POLLUTION-INDUCED COMMUNITY TOLERANCE AND FUNCTIONAL REDUNDANCY IN A DECOMPOSER FOOD WEB IN METAL-STRESSED SOIL

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Abstract—Pollution may lead to the development of pollution-induced community tolerance (PICT) in a stressed community. We studied the presence of PICT in soil food webs using soil microcosms. Soil microcosms containing soil invertebrates and microbes were collected from polluted and unpolluted areas and exposed to a range of soil zinc concentrations. A pine seedling was planted in each microcosm to measure the effects of the origin of the community and Zn pollution on above-ground plant production. The effects of the treatments on nutrient content in the soil were also measured. The diversity of soil microarthropods and the soil’s mineral nutrient content were low at the Zn-polluted site. We did not observe an increasing Zn tolerance among the soil organisms in the polluted soil. However, low population growth rates of soil invertebrates from the polluted site may indicate the deleterious effects on fitness of long-lasting pollution. In the soil from the nonpolluted site, Zn additions caused changes in the invertebrate food web structure. These changes were explained by the good physiological condition of the animals and their insensitivity to Zn. The fact that the food web structure in soil from the polluted site did not change can be used as a rough indicator of PICT. Structural stability is preserved by the lack of Zn-sensitive species at this site and the inability of populations to acclimate by altering their growth or reproduction patterns in response to changing soil conditions. Although microbial-based soil decomposer systems may have a high functional redundancy, our results indicate that metal stress at the polluted site exceeds the tolerance limits of the system. As a consequence, ecosystem function at this site is endangered. This study also shows that the evolution of metal tolerance by soil decomposer organisms may not be a common reaction to soil pollution, although changes of population and community structure indicated severe metal stress on organisms.

Keywords—Ecosystem functioning Fitness reduction Soil organisms Species interaction Zn resistance

INTRODUCTION

Environmental pollution may lead to the development of pollution-induced community tolerance (PICT) in a stressed community [1,2]. An increase in tolerance to pollution at the community level may develop by two different processes. First, pollution-induced selection in populations may allow an increase in the number of tolerant individuals in the community (may include phenotypic acclimatization to the stress) [3–6]. Second, pollution may lead to the replacement of pollution-sensitive species by less sensitive species [1,6].

The PICT analysis applies to a group or community of organisms and integrates both direct toxic and indirect effects of a contaminant. The induced tolerance, at a community level, lowers sensitivity of the community to the pollutant [1]. Therefore, the presence of PICT demonstrates that the contaminants in a polluted area really affect organisms [7].

Earlier studies on PICT focused on organisms at one trophic level or in one functional group within a food web (e.g., marine periphyton, unidentified sediment-living nematodes, or soil bacteria [1,8–10]). Predators of the studied organisms were not present in these test systems, and the responses of organisms at higher trophic levels have not been studied [6]. An increased pollution tolerance of individuals in the food web may, however, be widespread among organisms in various trophic positions [5,11]. A pollution-induced stress itself [12] and the evolution of an increased tolerance to pollution may indirectly affect the interactions between species and hence alter the community structure [3,6]. On the other hand, pollution directly affects a small number of organisms. In particular, species that have functions not readily replaced by other species such as keystone species [13] may have large effects on a food web. Hence, pollution may significantly alter ecosystem functions, such as decomposition and nutrient cycling [14]. To understand the ecological significance of PICT on the performance of stressed communities and ecosystem functioning in polluted areas, experiments on whole communities (i.e., food webs with a realistic trophic structure) are required.

The PICT in terrestrial decomposer communities can be studied in microcosms containing a food web composed of soil fauna, microbes, and plants [15,16]. In soil, microbes are a major factor in decomposition and nutrient mineralization. Feeding activity of soil fauna, however, may contribute significantly to soil microbial activity and thus indirectly affect ecosystem processes such as the rate of nutrient mineralization and plant production [16–20]. On the other hand, recent studies on soil food webs have shown that microbial-based decomposition systems may have much functional redundancy [21–23]. In these studies, faunal species were replaced by other species of the same functional group and trophic levels, which resulted in only small changes in system function [21–23]. When applying PICT, functional redundancy in the soil implies that a small proportion of the community, belonging to critical functional groups, is able to maintain ecosystem processes. Hence, responses of these species or groups of species to soil pollution should be studied with care. Bacterial communities in metal-polluted soil (or after experimental metal stress) have increased metal tolerance [8,9,24,25], but there are no studies...
on metal tolerance in realistic soil food webs and on concomitant changes in ecosystem functioning.

We studied the presence of PICT in a soil food web in a coniferous forest in The Netherlands, which was polluted by a zinc factory for more than 100 years. We constructed food webs using soil organisms collected from polluted and unpolluted areas and exposed these communities to various concentrations of Zn. The communities contained microbes, microbial–detritivorous invertebrates, and predatory mites as the first, second, and third trophic levels. In each microcosm, a pine seedling was planted to measure the effects of the origin of the community and artificial Zn pollution on above-ground plant production. Our hypotheses were that the soil community in the polluted area is more tolerant to additional Zn stress than the community in the reference area and that, because of its increased Zn tolerance, the community can better maintain ecosystem functions (plant production) in a contaminated microcosm. On the other hand, because of the high functional redundancy of the soil food web, altered species composition may not affect plant production if nonredundant functional groups, such as enchytraeids [22], have not been affected.

MATERIALS AND METHODS

Field sites and samples

At the end of September 1998, soil samples were taken near Budel in The Netherlands from a polluted site situated 2 km northeast of the Zn factory (a typical down-wind direction in the area) and a reference site (10 km in the same direction). Soil samples were also taken from an unpolluted pine forest 12 km northwest of the factory (referred to as the third site). The dominant tree species at the polluted and reference sites was Scots pine (Pinus sylvestris, L.). In addition, some oak (Quercus robur, L.) and birch (Betula pendula, Roth) grow at the third site. Heather (Calluna vulgaris, (L.) Hull) grows on the polluted and reference sites, and wavy hare grass, Deschampsia flexuosa, (L.) Trin., grows at the polluted and third sites. Five samples were taken from the organic soil layer (4–10 cm thick) of all three sites using a split corer (inner diameter 10 cm). The samples were divided into subsamples for faunal, microbial, and soil chemical analyses.

Soil microcosms

Microcosms were open-top 7.5-cm-high opaque plastic containers of 0.01 m² area. The bottom was fine-meshed gauze. On the top of these containers, a transparent plastic wall (20 cm high) was made to prevent the escape of soil arthropods. Microcosms were filled with a soil layer of 4 cm (∼59 g of dry soil) and then covered with a pine needle litter layer of 0.5 cm (8 g dry needles).

To construct the microcosms, soil and litter (pine needles) were collected from the third site to produce a novel soil habitat for the communities of both polluted and reference soils. The soil from the third site was sieved through a 5-mm sieve and contaminated with ZnCl₂ (Merck, Darmstadt, Germany; >95% pure) of five nominal concentrations, which were 0, 9.6, 48, 240, and 1,200 mg Zn/kg of dry soil. ZnCl₂ dissolved in de-ionized water (50 ml) was added to a 30-g portion of soil and carefully mixed in with the rest of the soil using an electric kitchen mixer. In the negative controls, only deionized water was used.

After adding Zn, the soil and noncontaminated needles were sterilized by autoclaving two times at 120°C (4 h each time).

To collect soil organisms in the polluted and reference sites, topsoil with mosses was collected from an area of approximately 2 m². Microbes were extracted from the soils by shaking soil portions with demineralized water (soil-to-water ratio 1:10) for 30 min. The sterilized soils were reinoculated with microbes from either site by adding the resulting filtered (35-μm) soil–water suspensions. The soils and microbes were incubated for one week before they were placed in the microcosms. Half of the microcosms received soil containing microbes from the polluted site; the other half received microorganisms from the reference site. For each Zn concentration, five replicate microcosms were prepared, resulting in a total of 50 microcosms. The rest of the soils with microbes were put in small plastic bags (n = 5 per treatment) and stored at 5°C for microbial analyses.

Nematodes and one species of enchytraeids (Cognettia sphagnetorum Vejd.) were extracted from the polluted and reference soils using wet funnel techniques [26, 27]. Nematodes in a small amount of water were put into the microcosms (three times, producing an initial density of 413–456 individuals/microcosm). Enchytraeids were added individually into the microcosms using a small hook (23 individuals/microcosm). Microarthropods (mites and springtails) were extracted from the polluted and reference soils in a Tullgren apparatus (Vrije Universiteit, Amsterdam, The Netherlands) using collecting tubes with moist plaster of Paris and paper. Before extraction, large amounts of soil from both sites were separately and gently mixed to increase the homogeneity of species composition and their abundance in the inoculums. Extraction for an individual microcosm was made from a soil volume equal to that of the microcosm. Extractions were made three times to get an initial density corresponding to the observed field density. Animals were transferred from the collecting tubes into the microcosms using a brush; large ants, spiders, and coleopterans were removed.

To measure above- and below-ground plant production, a small pine seedling (P. sylvestris) was planted in each microcosm five weeks after beginning the experiment. For that purpose, pine seedlings were collected at the end of October 1998 from a reference forest near Hilversum, The Netherlands. Seedlings were acclimatized to climate room conditions (see below) in native soils for 11 d. Before seedlings were transferred into the microcosms, their roots were carefully washed in deionized water to reduce microbial and animal contamination. Seedlings were weighed before they were planted in the microcosms. To estimate the average dry weight, five seedlings were dried at 60°C for 24 h.

Microcosms were incubated in a climate room at an air temperature of 21 ± 3°C for five months (encompassing several life cycles for most faunal species), a relative air humidity of 70 ± 10%, a daily cycle of 14 h light, and a photosynthetically active radiation of 0.25 μE/m²/s. The temperature in the soil was kept constant at 20 ± 1°C by using a cooling system under the microcosms. Water that evaporated was replaced weekly by artificial rainwater containing a low concentration of salts [28].

Analyses

Nine weeks after the soils had been inoculated with microbes, at the end of the microcosm experiment, basal respiration (as CO₂ evolution, indicating the activity of microbes) and substrate-induced respiration after adding glucose (indicating microbial biomass) were measured in the soils without
animals and in the soils of the microcosms. To measure the respiration, 10-g quantities of fresh soil were put into a flow chamber. After 30 min continuous airflow through the sample (8.1 L/h), the air was analyzed for \( \text{CO}_2 \) with an infrared \( \text{CO}_2 \) analyzer (IRGA, ADC-225-MK3, Analytical Development, Hoddeston, UK). Respiration (\( \mu \text{g} \text{CO}_2/\text{h/g dry soil} \)) was calculated as the product of the flow rate and the mean concentration of \( \text{CO}_2 \); this was then normalized to the dry mass of soil. Basal soil respiration was measured at 22 \(^\circ\text{C}\).

Substrate-induced respiration was measured in the same samples used for basal respiration analysis [29]. Glucose (200 mg, corresponding with 2% fresh weight of soil) in 1 mL of deionized water was mixed vigorously with the sample. After 4 h of incubation at 22 \(^\circ\text{C}\), \( \text{CO}_2 \) production was measured. Microbial biomass \( \text{C} \) was calculated from the amount of respired \( \text{CO}_2\)-\( \text{C} \) using the equation given by Anderson and Domsch [29]. Metabolic quotient (respired \( \text{C} \) to biomass \( \text{C} \) for 24 h). Dry weights of each pine seedling was ascertained by visual inspection and the condition of each pine seedling was ascertained by visual inspection and by microscopic examination. We noted the color of the needles and the mycorrhizal infection of the roots. To make these observations, seedlings were removed from the soil, roots were washed, and the above- and below-ground parts of the seedlings were separated and dried at 60°C for 24 h. Dry weights of roots and above-ground parts were determined and the root-to-shoot ratio was calculated. Soil Zn concentration was analyzed for samples taken at the end of the experiment. These measurements were made by flame atomic absorption spectrometry using a Perkin-Elmer 1100B AAS (Norwalk, CT, USA). To determine total Zn levels, 1-g soil samples were digested in a concentrated \( \text{HNO}_3/\text{HCl} \) mixture heated by microwave. To determine extractable Zn levels, the soil samples were shaken with 0.01 M CaCl\(_2\) (1:5) at 200 rpm for 2 h. The extracts were filtered through a paper filter (pore size 0.45 \( \mu \text{m} \)). The analyses were performed in duplicate. Furthermore, Zn, Cd, Cu, Pb, and Ca concentrations in the soil samples collected from the field sites were also determined (\( n = 5 \)).

Soil samples were shaken with 1 M KCl at 200 rpm (5:1) for 2 h to determine their \( \text{NO}_3^-\)-\( \text{N} \) and \( \text{NH}_4^+\)-\( \text{N} \) content. After filtering over a paper filter, nutrient concentrations were measured using an autoanalyzer (Skalar SA 400, Breda, The Netherlands).

Statistical analyses

The differences between the variables measured on the three field sites were analyzed by analysis of variance (ANOVA) and pairwise comparisons were made by Tukey’s test. In the microcosm experiment, Zn concentration was treated as an ordered factor and expressed as orthogonal polynomials in the statistical analysis. This was justified as the Zn levels increased log linearly (apart from the zero level) due to the chosen spacing factor of five [31]. Significant responses are marked only for the highest order probability level in the results, effectively ignoring the nonsignificant higher order effects. To meet the assumptions of ANOVA, square root (faunal and pine data) and log (microbial and chemical data) transformations were used. Statistical analyses were performed using the linear model functions in \( R \) [31].

RESULTS

Field sites

The soil of the polluted site contained more Zn than that of the two other sites (\( F_{2,12} = 35.9, p < 0.001 \)) for total Zn and \( F_{2,12} = 9.97, p = 0.003 \) for CaCl\(_2\)-extractable Zn and more Cd than the reference soil (\( F_{2,12} = 5.41, p = 0.021 \)) (Table 1). At the polluted site, soil contained less \( \text{NH}_4^+\)-\( \text{N} \) compared with both unpolluted sites (\( F_{2,12} = 14.9, p = 0.001 \)) and less \( \text{NO}_3^-\)-\( \text{N} \) than the soil of the reference site (\( F_{2,12} = 5.27, p = 0.023 \)) (Table 1). No significant differences in the densities of soil animals and microbial biomass and activity were found (Table 2). The diversity of microarthropods was

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Site</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Zn (mg/kg of dry soil)</td>
<td>Polluted</td>
</tr>
<tr>
<td>Total Cd</td>
<td>6.1 ± 1.3A</td>
</tr>
<tr>
<td>Total Pb</td>
<td>241 ± 66</td>
</tr>
<tr>
<td>Total Cu</td>
<td>35 ± 9</td>
</tr>
<tr>
<td>Total Ca</td>
<td>2,637 ± 826</td>
</tr>
<tr>
<td>CaCl(_2) extractable Zn (mg/kg of dry soil)</td>
<td>14.4 ± 2.5A</td>
</tr>
<tr>
<td>Soil respiration (( \mu \text{g} \text{CO}_2/\text{h/g dry soil} ))</td>
<td>6.7 ± 2.1</td>
</tr>
<tr>
<td>( \text{NO}_3^-)-N (( \mu \text{g/g dry soil} ))</td>
<td>30 ± 4.7A</td>
</tr>
<tr>
<td>( \text{NH}_4^+)-N (( \mu \text{g/g dry soil} ))</td>
<td>54 ± 6.4A</td>
</tr>
<tr>
<td>Loss on ignition (%)</td>
<td>82 ± 7</td>
</tr>
<tr>
<td>pH (in 1 M KCl)</td>
<td>2.93 ± 0.08</td>
</tr>
</tbody>
</table>

* Third refers to a nonpolluted site, where the soil for the microcosm experiment was collected.

\(^{\dagger}\) Third versus polluted in Tukey’s test, \( p = 0.052 \).
lower at the polluted site than at the nonpolluted reference and third sites ($F_{3,12} = 6.99, p = 0.010$) (Table 2).

**Physicochemical and chemical properties of the test soils**

Two weeks after the microbes were added, the soil organic matter content of the test soil was $79 \pm 0.1\%$ (mean $\pm$ standard error, loss on ignition at 600°C), water content was $68 \pm 0.2\%$ (weight loss at 60°C, 24 h), and pH in 1 M KCl was $3.34 \pm 0.01$ ($n = 3$).

At the end of the experiment, the total Zn concentrations measured were 439, 430, 450, 601, and 1,463 mg/kg dry soil, and corresponding 0.01 M CaCl$_2$ extractable Zn concentrations amounted to 179, 168, 211, 292, and 855 mg/kg dry soil. The overall coefficient of variation for the measurements of total Zn concentrations was 5.4%; for the extractable levels, it was 2.8% ($n = 2$).

**Soil fauna in microcosms**

Microbivorous-detrivorous microarthropods with low initial densities and also the oribatid mite *Phthiracarus* spp., a numerous group in the field soil, failed to establish populations in the microcosms, and taxa numbers in both communities decreased during the experiment. Microarthropods that survived in the microcosms, however, had higher population densities than in the field (Fig. 1 and Table 2).

Large predatory mites (*Pergamasus* spp. and *Lysigamasus* spp.) from the polluted site had a higher population density in the microcosms than those of the reference site and vice versa for *Zercon vagabundus* and *Eviphis ostrianus* (Fig. 1). Soil Zn contamination did not affect the population density of these predatory mites. The population densities of *Veigaia nemorensis* varied among Zn concentrations, and the origin of the population seemed to affect this variation (Fig. 1). The population density of the predatory mite *Rhodacarus calcavalatus* in the reference site increased along with increasing Zn concentration, but populations from the polluted site showed the opposite trend (Fig. 1). Relative growth rates for predatory mite populations indicated the same overall patterns as observed in the absolute densities (Fig. 2). In some predators, negative growth rate values were calculated, reflecting a reduction of population size during the microcosm experiment. In conclusion, at low Zn concentrations, the densities of predators were roughly similar in both communities, but different species were dominant. At the highest Zn concentration (1,200 mg/kg), the density of predatory mite species of the reference site increased, but the density of predators of the polluted site remained stable or even decreased with increasing Zn concentrations.

In the community of the polluted site, the densities of microbivorous-detrivorous microarthropods were much lower than in the community of the reference site. The population density of the oribatid mite *Tectocephus velatus* in the polluted site decreased below the initial density but tended to increase with increasing Zn concentrations in the reference site (Fig. 1). The Orbatida family Archeonothridae (most probably one species) had a high population density in the reference community, although the observed field density was low (Fig. 1 and Table 2). The density of Archeonothridae species was reduced at Zn concentrations of 240 and 1,200 mg/kg of dry soil (Fig. 1). The population density of Archeonothridae of the polluted site was lower than that of the reference site. The population growth rates of *T. velatus* and Archeonothridae species were lower in mites of the polluted site than of the reference site (Fig. 2). Zinc contamination decreased the growth rate of Archeonothridae of both sites but had no significant effect on the growth rate of *T. velatus* populations (Fig. 2). The oribatid mite group Oppioidea of the reference site had a density peak at 240 mg Zn/kg of dry soil, whereas in mites of the polluted site, the density decreased with increasing Zn concentrations (Fig. 1). Prostigmatid mites of the reference site increased their densities along with an increasing soil Zn concentration, unlike the mites of the polluted site (Fig. 1).

The density of Collembola (*Isotoma* spp., *Hypogastrura* spp., *Onychiurus* spp., and *Folsomia* spp.) of the reference site increased with increasing soil Zn concentrations, but individuals of the polluted site showed the opposite trend (Fig. 1).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Polluted</th>
<th>Reference</th>
<th>Third$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pergamasus</strong> spp. and <em>Lysigamasus</em> spp.</td>
<td>0.4 ± 0.07</td>
<td>0.6 ± 0.08</td>
<td>0.4 ± 0.04</td>
</tr>
<tr>
<td><em>Rhodacarus calcavalatus</em></td>
<td>0.8 ± 0.08</td>
<td>0.9 ± 0.4</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td><em>Zercon vagabundus</em></td>
<td>0.06 ± 0.06</td>
<td>0.4 ± 0.03</td>
<td>0.08 ± 0.05</td>
</tr>
<tr>
<td><em>Veigaia nemorensis</em></td>
<td>0.06 ± 0.03</td>
<td>0.02 ± 0.02</td>
<td>0 ± 0.01</td>
</tr>
<tr>
<td><em>Eviphis ostrianus</em></td>
<td>0.02 ± 0.02</td>
<td>0.11 ± 0.09</td>
<td>0.08 ± 0.03</td>
</tr>
<tr>
<td><em>Tectocephus velatus</em></td>
<td>0.9 ± 0.3</td>
<td>1.3 ± 0.1</td>
<td>1.1 ± 0.4</td>
</tr>
<tr>
<td>Oppioidea</td>
<td>0.3 ± 0.07</td>
<td>0.4 ± 0.1</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>Phthiracaridae</td>
<td>12 ± 6</td>
<td>4 ± 2</td>
<td>7 ± 2</td>
</tr>
<tr>
<td>Archeonothridae</td>
<td>0 ± 0.01</td>
<td>0.1 ± 0.03</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>Prostigmatid mites</td>
<td>1.4 ± 0.1</td>
<td>1.9 ± 0.4</td>
<td>0.8 ± 0.1</td>
</tr>
<tr>
<td>Collembola</td>
<td>1.3 ± 0.4</td>
<td>2.4 ± 0.3</td>
<td>1.2 ± 0.4</td>
</tr>
<tr>
<td>Total microarthropods</td>
<td>22 ± 7</td>
<td>20 ± 2</td>
<td>25 ± 4</td>
</tr>
<tr>
<td>Number of microarthropod taxa</td>
<td>14.4 ± 0.9A</td>
<td>19.4 ± 1.2B</td>
<td>18.8 ± 1.2B</td>
</tr>
<tr>
<td><em>Cognetta sphagnetorum</em></td>
<td>1.0 ± 0.5</td>
<td>1.2 ± 0.2</td>
<td>1.6 ± 0.5</td>
</tr>
<tr>
<td>Nematoda</td>
<td>43 ± 4</td>
<td>59 ± 12</td>
<td>74 ± 14</td>
</tr>
<tr>
<td>Microbial biomass</td>
<td>0.8 ± 0.1</td>
<td>0.7 ± 0.1</td>
<td>0.7 ± 0.1</td>
</tr>
</tbody>
</table>

* Third refers to a nonpolluted site, where the soil for the microcosm experiment was collected.
In conclusion, density, diversity, and population growth rates of microarthropods tended to be lower in the communities of the polluted site than those of the reference site. This indicated that microarthropods of the polluted site did not have an increased Zn tolerance (Fig. 1). On the other hand, species composition of microbivores-detritivores of the reference site was changed because of increasing soil Zn concentrations so that dominant oribatid mites were replaced by prostigmatid mites and springtails. In the community of the polluted site, no changes in the species composition were observed among Zn concentrations.

The density of the enchytraeid *C. sphagnetorum* was similar to field densities observed at low soil Zn concentrations, but the worms were extirpated at a concentration of 1,200 mg Zn/kg of dry soil (Fig. 1). No significant differences were observed in population growth rates and population densities of enchytraeids in the soils from the polluted site or the reference site (Figs. 1 and 2). The total number of nematodes in the microcosms was high compared with the field soils and did not differ statistically in relation to site or Zn treatments (Fig. 1).

**Microbes in microcosms**

After nine weeks of incubation in the soil without fauna, the microbes of the polluted site had a lower biomass but a higher metabolic activity than the microbes of the reference site (Fig. 3). Soil spiking (Zn contamination) did not initially affect microbial biomass. At the highest Zn concentration
(1,200 mg/kg dry soil), basal respiration and metabolic quotient were reduced in the case of microbes of the polluted site, indicating that microbes did not have a higher Zn tolerance than microbes of the reference site.

At the end of the experiment, the microbial biomass was increased in both communities and metabolic quotients were decreased with increasing Zn concentrations (Fig. 3). There were no site-dependent differences in microbial response to Zn contamination, i.e., evidence of an increased Zn tolerance of microbes was not found.

**Mineral nitrogen and pine growth in microcosms**

Two weeks after the microbes were inoculated into the autoclaved soil, NH$_4$-N content of the soil was higher when inoculated with microbes from the polluted site than with microbes from the reference site (Fig. 4). Soil NO$_3$-N content was high at the 1,200 mg Zn/kg of dry soil level with microbes from the polluted site (Fig. 4). At the end of the experiment, soil NH$_4$-N content differed between communities and among Zn concentrations (the quadratic effect of site x Zn $p = 0.035$, but the main effects of treatments were not statistically significant). We did not observe significant differences between treatments in soil NO$_3$-N content at the end of the experiment (Fig. 4). Water content and loss on ignition of the soil in the microcosms were at the same level as at the beginning of the experiment, but the soil pH was reduced to 3.03 ± 0.03 (mean ± standard error, $n = 50$).

There were no statistically significant effects of treatments on the total pine biomass (Fig. 3). The root-to-shoot ratio was high in the community of the polluted site and was not affected by Zn, whereas in the community of the reference site, this ratio was reduced at high Zn concentrations (Fig. 4).

**DISCUSSION**

**Soil organisms and evolution of metal tolerance**

Contrary to our first hypothesis, we found no evidence for increased Zn tolerance among soil-living microbes and fauna of the polluted site. However, earlier studies have shown that conditions in the polluted site were stressful enough to induce the evolution of increased metal tolerance in the springtail Orchesella cincta [32], the isopod Porcellio scaber [33], and community tolerance of microorganisms [34].

The densities and population growth rates of soil invertebrates and microbes showed negative and positive responses at high Zn concentrations, indicating that the Zn levels used were high enough to cause numerous direct and indirect effects. Hence, adaptive Zn tolerance in organisms of the polluted site should be seen as higher densities or biomass in contaminated microcosms compared with nonadapted organ-
long-lasting metal stress has really operated as a selection force. This would lead to reduced capability to respond to environmental changes [35]. Indications of reduced genetic variation (according to an allozyme analysis) in the asexual mite *Tectocephus velatus* have been observed at polluted sites [5]. However, our results indicate that the reduced variance of the response of *T. velatus* was not caused by an increasing frequency of Zn-tolerant genotypes in this clonal species (compare with [5]). Hence, we concluded that there was no increased Zn tolerance of soil organisms living in the metal-polluted soil but that some animals may have a lowered level of fitness because of the long-lasting metal stress at the site. Our results emphasize that the evolution of metal tolerance by soil decomposer organisms may not be a common reaction to soil pollution even under severe metal stress.

Pollution-induced community tolerance and species replacement in stressed decomposer food webs

A reduced number of species, altered population growth rates, and changes in N mineralization rates in the polluted field site indicate that Zn affected the soil decomposer food web function [see also 37,38]. The PICT in a stressed decomposer food web can be seen as a simple, sparse but more stable species composition when artificially stressed by Zn (in the sense of Luoma [3]). Long-term metal stress at the polluted site has led to reduced growth rates of populations, reduced species numbers, and a lower abundance of species. Hence, the community did not contain populations capable of altering their population dynamics upon changing conditions in their habitat (e.g., reduced metal toxicity or increased microbial biomass; see below). On the other hand, the term PICT may be wrong here because organisms in the community from the polluted site were markedly depressed by the metal stress and could not respond to the changed environmental conditions (no evidence of a real adaptive tolerance was found) [39].

In the community of the reference site, the density of predatory and microbivorous-detritivorous arthropods increased and the biomass of microbes increased at the highest Zn concentrations. These results indicate that, because of the better physiological condition and insensitivity to Zn, the predators and microbial-detritivores in the reference site benefited from the increasing microbial biomass and had higher population densities in the most polluted microcosms. The increased microbial biomass may be a consequence of structural changes in the microbial community due to Zn exposure. For instance, microbes were not only less sensitive to Zn but were also metabolically inactive (low carbon use efficiency), as indicated by the reduced metabolic quotient [40,41]. Altered dominance among microbivorous-detritivorous arthropods could be an indirect consequence of Zn pollution. For example, it is possible that the remaining microbes were unpalatable to oribatid mites but palatable to prostigmatid mites and springtails. However, another indirect mechanism is also plausible. In particular, Zn might directly reduce the number of oribatids (negative effects of Zn on the population growth of Archeonothridae gen. sp.) and *Cognettia sphagnetorum* (extinction), after which less competition within a trophic level could have increased the growth of less Zn-sensitive microbial-detritivores, leading to an altered dominance pattern. Finally, an increased biomass in microbivores-detritivores seemed to cascade up to the predators. The results are consistent with studies indicating that soil decomposer food webs are strongly controlled from the

![Graph](https://example.com/graph.png)
bottom up [21,42,43] and that contaminants may have important indirect effects on stressed communities [6,12].

**Plant growth and functional redundancy in the stressed decomposer food web**

No differences in Zn tolerance were found. However, we did note indications that the soil Zn concentration had different effects on root-to-shoot ratio of pine seedlings in the communities. The larger and Zn-indifferent root-to-shoot ratios in microcosms from the community of the polluted site may be caused by a low amount of nutrients (NH₄⁺-N) in the soil. Plants invest more energy in below-ground parts when they grow in soils with limited nutrient supply [44]. Because there were no significant differences in microbial biomass and activity between the two communities, the observed alteration in nitrogen dynamics can be attributed to the activity of the soil fauna. It is possible that, in the microcosms with high Zn concentrations, the less-abundant soil invertebrates in the soil from the polluted site were unable to mineralize nutrients as effectively as the dense fauna in the soil from the reference site [see 21]. These results refute our second hypothesis, i.e., that the polluted community can better maintain ecosystem function in an artificially polluted soil.

The overall faunal density was stable in both communities (except for enchytraeids), but we noted changes in dominance relationships among species in the reference community. A Zn-mediated change in species composition may partly explain the observed resource allocation of pines and contradicts earlier results indicating high functional redundancy in the soil food web [16,21,22]. Hence, springtails and prostigmatic mites seemed to behave as nonredundant functional groups affecting the resource allocation of pines [22]. Earlier results show that the microbivorous-detritivorous enchytraeid *C. sphagnetorum* is a crucial species for nutrient cycling and plant production in coniferous forest soil [22]. However, contrary to our third hypothesis, the extinction of worms at the highest Zn concentrations did not clearly affect the function of the microcosm communities. Finally, the results may indicate that, at the polluted field site, changes in the food web structure have exceeded the limits of the functional redundancy of the decomposer system, and the reduced nutrient mineralization rates indicate that the ecosystem functioning has been impaired.

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