GSA Data Repository 2016094

Cellular dissolution at hypha- and spore-mineral interfaces revealing unrecognized mechanisms and scales of fungal weathering

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SI Methods

Detection of siderophore activity. The CAS-Fe-HDTMA dye solution was prepared as follows: for 100 mL of solution, 0.0027 g of ferric chloride ($FeCl_3 \cdot 6H_2O$) was dissolved in 10 mL of 10 mM HCl as solution 1; 0.0605 g of Chromeazurol S (CAS; Sigma-Aldrich 72687, Japan) was dissolved in 49 mL of ddH_2O as solution 2; 0.0729 g of hexadecyl-trimethyl-ammonium bromide (HDTMA; ≥ 99%, Sigma-Aldrich, USA) was dissolved in 40 mL of ddH_2O with constant stirring over low heat as solution 3. Solutions 1 and 2 were mixed to create the Fe-CAS solution, which was then mixed with solution 3 to obtain the CAS-Fe-HDTMA dye solution (blue in color). This solution was stored in the dark at room temperature until use. A calibration curve was constructed using siderophore standard solutions containing 0-100 μM DFAM ($r^2 > 0.992$). To detect the siderophore concentration in the samples, 99 μL of the filtrate was mixed in a 1:1 ratio with the liquid CAS-Fe-HDTMA dye solution, and then mixed with 2 μL of 0.2 M 5-sulfosalicylic acid (shuttle solution). After incubation in dark for 15 min, the mixtures were measured at a wavelength of 630 nm. In the presence of siderophores, the absorbance of the dye is quenched.

Absorbance in all the analyses was done by a SpectraMax® M2 Microplate Reader (Molecular Devices, USA).

Calculations for the mass of lizardite dissolved by the fungus

(i) Cellular removal of materials from the CMI

Material loss by the channels ($Mass_{channel}$) or pits ($Mass_{pit}$) can be estimated as:

$$Mass_{channel/pit} = \frac{SSA \times P \times H}{2} \times D$$

Where

SSA = specific surface area (m^2)

P = percentage of the surface channel or pit (%)

H = height of the channel or pit (10^{-9} m)

D = density of the mineral ($kg \cdot m^{-3}$)

(ii) Mineral selective dissolution below CMI

Mineral selective dissolution below channel ($Selective\ dissolution_{channel}$) or pit ($Selective\ dissolution_{pit}$) can be estimated as:

$$Selective\ dissolution_{channel/pit} = \frac{SSA \times P \times (H_x - H_y)}{2} \times D \times [1 - \frac{\frac{Counts_{S1}}{Counts_{Fresh}} + \frac{Counts_{S2}}{Counts_{Fresh}} + \frac{Counts_{S3}}{Counts_{Fresh}} + \ldots + \frac{Counts_{Sn}}{Counts_{Fresh}}}{n}]$$

Where

SSA = specific surface area (m^2)

P = percentage of the surface channel or pit (%)

H_x = depth of altered layers below the channel or pit (10^{-9} m)

H_y = height of the channel or pit (10^{-9} m)

D = density of the mineral ($kg \cdot m^{-3}$)

$Counts_{Si}$ = raw counts of Mg at the site of *i* (a.u.)

$Counts_{Fresh}$ = raw counts of Mg at the fresh site (a.u.)

Table DR1 Chemical (from XRF) compositions (%) of the lizardite sample

MgO	SiO_2	Fe_2O_3	CaO	Al_2O_3	K_2O	NiO	Cr_2O_3	MnO	TiO_2
37.35	40.70	4.61	1.16	1.51	0.19	0.17	0.21	0.07	0.03

Table DR2 Summary of the experimental settings.

Reactor	Experiment	Lizardite	*T. flavus*	Dialysis bag (0.22 um)	Solution
Erlenmeyer flask	Exp-mix	+	+	-	Cza+Glu
	Exp-bag	+	+	+	Cza+Glu
	Exp-Czapek	+	-	-	Cza+Glu
	Exp-DDW	+	-	-	DDW
	Exp-fungi	-	+	-	Cza+Glu
Microplate	Exp-mixP	+	+	-	Cza-M-F+Glu
	Exp-fungiP	-	+	-	Cza-M-F+Glu

Cza+Glu: Czapek mineral medium supplemented with 2% (w/v) glucose;
DDW: distilled deionized water;
Cza-M-F+Glu: Mg- and Fe-free Czapek mineral medium supplemented with 2% (w/v) glucose

Table DR3 Percentage of surface pitting in selected regions on fungal dissolved mineral flake.

Area	Channel Area	Pit Area	Regular Area	Total Area
A1	6,388[a] (6.00%)[b]	26,914 (25.28%)	73,160 (68.72%)	106,462 (100%)
A2	13,152 (12.87%)	59,129 (57.86%)	29,913 (29.27%)	102,194 (100%)
A3	5,472 (8.54%)	7,907 (12.34%)	50,695 (79.12%)	64,074 (100%)
Average	8,337 (9.17%)	31,317 (34.45%)	51,256 (56.38%)	90,910 (100%)

[a] Area in μm^2.
[b] Percent relative to the total area of the selected region.

Li et al., Figure DR1, TIFF

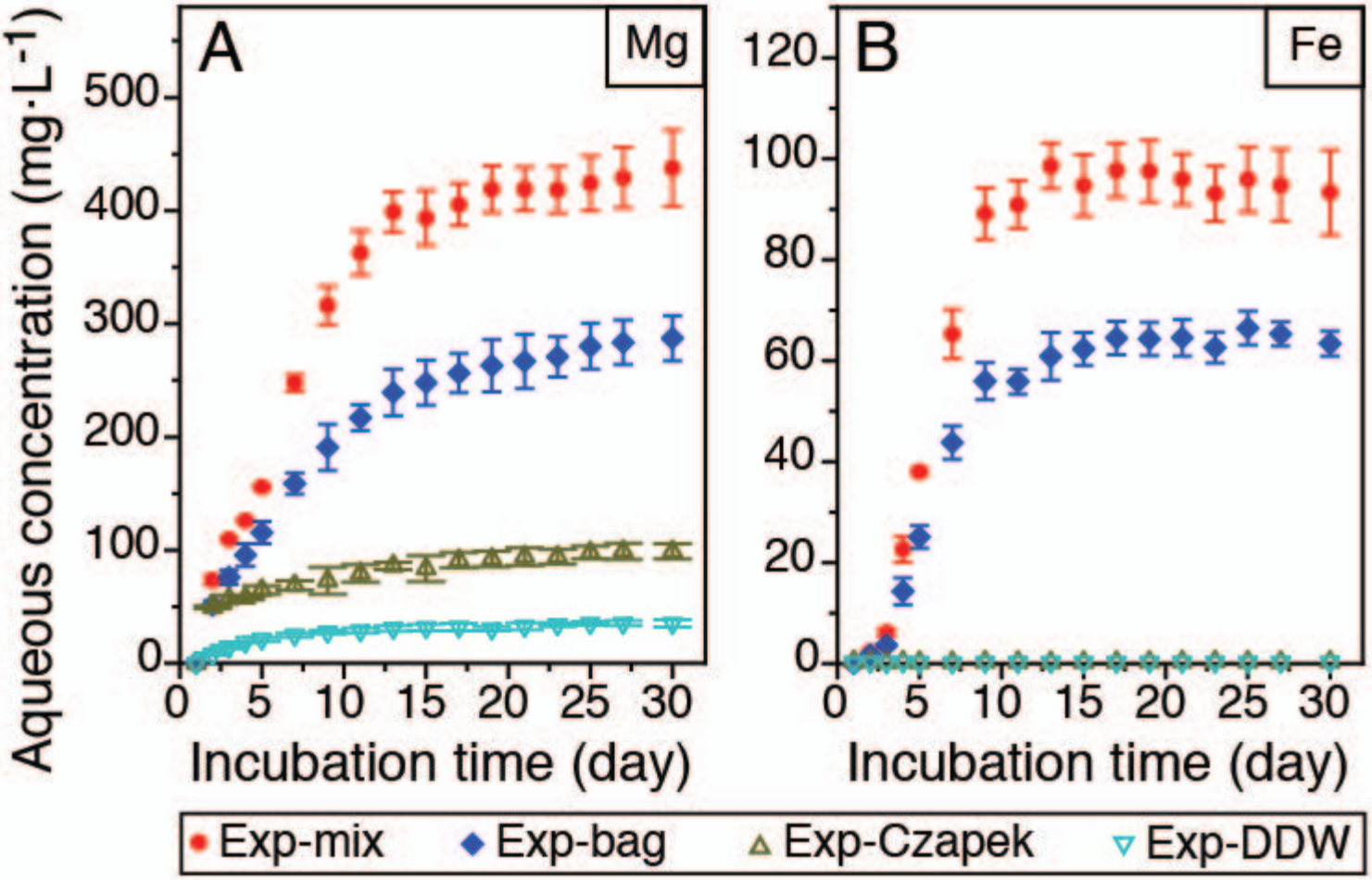

Fig. DR1 Accumulative release of Mg and Fe over 30-day incubation period during bulk dissolution. Error bars indicate the standard deviations (SD) of three replicas for individual measurements.