https://doi.org/10.5194/bg-2021-146
Preprint. Discussion started: 8 July 2021
© Author(s) 2021. CC BY 4.0 License.

Biogeosciences Discussions

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

Refat Abdel-Basset[1]*, Elhagag Ahmed Hassan[1^] and Hans-Peter Grossart[2,3+]

[1]* Botany and Microbiology Department, Faculty of Science, Assiut University, 71516 Assiut (Egypt)

[2]Dept. Experimental Limnology, Leibniz Institute for Freshwater Ecology and Inland Fisheries, D-16775 Stechlin, Germany

[3]Dept. of Biochemistry and Biology, Potsdam University, 14469 Potsdam, Germany

^ elhagaghassan@aun.edu.eg

* hgrossart@igb-berlin.de

*Correspondance to: rbasset@aun.edu.eg

Abstract. The aim of this work is to explore the ability and magnitude of the temperate cyanobacterium *Dolichospermum flosaquae* in microbially-induced calcium carbonate precipitation (MICP). Environmentally, MICP controls the availability of calcium, carbon and phosphorus in freshwater lakes and simultaneously controls carbon exchange with the atmosphere. Cultures of *flosaquae* were grown in BG11 medium containing 0, 1, 1.5, 2 and 4 mg Ca^{2+} L^{-1}, as cardinal concentrations previously reported in freshwater lakes, in addition to a control culture (BG11 containing 13 mg Ca^{2+} L^{-1}). Growth (cell number, chlorophyll a, and protein content) of *D. flosaquae* was generally reduced by elevating calcium concentrations of the different salts used (chloride, acetate, or citrate). *D. flosaquae* exhibited its ability to perform MICP as carbonate alkalinity was sharply increased up to its highest level (six times that of the control) at a citrate concentration of 4 mg Ca^{2+} L^{-1}. Calcium carbonate was formed at a pre-precipitation stage as the minimum pH necessary for precipitation (8.7) has been scarcely approached under such conditions. In this work, MICP took place mostly empowered by photosynthesis and respiration. Residual calcium exhibited its lowest value at 4 mg Ca^{2+} citrate L^{-1}, coinciding with the highest alkalinity level. Precipitated calcium was increased with chlorophyll a content, but not with increasing cell numbers.

https://doi.org/10.5194/bg-2021-146
Preprint. Discussion started: 8 July 2021
© Author(s) 2021. CC BY 4.0 License.


Key Words: *Dolichospermum flosaquae* – MICP - Photosynthesis – Respiration – Urease – Alkalinity – Calcium

1 Introduction

Microbially-induced calcium carbonate precipitation (MICP) depicts an exogenous or endogenous microbial activity that takes place during heterotrophic growth of numerous fungi and bacteria or during photoautotrophic growth of cyanobacteria in their natural environments including water, soils, tufas, biofilms or geological formations. Furthermore, bacterial, and cyanobacterial mucilaginous sheath (capsular polysaccharides or exopolysaccharides) as well as fungal chitin act as nucleation sites for $CaCO_3$ crystallization by binding Ca^{2+} onto their carboxylic groups. MICP requires sufficient Ca^{2+}, an alkaline pH and suitable microorganisms. Availability of nucleation sites is very important for stable and continuous calcium carbonate bio-mineralization (Phillips et al 2013). In particular, cyanobacteria are active prokaryotes performing MICP (Payandi-Rolland 2019; Xu et al 2019). Furthermore, morphology of the calcite crystal is strain-specific (Hammes et al 2003) and depends on the type of the calcium salt present (Achal and Pan 2014). Several metabolic processes such as photosynthesis, respiration, sulfate, nitrate or sulfide reduction and ureolytic activity have been recorded as driving mechanisms for MICP (e.g. Seifan et al 2019; Castro-Alonso et al 2019). Although these metabolic pathways are diverse, either of them empowers MICP by conserving CO_2, as a component of calcium carbonate, and ammonia to shift the pH into alkalinity as a prerequisite environmental feature for the process.

Subsequent to coprecipitation of calcium and carbon(ate), chemically and/or microbially to form calcium carbonate, the bioavailability of both calcium and carbon becomes limited. Calcium and phosphate also coprecipitate and thus get lowered at these conditions. Limited bioavailability of these elements, in turn, arises as rate-limiting to growth and nutrition of aquatic microbiota, e.g. via photosynthetic activity in the case of cyanobacteria. This MICP process is widely explored and regarded as a promising phenomenon for use in various industrial applications. MICP may be responsible (at least in part) for the widespread threat of calcium decline in freshwater lakes around the globe, as calcium co-precipitates simultaneously with carbonate

https://doi.org/10.5194/bg-2021-146
Preprint. Discussion started: 8 July 2021
© Author(s) 2021. CC BY 4.0 License.


(Jeziorski and Smol 2017). After studying 440,599 water samples from 43,184 inland water sites in 57 American and European countries, Weyhenmeyer et al (2019) concluded that the global median calcium concentration was 4.0 mg L^{-1} with 20.7% of the water samples showing Ca^{2+} concentrations ≤ 1.5 mg L^{-1}, a threshold considered critical for the survival of many Ca^{2+} dependent organisms, e.g. *Daphnia* (Jeziorski et al 2014). Anthropogenic activities, namely acid depositions, are detrimental to calcium decline. Since some time ago, governments determined to prevent acid deposition into lakes; acid deposition solubilizes calcium **(**Korosi et al 2012), i.e. no acid deposition means less calcium dissolution. Another explanation is that the acid deposition preceded such measures may have led to depletion of calcium in soil catchments leaving no more of the element to dissolve.

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

2 Materials and Methods

2.1 Experimental Set up

Cultures of the local strain of the cyanobacterium *Dolichospermum flosaquae* were incubated at different Ca^{2+} concentrations (0, 1, 1.5, 2 and 4 mg Ca^{2+} L^{-1}) of different salts (chloride “Cl”, acetate “Ac”, or citrate “Cit”) supplemented into calcium free BG11 medium specific for cyanobacteria (Rippka and Herdman 1993). *D. flosaquae* was also grown in full BG11 medium (containing 13 mg Ca^{2+} L^{-1}, which is considered the control culture “Con+”) in addition to a reference calcium-deprived culture (BG11 devoid of any supplemental calcium “Con-”). Culture media were inoculated with 10 ml of 5 days old cells of *D. flosaquae* in conical flasks capped with aluminum foil. Cultures were shaken for 4 weeks at 22±1 °C and white light intensity of 25 µmole m^{-2} sec^{-1} (14h light:10h dark cycle). *D. flosaquae* exhibited a relatively long lag phase, most probably

https://doi.org/10.5194/bg-2021-146
Preprint. Discussion started: 8 July 2021
© Author(s) 2021. CC BY 4.0 License.


because of relatively low temperature for cyanobacteria, which grow optimally at higher temperatures. The temperature was set up to be close as much as possible to the average of Lake Stechlinsee.

2.2 Analytical Methods

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

- Cell number and chlorophyll a were simultaneously assessed using a YSI-multiparameter probe.
- Protein contents were estimated according to the method of Bradford (1976). Cells were extracted in boiled water, centrifuged and soluble proteins were assessed in the supernatant. The binding of protein molecules with the Coomassie Brilliant Blue dye under acidic conditions results in a color change from brown to blue, measured at a wavelength of 595 nm using a BioTek Synergy 2, multidetector microplate reader (Vermont, USA).

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

$$CaCO_3 + 2HCl \rightarrow CaCl_2 + H_2O + CO_2$$

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

- Photosynthetic activity: The light-induced O_2 evolution by *D. flosaquae* in different cultures was followed by means of an oxygen sensor (PreSens MicroXTX3O_2 sensor, SoftwareTx3v6O_2, Presens, Germany) at the same growth conditions (white light intensity of about 25 µmole m^{-2} sec^{-1} at room temperature, i.e. 22±1ºC). Respiration (O_2 uptake) was also monitored using the same oxygen sensor, but in the dark.

- Assessment of residual free [Ca^{2+}] in the growth media: At the end of the experiment, calcium was assayed by calcium kits (ab102505, Calcium Detection Assay Kit-

https://doi.org/10.5194/bg-2021-146
Preprint. Discussion started: 8 July 2021
© Author(s) 2021. CC BY 4.0 License.


colorimetric, abcam) and determined at a wavelength of 575 nm using a BioTek Synergy 2, multidetector microplate reader (Vermont, USA). Consumed calcium was then calculated by subtracting residual from total calcium.

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

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

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

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

3 Results

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

https://doi.org/10.5194/bg-2021-146
Preprint. Discussion started: 8 July 2021
© Author(s) 2021. CC BY 4.0 License.


The pH of 7.0 was set for all *D. flosaquae* cultures at the beginning of the experiment; thereafter, it was elevated to levels ranging between pH 8.0 - 8.7, depending on calcium treatment (Fig. 2). A certain calcium concentration of each salt induced a higher pH than control or calcium-deprived cultures. The highest pH elevation (up to 8.7) occurred at calcium chloride and calcium citrate concentrations of 1 mg Ca^{2+} L^{-1} but decreased at higher concentrations. However, Ca^{2+} acetate resulted in the highest pH elevation (up to 8.7) at the highest added concentration (4 mg Ca^{2+} L^{-1}). Accordingly, the pH elevation depended on the calcium concentrations and type of Ca^{2+} salt (chloride, acetate or citrate) added.

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

Total alkalinity (T alkalinity), ammonia as well as corrected carbonate alkalinity values (C alkalinity), calculated by subtracting ammonia concentration from total alkalinity of the differently treated *D. flosaquae* cultures, are shown in Fig. (4). Total alkalinity exhibited its absolutely lowest value in the control culture of *D. flosaquae*, despite it contained the highest Ca^{2+} concentration (13 mg Ca^{2+} L^{-1}) while calcium deprivation remarkably enhanced alkalinity up to three times that of the control cultures (5 to 15 mmol carbonate µg Chl^{-1}). Furthermore, alkalinity level in any of the calcium treated cultures was markedly higher than that of the control or calcium-deprived cultures, with a maximum alkalinity level at calcium citrate concentration of 4 mg Ca^{2+} L^{-1}. All calcium acetate concentrations induced more or less similar alkalinity levels whereas calcium

https://doi.org/10.5194/bg-2021-146
Preprint. Discussion started: 8 July 2021
© Author(s) 2021. CC BY 4.0 License.

chloride induced its highest stimulation at 2 mg Ca^{2+} L^{-1}. As ammonia may interfere with carbonate alkalinity, ammonia has been assessed and detected in trace amounts not affecting total alkalinity (Fig. 4).

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

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

Urease enzyme (UE) activity is presented in Fig (6); specific activity “SA” represents the rate of enzyme activity as µmole ammonia released μg^{-1} protein min^{-1} while total activity “TA” represents the rate of enzyme activity as µmole ammonia released mL^{-1}

https://doi.org/10.5194/bg-2021-146
Preprint. Discussion started: 8 July 2021
© Author(s) 2021. CC BY 4.0 License.


algal suspension min ^{1}. Total activity is the product of specific activity per µg protein multiplied by the amount of protein per unit volume (mL) of algal cultures. Calcium deprivation inhibited UE activity; the magnitude of inhibition on a volume basis "TA" was more pronounced than the enzyme specific activity "SA" because enzyme (protein) contents were also lower. Calcium chloride induced the highest rates of UE, total and specific activity, at 1.5 mg Ca^{2+} L^{-1}; otherwise, it was inhibitory at lower or higher concentrations. Calcium acetate induced the highest rates of "TA" and "SA" at moderate concentrations of 1.5 and 2 mg Ca^{2+} L-1, both lowest and highest concentrations of 1 and 4 mg Ca^{2+} L^{-1} severely inhibited the enzyme activity. Calcium citrate induced a continuous increase in urease activity (SA) up to its "absolutely" highest rate at 4 mg Ca^{2+} L^{-1} among other concentrations and salts; such highest rate of urease activity was in accordance with the highest level of calcium consumption, i.e. calcium may be inductive to urease activity in *D. flosaquae*. The order of UE enhancement was as follows citrate > acetate > chloride.

4 Discussion

Our results indicate, for the first time, that *Dolichospermum flosaquae* is able to perform microbially-induced calcium carbonate precipitation (MICP). Therefore, the intensive blooms of this organism have the potential to contribute to controlling the overall biogeochemical dynamics in freshwater bodies, depending on the availability of calcium, carbon, and phosphorus, in addition to controlling carbon emissions into the atmosphere. Calcium precipitates carbon in the form of calcium carbonate either chemically or microbially and precipitates phosphorus in the form of calcium phosphate. Furthermore, any calcium carbonate precipitate is a good binder of phosphate (Yanamadala 2005). In this work, the capability of the cyanobacterium *D. flosaquae* in freshwater MICP was studied at different concentrations of three calcium salts (chloride, acetate, and citrate). It is proved that different salt types and calcium concentrations exerted different impacts on *D. flosaquae* growth and metabolism. The studied concentrations (0, 1, 1.5, 2 and 4 mg Ca^{2+} L^{-1}) were chosen from previous records in the literature (Weyhenmeyer et al 2019). These authors reported that the global median calcium concentration was 4.0 mg L^{-1} with 20.7% of the water samples showing Ca^{2+} concentrations of ≤ 1.5 mg L^{-1}, a threshold considered critical for the survival of many organisms. Growth of *D. flosaquae* in terms of cell number, protein –

https://doi.org/10.5194/bg-2021-146
Preprint. Discussion started: 8 July 2021
© Author(s) 2021. CC BY 4.0 License.


and chlorophyll a content, was inhibited by calcium deprivation as well as by higher concentrations of calcium. However, concentrations of only 1.5 mg Ca^{2+} L^{-1} in the form of acetate and citrate were stimulatory for *D. flosaquae* growth.

D. flosaquae seems able to perform MICP, as inferred from alkalinity levels in the growth media, elevated pH values, and residual vs. consumed calcium levels. However, MICP occurred but at a pre-precipitation stage since no precipitation has been seen by naked eyes, due to the inability of the organism to surpass the minimum pH threshold under our experimental conditions of inactive urease due to absence of urea (discussed later). Therefore, ammonia concentrations were found to be marginal in the culture media; its interference with carbonate alkalinity can be thus ruled out indicating that the assessed alkalinity levels are substantially carbonate alkalinity. Carbonate alkalinity exhibited the lowest levels at control cultures but increased upon calcium deprivation. However, it was induced up to its maximum level (six times that of the control) by the highest calcium concentration of the citrate salt (4 mg Ca^{2+} L^{-1}). This notion suggests that the capacity of *D. flosaquae* for carbonate formation depends on both salt type as well as Ca^{2+} concentration. In this respect, calcium chloride has been recorded to be the best salt for the production of calcite by *Bacillus* sp. among several other calcium sources used (Achal and Pan 2014). In this work, however, calcium citrate seems fitting to the studied organism more than chloride or acetate.

Alkaline pH is a prerequisite for calcium carbonate formation and stability. Most calcite precipitation occurs under alkaline conditions of pH values from 8.7 to 9.5 **(**Ferris et al 2003; Dupraz et al 2009). When pH levels decrease, carbonates tend to dissolve rather than precipitate **(**Loewenthal and Marais 1982). *D. flosaquae* exhibited a continuous ability of elevating the pH of the culture medium to high pH values, which in turn, may have caused a slow and long lag phase of growth, but favorable conditions for MICP. However, as long as the pH of the cultures did not surpass 8.7, i.e. the minimum pH for precipitation, carbonate has been formed but did not precipitate (Loewenthal and Marais 1982; Ferris et al 2003; Dupraz et al 2009; Gebauer et al 2010). In addition, it has been stated that calcium carbonate can be formed at very low solubility levels in pure water before precipitation; its solubility in pure water is as low as 13 mg L^{-1} at 25°C (Aylward et al 2008); it increases relatively with decreasing temperature and increases in rainwater saturated with carbon dioxide, due to the

https://doi.org/10.5194/bg-2021-146
Preprint. Discussion started: 8 July 2021
© Author(s) 2021. CC BY 4.0 License.


formation of more soluble calcium bicarbonate. For the great majority of calcium carbonate precipitations, qualitative and descriptive assessments are dominant because most studies are carried out on calcium carbonate structures formed decades or hundreds of years ago while quantitative assessments are scarce. In this respect, MICP quantities of precipitated calcium after six treatments to *Bacillus* sp. were 0.15 and 0.60g of Ca per cm^2 of treated sand surface for bulk or surface MICP, respectively (Chu et al 2012). Also, a putative calcium carbonate mineral mass of 2.5 mg/OD 660 has been reported in *Bacillus* sp. JH7 (Kim et al 2017). In this concert, Declet et al (2016) reported that synthesis of the particles involves some environmental variables including pH, temperature, concentration of solutions, concentration and type of additives (organic or inorganic) and the substrate surface roughness, which play a decisive role in the formation of calcium carbonate particles.

While multiple microbial metabolic activities have been described in the literature to empower MICP by providing a potentially major source of ammonia (shifting the pH around the cell to the alkaline side) and CO_2, which precipitates with calcium as carbonate precipitates (e.g. Anbu et al 2016), MICP of *D. flosaquae* mostly relied on or empowered by photosynthesis and respiration under conditions of this work. Urease activity, the most universal metabolic process supporting MICP, is not participating in this case, as the growth media was not supplemented with urea; despite the organism under test *D. flosaquae* exhibited potential urease specific activity in *in vitro* assays (four times that of the control at 4 mg Ca^{2+} citrate L^{-1}). However, the high ability of *D. flosaquae* to shift the pH to alkalinity, without urea and urease being included in the culture medium, indicates sources of alkalinity other than the urease-dependent ammonia production, i.e. photosynthesis and respiration in the present case. In this context, aerobic bacteria release CO_2 via cell respiration, which is paralleled by an increase in pH due to ammonia production (Ng et al 2012). Hamilton et al (2009) stated that lakes in carbonate-rich watersheds commonly precipitate calcium carbonate as calcite; this is accelerated by photosynthetic uptake of carbon dioxide, elevating the pH to 9–10 and reducing concentrations of calcium and alkalinity by up to 60%. However, urea hydrolytic strains showed higher calcite precipitation (~20–80%) in comparison with other metabolic pathways (Achal et al 2009); despite the amount of $CaCO_3$ precipitates depends more on Ca^{2+} concentrations (Okwadha and Li 2010).

https://doi.org/10.5194/bg-2021-146
Preprint. Discussion started: 8 July 2021
© Author(s) 2021. CC BY 4.0 License.


323 The *in* vitro assay of UE (EC 3.5.1.5), per se, was also affected by calcium
324 concentration and salt type i.e. it was inhibited by calcium deprivation while it exhibited
325 maxima at 1.5, 2 and 4 mg Ca^{2+} L^{-1} for chloride, acetate, and citrate, respectively.
326 Calcium induces UE activity; Hammes et al (2003) found that UE activity increased by
327 tenfold in the presence of 30 mM Ca^{2+} relative to its absence. UE activity is related to
328 cell (Ng et al 2012), urea and calcium concentrations (De Muynck et al 2010), and high
329 pH (Jones et al 1982)**.** In addition, UE is only active at high pH values specific for urea
330 hydrolysis. It has been reported that the optimum pH for UE is 8.0, above which the
331 enzyme activity decreases (Stocks-Fischer et al 1999; Gorospe et al 2013). In this
332 work, the results indicate that UE activity of *Dolichospermum flosaquae* was enhanced
333 due to an increase in specific activity of the enzyme rather than to higher cell numbers
334 or biomass. Urease and carbonic anhydrase expression and activities are genetically
335 and synergistically co-regulated for MICP (Dhami et al 2014; Castro-Alonso et al
336 2019).

337 Residual calcium was the least in the citrate-treated cultures, compared with other
338 salts (chloride or acetate). At 4 mg Ca^{2+} L^{-1} of citrate, in particular, the lowest residual
339 Ca^{2+} level coincided with the highest alkalinity level, indicating its transformation to
340 calcium carbonate. Consumed calcium per unit chlorophyll a was increased to its
341 highest level also at 4 mg Ca^{2+} L^{-1} of citrate treated cultures. In this respect, Kim et al
342 (2017) reported that *Bacillus* sp. JH7 decreased the remaining ionized calcium to zero,
343 which suggested that Ca^{2+} was either precipitated as $CaCO_3$ or simply absorbed onto
344 the exopolysaccaride (EPS) mucilaginous sheath of strain JH7. In conclusion, not all
345 the disappearing calcium from the outer media is precipitated as MICP but it can be
346 hidden onto the EPS sheaths of the cyanobacterium. In accordance with this, Silver et
347 al (1975) reported that it is unlikely that Ca^{2+} is utilized by metabolic processes but
348 accumulates outside the cells where it is readily available for $CaCO_3$ precipitation. In
349 this work, however, the unique and superior stimulating effect of 4 mg Ca^{2+} L^{-1} calcium
350 citrate compared with other salts (chloride or acetate) implies intracellular intervention
351 of calcium ions as well as the accompanying anion in the intracellular metabolism. In
352 this respect, citrate may serve at the same time as a carbon source and internal buffer.
353

354 Overall, calcium carbonate precipitation has been studied in natural (modern and
355 ancient) geological structures as well as at controlled laboratory experiments. Natural

https://doi.org/10.5194/bg-2021-146
Preprint. Discussion started: 8 July 2021
© Author(s) 2021. CC BY 4.0 License.


deposits represent a collective mixture of interfered multiple factors, not at least because of space, time, and interconnected microbes (products and processes). In contrast, experimental modelling in MICP biogenesis uses pure microbial cultures, defines the role a specific microorganism plays at a specific environmental condition. Although calcium carbonate precipitation occurs chemically, a microorganism is essential for durable and stable calcium carbonate structures. In line with this notion, Stocks-Fischer et al (1999) reported that at pH 9.0, only 35 and 54 % of the initial Ca^{2+} concentrations precipitated chemically in water and medium, respectively while 98 % were precipitated microbially. Berry et al (2002) reported that though the oceans are supersaturated with Ca^{2+} and CO_3^{2-}, spontaneous precipitation of $CaCO_3$ in the absence of calcifying (micro)- organisms is rare owing to various kinetic barriers**.** Thus, the process in nature is inefficient and the presence of a microorganism or part of it (cell walls, spores, chitin or mucilage) is indispensable for efficient calcification. It has also been reported that the largest share of global calcification takes place via biotic processes in the oceans (Olajire 2013). Microbially mediated calcification can be traced back for at least 2.6 billion years (Altermann et al 2006). The authors proposed that the interplay of cyanobacteria and heterotrophic bacteria has been the major contributor to the carbonate factory for roughly the last 3 billion years of Earth history. It is very hard to compare the MICP activity of a uni-cyanobacterial culture (*Dolichospermum flosaquae*), grown at laboratory conditions for a limited period of time, with a process occurring 1) in nature 2) by numerous consortia of microorganisms, 3) for a long-lasting time (billions of years), 4) under variable conditions over time, e.g. temperature, competition, synchronization and/or allelopathy. Under natural conditions, the precipitation of carbonates takes place very slowly over long geological times but in order to produce large amounts of carbonates shortly there is a need to focus on microorganisms that have the ability to create conditions for carbonate precipitation at much shorter time scales (Dhami et al 2013).

5 Conclusions

- *Dolichospermum flosaquae*, a major representative of temperate freshwater cyanobacteria, contributes to the microbially-induced calcium carbonate

https://doi.org/10.5194/bg-2021-146
Preprint. Discussion started: 8 July 2021
© Author(s) 2021. CC BY 4.0 License.


precipitation (MICP) with pronounced consequences in Ca^{2+} availability in freshwater lakes as well as carbon emissions to the atmosphere.

- Carbonate was formed in the range of 10-30 (mmol μg^{-1} Chl) throughout the life span of the cyanobacterium. However, it did not precipitate, as the organism could not elevate the pH value of the cultures beyond 8.7, which is the minimum pH for calcite precipitation. Although it is not a precipitate, the formed calcium carbonate proves CO_2 and calcium sequestration.
- The mechanism(s) empowering MICP seem to be photosynthesis and respiration without the participation of urease activity (as urea was not supplemented). However, UE activity elucidated a strong activity at our in vitro assays, which might maximally operate for MICP in cases of urea supplementation.
- Calcium citrate, particularly at 4 mg Ca^{2+} L^{-1} was the most inductive for MICP. For the emerging MICP-dependent technologies, it is therefore, recommended to apply calcium citrate because it shows outstanding enhancement of the process.
- The results can be used in modelling the environmental implications of MICP for biogeochemical cycles of calcium, carbon and phosphorus in freshwater lakes.

Author contribution

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

Competing interests

There are no competing interests among authors.

Acknowledgment

Prof. Dr. R. Abdel-Basset sincerely thanks the Alexander von Humboldt Stiftung for the generous financial coverage of his research stay in the lab of Prof. Dr. H. P. Grossart, Leibiniz Institute for Freshwater Ecology and Inland Fisheries (IGB), and thanks to the MIBI group for their help.

References

https://doi.org/10.5194/bg-2021-146
Preprint. Discussion started: 8 July 2021
© Author(s) 2021. CC BY 4.0 License.

412 Achal, V. and Pan, X.: Influence of calcium sources on microbially induced calcium
413 carbonate precipitation by *Bacillus* sp. CR2. Appl Biochem. Biotechnol.,173:307–
414 317. doi: 10.1007/s12010-014-0842-1, 2014.

415 Achal, V., Mukherjee, A., Basu, P.C. and Reddy, M.S.: Strain improvement of
416 *Sporosarcina pasteurii* for enhanced urease and calcite production. J. Ind.
417 Microbiol. Biotechnol., 36:981–988. doi: 10.1007/s10295-009-0578-z, 2009.

418 Alderkamp, A.-C., Mills, M. M., van Dijken, G. L., Laan, P., Thuroczy, C.-E., Gerringa,
419 L. J. A., de Baar, H. J. W., Payne, C. D., Visser, R. J. W., Buma, A. G. J., and
420 Arrigo, K. R.: Iron from melting glaciers fuels phytoplankton blooms in the
421 Amundsen Sea (Southern Ocean): Phytoplankton characteristics and
422 productivity, Deep-Sea Res. Pt. II, 71–76, 32–48,
423 https://doi.org/10.1016/j.dsr2.2012.03.005, 2012.

424 Allen, C. S., Peck, V. L., Graham, A. G. C., Blagbrough, H., Robinson, M. W., and
425 McClymont, E.: RRS James Clark Ross Marine Science Cruises JR257 and
426 JR254e, March–April 2012, British Antarctic Survey, Cambridge, UK,
427 https://www.bodc.ac.uk/resources/inventories/cruise_inventory/reports/jr257_25
428 4e.pdf, 2012.

429 Altermann, W., Kazmierczak J., Oren A., Wright D.T. (2006): Cyanobacterial
430 calcification and its rock-building potential during 3.5 billion years of Earth
431 history, Geobiology 4, 147-166. Doi: - 10.1111/j.1472-4669.2006.00076.x.

432 Anbu P., Kang C.H., Shin Y.J. and So J.S. (2016): Formations of calcium carbonate
433 minerals by bacteria and its multiple applications. Springer Plus 5:250. doi:
434 10.1186/s40064-016-1869-2.

435 Aylward, Gordon; Findlay, Tristan (2008). SI Chemical Data Book (4th ed.). John Wiley
436 & Sons Australia. ISBN 978-0-470-81638-7.

437 Berry L., Taylor A.R., Lucken U., Ryan K.P. and Brownlee C. (2002): Calcification and
438 inorganic carbon acquisition in coccolithophores. Funct. Plant Biol. 9:289-299.
439 Bradford M. M. (1976): A rapid and sensitive method for the quantitation of microgram
440 quantities of protein utilizing the principle of protein-dye binding. Anal Biochem.
441 72, 248-254.
442 Castro-Alonso M.J., Montañez-Hernandez L.E., Sanchez-Muñoz M.A., Franco
443 M.R.M., Narayanasamy R. and Balagurusamy N. (2019): Microbially induced
444 calcium carbonate precipitation (MICP) and its potential in bioconcrete:

https://doi.org/10.5194/bg-2021-146
Preprint. Discussion started: 8 July 2021
© Author(s) 2021. CC BY 4.0 License.


445 Microbiological and Molecular Concepts. Front. Mater., 6(126):
446 https://doi.org/10.3389/fmats.2019.00126.
447 Choi S.-G., Park S.-G., Wu S. and Chu J. (2017): Methods for calcium carbonate
448 content measurement of biocemented soils. J. Mater. Civ. Eng. 29(11):
449 06017015(1-4).

450 Chu J., Stabnikov V. and Ivanov V. (2012): Microbially induced calcium carbonate
451 precipitation on surface or in the bulk of soil. Geomicrobiology Journal, 29:544–
452 549.
453 De Muynck W., De Belie N. and Verstraete W. (2010): Microbial carbonate
454 precipitation in construction materials: a review. Ecol Eng. 36:118–136. doi:
455 10.1016/j.ecoleng.2009.02.006.

456 Declet, A., Reyes, E., and Suárez, O. M. (2016). Calcium carbonate precipitation: a
457 review of the carbonate crystallization process and applications in bioinspired
458 composites. *Rev. Adv. Mater. Sci.* 44, 87–107.

459 Dhami N. K., Reddy M. S. and Mukherjee A. (2014): Application of calcifying bacteria
460 for remediation of stones and cultural heritages. Front. Microbiol. Vol. 5:304. doi:
461 10.3389/fmicb.2014.00304.

462 Dhami N.K., Reddy M.S. and Mukherjee A. (2013): Biomineralization of calcium
463 carbonate polymorphs by the bacterial strains isolated from calcareous sites. J
464 Microbiol Biotechnol. 23:707–714. doi: 10.4014/jmb.1212.11087.

465 Dupraz S., Menez B., Gouze P., Leprovost R., Benezeth P., Pokrovsky O.S. and
466 Guyot F. (2009): Experimental approach of CO_2 biomineralization in deep saline
467 aquifers. Chem Geol. 265:54–62. doi: 10.1016/j.chemgeo.2008.12.012.

468 Ferris F.G., Phoenix V., Fujita Y. and Smith R.W. (2003): Kinetics of calcite
469 precipitation induced by ureolytic bacteria at 10 to 20 °C in artificial groundwater.
470 Geochim. Cosmochim. Acta. 67:1701–1710.

471 Gebauer D., Gunawidjaja P.N., Peter Ko J.Y., Bacsik Z., Aziz B., Liu L., Hu Y.,
472 Bergstrom L., Tai C.W., Sham T.K., Eden M. and Hedin N. (2010): Proto-calcite
473 and proto-vaterite in amorphous calcium carbonates. Angew Chem Int Ed.
474 49:8889–8891. doi: 10.1002/anie.201003220.

https://doi.org/10.5194/bg-2021-146
Preprint. Discussion started: 8 July 2021
© Author(s) 2021. CC BY 4.0 License.

475 Gorospe C.M., Han S.H., Kim S.G., Park J.Y., Kang C.H., Jeong J.H. and So J.S.
476 (2013): Effects of different calcium salts on calcium carbonate crystal formation by
477 *Sporosarcina pasteurii* KCTC 3558. Biotechnol. Bioproc. Eng. 18:903–908. doi:
478 10.1007/s12257-013-0030-0.

479 Hamilton S.K., Bruesewitz D.A., Horst G.P., Weed D.B. and Sarnelle O. (2009):
480 Biogenic calcite–phosphorus precipitation as a negative feedback to lake
481 eutrophication. Can. J. Fish. Aquat. Sci., 66: 343–350.

482 Hammes F, Boon N, de Villiers J, Verstraete W and Siciliano SD. (2003): Strain-
483 specific ureolytic microbial calcium carbonate precipitation. Appl Environ
484 Microbiol. 69:4901–4909. doi: 10.1128/AEM.69.8.4901-4909.2003.

485 Jeziorski A. and Smol J.P. (2017): The ecological impacts of lake water calcium
486 decline on soft water boreal ecosystems. Environ. Rev., 25: 245-253.
487 Jeziorski A., Paterson A.M., Watson I., Cumming B.F. and Smol J.P. (2014): The
488 influence of calcium decline and climate change on the cladocerans within low
489 calcium, circumneutral lakes of the experimental lakes area. Hydrobiologia 722:
490 129-142.
491 Kim H.J., Shin B., Lee Y.S. and Park W. (2017): Modulation of calcium carbonate
492 precipitation by exopolysaccharide in *Bacillus* sp. JH7. Appl Microbiol Biotechnol
493 101**,** 6551–6561. https://doi.org/10.1007/s00253-017-8372-8.
494 Korosi J.B., Burke S.M., Thienpont J. R. and Smol J. P. (2012): Anomalous rise in
495 algal production linked to lake water calcium decline through food web interactions
496 Proc Biol Sci. Mar 22; 279(1731): 1210–1217. doi: 10.1098/rspb.2011.1411.

497 Loewenthal R. E., and Marais G.V.R. (1982): Carbonate chemistry of aquatic systems,
498 In H.-J. Walther (ed.). Theorie and Application, V.1, 433 pp. USA, Michigan,
499 Collingwood, Ann Arbor Scienc Publishers, Third Printing 1982. ISBN 0 – 250 –
500 40141 - X
501 Mobley H.L.T., Cortesia M.J., Rosenthal L.E. and Jones B.D. (1988): Characterization
502 of urease from Campylobacter pylori. Journal of Clinical Microbiology, 26(5): 831-
503 836.
504 Ng S.W., Lee M.L. and Hii S.L. (2012): An overview of the factors affecting microbial-
505 induced calcite precipitation and its potential application in soil improvement.
506 World Acad Sci Eng Technol. 62:723–729.

https://doi.org/10.5194/bg-2021-146
Preprint. Discussion started: 8 July 2021
© Author(s) 2021. CC BY 4.0 License.

507 Okwadha GDO and Li J. (2010): Optimum conditions for microbial carbonate
508 precipitation. Chemosphere 81:1143–1148. doi:
509 10.1016/j.chemosphere.2010.09.066.

510 Payandi-Rolland D., Roche A., Vennin E., Visscher P.T., Suchet P.A., Camille Thomas
511 C. and Bundeleva I.A. (2019): Carbonate precipitation in mixed cyanobacterial
512 biofilms forming freshwater microbial tufa. Minerals 2019, 9, 409: 1-20;
513 doi:10.3390/min9070409.

514 Phillips A.J., Gerlach R, Lauchnor E, Mitchell AC, Cunningham AB, Spangler L.
515 (2013): Engineered applications of ureolytic biomineralization: a review.
516 Biofouling.; 29(6):715-33. doi: 10.1080/08927014.2013.79655.

517 Salek S.S., Bozkurt O.D., van Turnhout A.G., Kleerebezem R. and van Loosdrecht
518 M.C.M. (2016): Kinetics of $CaCO_3$ precipitation in an anaerobic digestion process
519 integrated with silicate minerals. Ecological Engineering 86: 105-112.

520 Seifan M., Berenjian A. and Aydin B. (2019): Microbially induced calcium carbonate
521 precipitation: a widespread phenomenon in the biological world. Applied
522 Microbiology and Biotechnology. 103. 10.1007/s00253-019-09861-5.

523 Silver S, Toth K. and Scribner H. (1975): Facilitated transport of calcium by cells and
524 subcellular membranes of Bacillus subtilis and *Escherichia coli*. J Bacteriol.
525 122:880–885.

526 Rippka R. and Herdman M. ((1993): Catalogue and Taxonomic Handbook, vol. 1,
527 Insitut Pasteur, Paris.

528 Stocks-Fischer S., Galinat J.K. and Bang S.S. (1999): Microbiological precipitation of
529 $CaCO_3$. Soil Biol. Biochem. 31:1563–1571. doi: 10.1016/S0038-0717(99)00082-
530 6.

531 Weyhenmeyer G. A., Hartmann J., Hessen D.o., Jiří Kopáček, Hejzlar J., Jacquet S.,
532 Hamilton S.K., Verburg P., Leach T.H., Schmid M., Flaim G., Nõges T., Nõges P.,
533 Wentzky V.C., Rogora M., Rusak J.A., Kosten S., Paterson A.M., Teubner K.,
534 Higgins S.N., Lawrence G., Kangur K., Kokorite I., Cerasino L., Funk C., Harvey
535 R., Moatar F., de Wit H.A. and Zechmeister T. (2019): Widespread diminishing
536 anthropogenic effects on calcium in freshwater. Scientific Reports, 9:10450.
537 https://doi.org/10.1038/s41598-019-46838-ws

https://doi.org/10.5194/bg-2021-146
Preprint. Discussion started: 8 July 2021
© Author(s) 2021. CC BY 4.0 License.


538 Xu H., Peng X., Bai S., Ta K., Yang S., Liu S., Jang H.B. and Guo Z. (2019).
539 Precipitation of calcium carbonate mineral induced by viral lysis of cyanobacteria:
540 evidence from laboratory experiments. Biogeosciences 16:949–960. doi:
541 10.5194/bg-16-949-2019.

542 Yanamadala V. (2005): Calcium carbonate phosphate binding ion exchange filtration
543 and accelerated denitrification improve public health standards and combat
544 eutrophication in aquatic ecosystems. Water Environ Res. 77(7): 3003–3012.

545

546 **Figure legends**

547 **Figure (1):** Growth as cell number (x10^3 cells mL^{-1}), chlorophyll (x10^3 µg mL^{-1}) and
548 protein contents (µg mL^{-1}) of the cyanobacterium *Dolichospermum flosaquae* as
549 influenced by various calcium treatments: Control (B), 0, 1, 1.5, 2 and 4 mM of calcium
550 chloride (Cl), calcium acetate (A) and calcium citrate (Ct). Control cultures were grown
551 in BG11 medium containing 13 mg Ca^{2+} L^{-1} (chloride), 0 is calcium deprived, i.e. not
552 supplemented with any external calcium.

553

554 **Figure (2):** Changes in the pH of the cyanobacterium *Dolichospermum flosaquae*
555 cultures as influenced by calcium treatments (as in figure 1).

556

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

559

560 **Figure (4):** Total alkalinity (carbonate and ammonia as mmol. µg Chl^{-1}) of the
561 cyanobacterium *Dolichospermum flosaquae* as influenced by calcium treatments (as
562 in figure 1).

563

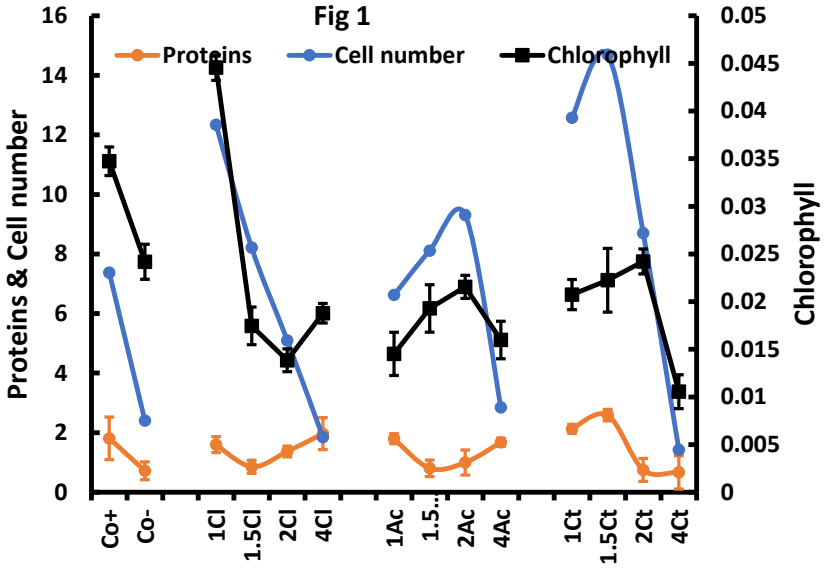
564 **Figure (5):** Residual (mg L^{-1}), total (mg L^{-1}) and consumed calcium (mg L^{-1} or mg. µg
565 Chl^{-1}) of the cyanobacterium *Dolichospermum flosaquae* as influenced by calcium
566 treatments (as in figure 1).

567

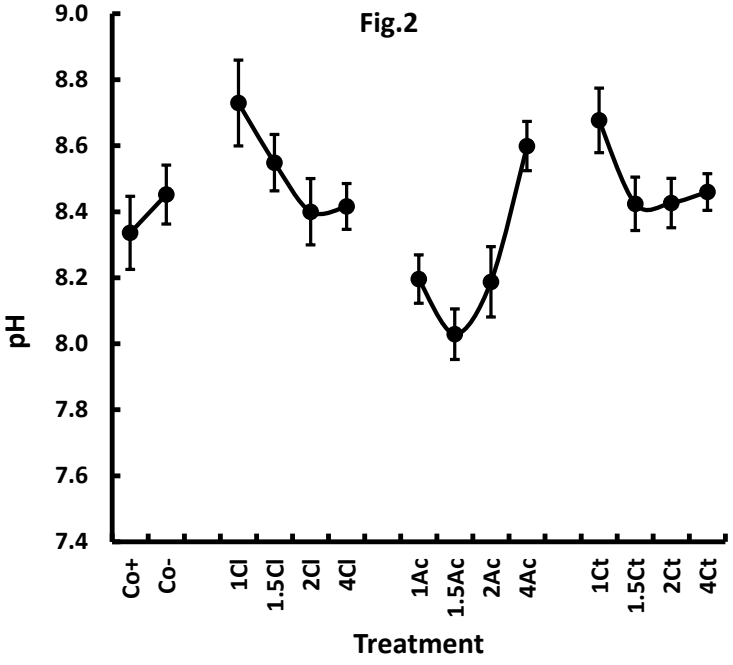
568 **Figure (6):** Urease activity, T (total) and SA (Specific Activity) of the cyanobacterium
569 *Dolichospermum flosaquae* as influenced by calcium treatments (as in figure 1).

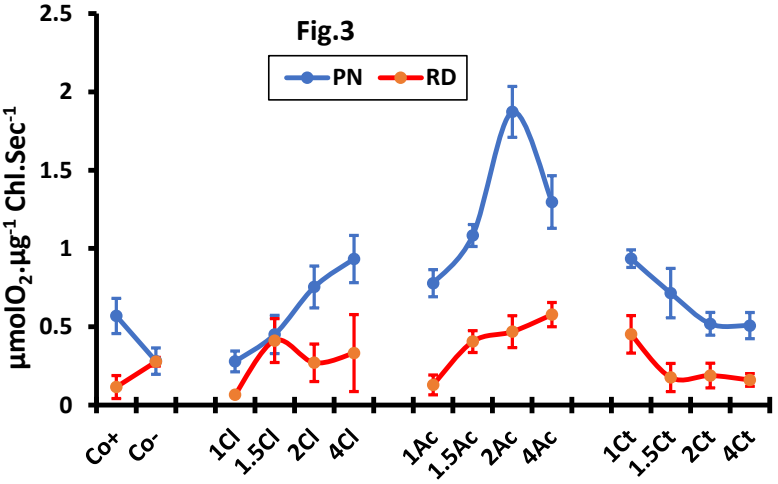
https://doi.org/10.5194/bg-2021-146
Preprint. Discussion started: 8 July 2021
© Author(s) 2021. CC BY 4.0 License.

Biogeosciences
Discussions
Open Access
EGU

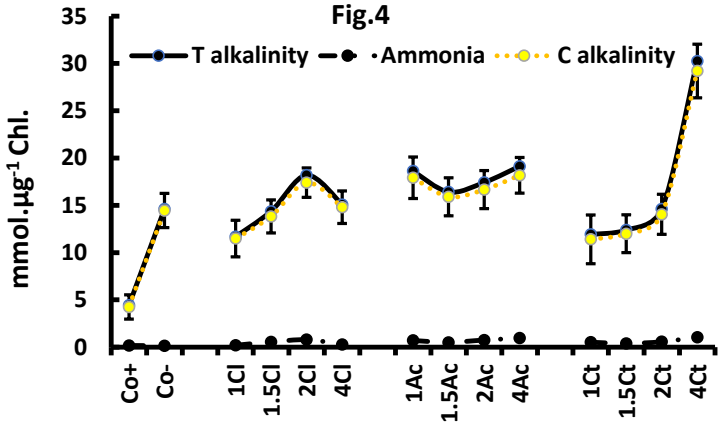


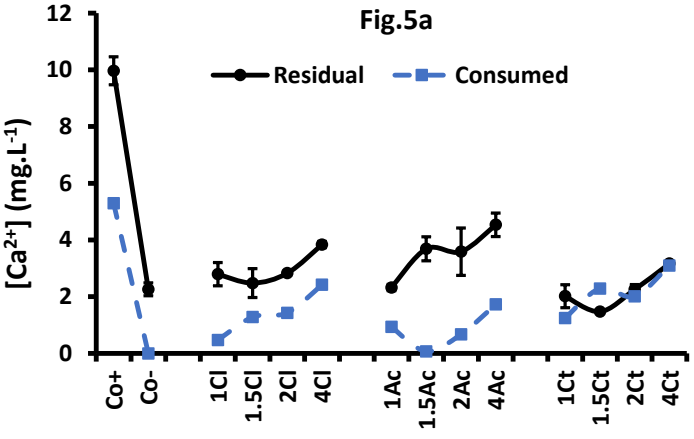
https://doi.org/10.5194/bg-2021-146
Preprint. Discussion started: 8 July 2021
© Author(s) 2021. CC BY 4.0 License.





https://doi.org/10.5194/bg-2021-146
Preprint. Discussion started: 8 July 2021
© Author(s) 2021. CC BY 4.0 License.





https://doi.org/10.5194/bg-2021-146
Preprint. Discussion started: 8 July 2021
© Author(s) 2021. CC BY 4.0 License.