Inorganic and Organic Carbon Dynamics of a South-Central Ontario Forest After the Application of Non-Industrial Wood Ash

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Abstract

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Forests play a critical role in the global carbon cycle, acting as carbon sinks that remove and store an estimated 0.4 to 4.1 Pg of atmospheric carbon annually. However, historical acid deposition and timber harvesting have disrupted nutrient cycles, leading to nutrient-deficient soils that hinder tree growth, particularly for sugar maple (*Acer saccharum*), a keystone species in Ontario sensitive to soil acidity and calcium (Ca) depletion. This study evaluates the effects of non-industrial wood ash (NIWA) applied at 0, 2, 4, 6, and 12 Mg ha^{-1} on soil chemistry, nutrient concentrations, microbial activity, CO_2 fluxes, and sugar maple growth over two years. NIWA increased soil pH and exchangeable base cations, especially in the organic horizon, with some increase in forest floor metals, though foliar levels remained safe. Enzyme activity responses were minimal, and fine root biomass declined at higher doses. Soil CO_2 fluxes rose sharply at 12 Mg ha^{-1}, suggesting pH-driven effects. NIWA improves soil chemistry, but long-term impacts remain uncertain.

Keywords: non-industrial wood ash, metal toxicity, sugar maple, carbon flux, extracellular soil enzyme

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Table of Contents

List of Figures

List of Tables

Abbreviations

Aluminum……………………………………………………………………………………Al
Analysis Of Variance……………………………………………………………………..ANOVA
Arsenic……………………………………………………………………………………...As
Basal Area Increment...BAI
Boron...B
Cadmium…………………………………………………………………………………...Cd
Calcium..……………………………………………………………………….…………...Ca
Calcium chloride...$CaCl_2$
Calcium oxide………………………………………………………………………………CaO
Carbon……………………………………………………………………………..……...C
Carbon dioxide……………………………………………………………………………CO_2
Content of Regulated Metals……………………………………………………………...CM
Copper……………………………………………………………………………………...Cu
Diameter at breast height…………………………………………………………………...DBH
Dissolved Organic Carbon…………………………………………………………………DOC
Dissolved Organic Matter…………………………………………………………………DOM
Dolomite……………………………………………………………………………$CaMg(CO_3)_2$
Diagnosis and Recommendation Integrated System..DRIS
Exchangeable Cations...EC
Fibrous, humus layer………………………………………………………………………FH
Friends of the Muskoka Watershed...FMW
Hydrogen……………………………………………………………………………………...H^+
Inductively Coupled Plasma-Optical Emission Spectrometry …………………………...ICP-OES
Industrial Wood Ash..IWA
Iron…………………………………………………………………………………………….Fe
Lead………………………………………………………………………………………...Pb
Leaf Litter…………………………………………………………………………………...L
Lime…………………………………………………………………………………$CaCO_3$
Loss on ignition……………………………………………………………………………...LOI
Manganese……………………………………………………………………………………Mn
Magnesium…………………………………………………………………………………..Mg
Mega grams per hectare……………………………………………………………$Mg\cdot ha^{-1}$
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
Phosphorus……………………………………………………………………………………P
Potassium………………………………………………………………………………………K
Sodium……………………………………………………………………………………..Na
Soil Organic Matter………………………………………………………………………...SOM
Standard Error………………………………………………………………………………SE

Sulphate……………………………………………………………………………………SO_4
Sulphur……………………………………………………………………………………….S
Tukey Honest Significant Difference………………………………………………Tukey HSD
Water………………………………………………………………………………………H_2O
Wollastonite……………………………………………………………………………$CaSiO_3$
Zinc…………………………………………………………………………………………Zn

1.0 General Introduction

1.1 Climate change and carbon sinks

Forests are essential to the global carbon cycle, serving as major carbon sinks by sequestering and storing carbon (Whitehead, 2011). Through the process of photosynthesis, forests sequester CO_2 and convert it into biomass (Nowak & Crane, 2002) and larger trees contribute more to carbon storage due to their greater biomass (Brack, 2002; Cox, 2012). In addition to above-ground biomass, forests and their soils collectively store over 800 Pg of carbon, nearly matching the amount found in the atmosphere (Pan et al., 2013). Soil organic matter (SOM) represents a significant carbon reservoir, containing more carbon than the atmosphere and vegetation combined (Scharlemann et al., 2014). Forest soils hold over 40% of the total organic carbon in terrestrial ecosystems, with soil carbon stocks comprising about 70% in boreal forests, 60% in temperate forests, and 30% in tropical forests (Pan et al., 2011). However, forests can transition from being carbon sinks to carbon sources if deforestation or tree die-off rates outpace growth (Natural Resources Canada, 2024; Ravin & Raine, 2007). Thus, effective forest management is essential for increasing carbon storage.

Historically, forest management has focused primarily on timber yield rather than broader ecological values (Cox et al., 2024). Additionally, land use changes, such as converting forests to agricultural land, disrupt ecosystems and can reduce soil carbon storage by 20–50% (Davidson & Ackerman, 1993). Factors contributing to soil carbon depletion include decreased biomass input, changes in soil moisture and temperature, the high decomposability of crop residues, tillage-induced disturbances, reduced soil aggregation, and increased erosion (Lal, 2005). Understanding soil carbon dynamics is essential for enhancing natural carbon sinks and mitigating climate change, especially considering the important role of forest soils in sequestering atmospheric CO_2 (Davidson & Ackerman, 1993).

While sustainably managed forests, where harvesting is balanced with regrowth, can continue to act as net sinks for atmospheric CO_2 (Kurz et al., 2013; Pan et al., 2011), deforestation and permanent land conversion result in long-term carbon losses and undermine a vital pathway for carbon removal (Houghton & Nassikas, 2018). Therefore, protecting and expanding forests emerges as one of the most effective strategies for limiting global warming to below 1.5–2 degrees Celsius (Griscom et al., 2017; Roe et al., 2019).

1.2 Acidic deposition in forested ecosystems

As international interest shifts away from using fossil fuels to aid in mitigating climate change, there is a growing focus on bioenergy (Popp et al., 2021). In 2019, biomass was the leading form of renewable energy globally, accounting for 12% of the renewable energy supply (WBA, 2022), and demand is projected to increase (Titus et al., 2021). This trend suggests that biomass will remain the most utilized renewable energy source (Popp et al., 2021). However, in addition to deforestation, urbanization, agriculture, and fossil fuel combustion, acidic deposition has significantly impacted the forest environment. This process has accelerated base cation leaching, resulting in nutrient-poor, acidified soils (Gauthier et al., 2015). This degradation hinders native plant life and biodiversity, and while reductions in sulfur dioxide (SO_2) and nitrogen oxides (NOx) emissions have been substantial since the 1990s, the long-term effects of acid deposition can still be observed in soils across North America, Europe, and Asia (Feng et al, 2020). The natural recovery of soils and surface waters is slower than anticipated, prompting the need for soil amendments to counteract acidification (Gauthier et al., 2015; Ott & Watmough, 2022; Watmough et al., 2016).

Nutrient depletion from soil acidification and timber harvesting further exacerbates tree stress and susceptibility to dieback from drought, insect defoliation, and other factors (Drohan et al., 2002; Watmough, 2002). Research in various regions, including Ontario, Quebec, Vermont, and Pennsylvania, has linked increased metal (mostly aluminum (Al) and manganese (Mn)) concentrations and nutrient loss to crown dieback and reduced tree growth (Liu et al., 1997; Miller & Watmough, 2009; Moore et al., 2012). As demand for biofuels continues to rise, addressing these soil nutrient deficiencies through amendments is critical for forest recovery and long-term sustainability, ensuring that forest ecosystems remain resilient and productive (Hannam et al., 2017; Joseph et al., 2022).

1.3 Evaluation of common soil amendments

Lime, wollastonite, biochar, and wood ash are commonly used soil amendments to counteract soil acidification and increase soil pH. Since the 1980s, lime has been widely applied in Europe and North America for this purpose, with its effectiveness well-documented (Hüttl & Zöttl, 1993; Long et al., 1997). Lime, available as limestone ($CaCO_3$) or dolomite ($CaMg(CO_3)_2$), effectively increases soil pH, boosts base cation availability, and immobilizes heavy metals (Kunhikrishnan et al., 2016). Long-term studies have demonstrated that lime maintains higher soil pH and improves calcium (Ca) and magnesium (Mg) levels several decades after application, as seen in the Allegheny Plateau (Long et al., 2011) and Quebec (Moore & Ouimet, 2021). However, lime application can lead to shallower roots, reduced mycorrhizal diversity, and increased nitrate leaching (Kreutzer, 1995; Huber et al., 2006). Additionally, the production of lime generates greenhouse gases and incurs high costs, prompting investigation into alternative materials (Zagvozda et al., 2022).

Wollastonite, another Ca-rich amendment, has a slower effect on soil chemistry because of its low solubility but has shown benefits in increasing soil acid-neutralizing capacity (ANC) and reducing aluminum (Al) concentrations (Johnson et al., 2014; Shao et al., 2016). The addition of wollastonite also improves foliar Ca and phosphorus (P) concentrations and supports mycorrhizal colonization (Green et al., 2013; Juice et al., 2006). Despite these benefits, its response can be moderate and slow, with significant effects taking up to 12 years (Lawrence et al., 2021). Wollastonite also has potential climate benefits through enhanced weathering, whereby calcium silicates react with atmospheric CO_2 to form stable carbonates, potentially contributing to long-term carbon sequestration and reducing net greenhouse gas emissions (Beerling et al., 2018; Kantzas et al., 2022).

Biochar is a carbon-rich material produced through the pyrolysis of organic biomass under low-oxygen conditions and is increasingly recognized for its potential to improve soil health and sequester carbon. It has been shown to raise soil pH, particularly in acidic soils, by neutralizing acidity and enhancing base saturation (Glaser et al., 2002; Jeffery et al., 2011). Biochar also improves cation exchange capacity (CEC), water retention, and microbial habitat structure, which can positively influence nutrient cycling and root development (Lehmann & Joseph, 2015). Additionally, due to its stable aromatic structure, biochar contributes to long-term carbon storage in soils, supporting climate change mitigation goals (Woolf et al., 2010). However, biochar’s properties and effects are highly variable depending on feedstock type and pyrolysis conditions, and in some cases, its impact on nutrient availability and plant growth may be limited or inconsistent (Spokas et al., 2012).

Wood ash, a by-product of biomass combustion, contains high levels of Ca and moderate amounts of Mg and potassium (K), with minimal sulfur (S), nitrogen (N), and P (Cairns et al., 2021; Hannam et al., 2019). It supports mycorrhizal relationships by limiting N and P availability, potentially reducing nutrient leaching and eutrophication (Schindler et al., 2008).

Given the growing global focus on bioenergy, and the widespread use of biomass as a renewable energy source, wood ash, which is produced as a by-product of biomass combustion, offers a sustainable option for soil amendment (Popp et al., 2021). It can help replenish soil nutrients and contribute to a circular economy by recycling waste from biomass production (Tan & Lamers, 2021).

1.4 Production and composition of wood ash

Globally, an estimated 476 million Mg of biomass ash is produced annually (Vassilev et al., 2013), with landfills being the most common disposal method (Lamers et al., 2018). In Finland, only 18% of the wood ash produced in 2004 was used as a forest amendment, while Sweden recycled just 5% of its 300,000 Mg annual production (Stupak et al., 2007). Austria landfilled about 44% of its biomass ash with limited recycling, and the US landfilled approximately 90% of its wood ash (Pitman, 2006). Canada produced about 420,000 Mg of wood ash annually as of 2013, with 63% landfilled (Elliott & Mahmood, 2015; Elliott et al., 2022). Although the cost of applying wood ash often exceeds landfilling in Canada (Hope et al., 2017), some jurisdictions still promote its use in forestry through policy incentives, C offset opportunities, and recognition of its ecological benefits. This suggests that a small but emerging market for wood ash recycling exists, particularly where long-term soil health and sustainability are prioritized (Emilson et al., 2018; Hannam et al., 2019; Hope et al., 2017; Pugliese et al., 2014).

In Ontario, wood ash is classified as a non-agricultural source material (NASM), requiring strict regulatory approval for use as a soil amendment (Government of Ontario, 2002; Hannam et al., 2018). Due to the high cost and time associated with obtaining this approval, landfilling remains a more feasible option. Research on the chemical composition of NASM, including wood ash, is crucial to ensure safe metal concentrations. Guidelines for NASMs are divided into categories (CM1 and CM2) based on metal content, with more restrictions as metal levels increase (Government of Ontario, 2002). Combining wood ash from multiple sources can help standardize nutrient and metal concentrations (Azan et al., 2019; Deighton & Watmough, 2020; Syeda et al., 2024), potentially reducing landfill waste and enhancing soil quality in nutrient-poor or acidified areas (Augusto et al., 2008).

Studies have shown that using wood ash can improve environmental indicators compared with landfilling and even reduce greenhouse gas emissions in spruce forests (Gaudreault et al., 2020; Klemedtsson et al., 2010). European countries, early adopters of soil amendments for decreasing soil acidity, have conducted long-term trials showing various benefits of wood ash. For instance, Finnish studies found lasting positive effects on soil pH and nutrient availability (Moilanen et al., 2002; Saarsalmi et al., 2001). In Sweden, wood ash improved fine root growth in mineral soil horizons but had mixed results in organic horizons (Clemensson-Lindell & Persson, 1995; Majdi & Viebke, 2004). Similar improvements in soil properties have been observed in Denmark, Switzerland, Norway, Slovakia, and Germany (Bang-Andreasen et al., 2021; Brunner et al., 2004; Clarke et al., 2018; Gömöryová et al., 2016; Huber et al., 2006; Rumpf et al., 2001), with Finland recommending applications of 5.0 Mg ha^{-1} every 30 years (Perkiömäki & Fritze, 2002).

1.5 Industrial and non-industrial wood ash properties

Wood ash is a by-product of burning woody biomass, such as woodchips, sawdust, and bark (Siddique, 2012). It can originate from both industrial and non-industrial sources. 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), Boron (B) and zinc (Zn) (Hannam et al., 2019; Majdi & Viebke, 2004).

1.5.1 Industrial wood ash

Industrial wood ash (IWA) primarily comes from forest residues and industrial processes such as pulp and paper mills (Elliott et al., 2022). Within industrial settings, wood ash is classified into two types based on its origin within the boilers: fly ash and bottom ash. Fly ash consists of fine particles captured from flue gases before they are released into the atmosphere (Hannam et al., 2017), whereas bottom ash is the material that settles at the bottom of the boilers (Elliott & Mahmood, 2006). Typically, fly ash contains higher levels of dioxins and heavy metals compared with bottom ash (Pitman, 2006). Conversely, metals with low volatility, such as nickel (Ni), chromium (Cr), and vanadium (V), tend to accumulate in bottom ash, along with nutrients like Ca, Mg, and P (Nardoslawsky & Obernberger, 1996).

1.5.2 Non-industrial wood ash

In contrast, non-industrial wood ash (NIWA) is generated from wood-fired stoves, furnaces, and fireplaces in homes and small businesses (Azan et al., 2019). Less is known about NIWA because industrial processes usually involve more consistent combustion conditions and source materials (Pitman, 2006), making the properties of industrial wood ash (IWA) generally more stable and less variable compared to NIWA. Variability in nutrient and metal concentrations in NIWA has been shown to depend on factors such as the burning temperature and tree species used (Deighton & Watmough, 2020; Misra et al., 1993; Pitman, 2006). For example, Deighton & Watmough (2020) found that ash from yellow birch contains 12 times more Zn, 9 times more arsenic (As), and 6 times more cadmium (Cd) and lead (Pb) compared with ash from sugar maple and white pine. These differences were observed in trees collected from the same general region and soil type, suggesting species-specific differences in metal accumulation. However, nutrient concentrations were generally more similar across these three tree species.

1.6 Wood ash effects below-ground

1.6.1 Wood ash effects on soil

The ability of wood ash to counteract soil acidity, which increases with application dose, typically leads to decreased acidity in the organic horizon shortly after application and reduced mineral soil acidity over the long term (Augusto et al., 2008; Demeyer et al., 2001; Pitman, 2006; Reid & Watmough, 2014). This increase in soil pH and base saturation in surface soil horizons has benefits lasting beyond the first year due to the gradual downward movement of base cations (Ca, Mg, K). The improvements in soil chemistry can persist for up to 30 years (Augusto et al., 2008; Jacobson et al., 2004; Saarsalmi et al., 2001; 2012). Significant increases in exchangeable Ca and Mg concentrations, as well as improvements in cation exchange capacity (CEC) and base saturation (BS), have been reported, with a combined increase of 411 kg ha^{-1} for Ca and 39 kg ha^{-1} for Mg observed in organic and mineral soils nineteen months after a 4.8 Mg ha^{-1} wood ash application (Ludwig et al., 2002). Wood ash typically does not directly contribute to nitrogen (N) availability due to its low N content, but it may enhance the decomposition of recalcitrant organic matter, potentially increasing soil N availability (Mortensen et al., 2019; Pitman, 2006).

Elevated levels of potentially toxic metals have been observed in other studies shortly after application, mainly in the upper soil horizons, and these effects usually diminish over time (Arvidsson et al., 2003; Ozolincius & Varnagiryte, 2005). For example, studies in Lithuania reported increases in chromium (Cr), copper (Cu), nickel (Ni), and Zn in the organic horizons, with significant downward transport observed for Zn and Ni (Ozolincius & Varnagiryte, 2005). Pugliese et al. (2014) found no significant changes in metal concentrations and a decrease in available lead (Pb), likely due to increased soil pH. Exchangeable Al concentrations also decreased in both humus and mineral layers after ash application (Saarsalmi et al., 2001).

Although elevated trace metal concentrations are reported, they are largely restricted to organic soil horizons due to reduced metal solubility with increased pH (Augusto et al., 2008; Bramryd & Fransman, 1995; Violante et al., 2010). Deighton et al. (2021) found no enhanced metal leaching even under simulated drought conditions, indicating that metal mobility may be limited despite reduced soil pH and any increase in soil metal content generally falls with the expected range for soils (Saarsalmi et al., 2012).

1.6.2 Wood ash effects on fine roots

Research indicates that wood ash application typically reduces fine root biomass (Helmisaari et al., 2009) and may limit fine root growth, especially when applied without N (Clemensson-Lindell & Persson, 1995; Persson & Ahlström, 1990). Nitrogen-free wood ash fertilizers have also been found to decrease fine root production and biomass in Norway spruce (Clemensson-Lindell & Persson, 1995; Majdi, 2005). However, responses vary: Genenger et al. (2003) reported increased fine root biomass within two years of applying loose wood ash in a 70-year-old Norway spruce stand in Switzerland, while Püttsepp et al. (2006) found no change in fine root biomass nine years after applying granulated wood ash. Clemensson-Lindell and Persson (1995) noted a decrease in fine root biomass and an increase in necromass three years after applying granulated wood ash. The variability in responses likely reflects the pore size of the wood ash used and changes in soil acidity, but the observed decreases in fine root biomass generally do not seem to significantly impact long-term tree growth (Helmisaari et al., 2009).

The effect of wood ash on fine root chemistry is closely related to changes in soil acidity. For example, higher concentrations of Ca and Mg in fine roots correspond to increased soil levels of these elements, while lower Mn in fine roots aligns with reduced soil Mn concentrations and increased soil pH (Brunner et al., 2004). Deighton and Watmough (2020) observed similar increases in Ca, Mg, and K in sugar maple (*Acer saccharum*) seedling roots following the application of wood ash from sugar maple, white pine (*Pinus strobus*), and yellow birch (*Betula alleghaniensis*). Although Brunner et al. (2004) found no significant rise in root metal concentrations, suggesting that while metals may accumulate in the soil, their availability for plant uptake is generally constrained by the increase in soil pH. The observed increases in metal concentrations in sugar maple roots were primarily attributed to the higher metal content in yellow birch wood ash (Deighton & Watmough, 2020).

1.6.3 Wood ash effects on microbial and enzyme activity

Microbial properties are valuable indicators for assessing the impact of soil treatments on forest soils due to their rapid response to changes in the ecosystem (Sparling, 1997). When wood ash is applied, it increases soil pH, which in turn stimulates microbial activity and respiration rates, potentially enhancing organic matter decomposition and mineralization (Bååth and Arnebrant, 1994; Fritze et al., 1994; Insam et al., 2009). This heightened microbial activity can also result in increased net nitrification and nitrogen leaching. Microorganisms are crucial for nutrient cycling and organic matter decomposition (Fekete et al., 2012; Veres et al., 2015). One method that is more cost effective and simple is studying microorganism's enzyme activity. The activities of β-glucosidase (a key enzyme in carbon cycling), phosphatase (involved in phosphorus mineralization), and N-acetylglucosaminidase (NAGase, which contributes to nitrogen cycling through the breakdown of chitin) are commonly studied in forest soils due to their roles in nutrient dynamics and their sensitivity to changes in pH and microbial community structure following wood ash application (Tabatabai, 1994; Allison & Vitousek, 2005). Enzymatic activity can serve as an early indicator of changes in soil properties (Błońska et al., 2017; Kotroczó et al., 2020). Soil enzymes are intimately involved in catalyzing reactions necessary for the stabilization of soil structure, organic matter (OM) decomposition and dissolved OM production (Allison and Vitousek, 2005), mineralization, and nutrient cycling (Tabatabai, 1994), energy transfer and environmental quality. Research shows variability in soil enzymatic responses depending on the study duration. For instance, Zimmermann and Frey (2002) observed a weak and short-term increase in microbial enzyme activity following the addition of 8 Mg ha^{-1} of wood ash over 460 days. In contrast, Błońska et al. (2023) reported a stronger response in soil microbial enzymatic activity with the application of 3, 4.5, and 6 Mg ha^{-1} of wood ash two years after application. This variation may be attributed to long-lasting changes in microbial community structures (Cruz-Paredes et al., 2017). Some studies suggest that an enzymatic approach may be more accurate than chemical approaches in assessing effective soil pH, especially when soils are amended with organic materials. The rational is that soil enzyme assays integrate soil chemical, soil physical and soil mineralogical parameters to express a single response (Dick et al., 2000).

1.6.4 Wood ash effects on soil CO_2 dynamics

Wood ash application has been shown to have variable effects on soil CO_2 fluxes, influenced by soil type and climate. For example, 5 Mg ha^{-1} of granulated wood ash had no effect on soil CO_2 fluxes after one year in boreal peatland forests (Maljanen et al., 2014). However, in a boreal coniferous forest with mineral soil, 3 and 8 Mg ha^{-1} of loose wood ash increased CO_2 fluxes after one year (Maljanen et al., 2006). Conversely, other research has reported reductions in CO_2 fluxes following wood ash application to drained organic soils (Klemedtsson et al., 2010).

Understanding the impact of wood ash on CO_2 dynamics involves distinguishing between soil respiration, which is the biological production of CO_2 by roots, mycorrhizal fungi, and microbial decomposers, and CO_2 efflux, the movement of CO_2 from the soil surface into the atmosphere. While total CO_2 efflux integrates these sources, it is influenced by both production and transport processes and thus does not always align directly with measured respiration (Zimmermann & Frey, 2002). For example, Lecki et al. (2022) emphasize that efflux reflects a mixture of autotrophic (root), mycorrhizal, and heterotrophic (microbial) respiration, and that the relative contributions and regulatory controls can shift in response to disturbance or amendment.

Several methods are available for measuring CO_2 efflux in forest soils, each with its own benefits and limitations. Static and dynamic chamber systems are widely used in field studies due to their portability and cost-effectiveness. Static chambers are simple to deploy and useful for capturing short-term fluxes, though they can be prone to pressure artifacts and offer limited temporal resolution (Pumpanen et al., 2004). Dynamic chambers, particularly those paired with infrared gas analyzers (IRGAs), provide continuous and high-precision flux measurements, but they require more technical expertise and maintenance (Knoepp & Vose, 2002). Automated chamber systems offer fine-scale temporal coverage and capture diurnal and seasonal variability but are more expensive and logistically demanding (Pumpanen et al., 2004). As highlighted by Lecki et al. (2022), method choice can substantially influence interpretations of wood ash effects, especially when differentiating between microbial and root respiration or when aiming to detect short-term vs. long-term flux changes.

Wood ash has a high pH of 9-13 and raises soil pH, which can affect carbon release and mineralization of organic matter. This shift in soil chemistry can enhance carbon mineralization and CO_2 production, particularly through increased microbial growth rates and organic matter decomposition (Corre et al., 2003; Persson et al., 1994; Bååth and Arnebrant, 1994; Persson et al., 1989; Weber et al., 1985; Zimmermann & Frey, 2002). However, soil CO_2 efflux is regulated by multiple factors, including soil temperature, moisture, substrate availability, pH, and plant root activity (Davidson & Janssens, 2006). Soil structure and gas diffusion rates also influence how rapidly CO_2 moves from the soil to the atmosphere (Lecki et al., 2022), making it important to consider both source contributions and transport dynamics when interpreting changes in efflux following wood ash application.

Additionally, CO_2 and moisture stored in wood ash can react to form carbonates, bicarbonates, and hydroxides (e.g., lime, CaO; calcite, $CaCO_3$; portlandite, $Ca(OH)_2$; calcium silicate, Ca_2SiO_4), further raising soil pH (Demeyer et al., 2001; Etiégni & Campbell, 1991). This process, known as carbonation, involves the reaction of alkaline compounds in wood ash with atmospheric or soil CO_2, leading to the formation of stable carbonate minerals such as $CaCO_3$ Viola et al., 2024). The rate and extent of carbonation are influenced by the mineral composition of the wood ash, as well as soil pH, moisture, and ambient CO_2 concentrations. While carbonation can contribute to long-term CO_2 storage (Koch et al., 2021), some pre-existing carbonates in wood ash may react with acidic forest soils and release CO_2 instead.

However, the impact of wood ash on soil CO_2 fluxes can be complex. While carbonation can store CO_2, the application of wood ash to soil can also stimulate microbial activity and enhance organic matter decomposition, leading to increased CO_2 emissions in some cases (Moilanen et al., 2012). Studies on forest soils have shown that wood ash can both enhance soil respiration and contribute to long-term carbon stabilization, with effects varying based on application rates and environmental conditions (Couch et al., 2020; Reed et al., 2017). While carbonation offers a potential pathway for carbon storage, careful management is required to optimize application rates and minimize potential increases in soil respiration and CO_2 release (Neeraj, & Yadav, 2020).

1.7 Wood ash effects above-ground

Wood ash application can lead to significant changes in forest ecosystems, with both positive and negative effects. In Finland, for instance, applying wood ash to an afforested, drained mire resulted in a complete substitution of ground vegetation after 50 years (Moilanen et al., 2002). This application has caused damage to mosses, lichens, and bryophytes (Økland et al., 2022; Ozolinčius et al., 2007), though these species often recover quickly (Jacobson & Gustafsson, 2001). Deighton & Watmough (2021) and Conquer et al (2024) observed significant increases in Ca and Mg concentrations and changes in understory vegetation composition. The impact on ground vegetation varies based on factors such as soil type, baseline fertility, ash dosage, and study duration (Aronsson & Ekelund, 2004; Augusto et al., 2008).

In terms of tree growth, wood ash application generally promotes increased growth, although results can be mixed (Moilanen et al., 2002; Reid & Watmough, 2014; Saarsalmi et al., 2006). A meta-analysis conducted by Reid & Watmough (2014) found that while wood ash application can enhance base cation availability and improve soil conditions, tree growth responses varied depending on species, site conditions, and ash application rates. Some studies within the meta-analysis reported increased diameter growth in hardwoods following wood ash additions, whereas others found neutral or even negative effects, particularly in nutrient-poor or highly acidic soils. The variability in response highlights the importance of site-specific considerations when applying wood ash as a forest soil amendment. Research has primarily focused on conifers (Pitman, 2006), but hardwoods, which have a higher demand for base cations like Ca, Mg, and K, might benefit more from ash application (Vance, 1996). Even so, Monterey pine (*Pinus radiata*) showed increased height and diameter after applying mixed wood bark ash at dosages of 5 and 10 Mg ha^{-1} for 5 years (Solla-Gullon et al., 2008). Similarly, Scots pines exhibited enhanced growth in the top and middle crown with combined wood ash and nitrogen applications (Ozolinčius et al., 2007). Long-term studies found substantial stem growth increases in Scots pine (*Pinus sylvestris*) with wood ash applications at rates of 8 and 16 Mg ha^{-1}, with wood production being 13 to 17 times greater in ash-treated plots compared to untreated ones. Untreated trees suffered from foliar deficiencies in P and K, whereas trees in ash-treated plots at 16 Mg ha^{-1} showed no nutrient shortages (Moilanen et al., 2002).

1.8 Sugar maple

Sugar maple, a keystone species in eastern North American forests, is crucial both economically and culturally. It plays a significant role in Canada's economy, contributing through timber and maple syrup production, which represented 78% of the global maple syrup supply in 2022 and generated an average of $512 million annually from 2018 to 2022 (AAFC, 2023). Despite its importance, sugar maple has faced considerable localized dieback since the 1940s, particularly in areas with acid-sensitive soils or poor base saturation (Bal et al., 2015; Watmough et al., 1999; Duchesne et al., 2002; Horsley et al., 2000). This decline is closely linked to soil acidification and nutrient imbalances, especially Ca depletion, made worse by decades of acid deposition. These processes increase the mobility of toxic metals like Al while reducing essential base cations (Horsley et al., 2000). While sugar maple decline is not uniform across its range, regions with thin, base-poor soils are especially vulnerable. In these areas, wood ash may help restore nutrient balance. However, on soils that already have good base status, adding wood ash could be unnecessary and might even disrupt soil chemistry or microbial activity due to its high pH and nutrient content (Deighton & Watmough 2020).

Research indicates that soil amendments can mitigate problems associated with acidic soils. For instance, the application of lime has been shown to reduce crown dieback and improve foliar nutrient concentrations (Wilmot et al., 1996; Moore & Ouimet, 2006). Wood ash, rich in Ca and other essential nutrients, offers a promising alternative. Studies have demonstrated that wood ash can enhance soil pH and base cation levels, leading to improved tree growth and reduced nutrient deficiencies (Arseneau et al., 2021; Deighton & Watmough, 2020). Although wood ash can increase metal concentrations in the soil, these have not been shown to adversely affect tree growth (Deighton et al., 2021). For example, Arseneau et al. (2021) found increased sugar maple growth within the first year of wood ash application, while Syeda (2023) reported significant increases in pH and exchangeable base cations in the organic horizon but no significant effects on tree growth.

Given sugar maple's sensitivity to nutrient depletion, particularly Ca, using wood ash as a soil amendment could counteract the effects caused by acidic deposition in soils where base cations have been depleted. Since Ca plays a critical role in tree physiological processes, including cell wall formation, photosynthesis, and root development (Lautner & Fromm, 2010), restoring Ca through wood ash application may influence C dynamics. Enhanced Ca availability could improve tree growth and foliar productivity, leading to increased C assimilation and storage in biomass. Understanding these interactions is essential for evaluating the broader implications of wood ash application on sugar maple health, and forest C cycling.

1.9 Objectives and Hypotheses

This study focused on evaluating the short-term effects of non-industrial wood ash additions in a sugar maple-dominated stand in Muskoka, Ontario through two separate research chapters. The first research chapter (Chapter 2) investigates the optimal dosage of NIWA and its effects on sugar maple over a short-term period of 2 years. In this chapter we analyzed how the various doses (Control, 2 Mg ha^{-1}, 4 Mg ha^{-1}, 6 Mg ha^{-1}, and 12 Mg ha^{-1}) influenced soil chemistry (pH, LOI, nutrients and metal concentrations), sugar maple root density, sugar maple foliar nutrient and metal chemistry, and sugar maple radial stem growth. The second research chapter (Chapter 3) assessed and compared the short-term effects of NIWA and dolomitic lime through field and column experiments using the lowest dose and highest dose (2 Mg ha^{-1} and 12 Mg ha^{-1}) identified in Chapter 2. This study focused on their effects on soil chemistry (pH, LOI, exchangeable cations), soil CO_2 fluxes, and soil microbial enzyme activities.

1.9.1 Chapter 2 hypotheses

The objective of this research chapter was to assess how varying doses of NIWA affect soil chemistry under sugar maple canopies growing on acid sensitive soils, as well as sugar maple root density, foliar chemistry, and radial stem growth. It was hypothesized that, following application, soils would show a dose-dependent gradient, with higher doses resulting in immediate increases in pH and nutrient concentrations (Ca, K, Mg, P) in the upper organic soil horizons. Conversely, increases in metal concentrations (Al, B, Cd, Cu, Fe, Mn, Ni, Pb, Zn) would be limited due to their retention in the organic layer. It was also anticipated that nutrient levels in sugar maple foliage would reflect the changes observed in the soil, without a corresponding rise in metals. Additionally, it was predicted that both sugar maple fine root growth and radial stem growth would increase along the gradient of NIWA doses, with higher doses leading to greater growth.

1.9.2 Chapter 3 hypotheses

The objective of this research chapter was to assess how soil responses to NIWA and dolomitic lime applications differ by examining soil chemistry, CO_2 fluxes, and microbial enzyme activity. It was hypothesized that both NIWA and dolomitic lime would cause rapid, short-term changes in soil microbial activity due to increased pH and nutrient availability. Additionally, it was anticipated that higher doses of NIWA and dolomitic lime would enhance atmospheric CO_2 drawdown into the soils, and that the mineralogical properties of the amendments would significantly impact the recorded CO_2 fluxes.

1.10 Significance of research

The conclusions of this research will shed light on the potential for enhancing CO_2 sequestration with NIWA and improve our understanding of carbon cycling both above and below ground. Non-industrial wood ash could serve as a fertilizer for forest soils without harming the forest ecosystem. However, because NIWA contains trace metals subject to regulatory limits for safe application, it is essential to first investigate these aspects to ensure the health of forest ecosystems. Ultimately, this research will offer valuable insights for effective forest management and enhancement in Ontario and similar forested regions.

2. The Short-Term Response of Sugar Maple Following the Application of Non-Industrial Wood Ash.

2.1 Abstract

Ongoing forestry practices along with legacy effects of acidic deposition have resulted in nutrient deficient soil that may hinder tree growth, particularly in sugar maple (*Acer saccharum* Marsh.), a keystone species in Ontario's hardwood forests. Wood ash, a byproduct of bioenergy production, offers a potential solution for restoring soil pH and replenishing key nutrients, including calcium (Ca), magnesium (Mg), and potassium (K). However, variability in tree growth responses and concerns about heavy metal accumulation have limited its use in Canada. This study evaluates the effects of varying wood ash dosages (0, 2, 4, 6, and 12 Mg ha^{-1}) on sugar maple growth, and soil, foliar and root chemistry up to 2 -years post wood ash application. There was a large and significant increase in soil pH and exchangeable base cations in the organic horizons immediately after ash application, with effects diminishing in the second year. Metal concentrations also increased in the forest floor, post application and by the second year were most enriched in the humus horizon. Foliar nutrient and metal concentrations remained within healthy ranges. No short-term growth response was observed in basal area increment, consistent with delayed effects seen in similar studies, but fine root biomass was reduced by wood ash application. This little or reduced growth response may be due to the delayed movement of growth limiting nutrients to mineral soils, pH shock, or due to nitrogen (N) being a limiting factor and is not present in wood ash.

2.2 Introduction

The acidification of forest soils results in the increased mobilization of metals such as aluminum (Al) and base cation depletion from the soil exchange complex can lower calcium (Ca) availability to trees (Lawrence et al., 1995; Li et al., 2022). Additionally, ongoing forest management practices, such as logging, have further influenced forest structure, diversity, and resilience, and intensified the acidification of soils (Camarero, 2017; De Schrijver et al., 2006). In regions where soils are dominated by silicate minerals, such as the Precambrian Shield, base cations are slowly replenished via weathering processes, resulting in a slow recovery of depleted base cations on the soil exchange complex. Base cation depletion has been associated with reduced growth and foliar nutrition in forested stands in Pennsylvania, Quebec, and Ontario (Duchesne et al., 2002; Horsley et al., 2000; Long et al., 2009; McLaughlin et al., 1987; Ouimet & Camire, 1995).

Sugar maple (*Acer saccharum* Marsh.) trees are highly sensitive to soil acidity and low soil Ca content and are a dominant hardwood species that is economically important to Ontario (Gradowski & Thomas, 2006; Little, 1971; Minorsky, 2003). Sugar maple decline has been notably documented across Ontario, Quebec, and the northeastern United States (McLaughlin, 1998). While some recovery has been noted in certain regions since that time, recent studies indicate that growth suppression and dieback continue to affect sugar maple populations. For example, Boakye et al. (2023) found that sugar maple growth in its southern range has been declining since the 1980s, with implications for forest structure and carbon storage. Numerous studies have reported decreased vigor, canopy condition, growth rates, and seedling recruitment in sugar maple growing on nutrient depleted soils (Driscoll et al., 2001; Duchesne et al., 2002; McLaughlin & Wimmer, 1999; Sullivan et al., 2013).

Soil amendments including lime, wood ash, biochar, and wollastonite have reported beneficial effects on sugar maple (Arseneau et al., 2021; Juice et al., 2006; Moore et al., 2015; Moore & Ouimet, 2021; Sifton et al., 2023; Wilmot et al., 1996). Reducing forest soil acidity with lime amendments has been practiced in Europe and North America since the 1980s (Hüttl & Zöttl, 1993; Long et al., 1997). Forest liming amendments typically consist of alkaline-earth metals, such as Ca that reduces soil acidity and improves plant growth (Reid & Watmough, 2014). Increases in soil pH and concentrations of essential macronutrients such as Ca, magnesium (Mg), potassium (K), and phosphorus (P) can persist in the soil and foliage up to 23 years after initial liming treatment (Juice et al., 2006; Long et al., 2011; Moore et al., 2012; Moore & Ouimet, 2021). Soil amendments have led to increased vigor, crown health, growth rate, recruitment of seedlings, and wound closure on sugar maple trees (Arseneau et al., 2021; Deighton & Watmough, 2020; Houle et al., 2002; Huggett et al., 2007; Juice et al., 2006; Long et al., 2011; Moore & Ouimet, 2010; Wilmot et al., 1996). However, due to the emission of greenhouse gases generated by lime production and its high cost, alternative materials are currently being investigated (Zagvozda et al., 2022). Thus, wood ash is garnering attention in a renewable energy context, and recycling of the ash produced has growing potential for use as a nutrient input in forest systems (Pitman, 2006).

While wood ash is commonly obtained from industrial sources such as Canadian pulp and paper mill boilers, which produced up to 0.75 million Mg of ash annually as of 2002 (Elliott & Mahmood, 2006), non-industrial wood ash (NIWA) is a comparatively underutilized resource. In Ontario alone, residential homes generate approximately 18,000 Mg of wood ash annually (Azan, 2017). Unlike industrial wood ash, which benefits from controlled combustion processes and feedstock management, NIWA often originates from a diverse range of tree species and components burned without consistent oversight, resulting in variable nutrient, metal concentrations, and pH (Azan et al., 2019; Deighton & Watmough, 2020). The effect of NIWA on forest soils is not solely determined by ash composition, but also by the local soil conditions into which it is applied (Deighton & Watmough, 2020). Soil pH, buffering capacity, organic matter content, and existing nutrient levels can all influence the mobility, solubility, and effectiveness of ash-derived elements (Demeyer et al., 2001). For instance, in acidic or base-poor soils, NIWA may neutralize acidity and improve nutrient availability, while in already alkaline or nutrient-rich soils, it could lead to nutrient imbalances or metal mobilization (Deighton & Watmough, 2020). As such, both the source characteristics of the ash and the chemical properties of the recipient soils must be considered when evaluating the suitability and potential risks of NIWA application. Despite the variability, the application of NIWA to forest soils has shown potential to boost soil pH, foliar Ca, tree growth, and overall forest productivity (Augusto et al., 2008; Reid & Watmough, 2014).

The expansion of forest biomass for energy in both residential heating (wood stoves and fireplaces) and industrial bioenergy facilities across Canada is increasing wood ash production. Despite its potential benefits, the widespread use of wood ash is limited by Canadian provincial and territorial regulations, which cite concerns about its effects on soil chemistry and forest nutrition, particularly the accumulation or mobilization of heavy metals. Combined with high costs for approval, transport, and application, landfilling remains the more economical choice (Hannam et al., 2018). However, research shows that wood ash can play a valuable role in forest management. It has been shown to replenish nutrients like P, Ca, magnesium (Mg), and potassium (K) removed during harvesting, mitigate soil and water acidification from atmospheric deposition, and may improve tree growth (Augusto et al., 2008; Huotari et al., 2015; Reid & Watmough, 2014).

Tree growth responses to wood ash application, however, are highly variable both among species and by study design (location and dosage) (Pitman, 2006; Reid & Watmough, 2014). While some studies found no significant growth response in sugar maple seedlings following wood ash application at a rate of 6 Mg ha^{-1} (Deighton & Watmough, 2020), there are relatively few studies that have examined mature sugar maple tree growth responses to wood ash addition. Arseneau et al. (2021) observed significant growth responses in mature sugar maple trees one to three years after applying 5, 10, and 20 Mg ha^{-1} of wood ash. Notably, this represents the first documented instance of a positive short-term (up to 3 years) growth response to wood ash application in mature sugar maple trees within an operational forest management context. While historically, forest decline and recovery patterns in these regions have been similar, differences in response may reflect variations in ash chemistry, application method (surface vs. incorporated), dosage, soil base status, or tree age class. Or if the localized soil conditions were more nutrient-depleted or responsive, allowing for a stronger benefit from ash inputs. These findings highlight a critical need for further research to identify the optimal wood ash dosage for maximizing tree growth and to develop precise and accurate methods for tracking tree growth over time.

To address short-term tree growth response to wood ash, accurately and precisely measuring growth responses is essential. Many studies rely on diameter tapes or calipers to assess stem growth (Long et al., 2011). However, these tools can introduce error due to difficulty in consistently measuring the same point on a tree trunk across different time intervals (Cattelino et al., 1986). Dendrometer bands, by contrast, offer a superior alternative. Permanently attached to the tree, they allow precise, repeatable measurements of diameter growth using digital calipers, providing a cost-effective and reliable method for long-term growth monitoring (Cheng et al., 2023; McMahon & Parker, 2015). Dendrometer bands have been successfully used to study pathogen impacts on tree growth, tropospheric ozone impacts, climate sensitivity to watershed acidification, and evaluate fertilizer effects on northern hardwoods (Lea et al., 1979; Malcomb et al., 2020; McLaughlin & Downing, 1996; Shaw & Toes, 1977). They are also valuable in forest inventory work when applied to permanent growth plots (Lea et al., 1979). For research on sugar maple growth following wood ash application, dendrometer bands offer significant advantages over diameter tapes, enabling more accurate assessments of growth responses across varying wood ash dosages. Using dendrometer bands could refine our understanding of the optimal wood ash application rate, supporting sustainable forestry practices and unlocking the full potential of this underutilized resource.

This study aims to examine the effects of non-industrial wood ash (NIWA) application over a two-year period on a sugar maple dominated hardwood stand in Bracebridge, Ontario. Specifically, it evaluated the response of sugar maple growth (fine root biomass and circumference), soil chemistry, and foliar nutrient levels to varying NIWA dosages: 0 (control), 2, 4, 6, and 12 Mg ha^{-1}. These dosages were selected to reflect a range of realistic application rates based on previous field studies in Ontario and Quebec (Reid & Watmough, 2014; Arseneau et al., 2021), while also capturing a dose–response gradient to evaluate potential thresholds for ecological benefit or risk. The 2 Mg ha^{-1} dose was included based on estimates from Azan et al. (2019), who proposed that this amount may be sufficient to replenish calcium losses in Muskoka forests caused by decades of acid deposition. The 6 Mg ha^{-1} dosage aligns with common trial rates in northern hardwood systems, while 12 Mg ha^{-1} represents a higher-end scenario to test for any negative effects at elevated application levels. It was hypothesized that wood ash application would enhance nutrient availability for sugar maple trees without causing harmful effects from metal toxicity. Increased nutrient availability was anticipated to promote radial tree growth. It was further predicted that wood ash application would significantly raise soil pH and elevate concentrations of key nutrients (Ca, Mg, K) and metals (Al, Fe, Zn, Mn) in the soil horizons. Additionally, foliar nutrient concentrations were expected to respond to these changes within one to two growing seasons following application. Finally, it was hypothesized that tree growth differences in the treated plots would become more pronounced following ash application with greater growth observed in ash-treated trees compared with the control. By examining these effects, this study seeks to contribute valuable insights into the potential of NIWA as a sustainable soil amendment for enhancing soil nutrients and tree vigour.

2.3 Methods

2.3.1 Study site

The study location is a 97-hardwood forest in the Great Lakes-St. Lawrence ecozone east of the town of Bracebridge, Ontario, Canada (45°04'14.5"N, 79°09'00.7"W). The study site is located within a recreational camp called Camp Big Canoe, and is an undisturbed, relatively even aged (~50-year-old) sugar maple dominated forest and includes white ash (*Fraxinus americana* L.), American beech (*Fagus grandifolia* Ehrh.), and yellow birch (*Betula alleghaniensis* Britt.). The study site has shallow, coarse-textured sandy loam soils that are classified as Sombric Brunisols overlying granitic gneiss Precambrian Shield bedrock (Soil Classification Working Group, 1998). The average annual precipitation (from 1981-2010) of the region is 832 mm. The average daily minimum and maximum temperatures between 1981-2010 were -15.8 °C and -4.8°C respectively in January and 9.9 °C and 22.5 °C in July (Environment and Climate Change Canada, 2021).

2.3.2 Plot setup and experimental design

Forty sugar maple trees, each measuring between 20 and 25 cm in diameter at breast height (DBH), were selected randomly within the study site. Each tree was designated as the central point of a 28 m^2 circular plot, featuring a 3 m radius. These plots were established with a 10 m buffer between them in the study forest in early July 2021. Each plot was selected based on the following characteristics: sugar maple trees were free of defects or decay, far from roads and urban locations, and plots had relatively flat slopes and be a minimum of 60 m away from any watercourse to minimize runoff after wood ash application. Volunteer residents from Muskoka contributed non-industrial wood ash, which was collected by the charitable organization Friends of the Muskoka Watershed (FMW, 2023). After collection, the ash from various sources underwent homogenization and sieving (<2 mm) to remove charcoal, nails, plastic, and other large debris. Following this, it was stored in large polyethylene containers in a cool, dark environment. Subsequently, the ash was weighed and transported in 10 kg buckets to each treatment plot, where it was applied by hand to ensure uniform distribution in October 2021. A total of 40 plots were established, with 8 replicate plots assigned to each of five treatment levels: 0 (control), 2, 4, 6, and 12 Mg ha^{-1}. Wood ash was applied to 32 plots, while 8 control plots received no ash (0 Mg ha^{-1}).

Sub-samples of ash were collected in November 2020 to determine the ash chemistry (Conquer et al., 2023). Levels of trace metals in NIWA were low compared with the unrestricted (CM1) and restricted (CM2) of the Nutrient Management Act (NASM) guidelines for unrestricted land use for non-agricultural non-aqueous source material (Table 2.1; Nutrient and Management Act, 2002).

Table 2. 1. Average pH, organic matter, nutrient, and metal concentrations of non-industrial wood ash (means ± SE) (Conquer et al., 2023). Ontario Regulation 267/03 of the Nutrient Management Act (NASM) limits for unrestricted (CM1) and restricted (CM2) use of wood ash for land application as a non-agricultural non-aqueous source material are also shown.

	Non-Industrial Wood Ash Properties	NASM limits	
		CM1	CM2
pH	13.0 (0.04)		
OM %	3.4 (0.3)		
C (%)	8.6 (0.1)		
N (%)	0.1 (0.0)		
Ca (g kg^{-1})	267 (3.0)		
K (g kg^{-1})	94.4 (2.9)		
Mg (g kg^{-1})	19.4 (0.3)		
Mn (g kg^{-1}	8.8 (0.3)		
P (g kg^{-1})	7.5 (0.1)		
Al (g kg^{-1})	3.8 (0.3)		
Fe (g kg^{-1})	2.2 (0.2)		
Zn (mg kg^{-1})	503 (18.5)	500	4200
Cu (mg kg^{-1})	164 (9.4)	100	1700
Cd (mg kg^{-1})	2.9 (0.2)	3	34
As (mg kg^{-1})	9.9 (2.2)	13	170
Ni (mg kg^{-1})	9.6 (0.6)	62	420
Pb (mg kg^{-1})	48.2 (16.1)	150	1100
B (mg kg^{-1})	265 (5.3)		

Government of Ontario, 2002, Conquer et al., 2023

2.3.3 Dendrometer bands

In August 2021 (prior to ash application), each central sugar maple in the study plots were fixed with a commercial precision dendrometer band (Series 5 Manual Band Dendrometer, Agricultural Electronics Corp., Tucson, AZ). The brass housing is attached to the tree with two stainless-steel bolts anchored 3 cm into the tree stem at breast height. This anchoring depth is standard in dendrometer band installations and is designed to provide stable support while minimizing damage to the cambial tissue. Previous studies have shown that shallow bolt anchoring at this depth has minimal impact on tree growth or physiology, particularly when limited to small diameter bolts and used in mature trees (Lea et al., 1979; DesRochers et al., 2003). Each dendrometer band has a 0.32-cm-wide stainless-steel strap attached to a brass housing consisting of a hinge, spring, and Vernier scale. The Vernier scale is fixed on the movable inner tube of the brass body, and the other tube was attached to the band and moves as the tree expands and contracts, being able to measure micrometer changes in the length of the band. A digital caliper (± 0.03 mm precision) was used to measure the growth (expansion or contraction) of the tree trunk. Monthly measurements tracked the progress of two growing seasons from 2022 to 2023.

Figure 2. 1. Series 5 Manual Band Dendrometer attached to a sugar maple tree in one of the study plots (A). One of the five soil root collars installed in each sugar maple plot in May 2022 (B).

2.3.4 Root ingrowth cores

Two hundred root ingrowth cores were created by cutting 15 × 18 cm rectangles from #5 plastic mesh canvas (Blue Ribbon Crafts – www.blueribboncrafts.com), shaping them into 5 × 5 cm cylinders, and sealing the sides and bottom with staples. At the beginning of the first growing season in May 2022, five root ingrowth cores were randomly distributed into each of the 40 circular plots. Using a trowel, a 5 cm hole was created in the upper mineral horizon beneath each central sugar maple in the study plots to insert the root ingrowth core, which was then filled with All Purpose Fine Granulated Washed Play Sand (Garden Club– www.canadiantire.ca) and covered with the surrounding leaf litter. At the end of the second growing season in October 2023 each ingrowth core was retrieved by cutting the roots around the core with a knife, carefully uprooting it using a trowel, and then storing it in polyethylene bags refrigerated at 4°C until analysis.

2.3.5 Field sampling

Baseline soil sampling was conducted in July 2021 and after ash application in June 2022 and 2023. Samples were collected beneath the sugar maple canopy within each plot. Grab samples were taken from the litter (L) and fibric-humic (FH) horizons, and an auger was used for the upper mineral (0 - 10 cm) soil that contained the Ah-horizon, and lower mineral (11 - 20 cm) soil that contained the upper B-horizon (AAFC 1998; The Canadian System of Soil Classification, 1998). Litter and FH samples were taken using a 10 cm ruler and a knife to form a set square. A soil auger was used to collect the 0-10 cm and 11-20 cm mineral horizons, and the L, FH, and mineral soil samples were stored in polyethylene bags and refrigerated at 4°C until analysis. Baseline and post application foliage samples were collected in September 2021, 2022, and 2023 using a 3.6 m pole pruner at the mid-crown, on the sun-facing side of the sugar maples canopy, and between 5 - 10 leaves were taken from each of the forty plots and stored in paper bags.

*2.3.*6 *Laboratory analysis*

2.3.6.1 Soil

Soil samples were oven-dried at 105 °C for 24 hours in a Grieve industrial oven. After drying, mineral soil was sieved to <2 mm. The L and FH layers were ground using a Wiley Mill. All soil layers were analyzed for pH and loss-on-ignition (LOI) and were analyzed for exchangeable cations (Ca, Mg, K, sodium (Na), P), and total metals (Al, arsenic (As), boron (B), cadmium (Cd), copper (Cu), iron (Fe), manganese (Mn), nickel (Ni), lead (Pb), and zinc (Zn).

Soil pH (0.01 M calcium chloride ($CaCl_2$)) was measured following Hendershot & Lalande (2008) method. A 1:5 ratio was used, shaken for 1 hour, and then measured using an OAKTON pH 510 series multimeter (Oakton Instruments, Vernon Hills, Illinois, USA). Organic matter content was analyzed using the loss of ignition (LOI) method by Hopkins (2008). Each sample was weighed to 1 g for LFH and 5 g for mineral soil and placed in porcelain crucibles to dry in the oven at 105 °C for 24 hours. The weight was recorded using an analytical balance and weighed again after the samples were ashed in the muffle furnace at 400 °C for 10 hours.

Metal concentrations were analyzed following acid digestion (United States Environmental Protection Agency Method 3050B, 1996). Briefly, 2.5 mL of nitric acid (HNO_3) was added to 0.2 g of foliage or soil. Samples were left to sit at room temperature to cold digest for 8 hours, then heated on a hotplate to 100°C for 8 hours. The digested samples were filtered through P8 Fast Flow Filter Paper and diluted to 25 mL in volumetric flasks and transferred to 50 mL FroggaBio conical tubes. Samples were additionally diluted to a 1:10 ratio using B-pure and analyzed by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) for concentrations of Ni, Al, Mn, Cd, As, B, Fe, Zn, Cu, and Pb. Reference samples for QA/QC were digested along with samples and blanks, using 0.2 g of EnviroMAT SS-2 Soil Standard for soil and 0.2 g of National Institute of Standards and Technology (NIST) SRM-1515 apple leaf for L, and FH.

Exchangeable cations (EC) were analyzed following the method described in Hendershot & Lalande (2008) by adding 25 mL of 1 M ammonium chloride (NH_4Cl) to 5 g of mineral soil or 1 g of LFH. Samples were shaken for 2 hours on a shaker table. They were then filtered through P8 Fast Flow Filter Paper. Samples were diluted 1:10 and acidified with 0.2 mL of trace metal grade HNO_3, and later analyzed using ICP-OES to measure concentrations of Ca, Mg, K, and Na.

2.3.6.2 Fine roots

Roots were extracted from their ingrowth cores by rinsing and sieving to separate them from the sand, followed by oven-drying at 105 °C for 24 hours using a Grieve industrial oven. The total dry mass of roots in each ingrowth core was measured using an analytical balance and summed for each tree. Subsequently, the roots were digested to determine total elemental concentrations (Ca, Mg, K, P, Al, As, B, Cd, Cu, Fe, Mn, Ni, Pb, and Zn) using HNO_3 digestion, following the same method as described earlier.

2.3.6.3 Foliage

Foliar samples were dried in a Grieve industrial oven at 105 °C for 24 hours, then ground using a coffee grinder. Nutrient and metal concentrations (Ca, Mg, K, P, Al, As, B, Cd, Cu, Fe, Mn, Ni, Pb, and Zn) were analyzed using HNO_3 acid digestion, following the method described earlier, and calibrated against NIST 1515-SRM apple leaf standards (SCP Science, Quebec, CA).

2.3.7 Statistical Analysis

Tree growth assessment was performed by converting ring width to basal area increment (BAI) to remove variation in radial growth attributable to increasing circumference. For comparing growth patterns, ring increments were converted to BAI (cm^2) with the formula:

$$\mathrm{BAI} = \pi(R^1{}_n - R^2{}_n) \tag{1}$$

To calculate BAI, the diameter of an individual tree is divided by 2 to obtain the radius in centimeters. The radius is multiplied by π to equal the area of the circle. To find the increment growth for each month R^1 is the tree radius at the beginning of the growing season and R^2 is the basal area of the tree measured at the end of each month. To ensure comparability, the mean (± SE) initial tree diameter prior to BAI calculations did not significantly differ among treatment groups (Control: 24.3 ± 1.0 cm; 2 Mg ha^{-1}: 21.8 ± 0.9 cm; 4 Mg ha^{-1}: 24.6 ± 1.3 cm; 6 Mg ha^{-1}: 22.2 ± 0.8 cm; 12 Mg ha^{-1}: 23.5 ± 1.3 cm; F=1.2, p=0.32).

Sugar maple foliar DRIS indices were calculated to determine whether nutrients were deficient (negative) or in excess (positive). Indices were determined by first calculating foliar P, N, Ca, Mg, and K DRIS ratios (Casson et al., 2012), $A/B \geq a/b$ (Eq. 2):

$$f\left(\frac{A}{B}\right) = \left(\frac{A/B}{a/b} - 1\right)\frac{1{,}000}{\mathrm{CV}} \tag{2}$$

or $A/B \leq a/b$ (Eq. 3):

$$f\left(\frac{A}{B}\right) = \left(1 - \frac{a/b}{A/B}\right)\frac{1{,}000}{\mathrm{CV}} \tag{3}$$

Foliar indices were then calculated (Eq. 4):

$$A \text{ index} = \frac{\left[f\left(\frac{A}{B}\right) + f\left(\frac{A}{C}\right) + f\left(\frac{A}{D}\right) + f\left(\frac{A}{E}\right)\right]}{z} \quad (4)$$

where A is defined as the foliar element that the index is being calculated for, B, C, D, and E refer to the other elements being measured, and z is the number of functions used within the nutrient index. The sum of all nutrient indices equals zero so that indices for each element can be compared relative to one another.

Statistical analysis was performed using R software version 4.2.2 (R Core Team, 2024). Soil and foliar chemistry were assessed between treatments within each year using a Wilcoxon rank-sum test with a Bonferroni correction to account for multiple comparisons (*rstatix package*). Fine root biomass and concentrations of nutrients and metal comparisons between treatments were conducted using a one-way analysis of variance (ANOVA). Differences in BAI over two growing seasons and among treatments were evaluated using Repeated measures ANOVA with treatment as a fixed effect and plot ID as a random effect. For variables showing significant effects, pairwise comparisons were conducted using Tukey's HSD with a Bonferroni correction (*multcomp package*). Significance was determined at $p < 0.05$ unless otherwise stated.

2.4 Results

2.4.1 Soil Chemical properties

Nonindustrial wood ash showed a strong treatment effect on soil pH that varied by dose and changed over time and by horizon, while reflecting a downward movement of ash over time (Figure 2.3). There was an immediate, large increase in pH in the L and FH horizons but by the second year the pH of the L horizon fell and only the highest NIWA dosage treatment remained significantly higher than the control, whereas the pH of the FH layer remained elevated. For instance, in the L horizon, plots treated with 12 Mg ha^{-1} reached a pH of up to 6.5, while the pH in the FH horizon increased to 7.2 by year 2. In the mineral horizons, a significant increase in pH was only evident in the 12 Mg ha^{-1} treatment (Figure 2.3). Organic matter content showed a significant decrease in the L horizon following NIWA application and increased back to control levels into the second year. By year 2, the organic matter content in the FH layer in the 12 Mg ha^{-1} dose was significantly lower than the control (Figure 2.3). Organic matter content in the 0 - 10 cm mineral horizon showed a significant increase after the first year but reverted to baseline levels in the second year and the lower mineral layer exhibited no change in organic matter content.

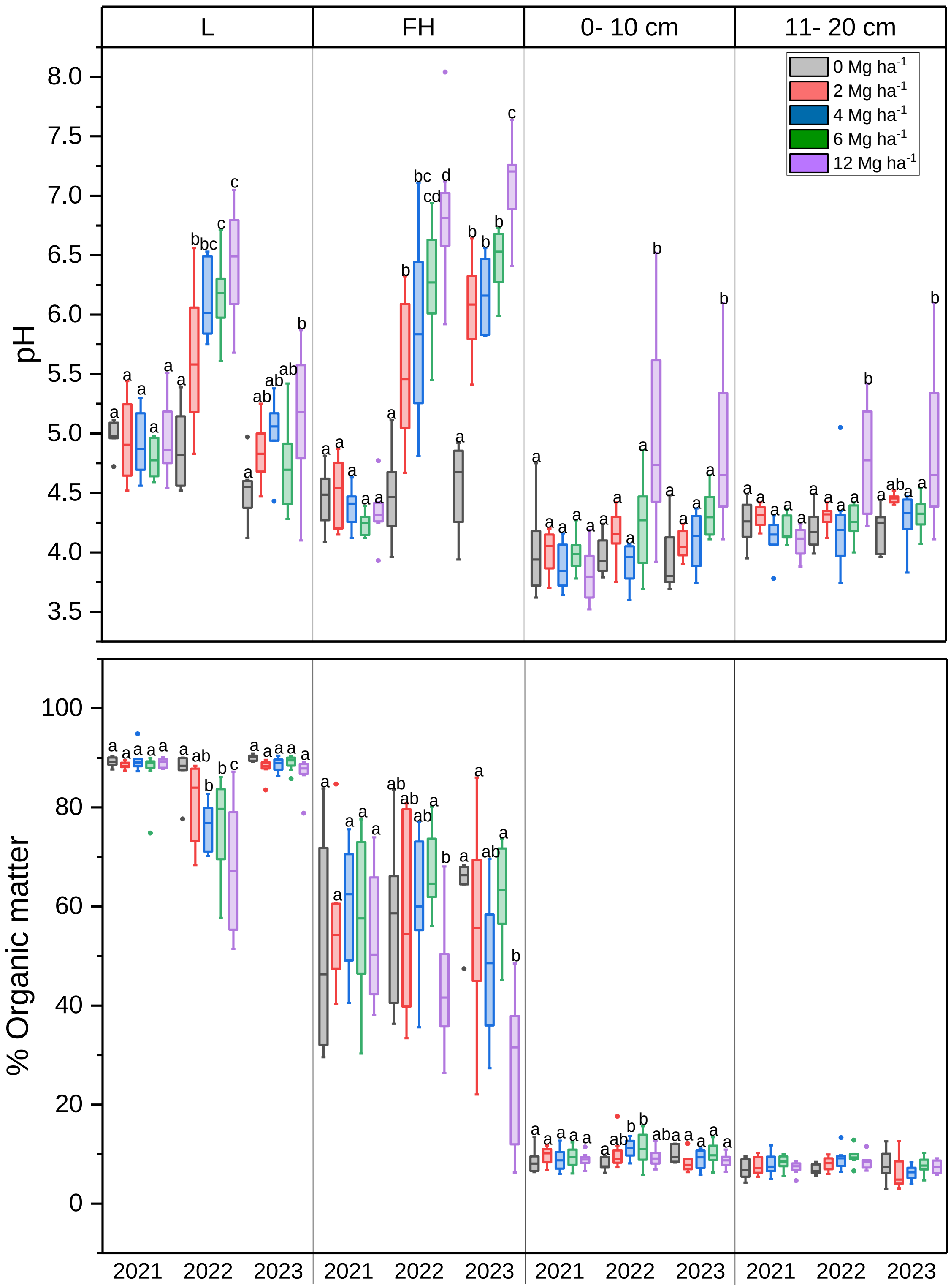

Figure 2. **2** Average pH and percent organic matter (OM) in the LFH, upper and lower mineral horizon sampled beneath the sugar maple tree in the control and NIWA treated plots prior to (2021) and two growing seasons after application (2022 & 2023). The letter display indicates significant differences between control and treatments ($p < 0.05$) determined by a Tukey HSD test.

Increases in soil exchangeable cation concentrations one year after application were observed primarily in the L and FH horizons (Figure 2.4). Calcium and Mg concentrations were significantly higher in the L and FH horizons of the treatment plots one year post application, whereas K was only significantly higher than controls in the FH layer (Figure 2.4). However, base cation concentrations measured in the L horizon were not significantly different from controls by the second year (FH data not available). Base cation concentrations in mineral soil horizons also increased post application with increases most evident for K and increased with ash dosage (Figure 2.5).

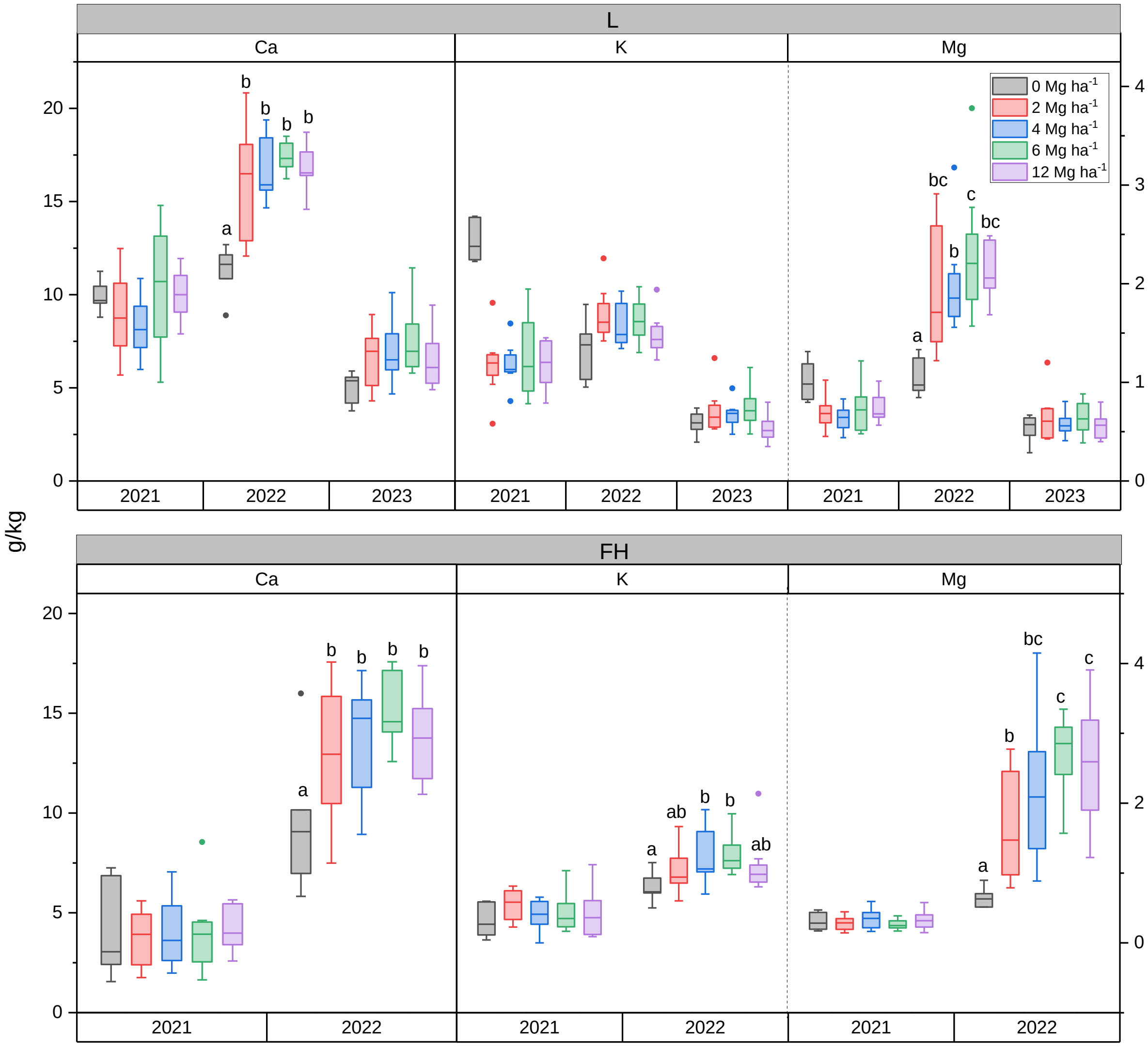

Figure 2. 3. Average exchangeable cations in the LFH horizon sampled beneath the sugar maple trees in the control and NIWA treated plots prior to application (2021) and two growing seasons after application (2022 & 2023). The letter display indicates significant differences between control and treatments ($p < 0.05$) determined by a Tukey HSD test.

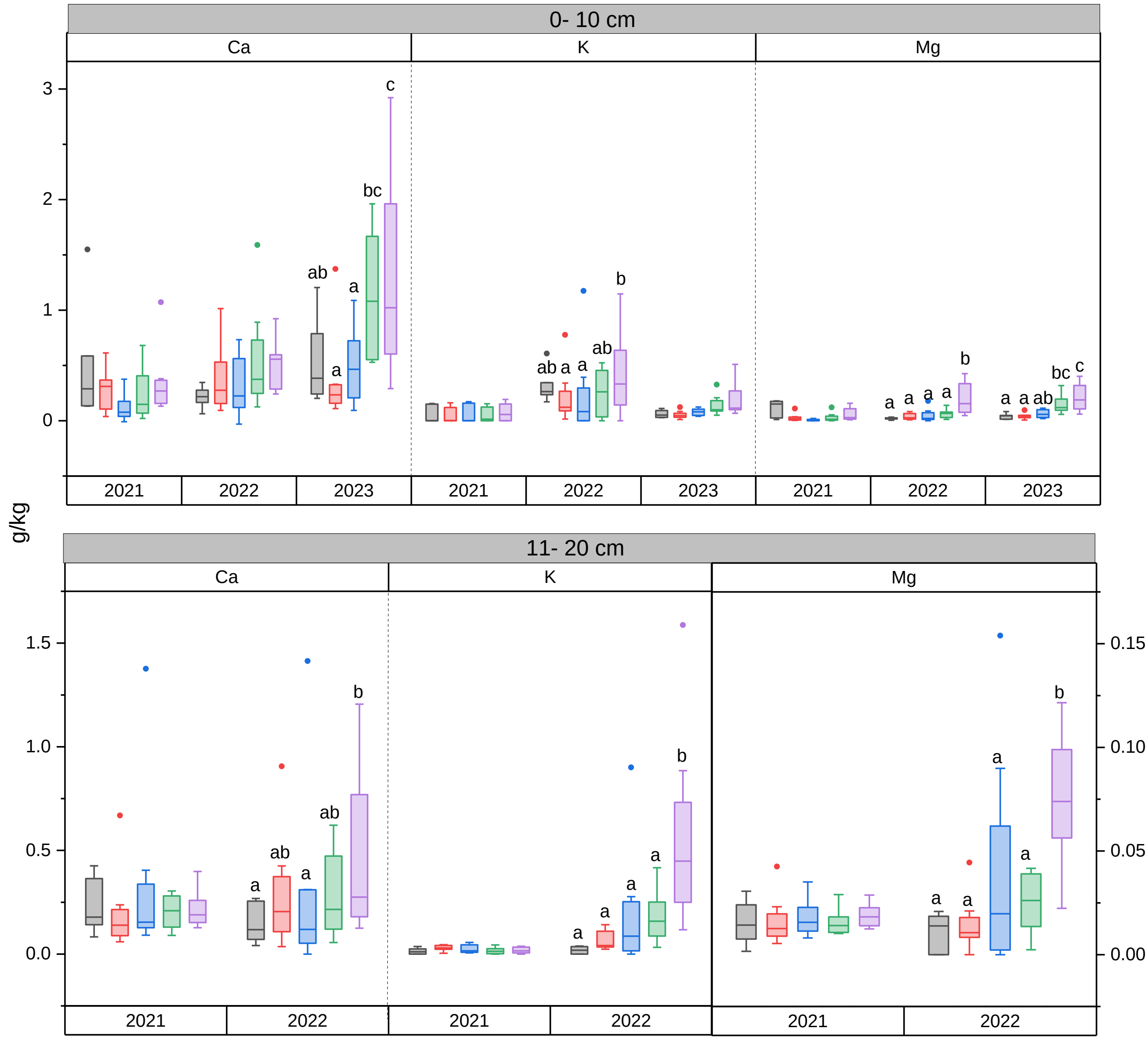

Figure 2. 4. Average exchangeable cations in the upper and lower mineral horizon sampled beneath the sugar maple trees in the control and NIWA treated plots prior to application (2021) and two growing seasons after application (2022 & 2023). The letter display indicates significant differences between control and treatments (p < 0.05) determined by a Tukey HSD test.

Following the NIWA application, concentrations of several metals increased significantly, but these changes were mostly confined to the L and FH horizons (Figures 2.6 and 2.7). Concentrations of Al, Mn, Cu, Pb, Cd, Ni, and Zn exhibited the largest increases in the L horizon of treated plots compared with the controls, but these elevated levels decreased by the second year (Figure 2.6). In the FH horizon, most (Cu, Zn, Ni, and Mn) of these metals and B remained elevated in both the first and second years after ash application (Figure 2.7). Metal concentrations in the upper and lower mineral horizons were unaffected one and two years after the application (Figures 2.8 & 2.9).

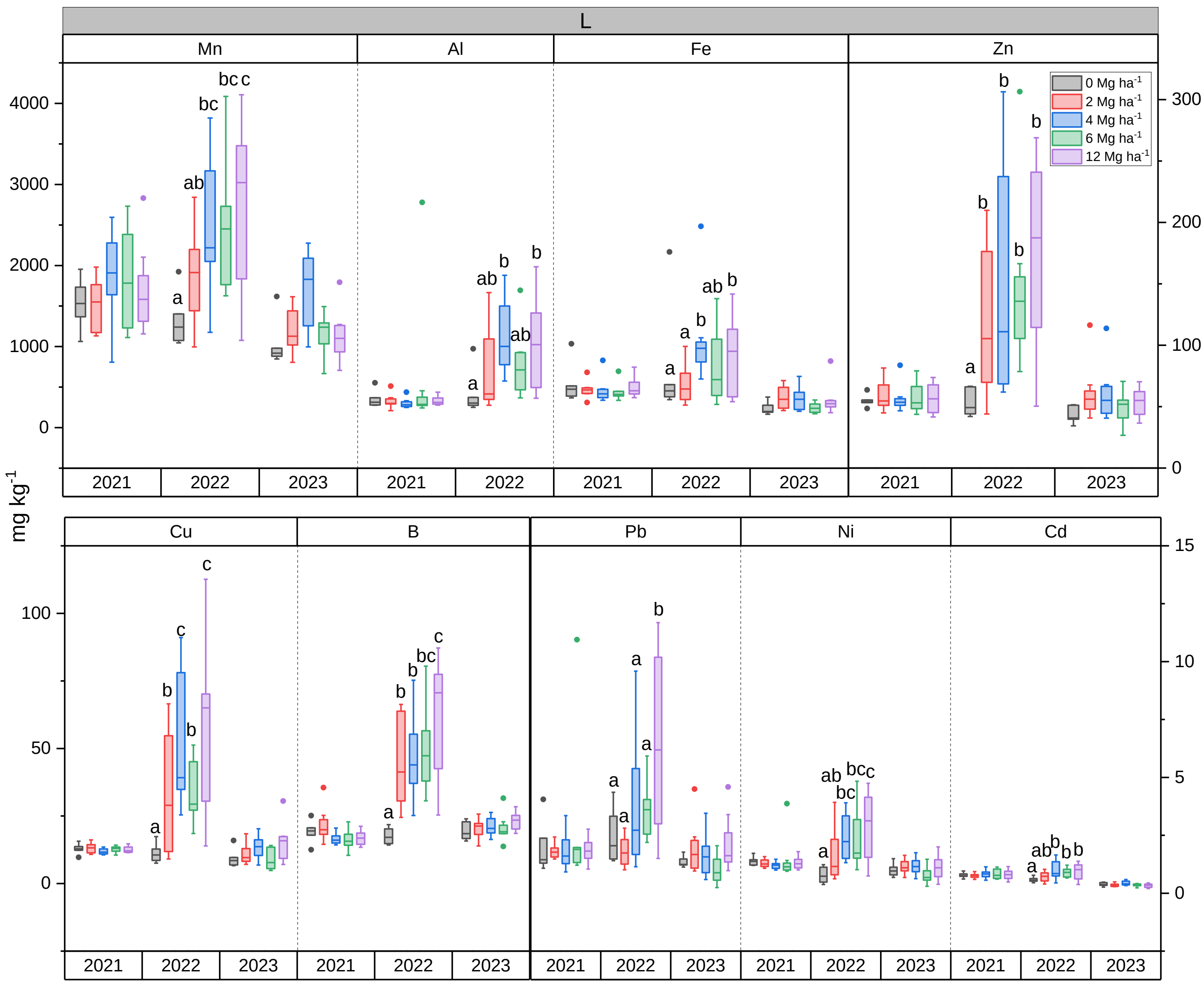

Figure 2. 5. Average elemental metal concentrations in the litter horizon sampled beneath the sugar maple tree in the control and NIWA treated plots prior to (2021) and two growing seasons after application (2022 & 2023). The letter display indicates significant differences between control and treatments ($p < 0.05$) determined by a Tukey HSD test.

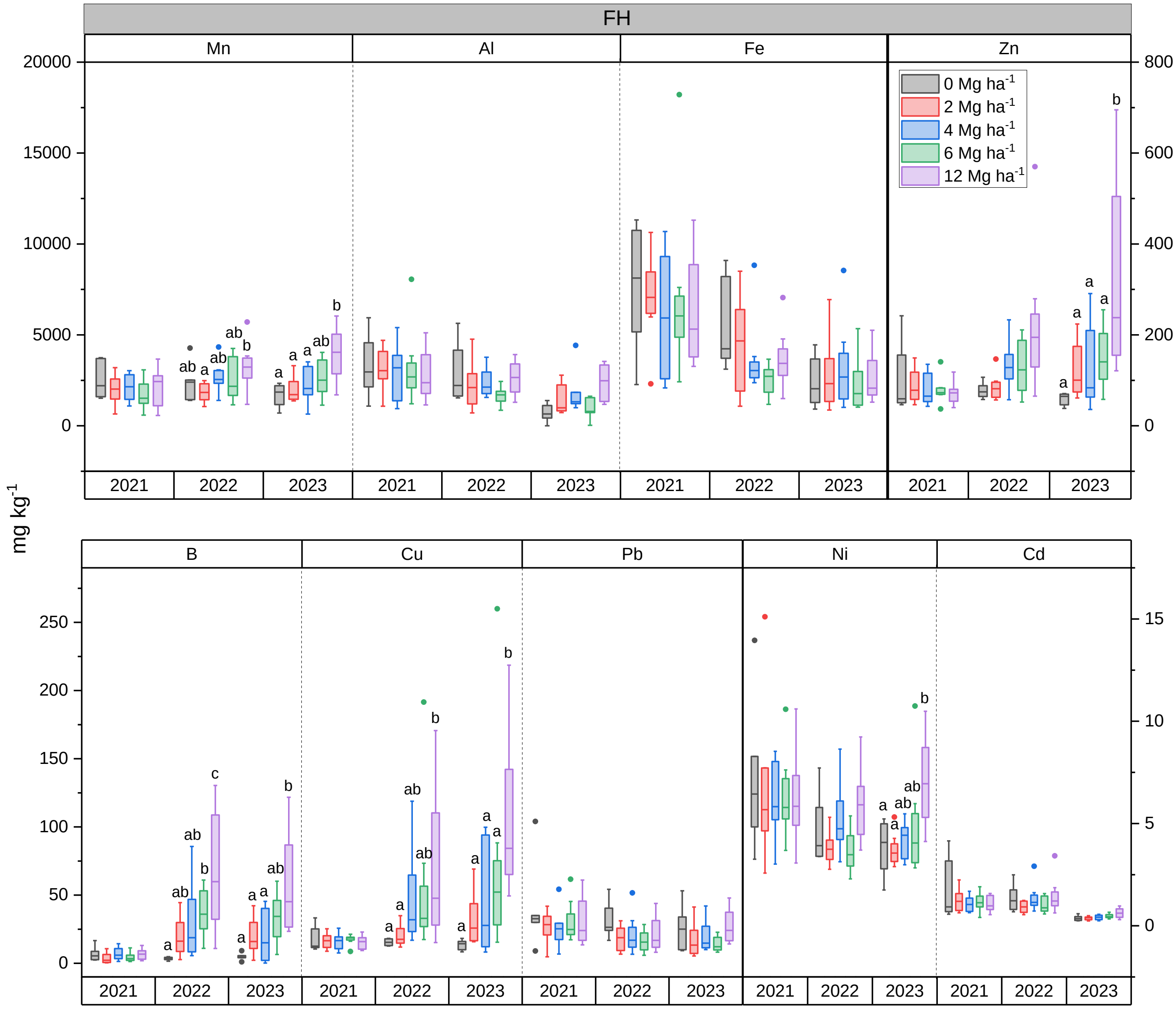

Figure 2. 6. Average elemental metal concentrations in the FH horizon sampled beneath the sugar maple tree in the control and NIWA treated plots prior to (2021) and two growing seasons after application (2022 & 2023). The letter display indicates significant differences between control and treatments ($p < 0.05$) determined by a Tukey HSD test.

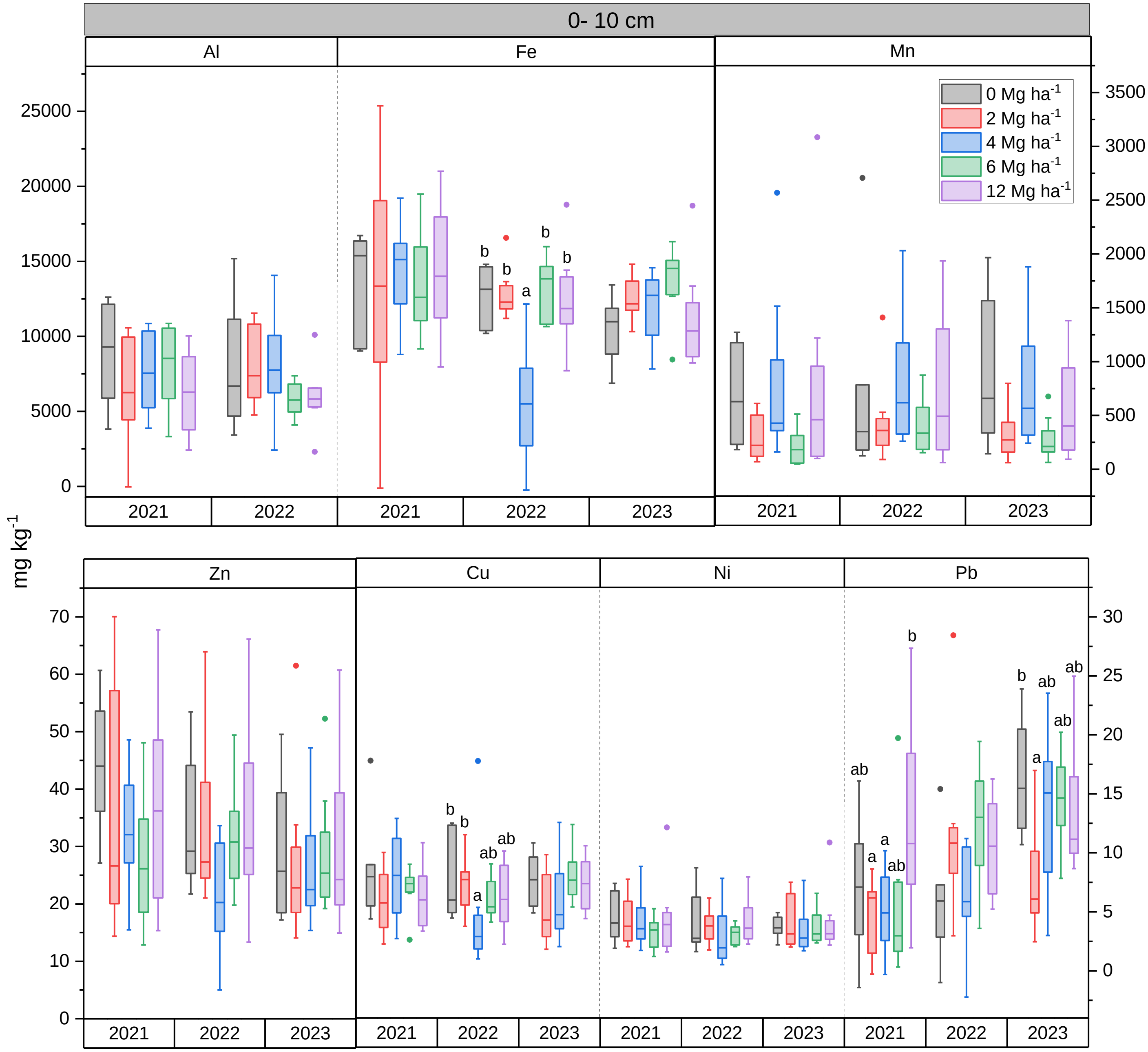

Figure 2. 7. Average elemental metal concentrations in the 0-10 cm upper mineral horizon sampled beneath the sugar maple tree in the control and NIWA treated plots prior to (2021) and two growing seasons after application (2022 & 2023). The letter display indicates significant differences between control and treatments (p < 0.05) determined by a Tukey HSD test.

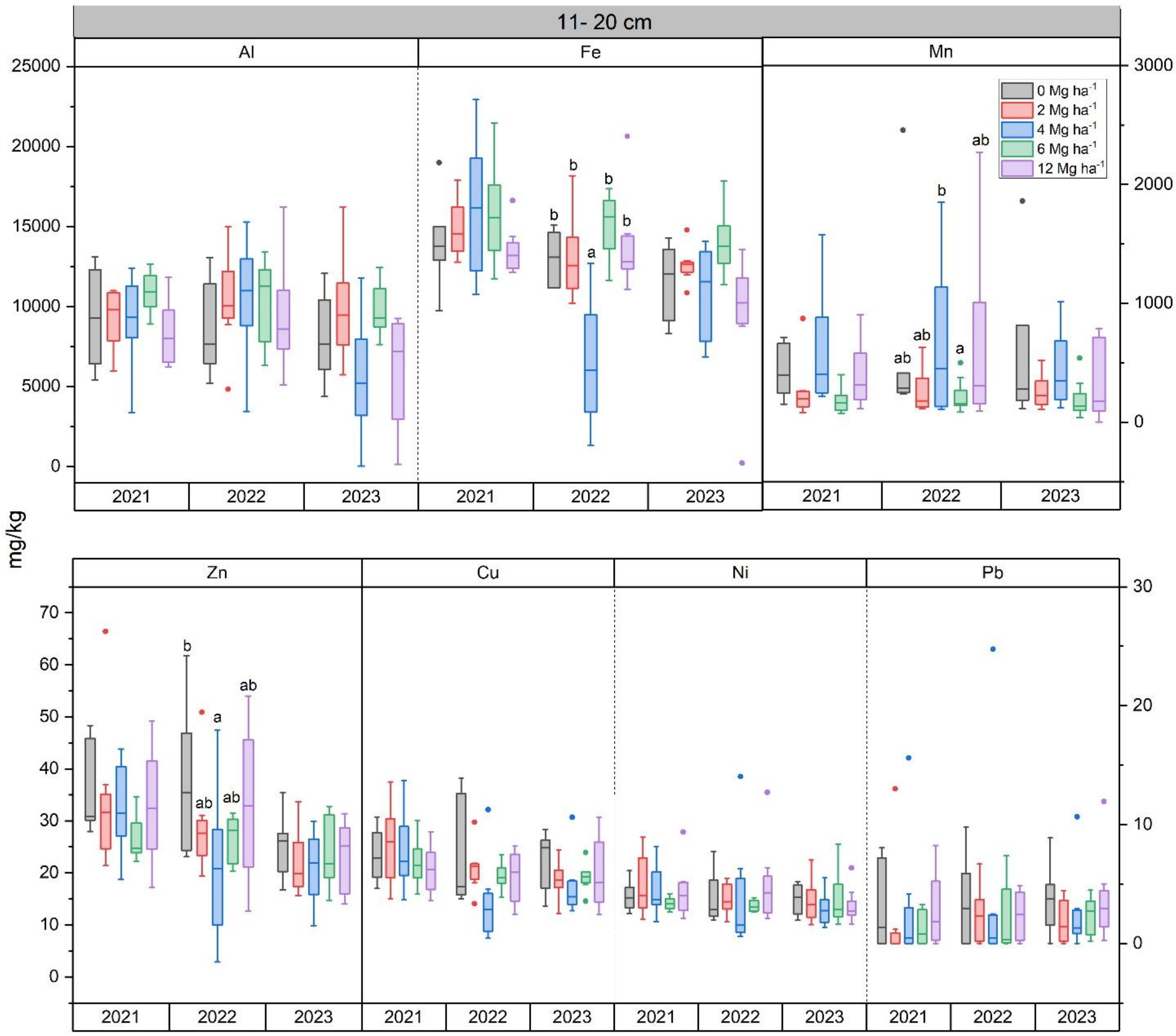

Figure 2. 8. Average elemental metal concentrations in the 11-20 cm lower mineral horizon sampled beneath the sugar maple tree in the control and NIWA treated plots prior to (2021) and two growing seasons after application (2022 & 2023). The letter display indicates significant differences between control and treatments ($p < 0.05$) determined by a Tukey HSD test.

2.4.2 Sugar maple root mass and chemical analysis

Significant differences in root mass density were observed between the control and treated plots collected beneath the sugar maple trees two years after NIWA application (Figure 2.10). Root mass density was lower in the higher ash treatments of 4, 6, and 12 Mg ha^{-1} compared with the control (Figure 2.10), but only the 6 Mg ha^{-1} treatment was significantly different from control plots with a decrease to 9.5 mg cm^{-3} compared with 21.5 mg cm^{-3} in the control. Significant differences in root elemental concentrations of P and Cd were also observed in the treated plots (Table 2.2). Specifically, P concentrations increased significantly in the 4, 6, and 12 Mg ha^{-1} plots, reaching up to 0.975 g kg^{-1} compared with 0.796 g kg^{-1} in the control. In contrast, Cd concentrations decreased slightly in all treated plots, falling to 0.2 mg kg^{-1} from 0.4 mg kg^{-1} in the control.

Table 2. 2. Average root nutrient and metal concentrations (± SE) beneath the control and treated sugar maple trees after two growing seasons (2022 & 2023). The letter display indicates significant differences between control and treatments (*p* < 0.05) determined by a Tukey HSD test.

		Control	2 Mg ha^{-1}	4 Mg ha^{-1}	6 Mg ha^{-1}	12 Mg ha^{-1}
g kg^{-1}	Ca	29.5 (2.4)	35.7 (3)	31.9 (1.8)	37.4 (4.2)	36.7 (2.2)
	Mg	6.8 (0.9)	8.6 (1)	6.3 (0.5)	8.2 (0.9)	7.4 (0.6)
	K	2.7 (0.3)	3.1 (0.4)	3.7 (0.2)	3.3 (0.3)	3.1 (0.4)
	P	0.79 (0.03)^{ab}	0.77 (0.04)^{a}	0.97 (0.04)^{b}	0.85 (0.02)^{ab}	0.92 (0.07)^{ab}
	Mn	0.99 (0.18)	0.61 (0.05)	1 (0.02)	0.69 (0.08)	0.95 (0.1)
	Al	2.8 (0.46)	2.3 (0.27)	2.9 (0.3)	2.3 (0.3)	2.7 (0.33)
	Fe	5.3 (0.68)	5.2 (0.57)	4.7 (0.25)	5.1 (0.47)	5.4 (0.47)
mg kg^{-1}	B	4 (1.2)	5 (1)	7 (0.5)	4 (0.9)	6 (1)
	Cd	0.4 (0)^{b}	0.3 (0.06)^{ab}	0.2 (0.03)^{ab}	0.2 (0.03)^{a}	0.2 (0.02)^{a}
	Cu	30 (1)	32 (2)	41 (3)	32 (2)	47 (9)
	Ni	6 (0.8)	6 (0.4)	8 (0.7)	7 (0.5)	7 (0.5)
	Pb	14 (2)	11 (1)	12 (2)	11 (1)	14 (1)
	Zn	109 (12)	123 (7)	121 (8)	150 (26)	128 (15)

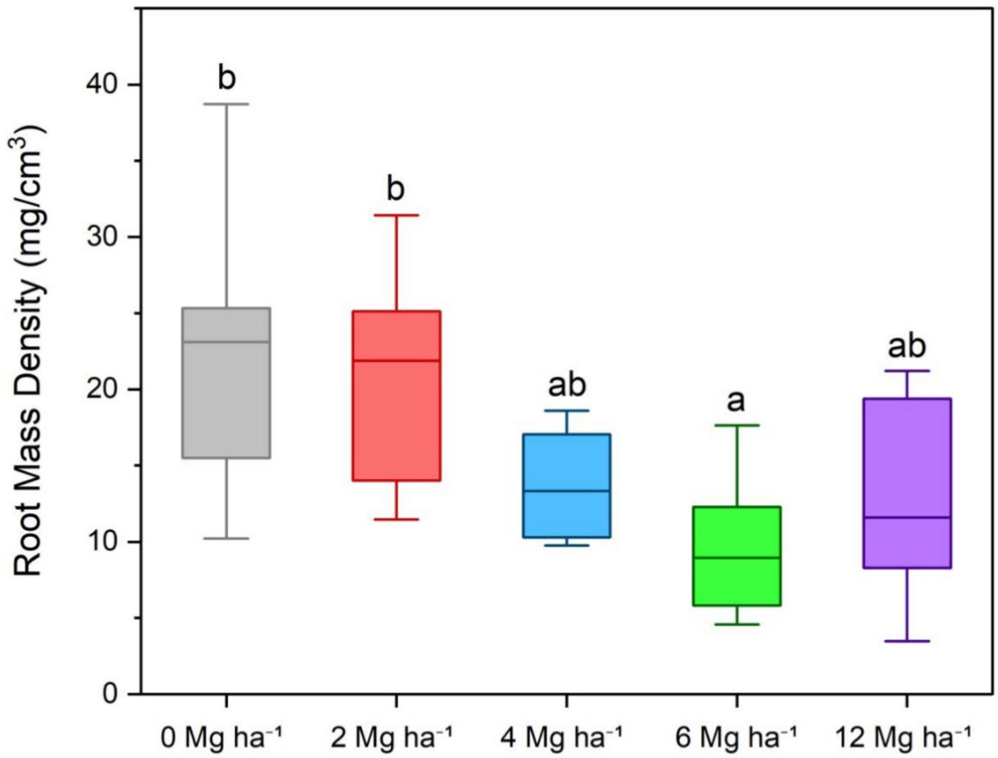

Figure 2. 9. Average root mass density (mg/cm^3) beneath the control and treated sugar maple trees after two growing seasons (2022 & 2023). The letter display indicates significant differences between control and treatments ($p < 0.05$) determined by a Tukey HSD test.

2.4.3 Sugar maple radial growth

Mean BAI of sugar maple trees increased in the two growing seasons following NIWA application. A significant treatment effect was observed in the 6 Mg ha^{-1} application resulting in significantly lower growth than the control and 4 Mg ha^{-1} treatments (Figure 2.10; Table 2.3). Control trees had the highest average BAI (59.6 ± 9.9 cm²/tree), while the 6 Mg ha^{-1} treatment exhibited the lowest (29.5 ± 9.5 cm²/tree).

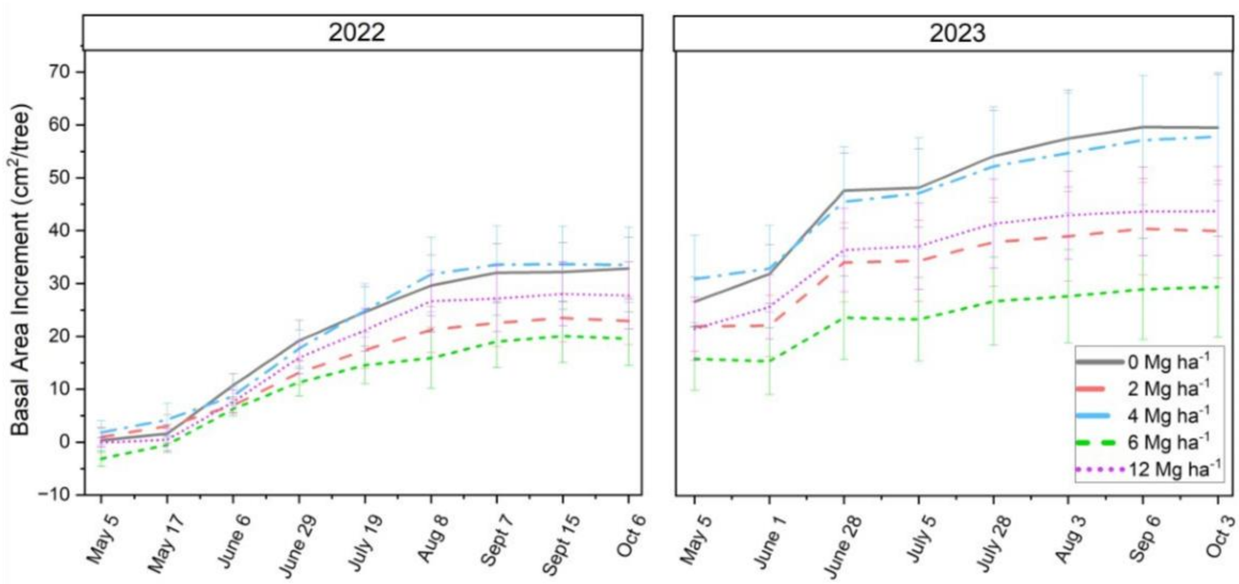

Figure 2. 10. Cumulative mean basal area increment (cm^2/ tree) in control and NIWA treated sugar maple trees during the 2022 and 2023 growing seasons.

Table 2. 3. Cumulative average (± SE) of basal area increment (cm^2/ tree) (BAI) in control and NIWA treated sugar maple trees during the 2022 and 2023 growing seasons. The letter display indicates significant differences between control and treatments ($p < 0.05$) determined by a Tukey HSD test.

	Control	2 Mg ha^{-1}	4 Mg ha^{-1}	6 Mg ha^{-1}	12 Mg ha^{-1}
BAI cm^2/tree	59.6 (9.9)^{b}	40 (8.8)^{ab}	57.9 (12.1)^{b}	29.5 (9.5)^{a}	43.8 (8.4)^{ab}

2.4.4 Sugar maple foliar chemistry

Over the three-year period (pre (2021) and post (2022 and 2023)), there were very few significant differences in sugar maple foliar chemistry among the treatment plots. The notable exception was Mn, which was significantly higher in the 4 Mg ha^{-1} treatment, but this difference was evident pre-treatment. While there were few significant differences in foliar chemistry, some patterns were evident. Calcium, K, Al, Fe, Cu, and B tended to be higher in the ash treated plots after the first year, with concentrations showing a decrease back to similar concentrations to the control plots in the second year. Except for B and Cu, which tended to consistently increase into the second year. However, these differences were small and insignificant and remained either within or below critical foliar concentrations reported for sugar maple trees (Burton et al., 1993; Kolb & McCormick, 1993). DRIS indices revealed distinct patterns in nutrient balance across treatments and years. All treatment trees showed consistently negative DRIS values for P across all years, suggesting P deficiency (Table 2.4). Nitrogen values in the treatments increased over time, indicating a relative N excess by 2023. In contrast, K was well balanced in the control treatment but became increasingly excessive in treated trees, particularly at 6 and 12 Mg ha^{-1}, with DRIS values reaching +23.1 and +21.0 in 2022, and +13.0 and +15.6 in 2023, respectively.

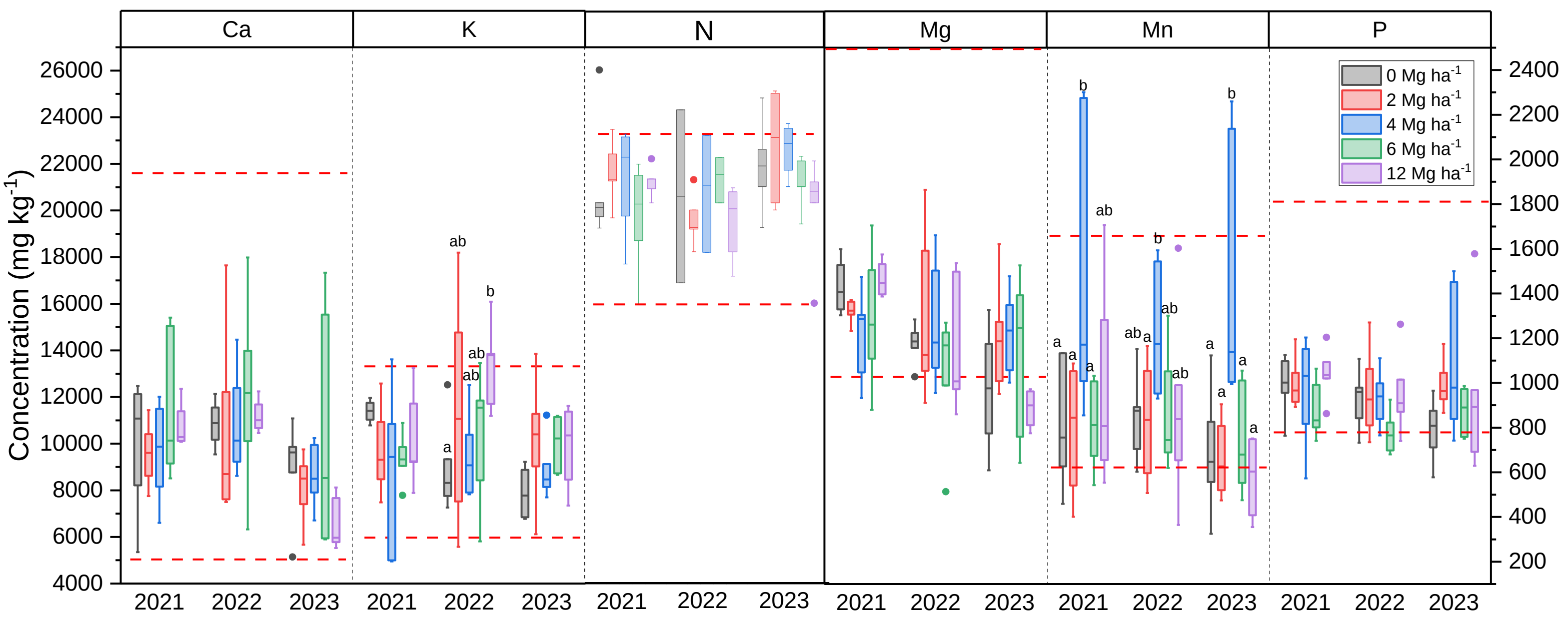

Figure 2. 11. Average foliar nutrient and metal concentrations in control and NIWA treated sugar maple trees prior to (2021) and two growing seasons after application (2022 & 2023). The letter display indicates significant differences between control and treatments ($p < 0.05$) determined by a Tukey HSD test. The published critical foliar range for healthy sugar maple trees is indicated by the red dashed lines (Burton et al., 1993; Kolb & McCormick, 1993). The primary and secondary Y-axis are separated by a solid black line.

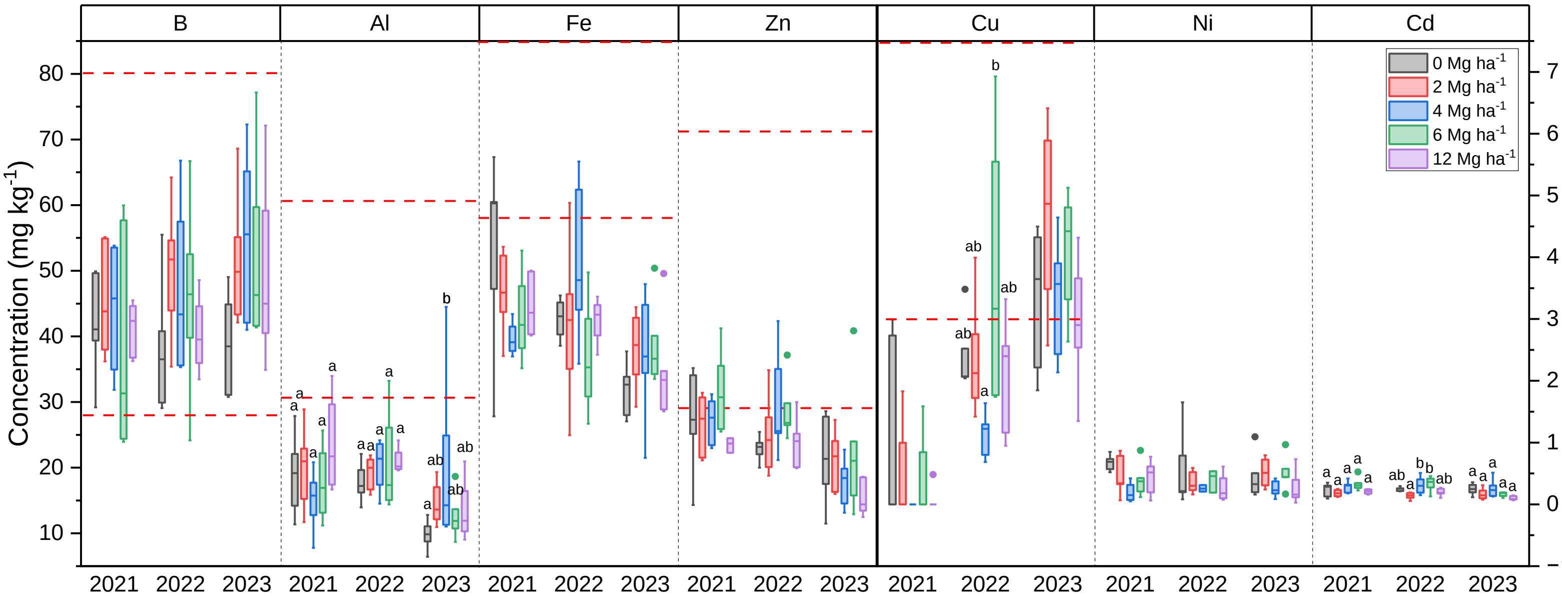

Figure 2. 12. Average foliar nutrient and metal concentrations in control and NIWA treated sugar maple trees prior to (2021) and two growing seasons after application (2022 & 2023). The letter display indicates significant differences between control and treatments ($p < 0.05$) determined by a Tukey HSD test. The published critical foliar range for healthy sugar maple trees is indicated by the red dashed lines (Burton et al., 1993; Kolb & McCormick, 1993). The primary and secondary Y-axis are separated by a solid black line.

Table 2. 4. Average sugar maple DRIS indices in control and NIWA treated sugar maple trees prior to (2021) and two growing seasons after application (2022 & 2023).

	P			N			Ca			K			Mg		
	2021	2022	2023	2021	2022	2023	2021	2022	2023	2021	2022	2023	2021	2022	2023
0 Mg ha^{-1}	-28.5	-28.9	-31.7	12.1	15.1	28.6	-6.9	7.8	2.6	13.8	10.1	5.2	9.5	-4.2	-4.8
2 Mg ha^{-1}	-23.5	-27.4	-21.3	15.4	12.4	21.2	-0.7	-1.9	-9.2	5.1	15.5	10.7	3.6	1.3	-1.4
4 Mg ha^{-1}	-25.0	-25.2	-21.5	20.7	18.2	21.3	0.5	2.1	-4.1	0.8	4.3	0.4	3.0	0.6	3.8
6 Mg ha^{-1}	-30.4	-38.1	-28.8	13.7	26.6	22.7	7.2	4.6	-3.0	6.2	23.1	13.0	3.3	-16.1	-4.0
12 Mg ha^{-1}	-21.7	-27.1	-17.8	12.3	6.8	20.9	0.5	3.2	-9.7	2.5	21.0	15.6	6.4	-3.9	-9.0

2.5 Discussion

This study evaluated the short-term response of soil and foliar chemistry along with tree growth and fine root production over a two-year period following the addition of various dosages of wood ash obtained from residential homes in the Muskoka region of Ontario. After one year, soil pH, exchangeable base cations (Ca, Mg), and most metals were significantly elevated in the upper organic soil horizons in ash treated plots compared with controls, but there were few differences in the mineral soil. Applications of NIWA generally had minimal impact on foliar elemental concentrations in relation to the published range for healthy sugar maple foliage (Burton et al., 1993; Kolb & McCormick, 1993), and few significant increases in metal concentrations in foliage and roots were observed relative to control plots. There was a significant effect of ash on sugar maple growth in the first two growing seasons following wood ash treatment and a reduction in fine root production in plots treated with the higher ash dosages.

2.5.1 Soil chemical properties

Before the application of wood ash, soils in the study area were acidic, with an average pH of around 4.5 in mineral soils. Following the application of NIWA, there was a large increase in the pH of the L and FH horizons, rising by as much as 1.0 to 2.0 pH units over the course of a year, while significant increases in the mineral horizons were only noted at the 12 Mg ha^{-1} treatment level. The rise in pH in the organic horizons can be attributed to the acid-neutralizing capacity of NIWA (Augusto et al., 2008; Pitman, 2006). Increases of 0.5 to 1.5 pH units in the organic horizon can persist for up to 30 years post-application (Arseneau et al., 2021; Fritze et al., 1994; Ozolinčius et al., 2007; Saarsalmi et al., 2001, 2004; Saarsalmi et al., 2012). The slow vertical leaching of soluble cations, retained in the organic horizons, typically delays the neutralization effects from reaching deeper soil layers. Consequently, significant increases in mineral soil pH are rarely observed within the first three years after application (Arseneau et al., 2021; Ozolinčius et al., 2007). Generally, researchers have found that, except for higher ash concentrations (greater than 6 Mg ha^{-1}), there is little to no impact on mineral horizon pH in short-term studies (Augusto, 2008; Jacobson et al., 2004). This increase in pH of upper organic soil horizons following ash application has been documented in various studies (Conquer et al., 2023; Deighton et al., 2020; Mandre et al., 2006; Zimmermann & Frey, 2002). For instance, Mandre et al. (2006) reported a rapid increase of 1 to 1.2 pH units after applying 2.5 and 5 Mg ha^{-1} to a young Scots pine (*Pinus sylvestris* L.) forest in northern Estonia. In Muskoka, Ontario, Deighton et al. (2020) found pH increases of 0.4 to 1.1 units after applying 6 Mg ha^{-1} of NIWA to two acidic forest sites dominated by sugar maple, eastern white pine (*Pinus strobus* L.), and yellow birch. Similarly, Conquer et al. (2023) observed pH increases in the forest floor of up to 2 units after applying 6 Mg ha^{-1} to sugar maple dominated acidic forest soils in Bracebridge, Ontario.

Soil organic matter content in the L and FH horizons decreased significantly as the ash treatment dose increased. In the second year, organic matter levels generally returned to baseline levels, except for the 12 Mg ha^{-1} treatment in the FH horizon. These results are consistent with other studies that found a change in organic matter content in the organic horizon following wood ash application. (Hansen et al., 2016). The lower organic matter content is most likely attributable to residual ash, which was observed on plots during sampling, and or to the nutrient content of ash, which may have stimulated microbial activity and organic matter decomposition (Hansen et al., 2016; Syeda et al., 2023). Given that the organic matter content of ash is only 3.4%, the observed decline from 90% to 75% OM suggests that ash accounted for approximately 15% of the total sample mass in Year 2, likely causing a dilution effect in the surface soil.

Soil exchangeable cations were significantly elevated in the L and FH horizon across all treatments one year after NIWA application. By the second year, concentrations in the LFH horizon declined, while those in the 0 – 10 cm and 11 – 20 cm mineral horizons increased, suggesting nutrient leaching through the soil profile. Previous studies have similarly reported increased exchangeable Ca, K, and Mg in upper organic horizons following wood ash application. For example, Domes et al. (2018) observed elevated base cation concentrations in the LFH horizon with no significant effects in the mineral soil one growing season after a 5 Mg ha^{-1} wood ash treatment. Jacobson et al. (2004) found a significant increase in Mg concentrations in the upper mineral soil horizon of 9 Mg ha^{-1} treatment plots. Exchangeable Ca, K, and Mg concentrations in the mineral soil were less affected during the first year, although higher concentrations were observed in the highest ash treatment. When wood ash is applied to the surface of forest soils, dissolved base cations gradually migrate downward, often requiring several years to influence mineral soil pH and base cation levels (Reid & Watmough, 2014). It is noteworthy that K showed no significant change in the L horizon but displayed significant increases in the FH and mineral horizons. The low K concentrations in the litter layer are likely due to its high solubility and displacement from soil exchange sites by other cations, such as Ca and Mg (Ohno, 1992; Reid & Watmough, 2014). However, Arseneau et al. (2021) found no significant differences in K concentrations between control and treated plots in the forest floor or upper mineral soil three years after applying 20 Mg ha^{-1} of wood ash in sugar maple stands in Quebec. These findings highlight the variability in wood ash effects based on dosage, soil properties, and site conditions.

Metal concentrations were primarily elevated in the L and FH horizons of the treatment plots one year after wood ash application but decreased by the second year in the L horizon and remained elevated in the FH horizon while concentrations in the mineral soil remained unchanged, similar to other studies (Arseneau et al., 2021; Conquer et al., 2023; Deighton & Watmough., 2020). These initial increases in metal concentration in the upper soil layers are consistent with the slow weathering rate of wood ash observed in other studies and align with findings from previous research (Deighton et al., 2021; Hansen et al., 2018). Metal mobility is likely further constrained by the reduced acidity in the upper soil horizons, as documented in other studies (Augusto et al., 2008; Violante et al., 2010). The metal concentrations of the NIWA measured in this study (As, Cd, Cu, Ni, Pb, and Zn) fell within or below the CM1 and CM2 guidelines for restricted metal land application limits, in agreement with findings from similar studies (Deighton et al., 2021; Syeda, 2023). Overall, increases in metal concentrations in the L and FH horizons appear to correlate with ash dosage rates (Hansen et al., 2018), as certain metals, including Zn, Cu, Pb, Ni, and B, showed a gradient increase proportional to the application rate in the first year. However, these concentrations declined by the second year in the L horizon, reflecting both the redistribution of metals through the soil profile and their reduced mobility over time along with burial of fresh litter that fell in the Fall post application. Holmberg (2000) suggests by mixing dolomite with the ash, high concentrations of heavy metals are avoided, and the required Ca and Mg concentrations are achieved. This study conducted by Holmberg et al (2000) on the chemical composition and leaching characteristics of granules made from wood ash and dolomite found that this combination effectively stabilized heavy metals, reducing their leachability. The granules also provided a balanced supply of Ca and Mg, beneficial for soil amendment applications.

2.5.2 Sugar maple root mass and chemical analysis

Fine root growth was reduced by approximately 40 % relative to the control in plots receiving 4, 6 and 12 Mg ha^{-1} two years after NIWA applications, although the response was only significant in the 6 Mg ha^{-1} treatment. Ash application has been reported to both increase and decrease fine root biomass. The form of ash, time since ash application, and nutrients added together with ash may all contribute to the overall effect of the treatment (Helmisaari et al., 2009). For example, Persson and Ahlström (1991) found areas in a Norway spruce stand treated with wood ash and peat ash decreased root development during the first 2 years after ash application. In a longer-term study, Helmisaari et al., (2009) found that root mortality was higher, and the production of new roots were lower in plots treated with 3 Mg ha^{-1} wood ash compared with no ash added controls 9 years following application. In contrast Genenger et al. (2003) applied loose wood ash in a 70-year-old Norway spruce stand in Switzerland and reported increased fine root biomass within 2 years of treatment. Additionally, as described by Steenari and Lindqvist (1997), reactive oxides in ash may produce undesirable effects such as pH shock and burning damage to plant tissues.

There were few changes in fine root chemistry. Large increases in Ca, Mg, K, and P concentrations are commonly observed in fine roots following wood ash application (Augusto et al., 2008). However, in this study only P significantly increased while Cd significantly decreased. The increase in root P concentrations is likely due to enhanced P availability from wood ash (Marius Tuyishime et al., 2022), while the decrease in Cd concentrations is likely due to pH-induced reductions in Cd bioavailability (Kindtler et al., 2019). The absence of significant changes in other elements suggests that their availability and uptake may not be as strongly influenced by wood ash treatments. However, due to wood ash significantly increasing soil pH, in turn affects nutrient availability. While this can enhance the uptake of certain nutrients, it may also cause imbalances that negatively impact root development (Joseph et al., 2022), as reflected in the decline in fine root biomass at higher application rates in this study. A study on Norway spruce in Finland found that wood ash and N fertilization altered soil acidity, causing a decrease in fine root biomass and altered growth dynamics (Helmisaari et al., 2009). Bang-Andreasen et al (2017) found observed increases in soil pH and electrical conductivity suggest that excessive wood ash application could potentially lead to conditions that impair soil functions and root health. Elevated soil pH can alter nutrient availability, potentially leading to nutrient imbalances that affect plant growth.

2.5.3 Sugar maple basal area increment

After two years, sugar maple basal area increment (BAI) showed significant variation among NIWA treatments. The 6 Mg ha^{-1} treatment exhibited significantly lower stem growth and fine root density overall. . These findings contribute to the growing body of evidence that tree responses to wood ash application can be highly variable and site-specific, particularly in the short term (Mandre et al., 2006; Reid & Watmough, 2014; Saarsalmi et al., 2014; Syeda, 2023). For instance, Mandre et al. (2006) reported no significant changes in diameter or height growth of Scots pine three years after wood ash application. Similarly, Saarsalmi et al. (2014) found no early growth response in Scots pine in southern Finland, although they observed significant increases in stem volume growth during the third, fourth, and fifth years. Conversely, Brais et al. (2015) observed decreased black spruce growth after wood ash application in the boreal forest. These mixed results suggest that tree and soil responses to wood ash application do not always align with predictions.

In this study site, sugar maple growth was generally lower than reported for more fertile forests in eastern North America. The control trees averaged ~60 cm^2 of BAI per tree over two years, which is at the lower end of the range typically observed in southern Quebec and Vermont (~80–100 cm^2/tree), where deeper soils and greater nutrient availability promote higher productivity. The shallow, acidic, and potentially nitrogen-limited soils at our central Ontario site may explain both the lower growth rates and the reduced efficacy of ash. In contrast, Arseneau et al. (2021) found positive BAI responses in sugar maple after applying 20 Mg ha^{-1} of ash in Quebec but also reported K-limited soils with high N availability, which likely contributed to stronger nutrient cycling.

Delayed growth responses may be attributed to factors such as P limitations that can emerge at both low and high soil pH (Pitman, 2006) or the low N content in wood ash, which reduces its potential to stimulate growth (Johansen et al., 2021). At low pH, P becomes unavailable due to fixation by iron and aluminum oxides. However, at elevated pH levels, P can also become less available through precipitation as calcium-phosphate compounds (e.g., hydroxyapatite), particularly when calcium levels rise following wood ash application (Maguire & Sims, 2002). Reid & Watmough (2014) also emphasized that the time elapsed since treatment is a key variable in detecting growth effects, and tree species respond differently to ash application (Emilson et al., 2019). Evidence suggests that positive growth responses to wood ash are most likely in N-rich sites, such as forests receiving high atmospheric N deposition or those established on naturally fertile peatlands or mineral soils (Jacobson et al., 2014; Huotari et al., 2015). For example, Arseneau et al. (2021) revealed an increase in BAI of sugar maple trees after the application of 20 Mg ha^{-1}. Their sites, located in southeastern Quebec, were characterized by K-limited but N-rich soils, where ash additions corrected cation imbalances without introducing N limitation. In contrast, the site in this study exhibited marginal N availability, as indicated by foliar N concentrations at the lower end of the sufficiency range (mean ~2.1% N, with ~20% of samples below 2.0%) and relatively modest DRIS N indices. These findings suggest that the site is not N-rich and may in fact be limited in available N. In these environments, wood ash can stimulate the decay of organic matter, releasing N for tree uptake (Augusto et al., 2008; Karltun et al., 2008; Huotari et al., 2015). Conversely, growth typically remains unchanged or even decreases when wood ash is applied to N-limited mineral soils where forest productivity is constrained by N availability (Augusto et al., 2008; Karltun et al., 2008; Huotari et al., 2015). This may explain why the 6 Mg ha^{-1} treatment exhibited the lowest growth, as the site conditions may have limited the availability of N, restricting the potential for tree growth.

2.5.4 Sugar maple foliar chemistry

While few significant differences were observed, certain elements, such as K, B, and Cu, tended to increase with dosage, reaching up to 40% higher than control plots at the highest treatment levels. In contrast, metals like Al, Zn, and Cd showed an initial increase at lower dosages but declined at higher dosages. This pattern may be attributed to the complex interaction between metal additions and pH changes, where shifts in soil chemistry influence metal solubility, mobility, and plant uptake (Pitman, 2006). There were few significant differences in sugar maple foliar chemistry among treatments two years after ash application, and all macronutrient concentrations fell within previously reported ranges for healthy sugar maple trees (Burton et al., 1993; Kolb & McCormick, 1993). Similar to findings from other studies (Deighton et al., 2021; Reid & Watmough, 2014; Conquer et al., 2023), foliar metals showed little to no significant changes across the wood ash dosages. For example, Deighton et al. (2021) found no significant increases in foliar metal concentrations four years after ash application, except for Fe, which remained within the range for healthy sugar maples. Except for K, Conquer et al. (2023) observed concentrations of Ca and several other metals (Mn, Fe, Zn, Al, Ni, and Cd) to be higher in ash-treated trees, but for the most part these differences were small and insignificant and remained either within or below healthy ranges. In contrast, Arseneau et al. (2021) reported significant increases in foliar Ca and Mg concentrations four years after applying higher doses of wood ash (20 Mg ha^{-1}) than those used in this study, suggesting that larger doses or longer timeframes may be needed for significant changes in foliar base cation concentrations (Augusto et al., 2008; Conquer et al., 2023; Reid & Watmough, 2014). These findings suggest that while some responses in sugar maple trees can occur within the first year following wood ash application, significant changes in foliar nutrient concentrations, particularly for elements like Ca and Mg, may take several years to become evident, in contrast to more soluble elements like K, which show quicker responses. The timeline for these changes depends on factors such as nutrient mobility in the soil, the rate of nutrient uptake by roots, and the tree's internal nutrient allocation processes (Kim et al., 2022). Importantly, the absence of substantial changes in foliar nutrient concentrations does not necessarily indicate a lack of treatment effect, it may reflect that baseline nutrient levels were already sufficient and tightly regulated by the trees (Miller et al., 2005). Once internal nutrient demands are met, further uptake may be physiologically restricted, particularly for base cations like Ca. As such, observable foliar changes may emerge only when trees are responding to true nutrient limitations or after longer periods of sustained treatment effects.

Among the elements analyzed, only K and Mn exceeded healthy sugar maple ranges. Potassium, essential for tree health, temporarily increased above the healthy range but returned to “healthy” levels by the second year. Manganese concentrations were notably elevated in the 4 Mg ha^{-1} treatment, possibly due to site-specific differences. Fernando et al. (2016) suggested that sugar maples on unbuffered, acidified soils upslope may over accumulate Mn, while those on buffered downslope soils are less affected. This site variability could contribute to the observed foliar Mn response in this study.

The lack of foliar response may in part be due to changes in mineral soil chemistry were very small, and this is where most of the fine roots of mature sugar maple are located at the study site. For example, the comparison of foliar Ca concentrations with exchangeable base cation levels in the upper A-horizon mineral soil of the study plots aligns with findings from the Ontario Forest Biomonitoring Network (OFBN) analyzed by Miller et al. (2005). Miller et al

(2005) identified a relationship between soil and foliar Ca, revealing a threshold beyond which increases in soil Ca do not translate into higher foliar Ca levels, and in some cases, foliar Ca may even decrease. This suggests that sugar maple trees may regulate calcium uptake once a sufficient threshold is reached, which could explain the limited response in foliar Ca concentrations observed in this study despite elevated soil Ca levels. Foliar N shows no response to ash and despite a long history of elevated N deposition in the region it may remain the key limiting nutrient for tree growth in the region (Conquer et al., 2023)

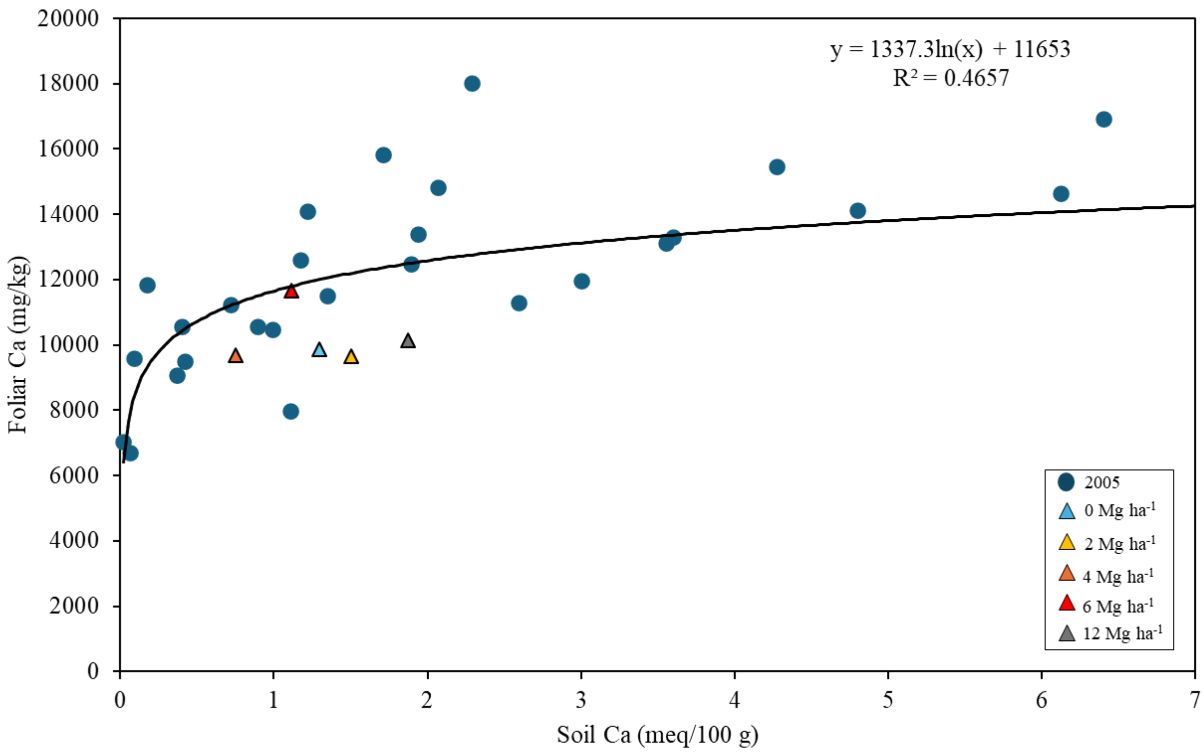

Figure 2. 13. Sugar maple foliar and A-horizon Ca of the control and NIWA treated sugar maple tree plots sampled in 2023 plotted against 35 Ontario Forest Biomonitoring Network plots sampled in 2005 by Miller et al (2005).

2.6 Conclusion

This study investigated the short-term effects of NIWA application on a sugar maple-dominated hardwood stand in Bracebridge, Ontario, focusing on soil chemistry, tree growth, and foliar nutrient dynamics. The findings indicate that wood ash significantly increased soil pH, base cations (Ca, Mg, K), and metal concentrations, particularly in the organic horizons, while having minimal impact on the mineral soil. Despite these soil changes, fine root growth declined, aboveground tree growth declined in the 6 Mg ha^{-1} treatment, suggesting possible nutrients imbalance or stress at this dose. Changes in foliar chemistry were generally small and within reported healthy ranges, and no evidence of metal toxicity was observed. The limited tree response suggests that these sites may be N-limited, with potential P constraints due to elevated soil pH. These results highlight that wood ash may be a safe and beneficial soil amendment when applied at lower dosages (< 4 Mg ha^{-1}), as it improves nutrient availability without causing negative effects, but its effectiveness may be enhanced with the addition of N. Longer-term studies are needed to determine whether repeated applications could lead to more pronounced ecological benefits, particularly in nitrogen-enriched environments.

3. Effects of Non-Industrial Wood Ash and Dolomitic Limestone on Soil Respiration and Microbial Enzyme Activity

3.1 Abstract

Forests exhibit large controls on the global carbon cycle, serving as significant carbon sinks with an estimated capacity of 0.4 to 4.1 Pg C per year. However, many forests are experiencing nutrient cycle disruptions due to historical human activities, such as acid deposition or timber harvesting. Soil amendments, including dolomite and non-industrial wood ash (NIWA), can be used to replenish lost soil nutrients and may aid in soil carbon sequestration. This study investigated the effects of applying 2 and 12 Mg ha^{-1} of NIWA and dolomite on soil transplants from a sugar maple (*Acer saccharum* Marsh.) dominated forest in south-central Ontario, using both field and laboratory trials. Compared with dolomite, NIWA had a stronger effect on soil chemistry and CO_2 fluxes from soil. Notably, the application of NIWA significantly increased soil pH and exchangeable base cations (calcium (Ca), magnesium (Mg), and potassium (K)) especially in the 12 Mg ha^{-1} treatment. The application of 12 Mg ha^{-1} resulted in a sharp intake of CO_2 immediately after application, but over the two-year study cumulative CO_2 fluxes were higher compared with the other treatments. Soil enzyme activity (β-1, 4-glucosidase, Phosphatase, and N-acetyl- β glucosaminidase) showed only weak responses to the highest treatments and only in the LFH layer. Overall, NIWA significantly improved soil pH and increased exchangeable cations with minimal effects on soil CO_2 fluxes and microbial activity.

3.2 Introduction

Terrestrial ecosystems play a vital role in the global carbon (C) cycle, acting as significant C sinks with an estimated capacity of 0.4 to 4.1 Pg C per year (Midgley et al., 2010). Canadian forests, covering nearly 362 million hectares, account for about 8.5% of the world's forested area. These forests are essential for absorbing CO_2 from the atmosphere, therefore helping to mitigate climate warming (Byrne et al., 2024). However, the role of forests as net carbon sinks is increasingly uncertain in the face of intensifying disturbances such as wildfires, insect outbreaks, and deforestation, which can cause forests to become temporary or long-term carbon sources (Kurz et al., 2008; Gauthier et al., 2015). In forests, soil contains more C than all terrestrial vegetation combined, with approximately 2,500 Pg C stored at a depth to one meter. This includes about 1,550 Pg of soil organic carbon (SOC) and 950 Pg of soil inorganic carbon (SIC) (Lal, 2004; Padarian et al., 2022). However, many temperate forests are experiencing disrupted and imbalanced nutrient cycles in its soils due to historical and ongoing human activities (Steffen et al., 2015). Acidic deposition is well-documented in its degradative effects on soil and water quality, causing harmful effects on vegetation, forests, and both aquatic and terrestrial wildlife (Bergström & Jansson, 2006; Driscoll et al., 2001; Pardo et al., 2011; Wright et al., 2018). Even with significant reductions in atmospheric emissions of sulfur dioxide (SO_2) and nitrogen oxides (NO_x), the long-term impacts of acidic deposition remain evident in soils across North America, Europe, and Asia (Driscoll et al., 2001; Feng et al., 2020). Over time, the loss of essential base cations causes increasing levels of hydrogen (H^+) and aluminum (Al) in soil lowering its acid-neutralizing capacity (Blake et al., 1999; Duchesne & Houle, 2008; Feng et al., 2020; Kunhikrishnan et al., 2016; Rengel, 2015). These chemical changes can negatively affect tree growth (Gilliam et al., 2019) and the acidification of soil increases the solubility of other metals that may also be harmful to biota (Moreno Marcos & Gallardo Lancho, 2002; Neina, 2019).

Sugar maple (*Acer saccharum* Marsh) trees are highly sensitive to soil acidity and low soil calcium (Ca) content and are a dominant hardwood species that are economically important to Ontario (Gradowski & Thomas, 2006; Little, 1971; Minorsky, 2003). Consequently, there is growing interest in applying soil amendments such as wood ash, dolomite [$CaMg(CO_3)_2$], limestone ($CaCO_3$), and wollastonite ($CaSiO_3$) to mitigate effects caused by acidic deposition (Haque et al., 2020; Kunhikrishnan et al., 2016; Lundstroem et al., 2003; Reid & Watmough, 2014). Additionally, these calcium-bearing minerals have the potential to serve as CO_2 storage materials during mineralization reactions, or the potential to release it (Adamczyk et al., 2010; Beerling et al., 2020; Yan et al., 2023; Zimmerman & Frey., 2002). Some amendments, particularly silicate- or oxide-based materials like wollastonite and wood ash, may act as carbon sinks by reacting with CO_2 through mineral carbonation. In these processes, CO_2 from the atmosphere or soil respiration dissolves into soil water and reacts with Ca-bearing minerals to form bicarbonate (HCO_3^-) and calcium ions (Ca^{2+}) (Dietzen et al., 2018). Under certain soil pH and moisture conditions, these may then combine to form calcium carbonate ($CaCO_3$) (Ferdush & Paul, 2021). This pathway is less relevant for carbonate-based amendments like limestone and dolomite, which are already in carbonate form and do not significantly contribute to additional CO_2 uptake through weathering (Beerling et al., 2020; Yan et al., 2023).

Applying dolomite to soils can enhance the fixation of both organic and inorganic carbon, serving as an effective climate change mitigation strategy (Xiao et al., 2016). However, dolomite is not always the best option for soil amendment due to the environmental impact of its mining and transport (Azan et al., 2019). This challenge also applies to wollastonite and other commonly used liming agents (Government of Canada, 2022). Azan et al. (2019) suggest that wood ash could help address Ca decline in Ontario, given its high Ca content, essential nutrients [e.g., magnesium (Mg), potassium (K), phosphorus (P)], and the opportunity to recycle a locally generated byproduct. While some wood ash sources may still require transport, the use of non-industrial wood ash from nearby operations or communities can significantly reduce associated emissions and costs, making it a more sustainable alternative. This practice not only returns nutrients to the soil but also helps reduce landfill waste and the environmental footprint of forest and biomass energy production. Studies from Finland suggest that adding wood ash to forest soils can also aid in climate change mitigation, as it may enhance C storage in the humus layer within just two to seven years (Saarsalmi & Malkonen, 2001). Unlike current energy-intensive and costly C-capture and storage technologies, using wood ash to decrease CO_2 release may present a promising low-tech alternative while diverting a classified waste product from the landfill. Several studies have investigated CO_2 fluxes from soil after the addition of wood ash, yielding mixed results. For example, Ernfors et al (2010) found no difference in forest floor CO_2 emissions during the 5 years following the application of wood ash. In contrast, Zimmerman & Frey (2002) found an increase in CO_2 fluxes 62 days after an 8 Mg ha^{-1} wood ash application in an acidic Norway spruce (*Picea* abies) stand while Klemedtsson et al. (2010) found a decreased CO_2 flux 2 years after wood ash additions of 3.3 and 6.6 Mg ha^{-1} from a spruce forest on a minerotrophic drained organic soil. These contrasting results highlight the complexity of factors influencing the effects of wood ash on CO_2 release, indicating that further investigation into the relationship between wood ash dose and CO_2 fluxes, as well as the resulting impacts on soil properties and ecological processes.

Soil amendments, both inorganic and organic, significantly influence soil pH and organic matter content, which in turn can affect the soil microbial community (Lima et al., 2009; Noyce et al., 2012; Zimmerman & Frey, 2002). Soil pH plays a critical role in shaping bacterial diversity and the fungi-to-bacteria ratio; fungi tend to thrive in acidic soils, while bacteria are more abundant at circumneutral pH (Strickland & Rousk, 2010). In addition to microbial composition, the activity of extracellular enzymes produced by microbes provides insight into nutrient availability and microbial metabolic strategies. Enzyme stoichiometry, the relative activity of enzymes targeting C, N, and P substrates, is increasingly used to infer microbial nutrient limitations and ecological responses to environmental change (Moorhead et al., 2013, 2016; Sinsabaugh et al., 2009). This approach assumes that microbial investment in enzyme production reflects the elemental imbalances between microbial demand and available resources, following principles similar to ecological stoichiometry. However, these tools rely on key assumptions that should be considered carefully. For example, enzyme activity assays measure potential rather than actual in situ activity, and the interpretation of enzyme ratios as indicators of nutrient limitation can be context-dependent, influenced by substrate availability, microbial community structure, and soil physicochemical conditions (Mori et al., 2022). Soil amendments can rapidly increase components such as organic C, potentially stimulating microbial nitrogen mineralization to maintain a balanced C:N ratio (Ding et al., 2024). Microbial properties and enzyme activities are particularly useful for assessing the effects of soil treatments, especially in forest soils, where they respond quickly to changes in soil chemistry (Sparling, 1997). Despite their importance in regulating soil C stocks and interacting with soil particles, the role of microbial communities is often underestimated. For example, while studies on enzymatic responses to wood ash application show mixed results, they highlight the need for more consideration of microbial activity. Błońska et al. (2023) found increased microbiological activity following the application of 6 Mg ha^{-1} of wood ash, whereas Zimmerman & Frey (2002) and Björk et al. (2010) observed negative or negligible effects at dosages of 8 Mg ha^{-1} and 2.5 – 3.3 Mg ha^{-1}, respectively.

The objective of this study was to compare the short-term effects of NIWA and dolomitic limestone applications at a rate of 2 Mg ha^{-1} (low end) and 12 Mg ha^{-1} (high end) on soil chemistry, CO_2 fluxes and soil extracellular enzymatic activity with a focus on β-glucosidase, acid phosphatase, and N-acetyl-β-D-glucosaminidase. This research included both a field trial and a controlled laboratory experiment using soil transplants from a sugar maple–dominated forest in South-central Ontario. The field trial was designed to assess soil CO_2 efflux responses under ambient environmental conditions, while the laboratory trial aimed to control for temperature and moisture in order to distinguish between abiotic and biotic processes contributing to CO_2 fluxes and soil enzyme activity.. Specifically, this study aimed to evaluate how NIWA application influences (1) soil exchangeable cations and pH, (2) soil CO_2 efflux, and (3) soil microbial extracellular enzyme activities. It was predicted that the addition of NIWA would result in more pronounced changes in soil chemistry, higher CO_2 efflux, and increased enzymatic activity compared with dolomitic limestone. By examining these effects, this study seeks to provide valuable insights into the potential of NIWA as a sustainable soil amendment for enhancing soil nutrients and carbon sequestration in forest ecosystems.

3.3 Methods

3.3.1 Study site

Soil transplants were extracted from a 97-ha mixed-wood forest in the Great Lakes-St. Lawrence ecozone east of the town of Bracebridge, Ontario, Canada (45°04'14.5"N, 79°09'00.7"W). The site is located within a recreational camp called Camp Big Canoe (CBC), and is an undisturbed, relatively even aged (~50-year-old) sugar maple dominated forest and includes white ash (*Fraxinus americana* L.), American beech (*Fagus grandifolia* Ehrh.), and yellow birch (*Betula alleghaniensis* Britt.). The site has shallow, coarse-textured sandy loam soils that are classified as Sombric Brunisols overlying granitic gneiss Precambrian Shield bedrock (Soil Classification Working Group, 1998). From the Bracebridge, ON weather station, the average annual precipitation (from 1991 - 2020) of the region is 1062.3 mm. The average daily minimum and maximum temperatures between 1991 - 2020 were -15.3 ℃ and -4.6 ℃ respectively in January and 12.8 ℃ and 25.3 ℃ in July (Environment and Climate Change Canada, 2024).

3.3.2 Experimental farm

The experimental farm is located on Trent campus lands, 3 km east of Trent University's east bank campus, Peterborough, Ontario, Canada. From the Peterborough, ON weather station, the average daily minimum and maximum temperatures in 2023 were –7.7 ℃ and -0.2 ℃ respectively in January and 13.7 ℃ and 26.9 ℃ in July (Environment and Climate Change Canada, 2024).

This site was selected for the field component of the study due to its proximity to the university, which enabled frequent and consistent measurement of soil CO_2 fluxes over time. The logistical feasibility of this site reduced travel time and allowed for greater sampling intensity and temporal resolution, which would not have been possible at the original Camp Big Canoe study site near Bracebridge, Ontario. While the soils were translocated from a sugar maple–dominated stand near Bracebridge, conducting the field trial at the Trent experimental farm ensured more controlled and repeated access to the experimental plots, improving the reliability of flux measurements. Additionally, the experimental farm provided the necessary infrastructure and space for setting up replicated treatments in a controlled outdoor environment.

3.3.3 Amendment acquisition and characterization

Volunteer residents from Muskoka contributed non-industrial wood ash, which was collected by the charitable organization Friends of the Muskoka Watershed (FMW, 2023). After collection, the ash from various sources was homogenized and sieved (< 2 mm) to remove charcoal, nails, plastic, and other large debris. Following this preparation, it was stored in large polyethylene containers in a cool, dark environment. Six sub samples were collected randomly from the wood ash kept separate for analysis (n = 24) of pH, OM, nutrient, and metal concentrations using the acid digestion method. (Table 3.1, Conquer et al., 2023). Qualitative mineralogical data of the NIWA and dolomite were obtained by powder X-ray diffraction (XRD) data analysis using a Bruker D2 Phaser X-ray diffractometer. The NIWA was comprised of a major abundance of calcite (>10 wt.%; $CaCO_3$), and minor abundances (1–10 wt.%) of portlandite [$Ca(OH)_2$], lime (Ca), fairchildite [$K_2Ca(CO_3)_2$], butschliite [$K_2Ca(CO_3)_2$], periclase (MgO), and monetite [$CaHPO_4$]. The dolomite used in this study was mainly comprised of dolomite [$CaMg(CO_3)_2$], calcite, and quartz (SiO_2).

Table 3. 1. Average pH, organic matter, nutrient, and metal concentrations of non-industrial wood ash (means ± SE) (Conquer et al., 2023). Ontario Regulation 267/03 of the Nutrient Management Act (NASM) limits for unrestricted (CM1) and restricted (CM2) use of wood ash for land application as a non-agricultural non-aqueous source material are also shown.

	Non-Industrial Wood Ash Properties	NASM Limits†	
		CM1	CM2
pH	13.0 (0.04)		
OM (%)	3.4 (0.3)		
C (%)	8.6 (0.1)		
N (%)	0.1 (0.0)		
Ca (g kg^{-1})	267 (3.0)		
K (g kg^{-1})	94.4 (2.9)		
Mg (g kg^{-1})	19.4 (0.3)		
Mn (g kg^{-1})	8.8 (0.3)		
P (g kg^{-1})	7.5 (0.1)		
Al (g kg^{-1})	3.8 (0.3)		
Fe (g kg^{-1})	2.2 (0.2)		
Zn (mg kg^{-1})	503 (18.5)	500	4200
Cu (mg kg^{-1})	164 (9.4)	100	1700
Cd (mg kg^{-1})	2.9 (0.2)	3	34
As (mg kg^{-1})	9.9 (2.2)	13	170
Ni (mg kg^{-1})	9.6 (0.6)	62	420
Pb (mg kg^{-1})	48.2 (16.1)	150	1100
B (mg kg^{-1})	265 (5.3)		

Government of Ontario, 2002, Conquer et al., 2023

3.3.4 Field trial: Plot setup and experimental design

Due to the study site located 175 km away, routine monitoring at the original location would have been logistically challenging. Therefore, relocating soil columns to a controlled setting ensured consistent data collection and minimized variability. Seventeen soil columns were created from 20 cm diameter polyvinyl chloride (PVC) pipes, each cut to a length of 20 cm. In May 2022, 15 soil collars were carefully driven into the soil beneath the canopy of sugar maple trees at Camp Big Canoeusing rubber mallets, ensuring preservation of the litter (L), fibric-humic (FH), and mineral horizons. These columns were then excavated with a shovel, capped with PVC disks, and transported to the experimental farm at Trent University for further analysis.

Figure 3. 1. The insertion of the soil column transplant in the forest floor (A) and the tools used (B) at CBC in Bracebridge, ON. The installation of the soil columns at the Trent University experimental farm (C).

The soil columns were inserted into a 5 × 5 m plot on Trent's experimental farm into rows with a 30 cm gap between each. An additional two soil columns were installed, one filled with 3 kg of NIWA only, and the other with only 5 kg of dolomitic lime were also inserted, resulting in the installation of 17 soil collars in the plot. The pure amendment plots were used to assess natural background weathering of NIWA and dolomite. Daily precipitation (mm) and air temperature (°C) data were obtained from the Trent University weather station. While we considered simulating forest canopy conditions (e.g., shading) to better mimic the microclimate of the original forest site, resource limitations prevented the installation of shading structures.

Soil CO_2 fluxes were monitored using a LI-8100 automated soil CO_2 flux system with a survey chamber, which was positioned on the PVC columns in the field trial. The system measured the diffusion rate of CO_2 (µmol/m^2/s) over a 2 min period, incorporating a 10 s purge before and after each reading. The exposed sample area in each soil column was 314 cm^2, and the chamber volume was 6283 cm^3. The offset, indicating the distance between the top of the PVC collar and the soil surface was adjusted to 4 cm to allow adequate air mixing for measurements.

Measurements were taken once per week over a two-month period prior to treatment initiation on July 27, 2022. Following treatment, CO_2 flux measurements were conducted twice a week for three weeks before returning to a once-per-week schedule for an additional two months. This monitoring continued into the following year. CO_2 fluxes were calculated using SoilFluxPro 4.0.1, which are based on the change in CO_2 concentration from the 120 s measurement, fitted to an exponential curve with the instantaneous rates extrapolated to 1 day.

The treatment included triplicates of 2 and 12 Mg ha^{-1} NIWA, 2 and 12 Mg ha^{-1} dolomite, and soil controls with no amendments added. At the end of the season in October 2022 and 2023, soil samples including the LFH, and mineral layers were collected from each soil column. The LFH was cut out of the column and a metal core (6.3 cm D × 12.7 cm L) was used to remove the mineral soil. Samples were stored in polyethylene bags and refrigerated at 4°C until analysis.

At the end of the 2022 growing season, all soil columns remained in place at the experimental farm over the winter months. No insulation or covering was added, allowing them to experience natural freeze–thaw and snow accumulation conditions. CO_2 flux measurements resumed in spring 2023 using the same soil columns.

3.3.5 Lab trial: Plot setup and experimental design

The lab trial was conducted to complement the field experiment by isolating and controlling environmental variables such as temperature and moisture, which influence soil CO_2 fluxes and enzyme activity. This trial aimed to help distinguish between abiotic and biotic reactions to CO_2 fluxes and to assess microbial responses under consistent conditions. Mirroring the methods from the field trial, nine soil columns were created from 20 cm diameter polyvinyl chloride (PVC) pipes, each cut to a length of 20 cm. In October 2023, the soil collars were carefully driven into the soil underneath sugar maple canopy at CBC using rubber mallets, ensuring preservation of the LFH and mineral horizons. These collars were then excavated with a shovel, capped with PVC disks, and transported to Trent University. They were then modified by fitting a PVC disk with holes to the bottom of the column to promote drainage and prevent material from being flushed after watering events. The soil columns were watered with reverse osmosis (RO) water once a week to maintain 60% field capacity. Soil CO_2 fluxes were monitored using the previous method for the field trial and measured the diffusion rate of CO_2 ($\mu mol/m^2/s$) over a 2 min period, incorporating a 10 s purge before and after each reading. Measurements were made at a frequency of three times per week over a three-week period prior to treatment initiation on November 20th, 2023. Subsequently, flux measurements were conducted twice daily for one week, followed by a return to the original three times per week frequency for an additional three weeks. The soil columns were treated with three replicates of 12 Mg ha^{-1} dolomite, and 12 Mg ha^{-1} NIWA, with three no-amendment control treatments. At the end of the six-week trial, soil samples including the LFH and mineral layers were collected from each soil column following the sampling methods from the field trial.

Figure 3. 2. The removal of the soil collar transplants in the field (A) and set up in their incubation study in the lab for analysis (B). An in-lab soil collar reading with the LICOR (C).

3.3.6 Soil microbial incubation

Separate columns were used for destructive sampling to avoid disturbing the soil structure and microbial communities in the larger columns used for CO_2 flux measurements, and to allow undisturbed leaching and vertical movement of the amendments over time. Fifty-one soil columns were created from a 15.2 cm diameter PVC pipe cut into 20 cm lengths. Each column was carefully driven into the soil at the study site using rubber mallets, ensuring preservation of the LFH and mineral horizons. These collars were then excavated with a shovel, capped with PVC disks, and transported to the laboratory. Once transported to the laboratory, the bottoms of columns were fitted with a PVC pipe reducer coupling and Quest Gold-Grade Landscape Fabric cut into 18 cm circles to fit over the bottom of the PVC pipe and to prevent material from being flushed away after watering and to allow water to drain from the columns. The soil columns were watered with RO water once a week to maintain 60% field capacity. This experiment included triplicates of three treatments: 12 Mg ha^{-1} dolomite, 12 Mg ha^{-1} NIWA, and soil controls. These treatments were hand spread on the surface of each column, with the amendments applied to the respective columns and controls left untreated. Destructive sampling of nine columns (three treatments in triplicates) was done by removing intact columns on days 0, 1, 3, 7, 14, and 42. At each sampling, LFH and mineral samples were collected and frozen at -20 °C for soil enzyme assays.

3.3.7 Laboratory analysis

3.3.7.1 Soil

Soil samples were oven-dried at 105 °C for 24 h in a Grieve industrial oven. After drying, mineral soil was sieved to < 2 mm. The LFH layers were ground using a Wiley Mill. All soil layers were analyzed for pH and loss-on-ignition (LOI) and were analyzed for exchangeable cations and metals (Ca, Mg, K, Al, Fe, Mn).

Soil pH (0.01 M calcium chloride ($CaCl_2$)) was measured following Hendershot & Lalande (2008) method. A 1:5 ratio was used, shaken for 1 h, and then measured using an OAKTON pH 510 series multimeter (Oakton Instruments, Vernon Hills, Illinois, USA). Organic matter content was analyzed using the loss of ignition (LOI) method by Hopkins (2008). Each sample was weighed to 1 g for LFH and 5 g for mineral soil and placed in porcelain crucibles to dry in the oven at 105 °C for 24 h. The weight was recorded using an analytical balance and weighed again after the samples were ashed in the muffle furnace at 400 °C for 10 h.

Exchangeable base cations (EC) were analyzed following the method described in Hendershot & Lalande (2008) by adding 25 mL of 1 M ammonium chloride (NH_4Cl) to 5 g of mineral soil or 1 g of LFH. Samples were mixed for 2 h on a shaker table. They were then filtered through P8 Fast Flow Filter Paper. Samples were diluted 1:10 and acidified with 0.2 mL of trace metal grade HNO_3 and later analyzed using inductively coupled plasma optical emission spectroscopy (ICP-OES) to measure concentrations of Ca, Mg, K, and Na (80-100% recovery). Soil standards (EnviroMAT SS-1) and blanks were tested periodically to ensure accuracy, and a standard curve was created for each analysis with the ICP-OES with elemental standards from SCP Science (SCP Science, Quebec, CA).

3.3.7.2 Soil microbial extracellular enzyme assay

The three extracellular enzymes assayed in this study were β-1, 4-glucosidase (BG), Phosphatase (Phos), and N-acetyl- β glucosaminidase (NAG). β-1, 4-glucosidase is involved in C-cycling and releases glucose from polysaccharides (i.e., cellobiose) for microbial C acquisition. Phosphatase is involved in P cycling and acts by releasing inorganic P from phosphate esters. N-acetyl- β glucosaminidase is involved in C and N cycling by hydrolyzing bonds in uridine diphosphate N-acetylglucosamine derived from chitin found in fungal cell walls

(Daunoras et al., 2024). While extracellular enzyme activity may originate from both plant roots and microbial sources, the soil columns were incubated in a low-light, controlled environment without growing plants. Under these conditions, root-derived enzyme activity is expected to decline rapidly due to the absence of photosynthetic activity and root exudation (Zhang et al., 2013). In contrast, microbial communities remain metabolically active and continue to produce extracellular enzymes in response to nutrient availability. Therefore, enzyme activity measured in this study is assumed to primarily reflect microbial processes.

Soil samples were thawed and air dried at room temperature for 24 h before being passed through a 2 mm sieve to remove course fragments and debris. Soil enzyme assays were conducted following the methods of Tabatabai (1994) and Parham and Deng (2000). A 1:10 ratio was used due to the high organic matter of the soil, by mixing 1 g of soil with 10 ml of 50 mM acetate buffer (pH 5.5) followed by pipetting 150 µL of the soil slurry into 1.5 mL centrifuge tubes and mixing it with 150 µL of pNP-1-D-glucopyranoside, pNP-phosphate, or pNP-1-N-acetylglucosaminide and incubated for 1 h (3 h for pNP-NAG) at 37 °C. After incubation, 100 µL of each sample was transferred to a clear 96 well microplate and 10 µL of 1 M NaOH with 190 µL of RO water was added to stop the reaction. Additional controls were performed by following the procedure described but without addition of soil to reaction mixtures. An Epoch Biotek microplate spectrophotometer reader at 410 nm absorbance was used to determine enzyme activity and the calibration was done with 4-nitrophenol. To calibrate enzyme activity measurements, a 4-nitrophenol (pNP) standard curve was prepared. A 1 mM 4-nitrophenol solution was created by dissolving 13.9 mg of 4-nitrophenol into 100 ml of 50 mM acetate buffer. A serial dilution was performed in 50 mM acetate buffer to achieve final concentrations of 0.5 mM, 0.25 mM, 0.1 mM, 0.05 mM, and 0.025 mM. Calibration was verified using a system test and an absorbance plate test. The plate absorbance was recorded at 410 nm. The concentration of each standard was multiplied by 0.3 to calculate µmoles of pNP per 300 µl reaction volume. A standard curve was generated by plotting µmoles of pNP against absorbance, with the slope of this line serving as a conversion factor to interpret the absorbance of the enzyme assay according to Beer's Law. Soil enzyme ratios C (BG), N (NAG), and P (Phos) acquiring enzymes were calculated to express the soil EEA stoichiometry (Sinsabaugh et al., 2008).

$$\textit{Soil enzyme C:N ratio} = \ln(BG)/\ln(NAG) \tag{5}$$

$$\textit{Soil enzyme C:P ratio} = \ln(BG)/\ln(Phos) \tag{6}$$

$$\textit{Soil enzyme N:P ratio} = \ln(NAG)/\ln(Phos) \tag{7}$$

3.3.8 Statistical analysis

Statistical analysis was conducted using R software version 4.2.2 (R Core Team, 2024). Soil pH, organic matter content, and exchangeable base cations were evaluated between treatments and across each year by soil horizon through a two-way analysis of variance (ANOVA) for the field trial. Cumulative CO_2 soil fluxes at the end of the study period were evaluated between treatments using a one-way ANOVA. For the lab trial, responses of soil pH, organic matter content, and exchangeable base cations to treatment were evaluated using a one-way ANOVA, categorized and separated by soil horizon. A two-way ANOVA was used to evaluate soil enzyme activities between treatments and across incubation time by soil horizon. When significant differences were identified, a post hoc test was performed using estimated marginal means and pairwise comparisons (*emmeans package*) with a Tukey adjustment to assess significant differences between treatment levels for each year. The normality of the model residuals was assessed with the Shapiro-Wilk normality test (*rstatix package*) and QQ plots (*ggpubr package*), while homogeneity of variances was evaluated using Levene's test (*car package*). Non-parametric data were tested using the Kruskal-Wallis test using the Bonferroni adjustment. A significance level of $p < 0.05$ was used, unless otherwise noted.

To account for missing data in cumulative CO_2 flux calculations a linear interpolation was used to estimate Fs values between two measurement dates, t1 and t2 in Microsoft Excel. This approach assumes a linear trend between Fs(t1) and Fs(t2), where Fs values for intermediate days (t1 < t < t2) were interpolated based on the following equation:

$$Fs\ (t) = Fs(t1) + \frac{Fs(t2) - Fs(t1)}{(t2 - t1)} \times (t - t1) \quad (8)$$

3.4 Results

3.4.1 Experimental farm trial: Soil chemical properties

In both horizons, the 12 Mg ha^{-1} NIWA had a stronger effect on soil pH compared to dolomite after application, the 2 Mg ha^{-1} rate did not significantly alter pH relative to dolomite (Figure 3.5). The pH of the soil controls was 4.7 in the LFH horizon. The soil pH value increased by almost 3 pH units in the 12 Mg ha^{-1} NIWA treatment after 3 months but decreased as the study progressed into the second year. In the 0 – 5 cm horizon, soil pH was elevated to 6.1 in the 12 Mg ha^{-1} NIWA treatment and 4.6 in the 2 Mg ha^{-1} NIWA treatment, while the control had a pH of 4.1 after 3 months. At the end of the trial in the first year, the pH values of the pure Dol and NIWA soil had a high pH of 8.7 and decreased to 7.6 in the second year. No significant differences in organic matter content were observed between treatments. However, there was a general trend of decreasing organic matter in the treatment plots following application, with noticeable reductions observed in the second sampling year.

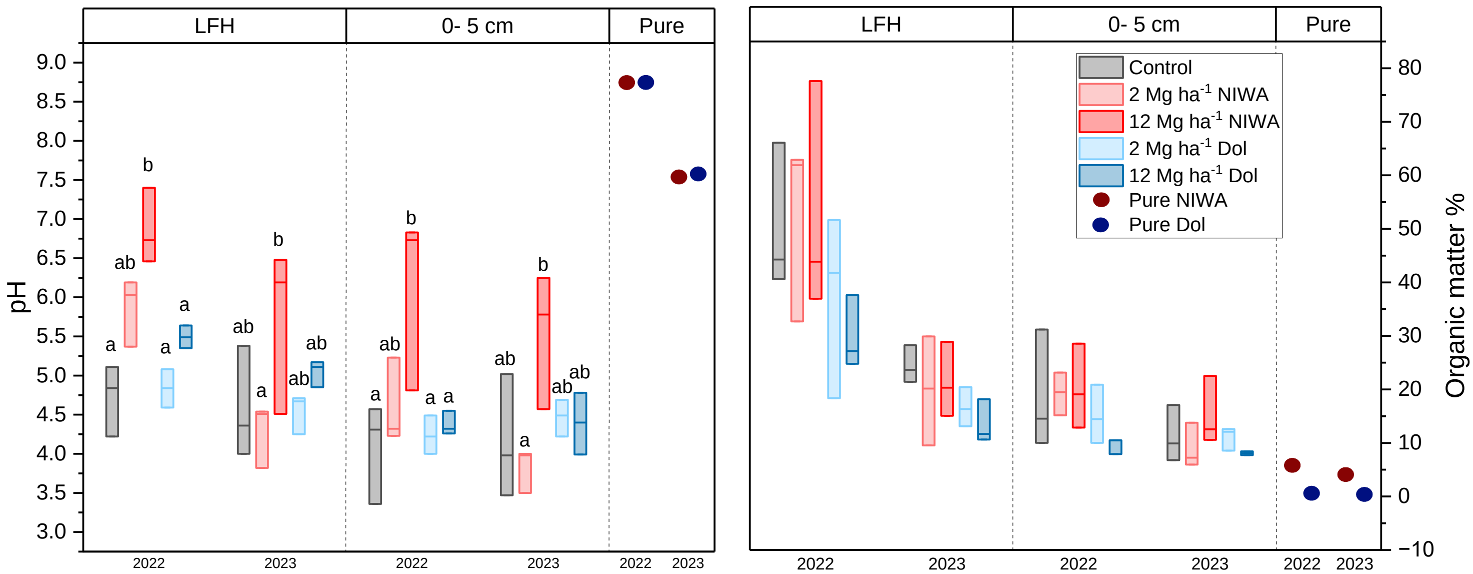

Figure 3. 3. Average pH (left) and percentage of organic matter (OM) (right) for each treatment including the soil control, 12 & 2 Mg ha^{-1} Non-industrial wood ash (NIWA), and 2 and 12 Mg ha^{-1} dolomite (Dol)—categorized by sampling year (2022 & 2023) and depth (LFH & 0-5 cm), including the pure NIWA and Dol controls. The letter display indicates significant differences between control and treatments ($p < 0.05$) determined by a Tukey HSD test.

Elevated levels in exchangeable soil base cation concentrations were observed following NIWA application that were most pronounced in the 12 Mg ha^{-1} treatment (Figure 3.6). Calcium and K concentrations in the LFH layer significantly increased in both the 2 and 12 Mg ha^{-1} NIWA plots, but in the 0 – 5 cm soil a significant increase was only observed in the 12 Mg ha^{-1} NIWA plots. Additionally, concentrations of Mg levels also increased significantly in the 12 Mg ha^{-1} NIWA plots in both the LFH and 0 – 5 cm soil horizon. However, these differences observed in soil K and Mg concentrations in the first year fell significantly by the second year, returning to control levels in both horizons. In contrast, Ca levels remained significantly elevated in the 12 Mg ha^{-1} treatment plots in the LFH horizon. Mn, Al, and Fe showed no significant differences among the treatments within each soil horizon (Figure 3.6). However, Mn and Al exhibited a tendency to decrease in concentration over both sampling years. Specifically, Mn concentrations decreased in both the LFH and 0 – 5 cm horizons, while Al concentrations decreased only in the

0 – 5 cm horizon. In the pure amendments, Ca concentrations showed a slight decrease in the second year for the pure NIWA treatment but increased slightly for the pure dolomite treatment. Potassium concentrations experienced a minor decline for the pure NIWA treatment, while Mn and Al concentrations increased in the second year for the pure dolomite treatment.

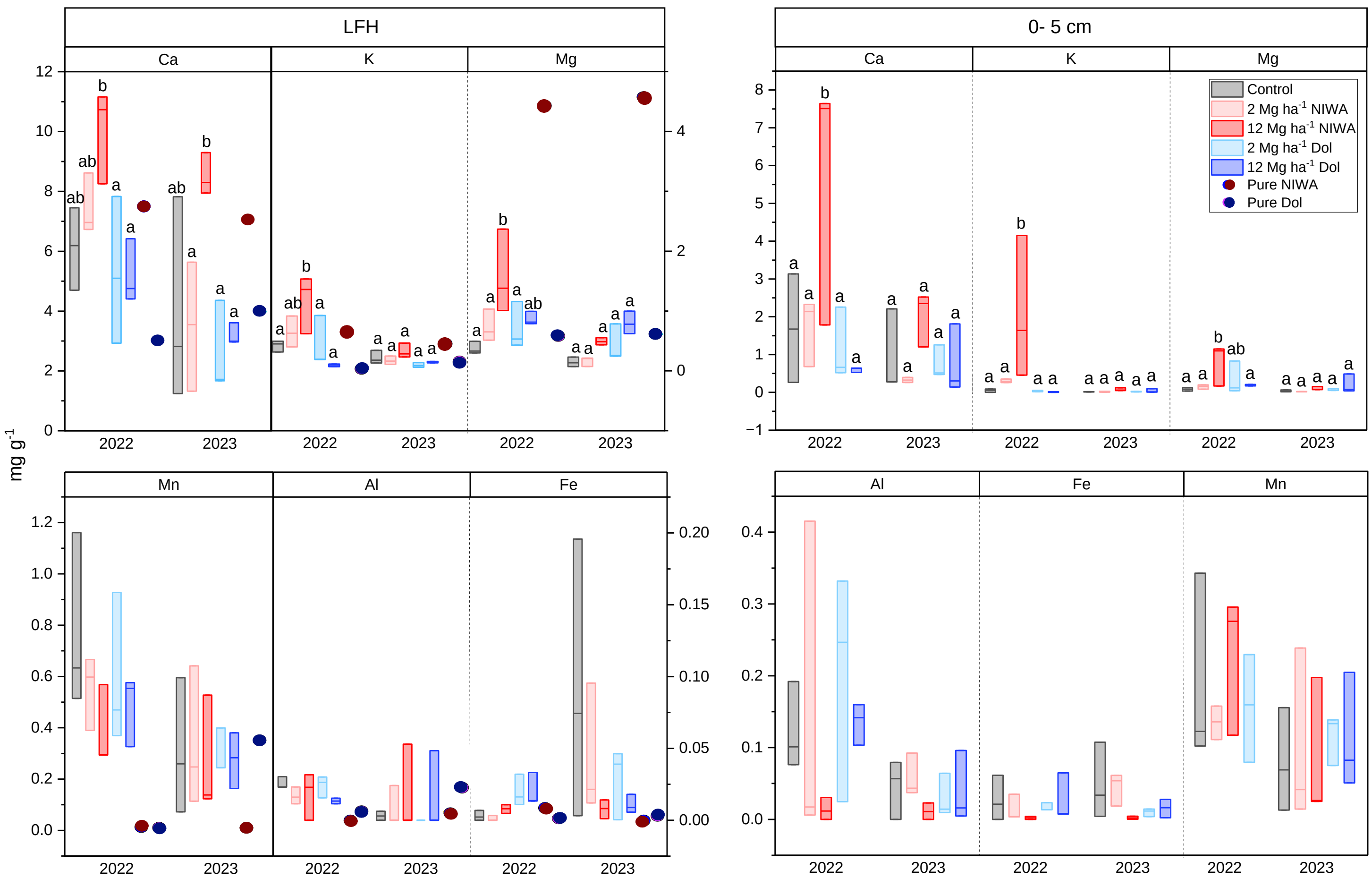

Figure 3. 4. Average exchangeable cations in the LFH, upper mineral horizon and pure non-industrial wood ash (NIWA) & dolomite (Dol) sampled from each soil collar in the control, NIWA, and Dol treated plots two seasons after application (2022 & 2023). The letter display indicates significant differences between control and treatments ($p < 0.05$) determined by a Tukey HSD test.

3.4.2 Field trial: CO_2 fluxes

The application of NIWA resulted in short-term changes in CO_2 fluxes, especially in the 12 Mg ha^{-1} treatment, while no changes were observed in the dolomite treatments (Figure 3.7). Prior to the addition of NIWA and dolomite treatments, the average *in situ* respiration was 17 g CO_2 m^2 day^{-1}. Twenty-four hours after treatments were applied loosely on the soils surface, CO_2 fluxes dropped to 0.4 g CO_2 m^2 day^{-1} in the 12 Mg ha^{-1} NIWA treatment plots whereas fluxes in the other treatments and control were relatively stable (Figure 3.7). Two days following the treatments, fluxes from the 12 Mg ha^{-1} NIWA increased to 23.6 g CO_2 m^2 day^{-1} and remained consistently higher than the other treatments and control plots throughout the study period while fluxes from the other treatments remained similar to the control plots after application (Figure 3.7 & 3.8). The pure NIWA control plot initially experienced a large CO_2 influx at -67.5 g CO_2 m^2 day^{-1}, and fluxes approached zero after about 2 months and remained fluctuating around zero for the duration of the study. CO_2 fluxes from the dolomite control were generally lower than CO_2 fluxes from the different treatments and soil control.

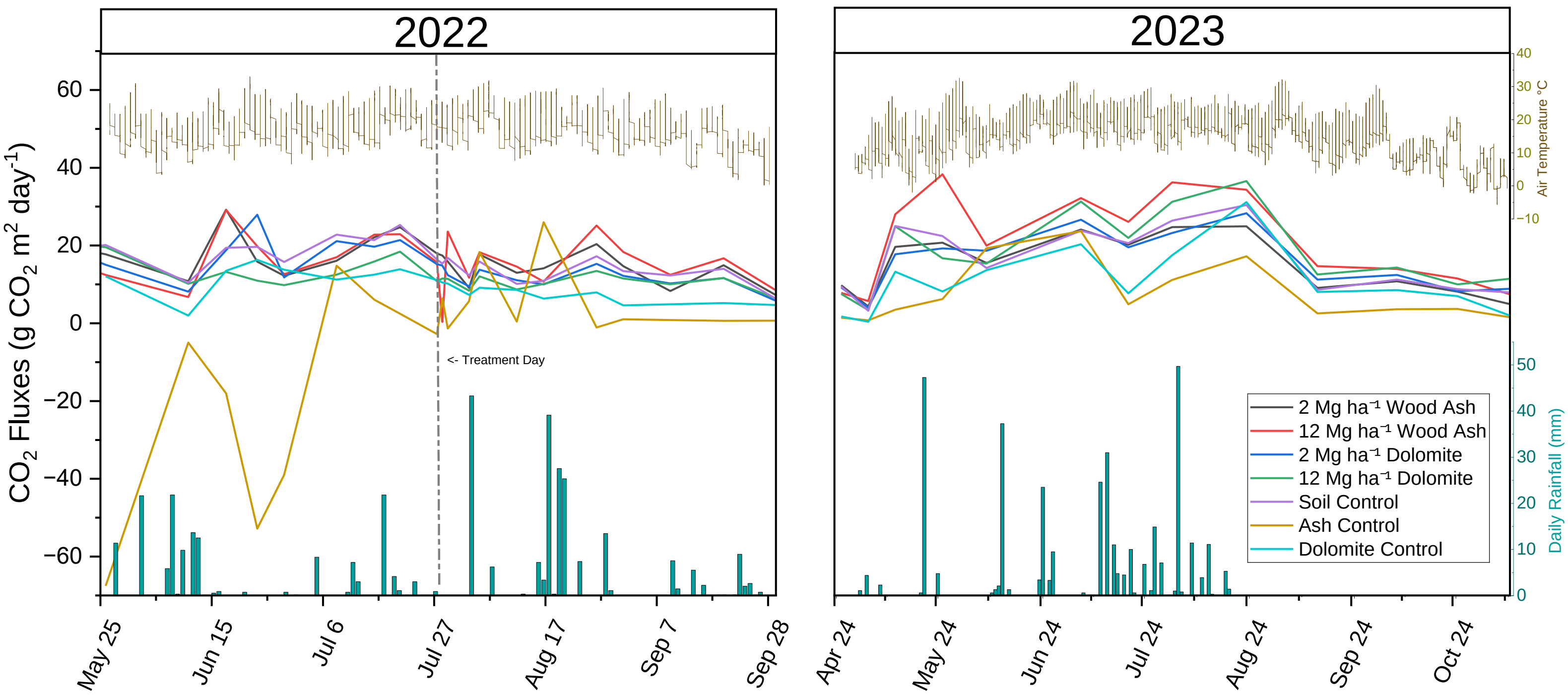

Figure 3. 5. Average CO_2 flux (g CO_2 m^2 day^{-1}) sampled from each treatment including the soil control, NIWA control, dolomite control, 2 & 12 Mg ha^{-1} NIWA, and 2 & 12 Mg ha^{-1} dolomite treatments during the sampling season of 2022 and 2023. With daily rainfall (mm), and air temperature (°C) recorded outside the plots. Positive and negative values represent effluxes and influxes of CO_2 respectively. The dashed line represents the treatment day of NIWA and dolomite.

Although not statistically significant (p: 0.4), the cumulative CO_2 efflux was highest in the 12 Mg ha^{-1} NIWA application compared with the other treatments (Figure 3.8). Before applying the NIWA and dolomite treatments, the average cumulative *in situ* CO_2 flux was 1177 g CO_2 m^2. By the end of the second year, the cumulative CO_2 efflux for the 12 Mg ha^{-1} NIWA treatment increased to 6889 g CO_2 m^2, surpassing the control, which measured 5733 g CO_2 m^2.

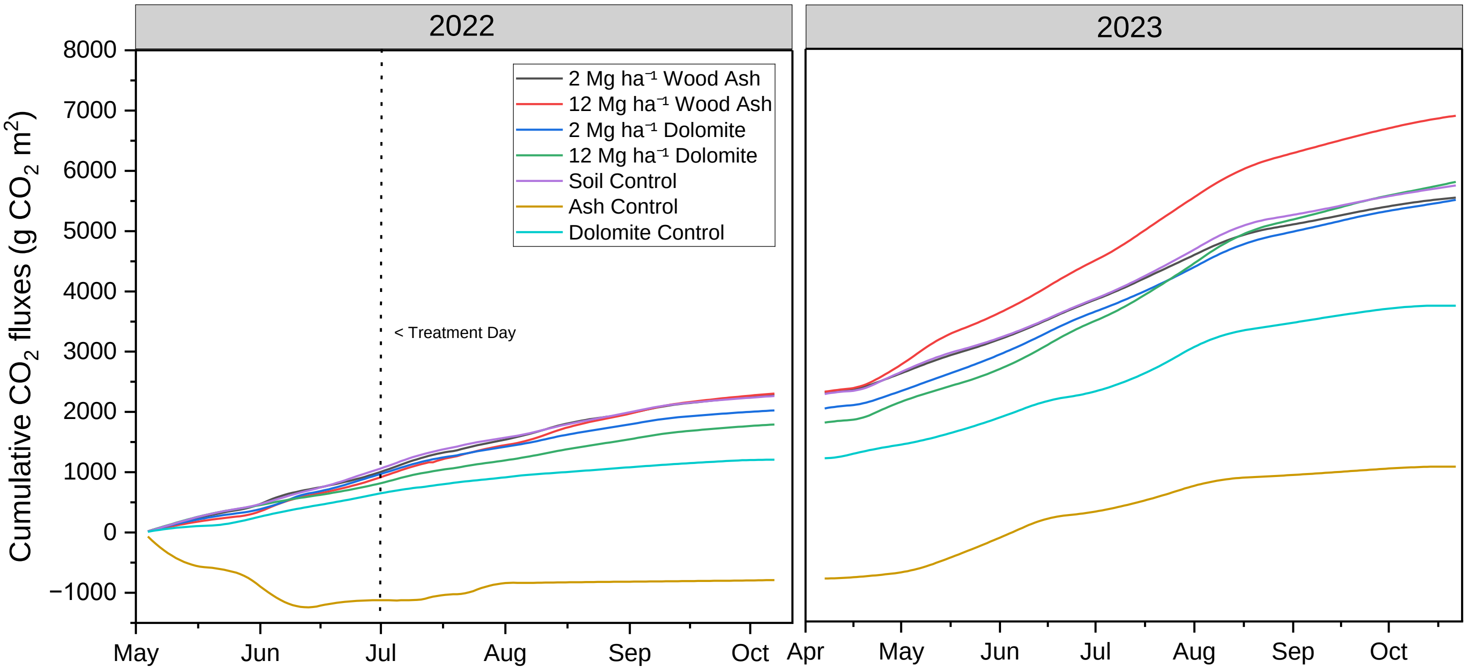

Figure 3. 6. Cumulative CO_2 flux (g CO_2 m^2) sampled from each soil collar that included control, NIWA, and dolomite treatments during the sampling season of 2022 and 2023. The dashed line represents the treatment day of NIWA and dolomite.

3.4.3 Lab trial: Soil chemical properties

The changes in soil chemistry observed in the laboratory study following the application of NIWA and dolomite were similar to those seen in the field study. Notably, the NIWA treatment resulted in a significant increase in pH, along with increased concentrations of exchangeable Ca in both the LFH and 0 – 5 cm soil horizons. Conversely, Al concentrations significantly decreased in both treatment plots (Table 3.2)

Table 3. 2. Average soil pH, organic matter, nutrient and metal concentrations (± SE) in the LFH and upper mineral horizon from each soil collar in the control, NIWA, and dolomite treated plots after the conclusion of the experimental farm field experiment. The letter display indicates significant differences between control and treatments ($p < 0.05$) determined by a Tukey HSD test.

	LFH			0- 5 cm		
	Control	12 Mg ha^{-1} NIWA	12 Mg ha^{-1} Dolomite	Control	12 Mg ha^{-1} NIWA	12 Mg ha^{-1} Dolomite
pH	4.3 (0.3)^{a}	6.3 (0.2)^{b}	4.6 (0.1)^{a}	3.7 (0.1)^{a}	5.0 (0.3)^{b}	3.8 (0.1)^{a}
OM %	32.2 (3.1)	24.5 (4.5)	18.3 (4.2)	13.0 (2.0)	10.5 (2.2)	10.1 (1.3)
(μg g^{-1})						
Ca	347 (49)^{a}	3794 (1104)^{b}	790 (131)^{a}	449 (91)^{a}	3853 (786)^{b}	1102 (150)^{a}
K	12 (1.3)	2061 (998)	48 (9)	51 (12)^{a}	1423 (302)^{b}	73 (13)^{a}
Mg	31 (19)^{a}	598 (325)^{b}	282 (68)^{a}	31 (4)^{a}	313 (72)^{ab}	509 (81)^{b}
Mn	38 (6)	128 (33)	100 (29)	56 (20)	309 (107)	172 (41)
Fe	20 (4)	13 (1)	13 (2)	178 (69)	92 (48)	43 (7)
Al	12 (16)	0 (0.0)	6 (28)	275 (44)^{a}	104 (45)^{ab}	34 (15)^{b}

3.4.4 Lab trial: CO_2 fluxes

In the laboratory study, the patterns in CO_2 fluxes were like those observed under field conditions. Prior to the addition of NIWA and dolomite treatments, the average *in situ* CO_2 flux was 7.05 g CO_2 m^2 day^{-1} (Figure 3.9). However, the laboratory study highlighted the significant drop in CO_2 fluxes more clearly. One hour after treatments were applied loosely on the soil surfaces, the CO_2 fluxes significantly dropped to –26.4 g CO_2 m^2 day^{-1} in the 12 Mg ha^{-1} NIWA treatment plots whereas fluxes in the other treatments stayed relatively similar to the control plots. Just three hours later, fluxes in the 12 Mg ha^{-1} NIWA increased to 0.7 g CO_2 m^2 day^{-1}. Two days following the treatments, fluxes in the 12 Mg ha^{-1} NIWA had increased to 10.7 g CO_2 m^2 day^{-1} before plateauing (6.3 g CO_2 m^2 day^{-1}) to values measured in the control plots (6.6 g CO_2 m^2 day^{-1}) three days later.

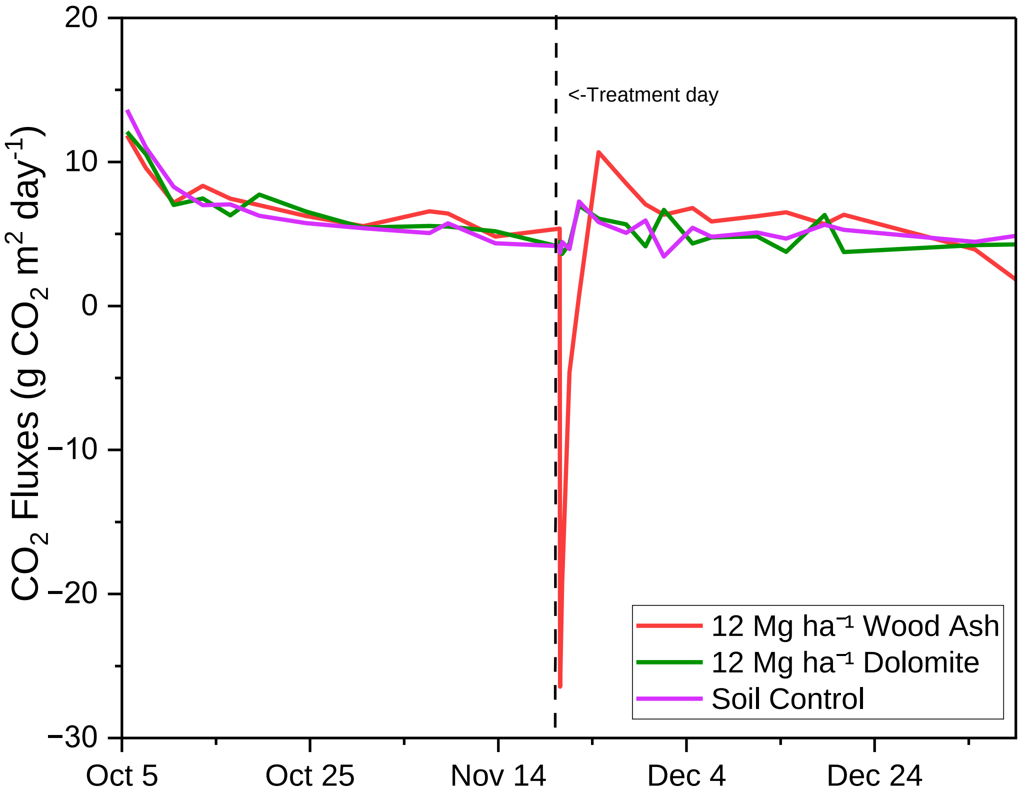

Figure 3. 7. CO_2 flux (CO_2 m^2 day^{-1}) sampled from each soil collar that included the control, NIWA, and dolomite treatments during the sampling season of 2023. Positive and negative values represent effluxes and influxes of CO_2 respectively. The dashed line represents the treatment day of NIWA and dolomite.

3.4.5 Lab trial: Soil extracellular enzyme activity

Statistically there was negligible impact of treatment effect and sampling day on soil enzyme activities over the full six-week study period (Figure 3.10). However, enzyme activities were quite variable during the first week after addition of the soil amendments. One day after treatment, acid phosphatase activity in the LFH decreased in both NIWA and dolomite treatments, but this was only significant in the dolomite treatment. Enzyme activity levels were more stable between weeks 1 and weeks 6, during which NAGase activity in the LFH was significantly (p= 0.004) lower in both NIWA and dolomite treatments compared with control (Figure 3.10). Similarly, application of NIWA significantly ($p < 0.001$) reduced acid phosphatase activity in the LFH layer compared with the control and dolomite treatments between weeks 1 and 6. There was no significant effect of treatments on β-1, 4-glucosidase activity in the LFH layer and there was no response of any enzyme to treatment in the upper mineral soil (Figure 3.10).

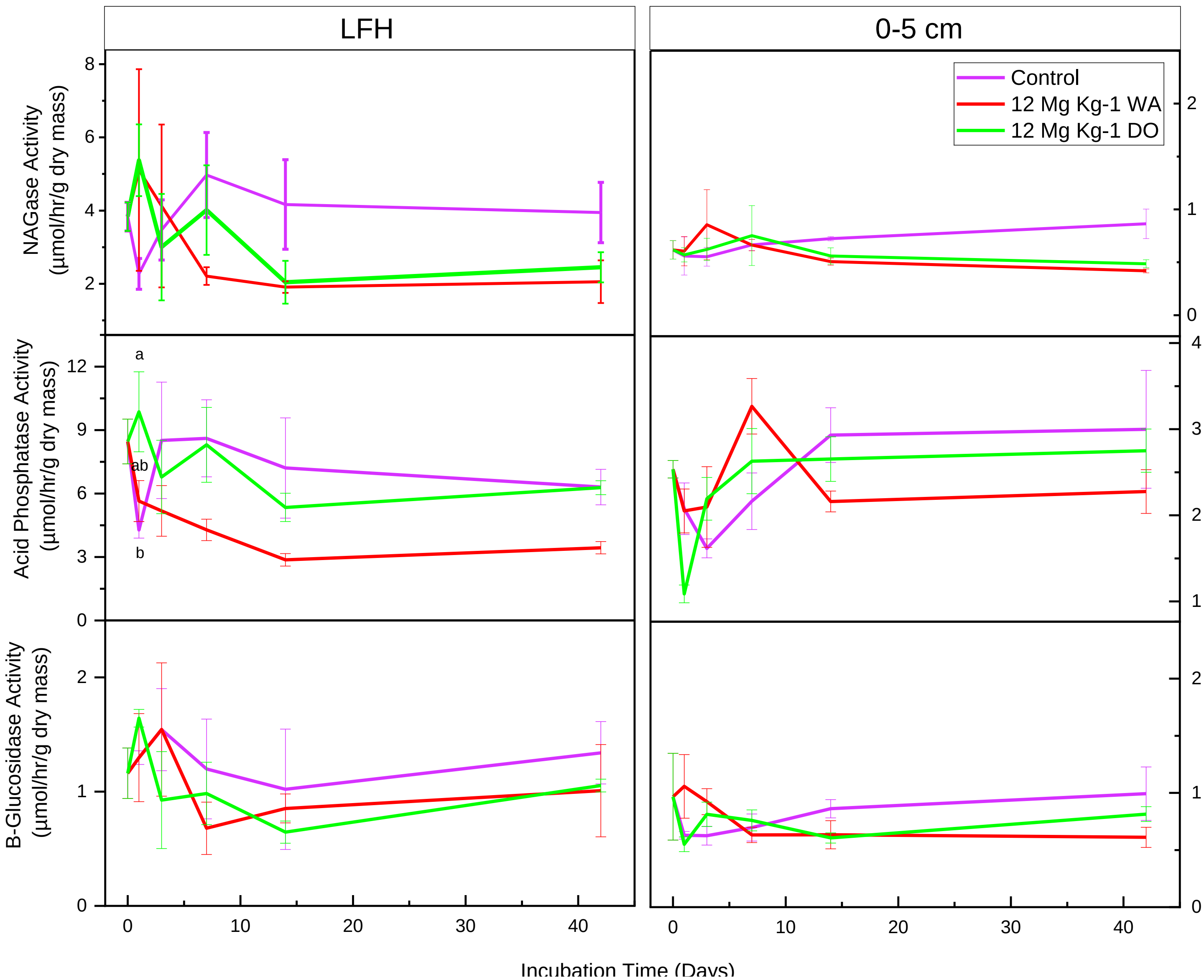

Figure 3. 8. Enzyme activities in the LFH and 0 – 5 cm mineral horizon in the control, NIWA, and dolomite treated plots. The enzyme activities were determined immediately prior to application on November 21st, 2023 (0 d) and 1, 3, 7, 14, and 42 days after application (mean values ± SE). The letter display indicates significant differences between control and treatments ($p < 0.05$) determined by a Tukey HSD test.

3.4.6 Lab trial: Soil enzyme BG:NAG:PHOS ratio

No consistent significant effects of treatments or sampling day on BG:NAG:PHOS ratios were observed (Table 3.3). The only significant response was measured in the mineral layer on day 1, with lower enzyme BG:NAG ratios in the NIWA and dolomite treatments compared with control, but this difference had disappeared by day 3. At the end of the 6-week trial, the average NAG:PHOS ratio in the LFH layer was higher than the BG:NAG and BG:PHOS ratio, whereas in the mineral layer the BG:NAG ratio was higher.

Table 3. 3. Soil enzyme BG:NAG:PHOS ratios in the LFH and 0-5 cm mineral horizon in the control, NIWA, and dolomite treated plots. The enzyme activities were determined immediately prior to application on November 21st, 2023 (0 d) and (0 d) and 1, 3, 7, 14, and 42 days after application (mean values ± SE). The letter display indicates significant differences between control and treatments (*p* < 0.05) determined by a Tukey HSD test.

		LFH			0- 5 cm		
		Control	12 Mg ha^{-1} NIWA	12 Mg ha^{-1} Dolomite	Control	12 Mg ha^{-1} NIWA	12 Mg ha^{-1} Dolomite
C:N	1 day	0.4 (0.1)	0.02 (0.16)	0.3 (0.01)	2.1 (1.6)^{b}	-0.11 (0.4)^{a}	1 (0.2)^{ab}
	3 days	0.2 (0.01)	-0.3 (0.7)	-0.94 (0.6)	0.8 (0.01)	0.2 (0.07)	0.49 (0.26)
	7 days	-0.15 (0.4)	-0.78 (0.5)	-0.46 (0.6)	0.86 (0.3)	1.2 (0.3)	0.3 (0.26)
	14 days	-0.35 (0.4)	-0.30 (0.3)	-2.3 (1.8)	0.48 (0.27)	0.7 (0.28)	0.88 (0.07)
	42 days	0.16 (0.1)	0.17 (0.34)	0.08 (0.08)	1.6 (0.7)	0.6 (0.19)	0.29 (0.1)
C:P	1 day	0.18 (0.05)	0.08 (0.15)	0.22 (0.002)	-0.69 (0.1)	-0.08 (0.5)	-0.7 (4.1)
	3 days	0.13 (0.13)	0.13 (0.21)	-0.2 (0.23)	-1 (0.3)	-0.36 (0.37)	-0.35 (0.2)
	7 days	-0.07 (0.3)	-0.4 (0.15)	-0.09 (0.19)	-0.68 (0.3)	-0.39 (0.06)	-0.35 (0.2)
	14 days	-0.24 (0.3)	0.2 (0.15)	-0.29 (0.1)	-0.1 (0.07)	-0.6 (0.25)	-0.5 (0.15)
	42 days	0.15 (0.11)	-0.16 (0.3)	0.03 (0.03)	-0.14 (0.2)	-0.6 (0.15)	-0.2 (0.07)
N:P	1 day	0.5 (0.09)	0.8 (0.2)	0.7 (0.02)	-1.2 (0.5)	-0.94 (0.47)	0.4 (4.7)
	3 days	0.6 (0.04)	0.6 (0.3)	0.4 (0.17)	-1.3 (0.39)	-1 (0.95)	-0.7 (0.35)
	7 days	0.7 (0.05)	0.6 (0.09)	0.6 (0.2)	-0.6 (0.2)	-0.36 (0.09)	-0.54 (0.4)
	14 days	0.7 (0.03)	0.6 (0.14)	0.4 (0.15)	-0.3 (0.03)	-0.9 (0.02)	-0.7 (0.25)
	42 days	0.7 (0.1)	0.5 (0.2)	0.5 (0.08)	-0.2 (0.15)	-1.1 (0.18)	-0.7 (0.11)

3.5 Discussion

This study investigated changes in soil chemistry, CO_2 fluxes and soil enzyme activities in a sugar maple-dominated forest floor in south-central Ontario, using field and laboratory methods to evaluate the short-term effects of NIWA and dolomite. The application of NIWA, particularly at the 12 Mg ha^{-1} rate, had a much greater influence on soil chemistry and CO_2 fluxes than dolomite. While overall reductions in enzyme activities were observed in the LFH horizon throughout most of the study period, these changes were not statistically significant. However, by one week after treatment application, NAGase and acid phosphatase activity were significantly lower in the NIWA-treated plots than the dolomite treated plots.

3.5.1 Field trial: Soil chemical properties

Non-industrial wood ash had a much stronger effect on soil chemistry compared with dolomite, with soil pH increasing by over 2.2 units in the LFH layer post application. Wood ash has a strong neutralizing capacity because of its hydroxide, and carbonate, components and its ability to buffer protons in the soil (Demeyer et al., 2001). Such large increases in pH tend to occur when pre-treatment conditions are more acidic as they were here (pH < 5.0, Reid & Watmough, 2014) and are consistent with increases observed by the application of similar doses in other short-term studies (5 – 6 Mg ha^{-1}, 1 – 5 years; Deighton & Watmough, 2020; Ozolinčius et al., 2007; Reid & Watmough, 2014). Similarly, Kahl et al. (1996) observed a pH increase of 0.6 to 2.2 units after applying 6 Mg ha^{-1} of wood ash and 1.3 to 2.3 units after applying 13 Mg ha^{-1} to acidic forest soils in Maine. In contrast to the upper organic soil horizons, the pH of the upper mineral soil only showed a slight pH increase relative to the control in the 12 Mg ha^{-1} treatment. After 4 years, Deighton et al. (2021) also reported a slight increase in mineral soil pH following wood ash (8 Mg ha^{-1}) application, but generally researchers have found that, except for high ash concentrations (~ > 6 Mg ha^{-1}), there is little to no effect on the mineral horizon pH in short-term studies (Augusto, 2008; Jacobson et al., 2004). In contrast dolomite was shown to have a weaker response on soil chemistry than NIWA. This may be due to dolomite acting more slowly in neutralizing soil acidity compared to wood ash. This slower reaction rate is due to its reliance on the gradual dissolution of carbonate ions (CO_3^{2-}), which interact with hydrogen ions (H^+) to raise soil pH (Wu et al., 2021).

Organic matter showed no significant change after the first year across all treatments and the control but began to decline after the second year in both soil horizons. While decreases in organic matter are not consistently reported in ash application studies (Deighton & Watmough, 2020; Fritze et al., 1994; Saarsalmi et al., 2001), the reductions observed in this study are primarily due to the observed loss of the LFH layer, which was exposed to wind erosion in the open field setting. Unlike in a forested environment, where seasonal litterfall replenishes the organic layer, the absence of tree inputs likely contributed to this decline. A study by Fritze et al. (1994) found that forest floor removal decreased microbial biomass carbon and nitrogen, likely due to reduced substrate availability for microbial metabolism. Similarly, Compton and Boone (2000) reported that the absence of the forest floor layer resulted in lower soil C and N content, suggesting that the loss of this layer can lead to increased mineralization of existing soil organic matter and can disrupt nutrient cycling. Soil exchangeable cations (Ca, K, Mg) were significantly higher after NIWA application and Ca remained high in the 12 Mg ha^{-1} after the second year of sampling in the LFH and 0-5 cm mineral horizon. The strong neutralizing and buffering capacity of ash is due to the hydroxyl ions that form because of the dissolution of hydroxides, oxides, and carbonates such as CaO, MgO, NaOH and $CaCO_3$, which neutralize the protons in soil solution and those bound on cation exchange sites in the soil (Saarsalmi et al., 2006). This increase in pH affects cation exchange capacity (CEC), as higher pH enhances the negative charge of soil colloids, increasing their ability to retain and exchange base cations (Komonweeraket et al., 2015; Spurgeon et al., 2006). Although not statistically significant, exchangeable Al, Mn, and Fe tended to decrease as base cations increased in the NIWA-treated plots because of either cation exchange reactions or a pH effect (Saarsalmi et al., 2001). This effect is commonly found in other studies (Augusto et al., 2008; Reid & Watmough 2014; Saarsalmi et al., 2001, 2004). Since pH shifts influence the relative adsorption of cations versus anions on soil exchange sites (Kunhikrishnan et al., 2016), the replenishment of base cations and subsequent pH rise should lead to decreased exchangeable concentrations of Al, Fe, Mn, and other trace metals (Gitari et al., 2009; Komonweeraket et al., 2015).

Despite ash containing high concentrations of Fe, Al, and Mn the initial decreases in exchangeable concentrations of Al, Fe, and Mn in soil are thought to be due to ash-induced increases in soil pH resulting in these elements being displaced from cation exchange sites and reducing the solubility (Kahl et al., 1996; Saarsalmi et al.,2001; Unger and Fernandez, 1990). In contrast, the dolomite-treated plots showed no such differences in exchangeable metal concentrations. This may be because wood ash, with its smaller particle size, alters soil pH more rapidly compared to dolomite (Clapham et al., 1992). Wood ash is more soluble and reactive than ground dolomite, which can take six months to a year to fully affect soil pH (Johan et al., 2021). As a result, the slower dissolution and reaction rate of dolomite may reduce its immediate impact on soil pH and its ability to displace Al, Mn, and Fe from cation exchange sites.

Additionally, soil nutrient availability and pH together control extracellular enzyme activities, which are crucial for organic matter decomposition and nutrient cycling (Sinsabaugh et al., 2008). Changes in pH can lead to the displacement of cations such as Al^{3+}, Fe^{3+}, and Ca^{2+}, affecting the fixation of nutrients like NH_4^+ and P, thereby influencing microbial activity and enzyme production (Stark et al., 2014). These shifts in nutrient availability may in turn alter microbial functioning, such as solubilization of P sources or the mineralization of organic matter containing essential nutrients like N, P, K, S, and C, which could be reflected in extracellular enzyme activities (Stark et al., 2014)

3.5.2 Field trial: CO_2 fluxes

The application of NIWA resulted in short-term changes in CO_2 fluxes, particularly in the 12 Mg ha^{-1} treatment, resulting in a higher CO_2 efflux within the second year of the trial while no differences relative to the controls were observed in the dolomite treatments. An estimate of the potential CO_2 flux increase can be made by considering the total C content of NIWA. NIWA contains approximately 8.6% carbon, and at an application rate of 12 Mg ha^{-1}, this equates to 1,032 kg C ha^{-1}. If fully mineralized, this C could contribute to 379 g CO_2 m^{-2} over the study period. However, the actual CO_2 release depends on factors such as the dissolution of carbonates, and enhanced microbial activity meaning that observed fluxes may be lower than the maximum estimates.

Soil CO_2 fluxes are primarily driven by microbial respiration and the decomposition of organic matter (Davidson and Janssens, 2006), but inorganic processes, such as mineral weathering and carbonation reactions, can also influence CO_2 dynamics in amended soils. The dissolution rates of calcium-bearing minerals vary across soil amendments and play a role in shaping the potential for inorganic carbon sequestration (Power et al., 2013; Stubbs et al., 2020). These rates depend on factors such as crystal and solution chemistry, temperature, and available reactive surface area (Power et al., 2013), and these must be considered when interpreting soil CO_2 fluxes. The high CaO content in wood ash rapidly hydrates upon exposure to water or humidity, forming $Ca(OH)_2$ (portlandite), which subsequently reacts with atmospheric or soil CO_2 to re-form $CaCO_3$ (calcite), influencing both short-term CO_2 fluxes and long-term carbon stabilization (Morales-Flórez et al., 2015; Pesce et al., 2017; Ridha et al., 2015).

$$CaCO_3 + heat(900 \circ C) \rightarrow CaO + CO_2 \; (Burning) \tag{9}$$

$$CaO + H_2O \rightarrow Ca(OH)_2 \ (Hydration) \tag{10}$$

$$Ca(OH)_2 + CO_2 \rightarrow CaCO_3 + H_2O \ (Carbonation) \tag{11}$$

However, under acidic conditions, carbonates can also act as a source of CO_2 rather than a sink. In this reaction, organic acids present in forest soils (e.g., from root exudates or decomposition) can react with carbonate minerals, releasing CO_2 (Ramnarine et al., 2012):

$$2R\text{–}COOH \ + \ CaCO_3 \ \rightarrow \ Ca^{2+} \ + \ CO_2 \ + \ H_2O \ + \ 2R\text{–}COO^{-} \tag{12}$$

This is particularly relevant given that the unamended forest soils in this study were acidic (LFH pH ~4.7), suggesting that some portion of the CO_2 efflux observed after amendment may be attributed to acid–carbonate reactions. After applying NIWA, CO_2 fluxes initially decreased, likely due to the rapid reaction of oxides and hydroxides in the wood ash with water and CO_2, which initiates the carbonation process and temporarily reduces CO_2 levels. This reaction is accompanied by a decrease in the soil pH value as the oxides and hydroxides are neutralized (Ohlsson, 2000). However, the drawdown effect was short-lived. Over time, a net increase in CO_2 fluxes is observed, which may be driven by a combination of abiotic factors, such as the continued dissolution of carbonates or re-release of CO_2 during carbonate mineral precipitation, and possible respiration from the soil below the collar. As ash derived alkaline ions leach downward, they can alter the chemistry of deeper soil layers, enhancing microbial activity and increasing CO_2 production (Baloch et al., 2024). Additionally, subterranean ventilation may allow CO_2 generated in the deeper soil to move upward, contributing to the observed increase in fluxes (Wang et al., 2020), especially if the initial crust formed by the ash degrades over time. In contrast, dolomite ($CaMg(CO_3)_2$) dissolves much more slowly than calcite ($CaCO_3$) due to its lower solubility and more stable crystal structure than the highly reactive components of wood ash, and they react with carbonic and organic acids in soil to release calcium, magnesium, and bicarbonate. (Lerman & Mackenzie, 2018; Plummer and Mackenzie, 1974). While carbonate precipitation can theoretically remove CO_2 under certain alkaline conditions, this process is highly unlikely in the acidic soils of this study (pH ~4.7). Instead, the dominant chemical pathway is the dissolution of existing carbonates by organic acids, which leads to CO_2 release rather than storage (Berner, 1995; van Hees et al., 2002). Therefore, some of the observed CO_2 efflux following amendment may be attributed to the acid-driven breakdown of carbonate phases present in NIWA or dolomite.

Studies also suggest that precipitation events reactivating microbial processes can elevate CO_2 fluxes (Andersson et al., 2004; Castaldi et al., 2010; van Straaten et al., 2019). Thus, the observed increase in CO_2 fluxes several days after application may reflect a complex interplay of abiotic and biotic processes. It is possible that the wood ash initially drew down CO_2 through carbonation reactions, but as those reactions progressed, the acidic soil environment may have promoted the dissolution of unreacted carbonate minerals, leading to subsequent CO_2 release. Given this dynamic, the wood ash likely has the potential to both store and release CO_2 abiotically, depending on local soil pH and reaction progress. Distinguishing these chemical effects from microbial respiration remains challenging in field settings, and the measured fluxes likely reflect a combination of both. . Similar research conducted by Zimmerman & Frey (2002) found a significant drawdown in CO_2 after wood ash application followed by a significant increase in CO_2 respiration becoming higher than the control plots 40 days later. However, Ernfors et al., (2010) found that during the 5 years following the application of wood ash on peatland soils, no differences in forest floor CO_2 emissions could be detected between the treatments in their study despite an increase in pH. Changes in soil pH can alter microbial community composition and C use efficiency, leading to increased CO_2 release. Malik et al. (2018) found that land-use-driven pH shifts significantly impacted microbial C cycling, while Wang et al. (2021) reported that bacterial diversity is highest in neutral pH soils, suggesting that pH-induced stress affects microbial efficiency and CO_2 emissions. The sudden increase in pH from highly alkaline amendments like wood ash may further stress microbes adapted to acidic conditions, lowering microbial C use efficiency and causing more C to be respired rather than used for growth, resulting in greater CO_2 loss (Aciego Pietri & Brookes, 2008; Rousk et al., 2010). This highlights that microbial activity might play a significant role in CO_2 fluxes, noting that soil respiration, which is defined as the production of CO_2 by microorganisms within the soil and can differ from CO_2 efflux, which is the movement of CO_2 from the soil to the atmosphere and is measured at the soil surface. Notably, increases in soil CO_2 efflux do not always correlate directly with increases in soil respiration. This is because CO_2 is produced throughout the soil profile, but not all of it reaches the atmosphere. Some CO_2 may be retained in pore spaces, dissolved in soil water, or even reabsorbed through carbonate reactions before it diffuses to the surface (Maier et al., 2011; Angert et al., 2015). As a result, efflux and respiration can be decoupled, especially in systems with variable moisture, temperature, or carbonate content.

Changes in soil pH induced by wood ash application further influence CO_2 dynamics. Elevated pH can enhance microbial growth and accelerate humus layer decomposition, potentially increasing N mineralization and CO_2 release (Corre et al., 2003; Bååth and Arnebrant, 1994; Persson et al., 1995; Zimmermann and Frey, 2002). Factors such as soil temperature and moisture also play a crucial role in regulating respiration rates (Buchmann, 2000; Raich and Schlesinger, 1992), adding complexity to the observed patterns.

In contrast, the dolomite treatments did not exhibit the same response as the NIWA treatments. Following application, neither the 2 and 12 Mg ha^{-1} dose, nor the dolomite control showed a significant initial drawdown in CO_2 flux. This lack of an immediate reduction in CO_2 flux suggests that dolomite may not trigger the same carbonation reactions or chemical changes in the soil as wood ash and thus does not have as strong an effect on CO_2 levels in the short term. The carbonation reaction is slower with dolomite, as it is more stable and less soluble than wood ash. It may take several months or even up to a year to noticeably alter the soil pH, leading to more gradual and prolonged changes in CO_2 flux (Wu et al., 2021). Over time, the dolomite treatments emitted less CO_2 compared to the NIWA treatments. This may be due to differences in how the two materials interact with soil, with dolomite likely exerting a more stable and less reactive influence on soil pH and microbial activity, resulting in lower long-term CO_2 emissions (Wu et al., 2021).

Notably, the pure wood ash control exhibited no net change in CO_2 flux when summed over two years. However, there was a large initial drawdown in CO_2 flux, followed by low emissions. By the end of the second year, CO_2 emissions began to rise slightly. In contrast, the pure dolomite control emitted less CO_2 than the treatments but still exhibited higher fluxes than the pure wood ash control. These processes may not be easily controlled or manipulated, and the stability of the resulting carbonates over geological time remains a significant consideration.

3.5.3 Lab trial: Soil properties and CO_2 fluxes

The lab trial showed similar trends in soil chemistry changes to the field trial, although the effects were less pronounced. Wood ash resulted in a more immediate and notable drawdown in CO_2 flux, which was short-lived and therefore not captured in the field trial due to less frequent flux measurements. Wood ash also had a stronger effect on soil pH, organic matter content, and exchangeable cations compared to dolomite. These changes were particularly evident in the LFH and 0 – 5 cm soil horizons, with pH increases attributed to the high alkalinity and neutralizing capacity of the wood ash (Augusto et al., 2008; Pitman, 2006). In contrast, dolomite did not significantly alter pH, likely due to its slower dissolution and smaller surface area for reaction (Clapham et al., 1992). This slower response aligns with field findings that dolomite had a less immediate effect on soil chemistry.

Both trials showed that NIWA application led to increased exchangeable cations, especially Ca, in the LFH and 0–5 cm horizons. However, the dolomite treatment did not exhibit the same increase, reinforcing its slower reaction rate (Wu et al., 2021). Despite these similarities, this lab trial highlighted the rapid initial effects of wood ash, while the dolomite treatment's slower dissolution led to more gradual changes.

3.5.4 Lab trial: Soil extracellular enzyme activity

Wood ash initially caused a decrease in soil CO_2 flux due to potential carbonation reactions, followed by a subsequent increase in flux, which was observed within hours. This pattern aligns with previous studies suggesting that microbial activity and carbonation processes influence short-term CO_2 dynamics (Andersson et al., 2004; Castaldi et al., 2010). However, enzyme activity in the lab trial (Figure 2.9 & 2.11) showed a weaker response compared to CO_2 flux changes, which is not unexpected given the difference in spatial scale between soil extracellular enzyme activity measurements and whole-soil CO_2 fluxes. Additionally, CO_2 flux is influenced by multiple abiotic and biotic factors beyond enzyme activity, such as carbonate dissolution, microbial respiration, and substrate availability (Tang et al., 2024). This aligns with findings by Zimmerman & Frey (2002), who also reported weak correlations between EEA and CO_2 flux, further emphasizing that enzyme activity alone does not directly predict CO_2 efflux in complex soil systems. In this study, enzyme activities exhibited high variability immediately after application, but by one-week post-treatment, activity levels appeared to stabilize, revealing differences in the LFH layer. After stabilization, acid phosphatase activity significantly decreased between weeks 1 and 6 following NIWA application, consistent with Zimmerman & Frey (2002). This decline aligns with the increase in P availability from NIWA, as microbes typically produce acid phosphatase when soil P is limited (Bargaz et al., 2012). When an easily accessible P source is present, microbes may reduce enzyme production to conserve energy. Additionally, the P in NIWA is likely in the inorganic form of orthophosphate, which can inhibit phosphatase activity by binding to the enzyme and preventing it from interacting with additional substrates (García-Gil et al., 2000; Pang & Kolenko, 1986). After the initial week of high activity variability, both N-acetyl-β-glucosaminidase (NAGase) and acid phosphatase activities were significantly lower in NIWA-treated plots, suggesting that the shifts in enzyme activity were driven by increased nutrient availability.

NAGase activity exhibited a similar pattern of response, with high variability in enzyme activities immediately after application but after 1-week differences between treatments were observed. NAGase activity significantly decreased between weeks 1 and 6 following NIWA application. Overall, the NIWA and dolomite treatments showed a decreasing pattern in all enzyme activities compared to the control. This decline may be driven by multiple factors, including shifts in nutrient availability due to pH changes, which can alter microbial demand for certain enzymes (Stark et al., 2014). For example, the decrease in N-acetyl-β-glucosaminidase (NAGase) activity may indicate that microbes had sufficient available nitrogen and no longer needed to invest energy in enzyme production. Since microbial biomass was not directly measured, it is also possible that changes in enzyme activity reflect shifts in overall microbial abundance rather than just metabolic adjustments (Daunoras et al., 2024). Additionally, pH-induced desorption of heavy metals from exchange sites could have influenced microbial communities, as some microbes and enzymatic processes are sensitive to metal toxicity (Hu et al., 2021). Increases in soil pH can change enzyme conformation and adsorption to soil colloids, in addition to modifying the solubility of substrates (Quiquampoix, 2000; Zimmerman & Ahn, 2010). Applying dry wood ash directly to soil often results in a sudden and significant increase in pH, known as a pH shock, which can negatively affect soil flora and fauna (Sarsaalmi, 2001). To mitigate these effects, pretreatment methods such as hardening or granulation (Åbyhammar et al., 1994; Eriksson, 1998; Hytönen, 1999) or wetting the ash before application (Ohlsson, 2000) are recommended. Wetting the ash promotes carbonation by enhancing its reaction with atmospheric CO_2, forming stable carbonates that lower its initial alkalinity and reduce the risk of abrupt pH shifts. This practice ensures a more controlled release of nutrients and minimizes disruptions to soil microbial communities.

This study also revealed that enzyme NAG:PHOS ratios were higher in the LFH layer compared to the mineral layer, while the BG:NAG ratios were higher in the mineral layer of NIWA and dolomite-treated soils, relative to the control mineral layers, after application. The higher BG:NAG ratios in the mineral layer of amended soils compared to the controls could indicate that microbes had sufficient N available and reduced the need for NAG production, potentially conserving energy for other metabolic processes. As a result, NAG would be lower than BG in these ratios as microbes become more focused on C acquisition rather than N (Fujita et al., 2018). In contrast, the higher NAG:PHOS ratios in the LFH layer indicate a greater N limitation or a higher demand for N acquisition in this layer. This difference could be due to varying nutrient availability between the layers, possibly influenced by the pH shift and changes in microbial community structure after treatments (Wang et al., 2019). Additionally, Mazzora et al (2019) examined how pH levels affect acid phosphatase activity, indicating that higher pH (above 6) can inhibit this enzyme, thereby reducing microbial access to organic P sources and in this study, the NIWA pH ranges rapidly increased to a pH level of 6.3. A higher abundance of P relative to C and N in soil suggests an increased microbial demand for N following P addition (Dong et al., 2019). Unlike P (7.6 g kg^{-1}), wood ash contains very little N concentration (0.1 %), as most N is volatilized during combustion (Pitman, 2005). However, the introduction of base cations to acidic soils can stimulate microbial growth, increasing microbial N demand and potentially leading to temporary N -depletion in the mineral soil (Horn et al., 2021; Zhang et al., 2019).

3.6 Conclusion

The application of NIWA significantly altered soil chemical properties and short-term CO_2 fluxes in both field and lab trials. Notable increases in soil pH, especially in the LFH and 0-5 cm horizons, indicate the strong acid-neutralizing capacity of wood ash. The rise in exchangeable cations, particularly Ca, K, and Mg, reflects the mineral composition of the ash. These changes in soil chemistry were associated with shifts in CO_2 fluxes, underscoring the complex interplay between microbial activity and abiotic factors after ash application. Enzyme activity responses also varied, suggesting that while microbial activity played a role in short-term changes, CO_2 drawdown may have been largely influenced by abiotic processes, such as pH-induced changes and mineral dissolution. Over time, microbial adaptation and organic matter decomposition could restore nutrient availability, but short-term shifts in enzyme activity may contribute to temporary nutrient limitations, particularly for phosphorus and nitrogen. To minimize potential negative impacts, such as pH shock to the ecosystem, it is recommended to wet and expose the ash to air prior to application. Wetting the ash will almost instantly convert any CaO to $Ca(OH)_2$ and exposing the ash to air to allow further conversion to $CaCO_3$. This approach enhances carbonation and stabilizes pH levels, ultimately fostering improved soil health and nutrient availability. Overall, these findings highlight the potential promising role of wood ash in sustainable soil management practices.

4. General Conclusions

The primary objective of this study was to evaluate Non-Industrial Wood Ash (NIWA) as a forest soil amendment and its potential to counteract soil acidification and potentially enhance C storage in Ontario forests. This was evaluated through two main approaches: by examining the effects of NIWA on soil chemistry, microbial activity, and C fluxes; and by assessing its influence on fine root biomass, tree growth, and foliar nutrient dynamics in a sugar maple-dominated forest. The findings indicate that NIWA effectively neutralizes soil acidity and replenishes essential base cations (Ca, Mg, K), but its broader ecological impacts, including its role in soil C cycling and potential trade-offs for tree nutrient uptake, warrant careful consideration.

One of the most notable effects of NIWA application was the rapid increase in soil pH, particularly in the LFH and upper mineral horizons, highlighting its strong acid-neutralizing capacity. This shift in pH may have influenced soil microbial activity and nutrient dynamics, with short-term increases in CO_2 fluxes suggesting that NIWA accelerates organic matter mineralization through both microbial and abiotic processes. Despite concerns regarding metal accumulation, NIWA application at rates below 4 Mg ha^{-1} did not result in metal toxicity, as total trace element concentrations remained below Canadian Soil Quality Guidelines (CM1) limits. However, since bioavailability can change over time with shifts in soil pH, organic matter, or redox conditions, careful long-term monitoring is recommended to ensure that trace metals do not become mobilized or taken up by plants at toxic levels.

In terms of forest productivity, NIWA had limited short-term effects on aboveground tree growth, with only minor changes in foliar nutrient concentrations. In fact, the control trees tended to exhibit the greatest increase in BAI. Further, the decline in fine root biomass, particularly in the 4 and 6 Mg ha^{-1} treatment, suggests a potential stress response, possibly due to pH shock following application. The rapid increase in soil pH may have disrupted root function, altered nutrient availability, or created physiological stress, leading to reduced root proliferation and tree growth. This indicates that while NIWA effectively improves soil chemistry, higher application rates may introduce trade-offs that constrain tree response, particularly in nutrient-limited stands. These findings show the importance of considering site-specific nutrient dynamics and the potential need for nitrogen amendments to maximize NIWA's benefits while mitigating unintended stress effects.

To optimize the benefits of NIWA and minimize potential negative effects, this research suggests that pre-wetting the ash and allowing limited air exposure prior to application may be beneficial. This approach can mitigate pH shock by slowing the release of base cations, improve nutrient retention in the soil, and reduce airborne dispersion, which is a common concern with dry ash application. The recommendation to wet the ash is supported by observed spikes in soil pH following application, as well as the potential for ash dust to negatively impact both soil microbial communities and for handling safety.

Overall, the results of this study suggest that NIWA is a viable soil amendment for restoring base cations and mitigating soil acidification in nutrient-poor forests typical of south-central Ontario and other regions underlain by Precambrian bedrock, if application rates are carefully controlled. While it effectively replenishes essential nutrients, its influence on forest productivity appears to be more complex, with potential trade-offs between nutrient availability and root biomass that require further investigation. Longer-term studies are needed to determine whether repeated NIWA applications could enhance tree growth and soil carbon sequestration, as well as to assess its potential role in nitrogen-limited ecosystems. Additionally, future research should explore how NIWA application influences metal mobility over time, particularly in relation to deeper soil horizons and groundwater quality. Further investigation is also needed to assess how weathered or pre-wetted NIWA interacts with the environment and whether these treatments could reduce pH shock, minimizing potential stress effects on fine roots and tree growth. By addressing these knowledge gaps, NIWA could be more effectively integrated into forest management strategies aimed at sustaining soil fertility and mitigating the impacts of historical acid deposition.

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