). After fixation in 4% formalin solution, nematodes were counted under an inverted microscope and the first 100 individuals encountered were identified into trophic groups and functional guilds. All nematodes were identified when the nematode were fewer than 100 individuals in a sample.

The Maturity Index (MI) was calculated as the weighted mean of the c–p values of the free-living nematodes (Bongers, 1990; Bongers and Bongers, 1998). MI is used to evaluate the functional responses of soil nematodes to resource and environmental change.

Nematode faunal analysis, based on a weighted matrix classification of life traits and feeding habits, provides qualitative measures of the soil food web. The structure index (SI) is based on the relative weighted abundance of disruption-sensitive guilds representing structure; the enrichment index (EI) is based on opportunistic bacterial- and fungal-feeding nematodes representing enrichment. At the base of both indices are taxa tolerant of adverse
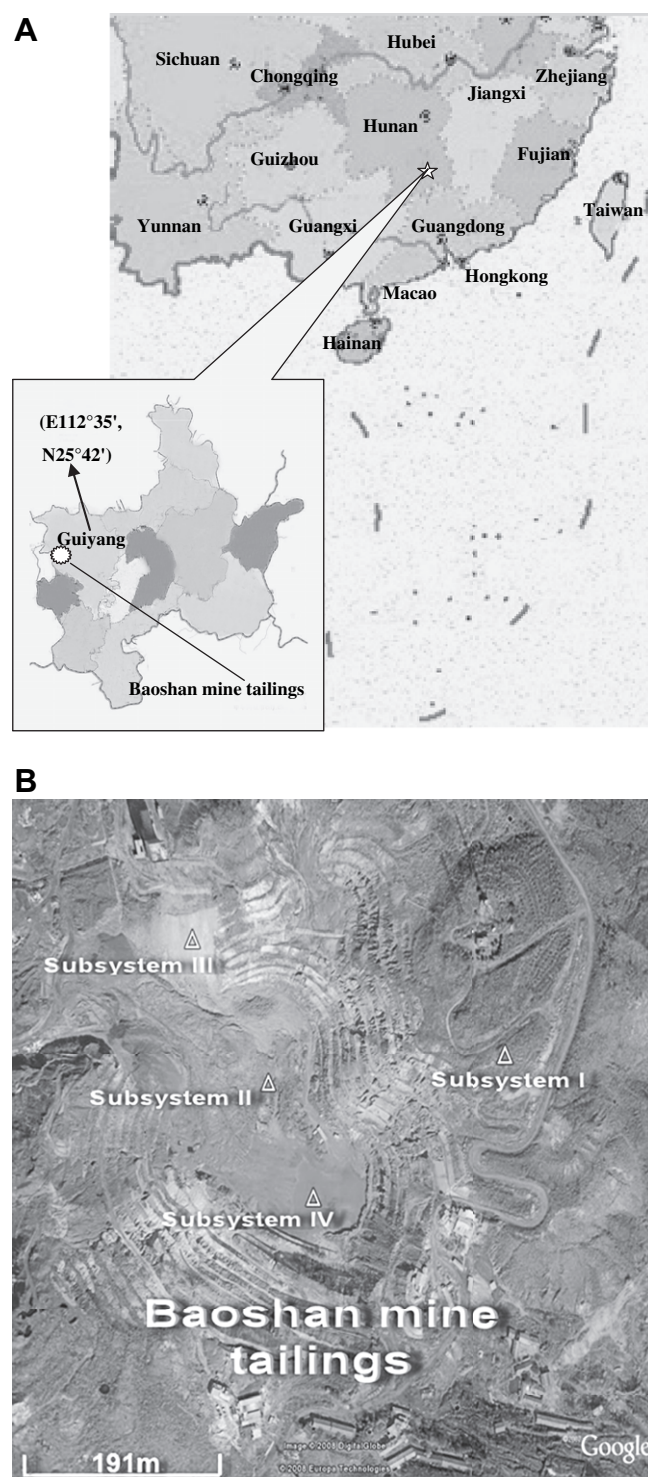
Fig. 1. Map of sampling site. (A): location of Baoshan mine tailings; (B): different subsystems in Baoshan mine tailings (modified from Google Earth).

conditions and basal to all nematode assemblages. When EI is plotted against SI for a sample of nematodes, the resulting graph can be divided into four quadrats which are descriptive of food web characteristics (Ferris et al., 2001; Ferris and Matute, 2003).

2.4. Statistical analysis

All data were analyzed by ANOVA using SPSS 15.0 statistical software (SPSS Inc., Chicago, IL). LSD was applied to test the

significant differences of variables between different subsystems. The abundance of nematode c–p groups and the environmental variables were analyzed by CCA (canonical correspondence analysis) using CANOCO software (ter Braak and Smilauer, 1998).

3. Results

3.1. Soil characteristics

Soil moisture was higher and soil pH was lower in subsystem IV than in other subsystems ($p < 0.05$). No significant differences of either soil moisture or soil pH were found among the other subsystems (Fig. 2A and B). Total soil organic C ranged from 0.28 to 1.59% among all subsystems, it was the lowest in subsystem III. Rhizosphere soil organic C was significantly higher than that of non-rhizosphere soil (Fig. 2C). Soil total N was only detectable in subsystem I, and there were no significant differences in physical and chemical characteristics of rhizosphere and non-rhizosphere soil (Fig. 2D).

3.2. Heavy metal concentrations in soils of different subsystems

The concentration of Pb was significantly higher in subsystem III than in other subsystems; it did not differ significantly among the other subsystems (Fig. 3A). The concentration of Zn was significantly higher in subsystem II than in other subsystems but was not significantly different among other subsystems (Fig. 3B). There were no differences in concentrations of Pb and Zn between rhizosphere and non-rhizosphere soil.

3.3. Soil nematodes and microbial biomass

Soil nematode density was significantly correlated with total soil organic C ($p < 0.01$, Figs. 2C and 4A). The density of total soil nematodes was significantly higher in vegetated subsystems (I and II) than in non-vegetated subsystems (III and IV), and it was higher in rhizosphere soil than in non-rhizosphere soil (Fig. 4A). The composition of nematode assemblages in terms of c–p values was similar in all vegetated subsystems where c–p2 nematodes (both bacteria- and fungi-feeders) were dominating. In non-vegetated subsystems, c–p2 (mostly bacteria-feeders) also dominated but no c–p4 and c–p5 nematodes were found (Table 1 and Fig. 4B). The MI values for rhizosphere soils Ir1 and Ir2 were 2.18 and 2.58, and for subsystems I–IV were 2.41, 2.16, 2.29 and 1.94, respectively. MI values for subsystems III and IV were the mean values calculated from less than half of the samples, while the MIs could not be calculated for the rest of samples due to the absence of nematodes (Table 1). Neither total soil nematode density nor MI was correlated with heavy metal Pb or Zn concentration.

Soil microbial biomass C (C_{mic}) was positively correlated with total soil organic C ($p < 0.05$). Rhizosphere C_{mic} was significantly higher than non-rhizosphere C_{mic}, but C_{mic} stays at the same level in the non-rhizosphere soil in all subsystems (Fig. 5). C_{mic}/C_{org} ratio from high to low was in the order of: Ir1 (0.024), III (0.021), Ir2 (0.020), I (0.013), II (0.009) and IV (0.005) (Figs. 2C and 5).

3.4. Nematode faunal analysis

The graphic presentation of "weighted faunal analysis" showed that only Ir2 was mapped in quadrat B with the rest mapped in quadrat D (Fig. 6). The EIs were 47.37, 50.94, 44.84, 25.80, 19.52 and 21.96 for Ir1, Ir2, I, II, III and IV, respectively. The SIs were 36.18, 57.78, 39.23, 28.58, 28.57 and 0 for Ir1, Ir2, I, II, III and IV, respectively. In the graph, EI and SI values were the means from uneven data points (5–15) for different subsystems because these indices could not be calculated if there were no nematodes or nematode groups of high c–p values in a sample. For example,
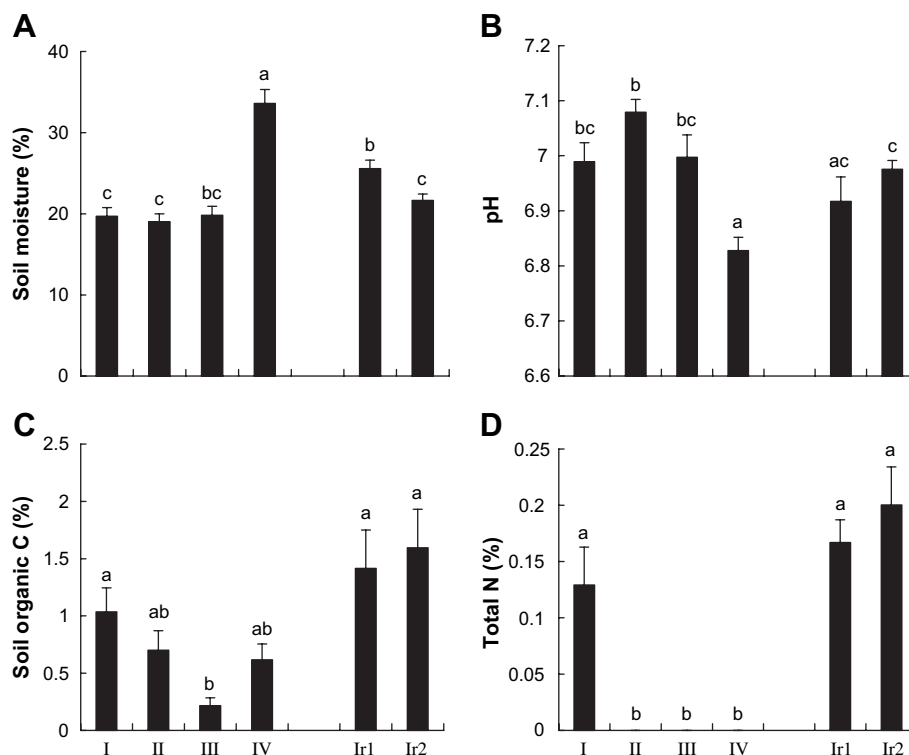

Fig. 2. Soil characteristics of different subsystems. (A): soil moisture; (B): soil pH; (C): total soil organic C; and (D): total soil N. I–IV refer to different subsystems of the sampling site, Ir1 and Ir2 refer to rhizosphere soils of two plant species in subsystem I; detailed descriptions can be found in Section 2. Each bar is the mean value and standard error of 15 replicates ($N = 15$). Different low-case letters above the bars indicate significant differences among the subsystems.
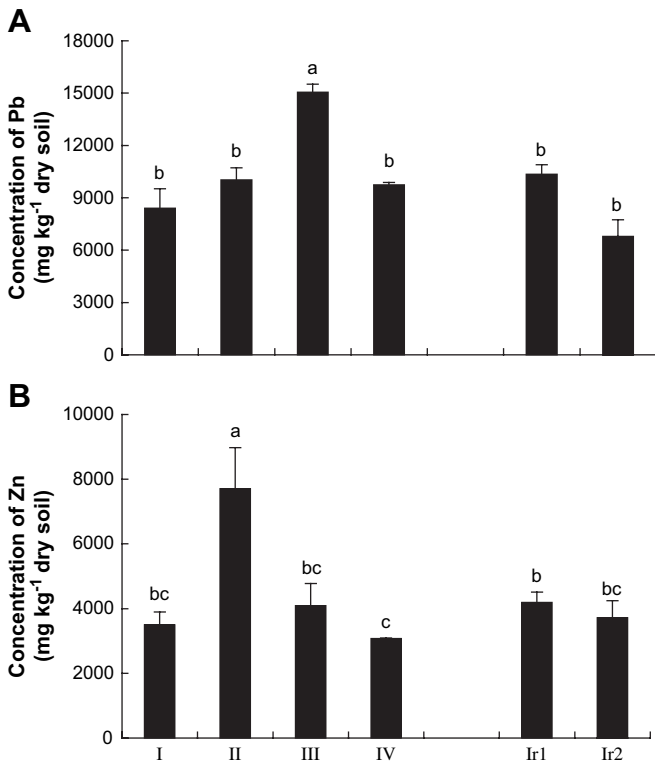
Fig. 3. Heavy metal concentrations in soils of different subsystems. (A): Pb and (B): Zn. For explanation of *x*-axis and the sampling replicates, refer to Fig. 2.

3.5. Ordination analyses

The ordination diagram (Fig. 7) is based on CCA of the absolute abundances of five nematode c–p groups and the data for six environmental variables in 90 samples. The eigenvalues of the first axis and the second axis were 0.051 and 0.034, respectively. The species–environment correlations for both the first and second axes were 0.60 and 0.65, respectively. The first axis explained 17.0% of the cumulative variance of the species data and 65.2% of the species–environment relationships; c–p3, c–p4 and c–p5 nematodes were negatively correlated with Pb and Zn concentrations (Fig. 7).

4. Discussion

4.1. Nematode as bioindicator of heavy metal contamination

In the present study, we did not find significant correlation between the density of total soil nematodes and heavy metal concentrations, but we found c–p3, c–p4 and c–p5 nematode groups were severely affected by heavy metal contamination. CCA analysis showed c–p3, c–p4 and c–p5 nematode groups were negatively correlated with heavy metal concentrations (Fig. 7). Our results were consistent with the findings of pioneer study by Zullini and Peretti (1986). They found that heavy metal (Pb) pollution did not affect the density of individuals, but significantly reduced the total biomass of nematodes, the number of species, and the Shannon diversity index. The nematode suborder Dorylaimina (mainly c–p4 and c–p5 omnivores) was the most sensitive group to Pb pollution in the study of Zullini and Peretti (1986). Similarly, Korthals et al. (1996) found that omnivorous and predatory nematodes were significantly affected by addition of 100 mg kg^{-1} Cu, Ni or Zn to the soil; c–p 4 and c–p5 groups usually include omnivores and predatory nematodes and they are considered to be highly sensitive to the environmental disturbance because of their large

nematodes were found in only 8 of the 15 samples in subsystem IV with only c–p1 and c–p2 groups but no c–p3 to c–p5 groups; therefore, EI was calculated based on eight samples and SI could not be calculated.
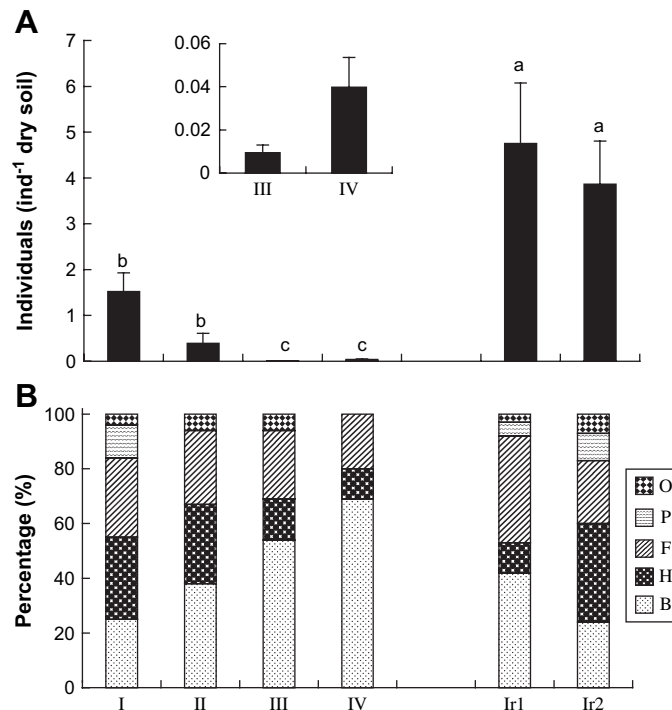

Fig. 4. Total soil nematode density and composition of trophic groups in different subsystems. (A): total soil nematode densities and (B): proportions of different trophic groups of soil nematodes. H, P, O, B and F refer to plant feeders, predators, omnivores, bacterial-feeders and fungal-feeders, respectively. For explanation of *x*-axis and the sampling replicates, refer to Fig. 2.

Table 1
Mean densities of non-plant-feeding nematodes in c–p groups and Maturity Index (MI) in different subsystems

	Mean densities (individuals^{-1} 100 g dry soil)					MI[b]
	c–p1	c–p2	c–p3	c–p4	c–p5	
I[a]	3.74 ± 1.12	99.0 ± 30.2	14.0 ± 5.05	14.2 ± 5.69	8.54 ± 3.37	2.41 ± 0.15
II	5.69 ± 2.73	110 ± 49.33	32.6 ± 19.2	8.23 ± 4.40	3.63 ± 2.02	2.16 ± 0.05
III	0	2.40 ± 1.28	0.34 ± 0.23	0	0	2.29 ± 0.18
IV	0.57 ± 0.42	4.19 ± 1.53	0	0	0	1.94 ± 0.05
Ir1	39.1 ± 14.3	500 ± 160	140 ± 50.4	25.4 ± 8.36	12.2 ± 4.29	2.18 ± 0.05
Ir2	11.9 ± 2.90	140 ± 32.6	47.5 ± 15.5	29.5 ± 8.27	17.9 ± 4.67	2.58 ± 0.12

[a] I–IV refer to different subsystems of the sampling site; Ir1 and Ir2 refer to rhizosphere soils of two plant species in subsystem I, detailed description is in Section 2.

[b] MI for subsystems III and IV was the mean values calculated from less than half of the samples, while the MI could not be calculated due to the absence of nematodes for the rest of samples.

body size, long life cycles and low reproduction rate (Bongers, 1990; Bongers and Ferris, 1999).

Although we did not find significant effect of heavy metal pollution on c–p2 nematodes, Sánchez-Moreno et al. (2006) found both c–p2 bacterial-feeding nematode (*Cephalobus persegnis*) and the c–p2 fungal-feeding nematode (*Aphelenchus avenae*) were negatively affected by Cu and Pb contamination in a short-term bioassay study. In contrast, Georgieva et al. (2002) reported that some c–p2 bacterial-feeding nematodes (i.e. *Eucephalobus*, *Acrobeloides*) increased in certain Cu and/or Zn treatments while some c–p4 bacterial-feeding nematodes (i.e. *Alaimus*) were negatively correlated with Zn and Cu concentrations. Such studies suggested that using the c–p2 nematodes to assess the effects of heavy metal contamination is in doubt, which is probably because this group is comprised of nematodes with different life strategies and resistant capacities to disturbance. It is worthy to notice that not only c–p4 and c–p5 nematodes but also c–p1 nematodes were absent in all 15 samples of subsystem III in our study; we postulated that heavy metal pollution might be responsible for the absence of c–p4 and c–p5 nematodes but poor nutrient status was likely the reason for the absence of c–p1 nematodes.

The MI has often been used to measure pollution-induced stress. Sanchez-Moreno and Navas (2007) found that the SI, Margalef's diversity index and the MI were appropriate indicators of the effect of soil pollution on nematode community. In the present study, the MI values were ranked in the following ascending order: IV (1.94), II (2.16), Ir1 (2.18), III (2.29), I (2.41) and Ir2 (2.58), which did not reflect the heavy metal contamination nor nutrient status of different subsystems. For example, the Pb concentration was the highest and the C_{org} was the lowest in subsystem III, but the MI value was medium. The absence of c–p1, c–p4 and c–p5 nematodes was the cause for a medium MI in subsystem III. Therefore, we conclude that MI is not a useful indicator of heavy metal

contamination in situations where there are extremely low numbers of soil nematodes. It is worthy to point out that in subsystems III and IV the MIs were calculated based on uneven number of samples because nematodes were absent in some samples where MIs cannot be calculated. The variability of sample information might have affected the indicative value of MI for soil health assessment.

We adopted the "graphic faunal analysis" system developed by Ferris et al. (2001) to assess if it can provide indicative information for soil health. We found that separate SI or EI was not indicative of soil health condition, but the graphic presentation which integrated SI and EI parameters provided clear information on that. In the graph, only Ir2 was mapped in quadrat B with the rest mapped in quadrat D, indicating the soil condition of the sampling site was both nutrient-poor and under high disturbance (high heavy metal contamination in this case). According to the notation of "weighted faunal analysis", the positions of I–IV in the graph were well matched with the general soil condition of the subsystems. Similarly, Hohberg (2003) found that the "weighted faunal analysis" was a good diagnostic tool to describe the impacts of various soil management practices on soil biological processes in an afforested coal mining site.

Soil microbial biomass (C_{mic}) is a small fraction of the soil organic carbon, and C_{mic}/C_{org} ratio rather than C_{mic} alone has been used as an indicator of soil health. Klumpp et al. (2003) reported a highly significant inverse relationship between heavy metal load and C_{mic}/C_{org} ratio. When the plant-available Cu concentrations were in the range of 0.18–99.78 mg kg^{-1} dry soil, C_{mic}/C_{org} ratio was in the range of 3.26–0.69%. In the present study, C_{mic}/C_{org} ratio was not indicative of general soil condition. More research is needed to demonstrate in what situations C_{mic}/C_{org} ratio can be used as an indicator for soil health.

4.2. Influence of plants

We found that the abundance of total nematodes, the composition of nematode community, the proportion and density of plant-feeding nematodes increased with the recovery of vegetation
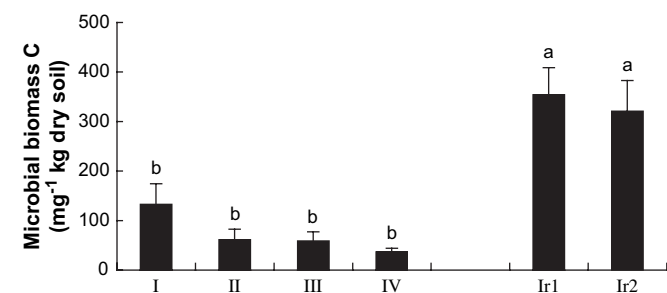

Fig. 5. Soil microbial biomass C (mean values and standard errors) of different subsystems. For explanation of x-axis and the sampling replicates, refer to Fig. 2.
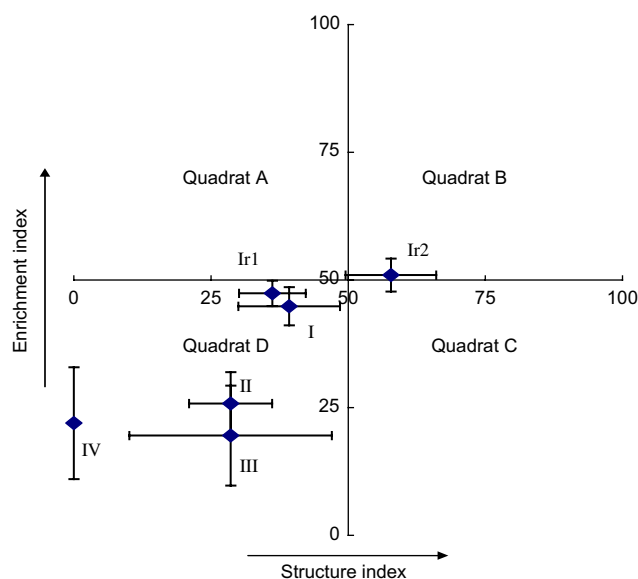

Fig. 6. Faunal analysis of different subsystems, means and standard errors for enrichment and structure indices. For explanation of I–IV, refer to Fig. 2. EI and SI values were the means of 5–15 points as the absence of some high c–p value groups led to zero values.
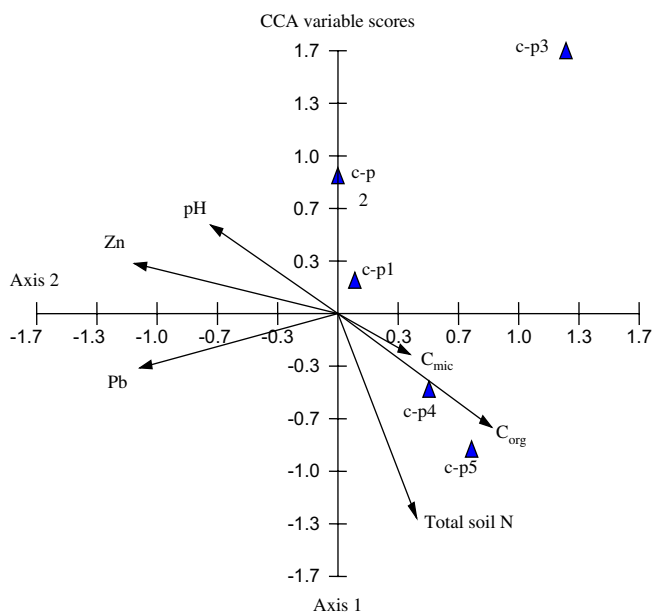
Fig. 7. Ordination diagram based on CCA of the data for the absolute abundance of the nematode taxa constituting c–p1, c–p2, c–p3, c–p4, c–p5, and the environmental variables: Pb, Zn, soil organic C (C_{org}), total soil N, soil microbial C (C_{mic}) and soil pH.

(Fig. 4A and B). Similar enrichment of nematode community structure occurred with vegetation recovery in industrial waste dumps (Hanel, 2004). Hohberg (2003) also found that the effect of mine spoil on soil biological succession is strongly modified by plant cover. Such studies may help to explain differences in response of nematodes and microbes among the subsystems of the present study.

Among the non-vegetated subsystems, soil microbial biomass and abundance of nematodes were low with very few omnivores and predators (Figs. 4 and 5). The physical and chemical properties of the largely "unknown" materials comprising mine tailings may exceed the tolerance capacity for survival of many soil organisms (Dmowska, 2001). Among the vegetated subsystems, total soil organic C, total soil N, microbial biomass C and soil nematode density were significantly higher in subsystem I than in subsystem II, indicating a better soil nutrient status and soil food web conditions in subsystem I. Although there were no differences in concentrations of Pb and Zn between rhizosphere and non-rhizosphere soil, the density of soil nematodes and soil microbial biomass were significantly higher in rhizosphere soil than in non-rhizosphere soil, indicating that microbial and nematode communities were affected by root exudates.

The positions of Ir2 and Ir1 in the graph of "weighted faunal analysis" suggested the soil food web was better developed in the rhizosphere of *P. villosa* than that of *V. baoshanensis* (Fig. 6). Although the *V. baoshanensis* is a plant that hyperaccumulates Cd, its usefulness in soil remediation is limited by its small size and slow growth rate (Liu et al., 2004). In contrast, *P. villosa* supported greater recovery of microbial and nematode communities probably due to its larger biomass and faster growth rate. In addition, the proportion and the density of plant-feeding nematodes were higher in the rhizosphere of *P. villosa* than that of *V. baoshanensis*, which was probably related to the differences in growth vigor of these two plants. Bongers and Ferris (1999) stated that the abundance of plant-feeding nematodes is largely determined by the community structure, biomass and vigor of plants. Based on the analysis of rhizosphere characteristics of nematode community, *P. villosa* seems superior to *V. baoshanensis* as a pioneer plant species for soil remediation.

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