

Supplementary Information for

## **Biofilms as agents of Ediacara-style fossilization**

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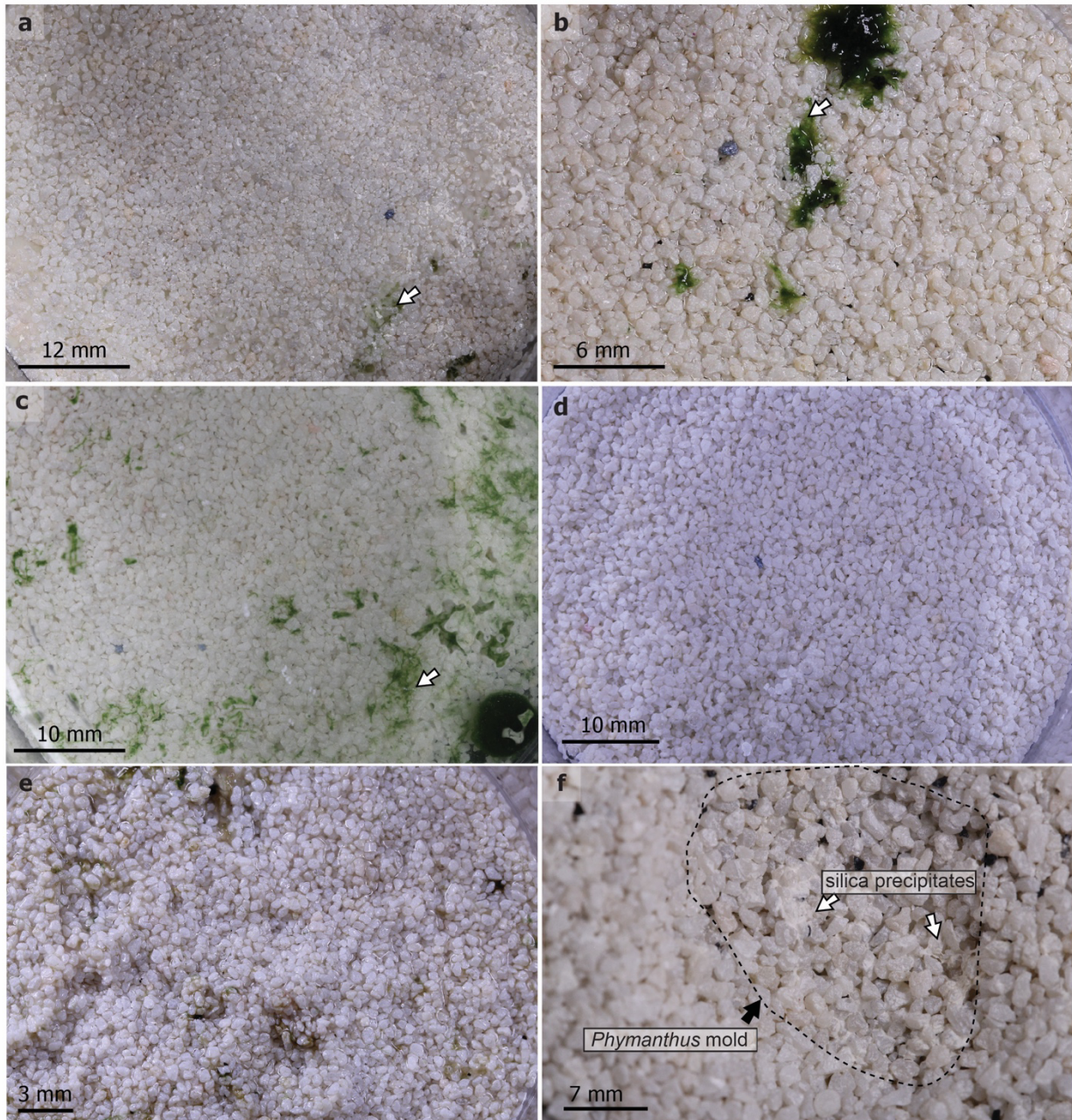
Table S2. Carbon content of experimental substrates through time.

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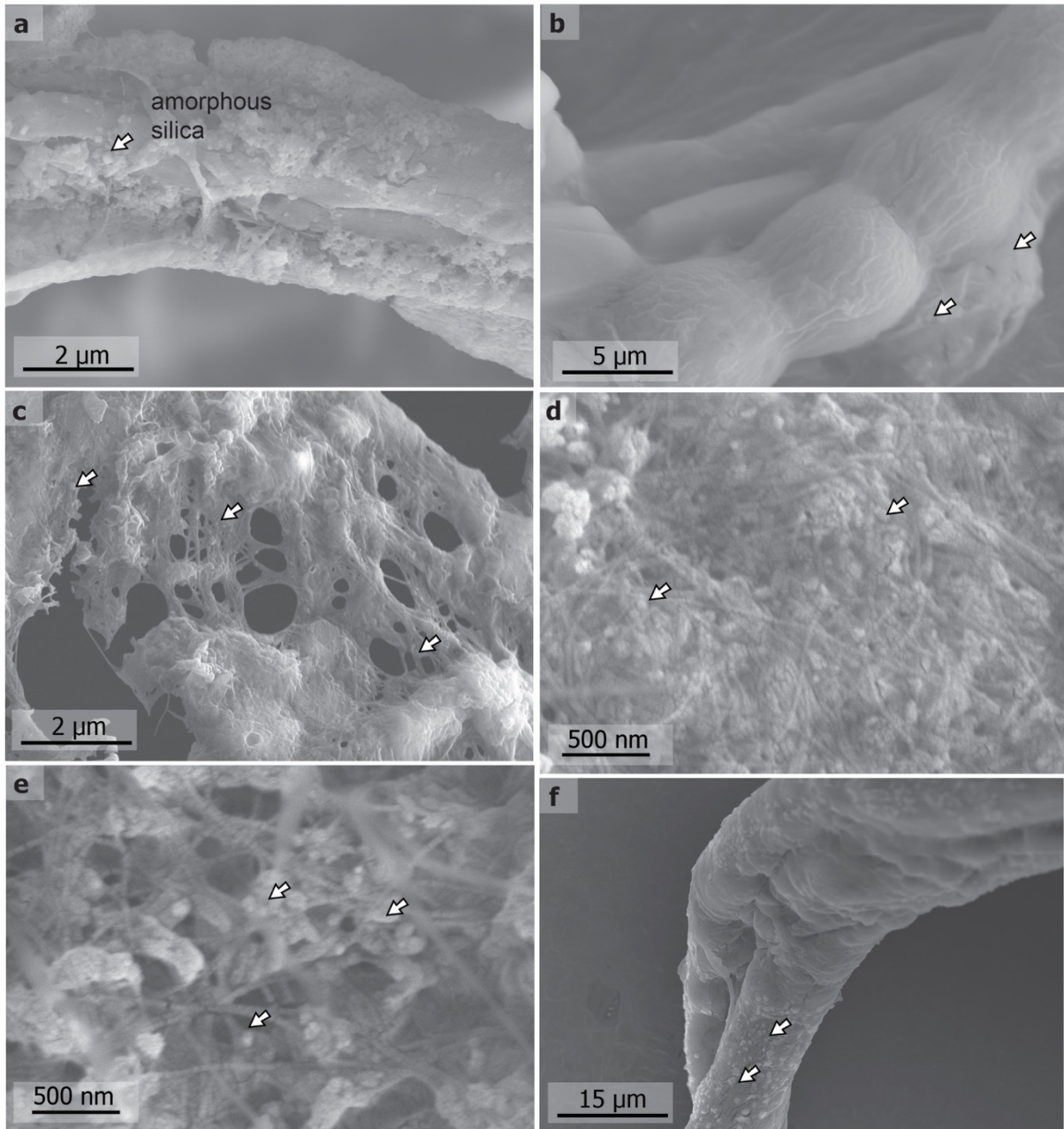
Table S5. Artificial seawater solution.

Table S6. Dissolved silica concentration in experiments with and without biofilms.

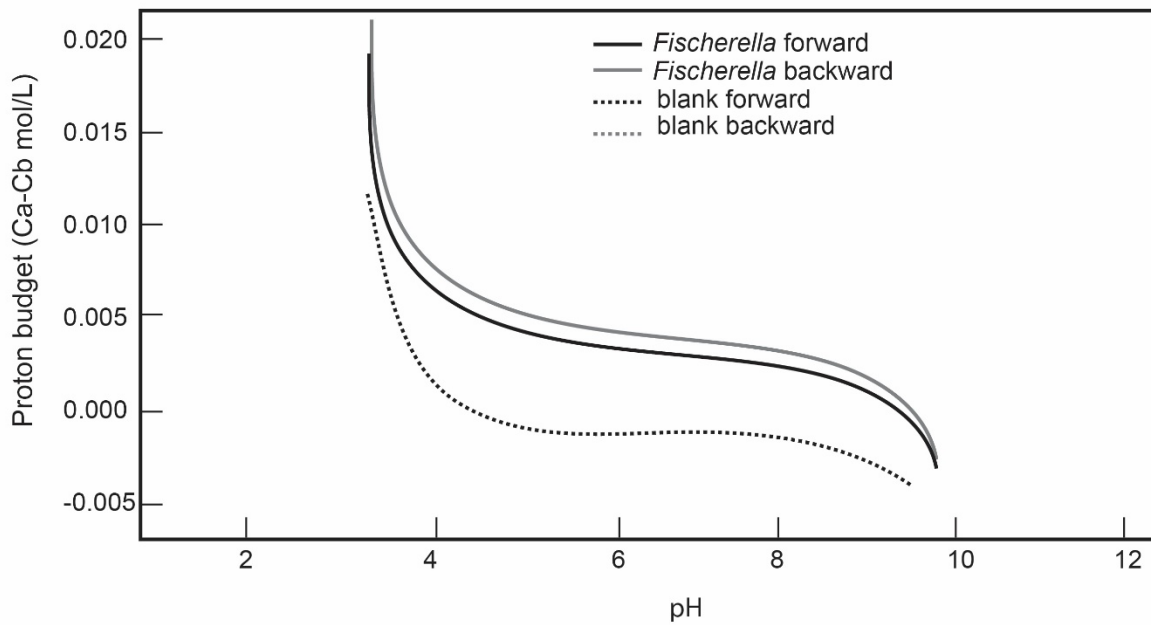


**Fig. S1. Representative experimental photographs.** a-d, Images of experiments after 48 hours and initial 2 mM DSi associated with *Fischerella* (experiment 4; a), *Spirogyra* (experiment 7; b), and *Anabaena* (experiment 5; c). Arrows denote examples of biofilm colonies; d-f, Images of experiments after removing *Phymanthus* (150 h), control experiment without DSi (experiment 2; d), initial 2 mM DSi (experiment 8; e) and initial 2 mM DSi and biofilms (experiment 11; f) where silica precipitates are visible in the ‘proto-fossil’ mold.

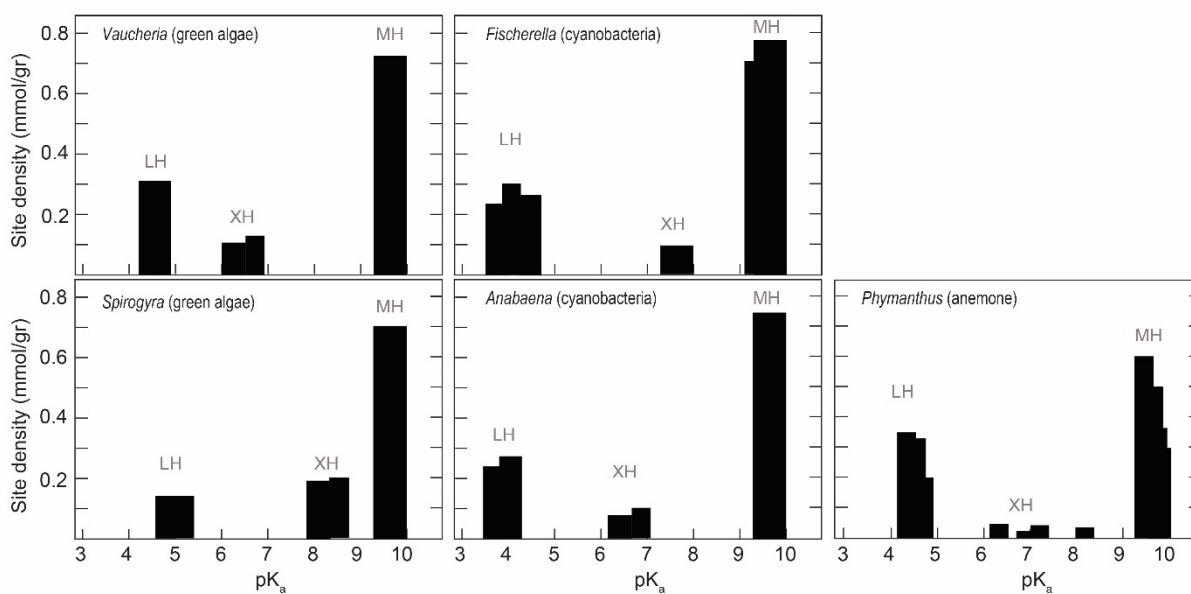




**Fig. S2. SEM-SE images of experiments associated with biofilms.** Images showing silica precipitation (examples indicated with arrows) associated with *Fischerella* (a; 150 h), *Anabaena* (b; 48 h), *Vaucheria* (c and d; 120 h), and *Spirogyra* (e and f; 96 h).



**Fig. S3. Acid-base titration curves.** Example of acid-base titration curves for *Fischerella*.



**Fig. S4. Modeled functional groups, identified as ligands  $\equiv\text{LH}$ ,  $\equiv\text{XH}$ , and  $\equiv\text{MH}$ , of each experimental organism, plotted as density of deprotonated sites (excess charge) *Phymanthus* data from ref. 1.**

**Table S1. Time series of dissolved silica concentrations (means of three replicates)**

		DSi (mM)						
	Experiment	8 h	26 h	48 h	72 h	120 h	138 h	150 h
3	DSi, Sa	2.000	1.999	1.999	1.959	1.987	2.016	1.999
4	DSi, <i>F</i> , Sa	1.550	0.630	0.589	0.525	0.515	0.450	0.439
5	DSi, <i>A</i> , Sa	1.690	0.433	0.433	0.433	0.528	0.510	0.447
6	DSi, <i>V</i> , Sa	1.849	0.442	0.531	0.348	0.584	0.784	0.436
7	DSi, <i>S</i> , Sa	1.529	1.485	1.375	1.350	1.200	0.629	0.684
8	DSi, <i>P</i>	1.014	0.880	0.665	0.699	0.631	0.753	0.677
9	DSi, <i>P</i> , Sa	1.318	0.870	0.876	0.685	0.773	0.793	0.788
10	DSi, Sa, B	1.234	0.702	0.746	0.782	0.699	0.552	0.518
11	DSi, <i>P</i> , B, Sa	1.230	0.784	0.584	0.426	0.342	0.325	0.206

DSi: dissolved silica; *P*: *Phymanthus*, B: biofilms, Sa: sand, *F*: *Fischerella*

*A*: *Anabaena*, *V*: *Vaucheria*, and *S*: *Spirogyra*. Experiments 1 and 2 are control experiments performed without DSi (see Main Text Table 1).

**Table S2. Final dissolved silica concentration (mM) in experiments conducted both with and without biofilms**

With biofilms (experiments 10 and 11)	Without biofilms (experiments 8 and 9)
0.875	1.014
0.784	0.880
0.584	0.665
0.426	0.589
0.342	0.631
0.325	0.753
0.204	0.677
0.023	1.014
0.784	0.980
0.584	0.665
0.444	0.589
0.342	0.631
0.329	1.753
0.106	0.477
0.230	0.114
0.641	1.180
0.311	1.565
0.426	0.789
0.942	0.611
0.625	0.753
0.206	0.671
0.875	1.014
0.812	0.967
0.581	0.665
0.426	0.699
0.356	0.231
0.355	0.743
0.121	0.617
0.023	1.001
0.784	1.000
0.554	0.632
0.426	0.765
0.686	0.636
0.325	1.753
1.106	0.456
0.366	0.114
0.640	1.180
0.384	1.678
0.426	0.656
0.342	0.351
0.325	0.749
0.200	0.687

**Table S3. Carbon content of experimental substrates through time**

Experiment	Time (h)	Weight (g)	% C
1	24	1.73	7.23
	48	1.08	2.43
	120	1.65	10.26
	144	0.45	17.54
2	72	0.31	33.79
	96	1.33	12.31
	120	0.76	9.41
	144	3.05	8.67
	150	3.8	5.4
4	24	4.8	5
	72	0.79	9.9
	96	2.9	6.5
	144	3.1	1.7
	150	3.3	0.6
5	24	2.8	5.2
	48	4.4	7.2
	120	1.2	3
	150	3.4	0.6
6	96	2.8	5
	150	5.5	2
7	48	2.19	12.1
	150	0.9	9
8	48	2.28	12.14
9	24	1.5	9.84
	150	2.7	1.8
10	48	1.2	7.23
	150	5	1.3
11	24	2.03	28.69
	72	2.29	12.75
	150	1	3.63



**Table S4. Silica sorbed normalized to biomass**

Experiment	Final DSi (mM)	Initial biomass (g)	Silica sorbed (mM)	Silica sorbed per gram of biomass
4	0.439	10	1.561	0.156
5	0.447	10	1.553	0.155
6	0.436	10	1.564	0.156
7	0.684	10	1.316	0.132
8	0.677	15.23	1.323	0.087
9	0.788	12.33	1.212	0.098
10	0.518	10	1.482	0.148
11	0.206	14.22	1.794	0.126

**Table S5. Acid-base titration and surface complexation model results**

Sample	Log(Ka)*			Site density (mol/L)**		
	<i>Ka1</i>	<i>Ka2</i>	<i>Ka3</i>	<i>LH</i>	<i>XH</i>	<i>MH</i>
<i>Vaucheria</i>	4.04 (carboxyl)	8.38 (carboxyl or phosphoryl)	10.83 (hydroxyl or amino)	3.04E-04	1.30E-04	8.02E-04
<i>Fischerella</i>	4.62 (carboxyl)	8.24 (carboxyl or phosphoryl)	10.8 (hydroxyl or amino)	2.52E-04	2.97E-04	9.80E-04
<i>Anabaena</i>	4.2 (carboxyl or phosphoryl)	7.75 (carboxyl or phosphoryl)	10.61 (hydroxyl or amino)	2.61E-04	1.22E-04	9.49E-04
<i>Spirogyra</i>	4.09 (carboxyl)	7.97 (carboxyl or phosphoryl)	10.36 (hydroxyl or amino)	2.22E-04	1.66E-04	7.03E-04
<i>Phymanthus</i> ***	5.31 (carboxyl or phosphoryl)	7.64 carboxyl or phosphoryl)	9.24 (hydroxyl or amino)	1.25E-04	4.86E-05	1.48E-04

\*Identification of functional groups is based on comparison with literature<sup>2</sup>.

\*\*Site density and pKa (i.e., Log(Ka)) values represent the mean values of all replicates.

\*\*\**Phymanthus* data from ref. 1

**Table S6. Artificial seawater solution**

Component	Concentration (g/kg)
NaCl	23.7034
NaSO <sub>4</sub>	4.008
KCl	0.865
NaHCO <sub>3</sub>	0.296
Na <sub>2</sub> SiO <sub>3</sub> ·9H <sub>2</sub> O	0.568
H <sub>2</sub> O	970.48

### Supplementary references

1. Slagter, S., Tarhan, L. G., Hao, W., Planavsky, N. J. & Konhauser, K. O. Experimental evidence supports early silica cementation of the Ediacara Biota. *Geology* **49**, 51–55 (2021).
2. Fein, J. B., Boily J.-F., Yee N., Gorman-Lewis, D. & Turner, B. F. Potentiometric titrations of *Bacillus subtilis* cells to low pH and a comparison of modeling approaches. *Geochim. Cosmochim. Acta* **69**, 1123-1132 (2005).