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Response of nematode communities to reclamation of agricultural soils following degradation through brown coal strip-mining processes

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Summary

The study assessed the impact on the soil nematodes during the first 3-years after reclamation of a brown coal mining area. Samples were taken from 5 fields: 1 year before excavation, right at the beginning of reclamation (Yr 0), and in fields 1, 2 and 3 years after reclamation. A total of 31 families of nematodes were recorded and the nematode community of field Yr 0 was significantly different from that of other four sampling sites. Nematode abundance decreased after excavation and then began to return to initial community levels at each of the three reclamation sites with bacterivore nematodes recovering faster than the fungivores, omnivore-predators or the plant parasites. A gradual but consistent regeneration of the nematode community to its original structure was seen from the beginning of sampling and this recovery process was detectable over the three successive years of experimentation. Open-pit mining, therefore, drastically disturbed nematode community structure initially but the community was able to recover and stabilized quickly after reclamation.

Keywords: nematode community composition; maturity index; nematode fauna; succession, ecological regeneration

Introduction

Brown coal mining is conducted in an open pit mining facility in Nordrhein-Westfalen (NRW), Germany. The Rheinland-facility consists of three active mines covering a total area of 200 km^2 mainly located in the Cologne-Aachen-Bucht or lowland plain. Due to restricted environmental laws and the need to maintain land use systems, land on 28 km^2 of former open pit mining was in the process of being reclaimed. Approximately 50 % of this reclaimed land is targeted for agricultural use. Characteristics of reclaimed soils differ from those formed by natural pedological processes in that excavated material is technically dumped back into the open pit and then covered by top soil material. Physical soil properties were studied in German mines to determine the interrelations between reclamation techniques and possible soil disturbances (Dumbeck, 1996). Improving soil health and fertility is a main factor that favors optimum plant production in reclaimed soils. Nutrient contents (C and N) were found to return to 75 % of the original levels only after 15 years of reclamation in a long term investigation (Thum *et al.*, 1990). Studies on the recovery of biological properties of reclaimed soils were mainly focused on microbial activity and earthworm abundance, both of which were shown to recover gradually within a period of 5 to 20 years (Insam, 1992; Westernacher-Dotzler & Dumbeck, 1992).

Nematodes are the most abundant metazoan in soil and generally regarded as an important bio-indicator of soil status (Bongers & Bongers, 1998; Renčo, 2013). Many of these studies concerned the recovery or succession of nematode communities in natural ecosystems or in disturbed habitats such as dunes (Wall *et al*., 2002; Zhi *et al*., 2009), wetlands (Wu *et al*., 2002; Wu *et al.*, 2005), forests (Panesar *et al*., 2001; Sohlenius, 2002), grasslands (Wasilewska, 2006), agricultural fields (Ferris & Matute, 2003; Biederman *et al*., 2008; Dupont *et al*., 2009), a dumps (Dmowska & Ilieva-Makulec, 2006).

Very little attention, however, has been given to studies on the chronosequence from coal-mining dumps to agriculture soils using nematode community structure as a bio-indicator. Most of the studies focused on the succession of soil nematodes in afforestation of open pit mining operations. For example, Háněl (2001, 2002, 2008) and Hohberg (2003) reported the short and long term succession of soil nematode communities under different afforestation prac-

tices including different tree species and restoration years. The results showed that nematode communities were highly diversified and abundant in the early stages of succession and stable after over the long term following restoration.

The processes of degradation after excavation and subsequent restoration after open pit coal mining is a secondary succession process that can be interpreted with nematode community analysis. In the present study we hypothesized that 1) soil excavation would dramatically decrease soil nematode abundance and change community composition and 2) that reclamation would result in the restoration of soil nematode assemblage to preexcavation status. The objectives of this study were to determine the: 1) impact of excavation on soil nematode communities; 2) response of soil nematode communities to reclamation of agricultural soils following massive degradation; and 3) relationship between soil environmental change and nematode communities.

Material and methods

The brown coal mining study area was located at Niederrheinische Bucht (51°03' N, 6°32' S), Germany. The annual mean air temperature is 9.8 °C and annual precipitation and sunshine are 716 mm and 1500 – 1600 h, respectively.

The reclamation process in brown coal strip-mining results in the soil to a depth of approximately 16 m, being excavated and transferred to the side of the area being mined. This soil is then used later for recultivation to create productive agricultural soil (Fig. 1). The soil prior to excavation was primarily loess and was usually planted in a rotation of field crops and legumes.

Five fields were selected as sampling sites, which represented the experimental field sites: 1 year before excavation (Yr –1), right at the beginning of reclamation (Yr 0), and in fields 1, 2 and 3 years after reclamation (Yr 1, Yr 2 and Yr 3) (Fig. 1). Sampling was conducted in May 2007, October 2007 and July 2008. Over time the Yr 0, Yr 1, Yr 2, Yr 3 fields became correspondingly Yr 1, Yr 2, Yr 3, Yr 4 in 2008. For convenience, we still call them Yr 0, Yr 1, Yr 2, Yr 3. In each field, six randomly selected subplots of approximately 2 m^2 were selected and acted as replicates. Ten soil cores within the subplots were sampled with an auger to a depth of 30 cm. The soil samples of each subplot were homogenized to constitute a composite sample and put in a plastic bag. All samples were taken to the laboratory of the Soil Ecosystem Phytopathology and Nematology Department, INRES, University of Bonn, Germany, where they were kept at 4 °C until processing. A 200 g sample of soil was air dried for 14 days and sieved to < 2 mm for determination of soil physical and chemical characters.

Total C and N were analyzed after dry combustion with an elemental analyzer of Fisons NA 2000. Soil organic carbon was determined as total C minus inorganic C with gas volumetric method. Soil pH was measured in 0.01 mol L^{-1} $CaCl_2$ slurry (soil : solution = 1 : 2.5) with a glass electrode. The texture was analyzed by wet sieving and sedimentation. Soil moisture was determined by weight loss after over-drying 24 h at 105 °C.

Nematodes were extracted from 300 g soil samples using a modified elutriation, sieving and flotation methods (Ingham, 1994). The soil suspension was passed through 2 mm, 150 µm, 100 µm, 45 µm and 20 µm aperture sieves and the retained material on the 100 µm, 45 µm and 20 µm sieves were collected. The suspension was centrifuged at 1000 rpm for 6 min to concentrate the nematodes in the sample to the bottom of the tube and then the upper water was discarded carefully without disturbing the nematodes at the bottom. The nematodes in the sediment were then floated with sucrose solution (1.18 g cm^{-3}) and centrifuged again for 20 seconds to separate the nematodes from the soil remaining particles. The nematode-sucrose-water solution was quickly poured onto a 20 µm sieve and then the nematodes collected from the sieve surface in a tube with a fine stream of tape water.

Nematode suspensions were collected in a total volume of 45 ml and a 5 ml random subsample was then taken for counting. The number of nematodes was counted and converted to individuals per 100 g dry soil. For detailed identification, the nematode specimens were fixed with double strength triethanolamine-formalin (TAF) solution (70 ml formaldehyde + 20 ml triethanolamine + 910 ml distilled water) whose volumes equaled to that of nematode suspensions. Between 100 and 150 nematodes were selected randomly for identification to family level according to the previous descriptions (Bongers, 1988) under a compound microscope at 100 × or 400 × magnification. All taxonomic families were assigned to one of the four tropic groups: bacterivores, fungivores, herbivores, omnivore-predator as described by Yardim and Edwards (1998).

The data collected was then analysed for nematode community composition and diversity with the following three ecological indices: 1) H', Shannon-Weaver diversity Index, $H' = -\sum pi(\ln pi)$; 2) λ, Simpson dominance index, $\lambda = \sum pi^2$; 3) J', Pielou's eveness index, J' = H'/ln(S), where *pi* is the proportion of the *i*-th taxon and S is the total number of taxa identified. Nematode response to environment stress due to excavation and reclamation is determined with the maturity index (MI) for free-living nematodes and the plant parasitic index (PPI) (Bongers & Bongers, 1998). MI or PPI= $\sum vipi$, where *vi* is the c-p value, assigned by Bongers (1990), for free-living nematodes or plant parasitic nematodes of the *i*-th nematode family and *pi* is the frequency of the family in the nematode community. Impact of excavation and reclamation on ecosystem condition were evaluated with enrichment index (EI), structure index (SI) and basal index (BI) those based on functional guilds of nematodes (Ferris *et al.*, 2001). EI, SI, BI are calculated from weighted basal, enrichment and structural components (b, e, s) of the nematode assemblage: $b = (Ba_2 + Fu_2) \times W_2$, where $W_2 = 0.8$; $e = (Ba_1 \times W_1)+(Fu_2 \times W_2)$, where $W_1 = 3.2$; $s = (Ba_n \times W_n + Fu_n \times W_n + Om_n \times W_n + Pr_n \times W_n)$, where n = 3-5, $W_3 = 1.8$, $W_4 = 3.2$, $W_5 = 5.0$. Ba, Fu, Om, Pr indicate the abundance of bacterivores, fungivores,

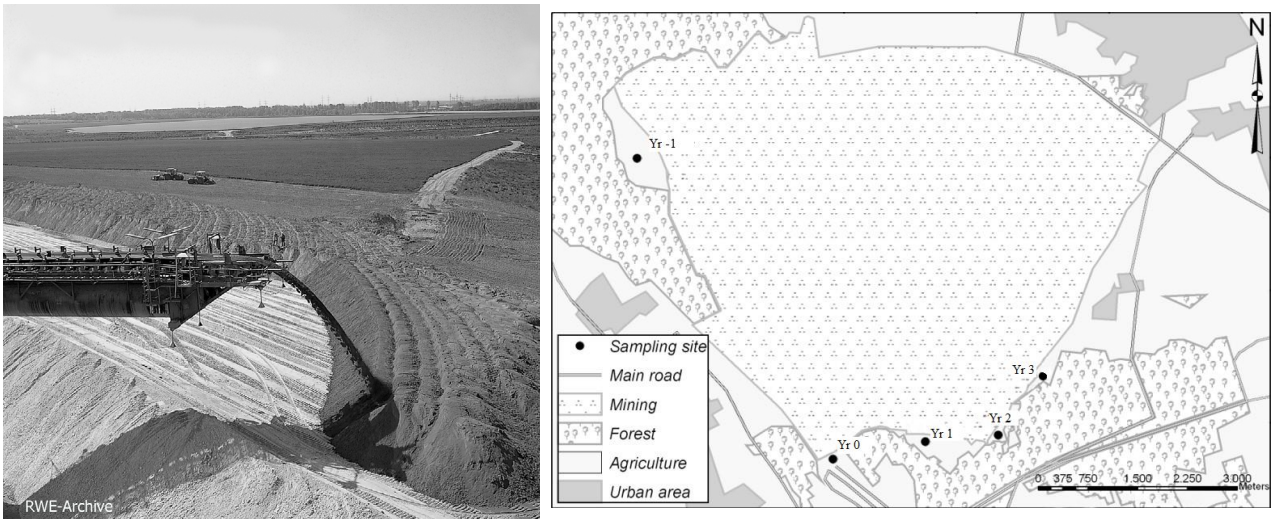

Fig. 1. Photograph in the left: stacker unit transfers excavated material to the new recultivation area. Carterpillers and tractor drawn machineries spread dumped material for land preparation. In the background first year after recultivation, drilled with lucerne for 3 years. Right: sampling sites at the brown coal mining. Yr –1, the field before excavation; Yr 0, the filed right after reclamation; Yr 1, 2 and 3 are fields after reclamation over 1, 2 and 3 years, respectively.

omnivores and predaceous nematode and n indicates the c-p value of nematode taxa. EI, SI, BI are calculated from the weighted faunal components: EI = 100 × (e / (e + b)), SI = 100 × (s / (b + s)), BI = 100 × (b / (e + b + s)).

One-way analysis of variance (ANOVA) was used to compare differences in soil characteristics, total nematode abundance, four trophic groups absolute/relative abundance and the ecological indices between the five sampling sites during the study period. The least significant difference (LSD) multiple comparison was performed to compare the means. For data that did not satisfy the assumption of equal variance, log(x + 1), square root transformation or 1 / x was used prior to analysis. If the data were still not homogenous, Tamhane's T2 multiple comparison was performed. All analyses were conducted with SPSS11.5 and the significant level is $P < 0.05$.

Non-metric multidimensional scaling (NMS) analysis, using the PC-ORD 5.0 program, was conducted to illustrate nematode community similarity along the reclamation sites. Mean nematode abundance was the input data. NMS autopilot mode was run at slow and thorough settings, and Bray-Curtis index was selected as a distance measure (McCune & Mefford, 1999). Canonical correspondence analysis (CCA) was conducted using CANOCO for windows 4.5 (Microcomputer Power, Ithaca, USA) in order to elucidate the nematode family distribution in relation to soil characteristics. Inter-species distance with Hill's scaling (ter Braak, 1986) was chosen and the data from the sampling time of May 2007 were log(x + 1) transformed prior to analysis.

Results

Soil characteristics, area and management practices of the 5 sampling sites are listed in Table 1. Excavation (Yr 0) and land reclamation (Yr 1, Yr 2 and Yr 3) did not alter the soil texture. Total N and organic C of Yr 0 field decreased significantly compare to the field before excavation (Yr –1), and then increased significantly after reclamation. However, no significant difference was observed between the years of reclamation and they did not recover completely to the original level of Yr –1. The change trend of total C was similar to that of total N whereas the organic C after reclamation was higher than that of Yr –1. Soil pH and $CaCO_3$ increased directly after excavation and reclamation and reached the highest level in Yr 0 and Yr 2, respectively.

A total of 31 nematode families were recorded at the three sampling times. Eight families were bacterivores, 6 were fungivores, 10 were plant-parasites and 7 were omnivores-

Table 1. Soil characters, area and management practices of sampling sites

Treatments	pH_{CaCl_2}	Total N (%)	Total C (%)	Organic C (%)	$CaCO_3$ (%)	Soil Texture			Area (ha)	Management practices
						Sand (%)	Silt (%)	Clay (%)		
Yr -1	6.82 c*	0.095 a	1.055 bc	0.92 a	2.23 c	6.16 a	90.60 a	3.24 a	about 2	Planted with cabbage
Yr 0	7.54 a	0.018 c	0.733 c	0.25 c	4.13 bc	7.39 a	89.02 a	3.59 a	1.86	Soil bulked directly after excavation
Yr 1	7.49 ab	0.025 b	1.388 a	0.48 b	7.53 a	4.91 a	92.09 a	3.00 a	13.92	Planted with lucerne for 1 year
Yr 2	7.47 b	0.031 b	1.498 a	0.57 b	7.76 a	7.02 a	88.95 a	4.03 a	22.46	Planted with lucerne for 2 years
Yr 3	7.52 ab	0.030 b	1.275 ab	0.49 b	6.57 ab	6.84 a	89.00 a	4.16 a	31.24	Planted with lucerne for 3 years

Yr –1 = 1 year before excavation; Yr 0 = right at the beginning of reclamation; Yr 1, 2 and 3 are fields 1, 2 and 3 years after reclamation

*The same letters in a column indicates no significant difference at $P < 0.05$

Table 2. Relative abundance (%) of various nematodes to the nematode assemblage at different reclamation sites during the study period

Trophic groups/family	May 2007					October 2007					July 2008				
	Yr -1	Yr 0	Yr 1	Yr 2	Yr 3	Yr -1	Yr 0	Yr 1	Yr 2	Yr 3	Yr -1	Yr 0	Yr 1	Yr 2	Yr 3
Bacterivores	**9.5c***	**24.3bc**	**42.5a**	**41.4a**	**28.8a**	**28.9b**	**12.4c**	**56.1a**	**45.8a**	**25.3bc**	**74.7a**	**77.6a**	**37.6b**	**77.3a**	**70.5a**
Alaimidae	0.0	0.0	0.0	1.3	0.2	0.2	0.2	0.0	0.0	0.2	0.3	0.0	0.0	0.0	0.0
Cephalobidae	4.3	9.2	19.5	21.1	10.4	11.3	6.7	20.4	25.4	14.2	20.5	45.0	29.8	29.4	25.4
Diplogasteridae	0.2	0.0	0.2	0.0	0.0	0.0	0.0	0.2	1.0	0.7	0.0	0.0	0.0	0.0	0.0
Leptolaimidae	0.5	0.0	10.8	5.7	8.5	0.5	0.0	0.0	0.3	0.8	0.0	0.0	0.0	0.0	0.0
Panagrolaimidae	0.8	3.5	1.5	0.8	1.3	3.8	0.8	2.8	2.4	2.6	0.0	0.3	3.3	29.0	2.1
Plectidae	0.0	0.0	1.2	0.7	1.5	0.0	0.0	0.2	0.0	0.2	1.2	1.4	0.0	0.0	0.8
Prismatolaimidae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0
Rhabditidae	3.7	11.6	9.3	11.8	6.9	13.1	4.7	32.6	16.6	6.7	52.6	30.9	4.5	18.7	42.2
Fungivores	**38.0a**	**24.7ab**	**26.5ab**	**15.4b**	**17.8b**	**36.7a**	**23.6a**	**12.4b**	**10.9b**	**25.4a**	**1.9c**	**1.1 c**	**20.7a**	**14.2ab**	**7.9bc**
Anguinidae	35.0	17.0	8.2	3.0	4.0	34.7	13.7	5.2	2.8	15.3	0.5	0.4	1.3	1.6	1.3
Aphelenchidae	0.0	2.3	0.3	0.2	0.5	0.2	0.3	0.2	0.0	0.0	0.9	0.5	2.3	7.4	3.8
Aphelenchoididae	2.8	5.4	17.3	11.8	12.6	1.8	9.4	7.1	8.1	10.1	0.3	0.3	16.7	5.3	2.9
Diphtherophoridae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.2	0.0	0.0
Paraphelenchidae	0.2	0.0	0.7	0.5	0.7	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Tylencholaimidae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0
Plant-parasites	**50.8a**	**50.5a**	**22.3b**	**29.1b**	**25.7b**	**32.3bc**	**59.1a**	**26.1c**	**40.7bc**	**43.4b**	**22.0ab**	**18.7ab**	**29.2a**	**4.8 b**	**17.2ab**
Belonolaimidae	6.7	0.6	12.3	2.5	5.2	5.1	4.1	7.2	4.4	9.3	0.0	0.0	0.0	0.0	0.0
Criconematidae	0.0	0.0	0.0	0.0	0.0	0.0	12.8	0.5	1.8	14.1	0.2	0.2	0.0	0.0	0.0
Dolichodoridae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	10.9	1.0	4.2
Heteroderoidea	38.2	6.7	0.8	0.7	0.8	1.1	0.5	0.5	0.0	0.3	3.2	0.2	1.9	0.0	0.0
Hoplolaimidae	1.8	11.2	2.8	0.2	0.0	4.6	0.0	0.2	0.0	0.3	4.6	4.7	0.3	0.2	0.4
Meloidogynidae	0.0	11.7	3.0	3.0	6.9	7.3	22.4	0.8	0.8	1.2	0.0	0.0	0.0	0.0	0.0
Paratylenchidae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.0	2.0	6.3	0.0	4.3
Pratylenchidae	1.3	4.3	0.5	0.2	0.3	4.0	14.5	1.4	6.7	2.4	0.2	5.5	0.2	0.8	3.5
Psilenchidae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0
Tylenchidae	2.8	16.0	2.8	22.6	12.5	10.2	4.8	15.6	27.1	15.7	8.9	5.8	9.6	2.6	4.8
Omnivore-predators	**1.7c**	**0.6c**	**8.7b**	**14.1b**	**27.7a**	**2.1a**	**4.9a**	**5.4a**	**2.6a**	**5.9a**	**1.4b**	**2.6b**	**12.6a**	**3.6b**	**4.4b**
Anatonchidae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0
Aporcelaimidae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.4	5.8	0.2	1.1
Dorylaimidae	1.7	0.6	8.7	14.1	27.7	1.3	4.4	5.4	2.6	5.9	0.2	0.0	1.7	1.4	0.8
Mononchidae	0.0	0.0	0.0	0.0	0.0	0.8	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Nordiidae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	2.2	4.9	2.0	2.2
Nygolaimidae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.3
Qudsianematidae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0

Yr -1 = 1 year before excavation; Yr 0 = right at the beginning of reclamation; Yr 1, 2 and 3 are fields 1, 2 and 3 years after reclamation.
*The same letters in a row indicates no significant difference at $P < 0.05$.

predators (Table 2). Of all nematodes, Cephalobidae and Rhabditidae were found to be the dominate families, whose relative abundance were 16.7 % and 16.5 % respectively. However, the dominant family groupings were different among the different sampling sites and at the different sampling dates. For the May, 2007 sampling, the dominant nematode family proportion changed over 10 % with excavation and reclamation. Before excavation (Yr –1), the relative abundance of Anguinidae and Heteroderidae were 35.0 % and 38.2 % respectively, which indicated that the cabbage field suffered serious plant-parasite nematode infestations. When excavation began, nematode community composition changed significantly. Nematode families of Rhabditidae, Anguinidae, Hoplolaimidae, and Tylenchidae were the dominant groups whose relative abundance were over 10 % as well as proportion of Cephalobidae was close to 10 %. The relative abundance of each family in Yr 1, Yr 2 and Yr 3 were similar but more dispersive when compared to those of Yr –1 and Yr 0. In October 2007, similar trends were observed.

NMS ordination depicted the responses of the nematode community to excavation and reclamation (Fig. 2). In the May 2007 sampling, five sites can be assigned to three groups according the distance of sites: before excavation (Yr –1), right after reclamation (Yr 0) and in reclamation (Yr 1, Yr 2, Yr 3). The distance between reclamation sites and Yr –1 was near than the distance between reclamation sites and Yr 0. In the October 2007 sampling, five sites can be assigned to two groups: Yr 0 and the other four sites. Of the four sites, Yr 1 was close to Yr 2 and Yr 3 was close to Yr –1. In 2008, Yr 0, Yr 1 and Yr 3 clustered with an approximate equilateral triangle and the other two sites clustered. Considering the distribution of 15 points, it can be seen that the reclamation sites and Yr –1 were concentrated together. NMS ordination showed that excavation altered seriously the nematode community and that with time the nematode community was restored to near original levels during the reclamation process.

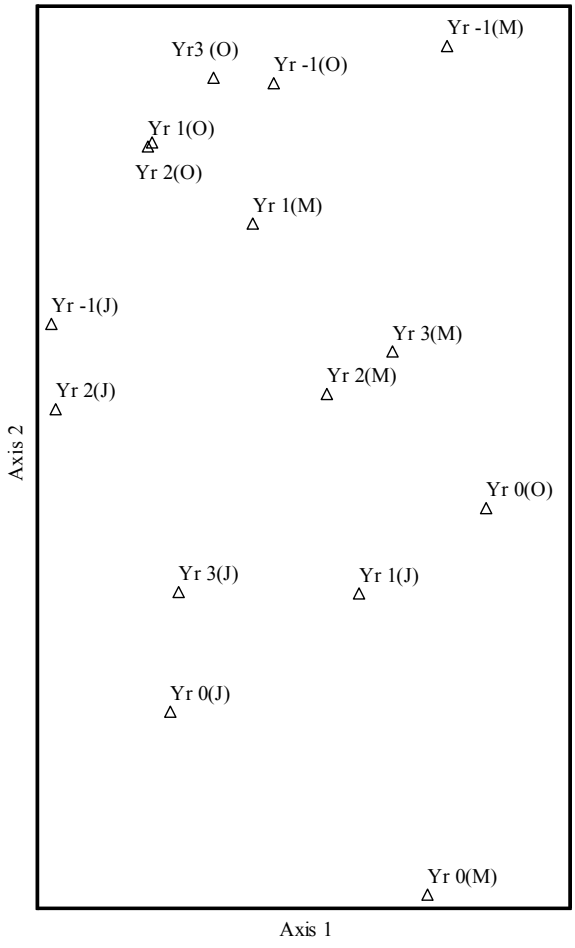

Fig. 2. Non-metric multidimensional scaling ordination of nematode community composition at different reclamation sites. (Yr –1: the field before excavation; Yr 0 : the field right after reclamation; Yr 1, 2 and 3 are fields after reclamation over 1, 2 and 3 years, respectively; M: May 2007; O: October 2007; J: July 2008.)

Of the five sampling sites, Yr 0 had the lowest total nematode number, so did for the four trophic groups. The total number was significantly lower than those of Yr –1 during the three sampling times (Table 3). The three reclamation sites had intermediate numbers with the exception of Yr 3 in October 2007 and Yr 2 in July 2008. During the three sampling times, the bacterivore nematodes were the most abundant group and increased dramatically in Yr 1 to a point that the numbers exceeded those of Yr –1. They then decreased at Yr 2 and Yr 3 in the sampling of May 2007, as well as in the sampling of October 2007 (Table 3). In July 2008, the bacterivores reached their maximum in Yr 2. The fungivores nematode numbers in July 2008 were lower than the other two sampling times. Nematode abundance of the reclamation sites did not recover to the level of Yr –1 in the two sampling times during 2007, but were higher than that of Yr –1 in July 2008 (Table 3). Plant-parasite nematodes were the predominant group in the 2007 sampling and the differences between the 5 sampling sites were similar to that seen in total nematode abundance (Table 3). The omnivore-predator group was scarce, and recovered after excavation to reach or exceeded the level of Yr –1 (Table 3). Furthermore, the bacterial-feeder and omnivore-predator nematodes increased quickly and even exceeded that in Yr –1. Since omnivore-predator nematodes had very low relative abundance, it can be inferred that the bacterial-feeder nematodes resulted in the quick recovery in overall nematode abundance.

The data on nematode community composition were evaluated with three ecological indices of H', λ and J' (Table 3). The results showed that the H' of the three reclamation sites led to no significant differences between the sites but that it was higher than that of Yr 0 and Yr -1 in May 2007. The H' difference among the five sites of 2008 was consistent with that of bacterial-feeder nematodes abundance in the sampling of May 2007. The change trend of λ was opposite to H'. Regarding the J', the change trend of May 2007 and July 2008 was consistent, which the reclamation sites were higher than Yr –1 but lower than Yr 0. All the three indices indicated that excavation alter sharply community composition. However, the nematode community was restored quickly to the earlier level following the reclamation process.

Table 3. Abundances (individuals per 100 g dry soil) of nematode trophic groups and ecological indices for nematode community at different reclamation sites during the study period

	May 2007					October 2007					July 2008				
	Yr -1	Yr 0	Yr 1	Yr 2	Yr 3	Yr -1	Yr 0	Yr 1	Yr 2	Yr 3	Yr -1	Yr 0	Yr 1	Yr 2	Yr 3
Abundance															
BF	39.2b	3.3b	114.7a	36.1b	35.5b	123.8a	9.7b	215.6a	159.3a	112.6ab	120.4a	19.1c	17.7bc	136.2a	33.3b
FF	155.1a	2.2c	60.2b	13.3c	21.6bc	139.4a	12.3c	40.1b	37.7b	88.6a	2.3bc	0.7c	9.8a	16.2a	4.3b
PP	215.0a	5.1c	41.6b	26.7b	32.3b	109.5a	24.5b	91.5a	133.4a	201.0a	28.7a	3.7b	13.0b	4.9b	6.1b
OP	7.5b	0.1c	20.6ab	12.6ab	33.8a	7.6b	5.2b	17.5ab	9.3b	23.7a	3.5ab	1.3b	5.4a	5.9a	1.9ab
TA	416.7a	10.7c	237.0b	88.6b	123.3b	380.3a	51.7b	364.6a	339.7a	425.9a	154.8a	24.8b	45.9b	163.2a	45.5b
Ecological indices															
H'	1.56b	1.65b	1.95a	1.97a	2.04a	1.91a	1.78a	1.80a	1.75a	2.01a	1.36ab	1.09b	1.89a	1.48ab	1.56ab
λ	0.30a	0.24b	0.19b	0.18b	0.17b	0.22a	0.24a	0.22a	0.23a	0.18a	0.38ab	0.45a	0.20b	0.33ab	0.31ab
J'	0.67c	0.89a	0.81ab	0.80b	0.82ab	0.77a	0.76a	0.80a	0.78a	0.81a	0.62b	0.83a	0.78ab	0.65ab	0.69ab
MI	1.98bc	1.71c	2.24b	2.33b	2.77a	1.82ab	2.04a	1.69b	1.75b	2.05a	1.39b	1.69b	2.33a	1.59b	1.61b
PPI	2.80a	2.67a	2.27b	2.14b	2.32b	2.47b	2.77a	2.11c	2.26bc	2.36bc	2.76a	2.88a	2.76a	2.98a	2.75a
SI	15.8b	3.7 c	51.4 a	62.6 a	78.3 a	16.1b	25.5ab	36.0a	19.4ab	35.7a	23.1a	22.0a	52.2a	26.5a	28.6a
EI	57.4a	66.2a	60.2a	61.7 a	59.7 a	61.7b	57.7b	77.4a	65.5ab	54.8b	88.2a	64.6ab	48.0b	77.1a	78.6a
BI	39.2a	33.2ab	27.3abc	21.3bc	14.6c	35.5a	35.4a	19.1b	30.7b	35.8a	11.4a	34.1a	32.9a	20.5a	20.3a

Yr -1 = 1 year before excavation; Yr 0 = right at the beginning of reclamation; Yr 1, 2 and 3 are fields 1, 2 and 3 years after reclamation; BF = Bacterivores; FF = Fungivores; PP = Plant parasites; OP = Omnivores-predators, TA = Total nematode individuals; H' = Shannon-Weaver diversity Index ; λ = dominance index; J' = evenness index; MI = maturity index, PPI = plant-parasitic maturity index; SI = structural index; EI = enrichment index; BI = basal index.

*The same letters in a column indicates no significant difference at $P < 0.05$.

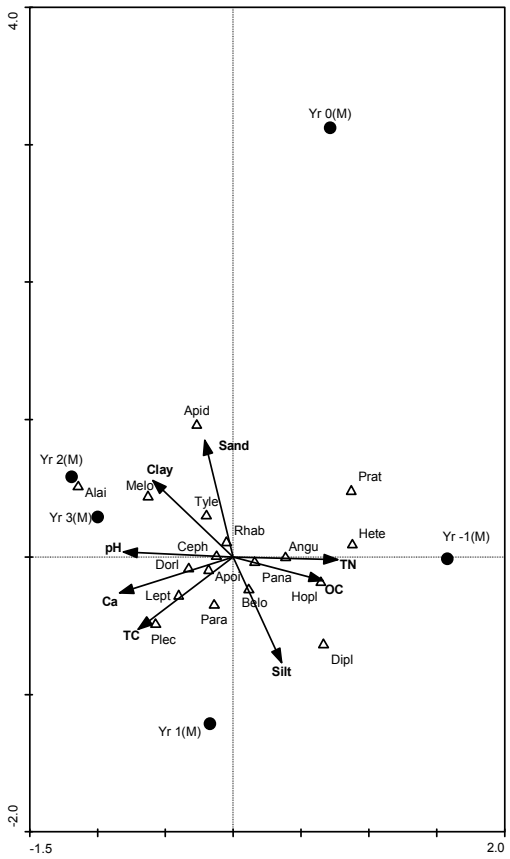

Fig. 3 Canonical correspondence analysis bi-plot of nematode family (△, sampling sites (●) and soil characters (→). Yr -1: the field before excavation; Yr 0: the field right after reclamation; Yr 1 ,2 and 3 are fields after reclamation over 1, 2 and 3 years, respectively. Sand: sand (%); Silt: silt%; Clay: clay(%); TN: total N;TC: total C;OC: organic C;Ca,$CaCO_3$; Soil nematode family abbreviated with the former 4 letters, see full name in table 2, except Aphelenchoididae and Aphelenchoididae abbreviated with Apid and Apoi.

In the May 2007 sampling, MI of Yr 0 was significantly lower than the three reclamation sites and also lower than Yr –1. However, there was no obvious trend in the other two sampling times. The MI of Yr 3 from the October 2007 sampling and Yr 1 of the 2008 sampling was the highest compared with the other sampling times. (Table 3). Most PPI were inversely related to MI especially in the sampling of May 2007 (Table 3). In the samples taken in 2007, PPI decreased significantly in the reclamation sites because the relative abundance of Heteroderoidea, Hoplolaimidae, Meloidogynidae decreased greatly. However, in 2008, there was no significant difference between the five sites (Table 3).

In the three sampling times, the SI of the three reclamation sites was not significantly in between, whereas higher than that of Yr –1, especially reached the significant level in the May 2007 sampling. The SI of Yr 0 from the May 2007 sampling and 2008 sampling was lower than that of the other four sampling sites. There were no obvious trends among the EI of the five sampling sites in the three sampling times. The BI of the three reclamations sites was lower than that of Yr 0 in the three sampling times except the SI of Yr 3 the October 2007 sampling.

Canonical correspondence analysis (CCA) was used to explore the relationship between soil environment, nematode family distribution and successive stage in the samplings of May 2007 (Fig. 3). The vectors for sand percentage, clay percentage, pH value, $CaCO_3$, total carbon were closed, which closely associated with the families of Rhabditidae, Tylenchidae Meloidogynidae, Cephalobidae, Dorylaimidae, Aphelenchoididae, Leptolaimidae, and Plectidae. Similarly, the vectors for total N, organic carbon, and silt lied near each other and were closely related with the families of Heteroderidae, Anguinidae, Panagrolaimidae, Hoploaimidae and Belonolaimidae. The length of 8 soil character vectors was similar, which showed that each soil characteristic had similar effects on the nematode community. Considering the relationship between nematode families with successive stage, it can be concluded that most nematode families had the lowest relative abundance in Yr 0, because the distance between them and Yr 0 was farther than the distance between the nematode families at the other 4 sites.

Discussion

The current investigations showed that excavation significantly decreased C and N and that the levels were subsequently restored in the 3-years of reclamation. The results were similar to that observed in the research conducted by Dumbeck (1996). Reclamation also led to an increase in pH, which was similar to that results obtained during the first 18 years of reclamation of coal-mining dumps near Cottbus, Germany (Háněl, 2002).

In this study, the 31 identified nematode families concurred with most agricultural soils (Yeates & Bongers, 1999). Excavation disturbed the soil environment and resulted in lowering the total nematode numbers in Yr 0 that ranged from 11 to 52 with an average of 29 individuals per 100 g dry soil. The reduction was lower than detected in soil treated with systemic nematicides (> 100 individuals per 100 g dry soil) (Timpera *et al*., 2012) or with a soil fumigant (> 60 individuals per 100 g dry soil) (Sánchez-Moreno *et al*., 2010). The mean number of nematodes in the reclamation sites was 204 individuals per 100 g dry soil, which was lower than that observed in long-term reclamation of coal-mining dumps soil near Cottbus (> 500 individuals per 100 g dry soil) (Háněl, 2002). Comparing results from Yr –1 and Yr 3 across all sampling dates and with four trophic groups in May 2007 demonstrated significant differences as shown in table 3. A similar trend in trophic groups was also seen in October 2007 and July 2008. These trends are probably related more to sampling date rather than to the years after reclamation, and were also seen in the studies of Háněl (2001). This indicates that sampling date changes in trophic groups was significant and concealed the effects of length of restoration.

Bacterial-feeding nematodes were the dominant groups detected and the results are in agreement with previous

reports conducted with soil agro-ecosystem (Yeates & Bongers, 1999). The numbers of bacterivore nematodes in the reclamation sites increased drastically and resulted in the higher total number of nematode detected after reclamation. However, fungivore nematodes increased very slowly over time. The main reason for these differences was the fact that the two groups have different sources of food intake and the availability of their food source changes as soil organic matter is decomposed over time. When lucerne was the source of the organic matter in the soil (Table 1) it released primarily labile organic matter which is first exploited by bacteria (Holtkamp *et al*., 2011). Therefore, bacteriovore nematodes have an advantage and their numbers increase rapidly.

The bacterivore nematodes in the present study were mainly composed of the Ba 1 and Ba 2 guild (Bongers & Bongers, 1998). The Ba1 guild is composed of the bacterivore nematodes families: Rhabditidae, Diplogasteridae and Panagrolaimidae and the Ba 2 guild of bacterivore families is represented by Cephalobidae and Plectidae. Both guilds are enrichment-opportunists or general-opportunists. These families respond rapidly to increases in bacterial densities in the soil and are relatively tolerant to soil ecosystem disturbances (Bongers & Bongers, 1998; Ferris *et al*., 2001). The recalcitrant organic matter remaining in the soil after bacterial decomposition is exploited by saprophytic fungi and shift in microbial community structure leads to the subsequent increases in the abundance of fungivore nematodes. These obtained in the present study are similar to those observed by Ferris and Matute (2003), who found that enrichment-opportunist and general-opportunist bacterivore nematodes increased rapidly in response to low C/N and simple organic materials (compost and alfalfa). They also reported that fungivore nematodes increased rapidly in those soils amended with higher C/N and more complex materials (wheat straw).

The fact that there was a sudden increase in plant-parasite nematode levels indicates that the lucerne may have been seriously infected with these parasites prior to incorporation. Plant parasite nematodes also increased quickly that maybe the lucerne was infected seriously. Omnivore-predator nematodes occupy a higher trophic level (Holtkamp *et al*., 2011) and any increase in number is slower than that of the other three trophic groups observed in this study.

The H' ranged from 1.09 to 2.04 with an average of 1.72, which were lower than Háněl's study on the open-cast coal-mining dumps where he reported the H' of most successive stages to be over 2 (Háněl, 2001; Háněl, 2002). Reclamation increased the H' compare to excavation which is in accord with the study of Háněl (2002). Usually, the λ is inversely related to H', thus reclamation decreased λ. In addition, λ value in reclamation sites (0.17 – 0.33, mean = 0.22) was found to be higher than some agricultural soils that received moderate disturbance due to fertilization (0.08 – 0.22, mean = 0.14) (Li *et al*., 2007; Hu & Qi, 2010). Similar results were obtained with disturbances due to tillage and residue management (0.20 – 0.29, mean = 0.24) (Zhang *et al*., 2012). The J' value in reclamation (0.77) was also similar to that of soil under long-term application of different types of organic manure (J' = 0.81) (Hu & Qi, 2010) where reclamation decreased J' twice at the three sampling times. The change trend of the three diversity indices demonstrated that the nematode community in reclamation sites were getting more diverse and evolving to the community of the agricultural soils.

The MI family including MI, ∑MI, MI25, ∑MI25, PPI, PPI / MI was used to analyse the successive stages of a soil community (Neher and Darby 2006). In the present study the mean value of MI was 1.9 and lower than the data reported by Háněl (2002), whereas the mean PPI was 2.8 and higher than that report by Háněl (2002). In this study, the MI and PPI were inversely related which in accord with the study of Bongers *et al*. (1997). In the three sampling sites, most of the reclamation sites showed an increase in the MI and a decrease in the PPI compare to Yr 0 and Yr –1. This also demonstrated that the nematode community during reclamation were being restored to former levels.

SI indicates the soil food web connectance and length, and higher value means more abundance of trophic linkages (Ferris, 2001). In the three sampling times, the SI of three reclamation sites exceed that of Yr –1 indicated soil food web was more structured, which agreed with the analysis of diversity indices. In addition, the SI in our agriculture soils (3.7 – 78.3, mean = 33.1) was lower than the value (4.5 – 99.4, mean = 72.7) of 20-32-year-old afforested soils (Háněl, 2008), which indicated soil food web structure in our study need more time to restore. EI reflects the food web response to available resources (Ferris, 2001). Almost all the value of EI in our study was more than 50, which inferred that the soil food web was N-enriched (Ferris, 2001). BI is an indicator of a food web diminished by stress (Ferris, 2001). In this study, the BI of reclamations sites was lower than Yr 0 indicated that the soil food webs of reclamation sites were more diminished compared to excavation sites.

With regards to the nematode family-environment correlation in this study, it was shown that the nematode community was affected by all soil characteristics (Fig. 3). The results were similar to those found in the study of Wu *et al*. (2005). However, the relationship between specific soil characteristics and nematode family was different between the three studies. Other studies also showed similar results in correlation coefficients between nematode genus/groups and soil characteristics (Liang *et al*., 2009; Hu & Qi, 2010). The relationship between nematode family and successive stage showed that most families had a low relative abundance in the massive degradation stage and this was restored in the recovery stage. The findings were again similar to those in the study of Sánchez-Moreno *et al.* (2010).

In this study, just three years of reclamation were monitored, and therefore should be regarded as a short-time successive since long-time successive investigations last several decades. The analysis results of nematode abun-

dance, composition, guilds showed that the soil nematode community during the three years successive process were similar to each other. This indicated that the recovery of soil nematode community abundance was relatively fast. The results obtained by during 40 and 70-years successive process investigations from two open-cast coal-mining dumps by Háněl (2001; 2002) also showed that the nematode community abundance recovered quickly but also became more diverse over years. This indicates that the nematode communities in our study were in an early recovery stage and required more time to recover original levels of diversity.

In conclusion, the analysis of nematode community composition, abundance and ecological indices indicated nematode community was drastically changed after excavation and that the process should be regarded as intense disturbance. However, attempts to return the soil to conditions conducive for agriculture resulted in quick restoration as seen in nematode community recovery within a 3-years reclamation process.

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