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 rational 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, and 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 interannual 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/426a91e1b2d21200718b334d3295fe40a1ea6) 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.


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.


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.

   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 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.
Temperature and moisture effects on greenhouse gas emissions from deep active-layer boreal soils

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Abstract

Rapid climatic changes, rising air temperatures, and increased fires are expected to drive permafrost degradation and alter soil carbon (C) cycling in many high-latitude ecosystems. How these soils will respond to changes in their temperature, moisture, and overlying vegetation is uncertain, but critical to understand given the large soil C stocks in these regions. We used a laboratory experiment to examine how temperature and moisture control CO₂ and CH₄ emissions from mineral soils sampled from the bottom of the annual active layer, i.e. directly above permafrost, in an Alaskan boreal forest. Gas emissions from thirty cores, subjected to two temperatures and either field moisture conditions or experimental drought, were tracked over a 100-day incubation; we also measured a variety of physical and
chemical characteristics of the cores. Gravimetric water content was 0.31 ± 0.12 (unitless) at the beginning of the incubation; cores at field moisture were unchanged at the end, but drought cores had declined to 0.06 ± 0.04. Daily CO$_2$ fluxes were positively correlated with incubation chamber temperature, core water content, and percent soil nitrogen, and had a temperature sensitivity (Q$_{10}$) of 1.3 and 1.9 for the field moisture and drought treatments, respectively. Daily CH$_4$ emissions were most strongly correlated with percent nitrogen, but neither temperature nor water content was a significant first-order predictor of CH$_4$ fluxes. The cumulative production of C from CO$_2$ was over six orders of magnitudes higher than that from CH$_4$; cumulative CO$_2$ was correlated with incubation temperature and moisture treatment, with drought cores producing 52% - 73% lower C. Cumulative CH$_4$ production was unaffected by any treatment. These results suggest that deep active-layer soils may be sensitive to changes in soil moisture under aerobic conditions, a critical factor as discontinuous permafrost thaws in interior Alaska. Deep but unfrozen high-latitude soils have been shown to be strongly affected by long-term experimental warming, and these results provide insight into their future dynamics and feedback potential with future climate change.

1 Introduction

High latitude ecosystems are being subjected to rapid changes in climate (IPCC, 2013) and increases in fire frequency and intensity (Kasischke et al., 2010), notably in northwestern North America and Alaska (Hinzman et al., 2005; Ju and Masek, 2016). This will have a wide variety of ecosystem effects (Alexander and Mack,
2016): in particular, rising temperatures and increasing fire will likely result in changes in soil temperature and permafrost degradation (Pastick et al., 2015; Zhang et al., 2015; Genet et al., 2013; Helbig et al., 2016), with subsequent hydrology changes that will influence soil greenhouse gas (GHG) fluxes to the atmosphere (Schädel et al., 2014). Such fluxes are a large component of the global C cycle and could result in a significant and positive climate feedback (Treat et al., 2015; Koven et al., 2011; Schaefer et al., 2014).

The magnitude, timing, and form-in particular as methane (CH\textsubscript{4}) or carbon dioxide (CO\textsubscript{2})-of such any such feedback remain highly uncertain (Schuur et al., 2015). While northern soils hold enormous quantities of potentially mineralizable soil organic carbon (SOC) (Hugelius et al., 2014), vegetation and succession dynamics (for example, thermal insulation by mosses) promote permafrost resilience to even large temperature changes (Jorgenson et al., 2010; Turetsky et al., 2012). Vegetation type also influences SOC quality and quantity, with microbial communities (Högberg et al., 2007), soil respiration (Raich and Tufekcioglu, 2000), and SOC all linked to aboveground factors such as woody versus nonwoody stems, deciduous versus evergreen canopies, and the presence of nitrogen-fixing plants. A number of factors may however disrupt these these feedbacks between vegetation type, ground cover, permafrost, and SOC stability. Climate changes, in particular regional warming and drying, cause vegetation stress (Ju and Masek, 2016; Barber et al., 2000) and increased mortality. Conversely, increasing plant productivity in some regions can stimulate the decomposition of older SOC (Hartley et al., 2012). Climate also drives fire regime changes, and ecosystem disruption is particularly likely after intense
fires (Johnstone et al., 2010; Genet et al., 2013). Even absent disturbance, the
stability of SOC is highly uncertain, as it depends on soil temperature and moisture,
the ages of and ratio between the carbon (C) and nitrogen (N) pools (Weiss et al.,
2015; Karhu et al., 2014), and its protection (whether by organomineral sorption,
chemical lability, or physical location) from competent microorganisms, enzymes,
and resources (Bailey et al., 2012; Schmidt et al., 2011).

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

Most previous studies have focused on surface soils or permafrost soils, neglecting
deep active-layer soils that were identified as subject to strong effects from a two-
Such deeper soils have particular characteristics distinguishing them from both shallow active layer soils and underlying permafrost: they are most affected by interannual variability in thaw depth, potentially flipping the C source/sink status of entire ecosystems (Goulden et al., 1998; Harden et al., 2012); they are subject to distinctive freeze-thaw, cryoturbation, and microbial dynamics, which are likely to change their sensitivity to climate change and feedback potential (Schuur et al., 2008); and they are known to pose particular problems for accurate modeling of high-latitude carbon dynamics (Nicolsky et al., 2007). These soils are likely to be a highly important contributor to future climate feedbacks, with modeling studies suggesting that one-third of 21st century climate-induced carbon loss may originate from seasonally frozen soil carbon (Koven et al., 2015).

The goal of this study was to examine how temperature and moisture control GHG (CO$_2$ and CH$_4$) emissions from soils sampled from the bottom of the annual active layer–i.e., directly above permafrost–in an Alaskan boreal forest. We also aimed to characterize the chemical and structural properties of these soils following a 100-day incubation at different temperatures, subjecting some cores to drying treatments. We hypothesized that (i) CO$_2$ would be the dominant pathway for C loss in these largely aerobic soils; (ii) soils maintained at field moisture and high (20°C) temperature would lose more C-CO$_2$ than cores incubated at 4°C, due to increased aerobic and anaerobic microbial activity; and (iii) core CH$_4$ fluxes would be small and sensitive only to temperature, as no anaerobic conditions were imposed on the cores.
2 Methods

2.1 Field sampling

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

We sampled soils from a southeast slope (65.1620 °N, 147.4874 °W) at CPCRW, in a 60 m transition zone between lowland Picea mariana and upland Betula neoalaskana, with significant white spruce (Picea glauca) presence as well. Stand density in this transition zone was 4060 ± 2310 trees ha⁻¹, with basal area of 27.9 ± 7.0 m² ha⁻¹. The forest was at least 90 years old (cf. Morishita et al., 2014) according to tree cores taken at the base of several of the largest white spruce. The soil is characterized as a poorly-drained silt loam, and on average had ~20 cm of organic material over the mineral soil.
Thirty-nine soil cores, each 30 cm high by 7.5 cm wide, were taken using a soil recovery augur (AMS Inc., American Falls, ID) on 3-5 August 2015. We sampled from the bottom (within 0-2 cm of permafrost) of the active layer, which averaged 80 cm depth. Sample points were randomly located in the transition zone described above, and separated by 2-5 m. Cores were kept cool in the field before being packed in dry ice and shipped to Richland, WA within 48-72 hours of collection.

2.2 Laboratory incubation

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

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

For each measurement, a six-core treatment group was connected to a Picarro A0311 multiplexer that was in turn connected to a Picarro G2301 GHG Analyzer (Picarro Inc., Santa Clara, CA, USA). Dry CH₄ and CO₂ concentrations were monitored for 2 minutes, and this was repeated 2-3 times before moving on to a new treatment group. Cores were weighed immediately after gas measurements. Ambient air was measured between treatment groups, and before starting measurements in a chamber, as a check on ambient CO₂ conditions and instrument stability.
The incubation experiment concluded on 9 December 2015, following the final CO\textsubscript{2} and CH\textsubscript{4} readings. Each soil core was maintained at the treatment-dependent temperature and moisture content (by mass) until removed for destructive sampling, December 14-18, 2015. Sub-samples were collected and composited throughout each soil core for dissolved organic carbon analysis (110 ± 24 g dry mass equivalent) and dry-mass calculations (~28 g each). The remaining core material was air-dried and separated into particles (>2 mm diameter) and soil (≤2 mm) using a U.S. Standard Test Sieve No. 10 (Fisherbrand, Pittsburg, PA, USA). The dry mass and volume of soil were used in calculations of gravimetric and volumetric soil moisture content, respectively (Gardner, 1986). Soil volume was calculated as the total core volume minus the volume of particles >2 mm diameter, with the latter determined by water displacement. Air-dried soil and sub-samples stored at -20 °C were sent to the Agricultural and Environmental Services Laboratory at the University of Georgia Extension in February 2016 for total C, N, and dissolved organic carbon (DOC). Samples were combusted in an oxygen atmosphere at 1350 °C, and measured for gaseous C and N using an Elementar Vario Max CNS (Langenselbold, Germany). DOC was measured using a Shimadzu 5000 TOC Analyzer (Columbia, Maryland, USA).

2.3 Data and statistical analysis

For each measurement of each sample throughout the 100-day incubation (i.e., each gas, core, and date/time), we used the rise in gas concentrations to calculate a flux rate in ppm s\textsuperscript{-1} (CO\textsubscript{2}) or ppb s\textsuperscript{-1} (CH\textsubscript{4}), a linear rate of change (δc/δt) based on the
concentration rise from a minimum (up to 10 seconds after measurement began) to a maximum (at 10-45 seconds). Each core's respiration flux ($F$) was then calculated as $F = \frac{\delta V P_a}{\delta M R}$ where $V$ is the core-specific system volume, $M$ the core dry mass as determined at the end of the incubation, $P_a$ atmospheric pressure (101 kPa; the incubation chambers were ~120 m a.s.l.), $R$ the universal gas constant (8.3 x 10$^{-3}$ m$^3$ kPa mol$^{-1}$ K$^{-1}$) and $T$ the chamber air temperature (K) at time of measurement. The final respiration rate was expressed on a soil C basis (µg or ng C g C$^{-1}$ day$^{-1}$).

Anomalous data were excluded based on their gas fluxes being more than 5 (for CO$_2$) or 10 (for CH$_4$) mean absolute deviations (Davies and Gather, 1993) from the treatment mean within a 10-day period, for a given treatment and temperature. We excluded 172 of 2686 (6.4%) measurements for this reason. If the coefficient of variability (CV) of fluxes from any core on a single day exceeded 140%, a value chosen based on the distribution of CVs across all cores, the entire core was excluded for that day (90 data points, 3.4%). Other data (4.8%) were removed because of known instrument problems, e.g. the analyzer was left running after leaving a chamber. The final number of valid flux samples from the 100-day incubation was 2198.

The effects of temperature, gravimetric water content, percent C, percent N, and DOC concentration on instantaneous gas fluxes were evaluated using a linear mixed-effects model fit by the R function lme in the R 'nlme' package, version 3.1.128. Because the dependent variable (CO$_2$ or CH$_4$ flux) was non-normally distributed, it was transformed using a natural-logarithm (+0.1 µg C g C$^{-1}$ day$^{-1}$) to ensure all
positive fluxes, following Treat et al. 2015) transformation. Soil core was treated as
a random effect in the model. We then performed stepwise model selection by
Akaike's information criterion (AIC) using the stepAIC function in the R 'MASS'
package, version 7.3.45. A linear mixed-effects model was also used to evaluate the
effect of treatment on core water content.

Cumulative respiration for each core and gas was calculated by linearly
interpolating flux rates between measurement dates and summing respired C over
the entire incubation. The effect of temperature and treatment (drought, controlled
drought, or field moisture conditions) on cumulative gas fluxes was evaluated with a
post-hoc Tukey Honest Significant Differences test. Temperature sensitivity (Q_{10})
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
cumulative gas fluxes (mg C g C^{-1}) at temperatures \( T_1 \) and \( T_2 \) (°C), respectively.

All data analysis and statistics were performed using R version 3.3.1 (2016-06-21)
(R Development Core Team, 2016). This experiment was run as an 'open
experiment' (Bond-Lamberty et al., 2016b) with all analysis code, data (from raw
instrument data to final summaries), diagnostics, etc., available at
https://github.com/bpbond/cpcrw_incubation. The summarized flux data backing
the main results have been archived under the Digital Object Identifier
The 30 experimental cores had a bulk density of 1.00 ± 0.18 (mean ± sd) g cm\(^{-3}\).

Large (>2 mm) particles, primarily schist, comprised 41% ± 11% of the cores' total mass. Soil (≤2 mm) dry mass was 886 ± 154 g. Sample DOC was 157.93 ± 55.74 mg kg\(^{-1}\). Carbon content was 1.20% ± 1.19%, while N content was 0.06% ± 0.06%. Mean C:N was 20.7. Neither temperature nor moisture treatment exerted any significant effect (P > 0.1 for all) on these highly variable properties (Table 1).

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

Carbon dioxide fluxes during the incubation ranged from 1.1 µg C g C\(^{-1}\) day\(^{-1}\) to a maximum of 5245.1 µg C g C\(^{-1}\) day\(^{-1}\), with a mean of 248.9 µg C g C\(^{-1}\) day\(^{-1}\) over the 100 days. CH\(_4\) rates ranged from 0.00 ng C g C\(^{-1}\) day\(^{-1}\) to a maximum of 1.31 ng C g C\(^{-1}\) day\(^{-1}\), with a mean of 0.06 ng C g C\(^{-1}\) day\(^{-1}\). These means conceal considerable variability over the course of the incubation (Table 1, Figures 2 and 3).
In the linear mixed-effects model (AIC = 2992.6), instantaneous CO$_2$ flux was positively correlated with incubation chamber temperature, core gravimetric water content, and percent soil N (all P < 0.05, and the latter two P < 0.001; Table 2). Temperature sensitivity decreased significantly (P < 0.001) over the course of the incubation, while moisture sensitivity was unaffected by time. Percent C and percent N were highly correlated (r = 0.99) for these cores. Because percent N was a slightly stronger predictor, it was retained in the model while percent C was excluded; cf. Colman and Schimel (2014). The interaction between water content and percent N was also highly significant (P < 0.001), although cores with N > 0.2% exhibited little relationship between water content and CO$_2$ flux (data not shown). Instantaneous CH$_4$ fluxes were positively correlated with percent N, while water content exhibited significant interactions with percent N and DOC as a predictor (Table A1). This model had little predictive power (AIC = -10879.2), however, and neither temperature nor water content was a significant first-order predictor of CH$_4$ fluxes.

The cumulative production of C from CO$_2$ (Figure 4) was over six order of magnitudes higher than that from CH$_4$, with CO$_2$:CH$_4$ C ratios ranging from 1.4 million in the 4 °C "Field moisture" treatment, to 6.2 million in the 20 °C "Field moisture" treatment. Cumulative CO$_2$ evolved was highly affected by temperature (P = 0.003), and "field moisture" cores emitted significantly more CO$_2$ than the other two moisture treatments at both temperatures (P < 0.001 for both, with no significant interactive effect). There was no difference between fluxes from the 20 °C "drought" and "controlled drought" treatments (P = 0.377). "Drought" cores’ cumulative production was 73% (4 °C) and 52% (20 °C) lower than the cores kept at...
field moisture. Neither temperature (P = 0.200) nor moisture treatment (mean P = 0.975) was a significant factor in predicting cumulative CH$_4$ fluxes.

The cumulative flux numbers above result in CO$_2$ temperature sensitivity ($Q_{10}$) values of 1.3 and 1.9 for the field moisture and drought treatments, respectively; the corresponding $Q_{10}$ values based on cumulative CH$_4$ were 1.2 and 1.3. Computing $Q_{10}$ values based on fluxes normalized by water filled pore space changed these values only slightly: to 1.2 and 1.7 for CO$_2$, for the field moisture and drought treatments respectively, and 1.1 and 1.2 for CH$_4$.

4 Discussion

Rises in boreal air temperatures, and unpredictable precipitation changes, will change fire disturbance regimes, warm and dry many soils, increase vegetation stress, degrade permafrost, and deepen the active layer (Schuur et al., 2015), all with uncertain consequences for soil dynamics and GHG fluxes. In this laboratory experiment we found that CO$_2$, but not CH$_4$, fluxes from these oxic active-layer mineral soils were sensitive to temperature and, in particular, moisture.

A number of studies have measured microbial respiration and GHG fluxes very close to our study site. Morishita et al. (2014) quantified GHG fluxes at CPCRW and nearby forests, and found CO$_2$ production to be correlated with both temperature and moisture in upland Cryosols, consistent with our results. Waldrop et al. (2010) incubated active-layer and permafrost soils from *Picea mariana* sites near Fairbanks, AK, observing aerobic $Q_{10}$ values of 9.0 (active layer) and 2.3 (permafrost) from -5 to 5 °C, and flux rates of 0.001-0.10 μmol CH$_4$ day$^{-1}$ g$^{-1}$
(~0.001-0.133 ng C g C^{-1} day^{-1}), and ~1-5 µg C-CO_2 hr^{-1} g^{-1} (~2000-10000 µg C g C^{-1} day^{-1}), considerably higher than the CO_2 rates observed here. During the first 100 days of an incubation of Fairbanks-area 0-10 cm mineral soils, Neff and Hooper (2002) observed fluxes of ~55-409 µg C-CO_2 g C^{-1} day^{-1}, in line with the results here, while Wickland and Neff (2008) reported that temperature and moisture exhibited interactive effects, of similar magnitude, on decomposition in P. mariana soils.

A number of synthesis studies have documented dynamics and C feedback potential of Arctic and boreal soils more generally; comparing to these results is useful because although the response of soil biota to stresses such as drought tends to differ between soil types, organisms, and vegetation, it is often broadly similar across biomes and climatic conditions (Manzoni et al., 2012). Using two meta-analyses of aerobic and anaerobic permafrost soil incubations, Schädel et al. (2016) showed that C release was highly sensitive to temperature, and that soils released far more (220-520%) C under aerobic conditions. Our incubation was fully aerobic, but its results are consistent with the conclusion that respiration in the form of CO_2 is likely to dominate the high latitude C feedback, and that aerobic soils, and the conditions under which currently waterlogged soils may drain, deserve particular attention. In terms of absolute flux rates, Treat et al. (2015) reported mean CO_2 rates of 47 (all mineral soils) and 101 (for 20-100 cm soils) µg C-CO_2 g C^{-1} day^{-1} from a pan-Arctic synthesis of anaerobic soil incubations, somewhat lower than our aerobic incubation results. Treat et al. (2014) also found CO_2 and CH_4 emissions to be strongly correlated with temperature and moisture based on an incubation of
Alaskan peats. Whether climate change makes northern regions wetter or drier is thus a critical factor affecting the quantity and form of C release.

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

We observed very low but positive CH$_4$ production from these upland mineral soils. This is contrast to many field studies that have observed CH$_4$ uptake (oxidation) in dry boreal sites (Matson et al., 2009; Schaufler et al., 2010). Anoxic microsites in soil can however provide enough CH$_4$ production to balance low-level consumption in otherwise aerobic soils (Kammann et al., 2009). In addition, our results are broadly consistent with data from 65 studies summarized by Olefeldt et al. (2013), who found that CH$_4$ emissions were more sensitive to soil temperature in wetter ecosystems; it would have been a surprise if the little methanogenic activity in our
upland, well-drained soils was temperature-sensitive at all. Methane was also a far smaller C flux than CO₂ from these soils, in particular at higher temperatures (as CO₂ was responsive to temperature, but CH₄ was not). This is true more generally: for example, Treat et al. found a median CO₂:CH₄ production ratio of 387 for anaerobic incubations of boreal soils. This is naturally far lower than our observed aerobic (and thus high-CO₂) ratios, but nonetheless consistent with them. Thus we see little opportunity for CH₄ to be a significant contributor to these upland soils' C fluxes and climate feedback risk, even accounting for the 25x stronger radiative forcing of this gas over a 100-year time horizon (Lee et al., 2012).

4.1 Temperature versus moisture sensitivity for cumulative emissions

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

The Q₁₀ values observed in this experiment were low (all less than 2.0, even when controlling for changes in soil moisture). Temperature sensitivities of ~2 are more
typical (Dutta et al., 2006; Schädel et al., 2016), although the temperature sensitivity
of C release can change over time of incubation (Dutta et al., 2006) and vary
between soil fractions cycling over different time horizons (Karhu et al., 2010;
Schädel et al., 2014). Observed surface CO$_2$ fluxes at this CPC RW site exhibited a $Q_{10}$
of 5.1 ± 1.4 over a temperature range of 3.5-15 °C (personal communication, C.
Anderson); these surface fluxes were measured over multiple months and include
root respiration, however, preventing any direct comparison. While increased
temperature does not always drive C mineralization rates in forest mineral soils
(Giardina and Ryan, 2000), it is linked with increases in soil moisture content can
lead to changes in microbial community structure and GHG fluxes (Xue et al., 2016).

Interestingly, $Q_{10}$ values were lower in the drought treatment cores, a mathematical
corollary of the fact that drought restricted CO$_2$ respiration more at 4 °C than at
20 °C. There is evidence that climate warming changes the microbial decay
dynamics of soil organic C compounds generally considered to be stable (Frey et al.,
2013; Bond-Lamberty et al., 2016a). Conditions such as drought can change the
amount and quality of DOC available to microbes (1999), but we observed no DOC
changes between treatments here. Deep active layer soils store large quantities of
soil C (Mueller et al., 2015) but are not subject to abundant inputs of fresh C from
vegetation, so the starting quality of the native soil C in active layer soils is older,
more microbially processed, and dominated by more stable "heavy" organic C
(Karlsson et al., 2011). Thus, it may not be surprising that these more stable C
compounds would be metabolized by processes that have been reported to be less
temperature-sensitive.
4.2 Soil nitrogen

Somewhat unexpectedly, percent soil N was very significantly and positively correlated with both CO$_2$ and CH$_4$ fluxes (Tables 2 and 3). Nitrogen interacts with microbial respiration via a number of complex, interactive, and still unclear mechanisms (Luo and Zhou, 2006), including reductions in belowground plant allocation, shifts in energy source or population of the saprotrophic community (Saiya-Cork et al., 2002) that leave it less capable of decomposing recalcitrant compounds, and perhaps abiotic stabilization mechanisms (Janssens et al., 2010).

Meta-analyses have generally shown negative to neutral effects of N deposition on microbial biomass (Treseder, 2008) and respiration (Ramirez et al., 2012), and total soil respiration across ecosystems and biomes (Janssens et al., 2010; Zhou et al., 2014). These effect are likely due to several one or more mechanisms involving soil pH, ligninase enzymes, and phenol oxidase activity (Luo and Zhou, 2006), and incubation results examining N effects can be highly variable (Lavoie et al., 2011; Sistla et al., 2012). Some studies have however observed positive correlations between ambient soil N and microbial respiration. For example, Weiss et al. (2015) found CO$_2$ production from Siberian Yedoma permafrost samples to be correlated with both percent C and N, consistent with our active-layer results (Table 2).

The C:N ratio was not a significant predictor of GHG fluxes in this study, although this ratio has been found to be important in meta-analyses (Sistla et al., 2012; Schädel et al., 2014). In situ respiration rates have also been shown to be negatively correlated with C:N at large spatial scales (Allaire et al., 2012). Percent C and N both
varied widely in our soil cores (Table 1), and were highly correlated with each other, even though the cores were collected within tens of meters of each other. This suggests that active-layer SOC response to temperature and moisture may also be highly spatially variable, even in a mixed-species boreal forest *that we expected, a priori*, to provide spatial variation in litter and SOC quality (Fierer et al., 2005).

Spatially explicit analyses of soil biochemistry, temperatures (Bond-Lamberty et al., 2005), and respiration (Allaire et al., 2012) are likely necessary to accurately constrain and predict soil fluxes in this ecosystem.

### 4.3 Limitations and weaknesses

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

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

The soils studied here were from an upland, mixed conifer-deciduous boreal forest, and care needs to be taken before drawing regional inferences, or about other
ecosystem types. We focused on an experimental drought, rather than flooding, because of the well-drained nature of the field site: it is unlikely that the mid-slope forest we sampled in will ever suffer from thermokarst or excessive soil moisture, but too-dry conditions are a serious possibility in this relatively low-precipitation ecosystem (Barber et al., 2000).

Finally, the soils here are not surface layer soils (where the majority of microbial activity and C mineralization of labile C takes place); removing them from in situ conditions (where they are less exposed to O$_2$, for example) may significantly change the abiotic conditions to which the microbial community is adapted. However, focusing on the active layer provides crucial information about the potential loss of C from these soils, a risk that needs to be well understood as permafrost degradation leads to expansions in the depth of the active layer across the Arctic.

5 Conclusions

In this laboratory experiment, we found that CO$_2$ fluxes were strongly influenced by temperature and water content, and correlated with soil C and N, while CH$_4$ fluxes were much smaller and not sensitive to temperature or water content in these well-drained mineral soils. These results add to a growing body of Arctic permafrost and active layer incubation literature, and underscore the importance of understanding moisture effects on CO$_2$ fluxes in particular. How soil moisture might change with spatially variable permafrost degradation, how soil biota will respond to these changes, and how models should treat soil organic matter decomposition with
respect to multiple and interacting drivers are all critical areas of research going
forward. Further controlled field and laboratory studies, ideally tightly integrated
with modeling experiments, are important to understand GHG emission dynamics
from high-latitude soils.

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Author contributions

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

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Table 1. Summary of dissolved organic carbon (DOC), percent C, percent N, bulk density (BD), and CO$_2$ and CH$_4$ fluxes by treatment. The "Field moisture" and "Drought" columns summarize (mean ± s.d.) 12 cores, combining two groups of N=6 at each incubation temperature, while the "Controlled drought" and "Pre-incubation" columns are N=6.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Field moisture</th>
<th>Controlled drought</th>
<th>Drought</th>
<th>Pre-incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOC (mg kg$^{-1}$)</td>
<td>173.62 ± 46.67</td>
<td>165.68 ± 66.46</td>
<td>154.60 ± 57.15</td>
<td>125.43 ± 49.07</td>
</tr>
<tr>
<td>C (%)</td>
<td>1.67 ± 1.60</td>
<td>0.87 ± 0.50</td>
<td>0.76 ± 0.60</td>
<td>1.44 ± 1.32</td>
</tr>
<tr>
<td>N (%)</td>
<td>0.08 ± 0.08</td>
<td>0.04 ± 0.03</td>
<td>0.03 ± 0.03</td>
<td>0.07 ± 0.06</td>
</tr>
<tr>
<td>BD (g cm$^{-3}$)</td>
<td>0.89 ± 0.18</td>
<td>1.06 ± 0.17</td>
<td>1.08 ± 0.14</td>
<td>1.13 ± 0.29</td>
</tr>
<tr>
<td>CO$_2$ (µg C g C$^{-1}$ day$^{-1}$)</td>
<td>456.40 ± 543.91</td>
<td>159.77 ± 116.41</td>
<td>97.03 ± 96.38</td>
<td>-</td>
</tr>
<tr>
<td>CH$_4$ (ng C g C$^{-1}$ day$^{-1}$)</td>
<td>0.10 ± 0.00</td>
<td>0.10 ± 0.00</td>
<td>0.10 ± 0.00</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 2. Linear mixed-effects model parameters, testing effects of temperature (°C), gravimetric water content (unitless), soil C (%), soil N (%), and dissolved organic carbon (mg kg\(^{-1}\)) on individual core CO\(_2\) fluxes (+0.1 µg C g C\(^{-1}\) day\(^{-1}\)); a colon ("::") indicates an interaction. Dependent variable has units of log(µg C g C\(^{-1}\) day\(^{-1}\)). Columns include parameter value; standard error (SE); degrees of freedom (DF); T statistic; and P value.

<table>
<thead>
<tr>
<th>Parameter</th>
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<th>SE</th>
<th>DF</th>
<th>T</th>
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<tr>
<td>(Intercept)</td>
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<td>0.354</td>
<td>1153</td>
<td>4.839</td>
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<td>WC_gravimetric</td>
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Figure 1. Core water content across the course of the incubation experiment by temperature (left panel 4 °C, right panel 20 °C) and treatment.
Figure 2. Mass-normalized CO$_2$ fluxes over the 100-day incubation, by temperature (4 and 20 °C, rows) and treatment (field moisture, drought, and controlled drought; columns). Error bars show core-to-core standard deviation. The "controlled drought" treatment, for 20 °C only, was meant to dry cores at roughly the same rate as the drought cores at 4 °C.
Figure 3. Mass-normalized CH$_4$ fluxes over the 100-day incubation, by temperature (4 and 20 °C, rows) and treatment (field moisture, drought, and controlled drought; columns). Error bars show core-to-core standard deviation. The "controlled drought" treatment, for 20 °C only, was meant to dry cores at roughly the same rate as the drought cores at 4 °C.
Figure 4. Cumulative mass-normalized C fluxes (mg g C^{-1}) over the incubation, by gas (CO₂ and CH₄, top and bottom panels respectively), treatment (columns), and temperatures (x-axis, °C). Letters within a panel indicate significant differences based on Tukey’s HSD.
Table A1. Linear mixed-effects model parameters, testing effects of temperature (°C), gravimetric water content (unitless), soil N (%), and dissolved organic carbon (DOC, mg kg$^{-1}$) on log-transformed, individual core CH$_4$ fluxes (+0.1 µg C g C$^{-1}$ day$^{-1}$); a colon (":") indicates an interaction. Dependent variable has units of log(µg C g C$^{-1}$ day$^{-1}$). Columns include parameter value; standard error (SE); degrees of freedom (DF); T statistic; and P value.

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