

Response to Referee #1

1.3. *I think that the work would benefit from a more thorough comparison with boreal forest incubations across the Arctic.*

This point was raised by Referee #2 as well (comment 2.4). We now provide a more complete comparison, citing for example studies such as the Schädel et al. (2016) meta-analysis, Dutta et al. (2006), Lavoie et al. (2011), Karhu et al. (2010), and Wickland and Neff (2008). **See new lines 308-337, 368-372, 401-408, 427-432.**

1.4. *N section would benefit from more Arctic-centric comparisons of N limitations and in particular of boreal forest N dynamics. Q10 can be temperature dependent, also depending on N limitation in the system.*

We have supplemented this section with a better comparison to relevant literature, for example, Lavoie et al. (2011), Sistla et al. (2012), and Bouskill et al. (2014). **See new lines 391-408.**

1.5. *This study raises interesting questions. In mineral soils, under woody vegetation that might be of low C quality, and slower C pool, one might expect higher temperature sensitivity. I think that these questions, even if not addressed directly by the data presented, should have been discussed more explicitly. Comparison with other Arctic woody plant systems would be instructive.*

This is similar to the referee's comment 1.9 (please see our response to that below), with the added factor of C quality. We have addressed this more explicitly in our revision, referring for example to incubation studies on this question (Fierer et al. 2005). **See new lines 414-420.**

1.6. *Studies have shown that moisture can have a weaker effect on temperature sensitivity early on during an incubation experiment, in the presence of more labile C. This relative to the effect on moisture on the Q-10 of cumulative respiration, reflecting slow turning over C - this could be an interesting analysis to include here, and would help to assess how*

This is an interesting suggestion. We do not observe any evidence for changes in CO₂ moisture sensitivity with time, and weak changes in CO₂ temperature sensitivity; CH₄ emissions show a weak decline in moisture sensitivity with time. This is now discussed in the text. **See new lines 260-261, 366-371.**

1.7. *How do your results in terms of temperature and moisture sensitivity (especially under drought conditions) scale with Alaskan climate change predictions from modelers? How does it compare with deep soils incubations (mineral soils) from the Arctic, and from boreal ecosystems?*

The first question is similar to a point raised by Referee #2 (comment 2.3). We have added this as a paragraph in the discussion, noting e.g. observable anthropogenic influences on high-latitude precipitation, drier and warmer conditions in boreal Eurasia, and growing season length increases in interior Alaska with no increase in precipitation. **See new lines 326-337.**

The second question largely repeats, we think, both referees' suggestions to better compare our results to previous work, in particular boreal and Arctic incubations; see our responses to 1.3 and 1.4 above.

1.8. Line 31-34: I cannot find discussion of this point in the rest of the text, and while important, this statement is relatively vague and there are no cited references. Since it underpins the rationale for studying deep, unfrozen Arctic soils, it would be helpful to expand on this more in the manuscript.

Referee #2 raised this point as well (comment 2.6), and it's a good one. We now better describe why deep active-layer soils, such as those studied here, are important and distinctive relative to permafrost or shallow active layer soils. **See new lines 87-98.**

1.9. Lines 48-60: I think that this section would benefit from an introduction of the interactions between the specific ecosystem (upland boreal forest) you are studying, and its interaction with soil chemistry, since vegetation type is influential in terms of soil carbon quality and quantity. Woody plant biomass tends to have a higher C:N ratio relative to herbaceous dominated systems, and this tends to result in lower quality resources for microbial communities.

Vegetation and ecosystem type is a significant factor that is not well explored here, we agree. We have added some points about this in the introduction and discussion. **See new lines 51-63, 433-439.**

1.10. Lines 70-72: These are really important considerations, and it seems appropriate to discuss them more explicitly. How is the temperature and precipitation regime of the boreal forest of interior AK expected to change? There are also indirect effects of vegetation type on soil temperatures that could be discussed here.

We agree, although this largely echoes comments 1.7 and 1.9; please see our responses above.

1.11. Lines 72-74: While these are important questions, they are not really addressed in this study, and so either it might make sense to leave it out, or to discuss the particulars as they apply to this study, ie: the importance and questions related to C:N ratios.

Referee 3 made this point as well, and this sentence has been removed.

1.12. Lines 77-80: I think a stronger argument for why deep active-layer soils can be made, and it would be helpful to clarify what are the 'strong effects' of warming.

Agreed. See our response to comment 1.8 above.

1.13. I cannot tell if C:N, %C and %N were measured at the end of the incubation. Could these results be collated in a table in the manuscript? Otherwise the methods section appears to be detailed and well written.

C and N were measured for all samples post-incubation, and in the 'extra' group (l. 128-129) pre-incubation. This will be clarified in the methods, particularly lines 162-, which we agree were ambiguous. A new Table 1 now summarizes a variety of physical and

flux data by treatment. Reviewer 2 also raised the idea of looking at C/N, and we have responded to that suggestion in detail (see comment 2.9).

1.14. Line 232: In this section it would also be interesting to know the soil respiration decay rate per treatment over the course of the incubation experiment.

This has been included in the new Table 1 (see comment 1.13 above).

1.15. Line 238-240: Confusingly worded sentence.

This has been clarified. **See new lines 264-266.**

1.16. I don't think that the summary of nearby respiration studies add very much to the discussion section. Perhaps if the similarities and discrepancies were more integral to the central findings of the paper or integrated differently into the discussion they would seem more meaningful here. Perhaps comparing with other boreal incubations (eg: Lee et al., 2012; Lavoie et al., 2011) would help to provide some additional context.

We agree that the comparison to other boreal respiration studies needs improvement, and this echoes Referee #2's comment 2.4. First, although we think the paragraph about nearby studies provides useful context, we have tightened it considerably. Second, we have restructured and improved the subsequent comparison section, discussing a variety of studies suggested by all the reviewers to better put our results in the context of previous work. **See new lines 295-307 for nearby studies, and expanded context in lines 308-337, 368-372, 401-408, 427-432.**

1.17. Line 270: There is missing punctuation after the word 'results'.

This will be fixed. **See new line 298.**

1.18. Line 286-293: Perhaps the new synthesis by Schadel et al., 2016, would also be a useful comparison here.

This point was also made by Referee #2 (comment 2.2). The fact that we didn't cite the Schädel et al. (2016) meta-analysis was a quirk of timing, as it appeared after our manuscript was submitted. In our revision, we have significantly expanded this paragraph, discussing and comparing to Schädel et al. (2016) in depth, particularly their findings of higher aerobic than anaerobic respiration; respiration dominance of CO₂ versus CH₄; and Q₁₀ values. We also cite and discuss a variety of other studies. **See new lines 46, 81, 313, 368-371, 411.**

1.19. Line 293: That soil moisture may be as important a control on microbial respiration as temperature is an important finding in recent incubation studies, and the potential to define its interaction with temperature will help modelers of soil decomposition better constrain the physical parameters of microbial respiration rates. This feels buried in the manuscript, and I think that it would improve the paper if it were highlighted better throughout the text.

Thanks for the useful suggestion; this point is now brought out more clearly. **See new lines 333-337, 356-365, 454-458.**

1.20. Line 311-317: *This section could be better explained in the context of the discussion or omitted altogether. It seems less important to defend the plausibility of relatively low temperature sensitivity, but instead to try to explain it in the context of these soil characteristics. Could low temperature sensitivity be the result of low C quality in this deep soil environment?*

We appreciate this useful advice and question. We have diminished the emphasis on defending this finding, and instead try to place it in the context of soil characteristics in this mixed-species boreal forest, SOC quality, etc. **See new lines 366-390.**

1.21. Line 322-332: *This section, which lays out the crux of the paper, the interaction between temperature and moisture sensitivity in driving microbial respiration is relatively vague. It would be good to describe the less-temperature-sensitive processes that would be important to consider for more stable-C metabolism. And how does moisture play a role here? Perhaps DOC becomes more limiting in the drought conditions?*

This is interesting to consider: what mechanisms might produce a Q10 increase under drought conditions? This is opposite to what is usually observed (e.g. Jassal et al. 2008), but the field is rife with contradictory results (von Lützow and Kögel-Knabner 2009). We have made this paragraph more specific in this area. **See new lines 378-390.**

1.22. Line 356: *The Janssens et al., 2010, citation refers to a meta-analysis of temperate forest soils that are not nitrogen limited. There are studies focusing on Arctic N cycling that would be more appropriate, and many Arctic studies have shown that N availability can limit C mineralization rates. Is this site considered to be N limited in the deep active layer?*

We agree that referring and comparing to studies such as Lavoie et al. (2011) and Bouskill et al. (2014), which focused specifically on high latitudes, would be a useful addition. We don't know of any studies examining the N limitation of deep soils at this site. We now discuss these results in our revision, along with other studies examining the relationship between N availability and C mineralization. **See new lines 392-408.**

1.23. Line 367: *Is this comparison, with North American soils, relevant to this study?*

It's true that Colman and Schimel (2014) include only a few studies that could be termed boreal (from Maine, USA). We have removed this comparison.

1.24. Line 383-384: *Can you be more explicit in your meaning here? How do you mean that there is weakness in what can be inferred about temperature sensitivity from experiments?*

We basically meant what the title of the Podrebarac et al. (2016) paper says: "Soils isolated during incubation underestimate temperature sensitivity of respiration and its response to climate history". I.e., incubation soils are isolated from their natural environment, and as a result we need to be cautious about extrapolating incubation results to *in situ* responses. We have clarified this in the text. **See new lines 425-426.**

Response to Referee #2

2.2. *It seems like this paper was published as a discussion paper before Schädel et al. 2016 was published and hence a discussion of the meta-analysis was not possible but should be addressed in the revisions.*

This point was also made by Referee #1 (comment 1.18). Yes, the fact that we didn't cite the Schädel et al. (2016) meta-analysis was a quirk of timing, as it appeared after our manuscript was submitted. We have significantly expanded the discussion on this point, comparing our results to Schädel et al. (2016) in depth, particularly their findings of higher aerobic than anaerobic respiration; respiration dominance of CO₂ versus CH₄; and Q₁₀ values. **See new lines 46, 81, 313, 368-371, 411.**

2.3. *The importance of the results would be more obvious if the discussion also contained an upscaling or circumpolar aspect of drought in the Arctic. It would be useful to have some discussion about the area that is expected to be most affected by drought. This is important as changes in temperature will affect most of the Arctic, whereas drought effects or dry soils will occur more locally.*

We have added this as a paragraph in the discussion, noting e.g. observable anthropogenic influences on high-latitude precipitation, drier and warmer conditions in boreal Eurasia, and growing season length increases in interior Alaska with no increase in precipitation. **See new lines 326-337.**

2.4. 1) *Throughout the manuscript, I have noticed that important papers from the permafrost literature are missing. This applies to C stocks in the permafrost area, Tarnocai et al. 2009 is a good paper but there are more recent and more accurate estimates of permafrost C stocks described in Hugelius et al. 2014 and Schuur et al. 2015 that should be cited. When it comes to the permafrost C feedback, Schuur et al. 2015 is currently the best and most up to date review. In addition, Koven et al. 2015 is a good one too. The discussion on incubation literature should include papers like Lavoie et al. 2011, Dutta et al. 2006, and Schädel et al. 2014.*

We appreciate the referee drawing our attention to these omissions. While Schuur et al. (2015) is already cited, Hugelius (2014) and Koven (2015; though we do cite his 2011 paper) are useful additions. The Lavoie paper is very useful with respect to N and microbial respiration, while Dutta et al. (2006), although it concerns Siberian soils, is also a good comparison. We had not included Schädel et al. (2014) simply because of its focus on permafrost, versus the active-layer soils studied here, but we agree it is also be a reasonable addition. All these references are now cited throughout the manuscript. **See new lines 308-337, 368-372, 401-408, 427-432.**

2.5. 2) *L. 31: Permafrost thaws and does not melt*
Fixed. **See new line 33.**

2.6. 3) *A better explanation is needed why deep-active layer soils are different to active layer or permafrost soils, I couldn't find a strong argument for why they would behave*

differently. Also, deep-active layer soils are those that are the most impacted by inter annual variability in thaw depth and so they might switch between active layer in one year to permafrost in another, that's worth some discussion as well.

This is a good point. We now better describe why deep active-layer soils, such as those studied here, are important and distinctive relative to permafrost or shallow active layer soils. **See new lines 87-98.**

2.7. 4) *The statistics in this paper are generally good and I would like to compliment the authors on making the entire data set and analysis available online. I would still suggest that the manuscript would profit from some additional details on collinearity of the tested variables as well as model outputs such as AIC.*

Thank you. We appreciate the useful suggestions, and now provide these additional details in our revised manuscript. **See new lines 257-270.**

2.8. 5) *Add a table with soil properties such as bulk density, %C etc.*

This useful suggestion was also made by Referee #1 (comment 1.13). We have done so, in a new Table 1.

2.9. 6) *Why not include C/N as a variable in the statistical analysis? Schädel et al. 2014 showed that C/N is a good predictor of C release and can be used as a scaling factor. It would be interesting to see if C release from short-term incubations show the same result*

This is an interesting suggestion. We added code (see https://github.com/bpbond/cpcrw_incubation/commit/426a91e1bbd21200718b334d3295fbef40a1ea6) to compute C/N and examine its significance as a predictor. Currently C/N seems to be a poorer predictor than %N. We now discuss this issue, referencing previous work such as Schädel et al (2014). **See new lines 409-420.**

2.10. 7) *In the discussion, it would be good to also include the warming potential of CO₂ and CH₄ especially when making assumptions about the permafrost C feedback, it is briefly mentioned in line 348 but a more in depth discussion would be good*

That's a very good point-thank you-and integrates well with an expanded comparison to the Schädel et al. (2016) paper (cf. comment 2.2 above) and other publications (comment 2.4 above). **See new lines 350-354.**

2.11. 8) *the conclusions might be a bit strong given the data and previous results published*

We have added caveats, noting in particular the useful but incremental nature of this study. **See new lines 449-460.**

Response to Referee #3

3.2. *My main criticism is that I think that the authors over-emphasize the results of the daily emissions and that the authors should further explore (or report) the results of the controls of the cumulative C emissions. I'm curious as to whether the relationships with soil C/N and %N observed in daily emissions still hold on cumulative emissions. The comparison between these soil parameters (i.e. ones that probably don't change much throughout the course of the incubation, including temperature) and the cumulative fluxes is perhaps more appropriate. Perhaps modelers find the controls on daily fluxes interesting and these are likely quite useful in regards to the relationship between moisture and C production (i.e. changes on a daily basis), but I think that the controls on cumulative fluxes are quite interesting and could be further explored.*

We agree that rebalancing the manuscript, focusing a bit more on controls on cumulative emissions and a bit less on the instantaneous fluxes, would strengthen it. Accordingly, we now more fully explore controls on the cumulative emissions, and have moved the table summarizing the instantaneous CH₄ flux model, to an appendix. **See new lines 826-832.**

3.3. *For example, how do the results of soil properties vs. emissions compare to those of Schädel et al. (2014) and Schädel et al. (2016)? How do the moisture results compare to those of Wickland et al. (2008)?*

The other referees both mentioned this as well. The fact that we didn't cite the Schädel et al. (2016) meta-analysis was a quirk of timing, as it appeared after our manuscript was submitted. We have significantly expanded this, discussing and comparing to Schädel et al. (2014, 2016) and Wickland et al. (2008). **See new lines 46, 81, 313, 368-371, 411, and line 306 for Wickland.**

3.4. *I do think that the time series of fluxes could be moved to the supplemental materials if the cumulative fluxes are explored in greater detail. I think this paper could be shortened a little bit although I didn't find the length of the paper onerous. Along these lines, I think that the results summarized above from the cumulative emissions should be included in the abstract.*

We have moved one table to supplementary material (see response to comment 3.2 above), and now summarize cumulative emissions results in the abstract. **See new lines 27-31.**

3.5. *22: Daily CO₂ fluxes? 26: positive or negative correlation? 27: daily CH₄ flux? 28: cumulative production as CO₂-as CH₄.*

These points have been clarified, except for the last, as we feel it's already clear and unambiguous. **See new lines 20-36.**

3.6. *29: Not really sure how the comparison as to the relative controls of T and moisture are evaluated.*

This statement has been reworded to remove the comparison. **See new lines 31-33.**

3.7. 50: see also updates in Hugelius et al. (2014) 63: Under some conditions (Olefeldt et al 2013): vague and confusing. Please clarify.

Reviewer 2 also raised the issue (comment 2.4) of our incomplete citation of relevant literature. The Tarnocai reference has been replaced by one to Hugelius et al. (2014), and the Olefeldt sentence clarified. **See new lines 52 and 74.**

3.8. 67: 'substantial variabilities between studies' WHY?

We have expanded on this point, pointing out that such variability originates from factors such as differences in soil type, antecedent conditions, phase changes, experimental protocols, etc. **See new lines 75-77.**

3.9. 72: Yes, this is an important question, but given that this isn't measured in this study, perhaps this sentence should be omitted or re-written.

This sentence has been removed.

3.10. 101: When did sampling occur? 112: Specify at the time of sampling 140: How frequently was moisture adjusted? Requires a bit more explanation. Were instantaneous moisture values used in analysis?

Sampling date is reported in line 110. We have clarified 80 cm at the time of sampling. Moisture adjustment was done after every mass measurement, i.e. every timepoint shown in Figure 1; this has been clarified. **See new lines 164-165.**

3.11. 211: Please remember to complete DOI

Done. **See new lines 234-235.**

3.12. 215: Not sure what this value for soil dry mass indicates

It's just useful, we think, to give readers a good sense of sample size.

3.13. 216: Standard deviation for %C and %N is nearly 100%. Check values.

Thanks. There was a great of variability (obviously), but distributed throughout the data set-i.e., this isn't driven by one or two outliers.

3.14. 229: add units 231: add units 233: positively correlated 241: positively correlated 245-246: 106

These have all been fixed. **See new lines 252-270.**

3.15. 253-254: So what variables were significant in predicting cumulative C emissions?

Please see our response to comment 3.2 above.

3.16. 262: First mention of vegetation stress, remove, not clear how it's related.

We now better integrate this point, mentioning it in the introduction and clarifying its relationship to the study goals. **See new lines 58-62, 291, 310-315.**

3.17. 270. Add '.'

This has been fixed. **See new line 298.**

3.18. 271: *Specify soil type in which these measurements were made (results not surprising for a forest soil)*

Upland Cryosols; we have clarified this. **See new line 298.**

3.19. 272: *What about results from Wickland et al. (2008). Study found threshold for moisture importance 305-307: again, see Wickland et al. (2008)*

Please see our response to comment 3.7 above.

3.20. 322-324: *cool!*

Agreed!

3.21. 344-345: *Specify that the results in Treat et al. (2015) were for anaerobic incubations and were thus likely to be much smaller.*

Thanks; we have done so. **See new line 321.**

3.22. 347-348: *See also Lee et al. (2012) 364-365: See also Schadel et al. (2014). Also, I thought this section was a bit vague, probably could be shortened slightly.*

Thanks for the Lee et al. reference, which we had not considered (see our response to 3.7 above) but is now cited. We have also reworked and tightened section 4.2. **See new lines 354, 391-420.**

3.23. 383-384: *'specific weaknesses': vague 384: See also lag effects found in Treat et al. (2015)*

This awkward language has been removed, and a note about lag effects added. **See new lines 425-429.**

3.24. 393: *'taking them out of depth' rephrase. Also could use this argument for the section on CH₄ production.*

We have reworded this. **See new lines 440-447.**

3.25. *Fig.1 : Edit figure to be color-blind friendly.*

We thought we were already doing so in using the `RColorBrewer` package, not the default palette of `ggplot2`, but have shifted to using a color-blind friendly palette from [http://www.cookbook-r.com/Graphs/Colors_\(ggplot2\)/#a-colorblind-friendly-palette](http://www.cookbook-r.com/Graphs/Colors_(ggplot2)/#a-colorblind-friendly-palette) in all figures.

3.26. *Fig. 2,3: When did watering / moisture adjustment occur? Consider indicating with arrows and specifying in text.*

Moisture adjustment was done after every mass measurement, i.e. every timepoint shown in Figure 1. This has been clarified. **See new lines 164-165.**

3.27. *Fig. 4: Switch top and bottom panels as CO₂ is always discussed before CH₄. Also edit colors and patterns to be color-blind friendly.*

Good point-fixed. Re colors, see our response to 3.25 above.

1 **Temperature and moisture effects on greenhouse gas**
2 **emissions from deep active-layer boreal soils**

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9 **Abstract**

10 Rapid climatic changes, rising air temperatures, and increased fires are expected to
11 drive permafrost degradation and alter soil carbon (C) cycling in many high-latitude
12 ecosystems. How these soils will respond to changes in their temperature, moisture,
13 and overlying vegetation is uncertain, but critical to understand given the large soil
14 C stocks in these regions. We used a laboratory experiment to examine how
15 temperature and moisture control CO₂ and CH₄ emissions from mineral soils
16 sampled from the bottom of the annual active layer, i.e. directly above permafrost, in
17 an Alaskan boreal forest. Gas emissions from thirty cores, subjected to two
18 temperatures and either field moisture conditions or experimental drought, were
19 tracked over a 100-day incubation; we also measured a variety of physical and

20 chemical characteristics of the cores. Gravimetric water content was 0.31 ± 0.12
21 (unitless) at the beginning of the incubation; cores at field moisture were unchanged
22 at the end, but drought cores had declined to 0.06 ± 0.04 . Daily CO₂ fluxes were
23 positively correlated with incubation chamber temperature, core water content, and
24 percent soil nitrogen, and had a temperature sensitivity (Q_{10}) of 1.3 and 1.9 for the
25 field moisture and drought treatments, respectively. Daily CH₄ emissions were most
26 strongly correlated with percent nitrogen, but neither temperature nor water
27 content was a significant first-order predictor of CH₄ fluxes. The cumulative
28 production of C from CO₂ was over six orders of magnitudes higher than that from
29 CH₄; cumulative CO₂ was correlated with incubation temperature and moisture
30 treatment, with drought cores producing 52% - 73% lower C. Cumulative CH₄
31 production was unaffected by any treatment. These results suggest that deep active-
32 layer soils may be sensitive to changes in soil moisture under aerobic conditions, a
33 critical factor as discontinuous permafrost thaws in interior Alaska. Deep but
34 unfrozen high-latitude soils have been shown to be strongly affected by long-term
35 experimental warming, and these results provide insight into their future dynamics
36 and feedback potential with future climate change.

37 1 Introduction

38 High latitude ecosystems are being subjected to rapid changes in climate (IPCC,
39 2013) and increases in fire frequency and intensity (Kasischke et al., 2010), notably
40 in northwestern North America and Alaska (Hinzman et al., 2005; Ju and Masek,
41 2016). This will have a wide variety of ecosystem effects (Alexander and Mack,

42 2016): in particular, rising temperatures and increasing fire will likely result in
43 changes in soil temperature and permafrost degradation (Pastick et al., 2015; Zhang
44 et al., 2015; Genet et al., 2013; Helbig et al., 2016), with subsequent hydrology
45 changes that will influence soil greenhouse gas (GHG) fluxes to the atmosphere
46 (Schädel et al., 2014). Such fluxes are a large component of the global C cycle and
47 could result in a significant and positive climate feedback (Treat et al., 2015; Koven
48 et al., 2011; Schaefer et al., 2014).

49 The magnitude, timing, and form-in particular as methane (CH₄) or carbon dioxide
50 (CO₂)-of such any such feedback remain highly uncertain (Schuur et al., 2015).

51 While northern soils hold enormous quantities of potentially mineralizable soil
52 organic carbon (SOC) (Hugelius et al., 2014), vegetation and succession dynamics
53 (for example, thermal insulation by mosses) promote permafrost resilience to even
54 large temperature changes (Jorgenson et al., 2010; Turetsky et al., 2012). Vegetation
55 type also influences SOC quality and quantity, with microbial communities (Högberg
56 et al., 2007), soil respiration (Raich and Tufekcioglu, 2000), and SOC all linked to
57 aboveground factors such as woody versus nonwoody stems, deciduous versus
58 evergreen canopies, and the presence of nitrogen-fixing plants. A number of factors
59 may however disrupt these these feedbacks between vegetation type, ground cover,
60 permafrost, and SOC stability. Climate changes, in particular regional warming and
61 drying, cause vegetation stress (Ju and Masek, 2016; Barber et al., 2000) and
62 increased mortality. Conversely, increasing plant productivity in some regions can
63 stimulate the decomposition of older SOC (Hartley et al., 2012). Climate also drives
64 fire regime changes, and ecosystem disruption is particularly likely after intense

65 fires (Johnstone et al., 2010; Genet et al., 2013). Even absent disturbance, the
66 stability of SOC is highly uncertain, as it depends on soil temperature and moisture,
67 the ages of and ratio between the carbon (C) and nitrogen (N) pools (Weiss et al.,
68 2015; Karhu et al., 2014), and its protection (whether by organomineral sorption,
69 chemical lability, or physical location) from competent microorganisms, enzymes,
70 and resources (Bailey et al., 2012; Schmidt et al., 2011).

71 Temperature and moisture typically have strong and often interactive influences on
72 soil GHG emissions. Laboratory incubations, field observations, and meta-analyses
73 have documented changing greenhouse gas (GHG) fluxes with rising temperature
74 (Olefeldt et al., 2013; Davidson and Janssens, 2006; Hashimoto et al., 2015; Treat et
75 al., 2015). GHG responses to wetting and thawing dynamics exhibit substantial
76 variability between studies, probably due to differences in soil type, antecedent
77 conditions, phase changes, experimental protocols, etc. (Kim et al., 2012). The
78 anaerobic conditions common following permafrost thaw are expected to lower CO₂
79 emissions but increase those of CH₄ (Treat et al., 2015; Treat et al., 2014), but
80 emissions from aerobic soils will likely dominate the permafrost C feedback
81 (Schädel et al., 2016). Decadal warming and drying trends in Alaska (Bieniek et al.,
82 2014) thus seem likely to increase GHG emissions from soils, and laboratory
83 incubation experiments are critical to understand these dynamics (Elberling et al.,
84 2013).

85 Most previous studies have focused on surface soils or permafrost soils, neglecting
86 deep active-layer soils that were identified as subject to strong effects from a two-

87 decade warming experiment in the Alaskan Arctic (Sistla et al., 2013). Such deeper
88 soils have particular characteristics distinguishing them from both shallow active
89 layer soils and underlying permafrost: they are most affected by interannual
90 variability in thaw depth, potentially flipping the C source/sink status of entire
91 ecosystems (Goulden et al., 1998; Harden et al., 2012); they are subject to distinctive
92 freeze-thaw, cryoturbation, and microbial dynamics, which are likely to change their
93 sensitivity to climate change and feedback potential (Schuur et al., 2008); and they
94 are known to pose particular problems for accurate modeling of high-latitude
95 carbon dynamics (Nicolsky et al., 2007). These soils are likely to be a highly
96 important contributor to future climate feedbacks, with modeling studies suggesting
97 that one-third of 21st century climate-induced carbon loss may originate from
98 seasonally frozen soil carbon (Koven et al., 2015).

99 The goal of this study was to examine how temperature and moisture control GHG
100 (CO₂ and CH₄) emissions from soils sampled from the bottom of the annual active
101 layer—i.e., directly above permafrost—in an Alaskan boreal forest. We also aimed to
102 characterize the chemical and structural properties of these soils following a 100-
103 day incubation at different temperatures, subjecting some cores to drying
104 treatments. We hypothesized that (i) CO₂ would be the dominant pathway for C loss
105 in these largely aerobic soils; (ii) soils maintained at field moisture and high (20°C)
106 temperature would lose more C-CO₂ than cores incubated at 4°C, due to increased
107 aerobic and anaerobic microbial activity; and (iii) core CH₄ fluxes would be small
108 and sensitive only to temperature, as no anaerobic conditions were imposed on the
109 cores.

110 2 Methods

111 2.1 Field sampling

112 The field component of this research took place in Caribou-Poker Creeks Research
113 Watershed (CPCRW), part of the Bonanza Creek LTER
114 (<http://www.lter.uaf.edu/research/study-sites-cpcrw>). CPCRW is located in the
115 Yukon-Tanana Uplands northeast of Fairbanks, AK, a part of the boreal forest that
116 has seen strong increases in air temperature and forest browning (Ju and Masek,
117 2016) over several decades. Annual average air temperature is $-2.5\text{ }^{\circ}\text{C}$, and annual
118 average precipitation 400 mm (Petrone et al., 2006). The watershed's lowlands and
119 north-facing slopes are dominated by black spruce (*Picea mariana* (Mill.) BSP),
120 feathermoss (*Pleurozium schreberi* and others), and *Sphagnum* spp.; the drier south
121 slopes tend to be deciduous with a mixture of trembling aspen (*Populus tremuloides*
122 Michx.), paper birch (*Betula neoalaskana*), and patches of alder (*Alnus crispa*).

123 We sampled soils from a southeast slope ($65.1620\text{ }^{\circ}\text{N}$, $147.4874\text{ }^{\circ}\text{W}$) at CPCRW, in a
124 60 m transition zone between lowland *Picea mariana* and upland *Betula*
125 *neoalaskana*, with significant white spruce (*Picea glauca*) presence as well. Stand
126 density in this transition zone was 4060 ± 2310 trees ha^{-1} , with basal area of $27.9 \pm$
127 $7.0\text{ m}^2\text{ ha}^{-1}$. The forest was at least 90 years old (cf. Morishita et al., 2014) according
128 to tree cores taken at the base of several of the largest white spruce. The soil is
129 characterized as a poorly-drained silt loam, and on average had ~ 20 cm of organic
130 material over the mineral soil.

131 Thirty-nine soil cores, each 30 cm high by 7.5 cm wide, were taken using a soil
132 recovery augur (AMS Inc., American Falls, ID) on 3-5 August 2015. We sampled from
133 the bottom (within 0-2 cm of permafrost) of the active layer, which averaged 80 cm
134 depth. Sample points were randomly located in the transition zone described above,
135 and separated by 2-5 m. Cores were kept cool in the field before being packed in dry
136 ice and shipped to Richland, WA within 48-72 hours of collection.

137 **2.2 Laboratory incubation**

138 In the lab, the soil cores were stored at 4 °C for several days until they were weighed
139 and prepared for incubation. At that point (11-12 August 2015), three fragmented
140 or otherwise damaged cores were discarded, and the remaining cores were
141 randomly assigned to one of six groups (N=6 in each group). These included two
142 incubation temperatures of 4 and 20 °C, following the protocol of a number of
143 previous boreal incubation studies (Treat et al., 2015). Within each temperature
144 there were two moisture treatments: one in which soil moisture was maintained at
145 field conditions (~28% moisture by volume), and a drought treatment in which no
146 water was added and cores were allowed to dry down to ~5% moisture by volume.
147 The fifth group was a 20 °C "controlled drought" one, in which water was added so
148 that these cores' moisture status would close match those of the 4 °C "drought"
149 cores, which we anticipated would dry more slowly than their 20 °C counterparts.
150 The final 6-core group was used for destructive, pre-incubation measurements
151 including moisture content, pH, soil carbon and N, and bulk density. Subsamples

152 were collected and stored at -20 °C for dissolved organic carbon measurements or
153 air-dried for soil C and N (see below).

154 On 18 August 2015 cores were placed into one of two growth chambers (Convion
155 Control Systems BDW80, Winnipeg, Canada) maintained at 4 and 20 °C
156 temperatures and 70% relative humidity and allowed to equilibrate for two weeks.
157 Starting on 31 August 2015 we measured the cores' mass and GHG emissions four
158 times in the first week, then twice per week for the first month, and then once per
159 week for the rest of the 100-day incubation. Throughout the incubation, cores had a
160 200 µm mesh screen fit to the base and were mounted on porous ceramic plates
161 (Soil Moisture Equipment Corp., Santa Barbara, CA, USA) so that, when the plates
162 were placed in contact with water, water would move up into the cores via capillary
163 action. The "drought" cores were mounted on dry plates, but not allowed to drop
164 below 5% water content. **After each flux measurement, cores received additional**
165 **wetting from the top to maintain their desired water status.**

166 For each measurement, a six-core treatment group was connected to a Picarro
167 A0311 multiplexer that was in turn connected to a Picarro G2301 GHG Analyzer
168 (Picarro Inc., Santa Clara, CA, USA). Dry CH₄ and CO₂ concentrations were monitored
169 for 2 minutes, and this was repeated 2-3 times before moving on to a new treatment
170 group. Cores were weighed immediately after gas measurements. Ambient air was
171 measured between treatment groups, and before starting measurements in a
172 chamber, as a check on ambient CO₂ conditions and instrument stability.

173 The incubation experiment concluded on 9 December 2015, following the final CO₂
174 and CH₄ readings. Each soil core was maintained at the treatment-dependent
175 temperature and moisture content (by mass) until removed for destructive
176 sampling, December 14-18, 2015. Sub-samples were collected and composited
177 throughout each soil core for dissolved organic carbon analysis (110 ± 24 g dry
178 mass equivalent) and dry-mass calculations (~28 g each). The remaining core
179 material was air-dried and separated into particles (>2 mm diameter) and soil (≤2
180 mm) using a U.S. Standard Test Sieve No. 10 (Fisherbrand, Pittsburg, PA, USA). The
181 dry mass and volume of soil were used in calculations of gravimetric and volumetric
182 soil moisture content, respectively (Gardner, 1986). Soil volume was calculated as
183 the total core volume minus the volume of particles >2 mm diameter, with the latter
184 determined by water displacement. Air-dried soil and sub-samples stored at -20 °C
185 were sent to the Agricultural and Environmental Services Laboratory at the
186 University of Georgia Extension in February 2016 for total C, N, and dissolved
187 organic carbon (DOC). Samples were combusted in an oxygen atmosphere at 1350
188 °C, and measured for gaseous C and N using an Elementar Vario Max CNS
189 (Langensfeld, Germany). DOC was measured using a Shimadzu 5000 TOC
190 Analyzer (Columbia, Maryland, USA).

191 **2.3 Data and statistical analysis**

192 For each measurement of each sample throughout the 100-day incubation (i.e., each
193 gas, core, and date/time), we used the rise in gas concentrations to calculate a flux
194 rate in ppm s⁻¹ (CO₂) or ppb s⁻¹ (CH₄), a linear rate of change ($\delta c/\delta t$) based on the

195 concentration rise from a minimum (up to 10 seconds after measurement began) to
196 a maximum (at 10-45 seconds). Each core's respiration flux (F) was then calculated
197 as $F = \frac{\delta}{\delta} \frac{V}{M} \frac{P_a}{R}$ where V is the core-specific system volume, M the core dry mass as
198 determined at the end of the incubation, P_a atmospheric pressure (101 kPa; the
199 incubation chambers were ~120 m a.s.l.), R the universal gas constant ($8.3 \times 10^{-3} \text{ m}^3$
200 $\text{kPa mol}^{-1} \text{ K}^{-1}$) and T the chamber air temperature (K) at time of measurement. The
201 final respiration rate was expressed on a soil C basis (μg or $\text{ng C g C}^{-1} \text{ day}^{-1}$).

202 Anomalous data were excluded based on their gas fluxes being more than 5 (for
203 CO_2) or 10 (for CH_4) mean absolute deviations (Davies and Gather, 1993) from the
204 treatment mean within a 10-day period, for a given treatment and temperature. We
205 excluded 172 of 2686 (6.4%) measurements for this reason. If the coefficient of
206 variability (CV) of fluxes from any core on a single day exceeded 140%, a value
207 chosen based on the distribution of CVs across all cores, the entire core was
208 excluded for that day (90 data points, 3.4%). Other data (4.8%) were removed
209 because of known instrument problems, e.g. the analyzer was left running after
210 leaving a chamber. The final number of valid flux samples from the 100-day
211 incubation was 2198.

212 The effects of temperature, gravimetric water content, percent C, percent N, and
213 DOC concentration on instantaneous gas fluxes were evaluated using a linear mixed-
214 effects model fit by the R function *lme* in the R 'nlme' package, version 3.1.128.
215 Because the dependent variable (CO_2 or CH_4 flux) was non-normally distributed, it
216 was transformed using a natural-logarithm ($+0.1 \mu\text{g C g C}^{-1} \text{ day}^{-1}$ to ensure all

217 positive fluxes, following Treat et al. 2015) transformation. Soil core was treated as
218 a random effect in the model. We then performed stepwise model selection by
219 Akaike's information criterion (AIC) using the *stepAIC* function in the R 'MASS'
220 package, version 7.3.45. A linear mixed-effects model was also used to evaluate the
221 effect of treatment on core water content.

222 Cumulative respiration for each core and gas was calculated by linearly
223 interpolating flux rates between measurement dates and summing respired C over
224 the entire incubation. The effect of temperature and treatment (drought, controlled
225 drought, or field moisture conditions) on cumulative gas fluxes was evaluated with a
226 post-hoc Tukey Honest Significant Differences test. Temperature sensitivity (Q_{10})

227 was calculated for each gas and treatment as $\frac{F_2}{F_1} \left(\frac{1}{T_2 - T_1} \right)$ where F_1 and F_2 are the
228 cumulative gas fluxes (mg C g C^{-1}) at temperatures T_1 and T_2 ($^{\circ}\text{C}$), respectively.

229 All data analysis and statistics were performed using R version 3.3.1 (2016-06-21)
230 (R Development Core Team, 2016). This experiment was run as an 'open
231 experiment' (Bond-Lamberty et al., 2016b) with all analysis code, data (from raw
232 instrument data to final summaries), diagnostics, etc., available at
233 https://github.com/bpbond/cpcrw_incubation. The summarized flux data backing
234 the main results have been archived under the Digital Object Identifier
235 10.6084/m9.figshare.4240436.v1.

236 **3 Results**

237 The 30 experimental cores had a bulk density of 1.00 ± 0.18 (mean \pm sd) g cm^{-3} .
238 Large (>2 mm) particles, primarily schist, comprised $41\% \pm 11\%$ of the cores' total
239 mass. Soil (≤ 2 mm) dry mass was 886 ± 154 g. Sample DOC was 157.93 ± 55.74 mg
240 kg^{-1} . Carbon content was $1.20\% \pm 1.19\%$, while N content was $0.06\% \pm 0.06\%$. Mean
241 C:N was 20.7. Neither temperature nor moisture treatment exerted any significant
242 effect ($P > 0.1$ for all) on these highly variable properties (**Table 1**).

243 Gravimetric water content was 0.31 ± 0.12 (min 0.19, max 0.77) at the beginning of
244 the incubation (**Figure 1**). "Field moisture" cores were on average unchanged (0.33
245 ± 0.13) at the end of the incubation, but both the drought treatments, which did not
246 differ from each other in their effect on gravimetric water content ($P = 0.880$), had
247 declined to 0.06 ± 0.04 . Volumetric water content values ranged from 0.29 ± 0.05
248 (min 0.23, max 0.43) at the beginning of the experiment to 0.15 ± 0.11 (min 0.03,
249 max 0.38) at the end across all cores. Water filled pore space, assuming a particle
250 density of 2.65 g cm^{-3} , was 22-65% over all cores, moisture treatments, and
251 temperatures.

252 Carbon dioxide fluxes during the incubation ranged from $1.1 \mu\text{g C g C}^{-1} \text{ day}^{-1}$ to a
253 maximum of $5245.1 \mu\text{g C g C}^{-1} \text{ day}^{-1}$, with a mean of $248.9 \mu\text{g C g C}^{-1} \text{ day}^{-1}$ over the
254 100 days. CH_4 rates ranged from $0.00 \text{ ng C g C}^{-1} \text{ day}^{-1}$ to a maximum of $1.31 \text{ ng C g C}^{-1}$
255 day^{-1} , with a mean of $0.06 \text{ ng C g C}^{-1} \text{ day}^{-1}$. These means conceal considerable
256 variability over the course of the incubation (**Table 1, Figures 2 and 3**).

257 In the linear mixed-effects model (AIC = 2992.6), instantaneous CO₂ flux was
258 positively correlated with incubation chamber temperature, core gravimetric water
259 content, and percent soil N (all P < 0.05, and the latter two P < 0.001; **Table 2**).

260 **Temperature sensitivity decreased significantly (P < 0.001) over the course of the**
261 **incubation, while moisture sensitivity was unaffected by time.** Percent C and percent
262 N were highly correlated (r = 0.99) for these cores. Because percent N was a slightly
263 stronger predictor, it was retained in the model while percent C was excluded; cf.
264 Colman and Schimel (2014). The interaction between water content and percent N
265 was also highly significant (P < 0.001), **although cores with N > 0.2% exhibited little**
266 **relationship between water content and CO₂ flux (data not shown).** Instantaneous
267 CH₄ fluxes were positively correlated with percent N, while water content exhibited
268 significant interactions with percent N and DOC as a predictor (**Table A1**). **This**
269 **model had little predictive power (AIC = -10879.2), however,** and neither
270 temperature nor water content was a significant first-order predictor of CH₄ fluxes.

271 The cumulative production of C from CO₂ (**Figure 4**) was over six orders of
272 magnitude higher than that from CH₄, with CO₂:CH₄ C ratios ranging from 1.4
273 million in the 4 °C "Field moisture" treatment, to 6.2 million in the 20 °C "Field
274 moisture" treatment. Cumulative CO₂ evolved was highly affected by temperature (P
275 = 0.003), and "field moisture" cores emitted significantly more CO₂ than the other
276 two moisture treatments at both temperatures (P < 0.001 for both, with no
277 significant interactive effect). There was no difference between fluxes from the 20 °C
278 "drought" and "controlled drought" treatments (P = 0.377). "Drought" cores'
279 cumulative production was 73% (4 °C) and 52% (20 °C) lower than the cores kept at

280 field moisture. Neither temperature ($P = 0.200$) nor moisture treatment (mean $P =$
281 0.975) was a significant factor in predicting cumulative CH_4 fluxes.

282 The cumulative flux numbers above result in CO_2 temperature sensitivity (Q_{10})
283 values of 1.3 and 1.9 for the field moisture and drought treatments, respectively; the
284 corresponding Q_{10} values based on cumulative CH_4 were 1.2 and 1.3. Computing Q_{10}
285 values based on fluxes normalized by water filled pore space changed these values
286 only slightly: to 1.2 and 1.7 for CO_2 , for the field moisture and drought treatments
287 respectively, and 1.1 and 1.2 for CH_4 .

288 4 Discussion

289 Rises in boreal air temperatures, and unpredictable precipitation changes, will
290 change fire disturbance regimes, warm and dry many soils, increase vegetation
291 stress, degrade permafrost, and deepen the active layer (Schuur et al., 2015), all
292 with uncertain consequences for soil dynamics and GHG fluxes. In this laboratory
293 experiment we found that CO_2 , but not CH_4 , fluxes from these oxic active-layer
294 mineral soils were sensitive to temperature and, in particular, moisture.

295 A number of studies have measured microbial respiration and GHG fluxes very close
296 to our study site. Morishita et al. (2014) quantified GHG fluxes at CPCRW and nearby
297 forests, and found CO_2 production to be correlated with both temperature and
298 moisture in upland Cryosols, consistent with our results. Waldrop et al. (2010)
299 incubated active-layer and permafrost soils from *Picea mariana* sites near
300 Fairbanks, AK, observing aerobic Q_{10} values of 9.0 (active layer) and 2.3
301 (permafrost) from -5 to 5 °C, and flux rates of 0.001 - 0.10 $\mu\text{mol CH}_4 \text{ day}^{-1} \text{ g}^{-1}$

302 (~0.001-0.133 ng C g C⁻¹ day⁻¹), and ~1-5 μg C-CO₂ hr⁻¹ g⁻¹ (~2000-10000 μg C g C⁻¹
303 day⁻¹), considerably higher than the CO₂ rates observed here. During the first 100
304 days of an incubation of Fairbanks-area 0-10 cm mineral soils, Neff and Hooper
305 (2002) observed fluxes of ~55-409 μg C-CO₂ g C⁻¹ day⁻¹, in line with the results here,
306 while Wickland and Neff (2008) reported that temperature and moisture exhibited
307 interactive effects, of similar magnitude, on decomposition in *P. mariana* soils.

308 A number of synthesis studies have documented dynamics and C feedback potential
309 of Arctic and boreal soils more generally; comparing to these results is useful
310 because although the response of soil biota to stresses such as drought tends to
311 differ between soil types, organisms, and vegetation, it is often broadly similar
312 across biomes and climatic conditions (Manzoni et al., 2012). Using two meta-
313 analyses of aerobic and anaerobic permafrost soil incubations, Schädel et al. (2016)
314 showed that C release was highly sensitive to temperature, and that soils released
315 far more (220-520%) C under aerobic conditions. Our incubation was fully aerobic,
316 but its results are consistent with the conclusion that respiration in the form of CO₂
317 is likely to dominate the high latitude C feedback, and that aerobic soils, and the
318 conditions under which currently waterlogged soils may drain, deserve particular
319 attention. In terms of absolute flux rates, Treat et al. (2015) reported mean CO₂
320 rates of 47 (all mineral soils) and 101 (for 20-100 cm soils) μg C-CO₂ g C⁻¹ day⁻¹ from
321 a pan-Arctic synthesis of anaerobic soil incubations, somewhat lower than our
322 aerobic incubation results. Treat et al. (2014) also found CO₂ and CH₄ emissions to
323 be strongly correlated with temperature and moisture based on an incubation of

324 Alaskan peats. Whether climate change makes northern regions wetter or drier is
325 thus a critical factor affecting the quantity and form of C release.

326 The drought treatment imposed in this experiment reduced soil C fluxes by 52% -
327 73%. The importance of this result depends, in part, on the spatial extent and
328 intensity of precipitation changes across the boreal and Arctic this century. There is
329 a detectable anthropogenic influence in high latitude precipitation changes (Wan et
330 al., 2015), but these changes are inconsistent: drier and warmer conditions in boreal
331 Eurasia (Buermann et al., 2014), for example, but growing season length increases
332 in interior Alaska with no increase in precipitation (Wendler and Shulski, 2009).
333 This spatial variability will interact with permafrost thaw dynamics to produce a
334 complex patchwork of soil moisture changes (Zhang et al., 2012; Watts et al., 2012).
335 The high uncertainty in this area makes it all the more important to understand the
336 interactive effects of soil moisture and temperature on decomposition and GHG
337 emissions (Sierra et al., 2015).

338 We observed very low but positive CH₄ production from these upland mineral soils.
339 This is contrast to many field studies that have observed CH₄ uptake (oxidation) in
340 dry boreal sites (Matson et al., 2009; Schaufler et al., 2010). Anoxic microsites in soil
341 can however provide enough CH₄ production to balance low-level consumption in
342 otherwise aerobic soils (Kammann et al., 2009). In addition, our results are broadly
343 consistent with data from 65 studies summarized by Olefeldt et al. (2013), who
344 found that CH₄ emissions were more sensitive to soil temperature in wetter
345 ecosystems; it would have been a surprise if the little methanogenic activity in our

346 upland, well-drained soils was temperature-sensitive at all. Methane was also a far
347 smaller C flux than CO₂ from these soils, in particular at higher temperatures (as CO₂
348 was responsive to temperature, but CH₄ was not). This is true more generally: for
349 example, Treat et al. found a median CO₂:CH₄ production ratio of 387 for **anaerobic**
350 incubations of boreal soils. This is naturally far lower than our observed aerobic
351 (and thus high-CO₂) ratios, but nonetheless consistent with them. Thus we see little
352 opportunity for CH₄ to be a significant contributor to these upland soils' C fluxes and
353 climate feedback risk, **even accounting for the 25x stronger radiative forcing of this**
354 **gas over a 100-year time horizon (Lee et al., 2012).**

355 **4.1 Temperature versus moisture sensitivity for cumulative emissions**

356 **The cumulative GHG fluxes (Figure 4) integrate the entire 100-day incubation,**
357 **eliminating the day-to-day variability of instantaneous fluxes and are thus more**
358 **generalizable. Our results suggest that moisture limitation could exert a large effect**
359 **on CO₂ production for deep active-layer soils: "drought" cores' cumulative**
360 **production was 73% (4 °C) and 52% (20 °C) lower than the cores kept at field**
361 **moisture. This effect was highly significant, and suggests that moisture limitations**
362 **could exert a significant constraint on deep active-layer soils as they slowly warm.**
363 Such moisture constraints are thought to be already exerting effects on vegetation
364 and soil fluxes at large scales (Ju and Masek, 2016; Bond-Lamberty et al., 2012), but
365 our understanding of the interactive effects involved is poor.

366 **The Q₁₀ values observed in this experiment were low (all less than 2.0, even when**
367 **controlling for changes in soil moisture). Temperature sensitivities of ~2 are more**

368 typical (Dutta et al., 2006; Schädel et al., 2016), although the temperature sensitivity
369 of C release can change over time of incubation (Dutta et al., 2006) and vary
370 between soil fractions cycling over different time horizons (Karhu et al., 2010;
371 Schädel et al., 2014). Observed surface CO₂ fluxes at this CPCRW site exhibited a Q₁₀
372 of 5.1 ± 1.4 over a temperature range of 3.5-15 °C (personal communication, C.
373 Anderson); these surface fluxes were measured over multiple months and include
374 root respiration, however, preventing any direct comparison. While increased
375 temperature does not always drive C mineralization rates in forest mineral soils
376 (Giardina and Ryan, 2000), it is linked with increases in soil moisture content can
377 lead to changes in microbial community structure and GHG fluxes (Xue et al., 2016).

378 Interestingly, Q₁₀ values were lower in the drought treatment cores, a mathematical
379 consequence of the fact that drought restricted CO₂ respiration more at 4 °C than at
380 20 °C. There is evidence that climate warming changes the microbial decay
381 dynamics of soil organic C compounds generally considered to be stable (Frey et al.,
382 2013; Bond-Lamberty et al., 2016a). Conditions such as drought can change the
383 amount and quality of DOC available to microbes (1999), but we observed no DOC
384 changes between treatments here. Deep active layer soils store large quantities of
385 soil C (Mueller et al., 2015) but are not subject to abundant inputs of fresh C from
386 vegetation, so the starting quality of the native soil C in active layer soils is older,
387 more microbially processed, and dominated by more stable "heavy" organic C
388 (Karlsson et al., 2011). Thus, it may not be surprising that these more stable C
389 compounds would be metabolized by processes that have been reported to be less
390 temperature-sensitive.

391 **4.2 Soil nitrogen**

392 Somewhat unexpectedly, percent soil N was very significantly and positively
393 correlated with both CO₂ and CH₄ fluxes (**Tables 2 and 3**). Nitrogen interacts with
394 microbial respiration via a number of complex, interactive, and still unclear
395 mechanisms (Luo and Zhou, 2006), including reductions in belowground plant
396 allocation, shifts in energy source or population of the saprotrophic community
397 (Saiya-Cork et al., 2002) that leave it less capable of decomposing recalcitrant
398 compounds, and perhaps abiotic stabilization mechanisms (Janssens et al., 2010).
399 Meta-analyses have generally shown negative to neutral effects of N deposition on
400 microbial biomass (Treseder, 2008) and respiration (Ramirez et al., 2012), and total
401 soil respiration across ecosystems and biomes (Janssens et al., 2010; Zhou et al.,
402 2014). These effect are likely due to several one or more mechanisms involving soil
403 pH, ligninase enzymes, and phenol oxidase activity (Luo and Zhou, 2006), and
404 incubation results examining N effects can be highly variable (Lavoie et al., 2011;
405 Sistla et al., 2012). Some studies have however observed positive correlations
406 between ambient soil N and microbial respiration. For example, Weiss et al. (2015)
407 found CO₂ production from Siberian Yedoma permafrost samples to be correlated
408 with both percent C and N, consistent with our active-layer results (**Table 2**).
409 The C:N ratio was not a significant predictor of GHG fluxes in this study, although
410 this ratio has been found to be important in meta-analyses (Sistla et al., 2012;
411 Schädel et al., 2014). *In situ* respiration rates have also been shown to be negatively
412 correlated with C:N at large spatial scales (Allaire et al., 2012). Percent C and N both

413 varied widely in our soil cores (**Table 1**), and were highly correlated with each
414 other, even though the cores were collected within tens of meters of each other. This
415 suggests that active-layer SOC response to temperature and moisture may also be
416 highly spatially variable, even in a mixed-species boreal forest **that we expected, a**
417 **priori, to provide spatial variation in litter and SOC quality (Fierer et al., 2005).**
418 Spatially explicit analyses of soil biochemistry, temperatures (Bond-Lamberty et al.,
419 2005), and respiration (Allaire et al., 2012) are likely necessary to accurately
420 constrain and predict soil fluxes in this ecosystem.

421 **4.3 Limitations and weaknesses**

422 There were weaknesses in our approach and experimental design that should be
423 considered. Laboratory experiments offer precise control, but lack the *in situ* nature
424 of field manipulations (Sistla et al., 2013), raising uncertainties to what degree their
425 results can be extrapolated. **Soils isolated during incubation may, for example,**
426 **underestimate temperature sensitivity of respiration (Podrebarac et al., 2016) or**
427 **exhibit lag effects (Treat et al., 2015). It should also be noted that our 100-day**
428 **incubation was not long enough to observe slowly-cycling soil fractions, which may**
429 **vary in their response to experimental manipulation (Karhu et al., 2010).**

430 Nonetheless, the controlled environments of incubations provide an important way
431 to elucidate the key mechanisms controlling GHG from high-latitude soils (Schuur et
432 al., 2015).

433 **The soils studied here were from an upland, mixed conifer-deciduous boreal forest,**
434 **and care needs to be taken before drawing regional inferences, or about other**

435 **ecosystem types.** We focused on an experimental drought, rather than flooding,
436 because of the well-drained nature of the field site: it is unlikely that the mid-slope
437 forest we sampled in will ever suffer from thermokarst or excessive soil moisture,
438 but too-dry conditions are a serious possibility in this relatively low-precipitation
439 ecosystem (Barber et al., 2000).

440 **Finally,** the soils here are not surface layer soils (where the majority of microbial
441 activity and C mineralization of labile C takes place); removing them from *in situ*
442 conditions (where they are less exposed to O₂, for example) may significantly
443 change the abiotic conditions to which the microbial community is adapted.
444 However, focusing on the active layer provides crucial information about the
445 potential loss of C from these soils, a risk that needs to be well understood as
446 permafrost degradation leads to expansions in the depth of the active layer across
447 the Arctic.

448 **5 Conclusions**

449 In this laboratory experiment, we found that CO₂ fluxes were strongly influenced by
450 temperature and water content, and correlated with soil C and N, while CH₄ fluxes
451 were much smaller and not sensitive to temperature or water content in these well-
452 drained mineral soils. **These results add to a growing body of Arctic permafrost and**
453 **active layer incubation literature, and underscore the importance of understanding**
454 **moisture effects on CO₂ fluxes in particular. How soil moisture might change with**
455 **spatially variable permafrost degradation,** how soil biota will respond to these
456 changes, and how models should treat soil organic matter decomposition with

457 respect to multiple and interacting drivers are all critical areas of research going
458 forward. Further controlled field and laboratory studies, ideally tightly integrated
459 with modeling experiments, are important to understand GHG emission dynamics
460 from high-latitude soils.

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468 **Author contributions**

469 B.B.-L., A.P.S., and V.L.B. designed this experiment. B.B.-L. and A.P.S. performed field
470 sampling, and A.P.S. led the laboratory incubation and analyses. B.B.-L. wrote the
471 manuscript, with contributions from all authors.

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787 **Table 1.** Summary of dissolved organic carbon (DOC), percent C, percent N, bulk
788 density (BD), and CO₂ and CH₄ fluxes by treatment. The "Field moisture" and
789 "Drought" columns summarize (mean ± s.d.) 12 cores, combining two groups of N=6
790 at each incubation temperature, while the "Controlled drought" and "Pre-
791 incubation" columns are N=6.

Variable	Field moisture	Controlled drought	Drought	Pre- incubation
DOC (mg kg ⁻¹)	173.62 ± 46.67	165.68 ± 66.46	154.60 ± 57.15	125.43 ± 49.07
C (%)	1.67 ± 1.60	0.87 ± 0.50	0.76 ± 0.60	1.44 ± 1.32
N (%)	0.08 ± 0.08	0.04 ± 0.03	0.03 ± 0.03	0.07 ± 0.06
BD (g cm ⁻³)	0.89 ± 0.18	1.06 ± 0.17	1.08 ± 0.14	1.13 ± 0.29
CO ₂ (μg C g C ⁻¹ day ⁻¹)	456.40 ± 543.91	159.77 ± 116.41	97.03 ± 96.38	-
CH ₄ (ng C g C ⁻¹ day ⁻¹)	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	-

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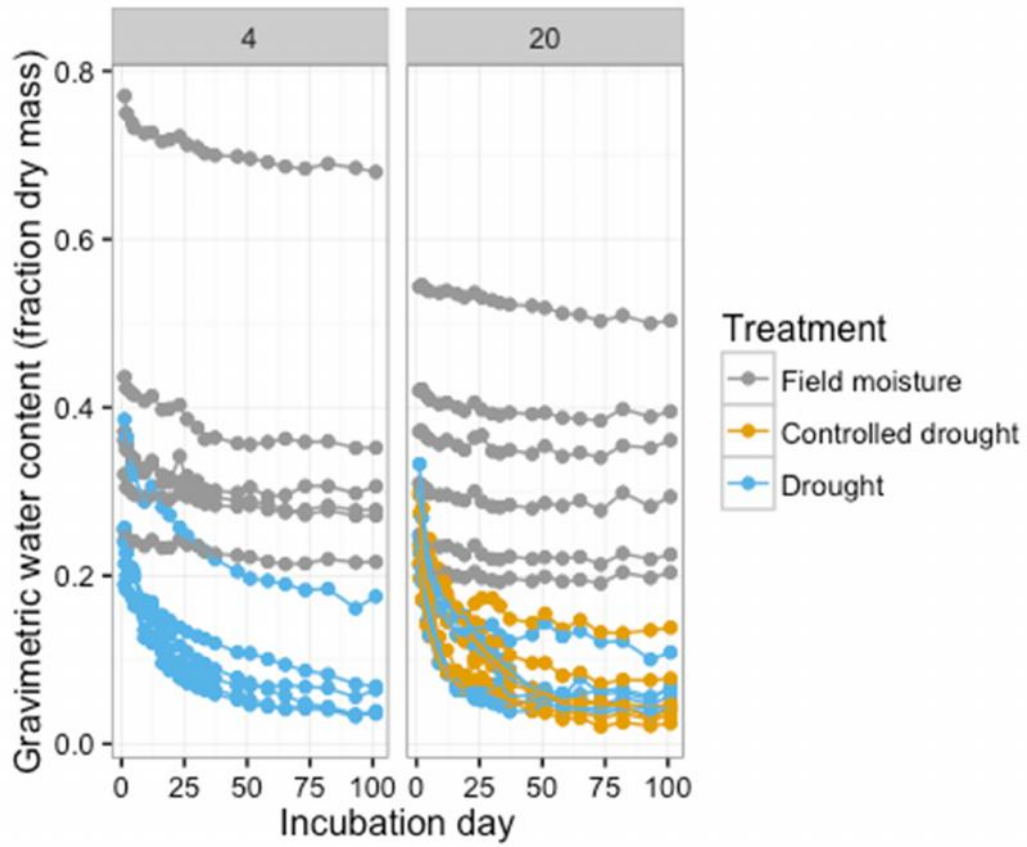
794 **Table 2.** Linear mixed-effects model parameters, testing effects of temperature (°C),
795 gravimetric water content (unitless), soil C (%), soil N (%), and dissolved organic
796 carbon (mg kg⁻¹) on individual core CO₂ fluxes (+0.1 μg C g C⁻¹ day⁻¹); a colon (":")
797 indicates an interaction. Dependent variable has units of log(μg C g C⁻¹ day⁻¹).
798 Columns include parameter value; standard error (SE); degrees of freedom (DF); T
799 statistic; and P value.

	Value	SE	DF	T	P
(Intercept)	1.713	0.354	1153	4.839	< 0.001
Temperature	0.046	0.020	26	2.336	0.027
WC_gravimetric	3.496	1.052	1153	3.322	0.001
N_percent	37.976	6.810	26	5.576	< 0.001
Temperature:WC_gravimetric	0.116	0.061	1153	1.905	0.057
Temperature:N_percent	-0.507	0.300	26	-1.690	0.103
WC_gravimetric:N_percent	-37.347	8.425	1153	-4.433	< 0.001

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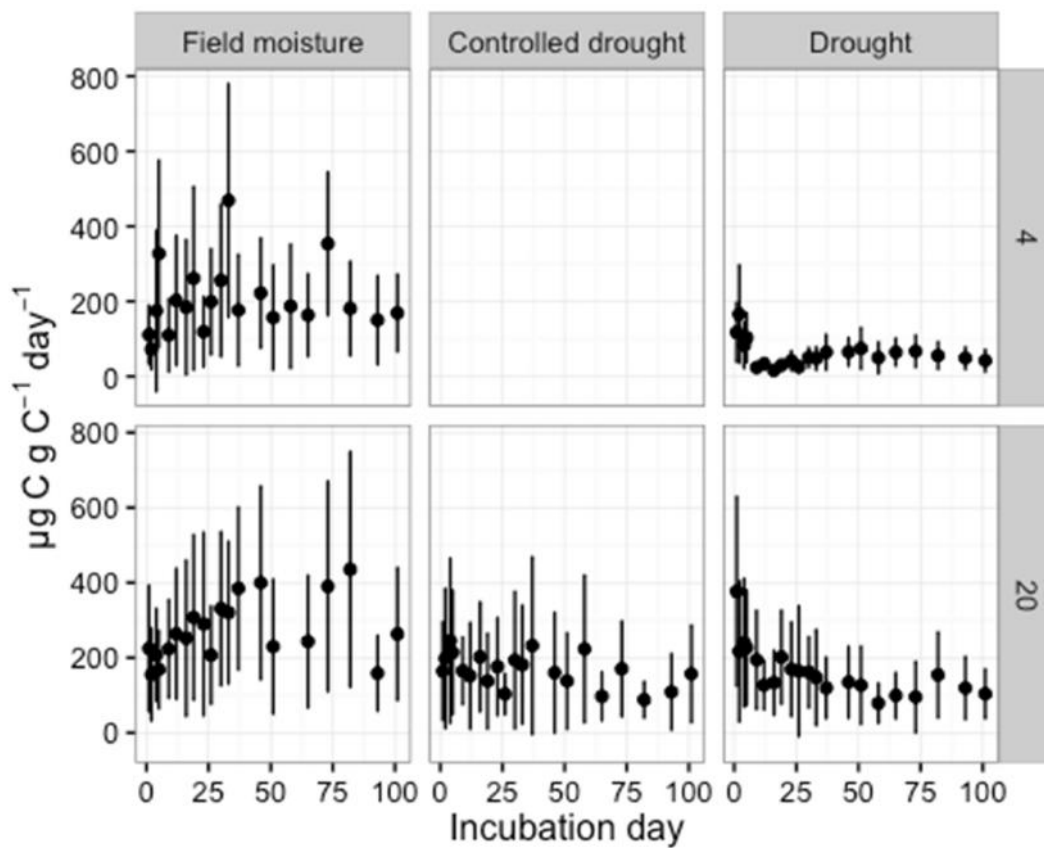
802 **Figure 1.** Core water content across the course of the incubation experiment by
803 temperature (left panel 4 °C, right panel 20 °C) and treatment.



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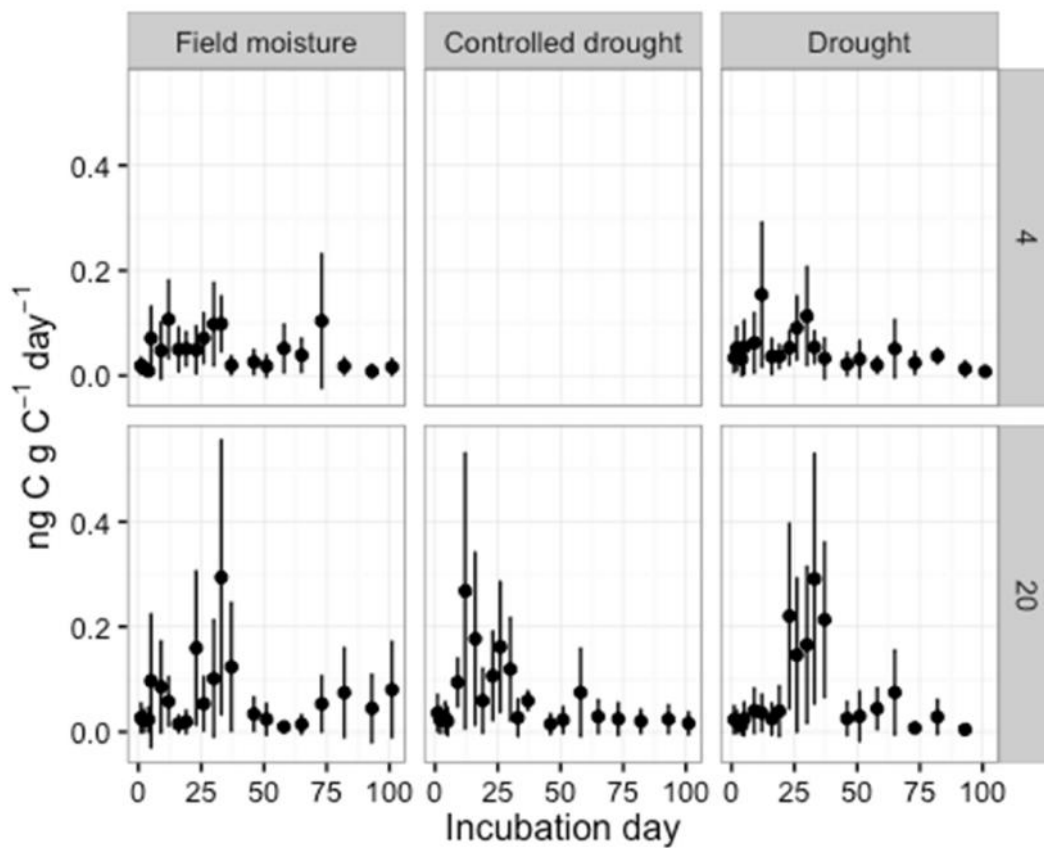
806 **Figure 2.** Mass-normalized CO₂ fluxes over the 100-day incubation, by temperature
807 (4 and 20 °C, rows) and treatment (field moisture, drought, and controlled drought;
808 columns). Error bars show core-to-core standard deviation. The "controlled
809 drought" treatment, for 20 °C only, was meant to dry cores at roughly the same rate
810 as the drought cores at 4 °C.



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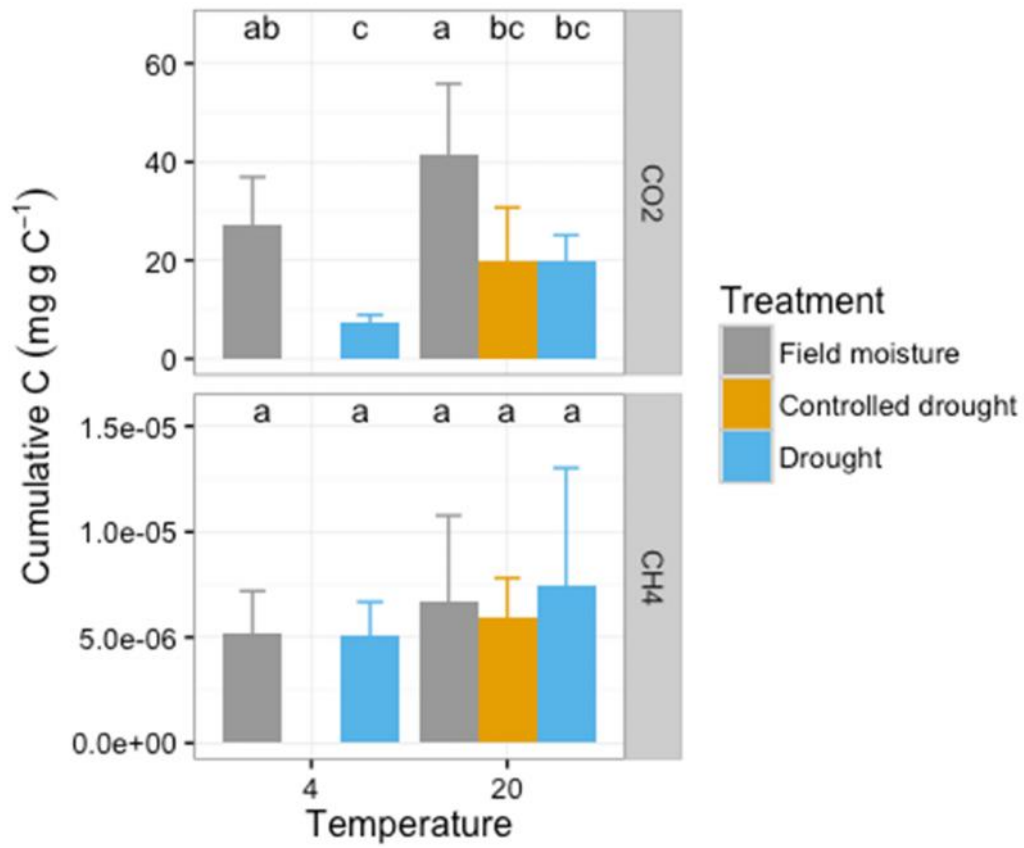
813 **Figure 3.** Mass-normalized CH₄ fluxes over the 100-day incubation, by temperature
814 (4 and 20 °C, rows) and treatment (field moisture, drought, and controlled drought;
815 columns). Error bars show core-to-core standard deviation. The "controlled
816 drought" treatment, for 20 °C only, was meant to dry cores at roughly the same rate
817 as the drought cores at 4 °C.



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820 **Figure 4.** Cumulative mass-normalized C fluxes (mg g C^{-1}) over the incubation, by
 821 gas (CO_2 and CH_4 , top and bottom panels respectively), treatment (columns), and
 822 temperatures (x-axis, $^\circ\text{C}$). Letters within a panel indicate significant differences
 823 based on Tukey's HSD.



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825

826 **Table A1.** Linear mixed-effects model parameters, testing effects of temperature
 827 (°C), gravimetric water content (unitless), soil N (%), and dissolved organic carbon
 828 (DOC, mg kg⁻¹) on log-transformed, individual core CH₄ fluxes (+0.1 µg C g C⁻¹ day⁻¹);
 829 a colon (":") indicates an interaction. Dependent variable has units of log(µg C g C⁻¹
 830 day⁻¹). Columns include parameter value; standard error (SE); degrees of freedom
 831 (DF); T statistic; and P value.

	Value	SE	DF	T	P
(Intercept)	1.713	0.354	1153	4.839	< 0.001
Temperature	0.046	0.020	26	2.336	0.027
WC_gravimetric	3.496	1.052	1153	3.322	0.001
N_percent	37.976	6.810	26	5.576	< 0.001
Temperature:WC_gravimetric	0.116	0.061	1153	1.905	0.057
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WC_gravimetric:N_percent	-37.347	8.425	1153	-4.433	< 0.001

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