Biogeosciences Discuss.,
https://doi.org/10.5194/bg-2018-479-AC2, 2019

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.

Hai-Yan Du et al.

yuguanghui@njau.edu.cn

Received and published: 16 February 2019

Reply to the Comments Anonymous Referee #2 The authors appreciate the report of Referee #2 and respond as follows. I. General comments: This study aimed to study (i) the impact of Al- and Fe-containing minerals (montmorillonite, kaolinite, hematite, goethite and ferrihydrite) on bacterial growth using cultural approach on Pseudomonas brassicacearum J12 and (ii) the involvement of ROS, produced via fenton reactions, on Pseudomonas brassicacearumJ12 growth. The subject is clearly interesting and is in accordance with researches published in Biogeosciences journal. Such researches on interactions between biotic and abiotic compartments are essential for our under-

standing of nutrients fluxes in soils and I encourage the publication of this manuscript in Biogeosciences journal. However, some points need to be clarified before publication. II. Major comments: - Major comment 1: Title: "Iron minerals inhibit the growth of bacteria via a free-radical mechanism: Implication for soil carbon storage": You cannot generalize your results to the domain of bacteria. I recognize that we will never be satisfied enough with the number of species studied, but I think that before expanding your results to the domain of bacteria, you should confirm them on other species from different phylum which show important genetic and phenotypic distances. Response (R): Thanks! In the revised manuscript, we revised "bacteria" to "Pseudomonas brassicacearum J12" in the Title. Diaz et al. (2013) showed that other species, e.g., Pseudomonas putida GB-1, could produce approximately 1 and 10 amol O2âĂćïij■ cell-1 h-1 during mid-exponential growth or stationary phase, respectively. Except for Pseudomonas, taxonomically and ecologically diverse heterotrophic bacteria from both aquatic and terrestrial environments were a vast source of superoxide (O2âĂćïij■) and H2O2 (Diaz et al., 2013). Based on the suggestion of Referee #2 and the results from Diaz et al. (2013), we think that "iron minerals inhibit the growth of heterotrophic bacteria via a free-radical mechanism" should be no problem. The revised part was colored in red in the revised manuscript and also listed as follows. "Iron minerals inhibit the growth of bacteria via a free-radical mechanism: Implications for soil carbon storage" (Page 1, Lines 1-2 in the original manuscript) was changed to "Iron minerals inhibit the growth of Pseudomonas brassicacearum J12 via a free-radical mechanism: Implications for soil carbon storage" (Page 1, Lines 1-3 in the revised manuscript) The reference is listed as follows: Diaz, J. M., Hansel, C. M., Voelker, B. M., Mendes, C. M., Andeer, P. F., and Zhang, T.: Widespread production of extracellular superoxide by heterotrophic bacteria, Science, 340, 1223-1226, 2013. - Major comment 2: Integrate your statistical results in the description of the results and in your figures. R: Good comments! In the revised manuscript, we added the statistical results in both the description of the results and figures. The revised parts were colored in red in the revised manuscript and also seen in response to specific comments below. Spe-

cific Comments: Abstract - “Together, these findings indicate that the reduced surface Fe(II) derived from Fe(III)- containing minerals inhibit bacteria via a free-radical mechanism, which may further contribute to soil carbon storage.” : see Major comment 1. Free-radicals may lead to organic matter degradation-mineralization, you do not develop this idea in the manuscript. R: In the revised manuscript, we changed "bacteria" to "J12" and then deleted "which may further contribute to soil carbon storage". The revised part was colored in red in the revised manuscript and also listed as follows. "Together, these findings indicate that the reduced surface Fe(II) derived from Fe(III)-containing minerals inhibit bacteria via a free-radical mechanism, which may further contribute to soil carbon storage." (Page 2, Lines 33-35 in the original manuscript) was changed to “Together, these findings indicate that the reduced surface Fe(II) derived from Fe(III)-containing minerals inhibit the growth of Pseudomonas brassicacearum J12 via a free-radical mechanism, which may serve as an ubiquitous mechanism between iron minerals and all of the heterotrophic bacteria in view of taxonomically and ecologically diverse heterotrophic bacteria from terrestrial environments as a vast source of superoxide.” (Page 2, Lines 35-40 in the revised manuscript) Introduction - This is a clear introduction which provide a good representation to the overall situation. R: Thanks! - “The bacterial inhibition property of a mineral is associated with the particular chemistry and with the mineral properties, resulting in the various bacterial inhibition mechanisms of minerals” [l. 43-45]: can you please give more precisions on the various inhibition mechanisms? R: Yes! In the revised manuscript, the various inhibition mechanisms were added. The revised part was colored in red in the revised manuscript and also listed as follows. “The bacterial inhibition property of a mineral is associated with the particular chemistry and with the mineral properties, resulting in the various bacterial inhibition mechanisms of minerals” (Page 3, Lines 42-45 in the original manuscript) was changed to “The bacterial inhibition property of a mineral is associated with the particular chemistry and with the mineral properties, resulting in the various bacterial inhibition mechanisms of minerals such as increase of membrane permeability and oxidative damage.” (Page 3, Lines 52-55 in the revised manuscript)

- Please, name the Al- and the Fe-contaning minerals that you used in this study, it is hard to understand for non-chemists to which category belong kaolinite, montmorillonite, hematite, goethite, ferrihydrite. R: Good suggestion. In the revised manuscript, we added the explanation of the Al- and the Fe-contaning minerals. The added part was colored in red in the revised manuscript and also listed as follows. “Specifically, montmorillonite and kaolinite are Al(III)-containing minerals, while hematite, goethite and ferrihydrite belong to Fe(III)-containing minerals.” (Page 5, Lines 104-105 in the revised manuscript) Material and Methods - I suggest to separate the paragraphe 2.1 into two parts: “2.1 Mineral preparation” [l.106], “2.2 Pseudomanas cultivation experiments” [l.121] R: In the revised manuscript, we separated the paragraph 2.1 into two parts: “2.1 Mineral preparation”, “2.2 Pseudomanas cultivation experiments” (Page 6, Lines 126-141 in the revised manuscript) - Suppress “which is a major group of rhizobacteria that aggressively colonize plant roots, has been considered an important group for sustainable agriculture” [l.122-123]: the information is already given [l.90]. R: Agree! In the revised manuscript, this sentence “which is a major group of rhizobacteria that aggressively colonize plant roots, has been considered an important group for sustainable agriculture” was deleted (Page 6, Line 142 in the revised manuscript). - Why didn’t you chose to have a control [NB + Mineral]? The OD of this control can be subtracted from the OD measured in [NB + Mineral + Bacteria] and give you the OD of your bacteria without the disturbance induced by the mineral? R: Good comment! In this study, we removed the effect of the OD of mineral on the Pseudomanas cultivation experiments based on the protocol of McMahon et al. (2016). In the future, we would like to compare the suggested method by Referee #2 to the protocol of McMahon et al. (2016). The mentioned reference is listed as follows. McMahon, S., Anderson, R. P., Saupe, E. E., and Briggs, D. E. G.: Experimental evidence that clay inhibits bacterial decomposers: Implications for preservation of organic fossils, Geology, 44, 867-870, 2016. - Did you measure the kinetic of bacterial growth during the 12 h? Are you sure that the bacteria is still in exponential phase of growth? Why did you chose 12 h for the first incubation and 8 h for the second one? R: Yes, we had measured the kinetic

of bacterial growth and shown as follows. Therefore, we confirm that the bacteria are still in exponential phase of growth from the figure. The protocol we used, i.e., 12 h for the first incubation and 8 h for the second one, is based on the results from McMahon et al. (2016), which is listed in the response of the above question.

Fig. The kinetic of bacterial growth within 15 h (n = 6). - pH measurement should be explain in “2.6 Chemical analysis” R: Thanks! In the revised manuscript, we added the pH measurement in the section of “2.7 Chemical analysis” and also shown as follows. “The pH of Pseudomonas brassicacearum J12 cultivated with different minerals or without mineral (control) was detected after 12 h.” (Page 11, Lines 257-258 in the revised manuscript) - I do not understand the choice of an ANOVA, when did you used that test. R: One-way ANOVA is a technique that can be used to compare means of two or more samples (using the F distribution). Typically, the one-way ANOVA is used to test for differences among at least three groups. When the conditions of normality and homogeneity of variance were met, we considered use this test. In this study, one-sample Kolmogorov-Smirnov Test was used to analyze the distribution of data. - Figure 1, 3, S5, S6, S7, S8 should integrate your statistical analysis. R: Thanks! We integrated the statistical analysis in the revised manuscript. The revised figures can be seen in the revised manuscript and not listed here for brevity. - Explain/describe the “one-sample Kolmogorov-Smirnov Test” R: The one sample Kolmogorov-Smirnov test is used to test whether a sample comes from a specific distribution. In this study we used this procedure to determine whether the data set was normally distributed. The added part was colored in red in the revised manuscript and also listed as follows. “The one sample Kolmogorov-Smirnov test is used to test whether a sample comes from a specific distribution. In this study we used this procedure to determine whether the data set was normally distributed.” (Page 11, Lines 266-268 in the revised manuscript) - Which software did you use to find and represent the model that best fits with your data (Fig.4)? R: In this study, we used SPSS 18.0 to find and represent the model that best fits with our data in Fig.4. Results - In this part you should not interpret your results: [l.230-231], [l.244-247], [l.294-295], [l.268-269], [l.280-281], [l.313], [l.318-319],

[l.327-328]. R: Agree! In the revised manuscript, we deleted these parts that interpret the results. The revised figures could be seen in the revised manuscript and not listed here for brevity. - Here, we are waiting for an exhaustive description of the results obtained during the study: give some values (mean $\pm$ SE) and precise when values are significantly (or not) different between the different conditions tested. R: Great comment! We integrated the description of the results and the statistical analysis in the revised manuscript. The revised parts could be seen in the revised manuscript and not listed here for brevity. Paragraph [l.224-234]: - “3.1. Bacterial inhibition by minerals” [l.223]: This title does not correspond in case of montmorillonite. I suggest something like: “Effect of mineral nature and their concentrations on P. brassicacearum J12 development”. R: Good suggestion! The revised part was colored in the revised manuscript and also listed as follows. "3.1. Effect of mineral nature and their concentrations on J12 development " (Page 12, Lines 275 in the revised manuscript). - Suppress “The effects of the nature and content of tested minerals on the OD 600 of Pseudomonas brassicacearum J12 subcultures taken after 12 h growth are shown in Fig. 1.”: it should be in the “Material and Methods” part. R: Agree! In the revised manuscript, we deleted this sentence. (Page 12, Lines 276 in the revised manuscript) - “Compared to Control (i.e., no minerals), the presence of montmorillonite significantly increased OD 600.”: give values. R: We added the values in the revised manuscript. The revised part was colored in the revised manuscript and also listed as follows. "Compared to Control (0.34 $\pm$ 0.01), the presence of montmorillonite significantly (pïijlJ0.05) increased OD600 (Fig. 1). Specifically, the OD600 values of samples were 0.43 $\pm$ 0.01, 0.44 $\pm$ 0.02 and 0.43 $\pm$ 0.01 at the concentration of 5, 10 and 25 mg mL-1, respectively." (Page 12, Lines 276-279 in the revised manuscript) - Suppress “On the other hand,” [l. 227] R: Done. - [l.227-230]: “Presence of all other investigated minerals decreased OD 600 in the following order: ferrihydrite > goethite > hematite > kaolinite at 5 and 25 mg mL-1, and ferrihydrite > goethite > kaolinite > hematite at 10 mg mL-1”: Please give some values. R: Done. The revised part was colored in the revised manuscript and also listed as follows. "Presence of all other investigated minerals decreased OD600 in the following

order: ferrihydrite (0.24 ± 0.04 and 0.09 ± 0.01) > goethite (0.26 ± 0.02 and 0.14 ± 0.00) > hematite (0.30 ± 0.03 and 0.16 ± 0.02) > kaolinite (0.32 ± 0.01 and 0.20 ± 0.01) at 5 and 25 mg mL-1, respectively, and ferrihydrite (0.16 ± 0.02) > goethite (0.18 ± 0.02) > kaolinite (0.21 ± 0.02) > hematite (0.28 ± 0.02) at 10 mg mL-1. An increase in mineral concentration resulted in a significant decrease in OD600." (Page 12, Lines 279-285 in the revised manuscript) - Suppress “Meanwhile” [l.232] R: Done. - “An increase in mineral concentration resulted in a significant decrease in OD 600, except for montmorillonite” [l. 232-233]: Give some values. R: We added the values and shown as follows. “Compared to Control (0.34 ± 0.01), the presence of montmorillonite significantly (pïijlJ0.05) increased OD600 (Fig. 1)” (Page 12, Lines 276-277 in the revised manuscript) “An increase in mineral concentration resulted in a significant (pïijlJ0.05) decrease in OD600. However, in presence of montmorillonite the OD600 is stable at about 0.43 for all the mineral concentration studied.” (Page 12, Lines 284-287 in the revised manuscript) - Suppress “as the OD 600 seemed to be independent of its concentration” [l.233-234]: it is an interpretation. You can replace it by something like: “However, in presence of montmorillonite the OD600 is stable at 0.43 ± SE for all the mineral concentration studied” R: Agree! We revised “as the OD 600 seemed to be independent of its concentration” and shown as follows. “except for montmorillonite, as the OD600 seemed to be independent of its concentration” (Page 12, Lines 233-234 in the original manuscript) was changed to “However, in presence of montmorillonite the OD600 is stable at about 0.43 for all the mineral concentration studied” (Page 12, Lines 285-286 in the revised manuscript) - Fig.1: we do not see bottom bar of the SE R: In the revised manuscript, we added the bottom bar of the SE. - Fig.1 text/description: you should mention the mineral concentrations. R: In the revised manuscript, we added the description of the mineral concentrations and shown as follows. “Gray, magenta and cyan represent the mineral concentration of 5, 10 and 25 mg mL-1, respectively.” (Page 35, Lines 1114-1116 in the revised manuscript) Paragraph [l.235-247]: - It represents a new idea: you should give it a title (e.g. “chemical structure of minerals”) R: Agree! In the revised manuscript, we added the title as “3.2. Chemical structure of minerals”.

(Page 12, Line 287 in the revised manuscript) - [l.235-247]: why didn’t you describe the EPR profiles of ferrihydrite, goethite and hematite? R: Electron paramagnetic resonance (EPR) spectroscopy is a method for studying materials with unpaired electrons. Therefore, it cannot be applied to examine paramagnetic substances, e.g. iron oxides. In this study, we cannot describe the EPR profiles of iron oxides. Paragraph [l.249-258]: - “A 12 h cultivation of Pseudomonas brassicacearum J12 in the presence of different minerals revealed that generation of HOâĂć radicals in the cases of montmorillonite, kaolinite and hematite was almost similar to the control (Fig. 3)”: “Almost”? You should precise if the difference are significant or not. To precise my comment, you should study the significance of the difference between the control and montmorillonite for the three concentrations, and kaolinite 25 mg mL-1. Moreover, I think that the difference is significant between (i) montmorillonite 25 mg mL-1 and kaolinite 25 mg mL-1 and (ii) montmorillonite 25 mg mL-1 and hematite 25 mg mL-1. R: Agree! In the revised manuscript, we revised this sentence and also listed as follows. " A 12 h cultivation of J12 in the presence of different minerals revealed that generation of HOâĂć radicals in the cases of montmorillonite, kaolinite and hematite was similar ($p > 0.05$) to the control at low concentration (i.e., 5 mg mL-1) but significant different ($p < 0.05$) at high concentration (i.e., 25 mg mL-1) (Fig. 3)." (Page 13, Lines 360-364 in the revised manuscript) - [l.255]: replace “rapidly” by significantly: there is no notion of time. R: Done. - Fig.3 text/description: you should mention the mineral concentrations R: Done. The revised parts were colored in red in the revised manuscript and also listed as follows. "Figure 3. Generation of hydroxyl radical (HOâĂć) after 12 h growth of Pseudomonas brassicacearum J12 with different minerals and with no minerals (control). Al-containing minerals: K, kaolinite; M, montmorillonite. Fe-containing minerals: H, hematite; G, goethite; F, ferrihydrite. C, Control (i.e., no mineral). Gray, magenta and cyan represent the mineral concentration of 5, 10 and 25 mg mL-1, respectively. Values are the mean ± SE (n = 3)." (Page 37, Lines 1125-1130 in the revised manuscript) "A 12 h cultivation of J12 in the presence of different minerals revealed that generation of HOâĂć radicals in the cases of montmorillonite, kaolinite and

hematite was similar ($p > 0.05$) to the control at low concentration (i.e., 5 mg mL-1) but significant different ($p < 0.05$) at high concentration (i.e., 25 mg mL-1) (Fig. 3). However, presence of goethite and ferrihydrite significantly increased the production of HOâĂć radicals, which increased with an increase in their concentration. Specifically, in ferrihydrite treatments, the concentration of HOâĂć was approximately 260 nM at 5 and 10 mg mL-1 but increased significantly to 450 nM at 25 mg mL-1. In addition, the generation of HOâĂć at early growth (i.e., 2 h) was only detected with ferrihydrite at both 10 and 25 mg mL-1 and with goethite at 25 mg mL-1 (Fig. S6)." (Page 13, Lines 360-369 in the revised manuscript) Paragraph [l.259-295]: - Globally, I encourage the authors to reorganize this part of the manuscript. You should describe all your results (Fig. 4) not only those which are consistent with your interpretation. Just for example: I observe a significant increase of soluble Fe in the treatment containing goethite with the increase of goethite concentration but this results is missing from the test. R: Thanks! In the revised manuscript, we had reorganized this part and also listed as follows. "To explore the factors affecting the generation of HOâĂć and the inhibition of J12, we examined iron chemistry and its correlation with HOâĂć and OD600 (Fig. 5). Much more soluble Fe at 12 h was released from Fe(III)-containing minerals (6.7-27, 21-36 and 41-107 mg L-1 for hematite, goethite and ferrihydrite, respectively) than from montmorillonite ($\sim$0.3 mg L-1), kaolinite ($\sim$0.6 mg L-1), and control ($\sim$0.4 mg L-1) (Fig. 5a). With the increase of concentration, soluble Fe significantly ($p < 0.05$) increased at both 2 h and 12 h for ferrihydrite, only at 12 h for goethite. As for hematite, significant ($p < 0.05$) increase was only observed from 5 to 10 mg L-1 at 12 h (Fig. S7). The solubility of Fe was closely related to redox potential and pH value (Fig. S8). Results showed that Eh of bacteria-mineral mixture after incubation was generally lower than the suspension of minerals alone (Table S5), suggesting that the redox potential was decreased by the interaction between mineral and J12. Furthermore, the solution pH was determined after 12 h growth of J12 with different minerals and with no minerals (control) (Fig. 4). The range of solution pH varied from 4 to 6 for all of the treatments, except for ferrihydrite treatment with a pH near 7. For all of the examined minerals, the

trends at 12 h were similar in the following order (total Fe and Fe(II)): ferrihydrite (760-3588 and 182-488 mg L-1) » goethite (48-127 and 31-94 mg L-1) > hematite (15-82 and 9-35 mg L-1) > montmorillonite (5-10 and 4-8 mg L-1), kaolinite (10-12 and 4-9 mg L-1) or control (7 and 6 mg L-1) (Fig. 5b-5c). A significant difference of total Fe in solutions containing 25 mg mL-1 ferrihydrite between 2 h and 12 h may be attributable to the aging of a portion of ferrihydrite to its more crystalline counterparts, as revealed by micro X-ray fluorescence (μ-XRF). The more crystalline counterparts could not be dissolved by the modified 1,10-phenanthroline method. Furthermore, a positive correlation exists between HOâĂć and soluble Fe content ($R = 0.92$, $t = -3.49$, $p = 0.003$) and Fe(II) ($R = 0.98$, $t = -4.28$, $p = 0.001$) (Fig. 5d and 5f, Table S2). However, a significant but negative correlation between OD600 and soluble Fe ($R = -0.57$, $t = 2.99$, $p = 0.009$), and Fe(II) ($R = -0.81$, $t = 2.23$, $p = 0.038$) was found (Fig. 5g and 5i). Moreover, the correlation between HOâĂć and Fe(III) ($R = 0.94$, $t = 1.38$, $p = 0.19$), and between OD600 and Fe(III) ($R = -0.80$, $t = 1.67$, $p = 0.116$) were not significant (Fig. 5e and 5h). To test whether the release of Fe(III) to solution inhibit the growth of J12 via a free-radical mechanism, we replaced Fe(III)-containing minerals by adding a series of concentrations of Fe(NO3)3, i.e., 0, 50 and 100 mg L-1, in the cultivation experiments with the final pH of 7.2. The results showed that addition of Fe(III) can inhibit the growth of J12 (25-50%) by producing an additional HOâĂć concentration of 15 nM (Fig. S9), supporting the role of Fe(III) ion from solution in the initialization of a free-radical reaction. In addition, the inhibition of soluble Fe on J12 was more important in the concentration of 100 mg L-1 than that of 50 mg L-1 while HOâĂć production still kept the same between those two concentrations (Fig. S9). The reason of this phenomenon may attributable to the intracellular oxidative damage of soluble Fe that penetrated into cells and triggering of intracellular ROS generation. In addition, we also examined soluble Al during the cultivation experiments (Fig. 6a) and found a high concentration of Al in the montmorillonite and kaolinite solutions. However, almost no correlation was found between soluble Al and HOâĂć ($R = -0.35$, $t = -3.36$, $p = 0.004$) and OD600 ($R = 0.30$, $t = 2.24$, $p = 0.041$) (Fig. 6b-6c)." (Pages 13-15, Lines 371-484

in the revised manuscript) - Given the importance of the pH in the results description, I think that this result may be integrated in Fig.4. R: Agree! In the revised manuscript, pH results was added as Fig.4. - [l. 261-263]: “Much more soluble Fe was released from Fe(III)-containing minerals than from montmorillonite, kaolinite, and control (Fig. 4a)”: Please give some values. R: Done. The revised part was colored in red in the revised manuscript and also listed as follows. "Much more soluble Fe at 12 h was released from Fe(III)-containing minerals (6.7-27, 21-36 and 41-107 mg L-1 for hematite, goethite and ferrihydrite, respectively) than from montmorillonite ($\sim$0.3 mg L-1), kaolinite ($\sim$0.6 mg L-1), and control ($\sim$0.4 mg L-1) (Fig. 5a). " (Pages 13-14, Lines 372-397 in the revised manuscript) - “The solubility of Fe is closely related to pH value.”: Are you sure about that? The pH of goethite solution is equivalent to the pH of kaolinite, montmorillonite, hematite and goethite but the solubility of Fe in solution containing hematite and goethite seems to be more important. You should draw the graph showing the correlation between pH and the soluble Fe. R: Yes. In the revised manuscript, we draw the graph showing the correlation between pH and the soluble Fe and listed as Fig. S8.

Figure S8. Correlation between pH and the soluble Fe. - Fig.4.b: You have a surprising result: the significant decrease of Total Fe in solutions containing 25 mg mL-1 ferrihydrite between 2 h and 12 h. How do you explain that? R: In this study, total Fe was determined by a modified 1,10-phenanthroline method (Amonette, 1998). This method dissolved Fe by HCl. Therefore, this method may not enough to extract all of Fe from crystalline Fe minerals (e.g. hematite and goethite) that were detected in ferrihydrite samples after 12 h cultivation (Fig. 7 and Table S3). Therefore, we inferred that the aging of a portion of ferrihydrite to its more crystalline counterparts may be the possible reason about the significant decrease of Total Fe in solutions containing 25 mg mL-1 ferrihydrite between 2 h and 12 h. In the revised manuscript, the corresponding explanation was added and also listed as follows. "A significant difference of total Fe in solutions containing 25 mg mL-1 ferrihydrite between 2 h and 12 h may be attributable to the aging of a portion of ferrihydrite to its more crystalline

counterparts, as revealed by μ-XRF, which could not be dissolved by the modified 1,10-phenanthroline method." (Page 14, Lines 411-414 in the revised manuscript) - “For all of the examined minerals, the trends of total Fe and Fe(II) were similar in the following order: ferrihydrite Âż goethite > hematite > montmorillonite $\approx$ kaolinite $\approx$ control (Fig. 4b-4c)”: Please give some values. What do you mean with Âż and $\approx$? Is there a relation with a statistical analysis? R: In the original manuscript, "Âż" represents the former being far greater than the latter, while "$\approx$" indicates the former being a close to the latter. In the revised manuscript, we added the values and shown as follows. “For all of the examined minerals, the trends of total Fe and Fe(II) were similar in the following order: ferrihydrite Âż goethite > hematite > montmorillonite $\approx$ kaolinite $\approx$ control (Fig. 4b-4c)” (Page 13, Lines 264-268 in the original manuscript) was changed to “For all of the examined minerals, the trends at 12 h were similar in the following order (total Fe and Fe(II)): ferrihydrite (760-3588 and 182-488 mg L-1) > goethite (48-127 and 31-94 mg L-1) > hematite (15-82 and 9-35 mg L-1) > montmorillonite (5-10 and 4-8 mg L-1), kaolinite (10-12 and 4-9 mg L-1) or control (7 and 6 mg L-1) (Fig. 5b-5c).” (Page 14, Lines 407-411 in the original manuscript) - [l.274-275] “Furthermore, a positive correlation exists between OD600 and soluble Fe content (R = 0.92, t = -3.49, p = 0.003) and Fe(II) (R = 0.98, t = -4.28, p =0.001) (Fig. 4d and 4f, Table S2).”: I think that you wanted to say “a positive correlation between Hydroxyl radical content and soluble Fe content”. R: Yes! In the revised manuscript, we changed OD600 to HOâĂć and listed as follows. “Furthermore, a positive correlation exists between OD600 and soluble Fe content (R = 0.92, t = -3.49, p = 0.003) and Fe(II) (R = 0.98, t = -4.28, p =0.001) (Fig. 4d and 4f, Table S2).” (Page 13, Lines 274-276 in the original manuscript) was changed to “Furthermore, a positive correlation exists between HOâĂć and soluble Fe content (R = 0.92, t = -3.49, p = 0.003) and Fe(II) (R = 0.98, t = -4.28, p =0.001) (Fig. 5d and 5f, Table S2).” (Page 14, Lines 415-417 in the revised manuscript) - The interpretation of “R” and “t” should appear in the Material and Methods. R: Thanks! In the revised manuscript, the interpretation of parameters was added and also listed as follows. "In the regression

equation, the parameters R and t represent coefficient of determination and t-test." (Pages 11-12, Lines 269-272 in the revised manuscript) - [l.277]: “R=-0.75” and “t= 2.27” do not correspond to the values in the Fig.4. R: Thanks! We revised the values and shown as follows. “However, a significant but negative correlation between OD600 and soluble Fe (R = -0.75, t = 2.99, p = 0.009), and Fe(II) (R = -0.81, t = 2.27, p = 0.038) was found (Fig. 4g and 4i).” (Page 13, Lines 276-279 in the original manuscript) was changed to “However, a significant but negative correlation between OD600 and soluble Fe (R = -0.57, t = 2.99, p = 0.009), and Fe(II) (R = -0.81, t = 2.23, p = 0.038) was found (Fig. 5g and 5i).” (Page 14, Lines 417-419 in the revised manuscript) - Fig.S7 should appear in Fig.4 R: The results of Fig.S7 were derived from an independent experiment, which was totally different from those of Fig. 4. Therefore, we did not combined Fig.S7 into Fig.4. - Fig.S7: Can you explain why the inhibition of Pseudomonas is more important in Fe(III) 100mg L-1 than in Fe(III) 50mg L-1 while hydroxyl radical production still the same between those two concentrations? Is that not the sign of the existence of another process involved in the Pseudomonas growth inhibition? I find this result very important, it should be considered in your discussion. R: In this study, HOâĂć trapped by TPA is mainly extracellular. Structural Fe(II), not soluble Fe2+, was responsible for extracellular HOâĂć production. However, the toxicity of soluble Fe may also contribute to the observed cell killing by penetrating into cells and triggering of intracellular ROS generation (Williams et al., 2011). The reason of this phenomenon may due to the intracellular oxidative damage of Fe. Consistent with the recent study (Wang et al., 2017), inhibition activity of Fe minerals is a result of followed two factors: (1) HOâĂć production extracellularly from structural Fe(II); (2) intracellularly from soluble Fe. In the revised manuscript, we added the related explanation about Fig. S7 and also listed as follows. “In addition, the inhibition of soluble Fe on J12 was more important in the concentration of 100 mg L-1 than that of 50 mg L-1 while HOâĂć production still kept the same between those two concentrations (Fig. S9). The reason of this phenomenon may attributable to the intracellular oxidative damage of soluble Fe that penetrated into cells and triggering

of intracellular ROS generation.” (Page 15, Lines 476-480 in the revised manuscript) Paragraph [l.296-328]: - For non-chemist, this part is difficult to understand. Maybe the next comments will allow you to make it more accessible for biologists. R: Thanks! We try to explain it more accessible. - Fig.5.a: What do the colors mean? R: Colors in Fig.5a represent the different density of Fe in the selected area. Red color represents high density of Fe, followed by orange, yellow, green, little green, and purple. In the revised manuscript, we added the explanation of color in the caption of Fig. 5. The added part was colored in red in the revised manuscript and also listed as follows. "Figure 5. Correlative micro X-ray fluorescence (μ-XRF) and synchrotron-based Fourier transform infrared (SR-FTIR) analysis of the thin section from the cultures of the 25 mg/mL ferrihydrite treatment after 12 h cultivation. (a) μ-XRF map. (b) The LCF fitting of μ-X-ray absorption near-edge structure (XANES) analysis the selected regions of interest (ROI) region (i.e., A and B). (c) SR-FTIR maps. The color scale in (c) is a relative scale for each peak height and does not allow quantitative comparisons between peaks." (Page 37, Lines 689-695 in the original manuscript) was changed to "Figure 7. Correlative micro X-ray fluorescence (μ-XRF) and synchrotron-based Fourier transform infrared (SR-FTIR) analysis of the thin section from the cultures of the 25 mg/mL ferrihydrite treatment after 12 h cultivation. (a) μ-XRF map. (b) The LCF fitting of μ-X-ray absorption near-edge structure (XANES) analysis the selected regions of interest (ROI) region (i.e., A and B). (c) SR-FTIR maps. Red color in (a) represents high density of Fe, followed by orange, yellow, green, little green, and purple. Red color in (c) indicates the highest intensity of functional groups, followed by yellow, green, and blue. The color scale in (c) is a relative scale for each peak height and does not allow quantitative comparisons between peaks." (Page 41, Lines 1158-1166 in the revised manuscript) - Why did you select those regions of the spectra for XANES? R: Two spots represent the internal and external of the selected particles, respectively. We want to observe the changes of Fe species from outside to inside, thus the spots were selected for XANES analysis. - Fig.5.c: What do the colors mean? R: Blue represents the background (i.e., the intensity nears zero), while red color

represents the highest intensity of functional groups, followed by yellow and green. In the revised manuscript, we added the explanation of color in the caption of Fig. 5-c. The added part was colored in red in the revised manuscript and also listed as follows. "Figure 7. Correlative micro X-ray fluorescence (μ-XRF) and synchrotron-based Fourier transform infrared (SR-FTIR) analysis of the thin section from the cultures of the 25 mg/mL ferrihydrite treatment after 12 h cultivation. (a) μ-XRF map. (b) The LCF fitting of μ-X-ray absorption near-edge structure (XANES) analysis the selected regions of interest (ROI) region (i.e., A and B). (c) SR-FTIR maps. Red color in (a) represents high density of Fe, followed by orange, yellow, green, little green, and purple. Red color in (c) indicates the highest intensity of functional groups, followed by yellow, green, and blue. The color scale in (c) is a relative scale for each peak height and does not allow quantitative comparisons between peaks." (Page 41, Lines 1158-1166 in the revised manuscript) - Conserve the same colors between Fig.5.b Spot A and B. R: Thanks! We revised the colors in Fig.5-b. - [l.309]: Spot A or Spot B? R: Thanks! We revised them in the revised manuscript and also listed as follows. “with a lesser percentage ($\sim$17%) of FeC2O4 among the mineral particles (Spot A in Fig. 7b and Table S3). However, considerable percentages of hematite ($\sim$13%), goethite ($\sim$19%) and FeC2O4 ($\sim$25.9%) were present on the edge of these mineral particles (Spot B in Fig. 7b and Table S3).” (Page 16, Lines 515-518 in the revised manuscript) - [l.307-309]: why don’t you speak about FeC2O4 (25.9%) in spot B? R: We added the FeC2O4 ($\sim$25.9%) in the revised manuscript and also listed as follows. “considerable percentages of hematite ($\sim$13%) and goethite ($\sim$19%) were present on the edge of these mineral particles (Spot B in Fig. 5b and Table S3).” (Page 14, Lines 308-309 in the original manuscript) was changed to “considerable percentages of hematite ($\sim$13%) , goethite ($\sim$19%) and FeC2O4 ($\sim$25.9%) were present on the edge of these mineral particles (Spot B in Fig. 7b and Table S3).” (Page 16, Lines 516-518 in the revised manuscript) - Why is there goethite and hematite in sample which only contain ferrihydrite? R: During the incubation, a portion of ferrihydrite will transform to its more crystalline counterparts, such as hematite and goethite, by J12, owing to the

so-called "aging" process. - You should give different title to the paragraph [l.298-309], [l.310-320] R: Agree. In the revised manuscript, a different title was added and also listed as follows. “3.6. Effect of the presence of J12 on surface Fe species” (Page 16, Line 526 in the revised manuscript) - Paragraph [l.321-328] should be describe in paragraph [l.298-309]: it is the same figure and consequently the same idea. R: Done. - [l.317-320] “Interestingly, the area of the peak at 709.5 eV was bigger in the F + bacteria treatment than that in F - bacteria treatment (Fig. 6b-6c), suggesting that Fe(II) was generated on the surface of ferrihydrite during the cultivation with bacteria. Based on the reaction 1, HOâĂć should be the oxidant products.”: Is that reproducible between samples? Is that spectrum the mean representation of several spectra? R: No. The two spectra were not reproducible but ferrihydrite cultivated with (F + bacteria) and without (F - bacteria) bacteria. That spectrum was measured once rather than mean one of several spectra. However, the spectra should be representative, owing to the prepared samples for XPS measurement being uniform. - Fig.6 b and c: what do the colors mean? R: Dark line represents the raw spectrum, orange line represents the fitted spectrum, and other lines represent the component of fitted Fe species. The above explanation was added in the revised manuscript and also listed as follows. "Figure 8. (a) Fe 2p X-ray photoelectron spectroscopy (XPS) spectra of ferrihydrite samples, F+bacteria and F-bacteria; (b-c) Fe 2p 3/2 spectra of F+bacteria and F-bacteria, respectively, during the cultivation (12 h). F+bacteria, ferrihydrite with bacteria; F-bacteria, ferrihydrite without bacteria. In subfigure (b) and (c), dark, orange and other lines represents the raw spectrum, fitted spectrum and the component of fitted Fe species." (Page 42, Lines 1171-1176 in the revised manuscript) - Fig.6 a: Correct “Inyensity” by “Intensity” R: Done. - [l.325]: “good”, can you precise this term please? R: Thanks! We replaced “good” with “significant” in the revised manuscript. New paragraph: - Given the importance of Al in your discussion (half of the discussion), the Fig.S8 should appear in the main manuscript (not in Sup Mat) with the results presented in Fig.4. R: Agree! In the revised manuscript, we moved Fig.S8 to the main manuscript as Fig.6. - In Fig.1, Fig.3, Fig.4, Fig.S6, Fig.S8: you should

distinguish Al- from Fe- containing minerals. R: Agree! We added the description of Al- from Fe- containing minerals in the in the revised manuscript and distinguish them in the captions, which could be seen in the revised manuscript. Discussion Another time: I disapprove the use of the term “bacteria” which may refer to the domain of bacteria (see main comment 1). R: As the response to Comment 1, we changed "bacteria" to more specific "J12" throughout the whole manuscript. 4.1. Effect of Al(III)-containing minerals on the inhibition of bacterial growth - [l.336-343]: “It should be noted that the presence of minerals may potentially interfere with the measurement of cell numbers in Fig. 1. In this study, we subsampled the experimental cultures and diluted them in fresh medium so that both clay particles and bacteria were 200$\times$ less concentrated (Fig. S3), following the protocol of McMahon et al. (2016). As a result, the effect of mineral concentration may be minimal. In addition, plating the bacteria by evaluating populations by counting colonies may act as a complementary method for OD600 and needs to be investigated in the future.”: I am not waiting for a response to that comment: In your case, I would have chosen the association of a cell labeling with DAPI and a count of labeled cells with flow cytometry (or fluorescence microscopy). R: Thanks! In the revised manuscript, we added the association of a cell labeling with DAPI and a count of labeled cells with flow cytometry (or fluorescence microscopy) as an alternative choose. The added part was colored in red in the revised manuscript and also listed as follows. "Furthermore, the association of a cell labeling with DAPI and a count of labeled cells with flow cytometry (or fluorescence microscopy) is also an alternative choose." (Page 17, Lines 606-608 in the revised manuscript) - [l.355-357]: “Furthermore, the formation of some Al intermediates by the decreasing pH, such as Al13O4(OH)247+, is also suggested to be more toxic for bacterial growth (Amonette et al., 2003; Liu et al., 2016).”: what pH are you referring to? Is that in accordance with the pH measured in your study? R: Good comment! The pH was referred to the solution pH. In this study, we did not detect a significant decrease of pH (see Fig. 4 in the revised manuscript). Therefore, we added the corresponding discussion in the revised manuscript and also listed as follows. "However, we did not

detect a significant decrease of pH in this study (Fig. 4), suggesting that the formation of some Al intermediates may be slightly." (Page 18, Lines 638-640 in the revised manuscript) - The information given at [l.357-359] should appear after [l.345-349]. Then, you can discuss (i) on the results that you expected to observe and (ii) on the interpretation of the results that you obtained. R: Agree! Done. 4.2. Inhibition of bacteria by Fe(III)-containing minerals via a free-radical mechanism - If we take the two equations cited in your introduction: (1) $\equiv$Fe(III)-OH + H2O2 $\rightarrow$ Fe(II) + H2O + HO2 (2) $\equiv$Fe(II) + H2O2 $\rightarrow$ Fe(III)-OH + HOâĂć Where does H2O2 come from? Pseudomonas? If it come from the bacteria, the reduction of it development should induce a decrease of HOâĂć production in LB medium containing Fe minerals (if H2O2 is the limiting compound in the reaction, and it should be the case here), am I wrong? R: Yes. Pseudomonas J12 could produce H2O2. The reduction of H2O2 along with the oxidation of Fe induce an increase of HOâĂć based on the following equation: $\equiv$Fe(II) + H2O2 $\rightarrow$ Fe(III)-OH + HOâĂć - Correct the sentence [l.383-387]: “In line with other studies (Kwan and Voelker, 2003; Wang et al., 2017b), we deduced that HOâĂć may mainly generate on the mineral surface, partly due to the positive charge of mineral surface (Tombácz and Szekeres, 2006) but the negative charge of microbes (Jucket et al., 1996).” R: Thanks! This sentence was changed to "In our experiment, there was a lesser amount of HOâĂć produced with the different concentrations of aqueous Fe(NO3)3 (Fig. S9) than with the iron minerals (Fig. 3), which was in line with other studies (Kwan and Voelker, 2003; Wang et al., 2017b). Therefore, we deduced that HOâĂć may mainly generate on the mineral surface, partly due to the positive charge of mineral surface (Tombácz and Szekeres, 2006) but the negative charge of microbes (Jucket et al., 1996)." (Page 19, Lines 687-693 in the revised manuscript) - [l.401-404]: “High percentage of the less stable ferrihydrite (Table S3) may be attributable to the stabilization role of produced EPS (Fig. 5c) by bacteria to minerals, which had been shown during the cultivation of fungi with minerals (Li et al., 2016). Please, divide this sentence into two sentences in order to distinguish your contribution from the contribution of Li et al. (2016). Can you

precise your idea on the role of EPS on stabilization process please? R: AgreeïijĄWe divided this sentence into two sentences and shown as follows. “High percentage of the less stable ferrihydrite (Table S3) may be attributable to the stabilization role of produced EPS (Fig. 5c) by bacteria to minerals, which had been shown during the cultivation of fungi with minerals (Li et al., 2016).” (Page 18, Lines 401-404 in the original manuscript) was changed to “High percentage of the less stable ferrihydrite (Table S3) may be attributable to the stabilization role of produced EPS (Fig. 5c) by J12 to minerals. It is consistent with a previous finding in the cultivation of fungi with minerals (Li et al., 2016). The stabilization role of EPS was mainly identified as its combination into the network structure of minerals, which prevents the formation of crystalline minerals (Braunschweig et al., 2013)” (Page 20, Lines 716-722 in the revised manuscript) Braunschweig J., Bosch J., and Meckenstock R. U.: Iron oxide nanoparticles in geomicrobiology: from biogeochemistry to bioremediation, New Biotechnol., 30, 793-802, 2013. - [l.414]: suppress “cellular”. I do not understand the difference between cellular and free reductant? Free reductant such as FADH2 are intracellular, no? I think that you want to separate (i) cellular from (ii) non-cellular reactions, am I wrong? R: We agree with the comment! We revised the sentence and shown as follows. “In addition to Fenton-like reactions (Garrido-Ramírez et al., 2010), Fe(II) can also be generated by catalyzing a series of cellular intracellular (e.g., glutathione and NAD(P)H) and free (e.g., L cysteine and FADH2) reductants (Imlay, 2003).” (Page 19, Lines 413-415 in the original manuscript) was changed to “In addition to Fenton-like reactions (Garrido-Ramírez et al., 2010), Fe(II) can also be generated by catalyzing a series of intracellular reductants (e.g., glutathione, NAD(P)H, L cysteine and FADH2) (Imlay, 2003).” (Page 20, Lines 731-733 in the revised manuscript). - [l.413-418]: Are those reactions linked to HOâĂć production? R: Yes. These reactions promote the formation of Fe(II) which reacts with H2O2 through Fenton reactions that can accelerate the generation of HOâĂć. - [l.424]: Given that results in Fig.1 and Fig.S7 are produce by different experimental device, are you sure that you can give this interpretation to your results? R: Yes. According to the results

of soluble Fe, the concentrations were about 0-100 mg/L. In order to observing the effects of soluble Fe on the inhibition of bacteria, the concentrations of soluble Fe were set as 0, 50, 100 mg/L. - [l.427]: “simultaneously”? R: In the revised manuscript, we changed “simultaneously” with “also” and the revised sentence was also listed as follows. "Intracellular oxidative toxicity also caused by soluble Fe(III) played an important role in the inhibition activity (Schoonen et al., 2006)" (Page 21, Lines 756-758 in the revised manuscript). 4.3. Inhibition of bacterial growth by a free-radical mechanism and its implications for soil carbon storage - Fig.7: Can you please explain the figure in the caption? R: Yes. In the revised manuscript, we changed “simultaneously” with “also” and the revised sentence was also listed as follows. "Figure 9. Schematic of the heterotrophic bacterial inhibition by Fe(III)-containing minerals through a free-radical mechanism. Reactions 1-4 represent the processes occurring at heterotrophic bacteria-mineral interfaces and are detailed in the main text. ïĆĄ Production of HOâĂć through the Fenton or Fenton-like reactions; ïĆČ Direct inhibition of heterotrophic bacteria by HOâĂć; ïĆČ Indirect inhibition of heterotrophic bacteria by HOâĂć; ïĆĎ Intracellular inhibition of heterotrophic bacteria by soluble Fe." (Page 43, Lines 1180-1185 in the revised manuscript). - Can you please go further in the processes through which soluble Fe3+ and Fe2+ will have an inhibition effect on Pseudomonas? R: Yes. We added the description in the revised manuscript and shown as follows. “Soluble Fe(II) and Fe(III) released from minerals can penetrate into the cell membranes, thereby inducing intracellular oxidative damage (Williams et al., 2011).” (Page 21, Lines 767-769 in the revised manuscript) - Can you please go further in processes through which HOâĂć will have a “direct” inhibition power on Pseudomonas (modification of cell membrane physico-chemical properties?) R: Yes. HOâĂć can modify the cell membrane physico-chemical properties. We added the description in the revised manuscript and shown as follows. “Oxidative damage of extracellular HOâĂć may lead to bacterial inactivation, and protection of carbon from microbial degradation.” (Page 20, Lines 436-437 in the original manuscript) was changed to “Oxidative damage of HOâĂć may induce the damage of a membrane lipid

and cardiolipin that can lead to heterotrophic bacterial inactivation (Wang et al., 2017). In soil, heterotrophic bacteria are the main driver of soil carbon decomposition and greenhouse gas emission. As a result, the inactivation of heterotrophic bacteria results in protection of carbon from microbial degradation." (Page 21, Lines 769-774 in the revised manuscript) - [l.436-437]: "Oxidative damage of extracellular HOâĂć may lead to bacterial inactivation, and protection of carbon from microbial degradation." Please go further in this interpretation: HOâĂć have a role on Pseudomonas growth (it is your study), but HOâĂć can have other impacts in soils. What are they? How can HOâĂć and Fe act (i) on the soil C storage and (ii) on the soil C degradation-mineralization? R: Oxidative damage of HOâĂć may induce the damage of a membrane lipid and cardiolipin that can lead to heterotrophic bacterial inactivation. In soil, heterotrophic bacteria are the main driver of soil carbon decomposition and greenhouse gas emission. As a result, the inactivation of heterotrophic bacteria results in protection of carbon from microbial degradation. HOâĂć do have other impacts in soils. Except for decomposition of soil organic carbon (SOC), the presence of HOâĂć can also stabilize C in soil via a rapid formation of new intermolecular covalent bonds among soil components (Piccolo et al., 2011). In addition, the mobilized Fe can be easily transformed into the newly-formed reactive Fe (hydro)oxides (especially poorly crystalline Fe oxides) (Kleber et al., 2005; Yu et al., 2017), which will promote the formation of organo-mineral associations that are chemically more stable (Koegel-Knabner et al., 2008). In the revised manuscript, we added the interpretation and discussion about the effect of HOâĂć and Fe on the soil C storage. The revised parts were colored in red in the revised manuscript and also listed as follows. "Oxidative damage of HOâĂć may induce the damage of a membrane lipid and cardiolipin that can lead to heterotrophic bacterial inactivation (Wang et al., 2017). In soil, heterotrophic bacteria are the main driver of soil carbon decomposition and greenhouse gas emission. As a result, the inactivation of heterotrophic bacteria results in protection of carbon from microbial degradation. Except for decomposition of soil organic carbon (SOC), the presence of HOâĂć can also stabilize C in soil via a rapid formation of new intermolecular

covalent bonds among soil components (Piccolo et al., 2011). Formation of new intermolecular covalent bonds increases the recalcitrance of SOC." (Pages 21-22, Lines 769-790 in the revised manuscript) "The mobilized Fe can be easily transformed into the newly-formed reactive Fe (hydro)oxides (especially poorly crystalline Fe oxides) (Kleber et al., 2005; Yu et al., 2017), which will promote the formation of organo-mineral associations that are chemically more stable (Koegel-Knabner et al., 2008)." (Page 22, Lines 796-799 in the revised manuscript) - [l.439-442]: "In addition, the generation of free radicals may also have indirect effects on bacterial growth via substrate availability (Table S4). Substrate availability is improved in the presence of radicals, owing to the following two facts: 1) the depolymerization role of radicals on the complex substrates; 2) the inhibition role of radicals on bacteria indirectly increasing the amounts of available substrates.": Do you think that we can see an inhibition of bacterial growth through the reduction of nutrient availability induced by free radicals in a medium where nutrients are in excess? R: Our results did not confirmed an inhibition of bacterial growth through the reduction of nutrient availability induced by free radicals. In the revised manuscript, we deleted "the inhibition role of radicals on bacteria indirectly increasing the amounts of available substrates". - What about the role of minerals on the "stabilization-adsorption" of organic compounds of the NB medium? R: Minerals may interact with organic compounds of the NB medium, including adsorption (owing to a big specific surface area) and the formation of organo-mineral complexes (i.e., stablization). However, these interaction is not discussed/included in this manuscript. - [l.445-448]: Fe is one of the numerous processes regulating carbon cycle in soils. I suggest something like: "In this study, we suggest that soil carbon cycle is partly regulated by Fe minerals (i) by the formation of organo-mineral complexes and (ii) by the bacterial development inhibition (specify the processes)." R: Agree! In the revised manuscript, we changed "In this study, we suggest that soil carbon storage is regulated by Fe minerals, not only because of the formation of organo-mineral complexes (Kögel-Knabner, 2002; Kleber and Johnson, 2010; Schmidt et al., 2011) but also due to the bacterial inhibition activity of Fe minerals." (Page

20, Lines 445-448 in the original manuscript) to “In this study, we suggest that soil carbon cycle is partly regulated by Fe minerals (i) by the formation of organo-mineral complexes (Kögel-Knabner, 2002; Kleber and Johnson, 2010; Schmidt et al., 2011) and (ii) by the bacterial development inhibition.” (Page 22, Lines 799-802 in the revised manuscript) - l.451 replace “but” by “and” R: Done. Conclusions - [l. 467-458]: “effects on bacterial growth and the presence of minerals may potentially interfere with the measurement of cell numbers”: I do not think that it is necessary to speak about that here. R: In the revised manuscript, we deleted this sentence. The deleted part could be seen in the tracked changes of Marked Manuscript and did not listed here for brevity.

Please also note the supplement to this comment:
https://www.biogeosciences-discuss.net/bg-2018-479/bg-2018-479-AC2-supplement.zip

---

Interactive comment on Biogeosciences Discuss., https://doi.org/10.5194/bg-2018-479, 2018.

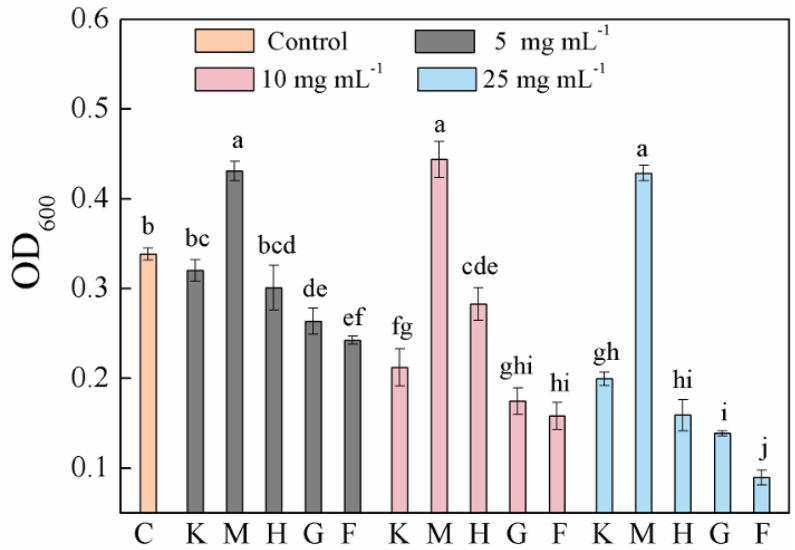


Fig. 1. Figure 1

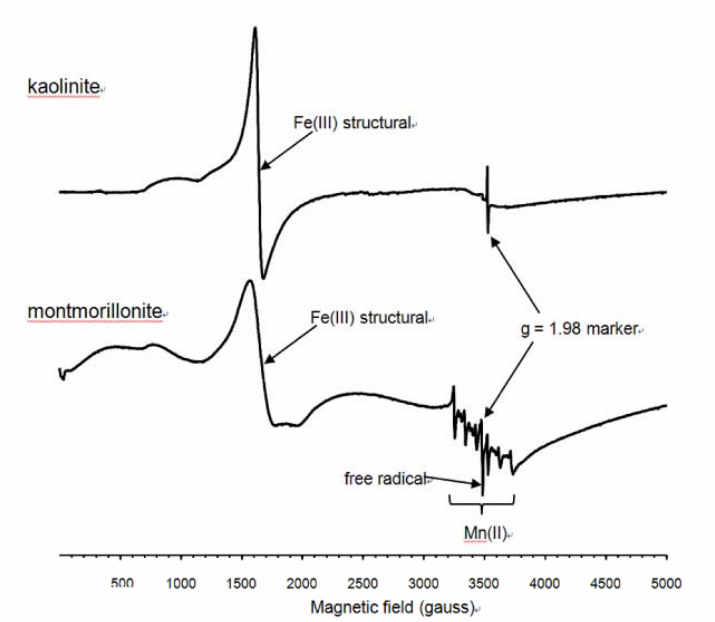


Fig. 2. Figure 2

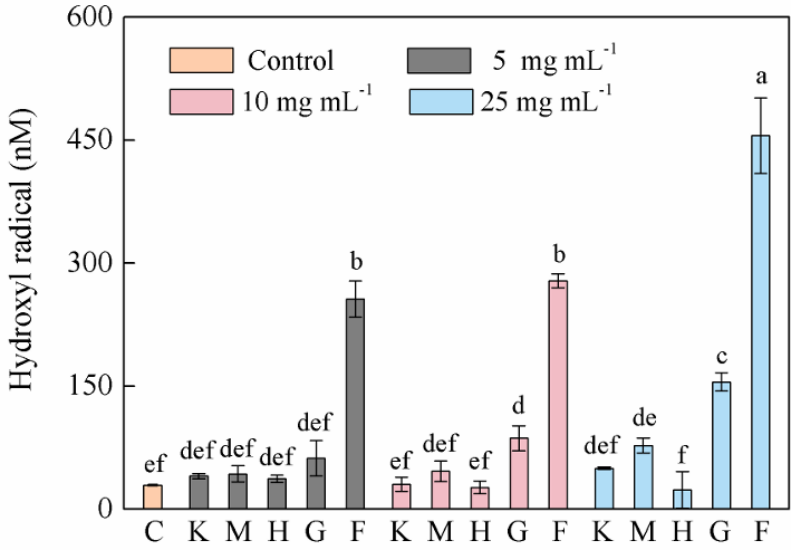


Fig. 3. Figure 3

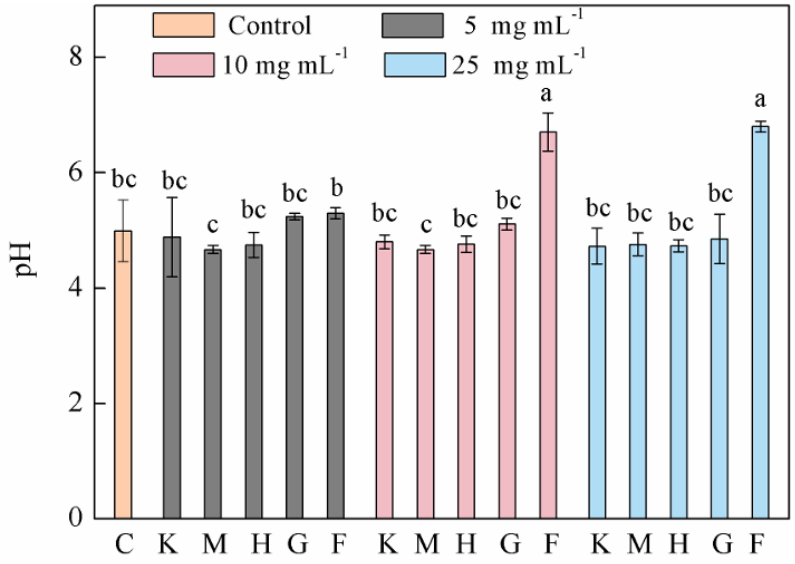


Fig. 4. Figure 4

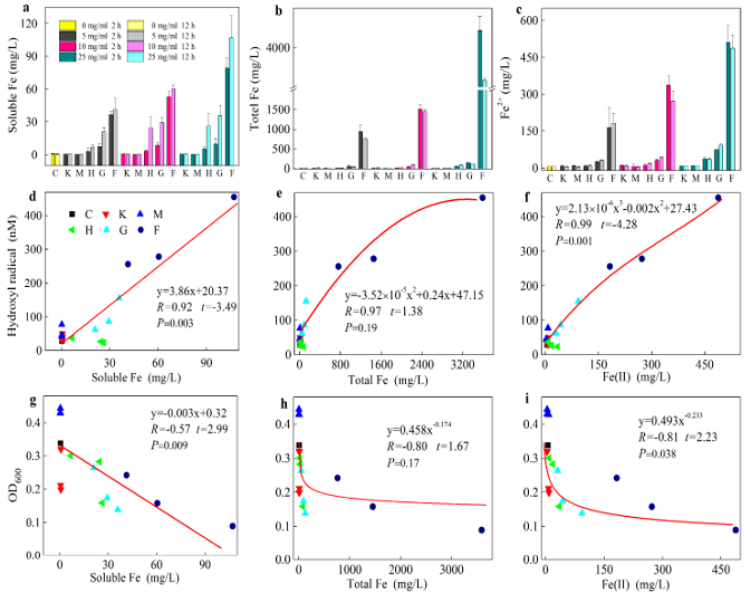


Fig. 5. Figure 5

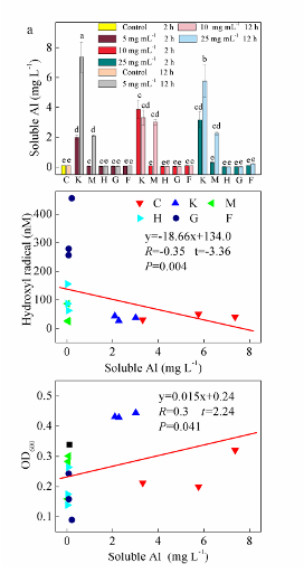


Fig. 6. Figure 6

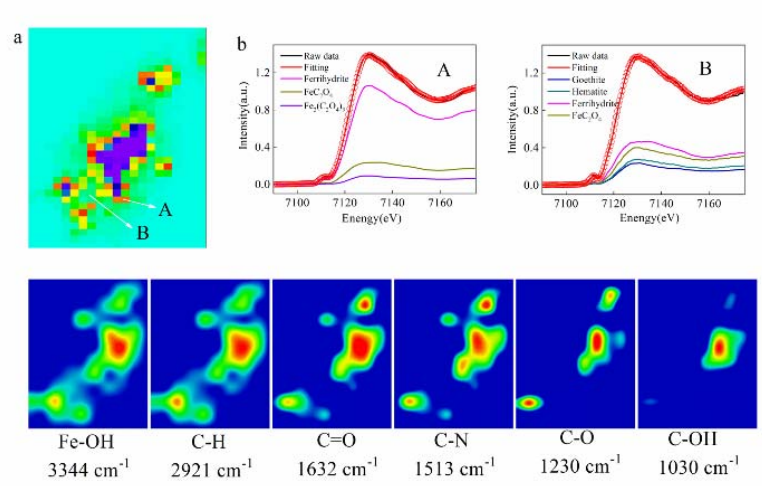


Fig. 7. Figure 7

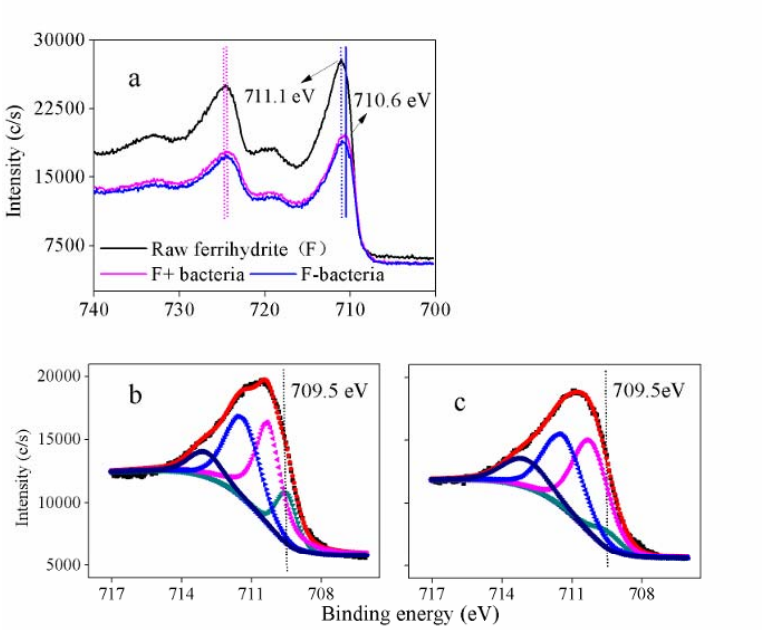


Fig. 8. Figure 8

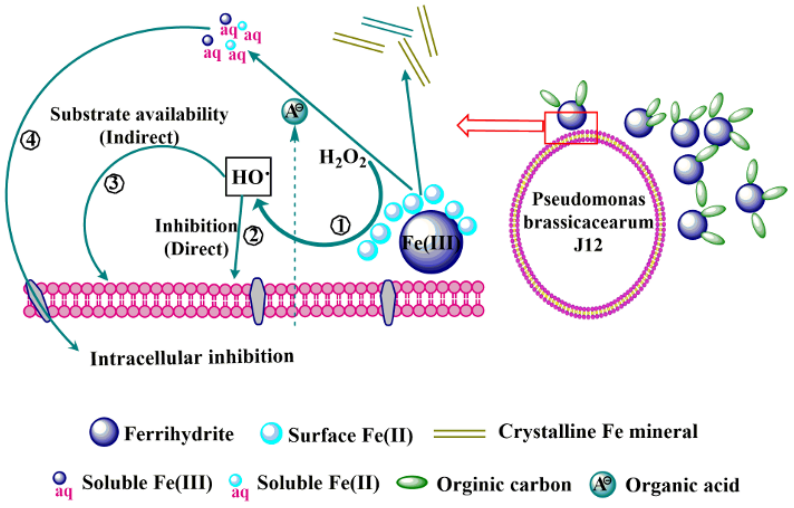


Fig. 9. Figure 9

Iron minerals inhibit the growth of *Pseudomonas brassicacearum* J12 via a free-radical mechanism: Implications for soil carbon storage

删除的内容: bacteria

Hai-Yan Du[1], Guang-Hui Yu[1, 2], Fu-Sheng Sun[1, 2], Muhammad Usman[3, 4], Bernard A. Goodman[5], Wei Ran[1], Qi-Rong Shen[1]

1. Jiangsu Provincial Key Lab for Organic Solid Waste Utilization, College of Resources & Environmental Sciences, Nanjing Agricultural University, Nanjing 210095, China

2. Institute of Surface-Earth System Science, Tianjin University, Tianjin 300072, China

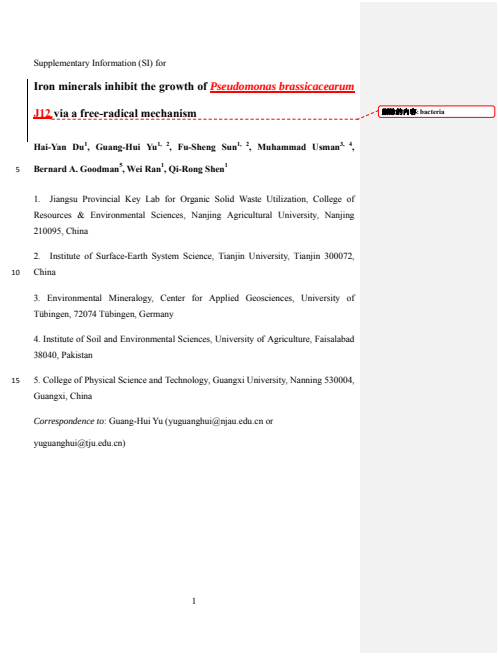
3. Environmental Mineralogy, Center for Applied Geosciences, University of Tübingen, 72074 Tübingen, Germany

4. Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad 38040, Pakistan

5. College of Physical Science and Technology, Guangxi University, Nanning 530004, Guangxi, China

Correspondence to: Guang-Hui Yu (yuguanghui@njau.edu.cn or yuguanghui@tju.edu.cn)

Fig. 10. Mark-manuscript



Supplementary Information (SI) for

Iron minerals inhibit the growth of *Pseudomonas brassicacearum* J12 via a free-radical mechanism

删除的内容: bacteria

Hai-Yan Du[1], Guang-Hui Yu[1, 2], Fu-Sheng Sun[1, 2], Muhammad Usman[3, 4], Bernard A. Goodman[5], Wei Ran[1], Qi-Rong Shen[1]

1. Jiangsu Provincial Key Lab for Organic Solid Waste Utilization, College of Resources & Environmental Sciences, Nanjing Agricultural University, Nanjing 210095, China

2. Institute of Surface-Earth System Science, Tianjin University, Tianjin 300072, China

3. Environmental Mineralogy, Center for Applied Geosciences, University of Tübingen, 72074 Tübingen, Germany

4. Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad 38040, Pakistan

5. College of Physical Science and Technology, Guangxi University, Nanning 530004, Guangxi, China

Correspondence to: Guang-Hui Yu (yuguanghui@njau.edu.cn or yuguanghui@tju.edu.cn)

Fig. 11. Marked-supplementary data

Reply to the Comments

Anonymous Referee #2

The authors appreciate the report of Referee #2 and respond as follows.

I. General comments: This study aimed to study (i) the impact of Al- and Fe-containing minerals (montmorillonite, kaolinite, hematite, goethite and ferrihydrite) on bacterial growth using cultural approach on Pseudomonas brassicacearum J12 and (ii) the involvement of ROS, produced via fenton reactions, on Pseudomonas brassicacearumJ12 growth. The subject is clearly interesting and is in accordance with researches published in Biogeosciences journal. Such researches on interactions between biotic and abiotic compartments are essential for our understanding of nutrients fluxes in soils and I encourage the publication of this manuscript in Biogeosciences journal. However, some points need to be clarified before publication.

II. Major comments:

- Major comment 1: Title: "Iron minerals inhibit the growth of bacteria via a free-radical mechanism: Implication for soil carbon storage": You cannot generalize your results to the domain of bacteria. I recognize that we will never be satisfied enough with the number of species studied, but I think that before expanding your results to the domain of bacteria, you should confirm them on other species from different phylum which show important genetic and phenotypic distances.

Response (R): Thanks! In the revised manuscript, we revised "bacteria" to "*Pseudomonas brassicacearum* J12" in the Title. Diaz et al. (2013) showed that other species, e.g., *Pseudomonas putida* GB-1, could produce approximately 1 and 10 amol $O_2^{\bullet-}$ $cell^{-1}$ h^{-1} during mid-exponential growth or stationary phase, respectively. Except for *Pseudomonas*, taxonomically and ecologically diverse heterotrophic bacteria from both aquatic and terrestrial environments were a vast source of superoxide ($O_2^{\bullet-}$) and H_2O_2 (Diaz et al., 2013). Based on the suggestion of Referee #2 and the results from Diaz et al. (2013), we think that "iron minerals inhibit the growth of heterotrophic bacteria via a free-radical mechanism" should be no problem. The revised part was colored in red in the revised manuscript and also listed as follows.

"**Iron minerals inhibit the growth of bacteria via a free-radical mechanism: Implications for soil carbon storage**" **(Page 1, Lines 1-2 in the original manuscript)**

was changed to

"**Iron minerals inhibit the growth of *Pseudomonas brassicacearum* J12 via a free-radical mechanism: Implications for soil carbon storage**" **(Page 1, Lines 1-3 in the revised manuscript)**

The reference is listed as follows:

Fig. 12. Detailed responses to Reviewer 2