

# The chemical conditions necessary for the formation of microbialites

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## Supplementary Information

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- Materials and Methods
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## Materials and Methods

**Setup of a new modern microbialite-hosting environment database and description of the database.** Modern microbialite localities were identified by searching the literature published between 1962 and 2022. For this purpose, the terms “microbialite”, “microbialite-forming mat”, “stromatolite” and “thrombolite” were used in search engines such as Web of Science or Google Scholar as well as the ScienceDirect, SpringerLink and ResearchGate platforms. Papers describing microbialites and containing chemical data on the aqueous environments where these microbialites form, were retrieved from papers listed in the database. Sedimentary structures referred to as “tufas” or “travertines” were not compiled in the database because of the significant differences sometimes attached to these terms (Pentecost and Viles, 1994). While there might be some subjectivity in choosing the term microbialites instead of another one, we did not add further subjective bias by reappraising ourselves the validity of the terms used in published studies.

The physicochemical parameters of the aqueous environments in which modern microbialites form were further searched when needed in additional publications not focusing on microbialites. Overall, we managed to compile 140 localities with 30 major variables into this database. The database is reported in Table S-1. Temperature, pH, conductivity, major ion and trace element concentrations, and some isotopic data (e.g.,  $\delta^{18}\text{O}$ ,  $\delta^{34}\text{S}$ ,  $\delta\text{D}$ ,  $\delta^{13}\text{C}_{\text{org}}$ ,  $\delta^{13}\text{C}_{\text{DIC}}$ ) were compiled when available. The type of environment (lacustrine, marine) as well as some details on the data collected from the literature and references were added. Moreover, the database contains the sampling dates and depth of microbialites, the major mineral phases composing them and the geological context. For aqueous environments of the GLEON database where both DIC and alkalinity were available, we checked that there was a 1:1 correlation between these parameters (Fig. S-2; Dickson, 1981). Then, for seven microbialite-hosting environments for which DIC was not available, DIC was equated to the alkalinity. Concentrations provided in  $\text{mg L}^{-1}$  or ppm were converted into mM in the database. For 81 localities, several water sample analyses were available and all of these data are reported, resulting in a total of 610 aqueous geochemistry analyses in the database.

Figure S-1 shows that modern microbialites can be found all over the world. The chemical types of all aqueous environments were determined according to the approach by Boros and Kolpakova (2018) by plotting them in  $\text{Ca}^{2+}$ – $\text{Na}^+$ + $\text{K}^+$ – $\text{Mg}^{2+}$  and  $\text{SO}_4^{2-}$ – $\text{Cl}^-$ – $\text{CO}_3^{2-}$ + $\text{HCO}_3^-$  ternary diagrams (Fig. S-3a). This shows that environments hosting modern microbialites are hydrochemically diverse and can be 'saline' for lakes with calcium and carbonate concentrations below 25 %, 'soda-saline' for lakes with  $\text{Ca}^{2+}$  and DIC contents >25 % and  $\text{Na}^{2+}$  as the dominant cation and carbonates not as the dominant anions, and 'soda' for lakes with  $\text{Ca}^{2+}$  and DIC contents >25 % and  $\text{Na}^{2+}$  and carbonates as the dominant cations and anions. In this classification, 53 % of microbialite-hosting environments are saline, 23 % soda-saline, and 24 % soda on a total of 95 environments. Microbialite-hosting aqueous solutions were also plotted in a ternary diagram defined as  $\text{Mg}^{2+}$ – $\text{SO}_4^{2-}$ – $\text{HCO}_3^-$ + $\text{CO}_3^{2-}$  (Deocampo and Jones, 2014) (Fig. S-3b). Red arrows indicate chemical evolutions upon evaporation and precipitation of calcite and dolomite. This shows that many saline microbialite-hosting environments may result from advanced evolution of an initially high  $\text{Ca}^{2+}/\text{CO}_3^{2-}$  solution. However, several saline environments may have had a low initial  $\text{Ca}^{2+}/\text{HCO}_3^-$  and became enriched in sulfates. By contrast, for soda-saline and soda microbialite-hosting environments, most may result from the evolution of an initially low  $\text{Ca}^{2+}/\text{CO}_3^{2-}$  solution and kept relatively sulfate poor.

#### ***Environmental Data Initiative (EDI) and Global Lake Ecological Observatory Network (GLEON) datasets.***

The dataset of freshwater environments from the Global Ecological Observatory Network (GLEON, <https://gleon.org/>) was previously analysed by Weyhenmeyer *et al.* (2019). It groups 160,660 analyses of lake samples and reports six parameters: water depth, pH, temperature,  $\text{Ca}^{2+}$ , and dissolved  $\text{CO}_2$  and DIC contents. Only data of lakes with available data for  $\text{Ca}^{2+}$  and DIC concentrations were extracted and used here, which represent 105,678 samples from 6,422 North European environments. Of those, at least 78 % had pH and/or temperature parameters.

The dataset from the Environmental Data Initiative (EDI, <https://portal.edirepository.org/nis/browseServlet?searchValue=limnology>) in the section “limnology” contains analyses of 29,176 samples from ~1600 environments, with 18 main variables: lake elevation, size, water depth, pH, temperature, conductivity, DIC, dissolved organic matter, dissolved oxygen,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ , Si,  $\text{Na}^+$ ,  $\text{Cl}^-$ ,  $\text{K}^+$ ,  $\text{SO}_4^{2-}$ ,  $\text{NO}_3^-$  and  $\text{NH}_4^+$  contents. Only 28,455 analyses from over 1,547 lakes from North America were selected as they included both  $\text{Ca}^{2+}$  and DIC contents. Interestingly, this database contained more parameters than the GLEON one, including the concentrations of several other major ions for over 40 % of the data. This allowed us to compare this database with the modern microbialite database on more parameters using PCA (Fig. 2 in the main text).

***Speciation calculations.*** The activities of major ions were calculated with the PHREEQC software (Parkhurst and Appelo, 2013) based on the Hückel-Debye method, and taking into account the temperature in the activity calculation for the solubility products of  $\text{CaCO}_3$ . The thermodynamic database “thermoddem.dat” (Blanc *et al.*, 2012) was used, with the solubility constants of calcite and aragonite from Plummer and Busenberg (1982), monohydrocalcite (MHC) from Hull and Turnbull (1973), and those of amorphous calcium carbonate (ACC) measured by Brečević and Nielsen (1989) at  $K_s = 10^{-6.4}$  (*i.e.* ACC2) and Kellermeier *et al.* (2014) at  $K_s = 10^{-7.63}$  (*i.e.* ACC1).

For the calculation of activities in the microbialite-hosting aqueous solutions, all available chemical species concentrations and temperature were considered. In the GLEON dataset only  $\text{Ca}^{2+}$  content, DIC content, temperature and pH were available to calculate the ion activities. The water chemistry analyses from all the databases were validated by verifying that the electric charge imbalance was between –10 % and +10 %.

***Statistical analyses.*** The distributions of the major ion concentrations in the GLEON and EDI datasets *vs.* in the microbialite dataset are shown in Figure S-6. The differences between these distributions in the GLEON and EDI datasets *vs.* in the microbialite dataset were statistically tested. As these values are not normally distributed, the non-parametric Wilcoxon-Mann-Whitney test was chosen with a *p*-value threshold of 5 %.



**Principal component analyses (PCA).** Similar to what was done by Zeyen *et al.* (2021), we conducted PCA to assess which parameters explain most of the variability in the datasets. In these analyses, each axis (or principal component) is a linear combination of variables defined so that they maximise the spatial discrimination of samples; coordinates of vectors on each axis indicate the coefficient of the variables in the linear combination. The longer the orthogonal projection of an arrow on a given axis, the stronger the contribution of the variable to this axis. The smaller the angle between arrows, the stronger the positive linear correlation between corresponding variables. Orthogonal arrows indicate no correlation, while arrows pointing in opposite directions indicate a negative correlation. The samples are plotted in two dimensions using their projections onto the first two principal components. The part of the total sample variability explained by each axis is indicated in the axis label (Zeyen *et al.*, 2021). We compared EDI and microbialite-hosting environments. One PCA was performed on the microbialite-hosting environments dataset (Fig. S-4). A second PCA was performed on the combined EDI and microbialite-hosting environments datasets (Fig. 2d, e). For each PCA dataset, the parameters missing for more than half of the environments were removed. The environments where more than half of the parameters were missing were then removed. The missing values were further imputed based on the nearest neighboring environments. Imputation of missing values and PCA were performed using the R packages missMDA, FactoMineR and Factoshiny (Lê, Josse and Husson, 2008; Josse and Husson, 2016). The GLEON dataset was not used in this analysis because most of the water chemistry parameters describing the microbialite-hosting environments are missing.

**Prediction of overlooked microbialite-hosting environments.** The present analysis may also help finding localities where modern microbialites have been potentially overlooked so far, by screening aqueous environments based on their SI with  $\text{CaCO}_3$ . Among the 7969 lakes in the GLEON and EDI databases, around 318 were on average saturated with ACC/MHC (Fig. 3; Table S-2) and therefore appear as potentially favorable for the development of microbialites. Looking back at the literature on some of these lakes, we found reports of (i) biogenic carbonate precipitation in Locknesjön lake (Sweden; Labuhn *et al.*, 2022), (ii) tufa precipitation in Sinijärv lake (Estonia; Laumets *et al.*, 2014), (iii) calcifying microbial mats in Deer lake (Canada; Zorz *et al.*, 2019), (iv) cyanobacterial calcareous mud in Storfjärden, Hållsjön and Käringsjön lakes (Sweden; Brunberg and Blomqvist, 1999), and (v) seasonal  $\text{CaCO}_3$  production in Ripley lake (US; Garrison and Pillsbury, 2009). Only future systematic and careful campaigns will be able to determine whether modern microbialites may form in these localities or not. If all of these lakes eventually prove to host microbialites, this will further support the conclusions of the present study. If some do not host microbialites, it will be important to understand what other parameters (including biological parameters) may prevent microbialite formation in an otherwise chemically favorable environment.



## Supplementary Tables

**Table S-1** Modern microbialite-hosting environments database.

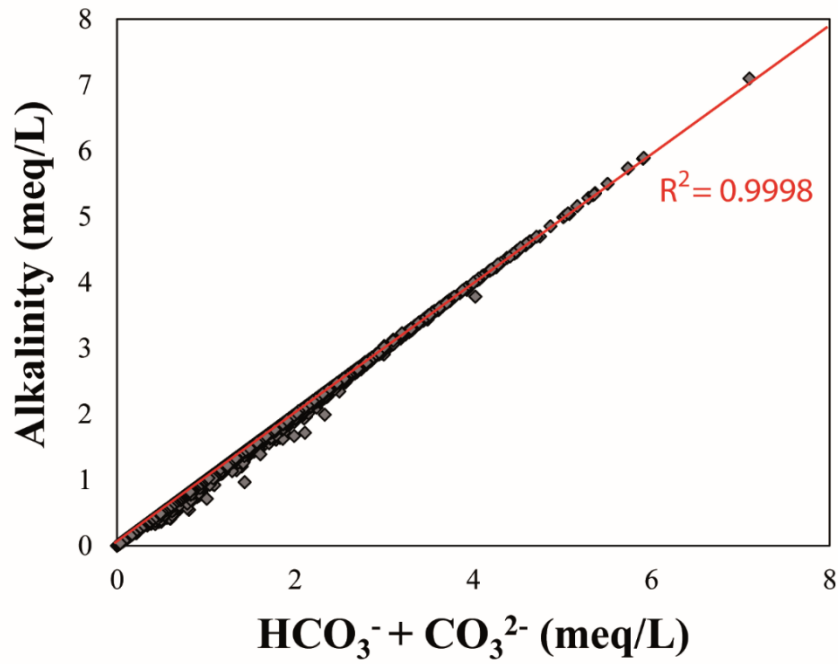
**Table S-2** List of the names of the lakes in GLEON and EDI datasets that are saturated with ACC as shown in Figure 3. Only six cases of microbially induced carbonates have been found in the literature in some of these lakes. Microbialites may have been overlooked in these environments. Some lakes may become artificially ACC-saturated, and possibly not prone to microbialite formation, following anthropic alkalinisation using liming to fight acidification. This is particularly the case in more than 20,000 rivers and lakes in Sweden (Svenson *et al.*, 1995; dataset used by Weyhenmeyer *et al.*, 2019).

Tables S-1 and S-2 (.xlsx) are available for download from the online version of this article at <https://doi.org/10.7185/geochemlet.2311>.

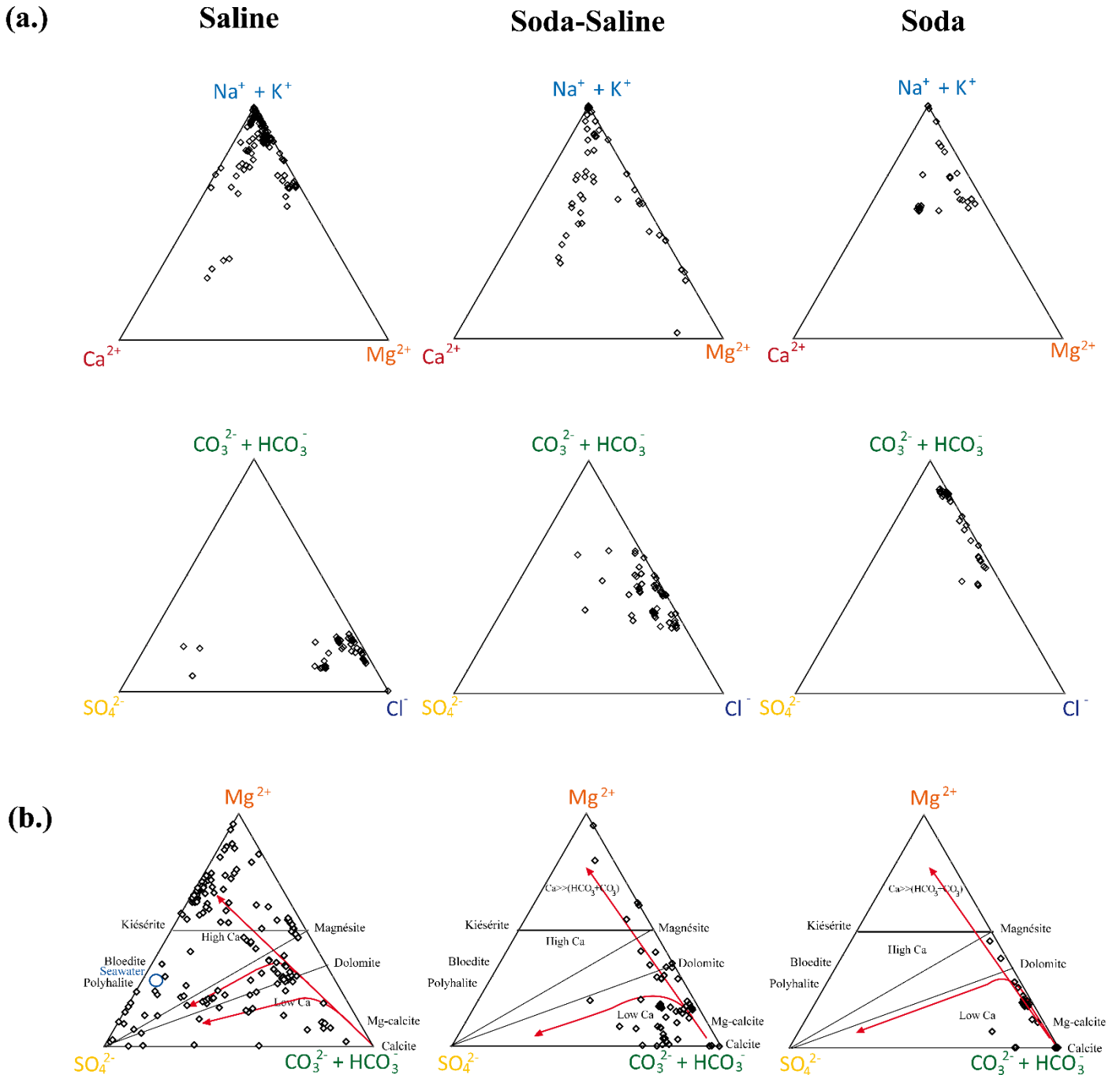
## Supplementary Figures



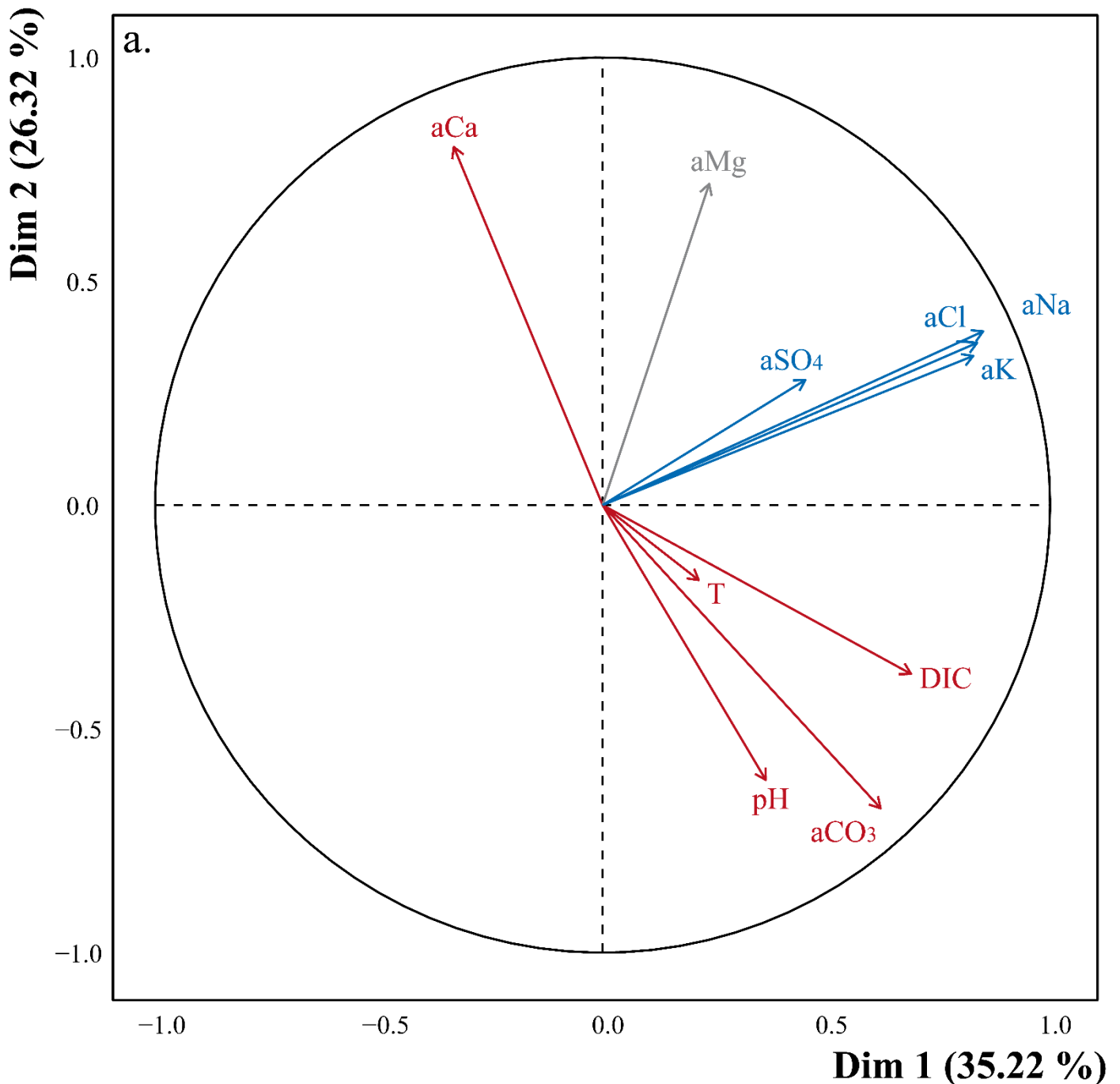
**Figure S-1** All modern microbialite-hosting environments listed in the database represented on a world map (provided by Microsoft Excel).



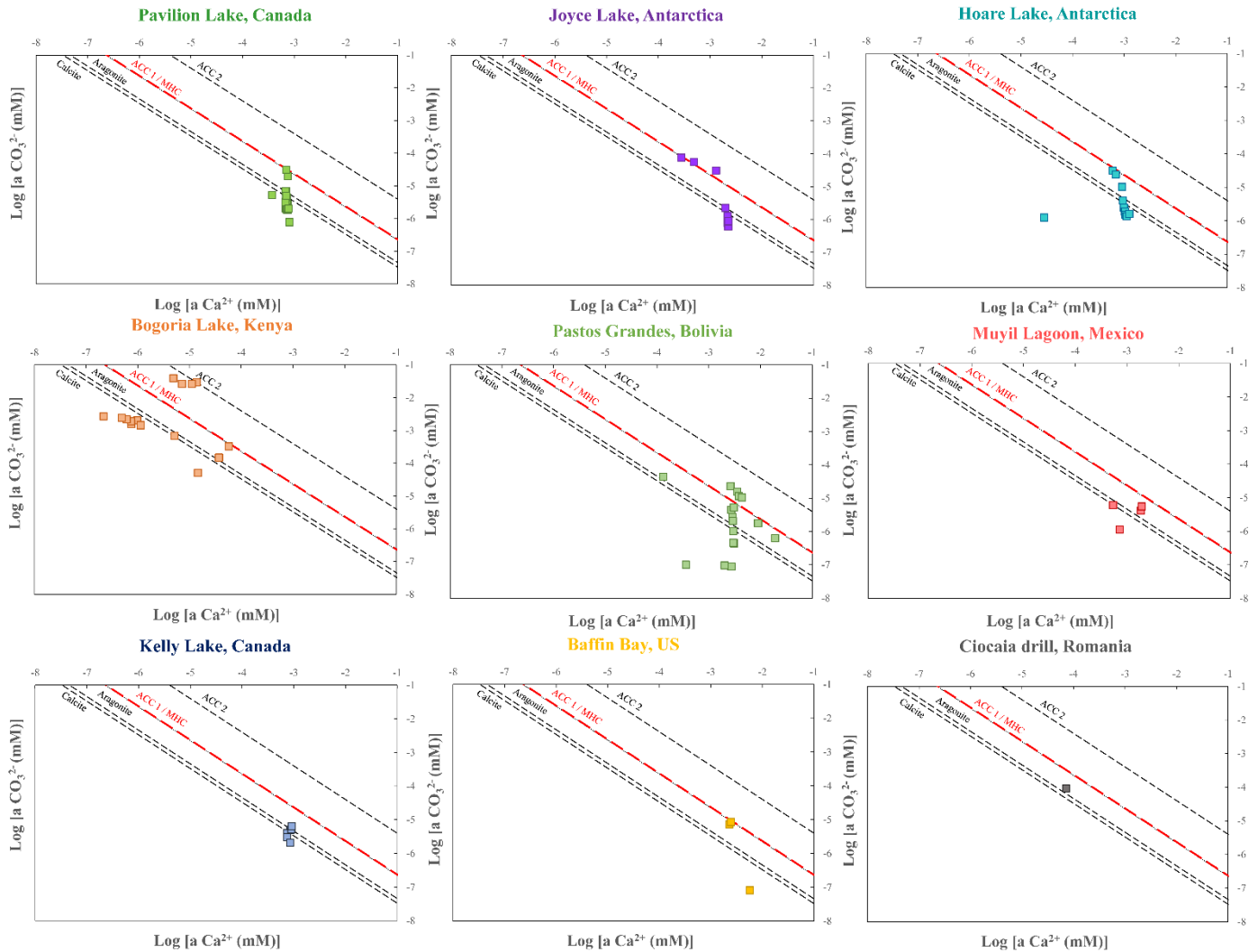
**Figure S-2** Linear correlation between the alkalinity and the DIC in freshwater environments from the GLEON dataset. We note that the pH range encompassed by these lakes is such that  $\text{H}_2\text{CO}_3$  and  $\text{CO}_2$  are not important contributors to DIC.



**Figure S-3** Ternary diagrams of microbialite environments. (a) Chemical types are assessed based on the approach of Boros and Kolpakova (2018). (b) Deocampo and Jones' (2014) triangles that show chemical evolution of aqueous environments by evaporation and precipitation depending on their initial  $\text{Ca}^{2+}/\text{HCO}_3^-$  ratio (red arrows) between modern microbialite-hosting environments.

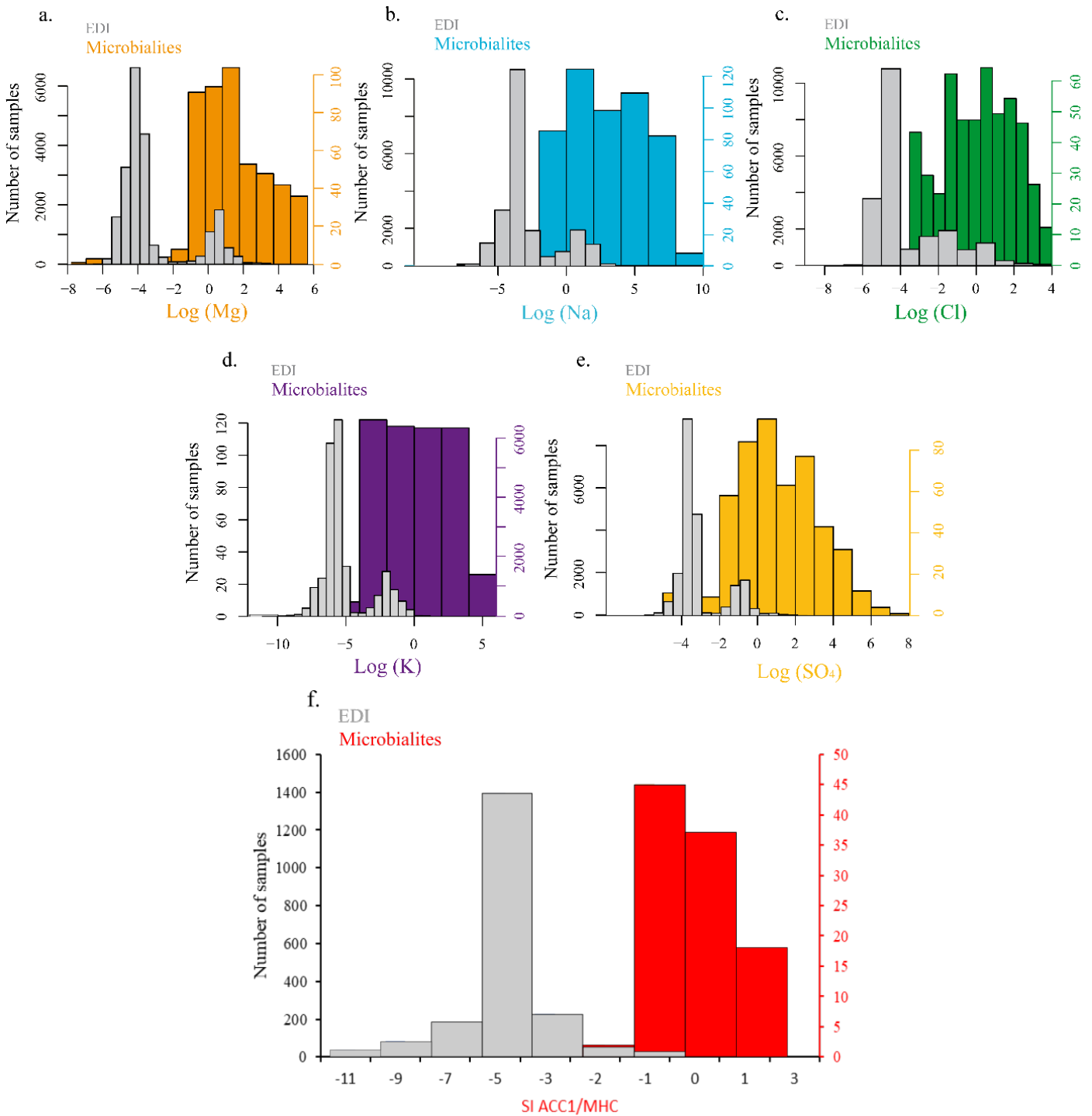


**Figure S-4** Correlation circle from the PCA on 10 physicochemical parameters from 545 aqueous samples collected from microbialite-forming environments. Ten variables were available, which are:  $\log(a\text{CO}_3)$ ,  $\log(\text{DIC})$ , pH,  $T$  ( $^{\circ}\text{C}$ ),  $\log(a\text{Ca})$ ,  $\log(a\text{Mg})$ ,  $\log(a\text{Na})$ ,  $\log(a\text{Cl})$ ,  $\log(a\text{K})$  and  $\log(a\text{SO}_4)$ . The variance of these environments is explained for almost 62 % by a dimension (blue arrows, explains ~35.22 % of variability) grouping the chemical species responsible for salinity as well as sulfates and by another dimension (red arrows, explains ~26.32 % of variability) characterising the carbonates contents, pH and temperature anticorrelated to the calcium and to a lesser extent magnesium contents.

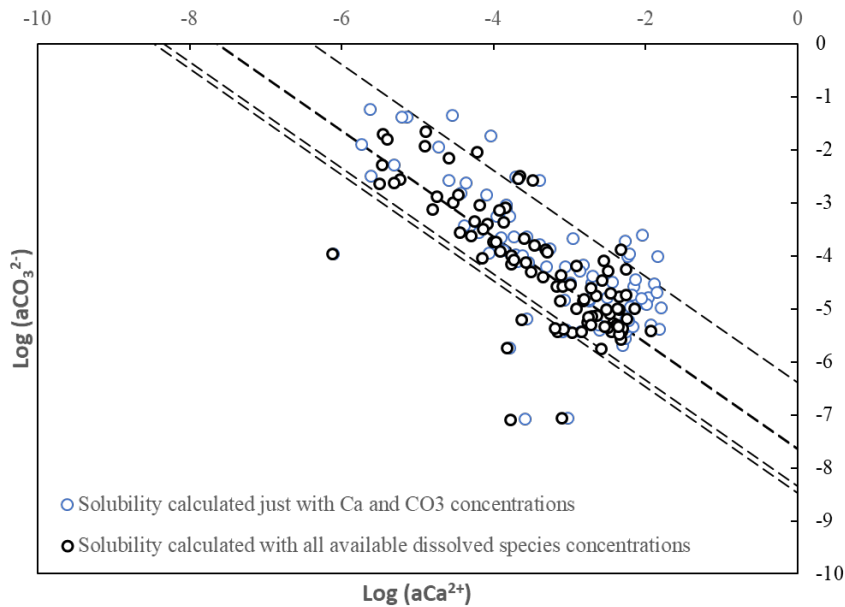


**Figure S-5** Plots of  $\text{CO}_3^{2-}$  vs.  $\text{Ca}^{2+}$  activities for aqueous environments undersaturated with ACC on average. Different squares represent analyses at different times or different locations along the aqueous system. Table S-2 lists these environments and summarises some of their characteristics. It appears that 73 % of these environments reach saturation with ACC at least once in the time and/or place of sampling; it is assumed the variability in their chemistry makes them outliers.

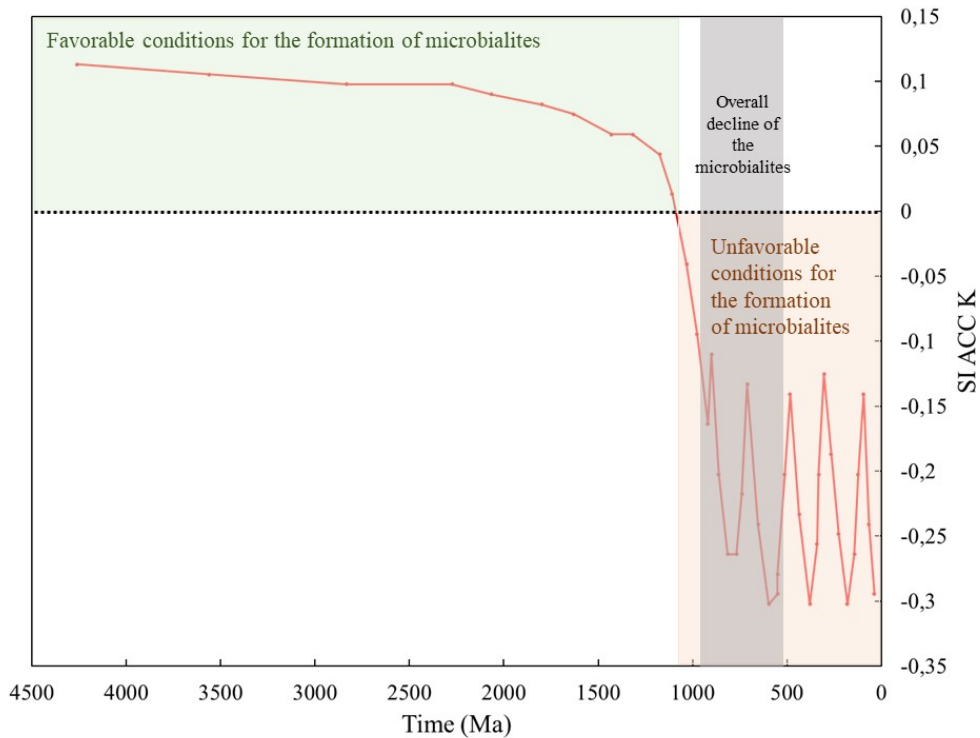




**Figure S-6** Compared distributions of the logarithms of the main dissolved chemical species (and saturation index (SI) of ACC1/MHC) in North American (grey) vs. microbialite-hosting (coloured) lakes. **(a)** Magnesium logarithm, **(b)** sodium logarithm, **(c)** chloride logarithm, **(d)** potassium logarithm, **(e)** sulfates logarithm, **(f)** SI of ACC1/MHC.



**Figure S-7** Comparison between activities in the microbialite-hosting environments (i) calculated based on the knowledge of all the dissolved species concentrations available (open black points; “standard” approach) vs. (ii) approximated based on the knowledge of pH, Ca and DIC concentrations only (open blue circles; “approximated” approach). The differences between the approximated and standard calculated activities appear minor. The mean difference based on Euclidean distances between the values of the  $\text{log}(\text{activities})$  calculated by the two approaches was 0.2.



**Figure S-8** Geological evolution of the saturation index (SI) of seawater with respect to MHC/ACC1 adapted from the model by Kempe and Kazmierczak (1994). The  $K_{s-ACC}$  was derived from Kellermeier *et al.* (2014; ACC1). The black dashed horizontal line indicates saturation with MHC/ACC1.



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