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Interactive comment on "Pyrite oxidization accelerates bacterial carbon sequestration in copper mine tailings Type of contribution" *by* Yang Li et al.

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1. Comments from Referees: The section of 2.1 should include more details about the soil sampling. Response: Thanks for this comment. I have rewritten the soil sampling details in the section of 2.1. Changes in manuscript: (Lines 66-68) "Samples of oxidized mine tailings on the surface (0-20 cm) were collected using a steel corer in October 2015. Mine tailing samples stored in sterilized plastic bags were transported to the laboratory in an ice cooler and stored at -20°C before analysis."

2. Comments from Referees: The experiment treatments was also not clear. From section 2.2, we can't judge how many treatments were set. Please specify. Moreover,

what's samples were conducted qPCR, sequencing and cloning were also confused. Response: Thanks for this comment. I have rewritten the experiment treatments in sections. And samples that conducted qPCR, sequencing and cloning were also written. Changes in manuscript: (1) For experiment treatment: (Lines 75-82) "There were a total of four treatments in the microcosms of the two mine tailings. For each mine tailing, fresh mine tailings (equivalent to 10.0 g d.w.s.) were mixed with a total of 2 g of sterile pulverized FeS2 at approximately 60% maximum water-holding capacity as the FeS2 treatment, followed by incubation at 25°C in the dark for 14 days. Yangshanchong mine tailing samples (YM) cultured with FeS2 are abbreviated as YM_FeS2, and Shuimuchong mine tailing samples (SM) cultured with FeS2 are abbreviated as SM_FeS2. In addition, fresh mine tailings at approximately 60% maximum water-holding capacity without any additive were used as the control groups and abbreviated as YM_ck and SM_ck. For each treatment, the microcosms were incubated with 10% 13C-CO2 or 12C-CO2, and both treatments were constructed in triplicate for DNA-SIP analysis." (2) For samples that conducted qPCR, sequencing and cloning: (Lines 107-109) "Real-time quantitative PCR analysis was performed on a CFX96 optical real-time detection system (Bio-Rad, Laboratories Inc., Hercules, CA, USA) to determine the copy numbers of the cbbL, cbbM and 16S rRNA genes in DNA gradient fractions from the YM_FeS2 and SM_FeS2 DNA-SIP microcosms."(Lines 122-124) "The composition of the bacterial communities in different samples was assessed by pyrosequencing of the 16S rRNA genes. The 16S rRNA gene from the 13C-labeled DNA fraction, which had CsCl buoyant densities of 1.738 in the heavy fraction in YM_FeS2 and 1.734 in the heavy fraction in SM_FeS2, was also amplified for pyrosequencing." (Lines 140-141) "Clone libraries of the cbbL and cbbM genes were also constructed from the 13C-labeled DNA fractions with CsCl buoyant densities of 1.738 in the heavy fraction in YM_FeS2 and 1.734 in the heavy fraction in SM_FeS2."

3. Comments from Referees: For section 4.1, authors only described how Carbon sequestration in mine tailings but without any discussion Combining with your own data. Response: Thanks for this comment. I have rewritten the discussion based on

this comments. Changes in manuscript: (Lines 259-286) “4.1 The effect of FeS2 on the whole bacterial community in mine tailings Acidic polymetallic mine tailings have strong potential for pyrite oxidation. In this study, a large amount of sulfuric acid was generated (increases of approximately 19.95 mg/g and 14.64 mg/g in YM and SM, respectively), and a persistent decline in pH was observed (pH decreased by 0.44 and 0.35 in YM and SM, respectively) in only 14 days. These changes clearly indicated oxidization of pyrite (i.e., acidification) in mine tailings. Previous studies have found that the some bacterial phyla, such as Firmicutes and Nitrospirae, significantly increase (Chen et al., 2013;Liu et al., 2014) the acidification process of mine tailings. In the present study, the bacterial composition in the different mine tailings varied greatly, and only the Firmicutes phylum increased in both tested mine tailings under pyrite addition. This group might participate in the oxidization of sulfide minerals (Chen et al., 2013), such as Sulfobacillus, which accounted for the majority of Firmicutes. Many other microorganisms might be inhibited under pyrite addition. Korehi et al. (2014) and Liu et al. (2014) also indicated that the ongoing oxidation process in mine tailings was accompanied by an increase in Firmicutes and a decrease in Actinobacteria and all classes of Proteobacteria except Gammaproteobacteria. In addition, Chen et al. (2013) and Liu et al. (2014) found that the relative abundances of Euryarchaeota belonging to archaea significantly increased with decreasing pH, which indicates that this taxon is an indicator of metal contamination (Hur et al., 2011). Euryarchaeota compete with β-Proteobacteria for ecological niches under such acidic conditions (Liu et al., 2014). However, in this study, only a few archaea were detected, which might be related to differences in primer affinities and samples. The growth of microorganisms in bare mine tailings is usually limited by the availability of organic carbon (Schimel and Weintraub, 2003). Pyrite oxidization in mine tailings further enhanced the acidity of the mine tailings (pH decreased to 2.77 and 2.57 in YM_FeS2 and SM_FeS2, respectively). As a result, only microorganisms that were resistant to infertility and/or acidophilic conditions could maintain high activities. In the present study, Conexibacter, Alicyclobacillus, Bacillus, Sulfobacillus, Leptospirillum, Rhodoplanes, Methylobacterium, Acidiphilium, Novosphingobium

and Methyloversatilis were the top genera with relative abundances above 0.2% in the mine tailings. Some specific taxa, including the genera Alicyclobacillus, Sulfobacillus, Leptospirillum and Acidiphilium, increased in both of the tested mine tailings under pyrite addition, indicating high consistency of dominant bacterial genera in different mine tailings. It is possible that in the case of pyrite oxidization and the availability of organic carbon, acidophilic and/or autotrophic bacteria could be stimulated (Deng et al., 2016;Antonelli et al., 2018), and the main carbon fixers found in these two mine tailings may be derived from the same groups.”

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