frontiers

# *Supplementary Material*

## 1 SUPPLEMENTARY TABLES AND FIGURES

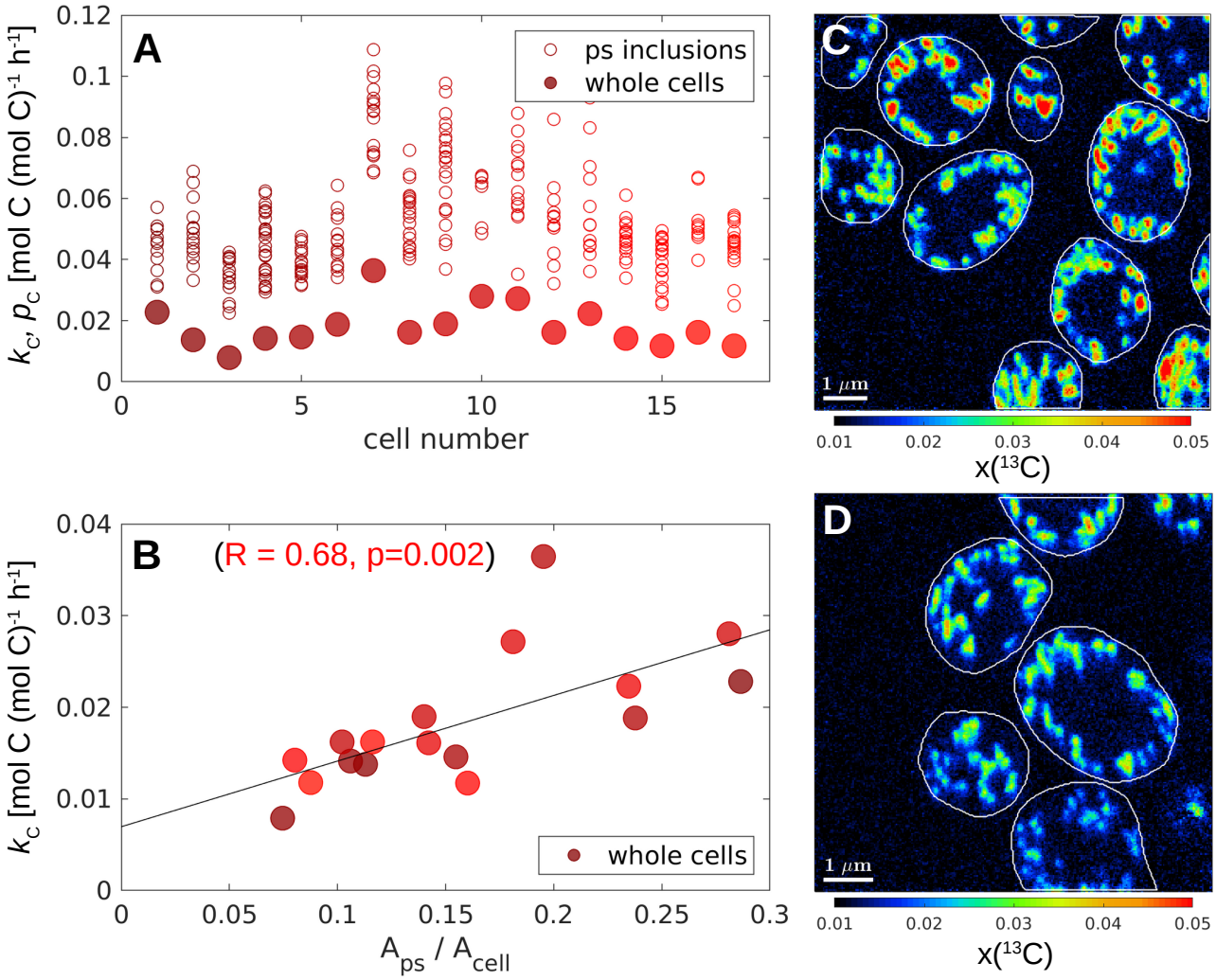
**Table S1.** Relevant chemical and isotopic characteristics of the incubation media used for SIP experiments.

| Incubation period | Culture | DIC[†] | | $N_2$ or $NO_3$ | |
|---|---|---|---|---|---|
| | | $[\mathrm{mmol\,L^{-1}}]$ | $x(^{13}C)_S$ | $[\mathrm{mmol\,L^{-1}}]$ | $x(^{15}N)_S$ |
| 07:30–09:30 (morning) | $N_2$ | 1.4 | 0.23 | 0.3 | 0.06 |
| | $NO_3$ | 1.2 | 0.28 | 22.7 | 0.07 |
| | SC-$N_2$ | 1.4[*] | 0.23 | 0.3 | 0.06 |
| 14:45–17:15 (afternoon) | $N_2$ | 0.5 | 0.67 | 0.3 | 0.06 |
| | $NO_3$ | 0.4 | 0.86 | 22.7 | 0.07 |
| | SC-$N_2$ | 0.3 | 0.99 | 0.3 | 0.06 |
| 21:45–17:15 (night+day) | $N_2$ | 0.6[§] | 0.53 | 0.3 | 0.06 |
| | SC-$N_2$ | 0.4[§] | 0.74 | 0.3 | 0.06 |
| 21:45–00:45 (early night) | $N_2$ | 0.6[§] | 0.53 | 0.3 | 0.06 |
| 02:00–07:00 (late night) | $N_2$ | 1.0[§] | 0.32 | 0.3 | 0.06 |
| 21:45–07:00 (full night) | $N_2$ | 0.6[§] | 0.53 | 0.3 | 0.06 |
| | $NO_3$ | 0.5[§] | 0.68 | 16.2 | 0.10 |
| | SC-$N_2$ | 0.4[§] | 0.74 | 0.3 | 0.06 |

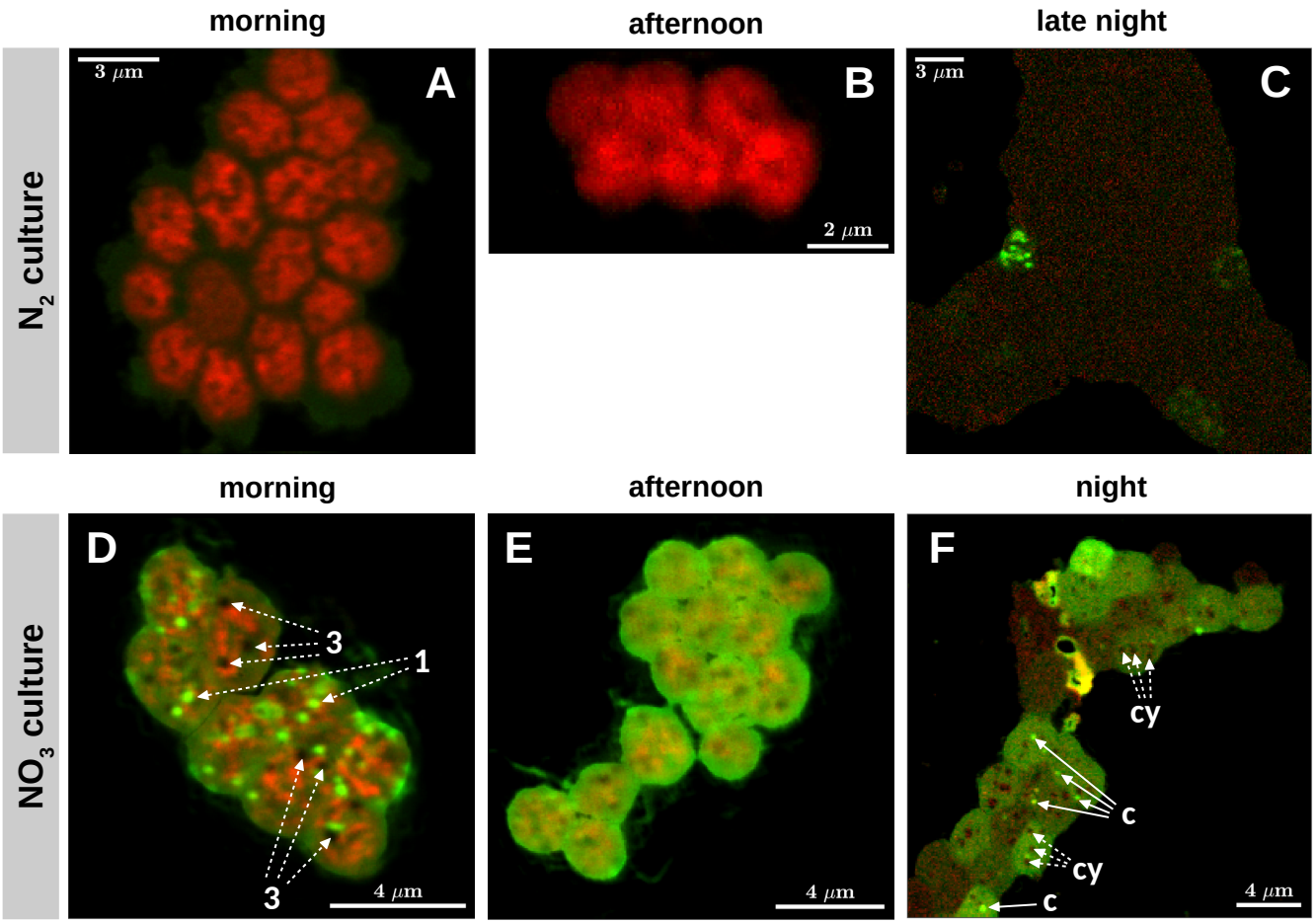
[†] DIC concentrations $[\mathrm{mmol\,L^{-1}}]$ and $^{13}C$ atom fractions $[x(^{13}C)_S]$ were calculated based on DIC measurements in samples from bioreactors taken at the same time point and the known amount of added $NaH^{13}CO_3$.

[*] Value was assumed to be the same as in the $N_2$ culture.

[§] Values were interpolated from DIC concentrations measured at 07:00, 09:00 and 14:00.




**Supplementary Figure S1. Intra-cellular heterogeneity of C assimilation in $N_2$-fixing *Cyanothece* 51142.** Shown are data for cells from the $N_2$ culture incubated in the morning (07:30–09:30). **(A)** Carbon-specific rates of C assimilation in individual polysaccharide (ps) inclusions ($p_C$; open symbols) and in whole cells ($k_C$; filled symbols), calculated for 279 inclusions in 17 selected cell sections. $p_C$ varied among polysaccharide inclusions (CV $= 32\%$), with 61% and 39% of the total variance explained by differences among and within cells, respectively ($\mathrm{SS_{groups}}/\mathrm{SS_{total}} = 0.0455/0.0746 = 0.61$, $\mathrm{SS_{error}}/\mathrm{SS_{total}} = 0.0291/0.0746 = 0.39$). Variation in $p_C$ values among cells was significant (ANOVA, $F(16, 262) = 25.6$, $p = 10^{-44}$). **(B)** Correlation between $k_C$ in whole cells and the relative area of the cell sections covered by polysaccharide inclusions, $A_{\mathrm{ps}}/A_{\mathrm{cell}}$ (coefficient of determination $R^2 = 0.46$). The correlation reveals that the cell-specific $^{13}$C enrichment depends on the cell volume probed by nanoSIMS. **(C–D)** Examples of $^{13}C$ atom fraction images from which data in panels A–B were calculated. White lines show approximate cell outlines, $^{13}C$-enriched spots correspond to polysaccharide inclusions.




**Supplementary Figure S2. Images of the isotopic composition of *Cyanothece* 51142 cells.** Shown are additional overlays of the $^{13}C$ (red) and $^{15}N$ (green) atom fractions measured in cells from the $N_2$ and $NO_3$ culture deposited on polycarbonate membrane filters. Note that panel C shows cells from the $N_2$ culture incubated during late night (02:00–07:00). In panels D and F, examples of cyanophycin inclusions (cy) and carboxysomes (c) are marked with dashed-line and solid-line arrows, respectively.