Supplementary Data and Methods for: Forfeiting the priority effect: turnover defines biofilm community succession

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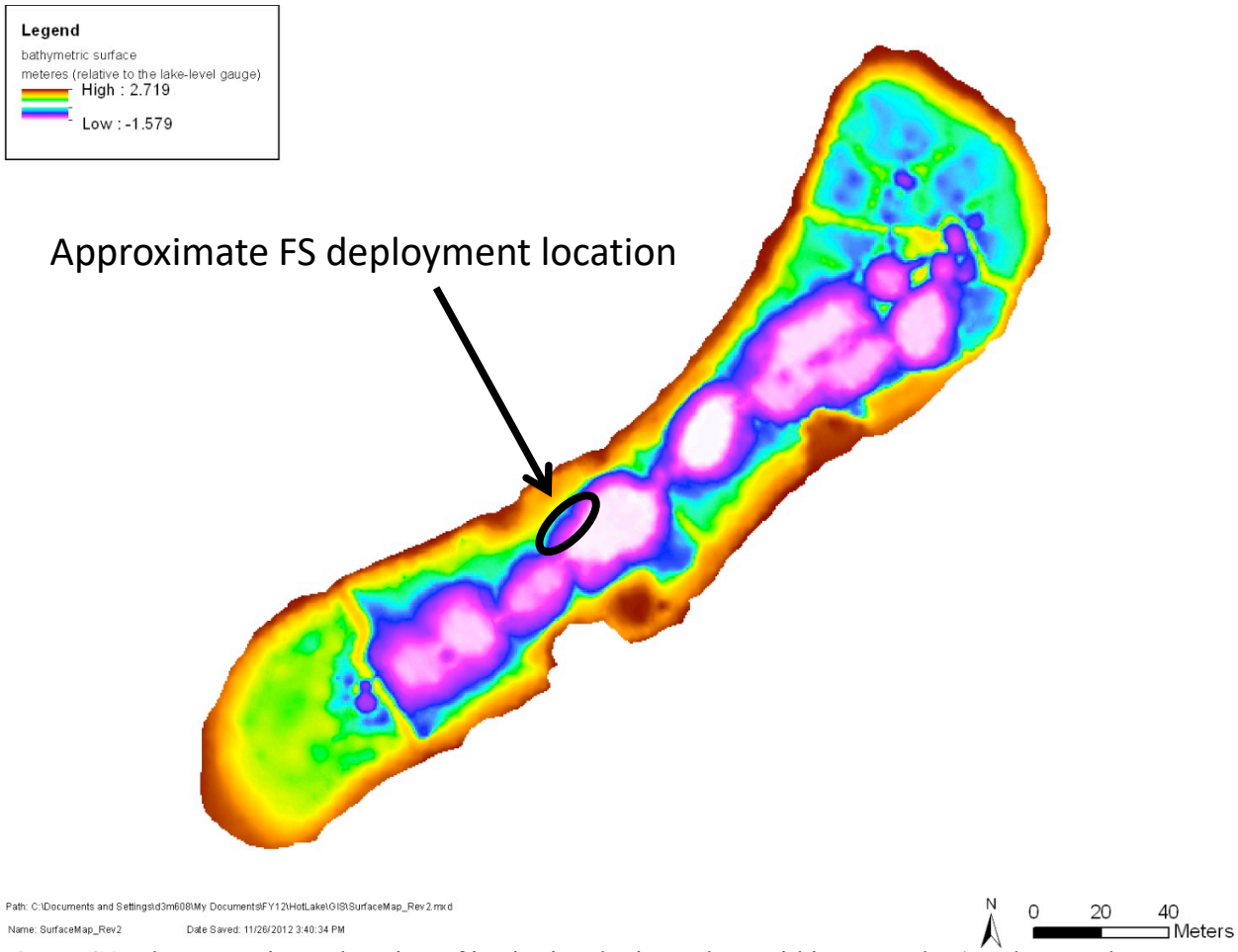


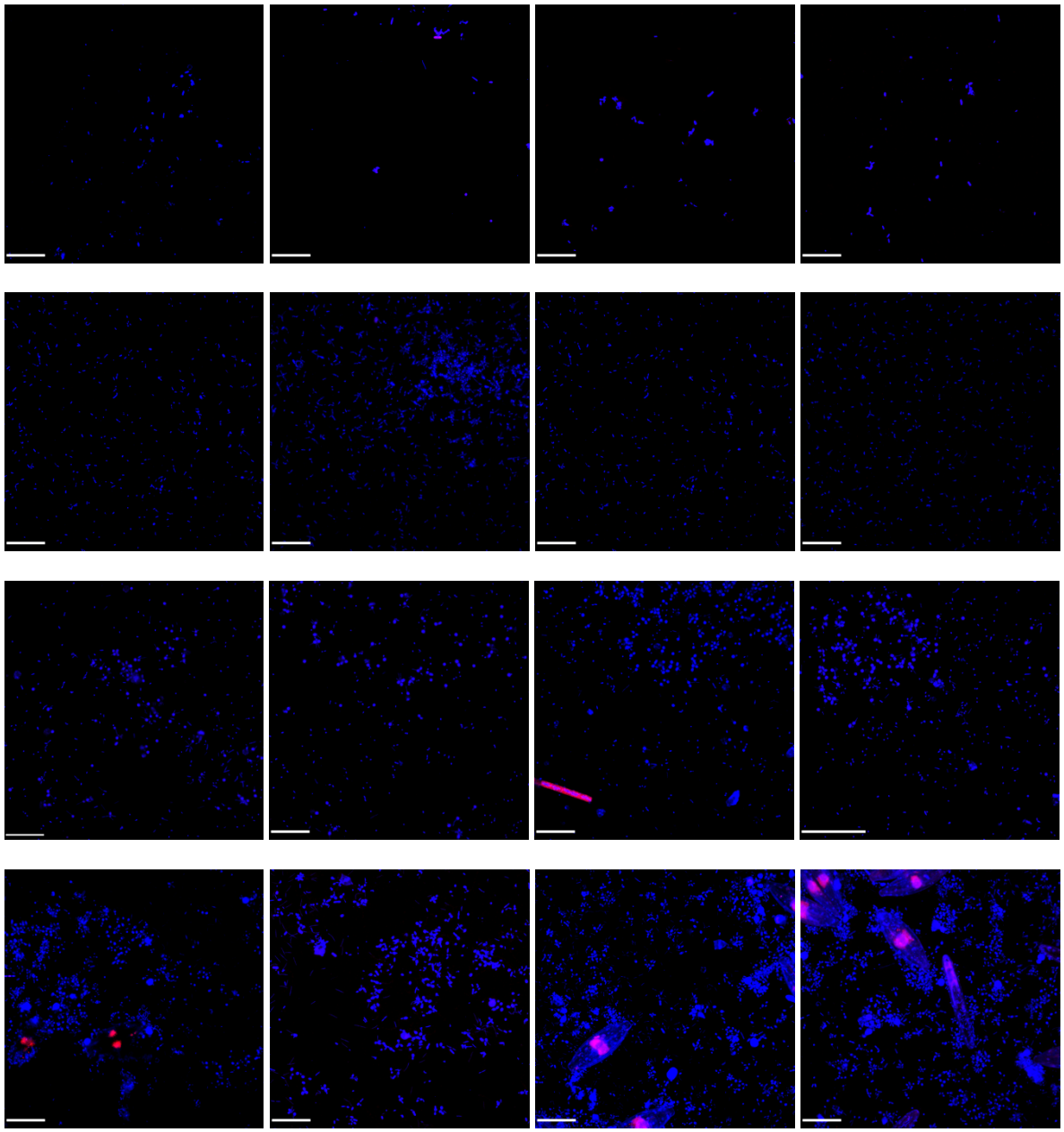
Figure S1. The approximate location of in situ incubations place within Hot Lake (north-central Washington State, USA; 48.973062°N, 119.476876°W). Incubations were performed on sterile, borosilicate surfaces deployed in 43-50 cm water depth.

Detailed methods for stable isotope probing. Petri dish containing glass coverslips were harvested from each time point and transferred (in 4°C coolers) to a mobile laboratory within 2-6 h of sampling. Incubations with stable isotope tracers were performed with ^{13}C labeled HCO_3- and ^{15}N labeled N_2 gas. The glass coverslips sampled from the lake at each time point were divided between two 950 mL sterile pyrex dishes, each charged with 500 mL of a previously described HLA-400 (Cole et al 2014) medium minus $NaHCO_3$, TES buffer and $NH_4Fe(III)$ citrate. A control incubation was established to account for the natural abundance of ^{13}C by adding 1.5 mL of a 63.1875 g L^{-1} $NaHCO_3$ solution. The labeled incubations were prepared by mixing 0.75 mL of this natural abundance solution with 0.75 mL of a 63.1875 g L^{-1} of ^{13}C labeled $NaHCO_3$ solution (lot number Pr-24217, Cambridge Isotope Laboratory, Tewksbury, MA). The ^{15}N labeled N_2 incubations were performed in the HLA-400 medium (minus TES and $NH_4Fe(III)$ citrate) containing 1 mM $NaHCO_3$; 100 mL glass bottles were charged with 20 mL of degassed medium and 1 coverslip sample. Each bottle was capped with a septum and purged with N_2 for 10 min; the head space was charged with 20 mL (1 ATM) of $^{15}N_2$ (98 atom % 15N, product number 364584, Lot: MBBB0968V, Sigma-Aldrich, St. Louis, MO) using a needle and gas-tight syringe. Natural abundance controls were prepared identically by omitting the 20 mL injection of $^{15}N_2$ labels. The N_2 incubation bottles were wrapped in aluminum foil to maintain darkness and anoxic conditions. All incubations were performed for 6 h under a T5 grow light (40 µmol photons PAR m^{-2} s^{-1}; Hydrofarm, Inc., Petaluma, CA, USA). Following the incubation, the $NaHCO_3$ incubated coverslips were removed

using sterile forceps and rinsed in 75 mL tissue culture dishes primed with the HLA-400 medium for 30 min to wash away unincorporated bicarbonate; rinse solution was removed. At the conclusion of all incubations and rinses each coverslip was placed in a 3.5 mL of fresh medium containing 4% paraformaldehyde and stored at 4°C.

Detailed methods for NanoSIMS imaging. High-resolution secondary ion intensity and isotope ratio maps were generated using a NanoSIMS 50L (CAMECA, Gennevilliers, France) at the Environmental Molecular Sciences Laboratory (EMSL) at Pacific Northwest National Laboratory (PNNL). Biofilm-coated coverslips were mounted on one-inch-diameter aluminum pin stubs and coated with 20 nm of high-purity gold or iridium to improve conductivity. Because of widely varying conductivities of the individual samples several different strategies were employed to maximize secondary signal. Samples with acceptable conductivity were presputtered with 5×10^{15} ions cm^{-2}, before 10 40 × 40 μm planes were acquired with a dwell time of 2.7 ms $pixel^{-1}$ and at a density of 256 × 256 pixels. Samples from day-8 and day-79 samples were very insulating and a different analysis strategy was employed. These samples were presputtered with 1×10^{16} ions cm^{-2}, followed by 2 planes of analysis employing 13.5 ms $pixel^{-1}$ dwell time, with a normal incidence electron gun employed for charge neutralization An in-house yeast standard ($\delta^{13}C$ = -11.0‰, $\delta^{15}N$ = +0.35‰) was analyzed daily using conditions identical to the analysis of the biofilms to correct for instrumental mass fractionation. Analysis conditions included a 16 keV, 2 pA Cs^+ primary ion beam with ~120 nm diameter, a D1 aperture of 200 μm, a 30 μm entrance slit, 200 μm aperture slit, 100 μm exit slits and a centered energy window of ~6 eV (FWHM). Electron multiplier (EM) detectors measured intensities of $^{12}C_2^-$, $^{12}C^{13}C^-$, $^{12}C^{14}N^-$, $^{12}C^{15}N^-$, $^{28}Si^-$, $^{31}P^-$, and $^{32}S^-$ secondary ions in multicollection mode with NMR magnetic field regulation employed. Particular care was taken to resolve $^{12}C^{15}N^-$ from $^{13}C^{14}N^-$, $^{12}C^{13}C^-$ from $^{12}C_2H^-$, and P^- from $^{30}SiH^-$. m/z scans were performed frequently during each analytical session to assure that instrumental drift did not compromise data integrity. Image processing and analysis were conducted in ImageJ using the OpenMIMS plugin, available from the National Resource for Imaging Mass Spectrometry (NRIMS, http://www.nrims.harvard.edu). All images were corrected for deadtime and quasi-simultaneous arrival (QSA) effects ($\beta = 0.5$) and stack-aligned using a best fit alignment algorithm within the plugin. Isotope ratio distributions were converted to Hue-Saturation-Intensity (HSI) image maps generated from the ratios of masses $^{12}C^{13}C^-$ /$^{12}C_2^-$ and $^{12}C^{15}N^-$ /$^{12}C^{14}N^-$ for carbon and nitrogen isotopes, respectively. Isotope values of individual cells were extracted from pixel-averaged Regions of Interest (ROIs) manually drawn around corresponding image areas and calibrated to cell-averaged yeast standard values obtained from the standard yeast image for that session. Isotope ratios are expressed in traditional delta notation relative to the Vienna Pee Dee Belemnite (VPDB) and atmospheric N_2 (AIR) international reference standards for carbon and nitrogen, respectively.

Environmental measurements collected from field. We took measurements of the lake and mat environment at every time point corresponding to the molecular measurements presented in the main text. The pH, temperature, conductivity, and incident irradiance was also measured using a Sond (MS5, Hydrolab, Loveland, CO,) and a radiometer (LI-COR 250-A light meter and Quantum sensor, LI-COR Biosciences, Lincoln, NE) in the lake water overlaying the sampling position at each time point.



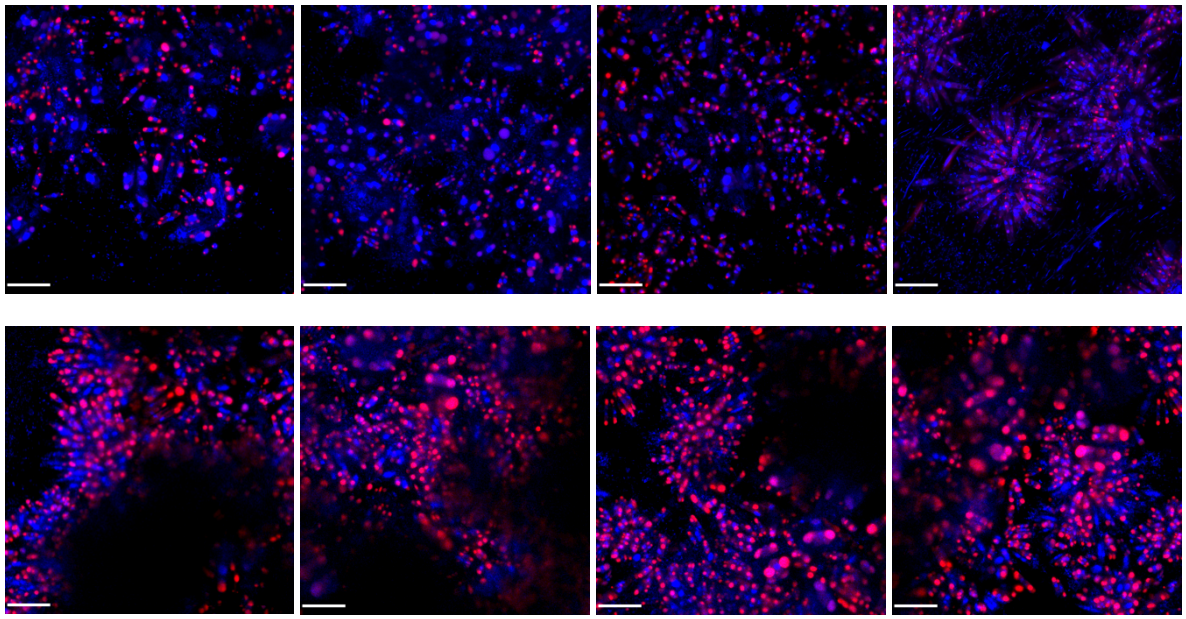
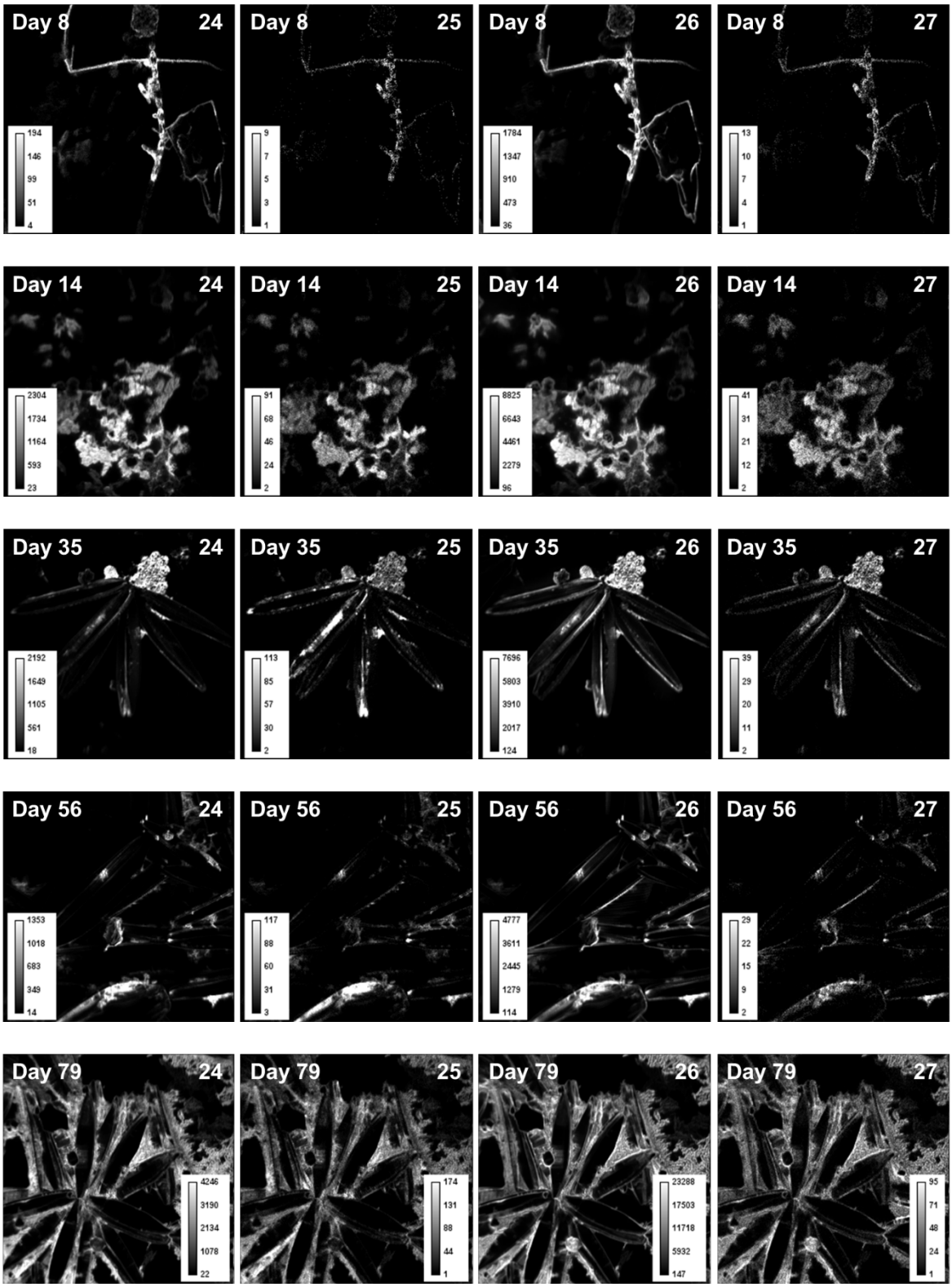
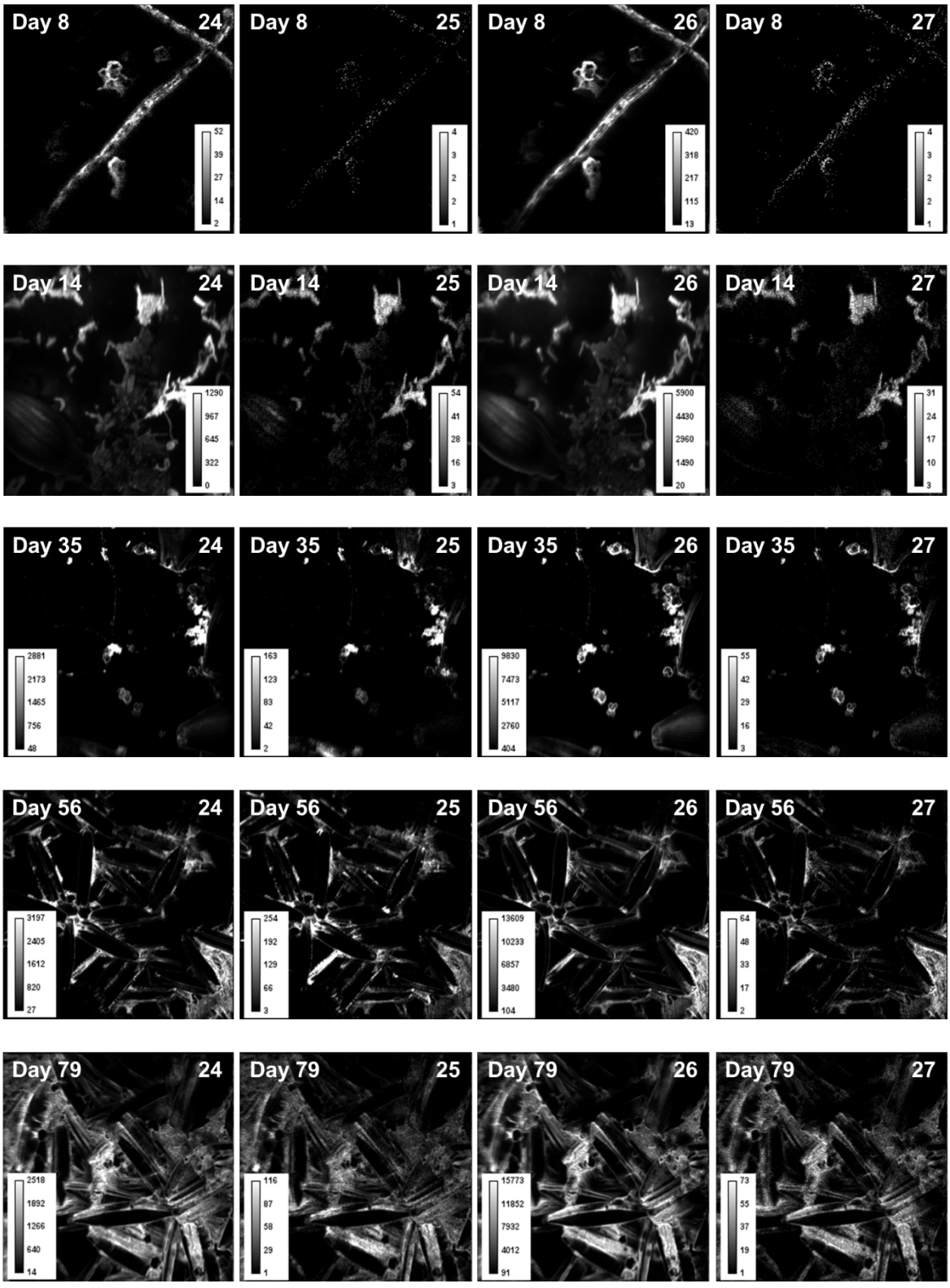


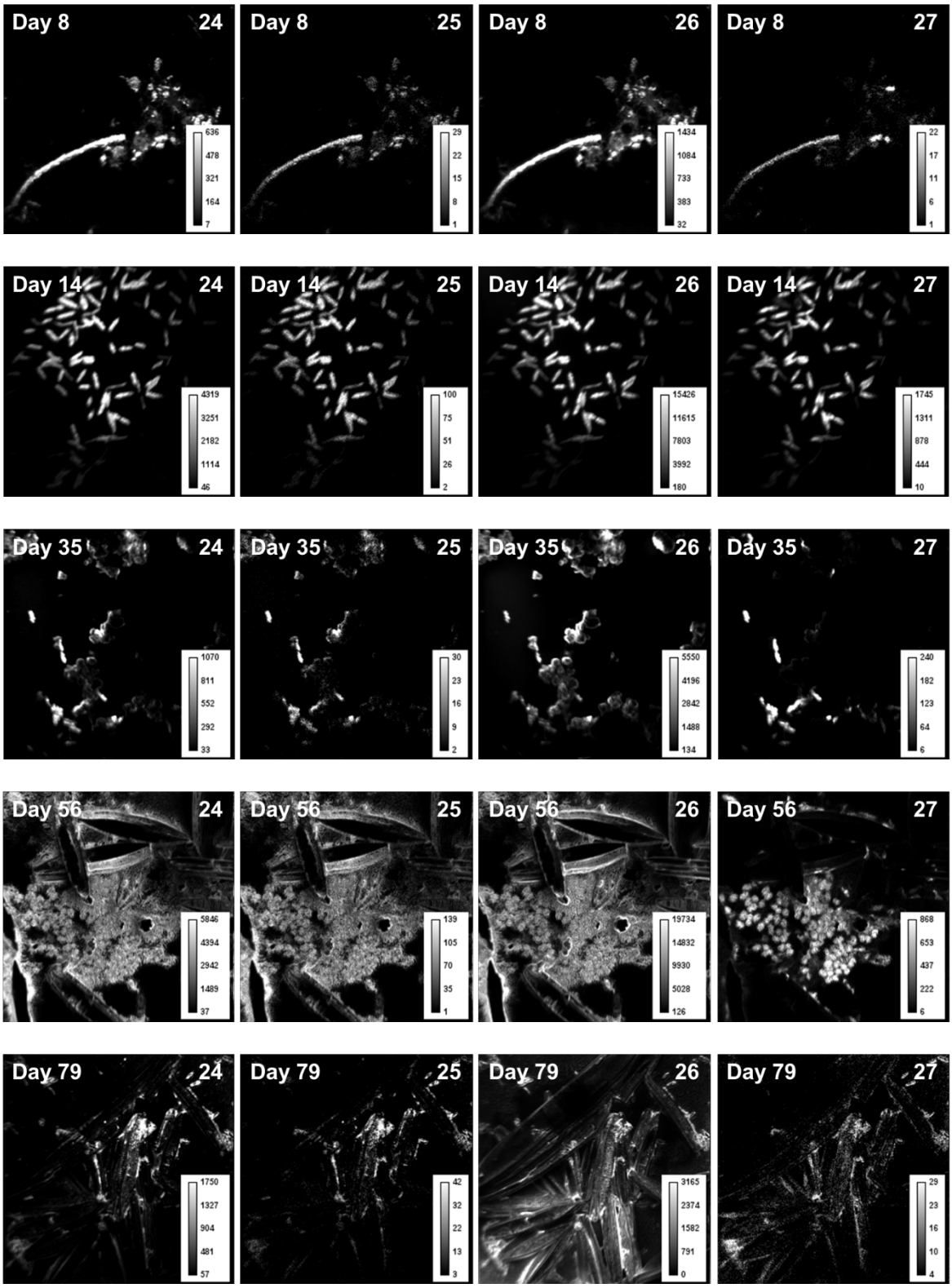
Figure S2. The comprehensive collection of confocal micrographs showing changes in biofilm community morphology as visualized by the DNA stain (Hoechst; blue) and chlorophyll autofluorescence (red); scale bars represent 20 µm. The rows of images or organized in descending order and correspond to the following experimental sampling points: 1-day, 8-day, 14-day, 35-day, 56-day and 79-day samples. Each image is 1 of 5 replicate frames; four are shown here and the remainder are shown in within Figure 2 of the main text.

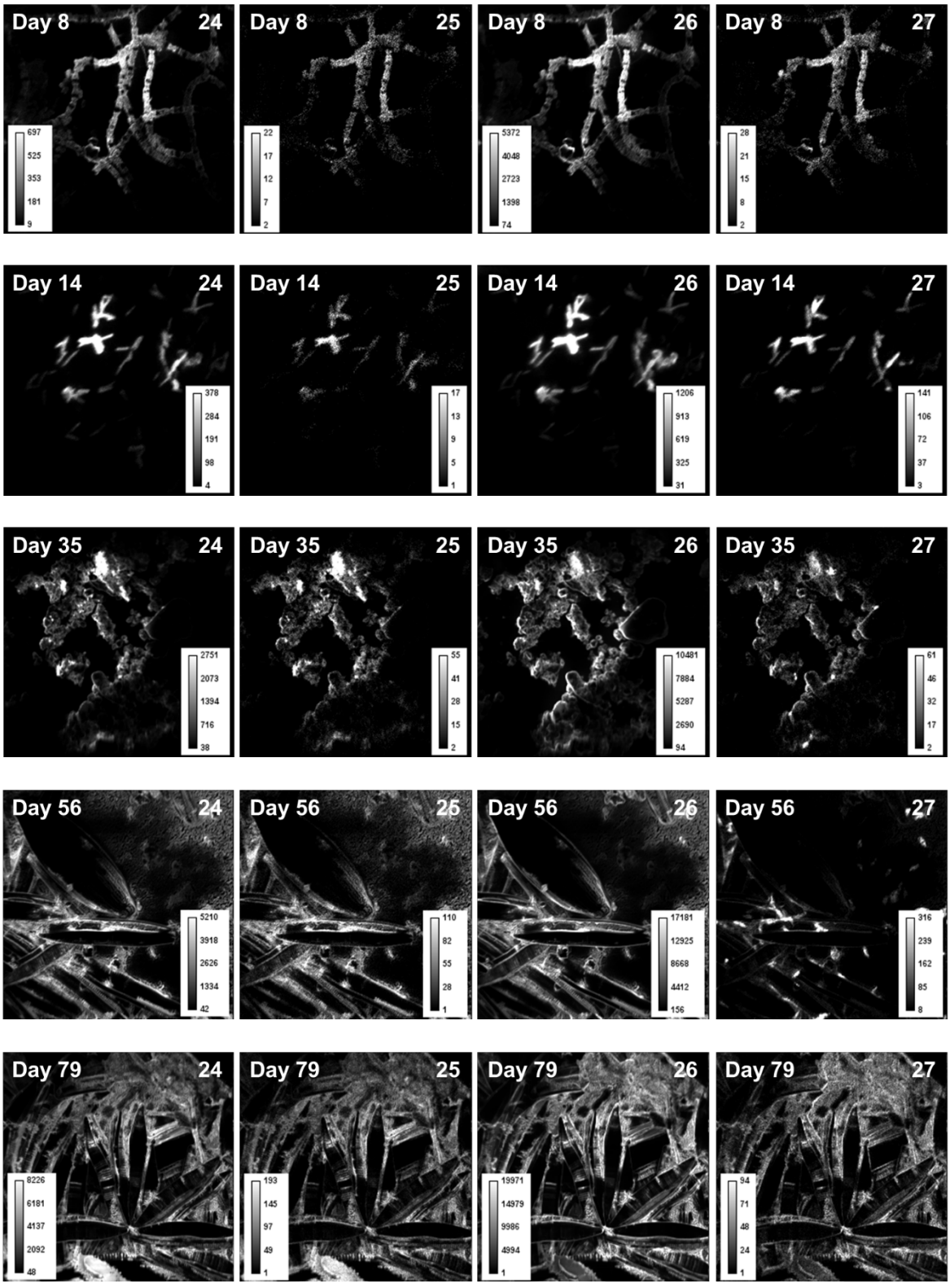
A) ^{13}C-labeled bicarbonate incubation



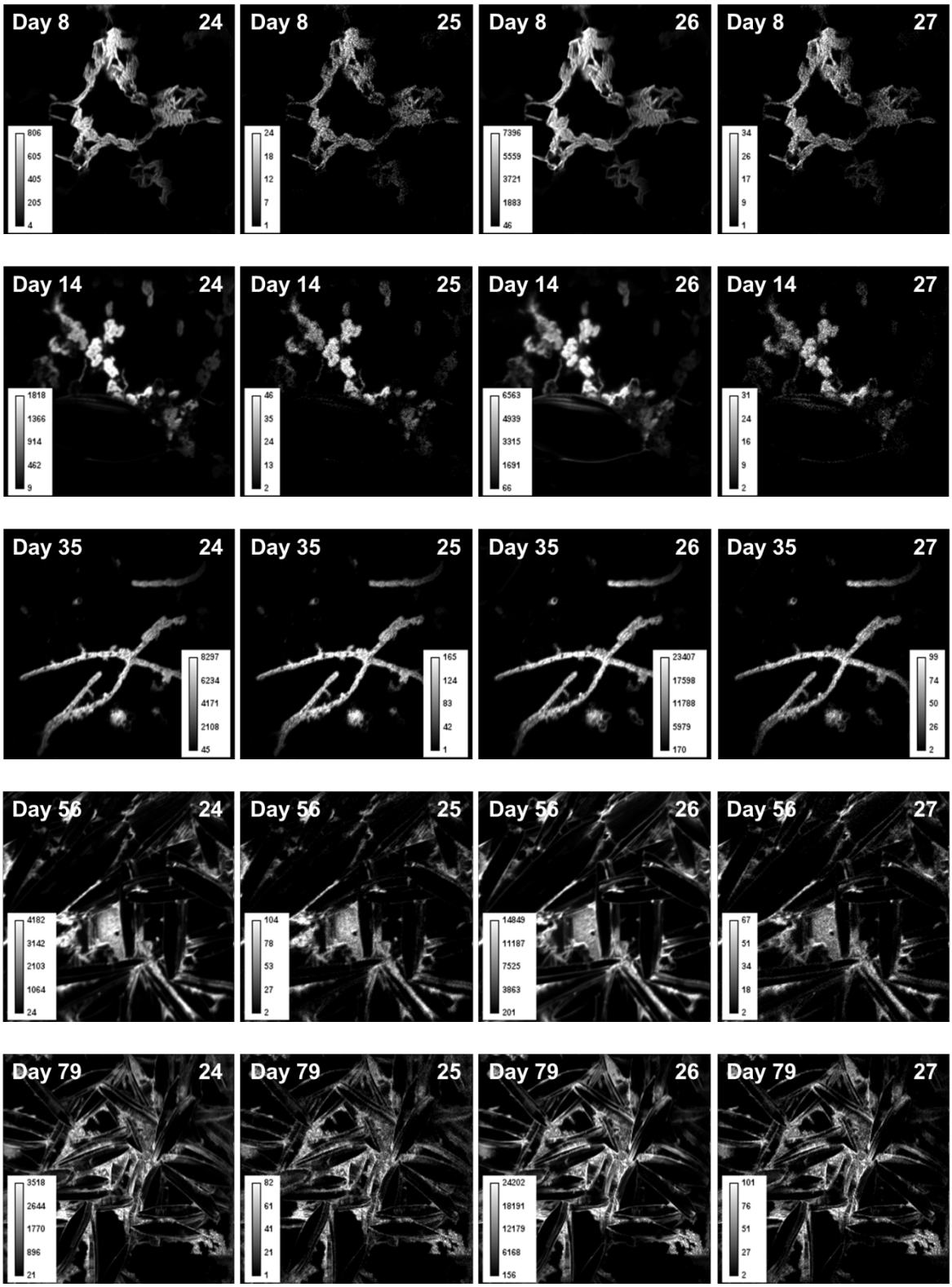

Day 8 24
52 39 27 14 2
Day 8 25
4 3 2 2 1
Day 8 26
420 318 217 115 13
Day 8 27
4 3 2 2 1
Day 14 24
1290 967 645 322 0
Day 14 25
54 41 28 16 3
Day 14 26
5900 4430 2960 1490 20
Day 14 27
31 24 17 10 3
Day 35 24
2881 2173 1465 756 48
Day 35 25
163 123 83 42 2
Day 35 26
9830 7473 5117 2760 404
Day 35 27
55 42 29 16 3
Day 56 24
3197 2405 1612 820 27
Day 56 25
254 192 129 66 3
Day 56 26
13609 10233 6857 3480 104
Day 56 27
64 48 33 17 2
Day 79 24
2518 1892 1266 640 14
Day 79 25
116 87 58 29 1
Day 79 26
15773 11852 7932 4012 91
Day 79 27
73 55 37 19 1

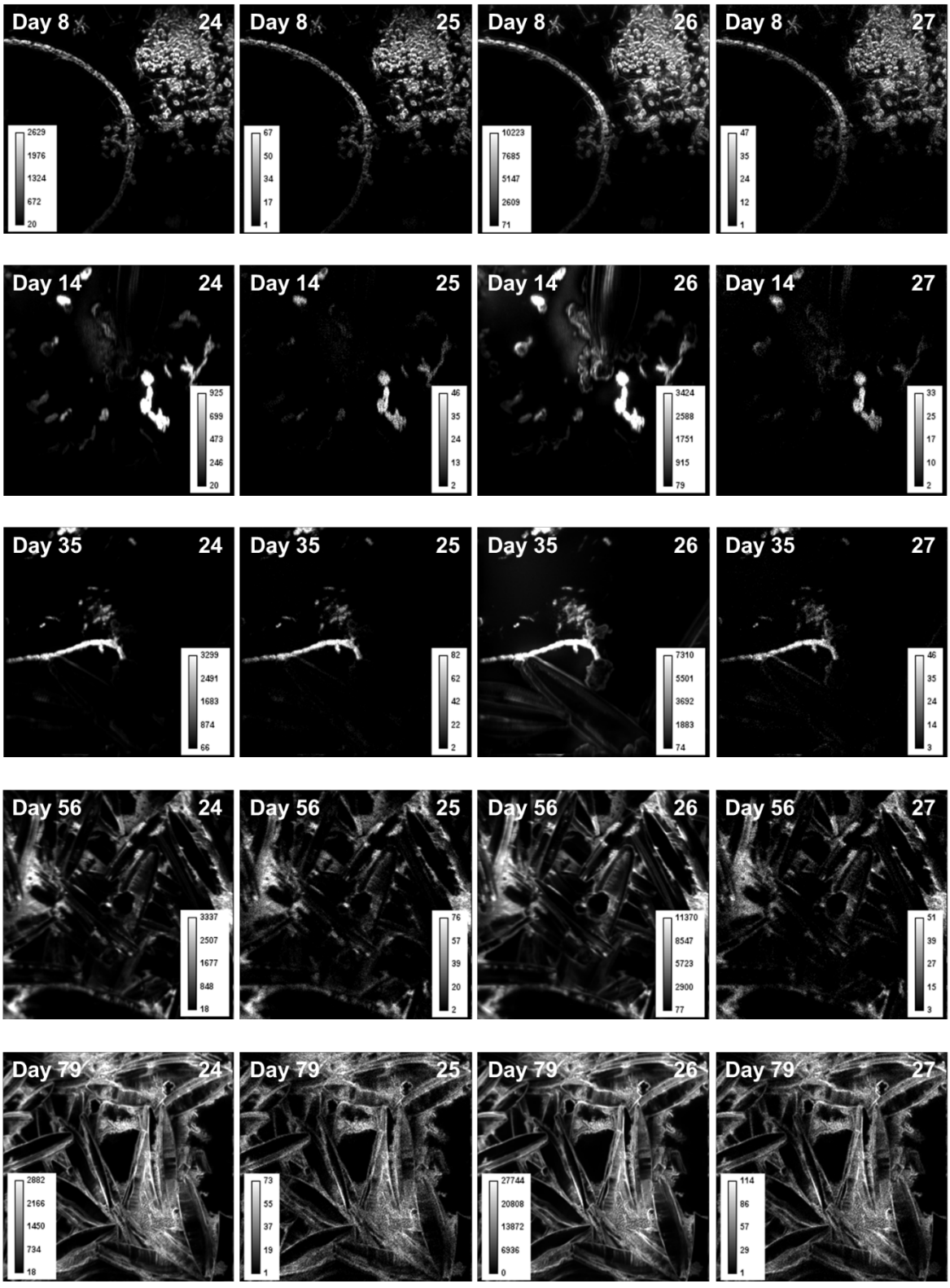
B) ^{15}N-labeled N_2 incubation



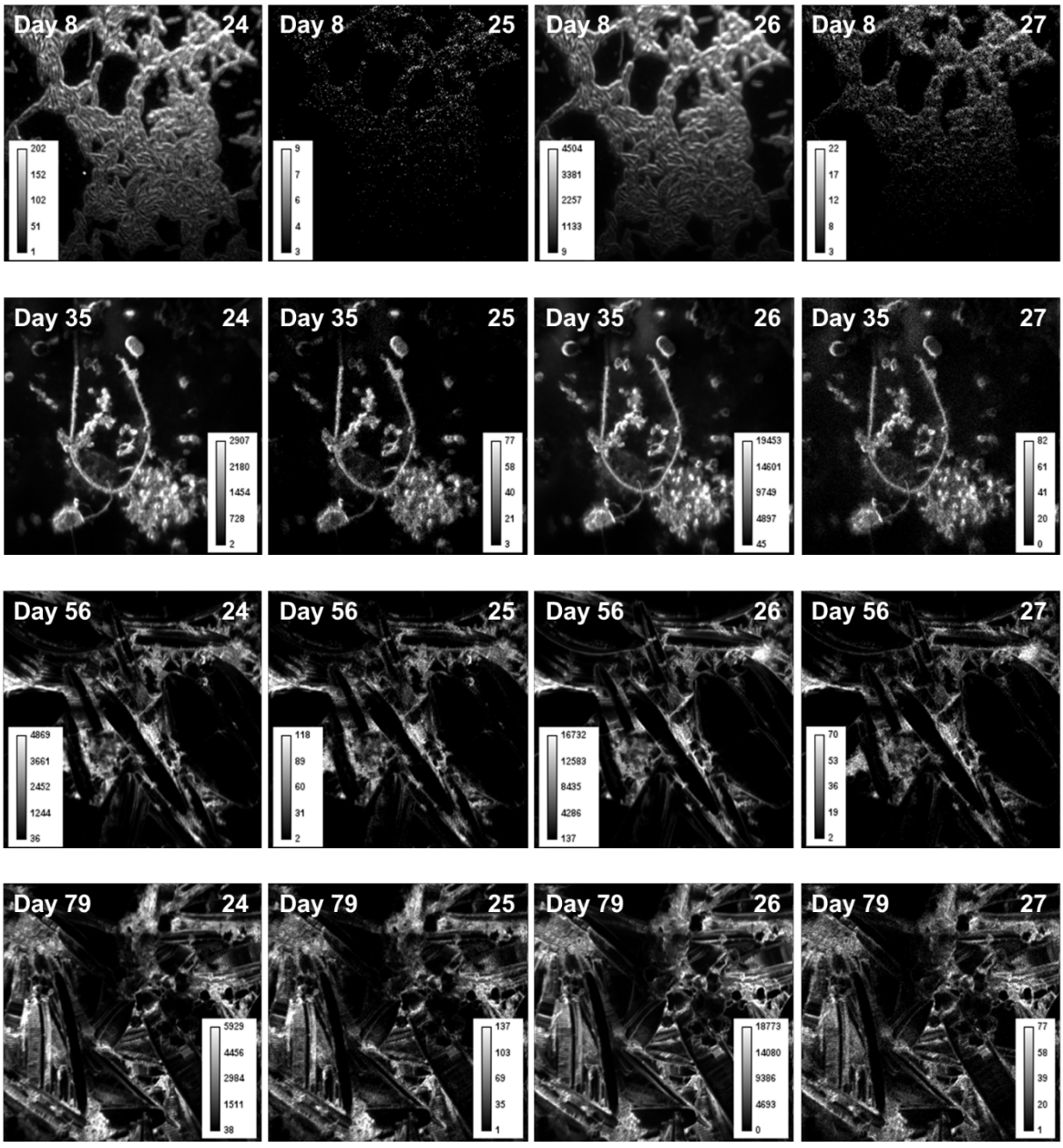

Day 8 24
697
525
353
181
9
Day 8 25
22
17
12
7
2
Day 8 26
5372
4048
2723
1398
74
Day 8 27
28
21
15
8
2
Day 14 24
378
284
191
98
4
Day 14 25
17
13
9
5
1
Day 14 26
1206
913
619
325
31
Day 14 27
141
106
72
37
3
Day 35 24
2751
2073
1394
716
38
Day 35 25
55
41
28
15
2
Day 35 26
10481
7884
5287
2690
94
Day 35 27
61
46
32
17
2
Day 56 24
5210
3918
2626
1334
42
Day 56 25
110
82
55
28
1
Day 56 26
17181
12925
8668
4412
156
Day 56 27
316
239
162
85
8
Day 79 24
8226
6181
4137
2092
48
Day 79 25
193
145
97
49
1
Day 79 26
19971
14979
9986
4994
1
Day 79 27
94
71
48
24
1

C) Natural abundance bicarbonate incubation




Day 8 24
2629
1976
1324
672
20
Day 8 25
67
50
34
17
1
Day 8 26
10223
7685
5147
2609
71
Day 8 27
47
35
24
12
1
Day 14 24
925
699
473
246
20
Day 14 25
46
35
24
13
2
Day 14 26
3424
2588
1751
915
79
Day 14 27
33
25
17
10
2
Day 35 24
3299
2491
1683
874
66
Day 35 25
82
62
42
22
2
Day 35 26
7310
5501
3692
1883
74
Day 35 27
46
35
24
14
3
Day 56 24
3337
2507
1677
848
18
Day 56 25
76
57
39
20
2
Day 56 26
11370
8547
5723
2900
77
Day 56 27
51
39
27
15
3
Day 79 24
2882
2166
1450
734
18
Day 79 25
73
55
37
19
1
Day 79 26
27744
20808
13872
6936
0
Day 79 27
114
86
57
29
1

D) Natural abundance N_2 incubation



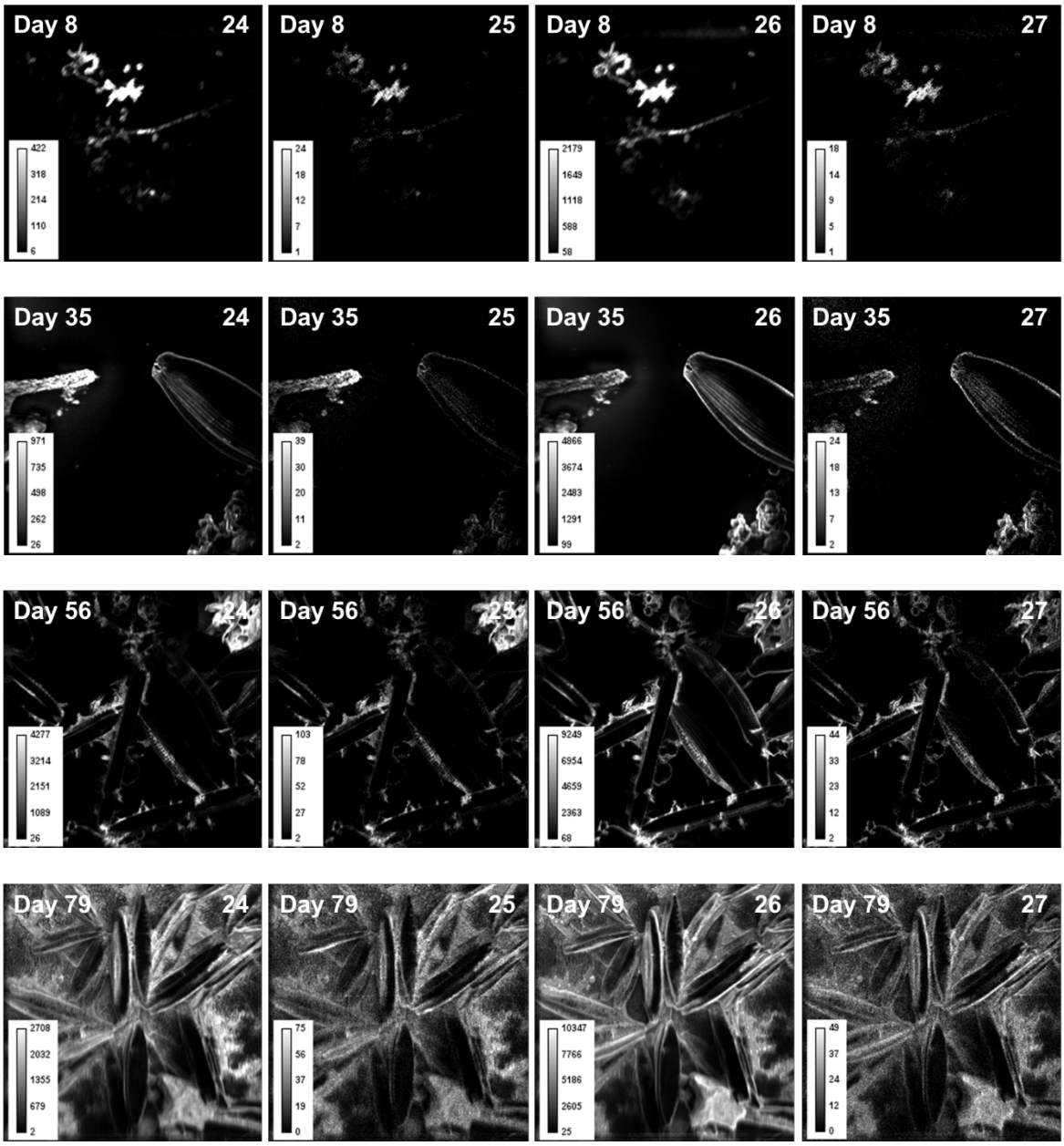
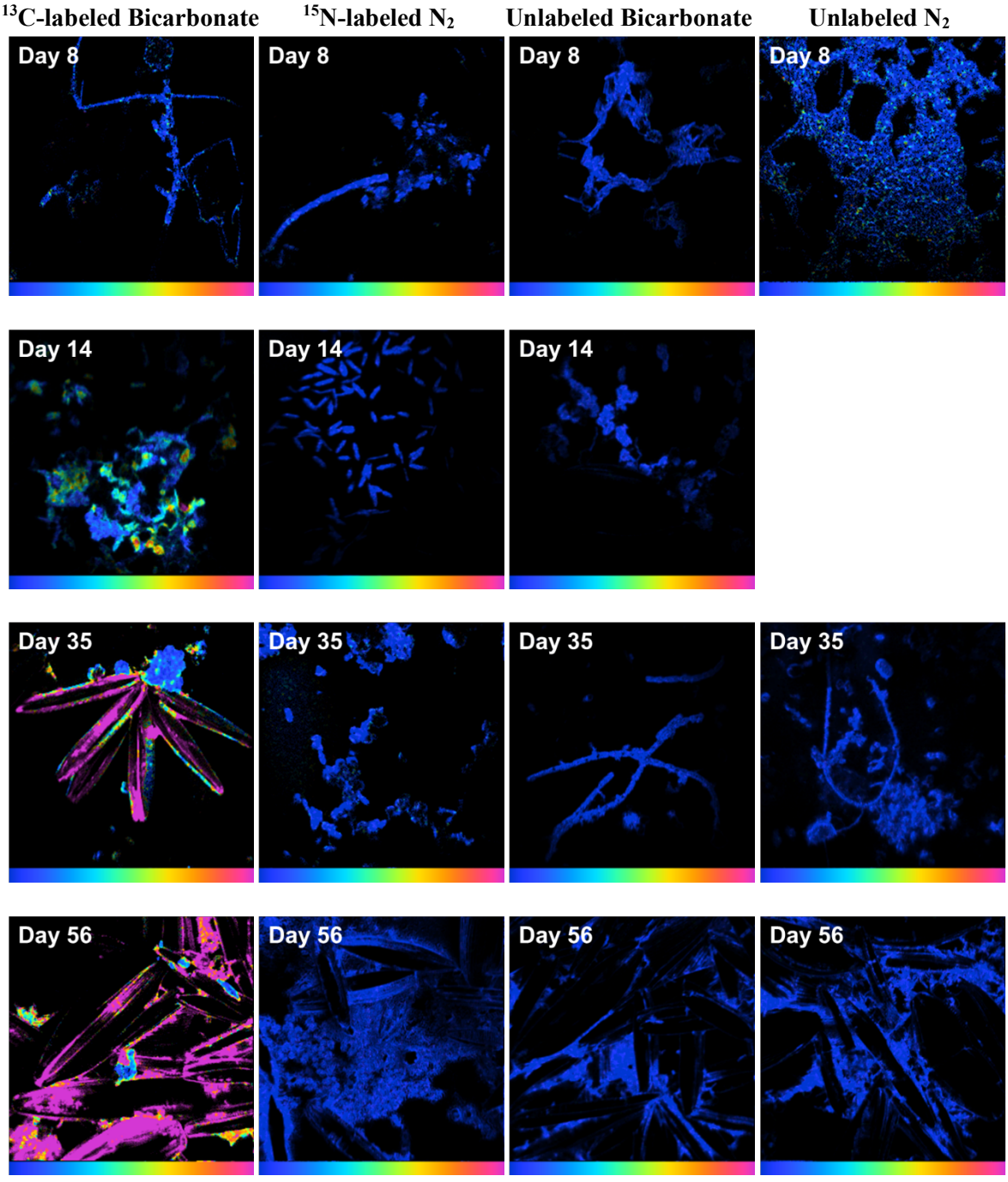
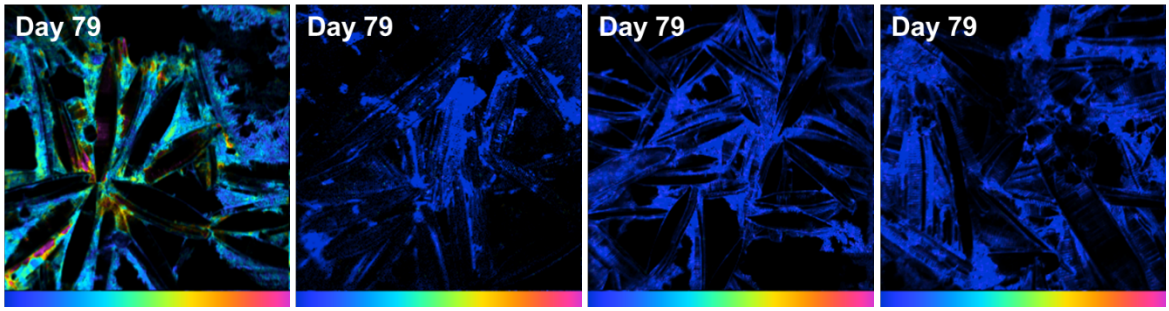


Figure S3. NanoSIMS ion intensity images of Hot Lake biofilm samples under different incubation conditions and durations. Image text denotes duration of incubation and m/z (species), where 24 represents $^{12}C_2^-$, 25, $^{12}C^{13}C^-$, 26, $^{12}C^{14}N^-$, and 27, $^{12}C^{15}N^-$. Scale bars correspond to absolute intensity of the given species across all pixels in the image. Ratios of these intensities are computed on a pixel-by-pixel basis to generate HSI maps (as in Fig. 2) and can be expressed in per mil notation relative to a standard. All images shown are 40 x 40 μm. Replicate analyses are shown for each sample type. A: Incubation with ^{13}C-labelled bicarbonate. B: Incubation with ^{15}N-labelled N_2. C: Incubation with natural abundance (unlabeled) bicarbonate. D: Incubation with natural abundance (unlabeled) N_2.


^{13}C-labeled Bicarbonate
^{15}N-labeled N_2
Unlabeled Bicarbonate
Unlabeled N_2
Day 8
Day 8
Day 8
Day 8
Day 14
Day 14
Day 14
Day 35
Day 35
Day 35
Day 35
Day 56
Day 56
Day 56
Day 56


Day 79
Day 79
Day 79
Day 79

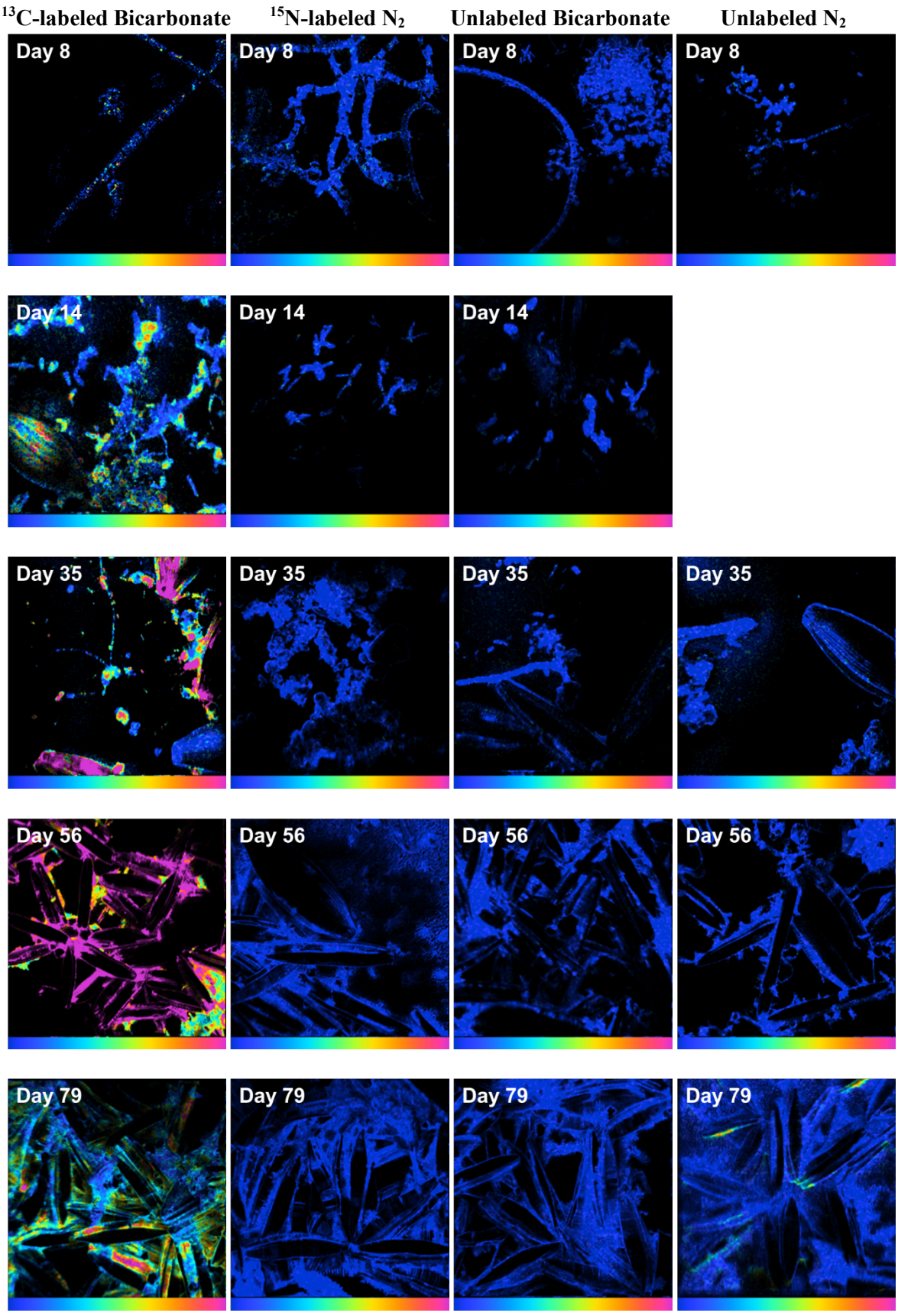
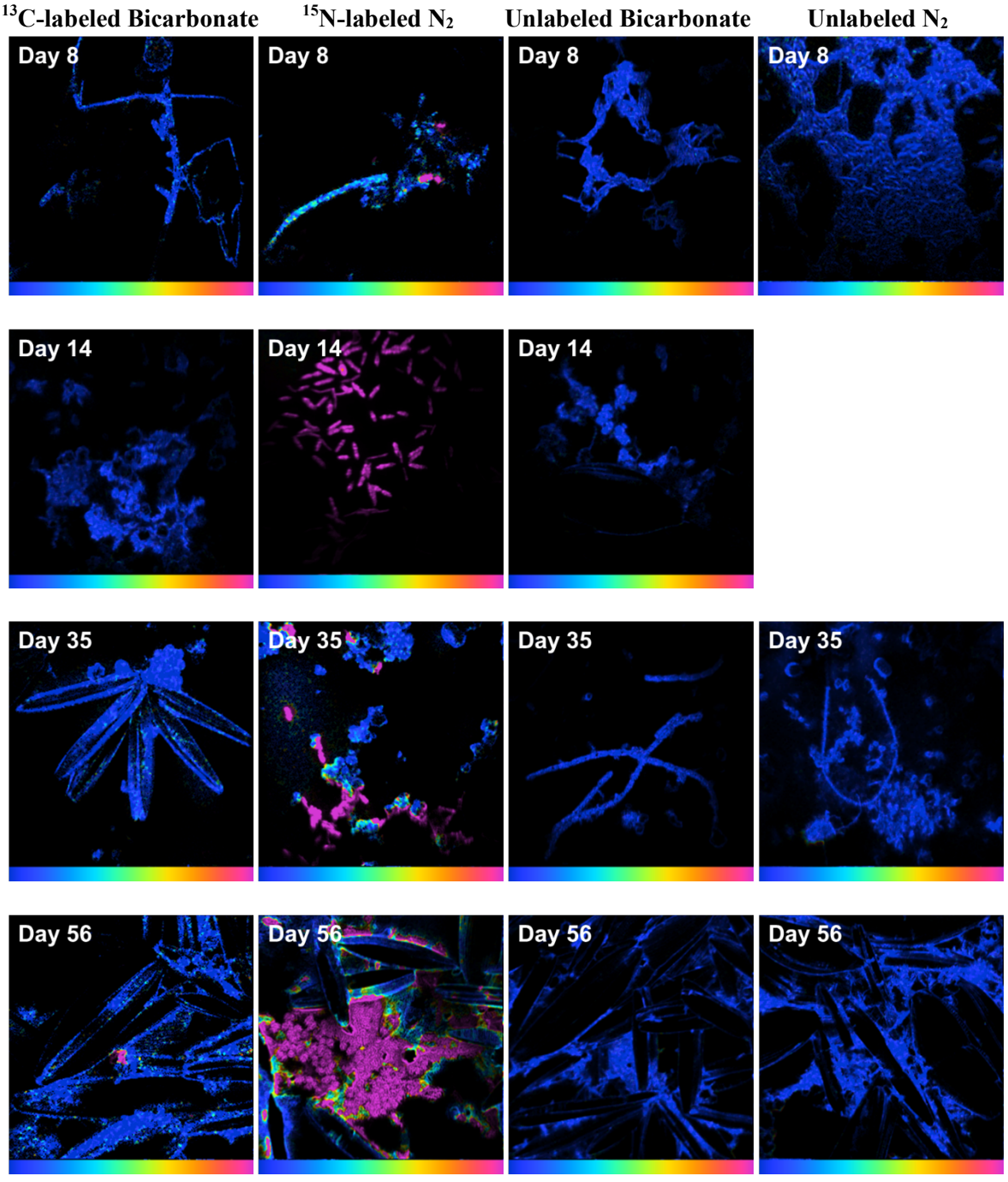
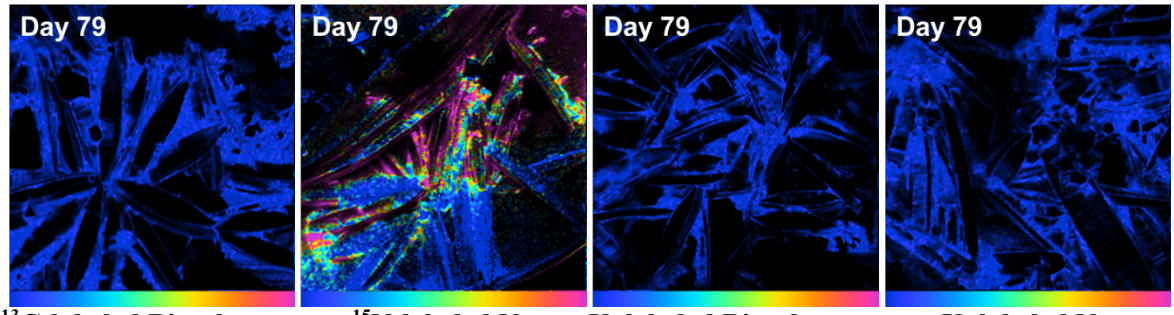

^{13}C-labeled Bicarbonate
^{15}N-labeled N_2
Unlabeled Bicarbonate
Unlabeled N_2
Day 8
Day 8
Day 8
Day 8
Day 14
Day 14
Day 14
Day 35
Day 35
Day 35
Day 35
Day 56
Day 56
Day 56
Day 56
Day 79
Day 79
Day 79
Day 79

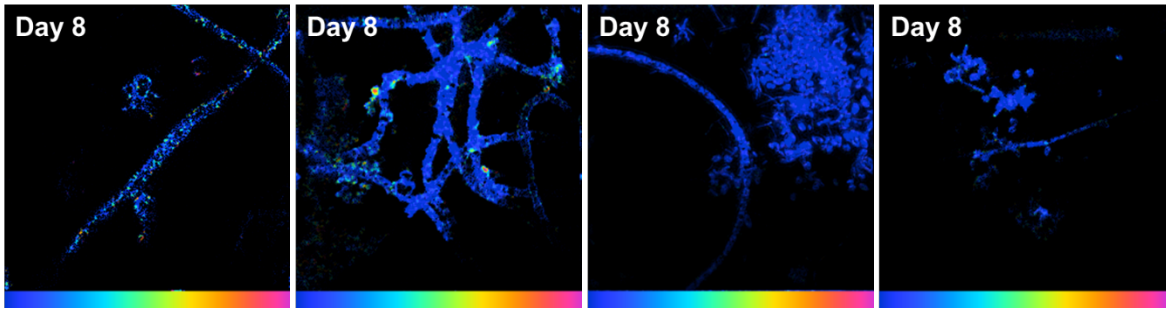
Figure S4. (above) NanoSIMS Hue-Saturation-Intensity (HSI) maps of $^{13}C^{12}C/^{12}C_2$ distribution in Hot Lake samples incubated under different conditions and durations. Image text denotes duration of incubation and columns indicate added substrate, as labelled. HSI color range correlates to $\delta^{13}C_{PDB}$ value, where dark blue (leftmost color on scale bar) is -11‰ and magenta (rightmost color) is >1700‰. Images are 40 x 40 µm. Replicate analyses shown for each sample type.

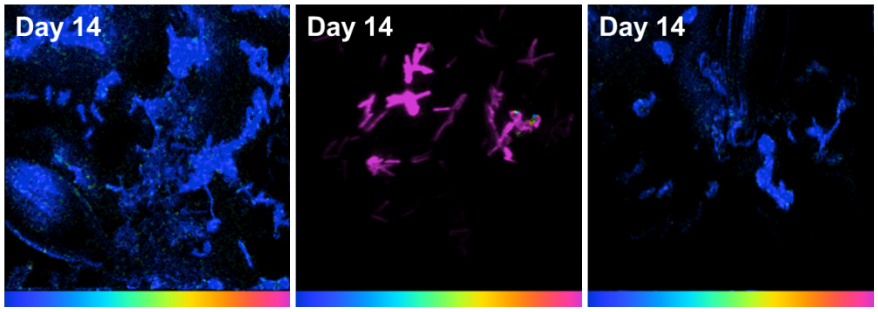


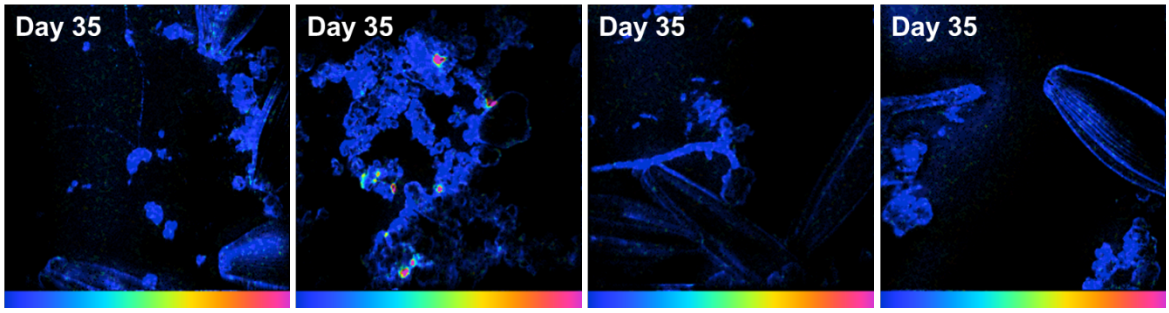


^{13}C-labeled Bicarbonate | ^{15}N-labeled N_2 | Unlabeled Bicarbonate | Unlabeled N_2

^{13}C-labeled Bicarbonate | ^{15}N-labeled N_2 | Unlabeled Bicarbonate | Unlabeled N_2







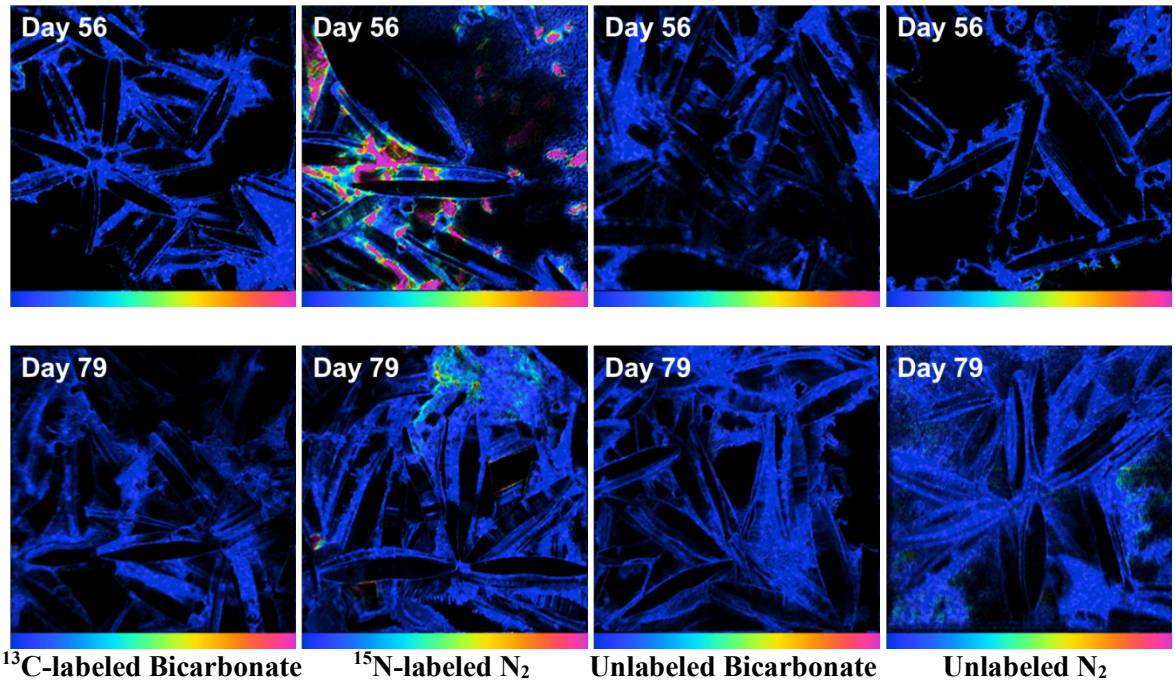


Figure S5. NanoSIMS Hue-Saturation-Intensity (HSI) maps of $^{12}C^{15}N/^{12}C^{14}N$ distribution in Hot Lake samples incubated under different conditions and durations. Image text denotes duration of incubation and columns indicate added substrate, as labelled. HSI color range correlates to $\delta^{15}N_{AIR}$ value, where dark blue (leftmost color on scale bar) is 0‰ and magenta (rightmost color) is >1700‰. Images are 40 x 40 μm. Replicate analyses shown for each sample type.

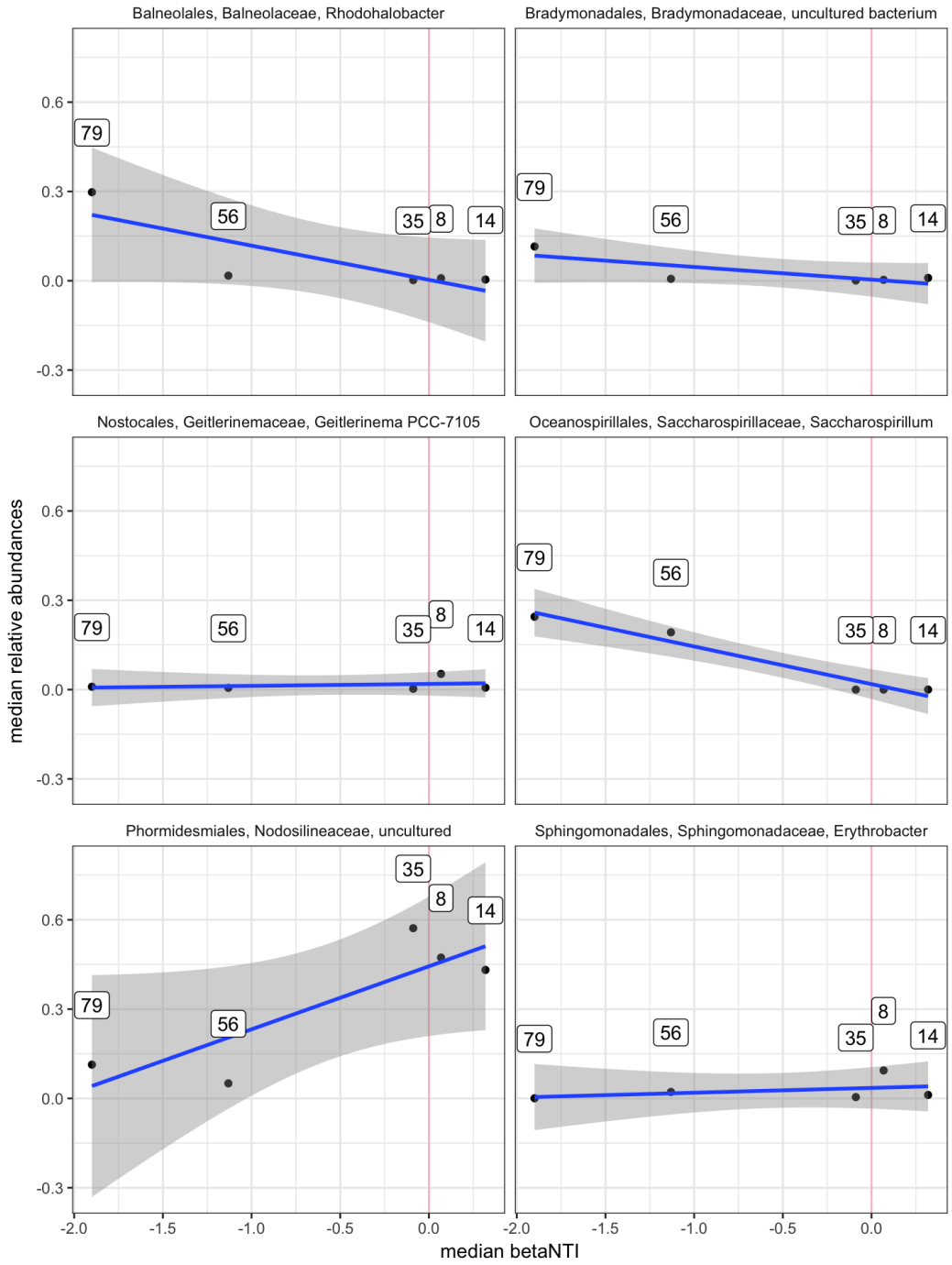


Figure S6. Correlation between bacterial founder species and selective pressure over time. Between all pairs of samples at each day, the median βNTI was calculated in order to measure if samples were under significant homogeneous (βNTI < -2) or heterogeneous (βNTI > +2) selective pressure. This is compared to the median relative abundance of each founder species at that day. A positive correlation between abundance and Beta NTI shows that a taxon has higher relative abundances during variable selection as observed on early days (i.e., the uncultured Nodosilineaceae). Anti-correlation indicates that a taxon thrives under homogeneous, stabilizing selection, as observed on later days (i.e., *Saccharospirillium*).

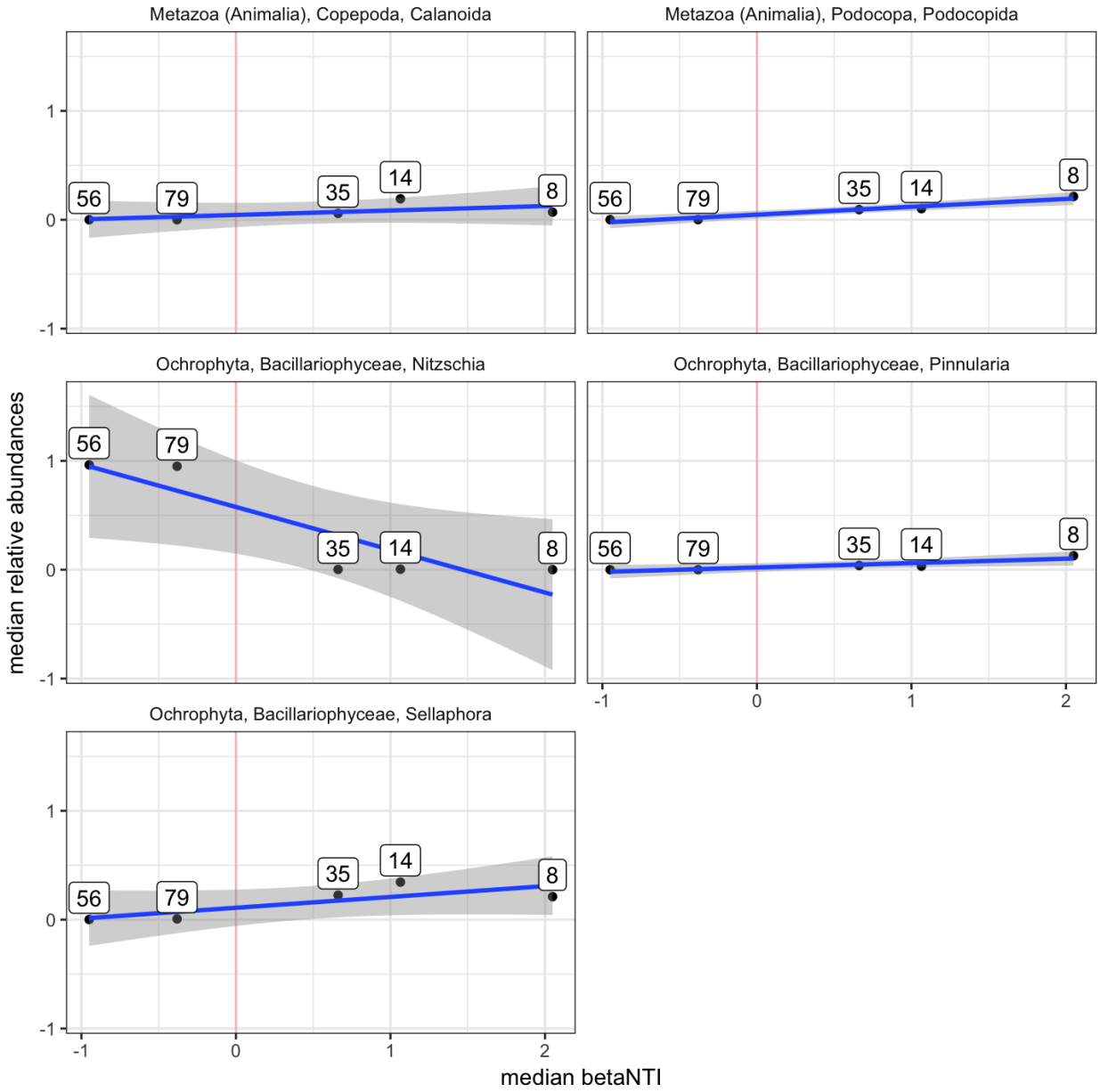


Figure S7. Beta NTI within each time point for eukaryotic founder species

Table S1. The adonis test is used to partition variation of nestedness and turnover by sampling day. The partial R-squared value shows what fraction of variation of binary Jaccard distances can be explained by Day, and p-values are calculated based on 999 permutations.

Microbe	Type	Df	SumsOfSqs	MeanSqs	F.Model	R2	P-value
Bacteria	Total	4	5.7921464	1.4480366	8.451756	0.3387239	0.001
Bacteria	Turnover	4	1.8001632	0.4500408	4.029431	0.1962758	0.001
Bacteria	Nestedness	4	0.8712268	0.2178067	19.328900	0.5394779	0.001
Eukaryotes	Total	4	4.2352656	1.0588164	5.373049	0.2543690	0.001
Eukaryotes	Turnover	4	4.1319385	1.0329846	6.908705	0.3049029	0.001
Eukaryotes	Nestedness	4	-0.1116551	-0.0279138	-4.206183	-0.3643667	1.000
Combined	Total	4	5.0666201	1.2666550	5.008877	0.2023063	0.001
Combined	Turnover	4	3.0729018	0.7682255	4.544901	0.1870722	0.001
Combined	Nestedness	4	0.1754401	0.0438600	2.657035	0.1185804	0.041

Table S2. Correlation between bacterial founder species and selective pressure over time. As shown in Fig. S6, the median daily βNTI was correlated with the median relative abundance of each founder species at that day. A positive or negative slope of the linear model indicates that taxa thrive under heterogeneous or homogeneous selective pressure, respectively, and the p value reports if this relationship is significantly different from zero.

Taxonomy	estimate	std.error	statistic	p.value
Balneolales, Balneolaceae, Rhodohalobacter	-0.1148079	0.0445916	-2.5746534	0.0821602
Bradymonadales, Bradymonadaceae, uncultured bacterium	-0.0424230	0.0180186	-2.3544014	0.0999059
Nostocales, Geitlerinemaceae, Geitlerinema PCC-7105	0.0064844	0.0123414	0.5254193	0.6356519
Oceanospirillales, Saccharospirillaceae, Saccharospirillum	-0.1264059	0.0157316	-8.0351814	0.0040252
Phormidesmiales, Nodosilineaceae, uncultured	0.2117093	0.0735351	2.8790222	0.0635798
Sphingomonadales, Sphingomonadaceae, Erythrobacter	0.0161410	0.0219005	0.7370162	0.5145199

Table S3. Analogous statistics from Table S2 but with respect to the eukaryotic OTU assignments and regressions shown in Figure S7.

Taxonomy	estimate	std.error	statistic	p.value
Metazoa (Animalia), Copepoda, Calanoida	0.0408648	0.0301152	1.356947	0.2678705
Metazoa (Animalia), Podocopa, Podocopida	0.0723646	0.0099350	7.283822	0.0053417
Ochrophyta, Bacillariophyceae, Nitzschia	-0.3928224	0.1155316	-3.400131	0.0424572
Ochrophyta, Bacillariophyceae, Pinnularia	0.0403338	0.0109043	3.698892	0.0343043
Ochrophyta, Bacillariophyceae, Sellaphora	0.0994120	0.0449143	2.213372	0.1137433

Table S4. Mean and median values for each βNTI score calculated. The full data sets are shown in Figure 3 of the main text.

Day	Type	Mean	Median
8	16S	-0.6626235	-0.5782877
14	16S	-0.4348574	-0.4097291
35	16S	-1.0244267	-1.2017599
56	16S	-1.9282180	-2.1575157
79	16S	-2.2495928	-2.4546289
8	18S	2.8101606	2.5030410
14	18S	1.6863899	2.0247967
35	18S	1.8270200	1.6889517
56	18S	0.9317534	1.5680731
79	18S	0.6671600	0.7790160