

The objectives of this study were to assess the impacts of time following permafrost thaw stage on methane emissions and methanogenic community composition. To do this, the authors identified two bog sites with permafrost that thawed 30 and 200 years ago. Analyses conducted at these sites included (1) metagenomic assessments, (2) dissolved gas concentrations (CO<sub>2</sub> and CH<sub>4</sub>), (3) surface emissions, (4) d<sup>13</sup>C signatures for both CH<sub>4</sub> and CO<sub>2</sub> (used to assess the relative contribution of acetoclastic methanogenesis to total methanogenesis).

Overall, this paper effectively approaches that goal.

I have two primary concerns:

- (1) My main concern with this paper is centered around the use of isotope d<sup>13</sup>C signatures. The alpha value is an accepted method for discerning the relative contributions of acetoclastic vs. hydrogenotrophic methanogenesis. While the authors used alpha values for dissolved gas analysis, they limited their assessments of acetoclastic input to <sup>13</sup>C signatures of methane (without concomitant <sup>13</sup>C-CO<sub>2</sub> signatures). Is the <sup>13</sup>C-CH<sub>4</sub> signature on its own a sufficient indicator of acetoclastic contribution? If so, please provide citations that indicate so.

Furthermore—I was not clear on whether the apparent difference in alpha values between mature vs. young sites was indeed statistically significant. This comparison needs to be made explicit. If significant differences between sites can only be found at specific depth intervals, then that should also be stated explicitly.

- (2) Your goal was to examine the effects of thaw stage on methane fluxes/methanogenic community composition. It is difficult to wrap my head around this goal since thaw succession causes shifts in so many different environmental factors (soil temperature, thickness of the unsaturated peat column, availability of labile organics). This makes your results difficult to build off of/apply to other settings. Perhaps you could perform an ordinary least squares regression analysis (OLS) to try to tease apart the relative influence of these numerous factors on a dependent variable of interest (perhaps total methane emissions, or acetoclastic methane emissions).

#### Specific Questions/Recommendations

- (1) I am unclear on what is meant by the term “ecological” in the context of this manuscript (e.g. L27-28). I get the impression that it references vegetation primarily. I am unsure about that, however, because “ecological” could also be used to describe microbial community composition. Please clarify.
- (2) Fig 1: I’d recommend explicitly stating how far apart the mature and young bog sites are from one another in panel f (or the caption). You list only one GPS coordinate from the whole site in the figure caption.
- (3) L158-159: How did you determine that this complex is representative? If the succeeding sentences are meant to serve as evidence for this claim, make that explicit.
- (4) L551-554: “Overall, the isotopic data indicates a general dominance of hydrogenotrophic methanogenesis in both sites, but a greater contribution of acetoclastic methanogenesis in the

young bog relative to the mature bog.” Was this difference statistically significant? I suggest adding a p-value after this statement.

- (5) L 572-575: *“The  $\delta^{13}\text{C}$ -CH<sub>4</sub> signature of CH<sub>4</sub> emissions (intercept values from Keeling plots), in the young bog were significantly greater than those observed in the mature bog (Figure 3c;  $F(1, 4) = 20.67, P < 0.05$ ), suggesting a greater influence of acetoclastic CH<sub>4</sub> production.”*

Why is there no alpha value for the flux measurements? Please provide a source indicating that  $^{13}\text{C}$ -CH<sub>4</sub> measurements alone (i.e. without concomitant  $^{13}\text{C}$ -CO<sub>2</sub>) are sufficient to discern the relative influence of acetoclastic methanogenesis on total methane production.

- (6) L704-706: *“Evidence of acetoclastic methanogens and CH<sub>4</sub> produced via the acetoclastic metabolic pathway was found in the young bog both near the surface and at depths below the thaw transition (i.e., in peat that accumulated prior to permafrost thaw).”*

I have two notes on this:

- (1) If the difference in alpha values was not significant in the subsurface (which I am not 100% clear on), this needs to be noted and discussed.
- (2) See my comments regarding L572-575. Make sure your methods for discerning the acetoclastic influence on surface CH<sub>4</sub> emissions is sound.

- (7) L765-766: *“The presence of hydrophilic vegetation, particularly graminoids, in the saturated young bog provides the precursors for fermentation..”*

I am confused by this statement. “Precursors” could be interpreted as “reactants”, which are primarily sugars. Sugars are ultimately delivered to porewater from other plants too (i.e. Sphagnum spp.). Are you saying that the sugars derived from graminoids are more labile than those derived from Sphagnum? I would agree with this, but it is necessary to clarify.

- (8) L805-813: I find the thread of this paragraph hard to follow. Please make the connections between sentences clearer.
- (9) Fig 2: Please add in a legend.