1 **The hidden role of dissolved organic carbon in the**
2 **biogeochemical cycle of carbon in modern redox-stratified**
3 **lakes**

4 Robin Havas[a,*], Christophe Thomazo[a,b], Miguel Iniesto[c], Didier Jézéquel[d], David Moreira[c], Rosaluz Tavera[e],

5 Jeanne Caumartin[f], Elodie Muller[f], Purificación López-García[c], Karim Benzerara[f]

7 [a] Biogéosciences, CNRS, Université de Bourgogne Franche-Comté, 21 000 Dijon, France

8 [b] Institut Universitaire de France, 75005 Paris, France

9 [c] Ecologie Systématique Evolution, CNRS, Université Paris-Saclay, AgroParisTech, 91190 Gif-sur-Yvette,
10 France

11 [d] IPGP, CNRS, Université Paris Cité, 75005 Paris, and UMR CARRTEL, INRAE & USMB, France

12 [e] Departamento de Ecología y Recursos Naturales, Universidad Nacional Autónoma de México, México

13 [f] Sorbonne Université, Muséum National d'Histoire Naturelle, CNRS, Institut de Minéralogie, de Physique des

14 Matériaux et de Cosmochimie (IMPMC), 75005 Paris, France.

17 * *Correspondence to*: Robin Havas (robin.havas@gmail.com)

19
20
21
22 *Keywords: Carbon cycle; isotopic fractionation; DOC; Precambrian analogs*

Abstract. The dissolved organic carbon (DOC) reservoir holds a critical role in the C cycle of marine and fresh water environments because of its large size and involvement in many biogeochemical reactions. Despite poor constraints, its importance in ancient Earth's C cycles is also commonly invoked. However, DOC remains rarely quantified and characterized in modern stratified analogs. Here, we investigated the DOC reservoirs of four redox-stratified alkaline crater lakes from Mexico. To achieve this, we analyzed the concentrations and isotopic compositions of DOC throughout the four water columns and compared them with existing data on dissolved inorganic and particulate organic C reservoirs (DIC and POC). The four lakes have high DOC concentrations with important variability between and within the lakes (averaging 2 ± 4 mM; 1SD, n=28; representing from ~ 15 to 160 times the amount of POC). $\delta^{13}C_{DOC}$ signatures also span a broad range of values from -29.3 to -8.7 ‰ (with as much as 12.5 ‰ variation within a single lake). The prominent DOC peaks (up to 21 mM), together with their associated isotopic variability, are interpreted to reflect oxygenic and/or anoxygenic primary productivity through the release of excess fixed-carbon in three of the lakes (Atexcac, La Preciosa and La Alberca de los Espinos). By contrast, the variability of [DOC] and $\delta^{13}C_{DOC}$ in Lake Alchichica was mainly explained by partial degradation of organic matter and accumulation of DOC in anoxic waters. Overall, DOC records detailed metabolic functioning such as active DIC-uptake and DIC-concentrating mechanism that cannot be inferred from DIC and POC analyses alone but that are critical to understand carbon fluxes from the environment to the biomass. Extrapolating our results to the geological record, we suggest that anaerobic oxidation of DOC may have caused the very negative C isotope excursions in the Neoproterozoic, but it is unlikely that a large oceanic DOC reservoir overweight the associated DIC reservoir. Overall, this study shows how the analysis of DOC in modern lakes deepens our understanding of the C cycle in stratified environments and how it can help to size boundary conditions to the Earth's past oceans.

1. INTRODUCTION

Dissolved organic carbon (DOC) is a major constituent of today's marine and fresh water environments (*e.g.* Ridgwell and Arndt, 2015; Brailsford, 2019). It generally represents the majority of freshwater organic matter (Kaplan et al., 2008; Brailsford, 2019), while the size of oceanic DOC equals the total amount of atmospheric carbon (Jiao et al., 2010; Thornton, 2014). DOC is (i) at the base of many trophic chains (Bade et al., 2007; Hessen and Anderson, 2008; Jiao et al., 2010; Thornton, 2014), (ii) key in physiological and ecological equilibria (Hessen and Anderson, 2008) and (iii) as a long-term C storage reservoir, it has a critical role for climate change (Jiao et al., 2010; Hansell, 2013; Thornton, 2014; Ridgwell and Arndt, 2015). The DOC reservoir has also been invoked to play a significant role in geological times, potentially reaching a much higher size and impacting various phenomena. These include the Neoproterozoic's climate and glaciations regulation (*e.g.* Peltier et al., 2007), the paleoecology of Ediacarian Biota and its early complex life forms (*e.g.* Sperling et al., 2011), the oxygenation of the ocean through innovations of eukaryotic life near the Neoproterozoic-Cambrian transition (*e.g.* Lenton and Daines, 2018) or some perturbations of the C cycle recorded in the δ^{13}C sedimentary archives from the Neoproterozoic to the Phanerozoic (*e.g.* Rothman et al., 2003; Fike et al., 2006; Sexton et al., 2011; Ridgwell and Arndt, 2015).

Nonetheless, the contribution of DOC reservoirs to the past and modern Earth's global climate and biogeochemical cycles remains poorly constrained (Jiao et al., 2010; Sperling et al., 2011; Dittmar, 2015; Fakhraee et al., 2021) and the existence and consequences of a large ancient oceanic DOC remain controverted (*e.g.* Jiang et al., 2010, 2012; Ridgwell and Arndt, 2015; Li et al., 2017; Fakhraee et al., 2021). Thus, in addition to modeling approaches (*e.g.* Shi et al., 2017; Fakhraee et al., 2021), the understanding of DOC-related processes in the past anoxic and redox-stratified oceans (Lyons et al., 2014; Havig et al., 2015; Satkoski et al., 2015) should rely on the characterization of DOC dynamics in comparable modern analogs (Sperling et al., 2011). Yet, although many studies explored the C cycle of modern redox-stratified environments (*e.g.* Crowe et al., 2011; Kuntz et al., 2015; Camacho et al., 2017; Posth et al., 2017; Schiff et al., 2017; Havig et al., 2018; Cadeau et al., 2020; Saini et al., 2021; Petrash et al., 2022), very few included the analysis of DOC and even fewer measured its stable isotope signature (Havig et al., 2018).

Here, we characterize the DOC reservoir of four modern redox-stratified alkaline crater lakes from the trans-Mexican volcanic belt (Ferrari et al., 2012) and its role within the C cycle of these environments. We report concentrations and isotopic compositions of DOC at multiple depths in the four water columns and discuss them together with physico-chemical characteristics (*e.g.* Vilaclara et al., 1993; Zeyen et al., 2021) and isotopic compositions of dissolved inorganic and particulate organic carbon (DIC, POC) measured in the same lakes (Havas et al., submitted). The four lakes exhibit distinct water chemistries aligning along an alkalinity/salinity gradient (Zeyen et al., 2021) and diverse planktonic microbial communities (Iniesto et al., 2022; Havas et al., submitted). This allows us to examine the effect of specific environmental and ecological constraints on the DOC reservoir, and how this latter influences the overall C cycle in redox-stratified environments.

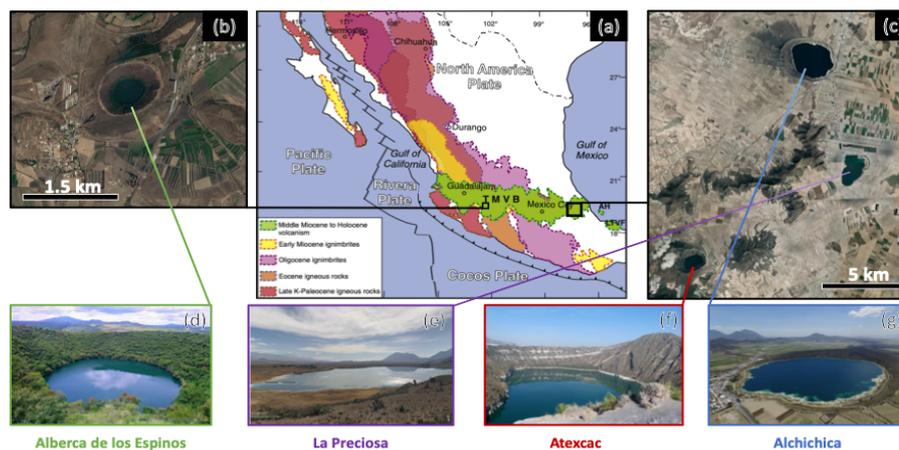
2. SITE DESCRIPTION

Here, the main characteristics of the geological, climatic and limnological context of the lakes under study are presented. A more detailed description is available in Havas et al. (submitted).

The four lakes are volcanic maars formed after phreatic, magmatic and phreatomagmatic explosions, and are located in the Trans-Mexican-Volcanic Belt (TMVB, Fig. 1). Three of them (Alchichica, Atexcac and La Preciosa) are located in a restricted area (~ 50 km²) of the Serdan-Oriental Basin (SOB), in the easternmost part of the TMVB (Fig. 1). By contrast, La Alberca de los Espinos is located at the margin of the Zacapu tectonic lacustrine basin in the Michoacán-Guanajuato Volcanic Field (MGVF), in the western-central part of the TMVB (Fig. 1). Lakes from the SOB experience temperate subhumid to temperate arid climates as reflected by much higher evaporation than precipitation fluxes in Alchichica for example (Silva-Aguilera et al., 2022). They overlie calcareous and basaltic/andesitic basement rocks (Carrasco-Núñez et al., 2007; Chako Tchamabé et al., 2020). La Alberca's climate is temperate to semi-humid and is underlain mostly by andesitic rocks (Siebe et al., 2012, 2014).

These geological contexts and hydrological processes generate a gradient of water chemical compositions, where alkalinity and salinity, notably, increase in the following order: (i) Lake La Alberca, (ii) La Preciosa, (iii) Atexcac and finally (iv) Alchichica (Zeyen et al., 2021). Nonetheless, the four lakes are alkaline with pH values around 9. This fosters the precipitation of microbialites deposits, which are found in the four systems but with an abundance increasing with alkalinity (Zeyen et al., 2021).

The four lakes are warm monomictic (one mixing period per year, during winter) with anoxic conditions prevailing in the bottom waters most of the year (Armienta et al., 2008; Macek et al., 2020; Havas et al., submitted). Importantly, they are all "closed lakes" with no inflow or outflow of surficial waters and thus receive water from rain and groundwaters only.

Finally, the trophic status of the lakes under study has only been assessed for the three SOB lakes, with Lake Atexcac being the most oligotrophic and La Preciosa and Alchichica being more or less oligotrophic than each other depending on the year of sampling (Lugo et al., 1993; Vilaclara et al., 1993; Sigala et al., 2017). Chlorophyll a data in May 2019 (Fig. 2) suggest, based on mean and maximum value categories (OECD, 1982), ultra-oligotrophic conditions in Atexcac (≤ 1 and 2 µg/L, respectively), oligotrophic conditions in Alchichica (≤ 2 and 6 µg/L, respectively), intermediate between oligo- and mesotrophic conditions in La Alberca (≤ 3 and 4.5 µg/L, respectively) and "low" mesotrophic conditions in La Preciosa (≤ 3 and 9 µg/L, respectively). Moreover, we note that total dissolved P concentrations in May 2019 show similar values for the three SOB lakes close to their surface (increasing in Alchichica anoxic zone) but much higher values in La Alberca (Havas et al., submitted). This same pattern has been observed during previous sampling campaigns (Zeyen et al., 2021). Thus, La Preciosa and Alberca appear overall as the least oligotrophic of the four lakes. Besides, La Alberca's is noticeably surrounded by more vegetation which could favor the input of nutrients to this lake.

120   Figure 1. Geographical location and photographs of the studied crater lakes from Havas et al. (submitted). (a)
121   Geological map from Ferrari et al. (2012) representing the location of the four lakes in the trans-Mexican volcanic
122   belt (TMVB). (b), (c) Close up © Google Earth views of lake Alberca de los Espinos and the Serdan-Oriental Basin
123   (SOB), respectively. (d-g) Pictures of the four lakes (d from © Google Image ['enamoratedemexicowebsite'], e
124   from © Google Earth street view, and g from © 'Agencia Es Imagen').

125

126

3.   METHOD

3.1. Sample Collection

129   All samples were collected in May 2019. Samples of DOC analyses were collected at different depths from the
130   surface to the bottom of the water columns with specific interests where the physico-chemical parameters showed
131   peculiar variations (*e.g.* at the chemocline, turbidity peaks; Figure 2 and Table 1). The main physico-chemical
132   vertical profiles are shown in Fig. 2. Water samples were collected with a Niskin bottle. For comparison with DIC
133   and POC data, the DOC analyses were carried out from the same Niskin sampling as in Havas et al. (submitted),
134   except when indicated (Fig. 4; Tables 1 and 2). Analyses of DOC, major, minor and trace ions were carried out
135   after water filtration at 0.22 μm directly on the field with Filtropur S filters that were pre-rinsed with the lake
136   water. Details about the sampling procedure and analysis of the physico-chemical parameters as well as DIC/POC
137   measurements are reported in Havas et al. (submitted).

138

3.2. Dissolved organic carbon (DOC) concentration and isotope measurements

140   Filtered solutions were first acidified to a pH of about 1-2 to degas all the DIC. DOC concentrations were measured
141   with a Vario TOC at the Laboratoire Biogéosciences (Dijon), calibrated with a range of potassium hydrogen
142   phthalate (Acros®) solutions. Before isotopic analyses, DOC concentrations of the samples were adjusted to match
143   our international standards at 5 ppm (USGS 40 glutamic acid and USGS 62 caffeine). Isotopic compositions were

144 measured at the Laboratoire Biogéosciences using an IsoTOC (running under He-continuous flow) coupled with

145 an IsoPrime stable isotope ratio mass spectrometer (IRMS; Isoprime, Manchester, UK). Samples were stirred with

146 a magnetic bar and flushed with He before injection of 1 mL sample aliquots (repeated 3 times). DOC was then

147 converted into gaseous CO_2 by combustion at about 850 °C, quantitatively oxidized by copper oxides and separated

148 from other combustion products in a reduction column and water condensers. Finally, it was transferred to the

149 IRMS via an open split device. In order to avoid a significant memory effect between consecutive analyses, each

150 sample (injected and measured three times) were separated by six injections of deionized water and the first sample

151 measurement was discarded from the isotopic calculations. Average reproducibility of $\delta^{13}C_{DOC}$ was 1 and 0.5 ‰

152 (1SD) for standards and samples, respectively. The average reproducibility for sample [DOC] measurements was

153 0.3 mM and blank tests were below the detection limit.

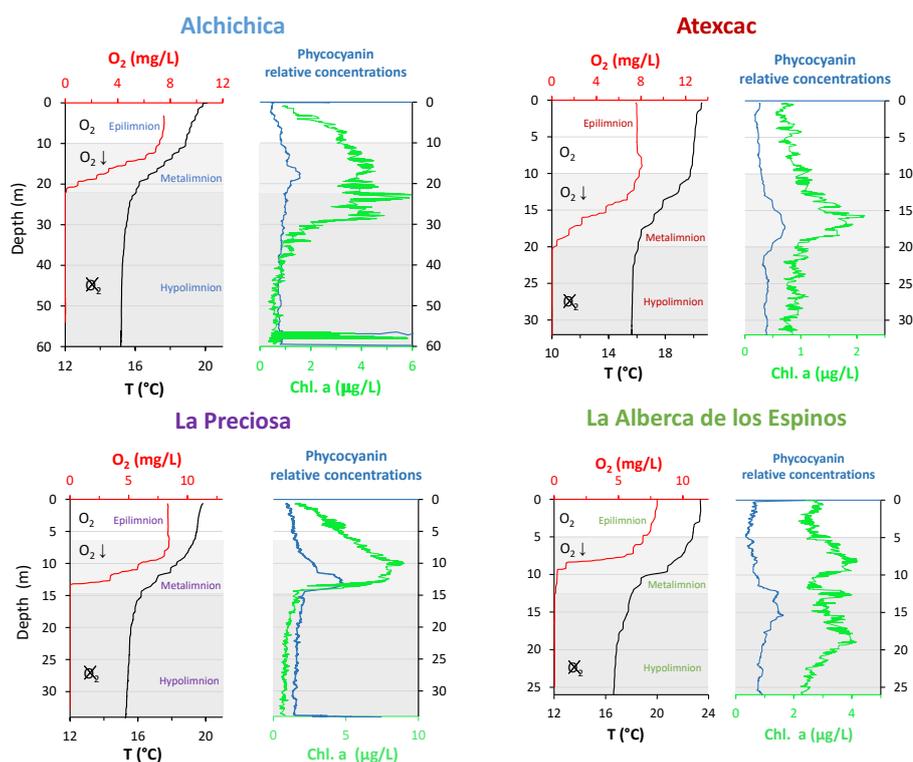



Figure 2. Main physico-chemical parameters depth profiles of Alchichica, Atexcac, La Preciosa and Alberca de los Espinos including: dissolved oxygen concentrations (mg/L), water temperature (°C), phycocyanin and chlorophyll a pigments (µg/L). Absolute values for phycocyanin concentrations were not determined; only relative variations are represented (with increasing concentrations to the right). Epi-, meta- and hypo-limnion layers are represented for each lake according to temperature profiles (and mostly correspond to oxygen-rich, -poor and intermediate zones). Original data from Havas et al. (submitted).

154

155

4. RESULTS

The water columns of the four lakes were clearly stratified in May 2019 (Fig. 2; Havas et al., submitted). The epi-, meta- and hypo-limnion layers of each lake (underlined by a light grey color gradient in the figures) were identified based on the thermocline depths.

4.1. Lake Alchichica

DOC had a concentration around 0.5 mM throughout the water column except in the hypolimnion, where it reached up to 5.4 mM. Its isotopic composition varied from -29.3 to -25.1 ‰, with maximum values found in the hypolimnion (Fig. 3).

The sum and weighted average of total carbon concentrations and isotopic compositions were calculated (considering the DIC and POC reservoirs). The DOC represented on average about 5 % of the total carbon and 93 % of the organic carbon present in the water column. The total carbon concentration depth profile roughly followed that of DOC, while $\delta^{13}C_{total}$ was roughly comprised between 0 and 2 ‰ through the water column, except in the lower part of the hypolimnion, where it decreased to -2.3 ‰ (Table 1). The isotopic difference between DOC and DIC ($\Delta^{13}C_{DOC-DIC}$) was slightly smaller in the hypolimnion and was overall comprised between -26.7 and -30.9 ‰. DOC isotopic compositions were more negative than those of POC with $\Delta^{13}C_{DOC-POC}$ values between -0.7 and -3.5 ‰ (Fig. 4; Table 2).

4.2. Lake Atexcac

DOC had a concentration of around 1.1 mM throughout the water column except at 16 and 23 m, where it reached 7.7 and 20.8 mM, respectively. Its isotopic composition showed values increasing from -20 to -9 ‰ between 5 and 23 m and decreasing to -11 ‰ at 30 m. On average, it represented about 16% of the total carbon and 98 % of the organic carbon present in the water column. Total C concentrations and $\delta^{13}C_{total}$ are centered around 27.7 mM and -0.6 ‰ with a clear increase to 38.9 mM and decrease to -2.7 ‰ at 23 m, respectively. The $\Delta^{13}C_{DOC-DIC}$ values significantly increased from the surface (-20 ‰) to the hypolimnion ($\sim$ -11 ‰). DOC isotopic compositions were strictly and significantly less negative than POC (*i.e.* enriched in heavy ^{13}C) reaching $\Delta^{13}C_{DOC-POC}$ values as high as +18 ‰ at the depth of 23 m (Fig. 4; Table 2).

4.3. Lake La Preciosa

DOC had a concentration around 0.5 mM throughout the water column except at 15 m, where it peaked at 1.6 mM. $\delta^{13}C_{DOC}$ was mostly around -26 ‰ except between 10 and 12.5 m, where it reached up to -20 ‰ (Fig. 3). It represented on average about 3% of the total carbon and 91% of the organic carbon present in the water column. The total C concentration was relatively stable at ~13.8 mM, while $\delta^{13}C_{total}$ was centered around -1 ‰ with a decrease down to -2.8 ‰ at 12.5 m (Table 1). The $\Delta^{13}C_{DOC-DIC}$ values were very stable with depth around -26 ‰ but markedly increased at 12.5 m up to -20 ‰. The $\Delta^{13}C_{DOC-POC}$ values were centered around 0 ‰ at all depths but 12.5 m where it increased to +7 ‰ (Fig. 4; Table 2).

10.5194/egusphere-2023-23
2023-01-12
EGUsphere
journal article preprint
en

4.4. Lake La Alberca de los Espinos

DOC had a concentration of around 0.4 mM throughout the water column except at 7 and 17 m, where DOC peaked at 1 and 1.7 mM, respectively (Fig. 3). Its isotopic composition was mostly comprised between -27 and -25 ‰ except at 7 m, where it reached -15 ‰ (Fig. 3). It represented about 8% of the total carbon and 93% of the organic carbon present in the water column. Total C concentration increased downward from about 7 to 9 mM. $\delta^{13}C_{total}$ decreased from -3.9 to -7.9 ‰ between 5 and 17 m and then increased up to -3.2 ‰ at 25 m (Table 1). The $\Delta^{13}C_{DOC-DIC}$ values were between -21 and -25 ‰ except at 7 m depth where it increased up to -12 ‰. The $\Delta^{13}C_{DOC-POC}$ values were comprised between -1 and +3 ‰ except at the same depth of 7 m where DOC was ^{13}C-enriched by ~12 ‰.

Lake	Sample	DOC	Total Carbon	$\delta^{13}C_{DOC}$	$\delta^{13}C_{Total}$
		mmol.L^{-1}		‰	
Alchichica	AL 5m	0.7	35.8	ND	ND
	AL 10m	0.4	33.5	-28.3	1.6
	AL 20m	0.4	35.0	-29.3	1.3
	AL 30m	0.4	35.1	-28.3	1.2
	AL 35m	2.3	37.2	-26.8	-0.2
	AL 40m	2.2	37.0	-25.8	-0.1
	AL 50m	5.0	39.8	-25.1	-1.8
	AL 55m	0.5	35.3	-27.6	1.1
	AL 58m	5.4	40.2	-27.7	-2.3
	AL 60m	0.7	35.3	-26.1	1.0
Atexcac	ATX 5m	0.92	27.4	-20.0	-0.4
	ATX 10m	1.8	28.1	-15.5	-0.7
	ATX 16m	7.8	34.7	ND	ND
	ATX 23m	21.0	45.2	-8.7	-3.6
	ATX 30m	0.7	26.4	-11.2	-0.1
La Preciosa	LP 5m	0.5	14.0	-25.4	-0.9
	LP 8m	0.9	ND	ND	ND
	LP 10m	0.3	13.7	-25.7	-0.4
	LP 12.5m	1.6	13.2	-20.0	-2.8
	LP 15m	0.5	13.9	-24.0	-1.3
	LP 20m	0.3	13.6	-26.2	-1.0
	LP 31m	0.3	13.6	-26.2	-0.9
La Alberca de Los Espinos	Albesp 5m	0.4	7.2	-26.7	-3.9
	Albesp 7m	1.0	8.1	-14.7	-3.9
	Albesp 10m	0.4	7.6	-25.2	-5.1
	Albesp 17m	1.7	9.0	-26.3	-7.9
	Albesp 20m	0.4	8.4	-25.1	-4.5
	Albesp 25m	0.4	9.2	-27.2	-3.2

Table 1

Concentrations and isotopic compositions of dissolved organic carbon (DOC). Total carbon concentration is the sum of DOC, DIC and POC reservoirs. For LP 8m, [DIC] was not measured, and therefore the total carbon concentration was not calculated. The latter two were determined by Havas et al. (submitted). $\delta^{13}C_{Total}$ is the weighted average of the three $\delta^{13}C$. ND: non-determined.

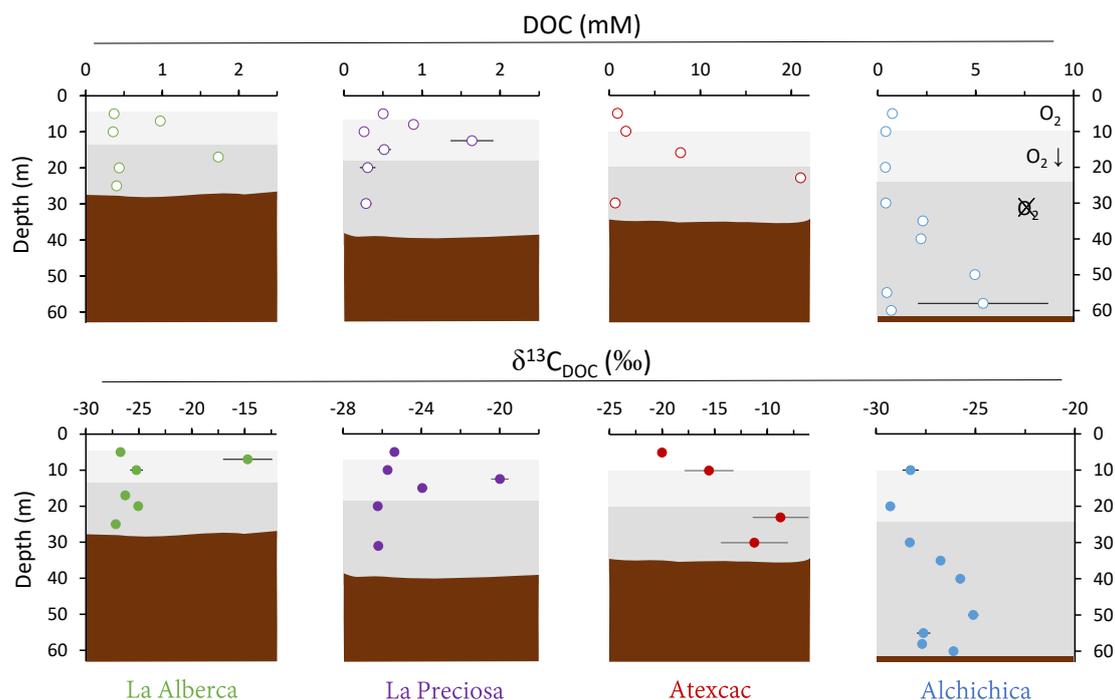



Figure 3. Vertical profiles of concentration and isotopic composition of dissolved organic carbon (DOC) throughout the water columns of the studied lakes: La Alberca de los Espinos, La Preciosa, Atexcac and Alchichica. Concentrations are in mmol.L^{-1} (mM) and isotopic compositions in ‰ *vs.* VPDB. Brown boxes represent the sediments at the bottom of the lakes, while boxes from white to dark grey color represent the water column layers from oxygen-rich to oxygen-poor conditions.

5. DISCUSSION

In all four Mexican lakes studied here, the DOC reservoir occupies a predominant role, but showed quite diverse dynamics and characteristics between the lakes. Indeed, the four lakes have a high DOC content but very different [DOC] or $\delta^{13}C_{DOC}$ profiles and signatures. In contrast, they show quite similar profiles and signatures for the DIC and POC reservoirs (Fig. 3; Havas et al., submitted). Evaporation may be one process that increases DOC concentrations (Anderson and Stedmon, 2007; Zeyen et al., 2021). However, it is likely marginal here because on the contrary to what was observed for DIC, there is no correlation between the average [DOC] in the Mexican lakes and their salinity. Moreover, evaporation would not explain the significant intra-lake DOC depth variability.

In the following, we explore the different patterns of DOC production and fate, which depend on slight environmental and biological variations between the Mexican lakes. Moreover, we describe how the analysis of DOC provides insights into microbial processes and overall C cycle dynamics in stratified water columns. Finally, we extrapolate our results to discuss the possible roles of DOC onto paleoclimate and sedimentary C isotopes variations at the geological scale.

5.1 Sources and fate of DOC

256

257   Dissolved organic carbon is an operationally defined fraction of aqueous organic carbon (here separated from

258   particulate organic carbon by filtration at 0.22 µm) within a continuum of organic molecules spanning a large

259   range of sizes, compositions, degrees of reactivity and bioavailability (Kaplan et al., 2008; Hansell, 2013; Beaupré,

260   2015; Carlson and Hansell, 2015; Brailsford, 2019). Importantly, the four lakes receive relatively little input of

261   allochthonous OM due to their endorheic nature (Alcocer et al., 2014b; Havas et al., submitted). This allows to

262   specifically focus on DOC-related processes occurring within the water columns, and notably on autochthonous

263   DOC primary production. Autochthonous DOC can form through multiple processes, including: higher-rank OM

264   degradation processes such as sloppy feeding by predators, UV photolysis or bacterial and viral cell lysis (Lampert,

265   1978; Hessen, 1992; Bade et al., 2007; Thornton, 2014; Brailsford, 2019) as well as passive (leakage) or active

266   (exudation) release by healthy cells (*e.g.* Baines and Pace, 1991; Hessen and Anderson, 2008; Thornton, 2014;

267   Ivanovsky et al., 2020). Generally, this C release (either "active" or "passive") tends to be enhanced in nutrient-

268   limited conditions because some recently fixed C is in excess compared with other essential nutrients such as N or

269   P (Hessen and Anderson, 2008; Morana et al., 2014; Ivanovsky et al., 2020) and that especially in high oxygenic

270   photosynthesis contexts, where photorespiration bolsters the excretion of DOC (Renstrom-Kellner and Bergman,

271   1989). Moreover, oligotrophic conditions tend to limit the heterotrophic bacterial activity and thus preserve the

272   DOC stocks (Thornton, 2014; Dittmar, 2015). This may partly explain the trend of increasing DOC concentrations

273   from the less oligotrophic La Alberca and La Preciosa's (0.7 mM on average) to the more oligotrophic Alchichica

274   (1.8 mM) and Atexcac's waters (6.5 mM).

275

5.1.1 DOC release by autotrophs

276

277   In the four Mexican lakes, [DOC] depth profiles exhibit one or several peaks standing out from low background

278   values and occurring both in oxic and anoxic waters (Fig. 3). In La Alberca and La Preciosa, they correlate with

279   chlorophyll a peaks. In the two other lakes, they do not match the chlorophyll increase. However, in Atexcac, a

280   remarkable DOC peak (over 10-fold increase, Fig. 3) occurs at the same depth as a peak of anoxygenic

281   photosynthesis (Havas et al., submitted). These co-occurrences support that a large portion of DOC in these three

282   lakes (at least at these depths) arise from the release of photosynthetic C fixed in excess. While it remains to be

283   shown for anoxygenic organisms, phytoplankton in aerobic conditions generally releases dissolved organic matter

284   by (i) an active "overflow mechanism" (DOM exudation) or (ii) passive diffusion throughout the cell membranes.

285   In the first case, DOM is actively released out of the cells as a result of C fixation rates higher than growth and

286   molecular synthesis rates (*e.g.* Baines and Pace, 1991). Hence, DOM exudation depends not only on the nature pf

287   primary producers (different taxa may display very different growth rate, photosynthetic efficiency or exudation

288   mechanisms), but also on environmental factors such as irradiance and nutrient availability (*e.g.* Morana et al.,

289   2014). Besides, it may serve "fitness-promoting purposes" such as storage, defense, or mutualistic goals (Bateson

290   and Ward, 1988; Hessen and Anderson, 2008). In the second case (passive diffusion), DOM release also depends

291   on cell permeability and the outward DOC gradient, and is more directly connected to the amount of phytoplankton

292   biomass (*e.g.* Marañón et al., 2004). Thus, any new photosynthate production drives a steady DOM release rate,

293   which is, to some extent, independent of the environmental conditions (Marañón et al., 2004; Morana et al., 2014).

294   The fact that lakes La Preciosa and Alberca have lower DOC but overall chlorophyll a concentrations higher than

295   Atexcac and Alchichica suggests that DOC production does not directly relate to phytoplankton biomass and is
296   not passively released. Alternatively, DOC isotopic signatures suggest an active DOC release (see below).
297   Furthermore, these Mexican lakes precisely correspond to environmental contexts (high irradiance and
298   oligotrophic freshwater bodies) where DOC exudation has been observed and is predicted (*e.g.* Baines and Pace,
299   1991; Morana et al., 2014; Thornton, 2014).

300   Release of DOC by primary producers can be characterized by the percentage of extracellular release (PER), which
301   corresponds to the fraction of DOC over total (dissolved and particulate) OM primary production (*e.g.* Thornton
302   et al., 2014). PER is highly variable and averages about 13% of C biomass over a wide range of environments (*e.g.*
303   Baines and Pace, 1991; Thornton, 2014). But values as high as 99% have been reported (see Bertilsson and Jones,
304   2003). At depths where oxygenic photosynthesis occurs, the DOC over total OC ratio averages approximately 85,
305   99, 94, and 95% for lakes Alchichica, Atexcac, La Preciosa and La Alberca, respectively. Thus, although these
306   ratios are likely higher than the actual PER (as some of the DOC measured in the Mexican lakes may correspond
307   to an older long-term DOC reservoir), these DOC fractions are consistent with extremely high phytoplankton-
308   release rates.

309   An interesting feature is that DOC peaks associated with primary production (mainly photosynthesis) are
310   characterized by very positive $\Delta^{13}C_{DOC-POC}$ (from +3 to +18 ‰, Fig. 4). It should be noticed that a switch from
311    $CO_{2(aq)}$ to HCO_3^- as an inorganic C source (and their 10‰ isotopic difference, *e.g.* Mook et al., 1974) could not
312   explain alone the isotopic difference between POC and DOC. The isotopic enrichment of DOC molecules
313   compared to POC could have different origins. First, it supports that DOC may correspond to new photosynthate
314   release instead of a product of cell lysis or zooplankton sloppy feeding since the latter would likely produce $\delta^{13}C_{DOC}$
315   close to $\delta^{13}C_{POC}$ values. Second, this heavy DOC could originate from photosynthetic organisms using a different
316   C-fixation pathway, inducing smaller isotopic fractionation (provided that these organisms contributed mostly to
317   the DOC but not to the POC fraction). In lakes Atexcac and La Alberca, anoxygenic phototrophic bacteria could
318   release important amounts of DOC, especially under nutrient-limiting conditions (Ivanovsky et al., 2020). By
319   contrast to cyanobacteria or purple sulfur bacteria (PSB), which use the CCB pathway, green sulfur bacteria (GSB;
320   another group of anoxygenic phototrophs belonging to the Chlorobi) use the reductive citric acid cycle or reverse
321   tricarboxylic-TCA cycle, which tends to induce smaller isotopic fractionations (between ~ 3–13 ‰, Hayes, 2001).
322   However, only the DOC isotopic signatures recorded in the hypolimnion of Lake La Alberca ($\epsilon_{DOC-CO2} \approx$ -13.5 ±
323   2 ‰) agree well with fractionations found for this type of organism in laboratory cultures and other stratified water
324   bodies (Posth et al., 2017), whereas $\epsilon_{DOC-CO2}$ in the hypolimnion of Lake Atexcac are slightly higher ($\epsilon_{DOC-CO2} \approx$
325   0 ‰). Moreover, GSB were identified in La Alberca only (Havas et al., submitted). Third, phytoplankton blooms
326   may specifically release isotopically heavy organic molecules. For example, carbohydrates could be preferentially
327   released under nutrient-limiting conditions as they are devoid of N and P (Bertilsson and Jones, 2003; Wetz and
328   Wheeler, 2007; Thornton, 2014). Carbohydrates typically have a ^{13}C-enriched (heavy) isotopic composition (Blair
329   et al., 1985; Jiao et al., 2010; Close and Henderson, 2020). Yet, this molecular hypothesis would barely explain
330   the full range of $\Delta^{13}C_{DOC-POC}$ variations measured in Atexcac and La Alberca according to isotopic mass balance
331   of cell specific organic compounds (Hayes, 2001). At last, such enrichments require otherwise that DOC and DIC
332   first accumulate in the cells. Indeed, if DOC molecules were released as soon as they were produced, their isotopic
333   composition should approach that of the biomass (*i.e.* $\delta^{13}C_{POC,}$ within the range of molecules-specific isotopic

334 compositions), which is not the case. If DIC could freely exchange between inner and outer cell media, maximum

335 "carboxylation-limited" fractionation (mostly between ~ 18 and 30 ‰ depending on RuBisCO form, Thomas et

336 al., 2019) would be expressed in all synthesized organic molecules as represented in Fig. 5a (*e.g.* O'Leary, 1988;

337 Descolas-Gros and Fontungne, 1990; Fry, 1996), which is also inconsistent with the DOC records (see $\varepsilon_{DOC-CO2}$

338 in Table. 2).

339
340
341
342

Lake	Sample	$\Delta^{13}C_{DOC-DIC}$	$\Delta^{13}C_{DOC-POC}$	$\varepsilon_{DOC-CO2}$
		‰		‰
Alchichica	AL 5m	ND	-1.6	ND
	AL 10m	-30.3		-20.1
	AL 20m	-30.9		-20.5
	AL 30m	-30.0	-2.0	-19.5
	AL 35m	-28.4	-1.0	-17.9
	AL 40m	-27.3	-0.7	-16.8
	AL 50m	-26.7		-16.2
	AL 55m	-29.1	-3.5	-18.7
	AL 58m	-29.3		-18.8
	AL 60m	-27.6		-17.1
Atexcac	ATX 5m	-20.4	8.4	-10.6
	ATX 10m	-16.0	12.6	-6.1
	ATX 16m	ND		
	ATX 23m	-9.7	17.9	0.6
	ATX 30m	-11.4	15.2	-1.2
La Preciosa	LP 5m	-25.5	1.0	-15.7
	LP 10m	-25.9	1.7	-16.0
	LP 12.5m	-19.8	7.1	-9.8
	LP 15m	-23.6	-0.4	-13.5
	LP 20m	-25.8	0.1	-15.7
	LP 31m	-25.8	-1.0	-15.7
La Alberca de Los Espinos	Albesp 5m	-24.2	0.2	-14.8
	Albesp 7m	-12.4	11.5	-3.0
	Albesp 10m	-21.2	3.1	-11.6
	Albesp 17m	-22.9	2.7	-13.1
	Albesp 20m	-21.8	1.5	-12.2
	Albesp 25m	-25.2	-1.5	-15.9

368
369 Table 2
370 Isotopic fractionations between DOC and DIC and POC where $\Delta^{13}C_{x-y} = \delta^{13}C_x - \delta^{13}C_y$ is the apparent fractionation
371 and ε is computed as the actual metabolic isotopic discrimination between CO_2 and DOC. In Alchichica, $\delta^{13}C_{DOC}$
372 was not measured at 5 m and its value at 10 m was used in this calculation of $\Delta^{13}C_{DOC-POC.}$ The full chemistry at
373 depths 35 and 58 m was also not determined, thus, the calculation of $\delta^{13}C_{CO2}$ for these samples is based on the
374 composition of samples above and beneath. Isotopic data for DIC, POC and CO_2 are from Havas et al. (submitted).

375

376 Under the environmental conditions of the studied lakes, *i.e.*, (i) low CO_2 quantities relative to HCO_3^-, (ii) local

377 planktonic competition for CO_2 and (iii) low nutrient availability, the activation of intracellular a DIC-

378 concentrating mechanism (DIC-CM) is expected (Beardall et al., 1982; Burns and Beardall, 1987; Fogel and

379 Cifuentes, 1993; Badger et al., 1998; Iñiguez et al., 2020). This mechanism is particularly relevant in oligotrophic

380   aqueous media (Beardall et al., 1982), where CO_2 diffusion is slower than in the air (O'Leary, 1988; Fogel and

381   Cifuentes, 1993; Iñiguez et al., 2020). A DIC-CM has been proposed to reduce the efflux of DIC from the cells

382   back to the extracellular solution. This internal DIC is eventually converted into organic biomass, thereby drawing

383   the cell isotopic composition closer to that of $\delta^{13}C_{DIC}$ (Fig. 5; Beardall et al., 1982; Fogel and Cifuentes, 1993;

384   Werne and Hollander, 2004). However, we suggest that the activation of a DIC-CM could preserve a large $\Delta^{13}C_{POC-}$

385    $_{DIC}$ while generating an apparent fractionation between the DOC and POC molecules instead. Indeed, initially fixed

386   OC would be discriminated against the heavy C isotopes and incorporated into the cellular biomass (Fig. 5c, 't_i').

387   Further, following the overflow mechanism scenario, high photosynthetic rates (due to high irradiance,

388   temperature and high DIC despite low CO_2) coupled with low population growth rates and organic molecule

389   synthesis (due to limited abundances of P, N, Fe) would result in the exudation of excess organic molecules with

390   heavy $\delta^{13}C_{DOC}$ as they are synthesized from residual internal DIC, which progressively becomes ^{13}C-enriched

391   (Fig. 5c, 't_{ii}'). This process could explain the formation of DOC with δ^{13}C very close to DIC/CO_2 signatures as

392   observed in Lake Atexcac. This suggests that oligotrophic conditions could be a determinant factor in the

393   generation of significantly heavy $\delta^{13}C_{DOC}$, and even more if they are coupled to high irradiance. It also underlines

394   that significant isotopic variability can exist between the different organic C reservoirs.

395

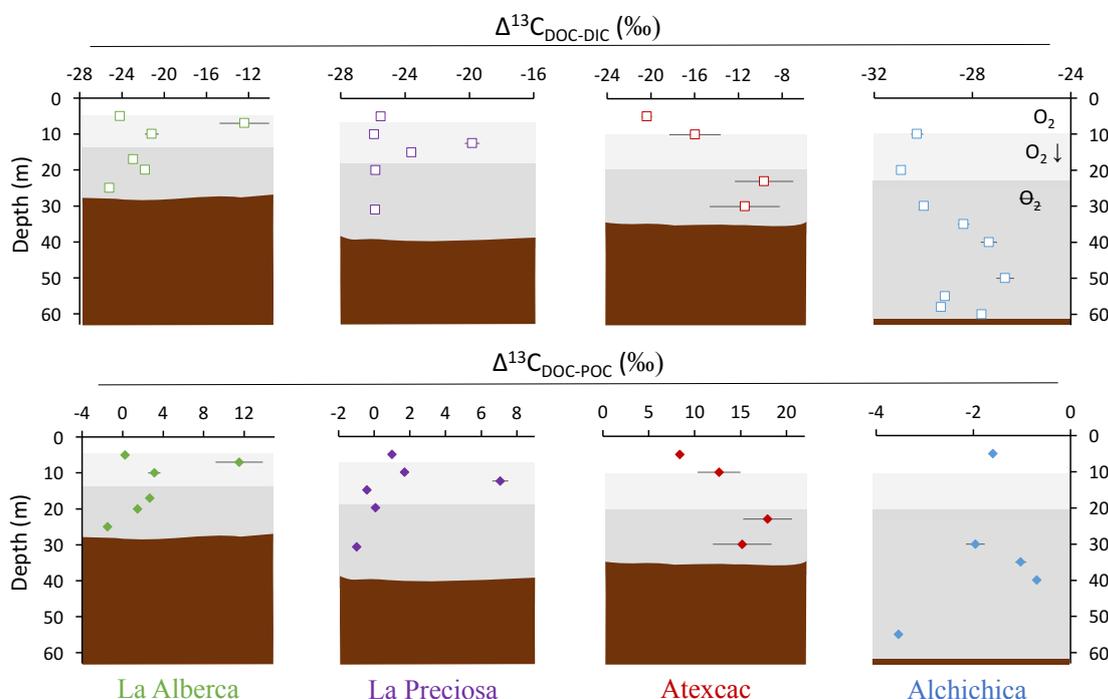



Figure 4. Vertical profiles of isotopic fractionations between the DOC and DIC (top) as well as between DOC and POC (bottom) throughout the water columns of the four lakes (all expressed in ‰ $vs.$ VPDB). In Alchichica, $\delta^{13}C_{DOC}$ was not measured at 5 m and its value at 10 m was used in this calculation of $\Delta^{13}C_{DOC-POC}$. Brown boxes represent the sediments at the bottom of the lakes, while boxes from white to dark grey color represent the water column layers from oxygen-rich to oxygen-poor conditions.

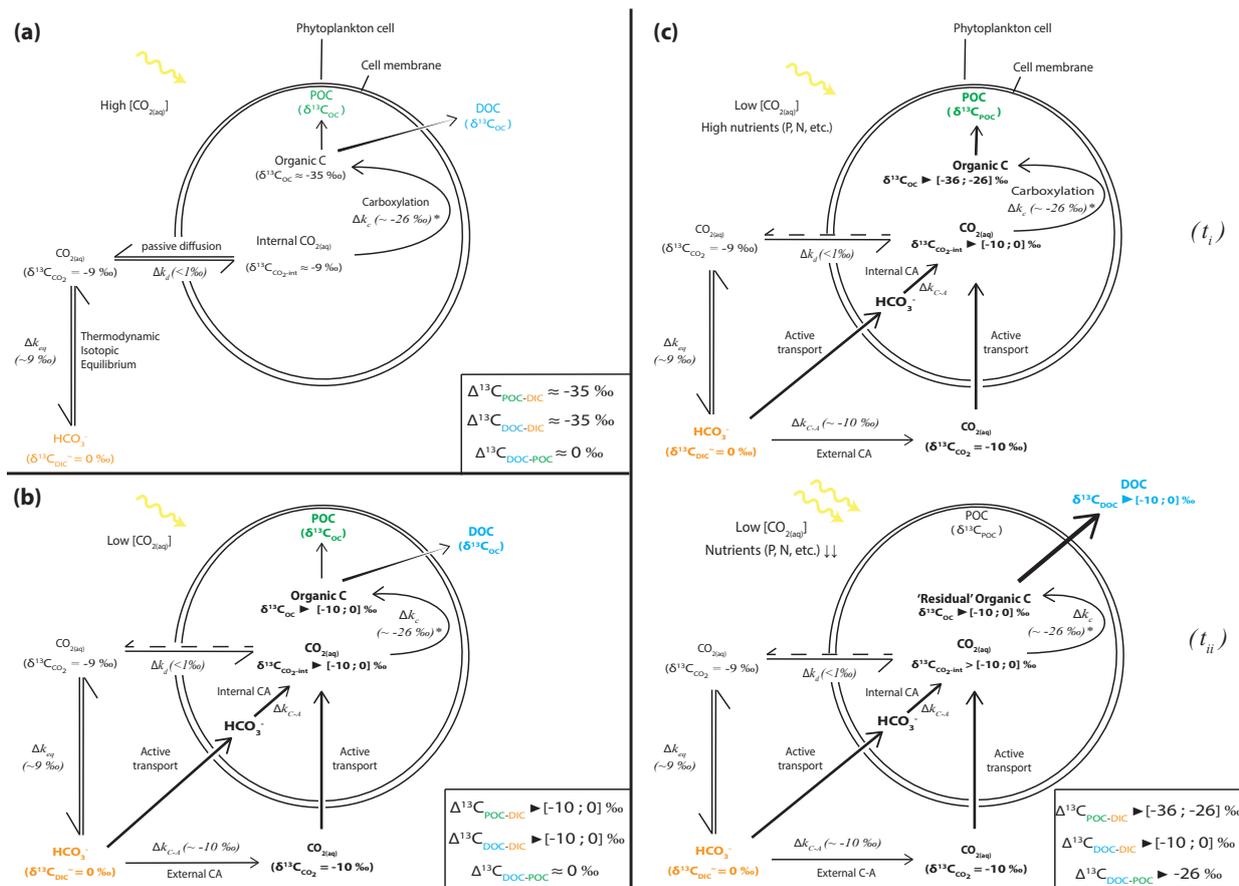



Figure 5. Schematic view of phytoplankton cells during autotrophic C fixation through different C supply strategies and associated apparent isotopic fractionation between DIC and POC/DOC and between DOC and POC. (a) Case where $[CO_{2(aq)}]$ is high enough to allow for a DIC supply by passive $CO_{2(aq)}$ diffusion through the cell membrane and $CO_{2(aq)}$ is at equilibrium with other DIC species. There, isotopic fractionation is maximum (minimum $\delta^{13}C_{OC}$) because C fixation is limited by the carboxylation step. DOC is released following an in- to outward cell concentration gradient and has a similar composition to POC. (b) "Classic" view of C isotopic cycling resulting from active DIC transport within the cell because of low ambient $[CO_{2(aq)}]$ (through a DIC-CM). Carbonic anhydrase (CA) catalyzes the conversion between HCO_3 and $CO_{2(aq)}$ inside or outside the cell with an isotopic fractionation close to equilibrium fractionation (~ 10 ‰). While inward passive $CO_{2(aq)}$ diffusion can still occur, the DIC-CM activation reduces the reverse diffusion, resulting in internal $CO_{2(aq)}$ isotopic composition approaching that of the incoming DIC (depending on the fraction of internal $CO_{2(aq)}$ leaving the cell). Acting as a "closed-system", most of internal DIC is fixed as OC and minimum isotopic fractionation is expressed for both POC and DOC. (c) Proposed model for C isotopic fractionation with active DIC transport including an isotopic discrimination between POC and DOC. (t_i) Initially fixed C is isotopically depleted and incorporates the cell's biomass as long as there are sufficient nutrients to enable "complex" organic molecules synthesis. (t_{ii}) In low nutrient conditions, but high photosynthetic activity – fixed OC is released out of the cell as DOC following the "overflow" hypothesis and inherits heavier isotopic compositions from the residual internal DIC. This leads to distinct POC and DOC isotopic signatures, with small fractionation between DOC and DIC, the amplitude of which will depend notably on the rate of CO_2 backward diffusion and ratio of biomass C (POC) and released C (DOC).

396
397

5.1.2 DOC accumulation in (the bottom water of) Lake Alchichica

398

399 From the previous discussion, it appears that environmental conditions of the Mexican lakes might favor an
400 important phytoplanktonic production of DOC. Alcocer et al. (2014a) also proposed that an early spring
401 cyanobacterial bloom in Lake Alchichica favored the production of DOC in the epilimnion. However, at the time
402 of sampling, the DOC reservoir in this lake was not correlated with any sizeable autotrophic activity at any depth.
403 Indeed, the large epilimnetic chlorophyll a peak did not correlate with any changes of [DOC] or $\delta^{13}C_{DOC}$ (Fig. 3).
404 Compared with the other lakes, the geochemical conditions at which chlorophyll a is produced in Alchichica could
405 have been incompatible with the activation of a DIC-CM and significant DOC exudation. For example, Alchichica
406 had similar [$CO_{2(aq)}$] as La Preciosa, but higher P and NH_4^+ concentrations (Havas et al., submitted); Lake La
407 Alberca had higher P concentrations, but presented similar [NH_4^+] and lower [$CO_{2(aq)}$]. In contrast with
408 measurements from 2013 (Alcocer et al., 2014a), we find a large DOC increase in the middle of the anoxic
409 hypolimnion of Lake Alchichica, but it did not correspond to any change in the DIC reservoir as observed for lakes
410 La Preciosa (at 12.5 m) or Atexcac (at 23 m) (Havas et al., submitted). Moreover, at these depths, photosynthetic
411 active radiation (PAR) is below 0.1% in Alchichica during the stratified season (Macek et al., 2020), which might
412 not be sufficient to trigger important anoxygenic phytoplankton DOC release.

413 The DOC reservoir in Alchichica is characterized by a $\delta^{13}C_{DOC}$ (and $\Delta^{13}C_{DOC-DIC}$) lower than in the other lakes and
414 systematically showing ^{13}C-depleted signatures relative to POC (i.e. $\delta^{13}C_{DOC} < \delta^{13}C_{POC}$; Fig. 4). Thus, if the DOC
415 increase in Alchichica's hypolimnion resulted from the release of photosynthetic OC as in some of the other lakes,
416 it was not associated with the same C isotopes fractionation (e.g. if anoxygenic phototrophs did not concentrate
417 intracellular DIC, cf. Fig. 5a). Some purple sulfur bacteria (anoxygenic phototrophs belonging to the
418 Proteobacteria) have been identified but they become significant towards the end of the stratification (from
419 July/August to December/January; Alcántara-Hernández et al., 2022; Iniesto et al., 2022).

420 Alternatively, this hypolimnetic DOC increase in Lake Alchichica may reflect the preservation and accumulation
421 of DOM over the years, consistently with higher [DOC] measured in 2019 than in the previous years (Alcocer et
422 al., 2014a). While alteration of the DOC reservoir by UV-photolysis would induce a positive isotopic fractionation
423 (Chomicki, 2009), the slightly negative $\Delta^{13}C_{DOC-POC}$ signatures support the possibility of DOC being mainly a
424 recalcitrant residue of primary OM degradation by heterotrophic organisms (Alcocer et al., 2014a). Indeed, the
425 preferential consumption of labile ^{13}C-enriched molecules by heterotrophic bacteria would leave the residual OM
426 with more negative isotopic signatures (Havas et al., submitted). The DIC and POM data were also consistent with
427 heterotrophic activity from the surface to the hypolimnion of Alchichica (Havas et al., submitted). Moreover,
428 degradation by heterotrophic bacteria leaves more recalcitrant DOM in the water column, which tends to
429 accumulate over longer periods of time (Ogawa et al., 2001; Jiao et al., 2010; Kawasaki et al., 2013). DOM content
430 is a balance between its production by autotrophs and consumption by heterotrophs, especially in environments
431 where both types of organisms compete for low-concentration nutrients (Dittmar, 2015). If Alchichica's DOC
432 actually represents a long-term reservoir, its presence might favor the development of bacterial populations
433 growing on it. A shift of the cyanobacterial DOC toward the hypolimnion of Lake Alchichica was described at the
434 end of the spring (Alcocer et al., 2014a; 2022). Thus, deeper and darker anoxic waters in Alchichica could better
435 preserve DOC from intense microbial and light degradation, hence allowing its accumulation over time.

436 In conclusion, Alchichica's DOC reservoir (notably in the hypolimnion) more likely represents an older and
437 evolved DOM pool. The time required for its accumulation and stability over the years remains to be evaluated.
438 Nevertheless, we cannot fully rule out that part of it this DOC was produced by anoxygenic photosynthetic
439 plankton. If so, the reasons why it did not bear the same isotopic enrichment as in the other lakes remain to be
440 elucidated.

441

442 **5.2 DOC analysis provides deeper insights into planktonic cell functioning and water column C cycle**
443   **dynamics than POC or DIC analyses**

444 Additional insights on the C cycles of the lakes can be provided by DOC analysis compared with the more classical
445 DIC and POC data described in Havas et al. (submitted). With concentrations ranging from 0.6 to 6.5 mM on
446 average, DOC amounts between 14 and 160 times the POC concentrations. It represents from about 5 to 16% of
447 the total C measured in the four lakes. In comparison, although DOC is the main organic pool in the ocean, its
448 concentration hardly exceeds 0.08 mM (Hansell, 2013) while in large-scale anoxic basins such as the Black Sea,
449 it remains under 0.3 mM (Ducklow et al., 2007). Hence, DOC is a major C reservoir in these Mexican lakes, by
450 its size.

451 The depth profiles of DOC concentration and isotopic composition differ significantly from those of POC. Notably
452 in Lake La Preciosa, the photosynthetic DOC production (+1.5 mM) at the Chl. a peak depth matches the decrease
453 of DIC (- 2 mM), while there is no change in [POC] or $\delta^{13}C_{POC}$ (Fig. 3; Havas et al., submitted). Just below, at a
454 15 m depth, the marked increase of $\delta^{13}C_{POC}$ interpreted to reflect heterotrophic activity is better evidenced when
455 considering the heavier DOC isotopes compositions as a C source between 12.5 and 15 m depth (Fig. 3; Havas et
456 al., submitted). Similarly, in Lake La Alberca, only a small portion of C is transferred from the inorganic to the
457 POC by primary productivity, while the DIC reservoir is largely influenced by methanotrophy, sediment-
458 associated methanogenesis and possible volcanic degassing in the bottom of the lake (Havas et al., submitted). In
459 Lake Atexcac, anoxygenic photosynthesis clearly stands out based on [DOC] and $\delta^{13}C_{DOC}$ data, but is not recorded
460 by the POC reservoir and only slightly by the DIC reservoir (Havas et al., submitted). Overall, it implies that
461 recently fixed OC is quickly released out of the cells as DOC, thereby transferring most C from DIC to DOC,
462 rather than POC. Therefore, POC is an incomplete archive of the biogeochemical reactions occurring in water
463 columns. Furthermore, this shows that the isotopic analysis of DIC and by extension authigenic carbonates,
464 especially in alkaline-buffered waters, might not be sensitive enough to faithfully record environmental and
465 biological changes.

466 The heavy $\delta^{13}C_{DOC}$ recorded in lakes La Preciosa, La Alberca and Atexcac provide important constraints on the
467 way planktonic cells deal with and cycle C: it may arise from the activation of a DIC-CM or from a specific
468 metabolism or C-fixation pathway. By contrast, the use of a DIC-CM is poorly captured by $\delta^{13}C_{POC}$ analyses,
469 although recognition of active DIC uptake has often been based on this signal (by reduced isotopic fractionation
470 with DIC; *e.g.* Beardall et al., 1982; Erez et al., 1998; Riebesell et al., 2000). Most interestingly, intra-cellular
471 amorphous Ca-carbonates (iACC) are formed in some of the cyanobacteria from Alchichica microbialites, possibly
472 due to supersaturated intra-cell media following active DIC uptake through a DIC-CM (Couradeau et al., 2012;

473   Benzerara et al., 2014). While the link between DIC-CM and iACC still needs to be demonstrated (Benzerara et

474   al., 2014), the active use of DIC-CMs in Mexican lakes is independently supported by the DOC isotopic signature.

475

476   **5.3 Implications for the hypothesis of a "big DOC" reservoir controlling past carbon cycling**

477

478   **5.3.1 Phanerozoic carbon isotope excursions (CIEs)**

479   The studied Mexican lakes have large DOC pools, allowing to draw comparisons with studies that have invoked

480   past occurrences of oceanic carbon cycles dominated by big DOC reservoirs (*e.g.* Rothman et al., 2003; Sexton et

481   al., 2011). Ventilation/oxidation cycles of a large deep ocean DOC reservoir have been inferred to explain

482   carbonate isotopic records of successive warming events through the Eocene (Sexton et al., 2011). Accordingly,

483   the release of carbon dioxide into the ocean/atmosphere system following DOC oxidation is suggested to trigger

484   both the precipitation of low $\delta^{13}C$ carbonates and an increase of the atmospheric greenhouse gas content. It was

485   assessed that the size of this DOC reservoir should have been at least 1600 PgC (about twice the size of the modern

486   ocean DOC reservoir) to account for a 2–4°C increase of deep ocean temperatures (Sexton et al., 2011). However,

487   the main counter argument to this hypothesis is that the buildup of such a DOC reservoir at modern DOC

488   production rates implies a sustained deep ocean anoxia over hundreds of thousand years, while independent

489   geochemical proxies do not support such a sustained anoxia during this time period (Rigwell and Arndt, 2015).

490   However, our study suggests, albeit at a different scale, that this kinetic argument may be weak. Indeed, in the

491   studied Mexican lakes, the lowest recorded [DOC] is 260 µM (Table 1), *i.e.*, about 6 times the deep modern ocean

492   concentrations (~ 45 µM; Hansell, 2013). Yet, the entire water columns of these lakes down to the surficial

493   sediments are seasonally mixed with di-oxygen, showing that high [DOC] (notably in Alchichica, which likely

494   harbors a "long-term" DOC reservoir) can be achieved despite frequent oxidative (oxygen-rich) conditions.

495   Besides, the oxidation of only half of the DOC in the lakes would generate average $\delta^{13}C_{DIC}$ deviations between -

496   0.6 and -1 ‰, corresponding to the C isotopes excursion magnitudes described by Sexton et al. (2011).

497   Similarly, Black Sea's deep anoxic waters hold about 3 times the amount of DOC found in the modern deep open

498   ocean (Ducklow et al., 2007; Sexton et al., 2011; Dittmar, 2015). In the Black Sea and Mexican lakes, the low

499   nutrient availability may limit sulfate-reduction despite high sulfate and labile organic matter concentrations,

500   thence favoring DOM preservation and accumulation (Dittmar, 2015 and references therein). Margolin et al.

501   (2016) argued that important DOM was sustained by important terrigenous inputs only. Our study attests the

502   possibility for "autochthonous systems" to reach DOC concentrations well above what is found in the Black Sea

503   and that terrigenous inputs are not needed for that. Therefore, it can be argued that the buildup of a large DOC

504   reservoir, which may have influenced the carbonates isotopic record of Eocene warming events is plausible.

505   Nonetheless, it remains to be proven how this can apply to larger oceanic-type basins, which notably harbor more

506   variable environmental conditions (*e.g.* tropical *vs.* polar latitudes), greater diversity of eukaryotic heterotrophs (in

507   Phanerozoic oceans) or yet, more active water currents and ventilation processes. In that regard, a better

508   characterization of the molecular composition of DOM in the Mexican lakes will help to understand how it can

509   accumulate over time. Furthermore, investigating the paleo-ecology and -geography of the CIE time period will

510   also help to constrain the potential applicability of a "big DOC" hypothesis (Sexton et al., 2011).

511

5.3.2 Neoproterozoic carbon isotope excursions (CIEs)

512

513   The presence of a large oceanic DOC reservoir has also been used to account for the Neoproterozoic C isotopic

514   record, where carbonates show δ^{13}C negative excursions of more than 10‰ over tens of Ma, while the paired

515   sedimentary organic carbon isotope signal remains stable (Rothman et al., 2003; Fike et al., 2006; Swanson-Hysell

516   et al., 2010; Tziperman et al., 2011). However, once again, this hypothesis has been questioned because of (i) the

517   too high DOC reservoir's size (10 times the contemporaneous DIC, *i.e.*, 10^2 to 10^3 times that of modern DOC) and

518   (ii) the amount of oxidants required to generate such a sustained DOC oxidation process (see Ridgwell and Arndt,

519   2015). Recent studies offered potential explanations for this latter issue showing that pulses of continental

520   weathering and an associated increase of sulfate supply would have provided sufficient oxidant material (Shields

521   et al., 2019; Chen et al., 2022) while lateral heterogeneity of the carbonate geochemical signatures – with a

522   restricted record of the CIEs on the continental shelves – would require lower amounts of oxidant (Li et al., 2017;

523   Shi et al., 2017).

524   Critically though, direct evidence for the existence of such high oceanic DOC levels in the past or present days

525   remains scarce (Li et al., 2017), although multiple studies have built on the Neoproterozoic big DOC scenario (*e.g.*

526   Sperling et al., 2011; Cañadas et al., 2022). Purported high oceanic DOC concentrations during the Ediacarian

527   period have been estimated from the Ge/Si ratio of diagenetic chert nodules (Xing et al., 2022) but they reflect the

528   sediments porewater geochemistry and remain difficult to directly relate to the ocean water itself. Besides, some

529   modeling approaches suggested that DOC abundance in the past Earth's oceans could not have significantly

530   departed from today's values (Fakhraee et al., 2021).

531   Modern analogous systems such as the Black Sea or Mexican lakes studied here support the possibility of important

532   DOC accumulation in anoxic waters (Ducklow et al., 2007) but those remain substantially lower than the levels

533   required to account for the Neoproterozoic events (minimum concentrations estimated between 25 and 100 mM;

534   Ridgwell and Arndt, 2015). One could argue that the development of larger DOC pools in the three Mexican lakes

535   from the SOB is hindered by relatively large sulfate reservoirs (especially in Alchichica ~10 mM). However, we

536   notice that Lake La Alberca does not show a larger DOC reservoir despite having the lowest availability of oxidant

537   (both oxygen- and sulfate-free at depth and the only one to present isotopic signatures associated with

538   methanogenesis or methanotrophy, Havas et al., submitted). It could also be argued that large OM particles in

539   Alchichica for example (Ardiles et al., 2012) favor the sedimentation of OC rather than DOC production, thereby

540   favoring the oxygenation of deep waters as suggested for the beginning of the Phanerozoic period with advent of

541   eukaryotic plankton (Lenton and Daines, 2018; Fakhraee et al., 2021). Yet, plankton of the studied Mexican lakes

542   is overall dominated by prokaryotic communities and not large organisms (Iniesto et al., 2022). Besides, rapid

543   sinking of OM particles in modern oceans does not necessarily preclude important fluxes of benthic DOC from

544   the sediments (around ~ 100 PgC.yr^{-1} in today's ocean; Burdige and Komada, 2015) and especially in anoxic

545   bottom waters (Dadi et al., 2017). Thus, even if the "oxidant paradox" may have found satisfactory explanations

546   (see above), the origin of the massive DOC reservoir required to generate these excursion remains to be elucidated

547   (Jiang et al., 2010; Li et al., 2017).

548

549

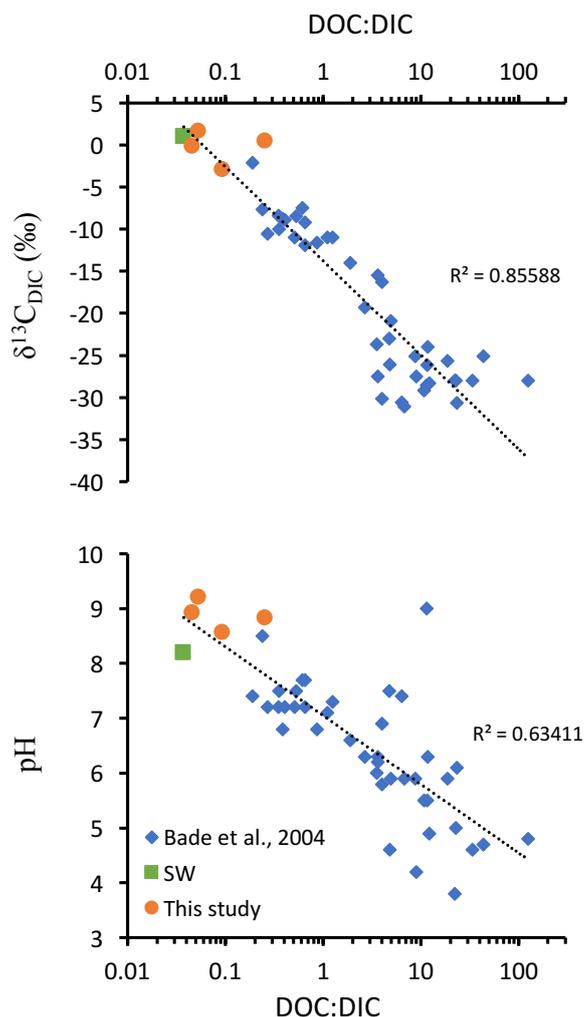



Figure 6. DOC:DIC ratios, pH and $\delta^{13}C_{DIC}$ values from different lakes compiled from Bade et al. (2004) and the four Mexican lakes from Havas et al. (submitted) as well as modern surface ocean values (from Kroopnick, 1985; Zeebe and Wolf-Gladrow, 2009 and Hansell, 2013).

Top: $\delta^{13}C_{DIC}$ as a function of DOC:DIC ratio represented with a logarithmic abscises scale and logarithmic trend line which combines the three datasets.

Bottom: pH as a function of DOC:DIC ratio, with a logarithmic abscises scale and logarithmic trend line which combines the three datasets.

550

In the studied alkaline lakes, oxidation of the DOC reservoir would generate a maximum $\delta^{13}C_{DIC}$ deviation of -2 ‰, in La Alberca de los Espinos, which has the lowest alkalinity. The other lakes $\delta^{13}C_{DIC}$ are less impacted, notably because they are largely buffered by high DIC content (Havas et al., submitted). Bade et al. (2004) showed that modern low alkalinity/low pH lakes generally show more negative $\delta^{13}C_{DIC}$ (down to ~ -30 ‰), partly due to a higher response to remineralization of OM and especially DOC. Compiling our data with those of Bade et al. (2004), we consistently show a clear negative trend of $\delta^{13}C_{DIC}$ with increasing DOC:DIC ratio over a broad range of lacustrine DOC and DIC concentrations (Fig. 6). This trend also matches modern ocean values (Fig. 6). These observations are consistent with the inference that systems where DOC:DIC >> 1 should drive $\delta^{13}C_{DIC}$ to very negative values (Rothman et al., 2003). However, in modern environments, the biomass is largely influenced by

560   aerobic heterotrophs and high DOC:DIC waters usually lean toward acidic pHs (Fig. 6; Bade et al., 2004), at which
561   carbonate precipitation is prevented. But in anoxic waters, remineralization of OM through sulfate- or iron-
562   reduction generates alkalinity instead (*e.g.* Tziperman et al., 2011). Hence, environmental conditions where
563   DOC:DIC $\gg$ 1 might be inconsistent with large carbonate deposits unless they are associated with anaerobic
564   remineralization. Besides, from the above discussion, increased terrigenous DOC inputs may be necessary to
565   generate sufficiently high DOC:DIC conditions and initiate the Neoproterozoic CIEs. Thus, this supports the
566   hypothesis that negative $\delta^{13}C_{Carb}$ excursions of the Ediacarian were triggered by continental sulfate addition to the
567   ocean (Li et al., 2017; Shields et al., 2019; Chen et al., 2022), but following the oxidation of DOC by anaerobic
568   (*e.g.* sulfate reduction) rather than aerobic (*e.g.* by free oxygen) pathways. The inferences from Fig. 6 also foster
569   the scenario proposed by Tziperman et al. (2011) where anaerobic respiration of a large DOM production leads to
570   the sequestration of newly produced C in carbonates – with very negative $\delta^{13}C$ – and thereby to the drawdown of
571   atmospheric pCO_2 and initiation of Cryogenian glaciations. Accordingly, we suggest that the climatic feedbacks
572   associated with the negative Neoproterozoic CIEs have been controlled by the total amount and balance between
573   terrigenous *vs.* marine DOC being oxidized and the nature of the oxidant (*e.g.* O_2 *vs.* SO_4^{2-}).

574   Therefore, Neoproterozoic carbonate carbon isotope excursions seem to require that DOC and DIC pools are
575   spatially decoupled (*e.g.* through terrestrial DOM inputs) and thus suggest that DOC was not necessarily larger
576   than DIC in the entire ocean. Moreover, the record of these excursions in carbonate deposits supports that the
577   oxidation of DOC should have occurred through anaerobic instead of aerobic pathways. Further characterization
578   of the nature and potential continental overprinting of the OM signatures in these sedimentary successions could
579   provide important insights into the triggers of these events.

580

581   **6. CONCLUSIONS AND SUMMARY**

582   The nature and role of DOC within the C cycle of four stratified alkaline crater lakes were characterized and
583   extensively compared, based on the concentration and isotopic signatures of this DOC reservoir, with previously
584   described DIC and POC data from these lakes. Despite similar contexts, the DOC reservoirs of the four lakes
585   exhibit important variability driven by environmental and ecological changes, as summarized below:

586   -   DOC is the largest OC reservoir in the water column of the studied lakes (> 90%). Its concentrations and
587   isotopic compositions bring precious, new and complementary information about the C cycle of these
588   stratified water bodies. Depending on environmental factors such as nutrients and DIC availability,
589   diverse photosynthetic planktonic communities appear to release more or less important amounts of DOC
590   depending on the lake, transferring most of the inorganic C to DOC rather than POC.
591   -   This process is marked by very heavy and distinct isotopic signatures of DOC compared with POC. They
592   reflect different metabolism/C fixation pathways and/or the activity of a DIC-CM coupled with an
593   overflow mechanism (*i.e.* DOM exudation) which could be active for both oxygenic and anoxygenic
594   phototrophs, and for which we propose a novel isotopic model of cell carbon cycling including the DOC.
595   -   Our results bring further constraints on the environmental conditions under which autochthonous DOM
596   can accumulate in anoxic water bodies and provides boundary conditions to the "big DOC reservoir"
597   scenarios. Specifically, our study of modern redox-stratified analogs supports the idea that a large oceanic

598   DOC reservoir may have generated the small Eocene C isotope excursions. However, while important

599   DOC oxidation could have favored locally – near the continental shelves – the precipitation of isotopically

600   light C carbonates, the existence of a globally very large oceanic DOC reservoir seems unlikely.

601

Author Contributions

602

603 RH and CT designed the study in a project directed by PLG, KB and CT. CT, MI, DJ, DM, RT, PLG and KB

604 collected the samples on the field. RH carried out the measurements for C data; DJ the physico-chemical parameter

605 probe measurements and EM provided data for trace and major elements. RH and CT analyzed the data. RH wrote

606 the manuscript with important contributions of all co-authors.

607

Competing Interests

608

609 The authors declare that they have no conflict of interest.

610

Disclaimer

611

612

Acknowledgements

613

614 This work was supported by Agence Nationale de la Recherche (France; ANR Microbialites, grant number ANR-

615 18-CE02-0013-02). The authors thank Anne-Lise Santoni, Elodie Cognard, Théophile Cocquerez and the GISMO

616 platform (Biogéosciences, University Bourgogne Franche-Comté, UMR CNRS 6282, France). We thank Céline

617 Liorzou and Bleuenn Guéguen for the analyses at the Pôle Spectrométrie Océan (Laboratoire Géo-Océan, Brest,

618 France) and Laure Cordier for ion chromatography analyses at IPGP (France). We thank Nelly Assayag and Pierre

619 Cadeau for their help on the AP 2003 at IPGP.

620

621 *References*

622 Alcántara-Hernández, R.J., Macek, M., Torres-Huesca, J., Arellano-Posadas, J., Valdespino-Castillo, P.M.

623 (2022). Bacterioplankton. In: Alcocer, J. (eds) Lake Alchichica Limnology. Springer, Cham.

624 https://doi.org/10.1007/978-3-030-79096-7_11

625 Alcocer, J., Guzmán-Arias, A., Oseguera, L.A., Escobar, E., 2014a. Dinámica del carbono orgánico disuelto y

626 particulado asociados al florecimiento de Nodularia spumigena en un lago tropical oligotrófico. Programma

627 Mex. Carbono 13.

628 Alcocer, J., Ruiz-Fernández, A.C., Escobar, E., Pérez-Bernal, L.H., Oseguera, L.A., Ardiles-Gloria, V., 2014b.

629 Deposition, burial and sequestration of carbon in an oligotrophic, tropical lake. J. Limnol. 73.

630 https://doi.org/10.4081/jlimnol.2014.783

631 Alcocer, J. *et al.* (2022). Physicochemical Characteristics. In: Alcocer, J. (eds) Lake Alchichica Limnology.

632 Springer, Cham. https://doi.org/10.1007/978-3-030-79096-7_7

633 Anderson, N.J., Stedmon, C.A., 2007. The effect of evapoconcentration on dissolved organic carbon

634 concentration and quality in lakes of SW Greenland. Freshw. Biol. 52, 280–289. https://doi.org/10.1111/j.1365-

635 2427.2006.01688.x

636 Ardiles, V., Alcocer, J., Vilaclara, G., Oseguera, L.A., Velasco, L., 2012. Diatom fluxes in a tropical,

637 oligotrophic lake dominated by large-sized phytoplankton. Hydrobiologia 679, 77–90.

638 https://doi.org/10.1007/s10750-011-0853-7

639 Armienta, M.A., Vilaclara, G., De la Cruz-Reyna, S., Ramos, S., Ceniceros, N., Cruz, O., Aguayo, A., Arcega-
640 Cabrera, F., 2008. Water chemistry of lakes related to active and inactive Mexican volcanoes. J. Volcanol.
641 Geotherm. Res. 178, 249–258. https://doi.org/10.1016/j.jvolgeores.2008.06.019

642 Bade, D.L., Carpenter, S.R., Cole, J.J., Hanson, P.C., Hesslein, R.H., 2004. Controls of δ13C-DIC in lakes:
643 Geochemistry, lake metabolism, and morphometry. Limnol. Oceanogr. 49, 1160–1172.
644 https://doi.org/10.4319/lo.2004.49.4.1160

645 Bade, D.L., Carpenter, S.R., Cole, J.J., Pace, M.L., Kritzberg, E., Van de Bogert, M.C., Cory, R.M., McKnight,
646 D.M., 2007. Sources and fates of dissolved organic carbon in lakes as determined by whole-lake carbon isotope
647 additions. Biogeochemistry 84, 115–129. https://doi.org/10.1007/s10533-006-9013-y

648 Badger, M.R., Andrews, T.J., Whitney, S.M., Ludwig, M., Yellowlees, D.C., Leggat, W., Price, G.D., 1998. The
649 diversity and coevolution of Rubisco, plastids, pyrenoids, and chloroplast-based CO2-concentrating mechanisms
650 in algae 76, 20.

651 Baines, S.B., Pace, M.L., 1991. The production of dissolved organic matter by phytoplankton and its importance
652 to bacteria: Patterns across marine and freshwater systems. Limnol. Oceanogr. 36, 1078–1090.
653 https://doi.org/10.4319/lo.1991.36.6.1078

654 Bateson, M.M., Ward, D.M., 1988. Photoexcretion and Fate of Glycolate in a Hot Spring Cyanobacterial Mat.
655 Appl. Environ. Microbiol. 54, 1738–1743. https://doi.org/10.1128/aem.54.7.1738-1743.1988

656 Beardall, J., Griffiths, H., Raven, J.A., 1982. Carbon Isotope Discrimination and the CO2 Accumulating
657 Mechanism in Chlorella emersonii. J. Exp. Bot. 33, 729–737. https://doi.org/10.1093/jxb/33.4.729

658 Beaupré, S.R., 2015. The Carbon Isotopic Composition of Marine DOC, in: Biogeochemistry of Marine
659 Dissolved Organic Matter. Elsevier, pp. 335–368. https://doi.org/10.1016/B978-0-12-405940-5.00006-6

660 Benzerara, K., Skouri-Panet, F., Li, J., Férard, C., Gugger, M., Laurent, T., Couradeau, E., Ragon, M., Cosmidis,
661 J., Menguy, N., Margaret-Oliver, I., Tavera, R., López-García, P., Moreira, D., 2014. Intracellular Ca-carbonate
662 biomineralization is widespread in cyanobacteria. Proc. Natl. Acad. Sci. 111, 10933–10938.
663 https://doi.org/10.1073/pnas.1403510111

664 Bertilsson, S., Jones, J.B., 2003. Supply of Dissolved Organic Matter to Aquatic Ecosystems: Autochthonous
665 Sources, in: Findlay, S.E.G., Sinsabaugh, R.L. (Eds.), Aquatic Ecosystems, Aquatic Ecology. Academic Press,
666 Burlington, pp. 3–24. https://doi.org/10.1016/B978-012256371-3/50002-0

667 Blair, N., Leu, A., Muñoz, E., Olsen, J., Kwong, E., Des Marais, D., 1985. Carbon isotopic fractionation in
668 heterotrophic microbial metabolism. Appl. Environ. Microbiol. 50, 996–1001.
669 https://doi.org/10.1128/aem.50.4.996-1001.1985

670 Brailsford, F.L., 2019. Dissolved organic matter (DOM) in freshwater ecosystems. Bangor University.

671 Burdige, D.J., Komada, T., 2015. Sediment Pore Waters, in: Biogeochemistry of Marine Dissolved Organic
672 Matter. Elsevier, pp. 535–577. https://doi.org/10.1016/B978-0-12-405940-5.00012-1

673 Burns, B.D., Beardall, J., 1987. Utilization of inorganic carbon by marine microalgae. J. Exp. Mar. Biol. Ecol.
674 107, 75–86. https://doi.org/10.1016/0022-0981(87)90125-0

675 Cadeau, P., Jézéquel, D., Leboulanger, C., Fouilland, E., Le Floc'h, E., Chaduteau, C., Milesi, V., Guélard, J.,
676 Sarazin, G., Katz, A., d'Amore, S., Bernard, C., Ader, M., 2020. Carbon isotope evidence for large methane
677 emissions to the Proterozoic atmosphere. Sci. Rep. 10, 18186. https://doi.org/10.1038/s41598-020-75100-x

678 Camacho, A., Walter, X.A., Picazo, A., Zopfi, J., 2017. Photoferrotrophy: Remains of an Ancient Photosynthesis
679 in Modern Environments. Front. Microbiol. 08. https://doi.org/10.3389/fmicb.2017.00323

680 Cañadas, F., Papineau, D., Leng, M.J., Li, C., 2022. Extensive primary production promoted the recovery of the
681 Ediacaran Shuram excursion. Nat. Commun. 13, 148. https://doi.org/10.1038/s41467-021-27812-5

682 Carlson, C.A., Hansell, D.A., 2015. DOM Sources, Sinks, Reactivity, and Budgets, in: Biogeochemistry of
683 Marine Dissolved Organic Matter. Elsevier, pp. 65–126. https://doi.org/10.1016/B978-0-12-405940-5.00003-0

684 Carrasco-Núñez, G., Ort, M.H., Romero, C., 2007. Evolution and hydrological conditions of a maar volcano
685 (Atexcac crater, Eastern Mexico). J. Volcanol. Geotherm. Res. 159, 179–197.
686 https://doi.org/10.1016/j.jvolgeores.2006.07.001

687 Chako Tchamabé, B., Carrasco-Núñez, G., Miggins, D.P., Németh, K., 2020. Late Pleistocene to Holocene
688 activity of Alchichica maar volcano, eastern Trans-Mexican Volcanic Belt. J. South Am. Earth Sci. 97, 102404.
689 https://doi.org/10.1016/j.jsames.2019.102404

690 Chen, B., Hu, C., Mills, B.J.W., He, T., Andersen, M.B., Chen, X., Liu, P., Lu, M., Newton, R.J., Poulton, S.W.,
691 Shields, G.A., Zhu, M., 2022. A short-lived oxidation event during the early Ediacaran and delayed oxygenation
692 of the Proterozoic ocean. Earth Planet. Sci. Lett. 577, 117274. https://doi.org/10.1016/j.epsl.2021.117274

693 Chomicki, K., 2009. The use of stable carbon and oxygen isotopes to examine the fate of dissolved organic
694 matter in two small, oligotrophic Canadian Shield lakes. University of Waterloo.

695 Close, H.G., Henderson, L.C., 2020. Open-Ocean Minima in δ13C Values of Particulate Organic Carbon in the
696 Lower Euphotic Zone. Front. Mar. Sci. 7, 540165. https://doi.org/10.3389/fmars.2020.540165

697 Couradeau, E., Benzerara, K., Gérard, E., Moreira, D., Bernard, S., Brown, G.E., López-García, P., 2012. An
698 Early-Branching Microbialite Cyanobacterium Forms Intracellular Carbonates. Science 336, 459–462.
699 https://doi.org/10.1126/science.1216171

700 Crowe, S.A., Katsev, S., Leslie, K., Sturm, A., Magen, C., Nomosatryo, S., Pack, M.A., Kessler, J.D., Reeburgh,
701 W.S., Roberts, J.A., González, L., Douglas Haffner, G., Mucci, A., Sundby, B., Fowle, D.A., 2011. The methane
702 cycle in ferruginous Lake Matano: Methane cycle in ferruginous Lake Matano. Geobiology 9, 61–78.
703 https://doi.org/10.1111/j.1472-4669.2010.00257.x

704 Dadi, T., Harir, M., Hertkorn, N., Koschorreck, M., Schmitt-Kopplin, P., Herzsprung, P., 2017. Redox
705 Conditions Affect Dissolved Organic Carbon Quality in Stratified Freshwaters. Environ. Sci. Technol. 51,
706 13705–13713. https://doi.org/10.1021/acs.est.7b04194

707 Descolas-Gros, C., Fontungne, M., 1990. Stable carbon isotope fractionation by marine phytoplankton during
708 photosynthesis. Plant Cell Environ. 13, 207–218. https://doi.org/10.1111/j.1365-3040.1990.tb01305.x

709 Dittmar, T., 2015. Reasons Behind the Long-Term Stability of Dissolved Organic Matter, in: Biogeochemistry of
710 Marine Dissolved Organic Matter. Elsevier, pp. 369–388. https://doi.org/10.1016/B978-0-12-405940-5.00007-8

711 Ducklow, H.W., Hansell, D.A., Morgan, J.A., 2007. Dissolved organic carbon and nitrogen in the Western Black
712 Sea. Mar. Chem. 105, 140–150. https://doi.org/10.1016/j.marchem.2007.01.015

713 Erez, J., Bouevitch, A., Kaplan, A., 1998. Carbon isotope fractionation by photosynthetic aquatic
714 microorganisms: experiments with *Synechococcus* PCC7942, and a simple carbon flux model. Can. J. Bot. 76,
715 1109–1118. https://doi.org/10.1139/b98-067

716 Fakhraee, M., Tarhan, L.G., Planavsky, N.J., Reinhard, C.T., 2021. A largely invariant marine dissolved organic
717 carbon reservoir across Earth's history. Proc. Natl. Acad. Sci. 118, e2103511118.
718 https://doi.org/10.1073/pnas.2103511118

719 Ferrari, L., Orozco-Esquivel, T., Manea, V., Manea, M., 2012. The dynamic history of the Trans-Mexican
720 Volcanic Belt and the Mexico subduction zone. Tectonophysics 522–523, 122–149.
721 https://doi.org/10.1016/j.tecto.2011.09.018

722 Fike, D.A., Grotzinger, J.P., Pratt, L.M., Summons, R.E., 2006. Oxidation of the Ediacaran Ocean. Nature 444,
723 744–747. https://doi.org/10.1038/nature05345

724 Fogel, M.L., Cifuentes, L.A., 1993. Isotope Fractionation during Primary Production, in: Engel, M.H., Macko,
725 S.A. (Eds.), Organic Geochemistry, Topics in Geobiology. Springer US, Boston, MA, pp. 73–98.
726 https://doi.org/10.1007/978-1-4615-2890-6_3

727 Fry, B., 1996. 13C/12C fractionation by marine diatoms. Mar. Ecol. Prog. Ser. 134, 283–294.
728 https://doi.org/10.3354/meps134283

729   Hansell, D.A., 2013. Recalcitrant Dissolved Organic Carbon Fractions. Annu. Rev. Mar. Sci. 5, 421–445.
730   https://doi.org/10.1146/annurev-marine-120710-100757

731   Havas, R., Thomazo, C., Iniesto, M., Jézéquel, D., Moreira, D., Tavera, R., Caumartin, J., Muller, E., Lopez-
732   Garcia, P., Benzerara, K., submitted. A comparative isotopic study of the biogeochemical cycle of carbon in
733   modern redox-stratified lakes. Biogeosciences preprint. https://doi.org/10.5194/bg-2022-149

734   Havig, J.R., Hamilton, T.L., McCormick, M., McClure, B., Sowers, T., Wegter, B., Kump, L.R., 2018. Water
735   column and sediment stable carbon isotope biogeochemistry of permanently redox-stratified Fayetteville Green
736   Lake, New York, U.S.A. Limnol. Oceanogr. 63, 570–587. https://doi.org/10.1002/lno.10649

737   Havig, J.R., McCormick, M.L., Hamilton, T.L., Kump, L.R., 2015. The behavior of biologically important trace
738   elements across the oxic/euxinic transition of meromictic Fayetteville Green Lake, New York, USA. Geochim.
739   Cosmochim. Acta 165, 389–406. https://doi.org/10.1016/j.gca.2015.06.024

740   Hayes, J.M., 2001. Fractionation of Carbon and Hydrogen Isotopes in Biosynthetic Processes*. Rev. Mineral.
741   Geochem. 43, 225–277. https://doi.org/10.2138/gsrmg.43.1.225

742   Hessen, D.O., 1992. Dissolved organic carbon in a humic lake: effects on bacterial production and respiration,
743   in: Salonen, K., Kairesalo, T., Jones, R.I. (Eds.), Dissolved Organic Matter in Lacustrine Ecosystems: Energy
744   Source and System Regulator, Developments in Hydrobiology. Springer Netherlands, Dordrecht, pp. 115–123.
745   https://doi.org/10.1007/978-94-011-2474-4_9

746   Hessen, D.O., Anderson, T.R., 2008. Excess carbon in aquatic organisms and ecosystems: Physiological,
747   ecological, and evolutionary implications. Limnol. Oceanogr. 53, 1685–1696.
748   https://doi.org/10.4319/lo.2008.53.4.1685

749   Iniesto, M., Moreira, D., Benzerara, K., Reboul, G., Bertolino, P., Tavera, R., López-García, P., 2022. Planktonic
750   microbial communities from microbialite-bearing lakes sampled along a salinity-alkalinity gradient. Limnol.
751   Oceanogr. lno.12233. https://doi.org/10.1002/lno.12233

752   Iñiguez, C., Capó-Bauçà, S., Niinemets, Ü., Stoll, H., Aguiló-Nicolau, P., Galmés, J., 2020. Evolutionary trends
753   in RuBisCO kinetics and their co-evolution with CO2 concentrating mechanisms. Plant J. 101, 897–918.
754   https://doi.org/10.1111/tpj.14643

755   Ivanovsky, R.N., Lebedeva, N.V., Keppen, O.I., Chudnovskaya, A.V., 2020. Release of Photosynthetically Fixed
756   Carbon as Dissolved Organic Matter by Anoxygenic Phototrophic Bacteria. Microbiology 89, 28–34.
757   https://doi.org/10.1134/S0026261720010075

758   Jiang, G., Wang, X., Shi, X., Xiao, S., Zhang, S., Dong, J., 2012. The origin of decoupled carbonate and organic
759   carbon isotope signatures in the early Cambrian (ca. 542–520Ma) Yangtze platform. Earth Planet. Sci. Lett. 317–
760   318, 96–110. https://doi.org/10.1016/j.epsl.2011.11.018

761   Jiang, G., Wang, X., Shi, X., Zhang, S., Xiao, S., Dong, J., 2010. Organic carbon isotope constraints on the
762   dissolved organic carbon (DOC) reservoir at the Cryogenian–Ediacaran transition. Earth Planet. Sci. Lett. 299,
763   159–168. https://doi.org/10.1016/j.epsl.2010.08.031

764   Jiao, N., Herndl, G.J., Hansell, D.A., Benner, R., Kattner, G., Wilhelm, S.W., Kirchman, D.L., Weinbauer, M.G.,
765   Luo, T., Chen, F., Azam, F., 2010. Microbial production of recalcitrant dissolved organic matter: long-term
766   carbon storage in the global ocean. Nat. Rev. Microbiol. 8, 593–599. https://doi.org/10.1038/nrmicro2386

767   Johnston, D.T., Macdonald, F.A., Gill, B.C., Hoffman, P.F., Schrag, D.P., 2012. Uncovering the Neoproterozoic
768   carbon cycle. Nature 483, 320–323. https://doi.org/10.1038/nature10854

769   Kaplan, L.A., Wiegner, T.N., Newbold, J.D., Ostrom, P.H., Gandhi, H., 2008. Untangling the complex issue of
770   dissolved organic carbon uptake: a stable isotope approach. Freshw. Biol. 53, 855–864.
771   https://doi.org/10.1111/j.1365-2427.2007.01941.x

772   Kawasaki, N., Komatsu, K., Kohzu, A., Tomioka, N., Shinohara, R., Satou, T., Watanabe, F.N., Tada, Y.,
773   Hamasaki, K., Kushairi, M.R.M., Imai, A., 2013. Bacterial Contribution to Dissolved Organic Matter in

774   Eutrophic Lake Kasumigaura, Japan. Appl. Environ. Microbiol. 79, 7160–7168.
775   https://doi.org/10.1128/AEM.01504-13

776   Kroopnick, P.M., 1985. The distribution of 13C of ΣCO2 in the world oceans. *Deep Sea Research Part A.*
777   Oceanographic Research Papers, *32*(1), pp.57-84. https://doi.org/10.1016/0198-0149(85)90017-2

778   Kuntz, L.B., Laakso, T.A., Schrag, D.P., Crowe, S.A., 2015. Modeling the carbon cycle in Lake Matano.
779   Geobiology 13, 454–461. https://doi.org/10.1111/gbi.12141

780   Lampert, W., 1978. Release of dissolved organic carbon by grazing zooplankton. Limnol. Oceanogr. 23, 831–
781   834. https://doi.org/10.4319/lo.1978.23.4.0831

782   Lenton, T.M., Daines, S.J., 2018. The effects of marine eukaryote evolution on phosphorus, carbon and oxygen
783   cycling across the Proterozoic–Phanerozoic transition. Emerg. Top. Life Sci. 2, 267–278.
784   https://doi.org/10.1042/ETLS20170156

785   Li, C., Hardisty, D.S., Luo, G., Huang, J., Algeo, T.J., Cheng, M., Shi, W., An, Z., Tong, J., Xie, S., Jiao, N.,
786   Lyons, T.W., 2017. Uncovering the spatial heterogeneity of Ediacaran carbon cycling. Geobiology 15, 211–224.
787   https://doi.org/10.1111/gbi.12222

788   Lugo, A., Alcocer, J., Sanchez, M.R., Escobar, E., 1993. Trophic status of tropical lakes indicated by littoral
789   protozoan assemblages. SIL Proc. 1922-2010 25, 441–443. https://doi.org/10.1080/03680770.1992.11900159

790   Lyons, T.W., Reinhard, C.T., Planavsky, N.J., 2014. The rise of oxygen in Earth's early ocean and atmosphere.
791   Nature 506, 307–315. https://doi.org/10.1038/nature13068

792   Macek, M., Medina, X.S., Picazo, A., Peštová, D., Reyes, F.B., Hernández, J.R.M., Alcocer, J., Ibarra, M.M.,
793   Camacho, A., 2020. Spirostomum teres: A Long Term Study of an Anoxic-Hypolimnion Population Feeding
794   upon Photosynthesizing Microorganisms. Acta Protozool. 59, 13–38.
795   https://doi.org/10.4467/16890027AP.20.002.12158

796   Marañón, E., Cermeño, P., Fernández, E., Rodríguez, J., Zabala, L., 2004. Significance and mechanisms of
797   photosynthetic production of dissolved organic carbonin a coastal eutrophic ecosystem. Limnol. Oceanogr. 49,
798   1652–1666. https://doi.org/10.4319/lo.2004.49.5.1652

799   Margolin, A.R., Gerringa, L.J.A., Hansell, D.A., Rijkenberg, M.J.A., 2016. Net removal of dissolved organic
800   carbon in the anoxic waters of the Black Sea. Mar. Chem. 183, 13–24.
801   https://doi.org/10.1016/j.marchem.2016.05.003

802   Morana, C., Sarmento, H., Descy, J.-P., Gasol, J.M., Borges, A.V., Bouillon, S., Darchambeau, F., 2014.
803   Production of dissolved organic matter by phytoplankton and its uptake by heterotrophic prokaryotes in large
804   tropical lakes. Limnol. Oceanogr. 59, 1364–1375. https://doi.org/10.4319/lo.2014.59.4.1364

805   Ogawa, H., Amagai, Y., Koike, I., Kaiser, K., Benner, R., 2001. Production of Refractory Dissolved Organic
806   Matter by Bacteria. Science 292, 917–920. https://doi.org/10.1126/science.1057627

807   O'Leary, M.H., 1988. Carbon Isotopes in Photosynthesis. BioScience 38, 328–336.
808   https://doi.org/10.2307/1310735

809   Organization for Economic Cooperation and Development (OECD), 1982, Eutrophication of waters. Monitoring,
810   assessment and control: Paris, Organization for Economic Cooperation and Development, 154 p.

811   Peltier, W.R., Liu, Y., Crowley, J.W., 2007. Snowball Earth prevention by dissolved organic carbon
812   remineralization. Nature 450, 813–818. https://doi.org/10.1038/nature06354

813   Petrash, D.A., Steenbergen, I.M., Valero, A., Meador, T.B., Pačes, T., Thomazo, C., 2022. Aqueous system-level
814   processes and prokaryote assemblages in the ferruginous and sulfate-rich bottom waters of a post-mining lake.
815   Biogeosciences 19, 1723–1751. https://doi.org/10.5194/bg-19-1723-2022

816   Posth, N.R., Bristow, L.A., Cox, R.P., Habicht, K.S., Danza, F., Tonolla, M., Frigaard, N. -U., Canfield, D.E.,
817   2017. Carbon isotope fractionation by anoxygenic phototrophic bacteria in euxinic Lake Cadagno. Geobiology
818   15, 798–816. https://doi.org/10.1111/gbi.12254

819 Renstrom-Kellner, E., Bergman, B., 1989. Glycolate metabolism in cyanobacteria. III. Nitrogen controls
820 excretion and metabolism of glycolate in Anabaena cylindrica. Physiol. Plant. 77, 46–51.
821 https://doi.org/10.1111/j.1399-3054.1989.tb05976.x

822 Ridgwell, A., Arndt, S., 2015. Chapter 1 - Why Dissolved Organics Matter: DOC in Ancient Oceans and Past
823 Climate Change, in: Hansell, D.A., Carlson, C.A. (Eds.), Biogeochemistry of Marine Dissolved Organic Matter
824 (Second Edition). Academic Press, Boston, pp. 1–20. https://doi.org/10.1016/B978-0-12-405940-5.00001-7

825 Riebesell, U., Burkhardt, S., Dauelsberg, A., Kroon, B., 2000. Carbon isotope fractionation by a marine
826 diatom:dependence on the growth-rate-limiting resource. Mar. Ecol. Prog. Ser. 193, 295–303.
827 https://doi.org/10.3354/meps193295

828 Rothman, D.H., Hayes, J.M., Summons, R.E., 2003. Dynamics of the Neoproterozoic carbon cycle. Proc. Natl.
829 Acad. Sci. 100, 8124–8129. https://doi.org/10.1073/pnas.0832439100

830 Saini, J.S., Hassler, C., Cable, R., Fourquez, M., Danza, F., Roman, S., Tonolla, M., Storelli, N., Jacquet, S.,
831 Zdobnov, E.M., Duhaime, M.B., 2021. Microbial loop of a Proterozoic ocean analogue (preprint). Microbiology.
832 https://doi.org/10.1101/2021.08.17.456685

833 Satkoski, A.M., Beukes, N.J., Li, W., Beard, B.L., Johnson, C.M., 2015. A redox-stratified ocean 3.2 billion
834 years ago. Earth Planet. Sci. Lett. 430, 43–53. https://doi.org/10.1016/j.epsl.2015.08.007

835 Schiff, S.L., Tsuji, J.M., Wu, L., Venkiteswaran, J.J., Molot, L.A., Elgood, R.J., Paterson, M.J., Neufeld, J.D.,
836 2017. Millions of Boreal Shield Lakes can be used to Probe Archaean Ocean Biogeochemistry. Sci. Rep. 7,
837 46708. https://doi.org/10.1038/srep46708

838 Sexton, P.F., Norris, R.D., Wilson, P.A., Pälike, H., Westerhold, T., Röhl, U., Bolton, C.T., Gibbs, S., 2011.
839 Eocene global warming events driven by ventilation of oceanic dissolved organic carbon. Nature 471, 349–352.
840 https://doi.org/10.1038/nature09826

841 Shi, W., Li, C., Algeo, T.J., 2017. Quantitative model evaluation of organic carbon oxidation hypotheses for the
842 Ediacaran Shuram carbon isotopic excursion. Sci. China Earth Sci. 60, 2118–2127.
843 https://doi.org/10.1007/s11430-017-9137-1

844 Shields, G.A., Mills, B.J.W., Zhu, M., Raub, T.D., Daines, S.J., Lenton, T.M., 2019. Unique Neoproterozoic
845 carbon isotope excursions sustained by coupled evaporite dissolution and pyrite burial. Nat. Geosci. 12, 823–
846 827. https://doi.org/10.1038/s41561-019-0434-3

847 Siebe, C., Guilbaud, M.-N., Salinas, S., Chédeville-Monzo, C., 2012. Eruption of Alberca de los Espinos tuff
848 cone causes transgression of Zacapu lake ca. 25,000 yr BP in Michoacán, México. Presented at the IAS 4IMC
849 Conference, Auckland, New Zeland, pp. 74–75.

850 Siebe, C., Guilbaud, M.-N., Salinas, S., Kshirsagar, P., Chevrel, M.O., Jiménez, A.H., Godínez, L., 2014.
851 Monogenetic volcanism of the Michoacán-Guanajuato Volcanic Field: Maar craters of the Zacapu basin and
852 domes, shields, and scoria cones of the Tarascan highlands (Paracho-Paricutin region). Presented at the Pre-
853 meeting field guide for the 5th international Maar Conference, Querétaro, México, pp. 1–37.

854 Sigala, I., Caballero, M., Correa-Metrio, A., Lozano-García, S., Vázquez, G., Pérez, L., Zawisza, E., 2017. Basic
855 limnology of 30 continental waterbodies of the Transmexican Volcanic Belt across climatic and environmental
856 gradients. Bol. Soc. Geológica Mex. 69, 313–370. https://doi.org/10.18268/BSGM2017v69n2a3

857 Silva-Aguilera, R.A., Escolero, Ó., Alcocer, J. (2022). Recent Climate of Serdán-Oriental Basin. In: Alcocer, J.
858 (eds) Lake Alchichica Limnology. Springer, Cham. https://doi.org/10.1007/978-3-030-79096-7_4

859 Sperling, E.A., Peterson, K.J., Laflamme, M., 2011. Rangeomorphs, Thectardis (Porifera?) and dissolved organic
860 carbon in the Ediacaran oceans: Rangeomorphs, Thectardis and DOC. Geobiology 9, 24–33.
861 https://doi.org/10.1111/j.1472-4669.2010.00259.x

862 Swanson-Hysell, N.L., Rose, C.V., Calmet, C.C., Halverson, G.P., Hurtgen, M.T., Maloof, A.C., 2010.
863 Cryogenian Glaciation and the Onset of Carbon-Isotope Decoupling. Science 328, 608–611.
864 https://doi.org/10.1126/science.1184508

865   Thomas, P.J., Boller, A.J., Satagopan, S., Tabita, F.R., Cavanaugh, C.M., Scott, K.M., 2019. Isotope
866   discrimination by form IC RubisCO from *Ralstonia eutropha* and *Rhodobacter sphaeroides* , metabolically
867   versatile members of ' *Proteobacteria* ' from aquatic and soil habitats. Environ. Microbiol. 21, 72–80.
868   https://doi.org/10.1111/1462-2920.14423

869   Thornton, D.C.O., 2014. Dissolved organic matter (DOM) release by phytoplankton in the contemporary and
870   future ocean. Eur. J. Phycol. 49, 20–46. https://doi.org/10.1080/09670262.2013.875596

871   Tziperman, E., Halevy, I., Johnston, D.T., Knoll, A.H., Schrag, D.P., 2011. Biologically induced initiation of
872   Neoproterozoic snowball-Earth events. Proc. Natl. Acad. Sci. 108, 15091–15096.
873   https://doi.org/10.1073/pnas.1016361108

874   Vilaclara, G., Chávez, M., Lugo, A., González, H., Gaytán, M., 1993. Comparative description of crater-lakes
875   basic chemistry in Puebla State, Mexico. SIL Proc. 1922-2010 25, 435–440.
876   https://doi.org/10.1080/03680770.1992.11900158

877   Werne, J.P., Hollander, D.J., 2004. Balancing supply and demand: controls on carbon isotope fractionation in the
878   Cariaco Basin (Venezuela) Younger Dryas to present. Mar. Chem. 92, 275–293.
879   https://doi.org/10.1016/j.marchem.2004.06.031

880   Wetz, M.S., Wheeler, P.A., 2007. Release of dissolved organic matter by coastal diatoms. Limnol. Oceanogr. 52,
881   798–807. https://doi.org/10.4319/lo.2007.52.2.0798

882   Xing, C., Liu, P., Wang, R., Li, C., Li, J., Shen, B., 2022. Tracing the evolution of dissolved organic carbon
883   (DOC) pool in the Ediacaran ocean by Germanium/silica (Ge/Si) ratios of diagenetic chert nodules from the
884   Doushantuo Formation, South China. Precambrian Res. 374, 106639.
885   https://doi.org/10.1016/j.precamres.2022.106639

886   Zeebe, R.E., Wolf-Gladrow, D.A. (2009). Carbon Dioxide, Dissolved (Ocean). In: Gornitz, V. (eds)
887   Encyclopedia of Paleoclimatology and Ancient Environments. Encyclopedia of Earth Sciences Series. Springer,
888   Dordrecht. https://doi.org/10.1007/978-1-4020-4411-3_30

889   Zeyen, N., Benzerara, K., Beyssac, O., Daval, D., Muller, E., Thomazo, C., Tavera, R., López-García, P.,
890   Moreira, D., Duprat, E., 2021. Integrative analysis of the mineralogical and chemical composition of modern
891   microbialites from ten Mexican lakes: What do we learn about their formation? Geochim. Cosmochim. Acta
892   305, 148–184. https://doi.org/10.1016/j.gca.2021.04.030

893