Analyzing Ants as Potential Bioindicators of Soil Quality at Friends Academy and a Local Farm

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Abstract:

Ants live on almost every continent in the world in great numbers, with the exception of Antarctica (Muller, 2024). The number of ants on Earth has been found to be around 20 quadrillion; clearly, ants must therefore play an impactful role in ecosystems (Schultheiss, 2022). Ants are known to contribute to soil health through aeration, a process that allows for the circulation of water and distribution of nutrients throughout soil. With this knowledge and interest in biodiversity, we wanted to try and find a relationship between biodiversity and soil health. Our team researched ants at a local farm and Friends Academy and hypothesized that there would be a larger composition of diverse ant species in areas of soil with higher nitrate levels compared to areas with lower nitrate levels. Our main objective was to compare the diversity of ants between the two locations while also comparing the amount of nitrate inside of the soil to determine a correlation between diversity and soil quality. The ant populations were collected using pitfall traps that contained a glycol and ethanol solution, killing and preserving the specimens. Ultimately, our findings, while limited by sample size and incomplete soil data from the farm, indicated slightly higher ant diversity at Friends Academy (SDI = 0.833) compared to the farm (SDI = 0.806), which disproved our initial hypothesis that higher nitrate levels would correlate with greater ant diversity.

Introduction:

In agriculture, nitrate is fundamental to life as it provides the ability to synthesize nucleic acids, the building blocks of DNA. Nitrate is replenished through the Earth through the nitrogen cycle. Nitrogen in the form of nitrate is a natural compound in soil, and an adequate supply of nitrate is required for optimal plant growth. Through the process of nitrification in the nitrogen cycle, nitrate is made from ammonia or ammonium by nitrifying bacteria. This cycle is important since nitrogen, specifically nitrate, helps with stem elongation, seed germination, flowering, and overall growth (Camut, 2021). Because the farm successfully grows, harvests, and sells crops throughout the fall, spring, and summer seasons, it's reasonable to assume that the practices that the farm uses, such as conservation tillage, keep nitrate levels in the soil at an adequate level (Youngs Farm, 2024). We can attribute the presence of these high nitrate levels to large ant populations. Ants play a significant role in maintaining and enriching the soil quality; "plant parts and other dead and decayed materials carried by ants for their food contribute to the topsoil, thus enriching soil organic carbon and other organic matter content… ants also enrich the nitrogen content of soil" (Raman, 2021). Ants also have bacteria in their gut which are transferred to soil during nest construction, which makes the topsoil and subsoil rich in nitrate. According to a study done by Scott W. Behie and Michael J. Bidochka, "many species of plants have evolved adaptations by which to exploit nitrogen present in insects," meaning ants directly support the growth of plant life (Behie, 2014). We hypothesized that, because of the positive correlation between ant population and nitrate levels, there would be more diversity in ant species at the farm, which had higher nitrate levels than our collection site at Friends Academy.

Materials/Methods:

In order to collect specimens for analysis, we planted ant traps at two locations within both our school and at a local farm. The trap itself was a 16-ounce plastic cup buried into the soil until its opening was at surface level. A smaller cup with a glycol and ethanol solution was put on the bottom of the cup to catch and preserve ants, with a funnel to make the ants slip into the trap (Figure 1). We left the traps out for around 5 days and then collected the ethanol cups from all locations. At the farm, we placed traps in tomato patches, in overturned soil, and in areas of soil with visible plant decomposition. We put five pitfall traps on a small forest trail on our school grounds and five more near the turf field. Another 10 cups were placed at a local farm near the tomatoes and the overturned soil with the same pitfall setup. After we collected our samples, we tried to pick out ants that didn't look the same using our knowledge of ant anatomy and species. Out of the collected 31 total specimens, we selected 21 ant specimens to barcode, and 18 specimens resulted in sequences that could be used for identification in DNA Subway. We specifically collected 5 specimens from the turf field, 5 from forest school, 6 from the area near the tomato plants, and 4 from the turned-over soil. The species diversity in all samples was compared through an analysis of the ants' genes to differentiate the species. We did this by cutting off a 0.5–0.7 mm piece of the ant and extracting DNA from the fragment using PCR to amplify part of a gene called COI and checking the amplification with gel electrophoresis. We sent the amplified DNA out for sequencing and then put the results into DNA Subway to create a DNA barcode. In DNA Subway, we trimmed the sequences, ran BLAST searches to find genetic matches, and finalized the barcodes based on the COI gene region to lead to our conclusion. To find a quantitative measure of diversity, we used the Simpson's Diversity Index. Additionally, we took soil samples from our school and used a YG-09 nitrogen electric tester to see how much nitrate was present in the samples.

Results:

At the turf field, we identified three unique species of ants: *Prenolepis imparis*, *Aphaenogaster rudis*, and *Formica subaenescens*. The five specimens from the forest school were of two different species, *Pachycondyla chinensis* and *Nylanderia parvula*. The Simpson's Diversity Index (SDI) for the ant species at Friends Academy (the turf and the forest school) was 0.833. Meanwhile, at the farm, we collected six specimens from the tomato field area, which were of four different species: *Lasius niger*, *Lasius neoniger*, *Nylanderia parvula*, and *Tetramorium caespitum*. We collected four specimens from the turned over soil area, which were of two different species, *Tetramorium caespitum* and *Nylanderia parvula*. The SDI for the ant species at the farm (the tomato field and the turned over soil field) was 0.806.

Discussion:

Our locations were initially chosen with the intent of collecting samples from multiple locations at both Friends Academy and the local farm. A problem we encountered was that an ant colony would live in the same general area, meaning that the ants that would fall into the trap would likely be ants from the same colony and hence, would be the same species (Evesham, 2021). This would cause the sample size to not accurately represent biodiversity as we would be collecting the same species in one site. As a result, we decided to take samples from two separate sub-locations within the two different locations. Once we collected the samples from the four sites, we combined them into two big data sets which had Friends Academy as one location and the farm as the other location. The reason we did this was so that we could make our research compare the farm against our school rather than comparing 4 separate locations that had very few species to analyze. While our sample size was relatively small, making it difficult to make broad generalizations, there are a few things worth noting.

Our data shows more diversity at Friends Academy. However, the nitrate readings from the turf field and forest school read 1 mg/kg and 5 mg/kg, respectively. We were unable to collect soil samples from the farm due to weather conditions not improving in time and the ground becoming too hard from the cold. Therefore, we cannot definitively infer the nitrate levels at the local farm, and further sampling would be required to assess this important data point. It is likely that a farm with decomposing organic matter, such as tomatoes, could have higher nitrate levels compared to school grounds with sparse woody debris. This is due to the higher nitrate content and faster decomposition rate of fresh plant material, which accelerates the release of nitrate into the soil through processes like nitrification. Ultimately our data indicates that Friends Academy, which notably included a site with lower nitrogen readings, the turf field, nevertheless showed more ant diversity compared to the farm. This finding directly conflicted with our hypothesis. Considering the difference in biodiversity was small when comparing the two locations' SDIs it is too small of a margin to make a definite conclusion. While our hypothesis was not supported by the available data, the extremely limited sample size and, more critically, the inability to collect soil samples from the farm severely compromised the generalizability and conclusive nature of these findings.

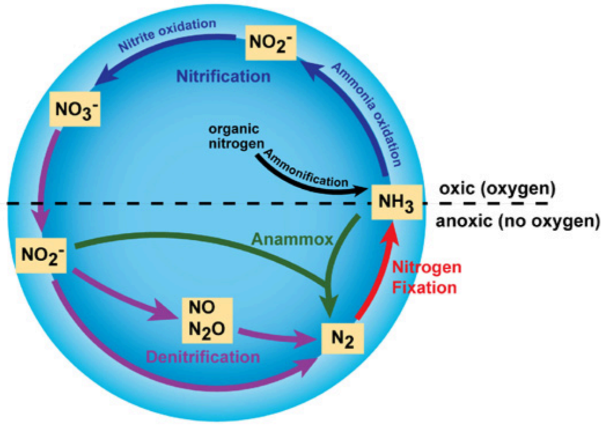

Figure 1 - The nitrogen cycle

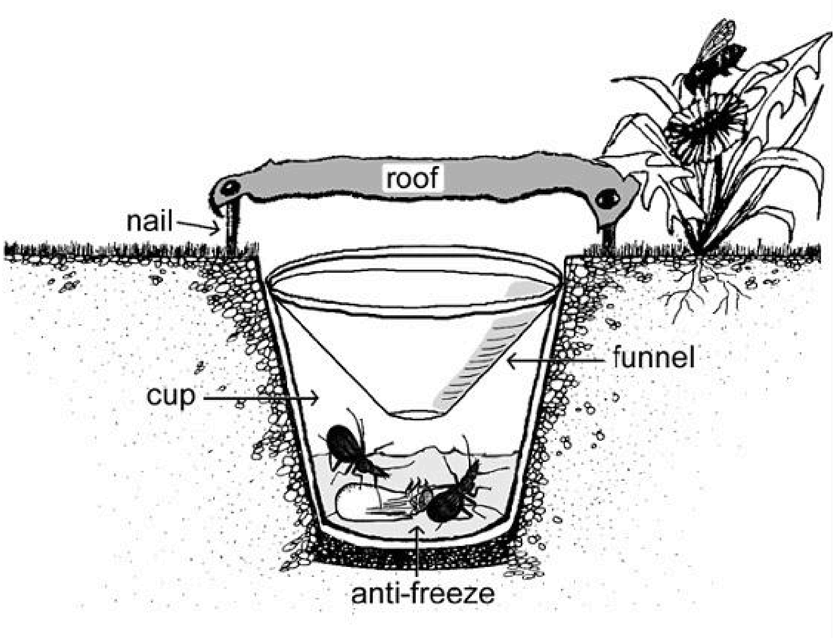

Figure 2 - Diagram of the traps used for specimen collection

Source: ResearchGate

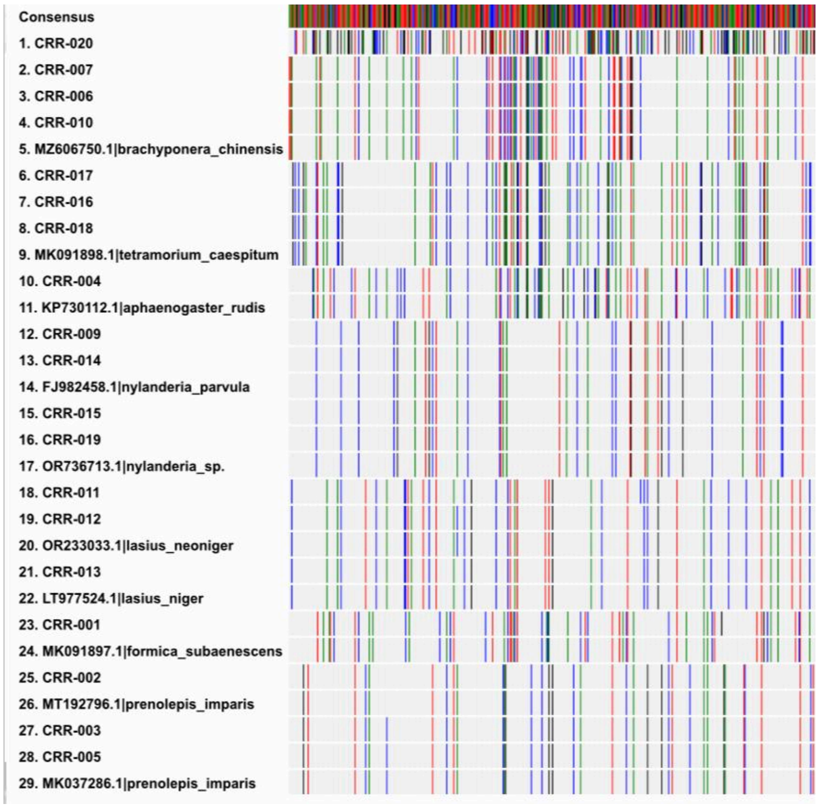

Figure 3 - Image of MUSCLE data; 19/20 ant specimens taken from DNA Subway compared with known specimens to find the species our specimens are.

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