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Long- and short-term effects of mercury pollution on the soil microbiome

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ABSTRACT

Despite the toxicity of mercury (Hg) for organisms in the environment, little is known on its impact on the soil microbiome, especially its chronic effect. Here, we assessed the effects of a long-term contamination of Hg in soils on the bacterial and fungal communities along a gradient of contamination from no to high contamination. Short-term reactions (30 days) of the microbial communities in these soils having different levels of historic Hg contamination were further evaluated in microcosm experiments where soils were either spiked with dissolved $HgCl_2$ or not. Results show a clear impact of a long-term Hg contamination on both bacterial and fungal community structures and diversity but only a weak effect was observed on their activities (basal respiration and growth rates). No short-term effects of Hg were observed on the microbial community structures and activities. Taxa from the *Chthoniobacteraceae* (bacteria) and *Trichosporon* sp. (fungi) were associated with high Hg contaminated soils, implying they possess capabilities to tolerate Hg in soils. Abundance of mercury reductase (*merA)* gene copies increased with higher Hg concentrations in soils both during short and long-term exposure to Hg pointing to potential mechanisms within microbial cells to tolerate higher amounts of Hg in soils.

1. Introduction

Mercury (Hg) is a naturally occurring heavy metal, mainly released on the surface of the earth by volcanic and other geothermal activities (Pirrone et al., 2010). However, anthropogenic activities (such as industrial waste management activities, fossil-fuel power plant use, production and manufacturing of metals and chemicals), released an average of 30% of the total Hg emission leading to increased Hg levels on Earth (Selin, 2009). The toxicity of Hg in soil is highly dependent on its chemical speciation. Methyl-Hg, the most poisonous chemical form of Hg, has a high affinity for the sulfhydryl ligands in amino acids, which induces alterations in protein structures and leads to a loss of function (Nies, 2003).

Bacteria possess various mechanisms to cope with high Hg concentrations in soils (Barkay et al., 2003). The *mer* operon system present in certain bacterial genomes, coding for detoxification proteins, is a known bacterial defense system against Hg (Dash and Das, 2012b). The central gene for Hg resistance in the *mer* operon system is *merA*. This gene codes for the mercuric reductase enzyme, a flavoprotein located in the cytoplasm using NADPH as electron donor, which catalyzes the conversion of Hg^{2+} to volatile Hg^0 (Barkay et al., 2003). These mercury resistance genes are often carried on plasmids or other genetic elements, which are widely spread in various ecosystem types (Barkay et al., 2003). Moreover, the proportion of bacteria with Hg-resistance abilities was directly proportional to the level of mercury contamination in the environment (Dash and Das, 2012a).

Hg was reported to strongly affect soil bacterial and fungal community structures and diversities (Frey and Rieder, 2013; Frossard et al., 2017). Moreover, basal respiration, used as a proxy for soil microbial activity, was shown to be highly affected by higher concentrations of methyl-Hg (Rieder and Frey, 2013). These studies showed that tolerance of bacterial communities mainly depends on the solubility of Hg in soil and that this is directly influenced by pH and organic matter (Frossard et al., 2017). However, these studies were conducted in short-term laboratory experiments, whereas knowledge gaps remain about the effects of long-term Hg contamination under field situations. Investigations on long-term effects of heavy metal contamination on soil microorganisms revealed that metal-tolerance may select for tolerant microbial taxa within a few weeks or months, but a complete community adaptation to metal pollution can take up to years to develop (McLaughlin and Smolders, 2001; Azarbad et al., 2015). Therefore, long-term field experiments are especially important and needed to improve our understanding of the effect of chronic exposure to mercury on the soil microbiome (Giller et al., 2009).

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Soil sampling along a Hg-pollution gradient in the field is a promising way to study the impact of a long-term Hg contamination on the soil microbiome (Liu et al., 2014). Bacterial diversity (observed richness) was observed to be the highest in moderate Hg pollution (6 μg Hg g^{-1} dry soil). Moreover, by using pyrosequencing of bacterial 16S rRNA gene amplicons, they found that OTUs from the *Gemmatimonadetes* phylum were increased under long-term Hg exposure while *Nitrospirae* sp. OTUs declined. Fungi are generally more tolerant than bacteria towards heavy metals (Rajapaksha et al., 2004) and were observed to be less affected by Hg than bacterial communities in forest soils (Rieder and Frey, 2013; Frossard et al., 2017). Moreover, while most of the studies on the effect of Hg in soil are conducted as short-term laboratory experiments, long-term studies in the field are still scarce.

The main aim of this study was first to assess the impact of a long-term Hg pollution on the soil microbiome (i.e. bacterial and fungal communities) under field conditions. We hypothesized that long-term contamination with high Hg concentrations in soils will impact both bacterial and fungal community structures and diversities as well as their activities. The microbial communities in high Hg contaminated soils are assumed to have developed mechanisms to tolerate higher amounts of Hg. Secondly, we aimed to assess the legacy effect of a long-term Hg contamination towards an additional Hg short-term exposure. We hypothesized that the legacy effect of Hg on the soil microbiome will be particularly pronounced in these soils which received high amounts of Hg in the past, thereby increasing their capabilities to tolerate higher concentrations of Hg in soils.

2. Material and methods

2.1. Long-term Hg contaminated soils: field sampling

Soils were collected in October 2015 in a field near the town of Raron (CH; 46°18′10.6″N, 7°48′34.2″E) where high Hg pollution had been reported (Gazut and Preiswerk, 2014). The pollution originated from the Grossgrundkanal (Supp. Figure 1), which received a large amount of mercury (estimation of 50–250 tons) from ca. 1930 till 1976, released from industrial wastes located upstream along this canal. The sediment from the Grossgrundkanal were then used to flatten and improve the fluvial soils for pasture and were laid on these fluvial soils at several occasions between ca. 1930 to 1990. This resulted in a gradient of Hg contamination along the Grossgrundkanal (Supp. Figure 1) with the highest Hg soil concentration close to the canal and decreasing with distance from the canal. A total of 12 soil samples were sampled along this Hg pollution gradient at 3 different levels of Hg (with 4 replicates for each Hg concentration level; Supp. Figure 1): 1) *high* Hg contamination, 2) *moderate* Hg pollution and 3) *low* Hg contamination (corresponding to natural background values in Switzerland, Rieder et al., 2011). Soils were collected from a soil depth of 0–10 cm (A-horizon) with a soil corer (diameter 7 cm). The fresh soil samples were mixed and sieved (2 mm) before being stored at −20 °C (for DNA extraction) or at 4 °C in the dark for one week before use.

2.2. Short-term Hg contaminated soils: microcosm set-up and mercury additions

Soil samples collected in the field along the Hg pollution gradient were incubated in microcosms spiked with (*+Hg*) or without *(-Hg)* Hg in order to test the legacy effect of Hg on the soil microbiome. Subsamples (10 g dry weight) of the 12 soil samples were air-dried at room temperature (around 18 °C) for three days, transferred into small plastic bags (minigrip®, 100 × 100 mm) and were then uniformly re-wetted to a moisture content of 30% (dry weight equivalent) either with mercuric-chloride ($HgCl_2$ 10 μg Hg g^{-1} dry soil) for the treated samples (Hg spiked), or only with sterile water for the samples used as incubation controls. Mercury was supplied to the microcosms as aqueous $HgCl_2$ solutions, as done in previous studies (Frey and Rieder, 2013; Frossard et al., 2017). The bags were stored 24 h at room temperature in order to let the Hg bind to the soil matrix before being transferred into soil containers (100 ml erlenmeyer flasks) and incubated in the dark at 20 °C with 60% external humidity in climatic chambers. Water loss, determined daily by weighing, was minimal. If necessary, sterile water was added to compensate for any loss. The microcosms were harvested after 30 days of incubation.

2.3. Soil physico-chemical parameters

Soil samples from the field were dried overnight at 105 °C to measure their gravimetric water content. Soil texture was determined by the hydrometer technique according to Gee and Bauder (1986). The pH was measured in each sample with a soil-extractant ratio of 1:2 using a glass electrode linked to a pH meter (FEP20-FiveEasy Plus, Mettler-Toledo GmbH, Switzerland). Around 2 g of well-homogenized soil was milled with a Teflon ball mill, and around 40 mg of soil was subsequently weighed into tin caps for measurement of the total carbon (TC) and total nitrogen (TN) concentrations with a CHN analyzer (Shimadzu, Tokyo, Japan). Organic carbon (Corg) was separated from inorganic carbon and was quantified according to Walthert et al. (2010). Water extractable Hg was extracted with milli-Q water for only 16 h in a slurry with a ratio of 1:10 g soil ml^{-1} (Lazzaro et al., 2006; Rieder and Frey, 2013). Total Hg concentrations in soils were analyzed using a direct mercury analyzer (AMA 254 Mercury Analyzer, LECO Corporation, St. Joseph, MI, USA; detection limit 0.001 μg Hg g^{-1} dw) and water-extractable Hg concentrations were determined using an Inductively Coupled Plasma Mass Spectrometer (ICP-MS, 7700x, Agilent Technologies, Japan).

2.4. Basal respiration

Basal respiration (BR) was measured in a closed soil-chamber system connected to a Li-8100 infrared gas analyzer (LI-COR Inc., Lincoln, NE, USA). The soil containers were connected to the CO_2 analyzer. CO_2-free air flowed at a rate of about 0.16 L min^{-1} through the containers, and entrained the CO_2 just released from the soil to the infrared gas analyzer. After reaching a steady state situation, the gas flow and the CO_2 concentration were recorded and the BR was calculated according to Rieder and Frey (2013). Fluxes are reported as μg CO_2 per day per g Corg.

2.5. Bacterial and fungal growth

Bacterial growth was estimated using the leucine (Leu) incorporation method adapted for soil (Bååth, 1994; Bååth et al., 2001). Briefly, soil samples were mixed with distilled water before being centrifuged to create a bacterial supernatant which was transferred into micro-centrifugation tubes. Labelled [^{3}H]Leu (37MBq ml^{-1} and 5.74 TBq $mmol^{-1}$; Perkin Elmer, USA) and unlabelled Leu (final concentration of Leu 275 nM) were then added and the tubes were incubated for 2 h at 22 °C. Growth was terminated by adding trichloroacetic acid, and subsequent washing steps and measurement of radioactivity were performed as in Bååth et al. (2001). The amount of Leu incorporated into extracted bacteria per hour and per g Corg was used as an estimate of bacterial growth.

Fungal growth was assessed using the acetate in ergosterol incorporation method adapted for soil with modifications (Rousk et al., 2009). Briefly, soil samples were mixed with distilled water, [1–^{14}C]-acetic acid sodium salt (7.4 Mbq ml^{-1} and 2.04 GBq $mmol^{-1}$; Perkin Elmer, USA) and unlabelled sodium acetate (final concentration 220 μM) and then incubated for 4.5 h at 22 °C in the dark. Formalin was added to terminate growth. Ergosterol was then extracted and quantified using HPLC and a UV detector, as described by Rousk et al. (2009). The amount of acetate incorporated into fungal ergosterol per hour and per g Corg was used as a measure of fungal growth.