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Long-term effects of charcoal on nematodes and other soil meso- and microfaunal groups at historical kiln-sites – a pilot study

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ABSTRACT

Charcoal additions considerably alter the chemical and physical properties of soil. However, direct and indirect charcoal-induced effects on soil biota, particularly on meso- and micro-invertebrates are scarcely assessed. This pilot study aimed to investigate abundances of meso- and micro-invertebrates, particularly free-living nematodes, in soil and decaying leaf litter on ancient charcoal kiln sites in comparison to adjacent control sites. For nematodes we additionally investigated feeding type distribution. The abundance of nematodes tended to be lower in kiln-soils and reduced in litter on kiln sites, while the abundance of most groups remained unaffected by charcoal. Additionally, the proportion of fungivorous nematodes was higher in litter, but remained unaffected in soil. In conclusion our pilot study indicates that charcoal additions affected nematode communities whereas other mesofaunal groups seem to remain unaffected.

1. Introduction

In the last decade the application of biochar has received growing interest due to potential sequestration of considerable amounts of atmospheric carbon in the soil while improving soil fertility simultaneously [1–4]. Adding thermally decomposed or charred biomass to soil (i.e. biochar), however, alters the soil's physiochemical properties immediately after application. These alterations could be of transient nature or last for decades because of the long resident times of charred biomass in soil [5–7]. Evidently, additions of charred biomass affect soil biota in the short term [8], but long-term effects on soil biota communities and ecosystem functioning are still largely unknown [9]. So far mainly char-induced impact on microbial activity and biomass have been studied [10–12], but effects on soil-invertebrates are scarcely assessed [13,14]. The aim of this pilot study was a first assessment of long-term impacts of char additions on the soil's meso- and micro faunal community with a focus on nematodes. For this we investigated abandoned kiln sites used to produce charcoal (i.e. fuel) until the mid-20th century and untreated control sites in the Siegerland (North-Rhine-Westphalia; Germany). Assuming inter-relations between soil and litter we additionally investigated abundance of the meso- and micro-fauna in decomposing leaf litter. Due to the evident effects of former charcoal production on soil physiochemical properties such as bulk density, carbon and nitrogen concentration and water holding capacity on our study sites [5], we expected that at least some meso- and micro-faunal groups would differ in abundance between kilns and control sites. It has been found that the effect of charcoal application was more pronounced on nematode trophic groups with a higher number of fungivore and a lower number of plant parasitic nematodes. Total abundance was less effected [15].We therefore analyzed the feeding type composition of the nematode community in soil and in decaying litter at kiln and control sites. Differences in feeding type composition may point to shifts in the soil food web for which nematodes are known to be good indicators [16,17].

2. Material and methods

We studied meso- and microfauna on abandoned kiln sites (n = 5) used to produce charcoal until the mid-20th century and adjacent control sites (n = 5) without charcoal in a beech timber forest (Luzolo-Fagetum formation with scarce understory) located in the Siegerland (North-Rhine-Westphalia; Germany; Table S1). For a description of study sites and their physicochemical properties see Table S2 and [5]. Kiln sites and control sites were about 5 m in diameter (approx. 20 m^2) and about 10 m apart. To assess the abundance of meso- and microfauna in uppermost topsoil (0–5 cm) we took 50 g fresh soil samples

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(n = 1 per kiln and control site). To collect meso- and microfauna from decomposing litter, we collected freshly fallen beech leaf litter within the study area, but not on the study sites to prevent home-site effects. We randomly filled 5 g of beech leaves into nylon litter bags (20 cm × 20 cm) with a mesh size of 5 mm and placed them on bare ground on the kiln and control sites (5 bags each) at 26th February 2014 for 100 days. Immediately after sampling, soil and litter were preserved in a 50 ml falcon tube in 4% formaldehyde solution and stored in a refrigerator at 4 °C for a maximum of 30 days. Total meso- and microfauna were extracted from soil using a density-centrifugation procedure involving Ludox HS-40 [18]. To extract total meso- and microfauna from litter, we carefully rinsed leaves with distilled water. The organic supernatant containing meso- and microfauna was poured through a 10 μm gaze, preserved in 4% formaldehyde solution, stained with a few drops of Rose Bengal (0.3 g/L), and counted in total under a stereomicroscope (Leica MZ 9.5; 40 × magnification).

For the feeding-type analysis approximately 50 randomly chosen nematodes were sorted from each soil sample and each litterbag (i.e. 50 nematodes per site from soil and 250 nematodes per site from litter; in total 3000 nematodes) and gradually transferred to anhydrous glycerol before mounting on slides [19]. Nematodes were classified into different feeding-types based on the morphology of their buccal cavity under a stereomicroscope (Zeiss Primo Star; 1000× magnification with oil immersion) [20]. We calculated the fungivore/bacterivore ratio (F/B) as an indicator for the dominant decomposition pathway [21,22].

Besides nematodes we additionally extracted a mentionable number of individuals of wheel animals (*Rotifera*) and ringed worms (*Annelida* (mainly *Enchytraeids*)). Other faunal groups (i.e. mites (*Arachnida*), springtails (*Collembola*) and water bears (*Tardigrada*)) were present but underrepresented and will not be considered in the following.

For statistical analysis, abundance of meso- and microfauna on leaf litter were pooled for each site. For comparisons of total abundances, F/B ratios, and feeding type abundances of nematodes between kiln sites and control sites, we used Kruskal-Wallis-Test because the data were not normally distributed. All statistics were performed using R [23]. All p-values are two-tailed.

3. Results

The abundance of nematodes, rotifer and annelida extracted from soil did not differ significantly between kiln and control sites (Table 1a; $p > 0.05$). However, if we excluded plant-parasitic nematodes and data of one control site characterized by very low nematode abundance, we found a significantly lower number of nematodes on kiln sites than on controls (Table 1a; $p = 0.01$). We are aware that such a data reduction is critical, but we think it is justifiable in this case especially for a comparison with the results from the litter samples in which plant-parasitic nematodes were absent. Similar to our results from soil, the abundance of nematodes in litter was significantly lower on kiln sites than on control sites (Table 1a; $p = 0.02$), whereas the abundance of ringed worms and wheel animals did not differ between kiln sites and control sites in litter (Table 1a; $p > 0.05$). Looking at the nematode feeding types, we found no differences between kiln sites and control sites for soil dwelling nematodes (Table 1b; $p > 0.05$) but significant differences for litter dwelling ones (Table 1b; $p = 0.03$). Here the proportion of fungivorous nematodes was significantly higher on kiln sites than on control sites (Table 1b; $p = 0.03$). This shift leads to a significantly higher F/B ratio of litter-dwelling nematodes on kiln sites (Table 1b; $p = 0.03$).

4. Discussion

Our results point to some considerable long-term effects of charcoal addition on the total abundance of nematodes, whereas the abundances of rotifera and annelida remained unaffected. Additionally, the detected differences in nematode feeding type composition may point to a shift in the soil food web with an increased role of fungi. Reduced nematode abundances as well as changes in feeding type composition at kiln sites could probably be explained by changes in soil properties due to charcoal addition that were found in our study area [5]. However, nematode community responses to soil property changes are complex [24] and a more comprehensive study is needed to analyze this relationship in detail. The nematode feeding type distribution indicates that charcoal addition promotes fungi rather than bacteria within the litter microbial community, although bacteria still dominate. This is in line with other studies that also suggest a charcoal-induced promotion of fungi indicated by an increase of fungivorous nematodes [15,25]. The fact that we found charcoal effects on microbivore nematodes in litter but not in soil is surprising, because direct effects of charcoal on litter dwelling microbes are unlikely and any effect should be due to cascading effects from soil to litter. Fungi abundance was probably also raised in kiln soil, but fungivorous nematodes were improper indicators due to a possible fungi shelter effect of charcoal micro-pores that leads to a reduced accessibility of fungal prey [8,26,27]. Consequently, the nematode community in kiln soil was unable to respond on possibly increased fungi abundance. As fungi in soil should be a major source of fungi colonizing litter, the increase of fungi abundance in soil due to some charcoal effects should lead to increased fungi abundance in litter as well. Due to lacking porous charcoal pieces, fungi in litter are accessible for fungivorous nematodes that adapt to the increased food

Table 1

a) Abundance of meso- and microfauna extracted from 50 g soil (n = 5) and 5 × 5 g litter (n = 5) presented as individuals per gram; note that data on non-plant parasitic nematodes correspond to total nematodes in litter, because plant parasites were absent here b) Nematode feeding type distribution in percent and ratio of fungivore and bacteriovore nematodes (F/B); all data presented as median with range in parentheses.

a)Abundance	Soil			Litter		
	Kiln	Control	p-value	Kiln	Control	p-value
Total Nematodes	20 (41)	43 (36)	0.35	9 (8)	24 (13)	**0.02**
Non-plant parasitic Nematodes	20 (23)	38 (8)	**0.01**			
Rotifera	6 (20)	2 (14)	0.92	42 (22)	40 (52)	0.92
Annelida	1 (1)	1 (1)	0.75	ND	ND	
b)Feeding type	**Soil**			**Litter**		
	Kiln	Control	p-value	Kiln	Control	p-value
Bacteriovore	63 (30)	58 (32)	0.91	67 (37)	85 (21)	0.17
Fungivore	2 (15)	4 (10)	0.58	23 (22)	6 (7)	**0.03**
Predatory	4 (19)	4 (11)	0.75	9 (15)	3 (7)	0.35
Omnivore	32 (18)	32 (18)	0.75	1 (2)	3 (21)	0.12
F/B ratio	0.04 (0.23)	0.06 (0.24)	0.75	0.35 (0.46)	0.07 (0.09)	**0.03**

ND: not detected; significant differences in bold face.

source at charcoal-enriched sites. Of course, this remains speculative due to a lack of information about the microbial community.

Because we did not analyze rotifers and annelids in more detail we cannot rule out charcoal effects on the community of these groups. Such effects did not necessarily lead to changes in total abundance but rather change the species composition. Our preliminary results on the nematode community underline that further studies of possible charcoal effects on the community of soil faunal groups would be valuable.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ejsobi.2019.103095.

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