Supporting Information

To Accompany

Cyanobacteria and Algae Meet at the Limits of their Habitat Ranges in Moderately Acidic Hot Springs

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Procedures

1. Ion chromatography. Major ions were quantified using two Dionex DX-600 ion chromatography systems operated by Chromeleon software (version 6.8). The anion system employs a potassium hydroxide eluent generator, a carbonate removal device, and AS11-HC/AG11-HC columns. The hydroxide concentration of the eluent is held isocratically at 5 mM for 5 minutes, followed by a non-linear (Chromeleon curve 8) hydroxide concentration gradient to 55 mM applied over 31 minutes, after which the column is reequilibrated at 5 mM hydroxide for 10 minutes before the next sample injection. The eluent flow rate is held constant at 1.0 mL/minute. The cation system is equipped with CS-16 and CG-16 columns and cations are eluted isocratically with 19 mM methanesulfonic acid (MSA) at 0.5 mL/minute. Samples for cations were acidified with 6 N MSA to approximately 19 mM final concentration. Both systems are plumbed with an external source of deionized water for suppressor regeneration to improve the signal-to-noise ratio of the analyses and suppressor currents were 137 mA and 28 mA for anions and cations, respectively. Samples were delivered to the instruments from 5 mL vials via AS-40 autosamplers (2 injections per vial) onto 100 µL and 75 µL sample loops for anions and cations, respectively. Quantification is achieved externally via calibration curves constructed from a series of dilutions of mixed-ion standards (Environmental Express, Charleston, SC, USA). Quantification accuracy is verified daily by analysis of an independent mixed ion standard (Thermo Scientific, Waltham, MA, USA). Uncertainties in reported ion concentrations are estimated to be ± 5%.

2. Correction of pH and speciation. The major ion and DIC data were used to calculate the speciation of inorganic carbon and assess charge balance in each sample with the geochemical speciation code EQ3/6 (Wolery and Jarek, 2003) using activity coefficients calculated with an extended Debye-Hückel equation and equilibrium constants derived from the revised Helgeson-

Kirkham-Flowers equation of state (Shock *et al.*, 1997; Sverjensky *et al.*, 1997). Two of the samples (RN1-2011 and RS4) exhibited charge imbalances (expressed as percent of the mean charge, as defined by Nordstrom *et al.*, 2009) in excess of 20%, which is believed to indicate faulty pH probes in use at the time of sampling. Since hydrogen ions are nearly at the same order of magnitude concentration as the major solutes in these samples, the pH measurements were corrected to achieve charge balance, as indicated in Table S1. These corrected values are believed to be closer to the actual pH of each hot spring at the time of sampling than the pH value determined in the field. The speciation of inorganic carbon was calculated using the corrected pH values.

3. Carbon uptake assays. Microcosms were prepared in pre-sterilized, N_2-purged 24 mL serum bottles. Ten mL of water was sampled directly from the spring source and added to each serum bottle using a syringe and needle. Mat samples were collected aseptically using a sterile spatula and placed in 50 mL falcon tubes. Twenty mL of spring water was added to each tube and each was shaken vigorously to create a homogenized slurry. One mL of this slurry was added to each serum bottle. The gas phase of all microcosms was equalized to atmospheric pressure using a sterile needle prior to injection of 5.0 µCi (10 µM final concentration) of radiolabeled sodium bicarbonate ($NaH^{14}CO_3$). Light, dark (foil-wrapped), and killed (500 µM $HgCl_2$ final concentration) experimental treatments were conducted in triplicate. All microcosms were placed in a sealed bag (secondary containment) and incubated in the source of the spring for < 60 minutes. Microcosms were terminated by freezing on dry ice and were stored at -20°C until processed.

In the laboratory, sealed microcosm assays were thawed at room temperature for approximately 2 hours, uncapped, and acidified to pH ~2 by injection of 1.0 mL of 1 N HCl to volatize unreacted CO_2. After acidification, microcosms were allowed to equilibrate for an additional 2 hours. Acidified samples were filtered onto white 0.22 µm polycarbonate membranes,

washed with 5 mL of sterile deionized water, and dried overnight at 80°C. Dried filters were placed in scintillation vials and overlain with 10 ml of CytoScint ES™ liquid scintillation fluid. Radioactivity associated with each of the samples was measured on a Beckman LS 6500 liquid scintillation counter (Beckman Coulter, Inc., Indianapolis, IN, USA).

4. Pigment extraction and analyses. Samples (~1 g) preserved for analysis of pigments were thawed, transferred aseptically to Lysing Matrix A tubes (MP Biomedicals, Irvine, CA, USA), and centrifuged at 21000 x *g* for 5 minutes at 4°C. After removal of the supernatant, 250 µL of 7:2 acetone:methanol (v/v) that had been stored over 4 Å molecular sieves (Sigma-Aldrich, St. Louis, MO, USA) was added and the samples were subjected to ballistic bead beating (FastPrep 24; 6.5 speed, 40 s). The samples were subsequently centrifuged and the organic supernatant was transferred to a microcentrifuge tube. Additional aliquots of 7:2 acetone:methanol and pure methanol were homogenized with the solid sample by additional rounds of bead beating and subsequently collected by centrifugation, whereupon the supernatants were pooled. This process was continued until the supernatant became clear and no obvious signs of methanol-soluble pigments remained in the solid sample, typically requiring ~1.5 mL of total solvent. The pooled supernatants were centrifuged and the top 500 µL were transferred to Teflon-sealed amber autosampler vials (Agilent Technologies, Inc., Santa Clara, CA, USA) for analysis. To minimize pigment degradation, all manipulations were conducted in a cold room (4 °C) without direct lighting and the samples were transported on ice in a closed container.

Pigment samples were analyzed immediately after extraction via high pressure liquid chromatography (HPLC) equipped with a photodiode array absorbance detector (Thermo Surveyor) coupled with atmospheric pressure chemical ionization mass spectrometry (Thermo Quantum Discovery MAX triple-quadrupole) operating in positive ion, single quadrupole

scanning mode from 200 to 1500 m/z with a scan rate of 1 Hz. Parameters for mass spectrometry were based on those employed by van Breemen *et al.* (2012), specifically a corona discharge of 8 µA, vaporizer temperature of 350°C, and capillary temperature of 300°C. The diode array detector monitored signal intensity at 360, 475, and 665 nm and collected spectra from 325–800 nm at 5 Hz. Samples were injected via a 50 µL sample loop onto a YMC Carotenoid C-30 reverse phase column (3 x 250 mm). HPLC conditions were modified from those described by Sander *et al.* (1994). The solvent system was initially isocratic at 81:15:4 methanol:methyl *tert*-butyl ether:water for 30 minutes, followed by a linear gradient to 6:90:4 methanol:methyl *tert*-butyl ether:water at 90 minutes. The solvent system was then returned to initial conditions over 5 minutes and held isocratically for 15 minutes to re-equilibrate the column for the next sample.

Insights into Other Microbial Populations in Moderately Acidic Hot Springs

1. Anoxygenic phototrophs. Ribosomal gene sequences affiliated with putative anoxygenic phototrophs were observed in most samples. Two sites (RS1 and RS4) yielded 16S rRNA gene sequences most closely associated with *Chloracidobacterium thermophilum* (13% and 2% of 16S rRNA gene OTUs, respectively), an organism first cultivated from an alkaline hot spring and the only known phototrophic member of the Acidobacteria (Bryant *et al.*, 2007; Tank and Bryant, 2015). Sequence similarity ranged from 86–99%, yet the most abundant OTU associated with *C. thermophilum* in both sites is 99% identical to the cultured representative. *C. thermophilum* is a microaerophilic photoheterotroph (Tank and Bryant, 2015a,b), as its genome lacks key genes for known carbon fixation pathways (Garcia Costas *et al.*, 2012b). Most samples also contained sequences of the aerobic anoxygenic phototrophic bacteria (AAPB) *Acidiphilium* and *Acidisphaera*, which have previously been detected by ribosomal gene sequencing of other

acidic Yellowstone hot spring mats (Macur *et al.* 2004; Hamamura *et al.*, 2005; Hamilton *et al.*, 2019). The AAPB can be characterized as aerobic heterotrophs that synthesize bacteriochlorophylls and reaction centers for light harvesting to supplement energetic requirements (Yurkov and Beatty, 1998; Rathgeber *et al.* 2004) and are important constituents of marine phytoplankton (Eiler, 2006). Energy gained via photophosphorylation enables greater partitioning of organic carbon to biomass (anabolism) than to respiration, which is advantageous when organic carbon is limited (Hauruseu and Koblížek, 2012). *Acidiphilium* sequences were detected in five samples (FF1, IG1, RS1, RS2, RS4), comprising 0.1–0.5% of the 16S rRNA gene OTUs in these samples. Sequences affiliated with *Acidisphaera* were more widespread, detected in all samples except RS1, RS3, and RS5-2012, ranging in abundance from 0.1–1.4% of the 16S rRNA gene OTUs. The sequences are most closely related to isolates from acid mine drainage environments, including *Acidiphilium* sp. CCP3 (100% similarity; Hallberg *et al.* 2006), *Acidiphilium* sp. NO-13 (94–100% similarity; Johnson *et al.* 2001), and *Acidisphaera* MS-Y2 (90–96% similarity; Okamura *et al.* 2015).

Chromatographic pigment analyses for several samples (FF1, RS1, RS2, RS4) yielded peaks thought to represent small amounts of bacteriochlorophyll *a* or its derivatives, based on absorbance spectra associated with these peaks exhibiting Q_y (*i.e.*, longest-wavelength absorption) maxima of >750 nm. One such peak was identified in two mat extracts (RS1, RS4) with a retention time very close to that of bacteriochlorophyll *a* in a pigment extract of *Rhodobacter sphaeroides*, but maxima in the absorption spectrum of the peak were slightly blue-shifted relative to that derived from *R. sphaeroides*, and no molecular ion corresponding to bacteriochlorophyll *a* was observed in the mass spectrum. Instead, the mass spectrum corresponding to this peak in each sample showed a molecular ion (M+H) at 951 m/z with predominant M+H+2 and M+H+4 peaks

(Figure S1), which is consistent with bacteriochlorophyll *a* containing zinc instead of magnesium as the central metal. Zinc-bacteriochlorophyll *a* is the primary chlorophyll in *Acidiphilium* spp. (Wakao *et al.*, 1996; Hiraishi and Shimada, 2001), which is thought to afford greater acid tolerance to the organism due to its retarded pheophytinization rates relative to those of Mg-bacteriochlorophyll *a* (Kobayashi *et al.*, 1998). Ribosomal gene sequences associated with *Acidiphilium* were detected in these two samples, in addition to three others where Zn-bacteriochlorophyll *a* was not detected. The epimer of Zn-bacteriochlorophyll *a* is found in the reaction centers of *C. thermophilum* (Tsukatani *et al.*, 2012; He *et al.*, 2019), sequences of which were found only in samples from the two sites where Zn-bacteriochlorophyll *a* was detected. Therefore, this pigment could alternatively be associated with *C. thermophilum*, as it is not possible to distinguish between the two epimers with the present data. Magnesium-bacteriochlorophyll *a* was not detected in any sample, which is the primary chlorophyll of *Acidisphaera* (Hiraishi *et al.*, 2000), despite *Acidisphaera* being more widely distributed and often at larger relative abundances than *Acidiphilium*. Bacteriochlorophyll *a* was previously observed in pigment extracts from other hot springs in YNP with pH values in the range of 3–6 (Hamilton *et al.*, 2012), yet mass spectra for these analytes were not collected to assess the central metal. Bacteriopheophytin *a* was detected in pigment extracts from 3 sites (FF1, RS1, RS2), which likely is the primary degradation product of both Zn- and Mg-bacteriochlorophyll *a*.

In contrast to the observation of bacteriochlorophyll *a* derivatives, no bacteriochlorophyll *c* homologues were detected in any of the samples. *C. thermophilum* employs a variety of bacteriochlorophyll *c* structures as the major chlorosome antenna pigments (Garcia Costas *et al.*, 2012a) so it is surprising that none were identified in samples where *C. thermophilum* sequences were found. While the similar absorbance spectra of bacteriochlorophyll *c* and chlorophyll *a* make

identifying bacteriochlorophyll *c* compounds more ambiguous, a search of unassigned analyte peaks from sample RS1, where *C. thermophilum* was most abundant, did not offer strong evidence for bacteriochlorophyll *c* homologues in the mass spectra. Given the complexity of the mixture of bacteriochlorophyll *c* species produced by *C. thermophilum*, perhaps each individual homologue is below detection in these samples.

Some preliminary assessments can be made regarding the potential for active anoxygenic phototrophs, all of which putatively grow photoheterotrophically, in the moderately acidic hot springs studied herein. *C. thermophilum* was identified at only two sites, though these sequences represented significant relative abundances at these locations. Since the presence of bacteriochlorophyll *c* homologues specific to this organism could not be confirmed, it is difficult to assess whether *C. thermophilum* was active in these springs at the time of sampling. Though originally isolated from an alkaline hot spring outflow, *C. thermophilum* sequences were also recovered from an acidic, lower temperature spring in YNP (Hamilton *et al.*, 2012), so these phototrophs do not appear to be limited to alkaline habitats. The detection of Zn-bacteriochlorophyll *a* at two sites might indicate the *Acidiphilium* sequences detected at those sites represented active populations, yet the weak signals of this pigment in samples from these sites do not lend themselves to the conclusion that the absence of this pigment indicates a lack of activity by *Acidiphilium* populations observed at other sites. The activity of *Acidisphaera* is also inconclusive in light of the sporadic detection of bacteriochlorophyll *a* chromophores, such as bacteriopheophytin *a*, that might be derived from these cells.

2. Fungi and other eukaryotes. Fungi represented a significant proportion of the 18S rRNA gene OTUs in most samples, with the exception of the three sites near Imperial Geyser. The diversity of the fungal OTUs is large, encompassing 6 of the 7 recognized phyla of fungi.

Thermotolerant fungi are thought to be more abundant in lower pH environments, and likely represent the most thermotolerant Eukarya, having a temperature maximum of ~60°C (Tansey and Brock, 1972; Brock, 1978). Though the Ascomycote *Ochroconis* (*i.e.*, *Dactylaria*) has been reported in acidic hot springs at temperatures consistent with those of this study (Tansey and Brock, 1973), OTUs associated with this organism were only detected at RS3 in very low abundance. Other 18S rRNA gene sequences are affiliated with protists, arthropods, and land plants; these sequences in many cases may represent exogenous surface input of biomass rather than indigenous members of the hot spring community. Fungi and protists were also observed in hot spring samples from Lassen Volcanic National Park, California, again with uncertainty regarding to what extent they represent autochthonous organisms (Brown and Wolfe, 2006). Nevertheless, putatively indigenous amoebae have been reported in Nymph Creek and other locations in YNP at somewhat lower temperatures (≤ 40°C) than those of this study (Sheehan *et al.*, 2003; Amaral-Zettler, 2013). A large (n = 160) study of thermal springs in New Zealand revealed extensive protist diversity across the planktonic samples, and the observation that communities in springs with higher temperatures (50–65°C) were different from those of lower temperature springs is suggestive of indigenous organisms inhabiting springs within this temperature range (Oliverio *et al.*, 2018).

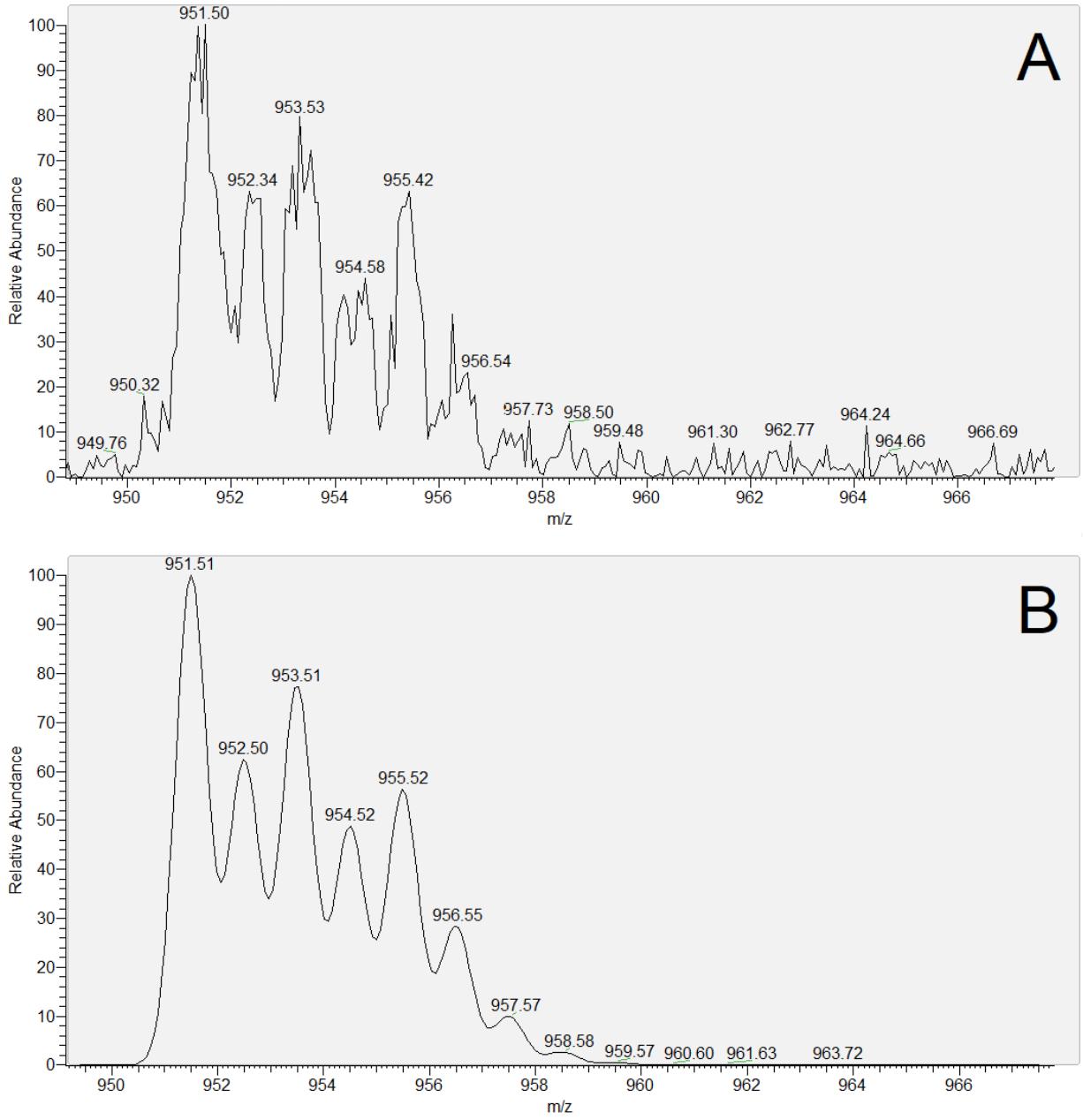


Figure S1. Mass spectrum of zinc-bacteriochlorophyll *a*. A) Uncorrected partial mass spectrum of the pigment extract for sample RS4 subjected to LC-MS analysis averaged over the retention time where light absorbance in the 700–800 nm region was observed in the diode array spectrum, which is indicative of bacteriochlorophylls. B) Predicted profile mass spectrum for $C_{55}H_{75}N_4O_6Zn^+$, the molecular formula for the putative protonated molecular ion (M+H) of zinc-bacteriochlorophyll *a*. The mass spectrum was calculated with a resolution of 0.7 Da at full width at half maximum using Thermo Xcalibur software. The high relative abundances and distribution pattern of the isotopic peaks are in large part attributed to the multiple stable isotopes of zinc; the predominance of the M+H+2 and M+H+4 peaks in particular is attributed to the significant natural abundances of ^{66}Zn and ^{68}Zn, respectively.

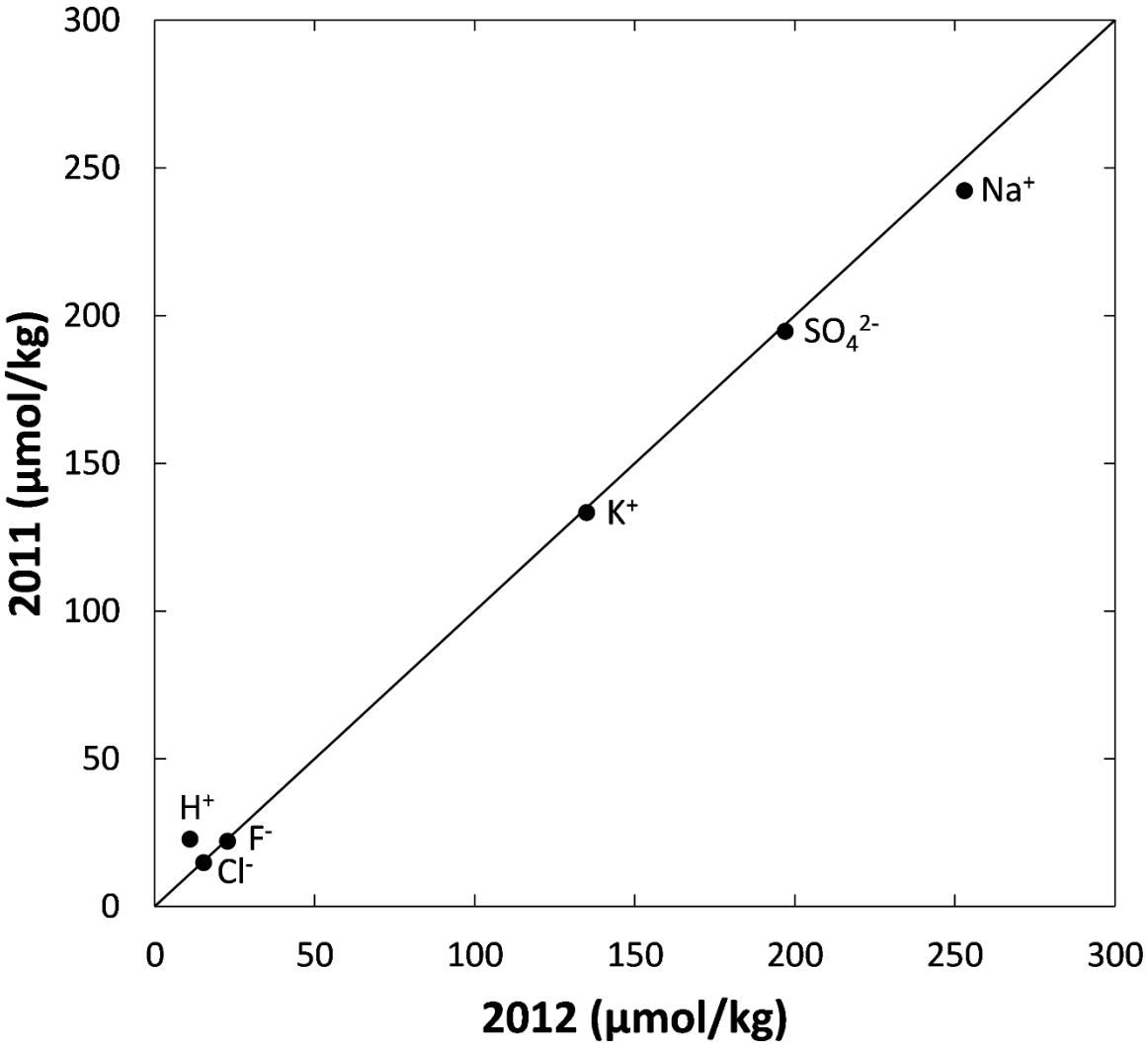
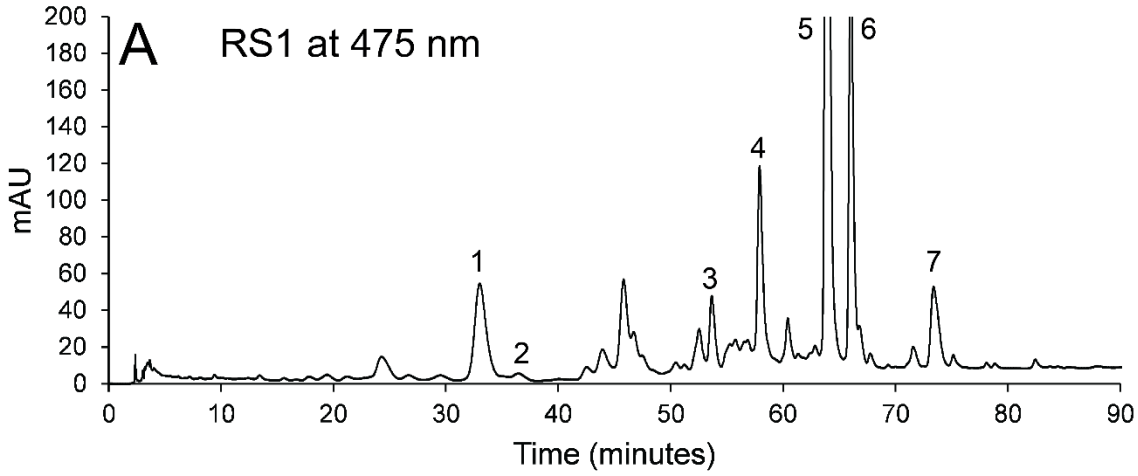
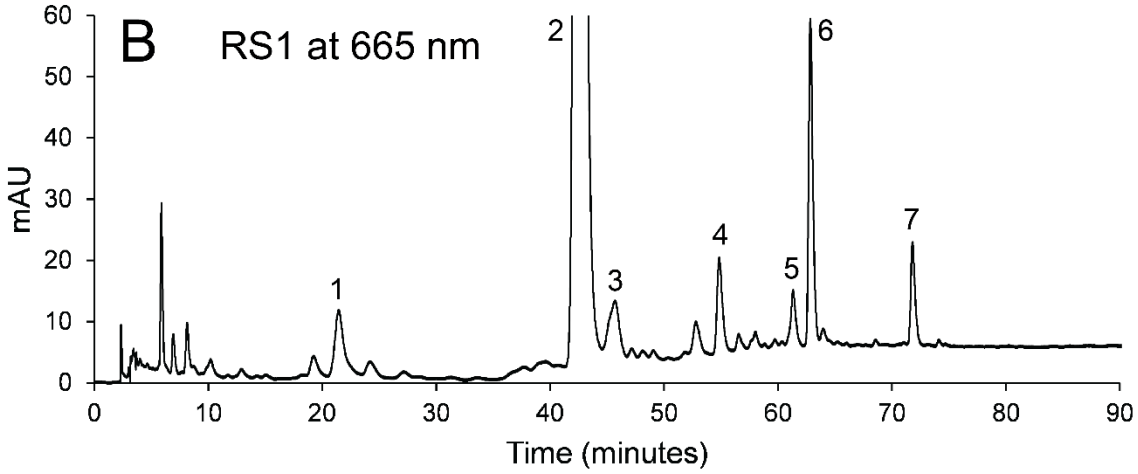
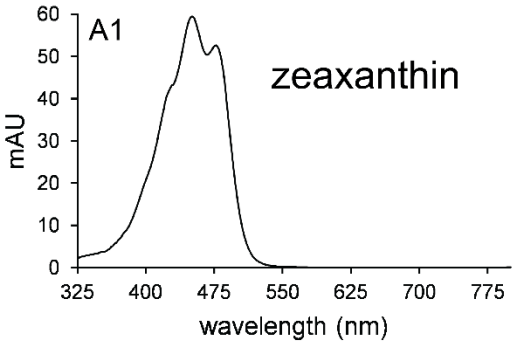
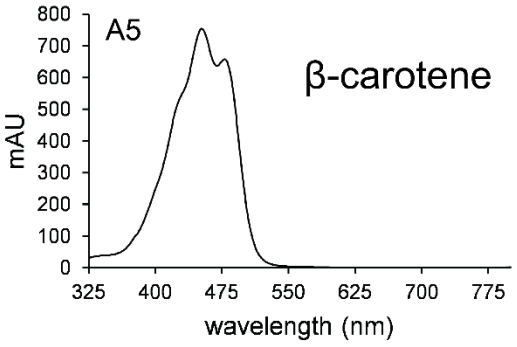


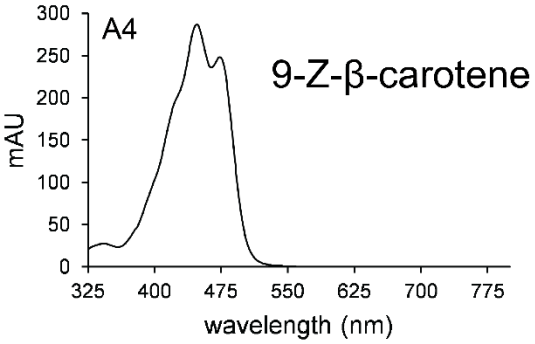
Figure S2. Comparison of major solutes at site RN1 for 2011 and 2012 samples. Corrected H^+ concentrations are shown.

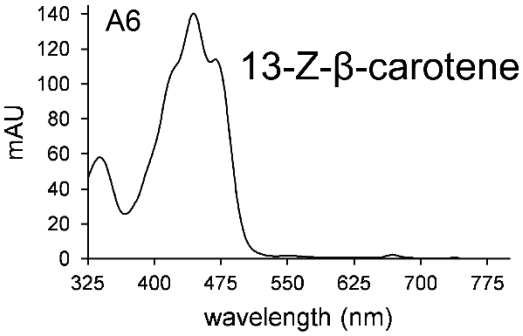

A
RS1 at 475 nm
mAU
Time (minutes)
1
2
3
4
5
6
7


B
RS1 at 665 nm
mAU
Time (minutes)
1
2
3
4
5
6
7


A1
zeaxanthin
mAU
wavelength (nm)


A5
β-carotene
mAU
wavelength (nm)


A4
9-Z-β-carotene
mAU
wavelength (nm)


A6
13-Z-β-carotene
mAU
wavelength (nm)

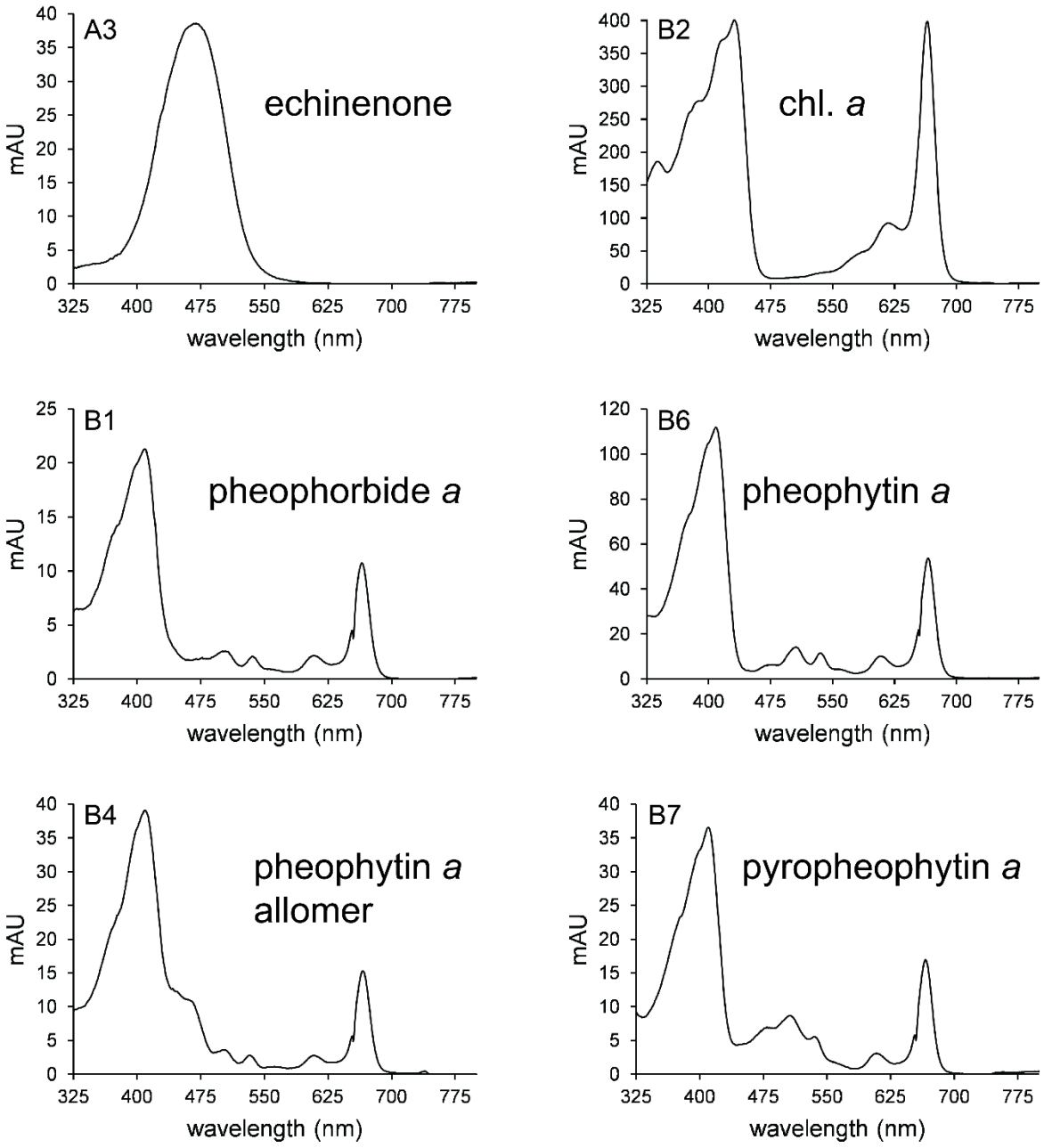


Figure S3. Typical chromatograms recorded at 475 nm (A) and 665 nm (B) with major peaks numbered (sample RS1 shown) as well as baseline-corrected diode array spectra for selected major pigments. The chromatogram and peak number are indicated in the upper left of each spectrum. Peak A7 is hypothesized to be lycopene (spectrum and data not shown).

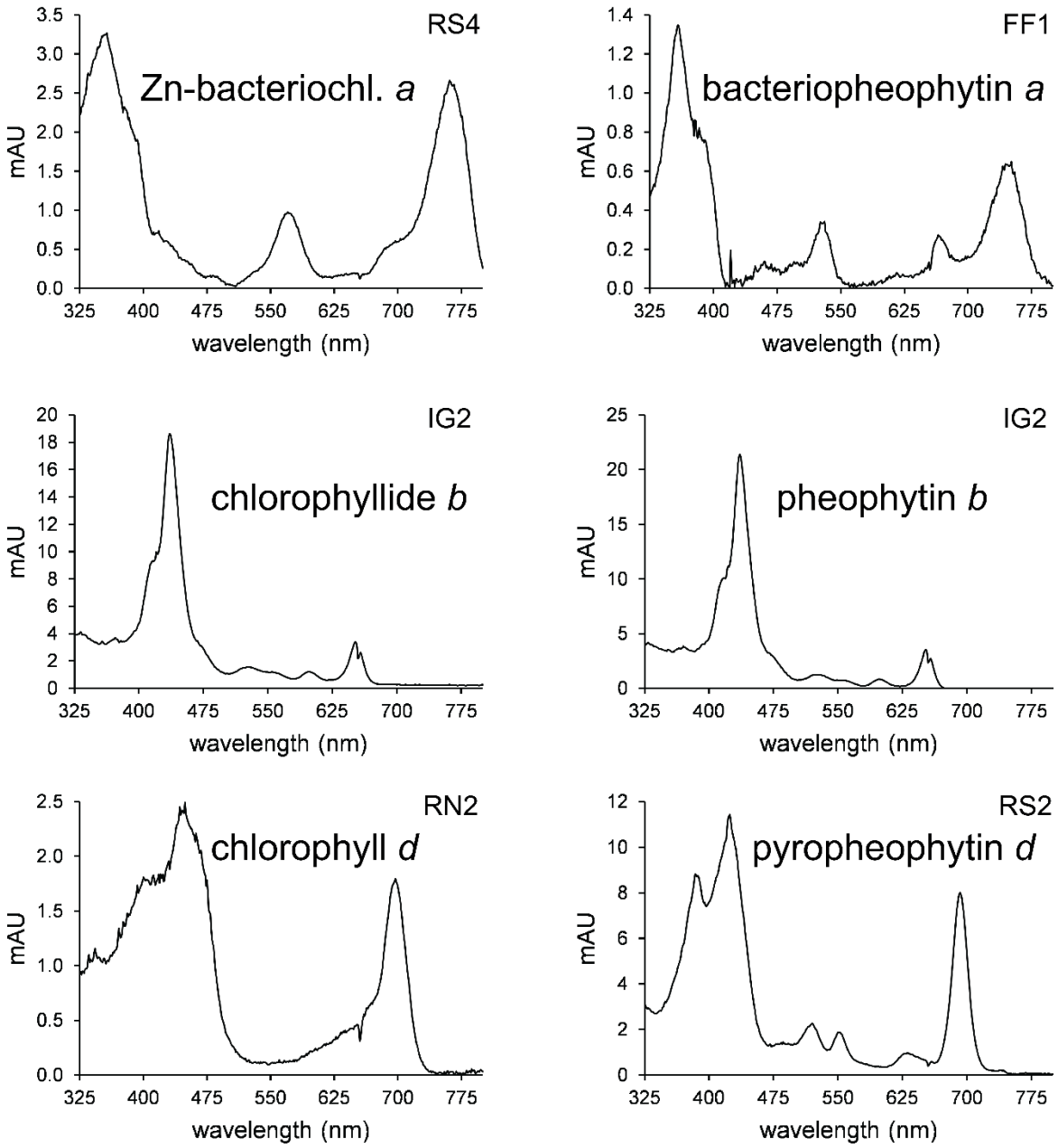


Figure S4. Baseline-corrected diode-array spectra of other detected chlorophylls. The sample from which each spectrum arises is indicated in the upper right corner.

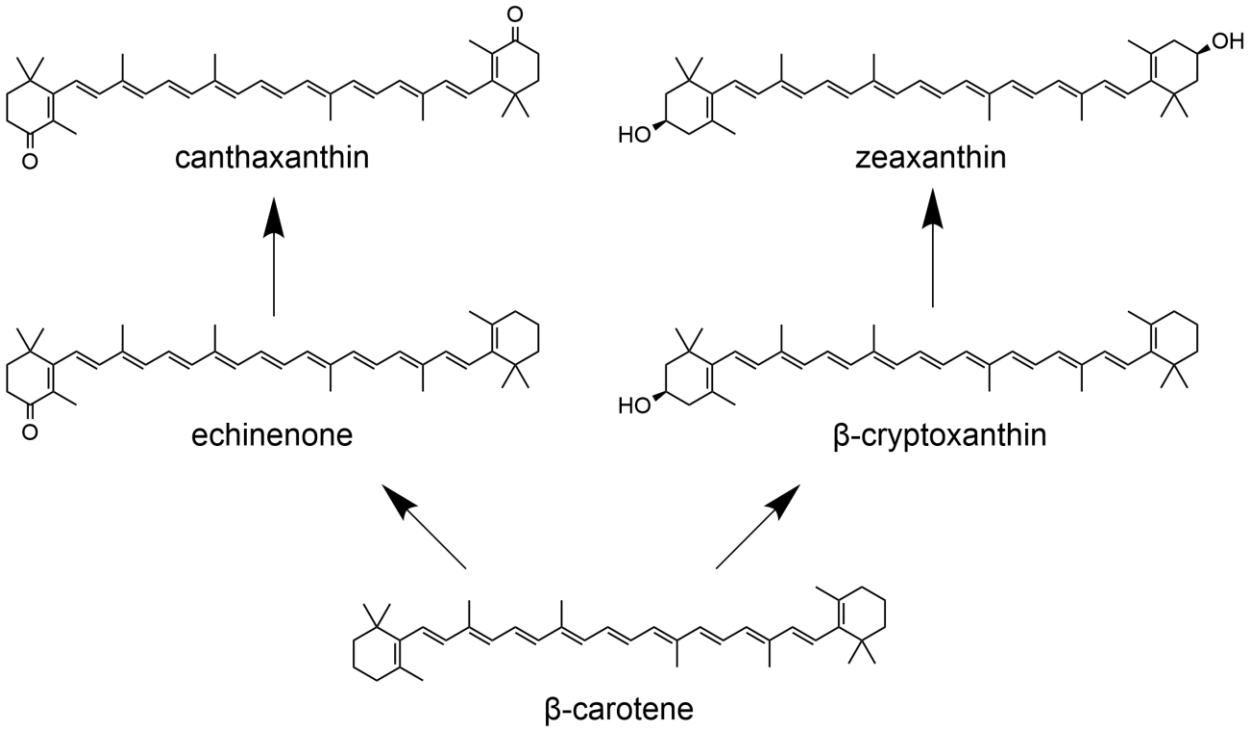


Figure S5. Structures of carotenoids discussed in this study arranged according to putative biosynthetic pathways.

Table S1. Carbon and nitrogen content of biofilms and dissolved inorganic carbon (DIC) speciation in hot spring waters.

Sample	Biofilm wt. % C	SD [a] wt. % C	Biofilm wt. % N	SD wt. % N	Biofilm C:N [b]	DIC µM	SD µM	CO_2 µM	HCO_3^- µM	CO_2/HCO_3^-
FF1	3.37	0.01	0.439	0.001	8.95	573	4	572	0.63	910
IG1	3.15	0.06	0.45	0.01	8.2	537	ND [c]	534	3.1	170
IG2	6.02	0.02	0.707	0.002	9.93	1830	30	1830	2.2	830
IG3	4.97	0.06	0.859	0.005	6.74	92	3	92	0.05	1800
RN1-2011	2.87	0.01	0.545	0.001	6.14	*140* [d]	30	140	3.3	42
RN1-2012	6.9	0.1	1.35	0.04	5.9	73	4	69	4.0	17
RN2	1.08	0.02	0.189	0.003	6.66	*180*	40	180	3.6	50
RN3	0.66	0.01	0.106	0.001	7.3	83	5	83	0.49	170
RS1	1.4	0.2	0.22	0.03	7.4	1120	20	1050	71	15
RS2	1.57	0.01	0.2080	0.0008	8.80	680	20	680	7.6	89
RS3	2.89	0.06	0.300	0.003	11.2	1000	5	869	130	6.7
RS4	6.1	0.1	0.596	0.008	12	1708	2	1430	280	5.1
RS5-2011	9.8	0.3	1.27	0.05	9.0	1320	20	1310	4.9	270
RS5-2012	0.29	0.01	0.038	0.003	8.9	2060	ND	1920	140	14

[a] Standard deviation. [b] mol:mol ratio. [c] Not determined; only a single analysis could be completed. [d] DIC data in italics were below the lowest calibration standard.

Table S2. Composition of enrichment medium.

Component	mg/L
Main Solution	
ammonium sulfate	1.39
sodium nitrate	0.32
potassium sulfate	4.96
magnesium sulfate heptahydrate	0.49
sodium sulfate	15.30
calcium chloride	0.94
sodium fluoride	0.92
Trace A Solution (1 mL/L in final media)	
disodium EDTA	130.28
potassium phosphate monobasic	51.71
ferrous sulfate heptahydrate	75.07
manganese (II) chloride tetrahydrate	96.98
boric acid	80.38
zinc sulfate heptahydrate	89.13
Trace B Solution (0.1 mL/L in final media)	
copper (II) sulfate pentahydrate	4.00
sodium molybdate dihydrate	30.24
vanadium (IV) oxide sulfate hydrate	5.71
cobalt (II) chloride hexahydrate	1.93
chromium (III) chloride hexahydrate	1.13
nickel (II) chloride hexahydrate	5.71
sodium selenate	1.89
sodium tungstate dihydrate	2.34

Table S3. Sample dates, locations, charge balance results, conductivities, and isotopic ratios of water.

Sample	Sample ID[a]	Easting[b]	Northing	Field pH	Corrected pH[c]	% charge imbalance	Specific Conductivity (µS/cm)	$\delta^{18}O$ vs. VSMOW (‰)	$\delta^{2}H$ vs. VSMOW (‰)
FF1	120720KF	513469	4929044	3.30	3.28	-1.02	462	-4.8	-108.3
IG1	110918R	509797	4930939	3.78	4.00	3.55	365	-8.5	-123.2
IG2	120722TE	509794	4930931	3.13	3.33	13.6	606	-5.4	-111.0
IG3	120722TK	510064	4930948	3.26	2.98	-9.24	914	-14.1	-127.4
RN1-2011	110708C	515110	4929721	3.94	4.62	22.0	69.5	-14.9	-137.7
RN1-2012	120718TJ	515110	4929721	4.91	5.02	0.874	96.7	-15.3	-138.2
RN2	110708D	515110	4929727	4.64	4.55	-1.10	59.0	-12.7	-133.2
RN3	120718TL	515045	4929810	4.43	4.02	-4.12	351.8	-11.7	-131.0
RS1	120713TI	515138	4928593	4.80	5.07	3.09	274.4	-9.1	-124.0
RS2	120713TL	515125	4928566	4.50	4.30	-2.49	171.4	-9.3	-123.4
RS3	110710Y	515146	4928521	4.87	5.42	9.18	155.3	-12.9	-127.8
RS4	110710D	515145	4928523	4.24	5.54	39.9	117.1	-15.3	-139.8
RS5-2011	110720F1	515129	4928550	3.80	3.82	0.916	165.8	-13.5	-134.2
RS5-2012	120713TJ	515129	4928550	4.52	5.13	14.4	224.3	-13.3	-134.8

[a] Sample IDs begin in YYMMDD format. [b] UTM coordinates, all in zone 12T, using the WGS84 datum. [c] Field pH corrected to achieve charge balance (see procedures above).

Table S4. Semi-quantitative abundance data for chlorophyll *a* and derivatives.[a]

Assignment	chl. *a*	chl. *a*'	phe. *a*	phe. *a*'	phe. *a* allomer	pyrophe. *a*	phede. *a*
Peak number [b]	B2	B3	B6	B5	B4	B7	B1
Soret band (nm)	431	432	409	409	409	410	408
Q_y band (nm)	665	665	666	666	666	666	665
molecular ion (m/z)	893.4	893.5	871.5	871.5	887.5	813.5	593.2
FF1	nd [c]	nd	20674	2898	3872	10418	28521
IG1	30452	396	1214	174	231	157	6180
IG2	62118	2475	1766	325	nd	572	3328
IG3	90981	1707	35160	5301	5152	586	2388
RN1-2011	12749	144	528	65	817	1894	nd
RN1-2012	93304	1479	2747	515	2494	286	nd
RN2	100392	3310	33731	6256	22130	15701	3863
RN3	223066	2857	14790	2512	9688	5654	nd
RS1	144631	4956	9389	2041	3534	3000	4786
RS2	41007	1158	7702	1435	5461	3388	nd
RS3	73171	1806	16080	2516	15697	3344	2303
RS4	83014	980	3615	385	1265	172	nd
RS5-2011	73321	1193	7088	1303	1259	1288	3115
RS5-2012	nd	nd	446	199	307	999	nd

[a] Data from observation at 665 nm. [b] Figure S3. [c] Not detected. Abbreviations: chl, chlorophyll; phe, pheophytin; pyrophe, pyropheophytin; phede, pheophorbide.

Table S5. Major carotenoid semi-quantitative abundance data.[a]

assignment	β-carotene	β-crypto-xanthin	zeaxanthin	echinenone	cantha-xanthin	13-Z-β-carotene	9-Z-β-carotene
peak number [b]	A5	not shown [c]	A1	A3	A2	A4	A6
absorbance maxima (nm)	452, 478	452, 478	451, 477	465	477	338, 444, 470	448, 474
% III/II	22	28	31	undefined [d]	undefined [d]	4	25
molecular ion (m/z)	537.4	553.4	569.5	551.4	565.4	537.4	537.4
FF1	4457	nd [e]	7468	nd	nd	nd	1646
IG1	4371	1263	15303	nd	nd	621	1353
IG2	9709	3226	43092	nd	nd	1704	2986
IG3	12971	nd	11147	9416	3857	1774	3540
RN1-2011	4683	nd	990	1668	384	nd	1421
RN1-2012	21792	nd	8497	8198	2711	3464	5803
RN2	25869	nd	9258	21075	5877	nd	9186
RN3	52709	nd	13335	15340	4993	nd	14022
RS1	119548	nd	27570	9347	2336	26504	44259
RS2	6530	nd	2329	2404	424	nd	2236
RS3	7704	nd	3675	8885	17671	1037	2580
RS4	10565	nd	4573	4073	593	1433	3149
RS5-2011	17659	nd	5357	7845	1618	1856	4395
RS5-2012	398	nd	394	408	nd	nd	208

[a] Data from observation at 475 nm. [b] Figure S3. [c] Not present in Figure S3; retention time is 54 minutes. [d] Only one absorbance maximum, so value undefined. [e] Not detected.

Table S6. Semi-quantitative abundance data for other chlorophylls and their derivatives.[a]

Assignment	Zn-bacterio-chlorophyll *a*	bacterio-pheophytin *a*	chloro-phyllide *b*	pheophytin *b*	pheophytin *b* allomer	chlorophyll *d* [d]	pyro-pheophytin *d* [d]
Retention time (min.)	16	32	18	61	53	24	71
Soret band (nm)	356	359	436	436	435	443	424
Q_y band (nm)[b]	761	746	652	653	652	697	692
molecular ion (m/z)	951.4	889.6	628.5	885.6	901.4	895.7	815.5
FF1	nd [c]	416	nd	1774	310	nd	nd
IG1	nd	nd	166	338	nd	nd	nd
IG2	nd	nd	339	231	nd	nd	nd
IG3	nd	nd	nd	nd	nd	nd	nd
RN1-2011	nd	nd	nd	nd	nd	160	nd
RN1-2012	nd	nd	nd	nd	nd	nd	nd
RN2	nd	nd	nd	nd	nd	1207	nd
RN3	nd	nd	nd	nd	nd	nd	464
RS1	152	986	nd	nd	nd	36	nd
RS2	nd	1265	nd	nd	nd	nd	631
RS3	nd	nd	nd	nd	nd	nd	nd
RS4	385	nd	nd	nd	nd	nd	nd
RS5-2011	nd	nd	nd	nd	nd	nd	nd
RS5-2012	nd	nd	nd	nd	nd	nd	nd

[a] Data from observation at 360 nm. [b] Longest-wavelength absorption maximum. [c] Not detected. [d] It is likely that chlorophyll *d* and pyropheophytin *d* are derived from enzymatic oxidation of chlorophyll *a* and pyropheophytin *a* during extraction (*e.g.*, Kadowaki *et al.* 2005), though it is possible that chlorophyll *d* is biosynthesized by *Chlorogloeopsis* sp., which *C. fritschii* is known to do under natural light (Airs *et al.*, 2014), albeit concomitantly with chlorophyll *f* which was not detected here.

Table S7. Quantitative abundances for selected pigments expressed as µmol/g N.

	chlorophyll a	β-carotene	zeaxanthin [a]	β-cryptoxanthin [a]
FF1	nd [b]	2.2	3.5	nd
IG1	16.7	2.2	7.2	0.6
IG2	34.0	4.8	20.2	1.6
IG3	49.7	6.4	5.2	nd
RN1-2011	7.0	2.3	0.5	nd
RN1-2012	51.0	10.8	4.0	nd
RN2	54.9	12.8	4.3	nd
RN3	122.0	26.2	6.2	nd
RS1	79.1	59.3	12.9	nd
RS2	22.4	3.2	1.1	nd
RS3	40.0	3.8	1.7	nd
RS4	45.4	5.2	2.1	nd
RS5-2011	40.1	8.8	2.5	nd
RS5-2012	nd	0.2	0.2	nd

[a] Quantified using the response factor for β-carotene and not corrected for slight differences in molar absorptivity. [b] Not detected.

Table S8. Abundance of bacterial 16S rRNA and eukaryal 18S rRNA gene templates as determined by quantitative PCR.

	Bacterial 16S rDNA		**Eukaryal 18S rDNA**	
	copies/ng DNA	uncertainty [a]	copies/ng DNA	uncertainty [a]
IG1	9×10^6	3×10^6	2.5×10^5	0.4×10^5
IG2	5.8×10^5	0.6×10^5	8×10^1	3×10^1
IG3	4×10^5	1×10^5	4.6×10^3	0.6×10^3
RN1-2012	1.2×10^6	0.2×10^6	4	1
RS1	7.7×10^5	0.3×10^5	2.3×10^2	0.3×10^2
RS2	5×10^5	1×10^5	5	1
RS4	4.0×10^5	0.6×10^5	1.31×10^2	1
RS5-2011	1.41×10^6	0.02×10^6	1.4×10^1	1

[a] Uncertainty is the standard deviation of three replicate qPCR assays.

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APPENDIX A: Relative abundances, affiliations, and percent identities of the best BLASTn query for the five most abundant 16S rRNA gene OTUs (left) and 18S rRNA gene OTUs (right) in each of the fourteen samples in this study.

FF1

Bacteria (16S)			Identity	Abundance	**Eukarya (18S)**			Identity	Abundance
Best BLASTn Hit	Phylum	Order	(%)	(%)	Best BLASTn Hit	Phylum	Order	(%)	(%)
Hydrogenobaculum sp. Y04ANC1	Aquificae	*Aquificales*	97	46	*Cryptococcus albidus*	Bastidiomycota	*Filobasidiales*	100	39
Desulfotomaculum kuznetsovii DSM 6115	Firmicutes	*Clostridiales*	93	9	*Cyanidioschyzon merolae* strain 10D	Rhodophyta	*Cyanidiales*	100	18
Thermoanaerobacterium thermosaccharolyticum DSM 571	Firmicutes	*Thermoanaerobacterales*	99	9	*Cyanidioschyzon merolae* strain 10D	Rhodophyta	*Cyanidiales*	100	11
Desulfoglaeba sp. Lake	Proteobacteria	*Syntrophobacterales*	89	6	*Cyanidioschyzon merolae* strain 10D	Rhodophyta	*Cyanidiales*	100	7
Ktedonobacteria bacterium Hsw-67	Chloroflexi	*Ktedonobacterales*	94	5	*Cyanidioschyzon merolae* strain 10D	Rhodophyta	*Cyanidiales*	100	5

IG1

Bacteria (16S)			Identity	Abundance	**Eukarya (18S)**			Identity	Abundance
Best BLASTn Hit	Phylum	Order	(%)	(%)	Best BLASTn Hit	Phylum	Order	(%)	(%)
Thermodesulforhabdus norvegica strain A8444	Proteobacteria	*Syntrophobacterales*	90	23	*Cyanidioschyzon merolae* strain 10D	Rhodophyta	*Cyanidiales*	100	35
Ktedonobacteria bacterium Hsw-67	Chloroflexi	*Ktedonobacterales*	94	19	*Cyanidioschyzon merolae* strain 10D	Rhodophyta	*Cyanidiales*	100	22
Hydrogenobaculum sp. SN	Aquificae	*Aquificales*	100	9	*Cyanidioschyzon merolae* strain 10D	Rhodophyta	*Cyanidiales*	100	14
Acidobacteria bacterium WSF1-34	Acidobacteria	Gp2	97	5	*Cyanidioschyzon merolae* strain 10D	Rhodophyta	*Cyanidiales*	100	8
Desulfotomaculum thermobenzoicum	Firmicutes	*Clostridiales*	92	4	*Cyanidioschyzon merolae* strain 10D	Rhodophyta	*Cyanidiales*	100	7

IG2

Bacteria (16S)			Identity	Abundance	**Eukarya (18S)**			Identity	Abundance
Best BLASTn Hit	Phylum	Order	(%)	(%)	Best BLASTn Hit	Phylum	Order	(%)	(%)
Ktedonobacteria bacterium Hsw-67	Chloroflexi	*Ktedonobacterales*	94	52	*Cyanidioschyzon merolae* strain 10D	Rhodophyta	*Cyanidiales*	100	26
Ktedonobacteria bacterium Hsw-67	Chloroflexi	*Ktedonobacterales*	93	10	*Cyanidioschyzon merolae* strain 10D	Rhodophyta	*Cyanidiales*	100	16
Thiomonas sp. 6C	Proteobacteria	*Burkholderiales*	99	6	*Chlamydomonadaceae* sp. RT1n14cul	Chlorophyta	*Chlamydomonadales*	99	10
Hydrogenobaculum sp. SN	Aquificae	*Aquificales*	100	5	*Cyanidioschyzon merolae* strain 10D	Rhodophyta	*Cyanidiales*	100	9
Chloroflexi bacterium T81	Chloroflexi	unclassified	87	3	*Cyanidioschyzon merolae* strain 10D	Rhodophyta	*Cyanidiales*	100	5

IG3

Bacteria (16S)			Identity	Abundance	**Eukarya (18S)**			Identity	Abundance
Best BLASTn Hit	Phylum	Order	(%)	(%)	Best BLASTn Hit	Phylum	Order	(%)	(%)
Chlorogloeopsis sp. Greenland 5	Cyanobacteria	*Stigonematales*	100	48	*Cyanidioschyzon merolae* strain 10D	Rhodophyta	*Cyanidiales*	100	32
Ktedonobacteria bacterium Hsw-67	Chloroflexi	*Ktedonobacterales*	94	16	*Cyanidioschyzon merolae* strain 10D	Rhodophyta	*Cyanidiales*	100	24
Acidobacteria bacterium IGE-010	Acidobacteria	unclassified	91	5	*Cyanidioschyzon merolae* strain 10D	Rhodophyta	*Cyanidiales*	100	14
Ktedonobacteria bacterium Hsw-67	Chloroflexi	*Ktedonobacterales*	93	3	*Cyanidioschyzon merolae* strain 10D	Rhodophyta	*Cyanidiales*	100	9
Meiothermus granaticus	Deinococcus-Thermus	Thermales	100	3	*Cyanidioschyzon merolae* strain 10D	Rhodophyta	*Cyanidiales*	100	7

RN1-2011

Bacteria (16S)			Identity	Abundance	**Eukarya (18S)**			Identity	Abundance
Best BLASTn Hit	Phylum	Order	(%)	(%)	Best BLASTn Hit	Phylum	Order	(%)	(%)
Chlorogloeopsis sp. Greenland 5	Cyanobacteria	*Stigonematales*	100	58	*Cochliobolus kusanoi*	Ascomycota	*Pleosporales*	99	36
Acidobacteriaceae bacterium K22	Acidobacteria	*Acidobacteriales*	100	11	*Penidiella columbiana*	Ascomycota	*Capnodiales*	100	24
Acidobacteria bacterium WSF1-34	Acidobacteria	Gp2	96	10	*Chironomus plumosus*	Arthropoda	*Diptera*	99	11
Chlorogloeopsis sp. Greenland 5	Cyanobacteria	*Stigonematales*	96	3	*Cryptococcus albidus*	Bastidiomycota	*Filobasidiales*	100	5
Hydrogenobaculum sp. Y04ANC1	Aquificae	*Aquificales*	97	2	*Surculiseries rugispora*	Ascomycota	*Xylariales*	99	4

RN1-2012

Bacteria (16S)			Identity	Abundance	**Eukarya (18S)**			Identity	Abundance
Best BLASTn Hit	Phylum	Order	(%)	(%)	Best BLASTn Hit	Phylum	Order	(%)	(%)
Chlorogloeopsis sp. Greenland 5	Cyanobacteria	*Stigonematales*	100	79	*Cyanidioschyzon merolae* strain 10D	Rhodophyta	*Cyanidiales*	100	23
Chlorogloeopsis sp. Greenland 5	Cyanobacteria	*Stigonematales*	96	4	*Cyanidioschyzon merolae* strain 10D	Rhodophyta	*Cyanidiales*	100	16
Rudea sp. YC6842	Proteobacteria	Xanthomonadales	93	3	*Penidiella columbiana*	Ascomycota	*Capnodiales*	100	15
Sulfurihydrogenibium sp. Y03AOP1	Aquificae	*Aquificales*	100	2	*Cyanidioschyzon merolae* strain 10D	Rhodophyta	*Cyanidiales*	100	9
Hydrogenobaculum sp. Y04ANC1	Aquificae	*Aquificales*	97	2	*Cochliobolus kusanoi*	Ascomycota	*Pleosporales*	99	5

RN2

Bacteria (16S)			Identity	Abundance	**Eukarya (18S)**			Identity	Abundance
Best BLASTn Hit	Phylum	Order	(%)	(%)	Best BLASTn Hit	Phylum	Order	(%)	(%)
Chlorogloeopsis sp. Greenland 5	Cyanobacteria	*Stigonematales*	100	31	*Pichia kudriavzevii* strain IPE100	Ascomycota	*Saccharomycetales*	100	31
Acidobacteria bacterium IGE-016	Acidobacteria	unclassified	94	16	*Cyanidioschyzon merolae* strain 10D	Rhodophyta	*Cyanidiales*	100	22
Hydrogenobaculum sp. Y04ANC1	Aquificae	*Aquificales*	97	7	*Cyanidioschyzon merolae* strain 10D	Rhodophyta	*Cyanidiales*	100	18
Thermolithobacter ferrireducens strain KA2	Firmicutes	*Thermolithobacterales*	89	7	*Cyanidioschyzon merolae* strain 10D	Rhodophyta	*Cyanidiales*	100	9
Sulfurihydrogenibium sp. Y03AOP1	Aquificae	*Aquificales*	100	4	*Cyanidioschyzon merolae* strain 10D	Rhodophyta	*Cyanidiales*	100	5

RN3

Bacteria (16S)			Identity	Abundance	**Eukarya (18S)**			Identity	Abundance
Best BLASTn Hit	Phylum	Order	(%)	(%)	Best BLASTn Hit	Phylum	Order	(%)	(%)
Chlorogloeopsis sp. Greenland 5	Cyanobacteria	*Stigonematales*	100	64	*Chlamydomonadaceae* sp. RT1n14cul	Chlorophyta	*Chlamydomonadales*	99	32
Hydrogenobaculum sp. Y04ANC1	Aquificae	*Aquificales*	97	9	*Cyanidioschyzon merolae* strain 10D	Rhodophyta	*Cyanidiales*	100	11
Hydrogenobaculum sp. SN	Aquificae	*Aquificales*	100	3	*Metopus palaeformis*	Ciliophora	*Armophorida*	96	10
Chlorogloeopsis sp. Greenland 5	Cyanobacteria	*Stigonematales*	96	3	*Cyanidioschyzon merolae* strain 10D	Rhodophyta	*Cyanidiales*	100	7
Melioribacter roseus P3M	Ignavibacteria	*Ignavibacteriales*	100	2	*Cyanidioschyzon merolae* strain 10D	Rhodophyta	*Cyanidiales*	100	5

RS1

Bacteria (16S)			Identity	Abundance	**Eukarya (18S)**			Identity	Abundance
Best BLASTn Hit	Phylum	Order	(%)	(%)	Best BLASTn Hit	Phylum	Order	(%)	(%)
Chlorogloeopsis sp. Greenland 5	Cyanobacteria	*Stigonematales*	100	48	*Cyanidioschyzon merolae* strain 10D	Rhodophyta	*Cyanidiales*	100	28
Chloracidobacterium thermophilum	Acidobacteria	Unclassified	99	12	*Cyanidioschyzon merolae* strain 10D	Rhodophyta	*Cyanidiales*	100	15
Synechococcus sp. C9	Cyanobacteria	*Chroococcales*	100	9	*Cyanidioschyzon merolae* strain 10D	Rhodophyta	*Cyanidiales*	100	9
Acidobacteria bacterium IGE-016	Acidobacteria	unclassified	94	4	*Neolecta irregularis* strain ZW-Geo79-Clark	Ascomycota	*Neolectales*	92	6
Sulfurihydrogenibium sp. Y03AOP1	Aquificae	*Aquificales*	100	4	*Parasonderia vestita*	Ciliophora	*Plagiopylida*	87	6

RS2

Bacteria (16S)			Identity	Abundance	**Eukarya (18S)**			Identity	Abundance
Best BLASTn Hit	Phylum	Order	(%)	(%)	Best BLASTn Hit	Phylum	Order	(%)	(%)
Chlorogloeopsis sp. Greenland 5	Cyanobacteria	*Stigonematales*	100	42	*Ascoidea hylecoeti* strain NRRL Y-17634	Ascomycota	*Saccharomycetales*	83	22
Bacterium Ellin5258	Acidobacteria	*Acidobacteriales*	97	24	*Cyanidioschyzon merolae* strain 10D	Rhodophyta	*Cyanidiales*	100	14
Ktedonobacteria bacterium Hsw-67	Chloroflexi	*Ktedonobacterales*	94	3	*Cyanidioschyzon merolae* strain 10D	Rhodophyta	*Cyanidiales*	100	7
Thiomonas sp. 6C	Proteobacteria	*Burkholderiales*	99	3	*Pseudostichococcus monallantoides*	Chlorophyta	*Trebouxiophyceae*	100	5
Paludibacter propionicigens WB4	Bacteroidetes	*Bacteroidales*	93	2	*Cyanidioschyzon merolae* strain 10D	Rhodophyta	*Cyanidiales*	100	4

RS3

Bacteria (16S)			Identity	Abundance	**Eukarya (18S)**			Identity	Abundance
Best BLASTn Hit	Phylum	Order	(%)	(%)	Best BLASTn Hit	Phylum	Order	(%)	(%)
Chlorogloeopsis sp. Greenland 5	Cyanobacteria	*Stigonematales*	100	58	*Neolecta irregularis* strain ZW-Geo79-Clark	Ascomycota	*Neolectales*	92	24
Sulfurihydrogenibium sp. Y03AOP1	Aquificae	*Aquificales*	100	13	*Uronema* sp. CCAP 334/1	Chlorophyta	*Chaetophorales*	100	20
Meiothermus granaticus	Deinococcus-Thermus	Thermales	100	8	*Pinus wallichiana*	Streptophyta	*Coniferales*	99	13
Meiothermus granaticus strain AF-68	Deinococcus-Thermus	Thermales	91	5	*Cyanidioschyzon merolae* strain 10D	Rhodophyta	*Cyanidiales*	100	5
Chlorogloeopsis sp. Greenland 5	Cyanobacteria	*Stigonematales*	96	3	*Codosiga minima* strain IOW73	Choanoflagellida	*Codonosigidae*	87	4

RS4

Bacteria (16S)			Identity	Abundance	**Eukarya (18S)**			Identity	Abundance
Best BLASTn Hit	Phylum	Order	(%)	(%)	Best BLASTn Hit	Phylum	Order	(%)	(%)
Chlorogloeopsis sp. Greenland 5	Cyanobacteria	*Stigonematales*	100	60	*Neolecta irregularis* strain ZW-Geo79-Clark	Ascomycota	*Neolectales*	92	54
Tepidimonas sp. AA2	Proteobacteria	*Burkholderiales*	98	4	*Chlorella protothecoides* var. acidicola	Chlorophyta	Chlorellales	100	12
Chlorogloeopsis sp. Greenland 5	Cyanobacteria	*Stigonematales*	96	3	*Chlamydomonadaceae* sp. RT1n14cul	Chlorophyta	*Chlamydomonadales*	99	5
Rudea sp. YC6842	Proteobacteria	Xanthomonadales	93	2	*Metopus palaeformis*	Ciliophora	*Armophorida*	96	4
Bacterium Ellin5258	Acidobacteria	*Acidobacteriales*	97	2	*Mesotaenium caldariorum*	Streptophyta	Zygnematales	98	3

RS5-2011

Bacteria (16S)			Identity	Abundance	**Eukarya (18S)**			Identity	Abundance
Best BLASTn Hit	Phylum	Order	(%)	(%)	Best BLASTn Hit	Phylum	Order	(%)	(%)
Chlorogloeopsis sp. Greenland 5	Cyanobacteria	*Stigonematales*	100	77	*Talaromyces purpurogenus* isolate BMC1	Ascomycota	*Eurotiales*	100	20
Sulfurihydrogenibium sp. Y03AOP1	Aquificae	*Aquificales*	100	3	*Pseudostichococcus monallantoides*	Chlorophyta	*Trebouxiophyceae*	100	8
Chlorogloeopsis sp. Greenland 5	Cyanobacteria	*Stigonematales*	96	2	*Cyanidioschyzon merolae* strain 10D	Rhodophyta	*Cyanidiales*	100	8
Sparobacteria bacterium NM5	Verrucomicrobia	*Spartobactieria*	93	2	*Penidiella columbiana*	Ascomycota	*Capnodiales*	100	6
Chthonomonas calidirosea	Armatimonadetes	*Chthonomonadales*	92	2	*Cyanidioschyzon merolae* strain 10D	Rhodophyta	*Cyanidiales*	100	5

RS5-2012

Bacteria (16S)			Identity	Abundance	**Eukarya (18S)**			Identity	Abundance
Best BLASTn Hit	Phylum	Order	(%)	(%)	Best BLASTn Hit	Phylum	Order	(%)	(%)
					Fibraurea tinctoria	Streptophyta	*Ranunculales*	100	>99
					Fibraurea tinctoria	Streptophyta	*Ranunculales*	96	<1
No 16S rRNA gene amplicons obtained					*Fibraurea tinctoria*	Streptophyta	*Ranunculales*	97	<1
					Fibraurea tinctoria	Streptophyta	*Ranunculales*	97	<1
					Fibraurea tinctoria	Streptophyta	*Ranunculales*	96	<1

APPENDIX B: Photographs of sample sites taken on the day of sampling (unless otherwise noted).

FF1

IG1


Research conducted under Yellowstone Research Permit YELL-2011-5434

IG2


Research conducted under Yellowstone Research Permit
YELL-2012-5434

IG3


Research conducted under Yellowstone Research Permit
YELL-2012-5434

RN1-2011


Research conducted under Yellowstone Research Permit
YELL-2011-5434

RN1-2012


Research conducted under Yellowstone Research Permit
YELL-2012-5434

RN2


Research conducted under Yellowstone Research Permit YELL-2011-5434

RN3


Research conducted under Yellowstone Research Permit
YELL-2012-5434

RS1


Research conducted under Yellowstone Research Permit
YELL-2012-5434

RS2*

*Photo taken July 2011

RS3


Research conducted under Yellowstone Research Permit YELL-2011-5434

RS4


Research conducted under Yellowstone Research Permit
YELL-2011-5434

RS5-2011


Research conducted under Yellowstone Research Permit
YELL-2011-5434

RS5-2012


Research conducted under Yellowstone Research Permit
YELL-2012-5434