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HEADWATERS SCIENCE INSTITUTE
Student Research Journal

Volume 1 • Issue 2 • Fall 2020

Kayla Brand collects water quality data during the summer research program.

Forward

The articles you see in this publication are the result of months of hard work from students in Headwaters Science Institute's 2020 Summer Research Program. While they are not peer-reviewed in typical journal fashion, they have been reviewed by Headwaters staff and professional scientists. The caliber of these projects speaks loudly to these students' accomplishments. Allow us to also highlight a few of the most special parts of this program here.

Part of what made this program possible was participation by a great array of professional scientists, post-docs, and graduate students from institutions across the country. These scientists not only brought great technical knowledge to the program but also shared with students their own invaluable experience in navigating the winding path of original scientific research. Thank you to all of the scientist mentors who made these student projects possible.

One of the unique outcomes of this course was that all of the articles in this text are the students' own original ideas. Each one of these manuscripts started as part of a list of scientific questions brainstormed by students. From there, students shaped and molded the questions they were interested in based on current scientific knowledge and access to field sites or existing datasets. Students revised their questions, collected preliminary data, and revised again, honing their scientific skills along the way. The diversity of research questions in this publication is not only evidence of the incredible creativity inside all students but also honors the wonderful job the scientist mentors did in nurturing this curiosity.

To the students who authored these papers: congratulations! All of you have worked incredibly hard to complete these manuscripts. More than having your name on this paper, be proud of all that it represents. Each one of you overcame setbacks, roadblocks, time crunches, and uncertainty to get to this point. The skills you practiced along the way will serve you well in whatever you choose to do next.

Spencer Eusden

Program Director - Headwaters Science Institute

Headwaters Science Institute Journal Staff

Editor
Spencer Eusden
spencer@headwatersscienceinstitute.org

Graphic Design and Production
Megan Seifert
meg@headwatersscienceintitute.org

Assistant Editors
Le'a Gleason, Daniel Dudek, Jack Holmes

Mentors
Spencer Eusden, Daniel Dudek,
Hana Khal, Kerri Spuller

For any questions please contact
meg@headwatersscienceinstitute.org
P.O. Box 913, Soda Springs, California 95728
(530) 426-3063

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STUDENT RESEARCH JOURNAL

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Trampling the Crop: How Proximity to Trails Reduces Plant Biodiversity

Vivan Sinha[1], Spencer Eusden[2]

[1]Gunn High School, [2]Headwaters Science Institute

ABSTRACT

Trails are a popular way to experience nature, but many refuse to follow them. This is a phenomenon known as social trails, trails created when more people follow an unofficial trail, the more plants die, and the more official it starts to look until visitors cannot distinguish them from official trails. Actions have been taken to close these social trails off, but the same can happen in terms of trail width. Chances are, many people step slightly off a trail and as the trail widens, more and more plant life is lost. This experiment investigated the impact high use trails in and around Yellowstone National Park had on the surrounding biodiversity of plants. It was hypothesized that as the distance from a trail increases, biodiversity will also increase.

To measure the extent of this unintended loss, plant life was surveyed at 3 transects along 5 trails. Species were identified and counts were conducted for 8-meter long transects. Biodiversity was calculated with the Simpson's Index of Biodiversity for each meter as well as an overall count of the number of plants found.

These results showed a significant increase in biodiversity as the distance from the trail increased. Additionally, shrubs and brush were less impacted by proximity to the trails compared to flowers and grasses, suggesting foot traffic was the source of the decline in plant biodiversity and population. These sturdier plants are less likely to be killed by hikers, not only for their resilience but also due to the deterrence strong, sometimes sharp, branches provide.

INTRODUCTION

Trails have long been considered a non-intrusive way of experiencing nature, requiring relatively little space and keeping visitors away from wildlife. However, many visitors don't abide by the trails laid out before them. Throughout my stay at Yellowstone National Park during my participation in the Youth Conservation Corps in the summer of 2020, I've seen many examples of this called "social trails," clearings in vegetation made by repeated travel over a certain area (1). This can cause a loss of natural biodiversity in areas near trails and can not only harm the experience of visitors but also have an impact on wildlife through the trophic cascade, the passing down of problems from one level in the food chain to the next. For example, lower biodiversity in plant life can cause a specific animal that is better adapted for an abundant plant to outcompete other herbivores in the area. As these other herbivores lower in number due to lack of nutrition, the predators which rely on them are also impacted. With less available prey, their carrying capacity, and subsequently number, will decrease (2). As this works its way up the food chain, a large number of organisms can be hurt, and the area as a whole will lose lots of its plants and animals (2). Yellowstone is a prime example of this idea, most famously in its wolf reintroduction program. After wolves were hunted out of the park, the effects on Yellowstone could be seen in the land itself (3). Riverbanks began to widen due to elk overgrazing, and many species were affected. After this loss, many realized the importance of allowing the natural order to continue, and as wolves were reintroduced, the issues that came with their absence slowly halted. Although this example the origin of the loss of biodiversity was hunting, the same could happen due to any other source. If enough of the lowest level, the plants, are destroyed by human foot traffic, a similar trophic cascade, this time working its way up the food chain, could harm the entire ecosystem. This is one reason fences have been built to close off some of these social trails in the park, but no work has been done to test whether a similar loss of biodiversity or plant populations could occur due to high foot traffic on and around trails. In Yellowstone, there are many such high foot traffic trails, and they provide the perfect location to gauge how much human activity on sanctioned trails affects nearby wildlife, how far those effects reach, and what preventative measures can be put in place to reduce these impacts while still allowing human enjoyment. The hypothesis is that as proximity to a trail increases, the biodiversity will decrease.

METHODS

First, 5 trails were chosen for this study(Gallatin National Forest Trails 215 & 313, North Fork Bear Creek, Bunsen Peak, and Love Lake). These trails were chosen for their popularity to better demonstrate the effects of human use. Another consideration was the richness of vegetation apart from grasses. They also required a negligible slope tangential to the trail to ensure access to water and sunlight was consistent across the transect. Next, plant identification was conducted with resources from the Yellowstone National Park website.

A tape measure was used to measure out four 1m strips of cardboard. These strips were attached at the edges to create a square. This structure would be used at the site to mark out each area of data to be collected. A randomizer was used to ensure that distances between transects were well spaced. Lastly, a 12-megapixel camera was used to photograph individual flowers to allow for identification not in the field.

At each location, 10 meters were measured from the beginning of the trail to ensure proper distance from the road so exhaust and pollution played a negligible effect on the data. The variables in the experiment were biodiversity and distance from the trail so three transects were chosen for each trail, with a distance of 1 to 15 meters between them, decided by the randomizer. In each of these transects, the square was placed 8 times each time moving only further away only perpendicular to the direction of the trail. A picture was taken at each of these intervals for analysis afterward. With pictures for 5 trails, three transects each, with distances ranging from 1 to 8 meters from the trail, the count of each species (apart from grass species with too many individuals to count) was recorded. Simpson's index of biodiversity was calculated for each 1 square meter and a linear regression was made showing the relationship between distance from a trail and biodiversity.

Figure 1: Simpson's index of biodiversity(average of 15 sets per meter) increases with distance from the trail. Linear Regression: $r^2 = 0.65$

Meter	1	2	3	4	5	6	7	8
1		0.9000	0.9000	0.9000	0.9000	0.6760	0.5410	0.0014
2	0.9000		0.9000	0.9000	0.9000	0.9000	0.9000	0.0263
3	0.9000	0.9000		0.9000	0.9000	0.9000	0.9000	0.0487
4	0.9000	0.9000	0.9000		0.9000	0.9000	0.9000	0.0259
5	0.9000	0.9000	0.9000	0.9000		0.9000	0.9000	0.0329
6	0.6760	0.9000	0.9000	0.9000	0.9000		0.9000	0.2047
7	0.5410	0.9000	0.9000	0.9000	0.9000	0.9000		0.3175
8	0.0014	0.0263	0.0487	0.0259	0.0329	0.2047	0.3175	

Table 1: Tukey post hoc test from one-way ANOVA of Simpson's diversity index to distance. (blue = statistically significant).

RESULTS

To aggregate the results I averaged the Simpson's Index of Biodiversity for each of the distances. The mean diversity increased as we got farther away from the trail. A linear regression r2 (Figure 1) of mean biodiversity and distance from was 0.65. Additionally, a one-way ANOVA test comparing the biodiversity at each meter had a p-value of 0.00484, and an f-value of 3.115455. In Table 1 we can see the relatively constant increase in biodiversity as distance increases, however, the real difference starts to be seen after the 5th meter.

The bar graph (Figure 2) shows the split between flowers and more sturdy plants like shrubs. The latter group is affected far less by proximity to a trail, having a lower decrease in number.

DISCUSSION

The greatest loss of biodiversity is within the first five meters (Figure 1), indicative of foot traffic. Even

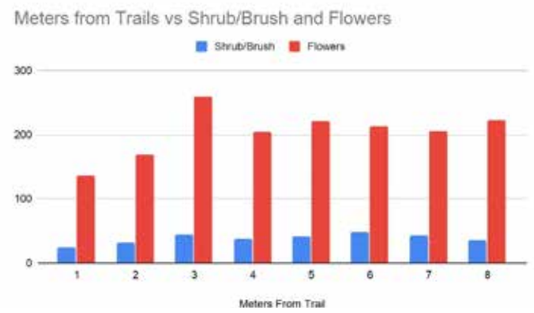

Figure 2: The total number of shrubs and brush varies less with distance than the number of flowers.

within those five, the biodiversity increased with distance from the trail. This seems reasonable as the amount of foot traffic on each meter outside the trail should decrease with distance. The diversity index used (Simpsons) penalizes areas with a low total number of species, but also areas with high variability in the counts of species present. Therefore the average biodiversity isn't the only measure used. The counts of plants are shown in a split bar graph (Figure 2). However, each type of data provides a different warning. The loss of biodiversity has a huge consequence on an ecosystem's inability to withstand natural disasters (4). These can include earthquakes and volcanic eruptions, something Yellowstone is known for, but now can also include climate change, which the ecosystem needs diversity to be able to handle and recover from. The decrease in absolute count of plants, especially flowers, (Figure 2) can also show the start of a chain reaction, harming first pollinators, then their predators, and working its way across the ecosystem, as discussed in the introduction. The idea that the reduction in one species can have effects across the ecosystem is powerful and makes it important to investigate every impact humans can have, no matter how small they might seem. The difference between the flowers and shrubs in Figure 2 can be explained by a variety of reasons, the most obvious being their ability to withstand trampling. This can be for one of two reasons, either the plant itself is made of a woodier stem, making it more resilient to damage and less desirable to trample over, or the plant's infrastructure is less dependent on the parts above ground. Both of these are true in the case of the most prevalent of these, the sagebrush. The hard stem and large area covered make it both difficult to bend and impossible to go through or over, especially compared to a flower.

These findings show that currently, even official trails are not completely successful in keeping visitors off of vegetation. To mitigate this issue, the simplest response would be education. When entering a park, signage defining rules for trail use and how to mitigate plant damage when straying off the trail could have a large impact on this loss of biodiversity seen. Another simple, yet likely extremely effective response could be to install railings on parts that receive a lot of foot traffic to reduce its impact (5). A physical barrier is something impossible for a visitor to forget and could decrease in the severity of the issue (5).

In future experiments, this study could be further improved by having a larger team to identify and count plants and species. This would allow for better accuracy while counting, and more locations in general. It would also allow for the accounting of grass species which could not be done in the interest of time and equipment in this experiment. I'd also want to have a larger dataset with more varied locations to ensure the results aren't specific to an area, and instead speak on trails as a whole.

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The Effect of Different Fertilizer Treatments on the Sprouting Time and Growth of *Cosmos* seeds, seeds of the genus *Myosotis* Forget-Me-Not seeds, and Cruciferae *Brassica nigra* Mustard seeds

Charlotte Ferguson[1], Daniel Dudek[2]

[1]Branson School, [2]University of Texas Arlington

ABSTRACT

During the past decade, climate change has become an important topic. A few ramifications include an excess amount of carbon dioxide and rising temperatures. It is predicted that ecosystems may change due to climate change and its effects on soil communities, resulting in a decrease in plant biodiversity. Soil communities contain tons of components with minerals being one. For this study, different treatments were formed, each containing varying levels of nitrogen, phosphorus, sulfur, and lime and altering the pH. I sowed three different species: *Cosmos* seeds, *Myosotis* Forget-Me-Not seeds, and Cruciferae *Brassica nigra* Mustard seeds. I hypothesize that as the treatment levels decrease in pH the sprouting time and plant growth will increase. Additionally, I predict that as the treatments increase pH, sprouting time and plant growth will increase as well. The results did not support my hypothesis. Only two pots have sprouts. The ANOVA results for the sprouting time in response to the treatment types was 0.042, a rejection of the null hypothesis. Moreover, the ANOVA p-value for the treatment type effect on plant height was 0.000024. The final ANOVA test conducted, which examined the difference in mean plant height between the three species, was 0.00441. All of these ANOVA results are significant, demonstrating that climate change may have a great impact on plant biodiversity.

INTRODUCTION

Over the past decade, climate change has become an increasingly pertinent global issue. Although significant data has been recorded in the last century, this phenomenon began thousands of years prior. Since the 1880s, the global temperature has risen 1.8°F (Callery, S. et al. Accessed 30 Jul 2020). Additionally, carbon dioxide levels are drastically increasing: "reaching its highest amount in the last 650,000 years" (Callery, S. et al. Accessed 30 Jul 2020). Both are ramifications of climate change, an issue that greatly affects the climate in many regions and subsequently the composition of soil (Classen, A. T. et al. 2015).

There are numerous components that constitute soil composition including, water, microorganisms, and minerals (Classen, A. T. et al. 2015). Water plays an important role in both chemical and biological decomposition (DeGomez, Tom, et al. 16 May 2019). The decomposition of materials releases nutrients and carbon for plants (Kleinman, S. 16 May 2019). With only a little water, soil biota struggles to decompose material and get nutrients (Kleinman, S. 16 May 2019). On the other hand, an excess amount of water results in a loss of oxygen for microorganisms and an increase in soil methane production (Kleinman, S. 16 May 2019). Microorganisms decompose raw organic matter (DeGomez, Tom, et al. 16 May 2019). Nitrogen-fixing bacteria, a specialized microorganism, facilitates the extracting of the necessary nutrient (DeGomez, Tom, et al. 16 May 2019). Plant growth and health can be restricted without microbes (DeGomez, Tom, et al. 16 May 2019). Lastly, minerals release ions that are essential for nutrient retainment (DeGomez, Tom, et al. 16 May 2019). Each plays a vital role in the composition of soil. Previous evidence suggests that community compositions will transform, and the productivity of various ecosystems will decrease due to an altercation in rainfall (Porporato, A. et al. Nov 2004). It is hypothesized that ecosystems may react differently based on the relationship between soil components and plants provided that

climate change has a significant impact on microbial communities in soil, ultimately affecting plant biodiversity and development (Classen, A. T. et al. 2015). Altercations to any of these vital components of soil, may cause a decrease in plant biodiversity (Classen, A. T. et al. 2015).

Narrowing the focus to minerals, it is evident that an increased level of nitrogen greatly minimized the growth period of plant growth (Ingestad, T. 1977). Additionally, the utilization of NPK-fertilizers [fertilizers containing Nitrogen, Phosphorous, and Potassium] at its most potent level proved to produce the highest plant growth compared to areas that only received a fraction of the recommended dose of fertilizer (El-Bassiony, A.M., et al. 2010). Another mineral, Sulfur, that is said to increase Nitrogen uptake, does not have an effect on plant growth as being the sole soil fertilizer applied (Salvagiott, Fernando, et al. 2 Aug 2009, Saskatchewan Inst. of Pedology. Jan. 1984). However, another study stated that Sulfur fertilization had a significant effect on the yield of crops, increasing the mean range 60 percent (Aulakh M.S. 2003). Another study illustrates that all characteristics of plants, including, plant height, growth rate, and number of branches increased as a result of greater levels of sulfur (Piri, Issa, et al. Oct. 2011). Agricultural limestone, a fertilizer that increases pH, drastically enhanced yield more than 200% in comparison to locations that were not limed (Buerkert, A., et al. 21 Mar. 1907). Lastly, the addition of phosphorus fertilizer considerably increased plant height (Nkaa, F.A., et al. Sept. 2014).

Along with plant height, sprouting time is another important factor to examine. According to one study, the soil type with the most Nitrogen available had the fourth highest percentage of germination compared to the soil type with the least Nitrogen available, having the greatest average percent (Sladonja, Barbara, et al. 2014). The amount of Phosphorus in each soil was tested as well in the same study. The soil type with the most Phosphorus had the second greatest percentage of germination (Sladonja, Barbara, et al. 2014). Conversely, the second to lowest average percent of germination occurred in the soil type that had the least amount of Phosphorus available (Sladonja, Barbara, et al. 2014). Lastly, the pH of the soils was also measured. The soil type that was the most alkaline had the greatest percent of average germination, while the most acidic soil had the fourth highest average.

From learning and reading about other studies, I hypothesize that as the treatment types decrease the soil pH, the percentage of germination will increase as well. As the treatment types increase in pH, I predict that the germination percentage will increase. Additionally, I hypothesize that the plant height and growth will increase as the treatment types increase pH and as the pH of the soil decreases, plant height will as well.

METHODS

I gathered 12 small clay pots. Next, I researched the materials needed to increase or decrease the pH of soil. I researched plants with short germination times as well. The types I chose include blood meal, rose and flower mix, limestone, and sulfur. Each of these had varying levels of nitrogen and phosphorus: both are minerals that alter the pH of a soil. I bought *Cosmos* seeds, seeds of the genus *Myosotis* Forget-Me-Not seeds, and Cruciferae *Brassica nigra* Mustard seeds and then I prepared 6 different soil types. The first soil treatment consisted of 50% agricultural lime and 50% potting soil. 100% potting soil was then measured to create the second soil. The third soil treatment was formed with 67% potting soil and 33% blood meal. Then, 33% rose mix and 67% potting soil was utilized for the fourth soil treatment. The fifth treatment consists of 17% and 83% rose mix. Finally, 50% sulfur and 50% potting soil was utilized to make the sixth soil treatment. After making the treatments, I tested the pH of the soils. The levels of pH are 7.5, 7.0, 6.0, 5.5, 4.5, and 4.0, respectively. Each of these 6 unique soil types were created twice to fill the 12 pots. After placing the soil in the pots, I then moved all the pots to a location that receives good sunlight throughout the day. I then planted the seeds. For one group of six pots, I placed 2 groups of four *Cosmos* seeds in each pot. The other group of six pots contained 2 groups of four Forget-Me-Not seeds and 2 groups of four Mustard seeds. Next, I watered each pot until the soil was saturated. Data was then collected, using a centimeter ruler to measure height and water was given to the plants each day. After two and a half weeks of data had been collected, an ANOVA test was performed to test the difference among the mean responses in sprouting time for each treatment type. Since the results of the ANOVA test were significant, I then proceeded to complete a Tukey Post Hoc test to compare the difference between all of the means.

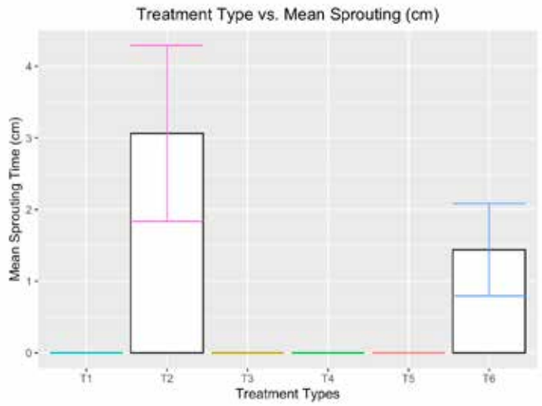

Figure 1: The mean sprouting time of the *Coreopsideae Cosmos* seeds, *Myosotis* Forget-Me-Not seeds, and *Brassica Nigra* Mustard seeds in the 6 different treatment types.

For plant height, an ANOVA test was done to examine the difference between the mean height for all sprouts. Because the p-value of ANOVA test was significant, the Tukey Post Hoc test was performed. Lastly, I completed an ANOVA test for plant height of the three different species all in Treatment two. Again, since the results were significant, a Tukey Post Hoc test was executed.

Sample No.	1	2	3	4	5	6
Treatment Types	Agricultural Lime and Potting Soil	Potting Soil	Blood Meal and Potting Soil	Rose Mix and Potting Soil	Rose Mix and Potting Soil	Sulfur and Potting Soil
Nitrogen (%)	10	10	22	14	20	10
Phosphate (%)	12	12	12	20	32	12
Potash (%)	6	6	6	10	16	6
Magnesium (%)	10.39	0.04	0.04	2.04	5.04	0.04
Calcium (%)	26.95	7	7	7	7	7
Sulfur (%)	6	6	6	6	6	96
Calcium Carbonate (%)	49.82					
Magnesium Carbonate (%)	42. 60					
Fir Bark (%)	25	25	25	25	25	25
Perlite (%)	25	25	25	25	25	25
Mushroom Compost (%)	25	25	25	25	25	25
Earthworm Castings (%)	25	25	25	25	25	25

Table 1: Chemical composition and soil composition of all six treatment types.

RESULTS

As seen in Figure 1, treatment two (T2) and treatment six (T6) were the only soil types in which seeds germinated. The ANOVA test produced a significant p-value indicating a rejection of the null hypothesis of there being no difference between treatments and sprouting time. Treatment two had the most sprouts; however, the mean sprouting time was greater than treatment six. The average sprouting time for treatment two (T2) was 6.15 days compared to treatment six (T6), in which the mean was 1.4375 days. The standard deviation for treatment two and six was 4.918 and 2.581, respectively. For the mean sprouting time data, the p-value was 0.0042, so it is significant. In Figure 2, the mean height for treatment two (T2) is 2.762 with a standard deviation of 4.256. The p-value was 0.0000224, which is of significance. Finally, in Figure 3, species A and species B were the only groups to have sprouts. The ANOVA test resulted in a significant p-value, that being 0.00441. Additionally, the average plant height for species A and species B was 1.5125 and 8.025, respectively. The standard deviation for species A was 1.065 and for species B it was 6.056.

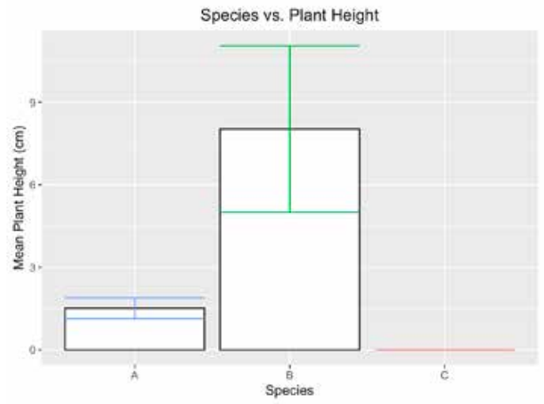

Figure 2: The mean height of the *Coreopsideae Cosmos* seeds, *Myosotis* Forget-Me-Not seeds, and *Brassica Nigra* Mustard seeds in the different treatments.

ANOVA test p-value: 0.0042	
Treatment Comparisons	P-value adj
T2-T1	0.0032596
T3-T1	1.0000000
T4-T1	1.0000000
T5-T1	1.0000000
T6-T1	0.4755294
T3-T2	0.0032596
T4-T2	0.0032596
T5-T2	0.0032596
T6-T2	0.3356747
T4-T3	1.0000000
T5-T3	1.0000000
T6-T3	0.4755294
T5-T4	1.0000000
T6-T4	0.4755294
T6-T5	0.4755294

Table 2: Tukey Post Hoc test values for all different combinations among the treatment types.

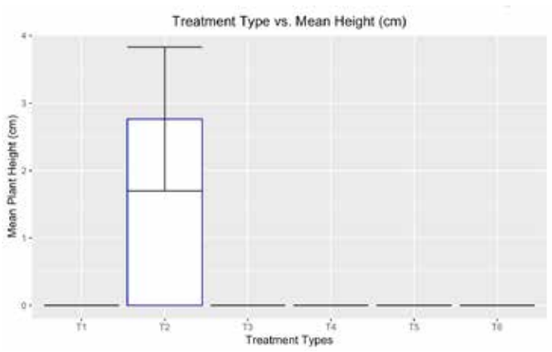

Figure 3: The mean height of each species in Treatment 2. "A" is *Myosotis* Forget-Me-Not seeds, "B" is Coreopsideae *Cosmos*, and "C" is Brassica Nigra Mustard seeds.

DISCUSSION

The results do not support my hypothesis. Before conducting the experiment, I anticipated that the seeds planted in soils that had the mineral fertilizers, which contained nitrogen and phosphorus, would have the shortest germination and greatest growth; however, only the plain potting soil and the treatment with sulfur had sprouts. While the plants in treatment six, the soil with sulfur, had a shorter germination period, the sprouts quickly died. This resulted in treatment two having the most seeds germinate and greatest mean growth.

While this study does not align with the conclusions of other researchers, the results are significant in that it demonstrates that mineral fertilizer may have harmful effects on the germination period and the growth of plants (Ingestad, T. 1977, El-Bassiony, A.M., et al, Buerkert, A., et al. 21 Mar. 1907, Aulakh M.S. 2003, Nkaa, F.A., et al. Sept. 2014). Furthermore, the data shows that treatment types with an increase in supply of nitrogen and phosphorus, decreasing the acidity, will lead to a reduction in the germination percent (Roem, W. J., et al. 11 Dec. 2002). It is evident that the different soil treatments had a great impact on the sprouting time of the different plants. It is unknown if the treatment types had any effect on the plant height, since no sprouts grew in soils with fertilizers. The results of this study suggest that the changing relationship between minerals and plants and changes to soil compositions may have more drastic effects than expected. Lastly, the results may be due to the levels of pH and other minerals in the fertilizer being too high for the plants.

The results of the experiment may not support other studies due to human error and other factors. To begin, I may not have sowed the seeds in the way that was recommended, however, I did follow the instructions for planting on each seed packet. As the study continued, I may not have been giving the plants enough water each day. Additionally, a few variables may not have been controlled. For instance, the amount of sunlight provided could not be controlled. Lastly, the temperature of the soil may have been altered during the duration of the experiment.

For future investigations, it would be interesting to study the effect of extreme levels of carbon dioxide on growing plants. This would be done to study another ramification of climate change: greenhouse gases. Investigating the sprouting time and growth of plants in soil with varying temperatures would be another intriguing future study.

ANOVA test p-value: 0.00441	
Species Comparisons	P-value adj
B-A	0.0096293
C-A	0.6977923
C-B	0.0061873

Table 3: Tukey Post Hoc test values for each the three different species all in Treatment two.

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Human Activity and Soil Hostility

Jonathan Choi[1] and Daniel Dudek[2]

[1]Mission San Jose High School, [2]University of Texas Arlington

ABSTRACT

The composition of soil can be broken down to a mixture of both abiotic and biotic matter. This composition can include animal waste, minerals, water, and even air. The complex nature of soil can consequently be affected by a large number of factors. These factors may range from simple weather alterations to complex human alterations to environments that could easily impose effects on soil. To see the effects humans can have on soil, this experiment was conducted in which three counties with varying levels of human activity (measured in population) had samples of soil collected to be tested for varying levels of pH and nutrients. Correlation tests were conducted to capture any significant correlation between the different levels of human activity and the variables tested in the soil. The results of these tests suggested that there was no significant correlation between varying degrees of human activity and soil pH and nutrient levels.

INTRODUCTION

Soil has undoubtedly structured and shaped the basis of countless human civilizations since the beginning of time. It molded agriculture and was a necessity for human survival amidst our Neolithic Period (1). This would lay a blueprint for an incredibly dependent relationship in which humans would look to the Earth's soil for crops to bear, for both survival and even economic purposes. It is also undeniable that even during contemporary times, soil is an important attribute for nations' agriculture to be successful and a critical part in determining the quality of human life (2). Additionally, it is stated that around 78% of the average per capita calorie consumption worldwide sources back to crops that were grown directly in soil, and the remaining 20% sourcing back to foods that depend on soil indirectly (1). Thus, soil can be seen as a crucial determinant in the health of nations and even ecosystems. In its healthiest form, soil is an extremely dense source of nutrition and an area for many plants to thrive (3). As a result, being able to gauge the health of soil is essential as soil can also be seen as the spine of nations' economies.

On the manner of soil health, it is imperative to answer how soil can be maintained in the healthiest state possible. Soil health and conditions can be monitored through a superfluous number of ways, but measurement of pH, N, P, and K should be sufficient. The pH of soil, determines numerous things, soil bacteria, nutrient leaching, nutrient availability, toxic elements, and soil structure (4), and a measurement of the pH level will indicate if the soil is adequate for healthy plant growth. This measurement does not represent how fertile the soil is but can affect the nutrient composition and concentration in the soil (4). As a result, soils can have all the nutrients a plant may need for growth but have a hostile pH level (4). Moreover, it is useful to test levels of N, P, and K which respectively represent nitrogen, phosphorus, and potassium. Nitrogen benefits soil health by means of being a nutrient that plants can absorb and utilize to promote the growth of leaves on the plant itself (5). While phosphorus is responsible for the plant's development of roots and growth of its flower or fruit (5). Likewise, potassium is responsible for all functions of the plant to work efficiently and correctly (5). The pH and the nutrients of the soil can, therefore, determine which plants are able to be grown and if the soil is deemed to have a pH in the extremities, it can be considered to be hostile (6). As conducted in an experiment by Jaswant et al., they gauged various soil conditions from a pH range of 4.5 to 9.5 (6), thus insinuating that hostile soil conditions can be determined by extremities. Moreover, the ideal soil pH is considered to range from 6.5 to 7.5, as in this range plant ranges are most readily available 6). To clarify, nutrient availability is partially due to pH levels, as levels greater than 7.5 have been known to induce reactions between phosphate ions, and calcium and magnesium, creating less soluble compounds (6). On the other hand, more acidic values will result in phosphate ions readily reacting with aluminum and iron, creating less soluble compounds (6). As a result, in experimentation, hostile soil qualities should be noted as soils with pH levels lesser than 6.5, greater than 7.5, or in the extremities of the pH scale.

This leads to solving the grand question of how human activity can impact the health of the soil around them. Examples of human activity include, but are not limited to, trampling, the increased intensity in agriculture, and acidification (7). In a study conducted in Scotland, it was noted that soil change was revealed due to the presence of human activity over a span of a few decades (7). Although this study acknowledged that these gradual changes are unable to be studied by means of traditional experimental science, the study acknowledged that there were two linked issues due to the approach of being reliant on observing changes in the field. The first problem was that human impacts could potentially have the same results as naturally occurring processes and the second problem being that human impacts could interact with each other, thus making it difficult to differentiate the effects of each activity (7). Despite the issues brought up in the study, it is predicted in our experiment that capturing the effects humans have on soil by gathering this information at one snapshot in time would be more useful in solving this question. Rather than pulling information over a gradual change in time, making observations between areas with varying degrees of human population density in one short time period could prove to show a result more representative to answer our question. This will make it possible to capture any statistically significant differences between areas with higher population numbers and lower population numbers. The differing levels of population numbers correlate to human activity due to the assumption that in areas with a greater number of humans present will surely have a higher level of activity, and vice versa with areas with lower populations of humans.

It is hypothesized that areas with higher human activity will have more hostile soil qualities for plants rather than areas with lower human activity. In support of finding an answer to this hypothesis, this experiment tested for variability and correlation of soil nutrient and pH among three counties which varied in human activity. As a result of this experimentation, the analyzed data insinuated that there was little to no correlation between human activity and soil health. Thus, there is insufficient evidence to confidently say that higher human activity does not necessarily result in more hostile soil quality.

METHODS

Area Selection

Firstly, I believed that the best way to sample the soil for testing was through a system of randomly generated locations. The ideal way, I thought, to avoid any experimental flaws while choosing locations to sample soil, was through a random selection of counties with varying degrees of human activity (low, moderate, and high human activity), and within those counties a set number of zip codes I could randomly pull. This was done through a random selection of three counties (Alameda, Santa Clara, and San Joaquin) that was accessible near me. These three counties would be representative of the varying degrees of human activity, as Alameda county had the highest population, thus high human activity, San Joaquin with the lowest high population, thus lowest human activity, and Santa Clara in between, with moderate human activity. Within each chosen county, three zip codes were randomly selected from the county's pool of zip codes. As a result, I had nine total zip codes (94555, 94544, 94539, 95121, 94087, 95126, 95202, 95385, and 95377) that were randomly selected to avoid any bias that could skew the results of this experiment. Within each zip code that was pulled, a grid overlay was placed over an image of the zip code's area on a map. Each unit of the grid was assigned a specific number, and then a random number was pulled from the grid three times. This was to further reduce any chances of selection bias. With 27 randomly-selected locations, I had pinpointed exactly where to extract soil samples from.

Sampling

At each of these locations, I had used a 21" steel soil probe to reach the ideal 6" depth (11) for extracting soil samples. These samples pulled by the probe were placed into air-tight plastic bags and ready to be tested.

Sample Testing

Testing the samples proved to be fairly stream-line as a soil test kit for pH, N, P, and K provided crucial solutions and papers to test the samples. Each soil sample was allowed to air dry, in which then it was run through respective tests which indicated the pH level, and the varying levels of N, P, and K. Data pulled from each sample was transferred

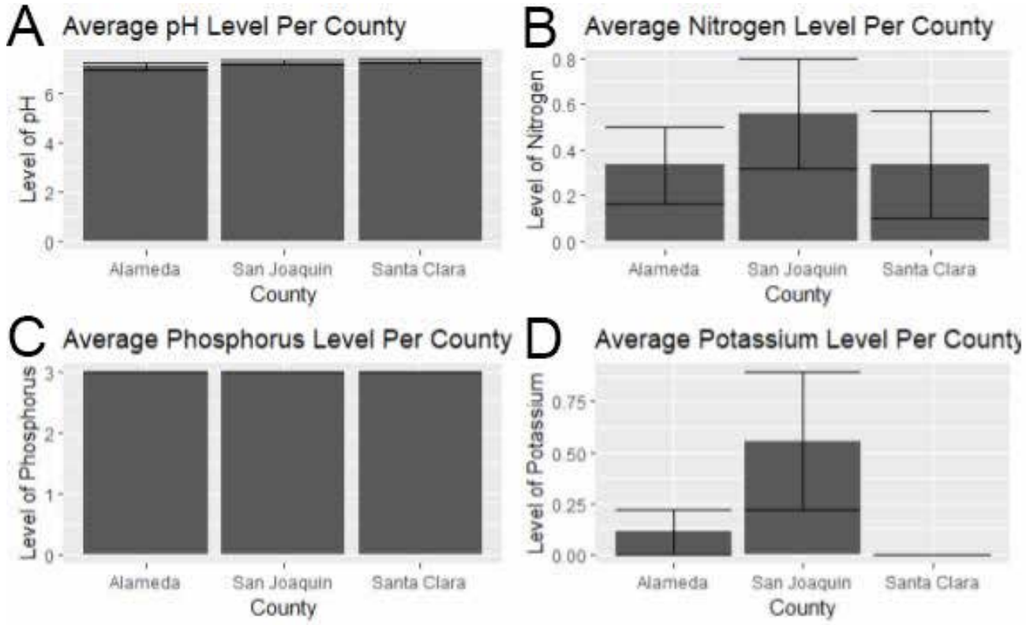

Figure 1: These graphs depict the average pH, Nitrogen (N), Phosphorus (P), and Potassium (K) levels per each county sampled from.

A. Mean +/- SEM pH for Alameda (7.111 +/- 0.138), San Joaquin (7.277 +/- 0.087), and Santa Clara County (7.333 +/- 0.083), with N=9, showed overlapping bars.

B. Mean +/- SEM N for Alameda (0.333 +/- 0.166), San Joaquin (0.555 +/- 0.242), and Santa Clara County (0.333 +/- 0.235), with N=9, showed overlapping bars.

C. Mean +/- SEM P for Alameda, San Joaquin, and Santa Clara County (3 +/- 0), with N=9, showed overlapping bars.

D. Mean +/- SEM K for Alameda (0.111 +/- 0.111), San Joaquin (0.555 +/- 0.337), and Santa Clara County (0.000 +/- 0.000), with N=9, showed overlapping bars between Alameda and Santa Clara, and San Joaquin and Alameda.

to a spreadsheet, where data would be grouped depending on which county it was sampled from and the variable it was testing for.

Data Analysis

With all the data regarding pH, N, P, and K respective to each county and variable being tested, an ANOVA test was run to examine for any differences in group means. In this experiment, the ANOVA test is used to see if there were any significant differences in pH, N, P, and K averages between the different counties. To further analyze data, a Pearson test was run to examine any correlations between human activity and soil parameters. The Pearson test would reveal coefficient correlation values between each variable (population, N, P, and K), and as a result, indicate how strong of a relationship one variable has with another. Data organization and test calculations took place in the software, R. From the results of the ANOVA and Pearson test, I was able to present a statement reflective of the relationship between human activity and soil health.

RESULTS

The impact of human activity (measured in population) on soil was investigated by methods in which soils in varying locations were examined to see any significant results. Across three counties (Alameda, San Joaquin, and Santa Clara), samples of soil were collected and tested to capture the differing levels of pH, nitrogen, phosphorus, and potassium. Three zip codes per each county were selected to sample soil from to create a fair experiment. The

pH	Df	Sum Sq	Mean Sq	F value	Pr(>F)
County	2	0.2407	0.1204	1.182	0.324
Residuals	24	2.4444	0.1018		
Nitrogen (N)	Df	Sum Sq	Mean Sq	F value	Pr(>F)
County	2	0.296	0.1481	0.348	0.71
Residuals	24	10.222	0.4259		
Phosphorus (P)	Df	Sum Sq	Mean Sq	F value	Pr(>F)
County	2	6.31E-30	3.16E-30	1	0.383
Residuals	24	7.57E-29	3.16E-30		
Potassium (K)	Df	Sum Sq	Mean Sq	F value	Pr(>F)
County	2	1.556	0.7778	2.049	0.151
Residuals	24	9.111	0.3796		

Figure 2: ANOVA test results with p values per each variable of soil tested between the three counties (Alameda, San Joaquin, and Santa Clara). pH p value (0.324), Nitrogen p value (0.71), Phosphorus p value (0.383), and Potassium p value (0.151), all under the 0.05 significance level.

data were then averaged per respective county and were organized and analyzed by R.

Of the four bar graphs created, the pH bar graph (Figure 1A) yielded results that were considered to be non-significant. Moreover, the nitrogen (Figure 1B), phosphorus (Figure 1C), and potassium (Figure 1D) bar graphs yielded results that too were considered to be non-significant. Bar graphs, with error bars representing the standard error of the mean (SEM) included, respective to each variable being tested in the soil resulted in data that overlapped among each other, thus suggesting non-significance.

Furthermore, the ANOVA test (Figure 2) indicated that results were also non-significant based on a 0.05 significance level. Due to non-significant results, a Pearson correlation test was conducted where it would test for any correlations between population numbers, pH, nitrogen, phosphorus, and potassium.

This test (Figure 3) resulted in correlation coefficient values that did not exceed a relationship that is considered to be a strong relationship but rather a moderate relationship, in terms of the correlation coefficient that is +/-0.6 being a moderate relationship (8). Pearson correlation test (Figure 3) also indicated that all p values were greater than the 0.05 significance level, thus results are non-significant.

DISCUSSION

After analysis of the data, the data reveals a failure to reject the null hypothesis thus suggesting that there is no significant relationship between human activity (measured by population) and the quality of soil. This does not support the initial hypothesis in which it was predicted that areas with higher human activity will have more hostile soil qualities for plants rather than areas with lower human activity.

In the bar graphs representing the data collected between the three counties (Figure 1), they are all cases of non-significant results, as error bars overlap. Although we can not necessarily conclude that there is no significance due to overlaps, it is a clue that non-significance is of great possibility. Furthermore, ANOVA test results also suggested non-significant results, based on a significance level of 0.05 as all p values dramatically exceeded that level. Due to a non-significant suggestion from ANOVA (Figure 2), a closer inspection to analyze variable to variable correlation was conducted by means of Pearson's r (Figure 3). By utilizing R, data organization was easily computed and resulted in values revealing the strengths of each variable's correlation to another variable's. This further resulted in having no strong correlations as the strongest relationship was determined to be moderate while having no significant variation between some variables, phosphorus, and others. While having no significant results or particularly strong correlation, there were some pertinent results that were acknowledged.

Soil is a dynamic resource, meaning that it is always changing and responding to environmental factors. As seen in the experiment completed in 2011 by Zhipeng et al., soil responded very significantly due to land use, precipitation, temperature, and even elevation (9). Therefore, suggesting that soil is highly susceptible to changes, and thus varying human populations could induce changes in soil. This

r / p value	Population	pH	N	P	K
Population	1 / _	-0.56 / 0.1139	-0.6 / 0.0859	NaN / _	-0.34 / 0.3739
pH	-0.56 / 0.1139	1 / _	0.27 / 0.4802	NaN / _	0.23 / 0.5438
N	-0.6 / 0.0859	0.27 / 0.4802	1 / _	NaN / _	0.05 / 0.8977
P	NaN / _	NaN / _	NaN / _	1 / _	NA / _
K	-0.34 / 0.3739	0.23 / 0.5438	0.05 / 0.8977	NaN / _	1 / _

Figure 3: Pearson correlation coefficient test results between the variables: population, pH, N (Nitrogen), P (Phosphorus), and K (Potassium). Correlation coefficient results found there to be no significant variation between P with any other variables tested. Correlation coefficient relationship between population and pH, N, and K resulted in -0.56, -0.6, and -0.34 respectively. Correlation coefficient relationship between pH, and N and K resulted in 0.27 and 0.23 respectively. Correlation coefficient relationship between N and K resulted in 0.05

leads to potential errors that could have occurred in experimentation.

A lack of support could potentially be due to a multitude of reasons. Firstly, a lack of a large sample size could have influenced the result of non-significance as smaller sample sizes will generally mean more variability (10). Thus, creating results that are not fully representative.

Additionally, there may have been an overwhelming number of confounds (e.g., varying organic matter and pre-mixed soils with designated nutrient and pH levels) that could have influenced the results of the experiment. These confounds could possibly be controlled in future experimentation through longer time spans of testing and use of replicates, as this experiment was done under tight time constraints. There could also be simple errors in testing (e.g., soil cross-contamination and lack of professional lab equipment) that could have skewed test results, thus leading to non-significance.

In future experimentation, sampling should be done over a wider variety of locations, potentially world-wide, and over greater lengths of time. The need for taking samples of greater lengths of time was demonstrated in Ian C. Grieve's study conducted in 2001 in which it is mentioned that soil can change slowly and as a result cannot be studied by means of "classical experimental science" (7). Soil can change drastically over a span of just days to a span of multiple years (7) and so it is important to capture the full effect humans can have on soil through a wider scope of experimentation length.

Connecting Ian's study done in 2001 (7), Zhipeng et al.'s study in 2011 (9), and this experiment, it is crucial to see the conditions in which soil can be tested to show valid and accurate results are delicate. Time must be taken into consideration, with respect to variations in weather and even elevation. This experiment may have shown non-significant results, but between errors made and past experimentations, it paves a way for successful future experimentation.

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HEADWATERS SCIENCE INSTITUTE
Student Research Journal

Aeroponic Experiment: The Efficiency of Aeroponics in Non-ideal Farming Conditions

Pascal Wettermark[1] and Spencer Eusden[2]
[1]Sugarbowl Academy, [2]Headwaters Science Institute

ABSTRACT

This experiment is about the efficiency of aeroponics in nonideal farming conditions. It was conducted in Truckee, California around 6,700 feet above sea level in very dry farming conditions. Studying aeroponics in harsh environments can be useful. Farming has never been considered in harsh environments, instead, we import our goods. Studying aeroponics in harsh conditions can prove to farmers that aeroponics can be used in harsh conditions, decreasing the need for imported goods. We hypothesized that aeroponics would use water more efficiently than soil-based farming. Results show that the aeroponics system used a lot less water but produces the same amount of plant growth. Aeroponics is a promising substitute for soil-based farming at high altitude in dry farming conditions. Aeroponics uses 90% less space therefore it can be applied in urban settings. Since aeroponics can be grown near urban and rural areas decreasing the need of transporting goods. This can reduce the amount of carbon emissions released by transportation. Most studies on aeroponics are done indoors therefore there isn't much data on outdoor aeroponics. My experiment provides evidence that aeroponics can be used outdoors at high altitudes in dry conditions. Future research should be focused on if aeroponics works in other unfavorable conditions. It will be really interesting to see if the water that isn't being used in the aeroponics system can be used over and over again or if it starts to get toxic for the plants.

INTRODUCTION

To explore the potential of aeroponics as a farming method, aeroponics was tested for how it would fare in terms of water usage and growth productivity, when used in high altitude, high desert (dry) climate. A traditional soil farming method was juxtaposed as comparison to aeroponics. The experiment was conducted in Tahoe, California at altitude 6,700 ft in planting zone 6b -7a (1), June - July, 2020. It included the planting, growing, and studying of spinach, arugula and radish seeds.

The world's population has increased from 1 billion in the 1800's to 7.7 billion today (2). The rapid increase in population has created a huge demand for food. Farmers have been able to meet this demand, but at a cost; space, water. In 2017, 23% of habitable land was used to grow crops (3) and 70% of freshwater is used for agriculture (4). Current farming methods are unsustainable given population growth and water and space intensive farming methods. One way to address this problem could be the use of new farming methods.

Aeroponics holds promise in this area. It is claimed to use 98% less water and 90% less space (5, 6). Some businesses, such as Plenty, have become very successful using aeroponics. With the use of aeroponics, these businesses can help reduce the likelihood droughts and deforestation and in the extension reduces wildfires and vegetation loss.

To date, most aeroponics systems are indoors primarily because it gives the farmer better control of the environment. Building indoor farms can be very costly (7). There are some aeroponic farming experiments taking place outdoors too. For example, one aeroponics experiment took place in Mindanao, an island in southern Philippines. Mindanao has ideal farming conditions with warm temperatures, regular rainfall, and it is generally protected from typhoons (8).

The research question at hand, focuses on aeroponics in non ideal conditions. What farming method, aeroponics or soil-based farming uses water more efficiently in high mediterranian conditions? Considering the successes of aeroponics in controlled or favorable farming conditions, the

hypothesis is that aeroponics would use water more efficiently than soil-based farming. This experiment was conducted outdoors in Truckee CA about 6,700 ft above sea level, with dry farming conditions. The experiment took an estimate of about five weeks to complete. Data from the experiment shows that outdoor aeroponics used water more efficiently than the soil system and provided the same amount of growth, hereby supporting the hypothesis. However, it does also pose some risks to the plants, as careful monitoring of weather conditions are required and soil appears to provide some protection from drying out the plants. If costs and risks can be mitigated, aeroponics proves to be highly efficient in non- ideal farming conditions compared to soil-based farming and should be further considered as a replacement of less efficient methods.

METHODS

An aeroponics system was created with common materials from a hardware store using a design from a video found online (citation of video). Once the rough draft of the design was complete, the parts for the design were purchased.

The materials used for the experiment are listed below. 10 sprayers: $4.72, Submersible Pump: $30, 8ft. of ¾ inch PVC: $6.45, ACE Blue Bucket: $28, 3 Packets of Radish Seeds: $3, Plastic, Lid and Pods: $5, Coconut Pods: $1, ACE Potting Soil: $5, Soil Bucket: $20, 3 Packets of Seeds: $3. The total cost of materials for the experiment was $106.17

The aeroponics pump was connected to electricity and tested. For the comparison system, i.e. the soil system, the potting soil was added to a planter box. Once the systems were complete, water was mixed with nutrients and added to the aeroponic system such that the pump was submerged in water. The amount of water added to the system was measured. Nutrients were also added to the water that would be used for the soil system. The amount of nutrients used was one tablespoon per gallon of water, per the recommendation on the packaging.

Coconut-based growing pods were placed into plant holders and seeds were planted both into the coconut pods (for the aeroponics) and directly into the soil (for the soil system). The different types of seeds used were arugula, spinach and radish. These three types of seeds were in different rows for monitoring purposes. Transparent, plastic lids were placed over each of the systems to control temperature, capture moisture and keep the sprouting seeds protected

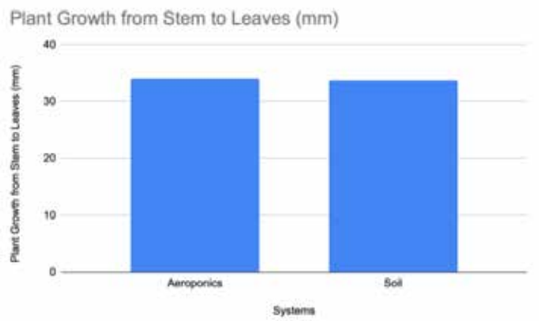

Figure 1: The average height that the plants grew in the aeroponics system was 34.00 mm compared to 33.72 mm for the soil system. Therefore there is no statistical difference between the systems t-test p = 0.7186. The N = 4 for aeroponics and N = 5 for the soil system.

from the environments (rain, wind, squirrels or birds).

Initial sprouting was largely unsuccessful for both treatments and an alternative sprouting technique was used. Instead of planting the seeds directly into pods and soil, seeds were left to soak in a wet paper towel placed in a ziplock bag. Within a couple of days, seeds had sprouted and were then placed in pods and soil.

Seeds were watered two or three times a day. In the soil system, water was poured onto the surrounding soil and not directly onto the sprouting seed / plant, to avoid stunting growth. Good drainage was provided so that the premature plant didn't rut. The aeroponics system sprayed a mist onto the pods for

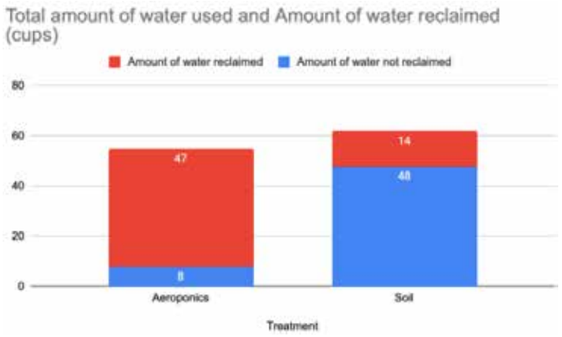

Figure 2: The aeroponics system used to water more efficiently in growing the plants. The aeroponics system was able to reclaim a lot more water compared to the soil system.

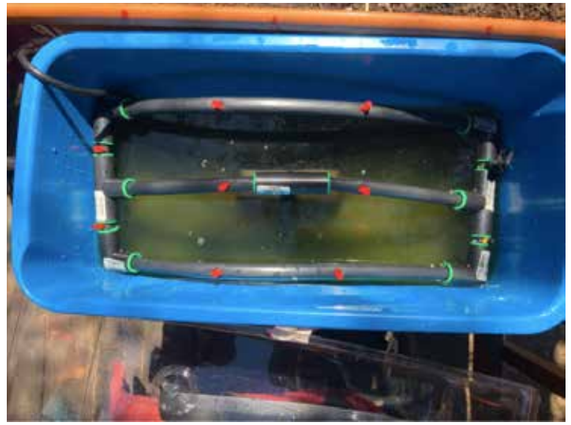

Figure 3: This is the inside of the aeroponics system, the red pieces are the sprays, the black tubes are PVC pipes and the pump is in between the two red sprayers in the middle row.

a duration of five minutes, two or three times per day. Sprouted seeds and later plants were sitting above the water and may also have benefitted from water condensation outside of the watering.

Once the plants were established, daily recording of waterings, growth and conditions provided data for the comparison of the two systems. Growth was measured in millimeters.

Weather impacts the growth of the plants. In this experiment, strong sun was most impactful. When the sun made the environment too hot and plants showed signs of wilting, the systems were brought inside. In other experiments, rain or wind may impact the systems and actions may have to be taken to protect the plants.

The experiment ran for 2-3 weeks. At the end of the experiment, water usage was measured. For the aeroponic system, the amount of water left in the system at the end was subtracted from the total amount of water used. In the soil system, runoff water was collected in a chamber at the bottom and measured at the end. For the soil system, "water used" was total water used for watering minus runoff water.

RESULTS

The key result metrics of this experiment are plant growth and water usage. If one system produced vastly larger plants, more water usage in that system may be warranted. However, the results of this study concludes that the amount of plant growth in the aeroponic and soil system was very similar (Figure 1). The growth of each seed that successfully sprouted in each system, was not statistically significant. Therefore, we cannot say that aeroponic or traditional farming methods help a seed and young plant grow faster or better. To grow the same size plants, the aeroponic system used significantly less water; aeroponics used 8 cups and soil used 48 cups.

Figure Two below shows how much water was added to the experiment and how much of that was reclaimed. The amount of water reclaimed is the water that still remained in each system at the end of the experiment. The water that was not reclaimed can be considered "water used". The aeroponics system used 8 cups out of the 55 cups added to it initially. 47 cups were reclaimed in the system. The soil system used 48 cups of water out of the 62 cups added ongoing. Only 14 cups were reclaimed in the soil system. This shows that aeroponics used less water while growing the same sized plants as the soil system.

DISCUSSION

My experiment shows that aeroponics uses a lot less water for the same amount of growth compared to the soil based system. In figure one above, it shows

Figure 4: The top layer of the aeroponics system is made up of this black sheet of plastic is the plant holders, and what is inside those holders is the non-soil based mix that holds the plants in place. Underneath the sheet of plastic is where the roots hang over the sprayers.

that there was no statistical difference between the two systems. In figure two, it shows that aeroponics reclaimed 47 cups of water and the soil system reclaimed 14 cups of water.

The study shows that aeroponics can be a far more water efficient method of growing seeds in high elevation mediterranean climate like the area around the northern sierra. By using less water, it reduces the likelihood of a drought and since we are using a lot less water we can grow crops during a drought. With fewer droughts, there will be more freshwater for living creatures in the Sacramento River delta. Fresh water can also be preserved for other, higher-value activities (9).

The study focused on water usage, and didn't take into account all aspects of energy consumption and pollution or other effects that farming has on the environment. For example, electricity was used to run the aeroponics pump, while no electricity was used for the soil system. The study was conducted at a small scale which allowed for mitigation techniques, such as moving the plants inside when the weather was unfavorable. In a larger scale study, such proactive or reactive actions may not be feasible. If mitigations can't be undertaken, it may be unreasonable to conclude that aeroponics (or any farming) can be sustained at high altitude, high desert. The study was conducted outside. In areas like the northern sierra, indoors may provide a better alternative to farming as it could prolong the growing season and protect against a cold environment. The study only considered the growth of the radish seeds since other seeds planted did not sprout.

Future studies should go deeper into water usage, but should also go beyond water usage. To learn more about water usage, different seeds should be used, the experiment should be longer to cover a whole growth period from sprouting to harvest, and be done at a larger scale as water consumption may change. To go beyond water usage, future studies should take into account all environmental, capital and operational impacts and costs to determine the holistic impact of different growing systems. Future studies should also be done at a larger scale to learn about the implications of scaling up aeroponics. In this study, radish, spinach and arugula seeds were used and only the radish sprouted and grew successfully. Understanding what seeds do well in these environments would be critical to future studies. Finally, testing aeroponics in indoor, vertical settings would be important since indoor farming can extend the growing season in mountainous regions, use less space, and provide for a more controlled environment, which may not require any use of pesticides.

It is well known that agriculture has tremendous effects on our earth and that current food production is not sustainable. Finding methods of growing crops more efficiently is an important scientific priority.

ACKNOWLEDGEMENTS

I'd like to thank my mentor Spencer Eusden for all his input and efforts to inform this study and for his practical help keeping the seedlings stay alive! It wouldn't have been possible without you. Thank you Spencer!

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Effects of Urbanization-related Soil Chemistry Changes on Bark Beetle Infestations

Lee Maskal[1], Hana Khal[2]

[1]Midland school, [2]University of California Davis

ABSTRACT

Trees damaged by bark beetle infestation have been shown to create significantly higher risk of damage and mortality due to fires. (3) Study was conducted in 2020 to investigate the relationship between soil salinity and urbanization on bark beetle infestation rates in evergreen trees in wooded areas. The hypothesis was that trees in more urbanized areas, would be in soil with higher salinity, due to pollution from runoff, and due to the raised salinity, there would be a higher rate of bark beetle infestation. Trees at a series of urban and remote locations were assigned a beetle-infestation score, based on the much evidence of bark beetle infestation. At each site, a soil sample was collected. A solution of soil and water was made and filtered, then was tested with a water conductivity meter to record the salinity. Salinity data was then compared to the data of tree condition. The data showed that trees in soils with higher salinity levels exhibited more bark beetle infestation than those in lower salinity soils. The data did not suggest that the salinity in urban areas was any higher than in remote forested areas. This data shows that, regardless of urbanization, trees in areas with increased soil salinity, may be more susceptible to bark beetle infestation than trees in areas with lower soil salinity.

INTRODUCTION

Trees that have been damaged by bark beetles have been shown to create significantly higher risk of damage and mortality due to fires, and fallen wood killed by beetles, increases the available fuel for a potential wildfire (3) In order to understand the relationship between soil salinity due to urbanization, and bark beetle infestation rates in trees, we investigated two interconnected questions: Whether salinity is higher in urban areas compared vs remote areas; and whether bark beetle infestation rates in trees is more severe in areas with more saline soils. This study was conducted to better understand whether salinity content in soils has an effect on tree population susceptibility to bark beetle infestation. A total of 75 trees were surveyed within the southern Lake Tahoe Basin of California and Nevada at 3 urban sites and 3 remote sites. Each tree was assigned a score from 1 to 6 to quantify the level of infestation. There is evidence that soil chemistry differences, such as nitrogen and moisture content, change the infestation rate of bark beetles in forests (2,4). It was therefore hypothesized that trees in urbanized areas will have higher incidences of bark beetle infestation due to soil pollution from runoff and other factors that increase soil salinity.

METHODS

At each site surveyed, a tree was chosen arbitrarily. Once the tree was chosen, the selected tree and all of the trees within 10 meters of the selected tree (including stumps) are surveyed and assigned a condition score. The conditions scores used increased from best or healthiest condition, to most evidence of beetle infestation:

Unaffected: Tree is healthy, and shows no signs of beetle infestation, (i.e. galleries, pitch tubes in the bark);

Repelled: Tree shows signs of infestation, but managed to repel the infestation. The main identifying factor being pitch tubes in the bark that are white, clear or yellow;

Infested (light): Tree was successfully infested, but shows minimal bark damage. Successful infestation is evidenced by red or dark orange colored pitch tubes, and galleries;

Infested (medium): Tree shows extensive damage of the bark, but is otherwise healthy.

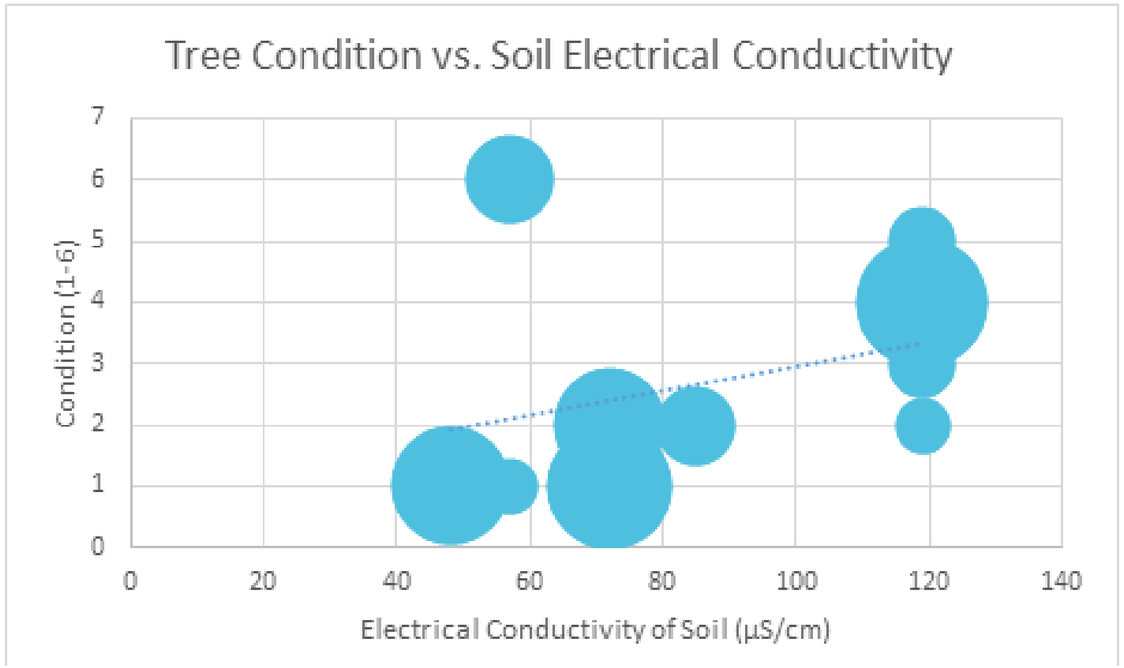

Figure 1: Salinity vs. Condition Score Graph With Outliers, R2 = 0.193

Infested (heavy): Tree shows extensive damage to all parts of the tree, tree is dying.

Killed: Tree is dead, shows no signs of moisture in the phloem, and the leaves are brown.

Each condition is represented by its number on the list in the data in the data.

After trees in the area are surveyed, a patch of soil near the fist tree is cleared to expose bare soil. Then, at least 80 grams of soil is collected and stored in a resealable plastic bag that is labeled to identify the sampling location.

Eighty grams of soil is measured and placed in a container; 250 grams of distilled water is added to the container with the soil sample; Soil and water are mixed thoroughly with a wooden skewer to form a slurry; solids are removed from the slurry by filtering through a 10-micron filter to produce a leachate with dissolvable solids from the soil; the resulting leachate is filtered a second time through a new filter. Electrical conductivity and total dissolved solids content of leachate are measured using a handheld meter. Samples are sealed in labeled 250ml glass jars, and retained for any additional testing needed. Soil conductivity and Total Dissolved Solids content are recorded for comparison with tree condition scores.

RESULTS

The effect of soil salinity on bark beetle infestation rates in urbanized areas was studied by surveying trees and soil salinity at sites in urban and remote areas. Analysis of the data showed correlation between increased soil salinity and increased bark beetle infestation score (Figure 1). A much stronger correlation was found when three outliers were removed from the data set as outliers. Figure 1 shows the data set with the outliers having an R-squared value of 0.193. Figure 2 shows the data set with the outliers removed, having an R-squared value of 0.778 (figure 2). These outliers were three small trees all within 1 meter of each other. These trees showed some damage from bark beetle infestation. However, these trees were on the edge of the trail,

Sampling Location	TDS (ppm)	Remote/Urban
Harrah's Parking Lot	23	Urban
Bijou #2	27	Urban
Van Sickle #1	28	Remote
Bijou #1	34	Urban
Van Sickle #2	40	Remote
Spooner Summit	55	Remote

Table 1: Soil Salinity and Location

and their root systems were likely damaged due to the construction of the trail.

The P-value for the data set without the outliers was 7.65E-18. This suggests that the variability in tree infestation scores is much more likely to be predicted by salinity than by random chance..

Table 1 shows each sampling location in order of increasing soil salinity and whether each location was remote or urban. There was no apparent trend of soil salinity vs remote or urban location (Table 1).

One possible source of internal error was the randomness of tree selection. Trees in sites surveyed were chosen arbitrarily, and not truly at random. Regarding external sources of error, there may have been other soil components that were

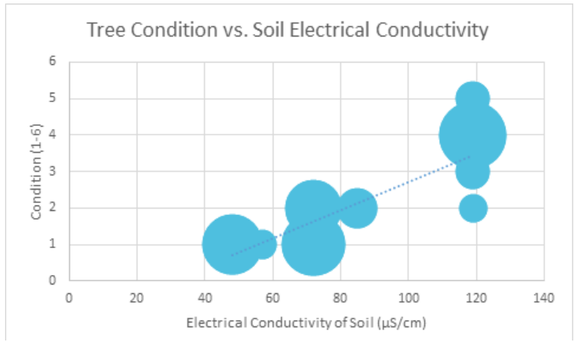

Figure 2: Salinity vs Condition Score Graph Without Outliers, R2 = 0.778

affecting the salinity and amount of infestation . In the urban areas tested, the trees were thinned, meaning beetle susceptible trees may have been cut down. Therefore, in those areas the tree density was considerably less, which has been shown to lower infestation rate, as trees in areas of higher density are closer, meaning beetles can transfer between trees more easily (1).

Questions for further study: Are there other soil components that could show such a strong correlation with infestation rate? Would a larger and more diverse sample size show different trends? Along with larger sample sizes could larger differences in terrain change the outcome of the study?

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Detergent pollutants decrease nutrient availability in soil

Sarah Ryu[1] Kerri Spuller[2]

[1]Menlo-Atherton High School, [2]Boise State University

ABSTRACT

Household detergents contain potentially harmful surfactants that can alter microbial activity and interfere with biogeochemical cycles (3). This study investigated the short-term effects of detergent pollution on pH and nutrient availability in soil through a controlled experiment on silty clay loam soil. Trends in pH, phosphorus, nitrogen, and potassium levels in soil polluted with a plant-based "green" detergent and soil polluted with a "regular", less eco-friendly detergent were observed over a 3-week study period. pH in both polluted groups both fluctuated during the first 60 hours, then returned to the initial level. Nitrogen and potassium availability levels decreased in the polluted groups. Detergent pollution did not have a clear impact on phosphorus availability. The results also support the idea that "green" products are an environmentally safer choice; however, even these "eco-friendly" products have an effect on nutrient availability when introduced into soil. Overall, this study concludes that proper disposal of household products is crucial, as pollutants released into the environment can have an impact on nutrient cycling in ecosystems even in a short time period.

INTRODUCTION

Household products are a major source of harmful organic pollutants (7). Through waste disposal and effluent discharge, products such as detergents and other cleaning products are dispersed throughout different environmental compartments including soil, water, and sediment, posing a threat to ecosystems (7). The main environmental sink for persistent organic pollutants is soil, where pollutants form stable bonds with organic matter(10). Soil pollution has been described as a "hidden danger" because it often cannot be directly assessed or visually perceived, causing growing concern worldwide (10). Understanding pollutants and their sources is important for pollution prevention, which greatly reduces both financial and economic costs (4).

The EPA lists 'switching to "green" cleaners' as a method of pollution prevention for homes and schools (4), and detergent products contribute to about 10% of organic matter in domestic wastewater (11). Historically, phosphate detergents have been known to cause eutrophication; however, regulations banning phosphate in detergents have caused companies to turn to surfactant detergents, which have high consumption rates and potential toxic effects connected with their surface-active properties (11). Toxic soil pollutants can severely degrade the major ecosystem services provided by soil by directly harming soil microorganisms, and chemically active pollutants can also initiate chemical changes (10). Additionally, surfactants have been found to alter enzyme activity in soil. In particular, alcohol ethoxylate, a surfactant in common detergents, including the "regular" detergent used in this study, has an inhibitory effect on soil enzyme activity and nutrient cycling (3). This study focuses on the changes in nutrient availability, as nutrients must be in available forms for plants and animals to support ecosystems.

Risk assessment of pollutants is still difficult because their specific environmental fates and impacts on ecosystems are not fully understood (10). To address this knowledge gap, this study investigates the question: how do pH, nitrogen, phosphorus, and potassium levels in soil change over time after organic pollutants (detergents) are introduced? In a sample of silty clay loam soil, the effects of both a detergent labeled as "green" and "plant-based" and another detergent containing less "green" surfactants on soil nutrient availability were measured 9 times over a 3-week time period. This study involved both a "green" and conventional detergent to be representative of different types of detergents and to test the environmental benefits of using "green" surfactants in terms of their effects on the biogeochemical cycles. We took measurements over time to understand how quickly the environmental effects of detergents begin to reveal themselves.

We hypothesized that both detergents would increase pH and decrease the availability of all essential nutrients, but the non-"green" detergent would cause a more extreme and longer-term decrease. The results show that the addition of household detergents decreases the availability of nitrogen and potassium and causes short-term fluctuation in pH in silty clay loam soil.

METHODS

In this controlled study, two containers of soil were each contaminated with a different brand of laundry detergent, and changes in nutrient availability and pH were observed over the following three weeks. This study was conducted under controlled conditions. A silty clay loam soil sample (10YR 4/1 on Munsell color chart) was collected from a level, grassy area away from human fertilizer input in Menlo Park, California using a soil sampling tube and divided into three 120g containers with lids. 0.6g of Persil ProClean Liquid Laundry Detergent (a "regular" detergent) was dissolved in about 10g of water, and 0.6g of Seventh Generation Natural Laundry Detergent (a "green" detergent, labeled as plant-based and eco-friendly) was dissolved in an equal amount of water in a separate container, and each diluted contaminant was mixed evenly throughout one of the soil samples to simulate a pollution site, at about 5000 ppm. The amount per load was 22ml for the green detergent and 46ml for the regular detergent, but equal concentrations were used for this experiment. As a control, an equal amount of pure water was mixed into one of the soil samples to keep soil moisture constant. The soil samples were stored together in a controlled environment with

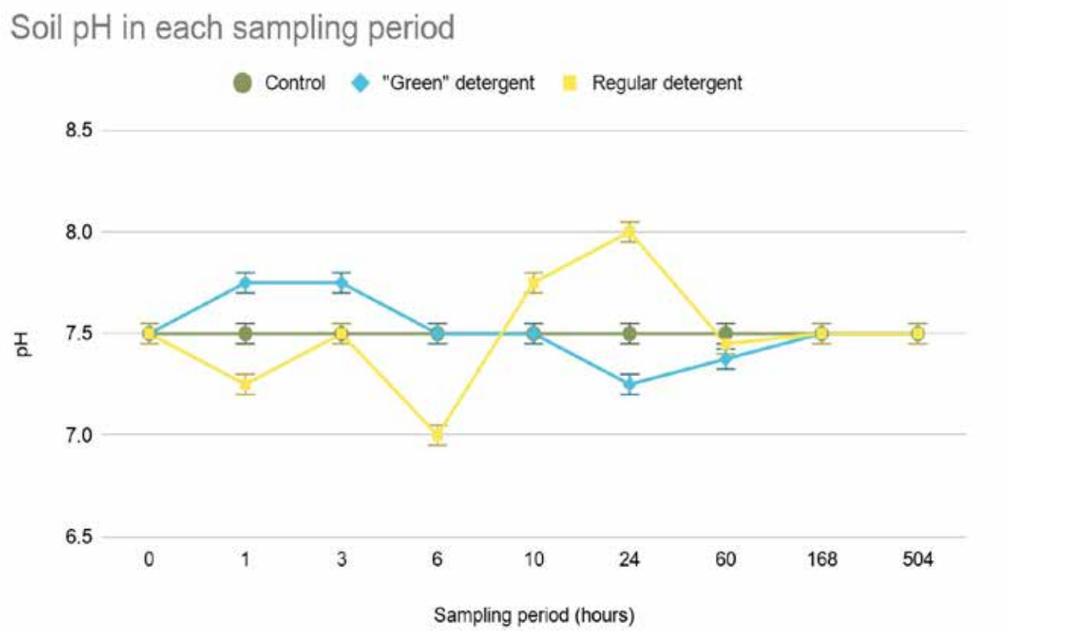

Figure 1: The pH of each contaminated group fluctuated within the first 60 hours before returning to its original level, while the pH of the control group remained constant for the entire study period.

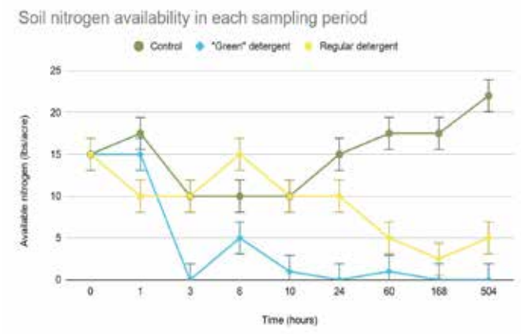

Figure 2: Nitrogen concentrations in the contaminated soils decreased over the course of the study period, with the "green" detergent causing a faster and more extreme decrease.

lids on to minimize outside factors influencing the results.

Using a LaMotte soil nutrient test kit, pH and phosphorus, nitrogen, and potassium availability in each sample were tested 0, 1, 3, 6, 10, 24, 60, 168, and 504 hours after the initial contamination to study both the short-term and longer-term trends in nutrient availability. Measurements were taken by using nutrient extracting solutions on small amounts of each sample, using nutrient indicators, and comparing the tests to a color chart. During testing, a random 20% of the tests were repeated to ensure precision and calculate estimated standard deviations in the results.

RESULTS

A soil sample was collected and divided into 3 containers as separate experimental groups; one remained uncontaminated, one was contaminated with a detergent labeled as a "green" detergent, and one was contaminated with a "regular" detergent to simulate pollution sites. Soil nutrient tests were used to measure pH, phosphorus, nitrogen, and potassium levels in each group over the study period. The study was limited by the number of available nutrient tests, about 40 tests. Estimated standard deviations were calculated from the 20% of measurements that were randomly repeated. The average standard deviations for pH, nitrogen, phosphorus, and potassium tests were 0.495, 1.919 lbs/acre, 4.041 lbs/acre, and 9.899 lbs/acre, respectively.

In Figures 1 to 4, the results of each nutrient test are displayed on a scatter plot with error bars showing the estimated standard deviation of the replicates in each dataset to represent the level of precision. Data points are equally spaced instead of being represented on a linear scale to clearly show each individual data point, since the sampling intervals changed over the course of the study period. The lines connecting the points represent an estimate of data between the sampling periods of and to help visualize trends over time.

The pH of each contaminated group fluctuated within the first 60 hours before returning to its original level, while the pH of the control group remained constant at pH 7.5 for the entire study period (Figure 1). The initial soil pH was 7.5 for all groups. The pH of the soil polluted with "green" detergent increased to 7.75 and decreased to 7.25 within the first day, and stabilized and returned to its original pH after 168 hours. The pH of the soil polluted with the regular detergent decreased as low as 7, increased up to 8, and also returned to the original pH of 7.5 after 168 hours.

Overall, nitrogen concentrations in the contaminated soils decreased over the course of the study period (Figure 2). Each soil sample initially contained 15 lbs/acre available nitrogen. Nitrogen availability in the control group decreased to as low as 10 lbs/acre in the first six hours but remained close to the original level for most of the experiment and increased up to 22.5 lbs/acre by the end of the study period. In the soil contaminated with "green" detergent, nitrogen availability began dropping between 1 and 3 hours, reaching 0 lbs/acre after 3 hours and

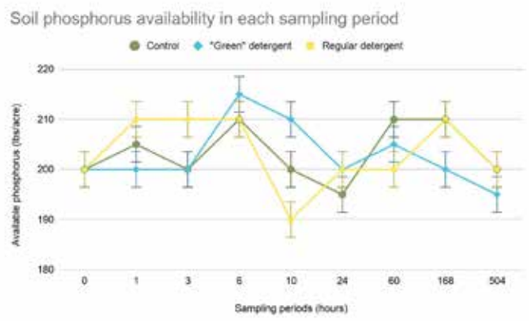

Figure 3: In all soil samples, the phosphorus availability fluctuated between 215 and 190 lbs/acre, with an average value of about 203 lbs/acre.

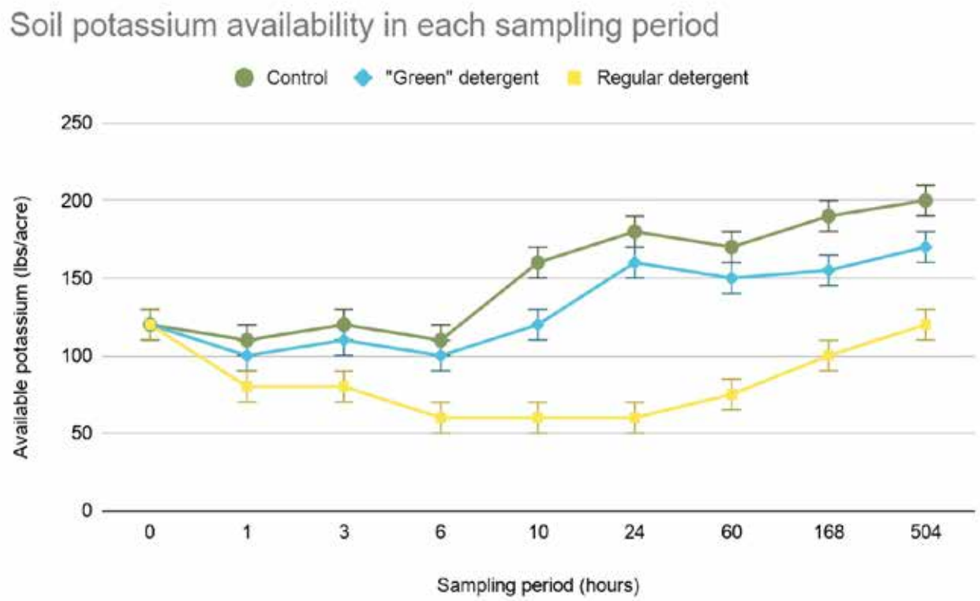

Figure 4: Potassium availability increased in the control after 6 hours. The soil polluted with green detergent showed a similar trend but with lower potassium levels. In the soil polluted with regular detergent, potassium availability decreased by 50% in the first 6 hours and started increasing after 24 hours.

staying under 5 lbs/acre for the remainder of the study period. Both sampling periods after 60 hours measured the nitrogen availability of this group to be 0. In the soil contaminated with regular detergent, nitrogen availability fluctuated between 15 lbs/acre and 10 lbs/acre in the first 24 hours before beginning to decrease; the lowest concentration of nitrogen measured was 2.5 lbs/acre 168 hours after contamination.

In all soil samples, the phosphorus availability was initially measured to be 200 lbs/acre and over the study period, fluctuated between 215 and 190 lbs/acre, with an average value of about 203 lbs/acre (Figure 3). Phosphorus availability in the control group increased as high as 210 lbs/acre and decreased as low as 195 lbs/acre. In the soil contaminated with "green" detergent, phosphorus levels reached as high as 215 lbs/acre and in the soil contaminated with regular detergent, phosphorus levels reached as high as 210 lbs/acre and as low as 190 lbs/acre. The control group and the regular detergent group returned to 200 lbs/acre by the end of the study period, while the measurements of the "green" detergent group ended at 195 lbs/acre, less than one standard deviation from the original level.

The addition of detergent pollutants, particularly the regular detergent, lowered potassium availability (Figure 4). Each soil sample initially contained 120 lbs/acre available potassium. Potassium availability in the control group stayed between 110 and 120 lbs/acre in the first 6 hours before increasing to 180 lbs/acre by 24 hours and then gradually increasing to 200 lbs/acre by the end of the study period at 504 hours. Potassium availability in the group contaminated with "green" detergent followed a similar trend but with lower amounts of potassium, first fluctuating between 120 and 100 lbs/acre in the first 24 hours, rapidly increasing to 160 lbs/acre in 24 hours, and gradually increasing to 170 lbs/acre by 504 hours. In the soil polluted with regular detergent, potassium availability immediately began decreasing, reached 60 lbs/acre after 6 hours, and remained at the same level for at least 18 hours. After 24 hours, the amount of potassium in this group began gradually increasing and reached 120 lbs/acre after 504 hours.

DISCUSSION

The findings of this study were that the addition of household detergents decreased the availability of nitrogen and potassium, caused short-term fluctuations in pH, and did not have a clear impact on phosphorus in silty clay loam soil. While we expected to see an increase in pH with the addition of detergents, detergent pollution in our experiment resulted in both short-term increases and decreases in soil pH and no long-term effects, with the regular detergent causing larger increases and decreases than the "green" detergent. Detergents are mixtures of compounds with varying pH levels along with pH buffers, so each compound in the mixture may have taken a different amount of time to interact with the soil and change its pH, explaining the observed fluctuations. (Information on the chemical compositions of each detergent was taken from the ingredients lists provided by the manufacturers.) Soil pH was important to observe in study since both high and low levels can lead to deficiencies of many nutrients and deterioration of soil health (8). Clay soils and soils with more organic matter content are more able to resist drops and rises in pH than sandy soils (8), so these rapid changes in pH may have had more severe effects if this experiment was performed using less resistant soil. Additionally, higher concentration of detergent could have overpowered the soil's ability to buffer.

As hypothesized, the addition of detergent pollutants decreased nitrogen and potassium availability. The observed trends in both nitrogen and phosphorus availability were likely to be caused by two main factors: chemical interactions between the detergents and soil and the effects of the detergents on microbes involved in nutrient cycling. Because chemical reactions take place relatively quickly, we believe they had a considerable effect on the shorter-term trends. Microbial degradation of surfactants takes several days or weeks, and the effect of surfactants on enzyme activity in previous studies have been measured over several weeks to months (3); thus, microbial activity was likely the main cause of the longer-term (168+ hours) trends in nutrient availability.

The addition of surfactants in soil may cause chemical reactions that may interfere with the transport of nutrients and reduce soil quality for microorganisms (1). Specifically, both detergents from this experiment contain sodium salts, which have been found to be detrimental to soil physicochemical properties (13). Soils affected by sodium deteriorate because of changes in the proportions of certain cations and anions, which may include potassium and nitrate ions, present in the soil solution and on the exchange sites (12). Because the nutrient tests used in this experiment measure the concentrations of nutrients in soluble, exchangeable form, sodium may have interfered with both potassium and nitrogen availability. In particular, due to their chemical similarities, Na^+ ions prevent the uptake of K^+ ions (13). Additionally, in previous studies, the use of surfactants has been recognized as a cause in decreased soil hydraulic conductivity as surfactants are adsorbed into clays and organic materials (1), which may have slowed down the overall flow of nutrients.

Nitrogen availability decreased following the addition of detergents, with the "green" detergent causing a faster and more extreme decrease, contrary to the hypothesis that the regular detergent would cause a larger decrease. A possible explanation for the trends after 168 hours is that higher carbon content in soil from the detergents biodegrading caused a nitrogen deficiency as nitrogen is used up when microorganisms decompose organic matter (2). Knowing that most surfactants have halflives of about 3 weeks or less (1), we believe that this could have affected microbial activity during the two last measurements of the study period. The regular detergent included more inorganic compounds and less anaerobically biodegradable surfactants, such as alkylbenzene sulfonate (6), while the green detergent was more readily biodegradable, using surfactants such as sodium lauryl sulfate, which mostly degrades in 50-140 hours (9), explaining the faster and larger-scale decrease in nitrogen availability.

In the last few sampling periods, potassium availability showed a relative increase but still stayed lower than the levels in the control group, with the group contaminated by the conventional detergent showing the lowest levels of potassium. Surfactants in silty clay loam soil have been previously found to inhibit the activity of enzymes involved in nutrient cycling (3). Also, surfactants serve as carbon sources for certain microbes and are toxic to others, interfering with the composition and structure of microbial communities (1). Since most potassium in soil is in unavailable forms, potassium availability depends on the activity of potassium-solubilizing bacteria (5), which may have been affected by the addition of surfactants.

Although it was hypothesized that adding detergent pollutants would decrease phosphorus availability, there were no clear differences between the control group and the contaminated groups; each group seemed to fluctuate around the same range, usually less than one standard deviation away from each other, suggesting that surfactants do not significantly increase or decrease the amount of phosphorus in soil. However, adding a higher concentration of detergent or using more precise nutrient tests could yield different results.

The results support the idea that even "eco-friendly" products have an effect on nutrient availability when introduced into soil. Overall, this study concludes that proper disposal of household products is crucial, as pollutants released into the environment can have an impact on nutrient cycling in ecosystems even in a short time period, showing the importance of pollution prevention.

Possible sources of error in this study are human error in using nutrient tests and potential outside factors affecting the soil, such as additional chemical pollutants existing in the soil, and the microbial community was not assessed. The equal concentrations of the detergents may have made this study less applicable to real pollution, since the amount per load for the regular detergent was about twice that of the green detergent. The sample size and scope of this experiment were relatively small due to limited resources, so further experimentation in this field would be helpful in confirming and expanding these results. Further research on surfactant pollution and nutrient availability in soil could focus on a broader range of household products and how they affect soil, the long-term effects of detergents in soil, comparing the severity of the effects of different concentrations of surfactants in soil, the impact of detergent pollution on soil-based ecosystems, and reexamining the effect of detergents on phosphorus availability.

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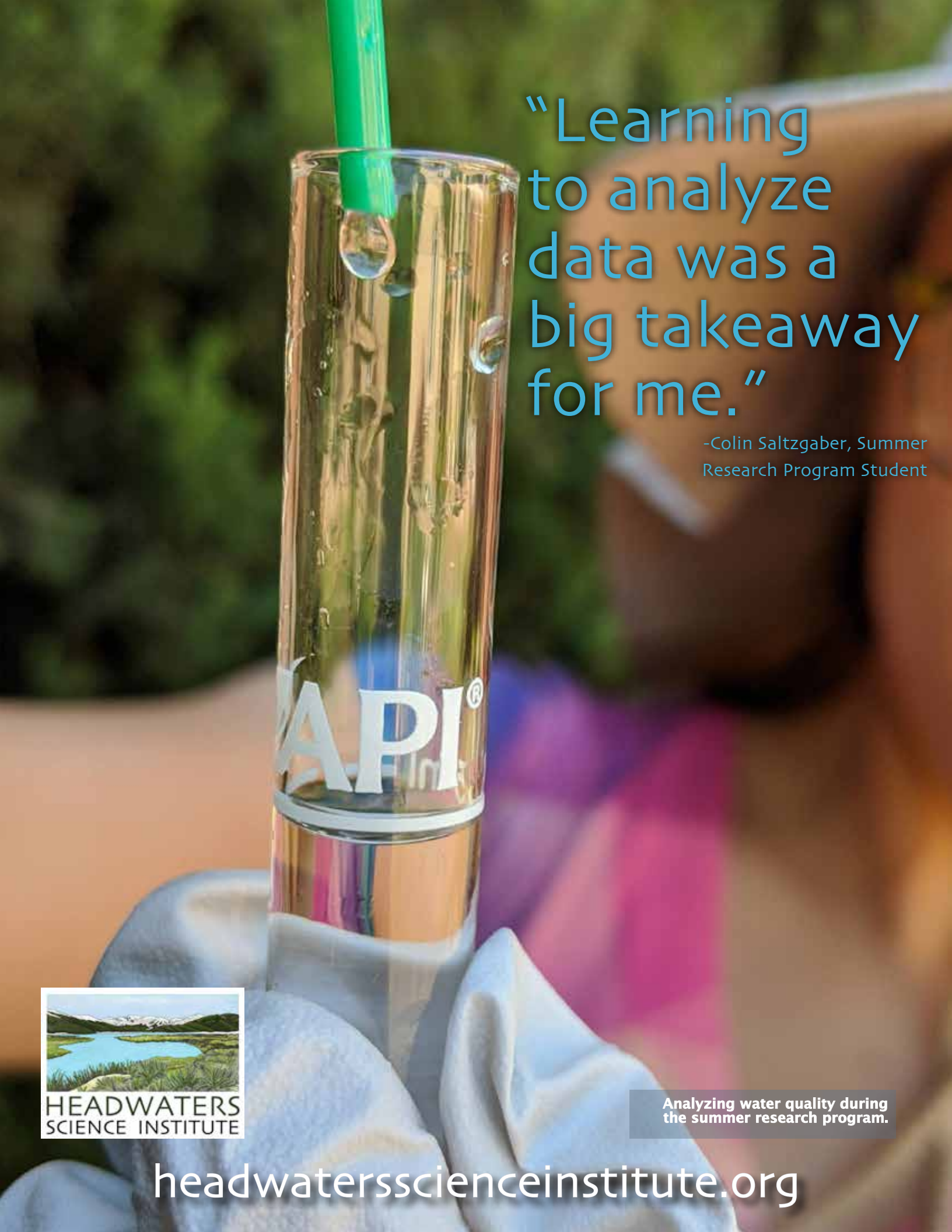

"Learning to analyze data was a big takeaway for me."

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