Effects of fertilization and seasonality on phosphorus pools in an ombrotrophic bog

Camille Chaussier Jones
Department of Geography
McGill University, Montreal

December 2024

A thesis submitted to McGill University in partial fulfillment of the requirements of the degree of Master of Science

Contents

Contents 1

Abstract 4

Résumé 5

Acknowledgements 8

Contribution of Authors 9

Figures 10

Tables 12

1. Introduction 13
   - 1.1. A Note on Nomenclature 14

2. Literature Review 15
   - 2.1. Peatlands 15
   - 2.2. Phosphorus Cycling in Ombrotrophic Peatlands 16
   - 2.3. Physical Disturbance and Diversity 17
   - 2.4. Nutrient Addition and Mer Bleue Bog 18
   - 2.5. Hedley Sequential Fractionation 19
   - 2.6. Benefits and Limitations of Sequential Fractionation 22
   - 2.7. Microbial Phosphorus 24
   - 2.8. Forms of Phosphorus in Microorganisms 25

3. Seasonal Changes in Labile and Microbial Phosphorus Concentrations in an Ombrotrophic Bog in Eastern Ontario, Canada 28
   - Abstract 28
   - 3.1. Introduction 29
     - 3.1.1. Environmental Variables 31
     - 3.1.2. Hydrology 32
     - 3.1.3. Objectives and Hypotheses 33
   - 3.2. Methods 34
     - 3.2.1. Field Site 34
     - 3.2.2. Sampling 34
     - 3.2.3. Characteristics of Peat Soils Affecting Phosphorus Extraction 35
     - 3.2.4. Anion Exchange Resin Extraction 38
     - 3.2.5. Phosphorus Quantification by Malachite Green Colorimetry 38
     - 3.2.6. Data Analysis & Visualization 39

3.3. Results 39
3.4. Discussion 41
3.5. Conclusion 44
Figures 46
References 55
A Deeper Look at Peat Phosphorus Pools 62
4. Nutrient Addition Increases Available and Residual Phosphorus Concentrations in an Ombrotrophic Bog 63
Abstract 63
4.1. Introduction 64
4.1.1. Objectives and Hypotheses 66
4.2. Methods 67
4.2.1. Site Description 67
4.2.2. Sample Preparation 68
4.2.3. Hedley Sequential Fractionation 69
4.2.4. Statistics 70
4.3. Results 71
4.4. Discussion 73
4.4.1. Resin-extractable P 73
4.4.2. Bicarbonate-extractable P 74
4.4.3. NaOH-extractable P 74
4.4.4. 1 M HCl-extractable P 75
4.4.5. Residual P 76
4.4.6. Total P 76
4.4.7. Patterns 78
4.4.8. Comparison to Published Values 78
4.4.9. Mer Bleue Fertilization Plots P Budget 80
4.5. Implications 81
4.6. Conclusions 82
Tables and Figures 83
Supplemental Materials 92
References 94
5. Discussion of Phosphorus Movement 101
5.1. Movement of Phosphorus Aboveground 101

5.2. Movement Between Labile Phosphorus Pools 102
5.3. Phosphorus Pulses and Disturbance 103
6. Conclusions 105
References 108

Abstract

Nutrient cycling and nutrient status control primary productivity, decomposition, plant community composition, and microbial community composition in ombrotrophic bogs. Ombrotrophic bogs in Canada are often nitrogen and phosphorus co-limited, meaning that changes in phosphorus concentrations have dramatic effects on ombrotrophic bog ecology. To study the current state of biotic phosphorus cycling, I measured microbial and labile phosphorus concentrations in Mer Bleue Bog near Ottawa, ON over a period of 14 months. Both microbial and labile phosphorus concentrations varied greatly through the year. This variation is linked to changes in water table depth and season. High water tables were associated with higher microbial phosphorus concentrations, while low water tables were associated with lower microbial phosphorus concentrations, even in layers that remained saturated during the entire year. During a year with a mild winter, microbial phosphorus concentrations were high during late winter and during spring snowmelt, averaging 115 to 130 mg kg^{-1} P in layers above the water table. Microbial phosphorus concentrations then declined through the growing season as plants became more competitive, averaging 45 to 105 mg kg^{-1} P in layers above the water table. Labile phosphorus concentrations were often low, averaging 2 to 12 mg kg^{-1} P, but increased sharply to 45 mg kg^{-1} P in the upper layers of peat for short periods of time before being taken up by microorganisms and plants. Both microbial and labile phosphorus concentrations were highest near the surface and decreased with depth from 0 to 75 cm.

To study the effects of nutrient addition and nutrient limitation on peatland soil phosphorus pools, samples were taken from the long-term fertilization experiment at Mer Bleue Bog. Samples were taken in 2021 following nearly twenty years of fertilizer application. These samples were subjected to a Hedley sequential fractionation to extract phosphorus along a chemical gradient of biological availability. In unfertilized peat, phosphorus concentrations were highest in the available and highly recalcitrant pools, with little between them. This U-shaped distribution of phosphorus along the gradient of availability contrasts with established patterns in mineral soils. In plots which received PK and NPK fertilizers, concentrations of both available phosphorus and highly recalcitrant phosphorus doubled. In plots receiving N fertilization alone, available and total P concentrations decreased, which may indicate increased demand for P by plants and microorganisms when N status is high. In all plots receiving fertilizer, concentrations of highly recalcitrant phosphorus increased, which may indicate increased decomposition of peat. In addition, fertilization led to changes aboveground. *Chamaedaphne calyculata* leaves in plots receiving PK and NPK were enriched in phosphorus compared to *C. calyculata* leaves in unfertilized plots and plots receiving N alone.

Résumé

Les cycles et les niveaux des nutriments contrôlent la productivité primaire, la décomposition, la composition de la communauté botanique et la composition de la communauté microbienne dans les tourbières ombrotrophiques. Les tourbières ombrotrophiques au Canada sont souvent co-limitées en azote et en phosphore, ce qui signifie que les changements des concentrations de phosphore ont des effets profonds sur l'écologie des tourbières ombrotrophiques. Pour étudier l'état actuel du cycle biotique du phosphore, j'ai mesuré les concentrations microbiennes et labiles de phosphore dans la tourbière Mer Bleue près d'Ottawa, en Ontario, sur une période de 14 mois. Les concentrations de phosphore microbien et labile variaient considérablement au cours de l'année. Cette variation est liée aux changements de

profondeur et de saison de la nappe phréatique. Les nappes phréatiques élevées étaient associées à des concentrations microbiennes de phosphore plus élevées, tandis que les nappes phréatiques profondes étaient associées à des concentrations microbiennes de phosphore plus faibles, même dans les couches qui restaient saturées pendant toute l’année. Au cours d’une année où l’hiver était doux, les concentrations de phosphore microbien étaient élevées à la fin de l’hiver et pendant la fonte des neiges printanière, avec une moyenne de 115 à 130 mg kg^{-1} P dans les couches au-dessus de la nappe phréatique. Les concentrations microbiennes de phosphore ont ensuite diminué au cours de la saison de croissance à mesure que les plantes devenaient plus compétitives, atteignant en moyenne 45 à 105 mg kg^{-1} P dans les couches au-dessus de la nappe phréatique. Les concentrations de phosphore labile étaient souvent faibles, atteignant en moyenne 2 à 12 mg kg^{-1} P, mais augmentaient fortement pour atteindre 45 mg kg^{-1} P dans les couches supérieures de tourbe pendant de courtes périodes avant d’être absorbées par les micro-organismes et les plantes. Les concentrations de phosphore microbien et labile étaient les plus élevées près de la surface et diminuaient avec la profondeur de 0 à 75 cm.

Pour étudier les effets de l’ajout de nutriments et de la limitation des nutriments sur les tipes de phosphore des tourbières, des échantillons ont été prélevés à partir de l’expérience de fertilisation à long terme à la tourbière Mer Bleue. Des échantillons ont été prélevés en 2021 après près de vingt ans d’application d’engrais. Ces échantillons ont été soumis à un fractionnement séquentiel de Hedley pour extraire le phosphore le long d’un gradient chimique de disponibilité biologique. Dans la tourbe non fertilisée, les concentrations de phosphore étaient les plus élevées dans les tipes disponibles et très récalcitrants, avec peu entre eux. Cette distribution en forme de U du phosphore le long du gradient de disponibilité contraste avec les schémas établis dans les sols minéraux. Dans les parcelles qui ont reçu des engrais PK et NPK, les concentrations de phosphore disponible et de phosphore très récalcitrant ont doublé. Dans les parcelles recevant uniquement de l’azote, les concentrations disponibles et totales de P ont diminué, ce qui peut indiquer une demande accrue de P par les plantes et les micro-organismes lorsque le statut en azote est élevé. Dans toutes les parcelles recevant de l’engrais, les concentrations de phosphore très récalcitrant ont augmenté, ce qui peut indiquer une décomposition accrue de la tourbe. En outre, la fertilisation a conduit à des changements en surface. Les feuilles de *Chamaedaphne calyculata* dans les parcelles recevant du PK et du NPK ont été enrichies en phosphore par rapport aux feuilles de *C. calyculata* dans les parcelles non fertilisées et les parcelles recevant du N seul.

Acknowledgements

My sincere thanks to all of the members of the Environmental Biogeochemistry lab, past & present: Kathrin Listl, Calder Jones, Shir Gruber, Janice Neumann, Melody Lynch, Camille Brais, Autumn Pereira, Charles Duncan, Noa Caspi, Olivier Grégoire, Julie Cagnard, Maya Berman, and Lev Stefanovich-Thomson. Many thanks for your excellent feedback on my presentations, advice on laboratory issues, and good cheer while helping me collect many kilograms of peat. I am so excited to see where research takes you.

To Geography's fantastic laboratory & field technicians, Mike Dalva & Paula Kestelman, who have prevented the loss of fingers, limbs, and data too many times to count,

To Environment & Climate Change Canada, NSERC, Mr. Roger Warren, and the McGill Department of Geography for making this work possible through their generosity,

To my partner, Elias Andraos, for his kindness, excellent advice, and frightening enthusiasm wielding the auger,

To my family, for their support and joy,

To professors Gail Chmura, Cynthia Kallenbach, Anthony Williams-Jones, Vincent van Hinsberg, Graham Macdonald, and Bernhard Lehner for their superb teaching during this degree. My writing and presentation skills are immensely improved thanks to your care.

To Professor Chandra Matramootoo for his superb feedback on this manuscript,

And to my supervisor, Christian von Sperber, for his support and excellent feedback, which pressed the unconsolidated *Sphagnum* stems of my writing into information-dense peat.

Contribution of Authors

Authors: Camille Jones[1], Tim Moore[1], Cynthia Kallenbach[2], Elyn Humphreys[3], Autumn Pereira[4], Camille Brais[1], Meng Wang[5], Christian von Sperber[1]

1 Department of Geography, McGill University, Montreal, QC

2 Department of Natural Resource Sciences, McGill University, Sainte Anne de Bellevue, QC

3 Department of Geography and Environmental Studies, Carleton University, Ottawa, ON

4 School of Integrative Plant Science, Cornell University, Ithaca, NY, USA

5 School of Geographical Sciences, Northeast Normal University, Changchun, Jilin

C.J., A.P., C.B., and C.v.S. collected samples. C.J. and A.P. prepared the samples and conducted lab analyses. C.J. analyzed the data and wrote the entirety of this thesis. T.M., C.K., and C.v.S. provided feedback on the manuscripts. M.W. provided inspiration for the sequential fractionation experiment. E.H. kindly provided data for the water table level and temperature at Mer Bleue. C.v.S supervised the work. All authors have consented for their names and contributions to appear as such in this thesis.

Figures

Figure 2-1 Flow chart of the steps of the sequential P extraction. 27
Figure 3-1 Simplified phosphorus cycle in an ombrotrophic bog. Processes and pools in green are mediated by microbes. 46
Figure 3-2 The von Post humification scale with colors found in ombrotrophic bog peat. 47
Figure 3-3 July 2022 microbial and labile phosphorus by layer reported in mg kg-1 P. Error bars represent one standard deviation above and below. 48
Figure 3-4 August 2022 microbial and labile phosphorus by layer reported in mg kg-1 P. Error bars represent one standard deviation above and below. 48
Figure 3-5 October 2022 microbial and labile phosphorus by layer reported in mg kg-1 P. Error bars represent one standard deviation above and below. 49
Figure 3-6 March 2023 microbial and labile phosphorus by layer reported in mg kg-1 P. Error bars represent one standard deviation above and below. 49
Figure 3-7 April 2023 microbial and labile phosphorus by layer reported in mg kg-1 P. Error bars represent one standard deviation above and below. 50
Figure 3-8 May 2023 microbial and labile phosphorus by layer reported in mg kg-1 P. Error bars represent one standard deviation above and below. 50
Figure 3-9 June 2023 microbial and labile phosphorus by layer reported in mg kg-1 P. Error bars represent one standard deviation above and below. 51
Figure 3-10 July 2023 microbial and labile phosphorus by layer reported in mg kg-1 P. Error bars represent one standard deviation above and below. 51
Figure 3-11 August 2023 microbial and labile phosphorus by layer reported in mg kg-1 P. Error bars represent one standard deviation above and below. 52
Figure 3-12 Microbial and labile phosphorus in layer H1 plotted with water table depth from July 2022 to August 2023. Water table depth reported as the mean of the week preceding sampling. Error bars represent one standard deviation above and below. 53
Figure 3-13 Microbial and labile phosphorus in layer H4 plotted with water table depth from July 2022 to August 2023. Note that the values for March 2023 come from the H3 layer, not H4 as in the rest of the months sampled. Water table depth reported as the mean of the week preceding sampling. Error bars represent one standard deviation above and below. 54
Figure 4-1 Map of Mer Bleue Bog. Made with OpenStreetMap. 84
Figure 4-2 Peat core sampled with Russian auger from water table to 48 cm below water table surface. 85
Figure 4-3 Flow chart of the steps of the sequential P extraction. 86
Figure 4-4 Hedley Resin Pt by Layer in mg kg-1 P. Boxplots are grouped by sampling depth. Color corresponds to treatment. Compact letter displays (clds) separate the treatments within each layer at 95% confidence. 87

Figure 4-5 Hedley Bicarbonate-Extractable Pi by Layer in mg/kg P. Boxplots are grouped by sampling depth. Color corresponds to treatment. Compact letter displays (clds) separate the treatments within each layer at 95% confidence. 87
Figure 4-6 Hedley Bicarbonate-Extractable Po by Layer in mg/kg P. Boxplots are grouped by sampling depth. Color corresponds to treatment. Compact letter displays (clds) separate the treatments within each layer at 95% confidence. 88
Figure 4-7 Hedley NaOH-Extractable Pt by Layer in mg/kg P. Boxplots are grouped by sampling depth. Color corresponds to treatment. Compact letter displays (clds) separate the treatments within each layer at 95% confidence. 88
Figure 4-8 Hedley 1 M HCl-Extractable Pi by Layer in mg/kg P. Boxplots are grouped by sampling depth. Color corresponds to treatment. Compact letter displays (clds) separate the treatments within each layer at 95% confidence. Outliers are shown as dots. 89
Figure 4-9 Hedley Concentrated HCl-Extractable Pt by Layer in mg/kg P. Boxplots are grouped by sampling depth. Color corresponds to treatment. Compact letter displays (clds) separate the treatments within each layer at 95% confidence. Outliers are shown as dots. 89
Figure 4-10 Hedley Residual Pt by Layer in mg/kg P. Boxplots are grouped by sampling depth. Color corresponds to treatment. Compact letter displays (clds) separate the treatments within each layer at 95% confidence. 90
Figure 4-11 Hedley Total P by Layer in mg/kg P. Sum of resin-, bicarbonate-, NaOH-, 1 M HCl-, conc. HCl-extractable and residual P concentrations. Boxplots are grouped by sampling depth. Color corresponds to treatment. Compact letter displays (clds) separate the treatments within each layer at 95% confidence. 90
Figure 4-12 Percent of total P made up by the residual fraction. Boxplots are grouped by sampling depth. Color corresponds to treatment. Compact letter displays (clds) separate the treatments within each layer at 95% confidence. 91
Figure 4-13 Aluminum concentration as measured by XRF. Color corresponds to layer. Outliers are shown as dots. 91
Figure 4-14 Total P concentrations in Chamaedaphne calyculata leaves in the control plot and fertilized plots as measured by XRF. Compact letter displays (clds) separate the treatments within each layer at 95% confidence. 92
Figure 4-15 Calcium concentration as measured by XRF. Color corresponds to layer. Outliers are shown as dots. 92
Figure 4-16 Iron concentration as measured by XRF. Color corresponds to layer. Outliers are shown as dots. 93

Tables

Table 3-1 . Range of mean values for each extraction from this experiment, from unfertilized mollisols, and from fertilized mollisols. The highest mean ranges for each extract are shown in bold. *Note that Schlichting et al. 2002 used 1 M H¬2SO4 for the acid extraction, where Negassa & Leinweber (2009), Cross & Schlesinger (1995), and this experiment used 1 M HCl.83
Table 3-2 Mer Bleue phosphorus budget 2000-2021.. 83

1. Introduction

Peatlands store approximately one-third of our planet's total soil carbon despite making up less than one-twentieth of the land surface area (Gorham, 1991; Hugelius et al., 2020). For comparison, this is five times as much carbon as China's entire coal reserve, which is the largest of any nation (Dai & Finkelman, 2020). However, unlike buried fossil carbon, peat is found on the planet's surface, making it vulnerable to environmental change. Permafrost thaw, drought, fire, and human disturbance represent some of the largest threats. All of these forms of disturbance change the nutrient status of the peat, and thus the rate of decomposition and carbon emission. In order to understand some of the potential changes that may occur with disturbance, we must first understand the current nutrient cycle in peatlands and the effects of different nutrient levels on peat. My contribution to these goals was to answer the following questions:

1. How do microbial and labile phosphorus concentrations in an ombrotrophic bog vary throughout the year?
2. Which key variables control changes in microbial and labile phosphorus concentrations?
3. What is the distribution of phosphorus between labile, moderately labile, and recalcitrant pools of phosphorus in an undisturbed peatland?
4. How does nutrient addition alter the distribution of phosphorus between those same phosphorus pools?

1.1. A Note on Nomenclature

Unfortunately, peat chemistry is more complex than we would perhaps like it to be. In the following pages, I will frequently discuss labile, microbial, and recalcitrant pools of phosphorus. There is debate about the exact definitions of these pools. By labile phosphorus, I am generally referring to molybdate-reactive phosphorus. By some definitions, labile phosphorus is exclusively free orthophosphate in solution. As molybdate-reactive phosphorus often includes acid-labile colloidal and organic phosphates along with free orthophosphate, it does not perfectly fit the definition (Turner et al., 2003). However, I would argue that this acid-labile phosphorus is still easily taken up by plants and microbes and is thus similar to orthophosphate in both its degree of lability and role in the ecosystem. As the focus of this thesis is the potential participation of various forms of phosphorus in ecological cycling, acid-labile colloidal and organic phosphorus will be lumped in with orthophosphate as the labile pool. To avoid confusion in the sequential extraction chapter, I will generally use operational definitions like bicarbonate-extractable phosphorus or resin-extractable phosphorus.

2. Literature Review

2.1. Peatlands

Peatlands are wetlands that have accumulated organic matter to a depth of at least forty centimeters (Warner & Rubec, 1997). Given time, many accumulate far more than that, occasionally reaching twenty or thirty meters thick (Warner & Rubec, 1997). Peatlands accomplish this feat despite low rates of primary productivity and low soil nutrient concentrations. Instead, massive stocks of organic matter accumulate in peatlands due to low rates of decomposition.

The term "peatland" includes diverse ecosystems with a wide range of soil conditions. The first distinction within peatlands is between ombrotrophic, or cloud-fed, and minerotrophic, or mineral-fed peatlands. These divide peatlands by their source of water and nutrients. Minerotrophic peatlands receive inflow from groundwater or surface water, while ombrotrophic peatlands do not (Mitsch & Gosselink, 2015). Instead, ombrotrophic peatlands receive water and nutrients from precipitation exclusively (Mitsch & Gosselink, 2015). As a consequence, nutrient concentrations in ombrotrophic peatlands are much lower than in minerotrophic peatlands, selecting for plant and microbial communities with adaptations to survive high C:N and C:P ratios (Mitsch & Gosselink, 2015).

Minerotrophic peatlands may slowly become ombrotrophic as organic matter accumulates and moves the living surface further away from bedrock, groundwater, and surface water. This transformation occurs slowly due to the slow pace of peat accumulation. For example, Mer Bleue Bog, the site studied in the following chapters, spent about two and a half thousand years post-glaciation as a minerotrophic fen (Elliott et al., 2012). At about six thousand years ago, the peat had accumulated enough that the surface was no longer chemically influenced by groundwater, and the oxbow of the Saint Lawrence that had been providing surface water changed course away from the bog (Elliott et al., 2012). At this point, plant residues preserved in the peat indicate a transition toward bog species with lower nutrient requirements (Elliott et al., 2012).

2.2. Phosphorus Cycling in Ombrotrophic Peatlands

Phosphorus is critical for all life on earth. Nucleic acids, cell membranes, and ATP all contain phosphorus; without it, cells could not carry out their basic functions. However, it is not universally available. Some soils have a surplus of phosphorus, while others are phosphorus-limited (Ellsworth et al., 2022; MacDonald et al., 2011). Ombrotrophic bogs are particularly nutrient-limited due to the process by which they form (Aerts et al., 1992; Jirousek et al., 2015; Mitsch & Gosselink, 2015). Without connection to underlying mineral soils or parent material, the only input of phosphorus in ombrotrophic peatlands is atmospheric deposition, either dry or with precipitation. This is generally in the form of phosphate, an inorganic form of phosphorus that is readily taken up by microorganisms and plants. Upon uptake, plants and microorganisms convert labile phosphate into organic forms of phosphorus like nucleic acids, phospholipid cell membranes, ATP, or other cell components. When these organisms die, their organic phosphorus remains in their tissues, requiring enzymatic breakdown into inorganic phosphate in order to be taken back up by living microorganisms and plants. If not converted to inorganic phosphate and taken up, organic phosphorus in plant litter and dead microorganisms becomes humified, and may eventually enter the residual pool as phosphonates or phosphate esters (Barbanti et al., 1994).

While the phosphorus cycle in peatlands is dominated by the organic phase, labile phosphate may sorb to the inorganic phase in some soils. In acid soils, phosphate availability may be lowered through sorption by iron, aluminum, and manganese oxides, hydroxides, and sesquioxides, while carbonate and calcium (apatite) are generally responsible for lowering phosphate availability through precipitation in more alkaline soils. Where clays are present, they may sorb phosphate as well.

2.3. Physical Disturbance and Diversity

Without connection to groundwater, the top layers of an ombrotrophic bog experience profound drying and re-wetting during the growing season (Yevdokimov et al., 2016), likely causing large changes in microbial phosphorus cycling. Drying can kill bacteria and fungi, and those that survive often release some of their cytoplasm and phosphates to their surroundings (Birch, 1961). Due to the scarcity of phosphorus and the variable wetness of the peat, I expect that microbial phosphorus cycling in this environment is opportunistic and responds rapidly to changes in external conditions. Other factors affecting microbial phosphorus cycling in bogs are their low pH and oxygen levels, depending on depth. While the uppermost layer is well oxygenated, anoxia in lower layers prevents aerobic respiration. This restricts the types of microorganisms found in deeper bog soils, as many are unable to survive these conditions. In particular, the fungal species present in bogs are very different than fungal species found in neighboring forests or agricultural soils since most fungi cannot survive anoxic conditions as well as bacteria and archaeans can (Golovchenko et al., 2010). Still, fungal biomass in bog soils is often higher than bacterial biomass despite limits on diversity (Andersen et al., 2006). In this way, bog microbial communities are similar to plant species communities in bogs and other marginal ecosystems; local biodiversity is low, but the species found there are highly specialized and often rare elsewhere, making them of great importance for global biodiversity (Howie et al., 2016; Mitsch & Gosselink, 2015). Low nutrient content is part of what makes this ecosystem marginal and precludes many generalist species. Instead, strategies of slow growth and nutrient-holding prevail (Howie et al., 2016). Their low competitiveness in environments with high nutrient concentrations means that nutrient contamination of ombrotrophic bogs may dramatically change species composition across all taxonomic groups.

2.4. Nutrient Addition and Mer Bleue Bog

Finally, while ombrotrophic bogs are generally nutrient-limited, nutrient deposition in these ecosystems increased significantly during the twentieth century. While nitrogen deposition in eastern Canada has slowed during the past two decades, it remains at least double the pre-industrial deposition rate (Kanakidou et al., 2016). Phosphorus deposition is also higher than pre-industrial levels and varies strongly from year to year (Tipping et al., 2014). Increased nutrient deposition has been shown to change the vegetation structure at Mer Bleue Bog, increasing vascular plant growth and decreasing non-vascular plant growth (Bubier et al., 2007; Larmola et al., 2013). This generally increases the level of the water table, making nutrient-rich areas wetter in the short-term (Larmola et al., 2013). However, little is known about long-term effects, and even less is known about changes below vegetation in the peat itself (Loisel et al., 2021).

Mer Bleue has been the site of a long-term fertilization trial since 2001. 3m x 3m plots were set up in triplicate and received nitrogen fertilizer (NH_4NO_3) with or without potassium and phosphate (KH_2PO_4) dissolved in distilled water. Ammonium nitrate was applied at 16, 32, or 64 $kgha^{-1}$. Potassium and phosphorus were applied at 50 $kgha^{-1}$ per year and 63 $kgha^{-1}$ per year

(Juutinen et al., 2010). Control plots received 2-mm distilled water with no fertilizer until 2021, at which point all fertilizers were applied as a dry powder due to logistical constraints during the COVID-19 pandemic. The control plots continued to receive nothing (Brais, 2022). Treatments were applied every third week from May until the end of August (Juutinen et al., 2010). In this analysis, the 10N, PK, 10NPK, and control (O) plots were sampled.

2.5. Hedley Sequential Fractionation

In order to understand how nutrient addition affects peat soil nutrients, I performed a Hedley sequential fractionation on samples taken from the Mer Bleue fertilization trial. This sequential fractionation measures soil phosphate concentrations by subjecting samples to a series of different extraction solutions moving from weak and alkaline to strong and acidic (Fig. 2-1) (Hedley & Stewart, 1982). This follows a gradient of phosphorus bioavailability – phosphorus found in the first two fractions is of high bioavailability, while phosphorus extracted in the last fraction is of very low bioavailability. See "Nutrient Addition Increases Labile and Residual Phosphorus Concentrations in an Ombrotrophic Bog" for detailed analysis of the fractions. Of the several different phosphorus sequential fractionation methods that exist, Tiessen & Moir's (1993) update to Hedley & Stewart's (1982) fractionation scheme was chosen because it is well-suited to peat soils and has a long history of use, allowing for comparison with a diverse range of soil types and land uses.

Soil phosphorus fractionation has been practiced for nearly a century, though methods and interpretation have changed over time (Chang & Jackson, 1957; Chen et al., 2000; Hedley & Stewart, 1982; Jiang & Gu, 1989; Ruttenberg, 1992; Tiessen & Moir, 1993). Early methods developed for mineral soils focus mainly on inorganic phosphates and extract fewer fractions than later methods (Dean, 1938; Fisher & Thomas, 1935). Chang & Jackson's (1957) sequential fractionation of soil phosphorus elaborates on these earlier methods by using ammonium fluoride to separate aluminum phosphate from iron phosphate, though later work has shown that there is significant overlap between these pools (Barrow et al., 2021). Sequential fractionation schemes from the latter half of the twentieth century included more extracts in an attempt to distinguish between a higher number of phosphorus pools, often targeting specific soil characteristics. For example, Jiang & Gu (1989) developed a fractionation scheme for calcareous soils, which are dominated by calcium phosphates. While Chang & Jackson's (1957) sequential fractionation involves modifications for calcareous soils, Jiang & Gu divide the Ca-P pool into subsections according to their availability to plants (Jiang & Gu, 1989). In addition, Ruttenberg (1992) independently developed a sequential fractionation scheme for marine sediments. The novel contribution of this fractionation scheme is its separation of carbonate minerals formed on-site and those that were formed elsewhere and then deposited in sediment (Ruttenberg, 1992). This scheme does not include separation between different organic forms of phosphorus since they are of less importance in deep marine sediments (Ruttenberg, 1992).

Later methods tend to involve longer extraction periods and define fractions operationally to avoid interpretation errors (Tiessen & Moir, 1993). In addition, Tiessen & Moir's (1993) procedure includes more thorough washing of soils between steps, which helps prevent mixing between fractions. These later fractionation schemes are also more often validated by other methods like XANES, ^{31}P NMR, or other spectroscopic techniques (Chen et al., 2000; Helfenstein et al., 2018; Turner et al., 2003). In this way, while authors continue to define fractions operationally to avoid error, they take steps to define fractions functionally by comparison with other methods.

In a survey of phosphorus sequential fractionation data from natural sites, Cross & Schlesinger (1995) found that the distribution of phosphorus among the different pools extractable in the Hedley fractionation reflected the age and degree of weathering of the soil. Older and more weathered soils had relatively larger amounts of phosphorus in the organic and residual pools, while younger and less weathered soil had relatively larger amounts of phosphorus in the 1 M HCl-extractable pool (Cross & Schlesinger, 1995). This can be explained in part by the larger concentration of calcium in the young soils. Over time, precipitation and organic acids dissolve calcium minerals, allowing calcium to be subsequently leached from the soil (Dijkstra et al., 2001; Sagoe et al., 1998; Walker & Syers, 1976). Considering that Ca-bound P makes up a large portion of the phosphorus extracted in the 1 M HCl step, it is no surprise that this step extracts larger amounts of phosphorus in younger soils with larger amounts of calcium available to bind phosphates (Cross & Schlesinger, 1995; Walker & Syers, 1976). Age and degree of weathering play a large role in determining forms of phosphorus in fertilized soils as well, though specific soil properties moderate these effects (Negassa & Leinweber, 2009). In their review of phosphorus sequential fractionation data from fertilized soils, Negassa & Leinweber (2009) found that fertilizer application increased resin- and bicarbonate-extractable phosphorus in both long-term and short-term studies, but whether or not phosphorus from labile pools would be retained in more recalcitrant pools was determined by the interaction of soil drainage and precipitation patterns (Leinweber et al., 1997; Zheng et al., 2002). Soils with good infiltration, deep water tables, and lower precipitation tended to have more movement of phosphorus from labile pools to more recalcitrant pools, while soils with poor drainage, shallow water tables, and higher precipitation tended to have higher losses of fertilizer and less movement of labile P to the more recalcitrant pools (Negassa & Leinweber, 2009). Bogs are likely to have characteristics of both groups due to their waterlogging without loss of nutrients (Ingram, 1987).

2.6. Benefits and Limitations of Sequential Fractionation

In recent years, the merits and limitations of sequential fractionation schemes for soil phosphorus received much discussion (Barrow, 2021; Barrow et al., 2021; Condron & Newman, 2011; Gu et al., 2020; Gu & Margenot, 2021). In particular, the interpretation of the different fractions has come under considerable scrutiny (Barrow et al., 2021; Gu & Margenot, 2021). Fractionation methods such as Chang & Jackson (1957) and Zhang & Kovar (2009) which attempt to separate Fe-bound P and Al-bound P fail to do so (Barrow et al., 2021). Additionally, strict interpretation of all dilute acid-extractable phosphorus as representing Ca-bound P, whether dilute sulfuric or hydrochloric acid is used, is inaccurate (Barrow, 2021). That being said, sequential fractionation schemes are not all created equally. Schemes similar to the Hedley fractionation correlate well with the P pools expected due to soil age and degree of weathering in chronosequences, showing that this scheme does indeed extract P over a gradient of availability (Cross & Schlesinger, 1995; Helfenstein et al., 2018; Walker & Syers, 1976).

Another potential issue in the method is that humic acids may precipitate with colorimetric agents (Condron & Newman, 2011; Tiessen & Moir, 1993). When working with molybdenum blue as the colorimetric agent, dissolved organic macromolecules often clump together and prevent accurate reading. Condron and Newman (2011) suggest a pre-treatment with acid and centrifugation to remove this precipitate, but this is likely to remove orthophosphate as well and thus artificially decrease P concentrations in fractions that require this step. For samples where humic acid precipitation is low or moderate, the issue can be circumvented by the use of a blank correction. In addition, malachite green colorimetry involves the addition of an acid pretreatment before the colorimetric agent, which can "temper" the dissolved organic matter and prevent its precipitation. If the acid pretreatment is added and mixed thoroughly, the plate can be read before the addition of the colorimetric agent and the value subtracted from the final reading. Additionally, thorough mixing of the acid-treated sample with the colorimetric agent prevented precipitation. This is likely insufficient in soils with very high amount of humic acid precipitation such as deep peat more than a meter below the surface.

While there are significant limitations to be overcome, there is still merit in sequential fractionation for soil phosphorus. Rigid interpretation of each fraction according to the precipitate-particulate theory should be avoided, as it is clear that NaOH does not solely extract Fe-P, dilute H_2SO_4 or dilute HCl does not solely extract Ca-P, and ammonium persulfate cannot digest every form of organic P that may be present in soil (Barrow, 2021; Barrow et al., 2021; Benzing & Richardson, 2005; Schlesinger et al., 1998). That said, Tiessen & Moir's (1993) sequential fractionation scheme still bears merit even after these limitations are considered. The order of the fractions may be seen as a gradient of plant availability instead of rigid, distinct pools. This is well-supported by Negassa & Leinweber's (2009) review of sequential fractionations of agricultural soils, as growing crops without P fertilization decreased P concentrations in the resin- and bicarbonate-extractable fractions first, then the NaOH- and dilute-HCl fractions, and only rarely affected P concentrations in the final, most recalcitrant fractions (Campbell et al., 2017; Kashem et al., 2004; von Sperber et al., 2017). As such, sequential fractionations must be wielded with specific goals in mind. If the goal is to understand P availability to plants, Tiessen & Moir's (1993) sequential fractionation is of use. If the goal is to discover specific forms of phosphorus in soil or what soil phosphates may be bound to, XANES, ^{31}P NMR, or other forms of analysis are better-suited to the task (Barrow et al., 2021; Gu & Margenot, 2021).

2.7. Microbial Phosphorus

Microorganisms drive the movement of phosphorus between the surface and soil and between soil phosphorus pools in ombrotrophic bogs. This, in turn, controls nitrogen movement, as nitrogen uptake and fixation by soil microorganisms, freely or in symbiosis with plants, requires phosphate. Since microbial activity controls access to both of the limiting or co-limiting nutrients in this ecosystem, it is of great ecological importance.

Microbial turnover of soil phosphorus is relatively fast – in a matter of days, a phosphate molecule may be taken up by a microorganism and released again upon its death. Plants tend to retain nutrients longer, with many bog shrubs taking up large amounts of phosphorus every year in late summer and autumn and retaining it for months or years (Jonasson & Stuart Chapin, 1991). In contrast, microbial phosphorus uptake tends to peak in spring, making use of their faster metabolisms before plants gain the competitive advantage in summer (Jonasson & Stuart Chapin, 1991). In times of low nutrient availability, be it N, P, or C, plants may stimulate their symbionts or free-living soil microorganisms to increase the rate of decomposition by exuding a pulse of labile C or P (Spohn et al., 2013; Spohn & Kuzyakov, 2013). The plants then intercept some of the nutrients liberated by microorganisms during decomposition (Spohn et al., 2013; Spohn & Kuzyakov, 2013).

2.8. Forms of Phosphorus in Microorganisms

Within the cell, phosphorus is found in several compartments. In bacterial and fungal cells sampled from temperate mineral soils, the nucleus contains 5-10% of the cell's phosphorus in the form of DNA, slightly less than 10% is found in cell membrane phospholipids, 15-20% is found in phosphorylated enzymes and organic phosphorus compounds, and 30-50% of the cell's total phosphorus is found in RNA throughout the cell (Alexander, 1977). A study of Ontario muck soils similarly found that nucleic acids make up the bulk of organic phosphorus in soil, with phospholipids making up between 5% and 20% of the total organic phosphorus (De Sena et al., 2022). The amount of phosphorus that a cell may devote to each compartment varies with cell age and external conditions, among other factors. In the first days or weeks of their life cycle, young and highly metabolically active microorganisms devote a higher proportion of their phosphorus to nucleic acids (Bakken & Frostegård, 2006). When conditions are unfavorable, microorganisms may enter into a low-metabolic-activity stationary stage, in which cells tend to devote a higher proportion of their phosphorus to cell membranes (Bakken & Frostegård, 2006). When phosphorus is scarce, microorganisms tend to devote a larger amount of their limited phosphorus resources to RNA (Elser et al., 2003). In environments where phosphorus is more abundant, a greater diversity of phosphorus-containing biomolecules can be found, including storage polyphosphates (Elser et al., 2003).

This has a few implications for the extraction of microbial phosphorus. Microbial cells must be lysed in order to analyze their contents. Young, growing cells tend to lyse more easily. Since older cells may be tougher, a sample with a large proportion of older cells may require a more thorough lysis procedure. Mature fungal cells are especially difficult to lyse, which can lead to underestimations of microbial phosphorus (Bakken & Frostegård, 2006). Phosphorus from nucleic acids and phosphorylated enzymes are readily measured using the methods described below, but phosphorus from cell membranes requires further analysis (Bünemann et al., 2011).

Given the importance of microorganisms to the availability and movement of phosphorus in ombrotrophic peatlands, it is of great importance to understand expected microbial phosphorus concentrations and how they are controlled by environmental variables. In order to do so, I carried out a fourteen-month long sampling campaign taking place at Mer Bleue Bog. The results of this study are detailed below.

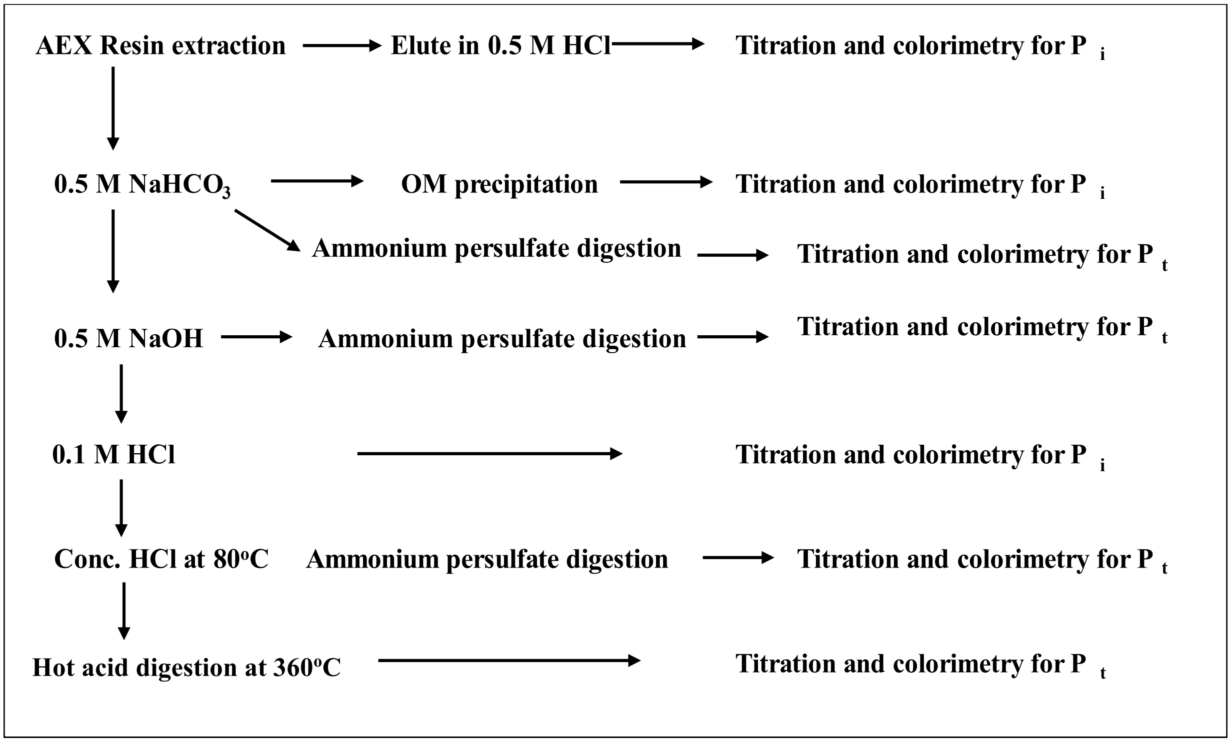

Figure 2-1 Flow chart of the steps of the sequential P extraction.

3. Seasonal Changes in Labile and Microbial Phosphorus Concentrations in an Ombrotrophic Bog in Eastern Ontario, Canada

Camille Jones[1], Cynthia Kallenbach[2], Tim Moore[1], Elyn Humphreys[3], Autumn Pereira[4], Christian von Sperber[1]

1 Department of Geography, McGill University, Montreal, QC; camille.jones@mail.mcgill.ca

2 Department of Natural Resource Sciences, McGill University, Sainte Anne de Bellevue, QC

3 Department of Geography and Environmental Studies, Carleton University, Ottawa, ON

4 School of Integrative Plant Science, Cornell University, Ithaca, NY, USA

Abstract

Phosphorus is a limiting or co-limiting nutrient in ombrotrophic bogs. Its cycling is largely controlled by microbial activity, meaning that microbial activity in this ecosystem may determine both net primary productivity and decomposition. The balance between NPP and decomposition is of critical importance in ombrotrophic bogs due to the enormous amount of carbon stored in peat. To study microbial phosphorus cycling, labile and microbial phosphorus concentrations were measured from June 2022 to August 2023 at Mer Bleue Bog near Ottawa, ON. Labile and microbial phosphorus concentrations were measured through anion-exchange resin extraction and fumigation. Microbial and labile phosphorus concentrations in an ombrotrophic bog vary greatly through the year. This variation is linked to changes in water table depth, temperature, and plant activity. High water tables were associated with higher microbial phosphorus concentrations, while low water tables were associated with lower microbial phosphorus concentrations. During a year with a mild winter, microbial phosphorus concentrations were high during late winter and snowmelt, averaging 115 to 130 mg kg^{-1} P in

layers above the water table. Microbial phosphorus concentrations then declined through the growing season as plants became more competitive, averaging 45 to 105 mg kg^{-1} P in layers above the water table. Labile phosphorus concentrations were often low, averaging 2 to 12 mg kg^{-1} P, but increased briefly to 45 mg kg^{-1} P in the upper layers of peat before being taken up by microorganisms and plants. Both microbial and labile phosphorus concentrations were highest near the surface and decreased with depth from 0 to 75 cm. The total size of the actively cycled pools varied through the sampling period, showing that microorganisms make use of lower-availability forms of phosphorus when there is sufficient heat and moisture.

3.1. Introduction

Peatlands store more than 500 petagrams of carbon, or approximately one-third of the planet's total soil carbon (Gorham, 1991; Hugelius et al., 2020). Peatlands' function as a carbon store is controlled in part by nutrient content and availability (Andersen et al., 2006; Gunnarsson et al., 2000; Turetsky, 2004). Ombrotrophic bogs are peatlands that are hydrologically disconnected from the underlying mineral soils and bedrock. Therefore, these ecosystems rely on atmospheric inputs of rock derived nutrients such as calcium, potassium or phosphorus either through wet or dry deposition.

Phosphorus is of particular interest in ombrotrophic bogs because it is often the limiting or co-limiting nutrient (Aerts et al., 1992; M. Wang et al., 2014). Organic forms of phosphorus usually make up the largest proportion of phosphorus in ombrotrophic bogs, and may be found in plant litter, microorganisms, humified material, and residual organics such as phosphate esters and phosphonates (Barbanti et al., 1994; Turner et al., 2005). Microorganisms can mineralize organic forms of phosphorus, enzymatically cleaving orthophosphate from the organic moieties.

In so doing, microorganisms transform organic phosphorus into available inorganic phosphate. Available inorganic phosphate tends to be quickly taken up by plants and microorganisms or may be sorbed to soil minerals. However, other than in mineral soils, peat soils usually do not contain many soil minerals and have only low concentrations of soil cations (e.g. calcium, iron, aluminum, and manganese). Therefore, the inorganic phosphorus pool is usually much smaller than the organic phosphorus pool in ombrotrophic bogs. For this reason, microorganisms are the primary drivers of the movement of phosphorus between different phosphorus pools in ombrotrophic bogs (Fig. 3-1). As layers of peat moss build up in a bog, the upper layer becomes disconnected from mineral soils and rocky material beneath (Mitsch & Gosselink, 2015). Since parent material is the primary source of phosphate, ecosystems with deep deposits of peat and little connection to underlying minerals or groundwater must survive on very little phosphorus.

As a limiting or co-limiting nutrient, phosphorus plays a role in determining carbon inputs through primary productivity and carbon outputs through decomposition. The relationship between decomposition and available phosphorus concentrations is bi-directional. While available phosphorus concentration plays a role in controlling decomposition, decomposition also liberates recalcitrant organic phosphorus, increasing available phosphorus concentrations. Rates of primary productivity and decomposition vary through the year with seasonal change, causing seasonal changes in available phosphorus concentrations (Zeppel et al., 2014). A deeper understanding of these relationships and the other variables that affect them is necessary to accurately predict future carbon storage in peatlands.

To survive in such a phosphorus-limited system, microbes have several strategies to take up phosphorus. Some phosphates are readily available in soil solution, and are taken up quickly at low energy cost to the microbe (Richardson, 2001). This is the Labile P fraction in Fig 1.

However, most of a soil microorganism's intracellular phosphorus comes from poorly-available sources (Richardson, 2001). Phosphorus may be found in plant litter, humified organic matter, and soil microorganisms in the organic pool or bound to metals and soil minerals in the inorganic pool (McGill & Cole, 1981). In order to make use of phosphorus from these pools, soil microorganisms use a suite of enzymes to solubilize phosphate from the inorganic pool or mineralize phosphate from the organic pool (Richardson, 2001). Upon death, microbial cells undergo lysis, releasing their cell contents into their surroundings. While most of their intracellular phosphorus is in an organic form, free extracellular phosphatases and the enzymes released during lysis hydrolyze organic phosphates into readily available mineral phosphate (Brookes et al., 1982). In this way, large microbial die-offs may be an opportunity for surviving organisms to take advantage of the resources left behind (Oberson & Joner, 2005).

3.1.1. Environmental Variables

Drying and rewetting releases a pulse of labile phosphorus from soil (Brödlin et al., 2019; Yevdokimov et al., 2016). While soil scientists have been aware of the phenomenon of nutrient release following drying and rewetting for more than eighty years, the mechanisms behind this process remain an area of active scholarship (Birch, 1961; Celi et al., 2022; Kitson & Bell, 2020). In addition to microbial lysis, drying and rewetting breaks down plant tissue, disturbs aggregates, and cleaves loosely sorbed phosphate from its binding partners, all of which release labile phosphorus into the soil solution (Celi et al., 2022). The relative importance of each of these mechanisms varies with soil type. For example, it is likely that aggregate breakup plays a smaller role in peatlands than in mineral soils, while microbial lysis and plant tissue breakdown may be more important. Freezing and thawing causes similar disruption of affected soil and litter, leading to similar nutrient release (Yevdokimov et al., 2016). The impact of both of these forms of disruption vary greatly from year to year due to variation in intensity of drying, amount of water received during rewetting, depth of freezing, number of cycles, and the point in the year at which disturbance occurs.

3.1.2. Hydrology

Given that precipitation is the only source of water input in an ombrotrophic bog, the timing and intensity of precipitation are of paramount importance (Fraser et al., 2001). At Mer Bleue, nearly half of the year's precipitation falls as snow (Hutchins, 2018). As a result, spring snowmelt is a major saturation event and is responsible for approximately 80% of the bog's annual runoff (Hutchins, 2018).

While total growing season precipitation has remained steady for at least the past sixty years, precipitation distribution has become more sporadic – that is, rain comes in large volumes less frequently, as opposed to smaller volumes more frequently (Fraser et al., 2001; Vincent et al., 2018). This is not to say that every year experiences long droughts and large storms, but they are both becoming more common (Zeppel et al., 2014). At Mer Bleue, precipitation volume tends to be higher in spring and autumn and low in late summer, though large summer storms can briefly raise the water table above the surface of the peat and cause some runoff (Hutchins, 2018).

Water moves both vertically and laterally in peat soils. For example, the massive flush of meltwater in freshet soaks down vertically into the peat until saturation, then moves laterally across the top and through the upper layers from upslope to downslope. When the water table is closer to the surface, lateral flow increases (Wilson, 2012). In contrast, vertical flow increases when the water table is deeper (Wilson, 2012). That said, peat density is an important factor to contend with, as deep layers of catotelm are much denser and less porous than upper layers. While water may move relatively quickly through upper layers, it may take months to years to move 100 m laterally through compressed catotelm (Morris et al., 2022). This has large implications on the movement of phosphorus within the bog – if the water table is low, a rainfall event may cause phosphorus from precipitation or lysate may move fairly quickly through a column, but move slowly across the surface of the peat (Fraser et al., 2001). In contrast, rainfall onto a bog with a high water table likely causes less vertical movement, but more movement across the surface of the peat (Fraser et al., 2001). However, once water and phosphorus reach the lower layers of older, denser peat, lateral movement with water is much slower.

3.1.3. Objectives and Hypotheses

Knowing the importance of microorganisms in ombrotrophic peatlands and the great potential variation in microbial activity with the seasons, my goal is to answer the following questions.

1. How do microbial and labile phosphorus concentrations in an ombrotrophic bog vary through the year?
2. What key variables control changes in microbial and labile phosphorus concentrations?

I hypothesize i) that microbial phosphorus concentrations will be highest in early spring, because microbial phosphorus uptake will outcompete plants' uptake early in the season when leaves have not yet fully developed, ii) that microbial phosphorus concentrations decrease in summer and autumn as plant uptake increases, iii) that microbial phosphorus levels decrease in the winter with dormancy and iv) that overall phosphorus availability in peat will be controlled primarily by moisture content. Labile phosphorus concentrations will likely remain low, though there may be brief spikes of labile phosphorus due to changes in water availability and plant activity.

3.2. Methods

3.2.1. Field Site

Samples were taken from Mer Bleue Bog near Ottawa, Ontario. The bog is domed and surrounded by a lagg which varies in size due to beaver activity. The bog center has hummock-and-hollow microtopography, which flattens at the edge of the lagg. Dominant vegetation consists of *Kalmia angustifolia, Chamaedaphne calyculata, Rhododendron groenlandicum,* and *Vaccinium myrtilloides*. Trees (*Picea mariana, Larix laricinia,* and *Betula populifolia*) and sedges (*Eriophorum vaginatum*) are scattered on hummocks in the bog center and grow more densely near the lagg. The average temperature for the area from 1991-2020 was 6.5°C, and average precipitation over the same time period was 930 mm, of which 404 mm fell as snow (*Canadian Climate Normals 1991-2020 Ottawa (Station Data)*, 2023).

3.2.2. Sampling

Samples were taken from July 2022 to the end of August 2023. Samples were taken monthly during the two growing seasons, once in the winter, and once during snowmelt. Samples were taken exclusively from hollows, as the water table, atmospheric nitrogen & phosphorus deposition, microbial community, and plant cover of hummocks and hollows differ (Golovchenko et al., 2010; Pinsonneault et al., 2016; Turunen et al., 2004). Trees and sedges were avoided, as their longer roots can bring phosphorus from deep in the catotelm up to surface layers (Rankin, 2022). Samples were taken with a Russian auger and separated according to their level of humification on the von Post humification scale (Von Post, 1922). Samples were taken from dead, undecomposed moss (H1), peat where fascicles had broken off but fibers were intact (H4), pulpy peat that exuded brown water (H7), and uniform paste (H9) (Fig. 3-2) (Von Post, 1922). Water table depth and depth of each layer of humification from surface were measured in the field. After sampling, soil water content was measured gravimetrically.

3.2.3. Characteristics of Peat Soils Affecting Phosphorus Extraction

Peat soil mainly consists of organic matter. Dissolved organic material may discolor sample solutions, leading to challenges detecting color changes during titration, precipitation of proteins during acidification, or skew results during colorimetry. To avoid this issue, discolored samples were diluted and re-plated.

In mineral soils, sesquioxide formation with Fe, Al, Mn, or sorption by clay particles, can be a large concern when performing a resin extraction, as these decrease the amount of phosphorus that binds to the resin strip (Bortoluzzi et al., 2015; Turner et al., 2005). While samples from 253 agricultural topsoils in the United States and Canada average 1.8% Fe, 4.5% Al, and 2.1 % Ca by dry weight (Smith et al., 2005), dried peat samples from an ombrotrophic bog in Minnesota averaged 0.2% Fe, 0.8% Al, and 0.7% Ca (Papp & Harms, 1985). As such, the peat soils I work with are unlikely to cause this issue, and resin extraction may yield a more accurate measure of phosphorus content.

Peat soils also have a lower pH than most mineral soils. At Mer Bleue, surface pH in hollows in summer 2022 ranged from 3.6-4.4 (unpublished data), with lower layers tending towards a higher pH. The mechanisms lowering bog pH are not fully understood, but the effects of precipitation, humic acids from decomposition, and *Sphagnum*'s cation exchange capacity likely play large roles (Mitsch & Gosselink, 2015). This can have effects on colorimetric methods, which can be sensitive to changes in pH (Ohno & Zibilske, 1991). As such, all samples will be titrated to keep pH relatively consistent. In addition, it is best to compare colorimetric results to samples of the same pH, as comparison to samples of varying acidity may require conversion (Bilyera et al., 2018).

In addition, samples need to be analyzed as quickly as possible. Since oven-drying or freezing would change the microbial environment greatly, long-term storage is not possible. To keep conditions representative of the field, samples were kept cool and analyzed within a few days of sampling. Due to these time constraints, homogenization of samples was not possible. To prevent under-lysis of microbes, liquid fumigants were chosen over gaseous fumigants, as they tend to penetrate the tough plant materials and bulky textures found in peat soil more effectively (Kouno et al., 1995; McLaughlin et al., 1986).

Even with liquid fumigants, a portion of microbial phosphorus may not be recovered (Hedley & Stewart, 1982). This can be explained by the loss of the cell membrane fraction, sorption, & loss in other small unextractable fractions (Brookes et al., 1982). As such, the conversion factor K_p is used to prevent microbial phosphorus underestimation (Brookes et al., 1982). While a generic K_p value of 0.4 was once used for all soils, it has become clear in recent years that a universal value is often inaccurate (Bilyera et al., 2018). Instead, it is more accurate to calculate an individual K_p based on soil properties such as soil organic carbon, clay content, and total phosphorus content (Bilyera et al., 2018). In the case of peat soil, only soil organic carbon and total phosphorus are relevant, as phosphorus immobilization by clays is negligible (see above).

Previous work in peatland microbial phosphorus has found a wide range of microbial phosphorus levels. Brake et al. (1999) found extremely high levels of microbial phosphorus in the topmost 5 cm of peat (300-900 mg kg-1 P), while samples from 30-50 cm deep had lower levels of microbial phosphorus (20-30 mg kg-1 P). These samples were taken in November from three restored peatlands in Lower Saxony, then subjected to fumigation-extraction to measure microbial phosphorus content (Brake et al., 1999). Microbial phosphorus made up 32% of total phosphorus in these samples on average (Brake et al., 1999). Baum et al. (2003) studied peatlands in a nearby area of Germany and used the same method of fumigation-extraction, but found 20-60 mg kg-1 P throughout the column. However, microbial phosphorus in these samples often made up less than 10% of the total phosphorus, meaning that it is likely that a large amount of the phosphorus measured in these samples was not available to microbes (Baum et al., 2003). Williams and Sparling (1984) used chloroform vapour fumigation and found similar levels of microbial phosphorus (25-66 mg/kg, not mentioned whether dry or wet mass, but I expect dry due to how well it falls in with the previous measurements). However, microbial phosphorus in these samples makes up a much larger percentage of total P, as total P was just 63+/- 10 mg kg^{-1} P (Williams & Sparling, 1984). Neither Baum et al. (2003) nor Williams & Sparling (1984) mentioned season or weather conditions at sampling, which might have had a large effect on microbial biomass phosphorus measurements.

3.2.4. Anion Exchange Resin Extraction

Anion exchange resins were used to extract labile and microbial phosphorus. To measure labile phosphorus, 7 grams of peat were shaken with 25 mL of deionized water and a 1.5 x 4 cm anion-exchange resin strip, which sorbs phosphates that are freely available in the soil solution (Kouno et al., 1995). Afterwards, the strip was removed from the sample and shaken for 22-24 hours in 25 mL of 0.5 M HCl to dissociate the phosphates from the resin strip (Kouno et al., 1995). This method avoids problems that may arise from the discoloration caused by peat samples, as the discolored peat solution does not come in contact with the final colorimetry solution. After using a resin strip to extract freely available phosphates, microbial phosphorus was measured through simultaneous fumigation-resin extraction (Kouno et al., 1995). 7 mL of liquid hexanol were added to the solution to lyse microbial cell membranes, releasing microbial phosphates into the surrounding solution (McLaughlin et al., 1986). Once released, phosphate from the cell contents was sorbed by the resin strip. Liquid hexanol was chosen over chloroform in order to prevent damage to resin strips and lab personnel (Bünemann et al., 2011; McLaughlin et al., 1986). Fumigation (with shaking) occurred for 24 hours to fully lyse microbial cells (Bakken & Frostegård, 2006; Turner et al., 2005).

3.2.5. Phosphorus Quantification by Malachite Green Colorimetry

Results from anion-exchange resin extraction were quantified using malachite green colorimetry. While Milin (2012) found that molybdenum blue gave higher accuracy with a larger range of P_i concentrations, experience in our lab has found that malachite green may give higher accuracy with peat samples, as it is applicable to a wider range of pH values.

While malachite green colorimetry is applicable to a wide range of pH values, from 1.5 to 9.5, but intensity of color can vary if the pH of samples varies strongly. For example, samples from a hot acid digestion with variable amounts of peroxide vary quite a bit, which may affect colorimetry results. For this reason, the pH of all samples was adjusted to approximately 5.5 by titration with *p*-nitrophenol as an indicator. Afterwards, 200 microliters of sample solution was added to a 96-well microplate with 40 microliters of acidic Malachite I solution (Ohno & Zibilske, 1991). The Malachite I solution converts the various forms of phosphate in the sample to inorganic phosphate, which reacts with 40 microliters of Malachite II solution to make a blue-green color (Ohno & Zibilske, 1991). The intensity of the color is then measured using a spectrophotometer, which analyzes how much of a particular wavelength of light is absorbed by the liquid in the well of the plate.

3.2.6. Data Analysis & Visualization

Data analysis and visualization were accomplished in Excel version 16.0.17 with the Analysis ToolPak add-in. Differences in mean phosphorus concentrations due to water table were evaluated using one-way ANOVA. Samples were binned by water table depth (15-25 cm, 25-35 cm, and 35-45 cm below surface), evaluated for normalcy using Q-Q plots, and significant differences between the groups were tested with the Anova: Single Factor tool from the Analysis ToolPak.

3.3. Results

In July 2022, microbial phosphorus concentrations greatly exceeded labile phosphorus concentrations (Fig. 3-3). Microbial phosphorus concentrations generally decreased with depth, though not in the deepest layer. In August 2022, microbial phosphorus concentrations dropped and labile phosphorus concentrations increased sharply in all layers (Fig. 3-4). Both decreased with depth. In October 2022, phosphorus concentrations returned to previous levels of high microbial phosphorus concentrations and low labile phosphorus concentrations as seen in July 2022 (Fig. 3-5). In early March of the following winter, microbial phosphorus concentrations increased substantially, though the pattern of decrease with depth remained (Fig. 3-6). Microbial phosphorus concentrations in all layers were nearly three times higher than they had been in July and October 2022. Labile phosphorus concentrations remained low in the winter samples. In April 2023, directly after snowmelt, microbial phosphorus concentrations in the uppermost layer increased compared to the winter measurement, but decreased in the deeper layers (Fig. 3-7). Labile phosphorus concentrations increased slightly but remained low. In May 2023, labile and microbial phosphorus concentrations dropped, but were not as low as they had been the previous summer (Fig. 3-8). By June 2023 microbial and labile phosphorus concentrations more closely resembled June and October 2022, though variability was high (Fig. 3-9). In July 2023, microbial phosphorus concentrations in the uppermost layer of peat increased, but changes in deeper layers were small (Fig. 3-10). In August 2023, labile and microbial phosphorus concentrations had dropped in all layers compared to the previous month (Fig. 3-11).

Many of the increases and decreases in microbial and labile phosphorus concentrations in the H1 and H4 layers correlate with water table depth (Figs. 3-12 and 3-13). H7 and H9 remained submerged year-round, while H1 and H4 were variably dry or submerged depending on water table depth. Accordingly, water table depth had a statistically significant correlation with microbial phosphorus in the H1 and H4 layers using one-way ANOVA ($p < 0.05$). A lowering of the water table, such as those between July and August 2022, April and May 2023, and May and

June 2023 correspond to a decrease in microbial phosphorus concentration. Inversely, a rise of the water table, such as between August and October 2022, October 2022 and March 2023, March and April 2023, and June and July 2023 corresponds to increases in microbial phosphorus concentration. These patterns are not universal, as both water table depth and microbial phosphorus concentrations decreased between July and August 2023.

3.4. Discussion

While three of my hypotheses were supported by this data, one was not. Those supported were hypothesis i) that microbial phosphorus would be highest in early spring, ii) that microbial phosphorus concentrations would decrease through the growing season and autumn, and iv) that water table level is the key variable controlling microbial and labile phosphorus concentrations. However, hypothesis iii), that microbial phosphorus concentrations would decrease through the winter, was not supported by this data. During a mild winter like that experienced during 2022-2023, only the unsaturated layers of peat froze. Snow and moss insulated the peat, allowing microbial activity to continue and microbial phosphorus concentrations to remain high (Figs. 3-12 and 3-13).

Labile and microbial phosphorus concentrations decreased with depth throughout the year (Figs. 3-3 to 3-11). The only exceptions to this trend occurred in July 2022 and June 2023, which were both dry periods with very low water tables (Figs. 3-3, 3-9, and 3-12). During these dryer months, it is possible that labile phosphorus from the upper layers is carried down with water table drawdown, causing the small increases in H4 labile and microbial phosphorus (Figs. 3-12 and 3-13).

A rise of the water table was generally associated with increases in microbial phosphorus concentrations, while a lowering of the water table was generally associated with decreases in microbial phosphorus concentrations (Figs. 3-12 and 3-13). In particular, the rise in microbial phosphorus with the rise in water table level in July 2023 may be an example of microorganisms taking advantage of the pulse of nutrients following drying and rewetting (Fig. 3-12). However, while the water table rose between July and August 2023, microbial phosphorus concentrations decreased in both the H1 and H4 layers (Figs. 3-12 and 3-13). In addition, water table rose substantially from August to October 2022, but microbial phosphorus concentrations in H1 and H4 showed only slight increases (Figs. 3-12 and 3-13). In the springtime, a similar rise of the water table was associated with much larger increases in microbial phosphorus levels (Figs. 3-12 and 3-13). The low late summer and autumn microbial phosphorus concentrations may be explained by plant uptake of phosphorus. In treed swamps of a similar latitude, plant phosphorus uptake peaks in late summer and autumn, then slows or stops under snow cover and remains low in the following spring (Yanai, 1992). This matches with the patterns of microbial phosphorus concentration found in this study. Here, microbial phosphorus concentrations are high in winter, spring, and early summer, when there is less competition with plants for labile phosphorus. In late summer and autumn, when bog plants are expected to increase their phosphorus uptake, microbial phosphorus concentrations are lower. However, these assertions cannot stand on my data alone. Investigation of seasonal change in aboveground phosphorus concentrations and an estimate of total biomass phosphorus above and belowground through the year would confirm or refute this hypothesis more conclusively.

Perhaps the most intriguing question raised by this experiment is why microbial and labile phosphorus concentrations generally rise and fall in tandem. Based on the literature, decreases in microbial phosphorus concentrations are associated with increases in labile phosphorus concentrations, as microbial phosphorus is quickly mineralized to orthophosphate once exposed to extracellular phosphatase (Richardson, 2001; Saa et al., 1993). This can be seen in my results from July to August 2022 (Figs. 3-3, 3-4, 3-12, and 3-13). However, the total amount of microbial plus labile phosphorus was not constant through the year, so movement back and forth between the microbial and labile pools cannot be the only explanation. If extracellular phosphatase mineralized the released microbial phosphorus, where else might the phosphate have gone? I propose three destinations: upward, downward, and loose sorption. As mentioned above, plant demand for phosphorus in late summer and autumn may be responsible for a large amount of the decrease in total microbial and labile P. Plant uptake of phosphorus leads to an upward translocation from the rhizosphere to the above ground biomass. Downward movement into the peat profile is another possibility, but probably one of less importance. Infiltration rates are low, and H9 microbial and labile phosphorus concentrations remained low throughout the fourteen-month sampling period, making it unlikely to find a second peak of phosphorus deeper in the profile. Instead, movement into another soil phosphorus pool is likely. During a sequential fractionation of peat taken from the same bog, I found that concentrations of phosphorus in the bicarbonate- and NaOH-extractable pools were high enough to be comparable to the microbial pool in Chapter 3. Movement between these pools can take place over a span of weeks or months, making this a likely explanation (Negassa & Leinweber, 2009). See "Nutrient Addition Increases Available and Residual Phosphorus Concentrations in an Ombrotrophic Bog" for further detail.

The large variation in microbial and labile phosphorus concentrations over the year show the importance of conducting a thorough sampling campaign. A single day of sampling is insufficient to accurately draw conclusions about the size of the microbial phosphorus pool, or indeed any microbial nutrient pool. While this study is the most thorough of its kind, it still falls short of measuring the full variation in microbial and labile phosphorus spikes through the year. While the August 2022 sample shows a clear decrease in microbial phosphorus and increase in labile phosphorus with drying, this is the only such spike measured over the two growing seasons. Labile phosphorus is taken up by soil microbes on a scale of days, thus the gaps between sampling are long enough to have missed “hot moments” in labile phosphorus concentrations (Oehl et al., 2001). To more accurately capture these “hot moments,” sampling should be conducted more frequently, and future studies should monitor temperature, precipitation, and evapotranspiration potential to more accurately target their sampling campaigns. That said, temperature and moisture are not the only factors contributing to these “hot moments,” especially in fens, swamps, and other organic wetlands. To account for patterns of nutrient availability in these ecosystems, the above variables plus nutrient concentration of inflows, pH, plant phenology, and disturbance should be monitored (Yanai, 1992).

3.5. Conclusion

Microbial and labile phosphorus concentrations in ombrotrophic bogs vary widely with the seasons. A low water table, >45 cm below surface, was associated with decreases in microbial and labile phosphorus concentrations, while higher water tables were associated with higher microbial and labile phosphorus concentrations (Fig. 13). These patterns were affected by seasonal variation as well. During a mild winter, microbial phosphorus concentrations remained high under the snow cover. The total size of the actively cycled pool varied widely as well, showing that organisms in a nutrient-poor ecosystem make use of lower-availability pools of soil nutrients when necessary. Future work should include seasonal patterns of phosphorus uptake by bog plants and seasonal changes in concentrations of phosphorus in lower-availability pools.

Figures

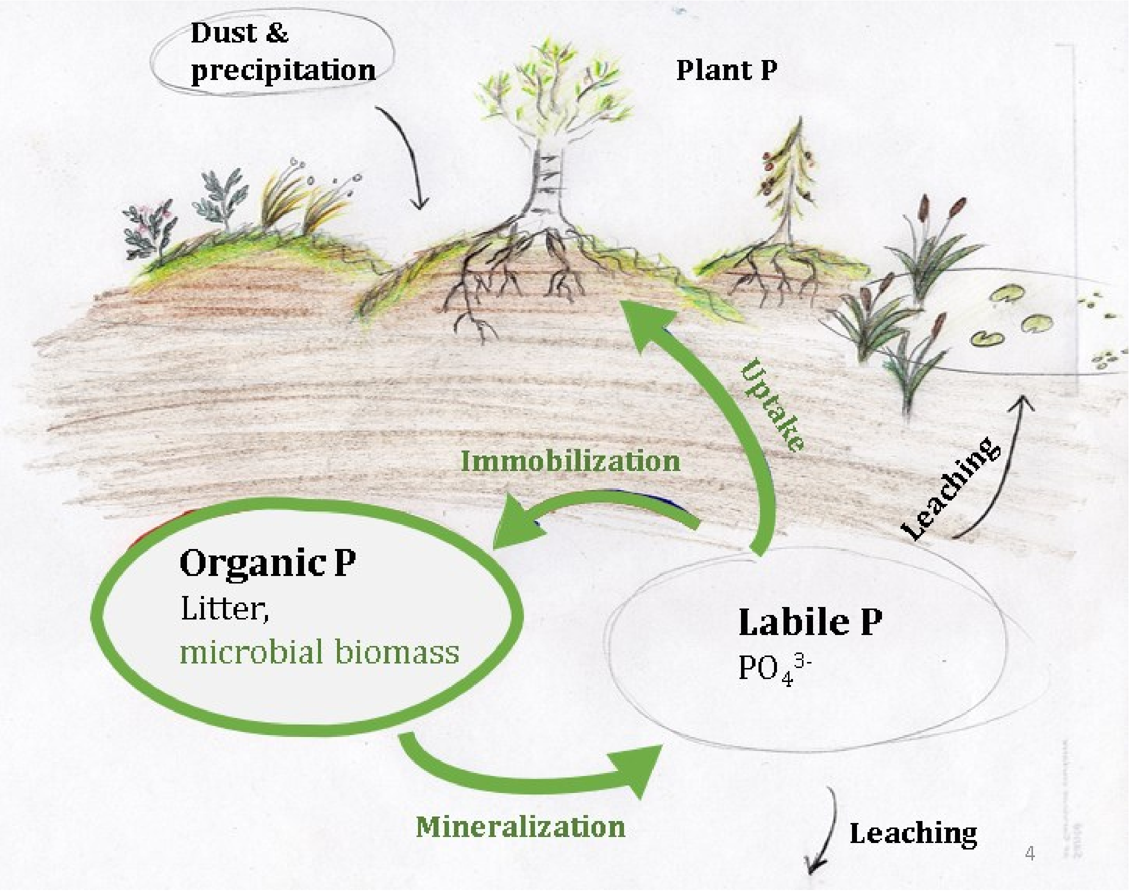

Figure 3-1 Simplified phosphorus cycle in an ombrotrophic bog. Processes and pools in green are mediated by microbes.

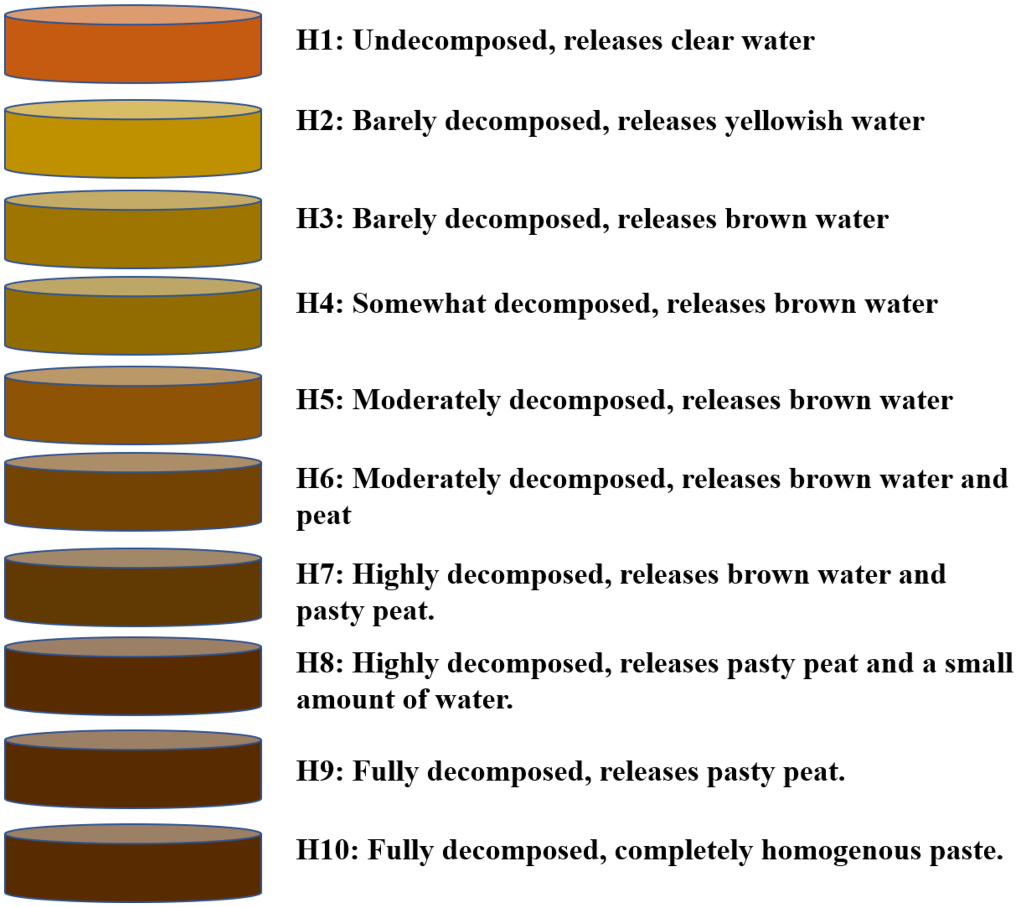

Figure 3-2 The von Post humification scale with colors found in ombrotrophic bog peat.

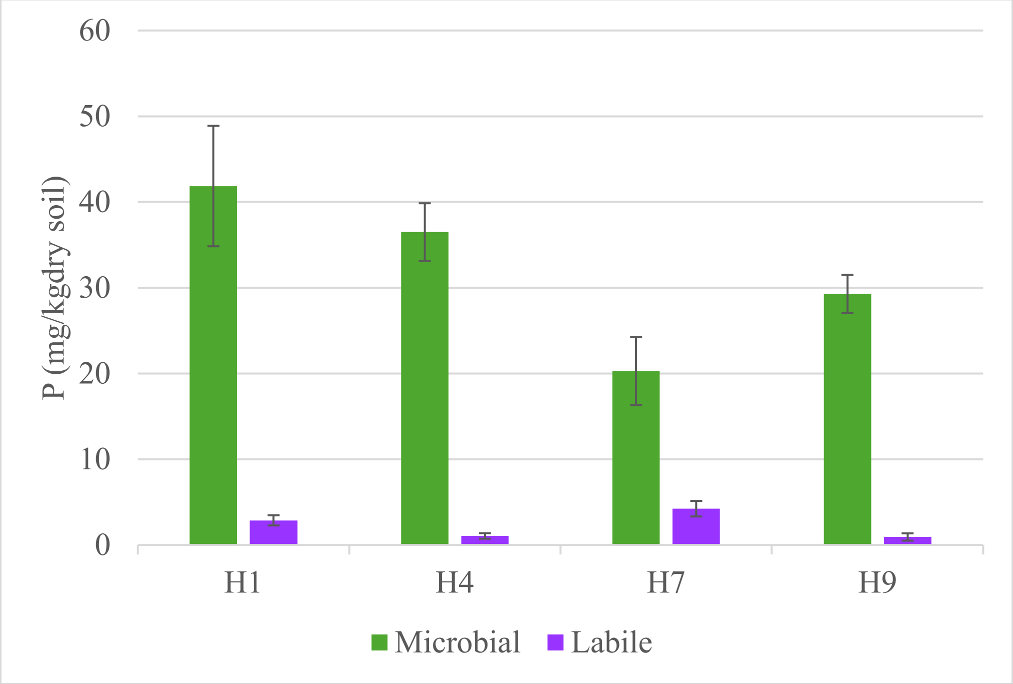

Figure 3-3 July 2022 microbial and labile phosphorus by layer reported in mg kg-1 P. Error bars represent one standard deviation above and below.

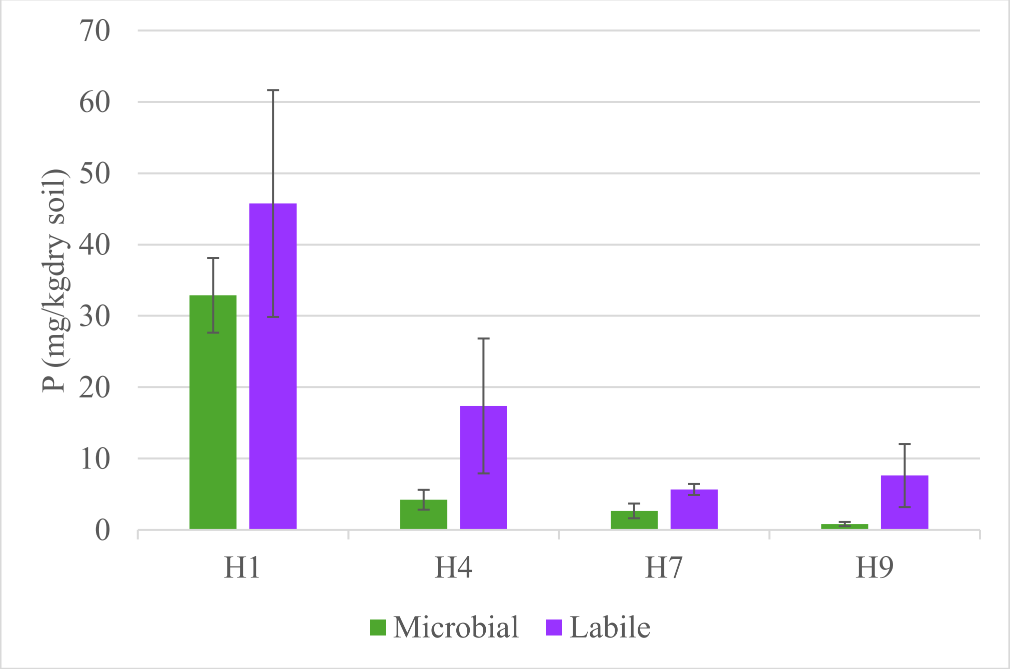

Figure 3-4 August 2022 microbial and labile phosphorus by layer reported in mg kg-1 P. Error bars represent one standard deviation above and below.

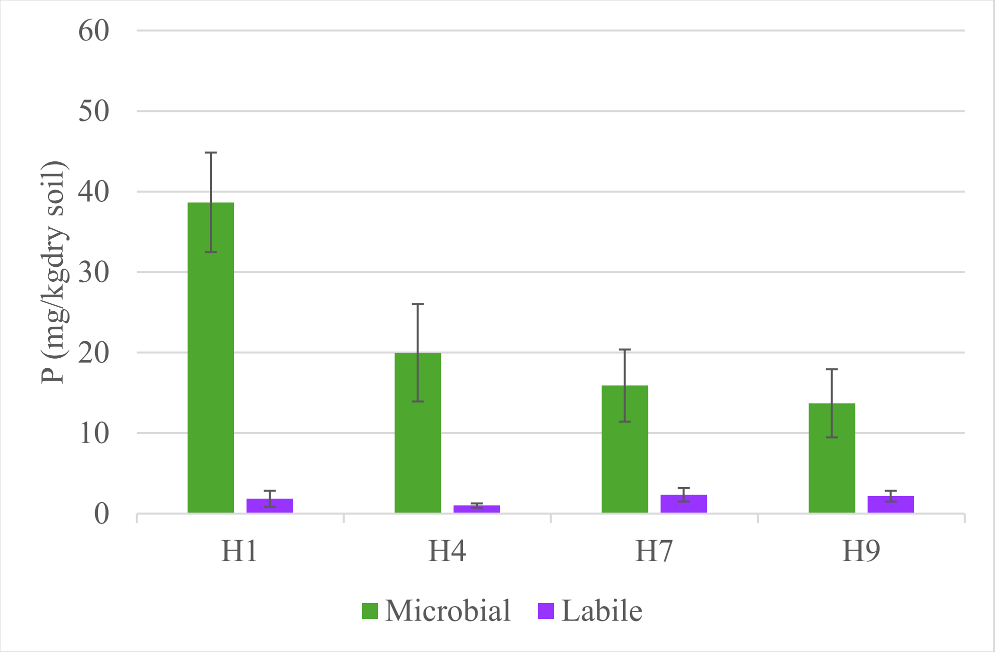

Figure 3-5 October 2022 microbial and labile phosphorus by layer reported in mg kg-1 P. Error bars represent one standard deviation above and below.

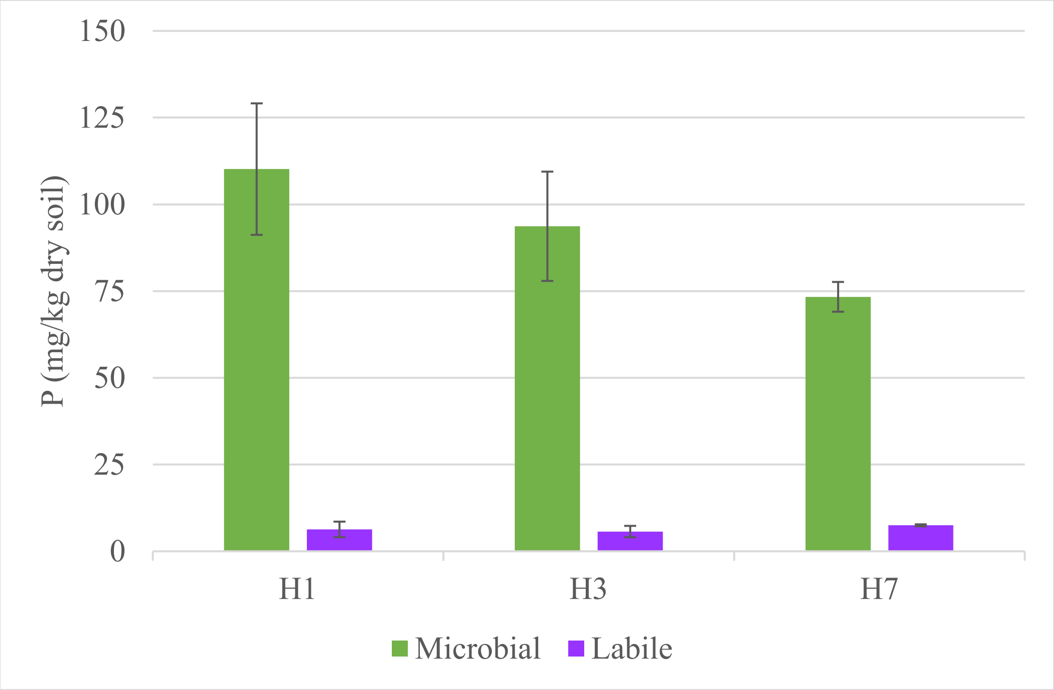

Figure 3-6 March 2023 microbial and labile phosphorus by layer reported in mg kg-1 P. Error bars represent one standard deviation above and below.

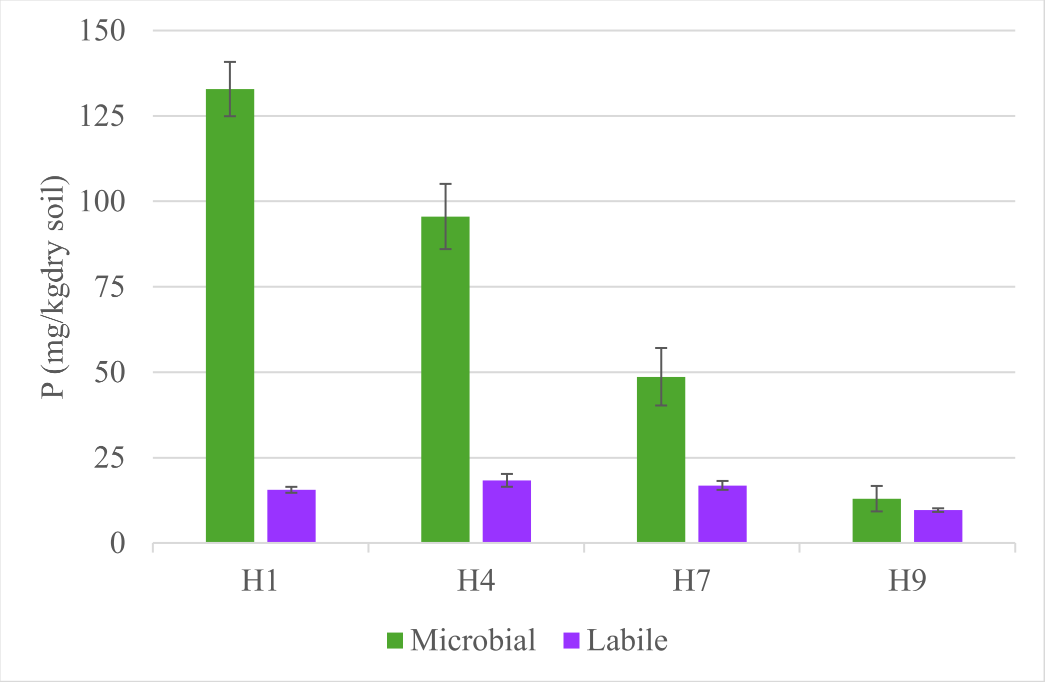

Figure 3-7 April 2023 microbial and labile phosphorus by layer reported in mg kg-1 P. Error bars represent one standard deviation above and below.

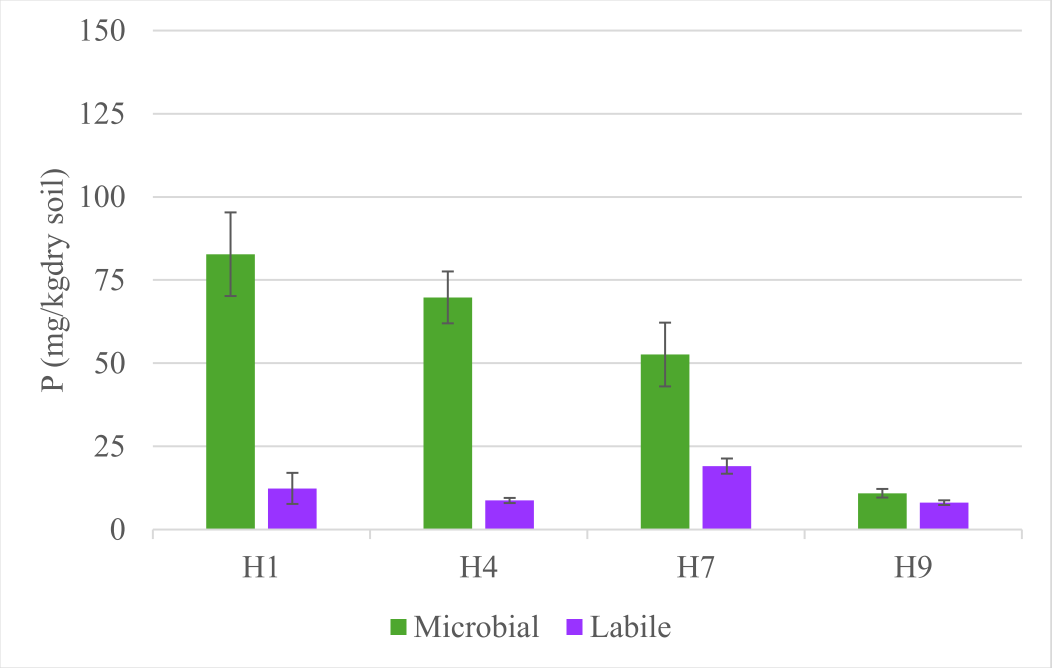

Figure 3-8 May 2023 microbial and labile phosphorus by layer reported in mg kg-1 P. Error bars represent one standard deviation above and below.

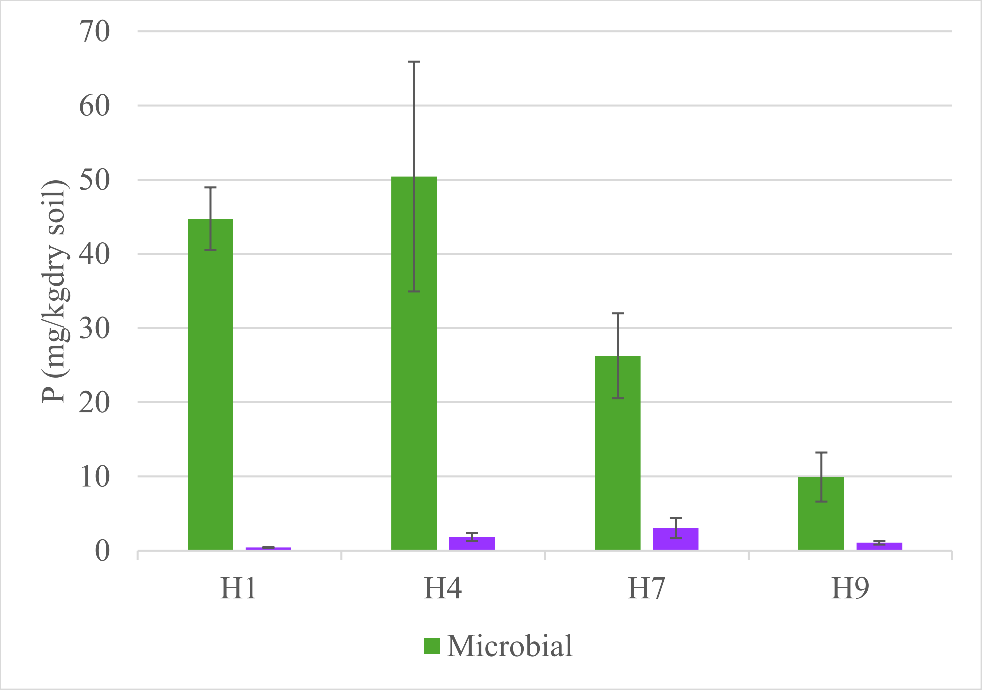

Figure 3-9 June 2023 microbial and labile phosphorus by layer reported in mg kg-1 P. Error bars represent one standard deviation above and below.

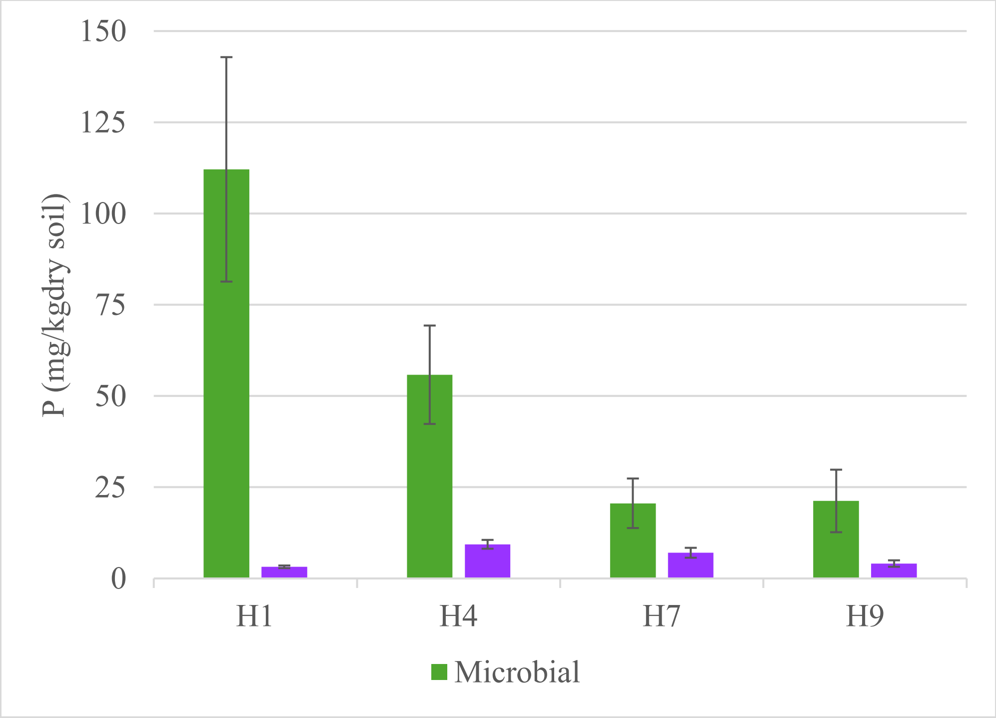

Figure 3-10 July 2023 microbial and labile phosphorus by layer reported in mg kg-1 P. Error bars represent one standard deviation above and below.

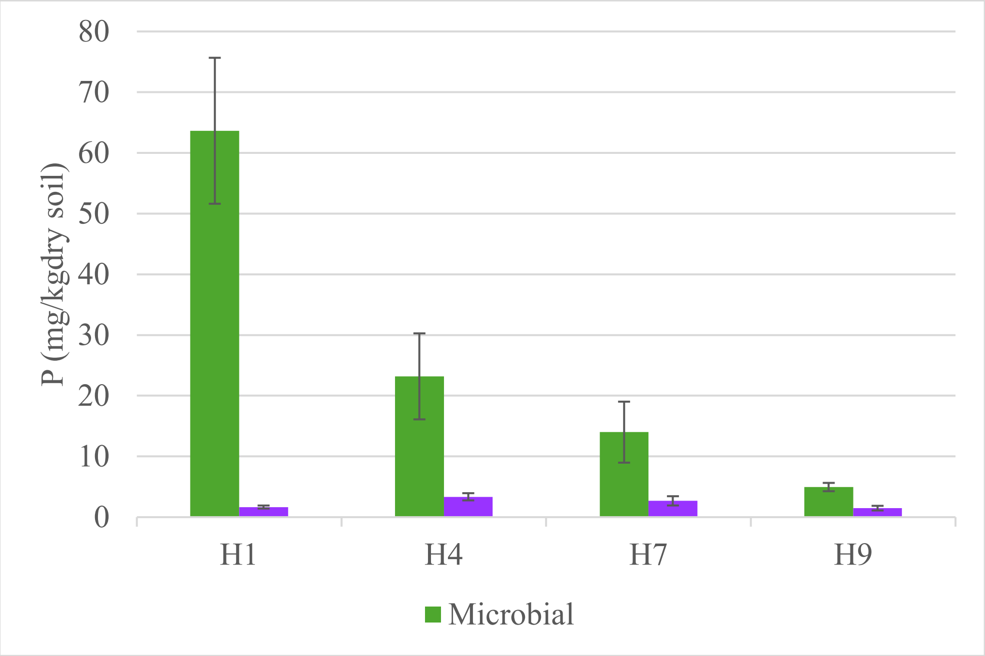

Figure 3-11 August 2023 microbial and labile phosphorus by layer reported in mg kg-1 P. Error bars represent one standard deviation above and below.

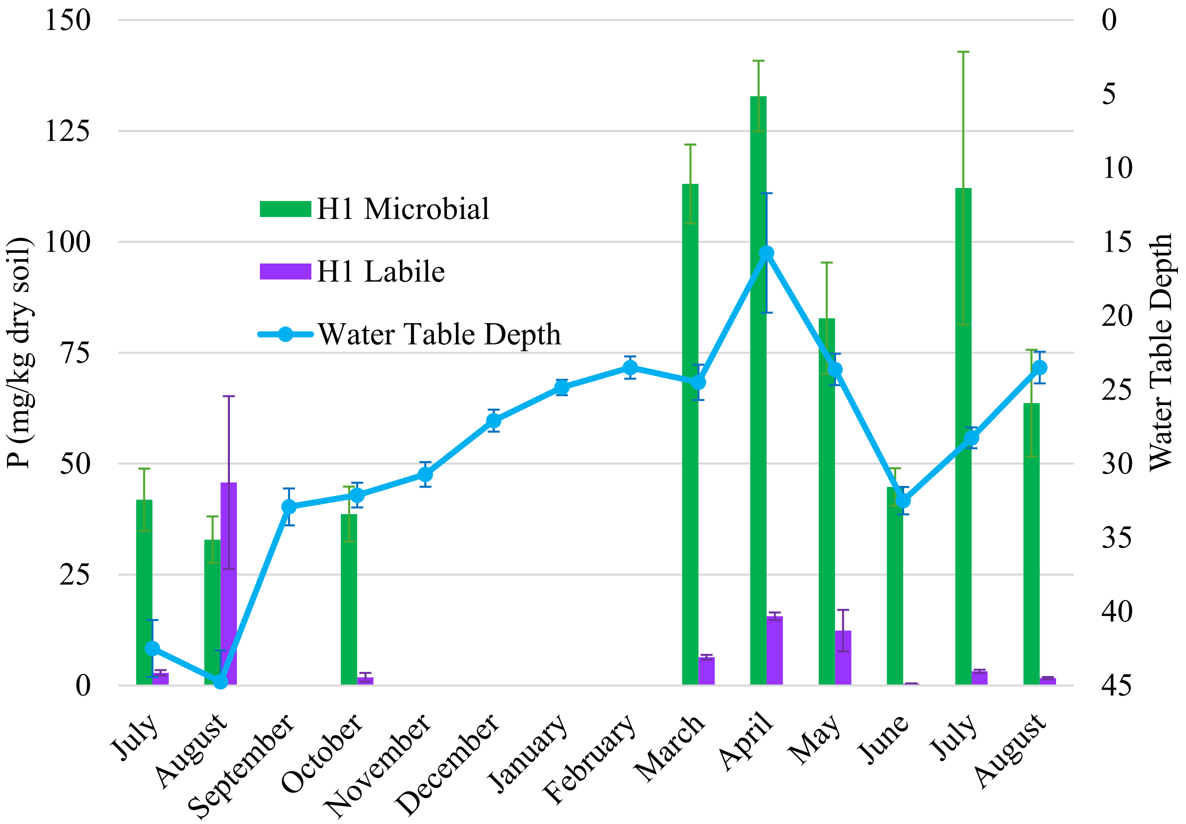

Figure 3-12 Microbial and labile phosphorus in layer H1 plotted with water table depth from July 2022 to August 2023. Water table depth reported as the mean of the week preceding sampling. Error bars represent one standard deviation above and below.

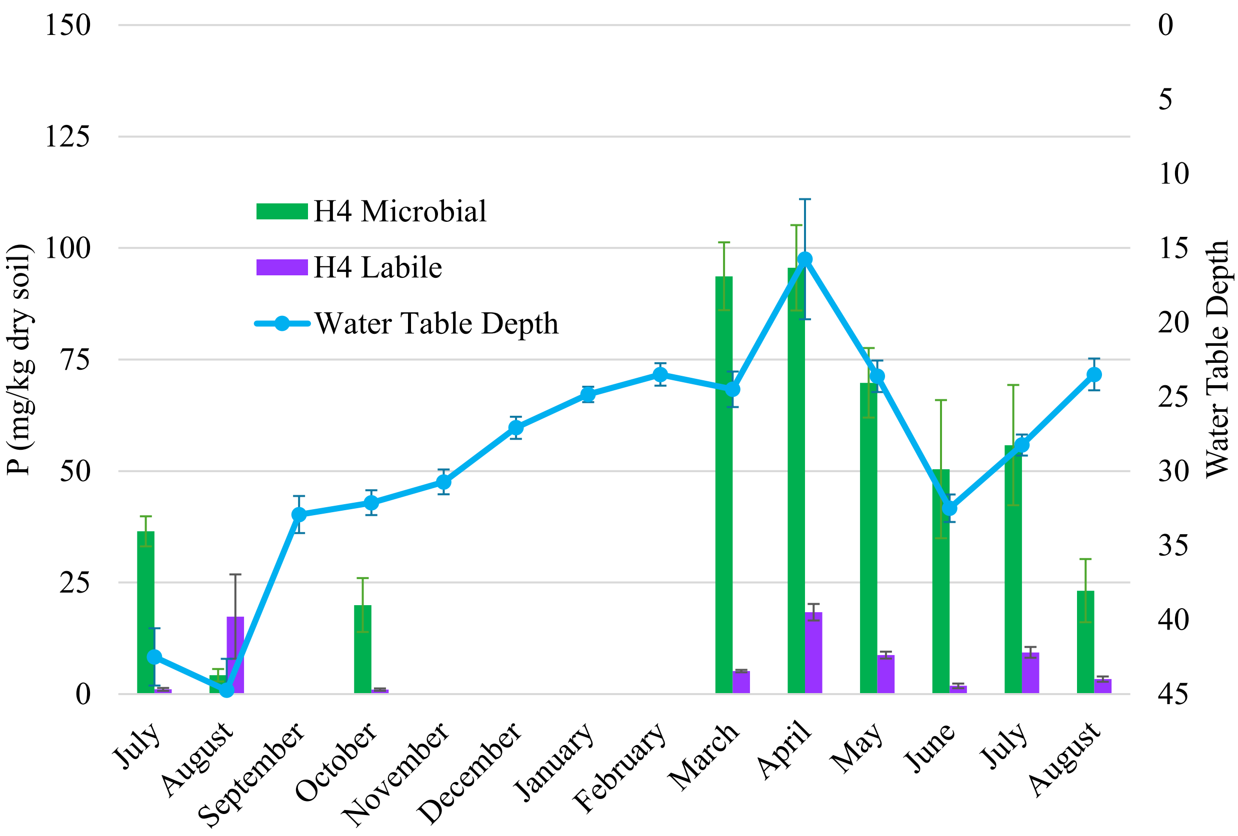

Figure 3-13 Microbial and labile phosphorus in layer H4 plotted with water table depth from July 2022 to August 2023. Note that the values for March 2023 come from the H3 layer, not H4 as in the rest of the months sampled. Water table depth reported as the mean of the week preceding sampling. Error bars represent one standard deviation above and below.

References

Aerts, R., Wallen, B., & Malmer, N. (1992). Growth-Limiting Nutrients in Sphagnum-Dominated Bogs Subject to Low and High Atmospheric Nitrogen Supply. *The Journal of Ecology*, *80*(1), 131. https://doi.org/10.2307/2261070

Andersen, R., Francez, A.-J., & Rochefort, L. (2006). The physicochemical and microbiological status of a restored bog in Québec: Identification of relevant criteria to monitor success. *Soil Biology and Biochemistry*, *38*(6), 1375–1387. https://doi.org/10.1016/j.soilbio.2005.10.012

Bakken, L. R., & Frostegård, Å. (2006). Nucleic Acid Extraction from Soil. In *Soil Biology* (Vol. 8, pp. 49–73). Springer-Verlag. http://www.umb.no/statisk/nitrogengroup/publications/Bakken-Frostegard2006_extraction.pdf

Barbanti, A., Bergamini, M. C., Frascari, F., Miserocchi, S., & Rosso, G. (1994). Critical Aspects of Sedimentary Phosphorus Chemical Fractionation. *Journal of Environmental Quality*, *23*(5), 1093–1102. https://doi.org/10.2134/jeq1994.00472425002300050035x

Baum, C., Leinweber, P., & Schlichting, A. (2003). Effects of chemical conditions in re-wetted peats on temporal variation in microbial biomass and acid phosphatase activity within the growing season. *Applied Soil Ecology*, *22*(2), 167–174. https://doi.org/10.1016/S0929-1393(02)00129-4

Bilyera, N., Blagodatskaya, E., Yevdokimov, I., & Kuzyakov, Y. (2018). Towards a conversion factor for soil microbial phosphorus. *European Journal of Soil Biology*, *87*, 1–8. https://doi.org/10.1016/j.ejsobi.2018.03.002

Bortoluzzi, E. C., Pérez, C. A. S., Ardisson, J. D., Tiecher, T., & Caner, L. (2015). Occurrence of iron and aluminum sesquioxides and their implications for the P sorption in subtropical soils. *Applied Clay Science*, *104*, 196–204. https://doi.org/10.1016/j.clay.2014.11.032

Brake, M., Höper, H., & Joergensen, R. G. (1999). Land use-induced changes in activity and biomass of microorganisms in raised bog peats at different depths. *Soil Biology and Biochemistry*, *31*(11), 1489–1497. https://doi.org/10.1016/S0038-0717(99)00053-X

Brookes, P. C., Powlson, D. S., & Jenkinson, D. S. (1982). Measurement of microbial biomass phosphorus in soil. *Soil Biology and Biochemistry*, *14*(4), 319–329. https://doi.org/10.1016/0038-0717(82)90001-3

Bünemann, E. K., Oberson, A., & Frossard, E. (2011). *Phosphorus in action: Biological processes in soil phosphorus cycling*. Springer. https://doi.org/10.1007/978-3-642-15271-9

Canadian Climate Normals 1991-2020 Ottawa (Station Data). (2023). [..Csv]. Canadian Climate Normals. https://climate.weather.gc.ca/climate_normals/results_1991_2020_e.html?searchType=stnName_1991&txtStationName_1991=Ottawa&searchMethod=contains&txtCentralLatMin=0&txtCentralLatSec=0&txtCentralLongMin=0&txtCentralLongSec=0&stnID=191000000&dispBack=1#normals-data

Fraser, C. J. D., Roulet, N. T., & Moore, T. R. (2001). Hydrology and dissolved organic carbon biogeochemistry in an ombrotrophic bog. *Hydrological Processes*, *15*(16), 3151–3166. https://doi.org/10.1002/hyp.322

Golovchenko, A. V., Bogdanova, O. Yu., Stepanov, A. L., Polyanskaya, L. M., & Zvyagintsev, D. G. (2010). Functioning of microbial complexes in aerated layers of a highmoor peat bog. *Eurasian Soil Science*, *43*(9), 1022–1029. https://doi.org/10.1134/S1064229310090097

Gorham, E. (1991). Northern Peatlands: Role in the Carbon Cycle and Probable Responses to Climatic Warming. *Ecological Applications*, *1*(2), 182–195. https://doi.org/10.2307/1941811

Gunnarsson, U., Rydin, H., & Sjörs, H. (2000). Diversity and pH changes after 50 years on the boreal mire Skattlösbergs Stormosse, Central Sweden. *Journal of Vegetation Science*, *11*(2), 277–286. https://doi.org/10.2307/3236808

Hedley, M. J., & Stewart, J. W. B. (1982). Method to measure microbial phosphate in soils. *Soil Biology and Biochemistry*, *14*(4), 377–385. https://doi.org/10.1016/0038-0717(82)90009-8

Hugelius, G., Loisel, J., Chadburn, S., Jackson, R. B., Jones, M., MacDonald, G., Marushchak, M., Olefeldt, D., Packalen, M., Siewert, M. B., Treat, C., Turetsky, M., Voigt, C., & Yu, Z. (2020). Large stocks of peatland carbon and nitrogen are vulnerable to permafrost thaw. *Proceedings of the National Academy of Sciences*, *117*(34), 20438–20446. https://doi.org/10.1073/pnas.1916387117

Hutchins, K. (2018). *Water Balance and Solute Export in a South-East Ontario Ombrotrophic Peatland*. McGill University.

Kouno, K., Tuchiya, Y., & Ando, T. (1995). Measurement of soil microbial biomass phosphorus by an anion exchange membrane method. *Soil Biology and Biochemistry*, *27*(10), 1353–1357. https://doi.org/10.1016/0038-0717(95)00057-L

McGill, W. B., & Cole, C. V. (1981). Comparative aspects of cycling of organic C, N, S and P through soil organic matter. *Geoderma*, *26*(4), 267–286. https://doi.org/10.1016/0016-7061(81)90024-0

McLaughlin, M., Alston, A., & Martin, J. (1986). Measurement of phosphorus in the soil microbial biomass: A modified procedure for field soils. *Soil Biology and Biochemistry*, *18*(4), 437–443. https://doi.org/10.1016/0038-0717(86)90050-7

Milin, S. (2012). *Comparaison de deux méthodes spectrophotométriques de dosage de l'acide phosphorique. Application à des sols et des végétaux*. INRA Bordeaux Sciences Agro.

Mitsch, W. J., & Gosselink, J. G. (2015). *Wetlands* (Fifth edition). John Wiley and Sons, Inc.

Negassa, W., & Leinweber, P. (2009). How does the Hedley sequential phosphorus fractionation reflect impacts of land use and management on soil phosphorus: A review. *Journal of Plant Nutrition and Soil Science*, *172*(3), 305–325. https://doi.org/10.1002/jpln.200800223

Oberson, A., & Joner, E. J. (2005). Microbial turnover of phosphorus in soil. In *Organic phosphorus in the environment* (pp. 133–164). CABI. https://doi.org/10.1079/9780851998220.0133

Oehl, F., Oberson, A., Probst, M., Fliessbach, A., Roth, H.-R., & Frossard, E. (2001). Kinetics of microbial phosphorus uptake in cultivated soils. *Biology and Fertility of Soils*, *34*(1), 31–41. https://doi.org/10.1007/s003740100362

Ohno, T., & Zibilske, L. M. (1991). Determination of Low Concentrations of Phosphorus in Soil Extracts Using Malachite Green. *Soil Science Society of America Journal*, *55*(3), 892–895. https://doi.org/10.2136/sssaj1991.03615995005500030046x

Papp, C. S. E., & Harms, T. F. (1985). Comparison of digestion methods for total elemental analysis of peat and separation of its organic and inorganic components. *The Analyst*, *110*(3), 237. https://doi.org/10.1039/an9851000237

Pinsonneault, A. J., Moore, T. R., & Roulet, N. T. (2016). Effects of long-term fertilization on peat stoichiometry and associated microbial enzyme activity in an ombrotrophic bog. *Biogeochemistry*, *129*(1), 149–164. https://doi.org/10.1007/s10533-016-0224-6

Rankin, T. (2022). *Controls on autotrophic and heterotrophic respiration in an ombrotrophic bog*. McGill University.

Richardson, A. E. (2001). Prospects for using soil microorganisms to improve the acquisition of phosphorus by plants. *Functional Plant Biology*, *28*(9), 897. https://doi.org/10.1071/PP01093

Saa, A., Trasar-Cepeda, M. C., Gil-Sotres, F., & Carballas, T. (1993). Changes in soil phosphorus and acid phosphatase activity immediately following forest fires. *Soil Biology and Biochemistry*, *25*(9), 1223–1230. https://doi.org/10.1016/0038-0717(93)90218-Z

Smith, D. B., Cannon, W. F., Woodruff, L. G., Garrett, R. G., Klassen, R., Kilburn, J. E., Horton, J. D., King, H. D., Goldhaber, M. B., & Morrison, J. M. (2005). *Major- and Trace-Element Concentrations in Soils from Two Continental-Scale Transects of the United States and Canada* (Open File Report 2005–1253; p. 22). U.S. Geological Survey.

Turetsky, M. R. (2004). Decomposition and Organic Matter Quality in Continental Peatlands: The Ghost of Permafrost Past. *Ecosystems*, *7*(7), 740–750. https://doi.org/10.1007/s10021-004-0247-z

Turner, B. L., Frossard, Emmanuel., & Baldwin, D. S. (2005). *Organic phosphorus in the environment* (1–1 online resource (xiii, 399 pages) : illustrations). CABI Publishing. https://doi.org/10.1079/9780851998220.0000

Turunen, J., Roulet, N. T., Moore, T. R., & Richard, P. J. H. (2004). Nitrogen deposition and increased carbon accumulation in ombrotrophic peatlands in eastern Canada: N deposition and peat accumulation. *Global Biogeochemical Cycles*, *18*(3), n/a-n/a. https://doi.org/10.1029/2003GB002154

Vincent, L. A., Zhang, X., Mekis, É., Wan, H., & Bush, E. J. (2018). Changes in Canada's Climate: Trends in Indices Based on Daily Temperature and Precipitation Data. *Atmosphere-Ocean*, *56*(5), 332–349. https://doi.org/10.1080/07055900.2018.1514579

Von Post, L. (1922). Swedish geological peat survey with the results obtained so far. *Sven. Mosskult. Tidskr*, *36*, 1–27.

Wang, M., Moore, T. R., Talbot, J., & Richard, P. J. H. (2014). The cascade of C:N:P stoichiometry in an ombrotrophic peatland: From plants to peat. *Environmental Research Letters*, *9*(2), 024003. https://doi.org/10.1088/1748-9326/9/2/024003

Williams, B. L., & Sparling, G. P. (1984). Extractable N and P in relation to microbial biomass in UK acid organic soils. *Plant and Soil*, *76*(1–3), 139–148. https://doi.org/10.1007/BF02205574

Wilson, P. G. (2012). *The relationship among micro-topographic variation, water table depth and biogeochemistry in an ombrotrophic bog* [Masters of Science, McGill University]. https://escholarship.mcgill.ca/downloads/1g05fg587?locale=en

Yanai, R. D. (1992). Phosphorus budget of a 70-year-old northern hardwood forest. *Biogeochemistry*, *17*(1). https://doi.org/10.1007/BF00002757

Yevdokimov, I., Larionova, A., & Blagodatskaya, E. (2016). Microbial immobilisation of phosphorus in soils exposed to drying-rewetting and freeze-thawing cycles. *Biology and Fertility of Soils*, *52*(5), 685–696. https://doi.org/10.1007/s00374-016-1112-x

Zeppel, M. J. B., Wilks, J. V., & Lewis, J. D. (2014). Impacts of extreme precipitation and seasonal changes in precipitation on plants. *Biogeosciences*, *11*(11), 3083–3093. https://doi.org/10.5194/bg-11-3083-2014

A Deeper Look at Peat Phosphorus Pools

In the first chapter, I have established that microbial and labile phosphorus concentrations in an ombrotrophic bog vary greatly with changes in water table level and season. Despite this wide variation, negative feedback loops prevent either the labile or microbial pools from becoming permanently depleted under normal conditions. Disturbance, however, is becoming the new normal. Increased nutrient concentrations from higher rates of decomposition or nutrient-rich ash post-fire could push the system into a new equilibrium. To examine some of the potential characteristics and consequences of nutrient addition to peat soils, I analyzed samples from the Mer Bleue long-term fertilization experiment. This fertilization trial occurs in the same bog from which I took samples for "Seasonal Changes in Labile and Microbial Phosphorus Concentrations in an Ombrotrophic Bog in Eastern Ontario, Canada." Samples were taken from the unfertilized center of the bog for the previous chapter, and from plots to which ammonium nitrate, phosphate + potassium, or all three together have been added for nineteen to twenty years.

The sequential fractionation allows me to look more closely at moderate- and low-availability forms of phosphorus in soils. This, in turn, can help predict how much of the added phosphorus will be taken up by plants and microorganisms at different time scales. In addition, looking at the available and moderate-availability phosphorus pools can explain some of the variation in the sum of labile and microbial phosphorus over the seasons. Samples were taken for the sequential fractionation at a single point in time, meaning that the speed of movement between the pools cannot be determined with any exactness, but the size of the available and moderately available pools prevent them from being ruled out as an explanation.

4. Nutrient Addition Increases Available and Residual Phosphorus Concentrations in an Ombrotrophic Bog

Camille Jones[1], Tim Moore[1], Cynthia Kallenbach[2], Camille Brais[1], Meng Wang[3], Christian von Sperber[1]

1 Department of Geography, McGill University, Montreal, QC; camille.jones@mail.mcgill.ca

2 Department of Natural Resource Sciences, McGill University, Sainte Anne de Bellevue, QC

3 School of Geographical Sciences, Northeast Normal University, Changchun, Jilin

Abstract

Nutrient cycling and nutrient status control primary productivity, decomposition, plant community composition, and microbial community composition. To study the effects of nutrient addition and nutrient limitation on peatland soil phosphorus pools, samples were taken from the long-term fertilization experiment at Mer Bleue Bog near Ottawa, ON. Samples were taken in 2021 following nearly twenty years of fertilizer application. These samples were subjected to a Hedley sequential fractionation to extract phosphorus along a gradient of biological availability. In unfertilized peat, phosphorus concentrations were highest in the available and highly recalcitrant pools, with little between them. This U-shaped distribution of phosphorus along the gradient of availability contrasts with established patterns in mineral soils. In plots which received PK and NPK fertilization, concentrations of both available phosphorus and highly recalcitrant phosphorus doubled. In plots receiving N fertilization alone, available and total P concentrations decreased, which may indicate increased demand for P by plants and microorganisms when N status is high. In addition, fertilization led to changes aboveground. *Chamaedaphne calyculata* leaves in plots receiving PK and NPK were enriched in phosphorus compared to *C. calyculata* leaves in unfertilized plots and plots receiving N alone. In all plots

receiving fertilizer, concentrations of highly recalcitrant phosphorus increased, which may indicate increased decomposition of peat.

4.1. Introduction

Northern peatlands store approximately one-third of global soil carbon despite covering only 3% of the land surface (Gorham, 1991; Yu, 2012). This is due to slow peat decomposition in undisturbed bogs and fens. A high water table, low pH, and low nutrient content preserve organic matter, allowing it to accumulate over thousands of years (Aerts et al., 1992; Ingram, 1987). As a result of this extensive peat accumulation, surface peat becomes far removed from the underlying soil and groundwater. In consequence, the peat becomes ombrotrophic, receiving water and nutrients from precipitation and atmospheric deposition alone (Mitsch & Gosselink, 2015). As a result, soil nutrient concentrations in ombrotrophic bogs are low, and they are considered to be nitrogen (N) and phosphorus (P) co-limited (M. Wang et al., 2014).

Due to the lack of connection to underlying mineral soils and groundwater, phosphorus in ombrotrophic bogs is dominated by the organic phase, though peat soil phosphorus is found in both organic and inorganic forms (Williams & Sparling, 1984). While organic phosphorus as a whole is a large P reservoir, it is often of low availability to plants and soil microorganisms (Alexander, 1977; Chen et al., 2003; Walker & Syers, 1976). Plants and microbes preferentially take up labile inorganic phosphate over organic phosphates when both are available, but may take organic phosphates directly when phosphorus-limited (Celi et al., 2022). Both organic and inorganic phosphates may be sorbed by soil minerals, reducing their biological availability. This is often the fate of the bulk of phosphates in mature mineral soils, but peat’s low mineral content greatly decreases the importance of this pool (Velásquez et al., 2016). After extensive decomposition, weathering, and aggregation, particularly strongly sorbed, well-protected, or otherwise low-availability phosphorus is known as residual or recalcitrant (Celi et al., 2022; Dean, 1938; Velásquez et al., 2016). The residual fraction may contain both organic and inorganic forms of phosphorus (Velásquez et al., 2016).

In times of high nitrogen demand, plants may promote microbial nitrogen mineralization by increasing their exudation of easily digestible sugars (Carrara et al., 2021; Meier et al., 2017). This favors high-turnover microbial populations that mineralize fast-cycling organic nitrogen (Carrara et al., 2021; Meier et al., 2017). When this occurs, the plants may intercept and take up some of the newly mineralized nitrogen (Carrara et al., 2021). While this phenomenon is most clearly shown during nitrogen limitation, it may occur in response to phosphorus limitation as well. For example, plants in some ecosystems may increase their labile carbon exudation when phosphorus is limited in an effort to stimulate microbial mining of soil organic matter, then intercept some of the newly released phosphorus (Spohn & Kuzyakov, 2013). While this phenomenon is not as well-established for phosphorus as it is for nitrogen, it merits discussion as a major potential plant controls on soil biogeochemistry in the living layer.

While rates of total nitrogen deposition in North America have slowed since their peak at the turn of the millennium, nitrogen deposition rates are still higher than they were in the pre-industrial era (Gilliam et al., 2019; Kanakidou et al., 2016). The ecosystem response to increased atmospheric nitrogen deposition may lag behind by years or decades (Gilliam et al., 2019). As such, ombrotrophic bogs may move from being nitrogen and phosphorus co-limited to phosphorus limited. Nutrient addition affects key ecological variables like primary productivity and net ecosystem exchange of CO_2 through changes in plant community composition and decomposition rate through changes microbial community composition and activity. Long-term fertilization experiments in upland ecosystems have shown that fertilization changes phosphorus biogeochemistry (Grunes, 1959; Negassa & Leinweber, 2009; Y. Wang et al., 2021). Similar experiments in wetlands have revealed changes in plant and microbial communities, but studies that investigated the belowground peat biogeochemistry are lacking. As such, this thesis aims to investigate the effects of fertilization on peat phosphorus biogeochemistry. To do so, I have used Hedley sequential fractionation to extract different pools of phosphorus along a gradient of bioavailability.

4.1.1. Objectives and Hypotheses

The broad objective of this analysis is to examine the effect of long-term nutrient addition on peat phosphorus pools. Analysis of fertilized peat plots allowed me to compare the effects of nitrogen addition, phosphorus addition, and the addition of nitrogen and phosphorus together on peat soil phosphorus pools. Furthermore, I was able to compare these effects to the effects of fertilization on soil phosphorus pools in other soil types. This granted me a better understanding of the effects of fertilization on peat biogeochemistry and potential ecological responses.

The addition of labile phosphorus in the form of orthophosphate will increase the concentration of labile phosphorus in fertilized plots. The addition of ammonium nitrate fertilizer may lead to small decreases in labile phosphorus concentrations as plants increase belowground phosphorus mining and move liberated phosphorus aboveground (Carrara et al., 2021). Neither nitrogen nor phosphorus addition is unlikely to cause large changes to the NaOH-extractable, 1 M HCl-extractable, or concentrated HCl-extractable fractions due to peat's low mineral content. Considering the increased respiration and subsidence of fertilized peat, there is likely higher decomposition and thus movement from labile to recalcitrant forms of phosphorus in all of the fertilized plots (Moore et al., 2019).

Hypotheses:

1. Addition of P fertilizer will increase both labile and recalcitrant P concentrations in peat.
2. Addition of N fertilizer will decrease labile P concentrations and increase the proportion of recalcitrant P concentrations in peat.
3. Addition of labile N and P fertilizers together will increase both labile and recalcitrant P concentrations in peat.

4.2. Methods

4.2.1. Site Description

Samples were taken from Mer Bleue Bog (45°24'39.6"N 75°31'15.6"W) near Ottawa, Ontario (Fig. 4-1). The bog is domed and surrounded by a lagg consisting of multiple narrow beaver ponds. The bog center has hummock-and-hollow microtopography, which flattens at the edge of the lagg. Dominant vegetation consists of *Kalmia angustifolia, Chamaedaphne calyculata, Rhododendron groenlandicum,* and *Vaccinium myrtilloides*. Trees (*Picea mariana, Larix laricinia,* and *Betula populifolia*) and sedges (*Eriophorum vaginatum*) are scattered on hummocks in the bog center and grow more densely near the lagg. The average temperature for the area from 1991-2020 was 6.5°C, and average precipitation over the same time period was 930 mm, of which 404 mm fell as snow (*Canadian Climate Normals 1991-2020 Ottawa (Station Data)*, 2023).

Mer Bleue is the site of a long-term fertilization trial (Juutinen et al., 2010). 3m x 3m plots were set up in triplicate and received nitrogen fertilizer (NH_4NO_3) with or without potassium and phosphate (KH_2PO_4) dissolved in 2-mm distilled water (Juutinen et al., 2010). Nitrogen was applied at 10 times the annual rate of summer wet deposition (Juutinen et al., 2010). Potassium and phosphorus were applied at 6.3 g P per m^2 per year and 5.0 g K per m^2 per year, approximately 50 times the background rate of deposition (Juutinen et al., 2010). Phosphorus and potassium have been added to the PK plots since 2000, nitrogen has been added to the N plots since 2005, and all three nutrients together have been added to the NPK plots since 2001. Control plots received 2-mm distilled water with no fertilizer until 2021, at which point all fertilizers were applied as a dry powder and the control plots received nothing (Brais, 2022). Treatments were applied every third week from May until the end of August (Juutinen et al., 2010). In this analysis, the 10N, PK, 10NPK, and Control O plots were sampled. These will henceforth be referred to as N, PK, NPK, and control.

4.2.2. Sample Preparation

Samples were collected in October 2021. Two peat cores were taken from each plot with a Russian auger (Fig. 4-1). Layers were separated primarily by depth, but exact boundaries were determined by color and physical characteristics of the layers so as to account for variations in microtopography, especially in the topmost layers. Separation by depth exclusively would prevent accurate comparison between the plots, as the peat surface subsided by more than 10cm in the center of the highly fertilized plots (Moore et al., 2019). As such, for ease of displaying figures, these layers will be reported by their corresponding depth in the control plots. For example, Layer X has consistent physical characteristics and level of humification across all plots, and is found at 20-35 cm in the control plots and 10-20 cm in the heavily fertilized plots. It will be reported as 20-35 cm since that is its depth in the control plots. The depths of the layers in the control plots roughly correspond to 0-15 cm, 20-35 cm, and 40-55 cm in depth and will be referred to as such in the following figures. Corresponding layers from the cores were bulked, oven-dried at 40°C for 96 hours, and ground using a size 40 mesh (Wiley Mini Mill 3383-L10, Thomas Scientific, USA).

4.2.3. Hedley Sequential Fractionation

Tiessen and Moir's (1993) update to the fractionation scheme devised by Hedley et al. (1982) was followed with minimal deviations. 0.5g of soil were subjected to a resin extraction with anion exchange resin membranes for 16 hours (VWR Chemicals, Leuven, Belgium). Afterwards, the samples were filtered and set in 30 mL 0.5 M $NaHCO_3$ for 16 hours, then centrifuged and filtered. After filtering, a subsample of bicarbonate extraction solution was subjected to an ammonium persulfate digestion to determine organic P. Next, 30 mL 0.1 M NaOH was added and samples were shaken for 16 hours before centrifugation and filtration. A subsample of NaOH extraction solution was subjected to an ammonium persulfate digestion to determine organic P in the NaOH fraction. After that, 30 mL 1 M HCl was added and samples were shaken for a further 16 hours before centrifugation and filtration. Next, the filtered samples were subjected to 10 mL conc. HCl and set in a hot water bath until they reached 80°C, at which point they were removed from the hot water bath and a further 5 mL of concentrated HCl was added to each sample. After cooling, the samples were centrifuged, supernatant was collected, samples were washed with DI water, and centrifuged again. After a final round of filtration, the samples were subjected to a hot acid digestion with piranha solution. This represents a small deviation from the Tiessen & Moir (1993) protocol. Tiessen & Moir call for digestion with concentrated sulfuric acid and hydrogen peroxide, to which I added lithium sulfate to increase digestion temperature, and selenium as a catalyst as in Parkinson & Allen (1975). Soils were added to quartz tubes with a boiling chip, 5 mL concentrated sulfuric acid, and 2.5 mL of the peroxide, lithium sulfate, and selenium mixture. Samples were heated to 360ºC for 4.5 hours with small amounts of peroxide added every 90 minutes if samples were not yet fully digested.

To prepare for quantification, all samples of extraction solution were titrated to pH 5.5. Malachite green was used for colorimetry in place of the molybdenum blue used by Tiessen and Moir (1993). Malachite green was chosen for its superior accuracy at low phosphorus concentrations and for its potential to avoid inaccuracy from humic acid precipitation (Ohno & Zibilske, 1991). Samples were read with a BioTek Epoch microplate spectrophotometer (Agilent, Santa Clara, USA).

4.2.4. Statistics

All statistical analyses were carried out in RStudio version 2023.12.0 +369, running R version 4.2.1. The resin-, bicarb-, NaOH-, 1 M HCl-, concentrated HCl-extractable, residual, and total phosphorus data were tested for normality using Q-Q plots. All were normally distributed, allowing me to calculate one-way analysis of variance (ANOVA) between treatments within each layer of sampling depth. The results are displayed on the figures below as compact letter displays (clds) at 95% confidence.

4.3. Results

The amount of total P extractable by anion-exchange resin was high, especially in the top layer of peat (Fig. 4-4, Table 4-1). There is a clear negative relationship between resin P concentration and depth across all treatments (Fig. 4-4). Mean P concentrations in the PK and NPK treatment were higher than the control, while P concentrations in the N treatment were slightly lower than in the control in the top two layers of sampling depth ($p < 0.05$). In the deepest layer, the control treatment overlaps with the fertilized treatments ($p > 0.05$).

Bicarbonate-extractable inorganic P (P_i) and organic P (P_o) concentrations were much lower than resin-extractable P concentrations (Figs. 4-5 and 4-6). The negative relationship between P concentration and depth is clear in the NPK treatment for both bicarb-extractable P_i and P_o, but not present for the other treatments. In the bicarb-extractable inorganic P fraction, the PK and NPK treatments are distinct from the control and N treatments in all layers ($p < 0.05$). In the organic fraction, this is only true in the middle layer (sampling depth 20-35 cm).

NaOH-extractable P levels varied by treatment, with distinctions between the control, PK, and NPK treatments in the first two layers of sampling depth ($p < 0.05$) (Fig. 4-7). Similar to the resin- and bicarbonate-extractable P fractions, there is less separation between the treatment groups in the deepest layer sampled. The negative relationship between P concentration and depth seen in resin-extractable and NPK bicarb-extractable P does not occur in NaOH-extractable P pool. In the control, N, and PK treatments, NaOH-extractable P decreases between 0-15 cm and 20-35 cm, but increases between 20-35 and 40-55 cm (Fig. 4-7). Similar to bicarbonate-extractable P, the top layer of the NPK treatment has a much higher P concentration than the rest of the layers.

P concentrations in the 1 M HCl-extractable fraction were lower than in all other fractions (Fig. 4-8). Variations between the treatment groups were relatively small, with the largest separation between the control, N, and NPK treatments ($p < 0.05$). Similar to the above fractions, differences between treatment groups were greater in the top two sampling depths than they were in the deepest layer.

Concentrated HCl-extractable P levels were much higher than 1 M HCl-extractable P levels (Fig. 4-9). P levels in this fraction generally decrease with depth, following the pattern seen in the resin- and bicarbonate-extractable P fractions. The separation between treatment groups is larger in the top two layers of sampling depth than in the deepest layer.

P concentrations in the residual fraction are relatively high and decrease with depth (Fig. 4-10). The control and N treatments are distinct from the PK and NPK treatment in the top layer ($p < 0.05$), but not in the two deeper layers ($p > 0.05$). Patterns seen in this layer are similar to patterns seen in the resin- and concentrated HCl-extractable fractions, which also had relatively high P concentrations.

Total P concentrations are the sum of all of the fractions. The negative relationship between P concentrations and depth is present in all treatments and is most dramatic in the PK and NPK treatments (Fig. 4-11). Similar to many of the above fractions, the control and N treatments are distinct from the PK and NPK treatments in the top two layers of sampling depth ($p < 0.05$), but not in the deepest layer ($p > 0.05$).

4.4. Discussion

4.4.1. Resin-extractable P

Resin-extractable P increased with phosphorus addition and decreased with depth of sampling (Fig. 4-4). One potential explanation for the negative relationship between depth and resin-P concentration (Fig. 4-4) is the "pump" effect of plant roots, which take labile phosphorus from deeper layers of peat and tend to move it closer to the surface (Chen et al., 2000; Richardson & Marshall, 1986). Another explanation is the effect of microbial phosphorus. Since these samples were dried and ground, widespread microbial lysis and potential mineralization of microbial P occurred (Bünemann et al., 2011), which would be extracted by the anion exchange resin. To determine how much of the trend could be explained by microbial phosphorus, the data for the control plots can be compared to my work on seasonal dynamics in microbial phosphorus. Resin-extractable P ranged from approximately 5 to 40 mg kg^{-1} P, and microbial P ranged from approximately 40 to 150 mg kg^{-1} P, which is large enough to make up the difference. For further information about microbial phosphorus, see Chapter 3.

In comparison to the control, the N-addition trial experienced a small dip in resin-extractable P concentrations (Fig. 4-4). This may be explained by the scavenging hypothesis (Carrara et al., 2021; Meier et al., 2017). Since plant and microbial N acquisition and use require P, increased P scavenging is often required in order for plants and organisms to acquire and make use of the large influx of N. In so doing, plants move a large amount of P aboveground, causing the slight decrease in soil resin-extractable P.

Resin-extractable P concentrations in the top layer (0-15 cm) are higher than resin-P concentrations in both fertilized agricultural soils and unfertilized mineral soils (Cross & Schlesinger, 1995; Negassa & Leinweber, 2009). In deeper layers (20-35 cm and 40-55 cm), resin-extractable P concentrations more closely resemble less-fertilized agricultural soils and unfertilized mineral soils (Cross & Schlesinger, 1995; Negassa & Leinweber, 2009).

4.4.2. Bicarbonate-extractable P

These bicarb-extractable P_i concentrations are slightly higher than reported concentrations for bicarbonate-extractable P in unfertilized mineral soils (Cross & Schlesinger, 1995), but slightly lower than reported concentrations for fertilized agricultural soils (Negassa & Leinweber, 2009). Bicarbonate-extractable P_o, on the other hand, was higher than concentrations in mineral soils regardless of fertilization. This falls in line with expected patterns, as the organic phase of the phosphorus cycle dominates in peatlands (Celi et al., 2022).

4.4.3. NaOH-extractable P

In comparison to the resin- and bicarbonate-extractable P fractions, the concentrations of NaOH-extractable P fraction were low and less affected by P application (Fig. 4-7). This supports Tiessen & Moir's interpretation of this fraction as being associated with iron and aluminum oxides and hydroxides (1993). Since iron and aluminum are found in extremely low concentrations in peat, this fraction is unlikely to be affected by P application (Fig. 4-13, Fig. 4-15).

NaOH-extractable P concentrations here are comparable to NaOH-extractable P concentrations in unfertilized mineral soils, with the exception of the top layer of the NPK treatment (Cross & Schlesinger, 1995). Concentrations are lower than NaOH-extractable P concentrations in fertilized agricultural soils, which also included a few anomalously high values, see Table 1 (Negassa & Leinweber, 2009).

There is a large degree of uncertainty in the separation between organic and inorganic forms of P within the same extract (Condron & Newman, 2011; Turner et al., 2003). In particular, Turner et al. (2003) showed that NaOH extracts contain both organic and inorganic P, meaning that the inorganic P pool for this step is often inflated and the organic P pool is underestimated. As such, only NaOH-extractable total P is presented here. Caution should be taken in the interpretation of organic and inorganic bicarbonate-extractable P in these results, as it is likely that there is more uncertainty in the division between them than in the divisions between the different extraction solutions (Turner et al., 2003). For example, inorganic pyrophosphates and other polyphosphates will not react during colorimetry, and will instead be converted to orthophosphate during the organic matter digestion in the bicarbonate and NaOH extractions, artificially increasing the organic P pool for these fractions (Reina et al., 2011). It is unclear how much polyphosphate may be present in peat soils, but it may make up more than 10% of total P in agricultural soils (Prüter et al., 2023). Polyphosphate-accumulating organisms are particularly abundant in freshwater ecosystems, meaning that wetlands may have an even higher abundance of polyphosphates (Saia et al., 2021).

4.4.4. 1 M HCl-extractable P

1 M HCl-extractable P concentrations were low and less affected by nutrient addition than the resin- and bicarbonate-extractable P fractions (Fig. 4-8). These concentrations are much smaller than 1 M HCl-extractable P concentrations in unfertilized mineral soils and fertilized agricultural soils (Cross & Schlesinger, 1995; Negassa & Leinweber, 2009). The 1 M HCl pool is

commonly interpreted as Ca-associated P, though this is not true in all cases (Barrow et al., 2021; Tiessen & Moir, 1993). However, considering that 1 M HCl-extractable P tends to be high in calcareous soils, it is not an unreasonable interpretation in Ca-rich soils (Cross & Schlesinger, 1995). Considering the extremely low concentration of Ca in peat soils, it is not surprising that this fraction was the least concentrated of all and, like the NaOH-extractable fraction, be relatively unaffected by P addition (Fig. 4-16).

4.4.5. Residual P

Residual P concentrations are quite high in comparison to the other fractions studied in this experiment (Fig. 4-10). As such, peatlands deviate from the large-scale pattern of P availability in mineral soils set forth by Walker and Syers (1976). In mineral soils, high residual and organic fractions, are associated with low concentrations of labile phosphorus and vice-versa (Walker & Syers, 1976). In mature peat, it appears that both labile and residual P concentrations are relatively high (Figs. 4-4 and 4-10). Residual P concentrations from this experiment are comparable to residual P concentrations in both unfertilized mineral soils and fertilized agricultural soils with the exception of the top layer in the PK treatment (Fig. 4-10) (Cross & Schlesinger, 1995; Negassa & Leinweber, 2009). As such, it appears that phosphorus fertilization can increase residual P concentrations after 20 years of fertilization.

4.4.6. Total P

The proportion of total P made up by the residual fraction increased with all three fertilization treatments in comparison to the control (Fig. 4-12). Between layers, the proportion of residual P increased with depth in the N and NPK treatments. The control and N treatments were distinct from each other in all three layers, while the PK and NPK treatments were only distinct in the top layer ($p > 0.05$). An increase in the proportion of residual P can be an indication of increased decomposition (Moore et al., 2019). Fertilization increases the scavenging activity of soil microbes, which take the most easily accessible nutrients first, proceeding from labile pools to slightly more recalcitrant pools, making some available to plants as they do so (Carrara et al., 2021; Tian et al., 2021). As plant roots pull labile phosphate from deeper layers to the surface, recalcitrant P makes up a greater share of the remaining P in deeper layers (Moore et al., 2019).

Figure 4-13 provides further evidence for the enhanced decomposition theory put forward by Figure 4-12. Aluminum is not of relevance as a plant nutrient, has not undergone the "pump" effect mentioned above, and is deposited relatively evenly within peat soils (Poschenrieder & Barceló, 2013). As such, it can be used as an indicator of decomposition; as more carbon and nutrients are removed, the relative concentration of aluminum increases. This is seen in Figure 4-13, as concentrations of aluminum increase with depth and show a marked increase with fertilization compared to the control (O). Neither the proportion of residual P nor aluminum concentration alone is sufficient evidence for increased decomposition due to fertilization, but taken along with higher respiration rates recorded by Moore et al., peat subsidence, increased bulk density, and changes in plant community composition as a result of fertilization, the link between nutrient addition and increased peat decomposition becomes clearer (2019).

Total P concentrations from this experiment are higher than total P concentrations in unfertilized mineral soils (Cross & Schlesinger, 1995) and lower than most total P concentrations in fertilized agricultural soils (Negassa & Leinweber, 2009). This contrasts with the popular idea that ombrotrophic bogs are low in phosphorus. However, peat's low dry mass makes phosphorus concentrations in the field much lower. In addition, a large proportion of the total is made up of low-availability phosphorus, keeping the ecosystem relatively low in P (Figs. 4-10 to 4-12).

4.4.7. Patterns

Plant activity may have caused the decrease in P concentrations in the N treatment of several fractions (Figs. 4-4, 4-7, 4-8, 4-9, 4-11). In order to make use of the excess nitrogen applied in the N treatment, plants may increase uptake of soil phosphorus, potassium, and micronutrients (Carrara et al., 2021; Grunes, 1959). As plants take up P, a larger amount of it is held in aboveground biomass, and soil P concentrations are slightly decreased. This phenomenon is not limited to nitrogen alone. A bolus of available C, N, or P can stimulate decomposition of soil organic matter (Bodker et al., 2015; Carrara et al., 2021; Grunes, 1959; Moore et al., 2019; Qualls & Richardson, 2000). This then liberates significant amounts of other nutrients necessary to take up and make use of the bolus (Carrara et al., 2021; Meier et al., 2017; Spohn & Kuzyakov, 2013). This would also the residual fraction makes up a larger proportion of the total P in all depths of the N treatment (Fig. 4-12). In fact, in the two deeper layers, where most of the enhanced decomposition would be expected, the N treatment had a higher proportion of residual P than the PK or NPK treatments (Fig. 4-12). Labile P would be taken up preferentially, leaving a smaller total P pool and increasing the proportion of residual P (Oehl et al., 2001).

4.4.8. Comparison to Published Values

Total P values for this study fall within the established range for soils with high organic matter content, but the distribution of phosphorus between fractions is different than that seen in studies of mollisols and histosols (Table 4-1). In broad strokes, the patterns are as follows: in unfertilized mollisols and highly degraded histosols, there was relatively little readily available phosphorus, relatively little phosphorus of moderate availability, and relatively large amounts of low-availability residual phosphorus (Cross & Schlesinger, 1995; Schlichting et al., 2002). In fertilized mollisols, phosphorus of moderate availability prevailed, though the residual pool made up a relevant share of the total in some samples (Negassa & Leinweber, 2009). In the surface peat samples studied here, readily available phosphorus and low-availability residual phosphorus made up the bulk of the total, with less contribution from phosphorus of moderate availability. In fact, the peat soils studied here had the highest resin-extractable and residual P concentrations of all the soils shown in Table 4-1. Soil characteristics may explain some of the differences seen. For example, iron, aluminum, and calcium concentrations are likely much higher in the mollisols studied by Cross & Schlesinger (1995) and Negassa & Leinweber (2009) than in the peat soils studied here, explaining the higher concentrations of phosphorus in the NaOH- and 1 M-HCl-extractable pools in these mineral soils. It is also likely that the histosols studied by Schlichting et al. also had higher mineral concentrations than the surface peat studied in this experiment (2002). The histosols studied by Schlichting et al. were highly degraded, as they were likely more than a meter below the living layer before being drained and used for cultivation (2002). As such, they would be expected to have slightly higher mineral concentrations and a much higher proportion of residual P. Indeed, this appears to be the case; residual P makes up between 50% and 75% of the total phosphorus in all of the degraded histosols studied by Schlichting et al., and roughly 20% to 50% of the total phosphorus in the unfertilized peat studied in this experiment (Schlichting et al., 2002). It is worth noting that Schlichting et al. used 1 M H_2SO_4 where 1 M HCl was used by the other three fractionation experiments displayed in Table 4-1 (2002).

4.4.9. Mer Bleue Fertilization Plots P Budget

The total phosphorus measured in this experiment does not account for all of the phosphorus that was applied to these plots since the beginning of the fertilization trial (Table 4-2). 25% of the phosphorus applied to the PK and 27% of the phosphorus applied to the NPK trial was not recovered in this sequential fractionation (Table 4-2). One potential explanation for P losses from peat in the fertilized plots is that it has moved aboveground. *Chamaedaphne calyculata* leaf P concentrations in the fertilization trial ranged between 500 and 700 mg kg^{-1} P in the control and N plots and 1100 to 1300 mg kg^{-1} P in the PK and NPK plots (Fig. 4-14). There are no recent studies quantifying the total mass of leaves growing in the plots, *C. calyculata* or otherwise, preventing analysis of the shrub's contribution to the P budget in the plots. That said, vascular plant growth in the fertilized plots is much higher than in the control plots (Juutinen et al., 2010). As such, P uptake by plants may be an explanation for some of the missing phosphorus. This would eventually return to the peat with decomposition but was not measured in this experiment and may thus be counted as a loss.

P translocation below and around the area studied may also explain P losses. Translocation downward is unlikely to be of great consequence here, as P concentrations in the lowest layers (40-55 cm) did not vary between the control plot and fertilized plots (Fig. 4-11). More interesting, perhaps, is lateral diffusion of labile P from the plots, in particular during the spring when the water table is high. Increases in phosphate concentrations of 1.2 ug/cm^2/month in control plot Ob were detected in 2023 (Tim Moore, personal communication). This is a relatively small amount, but given its distance from a plot where phosphorus was applied, the areas just outside of the borders of the fertilized plots may hold a large amount of the missing phosphorus.

4.5. Implications

Taken together with studies recording the effects of nutrient addition on plant community composition, NPP, and decomposition rate in peatlands, we may predict large changes in peatland biogeochemistry. As the plant community shifts towards vascular plants and away from *Sphagnum*, the “nutrient pump” effect may increase as vascular plants lay down higher-quality litter than mosses (Chen et al., 2000; Juutinen et al., 2010; Richardson & Marshall, 1986). In particular, nitrogen addition increases the nitrogen content of litter, increasing the speed at which it is decomposed (Bodker et al., 2015; Bragazza et al., 2012). Increases in vascular plant growth may also draw down the water table thanks to their poorer moisture retention and higher evapotranspiration rate (Ingram, 1987; Verry & Timmons, 1982). This causes further decomposition of the dry layer, providing more nutrients to plants and microbes and potentially leading to a positive feedback loop of decomposition, vascular plant growth, and drying (Fraser et al., 2001; Ingram, 1987). That said, precipitation in the Saint Lawrence region is predicted to increase in total, which may keep the water table level high enough to prevent this positive feedback loop (Kanakidou et al., 2016). However, precipitation variability will increase, which could involve dry periods and fires (Chen et al., 2023). Fire increases P availability and often increases drying in the years afterwards, further changing the biogeochemistry of the affected area (Turetsky et al., 2015). In addition, nitrogen deposition levels are projected to increase (Kanakidou et al., 2016).

The magnitudes of these variables and their effects on each other are not yet clear. Soil nutrient content is one of many controls on decomposition, and, as such, is unlikely to greatly change decomposition rates without concordant changes to the other key variables. That said, nutrient content's connection to plant community composition and ET opens the possibility of a positive feedback loop of increasing peat decomposition. This would represent a massive loss of peat carbon and could push the ecosystem from an ombrotrophic bog to a fen or shallow peat system.

4.6. Conclusions

Fertilization with N, P, or a combination of the two increases the proportion of residual P in peat soils, potentially indicating increased peat decomposition. As such, ombrotrophic peatland conservation must prevent nutrient addition to these unique systems if their carbon stocks are to be protected. In addition, Canadian carbon budgets need to consider increased nitrogen deposition's effect on peatland decomposition and subsequent emissions.

The distribution of phosphorus between the different forms extracted in a sequential fractionation differs greatly between peat soils and mineral soils, just as it does between different types of mineral soils. In this experiment, the majority of peat soil P was found in the residual and resin-extractable fractions with less in the bicarbonate-, NaOH-, 1 M HCl-, and concentrated HCl-extractable fractions. With the bulk of their phosphorus is found in both the residual and labile pools, peat soils do not resemble any stage in the normal aging of mineral soils. In this way, peat soil development and nutrient dynamics are unique.

Tables and Figures

*Table 4-1 . Range of mean values for each extraction from this experiment, from unfertilized mollisols, and from fertilized mollisols. The highest mean ranges for each extract are shown in bold. *Note that Schlichting et al. 2002 used 1 M H¬2SO4 for the acid extraction, where Negassa & Leinweber, Cross & Schlesinger, and this experiment used 1 M HCl.*

	This experiment *mg kg^{-1} P*	Unfertilized temperate mollisols *mg kg^{-1} P* (Cross & Schlesinger, 1995)	Fertilized temperate mollisols *mg kg^{-1} P* (Negassa & Leinweber, 2009)	Highly degraded Histosols *mg kg^{-1} P* (Schlichting et al., 2002)
Resin Pt	**48 - 507**	5 - 59	19 - 165	33 - 77
Bicarb P_i	17 - 106	2 - 29	**11 - 479**	3 – 26
Bicarb Po	26 - 124	5 - 68	10 - 52	**37 - 166**
NaOH Pt	44 - 206	55 - 156	**17 - 1303**	150 - 384
1 M HCl P_i	10 - 35	48 - 280	**101 - 530**	5 - 20*
Residue Pt	**173 - 840**	191 - 378	158 - 749	294 - 481
Total P	502 - 1760	382 - 768	**623 - 2848**	446 - 949

Table 4-2 Mer Bleue phosphorus budget 2000-2021.

	Control	N	PK	NPK
P applied per year (g/m^2)	0	0	5	5
Total P applied from 2000 to 2021 (g/m^2)	0	0	105	100
Total amount recovered in sequential fractionation (g/m^2)	114	111	193	197
Percent recovery	100%	103%	**75%**	**73%**

Figure 4-1 Map of Mer Bleue Bog. Made with OpenStreetMap.

Figure 4-2 Peat core sampled with Russian auger from water table to 48 cm below water table surface.

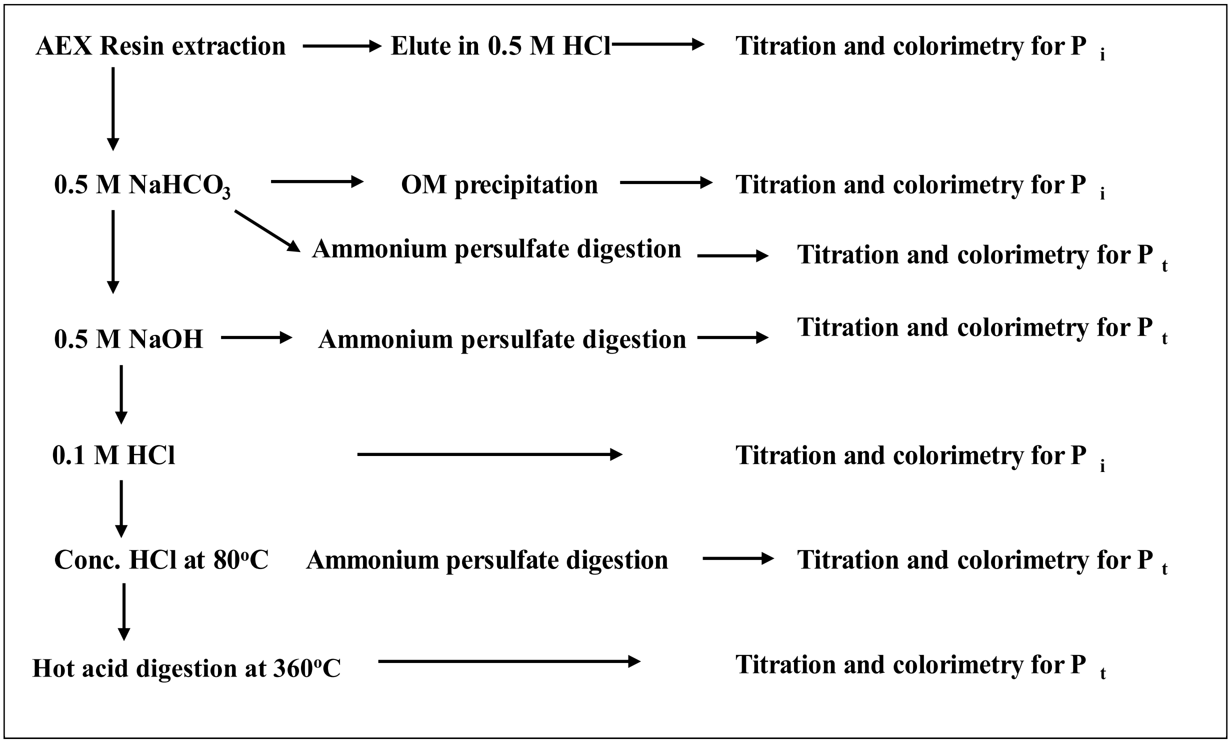

Figure 4-3 Flow chart of the steps of the sequential P extraction.

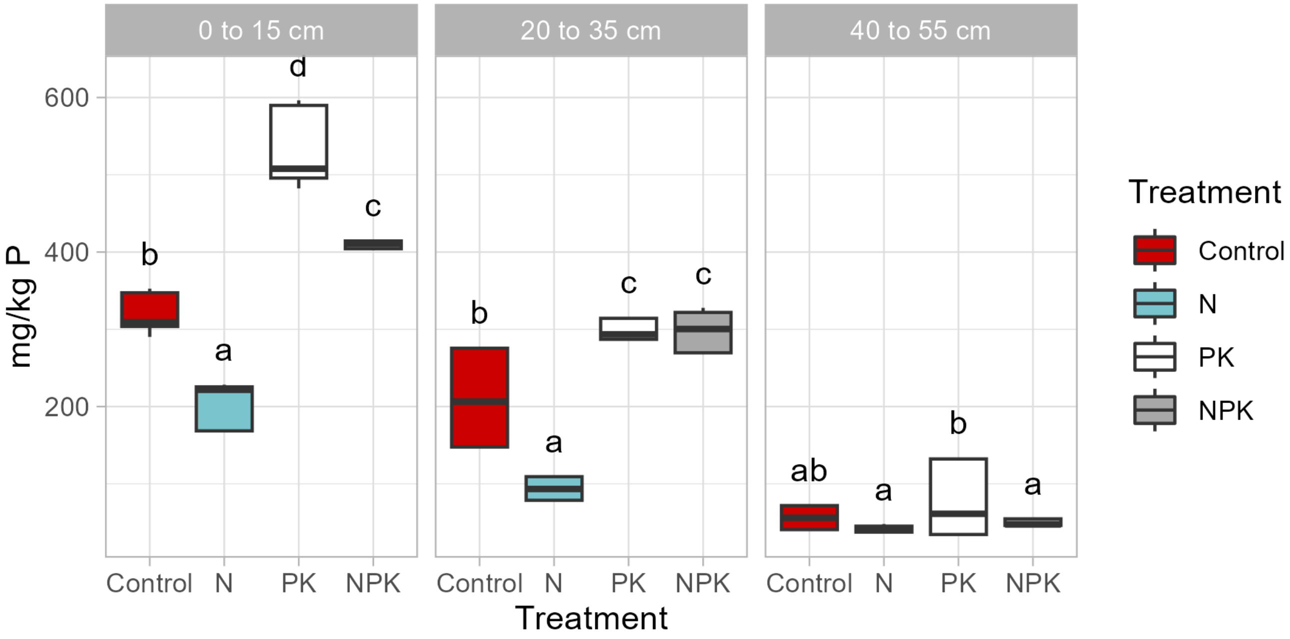

Figure 4-4 Hedley Resin Pt by Layer in mg kg-1 P. Boxplots are grouped by sampling depth. Color corresponds to treatment. Compact letter displays (clds) separate the treatments within each layer at 95% confidence.

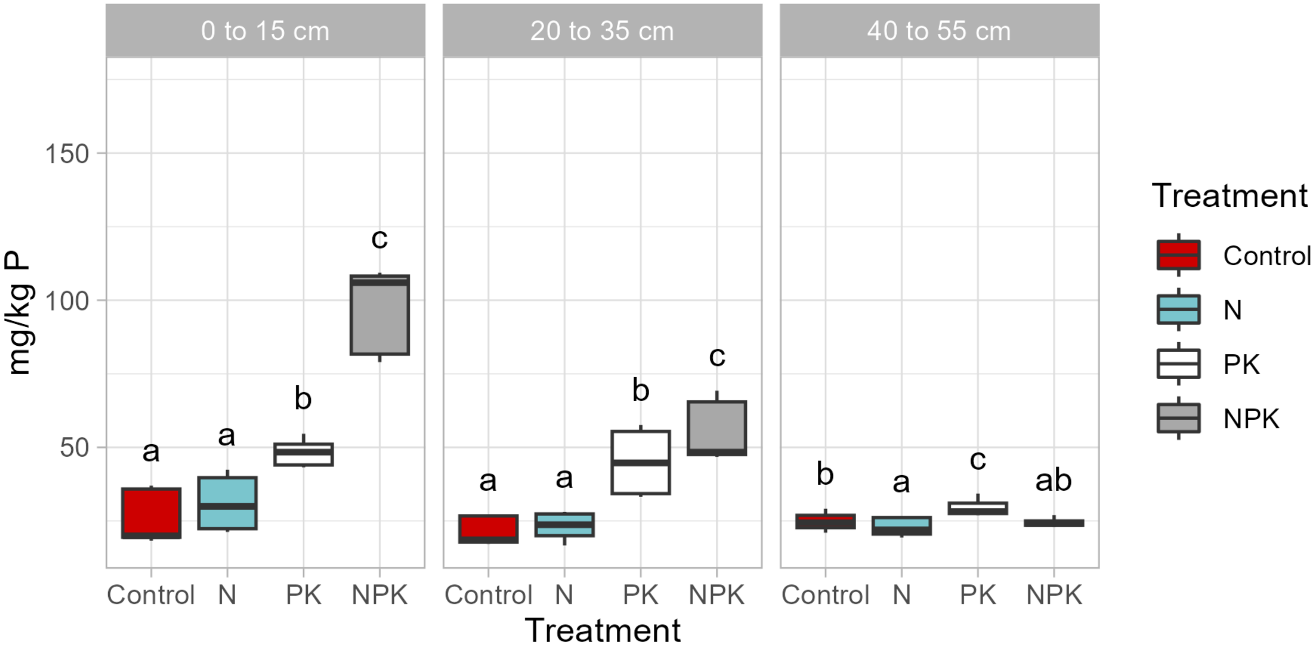

Figure 4-5 Hedley Bicarbonate-Extractable Pi by Layer in mg/kg P. Boxplots are grouped by sampling depth. Color corresponds to treatment. Compact letter displays (clds) separate the treatments within each layer at 95% confidence.

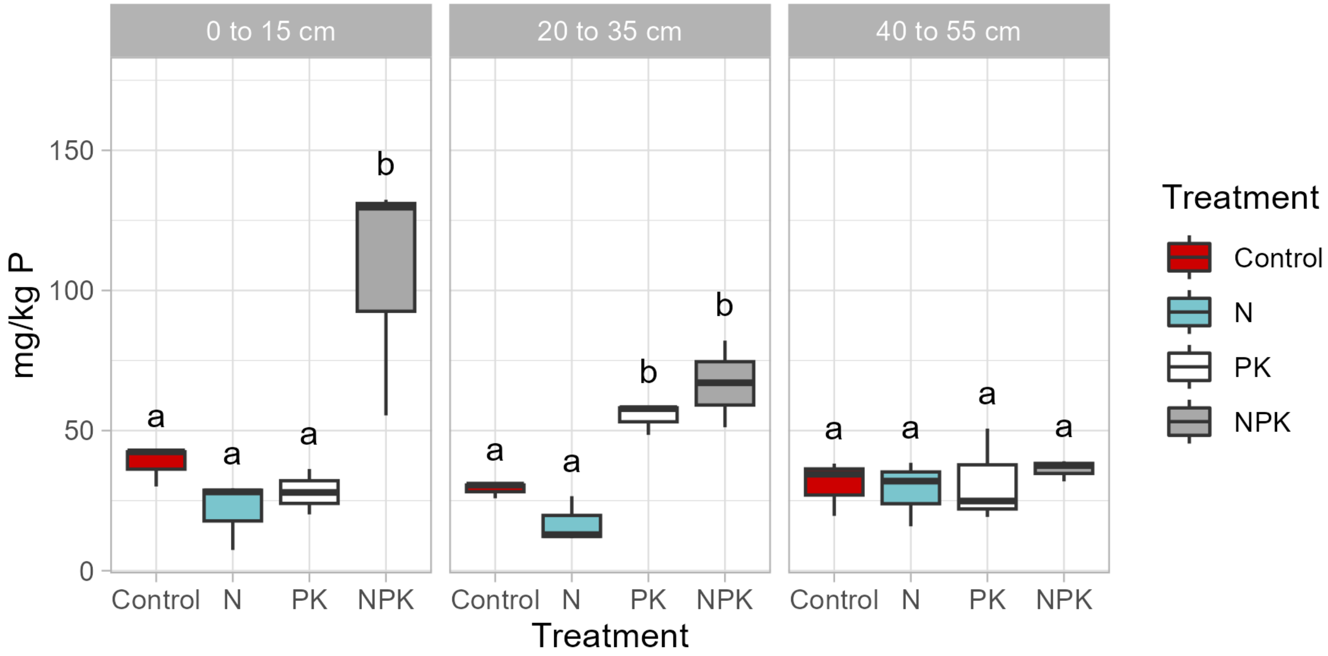

Figure 4-6 Hedley Bicarbonate-Extractable Po by Layer in mg/kg P. Boxplots are grouped by sampling depth. Color corresponds to treatment. Compact letter displays (clds) separate the treatments within each layer at 95% confidence.

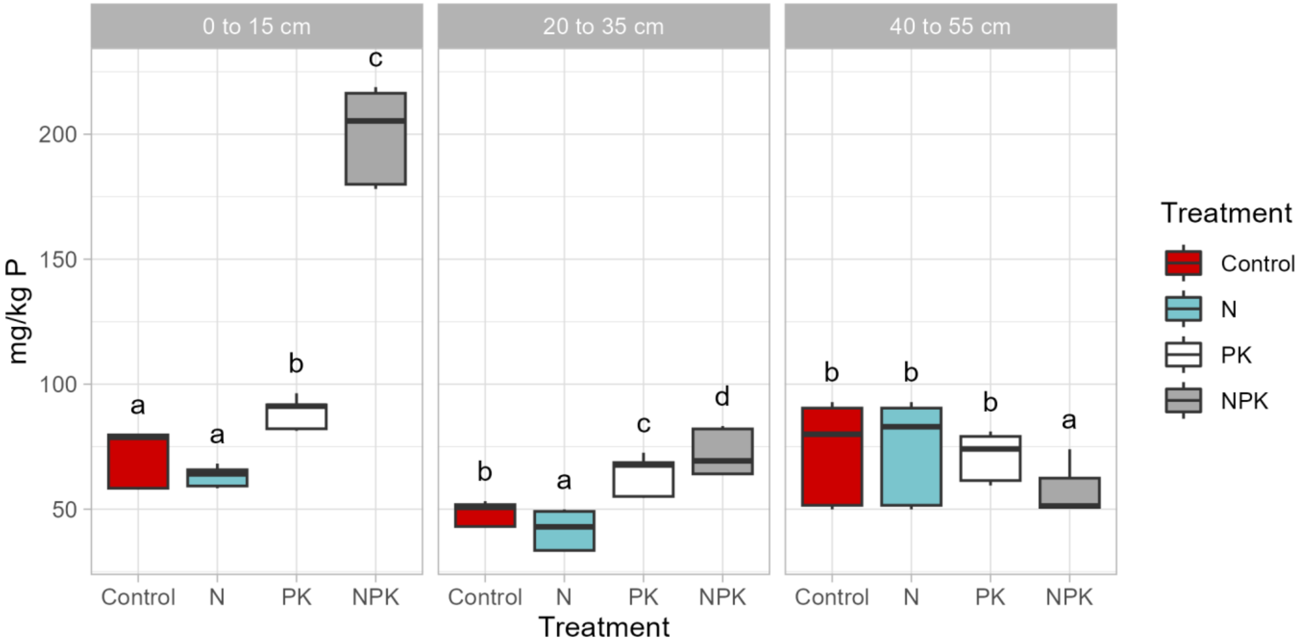

Figure 4-7 Hedley NaOH-Extractable Pt by Layer in mg/kg P. Boxplots are grouped by sampling depth. Color corresponds to treatment. Compact letter displays (clds) separate the treatments within each layer at 95% confidence.

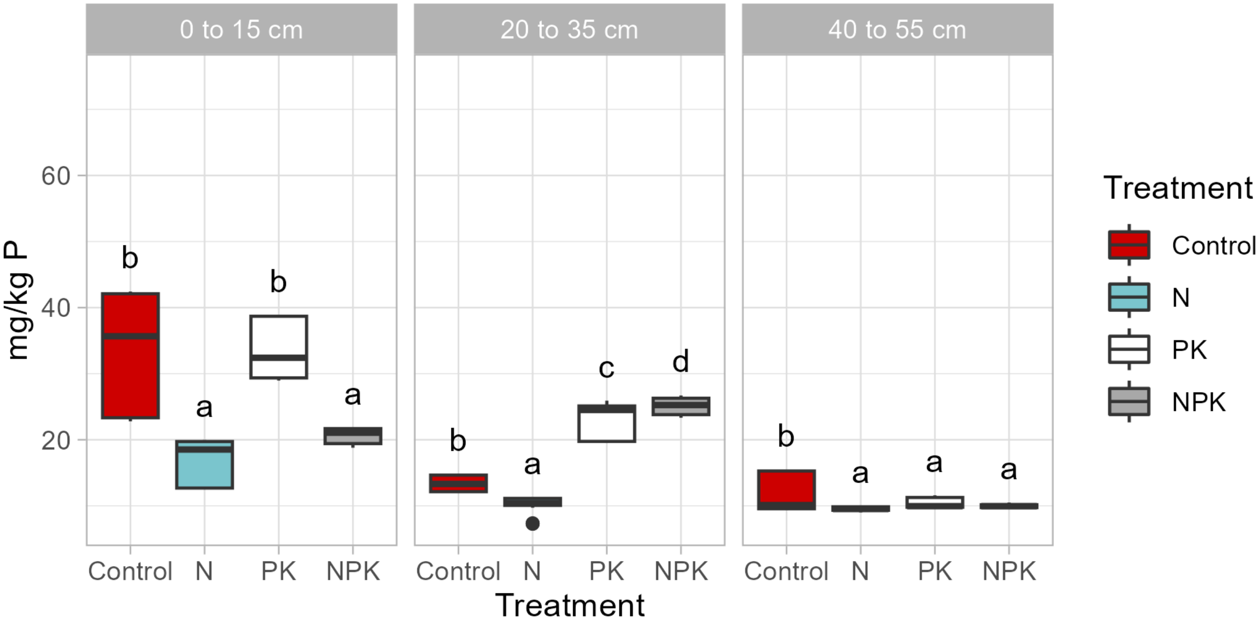

Figure 4-8 Hedley 1 M HCl-Extractable Pi by Layer in mg/kg P. Boxplots are grouped by sampling depth. Color corresponds to treatment. Compact letter displays (clds) separate the treatments within each layer at 95% confidence. Outliers are shown as dots.

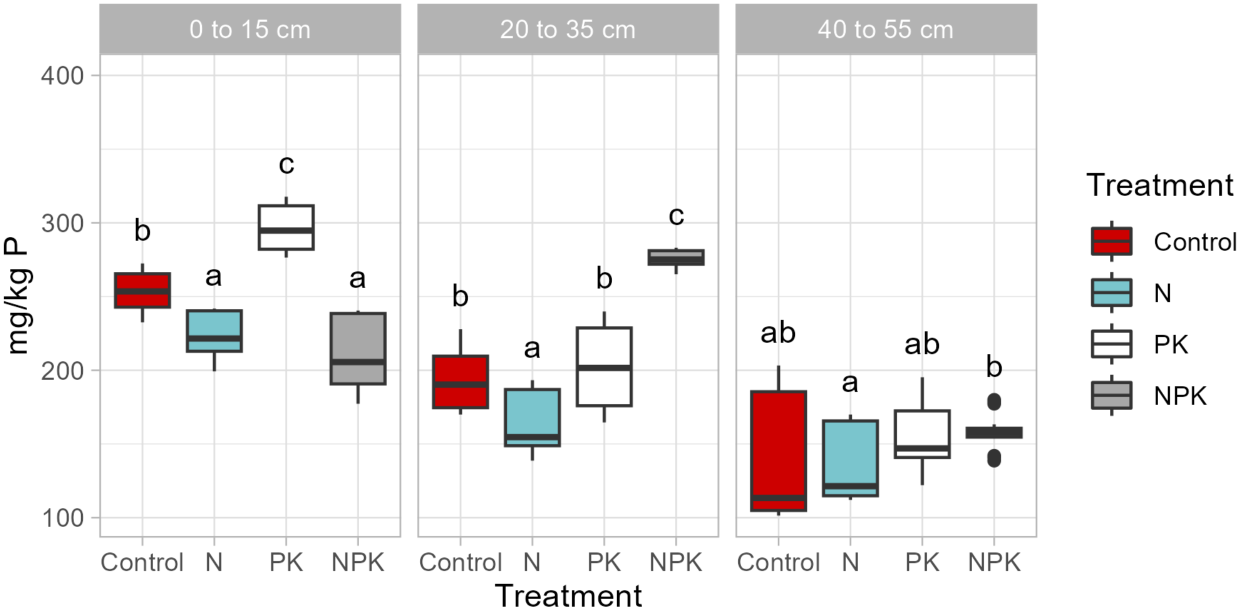

Figure 4-9 Hedley Concentrated HCl-Extractable Pt by Layer in mg/kg P. Boxplots are grouped by sampling depth. Color corresponds to treatment. Compact letter displays (clds) separate the treatments within each layer at 95% confidence. Outliers are shown as dots.

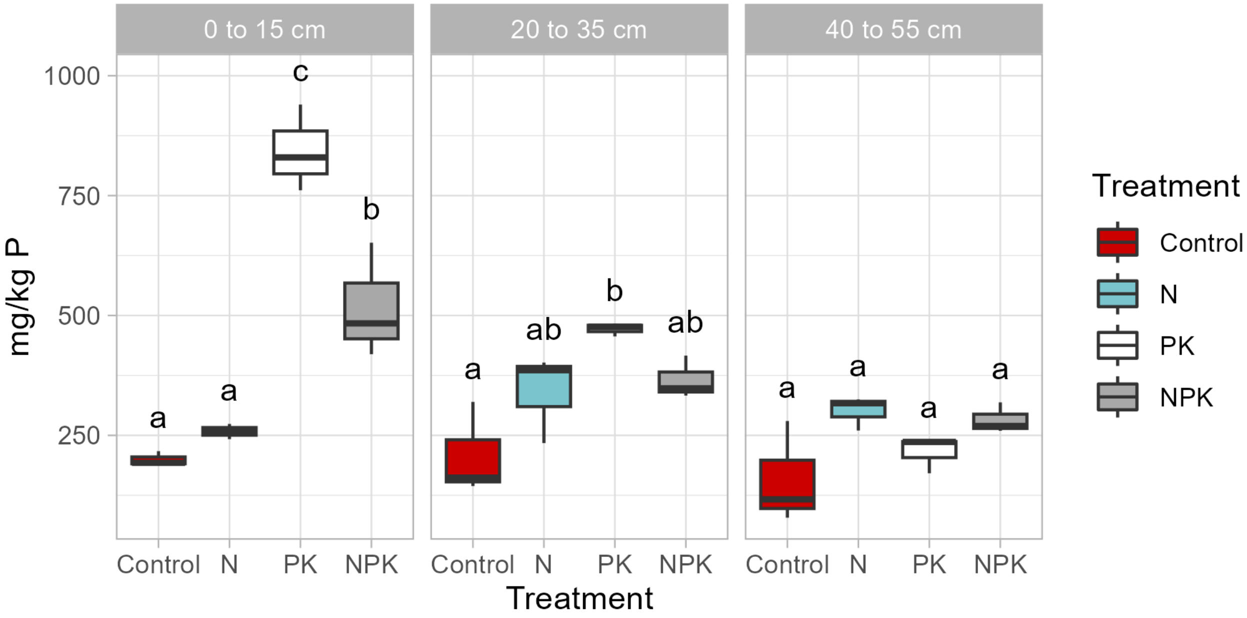

Figure 4-10 Hedley Residual Pt by Layer in mg/kg P. Boxplots are grouped by sampling depth. Color corresponds to treatment. Compact letter displays (clds) separate the treatments within each layer at 95% confidence.

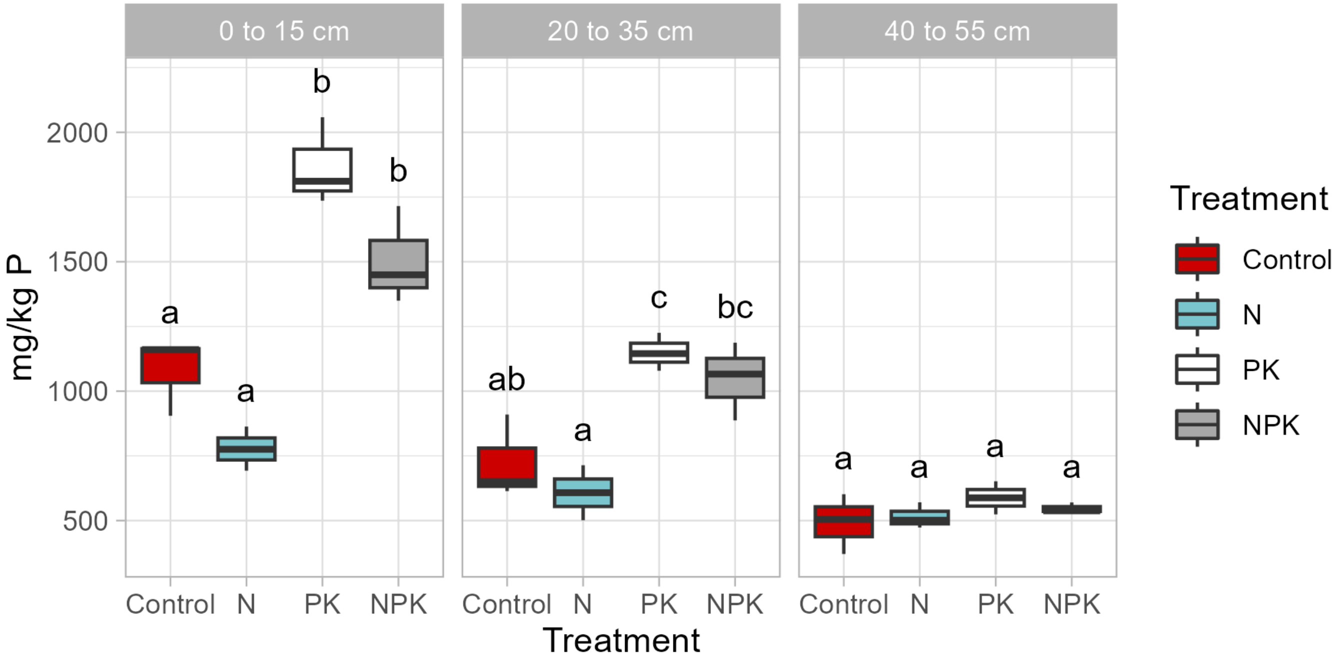

Figure 4-11 Hedley Total P by Layer in mg/kg P. Sum of resin-, bicarbonate-, NaOH-, 1 M HCl-, conc. HCl-extractable and residual P concentrations. Boxplots are grouped by sampling depth. Color corresponds to treatment. Compact letter displays (clds) separate the treatments within each layer at 95% confidence.

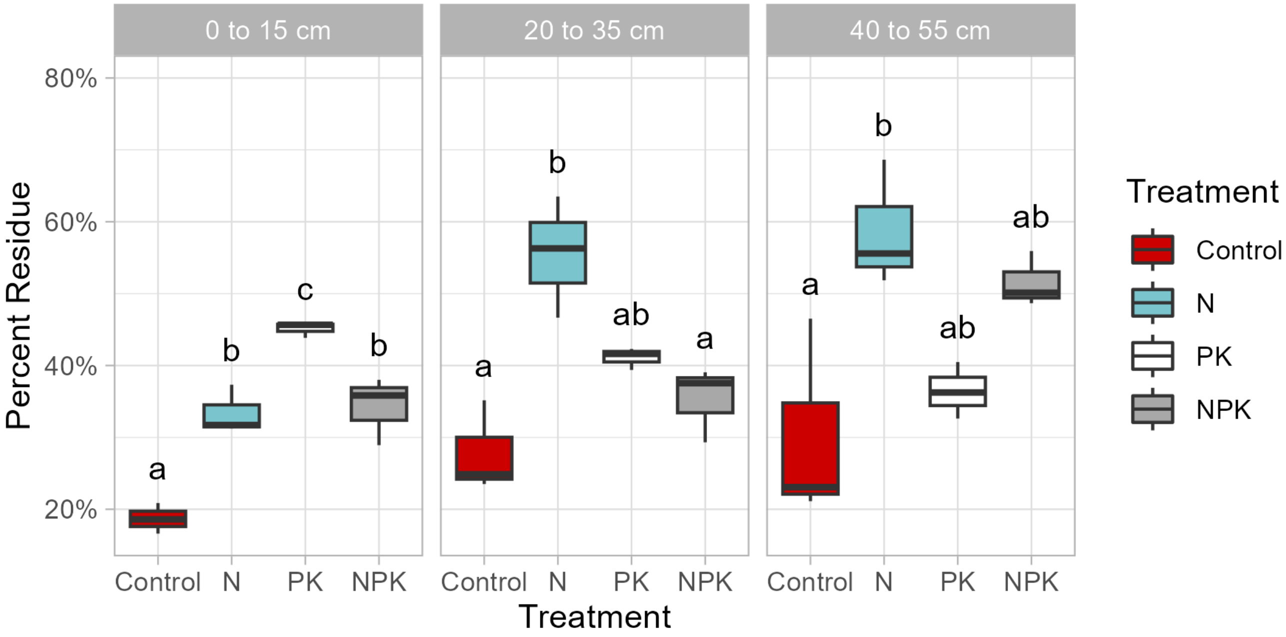

Figure 4-12 Percent of total P made up by the residual fraction. Boxplots are grouped by sampling depth. Color corresponds to treatment. Compact letter displays (clds) separate the treatments within each layer at 95% confidence.

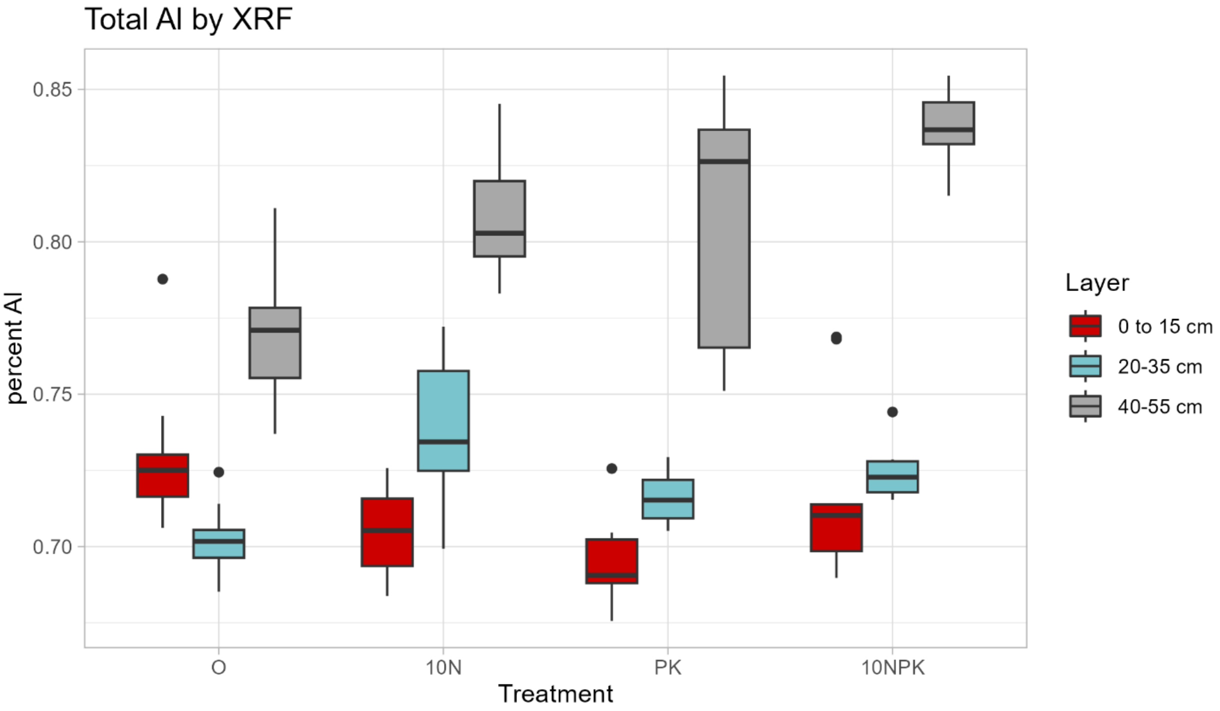

Figure 4-13 Aluminum concentration as measured by XRF. Color corresponds to layer. Outliers are shown as dots.

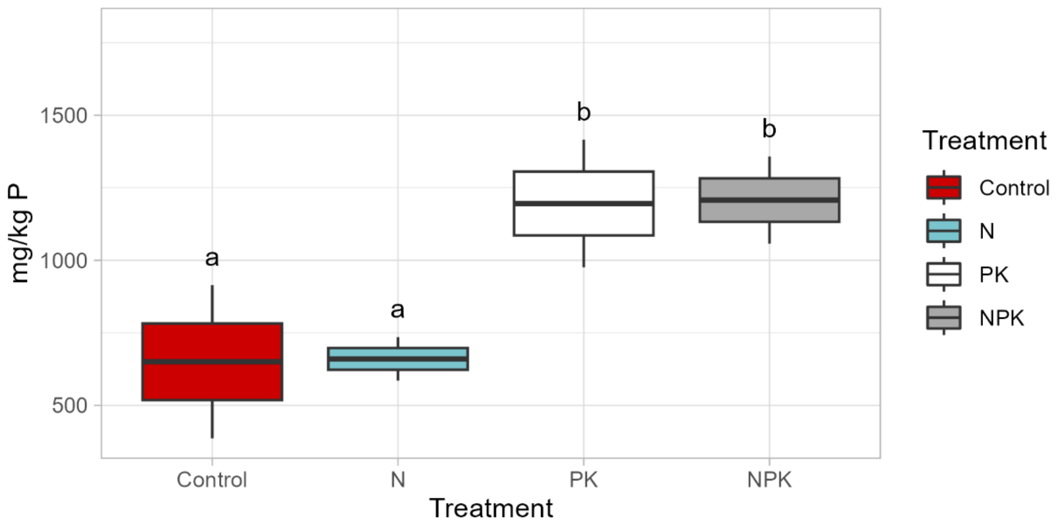

Figure 4-14 Total P concentrations in Chamaedaphne calyculata leaves in the control plot and fertilized plots as measured by XRF. Compact letter displays (clds) separate the treatments within each layer at 95% confidence.

Supplemental Materials

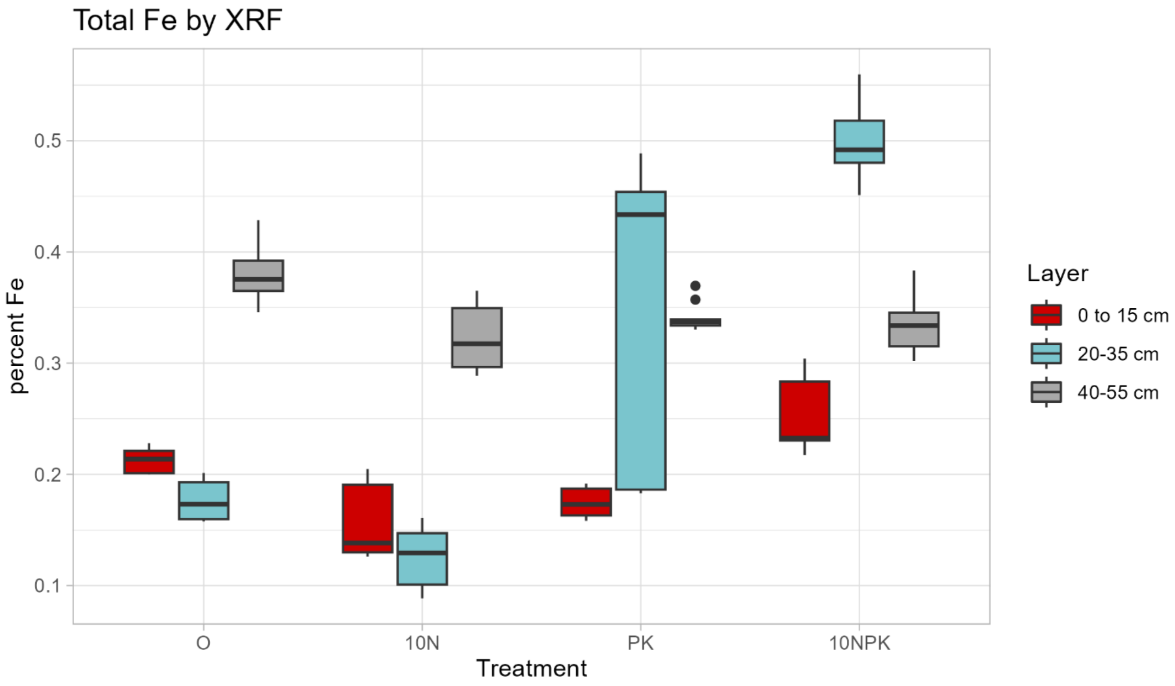

Figure 4-15 Calcium concentration as measured by XRF. Color corresponds to layer. Outliers are shown as dots.

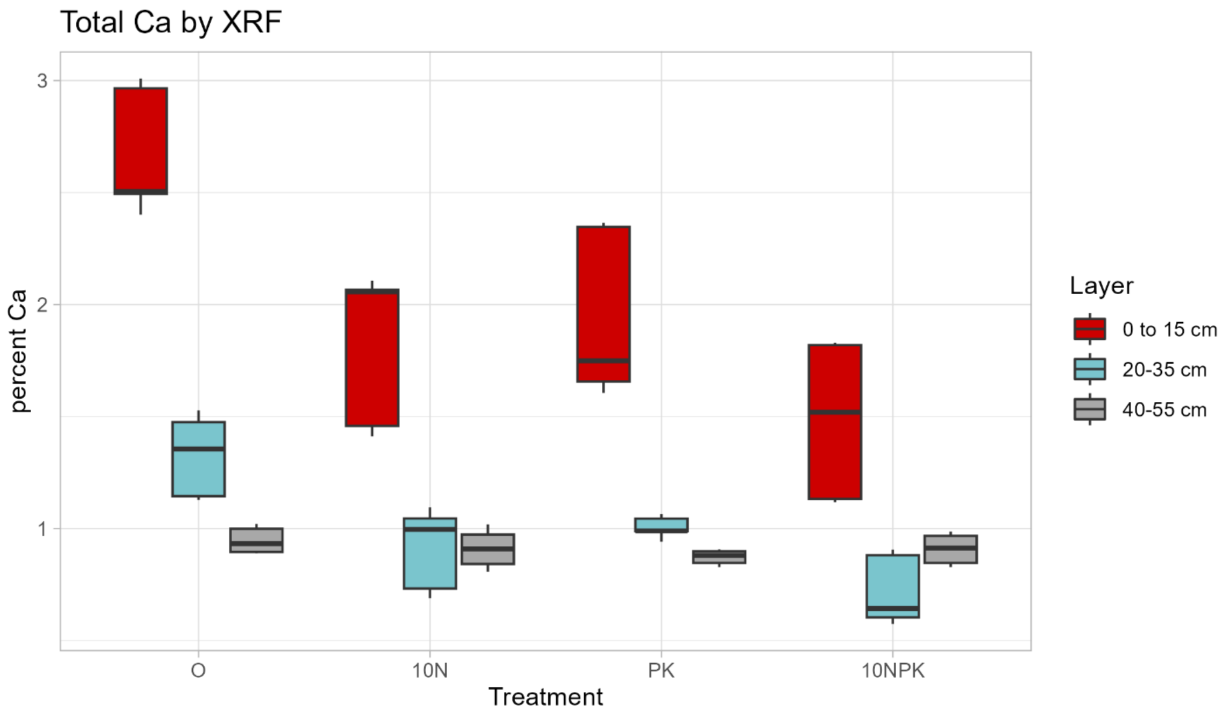

Figure 4-16 Iron concentration as measured by XRF. Color corresponds to layer. Outliers are shown as dots.

References

Aerts, R., Wallen, B., & Malmer, N. (1992). Growth-Limiting Nutrients in Sphagnum-Dominated Bogs Subject to Low and High Atmospheric Nitrogen Supply. *The Journal of Ecology*, *80*(1), 131. https://doi.org/10.2307/2261070

Alexander, M. (1977). Microbiology of Organic and Inorganic Phosphorus. In *Introduction to Soil Microbiology* (Second, pp. 465–477). John Wiley & Sons, Ltd.

Barrow, N. J., Sen, A., Roy, N., & Debnath, A. (2021). The soil phosphate fractionation fallacy. *Plant and Soil*, *459*(1–2), 1–11. https://doi.org/10.1007/s11104-020-04476-6

Bodker, J. E., Turner, R. E., Tweel, A., Schulz, C., & Swarzenski, C. (2015). Nutrient-enhanced decomposition of plant biomass in a freshwater wetland. *Aquatic Botany*, *127*, 44–52. https://doi.org/10.1016/j.aquabot.2015.08.001

Bragazza, L., Buttler, A., Habermacher, J., Brancaleoni, L., Gerdol, R., Fritze, H., Hanajík, P., Laiho, R., & Johnson, D. (2012). High nitrogen deposition alters the decomposition of bog plant litter and reduces carbon accumulation. *Global Change Biology*, *18*(3), 1163–1172. https://doi.org/10.1111/j.1365-2486.2011.02585.x

Brais, C. (2022). *Effect of nutrient additions on phosphorus biogeochemistry in an ombrotrophic bog* [Undergraduate Honors Research Project].

Bünemann, E. K., Oberson, A., & Frossard, E. (2011). *Phosphorus in action: Biological processes in soil phosphorus cycling*. Springer. https://doi.org/10.1007/978-3-642-15271-9

Canadian Climate Normals 1991-2020 Ottawa (Station Data). (2023). [..Csv]. Canadian Climate Normals. https://climate.weather.gc.ca/climate_normals/results_1991_2020_e.html?searchType=stn

Name_1991&txtStationName_1991=Ottawa&searchMethod=contains&txtCentralLatMin=0&txtCentralLatSec=0&txtCentralLongMin=0&txtCentralLongSec=0&stnID=191000000&dispBack=1#normals-data

Carrara, J. E., Walter, C. A., Freedman, Z. B., Hostetler, A. N., Hawkins, J. S., Fernandez, I. J., & Brzostek, E. R. (2021). Differences in microbial community response to nitrogen fertilization result in unique enzyme shifts between arbuscular and ectomycorrhizal-dominated soils. *Global Change Biology*, *27*(10), 2049–2060. https://doi.org/10.1111/gcb.15523

Celi, L., Said-Pullicino, D., Bol, R., Lang, F., & Luster, J. (2022). Interconnecting Soil Organic Matter with Nitrogen and Phosphorus Cycling. In Y. Yang, M. Keiluweit, N. Senesi, & B. Xing (Eds.), *Multi-Scale Biogeochemical Processes in Soil Ecosystems* (1st ed., pp. 51–77). Wiley. https://doi.org/10.1002/9781119480419.ch3

Chen, C. R., Condron, L. M., Davis, M. R., & Sherlock, R. R. (2000). Effects of afforestation on phosphorus dynamics and biological properties in a New Zealand grassland soil. *Plant and Soil*, *220*(1/2), 151–163. https://doi.org/10.1023/A:1004712401721

Chen, C. R., Sinaj, S., Condron, L. M., Frossard, E., Sherlock, R. R., & Davis, M. R. (2003). Characterization of phosphorus availability in selected New Zealand grassland soils. *Nutrient Cycling in Agroecosystems*, *65*(1), 89–100. https://doi.org/10.1023/A:1021889207109

Chen, Y., Hall, J., Van Wees, D., Andela, N., Hantson, S., Giglio, L., Van Der Werf, G. R., Morton, D. C., & Randerson, J. T. (2023). *Multi-decadal trends and variability in burned area from the 5th version of the Global Fire Emissions Database (GFED5)* [Preprint]. ESSD – Land/Energy and Emissions. https://doi.org/10.5194/essd-2023-182

Condron, L. M., & Newman, S. (2011). Revisiting the fundamentals of phosphorus fractionation of sediments and soils. *Journal of Soils and Sediments*, *11*(5), 830–840. https://doi.org/10.1007/s11368-011-0363-2

Cross, A. F., & Schlesinger, W. H. (1995). A literature review and evaluation of the Hedley fractionation: Applications to the biogeochemical cycle of soil phosphorus in natural ecosystems. *Geoderma*, *64*(3–4), 197–214. https://doi.org/10.1016/0016-7061(94)00023-4

Dean, L. A. (1938). An attempted fractionation of the soil phosphorus. *The Journal of Agricultural Science*, *28*(2), 234–246. https://doi.org/10.1017/S0021859600050644

Fraser, C. J. D., Roulet, N. T., & Moore, T. R. (2001). Hydrology and dissolved organic carbon biogeochemistry in an ombrotrophic bog. *Hydrological Processes*, *15*(16), 3151–3166. https://doi.org/10.1002/hyp.322

Gilliam, F. S., Burns, D. A., Driscoll, C. T., Frey, S. D., Lovett, G. M., & Watmough, S. A. (2019). Decreased atmospheric nitrogen deposition in eastern North America: Predicted responses of forest ecosystems. *Environmental Pollution*, *244*, 560–574. https://doi.org/10.1016/j.envpol.2018.09.135

Gorham, E. (1991). Northern Peatlands: Role in the Carbon Cycle and Probable Responses to Climatic Warming. *Ecological Applications*, *1*(2), 182–195. https://doi.org/10.2307/1941811

Grunes, D. L. (1959). Effect of Nitrogen on the Availability of Soil and Fertilizer Phosphorus to Plants. In *Advances in Agronomy* (Vol. 11, pp. 369–396). Elsevier. https://doi.org/10.1016/S0065-2113(08)60127-3

Ingram, H. A. P. (1987). Ecohydrology of Scottish peatlands. *Transactions of the Royal Society of Edinburgh: Earth Sciences*, *78*(4), 287–296. https://doi.org/10.1017/S0263593300011226

Juutinen, S., Bubier, J. L., & Moore, T. R. (2010). Responses of Vegetation and Ecosystem CO2 Exchange to 9 Years of Nutrient Addition at Mer Bleue Bog. *Ecosystems*, *13*(6), 874–887. https://doi.org/10.1007/s10021-010-9361-2

Kanakidou, M., Myriokefalitakis, S., Daskalakis, N., Fanourgakis, G., Nenes, A., Baker, A. R., Tsigaridis, K., & Mihalopoulos, N. (2016). Past, Present, and Future Atmospheric Nitrogen Deposition. *Journal of the Atmospheric Sciences*, *73*(5), 2039–2047. https://doi.org/10.1175/JAS-D-15-0278.1

Meier, I. C., Finzi, A. C., & Phillips, R. P. (2017). Root exudates increase N availability by stimulating microbial turnover of fast-cycling N pools. *Soil Biology and Biochemistry*, *106*, 119–128. https://doi.org/10.1016/j.soilbio.2016.12.004

Mitsch, W. J., & Gosselink, J. G. (2015). *Wetlands* (Fifth edition). John Wiley and Sons, Inc.

Moore, T. R., Knorr, K.-H., Thompson, L., Roy, C., & Bubier, J. L. (2019). The effect of long-term fertilization on peat in an ombrotrophic bog. *Geoderma*, *343*, 176–186. https://doi.org/10.1016/j.geoderma.2019.02.034

Negassa, W., & Leinweber, P. (2009). How does the Hedley sequential phosphorus fractionation reflect impacts of land use and management on soil phosphorus: A review. *Journal of Plant Nutrition and Soil Science*, *172*(3), 305–325. https://doi.org/10.1002/jpln.200800223

Oehl, F., Oberson, A., Probst, M., Fliessbach, A., Roth, H.-R., & Frossard, E. (2001). Kinetics of microbial phosphorus uptake in cultivated soils. *Biology and Fertility of Soils*, *34*(1), 31–41. https://doi.org/10.1007/s003740100362

Ohno, T., & Zibilske, L. M. (1991). Determination of Low Concentrations of Phosphorus in Soil Extracts Using Malachite Green. *Soil Science Society of America Journal*, *55*(3), 892–895. https://doi.org/10.2136/sssaj1991.03615995005500030046x

Parkinson, J. A., & Allen, S. E. (1975). A wet oxidation procedure suitable for the determination of nitrogen and mineral nutrients in biological material. *Communications in Soil Science and Plant Analysis*, *6*(1), 1–11. https://doi.org/10.1080/00103627509366539

Poschenrieder, C., & Barceló, J. (2013). Aluminum in Plants. In R. H. Kretsinger, V. N. Uversky, & E. A. Permyakov (Eds.), *Encyclopedia of Metalloproteins* (pp. 34–38). Springer New York. https://doi.org/10.1007/978-1-4614-1533-6_109

Prüter, J., McLaren, T. I., Pätzig, M., Hu, Y., & Leinweber, P. (2023). Phosphorus speciation along a soil to kettle hole transect: Sequential P fractionation, P XANES, and 31P NMR spectroscopy. *Geoderma*, *429*, 116215. https://doi.org/10.1016/j.geoderma.2022.116215

Qualls, R. G., & Richardson, C. J. (2000). Phosphorus Enrichment Affects Litter Decomposition, Immobilization, and Soil Microbial Phosphorus in Wetland Mesocosms. *Soil Science Society of America Journal*, *64*(2), 799–808. https://doi.org/10.2136/sssaj2000.642799x

Reina, M., Serrano, L., & Golterman, H. L. (2011). A sequential procedure for the quantification of biologically produced polyphosphate in sediment samples. *Limnetica*, *30*(1), 0017–0026.

Richardson, C. J., & Marshall, P. E. (1986). Processes Controlling Movement, Storage, and Export of Phosphorus in a Fen Peatland. *Ecological Monographs*, *56*(4), 279–302. https://doi.org/10.2307/1942548

Saia, S. M., Carrick, H. J., Buda, A. R., Regan, J. M., & Walter, M. T. (2021). Critical Review of Polyphosphate and Polyphosphate Accumulating Organisms for Agricultural Water Quality Management. *Environmental Science & Technology*, *55*(5), 2722–2742. https://doi.org/10.1021/acs.est.0c03566

Schlichting, A., Leinweber, P., Meissner, R., & Altermann, M. (2002). Sequentially extracted phosphorus fractions in peat-derived soils. *Journal of Plant Nutrition and Soil Science*, *165*(3), 290–298. https://doi.org/10.1002/1522-2624(200206)165:3%3C290::AID-JPLN290%3E3.0.CO;2-A

Spohn, M., & Kuzyakov, Y. (2013). Phosphorus mineralization can be driven by microbial need for carbon. *Soil Biology and Biochemistry*, *61*, 69–75. https://doi.org/10.1016/j.soilbio.2013.02.013

Tian, J., Ge, F., Zhang, D., Deng, S., & Liu, X. (2021). Roles of Phosphate Solubilizing Microorganisms from Managing Soil Phosphorus Deficiency to Mediating Biogeochemical P Cycle. *Biology*, *10*(2), 158. https://doi.org/10.3390/biology10020158

Tiessen, H., & Moir, J. O. (1993). Characterization of Available P by Sequential Extraction. In *Soil Sampling and Methods of Analysis* (pp. 75–87). Lewis Publishers.

Turetsky, M. R., Benscoter, B., Page, S., Rein, G., Van Der Werf, G. R., & Watts, A. (2015). Global vulnerability of peatlands to fire and carbon loss. *Nature Geoscience*, *8*(1), 11–14. https://doi.org/10.1038/ngeo2325

Turner, B. L., Mahieu, N., & Condron, L. M. (2003). The phosphorus composition of temperate pasture soils determined by NaOH–EDTA extraction and solution 31 P NMR spectroscopy. *Organic Geochemistry*, *34*(8), 1199–1210. https://doi.org/10.1016/S0146-6380(03)00061-5

Velásquez, G., Ngo, P.-T., Rumpel, C., Calabi-Floody, M., Redel, Y., Turner, B. L., Condron, L. M., & Mora, M. D. L. L. (2016). Chemical nature of residual phosphorus in Andisols. *Geoderma*, *271*, 27–31. https://doi.org/10.1016/j.geoderma.2016.01.027

Verry, E. S., & Timmons, D. R. (1982). Waterborne Nutrient Flow Through an Upland-Peatland Watershed in Minnesota. *Ecology*, *63*(5), 1456–1467. https://doi.org/10.2307/1938872

Walker, T. W., & Syers, J. K. (1976). The fate of phosphorus during pedogenesis. *Geoderma*, *15*(1), 1–19. https://doi.org/10.1016/0016-7061(76)90066-5

Wang, M., Moore, T. R., Talbot, J., & Richard, P. J. H. (2014). The cascade of C:N:P stoichiometry in an ombrotrophic peatland: From plants to peat. *Environmental Research Letters*, *9*(2), 024003. https://doi.org/10.1088/1748-9326/9/2/024003

Wang, Y., Bauke, S. L., Von Sperber, C., Tamburini, F., Guigue, J., Winkler, P., Kaiser, K., Honermeier, B., & Amelung, W. (2021). Soil phosphorus cycling is modified by carbon and nitrogen fertilization in a long-term field experiment. *Journal of Plant Nutrition and Soil Science*, *184*(2), 282–293. https://doi.org/10.1002/jpln.202000261

Williams, B. L., & Sparling, G. P. (1984). Extractable N and P in relation to microbial biomass in UK acid organic soils. *Plant and Soil*, *76*(1–3), 139–148. https://doi.org/10.1007/BF02205574

Yu, Z. C. (2012). Northern peatland carbon stocks and dynamics: A review. *Biogeosciences*, *9*(10), 4071–4085. https://doi.org/10.5194/bg-9-4071-2012

5. Discussion of Phosphorus Movement

5.1. Movement of Phosphorus Aboveground

The variation in the total of microbial and labile phosphorus over my 14-month sampling period can likely be explained in part by plant uptake of phosphorus. Wetland plant phosphorus uptake tends to peak in late summer and autumn, then drops sharply with freezing, remains low during the spring, and increases over the summer before another peak in autumn (Jonasson & Stuart Chapin, 1991). However, in a highly phosphorus-limited ecosystem, it is possible that plant phosphorus uptake is more opportunistic (Jonasson & Stuart Chapin, 1991). In this case, phosphorus movement aboveground could explain sudden drops in total microbial plus labile phosphorus. Phosphorus concentrations in *Chamaedaphne calyculata* leaves nearly doubled due to phosphorus addition at Mer Bleue (see Chapter 4). However, this was measured after twenty years of phosphorus addition. Further work is needed to determine the rate of plant phosphorus uptake in ombrotrophic bogs through the seasons.

Increases in aboveground phosphorus can lead to large ecological changes. Small amounts return to the soil surface as litter, though plants in phosphorus-limited environments generally resorb most of phosphorus from their leaves during senescence (Veneklaas, 2022). Globally, leaf phosphorus resorption varies between 20% and 90%, with plants in more phosphorus limited environments falling at the higher end of the scale (Veneklaas, 2022). Once on the ground, phosphorus from vascular leaf litter is recycled more quickly than phosphorus in dead *Sphagnum* moss, contributing to further plant growth. Further shrub growth causes peat loss due to increasing peat decomposition and decreasing peat accumulation. Vascular plant evapotranspiration is higher than *Sphagnum* evapotranspiration, which can lead to drying and increased decomposition. In addition, shrubs and their leaf litter can shade out *Sphagnum* and dramatically decrease its growth, thus decreasing peat accumulation. Given enough decomposition, this could cause changes in the species composition and nutrient cycling of the ecosystem such that it more closely resembles a poor fen than an ombrotrophic bog. In such a situation, the above and belowground conditions might resemble those seen in the Mer Bleue long-term fertilization plots. In these plots, nutrient addition has caused shrub growth to greatly increase, moss growth to decrease, and decomposition rate to increase (Moore et al., 2019). One difference at the fertilization trial is that soil wetness has increased (Moore et al., 2019). Since the fertilized plots are surrounded by unaltered peat, the higher evapotranspiration from the fertilized plots cannot overcome moisture seeping in from the surrounding area. Higher plot wetness decreases decomposition, meaning that vascular plant growth would have to surpass a high threshold before their increased evapotranspiration would lead to drying and large-scale changes.

5.2. Movement Between Labile Phosphorus Pools

Fertilizer application can increase the size of every soil P pool extracted in the Hedley fractionation. Changes appear within months in the labile and moderately labile fractions, and generally take more than a decade of application above recommended rates to cause changes in the recalcitrant fractions (Negassa & Leinweber, 2009). This may explain the variation in the sum of microbial and labile phosphorus in Mer Bleue through the 14-month sampling period. If phosphorus was moving from the resin-extractable to bicarbonate- and NaOH-extractable fractions over periods of weeks to months, it could lead to decreases in the sum of the microbial and labile phosphorus concentrations. The reverse is also true, as movement from the bicarbonate- and NaOH-extractable fractions to the resin-extractable fractions might explain increases in the sum of the microbial and labile phosphorus concentrations. However, these phenomena were observed in fertilization trials in mineral soils in agricultural fields. It is possible that the speed and magnitude of the movement between fractions is different in organic soils and when smaller quantities of phosphorus are involved. For example, phosphorus mineralization rates in phosphorus-limited pocosin soils were found to be five times lower than phosphorus mineralization rates in nearby forest soils which were not phosphorus-limited (Walbridge & Vitousek, 1987). In the more phosphorus-limited pocosin soils, plant phosphorus use was much more conservative, and there appeared to be some uptake of labile organic phosphorus (Walbridge & Vitousek, 1987). In this case, bicarbonate-extractable organic phosphorus might be moved aboveground directly without passing through the resin-extractable phosphorus pool.

Since the potential speed and magnitude of the movement of P between the labile and moderately labile fractions remains unknown in peat, I cannot say with certainty how much of the seasonal variation total microbial plus labile phosphorus can be explained by movement between pools. To answer this question, future work could involve Hedley fractionations at frequent points after applying ^{33}P labelled phosphate to see exactly how long it takes to move between pools.

5.3. Phosphorus Pulses and Disturbance

Phosphorus availability is variable in both space and time, resulting in “hot spots” and “hot moments.” This has been observed for decades, but the drivers remain poorly understood (Alexander, 1977). Pulses often occur after rewetting and thawing (Yevdokimov et al., 2016). While this has often been attributed mainly to microorganisms, the largest contributor to this phenomenon is likely physical disruption of aggregates (Butterly et al., 2009). Peat, being exclusively organic matter, does not form aggregates as readily as most mineral soils. However, freeze-thaw and drying-rewetting cycles contribute to organic matter breakdown and may release phosphorus from peat as it becomes further humified (Kaila et al., 2016). The extent of breakdown and nutrient release from peat due to freeze-thaw and drying-rewetting has not yet been quantified. However, due to the lack of other sources of phosphorus, I would expect that physical breakdown of organic matter due to freeze-thaw and drying-rewetting cycles is one of the major drivers of labile phosphorus pulses in this ecosystem. To better understand this phenomenon, peat mesocosms could be subjected to controlled temperature and moisture conditions before measuring labile phosphate concentrations.

6. Conclusions

In the preceding chapters, I have fulfilled my research objectives to the best of my ability. In doing so, I answered the following questions:

1. How do microbial and labile phosphorus concentrations in an ombrotrophic bog vary through the year?
2. What key variables control changes in microbial and labile phosphorus concentrations?
3. How does the long-term addition of N, PK, or all three together change the distribution of phosphorus in soil phosphorus pools in an ombrotrophic bog?

In answer to Question 1, variation in the labile and microbial pools is large, and in answer to Question 2, seasonality appears to be the largest explanatory variable, though water table level and winter temperature also play key roles. The variation in labile and microbial phosphorus concentrations follow patterns and do not generally exceed certain upper and lower bounds. The lower bound is likely a minimum threshold needed for survival at low levels of microbial and plant activity. The upper bound is likely the point at which phosphorus acquisition through enhanced decomposition is no longer worth the metabolic expense of enzyme production. The upper boundary for labile phosphate may be increased through phosphate fertilization, as shown in my second manuscript, “Nutrient Addition Increases Available and Residual Phosphorus Concentrations in an Ombrotrophic Bog.” The implications of this change in nutrient status are large. Since phosphorus is a limiting or co-limiting nutrient in this ecosystem, increases in labile phosphorus concentrations can be expected to increase vascular plant growth, in turn decreasing moss growth and increasing evapotranspiration. Increases in vascular plant growth and surface drying and decreases in *Sphagnum* growth increase peat decomposition and cause changes in microbial community composition. With increased decomposition, however, the peat surface subsides, bringing the surface nearer to the water table and slowing decomposition. It remains to be seen whether the water table is then lowered by further vascular plant growth and drying, or if the peat surface subsides to a new steady state. The balance between the two likely depends on precipitation and temperature, among other environmental variables. Dry years could lead to more drying, decomposition, and vascular plant growth. Given sufficient drought, the area could experience fire. With fire, recalcitrant forms of phosphorus are often pyrolized or hydrolyzed into orthophosphate, greatly increasing its availability to plants and microorganisms. This is not nutrient addition, but this sharp increase in nutrient availability may have similar effects on decomposition and vascular plant growth. Future work should involve continued monitoring vascular plant growth and peat decomposition in bogs exposed to nutrient addition in order to better inform carbon budgets and climate models.

In answer to Question 3, it appears that N addition causes small decreases in labile phosphorus concentrations and increases in recalcitrant phosphorus concentrations, while PK and NPK addition increase both labile and recalcitrant phosphorus concentrations. The increase in recalcitrant phosphorus concentrations in the fertilized plots was seen after fifteen to twenty years, but may have begun earlier. Further work is needed to determine the effects of this increase in recalcitrant phosphorus concentrations on plant growth and microbial activity both with and without increases in labile phosphorus concentrations. In addition, further work could determine the difference between the effects of adding the same quantity of fertilizer in a single large dose or over the long-term. The difference between the effects of the long-term low-dose and short-term large-dose nutrient addition would help determine potential ecological responses to different types of disturbances. For example, fire and flooding may add a single large dose of available nutrients, while drought and aeolian deposition may lead to regular small increases in available nutrient concentrations. In addition, this work could be expanded to fen peatlands, which are more at risk of eutrophication from nutrient-rich inflow.

References

Aerts, R., Wallen, B., & Malmer, N. (1992). Growth-Limiting Nutrients in Sphagnum-Dominated Bogs Subject to Low and High Atmospheric Nitrogen Supply. *The Journal of Ecology*, *80*(1), 131. https://doi.org/10.2307/2261070

Alexander, M. (1977). Microbiology of Organic and Inorganic Phosphorus. In *Introduction to Soil Microbiology* (Second, pp. 465–477). John Wiley & Sons, Ltd.

Andersen, R., Francez, A.-J., & Rochefort, L. (2006). The physicochemical and microbiological status of a restored bog in Québec: Identification of relevant criteria to monitor success. *Soil Biology and Biochemistry*, *38*(6), 1375–1387. https://doi.org/10.1016/j.soilbio.2005.10.012

Bakken, L. R., & Frostegård, Å. (2006). Nucleic Acid Extraction from Soil. In *Soil Biology* (Vol. 8, pp. 49–73). Springer-Verlag. http://www.umb.no/statisk/nitrogengroup/publications/Bakken-Frostegard2006_extraction.pdf

Barbanti, A., Bergamini, M. C., Frascari, F., Miserocchi, S., & Rosso, G. (1994). Critical Aspects of Sedimentary Phosphorus Chemical Fractionation. *Journal of Environmental Quality*, *23*(5), 1093–1102. https://doi.org/10.2134/jeq1994.00472425002300050035x

Barrow, N. J. (2021). Comparing two theories about the nature of soil phosphate. *European Journal of Soil Science*, *72*(2), 679–685. https://doi.org/10.1111/ejss.13027

Barrow, N. J., Sen, A., Roy, N., & Debnath, A. (2021). The soil phosphate fractionation fallacy. *Plant and Soil*, *459*(1–2), 1–11. https://doi.org/10.1007/s11104-020-04476-6

Baum, C., Leinweber, P., & Schlichting, A. (2003). Effects of chemical conditions in re-wetted peats on temporal variation in microbial biomass and acid phosphatase activity within the growing season. *Applied Soil Ecology*, *22*(2), 167–174. https://doi.org/10.1016/S0929-1393(02)00129-4

Benzing, P., & Richardson, C. J. (2005). CaCO3 Causes Underestimation of NaOH Extractable Phosphorus in Sequential Fractionations. *Soil Science*, *170*(10), 802–809. https://doi.org/10.1097/01.ss.0000190501.98437.d1

Bilyera, N., Blagodatskaya, E., Yevdokimov, I., & Kuzyakov, Y. (2018). Towards a conversion factor for soil microbial phosphorus. *European Journal of Soil Biology*, *87*, 1–8. https://doi.org/10.1016/j.ejsobi.2018.03.002

Birch, H. F. (1961). Phosphorus transformations during plant decomposition. *Plant and Soil*, *15*(4), 347–366.

Bodker, J. E., Turner, R. E., Tweel, A., Schulz, C., & Swarzenski, C. (2015). Nutrient-enhanced decomposition of plant biomass in a freshwater wetland. *Aquatic Botany*, *127*, 44–52. https://doi.org/10.1016/j.aquabot.2015.08.001

Bortoluzzi, E. C., Pérez, C. A. S., Ardisson, J. D., Tiecher, T., & Caner, L. (2015). Occurrence of iron and aluminum sesquioxides and their implications for the P sorption in subtropical soils. *Applied Clay Science*, *104*, 196–204. https://doi.org/10.1016/j.clay.2014.11.032

Bragazza, L., Buttler, A., Habermacher, J., Brancaleoni, L., Gerdol, R., Fritze, H., Hanajík, P., Laiho, R., & Johnson, D. (2012). High nitrogen deposition alters the decomposition of bog plant litter and reduces carbon accumulation. *Global Change Biology*, *18*(3), 1163–1172. https://doi.org/10.1111/j.1365-2486.2011.02585.x

Brais, C. (2022). *Effect of nutrient additions on phosphorus biogeochemistry in an ombrotrophic bog* [Undergraduate Honors Research Project].

Brake, M., Höper, H., & Joergensen, R. G. (1999). Land use-induced changes in activity and biomass of microorganisms in raised bog peats at different depths. *Soil Biology and Biochemistry*, *31*(11), 1489–1497. https://doi.org/10.1016/S0038-0717(99)00053-X

Brookes, P. C., Powlson, D. S., & Jenkinson, D. S. (1982). Measurement of microbial biomass phosphorus in soil. *Soil Biology and Biochemistry*, *14*(4), 319–329. https://doi.org/10.1016/0038-0717(82)90001-3

Bubier, J. L., Moore, T. R., & Bledzki, L. A. (2007). Effects of nutrient addition on vegetation and carbon cycling in an ombrotrophic bog. *Global Change Biology*, *13*(6), 1168–1186. https://doi.org/10.1111/j.1365-2486.2007.01346.x

Bünemann, E. K., Oberson, A., & Frossard, E. (2011). *Phosphorus in action: Biological processes in soil phosphorus cycling*. Springer; WorldCat.org. https://doi.org/10.1007/978-3-642-15271-9

Butterly, C. R., Bünemann, E. K., McNeill, A. M., Baldock, J. A., & Marschner, P. (2009). Carbon pulses but not phosphorus pulses are related to decreases in microbial biomass during repeated drying and rewetting of soils. *Soil Biology and Biochemistry*, *41*(7), 1406–1416. https://doi.org/10.1016/j.soilbio.2009.03.018

Campbell, B. M., Beare, D. J., Bennett, E. M., Hall-Spencer, J. M., Ingram, J. S. I., Jaramillo, F., Ortiz, R., Ramankutty, N., Sayer, J. A., & Shindell, D. (2017). Agriculture production as a major driver of the Earth system exceeding planetary boundaries. *Ecology and Society*, *22*(4). https://www.jstor.org/stable/26798991

Canadian Climate Normals 1991-2020 Ottawa (Station Data). (2023). [..Csv]. Canadian Climate Normals. https://climate.weather.gc.ca/climate_normals/results_1991_2020_e.html?searchType=stn

Name_1991&txtStationName_1991=Ottawa&searchMethod=contains&txtCentralLatMin=0&txtCentralLatSec=0&txtCentralLongMin=0&txtCentralLongSec=0&stnID=191000000&dispBack=1#normals-data

Carrara, J. E., Walter, C. A., Freedman, Z. B., Hostetler, A. N., Hawkins, J. S., Fernandez, I. J., & Brzostek, E. R. (2021). Differences in microbial community response to nitrogen fertilization result in unique enzyme shifts between arbuscular and ectomycorrhizal-dominated soils. *Global Change Biology*, *27*(10), 2049–2060. https://doi.org/10.1111/gcb.15523

Celi, L., Said‐Pullicino, D., Bol, R., Lang, F., & Luster, J. (2022). Interconnecting Soil Organic Matter with Nitrogen and Phosphorus Cycling. In Y. Yang, M. Keiluweit, N. Senesi, & B. Xing (Eds.), *Multi‐Scale Biogeochemical Processes in Soil Ecosystems* (1st ed., pp. 51–77). Wiley. https://doi.org/10.1002/9781119480419.ch3

Chang, S. C., & Jackson, M. L. (1957). Fractionation of soil phosphorus. *Soil Science*, *84*(2), 133–144. https://doi.org/10.1097/00010694-195708000-00005

Chen, C. R., Condron, L. M., Davis, M. R., & Sherlock, R. R. (2000). Effects of afforestation on phosphorus dynamics and biological properties in a New Zealand grassland soil. *Plant and Soil*, *220*(1/2), 151–163. https://doi.org/10.1023/A:1004712401721

Chen, C. R., Sinaj, S., Condron, L. M., Frossard, E., Sherlock, R. R., & Davis, M. R. (2003). Characterization of phosphorus availability in selected New Zealand grassland soils. *Nutrient Cycling in Agroecosystems*, *65*(1), 89–100. https://doi.org/10.1023/A:1021889207109

Chen, Y., Hall, J., Van Wees, D., Andela, N., Hantson, S., Giglio, L., Van Der Werf, G. R., Morton, D. C., & Randerson, J. T. (2023). *Multi-decadal trends and variability in burned* *area from the 5th version of the Global Fire Emissions Database (GFED5)* [Preprint]. ESSD – Land/Energy and Emissions. https://doi.org/10.5194/essd-2023-182

Condron, L. M., & Newman, S. (2011). Revisiting the fundamentals of phosphorus fractionation of sediments and soils. *Journal of Soils and Sediments*, *11*(5), 830–840. https://doi.org/10.1007/s11368-011-0363-2

Cross, A. F., & Schlesinger, W. H. (1995). A literature review and evaluation of the Hedley fractionation: Applications to the biogeochemical cycle of soil phosphorus in natural ecosystems. *Geoderma*, *64*(3–4), 197–214. https://doi.org/10.1016/0016-7061(94)00023-4

Dai, S., & Finkelman, R. B. (Eds.). (2020). Coal production in China: Past, present, and future projections. In *Coal geology of China* (1st ed.). Routledge.

De Sena, A., Madramootoo, C. A., Whalen, J. K., & Von Sperber, C. (2022). Nucleic acids are a major pool of hydrolyzable organic phosphorus in arable organic soils of Southern Ontario, Canada. *Biology and Fertility of Soils*, *58*(1), 7–16. https://doi.org/10.1007/s00374-021-01603-y

Dean, L. A. (1938). An attempted fractionation of the soil phosphorus. *The Journal of Agricultural Science*, *28*(2), 234–246. https://doi.org/10.1017/S0021859600050644

Dijkstra, F. A., Geibe, C., Holmström, S., Lundström, U. S., & Van Breemen, N. (2001). The effect of organic acids on base cation leaching from the forest floor under six North American tree species: Organic acidity in forest soils. *European Journal of Soil Science*, *52*(2), 205–214. https://doi.org/10.1046/j.1365-2389.2001.00369.x

Elliott, S. M., Roe, H. M., & Patterson, R. T. (2012). Testate amoebae as indicators of hydroseral change: An 8500 year record from Mer Bleue Bog, eastern Ontario, Canada. *Quaternary International*, *268*, 128–144.

Ellsworth, D. S., Crous, K. Y., De Kauwe, M. G., Verryckt, L. T., Goll, D., Zaehle, S., Bloomfield, K. J., Ciais, P., Cernusak, L. A., Domingues, T. F., Dusenge, M. E., Garcia, S., Guerrieri, R., Ishida, F. Y., Janssens, I. A., Kenzo, T., Ichie, T., Medlyn, B. E., Meir, P., … Wright, I. J. (2022). Convergence in phosphorus constraints to photosynthesis in forests around the world. *Nature Communications*, *13*(1), 5005. https://doi.org/10.1038/s41467-022-32545-0

Elser, J. J., Acharya, K., Kyle, M., Cotner, J., Makino, W., Markow, T., Watts, T., Hobbie, S., Fagan, W., Schade, J., Hood, J., & Sterner, R. W. (2003). Growth rate-stoichiometry couplings in diverse biota. *Ecology Letters*, *6*(10), 936–943. https://doi.org/10.1046/j.1461-0248.2003.00518.x

Fisher, R. A., & Thomas, R. P. (1935). The Determination of the Forms of Inorganic Phosphorus in Soils. *Agronomy Journal*, *27*(11), 863–873. https://doi.org/10.2134/agronj1935.00021962002700110002x

Fraser, C. J. D., Roulet, N. T., & Moore, T. R. (2001). Hydrology and dissolved organic carbon biogeochemistry in an ombrotrophic bog. *Hydrological Processes*, *15*(16), 3151–3166. https://doi.org/10.1002/hyp.322

Gilliam, F. S., Burns, D. A., Driscoll, C. T., Frey, S. D., Lovett, G. M., & Watmough, S. A. (2019). Decreased atmospheric nitrogen deposition in eastern North America: Predicted responses of forest ecosystems. *Environmental Pollution*, *244*, 560–574. https://doi.org/10.1016/j.envpol.2018.09.135

Golovchenko, A. V., Bogdanova, O. Yu., Stepanov, A. L., Polyanskaya, L. M., & Zvyagintsev, D. G. (2010). Functioning of microbial complexes in aerated layers of a highmoor peat bog. *Eurasian Soil Science*, *43*(9), 1022–1029. https://doi.org/10.1134/S1064229310090097

Gorham, E. (1991). Northern Peatlands: Role in the Carbon Cycle and Probable Responses to Climatic Warming. *Ecological Applications*, *1*(2), 182–195. https://doi.org/10.2307/1941811

Grunes, D. L. (1959). Effect of Nitrogen on the Availability of Soil and Fertilizer Phosphorus to Plants. In *Advances in Agronomy* (Vol. 11, pp. 369–396). Elsevier. https://doi.org/10.1016/S0065-2113(08)60127-3

Gu, C., Dam, T., Hart, S. C., Turner, B. L., Chadwick, O. A., Berhe, A. A., Hu, Y., & Zhu, M. (2020). Quantifying Uncertainties in Sequential Chemical Extraction of Soil Phosphorus Using XANES Spectroscopy. *Environmental Science & Technology*, *54*(4), 2257–2267. https://doi.org/10.1021/acs.est.9b05278

Gu, C., & Margenot, A. J. (2021). Navigating limitations and opportunities of soil phosphorus fractionation. *Plant and Soil*, *459*(1–2), 13–17. https://doi.org/10.1007/s11104-020-04552-x

Gunnarsson, U., Rydin, H., & Sjörs, H. (2000). Diversity and pH changes after 50 years on the boreal mire Skattlösbergs Stormosse, Central Sweden. *Journal of Vegetation Science*, *11*(2), 277–286. https://doi.org/10.2307/3236808

Hedley, M. J., & Stewart, J. W. B. (1982). Method to measure microbial phosphate in soils. *Soil Biology and Biochemistry*, *14*(4), 377–385. https://doi.org/10.1016/0038-0717(82)90009-8

Helfenstein, J., Tamburini, F., Von Sperber, C., Massey, M. S., Pistocchi, C., Chadwick, O. A., Vitousek, P. M., Kretzschmar, R., & Frossard, E. (2018). Combining spectroscopic and isotopic techniques gives a dynamic view of phosphorus cycling in soil. *Nature Communications*, *9*(1), 3226. https://doi.org/10.1038/s41467-018-05731-2

Howie, S. A., van Meerveld, H. J., & Hebda, R. J. (2016). Regional patterns and controlling factors in plant species composition and diversity in Canadian lowland coastal bogs and laggs. *Mires and Peat*, *18*, 1–13. https://doi.org/10.19189/MaP.2016.OMB.242

Hugelius, G., Loisel, J., Chadburn, S., Jackson, R. B., Jones, M., MacDonald, G., Marushchak, M., Olefeldt, D., Packalen, M., Siewert, M. B., Treat, C., Turetsky, M., Voigt, C., & Yu, Z. (2020). Large stocks of peatland carbon and nitrogen are vulnerable to permafrost thaw. *Proceedings of the National Academy of Sciences*, *117*(34), 20438–20446. https://doi.org/10.1073/pnas.1916387117

Hutchins, K. (2018). *Water Balance and Solute Export in a South-East Ontario Ombrotrophic Peatland*. McGill University.

Ingram, H. A. P. (1987). Ecohydrology of Scottish peatlands. *Transactions of the Royal Society of Edinburgh: Earth Sciences*, *78*(4), 287–296. https://doi.org/10.1017/S0263593300011226

Jiang, B., & Gu, Y. (1989). A suggested fractionation scheme of inorganic phosphorus in calcareous soils. *Fertilizer Research*, *20*(3), 159–165. https://doi.org/10.1007/BF01054551

Jirousek, M., Tuma, I., Zahora, J., Holub, P., Kintrova, K., & Hajek, M. (2015). Decomposition patterns, nutrient availability, species identities and vegetation changes in central-european summit bogs. *Boreal Environment Research*, *20*, 571–586.

Jonasson, S., & Stuart Chapin, F. (1991). Seasonal uptake and allocation of phosphorus in Eriophorum vaginatum L measured by labelling with 32 P. *New Phytologist*, *118*(2), 349–357. https://doi.org/10.1111/j.1469-8137.1991.tb00987.x

Juutinen, S., Bubier, J. L., & Moore, T. R. (2010). Responses of Vegetation and Ecosystem CO2 Exchange to 9 Years of Nutrient Addition at Mer Bleue Bog. *Ecosystems*, *13*(6), 874–887. https://doi.org/10.1007/s10021-010-9361-2

Kaila, A., Asam, Z., Koskinen, M., Uusitalo, R., Smolander, A., Kiikkilä, O., Sarkkola, S., O'Driscoll, C., Kitunen, V., Fritze, H., Nousiainen, H., Tervahauta, A., Xiao, L., & Nieminen, M. (2016). Impact of Re-wetting of Forestry-Drained Peatlands on Water Quality—A Laboratory Approach Assessing the Release of P, N, Fe, and Dissolved Organic Carbon. *Water, Air, & Soil Pollution*, *227*(8), 292. https://doi.org/10.1007/s11270-016-2994-9

Kanakidou, M., Myriokefalitakis, S., Daskalakis, N., Fanourgakis, G., Nenes, A., Baker, A. R., Tsigaridis, K., & Mihalopoulos, N. (2016). Past, Present, and Future Atmospheric Nitrogen Deposition. *Journal of the Atmospheric Sciences*, *73*(5), 2039–2047. https://doi.org/10.1175/JAS-D-15-0278.1

Kashem, Md. A., Akinremi, O. O., & Racz, G. J. (2004). Phosphorus fractions in soil amended with organic and inorganic phosphorus sources. *Canadian Journal of Soil Science*, *84*(1), 83–90. https://doi.org/10.4141/S03-018

Kouno, K., Tuchiya, Y., & Ando, T. (1995). Measurement of soil microbial biomass phosphorus by an anion exchange membrane method. *Soil Biology and Biochemistry*, *27*(10), 1353–1357. https://doi.org/10.1016/0038-0717(95)00057-L

Larmola, T., Bubier, J. L., Kobyljanec, C., Basiliko, N., Juutinen, S., Humphreys, E., Preston, M., & Moore, T. R. (2013). Vegetation feedbacks of nutrient addition lead to a weaker carbon sink in an ombrotrophic bog. *Global Change Biology*, *19*(12), 3729–3739. https://doi.org/10.1111/gcb.12328

Leinweber, P., Lünsmann, F., & Eckhardt, K. U. (1997). Phosphorus sorption capacities and saturation of soils in two regions with different livestock densities in northwest Germany. *Soil Use and Management*, *13*(2), 82–89. https://doi.org/10.1111/j.1475-2743.1997.tb00562.x

Loisel, J., Gallego-Sala, A. V., Amesbury, M. J., Magnan, G., Anshari, G., Beilman, D. W., Benavides, J. C., Blewett, J., Camill, P., Charman, D. J., Chawchai, S., Hedgpeth, A., Kleinen, T., Korhola, A., Large, D., Mansilla, C. A., Müller, J., van Bellen, S., West, J. B., … Wu, J. (2021). Expert assessment of future vulnerability of the global peatland carbon sink. *Nature Climate Change*, *11*(1), 70–77. https://doi.org/10.1038/s41558-020-00944-0

MacDonald, G. K., Bennett, E. M., Potter, P. A., & Ramankutty, N. (2011). Agronomic phosphorus imbalances across the world's croplands. *Proceedings of the National Academy of Sciences*, *108*(7), 3086–3091. https://doi.org/10.1073/pnas.1010808108

McGill, W. B., & Cole, C. V. (1981). Comparative aspects of cycling of organic C, N, S and P through soil organic matter. *Geoderma*, *26*(4), 267–286. https://doi.org/10.1016/0016-7061(81)90024-0

McLaughlin, M., Alston, A., & Martin, J. (1986). Measurement of phosphorus in the soil microbial biomass: A modified procedure for field soils. *Soil Biology and Biochemistry*, *18*(4), 437–443. https://doi.org/10.1016/0038-0717(86)90050-7

Meier, I. C., Finzi, A. C., & Phillips, R. P. (2017). Root exudates increase N availability by stimulating microbial turnover of fast-cycling N pools. *Soil Biology and Biochemistry*, *106*, 119–128. https://doi.org/10.1016/j.soilbio.2016.12.004

Milin, S. (2012). *Comparaison de deux méthodes spectrophotométriques de dosage de l'acide phosphorique. Application à des sols et des végétaux*. INRA Bordeaux Sciences Agro.

Mitsch, W. J., & Gosselink, J. G. (2015). *Wetlands* (Fifth edition). John Wiley and Sons, Inc.

Moore, T. R., Knorr, K.-H., Thompson, L., Roy, C., & Bubier, J. L. (2019). The effect of long-term fertilization on peat in an ombrotrophic bog. *Geoderma*, *343*, 176–186. https://doi.org/10.1016/j.geoderma.2019.02.034

Morris, P. J., Davies, M. L., Baird, A. J., Balliston, N., Bourgault, M.-A., Clymo, R. S., Fewster, R. E., Furukawa, A. K., Holden, J., Kessel, E., Ketcheson, S. J., Klove, B., Larocque, M., Marttila, H., Menberu, M. W., Moore, P. A., Price, J. S., Ronkanen, A.-K., Rosa, E., Strack, M., Surridge, B. W. J., Waddington, J. M., Whittington, P., Wilkinson, S. L. (2022). Saturated Hydraulic Conductivity in Northern Peats Inferred From Other Measurements. *Water Resources Research, 58,* 11-37. https://doi.org/10.1029/2022WR033181

Negassa, W., & Leinweber, P. (2009). How does the Hedley sequential phosphorus fractionation reflect impacts of land use and management on soil phosphorus: A review. *Journal of Plant Nutrition and Soil Science*, *172*(3), 305–325. https://doi.org/10.1002/jpln.200800223

Oberson, A., & Joner, E. J. (2005). Microbial turnover of phosphorus in soil. In *Organic phosphorus in the environment* (pp. 133–164). CABI. https://doi.org/10.1079/9780851998220.0133

Oehl, F., Oberson, A., Probst, M., Fliessbach, A., Roth, H.-R., & Frossard, E. (2001). Kinetics of microbial phosphorus uptake in cultivated soils. *Biology and Fertility of Soils*, *34*(1), 31–41. https://doi.org/10.1007/s003740100362

Ohno, T., & Zibilske, L. M. (1991). Determination of Low Concentrations of Phosphorus in Soil Extracts Using Malachite Green. *Soil Science Society of America Journal*, *55*(3), 892–895. https://doi.org/10.2136/sssaj1991.03615995005500030046x

Papp, C. S. E., & Harms, T. F. (1985). Comparison of digestion methods for total elemental analysis of peat and separation of its organic and inorganic components. *The Analyst*, *110*(3), 237. https://doi.org/10.1039/an9851000237

Parkinson, J. A., & Allen, S. E. (1975). A wet oxidation procedure suitable for the determination of nitrogen and mineral nutrients in biological material. *Communications in Soil Science and Plant Analysis*, *6*(1), 1–11. https://doi.org/10.1080/00103627509366539

Pinsonneault, A. J., Moore, T. R., & Roulet, N. T. (2016). Effects of long-term fertilization on peat stoichiometry and associated microbial enzyme activity in an ombrotrophic bog. *Biogeochemistry*, *129*(1), 149–164. https://doi.org/10.1007/s10533-016-0224-6

Poschenrieder, C., & Barceló, J. (2013). Aluminum in Plants. In R. H. Kretsinger, V. N. Uversky, & E. A. Permyakov (Eds.), *Encyclopedia of Metalloproteins* (pp. 34–38). Springer New York. https://doi.org/10.1007/978-1-4614-1533-6_109

Prüter, J., McLaren, T. I., Pätzig, M., Hu, Y., & Leinweber, P. (2023). Phosphorus speciation along a soil to kettle hole transect: Sequential P fractionation, P XANES, and 31P NMR spectroscopy. *Geoderma*, *429*, 116215. https://doi.org/10.1016/j.geoderma.2022.116215

Qualls, R. G., & Richardson, C. J. (2000). Phosphorus Enrichment Affects Litter Decomposition, Immobilization, and Soil Microbial Phosphorus in Wetland Mesocosms. *Soil Science Society of America Journal*, *64*(2), 799–808. https://doi.org/10.2136/sssaj2000.642799x

Rankin, T. (2022). *Controls on autotrophic and heterotrophic respiration in an ombrotrophic bog*. McGill University.

Reina, M., Serrano, L., & Golterman, H. L. (2011). A sequential procedure for the quantification of biologically produced polyphosphate in sediment samples. *Limnetica*, *30*(1), 0017–0026.

Richardson, A. E. (2001). Prospects for using soil microorganisms to improve the acquisition of phosphorus by plants. *Functional Plant Biology*, *28*(9), 897. https://doi.org/10.1071/PP01093

Richardson, C. J., & Marshall, P. E. (1986). Processes Controlling Movement, Storage, and Export of Phosphorus in a Fen Peatland. *Ecological Monographs*, *56*(4), 279–302. https://doi.org/10.2307/1942548

Ruttenberg, K. C. (1992). Development of a sequential extraction method for different forms of phosphorus in marine sediments. *Limnology and Oceanography*, *37*(7), 1460–1482. https://doi.org/10.4319/lo.1992.37.7.1460

Saa, A., Trasar-Cepeda, M. C., Gil-Sotres, F., & Carballas, T. (1993). Changes in soil phosphorus and acid phosphatase activity immediately following forest fires. *Soil Biology and Biochemistry*, *25*(9), 1223–1230. https://doi.org/10.1016/0038-0717(93)90218-Z

Sagoe, C. I., Ando, T., Kouno, K., & Nagaoka, T. (1998). Relative importance of protons and solution calcium concentration in phosphate rock dissolution by organic acids. *Soil*

Science and Plant Nutrition, *44*(4), 617–625. https://doi.org/10.1080/00380768.1998.10414485

Saia, S. M., Carrick, H. J., Buda, A. R., Regan, J. M., & Walter, M. T. (2021). Critical Review of Polyphosphate and Polyphosphate Accumulating Organisms for Agricultural Water Quality Management. *Environmental Science & Technology*, *55*(5), 2722–2742. https://doi.org/10.1021/acs.est.0c03566

Schlesinger, W. H., Bruijnzeel, L. A., Bush, M. B., Klein, E. M., Mace, K. A., Raikes, J. A., & Whittaker, R. J. (1998). The biogeochemistry of phosphorus after the first century of soil development on Rakata Island, Krakatau, Indonesia. *Biogeochemistry*, *40*(1), 37–55. https://doi.org/10.1023/A:1005838929706

Schlichting, A., Leinweber, P., Meissner, R., & Altermann, M. (2002). Sequentially extracted phosphorus fractions in peat-derived soils. *Journal of Plant Nutrition and Soil Science*, *165*(3), 290–298. https://doi.org/10.1002/1522-2624(200206)165:3%3C290::AID-JPLN290%3E3.0.CO;2-A

Smith, D. B., Cannon, W. F., Woodruff, L. G., Garrett, R. G., Klassen, R., Kilburn, J. E., Horton, J. D., King, H. D., Goldhaber, M. B., & Morrison, J. M. (2005). *Major- and Trace-Element Concentrations in Soils from Two Continental-Scale Transects of the United States and Canada* (Open File Report 2005–1253; p. 22). U.S. Geological Survey.

Spohn, M., Ermak, A., & Kuzyakov, Y. (2013). Microbial gross organic phosphorus mineralization can be stimulated by root exudates–a 33P isotopic dilution study. *Soil Biology and Biochemistry*, *65*, 254–263.

Spohn, M., & Kuzyakov, Y. (2013). Phosphorus mineralization can be driven by microbial need for carbon. *Soil Biology and Biochemistry*, *61*, 69–75. https://doi.org/10.1016/j.soilbio.2013.02.013

Tian, J., Ge, F., Zhang, D., Deng, S., & Liu, X. (2021). Roles of Phosphate Solubilizing Microorganisms from Managing Soil Phosphorus Deficiency to Mediating Biogeochemical P Cycle. *Biology*, *10*(2), 158. https://doi.org/10.3390/biology10020158

Tiessen, H., & Moir, J. O. (1993). Characterization of Available P by Sequential Extraction. In *Soil Sampling and Methods of Analysis* (pp. 75–87). Lewis Publishers.

Tipping, E., Benham, S., Boyle, J. F., Crow, P., Davies, J., Fischer, U., Guyatt, H., Helliwell, R., Jackson-Blake, L., Lawlor, A. J., Monteith, D. T., Rowe, E. C., & Toberman, H. (2014). Atmospheric deposition of phosphorus to land and freshwater. *Environ. Sci.: Processes Impacts*, *16*(7), 1608–1617. https://doi.org/10.1039/C3EM00641G

Turetsky, M. R. (2004). Decomposition and Organic Matter Quality in Continental Peatlands: The Ghost of Permafrost Past. *Ecosystems*, *7*(7), 740–750. https://doi.org/10.1007/s10021-004-0247-z

Turetsky, M. R., Benscoter, B., Page, S., Rein, G., Van Der Werf, G. R., & Watts, A. (2015). Global vulnerability of peatlands to fire and carbon loss. *Nature Geoscience*, *8*(1), 11–14. https://doi.org/10.1038/ngeo2325

Turner, B. L., Frossard, Emmanuel., & Baldwin, D. S. (2005). *Organic phosphorus in the environment* (1–1 online resource (xiii, 399 pages) : illustrations). CABI Publishing; WorldCat.org. https://doi.org/10.1079/9780851998220.0000

Turner, B. L., Mahieu, N., & Condron, L. M. (2003). The phosphorus composition of temperate pasture soils determined by NaOH–EDTA extraction and solution 31 P NMR spectroscopy. *Organic Geochemistry*, *34*(8), 1199–1210. https://doi.org/10.1016/S0146-6380(03)00061-5

Turunen, J., Roulet, N. T., Moore, T. R., & Richard, P. J. H. (2004). Nitrogen deposition and increased carbon accumulation in ombrotrophic peatlands in eastern Canada: N deposition and peat accumulation. *Global Biogeochemical Cycles*, *18*(3), n/a-n/a. https://doi.org/10.1029/2003GB002154

Velásquez, G., Ngo, P.-T., Rumpel, C., Calabi-Floody, M., Redel, Y., Turner, B. L., Condron, L. M., & Mora, M. D. L. L. (2016). Chemical nature of residual phosphorus in Andisols. *Geoderma*, *271*, 27–31. https://doi.org/10.1016/j.geoderma.2016.01.027

Veneklaas, E. J. (2022). Phosphorus resorption and tissue longevity of roots and leaves – importance for phosphorus use efficiency and ecosystem phosphorus cycles. *Plant and Soil*, *476*(1–2), 627–637. https://doi.org/10.1007/s11104-022-05522-1

Verry, E. S., & Timmons, D. R. (1982). Waterborne Nutrient Flow Through an Upland-Peatland Watershed in Minnesota. *Ecology*, *63*(5), 1456–1467. https://doi.org/10.2307/1938872

Vincent, L. A., Zhang, X., Mekis, É., Wan, H., & Bush, E. J. (2018). Changes in Canada's Climate: Trends in Indices Based on Daily Temperature and Precipitation Data. *Atmosphere-Ocean*, *56*(5), 332–349. https://doi.org/10.1080/07055900.2018.1514579

Von Post, L. (1922). Swedish geological peat survey with the results obtained so far. *Sven. Mosskult. Tidskr*, *36*, 1–27.

von Sperber, C., Stallforth, R., Du Preez, C., & Amelung, W. (2017). Changes in soil phosphorus pools during prolonged arable cropping in semiarid grasslands: Changes in soil phosphorus pools. *European Journal of Soil Science*, *68*(4), 462–471. https://doi.org/10.1111/ejss.12433

Walbridge, M. R., & Vitousek, P. M. (1987). Phosphorus mineralization potentials in acid organic soils: Processes affecting 32PO43− isotope dilution measurements. *Soil Biology and Biochemistry*, *19*(6), 709–717. https://doi.org/10.1016/0038-0717(87)90053-8

Walker, T. W., & Syers, J. K. (1976). The fate of phosphorus during pedogenesis. *Geoderma*, *15*(1), 1–19. https://doi.org/10.1016/0016-7061(76)90066-5

Wang, M., Moore, T. R., Talbot, J., & Richard, P. J. H. (2014). The cascade of C:N:P stoichiometry in an ombrotrophic peatland: From plants to peat. *Environmental Research Letters*, *9*(2), 024003. https://doi.org/10.1088/1748-9326/9/2/024003

Wang, Y., Bauke, S. L., Von Sperber, C., Tamburini, F., Guigue, J., Winkler, P., Kaiser, K., Honermeier, B., & Amelung, W. (2021). Soil phosphorus cycling is modified by carbon and nitrogen fertilization in a long-term field experiment. *Journal of Plant Nutrition and Soil Science*, *184*(2), 282–293. https://doi.org/10.1002/jpln.202000261

Warner, B. G., & Rubec, C. D. A. (1997). *The Canadian Wetland Classification System*. Wetlands Research Branch, University of Waterloo. https://books.google.ca/books?id=nL4WnwEACAAJ

Williams, B. L., & Sparling, G. P. (1984). Extractable N and P in relation to microbial biomass in UK acid organic soils. *Plant and Soil*, *76*(1–3), 139–148. https://doi.org/10.1007/BF02205574

Wilson, P. G. (2012). *The relationship among micro-topographic variation, water table depth and biogeochemistry in an ombrotrophic bog* [Masters of Science, McGill University]. https://escholarship.mcgill.ca/downloads/1g05fg587?locale=en

Yanai, R. D. (1992). Phosphorus budget of a 70-year-old northern hardwood forest. *Biogeochemistry*, *17*(1). https://doi.org/10.1007/BF00002757

Yevdokimov, I., Larionova, A., & Blagodatskaya, E. (2016). Microbial immobilisation of phosphorus in soils exposed to drying-rewetting and freeze-thawing cycles. *Biology and Fertility of Soils*, *52*(5), 685–696. https://doi.org/10.1007/s00374-016-1112-x

Yu, Z. C. (2012). Northern peatland carbon stocks and dynamics: A review. *Biogeosciences*, *9*(10), 4071–4085. https://doi.org/10.5194/bg-9-4071-2012

Zeppel, M. J. B., Wilks, J. V., & Lewis, J. D. (2014). Impacts of extreme precipitation and seasonal changes in precipitation on plants. *Biogeosciences*, *11*(11), 3083–3093. https://doi.org/10.5194/bg-11-3083-2014

Zheng, Z., Simard, R. R., Lafond, J., & Parent, L. E. (2002). Pathways of Soil Phosphorus Transformations after 8 Years of Cultivation under Contrasting Cropping Practices. *Soil Science Society of America Journal*, *66*(3), 999–1007. https://doi.org/10.2136/sssaj2002.9990