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Abstract
Soil processes, like C mineralisation, can be affected by soil contamination through disturbance of ecological interactions between soil fauna and microorganisms. In this study we have tested the hypotheses that vertically heterogeneous contamination affects soil fauna and microbial interactions and thereby soil process rates. The study consisted of a laboratory microcosm experiment with an epigeic and endogeic earthworm and an isopod species. Vertical heterogeneity in contamination did not affect behaviour of species which differed in their life history traits. Furthermore, interactions between macrofauna and microorganisms did not seem to be affected. Results indicated that microorganisms were more sensitive to the contaminants than soil fauna. The adverse effects of the contaminants on microbial activity were mitigated by soil fauna stimulating microbial activity.

Keywords: Heavy metals, soil processes, soil ecosystem functioning, soil fauna, microorganisms

Introduction

As soil is a very heterogeneous environment, it creates a great variety of microsites with specific environmental conditions along horizontal and vertical scales providing favourable conditions for many species (Faber and Joosse, 1993; Berg et al., 1998; Sadaka and Ponge, 2003; Berg and Bengtsson, 2007). Soil animals and microflora specifically interact at various depths with respective differentiation in the resulting direction and magnitude of organic matter and nutrient fluxes (Faber, 1991; Berg et al., 2001). These interactions include microbial grazing by soil animals, incorporation of leaf litter at certain depths in the soil profile, bioturbation

of the soil substrates, thereby affecting the physico-chemical characteristics of soils and dispersal of microbial community (Petersen and Luxton, 1982; Edwards and Bohlen, 1996; Hättenschwiler et al., 2005).

Soil contamination can affect the functioning of soil organisms in various ways. Besides having a direct toxic effect on their physiology and hence their functioning, it can also affect behavioural patterns of species and thereby adversely affect important ecological interactions between species. River floodplains may show vertical heterogeneity in soil contamination of inorganic and organic contaminants, due to different contaminant load of the sediments deposited over the years (Middelkoop, 1997; Thonon, 2006). The distribution of groups of organisms and their ecological functioning may be specifically affected by the presence of contaminants, given this vertical heterogeneity, either through exposure at specific microsites, or by avoidance of the contaminated soil layer. If the contamination is situated deeper in the soil profile, as is the case for older sediments, surface dwelling species and epigeic earthworms may not be affected, whereas burrowing and deep living species (i.e. anecic and endogeic earthworms, and some enchytraeid species) could still be exposed.

Avoidance of contaminated patches has been reported for earthworms (Slimak, 1997; van Zwieten et al., 2004; Eijsackers et al., 2005; Natal-da-Luz et al., 2008), while isopods have been shown to avoid contaminated litter (van Cappelleveen, 1986; Weiβenburg and Zimmer, 2003). By vertical avoidance, however, soil organisms may encounter suboptimal conditions, e.g. with respect to food availability or abiotic conditions. Therefore, soil processes such as the redistribution and decomposition of organic matter may be affected if species dysfunction or avoid unfavourable microsite habitats (Cortet et al., 1999; Salminen et al., 2001). Furthermore, species can redistribute contaminants by bioturbation and thereby introduce the contamination to formerly unexposed microsites (Zorn, 2004). Earthworms can also introduce uncontaminated litter into the profile thereby altering microbial functioning. Litter incorporation is known to stimulate microbial biomass carbon (e.g. Sheehan et al., 2008). Furthermore, uncontaminated leaf litter can stimulate microbial activity if microbial activity is inhibited by contaminated soil organic matter (Chaudri et al., 2008).

In this study we have tested the hypotheses that vertically heterogeneous contamination affects soil fauna and microbial interactions and thereby soil process rates. We have performed a laboratory experiment in which artificial soil profiles in microcosms were created, using river floodplain soil to mimic various situations of vertical distribution of

contamination found in the field. Organic matter breakdown was studied in the presence of decomposer fauna, and differences in decomposition rate were analysed in response to the presence and depth distribution of contaminants.

Materials en Methods

Experimental design

To assess the effect of vertically heterogeneous soil contamination on soil fauna and microbial interactions, we conducted a microcosm experiment in which four different soil profiles were made. Soil columns of 10 cm height were created of 1) 0-10 cm reference soil, 2) 0-5 cm reference and 5-10 cm contaminated soil, 3) 0-5 cm contaminated and 5-10 cm reference soil and 4) 0-10 cm contaminated soil (see Figure 1).

We assessed the impact of soil contaminants on three species that belong to the same functional group of macrodetritivores and that differ in their vertical microhabitat choice and preference in feeding mode; a surface dwelling isopod *Oniscus asellus* (Linnaeus, 1758), an epigeic earthworm *Lumbricus rubellus* (Hoffmeister, 1843), and an endogeic earthworm *Aporrectodea caliginosa* (Savigny, 1826). *Lumbricus rubellus* and *O. asellus* feed on leaf litter. *Aporrectodea caliginosa* feeds mostly on soil organic matter although it can change its food source to leaf litter (Jégou et al., 2001). We assessed their burrowing behaviour and their interactions with microorganisms by measuring microbial and fungal biomass, litter mass loss, soil organic matter content, soil respiration (CO_2 production) and nitrogen mineralisation (total net NH_4^+ and NO_3^- production).

Reference soil was retrieved in May 2001 from a floodplain, called the Afferdense en Deestse Waarden, along the river Waal, the Netherlands (longitude 51˚54'N, latitude 5˚39'E). Contaminated soils were collected in October 2002 in the Biesbosch floodplain (longitude 51˚45'N, latitude 4˚44'E) and the Heesseltse floodplain (longitude 51˚49'N, latitude 5˚20'E). A mixture of the latter two soils was used to produce heavily contaminated soil, which had organic content similar to the reference soil (Table 1). Alder leaf litter (*Alnus glutinosa,* Linnaeus 1753) was collected two weeks after leaf fall in autumn 1997 from a reference site, Roggebotzand, the Netherlands (longitude 52˚34'N, latitude 05˚47'E). The litter was air dried at 20 ˚C for three days and stored at room temperature till further use.

Figure 1. Experimental design of the four artificial soil profiles ***a****, profile of 0-10 cm reference soil;* ***b****, profile of reference topsoil 0-5 cm and contaminated sub-layer 5-10 cm;* ***c****, profile contaminated topsoil 0-5 cm over a reference sub-layer 5-10 cm and;* ***d****, profile of 0-10 cm contaminated soil.*

Table 1. Soil characteristics as measured at start of experiment; Bulk density, organic matter, clay content, water content, pH (H_2O), C and N content, zinc, and copper concentrations. Soils contained other contaminants including PAHs, PCBs and other trace metals. Abbreviation: WHC, water holding capacity; nd, not determined.

Soil characteristics		Reference soil	Heavily contaminated soil
Bulk density (kg/dm^3)		1.58	1.50
Organic matter content (% DW)		11.6	12.6
Clay content (% DW)		19.3	19.3
Water content (% DW) at 62.5% WHC		48.3	55.8
pH (H_2O)		7.5	7.6
C (% DW)		4.6	7.6
N (% DW)		0.40	0.43
Zinc	total (mg/kg)	550	1175
	$CaCl_2$-extractable (mg/kg)	0.5	1.2
Copper	total (mg/kg)	78	125
	$CaCl_2$-extractable (mg/kg)	< 0.010	nd

Macrofauna was removed from soils by hand sorting. To obtain similar contents in organic matter and clay in the contaminated soil and the reference soil, the two heavily contaminated soils were mixed (volume ratio 1:1) using a concrete mixer (volume: 160 L) for 1 minute to homogenise. Homogenisation of reference soil was also done using the same concrete mixer to obtain a similar pre-treatment; i.e. disturbance of the soil matrix. Reference and mixed contaminated soils were stored at 2 ºC till further use. Table 1 shows the soil characteristics and heavy metal load of both soil types. The contaminated soil used contained 1175 mg zinc /kg, which exceeds the Dutch intervention values for soil remediation (VROM, 2000)

Microcosms (∅ 12.5, 20 cm height, poly-ethylene) were filled with two 5 cm thick layers of soil. The differences in bulk density of the two soils resulted in 400 ± 5 g (FW) of contaminated soil and 445 ± 5 g of reference soil. Both layers were brought to a moisture content of 62.5% of the water holding capacity (WHC) (see Table 1 for corresponding water contents), and topped with a 1 cm thick layer of Alder leaf litter as a food source (10 g moisture content 74.2% FW). As *O. asellus* is a litter dwelling species, we started with a thin layer of litter to increase the exposure of *O. asellus* to the soil. After a pre-incubation period of 10 days, macrodetritivores were introduced into each of the microcosms: three adults of *L. rubellus* (0.37 ± 0.04 g DW per microcosm), five adults of *A. caliginosa* (0.56 ± 0.01 g DW per microcosm), or twelve adults of *O. asellus* (0.20 ± 0.01 g DW per microcosm). Ten microcosms were destructed after the pre-incubation period to assess initial organic matter and NO_3^- and NH_4^+ content. Additional 10 g portions of leaf litter were added after 10 days and 21 days of detritivore introduction to prevent starvation by food shortage.

The microcosm was airtight and suitable for continuous monitoring of soil respiration. During the experiment lids were removed once a week to refresh the air. Microcosms were randomly distributed over two water baths (15 °C) to buffer possible temperature fluctuations in the climate room. The climate conditions were 15 °C, 80% relative humidity, and a light/dark regime of 12/12 hours.

Soil properties

Moisture content (w/w) was determined gravimetrically by drying moist soil for 24 hours at 60 ºC, and weighing the mass loss to the nearest mg. To determine the water holding capacity and soil bulk density, 100 cm^3 sample-rings were inserted into undisturbed layers of the column, until entirely filled. Dry weight of the soil was determined by drying the rings for 48 hours at 70 ºC. For water holding capacity, a mesh-cloth (0.2 µm) was placed at the bottom of the samples to avoid sample loss. Rings were emerged in water, until saturated (± 2 hours), and placed on a rack for one hour before weighing. The pH (H_2O) was measured in a suspension of 5 g dry soil in 25 mL demineralised water.

Organic matter content of the soil was measured by ash furnacing of 20 g soil at 550 ˚C for three hours. Total metal concentrations were determined by digesting 1 g dry soil in a mixture of H_2O, concentrated HNO_3 (65%) and HCl (37%) at a volume of 1:1:4 using a MARS5 microwave (Bongers, 2007). Quality control was maintained by digesting reference samples (SETOC, Wepal), of which the measured Zn

concentrations did not deviate more than 10% from the certified reference value. Extractable concentrations were determined by shaking 5 g soil in 25 mL 0.01 M $CaCl_2$ solution for 4 hours. Tubes were centrifuged at 1300 *g* for 20 minutes and the supernatant passed through a 0.45 µm filter. Samples were stored at 4 ºC until analysed. All metal extracts were analysed using flame Atomic Absorption Spectrometry (Perkin Elmer 1100B AAS). Soil samples for soil particle size analyses were pre-treated to remove organic matter and carbonates to obtain only mineral soil particles (Konert and van den Berghe 1997). Clay fraction of the soil was determined using laser diffraction size analysis (Konert and van den Berghe, 1997). Carbon and nitrogen content of soils (dried at 50 ºC for 12 hours and milled using a mortar) were analysed using an element analyser (Carlo Erba Strumentazione elemental analyser, model 1106).

Soil organisms and processes

Soil processes measured were litter mass loss, nitrogen mineralisation (total NH_4^+ and NO_3^- production), and soil respiration (CO_2 production). After five weeks all the remaining microcosms were destructively sampled. Animal numbers and biomass were determined. Animal survival during the experiment was assessed visually, every week without disturbing the litter. If dead animals were observed in the microcosm, they were not replaced, nor removed.

Before the soil columns were destructively sampled, the number of earthworm burrows was counted against the side wall of the microcosms. By using a 5x5 cm grid, divided in 1x1 cm sections, we quantified the number of burrows crossing the section lines, at both 0-5 cm depth and 5-10 cm depth, with two replicates per depth (Giovannetti and Mosse, 1980). Soil columns were then destructively sampled. Samples were taken at three layers; the litter layer, and the two soil layers (0–5 cm and 5–10 cm). Samples were stored at 2 °C and individually processed.

Extraction of NO_3^- and NH_4^+ was done by shaking 20 g soil in 200 mL 1 M KCl solution for 12 hours. The suspension was centrifuged (10 min at 3000 rpm) and analysed on an auto-analyser (Skalar SA 40). Organic matter content of the soil was measured by ash furnacing of 20 g soil at 550 ˚C for three hours. Leaf litter fragment size distribution was measured by sieving leaf litter after air-drying of leaves. Sieve mesh sizes were 16, 8, 4, and 2 mm, respectively. Soil respiration in the microcosms was measured every two hours using conductrometry (respirometer filled with 0.5 M KOH) after Doelman and Haanstra (1979). A reference cell was included to measure CO_2 leaching during opening of microcosms.

Suspensions for bacterial and fungal analyses were made by blending 20 g soil with 190 mL demiwater for one min. Directly after blending, a 9 mL sample was taken and 1 mL formalin (40%) was added to the suspension for fixation. Suspensions were stored at 2 ºC. Bacteria were stained using vital-staining method of DTAF (Bloem et al., 1995). Bacteria counts were done using confocal laser scanning microscopy (image analyses by computer, Bloem et al., 1995). Fungi were differential fluorescent stained using fluorescein diacetate and differential fluorescent stain, a vital stain method that differentiates between active and inactive mycelia (Morris et al., 1997). Active, inactive and total fungi mycelia were counted under a microscope, using an intersection method (100x10 grid cells, UV-light).

Data analyses

Survival and changes in biomass of soil fauna were analysed using ANOVA. To assess net NO_3^- production, the NO_3^- content at the start of the experiment was subtracted from the final NO_3^-. Ammonium production was calculated in the same fashion as net NO_3^- production. NO_3^- and NH_4^+ production were expressed per g N in the soil, to correct for differences in soil moisture and organic matter content between the reference soil and contaminated soil. Soil respiration data was summed to a cumulative value over five weeks. To correct for differences in organic C content in the soil, CO_2 values were expressed as mg CO_2 per g soil C. Changes in litter fragment size distributions were analysed with the Bray-Curtis index of similarity (Legendre and Legendre, 1998). The reference distribution in litter fragment sizes was expressed in a reference Bray-Curtis value using the litter fragment sizes of the reference soil with no soil macrofauna present. Because we sampled at 0-5 cm and 5-10 cm depth, we tested the effect of contamination on species distribution and soil process rates using a three factor general linear model with species, location of contaminant and depth in soil profile as factors. Because these analyses showed interactions between the 3 factors, we ran two-factor general linear model (GLM) with species and location of contaminant treatment as factors on the individual depth sampling layers (0-5 and 5-10 cm separately). If the interaction between contaminant and species was significant, one way ANOVAs were used for detailed analyses. Correlations between soil processes and soil characteristics were analysed using Pearson's two-tailed correlation. All statistical analyses were done using SPSS v16.0.

Results

Survival and reproduction

Survival of *A. caliginosa* and *L. rubellus* was higher than 95%. Both species increased in weight during the experiment, being 19-46% for *L. rubellus* and 49-61% for *A. caliginosa*. Increases in earthworm biomass were similar in all treatments (ANOVA, *L. rubellus* $F_{3, 16} = 1.18$, $P = 0.35$, *A. caliginosa* $F_{3, 16} = 0.185$, $P = 0.18$). Survival of *O. asellus* in microcosms ranged from 0-100%, averaging 50%. Mortality occurred mainly after three weeks. Collembola and Nematoda were observed in low numbers.

Microorganism activity and biomass

In general, reference soil showed a higher CO_2 production than treatments containing contaminated soil (Tukey HSD post hoc, $P < 0.001$), both in the presence and absence of soil animals (Figure 2a). As the dry weight of the macrofauna species varied, the net effect of species (to the control) is shown per g dry weight species (Figure 2b). *Lumbricus rubellus* and *A. caliginosa* induced a significant increase in CO_2 production in all treatments compared to the control (Tukey HSD, $P < 0.001$). Increase in respiration rates in treatments containing *A. caliginosa* (compared to microcosms without animals) was significantly higher in the completely contaminated profile than the reference soil (ANOVA Tukey HSD post hoc, $P = 0.035$) being 57% increase in the contaminated profile, while the reference profile was increased by 17%. Mixture soil treatments showed intermediate values with a 29-31% increase in CO_2 production. Relative increase in CO_2 production for *L. rubellus* was similar for all soil treatments (ANOVA, $F_{3,16} = 1.1$, $P = 0.388$). *O. asellus* did not induce significant increases in CO_2 production (Tukey HSD, $P = 0.526$).

Bacterial biomass varied between 10 and 37 μg C/g soil, irrespective of soil fauna presence, and did not differ between treatments (all $P > 0.05$, Table 2). Fungal hyphal length varied between 27 and 89 m/g soil, of which on average 47% was metabolically active. No significant differences in active hyphal length were observed between neither species treatments nor soil treatments (all $P > 0.05$, Table 2).

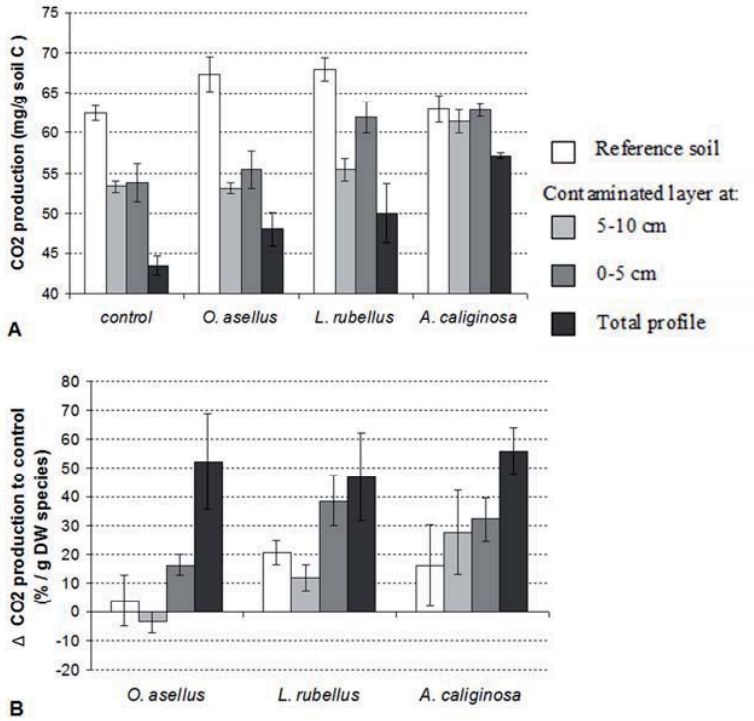

Figure 2. Accumulated CO_2 production in five weeks expressed as ***A****, total mg CO_2/g C in soil and;* ***B****, a percentage of the production in the control (%/g DW species). Bars indicate different contaminant stratification treatments, see legend. Error labels represent standard errors (n = 5).*

Soil organic matter and leaf litter

There were no differences in remaining litter mass between soil treatments (P = 0.62, Table 2). The presence of earthworms significantly reduced leaf litter mass due to consumption and incorporation of leaf litter fragments into the soil (Figure 3, Tukey HSD post hoc *L. rubellus,* $P < 0.001$; Tukey HSD post hoc *A. caliginosa*, $P < 0.001$). Highest values for litter mass loss were found in the presence of *L. rubellus,* namely 4.31 ± 0.42 mg DW. Presence of *A. caliginosa* resulted in litter mass losses of 2.5 ± 0.33 mg DW, while no significant increase in litter mass loss occurred in the presence of *O. asellus* compared to the control.

Changes in leaf litter fragments size distribution towards smaller fractions were observed for soil fauna; Bray-Curtis values were 0.81 ± 0.02 and 0.83 ± 0.02, respectively ($P < 0.001$, Table 2). However, no differences between soil treatments could be detected (P = 0.144, Table 2). Changes in fragment size distribution in presence of *L. rubellus* and *A. caliginosa* were

mainly due to incorporation of leaf fragments of 2-16 mm, smaller and larger pieces were similar to the control. No decrease in Bray-Curtis values was observed for *O. asellus*, indicating that litter fragmentation by the isopod was relatively low.

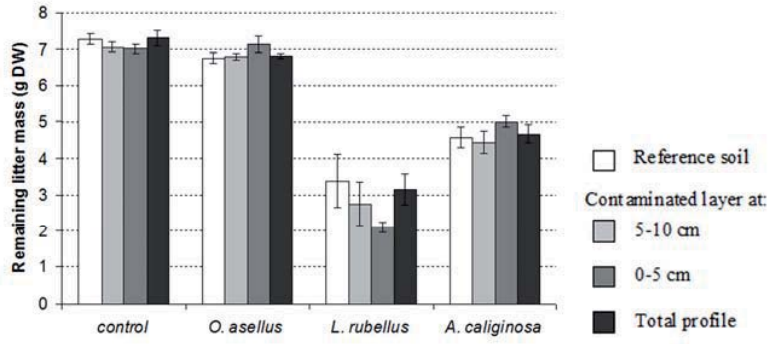

Figure 3. Remaining litter mass (g DW) in the litter layer at the end of the experiment (five weeks). Error labels represent standard errors (n = 5).

Soil organic matter (SOM) contents of the two soils in the control did not change (Table 1, Figure 4a), being 11.6 and 12.7% DW. *Oniscus asellus* did not affect SOM content (Figure 4b). *Lumbricus rubellus* significantly increased the SOM content of soil layers (Tukey HSD post hoc, $P < 0.001$) with 0.8–1.4% (Figure 4c), except for the 0-5 cm layer in the completely contaminated profile. In presence of *A. caliginosa* the surface 0-5 cm layer had a SOM content of 12.5%, regardless of the initial SOM content. Therefore the net difference in SOM was both negative and positive in the presence of *A. caliginosa* at 0-5 cm (Figure 4d). Soil organic matter content of the 5-10 cm layer was increased by 0.6–1.0% in presence of *A. caliginosa*.

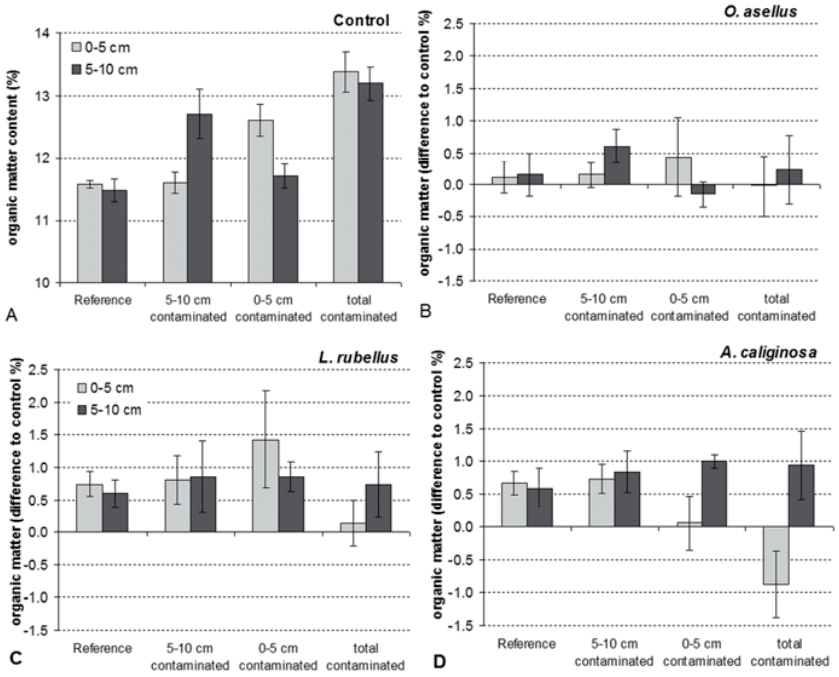

*Figure 4. Soil organic matter content (% DW) of **A**, control (no macrofauna added) and expressed as difference to control (% DW); for microcosms with **B**, Oniscus asellus; **C**, Lumbricus rubellus and; **D**, Aporrectodea caliginosa. Error labels represent standard errors (n = 5).*

Soil water content in the soil of the control reference soil was lower than the contaminated soil thereby reflecting the differences in the initial water content of the two soils (Figure 5a). An increase in water content was observed in the control group, which was most probably due to the addition of water saturated Alder litter during the experiment. At 0-5 cm fauna and soil treatment both were significant factors but also the interaction was significant (Table 2). *Oniscus asellus* did not affect water content at 0-5 cm in all soil treatments (ANOVA Tukey HSD post hoc all $P > 0.40$). *Lumbricus rubellus* increased water content in the 0-5 cm layer in all treatments except for the fully contaminated profile (Tukey HSD post hoc, Reference $P = 0.001$, 5-10 cm contaminated $P = 0.005$, 0-5 cm contaminated $P = 0.001$, totally contaminated profile $P = 0.637$). *Aporrectodea caliginosa* increased water content in the 0-5 cm layer only in the reference profile ($P = 0.001$). At 5-10 cm, only *L. rubellus* significantly increased soil water content on average by 1.2% (Tukey HSD post hoc, $P = 0.022$.

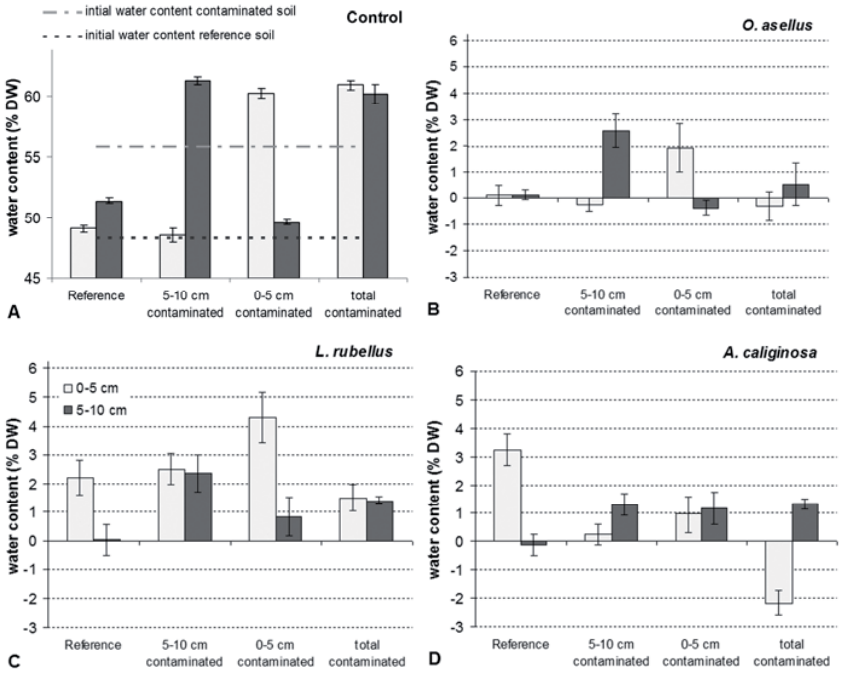

Figure 5. Soil water content (% DW) of ***A****, control (no macrofauna added) and expressed as difference to control (absolute difference % DW); for microcosms with* ***B****, Oniscus asellus;* ***C****, Lumbricus rubellus and;* ***D****, Aporrectodea caliginosa. Error labels represent standard errors (n = 5).*

Table 2. Two-factor GLM statistical analyses for soil processes measured in the microcosms at the end of the experiment: Soil respiration (mg CO_2 /g soil C), Bacterial biomass, metabolically active fungal hyphal length, remaining litter mass, Litter fragmentation (Bray-Curtis similarity index), water content, net NO_3^- production for total column and individual soil layers. Abbreviations: n, number of samples, df, degrees of freedom; SS, sum of squares; F, Fisher ratio; P, probability. Soil refers to the 4 different profiles of contamination. SxF refers to the interaction between the factors soil and fauna

Variable	n	Treatment	df	Type III SS	F	*P*
CO_2 production	79	Soil	3	**2496**	**53.9**	**<0.001**
		Fauna	3	**980**	**21.1**	**<0.001**
		SxF	9	157	1.13	0.355
Δ CO_2 to control (% / g DW species)	59	Soil	3	**15070**	**9.10**	**<0.001**
		Fauna	2	2666	2.41	0.101
		SxF	6	1945	0.59	0.739
Bacterial biomass at 0-5 cm	80	Soil	3	51.5	0.16	0.920
		Fauna	3	236	0.77	0.518
		SxF	9	427	0.46	0.895
Bacterial biomass at 5-10 cm	80	Soil	3	91.9	0.60	0.619
		Fauna	3	15.7	0.10	0.958
		SxF	9	200	0.43	0.912
Fungal active hyphal length at 0-5 cm	43	Soil	3	32.7	0.18	0.91
		Fauna	3	53.3	0.65	0.59
		SxF	9	59.2	0.90	0.54
Fungal active hyphal length at 5-10 cm	38	Soil	3	115	0.482	0.698
		Fauna	3	335	1.399	0.270
		SxF	9	388	0.541	0.829
Remaining litter mass	80	Soil	3	849762	0.60	0.620
		Fauna	3	**2.466 E8**	173	**<0.001**
		SxF	9	5440905	1.27	0.270
Litter fragmentation	80	Soil	3	0.009	0.601	0.62
		Fauna	3	**0.237**	**15.5**	**<0.001**
		SxF	9	0.068	1.49	0.17

Table 2. Two-factor GLM statistical analyses for soil processes - continued.

Variable	n	Treatment	df	Type III SS	F	*P*
Water content (% DW)\ 5-10 cm	80	Soil	3	**2564**	**196**	**<0.001**
		Fauna	3	**15.5**	**3.36**	**0.024**
		SxF	9	23.8	1.72	0.103
OM content (% DW) 0-5 cm	80	Soil	3	**26.4**	**627**	**<0.001**
		Fauna	3	**7.24**	**12.63**	**<0.001**
		SxF	9	**7.29**	**4.24**	**<0.001**
OM content (% DW) 5-10 cm	80	Soil	3	**47.3**	**130**	**<0.001**
		Fauna	3	**10.3**	**28.3**	**<0.001**
		SxF	9	1.60	1.47	0.18
NO_3^- production (mg NO_3^- /g DW litter) Alder litter	79	Soil	3	0.51	0.17	0.25
		Fauna	3	**8.9**	**24.0**	**<0.001**
		SxF	9	1.7	1.6	0.14
NO_3^- production (mg NO_3^- /g N soil) 0-5 cm	79	Soil	3	**14970**	**32.8**	**<0.001**
		Fauna	3	**8390**	**18.4**	**<0.001**
		SxF	9	1465	1.07	0.40
NO_3^- production (mg NO_3^- /g N soil) 5-10 cm	80	Soil	3	**12338**	**20.3**	**<0.001**
		Fauna	3	**153123**	**252**	**<0.001**
		SxF	9	**10575**	**5.80**	**<0.001**

NO_3^- and NH_4^+ production

In the litter layer NO_3^- production was not affected by soil treatment and the average NO_3^- production in the control was 1.1 mg/g DW litter. Soil fauna affected the NO_3^- and NH_4^+, by which *L. rubellus* showed an average net decrease of -0.36 mg NO_3^-/ g DW litter compared to control (Tukey HSD post hoc, $P = 0.008$). Microcosms with *O. asellus* showed an average increase of 0.56 NO_3^-/g DW litter (Tukey HSD post hoc, $P < 0.001$). Nitrate production in the litter was not significantly affected by *A. caliginosa* (Tukey HSD post hoc, $P = 0.71$).

Extractable NH_4^+ levels were low, being less than 5% of total N contents in the soil (data not shown). Analyses of the individual soil layers showed that both soil and fauna treatment affected NO_3^- production significantly in both soil layers, but the effect at 5-10 cm showed a significant interaction between soil and fauna treatments (Table 2). At 0-5 cm, NO_3^- production

in the reference treatment was significantly lower than in the 0-5 cm contaminated treatment (Tukey HSD post hoc, $P = 0.014$), and significantly higher than in the 5-10 cm treatment (Tukey HSD post hoc, $P < 0.001$). Soil fauna increased NO_3^- production at 0-5 cm in all treatments (Figure 6). At 5-10 cm the NO_3^- production for *O. asellus* was similar to the control (no macrofauna added) except for the 5-10 cm contaminated soil treatment. Nitrate production was high in treatments containing earthworms (Figure 6c-d). Both *A. caliginosa* and *L. rubellus* increased NO_3^- production at 5-10 cm in all soil treatment ($P < 0.001$). This increase was higher in treatments containing contaminated soil at 5-10 cm than in the reference soil (Tukey HSD post hoc, *L.rubellus*, $P = 0.006$, $P = 0.008$; *A. caliginosa*, $P < 0.001$, $P < 0.001$).

Pearson correlation analyses showed significant negative correlations between sum NO_3 with remaining litter mass loss ($r = -0.79$, $P < 0.01$) and litter fragmentation ($r = -0.35$, $P < 0.01$) and positive correlations with soil respiration ($r = 0.36$, $P < 0.01$) and total soil organic matter ($r = 0.41$, $P < 0.01$). Pearson correlation analyses showed a significant correlation between water content and SOM ($r = 0.87$, $P < 0.01$) and soil respiration and water content ($r = -0.67$, $P < 0.01$, Table 3 for all correlation analyses).

Table 3. Pearson correlation analyses for soil processes soil respiration (mg CO_2/g soil C), remaining litter mass, litter fragmentation (Bray-Curtis index), water content (% DW, sum of 0-5 and 5-10 cm), net total NO_3^- production for total soil, soil organic matter (SOM, in % DW, sum of 0-5 and 5-10 cm).

	NO_3 production	Soil respiration	Soil moisture content	Remaining litter	total SOM	Litter fragmentation
Soil respiration	0.364**	1.000				
Soil moisture content	0.205	-0.674**	1.000			
Remaining litter	-0.792**	-0.277*	-0.202	1.000		
total SOM	0.413**	-0.393**	0.874**	-0.423**	1.000	
Litter fragmentation	-0.351**	-0.203	-0.051	0.516**	-0.228*	1.000

* Correlation is significant at the 0.05 level (2-tailed); ** Correlation is significant at the 0.01 level (2-tailed)

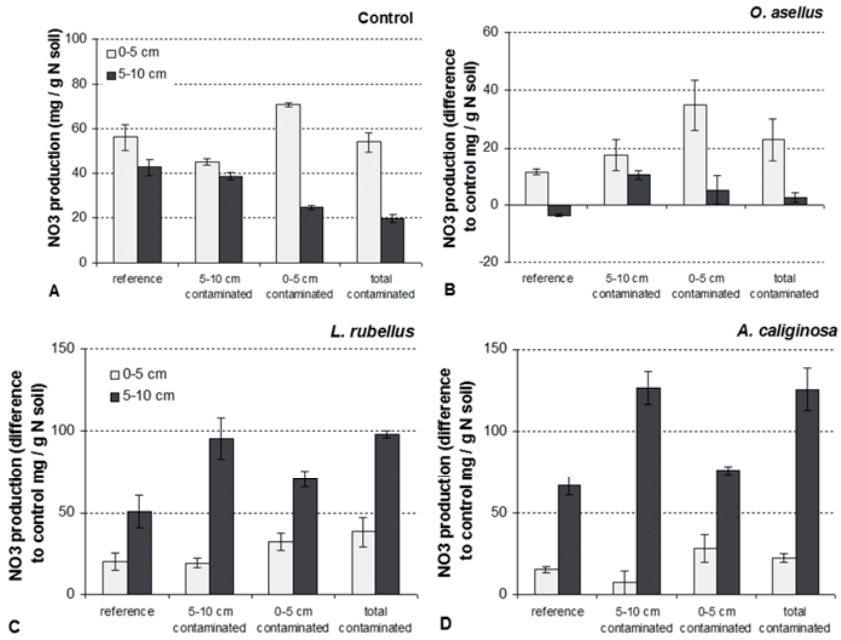

*Figure 6. Total NO_3^- (mg NO_3^- /g N in soil) in soils measured at two depths 0-5 and 5-10 cm of **A**, control; presence of **B**, Oniscus asellus; **C**, Lumbricus rubellus and; **D**, Aporrectodea caliginosa. Error labels represent standard errors (n = 5).*

Bioturbation

Burrows were observed along the transparent microcosm wall, indicating active bioturbation by earthworms. Burrow length of both earthworm species did not differ between treatments but burrow length of *A. caliginosa* was longer than *L. rubellus* (2-factor GLM: 0-5 cm contamination $P = 0.67$, fauna $P = 0.003$, soilxfauna $P = 0.90$; 5-10 cm, contamination $P = 0.85$, fauna $P < 0.001$; soilxfauna $P = 0.97$) (Figure 7). *Lumbricus rubellus* made permanent burrows, while *A. caliginosa* showed active re-burrowing through the soil which is reflected in the burrow length measured in treatments containing *A. caliginosa*. *Lumbricus rubellus* showed higher bioturbation in 0-5 cm than for 5-10 cm while *A. caliginosa* had similar burrow lengths for both layers (Figure 7).

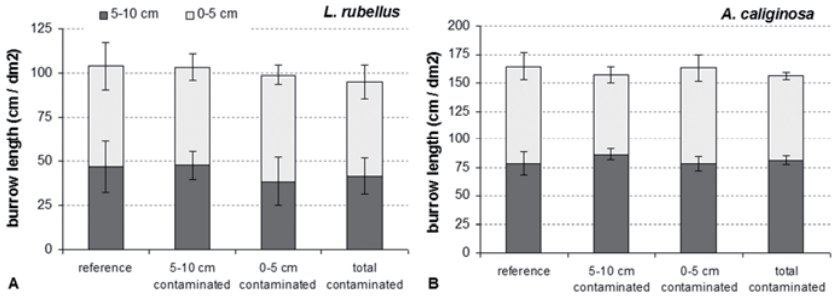

*Figure 7. Burrow lengths observed on microcosm wall (cm burrow / dm² microcosm) of **A**, Lumbricus rubellus and; **B**, Aporrectodea caliginosa. Error labels represent standard errors (n = 5).*

Discussion

The interactions between soil fauna and microorganisms as a function of vertically heterogeneous soil contamination were assessed using various soil processes. One of the main pathways by which these interactions can be disrupted is by avoidance behaviour of soil fauna in the vertical heterogeneous profile. There were no indications of avoidance of contaminated soil layers by earthworms in direct burrow length observations on the surface of the microcosm wall. Although it is known that burrow activity of earthworms can be more extensive at the column walls (Capowiez et al., 2001), we assumed that this border effect occurred to a similar extent in all soil treatments. Furthermore, litter mass loss and changes in soil organic matter content did not support the occurrence of avoidance. Hypothetically, the deeper contaminated layer could well have been avoided, especially by the litter dweller *O. asellus* and the epigeic earthworm *L. rubellus* as they both are predominantly litter feeders. The contaminants did not seem to affect *O. asellus*. For the earthworms, it did not seem that stratified contamination treatments affected the epigeic earthworm in a different way than the endogeic earthworm. It is therefore likely that the contaminated layer did not elicit avoidance. Although the contaminated soil showed a high total metal content, the $CaCl_2$ extractable metal content was relatively low (Table 1) and comparable to relatively uncontaminated locations in The Netherlands (van Gestel et al., 1992). In aged contaminated soils $CaCl_2$ extractable concentrations are in general a better indicator of the bioavailable fraction of contaminants to earthworms than total metal concentrations (Lock and Janssen, 2001a; Lock et al., 2006; Oorts et al., 2006)

In the absence of macrofauna, lower soil respiration rates were observed in the completely contaminated profile compared to the reference (Figure 2), which may indicate that contamination had a direct negative effect on the activity of microbes. The presence of macrofauna increased soil respiration rates, although no increases in microbial biomass and metabolically active hyphal length were observed. Positive effects of earthworms on microbial activity have been observed in contaminated soils (Cortet et al., 1999; Lukkari et al., 2005; Lahr et al., 2008). Whilst the microbial biomass may remain similar, earthworms increase the turnover or basal activity of the microbial community in the soil (Aira et al., 2008; Sen and Chandra, 2009).

Interestingly, in contaminated profiles the stimulation of microbial activity by earthworms was relatively higher than in reference soil layers, especially in the presence of *A. caliginosa*, though rates remained below reference soil level. This large increase in respiration rates in contaminated soil layers by earthworms suggests that microbial activity was restricted by some limiting factor, and that particularly in the contaminated soil earthworm mediation was enhanced, either by microbial grazing or by affecting physico-chemical characteristics of soil and litter through bioturbation, or incorporation of leaf litter in the soil profile (Petersen and Luxton, 1982; Brown, 1995, Edwards and Lofty, 1972).

If bioturbation was similar in all treatments, one can assume that grazing on microorganisms and the effects on the physico-chemical structure of the soil were similar in all treatments and therefore were an unlikely cause for the increased CO_2 production. There were no indications that the behaviour and activity of *O. asellus* was affected by the soil treatments, as fragmentation of leaf litter was similar in all different soil types (Figure 3). This was expected because even though a thin litter layer was created, the isopod is a soil surface dweller clinging to the litter (Paoletti and Hassall, 1999), thus escaping exposure. The two earthworm species incorporated similar amounts of leaf litter into the soil profile in all soil treatments. Therefore litter incorporation is the most likely factor for the relatively high microbial stimulation. The incorporated litter might have increased the water content of the soil as well. However, correlation analyses showed that the CO_2 production was negatively correlated to water content; thus, additional soil moisture was not likely to be the mechanism to enhance C mineralisation

Soils, sediments and biosolids may show high concentrations of metals through complexation of metals to soil organic matter and clay minerals (Alloway, 1995; McBride et al., 1997; Chaudri et al., 2008). The breakdown of SOM will mobilize heavy metals in time and thereby potentially increase bioavailability. Chaudri et al. (2008) showed that SOM associated metals in biosolids had a larger impact on rhizobia counts than their soluble salts due to association with SOM. The scale at which the SOM with high concentrations of contamination occur is crucial for a realistic assessment of microbial exposure due to their strong association with SOM (Chaudri et al. 2008). Larger soil fauna will be exposed through a larger surface, thereby integrating exposure over heterogeneous microsites. Because of this scale effect soil microorganisms may tend to be more sensitive to metals than soil fauna or plants, as established e.g. by Giller et al. (1998) and Broos et al. (2005).

If contaminated soil organic matter is suppressing microbial activity, the incorporation of fresh uncontaminated litter into the soil profile offers an alternative substrate and can stimulate the microbial community. Bioturbation of earthworms can thereby dilute contaminated SOM by mixing the SOM with fresh leaf litter of better quality. This effect will be larger in the contaminated soil than in the reference soil, thereby leading to a relative larger microbial stimulation in the contaminated soil than in the reference soil. Alternatively, the gut passage in the earthworms stimulating microbial activity (Brown, 1995; Drake and Horn, 2007) might be more important for less vital microbial communities like those in contaminated soils.

While contaminated soil and reference soil differed in microstructure and C:N-ratio, this is insufficient as an alternative explanation for the difference in CO_2 production in the control soil as this does not account for the disproportional increase of CO_2 production by the addition of earthworms. Therefore, we believe that N-content of the soils did not limit C-mineralisation, but that earthworms were the prime factor of mediation for microbial mineralisation of C and N.

NO_3^- production is the resultant of organic matter assimilation followed by N excretion by earthworms and the ammonification and nitrification by microorganisms. Especially earthworm casts are relatively rich in NH_4^+ (Edwards and Bohlen, 1996; Aira et al., 2003; Haynes et al., 2003). Therefore, data on NO_3^-production are less conclusive as it is affected by earthworm behaviour (cast deposition) and leaf litter degradation.

Furthermore, the NO_3^- production data of specific soil layers were affected by leaching of water through the soil profile; leachates may have flushed nitrate from the litter layer, into the soil and downwards. However, the results of NH_4^+ and NO_3^- production show that NH_4^+ was less than 5% of the mineral N, thereby indicating that nitrification was not affected by the contamination.

Conclusions

Vertically heterogeneous contamination did not affect behaviour of species which differed in their life history traits. Furthermore, interactions between macrofauna and microorganisms were not affected. The results of this research indicate that soil macrofauna can mitigate negative effects of soil contaminants on soil microbes. In soils rich in clay and organic matter showing a high total metal concentration, the negative effects of soil contaminants to microbial activity were compensated for by soil fauna stimulating microbial activity.

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