Differential Impacts of pH and Acid Type on Brassicaceae Seed Germination and Seedling Growth

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Abstract

Elevated acidity can be a considerable stressor for plants. Acids can arise from anthropogenic sources such as acid rain, characterized by inorganic acids like sulphuric acid. Beyond anthropogenic influences, various habitats exhibit inherent acidity due to bedrock properties, such as granite's limited buffering capacity or low pH resulting from biological activity, featuring an abundance of weak organic acids. While low pH (<4) is generally considered stressful for plants, differential impacts of distinct acid types—organic versus inorganic, strong versus weak—on plant growth and function remain unclear. To address this knowledge gap, I conducted a controlled experiment to determine whether acid responses are solely pH-dependent or if acid type specificity plays a role in plant functional responses. I grew two Brassicaceae species under factorial combinations of four pH levels (pH 1-5.5) and three acids (hydrochloric, sulfuric, and acetic) with distilled water as control, and measured seed germination, seedling growth, leaf size, shoot and root length. Seed germination, growth, and morphology increased with increasing pH across all acids. However, acid type also influenced plant responses. Hydrochloric acid promoted higher seed germination but hindered seedling growth, while acetic and sulphuric acids had the opposite effects. These results highlight the influence of acid types on plant functions, specifically affecting distinct developmental stages. Understanding the differential effects of acid types on various growth parameters provides insight into acid stress and potential tolerance mechanisms. My study has implications for restoration efforts in acid-impacted environments, challenging the assumption that pH alone dictates plant stress responses.

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1 Introduction

1.1 Plant stressors

Plants are sessile organisms bound by their habitat. In a natural environment, they are subjected to various unfavourable conditions or external factors that can disrupt their growth and development. Such factors, or 'stressors', can be divided into biotic, abiotic, natural and anthropogenic stressors (Lassalle, 2021; Lichtenthaler, 1998). Biotic stressors involve interactions with living organisms such as bacteria, insects, weeds, while abiotic stressors involve environmental factors such as water limitation, temperature fluctuations or UV radiation, inducing stressful conditions on plants (Georgieva and Vassileva, 2023). These stressors may exist naturally in the environment or may result from anthropogenic sources (Zinnert et al., 2013). For instance, the natural mineral deficiencies of low-nutrient ecosystems such as tundra and boreal typically have low plant growth rates (Hobbie, 1992). On the other hand, anthropogenic stressors resulting from human activities, including the release of air pollutants leading to acid rain and increasing CO_2 emissions contributing to global climate change (Lafuente et al., 2023; Lichtenthaler, 1998). At lower intensities, plants might acclimatise to these stressors, but at higher levels these stressors are more likely to cause plant damage and induce early senescence or mortality (Lichtenthaler, 1998).

1.2 Effects of acid on plants

Acidity, an abiotic stress caused by low pH can cause several essential mineral elements deficiencies such as phosphorus (P), calcium (Ca), and increase aluminium (Al) and manganese (Mn) toxicities (Shavrukov and Hirai, 2016; Zhang et al., 2015). This occurs due to increased levels of hydrogen (H^+) ions in the soil, leading to decreased availability of essential nutrients while metals such as zinc (Zn), aluminium (Al) and manganese (Mn) become more soluble at lower pH levels, and can negatively impact plant growth (Zhu and Shen, 2023).

Exposure of plants to strong acidic conditions can greatly impact plant cell structures and functions (Shavrukov and Hirai, 2016). Low pH levels can directly impact plant growth and reduce plant productivity through high H^+ activity (Zhang et al., 2015). For example, soil acidity stress significantly decreased the biomass of rice (*Oryza sativa L.*) seedlings, and reduced the root growth and root diameter as well (Zhang et al., 2015). Similarly, reduced root growth was observed in *Lotus corniculatus* after 5 days at pH 4 treatment (Pavlovkin et al., 2009). Under pH 2.5, Zhang et al. 2020 observed that tea leaves (*Camellia sinensis*) had total leaf tissue damage and 30-65% of the leaves had lesions.

Increasing evidence in the literature suggests that low-pH conditions reduces plant root growth via complex mechanisms such as affecting root water conductance, changing plasma membrane calcium (Ca^{2+}) fluctuations, and changing cytosol pH homeostasis (Chen et al., 2019). Low-pH stress can also disturb plant mineral nutrient uptake by disrupting the proton gradients across plasma membranes (Xu et al., 2020). Overall, low pH can lead to essential nutrient unavailability, ion imbalances, damage to plant membranes, and osmotic stress; hindering nutrient absorption, affecting plant growth, photosynthesis, and plant disease resistance (Aung et al., 2022).

1.3 Anthropogenically induced acid stress: acid rain

One of the forms of acid stress is caused by acid rain, an anthropogenic stress. Acid rain is rain, snow or hail that has a pH less than 5.6 (Ashenden and Bell, 1987; Singh and Agrawal, 2008). Human activities such as burning of fossil fuels, industrialization, and urbanization, release anthropogenic emissions, particularly sulfur dioxide (SO_2) and nitrogen oxides (NOx) into the environment (Zhang et al., 2023; Singh and Agrawal, 2008). These emissions react with the atmospheric components to produce acid rain composed primarily of strong inorganic sulfuric and/or nitric acids (Dong et al., 2017; Zhang et al., 2021).

The consequences of these acid depositions are soil and ecosystem acidification, resulting in loss of forested habitats and impacting plant morphological traits such as leaf area, root length and necrotic spot appearance, and physiological traits such as decreases in photosynthetic rate and chlorophyll contents (Dong et al., 2017; Singh and Agrawal, 2008). Under simulated acid rain conditions (pH 3.4 or pH 4.5), Liu et al. (2018) observed that the photosynthetic ability of rice (*Oryza sativa*) decreased, resulting in smaller allocation to root formation. When *Liquidambar styraciflua* and *Fraxinus uhdei* were exposed to pH below 5 under simulated acid rain conditions, the researchers observed necrotic spots on the leaf surface and structural damage to stomatal guard cells (Rodríguez-Sánchez et al., 2020).

Long-term exposure to acid rain can promote the accumulation of organic carbon in the soil, which inhibits microbial decomposition of organic carbon (Zhang et al., 2023). Acid rain also causes excessive release of H^+ into the soil solution, which can be beyond the buffering capacity of many soils and thus negatively impacts plants and other organisms (Ramlall et al., 2015). The toxic nature of excessive H^+ ions to soil microorganisms and plant roots, as highlighted by Zhang et al. (2023), leads to adverse effects on the soil microbial community.

Overall, acid rain has both direct and indirect impact on vegetation (Zhang et al., 2023). It directly impacts the plant growth and development, and the whole-plant biomass and indirectly impacts the plant-soil dynamics either through microorganisms-plant interactions or nutrient cycling (Zhang et al., 2023).

1.4 Naturally acidic environments

Natural acidity can occur as a result of poorly buffered soils and bedrock in the region, and biological activity (Bowman et al., 2014). Soils can be naturally acidic because of various process such as mineral weathering events, adsorption of protons by soil organic matter, microbial respiration, and production of organic acids (Hodson et al., 1998; Zoca and Penn, 2017). Soils with a higher content of organic matter or clay may have a stronger acid buffering capacity, and are thus less susceptible to acidification (Hodson et al., 1998; Jiang et al., 2018). However, the absence of lime, a natural acid-neutralizing base, in hard rocks such as granite and slate bedrock, contributes to soil inability to neutralize acid in many soils (Canada, 2004). For example, the Canadian province of Nova Scotia has predominately granite and slate bedrock, resulting in low buffering capacity for acidification and acid soils (Bowman et al., 2014; Clair et al., 2011).

In terms of naturally acidic environments from biological activity, a popular example is acidic peat bogs, a type of wetland that is a dominant terrestrial ecosystem in the temperate and boreal forest zones of North America and Eurasia (Dedysh et al., 1998). Bogs are naturally occurring highly acidic environments with a pH range of 3.5 to 4.5 (Dedysh et al., 1998; Ye et al., 2012). Bog acidity comes from organic acids from plants, particularly peat mosses (*Sphagnum*) that directly release organic acids such as polygalacturonic acid and hydrogen (H^+) ions into the peat soils through their cation exchange capacity (Bowman et al., 2014; Clymo, 1963, 1987).

1.4.1 Plants responses to naturally acidic environments

Although acidity has detrimental impacts on many plants, some plants are capable of surviving in acidic growth conditions and some species specifically prefer acidic environments.

Plants mainly use two mechanisms - tolerance and avoidance, to adapt to the negative conditions of acid soils (Lu et al., 2020). Tolerance reflects the ability to maintain plant functions by implementing modifications that neutralize the detrimental effects of stress occurrence and subsequently repair damages once the stress is relieved (Bandurska, 2022). Tolerance mechanisms typically involve low inherent nutrient demand, redistribution, and isolation of mineral nutrients or ionic stressors (Horst, 1983; Memon et al., 1981). Due to this, the plants develop a higher tolerance toward the otherwise adverse environmental condition (Lu et al., 2020). Avoidance involves modifying traits such as stomatal closure to hinder or weaken the impact of ionic stressors, thereby reducing the uptake of ionic stressors from the soil (Bandurska, 2022). Plants may also form symbiotic relationships with microorganisms, such as mycorrhizal fungi, that assist in nutrient acquisition through the ability to bind to toxic ions, secrete organic acids and glomalin (Msimbira and Smith, 2020). Plant roots also undergo structural and physiological adaptations to enhance nutrient uptake in acidic soils (Marschner, 1991). This can involve the development of specialized root structures or associations with mycorrhizal fungi that assist in nutrient absorption (Marschner, 1991).

1.5 pH variation and acid type

Acids are categorised based on sources (organic acid or mineral acid), strength (strong acids or weak acids) and their concentration (concentrated acid or diluted acid) (Hein et al., 2007). Low pH levels (pH < 5) is generally considered stressful for plants (Borhannuddin Bhuyan et al., 2019). However, the specific effects of different acid types such as organic versus inorganic, and strong versus weak, on plant growth and function remain unclear. This knowledge gap represents a crucial area of research because understanding how different acid types influence plant responses to acidic conditions is essential for managing the adverse effects of acidity on plant productivity. My thesis aims to contribute to a more comprehensive understanding of plant responses to acid stress by examining the relative impacts of pH level, acid type, and their interactions on plant growth and development.

1.6 Objectives and approach

The objectives of my thesis are to understand how plants respond to different acids across similar pH levels, and to investigate the relative effects of varying pH and acid types on a) seed germination, b) seedling growth, and c) seedling morphology.

To address these research questions, I employed an experimental approach. The plants were cultivated hydroponically with an intended focus on controlling both the acid type and the pH level administered to the seeds and seedlings.

My species of interest were Brown mustard (*Brassica juncea)* and Daikon Radish (*Raphanus sativus*). Both are members of the Brassicaceae family (Shekhawat et al., 2012; Singh, 2021). The focal acids were sulfuric acid (H_2SO_4), hydrochloric acid (HCl), and acetic acid (CH_3COOH). The four levels of pH used for this study were pH 1, pH 2.5, pH 4 and pH 5.5.

The pH values were selected to systematically explore the varying effects of acidity on plant growth across different environmental scenarios. Additionally, I used a control of distilled water with a pH of 7.

2 Methods

2.1 Focal Species

Brown mustard (*Brassica juncea)* is a member of the Brassicaceae family and is a cool weather plant that thrives predominantly in temperate climates (6°C to 27°C) (Serdyuk et al., 2022; Shekhawat et al., 2012). This plant has a short growing period and grows in pH levels between 5.5 and 6.8 and is moderately acid tolerant (Shekhawat et al., 2012). Daikon Radish (*Raphanus sativus*) is another member of the Brassicaceae family, that is also a cool weather plant that thrives in various climate zones ranging from temperate to tropical regions (Singh, 2021). This crop is adapted to wide range of temperatures (18°C to 30°C) and is not acid tolerant, thriving in soils with a pH between 6.0–6.6 (Singh, 2021). The seeds used in this experiment are organic sprouting seeds from Mumm's sprouting seeds (Parkside, Saskatchewan, Canada).

2.2 Focal acids and preparation

Sulfuric acid (H_2SO_4) is a strong inorganic acid that is formed by the reaction of sulfur dioxide (SO_2), water (H_2O) and oxygen (O_2) (Speight, 2017). SO_2 is one of the primary emissions from industries causing acid rain (Canada, 2004). While sulfur is an essential nutrient for plant growth, excessive concentrations can damage the plant's root system, inhibit plant growth, and lead to soil acidification (Likus-Cieślik et al., 2018; Narayan et al., 2022). Sulfuric acid (H_2SO_4) is a highly corrosive, colorless, or sometimes slightly yellow liquid soluble in water at any concentration (Speight, 2017).

Hydrochloric acid (HCl) is a colourless, corrosive, strong inorganic acid (Speight, 2017) which completely dissociates to give H^+ ions and Cl^- ions (Heald, 1896). HCl emissions are released into the environment through combustion of coal and also contribute to acid rain (Evans et al., 2011).

Acetic acid (CH_3COOH) is a colourless, weak organic acid (Chatveera and Lertwattanaruk, 2014). Most organic acids in the naturally acidic environments are linked to plant, microbial and organic matter decomposition (Adeleke et al., 2017). Acetic acid is one of the low molecular weight organic acids (LMWOAs) that plays a significant role in soil productivity and is similar in properties to other organic acids found in naturally acidic environments (Adeleke et al., 2017; St. James et al., 2021).

All the acids were obtained from Sigma-Aldrich Chemical Company (St. Louis, Missouri, United States). For H_2SO_4 and HCl, four pH solutions (pH 1, pH 2.5, pH 4 and pH 5.5) and for CH_3COOH three pH solutions (pH 2.5, pH 4 and pH 5.5) were prepared by diluting the acids with distilled water until the desired pH level was attained. The pH values of the acid were determined by Orion Star A211 benchtop pH meter from Thermo Fisher Scientific (Waltham, Massachusetts, United States). Overall, there were 11 acid treatment solutions and distilled water (pH 7) used as the control. The pH 1 and pH 2.5 levels were purely used to investigate the plant responses to high levels of acidic toxicity. The pH 4 and pH 5.5 levels were used to simulate conditions similar to those found in naturally acidic environments and acid rain (Dedysh et al., 1998; Singh and Agrawal, 2008; Ye et al., 2012).

2.3 Experiment 1: Seed germination

To germinate seeds from both species, Petri dishes were lined with a single layer of kitchen tissue paper at the bottom and another single layer on top of the seeds just before adding the solution (acid treatment or water control). For each treatment and control, approximately 30 sprouting seeds of each species were placed in individual Petri dishes (n =3, replicates per species, per treatment). I added one millilitre (ml) of solution (either the treatment solution or distilled water) into each Petri dish and sealed the dish with Parafilm from Uline (Wisconsin, United States). The damp tissue paper layers provide the initial germination conditions by retaining moisture. To maintain the moisture in tissue papers, an extra 0.3 ml of solution was added into Petri dishes every 3-4 days as required to maintain moisture. Seed germination was observed over 10 days and I collected data daily for germination rate (%) by counting the number of germinated seeds relative to the total number of seeds in the dish.

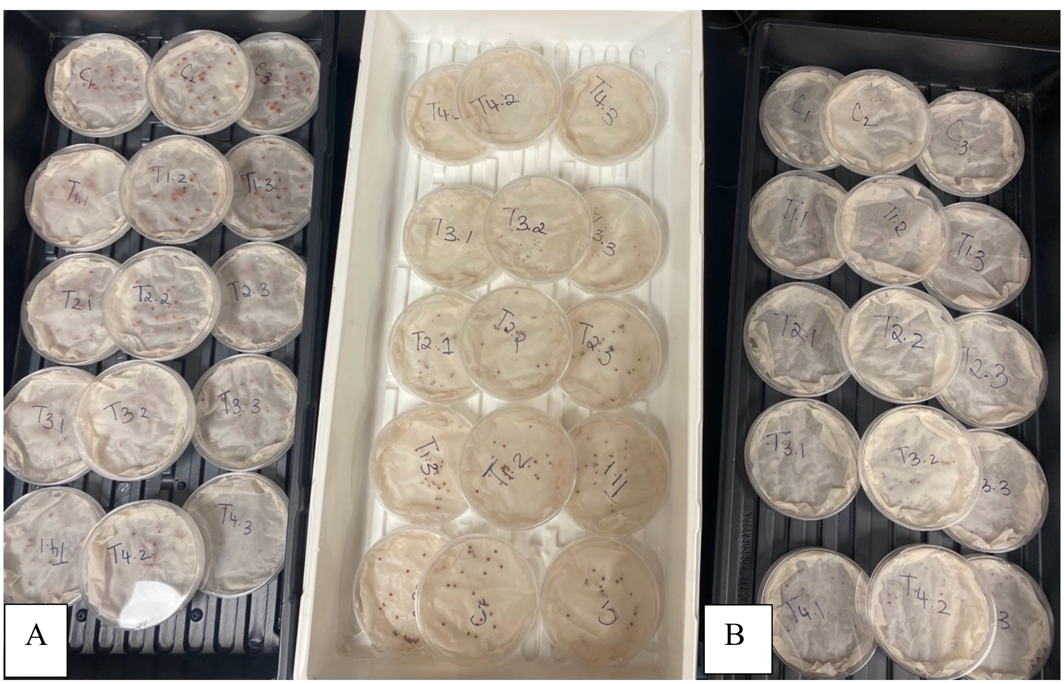

Figure 1: Petri dishes set up for seed germination experiment. (n =3 replicates per acid treatment, per species). (A) Tray containing Petri dishes filled with daikon radish seeds

treated with control (pH 7) and acetic acid solutions with a pH 2.5, 4 and 5.5.

(B) Tray containing Petri dishes filled with brown mustard treated with control (pH 7) and acetic acid solutions with a of pH of 2.5, 4 and 5.5.

2.4 Experiment 2: Seedlings from acid-treated seeds

Experiment 2 involved seeds subjected to acidic treatments during germination to study seedling growth and development. Similar to experiment 1, approximately 30 new seeds for each species were arranged in individual Petri dishes and one millilitre of treatment solution was added (n =3). In contrast to experiment 1, seed germination was observed for only 1-3 days to ensure seeds had a similar length of emerging hypocotyl. I chose a consistent hypocotyl length to reduce variability due to starting differences in growth to obtain a more accurate assessment of the effects of treatments on seedling growth and development.

Five germinated seeds of similar hypocotyl length were picked from the three replicates of each treatment and transferred to CYG Seed Germination Pouches from Mega International (Roseville, Minnesota, United States) (n = 5 per species, per treatment). The germinated seeds were positioned with the hypocotyl facing down which allowed roots to grow through small perforations and made root development visible. 20 mL of each control or acid treatment solution was added to each pouch using a 25 ml graduated cylinder, and seeds were placed equidistant from each other. These pouches containing seeds and treatment solutions were then transferred to growth light shelves where they received 16 hours of daylight. Seedling growth was observed for 11 days at room temperature (22°C). An additional 2.5 mL of treatment solution added on day 5 or day 7 into pouches depending on the amount of solution leftover to prevent the seedling from dying.

Various traits were measured to investigate the effect of pH and acid type on seedlings – leaf area (cm^2) using a LI-3000C Portable Leaf Area Meter with a conveyor belt attachment from LI-COR (Lincoln, Nebraska, United States), shoot and root lengths using a ruler. The number of cotyledons and true leaves of seedlings were also recorded. Fresh leaf and root mass were measured using a high-precision analytical balance from Sartorius ED124S (Göttingen, Germany). The samples were dried in a drying oven at 70°C for 48 hours. Dried samples total plant biomass was weighed using the analytical balance.

Figure 2: Daikon radish seedlings germinated in treatment and developed in germinating pouch with 20 mL of treatment solution (acetic acid. – pH 5.5) on day 11.

2.5 Experiment 3: Seedlings from control seeds

Seeds in experiment 3 were germinated similarly to those in experiment 2 (Section 2.4) and the same traits were measured: leaf area, shoot and root length, fresh leaf mass and root mass, total plant biomass, number of cotyledons and true leaves. The main difference between experiment 2 and experiment 3 is the type of seeds transferred into germinating pouches. In experiment 3, seeds were germinated in control (distilled water) to study seedling growth and development. The seed germination set up was akin to experiment 2, with the only difference being that one millilitre of distilled water was added into individual Petri dishes instead of treatment solution. Therefore, this experiment focuses on seeds germinated in the control transferred to germinating pouches containing treatment solutions.

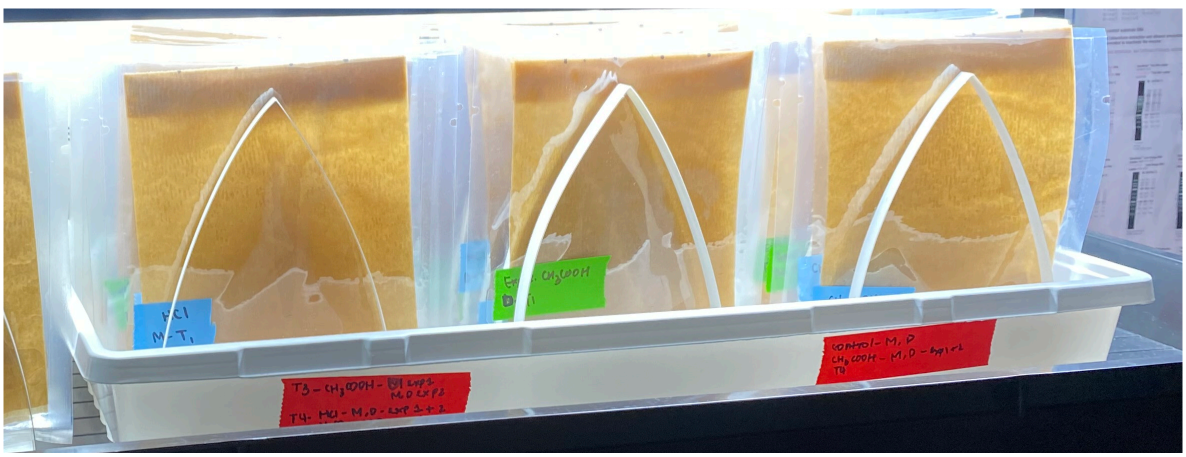

Figure 3: Germinating pouches set up under daylight conditions in growth shelf. (n = 5, three replicates per treatment, per specie).

2.6 Data collection and statistical analysis

All data analysis was performed in R version 4.3.1 and R studio (R Core Team, 2023). The impact of each pH level, acid type, and their interaction for each species was analyzed using multi–factor analysis of variance (ANOVA) to test the effects of acid type (fixed effect: H_2SO_4 , HCl, CH_3COOH) and pH (fixed effect: pH 1, pH 2.5, pH 4 and pH 5.5) on seed germination rate (experiment 1),leaf area, shoot length, root length and total plant biomass (experiments 2 and 3). To understand which cases had a significant effect of acid type, pH or a significant interaction, I conducted post–hoc Tukey's test using the package 'agricolae' to examine which treatments significantly differed (Mendiburu, 2023).

3 Results

3.1 Seed germination

The seed germination of brown mustard and daikon radish was significantly influenced by the different pH levels ($F = 1420.50$, $p < 0.001$ for brown mustard; $F = 1135.20$, $p < 0.001$ for daikon radish). Similarly, the three types of acids also had a significant effect on both species ($F = 409.60$, $p < 0.001$ for brown mustard; $F = 391.60$, $p < 0.001$ for daikon radish). Additionally, the interaction of pH and acids significantly affected the seed germination of both brown mustard and daikon radish ($F = 283.30$, $p < 0.001$ for brown mustard; $F = 298.20$, $p < 0.001$ for daikon radish). Seed germination was highest at pH 5.5 (all three acids types) and at pH 7 (water), and lowest at pH 2.5 across all acids (Figure 4). Seed germination rate was highest in HCl and lowest in CH_3COOH (Figure 4).

3.2 Seedling growth

In experiment 2, focusing on seedlings grown from seeds that had germinated in the acid treatments, the four pH levels had a significant impact on the seedling biomass of brown mustard ($F = 210.02$, $p < 0.001$) and daikon radish ($F = 57.40$, $p < 0.001$). Seeds failed to grow at pH 1 (Figure 5). Hence, the lowest biomass was observed at pH 2.5 and the highest was recorded at pH 5.5 for CH_3COOH and H_2SO_4, which was greater than the control (pH 7) in both species (Figure 5).

While the effect of acid type significantly influenced the total plant biomass of brown mustard seedlings ($F = 61.63$, $p < 0.001$), the impact was not significant for daikon radish ($F = 2.38$, $p = 0.103$). The total plant biomass of brown mustard seedlings was higher at pH 5.5 of CH_3COOH and H_2SO_4 than in control (pH 7) (Figure 5). In contrast, daikon radish had a lower plant biomass than the control (pH 7) at pH 5.5 of CH_3COOH and H_2SO_4 (Figure 5). The lowest biomass was observed in HCl at all pH levels in both the species (Figure 5). However, the interaction between pH and acid type significantly affected the total plant biomass of brown mustard ($F = 1.36E-14$, $p < 0.001$) and daikon radish ($F = 5.81$, $p = 0.0003$).

In experiment 3, focusing on seedlings grown from seeds that had germinated in water, the four pH levels had a significant impact on the seedling biomass of brown mustard ($F = 60.23$, $p < 0.001$) and daikon radish ($F = 196.58$, $p < 0.001$). Similar to experiment 2, no seedling growth was observed at pH 1; therefore, the lowest biomass was recorded at pH 2.5 and the highest biomass was recorded at pH 5.5 across all acid types in both the species (Figure 9).

Unlike experiment 2, the variation in acid type had the opposite effects on the total plant biomass here. Brown mustard ($F = 4.28$, $p = 0.02$) and daikon radish ($F = 9.22$, $p < 0.001$) seedlings biomass were significantly affected by different acids but significantly affected biomass The interaction between pH and acid type significantly influenced the total plant biomass of both brown mustard ($F = 4.61$, $p = 0.002$) and daikon radish ($F = 15.22$, $p < 0.001$).

3.3 Leaf area

The leaf area of brown mustard and daikon radish seedlings was significantly influenced by different pH levels in both the experiments ($F = 80.09$, $p < 0.001$ for brown mustard experiment 2; $F = 88.23$, $p < 0.001$ for brown mustard experiment 3; $F =58.22$, $p < 0.001$ for daikon radish experiment 2; $F = 241.65$, $p < 0.001$ for daikon radish experiment 3). In experiment 2 and 3, seedlings did not grow at pH 1 and leaf area was significantly reduced at pH 2.5 compared to control due to minimal seedling growth for both species (Figure 6 for experiment 2 and Figure 10 for experiment 3).

In experiment 2, the leaf area at pH 5.5 of H_2SO_4 was similar to the control and pH 5.5 of CH_3COOH was greater than the control in brown mustard ($F = 42.73$, $p < 0.001$). However, the leaf area in CH_3COOH is not statistically different from the control in brown mustard, as observed in Figure 6. Conversely, in daikon radish, at pH 5.5, H_2SO_4 had a larger leaf area than the control, and the leaf area of CH_3COOH was not statistically different from control ($F = 8.67$, $p = 0.001$; Figure 6).

Similar to experiment 2, the three types of acids also had a significant impact on the leaf area of brown mustard and daikon radish ($F = 19.56$, $p < 0.001$ for brown mustard; $F = 12.93$, $p < 0.001$ for daikon radish). Additionally, the interaction between pH and acid type had a significant impact on the leaf area of brown mustard and daikon radish in both experiments. ($F = 19.23$, $p < 0.001$ for brown mustard experiment 2; $F = 13.91$, $p < 0.001$for brown mustard experiment 3; $F = 9.85$, $p = 1.60E-06$ for daikon radish experiment 2; $F = 11.85$, $p < 0.001$ for daikon radish experiment 3). In experiment 3, brown mustard seeds grown in CH_3COOH had the largest leaf area relative to other treatments.H_2SO_4 had the largest leaf area in daikon radish (Figure 10).

3.4 Shoot length

In experiment 2, the shoot length of seedlings from both species was significantly influenced by the pH levels ($F = 88.81$, $p < 0.0001$ for brown mustard; $F = 59.96$, $p < 0.001$ for daikon radish). At pH 1 there was no seedling growth; therefore, the lowest shoot length was recorded at pH 2.5 and the highest was recorded at pH 5.5 across all acid types and at pH 7 (control) in both species (Figure 7).

Brown mustard seedlings exhibited a significant response to acid treatment solutions, impacting the shoot length ($F = 58.48$, $p < 0.001$). The shoot length of the seedlings was higher than the control (pH 7) in all the acids, HCl yielded the shortest shoots, followed by H_2SO_4 and CH_3COOH (Figure 7).

In contrast, daikon radish seedlings shoot length was not significantly influenced by acid type alone ($F = 0.03$, $p = 0.97$). The shoot length was relatively similar across all the acids but slightly taller in HCl (Figure 7). However, the interaction between pH and acid type had a significant impact on the shoot length of brown mustard seedlings ($F = 58.48$, $p < 0.001$) and daikon radish seedlings ($F = 16.95$, $p < 0.001$).

In experiment 3 similar to experiment 2, the shoot length of brown mustard and daikon radish seedlings was significantly affected by the four pH levels ($F = 33.05$, $p < 0.001$ for brown mustard; $F = 79.68$, $p < 0.0001$ for daikon radish). Shoot length was the tallest at pH 5.5 across all acid types in both the species but shorter than the control (pH 7) (Figure 11).

Acid types significantly affected brown mustard seedlings shoot length ($F=3.71$, $p = 0.03$), and daikon radish seedling ($F = 11.21$ $p < 0.001$). The combined effects of pH and acid type had a significant impact on the shoot length of brown mustard seedlings ($F=4.58$, $p = 0.002$) and daikon radish seedlings ($F = 13.07$, $p < 0.001$)

3.5 Root length

In experiment 2, the root length of brown mustard and daikon radish seedlings was significantly influenced by the four pH levels ($F = 32.56$, $p < 0.001$ for brown mustard; $F = 75.55$, $p < 0.0001$for daikon radish). Root length was the longest at pH 5.5 across all acid types in both species and similar to concctrol (pH 7) (Figure 8).

Similarly, the three types of acids also exhibited a significant impact on the root length of brown mustard and daikon radish seedings ($F = 16.42$, $p < 0.001$ for brown mustard; $F = 9.25$, $p < 0.001$ for daikon radish). The root length of brown mustard seedlings in H_2SO_4 appears relatively similar to the control, yet they are statistically different (Figure 8). Growth in HCl yielded the shortest root length contrary to shoot length; H_2SO_4 and CH_3COOH comparatively have a greater root length in brown mustard (Figure 8). Similarly in daikon radish, HCl had the shortest root length and, H_2SO_4 followed by CH_3COOH had longer roots.

The interaction between pH and acid type significantly affected the root length of brown mustard and daikon radish seedlings ($F = 7.40$, $p < 0.001$ for brown mustard; $F = 7.96$, $p < 0.001$ for daikon radish).

The four pH levels significantly influenced the root length of brown mustard and daikon radish seedlings in experiment 3, similar to that of experiment 2 ($F = 32.56$, $p < 0.001$ for brown mustard; $F = 32.56$, $p < 0.0001$ for daikon radish).The shortest root length was recorded at pH 2.5 and the longest was recorded at pH 5.5 and pH 7 (Figure 12).

However, there is a contrasting pattern considering the impact of acid types (Figure 12). Different acids did not significantly influence the root length of brown mustard seedlings ($F = 0.74$, $p = 0.48$) and daikon radish seedlings ($F = 86.43$, $p = 0.29$). H_2SO_4 had the lowest root length compared to CH_3COOH and HCl in brown mustard, and it is statistically distinguishable from the other two acids (Figure 9). On the other hand, HCl had the shortest root length and CH_3COOH had the longest root length in daikon radish, as shown in Figure 12.

Although the combined effects of pH and acids had a significant effect on the root length of daikon radish seedlings ($F = 1.29$, $p < 0.001$), but no significant effect was observed on brown mustard seedlings root length ($F = 1.52$, $p < 0.001$).

Table 1: Results from multi-factor analysis of variance (ANOVA) with pH levels, acid type, and their interaction as dependent variables and seed germination rate (%), total plant biomass (g), leaf area (cm^2), shoot length (cm) and root length (cm) as independent variables for Brown mustard (*B. juncea.)*. Data are from individual plants (n = 5 per species, per treatment).

Experiment 1: seed germination						
Seed germination rate (%)	Model	Df	Sum sq	Mean sq	F-value	P value
	pH	4	36033	9008	1420.50	< 0.001
	Acid	2	5196	2598	409.60	< 0.001
	pH:Acid	5	8983	1797	283.30	< 0.001
	Residuals	24	152	6		
Experiment 2: seedlings from treatment seeds						
Total plant biomass (g)	Model	Df	Sum sq	Mean sq	F-value	P value
	pH	4	2.90E-04	7.25E-05	210.02	< 0.001
	Acid	2	4.26E-05	2.13E-05	61.63	< 0.001
	pH:Acid	5	5.92E-05	1.19E-05	34.30	< 0.001
	Residuals	47	1.62E-05	3.50E-07		
Leaf area (cm^2)	pH	4	5.27	1.32	80.09	< 0.001
	Acid	2	1.41	0.71	42.73	< 0.001
	pH:Acid	5	1.59	0.32	19.23	< 0.001
	Residuals	47	0.78	0.02		
Shoot length (cm)	pH	4	28.93	7.23	88.81	< 0.0001
	Acid	2	9.53	4.76	58.48	< 0.001
	pH:Acid	5	6.70	1.34	16.46	< 0.001
	Residuals	47	3.83	0.08		
Root length (cm)	pH	4	1160.10	290.03	34.94	< 0.001
	Acid	2	272.50	136.26	16.42	< 0.001
	pH:Acid	5	307.10	61.42	7.40	< 0.001
	Residuals	47	390.10	8.30		

Experiment 3: seedlings from control seeds						
Total plant biomass (g)	Model	Df	Sum sq	Mean sq	F-value	P value
	pH	3	2.76E-04	9.18E-05	60.23	< 0.001
	Acid	2	1.31E-05	6.53E-06	4.28	0.02
	pH:Acid	5	3.52E-05	7.03E-06	4.61	0.002
	Residuals	44	6.71E-05	1.52E-06		
Leaf area (cm ^{2})	pH	3	2.76	0.92	88.23	< 0.0001
	Acid	2	0.41	0.20	19.56	< 0.001
	pH:Acid	5	0.73	0.15	13.91	< 0.001
	Residuals	44	0.46	0.01		
Shoot length (cm)	pH	3	26.50	8.83	33.05	< 0.001
	Acid	2	1.99	0.99	3.71	0.03
	pH:Acid	5	6.12	1.22	4.58	0.002
	Residuals	44	11.76	0.27		
Root length (cm)	pH	3	1575.20	525.10	32.56	< 0.001
	Acid	2	23.80	11.90	0.74	0.48
	pH:Acid	5	122.30	24.50	1.52	0.20
	Residuals	44	709.20	16.10		

Table 2: Results from multi-factor analysis of variance (ANOVA) with pH levels, acid type, and their interaction as dependent variables and seed germination rate (%), total plant biomass (g), leaf area (cm^2), shoot length (cm) and root length (cm) as independent variables for Daikon Radish (*R. sativus*). Data are from individual plants (n = 5 per species, per treatment).

Experiment 1: seed germination						
Seed germination rate (%)	Model	Df	Sum sq	Mean sq	F-value	P value
	pH	4	33103	8276	1135.20	< 0.0001
	Acid	2	5709	2855	391.60	< 0.0001
	pH:Acid	5	10871	2174	298.20	< 0.0001
	Residuals	24	175	7		
Experiment 2: seedlings from treatment seeds						
Total plant biomass (g)	Model	Df	Sum sq	Mean sq	F-value	P value
	pH	4	0.005	0.0014	57.40	< 0.0001
	Acid	2	0.0001	0.0001	2.38	0.103
	pH:Acid	5	0.0007	0.0001	5.81	0.0003
	Residuals	48	0.0011	0.00002		

Leaf area (cm^2)	pH	4	32.50	8.17	58.22	< 0.0001
	Acid	2	2.42	1.21	8.67	0.001
	pH:Acid	5	6.87	1.37	9.85	< 0.001
	Residuals	48	6.70	0.14		
Shoot length (cm)	pH	4	80.820	20.206	59.96	< 0.0001
	Acid	2	0.02	0.01	0.03	0.97
	pH:Acid	5	28.69	5.74	16.95	< 0.001
	Residuals	48	16.25	0.34		
Root length (cm)	pH	4	1917.70	479.40	75.55	< 0.0001
	Acid	2	117.40	58.70	9.25	< 0.001
	pH:Acid	5	252.40	50.50	7.96	< 0.001
	Residuals	48	304.60	6.30		
Experiment 3: seedlings from control seeds						
Total plant biomass (g)	Model	Df	Sum sq	Mean sq	F-value	P value
	pH	3	0.005	0.002	196.58	< 0.0001
	Acid	2	0.0002	0.0001	9.22	< 0.001
	pH:Acid	5	0.001	0.0001	15.22	< 0.001
	Residuals	44	0.0004	0.00001		
Leaf area (cm^2)	pH	3	32.17	10.72	241.65	< 0.0001
	Acid	2	1.15	0.57	12.93	< 0.001
	pH:Acid	5	2.63	0.53	11.85	< 0.001
	Residuals	44	1.95	0.04		
Shoot length (cm)	pH	3	62.34	20.78	79.68	< 0.0001
	Acid	2	5.85	2.92	11.21	< 0.001
	pH:Acid	5	17.04	3.41	13.07	< 0.001
	Residuals	44	11.48	0.26		
Root length (cm)	pH	3	1575.20	525.10	32.56	< 0.0001
	Acid	3	2103.60	701.20	86.43	0.29
	pH:Acid	2	20.90	10.50	1.29	< 0.001
	Residuals	5	408.80	81.80	10.08	

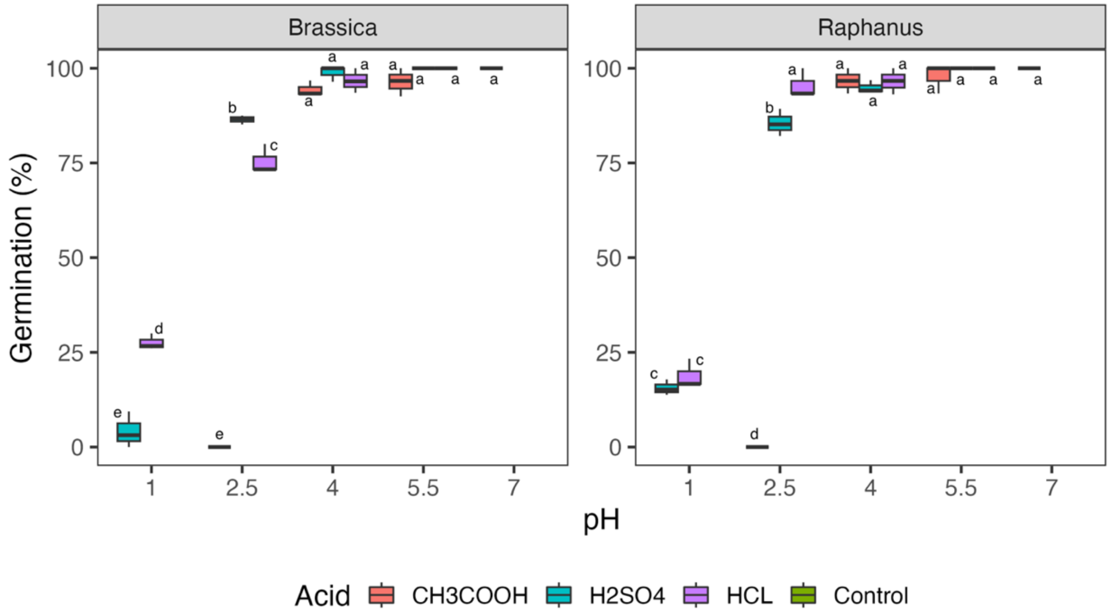

Figure 4: Variation in seed germination rate (%) across four pH levels for acetic (CH_3COOH), sulphuric (H_2SO_4) and hydrochloric (HCl) acids between brown mustard (*Brassica*, left) and daikon radish (*Raphanus*, right). Data are from individual seeds (n =3 per species, per treatment). Boxes sharing the same letter are statistically indistinguishable based on Tukey post-hoc tests.

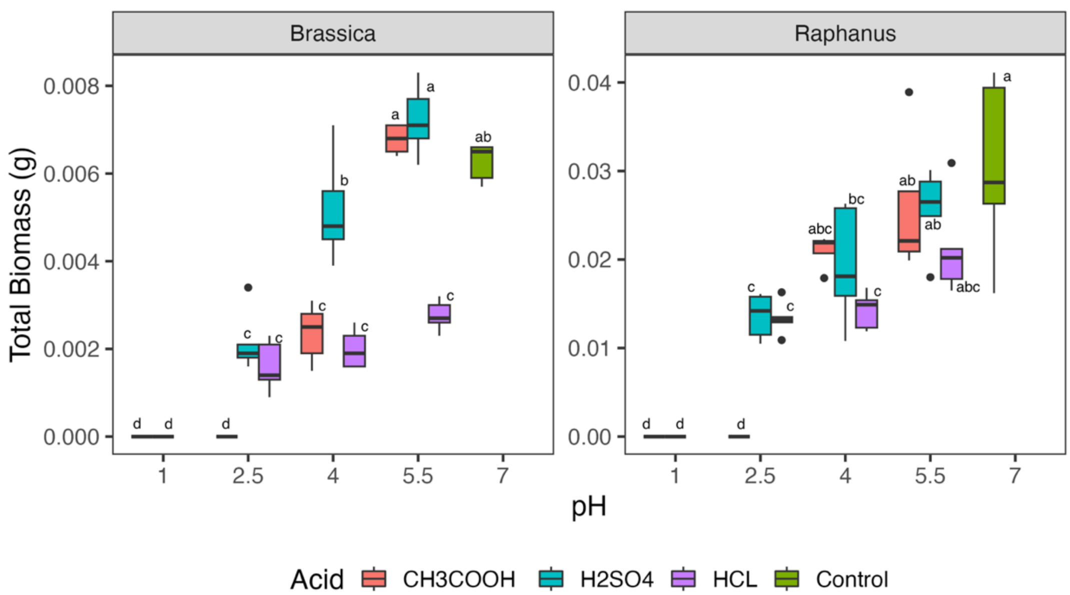

Figure 5: Variation in total plant biomass (g) across four pH levels for acetic (CH_3COOH), sulphuric (H_2SO_4) and hydrochloric (HCl) acids between brown mustard (*Brassica*, left) and daikon radish (*Raphanus*, right). Data are from individual plants (n = 5 per species, per treatment). The plants included in this analysis are seedlings that germinated from acid-treated seeds (experiment 2). Boxes sharing the same letter are statistically indistinguishable based on Tukey post-hoc tests.

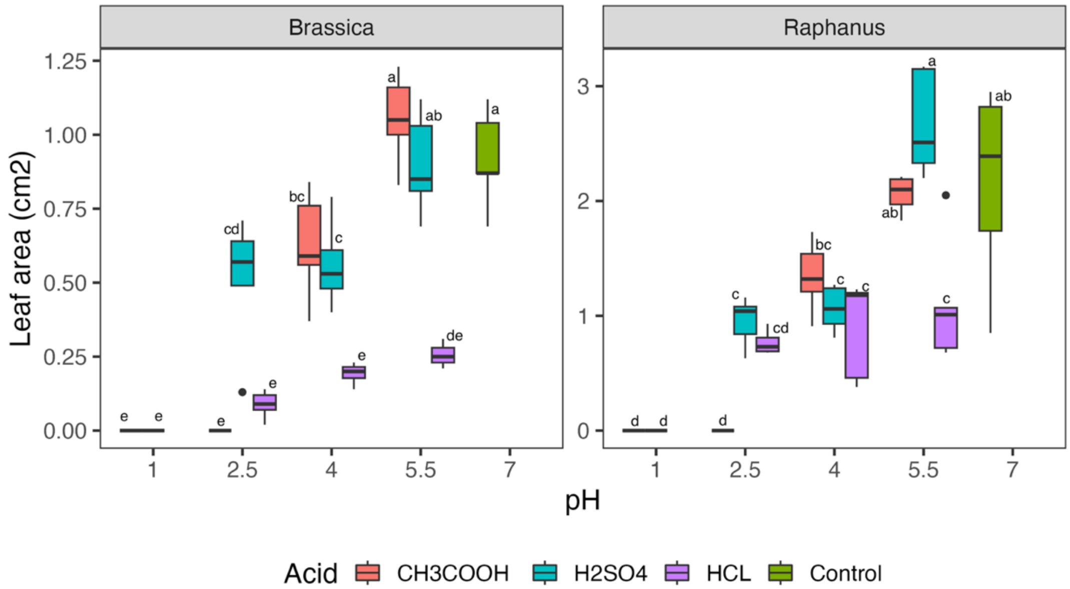

Figure 6: Variation in leaf area (cm ^{2}) across four pH levels for acetic (CH_3COOH), sulphuric (H_2SO_4) and hydrochloric (HCl) acids between brown mustard (*Brassica*, left) and daikon radish (*Raphanus*, right). Data are from individual plants (n = 5 per species, per treatment). The plants included in this analysis are seedlings that germinated from acid-treated seeds (experiment 2). Boxes sharing the same letter are statistically indistinguishable based on Tukey post-hoc tests.

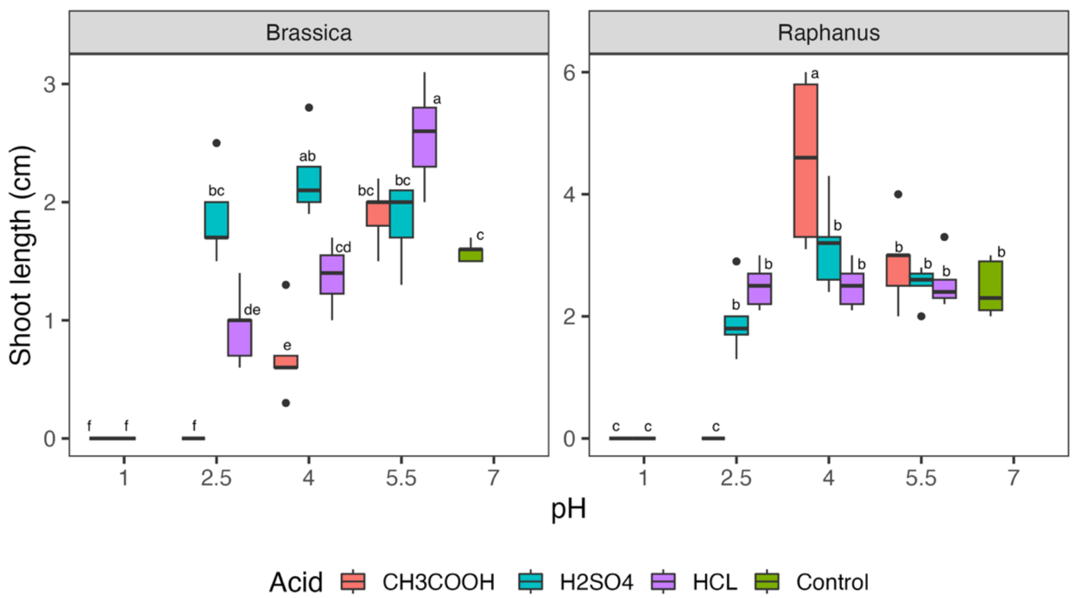

Figure 7: Variation in seedling shoot length (cm) across four pH levels for acetic (CH_3COOH), sulphuric (H_2SO_4) and hydrochloric (HCl) acids between brown mustard (*Brassica*, left) and daikon radish (*Raphanus*, right). Data are from individual plants (n = 5 per species, per treatment). The plants included in this analysis are seedlings that germinated from acid-treated seeds (experiment 2). Boxes sharing the same letter are statistically indistinguishable based on Tukey post-hoc tests.

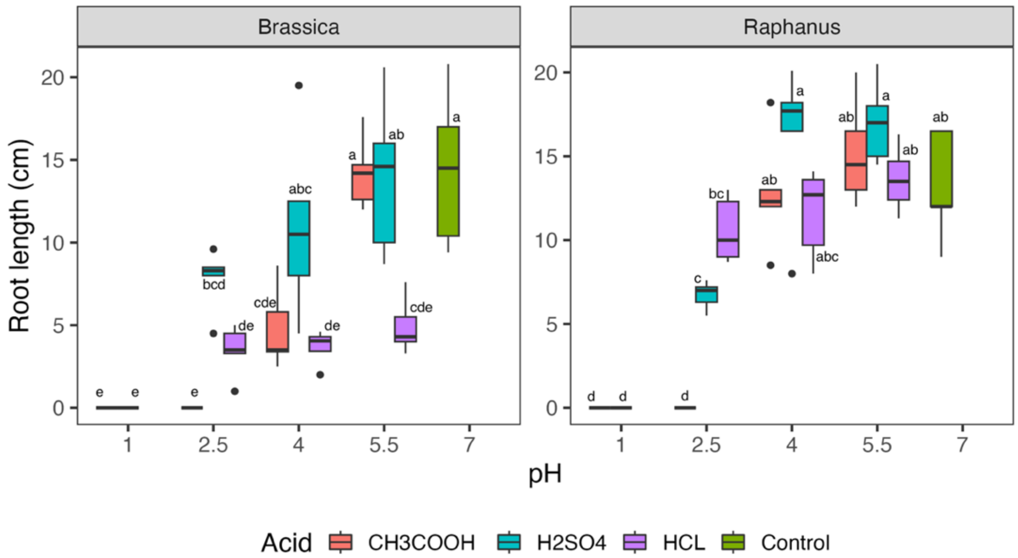

Figure 8: Variation in seedling root length (cm) across four pH levels for acetic (CH_3COOH), sulphuric (H_2SO_4) and hydrochloric (HCl) acids between brown mustard (*Brassica*, left) and daikon radish (*Raphanus*, right). Data are from individual plants (n = 5 per species, per treatment). The plants included in this analysis are seedlings that germinated from acid-treated seeds (experiment 2). Boxes sharing the same letter are statistically indistinguishable based on Tukey post-hoc tests.

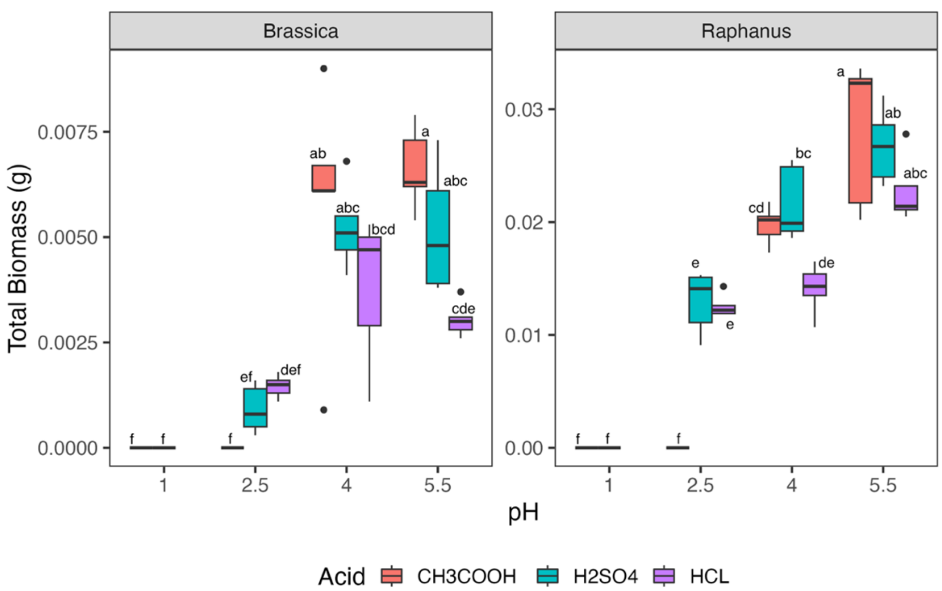

Figure 9: Variation in total plant biomass (g) across four pH levels for acetic (CH_3COOH), sulphuric (H_2SO_4) and hydrochloric (HCl) acids between brown mustard (*Brassica*, left) and daikon radish (*Raphanus*, right). Data are from individual plants (n = 5 per species, per treatment). The plants included in this analysis are seedlings that germinated from water-treated seeds (experiment 3). Boxes sharing the same letter are statistically indistinguishable based on Tukey post-hoc tests.

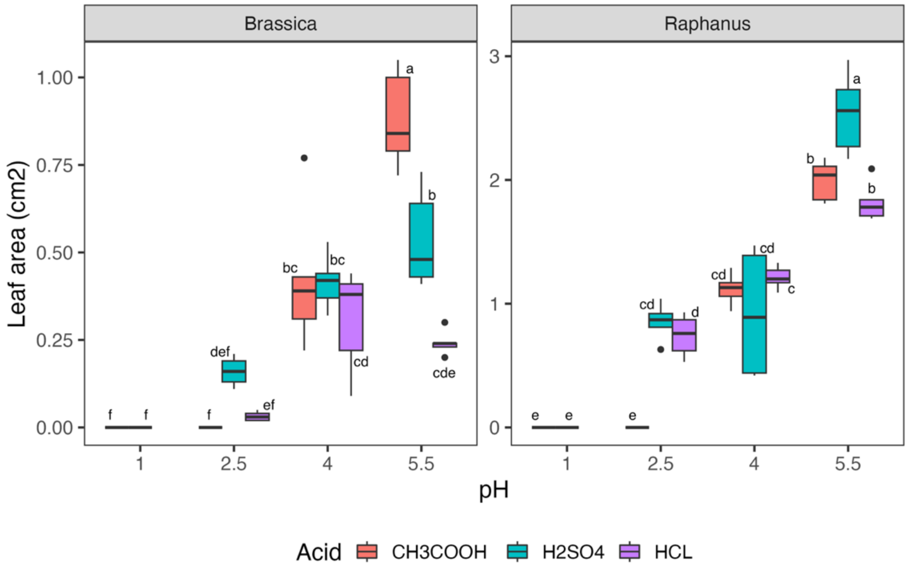

Figure 10: Variation in leaf area (cm ^{2}) across four pH levels for acetic (CH_3COOH), sulphuric (H_2SO_4) and hydrochloric (HCl) acids between brown mustard (*Brassica*, left) and daikon radish (*Raphanus*, right). Data are from individual plants (n = 5 per species, per treatment). The plants included in this analysis are seedlings that germinated from water-treated seeds (experiment 3). Boxes sharing the same letter are statistically indistinguishable based on Tukey post-hoc tests.

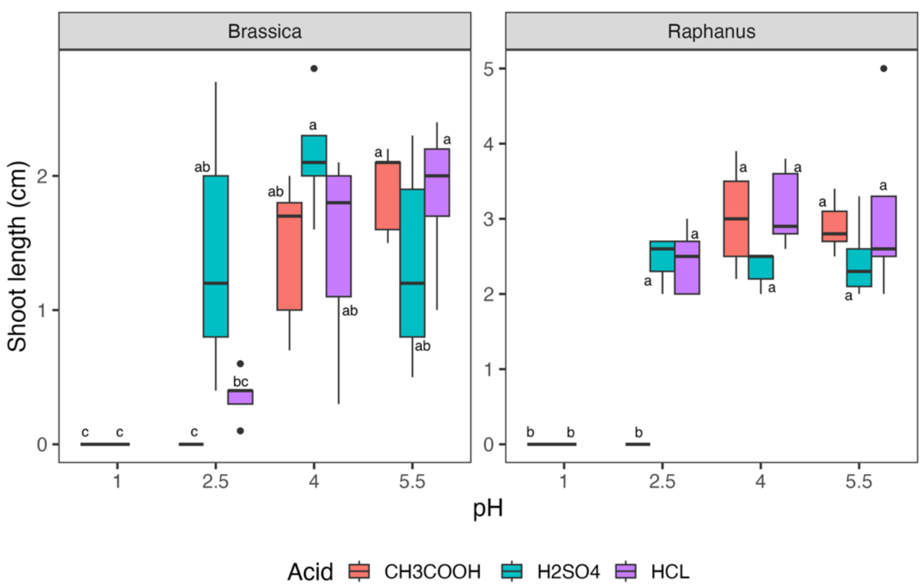

Figure 11: Variation in seedling shoot length (cm) across four pH levels for acetic (CH_3COOH), sulphuric (H_2SO_4) and hydrochloric (HCl) acids between brown mustard (*Brassica*, left) and daikon radish (*Raphanus*, right). Data are from individual plants (n = 5 per species, per treatment). The plants included in this analysis are seedlings that germinated from water-treated seeds (experiment 3). Boxes sharing the same letter are statistically indistinguishable based on Tukey post-hoc tests.

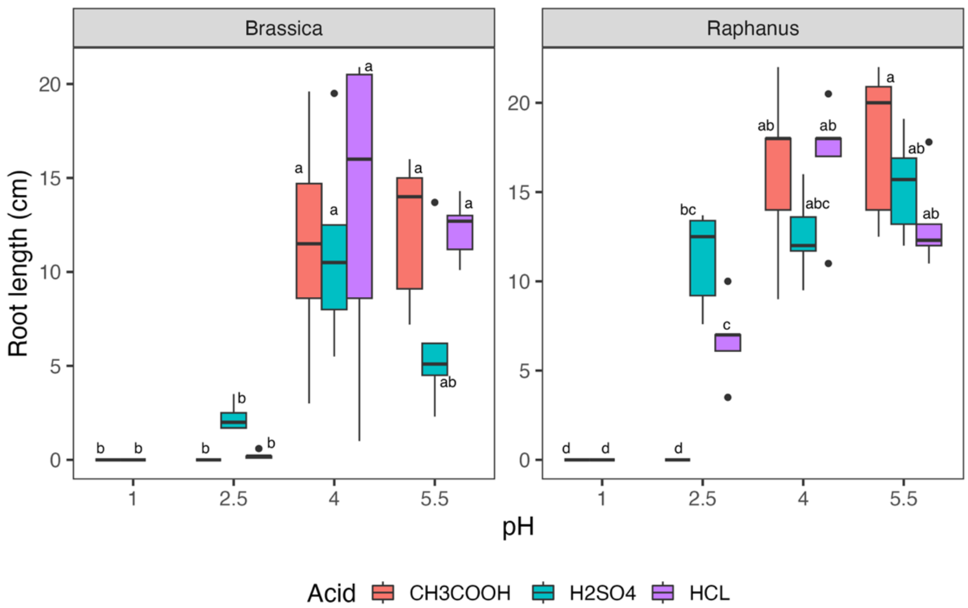

Figure 12: Variation in seedling root length (cm) across four pH levels for acetic (CH_3COOH), sulphuric (H_2SO_4) and hydrochloric (HCl) acids between brown mustard (*Brassica*, left) and daikon radish (*Raphanus*, right). Data are from individual plants (n = 5 per species, per treatment). The plants included in this analysis are seedlings that germinated from water-treated seeds (experiment 3). Boxes sharing the same letter are statistically indistinguishable based on Tukey post-hoc tests.

4 Discussion

I delve into the complex dynamics of pH and acid type impacts on seed germination, seedling growth, and seedling morphology in two Brassicaceae species that are known to differ in their growth form and acid tolerance. By investigating brown mustard and daikon radish responses to varying pH and acid types, my study provides insights into species-specific patterns of plant responses to acidic conditions.

4.1 Seed germination

My findings reveal significant impacts of different pH levels and acid types on seed germination in both brown mustard and daikon radish. Seed germination is a critical stage in plant development, influenced by various environmental factors such as soil pH, light, and temperature (Humphries et al., 2018). I observed that seed germination increased as pH increased, which is similar to the results of Ryan et al. (1975) for four different grasses and alfalfa (*Medicago sativa*). The highest germination rates were observed at pH 5.5 across all acid types, which was not statistically different from the control group (Figure 4). This is similar to the findings of a study which demonstrated that pH 5 did not affect the germination of pepper (*Capsicum annum L.)* (Ünlü and Ünlü, 2010). Additionally, the results are within the tolerable pH conditions of both species (Shekhawat et al., 2012; Singh, 2021). The germination rate was inhibited at pH 1 for inorganic acids (HCl, H_2SO_4) and at pH 2.5 for the organic acid (CH_3COOH) (Figure 4). My results are similar to Ryan et al. (1975) who observed no seed germination for four different grasses and alfalfa (*Medicago sativa*) treated with dilute sulfuric acid of pH 1. Zhao et al. (2020) also found that *Neyraudia reynaudiana* seed germination was moderately inhibited at pH 3.5 for CH_3COOH, consistent with my study's findings where no seed germination occurred at pH 2.5 for CH_3COOH, while at pH 4.0 of CH_3COOH, both daikon radish and brown mustard exhibited a germination rate of at least over 75%.

I observed considerable differences in the effects of different acid types on seed germination (Figure 4). Seed germination rates were higher in HCl and H_2SO_4 and lowest in CH_3COOH for both brown mustard and daikon radish seeds. Evidence from the literature suggests that HCl is effective in breaking seed dormancy and inducing seed germination of hard seed coats, irrespective of the acid concentration (Sharma et al., 2019; Šoch et al., 2023). This possibly explains the higher seed germination rate in HCl at pH 1 compared to the other acid types.

The lowest germination rates at pH 2.5 and pH 4 were observed for acetic acid. This may be explained by differences in solute concentrations between the different acid types that affected water potential. The solute concentrations of the acids varied based on differences in their chemical composition. At the same pH level, CH_3COOH has the highest solute concentration because of the contribution of CH_3COO^-. Higher solute concentrations may negatively impact seed germination if water uptake via osmosis is reduced by decreases in water potential (Taylor et al., 2021). This has been reported in high saline conditions, where seed germination is inhibited by low water potential caused by higher solute concentrations. This hinders with water uptake by the seeds, and inhibits seed germination (Guo et al., 2020).

The findings of my study suggest that variation in seed germination is not solely due to different pH levels but also due to the different acid types, each characterized by varying acid strength and chemical properties.

4.2 Seedling growth

Experiments 2 and 3 were conducted to examine seedling growth and development under acidic treatments. However, the seeds were exposed to different solutions during germination and then continued to grow in acidic treatments during their development. For experiment 2, seeds germinated in acidic treatments mimicking plants that are germinating in acidic environments, whereas in experiment 3, seeds germinated in control (distilled water) and later exposed to acids, simulating plants exposed to acidity at a later developmental stage.

Plant growth manifested as biomass accumulation is important to both agricultural and ecological productivity (Buxbaum et al., 2022). Biomass is directly related to traits such as productivity and stress response (Muchow, 1988; Scully and Wallace, 1990). Additionally, growth rate is closely linked to crop productivity and yield for grain, fruit, and vegetable crops (Buxbaum et al., 2022). The findings from experiments 2 and 3 highlight the influence of pH levels and acid types on the biomass of brown mustard and daikon radish seedlings. I observed that as pH increased plant biomass increased in all acid types (Figures 5 and 9). My findings align with the findings of reduced seedling growth of *Paulownia tomentosa* with increasing acidic substrates (Turner et al., 1988). Due to absence of seedling growth, the lowest biomass was observed at pH 1, followed by pH 2.5. I expected that control (pH 7) would have the highest biomass, which stands true for daikon radish in all three acid types. However, for brown mustard the highest biomass was consistently recorded at pH 5.5 for CH_3COOH and H_2SO_4, which was greater than control (pH 7). My results provide evidence for species-specific responses to acid variation similar to that of results seen in the literature (Ashenden and Bell, 1989; Edge et al., 1994).

My study also provides significant evidence indicating that plant responses are influenced by the developmental stage at which they encounter acid exposure, in addition to species-specific effects. In experiment 2, seedling growth was higher in H_2SO_4, while in experiment 3, it was higher in CH_3COOH; the lowest seedling growth was observed in HCl (Figures 5 and 9). Edge et al. (1994) suggest that the plants are likely utilizing the low levels of sulphur present in H_2SO_4 as essential nutrients, thus benefiting from it and overcoming the negative impact of relatively high concentrations of H^+ ions in pH (Edge et al., 1994). H_2SO_4 dissociates to give SO_4^{2-}, which is typically the primary source of sulfur for plants (Narayan et al., 2022; Pavlov, 2017). There is a possibility that these seedlings are likely utilising SO_4^{2-} as a nutrient and hence improving the overall plant growth and development. Mahmud et al. (2023) observed that CH_3COOH in low concentrations (500 µM) had positive effects on maize and arabidopsis growth and enhanced resistance towards polyethylene glycol-induced drought stress. Mahmud et al. (2023) suggests that CH_3COOH in low concentrations might have stimulated the accumulation of endogenous proline in these plants. Under stressful environmental conditions, proline assists in maintaining the cell turgidity and prevents oxidative bursts in plants (Msimbira and Smith, 2020). Rahman et al. (2019) also found that CH_3COOH in low concentrations (20 mM) could potentially be beneficial to mitigate salt stress impact on Mung beans. Rahman et al. (2019) suggests that CH_3COOH might increase the uptake of beneficial ions such as potassium (K^+), calcium (Ca^{2+}) and magnesium (Mg^{2+}), while reducing the accumulation of toxic ions such as sodium (Na^+). This helps in maintaining the ion balance within plant cells and prevents ion toxicity.

4.3 Leaf area

Leaf area is a key morphological trait that helps understand photosynthesis, water and nutrient use, and overall plant growth (Cho et al., 2007). Similar to biomass, I observed an increasing leaf area in all acid types with increasing pH. In both experiments, the leaf area of brown mustard and daikon radish seedlings was impacted by different pH levels, highlighting the importance of pH regulation in plant growth (Figures 6 and 10). Due to unsuccessful seedling growth at pH 1, no leaf area was observed, while pH 2.5 in both species had the smallest leaf area with considerable necrotic spots in experiments 2 and 3 for all three acids (Figures 6 and 10). At pH 4, brown mustard had no necrotic spots and daikon radish had significantly decreased necrotic spots in all three acids. My study findings align with observations of leaf morphology in *Phaseolus vulgaris* (Ferenbaugh, 1976). They observed small leaf areas at low pH values such as 2.5, along with noticeable necrotic spots at low pH. Conversely, at pH 5.5, I observed a large leaf area with no necrotic spots in both experiments. Additionally, leaf area at pH 5.5 was similar or larger than control for CH_3COOH and H_2SO_4 .

The impact of different acid types on leaf area further highlights the relationship between acidity and plant growth responses. The leaf area was the smallest in HCl for both species across all pH levels in experiments 2 and 3 (Figures 6 and 10). HCl is a strong acid and dissociates fully into H_3O^+ and Cl^- (Cheremisinoff and Rosenfeld, 2010). This could likely contribute to the reduced leaf area in both species since, as discussed in the previous section, it is likely that the seedlings were utilising SO_4^{2-} and CH_3COO^- as nutrients in the sulfuric and acetic acid treatments while the seedlings would not have had access to such essential nutrients in the HCl treatments. In support of this idea, brown mustard exhibited the largest leaf area in CH_3COOH, while daikon radish exhibited the largest leaf area in H_2SO_4, in both experiments (Figures 6 and 10). These findings highlight the species-specific nature of plant responses to acid-type variations.

4.4 Shoot length and root length

Shoot and root morphology are some of the many aspects that influence plant productivity (Walne and Reddy, 2022). Both experiments demonstrated a significant influence of pH levels on shoot and root length across all acid treatments, with the lowest lengths observed at pH 1 due to no growth, at pH 2.5 due to minimal growth, and the highest at pH 5.5, which was similar to control (Figures 7, 8,11,12).

The impact of different acid types on shoot and root length varied significantly between brown mustard and daikon radish seedlings. For experiment 2, brown mustard seedlings shoot length increased when exposed to HCl compared to other acid types and control, regardless of pH level, while root length remained relatively similar across the same conditions (Figures 7 and 8). Both H_2SO_4 and CH_3COOH resulted in increased shoot and root lengths as pH increased, indicating there might have been some nutrient utilisation by plants growing in these acidic environments. On the contrary, daikon radish seedlings shoot length was relatively similar across all acid types and control, with an exception of CH_3COOH at pH 4, but root length increased with increasing pH across all acid types, highlighting a species-specific response (Figures 7 and

8). There was considerable variation observed in shoot length, which did not follow the clear patterns observed for biomass and leaf area (Figure 7). On the contrary, root length had an increasing pattern similar to biomass and leaf area (Figure 8). This could possibly indicate that the resources are likely being more utilised by roots in order to grow away from the acidic treatments in search for better nutrient uptake.

For experiment 3, brown mustard seedlings shoot length increased in HCl but decreased in root length. Both H_2SO_4 and CH_3COOH have a similar pattern, as shoot lengths increased the root lengths increased as well (Figures 11 and 12). For daikon radish however, an obvious increase in root lengths and decrease in shoot lengths across all acid types was observed (Figures 11 and 12). There could possibly be a resource allocation shift, with resources being more favourable towards root development to improve survival and nutrient uptake in acidic conditions.

Although my study focuses on shoot and root lengths over root/shoot mass, I found similar patterns of resource allocation based on the literature. Troiano et al. (1982) suggested an increasing shoot mass and a decreasing root mass of radish (*Raphanus sativus*) at pH 5.6 under simulated acid rain conditions. They also observed that root mass was positively correlated with an increase in acidity but radish (*Raphanus sativus*) shoot mass was unaffected even at pH 2.8 (Troiano et al., 1982). These findings from the literature could possibly explain the increased root length of species in acid due to the tolerance mechanism and the increase in shoot length due to the avoidance mechanism. However, it is important to consider that the root/shoot mass is influenced by other factors such as density and thickness.

4.5 Limitations

My study was in a controlled lab environment with controlled pH levels. The seedlings grew hydroponically in the control and the treatments. Although the morphological traits used in this study (leaf area, shoot and root lengths) are great indicators of plant productivity and performance, measuring leaf area of some withered leaves could have impacted those leaf area measurements. It would also have been informative to have measured leaf, shoot and root mass individually to assess resource allocation among these organs and to assess shoot/root ratio.

If the study is to be repeated, acid variation concentration should be considered. This could involve testing different concentrations of acids to observe their effects on resource allocation and to specifically test whether increasing acid concentrations limits water uptake by seeds during seed germination. Additionally, the pH of the treatment solution throughout the experiment and at the end of the experiment should be measured, to determine whether the pH is changing from any seed-or root-induced buffering that may have resulted in a less acidic environment and thereby promoting plant growth and development.

5 Implications

The findings of my study challenge the assumption that pH alone dictates plant stress responses to acidic growth conditions. This has implications for natural environments, especially those suffering from elevated acid stress from anthropogenic sources. Additionally, tailored restoration approaches may be considered for environments impacted by different acid types, as my study provides evidence that acidic environments are influenced by factors beyond pH. Other factors, such as variations in acid composition and concentration, also significantly influence plant responses. The findings of my study also enhance our understanding of acid stress and potential avoidance or tolerance mechanisms as observed in shoot and root morphological responses. Based on the findings of my study, there is a need to understand how different acid types impact various growth parameters in the field, guiding the development of targeted restoration techniques and land management efforts.

6 Appendices

Appendix 1: Results from multi-factor analysis of variance (ANOVA) with species, pH levels, acid type, and their interaction as dependent variables and seed germination rate (%), total plant biomass (g), leaf area (cm^2), shoot length (cm) and root length (cm) as independent variables. Data are from individual plants (n = 5 per species, per treatment).

Seed Germination						
Seed germination rate (%)		Df	Sum sq	Mean sq	F-value	P-value
	pH	4	69003	17251	2530.99	< 0.0001
	Acid	2	10896	5448	799.29	< 0.0001
	Species	1	57	57	8.43	0.01
	pH:Acid	5	19109	3822	560.724	< 0.0001
	pH:Species	4	133	33	4.87	0.002
	Acid:Species	2	9	5	0.66	0.52
	pH:Acid:Species	5	745	149	21.85	< 0.0001
	Residuals	48	327	7		
Seedling growth and development						
Total plant biomass		Df	Sum sq	Mean sq	F-value	P-value
	pH	4	0.008	0.002	238.30	< 0.0001
	Acid	2	0.0002	0.0001	12.66	< 0.0001
	Species	1	0.008	0.008	1004.96	< 0.0001
	pH:Acid	5	0.0009	0.0002	22.99	< 0.0001
	pH:Species	4	0.003	0.001	94.70	< 0.0001
	Acid:Species	2	0.0001	0.00004	5.25	< 0.0001
	pH:Acid:Species	5	0.0004	0.0001	10.78	< 0.0001
	Residuals	205	0.002	0.00001		
Leaf area (cm^2)		Df	Sum sq	Mean sq	F-value	P-value
	pH	4	58.99	14.75	240.38	< 0.0001
	Acid	2	2.97	1.49	24.21	< 0.0001
	Species	1	28	28	456.40	< 0.0001
	pH:Acid	5	8.63	1.73	28.12	< 0.0001
	pH:Species	4	13.89	3.47	56.59	< 0.0001
	Acid:Species	2	1.29	0.65	10.55	< 0.0001
	pH:Acid:Species	5	2.22	0.45	7.25	< 0.0001
	Residuals	205	12.58	0.06		
Root length (cm)		Df	Sum sq	Mean sq	F-value	P-value
	pH	4	6246	1561.5	117.74	< 0.0001
	Acid	2	140	70.1	5.29	0.01
	Species	1	659	659.4	49.72	< 0.0001
	pH:Acid	5	563	112.6	8.49	< 0.0001

	pH:Species	4	230	57.5	4.34	0.002
	Acid:Species	2	40	20.1	1.51	0.22
	pH:Acid:Species	5	121	24.1	1.82	0.11
	Residuals	205	2719	13.3		
Shoot length (cm)		Df	Sum sq	Mean sq	F-value	P-value
	pH	4	180.36	45.09	159.56	< 0.0001
	Acid	2	10.23	5.12	18.105	< 0.0001
	Species	1	42.71	42.71	151.16	< 0.0001
	pH:Acid	5	35.46	7.09	25.10	< 0.0001
	pH:Species	4	14.74	3.69	13.04	< 0.0001
	Acid:Species	2	2.8	1.4	4.95	0.01
	pH:Acid:Species	5	18.18	3.64	12.87	< 0.0001
	Residuals	205	57.93	0.28		

References

Acar, R., and Osman, I. M. (2022). Some Classical Methods of Vegetation Attributes Measurements in Rangelands. *Selcuk Journal of Agriculture and Food Sciences*, *36*(0), Article 0. https://doi.org/10.15316/SJAFS.2022.084

Adeleke, R., Nwangburuka, C., and Oboirien, B. (2017). Origins, roles and fate of organic acids in soils: A review. *South African Journal of Botany*, *108*, 393–406. https://doi.org/10.1016/j.sajb.2016.09.002

An overview of the direct and indirect effects of acid rain on plants: Relationships among acid rain, soil, microorganisms, and plants. (2023). *Science of The Total Environment*, *873*, 162388. https://doi.org/10.1016/j.scitotenv.2023.162388

Ashenden, T. W., and Bell, S. A. (1987). The effects of simulated acid rain on the growth of three herbaceous species grown on a range of British soils. *Environmental Pollution*, *48*(4), 295–310. https://doi.org/10.1016/0269-7491(87)90110-2

Ashenden, T. W., and Bell, S. A. (1989). Growth responses of three legume species exposed to simulated acid rain. *Environmental Pollution*, *62*(1), 21–29. https://doi.org/10.1016/0269-7491(89)90093-6

Aung, T. T., Shi, F., Zhai, Y., Xue, J., Wang, S., Ren, X., and Zhang, X. (2022). Acidic and Alkaline Conditions Affect the Growth of Tree Peony Plants via Altering Photosynthetic Characteristics, Limiting Nutrient Assimilation, and Impairing ROS Balance. *International Journal of Molecular Sciences*, *23*(9), 5094. https://doi.org/10.3390/ijms23095094

Bandurska, H. (2022). Drought Stress Responses: Coping Strategy and Resistance. *Plants*, *11*(7), 922. https://doi.org/10.3390/plants11070922

Borhannuddin Bhuyan, M. H. M., Hasanuzzaman, M., Nahar, K., Mahmud, J. A., Parvin, K., Bhuiyan, T. F., and Fujita, M. (2019). Plants Behavior Under Soil Acidity Stress: Insight into Morphophysiological, Biochemical, and Molecular Responses. In M. Hasanuzzaman, K. R. Hakeem, K. Nahar, and H. F. Alharby (Eds.), *Plant Abiotic Stress Tolerance: Agronomic, Molecular and Biotechnological Approaches* (pp. 35–82). Springer International Publishing. https://doi.org/10.1007/978-3-030-06118-0_2

Bowman, M. F., Nussbaumer, C., and Burgess, N. M. (2014). Community composition of lake zooplankton, benthic macroinvertebrates and forage fish across a ph gradient in Kejimkujik National Park, Nova Scotia, Canada. *Water, Air, andamp; Soil Pollution*, *225*(12). https://doi.org/10.1007/s11270-014-2211-7

Buxbaum, N., Lieth, J. H., and Earles, M. (2022). Non-destructive Plant Biomass Monitoring With High Spatio-Temporal Resolution via Proximal RGB-D Imagery and End-to-End Deep Learning. *Frontiers in Plant Science*, *13*. https://doi.org/10.3389/fpls.2022.758818

Canada, E. and C. C. (2004, June 3). *Acid rain: Causes and effects* [Program descriptions]. https://www.canada.ca/en/environment-climate-change/services/air-pollution/issues/acid-rain-causes-effects.html

Chatveera, B., and Lertwattanaruk, P. (2014). Evaluation of nitric and acetic acid resistance of cement mortars containing high-volume black rice husk ash. *Journal of Environmental Management*, *133*, 365–373. https://doi.org/10.1016/j.jenvman.2013.12.010

Chen, Q., Wu, W., Zhao, T., Tan, W., Tian, J., and Liang, C. (2019). Complex Gene Regulation Underlying Mineral Nutrient Homeostasis in Soybean Root Response to Acidity Stress. *Genes*, *10*(5), 402. https://doi.org/10.3390/genes10050402

Cheremisinoff, N. P., and Rosenfeld, P. E. (2010). Chapter 6—Sources of air emissions from pulp and paper mills. In N. P. Cheremisinoff and P. E. Rosenfeld (Eds.), *Handbook of Pollution Prevention and Cleaner Production* (pp. 179–259). William Andrew Publishing. https://doi.org/10.1016/B978-0-08-096446-1.10006-1

Cho, Y. Y., Oh, S., Oh, M. M., and Son, J. E. (2007). Estimation of individual leaf area, fresh weight, and dry weight of hydroponically grown cucumbers (*Cucumis sativus* L.) using leaf length, width, and SPAD value. *Scientia Horticulturae*, *111*(4), 330–334. https://doi.org/10.1016/j.scienta.2006.12.028

Clair, T. A., Dennis, I. F., and Vet, R. (2011). Water chemistry and dissolved organic carbon trends in lakes from Canada's Atlantic Provinces: No recovery from acidification measured after 25 years of lake monitoring. *Canadian Journal of Fisheries and Aquatic Sciences*, *68*(4), 663–675. https://doi.org/10.1139/F11-013

Clymo, R. S. (1963). Ion Exchange in Sphagnum and its Relation to Bog Ecology. *Annals of Botany*, *27*(106), 309–324.

Clymo, R. S. (1987). Interactions of Sphagnum with Water and Air. In T. C. Hutchinson and K. M. Meema (Eds.), *Effects of Atmospheric Pollutants on Forests, Wetlands and Agricultural Ecosystems* (pp. 513–529). Springer. https://doi.org/10.1007/978-3-642-70874-9_37

Dedysh, S. N., Panikov, N. S., and Tiedje, J. M. (1998). Acidophilic methanotrophic communities from Sphagnum peat bogs. *Applied and Environmental Microbiology*, *64*(3), 922–929. https://doi.org/10.1128/AEM.64.3.922-929.1998

Dong, D., Du, E., Sun, Z., Zeng, X., and de Vries, W. (2017). Non-linear direct effects of acid rain on leaf photosynthetic rate of terrestrial plants. *Environmental Pollution*, *231*, 1442–1445. https://doi.org/10.1016/j.envpol.2017.09.005

Edge, C. P., Bell, S. A., and Ashenden, T. W. (1994). Contrasting growth responses of herbaceous species to acidic fogs. *Agriculture, Ecosystems and Environment*, *51*(3), 293–299. https://doi.org/10.1016/0167-8809(94)90141-4

Evans, C. D., Monteith, D. T., Fowler, D., Cape, J. N., and Brayshaw, S. (2011). Hydrochloric Acid: An Overlooked Driver of Environmental Change. *Environmental Science and Technology*, *45*(5), 1887–1894. https://doi.org/10.1021/es103574u

Ferenbaugh, R. W. (1976). Effects of Simulated Acid Rain on Phaseolus Vulgaris L. (fabaceae). *American Journal of Botany*, *63*(3), 283–288. https://doi.org/10.1002/j.1537-2197.1976.tb11813.x

Georgieva, M., and Vassileva, V. (2023). Stress Management in Plants: Examining Provisional and Unique Dose-Dependent Responses. *International Journal of Molecular Sciences*, *24*(6), 5105. https://doi.org/10.3390/ijms24065105

Guo, J., Du, M., Tian, H., and Wang, B. (2020). Exposure to High Salinity During Seed Development Markedly Enhances Seedling Emergence and Fitness of the Progeny of the Extreme Halophyte Suaeda salsa. *Frontiers in Plant Science*, *11*. https://doi.org/10.3389/fpls.2020.01291

Hobbie, S. E. (1992). Effects of plant species on nutrient cycling. *Trends in Ecology and Evolution*, *7*(10), 336–339. https://doi.org/10.1016/0169-5347(92)90126-V

Hodson, M. E., Langan, S. J., and Lumsdon, D. G. (1998). A Comparison of Soil Sensitivity to Acidification Based on Laboratory-Determined Short-Term Acid Buffering Capacity and the Skokloster Classification. *Water, Air, and Soil Pollution*, *105*(1), 53–62. https://doi.org/10.1023/A:1005035610525

Horst, W. J. (1983). Factors responsible for genotypic manganese tolerance in cowpea (Vigna unguiculata). *Plant and Soil*, *72*(2), 213–218. https://doi.org/10.1007/BF02181959

Humphries, T., Chauhan, B. S., and Florentine, S. K. (2018). Environmental factors effecting the germination and seedling emergence of two populations of an aggressive agricultural weed; Nassella trichotoma. *PLoS ONE*, *13*(7), e0199491. https://doi.org/10.1371/journal.pone.0199491

Jiang, J., Wang, Y.-P., Yu, M., Cao, N., and Yan, J. (2018). Soil organic matter is important for acid buffering and reducing aluminum leaching from acidic forest soils. *Chemical Geology*, *501*, 86–94. https://doi.org/10.1016/j.chemgeo.2018.10.009

Lafuente, E., Carles, L., Walser, J.-C., Giulio, M., Wullschleger, S., Stamm, C., Randauml, K., sandauml, and nen. (2023). Effects of anthropogenic stress on hosts and their microbiomes: Treated wastewater alters performance and gut microbiome of a key detritivore (Asellus aquaticus). *Evolutionary Applications*, *16*(4), 824–849.

Lassalle, G. (2021). Monitoring natural and anthropogenic plant stressors by hyperspectral remote sensing: Recommendations and guidelines based on a meta-review. *Science of The Total Environment*, *788*, 147758. https://doi.org/10.1016/j.scitotenv.2021.147758

Lichtenthaler, H. K. (1998). The Stress Concept in Plants: An Introduction. *Annals of the New York Academy of Sciences*, *851*(1), 187–198. https://doi.org/10.1111/j.1749-6632.1998.tb08993.x

Likus-Cieślik, J., Pietrzykowski, M., and Chodak, M. (2018). Chemistry of Sulfur-Contaminated Soil Substrate from a Former Frasch Extraction Method Sulfur Mine Leachate with

Various Forms of Litter in a Controlled Experiment. *Water, Air, and Soil Pollution*, *229*(3), 71. https://doi.org/10.1007/s11270-018-3716-2

Liu, H., Ren, X., Zhu, J., Wu, X., and Liang, C. (2018). Effect of exogenous abscisic acid on morphology, growth and nutrient uptake of rice (Oryza sativa) roots under simulated acid rain stress. *Planta*, *248*(3), 647–659. https://doi.org/10.1007/s00425-018-2922-x

Lu, H., Nkoh, J. N., Abdulaha-Al Baquy, M., Dong, G., Li, J., and Xu, R. (2020). Plants alter surface charge and functional groups of their roots to adapt to acidic soil conditions. *Environmental Pollution*, *267*, 115590. https://doi.org/10.1016/j.envpol.2020.115590

Mahmud, S., Kamruzzaman, M., Bhattacharyya, S., Alharbi, K., Abd El Moneim, D., and Mostofa, M. G. (2023). Acetic acid positively modulates proline metabolism for mitigating PEG-mediated drought stress in Maize and Arabidopsis. *Frontiers in Plant Science*, *14*, 1167238. https://doi.org/10.3389/fpls.2023.1167238

Marschner, H. (1991). Mechanisms of adaptation of plants to acid soils. *Plant and Soil*, *134*(1), 1–20.

Memon, A. R., Chino, M., Hara, K., and Yatazawa, M. (. (1981). *Microdistribution of manganese in the leaf tissues of different plant species as revealed by X-ray microanalyzer*. *55*(3), 225–232. https://doi.org/10.1111/j.1399-3054.1981.tb04491.x

Mendiburu, F. de. (2023). *agricolae: Statistical Procedures for Agricultural Research* (1.3-7) [Computer software]. https://cran.r-project.org/web/packages/agricolae/index.html

Msimbira, L. A., and Smith, D. L. (2020). The Roles of Plant Growth Promoting Microbes in Enhancing Plant Tolerance to Acidity and Alkalinity Stresses. *Frontiers in Sustainable Food Systems*, *4*. https://www.frontiersin.org/articles/10.3389/fsufs.2020.00106

Muchow, R. C. (1988). Effect of nitrogen supply on the comparative productivity of maize and sorghum in a semi-arid tropical environment III. Grain yield and nitrogen accumulation. *Field Crops Research*, *18*(1), 31–43. https://doi.org/10.1016/0378-4290(88)90057-3

Narayan, O. P., Kumar, P., Yadav, B., Dua, M., and Johri, A. K. (2022). Sulfur nutrition and its role in plant growth and development. *Plant Signaling and Behavior*, 2030082. https://doi.org/10.1080/15592324.2022.2030082

Pavlov, D. (2017). H2SO4 Electrolyte—An Active Material in the Lead–Acid Cell. In D. Pavlov (Ed.), *Lead-Acid Batteries: Science and Technology (Second Edition)* (pp. 133–167). Elsevier. https://doi.org/10.1016/B978-0-444-59552-2.00003-1

Pavlovkin, J., Pal'ove-Balang, P., Kolarovič, L., and Zelinová, V. (2009). Growth and functional responses of different cultivars of *Lotus corniculatus* to aluminum and low pH stress. *Journal of Plant Physiology*, *166*(14), 1479–1487. https://doi.org/10.1016/j.jplph.2009.03.005

R Core Team. (2023). *R: A Language and Environment for Statistical Computing* (Vienna, Austria). R Foundation for Statistical Computing. https://www.R-project.org/

Rahman, M. M., Mostofa, M. G., Rahman, M. A., Islam, M. R., Keya, S. S., Das, A. K., Miah, M. G., Kawser, A. Q. M. R., Ahsan, S. M., Hashem, A., Tabassum, B., Abd Allah, E. F., and Tran, L.-S. P. (2019). Acetic acid: A cost-effective agent for mitigation of seawater-induced salt toxicity in mung bean. *Scientific Reports*, *9*(1), 15186. https://doi.org/10.1038/s41598-019-51178-w

Ramlall, C., Varghese, B., Ramdhani, S., Pammenter, N. W., Bhatt, A., Berjak, P., and Sershen. (2015). Effects of simulated acid rain on germination, seedling growth and oxidative metabolism of recalcitrant-seeded Trichilia dregeana grown in its natural seed bank. *Physiologia Plantarum*, *153*(1), 149–160. https://doi.org/10.1111/ppl.12230

Rodríguez-Sánchez, V. M., Rosas, U., Calva-Vásquez, G., and Sandoval-Zapotitla, E. (2020). Does Acid Rain Alter the Leaf Anatomy and Photosynthetic Pigments in Urban Trees? *Plants*, *9*(7), Article 7. https://doi.org/10.3390/plants9070862

Ryan, J., Miyamoto, S., and Stroehlein, J. L. (1975). Effect of Acidity on Germination of Some Grasses and Alfalfa. *Journal of Range Management*, *28*(2), 154–155. https://doi.org/10.2307/3897451

Scully, B. T., and Wallace, D. H. (1990). Variation in and Relationship of Biomass, Growth Rate, Harvest Index, and Phenology to Yield of Common Bean. *Journal of the American Society for Horticultural Science*, *115*(2), 218–225. https://doi.org/10.21273/JASHS.115.2.218

Serdyuk, O., Trubina, V., and Gorlova, L. (2022). Breeding and chemical methods of brown mustard (Brassica juncea L.) protection from Fusarium blight. *BIO Web of Conferences*, *43*, 02018. https://doi.org/10.1051/bioconf/20224302018

Sharma, N., Sharma, J. R., Malik, A., and Sharma, A. (2019). *Influence of Hydrochloric Acid Concentration and Duration on Seed Germination in Guava*.

Shavrukov, Y., and Hirai, Y. (2016). Good and bad protons: Genetic aspects of acidity stress responses in plants. *Journal of Experimental Botany*, *67*(1), 15–30. https://doi.org/10.1093/jxb/erv437

Shekhawat, K., Rathore, S. S., Premi, O. P., Kandpal, B. K., and Chauhan, J. S. (2012). Advances in Agronomic Management of Indian Mustard (*Brassica juncea* (L.) Czernj.

Cosson): An Overview. *International Journal of Agronomy*, *2012*, e408284. https://doi.org/10.1155/2012/408284

Singh, A., and Agrawal, M. (2008). *Acid rain and its ecological consequences*.

Singh, B. K. (2021). Radish (Raphanus sativus L.): Breeding for Higher Yield, Better Quality and Wider Adaptability. In J. M. Al-Khayri, S. M. Jain, and D. V. Johnson (Eds.), *Advances in Plant Breeding Strategies: Vegetable Crops: Volume 8: Bulbs, Roots and Tubers* (pp. 275–304). Springer International Publishing. https://doi.org/10.1007/978-3-030-66965-2_7

Singh, M. P., Lallu, and Singh, N. B. (2014). Thermal requirement of indian mustard (Brassica juncea) at different phonological stages under late sown condition. *Indian Journal of Plant Physiology*, *19*(3), 238–243. https://doi.org/10.1007/s40502-014-0072-0

Šoch, J., Šonka, J., and Ponert, J. (2023). Acid scarification as a potent treatment for an in vitro germination of mature endozoochorous Vanilla planifolia seeds. *Botanical Studies*, *64*(1), 9. https://doi.org/10.1186/s40529-023-00374-z

Speight, J. G. (2017). Chapter Three—Industrial Inorganic Chemistry. In J. G. Speight (Ed.), *Environmental Inorganic Chemistry for Engineers* (pp. 111–169). Butterworth-Heinemann. https://doi.org/10.1016/B978-0-12-849891-0.00003-5

St. James, A. R., Yavitt, J. B., Zinder, S. H., and Richardson, R. E. (2021). Linking microbial Sphagnum degradation and acetate mineralization in acidic peat bogs: From global insights to a genome-centric case study. *The ISME Journal*, *15*(1), 293–303. https://doi.org/10.1038/s41396-020-00782-0

Taylor, M. R., Simon, E., Dickey, J. L., Hogan, K., Reece, J. (2021). *Campbell Biology: Concepts and Connections, Global Edition.* Pearson Education.

Troiano, J., Heller, L., and Jacobson, J. S. (1982). Effect of added water and acidity of simulated rain on growth of field-grown radish. *Environmental Pollution Series A, Ecological and Biological*, *29*(1), 1–11. https://doi.org/10.1016/0143-1471(82)90050-2

Turner, G. D., Lau, R. R., and Young, D. R. (1988). Effect of Acidity on Germination and Seedling Growth of Paulownia tomentosa. *Journal of Applied Ecology*, *25*(2), 561–567. https://doi.org/10.2307/2403844

Ünlü, H., and Ünlü, H. (2010). *pH, Nitrogen and Calcium Concentration Affect Germination and Seedling Growth In Pepper (Capsicum annuum L.)*.

Walne, C. H., and Reddy, K. R. (2022). Temperature Effects on the Shoot and Root Growth, Development, and Biomass Accumulation of Corn (Zea mays L.). *Agriculture*, *12*(4), Article 4. https://doi.org/10.3390/agriculture12040443

Xu, F., Vaziriyeganeh, M., and Zwiazek, J. J. (2020). Effects of pH and Mineral Nutrition on Growth and Physiological Responses of Trembling Aspen (Populus tremuloides), Jack Pine (Pinus banksiana), and White Spruce (Picea glauca) Seedlings in Sand Culture. *Plants*, *9*(6), 682. https://doi.org/10.3390/plants9060682

Ye, R., Jin, Q., Bohannan, B., Keller, J. K., McAllister, S. A., and Bridgham, S. D. (2012). pH controls over anaerobic carbon mineralization, the efficiency of methane production, and methanogenic pathways in peatlands across an ombrotrophic–minerotrophic gradient. *Soil Biology and Biochemistry*, *54*, 36–47. https://doi.org/10.1016/j.soilbio.2012.05.015

Zhang, C., Yi, X., Zhou, F., Gao, X., Wang, M., Chen, J., Huang, J., and Shen, C. (2020). Comprehensive transcriptome profiling of tea leaves (Camellia sinensis) in response to simulated acid rain. *Scientia Horticulturae*, *272*, 109491. https://doi.org/10.1016/j.scienta.2020.109491

Zhang, Y., Yu, T., Ma, W., Dayananda, B., Iwasaki, K., and Li, J. (2021). Morphological, Physiological and Photophysiological Responses of Critically Endangered Acer catalpifolium to Acid Stress. *Plants*, *10*(9), 1958. https://doi.org/10.3390/plants10091958

Zhang, Y.-K., Zhu, D.-F., Zhang, Y.-P., Chen, H.-Z., Xiang, J., and Lin, X.-Q. (2015). Low pH-Induced Changes of Antioxidant Enzyme and ATPase Activities in the Roots of Rice (Oryza sativa L.) Seedlings. *PLoS ONE*, *10*(2). https://doi.org/10.1371/journal.pone.0116971

Zhao, Y., Hou, X., Tigabu, M., Chen, S., Li, Q., Li, Z., and Cai, L. (2020). Effects of acid stress on germination, plasma membrane integrity and subcellular structure of *Neyraudia reynaudiana* seeds. *Flora*, *263*, 151549. https://doi.org/10.1016/j.flora.2020.151549

Zhu, X. F., and Shen, R. F. (2023). Towards sustainable use of acidic soils: Deciphering aluminum-resistant mechanisms in plants. *Fundamental Research*. https://doi.org/10.1016/j.fmre.2023.03.004

Zinnert, J. C., Via, S. M., and Young, D. R. (2013). Distinguishing natural from anthropogenic stress in plants: Physiology, fluorescence and hyperspectral reflectance. *Plant and Soil*, *366*(1), 133–141. https://doi.org/10.1007/s11104-012-1414-1

Zoca, S. M., and Penn, C. (2017). Chapter One - An Important Tool With No Instruction Manual: A Review of Gypsum Use in Agriculture. In D. L. Sparks (Ed.), *Advances in Agronomy* (Vol. 144, pp. 1–44). Academic Press. https://doi.org/10.1016/bs.agron.2017.03.001