The Effects of Acid Rain and Liming on Select Members of the Ericaceae Family

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Abstract

Acid rain, characterized by sulphuric acid deposition, poses threats to ecosystems, plants, and animals across Canada. Nova Scotia has a longstanding history of acid rain, influenced by both local sources from industry and air pollution, as well as atmospheric contributions from the northeastern United States. This situation is compounded by the natural acidity of the region's soils, primarily derived from the prevalence of granite bedrock. Greater Sudbury, Ontario is a historical mining town with a long history of acid rain deposition from metal mining that destroyed local flora.

Lime, administered as calcium carbonate successfully brought back Lifetree cover to Sudbury, and offers restoration potential elsewhere by neutralizing soil acidity. Despite successful applications in many Canadian forests, Nova Scotia's liming applications are limited. To address this, I applied sulphuric acid and lime to forest plots and monitored chemical, morphological and physiological responses of understory shrubs over a single growing season. A case study was conducted in Sudbury and compared to the experimental results obtained in Nova Scotia.

Responses to the treatments were species-specific, with a general reduction of functional traits for the acid treatments. Liming in the short-term showed indicators of being a positive driver for both teaberries and sheep laurel. Comparative analysis with data from Sudbury, Ontario, plants subjected to long-term liming showed more favorable growth outcomes in limed sites. Here, liming in the long-term increased the leaf pH, calcium (Ca^{2+}) content, and leaf dry matter content (LDMC). The long-term effects of liming were also evident in the lowbush blueberry, as shown in the increase in leaf pH and LDMC. This suggests the potential for positive lime effects on understory shrubs, possibly requiring extended periods for establishment. This study, though preliminary, provides valuable insights into using liming as a restoration strategy in regions impacted by acid rain in Nova Scotia.

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1.1. Introduction

1.2. Acid rain

Industrialization and urbanism are known to be two of the major contributors to global emissions of many different pollutants, especially air pollutants (McDuffie et al., 2020; Markham, 1994; Collins et al., 2013). Most cities use energy derived from burning fossil fuels to power cities, which releases pollutants known as greenhouse gases (Höök & Tang, 2013). Normally, atmospheric chemicals are naturally removed through various biogeochemical cycles – such as the carbon cycle, nitrogen cycle, and sulphur cycle (Fisher, 2018). However, due to the mass amounts of pollutants being released into the atmosphere both during and after the industrial revolution, natural biogeochemical cycling cannot get rid of pollutants as fast as they are being produced (Collins, et al., 2013). As a result, these gases remain in the atmosphere at dangerous levels.

Pollution can occur in many different forms, such as direct or indirect pollution. Direct pollution is when a pollutant is directly deposited into the environment. Indirect pollution occurs when contaminants are used for one non-pollutant purpose, but later enter the environment as a pollutant or something that increases the chances of pollutants entering the environment (Roberts et al., 1976). A form of direct pollution is air pollution, with one very common atmospheric pollutant being sulphur dioxide gas (SO_2), which is produced through the combustion of fossil fuels (Vestreng et al., 2007).

Emissions of SO_2 can pose long-lasting problems, such as acid rain. Acid rain is created when SO_2 and nitrogen oxide gases (NO_x) – another common group of air pollutants – rise into the atmosphere and mix with the water in the clouds to form acids (Singh & Agrawal, 2008). Sulphur dioxide, through a series of chemical reactions, becomes sulphuric acid (H_2SO_4), a secondary pollutant, which comes to ground-level in various forms of acidic precipitation, such as acidic rainwater, fog, and snow (Shukla, Sundar, Shivangi, & Naresh, 2013; Singh & Agrawal, 2008). This form of wet deposition is known as acid rain. Nitrous oxides undergo the same processes as SO_2, becoming nitric acids in the atmosphere. Depending on the region, the composition of acid rain could be mainly nitric acids or mainly H_2SO_4.

Sulphur is an important plant nutrient, primarily aiding in the synthesis of plant proteins, but also being essential for structural and regulatory functions (Jamal et al., 2010; Rennenberg, 1984). However, the wet deposition of H_2SO_4 via acid rain can have a negative effect on aquatic and terrestrial ecosystems (Wright & Schindler, 1995). When soils exceed their natural acid buffering capacity, there is a depletion of important base cations (e.g., Ca^{2+}, Mg^{2+}, K^+) and an increase in soil sulphur (S) and nitrogen (N), which can become harmful for plants in excess (Driscoll, et al., 2001).

1.2.1. Effects of acid rain on plants

Many parts of terrestrial systems are affected by acid rain, such as wildlife and abiotic factors like soils, but the effects are especially pronounced in plants. Leaves are the first part of the plant to be hit by acid rain, followed by the roots, which interact with the acid deposited into the soil. Aluminum (Al) is an abundant element in soils that is considered phytotoxic, but it is immobile in non-acidic soils (soil pH ≥ 5.0; Bojórquez-Quintal et al., 2017; Panda, Baluška, & Matsumoto, 2001). Through acid rain, soil pH becomes more acidic due to the increase in hydrogen (H^+) ions in the soil, allowing Al to move freely through the soil (Panda, Baluška, & Matsumoto, 2001).

Al cations block Ca^{2+} receptors on plant roots, preventing the uptake of calcium, an essential plant nutrient for cell structure as well as the growth of root and shoot cells (McLaughlin

& Wimmer, 1999; Rengel & Elliot, 1992; White & Broadley, 2003). On top of this, when soil conditions become too acidic, Ca^{2+} dissolves into rainwater and gets washed away creating calcium deficient soils (Seigneur, 2019). This calcium deficiency can prevent essential plant processes from occurring (McLaughlin & Wimmer, 1999).

Two common metrics of determining the effect acidic soils have on plants are above-ground biomass (AGBM) and height (Balasubramanian et al., 2007). AGBM measures the total dry weight of plant above-ground tissues (stems, leaves, reproductive organs) and is used as an indicator of how much a plant grew over a given period. Height similarly measures how much a plant has grown but focusses on upward growth rather than overall cellular growth.

When acid rain interacts with leaves, it has been shown to negatively affect leaf chemistry, physiology, and morphology in many species. In cases of low pH ($\leq$ 3.0 pH) acid rain, small sections of leaf tissue have been shown to undergo tissue death (Caporn & Hutchinson, 1986; Hogan, 1998). Leaf size (LS) is the average surface area of a leaf and can be affected by acid rain, and leaf density can be represented by leaf dry matter content (LDMC; Shipley & Vu, 2002). Specific leaf area (SLA) is a leaf characteristic that represents the tradeoff that a plant makes between expanding its leaves and investing energy in structural components (Poorter et al., 2009). Leaf morphological traits such as LS and LDMC have been shown to respond to acid rain by becoming smaller and/or less dense leaves when exposed to pH 3.0 simulated acid rain, which may be connected to this loss of cell tissue due to H_2SO_4 (Balasubramanian et al., 2007; Hogan, 1998). If exposure to low pH solutions can destroy leaf tissue, as well as make leaves smaller and less dense, an effect on plant fitness could also be occuring.

Foliar Ca^{2+} is one of the primary leachates that are exuded when leaves are exposed to acid rain (Fairfax & Lepp, 1975). Other cations that commonly leach out from leaves under acid stress are potassium ions (K^+) and magnesium ions (Mg^{2+}; Fairfax & Lepp, 1975; Hogan, 1992). As these ions are leaving the leaves, it is decreasing the total ion concentration within the leaf, which can negatively impact plant health (Samarakoon et al., 2006). This is commonly measured as electrical conductivity (EC).

Leaf pH is a plant trait that varies by species and environment, and it is a key factor in the uptake of ions into the leaves, as well as plant physiological traits (Cornelissen et al., 2011; Liu et al., 2021). It has also been shown to be affected by the acidity of the medium the plant is grown in, but this trait is predominantly species driven, so normal leaf pH ranges and responses to the environment vary from species to species (Cornelissen et al., 2011).

Leaf physiology is also affected by acid rain interacting with leaves. Chlorophyll content is often used as an indicator to determine the state of leaf physiology (Du et al., 2017; Dungarwal et al., 1974; Fan & Wang, 2000). Generally, acid rain decreases the chlorophyll content in leaves, which restricts the plant's ability to photosynthesize and create food for itself (Du et al., 2017). Stomatal conductance (g_{sw}) is a measurement of stomatal openness, which can provide information on leaf water gas exchange(Gimenez et al., 2005). Stomatal conductance rates have been found to decrease in leaves exposed to acid rain (Banhos et al., 2016; Khpalwak et al., 2017; Martens et al., 1989). In some cases, this is caused by the mobilization or increase in ion concentrations, such as phytotoxic aluminum ions (Al^{3+}) or H^+ in the soil solution, which reduces soil water potential and water availability for root uptake, consequently reducing g_{sw} from stomatal closure (Vitorello et al., 2005).

1.2.2. Acid tolerance in plants

As listed above, acid rain has been shown to have serious negative effects on the way plants function at a morphological, chemical, and physiological level. However, not all plants are equally as sensitive to acid, some are acid tolerant. Acid-tolerant plants are also known as calcifuges because they grow in soils with low levels of calcium and other nutrients (de Silva, 1934). One group of acid-tolerant plants are the Ericaceae (Heath), a plant family composed mainly of shrubs with some tree and herbaceous species. This large family consists of approximately 4100 species and members appear around the globe in a wide range of habitats including acidic wetlands (e.g., bogs), nutrient-poor shrublands (e.g., barrens) and a wide variety of forest types (Tucker, 2008).

1.2.3. Liming as a treatment for acid rain

Efforts to restore acid rain affected lands have been using lime, an umbrella term referring to a product made from the mineral limestone which is composed primarily of calcium carbonate ($CaCO_3$; Kumar, Ramakrishnan, & Hung, 2007). Different types of lime are composed of different minerals, such as quicklime, slaked lime and dolomitic lime. Quicklime is formed by exposing $CaCO_3$ to very high heats to form calcium oxide (CaO); slaked lime, also known as hydrated lime, is composed mostly of calcium hydroxide ($Ca(OH)_2$); and dolomitic lime is made up of CaO and magnesium oxide (MgO; Dowling et al., 2015). Different types of lime are used for soils with different needs, for example, dolomitic lime is typically used for soils that are deficient in both Ca and Mg (Dowling et al., 2015).

Lime is applied on top of the soil and water allows Ca^{2+} to diffuse into the soil. In the case of sulphuric acid rain, $CaCO_3$ also interacts with the sulphate in H_2SO_4 and produces water as a byproduct, which neutralizes the acids in soil, thus raising soil pH, aiding in the restoration of acidified soils (Kumar, Ramakrishnan, & Hung, 2007; Driscoll, et al., 2001; Gatiboni & Hardy, 2023). Through the raising of soil pH, Ca^{2+} also reduces Al^{3+} toxicity by immobilizing it (Kruger & Sucoff, 1989; Watanabe & Okada, 2005).

Liming has helped plant life in areas with acid deposition, such as improving the health of sugar maple crowns and increasing foliar Ca^{2+} in New Hampshire and Vermont, USA, but the full effects of liming are still not known as plants can respond to liming treatments differently in different regions (Juice et al., 2006; Pabian et al, 2012; Schaaf & Hüttl, 2006; Wilmot et al., 1996). Some studies in France, Germany, China and the United States have been conducted regarding the long-term effects that liming has on plants in forest ecosystems (Forey et al., 2015; Huettl & Zoettl, 1993; Li et al., 2014; Long et al., 2011; Schaaf & Hüttl, 2006). However, the results of liming differ depending on the type of lime used, as well as tree and forest types (Schaaf & Hüttl, 2006).

As found by Schaaf and Hüttl (2006), the short and long-term effects of lime in one forest do not necessarily translate to another forest, thus there is still a lot we do not know. For example, Nova Scotian forests have been subject to acid rain for decades, yet hardly any studies have tried liming these forests. Since the response of other forests to acid rain and lime cannot be applied to forests in Nova Scotia, experimental liming trials must be done within the province.

In Nova Scotia, the effects of lime on plants tends to be a secondary focus of scientific studies, as most studies to date have primarily focused on restoring aquatic ecosystems (Clair & Hindar, 2011; Pabian, Rummel, Sharpe, & Brittingham, 2012). Clair & Hindar (2011) found that multiple European lake studies have shown that any return of aquatic organisms is not sustainable once liming of the site ends. Initial effects of liming are shown to successfully detoxify watersheds by raising pH (Appelberg & Svenson, 2001; Keller, Gunn, & Yan, 1992). The long-term effects of lime application have been shown to be less prominent than the effects upon the first application, with the full effects of liming being unknown (Appelberg & Svenson, 2001; Keller, Gunn, & Yan, 1992).

1.3. Sites of interest

1.3.1. Halifax, Nova Scotia

Nova Scotia (NS) is a province that has naturally acidic soils. This is because most of NS is composed of non-calcareous siliceous sedimentary bedrock, which has a high-intermediate sensitivity to acid loading and few base cations. This type of bedrock includes shales, siltstone, and sandstone. Granite bedrock also makes up a large portion of the province's south-central region. Granitoid rocks have a very high sensitivity to acid loading, making the soils in this region both quite naturally acidic and sensitive to acid deposition (Shilts, 1981).

Nova Scotia has also historically received a lot of wet acid deposition from acid rain. This comes from both the province itself and the United States. Urbanization in New England (Maine, Massachusetts, Rhode Island, New Hampshire, Connecticut, and Vermont) causes a lot of air pollution which travels across the Gulf of Maine and the Bay of Fundy towards NS due to the prevailing northwesterly winds (Knapp et al., 1998; Millet, et al., 2006). Since most of the soils in NS are sensitive to acid loading, they can only buffer very little additional soil inputs (Shilts, 1981). When pollution comes to NS from New England from across the water and deposits H_2SO_4 into the soils, the soils are unable to buffer the acid being deposited, compounding the effect of the acid rain (Knapp et al., 1998; Millet, et al., 2006).

Acid rain has caused a lot of damage to NS soils and waterways by reducing the amount of beneficial ions and increasing the acidity of both waterways and soils (Gorham, 1957; Schindler et al., 1989; Seigneur, 2019). The increase in acidity combined with ion deficiency makes it more difficult for many organisms to live in soils and waterways (McLaughlin & Wimmer, 1999; Schindler et al., 1989). As a result, many studies have been conducted in Nova Scotia to try and restore aquatic habitats that have been degraded by acid rain, with many focussing on restoring fish populations (Laudon, Clair, & Hemond, 2002; Sterling, et al., 2014; Hart, Halfyard, & Sterling, 2023; Lacroix & Knox, 2005; Watt, 1987). However, the heavy focus on aquatic systems leads to a gap in our knowledge when it comes to terrestrial systems affected by acid rain in NS.

Warman, Walsh and Rodd (2000) performed the only published study focused on the effects of lime on soils and plants in Nova Scotia. In this study, they focused on crops of barley, sweet corn, wheat and turfgrass, as well as lime requirement tests (LRTs). LRTs are formulaic ways to determine how much lime is required to bring soil pH up to 6.0 in tonnes per hectare. Since this liming study was based around agricultural plants in NS, much remains unknown about how lime could affect the various natural ecosystems and native plant species impacted by acid rain across the province.

Restorative measures for species in acidified forests is largely unknown in NS. Through my research, I aim to contribute to and expand the knowledge we have about forest understory plants that are exposed to acid rain. I also aim to contribute to potential restorative measures that can be taken to reduce the negative effects of acid rain on plants in NS. Specifically, I am interested in three of the ericaceous shrubs that grow in many of the forests across Nova Scotia.

1.3.2. Sudbury, Ontario

One example of liming being used as a restorative measure in an acid-rain affected area is in Greater Sudbury, Ontario. Greater Sudbury is a city in Northeastern Ontario that has historically experienced significant amounts of pollution from copper and nickel metal mining and smelting. As a part of the “Southern province” of the Canadian Shield, this area is rich in minerals, leading to Sudbury becoming a large mining and smelting town in the 19th century (Pearson & Pitblado, 1995; Winterhalder, 1995). Part of the smelting process involved the roasting of copper (Cu) and nickel (Ni) ore in large, outdoor fires (Winterhalder, 1995). The amount of smelting activity increased rapidly and by the early 20th century large numbers of nearby trees had been, and were being, cut down to fuel the roasting of Cu and Ni in open roast yards (Winterhalder, 1995; SARA, 2008).

Most copper ores contain sulphides, so when the copper was roasted in the open yards, a large amount of sulphur gas was released as sulphur dioxide, SO_2 (Winterhalder, 1995; Chen, Xu, & Chen, 2020). Since these roast yards were open fires on the ground, there was no way to funnel the fumes towards the sky, thus the fumes remained on ground-level (Winterhalder, 1995). Although the invention and implementation of smelter stacks effectively reduced SO_2 emissions by raising the gases above ground-level and allowing them to be diluted in the atmosphere, the smelting process remains a source of SO_2 emissions. Smelting also released toxic Cu and Ni metal particulate matter, which are still present in the soils of Sudbury in high concentrations near the smelters (SARA, 2008). These metals cause severe damage to vegetation and have since proven to be even more dangerous to Sudbury vegetation than sulphur dioxide (Winterhalder, 1995).

The regreening of Sudbury began in 1978 as a part of the Sudbury Land Reclamation Programme to bring back the vegetation, primarily trees, that had been decimated by heavy metal and sulphuric acid pollution (Beckett & Negusanti, 1990). First, dolomitic limestone was applied, followed by the establishment of a grass and legume understory at a cover of 10 – 30% (Beckett & Negusanti, 1990). Without this understory, the introduction of trees would not have been possible (Beckett & Negusanti, 1990). Liming and seeding have been done many times since 1990 with different types of lime and many different plant seeds (Greater Sudbury, 2023) and the regreening program has proven to be successful in bringing back forest vegetation.

For this project, I am interested in the forest understory plants that now grow in Sudbury and how they have reacted to the long-term sulphuric acid deposition and liming treatment. With the data collected in Sudbury, I compared the long-term effects of liming on acid-tolerant shrubs to the short-term effects seen in the Halifax field experiment.

1.4. Plants of interest

Ericaceous species prefer acidic soils, thus making them a good plant group to study in Nova Scotia and Greater Sudbury (Tucker, 2008). Many species of Ericaceae grow abundantly across Eastern Canada, including Sudbury, Ontario, and most of NS. Because of this, I chose to conduct my research on members of the Ericaceae family. The species studied were lowbush blueberry (*Vaccinium angustifolium*), sheep laurel (*Kalmia angustifolia*) and teaberry (*Gaultheria procumbens*).

These three plant species each represent a different type of leaf longevity, which are deciduous, semi-deciduous, and evergreen, respectively. Deciduous leaves all fall off the plant every year and new leaves are produced the next growing season. Evergreen leaves are present on a plant year-round and only die after multiple years. Semi-deciduous plants will lose some of their leaves every year but maintain about half of their leaves (Kikuzawa & Lechowicz, 2011). Semi-deciduous plants are more prone to losing leaves than evergreen plants are, but less prone to losing leaves than deciduous plants are.

Vaccinium angustifolium, commonly known as the lowbush blueberry, is a deciduous shrub (Kloet, 2020). In Nova Scotia, this plant generally flowers in early spring and fruits in midsummer. *Kalmia angustifolia*, sheep laurel, is a semi-deciduous shrub that produces pink inflorescences in early summer (Liu et al., 2020). *Gaultheria procumbens*, teaberry, is an evergreen dwarf shrub. Teaberry plants are connected by a creeping sub-soil structure that links one leaf-body to another. It flowers in early summer and fruits from early fall to early winter. Flowers are white and bell shaped, while fruits are bright red. The leaves and fruits of the teaberry have a wintergreen taste (Trock, 2008).

1.5. Research objectives

My goals with this honour's thesis research are to better understand how lime interacts with acid tolerant ericaceous shrubs and if liming should be used as a restorative measure in acid rain-affected forests in Nova Scotia. By proxy this will also offer insight into how acid-tolerant shrubs interact with H_2SO_4. To do this, four types of plant characteristics were measured: plant growth, leaf morphology, leaf chemistry, and leaf physiology. From these four characteristics, ten plant traits were measured:1) above-ground biomass (g); 2) plant height (cm); 3) leaf size (LS, cm^2); 4) specific leaf area (SLA, cm^2/g); 5) leaf dry matter content (LDMC, g/g); 6) leaf pH; 7) leaf calcium content (Ca^{2+}, ppm); 8) leaf electrical conductivity (EC, µS/cm); 9) leaf chlorophyll concentration (µg/mL); and 10) stomatal conductance (g_{sw}, µmol $H_2O/m^2/s$).

The questions I wanted to answer with this thesis are as follows:

1) Is plant growth of acid-tolerant shrubs affected by liming and acid rain?

2) What are the effects of acid rain and liming on the leaf morphology of acid-tolerant plants (i.e., LS, SLA, LDMC)?

3) What are the effects of acid rain and liming on the leaf chemistry of acid-tolerant plants (i.e., pH, chemistry, EC)?

4) What are the effects of acid rain and liming on the leaf physiology of acid-tolerant shrubs (i.e., chlorophyll content, g_{sw})?

5) What are the short-term and long-term effects of acid rain and liming on acid-tolerant shrubs?

6) Do different leaf longevity types of Ericaceae (i.e., deciduous, semi-deciduous, evergreen) have different responses to acid rain and liming?

1.6. Approach

To answer these questions, I conducted an experimental field study in Halifax, Nova Scotia and a field study in Greater Sudbury, Ontario. The data collected were for a comparative analysis between my short-term liming experiment in Halifax and the long-term effects of liming in Sudbury.

2.1. Materials and Methods

2.2. Experimental design in the Oaks Forest, Halifax, NS

The field experiment was performed from June 8, 2023, to August 9, 2023, in the Oaks Forest, Halifax, NS, which is located partly on Saint Mary's University (SMU) campus (44.628545°N, -63.581374°W). A randomized block design was used with four blocks, two of which were under a more open tree canopy, and the other two under a relatively closed canopy. Each block contained four plots, each having one of four treatments applied and measuring 1 x 1 m. The four treatments used were: an acid treatment (A), an acid rain treatment (AR), a lime treatment (L), and a control (C). There were 16 plots total with four plots for each replicate (Figure 1)

Plots were made by planting four stakes into the ground 1 m apart from each other and connected using green string. Each plot contained at least three teaberries and three sheep laurel individuals. While lowbush blueberries did occur in the Oaks, they did not appear near the teaberries and sheep laurel in a high enough frequency to be included in this field experiment.

For the A and AR treatments, an H_2SO_4 solution with a pH range of 3.00 to 3.06 was used and 2 litres were applied to each replicant biweekly with a backpack sprayer. The AR treatments had H_2SO_4 applied from above the plot to simulate acid rain on the plants. The A treatments had H_2SO_4 applied directly to the soil to simulate acid that had already been deposited into the soil. Treatments of H_2SO_4 were broken up into two to see if plants responded differently to the different methods of acid application. The L treatment used commercially available dolomitic limestone (Golfgreen; Toronto, CA) which was applied once at the beginning of the experiment and was given 2 litres of water the first day. The amount of dolomitic lime applied was 1 kg/m^2 because that is the same ratio of weight to area that is applied in Greater Sudbury, ON. The C treatment was given 2 litres of water on the first day and was left alone for the rest of the experiment.

After plots were set up, two plant root simulator (PRS) probes (Western Ag; Saskatoon, CA) were placed in the ground near the middle of the plot. One probe was an anion PRS probe, and the other was a cation PRS probe. These probes measured the amounts of specific cations and anions in the soil, allowing for a more accurate analysis of soil ion concentrations that are available for root uptake. On June 25th, two additional probes were placed in each plot (one cation probe and one anion probe). All probes were removed on September 28th, 2023, cleaned with dH_2O in lab and sent to Western Ag for analysis.

In addition to the soil chemistry obtained from the PRS probes, one soil sample (approximately 10cm deep) was collected from each plot for soil pH, Ca^{2+} and electrical conductivity (EC) two weeks after the initial set-up of the field experiment. Soil samples were oven-dried at 75°C for 48 hours. For each soil sample, water-extractable soil pH, Ca, and EC were measured from a solution prepared from a 1:3 ratio of dried soil to deionized H_2Othat had. been mixed and then steeped for 20 minutes at room temperature and then centrifuged for 10 minutes for a total of 30 min of steeping time. The centrifuge helped to separate the liquids from the solids, which made pipetting extract out of the tube easier. The soil extract was analyzed for pH, Ca, and EC using ion-selective electrodes (Horiba LAQUAtwin pocket meters, Irvine, CA, USA).

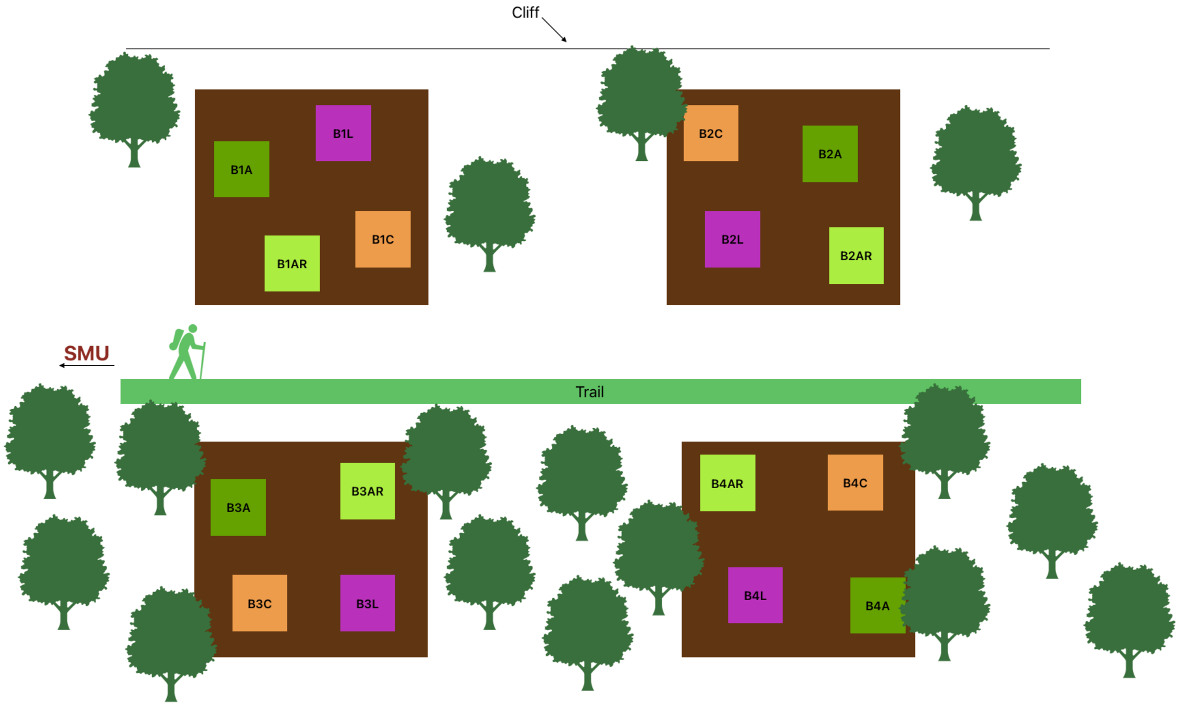

Figure 1. Layout of the field experiment with four treatments in the Oaks Forest, Halifax, NS, which follows a random block design with two blocks under a heavier tree canopy and two under a lighter tree canopy. Plots (1m^2 each) were randomly assigned to one of four treatments: A (acid), AR (acid rain), L (lime), C (control).

2.2.1. Harvesting and sample collection

Stomatal conductance (g_{sw}) was measured on June 20, July 7, and August 8, 2023, using an LI-600 porometer (LI-COR; Lincoln, NE, US). On each of the three sampling days, the porometer was used to take five g_{sw} samples from individual plants from each species. This was done for each plot, for a total of 160 g_{sw} measurements per sampling day.

On August 9, 2023, three individual plants per species per plot were harvested for aboveground biomass (AGBM) and plant traits. Samples were placed into brown paper bags, which were then placed into storage bins. The storage bins were then placed into a cooler bag containing ice packs. This kept samples cool, preventing leaf desiccation before they could be weighed for fresh mass – the mass of leaves immediately after collection in grams – in the lab. Plants were chosen as randomly as possible from a wide range of sizes to reduce sampling bias. In addition to the AGBM samples, one large leaf sample per species per plot was taken for chlorophyll extraction. Leaf samples for chlorophyll extraction contained several leaves from across the plot and were taken separately from the AGBM samples. Plants of each species were visually assessed for healthy, green leaves which were then picked and promptly wrapped in aluminum foil before placed in a cooler bag to prevent the degradation and loss of chlorophyll. No specific number of leaves was picked for these samples, but enough had to be picked for chlorophyll extraction (≥ 1 g). Leaf samples taken for chlorophyll extraction were frozen until they were analyzed.

2.2.2. Lab analysis of samples

The maximum height of each plant sample (in cm) was measured and ten leaves from each sample were collected for leaf morphological trait measurements. Leaves were chosen at random from a variety of sizes and ages to represent the plant as a whole. The remaining extra leaves, stems and if present, flowers and fruits were collected in separate envelopes and oven-dried at 75 °C for 48 hours.

The 10 separate leaves were measured for leaf area using a LI-3000C portable leaf area meter with a conveyor belt attachment (LI-COR, Lincoln, NE). After being measured for leaf area, the leaves were weighed for fresh mass and then placed into the drying oven along with the other harvested samples. After all samples were taken out of the oven, they were weighed for dry mass in grams. All sampled leaves were combined into one envelope after being weighed. Leaves were then ground into a fine powder using liquid nitrogen and a mortar and pestle.

Leaf area, fresh mass, dry mass, and number of leaves per sample were used to determine leaf morphological traits. These traits were leaf size (LS), leaf dry matter content (LDMC), and specific leaf area (SLA). LS was calculated by dividing the total leaf area for a sample by the number of leaves in that sample. LDMC was calculated by dividing the leaf dry mass by the fresh mass. SLA was calculated by dividing the total leaf area of a sample by its leaf dry mass.

Leaf pH, water-extractable calcium content, and EC were measured using ion-selective electrodes (Horiba LAQUAtwin pocket meters, Irvine, CA, USA). For each sample, a leaf extract was made using a 1:2 ratio of dried leaf material to deionized water in a 1.5 mL micro-centrifuge tube. The tube was mixed using a vortex mixer (FineVortex, FINEPCR; Gunpo, KR), after which the mixture was left to steep for 15 minutes at room temperature. 1 mL of the extracted solution was pipetted onto the meter's sensor, and the readings were written down. Calibration was performed before measurements began using one of two calibration solutions and again after every third sample measured, alternating between the high and low calibration solutions. The calibration solutions were 150 ppm (low concentration) and 2000 ppm (high concentration) for Ca^{2+}; pH 4.01

(low) and pH 7.00 (high) for pH; 1.41 mS/cm (low) and 12.9 mS/cm (high) for EC measurements. Small amounts

Chlorophyll extraction from frozen leaf samples was performed in the dark so that the fluorescent bulbs in the lab did not degrade the chlorophyll. Leaves were ground into a fine powder using liquid nitrogen, re-wrapped into their labelled aluminum foil, and placed back in the freezer at about -18 °C until analysis. Chlorophyll extraction was done using a mixture of solvents consisting mainly of ethanol, as well as lesser amounts of acetone and methanol. Leaf material and solvent were mixed in 15 mL centrifuge tubes, in a 1:10 ratio, with 1 g of sample to 10 mL of solvent and placed in a light-impermeable box for 24 hrs to extract in darkness. After 24 hours, the solution was then gravity filtered through 150 mm filter papers (Whatman; Marlborough, MA, US) into a clean 15 mL centrifuge tube. Extracts were placed into the freezer at -18 °C until they were to be analyzed in a spectrophotometer (Pharmacia LKB, Novaspec II; Uppsala, SE).

Chlorophyll extracts were analyzed at wavelengths of 663 nm (the red region of light; A663) and 645 nm (the blue region of light; A645). This provided absorbance readings for both chlorophyll a and b, where a absorbs the former, and b absorbs the latter. The spectrophotometer was calibrated at both wavelengths using deionized H_2Oin between each sample. Chlorophyll concentration was then found using the following formula: Chlorophyll concentration (μg/mL) = $[(20.2 \times A645) + (8.02 \times A663)] \times V/(1000 \text{ x } W)$, where A is the absorbance recorded at a particular wavelength, V is volume of solvent used (in mL), and W is the weight of leaf material used (in g).

2.3. Case study sites: Greater Sudbury, Ontario

In Greater Sudbury, Ontario, sites at Garson, Laurentian University Transplant (LUT), and St. Joseph Road (near Temagami River Provincial Park) were selected for sample collection. LUT (46.465850 °N, -80.963196 °W) is near Laurentian University and has been the site of previous *Sphagnum* moss transplants to see if they would grow in a contaminated wetland (Seward, 2023). Garson (46.554995 °N, -80.855381 °W) and LUT are both highly contaminated sites near smelters in Greater Sudbury (Fig. 2 – 3), with only the former having been treated with lime (Greater Sudbury, 2023). The St. Joseph Road site (46.43342 °N, 80.09378 °W) was used as a reference site in this study since it is far enough from Sudbury that contamination is considerably lower compared to LUT and Garson (Seward, 2023).

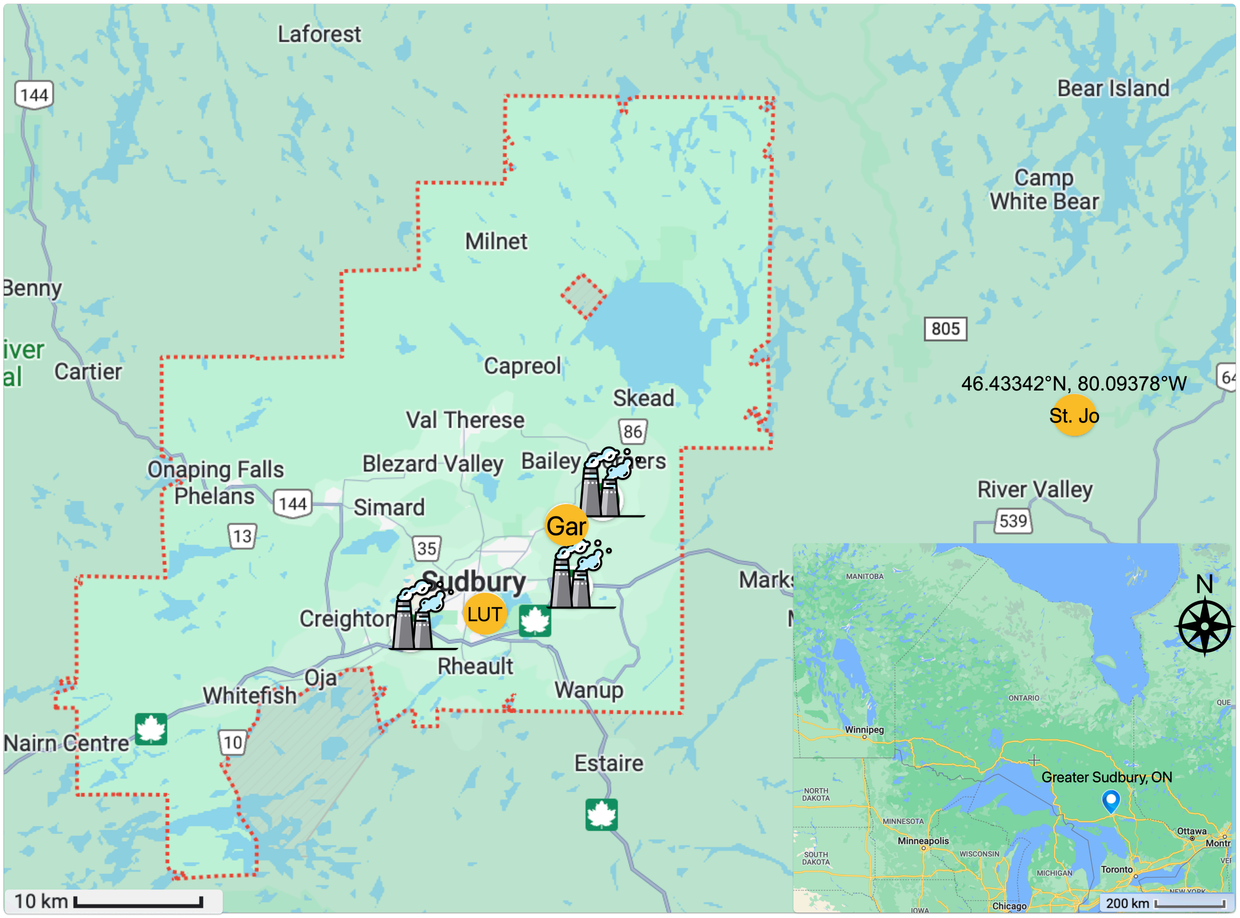

Figure 2. A map showing where Greater Sudbury is in Ontario, zoomed in to show the boundaries of the city, showing the locations of the smelters (represented by smokestacks), and the field sites both within (LUT, Gar), and outside the city limits (St. Jo); maps, scale bars and coordinates provided by Google Maps (2024).

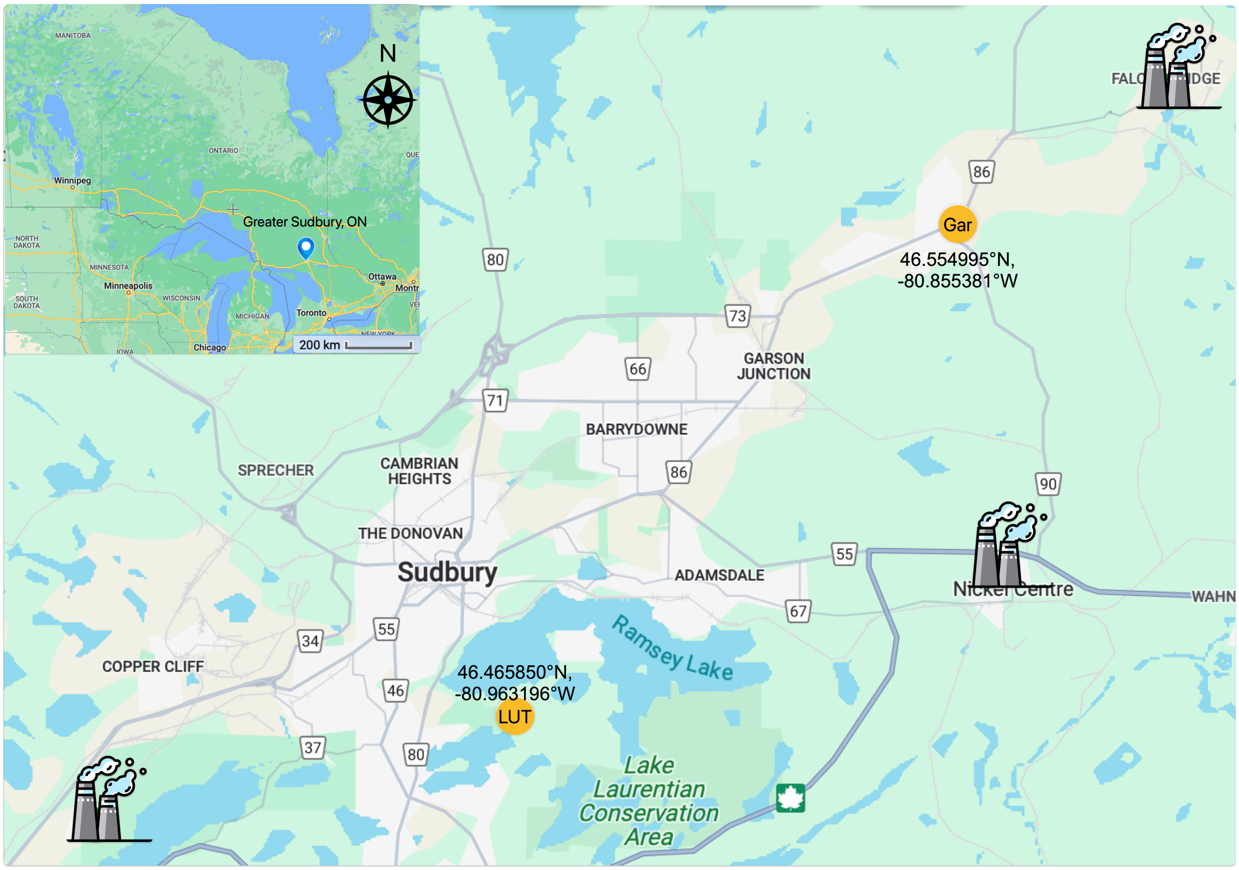

Figure 3. A map showing where the city of Greater Sudbury is in Ontario, zoomed into the city to show the proximity between the field sites (LUT and Gar) and the smokestacks (represented by smokestacks) within Greater Sudbury; maps, scale bars and coordinates provided by Google Maps (2024).

2.3.1. Sample collection in Greater Sudbury

The aim of this field research was to sample three Ericaceous plant species at each of the three sites to see how liming affects leaf chemistry (pH, Ca^{2+}, EC) and morphology (LS, LDMC, and SLA). These three study species for Sudbury were *Vaccinium angustifolium* (lowbush blueberry), *Kalmia angustifolia* (sheep laurel) and *Gaultheria procumbens* (teaberry). Teaberry was only found at LUT, but the difficulty in finding every species at each site led to the exclusion of teaberry as a sample from the LUT site. Soil samples were also collected and analyzed for pH, Ca^{2+}, and EC with the goal of understanding the characteristics of the soils at the different sites.

I collected 10 leaves from 10 plant samples per species at each site. Each of the 10 leaves collected in each sample were from the same plant, or from adjacently growing plants in the case of teaberry which typically has approximately three leaves per individual plant. The leaves in a sample were of varying sizes and ages and were free of obvious damage from disease or herbivory. The leaves were placed in a small coin envelope which was then put into a cooler bag to prevent leaf desiccation.

At each site, at least five soil samples were collected from two inches below the soil using a trowel. Each sample, collected from the soil in which the plant samples were growing, was taken from a different area of the site to provide a representation of the soil conditions at each site. These areas within the site were selected at random wherever a group of healthy focal plants were growing. Every sample was weighed for fresh mass in Sudbury and stored in a fridge at ~ 2 °C to prevent water loss.

2.3.2. Lab analysis of Sudbury samples

Leaf morphological traits measured on the Sudbury samples included LS, SLA, and LDMC and leaf chemical traits included leaf pH, Ca^{2+} and EC using the same procedure as the chemical analysis for Halifax samples in section 2.2.2. Morphological traits were also measured using the same protocols outlined in the previous lab analysis section for the Halifax samples.

Sudbury soil samples were extracted in the same way as the Halifax soil samples If the soil extracts were not going to be analyzed right away, they were placed in the fridge at ~2 °C overnight to be analyzed the next day.

For soil pH analysis, the Orion Star A211 Benchtop pH Meter (Thermo Scientific; Chelmsford, MA, US) was used with the ROSS Ultra pH/ATC Probe (Thermo Scientific). Before being used, the probe was calibrated using buffer solutions at pH 4.01, 7.00 and 10.01. Soil EC analysis was also done using the Orion Star A211 Benchtop Meter along with the 4-Cell Conductivity Probe attachment (Thermo Scientific). Calibration was done twice before samples were measured, once at 1413 µS/cm and once at 1000 µS/cm.

2.4 Statistical data analysis

All data analysis, transformation and visualization were performed using R version 4.3.1 and RStudio version 2023.12.0.369 (R Core Team, 2023; Posit Team 2023). Packages used were ggplot2, lme4, stats, dplyr, patchwork, tidyverse, lmerTest and agricolae (Bates et al., 2015; de Mendiburu, 2023; R Core Team, 2023; Kuznetsova, Brockhoff & Christensen, 2017; Pederson, 2024; Wickham, 2016; Wickham et al., 2019; Wickham et al., 2023). Halifax data was analyzed using linear mixed effects models with block as a random effect, plant or soil characteristics as independent variables, and treatment levels as the dependent variable. Sudbury data was analyzed using one-factor analysis of variance (ANOVA) with plant or soil characteristics as independent variables, and site type as the dependent variable. Two different post-hoc tests were used to determine significance of differences between groups. Tukey HSD tests were used for the ANOVAs (Sudbury data), and least squares means tests were done for the linear mixed effects models (Halifax data). Histograms were used to determine if data was approximately normal and if needed, data was transformed by using either a logarithm or by square rooting the data to reduce non-normality.

3.1 Results

3.2. Field experiment in Halifax, NS

3.2.1. Soil characteristics for the Oaks Forest

Two weeks after application, the treatments had a significant effect on soil pH (df = 3; F = 3.83; p = 0.04) in the Oaks Forest, Halifax, NS (Table 1). Soil pH was lowest in the acid rain and limed treatment (Fig. 4). After performing the Tukey post-hoc tests, the acid rain (df = 12; t = -2.62; p = 0.02) and lime (df = 12; t = 3.04; p = 0.01) treatments were found to significantly different from the control (Table 3). Soil Ca^{2+} at the two-week point (Fig. 4) was not significantly affected by treatment (df = 3; F = 0.43; p = 0.74) or by block (p = 0.81). Soil electrical conductivity (EC) was not significantly affected by treatment (df = 3; F = 0.38; p = 0.77) or block (0.61). The soil data collected two weeks after the field experiment started does not show the calcium levels in the soil at the end of the experiment, but the PRS probe data does. The data from the PRS probes showed that there was more soil Ca^{2+} supplied in the limed treatment than in the acid treatment (Fig. 5). The probes also showed that soil phosphorus (P) supply rates were highest in the acid treatments and lowest in the control and lime treatments (Fig. 6). Soil ammonium (NH_4) supply rates were highest in the acid rain treatment and lowest in the limed treatment (Fig. 7).

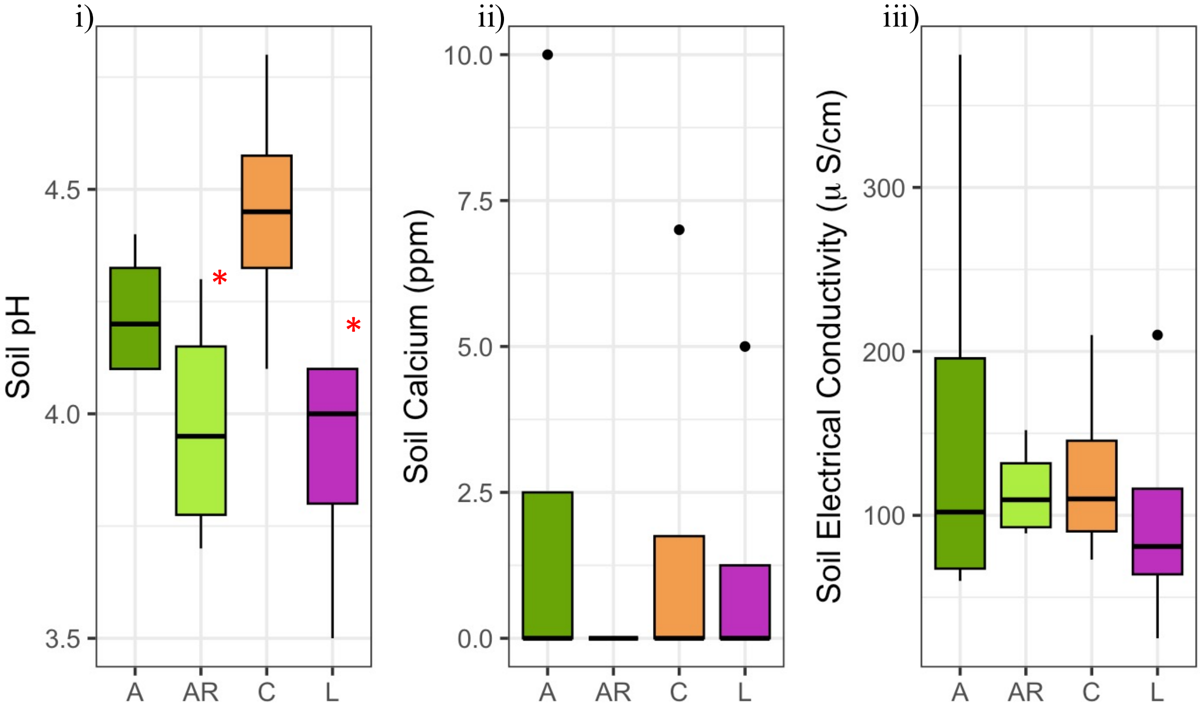

Figure 4. Variation in soil characteristics 2 weeks after application of four treatments: acid (A), acid rain (AR), control (C) and lime (L), with four replicates each in the Oaks Forest, Halifax, NS. Characteristics were i) soil pH ii) soil Ca^{2+} iii) soil EC. The red asterisk represents a significant difference relative to the control based on Tukey post-hoc tests ($0.001 < p < 0.01$).

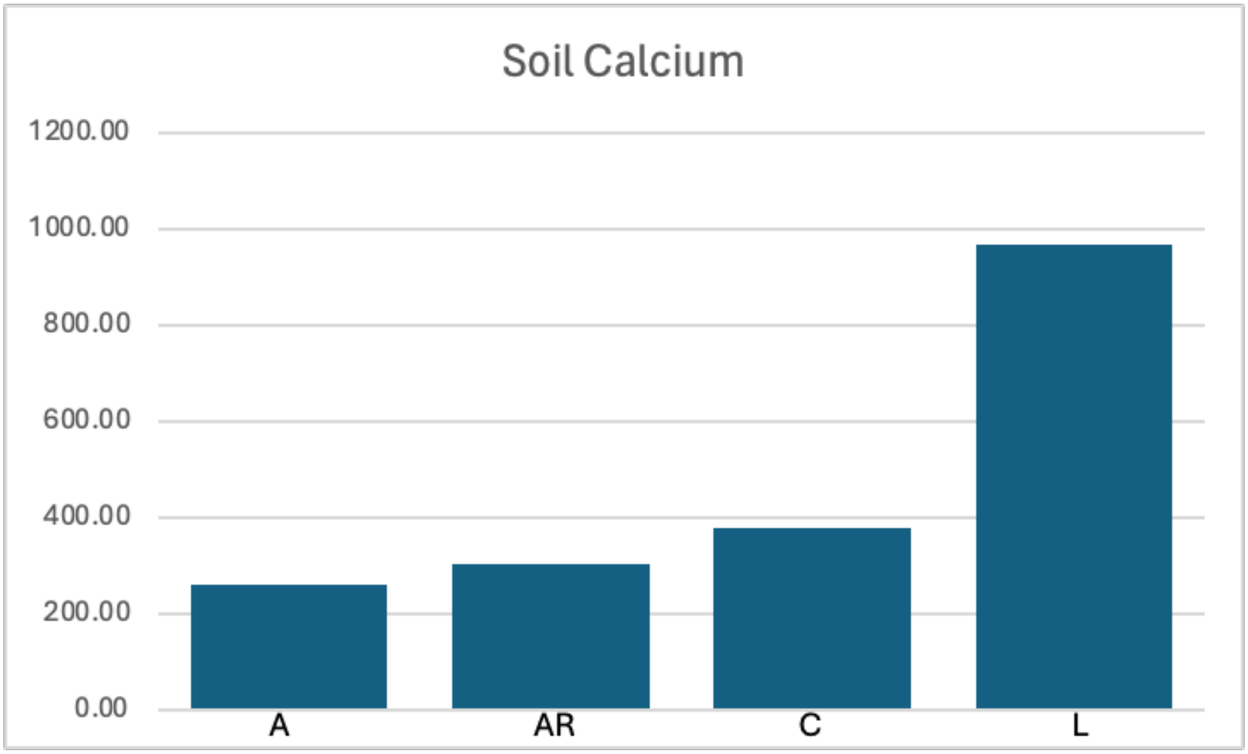

Figure 5.1. Variation in soil calcium ion supply rate (µg Ca/10cm^2/90 days) obtained from PRS probes across four treatments (n = 4 samples per treatment) in the Oaks Forest, Halifax, NS. These treatments were acid (A), acid rain (AR), control (C) and lime (L).

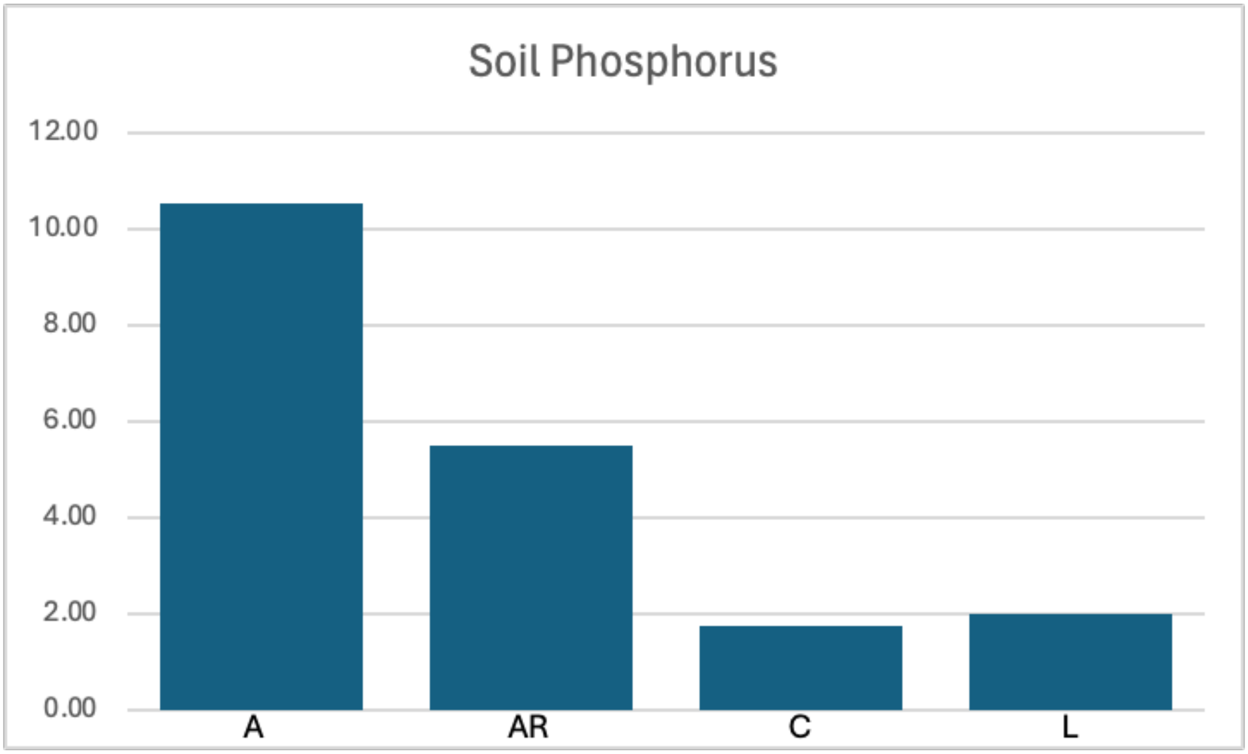

Figure 6.1. Variation in soil phosphorus ion supply rate (µg P/10cm^2/90 days) obtained from PRS probes across four treatments (n = 4 samples per treatment) in the Oaks Forest, Halifax, NS. These treatments were acid (A), acid rain (AR), control (C) and lime (L).

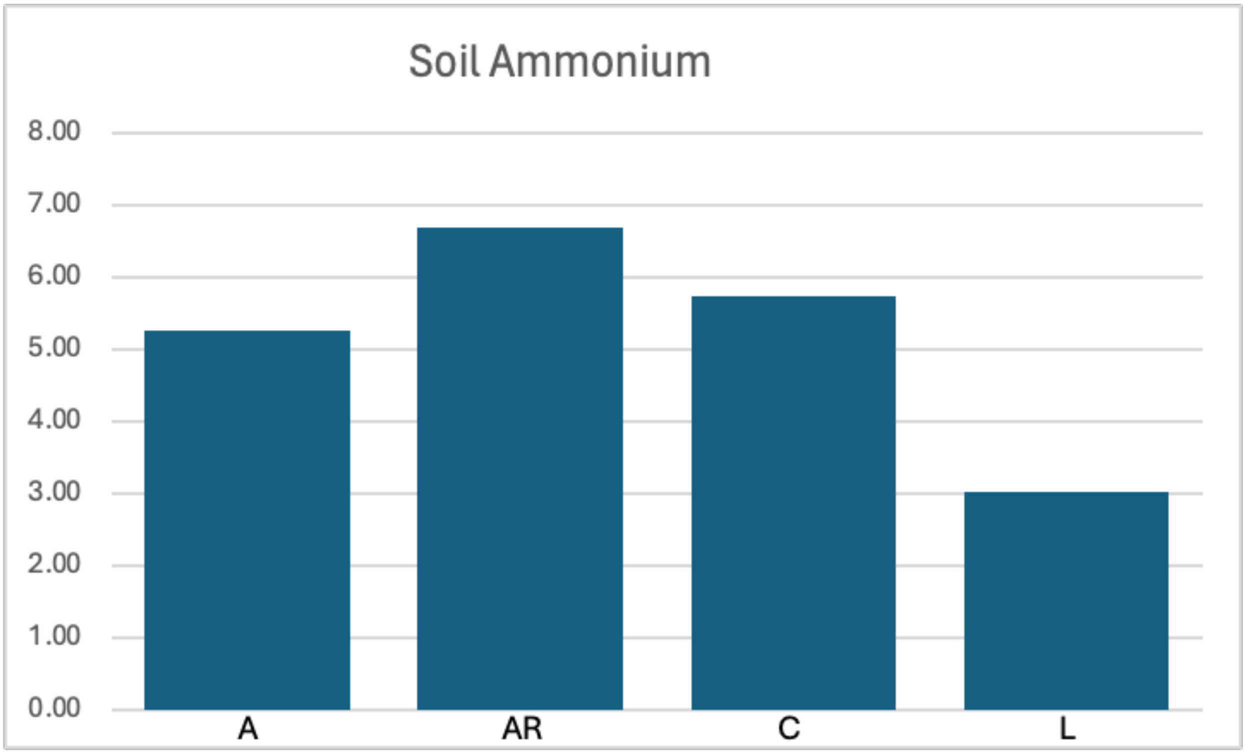

Figure 7.1. Variation in soil ammonium ion supply rate (µg NH_4/10cm^2/90 days) obtained from PRS probes across four treatments (n = 4 samples per treatment) in the Oaks Forest, Halifax, NS. These treatments were acid (A), acid rain (AR), control (C) and lime (L).

Table 1. Results from a linear mixed-effects model showing the significance of treatment on soil characteristics in the Oaks Forest experiment in Halifax, NS with block as a random effect. The independent variables are the four experimental treatments: acid (A), acid rain (AR), lime (L) and control (C), and the dependent variables are soil pH, Ca, and EC measured 2 weeks after the treatments were initially applied.

Trait			**Df**	**Sum Sq**	**F value**	**Pr (>F)**	**Pr(>Chisq)**
pH	Soil	Treatment	3	0.7525	3.8344	0.04	-
		Block	1	-	-	-	1
Ca^{2+}	Soil	Treatment	3	13.25	0.4309	0.74	-
		Block	1	-	-	-	0.81
EC	Soil	Treatment	3	8309.2	0.3841	0.77	-
		Block	1	-	-	-	0.61

3.2.2. Leaf traits of teaberries and sheep laurel

There was an increasing trend in above-ground biomass (AGBM)for both species across treatments, with the largest AGBM observed in the limed treatments and the smallest AGBM observed in the acid treatments relative to the control (Fig. 8). However, this trend was not significant for either teaberry (df = 3; F = 0.23; p = 0.87) or sheep laurel (df = 3; F = 0.48; p = 0.70). Both species also showed a general increase in height across treatments, with height being the highest in the limed treatment for teaberry, and highest in the control for sheep laurel (Fig. 8) but this trend was also not significant for either teaberry (df = 3; F = 0.70; p = 0.56) or sheep laurel (df = 3; F = 0.51; p = 0.68).

Leaf morphology in teaberries was affected by the acid rain treatment. According to the post-hoc tests used (Table 3), this treatment produced the largest leaf areas in teaberry as measured by both SLA (df = 44; t = 2.03; p = 0.048) and LS (df = 41; t = 2.33; p = 0.025). The ANOVA results for SLA (df = 3; F = 1.98; p = 0.13) and LS (df = 3; F = 2.34; p = 0.08) were not significantly affected by treatment, despite the post-hoc showing that the acid rain treatment was significantly different from the control. There were no trends for teaberry LS or sheep laurel LS (Fig. 9). Sheep laurel LS was not significantly affected by treatments (df = 3; F = 1.25; p = 0.30), nor were any of the post-hoc results (Table 3). Both species had the lowest value in the lime treatment for SLA, with the highest value for sheep laurel SLA being in the acid treatment (df = 3; F = 3.04; p = 0.04), showing a decreasing trend across treatments. No significance or trend was present in either teaberry (df = 3; F = 0.98; p = 0.41) or sheep laurel (df = 3; F = 0.55; p = 0.65) for LDMC.

The effects of treatments on leaf chemistry varied by species (Table 2). In teaberries, the lime treatment had the highest values for leaf pH (df = 3; F = 5.85; p = 0.33), Ca^{2+} (df = 3; F = 1.95; p = 0.13) and EC (df = 3; F = 2.09; p = 0.12), with the acid treatment having the lowest value for all three traits (Figure 10). This created a positive trend, showing an increase across treatments in all three teaberry leaf chemistry traits. In sheep laurel, leaf pH was highest in the acid treatment followed by lime treatment (df = 3; F = 5.85; p = 0.002). Leaf Ca^{2+} (df = 3; F = 12.95; p = 4.31E-06) and EC (df = 3; F = 2.30; p = 0.13) were highest in the control treatment for sheep laurel (p = 4.31E-06; p = 0.13), with the lowest Ca^{2+} and EC value for both traits being in the acid treatment. The post-hoc test (Table 3) revealed that teaberry EC was affected by the acid rain treatment (df = 41; F = -2.09; p = 0.04), but not the acid treatment and vice versa for sheep laurel (df = 41; F = -2.47; p = 0.01). Both sheep laurel leaf Ca^{2+} and EC showed a generally increasing trend across treatments, but with the values in the limed treatment being lower than that of the control.

Leaf physiology also varied between the two species (Figure 11). Both the acid and acid rain treatments had the two lowest values for stomatal conductance (g_{sw}) in teaberries (df = 3; F = 7.94; p = 4.58E-05), with the highest value being from the control treatment. This showed an increasing trend across treatments. Sheep laurel g_{sw} was not affected by treatment (df = 3; F = 1.81;

p = 0.15) but was affected by the block it was in (p = 1.72E-08) and showed no obvious trend across treatments. Chlorophyll content was not affected by any treatment for teaberries (df = 3; F = 0.44; p = 0.73) or for sheep laurel (df = 3; F = 1.94; p = 0.20). Though not significant, teaberry chlorophyll content showed a decreasing trend across treatments, while sheep laurel showed no trend. Absorption at 663nm was the only chlorophyll measure significantly affected in teaberries. This effect came from the location of the block (p = 0.03) rather than the treatments (df = 3; F = 1.07; p = 0.4; Table 2).

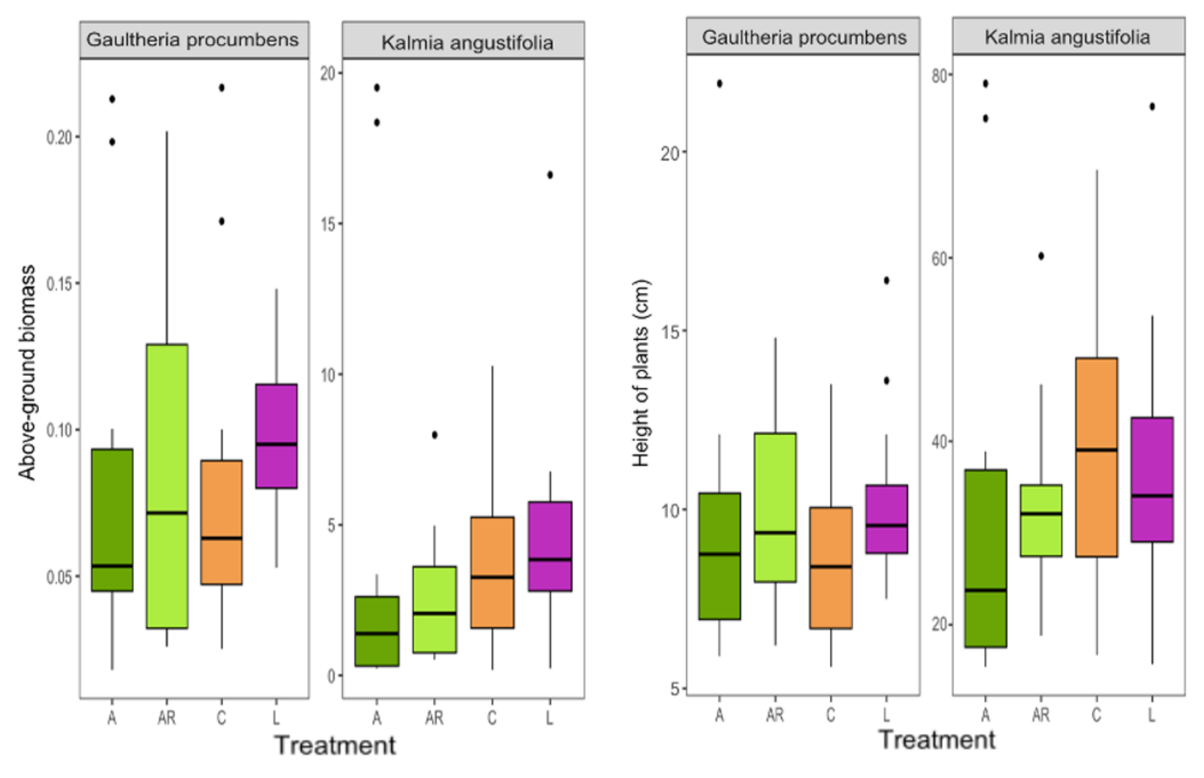

Figure 8. Variation in above-ground biomass (AGBM) and plant height for *G. procumbens* (teaberry) and *K. angustifolia* (sheep laurel) across four treatments in the Oaks Forest, Halifax, NS. These treatments are acid (A; dark green), acid rain (AR; light green), control (C; orange) and lime (L; purple). Significant differences relative to the control are based on the Tukey HSD post-hoc tests and are represented by either one ($p \leq 0.01$), two ($p \leq 0.001$) or three ($p \leq 0.0001$) red asterisks.

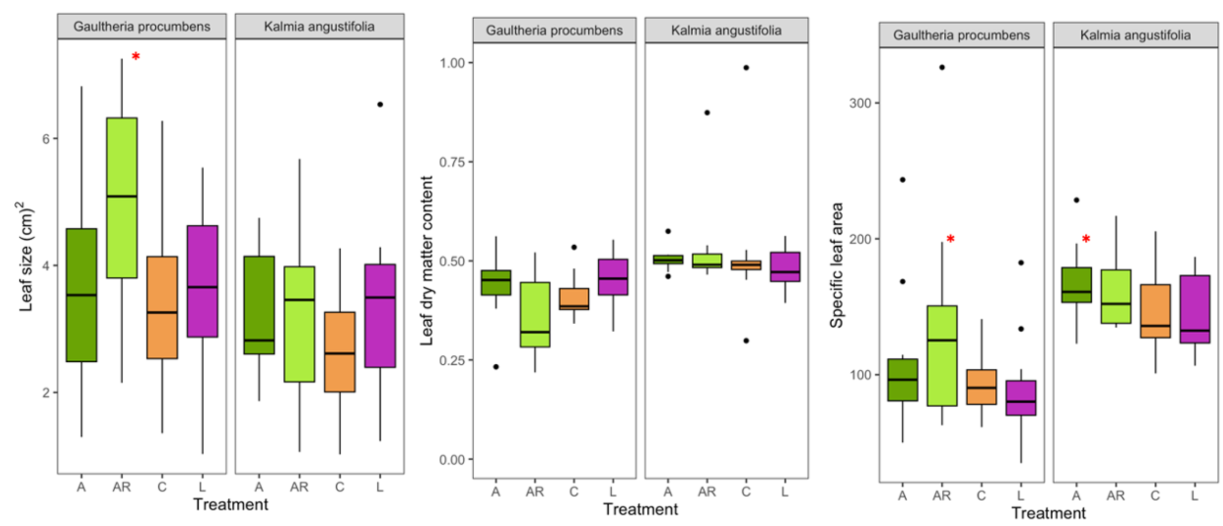

Figure 9. Variation in leaf size (LS), leaf dry matter content (LDMC) and specific leaf area (SLA)for *G. procumbens* (teaberry) and *K. angustifolia* (sheep laurel) across four treatments in the Oaks Forest, Halifax, NS. These treatments are acid (A; dark green), acid rain (AR; light green), control (C; orange) and lime (L; purple). Significant differences relative to the control are based on the Tukey HSD post-hoc tests and are represented by either one ($p \leq 0.01$), two ($p \leq 0.001$) or three ($p \leq 0.0001$) red asterisks.

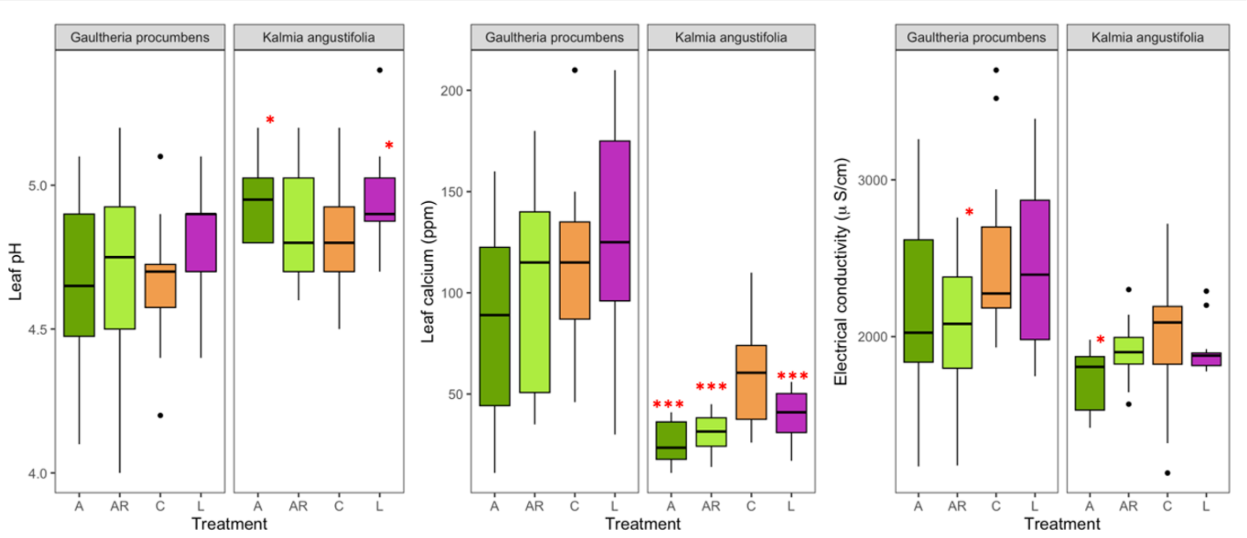

Figure 10. Variation in leaf pH, leaf Ca^{2+}, and leaf EC for *G. procumbens* (teaberry) and *K. angustifolia* (sheep laurel) across four treatments in the Oaks Forest, Halifax, NS. These treatments are acid (A; dark green), acid rain (AR; light green), control (C; orange) and lime (L; purple). Significant differences relative to the control are based on the Tukey HSD post-hoc tests and are represented by either one ($p \leq 0.01$), two ($p \leq 0.001$) or three ($p \leq 0.0001$) red asterisks.

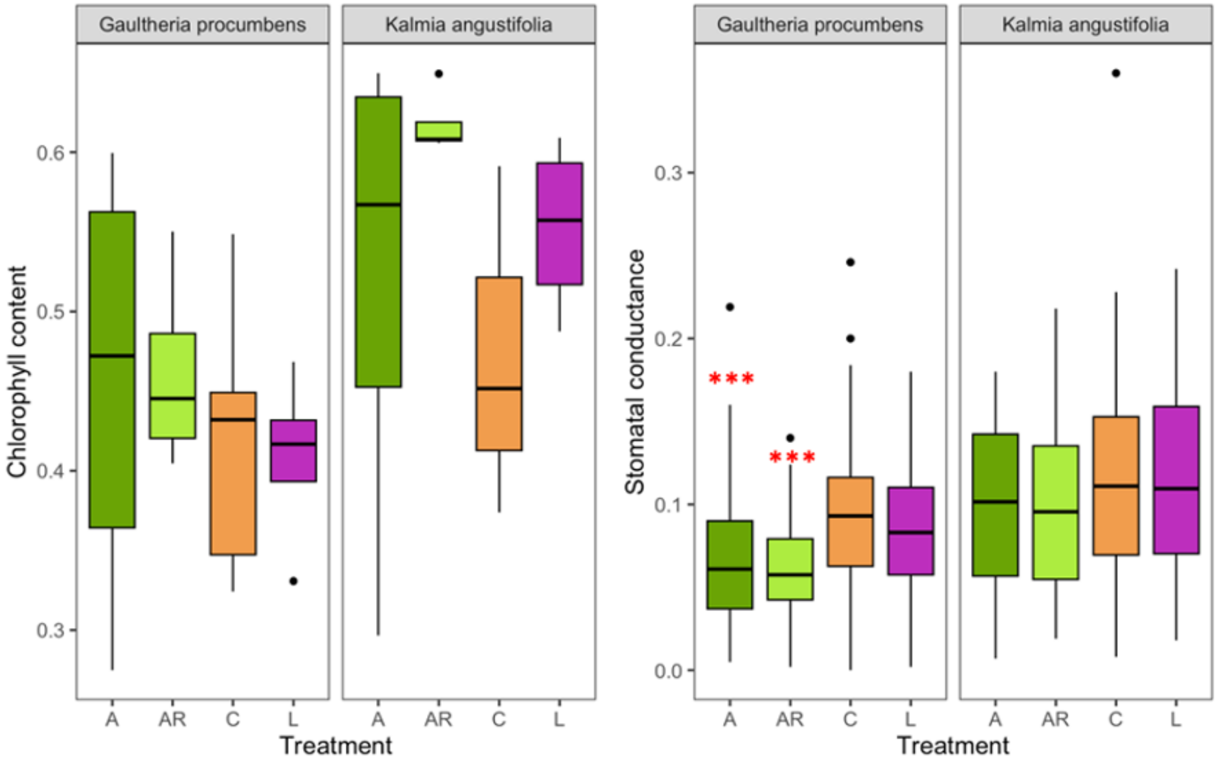

Figure 11. Variation in leaf chlorophyll content and stomatal conductance (g_{sw})for *G. procumbens* (teaberry) and *K. angustifolia* (sheep laurel) across four treatments in the Oaks Forest, Halifax, NS. These treatments are acid (A; dark green), acid rain (AR; light green), control (C; orange) and lime (L; purple). Significant differences relative to the control are based on the Tukey HSD post-hoc tests and are represented by either one ($p \leq 0.01$), two ($p\leq0.001$) or three ($p\leq0.0001$) red asterisks.

Table 2**.** Results from a linear mixed-effects model showing the significance of treatment on leaf traits in the Oaks Forest experiment in Halifax, NS with block as a random effect. The independent variables are the four treatments used for the experiment, which are acid (A), acid rain (AR), lime (L) and control (C), and the dependent variables are the plant traits. These traits are leaf pH, leaf calcium (Ca^{2+}), electrical conductivity (EC), chlorophyll content, absorbance at 663nm and 645 nm (A663; A645), stomatal conductance (g_{sw}), above-ground biomass (AGBM), leaf size (LS), leaf dry matter content (LDMC), and specific leaf area (SLA). The species are *K. angustifolia* (sheep laurel) and *G. procumbens* (teaberry).

Trait			Df	Sum Sq	F value	Pr (>F)	Pr(>Chisq)
pH	K. angustifolia	Treatment	3	0.18563	5.8515	0.002012	-
		Block	1	-	-	-	1.93E-10
	G. procumbens	Treatment	3	0.22562	1.165	0.3348	-
		Block	1	-	-	-	0.5448
Ca^{2+}	K. angustifolia	Treatment	3	8454.1	12.953	4.31E-06	-
		Block	1	-	-	-	0.009258
	G. procumbens	Treatment	3	14363	1.9532	0.1361	-
		Block	1	-	-	-	0.06761
EC	K. angustifolia	Treatment	3	444528	2.2973	0.09181	-
		Block	1	-	-	-	0.1265
	G. procumbens	Treatment	3	1928800	2.0853	0.117	-
		Block	1	-	-	-	0.8231
Chlorophyll content	K. angustifolia	Treatment	3	0.01685	1.9544	0.1985	-
		Block	1	-	-	-	0.09315
	G. procumbens	Treatment	3	0.0046244	0.4447	0.7264	-
		Block	1	-	-	-	0.1525
A663	K. angustifolia	Treatment	3	0.037161	0.74	0.5563	-
		Block	1	-	-	-	0.6726
	G. procumbens	Treatment	3	0.048152	1.0742	0.4035	-
		Block	1	-	-	-	0.02777
A645	K. angustifolia	Treatment	3	0.71148	2.5213	0.1307	-
		Block	1	-	-	-	0.05523
	G. procumbens	Treatment	3	0.20826	0.556	0.6559	-
		Block	1	-	-	-	0.282

g_{sw}	K. angustifolia	Treatment	3	0.014505	1.8138	0.1453	-
		Block	1	-	-	-	1.72E-08
	G. procumbens	Treatment	3	0.037632	7.9443	4.58E-05	-
		Block	1	-	-	-	0.2824
AGBM	K. angustifolia	Treatment	3	26.348	0.483	0.6959	-
		Block	1	-	-	-	0.1338
	G. procumbens	Treatment	3	0.0021238	0.2322	0.8734	-
		Block	1	-	-	-	1
Height	K. angustifolia	Treatment	3	309.91	0.5119	0.6763	-
		Block	1	-	-	-	0.00231
	G. procumbens	Treatment	3	19.791	0.6999	0.5575	-
		Block	1	-	-	-	0.5641
LS	K. angustifolia	Treatment	3	4.0579	1.2529	0.3031	-
		Block	1	-	-	-	0.003804
	G. procumbens	Treatment	3	16.686	2.4277	0.07912	-
		Block	1	-	-	-	0.9154
LDMC	K. angustifolia	Treatment	3	0.01693	0.5467	0.653	-
		Block	1	-	-	-	1
	G. procumbens	Treatment	3	1.0535	0.977	0.4122	-
		Block	1	-	-	-	1
SLA	K. angustifolia	Treatment	3	4525.2	3.0415	0.03955	-
		Block	1	-	-	-	9.23E-05
	G. procumbens	Treatment	3	14931	1.9757	0.1315	-
		Block	1	-	-	-	1

Table 3. Least squares means test performed with a confidence interval of 95%, showing the effects of treatments on the leaf and soil traits. The analysis compares the significance of each treatment relative to the other. The treatments are acid (A), acid rain (AR), control (C), and lime (L); the species are *Kalmia angustifolia* (sheep laurel) and *Gaultheria procumbens* (teaberry). Degrees of freedom were found using the Satterthwaite method.

Trait		**Treatment**	**Df**	**t value**	**Pr(>|t|)**
pH	K. angustifolia	A - AR	41	2.3821	0.021938
		A - C	41	3.3746	0.001626
		A - L	41	0	1
		AR - C	41	0.9925	0.326764
		AR - L	41	-2.3821	0.021938
		C - L	41	-3.3746	0.001626
	G. procumbens	A - AR	41	-0.482	0.63235
		A - C	41	-0.1607	0.87314
		A - L	41	-1.6871	0.09918
		AR - C	41	0.3214	0.74958
		AR - L	41	-1.2051	0.23509
		C - L	41	-1.5264	0.13458
Ca^{2+}	K. angustifolia	A - AR	41	-0.8442	0.4034713
		A - C	41	-5.7708	9.16E-07
		A - L	41	-2.2142	0.032432
		AR - C	41	-4.9267	1.42E-05
		AR - L	41	-1.3701	0.1781269
		C - L	41	3.5566	0.0009646
	G. procumbens	A - AR	41	-0.7215	0.47468
		A - C	41	-1.5791	0.122
		A - L	41	-2.2635	0.02896
		AR - C	41	-8576	0.39611
		AR - L	41	-1.542	0.13076
		C - L	41	-0.6844	0.49756
EC	K. angustifolia	A - AR	41	-1.851	0.07138
		A - C	41	-2.4707	0.01773
		A - L	41	-1.8743	0.06802
		AR - C	41	-0.6197	0.5389
		AR - L	41	-0.0233	0.98152
		C - L	41	0.5964	0.5542
		A - AR	41	0.5302	0.60574

	G. procumbens	A - C	41	-1.5734	0.12331
		A - L	41	-1.3521	0.18376
		AR - C	41	-2.0936	0.04252
		AR - L	41	-1.8723	0.06831
		C - L	41	0.2213	0.82595
Chlorophyll content	K. angustifolia	A - AR	8	-19084	0.09261
		A - C	8.2	0.4426	0.6695
		A - L	8	-0.7696	0.46355
		AR - C	8.2	2.1794	0.06003
		AR - L	8	1.1388	0.28762
		C - L	8.2	-1.143	0.28524
	G. procumbens	A - AR	9.9	-0.2656	0.7961
		A - C	10	0.6102	0.5553
		A - L	9	0.7044	0.4974
		AR - C	10	0.8885	0.3951
		AR - L	9.9	0.9698	0.3552
		C - L	10	0.1284	0.9004
A663	K. angustifolia	A - AR	8.1	-1.3527	0.2126
		A - C	8.6	-0.6482	0.5338
		A - L	8.2	-1.1996	0.2641
		AR - C	8.6	0.596	0.5665
		AR - L	8.1	0.153	0.8821
		C - L	8.6	-0.4553	0.6602
	G. procumbens	A - AR	10	-1.7788	0.1057
		A - C	10	-0.7418	0.4752
		A - L	10	-0.8966	0.391
		AR - C	10	1.1217	0.2882
		AR - L	10	0.8822	0.3985
		C - L	10	-0.1975	0.8474
A645	K. angustifolia	A - AR	8	-2.0047	0.07985
		A - C	8.2	0.7657	0.46537
		A - L	8	-0.5453	0.6004
		AR - C	8.2	2.5881	0.03164
		AR - L	8	1.4594	0.18248
		C - L	8.2	-1.2614	0.24196
	G. procumbens	A - AR	9.9	0.1111	0.9138
		A - C	10	0.8772	0.401
		A - L	9.9	1.0436	0.3215
		AR - C	10	0.7606	0.4644
		AR - L	9.9	0.9325	0.3732
		C - L	10	0.2178	0.832

g_{sw}	K. angustifolia	A - AR	233	-0.3855	0.70025
		A - C	233	-1.7204	0.08669
		A - L	233	-1.9078	0.05764
		AR - C	233	-1.335	0.1832
		AR - L	233	-1.5224	0.12927
		C - L	233	-0.1874	0.85149
	G. procumbens	A - AR	233	0.788	0.4315101
		A - C	233	-3.4598	0.0006428
		A - L	233	-2.3984	0.0172546
		AR - C	233	-4.2477	3.12E-05
		AR - L	233	-3.1864	0.0016378
		C - L	233	1.0614	0.2896257
AGBM	K. angustifolia	A - AR	41	0.8416	0.4049
		A - C	41	0.2566	0.7987
		A - L	41	-0.3194	0.751
		AR - C	41	-0.585	0.5618
		AR - L	41	-1.161	0.2523
		C - L	41	-0.5761	0.5677
	G. procumbens	A - AR	44	-0.5331	0.5966
		A - C	44	-0.142	0.8878
		A - L	44	-0.7361	0.4656
		AR - C	44	0.3911	0.6976
		AR - L	44	-0.203	0.8401
		C - L	44	-0.5941	0.5555
Height	K. angustifolia	A - AR	41	-0.1063	0.9158
		A - C	41	-1.0216	0.3129
		A - L	41	-0.8032	0.4265
		AR - C	41	-0.9153	0.3654
		AR - L	41	-0.6969	0.4898
		C - L	41	0.2184	0.8282
	G. procumbens	A - AR	41	-0.2726	0.7865
		A - C	41	0.8577	0.396
		A - L	41	-0.492	0.6253
		AR - C	41	1.1303	0.2649
		AR - L	41	-0.2194	0.8274
		C - L	41	-1.3497	0.1845
LS	K. angustifolia	A - AR	41	-0.2204	0.82664
		A - C	41	1.3758	0.17636
		A - L	41	-0.3295	0.74349
		AR - C	41	1.5962	0.11812
		AR - L	41	-0.109	0.91371

		C - L	41	-1.7052	0.09572
	G. procumbens	A - AR	41	-2.2269	0.03151
		A - C	41	0.099	0.92163
		A - L	41	-0.2166	0.82961
		AR - C	41	2.3259	0.02505
		AR - L	41	2.0103	0.05101
		C - L	41	-0.3156	0.75393
LDMC	K. angustifolia	A - AR	44	-0.5727	0.5697
		A - C	44	-0.2625	0.7941
		A - L	44	0.6566	0.5149
		AR - C	44	0.3102	0.7579
		AR - L	44	1.2294	0.2255
		C - L	44	0.9191	0.363
	G. procumbens	A - AR	44	1.3489	0.1843
		A - C	44	1.4993	0.1409
		A - L	44	1.3186	0.1941
		AR - C	44	0.1504	0.8812
		AR - L	44	-0.0303	0.976
		C - L	44	-0.1806	0.8575
SLA	K. angustifolia	A - AR	41	0.6367	0.52788
		A - C	41	2.3054	0.02628
		A - L	41	2.4982	0.01658
		AR - C	41	1.6687	0.10279
		AR - L	41	1.8616	0.06984
		C - L	41	0.1928	0.84804
	G. procumbens	A - AR	44	-1.3124	0.19621
		A - C	44	0.7248	0.47241
		A - L	44	0.8568	0.3962
		AR - C	44	2.0372	0.04768
		AR - L	44	2.1691	0.03552
		C - L	44	0.132	0.8956
pH	Soil	A - AR	12	1.3823	0.19206
		A - C	12	-1.2441	0.23722
		A - L	12	1.797	0.09753
		AR - C	12	-2.6264	0.02212
		AR - L	12	0.4147	0.68568
		C - L	12	3.0411	0.01025
Ca2+	Soil	A - AR	9	1.1043	0.2981
		A - C	9	0.3313	0.748
		A - L	9	0.5522	0.5943
		AR - C	9	-0.773	0.4593

		AR - L	9	-0.5522	0.5943
		C - L	9	0.2209	0.8301
EC	Soil	A - AR	9	0.7702	0.4609
		A - C	9	0.5912	0.5689
		A - L	9	1.0325	0.3288
		AR - C	9	-0.179	0.8619
		AR - L	9	0.2623	0.799
		C - L	9	0.4413	0.6694

3.3. Field study in Sudbury, ON

3.3.1. Soil characteristics in the Sudbury field study

Of the three soil characteristics measured in Greater Sudbury, ON, only pH and Ca^{2+} showed significant differences between sites (Table 4). The soil pH was highest in the high-contamination limed site (H-L; df = 2; F = 9.61; p = 0.001). Soil pH was lowest in the high-contamination un-limed site (H-U) and showed an increasing trend across sites. The amount of Ca^{2+} in soils was highest in the low contamination site (Low), and lowest in the H-L site (df = 2; F = 3.64; p = 0.04). There is no significant trend between sites for soil EC (df = 2; F = 0.168; p = 0.85).

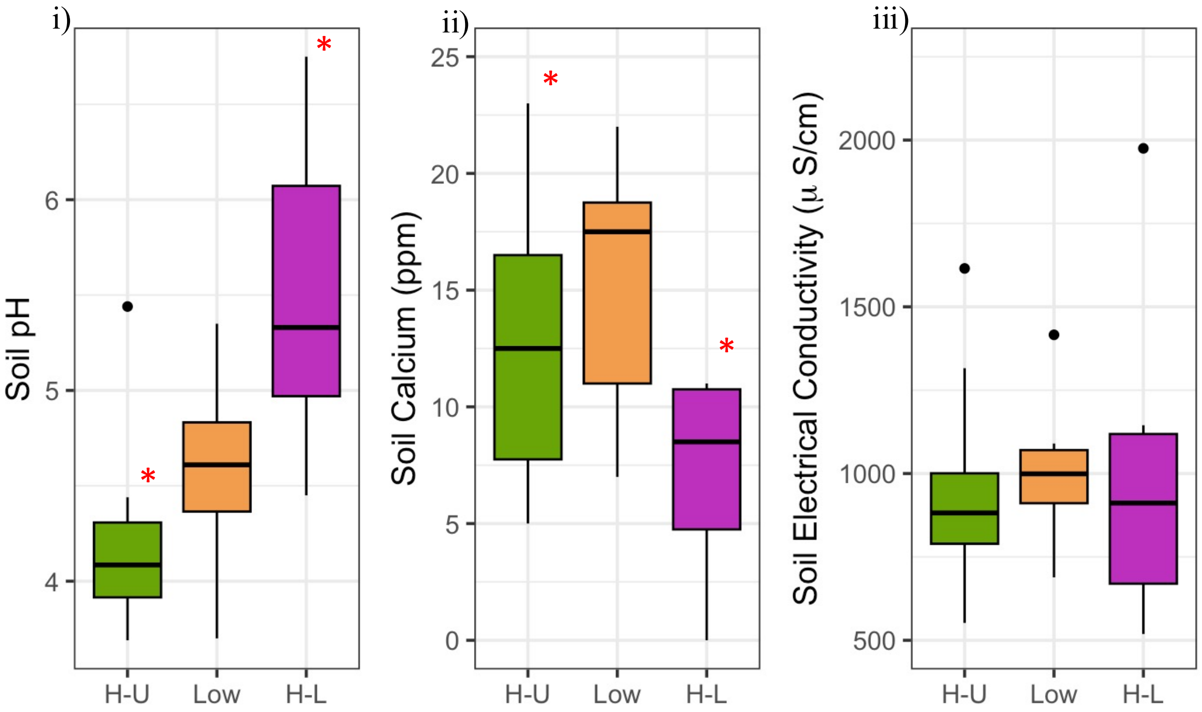

Figure 12. Variation in three soil characteristics across three sites: high contamination and unlimed (H-U), low contamination (Low), and high contamination and limed (H-L), in Greater Sudbury, ON. Data are from soil where plant samples were collected ($n \geq 5$ soil samples per site). The characteristics measured are (i) soil pH, (ii) soil calcium (Ca^{2+}), and (iii) soil electrical conductivity (EC). Significant differences relative to the control are based on the Tukey HSD post-hoc tests and are represented by either one ($p \leq 0.01$) or two ($p \leq 0.001$) red asterisks.

Table 4. Results from a one-way ANOVA showing the effect of site type on soil pH, Ca^{2+} and electrical conductivity (EC) of soil samples from Sudbury, ON. The independent variable is site, which had three levels: a high acid contamination and limed site (H-L), a high acid contamination and un-limed site (H-U), and a low acid contamination site (Low).

Traits			Df	Sum Sq	F value	Pr (>F)
pH	Soil	Site	2	7.057	9.611	0.001
		Residuals	21	7.71	-	-
Ca^{2+}	Soil	Site	2	215.1	3.644	0.04
		Residuals	21	619.8	-	-
EC	Soil	Site	2	0.0377	0.168	0.80
		Residuals	21	2.3508	-	-

3.3.2. Leaf traits of sheep laurel and lowbush blueberry

Trends between plant species and sites were generally similar for many leaf morphology traits (Figure 13). In sheep laurel, H-U had the largest LS (df = 2; F = 5.32; p = 0.01) and in lowbush blueberries, the highest LS value was in the Low site (df = 2; F = 4.73; p = 0.02). The H-L site had the lowest value for LS in both species. In sheep laurel, LS had a significant trend, showing a decrease across sites. LDMC values varied slightly between species, but the trends across sites were similar in both species. The H-L site had the highest LDMC for lowbush blueberry (df = 2; F = 19.47; p = 5.83E-06) and sheep laurel (df = 2; F = 7.84; p = 0.002), and the lowest values were from the Low site was for both species. Lowbush blueberry and sheep laurel share a similar pattern across site types for SLA. The Low site had the largest SLA, and the H-L site had the smallest SLA for lowbush blueberry (df = 2; F = 56.91; p = 2.07E-10) and sheep laurel (df = 2; F = 38.3; p = 1.31E-08).

Leaf chemistry traits collected in Greater Sudbury varied by species (Table 5). Leaf pH in the lowbush blueberry (df = 2; F = 2.74; p = 0.08) was highest in the H-L site and lowest in the H-

U site, showing a non-significant across sites (Fig. 14). The H-U site had the highest leaf pH for sheep laurel (df = 2; F = 0.40; p = 0.03), with the lowest being in the H-L site. For leaf Ca^{2+}, neither lowbush blueberry (df = 2; F = 3.27; p = 0.054), nor sheep laurel (df = 2; F = 3.31; p = 0.052) was significantly affected by site. There was significant difference between sites, with the highest leaf Ca^{2+} value at the H-U site in both lowbush blueberries and sheep laurel (Table 6), and lowest at the Low site. There was no significance or trend in leaf EC for lowbush blueberries (df = 2; F = 0.13; p = 0.88) between sites. The contamination level and treatment of the sites had a significant effect on leaf EC in sheep laurel (df = 2; F = 3.45; p = 0.046), but the leaf EC values between the sites was not significant (Table 6).

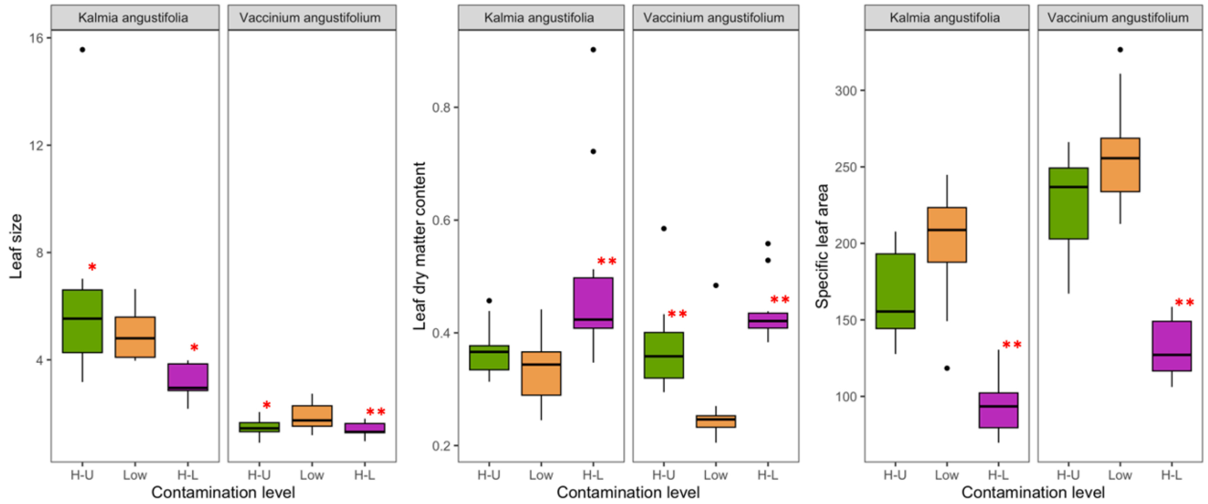

Figure 13. Variation in three leaf morphology traits for *K. angustifolia* (sheep laurel) and *V. angustifolium* (lowbush blueberry) across three sites varying in acid contamination and liming: high contamination and un-limed site (H-U), a low contamination site (Low), and a high contamination and limed site (H-L), in Sudbury, ON. Data are from individual plants (n = 10 per species, per site). The leaf traits measured are leaf size (LS), leaf dry matter content (LDMC), and specific leaf area (SLA). Significant differences relative to the control are based on the Tukey HSD post-hoc tests and are represented by either one ($p \leq 0.01$) or two ($p \leq 0.001$) red asterisks.

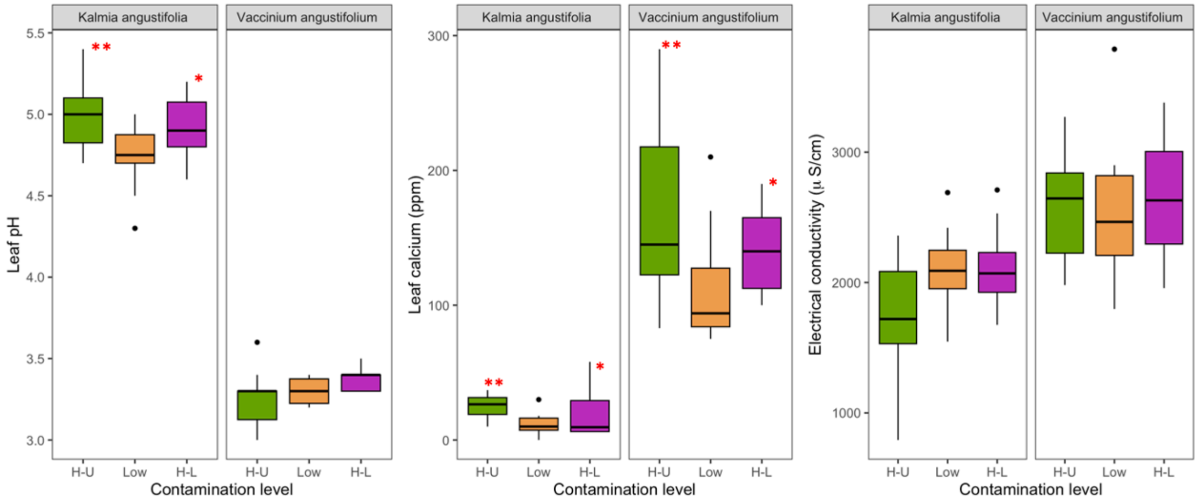

Figure 14. Variation in three leaf chemistry traits for *K. angustifolia* (sheep laurel) and *V. angustifolium* (lowbush blueberry) across three sites varying in acid contamination and liming: high contamination and un-limed site (H-U), a low contamination site (Low), and a high contamination and limed site (H-L), in Sudbury, ON. Data are from individual plants (n = 10 per species, per site). The leaf traits measured are leaf pH, leaf calcium (Ca^{2+}) and leaf electrical conductivity (EC). Significant differences relative to the control are based on the Tukey HSD post-hoc tests and are represented by either one ($p \leq 0.01$) or two ($p \leq 0.001$) red asterisks.

Table 5. Results from a one-way ANOVA showing the significance of the effect that site had on leaf traits across three sites in Greater Sudbury, ON. Sites are high acid contamination and limed (H-L), high acid contamination and un-limed (H-U), and low acid contamination (Low). Leaf traits are: (i) leaf pH, (ii) leaf calcium (Ca^{2+}), (iii) electrical conductivity (EC), (iv) leaf size (LS), (v) leaf dry matter content (LDMC), and (vi) specific leaf area (SLA). The species are *K. angustifolia* (sheep laurel) and *V. angustifolium* (lowbush blueberry).

Traits	**Species**		**Df**	**Sum Sq**	**F value**	**Pr (>F)**
pH	K. angustifolia	Site	2	0.01624	0.4009	0.03
		Residuals	27	0.05469	-	-
	V. angustifolium	Site	2	0.00709	2.744	0.08
		Residuals	27	0.03489	-	-
Ca^{2+}	K. angustifolia	Site	2	14.38	3.314	0.051
		Residuals	27	58.56	-	-
	V. angustifolium	Site	2	26.58	3.265	0.054
		Residuals	27	109.91	-	-
EC	K. angustifolia	Site	2	970154	3.452	0.046
		Residuals	27	3794591	-	-
	V. angustifolium	Site	2	67414	0.134	0.875
		Residuals	27	6788784	-	-
LS	K. angustifolia	Site	2	49	5.323	0.0112
		Residuals	27	124.3	-	-
	V. angustifolium	Site	2	1.546	4.726	0.0174
		Residuals	27	4.417	-	-
LDMC	K. angustifolia	Site	2	0.7282	7.836	0.00207
		Residuals	27	1.2546	-	-
	V. angustifolium	Site	2	1.4346	19.47	5.83E-06
		Residuals	27	0.9949	-	-
SLA	K. angustifolia	Site	2	3.117	38.3	1.31E-08
		Residuals	27	1.099	-	-
	V. angustifolium	Site	2	2.622	56.91	2.07E-10
		Residuals	27	0.6231	-	-

Table 6. Tukey HSD post-hoc test showing the individual effects that each of the Sudbury sites had on leaf and soil traits for two species. The species are *Kalmia angustifolia* (sheep laurel) and *Vaccinium angustifolium* (lowbush blueberry). The test was performed with an α-value of 0.05, treatments with the same letter are not significantly different from each other.

Trait	Species	Contamination level	Post-hoc Result
pH	K. angustifolia	High-Limed	ab
		High-Unlimed	a
		Low	b
	V. angustifolium	High-Limed	a
		High-Unlimed	a
		Low	a
Ca^{2+}	K. angustifolia	High-Limed	ab
		High-Unlimed	a
		Low	b
	V. angustifolium	High-Limed	ab
		High-Unlimed	a
		Low	b
EC	K. angustifolia	High-Limed	a
		High-Unlimed	a
		Low	a
	V. angustifolium	High-Limed	a
		High-Unlimed	a
		Low	a
LS	K. angustifolia	High-Limed	b
		High-Unlimed	a
		Low	ab
	V. angustifolium	High-Limed	b
		High-Unlimed	ab
		Low	a
LDMC	K. angustifolia	High-Limed	a
		High-Unlimed	b
		Low	b
	V. angustifolium	High-Limed	a
		High-Unlimed	a
		Low	b

SLA	K. angustifolia	High-Limed	b
		High-Unlimed	a
		Low	a
	V. angustifolium	High-Limed	b
		High-Unlimed	a
		Low	a
pH	Soil	High-Limed	b
		High-Unlimed	ab
		Low	a
Ca2+	Soil	High-Limed	b
		High-Unlimed	ab
		Low	a
EC	Soil	High-Limed	a
		High-Unlimed	a
		Low	a

4.1. Discussion

4.2. What were the effects of sulphuric acid deposition and lime on plant growth?

There were no significant effects on plant height or AGBM from any of the treatments in the Oaks experiment. However, there was a non-significant trend showing an increase in the AGBM and height of both species in the limed treatment compared the control and acid treatments. This increase could be explained by increased amounts of soil Ca^{2+} in the limed treatment (Fig. 5). Ingerslev and Hallbäcken (1999) found that AGBM of Norway spruce was not affected by various lime treatments. However, they did find that needles and bark had higher nutrient content, which may have occurred in teaberry and sheep laurel. Since they are much smaller than a Norway spruce, it is possible that the increase in soil Ca^{2+} had a more prominent effect on plant growth. More nutrients in the stem could also explain the slight increase in height observed in teaberry, as Ca is an essential plant nutrient.

A study done by Darendeh et al. (2011) found that the height of Asiatic lilies decreased when exposed to varying concentrations of H_2SO_4 solution. Jacobson et al. (1990) also found that an acidic mist reduced the shoot length in red spruce seedlings. Meanwhile, other studies provide data that height and AGBM are not affected by acid rain, which may be why the plant growth decreases seen in the acid treatments of both species was not significant (Dixon & Kuja, 1995; Shi et al., 2021).

Compared to the control, sheep laurel AGBM and height in the acid treatments were reduced more than those of the teaberry in the acid treatments (Fig. 8). This reduction could be linked to leaf longevity type. Du et al (2017) found that plant species reacted to simulated acid rain differently depending on if their leaves were deciduous or evergreen. Sheep laurel is semi-deciduous, and teaberries are evergreen, which may explain why sheep laurel seems to be less acid tolerant than teaberries.

It is also possible that the pH of the H_2SO_4 (3.0 pH) treatment was not acidic enough to cause a significant effect in the shrubs within a single growing season. This agrees with Neufeld et al. (1985), who found that when exposed to 3.0 pH sulphuric acid, the height and biomass of the seedlings of four deciduous tree species were not significantly affected by acid treatments. Shelburne et al. (1991) also found that H_2SO_4 treatments of pH 5.3, 4.3 and 3.3 had no significant effect on the height and AGBM of shortleaf pine trees. While these studies were not performed on shrubs, they indicate that the height and AGBM of both evergreen and deciduous woody plant species are not heavily affected by acid rain with a pH above 3.0 in the short-term.

4.3. What were the effects of sulphuric acid deposition and lime on leaf morphology?

LS is the average surface area of a leaf, LDMC often represents the density of a leaf (Shipley & Vu, 2002), while SLA represents the tradeoff that a plant makes between expanding its leaves and investing energy in structural components (Poorter et al., 2009). The higher the SLA value is, the more focus the plant is putting on expanding its leaf area, and the lower the SLA is, the more focus the plant is allocating to structural components. Compared to the control, the acid rain treatment in the Oaks increased both LS and SLA in the teaberry, but no treatment influenced LDMC. This size increase could be because of ammonium in the soil (Fig. 7). Nitrogen-deficient plants have been shown to exhibit a decrease in the LS (Chen et al., 2018). On the other hand, Pharis et al. (1963) found that when ammonium and calcium are present in soil together, loblolly pine seedlings grew to be much taller than if only ammonium was present. While soil Ca^{2+} levels were not high in the acid rain treatment (Fig. 5), the soil NH_4 levels were the highest of all the treatments (Fig. 7). The combining effect of these plant nutrients could be why teaberry LS and SLA were significantly larger in the acid rain treatment.

Sheep laurel had a larger SLA in the acid treatment compared to the control but had no significant responses to the treatments in LS or LDMC. Chen et al. (2018) found that a phosphorus (P) deficiency in Texas bluebells resulted in a decrease in LS, which was not seen here. However, if a plant is less able to focus on expanding leaf size due to a P-deficiency, this would likely decrease the SLA as well. This is what is seen in sheep laurel in the Oaks, as the SLA trend matches the trend seen in soil P-content (Fig. 6). Since there was no SLA peak for sheep laurel in the acid rain treatment, it could indicate that sheep laurel SLA is less dependent on ammonium than the teaberries are.

In the Oaks experiment, both the teaberry and the sheep laurel showed no clear trends in LS or in LDMC. Apart from teaberry LS, where there was a significant increase in the acid rain treatment, the treatments did not seem to have a large effect on LS and LDMC in either species. This lack of effect is supported by previous studies on simulated acid rain. These studies have shown that acid rain treatments of 3.0 pH or lower either decrease or have no significant effect on LS and LDMC (Balasubramanian et al., 2007; Evans & Lewin, 1981; Hogan, 1998; Lal & Singh, 2012; Paoletti & Manes, 2003; Shelburne et al., 1991).

The lowbush blueberries in Sudbury had smaller leaves in both the un-limed and limed sites and largest leaves in the reference site, while sheep laurel had smaller leaves in the limed site and larger leaves in the un-limed site compared to the low contamination reference site. The smaller leaves in the un-limed site is supported by previous studies (Evans & Lewin, 1981; Hogan, 1998; Lal & Singh, 2012; Paoletti & Manes, 2003; Shelburne et al., 1991).

However, the smaller LS for lowbush blueberry and sheep laurel in the limed site and larger leaves for sheep laurel in the un-limed site is unexpected. This could be explained by differences in soil pH between the limed and un-limed sites. The limed site was significantly less acidic compared to the other sites. It may be that the higher pH in the limed site is causing the decrease in LS and SLA seen in both sheep laurel and lowbush blueberry (Fig. 12). Kidd and Proctor (2000) found that soil pH plays a significant role in the size of common velvetgrass leaves. For them, making soils more alkaline increased leaf size and making soils more acidic decreased LS. However, acid-tolerant plants like the Ericaceae do not like to grow in alkaline, Ca-abundant soils and it can even hinder plant growth. It's possible that the higher soil pH found in the limed site could be hindering the growth of lowbush blueberry and sheep laurel leaves, which may be causing a decrease in both LS and SLA, while the un-limed site allows for the larger leaves seen in sheep laurel.

The larger sheep laurel leaves in the un-limed site could also be explained by differences in the soil Ca^{2+} levels found in this site. Despite not being limed, this site had the highest soil Ca^{2+} content. With the increase in essential growth nutrient availability, sheep laurel would likely be able to grow more.

LDMC was higher in the limed site for sheep laurel and in the un-limed and limed sites for lowbush blueberries. The LS of both species was also smaller in each site where LDMC was higher. Since these plants were not expanding their leaf size, it makes sense that any leaf growth occurring would be in structural components, as measured by dry matter content. Hence, the higher LDMC where there was a lower LS. Since more focus is being put on building structural components in these leaves rather than investing in larger leaf surface area, the SLA in the contaminated sites is also smaller compared to the low contamination site.

The similarity between sheep laurel and lowbush blueberry may be because of their leaf longevity type. Sheep laurel leaves are semi-deciduous, so it loses a large portion of its leaves during the year; lowbush blueberry leaves are deciduous and lose all of their leaves in the fall; and teaberry leaves are evergreen, so they only lose their leaves after multiple years (Kikuzawa & Lechowicz, 2011; Vander Kloet, 2008). Differences between how plants respond to acid rain treatments has been shown to be related to leaf longevity type, with evergreen leaves and deciduous leaves showing different responses (Du et al., 2017). Because sheep laurel and lowbush blueberries both lose leaves, their leaves may be more expendable than the evergreen teaberry leaves. If the expendability of their leaves is more similar, then it would make sense that the leaves are responding to the stressors more similarly, hence the similar patterns in LDMC and SLA across sites.

4.4. What were the effects on acid and lime on leaf chemistry?

Leaf pH is a plant trait that varies by species and environment, and is a key factor in the uptake of ions into the leaves, as well as plant physiological traits (Cornelissen et al., 2011; Liu et al., 2021). Leaf pH is often a species determined trait that is not always affected by the environment in the same ways as other plants, so teaberry leaf pH having no significant response to treatments could just mean that its leaf pH is more resistant to changes (Cornelissen et al., 2011). In the Oaks Forest experiment, sheep laurel leaf pH was raised in both the acid and lime treatments compared to the control. Similarly in Greater Sudbury, sheep laurel leaf pH was higher in the un-limed and limed sites relative to the reference site. The similarity between the short and long-term effects could mean that any lasting effects of acid rain and lime on sheep laurel leaf pH happen quickly. The increase in leaf pH due to liming was expected, but the increase in the acid treatment was not. None of the other plant traits analyzed offer any insight into why the leaf pH of sheep laurel increased in the acid treatment, so it could be that the leaf pH response to the environment is unique to sheep laurel (Cornelissen et al., 2011).

Calcium in leaves is essential for cell growth and aids in adding structure to cell walls (McLaughlin & Wimmer, 1999; White & Broadley, 2003). The acid treatment decreased the leaf Ca^{2+} in both species. Sheep laurel leaf Ca^{2+} was also decreased in the acid rain treatment. This was expected, as acids lower soil pH and allows for the mobilizations of Al^{3+} which can block Ca^{2+} uptake by the plant (Panda, Baluška, & Matsumoto, 2001; Rengel & Elliot, 1992). This is also supported by the soil Ca^{2+} levels from the PRS probes (Fig. 5), which shows that the acid treatments had the lowest amounts of Ca^{2+} in the soil. With less available Ca^{2+} in the soil, plants would have a decreased amount of Ca^{2+} in their leaves.

Sheep laurel leaf Ca^{2+} was also decreased in the lime treatment, but still had more Ca^{2+} in the leaves than in both acid treatments. Jeffries & Willis (1964) found that excess calcium in the soil hinders growth in some species of acid-tolerant plants and may illicit no response in other species. There was a lack of growth of sheep laurel (see section 4.2.), which would explain why there was less Ca^{2+} in leaves, as calcium is an essential plant growth nutrient and without it present, it is difficult for growth to occur (McLaughlin & Wimmer, 1999).

In the Oaks experiment, leaf Ca^{2+} in the teaberry was not significantly affected by any of the treatments. However, there was a clear increasing trend, with the acid treatments having the lowest leaf Ca^{2+} values and the lime treatment having the highest. The decrease seen in the acid treatments is supported by Scherbatskoy and Klein (1983) who found that the Ca^{2+} content in the leaves of yellow birch and white spruce decreased when exposed to simulated acid rain and continued to decrease as the rain became more acidic. The increase in the lime treatment may be linked to the soil Ca^{2+} levels being highest in this treatment (Fig. 5). With the increase of available Ca^{2+} in the soil, there is a higher chance that a plant will uptake that calcium, resulting in a higher leaf Ca^{2+} value.

Similar patterns were found between sheep laurel and lowbush blueberry for the leaf Ca^{2+} across the sites in Greater Sudbury, Ontario (Fig. 14). The leaf Ca^{2+} of these species was highest in the un-limed site, possibly because soil Ca^{2+} levels were highest in this site. The second highest leaf Ca^{2+} value for both species was in the un-limed site, which had the lowest soil Ca^{2+} levels. It may be that the Ca^{2+} is not present in the soil because it has been sequestered in the plant leaves. Ca^{2+} uptake in acid-tolerant plants, such as Ericaceous shrubs, is very efficient (Korcak, 1989).

This, combined with the observed higher density of plants in the limed site could explain why Ca^{2+} was lowest in the soil, but second highest in leaves.

Leaf EC is a measurement of total ion concentration in the leaves and can be used as an indicator of plant health (Samarakoon et al., 2006). The EC of both teaberry and sheep laurel was significantly lowered in the acid treatments compared to the control. Sheep laurel EC was lowered in the acid rain treatment and teaberry EC was lowered in the acid treatment. Calcium ions were likely one of the most exuded leachates for both species because the measurements for leaf Ca^{2+} content were quite low in the acid treatments. This is supported by studies showing that Ca^{2+} leaches in high amounts from needles/leaves in red pine trees and sugar maple seedlings exposed to 3.0 pH acidified mists (DeHayes et al., 1999; Scherbatskoy & Klein, 1983; Wood & Bormann, 1975). Other common foliar leachates are K^+ and Mg^{2+}, which could possibly account for some of the other ions lost from the total leaf ion concentration (i.e., EC) seen in the acid treatment (Scherbatskoy & Klein, 1983; Wood & Bormann, 1975). Sheep laurel EC in Sudbury was not affected by the soil contamination level or lime treatment, suggesting that the significant decrease in EC seen in the Oaks Forest may just be a temporary response to a novel stressor for this species.

4.5. What were the effects on acid and lime on leaf physiology?

Chlorophyll is the pigment that allows a plant to absorb light for photosynthesis, thus, chlorophyll content in leaves is essential in a plant's ability to photosynthesize. With long-term decreases in chlorophyll content, overall plant growth is likely to decrease (Du et al., 2017; Dungarwal et al., 1974; Fan & Wang, 2000). Treatments had no significant effect on chlorophyll content in either species but showed a decreasing trend in teaberries. Teaberry leaves in the acid treatment had the most chlorophyll, and teaberry leaves in the limed treatment had the least chlorophyll. This suggests that acid rain increases the chlorophyll content in teaberry leaves, at least initially. Kim (1987) performed a study on the effects of acid rain on the physiology of *Gingko biloba,* which showed an increase in leaf chlorophyll content. This was found to be a stress response to increases in acidity and was not a long-term effect. As the leaves were exposed to the acid rain for a longer period of time, leaf chlorophyll content began to decrease.

Stomatal conductance rate (g_{sw}) is a measurement of leaf water gas exchange that reflects stomatal openness (Gimenez et al., 2005). It is balanced by the plant to maximize gas exchange and minimize water loss, based on the environment around it (Sasaki et al., 2010). The acid and acid rain treatments had approximately the same effect on g_{sw} and were the only treatments to decrease g_{sw} in teaberries. It has been shown in various plants that exposure to sulphuric acid decreases g_{sw} (Khpalwak et al., 2017; Martens et al., 1989), but the opposite has also been shown, with the loblolly pine showing an increase in g_{sw} (Flagler et al., 1994). This reiterates that deciduous plants and evergreen plants likely respond differently to stressors and could help explain why the g_{sw} in sheep laurel did not change significantly, but it did in teaberry.

Variation in g_{sw} is linked to the amount of available water in the soil. Soil water content was approximately the same across treatments and all plots were exposed to the same environment, yet g_{sw} decreased in the acid treatment. This means that H_2SO_4 must be having some effect on plant physiology, possibly through an impact on soil water or solute availability. Through H_2SO_4, soil pH decreases, allowing Al^{3+} to move more freely in the soil (Panda, Baluška, & Matsumoto, 2001). Through H_2SO_4, soil pH decreases, allowing Al^{3+} to move more freely in the soil, increasing the likelihood of Al^{3+} being taken into the plant via the roots (Panda, Baluška, & Matsumoto, 2001). Al transporters have been associated with stomatal closing in thale cress, reducing g_{sw} despite the environment not requiring it (Sasaki et al., 2010). Aluminum ions in the soil have also been shown to interact with Rangpur lime plants, decreasing the g_{sw} in the leaves (Banhos et al., 2016). Since the acid treatments are having H_2SO_4 applied, this would likely make soils more acidic, thus potentially releasing more Al^{3+} into the soil, which may explain the decrease in g_{sw} seen in teaberries.

4.6. Do different leaf longevity types have different responses to acid rain and liming?

Leaf longevity seemed to play a role in how plants responded to the treatments. In the Oaks experiment, teaberries generally seemed to be more sensitive to acid treatments, but more responsive to liming treatments when compared to sheep laurel. Since teaberries are evergreen, they may have taken up more nutrients since it produces evergreen leaves, it may be more inclined to sequester as many ions in its leaves as possible during its growing season. This is supported by Chastain et al. (2006) who found that mountain laurel and great laurel, two evergreen understory shrubs, act as nutrient sinks in the Appalachian Mountains. The nutrients they studied were carbon, nitrogen, and phosphorus, but it could be possible that Ca^{2+} is also highly sequestered in evergreen understory shrubs, which would explain the observed responses in the teaberries in the Oaks.

Compared to teaberry, sheep laurel in the Oaks was more resistant to changes in acidity and less responsive to liming, but still exhibited growth with liming. Sheep laurel are the only species in this study that can have the short-term effects of acid rain and liming directly compared to the long-term effects. In Sudbury, sheep laurel and lowbush blueberry had very similar responses to the contamination levels and treatments of the sites. This similarity could be related to the types of leaf longevity these plants exhibit, as both are types of deciduous species, with sheep laurel being semi-deciduous and the blueberry being fully deciduous. Evergreen and deciduous species have different responses to acid rain, and since both sheep laurel and lowbush blueberry leaves are more expendable than the leaves of the evergreen teaberry, they would be more likely to share similar responses (Du et al., 2017). It could also be because they were both exposed to acid rain and liming over such a long period of time that eventually their responses to acid rain and liming became similar.

4.7. Caveats

During the summer that the Oaks Forest experiment was conducted, the weather was very extreme for Nova Scotia. Early in the summer, the province experienced raging forest fires, followed by major flooding which is very out of the ordinary. Throughout the summer there were days of extreme heat followed by heavy rain. These may have affected the responses of the plants by hindering the effects of the treatments, as the stressors in the natural environment may have taken priority over the treatments. It is also important to note that the control treatment has been exposed to the acidic rain in Nova Scotia for many years, as well as during the experiment through exposure to rainfall.

5.1. Conclusions

Some immediately obvious benefits of liming acid-tolerant plants in Nova Scotia were seen. Though not significant, plant height and AGBM both increased when exposed to the lime treatment. The effects of acid rain and liming were also found to vary by species, with liming increasing leaf Ca^{2+}, EC and LDMC in teaberries, but not sheep laurel. In Sudbury, the SLA and LDMC of sheep laurel and lowbush blueberry shared similar responses to contamination levels and treatments. Species-specific responses were also seen in Sudbury, as the leaves of lowbush blueberries took in more Ca^{2+} in the limed site, while the leaf EC of sheep laurel decreased in the un-limed site. When the Oaks experiment is compared to the field study in Sudbury, some long-term benefits to liming could be seen. Generally, plants in the limed sites of Sudbury had an increase in leaf pH, Ca^{2+}, EC and LDMC. This suggests the potential for positive lime effects on acid-tolerant understory shrubs when given multiple years for the lime to establish itself.

Future research should consider multi-year assessments to get a full understanding how liming impacts perennial understory shrubs. This study, though preliminary, provides valuable insights into using liming as a restoration strategy in regions impacted by acid rain in Nova Scotia.

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