The Impact of Plant Species on Soil pH

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Abstract

Soil pH is a key determinant of plant health, impacting everything from growth rate to germination rate (Gentili et al., 2018). Understanding soil pH, and the factors that influence it, is essential to ensuring optimal plant health and growth. While soil pH can change based on several abiotic factors, the plants growing within the soil can also have an impact on soil pH. To determine if the pH of forest soil would differ based on which species was grown in it, soil samples were collected from the roots of three species in different forested parks around Vancouver. The pH of the soil samples was then tested using a liquid anthocyanin solution. My analysis found that there was a statistically significant difference in soil pH based on which plant species was growing, regardless of the park in which the sample was collected. This indicates that even when grown in polycultures exposed to dynamic environmental conditions, the nutrient requirements of individual species are strong enough to change the pH of the nearby rhizosphere.

Introduction

While soil pH might seem somewhat inconsequential, it is incredibly impactful on the plants growing within that soil, as changes in pH can influence growth rate, shoot height and germination (Gentili et al., 2018). Soil pH is influenced by several internal factors, including the content of minerals, clay and organic matter within the soil (United States Department of Agriculture, 2014). Agricultural studies have shown that plants themselves can also have a large impact on soil pH. The pH of the rhizosphere, or the region of soil immediately adjacent to the root network of a plant, can change dramatically over time due to the root activity of that plant (Niena, 2019; Yan et al., 236).

This activity includes the leaching of certain nutrients from the soil by plants and the expulsion of waste, such as protons, from the roots of the plant (Yan et al., 236). The uptake of basic cations, such as Ca, Mg, K and Na by plants causes more acidic soil conditions as H^+ becomes more and more concentrated within the soil (Neina, 2019). Previous studies have shown

that monocultures have the most dramatic impact on soil pH, with pH changing based on the patterns of root activity of that particular species (Niena, 2019).

Additionally, these studies have shown that when two species are grown in close proximity, the rhizosphere is influenced by the nutrient requirements of both species, resulting in an intermediate soil pH that is in between the pH either species would cause on their own (Faget et al., 2013). However, these studies have focused on agricultural species grown in laboratory settings. Species used in agriculture commonly undergo artificial selection as farmers prefer to grow hearty plants, resulting in particularly robust species. Additionally, laboratory experiments often provide settings optimized for ideal plant growth, as most variables can be controlled within a laboratory setting.

With this in mind, I was curious to see if the varying nutrient requirements of different species would result in differences in soil pH when species naturally occur in a polyculture outdoors. In order to ensure that any trends that I found were not the result of random effects, I sampled soil from three different parks around Vancouver. My prediction was that while there would be some variation in soil pH between parks, due to slightly different environmental conditions and soil makeup, there would also be a difference in soil pH between species. This would indicate that even when co-exiting in a polyculture and exposed to rapidly changing conditions, the unique nutrient demands of different species are strong enough to impact soil pH.

Materials & Methods

Soil samples were collected from three different locations around the Vancouver area: Stanley Park, Pacific Spirit Park and Musqueam Park. These parks were chosen as all three contain dense forests with a wide variety of species, and are located in different areas within Vancouver, as illustrated by Figure 1. In each location, samples were taken from three plants of

each species as well as a control sample. Three replicates were collected for each plant, as well as the control, meaning thirty samples were collected from each park.

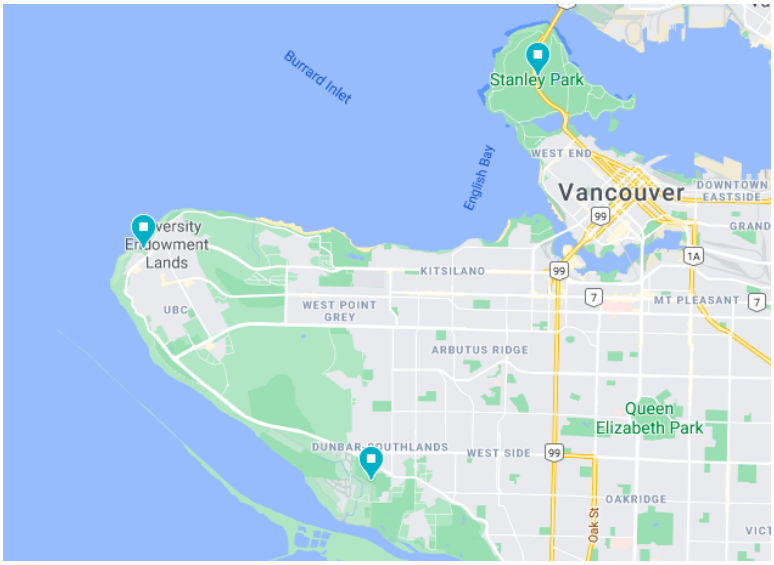

Figure 1. Map showing the collection points and how they are distributed around Vancouver. Figure generated using google maps.

Approximately two tablespoons of soil was collected from around the roots of three different species in each location: *Polystichum munitum* (Western Sword Fern), *Hedera helix* (English Ivy) and *Rubus armeniacus* (Himalayan Blackberry). All of the samples taken within each park were collected from the same large area, approximately 30 meters by 30 meters.

Once an appropriate plant had been identified, the collection spot was determined using a ruler. In order to ensure the collected soil was part of the rhizosphere, soil samples were taken from a collection point three centimeters away from the base of the plant and at a depth of two centimeters. A spoon was used to collect the samples, which were then placed into individually labelled plastic baggies. Control samples were taken at a depth of two centimeters in an area at least two meters from any plants. Sticks, debris and larger clumps were removed from samples. Samples were stored in labelled plastic baggies and kept in a cool, dark place until pH testing.

An aqueous anthocyanin solution was prepared by submerging one head of roughly chopped red cabbage in a large bowl with boiling water. The cabbage was left for around 30 minutes before being strained. The liquid was reserved and cooled to room temperature.

For each soil sample, two clear cups were labelled with the corresponding sample information. Approximately 5 tablespoons of anthocyanin solution was added to one cup, while the soil sample was added to the other cup. Two tablespoons of distilled water was added to the soil and thoroughly mixed. This cup was allowed to sit for 10 minutes to allow particles within the soil time to dissolve into the water. One tablespoon of this solution was then added to the cup with anthocyanin solution, and the resulting color change was recorded. The color was then matched with a pH value using an anthocyanin pH indicator chart, shown in Figure 2. The color could easily be determined by placing the cup directly under a light source and tilting it slightly to the side. This was repeated for all samples. Samples were collected over the course of two weeks, and all pH testing took place on the same day.

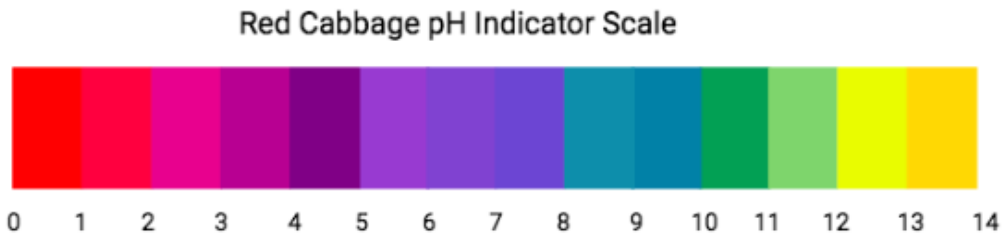

Figure 2. Liquid anthocyanin pH indicator scale, created with BioRender.

Testing pH using anthocyanin solution introduces a large potential for error, as color comparisons with the pH chart need to be made by eye. With this in mind, color classifications were made on a simplified scale. If the color of the anthocyanin solution matched one of the colors on the chart, it was given a pH that was half way between the two colors listed in the

chart. If the color of the solution was a mix between two adjacent colors on the chart, it was given the pH classification of the number between the two colors. As a result, the recorded pH values are either whole numbers or contain a fraction of exactly one half.

Once samples had been tested and pH had been recorded, the data was loaded into R Studio and a two-way ANOVA was carried out to determine if there was a statistically significant difference in soil pH between species, as well as between parks. A two way ANOVA was used as two categorical variables could be impacting soil pH: the park soil was sampled from, and the species soil was sampled from.

Results

The data was loaded into R Studio, version 1.4.1103, and was tested for normality. The skewness was determined to be -0.21, indicating that the data is relatively normally distributed. Bartlett's test was then conducted to assess variance homogeneity, and found that variances did not differ significantly between groups, meaning that the equal variance assumption of F-tests was met ($X^2_2 = 1.39$, $p = 0.40$). A mixed effects two way ANOVA was then carried out. There is a significant difference in soil pH between species ($F_{3,6} = 5.36$, $p = 0.03$), but there was not a significant difference in soil pH between parks ($F_{2,148} = 2.79$, $p = 0.06$). Additionally, there is no interaction effect between parks and species on soil pH ($F_{6,148} = 0.33$, $p = 0.92$). Figure 3 shows the distribution of soil pH across both species and parks.

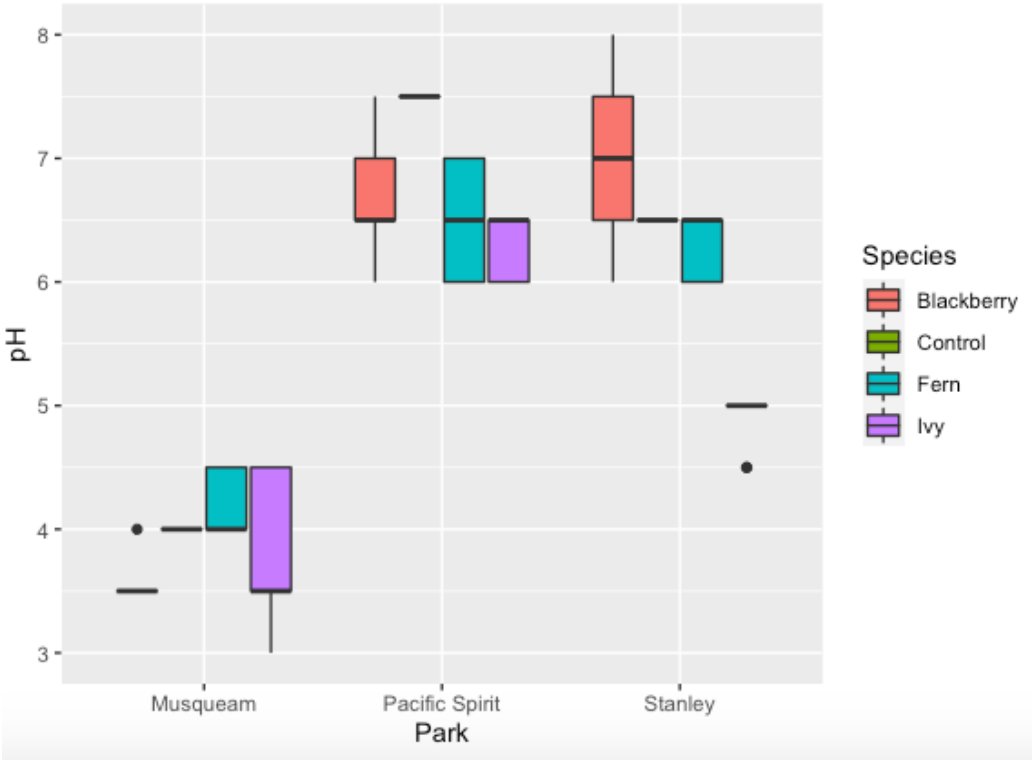

Figure 3. Box plot with outliers, minimum, maximum and median showing the range of pH values for each species, grouped by park. For each park, n=30, with 9 samples per species and 3 control samples. Data was analyzed using a two way ANOVA, which generated *p* values of 0.04 (between species), 0.06 (between parks) and 0.92 (interaction effects). Dots represent outliers while thick lines represent the median. The bounds of the boxes represent the upper and lower quartiles while the whiskers show the minimum and maximum.

Discussion

The results of the two way ANOVA show there is a statistically significant difference in soil pH between species, regardless of which park they were sampled from. This indicates that soil pH changes significantly based on which species is growing within it. This supports the conclusions of previous studies, and extends the findings of these studies beyond just monocultures. The species sampled during this experiment were growing in diverse polycultures,

and were coexisting with multiple other species in very close proximity. Additionally, these species were growing outdoors, and experienced very dynamic environmental conditions. Despite these factors, the species specific root activity of these plants was unique enough to create differences in soil pH.

Additionally, the two way ANOVA indicated that there was not a statistically significant difference in soil pH between the two parks. This finding is not directly applicable to discussions of the impact of species on soil pH, but should be examined nonetheless. Samples were collected from multiple parks in order to ensure that any perceived relationship between soil pH and species was due to the root activity of that particular species alone, and not due to random variation in soil pH throughout the park. The lack of a statistically significant difference in soil pH between parks shows only that there is no statistical difference in the range of pH values found within each park. This would seem to suggest that the park itself, and any differences in soil composition due to the location of the park, are not responsible for significant changes in soil pH.

It is important to note the *p* value for the interaction between species and parks. The results of the two way ANOVA showed that there was no interaction between parks or species, meaning that the effect of species on soil pH was not influenced by the park. This further strengthens the idea presented in the paragraph above, that the location is not what matters in determining soil pH. Instead, for this particular experiment, the species of the plant was the determining factor of soil pH. There are likely other possible confounding variables that may have influenced soil pH that were not addressed in this study.

The category of park is very broad and encompasses a vast array of biotic and abiotic variables that could be influencing soil pH. While sampling from three parks helped to eliminate

the effects of variation between parks, future studies should expand sampling even further. Additionally, this study focused on three species only, future studies could expand the number of species tested to see if the trends indicated are replicated with other species. In addition to increasing sampling locations and the number of species sampled, it would be interesting to see how much of a role the distance between coexisting species played in determining soil pH. This study focused exclusively on whether or not pH differed based on species when species are coexisting as opposed to growing in a monoculture, but did not look at how the distance between species might influence soil pH.

Finally, one major drawback to this study was the usage of liquid anthocyanin solution. Liquid anthocyanin solution has many benefits, mainly that it can be cheaply, quickly and easily produced in a normal kitchen, making it ideal for experiments done at home. However, liquid anthocyanin is not as sensitive as other pH testing methods, meaning that results are less accurate. Additionally, pH is assigned based on the comparison to two colors by the human eye, introducing a large amount of human error. Given the circumstances, liquid anthocyanin solution was an appropriate way to measure pH but future experiments would be more accurate if an alternative method of pH testing was used.

Conclusion

The results of this study supported the prediction that even when grown in a polyculture and subjected to volatile environmental conditions, the root activities of individual species would be distinct enough to generate differences in soil pH between species. I found that soil pH differs significantly based on which species is growing in it, and this difference is not impacted by the park in which the plant is growing. While further research is needed, these findings could have

important implications for maintaining the health and optimal growth of agricultural polycultures, home gardens and parks.

Acknowledgments

I would like to acknowledge that both the University of British Columbia, where this research was carried out, and the parks where soil was collected, are located on the ancestral and unceded land of the Musqueam, Squamish and Tsleil-Waututh nations. I would like to thank Dr. Celeste Leander for all of her help and the time that she dedicated to the BIOL 342 course, and the BIOL 342 teaching assistants: Sofya Langman, Tessa Blanchard and Jordan Hamden. I would like to thank the University of British Columbia for the opportunity to participate in the Integrative Biology Laboratory course.

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Appendix A - Summary Tables

Bartlett's Test:

Approximate X^2	1.39
Degrees of Freedom	2
p - value	0.40

Table 1. Results of Bartlett's test, conducted using R Studio.

Two-way ANOVA:

Source	Degrees of Freedom	Sum of Square	Mean Square	*F* Statistic	*p* value
Species	3	48.04	16.01	5.64	0.04
Parks	2	47.43	23.72	2.79	0.06
Interaction	6	17.03	2.84	0.33	0.92
Error	148	1257.05	8.49		

Table 2. Results of the two-way ANOVA, generated using R Studio. Three parks were sampled (n=30 for each park), with three species being sampled within each park (n=9 for each species within each park, along with 3 control samples per park).

Appendix B - Tidied Data

Musqueam Park			
	Replicate 1	Replicate 2	Replicate 3
Fern A	4.5	4.5	4.5
Fern B	4	4	4
Fern C	4	4	4
Ivy A	3	3	3.5
Ivy B	3.5	3.5	3.5
Ivy C	4.5	4.5	4.5
Blackberry A	3.5	4	3.5
Blackberry B	3.5	3.5	3.5
Blackberry C	3.5	3.5	3.5
Control	4	4	4

Stanley Park			
	Replicate 1	Replicate 2	Replicate 3
Fern A	6.5	6.5	6.5
Fern B	6.5	6.5	6.5
Fern C	6	6	6
Ivy A	5	5	5
Ivy B	4.5	5	4.5
Ivy C	5	5	5
Blackberry A	6.5	6	6
Blackberry B	7	7	7
Blackberry C	8	7.5	8
Control	6.5	6.5	6.5
Pacific Spirit Park			
	Replicate 1	Replicate 2	Replicate 3
Fern A	7	7	7
Fern B	6	6	6
Fern C	6.5	6	6.5
Ivy A	6	6	6
Ivy B	6.5	6.5	6.5
Ivy C	6.5	6.5	6.5
Blackberry A	7.5	7	7.5
Blackberry B	6.5	6.5	6.5
Blackberry C	6.5	6	6.5
Control	7.5	7.5	7.5

Appendix C - Raw Data

Park	pH	Species
Musqueam	4.5	Fern
Musqueam	4.5	Fern
Musqueam	4.5	Fern
Musqueam	4	Fern
Musqueam	4	Fern
Musqueam	4	Fern
Musqueam	4	Fern

Musqueam	4	Fern
Musqueam	4	Fern
Musqueam	3	Ivy
Musqueam	3	Ivy
Musqueam	3.5	Ivy
Musqueam	3.5	Ivy
Musqueam	3.5	Ivy
Musqueam	3.5	Ivy
Musqueam	4.5	Ivy
Musqueam	4.5	Ivy
Musqueam	4.5	Ivy
Musqueam	4	Blackberry
Musqueam	3.5	Blackberry
Musqueam	3.5	Blackberry
Musqueam	3.5	Blackberry
Musqueam	3.5	Blackberry
Musqueam	3.5	Blackberry
Musqueam	3.5	Blackberry
Musqueam	3.5	Blackberry
Musqueam	3.5	Blackberry
Stanley	6.5	Fern
Stanley	6.5	Fern
Stanley	6	Fern
Stanley	6.5	Fern
Stanley	6.5	Fern
Stanley	6	Fern
Stanley	6.5	Fern
Stanley	6.5	Fern
Stanley	6	Fern
Stanley	5	Ivy
Stanley	4.5	Ivy
Stanley	5	Ivy
Stanley	5	Ivy
Stanley	5	Ivy
Stanley	5	Ivy

Stanley	5	Ivy
Stanley	4.5	Ivy
Stanley	5	Ivy
Stanley	6.5	Blackberry
Stanley	7	Blackberry
Stanley	8	Blackberry
Stanley	6	Blackberry
Stanley	7	Blackberry
Stanley	7.5	Blackberry
Stanley	6	Blackberry
Stanley	7	Blackberry
Stanley	8	Blackberry
Stanley	6.5	Control
Stanley	6.5	Control
Stanley	6.5	Control
Pacific Spirit	7	Fern
Pacific Spirit	6	Fern
Pacific Spirit	6.5	Fern
Pacific Spirit	7	Fern
Pacific Spirit	6	Fern
Pacific Spirit	6	Fern
Pacific Spirit	7	Fern
Pacific Spirit	6	Fern
Pacific Spirit	6.5	Fern
Pacific Spirit	6	Ivy
Pacific Spirit	6.5	Ivy
Pacific Spirit	6.5	Ivy
Pacific Spirit	6	Ivy
Pacific Spirit	6.5	Ivy
Pacific Spirit	6.5	Ivy
Pacific Spirit	6	Ivy
Pacific Spirit	6.5	Ivy
Pacific Spirit	6.5	Ivy
Pacific Spirit	7.5	Blackberry
Pacific Spirit	6.5	Blackberry

Pacific Spirit	6.5	Blackberry
Pacific Spirit	7	Blackberry
Pacific Spirit	6.5	Blackberry
Pacific Spirit	6	Blackberry
Pacific Spirit	7.5	Blackberry
Pacific Spirit	6.5	Blackberry
Pacific Spirit	6.5	Blackberry
Pacific Spirit	7.5	Control
Pacific Spirit	7.5	Control
Pacific Spirit	7.5	Control