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Supplementary Materials for

Carbon recycling efficiency and phosphate turnover by marine nitrifying archaea

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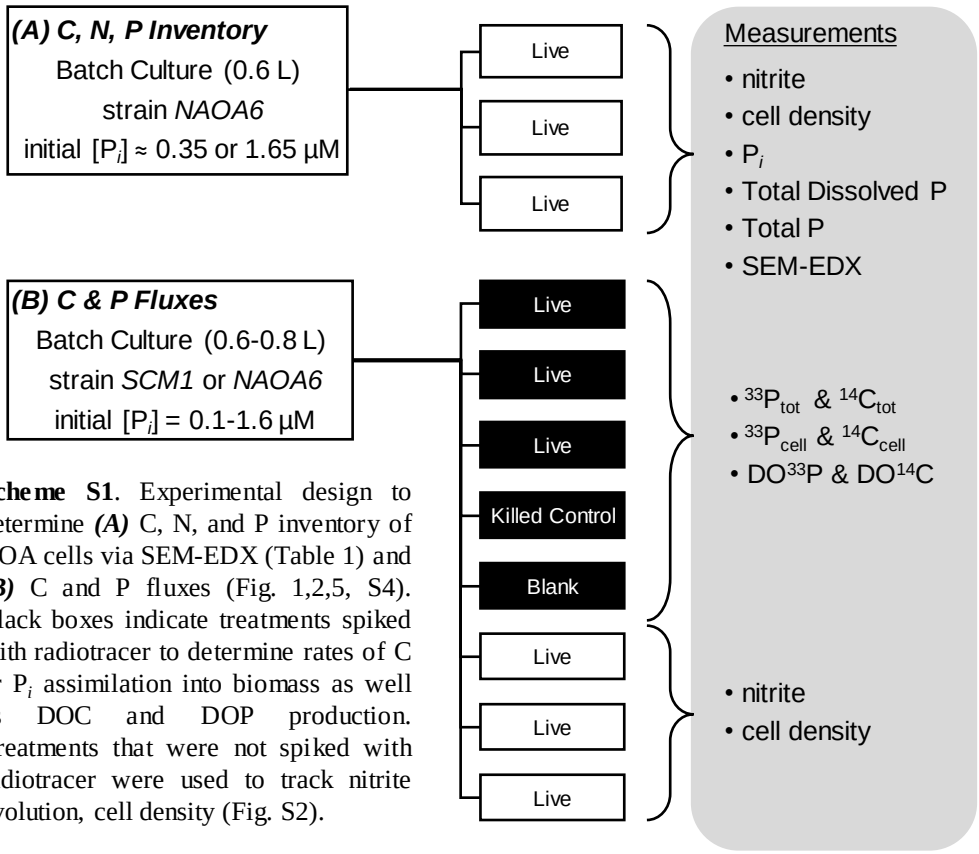
This PDF file includes:

Supplementary Material

Text S1. ***Calculation of global ocean C and P fluxes by Thaumarchaeota.***

Our global chemoautotrophy flux estimates are modeled after the depth regimes, ocean zonation, and calculation performed by (*2*) but now apply a higher estimate of the C yield from nitrification (0.32 or 0.22 for P-replete or -deplete conditions, respectively; Fig. 3), as opposed to a value of 0.1 assumed previously, after (*8*, *9*). Briefly, fluxes of organic C respiration (*31*, *38*) are converted to N by dividing by 6.6, according to the Redfield ratio. For example, the upper range of thaumarchaeal C-fixation in the dark ocean is calculated using the higher C-flux estimate (*38*): (330 Tmol N y^{-1}; Wutcher et al.) × 0.30 (C_o/N_n) × 12 g C mol^{-1} = 1188 Tg C y^{-1}. The entirety of this reduced N is assumed to be available for nitrification in the deep ocean; however, in the euphotic zone, nitrifiers competing with phytoplankton access only a portion of reduced N. We assume a conservative estimate of 43% of reduced N is available for nitrification in the euphotic zone (*2*, *12*), which was determined based on the relationship between specific nitrification rate and the ratio of "new" versus "regenerated" production in the surface ocean. Open ocean fluxes of C and P in the euphotic zone reported in the current study are further adjusted for the lower C yield expected in regions of the ocean characterized by P-limitation (Fig. S5); that is the N. Atlantic Subtropical Gyre and Equatorial Atlantic, which account or 13% of the surface area of the world's oceans (*13*, *31*). An annual global dark C-fixation flux of 0.6-1.1 Pg C y^{-1} was also extrapolated by combining volumetric DIC fixation rates reported by (*32*) for the subsurface oxygen minimum zone (~100-500 m; 40-100 µmol C m^{-3} d^{-1}), intermediate waters (~500-2000 m; 0.1 µmol C m-3 d-1), and deep waters (> 2000 m; 0.05 µmol C m^{-3} d^{-1}) with volumetric estimates of (*43*) for these regions (2, 20, and 1080 × 10^{15} m^3, respectively).

Supplementary Figures



Scheme S1. Experimental design to determine ***(A)*** C, N, and P inventory of AOA cells via SEM-EDX (Table 1) and ***(B)*** C and P fluxes (Fig. 1,2,5, S4). Black boxes indicate treatments spiked with radiotracer to determine rates of C or P_i assimilation into biomass as well as DOC and DOP production. Treatments that were not spiked with radiotracer were used to track nitrite evolution, cell density (Fig. S2).

Scheme S1. Experimental incubations of *N. maritimus NAOA6* and *SCM1* to measure cellular nutrient quotas (A) and rates of C-assimilation, P-uptake, and DOC and DOP release (B).

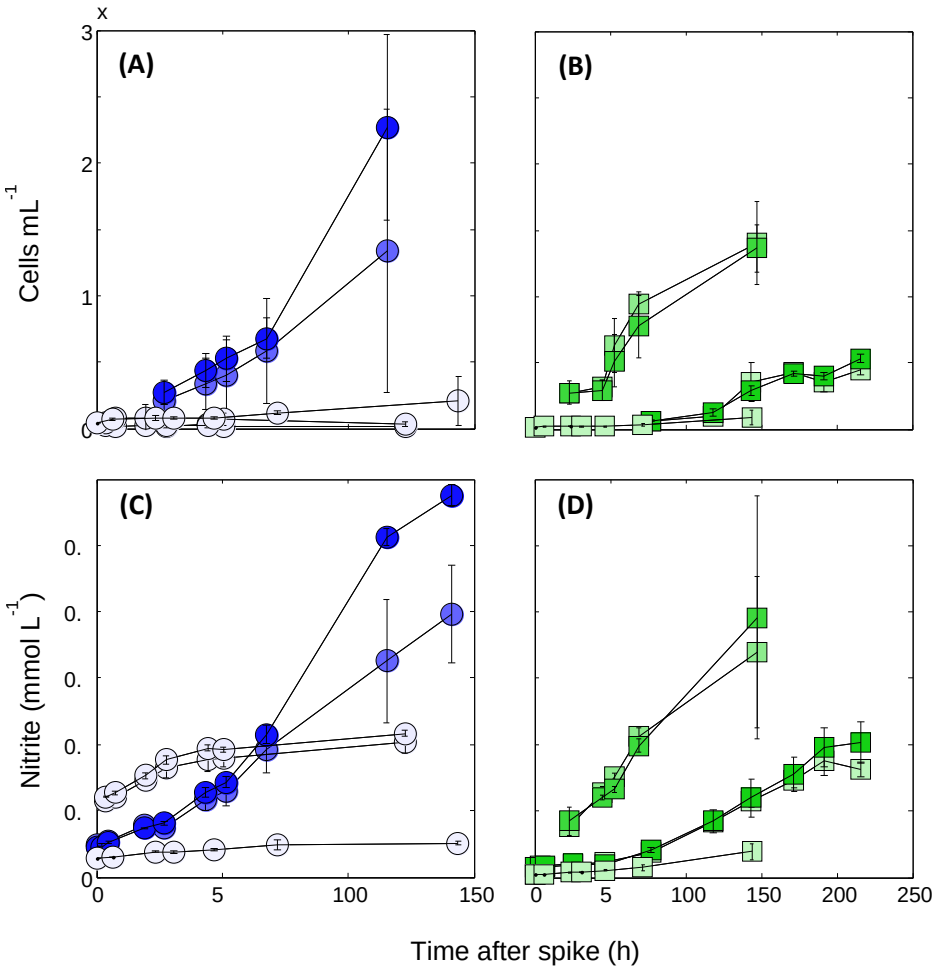


Figure S1. Growth curves of *N. maritimus* strains *NAOA6* (A) and *SCM1* (B) during radiotracer experiments at 0.12 < P_i < 2µM. For EDX and colorimetric analyses, independent batch cultures of *NAOA6* were grown in P-deplete (C; $P_i \approx 0.35$ µM) or P-replete SCM medium (D; $P_i \approx 1.5$ µM). The color of the symbols indicates the initial P_i concentration of the medium (cf. Fig. 1).

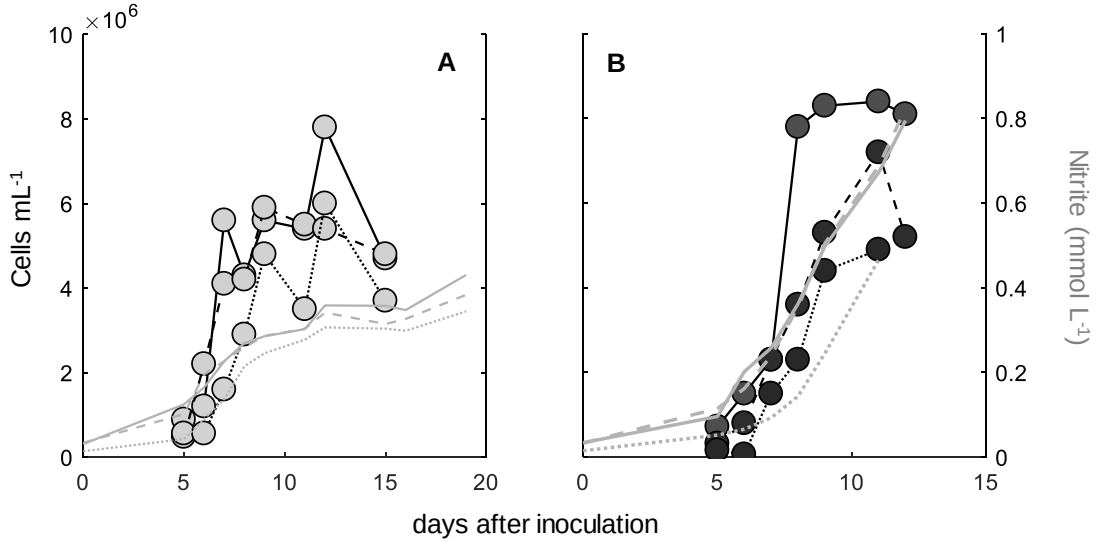


Figure S2. Growth curves (black lines) and nitrite evolution (grey lines) of replicate *N. maritimus NAOA6* batch cultures (*n* = 3) grown in P-deplete (A, $P_i \approx 0.35$ µM; grey circles) or P-replete (B, $P_i \approx$ 1.6 µM; black circles) SCM medium. Cell material for EDS measurements were collected from the replicates indicated by the solid line.

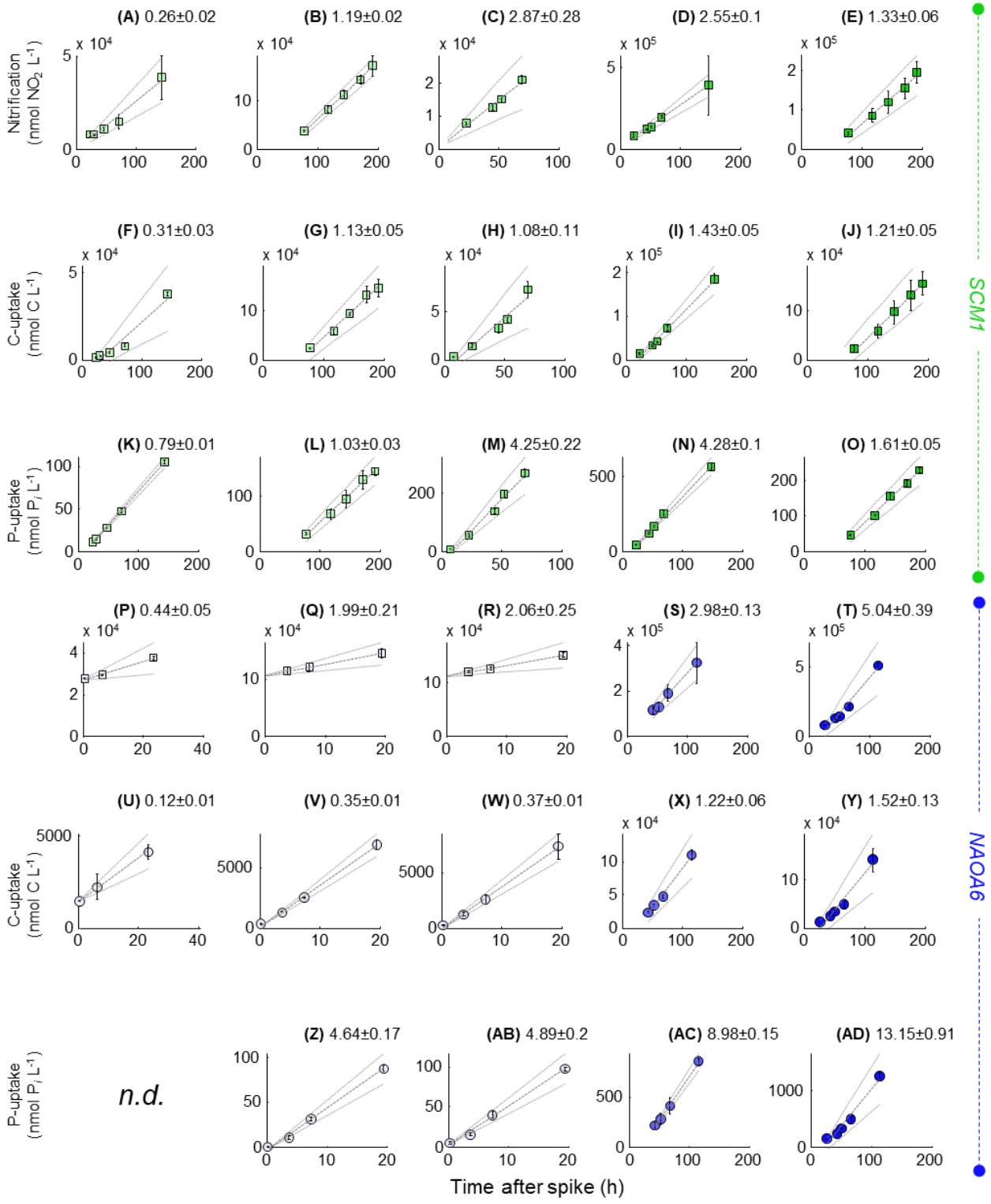


Figure S3. Linear rates of nitrite production, C-fixation, P-uptake by *N. maritimus SCM1* (A-O; squares) and *NAOA6* (P-AD; circles) in SCM media amended to different P_i concentrations. Initial P_i in SCM media increases from left to right (cf. Fig. 1, Table S1). The x-axis in all plots is time (in hours). Error bars represent the standard deviation of three replicate incubations. Dashed lines indicate the confidence interval ($\alpha = 0.05$). The title of each graph is the slope of the line ± standard error of the slope, with units of nmol L^{-1} h^{-1} for nitrification, C-fixation, and P_i-uptake. *n.d.* = no data

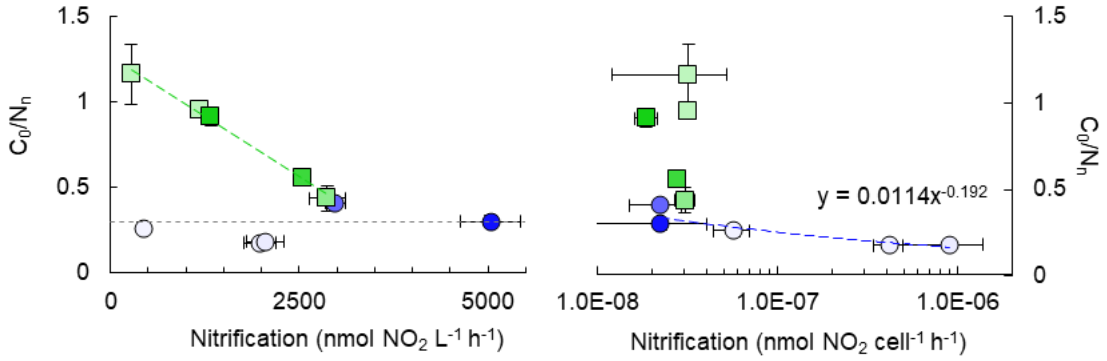


Fig. S4. Controls of absolute (A) or cell-specific (B) nitrification rates on C yield (C_0/N_n) by *N. maritimus* strains *SCM1* (green) and *NAOA6* (blue). P_i amendment of the growth medium is indicated by the shade of the symbol as defined in Fig. 1. Error bars indicate propagated error of the 95% confidence interval of the slopes of linear rates of radiotracer incorporation or nitrite production over 3-5 time points among triplicate incubations (N = 9-15 for each data point; cf. Fig. S3).

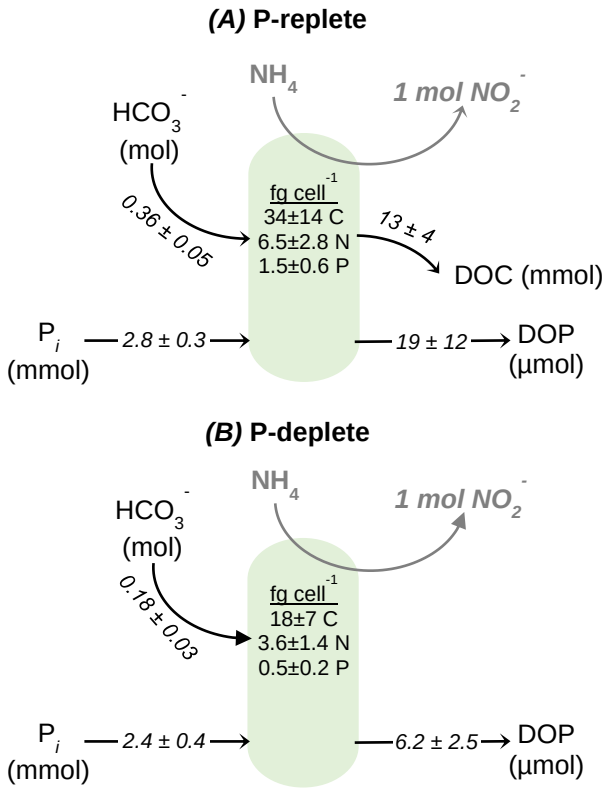


Fig. S5. Coupled biogeochemical fluxes of *N. maritimus NAOA6* and physiological responses to P-limitation

Supplementary Tables

Table S1. **Linear rates of nitrite production, C-fixation, P-uptake, as well as integrated DOC and DOP release rates by *N. maritimus*.** Rates are reported for strains *NAOA6* and *SCM1* in SCM media amended to different P_i concentrations (cf. Fig. S3). n.d. = no data

Strain	initial P_i (nM)	Nitrification (nmol L^{-1} h^{-1})	C-fixation (nmol L^{-1} h^{-1})	P_i uptake (nmol L^{-1} h^{-1})	DOC release (nmol L^{-1} h^{-1})	DOP release (pmol L^{-1} h^{-1})
SCM1	910	1327 ± 63	1212 ± 47	1.6 ± 0.0	57 ± 19	26 ± 4
	740	2551 ± 105	1426 ± 52	4.3 ± 0.1	108 ± 6	33 ± 4
	400	2874 ± 239	1253 ± 180	4.7 ± 0.3	130 ± 49	27 ± 1
	190	1191 ± 24	1135 ± 53	1.0 ± 0.0	106 ± 39	14 ± 0.5
	160	280 ± 24	326 ± 40	0.8 ± 0.0	*nd*	10 ± 5
NAOA6	1870	5035 ± 393	1517 ± 126	13.2 ± 0.9	47 ± 7	53 ± 16
	1220	2979 ± 131	1219 ± 57	9.0 ± 0.2	49 ± 11	79 ± 43
	160	2056 ± 251	375 ± 12	4.9 ± 0.2	*nd*	12 ± 3
	130	1988 ± 212	349 ± 10	4.6 ± 0.2	*nd*	13 ± 4
	< 100	442 ± 52	116 ± 6	*nd*[a]	*nd*	*nd*

[a] For this incubation, > 10% P_i was assimilated within 20 min of radiotracer addition, and therefore too quickly to presume the initial P_i concentration for subsequent calculations

Table S2. Concentration of P pools in *N. maritimus NAOA6* batch cultures initiated under P-deplete or P-replete conditions.

N. maritimus NAOA6 experiment		Batch Cultures					Cell concentrate			fg P cell^{-1} [B]
		initial P_i	final P_i	P_i assimilated	final DOP[A]	P released as DOP	cell density	P_i	total P	
		$\mu mol\ L^{-1}$	$\mu mol\ L^{-1}$	$\mu mol\ L^{-1}$	$\mu mol\ L^{-1}$		$\times 10^{10}$ cell L^{-1}	$\mu mol\ L^{-1}$	$\mu mol\ L^{-1}$	
P-deplete	1	0.35	0.00	0.35	0.025	7%	1.46	0.73	1.11	0.7
	2	0.37	0.00	0.37	0.012	3%	2.91	0.40	0.74	0.4
	3	0.35	0.00	0.35	0.011	3%	2.30	0.34	0.72	0.5
P-replete	1	1.39	0.19	1.20	0.023	2%	16.1	3.03	14.24	2.2
	2	1.65	0.48	1.17	0.040	3%	22.5	3.73	14.41	1.5
	3	1.69	0.73	0.97	0.029	3%	17.7	2.72	8.03	0.9

[A] DOP = TDP - P_i - blank; where blank is the value of DOP in sterile medium (0.07 µM)

[B] fg P cell^{-1} = total P of the cell concentrate minus final DOP of the batch culture, normalized by cell density.

Table S3. DO^{14}C and DO^{33}P release by *N. maritimus NAOA6* and *SCM1* in SCM medium amended with 0.2-2 µm P_i. *n.d.* = no data

N. maritimus strain	Initial P_i (nmol L^{-1})	min t_{PO4} (h)	Fixed C released as DOC (%)	Final [DOC] (nmol C L^{-1})	DOC release rate: pmol L^{-1} h^{-1}	DOC release rate: amol $cell^{-1}$ d^{-1}	Assimilated P released as DOP (%)	Final [DOP] (nmol P L^{-1})	Release rate: pmol P L^{-1} h^{-1}	Release rate: zmol P $cell^{-1}$ d^{-1}
NAOA6	*n.d.*	3	*n.d.*	*n.d.*	*n.d.*	*n.d.*	1.0 ± 0.5	*n.d.*	*n.d.*	*n.d.*
	127	16	*n.d.*	*n.d.*	*n.d.*	*n.d.*	1.8 ± 0.5	1.6 ± 0.4	13 ± 4	81 ± 30
	159	21	*n.d.*	*n.d.*	*n.d.*	*n.d.*	1.3 ± 0.3	1.4 ± 0.4	12 ± 3	28 ± 8
	1221	82	5.7 ± 1.3	7 ± 2	49 ± 11	9 ± 3	1.2 ± 0.7	11.1 ± 6.0	79 ± 43	14 ± 8
	1869	83	4.7 ± 1.4	7 ± 1	47 ± 7	5 ± 2	0.5 ± 0.2	7.5 ± 2.3	53 ± 16	6 ± 2
SCM1	179	159	*n.d.*	*n.d.*	*n.d.*	*n.d.*	1.5 ± 0.8	1.6 ± 0.8	11 ± 6	27 ± 15
	187	125	15.9 ± 5.9	23 ± 8	106 ± 39	55 ± 21	2.1 ± 0.2	3.1 ± 0.1	14 ± 0	7 ± 1
	388	59	18.8 ± 7.2	19 ± 7	130 ± 49	22 ± 10	1.4 ± 0.1	4.0 ± 0.2	27 ± 1	5 ± 1
	744	100	8.6 ± 0.8	16 ± 1	108 ± 6	19 ± 3	0.8 ± 0.1	4.8 ± 0.6	33 ± 4	6 ± 1
	910	661	9.3 ± 3.6	12 ± 4	57 ± 19	26 ± 9	2.5 ± 0.2	5.6 ± 0.8	26 ± 4	12 ± 2