Soil Biology & Biochemistry 64 (2013) 164–173

Contents lists available at SciVerse ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Methyl-mercury affects microbial activity and biomass, bacterial community structure but rarely the fungal community structure

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

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

ARTICLE INFO

Article history:
Received 19 November 2012
Received in revised form
16 April 2013
Accepted 17 April 2013
Available online 18 May 2013

Keywords:
Mercury
Hg
Methyl-Hg
Terminal restriction fragment length polymorphism (T-RFLP)
Basal respiration
Microbial biomass
16S rRNA gene
ITS gene

ABSTRACT

Monomethyl-mercury is one of the most toxic compounds. Methylation of Hg usually appears under anoxic conditions. In Swiss forest soils, methyl-Hg concentrations of up to 3 μg kg^{-1} soil dw have been observed, but the impact of methyl-Hg on soil microorganisms have rarely been examined so far. In this study, we investigated the effect of increasing concentrations of methyl-Hg (0, 5, 20, 90 μg kg^{-1} soil dw) on the microbial communities in various forest soils differing in their physico-chemical properties. Experiments were conducted in microcosms under controlled conditions and the basal respiration (BR), the microbial biomass carbon (MBC) and the bacterial and fungal community structures using T-RFLP-profiling were investigated. BR was significantly affected by methyl-Hg. In general, the BR increased with increasing methyl-Hg concentrations, whereas the MBC was significantly reduced. Bacterial communities were more sensitive to methyl-Hg than fungal communities. In five out of seven soils, the bacterial community structures differed significantly between the treatments whereas the fungal communities did not. The impact of methyl-Hg on the soil bacterial communities was site specific. In one soil, a methyl-Hg concentration of already 5 μg kg^{-1} soil dw significantly affected the relative abundance of 13% bacterial operational taxonomic units (OTU), whereas in other soils concentrations of even 90 μg kg^{-1} soil dw rarely affected the abundance of OTUs. In this study, for the first time, the impact of methyl-Hg on soil bacterial and fungal communities in forest soils was assessed. We showed that its impact strongly depends on the physico-chemical conditions of the soil and that bacterial communities were more sensitive to methyl-Hg than fungi.

1. Introduction

Mercury (Hg), and in particular monomethyl-mercury (CH_3Hg^+; methyl-Hg), are very toxic for most organisms. Mercury is a naturally occurring metal, which is emitted from natural and anthropogenic sources to the atmosphere (Schroeder and Munthe, 1998; Swain et al., 2007). Once emitted into the atmosphere, about half of the Hg is deposited locally [mainly as Hg(II)] (Mason et al., 1994) whereas the remaining Hg (mainly as Hg^0) enters to the global circulation with a residence time of approximately one year (Fitzgerald and Mason, 1997; Seigneur et al., 2004). Hg^0 in the atmosphere can be oxidized to Hg(II) with a much shorter lifetime in the atmosphere (days to weeks) than of Hg^0 (Slemr et al., 1981; Lindqvist and Rodhe, 1985), and oxidized Hg can be subjected to fall out and accumulate in the topsoil or photoreduced to Hg0 again (Selin, 2009). Thus Hg can be widely distributed over the globe before returning to the earth's surface, where it mostly accumulates in the top soil layer. The concentrations of Hg in the top layer of forest soils in Switzerland have been found range between 0.07 and 0.55 mg kg^{-1} soil dw (Ernst et al., 2008), with methyl-Hg percentages between 0.2 and 2.4% of total Hg (Boudou and Ribeyre, 1997; Rieder et al., 2011). In soils, methyl-Hg strongly binds to the thiol groups of organic matter (Qian et al., 2002). Mercury is methylated mainly through biotic processes in the soils, involving different groups of organisms, whereby sulphate-reducing bacteria (SRB) living under anoxic conditions are assumed to be mainly responsible for the methylation of Hg (Drott et al., 2007; Holloway et al., 2009). In addition, methyl-Hg may be transported into the soils through rain and litterfall (St Louis et al., 2001).

The toxicity of Hg strongly depends on its chemical species, with methyl-Hg compounds as the most poisonous species (Shao et al., 2012), mainly due to its affinity of Hg for thiol groups of proteins and enzymatic co-factors, Hg ions (Hg^{2+}) may affect proteins in three different ways: (1) by binding to proteins (e.g. to thiol groups)

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

0038-0717/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.soilbio.2013.04.017

and changing their functions, (2) by replacing essential ionic elements on proteins or (3) by inhibiting the refolding of denaturised proteins (Sharma et al., 2001). Several studies have investigated the effect of Hg(II) pollution on soil microbial processes and community structures. Van Faassen (1973) observed that very high concentrations of Hg compounds ($HgCl_2$, phenyl-Hg-acetate) in soils inhibited the basal respiration (BR), nitrification and the dehydrogenase activity. Previous research has shown that microbial respiration is reduced in soils treated with inorganic Hg (Tu, 1988; Bringmark and Bringmark, 2001; Palmborg et al., 2001), but still little is known about how toxic methyl-Hg is for soil microorganisms. We are aware of only three prior studies that described the impact of methyl-Hg on soil microorganisms. Bacterial cell numbers decreased on plating media containing methyl-Hg (Van Faassen, 1973) and fungal cell growth was reduced in soils with methyl-Hg twice as much as with $HgCl_2$ (Kungolos et al., 1999). Soils contaminated with methyl-Hg largely affected the bacterial community structures in earthworms (Rieder et al., 2013). The mercuric reductase enzyme *MerB* is known to be responsible for organic Hg tolerance in bacteria but no organic Hg detoxification mechanism is currently known for organic Hg in fungi. The *MerB* enzyme catalyses the degradation of organic Hg compounds to the less toxic form Hg(II) (Barkay et al., 2003). Both bacteria and fungi are important players in the decomposition of organic matter and thus in nutrient cycling and soil fertility (Berg and Mc Claugherty, 2007).

Here, we report on a study of the impact of increasing concentrations of methyl-Hg on soil microbial activities and community structures. Seven different forest soils were spiked with four rates of methyl-HgCl (0, 5, 20, 90 μg methyl-Hg kg^{-1} soil dw) in microcosms. The concentrations were chosen according to methyl-Hg concentrations in the top layers of Swiss forest soils (Rieder et al., 2011). We examined the impact of methyl-Hg by analysing the BR, the microbial biomass carbon (MBC) and bacterial and fungal community structures using T-RFLP profiling. As far as we know, this is the first study of the effects of increasing concentrations of methy-Hg on bacterial and fungal communities in forest soils differing in soil physico-chemical characteristics.

2. Material and methods

2.1. Study sites

Seven forest soils with a broad range of physical and chemical properties containing, with no or a thin litter layer (1 cm) were chosen from a soil profile database (Ernst et al., 2008) of the Swiss Federal Research Institute WSL, Birmensdorf, Switzerland (Table 1). After removal of the litter layer, soil was collected at a soil depth of 0–10 cm (A-horizon) with a soil corer (diameter 7 cm). At each site, ten samples of the mineral soil were collected at random locations from an area of approximately 5 × 5 m, then pooled in a plastic bag and returned to the laboratory. The collected soil was sieved (2 mm mesh) and stored at 4 °C for a week before use.

2.2. Experimental design

Four mg CH_3HgCl was dissolved in 100 ml sterile Milli-Q-water. The soils from each sampling site were filled in sterile plastic bags and small portions of a methyl-Hg solution (1 ml) were mixed with the soil to reach soil concentrations of 0, 5, 20 and 90 μg methyl-Hg kg^{-1} soil dw. During the treatment, the plastic bags were gently shaken several times to mix and homogenize the soils. The water content of each soil was adjusted to 35% by adding sterile Milli-Q water and the soils were gently shaken again. We chose these concentrations (0, 5, 20 and 90 μg methyl-Hg kg^{-1} soil dw) because forest top soils in Switzerland contain between <1 and 3 μg^{-1} methyl-Hg kg^{-1} soil dw, whereas soil biota may contain much higher methyl-Hg concentrations (Rieder et al., 2011). Soil microorganisms involved in the decomposing of dead fungi and earthworms are assumed to be exposed to such high concentrations of methyl-Hg. For this study, we therefore chose mean concentrations of methyl-Hg in mushrooms (20 μg kg^{-1} dw) and in earthworms (90 μg kg^{-1} dw) as the highest methyl-Hg rates. We also conducted control experiments with soils treated with $CaCl_2$ to examine the effect of Cl on the microbial communities. However, we did not observe any differences between the two control treatments ($CaCl_2$ versus water; data not shown).

A mixture of lyophilized powder of needle and leaf litter was mixed into the soils (1.5% of weight) as the food source for the microorganisms. 30 g soils of each methyl-Hg treatment were filled in air-permeable boxes (diameter 55 mm) and incubated at 15 °C in the dark. Each treatment was prepared in three replicates. During the incubation, the water content was determined gravimetrically every second day and, if necessary, any loss was compensated by adding sterilized Milli-Q water to retain the initial humidity. Soil aliquots of the microcosms were sampled at the beginning and at the end of the incubation (28 days). In a preliminary study, we found that approximately 30% of the methyl-Hg added to the soils was decomposed during a 28 day incubation period but that the total amount of Hg did not change (data not shown).

2.3. Basal respiration

The BR was measured in a closed soil-chamber system at 5 time points (after 1, 2, 8, 15, 28 days) during the incubation. The soil-boxes were connected to the CO_2-free air source, the red-y smart gas flow meter GSM-B5SA-BM00 (Vögtlin Instruments AG, Aesch, Switzerland) and the Li-8100 infrared gas analyzer (LI-COR Inc., Lincoln, NE, USA). CO_2-free air flowed at a rate of about 0.16 L min^{-1} through the boxes, and entrained the CO_2 just released from the soil to the infrared gas analyzer. After reaching a steady state situation

Table 1
Physico-chemical properties of the seven investigated forest soils (Lazzaro et al., 2006a,b; Rieder et al., 2011).

Location[a]	pH (H_2O)	BS [%]	CEC[b] [cmolc kg^{-1}]	C [%]	N [%]	DOC [mg C l^{-1}]	Sand [%]	Silt [%]	Clay [%]	Hg[c] [ppm]
Burgdorf	4.5	51	66	2.1	0.1	71	66	26	11	0.08
Gerlafingen	4.6	41	100	4.1	0.3	59	36	40	24	0.19
Laufen	6.8	100	383	5.0	0.3	24	6	39	55	0.10
Lausanne	4.6	29	77	3.5	0.2	23	62	25	13	0.08
Piotta	5.0	91	130	8.2	0.5	38	66	25	9	0.11
Schänis	5.7	96	118	3.2	0.3	17	43	35	22	0.32
Sihlwald	7.4	100	297	4.4	0.3	17	14	33	53	0.12

[a] Burgdorf (7°35′E, 47°02′N); Gerlafingen (7°33′E, 47°10′N); Laufen (7°25′E, 47°23′N); Lausanne (6°39′E, 46°34′N); Piotta (8°40′E, 46°30′N); Schänis (9°04′E, 47°09′N); Sihlwald (8°34′E, 47°14′N).
[b] CEC = cation exchange capacity.
[c] HNO_3 extractable concentrations.