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# Nematode Assemblages Development Twenty-One Years after the Introduction of Meadow Soil into Bare Post Mining Spoil Heap

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**Abstract:** The revitalisation of soil fauna in post-mining soils is one of the ways in which we can slow down biodiversity loss. To investigate the effect of a meadow soil transplanted directly into the spoil substrate on the colonisation of a spoil heap, we used nematodes as a tool for an assessment of success in soil fauna recolonisation. Three blocks of meadow topsoil ($10 \times 3 \times 0.4$ m) were dug out and transported as intact as possible into a bare substrate of spoil heaps near Sokolov (Czech Republic). The soil samples were taken at the beginning of the experiment (1997) and then, after 19 years (2016), were transported into blocks (B) in their surroundings in a 2 m distance (I) and, finally, 30 m from the transported blocks as a control (C), to compare and assess the complexity of soil food web. Nematode total abundances were highest in B plots and lowest in the nearby I plots at the beginning, whereas later, their abundances were highest in I plots and lowest in the control (C) plots. However, due to the high variability, abundances were statistically insignificant. The trophic composition of I plots became similar to the composition in B plots in the late phase; however, the high occurrence of predators in C plots showed a running succession even after 19 years. Our results together with previous works from the same experiment support the findings that the level of soil development has a larger impact on recolonisation by soil fauna than the migration barrier itself.

**Keywords:** spoil heaps; succession; soil fauna; restoration; soil introduction

## 1. Introduction

Mining and open cast mining, in particular, cause a severe destruction of ecosystems. Directly affected ecosystems are either completely erased by mining or covered by overburden dumped in heaps. This removes habitats as well as the soil and communities within. As a by-product, heaps of spoil material are created, and these heaps can serve as surrogate locations where previously destroyed or completely new habitats can be restored. Fresh heap substrates often differ substantially from contemporary soils. They are characterised by an extreme pH (either highly acidic or alkaline), extreme texture (too sandy or with a too high content of clay) and sometimes even toxic conditions [1,2]. Moreover, species colonising these locations must overcome conditions such as a lack of food, extreme weather and moisture fluctuations [3].

Besides adverse conditions, the post mining heaps are also large, which means that locations in these heaps are, to a large extent, very distant from surrounding ecosystems, which may serve as a source population for species colonising the heaps. Dispersal and local environmental conditions are important in the soil fauna community assembly [4,5]. Consequently, it has been repeatedly proposed that transplanting soil from well-developed

habitats may speed up soil fauna community development in the fragmented and heavily disturbed landscapes for which distance could be a significant barrier to colonization [3,6,7].

Soil nematodes are a very important part of soil biota. Despite the fact that their contribution to soil respiration is not dominant, their characteristics allow them to play an important role in key ecosystem processes. Those characteristics include worldwide distribution in almost all habitats, a body size ranging from very small to relatively big, different feeding habits, and sensitivity to toxic compounds such as heavy metals [8]. Their combination enables their occupation of various niches in the soil food web and, therefore, predestines them as useful indicators of its complexity [9–12].

In addition to serving as a source for soil fauna population, the application of topsoil also instantly increases soil conditions [13], which may form a refuge in which this transplanted soil population survives for the long term in an otherwise adverse heap environment. However, despite this idea being proposed by several researchers, there are not many studies that test this in the real world (Benetková et al., 2022, 2020; Moradi et al., 2018; van der Bij et al., 2018).

Some studies explored how various amounts of target soil (including soil fauna communities) introduced into substrate [14–16] affects nematode assemblages; however, so far none dealt with an addition of relatively large intact transported blocks. Here, we explore the long-term effects of soil transplant into post mining bare heaps on the development of the soil nematode community. We anticipated that transported blocks will, in the beginning, serve as the refugium for nematodes; thus, we formulated the hypothesis that total numbers as well as trophic distribution in the transported blocks will not be significantly different from those in the spoil substrate 21 years after the soil's introduction.

## 2. Materials and Methods

The study was conducted on Podkrušnohorská spoil heap, which was formed by the merging of several smaller heaps from opencast coal mining located near Sokolov (Czech Republic). Heaping lasted for several decades and was terminated in 2003. The altitude of the Podkrušnohorské heap is 550 m a.s.l. There is a mean annual temperature in the heap of 6.8 °C and a mean annual precipitation of 650 mm. The heap was formed by deposition of mudstones and by compacted clay, impregnated by carbonates and fossil organic matter [17]. During a landslide, which happened in 1992, approximately 2 m of the upper heap surface slid down, forming a strip of bare land about 200 m wide and about 1 km long. The surface of this area was formed by weathered overburden and had no vegetation.

Topsoil from a depth of 0–40 cm (including aboveground biomass) was sampled by heavy machinery in a nearby well-developed meadow in the fore field of mine Jiří and transported into the landslide area in the spoil heaps in April 1995, forming six blocks (10 m × 3 m × 0.4 m). The distance between the blocks was 10 m, and the blocks were organised in three rows and two columns (Figure 1). Over the years, *Calamagrostis epigeios* became the dominant plant species at the site, as is common for Central European post-mining spoil heaps' succession, but the transplanted block kept the plant species typical for donor meadows. For further details about the experimental site, see Háněl [18] and Moradi et al. [19].
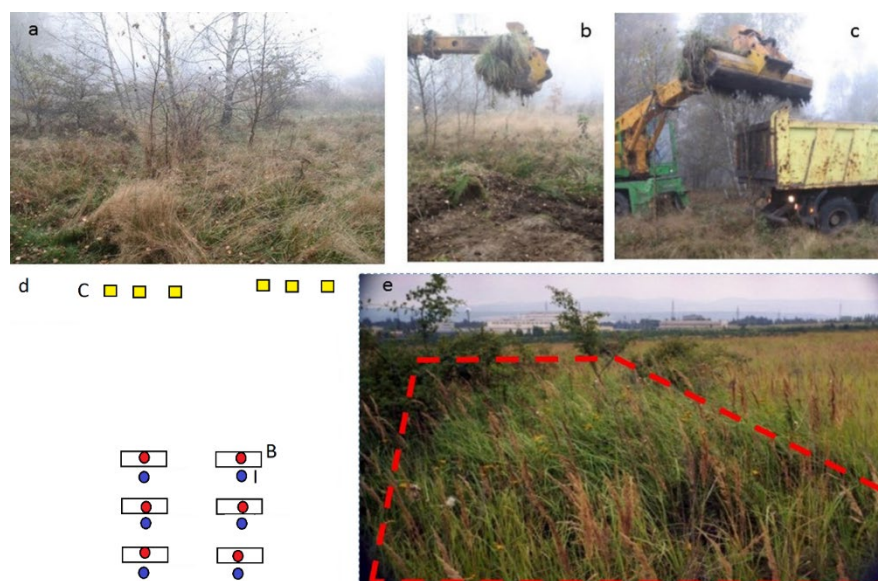
**Figure 1.** Sampling and transport of donor soil (**a**–**c**) scheme of transplanted block arrangement (**d**) showing position of samples in the transplanted block (B) in their immediate vicinity (I) and in control heap (C) and picture of transplanted block after 20 years (**e**).

Sampling in the early stages of an experiment in 1997 and detailed results of nematode communities in the initial phase are described in Háněl (2005). The same sampling design was followed in 2016: Soil samples were collected from the transported blocks—"B", in the soil in between the blocks in the immediate vicinity of the block (the distance from the block was 1 m)—"I", and in the control area of the heap unaffected by block transplant at a distance of 30 m from the blocks—"C". For each treatment, six sampling spots (six replicates) were used. Three Kopecki's rings (area 20 cm$^2$; depth 5 cm) were taken in each sampling spot and were mixed into one composite sample.

Soil was then transported into the laboratory and immediately prepared for the extraction of nematodes in Baermann's funnels. Twenty grams of fresh soil was left on a sieve in the water column for 36 h for an extraction of nematodes and their concentration at the bottom of a glass vial. The nematodes were then killed using hot formaldehyde, mounted into permanent slides, and thereafter sorted into classes and genera (based on morphological characteristics) under a light microscope according to mainly Bongers [20] and Andrássy [21–23] in both years 1997 and 2016. The allocation of genera to trophic and c–p groups has been updated according to recent papers.

Nematode-based indices are listed in Supplementary Table S1 and were calculated using NINJA interface [24] and statistical analyses (*t*-test, one-way ANOVA, factorial ANOVA and Tukey's HSD test). These were performed using the Statistica programme, ver. 14.0.0.15 (Tibco Software Inc., Palo Alto, CA, USA). The relative abundance of the trophic group was transformed by arcsin transformation prior to the analysis.

## 3. Results

The average abundance of the entire site was $616 \pm 275 \times 10^3$ ind.m$^{-2}$ in the initial phase of the experiment. *Aporcelaimellus* and *Ecumenicus* were the most abundant genera, with the latter being typical for spoil heaps and almost missing in transplanted blocks, whereas *Aporcelaimellus* was present in all samples (see Supplementary Table S2). The most abundant (both absolute and relative) trophic group was fungal feeders, with quite a high percentage of omnivores in second place, followed by plant feeders, bacterial feeders, and predators (Table 1 and Figure 2).
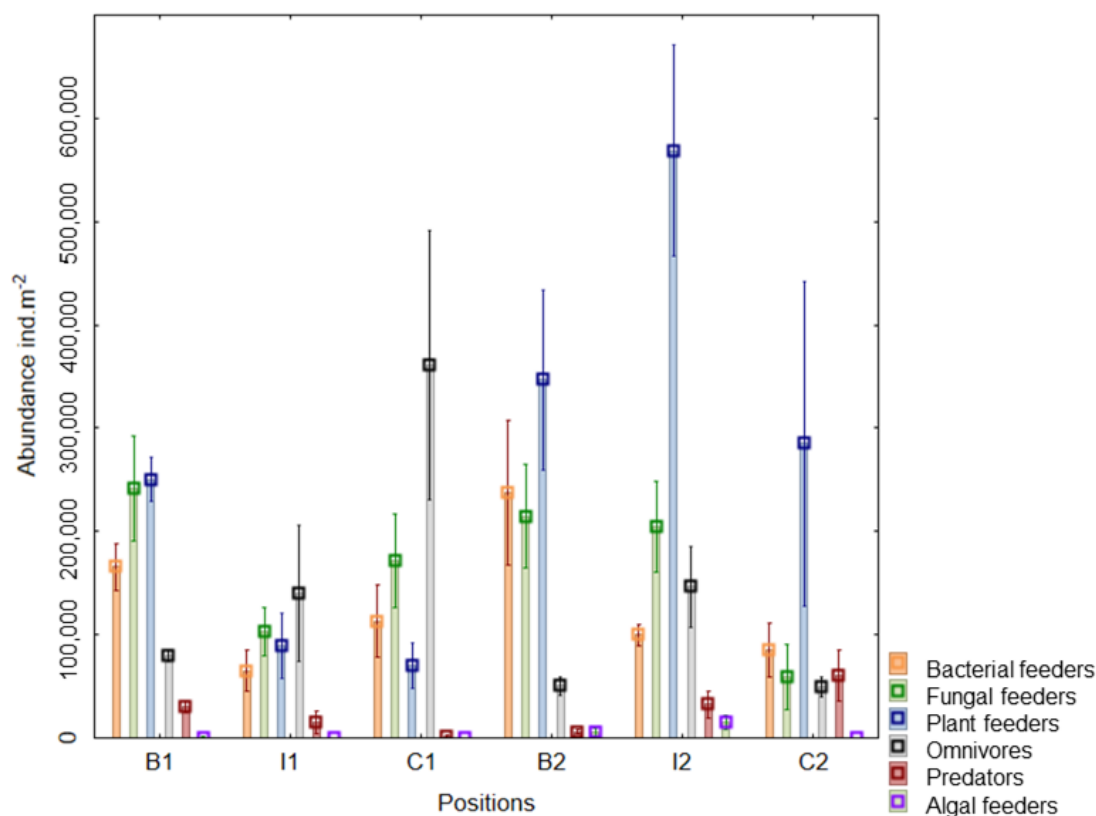
**Figure 2.** Abundance (ind.m$^{-2}$) of trophic groups in the nematode communities. B—Transported blocks; I—samples in between the blocks; C—control samples. Number 1 is for initial phase; 2 is for late phase. Points are means; whiskers are standard error.

In the late phase, the mean ($\pm$ st. dev.) of total abundance of nematodes in the transported Blocks was $861 \pm 448 \times 10^3$ ind.m$^{-2}$. In between the blocks, it was $1067 \pm 383 \times 10^3$ ind.m$^{-2}$, and in the control plots, it was $539 \pm 542 \times 10^3$ ind.m$^{-2}$ (see Supplementary Table S2). The average of the entire site was $823 \pm 488 \times 10^3$ ind.m$^{-2}$ and, therefore, slightly higher than in the initial phase. The most abundant genus overall was *Helicotylenchus*. In the transported blocks, it was the group of genera *Acrobeloides*, *Cephalobus* and *Eucephalobus* pooled together as "Cephalobids", followed by genus *Pratylenchus* (see Supplementary Table S2). In the in between plots, we found the most dominant genera *Helicotylenchus* and *Pratylenchus*. *Helicotylenchus* was also the most dominant genus for the control plots, together with *Mylonchulus* (see Supplementary Table S2). From the trophic group point of view, plant parasitic nematodes were the most abundant trophic group at the site, followed by fungal feeders, bacterial feeders, omnivores, predators and with algal feeders being the least abundant trophic group (Table 1 and Figure 2). Significant differences between positions in this late phase were in the trophic groups of bacterial feeders, fungal feeders and predators (one-way ANOVA, $p < 0.05$). For the determination of the factor important for community formation ("Position" $\times$ "Phase"), factorial ANOVA was used and showed that plant parasitic nematodes changed significantly over time, whereas bacterial feeders were more dependent on the distance from the transported blocks. For fungal feeders and omnivores, both factors showed importance and, for the latter the combination of these two variables, were also significant (see Table 1). The *t*-test comparison of means between the initial and late period showed significant differences for fungal feeders, plant feeders and omnivores, as well as for predators (Supplementary Table S3). The T-test did not show significant differences for plant feeders and predators in the initial phase and late phase for control plots, albeit Figure 2 clearly shows differences between means. This could be caused by the small number of samples in the control plot in the initial phase group (n = 2). There was a decrement of three major nematode indices (MI, ΣMI and PPI) in the transported

blocks over time, whereas those indices remained almost the same or even increased in the remaining two positions. MI as well as ΣMI tended to be highest in the control plots throughout the entire experiment (Table 2), which did not change over time, whereas SI remained lowest in the transported blocks in the beginning as well as in the late phase of the experiment.

**Table 1.** Relative abundance (%) of trophic groups. Mean ± SD, letters indicate significant differences between positions within one year (one-way ANOVA and Tukey/Unequal N HSD test), "a" being the lowest number. Below are *p* values of factors and their combination (Factorial ANOVA); B—transported blocks; I—samples in between the blocks; C—control samples; 1997—initial phase, 2006—late phase.

|  | Bacterial Feeders | Fungal Feeders | Plant Feeders | Omnivores | Predators | Algal Feeders |
|---|---|---|---|---|---|---|
| B1997 | 21.5 ± 4.5 | 30.5 ± 8.3 | 33.0 ± 5.9 b | 10.6 ± 2.7 a | 4.2 ± 1.7 | 0.1 ± 0.2 |
| I1997 | 16.8 ± 7.5 | 28.1 ± 11.3 | 21.9 ± 10.6 ab | 30.6 ± 8.9 b | 2.6 ± 2.6 | 0.0 |
| C1997 | 15.8 ± 0.4 | 24.5 ± 2.3 | 9.8 ± 0.3 a | 49.7 ± 2.8 b | 0.1 ± 0.2 | 0.0 |
| B2016 | 24.8 ± 7.7 b | 26.2 ± 10.1 b | 40.5 ± 12.7 | 6.7 ± 3.4 | 1.1 ± 0.9 a | 0.8 ± 1.6 ab |
| I2016 | 10.8 ± 5.6 a | 17.9 ± 5.3 ab | 52.1 ± 8.3 | 12.5 ± 5.9 | 4.4 ± 6.4 ab | 2.3 ± 3.6 b |
| C2016 | 19.0 ± 11.0 ab | 10.5 ± 4.6 a | 40.2 ± 23.3 | 13.8 ± 6.8 | 16.5 ± 16.0 b | 0.0 a |
| Position (B,I,C) | 0.04 | 0.04 | n.s. | <0.0005 | n.s. | n.s. |
| Year | n.s. | 0.007 | 0.0006 | <0.0005 | n.s. | n.s. |
| Position + Year | n.s. | n.s. | n.s. | <0.0005 | n.s. | n.s. |

**Table 2.** Various ecological indices (mean ± SD), N: number of samples; letters indicate significantly different treatments within one phase (ANOVA and unequal N HSD test), "a" being the lowest number. No letters mean not significant differences; *p* values are results of Factorial ANOVA; B—transported blocks; I—samples in between the blocks; C—control samples; MI—maturity index; ΣMI—sigma maturity index; PPI—plant parasitic index; CI—channel index; BI—basal index; EI—enrichment index; SI—structure index, N—number of samples.

| Treatment | Phase | MI | ΣMI | PPI | CI | BI | EI | SI | N |
|---|---|---|---|---|---|---|---|---|---|
| B | Initial | 2.64 ± 0.18 | 2.66 ± 0.11 a | 2.68 ± 0.16 | 45.45 ± 8.93 a | 18.93 ± 1.38 | 60.09 ± 4.55 | 72.93 ± 4.93 | 4 |
| I | Initial | 3.00 ± 0.32 | 2.80 ± 0.23 ab | 2.22 ± 0.23 | 72.06 ± 15.38 b | 17.02 ± 7.04 | 49.68 ± 7.71 | 79.19 ± 9.68 | 4 |
| C | Initial | 3.33 ± 0.09 | 3.24 ± 0.04 b | 2.42 ± 0.50 | 65.97 ± 0.30 ab | 11.26 ± 1.00 | 50.38 ± 0.98 | 87.28 ± 1.34 | 2 |
| B | Late | 2.38 ± 0.22 a | 2.41 ± 0.18 a | 2.50 ± 0.17 | 80.14 ± 15.23 | 39.09 ± 11.31 b | 37.08 ± 5.50 | 48.10 ± 18.59 a | 6 |
| I | Late | 2.96 ± 0.46 ab | 2.85 ± 0.28 ab | 2.74 ± 0.21 | 69.21 ± 18.4 | 18.75 ± 12.54 a | 41.14 ± 8.83 | 77.17 ± 16.91 b | 6 |
| C | Late | 3.32 ± 0.48 b | 3.05 ± 0.44 b | 2.72 ± 0.61 | 67.24 ± 20.05 | 13.63 ± 12.06 a | 22.81 ± 19.21 | 85.21 ± 13.74 b | 6 |
| Treatment | | 0.001 | 0.005 | n.s. | n.s. | 0.05 | 0.05 | 0.005 | |
| Phase | | n.s. | n.s. | n.s. | n.s. | n.s. | 0.0005 | n.s. | |
| Treatment * Phase | | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | |

## 4. Discussion

If we look at the trend of total abundance of nematodes recorded in the year 2016, we can see not only a very good survival rate of assemblages in the transported strips of meadow soil but also a thriving community in samples taken in close surroundings of those strips. However, abundances in the samples from the farther part of the spoil heap, being lower than in greater proximity to transported soil or even in the initial phase of the experiment, showed that the colonisation of the spoil heap did not reach a climax even after 20 years, which supports the findings of Moradi et al. [19]. Overall, we believe that the addition of fresh soil material of target habitats increases the chance of shifting a succession (both aboveground and belowground) to a desired pathway, as was proven by, e.g., Benetková et al. [14,15], Boyer et al. [25], Radujković et al. [26], or Wubs et al. [27]. These studies predicted that the introduction of fresh soil material will speed up the recovery of soil fauna in a disturbed soil material, especially in the first years after introduction. Frouz et al. [17] also states that this process is relatively faster than if the succession was spontaneous.

However, what differed significantly was the trophic composition of extracted samples in time. Whereas omnivorous nematodes represented by the most dominant genus of *Ecumenicus* were typical for spoil heap material in the initial phase [18], later on, plant parasitic nematodes seemed to be the more dominant trophic group throughout the entire site. *Helicotylenchus* with its relatively high density in the transported soil was apparently able to feed on established plant roots and successfully spread to the spoil heap, contrary to, e.g., *Paratylenchus* or *Pratylenchus*, which were practically absent in the control plots. Despite not being a dominant trophic group anymore, omnivores still kept an increasing trend with the distance from the transplanted soil, suggesting that we could still expect a running succession in the spoil substrate [28]. Predators could have superseded omnivores in the control plots in 2016, as there were enough food sources for them after 20 years of succession. Higher values of MI/ΣMI usually means larger nematodes creating more complex food webs. These nematodes enter new habitats relatively early in the succession [16,29] as there is enough food and an absence of predators in the soil; however, they may be constantly transported from the source habitats by wind in newly created habitats with scarcity of vegetation [30]. However, later in the succession, as food sources were being depleted, the above mentioned indices decreased and bacterial feeders again dominated nematode communities. Isolation and exploitation of transported blocks as an inoculum source can be seen in a radical drop in Structure Index in the B plots in the late phase.

With the increasing time of succession, the soil food web is more influenced by factors such as weathering of substrate, accumulation and bioturbation of organic matter, or the creation of a humus layer. Frouz and Nováková [31] found that physico-chemical as well as microbial parameters, measured in approximately 35-year-old unreclaimed spoil soils, resembled parameters obtained in undisturbed habitats. Based on this, we may assume that nematode communities in sites near the transported blocks will be more similar to the soil in the blocks, whereas the development of communities in the distant part of the heap will be more affected by migration from outside of the heap. This may prolong the succession or shift it to a different direction.

Despite the significant differences between positions in the late phase, we can clearly see the trend in the similarity of trophic composition between the blocks and adjacent spoil heap. The abundance of nematodes is in the range of abundances found in established habitats. Our results support the idea established by Rantalainen et al. [32] and was confirmed by the results of Moradi et al. [19] from the same experiment that not only the presence of the source of soil fauna alone is crucial for the successful colonisation of a disturbed soil habitat. The surrounding substrate should undergo an improvement of a certain degree to allow the rapid and stable establishment of soil fauna and, therefore, also support aboveground plant communities. Our data complete the jigsaw and prove that the statement above is valid for organisms across the entire range of the scale.

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