Review of manuscript bg-2018-444
Mineral formation induced by cable bacteria performing long-distance electron transport in marine sediments.

General comment:
This manuscript entitled "Mineral formation induced by cable bacteria performing long-distance electron transport in marine sediments" investigates the nature of minerals associated with cable bacteria from different marine settings. Microscopy and spectroscopy analyses reveal (1) intracellular poly-phosphate accumulations in "naked" bacteria, (2) association of cells with extracellular clay minerals, most probably mediated by EPS and (3) different levels of cellular encrustation by Fe-oxyhydroxides adsorbing phosphate.
The methodology is of quality, and the results are well discussed, without any excessive claim. These results are of high importance, as no previous study had been devoted to the analysis of minerals associated with these increasingly studied cable bacteria. They open wide and important issues to be explored. The manuscript is very well written, clearly organized and figures are of high quality. I recommend the publication of this manuscript in Biogeoscience, and have only minor comments.

Specific comments:

- In the introduction, you mention the fact that BIM leads to the formation of minerals that are indistinguishable of abiotic counterparts (l. 88-90). I would temper this claim, as actually, the simple fact that bacteria provide nucleation templates for mineral precipitation can lead to specific mineral textures (e.g. Mirvaux et al 2016) or even initiate mineral precipitation under conditions where this precipitation is kinetically hindered.
- I suggest to remove words like "unintended" (l. 86) or "inadvertent" (l. 538) that are not appropriate for the study of bacteria.
- I suggest not to refer to poly-phosphate as a "mineral".
- I am not sure (unless for the study of iron encrustation) which conditions the samples were exposed to (oxic vs. anoxic). This deserves to be indicated as you prepared samples for microscopy through rinsing in non-degassed (i.e. oxic) water. In case some samples were intially under anoxic conditions, this method would likely induce the formation of secondary minerals (e.g. Fe oxy-hydroxides). Please elucidate this point. In complement to this remark, I suggest the following modifications/discussions to be included:
    - Add the site of collection and depth/condition (oxic vs. anoxic) in each figure legend.
    - I wonder whether you observed different proportions of polyphosphates in samples collected in oxic vs. anoxic zones? This would be interesting to discuss if you have these informations, as part of a potential contribution of cable bacteria to the phosphorus cycle.
- Discussion:
    - Around l. 420: regarding the possibility to evaluate the similarity between poly-P granules and acidocalcisomes, you could also mention the use of fluorescent pH-probes as a potential method.
    - l. 467: regarding acidic micro-environments, you could instead or in addition cite:(Hegler *et al.*, 2010).

- L. 474 and l. 543: For the discussion about the impact of encrustation on metabolic activity, (Miot *et al.*, 2015) have quantified the impact of the level of Fe(III)-mineral encrustation on the uptake or organic molecules (acetate) in Fe(II)-oxidizing bacteria (BoFeN1). This should be discussed here. As an additional strategy to avoid cell encrustation (at the population level), you can thus mention as well the co-existence of bacteria at different levels of encrustation with the naked bacteria. Indeed, you mention that you systematically observe non-encrusted cells, which is consistent with observations by Miot et al (2015) with Fe(II)-oxidizing bacteria.
- L. 502-503: the nature of the minerals could be indeed evaluated by STXM, but also by TEM.
- The FIB-SEM images/videos are very impressive! However, you should mention somewhere in the discussion the potential artifacts that may be induced by the preparation (resin embedding) and analysis of the sample (e.g.(Dohnalkova *et al.*, 2011; Miot *et al.*, 2011; Bassim *et al.*, 2012). Cryo-methods could very interstingly complement your observations.
- You could summarize more clearly the potential role of cable bacteria in Fe-mineral formation: (1) their surface can provide a nucleation site for mineral precipitation, (2) their metabolism (in particular O_2 respiration in the oxic zone) may locally increase the pH (local pH gradient around the cells), which would be favorable to Fe-mineral precipitation and growth. The nano-sized globules observed on some cells could correspond to early stages of cell encrustation.

Minor corrections:

- Replace the wording “microscopic techniques” by “microscopy techniques” (e.g. l. 21, 103)
- Material and methods (l. 215 and next): indicate the modes of SEM imaging (backscattered vs secondary electron mode). This should be mentioned in figure legends as well if different modes have been applied.
- Fig. 4: mistake in the legend. Add (i): P and S …
- L. 400-401: “The storage of P […] the compounds”. I do not understand this sentence.

Bassim ND, De Gregorio BT, Kilcoyne ALD, Scott K, Chou T, Wirick S, Cody G, Stroud RM (2012) Minimizing damage during FIB sample preparation of soft materials: FIB SAMPLE PREPARATION OF SOFT MATERIALS. *Journal of Microscopy* **245**, 288–301.

Dohnalkova AC, Marshall MJ, Arey BW, Williams KH, Buck EC, Fredrickson JK (2011) Imaging Hydrated Microbial Extracellular Polymers: Comparative Analysis by Electron Microscopy. *Applied and Environmental Microbiology* **77**, 1254–1262.

Hegler F, Schmidt C, Schwarz H, Kappler A (2010) Does a low-pH microenvironment around phototrophic FeII-oxidizing bacteria prevent cell encrustation by FeIII

minerals?: Low-pH microenvironment prevents cell encrustation. *FEMS Microbiology Ecology* **74**, 592–600.

Miot J, Maclellan K, Benzerara K, Boisset N (2011) Preservation of protein globules and peptidoglycan in the mineralized cell wall of nitrate-reducing, iron(II)-oxidizing bacteria: a cryo-electron microscopy study: Persistence of organics in mineralized Fe-oxidizing bacteria. *Geobiology* **9**, 459–470.

Miot J, Remusat L, Duprat E, Gonzalez A, Pont S, Poinsot M (2015) Fe biomineralization mirrors individual metabolic activity in a nitrate-dependent Fe(II)-oxidizer. *Frontiers in Microbiology* **6**.