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Biogeosciences Discussions

Interactive comment on "Can Mg isotopes be used to trace cyanobacteria-mediated magnesium carbonate precipitation in alkaline lakes?" *by* L. S. Shirokova et al.

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Reply to the comment of the anonymous referee No 2 "Biogeosciences Discuss., 8, C3691–C3695, 2011"

Comment 1: Arguments on materials and methods The reviewer argued that the material and methods are incompletely described. In the original manuscript, we intentionally shortened the description of experimental methods because these techniques were thoroughly described in previous work conducted with Glooecapsa sp. cyanobacteria (Mavromatis et al., 2011, Geochim Cosmochim Acta, in press). We extended the materials and methods section in the revised version as requested.

(a) The reviewer requested us to produce a Table listing the starting and final chemical composition of all solutions. This is exactly what is given in the Electronic Supporting Information, the Table ESM-2 containing 3 pages of numbers. The initial composition of all the experiments is also stated in section 2.4 of the manuscript. Besides, we provide the intermediate solution composition to illustrate the evolution of experimental solution in the course of experiments. Note that the names of bacterial species are also given in Table 1 of the main text: all experiments were conducted with Synechoccocus sp. except S-Bio-8 and S-Bio-11 as indicated in the footnote of Table 1.

The reviewer pointed out that the results often refer to Gloeocapsa sp. whereas no experiments were performed with Gloeocapsa bacteria. This is not completely true. In end of section 2.3 we stated "Therefore, several experiments on the Lake Salda water were performed using a previously described model Gloeocapsa sp. culture (Pokrovsky et al., 2008; Mavromatis et al., 2011)." It is clearly stated in Table 1 footnote that Experiments S-Bio-8 and S-Bio-11 were performed with Gloeocapsa culture. We do agree that in several places in the text, the term "Gloeocapsa sp." was misused instead of "Synechoccocus sp." and we corrected this accordingly. We also added an explicatory sentence on the cyanobacterial species used in revised section 2.3.

(b) The reviewer requested that control experiments should have the same composition that the medium used for the culture experiments. This is exactly our approach for modeling the precipitation of Mg carbonates by cyanobacteria. The best way to preparer control solution was to use the cell growth media having the same temperature, ionic strength, pH, alkalinity and dissolved organic carbon (DOC) and this method was used in our work as clearly stated in the section 2.4. According to the reviewer, dead/autoclaved cells should be used in control experiments. The autoclaving of bacterial culture will inevitably change the physical properties of bacterial surface due to denaturation of membrane proteins thus making them very different from non-heated cells. Instead, sodium azide treatment of live cultures should be used, as was employed in our previous work on Gloeocapsa sp. cyanobacteria (Mavromatis et al.,

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Geochim Cosmochim Acta, in press). Conclusions achieved in our previous experimental work with both autoclaved cyanobacteria culture and cell-free reactors are very similar to those of the present study. As such, we think that sterile cell-free experiments may serve as an adequate model for abiotic Mg-carbonate precipitation. Note that the use of special inhibitors of photosynthesis to produce most realistic control cells (e.g., Merz, 1992. The biology of carbonate precipitation by cyanobacteria. Facies 26, 81–102) was beyond the scope of this study. We agree to rename "biotic experiments" to "bacterial culture experiments" and we did so in the revised version of the manuscript.

Comment 2: The reviewer requested presenting X-ray pattern of the mineral precipitates. We agree and in the revised version, the X-ray patterns of precipitated Mg-carbonates are given (Fig. ESM-2 of the Electronic Supporting Information). The reviewer made several remarks suggesting to rename "needle" to "elongated crystal" We corrected the text as following: "SEM images (see Fig. 2E-J) revealed that nesquehonite exhibited an acicular habit (see Fig. 2E), whilst the dypingite was present as 2 to 10 μm diameter aggregates that grow with time to 5-15 μm rosettes (see Fig. 2F)." Note that there is no Fig. 1E in the text, cited by the reviewer. The Fig 2G is a representative image of precipitate composed of mostly hydromagnesite as proven by X-ray analysis. We agree, however, that this hydromagnesite is represented by mineralized cells with rounded particles on their surfaces and we corrected the text accordingly. We do not agree with the reviewer that Fig. 2H represents organic matrix: As it is stated in section 2.5 prior to SEM analyses the organic material was removed by H2O2 treatment. Therefore, there is no organic material in Fig 2, only mineral phases. The reviewer argues that in Figures 3b and 3c, there is no mineral precipitate. We do not completely agree. The external envelope is absent in cells grown without Mg, HCO3- components, at low supersaturation (Fig. 3 F). This is stated in the end of section 3.2.1. In images 3 B and 3 C, the white color corresponds to highest electron density. We do agree that precipitation occurs at the external envelope, EPS or the organic films produced by bacterium and we corrected the text accordingly.

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Comment 3: The reviewer is confused that all experimental solutions are saturated with respect to hydrous Mg carbonate, because SI > 0. There is a misunderstanding. We used saturated states (ïĄŮ), not saturation indices (SI = log ïĄŮ) and we explained this in the revised text. In this work, the saturation state is defined as the ratio of the ion activity product of the mineral constituents (assuming aH2O = 1) to the solubility product of the mineral. All initial solutions were undersaturated with respect to the first precipitating phases, nesquehonite but the photosynthesis (or air bubbling) raised ïĄŮ to ∼1 or > 1 and then the precipitation occurs. Note that in cell-free experiments, there was no precipitation despite the fact that âĎęnesquehonite was similar or even higher than that in the bacterial culture experiments.

Comment 4: (a) The reviewer argues that the values of ïĄď25Mg and ïĄď26Mg of solutions should be the same because the source of Mg is the same in all experiments. This is simply not possible because Mg has 3 stable isotopes. The ïĄď value is calculated with respect to 24Mg (Eqn. 1).

The reviewer further argues that we should analyze the Mg isotopic composition of the MgCl2 powder used in experiments. This Mg salt has been analyzed in published work of Mavromatis et al. (GCA, 2011). In response to this comment, we added ïĄď25Mg and ïĄď26Mg values of MgCl2 salt in Table 3 of the revised manuscript. Note that the initial ïĄď26Mg is irrelevant to ïĄĎ26Mgmineral-solution used in our study to assess the fractionation in natural and experimental systems.

The reviewer argues that the values of ïĄď25Mg and ïĄď26Mg in precipitated minerals should be the same as those of the precipitating solutions. This is simply not possible because Mg has 3 stable isotopes and because ïĄĎ26Mgmineral-solution is not equal to zero.

The reviewer required to present the supersaturation index of hydromagnesite and dypingite. Unfortunately, there is no reliable thermodynamic data for these solid phases. However, based on experimental solution composition presented in ESM-

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2, an interested reader will be able to calculate ïĄŮdypingite once its K°sp becomes available.

The reviewer argues that the value of saturation state that we calculate in this work corresponds to bacterial cells and organic material rather than the mineral crystal. This is not true because we used the solubility product of nesquehonite, not the Mg-organic matrix (which is unknown anyway). At our experimental conditions, the Mg concentration in solution (10 mM) is an order of magnitude higher than that of DOC (on the order of 1 mM). Given that high molecular weight organic ligands capable of complexing Mg in solution contain several carbon atoms, the final metal to ligand ratio should be significantly higher than 10. At these conditions, less than 10% of total dissolved Mg may be complexed with dissolved organics and as such this complexation can be neglected for ïĄŮ calculation. We added necessary explanation in the revised text (section 3.2.2).

Finally, the last comment of reviewer – that the Mg isotopic composition should be the same in the solution and in the crystal precipitated and that isotopes cannot fractionate if the temperature is kept constant in abiotic experiments – demonstrates her/his certain misunderstanding of the basic principles of isotope fractionation and contradicts to numerous recent results on other stable metal isotope fractionation in experimental and natural settings.

(b) The reviewer inquires how we separated nesquehonite from dypingite and/or brucite and how we performed the respective Mg isotope analyses. We have not performed any physical separation of precipitated minerals. As described in the text (section 2.4), aliquots of the homogeneous suspension (containing the fluid, precipitated mineral phase, and cells if present) were sampled periodically from the reactors. The mineral identification was performed routinely by XRD analyses after cleaning of the mineral in H2O2. In many samples, one single mineral phase was identified and as such we attributed the measured ïĄď26Mg to this particular mineral. However, in other cases, two minerals were simultaneously present as indicated in Table ESM-2. For these, the

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isotopic analyses correspond to the mixture of minerals as indicated in Table 3.

Comment 5: The reviewer noticed several inconsistencies in sample description between tables. (1) Experiment S-Bio-1 produced dypingite after 13 days of exposure but we found only brucite as mineral phase at the last, 43rd day of sampling (Table ESM-2). The isotopic composition analysis was performed in this last sample, which was composed of brucite. We corrected the text accordingly. (2) Experiment S-ABio-1 always precipitated dypingite as indicated in Table ESM-2; isotopic analysis (Table 3) were performed on dypingite and the precipitation rates were measured (Table 1). We corrected Table 1 of the revised manuscript because the term "dypingite" was missing in the last column and we thank reviewer for pointing this out. (3) The reviewer stated the following: "In table 1 authors say that nesquehonite and dypingite were formed in S-ABIO-5, whereas for the same experiment in Table 3 only nesquehonite was formed;". This is not correct. No mineral precipitation occurred in experiment S-ABIO-5 as clearly stated in Table 1 and Table ESM-2. There is no data for S-ABIO-5 in Table 3. We have not corrected the text in response to this comment.

Further on, the reviewer continues: "...in Table 5 authors say that no precipitates were formed in experiment S-BIO-5, whereas in Table 5 the authors report that nesquehonite was formed in experiment S-BIO-5. In table 5 below S-BIO-5, the authors list a new experiment S-f-5 culture: which experiment is this?" Again, we do not understand this comment. There is no Table 5 in our manuscript. Experiment S-BIO-5 produced nesquehonite and, at the end, dypingite as clearly stated in Table ESM-2. Only nesquehonite intermediate samples were analyzed for isotopes as it is stated in Table 3. Mineral-free experiment S-f-5 is described in the end of section 2.4. We have not corrected the text in response to this comment.

Note again that detailed description of the mineral phase of collected solids at different time steps from each experiment in given in Table ESM 2.

Comment 6: The reviewer argues that the same units should be used to plot the data

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and criticized our Figure 7 where Mg concentration in millimol/L is plotted as a function of biomass in grams. We do not agree to present this figure in weight scale: Mg reacts in moles during mineral precipitation, where as the biomass is measured in grams and can hardly be presented in mol/L. More importantly, our eqn. 3 yields the molar inorganic Mg to organic C ratio in reaction product of 1.4 ïĆś 0.2 – this is the slope of dependence shown in Fig. 7. This is exactly the relationship requested by reviewer which adequately describes the biomass production – mineral precipitation process occurring in experimental system. Note that, when one plots element concentration as a function of time or pH, it is difficult to use the same units... We have not corrected the text in response to this comment. Further on, the reviewer requested to have the same physico-chemical composition and temperature between biotic and abiotic experiments. This is exactly what we did and the chemical compositions of initial solutions of both types of experiments are listed in Table ESM-2. Several distinct types of bacterial culture experiments were performed at 25 $\pm$ 2°C as stated in Section 2.4. We have no answer why the reviewer decided that the temperature was 21°C. We specified in the revised version that both temperature and initial chemical composition were similar among biotic and abiotic experiments.

Following recommendation of reviewer, we verified the consistency amongst methodology, analyses and results, and we checked that our interpretations and conclusions remain the same as in the first version.

Interactive comment on Biogeosciences Discuss., 8, 6473, 2011.