Short-term biogeochemical response of hardwood forest soils to wood ash additions in central Ontario

A Thesis Submitted to the Committee of Graduate Studies in Partial Fulfillment of the Requirements for the Degree of Master of Science in the Faculty of Arts and Science

Trent University

Peterborough, Ontario, Canada

Environmental and Life Sciences M.Sc. Graduate Program

May 2023

ABSTRACT

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The accelerated recovery of base-poor soils from the legacy effects of acidic deposition may be possible by applying industrial wood ash as a soil amendment. Wood ash may be an effective soil amendment due to its high alkalinity and concentrations of several essential nutrients, such as calcium, magnesium, potassium, and phosphorus, that are retained after the volatilization of the parent material. However, wood ash can also contain trace amounts of metals that could be released into the soil and soil solution. The short-term (<3 years) biogeochemical response of soils, microbial communities, and sugar maple (*Acer saccharum* Marsh.) trees were assessed following wood ash application at Porridge Lake, Ontario. The study design consisted of five blocks containing three treatment plots each (2.5, 5.0, 7.5 Mg ha^{-1}) and a control. Soil solution pH, base cation, and trace metal concentrations were monitored for three years, using tension lysimeters at depths of 30 and 60 cm and zero-tension lysimeters for forest floor percolate within each plot. In the last year of the trial, soil, foliage, and fine root samples were collected and analyzed for trace elements. Also, soil samples were analyzed for the abundance of 16S and ITS DNA through metabarcoding to ascertain the microbial response to wood ash. Significant changes in soil solution pH were measured within the forest floor horizon in the first year of the trial. Significant increases in calcium (Ca), magnesium (Mg) and calcium/aluminum (Ca/Al) ratios were also observed in the second year of the trial, along with decreases in dissolved organic carbon (DOC), sulphate (SO_4) and nitrate (NO_3) in the LFH horizon. By the third year of the trial, significant increases in soil solution pH and potassium (K) concentrations and decreases in Al were observed to a depth of 30 cm. Changes in trace metal concentrations in soil water were notably variable, with concentrations of chromium, copper, lead, nickel, and selenium remaining unresponsive, whereas concentrations of cadmium, manganese and zinc decreased by the third year. The metalloid arsenic showed a significant

increase in the third year of the trial but remained below regulatory guidelines, similar to all other trace metals. Soil measurements conducted in the third year of the trial showed positive pH responses in the FH horizon and increases in Ca and Mg in the Ah and Bm soil horizons, but foliar base cation and metal concentrations were unchanged. Diversity analysis on the soil prokaryotic and eukaryotic groups indicated increased bacterial alpha diversity in the FH horizon and bacterial dominance in the litter horizon. Analysis of relative abundance at the phylum level for prokaryotes and at the order for eukaryotes did not indicate any compositional shifts due to the wood ash treatments. Changes in the length and diameter of sugar maple and mycorrhizal fine root may point to pH shock being an issue at higher ash doses. The results from this study indicate that wood ash has a strong ameliorative effect on soil properties and does not pose a risk to soil communities.

Acknowledgements

I would like to start by thanking Dr. Paul Hazlett and Laura Hawdon and the team at the Great Lakes Forestry Centre along with Dr. Nathan Basiliko for setting up and supporting this project. I would especially like to thank Holly Deighton for supporting me in the initial months that I took over the project and offering my guidance when everything seemed overwhelming. To chase a dream so late in life is scary and I have nothing but love and respect for those that have helped me along the way. So, to those that ventured out into the field to brave the wild wilderness and the endless mosquitoes, I feel that I still owe you. Thank you Kaylen Foley, Jodi Newman, Edward Kellaway, Shelby Conquer, Anne-Sylvie Dasne, Chetwynd Osborne, Skylar Deraaf and William Humphrey. And Patrick Levasseur, you were the best fake best-friend and an amazing office mate. Life will shower you with joy as that is what you give to others, when you are not singing The Final Countdown. To the rest of my office and lab mates, we made memories, all of them were good to me and I thank you.

To my committee members Dr. Eric Sager and Dr. Catherine Eimers thank you for your insights and support, you help to keep from getting lost in the weeds and your opinions really mattered to me. I would especially like to thank my supervisor Dr. Shaun Watmough. You are an artist. You read me like a book, understood my weaknesses and gave me the freedom and guidance I needed to reach the finish line. Truly, thank you, I would have faltered so long ago without you being there.

Lastly, to the real motivation behind this dream, my beautiful wife and two terrible children (whom I absolutely unequivocable adore with all my heart). I have only ever thought of you when times were hard and used your support as motivation to jump each hurdle as they came. I want nothing more than for you to be proud of me.

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List of Abbreviations

Alpha Diversity……α
Aluminum……Al
Analysis Of Variance……ANOVA
Arbuscular mycorrhizae……AM
Arsenic……As
Bulk Density……BD
Below detection limit……BDL
Cadmium……Cd
Calcium……Ca
Calcium/Aluminum Ratio……Ca/Al
Calcium oxide……CaO
Canadian Environmental Quality Guidelines……CCME
Carbon……C
Carbon dioxide……CO_2
Carbon/Nitrogen Ratio……C/N
Chloride……Cl
Chromium……Cr
Content of Regulated Metals……CM
Cobalt……Co
Copper……Cu
Diameter at breast height……DBH
Diopside…… $MgCaSi_2O_6$
Dissolved Organic Carbon……DOC
Dolomite……$CaMg(CO_3)_2$
Ectomycorrhizal fungi……ECM
Eukaryote internal transcribed spacer……ITS
Fibrous, humus layer……FH
Hydrogen……H^+
Hydrogen chloride……HCl
Inductively Coupled Plasma-Mass Spectrometry ……ICP-MS
Inductively Coupled Plasma-Optical Emission Spectrometry ……ICP-OES
Iron……Fe
Lead……Pb
Leaf Litter……L
Lime……$CaCo_3$
Loss on ignition……LOI
Manganese……Mn
Magnesium……Mg
Mega grams per hectare……$Mg \cdot ha^{-1}$
Molybdenum……Mo
Multi-analysis of variance……MANOVA
National Institute of Standards and Technology……NIST

Nickel……Ni
Nitrate……NO_3
Nitric acid……HNO_3
Nitrogen……N
Nitrogen oxides……NO_x
Non-agricultural Source Material……NASM
Non-industrial wood ash……NIWA
Organic Matter……OM
Permutational Multivariate Analysis of Variance……PERMANOVA
Phosphorus……P
Potassium……K
Principal Component Analysis……PCA
Prokaryote 16S ribosomal DNA……16S
Relative Abundance……RA
Root Biomass……RB
Root Length Density……RLD
Root Tips……RT
Selenium……Se
Shannon-Weiner Index……H
Silica Dioxide……SiO_2
Simpson Index……D
Sodium……Na
Soil Organic Matter……SOM
Specific Root Length……SRL
Standard Error……SE
Strontium……Sr
Sulphate……SO_4
Sulphur……S
Sulphuric acid……H_2SO_4
Total Organic Carbon……TOC
Total Nitrogen……TN
Tukey Honest Significant Difference……Tukey HSD
Water……H_2O
Wollastonite…… $CaSiO_3$
Zinc……Zn

1. Introduction

1.1 Acidic deposition and soil acidification

Despite significant reductions in atmospheric emissions of sulphur dioxide (SO_2) and nitrogen oxides (NO_x), the long-term effects of acidic deposition can still be observed in soils throughout North America, Europe and Asia (Driscoll et al., 2001; Feng et al., 2020). Acidic deposition is caused by SO_2 and NO_3 oxidizing in the atmosphere and precipitating back down as wet deposition (sulphuric (H_2SO_4) and nitric acid (HNO_3)) (Feng et al., 2020; Kunhikrishnan et al., 2016) or as dry deposition through particulate matter or as gaseous SO_2 (Driscoll et al., 2001). When the deposition of strong acids exceeds the buffering capacity of the soil, the soil pH will decrease and trigger profound changes in ecosystem biogeochemistry (Watmough & Dillon, 2002). Decreases in soil pH have been shown to cause compositional changes in soil microbial communities (Rousk et al., 2009), negatively impact tree growth (Gilliam et al., 2019), and increase the solubility and mobility of nutrient cations and metals (Moreno Marcos & Gallardo Lancho, 2002; Neina, 2019). Over time, soils can become depleted of essential base cations, such as calcium (Ca), magnesium (Mg), and potassium (K), while hydrogen (H^+) and aluminum (Al) increase, effectively lowering the soil's acid-neutralizing capacity (ANC) (Blake et al., 1999; Duchesne & Houle, 2008; Feng et al., 2020; Hutchinson et al., 1997; Kunhikrishnan et al., 2016; Rengel, 2015).

Under acidic conditions, metallic elements such as iron (Fe), manganese (Mn) and Al become more soluble, and concentrations in soil solution can reach potentially phytotoxic levels that inhibit plant growth (Hutchinson et al., 1997; Marschner, 1991; St. Clair & Lynch, 2004). For instance, Fe and Mn have been shown to reduce photosynthesis and fine root growth in acidic soils (Li et al., 2019; St. Clair et al., 2005; St. Clair & Lynch, 2004). Studies have also reported higher plant uptake of trace metals at low pH, including cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), lead (Pb) and zinc (Zn) (Bradl, 2004) which can lead to compositional changes in plants (Lundström et al., 2003) and soil microbial communities (Rousk et al., 2009). However, the greatest concern associated with soil acidification is the mobilization of Al (Driscoll et al., 2001). Elevated concentrations of Al have been

shown to constrain the microbial oxidation of methane (CH_4) (Kunhikrishnan et al., 2016), increase the rigidity of cell walls and disrupt the acquisition of water and nutrients by fine roots (Foy, 1992), effectively reducing litter quality and nutrient cycling (Khabaz-Saberi & Rengel, 2010; Marschner, 1991; Mossor-Pietraszewska, 2001; Vanguelova et al., 2007). Further, Ca is essential for the growth and health of trees, prokaryote and eukaryote communities (Lawrence et al., 2021; Likens et al., 1998), and since Al can inhibit nutrient uptake, the molar ratio of Ca/Al is a strong indicator of root and nutrient impairment (Cronan & Grigal, 1995; Drohan et al., 2002; Lyon & Sharpe, 1999; Vanguelova et al., 2007). In a review of studies reporting Ca/Al ratios, Cronan & Grigal (1995) found that the chances of tree stress occurring increase by 50% below the critical ratio threshold of one.

1.2 Limitations on recovery from acidification

Nutrient loss from soils is also driven by the increased demand for biofuels in Canada to help reduce greenhouse gas emissions (GHGs) and meet our climate commitments for 2030 (Government of Canada, 2016). Exports of wood pellets in Canada for 2022 were estimated to be 3.3 million Mg, while in the province of Ontario, the Atikokan power generation station uses 90,000 Mg of wood pellets to help bolster the energy grid (Dampier et al., 2016). The most common and sustainable source of biofuel comes from timber harvesting (Joseph et al., 2022; Tubeileh et al., 2014), and as the global demand for biofuels increases, Canada's forestry sector has been at the forefront of production (Pugliese et al., 2014). The Canadian government defines timber as forest biomass, which includes the trunk, bark, branches, leaves and roots of trees (Government of Canada, 2020). For base-poor sites with thin soils and limited buffering capacity, such as those found on the Canadian Shield, the complete removal of biomass may be problematic for ecosystem recovery (Dillon et al., 2007; Duchesne & Houle, 2008; Majdi et al., 2008; Watmough, 2002). A study conducted in the Muskoka-Haliburton region of central Ontario showed that when the effects of timber harvesting were included in critical load models, the ANC of forest soils was predicted to decrease to a greater extent than unharvested scenarios due to the enhanced loss of nutrient cations (Watmough & Dillon, 2002). A similar analysis by Federer et al. (1989)

showed that nutrient loss caused by timber harvesting was roughly equal to leaching from soil acidification.

The net loss of nutrients from impaired ecosystems heightens trees' susceptibility to die-back following drought, insect defoliation, and the freeze-thaw cycle (Drohan et al., 2002; Horsley et al., 2000). Studies in Ontario (Miller & Watmough, 2009; Watmough, 2010), Quebec (Moore et al., 2012; Moore & Ouimet, 2010), Vermont (Liu et al., 1997), and Pennsylvania (Drohan et al., 2002) have linked crown dieback to increased metal availability, nutrient loss in the soil and foliar leaching. Research has also shown that with increases in canopy gaps, soil organic matter (SOM) breakdown by microbial communities also increases (Ballard, 2000), and although species like sugar maple (*Acer saccharum* Marsh.) are thriving (Treasure et al., 2019) they are not competitive in the long-term under base-poor conditions (Bannon et al., 2015). In addition, saplings have greater nutritional demands than mature trees, indicating that regeneration will be impaired as nutrient availability shifts with stand age (Likens et al., 1998; Naples & Fisk, 2010). Maintaining healthy bacterial and fungal communities is also crucial to maintaining tree growth as external inputs of Ca through atmospheric deposition are on the decline (Hedin, 1994; Kreutzweiser et al., 2008; Likens et al., 1998), and microbial decomposition drives SOM turnover, soil formation and nutrient allocation to trees (Condron et al., 2010; Huntington et al., 2000; Kennedy, 1999). Portions of nutrient uptake are stored in the foliage and recycled through the system as litterfall and microbial and fungal decomposition. Disruptions to soil cycling can also interrupt carbon (C) sequestration and inhibit the symbiotic relationship between mycorrhizal fungi and host trees (Clemmensen et al., 2013; Mahmood et al., 2001) and weaken microbial functionality (Smenderovac et al., 2017). Disruptions can further impact tree health as mycorrhizal fungi also help to alleviate plant stress from drought and the chelation of phytotoxic metals such as Al (Finlay, 2008). With the increasing demand for lumber and biofuels putting further pressure on nutrient-poor forest stands, soil amendments may be necessary to ensure long-term recovery and sustainability.

1.3 Evaluation of common soil amendments

Lime, wollastonite, and wood ash are some of the most common soil amendments used to increase soil pH and counteract soil acidification. Components of these amendments typically include alkaline-earth metals, such as Ca and Mg, that reduce soil acidity and improve plant growth (Haque et al., 2020b; Reid & Watmough, 2014). Lime is typically applied as either limestone ($CaCO_3$) or dolomite ($CaMg(CO_3)_2$) and has been proven effective in increasing soil pH and the availability of base cations while immobilizing heavy metals (Kunhikrishnan et al., 2016). For example, Majdi and Viebke (2004) found that liming increased soil pH, Ca, and Mg concentrations but not K, phosphorus (P), Mn or Zn. Similar improvements were also seen in Sweden following the application of dolomite, with concentrations of Ca and Mg being eight times greater than in control sites, but increases in Fe and NO_3 were also reported (Geibe et al., 2003).

The benefits of liming have been shown to persist over several decades. In a long-term study by Long et al. (2011) on the response of hardwood trees to lime in the Allegheny Plateau in Pennsylvania, the pH of the soil in treated plots was still higher after 23 years than in control plots (6.6, compared with 3.7, respectively). There were also lower Al, Fe and Mn concentrations, and the foliar concentration of Ca in sugar maple in treated plots was more than double the value in control plots. Because of the study's longevity, they also witnessed the effects of droughts and defoliation events on trees receiving lime amendments. They found that sugar maple responded more to the liming application than American beech (*Fagus grandifolia* Ehrh.) and displayed moderate increases in crown vigour despite the environmental stressors. Another study in Quebec also showed a sustained improvement in foliar concentrations of Ca and Mg in sugar maple and decreases in crown dieback after 20 years (Moore & Ouimet, 2021). While it has been suggested that lime has a more significant ameliorative effect on soil pH and foliar Ca concentrations compared with wood ash (Reid & Watmough, 2014; Van Hees et al., 2003), studies on lime have also shown a tendency towards shallower roots (Kreutzer, 1995), decreases

in mycorrhizal diversity (Taylor & Finlay, 2003), increases in root rot and the potential for greater nitrate leaching (Huber et al., 2006; Kreutzer, 1995; Kunhikrishnan et al., 2016).

Similar to lime, wollastonite is another Ca-rich amendment, typically in the form of $CaSiO_3$ or $MgCaSi_2O_6$ (diopside), that can improve soil Ca and Mg concentrations and, additionally, may enhance the sequestration of carbon dioxide (CO_2) (Haque et al., 2020a). Wollastonite is very alkaline (pH ca. 10.7) and, like lime, has low solubility, making it slow to reach rooting zones (Huijgen et al., 2006; Li et al., 2019). Experiments in the Hubbard Brook Experimental Forest (HBEF) in New Hampshire demonstrated improvements in the ANC of soil, decreases in Al in soil solution and greater retention of Ca in the organic horizons and slow percolation into the mineral horizon after seven years (Johnson et al., 2014; Shao et al., 2016). Other researchers at HBEF reported elevated concentrations of foliar Ca and P, decreased foliar Mn and higher fine root Ca/Al molar ratios, along with improved photosynthetic function, increased survivability of sugar maple saplings and greater mycorrhizal colonization and biomass (Green et al., 2013; Juice et al., 2006). However, soil response to the wollastonite application has not been as great, with only moderate increases in soil pH, which for specific sites took as long as 12 years to show a response (Lawrence et al., 2021). Wollastonite is a non-renewable resource, and its poor solubility may explain why there has been limited response seen in other studies and research on wollastonite in general, especially at higher dosage rates (Haque et al., 2020b). Compared with other Ca-based soil amendments, wood ash might be a better, more sustainable option, especially considering the increased demand for lumber and biofuel worldwide.

1.3.1 Wood ash as a soil amendment

Wood ash differs from lime and wollastonite as it is the by-product of timber combustion in biomass boilers, power plants and pulp and paper mills (Cairns et al., 2021; James et al., 2012), along with residential wood stoves for non-industrial wood ash (NIWA) (Azan et al., 2019). The combustion process does not completely remove the constituents of the parent material, and high concentrations of Ca are retained in wood ash (Khan et al., 2009). Other macronutrients (Mg, K) can also be found at

moderate levels, along with micronutrients like manganese (Mn) and zinc (Zn) (Hannam et al., 2019; Majdi & Viebke, 2004). Conversely, concentrations of sulphur (S), nitrogen (N), and P are often negligible (Chirenje & Ma, 2002; Khan et al., 2009). The symbiotic relationship between trees and mycorrhizae depends on the availability of N and P (Allen et al., 2003), and as such, wood ash may be beneficial to maintaining the relationship, as the Ca amendment can promote hyphae exploration and reduce the potential for leaching and eutrophication of water bodies (Schindler et al., 2008).

Wood ash may also contain high concentrations of trace metals, raising concerns about the safety of wood ash as a soil amendment (Olsson et al., 2017). Trace metal concentrations in wood ash are variable based on fuel type (stem, bark, wood, leaves), parent soil conditions, climate (Augusto et al., 2008; Pitman, 2006), and combustion temperature (Demeyer et al., 2001). Ultimately, wood ash amendments could potentially lead to increases in arsenic (As), Cd, Co, Cr, Mo, Ni, Pb, Se and Zn in soil and soil solution (Fritze et al., 2001; Hannam et al., 2016). Some of these elements are micronutrients at low concentrations, but others are non-essential (Vestergård et al., 2018) and at large doses, the application of wood ash could result in the bioaccumulation of trace metals that can reach phytotoxic levels and become detrimental to plant growth and microbial communities (Demeyer et al., 2001; Nieminen et al., 2005; Omil et al., 2007; Pandey & Singh, 2010).

The type of wood ash can also determine the concentrations of metals. For instance, fly ash is typically collected from the flue (Hannam et al., 2017) and is more lightweight and reactive (Olsson et al., 2017), whereas bottom ash is heavier yet lower in trace metal concentrations (Pitman, 2006; Rumpf et al., 2001). Cadmium is considered the most dangerous of the heavy metals due to its high mobility and presence in wood ash (Perkiömäki & Fritze, 2005). Cadmium has been shown to affect soil bacterial communities (Baldrian & Gabriel, 1997; Olsson et al., 2017), bioaccumulate in mushrooms and berries (Moilanen et al., 2006; Omil et al., 2007), vegetation (Huotari et al., 2011) and inhibit tree growth (Bilodeau-Gauthier et al., 2011). Fortunately, studies have shown that wood ash containing modest amounts of trace metals is safe for application but can depend on the feedstock's quality (Aronsson &

Ekelund, 2004; Brunner et al., 2004; Omil et al., 2007). In a report prepared for the International Energy Agency (IEA) bioenergy division, the average metal (As, Cd, Cr, Co, Cu, Pb, Mo, Ni, Se, Zn) concentrations of wood-ash across Canada for both fly and bottom ash were below the content of regulated metals (CM) 2 limits, and only As, Cd, Mo, Se and Zn were above CM1 limits (Lamers et al., 2018), making them safe for application. Wood ash must fall below CM2 standards which takes into account metal concentrations and factors such as proximity to surface water and groundwater depth (Hannam et al., 2016).

An extensive review of the effects of wood ash has shown that hardwood species (especially sugar maple) can be very responsive to improvements in soil conditions such as pH, nutrient concentrations, microbial decomposition and mycorrhizal infection (Reid & Watmough, 2014). Other

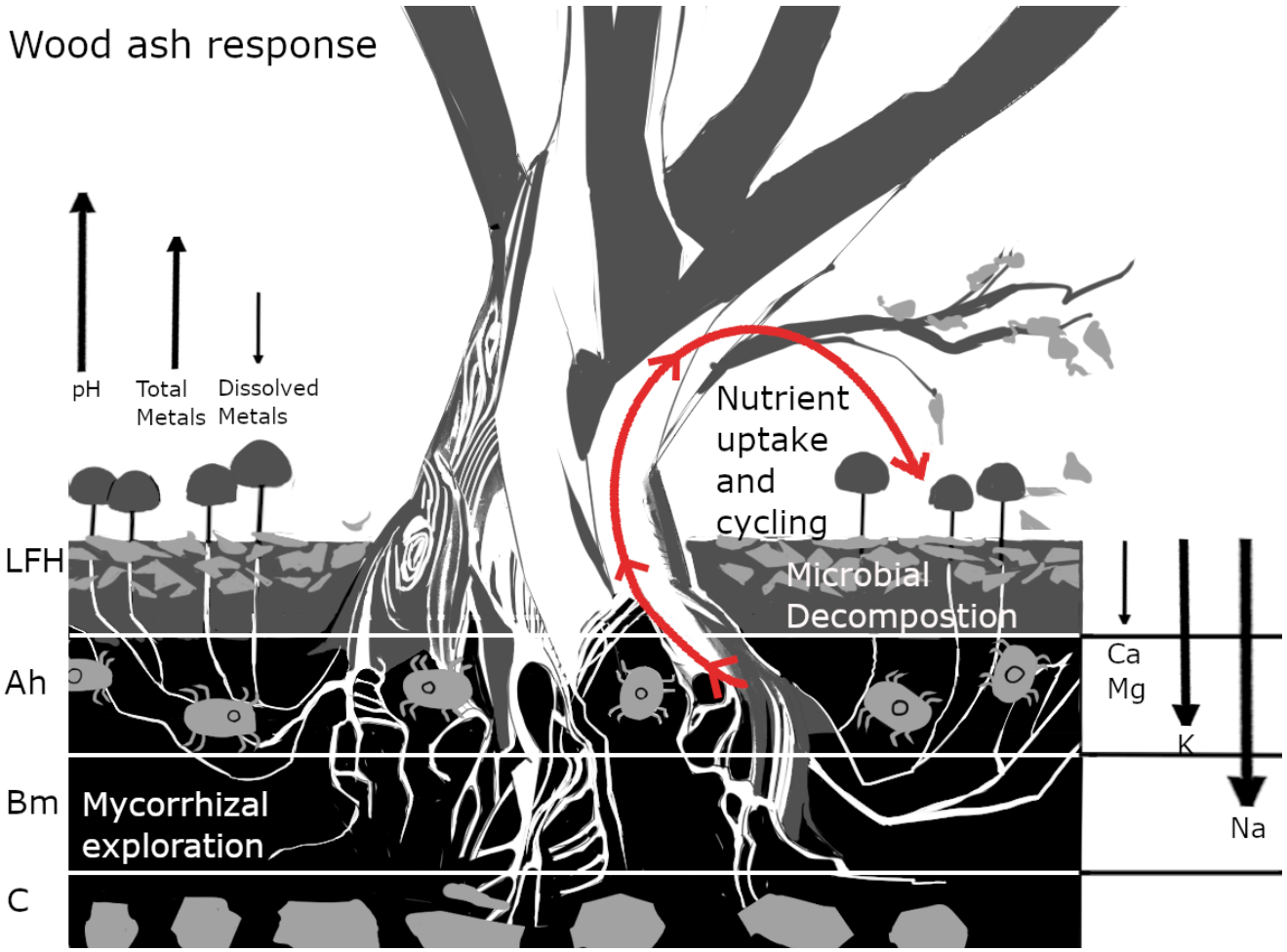

Figure 1.1 | System response to wood ash application. Microbial and bacterial decomposition increase nutrient cycling and litter quality. Arrows for Ca, Mg, K and Na represent solubility, while the arrows for pH, total and dissolved metals indicate strength.

research has shown that the ameliorative effect of wood ash can persist for decades (Pugliese et al., 2014). Wood ash can be applied in its loose form to enhance solubility or in a granulated form to prolong the effects and reduce the potential of pH shock by hardening the wood ash with water prior to application (Augusto et al., 2008; Huotari et al., 2015; Saarsalmi et al., 2001; Steenari et al., 1999). Wood ash contains many reactive oxides and salts, such as calcium oxide (CaO), which can cause pH shock in the form of burn damage on plant tissue and adversely affect soil flora and fauna if wood ash is applied in loose form without undergoing hardening (Bachmaier et al., 2021; Holmberg et al., 2000; Maresca et al., 2019). When amendments are applied to forest soils, the leaching of nutrients into the soil and soil solution depends on solubility, but increases in soil solution pH are typically seen immediately due to the high alkalinity of wood ash, which has pH values ranging from ca. 12 to 13 (Cruz-Paredes et al., 2019; Pitman, 2006). As the wood ash dissolves and the pH of the soil solution increases, microbial activity is stimulated by the leaching of macronutrients into the organic horizons and are absorbed by mycorrhizal and tree fine roots (Figure 1.1) (Brunner et al., 2004). Research has shown that applying wood ash can double ectomycorrhizal (ECM) biomass due to improved nutrient mobilization and plant acquisition (Hagerberg & Wallander, 2002; Mahmood et al., 2002). It can also increase the leaching of dissolved organic carbon (DOC) and NO_3 (Rumpf et al., 2001) and improve the water-holding capacity of soils while decreasing the solubility of metals and reducing potential leaching into the soil solution (Hagerberg & Wallander, 2002; Rieuwerts et al., 1998).

Currently, wood ash is underutilized in Canada, as the costs of applying wood ash exceed the costs of landfilling (Hope et al., 2017). It has been estimated that approximately 183,000 Mg of wood ash is landfilled each year (Lamers et al., 2018), which is a strong indication that large-scale application of wood ash is entirely possible if the costs of industrial application become more competitive (Emilson et al., 2018; Hannam et al., 2019; Hope et al., 2017; Pugliese et al., 2014). Nevertheless, to overcome the barriers to utilizing it as a soil amendment, research must prove its effectiveness and ensure it does not lead to phytotoxic concentrations of metals. Within Ontario, there are no regulations for the use of

wood ash; instead, the guidelines for its usage fall under the NASM composting guidelines for application to soil (Hannam et al., 2018). The NASM regulation covers all forms of recycled organic matter, such as compost, biosolids and wood ash and the content management levels are based on maximum metal concentrations allowable to reduce potential phytotoxic accumulation. The potential for metal contamination is probably one of the biggest concerns for using wood ash in Ontario, but it is commonly used in other countries.

1.3.2 Global use and response to wood ash

Europe was an early adopter of soil amendments to help with increased soil acidity, and a large body of research exists; detailed in this section are some long-term trials. In Finland, Saarsalmi et al. (2001) applied 3 Mg ha^{-1} of wood ash to plots of Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* L.) and found that 16 years post application, the pH of the LHF and mineral horizon still showed a positive response, along with extractable concentrations of Ca and Mg. At the same time, another study in Finland on a drained peatland showed that 50 years after wood ash application, the quality of nutrients in litter fall and subsequent improvements in cycling caused a shift to vegetation with more demanding nutrient requirements (Moilanen et al., 2002). A study in Sweden showed improved fine root growth eight years after applying wood ash in the mineral soil horizons but decreased growth in the organic horizons (Majdi & Viebke, 2004). It was hypothesized that shallower roots in the FH would benefit from increased nutrient availability, the decreased solubility of granulated wood ash and that increased root growth in the mineral horizons would help to protect trees from windthrow. Moreover, decreases in fine root biomass (RB), along with increases in specific root length (SRL), which are an indication of nutrient uptake per unit weight (Majdi & Viebke, 2004), were found in a study in Sweden (Clemensson-Lindell & Persson, 1995). This study looked at long-term changes following soil amendments and concluded that changes in fine root growth were only short-term responses (Clemensson-Lindell & Persson, 1993). Studies in Denmark (Bang-Andreasen et al., 2021), Switzerland (Brunner et al., 2004), Norway (Clarke et al., 2018), Slovakia (Gömöryová et al., 2016), and Germany

(Huber et al., 2006; Rumpf et al., 2001) have also noted improvements in soil properties from wood ash at varying application doses. The current recommendation in Finland is the application of 5.0 Mg ha^{-1} of wood ash every 30 years (Perkiömäki & Fritze, 2002).

The early adoption of wood ash in Europe has given them a significant jumpstart on research compared with North America. While wood ash is relatively understudied in North America, there has been a recent rise in research on wood ash in Canada and, to a lesser degree, the United States. For example, a study in New York applying varying doses (10 to 20 Mg ha^{-1}) of wood ash over three years noted an increase in soil base cations but decreased growth and biomass production in coppiced willow (*Salix purpurea* L.). The authors theorized that the decreased growth was not a treatment effect but likely due to the low concentrations of N in wood ash and in the soil (Park et al., 2005). A study in Hawthorne, Florida, using loading rates of 900 and 1800 Mg ha^{-1}, reported substantial increases in Ca, Mg, K, P, Cu, Fe, Mn and Zn and ultimately concluded that agronomic loading rates (in this instance 10 Mg ha^{-1}) should be used to avoid large concentrations of trace metal bioaccumulation in plants (Chirenje & Ma, 2002). The push towards wood ash research projects in Canada is partly due to AshNet (https://www.nrcan.gc.ca/science-and-data/research-centres-and-labs/forestry-research-centres/great-lakes-forestry-centre/ashnet/20279). AshNet is a collaboration between provinces, with 14 experimental sites and ten research groups, that seek to understand the effect of wood ash on forest soils to inform future policy development (Emilson et al., 2018; Hannam et al., 2018). A trial experiment in British Columbia using 5.0 Mg ha^{-1} of wood ash on a spruce (*Picea glauca* × *engelmannii* Parry × Engelm.) plantation reported positive responses in the pH and nutrient cation concentrations of the LFH horizon after just one year (Domes et al., 2018). In contrast, a collaborative review of AshNet studies across eight distinct geographical sites found no quantifiable change in SOM as a determinant of soil quality (Joseph et al., 2022). Using the same sites, Emilson et al. (2020) analyzed the growth response of various tree species and found a significant difference in response based on loading rates.

1.4 Below-ground response

Soil acidification can negatively impact mycorrhizal and fine tree roots through the depletion of nutrient cations and an increase in soluble metals, affecting the development of new roots and nutrient acquisition (Brunner et al., 2004; Park et al., 2008). The high alkalinity of wood ash is derived from the concentrations of Ca, Mg and K in the form of oxides and carbonates (Saarsalmi et al., 2001) and is the main characteristic controlling the solubility and mobility of most soil metals (Komonweeraket et al., 2015; Spurgeon et al., 2006). The dissolution of carbonate salts and subsequent increase in soil pH, along with increased microbial activity (Augusto et al., 2008), release nutrient cations into the solution, making them available to bind to the soil adsorption sites or leach into the mineral soil horizons (Zimmermann & Frey, 2002). Shifts in pH alter the adsorption of anions verses cations on soil exchange sites (Kunhikrishnan et al., 2016), so the replenishment of base cations and subsequent increase in pH should result in decreases of Al, Fe, Mn, and other trace metals concentrations (Gitari et al., 2009; Komonweeraket et al., 2015). Changes in Ca and Al concentrations in the soil solution should increase the Ca/Al ratio, reducing fine root impairment. Still, a body of evidence seems to indicate that wood ash does not always elicit responses in soil solution and microorganisms, or at least dosage may play an integral role in the process.

1.4.1 Soil water chemistry

An effective way to analyze the response of soils to a wood ash amendment is to measure analytes in the soil solution. The standard means to capture percolated water is by installing lysimeters before applying wood ash, but it is important to use both tension and zero-tension lysimeters. A zero-tension lysimeter collects the mobile portion of soil water (Makowski et al., 2020), while a tension lysimeter collects water bound within the soil matrix (Lindroos et al., 2018). Collecting soil water from the mineral horizons is essential to ascertain the concentrations of nutrients that can be utilized by tree roots (Smethurst, 2000) or leaching into groundwater, whereas within the organic horizons, this process is primarily led by mycorrhizal fungi exploration and decomposition (Finlay, 2004; Orwin et al., 2011).

The presence of high concentrations of Al and a Ca/Al (or, in some instances, Bc/Al) molar ratio ≤ 1.0 in soil solution can also act as an indicator of soil acidification (Cronan & Grigal, 1995). For example, a long-term study in Switzerland analyzed changes in soil solution chemistry using tension lysimeters over a ten-year period and observed incremental decreases in soil base cations and increases in Al. The long-term trends indicated that soil acidification increased the leaching of Al into the mineral horizons, and Bc/Al ratios in the Ae, B, and C horizons dropped below the critical threshold value (Blaser et al., 2008). Studies on the effect of wood ash amendments can sometimes be inconclusive, and characteristics like soil type, soil moisture, weather, and dosage can play a role. For example, Norström et al. (2012) observed no increase in soil solution pH after applying 3 Mg ha^{-1} of wood ash, whereas Rumpf et al. (2001) observed slight increases in pH from applying 4.8 Mg ha^{-1} but significant decreases at the 10 cm depth. More recently, a study in Ontario using 4 and 8 Mg ha^{-1} of wood ash found that soil solution pH did not significantly increase, while increases in Ca were moderate and short-lived (Deighton et al., 2021). Meanwhile, a short-term study in Norway found that after four years, it was unclear whether 3 Mg ha^{-1} of wood ash had any effect on soil solution pH at a depth of 40 cm, but it did have an effect on Ca, Mg, and K, Co, Ni and Zn concentrations (Clarke et al., 2018). Brunner et al. (2004) observed increases in the leaching of nitrates at a depth of 75 cm but also noted a strong correlation between extractable cations and fine root biomass in the upper horizons (Brunner et al., 2004). The author noted that this indicates fine root control over particular micro (Mn) and macronutrients (Ca, Mg). Other studies that measured leachate water suggest that microbial activity increases following wood ash application. In a study using controlled conditions to determine the effect of wood ash on soil microbial communities, Jokinen et al. (2006) found that pH and DOC affected microbial activity and that microorganisms could also use DOC in soil solution as a C source.

1.4.2 Fine root impact

As previously mentioned, analyzing Ca/Al ratios in soil solution is important due to the displacement of Ca ions by Al in acidic soils (Brunner et al., 2004; Park et al., 2008). The displacement of

Ca limits nutrient uptake and, in turn, decreases the allocation of C to root production; as such, Ca/Al ratios below critical values have been linked to decreased root turnover (Brunner et al., 2004; Coleman et al., 2000; Park et al., 2008). The allocation of C to fine root development is a balance between nutrient acquisition and the energy expended, and effects can be measured by looking at fine root biomass (Eissenstat, 1992). In acidified systems, roots have slower turnover rates, increased biomass and restricted diameters, further limiting nutrient uptake (Burton et al., 2000; Coleman et al., 2000). Research looking at the relationship between N and fine root development also found increases in fine root biomass due to decreased root turnover in the presence of elevated levels of N (Burton et al., 2000). Unlike Al, decreases in root turnover in N-rich soils resulted from the increased allocation of carbohydrates to roots to maintain N uptake at the expense of C sequestration (Burton et al., 2000). At the same time, soil acidification can also decrease the presence of cellulose-degrading enzymes and soil respiration (Li et al., 2018; Meng et al., 2019). Besides root biomass (RB), the health and productivity of fine roots can be measured by their specific root length (SRL) and root length density (RLD). Specific root length is determined by measuring the length of fine roots (m) and dividing by the dry weight (g) (Clemensson-Lindell & Persson, 1993) and determines how much energy (mass) is spent to acquire nutrients (length) (Pérez-Harguindeguy et al., 2016). The root length density is a measurement of root length (cm) in a volume of soil (cm^3) and gives insight into the development of fine roots into lower soil horizons (Dong et al., 2019). Root length density is an important metric as this can indicate increased C allocation as roots penetrate deeper into the soil layers, develop larger root diameters and survive longer (Coleman et al., 2000). A study using 5 and 10 Mg ha^{-1} of limestone found that root biomass, root growth and SRL decreased due to increased N mineralization, but stem growth increased (Clemensson-Lindell & Persson, 1993). They also found that wood ash (2.83 Mg ha^{-1}) was quite soluble, had a short treatment effect, and did not affect above or below-ground growth. Decreased root biomass seems to be a common effect of wood ash application in the short term (Persson & Ahlström, 1994). However, this may not always be the case, as there were no decreases in RB and SRL in a study conducted by

Majdi et al. (2008) using 3.2 Mg ha^{-1} of wood ash. Similar results were seen at the HBEF four years after applying 55 Mg ha^{-1} of wollastonite (Juice et al., 2006).

The only difference between fine root measurement of trees and mycorrhizal fungi is the inclusion of root tips per segment. Measuring root tips is restricted to ECM fungi, whereas arbuscular mycorrhizae (AM) generate hyphae and are generally less common in literature. Ectomycorrhizal root tips are more abundant but not limited to the organic horizons and are a good metric for exploration and nutrient uptake as they act as the primary nutrient source in forest soils (Smith & Read, 2008; Wallander et al., 2004). Studies have shown that applying Ca-based amendments can positively affect the number of ECM root tips in organic and mineral soil horizons. It has been suggested that increased ECM root tips could indicate increased uptake capacity in fine roots (Majdi et al., 2008). Despite fungi being more tolerant to changes in soil pH, they can still be affected by soil acidification, primarily due to the symbiotic relationship and the cost versus benefit of trees allocating C below ground. Nevertheless, they also play a role in alleviating nutrient stress in acidic soils by binding Al and metal to polyphosphates, attaching them to the ECM sheath and mitigating root impedance by Al (Cairney, 2011; Finlay, 1995). While this is beneficial in protecting roots from Al exposure, some forest systems in Ontario are showing signs of P limitations (Casson et al., 2012; Gradowski & Thomas, 2006), which may become more substantial as our climate changes (Cairney, 2011). It is also prudent to mention that climate change and decreases in snow accumulation will increase soil freezing events throughout winter, leading to shorter root lengths and lifespans (Coleman et al., 2000; Reinmann & Templer, 2016; Tierney et al., 2001).

1.4.3 Microbial Response

Prokaryotic (bacteria) 16S and eukaryotic (fungal) internal transcribed spacer (ITS) ribosomal RNA sequencing have become an essential tool for microbiology making it easier to study complex microbes. However, we still do not have a complete picture of these communities (Walters et al., 2016). Much research is now looking at the effects of wood ash on microbial communities, but many of the

microorganisms in soil cannot be easily cultured in laboratories (Gawad et al., 2016). Instead, researchers rely on metrics like relative abundance (RA), diversity and richness to determine the effects of wood ash and changes in pH and the availability of nutrients. Information gaps in the functionality of bacteria and redundancy amongst fungal communities (Dahlberg, 2001) have made it challenging to ascertain most microorganisms' role in the biogeochemical process (Vestergård et al., 2018). There is a smaller information gap regarding fungal communities, and much of their functionality is understood. For example, the fungal group ectomycorrhizal (ECM) facilitates the cycling of soil nutrients by releasing oxalic acid to chemically weather inorganic material (Wallander et al., 2003). They almost immediately colonize the roots of new saplings, improve host nutrient uptake (Dahlberg, 2001), and are important to N cycling (Cruz-Paredes et al., 2019).

Nevertheless, the microbial response to wood ash has been inconsistent, and there are strong indications that pH and dose play a crucial role in determining response. For instance, a study on the response of ECM fungi to dolomite in Sweden by Taylor & Finlay (2003) found that ECM species richness had not changed in the 15 years since the application of 8.75 Mg ha^{-1}, yet species composition in treatment sites was almost entirely different (ca. 80%). In comparison, Cruz-Paredes et al. (2021) found that the diversity and richness of fungal communities change at doses greater than 30 Mg ha^{-1}. Furthermore, they also noted a positive correlation between soil pH and bacterial communities' composition (RA) at doses between 9 to 15 Mg ha^{-1}, but no shifts in diversity or richness. The prevailing theory is that fungal communities are more dominant and tolerant to acidic conditions and less sensitive to changes in pH (Cruz-Paredes et al., 2021) and that bacterial communities have a narrower pH tolerance range (Rousk et al., 2010). Still, both fungal and bacterial communities drive decomposition and nutrient cycling in soils (Smith & Read, 2008) and can manipulate the solubility and mobility of nutrients and metals (Gadd & Sayer, 2014; Pandey & Singh, 2010). Under acidic conditions, acidophilic bacteria (autotrophic) can increase the availability and leaching of metals, especially when S-based compounds are abundant in the soil (Gadd, 2001). Gadd & Sayer (2014) also studied the influence of

fungi on mine waste and found that in acidic conditions (pH 2.4), many species of fungi were able to remove metal contaminants. Changes in microbial communities (or lack of) are caused by differences in pH, and the immediate and long-term changes may be hard to ascertain. In an analysis of AshNet projects, Smenderovac et al. (2022) corroborated other research showing that in the short-term, microbial communities are mainly unresponsive to wood ash (Aronsson & Ekelund, 2004; Noyce et al., 2016). They noted that while alpha (α) diversity changes occurred, those changes were not uniform across sites or dosage and were instead largely site-specific changes. Meanwhile, a study in Finland determined that changes in microbial composition were still apparent 18 years after applying 3 and 9 Mg ha^{-1} of wood ash (Perkiömäki & Fritze, 2002). To better understand the changes in soil fungal and bacterial communities, it may be prudent to continue looking at their short-term response to help determine when shifts in composition do or do not occur.

1.5 Acidic deposition impact in Ontario

Acidic deposition has led to the acidification of soil and the depletion of base cations vital to the health and growth of the forest. Critical load mapping shows that Ontario and Quebec have been hit the hardest, partly due to Canadian Shield soils' naturally low buffering capacity (Ouimet et al., 2006). The depletion of soil nutrients can increase tree susceptibility to disease, insect defoliation (Horsley et al., 2000; Kolb & McCormick, 1993), windfall (Braun et al., 2003), decreased snowpack (Tierney et al., 2001), and lower litter quality (McLaughlin, 2014). Determining the effects of acid deposition in Ontario is possible through studies conducted through the Ontario Forest Biomonitoring Network (OBFN), which encompasses a broad range of forest monitoring plots from the Carolinian forest in the south to the Great Lakes St. Lawrence region in the north (Ontario Ministry of Environment, Conservation and Parks, 2021). Evaluating crown conditions in OBFN plots, Tominaga et al. (2008) determined that historically, sites with the highest acidic deposition (Sudbury and Muskoka) had the greatest rate of tree decline. Higher concentrations of Cd, Cu, Pb and Zn and lower concentrations of Ca were present in the organic horizons amongst these sites (Watmough, 2010). The reductions in Ca in the forest floor corroborate

findings that show that over 20 years, foliar concentrations of Ca had significantly decreased at OBFN sites, despite reductions in S and N deposition (Miller & Watmough, 2009b). Lastly, a study on herbaceous plants in the OBFN plots showed that plant diversity is strongly correlated with soil pH and temperature, and the deposition of S and N has had a significant impact on diversity in sugar maple-dominated forests (McDonough & Watmough, 2015).

The effects of climate change may compound winter dieback events due to the shallower root systems found in acidified soils and the loss of protection from frost damage provided by deeper snowpacks (Berg & Matzner, 1997; Gaul et al., 2008; Meisner et al., 2021). The presence of snow cover on forest soils is crucial as it insulates the soil from freezing and allows for the continued cycling of C and N through the winter months (Groffman et al., 2001). A study on sugar maple and American beech dominated forests in Dorset, Ontario, revealed significant levels of litter decomposition and nitrification (between 23% and 49%) occurring during the winter months under the snowpack (Devito et al., 1999). Studies have also shown increased fine root development in the organic horizons with less snowpack coverage and increased mortality (Gaul et al., 2008). There is also evidence that decreases in litter quality due to base cation depletion can affect soil microorganism survivability during freeze/thaw events (Shimel & Clein, 1996) and changes in the drying/rewetting cycling as a consequence of climate change (Meisner et al., 2021). Cleavitt et al. (2008) hypothesized that these effects may determine the future composition of northern hardwood forests. Research conducted on sugar maple seedling response to wood ash treatments in Bracebridge, Ontario, found significant increases in foliar Ca and stem and root concentrations of Ca, Mg and K in sugar maple (Deighton & Watmough, 2020). The results strongly indicate that the prolonged effects of acid deposition and the resulting acidified soils will potentially decrease the health and survivability of tree species without amendments. These effects may be especially relevant for sugar maples, an economically significant hardwood species that dominants the temperate forests of Ontario (Casson et al., 2012; Davis et al., 2005; Lovett & Mitchell, 2004).

1.6 Sugar maple characteristics

The importance of sugar maple can not be underestimated for its cultural and economic value to Canada. The sugar maple is sacred to the First People (Marquis, 2021), and the leaf adorns our flag, representing our country. Economically, sugar maple contains medicinal properties (Bhatta et al., 2018; Marquis, 2021), and the production of lumber and maple syrup equates to an estimated $515 million annually to the Canadian economy (Agriculture and Agri-food Canada, 2021). It is abundant throughout Ontario and Quebec and is typically the dominant species (Casson et al., 2012; Moore et al., 2008). Beyond its economic and cultural values, sugar maple is a slow-growing hardwood that plays a vital role in the cycling of nutrients and the health of forests and watersheds; it has also been shown to be hardy to climate change (Moreau et al., 2020). For instance, sugar maples help to alleviate drought stress by raising water from deeper soil layers using hydraulic lift and reducing water scarcity in forests (Dawson, 1993). Sugar maple also exhibits control over Ca cycling to help alleviate nutrient stress through increased uptake in the deeper soil horizons (Dijkstra & Smits, 2002). Sugar maple has a high proclivity towards base-rich soils (Watmough & Dillon, 2001) since Ca is integral to cellular stability, seed production, and foliar and stem wood growth (Juice et al., 2006; Lawrence et al., 1995; Long et al., 1997). However, despite its effects on Ca cycling, under acidified conditions and increased base cation leaching, its vigour can be severely diminished (Hallett et al., 2006; Ouimet & Camiré, 1995; Watmough, 2010), and it can become susceptible to extreme weather events and climatic change (Moreau et al., 2020; Nolet & Kneeshaw, 2018). Studies conducted in Ontario have shown decreases in sugar maple growth (Watmough, 2002) and foliar nutrients (Miller & Watmough, 2009a) related to soil pH and nutrient depletion, with recovery from soil acidification being more difficult for soils underlain by Precambrian bedrock (Davis et al., 2005). Due to sugar maples' sensitivity to Ca decline (Bal et al., 2015), applying wood ash rich in Ca might be a viable soil amendment to help remediate forest systems impacted by acidic deposition and replace the lost nutrients. However, there is a need to understand the

potential benefits and drawbacks of using wood ash amendments and the effect on belowground systems.

1.7 Thesis objective

This study focused on determining the short-term (<3 years) effect of wood ash additions on soil biogeochemical properties in central Ontario. It was hypothesized that the addition of wood ash would illicit a range of responses in the short-term, including: (i) more pronounced leaching of base cation nutrients in the soil solution due to nutrient loading at higher ash applications, especially in shallower horizons, (ii) increases in the soil pH, base cations, and metal concentrations especially in the upper soil horizons three years post ash application, (iii) decreases in the relative abundance of prokaryote and eukaryote communities in soil horizons with increasing ash dosage, (iv) increases in sugar maple fine roots size and length, with decreases in mycorrhizal infection and hyphae production as a result of improved nutrient availability, and lastly, (v) increases in foliar nutrient concentrations in sugar maple three years post application as a result of increases in soil nutrient availability.

2. Methods

2.1 Study Area

Porridge Lake is situated within the Muskoka-Haliburton region of central Ontario, ecoregion 5E-9, approximately 250 km northeast of Toronto, near Dorset, Ontario (Figure 2.1). The Porridge Lake watershed is approximately 1.54 km^2 and is predominantly mixed hardwood forest, of which approximately 75% of the basal area is sugar maple and American beech (Ontario Ministry of Natural Resources and Forestry, 2022). The site is underlain by Precambrian Shield bedrock, comprising granitized biotite and hornblende gneiss, and the soil is characterized as a mixture of sandy loam podzols and brunisols (Canada Soil Classification Working Group, 1998). The ash treatment site slopes eastwards, down towards Porridge Lake, and has an approximate elevation of 452 m above sea level (Ontario Ministry of Natural Resources and Forestry, 2022). The average annual temperature is 5°C, ranging from -10.6°C in January to 17.7°C in August, with an average precipitation rate of 1058 mm per year (1981-2010, Environment Canada, 2022). In 2015, the site was selectively harvested, with approximately one-third of the tree basal area removed to help maintain high-value hardwoods and combat beech bark disease (Ontario Ministry of Natural Resources and Forestry, 2019). The monthly average temperature and precipitation during the soil water sampling periods from the closest weather station (Muskoka) are included in Table 2.1 (Environment and Climate Change Canada, 2023).

Table 2.1 | Average temperature and total precipitation during the collection of soil water during ice-free months (April-November) at Porridge Lake, Ontario.

		April	May	June	July	August	September	October	November
2019	Temp (°C)	3.4	9.6	15.0	20.6	17.5	13.9	7.8	-2.6
	Precip (mm)	129.5	101.0	86.9	20.7	84.4	58.8	151.5	87.8
2020	Temp (°C)	3.2	10.6	17.3	21.7	18.4	13.1	5.7*	3.9
	Precip (mm)	54.5	97.0	143.9	108.8	77.5	103.8	114.2*	-
2021	Temp (°C)	6.3	10.9*	18.6	18.7	21.0	14.1	11.7	0.6
	Precip (mm)	65.2	18.2	83.9	179.6	84.9	147.2	54.0	99.0
30-year Normals	Temp (°C)	4.8	11.4	16.2	18.7	17.8	13.4	7.1	0.8
	Precip (mm)	76.6	102.4	85.5	93.7	82.2	111.9	105.2	116.2

Months with incomplete data noted by *. Months lacking data noted by -.

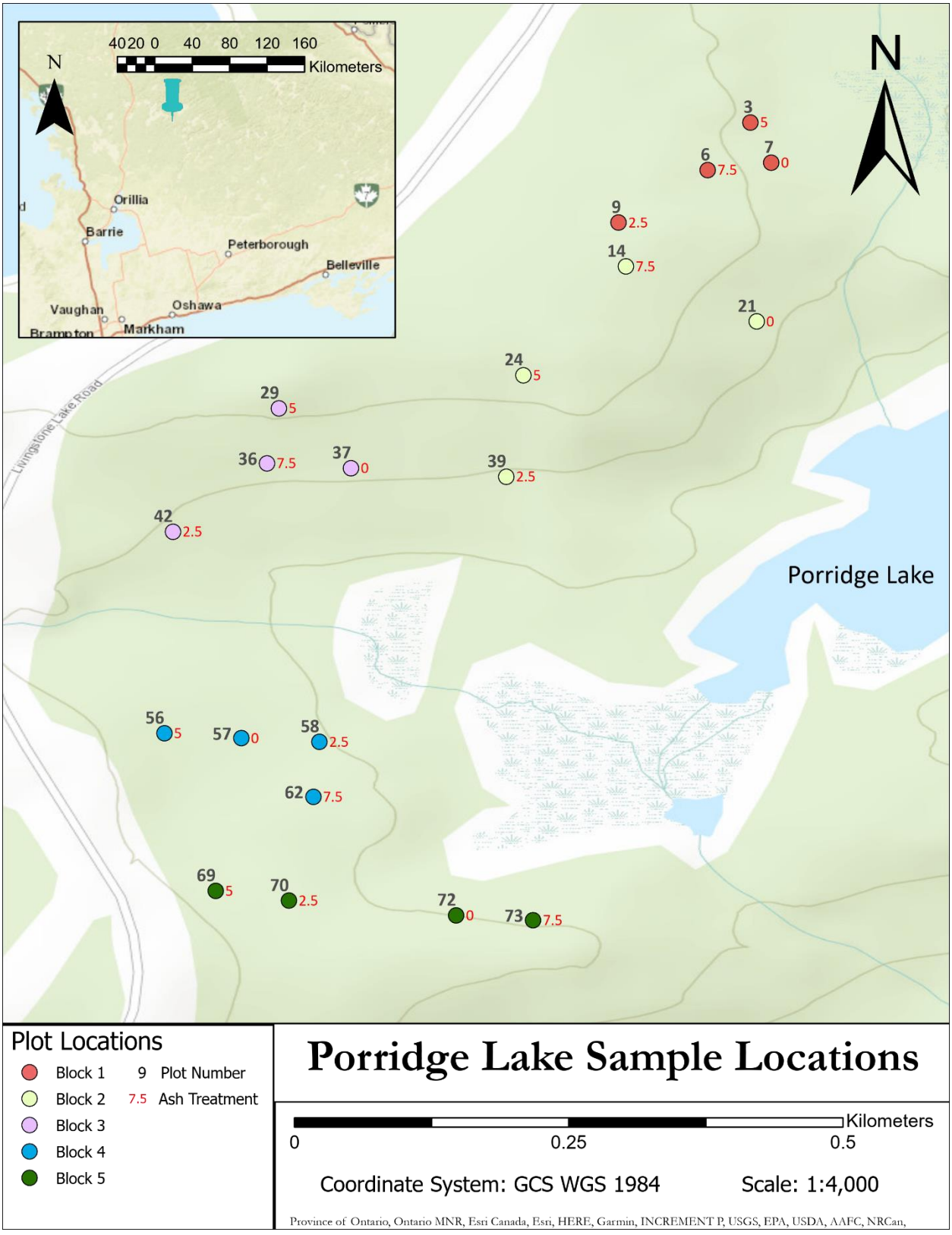

Figure 2.1 | Porridge Lake (45°19N, 78°51W), Ontario sampling locations, including plot numbers and treatments.

2.2 Plot Design

The Porridge Lake wood ash trial was established in 2019 in cooperation with Laurentian University, the Ontario Ministry of Natural Resources and Forestry (OMNRF) and Trent University as part of an AshNet trial. The study design consisted of five blocks with four replicate plots. Each plot is 625 m^2 (0.0652 ha), with one plot in each block acting as a control and three plots receiving a one-time treatment of 2.5, 5.0 or 7.5 Mg ha^{-1} of oven-dried loose wood ash in the summer of 2019 (Figure 2.1). The wood ash was a mixture of dry hardwood and softwood, fly and bottom wood ash, dried via kiln boilers, supplied by the Murray Brothers Lumber Company in Madawaska and was hand distributed over ten days, between July 9th to 16th, 2019. Treatment rates were based on the equivalent application of lime to increase soil pH by 0.5 (P. Hazlett, personal communication, April 20, 2020). The average temperature during the application period was 22.2°C, with minor rain events on July 11th (5.4 mm), 13th (2.1 mm) and 16th (8 mm); 30-year weather trends indicate this was a typical year (Environment Canada, 2022). Before application, the mixed wood ash (hereafter called wood ash) was tested to ensure it met the classification of NASM below the CM2 non-aqueous regulation (Table 2.2) for safe application to forest soil in Ontario, following limits set by Ontario Regulation 267/03 of the Nutrient Management Act of 2002 (Government of Ontario, 2002). The mean concentrations of all regulated metals were below CM1 standards, safe for application, having high alkalinity, and rich in Ca and K (Table 2.2).

Table 2.2| Nutrient and metal concentrations of Murray Brothers Lumber Company wood ash – Non-agricultural source material (NASM) Category 3, applied to the Porridge Lake AshNet trial plots, analyzed for the content of regulated metals (CM). CM1: Wood ash with a CM that falls below the limits for CM1 non-aqueous NASM. CM2: Wood ash with a CM that falls below the limits for CM2 but above CM1 limits for non-aqueous NASM.

Nutrient and Metal concentrations of Murray Bros mixed ash		NASM Limits CM1-CM2 (mg kg^{-1})
pH	13.2	n/a
Ca (%)	26.4	n/a
Mg (%)	0.5	n/a
P (%)	0.7	n/a
K (%)	6.8	n/a
Al (%)	1.1	n/a
Fe (%)	0.7	n/a
Mn (%)	1.0	n/a
Na (%)	0.4	n/a
As (mg kg^{-1})	<1.5	13 - 170
Cd (mg kg^{-1})	2.7	3 - 34
Co (mg kg^{-1})	5.2	34 - 340
Cr (mg kg^{-1})	11.1	210 - 2800
Cu (mg kg^{-1})	78	100 - 1700
Pb (mg kg^{-1})	4.5	150 - 1100
Mo (mg kg^{-1})	2.2	5 - 94
Ni (mg kg^{-1})	17.1	62 - 420
Se (mg kg^{-1})	0.1	2 - 34
Zn (mg kg^{-1})	222	500-4200

*The concentration of nitrogen and sulphur in the wood ash was not measured prior to application. The number of replicates and S.D. are currently unavailable. Analysis conducted externally.

2.3 Field Methods

2.3.1 Soil Water

In May 2019, three sets of tension lysimeters were installed at 30 and 60-cm depths, along with a zero-tension plate lysimeter (Figure 2.2) placed just below the LFH layer to collect soil water passing through the forest floor (n = 7 lysimeters per plot). The tension lysimeters were constructed with 2 bar standard ceramic cups with a 1.3 µm pore size (Model B02M2, Soil Moisture Equipment Corp., Santa

Barbara, California). Before installation, the lysimeters were acid washed with 1 M HCl and repeatedly rinsed with deionized water until the solution's pH, conductivity, base cation, and trace metal concentrations passed through the lysimeters equal to the values of the deionized water. After installation, the lysimeters were left to equilibrate for three months before sampling began. Soil water sampling began in August 2019. After that, sampling occurred at approximately monthly intervals during the ice-free seasons (April-November) until November 2021. During each sampling period, within each plot, all lysimeters at the same sampling depth were evacuated, composited, and stored in 500 ml pre-rinsed polyethylene bottles. The tension lysimeters were then primed to 50 kPa between sampling periods. The zero-tension lysimeters water was collected into two 20 L containers contained within an enclosure (Figure 2.3). When sampling the zero-tension lysimeters, the total volume of the two containers was recorded, and up to 500 mL of the consolidated water was collected in pre-rinsed polyethylene bottles. There were four sampling occasions in 2019 (August-November), five in 2020 due to COVID restrictions (June-October), and seven in 2021 (May-November). All soil water samples were transported to Trent University and temporarily stored at -10°C before being shipped on ice to the Great Lakes Forestry Centre in Sault Ste. Marie for analysis (see section 3.1 Soil Water).

2.3.2 Soil and Foliage Sampling

The Great Lakes Forestry Centre conducted pre-treatment soil sampling and analysis in Fall 2017. Their analysis showed that prior to the application of wood ash, soil chemistry was similar across treated and untreated plots (analyzed by one-way ANOVA) and that, in general, soils were acidic and low in exchangeable base cations and metals (Tables 2.3-2.7). As expected, soil nutrient concentrations and soil C/N ratio generally decreased with depth, while soil pH was lowest in the Ah horizon and increased with depth in all plots (Table 2.3-2.7).

For post-treatment analysis, litter, FH, and soil samples were collected in July 2021 at three randomly selected locations within each plot. Litter and FH samples were collected by cutting out a 4 x 4 cm section of each horizon, and then the mineral soil samples were collected from the Ah, Bm and C

horizons using a bulk density hammer (5 x 5 cm). Litter and FH samples were stored in paper bags, whereas the soil was separated by horizon, sealed in polyurethane bags, and shipped on ice to Trent University.

Within each plot, foliage samples were collected from a randomly selected mature sugar maple (DBH > 10 cm) using a pulley pruner at the mid-crown, on the sun-facing side of the canopy. All foliage samples were stored in paper bags for transport and at 4°C at Trent University before analysis. In addition, from the same randomly selected sugar maple, the L layer was removed, and the FH and Ah horizon were collected using a soil core (5 x 5 cm), 20 cm from the sampled sugar maple for mycorrhizal and sugar maple fine roots. At three randomly selected locations within each plot, replicate samples were collected to a depth of 5 cm, each stored in a polyurethane bag at -10°C.

Figure 2.2 | Zero-tension lysimeter water catchment just below the LFH horizon at Porridge Lake, Ontario.

Figure 2.3 | Containment box for drainage and capturing soil water percolate from the LFH horizon at Porridge Lake, Ontario.

Table 2.3 | Pre-treatment (2017) litter horizon characteristics, extractable cations (Ca, Mg, K, Na) and total elemental concentrations (means ± S.E.) from Porridge Lake, Ontario.

Pre-treatment	*n*	pH ($CaCl_2$)	Ca	Mg	K	Na	Al	Fe	Mn	C	N	C/N
			---------- ($g \cdot kg^{-1}$)----------							------%-----		
0	4	4.06	4.54	0.63	1.40	0.02	0.22	0.01	0.01	47.9	1.18	46.7
		(0.2)	(0.6)	(0.1)	(0.4)	(0.002)	(0.1)	(0.01)	(0.00)	(1.6)	(0.2)	(1.6)
2.5	5	3.95	4.10	0.60	1.10	0.02	0.17	0.00	0.01	48.3	0.98	47.4
		(0.3)	(0.5)	(0.1)	(0.3)	(0.002)	(0.04)	(0.01)	(0.01)	(0.6)	(0.2)	(0.5)
5.0	5	3.98	4.62	0.68	1.50	0.02	0.14	0.00	0.01	48.8	1.16	47.6
		(0.2)	(0.5)	(0.09)	(0.3)	(0.004)	(0.1)	(0.01)	(0.01)	(1)	(0.2)	(1.4)
7.5	4	3.83	4.40	0.63	1.22	0.02	0.02	0.01	0.01	48.5	1.02	47.5
		(0.2)	(0.3)	(0.0)	(0.1)	(0.001)	(0.1)	(0.01)	(0.00)	(0.7)	(0.1)	(0.6)

Table 2.4 | Pre-treatment (2017) FH horizon characteristics, extractable cations (Ca, Mg, K, Na) and total elemental concentrations (means ± S.E.) from Porridge Lake, Ontario.

Pre-treatment	*n*	pH ($CaCl_2$)	Ca	Mg	K	Na	Al	Fe	Mn	C	N	C/N
			---------- ($g \cdot kg^{-1}$)----------							------%-----		
0	4	4.40	4.89	0.46	0.71	0.02	0.22	0.01	0.01	40.1	1.51	38.6
		(0.1)	(1.3)	(0.1)	(0.2)	(0.01)	(0.1)	(0.01)	(0.00)	(9.9)	(0.4)	(9.5)
2.5	5	4.14	4.95	0.48	0.60	0.02	0.17	0.00	0.01	45.1	1.58	43.5
		(0.3)	(0.6)	(0.1)	(0.1)	(0.01)	(0.04)	(0.01)	(0.01)	(1.7)	(0.3)	(1.7)
5.0	5	4.23	5.03	0.50	0.81	0.02	0.14	0.00	0.01	44.2	1.79	42.4
		(0.2)	(0.5)	(0.04)	(0.1)	(0.01)	(0.1)	(0.01)	(0.01)	(3.5)	(0.3)	(3.3)
7.5	4	4.21	4.37	0.44	0.71	0.02	0.02	0.01	0.01	44.1	1.49	42.6
		(0.3)	(0.4)	(0.1)	(0.2)	(0.004)	(0.1)	(0.01)	(0.00)	(3.0)	(0.5)	(2.7)

Table 2.5 | Pre-treatment (2017) Ah soil horizon characteristics, extractable cations (Ca, Mg, K, Na) and total elemental concentrations (means ± S.E.) from Porridge Lake, Ontario.

Pre-treatment	n	pH ($CaCl_2$)	Ca	Mg	K	Na	Al	Fe	Mn	C	N	C/N	Cu	Zn
			($g \cdot kg^{-1}$)							%-			($mg \cdot kg^{-1}$)	
0	5	3.69	0.78	0.01	0.12	0.01	0.20	0.02	0.08	6.94	0.44	15.7	0.03	8.16
		(0.2)	(0.5)	(0.06)	(0.03)	(0.004)	(0.1)	(0.0)	(0.1)	(1.3)	(0.04)	(1.7)	(0.1)	(3.5)
2.5	5	3.69	0.73	0.07	0.10	0.01	0.18	0.01	0.09	7.34	0.46	16.1	0.02	5.42
		(0.4)	(0.5)	(0.03)	(0.04)	(0.004)	(0.1)	(0.0)	(0.1)	(1.2)	(0.1)	(3.0)	(0.0)	(2.4)
5.0	5	3.91	0.67	0.07	0.12	0.01	0.21	0.01	0.12	7.03	0.47	14.9	0.02	9.47
		(0.2)	(0.3)	(0.03)	(0.05)	(0.004)	(0.1)	(0.0)	(0.1)	(1)	(0.1)	(2.1)	(0.1)	(9.6)
7.5	5	3.76	0.70	0.08	0.12	0.01	0.24	0.02	0.08	8.43	0.54	15.4	0.05	4.93
		(0.3)	(0.1)	(0.02)	(0.03)	(0.002)	(0.1)	(0.0)	(0.1)	(2.1)	(0.1)	(2.0)	(0.1)	(1.2)

Table 2.6 |Pre-treatment (2017) Bm soil horizon characteristics, extractable cations (Ca, Mg, K, Na) and total elemental concentrations (means ± S.E.) from Porridge Lake, Ontario.

Pre-treatment	n	pH ($CaCl_2$)	Ca	Mg	K	Na	Al	Fe	Mn	C	N	C/N	Cu	Zn
			($g \cdot kg^{-1}$)							%-			($mg \cdot kg^{-1}$)	
0	5	3.93	0.28	0.04	0.05	0.01	0.28	0.02	0.02	4.63	0.29	16.3	0.01	3.92
		(0.2)	(0.2)	(0.04)	(0.01)	(0.002)	(0.1)	(0.03)	(0.01)	(1.2)	(0.1)	(1.8)	(0.01)	(2.0)
2.5	5	4.01	0.24	0.03	0.05	0.01	0.26	0.01	0.04	4.90	0.31	16.1	0.01	4.09
		(0.1)	(0.1)	(0.01)	(0.02)	(0.002)	(0.1)	(0.01)	(0.03)	(0.9)	(0.1)	(3.5)	(0.03)	(1.3)
5.0	5	4.18	0.22	0.03	0.05	0.004	0.22	0.01	0.03	4.92	0.32	15.7	0.05	4.09
		(0.2)	(0.1)	(0.01)	(0.01)	(0.002)	(0.1)	(0.01)	(0.03)	(0.9)	(0.1)	(2.2)	(0.1)	(3.6)
7.5	5	4.09	0.25	0.03	0.05	0.01	0.24	0.01	0.02	4.53	0.29	16.0	0.02	3.54
		(0.1)	(0.1)	(0.01)	(0.01)	(0.003)	(0.1)	(0.01)	(0.03)	(0.7)	(0.1)	(2.8)	(0.03)	(1.7)

Table 2.7 | Pre-treatment (2017) C soil horizon characteristics, extractable cations (Ca, Mg, K, Na) and total elemental concentrations (means ± S.E.) from Porridge Lake, Ontario.

Pre-treatment	n	pH ($CaCl_2$)	Ca	Mg	K	Na	Al	Fe	Mn	C	N	C/N	Cu	Zn
			($g \cdot kg^{-1}$)							%			($mg \cdot kg^{-1}$)	
0	5	4.24	0.23	0.03	0.03	0.004	0.22	0.01	0.01	4.48	0.25	18.1	0.01	2.82
		(0.1)	(0.3)	(0.04)	(0.01)	(0.001)	(0.1)	(0.01)	(0.00)	(1.2)	(0.03)	(2.9)	(0.02)	(1.5)
2.5	5	4.25	0.12	0.01	0.03	0.01	0.17	0.00	0.01	4.36	0.25	17.4	0.03	2.46
		(0.1)	(0.03)	(0.00)	(0.01)	(0.002)	(0.04)	(0.01)	(0.01)	(0.7)	(0.04)	(2.5)	(0.04)	(1.5)
5.0	5	4.37	0.10	0.01	0.02	0.004	0.14	0.00	0.01	3.86	0.23	16.6	0.07	1.56
		(0.1)	(0.1)	(0.01)	(0.01)	(0.002)	(0.1)	(0.01)	(0.01)	(1.2)	(0.1)	(2.5)	(0.2)	(0.4)
7.5	5	4.29	0.13	0.01	0.03	0.005	0.02	0.01	0.01	4.14	0.24	16.9	0.03	2.25
		(0.1)	(0.1)	(0.01)	(0.01)	(0.004)	(0.1)	(0.01)	(0.00)	(1.0)	(0.04)	(2.9)	(0.05)	(1.5)

3. Laboratory analysis

3.1 Soil Water

Lysimeter soil water samples were analyzed at the Great Lakes Forestry Centre in Sault Ste. Marie, Ontario, for conductivity, alkalinity, pH, nutrients, and metals. Conductivity, alkalinity, and pH were measured using a Man-Tech PC-Titrate (Mantech, Guelph, ON). Nitrate (NO_3), ammonium (NH_4), total nitrogen (TN) and total organic carbon (TOC) were measured with a Technicon Autoanalyzer II (SEAL Analytical Inc., Mequon, WI) by the ascorbic acid, cadmium reduction, sodium nitroprusside, autoclave digestion and potassium persulfate methods, respectively. Sulphate and chloride (Cl) concentrations were determined by ion chromatography. Base cations and metals were analyzed using an Agilent 7700X Inductively Coupled Plasma Mass Spectrometer (ICP-MS) (Agilent Technologies, Santa Clara, CA). Ion balances were conducted on all water samples for quality assurance purposes, with a difference of ± 20% considered acceptable.

3.2 Litter, FH, Soil and Foliage

All soil (L, FH, Ah, Bm, C) and foliage samples collected in the field were dried in a Grieve® industrial oven at 110°C for 24 hours. Dried soil samples were sieved to <2 mm, and the L, FH and foliage samples were ground using a Wiley Mill. The samples (L, FH, Ah, Bm, C, foliage) were consolidated by type, plot, and horizon prior to further analysis. Soil pH was measured in a 0.01M $CaCl_2$ slurry, with a 1:5 ratio for mineral soil and 1:10 for the L and FH layers. Each slurry was stirred frequently for 10 minutes and left to rest for 45 minutes before pH measurements were conducted using an OAKTON pH 510 series multimeter (Oakton Instruments, Vernon Hills, Il) under agitation, with the probe calibrated every 15 samples. Loss-on-ignition (LOI) was used to determine the percent of organic matter (OM) by placing five grams of oven-dried sample in a Fisher Isotemp® oven for 16h at 450°C.

Exchangeable cations (Ca, Mg, K, Al, Na, Fe, Mn) were determined in a 1:10 solution with 1M NH_4Cl for inorganic horizons (Ah, Bm, C) and 1:50 for organic soils (L, FH) and foliage, after shaking for 2h. Samples were then filtered, diluted, and acidified using concentration-grade nitric acid (Aristar® Plus, CAS 7697-37-2) before analysis by ICP-OES. The same samples were used to determine the cation exchange

capacity (NH_4^+) through extraction using 2M NaCl with a soil solution of 1:10, diluted with BPURE (1:100) and analyzed by a Bran and Luebbe Autoanalyzer 3.

Total base cation (Ca, K, Mg, Na), P and metal concentrations (Al, As, Cd, Ci, Cr, Cu, Fe, Mn, Ni, Pb, strontium (Sr), Zn) in foliage, organic and inorganic horizons (metals only) (L, FH, Ah, Bm, C) were determined by cold digesting 0.2 g of the sample treated with 2.5 mL of 100% (*v/v*) nitric acid at room temperature for 8h before heating at 110°C and digested for another 8h on a block digester. Samples were triple rinsed and filtered through P8 Fast Flow filter paper, adjusted to a final volume of 25 mL with B-Pure water (ThermoFisher Scientific, 2022a), and analyzed on a Perkin Elmer® ICP-OES. Reference samples for QA/QC were digested along with samples, using 0.15 g of EnviroMAT SS-2 Soil Standard for soil and 0.075 g of National Institute of Standards and Technology (NIST) SRM-1515 apple leaf for L, FH, and foliage. Soil standards were within 15% for Ca, Mg, K, Na, Al, Fe, Cr, Cu, Mn, Ni, Pb, Se, and Zn, and NIST standards were within 15% for Ca, Mg, K, Na, Al, Fe, Sr, Zn.

The carbon (C), nitrogen (N) and sulphur (S) quantities for the foliage, organic and inorganic horizons (L, FH, Ah, Bm, C) were determined using a CNS combustion analyzer (Elementar Vario MACRO cube CNS) (see Appendix F), with precision confirmed using soil standards (EnviroMAT SS-2) and apple leaf (NIST-1515-SRM).

3.3 Fine Roots and Mycorrhizae

Soil samples (L, FH, Ah) collected for fine root analysis were weighed and air-dried for seven days, then the soil and roots were separated using a 2 mm sieve. Fine roots were sorted by hand and analyzed using morphological features; for example, mycorrhizal hyphae can have round woolly tips, and nonmycorrhizal roots have either root hairs or pointed tips (Agerer et al., 2012; Majdi & Viebke, 2004). When root type was indeterminate, the roots were analyzed under a microscope (40x) to determine the presence of woolly tips and/or a mantle sheath; those with a damaged sheath were ignored, and roots lacking mycorrhizal indications were characterized as tree roots. Root diameter was measured using a vernier calliper, and the length of fine roots was determined using the line intersect method developed by Tennant (1975). Roots were placed on a 3 x 3 cm grid, and the total number of intersection points was counted and multiplied by 1.5714 to estimate the total root length (Tennant, 1975). Mycorrhizal roots

collected from each plot were placed in a beaker of deionized water and swirled. Root segments were chosen randomly until one meter was selected, and all root tips were counted under a dissecting microscope (40x) (Goodman & Trofymow, 1998). Afterwards, the root segments were dried in a Grieve® industrial oven at 110°C for 24 hours and weighed. Root (RB, $g \cdot m^{-2}$), root length density (RLD, $cm \cdot cm^{-3}$ soil), specific root length (SRL, $m \cdot g^{-1}$ DM), average length (cm) and average diameter (mm) were determined for all fine roots by soil layer and treatment, including root tips per segment (RT) for mycorrhizal roots. Total root nutrients were measured using 0.2 g of each sample per type (tree, mycorrhizal) for each plot and were acid digested and analyzed as described above.

3.4 Microbial Community Analysis

Approximately 0.25 g of soil (L, FH, Ah) was used for the prokaryote and eukaryote DNA extraction at the University of Guelph, following the protocols of the DNeasy Powersoil Pro Kits (Qiagen, 2013). All DNA extractions were performed in triplicate prior to the samples being bulked. In total, 61 samples were processed, 20 for the litter, 20 for humus, 20 for soil and one blank for contamination. All samples were quality-checked using a NanoDrop 8000 Spectrophotometer microanalyzer (Desjardins & Conklin, 2010), and DNA quantity was double-checked using a Qubit Fluorometer. Samples were stored at -80°C prior to being diluted to the specifications of and shipped to Metagenom Bio Life Sciences lab (Waterloo, ON, Canada) for prokaryote 16S ribosomal DNA (16S) and the eukaryote internal transcribed spacer (ITS) sequencing on an Illumina MiSeq Machine. Further details are provided in the Supplementary Information.

3.5 Statistical Analysis

To test the hypothesis that metal and nutrient leaching would increase post-wood ash application, an analysis of variance (ANOVA) was used to compare changes in soil water concentrations. Analysis was conducted yearly, with the treatment sites being compared to the control sites and against one another by soil horizon at depths that showed a treatment effect. Before analysis, the Lilliefors test for Normality (Gross & Ligges, 2015) was used to determine if the data followed a normal probability distribution. Non-parametric data were tested using the Kruskal-Wallis and Mann-Whitney U test using the Bonferroni adjustment. Normal data were analyzed by one-way ANOVA with blocking and a Tukey honestly significant

difference (HSD) test for pairwise comparison. All data were imported into R (R Core Team, 2022) for analysis.

A one-way ANOVA was used to test the hypothesis that wood ash would alter soil chemistry primarily in the upper organic horizons (L, FH, Ah, Bm, C). While a *p*-value of <0.05 was preferred, a *p-value* of <0.1 was also accepted due to the low replication level (n=5). Analysis of fine root data, including root length, diameter, SRL, RLD, RB and RT (mycorrhizal only), was conducted using a one-way ANOVA followed by a pairwise comparison of treatments (Tukey HSD). A *p*-value of <0.1 was considered significant and shown by letter display. Fine root elemental concentrations were analyzed using Levene's test to determine normality between sites, followed by a multivariate analysis of variance (MANOVA) to test whether fine root analyte concentrations differed by treatment and horizon.

Demultiplexed sequences were processed using the DADA2 pipeline (v1.8) (Callahan et al., 2016) in QIIME 2 (v2021.11) (Bolyen et al., 2019; Caporaso et al., 2010). Reads were truncated at decreasing quality and quantity filtered using default parameters. Taxonomy was assigned using a naïve Bayesian classifier in QIIME 2 with scikit-learn (v1.0.2). Taxonomy was assigned using SILVA (release 138) for 16S reads, and the Unite Database (Version 8) for ITS reads. The amplicon sequence variant (ASV) tables were imported into R and merged with soil metadata using phyloseq in R (McMurdie & Holmes, 2013). The sequencing had a total of 1,098,735 16S reads, and 2,736,946 ITS reads. All unassigned taxa at the kingdom level and organelles (mitochondria and chloroplasts) were trimmed from the phyloseq following rarefaction analysis through Vegan (Oksanen et al., 2013). A total of five unassigned taxa at the Kingdom level and 47 organelles at the Order and Family level were trimmed, leaving 8792 bacterial and 34 archaea taxa in the 16S group. There were no unassigned taxa at the Kingdom level for the ITS group, resulting in 5473 fungi taxa. Alpha diversity analysis included the Shannon-Wiener index (H), Simpson diversity (D), Chao1 and Pielou using the phyloseq package. Prokaryote and Eukaryote samples were subset by soil layer and tested using permutational multivariate analysis of variance (PERMANOVA) to determine if there was a significant difference in the microbial and fungal communities. Packages used for analysis include phyloseq (McMurdie & Holmes, 2013), genefilter (Gentleman et al., 2022), BIOM (D. McDonald et al., 2012), and vegan (Oksanen

et al., 2013). Functional guild for Eukaryotes was determined using FUNguild (Nguyen et al., 2016) and an ANOVA to determine significant differences between horizons.

4. Results

4.1 Soil Water

The addition of wood ash resulted in significant changes in the chemistry of soil leachate, but the level of response varied by analyte and differences from control plots were most pronounced in water draining the LFH horizon. More specifically, the addition of wood ash in 2019 resulted in a significant increase in soil percolate pH draining the LFH horizon that persisted over the three-year study (Figure 4.1). Within the first year of wood ash application, the pH of the soil water increased to 6.8, 6.8 and 7.0 in the 2.5, 5.0 and 7.5 $Mg \cdot ha^{-1}$ treatment plots, respectively, compared with 5.6 for control plots. By the second year, differences in pH in the LFH were more pronounced along the treatment gradient (Figure 4.1). Differences in soil percolate pH were also seen at 30 cm depth (tension lysimeter), with the strongest response occurring in the 7.5 $Mg \cdot ha^{-1}$ treatment plots (Figure 4.1). By 2021, the mean pH value of soil water in control plots was 5.4, whereas the pH values for the 2.5, 5.0 and 7.5 $Mg \cdot ha^{-1}$ treatment plots were 6.5, 6.9 and 7.2, respectively (Figure 4.1). The pH of soil water at 60 cm depth did not show any response to ash treatment throughout the study (Figure 4.1).

Similar to pH, Ca concentrations in soil water draining the LFH horizon increased following ash application, but Ca concentrations were very variable and significant increases relative to control plots were only observed in 2020 and 2021 (Figure 4.2). Calcium concentrations in the LFH water of control plots averaged 7.05 $mg \cdot L^{-1}$ in 2019 but fell to values around 5.62 $mg \cdot L^{-1}$ in 2020 and 3.19 $mg \cdot L^{-1}$ in 2021 (Figure 4.2). In contrast, by 2021, the mean Ca concentrations in the treated plots were 9.26, 11.8 and 16.6 $mg \cdot L^{-1}$ in the 2.5, 5.0 and 7.5 $Mg \cdot ha^{-1}$ amendments, respectively (Figure 4.2). Soil water Ca concentrations were notably lower at the 30 and 60 cm depths compared with concentrations in the LFH and were only significantly different in the 2.5 $Mg \cdot ha^{-1}$ treatment plots at the 30 cm depth in 2021 compared with all other treatments but not control, indicating a lack of treatment effect in the lower horizons (Figure 4.2).

Patterns in K and Na in soil water suggested these base cations were more mobile than Ca and Mg as soil water concentrations increased in the 30 cm and, in the case of Na, in the 60 cm horizon in the second and third year of the study, whereas Ca showed no treatment response and Mg decreased in the 30 cm and 60 cm horizons in the third year (Figure 4.3, Appendix A). For Mg, a treatment response was only

apparent in the LFH at the 7.5 Mg·ha^{-1} plots in 2019, whereas by 2020, there were significant increases in Mg in the LFH horizon across all treatments (Figure 4.3). The strongest response for Mg was measured in 2021, when mean Mg concentrations increased to 0.76, 0.93 and 1.20 mg·L^{-1} in the 2.5, 5.0 and 7.5 Mg·ha^{-1}, respectively, while the control plots had a mean concentration of 0.43 mg·L^{-1} (Figure 4.3). Potassium in soil percolate was elevated (not significant) in the treatment plots in LFH in the first two years, whereas significant increases were measured in 2021 for the 7.5 Mg·ha^{-1} treatment plots (Figure 4.3). The majority of the response from K occurred at 30 cm depth in 2020 for the 5.0 and 7.5 Mg·ha^{-1} treatments, but only the 5.0 Mg·ha^{-1} treatment plots showed a significant response in 2021 (Appendix A). There was a substantial and significant increase in Na in 2019 in the LFH, with the 7.5 Mg·ha^{-1} plots having a mean concentration of 4.59 mg·L^{-1} compared with 0.42 mg·L^{-1} in the control plots (Figure 4.3). After the initial increase, Na concentrations began to trend downwards in 2020, and significant responses were only seen in the 2.5 and 7.5 Mg·ha^{-1} plots and again in 2021, the strongest response was seen in the 7.5 Mg·ha^{-1} treatment plots (Figure 4.3). Sodium concentrations were significantly higher in the 30 and 60 cm horizons in 2020 and 2021, with the greatest response in the highest treatment plots (Appendix A).

Concentrations of DOC, NO_3 and SO_4 in the LFH horizon were highest in 2019 immediately after lysimeter installation and did not respond significantly to ash application (Figure 4.4). In 2020, the leachate of the anions tended to be lower in the ash-treated plots, especially in the 2.5 and 7.5 Mg·ha^{-1} plots (Figure 4.4). By 2021, DOC concentrations were significantly lower in all treatment plots compared with the control, while SO_4 concentrations were only significantly lower in the 2.5 and 7.5 Mg·ha^{-1} treated plots (Figure 4.4). Nitrate concentrations only responded in the LFH horizon in 2020, with concentrations significantly lower in the treatment plots (Figure 4.4). There was no response to ash treatment in soil water NO_3 concentrations at lower soil horizons (Appendix B). The only response in DOC concentration was measured at 30 cm and 60 cm in 2019, whereas concentrations in the control plots were significantly greater than the 7.5 Mg·ha^{-1} treatment at both depths. Sulphate concentration at 30 cm depth in the highest treatment regime (7.5 Mg·ha^{-1}) was significantly higher than the control plots in 2020 and 2021 (Appendix B). Concentrations of Cl remained unresponsive throughout the wood ash trial for all depths and treatments (Appendix B).

Concentrations of Al, Fe, Mn, and Zn generally declined in the LFH horizon in response to ash application, with all four showing a significant response in 2021 (Figure 4.5). The only metal to show a significant response to wood ash in the LFH horizon in 2019 was Mn, where concentrations in the 2.5 $Mg \cdot ha^{-1}$ plots were three times lower than in control plots (Figure 4.5). The treatment effect in the LFH horizon was more apparent in 2020 and 2021, with Mn concentrations in water draining the LFH layer in all ash treatments being significantly lower than in control plots. In fact, by the last year of the study, Mn concentrations in the control plots were 7 to 15 times greater than the treatment plots (Figure 4.5). Furthermore, Al showed the strongest decline in concentration in 2019 compared to control plots, where concentrations in the 30 cm horizon were 4 to 8 times lower than control plots in the 7.5 and 2.5 $Mg \cdot ha^{-1}$ treatments, respectively (Appendix C). While concentrations of Fe and Zn in LFH soil water were lower in treated plots in 2021 (Figure 4.5), there were no discernable treatment effects in the 30 and 60 cm horizons for both metals (Appendix C).

Concentrations of As, Cd, Co and Mo in solution showed a small but significant increase in response to wood ash addition, but in general, responses were most apparent in mineral horizons in 2020 and 2021 (Appendix D-E) and only As showed a significant increase in water draining the LFH horizon in ash-treated plots by 2021 (Figure 4.6). The remaining metals analyzed (Cr, Cu, Ni, Pb, Se) showed no significant response to wood ash treatment in any horizon (Figure 4.7, Appendix D-E). Overall, changes in metal concentrations were modest (Table 2.1).

Decreases in soil water Al concentrations in 2019 resulted in large increases in soil water Ca/Al ratios in the LFH horizon and a more modest increase at 30 cm depth (Figure 4.8). Soil water Ca/Al ratios in the treatment plots were 4 to 7 times greater than control plots in the LFH throughout the study. At 30 cm depth, the increase in Ca/Al was relatively modest (2x), and significant increases were only observed in 2020 and 2021 in the higher ash treatments (Figure 4.8). Soil leachate Ca/Al ratios at the 60 cm depth remained stable during all sampling years (Figure 4.8).

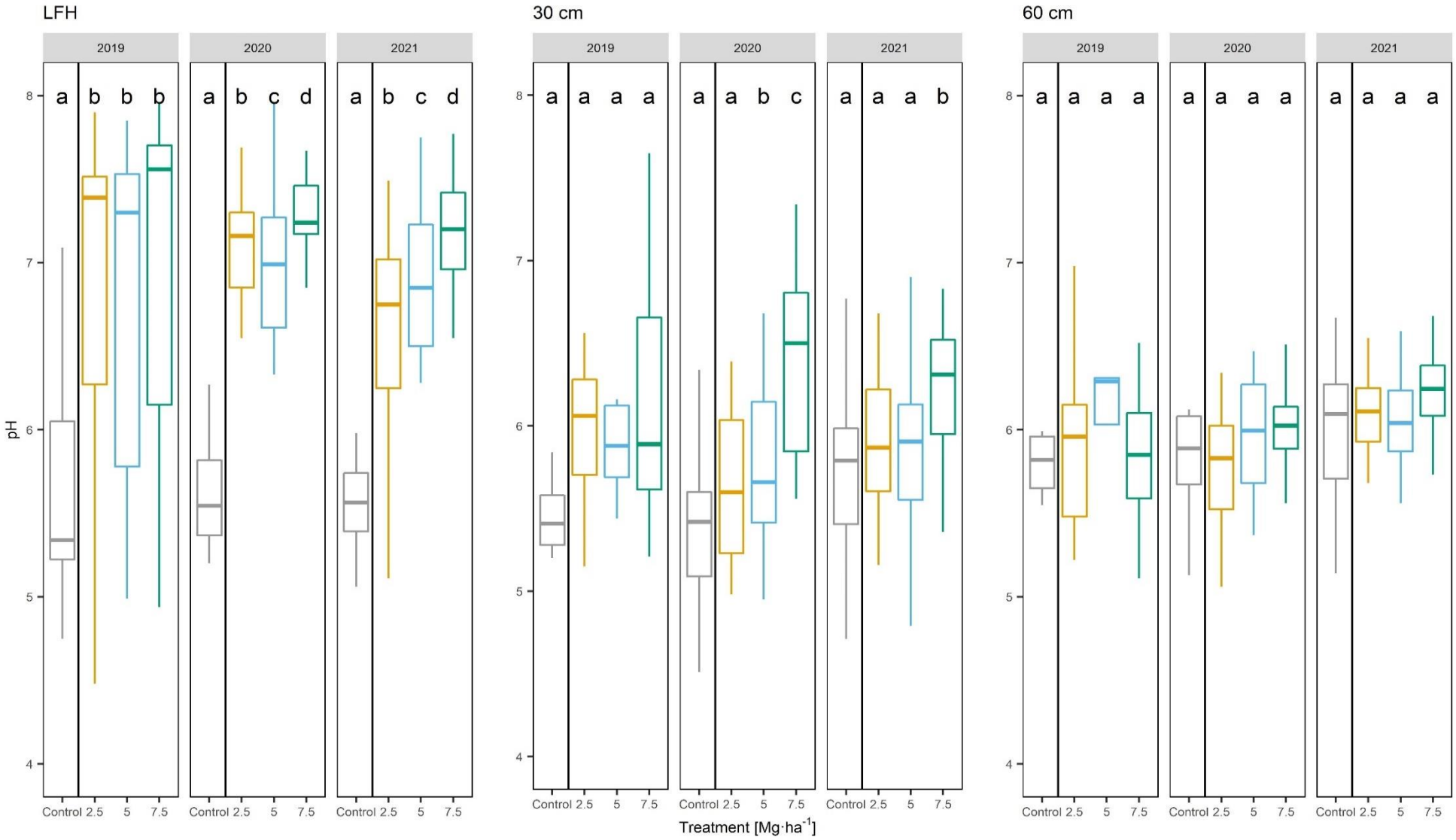

Figure 4.1 | pH of soil water percolate for three sampling depths (LFH, 30 cm, 60 cm) from 2019 to 2021 in Porridge Lake, Ontario. A letter display indicates significant differences ($p<0.05$) determined by the Tukey HSD test. Boxes represent the interquartile ranges, with the bottom marking the 25th and the top the 75th percentile. The whiskers above and below the box represent the 90th and 10th percentiles; the median trend is the line located within the box.

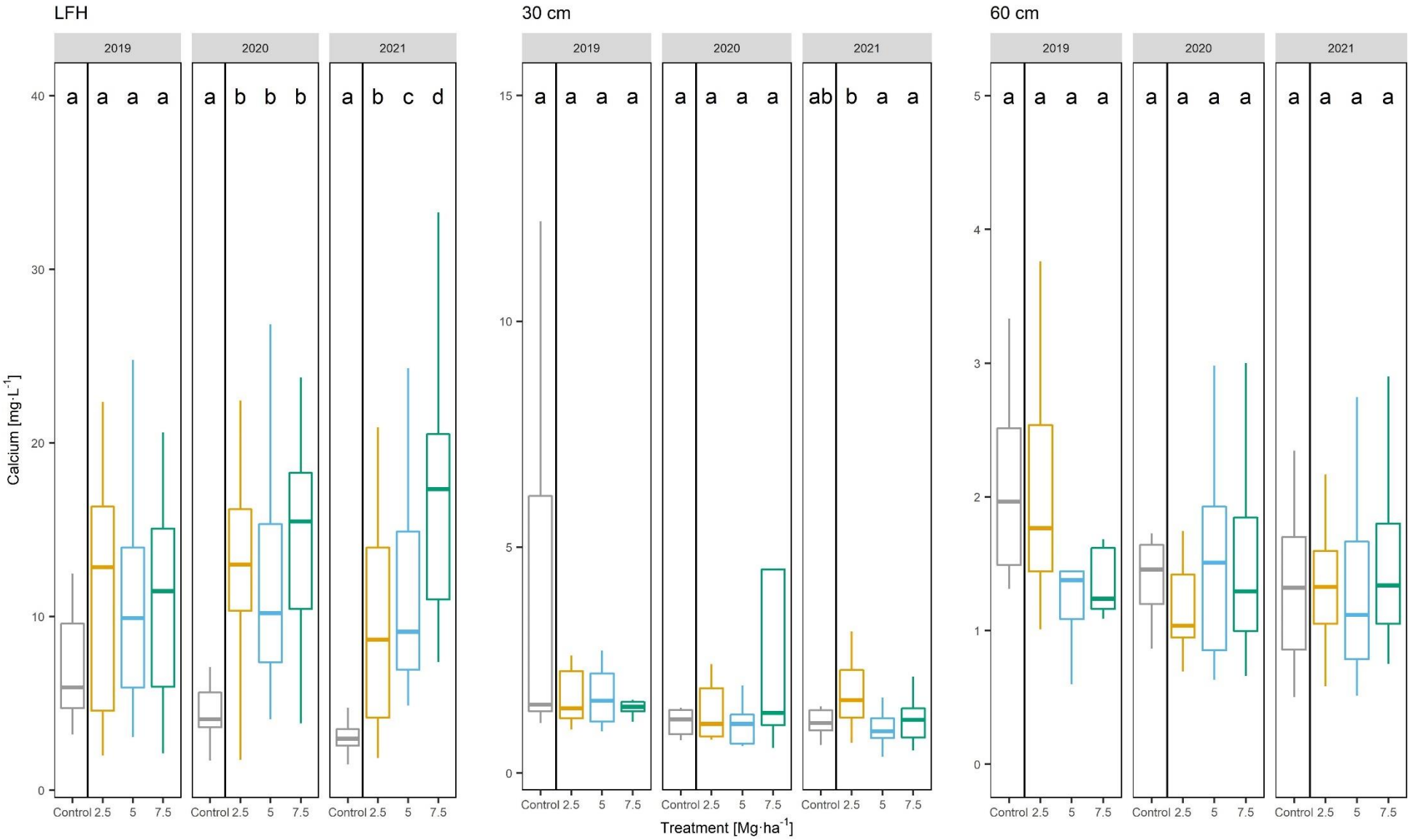

Figure 4.2 | Calcium concentrations in soil water percolate for three sampling depths (LFH, 30 cm, 60 cm) from 2019 to 2021 for Porridge Lake, Ontario. A letter display indicates significant differences ($p<0.05$) determined by the Tukey HSD test.

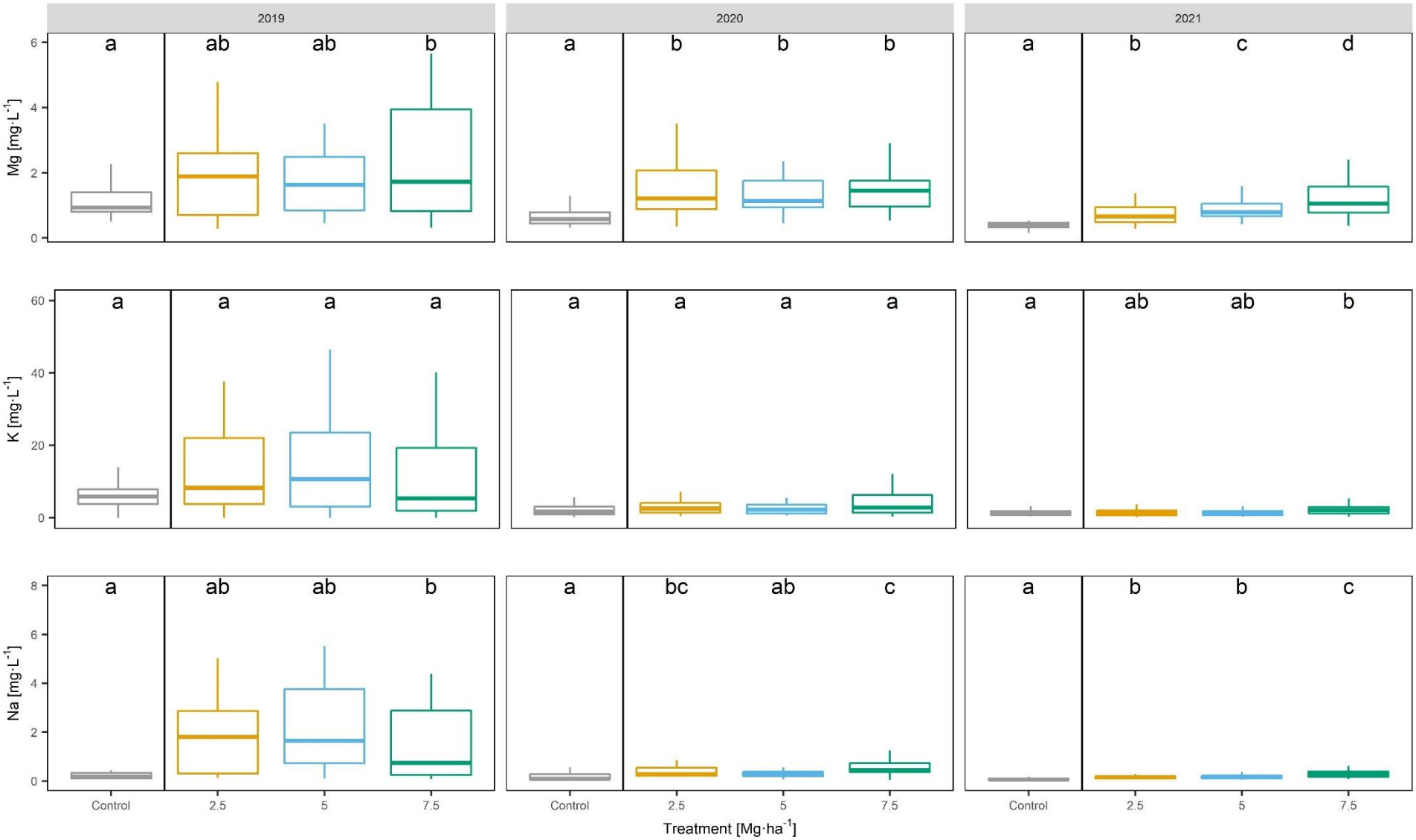

Figure 4.3 | Base cation (Mg, K, Na) concentrations (mg·L^{-1}) collected from the LFH with zero-tension lysimeters from 2019 to 2021 in Porridge Lake, Ontario. A letter display indicates significant differences ($p<0.05$) determined by the Tukey HSD test.

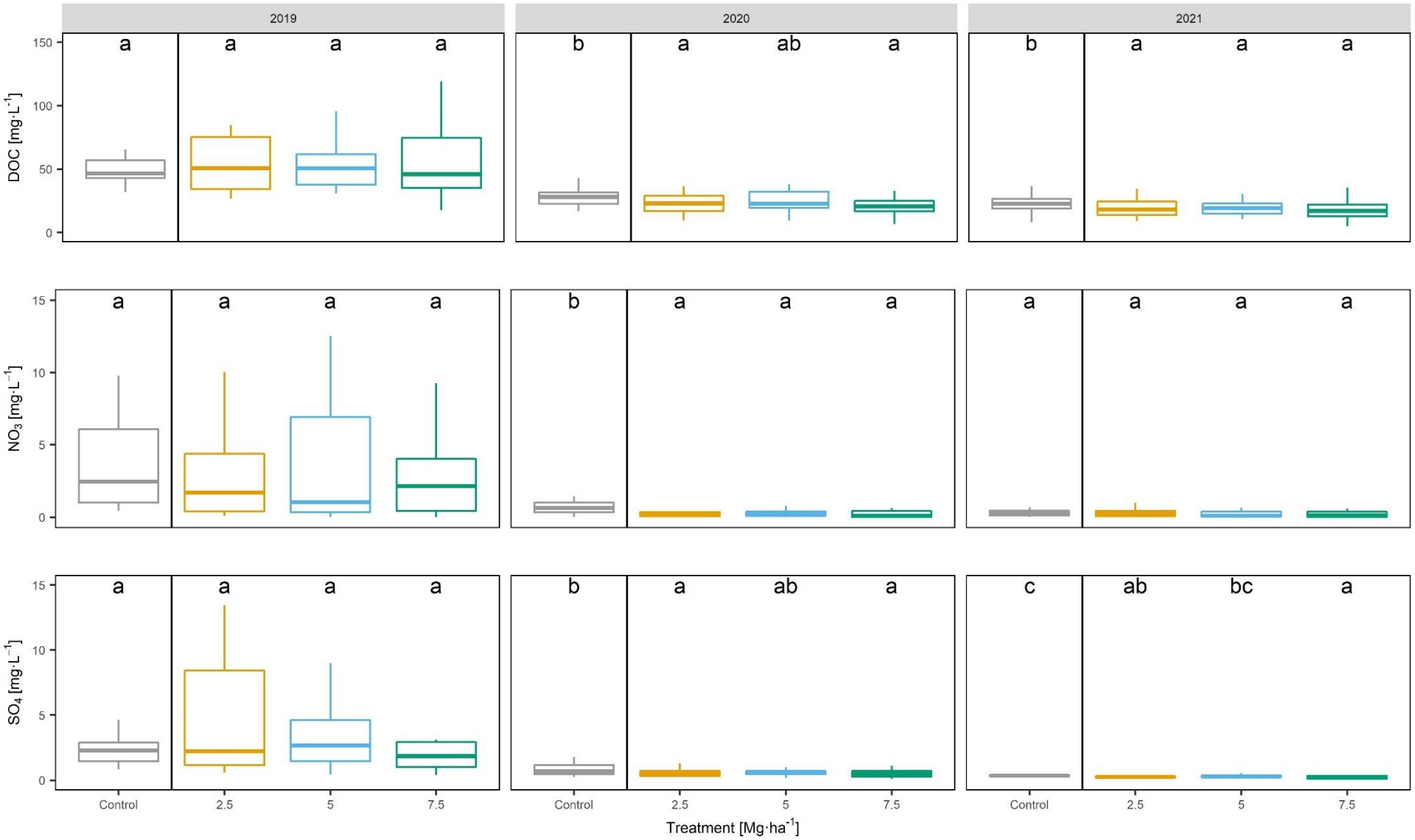

Figure 4.4 | Soil water concentrations of DOC, NO_3, SO_4 ($mg \cdot L^{-1}$) collected from the LFH with zero-tension lysimeters from 2019 to 2021 in Porridge Lake, Ontario. A letter display indicates significant differences ($p<0.05$) determined by the Tukey HSD test.

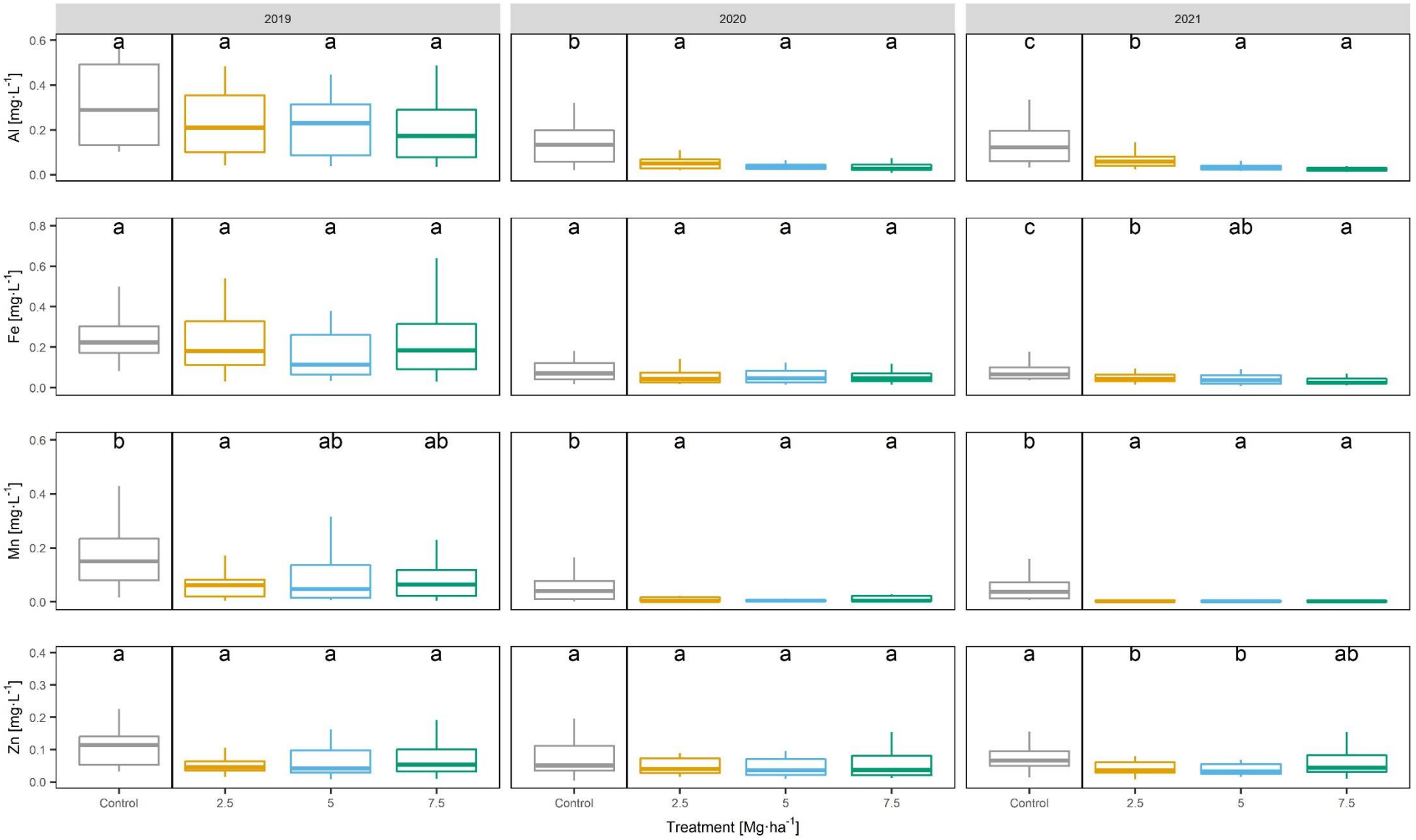

Figure 4.5 | Soil water concentrations of Al, Fe, Mn, and Zn (mg·L^{-1}) collected from the LFH with zero-tension lysimeters from 2019 to 2021 in Porridge Lake, Ontario. A letter display indicates significant differences ($p<0.05$) determined by the Tukey HSD test.

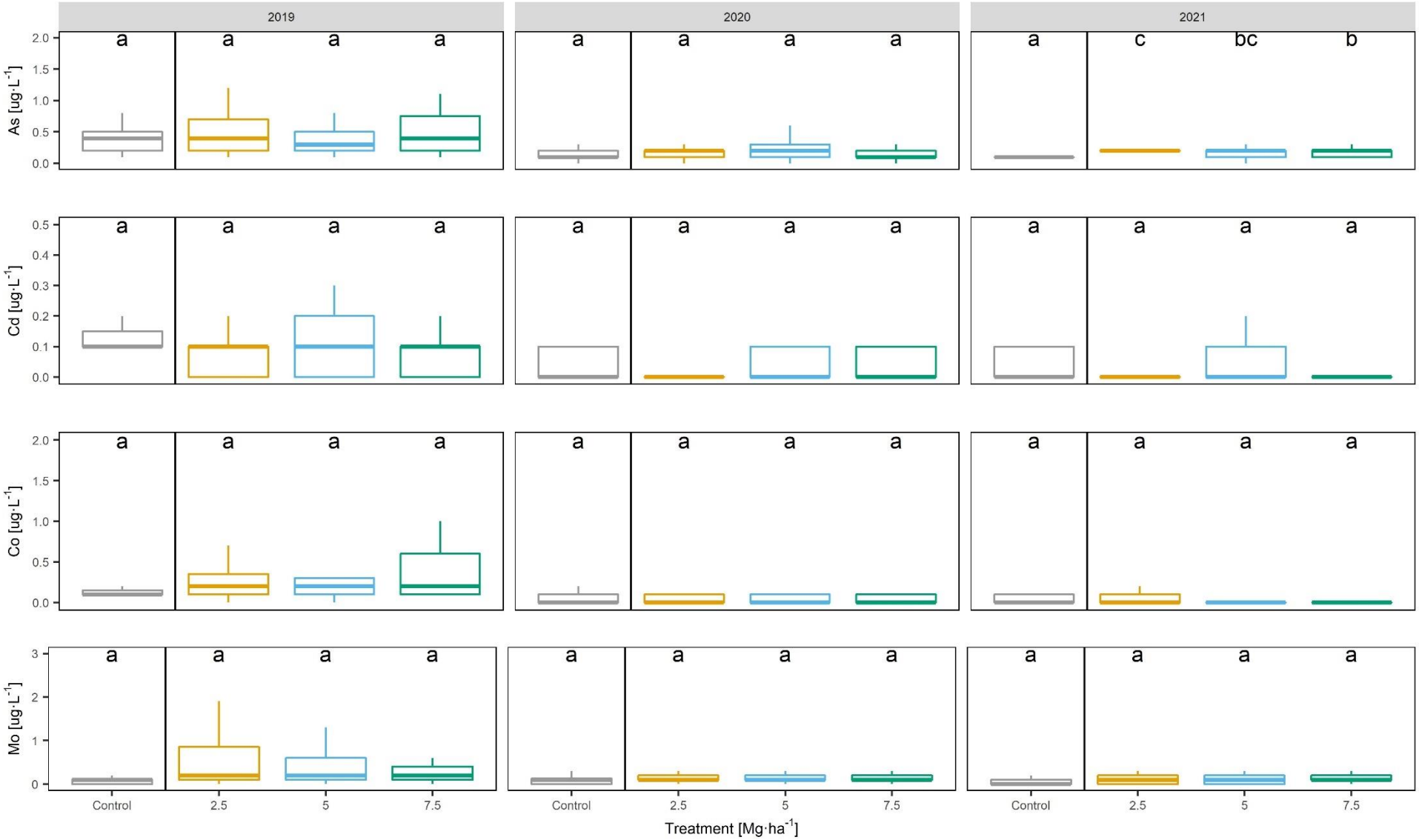

Figure 4.6 | Soil water metal concentrations of As, Cd, Co, and Mo (µg·L^{-1}) collected from the LFH with zero-tension lysimeters from 2019 to 2021 in Porridge Lake, Ontario. A letter display indicates significant differences ($p<0.05$) determined by the Tukey HSD test.

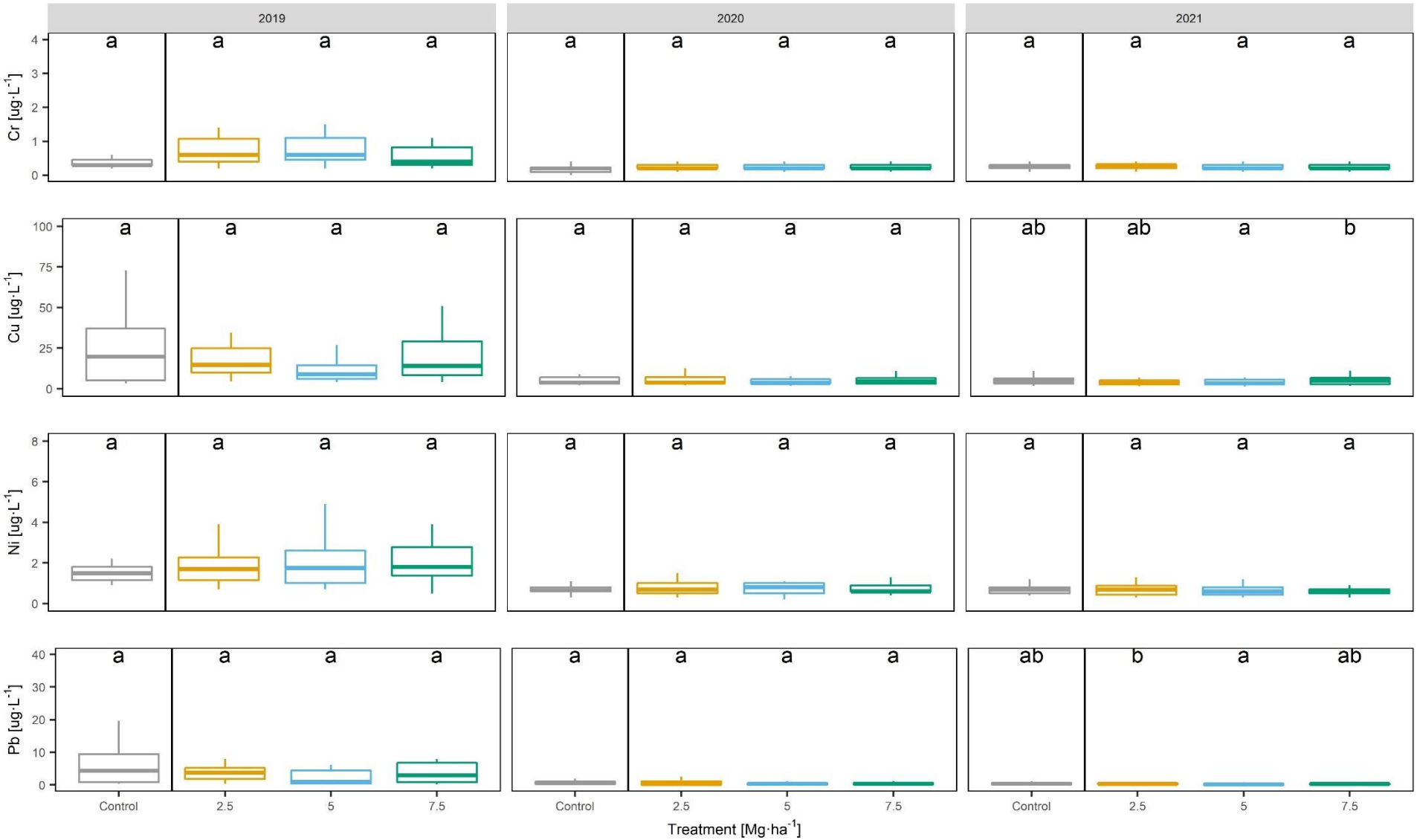

Figure 4.7 | Soil water metal concentrations of Cr, Cu, Ni and Pb (µg·L^{-1}) collected from the LFH with zero-tension lysimeters from 2019 to 2021 in Porridge Lake, Ontario. A letter display indicates significant differences ($p<0.05$) determined by the Tukey HSD test.

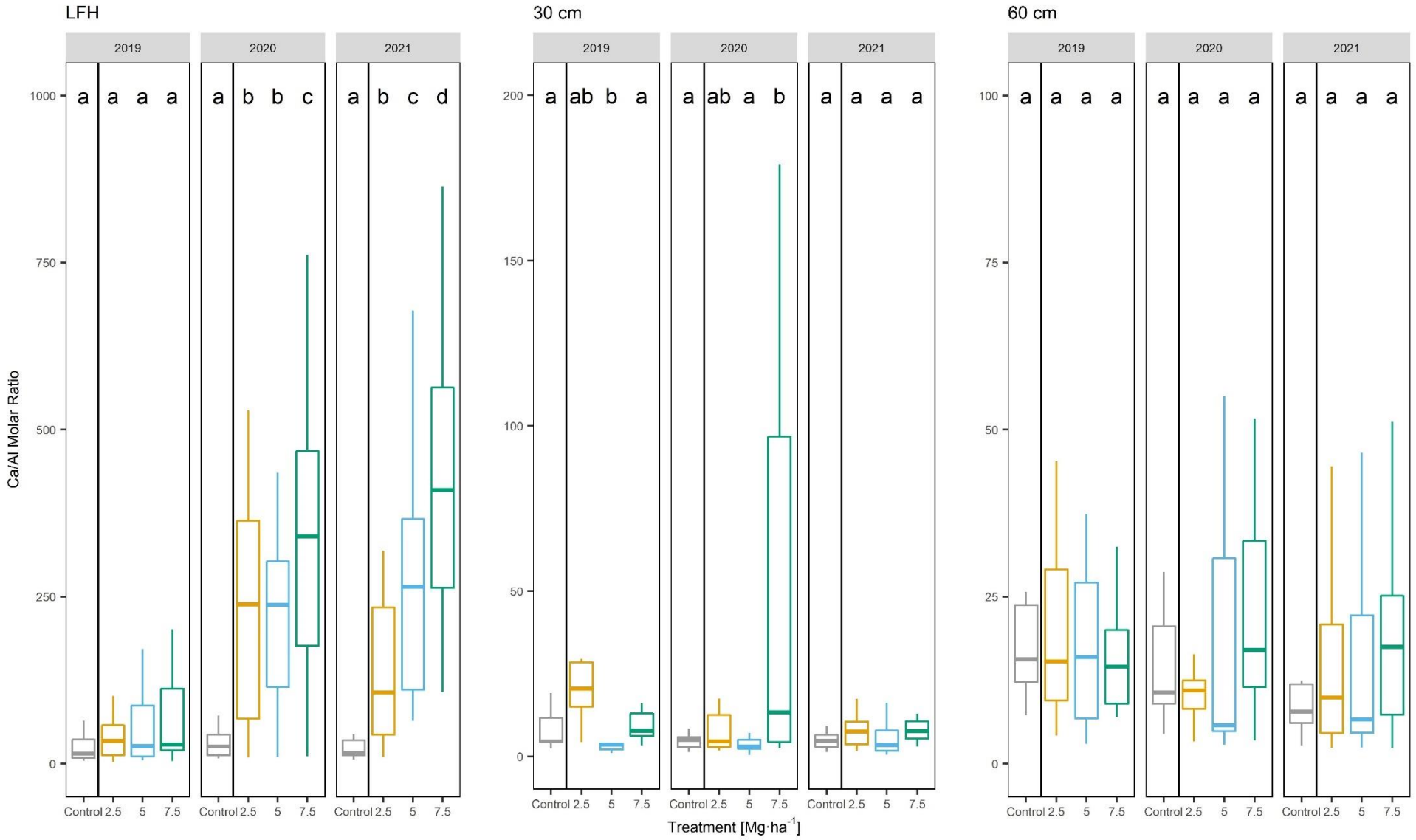

Figure 4.8 | Calcium/Al concentrations in soil water percolate for three sampling depths (LFH, 30 cm, 60 cm) from 2019 to 2021 for Porridge Lake, Ontario. A letter display indicates significant differences ($p<0.05$) determined by the Tukey HSD test.

4.2 Soil Chemical Responses to Ash Amendments

Three years after ash application, there were no significant differences among treatments in exchangeable Ca, Mg or Na concentrations in the Litter horizon, although pH, Ca, and Mg concentrations tended to be higher in ash-treated plots than control plots (Table 4.1). In contrast, deeper soil horizons (FH, Ah and Bm and C) generally had much higher pH and Ca and Mg concentrations in ash-treated plots, although the differences were not always significantly different from control plots (Table 4.2-4.5). For instance, in the FH horizon, the pH in the 5.0 and 7.5 $Mg \cdot ha^{-1}$ treatment plots averaged 5.88 and 5.95, respectively, compared with control plots that had a pH of just 3.95, while Ca concentrations were three to four times higher in the 5.0 and 7.5 $Mg \cdot ha^{-1}$ treatment plots (Table 4.2). Soil pH was also higher in mineral soil in ash-treated plots, but this difference was only significant in the C-horizon (Table 4.5), whereas increases in Ca were more apparent in treated plots, ranging from four to ten times greater than control plots for all soil horizons (Table 4.3-4.5). Similarly, concentrations of Mg in the Ah and Bm horizons were three to four times greater than in control plots (Table 4.3-4.4). There was no significant difference in soil C or N concentrations three years post-ash in all soil horizons, but the C/N ratio in the organic layer tended to be lower in ash-treated plots, but this was only significant in the FH horizon for the 2.5 $Mg \cdot ha^{-1}$ treatment plots (Appendix F). Exchangeable Mn concentrations were higher in the control plots compared with treatment plots in the FH horizon (Table 4.2). Similarly, exchangeable concentrations of Al were significantly higher in control plots in the Ah and C horizons (Table 4.3, 4.5). Molar Ca/Al ratios in the mineral soil horizons decreased with depth but increased significantly with ash treatment (Table 4.3-4.5). In control plots in the Ah horizon, 60% of the mean Ca/Al ratios were <1.0, whereas, in the ash-treated plots, the mean Ca/Al ratios ranged from 13.5 in the 2.5 plots to 62.6 in the 7.5 $Mg \cdot ha^{-1}$ treatment plots (Table 4.3). At deeper soil depths, all the Ca/Al ratios for the control plots were <1.0, while treated plots averaged between 1.45 and 19.9 in the Bm horizon and from ca. 1.0 to 4.45 in the C horizon (Table 4.4-4.5).

Total soil metal levels were quite variable, and while there were few significant differences amongst treatments, mean concentrations of some metals in the highest ash treatment plots were notably higher than in control plots (Table 4.6-4.10). For example, concentrations of Sr decreased with soil depth, but treatment values were only significantly greater than control plots in the FH, Ah and C soil horizons (Table

4.7-4.8, 4.10). The only other metal to show a treatment response was Pb, in the Bm horizon, with significantly higher concentrations in the treatment plots (Table 4.8).

Table 4.1 | Litter horizon characteristics, exchangeable base cations and metals (mean ± S.E.) three years post wood ash application at Porridge Lake, Ontario. The Lilliefors test was used to determine normality, and significant differences from control (p<0.05) were determined by pairwise comparison with the Tukey HSD test.

Treatment ($Mg \cdot ha^{-1}$)	*n*	pH ($CaCl_2$)	Ca	Mg	K	Na	Al	Fe	Mn
			---------- ($g \cdot kg^{-1}$)----------						
Control (0)	5	4.05	6.88	0.60	0.86^{b}	3.08	0.03	0.008	0.94
		(0.8)	(1.5)	(0.04)	(0.05)	(0.2)	(0.008)	(0.002)	(0.1)
2.5	5	4.55	7.92	0.55	0.65^{a}	2.96	0.03	0.01	0.86
		(0.6)	(1.5)	(0.04)	(0.05)	(0.2)	(0.008)	(0.002)	(0.1)
5.0	5	4.89	10.4	0.63	0.88^{b}	2.91	0.02	0.01	0.92
		(0.6)	(1.5)	(0.04)	(0.05)	(0.2)	(0.008)	(0.002)	(0.1)
7.5	5	4.84	10.4	0.65	0.90^{b}	2.94	0.03	0.01	0.091
		(0.7)	(1.5)	(0.04)	(0.05)	(0.2)	(0.008)	(0.002)	(0.1)
P-value (<0.01***, <0.05**)		0.14	0.26	0.28	**0.04** **	0.89	0.97	0.37	0.97

Table 4.2 | FH horizon characteristics, exchangeable base cations and metals (mean ± S.E.) three years post wood ash application at Porridge Lake, Ontario. The Lilliefors test was used to determine normality, and significant differences from control (p<0.05) were determined by pairwise comparison with the Tukey HSD test.

Treatment ($Mg \cdot ha^{-1}$)	*n*	pH ($CaCl_2$)	Ca	Mg	K	Na	Al	Fe	Mn
			---------- ($g \cdot kg^{-1}$)----------						
Control (0)	5	3.95^{a}	3.95^{a}	1.02	0.56	2.31	0.06	0.05	0.81^{b}
		(0.3)	(1.1)	(0.4)	(0.1)	(0.5)	(0.02)	(0.001)	(0.08)
2.5	5	4.50^{a}	7.01^{ab}	0.38	0.57	2.28	BDL	BDL	0.33^{a}
		(0.9)	(1.1)	(0.4)	(0.1)	(0.5)	(-)	(-)	(0.08)
5.0	5	5.88^{b}	9.77^{b}	0.42	0.54	2.11	BDL	BDL	0.44^{a}
		(0.3)	(1.1)	(0.4)	(0.1)	(0.5)	(-)	(-)	(0.08)
7.5	5	5.95^{b}	11.5^{b}	0.46	0.65	2.11	BDL	BDL	0.38^{a}
		(0.3)	(1.1)	(0.4)	(0.1)	(0.5)	(-)	(-)	(0.08)
P-value (<0.01***, <0.05**,<0.1*)		**<0.001** ***	**<0.001** ***	0.56	0.87	0.94	-	-	**0.002** ***

Table 4.3 | Ah soil horizon characteristics, exchangeable base cations and metals (mean ± S.E.) three years post wood ash application at Porridge Lake, Ontario. The Lilliefors test was used to determine normality, and significant differences from control (p<0.05) were determined by pairwise comparison with the Tukey HSD test.

Treatment ($Mg \cdot ha^{-1}$)	*n*	pH ($CaCl_2$)	OM (%)	Ca	Mg	K	Na	Al	Fe	Mn	Ca/Al
				($g \cdot kg^{-1}$)							
Control (0)	5	3.92	13.9	0.26^{a}	0.03^{a}	0.06	0.02	0.16^{b}	0.05	0.10	1.20^{a}
		(0.1)	(3.2)	(0.1)	(0.01)	(0.02)	(0.02)	(0.02)	(0.009)	(0.04)	(4.2)
2.5	5	4.03	13.1	0.81^{b}	0.07^{bc}	0.07	0.07	0.06^{a}	0.04	0.07	13.5^{ab}
		(0.5)	(3.0)	(0.4)	(0.03)	(0.03)	(0.02)	(0.02)	(0.009)	(0.04)	(4.2)
5.0	5	4.22	13.4	0.45^{ab}	0.05^{ab}	0.05	0.09	0.08^{a}	0.04	0.04	6.39^{ab}
		(0.2)	(2.4)	(0.2)	(0.01)	(0.02)	(0.02)	(0.02)	(0.009)	(0.04)	(4.2)
7.5	5	4.41	16.5	0.95^{b}	0.09^{c}	0.07	0.02	0.03^{a}	0.02	0.06	23.6^{b}
		(0.6)	(3.7)	(0.3)	(0.01)	(0.02)	(0.02)	(0.02)	(0.009)	(0.04)	(4.7)
P-value (<0.01***, <0.05**,<0.1*)		0.13	0.32	**0.006**	**<0.001**	0.46	0.23	**<0.01**	0.14	0.73	**0.005**
				***	***			***			***

Table 4.4 | Bm soil horizon characteristics, exchangeable base cations and metals (mean ± S.E.) three years post wood ash application at Porridge Lake, Ontario. The Lilliefors test was used to determine normality, and significant differences from control (p<0.05) were determined by pairwise comparison with the Tukey HSD test.

Treatment ($Mg \cdot ha^{-1}$)	*n*	pH ($CaCl_2$)	OM (%)	Ca	Mg	K	Na	Al	Fe	Mn	Ca/Al
				($g \cdot kg^{-1}$)							
Control (0)	5	4.06	10.8	0.06^{a}	0.007^{a}	0.01	0.10	0.15	0.04	0.03	0.28^{a}
		(0.3)	(3.3)	(0.1)	(0.006)	(0.02)	(0.04)	(0.03)	(0.009)	(0.02)	(5.7)
2.5	5	4.08	11.2	0.22^{ab}	0.02^{ab}	0.02	0.10	0.11	0.04	0.04	1.45^{ab}
		(0.4)	(0.7)	(0.1)	(0.006)	(0.01)	(0.04)	(0.03)	(0.009)	(0.02)	(5.7)
5.0	5	4.24	10.5	0.26^{ab}	0.03^{ab}	0.03	0.07	0.14	0.04	0.05	2.18^{ab}
		(0.2)	(1.7)	(0.2)	(0.006)	(0.02)	(0.04)	(0.03)	(0.009)	(0.02)	(5.7)
7.5	5	4.40	11.4	0.51^{b}	0.04^{b}	0.04	0.07	0.09	0.02	0.08	19.9^{b}
		(0.7)	(4.4)	(0.4)	(0.006)	(0.01)	(0.04)	(0.03)	(0.009)	(0.02)	(5.7)
P-value		0.20	0.96	**0.04**	**0.02**	0.13	0.94	0.54	0.47	0.44	**0.02**
(<0.01***, <0.05**,<0.1*)				**	**						**

Table 4.5 | C soil horizon characteristics, exchangeable base cations and metals (mean ± S.E.) three years post wood ash application at Porridge Lake, Ontario. The Lilliefors test was used to determine normality, and significant differences from control (p<0.05) were determined by pairwise comparison with the Tukey HSD test.

Treatment ($Mg \cdot ha^{-1}$)	*n*	pH ($CaCl_2$)	OM (%)	Ca	Mg	K	Na	Al	Fe	Mn	Ca/Al
				($g \cdot kg^{-1}$)							
Control (0)	5	4.24[a]	10.91	0.07[a]	0.007	0.01	0.04	0.16[b]	0.05	0.1	0.30[a]
		(0.2)	(2.2)	(0.04)	(0.006)	(0.01)	(0.05)	(0.02)	(0.01)	(0.04)	(1.1)
2.5	5	4.34[ab]	9.50	0.17[ab]	0.01	0.02	0.13	0.11[ab]	0.04	0.07	1.12[ab]
		(0.2)	(1.8)	(0.1)	(0.006)	(0.01)	(0.05)	(0.02)	(0.01)	(0.04)	(1.1)
5.0	5	4.16[a]	10.95	0.18[ab]	0.02	0.02	0.11	0.13[ab]	0.05	0.04	1.01[ab]
		(0.2)	(3.3)	(0.1)	(0.006)	(0.02)	(0.05)	(0.02)	(0.01)	(0.04)	(1.1)
7.5	5	4.64[b]	11.44	0.35[b]	0.02	0.03	0.09	0.08[a]	0.03	0.06	4.45[b]
		(0.2)	(4.0)	(0.2)	(0.006)	(0.02)	(0.05)	(0.02)	(0.01)	(0.04)	(1.1)
P-value (<0.01***, <0.05**,<0.1*)		**0.006** ***	0.76	**0.05** **	0.22	0.37	0.66	**0.01** ***	0.41	0.73	**0.05** **

Table 4.6 | Litter horizon total elemental concentrations (means ± S.E.) three years post wood ash application at Porridge Lake, Ontario. The Lilliefors test was used to determine normality, and significant differences from control (p<0.05) were determined by pairwise comparison with the Tukey HSD test.

Treatment ($Mg \cdot ha^{-1}$)	*n*	Al	Fe	Mn	As	Cd	Co	Cr	Cu	Ni	Pb	Sr	Zn
		($g \cdot kg^{-1}$)			($mg \cdot kg^{-1}$)								
Control (0)	5	0.52	0.60	1.04	0.60	0.43	0.23	0.51	10.9	0.24	0.31	70.2	48.6
		(0.3)	(0.2)	(0.2)	(0.2)	(0.1)	(0.2)	(0.3)	(3.6)	(0.3)	(0.2)	(32.0)	(7.2)
2.5	5	0.65	0.82	1.00	0.13	0.32	0.19	1.09	8.3	0.59	BDL	77.6	47.3
		(0.3)	(0.2)	(0.5)	(0.2)	(0.1)	(0.2)	(0.3)	(3.6)	(0.3)	-	(32.0)	(7.2)
5.0	5	1.18	0.90	1.61	0.14	0.48	0.38	0.77	10.6	0.25	0.01	175	54.4
		(0.3)	(0.2)	(0.5)	(0.2)	(0.1)	(0.2)	(0.3)	(3.6)	(0.3)	(0.2)	(32.0)	(7.2)
7.5	5	0.93	0.81	1.34	0.23	0.50	0.56	0.66	8.7	0.17	0.10	139	51.5
		(0.3)	(0.2)	(0.5)	(0.2)	(0.1)	(0.2)	(0.3)	(3.6)	(0.3)	(0.2)	(32.0)	(7.2)
P-value (<0.01***, <0.05**,<0.1*)		0.43	0.68	0.14	0.75	0.65	0.65	0.67	0.94	0.80	0.52	0.10	0.90

Table 4.7 | FH horizon total elemental concentrations (means ± S.E.) three years post wood ash application at Porridge Lake, Ontario. The Lilliefors test was used to determine normality, and significant differences from control (p<0.05) were determined by pairwise comparison with the Tukey HSD test.

Treatment ($Mg \cdot ha^{-1}$)	*n*	Al	Fe	Mn	Co	Cr	Cu	Ni	Pb	Sr	Zn
		---------- ($g \cdot kg^{-1}$)----------			---------- ($mg \cdot kg^{-1}$)----------						
Control (0)	5	2.55	4.33	0.93	0.84	4.88	8.01	2.48	15.5	35.9[a]	46.9
		(0.8)	(0.9)	(0.2)	(0.7)	(0.9)	(1.2)	(0.6)	(3.4)	(21.3)	(6.1)
2.5	5	2.23	3.68	0.63	0.46	3.14	8.41	1.89	12.1	69.2[ab]	45.1
		(0.8)	(0.9)	(0.2)	(0.7)	(0.9)	(1.2)	(0.6)	(3.4)	(21.3)	(6.1)
5.0	5	2.35	3.69	1.30	0.35	4.02	9.56	2.64	11.2	136[b]	48.4
		(0.8)	(0.9)	(0.2)	(0.7)	(0.9)	(1.2)	(0.6)	(3.4)	(21.3)	(6.1)
7.5	5	4.02	3.79	1.09	2.66	3.74	9.91	3.46	16.1	150[b]	68.4
		(0.8)	(0.9)	(0.2)	(0.7)	(0.9)	(1.2)	(0.6)	(3.4)	(21.3)	(6.1)
P-value		0.34	0.94	0.12	0.32	0.62	0.61	0.35	0.68	**0.004**	0.10

(<0.01***, <0.05**,<0.1*)

***Arsenic and Cadmium concentrations were below detection limits.**

Table 4.8 | Ah horizon total elemental concentrations (means ± S.E.) three years post wood ash application at Porridge Lake, Ontario. The Lilliefors test was used to determine normality, and significant differences from control (p<0.05) were determined by pairwise comparison with the Tukey HSD test.

Treatment ($Mg \cdot ha^{-1}$)	*n*	Al	Fe	Mn	As	Co	Cr	Cu	Ni	Pb	Sr	Zn
		---------- ($g \cdot kg^{-1}$)---------			---------- ($mg \cdot kg^{-1}$)----------							
Control (0)	5	5.09	9.39	0.30	0.19	2.40	6.72	4.83	3.55	24.9	4.65	26.6
		(0.8)	(1.3)	(0.1)	(1.1)	(2.4)	(0.9)	(2.3)	(1.0)	(3.9)	(5.9)	(6.2)
2.5	5	3.34	7.79	0.21	1.20	1.15	5.16	2.15	1.98	22.4	11.0	27.4
		(0.8)	(1.3)	(0.1)	(1.1)	(0.6)	(0.9)	(2.3)	(1.0)	(3.9)	(5.9)	(6.2)
5.0	5	4.72	9.77	0.21	1.08	1.35	6.30	1.82	2.59	23.9	18.9	20.9
		(0.8)	(1.3)	(0.1)	(1.1)	(0.9)	(0.9)	(2.3)	(1.0)	(3.9)	(5.9)	(6.2)
7.5	5	4.53	9.29	0.46	2.66	5.68	6.41	3.30	2.28	27.6	13.9	36.2
		(0.8)	(1.3)	(0.1)	(1.1)	(5.8)	(0.9)	(2.3)	(1.0)	(3.9)	(5.9)	(6.2)
P-value		0.43	0.62	0.43	0.47	0.27	0.63	0.79	0.73	0.81	0.41	0.40

(<0.01***, <0.05**,<0.1*)

***Cadmium concentrations were below detection limits.**

Table 4.9 | Bm horizon total elemental concentrations (means ± S.E.) three years post wood ash application at Porridge Lake, Ontario. The Lilliefors test was used to determine normality, and significant differences from control (p<0.05) were determined by pairwise comparison with the Tukey HSD test.

Treatment ($Mg \cdot ha^{-1}$)	*n*	Al	Fe	Mn	As	Co	Cr	Cu	Ni	Pb	Sr	Zn
		---------- ($g \cdot kg^{-1}$)----------			---------- ($mg \cdot kg^{-1}$)----------							
Control (0)	5	7.98 (1.2)	13.5 (1.5)	0.23 (0.1)	0.92 (2.8)	2.74 (2.2)	7.99 (0.9)	4.18 (1.6)	2.22 (0.6)	5.84^{a} (2.1)	3.27 (1.8)	21.8 (3.7)
2.5	5	5.43 (1.2)	10.1 (1.5)	0.17 (0.1)	5.36 (2.8)	1.63 (2.2)	5.99 (0.9)	1.44 (1.6)	2.01 (0.6)	13.8^{b} (2.1)	4.25 (1.8)	22.0 (3.7)
5.0	5	7.70 (1.2)	13.4 (1.5)	0.18 (0.1)	0.69 (2.8)	1.94 (2.2)	8.27 (0.9)	0.66 (1.6)	1.85 (0.6)	11.6^{ab} (2.1)	4.92 (1.8)	19.0 (3.7)
7.5	5	7.42 (1.2)	11.9 (1.5)	0.63 (0.1)	4.64 (2.8)	8.84 (2.2)	7.68 (0.9)	2.11 (1.6)	1.91 (0.6)	8.71^{ab} (2.1)	7.74 (1.8)	27.2 (3.7)
P-value (<0.01***, <0.05**,<0.1*)		0.46	0.36	0.39	0.52	0.10	0.27	0.47	0.97	**0.05** **	0.36	0.49

***Cadmium concentrations were below detection limits.**

Table 4.10 | C horizon total elemental concentrations (means ± S.E.) three years post wood ash application at Porridge Lake, Ontario. The Lilliefors test was used to determine normality, and significant differences from control (p<0.05) were determined by pairwise comparison with the Tukey HSD test.

Treatment ($Mg \cdot ha^{-1}$)	*n*	Al	Fe	Mn	Co	Cr	Cu	Ni	Pb	Sr	Zn
		---------- ($g \cdot kg^{-1}$)----------			---------- ($mg \cdot kg^{-1}$)----------						
Control (0)	5	10.0 (1.5)	16.7 (1.5)	0.21 (0.09)	2.94 (1.6)	9.43 (0.9)	3.92 (0.4)	2.37 (1.0)	3.61 (1.7)	2.79^{a} (0.6)	26.3 (5.0)
2.5	5	8.41 (1.5)	12.0 (1.5)	0.11 (0.09)	1.86 (1.6)	7.94 (0.9)	1.20 (0.4)	2.18 (1.2)	4.55 (1.7)	3.71^{ab} (0.6)	28.6 (5.0)
5.0	5	9.47 (1.5)	13.9 (1.5)	0.15 (0.09)	2.18 (1.6)	8.74 (0.9)	5.52 (0.4)	2.40 (0.4)	6.33 (1.7)	4.08^{ab} (0.6)	21.1 (5.0)
7.5	5	8.14 (1.5)	14.1 (1.5)	0.42 (0.09)	6.38 (1.6)	9.77 (0.9)	1.74 (0.4)	1.85 (0.8)	5.81 (1.7)	5.18^{b} (0.6)	29.8 (5.0)
P-value (<0.05**, <0.1*)		0.78	0.21	0.22	0.23	0.47	0.76	0.76	0.65	**0.09** *	0.62

***Arsenic and Cadmium concentrations were below detection limits.**

4.3 Soil Microbial Communities

Soil microbial communities varied considerably amongst the three soil horizons (L, FH and Ah) at both the phyla and order level, but there was minimal response to ash treatment three years after post-application. The only significant response in the diversity indices for soil prokaryote communities occurred in the 7.5 $Mg \cdot ha^{-1}$ treatment plots, where there was an increase in H diversity and Chao1 richness in the FH horizon relative to control plots (Table 4.11). At the same treatment regime, within the litter layer, there was a small but significant decrease in D diversity (Table 4.11). Diversity indices for the soil Eukaryote did not differ significantly between treatments (Table 4.12), and only the genera *Hygrocybe* showed a significant response amongst treatments in the FH layer (Appendix H).

Soil (litter, FH, Ah) prokaryote relative abundance (RA) was plotted based upon a RA of ≥1% at the phyla level and ≥3% at the order level across horizons (Appendix G). Three years post-ash application, there was no significant difference amongst phyla in the litter horizon (Appendix G). Within the litter horizon, *Proteobacteria* had the highest relative abundance across all treatments (58%), followed by *Acidobacteriota* (21%) (Figure 4.9A). Along with *Actinobacteriota* and *Bacteriodota*, these four phyla represented 98% of the total bacteria in the litter layer (Figure 4.9A). The dominant taxa (>10% mean relative abundance) at the order level were *Acidobacteriales* (21%), *Burkholderiales* (13%), *Rhizobiales* (13%), *Caulobacterales* (11%) and *Sphingomonadales* (10%) (Figure 4.9B). *Proteobacteria* (44%) and *Acidobacteriales* (23%) dominated at the phyla level in the FH horizon, with the rest of the phyla accounting for 10% or less (Figure 4.12A, Appendix G). At the order level, *Rhizobiales* was the dominant taxa (22%), followed by *Acidobacteriales* (10%), *Subgroup_2* (9%) and *Burkholderiales*. *Burkholderiales* was significantly greater in the 7.5 $Mg \cdot ha^{-1}$ plots compared to control plots but only accounted for 5% of relative abundance in the FH horizon (Figure 4.12B). Within the Ah horizon, *Proteobacteria* was again the dominant phyla with a mean relative abundance of 35%, then *Acidobacteriota* (28%), *Verrucomicrobiota* (13%) and *Planctomycetota* (6%) (Figure 4.13A). The average relative abundance of *RCP2-54* across all treatment regimes was low at 3%, but the RA in the Ah horizon for the 2.5 $Mg \cdot ha^{-1}$ plots was

significantly greater than the 7.5 Mg·ha^{-1} plots (Appendix G). The dominant taxa (>10%) at the order level were *Rhizobiales* (22%), *Subgroup_2* (13%), *Chthoniobacterales* (12%) and *Acidobacteriales* (11%) (Figure 4.13B).

Within Eukaryote communities, *Helotiales* dominated the litter layer, representing approximately 30% of the RA at the order level, followed by *Rhytismatales* (20%) and *Agaricales* (14%) (Figure 4.11C). The two dominant genera in the litter layer were *Coccomyces* (18%) and *Mycena* (9%), and while not significantly different, there was a precipitous drop in relative abundance between the control and 7.5 Mg·ha^{-1} plots (Figure 4.11D, Appendix H). The relative abundance of fungal communities greater than 10% in the FH horizon was dominated by the order *Agaricales* (34%) and *Mortierellales* (10%) (Figure 4.12C). The only significant response amongst the Eukaryote communities was the RA of *Hygrocybe* at the genus level, which showed a significantly strong response, increasing from 0.4% (control) to 37% relative abundance in the 2.5 Mg·ha^{-1} plots within the FH horizon (Figure 4.12D). *Hygrocybe* was also the dominant genera with an average relative abundance of 11%, followed closely by *Mortierella* at 10% (Figure 4.12D). Comparable to observations in the FH horizon, the dominant order in the Ah horizon was *Mortierellales* (27%) and *Agaricales* (17%) (Figure 4.13C). Still, the genera *Mortierella* was dominant in the Ah horizon, with an average relative abundance of 26% (Figure 4.13D). While not as strong as the response seen in the FH horizon, within the Ah horizon, there was a noticeable increase in the genus *Hygrocybe* in the 2.5 Mg·ha^{-1} treatment plots (12% greater than control) and *Inocybe* in the 7.5 Mg·ha^{-1} treatment plots (13% greater than control) (Figure 4.13D).

To further explore changes in fungal communities three years post wood ash application, all fungal data were separated into associated fungal guilds by soil horizons (Litter, FH, Ah) (Figure 4.14-4.16). In the litter horizon, the RA of saprotrophs was highest in the control plots (11%) and decreased in the 2.5 (8%), 5.0 (5%) and 7.5 Mg·ha^{-1} (7%) treatment plots. However, pathogen was the dominant fungal guild in the litter layer, with treatment plots (9-13%) being higher when compared with control plots (6%) (Figure 4.14). Again, the saprotroph guild was dominant in the FH horizon, but the highest relative abundance was in the 2.5 (15%) and 5.0 Mg·ha^{-1} (14%) treatment plots, whereas the control and 7.5 Mg·ha^{-1} treatment plots were considerably lower at 6% each (Figure 4.15). The second most abundant guild in the FH horizon was ectomycorrhizal (ECM),

with three times greater RA in control plots (9%) compared with treatment plots, averaging 3% (Figure 4.15). Despite the strong presence of ECM in the control plots in the FH layer, ECM dominance in the Ah layer shifted to the 5.0 $Mg \cdot ha^{-1}$ (11%) plots compared to the others that averaged between 4 and 5% relative abundance (Figure 4.16). The RA of saprotrophs was highest in the control plots (8%) and lowest in the 5.0 $Mg \cdot ha^{-1}$ (2%) treatment plots, while endophytes were greatest in the 7.5 $Mg \cdot ha^{-1}$ treatment plots and lowest in control and 5.0 $Mg \cdot ha^{-1}$ sites (6% and 5%, respectively (Figure 4.15)). The ratio of fungi to bacteria followed an increasing gradient by treatment in the litter horizon and decreased in the FH and Ah soil horizons; there was no significant treatment effect (Appendix I).

Table 4.11 Diversity indices for Prokaryotes from the litter, FH and Ah layers sampled at Porridge Lake, Ontario, in 2021. Taxa with less than 0.5% relative abundance trimmed prior to analysis. Statistical significance is indicated by letters.

		Shannon-Weiner (H)			Simpson (D)			Chao1			Pielou		
Treatment ($Mg \cdot ha^{-1}$)	*n*	Litter	FH	Ah	Litter	FH	Ah	Litter	FH	Ah	Litter	FH	Ah
		Diversity Indices						Richness			Evenness		
Control (0)	5	4.8 (0.4)	5.4[a] (0.5)	4.8 (1.6)	0.98[a] (0.01)	0.99 (0.01)	0.94 (0.1)	317 (95)	466[a] (163)	405 (209)	0.83 (0.3)	0.89 (0.04)	0.82 (0.2)
2.5	5	4.4 (0.7)	5.5[a] (0.5)	5.6 (0.2)	0.97[ab] (0.02)	0.99 (0.003)	0.99 (0.002)	247 (146)	500[a] (201)	523 (55)	0.82 (0.1)	0.90 (0.01)	0.89 (0.02)
5.0	5	4.8 (0.8)	5.8[a] (0.2)	5.5 (0.1)	0.98[a] (0.01)	0.99 (0.002)	0.99 (0.002)	329 (170)	608[ab] (58)	493 (70)	0.84 (0.05)	0.90 (0.02)	0.88 (0.02)
7.5	5	4.0 (0.3)	6.0[b] (0.2)	5.6 (0.1)	0.96[b] (0.02)	1.00 (0.001)	0.99 (0.001)	171 (52)	776[b] (130)	533 (52)	0.79 (0.02)	0.91 (0.01)	0.89 (0.004)
P-value (<0.05**, <0.1*)		0.16	**0.07** *	0.39	**0.08** *	0.14	0.41	0.21	**0.02** **	0.33	0.19	0.58	0.44

Table 4.12 | Diversity indices for Eukaryotes from the litter, FH and Ah layers sampled at Porridge Lake, Ontario, in 2021. Taxa with less than 0.5% relative abundance trimmed prior to analysis. Statistical significance is indicated by letters.

		Shannon-Weiner (H)			Simpson (D)			Chao1			Pielou		
Treatment ($Mg \cdot ha^{-1}$)	*n*	Litter	FH	Ah	Litter	FH	Ah	Litter	FH	Ah	Litter	FH	Ah
		Diversity Indices						Richness			Evenness		
Control (0)	5	3.0 (0.7)	3.5 (0.4)	3.5 (0.9)	0.85 (0.1)	0.92 (0.04)	0.85 (0.2)	212 (72)	298 (43)	243 (21)	0.56 (0.1)	0.62 (0.1)	0.63 (0.2)
2.5	5	3.2 (0.8)	2.7 (1.4)	3.6 (0.3)	0.86 (0.2)	0.72 (0.3)	0.92 (0.03)	236 (44)	325 (54)	277 (26)	0.58 (0.1)	0.46 (0.2)	0.63 (0.1)
5.0	5	3.0 (0.4)	3.0 (1.5)	3.9 (0.1)	0.85 (0.1)	0.74 (0.3)	0.95 (0.01)	234 (45)	330 (83)	286 (39)	0.55 (0.1)	0.52 (0.2)	0.69 (0.01)
7.5	5	3.3 (0.3)	3.7 (0.5)	3.5 (0.9)	0.92 (0.03)	0.93 (0.03)	0.88 (0.2)	216 (65)	372 (86)	248 (68)	0.62 (0.04)	0.63 (0.1)	0.63 (0.2)
P-value (<0.05**, <0.1*)		0.79	0.43	0.72	0.72	0.23	0.69	0.88	0.43	0.34	0.70	0.38	0.80

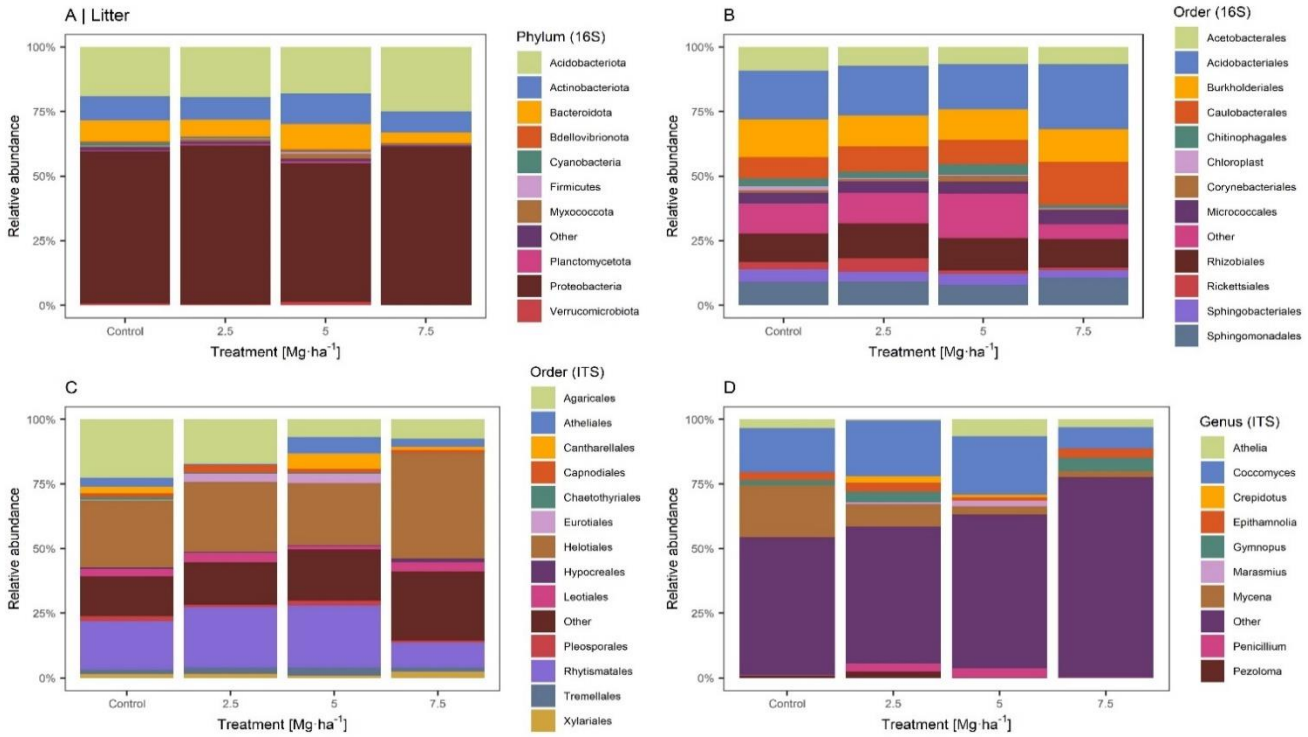

Figure 4.9 | Relative abundance (% of individual taxonomic groups) of bacterial (16S) microorganisms in the litter layer at the (A) phyla and (B) order level. Relative abundance of the most abundant fungal (ITS) (C) order and (D) genus in the litter layer. Samples were collected in 2021 from Porridge Lake, Ontario.

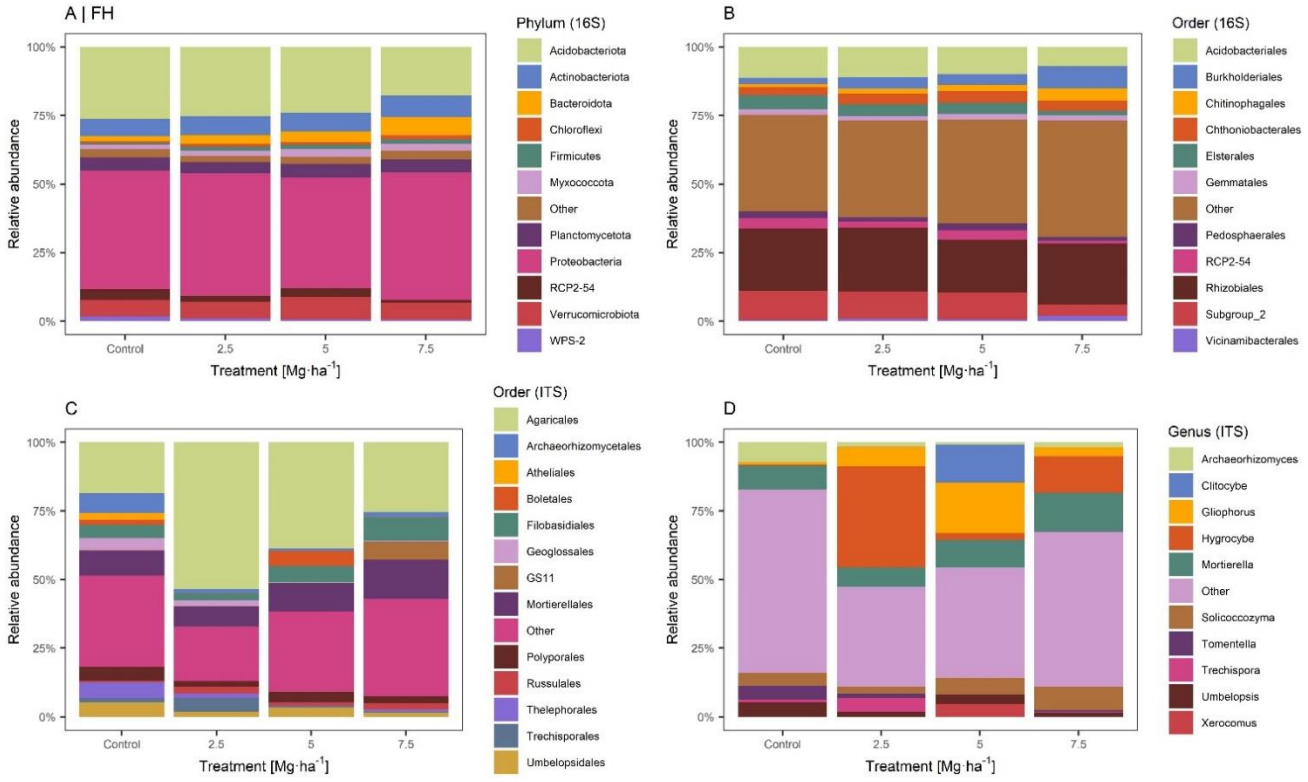

Figure 4.10 | Relative abundance (% of individual taxonomic groups) of bacterial (16S) microorganisms in the FH layer at the (A) phyla and (B) order level. Relative abundance of the most abundant fungal (ITS) (C) order and (D) genus in the FH layer. Samples were collected in 2021 from Porridge Lake, Ontario.

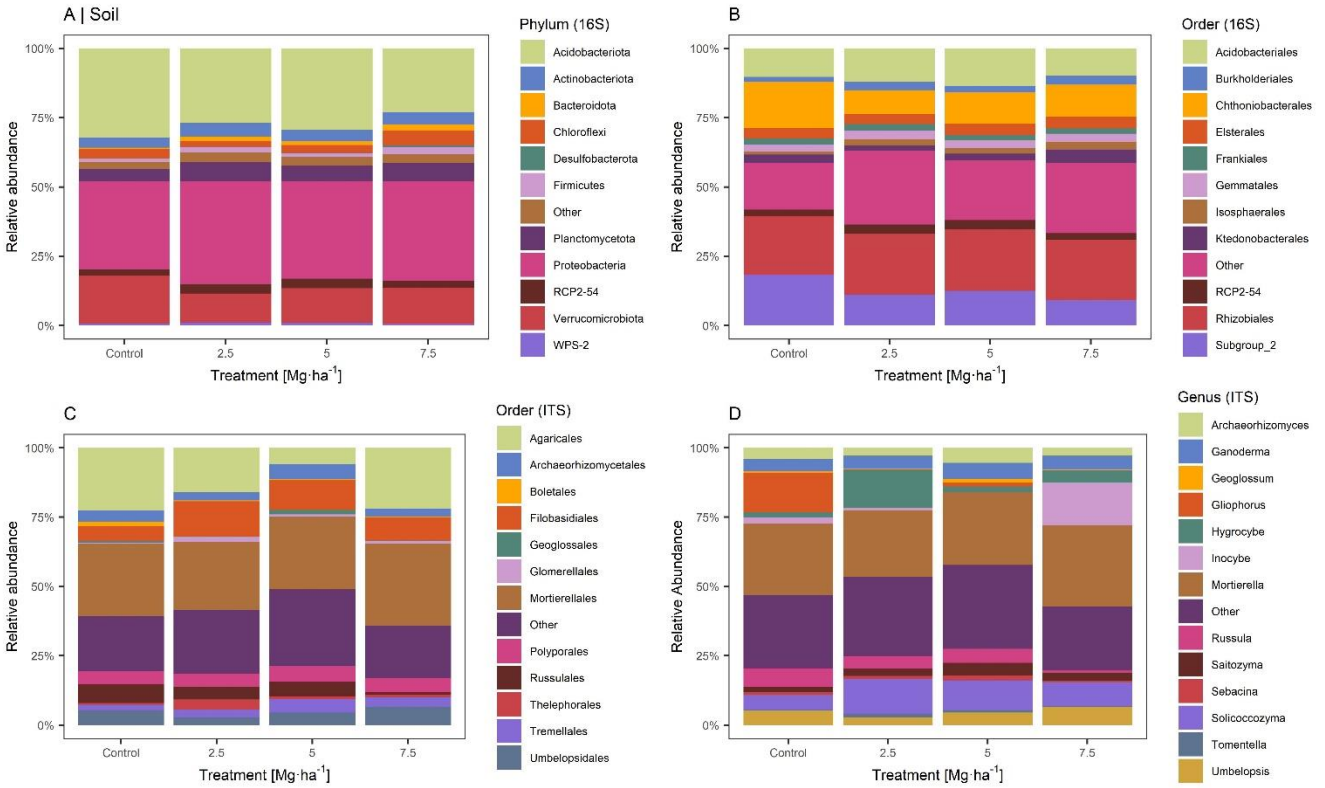

Figure 4.11 | Relative abundance (% of individual taxonomic groups) of bacterial (16S) microorganisms in the Ah layer at the (A) phyla and (B) order level. Relative abundance of the most abundant fungal (ITS) (C) order and (D) genus in the soil layer. Samples were collected in 2021 from Porridge Lake, Ontario

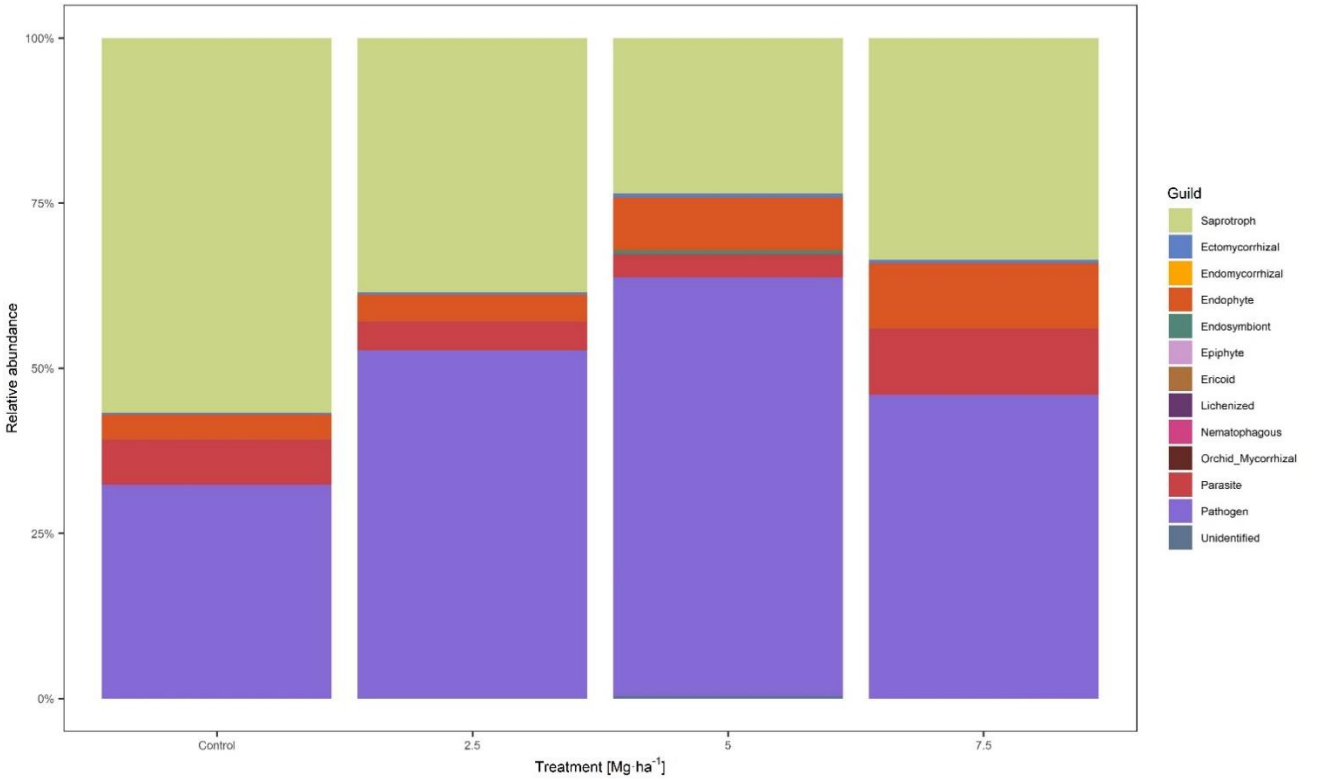

Figure 4.12 | Relative abundance of the various fungal guilds within the litter horizon with an identification confidence level of probable or greater. Samples were collected in 2021 from Porridge Lake, Ontario.

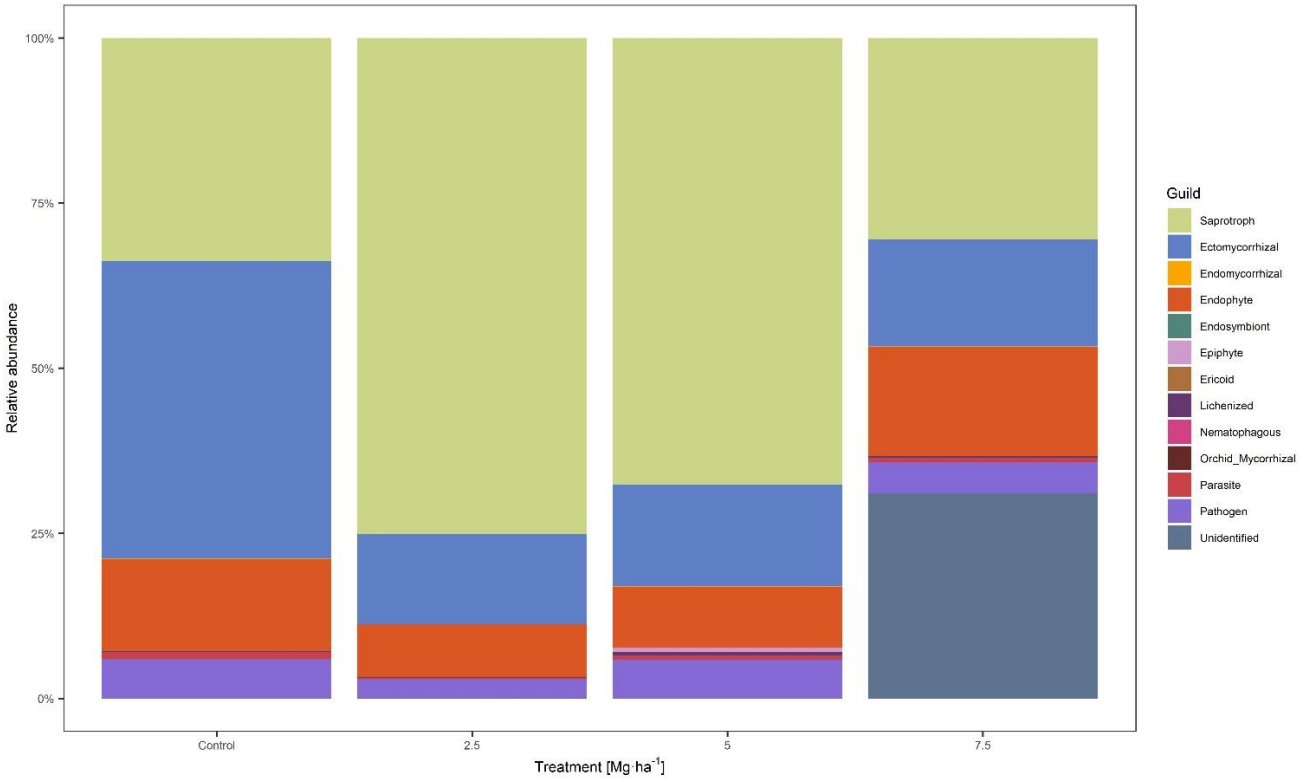

Figure 4.13 | Relative abundance of the various fungal guilds within the FH horizon with an identification confidence level of probable or greater. Samples were collected in 2021 from Porridge Lake, Ontario.

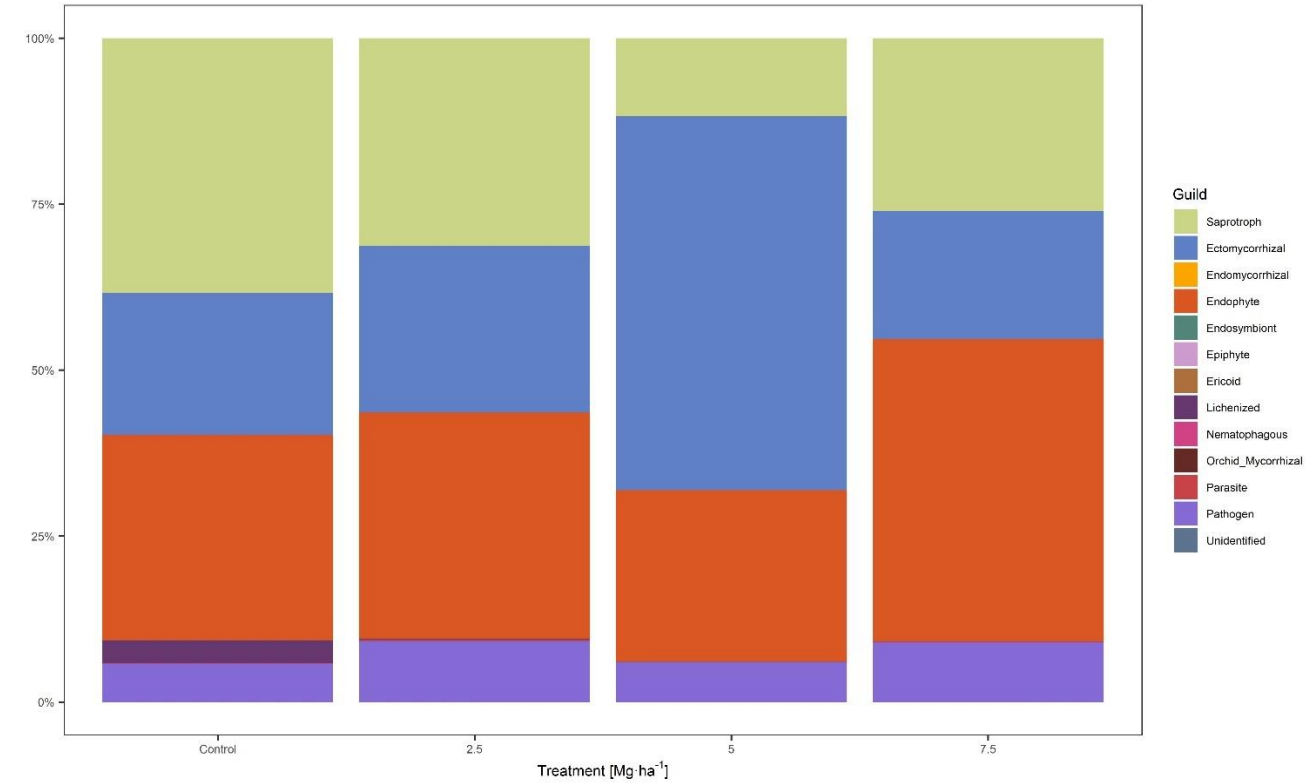

Figure 4.14 | Relative abundance of the various fungal guilds within the Ah horizon with an identification confidence level of probable or greater. Samples were collected in 2021 from Porridge Lake, Ontario.

4.4 Sugar Maple Foliar Chemistry

After three years, there were very few differences in sugar maple foliar chemistry among treatments, with the concentrations of Ca, Mg and K remaining slightly above the critical threshold for healthy sugar maples reported by Kolb & McCormick (1993) (Ca - 5 $g \cdot kg^{-1}$, Mg - 1.1 $g \cdot kg^{-1}$ and K - 5.5 $g \cdot kg^{-1}$) (Table 4.13). Whist not significant, concentrations of Ca in the 7.5 Mg ha^{-1} treatments were approximately 2 $g \cdot kg^{-1}$ (30%) higher than controls, corresponding with a tendency for C to be 0.3 % lower than control plots (Appendix F). The ash treatment did not affect concentrations of N, S, or C/N (Appendix F). Foliar metal concentrations showed no significant treatment response, with only marginal increases in Cu in control and Sr in the 7.5 $Mg \cdot ha^{-1}$ treatments plots (Table 4.13).

Table 4.13 | Sugar maple foliage characteristics, exchangeable base cations, and total metal (mean ± S.E.), three years post wood ash application. The Lilliefors test was used to determine normality, significant differences from control by pairwise comparison for normal distribution determined by Tukey HSD test, and Dunn's test for non-normal.

Treatment	*n*	Ca	Mg	K	Na	Al	Fe	Mn	As	Cu	Sr	Zn
($Mg \cdot ha^{-1}$)		---------- ($g \cdot kg^{-1}$)----------							---------- ($mg \cdot kg^{-1}$)----------			
Control (0)	5	5.68 (0.6)	1.28 (0.1)	5.52 (0.5)	0.14 (0.02)	0.02 (0.003)	0.08 (0.01)	0.69 (0.1)	0.63 (0.4)	7.24 (1.1)	31.2 (3.1)	20.9 (1.7)
2.5	5	6.46 (0.6)	1.44 (0.1)	5.87 (0.5)	0.18 (0.02)	0.02 (0.003)	0.05 (0.01)	0.69 (0.1)	0.86 (0.4)	6.53 (1.1)	35.3 (3.1)	21.6 (1.7)
5.0	5	6.54 (0.6)	1.45 (0.1)	5.83 (0.5)	0.14 (0.02)	0.02 (0.003)	0.05 (0.01)	0.58 (0.1)	0.64 (0.4)	5.79 (1.1)	32.3 (3.1)	19.3 (1.7)
7.5	5	7.27 (0.6)	1.45 (0.1)	5.81 (0.5)	0.15 (0.02)	0.01 (0.003)	0.05 (0.01)	0.50 (0.1)	0.88 (0.4)	4.23 (1.1)	41.4 (3.1)	20.6 (1.7)
P-value (<0.01***, <0.05**, <0.1*)		0.31	0.70	0.95	0.40	0.81	0.20	0.45	0.93	0.31	0.14	0.81

*** Concentrations of Cd, Co, Cr, Ni, Pb were below detection limits.**

4.5 Fine Root Response

When measured three years after application, fine roots were unaffected by ash application. Wood ash treatments did not significantly affect sugar maple fine root RB, RLD or SRL in the FH or the Ah horizon (Table 4.14). The length of fine roots in the FH layer was significantly shorter in control and 2.5 $Mg \cdot ha^{-1}$ plots compared with the 5.0 $Mg \cdot ha^{-1}$ treatment plots, but the differences did not persist into the mineral soil horizon (Table 4.14). The average diameter of sugar maple fine roots in the FH layer was significantly higher in the control plots, compared with the 2.5 and 7.5 $Mg \cdot ha^{-1}$ treatment plots, whereas within the mineral soil, at the 2.5 $Mg \cdot ha^{-1}$ plots, mean root diameter was almost twice that of the control (Table 4.14). The 2.5 $Mg \cdot ha^{-1}$ plots were again the most responsive for mycorrhizal fine root length and diameter increased in the FH layer, in contrast to the Ah layer, where there was a significant response in diameter, RLD and SRL (Table 4.14). In the Ah layer, mycorrhizal root lengths in the 5.0 $Mg \cdot ha^{-1}$ treatment plots were significantly longer than the 2.5 $Mg \cdot ha^{-1}$ treatment plots, even when RLD and SRL showed the strongest response in the 2.5 $Mg \cdot ha^{-1}$ treatment plots (Table 4.14). Only Ca concentrations in fine roots showed a significant response across treatments when compared by type (Mycorrhizal vs. Tree) and soil layer (FH vs. Ah). In the FH layer, sugar maple fine root Ca concentrations in plots that received 5.0 $Mg \cdot ha^{-1}$ of ash were significantly higher than the control and 2.5 $Mg \cdot ha^{-1}$ treatments plots (Figure 4.15), whereas there was no significant response in fine root metal concentrations (Figure 4.16). The Ca/Al molar ratios of mycorrhizal and sugar maple fine roots in the soil were, on average, 2 to 3 times greater in treatment plots but were also quite variable, and so there was no significant difference from control plots in both the FH and Ah horizons (Figure 4.17)

Table 4.14 | Mean values ± S.E. of fine root metrics, including biomass, root length density (RLD) and specific root length (SRL) in the FH and upper mineral soil layers, three years post wood ash application.

Classification & Soil Layer	Treatment ($Mg \cdot ha^{-1}$)				
	Control (0)	2.5	5.0	7.5	
Tree Fine Root					*P*-value
FH Layer					
Length (cm)	8.87 (0.5)^{a}	8.38 (0.5)^{a}	11.1 (0.5)^{b}	9.46 (0.5)^{ab}	**0.03**
Diameter (mm)	0.49 (0.02)^{b}	0.36 (0.02)^{a}	0.42 (0.03)^{ab}	0.37 (0.03)^{a}	**0.01**
Root Biomass ($g \cdot m^{-2}$)	0.25 (0.1)	0.17 (0.1)	0.22 (0.1)	0.15 (0.1)	0.38
RLD ($cm \cdot cm^{-3}$ soil)	1.00 (0.1)	1.67 (1.1)	1.76 (0.5)	1.95 (1.3)	0.40
SRL ($m \cdot g^{-1}$ DM)	5.19 (1.8)	7.94 (1.8)	4.77 (1.8)	8.95 (1.8)	0.32
Mineral Soil (Ah)					
Length (cm)	10.6 (4.5)	9.03 (3.1)	10.5 (4.7)	10.1 (4.2)	0.19
Diameter (mm)	0.35 (0.05)^{a}	0.60 (0.05)^{b}	0.42 (0.05)^{ab}	0.24 (0.05)^{a}	**<0.001**
Root Biomass ($g \cdot m^{-2}$)	0.24 (0.1)	0.28 (0.1)	0.31 (0.1)	0.21 (0.1)	0.50
RLD ($cm \cdot cm^{-3}$ soil)	1.07 (0.2)	0.95 (0.1)	1.24 (0.4)	1.15 (0.4)	0.50
SRL ($m \cdot g^{-1}$ DM)	4.59 (0.9)	4.54 (0.9)	3.30 (0.9)	5.55 (0.9)	0.39
Mycorrhizal Fine Root					
FH Layer					
Length (cm)	6.96 (0.3)^{a}	8.52 (0.3)^{b}	7.68 (0.3)^{ab}	7.21 (0.3)^{a}	**0.09**
Diameter (mm)	0.18 (0.07)^{ab}	0.23 (0.08)^{c}	0.17 (0.08)^{a}	0.20 (0.08)^{bc}	**0.02**
Root Tips per Segment	15.92 (1.0)	14.2 (1.1)	13.3 (1.1)	15.7 (1.0)	0.22
Root Biomass ($g \cdot m^{-2}$)	0.08 (0.02)	0.08 (0.02)	0.11 (0.02)	0.10 (0.02)	0.75
RLD ($cm \cdot cm^{-3}$ soil)	3.92 (1.0)	5.07 (1.0)	4.88 (1.0)	4.00 (1.0)	0.80
SRL ($m \cdot g^{-1}$ DM)	13.6 (4.3)	20.4 (4.2)	10.3 (4.0)	12.7 (4.2)	0.40
Mineral Soil (Ah)					
Length (cm)	9.62 (0.4)^{ab}	8.91 (0.4)^{a}	10.37 (0.4)^{b}	9.12 (0.4)^{ab}	**0.02**
Diameter (mm)	0.14 (0.1)	0.14 (0.1)	0.16 (0.1)	0.15 (0.1)	0.44
Root Tips per Segment	19.8 (1.7)	18.3 (1.6)	19.9 (1.7)	17.0 (1.6)	0.25
Root Biomass ($g \cdot m^{-2}$)	0.10 (0.01)	0.08 (0.02)	0.17 (0.1)	0.28 (0.3)	0.31
RLD ($cm \cdot cm^{-3}$ soil)	6.05 (2.6)^{ab}	7.54 (4.8)^{b}	3.08 (0.7)^{ab}	1.68 (0.9)^{a}	**0.02**
SRL ($m \cdot g^{-1}$ DM)	10.0 (1.8)^{ab}	13.6 (4.2)^{b}	6.75 (2.9)^{a}	8.04 (5.3)^{ab}	**0.06**

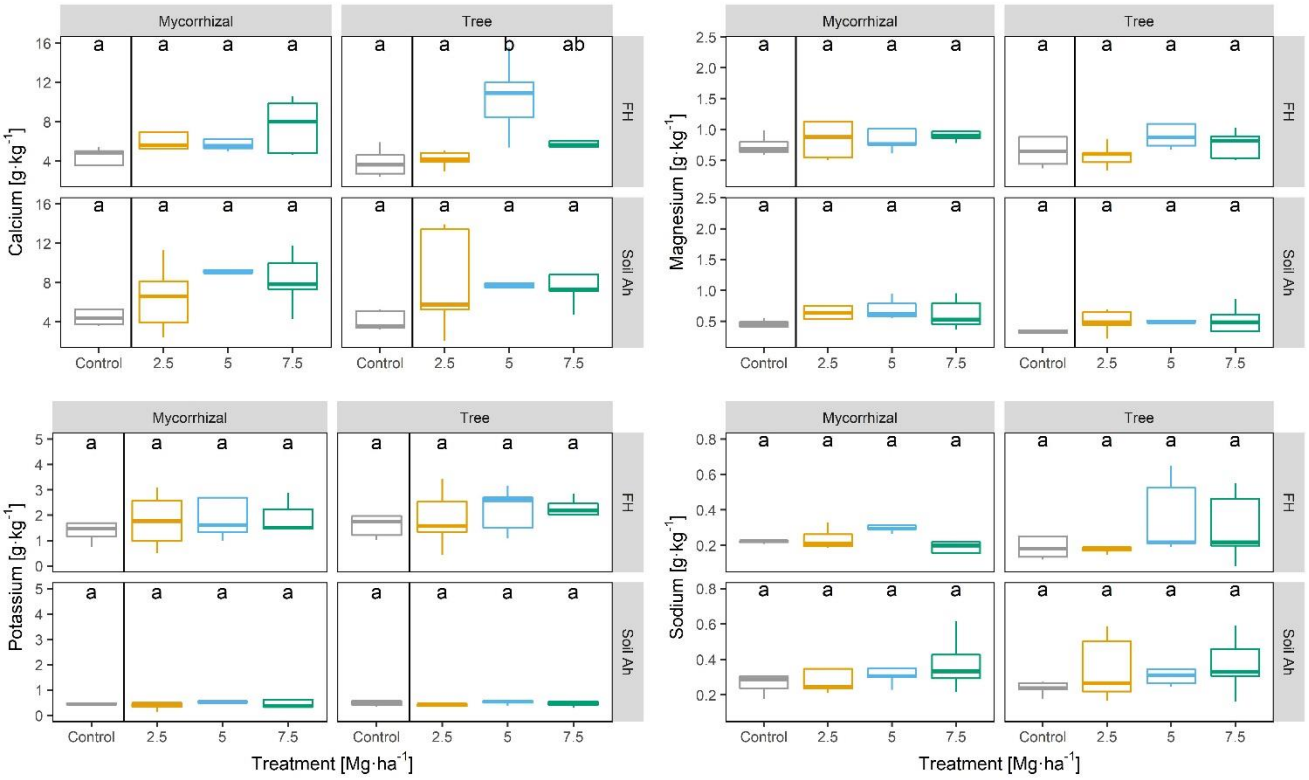

Figure 4.15 | Fine root elemental concentrations of Ca, Mg, K, and Na three years post wood ash application at Porridge Lake, Ontario. Significant difference from pairwise comparison (Tukey's HSD) indicated by letter display.

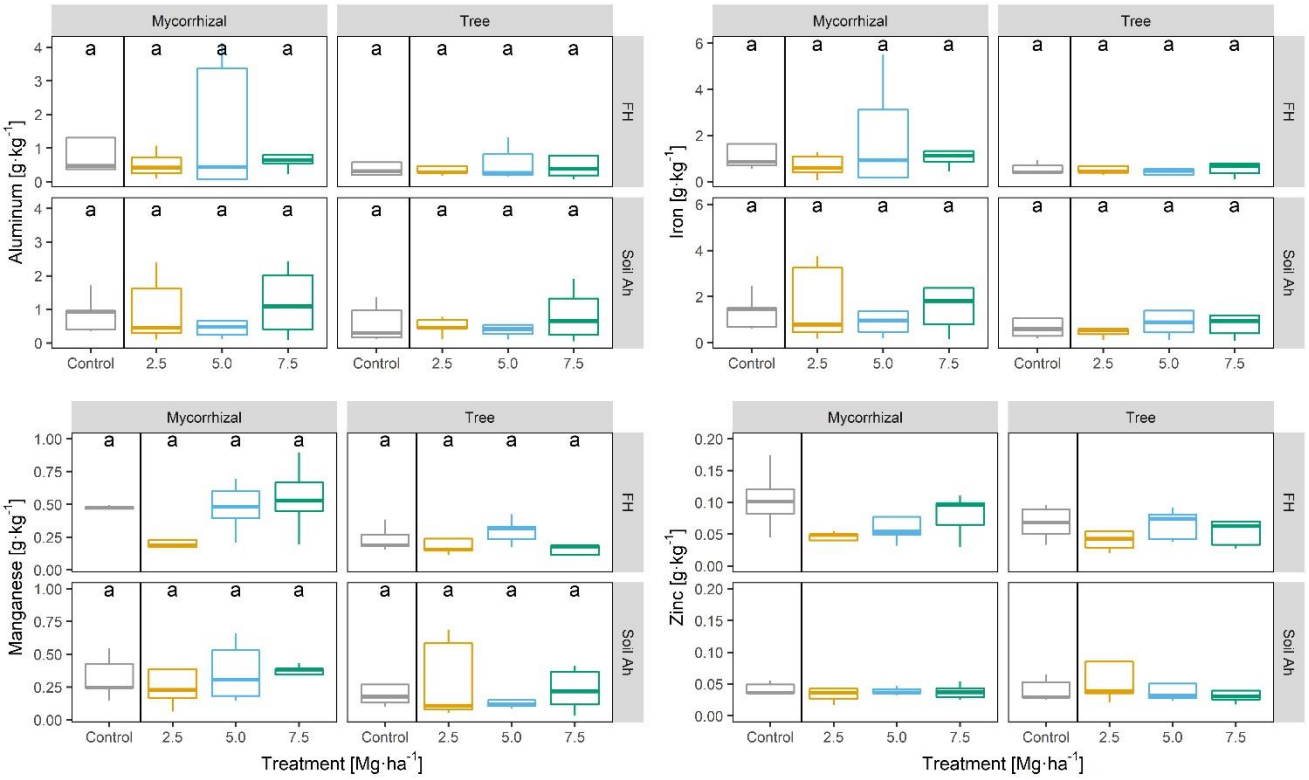

Figure 4.16 | Fine root elemental concentrations of Al, Fe, Mn, and Zn three years post wood ash application at Porridge Lake, Ontario. Significant difference from pairwise comparison (Tukey's HSD) indicated by letter display.

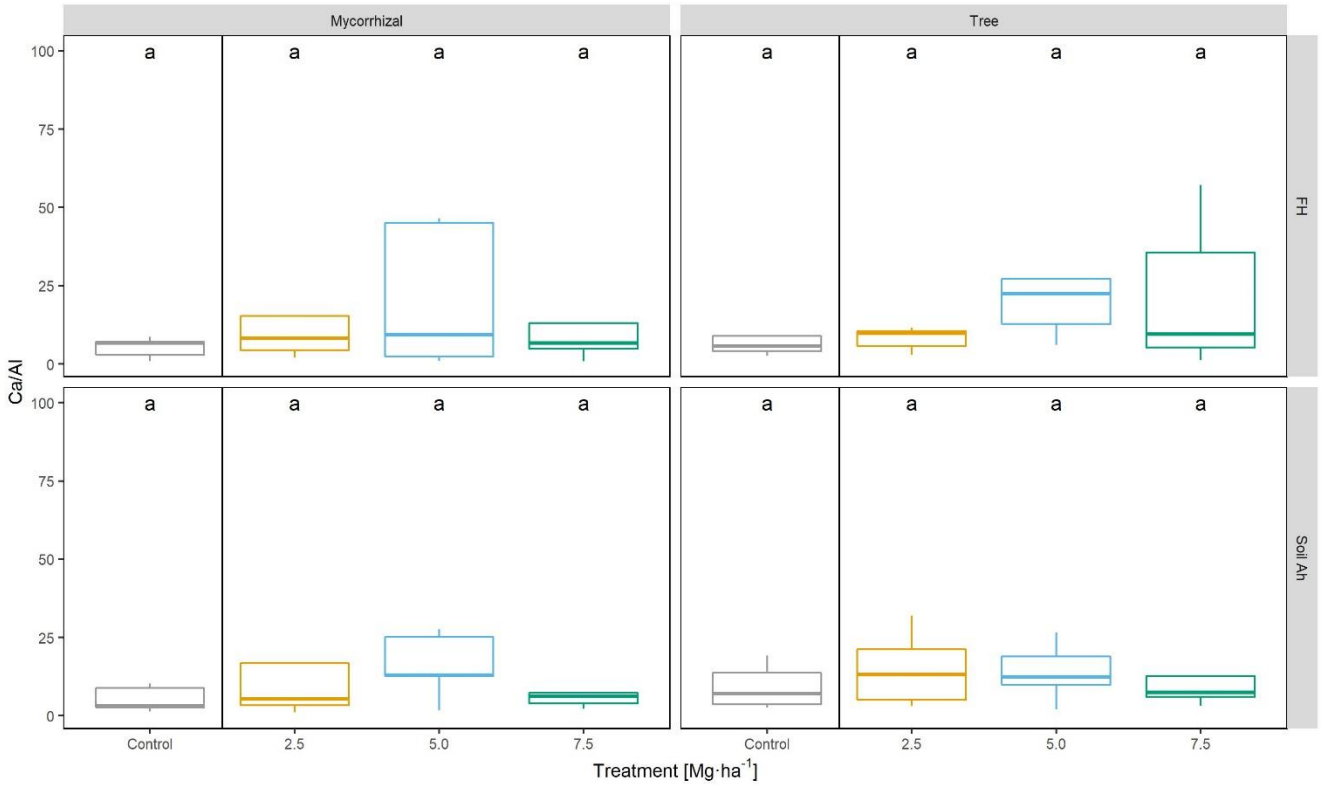

Figure 4.17 | Calcium/Aluminum molar ratios of mycorrhizal and tree fine roots, three post wood ash application from Porridge Lake, Ontario. Significant difference from pairwise comparison (Tukey's HSD) indicated by letter display.

5. Discussion

This study primarily evaluated the below-ground response of a hardwood forest in Central Ontario to mixed wood ash amendments of 2.5, 5.0 and 7.5 $Mg.ha^{-1}$ over a three-year period. The wood ash supplied by the Murray Bros Lumber Company in Madawaska, Ontario, was highly alkaline (pH: 13.2), with Ca (26%) and K (7%) being the most abundant base cations and concentrations of all metals analyzed were below NASM CM1 levels for non-agricultural compost. All three wood ash doses resulted in an immediate pH response in soil leachate draining the LFH horizon that grew stronger each subsequent year. Similar increases were seen in base cation (Ca, Mg, K, Na) concentrations in leachate draining the LFH horizon in response to wood ash amendment, but the timing and magnitude of the response varied amongst the four base cations. The response of DOC, NO_3 and SO_4 was quite variable in soil leachate, but the addition of wood ash tended to decrease concentrations in soil water, with DOC being the most responsive in the first year. Shifts in concentrations of Ca and Al translated into strong improvements in Ca/Al ratios in soil solution. Most of the analyzed metals (Cr, Cu, Ni, Pb, Se) did not change in response to ash treatment, although a few metals (As, Cd, Co, Mo) increased slightly in soil water following ash application, whereas concentrations of other metals (Al, Fe, Mn, Zn) decreased. Three years post ash application, soil pH and exchangeable Ca and Mg were generally elevated in ash-treated plots, especially in deeper soil horizons, whereas most trace metal levels in soil exhibited little difference amongst treatments and remained below regulations for the application of non-agricultural compost (NASM).

Minor shifts occurred in microbial communities, with increased diversity and richness for prokaryotic communities, whereas eukaryotic communities showed no change. Overall, there was no apparent change in the RA of prokaryotes, but there was an increase in the RA of the eukaryote *Hygrocybe* in the FH horizon plots receiving mixed wood ash. A breakdown of functional guilds showed that saprotrophic fungi are dominant in every horizon and that despite being a mixed hardwood forest of sugar maple and American beech, the ectomycorrhizal group had the highest relative abundance in upper mineral soil and the FH horizon. Sugar maple fine root growth and mycorrhizal growth were generally unaffected by ash treatment. Shifts were seen

in sugar maple and mycorrhizal fine root length and diameter, but the response was mostly limited to the 2.5 $Mg \cdot ha^{-1}$ plots in the FH and Ah horizon. There were very few significant differences in fine root chemistry (sugar maple or mycorrhizae), although Ca concentrations were two to four times higher in treated plots compared with controls. Three years after ash application, there were no significant differences in sugar maple foliar chemistry, although foliar Ca concentrations in ash-treated plots were 30-40% higher than in control plots.

5.1 Soil Solution

5.1.1 Response of base cations and pH

It was hypothesized that there would be increased leaching of nutrient cations at higher ash treatments and that leaching responses would be greatest in the upper (LFH) soil horizons. As predicted, in the first year of the study, the pH and concentrations of Ca and Mg in leachate in the LFH horizon increased with ash treatment, whereas there was no response to ash treatment measured in the 30 and 60 cm horizons. The pH response in soil solution in the LFH horizon was quite strong in the first year, and the trend continued in subsequent years. Other studies have shown that there can be a lot of variability in pH response, as site features along with wood ash composition and dosage seem to play a key role and have observed both short-term increases (Cairns et al., 2022; Clarke et al., 2018; Kahl et al., 1996; Meiwes, 1995) and decreases in soil solution pH (Geibe et al., 2003; Maresca et al., 2018; Ring et al., 2006; Rumpf et al., 2001). The muted response of base cations in deeper horizons may reflect the uptake of base cations by vegetation (Arseneau et al., 2021; Wilmot et al., 1996) or immobilization in soil, either by cation exchange or precipitation (Bockheim, 1997; Ohno, 1992). For example, Steenari et al. (1999) noted that when wood ash interacted with water and CO_2, Ca precipitated into calcite. Although not observed in this study, only research has reported that Ca and Mg can harden in the field to form calcite and dolomite, which reduces leaching through the soil profile (Holmberg & Claesson, 2001). There were three rain events at our sites during wood ash application in 2019, which could have been a catalyst for the formation of Ca-salts and reduced leaching of Ca and Mg into the 30 and 60-cm horizons. This is in line with an isotope tracing study by van der Heijden et al. (2013), where they showed that soil organic layers could retain considerable portions of Ca and Mg for up to a year, thus slowing the solubility

and vertical migration of these nutrients into deeper mineral layers (Brais et al., 2015; Gómez-Rey et al., 2012) via soil leachate (Pitman, 2006; Reid & Watmough, 2014). Furthermore, other studies have shown a positive correlation between soil pH and bacterial productivity (Rousk et al., 2009, 2010) and bacterial dependency on water-filled soil pores for movement and nutrient acquisition (Yang & van Elsas, 2018). Within our study, we measured a strong and sustained increase in soil solution pH that may have facilitated the microbial breakdown of the Ca-salts and released the Ca and Mg back into the soil solution in the second year. In fact, results from this study showed a 2x increase of Ca and Mg concentrations in the LFH horizon in 2020 and a 3x increase in 2021, but with minimal vertical movement. The dose size seems to play a large role in the magnitude and timing of the response, as we saw the greatest response in the 5.0 and 7.5 $Mg \cdot ha^{-1}$ treatments, similar to other studies. For example, Gómez-Rey et al. (2012) reported that the greatest leaching response in an 11 $Mg \cdot ha^{-1}$ wood ash trial occurred after the first year, whereas Kahl et al. (1996) found that in a trial using varying dosages, the application of wood ash greater than 13 $Mg \cdot ha^{-1}$ resulted in the leaching of Ca and Mg just one month after treatment. In particular, the study showed that when wood ash doses (and the subsequent concentrations of cations and anions) exceed the soil buffering capacity, there will be rapid leaching of both.

The response of Na and K in soil water to ash application differed somewhat from Ca and Mg. In the first year, there was a lot of within-treatment variability in the LFH layer, so few significant responses to treatment were seen. In later years, there was less variability, but differences amongst treatments were small and mostly insignificant. The three rain events may have caused the opposite effect for K and Na in the LFH and expedited their leaching, as concentrations were greater in the treated plots, but only Na showed a significant response in the 7.5 $Mg \cdot ha^{-1}$ plots. In research on the solubility of nutrients and metals from wood ash, Maresca et al. (2017) found a strong correlation between pH and the leaching of Ca and Mg, whereas K and Na were strongly correlated with rainfall due to their higher solubility. Other studies have also shown that K (Arseneau et al., 2021; Park et al., 2005; Silfverberg, 1998; Staples & Van Rees, 2001) and Na (Mellbo et al., 2008; Ring et al., 2006) have particularly high leaching rates immediately following the application of wood ash. In a study on wood ash leaching rates, Steenari et al. (1999) reported losses as high as 30% for K and 50% for Na from mixed

wood ash in the first year, while Staples & Van Rees (2001) found two times more K in soil solution at a depth of 60 cm within the first year of applying 5.0 $Mg \cdot ha^{-1}$ of wood ash. Given their increased solubility, the rain events and the lysimeter equalization period, we may have missed the initial leaching of K and Na at our sites. Still, by the second year, leaching of K and Na became more apparent, especially for Na in the lower horizons, despite K being the second most abundant element in the wood ash. This is similar to responses seen by Steenari et al. (1999), as Na concentrations were significantly higher at the 60 cm depth, whereas increases in K were restricted to the 30 cm depth.

5.1.2 Response of anions and metals

Concentrations of DOC, NO_3 and SO_4 were higher in 2019 compared with 2020 and 2021, but there was no treatment effect. In later years, concentrations of DOC, NO_3 and SO_4 were generally lower in ash-treated plots compared with controls. Still, it is difficult to assess the response of DOC, NO_3 and SO_4 in the first year (2019) due to the three-month equalization period following the lysimeter installation. The equalization period restricted sampling until fall abscission when the fungal and bacteria breakdown of OM would have been at its peak (Dijkstra & Fitzhugh, 2003). Decomposition of OM in the LFH horizon leads to increased concentrations of DOC and the mineralization of N into NO_3 and NH_4 (Johansen et al., 2021), which is evident in NO_3 concentrations in the first year being 7x greater than 2020 and 12x greater than 2021, while DOC was 2 to 3x greater in 2019 compared with 2020 and 2021. Like DOC and NO_3, SO_4 concentrations were initially high in the LFH and did not show a treatment effect, yet there was increased leaching in 2020 and 2021 which can be attributed to its solubility, as it behaves similarly to K and Na (Augusto et al., 2008; Rumpf et al., 2001). Overall, SO_4 concentrations measured in soil water were low compared to a study using Maritime sourced industrial wood ash in the nearby Haliburton Forest and Wildlife Reserve (Deighton et al., 2021). In contrast to our study, their study found that soil solution pH decreased at the highest loading rate (8 $Mg \cdot ha^{-1}$), with substantially higher concentrations of SO_4 leaching into the 30 cm horizon. The difference in response is most likely attributed to the fact that the wood ash applied at Haliburton forest was sourced from New Brunswick and was

very high in sulphur compared to other studies (Deighton et al., 2021; Pitman, 2006) and possibly because of greater deposition of sea salt sulphur in that region (Vet et al., 2014).

The decreased concentrations of DOC and NO_3 in the treated plots within the LFH horizon could be explained by the relatively low C and N contents of wood ash (Joseph et al., 2022) and altered rates of microbial decomposition, as Midgley & Phillips (2014) suggest that N losses are more closely related to the nutrient efficiency of the dominant mycorrhizae, whereas changes in DOC seems to be mixed. A review of wood ash and lime applications conducted by Lundström et al. (2003) found that for most European studies, there was a decrease in soil solution pH at lower depths (40-50 cm), followed by increased concentrations of Al, SO_4 and NO_3. Our study found the opposite effect, wherein pH increased in the soil solution in 2020 and 2021, while Al decreased, NO_3 remained unresponsive, and SO_4 showed variable responses at the 30 and 60 cm depths. Research conducted on the leaching rates of DOC found a strong connection between increased solution pH and the solubility and subsequent concentrations of DOC in soil solution (Tavakkoli et al., 2015). Furthermore, when Ca concentrations, particularly as $CaCO_3$, are present in the soil at high concentrations, they found a significant reduction in DOC leaching. Therefore, it was postulated that the three rain events would have caused the formation of calcite and helped to explain the lack of DOC leaching seen in other studies.

Vegetation surveys at our sites indicate a co-dominance between sugar maple and American beech, yet ECM had the greatest relative abundance amongst mycorrhizal types. Research on ECM distribution amongst forest composition in the Laurentide mountains of Quebec found that the forests were also ECM-dominated and positively correlated to the percent cover of ECM-associated trees (Villeneuve et al., 1989). Whereas, Carteron et al. (2021) found that ECM had a competitive advantage over AM in nutrient acquisition when saprotrophic fungi dominated the litter horizon. The researchers further noted a strong vertical difference in composition and that soil chemistry and soil horizon, but not forest type, influenced abundance. Ectomycorrhizal dominant forests prefer organic N through nutrient acquisition from litter decomposition (Phillips et al., 2013) and can increase N retention in the LFH and reduce leaching into the inorganic horizons

(Cruz-Paredes et al., 2019; Midgley & Phillips, 2014). Results from Lovett & Mitchell (2004) have shown that it is important to maintain co-dominance between sugar maple and American beech in eastern North America in order to reduce N leaching and inorganic N concentrations in the soil and soil solution (Midgley & Phillips, 2014) as high concentrations of N can further increase acidification of soil and water systems and damage fine roots (Lovett & Mitchell, 2004; Nadelhoffer, 2000; Nave et al., 2013). Similarly, the application of wood ash should have increased microbial activity and the turnover of the LFH horizons and led to an increase in NO_3 in the soil solution (Vestergård et al., 2018). However, this may depend on the ash dosage rate, as Vestergård et al. (2018) found a 10-fold increase in NO_3 leaching following the application of 9 $Mg \cdot ha^{-1}$ of wood ash, whereas our highest dosage was 7.5 $Mg \cdot ha^{-1}$. Based on their observations, lower NO_3 concentrations in soil solution and reductions in atmospheric deposition of N could cause a shift towards N-limitation in the forest.

Over three years, metals remained largely unresponsive to wood ash, but there were reductions in Al, Fe, Mn, and Zn in soil water seen up until the third year, and in contrast, concentrations of As, Co and Mo increased in soil water at various depths. Previous research has shown that wood ash often does not increase metal concentrations in soil solution (Cairns et al., 2021; Deighton et al., 2021; Norström et al., 2012; Pukalchik et al., 2018; Rumpf et al., 2001; Saarsalmi et al., 2005; Williams et al., 1996), as pH directly influences the solubility and adsorption of metals in soils (Bradl, 2004; Rieuwerts et al., 1998). In other studies, increases in metal concentrations have been observed (Chirenje et al., 2002a, 2002b; Ferreiro et al., 2011; Nabeela et al., 2015; Ring et al., 2006), so it appears the response varies and is likely caused by several factors. For example, Mo is a trace metal important for plant growth that becomes more mobile as pH increases (Kaiser et al., 2005), and while Mo in soil water increased slightly, concentrations at our sites were well below provincial guidelines. Similarly, Co concentrations were below EC_{50} value levels that have been proven to damage plant growth (Li et al., 2009).

The increase in pH in the soil solution and the tendency for metals to bind to organic matter (which also decreased in the soil solution) is likely responsible for the decrease in the concentration of some metals (Al, Fe, Mn, Zn) in the soil solution or the lack of response by other metals (Cr, Cu, Ni, Pb, Se). The ash quality used in

our study is also important, as no metal in wood ash applied in our study exceeded CM-1 guidelines. In a wood ash study using recycled wood ash treated with Cu, the researchers found that despite the high alkalinity of wood ash, the treatment resulted in elevated concentrations of heavy metals in the soil solution and, subsequently, plant tissue (Lucchini et al., 2014). Cadmium is another metal of concern, especially since it can bioaccumulate in berries, mushrooms and earthworms (McBride, 2003; Perkiömäki & Fritze, 2005). Perkiömäki & Fritze (2005) spiked wood ash with Cd and found an increase in concentration in mushrooms and, to a lesser degree, in berries, but the untreated wood ash had no effect, and Cd did not become bioavailable. In our study, both Cd and Cu concentrations in the wood ash were 2.7 and 78 $mg.kg^{-1}$, respectively, which is below the regulated levels (CM-1) for non-agricultural compost, and Cd significantly decreased in the 30 cm horizon in the third year for the 7.5 $Mg{\cdot}ha^{-1}$ treated plots. Overall, metal concentrations were either unresponsive or decreased in soil solution following wood ash application, except for As, Co and Mo, but all concentrations in soil solution were below provincial guidelines.

The application of wood ash increased Ca/Al ratios in soil solution, and subsequently, the LFH, 30 and 60 cm depths were not found to be at risk of Al stress (Vanguelova et al., 2007). A molar ratio of ≤ 1.0 in soil solution can be indicative of Al stress and can impede nutrient uptake and tree growth (Cronan & Grigal, 1995), especially for the Al-sensitive sugar maple (Halman et al., 2014). Several studies looking at the health and decline of sugar maples used changes in the basal area as an indication of poor site health (Duchesne et al., 2002; Kolb & McCormick, 1993; Schaberg et al., 2006) and subsequently found a correlation to Ca/Al ratios below critical thresholds of ≤ 1.0 (Long et al., 2009). A study comparing sugar maple to American beech response to cation deficiency found that American beech is more tolerant to Al concentrations in the soil and strongly influenced stand composition. In our study, we found that the decreased concentrations of extractable Al and the lack of response in total Al in the soil point to the application of Ca in the wood ash as having a strong ameliorative effect on the soil and soil solution.

5.2 Soil Chemistry

5.2.1 Organic Horizons

Analysis of the soil organic horizons three years after ash application revealed that wood ash had no treatment effect on litter concentrations for most analytes measured. The only exception was K, which was lowest in the 2.5 $Mg \cdot ha^{-1}$ treatment plots and did not exhibit a consistent treatment effect, suggesting that this response was unrelated to the wood ash treatment. Other studies have suggested that K concentrations in the litter can persist for several years, as Park et al. (2005) found that litter K concentrations increased in the two years following the application of 10 and 20 $Mg \cdot ha^{-1}$ of wood ash.

In contrast to the litter, residual effects of ash application were more evident in the FH horizon, where treated plots had higher pH, exchangeable Ca and lower exchangeable Mn compared with control plots, and concentrations of some metals (Sr, Zn, Co) were also higher in ash treated plots, especially the plots receiving 7.5 Mg ha^{-1}. If the application of wood ash caused the formation of calcite and dolomite, this would have most likely occurred in the FH horizon, and since dolomite dissolves faster (Holmberg & Claesson, 2001), this may explain the strong response of Ca and lack of response from Mg in the FH horizon. Furthermore, it was expected that improvements in the FH horizon would be sustainable, as research conducted by Brais et al. (2015) found that base cation concentrations in the forest floor were still elevated eight years after the application of wood ash. Indeed, treatment effects on pH and Ca concentrations in the FH horizon were still apparent 23 years after applying 5 $Mg \cdot ha^{-1}$ of wood ash in Finland (Saarsalmi et al., 2006). While some metals may have higher concentrations in the FH horizons of treated plots, they are unlikely to cause concern as all heavy metal concentrations in the litter layer are expected to decrease over time (Dimitriou et al., 2006). A study conducted by Saarsalmi et al. (2004) found that after five and ten years, there were still elevated levels of total Cd, Mn and Zn in the FH layer. We found elevated concentrations of total Zn, but Cd concentrations were below detection limits, and only extractable Mn was elevated in the control plots. Strontium concentrations also increased in the FH horizons, but Sr displays similar behaviour to Ca (Dijkstra & Smits, 2002) and is not a metal of concern as the preferential uptake of Ca by trees over Sr is determined by mycorrhizal association and

the presence of Ca-apatite in the soil. Lastly, there is concern that wood ash can contain toxic levels of Mn (Smenderovac et al., 2022), but based on the lack of response in fine root concentrations and the effects seen on yellow birch (Deighton & Watmough, 2020), this may be a species-specific concern and more closely linked to foliar concentrations (Watmough, 2010).

5.2.2 Inorganic Horizons

Treatment effects associated with wood ash application were evident in the mineral soil, where pH and exchangeable concentrations of Ca and Mg tended to be higher in wood ash treated plots, compared with control plots in all three (Ah, Bm, C) horizons. The changes in soil pH were less pronounced than the changes in base cation concentrations, but that may not indicate a lack of treatment response, as current research shows that changes in pH are initially limited to the uppermost soil layers. For example, a study looking at micro changes in soil pH and base cations after ash application found that soil pH can increase in the top 1 cm of the organic horizon, while base cations can increase in the top 5 cm within five months of application (Hansen et al., 2017). Within the first year of wood ash application, Bang-Andreasen et al. (2021) found that increases in soil pH were limited to the top 2 cm of the uppermost soil horizon, and a meta-analysis by Joseph et al. (2022) showed that response is largely limited to soil profiles closest to wood ash application. Within our study, we did not analyze changes in pH within soil horizons, and the eventual mixing of samples has most likely smoothed out increases in pH, yet there are improvements seen in pH, with all treatment values being greater than control values. It would seem that in the short-term, pH responses can be muted and, except in cases where wood ash doses are high, the soil pH response is more long-term (Augusto et al., 2008). Furthermore, it would seem that the less soluble base cations (Ca and Mg) will remain within the soil horizons, as Van Hees et al. (2003) found that the majority of the Ca applied from lime remained within the top 30 cm of the soil, especially in the organic horizon after 15 years. Soil C/N did not show a response in the inorganic horizons, but in a review of wood-ash literature, Reid & Watmough (2014) noted that, on average, there was very little response in soil C/N across wood-ash trials, but also that changes in either element might be obfuscated by equalizing changes in concentrations.

As part of the Ash-Net research group, one of the main underlying goals is to ensure that wood ash application has no adverse effect on the environment. This concern manifests in the composition of wood ash containing varying concentrations of heavy metals that may accumulate after application and become phytotoxic. The results from this study did not show increases in concentrations of metals (Al, As, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Se, Zn) in the soil horizons above levels set forth by the Nutrient Management Act of 2002 (Government of Ontario, 2002b). Other than Sr, only Pb significantly responded to wood ash, but concentrations were greatest in the 2.5 $Mg \cdot ha^{-1}$ plots and may not have come from the wood ash but from the soil (Lucchini et al., 2014). The increases in soil solution and soil pH limit the mobility of metals (Quina & Pinheiro, 2020), and while some concentrations may increase over time, research has shown that, for the most part, metals remain below set guidelines (Deighton et al., 2021; Forest et al., 1995; Moilanen et al., 2006; Saarsalmi et al., 2004). In a review of wood ash used across Canada, Hannam et al. (2018) found that concentrations of Cd, Co, Cr, Cu, Mo, Ni, Pb and Zn in wood ash remain below CCME guidelines and only As was of concern.

5.3 Microbial Response

Relative abundance analysis at the phyla level for both Prokaryote and Eukaryote communities indicates no large changes in soil microbial communities three years after wood ash application. These findings are consistent with a recent review of bacterial and fungal responses to wood ash across Canada conducted by Smenderovac et al. (2022), who noted that responses are primarily site-specific and limited to eukaryotic organisms. The most striking observation is the difference in microbial community composition among the tested soil horizons. Research conducted on microbial response to changes in litter type and tree dominance found that soil microbial communities are relatively similar among forest types and are resistant to change (Rodríguez Rodríguez et al., 2022). Still, there is a distinct difference between functions within each horizon; for instance, the litter horizon is potentially the most important, as this is where decomposition and the recycling of nutrient occurs and is strongly influenced by tree species (Laganière et al., 2010; Rodríguez Rodríguez et al., 2022). Generally, the first phase of decomposition occurs in the litter horizon and during the shift to the second

phase of decomposition, we move down into the FH horizon, where significant changes in bacterial communities can occur (Bray et al., 2012). The change in composition is much slower for fungal communities and is more strongly influenced by edaphic conditions (Haas et al., 2018).

5.3.1 Prokaryotic Response

While there was no evidence of a strong effect of wood ash on soil microbial communities, there were some significant differences in diversity in Prokaryotes, with increased diversity in the FH horizon in plots receiving the highest wood ash treatment and a tendency toward lower diversity in the litter layer. The Shannon-Weiner (H) index indicates that there were increases in alpha (α) diversity in the FH horizon at the highest application rates. Improvements in richness (Chao1) but not evenness (Pielou) at the same treatment intensity corroborates other research that shows that there are no adverse effects on microbial biomass following wood ash treatment (Fernández-Delgado Juarez et al., 2015; Pugliese et al., 2014), but rather, increases in diversity are often seen (Jenkins et al., 2017; Kim et al., 2017; Mason et al., 2021; Noyce et al., 2016). Whereas dominance increased at the expense of diversity in the litter horizon, as signified by the Simpson index (1-D). The change in α diversity in the 7.5 $Mg \cdot ha^{-1}$ sites may indicate compositional changes potentially occurring in the litter layer and may be a case of interspecific competition. Still, research has shown that wood ash applications can change prokaryotic communities, and the bulking of soil samples may be obscuring changes. Bang-Andreasen et al. (2021) found there were profound changes in prokaryote communities within the uppermost soil horizon (0.5 – 1.5 cm) in a mesocosm study using two doses (3 and 9 $Mg \cdot ha^{-1}$) of wood ash and a transition to copiotrophic species. At the same time, Gömöryová et al. (2016) found that changes in microbial communities do not simultaneously occur within a horizon but across a stratified gradient.

The increase in diversity within the FH horizon in the 7.5 $Mg \cdot ha^{-1}$ treatment sites coincides with significant shifts in the RA of the Prokaryote, *Burkholderiales* from the phylum *Proteobacteria* and *Chitinophagales* from the phylum *Bacteroidota*. *Proteobacteria* are characterized as being eutrophic bacterium that benefits from wood ash amendments, and *Burkholderiales* is described as being a potassium-solubilizing

bacteria (Teotia et al., 2016) and as a phosphate solubilizer (*Burkholderia* sp.) (Passos et al., 2014). *Chitinophagales* is a bacterium in soil known to breakdown chitin from recalcitrant organic sources, such as American beech litter, and increases in its RA have been seen in studies applying P-amendments to soils (Mason et al., 2021; Wang et al., 2017). A study by Noyce et al. (2016) hypothesized that the lack of increased microbial activity following the application of wood ash (0.7 to 5.7 $Mg \cdot ha^{-1}$) could be related to limited P availability and our study sites were located just outside Dorset, Ontario, near Haliburton forest, known to be P-limited (Gradowski & Thomas, 2006). This study did not examine P, but the increases in bacteria RA indicate that functional changes at the highest dosage rate are occurring, potentially alleviating P-limitations in the FH and soil. The dominance of ECM may also play a role through the release of organic acids, helping to release P from Al-oxides and increasing the solubility of Al (Fox et al., 1990). Long-term, this can be beneficial to soil, as P derived from the wood ash is not very soluble (Demeyer et al., 2001) and is slow to leach (Mahmood et al., 2002, 2003). Overall, there were no compositional changes in the RA of bacteria at the phylum level, and results remained similar to other studies (Janssen, 2006).

Although relative abundance analysis of functional guilds did not indicate significant changes in saprotrophs, they were most abundant in the 2.5 $Mg \cdot ha^{-1}$ sites, particularly in the FH, which along with ECM, are primarily responsible for the breakdown of SOM (Hansen et al., 2016; Joseph et al., 2022; Lin et al., 2017). There was a spike in C/N in the FH horizon, but only for the 2.5 $Mg \cdot ha^{-1}$ treatment plots and marginal increases in the other two treatments, and both Franklin et al. (2003) and Hyvönen et al. (2008) argue that sites that are N-rich will have decreased decomposition and subsequently higher C concentrations in the soil, especially in ECM-dominated forests with lower litter decomposition rates (Lin et al., 2017). However, since C/N levels were not elevated in the 5.0 and 7.5 $Mg \cdot ha^{-1}$ treatment plots, and the site is co-dominant between sugar maple and American beech, this may not be true for our sites. Furthermore, others have stated that nutrient loading onto forest soils will increase C mineralization (Saidy et al., 2020) and decrease the decomposition of the litter layer by microorganisms (Condron et al., 2010), which might be delayed at our sites if pH shock occurred under the higher treatments.

5.3.2 Eukaryotic Response

There was no significant difference in eukaryotic diversity indices, and with the exception of *Hygrocybe*, fungal communities did not respond significantly to wood ash three years after wood ash application. *Hygrocybe* was found to have a significantly higher RA in the FH of the 2.5 $Mg \cdot ha^{-1}$ sites, but it may not represent a true response as it only happened at the lowest treatment rate. Fungal diversity and composition play a large role in the decomposition of organic matter in the litter horizon and nutrient turnover in soil horizons (McGuire et al., 2013; Talbot et al., 2015), and while there was a treatment gradient for F:B, there was no real treatment effect.

The F:B ratios for treated sites in the litter horizon were ca. 1.5 to 2.0 times greater than the control sites, with the largest change occurring in the 7.5 $Mg \cdot ha^{-1}$ plots, whereas the opposite effect was seen in the FH horizon. As the most labile source of nutrients in forest ecosystems, litter decomposition is essential for nutrient cycling and is driven mainly by saprotrophic fungi and plant pathogens (Kyaschenko et al., 2017; Lindahl et al., 2007; Otsing et al., 2018). Wood ash treatment probably provided an easily accessible source of labile nutrients since wood ash is generally applied on top of the litter layer and explains the F:B gradient in the litter horizon. For the FH horizon, the decrease in F:B could be explained by the significant increases in pH (Fierer & Jackson, 2006), as the FH horizon proved to be the most responsive to wood ash, and bacteria the most responsive to changes in pH (Cruz-Paredes et al., 2019). Cruz-Paredes et al. (2019) pointed out that Acidobacteria decreases as nutrient supplies increase (oligotrophic), and although it was not significant, similar changes were seen in the FH horizon at our sites, which was countered by increases in α diversity. A study testing microbial responses to lime found no change in fungal biomass, but richness did increase in treated sites (Nkongolo & Narendrula-Kotha, 2023). The soil horizon may be trending toward a more copiotrophic system based on the changes occurring in the FH but at a slower pace. Research indicates that changes in the microbiome do not occur immediately after wood ash application, as seen in the study by Jenkins et al. (2017), where no changes in α occurred in the first year or in this study, where no changes were seen after three years.

Instead, it would seem that a compositional shift from oligotrophic species to more copiotrophic species will occur over time.

5.4 Sugar maple fine roots and mycorrhizae

When measured three years after wood ash application, there were few significant differences in sugar maple fine root or mycorrhizal growth metrics or chemistry. Calcium to aluminum molar ratios in the soil solution are often linked to fine root health (Wargo et al., 2003) due to the antagonistic competition for fine root bonding sites (Cronan & Grigal, 1995; Mossor-Pietraszewska, 2001), yet our results show no major changes in fine root metrics or Ca/Al ratios following wood ash application. In the present study, Ca concentrations in mycorrhizal roots in the upper mineral soil and the FH horizon were ca. 1.5 times greater in treated sites, and Ca concentrations (and Ca/Al ratios) in sugar maple fine roots tended to increase with ash application. Overall, results show that three-years post wood ash, there were no discernible increases in fine root biomass or indications of Al stress. The lack of response may be because soil conditions in control plots were not impeding growth as Ca/Al ratios were generally > 1.0, a value identified as a critical threshold for fine roots (Cronan & Grigal, 1995; Vanguelova et al., 2007). Additionally, this study only assessed roots three years after wood ash application, and the short-term responses may have been missed. A few responses were seen in the length and diameter of fine mycorrhizal roots in the FH layer, which corresponded with increased RLD and SRL (although insignificant). This is likely due to the increased concentrations of Ca in the FH horizon (Majdi & Viebke, 2004) and may not have been present in the higher treatments due to alkalinity shock (Couch et al., 2021; Huotari et al., 2015). The effect is only short-term as a wood ash study by Persson & Ahlström (1994), using root cores, showed that root biomass could initially decrease for the first two years before rebounding, followed by significant improvements in the fine root Ca/Al levels. In the long-term, improvements are expected, as Augusto et al. (2008) found improvements to fine root growth 4 to 9 years after wood ash application and similar research by Majdi & Viebke (2004) observed marked increases in root growth eight years after wood ash application.

Decreases in root length and SRL, along with increases in root diameter, are common indications of Al stress in forest soils (Ostonen et al., 2007; Vanguelova et al., 2007; Vardar & Ünal, 2007). Decreased nutrient uptake can lead to an increase in the turnover of fine roots in the soil, resulting in reductions in fine root SRL (Ostonen et al., 2007). However, this study found conflicting results, such as a decrease in mycorrhizal SRL in the mineral horizon but increases in root length in the 5.0 $Mg \cdot ha^{-1}$ sites and the opposite effect occurring at the 2.5 $Mg \cdot ha^{-1}$ sites. Similar conflicting results were also found by Clemensson-Lindell & Persson (1995) and are likely related to the short-term fertilization effects of pH shock at the higher treatments (Huotari et al., 2015).

5.5 Sugar maple foliage

Despite significant increases in base cation concentrations in soil solution and increased Mg and Ca levels in soil in ash treated plots, sugar maple foliar nutrient responses to ash treatment were muted when assessed three years after ash application. Nevertheless, foliar concentrations of Ca and Mg in wood ash treated plots were 20-40% higher in ash treated plots but remained at the lower end of critical thresholds for Ca and Mg in healthy sugar maples (Bal et al., 2015; Kolb & McCormick, 1993; Vizcayno-Soto & Côté, 2004), potentially due to dose size and solubility. Reid & Watmough (2014) found that forests that received lime amendments showed greater improvements in foliar Ca than wood ash, but the treatment size was also a factor. For instance, a study using 11 and 22 $Mg \cdot ha^{-1}$ of wood ash found that only the latter treatment improved concentrations of foliar Ca in hybrid larch (*Larix x marshlinsii)*, similar to results from McDonald et al. (1994) (Ca, Mg and K) using 25 $Mg \cdot ha^{-1}$ of wood ash and fish silage. Further to dosage, foliar response to wood ash application can vary as a function of nutrient, site condition, tree age and time since application (Reid & Watmough, 2014). Both Long et al. (1997) and Moore et al. (2012) found improvements in foliar concentrations of Ca and Mg and decreases in K, respectively, 9 and 15 years after the application of dolomitic lime. Moore & Ouimet (2010) saw a response in mature trees at their site after four years, whereas Deighton & Watmough (2020) found that increases in foliar Ca, Mg and K in sugar maple seedlings were evident ten months following wood ash application. These results correspond with a meta-analysis of wood-ash publications by Augusto et

al. (2008), who reported that foliar concentrations of Ca, Mg and K tend to increase, stabilize and decrease in the long term and by Reid & Watmough (2014) showing young trees (0-10 years) have the greatest response.

While there is evidence that Ca and Mg are elevated (insignificant) in sugar maple foliage (and fine roots), K does not show any trend toward higher values in wood ash treated plots. In a study on leaching tests of wood ash, it was found that even after hardening, K (and Na) were quickly leached from the wood ash (Steenari et al., 1999). In another leaching test, Holmberg et al. (2000) saw as much as 62% of the K in their wood ash lost to leaching within the first seven months due to K being monovalent, making it less likely to compete for exchange sites with Ca and Mg and is typically leached from soil horizons unless K concentrations are high in wood ash (Brais et al., 2015). So, ultimately, K remained closest to the lower limits of optimal foliar nutrition, with K deficiencies linked to a decline in hardwood species in Eastern Canada (Bernier & Brazeau, 1988; Ouimet et al., 2013; Ouimet & Camiré, 1995). Ontario has no recommended K concentrations for wood ash, but other countries, like Sweden, advise that K concentrations make up at least 3% of dry weight (d/w) for wood ash (Mellbo et al., 2008). This aligns with a meta-analysis by Pitman (2006), which reported that K constitutes an average of 2.93% of the total elemental content in wood ash. For reference, K was the second most abundant element making up 7% d/w (Table 2.1), yet this was insufficient to increase soil or foliar K.

The only treatment response in sugar maple foliage was lower C concentrations in the 7.5 $Mg \cdot ha^{-1}$ treatment plots. Studies have shown that wood ash treatments can increase trees' photosynthetic capabilities and sucrose production (Aguraijuja et al., 2015) and the mineralization of C in the FH layer (Saarsalmi et al., 2001). Since soil solution DOC was lowest in the 7.5 $Mg \cdot ha^{-1}$ treatment plots for all depths, there is no evidence of increased mineralization of C in the FH. Therefore, the most likely explanation is that the marginal increases in foliar Ca and K resulted in lower C concentrations in sugar maple foliage.

Although foliar metal concentrations did not show a significant treatment response, there were lower concentrations of Mn in the two highest wood ash treatment plots, while concentrations in all plots were much lower (ca. 0.62 $g \cdot kg^{-1}$) than the median values reported by Moore & Ouimet (2010) of 2.4 $g \cdot kg^{-1}$ for unhealthy stands in northeastern North America. While there was no treatment effect on Mn in soil, a study on Mn

cycling conducted in central Ontario showed a strong correlation between foliar Mn concentrations and soil Mn concentrations, especially in acidic soils (Watmough et al., 2007). In addition, a study in the Duchesnay Experimental Forest in Quebec found significant reductions in sugar maple foliar Mn concentrations 15 years after applying dolomitic lime (Moore et al., 2012), a good indication that foliar Mn concentrations will decrease over time, especially with the reduction in Mn concentrations seen in the soil solution.

6. Conclusion

This research thesis aimed to ascertain the short-term, below-ground response of forests that may have been affected by long-term acidic deposition to varying dosages of wood ash. The underlying goals were to analyze changes in soil solution chemistry, the organic and inorganic soil horizons and foliage, and potential shifts in microorganisms and fine root development. The general response across all sampling years is that wood ash increases pH in the soil solution and increases concentrations of base cations in both the soil and soil leachate. The magnitude of the effect on soil properties depended on dosage size, but temporal and spatial shifts seem to indicate that there may be a stronger treatment response in the long-term. Percolation of cations into lower horizons was primarily based on the propensity of the element to leach, apparent shifts were seen during the three-year study period, and significant improvements were seen in Ca/Al, reducing the risk of fine root damage. Although wood ash contains metals and their responses differed, no detectable risk was noted in the soil or the soil solution, and concentrations remained below current water quality guidelines.

Sugar maple response measured three years after application indicated a general improvement in root and foliar Ca status, but no notable differences in other chemical properties, and fine root growth were largely unaffected. Furthermore, in the short term, there was no strong compositional shift in bacteria or fungal communities, but there were shifts seen in diversity, but it remains to be seen what this means for ecosystem functionality. The fundamental responses seen throughout this research indicate that changes that come about by applying wood ash are moderate in the short term, while a review of long-term studies seems to indicate that it can be stronger over time.

The results from this thesis show that wood ash is an acceptable bio-amendment to forest systems and does not seem to impose any immediate risk to metal accumulation. However, determining the correct dosage and quality of the wood ash remains crucial in ensuring a positive response and more long-term monitoring is needed, as wood ash in Canada has mostly focused on short-term responses. Many of the responses at the microbial level are clear indications that the microcosm is much slower to respond to wood ash, but compositional shifts are possible in the long term.

7. References

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8. Appendix

Appendix A | Sample means (±SE) of the LFH, 30 and 60 cm lysimeter soil water pH and base cation concentrations. A block ANOVA was used to determine significant differences between all treatments and control. The letter display indicates significant differences ($p<0.05$) using the Tukey HSD.

Year	Treatment	Depth	pH	Ca	Mg	K	Na
	(Mg ha^{-1})	(cm)		----------mg·L^{-1}----------			
2019	Control	0	5.6 (0.2)^{a}	7.05 (1.2)	1.12 (0.3)^{a}	8.40 (4.5)	0.42 (0.9)^{a}
	2.5	0	6.8 (0.2)^{b}	10.5 (1.2)	1.79 (0.3)^{ab}	21.7 (5.3)	3.58 (0.9)^{ab}
	5.0	0	6.8 (0.2)^{b}	10.7 (1.2)	1.69 (0.3)^{ab}	19.3 (5.4)	2.59 (0.9)^{ab}
	7.5	0	7.0 (0.2)^{b}	10.7 (1.2)	2.36 (0.3)^{b}	21.8 (5.5)	4.59 (0.9)^{b}
	Control	30	5.4 (0.2)	3.61 (0.6)	1.09 (0.2)^{b}	0.52 (2.7)	0.62 (0.6)
	2.5	30	6.0 (0.2)	1.61 (0.5)	0.41 (0.2)^{a}	1.53 (2.4)	0.96 (0.5)
	5.0	30	6.1 (0.2)	1.72 (0.7)	0.29 (0.2)^{a}	5.26 (3.3)	1.93 (0.7)
	7.5	30	6.0 (0.2)	1.54 (0.6)	0.35 (0.2)^{ab}	6.40 (3.1)	2.65 (0.6)
	Control	60	5.9 (0.2)	2.94 (0.2)^{b}	0.60 (0.05)^{b}	1.04 (2.1)	0.92 (0.2)
	2.5	60	6.0 (0.1)	2.08 (0.2)^{a}	0.39 (0.04)^{a}	0.74 (1.7)	0.91 (0.2)
	5.0	60	6.3 (0.2)	1.34 (0.2)^{a}	0.36 (0.05)^{a}	6.56 (2.2)	1.53 (0.2)
	7.5	60	5.9 (0.2)	1.35 (0.2)^{a}	0.33 (0.05)^{a}	1.14 (2.2)	0.96 (0.2)
2020	Control	0	5.6 (0.05)^{a}	5.62 (1.1)^{a}	0.76 (0.1)^{a}	2.27 (0.6)	0.24 (0.05)^{a}
	2.5	0	6.8 (0.05)^{b}	13.3 (1.0)^{b}	1.44 (0.1)^{b}	3.37 (0.6)	0.46 (0.05)^{bc}
	5.0	0	7.0 (0.05)^{c}	13.2 (1.0)^{b}	1.36 (0.1)^{b}	2.40 (0.6)	0.31 (0.05)^{ab}
	7.5	0	7.3 (0.05)^{d}	14.8 (1.0)^{b}	1.61 (0.1)^{b}	4.38 (0.6)	0.57 (0.05)^{c}
	Control	30	5.4 (0.06)^{a}	1.32 (0.5)	0.32 (0.06)	1.23 (1.2)^{a}	0.59 (0.1)^{a}
	2.5	30	5.8 (0.07)^{a}	1.46 (0.6)	0.28 (0.07)	0.70 (0.2)^{a}	1.40 (0.1)^{b}
	5.0	30	5.8 (0.07)^{b}	1.04 (0.6)	0.31 (0.07)	3.07 (0.2)^{b}	1.71 (0.1)^{b}
	7.5	30	6.3 (0.08)^{c}	2.89 (0.6)	0.36 (0.07)	2.84 (0.2)^{b}	2.24 (0.1)^{c}
	Control	60	5.9 (0.1)	1.53 (0.2)	0.33 (0.03)	0.39 (0.1)	0.91 (0.1)^{a}
	2.5	60	5.7 (0.1)	1.15 (0.2)	0.22 (0.03)	0.38 (0.1)	1.08 (0.1)^{ab}
	5.0	60	6.0 (0.1)	1.56 (0.2)	0.32 (0.03)	0.70 (0.1)	1.47 (0.1)^{b}
	7.5	60	6.0 (0.1)	1.48 (0.2)	0.30 (0.03)	0.53 (0.1)	1.24 (0.1)^{ab}
2021	Control	0	5.4 (0.05)^{a}	3.19 (0.5)^{a}	0.43 (0.05)^{a}	1.43 (0.2)^{a}	0.09 (0.02)^{a}
	2.5	0	6.5 (0.05)^{b}	9.26 (0.5)^{b}	0.76 (0.05)^{b}	1.54 (0.2)^{ab}	0.17 (0.02)^{b}
	5.0	0	6.9 (0.05)^{c}	11.8 (0.5)^{c}	0.93 (0.05)^{c}	1.53 (0.2)^{ab}	0.17 (0.02)^{b}
	7.5	0	7.2 (0.05)^{d}	16.6 (0.5)^{d}	1.20 (0.05)^{d}	2.25 (0.2)^{b}	0.29 (0.02)^{c}
	Control	30	5.7 (0.08)^{a}	1.59 (0.1)^{ab}	0.39 (0.03)^{b}	0.52 (0.3)^{a}	0.55 (0.07)^{a}
	2.5	30	5.9 (0.08)^{a}	1.79 (0.1)^{b}	0.28 (0.03)^{ab}	0.36 (0.3)^{a}	0.67 (0.07)^{a}
	5.0	30	5.9 (0.08)^{a}	1.14 (0.1)^{a}	0.24 (0.03)^{a}	1.53 (0.3)^{b}	1.15 (0.07)^{b}
	7.5	30	6.3 (0.08)^{b}	1.23 (0.2)^{a}	0.28 (0.03)^{ab}	1.14 (0.3)^{ab}	1.66 (0.07)^{c}
	Control	60	6.0 (0.05)	1.57 (0.1)	0.35 (0.02)^{b}	0.28 (0.1)	0.71 (0.04)^{a}
	2.5	60	6.1 (0.05)	1.36 (0.1)	0.25 (0.02)^{a}	0.35 (0.1)	0.97 (0.04)^{b}
	5.0	60	6.1 (0.05)	1.21 (0.1)	0.26 (0.02)^{a}	0.80 (0.1)	0.98 (0.04)^{bc}
	7.5	60	6.2 (0.05)	1.62 (0.1)	0.23 (0.02)^{a}	0.71 (0.1)	1.10 (0.04)^{c}

Appendix B | Sample means (±SE) of the LFH, 30 and 60 cm lysimeter soil water concentrations of DOC, NO_3, SO_4 and Cl. A block ANOVA was used to determine significant differences between all treatments and control. The letter display indicates significant differences ($p<0.05$) using the Tukey HSD.

Year	Treatment	Depth	DOC	NO_3	SO_4	Cl
	(Mg ha^{-1})	(cm)	----------mg·L^{-1}----------			
2019	Control	0	51.4 (10)	4.59 (1.1)	2.31 (2.0)	0.95 (0.3)
	2.5	0	75.9 (10)	2.60 (1.1)	7.14 (2.0)	1.36 (0.3)
	5.0	0	60.7 (10)	4.19 (1.1)	5.56 (2.0)	1.28 (0.3)
	7.5	0	59.6 (10)	4.36 (1.1)	7.24 (2.0)	1.38 (0.3)
	Control	30	21.2 (4.3)^{b}	0.11 (0.05)	2.23 (0.5)	3.06 (0.9)
	2.5	30	4.46 (3.7)^{a}	0.19 (0.05)	2.00 (0.5)	3.43 (0.8)
	5.0	30	13.0 (5.0)^{ab}	0.05 (0.06)	2.47 (0.6)	4.53 (1.0)
	7.5	30	6.63 (4.8)^{ab}	0.17 (0.06)	2.91 (0.6)	4.52 (1.0)
	Control	60	7.81 (1.3)^{b}	0.39 (0.07)	2.64 (0.8)	3.19 (0.6)
	2.5	60	3.68 (1.0)^{ab}	0.34 (0.07)	2.18 (0.7)	2.88 (0.6)
	5.0	60	5.54 (1.3)^{ab}	0.23 (0.07)	4.48 (0.8)	3.40 (0.6)
	7.5	60	2.47 (1.3)^{a}	0.28 (0.07)	2.35 (0.8)	2.82 (0.6)
2020	Control	0	28.7 (1.5)^{b}	0.96 (0.1)^{b}	0.88 (0.1)^{b}	0.55 (0.07)
	2.5	0	22.8 (1.5)^{a}	0.48 (0.1)^{a}	0.57 (0.1)^{a}	0.32 (0.07)
	5.0	0	24.6 (1.5)^{ab}	0.34 (0.1)^{a}	0.64 (0.1)^{ab}	0.37 (0.07)
	7.5	0	21.8 (1.5)^{a}	0.26 (0.1)^{a}	0.54 (0.1)^{a}	0.34 (0.07)
	Control	30	9.73 (1.1)	0.03 (0.01)	1.13 (0.1)^{a}	2.78 (0.2)
	2.5	30	5.45 (1.3)	0.03 (0.01)	2.49 (0.2)^{bc}	1.97 (0.2)
	5.0	30	9.30 (1.2)	0.02 (0.01)	2.11 (0.2)^{b}	2.99 (0.2)
	7.5	30	6.54(1.3)	0.05 (0.01)	2.74 (0.2)^{c}	2.19 (0.2)
	Control	60	3.36 (0.6)	0.05 (0.02)	2.68 (0.2)	1.59 (0.2)
	2.5	60	2.55 (0.6)	0.13 (0.02)	2.12 (0.2)	1.40 (0.2)
	5.0	60	4.08 (0.6)	0.07 (0.02)	2.65 (0.2)	2.02 (0.2)
	7.5	60	2.89 (0.6)	0.06 (0.02)	2.61 (0.2)	1.90 (0.2)
2021	Control	0	23.8 (1.0)^{b}	0.39 (0.06)	0.36 (0.02)^{c}	0.16 (0.02)
	2.5	0	19.6 (1.0)^{a}	0.34 (0.07)	0.27 (0.02)^{ab}	0.15 (0.02)
	5.0	0	19.7 (1.0)^{a}	0.23 (0.07)	0.32 (0.02)^{bc}	0.13 (0.02)
	7.5	0	18.3 (1.0)^{a}	0.28 (0.07)	0.20 (0.02)^{a}	0.11 (0.02)
	Control	30	6.82 (0.8)	0.07 (0.06)	1.67 (0.1)^{a}	1.51 (0.2)
	2.5	30	7.25 (0.8)	0.15 (0.06)	1.76 (0.1)^{ab}	1.49 (0.2)
	5.0	30	7.21 (0.8)	0.02 (0.06)	1.74 (0.1)^{ab}	2.10 (0.2)
	7.5	30	4.66 (0.8)	0.04 (0.06)	2.12 (0.1)^{b}	1.83 (0.2)
	Control	60	3.46 (0.4)	0.08 (0.02)	2.61 (0.1)	0.88 (0.1)
	2.5	60	3.88 (0.4)	0.08 (0.02)	2.70 (0.1)	0.92 (0.1)
	5.0	60	3.52 (0.4)	0.03 (0.02)	2.56 (0.1)	1.15 (0.1)
	7.5	60	2.92 (0.4)	0.07 (0.02)	2.67 (0.1)	1.34 (0.1)

Appendix C | Sample means (±SE) of the LFH, 30 and 60 cm lysimeter soil water elemental concentrations, from Porridge Lake from 2019 to 2021. A block ANOVA was used to determine significant differences between all treatments and control. The letter display indicates significant differences ($p<0.05$) using the Tukey HSD.

Year	Treatment	Depth	Al	Fe	Mn	Zn	Ca/Al
	(Mg ha^{-1})	(cm)	----------mg·L^{-1}----------				
2019	Control	LFH	0.33 (0.04)	0.30 (0.05)	0.21 (0.03)^{b}	0.15 (0.04)	22.5 (18)
	2.5	LFH	0.23 (0.04)	0.24 (0.05)	0.07 (0.03)^{a}	0.08 (0.04)	70.0 (18)
	5.0	LFH	0.30 (0.04)	0.25 (0.05)	0.10 (0.03)^{ab}	0.09 (0.04)	50.2 (18)
	7.5	LFH	0.19 (0.04)	0.27 (0.05)	0.11 (0.03)^{ab}	0.13 (0.04)	77.4 (18)
	Control	30	0.49 (0.07)^{b}	0.04 (0.01)^{ab}	0.03 (0.006)^{a}	0.06 (0.01)	8.88 (7.9)^{a}
	2.5	30	0.06 (0.06)^{a}	0.02 (0.01)^{a}	0.05 (0.005)^{b}	0.05 (0.01)	32.6 (6.9)^{ab}
	5.0	30	0.31 (0.08)^{ab}	0.06 (0.01)^{b}	0.02 (0.007)^{a}	0.05 (0.01)	55.5 (9.4)^{b}
	7.5	30	0.12 (0.07)^{a}	0.03 (0.01)^{ab}	0.03 (0.007)^{ab}	0.04 (0.01)	13.4 (8.9)^{a}
	Control	60	0.11 (0.03)	0.01 (0.005)	0.14 (0.01)^{b}	0.04 (0.03)	18.5 (4.3)
	2.5	60	0.10 (0.02)	0.01 (0.004)	0.05 (0.01)^{a}	0.10 (0.03)	21.7 (3.5)
	5.0	60	0.09 (0.03)	0.02 (0.005)	0.04 (0.01)^{a}	0.03 (0.04)	15.0 (4.4)
	7.5	60	0.07 (0.03)	0.01 (0.005)	0.02 (0.01)^{a}	0.03 (0.04)	19.2 (4.3)
2020	Control	LFH	0.14 (0.01)^{b}	0.10 (0.01)	0.09 (0.01)^{b}	0.09 (0.01)	66.5 (48)^{a}
	2.5	LFH	0.07 (0.01)^{a}	0.07 (0.01)	0.02 (0.01)^{a}	0.06 (0.01)	247 (47)^{b}
	5.0	LFH	0.05 (0.01)^{a}	0.05 (0.01)	0.009 (0.01)^{a}	0.05 (0.01)	254 (47)^{b}
	7.5	LFH	0.04 (0.01)^{a}	0.06 (0.01)	0.01 (0.01)^{a}	0.07 (0.01)	445 (47)^{c}
	Control	30	0.26 (0.04)^{ab}	0.05 (0.005)^{c}	0.02 (0.01)	0.08 (0.01)	8.54 (5.1)^{a}
	2.5	30	0.15 (0.05)^{a}	0.02 (0.006)^{a}	0.05 (0.01)	0.06 (0.01)	14.2 (5.6)^{ab}
	5.0	30	0.35 (0.05)^{b}	0.04 (0.005)^{bc}	0.02 (0.01)	0.05 (0.01)	5.68 (5.5)^{a}
	7.5	30	0.13 (0.05)^{a}	0.02 (0.006)^{ab}	0.04 (0.01)	008 (0.01)	31.9(5.8)^{b}
	Control	60	0.09 (0.01)	0.009 (0.02)	0.07 (0.03)	0.04 (0.005)	23.0 (8.7)
	2.5	60	0.07 (0.02)	0.008 (0.02)	0.02 (0.03)	0.04 (0.005)	31.6 (8.5)
	5.0	60	0.12 (0.02)	0.05 (0.02)	0.04 (0.03)	0.03 (0.005)	26.3 (8.7)
	7.5	60	0.07 (0.02)	0.009 (0.02)	0.04 (0.03)	0.04 (0.005)	26.1 (8.7)
2021	Control	LFH	0.13 (0.004)^{c}	0.08 (0.04)^{c}	0.05 (0.04)^{b}	0.08 (0.006)^{b}	22.7 (27)^{a}
	2.5	LFH	0.07 (0.004)^{b}	0.06 (0.04)^{b}	0.007 (0.04)^{a}	0.04 (0.006)^{a}	136 (27)^{b}
	5.0	LFH	0.03 (0.004)^{a}	0.04 (0.04)^{ab}	0.004 (0.04)^{a}	0.06 (0.006)^{a}	276 (27)^{c}
	7.5	LFH	0.03 (0.004)^{a}	0.03 (0.04)^{a}	0.003 (0.04)^{a}	0..06 (0.006)^{ab}	525 (28)^{d}
	Control	30	0.27 (0.03)^{b}	0.03 (0.01)^{ab}	0.02 (0.01)	0.07 (0.01)^{ab}	14.7 (5.7)
	2.5	30	0.27 (0.03)^{b}	0.04 (0.01)^{ab}	0.02 (0.01)	0.07 (0.01)^{ab}	9.26 (5.8)
	5.0	30	0.28 (0.03)^{b}	0.02 (0.01)^{a}	0.04 (0.01)	0.05 (0.01)^{a}	13.3 (5.6)
	7.5	30	0.11 (0.03)^{a}	0.06 (0.01)^{b}	0.03 (0.01)	0.09 (0.01)^{b}	9.30 (5.9)
	Control	60	0.12 (0.02)	0.01 (0.003)	0.01 (0.007)	0.05 (0.009)	15.2 (3.2)
	2.5	60	0.13 (0.01)	0.01 (0.003)	0.02 (0.007)	0.04 (0.008)	17.3 (3.1)
	5.0	60	0.10 (0.01)	0.009 (0.003)	0.01 (0.007)	0.04 (0.009)	18.6 (3.2)
	7.5	60	0.09 (0.01)	0.02 (0.003)	0.03 (0.007)	0.05 (0.009)	18.3 (3.1)

Appendix D | Sample means (±SE) of the LFH, 30 and 60 cm lysimeter soil water metal concentrations. A block ANOVA was used to determine significant differences between all treatments and control. The letter display indicates significant differences (p<0.05) using the Tukey HSD.

Year	Treatment	Depth	As	Cd	Co	Cr	Cu
	(Mg ha^{-1})	(cm)	----------ug·L^{-1}----------				
2019	Control	0	0.37 (0.07)	0.13 (0.02)	0.13 (0.1)	0.37 (0.3)	67.0 (22)
	2.5	0	0.51 (0.07)	0.09 (0.02)	0.34 (0.1)	1.21 (0.3)	33.2 (22)
	5.0	0	0.41 (0.07)	0.11 (0.02)	0.32 (0.1)	0.93 (0.3)	33.2 (22)
	7.5	0	0.54 (0.07)	0.08 (0.02)	0.52 (0.1)	1.46 (0.3)	58.4 (22)
	Control	30	0.21 (0.06)	0.13 (0.05)	0.41 (0.1)^{a}	0.52 (0.09)	9.14 (2.3)
	2.5	30	0.09 (0.05)	0.17 (0.05)	0.65 (0.1)^{ab}	0.24 (0.08)	5.28 (2.0)
	5.0	30	0.18 (0.07)	0.12 (0.06)	0.54 (0.1)^{ab}	0.61 (0.11)	9.38 (2.7)
	7.5	30	0.13 (0.07)	0.10 (0.06)	0.99 (0.1)^{b}	0.40 (0.08)	6.12 (2.6)
	Control	60	0.11 (0.02)^{ab}	0.19 (0.04)	0.63 (0.1)	0.29 (0.08)	7.94 (1.1)
	2.5	60	0.08 (0.02)^{ab}	0.18 (0.03)	0.59 (0.1)	0.28 (0.06)	5.35 (0.9)
	5.0	60	0.15 (0.02)^{b}	0.14 (0.04)	0.68 (0.1)	0.41 (0.08)	5.38 (1.1)
	7.5	60	0.04 (0.02)^{a}	0.11 (0.04)	0.71 (0.1)	0.21 (0.08)	5.04 (1.1)
2020	Control	0	0.13 (0.02)	0.06 (0.01)	0.03 (0.01)	0.20 (0.03)	8.09 (1.6)
	2.5	0	0.20 (0.02)	0.03 (0.01)	0.04 (0.01)	0.22 (0.03)	6.86 (1.6)
	5.0	0	0.20 (0.02)	0.03 (0.01)	0.04 (0.01)	0.20 (0.03)	4.55 (1.6)
	7.5	0	0.16 (0.02)	0.03 (0.01)	0.05 (0.01)	0.26 (0.03)	8.68 (1.6)
	Control	30	0.19 (0.03)	0.10 (0.02)	0.30 (0.05)^{a}	0.33 (0.05)^{ab}	9.08 (1.5)
	2.5	30	0.07 (0.04)	0.08 (0.02)	0.69 (0.06)^{b}	0.25 (0.06)^{a}	9.21 (1.8)
	5.0	30	0.12 (0.03)	0.04 (0.02)	0.26 (0.06)^{a}	0.48 (0.06)^{b}	8.21 (1.7)
	7.5	30	0.07 (0.04)	0.08 (0.02)	0.38 (0.06)^{a}	0.33 (0.06)^{ab}	12.4 (1.7)
	Control	60	0.06 (0.05)	0.07 (0.02)	0.30 (0.1)	0.17 (0.04)	7.80 (1.0)
	2.5	60	0.06 (0.05)	0.06 (0.02)	0.53 (0.1)	0.18 (0.04)	7.71 (1.0)
	5.0	60	0.04 (0.05)	0.05 (0.02)	0.45 (0.1)	0.23 (0.04)	6.25 (1.0)
	7.5	60	0.04 (0.05)	0.05 (0.02)	0.47 (0.1)	0.15 (0.04)	5.66 (1.0)
2021	Control	0	0.12 (0.01)^{a}	0.05 (0.01)	0.03 (0.008)	0.26 (0.01)	5.16 (0.4)^{ab}
	2.5	0	0.21 (0.01)^{c}	0.03 (0.01)	0.04 (0.008)	0.26 (0.01)	4.12 (0.4)^{ab}
	5.0	0	0.18 (0.01)^{bc}	0.04 (0.01)	0.02 (0.008)	0.25 (0.01)	3.88 (0.4)^{a}
	7.5	0	0.17 (0.01)^{b}	0.03 (0.01)	0.03 (0.009)	0.24 (0.01)	5.44 (0.4)^{b}
	Control	30	0.20 (0.02)^{b}	0.12 (0.02)^{b}	0.32 (0.1)	0.49 (0.06)	8.36 (9.2)
	2.5	30	0.14 (0.02)^{ab}	0.10 (0.02)^{ab}	0.28 (0.1)	0.49 (0.06)	18.5 (9.4)
	5.0	30	0.15 (0.02)^{ab}	0.08 (0.02)^{ab}	0.44 (0.1)	0.54 (0.06)	6.34 (9.2)
	7.5	30	0.12 (0.02)^{a}	0.05 (0.02)^{a}	0.60 (0.1)	0.33 (0.06)	37.1 (9.7)
	Control	60	0.09 (0.01)	0.06 (0.02)	0.30 (0.05)^{a}	0.31 (0.03)	8.06 (6.5)
	2.5	60	0.09 (0.01)	0.09 (0.02)	0.48 (0.05)^{ab}	0.34 (0.03)	16.0 (6.3)
	5.0	60	0.09 (0.01)	0.07 (0.02)	0.29 (0.05)^{a}	0.32 (0.03)	7.94 (6.4)
	7.5	60	0.10 (0.01)	0.09 (0.02)	0.65 (0.05)^{b}	0.32 (0.03)	5.70 (6.3)

Appendix E | Sample means (±SE) of the LFH, 30 and 60 cm lysimeter soil water metal concentrations. A block ANOVA was used to determine significant differences between all treatments and control. The letter display indicates significant differences (p<0.05) using the Tukey HSD.

Year	Treatment	Depth	Mo	Ni	Pb	Se
	(Mg ha^{-1})	(cm)	----------ug·L^{-1}----------			
2019	Control	0	0.15 (0.3)	1.53 (0.5)	12.7 (2.6)	0.17 (0.06)
	2.5	0	0.66 (0.2)	2.32 (0.5)	5.47 (2.6)	0.30 (0.06)
	5.0	0	0.52 (0.2)	2.83 (0.5)	5.55 (2.6)	0.25 (0.06)
	7.5	0	0.56 (0.2)	2.82 (0.5)	8.67 (2.6)	0.26 (0.06)
	Control	30	0.18 (0.2)	1.54 (0.2)	0.24 (0.07)	0.07 (0.07)
	2.5	30	0.10 (0.1)	1.02 (0.1)	0.19 (0.06)	0.07 (0.07)
	5.0	30	0.38 (0.2)	1.28 (0.2)	0.39 (0.08)	0.17 (0.09)
	7.5	30	0.41 (0.2)	1.36 (0.2)	0.21 (0.07)	0.09 (0.09)
	Control	60	0.06 (0.2)	1.05 (0.2)	0.17 (0.04)	0.06 (0.03)
	2.5	60	0.15 (0.1)	1.01 (0.1)	0.23 (0.03)	0.04 (0.02)
	5.0	60	0.48 (0.2)	1.24 (0.2)	0.15 (0.04)	0.14 (0.03)
	7.5	60	0.15 (0.1)	1.00 (0.2)	0.27 (0.04)	BDL
2020	Control	0	0.11 (0.04)	0.72 (0.06)	1.35 (0.4)	0.06 (0.02)
	2.5	0	0.15 (0.04)	0.82 (0.06)	0.71 (0.4)	0.10 (0.02)
	5.0	0	0.24 (0.04)	0.71 (0.06)	0.44 (0.4)	0.10 (0.02)
	7.5	0	0.17 (0.04)	0.75 (0.06)	1.16 (0.4)	0.08 (0.02)
	Control	30	0.05 (0.03)^{a}	1.09 (0.07)	0.16 (0.04)	0.06 (0.03)
	2.5	30	0.05 (0.03)^{a}	0.85 (0.08)	0.15 (0.04)	0.03 (0.03)
	5.0	30	0.11 (0.1)^{ab}	0.89 (0.08)	0.15 (0.04)	0.08 (0.03)
	7.5	30	0.20 (0.2)^{b}	0.95 (0.08)	0.13(0.04)	0.09 (0.03)
	Control	60	0.05 (0.03)	0.81 (0.5)	0.10 (0.03)	0.05 (0.03)
	2.5	60	0.13 (0.03)	1.63 (0.5)	0.14 (0.03)	0.03 (0.03)
	5.0	60	0.12 (0.03)	0.79 (0.5)	0.13 (0.03)	0.07 (0.03)
	7.5	60	0.06 (0.03)	0.68 (0.5)	0.08 (0.03)	0.03 (0.03)
2021	Control	0	0.07 (0.03)	0.72 (0.04)	0.50 (0.06)^{ab}	0.19 (1.0)
	2.5	0	0.11 (0.03)	0.68 (0.04)	0.64 (0.06)^{b}	0.26 (1.0)
	5.0	0	0.13 (0.03)	0.65 (0.04)	0.34 (0.06)^{a}	0.30 (1.0)
	7.5	0	0.16 (0.03)	0.61 (0.04)	0.49 (0.06)^{ab}	0.28 (1.0)
	Control	30	0.12 (0.04)	1.01 (0.06)	0.29 (0.2)	0.15 (0.03)
	2.5	30	0.06 (0.04)	0.92 (0.06)	0.47 (0.2)	0.08 (0.03)
	5	30	0.08 (0.04)	0.90 (0.06)	0.25 (0.2)	0.12 (0.03)
	7.5	30	0.12 (0.04)	0.84 (0.06)	0.73 (0.2)	0.11 (0.03)
	Control	60	0.08 (0.02)	0.69 (0.04)	0.22 (0.08)	0.08 (0.03)
	2.5	60	0.05 (0.02)	0.65 (0.03)	0.26 (0.08)	0.07 (0.03)
	5.0	60	0.06 (0.02)	0.62 (0.03)	0.21 (0.08)	0.07 (0.03)
	7.5	60	0.06 (0.02)	0.70 (0.03)	0.17 (0.08)	0.11 (0.03)

Appendix F | Total carbon, nitrogen, sulphur, and C/N ratio (mean ± S.E.) for the litter, FH, soil (Ah, Bm, C) horizons and sugar maple foliage for Porridge Lake, Ontario. A block ANOVA was used to determine significant differences between all treatments and control. The letter display indicates significant differences ($p<0.05$) using the Tukey HSD.

Horizon	Treatment	C	N	S	C/N
	(Mg ha^{-1})	----------g·kg^{-1}----------			
Litter	Control	41.2 (0.7)	1.54 (0.7)	0.08 (0.005)	20.4 (1.0)
	2.5	41.2 (0.7)	1.43 (0.7)	0.07 (0.005)	20.9 (1.0)
	5.0	39.6 (0.7)	1.37 (0.7)	0.07 (0.005)	21.3 (1.0)
	7.5	41.2 (0.7)	1.44 (0.7)	0.07 (0.005)	20.2 (1.0)
	P-value	0.34	0.34	0.73	0.68
FH	Control	31.7 (2.8)	1.67 (0.2)	0.11 (0.02)	18.9^{a} (1.0)
	2.5	31.6 (2.8)	1.42 (0.4)	0.12 (0.02)	23.0^{b} (1.0)
	5.0	32.0 (2.8)	1.56 (0.3)	0.11 (0.02)	20.6^{ab} (1.0)
	7.5	29.3 (2.8)	1.38 (0.4)	0.10 (0.02)	21.2^{ab} (1.0)
	P-value	0.89	0.89	0.76	**0.05**
Ah	Control	7.34 (1.5)	0.53 (0.08)	0.03 (0.08)	13.9 (0.8)
	2.5	9.80 (1.5)	0.65 (0.08)	0.04 (0.08)	14.5 (0.8)
	5.0	6.68 (1.5)	0.48 (0.08)	0.04 (0.08)	14.2 (0.8)
	7.5	9.96 (1.5)	0.68 (0.08)	0.05 (0.08)	14.4 (0.8)
	P-value	0.35	0.25	0.54	0.93
Bm	Control	4.91 (1.0)	0.34 (0.1)	0.02 (0.02)	14.3 (0.8)
	2.5	5.47 (1.0)	0.38 (0.1)	0.04 (0.02)	14.6 (0.8)
	5.0	5.31 (1.0)	0.39 (0.1)	0.03 (0.02)	13.8 (0.8)
	7.5	6.06 (1.0)	0.42 (0.1)	0.05 (0.02)	13.9 (0.8)
	P-value	0.87	0.87	0.59	0.86
C	Control	5.33 (0.6)	0.37 (0.03)	0.03 (0.005)	14.6 (0.7)
	2.5	4.12 (0.6)	0.29 (0.03)	0.02 (0.005)	14.5 (0.7)
	5.0	4.77 (0.6)	0.34 (0.04)	0.02 (0.005)	13.8 (0.7)
	7.5	5.23 (0.6)	0.36 (0.03)	0.03 (0.005)	14.4 (0.7)
	P-value	0.52	0.40	0.55	0.84
Foliage	Control	47.8^{b} (0.7)	2.36 (0.1)	0.13 (0.1)	20.4 (1.0)
	2.5	46.6^{ab} (0.7)	2.25 (0.1)	0.12 (0.1)	20.9 (1.0)
	5.0	47.1^{ab} (0.7)	2.22 (0.1)	0.12 (0.1)	21.3 (1.0)
	7.5	44.8^{a} (0.7)	2.23 (0.1)	0.12 (0.1)	20.2 (1.0)
	P-value	**0.06**	0.72	0.19	0.85

Appendix G | Analysis of variance (ANOVA) of >1% relative abundance of Prokaryote phyla and >3% at the order level, based on sample layer. The letter display indicates significant differences (p<0.05) using the Tukey HSD.

			Treatment (Mg·ha^{-1})				
Layer	Group	*n*	Control	2.5	5.0	7.5	*P*-value
Litter	**Phyla >1%**						
	Proteobacteria	5	0.59 (0.05)	0.60 (0.05)	0.53 (0.06)	0.61 (0.04)	0.144
	Acidobacteriota	5	0.20 (0.05)	0.21 (0.08)	0.18 (0.11)	0.25 (0.01)	0.545
	Actinobacteriota	5	0.10 (0.03)	0.09 (0.04)	0.12 (0.07)	0.08 (0.03)	0.617
	Bacteriodota	5	0.09 (0.03)	0.07 (0.05)	0.10 (0.07)	0.04 (0.01)	0.241
	Myxococcota	5	0.01 (0.01)	0.01 (0.02)	0.02 (0.01)	0.003 (0.002)	0.240
	Order >3%						
	Acidobacteriales	5	0.20 (0.05)	0.20 (0.10)	0.18 (0.12)	0.25 (0.01)	0.538
	Burkholderiales	5	0.15 (0.05)	0.13 (0.03)	0.12 (0.003)	0.13 (0.02)	0.489
	Rhizobiales	5	0.12 (0.02)	0.14 (0.03)	0.13 (0.04)	0.11 (0.01)	0.292
	Caulobacterales	5	0.08 (0.04)	0.10 (0.07)	0.09 (0.03)	0.17 (0.04)	0.053
	Sphingomonadales	5	0.09 (0.03)	0.10 (0.04	0.08 (0.03)	0.11 (0.02)	0.600
	Acetobacterales	5	0.10 (0.05)	0.08 (0.04)	0.07 (0.04)	0.07 (0.02)	0.650
	Micrococcales	5	0.04 (0.02)	0.04 (0.02)	0.05 (0.02)	0.06 (0.03)	0.812
	Sphingobacteriales	5	0.05 (0.01)	0.04 (0.02)	0.04 (0.03)	0.03 (0.01)	0.579
	Chitinophagales	5	0.03 (0.02)	0.03 (0.02)	0.04 (0.04)	0.01 (0.01)	0.360
FH	**Phyla >1%**						
	Proteobacteria	5	0.43 (0.07)	0.45 (0.03)	0.40 (0.03)	0.46 (0.02)	0.292
	Acidobacteriota	5	0.26 (0.06)	0.25 (0.04)	0.24 (0.07)	0.18 (0.05)	0.143
	Actinobacteriota	5	0.06 (0.02)	0.07 (0.02)	0.07 (0.03)	0.08 (0.03)	0.825
	Verrucomicrobiota	5	0.06 (0.01)	0.06 (0.02)	0.08 (0.03)	0.06 (0.02)	0.417
	Planctomycetota	5	0.05 (0.02)	0.04 (0.01)	0.05 (0.01)	0.05 (0.009)	0.574
	Bacteriodota	5	0.04 (0.03)	0.04 (0.03)	0.04 (0.03)	0.04 (0.01)	0.973
	RCP2-54	5	0.02 (0.01)	0.02 (0.01)	0.04 (0.04)	0.02 (0.02)	0.591
	Myxococcota	5	0.02 (0.01)	0.03 (0.01)	0.02 (0.01)	0.02 (0.01)	0.657
	Firmicutes	5	0.02 (0.01)	0.01 (0.01)	0.02 (0.01)	0.01 (0.01)	0.382
	Chloroflexi	5	0.01 (0.004)	0.01 (0.003)	0.01 (0.01)	0.01 (0.01)	0.600
	WPS-2	5	0.01 (0.01)	0.02 (0.01)	0.005 (0.004)	0.006 (0.01)	2.585
	Order >3%						
	Rhizobiales	5	0.23 (0.07)	0.23 (0.04)	0.19 (0.05)	0.22 (0.07)	0.692
	Acidobacteriales	5	0.11 (0.02)	0.11 (0.02)	0.10 (0.02)	0.07 (0.04)	0.105
	Subgroup_2	5	0.11 (0.06)	0.10 (0.03)	0.10 (0.05)	0.04 (0.03)	0.134
	Burkholderiales	5	0.02 (0.01)^{a}	0.04 (0.02)^{ab}	0.04 (0.02)^{ab}	0.08 (0.03)^{ab}	**0.011**
	Elsterales	5	0.05 (002)	0.04 (0.03)	0.04 (0.02)	0.02 (0.01)	0.128
	Chthoniobacterales	5	0.03 (0.01)	0.04 (0.03)	0.04 (0.03)	0.04 (0.02)	0.816
	RCP2-54	5	0.04 (0.04)	0.02 (0.01)	0.03 (0.02)	0.01 (0.009)	0.219
Soil Ah	**Phyla >1%**						
	Proteobacteria	5	0.32 (0.15)	0.37 (0.03)	0.35 (006)	0.36 (0.06)	0.781
	Acidobacteriota	5	0.32 (0.12)	0.27 (0.05)	0.29 (0.05)	0.23 (0.05)	0.207
	Verrucomicrobiota	5	0.17 (0.13)	0.01 (0.05)	0.13 (0.05)	0.13 (0.04)	0.550
	Planctomycetota	5	0.04 (0.01)	0.07 (0.01)	0.06 (0.01)	0.07 (0.04)	0.471

Actinobacteriota	5	0.04 (0.01)	0.05 (0.01)	0.04 (0.01)	0.04 (0.02)	0.546
Chloroflexi	5	0.05 (0.04)	0.02 (0.01)	0.03 (0.01)	0.04 (0.02)	0.339
RCP2-54	5	0.03 (0.01)[ab]	0.04 (0.01)[b]	0.02 (0.01)[ab]	0.02 (0.01)[a]	**0.037**
Firmicutes	5	0.01 (0.01)	0.02 (0.01)	0.02 (0.01)	0.01 (0.01)	0.256
Bacteriodota	5	0.02 (0.01)	0.02 (0.01)	0.02 (0.01)	0.01 (0.004)	0.336
Order >3%						
Rhizobiales	5	0.21 (0.1)	0.022 (0.02)	0.22 (0.04)	0.22 (0.05)	0.998
Subgroup_2	5	0.18 (0.2)	0.11 (0.03)	0.12 (0.05)	0.09 (0.03)	0.416
Chthoniobacterales	5	0.17 (0.1)	0.09 (0.05)	0.11 (0.05)	0.12 (0.05)	0.453
Acidobacteriales	5	0.10 (0.06)	0.12 (0.02)	0.14 (0.02)	0.10 (0.02)	0.306
Elsterales	5	0.04 (0.01)	0.04 (0.01)	0.04 (0.01)	0.04 (0.01)	0.866
Ktedonobacterales	5	0.03 (0.02)	0.02 (0.01)	0.02 (0.02)	0.05 (0.03)	0.206
RCP2-54	5	0.02 (0.01)	0.03 (0.02)	0.03 (0.01)	0.02 (0.01)	0.330
Burkholderiales	5	0.02 (0.01)	0.03 (0.01)	0.02 (0.003)	0.03 (0.006)	0.171

Appendix H | Analysis of variance (ANOVA) of >2% relative abundance of Eukaryote order and >2% at the genera level, based on sample layer. The letter display indicates significant differences (p<0.05) using the Tukey HSD.

			Treatment (Mg·ha^{-1})				
Layer	Group	*n*	Control	2.5	5.0	7.5	*P*-value
Litter	**Order >2%**						
	Helotiales	5	0.26 (0.12)	0.27 (0.18)	0.24 (0.20)	0.41 (0.16)	0.411
	Rhytismatales	5	0.18 (0.19)	0.24 (0.28)	0.24 (0.24)	0.10 (0.08)	0.689
	Agaricales	5	0.23 (0.24)	0.17 (0.21)	0.07 (0.09)	0.08 (0.11)	0.437
	Atheliales	5	0.03 (0.06)	0.004 (0.01)	0.06 (0.14)	0.03 (0.06)	0.714
	Leotiales	5	0.03 (0.05)	0.03 (0.05)	0.01 (0.01)	0.04 (0.02)	0.796
	Cantharellales	5	0.03 (0.06)	0.001 (0.001)	0.06 (0.13)	0.01 (0.02)	0.609
	Tremellales	5	0.02 (0.008)	0.02 (0.03)	0.03 (0.05)	0.02 (0.01)	0.864
	Genus >2%						
	Coccomyces	5	0.17 (0.17)	0.22 (0.29)	0.23 (0.24)	0.08 (0.07)	0.697
	Mycena	5	0.20 (0.23)	0.09 (0.12)	0.03 (0.07)	0.02 (0.05)	0.190
	Athelia	5	0.03 (0.06)	0.004 (0.01)	0.06 (0.14)	0.03 (0.06)	0.714
	Epithamnolia	5	0.03 (0.05)	0.03 (0.05)	0.01 (0.006)	0.04 (0.02)	0.796
	Gymnopus	5	0.02 (0.05)	0.04 (0.09)	0.0001 (0.0001	0.05 (0.11)	0.730
FH	**Order >2%**						
	Agaricales	5	0.19 (0.11)	0.53 (0.25)	0.39 (0.38)	0.25 (0.20)	0.181
	Mortierellales	5	0.09 (0.05)	0.07 (0.04)	0.10 (0.07)	0.14 (0.12)	0.534
	Filobasidiales	5	0.05 (0.02)	0.03 (0.02)	0.06 (0.08)	0.08 (0.09)	0.494
	Polyporales	5	0.05 (0.05)	0.02 (0.02)	0.04 (0.04)	0.03 (0.02)	0.493
	Umbelopsidales	5	0.05 (0.10)	0.02 (0.02)	0.03 (0.02)	0.01 (0.01)	0.670
	Archaeorhizomycetales	5	0.07 (0.09)	0.02 (0.03)	0.01 (0.008)	0.02 (0.02)	0.148
	Tremellales	5	0.02 (0.01)	0.01 (0.009)	0.03 (0.04)	0.02 (0.01)	0.715
	Helotiales	5	0.02 (0.01)	0.01 (0.02)	0.02 (0.02)	0.03 (0.02)	0.421
	Thelephorales	5	0.06 (010)	0.02 (0.03)	0.003 (0.004)	0.008 (0.01)	0.402
	Genus >2%						
	Hygrocybe	5	0.004 (0.009)[a]	0.37 (0.36)[b]	0.02 (0.04)[a]	0.13 (0.11)[ab]	**0.027**
	Mortierella	5	0.09 (0.05)	0.07 (0.04)	0.10 (0.07)	0.14 (0.12)	0.531
	Gliophorus	5	0.009 (0.02)	0.07 (0.16)	0.18 (0.36)	0.03 (0.05)	0.530
	Solicoccozyma	5	0.05 (0.02)	0.03 (0.02)	0.06 (0.08)	0.08 (0.09)	0.497
	Clitocybe	5	0.0001 (0.0002)	0.001 (0.003)	0.14 (0.31)	0.0001 (0.0001)	0.421
	Ganoderma	5	0.04 (0.05)	0.02 (0.02)	0.04 (0.04)	0.02 (0.02)	0.676
	Umbelopsis	5	0.05 (0.1)	0.02 (0.02)	0.03 (0.02)	0.01 (0.01)	0.670
	Archaeorhizomyces	5	0.07 (0.09)	0.02 (0.03)	0.008 (0.008)	0.02 (0.02)	0.148
Soil Ah	**Order >2%**						
	Mortierellales	5	0.26 (0.12)	0.24 (0.11)	0.26 (0.13)	0.30 (0.19)	0.943
	Agaricales	5	0.23 (0.28)	0.16 (0.15)	0.06 (0.04)	0.22 (0.30)	0.606
	Filobasidiales	5	0.05 (0.04)	0.13 (0.10)	0.11 (0.04)	0.08 (0.03)	0.260
	Polyporales	5	0.05 (0.03)	0.05 (0.03)	0.06 (0.04)	0.05 (0.02)	0.958
	Umbelopsidales	5	0.05 (0.05)	0.03 (0.03)	0.04 (0.03)	0.07 (0.06)	0.593
	Russulales	5	0.07 (0.08)	0.05 (0.05)	0.05 (0.05)	0.01 (0.01)	0.355
	Archaeorhizomycetales	5	0.04 (0.03)	0.03 (0.03)	0.05 (0.01)	0.03 (0.03)	0.463

Tremellales	5	0.02 (0.02)	0.03 (0.02)	0.05 (0.04)	0.03 (0.02)	0.467
Genus >2%						
Mortierella	5	0.26 (0.01)	0.24 (0.10)	0.26 (0.13)	0.03 (0.18)	0.940
Solicoccozyma	5	0.05 (0.04)	0.13 (0.10)	0.11 (0.04)	0.08 (0.03)	0.259
Hygrocybe	5	0.02 (0.02)	0.14 (0.14)	0.02 (0.02)	0.04 (0.03)	0.066
Ganoderma	5	0.04 (0.03)	0.05 (0.03)	0.06 (0.04)	0.05 (0.02)	0.909
Umbelopsis	5	0.05 (0.05)	0.03 (0.03)	0.04 (0.03)	0.07 (0.06)	0.593
Inocybe	5	0.02 (0.04)	0.01 (0.01)	0.001 (0.003)	0.15 (0.28)	0.313
Russula	5	0.07 (0.08)	0.05 (0.05)	0.05 (0.05)	0.01 (0.01)	0.364
Gliophorus	5	0.14 (0.31)	0.002 (0.004)	0.01 (0.03)	0.001 (0.002)	0.420
Archaeorhizomyces	5	0.04 (0.03)	0.03 (0.03)	0.05 (0.01)	0.03 (0.03)	0.463
Saitozyma	5	0.02 (0.02)	0.03 (0.02)	0.05 (0.04)	0.03 (0.02)	0.521

Appendix I | Ratio of fungal to bacteria PLFA for three horizons, three years post wood ash treatment. The letter display indicates significant differences (p<0.05) using the Tukey HSD.

Treatment ($Mg \cdot ha^{-1}$)	*n*	Fungal:Bacteria		
		Litter	FH	Soil Ah
Control (0)	5	2.51	4.22	2.48
		(0.7)	(4.2)	(3.0)
2.5	5	3.99	5.76	1.26
		(1.9)	(3.7)	(0.5)
5.0	5	4.34	3.69	1.32
		(1.7)	(2.6)	(0.5)
7.5	5	4.59	1.79	1.22
		(2.4)	(0.6)	(0.7)
P-value		0.275	0.281	0.542

9. Supplementary

Polymerase chain reactions (PCR) were performed in triplicate for each sample (n=60), using a mixture of 2.5 µL of 10x standard Taq buffer, 0.5 µL of 10 mM dNTP, 0.25 µL of BSA (20 mg/mL), 5.0 µL of 1 µM forward primer, 5.0 µL of 1 µM reverse primer, 5.0 µL DNA, 0.2 µL of Taq DNA polymerase (5 µ/µL) and 6.55 µL of PCR-safe water. DNA was denatured at 95°C for 5 minutes, followed by 35 cycles of 95°C for 30 seconds, 30°C for 30 seconds, and 72°C for 50 seconds, then extended at 72°C for 10 minutes. The resulting PCR products were pooled, gel purified and quantified using the Qubit dsDNA HS Assay Kit (ThermoFisher Scientific, 2022). Library preparation was performed using Metagenom Bio Life Sciences lab internal protocols, and DNA was sequenced with a MiSeq Reagent Kit v2 (2 x 250 cycles). The V4 region of the 16S rRNA gene was sequenced using the primers 515F and 806R (Walters et al., 2016).