

Supplemental materials for: Permafrost thaw reshapes methane cycling across an interior Alaska peatland

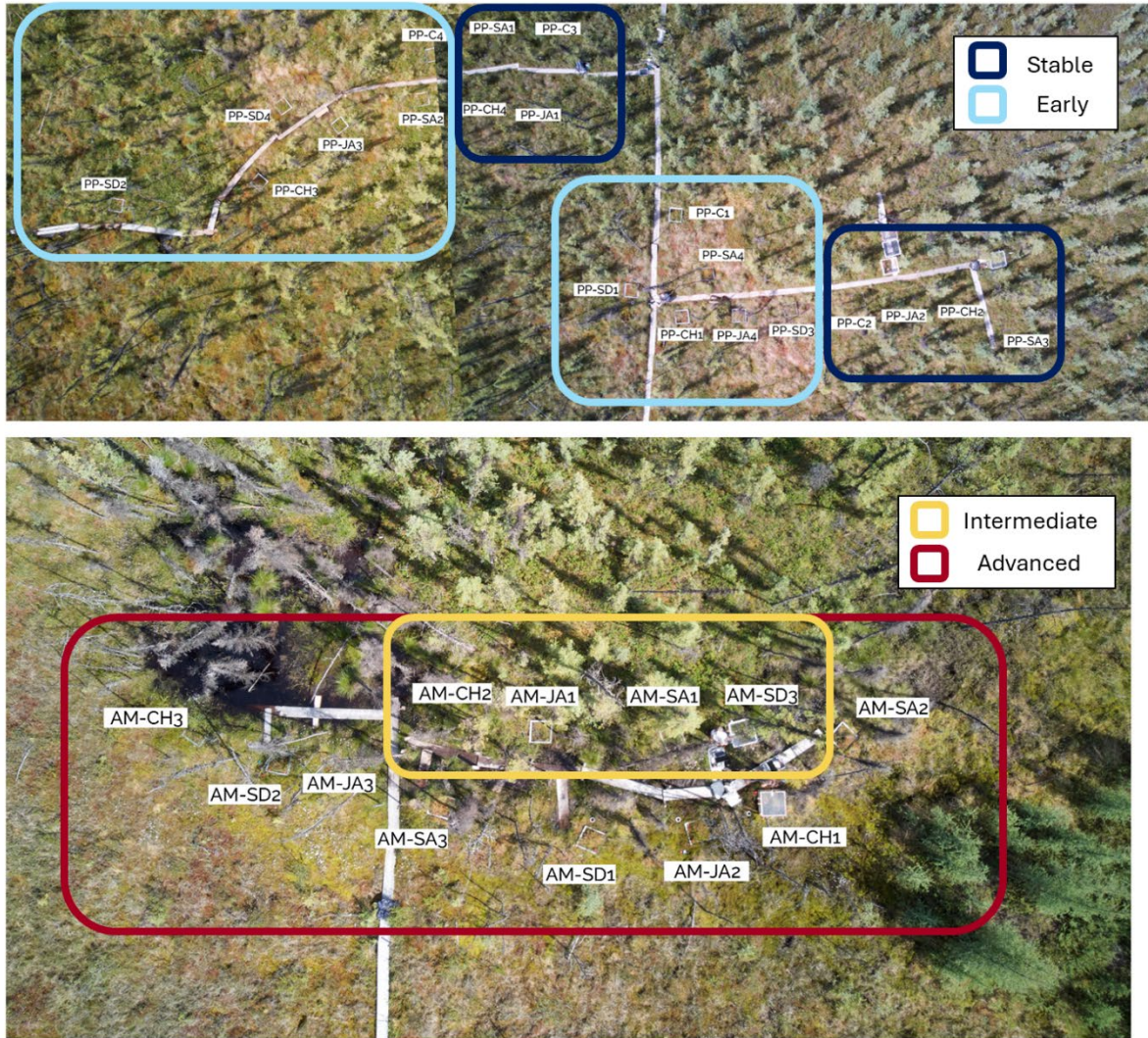


Figure S1. Drone image of the APEX Beta site showing soil collars experiencing stable to early-stage thawing permafrost in the PP (top) and intermediate- to advanced- stage permafrost thaw in the AM (bottom). Thaw stages are outlined to show the spatial distribution of stable, early, intermediate, and advanced permafrost thaw at the site. Cox et al. (2025) also used this photo in their publication. Photo credit: Evan Schijns.



Figure S2. Soil core from the permafrost plateau showing the three depth intervals (shallow, intermediate, and deep) used for sectioning each core.

Table S1. Summary of environmental variables across the permafrost plateau and active thaw margin grouped by thaw stages. Mean values are provided with the associated standard error.

Site	Thaw stage	Water table depth below the ground surface (cm)	Seasonal thaw depth (cm)	Soil temperature (°C)	Soil moisture (%)
Permafrost Plateau	Stable	22.4 ± 0.1	33.1 ± 0.1	11.0 ± 0.1	24.3 ± 2.3
	Early	24.7 ± 0.1	43.0 ± 0.2	12.3 ± 0.05	25.9 ± 3.6
Active Thaw Margin	Intermediate	1.8 ± 0.6	57.2 ± 0.9	12.8 ± 0.1	26.0 ± 2.6
	Advanced	4.7 ± 0.1	111.7 ± 0.7	15.1 ± 0.04	36.3 ± 7.9

Table S2. Summary of mean CO₂ and CH₄ flux values across the permafrost plateau and active thaw margin grouped by thaw stages. These represent mean flux values taken over three sampling campaigns in July 2022. Mean values are provided with the associated standard error.

Site	Thaw Stage	CO ₂ flux (μmol m ⁻² s ⁻¹)	CH ₄ flux (μmol m ⁻² min ⁻¹)
Permafrost Plateau	Stable	1.0 ± 0.8	-0.1 ± 0.02
	Early	0.3 ± 0.8	3.8 ± 1.0
Active Thaw Margin	Intermediate	-1.3 ± 1.1	19.3 ± 9.6
	Advanced	-1.6 ± 0.4	40.2 ± 12.0

1.1 – Basic PCR for inhibitor testing

A basic 16S rRNA PCR was performed on random samples to test for the presence of PCR inhibitors. The following PCR conditions were used:

PCR Conditions

Component	Vol per 25 µl reaction (µl)
2X Phusion Master Mix	12.5
515F (10 mM)	0.5
896R (10 mM)	0.5
Molecular Grade Water	9.5
Template*	2

*E. coli gDNA was used as a positive control and molecular grade water was used as a negative control.

Thermal Cycler Conditions

Temperature	Time	Cycles
95°C	4 min	1
95°C	45 sec	30
50°C	30 sec	
72°C	90 sec	
72°C	10 min	
4°C	---	1

The presence of a strong positive band was used to indicate that the DNA extracts did not contain any significant PCR inhibitor.

P5-P7 PCR Assay

A basic PCR assay was used with primers designed the anneal to the P5 and P7 Illumina Adapters

Illumina P5 primer:

5'-AATGATACGGCGACCACCGAGATC-3'

Illumine P7 primer:

5'-CAAGCAGAAGACGGCATAACGAG-3'

PCR Conditions

Component	Vol per 25 µl reaction (µl)
2X Phusion Master Mix	12.5
P5 (10 mM)	0.5
P7 (10 mM)	0.5
Molecular Grade Water	9.5
Template*	2

* Molecular grade water was used as a negative control

Thermal Cycler Conditions

Temperature	Time	Cycles
95°C	4 min	1
95°C	45 sec	30
50°C	30 sec	
72°C	90 sec	
72°C	10 min	
4°C	---	1

A strong band at the target size was used to indicate that the appropriate illumina primers were present.

iTru Adapters and Limit Cycle PCR

The mcrA_F and mcrA_R primers were modified by adding 5' iTru adaptors based on the Adapterama II: TaggiMatrix protocol (Glenn et al., 2019) to make iTru Fusion primers without internal indexes. See Glenn et al for details.

Barcoded iTru5 and iTru7 adapters were then ordered from BadDNA (<https://baddna.uga.edu/webstore>).

A 2 step PCR assay was then used to amplify and barcode each sample. The first PCR used the fusion primers and the second PCR attached the barcoded iTru adapters.

PCR 1

PCR Conditions

Component	Vol per 25 µl reaction (µl)
2X Phusion Master Mix	12.5
P5 (10 mM)	0.5
P7 (10 mM)	0.5
Molecular Grade Water	9.5
Template*	2

* Molecular grade water was used as a negative control

Thermal Cycler Conditions

Temperature	Time	Cycles
95°C	4 min	1
95°C	45 sec	30
50°C	30 sec	
72°C	90 sec	
72°C	10 min	1
4°C	---	

PCR 2

PCR Conditions

Component	Vol per 25 µl reaction (µl)
2X Phusion Master Mix	12.5
P5 (10 mM)	0.5
P7 (10 mM)	0.5
Molecular Grade Water	9.5
Template*	2

* Molecular grade water was used as a negative control

Thermal Cycler Conditions

Temperature	Time	Cycles
95°C	4 min	1
95°C	45 sec	8
50°C	30 sec	
72°C	90 sec	
72°C	10 min	1
4°C	---	

Qubit data results 16S

Sample ID	Site	Depth (cm)	DNA concentration (ng/ μ L)
AM_SD1_0_15	Active thaw margin	0-15	10.214
AM_SD1_15_30	Active thaw margin	15-30	15.826
PP_C4_0_15	Permafrost Plateau	0-15	14.557
PP_SD2_30_37	Permafrost Plateau	30-37	17.234
AM_JA1_42_48	Active thaw margin	42-48	18.614
PP_SD3_30_45	Permafrost Plateau	30-45	15.676
AM_SD1_30_42	Active thaw margin	30-42	20.461
PP_JA3_0_15	Permafrost Plateau	0-15	37.821
PP_SA1_15_30	Permafrost Plateau	15-30	71.648
PP_C2_15_25	Permafrost Plateau	15-25	61.921
PP_SD1_0_15	Permafrost Plateau	0-15	77.363
AM_CH1_0_15	Active thaw margin	0-15	72.927
AM_SA1_0_15	Active thaw margin	0-15	62.732
PP_C4_30_35	Permafrost Plateau	30-35	60.493
PP_C3_0_15	Permafrost Plateau	0-15	79.827
PP_C3_15_30	Permafrost Plateau	15-30	42.983
PP_JA3_30_38	Permafrost Plateau	30-38	53.781
PP_JA2_15_25	Permafrost Plateau	15-25	71.523
PP_C1_15_30	Permafrost Plateau	15-30	79.638
PP_C1_30_40	Permafrost Plateau	30-40	72.875
PP_CH4_0_15	Permafrost Plateau	0-15	77.328
PP_CH4_15_30	Permafrost Plateau	15-30	66.86
PP_CH4_30_35	Permafrost Plateau	30-35	72.746
PP_SA2_0_15	Permafrost Plateau	0-15	76.593
PP_SA2_15_30	Permafrost Plateau	15-30	72.334
PP_SA2_30_40	Permafrost Plateau	30-40	75.472
AM_JA2_15_30	Active thaw margin	15-30	77.31
PP_CH2_0_15	Permafrost Plateau	0-15	76.281
PP_CH2_15_30	Permafrost Plateau	15-30	59.542
PP_SD2_15_30	Permafrost Plateau	15-30	79.543
PP_JA1_0_15	Permafrost Plateau	0-15	69.574
AM_SA2_0_15	Active thaw margin	0-15	79.694
AM_SA2_30_45	Active thaw margin	30-45	59.27
PP_CH3_15_30	Permafrost Plateau	15-30	65.432
PP_CH3_30_40	Permafrost Plateau	30-40	79.694
AM_SD3_15_30	Active thaw margin	15-30	44.232
AM_SD3_30_40	Active thaw margin	30-40	42.367
AM_JA1_30_42	Active thaw margin	30-42	65.622

AM_SD2_0_15	Active thaw margin	0-15	42.054
AM_SD2_15_30	Active thaw margin	15-30	67.116
AM_SD2_30_45	Active thaw margin	30-45	46.989
PP_SA4_0_15	Permafrost Plateau	0-15	73.831
PP_SA4_15_30	Permafrost Plateau	15-30	69.629
PP_SA4_30_50	Permafrost Plateau	30-50	63.403
PP_SD4_0_15	Permafrost Plateau	0-15	54.675
PP_SD4_15_25	Permafrost Plateau	15-25	55.886
PP_JA4_0_15	Permafrost Plateau	0-15	66.742
PP_JA4_15_30	Permafrost Plateau	15-30	50.358
PP_JA4_30_40	Permafrost Plateau	30-40	59.872
PP_SD3_0_15	Permafrost Plateau	0-15	48.456
PP_SD3_15_30	Permafrost Plateau	15-30	70.931
PP_CH1_15_30	Permafrost Plateau	15-30	79.729
PP_CH1_30_50	Permafrost Plateau	30-50	76.663
AM_CH2_30_45	Active thaw margin	30-45	63.909
AM_CH1_15_30	Active thaw margin	15-30	6.203
PP_C4_15_30	Permafrost Plateau	15-30	8.664
PP_C2_0_15	Permafrost Plateau	0-15	97.604
PP_SD1_15_30	Permafrost Plateau	15-30	89.326
PP_SD1_30_45	Permafrost Plateau	30-45	94.024
AM_CH1_30_50	Active thaw margin	30-50	85.929
PP_JA3_15_30	Permafrost Plateau	15-30	87.656
PP_JA2_0_15	Permafrost Plateau	0-15	104.59
PP_C1_0_15	Permafrost Plateau	0-15	80.736
AM_SA3_0_15	Active thaw margin	0-15	82.41
AM_SA3_15_30	Active thaw margin	15-30	84.32
AM_SA3_30_40	Active thaw margin	30-40	85.42
AM_JA3_0_15	Active thaw margin	0-15	85.644
AM_JA3_15_22	Active thaw margin	15-22	89.136
AM_JA2_0_15	Active thaw margin	0-15	87.202
AM_JA2_30_40	Active thaw margin	30-40	82.171
PP_SD2_0_15	Permafrost Plateau	0-15	83.43
PP_JA1_15_30	Permafrost Plateau	15-30	88.472
PP_JA1_30_40	Permafrost Plateau	30-40	96.005
PP_SA3_0_15	Permafrost Plateau	0-15	90.624
PP_SA3_15_30	Permafrost Plateau	15-30	95.2
PP_SA3_30_40	Permafrost Plateau	30-40	89.482
AM_SA2_15_30	Active thaw margin	15-30	90.504
PP_CH3_0_15	Permafrost Plateau	0-15	93.775
AM_SD3_0_15	Active thaw margin	0-15	84.909
AM_JA1_0_15	Active thaw margin	0-15	88.469

AM_JA1_15_30	Active thaw margin	15-30	82.938
PP_CH1_0_15	Permafrost Plateau	0-15	119.628
AM_CH2_0_15	Active thaw margin	0-15	80.762
AM_CH2_15_30	Active thaw margin	15-30	85.987
AM_CH3_0_15	Active thaw margin	0-15	101.812
AM_CH3_15_30	Active thaw margin	15-30	88.081
AM_CH3_30_38	Active thaw margin	30-38	90.964
PP_SA1_0_15	Permafrost Plateau	0-15	19.8
AM_SA1_15_30	Active thaw margin	15-30	22.8
AM_SA1_30_42	Active thaw margin	30-42	27.4

Qubit data results mcra

Sample ID	Site	Depth (cm)	DNA concentration (ng/ μ L)
AMCH1 0-15	Active thaw margin	0-15	8.818
AMCH1 15-30	Active thaw margin	15-30	6.547
PPSA3 30-40	Permafrost plateau	30-40	2.731
PPSD1 15-30	Permafrost plateau	15-30	2.071
PPSD3 15-30	Permafrost plateau	15-30	5.447
AMCH1 30-50	Active thaw margin	30-50	4.808
PPSA4 30-50	Permafrost plateau	30-50	4.588
PPSD1 0-15	Permafrost plateau	0-15	2.415
PPSD1 30-45	Permafrost plateau	30-45	7.019
PPSA2 30-40	Permafrost plateau	30-40	2.845
PPC2 0-15	Permafrost plateau	0-15	2.248
PPCH1 15-30	Permafrost plateau	15-30	3.767
AMSD2 30-45	Active thaw margin	30-45	4.897
AMSD1 15-30	Active thaw margin	15-30	4.857
PPC1 15-30	Permafrost plateau	15-30	2.767
AMSA3 15-30	Active thaw margin	15-30	3.252
PPCH4 0-15	Permafrost plateau	0-15	2.518
PPC1 30-40	Permafrost plateau	30-40	5.542
PPJA1 30-40	Permafrost plateau	30-40	3.784
AMJA1 15-30	Active thaw margin	15-30	8.412
AMJA3 0-15	Active thaw margin	0-15	11.327
AMSD2 0-15	Active thaw margin	0-15	9.85
PPSA2 15-30	Permafrost plateau	15-30	2.4
AMCH2 30-45	Active thaw margin	30-45	3.139
AMJA3 15-22	Active thaw margin	15-22	16.039
AMCH3 15-30	Active thaw margin	15-30	10.614
AMJA1 30-42	Active thaw margin	30-42	7.853
AMSA1 30-42	Active thaw margin	30-42	14.089

PPSD4 0-15	Permafrost plateau	0-15	8.756
AMCH3 30-38	Active thaw margin	30-38	9.056
AMCH3 0-15	Active thaw margin	0-15	10.986
AMSA2 15-30	Active thaw margin	15-30	4.43
AMCH2 15-300	Active thaw margin	15-30	6.255
AMSA1 15-30	Active thaw margin	15-30	2.872
PPSD4 15-25	Permafrost plateau	15-25	2.312
AMSA3 0-15	Active thaw margin	0-15	3.52
PPJA3 15-30	Permafrost plateau	15-30	3.794
PPCH1 30-50	Permafrost plateau	30-50	2.946
AMSD2 15-30	Active thaw margin	15-30	6.069
PPC3 15-30	Permafrost plateau	15-30	3.837
PPJA4 15-30	Permafrost plateau	15-30	4.884
AMCH2 0-15	Active thaw margin	0-15	4.635
AMSD1 0-15	Active thaw margin	0-15	6.022
AMJA1 0-15	Active thaw margin	0-15	2.519
AMSA1 0-15	Active thaw margin	0-15	3.062
PPJA3 30-38	Permafrost plateau	30-38	2.128
PPCH4 30-35	Permafrost plateau	30-35	3.013
AMSD1 30-42	Active thaw margin	30-42	5.162
PPJA2 15-25	Permafrost plateau	15-25	4.992
PPSD2 15-30	Permafrost plateau	15-30	1.505
AMSD3 15-30	Active thaw margin	15-30	1.447
PPJA1 0-15	Permafrost plateau	0-15	1.779
AMSA3 30-40	Active thaw margin	30-40	4.914
AMJA2 30-40	Active thaw margin	30-40	4.149
PPC4 30-35	Permafrost plateau	30-35	2.217
AMSA2 30-45	Active thaw margin	30-45	3.891
PPJA4 30-40	Permafrost plateau	30-40	10.295
AMJA2 15-30	Active thaw margin	15-30	5.246
AMSD3 0-15	Active thaw margin	0-15	5.737
AMSA2 0-15	Active thaw margin	0-15	2.121
PPSD2 30-27	Permafrost plateau	30-37	2.684
PPCH3 30-40	Permafrost plateau	30-40	3.415
PPSD3 0-15	Permafrost plateau	0-15	3.165
PPJA1 15-30	Permafrost plateau	15-30	7.421
AMJA2 0-15	Active thaw margin	0-15	5.878
PPSD2 0-15	Permafrost plateau	0-15	3.783
PPC4 15-30	Permafrost plateau	15-30	7.299
PPSA1 0-15	Permafrost plateau	0-15	4.464
PPCH3 15-30	Permafrost plateau	15-30	2.299
PPSA3 0-15	Permafrost plateau	0-15	3.762

PPCH2 15-30	Permafrost plateau	15-30	1.783
PPSD3 30-45	Permafrost plateau	30-45	2.789
PPSA4 15-30	Permafrost plateau	15-30	4.733
PPSA3 15-30	Permafrost plateau	15-30	2.762
PPCH3 0-15	Permafrost plateau	0-15	2.928
PPSA1 15-30	Permafrost plateau	15-30	5.987
PPSA4 0-15	Permafrost plateau	0-15	2.654
PPJA2 0-15	Permafrost plateau	0-15	2.353
AMSD3 30-40	Active thaw margin	30-40	1.773
PPCH4 15-30	Permafrost plateau	15-30	5.565
PPC1 0-15	Permafrost plateau	0-15	2.042
PPJA3 0-15	Permafrost plateau	0-15	4.904
PPC3 0-15	Permafrost plateau	0-15	2.115
PPC4 0-15	Permafrost plateau	0-15	6.256
PPC2 15-25	Permafrost plateau	15-25	0.929
PPCH1 0-15	Permafrost plateau	0-15	0.967
PPJA4 0-15	Permafrost plateau	0-15	1.468
PPSA2 0-15	Permafrost plateau	0-15	2.127
PPCH2 0-15	Permafrost plateau	0-15	2.74

References

Glenn, T. C., Nilsen, R. A., Kieran, T. J., Sanders, J. G., Bayona-Vásquez, N. J., Finger, J. W., Pierson, T. W., Bentley, K. E., Hoffberg, S. L., Louha, S., Garcia-De Leon, F. J., Del Rio Portilla, M. A., Reed, K. D., Anderson, J. L., Meece, J. K., Aggrey, S. E., Rekaya, R., Alabady, M., Belanger, M., Winker, K., and Faircloth, B. C.: Adapterama I: universal stubs and primers for 384 unique dual-indexed or 147,456 combinatorially-indexed Illumina libraries (iTru & iNext), *PeerJ*, 7, e7755, <https://doi.org/10.7717/peerj.7755>, 2019.