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## Weak impact of microorganisms on Ca, Mg-bearing silicate weathering

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### Abstract

Assessment of the microbial impact on mineral dissolution is crucial for a predictive understanding of basic (Ca, Mg-bearing) silicate weathering and the associated CO<sub>2</sub> consumption, bioerosion and CO<sub>2</sub> storage in basaltic rocks. However, there are controversies about the mechanism of microbial effect, which ranges from inhibiting via nil to accelerating. Here we studied diopside interaction with the heterotrophic bacterium *Pseudomonas reactants* and the soil fungus *Chaetomium brasiliense* using a combination of mixed-flow and batch reactors and in-situ (AFM) and ex-situ (SEM) microscopy. The results provide new nano-level insights into the degree to which microorganisms modify silicate dissolution. Taking into account negligible effects of organic ligands on diopside dissolution as reported earlier, we conclude that the microbial effect on Ca-Mg silicates is weak and the acceleration of dissolution of “basic” silicate rocks in the presence of soil biota is solely due to pH decrease in porewaters.

### INTRODUCTION

Chemical weathering of basic (Ca- and Mg-bearing) silicates is a primary factor regulating the climate of our planet via a long-term (million year scale) CO<sub>2</sub> uptake from the atmosphere<sup>1</sup>. Both direct biological (enzymatic action of bacteria, fungi, and plant roots) and indirect physico-chemical (soil porewater pH, extracellular organic ligands and biomass degradation products) mechanisms have been identified for chemical weathering of primary silicate minerals in soil environments<sup>2-4</sup>. Among the “aqueous” factors, controlling the intensity of this process, solution pH, pCO<sub>2</sub> and organic compounds produced during enzymatic degradation of vegetation litter or the exudates of soil bacteria and plant roots are believed to be the most important<sup>5</sup>. The organic compounds capable to affect mineral dissolution rate include (but are not limited to) extracellular acid and neutral polysaccharides, simple carboxylic acids, uronic acids (galacturonic, guluronic), peptides and aminoacids<sup>6,7</sup>, as well as various lichen polyphenolic compounds<sup>8</sup>.

42 Positive effects of bacteria and organic ligands on aluminosilicate dissolution are fairly well established<sup>9-15</sup>.  
43 These effects are related to the capacity of biota to facilitate the Al-O-Si and Fe-O-Si rate-limiting bond  
44 breaking, due mostly to strong complexation of bacterially-originated organic moieties with rate-controlling  
45 aqueous  $\text{Al}^{3+}(\text{aq})$  cation<sup>16</sup>, surface  $\text{>AlOH}_2^+$  and  $\text{>FeOH}_2^+$  sites<sup>17</sup>, or  $\text{Fe}^{3+}(\text{aq})$  mobilization in solution<sup>18-20</sup>.  
46 The latter is known to prevent passivation of mineral surfaces by newly-formed Fe(III) hydroxides<sup>21,22</sup>.  
47 However, all the above mentioned mechanisms are not likely to operate for Fe- and Al-free, Ca and Mg  
48 dominant silicates. In fact, several experimental works performed over the past two decades unambiguously  
49 demonstrated the lack of any sizable impact of  $\text{CO}_2$  and organic ligands (at otherwise constant pH) on Ca-  
50 and Mg-bearing mineral dissolution at typical soil water environments. The reason for this is rather weak  
51 complexation of organic ligands with  $\text{>CaOH}_2^+$  and  $\text{>MgOH}_2^+$  surface sites responsible for the rate-  
52 controlling Mg-O and Ca-O bond breaking in these minerals<sup>23-30</sup>. According to these studies, unreasonably  
53 high concentrations of naturally-relevant organic ligands (0.01 – 0.1 M) are necessary to significantly  
54 enhance or inhibit mineral dissolution. As a result, the effect of extracellular organic products and in general  
55 soil biota on the weathering rate of Mg- and Ca-bearing minerals in natural settings is expected to be weak.  
56 Further, work with heterotrophic bacteria and basaltic glass<sup>31</sup>, wollastonite<sup>28</sup> and olivine<sup>32</sup> demonstrated that  
57 even unrealistically high concentration of live biomass (several gram per liter, regardless of the presence of  
58 nutrients) are not capable of substantially accelerating the Ca, Mg or Si release rate from the mineral. As a  
59 result, despite the dominant paradigm on the importance of microbes in chemical weathering of Ca-Mg-  
60 silicates in soil environments, the impact of biota on dissolution rates of these silicates might be lower than  
61 generally thought. This, in turn, may require revisiting the current understanding of biotic control on  
62 feedbacks in atmospheric  $\text{CO}_2$  consumption vs. silicate weathering processes. To further address the role of  
63 soil biota on mineral weathering process and to extend the previous results to other alkaline-earth bearing  
64 minerals (e.g., crystalline mixed calcium and magnesium silicates), in the present study we measured the  
65 dissolution rate of typical basic silicate diopside ( $\text{CaMgSi}_2\text{O}_6$ ) in the presence of aquatic and soil  
66 microorganisms. For these experiments, we were able to separate the effect of pH from that of bacterial  
67 presence using a mixed-flow and batch reactor systems coupled with an in-situ and ex-situ microscopic  
68 examination of reacted surfaces.

69 To provide a better understanding of the bacteria–mineral interaction in the context of chemical weathering  
70 in soils and  $\text{CO}_2$  geological storage, we selected a common soil and underground bacterium, *Pseudomonas*  
71 *reactans*. Bacterial strains of the genera *Pseudomonas*, thanks to their relatively easy culturing, have been  
72 frequently used as a model organisms in studying mineral weathering in the past<sup>33,34</sup> and recently<sup>15,28,35</sup>.  
73 Diopside was chosen as a typical model mineral for basic silicates, frequently used as a representative of  
74 silicate dissolution at the Earth surface<sup>36</sup>. Indeed, because hydrolysis of Ca-O and Mg-O bonds is often the  
75 rate-limiting step for Ca- and Mg-bearing mineral dissolution<sup>37</sup>, understanding of microorganism interaction  
76 with diopside may help to predict the reactivity of other basic silicates in the presence of microbes. In the  
77 present study, the experiments were designed in batch and flow-through reactors under constant biomass  
78 concentration, in the presence of both live and dead cells in both nutrient-free and nutrient-bearing media.  
79 We measured forward far from equilibrium dissolution rates and we microscopically examined reacted  
80 surfaces with respect to microorganism attachment, etch pits and corrosion features. The ensemble of  
81 obtained results demonstrated rather weak impact of both bacterium and fungus on diopside reactivity,  
82 regardless of the presence of nutrients and live status of microorganisms. Moreover, the passivation of  
83 mineral surfaces via formation of an exopolysaccharide layer (in case of bacteria) may act as protection  
84 mechanism in natural settings. Globally, these new findings challenge the dominant paradigm on dominant  
85 biotic control of basic silicates weathering at the Earth surface conditions. This may require revising the  
86 quantitative modeling of chemical weathering as main factor of biotically driven long term  $\text{CO}_2$  consumption  
87 (coupled to Ca and Mg release from silicates). Further, the results call for a need of *in-situ* assessment of soil  
88 porewater pH at high spatial and temporal resolution as a sole factor controlling the rate of Ca, and Mg  
89 bearing silicate weathering.

90

## 91 **RESULTS**

### 92 **Diopside interaction with *P. reactans***

93 In most experiments performed at pH of 6 to 9, stoichiometric release of Si, Ca and Mg was  
94 observed. In bacteria-free experiments, diopside dissolution rates decreased by ca. 0.5-1 order of magnitude  
95 during the pH decrease from 6 to 9 (Fig. 1, Table 1). The DOC concentration remained fairly stable in the  
96 course of given bacterial experiment and generally ranged from 7 to 25 mg L<sup>-1</sup> being the highest at highest  
97 biomass concentration. Considering certain scatter in the rates obtained in different experiments, the  
98 presence of bacteria in either nutrient-free or nutrient-rich media did not exhibit any significant (at p < 0.05)  
99 effect on Si release rate from diopside, or even led to a decrease of dissolution rates (Fig. 2). In fact, the  
100 effect of bacterial presence did not exceed 0.2 log  $R_{Si}$  units, whereas the reproducibility of rates measured in  
101 individual reactors at otherwise similar aqueous solution composition ranged from 0.1 to 0.3 log  $R_{Si}$  units.  
102 Thus it can be concluded that, within the resolution of our experiments, the impact of bacteria on diopside Si  
103 dissolution rate could not be quantified or it was below 0.2 log R unit. Furthermore, there was a net  
104 inhibiting effect of bacteria on Ca and Mg release rate from the diopside in nutrient solution at pH = 6.1 (Fig.  
105 2 A). In more alkaline solutions (pH = 8.5 to 9.0), the presence of bacteria (with or without nutrients) did not  
106 considerably (> 0.2 log R unit) impact the release rate of Si, Ca and Mg (Fig. 2 B).

107 The second remarkable result of BMFR experiments is that live bacteria with (D2-2, D2-3, D4-2,  
108 D4-3) and without (D1-2, D1-3, D3-2, D3-3) nutrients exhibited a negligible effect of biomass concentration  
109 (between 0.54 and 3.85 g<sub>wet</sub>/L) on Mg, Ca and Si release rates because there was no substantial (> 0.2 log R  
110 unit) change in rate during biomass increase by a factor of 7 (Table 1).

111 The third important observation is that there was a significant, by a factor of 3 to 5, decrease in Ca,  
112 Mg, Si concentrations and release rates between the beginning (exp. D1-1, D2-1, D3-1 and D4-1) and the  
113 end of bacteria-free experiments (exp. D1-4, D2-4, D3-4 and D4-4). This is illustrated in Supplementary  
114 Figure 1, where the Si concentration in the outlet solution corresponding to the sequence of inlet solutions  
115 “bacteria-free 0.1 M NaCl → 0.54 g<sub>wet</sub>/L bacteria → 3.85 g<sub>wet</sub>/L bacteria → bacteria-free 0.1 M NaCl”, at a  
116 constant flow rate, demonstrated a 3- to 5-fold decrease between the initial and the final bacteria-free  
117 experiment. Such a decrease could be related to passivation of mineral surface by bacterial cells and their  
118 exometabolites as evidenced from in-situ microscopic observations (see section on the AFM observations  
119 below).

120

### 121 **Ca, Mg and Si release in the presence of fungus**

122 Results of Ca, Mg and Si release from diopside in 0.1 M NaCl and in nutrient media in the presence  
123 of live and dead *Chaetomium brasiliense* demonstrated that concentrations of all mineral constituents  
124 increase quasi-linearly with time in all types of experiments (illustrated for Si in Supplementary Figure 2).  
125 The linear regression coefficients varied from 0.9 to 0.99 for Si and from 0.8 to 0.95 for Ca and Mg. The pH  
126 remained generally constant (±0.2 unit) or slightly increased (within 0.2 – 0.3 units) over the 19 days of  
127 exposure. In batch experiments with fungus, the DOC increased from 10 to 30 mg/L over the first 100 h of  
128 reaction and then remained constant over the following 300 h. Taking into account that 1) all solutions are  
129 strongly undersaturated with respect to diopside, all other possible Ca and Mg silicates and amorphous silica  
130 and 2) there is no effect of aqueous Ca, Mg and Si concentration on diopside forward dissolution rates in  
131 neutral solution<sup>24,38,39</sup>, one can quantify the far-from equilibrium dissolution rate ( $R$ ) for each individual  
132 experiment using the following equation which can be applied over the whole duration of the experiment:

133

$$R = (d[\text{Ca, Mg, Si/2}]_{\text{tot}}/dt)/s \quad (1)$$

134 where  $t$  (s) designates the elapsed time,  $[\text{Ca, Mg, Si}]_{\text{tot}}$  (mol/L) stands for the concentration of calcium,  
135 magnesium or silica released from the solid, and  $s$  (cm<sup>2</sup>/L) is the powder B.E.T. surface area. Results are

136 listed in Table 2. The uncertainties on these rates stem from duplicate experiments and range between  $\pm 10$   
137 and 30%. The rates measured in the presence of dead or live fungi generally agree (within  $\pm 0.2$  log units)  
138 with those of abiotic and biotic experiments in mixed-flow reactor (Fig. 1). The Si release rates were a factor  
139 of 1.5 higher in experiments with live fungus (with or without nutrients) compared to experiments with dead  
140 fungus (Fig. 3). However, there was no difference in Mg release rate between different treatments, whereas  
141 the Ca release rate, although more variable among replicates, exhibited a maximum in nutrient-free  
142 experiments with live fungus (Fig. 3). The element release rate in the presence of fungus was mostly  
143 stoichiometric (Table 2). The disagreement between Si and Mg release rate ranged from 0.1 to 0.05 log  $R$   
144 unit except in one experiment with dead biomass (0.2 units). Calcium release rate was in agreement with that  
145 of Si within 0.01-0.19 log  $R$  units, except in one experiment with live biomass (0.24 log  $R$  lower).

146

#### 147 **AFM observations in the presence of *P. reactans***

148 Freshly reacted diopside surfaces (2-5 h in sterile NaCl solution) largely preserved their cleavage  
149 plane morphology (Fig. 4 A). Occasionally, some etch pits and local surface roughness were observable (Fig.  
150 4 B). After 36 h reaction in bacteria-free nutrient solution, rounding of edges occurred (Fig. 4 C). After 1 to 2  
151 days of reaction with nutrient solution at pH = 8.5 or 6.5, we have observed a strong colonization of diopside  
152 surfaces by a biofilm-like bacterial assemblage (Fig. 4 D). This extracellular polymeric substances (EPS)  
153 coverage included individual elongated spheres and generally convex forms, less than 0.5  $\mu\text{m}$  in longest axis,  
154 although their size distribution was not uniform (Figs. 4 D, E, F). Groups of objects occasionally exhibited a  
155 distinct internally preferred orientation, which was, however, quite variable within a relatively small area  
156 ( $\sim 50 \mu\text{m}^2$ ). The obtained images are consistent with earlier reported bacterial EPS via AFM<sup>40,41</sup>. Extensive  
157 production of exopolymeric substances precluded imaging of individual bacterial cells within this thick  
158 biofilm coverage. In contrast, mineral interaction with bacteria in nutrient-free neutral electrolyte allowed  
159 recognizing individual cells attached to the diopside surface (Fig. 4 G); however, no site of preferential  
160 interactions such as steps or edges could be identified.

161

#### 162 **SEM imaging of diopside reacted with fungus**

163 The diopside grains reacted with dead fungus in 0.1 M NaCl demonstrated sharp borders and no  
164 clear dissolution features (Fig. 5 A). After reaction with live fungus in nutrient-free media, there was an  
165 appearance of specific dissolution features such as etching and rounding of edges (Fig. 5 B). The rounded  
166 edges were also observed in experiments with live fungus; these features were distinctly different from  
167 cleavage (Fig. 5 C). The diopside crystals were also imaged in the presence of fungal mycelium: the hyphae  
168 were strongly attached to the grain surfaces and not lost during sample preparation for SEM (Fig. 5 D). Some  
169 grains appeared significantly ( $> 50\%$ ) covered by or even embedded into network of fungal hyphae. There  
170 were no distinct sites of hyphae location at the diopside surface, and overall, the interaction appeared to be  
171 quite inert, non-specific and site-indifferent. With increasing the resolution, some organic spherical entities,  
172 2 to 3  $\mu\text{m}$  in diameter, were identified at the extremities of the hyphae (Fig. 5 D).

173

#### 174 **DISCUSSION**

175 Results of the present study demonstrated a weak (or non-measurable) impact of bacterium and  
176 fungus on Ca, Mg and Si release rate from diopside. Biologically accelerated weathering of silicates is  
177 thought to be controlled by microbial production of extracellular enzymes, chelates, simple and complex  
178 organic acids and exopolysaccharides<sup>5,11,42,43,44</sup>. However, there are serious controversies, whether such an  
179 effect is largely direct (metabolically-driven nutrient mobilization and specific ligand production) or indirect

180 (passive pH lowering and cation complexation via EPS excretion), given that it is largely dependent on the  
181 nature of mineral and the identity of microorganism<sup>9,45-48</sup>. Furthermore, considering basic silicates, some  
182 studies reported nil or inhibiting effect of bacteria on olivine<sup>32,49,50</sup> or wollastonite<sup>28</sup> dissolution, whereas  
183 other claimed strong accelerating effect of fungi on olivine<sup>21,22,51</sup>. However, in the latter works, all the effects  
184 of microorganisms were limited to their ability to sequester rate-inhibiting Fe(III) thus preventing the  
185 slowdown of dissolution, rather than directly promoting the breaking of structural bonds in the mineral  
186 lattice. Overall, in contrast to large number of works on microbial impact on aluminosilicate dissolution<sup>52-56</sup>,  
187 the interaction of aquatic microorganisms and relevant organic ligands with Ca or Mg-bearing silicates  
188 remained insufficiently characterized.

189 In the present study, the global rate uncertainty in the studied pH range (6 to 9) was between 20 and  
190 50%. This uncertainty stems from both the pH variation ( $\pm 0.2$ - $0.3$  units) during the course of an experiment  
191 and the reproducibility of steady state concentrations of diopside constituents in mixed-flow reactors with  
192 bacteria-free systems or among duplicates in batch reactors with dead fungus ( $\pm 20\%$ ). Therefore, we  
193 conclude that the difference between abiotic (bacteria-free or dead cells) and live bacteria experiments is  
194 within the experimental uncertainty. As such, the effect of selected live microorganisms on Ca, Mg and Si  
195 release rate from diopside is not significant, or cannot be resolved using the experimental design employed in  
196 the present study.

197 In this regard, the present result corroborates the former works of our group which demonstrated  
198 weak inhibiting or negligible impact of cultivable heterotrophic bacteria *P. aureofaciens* on wollastonite<sup>28</sup>  
199 and *P. reactans* on basaltic glass<sup>31</sup> and olivine<sup>32</sup>, in agreement with other studies<sup>15,47,49</sup>. The broad meaning of  
200 these results is that microbial impact on basic silicate mineral dissolution is limited by *i*) pH decrease, thus  
201 accelerating proton-promoted dissolution, which is relevant to all basic silicates; *ii*) release of Fe(III)-  
202 complexing organic ligands including siderophores. The latter is pertinent solely to minerals whose  
203 dissolution is limited by Fe(OH)<sub>3</sub> passivation of the surface. In all other cases of microbially-affected basic  
204 silicate dissolution, the concentrations of excreted organic ligands are not sufficient to sizably complex Mg  
205 and Ca at the surface or in the aqueous solution which is necessary for breaking of rate-controlling Mg-O or  
206 Ca-O bonds at the mineral surface. For example, gluconic acid and its derivatives are believed to be the main  
207 exometabolite of heterotrophic bacteria that use glucose as a substrate<sup>10,34</sup>. Gluconic acid can favor olivine  
208 dissolution in neutral waters<sup>32</sup>; however, this effect occurs at concentrations  $> 1$  mM, totally irrelevant to  
209 natural environments. Another example comes from a concerted study of ~40 various ligand impact on  
210 wollastonite dissolution<sup>28</sup>. These authors calculated, based on the results in mixed-flow reactors, the  
211 threshold concentration of ligands necessary to triple the rates of wollastonite at pH around 7. For most  
212 ligands, very high concentration (2 to 3 orders of magnitude higher than those encountered in natural  
213 settings) were necessary to appreciably affect the rates. Finally, Golubev and Pokrovsky (2006)<sup>24</sup>  
214 demonstrated that very high concentrations (0.01-0.1 M) of organic ligands originated from enzymatic  
215 degradation of organic matter or bacterial metabolic activity, are necessary to appreciably affect diopside  
216 dissolution. Considering these results and by analogy with olivine and wollastonite, we hypothesize that  
217 possible impacts of studied heterotrophic bacteria on the diopside dissolution via gluconic acid or other  
218 soluble exometabolite products are not detectable due to insufficient concentration of these compounds  
219 generated via metabolism of *P. reactans* in experimental reactors.

220

221 Below we discuss the mechanisms of diopside interaction with microbial cells and natural implications.  
222 Reactive surfaces in both bacterial and fungal experiments demonstrated lack of specific sites of dissolution,  
223 etch pit formation, surface amorphisation or new mineral occurrences. Rather, our in-situ (AFM) and ex-situ  
224 (SEM) microscopic observations suggested “passivation” of reacted surfaces by microbial metabolic  
225 products. We therefore hypothesize that the biofilm coverage either limited aqueous solution contact with  
226 mineral surface or merely did not produce sufficient amount of organic ligands capable to impact the  
227 diopside dissolution.

228 Microbial biofilm formation, dominated by exopolysaccharides (EPS), is known to protect the  
229 mineral surface from interaction with bulk fluid<sup>42,46,47,57</sup> thus inhibiting overall dissolution rate<sup>2,3,6,7,35,58</sup>. In the  
230 present experiments with diopside, the biofilm formation was also evidenced from indirect assessment of  
231 dissolution rate: the rates decreased by a factor of 2 to 3 after ~500 h treatment with bacteria; this decrease  
232 persisted even after changing back the inlet solution to sterile bacteria-free background electrolyte (see  
233 Supplementary Figure 1). Noteworthy that such an irreversible rate decrease was also observed after reaction  
234 with live bacteria in nutrient-free media and suggested substantial and long-lasting interaction of live *P.*  
235 *reactans* with diopside surface in both neutral (pH = 6) and slightly alkaline (pH = 9) solutions. At the same  
236 time, no decrease in diopside dissolution rate was observed over 400-800 h of experiments in the abiotic  
237 inorganic systems<sup>23,24</sup>.

238 As a result of lack of measurable rate increase in the presence of either bacteria or fungus in this  
239 range of pH, nutrient and biomass concentration, we suggest, in agreement with previous studies, that,  
240 regardless of nutrient regime, biomass concentration and vital status of microorganism, solely the solution  
241 pH can act as the main governing factor of mineral dissolution in the presence of aquatic microorganisms. A  
242 similar conclusion has been recently achieved for olivine dissolution in the presence of complex natural  
243 microbial communities<sup>59</sup>. Therefore, implementation of mineral–microorganism interaction in chemical  
244 weathering codes requires solely the information on porewater solution chemistry that can be directly  
245 assessed from macroscopic measurements of interstitial soil solutions.

246 Moreover, extremely high concentrations of organic ligands, necessary to affect the rates of basic  
247 silicate dissolution in laboratory experiments<sup>24,28,32</sup>, together with high bacterial and fungi concentrations,  
248 comparable with those used in the present study, can be encountered exclusively in surface (litter) soil  
249 horizons, and unlikely to ever occur in deep (mineral) soil horizons. Exceptions are specific  
250 microenvironments around fungal hyphae, or near decaying organic matter. For example, a strong  
251 acidification was reported with hyphae bound to the biotite surface<sup>60</sup>. To quantify these effects on a broader  
252 scale, high-resolution in-situ quantitative analysis of chemical composition of solution, bacteria and organic  
253 ligand concentration in soil pore water microenvironments are needed.

254 At the same time, the role of microorganisms under natural environment, especially in the non-  
255 saturated zone, might be more important than that in aqueous environment of mixed-flow experiments. It is  
256 generally understood that a lack of “wetted” mineral surface area, or mineral surfaces being exposed to the  
257 reactive fluid, could be a partial explanation for slower reaction rates observed in the field compared to those  
258 measured in the laboratory with unlimited water<sup>61,62</sup>. For example, via exerting hydrated exopolysaccharides,  
259 microbial cells are capable of prolonging the degree of mineral surfaces hydration during natural  
260 wetting/drying cycles. Therefore, although our experiments, performed under nutrient-free conditions with  
261 starved cells, are most similar to natural environments under short-term scale, there is sizable uncertainty in  
262 extrapolating these short-term laboratory results to long-term field conditions. The latter are known to exhibit  
263 one to three orders of magnitude lower weathering rates, due to different time scale of observation, diversity  
264 of microorganisms, and nutritional conditions.

265 For more general natural implications of the obtained results, we consider below all Ca, Mg-bearing  
266 ‘basic’ silicates including Ca aluminosilicates. Several lines of evidences allow us to suggest rather weak  
267 effect of dissolved organics on (Ca, Mg, Al)-bearing silicate dissolution in natural settings. First, the  
268 concentration range of various carboxylic ligands (oxalic, citric, malonic, succinic, lactic, formic, acetic),  
269 detected in soil solutions, spans from 10<sup>-6</sup> to 10<sup>-4</sup> M<sup>63</sup>. Laboratory experiments suggest that these  
270 concentrations are still not sufficient to appreciably affect feldspars dissolution rates. Second, the effect of  
271 organic ligands on Al release and thus on overall dissolution rates of feldspars and basaltic glass is absent or  
272 becomes negligible at pH ≥ 7<sup>16,64</sup>. The pH of rivers and streams draining ‘basic’ silicate rocks is between 7  
273 and 8<sup>65,66</sup> whereas the pH of soil solutions in organic rich soils typically ranges from 4 to 7<sup>67</sup>. However,  
274 surficial acid- and organic-rich soil horizons rarely contain primary silicates, whereas in deep (C) soil  
275 horizons, the interstitial soil solution pH is always above 7 (see ref. 65). At these conditions, the effect of  
276 organic ligands on Ca, Mg, Al and Si release rate from primary silicates should be negligible which

277 corroborates the conclusion of Drever (1994)<sup>68</sup> that vegetation should cause an increase in weathering rate  
278 through the pH effect only where the pH is below 4-5. As a results, the sole effect of soil organics on both  
279 acidic and felsic rock weathering seems to be in decreasing the aqueous activity of Al<sup>3+</sup> thus preventing  
280 formation of secondary minerals without significant modification of Ca and Mg ions release.

281 It has been widely argued that the presence of soluble minerals in trace amounts in the rocks (i.e.,  
282 carbonates<sup>69</sup>; phosphates<sup>70</sup> may strongly affect the intensity of chemical weathering and export fluxes of  
283 major ions. The effect of biological activity in this case is expected to be solely due to pH decrease in the  
284 vicinity of microorganisms or plant roots. Indeed, the data for dolomite<sup>25</sup>, calcite and magnesite<sup>27,29</sup>,  
285 brucite<sup>26</sup>, and calcite<sup>30</sup> dissolution in the presence of various organics demonstrate a negligible effect of  
286 organic ligands on Ca and Mg release from carbonates and oxides at environmentally-relevant conditions.  
287 The effect of soil and atmospheric pCO<sub>2</sub> is always considered to be an important factor accelerating rock  
288 weathering at the Earth surface. In this regard, we would like to underline that this effect stems solely from  
289 the decrease of soil solution pH since there is no chemical effect of CO<sub>2</sub>(aq) or dissolved H<sub>2</sub>CO<sub>3</sub> molecules  
290 on the main rock-forming silicates dissolution rate<sup>39</sup>, and in neutral and alkaline solutions the pCO<sub>2</sub> effect  
291 becomes even inhibiting<sup>71</sup>.

292 Overall, this work allows a better understanding of microbially-affected basic silicate dissolution and  
293 provides microscopic insights for quantifying the degree to which the heterotrophic bacteria and fungi are  
294 capable of modifying the dissolution rate of rock-forming silicates. Using bacterial-mixed flow and batch  
295 reactors combined with in-situ atomic force microscopy of bacteria-diopside surfaces and experiments on  
296 diopside dissolution in the presence of fungus combined with microscopic examination of reacted surfaces,  
297 we demonstrated a rather weak impact of microorganisms on Ca, Mg and Si release rate. Regardless of the  
298 presence or absence of nutrients, in a wide range of pH (from 6 to 9) and despite very high biomass  
299 concentration (up to 4-5 g<sub>wet</sub>/L), the rates were not considerably affected by microbial presence. Moreover,  
300 there was a detectable (ca. by a factor of 3) decrease in reactivity of diopside after its prolonged contact with  
301 live heterotrophic bacteria. This ‘passivation’ of the mineral surface was evidenced by extensive production  
302 of exopolymeric substances yielding a biofilm-like coverage already after 1-2 days of interaction in nutrient-  
303 rich media. Despite tight contact between fungus and diopside surfaces, no site of preferential interactions  
304 such as steps, kinks or edges, etch pits or surface amorphisation was detected. The obtained results are fully  
305 consistent with large body of evidences that any possible microbially produced organic ligands at  
306 concentrations relevant to live biotic systems or natural settings are not capable of noticeably impacting the  
307 reactivity of Mg-O and Ca-O sites on diopside surface. As such, this work challenges the dominant paradigm  
308 of an overwhelming role of biota on silicate mineral weathering in soils. At the same time, it is in line with a  
309 controversial opinion of Sverdrup (2009)<sup>72</sup> that physical and chemical conditions of forest soils do not allow  
310 any significant direct surface actions on minerals by microorganisms or tree roots. While we do not contest  
311 the importance of microbial organic matter in Fe and Al complexation and mobilization from silicate  
312 structure, the role of biota on basic (Ca and Mg) silicates, playing a pivotal role in atmospheric CO<sub>2</sub>  
313 sequestration via chemical weathering, may be much lower than traditionally thought. In fact, only drastic  
314 pH decrease (i.e., over several pH units), probably occurring in the microniches adjacent to plant roots, is  
315 capable of sizably (> a factor of 2 to 3) increase mineral dissolution rate. It is not excluded however that this  
316 promoting effect will be partially attenuated due to biofilm formation and passivation of silicate surfaces by  
317 microbial cells. Detecting such ‘active’ or ‘passive’ microniches within the complex plant-microbe-mineral  
318 systems and more importantly, characterizing chemical environment of the fluids with spatial resolution on  
319 the level of μm to mm may turn out to be a bottleneck for quantitative mechanistic modeling of chemical  
320 weathering. Currently, these gradients are not taken into account even in most sophisticated modeling  
321 approaches<sup>73,74</sup>. Further experimental and field studies are thus needed to answer the question whether a  
322 quantitative modelling of basic silicate mineral weathering and related CO<sub>2</sub> consumption require an explicit  
323 prediction for microbial activity or it can be merely approximated by the pH of bulk soil porewaters.

## 325 METHODS

### 326 Mineral, bacterial and fungal culture

327 Natural diopside ( $\text{Ca}_{0.99}\text{Mg}_{0.98}\text{Fe}_{0.02}\text{Cr}_{0.01}\text{Si}_2\text{O}_6$ ) crystals characterized in previous works<sup>24,39</sup> were  
328 used in this study. Ultrasonically-cleaned 100 to 200  $\mu\text{m}$  size fraction having a specific surface area of 1045  
329  $\pm 50 \text{ cm}^2/\text{g}$  was selected for dissolution rate measurements.

330 *Pseudomonas reactans* is a common rod-shape groundwater and soil bacteria averaging 2  $\mu\text{m}$  in size  
331 (see further description in ref. 31, 32). For experiments, cells were first cultured to a stationary stage on a  
332 shaker at 25°C in the darkness using 10% nutrient broth with the following composition: 0.1 g/L glucose, 1.5  
333 g/L peptone, 0.6 g/L NaCl and 0.3 g/L yeast extract. Cells were allowed starving for 2 days in nutrient-free  
334 media, prior the macroscopic dissolution and microscopic (AFM) experiments. For this, freshly-grown cell  
335 suspension (at the stationary stage) was rinsed by sterile 0.6 g L<sup>-1</sup> NaCl solution via centrifugation, and then  
336 placed in 0.6 g L<sup>-1</sup> NaCl without nutrients for 2 days. Before the experiment, this bacterial suspension was  
337 rinsed again with sterile 0.6 g L<sup>-1</sup> NaCl to remove, as possible, all cellular exometabolites.

338 Active bacteria number counts (colony forming-units, CFU/mL) were performed using Petri dish  
339 inoculation on a nutrient agar (0.1, 0.2, and 0.5 mL of sampled solution in three replicates) in a laminar hood  
340 box. Inoculation of blanks was routinely performed to assure the absence of external contamination. The  
341 biomass of live bacteria suspensions was also quantified by measuring wet (after it was centrifuged 15 min at  
342 10,000 rpm) and freeze-dried weights in duplicates. Before the experiments, cells were rinsed twice in either  
343 the appropriate fresh culture media or a sterile 0.1 M NaCl solution using centrifugation with ~ 500 mL of  
344 solution for 1 g of wet biomass, to remove adsorbed metals and cell exudates from the surface. The  
345 conversion ratio wet/freeze-dried weight of *P. reactans* was equal to 8.4±0.5. The conversion factor of  
346 optical density (600 nm, 10 mm path) and wet biomass (g<sub>wet</sub>/L) to the cell number (CFU/mL) was equal to  
347  $(5\pm 1)\times 10^8$  and  $(8.0\pm 1.5)\times 10^7$ , respectively, as determined by triplicate measurements. Typical live biomass  
348 concentration during experiments was 0.54 and 3.85 g<sub>wet</sub>/L.

349 Experiments on fungus were performed with *Chaetomium brasiliense*, belonging to the largest genus  
350 of saprophytic ascomycetes, inhabiting soils worldwide. The environmental habitats of genus *Chaetomium*  
351 include deserts, salterns, agricultural, mangrove, living plants and animals, air, decayed wood and stones<sup>75</sup>.  
352 *Chaetomium brasiliense* Bat. Et Pontual 1948, stumm F-3649 was obtained from All-Russian Collection of  
353 Microorganisms - VKM (<http://www.vkm.ru/Collections.htm>). Fungal biomass was produced on liquid  
354 Czapek medium (3 g/L NaNO<sub>3</sub>, 1 g/L K<sub>2</sub>HPO<sub>4</sub>, 0.5 g/L KCl; 0.5 g/L MgSO<sub>4</sub>×7H<sub>2</sub>O, 0.01 g/L FeSO<sub>4</sub>×7H<sub>2</sub>O,  
355 30 g/L saccharose at pH = 6.0). The biomass was centrifuged at 5000 rpm during 15 min, rinsed 4 times in  
356 0.1 M NaCl and allowed starving during 2 days in 0.1 M NaCl prior the experiment. Dead (heat-killed) cells  
357 were produced via autoclaving 1 h at 132°C the freshly grown fungal biomass followed by thorough rinsing  
358 in sterile 0.1 M NaCl. Cell concentration in the sample was analyzed by measuring optical density (OD) at  
359 580 nm using a spectrophotometer, with the culture medium used as a reference. Diopside dissolution  
360 experiments were performed in duplicates, in 0.1 M NaCl with dead biomass, and live biomass with and  
361 without 1% nutrient solution.

362

### 363 Bacterial mixed-flow reactor

364 The Bacterial Mixed-Flow Reactor (BMFR) system used in this work was similar to the one used for  
365 basaltic glass dissolution in the presence of bacteria<sup>31</sup>. The Teflon-made 25 mL reactor with a suspended  
366 Teflon-coated stirbar, immersed in a water bath at 25 ± 0.5 °C, was fitted with 20  $\mu\text{m}$  poresize Magna  
367 Millipore Nylon outlet filters (45 mm diameter) to allow bacteria to pass while retaining the 100-200  $\mu\text{m}$   
368 mineral powder in the reactor. This system therefore maintained a constant biomass concentration in the  
369 reactor, equal to that of the inlet fluid. The input fluids were kept in 1L polypropylene bottles closed with  
370 Biosilico® ventilated caps. These fluids were stirred continuously during the experiments and were changed  
371 typically each 7 days. This allowed maintenance of a constant and stable stationary phase bacterial culture in

372 the bacteria-bearing inlet fluid. Bacteria concentration was verified by periodical sampling of inlet and outlet  
373 fluids for cell optical density (total biomass) and live cell numbers (via agar plate counting). Prior to each  
374 experiment, all reactor system parts, including tubing, were sterilized at 130°C for 30 min and rinsed with  
375 sterile MilliQ water.

376 Steady-state dissolution rates ( $R_i$ , mol/cm<sup>2</sup>/s) were computed from measured solution composition  
377 using:

$$378 \quad R_i = -q \cdot 1/n \cdot \Delta[i(\text{aq})]_{\text{tot}} / s \quad (2)$$

379 where  $q$  (L/s) designates the fluid flow rate,  $\Delta[i(\text{aq})]_{\text{tot}}$  (mol/L) stands for the difference between the input  
380 and output solution concentration of  $i$ -th element,  $n$  is the stoichiometric coefficient of  $i$ -th element in  
381 diopside formula equaled to 2, 1, and 1 for Si, Mg and Ca, respectively, and  $s$  (cm<sup>2</sup>) refers to the total mineral  
382 surface area present in the reactor. The surface area used to calculate the rates was that measured on the fresh  
383 (unreacted) diopside powder. Note that during 600 h of reaction time, no systematic variation of the surface  
384 area for powder reacted at  $5 < \text{pH} < 10$  in bacteria-free solutions was observed<sup>24</sup>.

385 Experiments were performed in series at constant flow rate between 0.018 and 0.022 mL/min. This  
386 corresponded to a mechanical steady-state achievement (x 5 reactor volume renewal) after 4 to 5 days. At the  
387 beginning of each series, a quantity of fresh diopside powder was added to the reactor system. For each  
388 experimental series, a sterile bacteria-free 0.1 M NaCl with relevant buffer (10 mM NaMES (2-  
389 morpholinoethanesulfonic acid) or NaHCO<sub>3</sub>) was first prepared and injected into the reactor until a steady-  
390 state outlet fluid composition was attained (typically 2 weeks, D1 series). Once steady state was attained, live  
391 bacteria (0.54 g<sub>wet</sub>/L) with or without nutrients were added to this inlet solution (D2 series). The next series  
392 comprised higher concentration of live bacteria in the inlet fluid (3.85 g<sub>wet</sub>/L). The 2nd and 3rd series lasted  
393 7-10 days each. After that, the inlet solution was replaced by fresh sterile bacteria-free fluid; this experiment,  
394 which lasted > 3 weeks, was destined to assess the recovery of the mineral reactivity and to test the  
395 consequences of passivation of mineral surface by a bacterial biofilm.

396 The inlet solutions were composed of 0.1 M NaCl and 0.01 M NaMES (D1 and D2 series) and 0.1 M  
397 NaCl with 0.01 M NaHCO<sub>3</sub> (D3 and D4 series). The 1:10 diluted Aldrich nutrient broth (0.1 g/L glucose, 1.5  
398 g/L peptone, 0.6 g/L NaCl and 0.3 g/L yeast extract) was added to selected live bacteria experiments. These  
399 were compared to live bacteria experiments performed without nutrients. Overall, the experiments conducted  
400 in this study can be divided into three main categories: 1) bacteria- and nutrient-free, 2) live bacteria in  
401 nutrient-free media, and 3) live bacteria in 10% growth media.

402 All outlet sample fluids were additionally filtered through 0.45 μm Millipore acetate cellulose filters  
403 and acidified with ultrapure 2% HNO<sub>3</sub> before chemical analysis. The inlet fluids were routinely analyzed at  
404 the beginning, in the middle and at the end of each experiment and were filtered and processed using exactly  
405 the same protocol as the outlet fluids.

406 Mechanical steady-state was achieved after 24 h of reaction. For each steady-state condition,  
407 minimum 4 independent measurements of Mg, Ca and Si concentrations, bacterial optical density, pH and  
408 flow rate were performed (reproducibility of ± 10%) and used for calculating the dissolution rate. The cell  
409 count (CFU) on nutrient agar plates was performed at the beginning, in the middle and at the end of each  
410 experimental run and did not vary significantly throughout all experiments.

411

### 412 **Batch experiments on diopside dissolution in the presence of fungus**

413 The aim of these experiments was to compare, under identical solution conditions, the Ca, Mg and Si  
414 release rate from diopside placed in contact with dead and live fungus cultures. The batch reactors used to  
415 study diopside–fungus interaction consisted of sterile 250 mL polypropylene culture flasks with vented caps  
416 (Biosilico). All manipulations were conducted in a sterile laminar hood box (class 100). For experiments, 1.3  
417 g of sterilized 100-200 μm diopside were placed in 250 mL sterile solution. We conducted six experiments in  
418 duplicates, four involving live cultures, with and without 1% nutrient solution, and two with dead  
419 (autoclaved) biomass (in nutrient-free 0.1 M NaCl).

420 The reactors were inoculated with 4.2-5.6 g<sub>wet</sub>/L of fresh *Chaetomium brasiliense* culture or the  
421 equivalent amount of dead biomass and placed on rotative shaker (120 rpm) at 25 ± 1°C for 19 days. Sterile  
422 controls were routinely run both for nutrient media and 0.1 M NaCl solutions and did not demonstrate any  
423 fungal contamination. Periodically, 10 mL aliquots of homogeneous mineral suspension + fungus were  
424 collected using sterile serological pipettes and transferred in polystyrene vials for Ca, Mg, Si, pH and cell  
425 biomass measurements. The mineral/fluid ratio remained constant during experiments and the concentration  
426 of fungus was not affected by the sampling. Calcium, Mg and Si concentrations were analyzed in 0.45 µm  
427 filtrates and pH and cell biomass were measured in unfiltered samples.

428

## 429 **Analyses**

430 All input and output solutions were analyzed for Mg, Ca, Si, optical density and pH as a function of  
431 time. NIST buffers (pH = 4.008, 6.865, and 9.180 at 25°C) were used for calibration of a combination pH-  
432 electrode (Schott Geräte H62). Magnesium and Ca concentrations were measured by flame atomic  
433 absorption spectroscopy with an uncertainty of 1 and 2 % and a detection limit of 0.1 and 0.2 µM,  
434 respectively. Total silica concentration was determined by a Technicon automatic analyzer using the  
435 molybdate blue method with an uncertainty of 2% and a detection limit of 0.3 µM. The concentration of  
436 soluble organic exometabolites of bacteria and fungi was measured as total dissolved organic carbon (DOC)  
437 using a TOC Shimadzu VSCN analyzer with an uncertainty of 5% and a detection limit of 0.1 mg C<sub>org</sub> L<sup>-1</sup>.  
438 All experiments were performed at far from equilibrium conditions with respect to diopside as calculated via  
439 vMinteq program<sup>76</sup> with relevant solubility parameters<sup>77</sup>.

## 440 **Microscopic observations**

441 An AFM study using a biological instrument (JPK NanoWizard) allowed in-situ high-resolution  
442 characterization of the status of diopside surfaces in the presence of live bacteria with and without nutrients.  
443 Diopside cleavage planes were obtained by cleaving optically transparent crystals 5-10 mm in size in a  
444 laminar hood box (A100). Fresh cleavage surfaces were sterilized by alcohol and fixed within petri dishes for  
445 imaging with the AFM. For long-term (> 5 h) exposure, diopside cleavages were reacted under sterile  
446 conditions with 1 g<sub>wet</sub>/L of bacterial biomass in separate petri dishes. The petri dishes were mounted into the  
447 AFM for in-situ inspection of the diopside surfaces directly immersed within the bacteria containing  
448 medium, removed from the microscope for further storage, and re-investigated with the microscope  
449 whenever appropriate. Depending on the specific aim of the scanning process (attempt of potentially imaging  
450 all adhering matter on the surface vs. attempt of removing matter adhering to the surface), images were  
451 acquired with both intermittent-contact mode and contact mode using uncoated Si-cantilevers with integrated  
452 tips.

453 The diopside grains reacted with fungus were examined by Scanning Electron Microscopy (SEM).  
454 Prior to characterization by SEM, samples were centrifuged (3000 g, 30 min) and the organic matter was  
455 removed from the solid phase by rinsing them in nutrient-free sterile 0.1 M NaCl solution. The remaining  
456 solids were rinsed three times with MilliQ water and freeze-dried at -60 °C. They were observed after carbon  
457 film coated on the sample surface with a JEOL JSM 6360LV SEM coupled with a SDD PGT Sahara EDS  
458 analyzer operating at 30 kV.

459

## 460 **DATA AVAILABILITY**

461 Supplementary material is available. All measured dissolution rates are presented in Tables 1 and 2. Other  
462 relevant data are also available from the corresponding author of this paper upon a reasonable request.

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466 **AUTHOR CONTRIBUTIONS**

467 O.S.P. developed the concept of the study. L.S.S., S.A.Z. and O.S.P. performed dissolution experiments. G.J.  
468 performed AFM study. P.B. participated in laboratory experiments and SEM study. O.S.P. wrote the paper,  
469 with input from L.S.S., P.B., G.J. and S.A.Z. All the co-authors discussed the final version of the paper.

470

471 **Competing Interests**

472 The authors declare that they have no known competing financial interests or personal relationships that  
473 could have appeared to influence the work reported in this paper.

474

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650

## 651 **Figure Legends**

652

653 **Figure 1.** Calcium (a), Mg (b) and Si (c) release rates from diopside measured in the mixed-flow  
654 reactor with and without bacteria and 10% nutrient or nutrient-free solution.

655

656 **Figure 2.** Synthesis of obtained steady-state Ca, Mg and Si release rates (average  $\pm$  s.d.) from  
657 diopside at pH 6 (a) and 8.5-9.0 (b) with and without *Pseudomonas reactans* and in the presence or  
658 not of nutrients. The error bars represent average of 2 to 4 duplicates, each comprising 3 to 4  
659 independent sampling at the steady state.

660

661 **Figure 3.** Synthesis of obtained Ca, Mg and Si release rates (average  $\pm$  s.d., mol cm<sup>-2</sup> s<sup>-1</sup>) from  
662 diopside at pH 6.0-6.5 with and without *Chaetomium brasiliense* fungus (4.5-5.6g<sub>wet</sub>/L) and in the  
663 presence or not of nutrients (1% Czapek medium). The error bars represent average of 2 reactors,  
664 each comprising 9 to 10 independent sampling as a function of time over 19 days of exposure.

665 **Figure 4. A-C:** In-situ AFM images of diopside surfaces immersed within sterile electrolyte media  
666 for up to 5 hours (**A:** 70 x 70  $\mu\text{m}^2$ , deflection mode; **B:** 20 x 20  $\mu\text{m}^2$ , deflection mode; **C:** 12 x 12  
667  $\mu\text{m}^2$ , height mode - with the color scale spanning a total of 238 nm). **D-G:** In-situ AFM images of  
668 diopside surfaces immersed in nutrient media (**D:** 8.6 x 8.6  $\mu\text{m}^2$ , deflection mode; **E:** 10 x 10  $\mu\text{m}^2$ ,  
669 deflection mode; **F:** 5.5 x 5.5  $\mu\text{m}^2$ , deflection mode; **G:** 1.5 x 1.5  $\mu\text{m}^2$ , deflection mode.

670

671 **Figure 5.** SEM images of reacted diopside surfaces in the sterile electrolyte (a), live fungus in 0.1  
672 M NaCl (b, d) and in 1% nutrient media (c, d).

673

674 **Table 1.** Results of Bacterial MFR experiment. *R* units are mol cm<sup>-2</sup> s<sup>-1</sup>.

675

676 **Table 2.** Results of fungus batch experiments (0.1 M NaCl). *R* units are mol cm<sup>-2</sup> s<sup>-1</sup>.