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Biogeosciences Discussions

# *Interactive comment on* "Iron minerals inhibit the growth of bacteria via a free-radical mechanism: Implications for soil carbon storage" *by* Hai-Yan Du et al.

**Anonymous Referee #1**

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General Comments: This study is a nice contribution to the analysis of mineral-microbe interactions in soils, and it presents some new evidence for the role of iron in diminishing certain bacterial populations. Of particular interest to me was the X-ray photoelectron spectroscopy data demonstrating the presence of Fe(II) on the ferrihydrite surface, suggesting that the microbe (J12) reduces mineral bound Fe(III). This is important, because Fe(II) is then available for Fenton reactions that can produce radicals that damage cell membranes allowing soluble metals to enter the bacterial cell. The work suggests that iron bearing minerals in soils contribute to the preservation of organic C by limiting the productivity of bacteria that degrade carbon.



Specific Comments: Line 108. Your description of the clay minerals used is not adequate for determining the chemical composition and stability of the phyllosilicates. In particular montmorillonite has many chemical components in each crystallographic site that contribute to the mineral surface characteristics (charge distribution) and interlayer cations. Therefore, one must give the chemical formula for the mineral used. One montmorillonite might increase bacterial growth (if it provides nutrients) while another might decrease bacterial growth (if it provides toxins).

Line 128. How were the concentrations of minerals (5, 10, 25 mg/ml) decided? Did you measure the minimum inhibitory and minimum bactericidal concentrations for the J12 bacteria under the pH conditions of the experiment? Were the minerals only hydrated by the growth media? If so, what is the speciation of soluble metals with the components of the media solution?

Line 135. It is unclear why the particle size distribution is presented before and after incubation with bacteria. The increase in particle size after incubation (which would be better demonstrated in a graph than a table) probably results from agglomeration of mineral-bacteria clusters rather than a crystal growth. Is this important to the conclusions? If anything, a measurement of specific surface area of the minerals would be more important to the chemical interactions.

Line 209. For chemical analysis you have filtered the mineral-microbe suspension through 0.45 $\mu$m membrane, which may remove the bacteria, but allows clay size particles through. This is then analyzed by ICP and results reported as 'soluble' Al and Fe. However, the clay particles in this fraction will contribute to the elemental analysis.

Line 225. This analysis presumes that OD600 only reflects absorbance by bacteria, but what is the absorbance of the mineral suspension alone?

Line 245. Mn(II), being redox active, is more likely to produce hydroxyl radical than to scavenge (Zarate-Reyes et al., 2017 Appl Clay Sci; Shi et al, 2016 Nature Sci Rev.)

Line 267. Replace expect with except

Line 270. The production of acids by bacteria is plausible, but the surface area of the minerals is far greater (in general) than that of the bacteria, thus you might consider that each mineral surface attracts or repels H+ and OH- which is the major factor in buffering the fluid pH. You should test this by monitoring the pH (and Eh) of the suspension of minerals alone, compared to the suspension of bacteria alone.

Line 289. This requires H2O2, what is the source of that in the mineral-microbe suspension?

Line 295. Al3+ is not redox active, so why (or how) could it be correlated with production of hydroxyl radical? The toxicity of Al is from interactions with phospholipids, not production of radicals.

Line 305. This analysis is confusing to me. Did you add all of the minerals to this bacterial suspension and you are looking for which mineral dominates?

Line 320. This suggests that the bacteria reduce the mineral Fe, which in turn produces radicals that oxidize the bacteria. Why would bacteria not have a defense against such radicals?

Line 341. A simple measurement of the OD600 on a mineral suspension should answer this definitively.

Line 345. The inhibitory concentration of metals is pH dependent, so unless the work of Illmer and Schinner was at the same pH of your experiment (after 12 hrs) then this reported concentration may not be relevant. You need to determine the MIC and MBC concentrations at the pH of your mineral-microbe mixture (after incubation).

Line 385. The phyllosilicates generally have a negative charge on their more extensive basal surfaces. Positive charges are limited to broken edges of the structure. This may not be true for ferrihydrite and goethite. But remember that hydroxyl radicals only exist for 1ns, so for them to interact with bacteria, there must be an attractions between the



microbe and mineral surface where the radical is generated.

Line 403. It is unclear what you mean by 'stabilization role' here. What does the EPS do to stabilize the ferrihydrite?

Line 412. This is an important result of this study!

Line 434. Do you suggest that the H2O2 required is generated by the bacteria or by dissolved O2 in solutions? Have you monitored the Eh of the solutions?

Supplementary Figures Figure S6. How do you explain the drop in pH from 7.2 even in the control suspension? Figure S8. Why does the control have soluble Al even though there are no minerals in it?

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