The biogeochemical effects of non-industrial wood ash application on ecosystem regeneration in central Ontario

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Abstract

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Decades of sulphur and nitrogen deposition acidified forest ecosystems across northeastern North America causing declines in pH and exchangeable base cation concentrations, negatively affecting biota. To assist natural recovery, researchers are investigating using alkaline soil amendments such as wood ash. However, much remains unknown about its use. This thesis evaluated the effects of non-industrial wood ash application (between 0 – 12 Mg ha^{-1}) on soil chemistry, understory vascular plant communities and sugar maple (*Acer saccharum* Marsh.) regeneration in central Ontario. Wood ash increased soil pH and concentrations of calcium, magnesium and several metals. Vascular plant species abundance, richness, and diversity exhibited no consistent treatment effect. Sugar maple seedling survivorship was adversely affected by wood ash applications > 4 Mg ha^{-1}, while growth was unaffected. These results support related research regarding the ability for wood ash to increase soil pH and base cation status but raises uncertainty regarding consequences for vascular plants.

Key words: Acid deposition, *Acer saccharum*, Base cation, Ecosystem regeneration, Seedling survival, Soil amendment, Understory vegetation, Vascular plants, Wood ash

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List of abbreviations

Akaike information criterion	AIC
Aluminum	Al
Ammonium	NH_4
Ammonium chloride	NH_4Cl
Arsenic	As
Bicarbonate	HCO_3
Boron	B
Brooklands Farm	Brooks
Burnt lime/Calcium oxide	CaO
Calcium	Ca
Calcium chloride	$CaCl_2$
Cadmium	Cd
Carbon	C
Carbon dioxide	CO_2
Carbon trioxide	CO_3
Chromium	Cr
Concentration of regulated metals	CM
Copper	Cu
Diameter at breast height	DBH
Dolomitic lime	$CaCO_3 \bullet MgCO_3$
Dry weight	DW
Exchangeable cations	EC
Fibric-humic	FH
Friends of the Muskoka Watershed	FMW
Gypsum/phosphogypsum	$CaSO_4 \bullet 2H_2O$
Hydrogen ion	H^+
Hydroxide	OH
Inductively coupled plasma–optical emission spectrometer	ICP-OES
Industrial wood ash	IWA
Iron	Fe
Lead	Pb
Limestone/chalk	$CaCO_3$
Loss on ignition	LOI
Magnesium	Mg
Magnesium oxide	MgO
Manganese	Mn
Marks Muskoka Maple Sugarbush	Marks
Nickel	Ni
Nitrate	NO_3
Nitrite	NO_2
Nitric acid	HNO_3
Nitrogen	N
Nitrogen oxides	NO_x
Non-agricultural source material	NASM

Non-industrial wood ash..NIWA
Non-parametric maximum likelihood estimator..NPMLE
Organic matter..OM
Principal component analysis...PCA
Potassium..K
Potassium chloride...KCl
Potassium oxide..K_2O
Shannon diversity..H
Simpson's diversity..D
Slacked lime...$Ca(OH)_2$
Sodium...Na
Sodium hydroxide..NaOH
Standard error..SE
Sulphate..SO_4
Sulphur..S
Sulphur dioxide...SO_2
Sulphuric acid...H_2SO_4
Sulphur trioxide..SO_3
Phosphorous...P
Wilfrid Creasor Sugarbush..Wilfs
Wollastonite..$CaSiO_3$
Zinc...Zn

1.0 General introduction

1.1 Acid deposition and soil acidification

Sulphur dioxide (SO_2) and nitrogen oxides (NO_x) are primary pollutants that can be released by natural processes such as volcanic eruptions, but are more often emitted from power plants, vehicle exhaust, mining, and industry (Kolawole & Iyiola, 2023). Sulphuric oxides and NO_x react with sunlight and vapour (Singh & Agrawal, 2008) in the atmosphere and are oxidized and hydrolyzed to form sulphuric and nitric acid (Likens et al., 1979). More specifically, SO_2 reacts with ozone or hydrogen peroxide to form sulphur trioxide (SO_3), which reacts with water to form sulphuric acid (H_2SO_4), while nitrogen dioxide (NO_2) reacts with hydroxide (OH) to form nitric acid (HNO_3) (Shammas et al., 2020). When these acids accumulate in large enough quantities, they will cause the pH of precipitation to decrease below 5.6, constituting acid precipitation (Likens et al, 1979). Additionally, acid deposition could occur via gaseous and dry particulate deposition, along with any type of wet precipitation (Shammas et al., 2020).

Soil acidification occurs when prolonged acid deposition causes forest soils to be unable to buffer incoming acids (Driscoll & Wang, 2019) because the acidic deposition increases the concentration of sulphate (SO_4), nitrate (NO_3) and hydrogen (H^+) ions in soil (Driscoll et al., 2001). The H^+ ions and SO_4 are readily adsorbed into soil, but once saturation is reached, excess SO_4 is leached from the soil along with base cations due to charge balance (Adams et al., 2006). As the acidic precipitation moves through the soil, these compounds interact with the soil, causing a reaction in which nutrient cations (positively charged nutrient ions) separate and leach out of the soil (Driscoll et al., 2001; Tomlinson, 2003). This reaction occurs because the base cations exchange with H^+ ions that bind more tightly to the soil, causing the water moving through the soil to maintain a lower acidity than the surrounding soil (Hedin & Likens, 1996).

Simultaneously, trace metals become increasingly mobilized and available for uptake by the vegetation, which can result in negative growth effects (Watmough, 2002; Juice et al., 2006; Singh & Agrawal, 2008). For example, acidification of soil increases aluminum (Al) mobilization into soil water, along with a shift from organic forms to toxic inorganic forms (Driscoll & Wang, 2019). It is also worth noting that mobilization of Al can also cause accelerated base cation loss (Hedin & Likens, 1996). Nitrogen (N), which is typically limiting in forest systems, can contribute to acidification in other ways (Ok et al., 2008). Specifically, NO_3 is poorly adsorbed in soils (Adams et al., 2006), and elevated deposition can lead to N saturation, and NO_3 is leached out of the soil. During this, the presence of H^+ ions will replace base cations, which will instead leach out with the NO_3 (Likens et al., 1996; Ok et al., 2008). Additionally, processes such as the biological uptake of ammonium NH_4 and nitrification have also been shown to result in the release of additional H^+ ions within the soil rooting zone (Vitousek et al., 1997). For example, plant roots placed in ammonium chloride (NH_4Cl) solution will uptake ammonium ions in their roots, subsequently releasing H^+ ions from those roots and reducing the pH of the NH_4Cl solution (Becking, 1956).

These processes cumulatively resulted in the significant acidification of forest soils across the northeastern United States (Warby et al., 2009; Fenn et al., 2006), Ontario, and Quebec. In central Ontario, for example, soil pH values below 4.4 have been regularly reported (Gradowski & Thomas, 2006; Casson et al., 2012). Base cation concentrations have also been shown to be depleted in northeastern North America forest soils, and Al has been mobilized (Schaberg & Hawley, 2010). Concentrations of other metals, such as manganese (Mn) can also increase in soil under acidic conditions (Watmough et al., 2007). Timber harvesting further contributes to nutrient depletion as the removal of large amounts of forest biomass during harvesting results in the simultaneous removal of large amounts of nutrients such as calcium (Watmough et al., 2003).

Calcium depletion and increased Al mobility in soils have been linked to lower plant photosynthesis and respiration, which can impact carbon sequestration in the affected trees (Schaberg & Hawley, 2010). Soil acidification and the increased concentrations of Al and Mn in soil solution can also negatively impact plant growth (Bolan et al., 2023; Singh & Agrawal, 2008). Historical evidence of sugar maple (*Acer saccharum* Marsh.) dieback in the 1950's was attributed to acidic deposition and base cation loss (Driscoll et al., 2001). However, sugar maple does not appear to be showing signs of recovery following decreases in acid deposition (Driscoll & Wang, 2019). The lack of recovery could be due to its sensitivity to calcium (Ca) losses (Huggett et al., 2007; Halman et al., 2013). Additionally, ecosystem services such as soil and water quality, and plant diversity have also been identified as being adversely impacted by N and sulphur (S) deposition (Aherne & Posh, 2013). Despite this, broader signs of ecosystem recovery have begun to emerge in soils (Lawrence et al., 2015; Hazlett et al., 2020), waters (McHale et al., 2017; Watmough & Eimers, 2020), and some biota (Thomas et al., 2013). The signs of recovery can be attributed to reductions in SO_2 emissions that began in the 1970's (Driscoll et al., 2001) with declines in emissions being seen in Europe (Vesteng et al., 2007), and North America, however reductions in east Asia have not been as significant (Aas et al., 2019). Reductions in SO_2 and to a lesser extent

NO_2 emissions can be attributed to the implementation of environmental policy such as the 1970 U.S Clean Air Act (McKitrick, 2007) and the 1990 Clean Air Act Amendments (Butler et al., 2001; LaCount et al., 2021), including the Acid Rain cap- and trade- program (Ross et al., 2012). Canada also passed its own Clean Air Act in 1971 (Powell & Wharton, 1982). Reductions in NO_x have been much more recent, but evidence of NO_3 deposition is evident in the United States (Strock et al., 2014; Feng et al., 2020) and Canada (Feng et al., 2020; Watmough & Eimers, 2020). However, despite these signs of recovery and emission reductions, rates of recovery are slow, and modeling predicts soil recovery may take decades or centuries (Ott & Watmough, 2022). The slow rate of recovery is because Ca outputs continue to surpass natural Ca inputs or because natural replenishment of soil base cations through weathering is inherently slow (Huntington, 2000). Furthermore, the continued harvesting of these deciduous forests is only expected to further delay forest recovery (Ott & Watmough, 2022). Thus, researchers are looking for remediation strategies.

1.2 Soil amendments

To combat nutrient loss from acidic soils and assist natural recovery, researchers have tested various remediation strategies, such as the application of alkaline soil amendments. Generally, soil amendments are materials that alter the biological, chemical, or physical characteristics of the soil. In this instance, researchers have been looking at the use of Ca-rich amendments (liming) to help increase Ca in these impacted ecosystems, as these amendments can increase soil pH and base cation concentrations. Liming materials can also enhance biological activities in acidic soils including mineralization of N, phosphorus (P), and promote N fixation (Bolan, 2023). One such common Ca amendment is lime (Pabian et al., 2012; Goulding, 2016). Liming agents include lime (in various forms such as limestone/chalk ($CaCO_3$), dolomitic lime ($CaCO_3 \cdot MgCO_3$), burnt lime (CaO), and slaked lime ($Ca(OH)_2$)). The effectiveness of lime, however, is generally based on its rate of dissolution (Goulding, 2016). Lime has been readily used in Europe and shown to increase soil pH, increase base cation concentrations (Lundström, 2003; Homan et al., 2016), decrease Al concentrations (Geibe et al., 2003; Homan et al., 2016), and enhance microbial activity (Fuentes et al., 2006). However, negative effects of liming have included increased dissolved organic carbon and NO_3 soil (Lundström, 2003) and creating potentially more suitable environments for invasive earthworms (Homan et al., 2016). Other alkaline amendments include gypsum and phosphogypsum ($CaSO_4 \cdot 2H_2O$) (Goulding, 2016). Results of gypsum use as a soil amendment are similarly positive on soil properties and crops (Shruthi et al., 2024), and phosphogypsum has been shown to increase Ca availability for plants and decrease Al concentrations (Alva & Sumner, 1990).

Another example of an alkaline soil amendment is wollastonite ($CaSiO_3$) that was applied in 1999 at the Hubbard Brook Experimental Forest in New Hampshire, USA. This addition resulted in increased soil calcium concentrations, increased soil pH, and an improvement in the Ca/Al ratio in the treated soils (Cho et al., 2010). This ratio is an indicator of nutrient imbalance (high Al or low Ca) that can cause ecosystem stress and forest damage (Cronan & Grigal, 1995). The response by sugar maples during the Hubbard Brook experiment included increases in foliar (leaf) Ca concentrations and crown condition improvements (Juice et al., 2006). Furthermore, wollastonite additions at Hubbard Brook have shown the ability to reduce branch dieback and improve basal area growth while simultaneously improving wound closure and stress response in sugar maples (Schaberg & Hawley, 2010; Huggett et al., 2007). Increased carbon sequestration following wollastonite application has been reported both in agricultural fields (Haque et al., 2020a) and forests (Taylor et al., 2021). Carbon storage through increased biomass was also reported at Hubbard Brook (Schaberg & Hawley, 2010), along with improvements to flowering ability and seed production (Halman et al., 2013). Wollastonite, however, must be mined, meaning availability can be limited, with the material being potentially costly to procure.

Biochar is another potential soil amendment (Dai et al., 2017). Broadly, biochar is intentionally produced high-carbon char from the pyrolysis of plant-material feedstock for use as a soil amendment (Sohi et al., 2010). This material has been found to increase soil pH (Dai et al., 2017; Zhang et al., 2025), nutrient concentrations including K and P, water holding capacity (Piccolo et al., 2022), decrease Al concentrations, and potentially increase nitrification (Dai et al., 2017). Biochar also enhances soil microbial abundance, activity and diversity, leading to enhanced biochemical cycling, improved soil structure, and more (Palansooriya et al., 2019). This amendment has also been found to enhance early-stage seedling development (Thomas, 2021) and physiological and reproductive performance of certain pioneer species (Gale et al., 2017). Biochar is also capable of aiding denitrification and reducing N_2O emissions (Cayuela et al., 2013). However, biochar can increase carbon dioxide (CO_2) emissions after application in acidic soils (Sheng et al., 2016), as can calcite and dolomite (Bolan et al., 2023).

1.3 Wood ash as a soil amendment

Wood ash is another amendment highly regarded for its ability to treat forest soils impacted by acid deposition and harvesting, and its use is well documented in Europe and North America (Demeyer et al., 2001; Pitman, 2006). Generally, wood ash can be subdivided into two groups, industrial wood ash and non-industrial wood ash (NIWA). Industrial wood ash is produced from industrial processes, such as bottom and fly ash. Alternatively, NIWA is the ash produced from residential burning in household woodburning stoves, fireplaces and firepits. It is worth noting that wood ash is different from biochar in that biochar is produced from pyrolysis (low oxygen) while wood ash is formed through complete incineration at a higher temperature (Reed et al., 2017). Biochar also tends to have a higher carbon content than wood ash. Broadly, wood ash is a promising soil amendment given that it has been found to increase soil pH (Huotari et al., 2015; Deighton & Watmough, 2020), increase nutrient cations such as Ca and magnesium (Mg) (Saarsalmi et al., 2001; Brunner et al., 2004), and contain nutrients that are required by plants, such as P and potassium (K) (Azan et al., 2019). Additionally, wood ash can improve mature tree growth (Reid & Watmough, 2014; Arseneau et al., 2021). Wood ash has also been shown to cause minimal changes in arthropod (Smenderovac et al., 2022), bacterial, and fungal communities (Smenderovac et al., 2022; Smith et al., 2024). Interestingly, certain types of wood ash have also been shown to increase Eastern red-backed salamander (*Plethodon cinereus* Green.) abundance, while not limiting salamander movement, owing to changes in soil conditions (Gorgolewski et al., 2016). However, knowledge gaps still exist, and much is still unknown about its use. For example, the NIWA application may potentially increase the heavy metal concentrations in nearby vegetation (Deighton et al., 2021). Results of several studies suggest that concentrations of metals such as boron (B), copper (Cu), Mn, and zinc (Zn) increase following ash addition (Saarsalmi et al., 2004; Ozolinčius & Varnagirytė, 2005; Hansen et al., 2018; Deighton & Watmough, 2020), owing to their presence in the ash, though some metals also see increased availability with increasing soil pH. In connection to this, the potential impacts of these trace heavy metals present are also understudied. Because of this, the use of wood ash as a soil amendment in Canada remains restricted (Kim et al., 2022), meaning there are barriers to its use, including extensive regulatory approvals, and these approvals widely vary between provinces (Hannam et al., 2016).

1.4 Community composition and seedling response

Biodiversity, the diversity of living things, is critical for the plethora of significant benefits it provides (Alho, 2008; Rathore & Jasrai, 2013; Rawat & Agarwal, 2015; Sufiyan, 2022; Singh, 2024), including ecosystem services (Rawat & Agarwal, 2015; Singh, 2024), such as soil formation and maintenance (Rathore & Jasrai, 2013). However, biodiversity is in decline, with extinctions happening at 10 – 100 times the baseline rate (WHO, 2025), with a multitude of biodiversity threats being listed (Daszak et al., 2000; Altizer et al., 2003; Tilman et al., 2017; Bellard et al., 2022). A decline in biodiversity can reduce the function and productivity of ecosystems, including how they cycle nutrients, along with reducing ecosystem stability (Cardinale et al., 2012), and carbon sequestration (Weiskopf et al., 2024). Additionally, organic and inorganic pollutants have emerged as one of the most significant factors in biodiversity loss in many ecosystems (Rawat & Agarwal, 2015). Pollutants can alter vegetation and the environment, causing species to adapt to these changes caused by environmental pollutants (Singh, 2024). For instance, forest losses occur not just through direct destruction but through degradation resulting from human activity (Leuschner & Homeier, 2022). Examples of such adverse pollutants include persistent organic pollutants such as dichlorodiphenyltrichloroethane, polychlorobiphenyls, and dioxins (Finizio et al., 1998). Likewise, other pollutants of concern include SO_2 and NO_x produced from sources such as the burning of fossil fuels. These pollutants have been shown to impact vegetation and tree health and cause forest decline (Singh, 2024). Broadly, both S and N-based air pollutants have been shown to impact biodiversity and the function of ecosystems (Lovett et al., 2009) and have shown to influence forest vegetation communities (van Dobben &Vries, 2010). Specifically, SO_2 has been shown to impact biodiversity (Bhuiyan et al., 2018), through means such as soil acidification (Ataei et al., 2023) and similarly, NO_x has been shown to influence biodiversity (Ariño et al., 2000) and NO_x emissions have been identified as a significant risk to biodiversity (Badeck & Sterzel, 2010). For example, studies in Ontario found negative effects on lichen species richness with increasing N exposure (McDonough & Watmough, 2015) or a negative correlation with both S and N pollution and lichen richness (Miller & Watmough, 2009).

The response of plants and plant communities to soil amendments is far more variable than soil responses. Specifically, lime has been found to increase agricultural crop yields (Athanase et al., 2013), while potential benefits have been found for biodiversity (Goulding, 2016), though findings are mixed (Holland et al., 2018). Wollastonite has been found to increase seedling density and increase seedling survivorship with larger and healthier seedlings (Juice et al., 2006; Cleavitt et al., 2011) while also improving crop growth (Haque et al., 2020b) and yield in certain species

(Wang et al., 2024). Effects of biochar are mixed, with studies finding increases and decreases in the cover of various species, while ultimately not affecting species richness (Bieser & Thomas, 2019; Williams & Thomas, 2023). It was also found that biochar generally results in increased tree growth following application (Thomas & Gale, 2015; Piccolo et al., 2022) and that it can also potentially enhance early-stage seedling development (Thomas, 2021), and in certain situations improve tree survival (Williams & Thomas, 2024), and seedling survival (Muraro et al., 2025).

There have been far fewer studies on the effects of wood ash on understory plants and plant communities. Wood ash has been found to potentially improve seedling growth (Staples & Van Rees, 2001; Augusto et al., 2008; Muraro et al., 2025), while other studies have found no growth effects (Arseneau et al., 2021; Deighton et al., 2021) or inconsistent growth effects (Noyce et al., 2017) on seedlings. However, to date, there are no known studies on wood ash addition and sugar maple seedling survival, however studies on a coniferous species found no seedling survival on un-leached wood ash (Thomas & Wein, 1990), while a study on poplar species found increased survival following low-dose ash application (Muraro et al., 2025). Studies on understory plant communities are far more variable; however, as some suggest no effect, or very minimal impact to ash addition (Andreas Aronsson & Ekelund, 2004; Pitman, 2006; Agusto et al., 2008). Some studies have also suggested increases in community composition following ash addition (Arvidsson et al., 2002; Merzouki et al., Submitted). These results in Canadian studies are also quite mixed (Hart et al., 2019; Merzouki et al., Submitted). Additionally, negative effects have been seen on non-vascular plants such as bryophytes

(Ozolinčius et al., 2007b; Økland et al., 2022; Augusto et al., 2008) and lichen (Økland et al., 2022), raising uncertainties surrounding the effects of ash on plant communities.

1.5 Current research and the Friends of the Muskoka Watershed

In Canada, ASHNET is a federal program exploring wood ash application on forest soils in Canada (NRC, 2025a) and has led to several successful studies (NRC, 2025b) such as Arseneau et al. (2019), Deighton et al. (2021) and Kim et al. (2022). Alternatively, the Friends of the Muskoka Watershed is a not-for-profit charity group based out of Muskoka, Ontario that focuses on identifying and developing solutions to science-based threats, while working with policymakers to implement these solutions (FMW, 2025a). As a part of these efforts, one of their projects, entitled "HATSEO" looked to understand the reduction in soil Ca in the Muskoka region. This project then led to the "ASHMUSKOKA" project that looked to address the problem of soil and lake acidification (and corresponding calcium depletion) in the region through the application of NIWA and to illustrate that this wood ash is safe for use in these ecosystems (FMW, 2025b). This program has led to several studies on the topic (Syeda et al., 2024; Conquer et al., 2024; Conquer et al., 2025). This thesis is one of the two latest studies to come out of the ASHMUSKOKA project, with the other thesis looking at the above and below ground response to NIWA addition (Foley, 2025).

1.6 Objectives and hypothesis

The broad objective of this thesis is to assess the viability of NIWA as a soil amendment and the effects on ecosystem regeneration by looking at the short-term effects on soil, sugar maple seedling growth, survival, and understory vegetation community composition following wood ash addition in the nutrient-depleted Muskoka region of central Ontario. The subsequent findings are presented in two research chapters. The first chapter (Chapter 2) looks at understory vegetation community response to wood ash addition (0, 4, 8 Mg ha^{-1}) up to four years following application at three central Ontario sugarbush sites. This research was done by assessing a) species abundance, b) species richness, and c) species diversity through Shannon's (H) and Simpson's (D) diversity indices. Additionally, d) soil pH was also measured to represent soil chemistry changes within these sites. The second chapter (Chapter 3) looks at short-term (two-year) soil chemistry response and the short-term (two-year) growth and survival response of sugar maple seedlings at a sugar maple-dominated forest in Bracebridge, Ontario, following wood ash addition (0, 2, 4, 6, 12 Mg ha^{-1}). This research was completed by assessing a) soil chemistry (including pH, OM, nutrient concentrations, and metal concentrations), b) sugar maple seedling height and diameter growth for two separate cohorts, and c) sugar maple seedling survivorship for two separate cohorts.

The objective of Chapter 2 was to determine if the application of wood ash had any positive or negative effects on understory vascular plant communities within four years of application. It was hypothesised that wood ash addition would positively increase soil pH and increase species richness and diversity within the vascular plant communities following ash addition. The objective of Chapter 3 was to evaluate the short-term responses of wood ash addition to soil chemistry and sugar maple seedling performance. It was hypothesized that wood ash addition would increase pH, nutrient availability (Ca, Mg, K), and heavy metal concentrations (Al, B, Cu, iron (Fe), Mn, nickel (Ni), Zn) in soils. It was also hypothesized that wood ash would increase the growth and survivorship of sugar maple seedlings following application.

1.7 Research significance

Forests are considered critical to biodiversity preservation (Leuschner & Homeier, 2022), and ensuring they remain healthy is important. Decades of acid deposition and soil acidification are adversely impacting these ecosystems, and as such, methods of restoration are required. Non-industrial wood ash is an attractive option for soil amending given the available quantities, as in the Muskoka region alone, an estimated 235,000 kg of ash is produced every year (Azan et al., 2019). This number grows to 18,000,000 kg for all of Ontario (Azan, 2017). Diverting this ash from landfills to land restoration projects creates an opportunity to reduce inputs to aging landfills while simultaneously improving forest health in Canada in the wake of forest harvesting and long-term soil acidification. Given the abundance of wood ash available, there may also be economic benefits associated with its use compared to other Ca-rich amendments. Previous research surrounding wood ash use internationally has been promising. Studies in Europe have shown a multitude of benefits, however, there are concerns over heavy metals and trace substances. For example, ash samples from Conquer et al. (2025) found several metals to exceed the Canadian non-agricultural source material (NASM) concentration of regulated metal (CM) guidelines, which could negatively affect the ecosystems in which the ash is applied. Likewise, several European studies have shown negative effects on non-vascular plants (Ozolinčius et al., 2007b; Økland et al., 2022; Augusto et al., 2008). Overall, significant knowledge gaps still exist regarding the use of wood ash as a soil amendment for restoring acidified forests in Canada. Broadly, the impacts of wood ash application on forest ecosystems, especially their ability to regenerate, have not been fully determined. This material needs to be thoroughly studied in Canada before it can be safely implemented.

Ultimately, this research aims to determine the effects of wood ash application on soil chemistry along with ecosystem regeneration by looking at understory vegetation communities. This study will address under-researched areas in the literature by being one of only several (Hart et al., 2019; Merzouki et al., Submitted) to investigate the effects of wood ash on understory plants in Canada. Similarly, to date, there has been a limited number of studies that have explored how wood ash affects sugar maple seedling performance (Deighton et al., 2021; Arseneau et al., 2021), while this study will most likely be the first globally to investigate the effects of wood ash on sugar maple regeneration. By addressing these knowledge gaps, this study can contribute to policy changes and the potential deregulation of ash as a soil amendment in Canada. In turn, contributing to the remediation of soils and the soil health in acidified forest soils across Canada.

2.0 Understory vegetation community response to non-industrial wood ash application

2.1 Abstract

Wood ash is a common soil amendment used for counteracting the effects of acid deposition and soil acidification. However, in Canada, its use remains restricted due to uncertainties and environmental concerns. This study looked to evaluate the effects of non-industrial wood ash application (0, 4 and 8 Mg ha^{-1}) on understory vegetation communities at three sugarbush sites in Muskoka, Ontario, over four years. The addition of wood ash resulted in large, but transient increases in soil pH in the litter layer of each site, while a large and prolonged increase in pH was seen in the fibric humic (FH) layer at each site. Smaller but pronounced increases in pH were also seen in the upper 0 – 10 cm mineral soils. Understory species abundance, richness and diversity were largely variable between sites but there was no consistent treatment effect up to four years after wood ash application. Local site characteristics appeared to heavily drive community composition and presence of sugar maple (*Acer saccharum* Marsh.) was found to not be responsible for treatment differences between sites, despite contributing the highest level of dissimilarity between sites. Based on these findings, it appears that non-industrial wood ash, when applied at dosages up to 8 Mg ha^{-1}, does not affect understory plant communities in acidified forests up to four years after wood ash application and therefore may not be suitable for the restoration of these communities. These findings however, suggest that NIWA is suitable for use to restore soils impacted by decades of acid deposition and soil acidification.

2.2 Introduction

Decades of elevated sulphur (S) and nitrogen (N) deposition in conjunction with timber harvesting resulted in the acidification of forest soil across northeastern North America (Fenn et al., 2006; Warby et al., 2009), including Ontario, where soil pH values below 4 have regularly been reported on the Precambrian Shield (Deighton & Watmough, 2020; Conquer et al., 2024). Acidic deposition led to the loss of base cations such as calcium (Ca) and mobilization of aluminum (Al), which can negatively impact vegetation (Sverdrup & Warfvinge, 1993; Driscoll et al., 2001). Sugar maple (*Acer saccharum* Marsh.), a dominant species within these forests, is especially sensitive to Ca losses (Huggett et al., 2007; Halman et al., 2013). Understory plant communities have also been shown to be adversely affected by soil acidification (Baeten et al., 2009; McDonough & Watmough, 2015; Jung et al., 2018; Zafros et al., 2019), as acidification alters the availability of essential nutrients required by plants (Gilliam et al., 2019). Specifically, species diversity, richness, and occurrence can all be changed by soil acidification, which can affect ecosystem structure and function (Yadav et al., 2020). This is a concern as the herbaceous layer in the understory also drives ecosystem succession by filtering future tree composition (Nilsson & Wardle, 2005) and influencing early seedling competition of overstory species (Gilliam et al., 1994). Understory vegetation can also impact other biota by directly affecting soil microbial and microfauna composition (Mitchell et al., 2012) and providing habitat for pollinators (Landuyt et al., 2019) and birds (Rodewald & Yahner, 2001). Understory vegetation can also drive soil fertility (Nilsson & Wardle, 2005), nutrient cycling, evapotranspiration (Landuyt et al., 2019) and may even contribute to soil acidification recovery (Lawrence et al., 2012).

Significant reductions have been seen in sulphur dioxide (SO_2), and more recently nitrogen oxide (NO_x) emissions resulting in large decreases in sulphate (SO_4) and nitrate (NO_3) deposition in the United States (Strock et al., 2014; Feng et al., 2020), and Canada (Feng et al., 2020; Watmough & Eimers, 2020). As a result, affected ecosystems are beginning to show signs of recovery including increasing soil pH (Lawrence et al., 2012; Lawrence et al., 2015; Hazlett et al., 2020), reductions in soil Al concentrations (Lawrence et al., 2015), and improvements to forest stream water chemistry (McHale et al., 2017) and lakes (Watmough & Eimers, 2020). However, modelling suggests that soil base cation saturation may take decades, if not centuries, to fully recover to pre-disturbance 1900 levels, even with increased reductions to acid deposition (Ott & Watmough, 2022).

To assist and accelerate natural recovery, researchers globally are looking at Ca-rich soil amendments to increase soil pH and reintroduce base cations, with wood ash being presented as a viable option. Studies to date have shown that wood ash can increase soil pH (Deighton & Watmough, 2020), and improve tree growth (Reid & Watmough, 2014; Arseneau et al., 2021), without causing significant changes in arthropod (Smenderovac et al., 2022), bacterial, and fungal communities (Smenderovac et al., 2022; Smith et al., 2024). Wood ash also contains nutrients such as phosphorus (P) and potassium (K) that are required by plants (Azan et al., 2019), and studies have shown growth of plants improves from the nutrients in wood ash (Demeyer et al., 2001). Changes in soil pH can also alter nutrient availability for plants by altering both the sorption of nutrients by soils and the ability of plants to uptake these nutrients (Barrow & Hartemink, 2023). For instance, increasing soil pH reduces the availability of toxic Al

(Andreas Aronsson & Ekelind, 2004), which has been linked to physiological and morphological issues including root growth (Rout et al., 2001). Additionally, increasing soil pH influences other biogeochemical processes, including increasing nitrification and denitrification (Neina, 2019), which can impact N availability and plant growth (Johansen et al., 2021). Simultaneously however, wood ash also contains variable concentrations of heavy metals such as lead (Pb), chromium (Cr) and nickel (Ni) that may negatively affect biota (Azan et al., 2019; Deighton & Watmough, 2020; Deighton et al., 2021). Because of uncertainties over high heavy metal content, wood ash is currently restricted for use in Canada (Kim et al., 2022). Despite these concerns, studies in Europe on *Vaccinium* species have found little to no risk of heavy metal uptake following wood ash addition, despite the ash and surrounding soil (following application) containing several heavy metals, including cadmium (Cd), Cr and zinc (Zn) (Moilanen et al., 2006; Norström et al., 2012; Lazarenko et al., 2020). Additionally, it has been suggested that species composition and richness in North American hardwood forests may be positively associated with increased pH and base cation concentrations (Zarfos et al., 2019), indicative of potential positive responses to wood ash application.

Knowledge gaps remain regarding the effects of wood ash applications in Canadian forest ecosystems. Specifically, it is not fully known if the use of wood ash as a soil amendment has any positive or negative effects on understory vegetation communities in acidified forests. Research on the subject remains limited in Canada (Hart et al., 2019; Merzouki et al., Submitted) and the results globally appear conflicting with some studies showing adverse effects while others suggest minimal impact of wood ash on forest understory (Andreas Aronsson & Ekelund, 2004; Pitman, 2006; Agusto et al., 2008). Comparably however, similar amendments such as biochar have been shown to aid the physiological and reproductive ability of some early-successional species (Gale et al., 2017).

This study set out to assess soil chemical and understory vegetation community responses to a one-time wood ash application of 4 or 8 Mg ha^{-1} as three sugar bush sites in Central Ontario. It was hypothesized that the wood ash addition would result in increases in soil pH. It was also hypothesized that the addition of wood ash would result in significant increases in species richness and diversity of vascular understory communities.

2.3 Methods

2.3.1 Study sites

Three study sites (Brooks, Marks and Wilfs) located in the Muskoka region of Central Ontario, were included in the study and are located roughly 200 km northeast of Toronto, Ontario (Figure 2.1).

Figure 2.1 Map showing the location of the study sites, Brooks, Marks and Wilfs sugarbushes in Muskoka, Ontario, Canada, in proximity to Toronto, Ontario, Canada.

Each of the sites is either a functional (Brooks and Marks) or a retired sugarbush (Wilfs) characterized by sugar maple-dominated deciduous forests (> 90 % basal area). The first site, Brooklands Farm (45°04'56.2"N, 79°27'51.8"W), henceforth referred to as ‘Brooks’, is a 60–acre sugarbush located west of the town of Bracebridge. This site exhibited uneven topography with steep slopes. The forest was dominated by sugar maple but also contained black cherry (*Prunus serotina* Ehrh.), red oak (*Quercus rubra* L.), and American beech (*Fagus grandifolia* Ehrh.). The site sits at roughly 255 – 275 m above sea level. The second site, Wilfrid Creasor Sugarbush (45°13'02.8"N, 79°26'48.1"W**),** henceforth referred to as ‘Wilf’s’, is an 83–acre deciduous forest located west of Port Sydney, Ontario. This site exhibited gently undulating topography with some minor slopes. The site is sugar maple dominated, while exhibiting other deciduous species including black cherry, red maple (*Acer rubrum* L.), and American beech. The site sits about 305 – 320 m above sea level. The third site, Marks Muskoka Maple Sugarbush (45°16'47.0"N, 79°08'51.5"W), henceforth referred to as ‘Mark’s’, is a 49–acre deciduous forest located southeast of Huntsville, Ontario. This site exhibits gently undulating topography with minimal slopes. The site is dominated by sugar maples, with minimal other canopy species outside of American beech. The site sits about 355 – 375 m above sea level. Regionally, based on climate averages between 1991 – 2020 the annual average temperature of the site is 5.3 °C with mean daily averages between -10.0 °C (January) and 19.1 °C (July), and total annual average precipitation of 1062 mm, split between 689 mm of rainfall and 373 mm of snowfall (ECCC, 2025). The site sits on the southern tip of the Precambrian Shield, which possesses generally hard rock including granite and gneiss. The sites were found to have generally shallow Brunisolic and acidic podzol soils that are typically coarse-textured sandy loams (Soil Classification Working Group, 1998). The average pH of the organic soils was found to be between 3.3 – 5.09, while the mineral soils were around 3.33 – 4.96. The selection of three sites was based on a call for volunteers in the region with the intention of selecting sites spread across the Muskoka region to capture the effects of site variability in vascular community composition response.

2.3.2 Plot Setup, experimental design, and ash application

In August 2019, eighteen 10 x 10 m plots were randomly established across each of the three study sites. The criteria for plot location included relatively flat topography with at least two mature sugar maples (> 10 cm diameter at breast height (DBH)) and seedlings present, with a buffer zone of at least 10 m between plots. Following this, in October 2019, non-industrial wood ash (NIWA) was applied to the sites in three treatments (0, 4, or 8 Mg ha^{-1}) so that six replicates were produced per treatment dosage. Due to shortages in available ash at Brooks site, only seventeen plots were treated, with only five plots receiving 8 Mg ha^{-1} wood ash application instead of six.

The ash used in this study was collected and processed by the Friends of the Muskoka Watershed charity from residents and small businesses in the Muskoka region over several ash drives (FMW, 2025b). Once collected, the ash was sieved down to < 2 mm to remove coarse char and any unwanted items (nails, bottle caps) and stored in galvanized metal bins. Prior to application, the ash was homogenized using a large cement mixer before being distributed into several containers to facilitate transport to the sugarbush sites. In a questionnaire regarding the ash donated, residents indicated that predominantly bark (70.2 %) and trunk wood (85.1 %) were burned. Residents also indicated that predominantly hardwoods were burned, including maple (70.2 %), birch (51.1 %) and oak (27.7 %), while softwoods such as pine, spruce, and hemlock only made up about 25 % of what was burned (Syeda et al., 2024). The ash applied was highly variable, with Marks having a significantly lower pH than Brooks and Wilfs and significant differences in concentrations of Pb, copper (Cu) and iron (Fe) (Table 2.1, adapted from Conquer et al., 2025). Ash from all three sites exceeded the concentration of regulated metals (CM1) (Nutrient and Management Act, 2002) limits for Cu, while only Brooks and Wilfs exceeded limits for Zn, meaning the ash is suitable for land spreading, but under certain restrictions.

Table 2.1 The pH ($CaCl_2$), organic matter, nutrient and metal concentrations from samples of the NIWA collected in Muskoka, Ontario. Values are represented as means (±SE). Included are the CM1 and CM2 guidelines per the Nutrient and Management Act, 2002. Samples were reported as dry weight by mass. Table adapted from Conquer et al., 2025.

Properties pertaining to Sugarbush Non-Industrial Wood Ash Chemistry				Non-Agricultural Source Material Limits†	
	Brooks	Marks	Wilfs	CM 1	CM2
pH	13.5	11.5	13.3		
OM (%)	3.5 (0.8)	5.6 (1)	5.8 (1)		
C (%)	11.6 (4)	9.1 (0.9)	8.8 (0.8)		
N (%)	0.2 (0.2)	0.1 (NA)	0.1 (NA)		
Ca ($g.kg^{-1}$)	305 (15.2)	294 (46.4)	273 (48.4)		
K($g.kg^{-1}$)	109 (13)	104 (20.3)	112 (21.7)		
Mg ($g.kg^{-1}$)	24.2 (2.5)	22.5 (3.8)	22.1 (3.5)		
Mn ($mg \cdot kg^{-1}$)	6306 (683)	6329 (1215)	6837 (1023)		
P ($g.kg^{-1}$)	8.8 (1.2)	7.8 (1.2)	7.9 (1.2)		
Al ($mg \cdot kg^{-1}$)	4075.9 (1031.8)	3933.07 (1261.5)	3044.6 (1019.2)		
Fe ($mg \cdot kg^{-1}$)	2793 (1150)	1872 (634)	1322 (528)		
Zn ($mg \cdot kg^{-1}$)	523 (109)	439 (61.5)	516 (151)	500	4200
Cu ($mg \cdot kg^{-1}$)	140 (41.9)	106 (15.2)	154 (92.1)	100	1700
Cd ($mg.kg^{-1}$)	2.7 (0.4)	2.6 (0.4)	2.5 (0.6)	3	34
As ($mg \cdot kg^{-1}$)	3.9 (6)	3.7 (5.7)	3.1 (7.4)	13	170
Ni ($mg \cdot kg^{-1}$)	10.5 (3)	7.9 (1.5)	8.8 (2)	62	420
Pb ($mg \cdot kg^{-1}$)	24.3 (17)	48.5 (64.2)	12.7 (3.8)	150	1100
B ($mg \cdot kg^{-1}$)	235.58 (46)	213.15 (30.17)	239.3 (49.9)		

†Government of Ontario, 2002.

Application of the NIWA was completed by pre-weighing the required dosages into 8-L buckets in the field (40 or 80 kg for a 10 x 10 m plot at 4 or 8 Mg ha^{-1}, respectively). The ash was then carried to the plots and hand-spread with large scoops from each corresponding bucket, taking care to visually ensure even application.

2.3.3 Field sampling

2.3.3.1 Soil sampling

Soil samples were collected annually in late summer starting in 2019, with a reference sample taken prior to ash application, followed by four years of samples post-application. Samples of organic layers, including the surface leaf litter (L) and fibric humic (FH) soil, were collected at each plot (n = 53) by taking five grab samples, one in each corner of the plot and the centre. The surface litter was sampled first, followed by the FH, which was peeled back, both were placed into separate sealed plastic bags. The upper Ah mineral soil (0 – 10 cm) was subsequently sampled at the same locations using an Eijkelkamp Dutch soil auger (once the organic materials had been sampled to allow access to the mineral soil). Soils were placed in sealed plastic bags and transported to the laboratory, where they were stored in a fridge at 2.5 °C prior to analysis.

2.3.3.2 Vegetation surveys

After ash application, understory vegetation surveys were conducted annually in July of each year (2020 – 2023) by placing three 1 m^2 quadrats within each plot at random, with the only criterion for suitable quadrat location being that the quadrat had at least one vascular plant within it. The quadrats were then surveyed to determine the species composition and abundance. Vascular species under 2 m tall and less than 10 cm

DBH were included in the survey and were identified down to species level when possible, however, some species were only identified to genus, such as ash (*Fraxinus spp.*) or family, such as grasses/sedges (*Gramineae/Cyperaceae spp.*). Species growing by rhizome were counted by individual leaf above the soil surface. Bryophyte species were omitted from the study as the focus was intended to be on vascular plants (minimal bryophyte cover present).

2.3.4 Laboratory analysis

Soil samples were analyzed for pH to evaluate soil response to ash application, as other chemical variables have been described in Conquer et al. (2025). In the lab, each set of soil samples were amalgamated to make one sample per horizon per plot. Samples were then dried in an industrial oven (The Grieve Corporation, Round Lake, IL, US) for twenty-four hours at 105 °C. Afterwards, the litter and FH layers were milled using a Willey Mill (Arthur H. Thomas Corporation, Philadelphia, PA, US), while the mineral layers were sieved using a 2 mm sieve; anything larger than 2 mm was discarded. Samples were then stored in a plastic bag until analysis.

Either 1 g of organic soil (FH, litter) or 5 g of mineral soil were placed in a 50 ml conical Falcon Tube (FroggaBio Inc, Vaughan, ON, CA) with 15 ml of 0.01 M calcium chloride ($CaCl_2$) aqueous matrix. Samples were then placed on a shaker table (Eberbach Corporation, Van Buren Charter Township, MI, US) for two hours, and then left to rest for one hour. Samples were analyzed using an OAKTON pH 510 series multimeter (Oakton Instruments, Vernon Hills, Il, US). To ensure accuracy, the pH probe was calibrated every 25 samples.

2.3.5 Statistical analysis

Most statistical analyses were performed using parametric statistics, namely linear regression models. The only non-parametric tests performed were the Similarity Percentage (Simper) and Analysis of Similarities (Anosim) tests. All statistics were performed using R Statistical software version 4.2.2 (R Core Team, 2022).

2.3.5.1 Soil samples

Soil pH data was assessed for homoscedasticity using Levene's test and normality of dependent variables using both Shapiro-Wilk's test (*rstatix* package) and visually checking using a QQ plot (*ggpubr* package). Once the assumptions of normality were met, several generalized linear models (*stats* package) were fit to determine significant differences between treatments across years. Akaike Information Criterion (AIC) was used (*stats* package) to determine which model was the best fit (Akaike, 1974). Once significant differences were detected, a post-hoc test was performed with multiple means comparisons at an alpha value of 0.05 with a Bonferroni correction (*emmeans* package) to determine where the differences between treatments occurred.

2.3.5.2 Vegetation surveys

Species abundance was determined as the total frequency (counts) of observations within the six replicate plots of each treatment (for each site and year). Species that made up less than 1 % of the total observations within a treatment group for each site and year were grouped as "*Other spp.*" to be included in the general abundance figure and omitted from statistical analysis. Anosim tests were used to determine significant differences between species abundance, performed on the data that made up greater than 1 % of total treatment observations using the *Vegan* package in R. Simper (pairwise compassion) tests were also conducted to determine which species were most influential for differences between treatments using the *Vegan* package at an alpha value of 0.05. Principal component analysis (PCA) was performed on the abundance data (species frequency) that made up greater than 1 % of the total observations for a treatment group using the *factoMineR* package in R.

The species composition and abundance data were used to determine Shannon's Diversity Index (H), Simpson's Diversity Index (D), and species richness for each season of data at each separate site, performed on each replicate plot (n = 6 per treatment). Two diversity indices were used to capture the variation in diversity. Shannon's Index accounts for species richness more, while Simpson's Index accounts for species evenness more. Shannon's Diversity Index was calculated using (Eq. 2.2):

$$H = -\sum_{i=1}^{s} p_i \ln (p_i)$$

(2.2)

In this equation *s* refers to the number of species and pi to the proportion of individuals of one species across the cumulative of all individuals of all species. Simpson's Diversity Index was calculated using (Eq. 2.3):

$$D = 1 - (\sum n(n-1)/N(N-1))$$

(2.3)

In this equation *n* is the total number of individuals of a species and N is the total number of all individuals across all species. Species richness was calculated by determining the total number of species present within each plot divided by the number of surveys per plot, meaning the average number of species per 1 m^2 of area. Once the assumptions of normality were met, several Analysis of Variance (ANOVA) models were fit using H, D, and species richness data as the dependent variables. Candidate models included main effects and various combinations of two- and three- way interactions amongst year, site and dosage. Model selection was performed using Akaike Information Criterion (AIC) (*stats* package) with the model yielding the lowest AIC value considered the best-fitting model (Akaike, 1974). If the AIC values were within 2 – 3 points, the degrees of freedom (d.f.) were considered. If significant differences were detected, multiple means comparisons post-hoc tests were performed at an alpha value of 0.05 with a Bonferroni correction (*emmeans* package) to determine where the differences between treatments occurred.

2.4 Results

2.4.1 Soil pH response

Soil pH values ranged between 3.24 – 7.58 across all sites and treatments between 2019 – 2023. Before treatment (2019) soil pH was consistent within each soil horizon across all plots at the three sugarbush sites (Figure 2.2). AIC indicated that soil pH for each horizon was best modelled by the model that looked at the three-way interaction between dosage, site and year (L AIC = 47.310, d.f. =37, FH AIC = 113.815, d.f. = 37, Mineral 0 – 10 AIC = 142.220, d.f. = 37). Results of the model indicate significant treatment effect across the study sites and duration of the study (L d.f. = 2, F = 254.449, p

< 0.001, FH d.f. = 2, $F = 571.582$, $p = < 0.001$, Mineral 0 – 10 d.f. = 2, $F = 23.786$, $p < 0.001$). One year following NIWA application (2020) large, statistically significant increases in pH (2 – 3 units) were measured in the L and FH layers in the 4 and 8 Mg ha^{-1} treatments at all three sites. Only Marks exhibited a significant increase in the upper 0 – 10 cm soil horizon in the first year. Two years post-application (2021) significant increases in pH were seen across all sites and soil horizons (1 – 3 units). By 2021, the upper mineral exhibited larger increases in pH compared with the previous year in the 4 and 8 Mg ha^{-1} treatments at all three sites. Four years post application (2023) pH in the L layer had nearly returned to baseline values, but the levels in the treatment plots still differed significantly from the controls at Marks and Wilfs. The pH of the FH and mineral layers also remained significantly higher (except for the mineral soil at Brooks), but these values had also marginally decreased from 2021 (Figure 2.2).

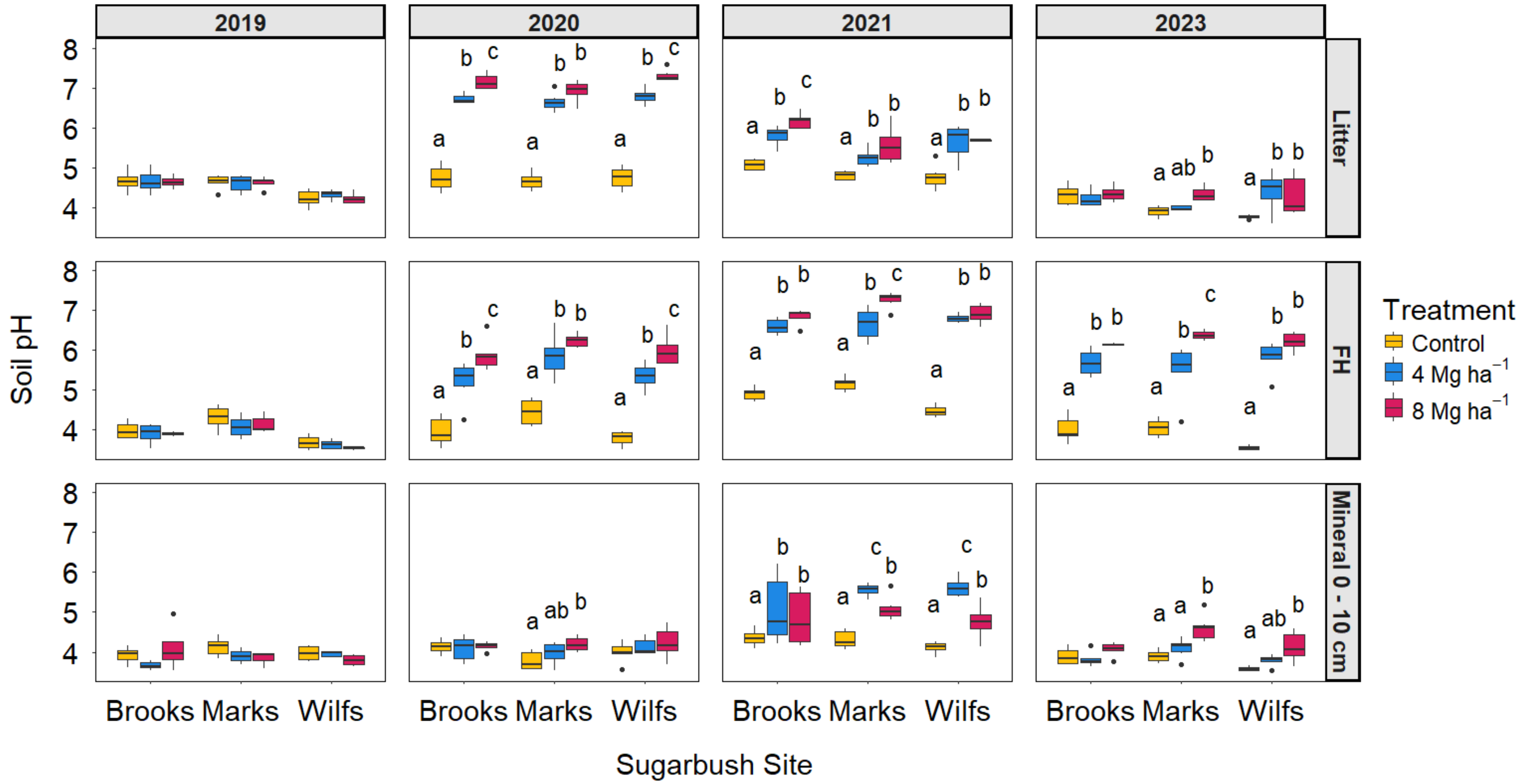

Figure 2.2 Average soil pH from 2019 – 2021, and 2023 in the L, FH, and upper mineral 0 – 10 cm soil horizons at Brooks, Marks and Wilfs sugarbushes using $CaCl_2$ matrix. Different letters indicate statistically significant differences ($p < 0.05$) using a post-hoc emmeans test between each treatment at each soil depth.

2.4.2 Understory community vegetation abundance

Across the study, 40 plant species/genus were observed among the three sites (including unclassified species; Appendix A). Species abundance was variable across sites and years within the plots (Figure 2.3).

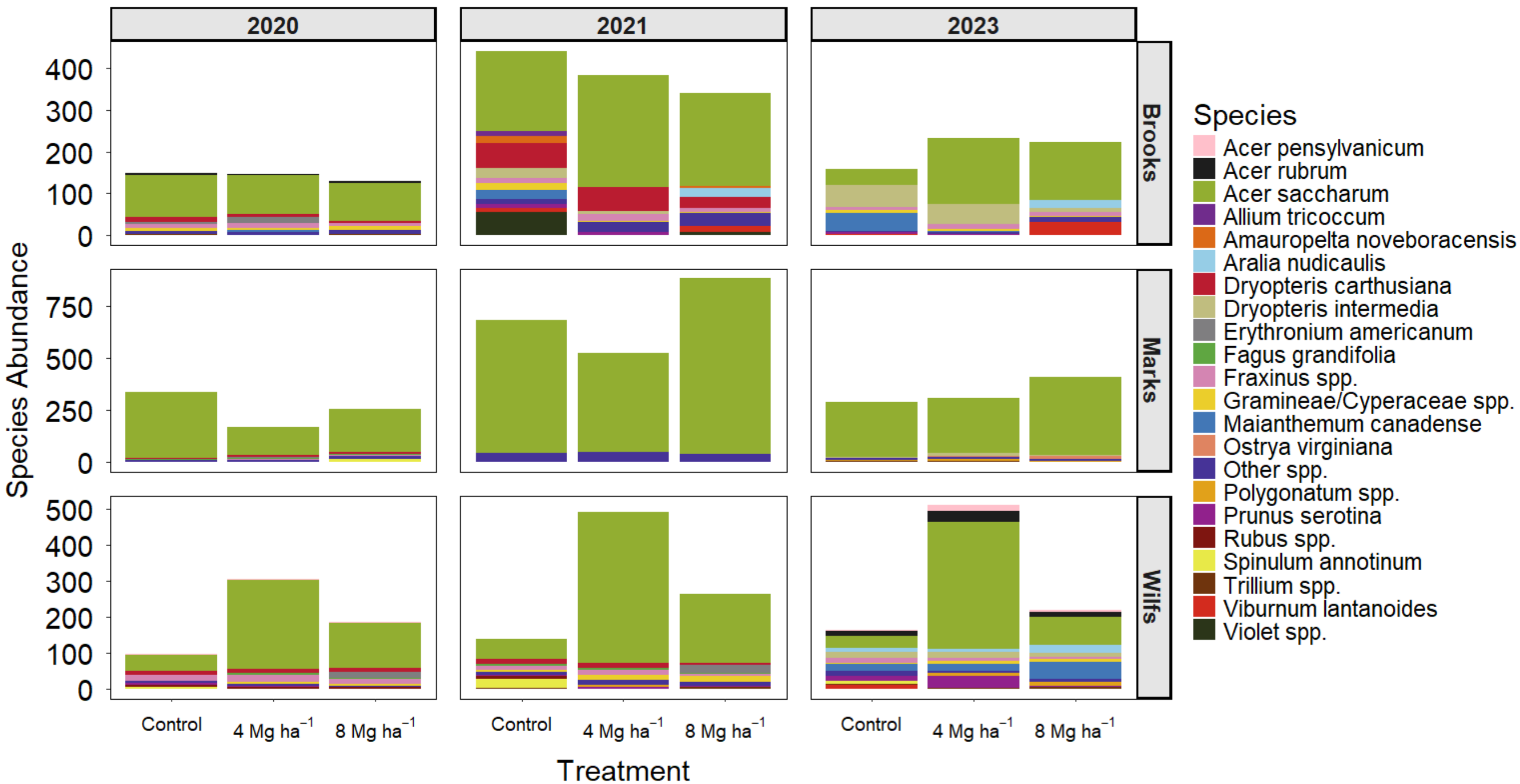

Figure 2.3 Understory vascular vegetation community composition (abundance) across 18 m^2 (15 m^2 for Brooks 8 Mg ha^{-1}) for 2020, 2021, and 2023 at Brooks, Marks and Wilfs sugar bushes. Species making up less than 1 % of each panel are listed as "*Other spp*".

Sugar maple exhibited the highest abundance and dominated the understory community composition at all three sites in all years. Simper tests indicated that sugar maple was not responsible for differences in vegetation community between treatments for each study year ($p < 0.05$), despite the largest contribution to dissimilarity (0.067 – 0.1430). Nonetheless, Anosim tests on species abundance showed no significant differences between treatments at all sugar bush sites for each year post-application (2020 $p = 0.96$, R = -0.276; 2021 $p = 0.70$, R = -0.119; 2023 $p = 0.56$, R = -0.062).

2.4.3 Understory community vegetation richness

AIC indicated that vegetation richness was best modelled by the model that looked at dosage as the main effect, with interaction between site and year (AIC = 256.509, d.f. = 12). There was no significant effect of wood ash treatment on species richness (d.f. = 2, $F = 1.616$, $p = 0.202$) but there were interaction effects between site and year (d.f. = 4, $F = 3.440$ $p < 0.001$), although Brooks tended to have higher richness at the 8 Mg ha^{-1} treatment in 2021 and 2023 (Figure 2.4). Average species richness ranged between 0.33 and 3.67 and was similar at all sites.

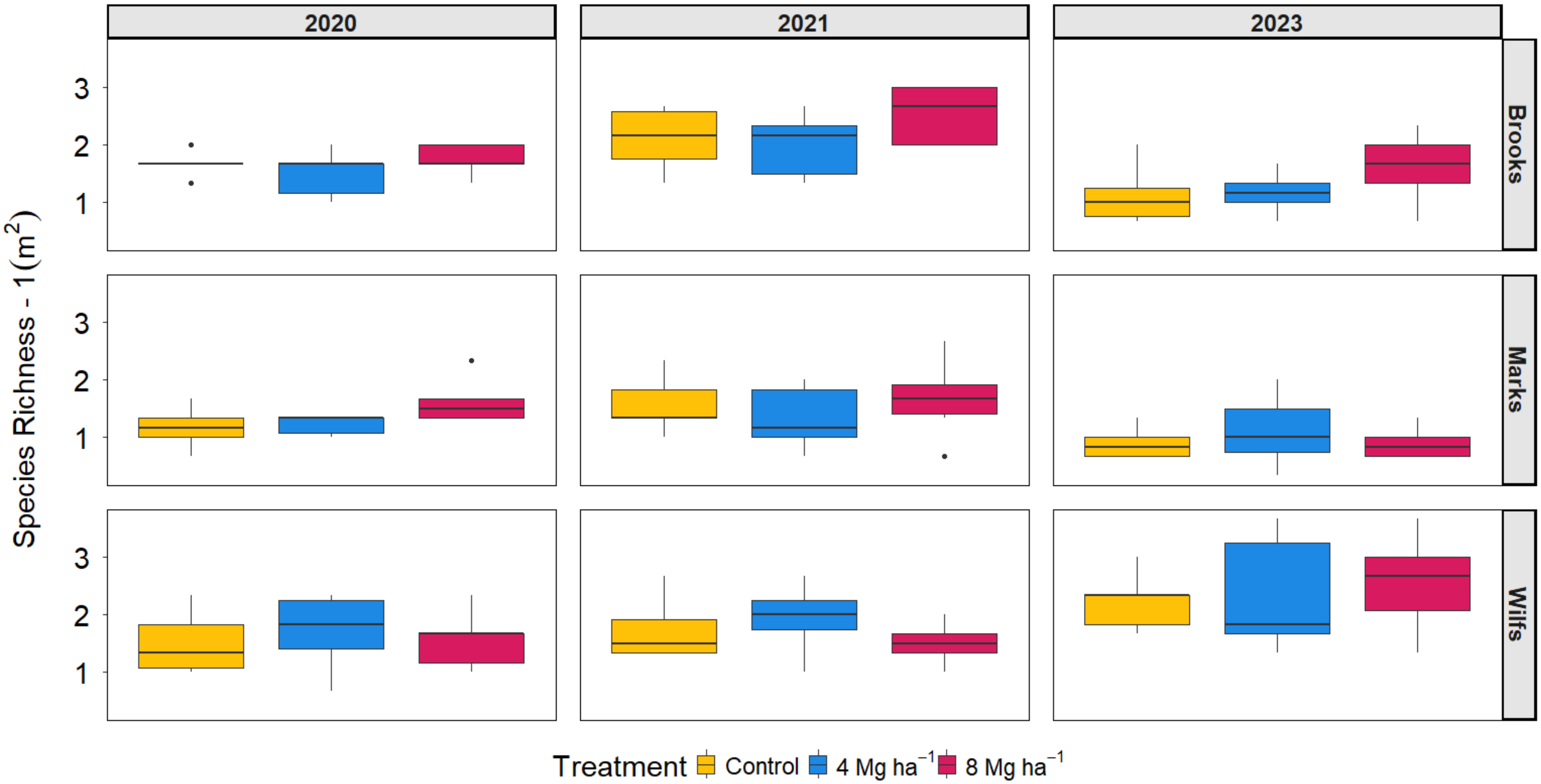

Figure 2.4 Species richness per m^2 of the three sugarbush sites Brooks, Marks, and Wilfs for the completed vegetation surveys in 2020, 2021, and 2023. Values were calculated using three 1 m^2 surveys per plot. All sites had non-significant differences between treatments

2.4.4 Understory community vegetation diversity

There were minimal and inconsistent changes in species diversity across treatments and sites. Shannon's Diversity Index (H) values were between 0 and 2.20, and AIC indicated that H was best modelled by the model that looked at dosage as the main effect, with interaction between site and year (AIC = 147.155, d.f. = 12). There was no significant effect of wood ash application on H diversity (d.f. = 2, $F = 2.110$, $p = 0.125$); however, there were interaction effects between site and year (d.f. = 4, $F = 6.732$, $p < 0.001$). Additionally, values at Marks tended to be lower than at the other two sites. Simpsons Diversity Index (S) values were between 0 and 0.95 and only showed significant differences in treatment at Wilfs across all years (df =2, $F = 3.831$, $p = 0.024$), and interaction effects between site and dosage (df = 4, $F = 3.871$, $p = 0.005$). Generally, one-year post-application (2020) Marks showed slightly increased diversity in treatments while Wilfs showed decreased diversity. Two years post-application (2021) Mark and Wilfs both showed marginal decreases in diversity in the treatment plots. Four years post-application (2023) Brooks showed marginal decreases in species diversity. Across the study duration, Marks generally exhibited lower overall diversity compared to Brooks and Wilfs (Figures 2.5 – 2.6).

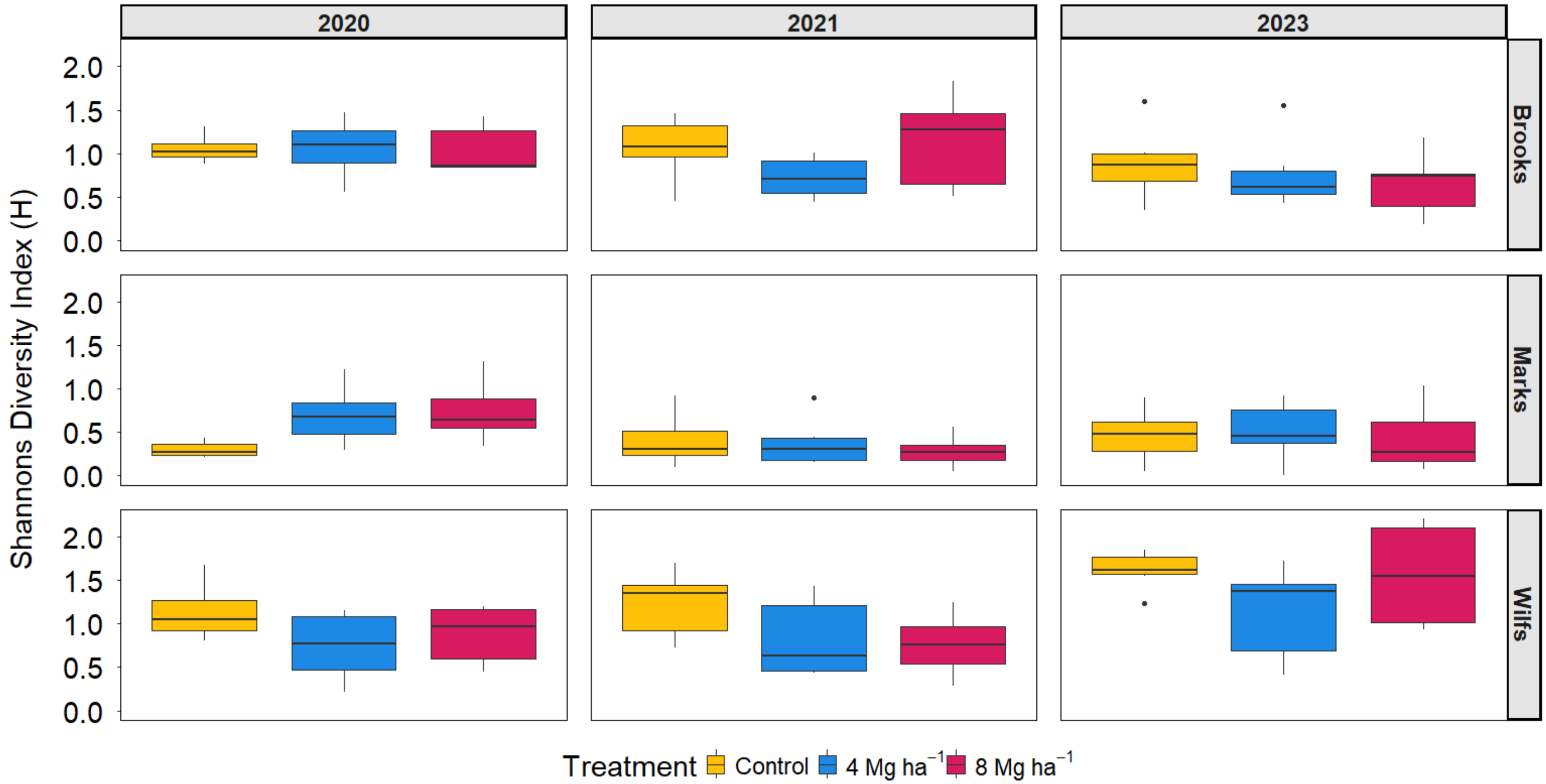

Figure 2.5 Shannon's Diversity Index (H) for the three sugarbush sites Brooks, Marks, and Wilfs for the completed vegetation surveys in 2020, 2021, and 2023. Values were calculated using three 1 m^2 surveys per plot. All sites had non-significant differences between treatments.

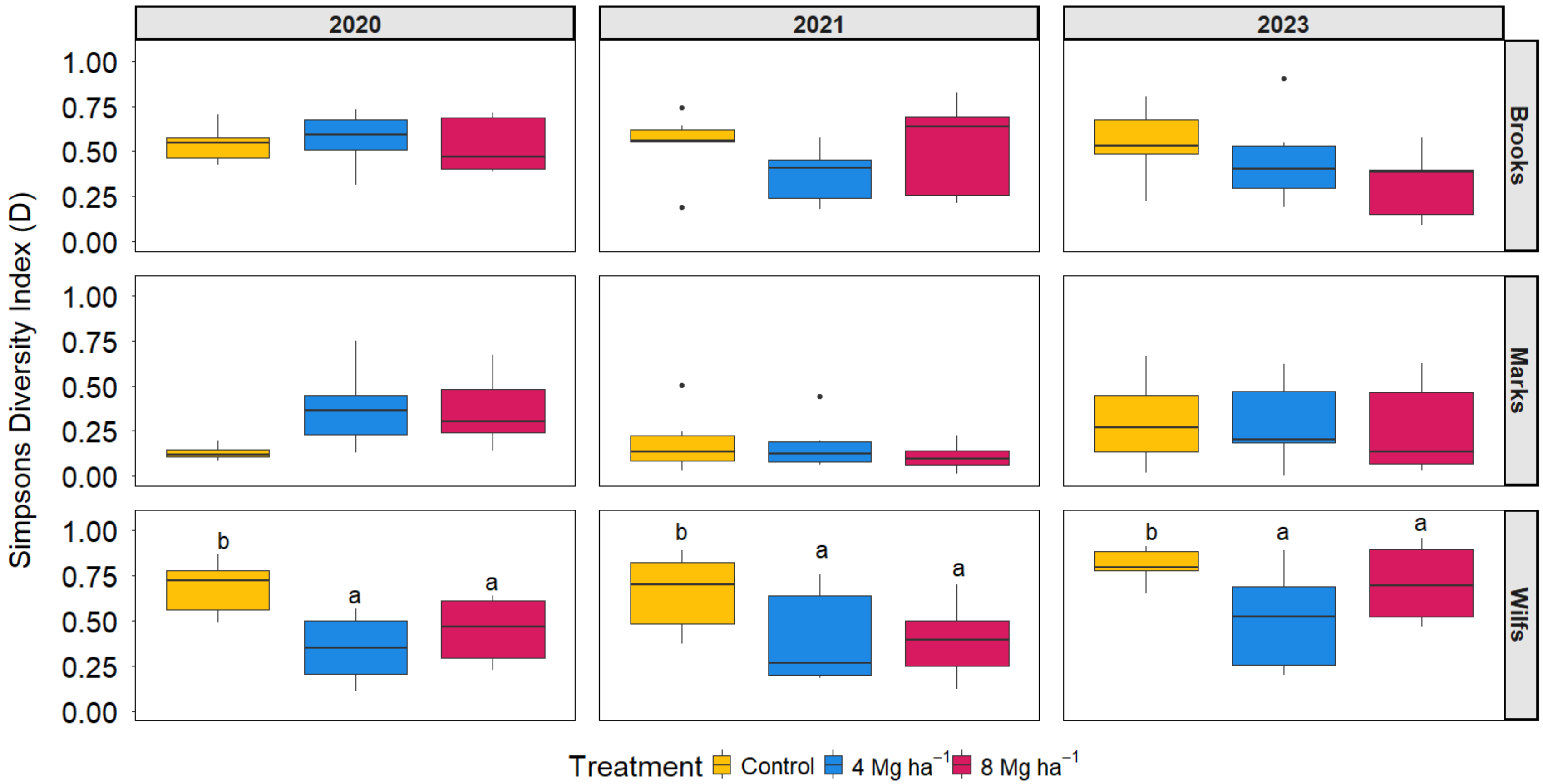

Figure 2.6 Simpson's Diversity Index (D) for the three sugarbush sites Brooks, Marks, and Wilfs for the completed vegetation surveys in 2020, 2021, and 2023. Values were calculated using three 1 m^2 surveys per plot. Letters indicate significance at $p < 0.05$. Only Wilfs site exhibited significant differences between treatments across all sampling dates.

2.4.4 Understory community PCA analysis

Principal component analyses indicated that community composition at each site in each year differed primarily by sites, with no influence of treatment on species composition (Figures 2.7 – 2.9). Community composition was variable among years at each of the sites. Marks site was typically driven by fewer dominant species than Brooks and Wilfs (lower species richness). Greater proportions of sugar maples were present at Marks regardless of year while greater proportions of the most abundant herbaceous species were present at either Brooks or Wilfs across all years, such as grasses/sedges, trilliums (*Trillium spp.* L.), solomon's seal (*Polygonatum spp.* Mill.) hobblebush (*Viburnum lantanoides* Michx.), wood ferns (*Dryopteris spp.* Adans.) and others. Greater proportions of tree seedlings were also present more often at Brooks and Wilfs across all years, including ash species, red maple, and striped maple (*Acer pensylvanicum* L.). Average pH values were also included in the analysis and showed that pH was closely associated with the Marks site and with sugar maple abundance.

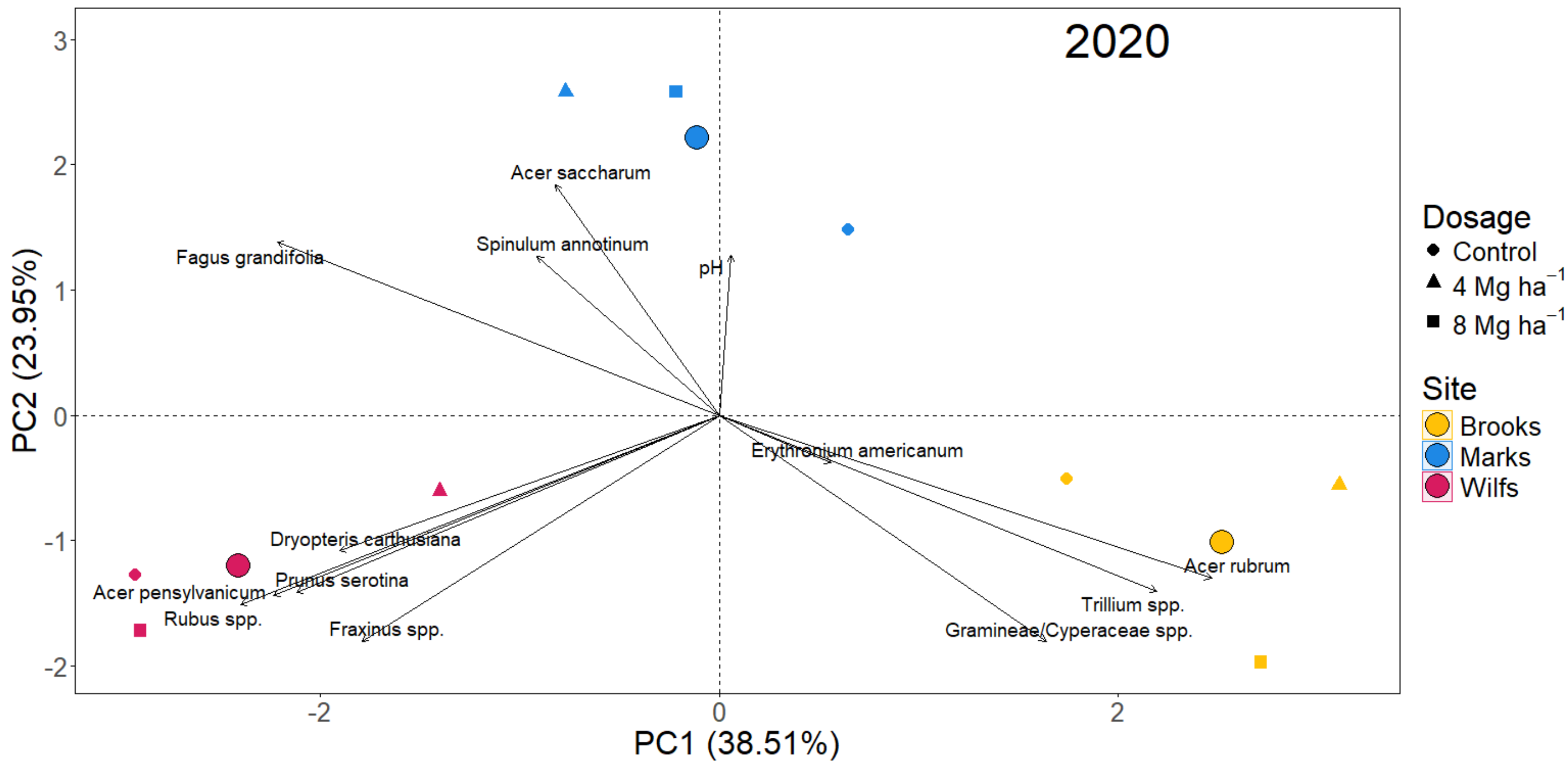

Figure 2.7 PCA bi-plot of species abundance and soil pH in the FH layer at Brooks, Marks and Wilfs in 2020. Shape corresponds to dosage and colour to site.

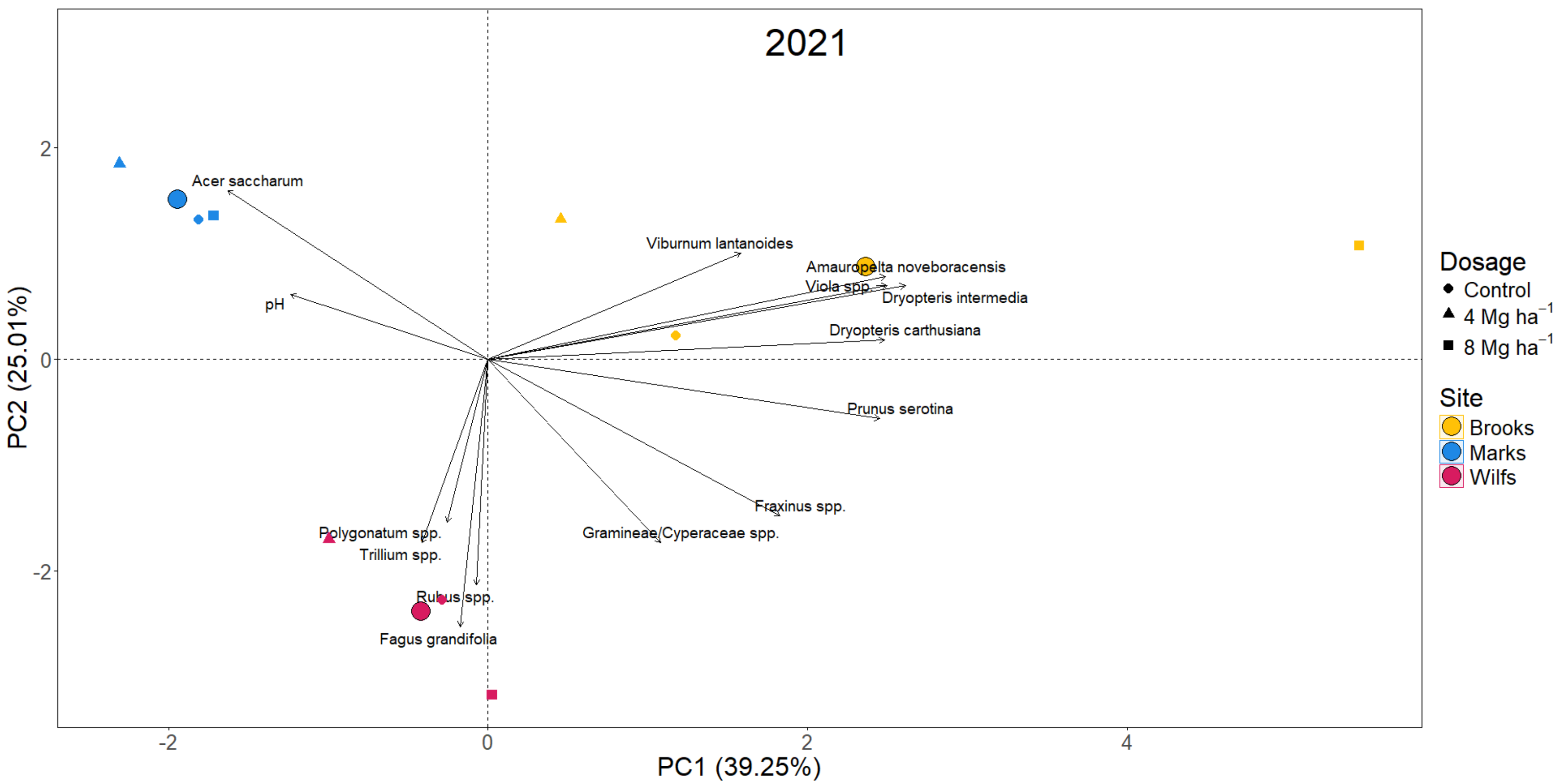

Figure 2.8 PCA bi-plot of species abundance and soil pH in the FH layer at Brooks, Marks and Wilfs in 2021. Shape corresponds to dosage and colour to site.

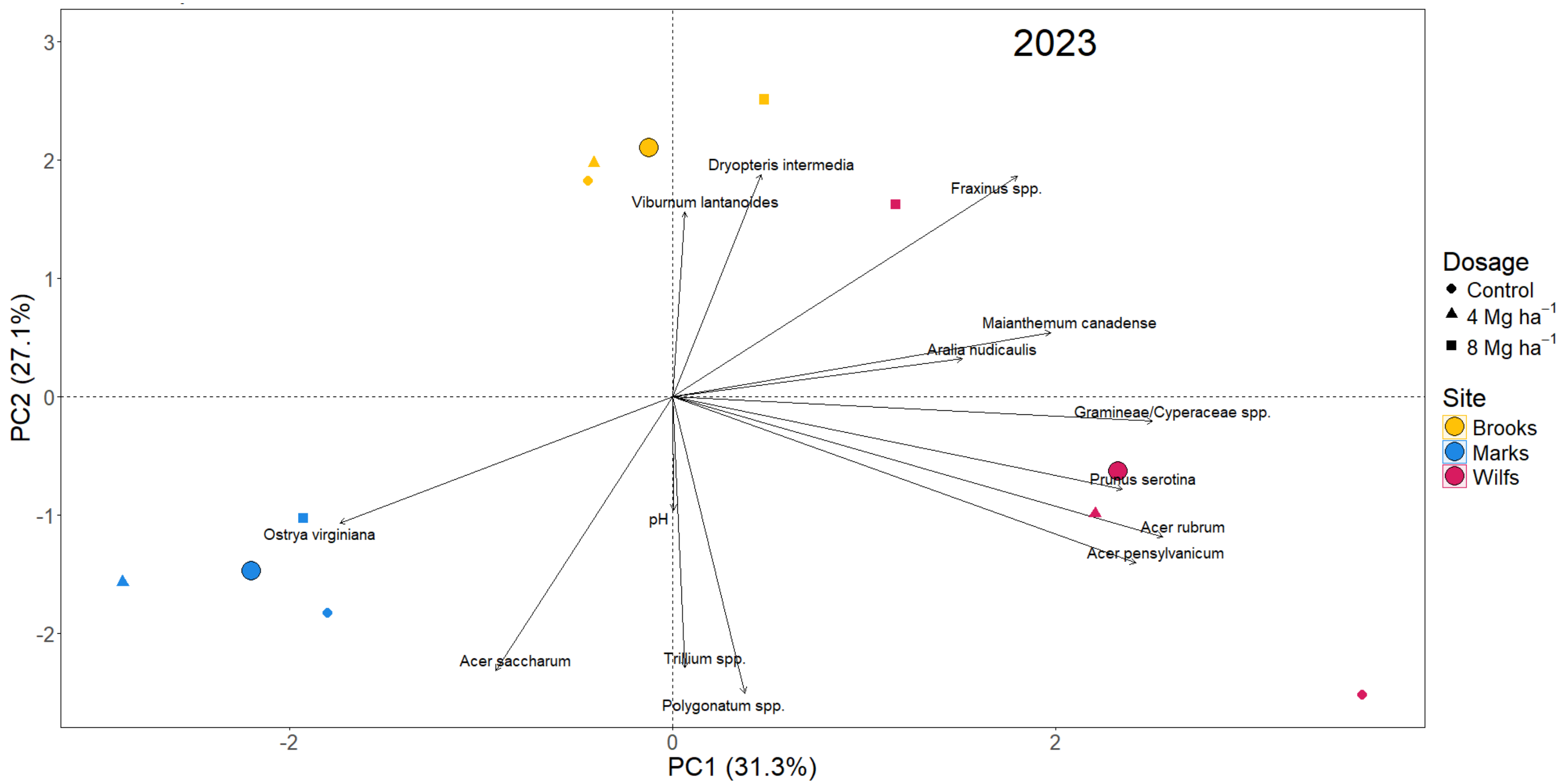

Figure 2.9 PCA bi-plot of species abundance and soil pH in the FH layer at Brooks, Marks and Wilfs in 2023. Shape corresponds to dosage and colour to site.

2.5 Discussion

The application of NIWA immediately increased soil pH following application in the upper organic horizons, followed by a more muted increase in upper mineral soil pH that emerged two years after application of 4 and 8 Mg ha^{-1} NIWA. The addition of up to 8 Mg ha^{-1} NIWA to each of the sugarbush sites generally had small effects on understory vascular plant composition as there was little-to-no effect of treatment on species abundance, richness and diversity within four years of application. Instead, site appears to be the driver of differences in understory vegetation communities.

2.5.1 Soil chemistry

In this study, soil pH was used as an indicator of soil chemical response to NIWA application. Other soil chemical responses to NIWA are reported in Conquer et al. (2025) and in Chapter 3. Following the application of NIWA, large increases in pH (2 – 3 units) were seen in the L and FH layers in both 2020 and 2021 compared with the controls at all three sugarbush sites. Effects in the upper mineral soils were muted in 2020, with only one site exhibiting slight increases in treatment plots compared to control. By the end of the study period, small increases in mineral soil pH were evident at all sites, while litter pH had almost returned to pretreatment values and were similar to control plots. The changes in soil pH occur because of the high neutralizing and buffering capacity of wood ash (Saarsalmi et al., 2006; Augusto et al., 2008), resulting from its high alkalinity (pH 8 – 13) (Vance, 1996; Augusto et al., 2008). Once applied to soil, the dissolution of metal oxides and hydroxides such as CaO, MgO, K_2O and NaOH produce hydroxyl ions that neutralize the protons present in the soil (Saarsalmi et al., 2006). These changes also result in increases in exchangeable soil Ca and Mg concentrations in upper soil horizons along with concentrations of several metals such as B, Cu, Mn, Ni and Zn (Conquer et al., 2025; Chapter 3). The soil pH response within two years (2020 – 2021) is comparable to other Ontario studies that found various rapid increases in the LFH horizons following wood ash addition (Deighton & Watmough, 2020; Conquer et al., 2024; Chapter 3). The lack of a response in mineral soil in 2020 was also anticipated, as the NIWA needs time to percolate through the soil profile, as seen in other studies (Reid & Watmough, 2014; Hansen et al., 2016). However, by 2021, the pH was significantly elevated in the upper 0 – 10 cm mineral soils. In other recent studies, soil pore water chemistry similarly showed that increases in pH at the 30 cm depth and base cations were only reported two years following wood ash application at similar (up to 7.5 Mg ha^{-1}) dosages (Smith et al., 2024).

The rapid and significant changes in soil pH were anticipated for these sites given their low base cation status and low starting soil pH, as soils with lower starting pH are expected to exhibit higher rates of change immediately following wood ash addition (Ohno, 1992). Generally, the higher treatment (8 Mg ha^{-1}) resulted in more pronounced increases in soil pH than the 4 Mg ha^{-1} treatment, regardless of site. Hansen et al. (2017) also showed soil pH increases with increasing wood ash application rates (from 3 Mg ha^{-1} to 9 Mg ha^{-1}). The increase in soil pH following wood ash addition does appear to be slowing four years after application (2023) as soil pH across all horizons was reduced from 2021 levels (despite still being significantly increased compared to controls). Despite this reduction, prolonged increases can still be expected for several additional years. Generally, long-term (> 5 years) wood ash studies are limited, especially in Canada, meaning longer-term pH response is under reported. Long-term studies in

Europe are more abundant and have found evidence of pH responses in mineral soil taking 5 – 7 years to be seen and persisting for up to 16 years following moderate wood ash applications of 3 Mg ha^{-1} (Saarsalmi et al., 2001; Saarsalmi et al., 2004). Studies have also found elevated pH in organic humus layers up to 10 years after applications of 2 – 10 Mg ha^{-1} (Bramryd & Fransman, 1995), suggesting soil pH in the treated plots at the sugarbushes should remain elevated for at least an additional 5 years.

The findings of this study suggest that wood ash suitably restores soil pH rapidly following application and that there is a positive relationship between soil pH and wood ash dosage. The implication of this finding is that the rate at which wood ash is applied can be tailored to individual site characteristics and needs, including starting soil pH and base cation status. This would facilitate the broader use of wood ash as a soil amendment in Canada across sites with varying degrees of acidification, both on the Canadian Shield and off. The rate of dissolution of wood ash will vary based on factors such as the form in which it is applied (loose or granulated), and soil conditions of the sites. Given the limited long-term studies on wood ash use in Canada there remains uncertainties surrounding how long this dissolution lasts and as a result how long pH changes in soil can be expected to occur. The lack of studies exemplifies the requirement of long-term studies in Canada to monitor soil condition between 5 – 15 years following various treatment dosages to determine how long the response persists in both the FH and mineral soil horizons. Once this is done a better understanding can be reached regarding whether wood ash addition is a one-time solution or if repeat applications will be needed, especially for sites on the Canadian Shield where precipitation remains acidic and soils exhibit low buffering capacity.

2.5.2 Understory community vegetation response

Understory vegetation at the three sugar bush sites showed no significant change in abundance and community composition up to three years post wood ash application, and only slight changes in diversity amongst treatments. The differences that existed in species composition seen in this study are almost entirely explained by differences among the three distinct sites. For example, the greatest proportion of sugar maple was seen at Marks site across all three years, while herbaceous plants and other tree seedlings were found in greater proportions at Brooks and Wilfs, though the same species were not always most abundant at the same site every year. Petaja & Zvaigzne, (2019) similarly found species composition to be driven by forest type (starting vegetation), regardless of wood ash treatment (at 3 Mg ha^{-1}). These differences in site illustrate the natural variability of forested sites, despite all the sites being central Ontario, sugarbushes. The results are therefore more robust as they provide a larger representation of vegetation present within these forests and thus how they respond within four years to wood ash addition. Generally, studies regarding understory vegetation communities and wood ash application are limited (Agusto et al., 2008). Several review papers on wood ash as a soil amendment offer a collection of mixed results regarding the effects of ash on understory plant communities (Andreas Aronsson & Ekelund, 2004; Pitman, 2006; Agusto et al., 2008). Studies within Canada are even rarer, however, several have emerged within the last 5 years. One study performed in British Columbia suggests there were minimal decreases in community composition based on ground vegetation cover, specifically in lichen and tree seedlings, one year following a 5 Mg ha^{-1} wood ash addition (Hart et al., 2019). Further, Rutherford et al. (2024) reported seeing no negative physiological effects such as burning (damage or death of plant tissue) on understory plants following applications of up to 10 Mg ha^{-1} equivalent at those same field sites. Results continue to be mixed, however, as a study in Quebec found significant changes in community composition following a 7 Mg ha^{-1} wood ash addition after two years, as the ash encouraged establishment of certain herbs and shrubs (Merzouki et al., Submitted). Studies in Europe tend to align with findings from this study. For example, Arvidsson et al. (2002) found limited increases in species composition (species cover) five years following 3 Mg ha^{-1} addition, suggesting that wood ash does not generally impact species abundance. Likewise, studies in Norway and Lithuania have found no changes in the general species abundance of vascular plant (groups) two years after 3 Mg ha^{-1} addition (Økland et al., 2022) or significant changes in vascular species cover, two years following application of 1.25 – 5 Mg ha^{-1} applications (Ozolinčius et al., 2007b). Results continue to be variable, though, as while changes in overall vegetation composition were considered small, Jacobson & Gustaffson, (2001) reported decreases in cover of dwarf shrub species after 3, 6 and 9 Mg ha^{-1} wood ash applications, which is attributed to the changes in soil chemistry. For example, changes in species composition that have been found have been suggested as being due to changes in soil fertility following ash application (Hart et al., 2019), which could be expected given the high concentrations of various nutrients present in ash. Nonetheless, given the lack of changes in species abundance in this study, increases in plant available nutrients do not appear to have influenced species abundance, which could also be expected if nutrients were not limiting at these locations.

Species richness was not significantly impacted across the duration of the study at all three study sites, but there was variation among sites. At Brooks, for example, species richness tended to be higher in the 8 Mg ha^{-1} treatment across the duration of the study compared to the control. Species diversity was also largely variable across the study and was generally unimpacted by wood ash treatment. The exception to this was Simpsons diversity at Wilfs in 2021 and 2023, in which the control was significantly higher than the 4 Mg ha^{-1} treatment, but not significantly higher than the 8 Mg ha^{-1}. Results appear similar to other findings, for example, high carbon wood ash (biochar) at doses of 5 Mg ha^{-1} has been found to increase the cover of several species, including *Solidago canadensis* L. and *Vicia cracca* L., and *Ranunculus acris* L., while decreasing the cover of *Symphyotrichum cordifolium* (L.) G.L. Nesom, but overall species richness was not affected (Bieser & Thomas, 2019). Similarly, in Quebec, wood ash amendments of 7 Mg ha^{-1} were not found to significantly alter species diversity up to two years following application (Merzouki et al., Submitted) and in British Columbia, a study also showed generally minor changes in species diversity following wood ash treatment (5 Mg ha^{-1}) (Hart et al., 2019). In Lithuania, Ozolinčius et al. (2007a) found no significant changes in ground vegetation diversity two years following application of 1.25 – 5 Mg ha^{-1} of wood ash but did note that Simpson's diversity was variable with values between 0.7 – 1.2. Hart et al. (2019) also note that no pre-treatment measurements of species diversity were taken, which they suggest could have been beneficial (Hart et al., 2019). This sentiment is supported in this study, as pre-treatment measures in species diversity would have been more informative given the spatial variation in species abundance, richness and composition within the sugarbush sites.

While understory vascular plants appear to be minimally affected by moderate wood ash applications, some non-vascular plant communities have shown potential negative impacts from wood ash additions. A review by Augusto et al. (2008) states that results appear similar regarding the negative effects on bryophytes. Ozolinčius et al. (2007b) also found negative effects on moss cover following wood ash application, while Økland et al. (2022) found decreases in bryophyte abundance following wood ash application. Decreases in bryophyte and lichen cover have also been reported following wood ash application (Jacobson & Gustaffson, 2001). This is supported by Hart et al. (2019), who found that ash application negatively impacted lichen (and tree seedling cover), though they attributed this to pre-treatment seedling density instead of treatment effect (Hart et al., 2019). Alternatively, most vascular plants root below the organic horizons and into the mineral layer, where soil responses such as pH are much more muted, while lichen and bryophytes are on the soil surface and receive direct exposure to surface spreading while tree seedlings root within the FH layer, which could create a direct exposure to the more drastic changes in pH, nutrients and metals. Notably, results in Chapter 3 also suggest that sugar maple seedling survivorship is decreased by high dosages of wood ash. Most trees are also seeded every year and therefore replaced, meaning that long-term effects of ash application may not be detectable by annual measurements of species presence. Likewise, many of the other species observed are perennial, reproducing through sexual (seeding) or asexual (rhizome splitting) reproduction, which could result in slower changes not seen within an annual measurement of species presence. It is also worth noting that ash application occurred in the late fall after senescence, meaning that perennial species should have been less affected by wood ash application and its immediate reactions given their dormancy. Lichen and bryophytes on the other hand do not cycle the same way, and would have been directly exposed to the ash via smothering in the fall, possibly accounting for the increased negative effects seen in many studies

Soil conditions between sites may potentially influence the response of vegetation communities (Augsto et al., 2008) and account for site-specific differences that are reported in the literature. For instance, a study on wood ash application (8 or 16 Mg ha^{-1}) to a peatland resulted in substantial changes to species composition, including increases in grasses and herbs (Moilanen et al., 2002). Ferm et al. (1992) found that high dose ash applications (up to 20 Mg ha^{-1}) favoured grasses in peatland systems. This is further corroborated by Maljanen et al. (2014) who found changes in species composition, namely increases in the coverage of various grasses, herbs and spinulose woodfern (*Dryopteris carthusiana* (Vill.) H.P. Fuchs) following 5 Mg ha^{-1} wood ash addition to peatland soils. It is believed that the presence of N in the surface peat may partially account for the strong growth response in vegetation (Moilanen et al., 2013). This would suggest that N limitation may be a factor contributing to muted understory vegetation effects in forested systems. Pitman (2006) supports this as they concluded that wood ash effects would be limited when N limitation occurs. This is supported by evidence of the DRIS indices completed on sapling and mature foliage at these sugarbush sites, which suggests that following wood ash application, N is limiting (Conquer et al., 2025). Furthermore. results of several studies (Jacobson & Gustaffson, 2001; Hart et al., 2019; Økland et al., 2022) suggest that ash application with N fertilization would have resulted in more significant effects on understory vegetation communities. Alternatively, light in peatland sites may also contribute to these findings as light is far more limiting in forested ecosystems than in peatlands. For example, results of research on understory plants and light have found herbaceous species to positively correlate with light availability (Ádám et al., 2018) while light availability influenced both vegetative and regenerative performance of herb species (Verstraeten et al., 2014).

Given the presence of minimal changes in species abundance, richness and diversity, it can be suggested that despite the caustic nature of wood ash, it can suitably be used as a soil amendment to restore forests impacted by acidification. The presence of no negative effect on vascular communities could be the result of several factors including natural forest resiliency, or the duration of time required to see change at the community level. The acidification of forests in this region has been a significant long-term pressure and it is possible that these communities are simply incapable or recovery within four years of application. Thus, long-term monitoring is suggested to determine if changes begin to occur. Additionally, attempts to monitor the response of individual target species may yield treatment effects lost in broader vegetation surveys. Ultimately, however, the result of no negative effects on vascular community composition should help facilitate changes to policy in Ontario and Canada in order to deregulate wood ash for use and facilitate upscaling of its use as a soil amendment.

2.6 Conclusions

The purpose of this study was to evaluate the effects of NIWA application on understory vegetation community abundance, richness and diversity to determine if it is a suitable amendment for restoration of acidified forest soils. Results on soil chemistry reaffirm years of previous research and show that NIWA increases soil pH in organic and upper mineral soils and increases base cation concentrations. At dosages of 4 and 8 Mg ha^{-1}, wood ash does not appear to significantly influence understory vascular species abundance, richness or diversity within four years of application, congruent with other literature. Instead, species composition appears primarily driven by site instead of NIWA treatment. Responses in understory vegetation communities may be delayed (> 5 years) or N limitation at the sites may prohibit vegetation response to ash addition. Ultimately, the results of this study continue to support related studies in that NIWA improves soil pH and base status without adversely impacting understory vascular plant communities.

3.0 Impact of non-industrial wood ash application on acidified soils and sugar maple regeneration

3.1 Abstract

Wood ash is a widely used soil amendment for neutralizing acidified soils, yet its use in Canada remains restricted owing to environmental concerns. This study aimed to evaluate the effects of non-industrial wood ash application (0, 2, 4, 6, or 12 Mg ha^{-1}) on sugar maple (*Acer saccharum* Marsh.) regeneration in a deciduous hardwood forest in Bracebridge, Ontario. Wood ash addition resulted in a large, but transient increase in soil pH in the litter layer, while the largest and most prolonged increase in pH was seen in the FH layer, with the pH response being dosage dependent. The mineral soils exhibited the smallest increases in pH, especially the lower 10 – 20 cm soil horizon. Calcium, magnesium, and concentrations of several metals (boron, copper, manganese and zinc) also increased significantly in the organic soils following application. Seedling survivorship was adversely affected by increasingly larger non-industrial wood ash treatments with no survivorship by mid-summer of the second year at treatments of 6 Mg ha^{-1} and above. The seedling cohort established in the second year of the study similarly experienced reduced survivorship at treatments > 4 Mg ha^{-1}. As a result of these findings, it appears that non-industrial wood ash, when applied at dosages ≤ 4 Mg ha^{-1}, is effective at improving soil base cation status with no short-term effects on sugar maple seedling regeneration.

3.2 Introduction

Forests across central Ontario, Quebec, and the northeastern United States have been acidified by the combined effects of acidic deposition and timber harvesting (Fenn et al., 2006; Warby et al., 2009). Base cation losses in these soils negatively impact aquatic and terrestrial ecosystems, with calcium (Ca) losses a major ecological concern. Sugar maple (*Acer saccharum,* Marsh) is especially sensitive to Ca and base cation losses (Huggett et al., 2007; Halman et al., 2013). This has resulted in this species showing symptoms of decline including reduced growth (Watmough, 2002; Juice et al., 2006), crown (branch and leaves) condition decline (Huggett et al., 2007; Watmough, 2010), and a reduced ability for the trees to cope and recover from stress events (Long et al., 2009). In addition, sugar maple seedling regeneration has decreased in harvested and undisturbed forest ecosystems (Juice et al., 2006; Cleavitt et al., 2018; Sullivan et al., 2018), owing most likely to acidification and low base cation status. Natural regeneration is vital to forest sustainability (Henry et al., 2021), and regeneration failure of the dominant tree species could result in significant ecological damage to the forest ecosystem (Barnes & Burton, 2021).

Since the 1970s, significant reductions in sulphur (S) and more recently nitrogen oxides (NO_x) emissions have occurred, and signs of recovery within these forest ecosystems are emerging (Lawrence et al., 2015; Hazlett et al., 2020). However, modelling predicts that the recovery of these ecosystems will be slow, taking decades or centuries to recover to pre-disturbance conditions (Ott & Watmough, 2022), with timber harvesting further delaying or arresting this recovery. This is because, despite reductions in acidic pollution, Ca losses through leaching and vegetation uptake are expected to continue to exceed Ca additions through natural atmospheric deposition and mineral weathering (Huntington, 2000).

To combat the loss of nutrient cations and increase soil pH researchers have been evaluating the introduction of Ca-rich soil amendments. One such amendment is wood ash. In European studies, positive results from wood ash use in forest ecosystems included increases in nutrient cations (both from ash content and changes in availability in soil) and decreases in aluminum (Al) in soil (Saarsalmi et al., 2001; Brunner et al., 2004), along with increases in tree production and reductions in soil acidity (Huotari et al., 2015). Responses to tree seedling growth and survival are mixed with either positive, negative or no effects (Augusto et al., 2008). However, wood ash also contains heavy metals such as lead (Pb) and chromium (Cr) that may negatively affect biota (Azan et al., 2019; Deighton & Watmough, 2020; Deighton et al., 2021). Because of these concerns wood ash is currently restricted for use in Canada (Kim et a., 2022) and most is disposed of as hazardous waste in landfills (Azan et al., 2019). Despite these concerns, experimental studies in Canada have found that wood ash increases soil pH (Deighton & Watmough, 2020), and improves mature tree growth (Reid & Watmough, 2014; Arseneau et al., 2021), while causing minimal changes in arthropod (Smenderovac et al., 2022), bacterial, and fungal communities (Smenderovac et al., 2022; Smith et al., 2024).

Significant knowledge gaps still exist regarding the use of wood ash as a soil amendment for restoring acidified forests in Canada. Broadly, the impacts of wood ash application on forest ecosystems, especially their ability for seedlings to regenerate, have not been fully determined. To date, there has been a limited number of studies that have explored how wood ash affects sugar maple seedling performance (Deighton et al., 2020;

Arseneau et al., 2021). There is, however, growing evidence of the benefit of soil amendments to sugar maple seedling regeneration. For example, biochar, a similarly alkaline soil amendment, has been shown to enhance early-stage seedling development in various species including sugar maple from increased soil pH and K availability (Thomas, 2021). Studies conducted in New Hampshire USA, using wollastonite ($CaSiO_3$), another Ca-rich amendment, found higher seedling survivorship with larger and healthier seedlings several years following application owing to the increased Ca following application (Juice et al., 2006; Cleavitt et al., 2011). While mineral wollastonite has a pH of 10 – 10.4 (Fernandez-Caliani et al., 2008; Chai et al., 2021), wood ash can have higher pH values of 12.5 – 13 (Syeda et al., 2024; Conquer et al., 2024; Conquer et al., 2025), contain higher concentrations of potentially toxic metals, and provides other nutrients required by plants such as phosphorus (P) and potassium (K) (Azan et al., 2019).

The objectives of this field study were to assess soil chemical responses along with sugar maple seedling growth and survivorship over two years post-wood ash addition at dosages ranging from 2 Mg ha^{-1} to 12 Mg ha^{-1}. It was hypothesized that wood ash would increase soil pH, nutrient availability, and the total metal concentrations in upper soil horizons. It was also hypothesized that wood ash would increase the growth and survivorship of sugar maple seedlings in nutrient-poor soils.

3.3 Methods

3.3.1 Study site

The study was conducted at a site located in the Muskoka region of central Ontario roughly 200 km northeast of Toronto, Ontario. The site is a privately owned summer camp called Camp Big Canoe (45° 3'43.77" N, 79° 8'15.19" W), situated just east of the town of Bracebridge (Figure 3.1).

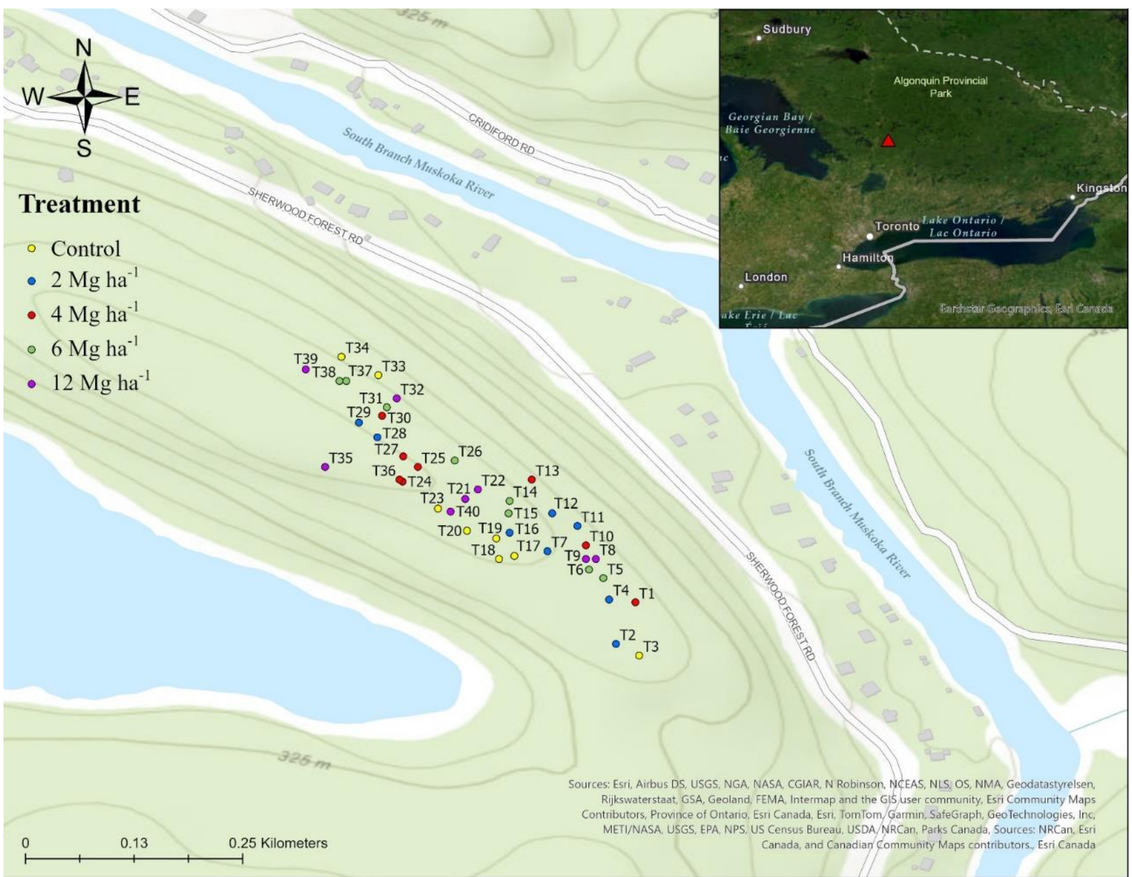

Figure 3.1 Map showing the plot layout at the study area, Camp Big Canoe in Bracebridge, Ontario, Canada, in proximity to Toronto Ontario, Canada.

This site is at an elevation ranging between 325 and 338 meters above sea level and exhibits gently undulating topography. Based on climate averages between 1991 – 2020 the annual average temperature of the site is 5.3 °C with mean daily averages between -10.0 °C (January) and 19.1 °C (July), and total annual average precipitation of 1062 mm, split between 689 mm of rainfall and 373 mm of snowfall (ECCC, 2025). The site sits on the southern tip of the Precambrian Shield which possesses generally hard rock, including granite and gneiss. The site exhibits shallow Brunisolic and acidic Podzolic soils that are typically coarse textured sandy loams (Soil Classification Working Group, 1998). The soils are generally acidic, with fibric humic (FH) layer pH between 3.9 – 4.9 and mineral soils having a pH of 3.5 – 4.8. The site is situated within the Great Lakes-St. Lawrence Forest and is dominated by sugar maple but also exhibits other deciduous species including yellow birch (*Betula alleghaniensis* Britt.), white ash (*Fraxinus americana* L.), and American elm (*Ulmus americana* L.) along with coniferous species including eastern hemlock (*Tsuga canadensis* (L.) Carrière) and balsam fir (*Abies balsamea* (L.) Mill.). The site was chosen because it is in a region historically impacted by acid deposition with negatively impacted soil conditions and nutrient concentrations, while also being relatively undisturbed in recent years. Specifically, this camp is situated on 100 ha of forest that has received minimal harvesting and disturbance since 1968 (Casey, 2021). The site also sits well enough away from major roadways to reduce the risk of potential site disturbance or contamination.

3.3.2 Plot setup, experimental design, ash chemistry, and ash application

In July 2021, forty circular plots (28.27 m^2) were established at the study site (Figure 3.1). Each plot was placed around a similarly sized (20 – 25 cm diameter at breast height) mature sugar maple with the trunk of the tree acting as the centre point of the plot (radius of 3 m) (Figure 3.2).

Figure 3.2 Left: diagram of plot and sup-plot layout at the study site. All plots were setup in a similar manner based on suitable topography and minimum distance. Right: plot setup in situ.

Plot locations were selected by selecting healthy sugar maples a suitable distance away from each other (minimum 16 m). A buffer zone of 10 m between plots was ensured, and plots were placed on relatively flat areas away from water courses (minimum 60 m) in case of accidental ash runoff. Following this, in October 2021, the plots were hand-treated with NIWA at 0, 2, 4, 6, or 12 Mg ha^{-1} so that eight replicates were produced per dosage. 2 Mg ha^{-1} served as estimated Ca replacement, while the 4 – 12 Mg ha^{-1} dosages represent scaling treatments to determine effects of higher wood ash additions.

The ash used in this study was collected and processed by the Friends of the Muskoka Watershed charity from residents and small businesses in the Muskoka region over several ash drives (FMW, 2025b). When collected, the ash was amalgamated and sieved down to < 2mm to remove course char and any unwanted items (nails, bottle caps) before being stored in plastic polyethylene containers before use (Conquer et al., 2024). In a questionnaire regarding the ash donated, residents indicated that predominantly bark (70.2 %) and trunk wood (85.1 %) were burned. Residents also indicated that predominantly hardwoods were burned, including maple (70.2 %), birch (51.1 %) and oak (27.7 %), while softwoods such as pine, spruce, and hemlock only made up about 25 % of what was burned (Syeda et al., 2024). Analysis of the ash found that it had an average pH of 13.0 and was made up of 27 % Ca and 9 % K (Conquer et al., 2024) while only zinc (Zn) and copper (Cu) concentrations were slightly higher than non-agricultural source material (NASM) concentration of regulated metals (CM) limits (CM1), meaning the ash is suitable for land spreading, but under certain restrictions. A full analysis of nutrients and metals was performed (Table 3.1 adapted from Conquer et al., 2024).

Table 3.1 The pH ($CaCl_2$), organic matter, nutrient and metal concentrations from samples of the NIWA collected in Muskoka, Ontario. Values are represented as means (±SE). Included are the CM1 and CM2 guidelines per the Nutrient and Management Act, 2002. Samples were reported as dry weight by mass. Table adapted from Conquer et al., 2024.

Properties pertaining to Non-Industrial Wood Ash (n = 24)		Non-Agricultural Source Material 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

Application of the NIWA was completed by pre-weighing the required dosage for each plot into buckets (5.56 kg for 2 Mg ha^{-1}, 11.31 kg for 4 Mg ha^{-1}, 16.96 kg for 6 Mg ha^{-1}, and 33.93 kg for 12 Mg ha^{-1} treatment). The perimeter of each plot was marked with wooden stakes, and the ash was then hand-spread with large scoops. The ash was spread first in a circular fashion to create an outline, then the interior was spread to fill the circle in, visually ensuring even application.

In June 2022, eighty 1 m^2 sub-plots were established (for the following two growing seasons) by randomly nesting two sub-plots within each of the original forty circular plots (Figure 3.2). These smaller square sub-plots were observational plots to assess sugar maple seedling growth and survivorship to the wood ash addition. During the first growing season, only first-year seedlings (2022 cohort) were monitored. First-year seedlings were identified as seedlings exhibiting one set of leaf nodes, typically with no more than two leaves. During the second growing season, two cohorts (2022 and 2023 cohorts) were monitored, the second years (identified as seedlings with two sets of leaf nodes and no more than four leaves), and the new first-year seedlings.

3.3.3 Field sampling

Soil samples were collected in June 2021 before ash application, followed by two annual sampling events post-application in June 2022, 2023. Samples (n = 40) were a composite of two sub-samples collected at random from each side of the center tree. Organic soils from the surface leaf litter (L) and fibric humic (FH) layer were collected using grab sampling. The surface litter was sampled first, followed by the FH, which was peeled back, both were placed into separate sealed plastic bags. The (upper) Ah mineral soil (0 – 10 cm) and (lower) B horizon mineral soil (10 – 20 cm) were sampled using an

Eijkelkamp Dutch soil augur. Soils were placed in sealed plastic bags and transported to the laboratory, where they were stored at 2.5 °C prior to analysis.

Field sampling and monitoring of seedlings occurred between June and October 2022 and between May and September 2023 to coincide with the growing seasons in central Ontario. Within the eighty observational sub-plots, presence of sugar maple seedlings was quantified, as well as height and diameter at ground level of each seedling. These measurements were completed using a Mastercraft Digital Caliper, 6-in, and were taken at roughly one-month intervals for four months each year. This method was replicated for each cohort separately during the 2023 season. Additionally, once yearly, destructive sampling of up to ten seedlings per plot was completed for biomass assessment as another indicator of seedling growth from regions outside the 1 m^2 plots. The number of samples was highly variable due to the natural variation in seedling density across the study site.

3.3.4 Laboratory analysis

3.3.4.1 Soil samples

In the lab, the soil samples from each plot per year were amalgamated to make one sample per horizon per plot. Samples were then dried in an industrial oven (The Grieve Corporation, Round Lake, IL, US) for twenty-four hours at 105 °C. Afterwards, the litter and FH layers were milled using a Willey Mill (Arthur H. Thomas Corporation, Philadelphia, PA, US), while the mineral layers were sieved using a 2 – mm sieve. Samples were then stored in a plastic bag until analysis. All analysis was conducted based on dry weight by mass.

To test for pH, either 1 g of organic soil or 5 g of mineral soil were placed in a 50 ml conical Falcon Tube (FroggaBio Inc, Vaughan, ON, CA) with 15 ml of 0.01 M calcium chloride ($CaCl_2$) aqueous matrix. Samples were then placed on a shaker table (Eberbach Corporation, Van Buren Charter Township, MI, US) for two hours, and then left to rest for one hour. Samples were analyzed using an OAKTON pH 510 series multimeter (Oakton Instruments, Vernon Hills, Il, US). To ensure accuracy, the pH probe was calibrated every 25 samples. Loss-on-ignition (LOI) was employed to test for organic matter content. Either 1g of organic soil or 5g of mineral soil was heated in a pre-weighed ceramic crucible in an industrial oven (The Grieve Corporation, Round Lake, IL, US) for twenty-four hours at 105 °C. Following this, samples were reweighed on an analytical balance and placed in an Isotemp muffle furnace (Fisher Scientific, Pittsburgh, PA, US) for ten hours at 400 °C. Afterward, warm samples were removed from the muffle furnace one at a time and reweighed. Care was taken to expose the samples to the air as briefly as possible to ensure that they did not absorb ambient moisture from the air. Once completed, the weights at each stage were used to calculate the percentage of organic matter.

Exchangeable cations (Ca, K, Mg) were tested by performing an extraction whereby either 1 g of organic soil or 5 g of mineral soil were weighed on an analytical balance and placed into a 50–ml conical Falcon Tube (FroggaBio Inc, Vaughan, ON, CA) with 25 ml of 1 M ammonium chloride (NH_4Cl) solution. The tubes were then placed on a shaker table (Eberbach Corporation, Van Buren Charter Township, MI, US) for two hours, then left to rest for one hour. Samples were filtered using vacuum filtration with a Buchner funnel and sidearm flask. Within the funnel, a P8 Fast Flow Filter Paper filter

(Thermo Fisher Scientific, Waltham, MA) was used to remove the solid soil. The mixed solutions were then poured through the filter paper, and the tube was rinsed with an additional 25 ml of NH_4Cl solution. The filtered samples were then placed into a new 50-ml conical Falcon Tube (FroggaBio Inc, Vaughan, ON, CA). The 50 ml solution was then further diluted at a 1:10 ratio with 1 ml sample, 8.8 ml of B-pure, and acidified using 0.2 ml nitric acid (HNO_3) (67 – 70% w/w; VWR Chemicals, PA, US) before being placed in a 15-ml conical Falcon Tube (FroggaBio Inc, Vaughan, ON, CA) and stored in the fridge at 2.5 °C prior to analysis. Samples were analyzed using a Perkin Elmer Optima 7000DV inductively coupled plasma optical emission spectrometer (ICP-OES; Waltham, MA, US). Soil standards (EnvrioMAT SS-1) and elemental standards (SCP Science, Quebec, CA) were used in the analysis.

Nutrient and metal content (Al, Boron (B), Cu, Iron (Fe), Manganese (Mn), and (Zn) were assessed by performing nitric acid digestion extractions before analysis with the ICP-OES. To prepare the samples 0.2 g of soil were weighed on an analytical balance and placed in 50-ml flat bottom digiTUBEs (SCP Science, Quebec, CA) with 2.5 ml of nitric acid (67 – 70% w/w; VWR Chemicals, PA, US). Tubes were then placed on a Thermolyne Cimarec 3 hotplate (Thermo Fisher Scientific, Waltham, MA) for digestion. The tubes were cold digested for the first eight hours and then heated to 100 °C for eight hours of hot digestion. Following this, samples were filtered into 25–ml graduated cylinders, using plastic funnels with a P8 Fast Flow Filter Paper (Thermo Fisher Scientific, Waltham, MA). Samples were then diluted to 25 ml using B-pure water before being placed into a 50 ml conical Falcon Tube (FroggaBio Inc, Vaughan, ON, CA). Before analysis, the sample was transferred to a 15-ml conical Falcon Tube (FroggaBio

Inc, Vaughan, ON, CA) and stored in the fridge at 2.5 °C. Soil standards (EnvrioMAT SS-1) and elemental standards (SCP Science, Quebec, CA) were used in the analysis.

3.3.4.2 Sugar maple seedling samples

The sugar maple seedling samples were first cut to separate the belowground (root) and aboveground (shoot and foliage) biomass. The cut was made at approximately the first cotyledon as described in (Menes & Mohammed, 1995). Following this, the shoots and foliage were dried in an industrial oven (The Grieve Corporation, Round Lake, IL, US) at 105 °C for twenty-four hours. The roots were gently washed with B-pure to remove any remaining soil and other debris before being dried in the industrial oven (The Grieve Corporation, Round Lake, IL, US) for twenty-four hours at 105 °C. Subsequently, both the belowground and aboveground samples were weighed to determine a dry weight (DWT) biomass.

3.3.5 Statistical analysis

All statistics were performed using R Statistical software version 4.2.2 (R Core Team, 2022). Significance was determined at an alpha value of 0.05. For each response variable, assumptions of homoscedasticity and normality were assessed using Levene's test, Shapiro-Wilk's test (*rstatix* package), and visually checking using a QQ plot (*ggpubr* package). Most data met assumptions, and statistical analyses were performed using parametric statistics, including two-way ANOVA tests and linear mixed effects models. Only seedling survivorship was assessed using non-parametric statistical tools.

3.3.5.1 Soil samples

Soil chemistry (including pH, organic matter, nutrients, and metals) was tested using a two-way ANOVA with interaction to determine differences produced by NIWA treatments and year on the soil chemistry. If the two-way ANOVA indicated significant differences, an emmeans post-hoc test was performed with multiple means comparisons with a Bonferroni correction (*emmeans* package) to determine where the differences between treatments occurred.

3.3.5.2 Sugar maple seedling survivorship

To analyze survival data using non-parametric statistics the analysis performed by Cleavitt et al. (2011) was followed. The first step was to apply the necessary censoring of the data. Two types of censoring were used, right censoring, to account for the number of seedlings still alive in each cohort at the end of the study period (27 in the 2022 cohort and 54 in the 2023 cohort). The second type of censoring, interval censoring, was applied because the exact date of death of a seedling was not known, only an interval (month) between the last living sighting and death. For the purposes of analysis, it was assumed that the rate of death from outside factors (herbivory, drought or human disturbance) was the same across treatments and controls, with any changes in mortality in the treatment plots being a treatment-induced effect. The intervals ranged from 29 to 238 days, depending on the growing season and feasibility of sampling. Then, a variation of Kaplan-Meier maximum likelihood estimation was employed based on the recommendation of Aljawadi et al. (2012) that employs Turnbull's (1976) generalization of the Kaplan-Meier. This likelihood estimator is called the non-parametric maximum likelihood estimator (NPMLE) and was used to analyze the data and build survivorship curves with step functions. The (*interval* package) was used following the explanation provided by Fay & Shaw (2010), which also required the use of the (*survival* package) and (*icens* package). Significance between curves was determined using weighed long-rank tests with permutation.

3.3.5.3 Sugar maple seedling growth

Below and above-ground biomass was analyzed separately. A two-way ANOVA test with interaction was used to determine differences produced by NIWA treatments on seedling biomass each year. If the two-way ANOVA indicated significant differences, an emmeans post-hoc test was performed with multiple means comparisons with a Bonferroni correction (*emmeans* package) to determine where the differences between treatments occurred. Seedling growth via height and diameter was assessed using a linear mixed model (*lme4* package). To determine the treatment effect, date and plot were used as the random effects, while treatment was used as the fixed effect. The 2022 and 2023 cohorts were run independently of each other to increase accuracy in analysis.

3.4 Results

3.4.1 Soil chemistry

Prior to application of the ash (2021) soil pH, organic matter (OM), and nutrient and metal concentrations were relatively consistent across each soil horizon in both the treatment and control plots (Figures 3.3 – 3.6). Results of the ANOVA test indicate significant treatment effect on soil pH across the duration of the study (L d.f. = 4, F = 10.15, $p < 0.001$, FH d.f. = 4, F = 41.58, p = < 0.001, Mineral 0 – 10 d.f. = 42, F = 9.467, $p < 0.001$, Mineral 11 – 20 d.f. = 4, F = 7.581, $p < 0.001$). One year following the NIWA application (2022), statistically significant increases in soil pH were measured in all soil horizons. The largest increases in pH (2 – 3 units) were seen in the L and FH layers immediately following application while statistically significant increases in the upper 0 – 10 cm mineral soil were not as large (1 – 2 units) but still present. Significant increases (about 1 unit) in the lower 11 – 20 cm mineral soil horizons only occurred with the highest treatment (Figure 3.3). By 2023, two years post-application, the pH in the L layer returned close to baseline values while elevated pH values (1 – 3 units) continued in the FH layer and upper 0 – 10 cm mineral soil. The lower 11 – 20 cm mineral soil still showed significantly elevated pH in the highest treatment (about 1 unit).

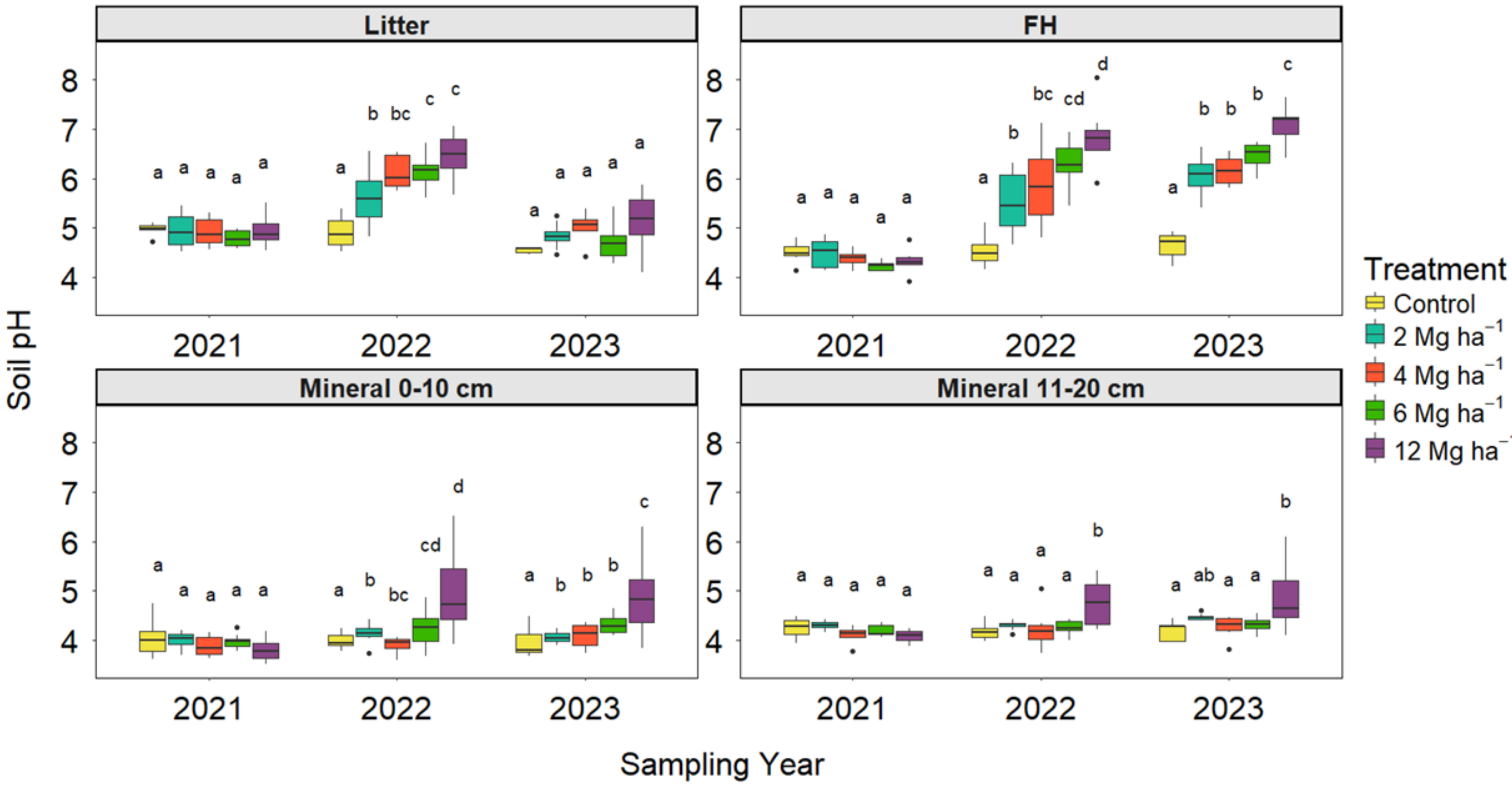

Figure 3.3 Average soil pH from 2021 – 2023 in the L, FH, upper mineral (0 – 10 cm) and lower (11 – 20 cm) horizons using $CaCl_2$ matrix. Different letters indicate statistically significant differences ($p < 0.05$) using a post-hoc emmeans test between each treatment at each soil depth.

Results of the ANOVA test on soil OM indicate the presence of significant treatment effect across the duration of the study in the organic horizons (L d.f. = 4, F = 6.184, $p < 0.001$, FH d.f. = 4, F = 6.852, $p = < 0.001$) while there were no significant differences in the mineral horizons (Mineral 0 – 10 d.f. = 4, F = 1.719, p = 0.152, Mineral 11 – 20 d.f. = 4, F = 1.088, p = 0.367). Soil OM in the L layer generally decreased (up to 40 %) with increasing treatment dosages following NIWA application. By 2023, OM returned to baseline in the L layer (86.31 – 90.90 %). Significant decreases in OM were also observed in the highest treatment in the FH in both 2022 and 2023, while changes in treatments < 12 Mg ha^{-1} were not consistent (values between 6.30 – 86.04 % reported). No treatment effects were observed in the upper 0 – 10 cm or lower 11 – 20 cm mineral soils in 2022 and 2023 (2.90 – 15.63 %) (Figure 3.4).

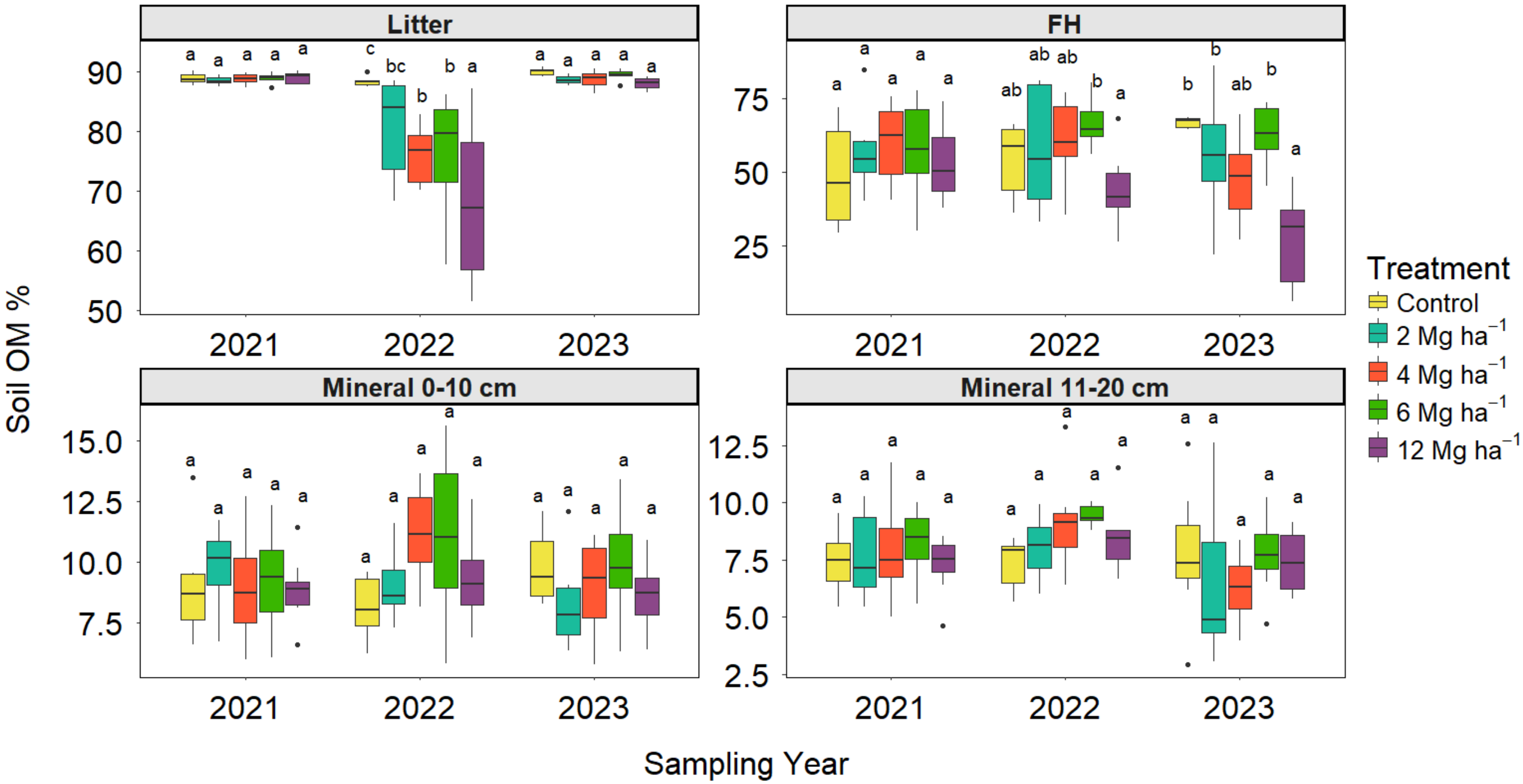

Figure 3.4 Average soil organic matter (OM) present in the L, FH, upper mineral (0 – 10 cm) and lower mineral (11 – 20 cm) horizons both before (2021), and two years post NIWA application (2022, 2023). Different letters indicate statistically significant differences ($p < 0.05$) using a post-hoc emmeans test between each treatment at each soil depth.

Soil base cation and metal data were only available for 2021 and 2022. Results of the ANOVA tests on soil cations indicate significant treatment effects post-application for Ca (d.f. = 4, $F = 15.307$, $p < 0.001$), Mg (d.f. = 4, $F = 15.957$, $p < 0.001$) and K (d.f. = 4, $F = 3.864$, $p = 0.0053$). Large dosage-dependent increases were seen in Ca and Mg concentrations following application in the L and FH layers in 2022, but this was not evident for K. One year after application, only K showed statistically significant increases in the FH and in the 12 Mg ha^{-1} treatment in the lower mineral soils (non-significant increase in upper mineral). There were no significant changes in mineral soil base cation concentrations one year after NIWA application for Ca and Mg, although both tended to be elevated in the 12 Mg ha^{-1} treatment. (Figure 3.5)

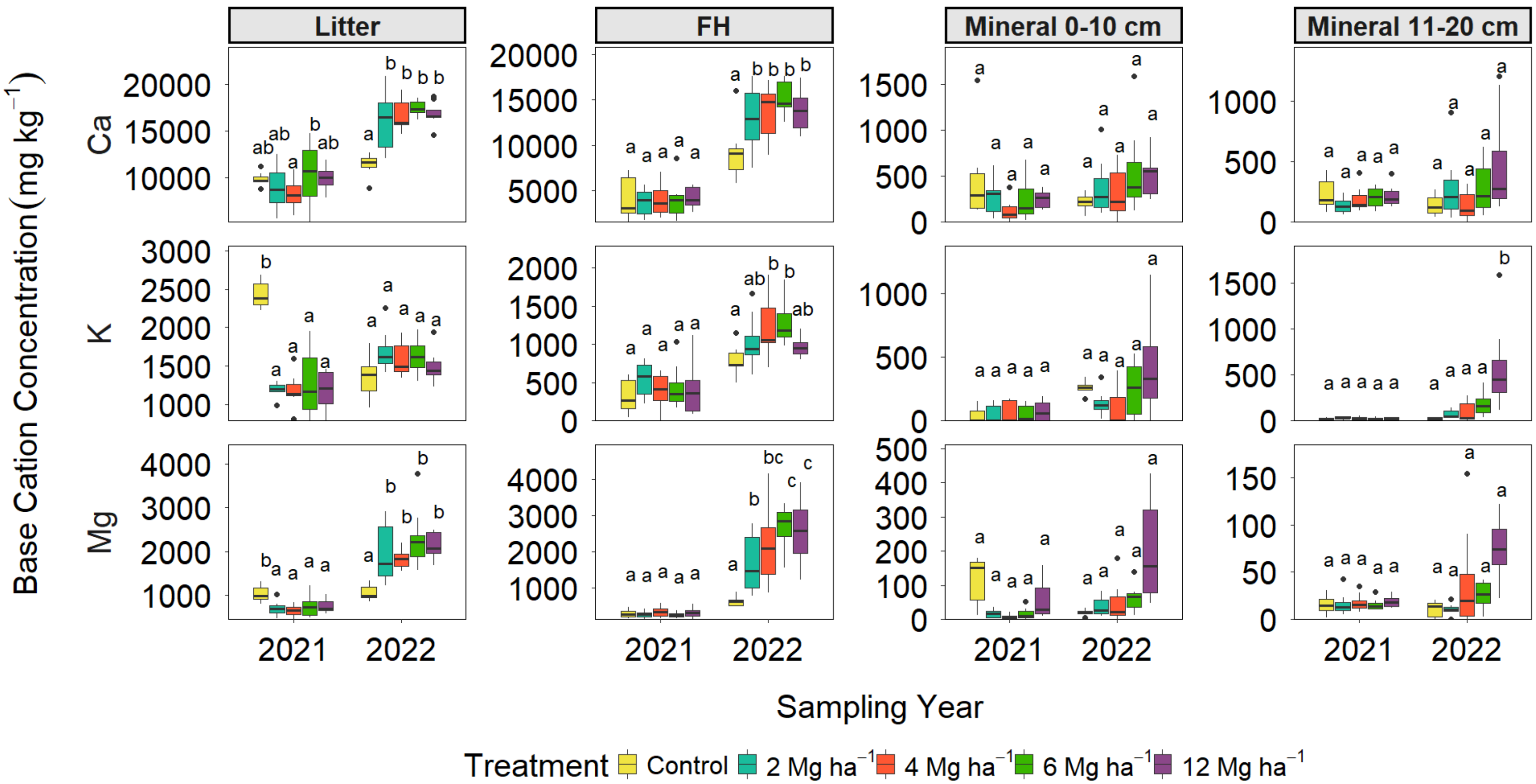

Figure 3.5 Average exchangeable soil base cation concentrations in L, FH, upper mineral (0 – 10 cm) and lower mineral (11 – 20 cm) horizons from baseline (2021) and one-year post NIWA application (2022). Different letters indicate significant differences ($p < 0.05$) using a post-hoc emmeans test between each treatment at each soil horizon.

Results of the ANOVA tests on soil metal concentrations indicate significant treatment effects post-application in the organic horizons for B (d.f. = 4, $F = 12.976$, $p < 0.001$), Cu (d.f. = 4, $F = 7.180$, $p < 0.001$), Mn (d.f. = 4, $F = 5.808$, $p < 0.001$) and Zn (d.f. = 4, $F = 6.302$, $p < 0.001$), but not for Al (d.f. = 4, $F = 0.929$, $p = 0.449$) or Fe (d.f. = 4, $F = 18.733$, $p < 0.001$), which displayed treatment effect only in the mineral soils. The addition of NIWA resulted in significant increases in several metals, including B, Cu, Mn and Zn in the L layer in 2022, one year after application. More muted increases were seen in the FH layer, predominantly for B, Cu and Zn, while Al, Fe and Mn changes were not elevated in response to NIWA treatment (Figure 3.6). There were no significant effects on mineral soil metal concentrations resulting from wood ash application for Al, B, Cu, Mn or Zn, however, there were small statistically significant treatment effects for Fe (Appendix Figure B).

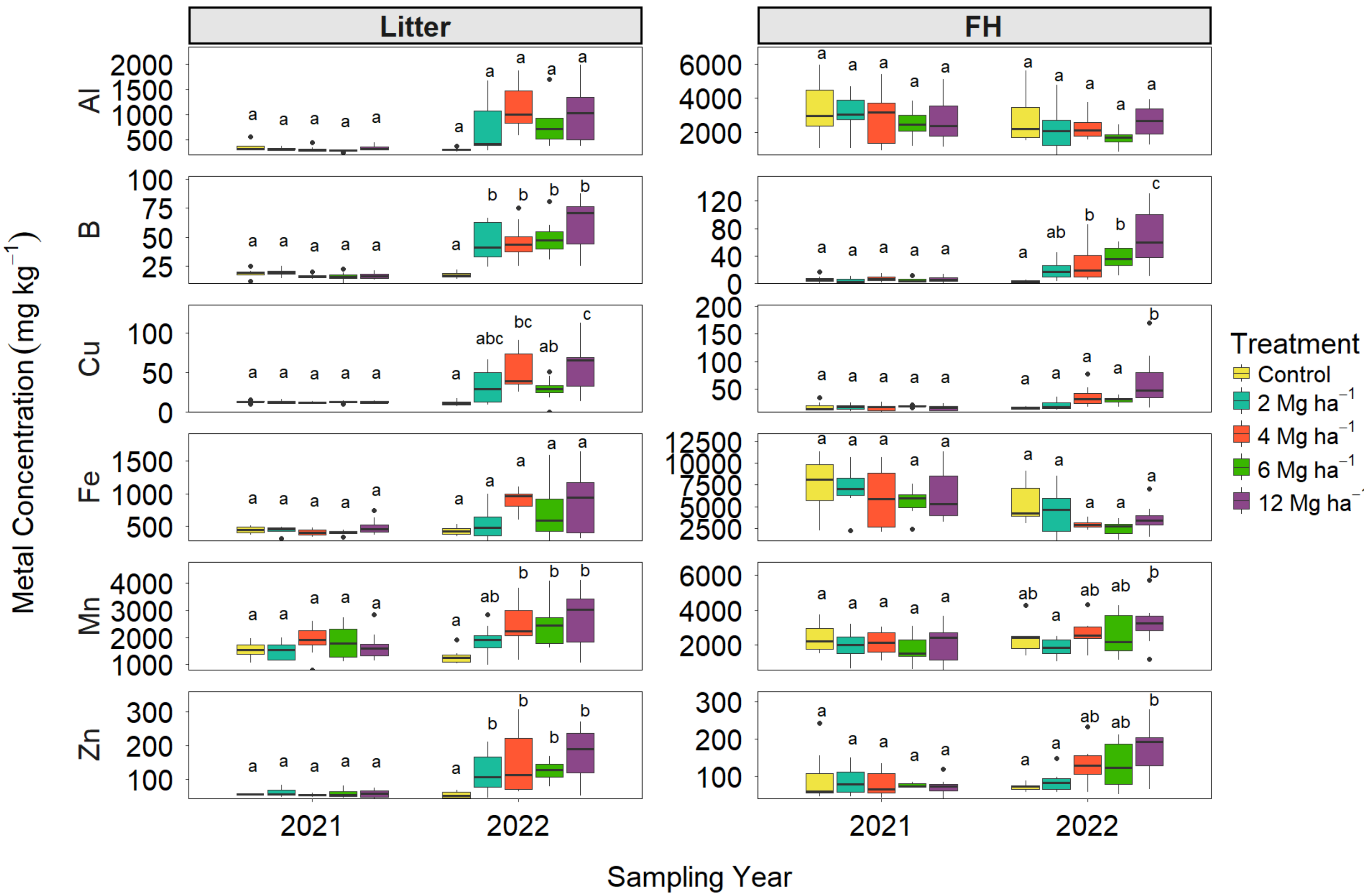

Figure 3.6 Average soil metal concentrations in L and FH horizons from baseline (2021) and one-year post NIWA application (2022). Different letters indicate significant differences ($p < 0.05$) using a post-hoc emmeans test between each treatment at each soil horizon.

3.4.2 Sugar maple seedling survivorship

There was a strong relationship between ash treatment and sugar maple seedling survivorship across both the 2022 and 2023 cohorts, with statistically significant differences amongst treatments ($Chi^2 = 37.2134$, $p < 0.001$ in 2022) and ($Chi^2 = 17.212$, $p < 0.002$ in 2023) (Figures 3.7 – 3.8). Across all treatments and cohorts, sugar maple seedling survivorship declined over time. However, sugar maple seedling mortality increased relative to controls at treatments > 4 Mg ha^{-1}. For the 2022 cohort, all seedlings in 6 and 12 Mg ha^{-1} treatments had died by July 2023 compared with 9.8 %, 8.5 % and 18.4 % survival in the control, 2, and 4 Mg ha^{-1} treatments respectively by September 2023 (Figure 3.7). Similarly, over the first year of growth for the 2023 cohort, the rate of seedling mortality was elevated in the 6 and 12 Mg ha^{-1} treatments with 15.4 % and 8.3 % survival in the first growing season compared with 63.3 %, 49.2 % and 20 % survival in the control, 2, and 4 Mg ha^{-1} treatments respectively (Figure 3.8). Weighted log-rank tests also suggested that in the 2022 cohort, there was earlier than expected mortality in the 6 and 12 Mg ha^{-1} treatments while in the 2023 cohort, there was earlier than expected mortality in the 4, 6, and 12 Mg ha^{-1} treatments.

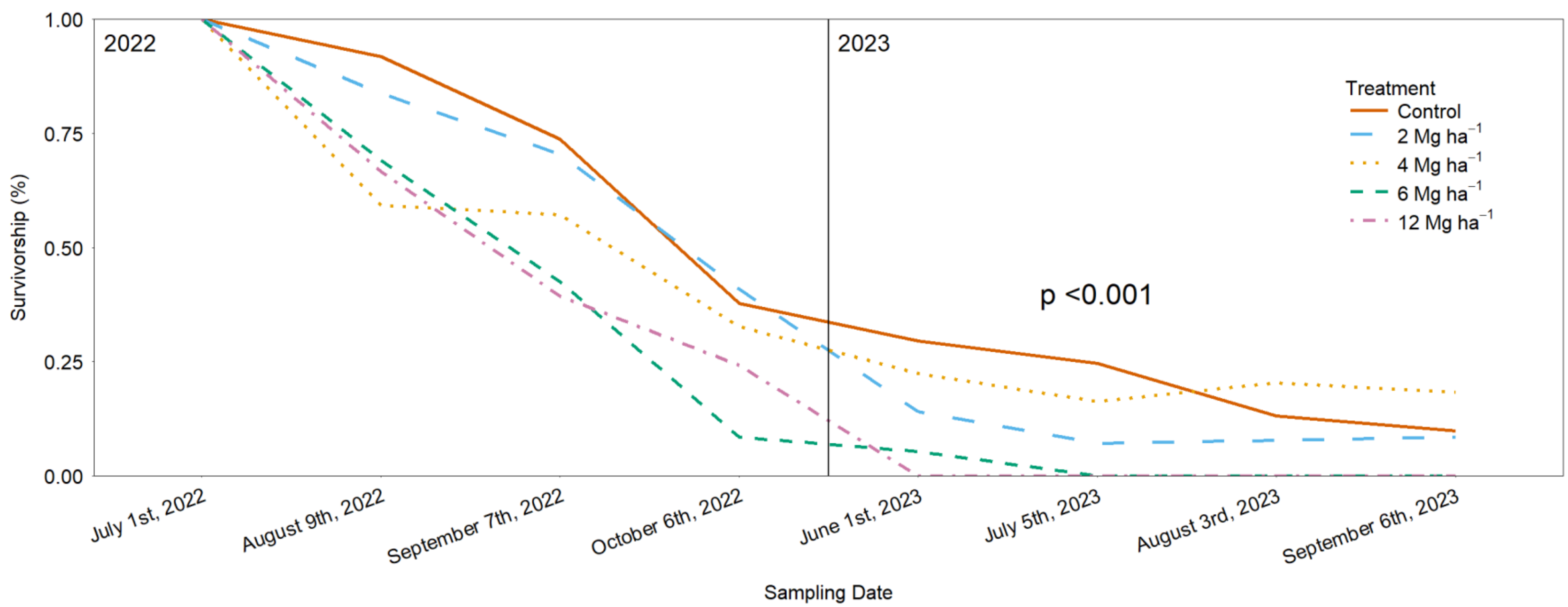

Figure 3.7 Sugar maple seedling survivorship for the 2022 cohort over two years (2022 – 2023). Survivorship is based on the average survival in two 1 m^2 subplots and then averaged for treatment effect. Significance was found using weighted long-rank tests with permutation.

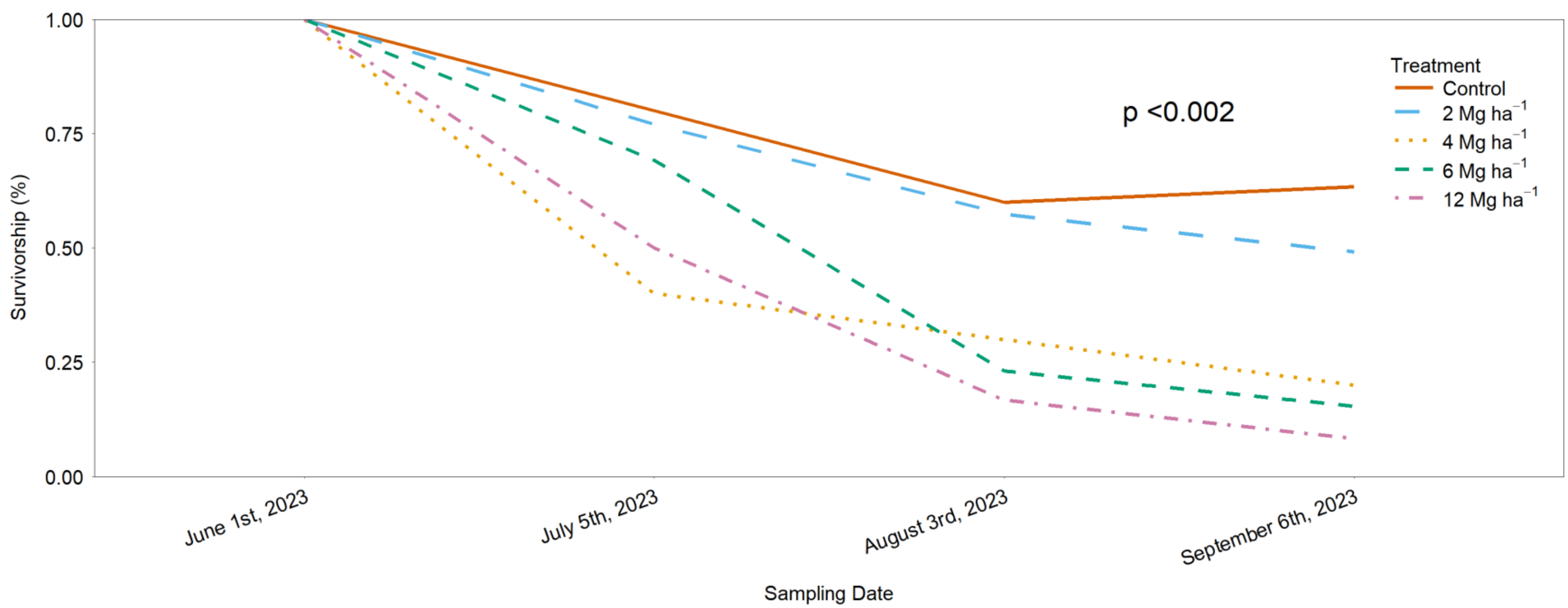

Figure 3.8 Sugar maple seedling survivorship for the 2023 cohort over one year (2023). Survivorship is based on the average survival in two 1 m^2 sub plots and then averaged for treatment effect. Significance was found using weighed long-rank tests with permutation.

3.4.3 Sugar maple seedling growth

Sugar maple growth was assessed by looking at the biomass of surviving first-year seedlings, and the height and diameter of seedlings. Biomass measurements were only available in 2022 because a poor seed year in the fall of 2022 resulted in insufficient seedlings for destructive sampling in 2023. Results of the ANOVA tests on sugar maple seedling biomass indicate no significant treatment effects for either above-ground biomass (d.f. = 4, $F = 1.916$, $p = 0.145$) or below-ground biomass (d.f. = 4, $F = 1.479$, $p = 0.244$), although biomass tended to be lower at treatments > 4 Mg ha^{-1} (Figure 3.9).

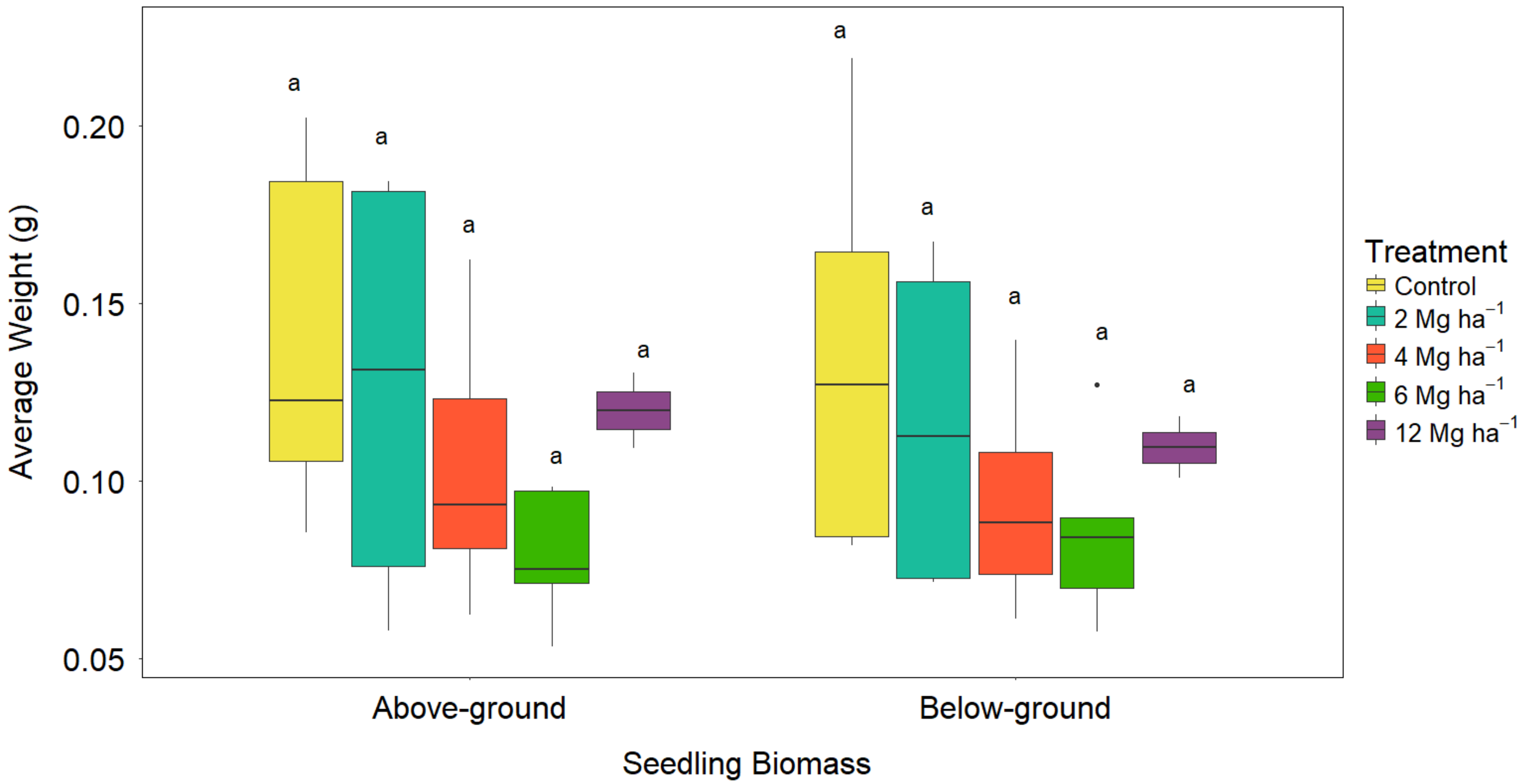

Figure 3.9 Average weight of seedling above-ground and below-ground biomass in 2022. Different letters indicate statistically significant differences ($p < 0.05$) using a post hoc emmeans test between each treatment each year.

Results of the linear mixed model using treatment as the fixed effect and plot and sampling date as the random effects found that there was no significant effect of each treatment on surviving seedling height in 2022 (left panel) (d.f. = 18.788 – 21.988, T = -1.725 – -0.379, p = 0.101 – 0.260), compared to control. Although by 2023, only treatments ≤ 4 Mg ha^{-1} were left growing. For the 2023 cohort (right panel), there was also no treatment effect on height (d.f. = 10.219 – 17.810, T = -1.783 – -0.027, p = 0.0947 – 0.979), compared to the control. For the 2023 cohort (right panel), there were no treatment effects on height at $p < 0.05$, similar to the first year of the 2022 cohort (Figure 3.10).

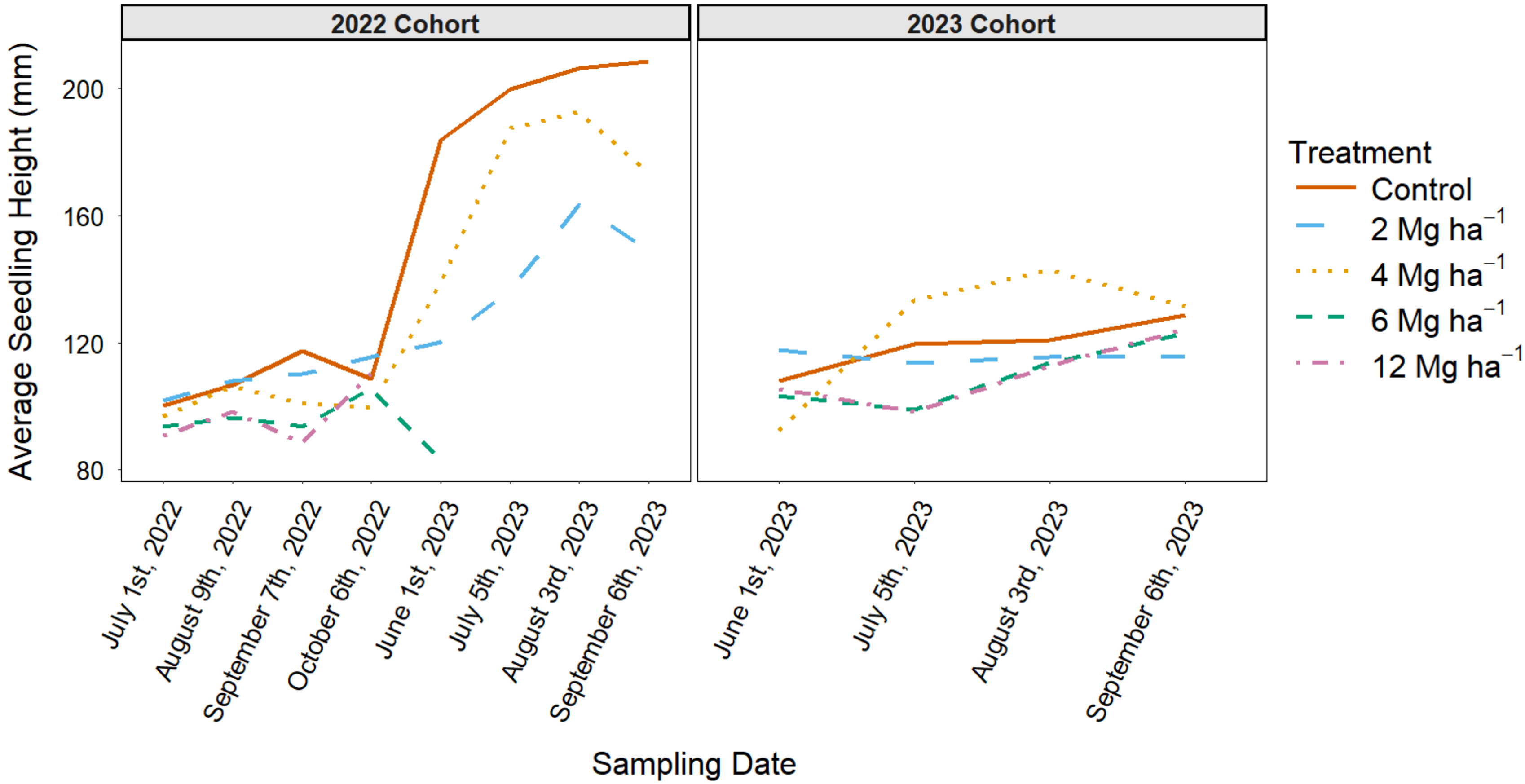

Figure 3.10 Average sugar maple seedling height of the 2022 and 2023 cohorts. The average of each subplot and plot is calculated followed by the average of each treatment. A linear mixed effects model was used to determine significance at $p < 0.05$.There was no significant effect of each treatment on seedling height in either cohort.

For seedling diameter measured at root collar, results of the linear mixed model using treatment as the fixed effect and plot and sampling date as the random effects found that there was no significant effect of each treatment on surviving seedling diameter in 2022 (left panel) (d.f. = 18.978 – 21.890, T = -1.120 – 0.083, p = 0.277 – 0.935), compared to control. Although by 2023, only treatments ≤ 4 Mg ha^{-1} were left growing. For the 2023 cohort (right panel), only the 6 Mg ha^{-1} plot showed a significant treatment effect (d.f. = 15.951, T = -2.510, $p < 0.05$), while the remaining treatments showed no treatment effect on diameter (d.f. = 11.100 – 18.677, T = -1.481 – -0.972, p = 0.286 – 0.352), compared to the control (Figure 3.11).

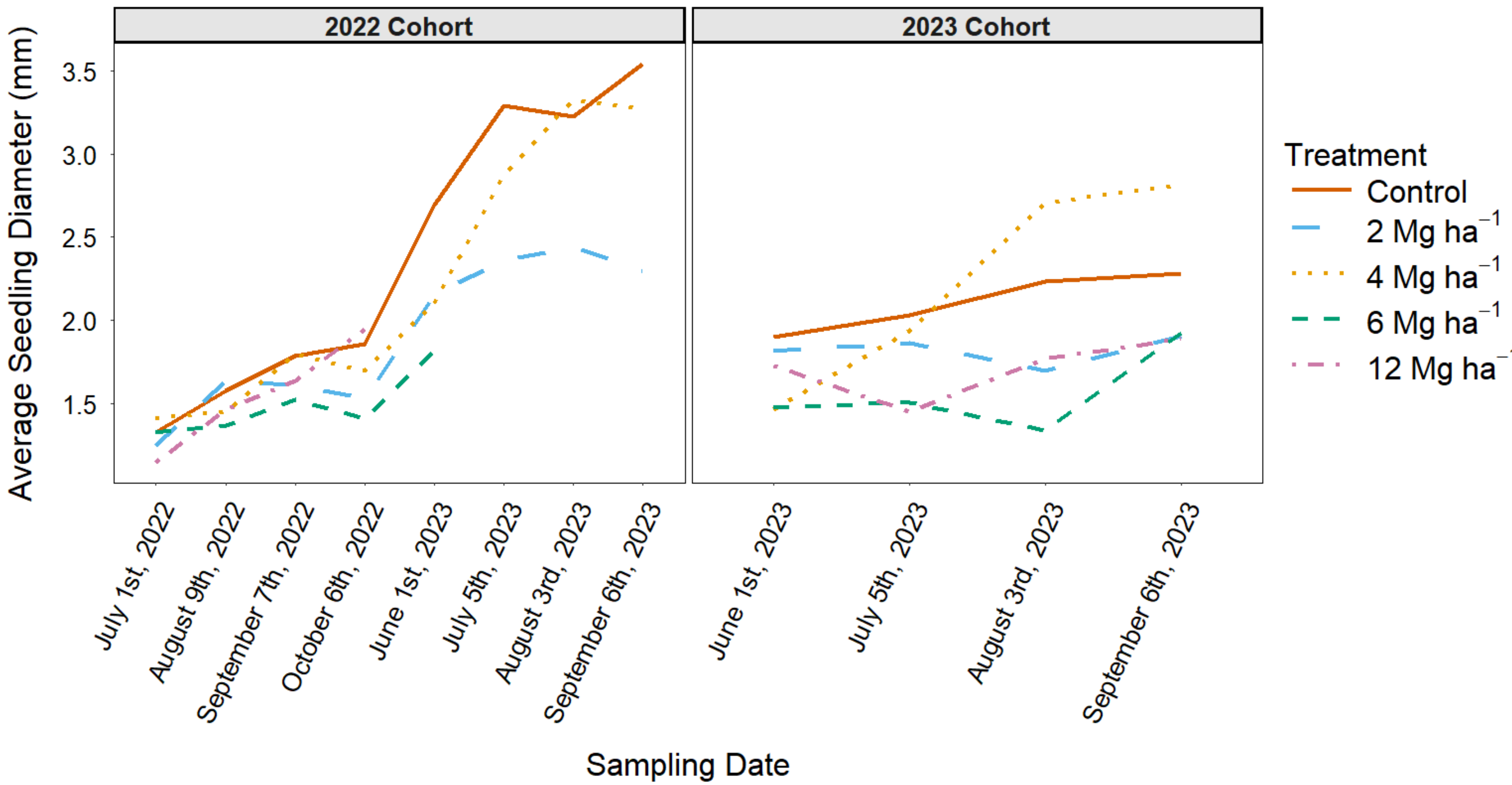

Figure 3.11 Average sugar maple seedling diameter of the 2022 and 2023 cohorts. The average of each plot is calculated followed by the average of each treatment. A Linear mixed effects model was used to determine significance at $p < 0.05$. There was no significant effect of each treatment on surviving seedling diameter in 2022. For the 2023 cohort, only the 6 Mg ha^{-1} plot showed a significant treatment effect.

3.5 Discussion

The addition of NIWA greatly altered soil chemistry following application, especially in the upper organic soil horizons where sugar maple seedlings are rooted. The application of NIWA negatively affected sugar maple seedling survival at dosages > 4 Mg ha^{-1}, with full mortality within two years in higher treatment doses, although the growth of surviving sugar maple seedlings was not affected by wood ash application.

3.5.1 Soil chemistry

Following the application of NIWA in 2022, there were large increases in soil pH (2 – 3 units) in both the L and FH layers compared with the control, while effects in mineral soil were muted and only apparent in the highest treatments. In 2023, the response of the L layer was transient as values returned to baseline after two years, whereas the pH of the FH layer remained greatly elevated. Effects remained muted in the mineral soil with increases only apparent in the highest treatments. The increase in soil pH can be attributed to the highly alkaline nature of wood ash resulting from the presence of metal oxides contained within the ash (Johansen et al., 2021). These metal oxides are largely derived from plant tissues. When the ash begins to weather the metal oxides react with water and carbon dioxide, producing carbonates and hydroxides (Kahl et al., 1996). The result is an exceedingly basic or alkaline amendment with high neutralizing ability (Demeyer et al., 2001), with wood ash pH ranging between 8 – 13 (Agusto et al., 2008). The variability in ash characteristics is attributed to variables like species of source material (Deighton & Watmough, 2020), type of plant tissue combusted (Werkelin et al., 2005), temperature at which the tissue was combusted, how the ash was stored (Etiégni & Campbell, 1991) and if the ash was weathered before use (Gori et al., 2011). These findings are comparable to other local central Ontario studies such as the one performed by Conquer et al. (2024) that found NIWA additions of 6 Mg ha^{-1} resulted in statistically significant increases to soil pH within one year of application in the L and FH layer. Conquer et al. (2025) found similar results with 4 Mg ha^{-1} and 8 Mg ha^{-1} additions at three nearby sugar bush sites within the L and FH layers one-year following application. It is noteworthy that these applications used ash that was also produced by the Friends of the Muskoka Watershed around the same time. Looking at other local projects that used different wood ash showed similar results, such as Deighton & Watmough, (2020) which found a roughly 0.4 – 0.7 pH unit increase three months following 6 Mg ha^{-1} ash addition. The significant and large changes in soil pH were to be anticipated as soils with lower starting pH and organic matter content are expected to exhibit higher rates of change immediately following wood ash addition (Ohno, 1992). The bulk of the pH response being limited to the organic LFH horizons was also anticipated, as the NIWA needs time to percolate through the soil profile, as seen in other studies (Hansen et al., 2016; Reid & Watmough, 2014). For example, European studies have found increases in mineral soil pH > five years after application, lasting sixteen years at dosages of only 3 Mg ha^{-1} (Saarsalmi et al., 2001; Saarsalmi et al., 2004). The highest treatment (12 Mg ha^{-1}) results in this study are comparable to a study conducted in southeastern Quebec, where a wood ash addition of 20 Mg ha^{-1} was again shown to increase soil pH three years post-application, with only slightly significant increases in the upper mineral soils (Arseneau et al., 2021). These results are also consistent in European studies such as a 2004 study in Lithuania that found a 5 Mg ha^{-1} wood ash addition increased organic horizon pH from 3.45 to 6.15 within three months of application (Ozolinčius et al., 2005).

Likewise, a microcosm study in Denmark using industrial wood ash (pH 12.9) dosages of 5, 22 and 167 Mg wood ash ha^{-1} were found to positively correlate with pH values in the soil and increase soil pH in the 0 – 10 cm mineral soil to roughly 5.5, 7.5 and 10.75 respectively after about 40 days (Bang-Andreasen et al., 2017). This supports this study's findings that wood ash addition increases soil pH following application. Given the results of this study and those reported in literature it can be expected that the bulk of the response in the mineral horizons at this site will be seen in roughly another three sampling seasons, and may persist for a decade or more beyond that. Given the results of Saarsalmi et al. (2001 and 2004) it can be anticipated that the response in some of the higher treatment plots > 4 mg ha^{-1} may persist even longer than 16 years. This illustrates the requirement of long-term studies that will inform if whether one application is suitable or repeat applications may be more beneficial to these forest ecosystems.

Following the application of NIWA in 2022 decreases in OM were observed in the L layer. The response was short-lived as OM returned to baseline in 2023. Changes in the FH layer were not consistent in 2022 and 2023, however reductions in OM were observed at the highest treatment. The changes in the organic horizons are most likely due to the wood ash itself as it possesses a low OM content (3.4 %). The effects of wood ash addition on the mineral soils were not consistent across 2022 and 2023. These results are comparable to other local NIWA applications whereby there were significant reductions in OM reported one year after 6 Mg ha^{-1} dosing in the L layer, however, only nonsignificant reductions were seen in the FH following this application (Conquer et al., 2024). Similarly, at three sugar bush sites in the Bracebridge and Muskoka ON, area, significant reductions in soil organic matter were seen one year following 4 and 8 Mg ha^{-1}

additions compared to controls (Conquer et al., 2025). Similarly, a European study in Finland found wood ash additions only resulted in SOM changes in the O–horizon of soils following 3 Mg ha^{-1} treatments. Comparably, a first-of-its-kind Canada-wide study on wood ash and soil organic matter found that wood ash addition did not result in consistent or measurable changes to OM content, however, when differences were seen they were typically closer to the surface (Joseph et al., 2022). These results support this study's findings of predominantly significant results in the upper organic soils along with otherwise inconsistent effects in the other mineral horizons.

Significant increases in Ca and Mg were observed in the L and FH layers of the soil one year following ash application, whereas no significant changes were observed in the upper and lower mineral soils at that time. Comparably, similar studies in Canada (Arseneau et al., 2021; Conquer et al., 2024) and in Europe (Rumpf et al., 2001; Arvidsson & Lundkvist, 2003; Saarsalmi et al., 2004) have shown Ca and Mg concentrations to increase in the upper soil layers following wood ash application. This increase in Ca and Mg in the organic horizons can be attributed to the high quantities of Ca and Mg present in the wood ash being directly applied to the soil surface. For example, the ash in this study contained 27 % Ca (267 g kg^{-1}) and 2 % Mg (19.4 g kg^{-1}), which are the first and third most abundant metals, with 9 % K (94.4 g kg^{-1}) being second, which is consistent with ash samples reported in Azan et al. (2019). This high concentration of Ca and Mg present in ash results in an immediate addition of these base cations that is localized to the organic horizons immediately following application. The reason for the limited response in Ca and Mg in the mineral soils is likely due to the surface applied ash not having adequate time or quantity to percolate down through the soil profile within a year of application. This can be attributed to the lower solubility of Ca and Mg in ash compared to other elements such as K, B, S, and sodium (Na) (Augusto et al., 2008). For example, Arseneau et al. (2021) found only minor increases in Ca and Mg in mineral soils (0 – 20 cm) compared with controls in their study using a 20 Mg ha^{-1} dosage nearly three years following application, suggesting that significant increases in soil Ca and Mg are only seen in mineral soils several years following application at high dosages. Conversely, K behaved differently, showing no observable trends in the organic layers but significantly increased concentrations in the 12 Mg ha^{-1} treatment in the lower mineral soils. This is comparable to findings in central Ontario that reported typically no effect on K concentrations in organic horizons twelve months following industrial ash addition (1, 4 and 8 Mg ha^{-1}) (Noyce et al., 2016) and significant increases in K concentrations in mineral soils following 6 Mg ha^{-1} ash additions (Conquer et al., 2024). Several studies outside of Canada have also found K to increase in the mineral horizons following application (Bramryd & Fransman, 1995; Kahl et al., 1996; Arvidsson & Lundkvist, 2003), with typically poor K recovery in soil samples compared with the proportion of K added (Jacobson et al., 2004). Interestingly, other studies have not found increases in K in mineral soils one and three years after application (Deighton & Watmough, 2020; Arseneau et al., 2021), suggesting that K is not being retained after application. Ohno (1992) found that a large and rapid release of K upon land spreading of wood ash is to be expected. This is because K is highly soluble, and large quantities can be released from the wood ash rapidly after application. Smith et al. (2024) found elevated levels of K in soil water in the LFH and 30 cm soil depths in the year immediately following 2.5, 5 and 7.5 Mg ha^{-1} ash applications compared to control, indicating K movement in soil water following ash addition. The reason for this solubility is partly due to K mainly existing in the form of soluble salts in wood ash (Steenari et al., 1999), such as the dissolution of KCl (Maresca et al., 2018), while Ca exists as carbonates (Steenari & Lindqvist, 1997) and Mg as MgO or magnesium silicates (Steenari et al., 1999). As a result, K is more soluble than Ca and Mg in wood ash (Meiwes, 1995). For example, a study of various types of ash addition to Mediterranean soils found that K leaching was substantially higher (34 – 68 %) than Ca (1.6 %) and Mg (3.3 %) over twenty-four months and increased with application rate (Gómez-Rey et al., 2012). Another reason for the low K recovery in the soil is that Ca is more attracted to soil exchange sites than K, leading to K being displaced from exchange sites (Ohno, 1992; Arvidsson & Lundkvist, 2003) and leached from the system.

Concentrations of some metals (B, Cu, Mn, and Zn) also increased following ash application in the organic soil layers, which is consistent with previous studies in Ontario (Deighton & Watmough, 2020; Conquer et al., 2024; Conquer et al., 2025). Studies in Europe corroborate these findings as metal concentrations including B, Cu, Mn, or Zn have shown varying increases in the organic horizons following application (Saarsalmi et al., 2004; Ozolinčius & Varnagirytė, 2005; Hansen et al. 2018). Most studies also report negligible effects on metal concentrations in mineral soils following application (Hansen et al., 2018; Conquer et al., 2024; Conquer et al., 2025). Interestingly, one study found that B and K are more easily released from wood ash (indicative of higher solubility), while heavy metals, Ca and P are highly insoluble (Nieminen et al., 2005). These solubility rates account for why there are consistent negligible effects in the mineral soils of wood ash studies, and why B also increase in the FH layer following application. One reason for low metal solubility is that metal compounds exhibit low chemical solubilities in high pH conditions (such as following ash application). It can be expected that in the long term, as the soil pH decreases or stabilizes, leaching of some metals may begin (Steeneri et al., 1999), suggesting the need for long-term monitoring of soil conditions. One laboratory experiment found that while metals tend to be immobile under alkaline conditions, B, Cu, Mn, and Zn aqueous concentrations from ash samples can increase when pH decreases (Rehl et al., 2022). This data is supported by the findings of this study as elevated B, Cu, and Zn concentrations were seen in the FH horizon following ash addition. Another suggested reason for the accumulation of metals in the organic soils could be because of increased metal binding to organic substances (Bramryd & Fransman, 1995). This observation is well documented with a laboratory batch experiment finding that sorption of heavy metals was 6 – 13 times higher on organic matter than mineral soils (Lair et al., 2007). Furthermore, a study in Sweden found that organic matter was a crucial sorbent for heavy metals in temperate and boreal climates in surface soil horizons (Gustafsson & Pechová, 2003).

It is worth noting that soil pH, base cation concentrations and metal concentrations can be highly variable within the same treatments. For example, in the 12 Mg ha^{-1} treatment soil pH values in the FH layer can be between 5.92 – 8.04 pH units, while Ca concentrations in the FH layer can be between 10, 941 – 17, 382 mg kg^{-1}. Wood ash exhibits a large variability in OH, CO_3 and HCO_3 ratios that result in variable alkalinity (Denmeyer et al., 2001), as shown by a pH range of 8 – 13 in wood ash (Agusto et al., 2008). Wood ash is also highly variable in terms of macro and micro elements (Denmeyer et al., 2001), both between different ash sources (Adotey et al., 2018;

Deighton & Watmough, 2020) and within amalgamated samples of the same source material (Syeda et al., 2024). The heterogeneity of ash coupled with the manual hand spreading application, as done in this study, can also result in localized doses far below or far over the target dosage, producing soil pockets exhibiting considerably variable pH and base cation concentrations. This significant variability in alkalinity and base cation saturation coupled with the rapid pH changes observed in the organic horizons can in turn affect other aspects of the forest ecosystem, including sensitive forest biota such as insects, microbes and mycorrhizae, and vascular plants such as seedlings which root within the organic layers of the soil. These populations can be subjected to significant and rapid pH changes which could result in adverse effects that require additional exploration before wood ash use can be derestricted for use and upscaled in Canada.

3.5.2 Sugar maple seedling survivorship

Weighted long-rank tests show statistical differences between treatments both in the 2022 and 2023 cohorts with $p < 0.001$ and $p < 0.002$ respectively. The 2022 cohort seedlings treated with NIWA at dosages > 4 Mg ha^{-1} experienced complete mortality by the end of the second growing season, most likely due to some physiological effect of the caustic ash such as pH or salt shock. The 2023 cohort appeared to show a similar trend with the 6 and 12 Mg ha^{-1} treatments experiencing the highest mortality by the end of the study period. Total first-year seedling recruitment in 2023 was also low, as the sugar maples exhibited a poor seed year during the fall of 2022. No studies have looked at the effect of wood ash on sugar maple survival, but studies have examined sugar maple seedling survival following another Ca amendment (wollastonite; a calcium silicate mineral) (Juice et al., 2006; Cleavitt et al., 2011). These studies applied 4.6 Mg ha^{-1} to a small watershed in New Hampshire (Peters et al., 2004) and found that sugar maple seedling survival did increase following treatment after several years (Cleavitt et al., 2011), but the effect was not immediate as early cohorts experienced low seedling density in treated plots (Juice et al., 2006). In addition to dosage, the main difference between the New Hampshire studies and this study is that wood ash contains higher concentrations of metals and other nutrients (Azan et al., 2019) and has a higher pH range (8 – 13 pH units) (Agusto et al., 2008), compared with 10 – 10.4 pH units for wollastonite (Fernandez-Caliani et al., 2008; Chai et al., 2021). This results in wollastonite dissolution producing smaller pH changes than calcium carbonates (Balaria et al., 2015) that are abundant in wood ash. For example, the wollastonite-treated studies of 4.6 Mg ha^{-1} (0.85 Mg Ca ha^{-1}) (comparable to 4 Mg ha^{-1} wood ash) resulted in soil pH values of roughly 4.21 in the Oie horizon, 3.24 in the Oa horizon, and 3.47 in the mineral soils one year after application (Cho et al., 2010) compared with values as high as 6.57 in the L layer, 7.11 in the FH layer, and 4.69 in the upper mineral soils following 4 Mg ha^{-1} wood ash addition. The Cleavitt et al. (2011) study was also conducted several years following treatment (with a relatively low calcium dosage), and thus, the authors hypothesized that the smaller amount of Ca had moved through the soil profile and into the deeper horizons. Their findings and the findings of this study suggests that immediate seedling mortality may be expected and that it may be a short-term result of using calcium-rich soil amendments under a certain dosage threshold. Comparably, the results of a biochar addition study in Ontario found enhanced seedling development in several coniferous and deciduous species, including sugar maple (Thomas, 2021). Further showing the beneficial results following Ca addition. Augusto et al. (2008) synthesized the results of several studies on the effects of wood ash addition to tree seedlings and found great variability in the results ranging from positive, no effect, or negative effect on growth and survival rate. The authors suggest that the variability may be due to factors such as species, time span, and soil type. The authors also state that part of the variability in results may be because the addition of high pH ash may induce biochemical soil changes and subsequent physiology changes in seedlings due to reduced resiliency (Augusto et al., 2008). Specifically, sugar maple seedlings have been shown to be sensitive to nutrient availability (St. Clair & Lynch, 2005; Park & Yanai, 2009). Sugar maple seedlings have shallow roots that are located below the L layer and above the mineral soils (Godman et al., 1990), which was evident during destructive sampling as most seedling fine roots were found within the FH layer of the soil, while the tap root descended into the upper mineral soils. The shallow roots allow the seedlings to extract water and nutrients from the humus layer until the root systems become more developed (Nyland, 1999). The location where sugar maple seedlings root is also where the most significant pH changes occur, with values in the 6 and 12 Mg ha^{-1} treatments over 6, and up to 8 potentially leading to pH shock. Wood ash has been shown to react quickly when applied to the soil surface (Kahl et al., 1996) but the large pH increase does not last long because the oxides, hydroxides and carbonates present in ash are highly soluble and do not persist in the soil as long as calcites (Ulery et al., 1993). These findings would suggest that the pH shock in the soil may be short-term and that survivorship of the seedlings in the 3 – 5 years following wood ash addition may become more consistent with lower doses and the control. Continuing the duration of this study, similar to Cleavitt et al. (2011) would be advantageous to determine if a similar effect occurs with wood ash treatment.

Sugar maples exhibit survival strategies consistent with R–type strategists. These strategists produce many offspring that suffer high mortality early in life. Therefore, it is expected that survivorship will decline rapidly within the first several years following seeding, especially after the first year. All treatments and the control exhibited this pattern with rapidly declining survivorship over the duration of the study. Other studies of sugar maple seedling survivorship have also shown this behaviour with high mortality in the first growing season (Cleavitt et al., 2014). However, alternative reasons for the increased mortality in the 6 and 12 Mg ha^{-1} treatments could be the elevated nutrient or metal content in the organic soils post-application. Sugar maple seedlings have been shown to be sensitive to nutrient availability (St. Clair & Lynch, 2005), and introduction of excess nutrients may be responsible for this mortality. For example, a study in Haliburton Ontario found that Ca levels in seedlings were significantly higher than in control plots following ash addition (Deighton et al., 2020) and foliar Ca levels were above the critical threshold for healthy trees as per (Kolb & McCormick, 1993). Plants are considered capable of tolerating varying amounts of Ca in their rhizosphere and excess Ca in some species can lead to calcium-induced toxicity (White & Broadley, 2003). This toxicity may have been exhibiting itself as pH shock, similar to effect seen on non-vascular plants. Looking beyond pH shock and base cation toxicity, an alternative causation may be metal toxicity. For example, sugar maple seedlings have been found to be sensitive to excess Mn (Schier & McQuattie, 2000; St. Clair & Lynch, 2005) which may result in symptoms of toxicity. Mn was found to be elevated in both L and FH soils following application, which is generally unexpected given the relationship between Mn and soil pH (Watmough et al., 2007), thus suggesting the Mn is coming from the ash itself and could have been accessible to the seedlings. Results of a repeat wood ash addition experiment found increases in soil exchangeable Mn along with increases Mn concentrations in mushroom species (Omil et al., 2007), indicating potential introduction and uptake of Mn following ash addition. Ultimately however, this mechanism remains unlikely. The much more likely mechanism impacting sugar maple seedling survival may be salt shock. Specifically wood ash contains a high level of soluble salts that are released upon surface spreading, especially K salts (Steenari et al., 1999). This increase in salts in the soil is observed by the increase in electrical conductivity seen in several studies (Staples & Van Rees, 2001; Deighton & Watmough, 2020) and by increased Sodium (Na) values seen in Pugliese et al. (2014). Furthermore, Augusto et al. (2008) reports that large increases in K, Na, and SO_4 concentrations can be seen as the result of the dissolution of salts, and this salt content can cause modification of plant physiology. Likewise, Nawaz et al. (2010) state that large amounts of salt in the plant rooting zone can cause osmotic stress, which can inhibit the plant from up taking water and nutrients. Excess Na can also lead to the opposite, inhibition of K uptake, and thus nutrition imbalance (Nawaz et al., 2010). Staples & Van Rees (2001) report significantly decreased seedling growth of white spruce (*Picea glauca* (Moench) Voss) seedlings following 5 Mg ha-1 wood/sludge ash addition, which they contribute to salt phytotoxicity.

These findings carry serious implications for the adoption of wood ash as a commonly used soil amendment and for its derestriction in Canada. To date, there have been a limited number of studies indicating that wood ash is unsafe for use, even the metal concentrations present in soil following application seen in this study provide little concern to its use. However, the significant levels of mortality seen in this study are cause for concern, as long-term mortality of sugar maple could significantly alter forest composition in these ecosystems. Therefore, further research is required to tease out the exact mechanisms resulting in this mortality and once found, if there is a way that they can be limited, such as applying wood ash in conjunction with another amendment or limiting dosages under 4 Mg ha^{-1}. Alternatively, research suggests that weathering or stabilization of ash can reduce some of its caustic nature (Steenari et al., 1999), potentially limiting its effects on biota. Similarly, applying it in pelleted form at dosages less than 5 Mg ha^{-1} as opposed to the loose ash used in this study may further reduce shock effects (Andreas Aronsson & Ekelund, 2004). However, weathering of ash does not necessarily reduce the release of soluble salts (Steenari et al., 1999). There must also be consideration for factors not considered in this study, as it is more likely to be a combination of factors influencing mortality. Factors such as fungal damage, herbivory, rodent tunnelling, and winter freeze are all important variables in early sugar maple seedling survival (Cleavitt et al., 2014). These variables were not tracked in this study and could influence sugar maple seedling survival independent of NIWA addition. Evidence of herbivory was observed throughout the duration of this study and drought conditions were readily seen in peak summer months, both of these would be worth examining in future research.

3.5.3 Sugar maple seedling growth

While NIWA application had a pronounced negative effect on survivorship, there were limited effect of ash on the growth of surviving seedlings with respect to height, diameter and biomass. Regarding biomass, the results of this study (no effect) were consistent with the findings of other Ontario studies that found no significant effects on sugar maple seedling biomass following ash application (Noyce et al., 2017; Deighton et al., 2020). In both diameter and height, there did not appear to be any significant effects following ash addition. The 6 and 12 Mg ha^{-1} treatments generally appeared to show the most limited growth. However, this is directly related to their decreased survivorship as seedlings in those treatment groups generally did not live long enough to get bigger. Similarly, wood ash additions of 4, 8, 12, 16 and 20 Mg ha^{-1} were studied on red maple (*Acer rubrum* L.) seedlings in a greenhouse experiment and no significant changes between treatments were seen in seedling diameter or height over eighteen weeks (Unger & Fernandez, 1990). In Quebec, Canada, a wood ash dosage of 5, 10 or 20 Mg ha^{-1} resulted in no significant changes in sugar maple seedling height and diameter up to four years after wood ash addition (Arseneau et al., 2021).

The simple reason for this outcome may be that sugar maple seedlings are not as nutrient-limited in these acidic forest ecosystems as previously believed, and this has been widely speculated. Instead, some other factor may be more important for growth, for instance, Arseneau et al. (2021) speculate that the presence and absence of light may be the more important factor for seedling growth. Numerous studies have shown sugar maple seedlings to experience increased growth with increased light addition (Beaudet & Messier, 1998; Marks & Gardescu, 1998; St. Clair & Lynch, 2005). Light availability could also be factoring into the results of this study, though treatments are randomized throughout the forest site, localized light availability could be influencing seedling growth. Interestingly, another study looked at sugar maple seedlings after both liming and releasing the canopy above them and found this combination to have the strongest effect on growth (114 %) compared to just canopy release (58 %) or liming (82 %) over seven years (Duchesne et al., 2013). Another study found that fifteen years post-lime application height and diameter of seedlings were inversely related to liming application rate. The authors speculate that the lime increased competition for other resources like light and space (Moore et al., 2012). This finding suggests that light release may be more beneficial to seedling growth than Ca addition. Another plausible explanation for the lack of treatment effect on growth may again relate to the notion of Ca toxicity discussed in seedling survivorship. In a study looking at Ca addition to Chinese poplar (*Populus simonii* Carrière) seedlings, Ca was shown to first improve growth and biomass and then decrease growth and biomass past a certain threshold. Thus, the author suggests there is an optimal Ca gradient for growing poplar seedlings (Weng et al., 2022). Comparably, a study on jack pine (*Pinus banksiana* Lamb.) seedlings and Ca sensitivity under varying pH showed that high Ca concentrations worsened the physiological effects of high pH on the seedlings such as inhibition of root cell elongation, meaning seedling growth (Zhang et al., 2015). These same mechanisms may be at play in sugar maple seedlings and may account for why seedling growth is not positively impacted by ash addition, contrary to the results of other studies that found increased seedling growth following Ca addition. For example, the results of a liming study showed surface liming to not significantly reduce growth compared to control, while incorporating the lime resulted in increased growth of two-year-old sugar maple seedlings compared to control (Burke & Raynal, 1998). Kobe et al. (2002) showed significantly increased seedling diameter growth in sugar maples with calcium chloride addition. These studies suggest that under the right conditions, calcium addition in general may in fact improve seedling growth and raises questions pertaining to why wood ash specifically does not follow this trend. This could be a result of the other nutrients and metals present within the ash. For instance, K toxicity under high dosages impacting growth, as suggested by Etiégni et al. (1991). The results of this study pertaining to sugar maple growth following wood ash addition suggest the need for further research. While this study did not yield a treatment effect on sugar maple growth, the results of other soil amendment studies such as that by Thomas (2021) suggest potential pathways where Ca addition should increase sugar maple growth. Perhaps the specific site conditions post application (such as a new limiting nutrient) are restricting growth. Further, the presence of some kind of multi-factor synergism between nutrient levels, light levels and Ca addition is likely, suggesting the need for exploration into the effects of wood ash application on sugar maple seedling growth when there is different light and nutrient availability within these sites in central Ontario.

3.6 Conclusions

The purpose of this study was to evaluate the effects of variable NIWA applications on sugar maple seedling survivorship and growth to determine if NIWA is a suitable soil amendment for acidified forest soils. Results on soil chemistry reaffirm years of previous research and show that NIWA increases soil pH in the organic and upper mineral soil and base cation concentrations and increases in metal content are restricted to upper soil layers. At dosages between 2 and 12 Mg ha^{-1} wood ash does not appear to influence seedling height or diameter within two years of application. However, sugar maple seedling mortality is elevated at dosages exceeding 4 Mg ha^{-1}. Mechanisms causing higher mortality could relate to pH shock and Ca toxicity due to seedling sensitivity and saturation of the rhizosphere in which the seedlings draw nutrients. More investigation is required to characterize these effects. Overall, these findings continue to support related studies in that NIWA, at dosages $\leq$ 4 Mg ha^{-1} can improve soil pH and base status without adverse impacts on sugar maple seeding growth and regeneration.

4.0 General conclusion

The broad objective of this thesis was to assess the viability of wood ash (NIWA) as a soil amendment and the effects on ecosystem regeneration. Findings from both chapters reaffirm years of previous research both in Europe and North America and shows that NIWA rapidly (within one year) increases soil pH in organic and upper mineral soils and that treatment increases base cation concentrations (Ca, K, Mg), and increases metal concentrations (especially B, Cu, Mn, Zn) in the upper soil layers, with these responses being positively correlated with increasing treatment. At dosages of 4 and 8 Mg ha^{-1}, NIWA does not appear to significantly influence species abundance, richness or diversity within four years of application, congruent with other literature. Understory vascular community composition was heavily driven by site as opposed to NIWA treatment. At dosages between 2 – 12 Mg ha^{-1} NIWA does not appear to influence seedling height or diameter within the first two years following application. However, dosages exceeding 4 Mg ha^{-1} resulted in increased sugar maple seedling mortality.

Ultimately, these findings continue to support related studies in that NIWA improves soil pH and base status without adversely impacting understory vascular plant communities within four years. Additionally, NIWA, at dosages $\leq$ 4 Mg ha^{-1}, do not result in adverse impacts on sugar maple seeding growth and regeneration within two years of application. Broadly, this suggests that NIWA, when used at certain dosages, can be applied to acidified forest soils in central Ontario without experiencing adverse short-term effects on ecosystem regeneration.

4.1 Recommendations and future research

This study only evaluated soil response up to four years after application and while decreases in pH began to be observed, it is not known exactly how long the dissolution of ash will persist and thus continue to neutralize acidity and support base cation status. This raises questions as to the efficacy of a large one-time dose or repeated applications at lower dosages. Overall, long-term studies are required in Canada to document these prolonged soil changes. Additionally, the addition of NIWA may shift which nutrients are limiting within these forest systems, as shown by Conquer et al. (2025). For instance, a shift may occur from Ca limitation to N limitation in these systems, which may account for limited vascular plant response. The addition of supplemental soil amendments such as N-rich fertilizers should be explored to determine if increased growth effects or community differences are found. Understory vegetation communities are dynamic and critical to forest ecosystems, and a thorough evaluation of the effects of NIWA application are required. Long-term monitoring of vegetation communities will determine if the response of vascular plants is slow (not observable within four years) or delayed as the ash percolates into the rooting layers of the soil. Further, due to the spatial variability of vegetation communities and the significant influence of site found in this study, the use of a fixed quadrant design would allow for repeated surveys of the same individual plants. This, along with a baseline assessment before application, may allow for a more detailed account of the NIWA effects and for the detection of more subtle (or rapid) changes in community composition. Lastly, this is the first study to show adverse effects on sugar maple seedling regeneration at high NIWA dosages, with the mechanisms facilitating this mortality not being completely determined.

It is possible that the mortality is owing to pH and/or salt shock and base cation saturation of the rhizosphere, where the seedlings draw nutrients, though not definitively. It is recommended that a more thorough investigation be conducted to ascertain the exact mechanisms contributing to this. Use of laboratory or greenhouse studies would allow for control over more external variables like extreme weather, herbivory, and disease that may have influenced mortality in the field. Simultaneously, it is recommended that long-term monitoring of seedling cohorts be conducted to determine if the elevated mortality is a temporary response, and if so, how long following large NIWA addition mortality would take to return to baseline. Finally, in this evaluation of ecosystem regeneration, sugar maple, the dominant overstory species in central Ontario deciduous forests, was used, however, a more thorough evaluation of seedling survival for other deciduous and coniferous species is also recommended, as this mortality may be due to the high nutrient sensitivity of sugar maple compared to other tree species.

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6.0 Appendix

A) Species removed from PCA analysis (< 1 % of total observations for that group (site and year). These species typically made up the "*Other spp.*" group in the species abundance figure (**Figure** 2.3). **BOLD** = Species that had to be removed from the dataset to facilitate PCA by year analysis.

2020		2021		2023	
Brooks	**Frequency**	**Brooks**	**Frequency**	**Brooks**	**Frequency**
Acer pensylvanicum	4	*Acer negundo*	3	*Acer pensylvanicum*	2
Dryopteris intermedia	4	***Allium tricoccum***	12	*Acer rubrum*	1
Maianthemum canadense	6	***Aralia nudicaulis***	22	*Corus spp.*	1
Ostrya virginiana	2	*Caulophyllum thalictroides*	3	*Epipactis spp.*	1
Polygonatum spp.	4	*Convolvulus*	2	*Fagus grandifolia*	6
Quercus rubra	1	*Corus spp.*	1	*Ostrya virginiana*	1
Ribes spp.	2	*Fagus grandifolia*	3	*Polygonatum spp.*	1
Rubus spp.	2	***Maianthemum canadense***	23	*Quercus rubra*	1
Unclassified spp.	2	*Ostrya virginiana*	2	*Sambucus spp.*	4
Viburnum lantanoides	2	*Polygonatum spp.*	11	*Trillium spp.*	1
Marks		*Quercus rubra*	6	**Marks**	
Abies balsamea	1	*Rubus allegheniensis*	2	Acer rubrum	1
Corus spp.	1	*Rubus spp.*	5	*Aralia nudicaulis*	1
Dalibarda repens	1	*Sambucus spp.*	8	*Fagus grandifolia*	4
Gramineae/Cyperaceae spp.	5	*Trillium spp.*	10	*Gramineae/Cyperaceae spp.*	7
Lysimachia borealis	1	*Unclassified spp.*	8	*Maianthemum canadense*	8
Ostrya virginiana	7	*Viburnum dentatum*	4	*Prunus serotina*	1
Polygonatum spp.	3	**Marks**		*Spinulum annotinum*	9
Prunus serotina	1	*Abies balsamea*	1	*Viola spp.*	2

Trillium spp.	5
Viola spp.	3
Wilfs	
Corus spp.	1
Dryopteris intermedia	3
Maianthemum canadense	1
Ostrya virginiana	2
Polygonatum spp.	2
Ribes spp.	3
Solidago spp.	3
Unclassified spp.	1
Aralia nudicaulis	2
Corus spp.	2
Dalibarda repens	1
Dryopteris intermedia	9
Erythronium americanum	15
Fagus grandifolia	17
Fraxinus spp.	4
Gramineae/Cyperaceae spp.	14
Maianthemum canadense	1
Mitchella repens	1
Polygonatum spp.	15
Sambucus spp,	1
Spinulous Wood Fern	4
Spinulum annotinum	14
Trillium spp.	14
Ulmus spp.	1
Unclassified spp.	4
Viola spp.	15
Wilfs	
Acer pensylvanicum	8
Acer rubrum	1
Dalibarda repens	1
Dryopteris intermedia	4
Erythronium americanum	24
Maianthemum canadense	7
Quercus rubra	2
Unclassified spp.	3
Viburnum lantanoides	5
Spinulum annotinum	26
Wilfs	
American Honeysuckle	3
Epipactis spp.	3
Fagus grandifolia	8
Marginal Wood Fern	5
Ostrya virginiana	2
Quercus rubra	2
Unclassified spp.	2
Viola spp.	6
Spinulum annotinum	10

B) Soil metal concentrations in the upper (0 – 10 cm) and lower (11 – 20 cm) mineral horizons at Camp Big Canoe in 2021 and 2022.