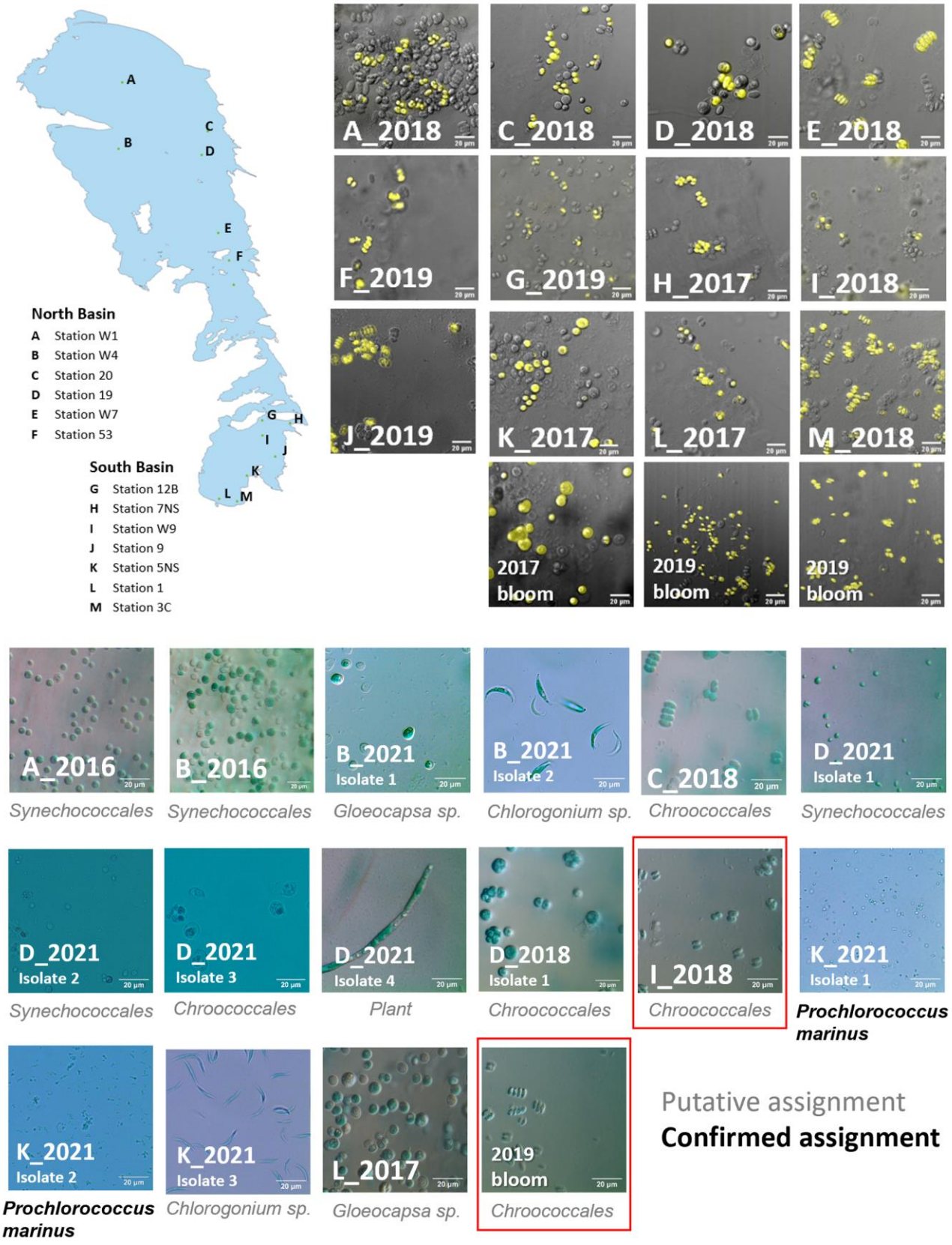

Figure S1. Microscopic images of cyanobacterial cultures. 40X magnification images of crude (greyscale images, cyanobacteria highlighted in yellow) and isolate (color images) cyanobacteria. Putative assignments based on morphological characteristics; confirmed assignments based on 16S rRNA taxonomic identification. Squares encapsulate putatively identical cyanobacterial species.

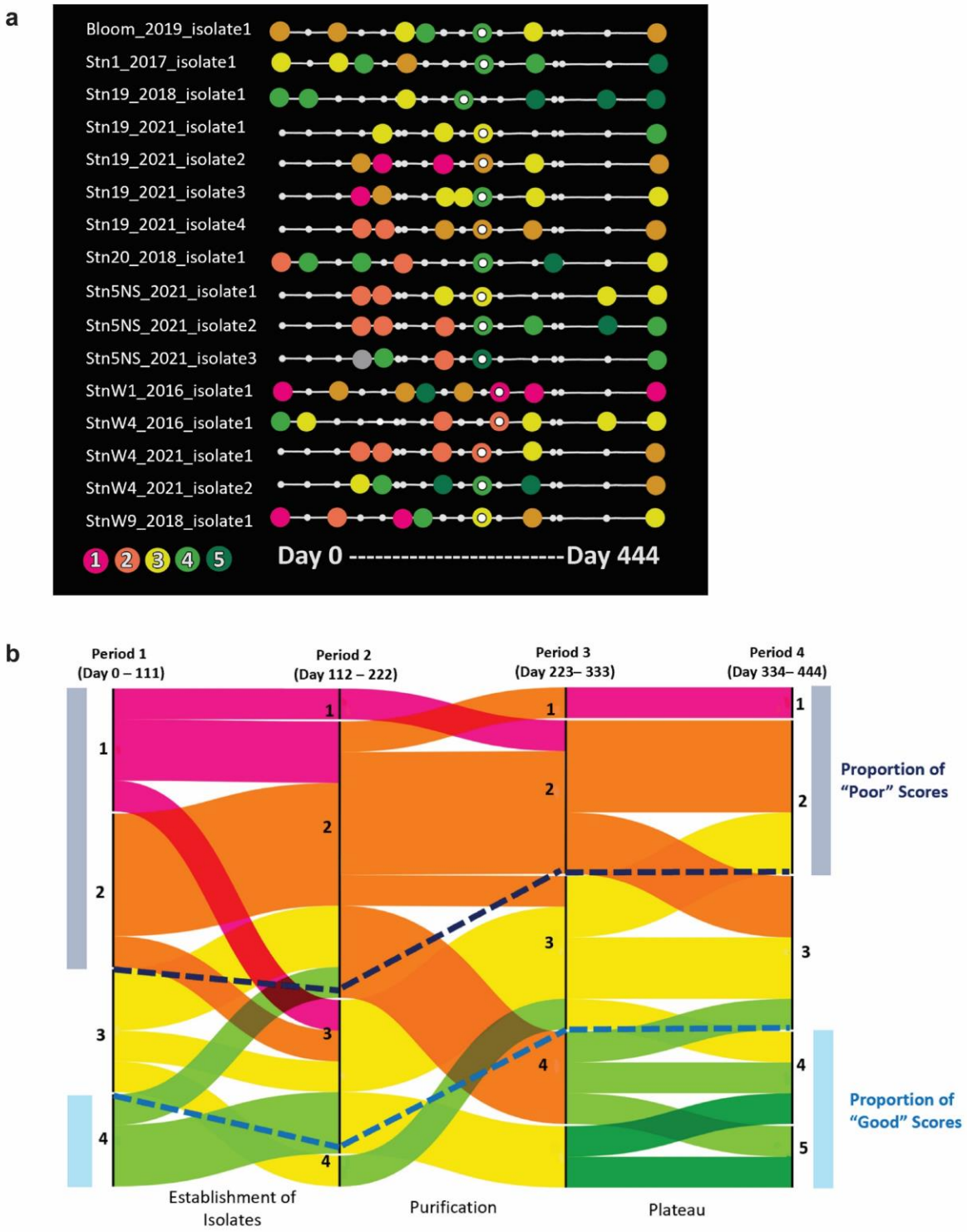

Figure S2. Culture quality tracking spanning a period of 445 days. (a) The lines are normalized to dates of subculturing to show progression over time. The colors used correspond to the qualitative 1-5 ranking. Grey indicates that a subculture was created, but no growth of any kind was observed. White circle denotes subculture used for non-protein amino acid quantification and 16S rRNA metagenomic sequencing. (b) Alluvial diagram showing median culture ranking during four 111 day periods over the course of the cyanobacterial culture purification process. "Good" scores defined as qualitative rankings of 4 or 5; "Poor" scores defined as qualitative rankings of 1 or 2.

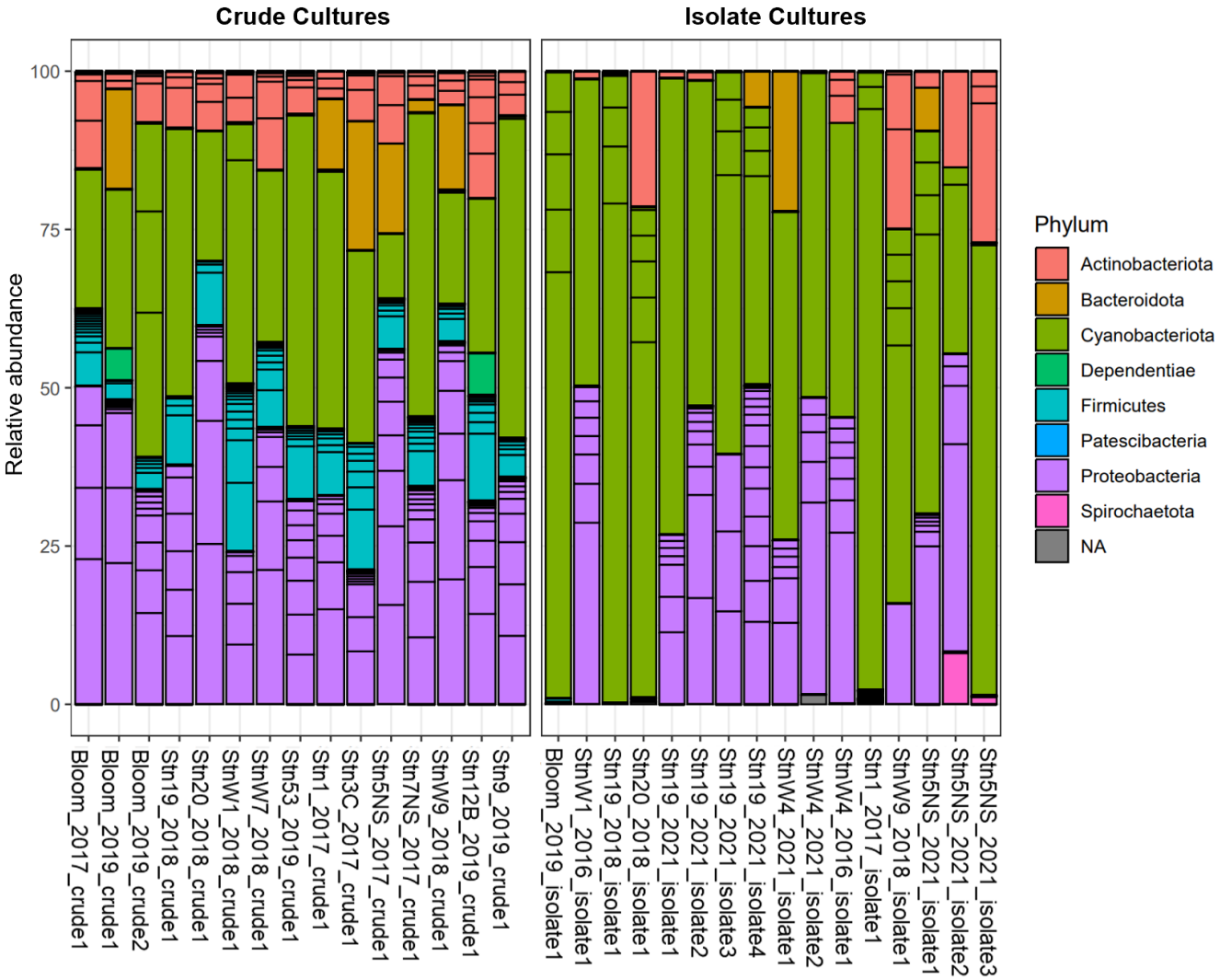

Figure S3. Metagenomic profiles of crude and isolate cultures. Profiles were computed using the DADA2 bioinformatic pipeline and taxonomy assigned using SILVA v138.1 and CyanoSeq v1.2 databases (bootstrap level = 50).

Table S1. Total β-*N*-methylamino-L-alanine (BMAA), 2,4-diaminobutyric acid (DAB), *N*-(2-aminoethyl)glycine (AEG) and β-aminomethyl-L-alanine (BAMA) concentrations for all bloom and isolate cyanobacterial cultures analyzed in this study, including collection sites and years. Values provided as averages ± standard error of the mean. ND means the compound was not detected in any replicates of the sample.

Lake Section	Location	Year Collected	Culture Type	BMAA (ng/g)		AEG (ng/g)		DAB (μg/g)		BAMA (ng/g)	
Blooms	Bloom	2017	Crude	ND		120.36 ±	11.92	2.02 ±	0.25	47.38 ±	15.24
		2019	Crude	ND		108.75 ±	5.28	0.41 ±	0.03	37.54 ±	16.71
			Crude	ND		141.08 ±	6.60	0.82 ±	0.06	34.12 ±	21.71
			Isolate	15.18 ±	11.02	23.73 ±	8.20	0.31 ±	0.06	9.95 ±	4/13
North Basin	North Basin	2018	Crude	347.39 ±	45.19	104.97 ±	3.17	3.65 ±	0.29	35.82 ±	16.82
			Crude	250.87 ±	27.51	55.19 ±	9.78	8.33 ±	0.92	48.42 ±	22.89
	Stn 19	2018	Crude	96.60 ±	22.35	52.56 ±	6.00	0.71 ±	0.04	17.11 ±	2.60
			Isolate	45.90 ±	5.90	23.73 ±	9.93	0.38 ±	0.10	19.40 ±	3.46
		2021	Isolate 1	33.48 ±	11.23	17.54 ±	8.20	0.62 ±	0.04	74.76 ±	26.09
			Isolate 2	49.84 ±	15.89	38.49 ±	15.39	1.21 ±	0.10	71.94 ±	58.45
			Isolate 3	ND		26.89 ±	10.57	0.28 ±	0.02	ND	
			Isolate 4	19.55 ±	5.66	27.96 ±	4.93	0.15 ±	0.02	86.80 ±	10.00
	Stn 20	2018	Crude	331.04 ±	13.03	56.83 ±	2.98	2.04 ±	0.17	ND	
			Crude	317.64 ±	25.83	111.68 ±	7.39	8.35 ±	0.55	25.74 ±	9.06
			Isolate	ND		18.17 ±	8.44	0.80 ±	0.23	6.32 ±	2.73
	Stn W1	2016	Isolate	73.13 ±	35.07	53.97 ±	19.82	0.36 ±	0.05	ND	
		2018	Crude	182.38 ±	40.25	69.28 ±	3.70	1.09 ±	0.03	ND	
			Crude	ND		54.63 ±	4.05	0.97 ±	0.10	11.64 ±	4.67
	Stn W4	2016	Isolate	ND		42.97 ±	17.72	0.11 ±	0.02	188.01 ±	97.30
		2021	Isolate 1	6.69 ±	1.60	14.40 ±	4.16	0.11 ±	0.01	57.53 ±	50.47
			Isolate 2	ND		16.65 ±	5.02	0.50 ±	0.02	26.28 ±	18.63
North Basin Narrows	Stn 53	2019	Crude	ND		127.36 ±	9.07	4.08 ±	0.34	51.46 ±	11.95
			Crude	ND		103.55 ±	9.25	0.36 ±	0.03	22.47 ±	7.64
	Stn W7	2018	Crude	399.84 ±	48.67	428.68 ±	15.33	1.45 ±	0.07	38.00 ±	2.66
			Crude	279.34 ±	42.49	42.42 ±	5.07	1.59 ±	0.09	ND	
South basin	Stn 1	2017	Crude	127.01 ±	20.68	35.07 ±	1.96	3.79 ±	0.43	13.79 ±	5.61
			Isolate	16.16 ±	4.26	20.64 ±	12.51	0.23 ±	0.01	38.51 ±	29.35
	Stn 12B	2019	Crude	ND		120.93 ±	7.72	5.36 ±	0.43	21.67 ±	8.08
			Crude	ND		176.22 ±	11.84	4.30 ±	0.32	21.05 ±	4.18
	Stn 3C	2017	Crude	483.96 ±	52.87	50.82 ±	5.44	1.89 ±	0.12	59.06 ±	24.75
	Stn 5 NS	2017	Crude	113.41 ±	21.30	193.55 ±	18.94	2.72 ±	0.26	31.98 ±	10.48
			Crude	26.78 ±	16.86	68.45 ±	4.19	1.57 ±	0.17	13.94 ±	2.14
		2021	Isolate 1	ND		34.10 ±	10.27	4.78 ±	0.73	39.80 ±	9.63
			Isolate 2	ND		247.66 ±	53.37	11.02 ±	1.42	67.41 ±	37.98
			Isolate 3	ND		43.01 ±	17.04	18.65 ±	2.37	84.78 ±	34.13
	Stn 7 NS	2017	Crude	465.18 ±	61.80	92.49 ±	5.26	1.97 ±	0.23	82.97 ±	27.14
			Crude	139.19 ±	20.82	28.69 ±	0.34	1.27 ±	0.08	28.04 ±	17.12
	Stn 9	2019	Crude	ND		369.39 ±	26.25	5.14 ±	0.44	47.36 ±	18.49
	Stn W9	2018	Crude	206.43 ±	13.96	94.78 ±	8.41	1.67 ±	0.16	21.65 ±	6.34
			Crude	ND		156.68 ±	12.91	1.08 ±	0.14	38.89 ±	7.40
			Isolate	41.65 ±	11.94	35.92 ±	11.75	0.69 ±	0.04	23.61 ±	14.13
		2019	Crude	ND		77.58 ±	2.05	2.91 ±	0.27	25.01 ±	11.62
			Crude	ND		680.98 ±	49.53	1.92 ±	0.13	54.98 ±	21.69

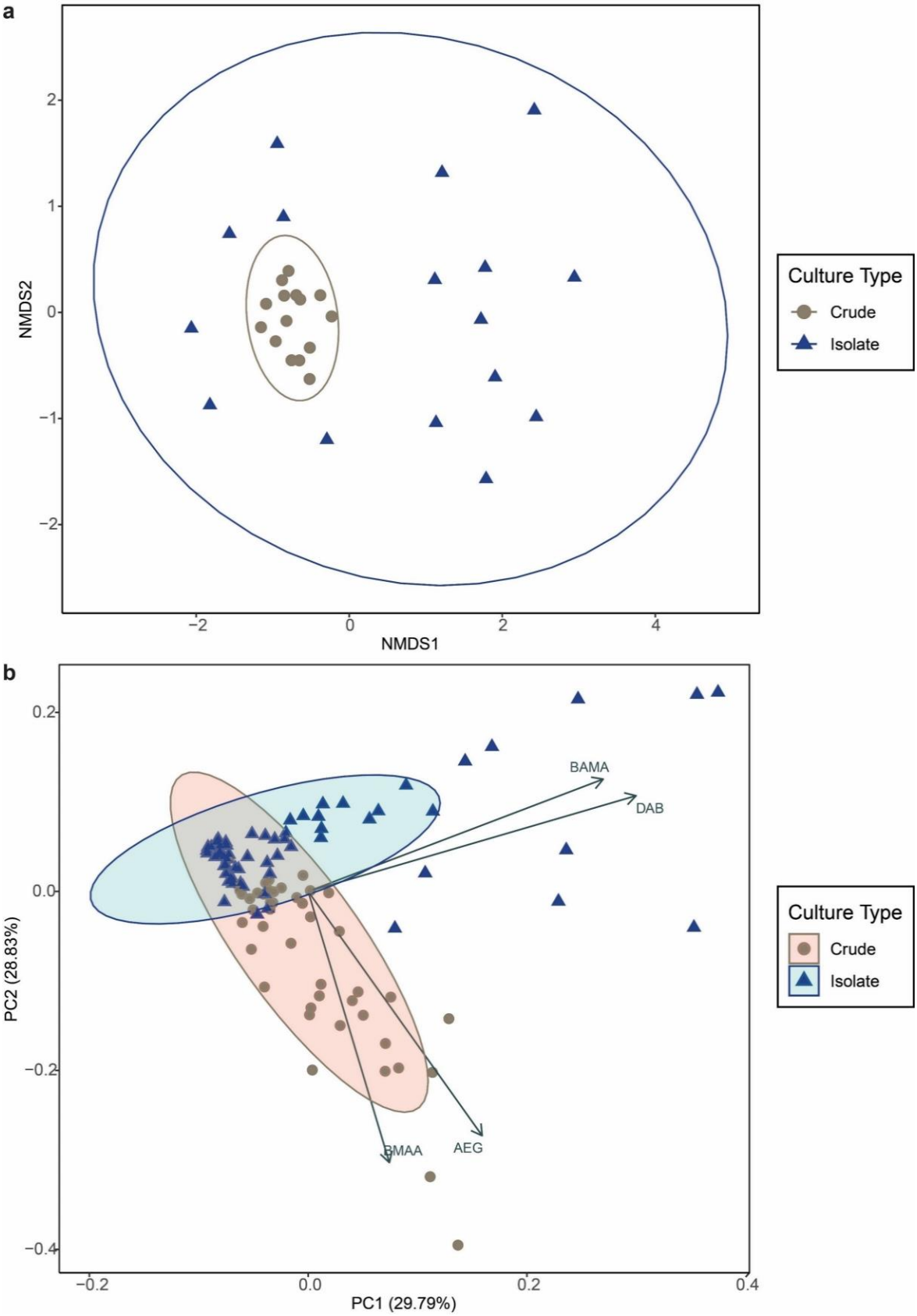

Figure S4. Correlations of non-protein amino acid (NPAA) concentrations to culture type. (a) Non-metric Multi-dimensional Scaling (NMDS) plot of beta diversity measure (Bray-Curtis distances) and (b) Principal Component (PC) Analysis biplot of auto-scaled NPAA concentrations showing clustering of crude cultures (brown circles) compared to isolate cultures (blue triangles). Ellipses show multivariate t-distribution at the 95% confidence level.

Table S2. Multiple reaction monitoring (MRM) transitions for validated ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) method with AccQ-Fluor reagent.

Analyte	Electrospray Ionization Mode	Retention Time (RT; min)	% Relative Standard Deviation (RT)	Transition (m/z)	Cone Voltage (V)	Collision Voltage (V)
β-aminomethyl-L-alanine (BAMA)	+	5.56	0.60%	459 > 171	16	30
				459 > 119	16	18
				459 > 289	16	15
N-(2-aminoethyl)glycine (AEG)	+	5.98	0.21%	459 > 171	16	30
				459 > 119	16	18
				459 > 289	16	15
				459 > 214 (qualifier)	16	20
β-*N*-methylamino-L-alanine (BMAA)	+	6.24	0.45%	459 > 171	16	30
				459 > 119	16	18
				459 > 289	16	15
				459 > 258 (qualifier)	16	20
2,4-diaminobutyric acid (DAB)	+	6.55	0.22%	459 > 171	16	30
				459 > 119	16	18
				459 > 289	16	15
				459 > 188 (qualifier)	16	20