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Indication of rapid soil food web recovery by nematode-derived indices in restored agricultural soil after open-cast lignite mining

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ABSTRACT

Open-cast lignite mining consumes arable land, which has to be restored and recultivated afterwards. This process strongly dilutes the organic carbon and nitrogen pool as well as soil biological parameters of the former soil due to mixing with the underlying subsoil and parent material before redeposition. Cultivation of alfalfa is commonly used to restore agricultural land and to refill diluted C and N pools and re-establish biologic functions. Based on nematode-derived indices, we here evaluate the development of the food web during the early recovery period with N-fixing alfalfa on post-mining soil substrates in the lignite mining district west of Cologne (Germany). Nematode-derived indices revealed a fast recovery of the soil food web during this initial alfalfa cultivation. We found evidence that the applied recultivation procedure lowers the stress and disturbance level in the soil-microbial food web and improves the trophic complexity. The fast maturing of the food web was indicated by *Thornenematidae* nematodes, which indicated a highly structured and stable food web already after three years of alfalfa cultivation. A declining $\delta^{15}N$ signal of the soil indicated a strong impact of N-fixation by alfalfa. Microbial and mineral N content increased during the alfalfa cultivation period. We concluded that the rapid recovery of the soil food web might not be paralleled by an equal increase of its capacity to retain N in the soil food web. This might have implications for nitrate leaching, nitrous oxide emission, and a later agricultural recultivation with common field crops.

1. Introduction

Recultivation is the re-establishment of a functioning soil-plant-microbial system after massive soil disturbance or soil removal (Schinner and Sonnleitner, 2013). In the open-cast lignite mining district in the triangle between the cities Aachen, Cologne and Düsseldorf, Germany, bucket-wheel excavators are removing the overburden layer by layer, including fertile former topsoil. While the mine is moving through the landscape, the removed mixed substrates are transported within an average time of 30 min via conveyor belts to the backside of the mine and deposited there on a coarse-grained and compacted layer. Before sowing, after three month resting time, soil substrate is leveled by chain-bulldozers. The upper layer contains the recultivation substrate, and is a mixture of the former agricultural soil, and the following 4–5 m of underlying subsoil and parent material, i.e. unweathered loess. This substrate is well suited for agricultural recultivation due to its loose structure and quantity of plant-available water (Blume et al., 2016a). Mixing of arable topsoil and loess parent material leads to a dilution of soil organic carbon, total nitrogen, soil biology, and to a shift of other parameters such as pH from weakly acid to alkaline (cf. original soil vs. soil restored 2016 of Table 1). Based on the organic carbon content before and after soil removal, the dilution ratio of arable soil to loess material was around 1:5. Hence, at first the restored soil substrate is of suboptimal quality for agricultural production, but contains the seeds for biological recolonization.

During recultivation, the operating company (RWE Power AG, Essen, Germany) manages the restored soil for five to seven years before the new arable land is handed over to the local farmers. After the deposition of soil substrate, the N-fixing legume alfalfa (*Medicago sativa*) is cultivated for three years to recover soil quality and biology, before typical crops such as barley and later wheat and sugar beet are grown.

Typical input of alfalfa-derived carbon and nitrogen has been reported as 2800 kg C and 150 kg N per hectare and year (Blume et al., 2016b; Autret et al., 2016). Alfalfa biomass has a narrow C/

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Table 1
Nematode-derived soil food web indices and supporting soil parameters. Median values of the nematode Enrichment Index (EI), nematode Structure Index (SI), microbial biomass carbon (MB-C), microbial biomass C/N, and potential level of CO_2-C and N_2O-N emissions. The right part contains median values of the following soil parameters: soil organic carbon (C_{org}), soil total nitrogen (N_t), soil C/N ratio, nitrate (NO_3-N), ammonium (NH_4-N), soil pH_{CaCl2}, and gravimetric water content (H_2O), and $\delta^{15}N$ of soil vs. air. Parameters marked "nd" were not determined. Statistical significant shifts are marked in columns by different letters.

Soil parameter Restoration year	EI (%)	SI (%)	MB-C* mg kg^{-1}	C/N* MB	CO_2 µg C kg^{-1} h^{-1}	N_2O ng N kg^{-1} h^{-1}	C_{org} %-dm	N_t %-dm	C/N Soil	NO_3 mg kg^{-1}	NH_4 mg kg^{-1}	pH $CaCl_2$	H_2O %-dm	$\delta^{15}N$ ‰ vs. air
2016 (deposition)	0 ^{a}	0 ^{a}	38 ^{a}	5.1	36 ^{a}	0 ^{a}	0.28 ^{a}	0.05 ^{a}	6.0	0.55 ^{a}	0.18 ^{a}	7.9 ^{a}	18 ^{a}	5.6 ^{a}
2015 (alfalfa)	67 ^{ab}	12 ^{a}	129 ^{b}	5.0	287 ^{b}	0.9 ^{a}	0.35 ^{a}	0.05 ^{a}	7.4	0.13 ^{a}	0.16 ^{a}	7.8 ^{a}	22 ^{b}	4.1 ^{b}
2014 (alfalfa)	73 ^{b}	66 ^{b}	112 ^{ab}	4.8	403 ^{bc}	198 ^{ab}	0.35 ^{a}	0.05 ^{a}	7.2	2.90 ^{b}	0.23 ^{a}	7.9 ^{a}	21 ^{b}	3.3 ^{b}
2013 (alfalfa)	80 ^{b}	57 ^{b}	176 ^{b}	6.4	458 ^{c}	260 ^{ab}	0.37 ^{a}	0.06 ^{a}	6.8	3.22 ^{bc}	0.12 ^{ab}	7.8 ^{a}	22 ^{b}	3.8 ^{b}
Original soil	nd	nd	nd	nd	1135 ^{d}	130 ^{b}	1.34 ^{b}	0.14 ^{b}	9.4	7.10 ^{c}	0.03 ^{b}	6.4 ^{b}	22 ^{b}	5.8 ^{a}

* Corrected for the MB-C and MB-N loss caused by soil freezing.

N ratio of 15 compared to crops like wheat with a wide C/N ratio of 75 (Rousk and Bååth, 2007).

Soil microorganisms are very competitive in obtaining mineral N in the short term (Näsholm et al., 2009), if C supply or other environmental variables are not limiting. Usually, more than two third of the living soil biomass consist of bacteria and fungi (Gobat et al., 2004) and thereby bind a significant amount of nitrogen in the soil. The immobilization potential depends on the microbial biomass size and if microbial predators foster a remineralization. Roughly, agricultural soils with typical cropping history contain a microbial biomass between 200 and 1000 mg C kg^{-1} (Wu et al., 1990). Microbivores such as protozoa and bacterivorous nematodes as part of the soil food web can significantly stimulate the remineralization of microbial N (Ferris et al., 1998, 2004). Free mineral N might be a source that promotes nitrous oxide emission (N_2O) (Nevison et al., 1996) and nitrate leaching (NO_3^-) from soil (Spalding and Exner, 1993).

The main aim of the study was to elucidate if the initial alfalfa restoration phase of three years allows a recovery of the soil food web, which might be needed in the following phase of agricultural recultivation. We further discuss if an improvement of the soil food web is equally paralleled by a rise of its capacity to retain N in the soil food web, which would mitigate environmentally hazardous N losses and might be of interest for later agricultural recultivation.

We examined indicative taxa of the free living nematode assemblage to evaluate the development of food web structure. Derived indices include trophic levels from bacterial and fungal grazers up to omnivorous predators. Nematodes are well suited for this analysis, since they are ubiquitous soil inhabitants, interact with other soil biota, and have both food specificity and a short response time (Bongers and Bongers, 1998).

2. Material and methods

The selected study sites were located within an area of 5 km^2, consisting of four fields with increasing age after restoration (Fig. S1 of the supplementary material). Soil substrate of 2016 had been deposited recently and was free of alfalfa plants. Fields restored in 2015, 2014, and 2013 had been permanently cultivated with alfalfa since one, two and three years, respectively. Sampling locations were defined in advance by GPS coordinates. From each field, three independent replicates were sampled to evaluate the indicative nematode taxa as well as to quantify the microbial biomass. Five replicates were used for all other parameters. Parameters were determined in samples of the main growing season (28 June 2016). Except for 2016 samples, parameters that do not change quickly, like soil organic carbon (C_{org}) and total nitrogen (N_t), were determined on samples from 1 March 2016. We sampled the biologically most active soil layer to a depth of 10 cm. For the nematode analyses, we used unsieved soil samples. For all other samples we pooled five sub-samples of about 300 g around each GPS location of one replicate. This soil was sieved to <2 mm before VDLUFA guidelines were applied to determine gravimetric water content, soil pH, and NH_4 and NO_3^- content (VDLUFA, 2017). C_{org} and N_t were determined according to DIN (2017). To determine the $\delta^{15}N$ value of air-dried and ground soil samples, nitrogen isotope analysis was conducted according to the detailed protocol of Zhu et al. (2013). Microbial biomass carbon (MB-C) and nitrogen (MB-N) were determined with the chloroform fumigation–extraction method using soil samples that had been stored at −20 °C prior to analysis (Vance et al., 1987; Joergensen, 1996). For the calculation of MB-C, we used a kEC of 0.45 for MB-C (C_{mic}) and a kEN of 0.4 for MB-N (N_{mic}; Joergensen, 1996). Tests revealed that freezing at −20 °C followed by direct extraction without thawing lowers the MB-C and MB-N on average by 10% and 25% respectively, compared to fresh soil samples (data not shown).

CO_2 and N_2O emission rates were determined as follows: a layer of 0.5 cm fresh soil was quickly dried using a continuous air stream before sieving to <2 mm. Three grams of root-free soil were remoistened to 60% water-holding capacity and kept in Parafilm®-sealed 22-ml GC vials for 28 h (VWR International, Darmstadt, Germany). Sixty minutes before the vials were crimped gas-tight with butyl rubber septa and aluminum caps, the Parafilm® was removed to ensure free air exchange. Closed incubation was performed at room temperature for 1, 4, 7, 10 h, respectively. The emission rate was calculated from the slope of the gas concentrations in the headspace of the GC vials, using a gas chromatograph (GC) equipped with FID and ECD (Clarus 580, PerkinElmer, Rodgau, Germany). For a more detailed description of the method see Liu et al. (2014). Based on tests, we are convinced that our highly standardized laboratory procedure reflects more the average greenhouse gas (GHG) emission potential in relation to soil development and nutrient status rather than to short-term field variabilities (Fig. S2). We here use the GHG emission rates as relative indicator for a soil immanent emission potential.

Free living nematodes were extracted from fresh soil, equivalent to 100 g dry soil, using a modified Cobb's method, i.e., a decanting and sieving procedure (van Bezooijen, 2006). We only counted individuals of indicative nematode families to determine the required relative composition (Table S1). This information was used for the c-p scaling after Bongers (1990) to rank the food web disturbance and stability, which relates to the concept of r- and K-strategists. The Nematode Faunal Profile (Fig. 1) summarizes all nematode indicator information, using the calculated Enrichment Index (EI) as measure of resource availability, and the Structure Index (SI) as indicator of stress recovery and trophic food web complexity (Ferris et al., 2001). The calculation of the nematode plant parasite index (Bongers et al., 1997) was beyond the topic of this study.

Statistics were performed including all available replications; however, we present median values to give a realistic picture, which is less susceptible to the influence of exceptional values