

Impact of Increased Soil Nitrogen Levels on *Pogonomyrmex occidentalis* Mortality

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Abstract

Ants serve critical ecological functions, acting as both prey and predator, and contributing to the decomposition of organic material. This study examines how varying soil nitrogen levels affect the mortality of Western Harvester ants (*Pogonomyrmex occidentalis*). It was hypothesized that elevated nitrogen concentrations in soil would significantly reduce ant survival rates. The experiment was conducted in a controlled laboratory environment at High Technology High School, where all variables such as temperature, food, and water were held constant across groups, with soil nitrogen levels being the sole variable. Ant mortality was recorded over a four-week period. Statistical analyses, including ANOVA and multiple t-tests, revealed significant differences in mortality between the control and nitrogen-treated groups. All experimental groups receiving nitrogen fertilizer exhibited 100% mortality by the end of the study. While the data were statistically significant, high mortality in the early weeks suggests that nitrogen concentrations may have been excessively high. Future research should focus on evaluating the effects of lower fertilizer concentrations to gain a more nuanced understanding of nitrogen's impact on subterranean insect populations. This research highlights the potential ecological risks posed by nitrogen-heavy fertilizers and underscores the need for more sustainable agricultural practices to mitigate environmental harm.

Keywords

Fertilizer, Ants, Pollution, Nitrogen

1. Introduction

Fertilizer is a common application used in farmland and even small gardens to help crops or plants grow, by supplying nutrients into the soil. There are many kinds of fertilizers. A common way to differentiate between fertilizers is to look at

their NPK values. NPK is a measure of the number of macronutrients in a specific fertilizer. The N stands for nitrogen, the P stands for phosphorus, and the K stands for potassium. For example, if a fertilizer has an NPK value of 2:20:30, there is 2% of nitrogen in the fertilizer along with 20% of phosphorus and 30% of potassium. The other 48% left is other nutrients that do not impact the soil as much, essentially filler content. However, some scientists have also noted inherent disadvantages to using fertilizers. One such study explains how fertilizer misuse can cause massive implications for air and water pollution. Specifically, in this study, the environmental consequences of the excessive use of Nr, a synthetic nitrogen fertilizer, were observed (Gu et al., 2015).

Another research group looked at crop production in China, particularly the influence on the environment and how efficient the process was. The results of the study showed that most non-point source pollution (NPS)—a type of water pollution—comes from nitrogen and phosphorus residue, both common elements in fertilizers (Liu et al., 2021). Also, nitrogen fertilizers can have long-term effects on the soil, such as soil erosion or changes in its properties (Daba et al., 2021). Lastly, one study also points out that increasing the amount of nitrogen in soil could be inversely proportional to the number of microbes in the soil. The study concluded that the increase in nitrogen decreased the variability of the microbes and changed the bacteria present in the soil (Zhou et al., 2017). “Microbes are tiny organisms in the soil, water, and air, and play a vital role in the food web” (National Library of Medicine, 2016). One should also consider how the decrease in microbes would affect animals in the ecosystem.

Inorganic fertilizers especially, have been seen to damage soil long-term rather than helping. In the study by Gupta & Hussain (2014), fertilizers are prone to leaching, a part of the nitrogen cycle in which nitrate goes with the water flow and potentially ends up somewhere else. Also, it was observed that chemical fertilizers can dry out the soil because of the salt commonly found inside them. These fertilizers allow for more soil erosion and create an imbalance between nitrogen and other necessary nutrients. This nitrogen accumulation can be deadly for everything near this soil (Hashimi & Hashimi, 2020).

Various methods have been used to limit the effect of fertilizer overuse on the environment. One group of researchers was straightforward, using technological guidance and subsidies to push the experimental group of 516 households in the Heyang County of Shaanxi Province in China (Wu & Ge, 2019). Other scientists proposed combining synthetic fertilizer with organic manure, which they put into a Denitrification Decomposition model (DNDC) (Lv et al., 2020). A DNDC model is a simulation model for N₂O, CO₂, and N₂ emissions from agricultural soils in the U.S. (Li et al., 1992). The study carried out by Lv et al. (2020) found that using the organic matter as a replacement only slightly affected the crop yield and significantly lowered the environmental impact. Both methods described above are useful in their respective manners; however, people still haven't taken significant steps to stop the damage the fertilizers are having on the environment.

On the other hand, one should consider the effects of fertilizer overuse on animals everywhere. Ants are important members of the environment that can be damaged because of this. In a study by [Lafleur, Hooper-Bùi, Mumma, & Geaghan \(2005\)](#), a species of red imported fire ant was used to discover if there was a connection between them and plant growth in pine forests. Because of how ants concentrate organic matter into their ant nests, the Louisiana State scientists thought that this would help plant growth. The results showed that ants efficiently disinfected their habitat and also increased the nitrogen content in the soil.

On the other hand, another study by [Green, Pettry, & Switzer \(1998\)](#) states that the same red imported fire ants enriched the soil and could positively impact an entire landscape in about a hundred years if needed. An analysis by [Farji-Brener & Werenkraut \(2017\)](#) showed the effects of ant nests on soil fertility. They concluded that the plants with ant nests nearby had higher nutrient and calorie content than the plants not near any ant nests. Moreover, plants showed greater biomass and fitness close to ant nest soils than vice versa.

All the previous studies examined the effects of ants on the environment, and most of the studies showed that ants played a significant part in soil fertility and plant growth. However, even with this realized importance, there is little to no research on the direct impact of the increased fertilizer amount on the animals specifically. This experiment measured how different amounts of nitrogen in soil affect the survival of *Pogonomyrmex occidentalis*. In doing so, this can shed light on the direct impacts of fertilizer use on organisms. This study provides crucial information for maintaining ecological balance amid ongoing environmental damage. Moreover, the study helps to educate others about the potential consequences of human activity. Lastly, this information is also crucial to biodiversity conservation, to keep these ants living and well.

Pogonomyrmex occidentalis was chosen for this experiment due to its known underground foraging behavior. It is also known for its ability and willingness to communicate with other ants ([Cole, 1968](#)). This species of ant has also been seen to affect the nitrogen and phosphorus levels in the soil ([Green et al., 1998](#)).

In essence, this study examines whether there is a connection between increasing nitrogen levels in the soil and the survival of *Pogonomyrmex occidentalis*. The only variable that differed among the experimental groups was the amount of nitrogenous fertilizer. It was noted how the independent variable would affect the survival of *Pogonomyrmex occidentalis*. The decrease in the population of *Pogonomyrmex occidentalis* can be attributed to nitrogen poisoning because of the added fertilizer. Based on the effects of fertilizer overuse that were described beforehand, the increase in nitrogen concentration in the soil would have a significant negative impact on the survival of the *Pogonomyrmex occidentalis*.

2. Materials and Methods

2.1. Experimental Setup

Pogonomyrmex occidentalis ants were purchased in bulk from Insect Sales.org.

Twelve plastic jars, each with a volume of 32 fluid ounces, were used in the experiment. The jars measured approximately 6.5 inches in height, with a bore radius of 1.75 inches. Each jar lid was modified with three holes to allow airflow, created using a Warrior 18V Cordless 3/4-inch drill, as shown in **Figure 1**.

The experiment included six groups: a control group and five groups treated with different concentrations of nitrogen fertilizer (1 mL, 2 mL, 5 mL, 10 mL, and 20 mL). Each group had two replicates to ensure accuracy. The experiment focused on how different nitrogen levels affected the survival of *Pogonomyrmex occidentalis*. All jars were stored in the Research Wing of High Technology High School, under controlled temperature (22°C) and lighting conditions, to maintain consistency.

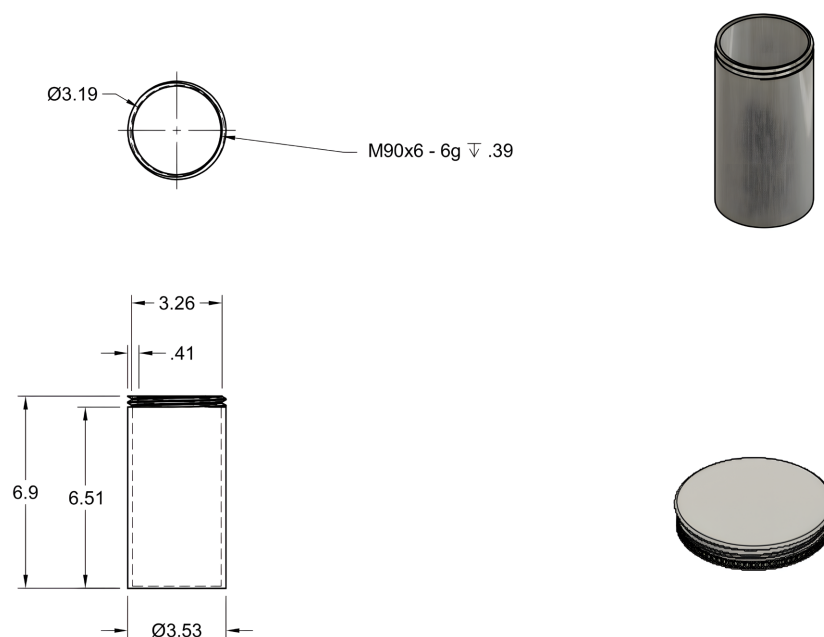


Figure 1. Different views of the 3.5" × 6.9" Jar.

2.2. Ant Habitat Preparation

Each jar, measuring 3.5" × 6.9", was filled with 16 ounces of organic topsoil from Gardenpro, using an Edward Tools Garden Trowel. The soil was kept uniform across all jars, with the only difference being the nitrogen content. Since the topsoil is organic, it contains minimal additional nutrients, making it a constant factor in the experiment.

Nitrogen was added using PetraTools Liquid Nitrogen Fertilizer. A mixture of 20 mL of water and varying amounts of nitrogen fertilizer was prepared for each experimental group, as shown in **Figure 2**. For the first experimental group, 1 mL of Petra-Tools Liquid Nitrogen Fertilizer was mixed with 20 mL of water. The amounts were carefully measured using a 10 mL glass serological pipette from PYREX, ensuring accurate proportions. This mixture was poured into a jar and thoroughly combined with the topsoil.

For the second experimental group, 2 mL of PetraTools Liquid Nitrogen Fer-

tilizer was mixed with 20 mL of water, then poured into a separate jar and blended with the topsoil. For the third experimental group, 5 mL of nitrogen fertilizer was added to 20 mL of water and mixed into another jar with topsoil. For the fourth experimental group, 10 mL of nitrogen fertilizer was combined with 20 mL of water, then mixed into a separate jar with topsoil. For the fifth experimental group, 20 mL of nitrogen fertilizer was mixed with 20 mL of water and added to another jar, which was then thoroughly mixed with the topsoil. These five experimental groups, along with the control group, were replicated to ensure accurate results. All jars were labeled accordingly for easy identification, as shown in **Figure 3**.

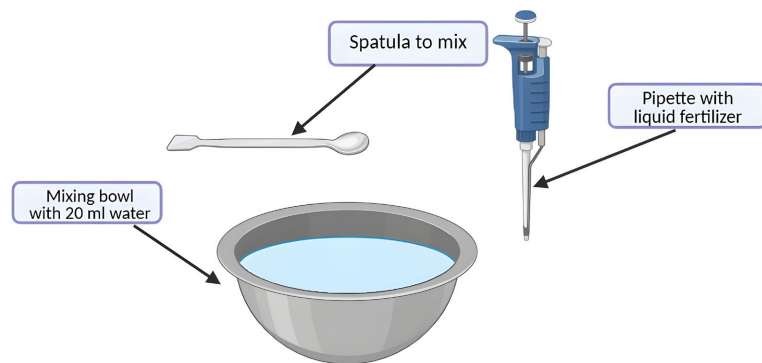


Figure 2. Liquid nitrogen fertilizer was measured with pipette, mixed with 20 mL water.

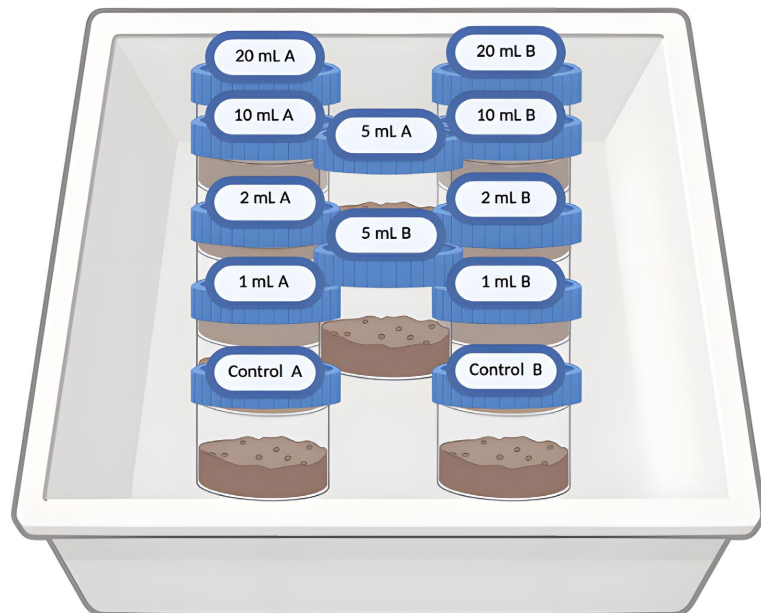


Figure 3. Experimental setup inside Research Lab at HTHS.

2.3. Experimental Procedure

The setup consists of 12 jars, with two control and ten experimental groups. Each experimental group has a mirror of itself, to limit outliers in the dataset. To summarize the ant Habitat preparation: there are two jars with no added nitrogen

content, two jars with 1 mL of added nitrogen content, two jars with 2 mL of added nitrogen content, two jars with 5 mL of added nitrogen content, two jars with 10 mL of added nitrogen content, and another two jars with 20 mL of added nitrogen content. Each of these jars has seven of the *Pogonomyrmex occidentalis* placed inside. The ants will be observed throughout four weeks, with the summation of ants in each jar at the end of every week. 5 mg of food will be provided two times a week through cracked corn, wheat, oats, barley, and milo. An equal amount of the food source will be provided to all jars. Next, to stimulate rain, moisten the soil by watering all of the jars with 5 mL every other day, except between the gap of Friday and Monday, which would be a two-day break. The water will be provided through the serological pipette and a Bel-Art Fast-Release Pipette Pump. The top layer of the soil should usually remain dry but everything under this layer should be kept moist.

3. Results

3.1. Working Data

This study focused on the effects of increased nitrogen levels in soil on *Pogonomyrmex occidentalis* over four weeks. The data below (Tables 1-8) only refers to the number of ants that decreased over time. There are Group A and B which are replicas of each other, they have the same experimental groups. This was done to ensure that the results were accurate.

Table 1. Raw data table for *Pogonomyrmex occidentalis* alive after being exposed to control or various amounts of nitrogenous fertilizer over four weeks.

Groups	Weeks Passed				
	Week 0	Week 1	Week 2	Week 3	Week 4
Control A	7.0	2.0	2.0	2.0	1.0
1 mL A	7.0	4.0	2.0	0.0	0.0
2 mL A	7.0	3.0	2.0	0.0	0.0
5 mL A	7.0	0.0	0.0	0.0	0.0
10 mL A	7.0	5.0	2.0	0.0	0.0
20 mL A	7.0	0.0	0.0	0.0	0.0
Control B	7.0	3.0	3.0	2.0	1.0
1 mL B	7.0	2.0	0.0	0.0	0.0
2 mL B	7.0	2.0	2.0	0.0	0.0
5 mL B	7.0	2.0	1.0	0.0	0.0
10 mL B	7.0	4.0	2.0	0.0	0.0
20 mL B	7.0	3.0	0.0	0.0	0.0
Average	7.0	2.5	1.3	0.3	0.1

a. All values are rounded to two significant figures. This table shows the number of ants that were still alive after a certain amount of time had passed. The last row shows the average amount of *Pogonomyrmex occidentalis* left across all experimental groups and controls each week.

Table 2. Calculated data table for *Pogonomyrmex occidentalis* decrease over time (% of day 1) due to control or various amounts of nitrogenous fertilizer.

Groups	Weeks Passed				
	Week 0	Week 1	Week 2	Week 3	Week 4
Control A	0.000	71.43	71.43	71.43	87.71
1 mL A	0.000	42.86	71.43	100.0	100.0
2 mL A	0.000	57.14	71.43	100.0	100.0
5 mL A	0.000	100.0	100.0	100.0	100.0
10 mL A	0.000	28.57	71.43	100.0	100.0
20 mL A	0.000	100.0	100.0	100.0	100.0
Control B	0.000	57.14	57.14	71.43	87.71
1 mL B	0.000	71.43	100.0	100.0	100.0
2 mL B	0.000	71.43	71.43	100.0	100.0
5 mL B	0.000	71.43	87.71	100.0	100.0
10 mL B	0.000	42.86	71.43	100.0	100.0
20 mL B	0.000	57.14	100.0	100.0	100.0
Average	0.000	64.29	81.12	95.24	98.00

a. The values seen in **Table 2** were taken from **Table 1** and applied the formula for percent increase/decrease. $((x_f - x_i)/x_i) * 100 = \text{Percent Change}$. All numbers are rounded to four significant figures. This shows the total decrease in the population of *Pogonomyrmex occidentalis* in each jar over the four weeks.

Table 3. Calculated data table for *Pogonomyrmex occidentalis* decrease over time (% of day 1) due to control or various amounts of nitrogenous fertilizer (mean of A and B).

Groups	Weeks Passed				
	Week 0	Week 1	Week 2	Week 3	Week 4
Control A	0.000	64.29	64.29	71.43	87.71
1 mL A	0.000	57.15	85.72	100.0	100.0
2 mL A	0.000	64.29	71.43	100.0	100.0
5 mL A	0.000	85.72	93.86	100.0	100.0
10 mL A	0.000	35.72	71.43	100.0	100.0
20 mL A	0.000	78.57	100.0	100.0	100.0

a. The formula for the calculated data table above is from averaging groups A and B. $(a + b/2)$ The data for the table was obtained from **Table 2**.

Table 4. Summative data table for *Pogonomyrmex occidentalis* decrease over time (% of day 1) by varying amounts of nitrogen (Group A).

Groups	Groups (A)					
	Control A	1 mL A	2 mL A	5 mL A	10 mL A	20 mL A
Mean	75.50	78.57	82.14	100.0	75.00	100.0
SD	8.140	27.35	21.43	0.000	33.76	0.000
Var	66.26	748.2	459.2	0.000	1140	0.000
n	4.000	4.000	4.000	4.000	4.000	4.000

a. All numbers are rounded to four significant figures. This is the summary of the decrease in the population of *Pogonomyrmex occidentalis* over time (% of Day 1) by Varying Amounts of Nitrogen for Group A.

Table 5. Summative data table for *Pogonomyrmex occidentalis* decrease over time (% of day 1) by varying amounts of nitrogen (Group B).

Groups	Groups (B)					
	Control A	1 mL A	2 mL A	5 mL A	10 mL A	20 mL A
Mean	75.50	78.57	82.14	100.0	75.00	84.67
SD	8.140	27.35	21.43	0.000	33.76	15.5
Var	66.26	748.2	459.2	0.000	1140	239
n	4.000	4.000	4.000	4.000	4.000	4.000

a. All numbers are rounded to three significant figures. This is the summary of the decrease in the population of *Pogonomyrmex occidentalis* over time (% of Day 1) by Varying Amounts of Nitrogen for Group B.

Table 6. Statistical table describing results of ANOVA test for *Pogonomyrmex occidentalis* decrease, week three.

P-Value	Alpha
0.002872821965	0.05

a. An ANOVA test was conducted to compare the twelve data sets, to week Three. The p-value for week Three is 0.002872821965.

Table 7. T-test results for *Pogonomyrmex occidentalis* decrease, week three (Group A).

T-test Comparison	P-Value
Control – 1 mL	0.008153917065
Control – 2 mL	0.005185066967
Control – 5 mL	0.009187730962
Control – 10 mL	0.009759577837
Control – 20 mL	0.009187730962

a. A two-tailed T-test was used for this Statistical Data Table. Two-tailed T-test can show if the control and the experimental group are different.

Table 8. T-test results for *Pogonomyrmex occidentalis* decrease, week three (Group B).

T-test Comparison	P-Value
Control – 1 mL	0.004108037084
Control – 2 mL	0.001920721572
Control – 5 mL	0.002017648658
Control – 10 mL	0.003357581280
Control – 20 mL	0.001114389600

a. A two-tailed T-test was used for this Statistical Data Table. Two-tailed T-test can show if the control and the experimental group are different.

3.2. Presentation of Results

The graphs and images (**Figures 4-5**) below show the data in more comprehensive matter, showing the negative correlation between increasing nitrogen fertilizer and ant population over time. They also illustrate the other unexpected variables in the experiment, suggesting that the correlation is not objectively clear.

Graph of *Pogonomyrmex occidentalis* Decrease Over Time (% of Day 1) Due to Control or Various Amounts of Nitrogenous Fertilizer

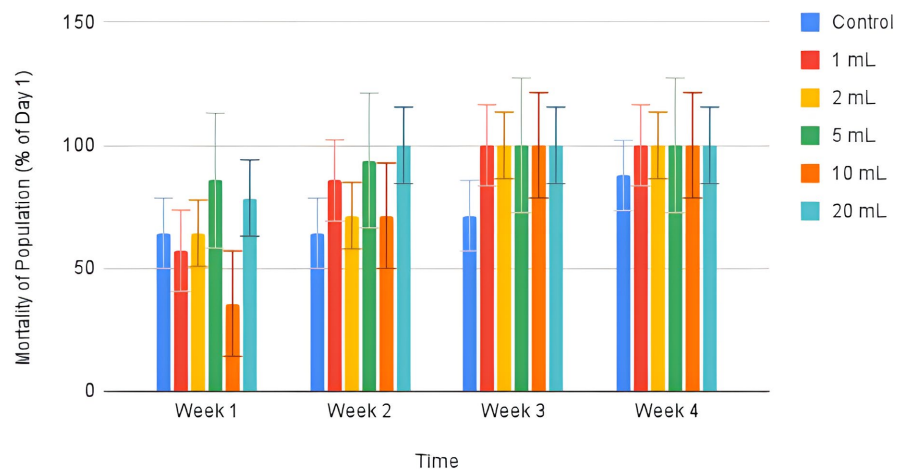


Figure 4. Graph showing the average of the experimental groups and the controls with one standard deviation. Mean values were taken from **Table 3** and the standard deviation values were taken from **Table 5**.



Figure 5. Control A (shown above) and others were found to have plants growing inside of them.

4. Summary of Results and Conclusions

4.1. Conclusions

This study specifically tested whether increasing nitrogen levels in the soil had a significant negative effect on the survival of *Pogonomyrmex occidentalis*. The results showed a negative relationship between increasing nitrogen levels and the survival of the ants. Within one week of being introduced into the experimental conditions, most ants in the higher nitrogen concentration groups (such as 20 mL A, 10 mL A and B, and 5 mL) exhibited immediate mortality as shown in **Table 1**. While it was expected that the nitrogen fertilizer would harm *Pogonomyrmex oc-*

cidental, the rapid and extensive mortality was unexpected.

As shown in **Table 4**, **Table 7**, and **Table 8**, a significant impact can be observed on the ant populations due to nitrogen levels. However, while the differences were statistically significant, the high mortality rates in the experimental groups made it difficult to assess the precise relationship between nitrogen levels and ant survival. As shown in **Table 6**, there was insufficient variability among the twelve experimental groups by Week 3, likely due to the high levels of mortality.

Table 2 and **Table 3** show the percentage of the ant population that died throughout the study. By the end of Week 4, all ants in the experimental groups were dead, with only a few remaining in the control groups. **Figure 4** illustrates the increasing mortality over the four weeks, with most experimental groups reaching 100% mortality by the end. These results suggest a strong negative effect of nitrogen levels on the survival of *Pogonomyrmex occidentalis*, though the high mortality rates likely resulted from the fertilizer doses being too high.

Several inconsistencies were noted during the study. The most significant unforeseen variable was the growth of plants in the jars (see **Figure 5**). Even by Week 1, plants began to grow in both the control and experimental jars. It was later determined that the food provided by InsectSales.org, which included seeds and honey, was likely the source of the plant growth. It is important to note that there were no light sources, but the seeds appeared resilient, germinating despite the lack of light. Interestingly, most plants grew in the control groups A and B.

Another inconsistency was the experimental habitat itself, which was somewhat unrealistic. The ants were confined to small jars with limited space to burrow—only about 3 inches deep—and a barren environment. These factors may have contributed to the high mortality. Given the large number of deaths, it is likely that the fertilizer doses were too high for the ants to survive.

4.2. Future Studies and Research Impact

Future research could entail the effects of supplementing organic matter with artificial fertilizer on the survival of *Pogonomyrmex occidentalis*. This could help determine if organic fertilizer is a better environmental option than synthetic fertilizers. In the future, lower concentrations of fertilizer should be utilized to assess more subtle effects on ant survival. This would help identify more precise effects of nitrogen exposure to ants or other organisms.

Other studies that could stem from this research include using a different ant species such as the red imported fire ant (*Solenopsis invicta*). This experiment would examine how increasing fertilizer levels affect the survival of *Solenopsis invicta* and thus would get closer to finding out if the negative effects of fertilizer are universal to all species of ants.

Additionally, another study could be the effects of phosphorus fertilizer on the survival of *Pogonomyrmex occidentali*, branching the type of fertilizer beyond just nitrogen-based fertilizer. Since nitrogen is relatively inert, with phosphorus reactivity, the study could be even more significant than the effects observed in this experiment.

The potential impacts of this study could be from many industries such as farming, agriculture, and even animal conversation. With the correct principles of what fertilizer to use, farmers could now use healthier alternatives and possibly reduce the damage artificial fertilizers have done to the environment. With agriculture, farming practices improved and more organisms, the industry could benefit from more plant growth and produce. The importance of ants could raise important questions about how human practices are affecting organisms and what can be done to solve this.

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Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

References

- Cole, A. C. (1968). *Pogonomyrmex Harvester Ants*. University of Tennessee Press.
- Daba, N. A., Li, D., Huang, J., Han, T., Zhang, L., Ali, S. et al. (2021). Long-Term Fertilization and Lime-Induced Soil Ph Changes Affect Nitrogen Use Efficiency and Grain Yields in Acidic Soil under Wheat-Maize Rotation. *Agronomy*, *11*, Article 2069. <https://doi.org/10.3390/agronomy11102069>
- Farji-Brener, A. G., & Werenkraut, V. (2017). The Effects of Ant Nests on Soil Fertility and Plant Performance: A Meta-Analysis. *Journal of Animal Ecology*, *86*, 866-877. <https://doi.org/10.1111/1365-2656.12672>
- Green, W. P., Pettry, D. E., & Switzer, R. E. (1998). Impact of Imported Fire Ants on the Texture and Fertility of Mississippi Soils. *Communications in Soil Science and Plant Analysis*, *29*, 447-457. <https://doi.org/10.1080/00103629809369957>
- Gu, B., Ju, X., Chang, J., Ge, Y., & Vitousek, P. M. (2015). Integrated Reactive Nitrogen Budgets and Future Trends in China. *Proceedings of the National Academy of Sciences*, *112*, 8792-8797. <https://doi.org/10.1073/pnas.1510211112>
- Gupta, A., & Hussain, N. (2014). Industrial Pollution Control. *EM International*, *30*, 191-194.
- Hashimi, R., & Hashimi, M. H. (2020). Effect of Losing Nitrogen Fertilizers on Living Organism and Ecosystem, and Prevention Approaches of Their Harmful Effect. *Asian Soil Research Journal*, *4*, 10-20. <https://doi.org/10.9734/asrj/2020/v4i230088>
- Lafleur, B., Hooper-Bùi, L. M., Paul Mumma, E., & Geaghan, J. P. (2005). Soil Fertility and Plant Growth in Soils from Pine Forests and Plantations: Effect of Invasive Red Imported

- Fire Ants *Solenopsis Invicta* (Buren). *Pedobiologia*, 49, 415-423.
<https://doi.org/10.1016/j.pedobi.2005.05.002>
- Li, C., Frohling, S., & Frohling, T. A. (1992). A Model of Nitrous Oxide Evolution from Soil Driven by Rainfall Events: 1. Model Structure and Sensitivity. *Journal of Geophysical Research: Atmospheres*, 97, 9759-9776. <https://doi.org/10.1029/92jd00509>
- Liu, Z., Wang, S., Xue, B., Li, R., Geng, Y., Yang, T. et al. (2021). Emergy-Based Indicators of the Environmental Impacts and Driving Forces of Non-Point Source Pollution from Crop Production in China. *Ecological Indicators*, 121, Article 107023.
<https://doi.org/10.1016/j.ecolind.2020.107023>
- Lv, F., Song, J., Giltrap, D., Feng, Y., Yang, X., & Zhang, S. (2020). Crop Yield and N₂O Emission Affected by Long-Term Organic Manure Substitution Fertilizer under Winter Wheat-Summer Maize Cropping System. *Science of The Total Environment*, 732, Article 139321. <https://doi.org/10.1016/j.scitotenv.2020.139321>
- National Library of Medicine (2016). *What Are Microbes?* National Center for Biotechnology Information.
- Wu, H., & Ge, Y. (2019). Excessive Application of Fertilizer, Agricultural Non-Point Source Pollution, and Farmers' Policy Choice. *Sustainability*, 11, Article 1165.
<https://doi.org/10.3390/su11041165>
- Zhou, J., Jiang, X., Wei, D., Zhao, B., Ma, M., Chen, S. et al. (2017). Consistent Effects of Nitrogen Fertilization on Soil Bacterial Communities in Black Soils for Two Crop Seasons in China. *Scientific Reports*, 7, Article No. 3267.
<https://doi.org/10.1038/s41598-017-03539-6>