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Tolerance of the forest soil microbiome to increasing mercury concentrations

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ABSTRACT

Mercury (Hg) occurs naturally in soil and can reach high concentrations in the environment due to anthropogenic activities; however, little is known about its impact on the soil microbiome. Here, we tested the impact of different concentrations of Hg on soil bacterial and fungal communities by carrying out microcosm experiments with seven different forest soils. The highest concentration of Hg (32 μg Hg g^{-1} dry soil) caused severe diversity loss and shifts in the bacterial and fungal community structures and composition. Bioavailable Hg was reduced in soils with the highest proportion of clay, but the impact of Hg on microbial community structures was still evident in these soils. Lower concentrations of Hg (≤3.2 μg Hg g^{-1} dry soil) had only a limited effect on the soil microbiome. Fungal communities were generally less affected than the bacterial communities. The bacterial Hg-detoxification capacity, as assessed by mercuric reductase gene abundance, was reduced in soils with the lowest amount bioavailable Hg. We found a wide range of Hg-responsive taxa in soils spiked with high amounts of Hg, although they were generally not specific to any soil type or taxonomic group. Overall, our data show that the impacts of Hg on the soil microbiome and its detoxification responses depend on soil characteristics.

1. Introduction

Mercury (Hg) occurs naturally in soil and is mainly released to the surface by volcanic and other geothermal activities (Pirrone et al., 2010). However, Hg levels have increased in the recent past due to anthropogenic activities (such as use of fossil-fuels in power plants, production and manufacturing of metals and chemicals, and industrial waste management activities) (Selin, 2009). Globally, it has been estimated that 7527 Mg of Hg is emitted to the atmosphere every year, about 2320 Mg of which is derived from anthropogenic sources (Pirrone et al., 2010).

Mercury is present in two different forms in the atmosphere: the neutral Hg^0 form, which can be oxidized to the ionic form, Hg^{2+}. Atmospheric transport of Hg^0, in the vapour phase represents an important route of global dissemination. By contrast, Hg^{2+} has a much shorter lifetime in the atmosphere and is rapidly deposited on the topsoil: this is the main form of Hg deposition on the earth's surface (Selin, 2009). While most of the deposited Hg has been shown to quickly re-volatilize to the atmosphere (Selin, 2009), the remaining ionic Hg is incorporated into the soil pool and the majority (>90%) becomes associated with organic matter and binds strongly to molecules containing reduced sulphur groups (Skyllberg et al., 2003).

The toxicity of Hg in soil is highly dependent on its chemical species. Methyl-Hg compounds have been recognized as the most toxic species (Clarkson and Magos, 2006). This is mainly due to the high affinity of methyl-Hg for the sulfhydryl ligands in amino acids, which induces alterations in protein structures, often leading to loss of function (Nies, 2003). Methylation of Hg in soil mainly occurs through biotic processes involving different groups of organisms. Sulphate-reducing bacteria (SRB) are now recognized as one of the main contributors to the methylation process in soils (Holloway et al., 2009), whereas iron-reducing bacteria have been observed to participate in the methylation process in freshwater systems (Yu et al., 2012). Moreover, earthworms, which can constitute up to 90% of the total soil fauna and play an important role in soil-forming process (Brown, 1995), have been shown to actively participate in the methylation of inorganic Hg, independently of the potential participation of the earthworm gut-associated microbial communities (Rieder et al., 2013).

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Environmental variables, such as temperature, pH and underlying geology, are known to affect uptake and turnover of Hg by organisms (Boening, 2000). However, studies on the effects of Hg on soil microbial communities are rare. In a recent study involving T-RFLP profiling of 16S rRNA genes in temperate forest soils, Frey and Rieder (2013) showed that Hg can strongly affect soil microbial activities and bacterial community structures. The effect of Hg on soil fungal community structure and composition is, however, currently unknown. Basal soil respiration, used as a proxy for soil microbial activity, was shown to be strongly impacted by high concentrations of methyl-Hg (Rieder and Frey, 2013). Fungi are generally more tolerant than bacteria towards heavy metals (Rajapaksha et al., 2004). The results of T-RFLP analysis also suggested that bacterial communities were more sensitive to the methyl-Hg concentration in soil than fungal communities (Rieder and Frey, 2013). However, the phylogenetic resolution and the inability to identify taxa of T-RFLP method is not sufficient to identify Hg-sensitive taxa.

In a recent study assessing the bacterial diversity across a Hg-polluted paddy soil gradient, Liu et al. (2014) identified the highest bacterial diversity at a moderate Hg concentration (6 μg Hg g^{-1} dry soil), suggesting a peak in biological diversity under intermediate disturbance. By using pyrosequencing of bacterial 16S rRNA gene amplicons, these researchers found that *Gemmatimonadetes* were stimulated under long-term Hg exposure while *Nitrospirae* declined. Nevertheless, evidence of detrimental effects of Hg to bacterial and fungal communities in forest soils with contrasting physico-chemical characteristics is scarce. In particular, little information is available regarding the evaluation of critical limits of Hg in temperate forest soils (Tipping et al., 2010). Knowledge is especially scarce concerning the soluble, but ecotoxicologically more relevant, fraction of Hg (Lazzaro et al., 2006b; Lazzaro et al., 2006a).

A broad range of bacteria have the capacity to resist the effects of Hg contamination. The most common Hg-resistance mechanism in bacterial cells involves the reduction of the highly reactive ionic form of mercury (Hg^{2+}) to the volatile and relatively inert monoatomic mercury vapour (Hg^0; Barkay et al., 2003). Bacterial resistance to Hg was shown to be associated with the presence of the *mer* operon system in the bacterial genome (reviewed in Robinson and Tuovinen, 1984; Barkay et al., 2003; Dash and Das, 2012b). Mercury resistance genes are often carried on plasmids or other genetic elements, which are widespread in various types of ecosystems (Barkay et al., 2003). Moreover, the proportion of bacteria showing resistance to mercury was found to be directly proportional to the level of mercury contamination in the environment (Dash and Das, 2012a).

The central gene for Hg resistance in the *mer* operon system is *merA*. This gene codes for the mercuric reductase enzyme, a cytoplasm-located flavoprotein that uses NADPH as electron donor, thus catalyzing the conversion of Hg^{2+} to volatile Hg^0 (Barkay et al., 2003). Very few studies have investigated the abundance of mercuric reductase genes in soil. In a Chinese agricultural soil, the *merA* gene diversity was influenced by long-term fertilization practices (Liu et al., 2012). The NH_4^+ and NO_3^- concentrations and organic matter content had the strongest effects on the *merA* gene diversity pattern.

The first aim of this study was to test the effects of different Hg^{2+} concentrations on soil bacterial and fungal community diversity and composition across different soils. We hypothesized that both bacterial and fungal diversity and community composition would be affected by increasing concentrations of Hg^{2+}. However, fungal communities are expected to be more tolerant to Hg than bacterial communities. A second aim of this study was to quantify the *merA* gene in order to assess the resistance capacity of the bacterial communities in relation to Hg concentration. We hypothesized that above a critical concentration of Hg^{2+} in soil, the *merA* copy number would increase due to shifts in community structure towards Hg-tolerant taxa. Finally, we also hypothesized that resistant or tolerant bacterial and fungal OTUs would be found in several type of soils, although soil conditions may affect their distribution.

2. Material and methods

2.1. Soil collection

Seven forest soils with different physical and chemical properties containing no or only a thin litter layer were chosen from the soil profile database of the Swiss Research Institute for Forest, Snow and Landscape Research (WSL), Birmensdorf, Switzerland (Ernst et al., 2008; Rieder et al., 2011). The soils chosen in this study represent a wide range with respect to parent material and composition of the soil (organic carbon: 2–8%; clay: 9–55%; pH 4.4–7.3; Table 1) and represent the most commonly found forest soil types in Switzerland (Lazzaro et al., 2006b; Lazzaro et al., 2006a; Ernst et al., 2008; Rieder et al., 2011). After removing the litter layer (less than 1 cm), soil material was collected from a soil depth of 0–10 cm (A-horizon) with a soil corer (diameter 7 cm) in spring 2010. At each site, three samples of the mineral soil were collected at random locations from an area of approximately 15 × 15 m and were treated separately. The fresh soil samples were passed through a 2-mm sieve and mixed thoroughly. The soil samples were stored at 4 °C in the dark for one week before use.

2.2. Microcosm setup and addition of Hg

Prior to setting up the microcosms, the soils were air-dried at room temperature (around 18 °C) for three days before being uniformly rewetted to a moisture content of 58% (dry weight equivalent with mercuric-chloride ($HgCl_2$, Sigma-Aldrich, Buchs, Switzerland) dissolved in sterile water to produce concentrations of 0.32, 3.2 and 32 μg Hg g^{-1} dry soil. A similar level of Hg was used in other studies (Philippot et al., 2008; Harris-Hellal et al., 2009; Frey and Rieder, 2013). Mercury was supplied to the microcosms as aqueous $HgCl_2$ solutions, as in previous studies (Ranjard et al., 2000; Bringmark and Bringmark, 2001a; Bringmark and Bringmark 2001b; Müller et al., 2001). The amounts of Hg added to the microcosm soils spanned a wide range of concentrations, from environmentally relevant concentrations to levels of extreme pollution. The lowest rates of addition of Hg used in this study (0.32 μg g^{-1} dry soil) were within the range of concentrations detected in soils without any surrounding Hg source (Schwesig and Matzner, 2000; Grigal, 2003; Rieder et al., 2011). Higher concentrations of Hg (3.2 μg g^{-1} dry soil) were within the range of concentrations found in contaminated soils (Wiersma et al., 1986). The highest concentration used in this study (32 μg Hg g^{-1} dry soil) corresponded to the amount applied in previous microcosm experiments with agricultural soils (Ranjard et al., 2000; Rasmussen

Table 1
Statistical differences of bioavailable Hg and the abundance of *merA* genes among soils and Hg treatments, analyzed for all soils together.

	DF[a]	Bioavailable Hg		*merA*	
		F	*P*	*F*	*P*
Soil	6, 83	0	1	341.9	<0.001
Hg treat.	3, 83	9.7	<0.001	101.4	<0.001
Soil × Hg treat.	18, 83	16.9	<0.001	14.8	<0.001

[a] DF = degrees of freedom (numerator, total).