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Supplementary material

Studying selenium and sulfur volatilisation by marine algae *Emiliania huxleyi* and *Thalassiosira oceanica* in culture

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DMSe sorption experiments

To test potential sorption of DMSe on biomass and excreted organic matter, in DMSe sorption assays, 10, 100 or 1000 nM DMSe was added to 10 mL of homogenous algal cultures (~$10^{5.7}$ cells mL^{-1}) via a glass microsyringe (Hamilton, Bonaduz, Switzerland) directly into gastight 10 mL glass headspace crimp vials (BGB Analytics, Boeckten, Switzerland). The vials were allowed to equilibrate at ambient light and temperature for five hours, and then the concentrations of volatile S and Se in the culture media were analyzed as reported previously using SPME-GC-MS.[1] Some DMSe sorption to *E. huxleyi* and *T. oceanica* cultures was observed, but this sorption was incomplete, i.e. would not have obscured a signal from the production of DMSe by these algae during the volatilization experiments had they produced DMSe (Figure S8).

Table S1. Chemical composition for the artificial seawater culturing media

The artificial seawater medium was identical for all species. For *T. oceanica*, the nutrient enrichment solution contained 1.06×10^{-4} M $Na_2SiO_3 \cdot 5H_2O$ as well. For many of the experiments, as described in the materials and methods, cultures were grown in phosphate limited medium. This medium contained an order of magnitude less phosphate, i.e. 3.62×10^{-6} M NaH_2PO_4

	Concentration (M)
Artificial seawater medium	
NaCl	4.09×10^{-1}
Na_2SO_4	2.82×10^{-2}
KCl	9.09×10^{-3}
$NaHCO_3$	2.34×10^{-3}
KBr	8.32×10^{-4}
H_3BO_3	4.42×10^{-4}
NaF	6.19×10^{-5}
$MgCl_2.6H_2O$	5.30×10^{-2}
$CaCl_2.2H_2O$	1.00×10^{-2}
$SrCO_3$	9.13×10^{-5}
Nutrient enrichment solution	
$NaNO_3$	8.82×10^{-4}
NaH_2PO_4	3.62×10^{-5}
$CoCl_2$	4.20×10^{-8}
$CuSO_4$	3.92×10^{-8}
$MnCl_2$	9.09×10^{-7}
Na_2MoO_4	2.60×10^{-8}
$ZnSO_4$	7.65×10^{-8}
Na_2EDTA	1.29×10^{-5}
$FeCl_3$	1.17×10^{-5}
Thiamine HCl	3.32×10^{-7}
Biotine	2.05×10^{-9}
Vitamin B12	3.69×10^{-10}

Table S2. Algal growth during volatilisation experiments

Experiment length and algal growth parameters during the volatilisation experiments V, Y and AB. The biomass accumulation factor is the ratio of the final to the initial cell densities, measured as cells mL^{-1}

ID	Species	Growth period (days)	Initial cell density	Final cell density	Biomass accumulation factor	Average diameter (μm)
V1	*C. reinhardtii*	6	4.41E+04	4.08E+05	9.2	5.89
V2	*P. globosa*	6	1.91E+05	8.90E+05	4.7	2.18
V4	*E. huxleyi*	6	7.41E+04	1.73E+05	2.3	5.50
V7	*E. huxleyi*	6	8.17E+04	2.59E+05	3.2	5.60
Y1	*E. huxleyi*	8	2.53E+04	2.06E+05	8.1	5.78
Y2	*E. huxleyi*	8	2.55E+04	1.70E+05	6.6	5.65
AB4	*E. huxleyi*	10	2.75E+04	1.49E+05	5.4	6.20
AB5	*E. huxleyi*	10	2.18E+04	1.42E+05	6.5	6.21
AB6	*E. huxleyi* (Se acclimated)	10	2.07E+04	1.88E+05	9.1	5.92
AB7	*E. huxleyi* (Se acclimated)	10	2.20E+04	1.89E+05	8.6	6.01
AB8	*E. huxleyi* (Se acclimated)	10	2.51E+04	2.18E+05	8.7	5.81
V3	*T. oceanica*	6	(not measured)	2.81E+05	(not measured)	6.47
Y7	*T. oceanica*	8	4.43E+04	2.61E+05	5.9	6.41
Y8	*T. oceanica*	8	4.03E+04	2.01E+05	5.0	6.85
AB9	*T. oceanica*	10	2.96E+04	1.07E+05	3.6	6.70
AB10	*T. oceanica*	10	3.19E+04	1.72E+05	5.4	6.30
AB11	*T. oceanica* (Se acclimated)	10	2.54E+04	8.86E+04	3.5	6.45
AB12	*T. oceanica* (Se acclimated)	10	2.84E+04	2.18E+05	7.7	6.17
AB13	*T. oceanica* (Se acclimated)	10	2.46E+04	2.06E+05	8.4	6.26

Table S3. Bacterial strains used in the bacterial Se volatilisation experiments

Taxonomic classifications of the 15 marine bacterial strains used in the bacterial Se volatilisation experiment (Experiment Y), reproduced from Datta et al.[2] The classifications were identified with the Ribosomal Protein Database, and classifications with <80% confidence are not shown

Strain	Taxonomic classification
5C01	Bacteria;Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
4F10	Bacteria;Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
3D05	Bacteria;Proteobacteria;Gammaproteobacteria;Alteromonadales;Pseudoalteromonadaceae;Pseudoalteromonas
4B03	Bacteria;Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;Alteromonas
6D02	Bacteria;Proteobacteria;Gammaproteobacteria;Alteromonadales;NB-1d
6C06	Bacteria;Proteobacteria;Gammaproteobacteria;Alteromonadales;Psychromonadaceae;Psychromonas
6E02	Bacteria;Proteobacteria;Gammaproteobacteria;Alteromonadales;Colwelliaceae;Colwellia
1A01	Bacteria;Proteobacteria;Gammaproteobacteria;Vibrionales;Vibrionaceae;Vibrio
6D03	Bacteria;Proteobacteria;Gammaproteobacteria;Vibrionales;Vibrionaceae
4A09	Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae
3B05	Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;Neptunomonas
4G03	Bacteria;Bacteroidetes;Flavobacteria;Flavobacteriales;Flavobacteriaceae;Tenacibaculum
5F06	Bacteria;Bacteroidetes;Flavobacteria;Flavobacteriales;Flavobacteriaceae;Dokdonia
6B07	Bacteria;Bacteroidetes;Flavobacteria;Flavobacteriales;Flavobacteriaceae;Maribacter
4C08	Bacteria;Bacteroidetes;Flavobacteria;Flavobacteriales;Flavobacteriaceae;Polaribacter

Table S4. Algal Se volatilisation

Concentrations of volatile S (S_{vol}) and Se (Se_{vol}) measured in the growth medium after 6–10 days of incubation with algal species exposed to 10 μM Se^{IV}. Intracellular S and Se concentrations are also included. Vol/Intracellular represents the amount of volatile Se produced relative to the total Se accumulated by each treatment; Intracellular/Exposed is the ratio of the total accumulated Se relative to the amount of exposed Se^{IV}. In AB4 and V3, leakage was noted during the acid trapping process; some digestate from Y1 was lost during dilution, leading to underestimation of intracellular concentrations. The values shown in italics represent lower bounds

ID	Species	Se_{vol} (nM)	S_{vol} (nM)	Se_{vol}/S_{vol}	Intracellular Se (mM)	Intracellular S (mM)	Intracellular Se/S	Vol/Intracellular (%)	Intracellular/Exposed (%)
V1	*C. reinhardtii*	386	25.7	15.02	3.3	331.5	0.01	23	2
V2	*P. globosa*	8.4	23.6	0.35	11.1	397.2	0.03	5.7	1
V4	*E. huxleyi*	0.4	15.3	0.02	11.8	624.6	0.02	0.2	2
V7	*E. huxleyi*	0.2	13.3	0.02	8.1	436.0	0.02	0.1	2
Y1	*E. huxleyi*	0.2	69.5	0.003	*5.0*	*292.7*	0.02	0.1	1
Y2	*E. huxleyi*	0.1	116.5	0.001	8.0	407.4	0.02	0.1	2
AB4	*E. huxleyi*	*0.0*	*13.4*	0.003	11.1	287.2	0.04	*0.0*	2
AB5	*E. huxleyi*	0.2	40.6	0.004	10.3	359.3	0.03	0.1	2
AB6	*E. huxleyi* (Se acclimated)	0.3	81.8	0.003	8.4	271.5	0.03	0.1	2
AB7	*E. huxleyi* (Se acclimated)	0.3	87.7	0.004	6.8	259.9	0.03	0.2	2
AB8	*E. huxleyi* (Se acclimated)	0.5	65.9	0.01	6.6	239.6	0.03	0.3	2
V3	*T. oceanica*	*0.5*	*9.0*	0.06	1.5	78.9	0.02	*0.7*	1
Y7	*T. oceanica*	0.3	26.5	0.01	6.0	216.3	0.03	0.1	2
Y8	*T. oceanica*	0.5	65.8	0.01	8.9	244.7	0.04	0.1	4
AB9	*T. oceanica*	1.4	46.0	0.03	13.1	237.6	0.06	0.5	3
AB10	*T. oceanica*	1.1	39.7	0.03	2.3	156.9	0.01	1.9	1
AB11	*T. oceanica* (Se acclimated)	0.8	38.1	0.02	11.8	260.2	0.05	0.5	2
AB12	*T. oceanica* (Se acclimated)	0.8	42.0	0.02	7.0	266.2	0.03	0.4	2
AB13	*T. oceanica* (Se acclimated)	0.4	27.9	0.02	3.6	173.5	0.02	0.4	1

Table S5. Standard recovery (%) using acid trapping

Recovery of volatile Se standards by acid trapping in concentrated nitric acid. The spike (theoretical) and trapped Se concentrations refer to the concentrations in the 1-L sample bottle. The recovery was calculated as the trapped concentration over the theoretical concentration. Samples Y16 and Y17 are identical spikes at, respectively, the beginning and end of the 8 day experiment Y. Likewise, AB14 and AB15 were spiked at the beginning, and AB16 and AB17 at the end, of the 10 day experiment AB. The differences between the samples spiked at the beginning and ends of the same experiment are within the scatter of our data, suggesting that degradation of the standards is limited or, if occurring, not obscuring any production of volatile Se during the experimental time period. Volatile standards were spiked into deioinzied water in experiments G and AC, and into the seawater medium with nutrients and Se^{IV} in experiments V, Y and AB

ID	Standard	Spike (nM S/Se)	Trapped (nM S/Se)	Recovery (%)
G1	DMS	1397	1093	78
G2	DMS	1397	998	71
G3	DMDS	3830	1322	35
G4	DMDS	3830	1091	28
V5	DMSe	200	122.7	61
Y16	DMSe	200	82.0	41
Y17	DMSe	200	84.9	42
AB14	DMSe	200	78.3	39
AB15	DMSe	200	108.0	54
AB16	DMSe	200	155.5	78
AB17	DMSe	200	113.7	57
AC1	DMSe	26	149.4	575[A]
AC3	DMSe	26	156.1	601[A]
AC4	DMDSe	42	28.2	67
AC5	DMDSe	42	25.7	61
AC6	DMDSe	42	12.0	29

[A]These recoveries are unrealistically high, which may be related to the relatively low concentrations of the spikes.

Table S6. Se volatilisation by algal-bacterial co-cultures

Volatile S and Se concentrations produced by cultures after 6–10 days of incubation, showing each individual treatment. The data for algae (axenic as determined by fluorescence microscopy) and algae which were acclimatised to Se^{IV} before the experiment (Se acclimatised) is reproduced from Table S4. Algae were cryogenically killed and added to bottles at concentrations of 5×10^5 cells mL^{-1}, alone ('Dead') and with bacteria ('Dead (with bacteria)'). Living algae were grown alongside the same bacterial mixture ('with bacteria'). Bacteria were grown in the absence of algae on rich medium ('Marine broth and bacteria')

ID	Sample information	Volatile Se (nM)	Volatile S (nM)	Volatile Se/S
E. huxleyi				
V4	Axenic	0.4	15	0.02
V7	Axenic	0.2	13	0.02
Y1	Axenic	0.2	69	0.00
Y2	Axenic	0.1	117	0.00
AB4	Axenic	0.0	13	0.00
AB5	Axenic	0.2	41	0.00
AB6	Se acclimated	0.3	82	0.00
AB7	Se acclimated	0.3	88	0.00
AB8	Se acclimated	0.5	66	0.01
Y5	Dead (with bacteria)	24.8	78	0.32
Y6	Dead (with bacteria)	3.2	56	0.06
Y3	with bacteria	0.0	48	0.00
Y4	with bacteria	0.2	37	0.01
AB2	Dead	2.5	27	0.09
AB3	Dead	8.4	31	0.27
Y14	Dead	4.7	100	0.05
T. oceanica				
V3	Axenic	0.5	9	0.06
Y7	Axenic	0.3	26	0.01
Y8	Axenic	0.5	66	0.01
AB9	Axenic	1.4	46	0.03
AB10	Axenic	1.1	40	0.03
AB11	Se acclimated	0.8	38	0.02
AB12	Se acclimated	0.8	42	0.02
AB13	Se acclimated	0.4	28	0.02
Y11	Dead (with bacteria)	0.1	93	0.00
Y12	Dead (with bacteria)	0.2	59	0.00
Y9	with bacteria	0.1	13	0.01
Y10	with bacteria	0.4	17	0.03
Y15	Dead	0.3	45	0.01

Marine Broth and Bacteria				
Y13	Marine Broth and Bacteria	52.5	90	0.58
AB1	Marine Broth Control	3.8	73	0.05

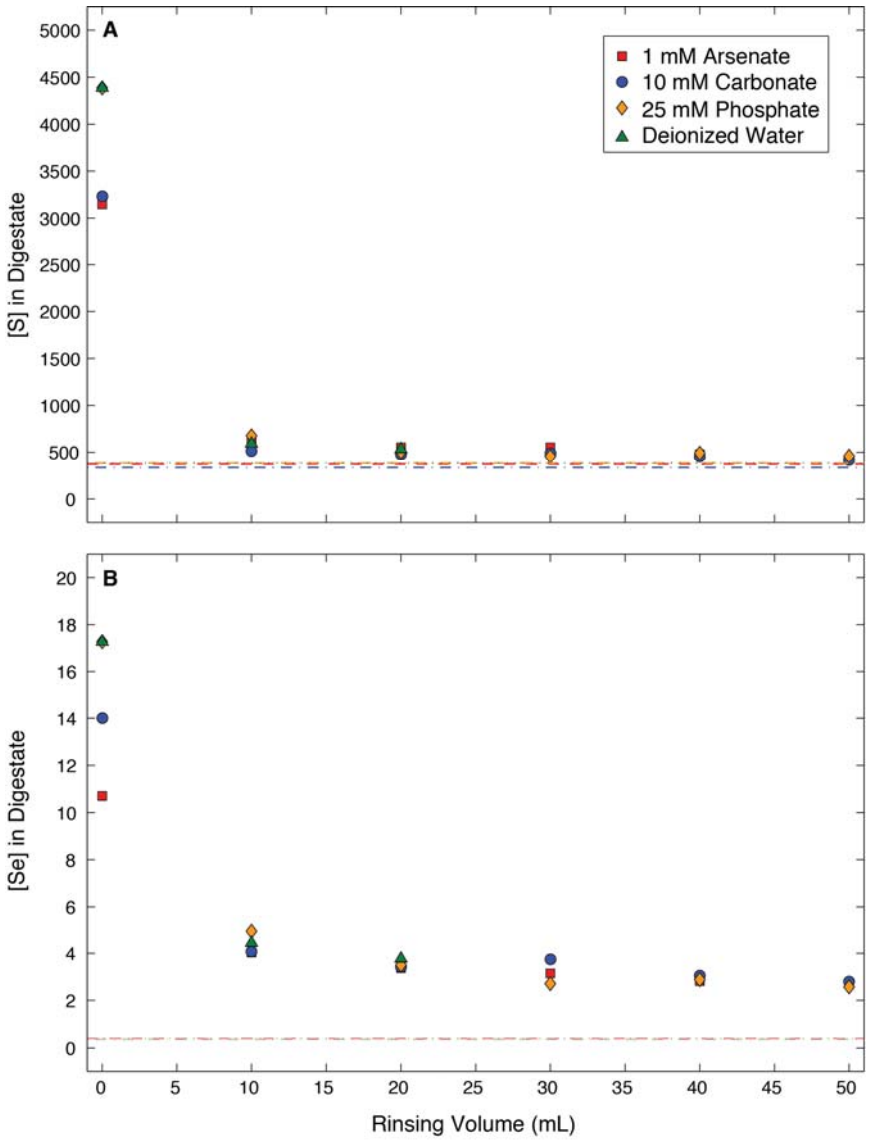

Fig. S1. Intracellular S ((A)) and Se ((B)) in algal cell digestate. A homogenous cell solution was filtered and rinsed with the buffers shown, varying the volume of buffer used. The blank values (filters rinsed with 50 mL) are shown as dashed lines. Based on this data, cells were rinsed with 20 mL of 10 mM carbonate buffer to obtain the non-carbonate, non-phosphate, non-arsenate exchangeable fraction of intracellular S and Se, referred to as intracellular S and Se, respectively.

Fig. S2. Batch culture system in Se Volatilisation Experiments. The bottles were gas tight until valves were opened for bubbling at the end of the experiment.

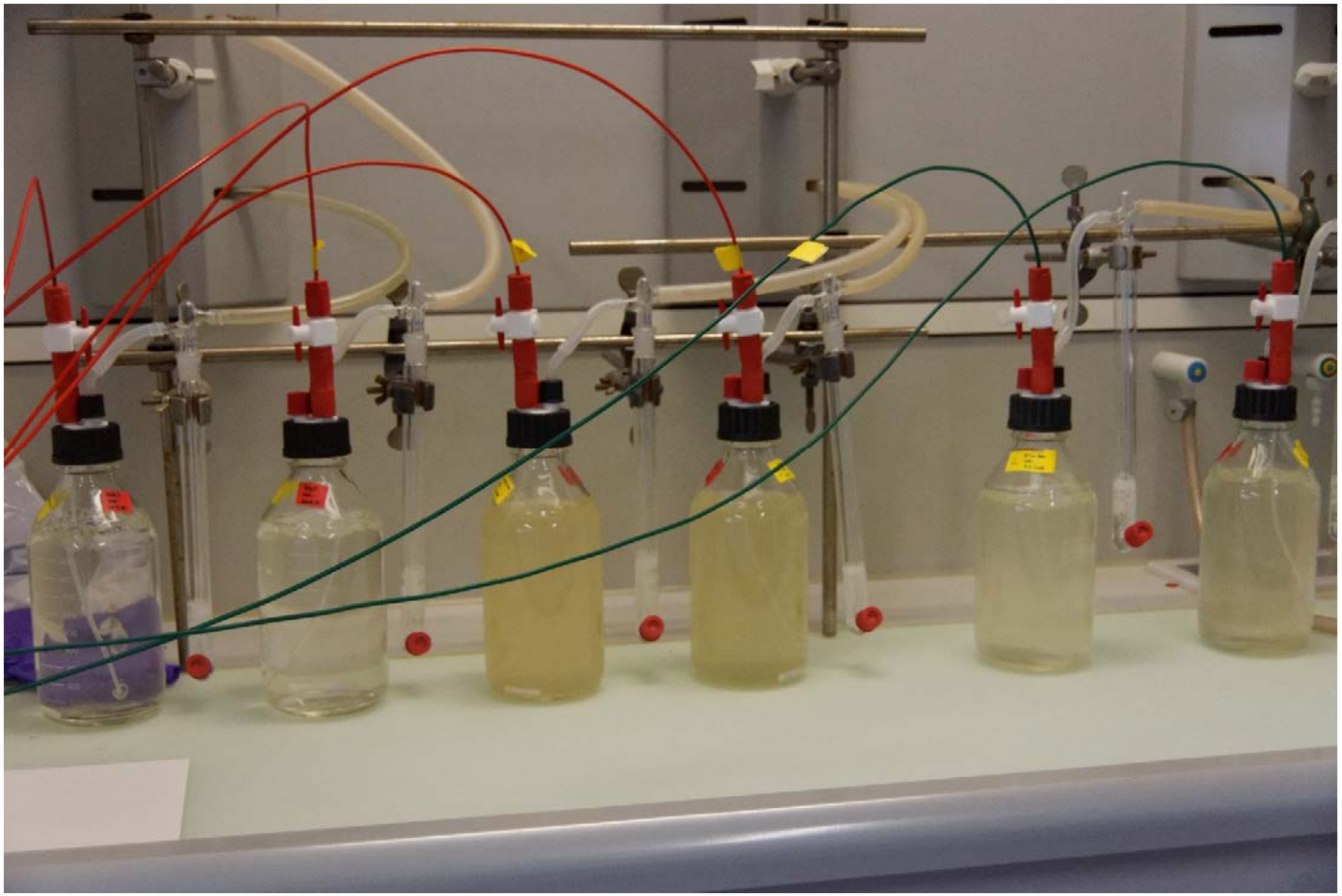

Fig. S3. Acid trapping system used to measure production of volatile S and Se, as described in the materials and methods.

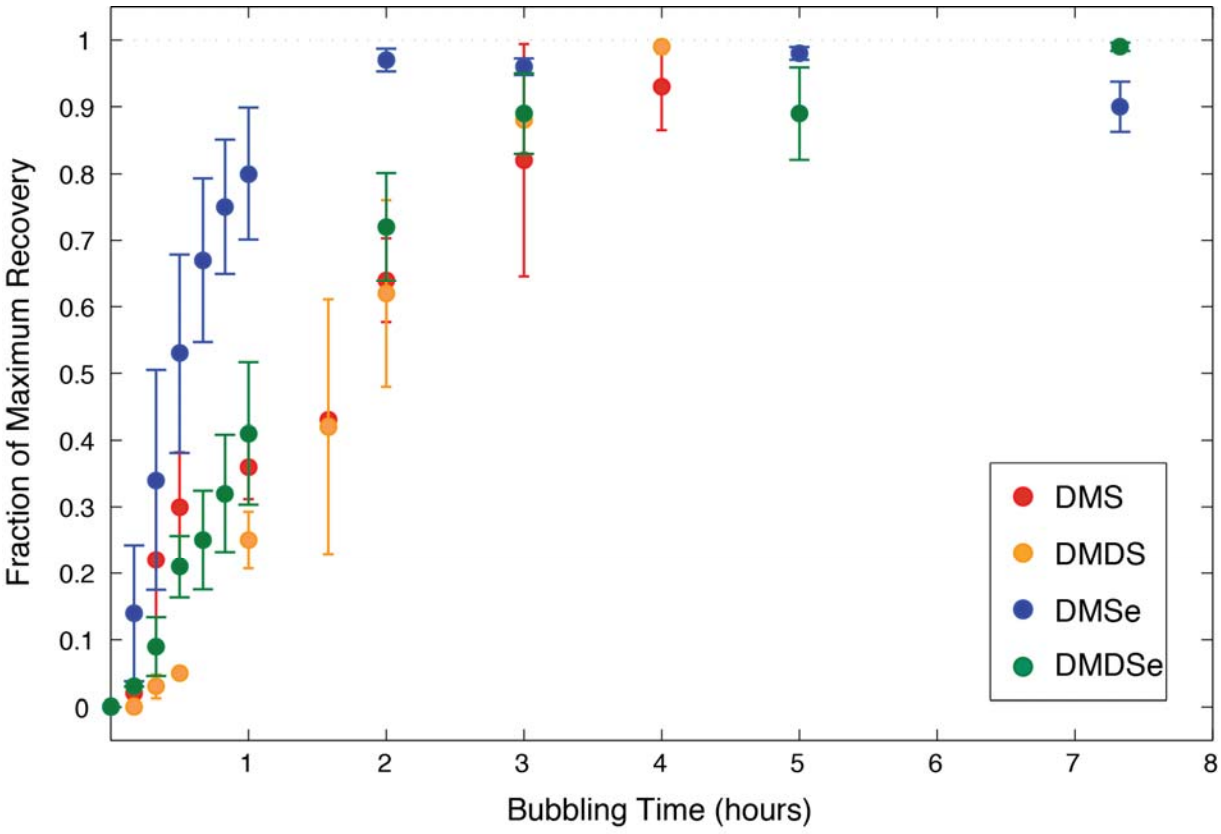

Fig. S4. Fraction of maximal volatile standard recovery in the acid trap as a function of bubbling time. The maximal volatile standard recovery was defined as the greatest Se concentration measured in the acid trap within each replicate. Samples were run in duplicate (DMS and DMDS) or triplicate (DMSe and DMDSe). The error bars show the standard error. Based on these data, a trapping time of 4.5 h was chosen for all experiments in this study.

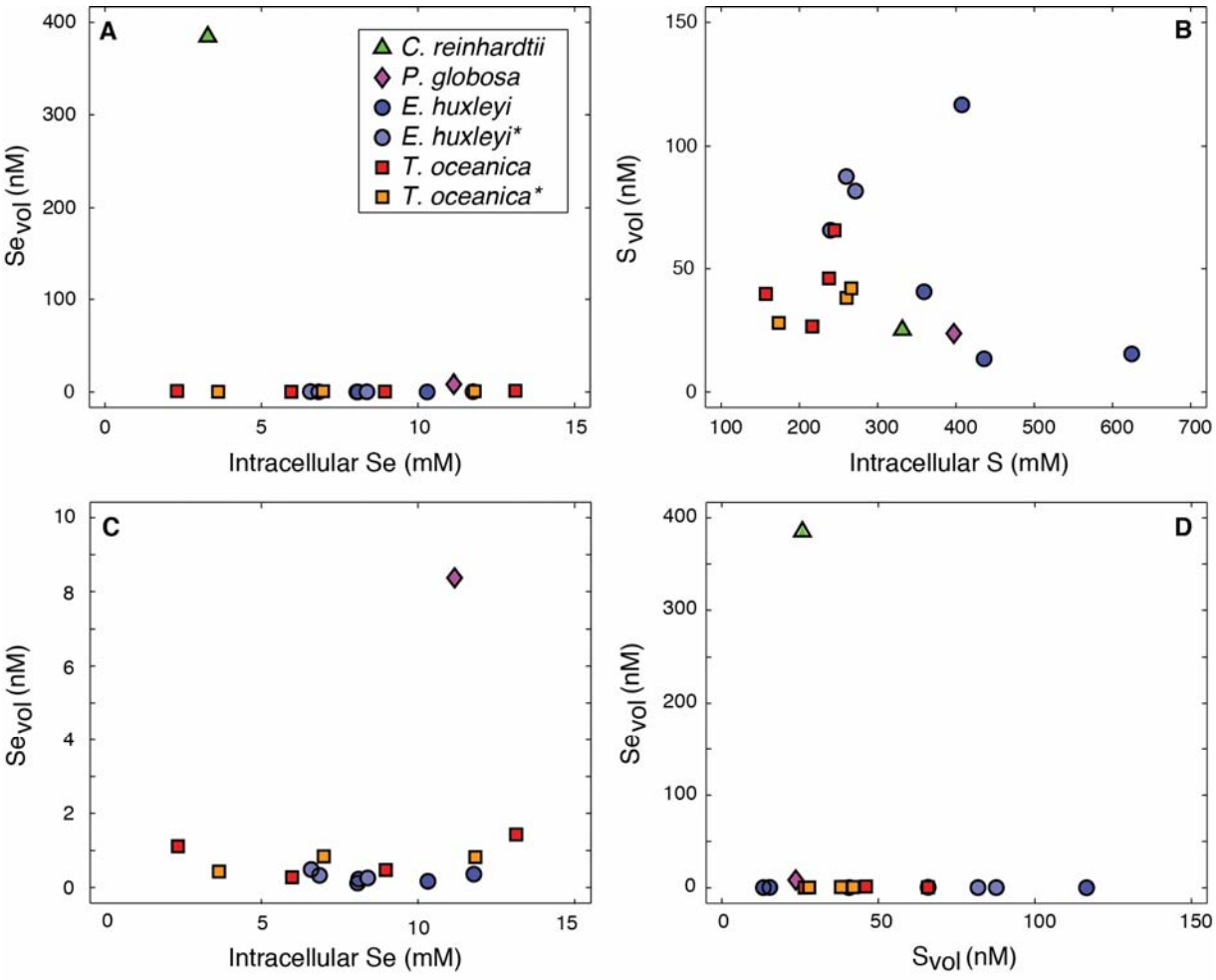

Fig. S5. Intracellular versus volatile Se ((A)) and S ((B)) concentrations produced by algal cultures after 6–10 day incubations. ((C)) shows the data from ((A)), excluding *C. reinhardtii*. ((D)) depicts the relationship between the concentrations of volatile S and Se produced during each incubation. The * indicates that algae were acclimated to 10 μM Se(IV) before the experiment.

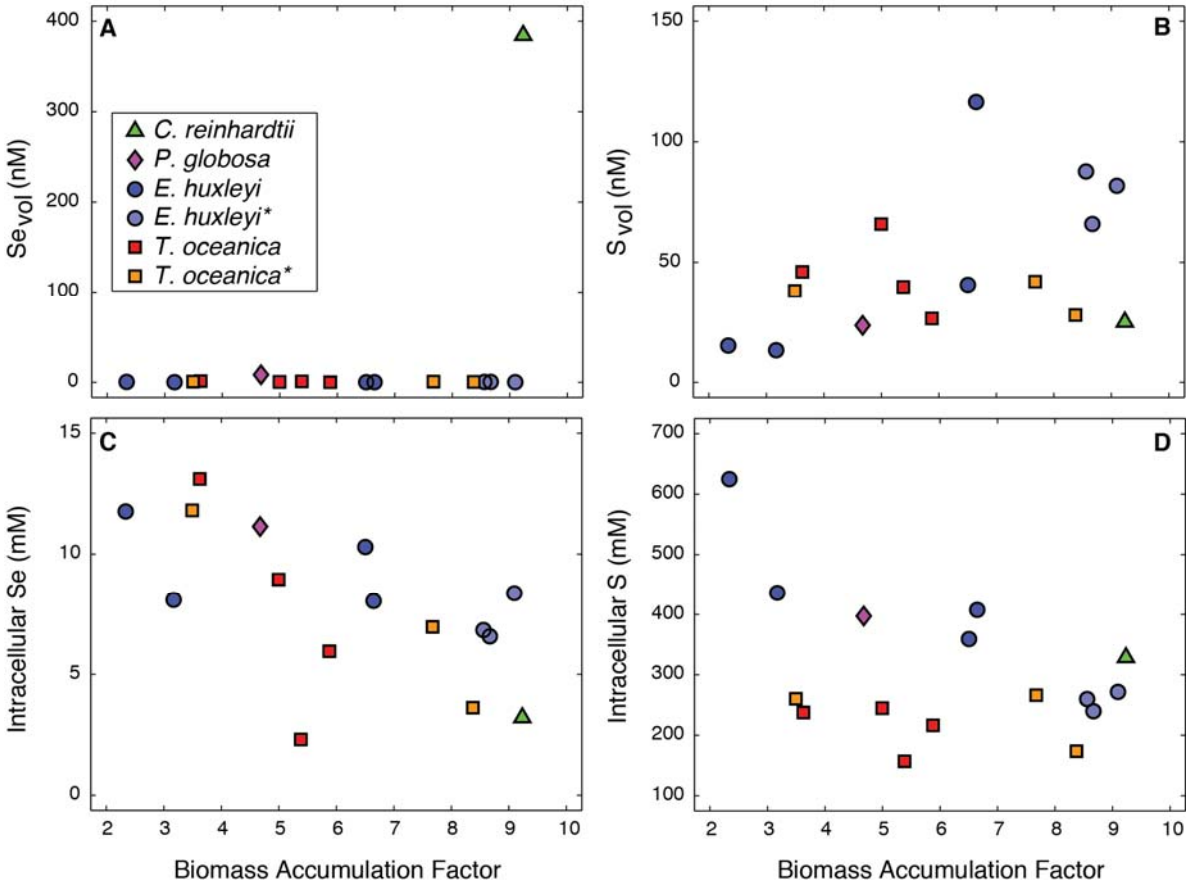

Fig. S6. Biomass accumulation factor versus volatile Se ((A)), volatile S ((B)), intracellular Se ((C)) and intracellular S ((D)) produced by algal cultures over 6–10 day incubations. The * indicates that algae were acclimated to 10 μM Se(IV) before the experiment.

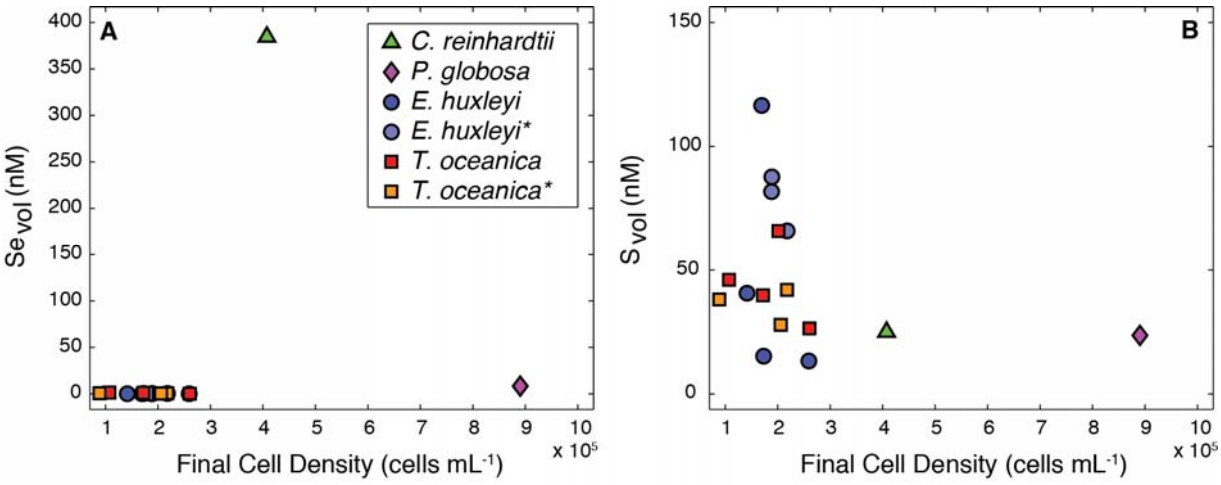

Fig. S7. Final cell density (cells mL^{-1}) versus volatile Se ((A)) and S ((B)) produced by algal cultures over 6–10 day incubations. The * indicates that algae were acclimated to 10 μM Se(IV) before the experiment.

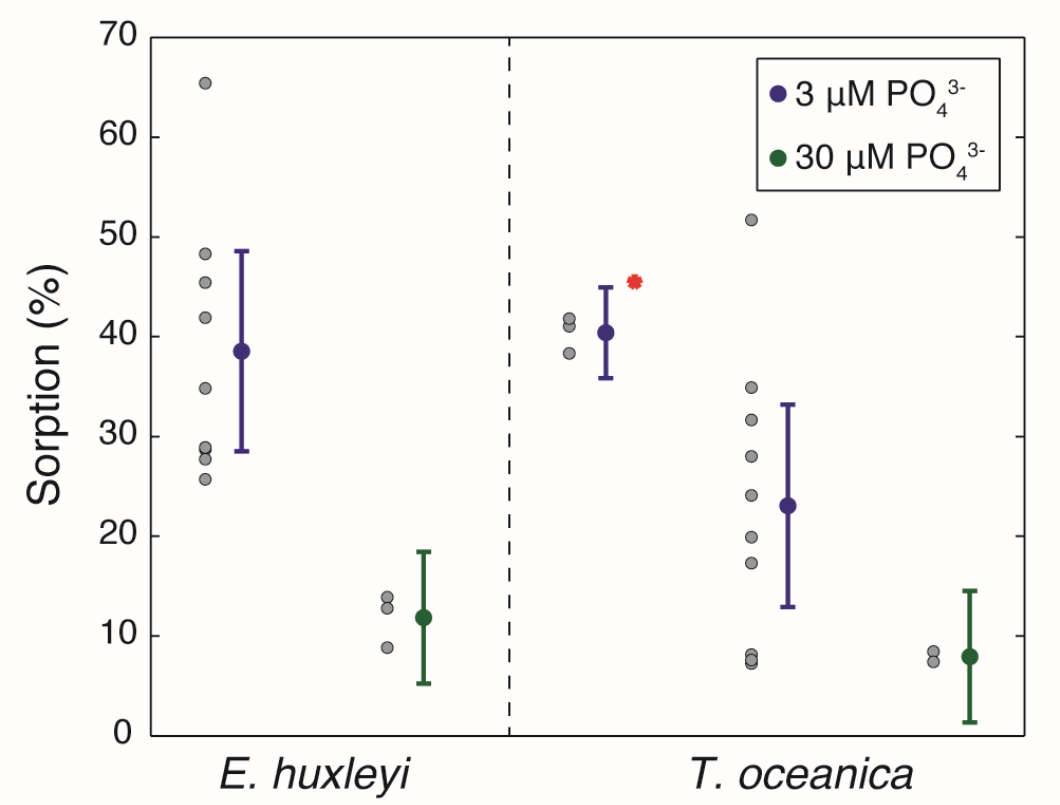

Fig. S8. Percent sorption of a DMSe standard to algal cells (~10^6 cells mL^{-1}). The average percent sorption for algae grown under phosphate replete conditions are in green, and those for cells grown under phosphate limited conditions are shown in blue. The error bars represent the 95 percent confidence interval for the mean sorption. The red * indicates that cells were killed by UV illumination before the experiment. Individual samples are shown in grey.

References

[1] B. Vriens, M. Mathis, L. H. E. Winkel, M. Berg, Quantification of volatile-alkylated selenium and sulfur in complex aqueous media using solid-phase microextraction. *Journal of Chromatography A*. **2015**, *1407*, 11.
[2] M. S. Datta, E. Sliwerska, J. Gore, M. F. Polz, O. X. Cordero, Microbial interactions lead to rapid micro-scale successions on model marine particles. *Nat Commun*. **2016**, *7*.