

Supplementary Information for:

Physicochemical controls on ancient carbon assimilation into ecosystem biomass in shallow-water hydrothermal systems

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Calculation of individual percentages and errors per fatty acid

To quantify the contribution of carbon uptake pathways and fatty acid synthesis, isotope-based calculations were performed. These percentages and associated errors were derived using the following equations:

Chemoautotrophy percentage

The percentage of chemoautotrophic carbon uptake (f_{chemo}) and the associated error (σ_{chemo}) were calculated using the measured hydrogen isotope values (δ^2H) as well as those for endmember values of photoautotrophy (δ^2H_{photo}) and chemoautotrophy (δ^2H_{chemo}):

$$f_{\text{chemo}} = \frac{\delta^2H - \delta^2H_{\text{photo}}}{\delta^2H_{\text{chemo}} - \delta^2H_{\text{photo}}} \times 100 \quad (\text{S1})$$

$$\sigma_{\text{chemo}} = \left| \frac{\sigma_{\delta^2H}}{\delta^2H_{\text{chemo}} - \delta^2H_{\text{photo}}} \right| \times 100 \quad (\text{S2})$$

rTCA percentage

The percentage of carbon uptake through the reductive tricarboxylic acid (rTCA) cycle (f_{rTCA}) was calculated using the measured stable carbon isotopic composition ($\delta^{13}C$) and the defined endmembers for carbon taken up by CBB ($\delta^{13}C_{\text{CBB}}$) and rTCA-derived carbon (C_{rTCA}):

$$f_{\text{rTCA}} = \frac{\delta^{13}C - \delta^{13}C_{\text{CBB}}}{\delta^{13}C_{\text{rTCA}} - \delta^{13}C_{\text{CBB}}} \times 100 \quad (\text{S3})$$

Fluid carbon uptake for fatty acid synthesis

The contribution of aged carbon to fatty acid synthesis ($f_{\text{old, d14C}}$) and the associated error ($\sigma_{\text{old, d14C}}$) were calculated using the measured radiocarbon values ($\Delta^{14}\text{C}$) and the defined endmembers for modern ($\Delta^{14}\text{C}_{\text{modern}}$) and aged carbon ($\Delta^{14}\text{C}_{\text{old}}$).

$$f_{\text{old, d14C}} = \frac{\Delta^{14}\text{C} - \Delta^{14}\text{C}_{\text{modern}}}{\Delta^{14}\text{C}_{\text{old}} - \Delta^{14}\text{C}_{\text{modern}}} \times 100 \quad (\text{S4})$$

$$\sigma_{\text{old, d14C}} = \frac{\sigma_{\Delta^{14}\text{C}}}{\Delta^{14}\text{C}_{\text{old}} - \Delta^{14}\text{C}_{\text{modern}}} \times 100 \quad (\text{S5})$$

The resulting values from calculations S1 to S5 are displayed in Supplementary Fig. 3.

Combining individual measurements within a sample

For measurements with multiple data points per sample (e.g., $\Delta^{14}\text{C}$ values of fatty acids), a weighted mean was calculated. The weights were determined as the inverse square of the measurement errors ($w_i = 1/\sigma_i^2$), where f is the individual percentage and w the weight for the measurements (i).

$$\bar{f} = \frac{\sum_i w_i f_i}{\sum_i w_i} \quad (\text{S6})$$

The combined error (standard error of the weighted mean) was calculated as follows:

$$\sigma_{\bar{f}} = \sqrt{\frac{1}{\sum_i w_i}} \quad (\text{S7})$$

The resulting values from calculations S6 and S7 are displayed in Fig. 5.

Supplementary Tables

Supplementary Table 1: Radiocarbon values ($\Delta^{14}\text{C}$), $\delta^{13}\text{C}$ values, and content of particulate organic carbon (POC). For the samples directly above the vent orifice (in cursive), POC in mg L^{-1} were taken from Lin et al.¹, while F^{14}C and $\delta^{13}\text{C}$ values of POC were taken from Wang et al.². For the sample inside the orifice of the yellow vent, the $\Delta^{14}\text{C}$ value was taken from a sample retrieved in 2016, while the carbon content was taken from a sample retrieved in 2015^{1,2}. All other values correspond to the same samples taken in 2015.

Lab code	Sample ID	Vent	horizontal distance to vent (m)	water depth (m)	POC mg L^{-1}	σ	$\delta^{13}\text{C}$ POC (‰)	σ	$\Delta^{14}\text{C}$	σ	Mass C (μg)	$\Delta^{14}\text{C}$ (mass weighted)
	<i>My0-0</i>	YV	0	0	0.58	0.21	-20.3	1.0	-268			
	<i>My0-5</i>	YV	0	5	0.94		-21.0		-125			
	<i>My0-8 (2015)</i>	YV	0	8	0.46	0.08						
	<i>My0-8 (2016)</i>	YV	0	8			-20.5	0.2	-509			
	<i>Mw0-0</i>	WV	0	0	0.28	0.03	-19.4	0.3	-116			
	<i>Mw0-5</i>	WV	0	5	0.42		-20.7		-237			
	<i>Mw0-10</i>	WV	0	10	0.23		-21.0		-567			
	<i>Mw0-10.5</i>	WV	0	10.5	0.55	0.24	-21.8		-393			
	<i>Mw0-11.5</i>	WV	0	11.5	1.03	0.53	-20.8	1.7	-679			
12484.1.1	My6_0m	YV	6	0	0.12	0.84	-23.3	3.0	-68	9	61	-55
12484.1.2	My6_0m	YV	6	0					-129	9	69	
12484.2.1	My6_0m	YV	6	0					-5	10	116	
12485.1.1	My6_8m	YV	6	8	0.12	0.00	-23.3	0.1	-72	8	70	-72
12485.2.1	My6_8m	YV	6	8					-72	10	105	
12486.1.1	My14_9m	YV	14	9	0.29	0.02	-23.2	0.2	-37	8	141	-31
12486.1.2	My14_9m	YV	14	9					-20	9	78	
12487.1.1	My20_0m	YV	20	0	0.17	0.03	-23.0	0.1	103	12	50	-85
12487.1.2	My20_0m	YV	20	0					-178	9	52	
12487.2.1	My20_0m	YV	20	0					-78	10	93	
12488.1.1	My20_10m	YV	20	10	0.10	0.00	-23.6	0.0	-50	9	66	-71
12488.2.1	My20_10m	YV	20	10					-83	9	109	

12489.1.1	Mw6_0m	WV	6	0	0.20	0.00	-22.7	0.1	-111	7	114	-114
12489.1.2	Mw6_0m	WV	6	0					-116	8	135	
12490.1.1	Mw6_13m	WV	6	13	0.17	0.06	-24.1	0.1	-90	19	34	-79
12490.2.1	Mw6_13m	WV	6	13					-72	13	57	
12491.1.1	Mw14_0m	WV	14	0	0.11	0.08	-22.7	1.3	-132	7	95	-261
12491.1.2	Mw14_0m	WV	14	0					-384	6	102	
12491.1.3	Mw14_0m	WV	14	0					-254	8	54	
12492.1.1	Mw14_13m	WV	14	13	0.12	0.01	-23.1	1.3	-91	11	79	-90
12492.1.2	Mw14_13m	WV	14	13					-89	12	64	
12493.1.1	Mw20_0m	WV	20	0	0.08	0.00	-21.8	0.0	-137	8	80	-135
12493.1.2	Mw20_0m	WV	20	0					-129	11	37	
12494.1.1	Mw20_13m	WV	20	13	0.19	0.03	-23.7	0.9	-91	11	74	-93
12494.1.2	Mw20_13m	WV	20	13					-95	11	76	
14992.1.1	Mw6_5m	WV	6	5	0.13	0.00	-22.4	0.2	-114	9	115	
14993.1.1	Mw14_5m	WV	14	5	0.18	0.00	-21.3	0.2	-148	8	148	
14994.1.1	Mw20_5m	WV	20	5	0.20	0.01	-22.1	0.6	-101	9	162	
14995.1.1	My6_5m	YV	6	5	0.17		-23.9		-132	11	63	
14996.1.1	My14_5m	YV	14	5	0.13	0.01	-23.8	0.1	-74	10	96	
14997.1.1	My20_5m	YV	20	5	0.18		-23.8		-130	11	73	

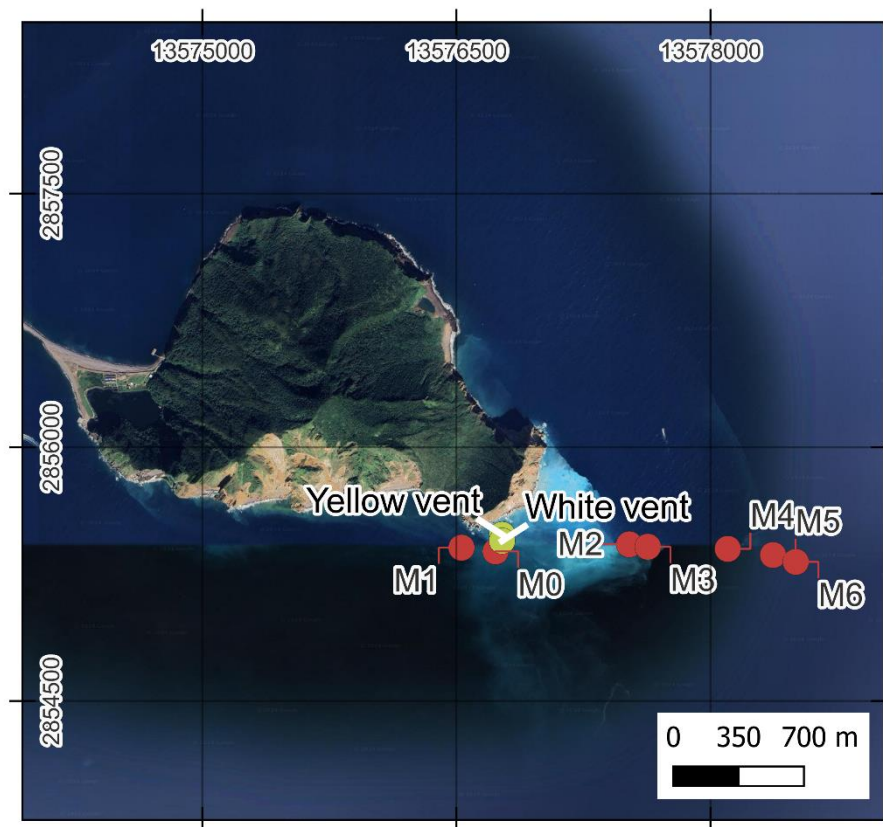
Supplementary Table 2: Hydrogen stable isotope compositions of vent fluids and water column samples from Kueishantao. Sampling locations are shown in Supplementary Fig. 1. The reproducibility of $\delta^2\text{H}$ is better than 0.5‰ (2σ , $n=6$), by repeating the analyses of selected samples.

Sample ID	$\delta^2\text{H}_{\text{VSMOW}}$ (‰)	horizontal distance to vent (m)
YV vent	0.3	0
WV vent	0.1	0
YV 0m	0.5	0
WV 0m	1.3	0
YV 20/8m (20 meters distance, 8 meters depth)	1.9	20
WV 19m	1.4	19
M0 0M	0.8	70
M1 0M	1.5	230
M2 0M	1.1	700
M3 0M	1.6	800
M4 0M	1.0	1200
M5 0M	1.3	1500
M6 0M	1.0	1600
M1 14M	1.0	230
M2 30M	0.0	700
M3 33M	0.8	800
M4 50M	1.3	1200
M5 70M	0.3	1500
M6 100M	0.6	1600
M6 160M	0.6	1600

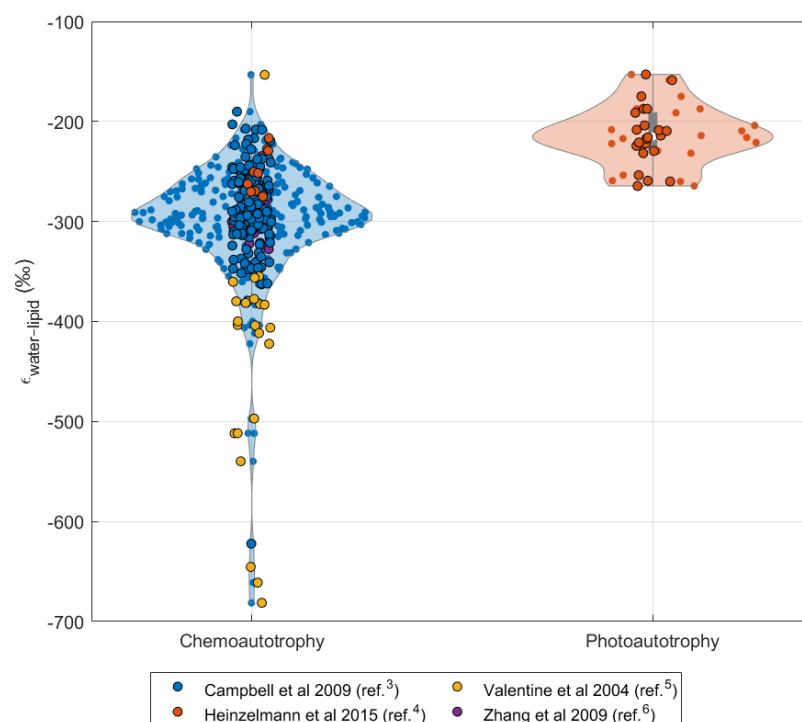
Supplementary Table 3: Radiocarbon content, measurement targets, mass (μgC), and $\delta^2\text{H}$ of measured fatty acids. $\text{C}_{16:1}$ from the White vent 20 m distance sample was lost during ^{14}C measurements.

Lab code	Sample	Fatty acid	$\Delta^{14}\text{C}$ (‰)	$\pm\sigma$ - $\Delta^{14}\text{C}$	target	Mass (μgC)	$\delta^2\text{H}$	error
13440.1.1	WV 1 m	$\text{C}_{14:0}$	-87	7	gas	111	-284	2
13440.2.1	WV 1 m	<i>i/ai</i> - $\text{C}_{15:0}$	-107	8	gas	107	-212	2
13440.3.1	WV 1 m	$\text{C}_{16:1}$	-126	7	gas	154	-265	1
13440.4.1	WV 1 m	$\text{C}_{16:0}$	-88	3	graphite	313	-262	1
13440.5.1	WV 1 m	$\text{C}_{18:1}$	-119	7	gas	149	-265	2
13440.6.1	WV 1 m	$\text{C}_{18:0}$	-118	9	gas	47	-216	0
13441.1.1	WV 20 m	$\text{C}_{14:0}$	-220	11	gas	35	-261	2
13441.2.1	WV 20 m	<i>i/ai</i> - $\text{C}_{15:0}$	-120	9	gas	43	-202	1
n.d.	WV 20 m	$\text{C}_{16:1}$	n.d.	n.d.	n.d.	n.d.	-229	1
13441.4.1	WV 20 m	$\text{C}_{16:0}$	-94	9	gas	50	-229	2
13441.5.1	WV 20 m	$\text{C}_{18:1}$	-98	8	gas	48	-230	1
13441.6.1	WV 20 m	$\text{C}_{18:0}$	-56	19	gas	14	-219	1
13442.1.1	YV 1 m	$\text{C}_{14:0}$	-208	10	gas	34	-296	5
13442.2.1	YV 1 m	<i>i/ai</i> - $\text{C}_{15:0}$	-208	11	gas	31	-216	1
13442.3.1	YV 1 m	$\text{C}_{16:1}$	-204	8	gas	65	-305	2
13442.4.1	YV 1 m	$\text{C}_{16:0}$	-123	7	gas	149	-263	0
13442.5.1	YV 1 m	$\text{C}_{18:1}$	-234	9	gas	42	-301	1
13442.6.1	YV 1 m	$\text{C}_{18:0}$	-54	11	gas	31	-259	2

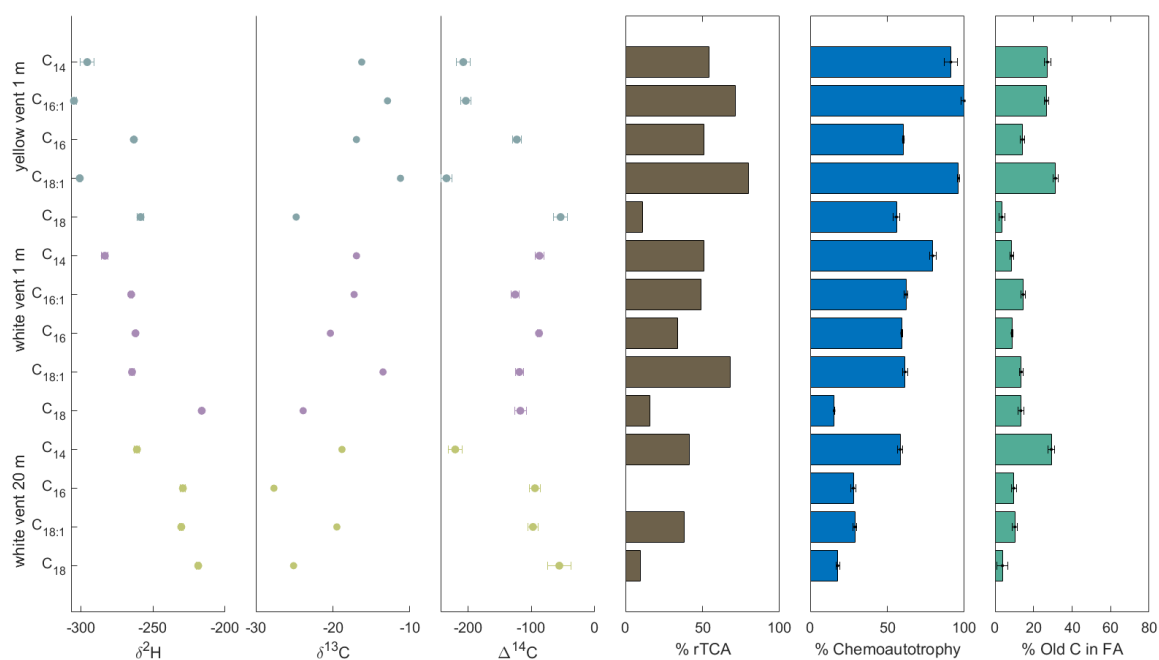
Supplementary Figures



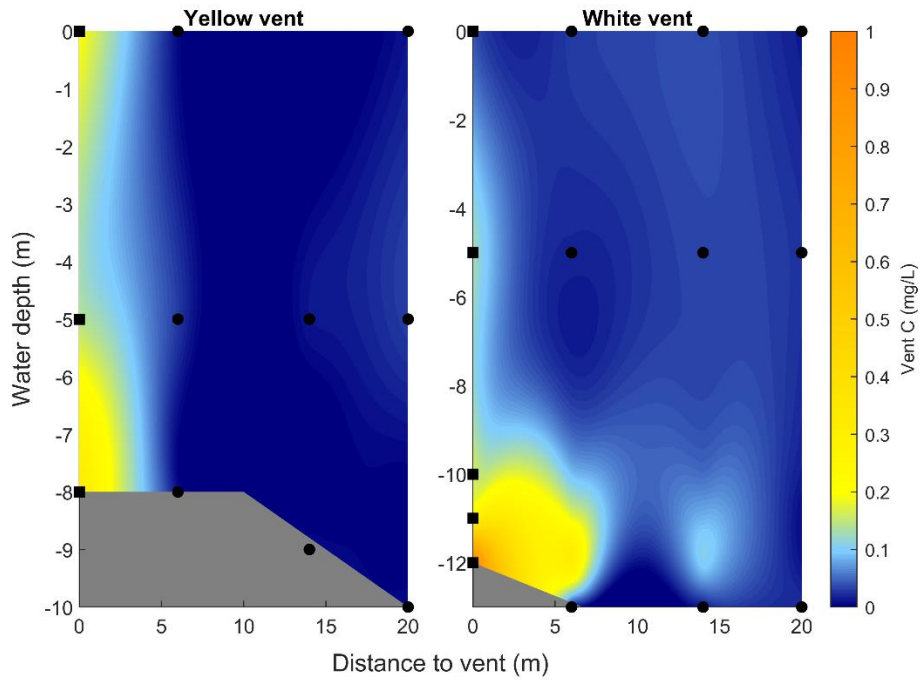
Supplementary Figure 1: Sampling locations of $\delta^2\text{H}$ measurements in vent fluids and in the water column. Map data © 2024 Google Satellite.



Supplementary Figure 2: Hydrogen isotopic fractionation of autotrophic bacterial lipids. References: blue³, orange⁴, yellow⁵, purple⁶. Values were found using ref.⁷ with the input parameter “Bacteria”.



Supplementary Figure 3: Measured isotope values of $\delta^2\text{H}$, $\delta^{13}\text{C}$, and $\Delta^{14}\text{C}$, as well as calculated percentages of individual fatty acids sources (excluding *i*- and *ai*- C_{15} , rTCA (%), chemoautotrophy (%), fluid C used for total FA synthesis (%)).



Supplementary Figure 4: Calculated vent carbon uptake in the water column in mgC L^{-1} . Vent carbon uptake in mg L^{-1} was calculated based on the endmember values defined in Tab. 1. Values above the vent orifice were calculated using the carbon content from ref.¹ and $\Delta^{14}\text{C}$ values from ref.² (cubic points). Black circles represent values derived from POC measurements conducted in this study. For surface and bottom water POC from this study, $\Delta^{14}\text{C}$ was measured two to three times, and the resulting average was weighted by the carbon content of each measurement to address uncertainties introduced by samples with low carbon content (see Supplementary Tab. 3). The dataset was interpolated onto a high-resolution 3000×3000 grid using the `inpaint_nans` toolbox⁸, which employs a least-squares approach to fill missing data. A biharmonic spline interpolation was applied to create a smooth, high-resolution visualization.

Supplementary References

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