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1 **Constraints on potential enzyme activities in thermokarst bogs: implications for the carbon**  
2 **balance of peatlands following thaw**

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18 **Abstract**

19 Northern peatlands store a globally significant amount of soil organic carbon, much of it found in  
20 rapidly thawing permafrost. Permafrost thaw in peatlands often leads to the development and  
21 expansion of thermokarst bogs, where microbial activity will determine the stability of the  
22 carbon storage and the release of greenhouse gases. In this study we compared potential enzyme  
23 activities between young (thawed ~30 years ago) and mature (~200 years) thermokarst bogs, for  
24 both shallow and deep peat layers. We found very low potential enzyme activities in deep peat  
25 layers, with no differences between the young and mature bogs. Peat quality at depth was found  
26 to be highly humified (FTIR analysis) in both the young and mature bogs. This suggests that  
27 deep, old peat was largely stable following permafrost thaw, without a rapid pulse of  
28 decomposition during the young bog stage. For near-surface peat, we found significantly higher  
29 potential enzyme activities in the young bog than in the mature – associated with differences in  
30 peat quality derived from different *Sphagnum* species. A lab incubation of near-surface peat  
31 showed that differences in potential enzyme activity was primarily influenced by peat type rather  
32 than oxygen availability. This suggested that the young bog can have higher rates of near-surface  
33 decomposition despite being substantially wetter than the mature bog. Overall, our study shows  
34 that peat chemistry is the dominant constraint on potential enzyme activity and that peatland site  
35 development (successional pathways and permafrost history) through its influence on peat type  
36 and chemistry is likely to determine peat decomposition following permafrost thaw.

37 **Keywords**

38 Permafrost, soil organic carbon, peatland, thermokarst, soil enzyme activities, organic matter  
39 quality, peat pore-water

40

## 41 **1. Introduction**

42 The northern permafrost region contains almost half of the global terrestrial soil organic carbon  
43 (C), and roughly a third of this ~1,300 Pg C store is found within the ~20% areal extent covered  
44 by peatlands (Hugelius et al., 2014; Schuur et al., 2015). Frozen, cold, and waterlogged  
45 conditions (Moore and Basiliko 2006; Jassey et al. 2016), coupled with the production of  
46 recalcitrant litter and the release of antimicrobial compounds by *Sphagnum* mosses (Chiapusio et  
47 al., 2018; Fudyma et al., 2019), limits microbial activity and protects this large C store. As higher  
48 latitude air temperatures rise at twice the speed of the global average (Mudryk et al., 2018)  
49 increased rates of permafrost thaw is leading to widespread thermokarst development (Kokelj &  
50 Jorgenson, 2013), particularly in high C density peatlands (Olefeldt et al., 2016). Thermokarst  
51 formation in ice-rich peatlands leads to ground subsidence, surface inundation, and an abrupt  
52 shift in vegetation communities (Beilman, 2001; Camill, 1999). This exposes vast stores of  
53 previously frozen C to microbial activity, potentially causing a positive feedback to climate  
54 warming through the release of greenhouse gases (Schuur et al., 2008; Tarnocai, 1999).

55 Previous studies using a space-for-time permafrost thaw chronosequence approach in peatlands  
56 to estimate the change in C stores following thaw have indicated both large (Jones et al., 2017)  
57 and significantly lower losses of C (Heffernan et al., 2020). However, the precision of this  
58 approach is often insufficient to accurately determine the net change in C stocks following thaw.  
59 Direct approaches measuring the impacts of thawing on the net C balance using eddy-covariance  
60 (Helbig, Chasmer, Desai, et al., 2017) or chamber based (Wickland et al., 2006) flux  
61 measurements are also limited due to spatial and temporal variability in the response of fluxes to  
62 environmental drivers (Myers-Smith et al., 2007). Also, inter-annual variability in greenhouse

63 gas fluxes often obscures long-term net ecosystem C balance trends in both permafrost-affected  
64 and non-permafrost peatlands (Euskirchen et al, 2014; Roulet et al., 2007). Heterotrophic  
65 respiration and organic matter decomposition responsible for greenhouse gas fluxes and the  
66 reduction of C stores following thaw is a result of microbial activity (Ernakovich et al., 2017;  
67 Voigt et al., 2019). Comparing microbial activity between areas that have thawed both recently  
68 and centuries ago may provide an indication of the long-term stability of these vast, previously  
69 frozen C stores. It is currently unknown to what degree permafrost thaw will impact the  
70 microbial activity that drives these surface greenhouse gas fluxes and net changes in C stocks.

71 Peat decomposition is governed directly by the impact of abiotic (water table position,  
72 temperature and oxygen availability), and biotic (vegetation community, litter inputs and  
73 substrate quality) factors, and indirectly by the interactions between these abiotic and biotic  
74 factors (Laiho, 2006). The water table position determines oxygen availability for respiration,  
75 with anoxic conditions often being found 1 – 3 cm beneath the water table (Blodau et al., 2004).  
76 Water table position influences the structure of the vegetation community (Robroek et al., 2007)  
77 which in turn impacts the quality of litter inputs (Laiho et al., 2003) and root exudates (Robroek  
78 et al., 2016). Litter type and its chemical composition are important controls on microbial  
79 decomposition and C mineralization into CO<sub>2</sub> and CH<sub>4</sub>, particularly that derived from *Sphagnum*  
80 spp. (Limpens et al., 2017; Sjögersten et al., 2016; Strakova et al., 2011). Litter and plant-root  
81 exudate inputs regulate the composition and activity of the microbial community as they provide  
82 the substrate and nutrients used as an energy source (Borga et al., 1994). The activity of  
83 extracellular enzymes produced by the microbial community control the rate limiting step of  
84 decomposition and at which solubilized substrates become available for microbial metabolism  
85 (Bell et al., 2013; Luo et al., 2017). The microbial community's activity is determined by the

86 sensitivity of these extracellular enzymes to the water table position (Jassey et al. 2018) and its  
87 influence on peat substrate and nutrient availability (Briones et al., 2014; Sinsabaugh et al.,  
88 2008). However, whether variations in water table position and oxygen availability or plant litter  
89 inputs is of greater importance to extracellular activity remains poorly known.

90 Drastic shifts in water table position, soil temperatures, and litter inputs, particularly in the initial  
91 decades following permafrost thaw, may have cascading effects on the microbial community  
92 structure and activity, potentially driving deep, old C losses and impacting the long-term stability  
93 of permafrost peatland C (Bragazza et al., 2015; Walker et al., 2016). A number of studies have  
94 addressed the acceleration of microbial activity due to climate warming causing a positive  
95 feedback to climate change (Allison et al., 2010; Frey et al., 2013; Walker et al., 2018). Recent  
96 modelling efforts predict that increased thaw is leaving potentially labile permafrost C vulnerable  
97 to enhanced mineralization and greenhouse gas emissions (Lawrence et al., 2015; Schaefer et al.,  
98 2014; Turetsky et al., 2020). However, current attempts lack mechanistic information on the  
99 contribution of various processes, such as the influence of shifting water table position and  
100 vegetation communities, i.e., ecological peatland succession, on the microbial decomposition of  
101 previously frozen permafrost C, to future C balances. Such understanding and input are urgently  
102 needed to predict future trajectories of permafrost C under climate change and improve global  
103 climate models.

104 Here, we assess the vulnerability of deep, previously frozen C to enhanced microbial  
105 decomposition and determine the main drivers of enzymatic activity along a post-permafrost  
106 thaw peatland successional gradient. To examine both the initial and long-term response of  
107 microbial decomposition to permafrost thaw and peatland succession we measured potential  
108 enzymatic activities along a space-for-time chronosequence in areas that thawed ~30 and ~200

109 years ago. In both areas we measured activity on shallow near-surface peat that accumulated  
110 post-permafrost thaw and deep peat that accumulated pre-permafrost thaw. This allowed us to  
111 determine whether previously frozen peat demonstrated evidence of elevated potential enzyme  
112 activities during the early stages of thaw that would be an indicator of rapid peat decomposition.  
113 We also used a laboratory-based peat mesocosms experiment on surface peat to evaluate whether  
114 water table position or peat type was the main control on enzyme activity and thus important  
115 constraints on the rate of new peat accumulation. We hypothesize that; (1) deep, previously  
116 frozen peat is more vulnerable to enhanced microbial decomposition in the early stages of thaw  
117 rather than following centuries of thaw due to higher substrate quality and soil temperatures, and  
118 (2) surface inundation and reduced oxygen availability in the early stages of thaw leads to lower  
119 rates of microbial decomposition on shallow peat than in the area where thaw occurred centuries  
120 ago, despite intrinsically more labile new peat substrate, leading to rapid new peat accumulation  
121 in the initial decades following thaw (Heffernan et al., 2020; Turetsky et al., 2007). To link these  
122 hypotheses, we built a path diagram of causal relationships subjected to structural equation  
123 modelling to show the importance of litter inputs and peat lability for microbial decomposition  
124 and the stability of previously frozen C following permafrost thaw.

125

## 126 **2. Methods**

### 127 *2.1 Study site*

128 This research was carried out at a permafrost peatland complex in the discontinuous permafrost  
129 zone of western Canada (59.5°N, 117.2°W) (Brown et al., 1997; Heginbottom et al., 1995)  
130 (Figure 1a). The site has a continental climate with an average annual rainfall of 391 mm and an

131 average annual temperature of  $-1.8^{\circ}\text{C}$ , as measured at a station 50 km south of the site (Climate-  
132 Data.org, 2019). Permafrost peatland complexes cover  $>25\%$  of the region (Hugelius et al., 2014;  
133 Vitt et al., 2000) and are a mix of permafrost-affected peat plateaus interspersed with non-  
134 permafrost thermokarst bogs, channel fens, and ponds (Quinton et al., 2011). Permafrost here is  
135 warm (near  $0^{\circ}\text{C}$ ), fragmented, and susceptible to thaw (Smith et al., 2005, 2009) and complete  
136 permafrost loss is predicted to occur over the next  $\sim 50$  years (Chasmer and Hopkinson, 2017).

137 Organic matter began accumulating in the studied peatland complex  $\sim 8,800$  years ago, and the  
138 site developed over time from a marsh, through a fen stage and a bog stage prior to permafrost  
139 aggradation (Heffernan et al., 2020). The site, including a peat plateau and thermokarst bog,  
140 currently has a 5 – 6 m peat profile that exhibits synchronous transition through these multiple  
141 developmental stages up until the timing of permafrost thaw. Hereafter, old peat that  
142 accumulated prior to this permafrost thaw is referred to as deep peat, while peat that accumulated  
143 after permafrost thaw and thermokarst bog development is referred to as shallow peat.

144 We established a transect within a thermokarst bog perpendicular to an adjacent a raised (1 – 2  
145 m) permafrost peat plateau. The thermokarst bog has a young and mature bog stage transitioning  
146 away from the thawing edge of the peat plateau, and these stages are differentiated with regards  
147 to the vegetation community and water table position (Figure 1b). The young bog thawed most  
148 recently,  $\sim 30$  years ago, and is directly adjacent to the thawing edge, whereas the mature bog  
149 thawed  $\sim 200$  years ago and is  $>10$  m from the thawing edge (Heffernan et al., 2020). The depth  
150 of shallow peat in the young bog and mature bog is 30 cm and 70 cm, respectively. The timing of  
151 this transition, and depth at which is currently found, was determined by  $^{14}\text{C}$  dating and  
152 macrofossil analysis (Heffernan et al., 2020). The young bog surface is saturated with an average  
153 growing season water table depth of  $1.1 \pm 3.5$  cm below the peat surface and its vegetation

154 community is dominated by hydrophilic species such as *Sphagnum riparium*, rannoch rush  
155 (*Scheuchzeria palustris*), and bog-sedge (*Carex limosa*) (Figure 1c). The drier mature bog has an  
156 average growing season water table depth of  $23.5 \pm 3.9$  below the peat surface and its vegetation  
157 community consists of *Sphagnum fuscum*, *Sphagnum magellanicum*, leather leaf  
158 (*Chamaedaphne calyculata*), bog rosemary (*Andromeda polifolia*), and Labrador tea  
159 (*Rhododendron groenlandicum*) (Figure 1d).

## 160 2.2 Field sampling

161 To assess seasonal variations in potential enzyme activities and peat chemistry depth profiles, we  
162 extracted peat cores from a 1.5 m  $\times$  3 m area in both the young and mature bog once a month  
163 from May – September 2018 to a depth of 160 cm. Surface cores for the top 0 – 30 cm were  
164 extracted manually using a cutting tool. Below 30 cm we used a Russian peat corer (4.5 cm  
165 inner-diameter, Eijkelkamp, Giesbeek, Netherlands) to extract cores from two boreholes located  
166 ~20 cm apart, alternating between both in 50 cm long sections with a 10 cm overlap. Each core  
167 section was then placed in a halved PVC tube and divided into 15 segments, with the first two  
168 segments including 0 – 5 cm and 5 – 10 cm, followed by 10 cm increments down to 120 cm, and  
169 two further segments from 130 – 140 cm and 150 – 160 cm. A total of 132 peat samples were  
170 extracted over the study period. Each peat sample segment was then placed in zipper plastic  
171 storage bags and transported back to the laboratory in a cooled container. Peat samples were  
172 stored at 4°C for less than one week in the laboratory before homogenization and analysis.

173 We categorized the 15 peat measurement depths into deep peat that accumulated pre-thaw and  
174 shallow peat that accumulated post-thaw. This resulted in four categories of peat layer based on  
175 whether the core was taken from either the young or mature bog, and whether the peat from each  
176 was from a shallow or deep peat depth. These four categories of peat layer are hereafter referred

177 to as (a) YB Shallow (young bog; depth 0 – 30 cm), YB Deep (young bog; depth 30 – 160 cm),  
178 MB Shallow (mature bog; depth 0 – 70 cm), MB Deep (mature bog; depth 70 – 160 cm). All  
179 measured extracellular enzyme activities, peat pore water chemistry, and environmental variables  
180 are presented in each of these four peat layer categories.

181 Peat pore water samples were taken at all 15 peat sampling depths mentioned above from pre-  
182 installed pore water peepers. Custom made plexiglass pore-water peepers that connected sample  
183 depths to the surface via silicone tubing allowed for repeated non-destructive retrieval of pore-  
184 water samples from the peat column. Sampling was done by applying a vacuum at the surface  
185 and collecting water with syringes via a three-way stopcock. Pore-water peepers were installed  
186 ~2 m from the peat coring location, in areas with similar vegetation, water table depth, and  
187 distance from the thawing edge to the coring location in both the young and mature bog. Three  
188 60 ml pore water samples were taken at the same time as peat extraction and each water sample  
189 was filtered immediately through 0.7  $\mu\text{m}$  pore size glass fiber filters (GF/F Whatman) into acid-  
190 washed amber glass bottles. One of the 60 ml samples was acidified with 0.6 mL 2N HCl to  
191 prevent further microbial activity during transport back to the laboratory. Pore water samples  
192 were transported in a cooled container and stored at 4°C prior to analysis.

193 We used Fourier transform infrared (FTIR) spectroscopy from peat elemental analysis to  
194 determine peat humification on peat samples collected from cores in 2015 (Heffernan et al.,  
195 2020). Peat coring locations for both FTIR and enzyme analysis had similar vegetation, water  
196 table depths, and distance from the thawing edge, and were located ~2 – 3 m apart in both the  
197 young and mature bog. We analyzed samples for FTIR using an Agilent 660 FT-IR spectrometer  
198 (Agilent Technologies Inc., Santa Clara, CA, USA) from 8 and 10 depths in young and mature  
199 bog respectively. Peak height ratios were calculated for absorption at ~1630  $\text{cm}^{-1}$  and 1090  $\text{cm}^{-1}$

200 to determine the abundance of aromatic and carboxylate moieties relative to polysaccharides  
201 (Artz et al., 2008; Broder et al., 2012), and higher values represent higher humification, i.e.  
202 degree of peat decomposition.

203 Continuous bi-hourly measurements of soil temperature ( $^{\circ}\text{C}$ ) at 5, 10, 40, 100, and 150 cm were  
204 taken using installed Hobo 8k Pendant (Onset Computer, Bourne, MA, USA) loggers in all bog  
205 stages throughout the measurement period.

### 206 *2.3 Pore water analyses*

207 Pore water pH was measured on filtered samples in the field using a ProfiLine pH 3310 meter  
208 (Xylem Analytics, Germany). We determined the concentrations of phosphate ( $\text{PO}_4^{3-}$ ;  $\mu\text{g L}^{-1}$ )  
209 from filtered but not acidified pore water using colorimetric methods on a SmartChem<sup>®</sup> 200  
210 discrete wet chemistry analyzer (Westco Scientific, Milford, MA, USA).

211 Dissolved organic carbon (DOC) and total dissolved nitrogen (TN) concentrations were  
212 determined from acidified pore water samples using the combustion catalyst method on a TOC-L  
213 combustion analyser with a TNM-L module (Shimadzu, Kyoto, Japan). We quantified the  
214 phenolic contents in a 3.5 mL aliquot of filtered sample using a Folin-Ciocalteu assay and  
215 standard curves based on gallic acid equivalent (GAE) (Jassey et al., 2011). Absorbance of each  
216 sample was measured at 760 nm ( $A_{760}$ ) wavelength on a Synergy<sup>™</sup> HT spectrophotometer  
217 (Biotek, Winooski, VT, USA) and results were expressed as GAE per litre of peat pore water  
218 ( $\text{GAE mg L}^{-1}$ ).

219 We measured the spectra of UV-vis absorbance, between 230 and 600 nm, to assess relative  
220 aromatic content and average molecular weight using the specific UV absorbance at 254 nm  
221 ( $\text{SUVA, L mg C}^{-1} \text{ m}^{-1}$ ) and spectral slope between 250 – 465 nm ( $S_{250-465, \text{ nm}^{-1}}$ ) respectively. A

222 4 mL aliquot of filtered sample was transferred to a 1 cm path-length quartz cuvette and analysed  
223 using a Flame-DA-CUV-UV-VIS light source and Flame-S spectrophotometer (Ocean Optics,  
224 Dunedin, FL, USA). The decadal absorbance at 254 nm ( $\text{cm}^{-1}$ ) was divided with DOC  
225 concentration ( $\text{mg C L}^{-1}$ ) and multiplied the value by 100 to calculate SUVA (Weishaar et al.,  
226 2003). Spectral slopes ( $S_{250-465}$ ) were estimated using a linear fit of the log-transformed  
227 absorption spectra between 250 – 465 nm. High SUVA values indicate a high aromatic content  
228 while high  $S_{250-465}$  values indicate a low average molecular weight or decreasing aromaticity  
229 (Hansen et al., 2016).

#### 230 *2.4 Enzyme activity assays*

231 We performed hydrolytic enzyme assays for four enzymes; phosphatase (PHOS),  $\beta$ -N-  
232 glucosaminidase (NAG),  $\beta$ -glucosidase (BG), and  $\beta$ -cellobiosidase (CB) using fluorogenic 4-  
233 methylumbelliferone labelled substrates (Dunn et al., 2014). These enzymes are involved in the  
234 degradation of carbohydrates and polysaccharides (carbon degradation; BG and CB), the  
235 mineralization of nitrogen from chitin and proteins (NAG), and phosphorus mineralization  
236 (PHOS) (Machmuller et al., 2016). We assayed oxidative enzyme activity by measuring laccase  
237 (LAC) activity using syringaldazine (Criquet et al., 2000; Jassej et al., 2012).

238 We extracted enzymes by adding 5 g of homogenised wet peat to 50 mL 0.1 M  $\text{CaCl}_2$ , 0.5 mL  
239 Tween 80 and 20 g polyvinylpyrrolidone and shaking at room temperature for 90 minutes  
240 (Criquet et al., 1999). This mixture was then centrifuged, and the supernatant was filtered  
241 through 0.2  $\mu\text{m}$  pore size glass fiber filters (GF/C Whatman). This filtrate was then placed inside  
242 cellulose dialysis tubing (10 kDa molecular mass cut-off) and covered with polyethylene glycol  
243 to concentrate the filtrate. Concentrated extracts were brought back to 40% of their initial  
244 volume by adding 20 ml of phosphate buffer (pH 5.6) and separated into two equal fractions.

245 One of these fractions was then stored at 4°C overnight and contained the active enzymatic  
246 extract, whereas the other fraction was boiled for 3 hours at 90°C and was used as the control  
247 assay. Hydrolytic enzyme activities were measured spectrophotometrically at 365 nm excitation  
248 and 450 nm emission in black 96 well microplates. Plates had 250 µL of enzymatic extract or  
249 control and 38 µL of enzymatic substrate added to each well and were then incubated for 3 hours  
250 at 25°C. Phenol oxidases were measured in clear 96 well plates. Each well contained 150 µL of  
251 enzyme extract with 2 µL of LAC (5 mM;  $\epsilon^M = 65,000 \text{ M}^{-1} \text{ cm}^{-1}$ ) and was monitored at 525 nm  
252 (Jassey et al., 2012; Criquet et al., 2000). To determine dry weight, peat material was dried at  
253 100°C for 24 hours. Hydrolytic enzyme activities were expressed as nanomole per gram of dry  
254 soil per hour ( $\text{nmol g}^{-1} \text{ hour}^{-1}$ ) and as micromole per gram of dry soil per hour ( $\mu \text{mol g}^{-1} \text{ hour}^{-1}$ )  
255 for oxidative enzyme activity.

### 256 *2.5 Peat mesocosms*

257 Six near-surface, shallow peat mesocosms were extracted in September 2018 from both the  
258 young and mature bog. Mesocosms were extracted as a single section to a depth of  $23 \pm 1$  cm  
259 using cutting tools and placed inside  $25 \times 20$  cm plastic buckets (Figure 1e). Care was taken  
260 when extracting each mesocosm to minimize damage to the vegetation. Mesocosms were then  
261 transported to the laboratory and stored out of direct sunlight and at room temperature (19 –  
262 23°C). All mesocosms were subsequently completely saturated with distilled water and  
263 waterlogged conditions were maintained by adding distilled water every 1 – 2 days for 4 months  
264 to develop and maintain anoxic conditions. We tested the dissolved oxygen concentration (%),  
265 temperature (°C), and pH of pore water at 5, 10, 15, and 20 cm depth in each mesocosm prior to  
266 draining. We consider pore water that has a dissolved oxygen concentration lower than 10% to  
267 be anoxic. After four months, three young bog and three mature bog mesocosms were drained.

268 To drain, multiple holes were drilled on the bottom and sides of each plastic mesocosm and left  
269 to drain for 72 hours. We then measured the dissolved oxygen, temperature, and pH of each  
270 mesocosm. This resulted in four groups of peat mesocosm conditions; (1) young bog peat under  
271 anoxic condition; YB Anoxic, (2) young bog peat under oxic conditions; YB Oxic, (3) mature  
272 bog peat under anoxic condition; MB Anoxic, (4) mature bog peat under oxic conditions; MB  
273 Oxic.

274 We extracted peat samples at 0 – 5 cm, 5 – 10 cm, and 10 – 20 cm in each mesocosm using  
275 cutting tools 96 hours after draining. A total of 36 peat samples were collected, nine samples  
276 from each peat mesocosm group, and stored at 4°C before homogenization and analysis. Assays  
277 for enzyme activity were carried out on all 36 mesocosm peat samples as described for field  
278 samples above. Peat pore water samples were collected at 5, 10, and 15 cm in every peat  
279 mesocosm. Pore-water from drained mesocosms was first squeezed out of peat into clean glass  
280 beakers and sampled from the beakers. These pore water samples were treated the same as pore  
281 water samples collected from the field (as described above) and used to determine phenolic  
282 concentrations, pH, DOC concentrations, SUVA, and  $S_{250 - 465}$ .

### 283 *2.6 Statistical analyses*

284 We performed ANOVAs and Bonferroni post-hoc tests on linear mixed effects models to test for  
285 significant seasonal trends and evaluate differences between the four different peat layers (YB  
286 Shallow, YB Deep, MB Shallow, MB Deep) in; a) site hydrology and pore water chemistry and  
287 b) enzymatic activity. In all models, sampling month and peat layer were included as fixed  
288 effects and sample depth was included as a random effect. We summarized the activity of all  
289 hydrolytic enzymes in the field study using a multifunctionality index based on z-scores  
290 (MultEnz<sub>Field</sub>) (Allan et al., 2015). This enzyme activity proxy summarizes the activity of

291 multiple enzymes so that high enzyme multifunctionality values indicate high activity of many,  
292 but not necessarily all, measured hydrolytic enzymes.

293 To evaluate the drivers of enzyme activity in field samples redundancy analysis (RDA) was  
294 applied to log-transformed and standardized enzymatic data using biotic and abiotic  
295 environmental data as explanatory variables. The selection of explanatory variables used were  
296 determined using automatic stepwise model selection permutation tests, that included 1,000  
297 permutations, and Akaike Information Criterion (AIC). The significance of our RDA model and  
298 each explanatory variable was tested using 1,000 permutations (Jassey et al., 2011).

299 Additionally, we performed variance partitioning using RDA and adjusted  $R^2$  to compare the  
300 individual effect of each explanatory variable on enzyme activity (Peres-Neto et al., 2006). The  
301 influence of peat quality on field enzyme activity measurements was tested using a linear  
302 regression of the response of enzyme activity to the peat humification index (FTIR).

303 Using the same approach as used for field data, peat mesocosm hydrolytic enzyme activities  
304 were also summarized to calculate a hydrolytic enzyme multifunctionality estimate  
305 ( $\text{MultEnz}_{\text{Meso}}$ ). To test for differences in  $\text{Meso}_{\text{enz}}$ , and individual potential enzyme activities,  
306 between mesocosms we performed ANOVAs and Bonferroni post-hoc tests on linear mixed  
307 effects models. In all model's oxic/anoxic conditions and peat type were included as fixed effects  
308 and mesocosm was included as a random effect. Furthermore, we built a path diagram of causal  
309 relationships subjected to structural equation modeling (SEM; Grace et al., 2014) to investigate  
310 the influence of peat type, pore-water quality, and oxidative enzyme activity on  $\text{Meso}_{\text{enz}}$  in our  
311 mesocosms. We first built *a priori* conceptual model among our descriptors (Figure S1. See  
312 Table S1 for detailed hypotheses). Second, we determined the main explanatory variables that  
313 were the drivers of  $\text{Meso}_{\text{enz}}$  in our SEM was based on step-wise selection of variables guided by

314 AIC scores (Jassey et al., 2018). The adequacy of our model was tested using AIC values and  
315 goodness of fit index (CFI), including Fischer's C statistic and p-values based on chi-square tests  
316 (Grace et al., 2010; Jonsson and Wardle, 2010). All pathways of our SEM model included  
317 mesocosm as a random effect.

318 All statistical analysis was carried out in R (Version 3.4.4) (R Core Team, 2015) using the nlme,  
319 vegan, piecewiseSEM, and ggpubr packages (Pinheiro, Bates, and DebRoy 2017; Oksanen et al.,  
320 2013; Lefcheck, 2016; Kassambara, 2018). For ANOVAs distribution of the data was inspected  
321 visually and with the Shapiro-Wilk test. We tested homogeneity of variances using the car  
322 package and the Levene test (Fox and Weisberg, 2011). We report uncertainty as  $\pm 1$  standard  
323 deviation, unless stated otherwise. We define the statistical significance level at 5%.

324

### 325 **3. Results**

#### 326 *3.1 Site hydrology and chemistry*

327 The average water table depth during the growing season was higher in the young bog than in the  
328 mature bog (Table 1). The young bog had higher seasonal mean and maximum soil temperatures  
329 than the mature bog,  $1.8 \pm 0.8^\circ\text{C}$  and  $1.1 \pm 0.9^\circ\text{C}$  respectively, at all depths measured (Table S2).  
330 Near-surface temperatures peaked in late July – early August, while deep temperatures peaked in  
331 late August – early September. Pore-water pH did not vary between the shallow and the deep  
332 layers, but the young bog had higher average seasonal pH than the mature bog, at  $4.19 \pm 0.31$   
333 and  $4.08 \pm 0.22$  respectively (see Table 1 for statistics).

334 Average pore-water DOC concentrations were generally lower in the young bog than in the  
335 mature bog ( $53.8 \pm 5.4$  and  $69.2 \pm 18.4$  mg C L<sup>-1</sup> respectively), while differences between

336 shallow and deep peat layers were much smaller (Table 1). In the young bog we found that pore-  
337 water DOC concentrations in both shallow and deep peat layers increased throughout the season.  
338 The young bog also had lower pore-water TN concentrations than the mature bog, at  $0.88 \pm 0.12$   
339 and  $1.53 \pm 1.34 \text{ mg L}^{-1}$  respectively, while there were no differences between shallow and deep  
340 peat layers. The young bog pore-water DOC had higher SUVA than the mature bog, and shallow  
341 peat layers had generally higher SUVA than deeper peat layers. The seasonal average spectral  
342 slope ( $S_{250-465}$ ) and concentrations of phenolics and  $\text{PO}_4^{3-}$  did not vary between the young and  
343 mature bog, or peat layers (Table 1).

### 344 *3.2 In situ potential enzyme activities and multifunctionality; field study*

345 Overall, we found that potential enzyme activities were highest in the young bog shallow peat  
346 layers, followed by the mature bog shallow peat layers, while enzyme activities in the deep peat  
347 layers were much lower and showed no difference between the young and mature bogs (Figure  
348 2; Table S3). Depth profiles of potential enzyme activity shows that there were distinct shifts in  
349 potential enzyme activity at the transition between shallow and deep peat, i.e. at the depth where  
350 there is a transition from sylvic peat accumulated during peat plateau stages and the *Sphagnum*  
351 peat that accumulated during the thermokarst bog stage that that follows permafrost thaw. The  
352 greater potential enzyme activity in the young bog shallow peat layer compared to the mature  
353 bog was evident throughout the season, with the greatest differences found for PHOS and BG,  
354 followed by NAG and CB, and a less pronounced difference for LAC (Figure 2). Potential  
355 enzyme activity did not vary throughout the season, with the exception of LAC which decreased  
356 through the season in the young bog shallow peat layer (Figure S2; Table S4).

357 Combining all data of hydrolytic enzyme activity (i.e., PHOS, NAG, BG, and CB) into an  
358 enzyme multifunctionality index further emphasized the difference between the highest activity

359 in the shallow young bog peat, followed by the shallow mature bog peat, which were greater than  
360 the deep peat in both the young and mature stages (Figure 3a). Overall, the multifunctionality  
361 index was over five times greater in the shallow young bog peat ( $0.33 \pm 0.25$ ) than in the deep  
362 peat layers ( $0.06 \pm 0.03$  for both young and mature bog).

363 In contrast to the hydrolytic multifunctionality index, we did not find any differences between  
364 bog stages or peat depths for laccase activity (Figure 3b; Table S4). However, for the shallow  
365 young bog we did find a positive relationship between laccase activity and the multifunctionality  
366 index ( $R^2 = 0.67$ ,  $P < 0.001$ ). This positive relationship was not found for the other three peat  
367 layers (Figure 3c).

368 We found that enzyme multifunctionality decreased with higher peat humification, as determined  
369 by the ratio of aromatics ( $1630 \text{ cm}^{-1}$ ) to polysaccharides ( $1090 \text{ cm}^{-1}$ ) from FTIR analysis (Figure  
370 4a). This negative relationship demonstrates that as the degree of decomposition of peat  
371 increases, i.e. as peat quality decreases, the rate of hydrolytic enzyme activity also decreases.  
372 Peat in the shallow young bog layer was the least humified and had the highest enzyme  
373 multifunctionality estimate, with enzyme multifunctionality decreasing with increasing FTIR  
374 ratios ( $1630 \text{ cm}^{-1}/1090 \text{ cm}^{-1}$ ) and peat humification, suggesting that peat quality is an important  
375 controlling factor on enzyme activity.

376 Overall, 30.3% of enzyme activity was explained by environmental variables (RDA analysis;  
377 Figure 4b). Following stepwise selection, FTIR ratios ( $1630 \text{ cm}^{-1}/1090 \text{ cm}^{-1}$ ), pore-water pH and  
378 DOC concentration were found to be significant environmental variables ( $P < 0.01$  for all)  
379 influencing enzyme activity and no collinearity was found between any of these explanatory  
380 variables. Our model, and both RDA biplot axes 1 and 2, were significant ( $P < 0.05$ ). Variation  
381 partitioning and adjusted  $R^2$  showed that FTIR alone explained 25.7% of the variation of enzyme

382 activity, further supporting the finding of greatest enzyme activity in the less humified young  
383 bog shallow peat layer. Whereas pH and DOC explained 1.2% and 0.8% respectively of the  
384 variation in enzyme activity (Figure 4b).

### 385 *3.3 Mesocosm incubation; constraints on enzyme activity*

386 The DO concentrations of peat mesocosms transferred to the lab were initially  $1.1 \pm 0.6\%$  and  
387  $4.4 \pm 3.7\%$  in the young bog and mature bogs, respectively, indicating anoxic conditions (i.e.  
388  $<10\%$ ). After draining half of the mesocosms, the average DO concentration over the 5 cm, 10  
389 cm, and 20 cm depth profile in the young bog oxic and mature bog oxic peat mesocosms  
390 increased to  $76 \pm 26\%$  and  $56 \pm 32\%$  respectively, and thus considered oxic (i.e.,  $>10\%$ ).

391 Analysis of pore-water at the end of the incubation, 96 hours after drainage, showed no  
392 differences in DOC concentrations, phenolic concentration, and  $S_{250-465}$  between either the  
393 young and mature bog mesocosms, or between the oxic and anoxic mesocosms. The only  
394 significant variation observed was a lower pH in the mature bog anoxic mesocosm than the  
395 young bog anoxic mesocosm (Table S5).

396 Hydrolytic enzyme activities measured in peat mesocosms at the end of the incubation  
397 experiment were summarized to an enzyme multifunctionality estimate ( $Meso_{enz}$ ), using the same  
398 approach as for field data.  $Meso_{enz}$  differed significantly ( $F_{(3, 30)} = 4.48$ ,  $P < 0.05$ ) between the  
399 four mesocosms. Higher hydrolytic enzyme activities were observed in the young than mature  
400 bog mesocosms, while anoxic/oxic conditions had no influence (Figure 5a). There was no  
401 difference between the young bog oxic and anoxic mesocosms, and similarly there was no  
402 difference between the mature bog oxic and anoxic mesocosms (Table S6). The activity of the  
403 majority of all four individual hydrolytic enzymes measured from peat mesocosms demonstrated  
404 similar patterns (Table S6).

405 Laccase activity was higher in the young bog peat mesocosms than that measured in the mature  
406 bog mesocosms ( $F_{(1,28)} = 6.8, P < 0.05$ ) (Fig. S2). Upon inspection of post-hoc test results, this  
407 difference was driven by significantly higher laccase in the young bog anoxic mesocosm than in  
408 the mature bog anoxic mesocosm (Table S6).

409 Our structural equation model (SEM) explained 59% of the variance in  $Meso_{enz}$  in the peat  
410 mesocosms (Figure 5b). Thermokarst bog stage was found to be the major control on  $Meso_{enz}$  in  
411 the model (path = 0.57,  $P < 0.05$ ). Similarly, both  $S_{250-465}$  and laccase activity were significantly  
412 influenced by thermokarst bog stage. Laccase activity and  $S_{250-465}$  were shown to have a  
413 positive, but not significant, effect on  $Meso_{enz}$  (path = 0.25,  $P = 0.12$  and path = 0.17,  $P = 0.21$   
414 respectively). Overall, thermokarst bog stage was the major control on enzymatic activity, but  
415 there was evidence of a negative influence of increasing pore water aromaticity and peat  
416 humification on hydrolytic enzyme activity.

417

#### 418 **4. Discussion**

419 Permafrost peatlands within the discontinuous permafrost zone are currently undergoing rapid  
420 warming-induced permafrost thaw (Chasmer and Hopkinson, 2017), exposing vast stores of soil  
421 C to microbial activity (Schuur et al., 2008). This study suggests that for many thawing peatlands  
422 it is unlikely that there is rapid mineralization of deep soil C in the early stages of thermokarst,  
423 and that the main difference in the C balance between young and mature thermokarst bogs rather  
424 is controlled by constraints on the accumulation of new peat near the surface. We draw these  
425 conclusions based on measures of potential enzyme activities, both from field measurements and  
426 from lab incubations. In the field we found no evidence of elevated potential enzyme activities at  
427 depth in a recently thawed (~30 years) thermokarst bog when compared to deep peat of the same

428 age in a bog stage that has been thawed for ~200 years. The field data further showed  
429 consistently higher potential enzyme activities in near-surface, shallow peat in the young bog  
430 compared to the mature bog, suggesting higher rates of potential decomposition and potentially  
431 lower rates of peat accumulation. However, complete inundation in the young bog compared to  
432 the lower water table in the mature bog put into question whether these potential enzyme  
433 activities are realized under field conditions. Here our lab experiment showed that enzyme  
434 activities in near-surface peat were not influenced by redox conditions, and thus unlikely to  
435 control the rate of surface-peat accumulation. Below we further explore the controls on potential  
436 enzyme activities in young and mature thermokarst bogs, and the inferences we can make  
437 regarding the carbon balance of peatlands following permafrost thaw.

#### 438 *4.1 Constraints on potential enzyme activity along a thermokarst bog transect*

439 In this study we found that potential enzymatic activities generally decreased with depth (Figure  
440 2), as has been shown elsewhere (Fisk et al., 2003; Preston et al., 2012). Microbial respiration  
441 and the potential for microbial respiration has previously been shown to decrease with depth  
442 even under aerobic conditions in thawing permafrost peatlands (Monteux et al., 2018). The  
443 greatest rates of potential enzyme activities were observed in the young bog shallow peat layer  
444 (Figure 3, Figure 5) regardless of *in situ* surface inundation (Table 1) or mesocosm treatment.  
445 Our results suggest that peat type and chemistry have an intrinsic control on potential enzyme  
446 activities (Figure 5).

447 We found a large increase in peat humification, indicated by FTIR ratios, with depth and the  
448 switch from near-surface *Sphagnum* peat to previously sylvic peat, and to a lesser extent with  
449 successional vegetation communities. Increased peat humification lead to decreased potential  
450 enzyme activities (Figure 4a), and peat humification was an important constraint on enzyme

451 activity, accounting for 25.7% variation in enzyme activity (Figure 4b). This finding supports the  
452 idea that peat humification is a major control on enzyme activity and highlights the importance  
453 of peat chemistry in governing decomposition following permafrost thaw. In the initial decades  
454 following permafrost thaw, there is a shift in thermokarst dissolved organic matter towards more  
455 biodegradable, lower molecular weight compounds with lower aromaticity (Burd, 2017) which  
456 can have a significant impact on potential peat mineralization and CO<sub>2</sub> and CH<sub>4</sub> production  
457 (Hodgkins et al., 2014). The decrease in enzyme activity with depth may be not be due to the  
458 distance from water table position but rather the distance from vegetation inputs, labile substrate  
459 availability, and the rhizosphere priming effect (Kaštovská et al., 2018; Keuper et al., 2020; Lin  
460 et al., 2014; Straková et al., 2012; Tfaily et al., 2014).

461 Oxidative enzyme activity has previously been shown to release hydrolytic enzyme activity from  
462 constraints by reducing phenolic concentrations in peat (Freeman et al., 2002; Freeman et al.,  
463 2004; Saraswati et al., 2016). Similar to previous studies (Romanowicz et al., 2015; Strickland et  
464 al., 2009), we find no evidence of constraints on oxidative and hydrolytic enzyme activity due to  
465 low oxygen availability or phenolic concentrations. The inundated and low oxygen conditions  
466 found in the young bog shallow layer may may have led to enhanced iron reduction, which has  
467 been shown to promote oxidative enzyme activity (Wen et al., 2019). Furthermore, as the young  
468 bog microbial community preferentially expends the plant-derived labile C source there is  
469 increased decomposition of recalcitrant peat DOM by oxidative enzyme activity. This leads to  
470 greater overall potential enzyme activity in the young bog shallow peat and the positive  
471 relationship between potential oxidative and hydrolytic enzyme activity (Figure 3c, Figure 5b).

472 Previous work has shown that in peat hummocks, with vegetation, water table position, and oxic  
473 conditions similar to those found in the mature bog, vascular plant rhizosphere inputs are an

474 important substrate for potential enzyme activity (Robroek et al., 2016). Whereas in peat lawns,  
475 with similar vegetation, water table position, and anoxic conditions to the young bog, these  
476 inputs are less important. Aerobic microbial respiration under oxic conditions occurs 1.2 – 7  
477 times faster than under anaerobic conditions (Blodau et al., 2004). As such the microbial  
478 community found in the mature bog have greater requirements to fuel this aerobic respiration.  
479 Due to the antimicrobial properties of peat DOM and *Sphagnum* leachates (Hamard et al., 2019;  
480 Tfaily et al., 2013) the microbial community in the oxic mature bog shallow peat is thus, highly  
481 dependent on labile vascular plant rhizosphere inputs. Whereas the young bog shallow peat  
482 microbial community involved in slower anaerobic degradation are less dependent on vascular  
483 plant leachates, and as a result hydrolytic enzyme activity increases as the availability of easily  
484 degradable vascular plant derived C decreases (Robroek et al., 2016).

485 We found no consistent seasonal trends in potential enzyme activity, neither across different  
486 enzymes or different peat types and depths (Figure 2; Figure S1a – e). This homeostasis in  
487 potential enzyme concentrations over the growing season and with depth is likely due to the  
488 regulatory response of the microbial community to substrate availability (Sinsabaugh et al.,  
489 2014). Our lack of a seasonal pattern in potential enzyme activities is consistent with previous  
490 studies which have had mixed results when assessing the seasonality of potential enzyme activity  
491 (Jassey et al., 2011; Pinsonneault et al., 2016). Seasonal temperatures have however been shown  
492 to be an important driver for *in situ* rates of enzyme activity in tundra soils (Wallenstein et al.,  
493 2009) and the temperature sensitivity of soil enzymes ( $Q_{10}$ ) can range from 1.2 – 2.4  
494 (Nottingham et al., 2016; Paz-Ferreiro, 2015). Applying these  $Q_{10}$  values to this study would  
495 suggest that average *in situ* soil enzyme activities in the warmer young bog (Table S2) could be  
496 up to 16% and 10% greater than in the mature bog at 45 cm and 105 cm, respectively. These

497 potentially higher *in situ* rates of enzyme activity may influence the response and vulnerability of  
498 permafrost peatland C stores and the net carbon balance following thermokarst formation  
499 (Hugelius et al., 2020). However, this moderate difference in soil temperatures is unlikely to  
500 cause rapid losses of deep, old, C during the early stages of thermokarst bogs – at least in sites  
501 where the thawed peat is highly humified (Heffernan et al 2020, Treat et al 2021).

502 However even at high temperatures *in situ* enzyme activity is limited by nutrient availability  
503 (Wallenstein et al., 2009). This suggests that while temperature is an important control on *in situ*  
504 enzyme activity (Wallenstein et al., 2010), the main constraint on rates of potential enzyme  
505 activities is likely to be peat chemistry.

#### 506 *4.2 Stability of deep peat following thaw*

507 Results from this study suggest that deep, previously frozen peat is unlikely to experience a  
508 period of rapid increases in rates of enzyme activity and mineralization during the early stages  
509 after thaw. The transportation of labile substrates to depths of 3 m can occur in peatlands and  
510 prime microbial decomposition at depth (Chanton et al., 2008). However, despite increased  
511 enzyme activities and labile DOM at the surface, along with higher soil temperatures at depth  
512 we found no evidence of greater potential enzyme activity in the young bog compared to the  
513 mature bog. While the lack of evidence of elevated enzyme concentrations at depth in the young  
514 bog could result from more rapid enzyme turnover we consider this unlikely due to the distance  
515 from fresh litter inputs, increased peat humification, and decreased oxidative enzyme activity.  
516 Our results have important implications for predicting the response of permafrost peatland C to  
517 rapidly increasing temperatures and permafrost thaw by suggesting that previously frozen C  
518 stores in many regions may be more stable than previously thought and proposes that peat type

519 and humification are important factors controlling decomposition following permafrost thaw  
520 (Keuper et al., 2020). Despite warmer temperatures, more labile litter inputs, and no constraint  
521 on enzymes due to oxygen unavailability, there is no evidence of a rapid pulse of increased  
522 potential enzyme activity of previously frozen peat in the initial decades following permafrost  
523 thaw. While warmer temperatures throughout the young bog peat profile may lead to somewhat  
524 higher *in situ* rates of enzymatic degradation indicated by the potential enzyme activities we  
525 measured, our data show that microbial activity is not enhanced due to the availability of an  
526 intrinsically more labile, previously frozen substrate. Our findings of stable deep peat following  
527 permafrost thaw are reinforced by previous studies demonstrating modest losses of C stores or no  
528 contribution of deep soil C to CO<sub>2</sub> and CH<sub>4</sub> respired at the surface at our study site and other  
529 sites with similar site histories (Cooper et al., 2017; Estop-Aragonés et al., 2018; Heffernan et  
530 al., 2020).

531 Contrary to previous studies (Bonnett et al., 2017), but supportive of others (Straková et al.,  
532 2012), we show that peat type and not water table position is the main driver of potential enzyme  
533 activity. Distance from labile litter inputs, and increasing peat humification, were key constraints  
534 on enzyme activity at depth and these findings suggest that peat chemistry following permafrost  
535 thaw is a major factor in controlling the potential rapid loss of C as has been described elsewhere  
536 (Jones et al., 2017).

537 Permafrost aggradation and site development history can lead to legacy effects on peat quality  
538 following thaw, influencing the degree of decomposition of the peat and peat chemistry  
539 associated with the vegetation community at time of accumulation (Treat et al., 2016). For  
540 example, sites where permafrost aggraded early in their development history and have not  
541 undergone centuries – millennia of anaerobic decomposition may have intrinsically more labile

542 organic matter available to the microbial community following thaw. This highlights the  
543 importance of site-specific responses in determining C loss following thaw and may help in  
544 reconciling the contrasting findings of rapid and slow C losses observed in thermokarst peatlands  
545 with syngenetic (Jones et al., 2017) and epigenetic (Heffernan et al., 2020) permafrost histories.  
546 Considering our findings, we suggest that future permafrost C trajectory modelling attempts  
547 should include; (1) peatland succession following thermokarst development, as not only do both  
548 early and mature stages of succession after thaw differ, but terrestrial thermokarst development  
549 in peatlands can result in differing early stage vegetation communities and peat types in the form  
550 of thermokarst bogs and fens; and (2) site developmental and permafrost aggradation history, as  
551 both will influence peat chemistry following thaw which we show to be the main constraint on  
552 potential enzyme activities. This joint consideration is an important step towards more accurate  
553 representation of the contribution of microbial processes to future C balances and the magnitude  
554 of the permafrost C feedback.

555

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560

## 561 **References**

562 Allan, E., Manning, P., Alt, F., Binkenstein, J., Blaser, S., Blüthgen, N., ... Fischer, M. (2015).

563 Land use intensification alters ecosystem multifunctionality via loss of biodiversity and  
564 changes to functional composition. *Ecology Letters*. <https://doi.org/10.1111/ele.12469>

565 Allison, S. D., Wallenstein, M. D., & Bradford, M. A. (2010). Soil-carbon response to warming  
566 dependent on microbial physiology. *Nature Geoscience*. <https://doi.org/10.1038/ngeo846>

567 Artz, R. R. E., Chapman, S. J., Jean Robertson, A. H., Potts, J. M., Laggoun-Défarge, F., Gogo,  
568 S., ... Francez, A. J. (2008). FTIR spectroscopy can be used as a screening tool for organic  
569 matter quality in regenerating cutover peatlands. *Soil Biology and Biochemistry*, 40(2), 515–  
570 527. <https://doi.org/10.1016/j.soilbio.2007.09.019>

571 Beilman, D. W. (2001). Plant community and diversity change due to localized permafrost  
572 dynamics in bogs of western Canada. *Canadian Journal of Botany*, 79(8), 983–993.  
573 <https://doi.org/10.1139/cjb-79-8-983>

574 Bell, C. W., Fricks, B. E., Rocca, J. D., Steinweg, J. M., McMahon, S. K., & Wallenstein, M. D.  
575 (2013). High-throughput Fluorometric Measurement of Potential Soil Extracellular Enzyme  
576 Activities. *Journal of Visualized Experiments*. <https://doi.org/10.3791/50961>

577 Blodau, C., Basiliko, N., & Moore, T. R. (2004). Carbon turnover in peatland mesocosms  
578 exposed to different water table levels. *Biogeochemistry*.  
579 <https://doi.org/10.1023/B:BIOG.0000015788.30164.e2>

580 Bonnett, S. A. F., Maltby, E., & Freeman, C. (2017). Hydrological legacy determines the type of  
581 enzyme inhibition in a peatlands chronosequence. *Scientific Reports*.  
582 <https://doi.org/10.1038/s41598-017-10430-x>

583 Borga, P., Nilsson, M., & Tunlid, A. (1994). Bacterial communities in peat in relation to

584 botanical composition as revealed by phospholipid fatty acid analysis. *Soil Biology and*  
585 *Biochemistry*. [https://doi.org/10.1016/0038-0717\(94\)90300-X](https://doi.org/10.1016/0038-0717(94)90300-X)

586 Bragazza, L., Bardgett, R. D., Mitchell, E. A. D., & Buttler, A. (2015). Linking soil microbial  
587 communities to vascular plant abundance along a climate gradient. *New Phytologist*, *205*(3),  
588 1175–1182. <https://doi.org/10.1111/nph.13116>

589 Briones, M. J. I., Mcnamara, N. P., Poskitt, J., Crow, S. E., & Ostle, N. J. (2014). Interactive  
590 biotic and abiotic regulators of soil carbon cycling: Evidence from controlled climate  
591 experiments on peatland and boreal soils. *Global Change Biology*, *20*(9), 2971–2982.  
592 <https://doi.org/10.1111/gcb.12585>

593 Broder, T., Blodau, C., Biester, H., & Knorr, K. H. (2012). Peat decomposition records in three  
594 pristine ombrotrophic bogs in southern Patagonia. *Biogeosciences*, *9*(4), 1479–1491.  
595 <https://doi.org/10.5194/bg-9-1479-2012>

596 Brown, J., Ferrians Jr., O. J., Heginbottom, J. A., & Melnikov, E. S. (1997). Circum-Arctic map  
597 of permafrost and ground ice conditions. *USGS Numbered Series*, 1.  
598 <https://doi.org/10.1016/j.jallcom.2010.03.054>

599 Burd, K. (2017). *Influence of wildfire and permafrost thaw on DOC in northern peatlands*.  
600 University of Alberta. <https://doi.org/https://doi.org/10.7939/R3FB4X10G>

601 Camill, P. (1999). Peat accumulation and succession following permafrost thaw in the Boreal  
602 peatlands of Manitoba, Canada. *Ecoscience*, *6*(4), 592–602.  
603 <https://doi.org/10.1080/11956860.1999.11682561>

604 Chanton, J. P., Glaser, P. H., Chasar, L. S., Burdige, D. J., Hines, M. E., Siegel, D. I., ... Cooper,

605 W. T. (2008). Radiocarbon evidence for the importance of surface vegetation on  
606 fermentation and methanogenesis in contrasting types of boreal peatlands. *Global*  
607 *Biogeochemical Cycles*, 22(4), 1–11. <https://doi.org/10.1029/2008GB003274>

608 Chasmer, L. E., & Hopkinson, C. (2017). Threshold loss of discontinuous permafrost and  
609 landscape evolution, 2672–2686. <https://doi.org/10.1111/gcb.13537>

610 Chiapusio, G., Jassey, V. E. J., Bellvert, F., Comte, G., Weston, L. A., Delarue, F., ... Binet, P.  
611 (2018). Sphagnum Species Modulate their Phenolic Profiles and Mycorrhizal Colonization  
612 of Surrounding *Andromeda polifolia* along Peatland Microhabitats. *Journal of Chemical*  
613 *Ecology*. <https://doi.org/10.1007/s10886-018-1023-4>

614 Climate-Data.org. (2019). ̂. Retrieved January 21, 2019, from [https://en.climate-data.org/north-](https://en.climate-data.org/north-america/canada/alberta/meander-river-11380/)  
615 [america/canada/alberta/meander-river-11380/](https://en.climate-data.org/north-america/canada/alberta/meander-river-11380/)

616 Cooper, M. D. A., Estop-Aragonés, C., Fisher, J. P., Thierry, A., Garnett, M. H., Charman, D. J.,  
617 ... Hartley, I. P. (2017). Limited contribution of permafrost carbon to methane release from  
618 thawing peatlands. *Nature Climate Change*, 7(7), 507–511.  
619 <https://doi.org/10.1038/nclimate3328>

620 Criquet, S., Farnet, A. M., Tagger, S., & Le Petit, J. (2000). Annual variations of phenoloxidase  
621 activities in an evergreen oak litter: Influence of certain biotic and abiotic factors. *Soil*  
622 *Biology and Biochemistry*. [https://doi.org/10.1016/S0038-0717\(00\)00027-4](https://doi.org/10.1016/S0038-0717(00)00027-4)

623 Criquet, S., Tagger, S., Vogt, G., Iacazio, G., & Le Petit, J. (1999). Laccase activity of forest  
624 litter. *Soil Biology and Biochemistry*. [https://doi.org/10.1016/S0038-0717\(99\)00038-3](https://doi.org/10.1016/S0038-0717(99)00038-3)

625 Dunn, C., Jones, T. G., Girard, A., & Freeman, C. (2014). Methodologies for extracellular

626 enzyme assays from wetland soils. *Wetlands*, 34(1), 9–17. <https://doi.org/10.1007/s13157->  
627 013-0475-0

628 Ernakovich, J. G., Lynch, L. M., Brewer, P. E., Calderon, F. J., & Wallenstein, M. D. (2017).  
629 Redox and temperature-sensitive changes in microbial communities and soil chemistry  
630 dictate greenhouse gas loss from thawed permafrost. *BIOGEOCHEMISTRY*, 134(1–2),  
631 183–200. <https://doi.org/10.1007/s10533-017-0354-5>

632 Estop-Aragonés, C., Czimczik, C. I., Heffernan, L., Gibson, C., Walker, J. C., Xu, X., &  
633 Olefeldt, D. (2018). Respiration of aged soil carbon during fall in permafrost peatlands  
634 enhanced by active layer deepening following wildfire but limited following thermokarst.  
635 *Environmental Research Letters*, 13(8). <https://doi.org/10.1088/1748-9326/aad5f0>

636 Euskirchen, E. S., Edgar, C. W., Turetsky, M. R., Waldrop, M. P., & Harden, J. W. (2014).  
637 Differential response of carbon fluxes to climate in three peatland ecosystems that vary in  
638 the presence and stability of permafrost. *Journal of Geophysical Research G:*  
639 *Biogeosciences*. <https://doi.org/10.1002/2014JG002683>

640 Fenner, N., Freeman, C., & Reynolds, B. (2005). Observations of a seasonally shifting thermal  
641 optimum in peatland carbon-cycling processes; implications for the global carbon cycle and  
642 soil enzyme methodologies. *Soil Biology and Biochemistry*, 37(10), 1814–1821.  
643 <https://doi.org/10.1016/j.soilbio.2005.02.032>

644 Fisk, M. C., Ruether, K. F., & Yavitt, J. B. (2003). Microbial activity and functional composition  
645 among northern peatland ecosystems. *Soil Biology and Biochemistry*.  
646 [https://doi.org/10.1016/S0038-0717\(03\)00053-1](https://doi.org/10.1016/S0038-0717(03)00053-1)

647 Fox, J., & Weisberg, S. (2011). *An R Companion to Applied Regression*, second ed.

648 <https://doi.org/10.1016/j.stomax.2010.07.001>

649 Freeman, C., Ostle, N. J., Fenner, N., & Kang, H. (2004). A regulatory role for phenol oxidase  
650 during decomposition in peatlands. In *Soil Biology and Biochemistry*.  
651 <https://doi.org/10.1016/j.soilbio.2004.07.012>

652 Freeman, Chris, Ostle, N., & Kang, H. (2002). An enzymic “latch” on a global carbon store.  
653 *Nature*. <https://doi.org/10.1038/35051650>

654 Frey, S. D., Lee, J., Melillo, J. M., & Six, J. (2013). The temperature response of soil microbial  
655 efficiency and its feedback to climate. *Nature Climate Change*.  
656 <https://doi.org/10.1038/nclimate1796>

657 Fudyma, J. D., Lyon, J., AminiTabrizi, R., Gieschen, H., Chu, R. K., Hoyt, D. W., ... Tfaily, M.  
658 M. (2019). Untargeted metabolomic profiling of *Sphagnum fallax* reveals novel  
659 antimicrobial metabolites. *Plant Direct*. <https://doi.org/10.1002/pld3.179>

660 Grace, J. B., Adler, P. B., Stanley Harpole, W., Borer, E. T., & Seabloom, E. W. (2014). Causal  
661 networks clarify productivity-richness interrelations, bivariate plots do not. *Functional*  
662 *Ecology*, 28(4), 787–798. <https://doi.org/10.1111/1365-2435.12269>

663 Grace, J. B., Michael Anderson, T., Han, O., & Scheiner, S. M. (2010). On the specification of  
664 structural equation models for ecological systems. *Ecological Monographs*.  
665 <https://doi.org/10.1890/09-0464.1>

666 Hamard, S., Robroek, B. J. M., Allard, P. M., Signarbieux, C., Zhou, S., Saesong, T., ... Jassey,  
667 V. E. J. (2019). Effects of *Sphagnum* Leachate on Competitive *Sphagnum* Microbiome  
668 Depend on Species and Time. *Frontiers in Microbiology*, 10.

669 <https://doi.org/10.3389/fmicb.2019.02042>

670 Hansen, A. M., Kraus, T. E. C., Pellerin, B. A., Fleck, J. A., Downing, B. D., & Bergamaschi, B.  
671 A. (2016). Optical properties of dissolved organic matter (DOM): Effects of biological and  
672 photolytic degradation. *Limnology and Oceanography*. <https://doi.org/10.1002/lno.10270>

673 Heffernan, L., Estop-Aragónés, C., Knorr, K.-H., Talbot, J., & Olefeldt, D. (2020). Long-term  
674 impacts of permafrost thaw on carbon storage in peatlands: deep losses offset by surficial  
675 accumulation. *Journal of Geophysical Research: Biogeosciences*, *2011*(2865),  
676 e2019JG005501. <https://doi.org/10.1029/2019JG005501>

677 Heginbottom, J. A., Dubreuil, M. H., & Harker, P. T. (1995). Canada, Permafrost. *National Atlas*  
678 *of Canada*.

679 Helbig, M., Chasmer, L. E., Desai, A. R., Kljun, N., Quinton, W. L., & Sonnentag, O. (2017).  
680 Direct and indirect climate change effects on carbon dioxide fluxes in a thawing boreal  
681 forest–wetland landscape. *Global Change Biology*, *23*(8), 3231–3248.  
682 <https://doi.org/10.1111/gcb.13638>

683 Hodgkins, S. B., Tfaily, M. M., McCalley, C. K., Logan, T. A., Crill, P. M., Saleska, S. R., ...  
684 Chanton, J. P. (2014). Changes in peat chemistry associated with permafrost thaw increase  
685 greenhouse gas production. *Proceedings of the National Academy of Sciences*, *111*(16),  
686 5819–5824. <https://doi.org/10.1073/pnas.1314641111>

687 Hugelius, G., Strauss, J., Zubrzycki, S., Harden, J. W., Schuur, E. A. G., Ping, C. L., ... Kuhry,  
688 P. (2014). Estimated stocks of circumpolar permafrost carbon with quantified uncertainty  
689 ranges and identified data gaps. *Biogeosciences*, *11*(23), 6573–6593.  
690 <https://doi.org/10.5194/bg-11-6573-2014>

691 Hugelius, Gustaf, Loisel, J., Chadburn, S., Jackson, R. B., Jones, M., MacDonald, G., ... Yu, Z.  
692 (2020). Large stocks of peatland carbon and nitrogen are vulnerable to permafrost thaw.  
693 *Proceedings of the National Academy of Sciences*, 117(34), 20438–20446.  
694 <https://doi.org/10.1073/pnas.1916387117>

695 Jassey, V. E. J., Chiapusio, G., Gilbert, D., Buttler, A., Toussaint, M. L., & Binet, P. (2011).  
696 Experimental climate effect on seasonal variability of polyphenol/phenoloxidase interplay  
697 along a narrow fen-bog ecological gradient in *Sphagnum fallax*. *Global Change Biology*,  
698 17(9), 2945–2957. <https://doi.org/10.1111/j.1365-2486.2011.02437.x>

699 Jassey, V. E. J., Chiapusio, G., Gilbert, D., Toussaint, M. L., & Binet, P. (2012). Phenoloxidase  
700 and peroxidase activities in *Sphagnum*-dominated peatland in a warming climate. *Soil*  
701 *Biology and Biochemistry*, 46, 49–52. <https://doi.org/10.1016/j.soilbio.2011.11.011>

702 Jassey, V. E. J., Chiapusio, G., Mitchell, E. A. D., Binet, P., Toussaint, M. L., & Gilbert, D.  
703 (2011). Fine-Scale Horizontal and Vertical Micro-distribution Patterns of Testate Amoebae  
704 Along a Narrow Fen/Bog Gradient. *Microbial Ecology*. [https://doi.org/10.1007/s00248-010-](https://doi.org/10.1007/s00248-010-9756-9)  
705 9756-9

706 Jassey, V. E. J., Lamentowicz, M., Bragazza, L., Hofsommer, M. L., Mills, R. T. E., Buttler, A.,  
707 ... Robroek, B. J. M. (2016). Loss of testate amoeba functional diversity with increasing  
708 frost intensity across a continental gradient reduces microbial activity in peatlands.  
709 *European Journal of Protistology*, 55, 190–202. <https://doi.org/10.1016/j.ejop.2016.04.007>

710 Jassey, V. E. J., Reczuga, M. K., Zielińska, M., Słowińska, S., Robroek, B. J. M., Mariotte, P.,  
711 ... Buttler, A. (2018). Tipping point in plant–fungal interactions under severe drought  
712 causes abrupt rise in peatland ecosystem respiration. *Global Change Biology*.

713 <https://doi.org/10.1111/gcb.13928>

714 Jones, M. C., Harden, J., O'Donnell, J., Manies, K., Jorgenson, T., Treat, C., & Ewing, S. (2017).  
715 Rapid carbon loss and slow recovery following permafrost thaw in boreal peatlands. *Global*  
716 *Change Biology*, 23(3), 1109–1127. <https://doi.org/10.1111/gcb.13403>

717 Jonsson, M., & Wardle, D. A. (2010). Structural equation modelling reveals plant-community  
718 drivers of carbon storage in boreal forest ecosystems. *Biology Letters*.  
719 <https://doi.org/10.1098/rsbl.2009.0613>

720 Kassambara, A. (2018). ggpubr: “ggplot2” Based Publication Ready Plots. R package version  
721 0.2. <https://CRAN.R-project.org/package=ggpubr>. [https://CRAN.R-](https://CRAN.R-Project.Org/Package=ggpubr)  
722 [Project.Org/Package=ggpubr](https://CRAN.R-Project.Org/Package=ggpubr). [https://doi.org/R package version 0.1.8](https://doi.org/R%20package%20version%200.1.8)

723 Kaštovská, E., Straková, P., Edwards, K., Urbanová, Z., Bárta, J., Mastný, J., ... Pícek, T.  
724 (2018). Cotton-Grass and Blueberry have Opposite Effect on Peat Characteristics and  
725 Nutrient Transformation in Peatland. *Ecosystems*, 21(3), 443–458.  
726 <https://doi.org/10.1007/s10021-017-0159-3>

727 Keuper, F., Wild, B., Kumm, M., Beer, C., Blume-Werry, G., Fontaine, S., ... Dorrepaal, E.  
728 (2020). Carbon loss from northern circumpolar permafrost soils amplified by rhizosphere  
729 priming. *Nature Geoscience*, 13(8). <https://doi.org/10.1038/s41561-020-0607-0>

730 Kokelj, S. V., & Jorgenson, M. T. (2013). Advances in thermokarst research. *Permafrost and*  
731 *Periglacial Processes*, 24(2), 108–119. <https://doi.org/10.1002/ppp.1779>

732 Laiho, R. (2006). Decomposition in peatlands: Reconciling seemingly contrasting results on the  
733 impacts of lowered water levels. *Soil Biology and Biochemistry*, 38(8), 2011–2024.

734 <https://doi.org/10.1016/j.soilbio.2006.02.017>

735 Laiho, R., Vasander, H., Penttilä, T., & Laine, J. (2003). Dynamics of plant-mediated organic  
736 matter and nutrient cycling following water-level drawdown in boreal peatlands. *Global*  
737 *Biogeochemical Cycles*. <https://doi.org/10.1029/2002gb002015>

738 Lawrence, D. M., Koven, C. D., Swenson, S. C., Riley, W. J., & Slater, A. G. (2015). Permafrost  
739 thaw and resulting soil moisture changes regulate projected high-latitude CO<sub>2</sub> and CH<sub>4</sub>  
740 emissions. *Environmental Research Letters*. <https://doi.org/10.1088/1748-9326/10/9/094011>

741 Lefcheck, J. S. (2016). piecewiseSEM: Piecewise structural equation modelling in r for ecology,  
742 evolution, and systematics. *Methods in Ecology and Evolution*, 7(5), 573–579.  
743 <https://doi.org/10.1111/2041-210X.12512>

744 Limpens, J., Bohlin, E., & Nilsson, M. B. (2017). Phylogenetic or environmental control on the  
745 elemental and organo-chemical composition of Sphagnum mosses? *Plant and Soil*, 417(1–  
746 2), 69–85. <https://doi.org/10.1007/s11104-017-3239-4>

747 Lin, X., Tfaily, M. M., Steinweg, J. M., Chanton, P., Esson, K., Yang, Z. K., ... Kostka, J. E.  
748 (2014). Microbial community stratification linked to utilization of carbohydrates and  
749 phosphorus limitation in a Boreal Peatland at Marcell Experimental Forest, Minnesota,  
750 USA. *Applied and Environmental Microbiology*. <https://doi.org/10.1128/AEM.00205-14>

751 Luo, L., Meng, H., & Gu, J. D. (2017). Microbial extracellular enzymes in biogeochemical  
752 cycling of ecosystems. *Journal of Environmental Management*.  
753 <https://doi.org/10.1016/j.jenvman.2017.04.023>

754 Machmuller, M. B., Mohan, J. E., Minucci, J. M., Phillips, C. A., & Wurzburger, N. (2016).

755 Season, but not experimental warming, affects the activity and temperature sensitivity of  
756 extracellular enzymes. *Biogeochemistry*, 131(3), 255–265. [https://doi.org/10.1007/s10533-](https://doi.org/10.1007/s10533-016-0277-6)  
757 016-0277-6

758 Monteux, S., Weedon, J. T., Blume-Werry, G., Gavazov, K., Jassey, V. E. J., Johansson, M., ...  
759 Dorrepaal, E. (2018). Long-term in situ permafrost thaw effects on bacterial communities  
760 and potential aerobic respiration. *ISME Journal*. <https://doi.org/10.1038/s41396-018-0176-z>

761 Moore, T., & Basiliko, N. (2006). Decomposition in Boreal Peatlands. In *Boreal Peatland*  
762 *Ecosystems*. [https://doi.org/10.1007/978-3-540-31913-9\\_7](https://doi.org/10.1007/978-3-540-31913-9_7)

763 Mudryk, L., Brown, R., Derksen, C., Luojus, K., Decharme, B., & Helfrich, S. (2018). *Surface*  
764 *Air Temperature [in Arctic Report Card 2018]*. Retrieved from  
765 <https://www.arctic.noaa.gov/Report-Card>

766 Myers-Smith, I. H., McGuire, A. D., Harden, J. W., & Chapin, F. S. (2007). Influence of  
767 disturbance on carbon exchange in a permafrost collapse and adjacent burned forest.  
768 *Journal of Geophysical Research: Biogeosciences*. <https://doi.org/10.1029/2007jg000423>

769 Nottingham, A. T., Turner, B. L., Whitaker, J., Ostle, N., Bardgett, R. D., McNamara, N. P., ...  
770 Meir, P. (2016). Temperature sensitivity of soil enzymes along an elevation gradient in the  
771 Peruvian Andes. *Biogeochemistry*, 127(2–3). <https://doi.org/10.1007/s10533-015-0176-2>

772 O'Donnell, J. A., Jorgenson, M. T., Harden, J. W., McGuire, A. D., Kanevskiy, M. Z., &  
773 Wickland, K. P. (2012). The Effects of Permafrost Thaw on Soil Hydrologic, Thermal, and  
774 Carbon Dynamics in an Alaskan Peatland. *Ecosystems*, 15(2), 213–229.  
775 <https://doi.org/10.1007/s10021-011-9504-0>

776 Oksanen, J., Blanchet, F. G., Kindt, R., Oksanen, M. J., & Suggests, M. (2013). Package ‘vegan.’  
777 *Community Ecology Package Version*.

778 Olefeldt, D., Goswami, S., Grosse, G., Hayes, D., Hugelius, G., Kuhry, P., ... Turetsky, M. R.  
779 (2016). Circumpolar distribution and carbon storage of thermokarst landscapes. *Nature*  
780 *Communications*, 7, 13043. <https://doi.org/10.1038/ncomms13043>

781 Paz-Ferreiro, J., Fu, S., Méndez, A., & Gascó, G. (2015). Biochar modifies the thermodynamic  
782 parameters of soil enzyme activity in a tropical soil. *Journal of Soils and Sediments*, 15(3).  
783 <https://doi.org/10.1007/s11368-014-1029-7>

784 Peres-Neto, P. R., Legendre, P., Dray, S., & Borcard, D. (2006). Variation partitioning of species  
785 data matrices: Estimation and comparison of fractions. *Ecology*.  
786 [https://doi.org/10.1890/0012-9658\(2006\)87\[2614:VPOSDM\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2006)87[2614:VPOSDM]2.0.CO;2)

787 Pinheiro J, Bates D, DebRoy S, S. D. and R. C. T. (2017). nlme: Linear and Nonlinear Mixed  
788 Effects Models. R package version 3.1-131, <https://CRAN.R-project.org/package=nlme>. *R*  
789 *Package Version 3.1-131*, <https://CRAN.R-Project.Org/Package=nlme>.  
790 <https://doi.org/10.1016/j.tibs.2011.05.003>

791 Pinsonneault, A. J., Moore, T. R., & Roulet, N. T. (2016). Temperature the dominant control on  
792 the enzyme-latch across a range of temperate peatland types. *Soil Biology and Biochemistry*,  
793 97, 121–130. <https://doi.org/10.1016/j.soilbio.2016.03.006>

794 Preston, M. D., Smemo, K. A., McLaughlin, J. W., & Basiliko, N. (2012). Peatland microbial  
795 communities and decomposition processes in the James Bay Lowlands, Canada. *Frontiers*  
796 *in Microbiology*. <https://doi.org/10.3389/fmicb.2012.00070>

797 Quinton, W. L., Hayashi, M., & Chasmer, L. E. (2011). Permafrost-thaw-induced land-cover  
798 change in the Canadian subarctic: Implications for water resources. *Hydrological Processes*,  
799 25(1), 152–158. <https://doi.org/10.1002/hyp.7894>

800 R Core Team. (2015). R: A language and environment for statistical computing. Vienna, Austria;  
801 2014. URL <Http://Www.R-Project.Org>. Vienna, Austria: R Foundation for Statistical  
802 Computing. <https://doi.org/10.1007/978-3-540-74686-7>

803 Robroek, B. J. M., Albrecht, R. J. H., Hamard, S., Pulgarin, A., Bragazza, L., Buttler, A., &  
804 Jassey, V. E. (2016). Peatland vascular plant functional types affect dissolved organic  
805 matter chemistry. *Plant and Soil*. <https://doi.org/10.1007/s11104-015-2710-3>

806 Robroek, B. J. M., Limpens, J., Breeuwer, A., & Schouten, M. G. C. (2007). Effects of water  
807 level and temperature on performance of four Sphagnum mosses. *Plant Ecology*.  
808 <https://doi.org/10.1007/s11258-006-9193-5>

809 Romanowicz, K. J., Kane, E. S., Potvin, L. R., Daniels, A. L., Kolka, R. K., & Lilleskov, E. A.  
810 (2015). Understanding drivers of peatland extracellular enzyme activity in the PEATcosm  
811 experiment: mixed evidence for enzymic latch hypothesis. *Plant and Soil*, 397(1–2), 371–  
812 386. <https://doi.org/10.1007/s11104-015-2746-4>

813 Roulet, N. T., Lafleur, P. M., Richard, P. J. H., Moore, T. R., Humphreys, E. R., & Bubier, J.  
814 (2007). Contemporary carbon balance and late Holocene carbon accumulation in a northern  
815 peatland. *Global Change Biology*, 13(2), 397–411. [https://doi.org/10.1111/j.1365-  
816 2486.2006.01292.x](https://doi.org/10.1111/j.1365-2486.2006.01292.x)

817 Saraswati, S., Dunn, C., Mitsch, W. J., & Freeman, C. (2016). Is peat accumulation in mangrove  
818 swamps influenced by the “enzymic latch” mechanism? *Wetlands Ecology and*

819           *Management*, 24(6), 641–650. <https://doi.org/10.1007/s11273-016-9493-z>

820   Schaefer, K., Lantuit, H., Romanovsky, V. E., Schuur, E. A. G., & Witt, R. (2014). The impact  
821           of the permafrost carbon feedback on global climate. *Environmental Research Letters*.  
822           <https://doi.org/10.1088/1748-9326/9/8/085003>

823   Schuur, E. A.G., McGuire, A. D., Schädel, C., Grosse, G., Harden, J. W., Hayes, D. J., ... Vonk,  
824           J. E. (2015). Climate change and the permafrost carbon feedback. *Nature*, 520(7546), 171–  
825           179. <https://doi.org/10.1038/nature14338>

826   Schuur, Edward A. G., Bockheim, J., Canadell, J. G., Euskirchen, E., Field, C. B., Goryachkin,  
827           S. V., ... Zimov, S. A. (2008). Vulnerability of Permafrost Carbon to Climate Change:  
828           Implications for the Global Carbon Cycle. *BioScience*. <https://doi.org/10.1641/B580807>

829   Sinsabaugh, R. L., Belnap, J., Findlay, S. G., Shah, J. J. F., Hill, B. H., Kuehn, K. A., ...  
830           Warnock, D. D. (2014). Extracellular enzyme kinetics scale with resource availability.  
831           *Biogeochemistry*, 121(2). <https://doi.org/10.1007/s10533-014-0030-y>

832   Sinsabaugh, R. L., Lauber, C. L., Weintraub, M. N., Ahmed, B., Allison, S. D., Crenshaw, C., ...  
833           Zeglin, L. H. (2008). Stoichiometry of soil enzyme activity at global scale. *Ecology Letters*.  
834           <https://doi.org/10.1111/j.1461-0248.2008.01245.x>

835   Sjögersten, S., Caul, S., Daniell, T. J., Jurd, A. P. S., O’Sullivan, O. S., Stapleton, C. S., &  
836           Titman, J. J. (2016). Organic matter chemistry controls greenhouse gas emissions from  
837           permafrost peatlands. *Soil Biology and Biochemistry*, 98, 42–53.  
838           <https://doi.org/10.1016/j.soilbio.2016.03.016>

839   Smith, S. L., Burgess, M. M., Riseborough, D., & Nixon, F. M. (2005). Recent trends from

840 Canadian permafrost thermal monitoring network sites. *Permafrost and Periglacial*  
841 *Processes*, 16(1), 19–30. <https://doi.org/10.1002/ppp.511>

842 Smith, S. L., Wolfe, S. A., Riseborough, D. W., & Mark Nixon, F. (2009). Active-layer  
843 characteristics and summer climatic indices, Mackenzie Valley, Northwest Territories,  
844 Canada. *Permafrost and Periglacial Processes*, 20(2), 201–220.  
845 <https://doi.org/10.1002/ppp.651>

846 Steinweg, J. M., Kostka, J. E., Hanson, P. J., & Schadt, C. W. (2018). Temperature sensitivity of  
847 extracellular enzymes differs with peat depth but not with season in an ombrotrophic bog.  
848 *Soil Biology and Biochemistry*. <https://doi.org/10.1016/j.soilbio.2018.07.001>

849 Strakova, P., Niemi, R. M., Freeman, C., Peltoniemi, K., Toberman, H., Heiskanen, I., ... Laiho,  
850 R. (2011). Litter type affects the activity of aerobic decomposers in a boreal peatland more  
851 than site nutrient and water table regimes. *Biogeosciences*, 8(9), 2741–2755.  
852 <https://doi.org/10.5194/bg-8-2741-2011>

853 Straková, P., Penttilä, T., Laine, J., & Laiho, R. (2012). Disentangling direct and indirect effects  
854 of water table drawdown on above- and belowground plant litter decomposition:  
855 Consequences for accumulation of organic matter in boreal peatlands. *Global Change*  
856 *Biology*, 18(1), 322–335. <https://doi.org/10.1111/j.1365-2486.2011.02503.x>

857 Strickland, M. S., Osburn, E., Lauber, C., Fierer, N., & Bradford, M. A. (2009). Litter quality is  
858 in the eye of the beholder: Initial decomposition rates as a function of inoculum  
859 characteristics. *Functional Ecology*. <https://doi.org/10.1111/j.1365-2435.2008.01515.x>

860 Tarnocai, C. (1999). The effect of climate warming on the carbon balance of Cryosols in Canada.  
861 *Permafrost and Periglacial Processes*. [https://doi.org/10.1002/\(SICI\)1099-](https://doi.org/10.1002/(SICI)1099-)

862 1530(199907/09)10:3<251::AID-PPP323>3.0.CO;2-5

863 Tfaily, M. M., Cooper, W. T., Kostka, J. E., Chanton, P. R., Schadt, C. W., Hanson, P. J., ...  
864 Chanton, J. P. (2014). Organic matter transformation in the peat column at Marcell  
865 Experimental Forest: Humification and vertical stratification. *Journal of Geophysical*  
866 *Research: Biogeosciences*. <https://doi.org/10.1002/2013JG002492>

867 Tfaily, M. M., Hamdan, R., Corbett, J. E., Chanton, J. P., Glaser, P. H., & Cooper, W. T. (2013).  
868 Investigating dissolved organic matter decomposition in northern peatlands using  
869 complimentary analytical techniques. *Geochimica et Cosmochimica Acta*.  
870 <https://doi.org/10.1016/j.gca.2013.03.002>

871 Treat, C. C., Jones, M. C., Camill, P., Gallego-Sala, A., Garneau, M., Harden, J. W., ...  
872 Välliranta, M. (2016). Effects of permafrost aggradation on peat properties as determined  
873 from a pan-Arctic synthesis of plant macrofossils. *Journal of Geophysical Research:*  
874 *Biogeosciences*, 121(1), 78–94. <https://doi.org/10.1002/2015JG003061>

875 Turetsky, M. R., Wieder, R. K., Vitt, D. H., Evans, R. J., & Scott, K. D. (2007). The  
876 disappearance of relict permafrost in boreal north America: Effects on peatland carbon  
877 storage and fluxes. *Global Change Biology*, 13(9), 1922–1934.  
878 <https://doi.org/10.1111/j.1365-2486.2007.01381.x>

879 Turetsky, Merritt R., Abbott, B. W., Jones, M. C., Anthony, K. W., Olefeldt, D., Schuur, E. A.  
880 G., ... McGuire, A. D. (2020). Carbon release through abrupt permafrost thaw. *Nature*  
881 *Geoscience*. <https://doi.org/10.1038/s41561-019-0526-0>

882 Vitt, D. H., Halsey, L. A., & Zoltai, S. C. (2000). The changing landscape of Canada's western  
883 boreal forest: the current dynamics of permafrost. *Canadian Journal of Forest Research*,

884 30(2), 283–287. <https://doi.org/10.1139/x99-214>

885 Voigt, C., Marushchak, M. E., Mastepanov, M., Lamprecht, R. E., Christensen, T. R.,  
886 Dorodnikov, M., ... Biasi, C. (2019). Ecosystem carbon response of an Arctic peatland to  
887 simulated permafrost thaw. *Global Change Biology*, 25(5), 1746–1764.  
888 <https://doi.org/10.1111/gcb.14574>

889 Walker, T. N., Garnett, M. H., Ward, S. E., Oakley, S., Bardgett, R. D., & Ostle, N. J. (2016).  
890 Vascular plants promote ancient peatland carbon loss with climate warming. *Global Change*  
891 *Biology*. <https://doi.org/10.1111/gcb.13213>

892 Walker, T. W. N., Kaiser, C., Strasser, F., Herbold, C. W., Leblans, N. I. W., Wuebken, D., ...  
893 Richter, A. (2018). Microbial temperature sensitivity and biomass change explain soil  
894 carbon loss with warming. *Nature Climate Change*. [https://doi.org/10.1038/s41558-018-](https://doi.org/10.1038/s41558-018-0259-x)  
895 [0259-x](https://doi.org/10.1038/s41558-018-0259-x)

896 Wallenstein, M., Allison, S. D., Ernakovich, J., Steinweg, J. M., & Sinsabaugh, R. (2010).  
897 Controls on the Temperature Sensitivity of Soil Enzymes: A Key Driver of In Situ Enzyme  
898 Activity Rates. [https://doi.org/10.1007/978-3-642-14225-3\\_13](https://doi.org/10.1007/978-3-642-14225-3_13)

899 Wallenstein, M. D., McMahon, S. K., & Schimel, J. P. (2009). Seasonal variation in enzyme  
900 activities and temperature sensitivities in Arctic tundra soils. *Global Change Biology*.  
901 <https://doi.org/10.1111/j.1365-2486.2008.01819.x>

902 Weishaar, J. L., Aiken, G. R., Bergamaschi, B. A., Fram, M. S., Fujii, R., & Mopper, K. (2003).  
903 Evaluation of specific ultraviolet absorbance as an indicator of the chemical composition  
904 and reactivity of dissolved organic carbon. *Environmental Science and Technology*.  
905 <https://doi.org/10.1021/es030360x>

906 Wen, Y., Zang, H., Ma, Q., Evans, C. D., Chadwick, D. R., & Jones, D. L. (2019). Is the  
 907 ‘enzyme latch’ or ‘iron gate’ the key to protecting soil organic carbon in peatlands?  
 908 *Geoderma*, 349. <https://doi.org/10.1016/j.geoderma.2019.04.023>

909 Wickland, K. P., Striegl, R. G., Neff, J. C., & Sachs, T. (2006). Effects of permafrost melting on  
 910 CO<sub>2</sub> and CH<sub>4</sub> exchange of a poorly drained black spruce lowland. *Journal of Geophysical*  
 911 *Research: Biogeosciences*, 111(2), 1–13. <https://doi.org/10.1029/2005JG000099>

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914 **Tables**

Table 1. Average ( $\pm 1$  SD) growing season (May – September 2018) pore-water chemistry between the young bog (YB) and mature bog (MB)

	YB Shallow <sup>1</sup>	YB Deep <sup>2</sup>	MB Shallow <sup>3</sup>	MB Deep <sup>4</sup>
Water Table (cm)	-1.1 $\pm$ 3.5 <sup>a</sup>		-23.5 $\pm$ 3.9 <sup>b</sup>	
Mean Temperature (°C)	11.0 $\pm$ 4.4	7.0 $\pm$ 3.7	8.4 $\pm$ 5.0	3.9 $\pm$ 2.8
Maximum temperature	16.4	12.4	16.0	6.8
pH	4.19 $\pm$ 0.31 <sup>a</sup>	4.08 $\pm$ 0.22 <sup>a*</sup>	3.89 $\pm$ 0.18 <sup>b*</sup>	3.96 $\pm$ 0.18 <sup>b</sup>
DOC (mg C L <sup>-1</sup> )	52.5 $\pm$ 3.3 <sup>a*</sup>	54.1 $\pm$ 5.8 <sup>b*</sup>	68.6 $\pm$ 23.1 <sup>c</sup>	70.0 $\pm$ 10.9 <sup>c</sup>
SUVA (L mg C <sup>-1</sup> m <sup>-1</sup> )	3.6 $\pm$ 0.1 <sup>a*</sup>	3.1 $\pm$ 0.4 <sup>b*</sup>	2.7 $\pm$ 0.4 <sup>c</sup>	2.4 $\pm$ 0.4 <sup>c</sup>
Phenolics (mg L <sup>-1</sup> )	0.60 $\pm$ 0.19 <sup>a</sup>	0.64 $\pm$ 0.19 <sup>a*</sup>	0.58 $\pm$ 0.21 <sup>a</sup>	0.56 $\pm$ 0.15 <sup>a</sup>
S <sub>250–465</sub> (nm <sup>-1</sup> )	-0.016 $\pm$ 0.001 <sup>a*</sup>	-0.016 $\pm$ 0.002 <sup>a*</sup>	-0.017 $\pm$ 0.003 <sup>a</sup>	-0.018 $\pm$ 0.003 <sup>a*</sup>
TN (mg L <sup>-1</sup> )	0.92 $\pm$ 0.09 <sup>a</sup>	0.87 $\pm$ 0.12 <sup>a*</sup>	1.52 $\pm$ 1.77 <sup>b</sup>	1.53 $\pm$ 0.49 <sup>b</sup>
PO <sub>4</sub> <sup>3-</sup> (µg L <sup>-1</sup> )	10.1 $\pm$ 14.4 <sup>a</sup>	8.6 $\pm$ 14.5 <sup>a</sup>	5.8 $\pm$ 2.3 <sup>a*</sup>	8.0 $\pm$ 3.4 <sup>a</sup>

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Negative values indicate water table is beneath the peat surface

Letters indicate significant variations between four peat types (Bonferroni:  $P < 0.05$ ).

\* indicates significant monthly variation within each peat type (Bonferroni:  $P < 0.05$ ).

<sup>1</sup> Young Bog Shallow peat layers defined as 0 – 30 cm

<sup>2</sup> Young Bog Deep peat layers defined as 30 – 160 cm

<sup>3</sup> Mature Bog Shallow peat layers defined as 0 – 70 cm

<sup>4</sup> Mature Bog Deep peat layers defined as 70 – 160 cm