Supporting information – Additional Text, Figures and Tables

High single cell diversity in carbon and nitrogen assimilation by a chain-forming diatom across a century

Malin Olofsson[1,*], Olga Kourtchenko[1], Eva-Maria Zetsche[1], Hannah K. Marchant[2], Martin J. Whitehouse[3], Anna Godhe[1], and Helle Ploug[1]

[1]Department of Marine Sciences, University of Gothenburg, Box 461, SE 405 30 Gothenburg, Sweden

[2]Max Planck Institute for Marine Microbiology, Celsiusstrasse 1, D-28359, Bremen, Germany

[3]Swedish Museum of Natural History, Box 50 007, SE 104 05 Stockholm, Sweden

[*]corresponding author: Malin Olofsson

Tel: +46 (0) 31-786 2629, Fax: +46 (0) 31-786 2560

E-mail: malin.olofsson@marine.gu.se

Text S1. Additional results

Clone-specific measurements

The clone-specific carbon- and nitrate-assimilation rates, assimilation ratios, and inorganic nitrate concentrations, indicated an early surplus uptake of nitrate relative to carbon by the young clones under nitrate-replete conditions (Figs. S1a, b, and S4a, c, and Table S3). However, neither of the significant differences between the ages detected during specific growth phases under nitrate-replete conditions occurred under nitrate-limited and with an overall large variability among clones (Fig. S4b, d). Under nitrate-limited conditions, high rates of nitrate-assimilation were followed by an increased carbon-assimilation. Overall, the carbon to nitrate assimilation ratios indicated a large flexibility, often deviating from Redfield, and partly related to a large variation in cell sizes and nutrient demands (Table S3).

The third experiment with high-resolution nitrate measurements under nitrate-limited conditions also revealed a larger difference within the ages between the clones as compared to across (Fig. S6 and Table S5). Also, it showed that 72-95 % and 80-110 % of the initial nitrate concentration was assimilated as particulate organic nitrogen after 4 and 6 d, respectively (Table S5). While particulate organic nitrogen increased by only 2-31 % from day 4 to day 6, particulate organic carbon increased 60-182 % during the same period. Consequently, the particulate organic carbon to nitrogen ratio increased by 46-155 % from day 4 to 6 (from 6.8 to 21.7), with no difference between young and old clones (Table S5).

Chain length

Based on relative fluorescence measurements (RFU; see Experimental procedure), the exponential growth phase of *Skeletonema marinoi* lasted between 2 and 3 days, and was slightly shorter under nitrate-limited as compared to nitrate-replete conditions (Fig. S2). The transition from the exponential growth phase to the stationary growth phase was accompanied by a significant reduction in chain length for all clones, independent of age. The average chain length of *S. marinoi*, based on microscopy analysis, during the exponential growth phase under nitrate-limited conditions was 9.35 cells chain^{-1}, and 4.26 cells chain^{-1} during the stationary growth phase (Fig. S5).

Nutrient conditions and stoichiometry

Dissolved nitrate and silicate were assimilated in a 1:1 ratio during the first days under both conditions. Dissolved nitrate concentrations were depleted under nitrate-limited conditions, while there was ~75 µM under nitrate-replete conditions, during stationary growth phase (Fig. S1). In addition, there was a low residual concentration of phosphate under nitrate-limited, and very low concentration under nitrate-replete conditions, with very low residual concentrations of silicate under both conditions (Fig. S1). As the dissolved nitrate concentration decreased during the experiments, a corresponding increase in particulate organic nitrogen was observed. It was twice as high in clones grown under nitrate-replete compared to nitrate-limited conditions, with maximum concentration reached at the end of the exponential growth phase (Table S3).

Under both nitrate conditions, the total concentration of particulate organic carbon (cells and exudates), number of cells, and RFU all increased by a factor between 4 and 6 (Fig. S2 and Table S3), with high particulate organic carbon to nitrogen ratios in all clones during the stationary growth phase (Table S3). The particulate organic nitrogen to phosphorus ratio was approx. 6 (below Redfield of 16:1) under nitrate-limited conditions during stationary growth phase, while it was > 21 under nitrate-replete conditions during the late stationary growth phase (Table S3).

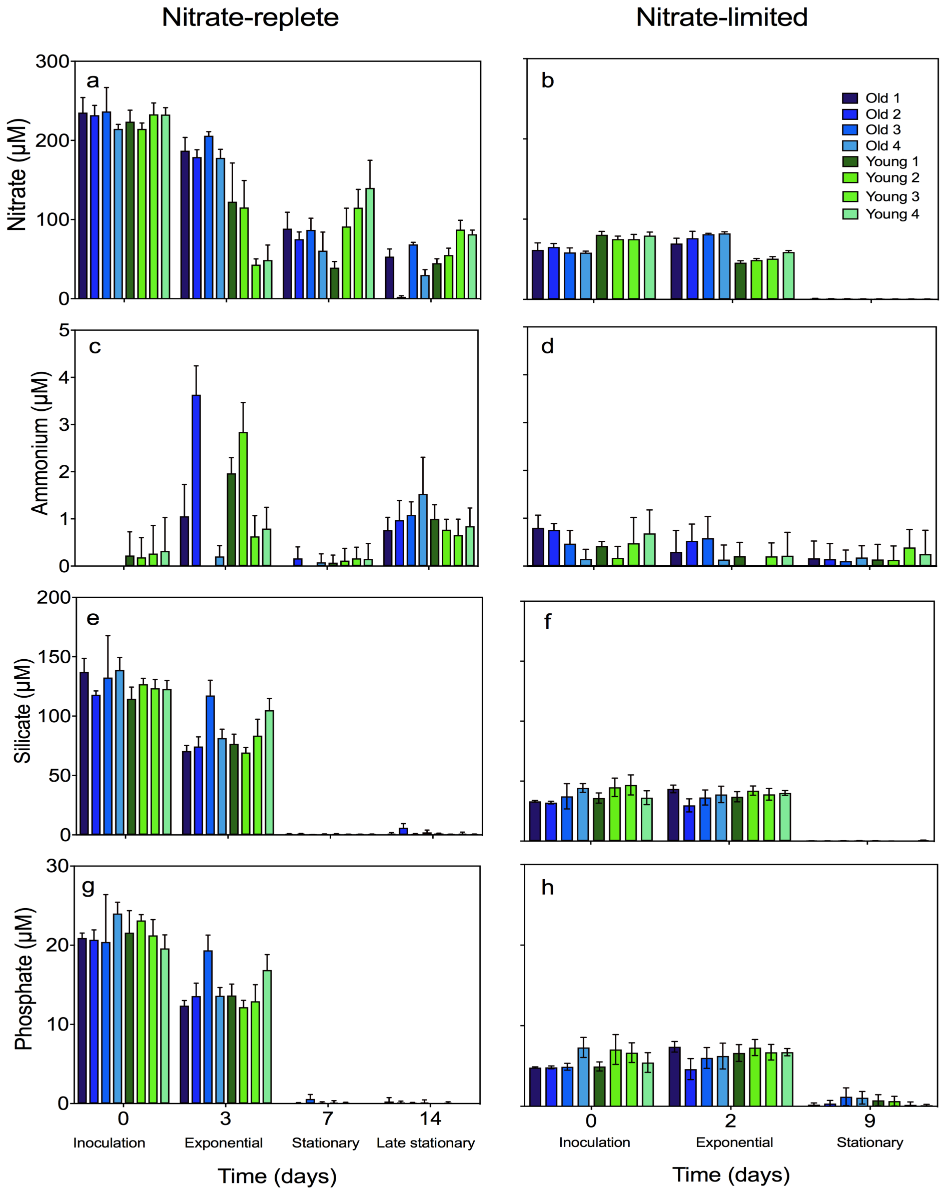

Fig. S1 Dissolved inorganic nitrate (a, b), ammonium (c, d), silicate (e, f) and phosphate (g, h) concentrations (µM) under nitrate-replete (a, c, e, g) and nitrate-limited conditions (b, d, f, h) for all clones. N = 5, error bars indicate standard deviation.

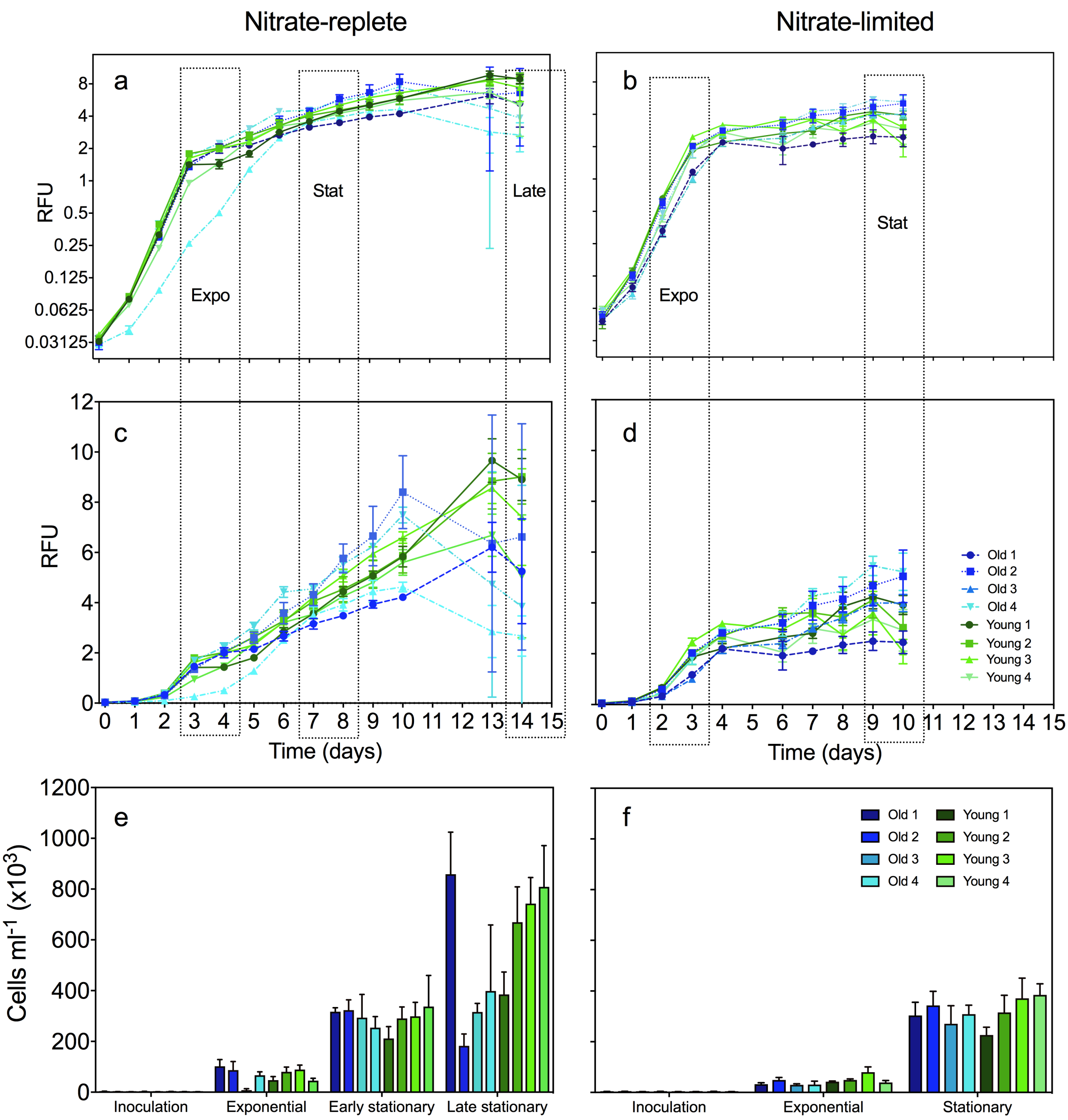

Fig. S2 Growth demonstrated by relative fluorescence unit (RFU) at $\log_2$ scale (a, b) and linear scale (c, d), and cells mL^{-1} x 10^3 (e, f), under nitrate-replete (a, c, e) and nitrate-limited (b, d, f) conditions for all clones, where dotted rectangles mark the 24-h isotope incubations, at exponential (Expo), stationary (Stat) and late stationary (Late) growth phases, N = 3-5, error bars indicate standard deviation.

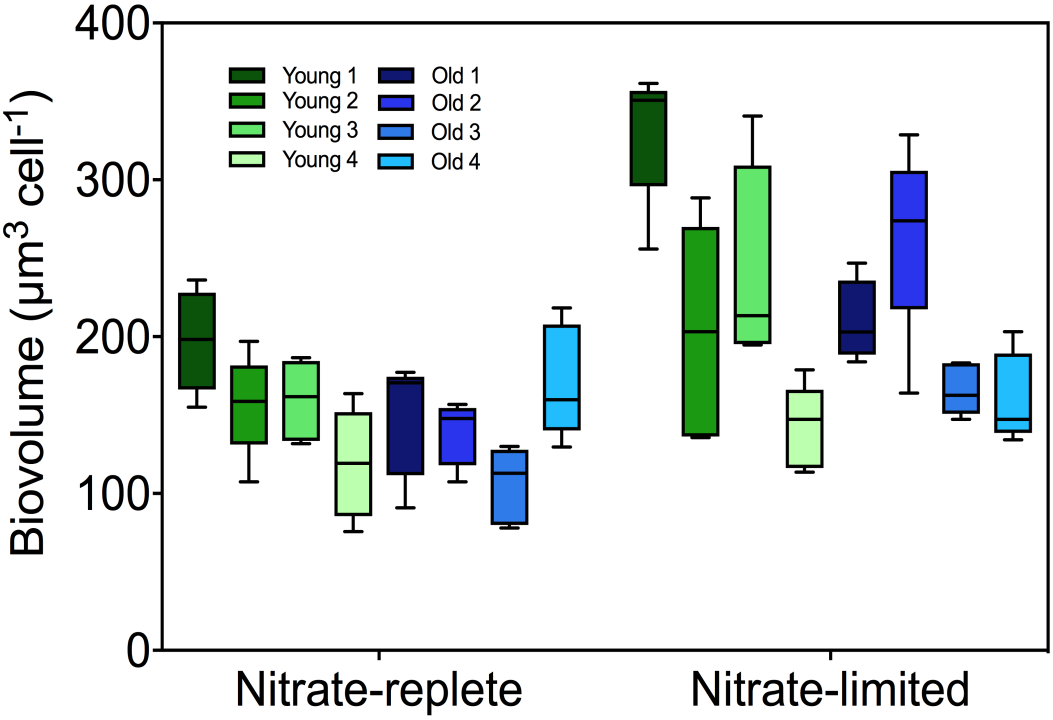

Fig. S3 Cellular biovolumes. Average biovolumes (μm^3 $cell^{-1}$) of *Skeletonema marinoi* from microscopy analyses under nitrate-replete and nitrate-limited conditions for old and young clones. N = 5 and the data are shown as box-whisker plots where median values are shown, with the lower and upper box limits showing the 25th and 75th percentile, respectively, and whiskers showing the range between min and max value.

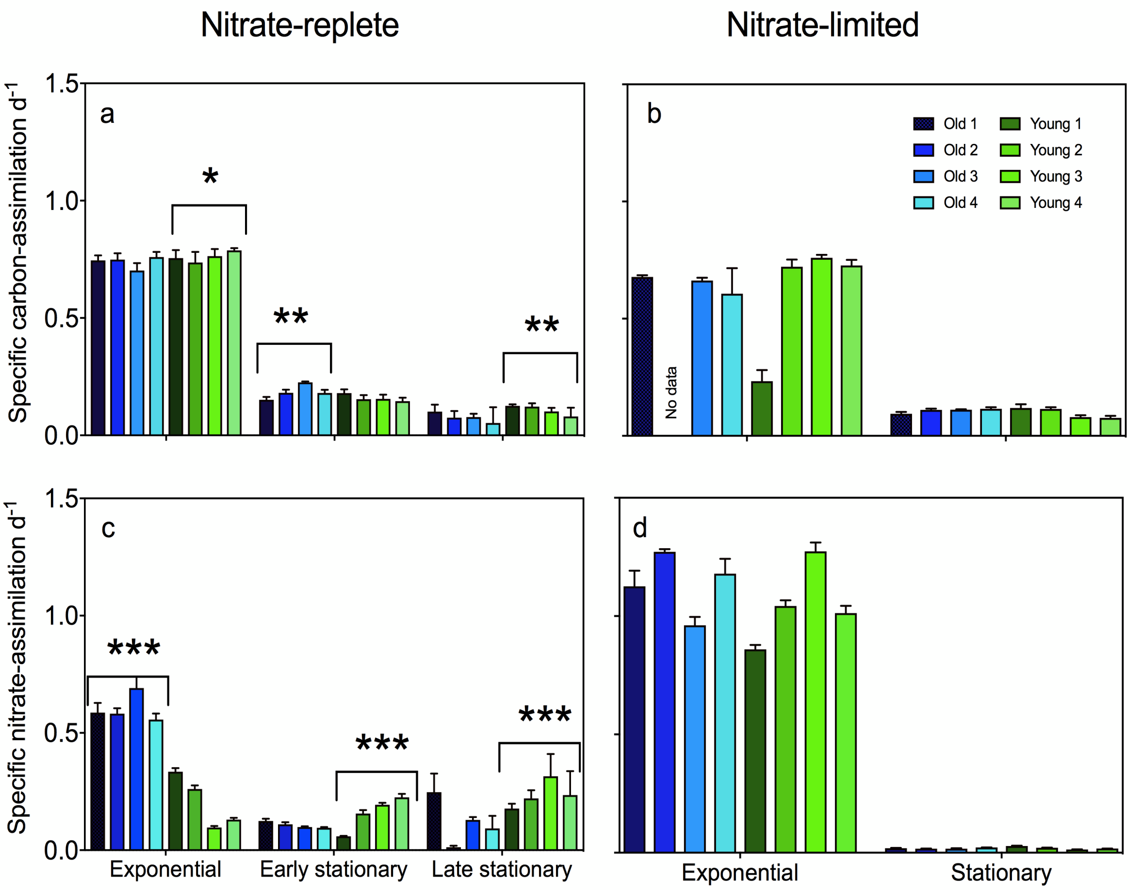

Fig. S4 The clone-specific carbon- and nitrate-assimilation rates d^{-1} under nitrate-replete (a, c) and nitrate-limited (b, d) conditions during exponential, early stationary and late stationary growth phases, measured by EA-IRMS. N = 5 and error bars indicate standard deviation. Asterisks mark significant differences (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$) between young and old clones when pooled together, whereby N = 20 for all except under nitrate-limited conditions during the exponential growth phase when N = 15 for old clones and carbon assimilation, and N = 16 for young clones for both carbon- and nitrate-assimilation, due to technical constraints.

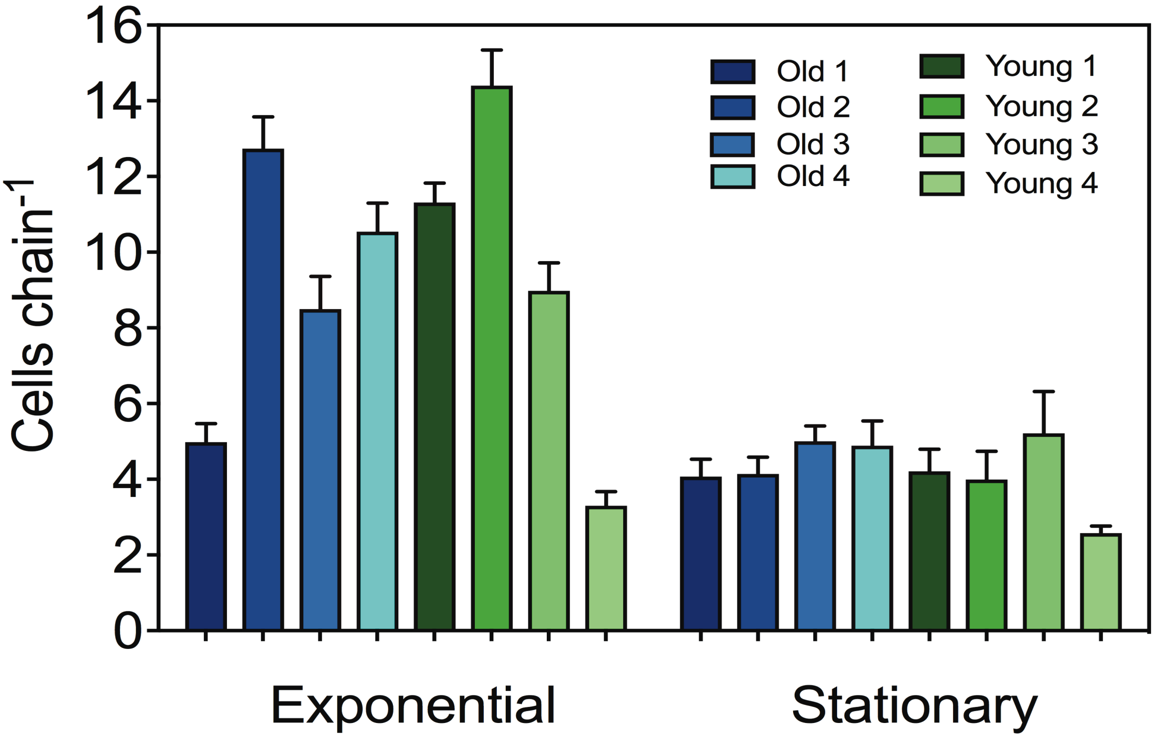

Fig. S5 Chain length of *Skeletonema marinoi.* Chain length (cells chain^{-1}) from microscopy analyses of old and young clones of *S. marinoi* under nitrate-limited conditions, during exponential and stationary growth phases. N = 5 and error bars indicate standard deviation.

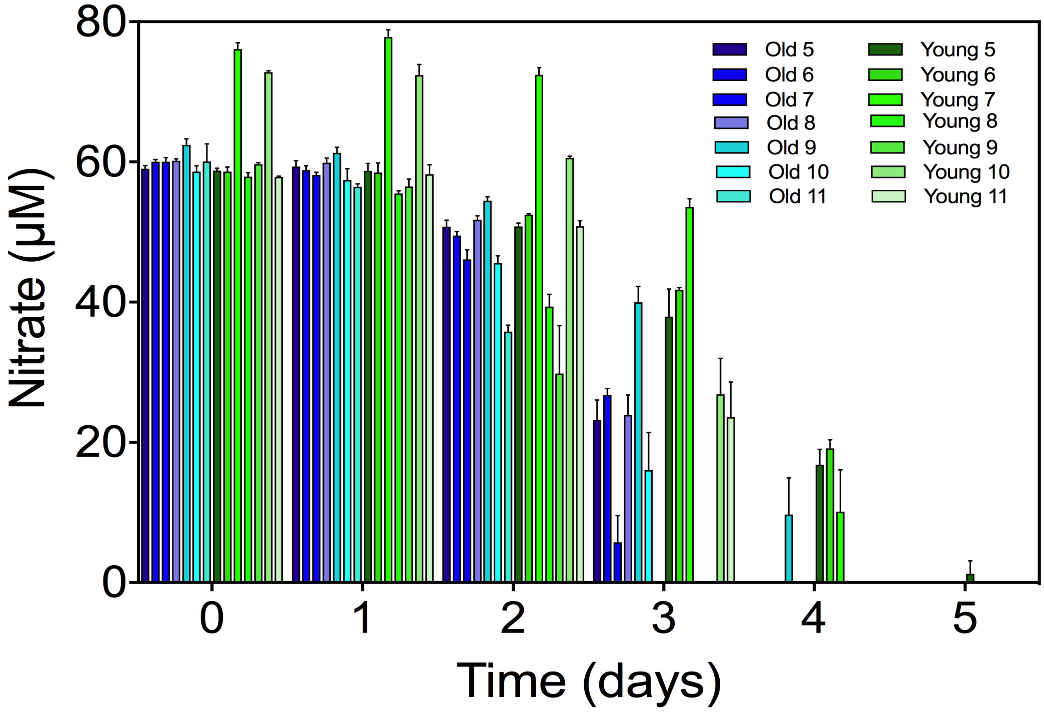

Fig. S6 Dissolved inorganic nitrate concentrations (µM) under nitrate-limited conditions at a high time-resolution for all clones until the nitrate concentration was close to non-detectable after 5 d. N = 3, error bars indicate standard deviation.

Table S1 Growth rates of particulate organic carbon (POC) and nitrogen (PON) at a clone-specific level measured by EAIRMS, and C- and N-growth rates at cell-specific levels during the exponential growth phase measured by SIMS. Rates are shown for old (O) and young (Y) clones under nitrate replete and limited conditions.

Clone/ N-condition.	Rel. POC increase+ (d^{-1})	Rel. PON increase+ (d^{-1})	POC-growth rate* (d^{-1})	PON-growth rate* (d^{-1})	C-growth rate (single cell level)° (d^{-1})	N-growth rate (single cell level)° (d^{-1})
O1-replete	2.40	1.68	0.88	0.52	1.08	0.69
O2- replete	2.00	1.65	0.69	0.50	1.08	0.56
O3- replete	2.27	1.62	0.82	0.48	1.14	0.21
O4- replete	2.77	2.33	1.02	0.85	1.10	0.26
Y1- replete	2.48	1.79	0.91	0.58	1.08	1.11
Y2- replete	2.24	1.78	0.81	0.58	1.13	1.16
Y3- replete	2.49	2.72	0.91	1.00	0.98	1.12
Y4- replete	2.06	1.52	0.72	0.42	1.1	1.14
O1-limited	3.27	2.44	1.18	0.89	1.23	1.75
O2- limited	2.38	2.69	0.87	0.99	1.12	2.17
O3- limited	2.40	2.24	0.88	0.81	1.16	2.41
O4- limited	2.79	3.81	1.02	1.34	1.11	2.0
Y1- limited	2.46	2.87	0.90	1.05	1.02	2.12
Y2- limited	3.26	2.64	1.18	0.97	1.29	2.62
Y3- limited	2.83	3.37	1.04	1.22	0.92	1.85
Y4- limited	3.39	3.71	1.22	1.31	1.27	2.69
Average	2.59	2.43	0.94	0.84	1.11	1.49
Stdev	0.42	0.75	0.16	0.30	0.10	0.82

+measured by EAIRMS
* calculated as ln(rel. POC or PON increase) during exponential growth
° measured by SIMS and calculated from Eq. 3

Table S2 Carbon (C)- and nitrogen (N)-specific growth rate under nitrate-replete conditions at exponential growth phase, and nitrate-limited conditions at exponential and stationary growth phase, based on individual cell measurements by SIMS (mean ± SD). N = 56-265 cells per strain.

	Nitrate-replete		Nitrate-limited			
	Exponential C-growth	Exponential N-growth	Exponential C-growth	Exponential N-growth	Stationary C-growth	Stationary N-growth
Young 1	0.54 ± 0.08	0.35 ± 0.06	0.51 ± 0.20	0.87 ± 0.08	0.04 ± 0.01	0.03 ± 0.02
Young 2	0.54 ± 0.08	0.28 ± 0.04	0.65 ± 0.07	1.09 ± 0.14	0.04 ± 0.01	0.03 ± 0.01
Young 3	0.57 ± 0.05	0.10 ± 0.01	0.58 ± 0.08	1.21 ± 0.15	0.03 ± 0.01	0.02 ± 0.01
Young 4	0.55 ± 0.06	0.13 ± 0.02	0.56 ± 0.10	1.00 ± 0.19	0.03 ± 0.01	0.02 ± 0.01
Young pooled	0.55 ± 0.07	0.20 ± 0.10	0.59 ± 0.09	1.07 ± 0.19	0.03 ± 0.01	0.02 ± 0.01
Old 1	0.54 ± 0.06	0.55 ± 0.07	0.51 ± 0.20	1.06 ± 0.39	0.03 ± 0.01	0.02 ± 0.03
Old 2	0.57 ± 0.08	0.58 ± 0.09	0.65 ± 0.07	1.31 ± 0.12	0.04 ± 0.01	0.02 ± 0.01
Old 3	0.49 ± 0.13	0.60 ± 0.17	0.46 ± 0.14	0.93 ± 0.20	0.03 ± 0.01	0.02 ± 0.01
Old 4	0.55 ± 0.05	0.57 ± 0.07	0.63 ± 0.06	1.35 ± 0.08	0.04 ± 0.01	0.02 ± 0.02
Old pooled	0.54 ± 0.09	0.57 ± 0.11	0.56 ± 0.15	1.17 ± 0.30	0.03 ± 0.01	0.02 ± 0.02

Table S3 Clone-specific particulate organic matter (POM) ratios for carbon (C), nitrogen (N) and phosphorus (P), and clone-specific carbon to nitrate assimilation ratios (mol). If available, ratios are included for nitrate-replete and nitrate-limited conditions for the exponential (Expo), stationary (Stat) and late stationary (Late) growth phases. Bold numbers with asterisks mark significantly higher ratios in old (O) or young (Y) clones pooled, where N = 20 for all except for numbers marked with plus (N = 15) or minus (N = 16) due to technical constraints.

		Nitrate-replete					Nitrate-limited			
		Expo C:N	Stat C:N	Late C:N	Late C:P	Late N:P	Expo C:N	Stat C:N	Stat C:P	Stat N:P
POM-ratios	Y1	**8.4****	10.2	10.2	217.3	21.4	7.2-	27.7	179.1	6.5
	Y2	**7.5****	9.4	9.4	235.2	22.9	5.8-	26.3	167.5	6.4
	Y3	**8.6****	10.5	10.5	234.1	23.4	6.5-	24.6	159.3	6.5
	Y4	**8.3****	9.9	9.9	175.3	22.0	7.3-	25.2	174.6	7.0
	O1	8.2	10.9	11.5	351.7	**30.8***	**7.7*****	23.5	158.5	6.8
	O2	7.3	8.3	7.9	213.9	**25.7***	**7.3*****	29.4	177.8	6.1
	O3	7.7	8.2	5.8	120.6	**21.0***	**9.1*****	24.0	140.0	5.8
	O4	7.9	9.8	7.0	172.1	**26.0***	**7.6*****	26.3	163.1	6.2
carbon:nitrate assimilation ratios	Y1	**19.0*****	30.6	7.2			2.0-	116.7		
	Y2	**21.1*****	9.3	5.7			4.1-	141.9		
	Y3	**68.5*****	8.5	3.2			3.9-	140.3		
	Y4	**60.6*****	6.4	2.8			5.3-	110.5		
	O1	10.4	13.3	**4.7***			**4.7*+**	**115.0****		
	O2	9.4	13.6	**50.1***			MD	**188.5****		
	O3	7.9	18.7	**3.5***			**6.3*+**	**157.8****		
	O4	10.8	18.5	**3.6***			**3.9*+**	**137.4****		

Bold * = p-value < 0.05, ** = p-value < 0.01, *** = p-value < 0.001; One-way ANOVA; MD, missing data due to technical constraints

Table S4 Particulate organic carbon (POC), nitrogen (PON) and phosphorus (POP) concentrations in µM for all clones under nitrate-replete and nitrate-limited conditions. If available, concentrations are included for the exponential (Expo), stationary (Stat) and late stationary (Late) growth phases. Bold numbers with asterisks mark significantly higher concentrations in young (Y) or old (O) clones when pooled, where N = 20 for all except for numbers marked with a plus (N = 15) or minus (N = 16) symbol due to technical constraints.

	Nitrate-replete							Nitrate-limited				
	Expo POC	Stat POC	Late POC	Expo PON	Stat PON	Late PON	Late POP	Expo POC	Stat POC	Expo PON	Stat PON	Stat POP
Y1	356.0	1274.8	2043.9	63.2	126.0	200.6	**9.8***	417.1-	1566.1	**58.0*-**	56.5	9.1
Y2	328.7	1277.6	2070.8	65.7	136.6	200.6	**9.1***	287.1-	1420.4	**49.6*-**	53.9	8.8
Y3	349.1	1121.8	1941.3	61.2	107.2	196.5	**8.8***	312.5-	1347.5	**48.4*-**	54.9	8.8
Y4	330.1	1069.9	1601.8	59.3	107.9	197.1	**9.4***	240.5-	1356.2	**32.9*-**	53.9	8.0
O1	**541.3***	1168.4	2521.9	66.4	107.4	219.9	7.5	266.2+	1361.6	34.7	**58.0***	**9.0***
O2	**522.8***	1171.2	1664.8	71.6	141.2	202.8	7.7	MD	1687.6	50.2	**57.5***	**9.9***
O3	**161.8***	930.3	1021.5	20.9	114.0	176.0	8.8	245.4+	1280.8	27.1	**53.4***	**9.5***
O4	**512.2***	1216.7	1470.4	64.9	124.3	206.2	8.5	309.0+	1510.6	40.9	**57.3***	**9.6***

Bold * = p-value < 0.05, ** = p-value < 0.01, *** = p-value < 0.001; One-way ANOVA; MD, missing data due to technical constraints

Table S5 Dissolved inorganic nitrate concentrations (μM) for day (D) 0, 4 and 6 under nitrate-limited conditions at a high time-resolution. The particulate organic nitrogen (PON) concentration at day 4 (D4) and day 6 (D6) (μM) and the %-increase PON concentration between the days. The particulate organic carbon (POC) concentration at D4 and D6 (μM), and the %-increase between the days, as well as POC:PON ratios (mol:mol) for D4 and D6 for young (Y) and old (O) clones (N= 3).

	Nitrate concentration			PON concentration		Change	POC concentration		Change	POC:PON	
	D0	D4	D6	D4	D6	PON %	D4	D6	POC %	D4	D6
Y5	58.8	16.8	0.0	42.4	49.0	15.6	475.4	963.6	102.7	11.3	19.7
Y6	58.6	19.1	0.0	47.0	54.2	15.3	328.3	846.0	157.7	7.0	15.6
Y7	76.1	10.1	0.0	70.7	72.3	2.3	486.9	1057.3	117.1	6.9	14.6
Y8	57.9	0.0	0.0	51.9	56.9	9.7	670.0	1074.2	60.3	12.9	18.9
Y9	59.7	0.0	0.0	52.1	60.2	15.5	673.6	1137.7	68.9	12.9	18.9
Y10	72.8	0.0	0.0	69.1	77.6	12.3	579.7	1129.2	94.8	8.4	14.5
Y11	57.9	0.0	0.0	55.0	58.4	6.1	569.2	1128.1	98.2	10.3	19.3
O5	59.0	0.0	0.0	50.2	MD	MD	577.6	MD	MD	11.5	MD
O6	60.0	0.0	0.0	53.1	MD	MD	495.2	MD	MD	9.3	MD
O7	60.0	0.0	0.0	50.3	MD	MD	638.7	MD	MD	12.7	MD
O8	60.2	0.0	0.0	50.1	53.2	6.2	547.8	1010.9	84.5	10.9	19.0
O9	62.4	9.7	0.0	53.7	59.3	10.4	366.6	1033.6	181.9	6.8	17.4
O10	58.6	0.0	0.0	49.3	64.8	31.4	559.4	1236.2	121.0	11.4	19.1
O11	60.1	0.0	0.0	49.8	58.3	17.1	663.0	1262.3	90.4	13.3	21.7

MD, missing data due to technical constraints