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# Understanding mercury methylation in the changing environment: Recent advances in assessing microbial methylators and mercury bioavailability

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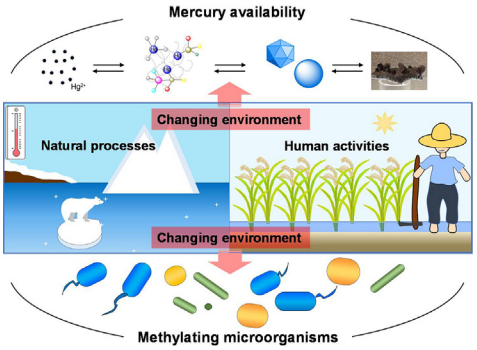
## HIGHLIGHTS

- Changing natural condition and human activity strongly affect mercury methylation.
- Microorganisms possessing hgcAB genes are present in diverse habitats.
- Mercury isotopes, whole-cell biosensor and DGT can assess mercury bioavailability.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Methylmercury (MeHg) is a neurotoxin, mainly derived from microbial mercury methylation in natural aquatic environments, and poses threats to human health. Polar regions and paddy soils are potential hotspots of mercury methylation and represent environmental settings that are susceptible to natural and anthropogenic perturbations. The effects of changing environmental conditions on the methylating microorganisms and mercury speciation due to global climate change and farming practices aimed for sustainable agriculture were discussed for polar regions and paddy soils, respectively. To better understand and predict microbial mercury methylation in the changing environment, we synthesized current understanding of how to effectively identify active mercury methylators and assess the bioavailability of different mercury species for methylation. The application of biomarkers based on the *hgcAB* genes have demonstrated the occurrence of potential mercury methylators, such as sulfate-reducing bacteria, iron-reducing bacteria, methanogen and syntrophs, in a diverse variety of microbial habitats. Advanced techniques, such as enriched stable isotope tracers, whole-cell biosensor and diffusive gradient thin film (DGT) have shown great promises in quantitatively assessing mercury availability to microbial methylators. Improved understanding of the complex structure of microbial communities consisting mercury methylators and non-methylators, chemical speciation of inorganic mercury under geochemically

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relevant conditions, and the pathway of cellular mercury uptake will undoubtedly facilitate accurate assessment and prediction of in situ microbial mercury methylation.



## 1. Mercury methylation in the changing environment

Mercury is a global pollutant that has raised great concerns worldwide (Hsu-Kim et al., 2018; Selin, 2018). Inorganic mercury in the environment can be converted to highly neurotoxic methylmercury (MeHg), which is bioaccumulated and biomagnified in the food chain and thus endangers human health (Driscoll et al., 2013; Mcnutt, 2013). Even at low level, MeHg may pose chronic toxicity to certain population due to long-term exposure (Zhao et al., 2019). For example, the neurocognitive functions of adults, such as fine-motor skills, verbal function, memory and concentration, appeared to be compromised after low level MeHg exposure (Yokoo et al., 2003). The presence and accumulation of MeHg in the environment is mainly a result of mercury methylation driven by anaerobic microorganisms (Parks et al., 2013), and this process is largely determined by the microbial activity and the mercury bioavailability in the aquatic environmental settings (Hsu-Kim et al., 2013).

A growing body of evidence has suggested that mercury methylation is subject to changing environmental conditions that strongly affect the occurrence and activity of the methylating microorganisms, as well as the bioavailability and methylation potential of inorganic mercury species (Ma et al., 2019; Paranjape and Hall, 2017; Regnell and Watras, 2019). The influence of global and local scale perturbations on mercury cycling and pollution management has been well documented in recently published reviews by Obrist et al. (Obrist et al., 2018) and Hsu-Kim et al. (Hsu-Kim et al., 2018). Here, we elaborate on two specific examples of environmental settings, where mercury methylation poses severe health risks and MeHg production are particularly susceptible to natural and anthropogenic perturbations. Knowledge regarding mercury methylation in these environments and how it responses to the changing conditions is critical to prevent and mitigate the risks of MeHg. Hence, we also synthesize the current understanding of the environmental occurrence of mercury methylating microorganisms (based on the newly discovered *hgc*AB genes) (Parks et al., 2013) and the techniques for assessing mercury bioavailability for methylation, so as to facilitate future research on mercury methylation in the changing environment.

### 1.1. Polar regions

The diet of the residents in polar regions heavily relies on marine organisms that are high on the aquatic food chain (St Pierre et al., 2015), and thus, mercury methylation in polar environment leads to potentially high exposure of MeHg to this population. In fact, MeHg and mercury methylation has been found to occur in polar sediment, water column, snow pack and sea ice (Table 1). The MeHg fraction of total mercury was relatively large in polar samples (e.g., up to 85% in snowpack (St Louis et al., 2005), up to 40.9% in sea ice (Beattie et al., 2014)). In particular, in situ mercury methylation appeared to be the main source of MeHg in high Arctic wetland systems, accounting for 80–91% of total MeHg inputs (Hammerschmidt et al., 2006). In recent research, Gionfriddo et al. identified a marine microaerophilic bacterium, *Nitrospina*, which likely facilitated mercury methylation in sea ice (Gionfriddo et al., 2016).

Due to the long lifetime of mercury in the atmosphere, polar environment receives mercury inputs globally. The 'newly' deposited mercury is generally considered more available for methylation compared with legacy mercury (Chadwick et al., 2013; Harris et al., 2007; Hintelmann et al., 2002) and the sources of these bioavailable mercury species in polar environment is dependent on the climatic conditions. For example, Atmospheric Mercury Depletion Events (AMDEs) regularly take place in the springtime and are a major contributor of 'new' mercury inputs to the polar aquatic environment. The occurrence of AMDEs is because photooxidation of atmospheric elemental mercury by halogen radicals and oxidized halogens is sharply enhanced after the polar nights end and the divalent inorganic mercury species generated from this process enter the aquatic environment via dry or wet deposition (Barkay et al., 2011;Schroeder et al., 1998; Steffen et al., 2007). Therefore, the abundance and speciation of the methylation precursors may vary with the changing schemes of solar irradiation and precipitation due to climate change.

Moreover, polar regions are covered by ice and snowpack for most of the year, and 95% of Antarctic is covered by ice permanently. Therefore, the fate of mercury in polar environment is particularly sensitive to climate change (Kramshoj et al., 2018; Oiffer and Siciliano, 2009; Schartup et al., 2019; Stern et al., 2012), considering that thawing of glacier and snow induces the release of mercury species to adjacent aquatic settings (Beattie et al., 2014; Poissant et al., 2008; Soerensen et al., 2016) that become precursors of microbial methylation. Increasing temperature also substantially promotes microbial activities in polar environment. On one hand, laboratory incubations of polar soils demonstrated that temperature increase of several degrees led to 1–2 orders of magnitude greater MeHg production (Loseto et al., 2004; Oiffer and Siciliano, 2009). On the other hand, the primary producers, such as marine phytoplankton, possibly provide methyl-donating compounds for the methylating microorganisms by direct or indirect means, and this process may be affected by the changing temperature. Recent research findings that point to this potential mercury methylation pathway in polar environment includes two main lines of evidence. First, marine phytoplankton produces dimethylsulfoniopropionate (DMSP) during growth, and demethylation of DMSP involves tetrahydrofolate and aminomethyltransferase (Kiene et al., 2000) that are both involved in microbial mercury methylation (Parks et al., 2013). Second, the concentration of methanesulfonate, a product of dimethylsulfate (DMSP derivative) photo-oxidation, appeared to correlate with the concentration of MeHg in snowpack (Larose et al., 2010).

### 1.2. Paddy soils

Rice is a staple food for >60% of the world population and ingesting rice is considered to be an important route of human exposure to MeHg (Gong et al., 2018; Zhang et al., 2010a). This is because rice grains tend to accumulate MeHg from paddy soils, with bioaccumulation factor ranging from 0.2 to 1100 (Rothenberg et al., 2016; Rothenberg et al., 2014). The concentration levels of MeHg in paddy soils were 0.14–67 μg $kg^{-1}$ in areas impacted by mercury mining and 0.01–4.5 μg $kg^{-1}$ in non-contaminated areas (Table 1). Although the total mercury abundance in non-contaminated paddy soil is relatively low, the methylation potential of inorganic mercury (represented as the concentration ratio of MeHg and total mercury) in this environment often exceeded that in mining areas (Table 1), suggesting that paddy soils, which are not impacted by mercury pollution, may also be environmental hotspots for MeHg production and accumulation.

Paddy soil, with a total harvested area of 163 million ha worldwide (United States Department of Agriculture, 2018), represents a specialized wetland environment heavily impacted by human activities, which alter the conditions dictating the microbial communities and mercury speciation in this environment. For increasing the efficiency of water utilization in response to the global water crisis, continuous flooding in paddy soil has been gradually switched to intermittent

**Table 1**
Reported mercury methylation potential in different environmental settings.

| Environment | Sample description | MeHg level (ng $L^{-1}$ or μg $kg^{-1}$)[a] | Methylation potential (%)[b] | Location | References |
|---|---|---|---|---|---|
| Polar regions | Snow | <0.02–0.03 | <0.2–3.0 | Antarctic | Gionfriddo et al., 2016 |
| | | ≤0.015–0.118 | 0 — ~85 | Canadian Arctic | St Louis et al., 2005, 2007 |
| | Sea ice | <0.02–0.17 | 0.05–3.1 | Antarctic | Gionfriddo et al., 2016 |
| | | <0.02–0.57 | 0–40.9 | Arctic | Beattie et al., 2014 |
| | Sea water | N.A.[c] | 13–33 | Antarctic | De Ferro et al., 2014 |
| | | <0.02–0.15 | <0.1–56 | Antarctic | Gionfriddo et al., 2016 |
| | | <0.01–0.18 | <5–78 | Southern Ocean | Cossa et al., 2011 |
| | | 0.057–0.095 | 30.4–45.2 | Canadian Arctic | St Louis et al., 2007 |
| | | 0.015–178 | 1–67 | Canadian Arctic | Kirk et al., 2008 |
| | | 0.021–0.126 | ~0–61 | Arctic | Wang et al., 2012 |
| | Lake water | <0.085–0.257 | <9–30 | Antarctic | Vandal et al., 1998 |
| | | 0.04–30 | 1.2–62 | Canadian Arctic | Lehnherr et al., 2012a, 2012b; St Louis et al., 2005 |
| | Sediment | 0.001–0.081 | <0.014 | Alaska, USA | Poissant et al., 2008; Naidu et al., 2003 |
| | | 0.26–3.4 | N.A. | Alaska, USA | Hammerschmidt et al., 2006 |
| | | 0.4–1.1 | N.A. | Ny-Ålesund, Norway | Jiang et al., 2011 |
| | Soil | 0.01–<9.6 | 0.01–9.4% | Canadian Arctic | Loseto et al., 2004; Oiffer and Siciliano, 2009; St Pierre et al., 2015 |
| Paddy soils | Non-contaminated | 0.02–1.76 | 0.016–2.54 | Main rice planting areas in China[d] | Unpublished data |
| | | 0.84–4.5 | N.A. | Chongqing, China | Tang et al., 2018 |
| | | 0.17–1.0 | 0.05–1.3 | California, USA | Tanner et al., 2018a, 2018b |
| | | 0.52–1.42 | 0.52–1.07 | California, USA | Marvin-Dipasquale et al., 2014 |
| | | 0.01–0.29 | 0.59–1.32 | Arkansas, USA | Rothenberg et al., 2016 |
| | Mining impacted | 0.14–67 | <0.1 | Guizhou, China | Rothenberg and Feng, 2012; Li et al., 2019; Meng et al., 2010; Meng et al., 2014; Zhang et al., 2010a, 2010b; Horvat et al., 2003 |
| | | 6.0–36.9 | 0.001–0.14 | Shaanxi, China | Tang et al., 2019; Qiu et al., 2012 |
| | | 2.8–10.9 | 0 < 0.03 | Hunan, China | Meng et al., 2014 |
| | | 0.3–8.5[e] | N.A. | Guangdong, China | Meng et al., 2014 |

[a] The MeHg level in snow, sea ice and sea/lake water is shown as ng $L^{-1}$, while that in sediment, wetland soil and paddy soils is shown as μg $kg^{-1}$.
[b] Methylation potential is calculated as the ratio of MeHg level/Total Hg level (%).
[c] N.A. means data are not available.
[d] The data are obtained from 64 sites in 12 provinces in China, i.e., Heilongjiang, Liaoning, Jiangsu, Anhui, Hubei, Jiangxi, Hunan, Guizhou, Sichuan, Guangdong, Guangxi and Hainan. The rice planting area in these provinces account for >80% of the total rice planting area in China.
[e] Data from Pb/Zn mining impacted area.

flooding (i.e., flooded during planting season and drained otherwise). This change of farming activity has been reported to markedly decrease the mercury content in porewater (Peng et al., 2012) and rice grains (Rothenberg et al., 2016; Tanner et al., 2018a; Wang et al., 2014), which is likely attributable to the inhibitory effect of aerobic conditions during dry periods on the anaerobic methylating microorganisms. The alternate aerobic-anaerobic conditions also greatly affect the metal oxide and metal sulfide mineral phases that control mercury speciation via adsorption/desorption and precipitation/dissolution processes (Wang et al., 2014), especially considering that nanoparticulate mercury available for microbial methylation can be produced from these processes (Rivera et al., 2019; Zhang et al., 2014).

Straw incorporation into paddy soil is another farming activity widely promoted for sustainable agriculture, given that it is an environmentally friendly measure of recycling solid waste (as opposed to straw burning as an alternative measure) while improving the soil texture and fertility (Tang et al., 2019). Nevertheless, both laboratory and field investigations showed that straw incorporation enhanced the risks of MeHg production in paddy soil because of the organic matter release during wet periods (Liu et al., 2016a; Wang et al., 2019b; Zhang et al., 2018; Zhu et al., 2016). The labile fraction of this organic matter serves as carbon and energy sources that can stimulate the growth and activity of heterotrophic methylating microorganisms (indicated by the larger copy numbers of *hgcA* genes) (Tang et al., 2019). The organic matter fraction rich in mercury binding groups may induce the production of bioavailable mercury species for methylation through ligand-promoting dissolution of bulk-scale minerals (e.g., metacinnabar, cinnabar) (Ravichandran et al., 1998; Waples et al., 2005) or formation of organic matter coated nanoparticles from kinetically-hindered precipitation (Graham et al., 2013; Zhang et al., 2012). Thus, cautions are needed when considering straw incorporation in paddy soils with high abundance of inorganic mercury and methylating microorganisms. Agricultural practice in such environment should balance between the needs of booting crop production and minimizing MeHg accumulation.

## 2. Identification of potential microbial methylators

The conversion of inorganic mercury into MeHg in the environment is mainly driven by anaerobic microorganisms (Hsu-Kim et al., 2013; Parks et al., 2013). Earlier studies suggested that *Desulfovibrio desulfuricans* LS synthesized MeHg via the acetyl-CoA pathway (Choi et al., 1994a; Choi et al., 1994b), where the methyl groups were originated from 3-C serine or formate according to the experiments using $^{14}C$-labeled substrates. However, a subsequent study argued that only complete-oxidizing sulfate reducing bacteria (SRB) used the acetyl-CoA pathway for basic carbon metabolism and mercury methylation, whereas incomplete-oxidizing strains transformed inorganic mercury to MeHg via other unknown pathway (Ekstrom et al., 2003). Until recently, the discovery of *hgcAB* genes provides genetic basis for mercury methylation (Parks et al., 2013), and the gene clusters were confirmed to be necessary for microbial methylation. Specifically, the *hgcA* gene encodes putative corrinoid protein that transfers a methyl group to inorganic mercury to form MeHg, and *hgcB* gene encodes [4Fe-4S] ferredoxin that is responsible for *hgcA* reduction (Fig. 1) (Parks et al., 2013; Smith et al., 2015).

The *hgcAB* gene clusters haven been considered as a reliable molecular marker to identify mercury methylating communities and predict MeHg production in the environment (Podar et al., 2015; Poulain and Barkay, 2013). Full-length *hgcAB* can be amplified using broad-range degenerate primers, while the fragment of *hgcA* gene is usually quantified using clade-specific primers as previously suggested (Christensen et al., 2016; Liu et al., 2014). These reported *hgcAB* genes mainly

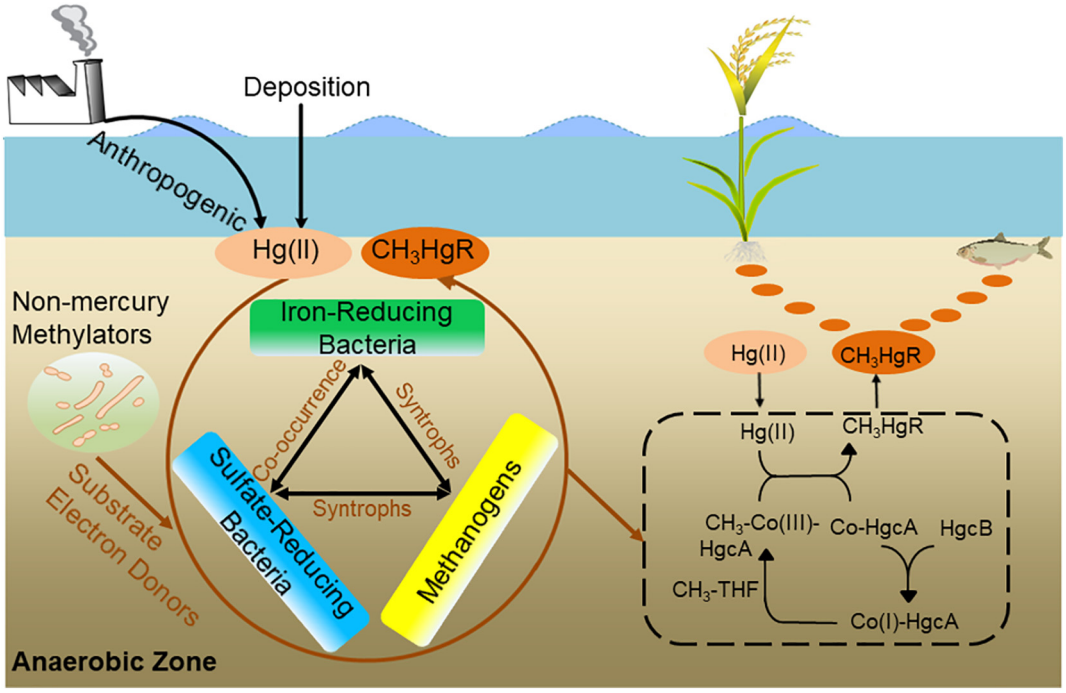


**Fig. 1.** Microorganisms and potential pathways involved in mercury methylation in natural aquatic environment. Co-HgcA: cobalamin-HgcA; $CH_3$-Co(III)-HgcA: formed after passing methyl to Co(I)-HgcA via $CH_3$-THF; HgcA: putative corrinoid proteins, encoded by *hgcA*; HgcB: putative corrinoid protein–associated 2[4Fe—4S] ferredoxins, encoded by *hgcB*; $CH_3$-THF: $CH_3$-$H_4$folate.

distributed within phyla of *Proteobacteria* (SRB, iron-reducing bacteria (FeRB), *Syntrophobacterales* and *Nitrospina*), *Chloroflexi*, *Firmicutes* (i.e., *Clostridiales*) and *Euryarchaeota* (i.e., *Methanomicrobia*) (Gilmour et al., 2013; Podar et al., 2015). A recent study showed that the phyla of *Aminicenantes*, *Spirochaetes* and *Kiritimatiellaeota* may also contain mercury methylators (Jones et al., 2019). The occurrence of the potential mercury methylating microorganisms has been investigated in a diverse variety of microbial habitats, such as polar regions, paddy soils, forest soils, wetland ecosystems, freshwater sediments, oceanic environment, bioreactors and animal hindgut, using multiple molecular biological tools designed to target *hgc*AB genes (Table 2).

### 2.1. Sulfate-reducing bacteria

SRB are members of *Deltaproteobacteria*, and usually utilize sulfate as electron accepter to generate energy (Dhillon et al., 2003; Thiele and Zeikus, 1988). SRB were first proposed to be mercury methylators through the inhibition of metabolic activity of SRB in estuarine sediments (Compeau and Bartha, 1985). Hereafter, SRB were considered to be the principal guilds for mercury methylation in aquatic systems (Acha et al., 2011; Devereux et al., 1996; Gilmour et al., 1992; King et al., 1999). SRB *D. desulfuricans* LS and *Desulfovibrio desulfuricans* ND132 are commonly used as model organisms to study the potential, kinetics and pathways of mercury methylation (Choi et al., 1994a; Gilmour et al., 2013; Liu et al., 2016b; Schaefer et al., 2011). However, not all SRB can convert inorganic mercury to MeHg, and the bacterial strains that carry *hgcAB* genes are potential mercury methylators (Gilmour et al., 2013; Parks et al., 2013). SRB capable of methylating mercury were ubiquitous in different environmental niches (Table 2), though their abundance varies across different ecosystems. For example, a recent study suggested that SRB were the dominant contributors to the production of MeHg in lake sediments (Ma et al., 2018). SRB was also found in other habitats, including lake biofilm and periphyton (Bouchet et al., 2018), paddy soils (Liu et al., 2018; Liu et al., 2014), boreal forest soil and boreal lake sediments (Bravo et al., 2018b; Xu et al., 2019), where SRB appeared to occur to a lesser degree, compared to the other major methylating groups (e.g., FeRB and methanogens). However, the relative contribution of SRB to MeHg production in these environmental settings is currently unknown.

### 2.2. Iron-reducing bacteria

FeRB belong to *Deltaproteobacteria* and are related to SRB, and can use Fe(III) as electron accepter for growth (Holmes et al., 2004; Kerin et al., 2006). *Geobacter* sp. strain CLFeRB was initially isolated from Clear Lake sediments and showed considerable ability to methylate mercury (Fleming et al., 2006). Further laboratory study suggested that *G. metallireducens*, *G.sulfurreducens* and *G. hydrogenophilus* can methylate inorganic mercury in the presence of ferric iron or other electron acceptors (i.e., nitrate and fumarate), however, the other FeRB strains, *Shewanella alga* and *Shewanella putrefaciens*, cannot convert inorganic mercury to MeHg (Kerin et al., 2006). To date, it has been known that almost all *Geobacter* sp. possess the *hgcAB* genes, whereas their capacity of mercury methylation differs among different species. Although FeRB often coexist with SRB in anaerobic environment, their important roles in driving in situ mercury methylation have been highlighted in recent research. *Geobacteraceae* were demonstrated to be the dominant mercury methylators in iron-enriched river sediments and boreal forest soils according to high-throughput sequencing of *hgcA* amplicon (Bravo et al., 2018a; Bravo et al., 2018b). Combined application of PacBio sequencing of *hgcAB* and shotgun metagenomic sequencing demonstrated that *Geobacteraceae* were the major contributors to MeHg production in paddy soils (Liu et al., 2018). Yet, the information regarding the prevalence and diversity of FeRB responsible for mercury methylation in diverse environments remains limited.

### 2.3. Methanogens

Methanogens affiliated within class *Methanomicrobia* are potential mercury methylators, which can use $CO_2$, acetate and C1 compounds as substrate for growth and metabolism (Liu and Whitman, 2008). Early on, methanogens were proposed to be mercury methylators

**Table 2**
Typical mercury methylators distributed in varied environments. SRB: sulfate-reducing bacteria; FeRB: iron-reducing bacteria.

| Habitats | Strategies for identification | Taxa | References |
|---|---|---|---|
| Wetland sediments | *hgcA* gene clone libraries<br>454-FLX Titanium of 16S rRNA amplicon<br>Shotgun metagenomic analysis | SRB (e.g., Desulfovibrionaceae, Desulfobulbaceae, Desulfobacteraceae)<br>FeRB (e.g., Geobacteraceae)<br>Methanogens (e.g., *Methanoregulaceae*, Methanomicrobiaceae, Methanosarcinaceae)<br>Syntrophs (e.g., *Syntrophaceae*, Syntrophorhabdaceae, Syntrophomonadaceae) | Bae et al., 2014<br>Schaefer et al., 2014a, 2014b<br>Bae et al., 2019<br>Christensen et al., 2019 |
| Lake/river sediments | *hgcA* gene clone libraries<br>Illumina sequencing of 16S rRNA amplicon<br>Illumina sequencing of *hgcA* amplicon<br>Shotgun metagenomic analysis | SRB (e.g., Desulfuromonadaceae, Desulfovibrionaceae, Desulfobulbaceae)<br>FeRB (e.g., Geobacteraceae)<br>Methanogens (e.g., Methanospirillaceae, Methanoregulaceae, Methanomassiliicoccus)<br>Syntrophs (e.g., *Syntrophaceae*, Syntrophobacteraceae, Syntrophomonadaceae) | Podar et al., 2015<br>Bravo et al., 2018a<br>Bravo et al., 2018b<br>Christensen et al., 2019<br>Jones et al., 2019<br>Yuan et al., 2019 |
| Paddy soils | *hgcA* gene clone libraries<br>Illumina sequencing of *hgcA* amplicon<br>PacBio sequencing of *hgcAB* genes<br>Shotgun metagenomic analysis | SRB (e.g., Desulfovibrionaceae, Desulfobulbaceae, Desulfomicrobiaceae)<br>FeRB (e.g., Geobacteraceae)<br>Methanogens (e.g., *Methanoregulaceae*, Methanocellaceae, Methanosarcinaceae)<br>Syntrophs (e.g., Syntrophorhabdaceae, Syntrophaceae) | Liu et al., 2014<br>Liu et al., 2018<br>Vishnivetskaya et al., 2018 |
| Forest soils | Illumina sequencing of 16S rRNA amplicon<br>Illumina sequencing of *hgcA* amplicon<br>Shotgun metagenomic analysis | SRB (e.g., Desulfovibrionaceae, Desulfobulbaceae, Clostridiaceae)<br>FeRB (e.g., Geobacteraceae)<br>Methanogens (e.g., Methanomassiliicoccaceae, Methanosarcinaceae, Methanoregulaceae)<br>Syntrophs (e.g., *Syntrophaceae*, Syntrophomonadaceae, Peptococcaceae) | Podar et al., 2015<br>Xu et al., 2019 |
| Biofilms and Periphyton | Illumina sequencing of 16S rRNA amplicon | SRB (e.g., Desulfobulbaceae)<br>FeRB (e.g., Geobacteraceae)<br>Syntrophs (e.g., *Syntrophaceae*, Syntrophorhabdaceae) | Bouchet et al., 2018 |
| Marine (snow, ice and seawater) | Shotgun metagenomic analysis | SRB (e.g., Desulfovibrionaceae, Desulfobulbaceae, Desulfobacteraceae)<br>FeRB (e.g., Geobacteraceae)<br>Methanogens (e.g., *Methanosarcinaceae*, Methanomicrobiaceae, Methanoregulaceae)<br>Syntrophs (e.g., *Syntrophaceae*, Syntrophomonadaceae,Syntrophobacteraceae) | Podar et al., 2015<br>Gionfriddo et al., 2016<br>Villar et al., 2019 |
| Extreme environments (e.g., Permafrost, Soda lakes and soils, Arctic coastal water and sediments) | 454-FLX Titanium of 16S rRNamplicon<br>Shotgun metagenomic analysis | SRB (e.g., Desulfobulbaceae, Desulfovibrionaceae, Desulfohalobiaceae)<br>FeRB (e.g., Geobacteraceae)<br>Methanogens (e.g., *Methanosarcinaceae*, Methanocellaceae, Methanoregulaceae)<br>Syntrophs (e.g., *Syntrophaceae*) | Podar et al., 2015<br>Christensen et al., 2019 |
| Bioreactor | Illumina sequencing of 16S rRNA amplicon<br>Shotgun metagenomic analysis | SRB (e.g., Desulfomicrobiaceae, Desulfovibrionaceae, Clostridiaceae)<br>FeRB (e.g., Geobacteraceae)<br>Methanogens (e.g., *Methanocellaceae*) | Podar et al., 2015<br>Wang et al., 2019a, 2019b |
| Animal hindgut | Shotgun metagenomic analysis | Syntrophs (e.g., *Peptococcaceae*) | Podar et al., 2015 |

based on MeHg production from cell extracts of *Methanobacterium bryantii* (Wood et al., 1968; Yu et al., 2013), but this theory was subsequently rejected, because later studies failed to observe mercury methylation by other pure cultures of methanogens (Pak and Bartha, 1998). Lately, Hamelin et al., revealed that mercury methylation in periphyton was likely attributable to methanogens (Hamelin et al., 2011), since MeHg production was diminished when a specific inhibitor of methanogens was added. The identification of the *hgcAB* genes in the genomes of *Methanomicrobia* further corroborated the roles of methanogens being potential mercury methylators and the methylating capacity of some *Methanomicrobia* strains were experimentally verified (Gilmour et al., 2013; Parks et al., 2013). For example, *Methanomassiliicoccus luminyensis*, *Methanosphaerula palustris and Methanospirillum hungatei* can convert >10% inorganic mercury to MeHg in batch incubations (Gilmour et al., 2018; Yu et al., 2013). It is interesting to note that in some lakes with high sulfate level, *Methanomicrobia* outcompeted SRB to be the dominant mercury methylators (Jones et al., 2019). Both metagenomic sequencing and qPCR analysis demonstrated that *Methanomicrobia* were the most abundant mercury methylators in paddy soils and a large percentage of the *hgcA* homologs of *Methanomicrobia* (57%) appeared to associate with *Methanofollis* and *Methanoregula* (Liu et al., 2018). Moreover, metagenomic analysis of *hgcA* homologs revealed that methanogens were the dominate microbial communities in thawing permafrost soils, pointing to the potential risks of in situ MeHg production in polar regions due to the enhanced mercury release and microbial activity induced by global warming (Podar et al., 2015). Hence, the contribution of methanogens to mercury methylation is expected to increase in the context of global climate change.

### 2.4. Syntrophs

Syntrophs are mainly assigned to class *Deltaproteobacteria* that are obligate anaerobic bacteria and can obtain energy from syntrophy with SRB or methanogens (Bae et al., 2014; Friedrich et al., 1991; Yu et al., 2018). Syntrophobacter-related species are ubiquitous in wetlands and freshwater ecosystems, and they may be the major propionate-dependent sulfate reducers and syntrophs in rice paddy soil (Liu and Conrad, 2017). Recent identification of *hgcAB* suggested that syntrophs have the potential to methylate mercury. For example, *Syntrophus aciditrophicus* was confirmed as a strong mercury methylator in pure culture experiments (Gilmour et al., 2013), while the

methylation capacity of *Syntrophobacter wolinii* was evidently increased when co-cultured with *Methanospirillum hungatei* (Yu et al., 2018). In particular, syntrophic microbial interactions have recently been suggested as a major source of MeHg in sulfate- and iron-limited anoxic environments (Yu et al., 2018). Additionally, *Syntrophobacterales* were found to be the most abundant mercury methylators in soils with low sulfate content in Florida Everglades (Bae et al., 2014). Recent metagenomic analysis also indicated the presence of *hgcA* syntrophs in marsh and organic carbon rich Arctic permafrost (Podar et al., 2015), though the activities and relative contribution to mercury methylation remains to be determined.

## 3. Assessment of mercury availability to microbial methylators

Over the past decades, a variety of methods to assess the availability of inorganic mercury to the methylating microorganisms, including theoretical (Liem-Nguyen et al., 2017; Town et al., 2019; Zhou et al., 2017; Zhu et al., 2018) and experimental approaches (Bjorn et al., 2007; Ndu et al., 2015; Regnell and Watras, 2019), have been proposed and examined. Given that mercury methylation often occurs in the environmental matrices (e.g., anaerobic sediments) that are intrinsically complex and subject to microbial and geochemical perturbations (Hsu-Kim et al., 2018; Obrist et al., 2018), classical tools for mercury speciation analysis, such as chemical equilibrium models and selective extraction assays were applied to evaluate mercury bioavailability with limited success (Hsu-Kim et al., 2013; Rivera et al., 2019; Ticknor et al., 2015). New analytical techniques, such as enriched stable isotope tracers (Jiskra et al., 2014; Jonsson et al., 2014; Liem-Nguyen et al., 2016), whole-cell biosensors (Chiasson-Gould et al., 2014; Ndu et al., 2015; Stenzler et al., 2017; Thomas et al., 2014) and diffusive gradient thin film (DGT) (Diez et al., 2016; Ndu et al., 2018; Pham et al., 2015; Wu et al., 2017), are becoming prominent in assessing mercury bioavailability, because these methods have the potential to allow proper simulation of the complex interaction between the methylating microbial cells and a diverse variety of inorganic mercury species that are geochemically relevant (Fig. 2).

### 3.1. Enriched stable isotope tracers

Methylmercury production is one of the most frequently used endpoint indicators of the bioavailability of inorganic mercury to methylating microorganisms. However, microbial mercury methylation and MeHg demethylation often simultaneously occur in pure cultures and environmental samples (Bridou et al., 2011; Figueiredo et al., 2018; Graham et al., 2012; Lu et al., 2016). Thus, it is crucial to monitor these two competing processes separately and simultaneously to discern the fraction of inorganic mercury that is available to methylation. In the mercury methylation assays, the addition of inorganic mercury should

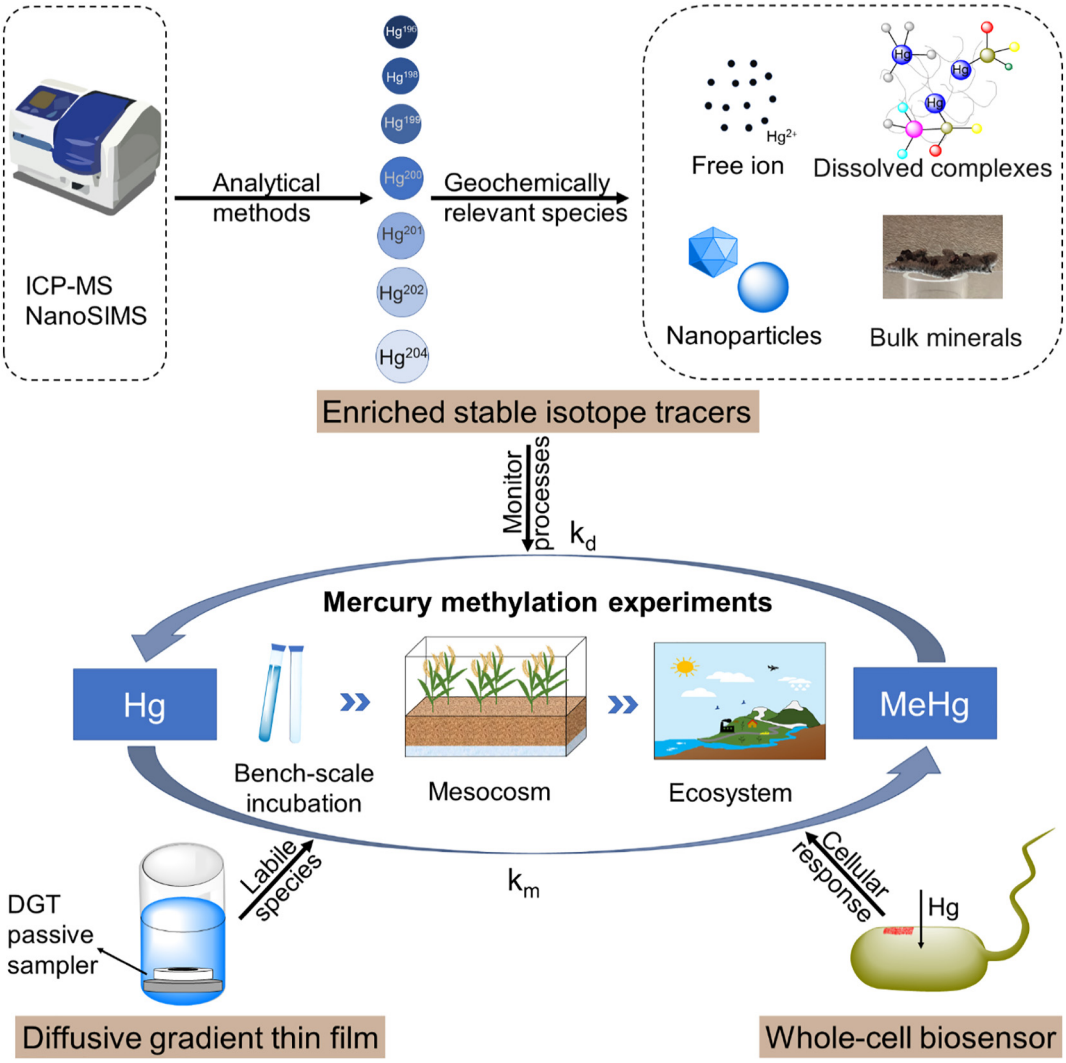


**Fig. 2.** Methods for assessing mercury availability to microbial methylators.

be easily differentiated from the background mercury species without imposing significant biological or chemical perturbations to the experimental systems. These requirements can be fulfilled by the application of multiple stable isotopically enriched mercury tracers in practice ranging from bench-scale pure culture (Bridou et al., 2011) and sediment slurry incubations (RodríGuez MartíN-Doimeadios et al., 2004) to mesocosm (Jonsson et al., 2014) and in situ systematic investigations (Hintelmann et al., 2000).

Mercury has seven naturally-occurring stable isotopes (i.e., $^{196}$Hg, $^{198}$Hg, $^{199}$Hg, $^{200}$Hg, $^{201}$Hg, $^{202}$Hg and $^{204}$Hg) with $^{202}$Hg being the most abundant (29.86%) (Bergquist and Blum, 2007; Blum and Bergquist, 2007). Enriched mercury isotopes predominantly consist of one specific stable isotope while the other six are present at trace levels (<5%). For assessing the mercury bioavailability, four enriched isotope tracers may be used for different purposes, including: (1) an inorganic mercury tracer added to the reaction matrices at the beginning of the experiments for tracking microbial methylation; (2) a MeHg tracer added to the reaction matrices at the beginning of the experiments for tracking microbial demethylation; (3) an inorganic mercury tracer added to the samples after sample collection for monitoring artifact methylation during subsequent sample work-up (e.g., distillation, acid or alkaline leaching, organic solvent extraction); (4) a MeHg tracer added to the samples as a species-specific internal standard after sample collection for monitoring the recovery of methylmercury during subsequent sample work-up and analysis. The first two tracers for simultaneous detection of mercury methylation and MeHg demethylation are often added at 10–100% of the ambient concentration levels, while the addition of the latter two tracers are kept similar to the inorganic mercury or methylmercury concentrations in the samples for data quality assurance (Bergquist and Blum, 2007; Bjorn et al., 2007). An additional stable mercury isotope, usually $^{202}$Hg, is measured from each sample to account for the contribution of native inorganic mercury to MeHg production. However, simpler approaches involving fewer isotope tracers may be used according to specific research needs. In most of the cases, only the isotopic composition of MeHg is analyzed using gas chromatography with inductively coupled plasma mass spectrometry (GC-ICP-MS) that offers superb instrumental sensitivity and can concomitantly quantify various mercury isotope tracers at environmentally relevant levels. The respective concentrations of the above-mentioned isotopic tracers in each sample are calculated using algebraic deconvolution (Hintelmann and Evans, 1997; Hintelmann et al., 1997; Hintelmann and Ogrinc, 2003; Sturup et al., 2005).

It has become consensus that the bioavailability of inorganic mercury is largely determined by its chemical speciation (Hsu-Kim et al., 2013). Hence, understanding the methylation potential of different mercury species that are prevalent in anaerobic aquatic environment is a prerequisite for accurately predicting environmental MeHg accumulation. The enriched mercury isotopes have been utilized to synthesize tracers that represent a spectrum of inorganic mercury species, including ionic, complexed, nanoparticulate mercury as well as mercury associated with bulk-scale mineral phases, for comparatively assessing the bioavailability of these geochemically relevant mercury species in the same experimental system (Jiskra et al., 2014; Jonsson et al., 2014; Jonsson et al., 2012; Liem-Nguyen et al., 2016). For example, model mercury compounds, including metacinnabar, cinnabar, mercury chemically adsorbed to mackinawite or natural organic matter and aqueous ionic mercury were synthesized using different enriched mercury isotopes and added to both bench-scale incubations (Jonsson et al., 2012) and mesocosm ecosystems containing estuarine sediments (Jonsson et al., 2014). These inorganic mercury tracers exhibited substantially variable methylation potential and demonstrated the previously underestimated contribution of solid-phase mercury to sedimentary methylmercury production (Jonsson et al., 2014; Jonsson et al., 2012). The dynamic exchange of inorganic mercury between aqueous phase and solid phase is therefore important for understanding mercury methylation and appeared to be kinetically controlled in the experiments using enriched isotopic tracers that allowed concurrent determination of adsorption and desorption rate coefficients (Jiskra et al., 2014).

In addition to mercury methylation and demethylation rates, the enriched isotopic tracers may also provide information regarding the mechanism and factors affecting the bioavailability and methylation of inorganic mercury. Recent research revealed that inorganic mercury was rapidly taken up by SRB and became unavailable for methylation within a short period of time, possibly due to the binding with biomolecules inside the cells (Liu et al., 2016b). Mercury tracers that represent a diverse range of bioavailabilities can be added to pure cultures of methylating microorganisms to monitor the cellular and subcellular distribution (Pedrero et al., 2012), which may shed light on the potentially dissimilar cell internalization pathways of these mercury species that constrain methylation. Moreover, mercury isotope tracers were added to a mesocosm that simulated pelagic environment to illustrate that the increased nutrient input apparently enhanced methylation of mercury with high bioavailability while having little effect on the methylation of mercury with low bioavailability (e.g., metacinnabar) (Liem-Nguyen et al., 2016). This application of enriched mercury isotope tracers can be extended to address questions regarding how mercury bioavailability and methylation potential respond to the changing environment.

### 3.2. Whole-cell biosensor

Intracellular uptake of inorganic mercury has long been considered a rate limiting process that dictates mercury methylation (Hsu-Kim et al., 2013; Regnell and Watras, 2019). Both passive permeation (An et al., 2019; Zhou et al., 2017) and active transport pathways (Schaefer and Morel, 2009; Schaefer et al., 2011; Schaefer et al., 2014b) may be involved in mercury uptake prior to methylation. Whole-cell biosensors offer a direct approach to interrogate this process and assess mercury bioavailability (Chiasson-Gould et al., 2014; Ndu et al., 2015; Stenzler et al., 2017; Thomas et al., 2014). In theory, the biosensors are constructed by transferring plasmids into a model methylating microorganism and the plasmids contain gene sequences of fluorescent proteins, whose expression is responsive to cellular uptake of mercury. In practice, a gram-negative bacterium (e.g., *Escherichia coli*) is often utilized as a model strain, with bioluminescence controlled by *merR*, a transcription regulator of the mercury resistance system (Chiasson-Gould et al., 2014; Stenzler et al., 2017; Thomas et al., 2014). Generation of elemental mercury, the transformation product of divalent mercury by the mercury resistance systems, may also be monitored in addition to bioluminescence for evaluating mercury bioavailability (Ndu et al., 2015).

Thus far, whole-cell biosensors have been applied to both aerobic and anaerobic systems to investigate the influence of environmental variables, including pH, salinity, cations, natural and synthetic ligands on the bioavailability of inorganic mercury (Chiasson-Gould et al., 2014; Jonsson et al., 2014; Kelly et al., 2003; Lin et al., 2015; Ndu et al., 2015; Stenzler et al., 2017; Thomas et al., 2018; Thomas et al., 2014). For example, Chiasson-Gould et al., used a *mer-lux* biosensor to demonstrate that natural organic matter markedly enhanced mercury uptake without altering the permeability of cell walls. This enhancing effect occurred within a limited time frame, pointing to the important kinetic constraints on DOM-controlled bioavailability of inorganic mercury (Chiasson-Gould et al., 2014). Another *mer*-R based whole-cell biosensor provided the first line of direct evidence exemplifying the bioavailability of negatively charged mercury complexes with halogen anions that are expected to dominate mercury speciation in polar environment (Stenzler et al., 2017).

Despite the valuable information derived from the current whole-cell biosensing systems, growing evidence reveals that mercury resistance and mercury methylation occur through two independent microbial pathways, and methylating microorganisms are not necessarily more

resistant to mercury than non-methylators (Diaz et al., 2013; Gilmour et al., 2011; Stenzler et al., 2017). Furthermore, mercury uptake and methylation rates appeared to vary with different microbial strains, even for the ones that have close phylogenetic relationship (Bridou et al., 2011; Graham et al., 2012). Conceivably, the biosensors constructed based on the mercury resistance system in non-methylating bacteria may not mimic cellular uptake of mercury precursors for methylation with sufficient accuracy. In fact, recent research has advanced the understanding of the mechanisms that govern mercury transport into methylating microbial cells. It has been repeatedly shown that cysteine promoted mercury uptake and methylation in both SRB and FeRB via an energy-dependent pathway (Bridou et al., 2011; Graham et al., 2012; Lu et al., 2016; Schaefer and Morel, 2009; Zhou et al., 2017). This pathway was later deduced to be divalent metal transporters since the co-occurrence of Zn(II), Cd(II), Cu(II) and Ni(II) all exhibited inhibitory effects on the uptake and methylation of mercury (Lu et al., 2018; Schaefer et al., 2014b). In particular, Zn(II) significantly decreased mercury methylation at environmentally relevant Zn(II):Hg(II) concentration ratios without inducing toxicity to methylating bacteria whether cysteine was present or not (Schaefer et al., 2014b). While the specific transport mechanism of mercury remains to be discovered, the competition between divalent mercury and other metals for the same membrane transporters suggests the feasibility of biosensors constructed with model methylating microorganisms equipped with divalent metal transporters for assessing mercury bioavailability. Owing to the recent advances in the development of analytical tools, such as nanosecondary ion mass spectroscopy (NanoSIMS), the application of whole-cell biosensor and stable isotope tracers may be coupled to monitor the cellular response to an array of specific mercury species (Butler et al., 2017).

### 3.3. Diffusive gradient thin film (DGT)

DGT technique is an in situ passive sampling approach that has widely been used to quantify kinetically labile species of inorganic mercury in anaerobic aquatic environment (Divis et al., 2005; Divis et al., 2009; Fernandez-Gomez et al., 2011; Hong et al., 2011; Merritt and Amirbahman, 2007). The DGT samplers usually have a three-layer structure, including a membrane filter, a diffusion phase and a ligand-functionalized binding phase (Zhang and Davison, 2015). Mercury species diffuse through a membrane and hydrogel (i.e., diffusion phase) and accumulate on a resin (i.e., binding phase) via chemical complexation with functional groups, typically thiol-containing ligands. The abundance of the accumulated mercury species within a specific incubation period presumably reflects the fraction of "chemically labile" mercury (Fernandez-Gomez et al., 2011; Ndu et al., 2018; Pham et al., 2015; Wu et al., 2017). Although selective binding of mercury in DGT device occurs through filtration and ligand exchange, DGT showed better performance in assessing mercury bioavailability for methylation, compared to conventional filtration and thiol-based extraction approach (Ndu et al., 2018; Rivera et al., 2019). Combined applications of DGT samplers and enriched stable isotope tracers (e.g., dissolved, DOM-complexed, nanoparticulate mercury, mercury sorbed to nanocrystalline FeS) in a recent mercury methylation study successfully predicted the bioavailability of these inorganic mercury species for microbial methylation in anaerobic sediment slurries (Ndu et al., 2018).

Two issues need to be addressed in order to improve the application of DGT in mercury bioavailability assessment. First, DGT measurements may be subject to errors if macromolecular organic ligands dominate mercury speciation, considering the large uncertainty in the diffusion coefficients and stability of mercury-organic complexes induced by the great diversity of the binding sites and molecular structure of the organic ligands (Hong et al., 2011; Zhang and Davison, 2015). Second, the transport and transformation behavior of nanoparticulate mercury in DGT samples is currently unclear. It is generally presumes that DGT only quantifies the dissolved mercury fraction, not solid-phase mercury (Zhang and Davison, 2015). However, mercury-containing particles with small sizes can penetrate the membrane layer and may dissolve or aggregate in DGT samplers, which interferes with the DGT measurements of other mercury species. For example, Pham et al., has shown that HgS nanoparticles aggregated on the surface of DGT samples and diminished the migration of dissolved mercury species through adsorption that led to underestimation of the DGT-labile mercury fraction (Pham et al., 2015).

The performance of DGT in assessing mercury bioavailability may be improved via modifying the design of diffusion phase as well as binding phase. Ideal diffusion phases, such as agarose diffusive gel and polyacrylamide gel, do not retard the transport of or compete with the binding phase for a wide variety of mercury species (e.g., Hg-DOM complexes) (Fernandez-Gomez et al., 2011). This is reflected by three parameters, including good linear regression of the accumulated mercury mass against time, large mercury uptake rate and low mercury residue in gel blanks. In DGT samplers, the solid binding phase may be replaced with liquid binding phase that contains nanomaterial suspension. These nanomaterials offer high specific surface area and abundant binding sites, and thus greatly enlarge the working capacity of DGT (i.e., detectable concentration range of mercury). With proper surface functionalization of carbon-based nanomaterials (Wu et al., 2017) or facet-engineering of metal-based nanomaterials (Yuan et al., 2017), the selectivity of the binding phase may be substantially enhanced, which is key to successful DGT applications in complex environmental matrices.

## 4. Concluding remarks

Even though the mechanistic understanding of microbial mercury methylation continued to grow in recent years, accurately predicting the "hotspots" of MeHg contamination in natural aquatic environment remains a great challenge, particularly considering the changing natural conditions and human activities that strongly influence the methylating microbial communities and mercury speciation. Overcoming the knowledge gaps regarding the specific pathways of mercury uptake and methylation along with specific forms of bioavailable mercury will undoubtedly lead to better understanding of the current variation in MeHg abundance among ecosystems, and enable predictions of future changes in MeHg abundance in response to local and global perturbations, ranging from environmental remediation, altered landscape to climate change. Therefore, future research efforts ought to focus on improving the mechanistic understanding of both microbial and geochemical factors that constrain environmental MeHg production.

It is not common to observe a significant correlation between the gene abundance or expression and MeHg production, though the *hgcAB* genes are greatly helpful as biomarkers for monitoring mercury methylation in natural and engineered systems. The prevalence and activities of mercury methylating microbes in the environment are modulated by many biotic and abiotic variables. For example, the co-occurrence between mercury methylators and non-methylators may dictate the capacity of mercury methylation by complex microbial communities, and thus the non-methylating taxa may also be useful indicators for predicting MeHg formation and accumulation (Liu et al., 2019). The changing environmental parameters, such as climatic conditions and geochemical indices, are also important regulators of the mercury methylating communities. New technologies such as RNA–based stable isotope probing combined metatranscriptomics allow us to identify specific metabolic processes of a target microbial group (Dumont et al., 2013; Moran et al., 2013), which is essential information for untangling the mechanisms of mercury methylation in complex environmental settings. Furthermore, statistical models, such as random forest and structural equation models (Chen et al., 2019; Delgado-Baquerizo et al., 2018; Jing et al., 2015; Wang et al., 2019a), are powerful tools for processing large-omics datasets to establish quantitative links between biomarkers and MeHg production.

Future research on developing and improving methods for assessing the bioavailability and methylation potential of mercury need to consider two important lines of evidence discovered in recent studies. First, kinetic constrains likely play a major role in controlling the bioavailability of mercury (Chiasson-Gould et al., 2014; Jiskra et al., 2014; Jonsson et al., 2012), which, as a result, needs to be investigated under non-equilibrium conditions of transformation reactions among different species of inorganic mercury. This is particularly important for understanding the "aging effect" of mercury bioavailability (Chadwick et al., 2013; Harris et al., 2007; Hintelmann et al., 2002; Pham et al., 2014; Zhang et al., 2012) and assessing the contribution of newly input as well as legacy mercury to in situ MeHg production. Second, ligand exchange between aqueous mercury species and specific transporters on the surfaces of methylating microorganisms may be a determining factor of mercury uptake and bioavailability (Liu et al., 2016b; Schaefer et al., 2014b; Thomas et al., 2014), and thus methods are needed to interrogate mercury speciation and binding configuration at the water-cell-mineral interfaces to supplement aqueous mercury speciation analysis. Ultimately, advanced methods with sufficient time and spatial resolution are desired to elucidate the dynamic changes of mercury speciation and bioavailability during interfacial interactions with methylating microorganisms.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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