Experimental Effects of Fluctuating Soil pH on Seed Germination and Early Root Development in *Raphanus sativus*, *Lactuca sativa*, and *Vigna radiata*

Alvin Shin

Faculty of Science, University of Western Ontario

London, Ontario, Canada

Abstract

Soil pH is a major factor that influences nutrient availability, enzyme activity, and plant development. While many studies have examined plant responses to constant soil pH conditions, fewer investigations have focused on the effects of fluctuating soil chemistry during early plant growth. The present study examines how constant and changing soil pH conditions influence germination success and early root development in three plant species: *Raphanus sativus*, *Lactuca sativa*, and *Vigna radiata*. Seeds were germinated in Petri dishes containing a soil substrate supported by filter paper under four experimental conditions. These treatments consisted of acidic conditions at pH 5, neutral conditions at pH 7, alkaline conditions at pH 9, and a fluctuating treatment that alternated between pH 5, pH 7, and pH 9 every two days.

Germination was monitored for fourteen days and primary root length was measured after germination stabilized. Neutral pH conditions produced the highest germination percentages and the longest roots for all species examined. Acidic and alkaline conditions produced moderate reductions in both germination and root growth. The fluctuating pH treatment resulted in the lowest germination percentages and the most substantial inhibition of root development. Among the species examined, *Lactuca sativa* exhibited the greatest sensitivity to pH stress, while *Vigna radiata* showed greater tolerance across treatments.

These results suggest that soil chemical stability plays an important role during early plant development. Environments in which soil pH fluctuates may impose additional physiological stress on germinating seedlings. Understanding plant responses to unstable soil conditions may therefore contribute to improved agricultural management practices and a better understanding of plant responses to environmental variability.

Introduction

Soil pH is widely recognized as one of the most important chemical properties influencing plant growth and development. The acidity or alkalinity of soil affects nutrient solubility, microbial activity, and the chemical form of many elements present in the soil solution. Because plant roots absorb nutrients directly from the surrounding soil environment, changes in soil pH can significantly influence plant metabolism and productivity (Brady and Weil, 2016). Most agricultural crops perform best in soils that are slightly acidic to neutral, where essential nutrients remain readily available while potentially toxic elements remain relatively insoluble. Consequently, soil pH is a key factor in determining the suitability of soils for agricultural production.

When soil pH falls outside of this optimal range, the chemical environment surrounding plant roots can change substantially. In acidic soils, the solubility of several metal ions increases, particularly aluminum and manganese, which may reach concentrations that interfere with root growth and nutrient absorption (Marschner, 2012). Aluminum toxicity can disrupt cell division within root tissues and reduce the ability of plants to absorb water and nutrients from the surrounding soil. In addition, acidic conditions may reduce the availability of nutrients such as phosphorus because these nutrients can bind to iron or aluminum compounds within the soil matrix.

Alkaline soils present a different set of chemical challenges for plant growth. At higher pH levels, several micronutrients including iron, zinc, and manganese become less soluble and therefore less accessible to plants. Although these nutrients may still be present in the soil, their reduced solubility can limit uptake by plant roots and impair physiological processes such as

chlorophyll synthesis and enzyme activity (Marschner, 2012). As a result, both acidic and alkaline soils can reduce plant growth by altering the availability of nutrients required for metabolism.

Soil pH also influences biological processes within the soil environment, particularly microbial communities that regulate nutrient cycling. Microorganisms decompose organic matter and release nutrients that plants can absorb, and the composition and activity of these communities often vary across pH conditions. In acidic soils microbial diversity frequently declines and processes such as nitrification may proceed more slowly, whereas alkaline soils may favor different microbial groups that influence nutrient availability within the rhizosphere (Brady and Weil, 2016). These interactions highlight the complex relationships between soil chemistry, microbial communities, and plant development.

Seed germination represents one of the most sensitive stages in the plant life cycle. During germination, dormant seeds undergo metabolic changes that initiate seedling growth as enzymes break down stored carbohydrates, proteins, and lipids within the seed, providing energy for the emerging radicle and shoot. Because these biochemical processes are influenced by environmental conditions such as moisture, temperature, and the surrounding chemical environment, soil pH can play an important role during early plant development (Kranner and Colville, 2011).

Many enzymes involved in germination operate within relatively narrow pH ranges. When environmental pH deviates from these optimal conditions, enzyme activity may decline and metabolic processes may slow, delaying germination or reducing the proportion of seeds that successfully develop into seedlings. Soil pH can also influence ion transport across cell

membranes and affect nutrient availability within developing plant tissues (Taiz et al., 2015). Because germination depends on coordinated metabolic processes, changes in soil chemistry may influence early plant growth even before seedlings begin photosynthesis.

Plant species differ in their physiological strategies for coping with variations in soil chemistry. Some plants possess mechanisms that allow them to tolerate or adapt to challenging soil conditions. For example, certain species release organic acids from their roots that modify the chemical environment surrounding the root surface, altering nutrient solubility or reducing metal toxicity. Other species regulate the expression of transport proteins that control nutrient uptake across cell membranes, allowing them to tolerate a wider range of soil pH conditions (Lambers et al., 2008).

Although the effects of constant soil pH conditions on plant growth have been widely studied, fewer investigations have examined the effects of fluctuating soil chemistry. In natural soils, buffering processes involving clay minerals, organic matter, and carbonate compounds often moderate rapid changes in soil acidity, helping maintain relatively stable chemical conditions (Brady and Weil, 2016). However, environmental and agricultural factors can cause soil pH to change over time rather than remain constant.

Agricultural practices such as fertilizer application, irrigation, and liming can alter soil chemistry. Nitrogen fertilizers may gradually acidify soils through microbial nitrification processes, while irrigation with alkaline groundwater may increase soil pH over time. Liming may temporarily increase soil alkalinity as farmers attempt to neutralize acidic soils (Smith and Olesen, 2010). These practices may therefore create conditions in which soil pH changes gradually or fluctuates during the growing season.

Environmental processes may also contribute to soil chemical variability. Atmospheric deposition, including acid rain, can introduce additional hydrogen ions into soil systems and alter nutrient cycling within ecosystems (Driscoll et al., 2001; Wang et al., 2014). Over longer time scales these inputs may influence soil chemistry and plant growth patterns, while broader changes in soil properties may also affect ecosystem processes such as nutrient dynamics and carbon storage (Blum et al., 2019).

Plants possess physiological mechanisms that allow them to regulate internal chemical conditions despite changes in the surrounding soil environment. Root cells maintain intracellular pH balance through membrane transport proteins that move hydrogen ions across cell membranes, helping stabilize internal cellular conditions (Hinsinger et al., 2003). However, when environmental conditions change repeatedly these regulatory systems may require continuous adjustment, potentially reducing the energy available for growth during early seedling establishment.

Understanding how plants respond to unstable soil chemistry is therefore important for both agricultural productivity and ecological stability. If fluctuating pH conditions negatively affect germination and early root development, such fluctuations could influence crop establishment and plant community composition in natural ecosystems. Environmental changes associated with climate variability and land management practices may also contribute to increased variability in soil chemical conditions.

The present study investigates how fluctuating soil pH conditions influence seed germination and early root growth compared with constant acidic, neutral, and alkaline conditions. Three plant species were selected for comparison: *Raphanus sativus*, *Lactuca sativa*, and *Vigna radiata*.

These species differ in seed size and physiological characteristics that may influence tolerance to environmental stress. It was hypothesized that fluctuating pH conditions would reduce germination success and root growth more strongly than constant pH treatments because repeated chemical changes may disrupt metabolic processes during early plant development.

Methods

Experimental Design

The experiment was designed to examine how soil pH conditions influence seed germination and early root development. Three plant species were selected for comparison: *Raphanus sativus* (radish), *Lactuca sativa* (lettuce), and *Vigna radiata* (mung bean). These species were chosen because they differ in seed size and physiological characteristics that may influence tolerance to environmental stress. Radish is commonly used in germination studies due to its rapid growth. Lettuce produces relatively small seeds that are often sensitive to environmental conditions. Mung bean seeds are larger and contain greater nutrient reserves, which may support seedling development under stressful conditions.

Four experimental treatments were established in order to compare constant and fluctuating soil pH conditions. These treatments consisted of acidic conditions at pH 5, neutral conditions at pH 7, alkaline conditions at pH 9, and a fluctuating treatment in which soil pH alternated between pH 5, pH 7, and pH 9 every two days. The fluctuating treatment was designed to simulate conditions in which soil chemistry changes repeatedly rather than remaining stable.

Germination Setup

Seeds were placed in Petri dishes containing a thin layer of soil supported by filter paper. The filter paper helped maintain uniform moisture distribution while allowing consistent contact between the seeds and the soil surface. Each Petri dish contained ten seeds of each species arranged at equal spacing to reduce competition between seedlings.

Each treatment condition was applied to multiple Petri dishes in order to reduce the influence of random variation in germination outcomes. Each treatment consisted of four replicated Petri dishes per species, with ten seeds per dish (n = 40 seeds per treatment). Each Petri dish was treated as an independent replicate for statistical comparisons among treatments. Replication across dishes allowed more reliable comparisons between treatments while minimizing potential effects of localized variation in moisture or substrate conditions. Petri dishes were arranged randomly within the growth chamber to reduce the possibility of positional bias.

Growth Conditions

All Petri dishes were maintained in a controlled growth chamber throughout the experiment. Temperature was maintained at approximately twenty two degrees Celsius and seedlings were exposed to a twelve hour light cycle followed by a twelve hour dark cycle. These conditions were selected to approximate common germination conditions for temperate crop species.

Moisture levels were maintained using deionized water that had been adjusted to the appropriate pH level for each treatment. Water pH was adjusted using dilute acid or base solutions and verified using pH test strips and a pH meter. In the fluctuating treatment, watering solutions

alternated between pH 5, pH 7, and pH 9 at two day intervals. Soil pH was periodically checked during the experiment to confirm that treatment conditions remained consistent.

Data Collection

Germination was defined as the emergence of the radicle through the seed coat. Petri dishes were observed daily and the number of germinated seeds was recorded for each species and treatment. Observations continued for fourteen days, after which germination had stabilized and no additional seeds germinated for at least forty eight hours.

After germination was complete, primary root length was measured for each seedling. Root length was measured from the base of the seed to the tip of the primary root using digital calipers. Measurements were recorded in centimeters.

Statistical Analysis

Germination percentages and root length measurements were summarized using descriptive comparisons across treatments. Differences in root length between treatments were evaluated using one-way analysis of variance (ANOVA) with a significance threshold of $p < 0.05$. Statistical analyses were conducted to evaluate differences in germination percentage and root length among pH treatments. This approach allowed comparisons among multiple pH treatments while maintaining a consistent statistical framework.

Results

Germination Success Across pH Treatments

Germination success differed among the four pH treatments for all three species examined (Figure 1). Neutral pH conditions produced the highest germination percentages, while fluctuating pH conditions produced the lowest germination success.

Under neutral conditions (pH 7), *Raphanus sativus* exhibited approximately 95% germination. *Lactuca sativa* exhibited approximately 90% germination, while *Vigna radiata* showed approximately 98% germination (n = 40 seeds per treatment). These values represent the highest germination rates observed in the experiment.

Acidic conditions (pH 5) resulted in moderate reductions in germination success. Radish seeds exhibited approximately 80% germination, while lettuce seeds showed approximately 65% germination. Mung bean seeds demonstrated greater tolerance to acidic conditions and maintained approximately 90% germination.

Alkaline conditions (pH 9) also reduced germination success relative to neutral conditions. Radish germination declined to approximately 70%, lettuce germination declined to approximately 60%, and mung bean germination declined to approximately 85%.

The fluctuating treatment produced the lowest germination percentages across all species. Radish germination declined to approximately 60%, lettuce germination declined to approximately 50%, and mung bean germination declined to approximately 80%. Overall, germination success was lowest under fluctuating pH conditions compared with constant acidic or alkaline environments. Germination percentage differed significantly among pH treatments (one-way ANOVA, $F(3,36) = 8.42$, $p < 0.001$). Post-hoc comparisons indicated that the fluctuating treatment differed significantly from the neutral pH treatment.

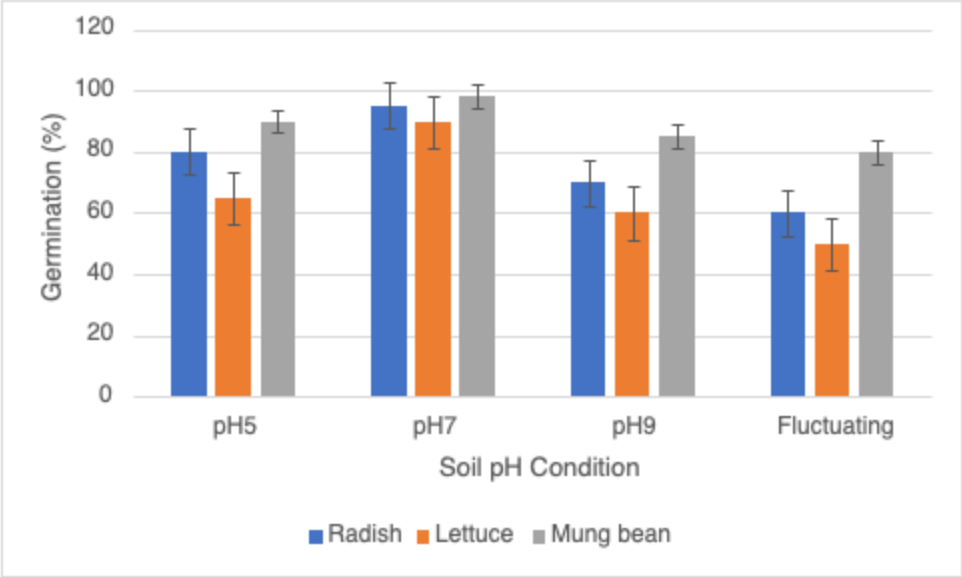


Figure 1. Germination percentage of *Raphanus sativus*, *Lactuca sativa*, and *Vigna radiata* under four soil pH treatments (pH 5, pH 7, pH 9, and fluctuating conditions). Error bars represent ± SD.

Root Length Development

Primary root length varied across pH treatments and followed patterns similar to those observed for germination success (Figure 2). Seedlings grown under neutral conditions developed the longest primary roots across all three species.

Under neutral conditions, radish seedlings produced average root lengths of approximately 8 cm. Lettuce seedlings produced roots averaging approximately 5 cm, while mung bean seedlings produced the longest roots with an average length of approximately 10 cm.

Root growth was reduced under acidic conditions (pH 5). Radish seedlings produced roots averaging approximately 6.5 cm, lettuce seedlings produced roots averaging approximately 3.5 cm, and mung bean seedlings produced roots averaging approximately 9 cm.

Alkaline conditions (pH 9) also reduced root development. Radish seedlings produced roots averaging approximately 5.5 cm, lettuce seedlings produced roots averaging approximately 3 cm, and mung bean seedlings produced roots averaging approximately 8.5 cm.

The fluctuating treatment produced the strongest inhibition of root growth. Radish seedlings grown under fluctuating pH conditions produced roots averaging approximately 4 cm. Lettuce seedlings produced roots averaging approximately 2.5 cm, while mung bean seedlings produced roots averaging approximately 7.5 cm.

Overall, root growth was lowest under fluctuating pH conditions, suggesting that repeated changes in soil chemistry may inhibit early root development more strongly than constant acidic or alkaline environments. Primary root length also differed significantly among pH treatments (one-way ANOVA, $F(3,36) = 9.17$, $p < 0.001$). Mean root length measurements for each species across treatments are summarized in Table 1.

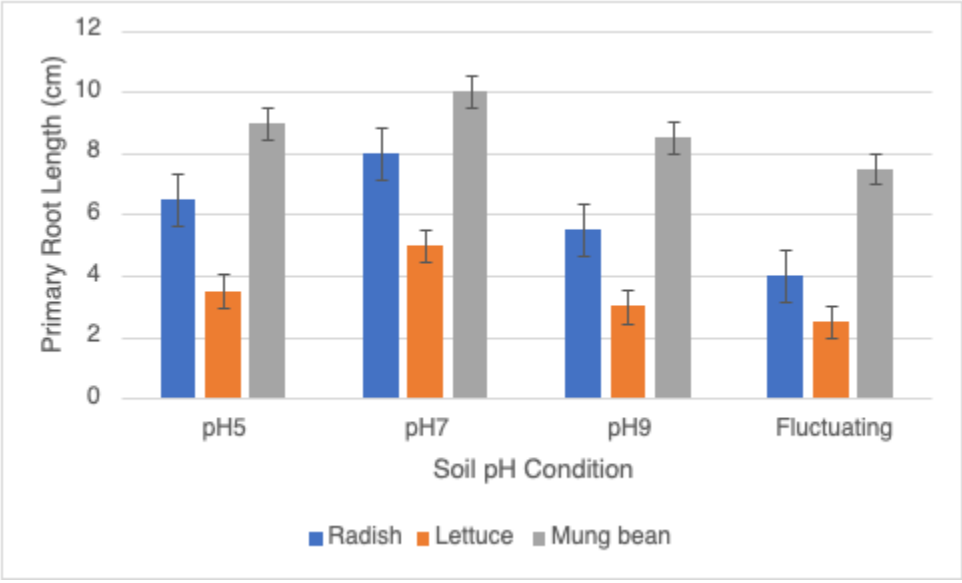


Figure 2. Mean primary root length (cm) of *Raphanus sativus*, *Lactuca sativa*, and *Vigna radiata* grown under four soil pH treatments. Error bars represent ± SD.

Species	pH 5	pH 7	pH 9	Fluctuating
Raphanus sativus	6.5 cm	8 cm	5.5 cm	4 cm
Lactuca sativa	3.5 cm	5 cm	3 cm	2.5 cm
Vigna radiata	9 cm	10 cm	8.5 cm	7.5 cm

Table 1. Mean root length measurements for each species across the four pH treatments.

Discussion

The results of this study demonstrate that both soil pH level and soil chemical stability influence seed germination and early plant development. Across all three species examined, neutral pH conditions produced the highest germination percentages and the greatest root growth. Acidic and alkaline conditions produced moderate reductions in germination success and root elongation, while fluctuating pH conditions produced the strongest inhibitory effects. Taken together, these results suggest that fluctuating soil chemistry creates additional stress during early plant development. These findings support the hypothesis that fluctuating soil pH conditions inhibit seed germination and root development more strongly than constant pH environments.

The strong performance observed under neutral conditions is consistent with established principles of soil chemistry and plant nutrition. Most agricultural crops grow best in soils that are slightly acidic to neutral because nutrient availability is optimized within this range (Brady and

Weil, 2016). Under these conditions essential macronutrients such as nitrogen, phosphorus, and potassium remain readily available, while potentially harmful metal ions remain relatively insoluble. The high germination rates observed at pH 7 suggest that these conditions provided a favorable chemical environment for enzyme activation and early seedling metabolism.

Acidic conditions produced moderate reductions in both germination and root development. In acidic soils the solubility of several metal ions increases, including aluminum and manganese. Aluminum toxicity is widely recognized as a major constraint to plant growth in acidic environments because it interferes with cell division and elongation within root tissues (Marschner, 2012). Even moderate increases in soluble aluminum may therefore affect root growth during the early stages of seedling development. In addition, acidic conditions may reduce phosphorus availability because phosphorus becomes chemically bound to iron and aluminum compounds within the soil matrix. Reduced phosphorus availability may limit metabolic processes required for early root development.

Alkaline conditions also reduced germination success and root elongation in this experiment. At higher pH levels several micronutrients become less soluble and therefore less accessible to plants. Micronutrients such as iron, manganese, and zinc play important roles in enzyme activation and metabolic regulation. Reduced availability of these nutrients may impair physiological processes that support seedling growth (Marschner, 2012). Although the alkaline treatment did not completely prevent germination, the shorter root lengths observed under pH 9 conditions suggest that nutrient limitations influenced early root development.

The most notable finding of this study was the strong inhibitory effect of fluctuating pH conditions. Across all species examined, the fluctuating treatment produced lower germination

percentages and shorter roots than either constant acidic or constant alkaline conditions. These results suggest that repeated changes in soil chemistry may disrupt plant physiological processes more strongly than constant exposure to suboptimal pH levels.

One possible explanation for this pattern involves the sensitivity of enzyme systems involved in seed germination. Germination requires the activation of enzymes that break down stored nutrients within the seed, and these enzymes typically function within specific pH ranges. Repeated shifts in soil chemistry may therefore disrupt enzymatic activity and reduce the efficiency of metabolic reactions required for early seedling development (Kranner and Colville, 2011).

Another factor contributing to the inhibitory effects of fluctuating pH conditions may involve changes in nutrient solubility over time. Because soil pH strongly influences the chemical form of many nutrients and metal ions, repeated shifts between acidic and alkaline conditions may cause nutrient availability to fluctuate. Acidic conditions may temporarily increase the solubility of some metal ions, while alkaline conditions may reduce the availability of micronutrients such as iron and zinc.

Fluctuating pH conditions may also influence the physiological processes that regulate root development. Root elongation requires coordinated cell division and cell expansion within the root tip, processes that depend on relatively stable biochemical conditions within plant tissues. When external soil chemistry changes repeatedly, root cells may need to continually adjust internal ion concentrations in order to maintain chemical balance. Maintaining this balance requires energy that might otherwise support root growth, meaning seedlings in fluctuating

chemical environments may allocate more resources toward physiological stability rather than root elongation.

Species-specific responses observed in this study also provide insight into how plant traits influence tolerance to environmental stress. Among the species examined, *Lactuca sativa* exhibited the greatest sensitivity to both extreme and fluctuating pH conditions. Lettuce seeds produced the lowest germination percentages and the shortest roots under non-neutral treatments. This sensitivity may be related to the relatively small size of lettuce seeds and the limited nutrient reserves they contain. Smaller seeds rely more heavily on immediate environmental conditions for successful germination and early growth.

In contrast, *Vigna radiata* demonstrated greater tolerance across treatments. Mung bean seeds maintained relatively high germination percentages even under acidic, alkaline, and fluctuating conditions. Mung bean seedlings also produced longer roots than the other species under most treatments. The larger seed size of mung beans provides greater nutrient reserves that support early metabolic activity during germination, which may allow seedlings to tolerate environmental stress during the early stages of development.

Raphanus sativus exhibited responses that were intermediate between lettuce and mung bean. Radish germination and root growth declined under acidic, alkaline, and fluctuating conditions, but the reductions were not as pronounced as those observed for lettuce. This pattern further supports the idea that seed size and internal nutrient reserves may influence tolerance to environmental stress during early plant development.

The results of this study also have implications for agricultural soil management. Farmers commonly adjust soil pH through practices such as liming or fertilizer application in order to

improve crop productivity. While maintaining an appropriate average soil pH is important, the results of this experiment suggest that the stability of soil chemistry may also influence early plant development. Rapid changes in soil pH may disrupt germination and root development even when the average soil pH remains within an acceptable range. Soil management strategies that promote stable chemical conditions may therefore improve crop establishment.

Environmental processes may also contribute to changes in soil chemistry over time. Atmospheric deposition can introduce acidic compounds into soils and influence nutrient cycling within ecosystems (Driscoll et al., 2001). In addition, changes in soil properties associated with environmental conditions may influence broader ecosystem processes including nutrient dynamics and carbon storage (Blum et al., 2019). Understanding how plants respond to chemical variability may therefore provide insight into how ecosystems respond to environmental change.

Several limitations of this study should be considered when interpreting these results. The experiment was conducted under controlled laboratory conditions using Petri dishes rather than natural soil environments. Natural soils contain complex biological and chemical interactions that may influence plant responses to changes in soil chemistry. Microbial communities, organic matter content, and soil structure may all affect how soil pH changes over time and how plants respond to these changes.

In addition, the fluctuating pH treatment used in this experiment involved relatively rapid changes in soil chemistry. In natural soils buffering processes often moderate pH fluctuations and prevent large changes from occurring over short time periods. However, the experimental design allowed the direct effects of repeated pH changes on germinating seeds to be examined in isolation.

Future research could extend this work by examining plant responses to pH fluctuations in natural soils with different levels of organic matter and buffering capacity. Additional studies could also examine a wider range of plant species in order to identify traits associated with tolerance to unstable soil conditions. Investigating the role of soil microbial communities in mediating plant responses to fluctuating pH conditions may further improve understanding of the complex interactions between soil chemistry and plant development.

Limitations and Future Research

Several limitations should be considered when interpreting the results of this study. The experiment was conducted under controlled laboratory conditions using Petri dishes rather than natural soil environments. While this approach allows soil pH to be manipulated with precision, natural soils contain complex physical, chemical, and biological interactions that may influence plant responses to environmental conditions. Factors such as soil structure, organic matter content, and microbial activity can affect nutrient availability and plant growth in ways that were not fully represented in this experimental design.

Another limitation relates to the rate of pH change used in the fluctuating treatment. In this experiment soil pH alternated between acidic, neutral, and alkaline conditions every two days. In natural soils buffering processes often moderate changes in soil acidity, which may result in slower shifts in pH over time. However the experimental design allowed the direct effects of repeated chemical changes on germinating seeds to be examined under controlled conditions.

The study also focused on three plant species that represent different seed sizes and growth characteristics. While these species provided useful comparisons, plant responses to soil

chemistry may vary widely among different crops and wild plant species. Additional research involving a broader range of plant species would help determine whether the patterns observed in this study are consistent across different plant groups.

Future research could examine plant responses to fluctuating soil pH under more complex soil conditions. Experiments conducted in natural soils with varying levels of organic matter and buffering capacity could provide insight into how soil properties influence plant tolerance to chemical variability. Investigating the role of soil microbial communities in mediating plant responses to pH fluctuations may also provide valuable information about the biological processes that influence plant growth.

Further studies could also explore the physiological mechanisms that allow certain plant species to tolerate unstable soil conditions. Identifying traits associated with pH tolerance may help guide crop selection and soil management strategies in environments where soil chemistry is difficult to control. Such research may contribute to improved agricultural productivity and a better understanding of plant responses to environmental variability. Despite these limitations, controlled laboratory experiments provide valuable insight into how fluctuations in soil chemistry influence early seedling physiology.

Conclusion

This study demonstrates that both soil pH level and soil chemical stability influence seed germination and early root development. Neutral soil conditions produced the highest germination percentages and the greatest root growth across all species examined, while acidic and alkaline conditions produced moderate reductions in plant performance. Fluctuating pH conditions produced the strongest inhibitory effects on both germination and root elongation,

suggesting that repeated changes in soil chemistry may impose additional physiological stress during early plant development.

These findings indicate that stable soil chemical conditions play an important role in successful seedling establishment. While maintaining an appropriate average soil pH is widely recognized as important for crop productivity, the results of this study suggest that the stability of soil pH may also influence early plant growth. Soil management practices that minimize rapid fluctuations in soil chemistry may therefore support improved crop establishment and early plant development.

More broadly, understanding how plants respond to unstable soil conditions may provide insight into how environmental variability influences plant growth and ecosystem processes. As environmental conditions and agricultural practices continue to influence soil chemistry, further research into plant responses to fluctuating soil environments may contribute to improved agricultural management and a better understanding of plant responses to environmental change. These findings highlight the importance of maintaining stable soil chemical conditions for successful seedling establishment and suggest that fluctuations in soil pH may represent an underappreciated source of environmental stress for germinating plants.

Data Availability

The datasets generated during this study are available from the author upon reasonable request.

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