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Interactive comment on "Application of clustering techniques to study environmental characteristics of microbialite-bearing aquatic systems" *by* R. Dalinana et al.

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The second reviewer made some very good points about our analysis, which we have addressed, but also made some errors with regard to basic geochemistry and geology, that we have tried to clarify as best as possible. Additionally, some of the review was confusing, though we have tried to accommodate the concerns to the best of our understanding. The reviewer's main concern was the relationship between the parameters and the different aspects involved in lithification:

"In the present version of this manuscript it is not clearly defined which process is being considered. The formation of microbialites involves several fundamentally different

processes, each of them responding to different factors. In a first step, a microbial biofilm or microbial mat may grow to a variety of shapes and sizes. Only a minority of microbial mats is in fact calcifying and results in the formation of a hard lithified microbialite. The lithification process may also occur via different mechanisms (microbially induced vs. microbially catalysed precipitation). A distinction between processes would be fundamental for this study."

In general, there are only two ways in which microbialites form. microbiallty-induced precipitation, and trapping and binding of grains. We are unsure of what the reviewer means by "microbially induced vs. microbially catalysed precipitation", which would appear to be the same thing. Perhaps they mean 'microbially induced', which refers to changes in alkalinity/saturation state due to biology, vs. 'microbially influenced', which refers to the mineralization of organic matter (either passively by ambient chemistry or through changes in chemistry through metabolism). Regardless, all the forms of microbialites in this study are thought to form via microbially-induced precipitation, which would not require further distinction.

"For example, microbial mat growth may respond to competitive advantages with other organisms, or to the availability of organic matter substrates, a redox gradient, light intensity or inorganic macro- or micronutrients. In contrast, microbially induced precipitation depends on carbonate supersaturation (as a result of pH, alkalinity, DIC, Ca and Mg concentration). Mixing these two processes is like comparing apples to pairs. Correlations are invalid as they can be assigned to the wrong processes."

While the reviewer is correct in saying that comparing these processes would be problematic, we are not doing so in this manuscript. All of the study sites have abundant microbial communities, and we have no reason to think these mats are limited in any way. We are not testing whether or not the microbes can grow – they are growing. We are testing whether or not there is a unique set of geochemical parameters between disparate sites that may account for presence vs. lack of microbialites.

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"Probably the most severe flaw is the confusion between cause and consequence of the investigated parameters. For example, pH is often more strongly altered by the microbial community than it varies in the surrounding environment. Therefore the main output of this study, the pH not being a controlling factor, is meaningless, since the pH is the result and not the cause of the process. The same is the case for several other parameters, such as alkalinity, DIC, Ca and all other metabolites within the diverse microbial communities."

While local changes in pH, alkalinity, etc do occur, and can affect the local microenvironment from which carbonate precipitation takes place, they are not permanent and fluctuate in the opposite direction when heterotrophy takes over during night cycles. The overall environment (such as the lake or the ocean pH) is a starting point that can be altered by microbial metabolism, but the local environment is not completely independent from the environment. It is the starting point from which dissolution and precipitation can take place and must be calculated, and therefore dismissing environmental pH seems misguided.

Additionally, in the sites studied for this manuscript, pH, alkalinity, etc would all indicate a propensity to precipitate, with or without the changes in local chemistry of a microbial mat. Therefore, local changes in carbonate saturation are not needed to explain microbialite formation.

“A further really fundamental shortcoming is the lack of a negative control. For a meaningful evaluation of the data, the authors should include datasets from sites showing microbial mat formation but lacking any calcification or microbialite growth. It should not be difficult to find such datasets, as non-calcifying mats are the majority. A possible study site would be Laguna Figueroa (Baja California).”

We are fairly confused by this comment, as we do include both Mono Lake, which has tufa but not microbialites, and open sea water as negative controls. They are described as being used specifically as an outgroup, and details of the sites are given

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in section 2.2, titled “Non-microbialite forming systems”. Additionally, we have the mats of Stinking Springs, which contain small amounts of carbonate grains, but cannot be classified as ‘microbialites’. In order to clarify this, we can change the text to specify that in the context of these analyses, an ‘outgroup’ is the same as a negative control.

"The study does include positive controls with abiotic microbialites. But the question is again, which process is being considered."

This comment is confusing, as there is no such thing as an ‘abiotic microbialite’, and we never refer to such in the text.

"These control sites will certainly resemble the microbialite sites in terms of increased saturation state. Thereby I suggest comparing saturation indices (SI = log IAP – log Ksp) between all of the sites. In terms of microbial growth conditions (nutrients etc.) the microbialite sites are likely rather different from the control sites"

Since parameters were pulled from the literature, we could not calculate IAP for all sites. However, in spirit, this comment is similar to the one made by Reviewer 1, asking us to consider the coupling the parameters of [Ca], pH, and alkalinity into one variable that could then be clustered with the others. As a substitute for SI, which we could not retrieve from the literature in all cases, we believe the extra PCA analysis, and the generation of new clusters based on these analyses address the concerns presented here. We would add a description of this to Section 3.2:

“To see whether not one, but a combination of features can lead to a different clustering we used principal component analysis to derive a linear combinations of variables at hand. Principal component analysis (PCA) is a standard statistical technique for reducing dimensionality of the dataset to a few components explaining most of the variability in the data. In order to be able to apply the technique we omit Ba from this portion of the analysis due to its many missing values. As seen from Table 3, 3 components explain 92% variability in the data. From magnitude of the loadings, we see that the first component contains information from all dimensions except pH, Si, and Alkalinity

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2nd component is mostly drive by pH and Alkalinity; 3rd component has the largest loading for Si. Table 4 contains the components and loadings. Then, aforementioned k-means with partial distance is performed on the 3 resulting components. The scree plot (Figure 7) shows that similarly to previous findings we see 2 or 3 clusters in the data. Cluster assignment also remains the same: Mono lake sample forming one cluster, Kiritimati samples forming second cluster, and all other observations forming 3rd. If repeat this exercise with two clusters, Kiritimati and all others form the two groups. This exercise confirmed clustering we have seen with using k-means directly on the features. “

We also added a new figure (Figure 7, attached), showing that the new parameters do not influence the overall clustering pattern. Additionally, we are unsure why the non-microbialite bearing sites would be different in terms of microbial growth conditions. All sites were specifically chosen because they have vibrant, healthy, and abundant microbial communities, yet some have carbonate microbialites, and some do not. If anything, the difference should be seen in the carbonate system.

"A further question is, whether abiotic and microbially influenced microbialites can be really distinguished. We may think that microbial mats sometimes just become calcified, without actually contributing to their calcification. Such mechanisms have been recently suggested by Castro-Contreras for Laguna Bacalar (Mexico) or by Birgel et al. for Lagoa Salgada (Brasil)."

We are unsure of what is meant by ‘abiotic microbialite’. In either case, the question of biogenicity is not in the scope of this manuscript. All of the forms included (aside from negative controls) are known to be biogenic.

"Consumption of CO2 by photosynthesis does not affect the alkalinity. It increases the pH and decreases the DIC content, resulting in an increase in saturation state with respect to calcium carbonate."

This is an odd simplification of carbonate chemistry. Uptake of CO2 by photosynthe-

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sis perturbs the carbonate equilibrium and leads to an increase in alkalinity – these processes cannot be decoupled. In the literature, CO2 uptake via photosynthesis is commonly referred to as the 'alkalinity pump'.

"P. 9, Line 15: Microbial carbonates that are not microbialites? This is not consistent with the definition of "microbialite" given in the introduction: "Organosedimentary deposits formed by trapping and binding or by microbially induced precipitation of minerals" (Burns and Moore, 1987). Is there an example of a microbial carbonate that is not a microbialite? Besides, it would be interesting to evaluate the cluster analysis in terms of type of microbialite (e.g. stromatolites vs. thrombolites, etc.)."

A microbialite is a rock (It is basic geology to use the suffix "-ite" to denote a fully lithified structure). A single carbonate grain within a mat cannot be termed a microbialite. It is just a mat with a grain in it. There is no researcher who would term the mats of Stinking Spring 'microbialites'. They are still unlithified microbial mats.

"The study lacks some essential literature references: A whole suite of papers has been published by Shiraishi et al. and Bissett et al. on microsensor measurements from the Deinschwanger and Westerhöfer Bach. These studies show very convincingly that precipitation is induced by the phototrophic action within cyanobacterial biofilms"

We would add these references to the text.

Interactive comment on Biogeosciences Discuss., 12, 10511, 2015.

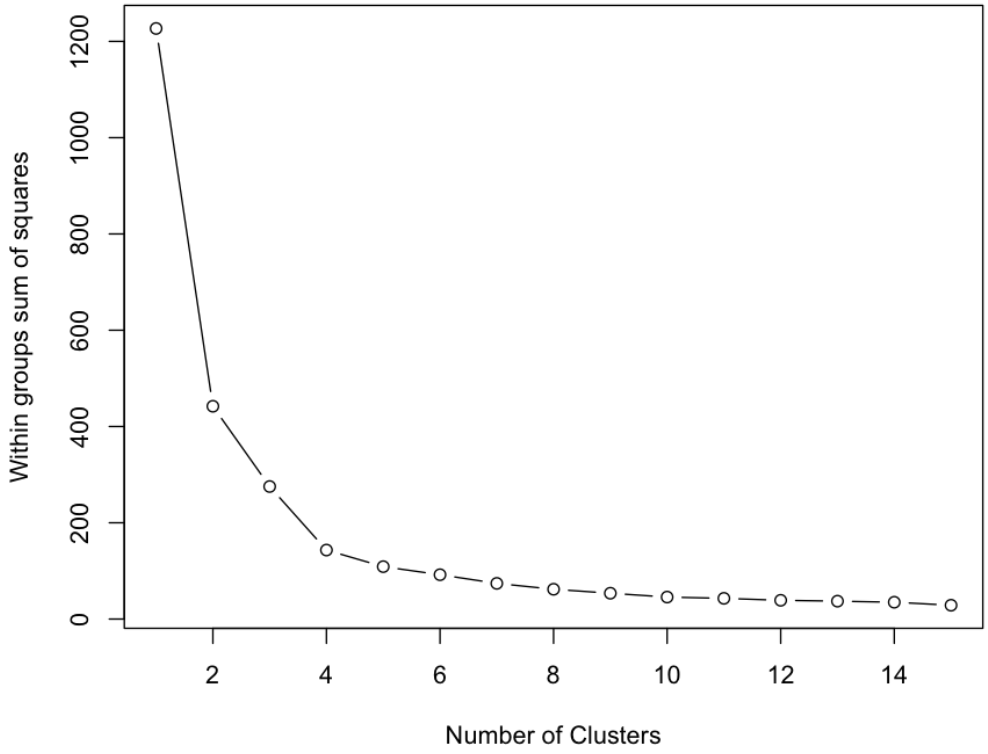

Fig. 1.

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