1   # Supporting Information

2   Unravelling metal speciation in the microenvironment surrounding

3   phytoplankton cells to improve predictions of metal bioavailability

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Table S1. A summary of the *in situ* measured pH and O$_2$ concentrations in the phycosphere of marine and freshwater phytoplankton cells.

Species (Reference)	Habitat	Cell sample	Light (μmol photons m^{-2} s^{-1})	pH			O$_2$ (mM)		
				Phycosphere	Bulk	difference	Phycosphere	Bulk	difference
Halimeda discoidea[1]	marine	segment	0	8.00	8.10	-0.10	0.15	0.21	-0.06
			12	8.10	8.10	0.00	0.21	0.21	0.00
			36	8.50	8.10	0.40	0.32	0.21	0.11
			93	9.00	8.10	0.90	0.50	0.21	0.29
			200	8.80	8.10	0.70	0.58	0.21	0.37
Odontella sinensis[2]	marine	individual	0	7.99	8.00	-0.01	0.24	0.24	0.00
			200	8.35	8.00	0.35	0.32	0.24	0.08
Coscinodiscus sp.[2]	marine	individual	200	8.35	8.00	0.35	--	--	--
Odontella mobiliensis[2]	marine	individual	200	8.10	8.00	0.10	--	--	--
Thalassiosira weissflogii[2]	marine	individual	200	8.05	8.00	0.05	--	--	--
Thalassiosira weissflogii[3]	marine	individual	160	8.75	8.55	0.20	--	--	--
			160	9.05	8.55	0.50	--	--	--
			160	7.93	7.88	0.05	--	--	--
			160	7.90	7.88	0.02	--	--	--
Coscinodiscus wailesii[4]	marine	individual	200	8.40	8.10	0.30	--	--	--
Coscinodiscus wailesii[5]	marine	individual	0	7.70	7.70	0.00	0.19	0.22	-0.03

Species (Reference)	Habitat	Cell sample	Light (μmol photons m^{-2} s^{-1})	pH			O$_2$ (mM)		
				Phycosphere	Bulk	difference	Phycosphere	Bulk	difference
Coscinodiscus wailesii[5]	marine	individual	170	8.60	7.70	0.90	0.38	0.22	0.16
Trichodesmium[6]	marine	colony	1000	8.40	8.10	0.30	0.32	0.21	0.11
			0	7.80	8.10	-0.30	0.18	0.21	-0.03
			1000	8.00	7.80	0.20	0.25	0.21	0.04
			0	7.60	7.80	-0.20	0.16	0.21	-0.05
Coscinodiscus granii[7]	marine	individual		8.32	8.03	0.29	--	--	--
				8.35	8.14	0.21	--	--	--
				8.48	8.22	0.26	--	--	--
Phaeocystis[8]	marine	natural colony	150	8.90	8.50	0.40	0.54	0.30	0.24
			0	8.46	8.50	-0.04	0.28	0.30	-0.02
			0	8.16	8.19	-0.03	0.28	0.30	-0.02
			20	8.23	8.19	0.04	0.32	0.30	0.02
			25	8.28	8.19	0.09	0.33	0.30	0.03
			50	8.35	8.19	0.16	0.36	0.30	0.06
			80	8.45	8.19	0.26	0.40	0.30	0.10
			130	8.51	8.19	0.32	0.45	0.30	0.15
Ankistrodesmus sp.[9]	freshwater	aggregate	85	>8.00	6.80	>1.20	--	--	--
Microcystis sp.[9]	freshwater	aggregate	85	>8.00	6.80	>1.20	--	--	--
Chlorella sp.[9]	freshwater	aggregate	85	>8.00	6.80	>1.20	--	--	--
Nitzschia sp.[9]	freshwater	aggregate	85	>8.00	6.80	>1.20	--	--	--
Anabaena sp.[9]	freshwater	individual	85	>8.00	6.80	>1.20	--	--	--
Scenedesmus sp.[9]	freshwater	individual	85	>8.00	6.80	>1.20	--	--	--

Species (Reference)	Habitat	Cell sample	Light (μmol photons m^{-2} s^{-1})	pH			O$_2$ (mM)		
				Phycosphere	Bulk	difference	Phycosphere	Bulk	difference
Unicellular green[9]	freshwater	individual	85	>8.00	6.80	>1.20	--	--	--
Vaucheria sp.[9]	freshwater	individual	85	>8.00	6.80	>1.20	--	--	--
Chlorella sp.[10]	freshwater	cell layer natural	108	10.30	7.50	2.80	0.36	0.14	0.22
Microcystis sp.[10]	freshwater	aggregate natural	sunlight	10.40	9.00	1.40	1.10	0.37	0.73
Microcystis sp.[10]	freshwater	aggregate	sunlight	9.25	9.00	0.25	0.40	0.20	0.20

57

58 Note, the Δ pH values in the Figure 1 were calculated as the differences between the bulk waters and the phycosphere, and we
59 didn't convert the numbers into Δ [H$^+$].

60 **Table S2. Basic chemical composition of freshwater and seawater used for the**

61 **calculation of metal speciation.**

Component	Freshwater (mol L^{-1})	Seawater (mol L^{-1})
Na	5.0×10^{-4}	4.8×10^{-1}
Mg	5.0×10^{-4}	5.5×10^{-2}
K	5.0×10^{-5}	1.0×10^{-2}
Ca	5.0×10^{-4}	1.0×10^{-2}
Cl	1.0×10^{-3}	5.6×10^{-1}
SO_4	5.0×10^{-4}	2.9×10^{-2}
CO_3	open to air	2.4×10^{-3}
NO_3	5.0×10^{-4}	--
Br	--	8.4×10^{-4}
BO_3	--	4.9×10^{-5}
F	--	7.2×10^{-5}
Sr	--	6.4×10^{-5}
Al	1.0×10^{-5}	1.0×10^{-7}
Cd	1.0×10^{-9}	1.0×10^{-10}
Co(II)	1.0×10^{-9}	1.0×10^{-9}
Cu(II)	2.0×10^{-8}	5.0×10^{-8}
Cu(I)	--	2.5×10^{-9}
Fe(III)	1.0×10^{-6}	1.0×10^{-8}
Fe(II)	--	2.4×10^{-9}
Sm*	5.0×10^{-11}	1.0×10^{-11}
Hg	--	1.0×10^{-12}
Mn(II)	--	1.0×10^{-7}
Ni	5.0×10^{-9}	1.0×10^{-8}
Pb	1.0×10^{-8}	1.0×10^{-10}
Zn	1.0×10^{-7}	1.0×10^{-8}
DOC (mg/L)	0.3-32	0.14-16

62 The concentrations of major cations/anions, trace metals, and DOC were taken from

63 several publications.[11-14] *The dissolved Sm concentrations refer to two studies.[15, 16]

Table S3. Equilibrium modelled relative distribution (%) of free ion, total inorganic complexes (-Inorg.) and total organic complexes (-Org.) of metals in the phycosphere of marine phytoplankton cells.

Metal species %	Bulk seawater pH 8.0	Phycosphere pH 8.3 (light)	pH 7.9 (dark)	Bulk seawater pH 8.0	Phycosphere pH 8.3 (light)	pH 7.9 (dark)
	in the presence of 0.14 mg L^{-1} DOC			in the presence of 16 mg L^{-1} DOC		
Al^{3+}	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Al-Inorg.	99.99	99.99	99.99	84.68	96.60	77.43
Al-Org.	<0.01	<0.01	<0.01	15.32	3.40	22.57
Cd^{2+}	3.49	3.48	3.49	2.96	2.66	3.05
Cd-Inorg.	96.50	96.50	96.50	81.90	73.58	84.34
Cd-Org.	<0.01	<0.01	<0.01	15.12	23.76	12.61
Co^{2+}	74.51	72.45	74.93	59.05	50.37	61.66
Co-Inorg.	25.48	27.54	25.06	20.20	19.15	20.63
Co-Org.	<0.01	<0.01	<0.01	20.75	30.48	17.71
Cu(I)$^{+}$	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Cu(I)-Inorg.	99.99	99.99	99.99	99.99	99.99	99.99
Cu(I)-Org.	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Cu^{2+}	4.45	1.93	5.66	<0.01	<0.01	<0.01
Cu-Inorg.	54.30	48.91	55.26	<0.01	<0.01	<0.01
Cu-Org.	41.25	49.14	39.07	99.99	99.99	99.99
Fe(II)$^{2+}$	73.80	72.89	73.98	73.57	72.67	73.75
Fe(II)-Inorg.	26.20	27.10	26.02	26.12	27.03	25.94
Fe(II)-Org.	<0.01	<0.01	<0.01	0.31	0.30	0.31
Fe^{3+}	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Fe-Inorg.	24.85	38.43	22.19	<0.01	<0.01	<0.01
Fe-Org.	75.15	61.56	77.81	99.99	99.99	99.99
Hg^{2+}	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Hg-Inorg.	96.49	94.79	96.87	<0.01	<0.01	<0.01
Hg-Org.	3.51	5.21	3.13	99.99	99.99	99.99
Mn^{2+}	67.44	63.97	68.19	67.36	63.90	68.11
Mn-Inorg.	32.56	36.03	31.81	32.53	36.00	31.78
Mn-Org.	<0.01	<0.01	<0.01	0.11	0.10	0.11
Ni^{2+}	72.26	69.16	72.92	67.97	65.29	68.54
Ni-Inorg.	27.69	30.79	27.03	26.05	29.07	25.41
Ni-Org.	0.05	0.04	0.05	5.97	5.63	6.04

64

| Metal species %	Bulk seawater pH 8.0	Phycosphere pH 8.3 (light)	pH 7.9 (dark)	Bulk seawater pH 8.0	Phycosphere pH 8.3 (light)	pH 7.9 (dark)
	in the presence of 0.14 mg L^{-1} DOC			in the presence of 16 mg L^{-1} DOC		
Pb^{2+}	5.29	3.73	5.74	0.12	0.07	0.14
Pb-Inorg.	91.92	92.87	91.71	2.03	1.65	2.29
Pb-Org.	2.78	3.39	2.54	97.84	98.27	97.55
Sm^{3+}	1.41	0.69	1.73	<0.01	<0.01	<0.01
Sm-Inorg.	42.18	54.97	38.85	<0.01	<0.01	<0.01
Sm-Org.	56.41	44.34	59.42	99.99	99.99	99.99
Zn^{2+}	49.35	44.04	50.31	8.19	4.97	9.78
Zn-Inorg.	50.59	55.85	49.63	8.39	6.31	9.64
Zn-Org.	0.07	0.08	0.06	83.41	88.72	80.57

65 Note, data are also shown for bulk seawater at pH 8.0. Here, the average phycosphere

66 pH change (i.e., a 0.3-unit increase in the light, and a 0.1-unit decrease in the dark) was

67 used to calculate the speciation change in the presence of low and high concentrations

68 of dissolved organic carbon (DOC).

69 **Table S4. Equilibrium modelled relative distribution (%) of free ion, total inorganic**

70 **complexes (-Inorg.) and total organic complexes (-Org.) of metals in the phycosphere**

71 **of freshwater phytoplankton cells.**

| Metal species %	Bulk freshwater pH 7.0	Phycosphere pH 8.3 (light)	pH 6.5 (dark)	Bulk freshwater pH 7.0	Phycosphere pH 8.3 (light)	pH 6.5 (dark)
	in the presence of 0.3 mg L^{-1} DOC			in the presence of 32 mg L^{-1} DOC		
Al^{3+}	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Al-Inorg.	99.27	99.99	97.45	10.39	91.47	1.87
Al-Org.	0.72	<0.01	2.54	89.61	8.53	98.13
Cd^{2+}	87.66	86.45	87.76	42.60	5.73	47.53
Cd-Inorg.	11.98	12.85	11.96	5.85	0.85	6.50
Cd-Org.	0.35	0.70	0.28	51.55	93.41	45.96
Co^{2+}	94.55	91.47	94.70	58.46	29.66	61.27
Co-Inorg.	5.18	8.13	5.08	3.24	2.66	3.32
Co-Org.	0.26	0.40	0.21	38.31	67.68	35.41
Cu^{2+}	59.07	3.08	80.46	0.08	<0.01	0.28
Cu-Inorg.	19.35	20.52	11.70	0.02	<0.01	0.04
Cu-Org.	21.58	76.39	7.84	99.89	99.99	99.68
Fe^{3+}	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Fe-Inorg.	66.36	68.81	74.72	<0.01	<0.01	<0.01
Fe-Org.	33.63	31.19	25.28	99.99	99.99	99.99
Ni^{2+}	93.76	91.33	94.05	23.52	17.85	25.89
Ni-Inorg.	5.11	6.94	5.06	1.29	1.36	1.39
Ni-Org.	1.13	1.73	0.90	75.18	80.78	72.70
Pb^{2+}	63.07	10.38	76.74	0.59	0.01	1.69
Pb-Inorg.	23.29	44.89	17.44	0.22	0.05	0.38
Pb-Org.	13.64	44.72	5.81	99.19	99.94	97.92
Sm^{3+}	2.35	0.09	10.81	<0.01	<0.01	<0.01
Sm-Inorg.	2.46	0.26	10.60	<0.01	<0.01	<0.01
Sm-Org.	95.19	99.65	78.58	99.99	99.99	99.99
Zn^{2+}	92.86	63.04	93.56	30.95	2.96	36.16
Zn-Inorg.	6.49	36.04	5.92	2.17	1.69	2.30
Zn-Org.	0.66	0.91	0.51	66.86	95.34	61.53

72 Note, data are also shown for bulk freshwater at pH 7.0. Here, the average phycosphere

73 pH change (i.e., a 1.3-unit increase in the light, and an assumed 0.5-unit decrease in the

74   dark) was used to calculate the speciation change in the presence of low and high

75   concentrations of dissolved organic carbon (DOC).
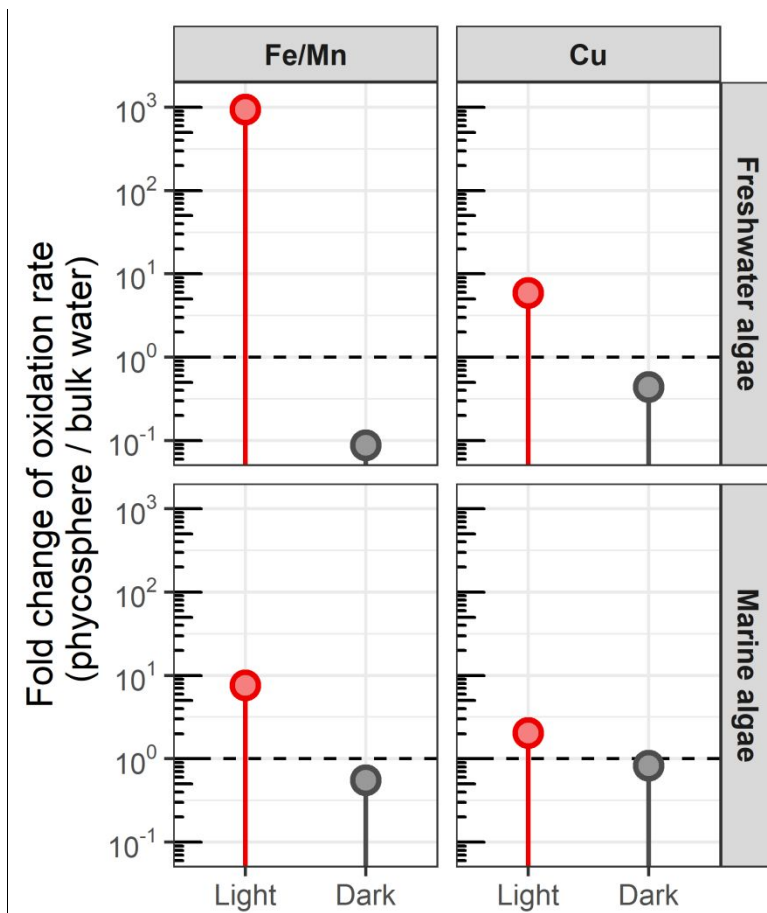
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78 **Figure S1. Fold changes in the calculated oxidation rate of Fe(II), Mn(II) and Cu(I) in the**

79 **phycosphere of freshwater or marine algae in the light (red) or in the dark (grey), in**

80 **comparison to the calculated rates in bulk solutions.** The data are shown on a log scale,

81 and the dashed line indicates no difference between the phycosphere and bulk water.

82 The details of the calculation are given in the Note S2.
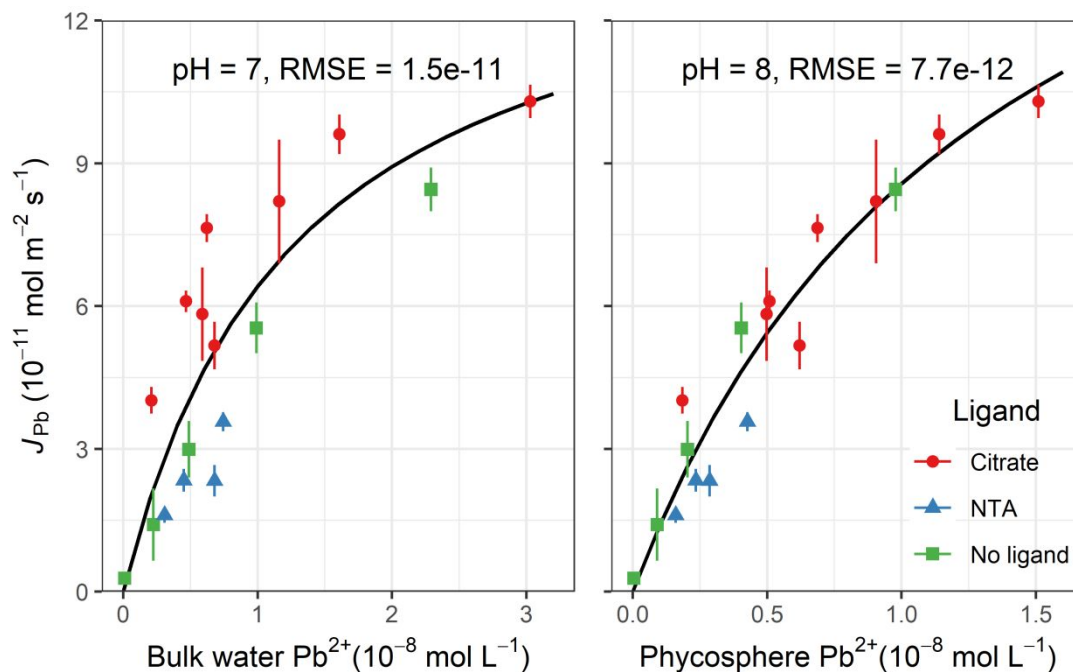
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Figure S2. The relationships between the calculated free Pb^{2+} concentration in the bulk water (left panel) or in the phycosphere (right panel) and the measured Pb uptake rate (J_{Pb}) by the freshwater alga *Chlamydomonas reinhardtii* CPCC11 under constant light (100 μmol photons m^{-2} s^{-1}). The data are replotted.[17]

89
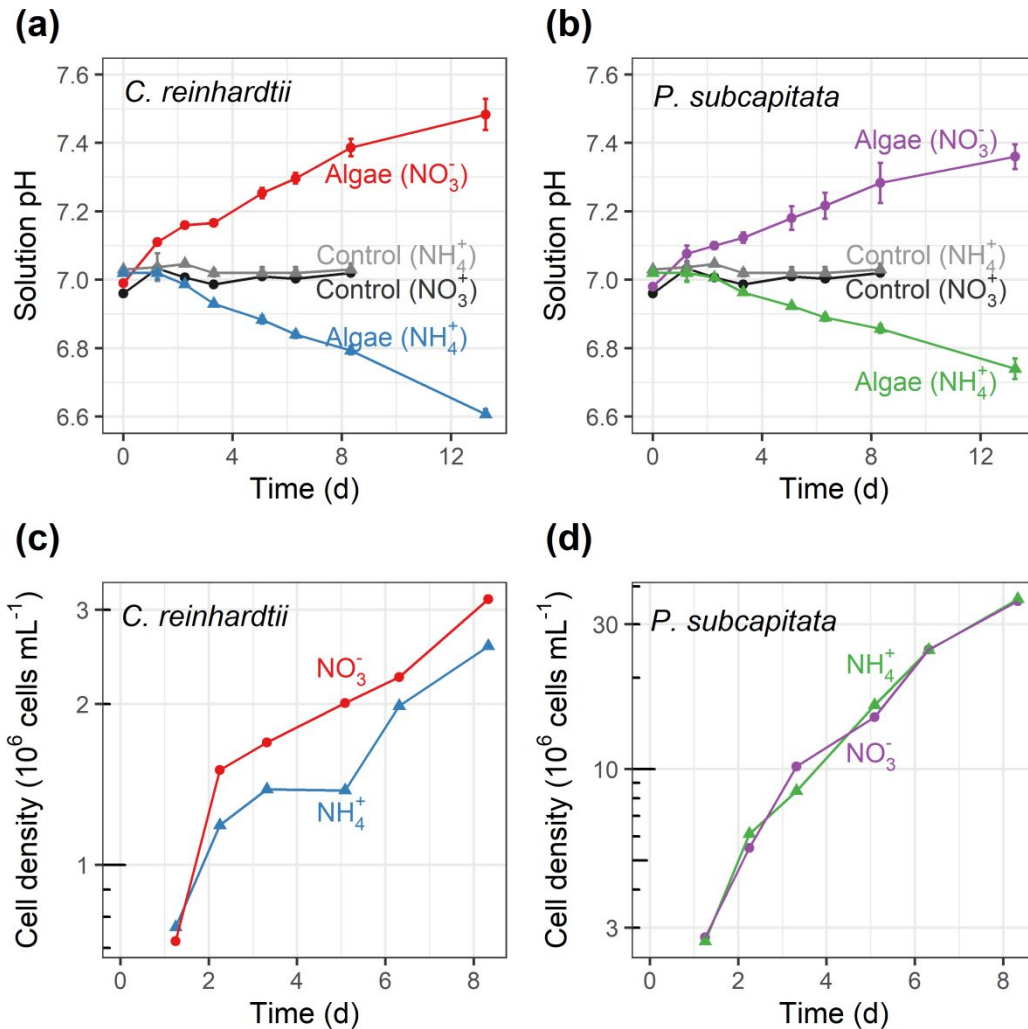
Figure S3. Bulk solution pH increase/decrease in the exposure solution, by nitrate NO_3^- or ammonium NH_4^+-fed algae *Chlamydomonas reinhardtii* CC1690 (a) and *Pseudokirchneriella subcapitata* CPCC37 (b). The growth of the two algae was simultaneously monitored (c & d). The dots are the means of three replicates, and the error bars representing standard deviation are very small. The growth medium MHSM-1 was buffered with 10 mM MOPS with either NO_3^- or NH_4^+ as the only N source; more details can be found in our previous study.[18] The present data, except for the result of the NO_3^--fed algae *C. reinhardtii* CC1690 in panel (a)[18], are not shown in our early papers.
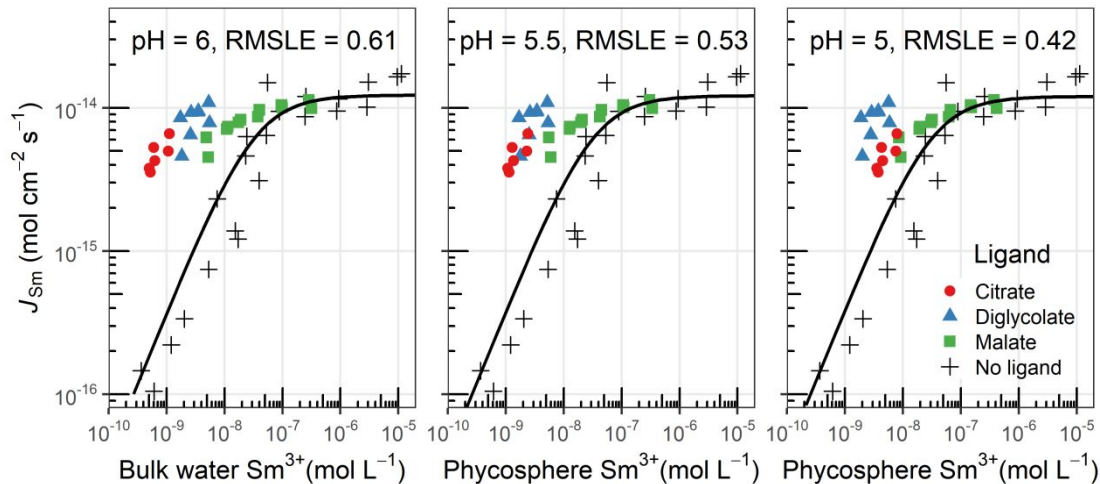
101
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103 **Figure S4. The relationships between the measured Sm uptake rate (J_{Sm}) by the**

104 **freshwater alga *C. reinhardtii* CPCC11 and the calculated free Sm^{3+} concentration in**

105 **the bulk water and in the phycosphere.** The bulk medium pH[19] was 6, and the

106 phycosphere Sm^{3+} concentration was calculated by assuming a phycosphere pH 5 or 5.5.

107 Here, the phycosphere pH was assumed to be lower than the bulk water, because the

108 uptake test was carried out under a low light intensity (< 20 µmol photons $m^{-2} s^{-1}$) and

109 the algae were cultured with NH_4^+ as the nitrogen source. The low light and the supply

110 of NH_4^+ would likely result in an overall lower phycosphere pH than that in the bulk

111 water (see Figure S5 for an example).

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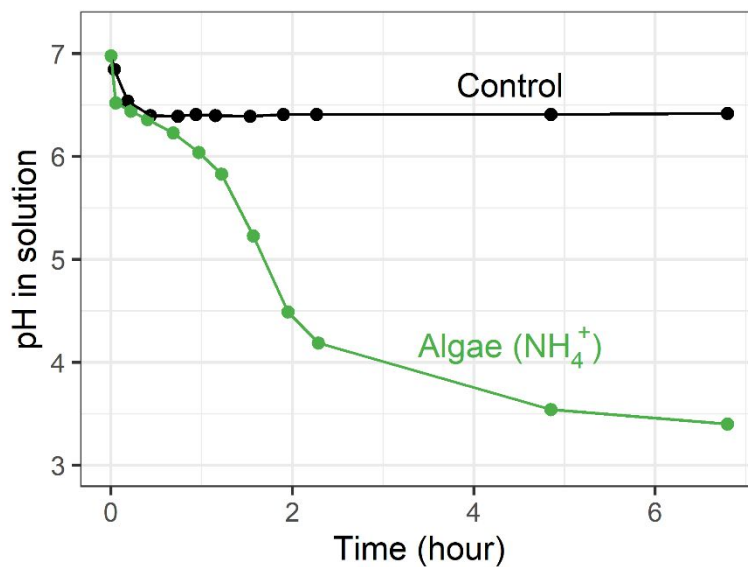
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115 **Figure S5. A significant pH decrease of the exposure solution by ammonium NH$_4^+$-fed**

116 **algae** *Chlamydomonas reinhardtii* CPCC11 at 2×10^7 cells mL^{-1}. The experimental details

117 can be found in our previous studies.[17, 18] The present data are not shown in those

118 papers.

119

Note S1. Potential influences of several physical/chemical/biological factors, other than pH and O_2, on metal behaviour in the phycosphere.

In addition to pH changes, redox dynamics in the phycosphere might differ from those in the bulk water. For example, phytoplankton release various reactive oxygen species (ROS) such as H_2O_2 and superoxide into the extracellular environment[20, 21]. Such solutes have slower diffusive rates (0.6-1.4 × 10^{-9} m^2 s^{-1} for H_2O_2)[22] than OH$^-$/H$^+$ (5.3-9.3 × 10^{-9} m^2 s^{-1}), and they could hence be locally enriched. The ROS-enriched micro-environment would facilitate redox transformation of redox-sensitive metals (e.g., Fe, Cu, and Mn) and ligands (e.g., thiol-containing ligands) before metals are taken up.[23, 24] At present, the level and dynamic of ROS in the phycosphere remain unknown, let alone their potential influence on the local metal speciation.

Nutritional status of algae might alter chemical conditions in the phycosphere as well. For example, Milligan et al.[3] observed that both Fe and carbon availability influenced the phycosphere pH of the marine diatom *Thalassiosira weissflogii*; the enhancement in phycosphere pH of Fe-replete cells was 0.3-unit higher than for Fe-limited cells. Moreover, the phycosphere pH change in high pCO$_2$ treatments was not as significant as in low pCO$_2$ treatments.[3]

Nitrogen sources for algae might be another factor in influencing both phycosphere pH and ROS levels. Lavoie et al. [25] modelled the effect of different N sources on the phycosphere pH of freshwater phytoplankton, and their results suggested that NO_3^--grown algae would have a higher phycosphere pH than NH_4^+-grown cells. On the other hand, studies with marine algae found that extracellular production of ROS was associated with nitrogen nutrition; nitrate could stimulate more extracellular ROS than ammonium in some algae species.[26]

Extracellular polymeric substances (EPS) secreted by certain phytoplankton species such

148 as *Phaeocystis*[27] might alter the chemical behaviour of metals in the phycosphere. For

149 example, the residence time of metals would be longer in the phycosphere of algae

150 having an abundance of EPS than for those without EPS, since diffusion coefficients (D)

151 of metal ions and complexes are lower in solutions of higher viscosity, η, such as in the

152 phycosphere of algae that produce EPS (i.e., due to the inverse relationship between D

153 and η in the Stokes-Einstein equation).

154

155 The local chemistry in the phycosphere might also be influenced by the microbial

156 community living in this micro-environment. In nature, a diversity of smaller

157 microorganisms such as bacteria and viruses frequently cluster near, or attach to,

158 phytoplankton.[28, 29] Thus, the metabolism of these microbes might alter phycosphere pH

159 and redox potential (e.g., CO_2 release and O_2 consumption during respiration), and

160 extracellular release of ROS from these bacteria[21] might influence chemical reactions

161 between metals and ligands. However, the potential influence of the microbes on local

162 metal speciation has been little examined.

163

164 Other than these biological factors, water turbulence can increase interfacial mixing of

165 nutrients and metabolites to and from an organism (> 100 μm) between bulk waters and

166 the biota. However, the effect from turbulence on the phycosphere conditions for

167 micro-organisms of < 100 μm in diameter would be weak, because modelling work has

168 suggested that even high levels of turbulence (e.g., at a dissipation rate 10^{-6} W kg^{-1})

169 produce a tiny increase (around 2%) of the flux in the tiny interfacial layer.[30]

170

171 Finally, total metal concentrations as well as competing metal interactions might not be

172 the same as those in the bulk water. For example, a higher pH and [O_2] in the

173 phycosphere could result in local precipitation of Fe and Mn[9, 31] and hence alter source

174 and bioavailability of the metals to algal cells. With respect to metal-metal interactions,

175 even if there was a simultaneous enhancement of free metal ions (e.g., Zn^{2+} and Cd^{2+}) in

176 the phycosphere, this would not necessarily result in an increase in uptake of both

177   metals since they might compete with each other for the same membrane transporter.[32]

178

Note S2. Calculation of the differences in oxidation rates of Fe(II), Cu(I) and Mn(II) between the phycosphere and bulk water.

Fe oxidation kinetics in both freshwater and marine waters follow the rate law:[33, 34]

$$\text{Fe(II) oxidation rate} = \frac{-d[\text{Fe(II)}]}{dt} = k_{Fe}[\text{Fe(II)}][O_2][OH^-]^2$$

Thus, the fold change in the Fe(II) oxidation rate can be calculated by the following equation, and it can be calculated based upon the numbers in Table S5:

$$\frac{\text{Fe(II) oxidation rate}_{\text{phycosphere}}}{\text{Fe(II) oxidation rate}_{\text{bulk water}}} = \frac{[O_2][OH^-]^2_{\text{phycosphere}}}{[O_2][OH^-]^2_{\text{bulk water}}}$$

Similarly, Mn(II) oxidation kinetics in seawater follow the rate law shown below[35] and it can be calculated based upon the numbers in the Table S5:

$$\text{Mn(II) oxidation rate} = \frac{-d[\text{Mn(II)}]}{dt} = k_{Mn}[\text{Mn(II)}][O_2][OH^-]^2$$

$$\frac{\text{Mn(II) oxidation rate}_{\text{phycosphere}}}{\text{Mn(II) oxidation rate}_{\text{bulk water}}} = \frac{[O_2][OH^-]^2_{\text{phycosphere}}}{[O_2][OH^-]^2_{\text{bulk water}}}$$

Thus, the fold change in the Mn(II) oxidation rate between phycosphere and bulk water is the same as that of Fe(II).

Cu oxidation kinetics in seawater follow the rate law shown below[36] and it can be calculated based upon the numbers in the Table S5:

$$\text{Cu(I) oxidation rate} = \frac{-d[\text{Cu(I)}]}{dt} = k^*_{Cu}[\text{Cu(I)}][O_2]$$

The k^*_{Cu} (apparent oxidation rate constant, M^{-1} s^{-1}) was calculated based on the measured Cu(I) oxidation kinetics at different pH levels.[37] The k^*_{Cu} values varied with solution pH. Specifically, the k^*_{Cu} values (M^{-1} s^{-1}) used for the seawater calculation are

204 2.45 at pH 7.9, 2.58 at pH 8.0, and 3.02 at pH 8.32, while in freshwater they are 5.0 at

205 pH 6.5, 10.0 at pH 7.0, and 22.9 at pH 8.28.

206

207 **Table S5. Values used for the calculation of relative changes in metal oxidation rates in**
208 **the phycosphere, in comparison to bulk waters.**

		bulk water	phycosphere light	dark
freshwater	pH	7.00	8.28	6.50
	O_2 (mM)	0.24	0.62	0.21
seawater	pH	8.00	8.32	7.90
	O_2 (mM)	0.23	0.40	0.20

209 **Note S3. Estimation of the amount of Cu(I) oxidation and Cu complexation in the**

210 **phycosphere.**

211 When the residence time of Cu(I) in the phycosphere is sufficiently long, all Cu(I) can be

212 oxidized by oxidants such as O_2 before reacting with membrane-bound Cu reductases.

213 However, since the phycosphere of micro-algae is a thin layer of micro-meter

214 dimensions, the local residence time might be very short and hence little Cu(I) would be

215 oxidized by O_2 within the phycosphere. Here, we calculated the residence time of Cu(I)

216 and the percentage of total Cu(I) being oxidized by O_2 in the phycosphere of two marine

217 diatoms *Thalassiosira pseudonana* and *Thalassiosira oceanica*.

218   To calculate the residence time of Cu(I) in the phycosphere, we follow:

219
$$phycosphere\ residence\ time = \frac{[Cu(I)\ concentration] \times [phycosphere\ volumn]}{[Cu(I)\ uptake\ rate] \times [cell\ surface\ area]}$$

220

221 For *T. pseudonana*, cell radius = 2.3 μm,[38] theoretical phycosphere thickness = cell radius

222 [39] = 2.3 μm; for *T. oceanica,* cell radius = 3.3 μm,[38] theoretical phycosphere thickness =

223 3.3 μm. The ratio between [Cu(I) concentration] in bulk seawater and [Cu(I) uptake rate]

224 were taken from a short-term algal Cu uptake study,[38] assuming that

225
$$\frac{[Cu(I)\ concentration]}{[Cu(I)\ uptake\ rate]} = \frac{[total\ Cu\ concentration]}{[total\ Cu\ uptake\ rate]}$$

226 since data for algal Cu(I) uptake are rare in the literature. The calculated residence time

227 of Cu ranged from 230 s to 3000 s in the phycosphere of the two species.

228

229   To calculate the percentage of total Cu(I) being oxidized by O_2 in the phycosphere,

230 we considered:

231
$$Oxidized\ Cu(I)_t\ \% = 100\% - e^{-\frac{K_{Cu}}{t}} \times 100\%$$

232 t = phycosphere residence time, and K_{Cu} = $k^*_{Cu} \times$ [phycosphere O_2]. Specifically,

233 k^*_{Cu} = 3 $M^{-1} s^{-1}$ (at pH 8.3)[37] and [phycosphere O_2] = 0.4 mM (Table S5). The

234 percentage of total Cu(I) being oxidized by O_2 in the phycosphere of two marine diatoms

235   ranged from 24% to 98% (Table S6).

236

237   **Table S6. Calculation of Cu residence time and percentage of total Cu(I) being**

238   **oxidized by O_2 in the phycosphere.**

Seawater Cu (mol L^{-1})	Cu uptake rate (mol cm^{-2} s^{-1})		Phycosphere residence time (s)		Phycosphere oxidized Cu (%)	
	T. oceanica	*T. pseudonana*	*T. oceanica*	*T. pseudonana*	*T. oceanica*	*T. pseudonana*
2.0×10^{-9}	5.6×10^{-18}	4.6×10^{-18}	273	232	28	24
3.0×10^{-8}	6.2×10^{-17}	5.5×10^{-17}	366	294	36	30
6.0×10^{-8}	1.0×10^{-16}	9.9×10^{-17}	442	326	41	32
1.2×10^{-7}	2.6×10^{-16}	9.5×10^{-17}	348	680	34	56
2.4×10^{-7}	1.8×10^{-16}	2.2×10^{-16}	1039	591	71	51
5.0×10^{-7}	2.6×10^{-16}	2.5×10^{-16}	1455	1086	83	73
1.0×10^{-6}	4.2×10^{-16}	2.9×10^{-16}	1806	1872	89	89
2.0×10^{-6}	4.8×10^{-16}	4.0×10^{-16}	3146	2716	98	96

239   Note, the Cu uptake data were taken from the literature.[38]

240

241   Note, the calculated residence time of Cu in the phycosphere above is much longer than

242   the time required for the metal diffusion from the bulk water to the cell surface in the

243   diffusive boundary layer. Specifically, the diffusive boundary layer (i.e., a concentration

244   gradient of Cu) would form when the algal metal uptake is very rapid but the ambient

245   Cu concentration is relatively low.

246   To calculate the diffusion time, we can follow

247   (https://www.physiologyweb.com/calculators/diffusion_time_calculator):

248    $$Diffusion\ time \approx \frac{thickness\ of\ diffusive\ boundary\ layer^2}{2 \times diffusion\ coefficient}$$

249   Here, the thickness of diffusive boundary layer was assumed comparable to the cell

250   radius (i.e., around 2 to 3 μm for the two diatoms), and the diffusion coefficient of Cu

251   ion and its complexes at the order of 10^{-6} cm^2 s^{-1}.[40] Thus, the diffusion time of Cu ions in

252   the phycosphere was around 8 ms.

253

254   Similarly, we calculated whether Cu complexation by DOC is fast enough to allow Cu

255   speciation to reach equilibrium in the phycosphere. Specially, we follow:

256    $$\frac{[Cu]_t}{[Cu]_0}\% = 100\% - e^{-\frac{K_{Cu-DOC}}{t}} \times 100\%$$

257   t = phycosphere residence time, $[Cu]_0$ = the initial Cu concentration, and K_{Cu-DOC} =

258    $k_{Cu-DOC} \times$ [phycosphere DOC]. Specifically, k_{Cu-DOC} = 6×10^3 M^{-1} s^{-1} and

259   [phycosphere DOC] = 80 µM (around 1 mg L^{-1}).[41] The percentage of Cu complexation in

260   the phycosphere of the two algae was 100%.

261

Note S4. Definition of Root Mean Square Logarithmic Error (RMSLE).

Root mean square error (RMSE) measures the difference between model fittings and observed values and is a measure of goodness of model fitting. In the Figures 3 and S4, where both the metal ion concentrations and the uptake rates spanned several orders of magnitude, the logarithmic version of RMSE, i.e., RMSLE, was thus used instead to achieve a more balanced measure of model performance.

$$\mathrm{RMSLE} = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(\log y_i - \log \hat{y}_i)^2}$$

where y_i is the ith observed value; $\hat{y}_i$ is the ith value predicted from the fitting; n is the number of data points.

$$\mathrm{RMSE} = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}$$

Note, the RMSE was used to assess the goodness of fit between algal Pb uptake and Pb^{2+} concentration (i.e., Figure S2), since the uptake test was carried out in a narrow range of ambient Pb^{2+} concentrations.

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