Assessing the Enzyme Latch Hypothesis in Arctic Peatlands: An Enzymatic Activity Approach

Introduction

Northern Arctic peatlands store vast amounts of partially decomposed organic carbon (C), representing 28% (390 Pg of the global C stock) of the terrestrial C stock in the world that has been accumulating for millennia (Freeman et al. 2004; Frolking and Roulet 2007). This accumulation of organic C in peat soils is the result of the peculiar characteristics of this ecosystem that cause higher rates of production than decomposition (Dunn and Freeman 2018). Arctic peatlands can be categorized as palsa, bog and fen. As illustrated in Figure 1, palsa soils are rich in permafrost, which is defined as ground that has been frozen for more than two consecutive years, and these freezing soil conditions limit microbial activity and decomposition (Koven et al. 2011; Swindles et al. 2015). Moving along the permafrost thaw gradient, bog soils have a thinner permafrost layer and a wider active layer, which thaws and refreezes every summer-winter period, and these soils are partially anoxic due to the presence of surface water from precipitation (Beilman, Vitt, and Halsey 2001). These anoxic conditions are more prevalent in fen due to its saturated soil conditions. Peatlands are critical in the global C cycle due to their role in C sequestration and storing (Romanowicz et al. 2015; Yu 2012). Nonetheless, permafrost is thawing at an accelerated rate due to global warming and increased snowfall, which results in more organic matter becoming available for microbes to decompose (Olefeldt and Roulet 2012). This results in the release of methane and carbon dioxide (Frolking and Roulet 2007; Treat et al. 2016), which could imply a change in peatlands' C accumulation to become sources of greenhouse gases to the atmosphere, and alter global C fluxes if the Arctic temperatures continue to increase (Freeman et al. 2004; Minayeva et al. 2017).

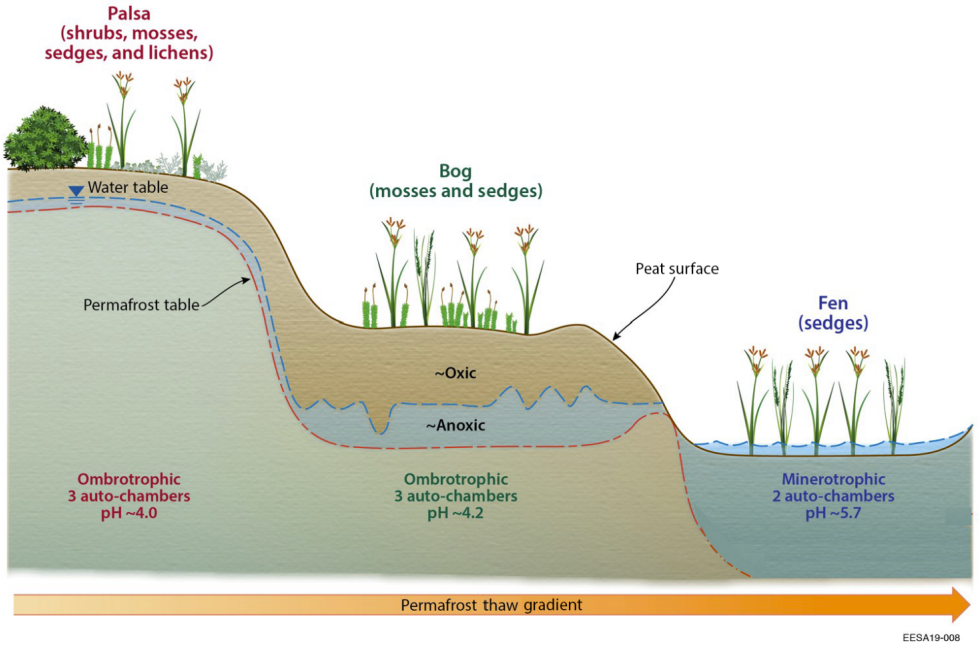

Figure 1: Visual representation of three types of Arctic peatland, i.e. palsa, bog and fen; including their corresponding vegetation, oxigenic conditions, and permafrost thawing gradient. From Chang et al. (2019).

Soil organic decomposition is carried out by microbial organisms that release enzymes into the soil matrix that break down large organic macromolecules and convert them into simpler molecules available to be assimilated by plants and microbes. Hydrolytic enzymes play an important role in the depolymerization of large polysaccharides to mineralize nitrogen and phosphorus, which are not oxygen-dependent (Urbanová

and Hájek 2021). On the other hand, oxidative enzymes (O2-dependent), such as phenol oxidases, are responsible for the decomposition of phenolic compounds that are produced by plants as secondary metabolites, e.g. flavonoids and tannins, which are commonly found in soil detritus (Dunn and Freeman 2018).

Low decomposition rates in Arctic peatlands are often attributed to the anoxic conditions that limit phenol oxidase activity. This statement has been defined as the "enzyme latch hypothesis" that proposes that peatland anoxia suppresses phenol oxidases, resulting in the accumulation of phenolics in soils, which are thought to be inhibitors of hydrolase activity. Thus, both oxidative and hydrolytic activities are constrained by phenols in anaerobic peat soils, reducing decomposition rates and increasing organic matter accumulation (Hall, Treffkorn, and Silver 2014).

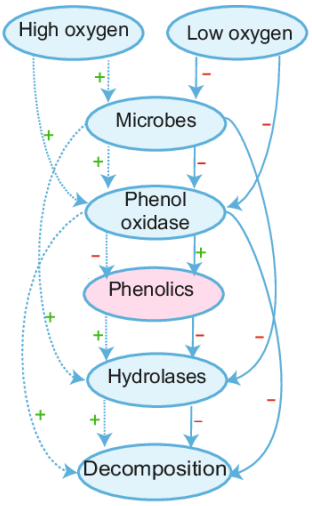

Figure 2: Diagram representation of the enzyme latch hypothesis. The oxygen levels in the peat environment are thought to affect phenol oxidase activity and result in the accumulation of phenolics, which in turn may affect hydrolase activity. From Belwase et al. (2016).

Despite the broadly acceptance of the enzyme latch hypothesis, there is not enough solid supporting evidence, and the low enzyme activity rates can be explained by other factors, such as low microbial biomass, seasonal changes in soil temperature, and wetland type (Urbanová and Hájek 2021). This study aims to examine the enzyme latch hypothesis along the thaw gradient (palsa, bog, fen) in Arctic peatland from Sweden through the analysis of oxidative and hydrolytic enzyme activities.

Methods

Sample Collection

27 samples were collected from Swedish palsa, bog and fen peat soils from three cores, at 5 cm, 10 cm and 20 cm depth each. These samples were shipped to the US for analysis.

Enzyme Assays

Soil microbes secrete extracellular enzymes into the soil matrix. These enzymes are responsible for breaking down large macromolecules into simpler compounds that can be easily assimilated by microbes and plants. Enzymes can be broadly categorized as hydrolytic and oxidative; a description of the type of assay used is found in Table 1. This study tested five hydrolytic enzymes, i.e. β-d-glucosidase, β-d-xylosidase, N-acetyl-β-d-glucosaminidase, arylsulphatase, and phosphatase, and the oxidative enzyme phenol oxidase. All enzymatic assays were performed at the University of New Hampshire.

Table 1: Summary of assay type and microplates used to perform hydrolytic and oxidative enzyme assays.

Enzyme Type	**Degrade**	**Assay**	**Microplate**
Hydrolytic	e.g. cellulose	Fluorimetric	Black
Oxidative	e.g. lignin	Colorimetric	Clear

A V-max test was performed on each enzyme to calculate the optimal incubation times and substrate concentrations. This information, along with the function of the six enzymes is found in Table 2. In addition, all enzymes tested required 50 μM sodium acetate (NaOAc) buffer with pH 4-5.6, and hydrolytic enzymes required 10 μM 4-Methylumbelliferone (MUB) as standard.

Table 2: List of enzymes tested in the study and their respective substrate, function, incubation time and substrate concentration.

Enzyme	**Substrate**	**Function**	**Incubation Time**	**Substrate Concentration (µM)**
Phenol oxidase	L-DOPA	Oxidation of benzenediols to semiquinones with O2	24 h	400
ß-d-glucosidase	4-MUB-ß-D-glucoside	Catalysis of hydrolysis of 1,4 linked ß-D-glucose residues from ß-D-glucosides	45 min	400
ß-d-xylosidase	4-MUB-ß-D-xyloside	Degradation of xylooligomers into xylose	30 min	400
N-acetyl-ß-d-glucosaminidase	4-MUB-N-acetyl-ß-D-glucosaminide	Catalysis of hydrolysis of 1,4 linked N-acetyl-ß-D-glucosaminide residues in chitooligosaccharides	45 min	400
Arylsulphatase	4-MUB-sulfate	Catalysis of desulfation of 3-O-sulfogalactosyl residues in glycosphingolipids	45 min	400
Phosphatase	4-MUB-phosphate	Mineralization of organic P into phosphate	45 min	600

All soils samples were homogenized by blending 1 g of soil and 60 mL of NaOAc buffer for 30 sec, and then adding additional 65 mL of buffer. The soil slurry was kept stirring on a stir plate before being transferred. Soil slurries were pipetted into their respective wells, along with MUB and NaOAc, including the control wells. Microplates were incubated (Table 2) before being read.

After incubation, microplates were read in the BioTek Synergy microplate reader using Gen5 software. Hydrolytic enzymes require an excitation wavelength of 360 nm and emission of 460 nm; whereas phenol oxidase requires 450 nm. This raw data was copied to an Excel file.

Enzyme activity was calculated as follows.

For oxidative enzyme activity:

$$Final\ abs = Mean\ Assay\ abs - (Sample\ Control\ abs + Substrate\ Control\ abs)$$

$$Final\ activity\ (\mu mol\ g^{-1}h^{-1}) = \frac{Final\ abs \cdot 125\ mL}{Emmision\ Coefficient \cdot 0.2\ mL \cdot h \cdot g}$$

For hydrolytic enzyme activity:

$$Final\ activity\ (\mu mol\ g^{-1}h^{-1}) = \frac{Net\ Fluorescence \cdot 125\ mL}{Emission\ Coefficient\ 0.2\ mL \cdot h \cdot g}$$

Results

The averaged enzyme activity per site showed highest activity for palsa and lowest for fen soils (Figure 3). Whereas surface (5 cm) samples showed highest enzyme values (Figure 4).

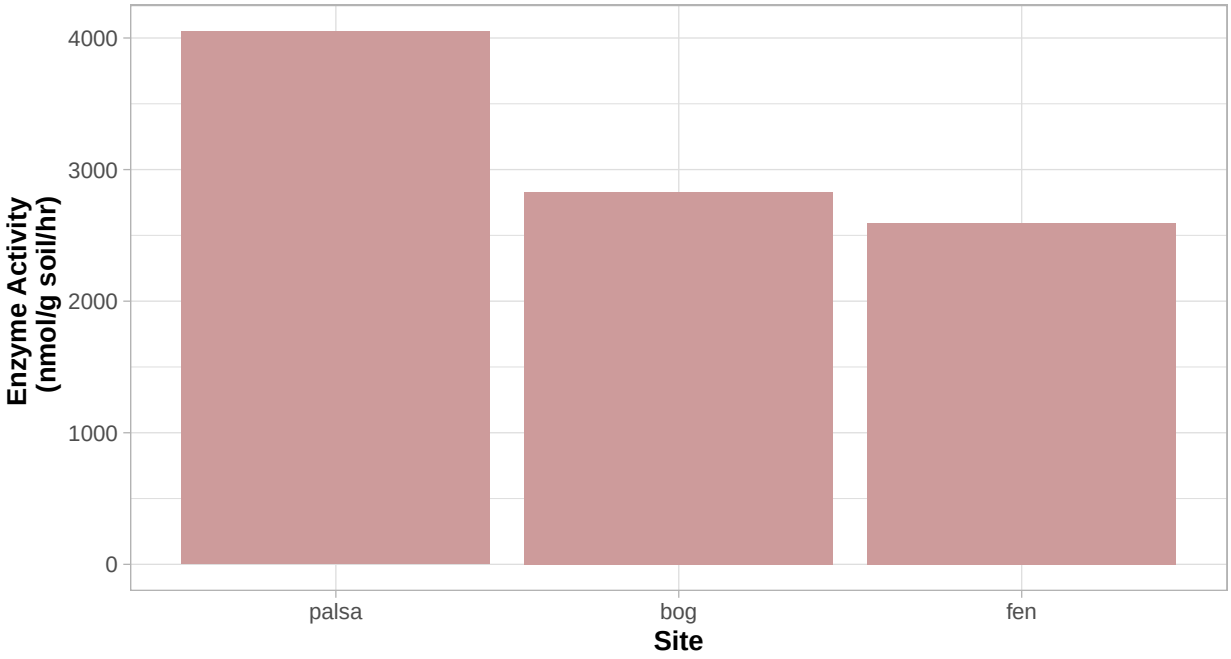

Figure 3. Average enzyme activity at palsa, bog and fen sites.

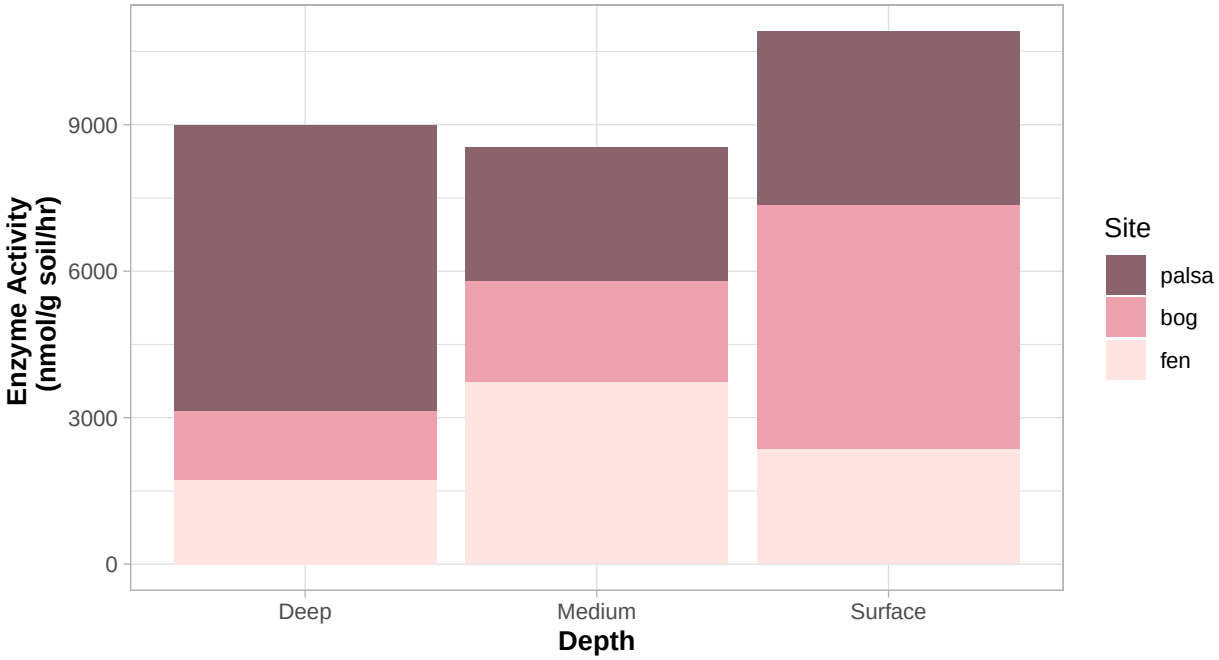

Figure 4. Average enzyme activity at the three different depths.

When evaluating the activity of individual enzymes, most enzymes showed values below 10,000 nmol/g/h in all soil sites. Nonetheless, phosphatase had values above 30,000 nmol/g/h for depth palsa. In addition,

phenol oxidase activity was below 1.5 nmol/g/h (Figure 5).

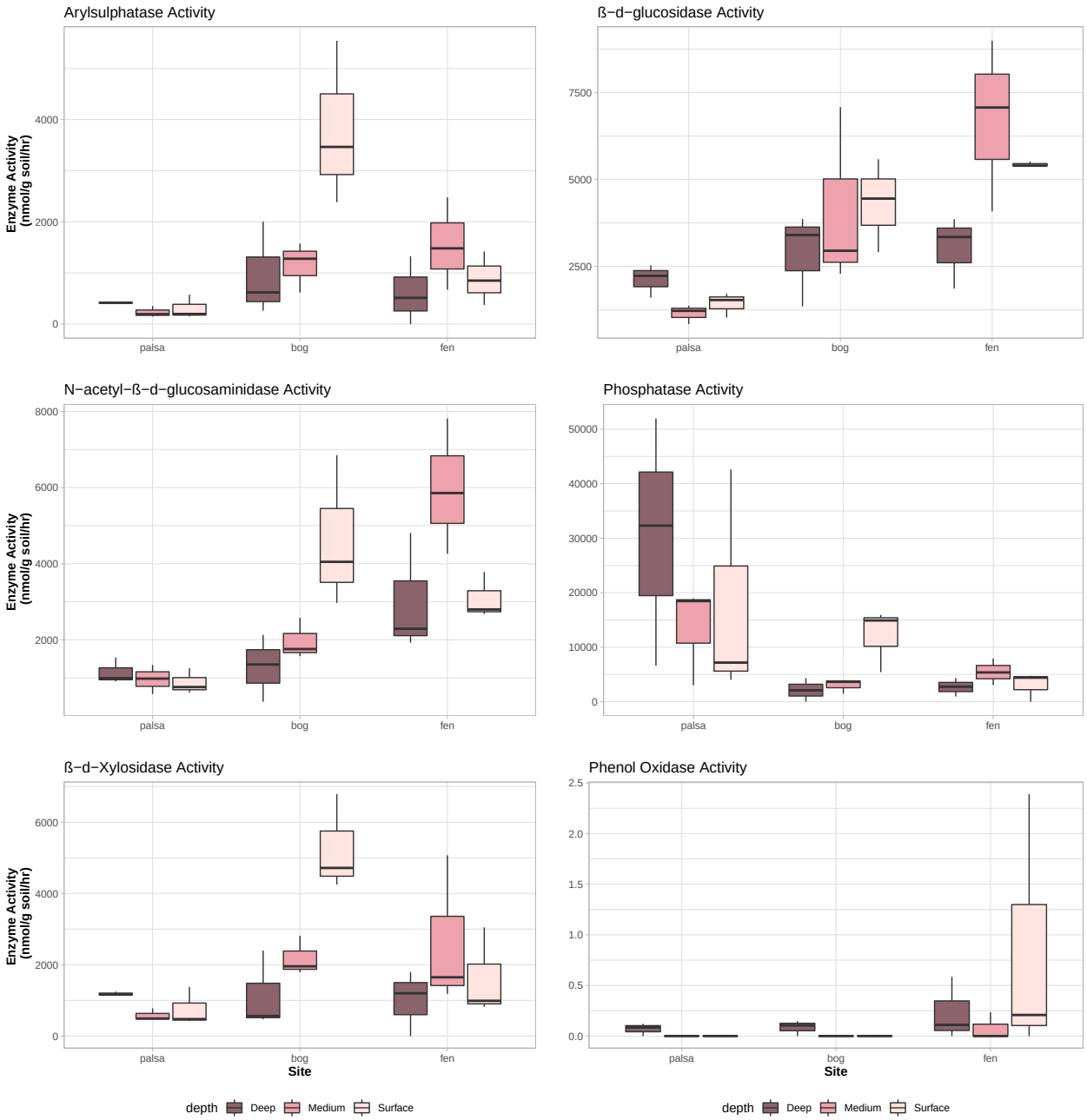

Figure 5. Average enzyme activity at the three different sites and depths.

Discussion

References

Beilman, David W., Dale H. Vitt, and Linda A. Halsey. 2001. "Localized Permafrost Peatlands in Western Canada: Definition, Distributions, and Degradation." *Arctic, Antarctic, and Alpine Research* 33 (1): 70–77. https://doi.org/10.1080/15230430.2001.12003406.

Belwase, Saraswati, Christian Dunn, William Mitsch, and Christopher Freeman. 2016. "Is Peat Accumu-

lation in Mangrove Swamps Influenced by the 'Enzymic Latch' Mechanism?" *Wetlands Ecology and Management* 24 (December). https://doi.org/10.1007/s11273-016-9493-z.

Chang, Kuang-Yu, William Riley, Patrick Crill, R. F. Grant, Virginia Rich, and Scott Saleska. 2019. "Large Carbon Cycle Sensitivities to Climate Across a Permafrost Thaw Gradient in Subarctic Sweden." *The Cryosphere* 13 (February): 647–63. https://doi.org/10.5194/tc-13-647-2019.

Dunn, Christian, and Chris Freeman. 2018. "The Role of Molecular Weight in the Enzyme-Inhibiting Effect of Phenolics: The Significance in Peatland Carbon Sequestration." *Ecological Engineering*, Wetlands and carbon revisited, 114 (April): 162–66. https://doi.org/10.1016/j.ecoleng.2017.06.036.

Freeman, C., N. J. Ostle, N. Fenner, and H. Kang. 2004. "A Regulatory Role for Phenol Oxidase During Decomposition in Peatlands." *Soil Biology and Biochemistry*, Enzymes in the environment: Activity, ecology and applications, 36 (10): 1663–67. https://doi.org/10.1016/j.soilbio.2004.07.012.

Frolking, Steve, and Nigel T. Roulet. 2007. "Holocene Radiative Forcing Impact of Northern Peatland Carbon Accumulation and Methane Emissions." *Global Change Biology* 13 (5): 1079–88. https://doi.org/10.1111/j.1365-2486.2007.01339.x.

Hall, Steven J., Jonathan Treffkorn, and Whendee L. Silver. 2014. "Breaking the Enzymatic Latch: Impacts of Reducing Conditions on Hydrolytic Enzyme Activity in Tropical Forest Soils." *Ecology* 95 (10): 2964–73. https://doi.org/10.1890/13-2151.1.

Koven, Charles D., Bruno Ringeval, Pierre Friedlingstein, Philippe Ciais, Patricia Cadule, Dmitry Khvorostyanov, Gerhard Krinner, and Charles Tarnocai. 2011. "Permafrost Carbon-Climate Feedbacks Accelerate Global Warming." *Proceedings of the National Academy of Sciences* 108 (36): 14769–74. https://doi.org/10.1073/pnas.1103910108.

Minayeva, Tatiana, Andrey Sirin, Peter Kershaw, and Olivia Bragg. 2017. "Arctic Peatlands." In *The Wetland Book: II: Distribution, Description and Conservation*, edited by C. Max Finlayson, G. Randy Milton, R. Crawford Prentice, and Nick C. Davidson, 1–15. Dordrecht: Springer Netherlands. https://doi.org/10.1007/978-94-007-6173-5_109-2.

Olefeldt, David, and Nigel T. Roulet. 2012. "Effects of Permafrost and Hydrology on the Composition and Transport of Dissolved Organic Carbon in a Subarctic Peatland Complex." *Journal of Geophysical Research: Biogeosciences* 117. https://doi.org/10.1029/2011JG001819.

Romanowicz, Karl J., Evan S. Kane, Lynette R. Potvin, Aleta L. Daniels, Randall K. Kolka, and Erik A. Lilleskov. 2015. "Understanding Drivers of Peatland Extracellular Enzyme Activity in the PEATcosm Experiment: Mixed Evidence for Enzymic Latch Hypothesis." *Plant and Soil* 397 (1): 371–86. https://doi.org/10.1007/s11104-015-2746-4.

Swindles, Graeme T., Paul J. Morris, Donal Mullan, Elizabeth J. Watson, T. Edward Turner, Thomas P. Roland, Matthew J. Amesbury, et al. 2015. "The Long-Term Fate of Permafrost Peatlands Under Rapid Climate Warming." *Scientific Reports* 5 (1): 17951. https://doi.org/10.1038/srep17951.

Treat, C. C., M. C. Jones, P. Camill, A. Gallego-Sala, M. Garneau, J. W. Harden, G. Hugelius, et al. 2016. "Effects of Permafrost Aggradation on Peat Properties as Determined from a Pan-Arctic Synthesis of Plant Macrofossils." *Journal of Geophysical Research: Biogeosciences* 121 (1): 78–94. https://doi.org/10.1002/2015JG003061.

Urbanová, Zuzana, and Tomáš Hájek. 2021. "Revisiting the Concept of 'Enzymic Latch' on Carbon in Peatlands." *Science of The Total Environment* 779 (July): 146384. https://doi.org/10.1016/j.scitotenv.2021.146384.

Yu, Z. C. 2012. "Northern Peatland Carbon Stocks and Dynamics: A Review." *Biogeosciences* 9 (10): 4071–85. https://doi.org/10.5194/bg-9-4071-2012.