The Effects of Invasive Trees on Protozoa Diversity and Soil Nutrients

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Abstract

Biodiversity and macronutrients play an important role in the fertility of soil ecosystems. Protozoa mineralizes nutrients for plants to absorb and feed on bacteria which break down nitrogen in soil; roughly 95% of the nitrogen in soil is organic material (Walworth, 2013) (Ingham). This is an important part of nutrient cycling in which nitrification transforms ammonium into nitrite and oxidizes nitrite into nitrate. Nitrate makes up a major portion of inorganic nitrogen which is the principal form for plant growth (Walworth, 2013) . Invasive plant species can change soil chemistry and decrease biodiversity. In this study, it was hypothesized that the soil surrounding an invasive tree would have less protozoa diversity and lower levels of nitrate and nitrite. To test this hypothesis, an experiment was conducted in which soil samples were extracted from two 5 m by 5 m sites: one around an invasive tree and one around a native tree. The experimental extractions were taken at random locations between 15 cm and 100 cm away from the tree, and the control extractions were taken at random locations between 2 meters and 3 meters away from the tree, which was calculated by measuring the canopy of the tree, corresponding to the extent of the root systems. This hypothesis was partially supported by the results of this experiment because in our protozoa data the invasive experimental soil had a smaller amount of protozoa diversity compared to the invasive control soil. However, our native experimental soil had a greater amount of protozoa diversity compared to the native control soil, proving our hypothesis. All of the soil that we tested had the same level of nitrite, proving this part of our hypothesis incorrect. Our nitrate data showed that there was a significant difference in nitrite levels between the soil from native experimental and invasive experimental. Our t-tests showed no significant difference in the nitrate levels between both the invasive experimental and invasive control soil and native experimental and native control soil.

Introduction

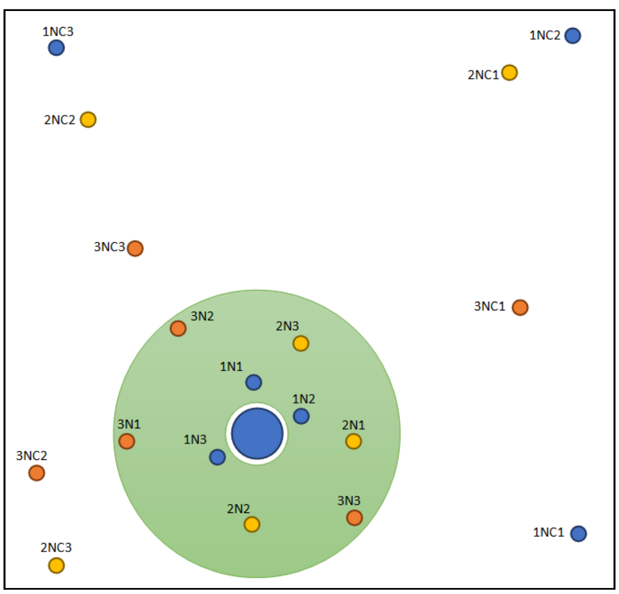
Biodiversity is fundamental for an ecosystem to stay healthy and productive. A more diverse ecosystem fosters high soil quality and healthy levels of nutrients and oxygen (). However, imposed life originating outside the ecosystem, such as invasive species, can cause significant changes to the effective functioning of ecosystems by competing with native species for the environment's limited resources, possibly outperforming native plants and animals, resulting in the extinction of those species (US Department of Commerce, 2019).

Protozoa are unicellular microorganisms that are an important part of the soil food web. A diverse population of protozoa ensures that there is an abundance of bacterial populations, and therefore an increase in decomposition rates and soil aggregation (Natural Resources Conservation Service). They also play an essential role in the mineralization of nutrients, a process that allows the nutrients to be absorbed by plants. Trees share these nutrients with each other by transferring them through fungal networks which grow in the root systems. In this way, native trees become interdependent with both each other and the protozoa. However, previous studies show that the introduction of invasive plants interrupts this process by altering the surrounding environment, by damaging the diversity of native species and reducing soil nutrient concentration (Clusella-Trullas, 2020) (Teixeira, Yannelli, Ganade, Kollmann, 2020).

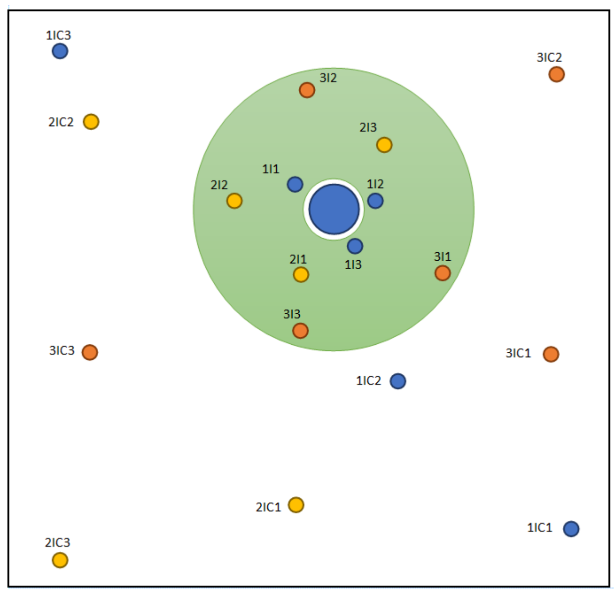
Nitrogen is an essential macronutrient which allows plants to produce protein for growth as well as photosynthesize (Nitrogen Cycle. Soil Science Society of America.). Therefore, the presence of invasive species could decrease the diversity of the protozoa population, because of the decrease in the population of native species, as well as decrease the presence of macronutrients such as nitrate and nitrite.

This information motivated the conduction of a study that researched the effects of invasive trees on the biodiversity of soil microbes by comparing the number of different species of protozoa in soil samples surrounding native and invasive trees and the levels of nitrogen in the soil. Nitrate and nitrite levels were measured as a possible indicator of sharing between trees, and therefore, the presence of a variety of fungi and protozoa (Ingham). The hypothesis of this experiment was that the presence of invasive trees would decrease protozoa diversity, nitrate and nitrite levels, while native trees would foster an environment with a higher protozoa diversity in nitrate and nitrite levels.

Methodology



Map of samples taken in relation to the tree in the native plot



Map of samples taken in relation to the tree in the invasive plot

Key	
	Day 1
	Day 2
	Day 3
	Tree
	Tree testing zone
	Control testing zone

On July 7th, 2021, one native tree (39.357834, -76.640034) and one invasive tree (39.357882, -76.639874) of similar ages in the same microclimate were identified in the woods behind Roland Park Country School, a 5 x 5 meter site was plotted around each tree. The invasive tree and the native tree were both located in Microclimate 1 (E.S.S.R.E. 2021). We set up the plots in the same microclimate to control for environmental factors that might impact our data. Each day for three days, a clean soil core extractor, with a depth of 15 cm and a diameter of 2 cm, was used to collect 3 soil samples each 15 centimeters from the base of both the native and invasive trees. Each day, 3 control samples were also taken in each plot between 2 to 3 meters away from the base of both the trees. Each soil sample was tested and observed to collect data about the diversity of the protozoa it contained. After each soil sample was dried, the soil was grinded and sifted to measure 9-10 grams of soil using a 1 mm^2 nylon screen. Once the samples were rehydrated with distilled water, they were left overnight in uhlig extractors. The filtrate was then filtered through a funnel and one drop of the 2nd filtrate from the pipette was mixed with 7 dots of methyl-green stain on a clean microscope slide and covered with a 18 x 18 mm^2 cover slip. The cover slip was then observed from 9 different areas and the number of each protozoa species in each area was counted. The average of the protozoa was then calculated to determine the levels of protozoa diversity throughout the soil. Soil samples were also tested for the levels of nitrate (ppm) and nitrite (ppm) using the LaMotte chemical test kit STH-14. All of these tests were statistically compared to each other to see how the diversity of protozoa in the soil of native versus invasive trees related to the levels of nitrate and nitrite.

Results/Data Analysis

` **Figure 1:** This bar graph shows the average nitrite and nitrate levels in native and invasive plots.

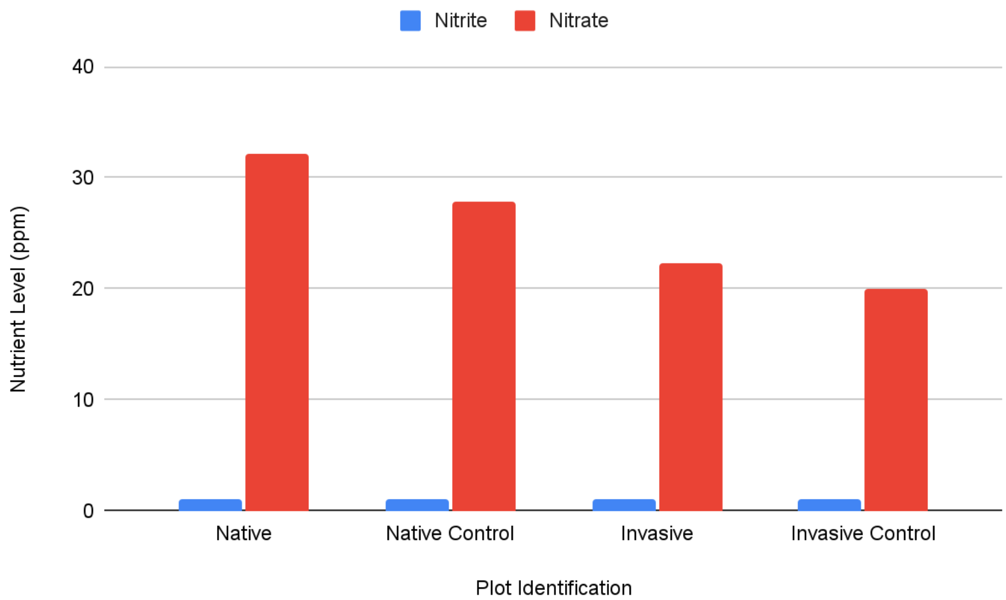


Table 1 shows the T-test data for our Nitrate analysis. The p-value represents the probability of error: the smaller the value, the higher the difference between the 2-sets of data. A p-value less than or equal to 0.2 suggests a significant difference in the compared data sets. The p-values suggest that there was a significant difference between the soil samples taken from native experimental and invasive experimental plots. The mean nitrate level for native experimental was significantly greater than the invasive experimental. Additionally, there was no significant difference in nitrate levels when comparing both invasive experimental and invasive control and native experimental and native control. All of the nitrite data was 1 ppm, so a t-test was not possible.

Table 1: This chart shows the results of t-tests performed for the nitrate data.

Comparison	Hypothesis Proved	p value
N vs. I	Alternate	0.056983
I vs. IC	Null	0.639052
N vs. NC	Null	0.401069

Table 2 shows the average number of the five types of protozoa (Mastigophora, Sporozoa, Ciliophora, Unshelled Amoeba, and Shelled Amoeba) found in the 9 fields of view for all of the soil samples that were taken. As the chart shows, the Unshelled Amoeba was the most common type of protozoa that was found overall. Additionally, the soil from the native experimental area had a much higher amount of Shelled Amoeba compared to any other area.

Table 2: This chart shows the average number of protozoa species counted in 9 fields of view at 100x magnification.

	Mastigophora	Sporozoa	Ciliophora	Shelled Amoeba	Unshelled Amoeba
Native	9.555555556	3.333333333	7.777777778	53.44444444	304.4444444
Native Control	12.77777778	1	25.11111111	36.88888889	129.5555556
Invasive	7	0.444444444	3.666666667	5.777777778	460.3333333
Invasive Control	3.111111111	2.111111111	3.888888889	24	476.4444444

Figure 2: This bar graph compares the protozoa species counted in native and invasive plots.

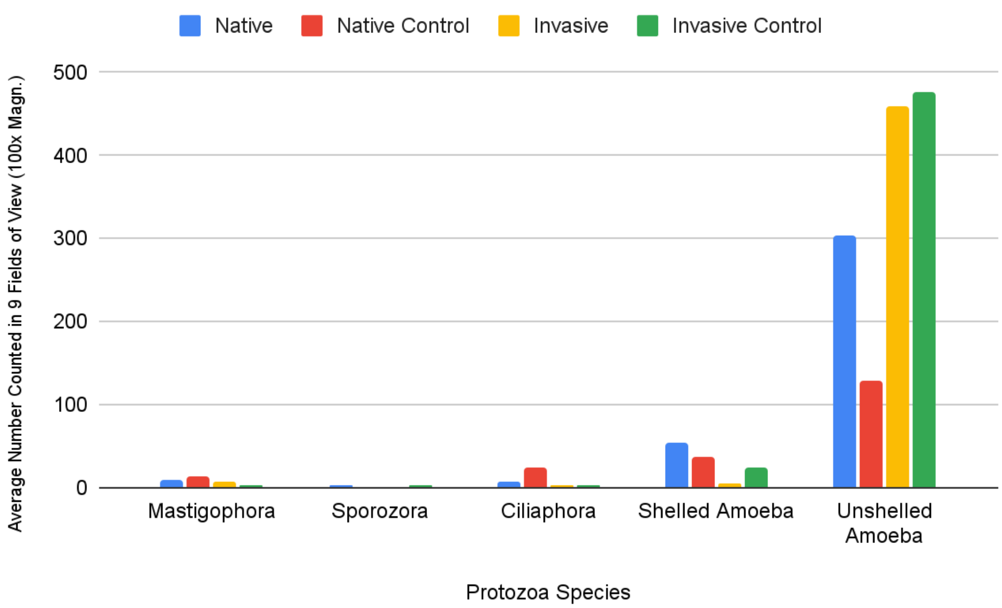


Table 3 shows the Chi-Squared Value and the p-Value that resulted from a Chi-Squared test on the protozoa diversity data. For both the comparisons of native to native control and invasive to invasive control, the p-value suggests a significant difference in the data sets. Further analysis was performed to determine the diversity of the plot by comparing the data to an even distribution of protozoa species, which would suggest high diversity.

The native and native control data do not support the hypothesis as the p-values suggest that the native control protozoa population is more diverse than the native tree protozoa population. The invasive and invasive control data do support the hypothesis as the p-values suggest that the invasive control protozoa population is more diverse than the invasive tree protozoa population.

Table 3: This chart shows the Chi-Squared Test Results for Protozoa Diversity.

Chi-Squared Test Protozoa			
Comparisons	Chi-Squared Value	Implication for data	p-Value
N-NC	12.71555	Significant Difference	p=0.05
I-IC	6.698364599	Significant Difference	p<0.2
N to 1/5	2.339245096	No difference	p > 0.5
NC to 1/5	1.246142431	No difference	p > 0.75
I to 1/5	0.7313282223	No difference	p > 0.9
IC to 1/5	0.6816946816	No difference	p > 0.95

Discussion

We hypothesized that because native trees are more immersed in an ecosystem, they would have a higher amount of protozoa diversity, causing there to also be a higher level of the macronutrients nitrite and nitrate in the soil. Table 1 and Figure 2 show the data for the average amount of the 5 types of protozoa (Mastigophora, Sporozoa, Ciliophora, Unshelled Amoeba, and Shelled Amoeba) in the soil samples taken from the Native and Invasive plots, including both experimental and control. Our results for protozoa diversity have proven that there is a significant difference between the protozoa diversity in the soil near native and invasive trees compared to the control plot located outside the tree root system. Table 3 demonstrates this relationship through the p-values resulting from a series of chi-squared tests. The invasive plot data supported our hypothesis as the p-value suggests that the invasive tree soil had less protozoa diversity than the invasive control soil. However, the native plot data contradicts our hypothesis because the p-values suggest that the native control soil had a greater amount of soil diversity than the native tree soil. In both cases, we found a significant difference in protozoa diversity between experimental samples and the control samples outside the tree root system.

We did not find a significant difference between any of our comparisons in our nitrite data, see Figure 1. The nitrite levels were the same for all of the soil samples that we collected. We feel that there was no difference in the nitrite levels because even though our plots contained different species of trees, they were near each other and were located in the same environment and the soil was affected by the same environmental factors.

We found a statistically significant difference between nitrate levels in the invasive experimental and native experimental soil (see table 1). Our t-tests showed no significant difference in the nitrate levels between both the invasive experimental and invasive control soil and native experimental and native control soil. While the fact that the difference between the nitrate levels in the native and invasive experimental soil proved our hypothesis, the fact that there was no significant difference in both of the plots between the experimental samples and control samples shows us that it might not have been the tree roots that affected the soil. Another reason why no significant difference was found could be that the control samples may have been taken too close to the roots of the tree, causing the tree roots to affect the soil in our control plots.

It is noted that there are environmental factors that are difficult to control that could have affected the two plots that the soil samples were taken from, such as rain, plants, animals, and other organisms. We made sure the two trees we chose were in the same microclimate and therefore affected by similar environmental factors. We also chose the trees that were around the same age so they had similar sized roots and canopy. In the lab, it was difficult to count the protozoa in fields of view with large numbers of protozoa. If we were to do this experiment again, we would ensure that there was only one of the tree species being studied in each plot.

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