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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. The manuscript explored the autotrophic microbes and the FeS2 facilitation role in acidic mine tailings using stable isotope and molecular methods. The results showed that FeS2 facilitated CO2-fixing by microbes and increased the abundances of relevant autotrophs. The study is very interesting, which could provide new insights into the autotrophic roles in extreme environments. However, the article writing is awful in logic, result description and interpretation. Reply: We thank the reviewer for pointing out the importance of this study and giving me a number of useful advices.

2. Comments from Referees: The introduction did not show some key points rele-

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vant to the research, such as possible CO2-fixing pathways and autotrophs in acidic sulfur-enriched environments. The introduction was not well structured and really needs rewrite. Response: Thanks for this comment. I have rewritten the introduction details according to this comment. Changes in manuscript: (Lines 29-61) “Terrestrial ecosystems have great potential as carbon sinks to stabilize CO2 and regulate climate change (White et al., 2000), and atmospheric CO2 can be fixed into plants by photosynthesis and assimilated into soils as soil organic carbon (SOC) by decomposition and microbial activity (Deng et al., 2016;Antonelli et al., 2018). Currently, chemolithoautotrophic organisms fix atmospheric CO2 by six pathways, including the widely distributed Calvin-Benson-Bassham (CBB) cycle, the reductive tricarboxylic acid (rTCA) cycle, the reductive acetyl-CoA pathway and the recently discovered 3-hydroxypropionate/4-hydroxybutyrate (HP/HB) cycles (Berg, 2011;Alfreider et al., 2017). The ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) enzyme is the most prominent enzyme on earth (Raven, 2013) and is important in the CBB cycle. The CBB cycle is the most prevalent means of CO2 fixation by autotrophs (Tabita, 1999;Berg, 2011). In addition, the genes encoding the large subunit of RuBisCO serve as autotroph markers (Alfreider et al., 2017;Berg, 2011). The cbbL and cbbM genes encode RuBisCO form I and form II, respectively, with 25 to 30% amino acid sequence identity (Tabita et al., 2008). Polymetallic mine tailings have considerable potential to stabilize levels of atmospheric CO2 (Harrison et al., 2013) through the carbonation of noncarbonate minerals, including the dissolution of silicates, hydroxides and oxides and the precipitation of carbonate minerals (McCutcheon et al., 2016;Meyer et al., 2014;McCutcheon et al., 2014). However, sequestered carbon is also present in the form of inorganic carbon. Compared with soil ecosystems, polymetallic mine tailings exhibit specific features, including a lack of organic matter, nutrients and nutrient-holding capacity (Lottermoser, 2010;Young et al., 2015), that restrict plant growth, and plant productivity is generally difficult to restore in mining wastelands (Hu et al., 2018;Li et al., 2017). Therefore, microbes may be the main source of organic carbon in mine tailings. The limited amount of organic matter in mine tailings also in-

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hibits the activities of heterotrophic microorganisms, and therefore the microorganisms in these environments are lithotroph-dominant (Li et al., 2015). Polymetallic mine tailings contain sulfide minerals (e.g., pyrite), and the oxidation of these sulfide minerals leads to a decrease in pH, also known as mine tailing acidification. Previous studies have noted that polymetallic mine tailings have lithotroph-dominant microbial compositions (Li et al., 2015) due to the limited amount of organic matter. Consequently, acidophilic, chemoautotrophic bacteria, including Acidithiobacillus, Leptospirillum and Sulfobacillus (Chen et al., 2013;Liu et al., 2014), can promptly participate in ferrous and sulfur oxidation in mine tailings, and these autotrophic taxa play leading roles in carbon cycling and energy flow during the mine tailing acidification process. However, the relationship between the oxidation of sulfide minerals and carbon sequestration by these acidophilic, chemoautotrophic bacteria is still unknown. In the present study, we conducted a microcosm experiment using mine tailings collected from two mines to determine the effects of sulfide mineral (pyrite) oxidation on carbon sequestration in mine tailings through the addition of pyrite. In addition, the main carbon fixers were analyzed through DNA-based stable isotope probing (DNA-SIP) and cbbL and cbbM gene analysis. Our objectives were to investigate whether sulfide mineral oxidation can stimulate carbon sequestration in mine tailings and to identify the key carbon sequestration groups in mine tailings during the acidification process."

3. Comments from Referees: The method section failed to describe key details: 1) weather the samples were washed by acid prior to measuring isotope compositions? 2) no descriptions on chemical analysis in samples, i.e. solutes for Fe2+,Fe3+, SO42-; 3) no citations for the primer sets, which were apparrently designed in the study; 4) no informations on PCR reactions 5) how did the authors determine the PCR efficiency? 6) how did the authors qualify gene abundance? standard curves? 7) no statistic software informations; 8) how many replicates were for each treatment? Response: Thanks for this comment. I have rewritten and added more information according to this comment. Changes in manuscript: (1) (Lines 85-86) "Carbon isotope composition was analyzed by a Delta V Advantage Mass Spectrometer (Thermo Fisher Scientific, Inc.,

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USA) coupled with an elemental analyzer (Flash2000; HT Instruments, Inc., USA) in continuous flow mode." (Lines 89-91) "The carbon isotope composition and TOC content were analyzed after performing a soil acidification pretreatment method to remove inorganic carbon as described previously (Wang et al., 2015)" (2) (Lines 92-97) "The Fe2+ and Fe3+ in the soils were extracted by HCl. The Fe2+ in the extract was measured by a spectrophotometric method after mixing with phenanthroline and trisodium citrate, and the Fe3+ in the extract was reduced to Fe2+ by hydroxylammonium chloride and measured by a spectrophotometric method (Heron et al., 1994). The total sulfate ion content was determined via ion chromatography after extraction by sodium hydroxide, as described previously (Yin and Catalan, 2003)." (3) (Lines 109-114) "The K2f/V2r primer pair (K2f: 5'-ACC AYC AAG CCS AAG CTS GG-3' and V2r: 5'-GCC TTC SAG CTT GCC SAC CRC-3') (Nanba et al., 2004), the cbbMF/cbbMR primer pair (cbbMF: 5'-TTC TGG CTG GGB GGH GAY TTY ATY AAR AAY GAC GA-3' and cbbM-R: 5'-CCG TGR CCR GCV CGR TGG TAR TG-3') (Campbell and Cary, 2004) and the 515F/907R primer pair (515F: 5'-GTG CCA GCM GCC GCG G-3' and 907R: 5'-CCG TCA ATT CMT TTR AGT TT-3') (Zhou et al., 2011) were used to amplify the cbbL, cbbM and 16S rRNA genes, respectively." (4) (Lines 116-117) "qPCR analysis of the cbbL, cbbM and 16S rRNA genes was performed under the following conditions: 40 cycles of 30 s at 95°C, 30 s at 55°C (cbbL and 16S rRNA genes) or 57°C (cbbM gene), and 45 s at 72°C." (5) (Lines 117-120) "Standard curves were obtained using 10-fold serial dilutions of linearized recombinant plasmids containing the cbbL, cbbM and 16S rRNA genes with known copy numbers. The amplification efficiencies were 90-100%, which were obtained with R2 values greater than 0.99." (6) (Lines 117-119) "Standard curves were obtained using 10-fold serial dilutions of linearized recombinant plasmids containing the cbbL, cbbM and 16S rRNA genes with known copy numbers." (7) (Lines 151-156) "Bray-Curtis distance matrices for the overall bacterial community composition among the given samples were calculated in R v.3.3.2 using the 'vegdist' function of the vegan package and visualized by nonmetric multidimensional scaling (NMDS) in Origin 8. A heatmap of dominant genera with relative abundances above

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0.2% was applied for plotting in the R environment with the pheatmap package. The translated cbbL and cbbM sequences from the heavy fractions were used to construct a phylogenetic tree by the neighbor-joining method using the MEGA package, version 7.0." (8) (Lines 175-182) "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."

4. Comments from Referees: Fig1 symbols are very confusing, and no descriptions on the above and bottom columns. Response: Thanks for this comment. I have redrawn the figure and have rewritten the figure legend. Changes in manuscript:

Fig. 1 pH values (a), 13C atom % (b), TOC (c) contents, SO42- (d), Fe2+ (e) and Fe3+ (f) in mine tailings. The error bars indicate the standard errors of three subsamples for each tailing sample. To determine 13C atom % (b), all analyzed samples were treated with 13C-CO2 in microcosms. YM_ck, control group of Yangshanchong mine tailings; SM_ck, control group of Shuimuchong mine tailings; YM_FeS2, Yangshanchong mine tailings treated with FeS2; SM_FeS2, Shuimuchong mine tailings treated with FeS2.

5. Comments from Referees: No specific legends or descriptions on the two inserts in Fig. 3, and the color differences are not clear Response: Thanks for this comment. I have redrawn the figure and have rewritten the figure legend. Changes in manuscript:

Fig. 3 Relative abundances (percentages) of the main identified bacterial taxonomic

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groups, i.e., the phyla Proteobacteria, Firmicutes, Acidobacteria, Actinobacteria, WPS-2, Planctomycetes, AD3 and Nitrospirae (a); and the classes Alphaproteobacteria, Betaproteobacteria, Deltaproteobacteria and Gammaproteobacteria (within the phylum Proteobacteria) (b). For each tailing sample, the relative abundances of the sequences assigned to a given taxonomic unit were calculated for each of three subsamples, and the average value was then used to represent the relative abundance of each tailing sample.

6. Comments from Referees: L252-261, 284-291, there are many super long sentences. A sentence usually contains maximum 22 words. Response: Thanks for this comment. I have rewritten these sentences. Changes in manuscript: (1) (Lines 190-197) "In the Yangshanchong mine tailings, pyrite addition significantly increased the relative abundances of AD3, Nitrospirae and unclassified Proteobacteria by 0.75% ($P=0.008$), 0.59% ($P=0.019$) and 6.33% ($P<0.001$), respectively. In parallel, in Shuimuchong mine tailings, FeS2 addition significantly increased the relative abundances of Firmicutes, Planctomycetes, unclassified Proteobacteria, Alphaproteobacteria, Betaproteobacteria, and Deltaproteobacteria by 15.69% ($P<0.001$), 0.97% ($P<0.001$), 5.88% ($P=0.002$), 4.35% ($P=0.001$), 8.61% ($P<0.001$) and 0.21% ($P=0.003$), respectively. However, in SM, the percentages of AD3, Acidobacteria, Actinobacteria and Gammaproteobacteria in SM by 0.97% ($P=0.002$), 7.43% ($P=0.002$), 1.35% ($P=0.016$) and 4.85% ($P=0.002$) were decreased under pyrite addition." (2) (Lines 214-220) "In the Yangshanchong mine tailings, pyrite addition significantly increased the relative abundances of the genera Alicyclobacillus, Leptospirillum, Sulfobacillus and Acidiphilium by 0.67% ($P=0.027$), 0.74% ($P=0.002$), 8.86% ($P=0.043$) and 1.57% ($P<0.001$), respectively. In the Shuimuchong mine tailings, FeS2 addition significantly increased the relative abundances of Alicyclobacillus, Bacillus, Sulfobacillus, Methylobacterium, Novosphingobium and Methyloversatilis by 0.57% ($P<0.001$), 9.94% ($P<0.001$), 5.99% ($P<0.001$), 0.15% ($P<0.001$), 2.06% ($P=0.004$) and 0.56% ($P<0.001$), respectively."

7. Comments from Referees: The CO2-fixing capacity by autotrophs should be calcu-

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lated. Response: Thanks for this comment. I have calculated the CO2-fixing capacity by autotrophs in the result. Changes in manuscript: (Lines 170-171) “The CO2-fixing capacities of autotrophs under FeS2 addition were 9.50±0.91 mg/kgÂůd in YM and 3.69±0.11 mg/kgÂůd in SM.”

8. Comments from Referees: L306-307, the statement is problematic: 12CO2 is a control relative to 13CO2, so the shift to heavy fractions should not be observed in 12CO2. L307-311, the statements are not correct: for the peak in 13CO2 occurred in the density of 1.72 rather than 1.73 in both 12CO2 and 13CO2. Response: Thanks for this comment. I have written the sentences. Changes in manuscript: (Lines 232-235) “cbbL and cbbM gene levels under 13C-CO2 treatment peaked at a density of 1.72 in both the 12C-CO2 and 13C-CO2 treatments. In addition, a shift toward heavy fractions was observed for the cbbL and cbbM gene abundances in the 13C-CO2 treatment (Fig. 5), with buoyant densities of 1.738 gÂůmL-1 in YM_FeS2 and 1.734 gÂůmL-1 in SM_FeS2.”

9. Comments from Referees: L311-314, the statements should go to discussion section Response: Thanks for this comment. I have move the works into the discussion section. Changes in manuscript: (Lines 301-306) “In addition, only a few archaea were detected based on 16S rRNA gene sequencing, and the clone libraries of the cbbL and cbbM genes in the 13C-labeled heavy fraction did not show archaeal sequences for Calvin cycle genes. These results indicated that bacterial carbon sequestration is mainly attributable to chemoautotrophic bacteria in pyrite oxidization of mine tailings. However, archaea may have higher activities in RuBisCO-mediated carbon metabolic pathways (Kono et al., 2017), which will require further study.”

10. Comments from Referees: Fig. 6, Cultured genus most related to OTU1, 2, 3 and 4 should be given for identifying purpose Response: Thanks for this comment. I have added more information about the cultured genus most related to OTU1, 2, 3 and 4 in the supply materials Changes in manuscript: Fig. 6 Phylogenetic tree of the translated cbbL and cbbM sequences in the heavy fractions from YM and SM treated

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with FeS2. Relative frequencies (%) are marked in the bar graph. Bootstrap values of >50% are indicated at branch points. The cbbL and cbbM gene copy numbers in the heavy fractions from FeS2-treated YM and SM are shown in the middle of the figure. The cultured genera most related to OTUs from the cbbL and cbbM clone libraries are shown in Table S1.

Table S1 Cultured genus most related to OTUs from cbbL and cbbM clone libraries

11. Comments from Referees: cbb is not a correct gene name, it should be cbbL or cbbM. Response: Thanks for this comment. I have rewritten the gene name across the manuscript. Changes in manuscript: (Lines 237-238) "The cbbL and cbbM gene sequences from the clone libraries in the 13C-DNA heavy fraction treated with 13C-CO2 were used for phylogenetic analysis (Fig. 6)." (Lines 230-232) "For the quantitative analysis of cbbL and cbbM gene abundances, the buoyant densities of the DNA in isopycnic centrifugation gradients were used to assess the labeling efficiencies of cbbL or cbbM gene-carrying carbon fixers in the DNA-SIP microcosms (Fig. 5)."

12. Comments from Referees: Is Fig. 5 for FeS2 treatments or raw mine tailings? Response: Thanks for this comment. The figure was for FeS2 treatment and I have redrawn the figure. Changes in manuscript:

Fig. 5 Quantitative distribution of cbbL and cbbM gene fragments across the entire buoyant density gradients of the DNA fractions from microcosms treated with FeS2 and incubated with 12C-CO2 or 13C-CO2. The normalized data consist of the ratio of the gene copy number for each DNA gradient to the maximum quantity for each treatment. The error bars represent the standard errors of triplicate microcosms, and each contains three technical replicates.

13. Comments from Referees: L351-371, the paragraph should go to introduction section. The discussion is far from the results, i.e. discuss why and how FeS2 facilitates microbial CO2-fixing and changes the whole bacterial community. Response: Thanks for this comment. I have rewritten the Introduction and Discussion

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according to this comment. Changes in manuscript: (1) Introduction: “Terrestrial ecosystems have great potential as carbon sinks to stabilize CO2 and regulate climate change (White et al., 2000), and atmospheric CO2 can be fixed into plants by photosynthesis and assimilated into soils as soil organic carbon (SOC) by decomposition and microbial activity (Deng et al., 2016;Antonelli et al., 2018). Currently, chemolithoautotrophic organisms fix atmospheric CO2 by six pathways, including the widely distributed Calvin-Benson-Bassham (CBB) cycle, the reductive tricarboxylic acid (rTCA) cycle, the reductive acetyl-CoA pathway and the recently discovered 3-hydroxypropionate/4-hydroxybutyrate (HP/HB) cycles (Berg, 2011;Alfreider et al., 2017). The ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) enzyme is the most prominent enzyme on earth (Raven, 2013) and is important in the CBB cycle. The CBB cycle is the most prevalent means of CO2 fixation by autotrophs (Tabita, 1999;Berg, 2011). In addition, the genes encoding the large subunit of RuBisCO serve as autotroph markers (Alfreider et al., 2017;Berg, 2011). The cbbL and cbbM genes encode RuBisCO form I and form II, respectively, with 25 to 30% amino acid sequence identity (Tabita et al., 2008). Polymetallic mine tailings have considerable potential to stabilize levels of atmospheric CO2 (Harrison et al., 2013) through the carbonation of noncarbonate minerals, including the dissolution of silicates, hydroxides and oxides and the precipitation of carbonate minerals (McCutcheon et al., 2016;Meyer et al., 2014;McCutcheon et al., 2014). However, sequestered carbon is also present in the form of inorganic carbon. Compared with soil ecosystems, polymetallic mine tailings exhibit specific features, including a lack of organic matter, nutrients and nutrient-holding capacity (Lottermoser, 2010;Young et al., 2015), that restrict plant growth, and plant productivity is generally difficult to restore in mining wastelands (Hu et al., 2018;Li et al., 2017). Therefore, microbes may be the main source of organic carbon in mine tailings. The limited amount of organic matter in mine tailings also inhibits the activities of heterotrophic microorganisms, and therefore the microorganisms in these environments are lithotroph-dominant (Li et al., 2015). Polymetallic mine tailings contain sulfide minerals (e.g., pyrite), and the oxidation of these sulfide minerals leads

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to a decrease in pH, also known as mine tailing acidification. Previous studies have noted that polymetallic mine tailings have lithotroph-dominant microbial compositions (Li et al., 2015) due to the limited amount of organic matter. Consequently, acidophilic, chemoautotrophic bacteria, including Acidithiobacillus, Leptospirillum and Sulfobacillus (Chen et al., 2013;Liu et al., 2014), can promptly participate in ferrous and sulfur oxidation in mine tailings, and these autotrophic taxa play leading roles in carbon cycling and energy flow during the mine tailing acidification process. However, the relationship between the oxidation of sulfide minerals and carbon sequestration by these acidophilic, chemoautotrophic bacteria is still unknown. In the present study, we conducted a microcosm experiment using mine tailings collected from two mines to determine the effects of sulfide mineral (pyrite) oxidation on carbon sequestration in mine tailings through the addition of pyrite. In addition, the main carbon fixers were analyzed through DNA-based stable isotope probing (DNA-SIP) and cbbL and cbbM gene analysis. Our objectives were to investigate whether sulfide mineral oxidation can stimulate carbon sequestration in mine tailings and to identify the key carbon sequestration groups in mine tailings during the acidification process." (2) Discussion: "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

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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. 4.2 The effect of FeS2 on bacterial carbon sequestration in mine tailings Previous studies have shown that mine tailings provide an excellent substrate for carbon sequestration through the formation of carbonate due to the large surface area of the material grains (McCutcheon et al., 2016). Compared to soils and natural bedrock, mine tailings may possess higher carbonate precipitation rates (Wilson et al., 2009). However, in this study, the 13C content and

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TOC content in mine tailings increased slightly. DNA-SIP analysis demonstrated that a considerable amount of 13C-CO2 was assimilated by carbon fixers in the 13C-CO2-labeled mine tailing samples, leading to a significant shift of cbbL or cbbM gene-carrying genomic DNA into the heavy fraction. Both of these results indicated the contribution of microbial activities to carbon sequestration in mine tailings. To the best of our present knowledge, this report is the first to elucidate carbon sequestration by autotrophic groups in mine tailings based on isotope tracers and DNA-SIP. Previous studies have found that microbial photosynthesis accelerates carbonate mineral precipitation and further induces mineralization (McCutcheon et al., 2014;McCutcheon et al., 2016). However, in the present study, the microcosms were not cultured in the presence of illumination, and as a result, chemoautotrophic microorganisms, particularly iron and/or sulfide oxidizers, were the dominant carbon fixers. In addition, only a few archaea were detected based on 16S rRNA gene sequencing, and the clone libraries of the cbbL and cbbM genes in the 13C-labeled heavy fraction did not show archaeal sequences for Calvin cycle genes. These results indicated that bacterial carbon sequestration is mainly attributable to chemoautotrophic bacteria in pyrite oxidization of mine tailings. However, archaea may have higher activities in RuBisCO-mediated carbon metabolic pathways (Kono et al., 2017), which will require further study. During pyrite oxidization in mine tailings, some acidophilic autotrophic microorganisms have very high activity levels. For example, the Sulfobacillus and Leptospirillum genera, both of which are vital ferrous and sulfur oxidizers, increased significantly during pyrite oxidization. Zhang et al. (2016) found genes for the CBB pathway and rTCA, but no other CO2 fixation pathways, in a copper bioleaching microbial community. For the CBB pathway in this study, the Sulfobacillus-like cbbL gene was the primary carbon fixing-associated gene. Ňancucheo and Johnson (2012) found that among acidophilic prokaryotes isolated from mine-impacted environments, the ability to metabolize glycolic acid appeared to be restricted to Firmicutes (e.g., Sulfobacillus). The glycolic acid in all of these acidophiles might be due to the activity of RuBisCO (Nancucheo and Johnson, 2012). Previous studies have confirmed

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the presence of Sulfobacillus in mine tailings (Coral et al., 2018;Yu et al., 2018); Sulfobacillus has the ability to oxidize or reduce Fe(III) and oxidize sulfur (Dold et al., 2005). This ability is important, as this genus likely leads to a high mineral dissolution rate by adhering to mineral surfaces and further enhancing sulfide mineral oxidation (Li et al., 2016;Becker et al., 2011). None of the cbbL or cbbM genes identified were highly homologous to genes in Leptospirillum, but this may be due to primer specificity. Nevertheless, Marín et al. (2017) found that the rTCA carbon fixation pathway genes were mainly found in by Leptospirillum spp. RuBisCO is the most prominent enzyme, and the gene coding for the large subunit of RuBisCO serves as a marker for the analysis of autotrophic organisms, including bacteria, using the CBB cycle (Berg, 2011). In addition, the Sulfobacillus-like cbbL gene dominated the 13C-labeled DNA of the carbon-fixing taxa. Furthermore, the higher relative abundance of Sulfobacillus than Leptospirillum, according to 16S rRNA analysis, demonstrates the contribution of the Sulfobacillus-like cbbL gene to carbon sequestration. Even so, while the number (or relative abundance) of autotrophs demonstrated their ability to sequester carbon, it did not reflect their ability to perform or their importance in ferrous and sulfur oxidation. For example, the reduced percentage of the genus Acidithiobacillus in the two mine tailings did not reflect the contribution of this genus to the oxidation of iron and sulfur. Falagán et al. (2017) highlighted the importance of thermotolerant acidophiles, such as Acidithiobacillus and the genus Sulfobacillus, in extracting and recovering metals from mine tailings. Furthermore, it has been known for many years that Acidithiobacillus can obtain energy by catalyzing the oxidation reaction of Fe2+ to Fe3+ from sulfites (Dold, 2014), and this may significantly speed up the rate of ferrous oxidization. The decreased level of total carbon, including low-molecular-weight carboxylic acids LMWCA, may also limit the activity of this bacterium. In conclusion, this study was the first to elucidate carbon sequestration by autotrophic groups in mine tailings based on isotope tracers and DNA-SIP. Our results demonstrated higher 13C atom % values with the addition of pyrite than in control groups after a 14-day incubation, as well as a significant increase in the total organic carbon content. The Sulfobacillus genus

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was dominant in the pyrite-treated bacterial communities and was also the primary carbon fixer with the RuBisCO form I-encoding gene cbbL. Finally, the cbbL gene may play a vital role in carbon sequestration in the sulfide mineral oxidation of mine tailings."

Please also note the supplement to this comment:
https://www.biogeosciences-discuss.net/bg-2018-370/bg-2018-370-AC2-supplement.pdf

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Interactive comment on Biogeosciences Discuss., https://doi.org/10.5194/bg-2018-370, 2018.

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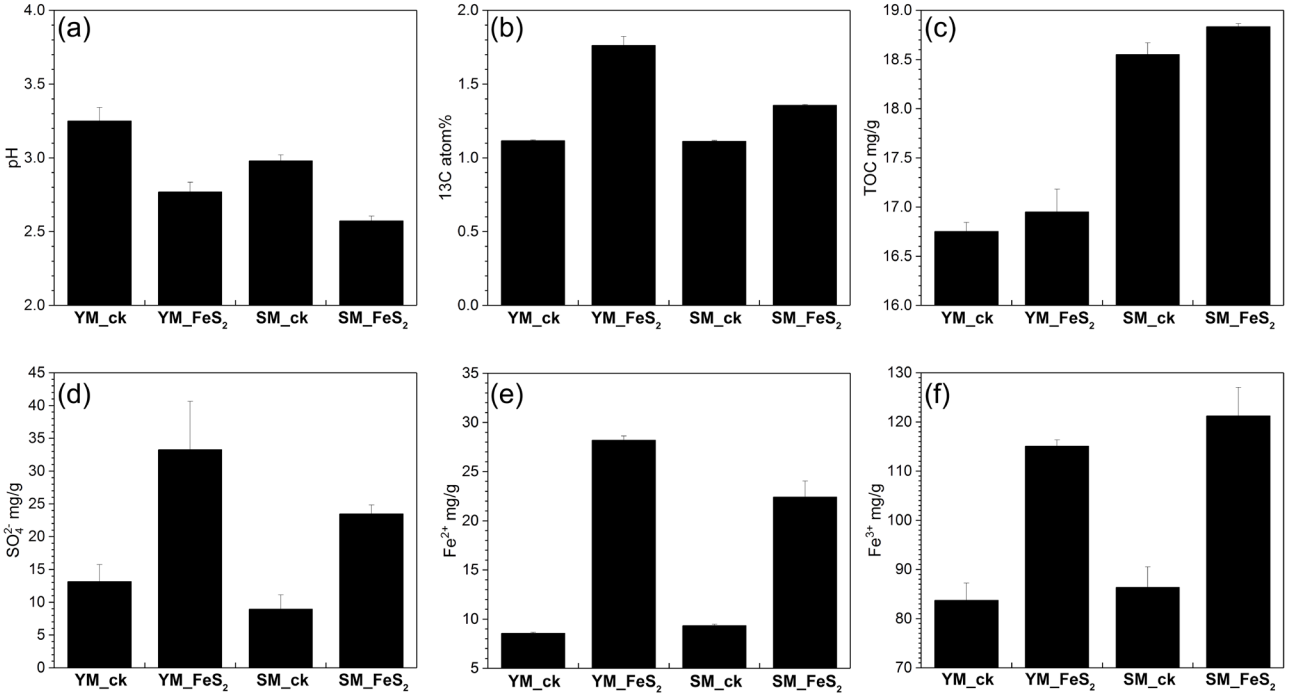

Fig. 1 pH values (a), ^{13}C atom % (b), TOC (c) contents, SO_4^{2-} (d), Fe^{2+} (e) and Fe^{3+} (f) in mine tailings. The error bars indicate the standard errors of three subsamples for each tailing sample. To determine ^{13}C atom % (b), all analyzed samples were treated with ^{13}C-CO_2 in microcosms. YM_ck, control group of Yangshanchong mine tailings; SM_ck, control group of Shuimuchong mine tailings; YM_FeS_2, Yangshanchong mine tailings treated with FeS_2; SM_FeS_2, Shuimuchong mine tailings treated with FeS_2.

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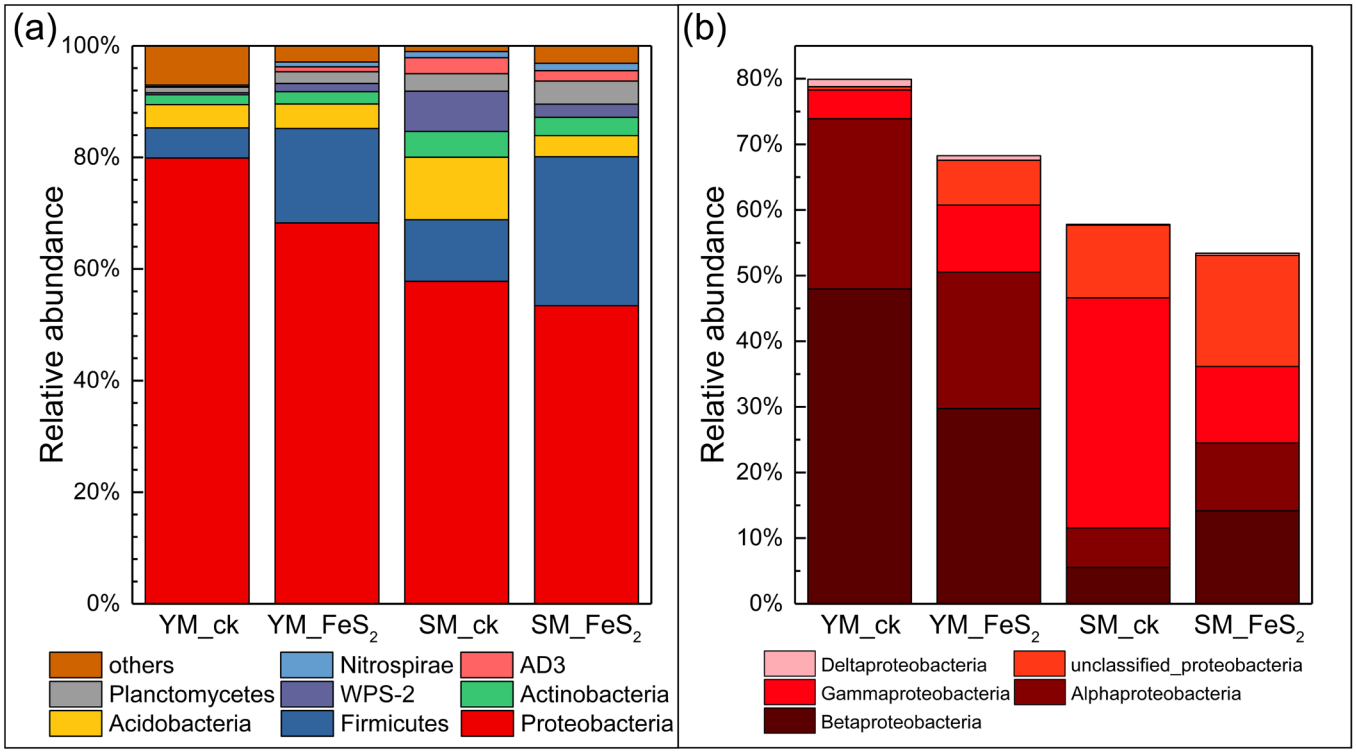

Fig. 3 Relative abundances (percentages) of the main identified bacterial taxonomic groups, i.e., the phyla Proteobacteria, Firmicutes, Acidobacteria, Actinobacteria, WPS-2, Planctomycetes, AD3 and Nitrospirae (a); and the classes Alphaproteobacteria, Betaproteobacteria, Deltaproteobacteria and Gammaproteobacteria (within the phylum Proteobacteria) (b). For each tailing sample, the relative abundances of the sequences assigned to a given taxonomic unit were calculated for each of three subsamples, and the average value was then used to represent the relative abundance of each tailing sample.

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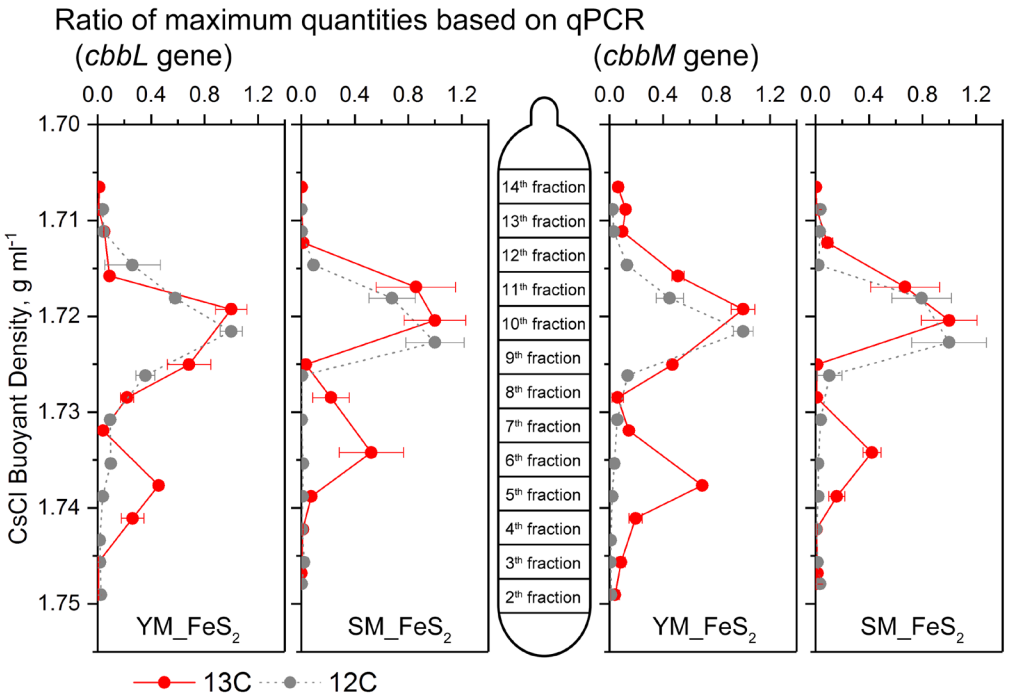

Fig. 5 Quantitative distribution of *cbbL* and *cbbM* gene fragments across the entire buoyant density gradients of the DNA fractions from microcosms treated with FeS_2 and incubated with ^{12}C-CO_2 or ^{13}C-CO_2. The normalized data consist of the ratio of the gene copy number for each DNA gradient to the maximum quantity for each treatment. The error bars represent the standard errors of triplicate microcosms, and each contains three technical replicates.

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