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Response of forest soil bacterial communities to mercury chloride application

Beat Frey [a,*], Stephan R. Rieder [a,b]

[a] Rhizosphere Processes Group, Swiss Federal Research Institute WSL, CH-8903 Birmensdorf, Switzerland
[b] Institute for Biogeochemistry and Pollutant Dynamics, ETH Zürich, CH-8092 Zürich, Switzerland

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ABSTRACT

This study evaluates the response of the bacterial communities to different mercury (Hg) amendments in temperate forest soils. Seven soils were spiked with increasing amounts of Hg [(0, 0.032, 0.32, 3.2 and 32 μg Hg(II) g^{-1} dry soil]. After 30 days, we examined the bioavailable Hg using bacterial biosenors (*merlux*), basal respiration, bacterial community structures and identified indicator OTUs which were responsive to Hg. In soils treated with at least 3.2 μg Hg g^{-1} dry soil, resulting in bioavailable Hg higher than 0.004 μg Hg g^{-1} dry soil, the basal respiration was strongly affected. High bioavailable Hg also caused significant changes in the bacterial T-RFLP profiles. Members of the *Alphaproteobacteria* (*Rhodospirillales*) and *Betaproteobacteria* (*Burkholderiales*) were found to be Hg-tolerant. Here, we propose a critical limit concentration for soluble Hg of 0.004 μg Hg g^{-1} soil.

1. Introduction

Present concentrations of mercury (Hg) over large areas in Europe are increased to levels that may exert some environmental impact (critical limits) (De Vries et al., 2005). Hg is emitted from anthropogenic and natural sources to the atmosphere, mainly in the gaseous form, and can be transported to remote and pristine locations (Pacyna et al., 2006; Harmens et al., 2008). In soils, Hg is highly immobile, bound to the soil organic matter in particular to thiol groups (Obrist et al., 2009; Skyllberg et al., 2010) and has the ability to interact with soil mineral components as well as to form precipitates with sulphide, carbonate, hydroxide, and other anions (Schuster, 1991; Skyllberg et al., 2006). Forest soils rich in organic matter can therefore be regarded as an efficient filter between the supply from the atmospheric deposition and soil layers at greater depths (Grigal, 2003; Meili et al., 2003; Akerblom et al., 2008). In boreal forest soils, the concentrations of Hg in the litter layer vary from 0.07 μg to 1.0 μg Hg g^{-1} dry soil (Meili et al., 2003; Akerblom et al., 2008; Klaminder et al., 2008) and may exceed 0.5 μg Hg g^{-1} dry soil in temperate forests in Germany (Schwesig et al., 2000) and in Switzerland (Ernst et al., 2008; Rieder et al., 2011).

Evidence of detrimental effects of Hg to soil microorganisms in general and in particular in forest soils is scarce. Although Hg is known to act as powerful toxicant, ecotoxicological data of Hg in temperate forest soils are very limited. Most of the studies have been performed in boreal forest soils (Bringmark et al., 2001a, 2001b; Akerblom et al., 2010), in agricultural soils (Landa et al., 1978; Ranjard et al., 1997, 2000; Müller et al., 2001; Rasmussen et al., 2001; Casucci et al., 2003) or in tropical soils (Harris-Hellal et al., 2009). Soil bacterial communities are responsible for many fundamental ecological processes, such as the biogeochemical cycling of chemical elements or the decomposition of plant and animal residues (Balser et al., 2005). Evaluating the ecotoxicity of Hg in soils can give valuable information about the sensitivity of the indigenous soil microorganisms. However, there is only limited data on critical limit concentrations below which there is no effect on soil bacterial communities and published threshold values were only given as total Hg concentrations in soils. For example, critical limit for total Hg contents to prevent ecological effects for Hg in organic soils have been set to 0.4 μg Hg g^{-1} dry soil (or 0.5 μg Hg g^{-1} organic matter) by an international expert group on effect-based critical limits for heavy metals working within the framework of UNECE Convention on Long-range Transboundary Air Pollution (Curlic et al., 2000; Meili et al., 2003; De Vries et al., 2005). At lower concentrations, it is assumed that there are no harmful effects on soil organisms. Data obtained for the estimation of these critical

* Corresponding author. Tel.: +41 44 7392 541.
E-mail address: beat.frey@wsl.ch (B. Frey).

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limits were mainly derived from podzolic soils with a high organic matter content (≥85%). Soil type is a very important factor to evaluate the response of soil microorganisms to heavy metal pollution (Lazzaro et al., 2006a, 2006b). Similarly, Tipping et al. (2010) reported a critical limit for total content of 3.3 μg Hg g^{-1} organic matter or 0.13 μg Hg g^{-1} dry soil. By applying this definition, 60% of 34 natural forest soils studied in Switzerland would exceed this critical limit (Rieder et al., 2011). In addition, regarding the evaluation of critical limits of Hg in temperate forest soils data of the soluble fraction instead the total content would be more ecotoxicological relevant (Lazzaro et al., 2006a, 2006b). Therefore, there is an urgent need to study the impact of Hg to soil microbial communities in forest soils with contrasting physico-chemical characteristics (e.g. low in organic matter and a broad pH range from acidic to calcareous soils) and to evaluate critical limit concentrations for soluble Hg in the soil water.

In the present study we determined the effects of increasing Hg concentrations on the soil bacterial communities of natural deciduous, spruce and mixed forests in Switzerland. Seven different forest soils with contrasting physico-chemical characteristics and within a pH range from 4.4 to 7.3 were spiked with a Hg(II) chloride solution in microcosms experiments. Short-term Hg exposure and employed microcosms minimize abiotic variances and enable good standardization (Lazzaro et al., 2008). We tested microbial activity variables (basal respiration) in soils and examined the bacterial community structure by using T-RFLP profiling of the 16S rRNA gene in relation to the increasing Hg concentrations in soils. Our overall aim was to relate effects on the bacterial communities to the bioavailable Hg concentrations and to determine threshold concentrations for soil types with low organic matter content below no negative effects on the bacterial communities emerge.

2. Material and methods

2.1. Soil types, location, soil characteristics

Seven forest soils with different physical and chemical properties containing no or only a small litter layer were chosen from a soil profile database (Ernst et al., 2008; Rieder et al., 2011) of the Swiss Research Institute WSL, Birmensdorf, Switzerland (Tables S1 and S2). Physico-chemical characteristics of the soils tested have been described elsewhere (Lazzaro et al., 2006a, 2006b; Ernst et al., 2008; Rieder et al., 2011). After removal of the litter layer (less than 1 cm), soil material was collected at a soil depth of 0–10 cm (A-horizon) with a shovel. At each site, ten samples of the mineral soil were collected randomly from an area of approximately 5 × 5 m, then pooled in a plastic bag. The collected soil was stored at 4 °C in the dark for a week before use.

2.2. Microcosm setup and mercury additions

Prior to setting up microcosms the soils were sieved (mesh size 2 mm) and air-dried at room temperature (about 15 °C) for three days. The soils were then uniformly rewetted to a moisture content of 58% (dry wt equiv.) with mercuric-chloride ($HgCl_2$, Sigma–Aldrich, Buchs, Switzerland) dissolved in sterilized Millipore water to obtain concentrations of 0.032, 0.32, 3.2 and 32 μg Hg g^{-1} dry soil. These concentrations were chosen by compiling data from literature dealing with Hg contaminations and metal effects on soil microbial communities (Ranjard et al., 2000; Müller et al., 2001). After addition of the Hg solution the soils were placed in plastic bags and mixed thoroughly. Control treatments received only water or $CaCl_2$ to test the ionic strength on the soil microbial communities. The soils were kept at room temperature for 24 h prior to filling in the microcosm to obtain a uniform distribution of the metal and equilibrium between Hg and soil binding sites. Then the soils (100 g) were transferred into 250 mL plastic jars (Stericup filter systems, Millipore, Billerica, MA, USA), and covered with the supplied air permeable caps throughout the experiment to minimize evaporation, but permitting aeration. The microcosms were incubated in the dark at 20 °C and 60% of external humidity in climatic chambers similar as described by (Lazzaro et al., 2006a, 2006b). Water loss was minimal (determined daily by weight), and, if necessary, was compensated by the addition of sterile water. All experiments were done in triplicate from both Hg-spiked and control soils (water and $CaCl_2$). The microcosms were harvested after 30 days of incubation. Part of the soil was immediately used for basal respiration and C biomass measurements, while aliquots were frozen at −80 °C for DNA extraction. The remaining soil was stored at 4 °C for determination of pH, DOC, and bioavailable Hg and/or oven-dried for 48 h at 105 °C to determine remaining water content.

2.3. Soil chemical characteristics

Water extractions were performed by shaking 20 g of fresh soil mixed with 200 mL of Millipore water for 16 h at 20 °C on an overhead shaker (Lazzaro et al., 2006b). The soil slurry was then centrifuged for 10 min at 1200 × *g* in a Sigma 6-15 centrifuge (Sigma–Aldrich, St. Louis, MO, USA), and subsequently vacuum-filtered through a 0.45 μm membrane filter (Millipore Stericup systems). Each extract was divided into two portions for pH and DOC analyses. DOC concentrations in the water extracts were measured with a Shimadzu TOC-500 apparatus (Shimadzu, Kyoto, Japan), while pH was measured with an Orion 520A pH meter (Thermo 5 Electron, Waltham, MA, USA).

The bioavailable Hg in soil samples was measured in duplicate by a *mer-lux* assay, applying a whole-cell bacterial biosensor as described previously (Lappalainen et al., 2000). The *mer-lux* biosensor has been shown to be a useful and sensitive tool for the estimation of bioavailable Hg in soil (Rasmussen et al., 2000; Ivask et al., 2002; Petänen et al., 2002). Briefly, 1 g of soil in 10 mL of sterile water was shaken at 20 °C in polyethylene tubes for 24 h on an overhead shaker. Soil particles were removed by centrifugation for 10 min at 12 000 × *g*. Appropriate soil leachate dilutions (100 μL) were mixed with 100 μL of a cell suspension of the biosensor strain *Escherichia coli* MC1061 (pTOO11) obtained from Aboatox Oy (Turku, Finland), which bioluminescences in the presence of Hg (Lappalainen et al., 2000). Light emission in soil samples, Hg standards, and blank assays were recorded as relative light units using a Luminescan (Labsystems, Waltham, MA, USA) luminometer. A standard curve was established using the regression equation for the relationship between the amount of bioavailable Hg and the expression factors obtained from a standard assay of known concentrations of Hg (Lappalainen et al., 2000). The detection limit was equal to 0.1 μg Hg L^{-1}. We calculated the bioavailable Hg concentration of the soil extract (g L^{-1}) back to the amount of bioavailable Hg per g soil (in μg Hg g^{-1} soil).

2.4. Basal respiration

Basal respiration (CO_2 evolution) was measured using the method described by (Frey et al., 2008) by incubating moist soil samples (approximately 20 g dry soil) for 3 days in gas tight vessels. The percentage of basal respiration by the Hg treatments, relative to the controls, was taken as an index of activity of the soil microbial communities.

2.5. Extraction of DNA

DNA extraction from each of the replicate microcosms (0.5 g of fresh soils) was performed using a bead-beating method described