

Supplementary Material for “**Monodeuterated Methane: An Isotopic Probe to Measure Biological Methane Metabolism Rates and Track Catabolic Exchange Reactions**”

Table S1: Conditions for the aerobic methanotrophy experiments. All sample types were set up in triplicate; pressures shown as partial pressures.

Sample Condition	p(CH ₃ D)	p(CH ₄)	¹⁴ CH ₄	p(O ₂)	p(Ar)	Inoculum Introduced	Killed Cells
1	0.1 MPa			1 x 10 ⁵ Pa		10% v/v	
2	0.1 MPa			1 x 10 ⁵ Pa		10% v/v	Yes
3	0.1 MPa			1 x 10 ⁵ Pa			
4	0.1 MPa				1 x 10 ⁵ Pa	10% v/v	
5		0.1 MPa		1 x 10 ⁵ Pa		10% v/v	
6	0.1 MPa		13 kBq (T1)	1 x 10 ⁵ Pa		10% v/v	
7	0.1 MPa		13 kBq (T2)	1 x 10 ⁵ Pa		10% v/v	
8	0.1 MPa		13 kBq (T3)	1 x 10 ⁵ Pa		10% v/v	
9	0.1 MPa			1 x 10 ⁵ Pa		10% v/v	
10	0.1 MPa		13 kBq (T3)	1 x 10 ⁵ Pa		10% v/v	Yes

Table S2: The experimental set-up for methane seep sediment pressurized rate measurement incubations. The samples ran for 38 days at 4 °C, and each sample was contained in a sealed Mylar bag. Pressure values indicate absolute pressure exerted on the incubated Mylar bags.

Sample #	Sediment	Labeled Nitrogen Source	Methane Source	Pressure (MPa)
1a	50 mL	500 uM ¹³ C ¹⁵ N Glycine	40 mL CH ₃ D	0.1
2a	50 mL	500 uM ¹⁵ NH ₄ Cl	40 mL CH ₃ D	0.1
3a	50 mL	500 uM ¹⁴ NH ₄ Cl	40 mL CH ₄	0.1
4a	50 mL, killed control	500 uM ¹³ C ¹⁵ N Glycine	40 mL CH ₃ D	0.1
1b	50 mL	500 uM ¹³ C ¹⁵ N Glycine	40 mL CH ₃ D	9.0
2b	50 mL	500 uM ¹⁵ NH ₄ Cl	40 mL CH ₃ D	9.0
3b	50 mL	500 uM ¹⁴ NH ₄ Cl	40 mL CH ₄	9.0
4b	50 mL, killed control	500 uM ¹³ C ¹⁵ N Glycine	40 mL CH ₃ D	9.0

Fig. S1: To assess the empirical resolving power of the D/H measurement technique, we determined the time points showing non-overlapping confidence intervals for triplicate incubations of A.Sed-5128. Distinct signals were seen at the 20-hour time point for replicate A (teal diamonds) and the 26-hour time point for replicates B (pink diamonds) and C (green diamonds).

