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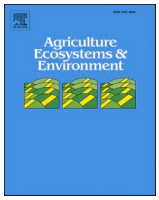

Comparing a real and pseudo chronosequence of mining soil reclamation using free-living nematodes to characterize the food web and C and N dynamics

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ABSTRACT

Space-for-time substitution, also known as pseudo chronosequence (PCS), is often used as a method to assess temporal changes in ecosystems without having to wait for the time span that the study is supposed to cover. However, the suitability of PCS studies is often questioned because convergent development of spatially separated plots over time is rarely guaranteed even under optimal conditions. To assess whether the PCS approach is justified and suitable, we studied the nematode soil food web development of free-living nematodes and biological and chemical soil components related to C and N mobilization (CO_2 and NO_3^-) and immobilization (microbial and soil organic carbon), in a PCS focusing on the critical early reclamation period of four years after open-cast lignite mining, and compared it with a real chronosequence (RCS) of a reclamation site monitored over four years. We hypothesized that: (I) nematode-derived indices indicate the same course of nematode soil food web development in the PCS and RCS, tolerating a range of variability in weather conditions, soil components, and management; (II) the development of nematode soil food web indicators can provide information on the status of C and N retention and cycling in the reclaimed soil. Our results show that the PCS and RCS approaches reach similar conclusions, indicating a rapidly developing nematode soil food web during the first four years after reclamation. They also suggest that the nematode faunal profile may indicate the status of C and N retention and cycling in reclaimed soil. Overall, it can be concluded that the PCS approach successfully predicts the temporal development of the nematode soil food web in loess-dominated reclaimed mining soils, even when there is a range of variability in soil components and management conditions.

1. Introduction

Space-for-time substitution approaches, also known as pseudo chronosequences (PCS) are used to study the long-term development of floral, faunal, and pedogenic processes in sand dunes, glacial moraines, lava flows, landslide scars, abandoned or burnt land, old mining areas, alluvial fans, floodplains of rivers, or marine terraces at manageable time scales (Chen et al., 2014; Huggett, 1998; Walker et al., 2010; Zhao et al., 2022). A basic requirement of the PCS approach is geological, geographical and topographical similarity in order to isolate the effect of time on ecosystem properties from other factors (Johnson and Miyanishi, 2008). Many authors stress that reliable PCS studies also require an identical historical development, as shown by comparisons with long-term monitoring trials (Foster and Tilman, 2000; Johnson and Miyanishi, 2008). Even if the latter criterion cannot be fully met, conclusions from long-term PCS studies over more than one century, could still be useful if weaknesses pertaining to different historical developments are openly discussed (Walker et al., 2010).

In North Rhine-Westphalia (NRW, Germany), open-cast lignite mining produced more than 250,000 ha of initially marginal soils over the last half century, with well-documented geological, geographic, and topographic characteristics. It is therefore an optimal area for understanding both short-term and long-term soil processes, such as organic carbon and nutrient storage, as well as the bacterial and fungal response

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to different reclamation phases (Roy et al., 2022; Schmid et al., 2020; Zhao et al., 2022). The reclamation substrates (loess-dominated silt loam) at the Inden mine in North Rhine-Westphalia (50°52′44.6″N, 6°19′04.4″E), selected for this study, have not changed much over the past decades and are based on an officially regulated reclamation procedure (Landesoberbergamt, 1986; Lössabkommen, 1961). Nevertheless, even under these optimal conditions, factors are not fully controlled and do not necessarily remain comparable over the years, which may affect the soil recovery, but also the validity of PCS. This raised the question if the PCS and the real chronosequence (RCS) monitoring approach come to comparable results on nematode soil food web development in the early reclamation phase of a lignite mine.

A nematode soil food web is a complex, interconnected network of organisms interacting through multiple feeding relationships, influencing the retention and cycling of C and N (de Vries and Caruso, 2016; de Vries et al., 2012; Potapov, 2022). Free-living bacterial, fungal, as well as carnivorous and omnivorous nematode families are associated with different levels of nematode soil food web quality, indicating the degree of soil disturbance and nutrient status (Bongers and Ferris, 1999; Ferris et al., 2001). Disturbances, such as increased salinity, tillage, fertilization, acidification, heavy metal pollution, and crop rotation frequency, affect decomposition, mineralization, nutrient cycling, and agricultural productivity, which is indicated by negative changes in the nematode food web structure (Biswal, 2022), loss of disturbance-sensitive K-strategists.

Disturbances can be visualized in the nematode faunal profile, which summarizes the overall status of a nematode soil food web. It consists of two indicator values: First the enrichment index (EI), which stands for the relative frequency of fast-growing, r-strategic nematodes, indicating increased resources availability (Ferris et al., 2001). Second, the structure index (SI) on the x-axis of the faunal profile represents more slow-growing, more K-strategic nematodes known as indicator of food web complexity, including predators at higher trophic levels (Ferris et al., 2001). Most free-living nematodes, which are not plant parasites, feed on soil bacteria and fungi regulating their activity as well as growth and death rate (Biswal, 2022; Shi et al., 2023). Thus, the complexity in the food web may relate to the necromass formation rate as basis for C and N cycling and retention, and thus soil fertility in the long term (Camenzind et al., 2023; Sokol et al., 2022). Therefore, we propose that the nematode-derived food web indicators can serve as an indicator of the C and N retention and cycling potential in the reclamation soil.

To address the above questions, we monitored the nematode soil food web development on one reclamation field over four years, i.e., a RCS and compared it with data from a PCS with one-time sampling at multiple plots of increasing reclamation age (cf. Reichel et al., 2017). We formulated the following hypotheses:

I. Nematode-derived indices indicate the same course of nematode soil food web development in the PCS and RCS, tolerating a range of variability in weather conditions, soil components, and management.
II. The development of nematode soil food web indicators can provide information on the status of soil C and N retention and cycling in reclaimed soil.

2. Material and methods

2.1. Site description

The sites of the PCS and RCS were both located in an area of 5 km^2 of the open-cast lignite mine "Zukunft/Inden" between Eschweiler and Jülich, Germany (Fig. 1). The climatic conditions of the PCS between 2010 and 2016 were: 10.5 °C mean annual temperature and 703 mm mean annual rainfall (German Weather Service, Aachen-Orsbach, Germany). The RCS site, reclaimed in 2018 and monitored until 2021, had a mean annual temperature of 11.4 °C (0.9 °C warmer than PCS) and a mean annual rainfall of 624 mm (79 mm less rainfall per year than PCS).

The characteristics of the original Luvisol soil (soil texture: silt loam, 12.5 % clay, 82.7 % silt, and 4.8 % sand) prior to excavation varied according to the previously applied soil management practices: 6.3–6.6 pH($CaCl_2$), 1.07–1.46 % organic carbon (C_{org}), 0.14–0.16 % total nitrogen (N_t), 3–57 mg kg^{-1} mineral N (N_{min}), 1–17 mg kg^{-1} mineral sulfate (S_{min}), 79–153 mg kg^{-1} plant-accessible P (P-CAL), 100–141 mg kg^{-1} plant-accessible K (K-CAL), and 130–160 mg kg^{-1}

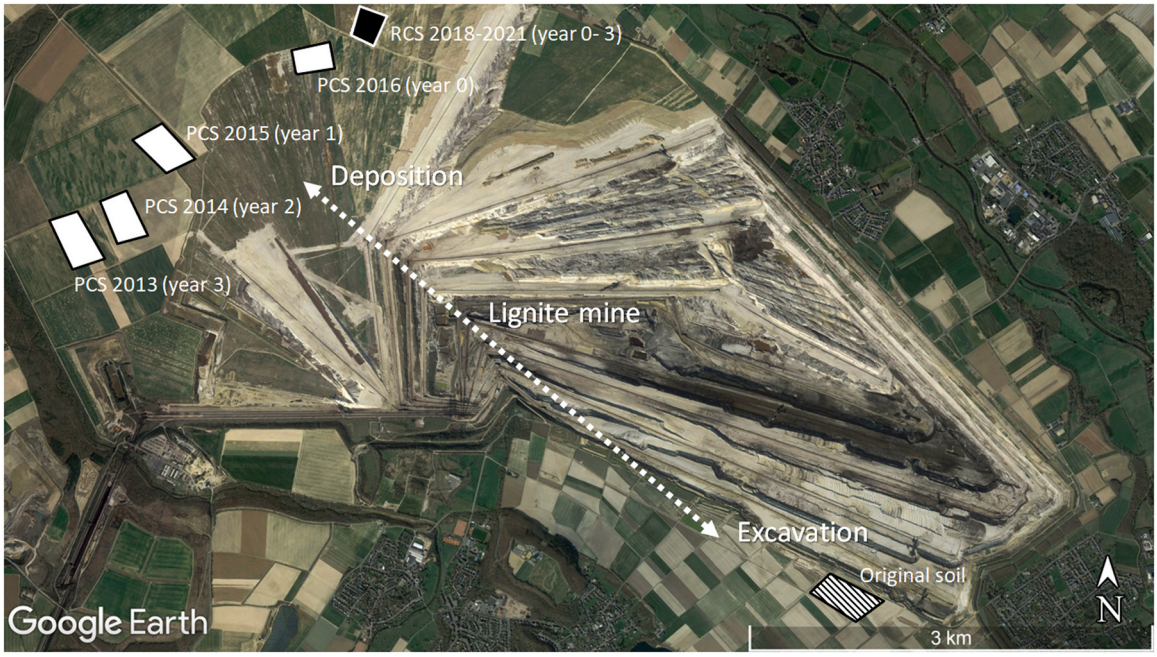

Fig. 1. Location of the real chronosequence site (RCS, black) and the pseudo chronosequence sites (PCS, white) relative to the Inden open-cast lignite mine of the RWE AG (Germany). The calendar year of soil reclamation is shown next to the sites, followed by the years after reclamation. The dashed arrow shows the distance between deposition of the freshly mixed substrate and excavation of the original soil and underlaying loess material (original soil, black striped).

plant-accessible magnesium (Mg). The properties were analyzed by the LUFA Speyer (Germany) using standard methods (VDLUFA, 1991). Prior to reclamation, lignite had been mined using bucket excavators that initially remove the first five to six meters, comprising the recent topsoil and the underlying calcium carbonate-rich loess material. The two substrates were mixed in a ratio of approximately 1:5 (v/v) during excavation, transport by conveyor, and dumping. After three to six months of settling, the substrate was levelled and constituted the top two meters of the reclaimed soil profile (Lössabkommen, 1961). After mixing, the properties of the reclamation substrates were significantly different from those of the original soil, as shown here (Reichel et al., 2017).

2.2. Chronosequence description

A summary of the management steps and differences between the RCS and PCS is shown in Table 1 and described in detail below.

Sampling of all PCS sites was conducted in June 2016 (Reichel et al., 2017). The PCS site established in 2016 (year 0; 50°52.996'N, 6°20.564'E) was between the deposition and levelling phase (Table 1). Directly after ploughing and sowing of alfalfa (*Medicago sativa* L.), the PCS site established in 2015 (year 1; 50°52.637'N, 6°19.648'E) received an initial fertilization with 200 kg compound fertilizer with 15 % N, 15 % P_2O_5 and 15 % K_2O to alleviate the high initial nutrient limitation. Alfalfa grew up to 8 cm until the time of sampling. The PCS site established in 2014 (year 2; 50°52.361'N, 6°19.384'E) and PCS 2013 (year 3; 50°52.267'N, 6° 19.115'E) were managed as described for the site established in 2015. Before soil sampling, the fields had been densely covered with alfalfa, which was later partially harvested and mulched.

All samplings of the RCS were conducted on a single reclamation field (50°53.098'N, 6° 20.933'E) in June and October 2018 (year 0), March and September 2019 (year 1), April and October 2020 (year 2), and May and September 2021 (year 3). The samplings in year 0 covered the state of deposition (June) and levelling (October). In contrast to the PCS, winter wheat (*Triticum aestivum* L., cultivar Benchmark) instead of alfalfa was sown after ploughing in year 0 to protect the soil during the cold and wet winter season. The fields also received the typical initial fertilization as mentioned above. During the March sampling of year 1, wheat roots reached around 15 cm soil depth. In June, the wheat was harvested in premature state and used for biogas production. Before sowing of alfalfa, wheat residues were plowed into the soil. Alfalfa reached up to 5 cm height at the time of sampling in September. In contrast to the PCS, the alfalfa growth was patchy and dominated by weeds such as *Chenopodium album* (L.). Mid-April of year 2, the vegetation was cleared in the RCS and plowed again. Afterwards, alfalfa was re-established until the next sampling in October. In May of year 3, alfalfa formed a dense cover. Prior to the following sampling in September, the aboveground biomass of alfalfa was mulched.

2.3. Soil sampling

The dimensions and age of the reclaimed sites was determined, using maps provided by the RWE (RWE Power AG, Germany). The first sampling spot was randomly selected at a distance of 20 m parallel to the country lane adjacent to the site. The other sampling spots of each site were placed in a diamond-shaped area 25 m apart, randomly labeled, and marked by GPS. Three replicates were used for nematode analysis on undisturbed fresh samples (RCS and PCS) and four (RCS) and five (PCS) for all other soil components, depending on the time required for analysis. The total number of samples is shown in Table S1 at the sum of reclamation years × samplings per year (one for PCS and two for RCS) × the number of replicates per parameter (three to five).

From each sampling spot, we took an undisturbed and a mixed soil sample from 0 to 10 cm soil depth at five spots. Sampling of undisturbed soil was carried out for determining the nematode soil food web indicators (nematode abundance, EI and SI) and the greenhouse gas emissions (CO_2, N_2O), using PP (polypropylene) tubes (HT drainpipes with 50 mm in diameter and 215 mm long), which were inserted to the depth of 10 cm using a rubber hammer. During transport and storage, the bottom was sealed with a plastic bag and tape, and the top was closed by a plug (HT drainpipe socket plug, 50 mm in diameter, PP). For all other soil components, soil was collected as a composite sample around the PP tube using four drills with a 6 cm diameter soil auger. Undisturbed and disturbed soil samples were transported on ice. Disturbed soil samples were sieved directly to 2 mm, aliquoted for the various analyses, and frozen at − 20 °C. The undisturbed samples in the PP tubes were stored at 4 °C until measurement of greenhouse gas emissions and nematode extraction. Note that all PCS data described below refer to Reichel et al. (2017).

2.4. Microbial soil analyses

C_{mic} and N_{mic} were extracted with 0.01 M $CaCl_2$ after chloroform fumigation and determined with a TOC/TN analyzer (TOC-VcPH + TNM-1 + ASIV, Shimadzu, Japan), and calculated and corrected with the factors kEC = 0.45 and kEN = 0.40 (Joergensen, 1996).

We quantified the abundance of N cycling marker genes in the soil of RCS autumn samples from reclamation years 0, 2 and 3, when the processes are most active, using the method described in detail here (Reichel et al., 2022). For nitrification, we used archaeal (AOA) and bacterial ammonia (AOB) monooxygenase (*amoA* gene) as markers, for denitrification the nitrate reductase (*nirS* and *nirK* genes), and for complete denitrification to N_2 the nitrous oxide reductase (*nosZ* gene).

2.5. Physicochemical soil analyses

For H_2O_{grav}, soil was dried at 105°C until constant weight, pH was determined in 0.01 M $CaCl_2$ at a dry soil-to-solution ratio of 1:2.5 (VDLUFA, 1991). C_{org} and N_t were determined by combustion according to DIN EN 15936:2012-11 and DIN EN 16168:2012-11 (DIN, 2017).

Table 1
Reclamation steps in the real chronosequence (RCS) and the pseudo chronosequence (PCS) in the phase between deposition (D) and levelling (L), L and ploughing/sowing (PS), and PS and fertilization (F) with winter wheat (W) or alfalfa (A).

Reclamation Year. Section		**0.1**	**0.2**	**0.3**	**0.4**	**1.1**	**1.2**	**1.3**	**1.4**	**2.1**	**2.2**	**2.3**	**2.4**	**3.1**	**3.2**	**3.3**	**3.4**
Deposition	RCS	D	D														
	PCS	D	D														
Leveling	RCS			L													
	PCS			L													
Ploughing/sowing	RCS				PS		PS				PS						
	PCS				PS												
Fertilization	RCS				F												
	PCS				F												
Cover plant (W/A)	RCS				W	W		A	A	A		A	A	A	A	A	A
	PCS				A	A	A	A	A	A	A	A	A	A	A	A	A

DOC and DN were determined using 0.45 µm filtrates of the unfumigated samples from the previously described C_{mic} and N_{mic} extraction procedure. VDLUFA extraction methods were applied for NH_4^+, NO_3^-, S_{min}, Mg, and plant accessible P-CAL plus K-CAL (VDLUFA, 1991).

2.6. Greenhouse gas emissions

Soil CO_2 and N_2O emissions of the PCS were determined with a gas chromatograph with flame ionization and electron capture detector (Clarus 580, PerkinElmer, Rodgau, Germany) using disturbed, recompacted soil (1.4 g cm^3) in glass vials (Reichel et al., 2017).

CO_2 and N_2O emissions of the RCS were measured using the PP tubes with undisturbed soil cores as described above. Tubes were left with the PP plugs removed for 24 h prior to analysis. For the measurement, a gas-tight PP headspace chamber (HT drainpipe coupler, 50 mm in diameter, 126 mm long, with two sockets and rubber washer, one side covered by a PP plug) was placed on top of the PP tube, covering 0.0016 m^2 soil. Headspace plus the empty space of the tube added up to 0.3674 L. The PP cap of the headspace chamber contained three Swagelok ® tube fittings. One for a polytetrafluoroethylene (PTFE) vent tube open to ambient air with 1.6 mm inner diameter and 1300 mm length. The other two fittings connected a PTFE tube with an internal diameter of 4 mm and a length of 630 mm to an infrared laser gas analyzer for 10 minutes in a closed-loop mode (G2508, Picarro, Inc., Santa Clara, CA, USA), with a volume of 0.0792 L. Increases in gas concentrations over time with $R^2 > 0.81$ were considered as significant and used in the calculation as described by Brümmer et al. (2008).

2.7. Nematode extraction and calculation of indices

Free-living nematodes were extracted from the whole undisturbed soil cores of 196 cm^3 (bulk density 1.4 g m^{-3}) of the PCS and RCS using a modified Cobb's method, i.e., a decanting and sieving procedure (Van Bezooijen, 2006), reducing the extract volume stepwise from 100 ml to 5 ml. The final concentrate was vortexed before transferring 3 × 50 µl to microscope slides for examination and counting of nematode families with different colonizer-persister (cp) values (Bongers and Bongers, 1998) and their affiliation to the basal, enrichment, or structure component (Ferris et al., 2001) as provided in Table 2. To avoid double counting, the entire slide was examined along vertical lines that visually overlapped. In addition, we counted all nematodes extracted from 2 g of undisturbed RCS soil from October 2020 (year 2) to provide an estimate of absolute nematode abundance during the period of apparent structural change.

We focused on EI and SI as the two main food web indicators, allowing a time-efficient assessment of the nematode soil food web status (Chen et al., 2014; Reichel et al., 2017). Enrichment (EI) and structure (SI) indices were calculated as described below and then summarized in the nematode faunal profile as described here (Ferris et al., 2001).

The EI was calculated by the basal and enrichment components as follows: $100\left(\frac{e}{(e+b)}\right)$, with $b = \sum k_b n_b$ (k_b: guild weighting; n_b: guild abundance) and $e = \sum k_e n_e$ (k_e: guild weighting; n_e: guild abundance), reflecting the responsiveness to resources availability.

The SI was calculated as follows: $100\left(\frac{s}{(s+b)}\right)$, with $b = \sum k_b n_b$ and $s = \sum k_s n_s$ (k_s: guild weighting; n_s: guild abundance), integrating parameters such as longevity, body size and perturbation sensitivity based on the life history of r and K strategists, reflected in the colonizer-persister (cp) concept (Bongers, 1990).

Nematode classification was based on existing ecological knowledge, representing the state of the art at the time of the PCS study (Reichel et al., 2017), to ensure maximum comparability. Please note that there may be more current nematode data available in the online tool Nematode INdicator Joint Analysis (NINJA: https://shiny.wur.nl/content/5/; (Sieriebriennikov et al., 2014).

2.8. Statistics

Statistics were performed with SPSS Statistics 21 (IBM, Ehningen, Germany) using all replicates of a parameter. Calculation of means with standard deviation and visualization in graphs and tables were performed using Excel 365 (Microsoft, Munich, Germany, 2023). For better

Table 2
Mean abundance (± standard deviation) of nematode families of the real chronosequence (RCS) and pseudo chronosequence (PCS) with different colonizer-persister (cp) values and their affiliation to the basal (b), enrichment (e) or structure (s) component and the guild weight used. The mean total abundances of nematodes related to EI and SI in 150 µl of 5 ml concentrated extract from 196 cm^3 soil are given in the last row. We also counted all nematodes extracted from 2 g of undisturbed soil from the October 2020 (year 2) RCS soil replicates to provide an estimate of the total nematode abundance during the period of apparent structural change. The absolute nematode numbers ranged from 1050 to 13,050 per 100 g dry soil. The PCS data refer to Reichel et al. (2017).

Year after reclamation				Year 0		Year 1		Year 2		Year 3	
Indicator values/approach	cp	Affiliation		RCS	PCS	RCS	PCS	RCS	PCS	RCS	PCS
Nematode families	value	*k*	weight								
Rhabditidae	1	*e*	3.2	**0.5** ± 0.8		**4.8** ± 5.6	**16.0** ± 9.2	**12.2** ± 5.6	**14.3** ± 6.8	**34.7** ± 24.0	**25.0** ± 7.1
Plectidae	2	*b*	0.8	**2.3** ± 2.6	**0.3** ± 0.5	**1.3** ± 2.2	**9.7** ± 6.8	**6.8** ± 4.5	**2.7** ± 3.8	**4.7** ± 6.2	**0.3** ± 0.5
Cephalobidae	2	*b*	0.8	**6.3** ± 4.9	**1.0** ± 0.8	**28.7** ± 6.7	**33.7** ± 8.1	**29.0** ± 10.9	**15.0** ± 4.5	**20.3** ± 24.8	**16.3** ± 2.1
Aphelenchidae/-choididae	2	*b/e*	0.8	**1.0** ± 1.0		**4.2** ± 3.2	**1.3** ± 1.2	**18.2** ± 17.4	**5.7** ± 4.2	**14.0** ± 9.3	**11.7** ± 6.3
Diphtherophoridae	3	*s*	1.8	**0.2** ± 0.4							
Prismatolaimidae	3	*s*	1.8				**1.0** ± 1.4		**15.0** ± 4.1		**0.3** ± 0.5
Nordiidae	4	*s*	3.2			**0.2** ± 0.4		**1.2** ± 0.9	**4.7** ± 4.1	**3.7** ± 2.4	**4.3** ± 1.7
Qudsianematidae	4	*s*	3.2					**0.7** ± 1.1			
Aporcelaimidae	5	*s*	5	**0.2** ± 0.4		**0.2** ± 0.4	**0.3** ± 0.5	**3.3** ± 1.9	**0.3** ± 0.5		**5.7** ± 4.1
Thornenematidae	5	*s*	5							**4.8** ± 3.5	**1.3** ± 1.9
Absolute EI and SI nematode abundance in 150 µl extract		-		**10.5** ± 5.6	**1.3** ± 1.2	**39.3** ± 10.9	**62.0** ± 7.1	**71.3** ± 10.3	**57.7** ± 6.6	**82.2** ± 32.8	**65.0** ± 7.1

comparison with the PCS, the two sampling times of the RCS have been averaged.

The Kolmogorov-Smirnov test was performed on each CS dataset/residuals to test for normal distribution. The Levene test was used to determine whether the data showed homogeneity of variance. Based on this, we decided which statistic (parametric Student's t-test or non-parametric Mann-Whitney U test) to use to check for statistically significant differences between the CS approaches. To compare the CO_2 and N_2O emissions between the CS approaches, which were measured using different methods, we used z-transformed data. Significance over reclamation time was tested separately using either one-way ANOVA with post-hoc tests (Tukey-B for homogeneous variance or Games-Howell for non-homogeneous variance) or the non-parametric Kruskal-Wallis test for multiple pairwise comparisons.

In addition, we performed stepwise multiple regressions to explore the significant predictors of the nematode soil food web indicators EI and SI, including the combined, z-transformed data of the PCS and RCS, where EI and SI can be directly related to the soil data ($n = 108$). The nitrogen cycle gene data ($n = 9$) from the RCS was used to explore their potential relationship with the EI and SI indicators. The method "stepwise forward" and the Akaike information criterion (AICC) were used to include significant predictors. The significance level was $p < 0.05$ for all tests.

3. Results and discussion

3.1. Nematode soil food web development of the PCS and RCS are comparable

In both approaches, the food web quality developed from a disturbed and nutrient-depleted (square D in the faunal profile) to a more mature and nutrient-enriched status (square A) over three years of reclamation (Fig. 2). A range of variability in weather conditions, soil components, and management (cf. Section 2, Tables S1 and Table 2) did not confound the direction of nematode soil food web development over time (Fig. 2) as both chronosequences have a comparable geological, geographic, and topographic setting. A review of successions comparing non-chronosequence methods, such as monitoring permanent plots over time, with space-time substitution (PCS) approaches, highlighted that PCS studies are often too uncritical without any justification of site similarity in terms of the substrate mineralogy, topography, and especially historical development (Johnson and Miyanishi, 2008). In this respect, reclamation studies are very attractive, as accurate maps and information on the above prerequisites are usually available, and management is similar over long periods of time (Lössabkommen, 1961). When the geology, geography, and topography are similar, some variation in weather, soil components, and management, as in the present study (Table 1, Table 2), does not seem to preclude the use of PCS to draw conclusions about time-dependent soil developments. Even the initial use of wheat instead of alfalfa and its subsequent clearing and ploughing in the RCS compared to the PCS (Table 1) did not prevent the overall recovery of the nematode soil food web (Fig. 2). Nevertheless, interventions as in the RCS may temporarily alter some biological dynamics, such as the development of the abundance of EI and SI indicator nematodes, which was linearly related to time in the RCS but more logarithmically in the PCS (Table 2). It is known that heavy metal contamination, salinity, acidity, fertilization, pesticides, and tillage can negatively affect the dynamics and structure of a nematode soil food web (Biswal, 2022), but for some stressors this is only temporary as long as a certain threshold and duration of disturbance is not exceeded. Despite some obvious differences, the RCS and PCS showed a time-dependent course of developments in the soil, especially of the biological components (Table S1). Our study also shows that PCS approaches are best suited for soil components that change in a linear manner with respect to time (Walker et al., 2010). This was validated for plant communities on marginal soil by resampling the same plots years later at the Cedar Creek Natural History Area, Minnesota, USA (Foster and Tilman, 2000). We agree with other authors that well-documented PCS studies are more likely to improve our understanding of temporal processes in soil, especially when they cannot be studied within a reasonable time frame (Huggett, 1998; Walker et al., 2010). Consistent with our first hypothesis, we can therefore confirm that the PCS and RCS approach show the same developmental trajectory of the nematode soil food web.

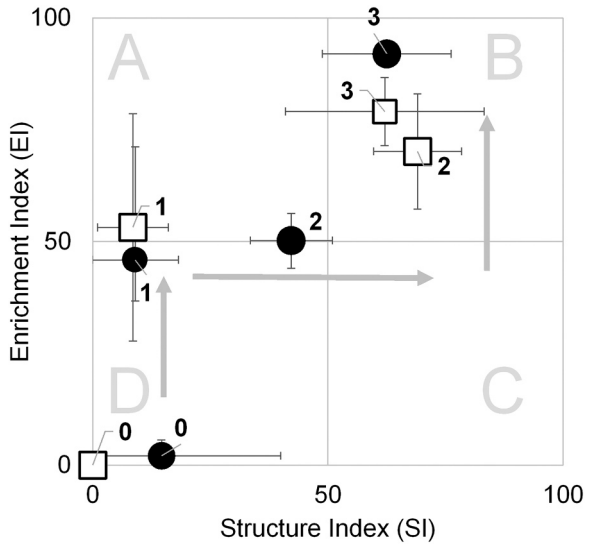

Fig. 2. Nematode faunal profile of the real (RCS, black circles) and pseudo chronosequence (PCS, white boxes). The year after reclamation is displayed next to the symbols. The y-axis is related to the nematode-derived enrichment index (EI), indicating the level of rapidly growing members of the nematode soil food web and the related resource availability. The x-axis displays the nematode-derived structure index (SI), indicating the trophic complexity of a food web. Arrows mark the dominant direction of the food web development between the reclamation years. Faunal profile square "A" indicates strongly disturbed food webs dominated by bacterial growth and related to high resource availability (typical for interrupted, annual cultivation); "B" indicates increasingly developed, more balanced bacterial-fungal food webs and increased resource availability (typical for perennial cultivation); square "C" represents undisturbed food webs dominated by fungi and moderate resource availability. "D" indicates highly disturbed food webs and low resource availability.

3.2. Nematode soil food web may indicate C and N retention and cycling status

Our data show that DN and the nematodes abundance have a positive while DOC and S_{min} have a negative relationship with the EI (Table 3, Table S1). The increase in nematode abundance was only significantly related to EI, but not to SI. The increased abundance of EI nematodes such as Rhabditidae (Table 2) with a wider biomass C:N ratio compared to their microbial food source may explain the contrasting change in DOC (negative = incorporation) and DN (positive = excretion; Table 3). The negative response of EI to S_{min} may be explained by a negative effect on some nematode groups as shown here (Zhang et al., 2021). Alternatively, some of the relationships could also be due to parallel, time-dependent developments, such as sulfate leaching and uptake, N accumulation due to N fixation by alfalfa, and increasing microbial C limitation.

Nevertheless, our data seem to show that the abundance of EI nematodes and their predation activities may have limited the development of the microbial biomass (Table 1). This was particularly evident for the PCS, where much of the accessible DOC and DN were not immobilized into new microbial biomass as might be expected (Table S1). This could

Table 3
Stepwise multiple linear regressions (SMLR) to explore the significant predictors ($p < 0.05$) of the nematode soil food web indicators EI (Enrichment Index) and SI (Structure Index), performed on the z-transformed data, including the combined data set of the pseudo chronosequence (PCS) and the real chronosequence (RCS), where EI and SI can be directly related to the soil data (# 1, #3; n = 108; Table S1). The nitrogen cycling gene data (#2, #4; n = 9; Fig. S1) from the RCS was used to explore their potential relationship with the EI and SI indicators. The direction of the relationship (+/-) and the importance of the predictor are indicated by the standardized regression coefficients.

SMLR# target	Significant predictors	P value	Standardized regression coefficient
#1 EI	DN	0.000	+ 0.56
	Nematode counts	0.003	+ 0.46
	DOC	0.035	− 0.27
	S_{min}	0.019	− 0.65
#2: EI	AOA (RCS)	0.001	+ 0.94
#3 SI	C_{org}	0.000	+ 0.53
	N_2O	0.038	+ 0.29
#4 SI	AOA (RCS)	0.003	+ 0.90

be explained by a temporal imbalance in the nematode soil food web, i.e. more microbial grazers than their predators, shifting the status more from retention to release of C and N. This is supported by an early desert ecosystem study, where the loss of predators of microbial grazers in the soil food web led to an overgrazing of the bacterial biomass and a halt in their nutrient cycling activities (Whitford et al., 1982).

Some microbial groups in the N cycle may have significantly benefited from N release through increased bacterial grazing, as indicated by the significant positive relationship between EI and AOA in the RCS soil (Table 3, Fig. S1).

Interestingly, we also found a positive relationship between food web structure (SI) and AOA in the RCS and with released N_2O in both approaches (Table 3), indicating nitrification activity in well-aerated soils (Mathieu et al., 2006). The release of N by grazing of microbial biomass, as described above, and its control by the soil food web could support higher levels of microbial biomass and its contribution to N cycling, but also C retention.

Overall, we found a significant positive relationship between SI and C_{org} in the reclaimed soil (Table 3). Also other authors proposed a relationship between SI and the size and activity of the microbial biomass affecting soil C retention processes (Shi et al., 2023). A shift to a fungal-dominated community may increase the C and N retention efficiency in soil approximately sixfold (Zhou et al., 2023), as indicated by the square C of the nematode faunal profile (Fig. 2). A consequence of this shift may be a more moderate nutrient availability, which may limit the rate of biomass and necromass formation and cycling. In a study using isotopically labeled microbial necromass, two-thirds of it was associated with soil minerals within three days (Buckeridge et al., 2022), a process that can also be expected in the marginal loess-dominated reclaimed soils, as the mineral surfaces are unlikely to be saturated. Other authors have shown that microorganisms in such reclaimed soils become more efficient at incorporating C into biomass when a critical value of 1 % C_{org} is reached (Clayton et al., 2021), which could be related to the saturation state of the mineral surface with microbial compounds such as proteins, reducing the investment in exoenzymes and increasing their efficiency in C acquisition. Inferred from our data, the optimal rate of formation of microbial compounds that can associate with mineral surfaces may be reached when reclaimed soils are located in square B of the nematode faunal profile, where EI and SI approach their maximum (Fig. 2). This is consistent with our second hypothesis and may help to optimize the organic C retention rate and counteract the often overlooked inorganic C losses due to natural acid buffering reactions from these marginal, carbonate-rich loess soil (Zhao et al., 2022).

4. Conclusions

In conclusion, the PCS approach successfully predicted the temporal development of the nematode food web during the early phase of soil reclamation at a lignite mine. The nematode soil food web indicators EI and SI reflected the status of C and N retention and cycling in the reclaimed soil. Further research is needed to determine the thresholds above which natural and anthropogenic variations within a PCS approach compromise the time factor.

CRediT authorship contribution statement

Benoit Renaud Martins: Methodology. **Michael Schloter:** Writing – review & editing, Supervision, Resources, Funding acquisition. **Nicolas Brüggemann:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Rüdiger Reichel:** Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Mathias Hänsch:** Writing – review & editing, Methodology, Conceptualization. **Stefanie Schulz:** Writing – review & editing, Validation, Project administration, Methodology.

Declaration of Competing Interest

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

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agee.2024.109234.

References

Biswal, D., 2022. Nematodes as ghosts of land use past: elucidating the roles of soil nematode community studies as indicators of soil health and land management practices [review]. Appl. Biochem. Biotechnol. 194 (5), 2357–2417. https://doi.org/10.1007/s12010-022-03808-9.

Bongers, T., 1990. The maturity index: an ecological measure of environmental disturbance based on nematode species composition. Oecologia 83, 14–19.

Bongers, T., Bongers, M., 1998. Functional diversity of nematodes. Appl. Soil Ecol. 10 (3), 239–251. https://doi.org/10.1016/S0929-1393(98)00123-1.

Bongers, T., Ferris, H., 1999. Nematode community structure as a bioindicator in environmental monitoring. Trends Ecol. Evol. 14 (6), 224–228. https://doi.org/10.1016/S0169-5347(98)01583-3.

Brümmer, C., Brüggemann, N., Butterbach-Bahl, K., Falk, U., Szarzynski, J., Vielhauer, K., Wassmann, R., Papen, H., 2008. Soil-atmosphere exchange of N_2O and

NO in near-natural Savanna and agricultural land in Burkina Faso (W. Africa). Ecosystems 11 (4), 582–600. https://doi.org/10.1007/s10021-008-9144-1.
Buckeridge, K.M., Mason, K.E., Ostle, N., McNamara, N.P., Grant, H.K., Whitaker, J., 2022. Microbial necromass carbon and nitrogen persistence are decoupled in agricultural grassland soils. Commun. Earth Environ. 3 (1), 114. https://doi.org/10.1038/s43247-022-00439-0.
Camenzind, T., Mason-Jones, K., Mansour, I., Rillig, M.C., Lehmann, J., 2023. Formation of necromass-derived soil organic carbon determined by microbial death pathways. Nat. Geosci. 16 (2), 115–122. https://doi.org/10.1038/s41561-022-01100-3.
Chen, Y., Mekete, T., Dababat, A., Daub, M., Cao, Z., Sikora, R., 2014. Response of nematode communities to reclamation of agricultural soils following degradation through brown coal strip-mining processes. Helminthologia 51 (1), 53–62. https://doi.org/10.2478/s11687-014-0208-9.
Clayton, J., Lemanski, K., Bonkowski, M., 2021. Shifts in soil microbial stoichiometry and metabolic quotient provide evidence for a critical tipping point at 1 % soil organic carbon in an agricultural post-mining chronosequence. Biol. Fertil. Soils 57 (3), 435–446. https://doi.org/10.1007/s00374-020-01532-2.
de Vries, F.T., Caruso, T., 2016. Eating from the same plate? Revisiting the role of labile carbon inputs in the soil food web. Soil Biol. Biochem. 102, 4–9. https://doi.org/10.1016/j.soilbio.2016.06.023.
de Vries, F.T., Liiri, M.E., Bjørnlund, L., Bowker, M.A., Christensen, S., Setälä, H.M., Bardgett, R.D., 2012. Land use alters the resistance and resilience of soil food webs to drought. Nat. Clim. Change 2 (4), 276–280. https://doi.org/10.1038/nclimate1368.
DIN, 2017. Determination of total organic carbon (TOC) by dry combustion (DIN EN 15936:2012-11). Determination of Total Nitrogen Using Dry Combustion Method (DIN EN 16168:2012-11). Beuth Verlag, Germany. German Institute for Standardisation (Deutsches Institut für Normung).
Ferris, H., Bongers, T., de Goede, R.G., 2001. A framework for soil food web diagnostics: extension of the nematode faunal analysis concept. Appl. Soil Ecol. 18 (1), 13–29.
Foster, B.L., Tilman, D., 2000. Dynamic and static views of succession: testing the descriptive power of the chronosequence approach. Plant Ecol. 146 (1), 1–10. https://doi.org/10.1023/a:1009895103017.
Huggett, R.J., 1998. Soil chronosequences, soil development, and soil evolution: a critical review. CATENA 32 (3), 155–172. https://doi.org/10.1016/S0341-8162(98)00053-8.
Joergensen, R.G., 1996. The fumigation-extraction method to estimate soil microbial biomass: Calibration of the kEC value. Soil Biology and Biochemistry 28 (1), 25–31. https://doi.org/10.1016/0038-0717(95)00102-6.
Johnson, E.A., Miyanishi, K., 2008. Testing the assumptions of chronosequences in succession. Ecol. Lett. 11 (5), 419–431. https://doi.org/10.1111/j.1461-0248.2008.01173.x.
Landesoberbergamt, N., 1986. Richtlinien des Landesoberbergamt Nordrhein-Westfalen für das Aufbringen von kulturfähigem Bodenmaterial bei landwirtschaftlicher Rekultivierung für die im Tagebau betriebenen Braunkohlenwerke vom 22.7.1986. Sammelblatt des Landesoberbergamtes Nordrhein-Westfalen, A2, 29.
Lössabkommen, N., 1961. Einigung über die Lößschütthöhe auf den landwirtschaftlich zur rekultivierenden Flächen im Bereich der bereits beendeten und der zurzeit beriebenen Tagebaue der der Rheinischen Braunkohlen AG einschließlich deren Außenkippen zwischen dem min. f. Wirtschaft, Mittelstand und Verkehr, dem Min. f. Ernährung, Landwirtschaft und Forsten, der Landesplanungsbehörde und der Rheinischen Braunkohlen AG vom 20.2.1961.
Mathieu, O., Hénault, C., Lévêque, J., Baujard, E., Milloux, M.J., Andreux, F., 2006. Quantifying the contribution of nitrification and denitrification to the nitrous oxide flux using ^{15}N tracers. Environmental Pollution 144 (3), 933–940. https://doi.org/10.1016/j.envpol.2006.02.005.
Potapov, A.M., 2022. Multifunctionality of belowground food webs: resource, size and spatial energy channels. Biol. Rev. 97 (4), 1691–1711. https://doi.org/10.1111/brv.12857.
Reichel, R., Hänsch, M., Brüggemann, N., 2017. Indication of rapid soil food web recovery by nematode-derived indices in restored agricultural soil after open-cast lignite mining. Soil Biol. Biochem. 115, 261–264. https://doi.org/10.1016/j.soilbio.2017.08.020 (M4 - Citavi.
Reichel, R., Kamau, C.W., Kumar, A., Li, Z., Radl, V., Temperton, V.M., Schloter, M., Brüggemann, N., 2022. Spring barley performance benefits from simultaneous shallow straw incorporation and top dressing as revealed by rhizotrons with resealable sampling ports. Biol. Fertil. Soils 58 (4), 375–388.
Roy, J., Reichel, R., Brüggemann, N., Rillig, M.C., 2022. Functional, not taxonomic, composition of soil fungi reestablishes to pre-mining initial state after 52 years of recultivation. Microb. Ecol. https://doi.org/10.1007/s00248-022-02058-w.
Schmid, C.A.O., Reichel, R., Schröder, P., Brüggemann, N., Schloter, M., 2020. 52 years of ecological restoration following a major disturbance by opencast lignite mining does not reassemble microbiome structures of the original arable soils. Sci. Total Environ. 745, 140955 https://doi.org/10.1016/j.scitotenv.2020.140955.
Shi, G., Luan, L., Zhu, G., Zeng, Z., Zheng, J., Shi, Y., Sun, B., Jiang, Y., 2023. Interaction between nematodes and bacteria enhances soil carbon sequestration under organic material amendments [original research]. Front. Microbiol. 14 https://doi.org/10.3389/fmicb.2023.1155088.
Sieriebriennikov, B., Ferris, H., de Goede, R.G.M., 2014. NINJA: An automated calculation system for nematode-based biological monitoring. Eur. J. Soil Biol. 61, 90–93. https://doi.org/10.1016/j.ejsobi.2014.02.004.
Sokol, N.W., Slessarev, E., Marschmann, G.L., Nicolas, A., Blazewicz, S.J., Brodie, E.L., Firestone, M.K., Foley, M.M., Hestrin, R., Hungate, B.A., Koch, B.J., Stone, B.W., Sullivan, M.B., Zablocki, O., Trubl, G., McFarlane, K., Stuart, R., Nuccio, E., Weber, P., Consortium, L.S.M., et al., 2022. Life and death in the soil microbiome: how ecological processes influence biogeochemistry. Nat. Rev. Microbiol. 20 (7), 415–430. https://doi.org/10.1038/s41579-022-00695-z.
Van Bezooijen, J., 2006. Methods and Techniques for Nematology. Wageningen University Wageningen.
VDLUFA, 1991. Method Book I. Determination of the Gravimetric Water Content (A 2.1.1). Determination of the Soil PH in CaCl2 Extracts (A 5.1.1). Determination of Mineral N (Nmin: NO3 and NH4) in Soil Profiles (A 6.1.4.1). Association of German Agricultural Analytic and Research Institutes, VDLUFA-Verlag, Germany.
Walker, L.R., Wardle, D.A., Bardgett, R.D., Clarkson, B.D., 2010. The use of chronosequences in studies of ecological succession and soil development. J. Ecol. 98 (4), 725–736. https://doi.org/10.1111/j.1365-2745.2010.01664.x.
Whitford, W.G., Freckman, D.W., Santos, P.F., Elkins, N.Z., Parker, L.W., 1982. The role of nematodes in decomposition in desert ecosystems. In: Diana, W.F. (Ed.), Nematodes in Soil Ecosystems. University of Texas Press, pp. 98–116. https://doi.org/10.7560/755260-009.
Zhang, A., Olatunji, O.A., Tariq, A., Li, T., Wang, R., Jiang, Y., 2021. Sulfur deposition changed the community structure of soil nematodes by affecting omnivores-predators. Sci. Total Environ. 771, 144912 https://doi.org/10.1016/j.scitotenv.2020.144912.
Zhao, Y., Reichel, R., Herbst, M., Sun, Y., Brüggemann, N., Mörchen, R., Welp, G., Meng, F., Bol, R., 2022. Declining total carbon stocks in carbonate-containing agricultural soils over a 62-year recultivation chronosequence under humid conditions. Geoderma 425, 116060. https://doi.org/10.1016/j.geoderma.2022.116060.
Zhou, R., Liu, Y., Dungait, J.A.J., Kumar, A., Wang, J., Tiemann, L.K., Zhang, F., Kuzyakov, Y., Tian, J., 2023. Microbial necromass in cropland soils: a global meta-analysis of management effects. Glob. Change Biol. 29 (7), 1998–2014. https://doi.org/10.1111/gcb.16613.