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Research paper

Long-term industrial metal contamination unexpectedly shaped diversity and activity response of sediment microbiome

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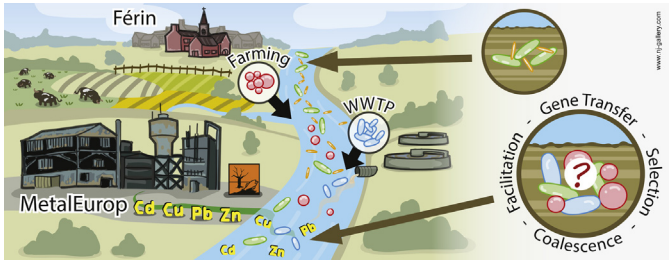
HIGHLIGHTS

- Combined DNA/RNA sequencing and FRGs accurately predicted microbial lifestyles.
- Metal pollution in sediment resulted in unexpected higher microbial diversity.
- Community coalescence, HGT and microbial facilitation explained this higher diversity.

GRAPHICAL ABSTRACT



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ABSTRACT

Metal contamination poses serious biotoxicity and bioaccumulation issues, affecting both abiotic conditions and biological activity in ecosystem trophic levels, especially sediments. The MetalEurop foundry released metals directly into the French river "la Deûle" during a century, contaminating sediments with a 30-fold increase compared to upstream unpolluted areas (Férin, Sensée canal). Previous metaproteogenomic work revealed phylogenetically analogous, but functionally different microbial communities between the two locations. However, their potential activity status *in situ* remains unknown. The present study respectively compares the structures of both total and active fractions of sediment prokaryotic microbiomes by coupling DNA and RNA-based sequencing approaches at the polluted MetalEurop site and its upstream control. We applied the innovative ecological concept of Functional Response Groups (FRGs) to decipher the adaptive tolerance range of the communities through characterization of microbial lifestyles and strategists. The complementing use of DNA and RNA sequencing revealed indications that metals selected for mechanisms such as microbial facilitation *via* "public-good" providing bacteria, Horizontal Gene Transfer (HGT) and community coalescence, overall resulting in an unexpected higher microbial diversity at the polluted site.

1. Introduction

Although known to naturally occur due to particular geological context, metal contamination of soils and sediments often origi-

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nates from anthropogenic activities such as mining activities [1,2], wood processing [3], shipping, dredging [4], urbanization [5] and industrial processes [6,7]. These metals constitute a serious risk because of their biotoxicity and bioaccumulation in the environment [8].

The peculiar characteristics of sedimentary environments, such as redox potential, pH, organic matter as well as biological activity, turn them into natural accumulation hotspots adsorbing and precipitating up to 90% of soluble metals and metalloids from water compartments [2,9]. Fresh water sediments are hosting thousands of microbial species [10], seating at the bottom of the trophic levels and often actively involved in metal movements in the biosphere [11]. Therefore, many studies have analyzed the link between metal accumulation and microbial communities in sediments. Investigating sediment microbiomes represents a good opportunity to understand resistance/tolerance adaptation and molecular mechanisms involved, with important applications in the field of bioremediation and biostimulation [12]. Metal-contaminated sediment bacteria highlighted by previous studies were mainly affiliated to Proteobacteria [1,7,13,14], Bacteroidetes [13,14], Firmicutes [12,13] and Actinobacteria [5,7]. Beta- and Gammaproteobacteria are the essential Proteobacterial classes, including respectively microbial members from Burkholderiales [2,7,13,14] and Pseudomonadales/Xanthomonadales [5,7,12–14].

Total microbial biomass, activity and phylogeny are the most studied traits when investigating the response of environmental microorganisms to metals. For instance, Gillan et al. [7] found no changes in biomass and activity in an 80-years metal contaminated fjord, but revealed community structure variations *via* DGGE fingerprints, implying long-term adaptation and functional recovery over time [15]. These observations were also reported for other systems, including river-connected lakes in the Rouyn-Noranda region, Canada [16], as well as long-term copper polluted grasslands, for which the microbial community composition was altered at DNA level with no consequences on species richness [17]. Conversely, Nayar and colleagues used mesocosms to show the negative impact of metals on crucial ecosystem components, such as primary producers (*e.g.* phytoplankton and autotrophic bacterial activity) [4]. They also pointed out that heterotrophic bacteria seem to be less affected by metals as a short-term stress [4]. Conversely, some recent studies have revealed strong negative impact of metals on microbial diversity in terms of richness and evenness [1,3,5]. These contrasting observations imply that other factors are involved in structuring the microbial response to metals. These might include site-specific physicochemical differences (*e.g.* pH, organic matter…), sediment sampling depth and associated metal bioavailability (*e.g.* anoxic gradient, ecosystem temporal dynamics…), the nature of social interactions between microbiome members (*e.g.* facilitation, exclusion, priority, biofilms, keystone species…) and genetic modalities of metal resistance/tolerance (chromosomes and/or plasmids) [18]. In addition, differences between molecular markers have been reported. For instance, Berg et al. found no richness loss after long-term copper pollution in grassland soil at the DNA level [17], while a recent study on the same site reported a clear diversity loss in the potentially active microbial fraction at the RNA level [3], both based on 16S rRNA amplicon sequencing. This suggests that some community members might display low activity profiles, explaining the maintained DNA diversity levels unlike RNA. This also implies that genetic diversity may not be lost due to metal pollution per se, but will still be present under latent state, suggesting potential reactivation of dormant ecosystem functions in case of disturbance removal. Overall, these contrasting observations call for better alternatives to investigate and understand the ecology and modalities of microbial adaptation to metals.

In the present study, we have revisited the sediments from the DeÛle river sites in northern France, previously investigated by a metaproteogenomic approach [7]. Sediments were exposed to long-term metal releases from the industrial site of MetalEurop, a former foundry operating from 1893 to 2003 in Noyelles-Godault [19]. Metal concentrations in these sediments are currently up to 30-fold higher compared to control upstream locations at the Sensée canal in Férin. Sediments are mainly contaminated with cadmium, copper, lead and zinc that respectively reach 38.1, 100, 913.8 and 3218.5 mg/kg (Table S1) [6,7,19]. Gillan et al. showed by shotgun metagenomics that microbiomes from Férin (FER) and MetalEurop (MET) were phylogenetically analogous but functionally different [7]. In this study, we aimed to investigate the sediment prokaryotic communities with a refined complementing approach using both DNA and RNA (cDNA) molecular levels by means of high throughput sequencing of the 16S rRNA gene. We hypothesized that the long-term pollution has impacted the prokaryote diversity and selected for different microbial strategies and lifestyles. We applied the ecological concept of functional response groups (FRGs), which aims to classify the response of microorganisms "as a function of" environmental parameters [3,20,21]. FRGs should be clearly differentiated from Functional Effect Groups (aka guilds), which are groups of organisms contributing to the same ecosystem function (*e.g.* nitrogen cycling or cellulose degradation). Defining response groups based on RNA/DNA abundance patterns in relation to environmental variables and without any phylogenetic a priori is a powerful method in ecology for detecting niches occupied by specific microbial strategists [3,20]. Although communities are sharing similarities as previously reported, the resolution of our analysis allowed identification of six microbial response groups with specific DNA and RNA molecular signatures linked to metal sensitivity/tolerance after long-term exposure. Our study adds a decisive and innovative contribution to the current knowledge regarding microbial adaptation to metals in sediments, with regards to the contrasting results often reported in the literature.

2. Materials and methods

2.1. Sampling, DNA and RNA extraction, cDNA synthesis

Sediments were sampled in May 2016 from the Sensée Canal and the DeÛle river sediment in Férin (FER) and Noyelles-Godault next to MetalEurop (MET) in France, respectively. Three sediment cores were collected at each station and two samples of 2 g were taken from the upper part of each core, representing a total of 12 samples (3 cores × 2 samples at FER and MET, respectively, Table S2). Samples were stored in Life-Guard RNA blocking solution (Mobio) at 4 °C during transport and −20 °C in the laboratory. For DNA/RNA extraction, 6 × 2 g of sediments per station were washed using the Fortin et al. (2004) procedure in order to remove potential PCR inhibitors [22]. From the 2 mL re-suspended and washed sediment, 500 μL were used for total DNA extraction (FastDNA® SPIN Kit for Soil, MP Biomedicals, Santa Ana, CA, USA) and 800 μL were used for total RNA extraction (FastRNA™ Pro Soil-Direct Kit, MP Biomedicals, Santa Ana, CA, USA). DNA was removed from the RNA solution with a DNaseI treatment using the Ambion® DNA-free™ DNase Treatment and Removal Reagents kit (ThermoFisher Scientific, Waltham, MA, USA). cDNA synthesis was performed using 10 ng of DNaseI treated RNA as template with Random Hexamer primers (Sigma, St. Louis, MO, USA) using the Roche Expand™ Reverse Transcriptase kit (Roche, Basel, Switzerland), according to manufacturer's instruction. Generated cDNA samples were stored at −20 °C until further processing.