



1 **Manifestations and environmental implications of microbially-induced calcium**
2 **carbonate precipitation (MICP) by the cyanobacterium *Dolichospermum***
3 ***flosaquae***

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12

13 **Abstract:**

14 The aim of this work is to explore the ability and magnitude of the temperate
15 cyanobacterium *Dolichospermum flosaquae* in MICP (microbially-induced calcium
16 carbonate precipitation). Environmentally, MICP controls the availability of calcium,
17 carbon and phosphorus in freshwater lakes and simultaneously controls carbon
18 exchange with the atmosphere. Cultures of *flosaquae* were grown in BG11 medium
19 containing 0, 1, 1.5, 2 and 4 mg Ca²⁺ L⁻¹, as cardinal concentrations previously
20 reported in freshwater lakes, in addition to a control culture (BG11 containing 13 mg
21 Ca²⁺ L⁻¹). Growth (cell number, chlorophyll a, and protein content) of *D. flosaquae* was
22 generally reduced by elevating calcium concentrations of the different salts used
23 (chloride, acetate, or citrate). *D. flosaquae* seems able to perform MICP as carbonate
24 alkalinity was sharply induced up to its highest level (six times that of the control) at a
25 citrate concentration of 4 mg Ca²⁺ L⁻¹. Calcium carbonate was formed at a pre-
26 precipitation stage as the minimum pH necessary for precipitation (8.7) has been
27 scarcely approached under such conditions. MICP took place mostly relying on
28 photosynthesis and respiration, but not on urease activity, as urea was not
29 supplemented in the growth media. However, *D. flosaquae* exhibited strong urease
30 specific activity in *in vitro* assays (four times that of the control at 4 mg Ca²⁺ citrate L⁻¹
31 ¹). Residual calcium exhibited its lowest value at 4 mg Ca²⁺ citrate L⁻¹, coinciding with
32 the highest alkalinity level. Consumed calcium was increasing with chlorophyll a



33 content, but not with increasing cell numbers. The experiments should be repeated in
34 a future study, but in the presence of urea, to evaluate the full potential of calcium
35 carbonate precipitation of *D. flosaquae*, its dynamics and impact on biogeochemical
36 cycles of calcium, carbon and phosphorus in freshwater lakes.

37 **Key Words:**

38 *Dolichospermum flosaquae* – MICP - Photosynthesis – Respiration – Urease –
39 Alkalinity – Calcium

40 **Introduction:**

41 Microbially-induced calcium carbonate precipitation (MICP) depicts an exogenous or
42 endogenous microbial activity that takes place during heterotrophic growth of
43 numerous fungi and bacteria or during photoautotrophic growth of cyanobacteria in
44 their natural environments including water, soils, tufas, biofilms or geological
45 formations. Furthermore, bacterial, and cyanobacterial mucilaginous sheath (capsular
46 polysaccharides or exopolysaccharides) as well as fungal chitin act as nucleation sites
47 for CaCO₃ crystallization by binding Ca²⁺ onto their carboxylic groups. MICP requires
48 sufficient Ca²⁺, an alkaline pH and suitable microorganisms. Availability of nucleation
49 sites is very important for stable and continuous calcium carbonate bio-mineralization
50 (Phillips et al 2013). In particular, cyanobacteria are active prokaryotes performing
51 MICP (Payandi-Rolland 2019; Xu et al 2019). Several metabolic processes such as
52 photosynthesis, respiration, sulfate, nitrate or sulfide reduction have been recorded as
53 driving mechanisms for MICP (e.g. Seifan et al 2019). However, ureolytic activity has
54 been also found as a major mechanism catalyzing MICP via CO₂ and ammonia
55 production during urea hydrolysis. Castro-Alonso et al (2019) reported on a series of
56 complex reactions of urease, calcium, and carbonate during MICP surrounding the
57 cells. Ca²⁺, beside being a component of CaCO₃, is inductive for urease activity
58 resulting in a pronounced upregulation. Furthermore, morphology of the calcite crystal
59 is strain-specific (Hammes et al 2003) and depends on the type of the calcium salt
60 present (Achal and Pan 2014).

61 Subsequent to precipitation of calcium carbonate, the bioavailability of both calcium
62 and phosphorus as well as CO₂ are lowered in lakes; which, in turn, arises as rate-
63 limiting to growth and nutrition of aquatic microbiota, e.g. via photosynthetic activity in
64 the case of cyanobacteria. This metabolic process is widely explored and regarded as



65 a promising phenomenon for use in various industrial applications. In addition to the
66 governmental control of acid deposition into lakes, MICP may be responsible (at least
67 in part) for the widespread threat of calcium decline in freshwater lakes around the
68 globe, as calcium co-precipitates simultaneously with carbonate (Jeziorski and Smol
69 2017). After studying thousands of water samples in tens of countries (spatially and
70 temporally), Weyhenmeyer et al (2019) concluded that the global median calcium
71 concentration was 4.0 mg L^{-1} with 20.7% of the water samples showing Ca^{2+}
72 concentrations $\leq 1.5 \text{ mg L}^{-1}$, a threshold considered critical for the survival of many
73 Ca^{2+} dependent organisms.

74 The hypothesis of this work is to explore whether *Dolichospermum flosaquae*, a major
75 temperate cyanobacterium, is able to perform MICP in freshwater lakes. Dependence
76 of MICP magnitude on Ca^{2+} concentration and salt type of chloride, acetate or citrate
77 as well as the powering metabolic process are also concerned within this study.
78 Photosynthesis, respiration, total alkalinity and urease activity of *D. flosaquae* are
79 measurables assessed to elucidate their role in mediating MICP and to detect the
80 effect of the applied treatments. The results obtained are discussed on the lights of
81 their anticipated environmental impact and implications.

82

83 **Materials and Methods:**

84 Experimental Set up:

85 Cultures of the cyanobacterium *D. flosaquae* were incubated at different Ca^{2+}
86 concentrations (0, 1, 1.5, 2 and $4 \text{ mg Ca}^{2+} \text{ L}^{-1}$) of different salts (chloride “Cl”, acetate
87 “Ac”, or citrate “Cit”) supplemented into calcium free BG11 medium specific for
88 cyanobacteria (Stanier et al 1971). *D. flosaquae* was also grown in full BG11 medium
89 (containing $13 \text{ mg Ca}^{2+} \text{ L}^{-1}$, which is considered the control culture “Con+”) in addition
90 to a reference calcium-deprived culture (BG11 devoid of any supplemental calcium
91 “Con-”). Culture media were inoculated with 10 ml of 5 days old cells of *D. flosaquae*
92 in conical flasks capped with aluminum foil. Cultures were shaken for 4 weeks at 22 ± 1
93 °C and white light intensity of $25 \mu\text{mole m}^{-2} \text{ sec}^{-1}$ (14h light:10h dark cycle).

94

95 Analytical Methods:



96 At the end of the experiment, i.e. after growth for 4 weeks, the following parameters of
97 the variously treated *D. flosaquae* cultures were analyzed and assessed as follows:

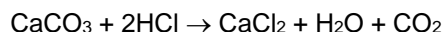
98 - Cell number and chlorophyll a were simultaneously assessed using a YSI-
99 multiparameter probe.

100 - Protein contents were estimated according to the method of **Bradford** (1976). Cells
101 were extracted in boiled water, centrifuged and proteins were assessed in the
102 supernatant. The binding of protein molecules with the Coomassie Brilliant Blue dye
103 under acidic conditions results in a color change from brown to blue, measured at a
104 wavelength of 595 nm using a BioTek Synergy 2, multidetector microplate reader
105 (Vermont, USA).

106

107 - Total alkalinity was assessed by titration of 50 mL algal culture media with 0.1M HCl
108 following **Choi** et al (2017) and **Xu** et al (2019), and then calculated using the
109 following equation:

110



111 Based on the reaction stoichiometry between CaCO_3 and HCl, the mole ratio of CaCO_3
112 to HCl is 1:2; by dividing the number of moles of HCl by 2, the product is the number
113 of moles of CaCO_3 . The number of moles of CaCO_3 would be multiplied with its
114 molecular weight to get the yielded respective CaCO_3 mass.

115 - Photosynthetic activity: The light-induced O_2 evolution by *D. flosaquae* in different
116 cultures was followed by means of an oxygen sensor (PreSens MicroXTX3O₂
117 sensor, SoftwareTx3v6O₂, Presens, Germany) at the same growth conditions (white
118 light intensity of about $25 \mu\text{mole m}^{-2} \text{sec}^{-1}$ at room temperature, i.e. $22 \pm 1^\circ\text{C}$).
119 Respiration (O_2 uptake) was also monitored using the same oxygen sensor, but in
120 the dark.

121

122 - Assessment of residual free $[\text{Ca}^{2+}]$ in the growth media: At the end of the experiment,
123 calcium was assayed by calcium kits (ab102505, Calcium Detection Assay Kit-
124 colorimetric, abcam) and determined at a wave length of 575 nm using a BioTek
125 Synergy 2, multidetector microplate reader (Vermont, USA). Consumed calcium was
126 then calculated by subtracting residual from total calcium.

127



128 - Urease enzyme (UE) activity was assayed spectrophotometrically following the
129 procedure of **Mobley** et al (1988) and quantified using a calibration curve of
130 ammonia. The in vitro assay mixture of UE contained intact cells of *D. flosaquae*,
131 urea (200 mM), phenol red ($7 \mu\text{g mL}^{-1}$) and phosphate buffer (pH 6.8). After 10 min,
132 the developed color, as a result of liberated ammonia from urea hydrolysis, was
133 determined at a wavelength of 500 nm using the same microplate reader (as
134 described above).

135

136 - Ammonia accumulated in the different culture media at the end of the experimental
137 period was assessed as mentioned above in urease-liberated ammonia.

138

139 - The pH values of the differently treated cultures were determined via a pH meter
140 (WTW3301, Germany).

141

142 All experiments and assessments were conducted in triplicates and the mean values
143 \pm SE (standard error) are presented in the figures.

144

145 **Results:**

146 Under culture conditions, growth indices (cell number, chlorophyll - and protein
147 contents) of *D. flosaquae* were variably affected in response to calcium concentration
148 as well as to its counter anion (chloride, acetate, or citrate). Growth of *D. flosaquae*
149 decreased as calcium concentrations of all salts were lowered, following a relative
150 growth enhancement (higher than the control) at a threshold value of $1.5\text{-}2.0 \text{ mg Ca}^{2+}$
151 L^{-1} of the calcium salts citrate and acetate, respectively, while continually lowered by
152 calcium chloride. Calcium-deprived cultures (Co-) exhibited markedly lower growth
153 rates than Ca^{2+} supplemented ones (Co+) in terms of cell number, chlorophyll a and
154 protein contents (Fig. 1).

155 The pH of 7.0 was set for all *D. flosaquae* cultures at the beginning of the experiment;
156 thereafter, it was elevated to levels ranging between pH 8.0 - 8.7, depending on
157 calcium treatment (Fig. 2). A certain calcium concentration of each salt induced a
158 higher pH than control or calcium-deprived cultures. The highest pH elevation (up to
159 8.7) occurred at calcium chloride and calcium citrate concentrations of $1 \text{ mg Ca}^{2+} \text{ L}^{-1}$



160 but decreased at higher concentrations. However, Ca^{2+} acetate resulted in the highest
161 pH elevation of 8.7 at the highest added concentration ($4 \text{ mg Ca}^{2+} \text{ L}^{-1}$). Accordingly,
162 the pH elevation depended on the calcium concentrations and type of Ca^{2+} salt
163 (chloride, acetate or citrate).

164

165 Fig. (3) presents the net photosynthetic oxygen evolution (P_N) and dark respiratory
166 oxygen uptake (R_D) of *D. flosaquae* in dependence on the imposed calcium
167 treatments. P_N was severely inhibited in calcium deprived cultures of *D. flosaquae*
168 relative to control cultures while R_D was enhanced. Different calcium salts exerted
169 different impacts, but in most calcium treatments, net photosynthetic oxygen evolution
170 was higher than in the control cultures. In calcium chloride and acetate treated cultures
171 of *D. flosaquae*, P_N and R_D enhanced with increased concentrations of calcium while
172 in citrate treated *D. flosaquae* cultures, both P_N and R_D decreased.
173 Photosynthesis:respiration ($P_N:R_D$) ratios, which represent the net productivity of cells
174 or cultures, were severely inhibited by calcium deprivation while calcium chloride,
175 acetate and citrate induced inhibition or stimulation of $P_N:R_D$, depending on the calcium
176 concentration.

177

178 Total alkalinity (T alkalinity), ammonia as well as corrected carbonate alkalinity values
179 (C alkalinity), calculated by subtracting ammonia concentration from total alkalinity of
180 the differently treated *D. flosaquae* cultures, are shown in Fig. (4). Total alkalinity
181 exhibited its absolutely lowest amount in the control culture of *D. flosaquae*, despite it
182 contained the highest Ca^{2+} concentration ($13 \text{ mg Ca}^{2+} \text{ L}^{-1}$) while calcium deprivation
183 remarkably enhanced alkalinity up to three times that of the control cultures (5 to 15
184 $\text{mmol carbonate } \mu\text{g Chl}^{-1}$). Furthermore, alkalinity level in any of the calcium treated
185 cultures was markedly higher than that of control or calcium-deprived cultures, with a
186 maximum alkalinity level at calcium citrate concentration of $4 \text{ mg Ca}^{2+} \text{ L}^{-1}$. All calcium
187 acetate concentrations induced more or less similar alkalinity levels whereas calcium
188 chloride induced its highest stimulation at $2 \text{ mg Ca}^{2+} \text{ L}^{-1}$. As ammonia may interfere
189 with carbonate alkalinity, ammonia has been assessed and detected in trace amounts
190 not affecting total alkalinity (Fig. 4).



191 Residual calcium was assessed while total and consumed fractions were calculated
192 (per mL culture and per unit chlorophyll) and presented in Fig. (5a&b); consumed
193 calcium means its incorporation into or precipitation as calcium carbonate. It is
194 important to mention that in calcium-deprived cultures, i.e. without any external
195 supplementation, calcium concentration was still $2.26 \text{ mg Ca}^{2+} \text{ L}^{-1}$, nevertheless. This
196 amount might have been released from cellular apoplastic regions as well as from
197 intracellular stores. Therefore, a virtual concentration of total calcium is given to
198 account for the externally supplemented concentration of calcium (0, 1, 1.5, 2 or 4 mg
199 $\text{Ca}^{2+} \text{ L}^{-1}$) and the amount of calcium found at calcium-deprivation (i.e. $2.26 \text{ mg Ca}^{2+} \text{ L}^{-1}$)
200 1), which was assumed to be equally released by each culture. Control cultures
201 displayed the highest levels of all calcium fractions as they started at the highest total
202 virtual concentration of $15.26 \text{ mg Ca}^{2+} \text{ L}^{-1}$ (i.e. $13 \text{ mg Ca}^{2+} \text{ L}^{-1}$ in BG11 plus 2.26 mg
203 $\text{Ca}^{2+} \text{ L}^{-1}$ released). On the contrary, calcium-deprived cultures exhibited the lowest
204 levels of all calcium fractions since no calcium had been added and thus the released
205 calcium was the only calcium resource.

206 Residual calcium (in the culture media) and consumed calcium (per unit chlorophyll
207 and per unit volume) increased with elevated calcium additions (Fig 5a). The lowest
208 amounts of residual calcium were recorded in citrate treated cultures (almost equal to
209 the consumed fraction and about 50% of total calcium). The concentration of 4 mg
210 citrate L^{-1} enhanced the calcium consumption nearly up to that of the control despite
211 the big difference in the externally supplemented calcium concentration (4 vs. 13 mg
212 $\text{Ca}^{2+} \text{ L}^{-1}$, respectively). In chloride and acetate, residual calcium was considerably
213 higher indicating less incorporation into calcium carbonate. Consumed calcium per
214 unit chlorophyll (C/Chl) was increasing with increasing supplemented calcium
215 concentration; the highest enhancement was recorded at citrate (Fig 5b).

216

217 Urease enzyme (UE) activity is presented in Fig (6); specific activity “SA” represents
218 the rate of enzyme activity as $\mu\text{mole ammonia released. } \mu\text{g}^{-1} \text{ protein. min}^{-1}$ while total
219 activity “TA” represents the rate of enzyme activity as $\mu\text{mole ammonia released. mL}^{-1}$
220 $\text{algal suspension. min}^{-1}$. Total activity is the product of specific activity per $\mu\text{g protein}$
221 multiplied by the amount of protein per unit volume (mL) of algal cultures. Calcium
222 deprivation inhibited UE activity; the magnitude of inhibition on a volume basis “TA”



223 was more pronounced than the enzyme specific activity “SA” because enzyme
224 (protein) contents were also lower. Calcium chloride induced the highest rates of UE,
225 total and specific activity, at 1.5 mg Ca²⁺ L⁻¹; otherwise, it was inhibitory at lower or
226 higher concentrations. Calcium acetate induced the highest rates of “TA” and “SA” at
227 moderate concentrations of 1.5 and 2 mg Ca²⁺ L⁻¹, both lowest and highest
228 concentrations of 1 and 4 mg Ca²⁺ L⁻¹ severely inhibited the enzyme activity. Calcium
229 citrate induced a continuous increase in urease activity (SA) up to its “absolutely”
230 highest rate at 4 mg Ca²⁺ L⁻¹ among other concentrations and salts; such highest rate
231 of urease activity was in accordance with the highest level of calcium consumption i.e.
232 calcium may be inductive to urease activity in *D. flosaquae*. The order of UE
233 enhancement was as follows citrate > acetate > chloride.

234

235 **Discussion:**

236 Our results indicate that *Dolichospermum flosaquae* is able to perform MICP
237 (microbially-induced calcium carbonate precipitation). Therefore, the intensive blooms
238 of this organism have the potential to control the overall biogeochemistry dynamics in
239 freshwater bodies, i.e. controlling the availability of calcium, carbon, and phosphorus
240 in addition to carbon emissions into the atmosphere. In this work, the capability of the
241 cyanobacterium *D. flosaquae* in freshwater MICP was studied at different
242 concentrations of three calcium salts (chloride, acetate, and citrate). It proved that
243 different salt types and calcium concentrations exerted different impacts on *D.*
244 *flosaquae* growth and metabolism. The studied concentrations (0, 1, 1.5, 2 and 4 mg
245 Ca²⁺ L⁻¹) are critical depending on previous records in the literature. In this context,
246 Weyhenmeyer et al (2019) reported that the global median calcium concentration was
247 4.0 mg L⁻¹ with 20.7% of the water samples showing Ca²⁺ concentrations of ≤ 1.5 mg
248 L⁻¹, a threshold considered critical for the survival of many organisms. Growth of *D.*
249 *flosaquae* in terms of cell number, protein – and chlorophyll a content, was inhibited
250 by calcium deprivation as well as by higher concentrations of calcium. However,
251 concentrations of only 1.5 mg Ca²⁺ L⁻¹ of acetate and citrate were stimulatory for *D.*
252 *flosaquae* growth.

253 *D. flosaquae* seems able to perform MICP, as inferred from alkalinity levels in the
254 growth media, elevated pH values, and residual vs. consumed calcium levels.



255 However, MICP occurred but at a pre-precipitation stage since no precipitation has
256 been seen by naked eyes, due to the inability of the organism to surpass the minimum
257 pH threshold under our experimental conditions of inactive urease due to absence of
258 urea (discussed later). Therefore, ammonia concentrations were found to be marginal
259 in the culture media. Its interference with carbonate alkalinity can be thus ruled out
260 indicating that the assessed alkalinity levels are substantially carbonate alkalinity.
261 Carbonate alkalinity exhibited the lowest levels at control cultures but increased via
262 calcium deprivation. However, it was induced up to its maximum level (six times that
263 of the control) by the highest calcium concentration of the citrate salt ($4 \text{ mg Ca}^{2+} \text{ L}^{-1}$).
264 This notion suggests that the capacity of *D. flosaquae* for carbonate formation
265 depends on the salt type as well as on the Ca^{2+} concentration. In this respect, calcium
266 chloride has been recorded to be the best salt for the production of calcite by *Bacillus*
267 sp. among several other calcium sources used (Achal and Pan 2014). In this work,
268 however, calcium citrate apparently fits the studied organism more than chloride or
269 acetate.

270 Alkaline pH is a prerequisite for calcium carbonate formation and stability. Most calcite
271 precipitation occurs under alkaline conditions of pH 8.7 to 9.5 (Ferris et al 2003;
272 Dupraz et al 2009). When pH levels decrease, carbonates tend to dissolve rather than
273 precipitate (Loewenthal and Marais 1982). *D. flosaquae* exhibited a continuous ability
274 of elevating the pH of the culture medium to high pH values, which in turn, may have
275 caused a slow and long lag phase of growth, but favorable conditions for MICP.
276 However, as long as the pH of the cultures did not surpass 8.7, i.e. the lowest pH for
277 precipitation, carbonate has been formed but did not precipitate (see references
278 above: Loewenthal and Marais 1982; Ferris et al 2003; Dupraz et al 2009; Gebauer
279 et al 2010).

280 Amongst the multiple microbial metabolic activities described in the literature to
281 support MICP (e.g. Anbu et al 2016), that of *D. flosaquae* mostly relied on
282 photosynthesis and respiration under conditions of this work. Urease activity, the most
283 universal metabolic process powering MICP, is unlikely in this case, as the growth
284 media was not supplemented with urea. UE activity is a potentially major source of
285 ammonia (shifting the pH around the cell to the alkaline side) and CO_2 (transforming
286 into calcium carbonate precipitates).



287 UE activity is a potentially major source of ammonia (shifting the pH around the cell to
288 the alkaline side); meanwhile CO_2 and Ca^{2+} transform into calcium carbonate
289 precipitates. Urea hydrolysis via UE activity is not complex (**Hammes** et al 2003;
290 **Achal** et al 2011; **Stabnikov** et al 2013). However, the high ability of *D. flosaquae* to
291 shift the pH to alkalinity, without urea being included in the culture medium, indicates
292 sources of alkalinity other than the urease-dependent ammonia production, i.e.
293 photosynthesis and respiration in our case. In this context, aerobic bacteria release
294 CO_2 via cell respiration, which is paralleled by an increase in pH due to ammonia
295 production (**Ng** et al 2012). **Hamilton** et al (2009) stated that lakes in carbonate-rich
296 watersheds commonly precipitate calcium carbonate as calcite; this is accelerated by
297 photosynthetic uptake of carbon dioxide, elevating the pH to 9–10 and reducing
298 concentrations of calcium and alkalinity by up to 60%. However, urea hydrolytic strains
299 showed higher calcite precipitation (~20–80%) in comparison with other metabolic
300 pathways (**Achal** et al 2009). **Okwadha** and Li (2010) reported that the amount of
301 CaCO_3 precipitation depends more on Ca^{2+} concentrations than urea concentrations.
302 The *in vitro* assay of UE (EC 3.5.1.5), per se, was also affected by calcium
303 concentration and salt type, i.e. it was inhibited by calcium deprivation while it exhibited
304 maxima at 1.5, 2 and 4 mg $\text{Ca}^{2+} \text{L}^{-1}$ for chloride, acetate, and citrate, respectively.
305 Calcium induces UE activity; **Hammes** et al (2003) found that UE activity increased by
306 tenfold in the presence of 30 mM Ca^{2+} relative to its absence. UE activity is related to
307 cell (**Ng** et al 2012), urea and calcium concentrations (**De Muyneck** et al 2010), and
308 high pH (**Jones** et al 1982). In addition, UE is only active at high pH values specific
309 for urea hydrolysis. It has been reported that the optimum pH for UE is 8.0, above
310 which the enzyme activity decreases (**Stocks-Fischer** et al 1999; **Gorospe** et al 2013).
311 In this work, the results indicate that UE activity of *Dolichospermum flosaquae* was
312 enhanced due to an increase in specific activity of the enzyme rather than to higher
313 cell numbers or biomass. Urease and carbonic anhydrase expression and activities
314 are genetically and synergistically co-regulated for MICP (**Dhami** et al 2014; **Castro-**
315 **Alonso** et al 2019).

316 Residual calcium was the least in citrate-treated cultures, compared with other salts
317 (chloride or acetate). At 4 mg $\text{Ca}^{2+} \text{L}^{-1}$ of citrate, in particular, the lowest residual Ca^{2+}
318 level coincided with the highest alkalinity level, indicating its transformation to calcium
319 carbonate. Consumed calcium per unit chlorophyll a was increased to its highest level



320 also at 4 mg Ca²⁺ L⁻¹ of citrate treated cultures. Actually, Ca²⁺ is not likely utilized by
321 metabolic processes, but accumulates outside the cells where it is readily available for
322 CaCO₃ precipitation (**Silver** et al 1975). In this work, however, the unique and superior
323 stimulating effect of 4 mg Ca²⁺ L⁻¹ calcium citrate compared with other salts (chloride
324 or acetate) implies intracellular intervention of calcium ions as well as the
325 accompanying anion in the intracellular metabolism. In this respect, citrate may serve
326 as a carbon source and internal buffer.

327

328 **Conclusions:**

- 329 • *D. flosaquae*, a major representative of temperate freshwater cyanobacteria,
330 contributes to the microbially-induced calcium carbonate precipitation (MICP) with
331 pronounced consequences for Ca²⁺ availability in freshwater lakes as well as
332 carbon emissions to the atmosphere.
- 333 • Carbonate was formed but did not precipitate, as the organism could not increase
334 the pH of the cultures beyond 8.7, which is considered the minimum pH value for
335 calcite precipitation. Although it is not a precipitate, the formed calcium carbonate
336 proves CO₂ and calcium sequestration.
- 337 • The mechanism(s) powering MICP seem to be photosynthesis and respiration
338 without the participation of urease activity (as urea was not supplemented).
339 However, UE activity elucidated a strong activity at our in vitro assays, which might
340 maximally operate for MICP in cases of urea supplementation.
- 341 • Calcium citrate, particularly at 4 mg Ca²⁺ L⁻¹ was the most inductive for MICP. For
342 the emerging MICP-dependent technologies, it is therefore, recommended to apply
343 calcium citrate because it shows outstanding enhancement of the process.
- 344 • The results can be used in modelling the environmental implications of MICP for
345 biogeochemical cycles of calcium, carbon and phosphorus in freshwater lakes.

346

347

348 **Figure legends:**

349 **Figure (1):** Growth (cell number (10³ cells mL⁻¹), chlorophyll (x10⁻³ µg mL⁻¹) and protein
350 contents (µg mL⁻¹) of the cyanobacterium *Dolichospermum flosaquae* as influenced



351 by various calcium treatments: Control (B), 0, 1, 1.5, 2 and 4 mM of calcium chloride
352 (C), calcium acetate (A) and calcium citrate (Ct). Control cultures were grown in BG11
353 medium containing 13 mg Ca²⁺ L⁻¹ (chloride), 0 is calcium deprived, i.e. not
354 supplemented with any external calcium.

355

356 **Figure (2):** pH changes of the cyanobacterium *Dolichospermum flosaquae* as
357 influenced by calcium treatments (as in figure 1).

358

359 **Figure (3):** Photosynthesis and respiration rates of the cyanobacterium
360 *Dolichospermum flosaquae* as influenced by calcium treatments (as in figure 1).

361

362 **Figure (4):** Total alkalinity (carbonate and ammonia as mmol. µg Chl⁻¹) of the
363 cyanobacterium *Dolichospermum flosaquae* as influenced by calcium treatments (as
364 in figure 1).

365

366 **Figure (5):** Residual (mg L⁻¹), total (mg L⁻¹) and consumed calcium (mg L⁻¹ or µg µg
367 Chl a⁻¹) of the cyanobacterium *Dolichospermum flosaquae* as influenced by calcium
368 treatments (as in figure 1).

369

370 **Figure (6):** Urease activity, T (total) and SA (Specific Activity) of the cyanobacterium
371 *Dolichospermum flosaquae* as influenced by calcium treatments (as in figure 1).

372

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377

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The first author (R. A.-B.), designed the work, implemented the experiments and wrote the drafts, the second author (E.A.H.), helped in the experiments and calculated the standard errors, the third author (H.P. G.) hosted the first two authors in his lab in IGB and revised the manuscript.

Competing interests

There are no competing interests among authors.

