Extended Data Tables and Figures

Extended Data Table 1. Q_{10} values for anaplerotic DIC fixation and heterotrophic carbon production by AltDE. The Q_{10} values were calculated from the cell-specific rates measured at 13°C and 23°C incubation conditions. Values are presented as mean and s.d. (n = 3).

	Q_{10} DICfix		Q_{10} heterotrophic carbon production	
	mean	s.d.	mean	s.d.
1×Ala	2.1	0.4	1.3	0.1
1×Glc	1.9	0.1	1.7	0.1
10×Glc	2.6	0.3	2.4	0.3

Extended Data Table 2. Percentage contribution of putative anaplerotic DIC fixation by Alteromonas to the total dark DIC fixation in the Atlantic and Pacific Ocean. The table shows the proportion of dark DIC fixation attributed to *Alteromonas* relative to the overall dark DIC fixation by prokaryotes, expressed as a percentage. Data are presented for different sampling locations and water masses.

Cruise	Station	WM	Depth (m)	DIC+Alt / DIC+Prok (%)
Geo1	St. 6	ASUW	250	22.7
			400	
	St. 11	WASIW	1250	37.5
		NADW	1750	11.1
			2500	
			3500	
	St. 16	WNACW	250	21.4
			500	
		WASIW	1250	0.0
		NADW	2000	42.9
			3000	
Rad15	St. 11	EASIW	1800	5.3
SO248	St. 6	PEW	200	0.0
			400	
			500	
		AAIW	1000	0.0
		CDW	2000	0.0
	St. 10	PSIW	500	36.8
			1000	
	St. 14	PSUW	200	0.0
		PSIW	500	0.0
		PDW	1000	12.0
			2000	

Abbreviations of the water masses (WM) according to ref[60]: Atlantic Subarctic Upper Water (ASUW), Western North Atlantic Central Water (WNACW), Western Atlantic Subarctic Intermediate Water (WASIW), North Atlantic Deep Water (NADW), Eastern North Atlantic Subarctic Intermediate Water (EASIW), Pacific Equatorial Water (PEW), Antarctic Intermediate Water (AIW), Circumpolar Deep Water (CDW), Pacific Subarctic Upper Water (PSUW), Pacific Subarctic Intermediate Water (PSIW), Pacific Deep Water (PDW).

Extended Data Table 3. Amino acid composition of AltDE proteins during the exponential phase under alanine and glucose incubations. The table lists the amino acid percentages in the proteins, with values corrected based on the complete genome sequence of *Alteromonas macleodii*. Corrected percentages are shown as g% corr.

AA	MW	*A. macleodii**		This study					
		mol%	g%	mol%		g%		g% corr.	
				Ala	Glc	Ala	Glc	Ala	Glc
Asparagine [a]	132.1	4.4	4.5	11.7 ± 0.0	13.5 ± 0.8	12.3 ± 0.0	14.2 ± 0.8	8.9 ± 0.0	10.3 ± 0.6
Aspartic acid [a]	133.1	5.4	5.6						
Glutamine [b]	146.2	4.1	4.6	27.2 ± 0.2	22.2 ± 0.2	31.4± 0.3	26.0 ± 0.3	22.7 ± 0.2	18.8 ± 0.2
Glutamic acid [b]	147.1	6.3	7.2						
Serine	105.1	6.8	5.6	6.1 ± 0.5	6.1 ± 1.4	5.0 ± 0.4	5.1 ± 1.2	3.6 ± 0.3	3.6 ± 0.8
Histidine [c]	155.2	2.3	2.7	4.1 ± 0.1	5.0 ± 0.4	5.0 ± 0.1	6.1 ± 0.5	3.6 ± 0.1	4.4 ± 0.4
Threonine [c]	119.1	5.5	5.1						
Glycine	75.1	6.5	3.8	13.8 ± 0.8	13.5 ± 1.7	8.1 ± 0.5	8.1 ± 1.0	5.9 ± 0.4	5.8 ± 0.7
Alanine	89.1	8.9	6.1	11.3 ± 0.4	13.9 ± 1.5	7.9 ± 0.2	9.8 ± 1.0	5.7 ± 0.2	7.1 ± 0.8
Arginine	174.2	4.5	6.1	5.0 ± 0.1	5.3 ± 0.0	6.9 ± 0.2	7.4 ± 0.1	5.0 ± 0.1	5.3 ± 0.0
Tyrosine	181.2	3.0	4.2	2.7 ± 0.0	2.9 ± 0.0	3.8 ± 0.0	4.1 ± 0.0	2.8 ± 0.0	3.0 ± 0.0
Valine	117.1	7.1	6.5	ND					
Phenylalanine	165.2	4.4	5.6	ND					
Isoleucine	131.2	6.1	6.2	ND					
Leucine	131.2	10.0	10.2	10.1 ± 0.5	10.5 ± 0.2	10.4 ± 0.5	11.0 ± 0.2	7.5 ± 0.4	7.9 ± 0.1
Cysteine	121.2	1.1	1.0	ND					
Lysine	146.2	5.8	6.6	8.0 ± 0.4	7.2 ± 0.0	9.1 ± 0.5	8.3 ± 0.0	6.6 ± 0.3	6.0 ± 0.0
Methionine	149.2	2.8	3.2	ND					
Proline	115.1	3.8	3.4	ND					
Tryptophan	204.2	1.2	1.9	ND					

[*] Amino acid percentages from a complete genome sequence of *A. macleodii*[53].

[a] sum of asparagine and aspartic acid in this study.

[b] sum of glutamine and glutamic acid in this study.

[c] sum of histidine and threonine in this study.

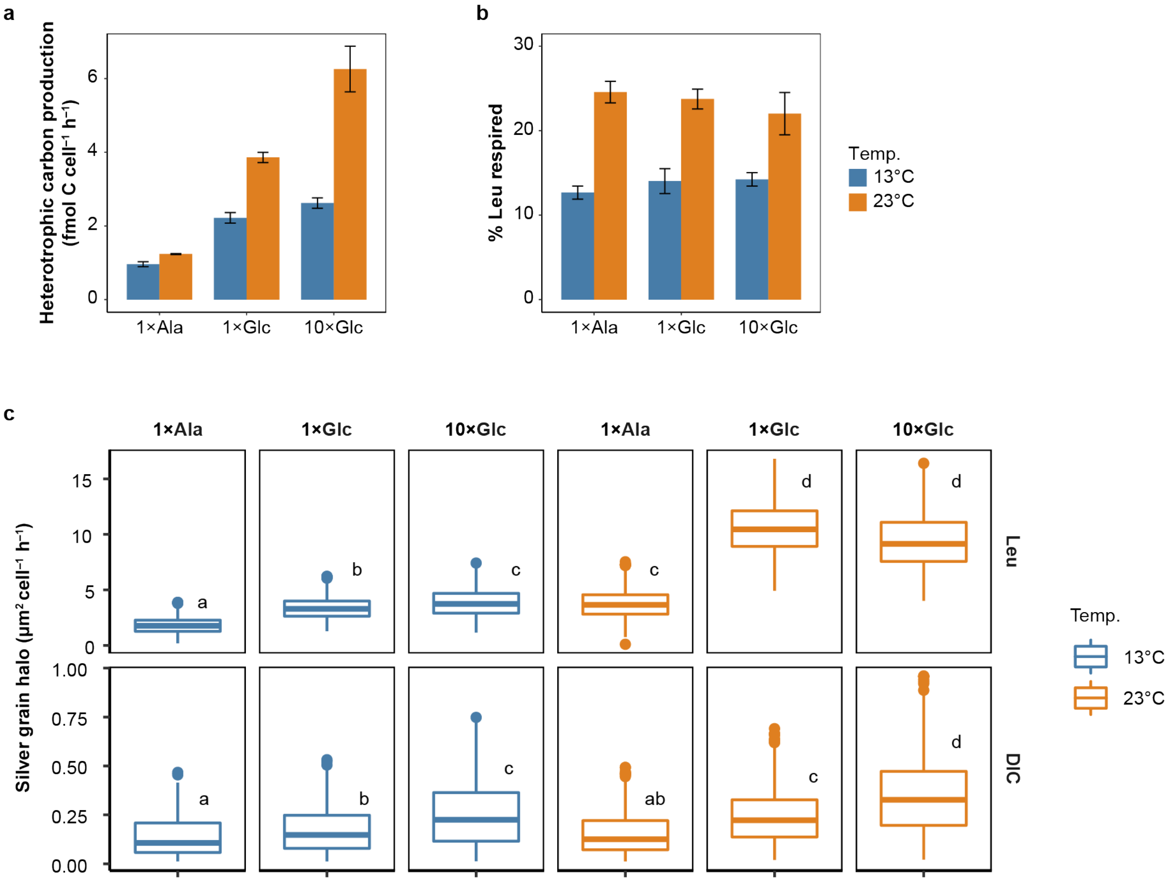
Values are shown as: mean ± |mean – replicate| (n = 2).

AA: amino acids; MW: molecular weight; Glc: 1×Glc, Ala: 1×Ala both at 23°C; ND: not determined.

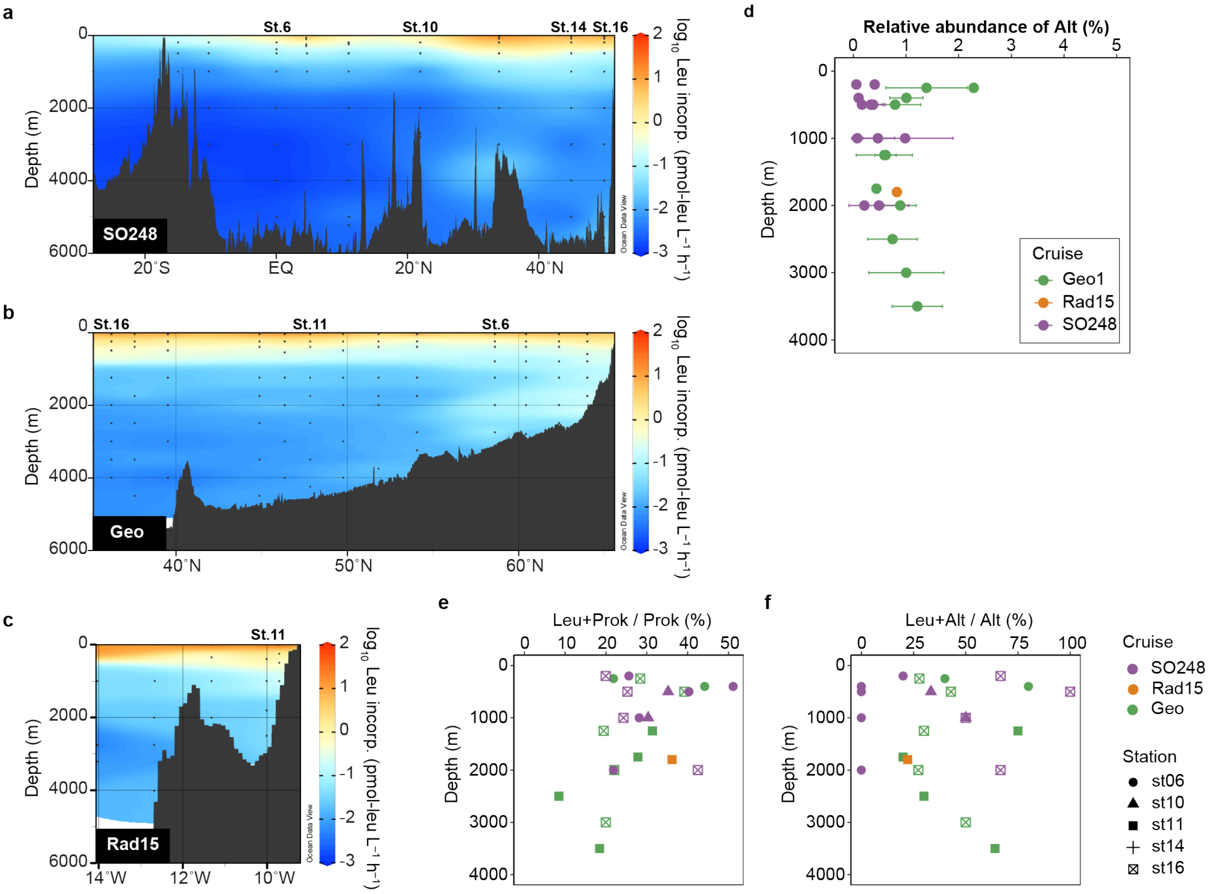
Extended Data Table 4. Empirical leucine to carbon conversion factors (CF_{leu}) for AltDE in incubations with alanine (Ala) or glucose (Glc).

	Ala	Glc
Leu (g%)	7.5 ± 0.4 (2)	7.9 ± 0.1 (2)
Protein (fg cell^{-1})	87.7 ± 0.0 (2)	103.2 ± 3.3 (2)
Carbon (fg cell^{-1})	51.8 ± 4.6 (3)	69.6 ± 8.0 (3)
CF_{Leu} (kg C [mol leu]$^{-1}$)	1.0 ± 0.1	1.1 ± 0.1

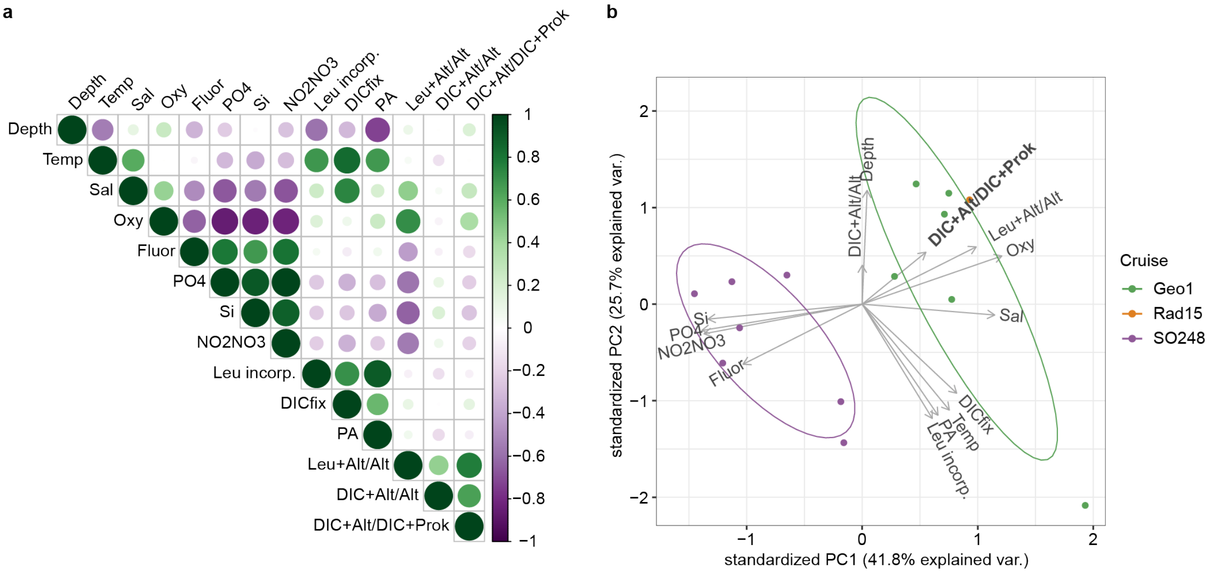
Values are shown as mean ± s.d. (n = 3) or mean ± |mean – replicate| (n = 2). Sample sizes are indicated in the brackets. Error propagated for CF_{leu}. Isotope dilution applied as 1.



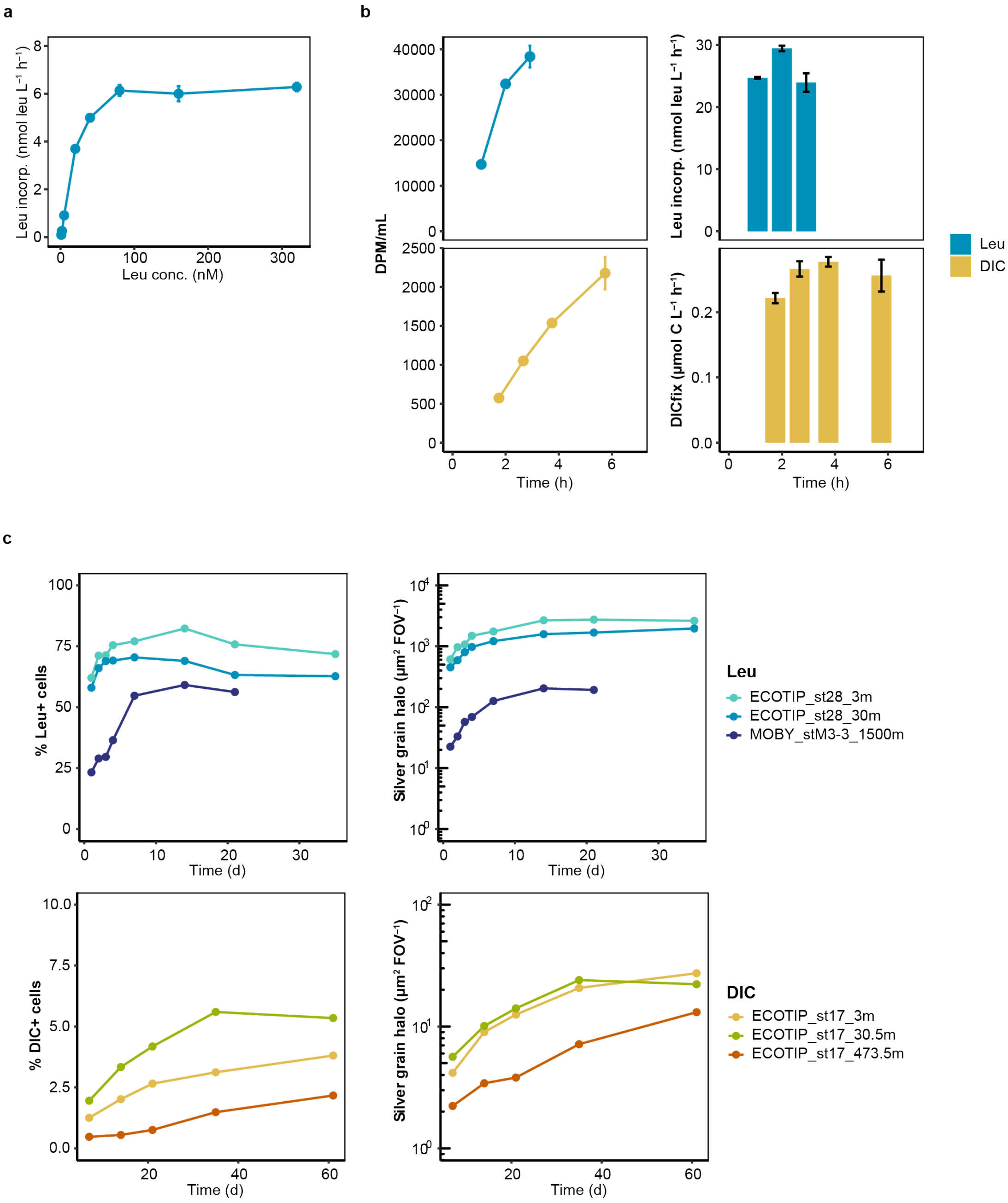
Extended Data Fig. 1. Heterotrophic activity and single-cell activity of AltDE under different organic carbon sources and temperatures. (a) Cell-specific heterotrophic carbon production rates of AltDE. (b) Percentage contribution of leucine respiration to total leucine uptake, calculated as Leu resp. / (Leu incorp. + Leu resp.) in %. (c) Silver grain halo sizes around individual AltDE cells, indicative of their activity levels[55]. Letters next to the boxplots represent results from a post hoc Dunn test following Kruskal-Wallis multiple comparisons, with Leu and DIC samples analyzed separately. Leu resp.: leucine respiration, Leu incorp.: leucine incorporation.



Extended Data Fig. 2. Distribution of prokaryotic heterotrophic activity, shown as leucine incorporation rates, in the Pacific and Atlantic Ocean. (a-c) Transect views of leucine incorporation rates (log scale) during SO248 in Pacific, and Geo and Rad15 cruises in Atlantic Ocean (refer to Fig. 3a). (d, e) Relative abundance of leucine-positive prokaryotes and leucine-positive *Alteromonas* to total prokaryotes and total *Alteromonas*, respectively, as determined by MICRO-CARD-FISH.



Extended Data Fig. 3. Correlation and principal component analysis (PCA) of Alteromonas and environmental parameters. (a) Correlation matrix showing the relationship between active Alteromonas and environmental parameters. Correlation coefficients are represented by the color and size of circles. (b) PCA plot based on standardized values, with variables also displayed as biplots. Temp: temperature, Sal: salinity, Oxy: dissolved oxygen, Fluor: chlorophyll fluorescence, PO4: dissolved inorganic phosphate, Si: dissolved silicate, NO2&NO3: sum of dissolved inorganic nitrite and nitrate, Leu incorp.: leucine incorporation, DICfix: bulk dark DIC fixation, PA: prokaryotic abundance. For example, Leu+Alt/Alt represents the relative abundance of leucine-positive Alteromonas in total Alteromonas.



Extended Data Fig. 4. Methodological adjustments and kinetics. (a) Concentration kinetics for AltDE cultures to determine the saturation concentration of tracer leucine, resulting in a final concentration of 140 nM. AltDE was incubated in 1×Glc at 23°C. (b) Duration kinetics for leucine incorporation (Leu incorp.) and dark DIC fixation (DICfix) rates, with optimal incubation times of 2 hours for leucine and 4 hours for DIC. (c) Detection of leucine-positive (upper panels) and DIC-positive (lower panels) cells under varying exposure times for microautoradiography. The left panels show the relative abundance of tracer-positive cells, while the right panels display the total silver grain halo sizes per field of view (FOV) of the microscope. Natural communities from stations at 69.1267°N, 52.8072°W (ECOTIP_st17), 70.410°N, 56.2697°W (ECOTIP_st28), and 68.0523°E, 50.7001°S (MOBY_stM3-3) were incubated with either ^{3}H-leucine or ^{14}C-bicarbonate (see Methods). Depths are indicated in the legends.