PLFA analysis of microbial biomass in soil treated with fish urea or compost-based liquid fertilizer

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ABSTRACT

The goal of this study was to determine the effects that fish urea and liquid fertilizer have on a soil's microbial biomass after its application to soil samples. We want to know if these applications increase or decrease the microbial biomass and also if the applications help provide a diverse group of microbes. Phospholipid fatty acid (PLFA) analysis is a method for measuring microbial biomass and determining the overall composition of the microbial community. PLFA analysis is gaining popularity among the farming community and many want to learn more about it. In this study, PLFA analysis was used to determine the composition of microbial biomass of soil samples. Soil samples were collected from the Oasis Project in Pittsburgh, Pennsylvania, which is an organization emphasizing sustainable community farming. The samples were divided into three categories: control, urea, and liquid fertilizer. Soils were tested after two and four weeks post-application of urea and liquid fertilizer. Despite the soils being stored in anaerobic conditions for over two months in glass jars between when they were collected and when the experiment was conducted, the initial soil samples had a total living biomass and fungi:bacteria ratio that qualified as very good or excellent. Surprisingly, the first test results at two-weeks post-application showed a decrease in all report categories. By the fourth week, indicators had improved in all three experimental groups with the urea sample doing better than the control and fertilizer sample with respect to total living microbial biomass, functional group diversity, and bacteria:fungi ratio. Although application of fish urea showed greater benefits by the fourth week in this limited study, these results may not be generalizable. This abbreviated laboratory-based experiment could be followed by a study during the growing season using soil in its natural environment. Future studies would include tracking moisture content, conducting the pH levels of both the fish urea and liquid fertilizer, and conducting a nutrient analysis to determine the available nutrients in the soil.

INTRODUCTION

When it comes to survival, soil is a crucial resource for both people and animals, because of its ability to help produce food, fiber, habitats, shelter, recreational space, and clean air and water. In order to take care of the soil in more sustainable ways, we need to understand what is composed in the soil and how it adapts to other ecosystems. Soil is a dynamic interface that lies between rock, air, water, and living things. Soil is composed of many things like mineral solids and organic matter. Mineral solids can be anything from silt, clay, sand, or stone fragments and can determine how soil functions. Organic matter is mainly made up of carbon and any other matter that has come from any living organism. Organic matter is vital to soil's composition due to its part in giving the soil the ability to hold nutrient ions. The most common nutrients, potassium, nitrogen, and phosphorus can be released back into plants once the organic matter decomposes (*1*). The gold-standard Comprehensive Soil Health Assessment from Cornell addresses all three major components of soils (solids, water, and gasses) through various measurements, such as minerals, water, and oxygen content (*1*). Although the Cornell assessment discusses these points, it does not differentiate between living and non-living organic matter; this assessment only quantifies total organic matter and not microbial biomass.

Microbes are essential to a soil's composition and health. As shown in Figure 1, microbes, such as nematodes, fungi, protozoa, and bacteria, are essential to the food web. It is important to know what the microbial community is made up of so there is a better understanding of how to manage both the soil and microbial communities. Bacteria and fungi are essential microbes because they produce digestive enzymes that can help release nutrient ions like potassium, nitrogen, and phosphorus (*1*) . There are laboratories that offer soil analysis of microbial communities. Companies like Ward Laboratories (https://www.wardlab.com), Prolific (https://microbiometer.com), and Woods End (https://woodsend.com) offer soil analysis. The most notable soil analysis that has gained popularity is PLFA analysis.

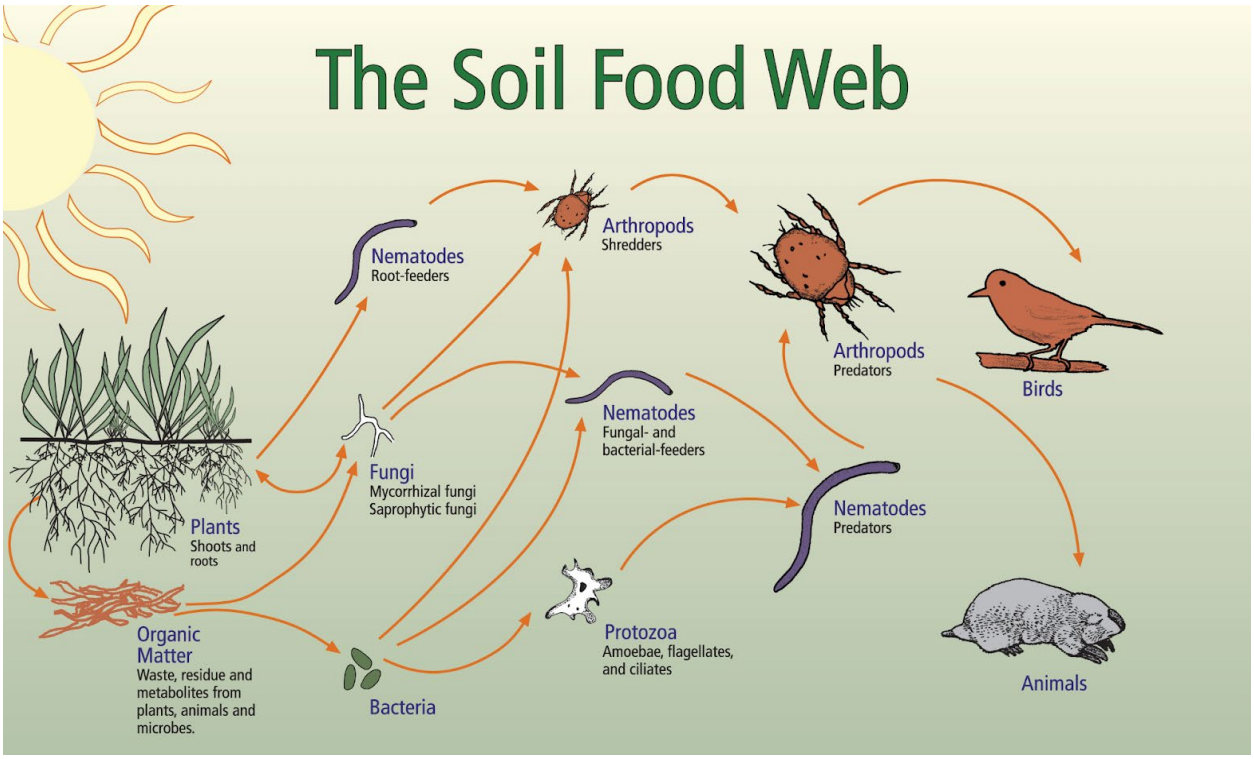


Figure 1. The Soil Food Web, Cornell University (*1*)

Phospholipid Fatty Acid (PLFA) Analysis

Phospholipid fatty acids (PLFAs) are present in cell membranes (Figure 2) and microbes can be identified by different characteristics of the hydrophobic tails (Figure 3), such as the length, saturation, and functional groups. Microbes within the soil can produce different chain lengths which can be used to characterize a specified type of microbe. According to Quideau, PLFAs that have a chain length that are between 14-20 carbon atoms are identified as bacteria and/or fungi (*2*).

Protocols used by Quideau were able to separate PLFAs based on their saturation. Saturated PLFAs can be straight-chained or single-bonded and are used to represent the gram-positive bacteria in the soil. Saturated PLFAs can also be terminally branched, meaning that there are other atoms bonded as side chains to the original branch. There are also mid-chain saturated PLFAs that can represent the actinomycetes within the soil. Unsaturated PLFAs contain double bonds and are used to represent gram-negative bacteria in the soil. The fungal PLFAs are primarily represented as unsaturated but can also be monounsaturated (2).

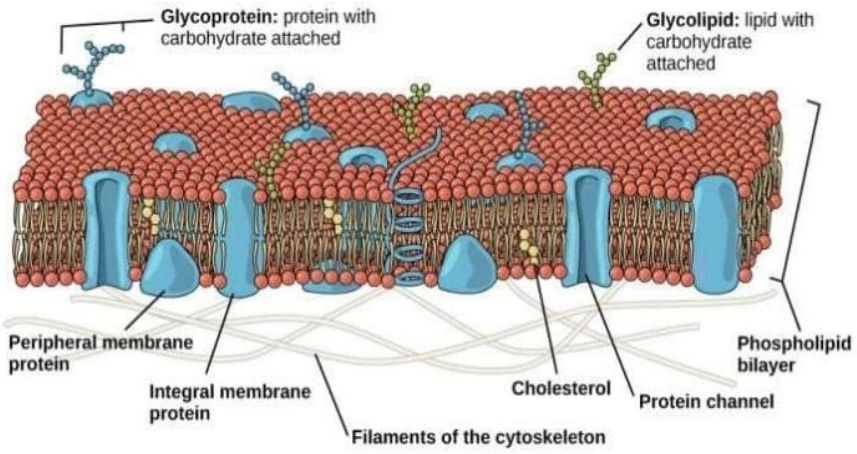


Figure 2. Phospholipid Bilayer (3)

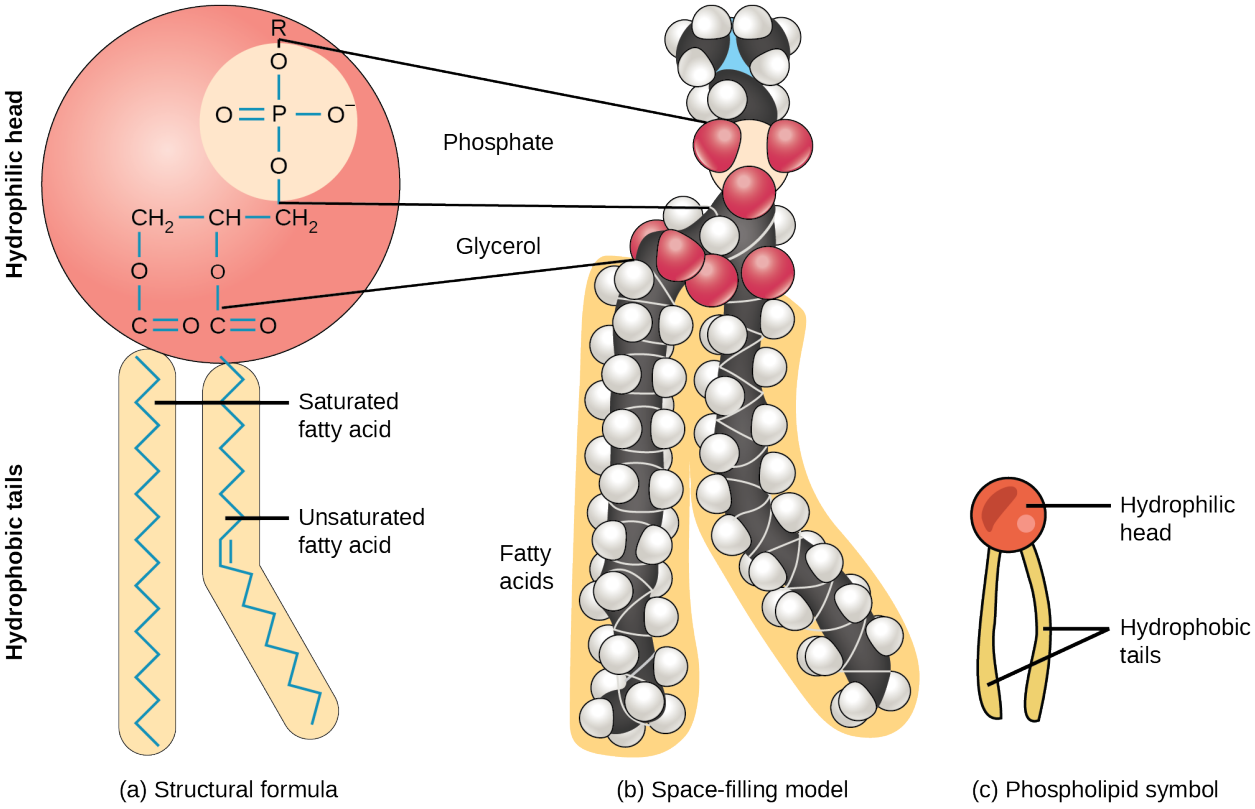


Figure 3. Phospholipid with molecular structure. (4)

To complete a PLFA analysis, scientists follow the general protocol shown in Figure 4 to prepare samples for analysis with gas chromatography-mass spectrometry (GC-MS). The results from the GC-MS are then compared to a database, such as the Sherlock Library. The Sherlock Library contains the profiles of over 1,500 bacterial species (5). PLFAs are matched to these profiles to quantify the total microbial biomass and differentiate between types of bacteria (e.g. gram-positive and gram-negative).

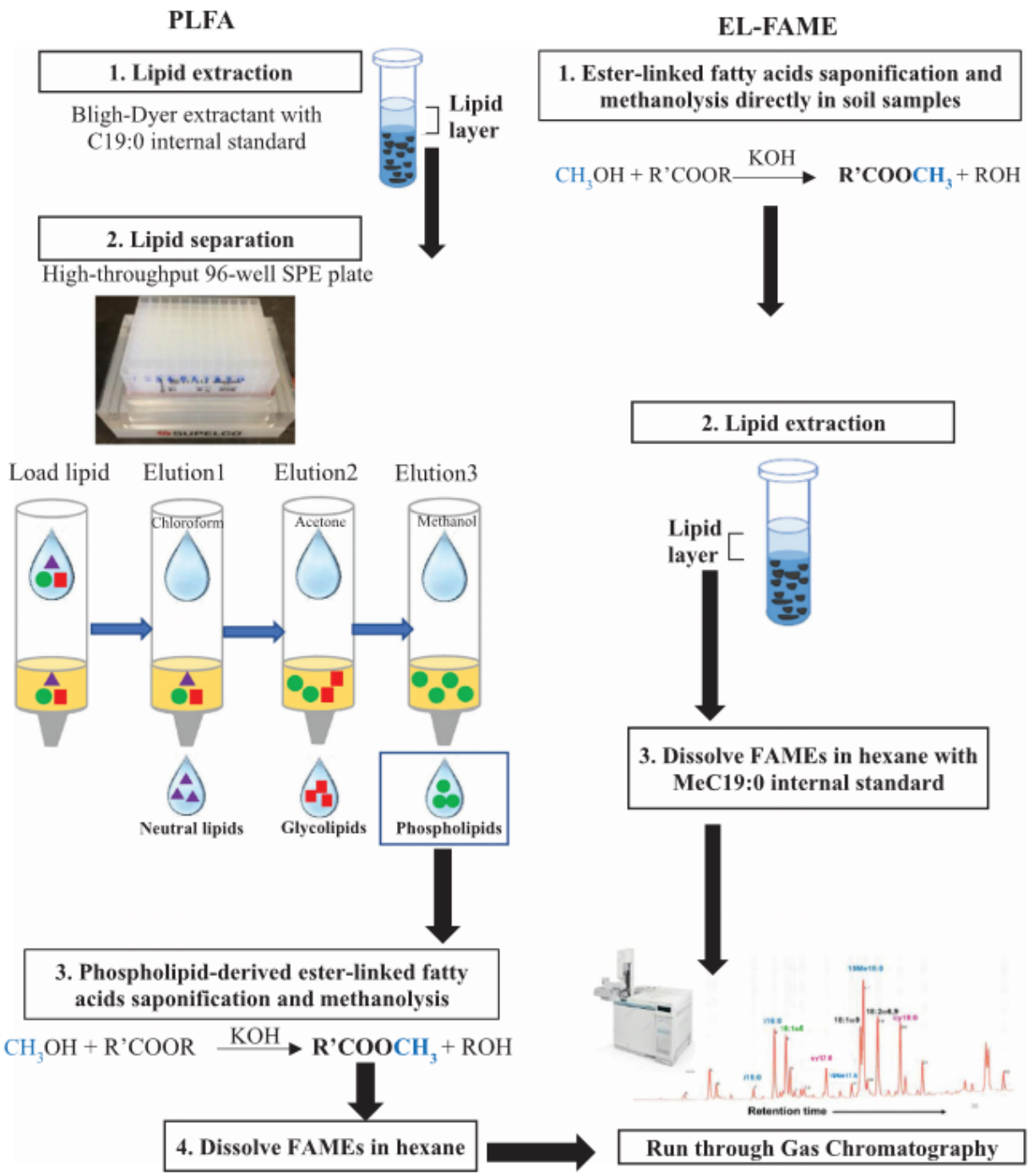


Figure 4. General protocol of PLFA analysis. (6)

Project Overview

In this study, we collaborated with The Oasis Project in Pittsburgh, Pennsylvania. The Oasis Project has recently experimented with making liquid fertilizer by using coffee grounds, fish urea, and produce. Fish urea is readily available because they have an aquaponics set-up. The Oasis Project staff provided soil samples which we treated with either compost-based liquid fertilizer or urea. A PLFA

analysis was used to determine if there are more microbial biomass in the treated soil compared to the untreated soil.

EXPERIMENTAL

Source and Storage of Soil Samples

Tacumba Turner (The Oasis Project) collected soil samples from outdoor garden beds used to grow leafy greens at the Oasis Project on Saturday, November 13, 2021. Soil samples were retrieved on January 19, 2022, and stored in four 32 oz. mason jars indoors at room temperature away from direct sunlight until 8 AM to 4 PM. Two of the jars contained regular soil, while the other two contained soil that was organically treated. The organic soil samples were treated with the compost layer before winter and then again during the winter season in order to prepare them for winterization.

Analysis of Original Soil Samples

The jars of original soil were sampled using a core soil sampler. The sampled soil was then put into solo cups and then weighed. The extracted amount was anywhere from 5-10 grams of soil. The samples were put in a plastic sample bag and stored with cooler packs and sent in the mail to Ward Laboratories using two-day shipping. The same process was used when sampling the post-organic soil samples.

Determination of Moisture Content of Soil Samples

The mixed soil (original soil) was placed on a watch glass and put in a ventilation hood for two days. The mixed organic soil was placed on two trays and also placed in the ventilation hood for the same period of time. The moisture content was determined by comparing the dry mass to the initial mass.

Preparation of Samples

Soil samples from all four jars were combined and manually mixed together. A total of seven worms were discovered. There were five small worms and two big worms. The control and urea pots were each given a large worm and a small worm; whereas the liquid fertilizer sample was given four worms. The treatments and water were not added until two days later after the trial setup.

Soil Watering

The soils were watered Monday, Wednesday, and Friday to return the initial water lost by calculating the mass of soil and the water added. The previous mass of soil was added to the previous amount of water added and was then subtracted by the current number of the mass of soil. The calculation gives us the amount of water that should be added that day. It is worth noting that each pot of soil loses, on average, 50 mL of water every two days.

PLFA Analysis

Samples were collected and sent to Ward Laboratories for analysis two and four weeks after application of fertilizer treatment (e.g. urea or liquid fertilizer).

RESULTS AND DISCUSSION

Garden bed soil with and without organic compost layer

The PLFA results for the original soil sample (original) collected prior to winterization with the organic compost layer and the soil sample taken after application of the compost layer (organic) are reported to in Table 1. Figure 5 shows the composition of the microbial community.

Table 1: PLFA results of original and organic soil sample

Sampling Date	soil sample	Total Living Microbial Biomass (ng/g)	Functional Group Diversity Index	Community Composition Ratios		
				Fungai: Bacteria (ng/g)	Predator: Prey	Gram(+) : Gram(-) (ng/g)
2/2	original	5519.17	1.437	0.3210	All Prey	0.7521
	organic	3529.40	1.548	0.4657	All Prey	2.1393
2/23	Control	1584.58	1.543	0.3744	0.00021	1.3127
	Urea	891.66	1.523	0.3668	All Prey	1.0731
	Liquid Fertilizer	1333.07	1.505	0.6714	0.0041	1.0908
3/9	Control	2135.33	1.49	0.2892	All Prey	1.1187
	Urea	2649.37	1.503	0.3171	All Prey	1.1667
	Liquid Fertilizer	2369.77	1.496	0.2991	0.0036	1.0484

Table 2: Color Key

Color	Rating		Color	Rating
Dark Red	Very Poor		Lighter green	Slightly Above Average
Medium red	Poor		Light green	Good
Light red	Slightly Below Average		Medium green	Very Good

Average	Blank		Dark green	Excellent

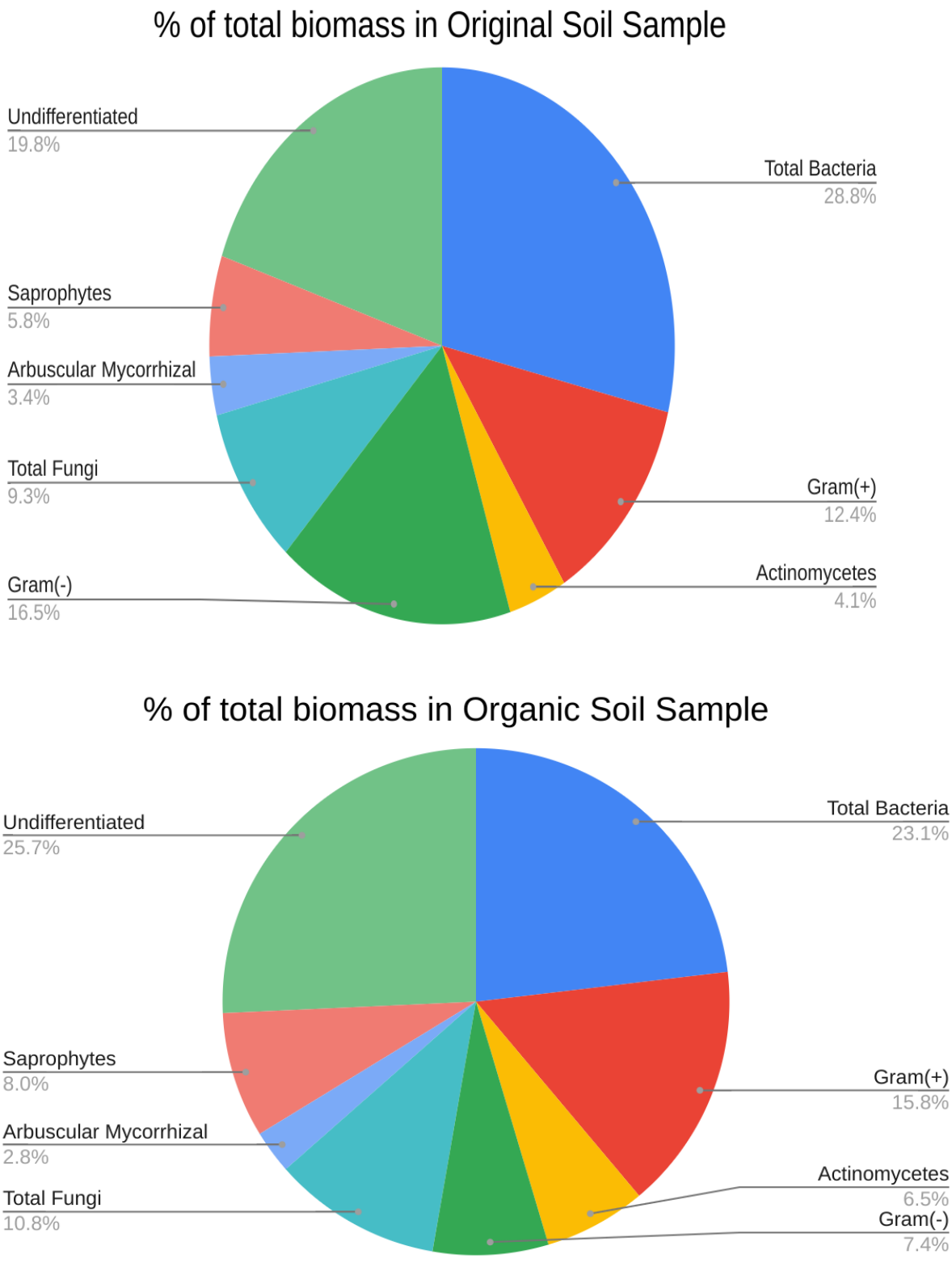


Figure 5. Composition of Microbial Community in original and organic soil sample

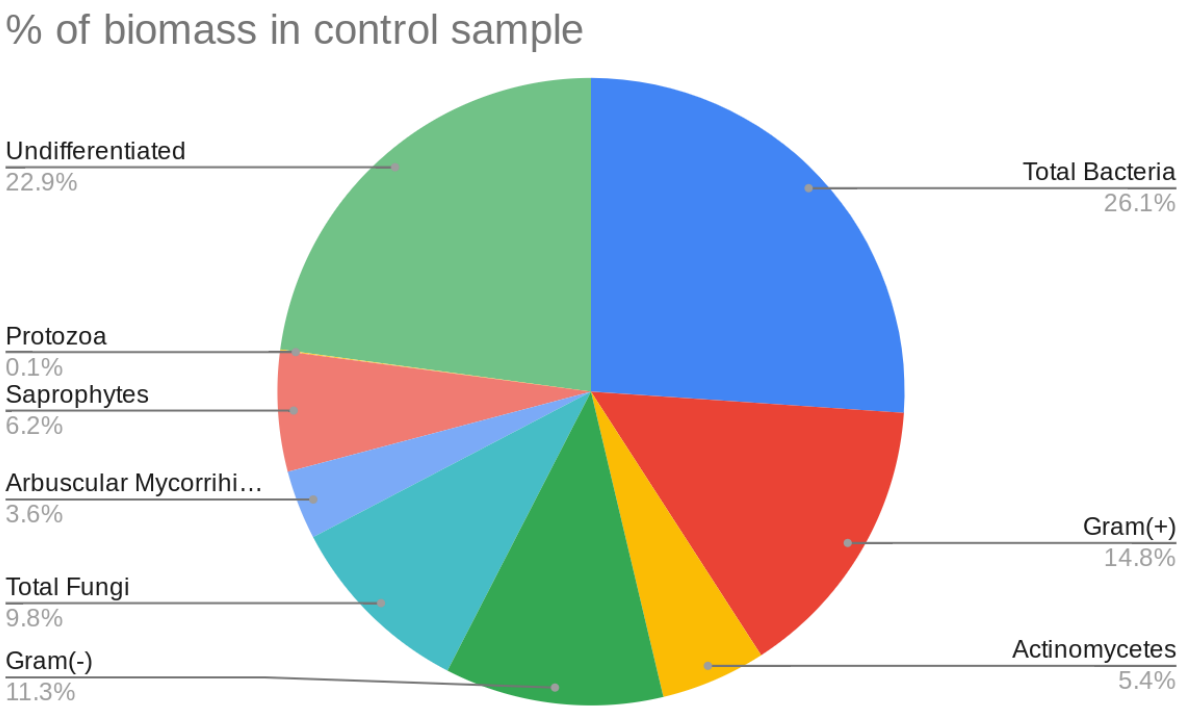
In Figure 5, the pie charts above show the makeup of the microbial community in each soil sample. In the organic soil sample, the gram (+) is greater (15.08%) than the gram (+) in the original soil sample (12.4%). However, the gram (-) bacteria was significantly higher in the original sample (16.5%) than in the organic sample (7.4%). It is worth noting that the undifferentiated is making up almost half of the soil samples. This is to be expected because, in the undifferentiated, there are all kinds of microbes that have yet to be identified.

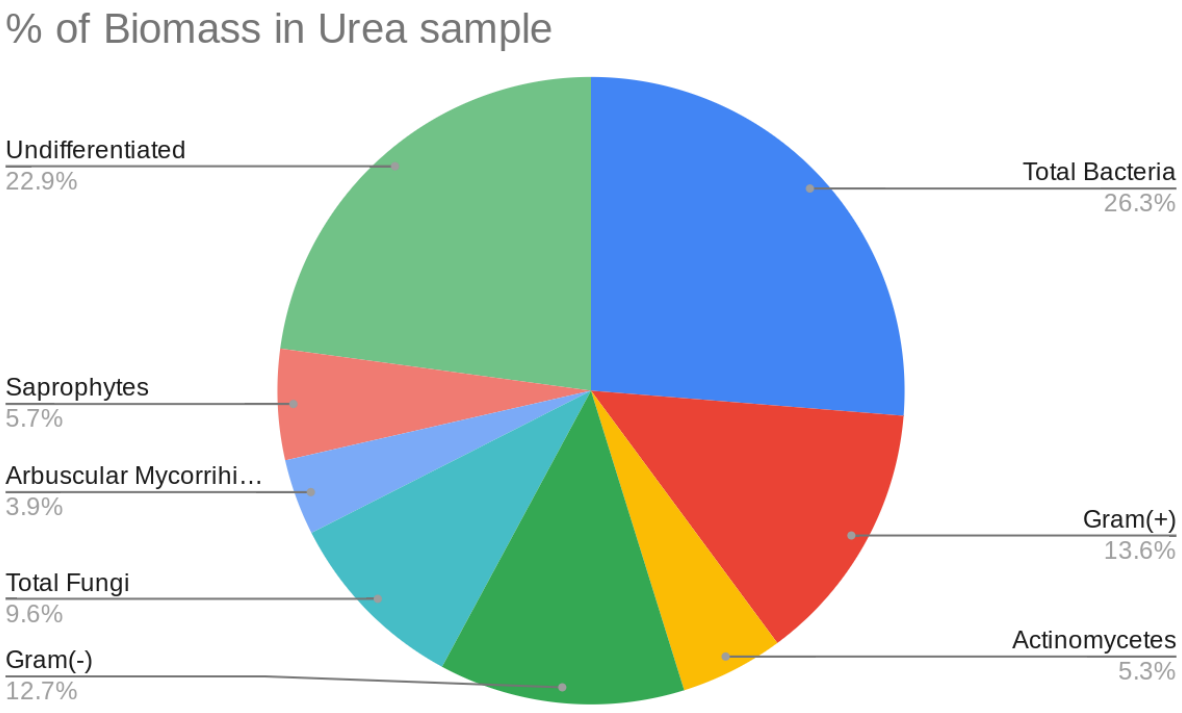
The soil samples were sent to Ward Laboratories for a PLFA Analysis. The results that were gathered showed significant differences between the two soils. In Table 1, the original soil sample had a biomass of 5519.17 ng/g. The rating given to this soil would be excellent because of the category it falls under and that there is also a healthy amount of microbes in that soil. The diversity of the soil was 1.43 ng/g, this means that there is a good amount of diversity among the microbes in the soil is good. The biomass of the organic soil was slightly lower than the original soil. The biomass was 3529.49 ng/g which means that the soil is very good. The diversity of the microbial community was 1.5 ng/g which meant that the diversity of the microbial community is very good. These are surprising results because it was initially believed that the organic sample would have better diversity. The organic soil was treated with an organic compost layer before and after winterization. It should be noted that the compost layer was made up of organic matter like fruit, coffee grounds, fish particulate, and water.

Garden Bed soil separated into groups: control, urea, and liquid fertilizer

The PLFA results for the soil samples (*control, urea, and liquid fertilizer)* were taken after soils were mixed and potted. The results are summarized in Table 1. Figure 6 shows the composition of the microbial community in each case.

Table 1 shows the results of each soil sample sent in. The results provided are surprising. The total living microbial biomass for each sample is not strong. The sample with the weakest microbial biomass was the urea sample. The biomass of that sample falls under the poor category. This was shocking because it was initially believed that the urea sample would have better results and be in the “good” or “very good” category because the urea contained resources like nitrogen that could have helped the soil’s microbial community. The two other samples, control, and fertilizer had better biomass with the control falling under the “average" category and the fertilizer falling under the “below average” category. It should also be noted that the urea sample is the only sample that contains only “prey” or bacteria. The control and urea samples only have small percentages of protozoans and also show the difference between the previous soil samples. The final soil analysis from 3/9 shows improvement from the last analysis (2/23). Each sample in the last analysis has reached the average or slightly above average category. The predator-to-prey ratio also changed. The control and urea samples are both containing all prey, while the fertilizer sample has a small number of protozoans present within the soil.


% of biomass in control sample
Undifferentiated
22.9%
Total Bacteria
26.1%
Protozoa
0.1%
Saprophytes
6.2%
Arbuscular Mycorrihi…
3.6%
Gram(+)
14.8%
Total Fungi
9.8%
Actinomycetes
5.4%
Gram(-)
11.3%


% of Biomass in Urea sample
Undifferentiated
22.9%
Total Bacteria
26.3%
Saprophytes
5.7%
Arbuscular Mycorrihi…
3.9%
Gram(+)
13.6%
Total Fungi
9.6%
Actinomycetes
5.3%
Gram(-)
12.7%

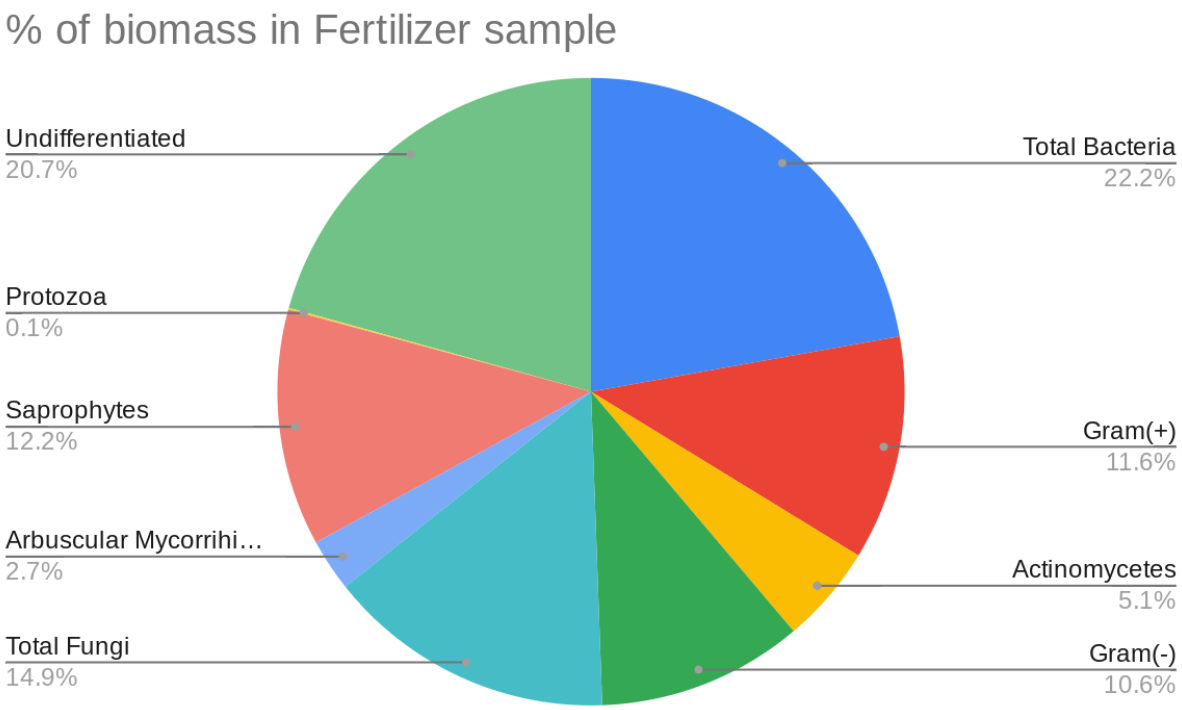
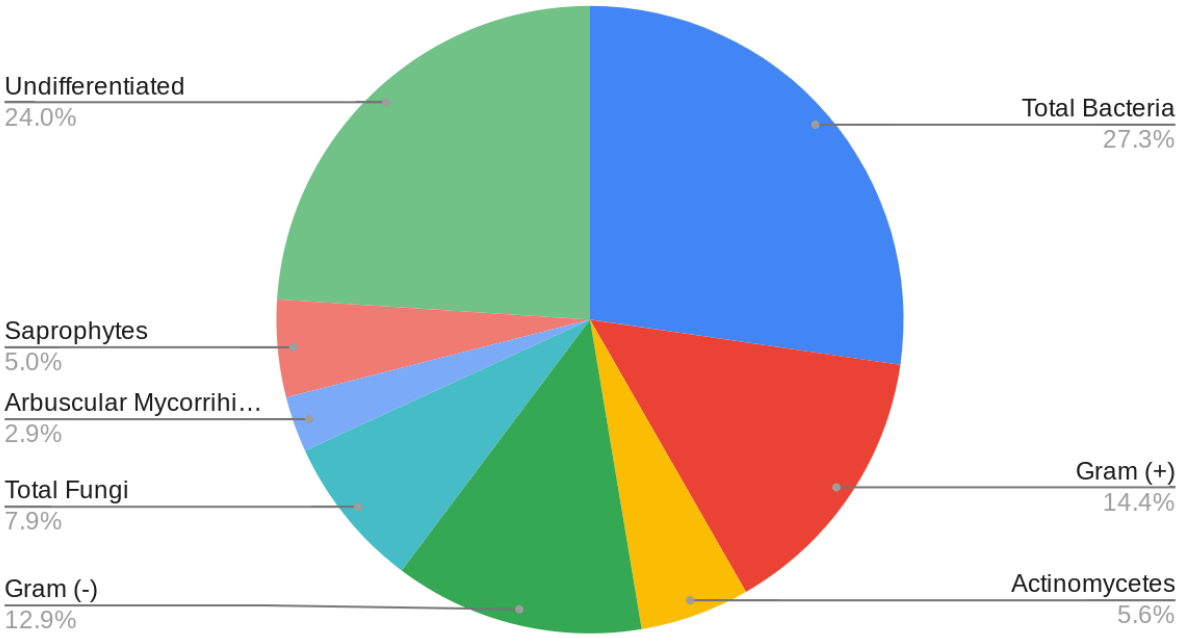


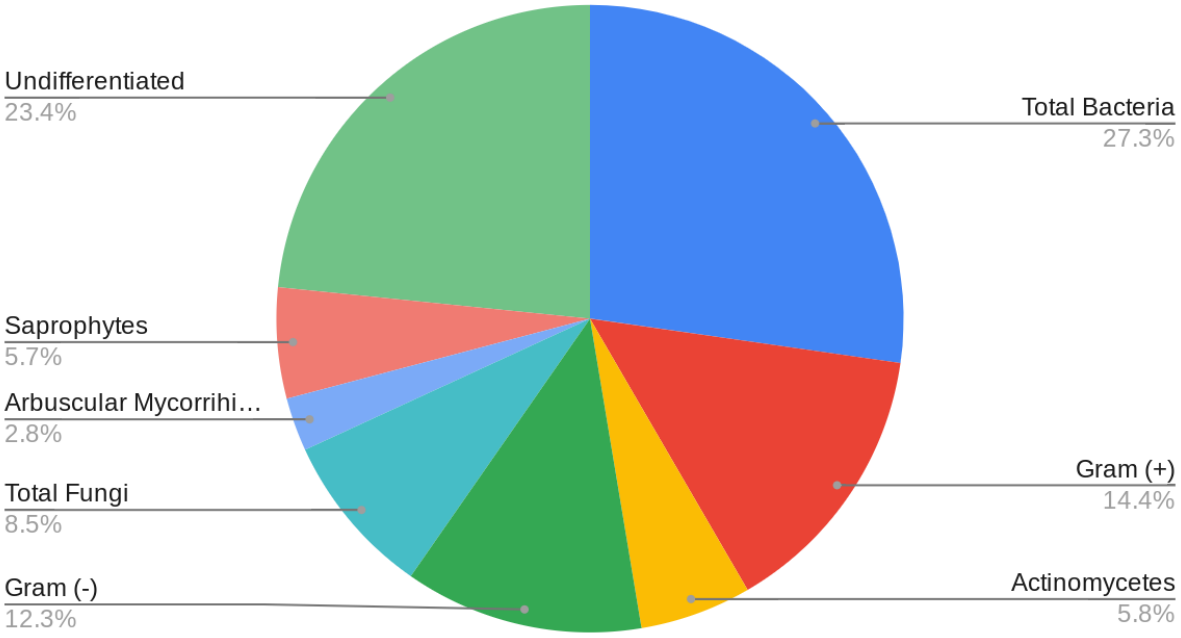
Figure 6. Composition of the microbial community in control, urea, and fertilizer samples

The figure above is a pie chart of the microbial community of each sample. The microbial composition of the control and fertilizer held a significant difference that was not present in the urea sample. The control and fertilizer samples had protozoans present in the soil. Although this is a small percentage, it is unlikely that protozoans are present in soil due to bacteria outweighing them.

% of biomass in Control Sample



% of biomass of Urea Sample



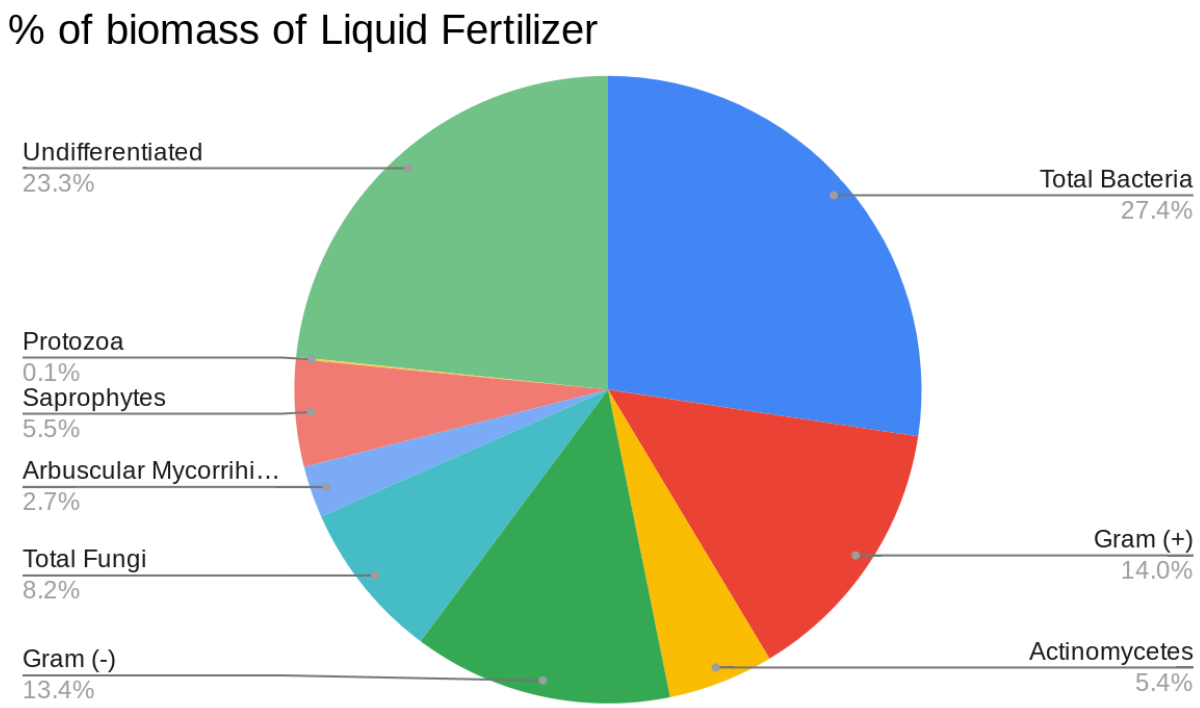


Figure 7: Composition of Microbial biomass from Control, Urea, and Liquid Fertilizer samples (2nd analysis)

The pie charts above show the microbial biomass of each sample. Each sample across the pie charts shows that the functional groups in each sample are fairly close to each other. The only noticeable difference is seen in the liquid fertilizer. The fertilizer sample is the only sample that contains a small number of protozoans. This is shocking to see because protozoa do not typically show up in a soil analysis because they are generally outnumbered by bacteria.

CONCLUSION

Although PLFAs can be used to see the microbial life in the soil, others have criticized the method as not providing enough information but the DNA analysis is the better option (5). According to Fierer, et al. paper, there are assumptions that come with the PLFA analysis that is not exactly correct. The assumptions give the idea that larger microbial biomass indicates healthier soil or that a higher fungal to bacteria means that there is a sustainable soil system. (5). While we know that these limits may cause obstacles to our study, we have found a different way to go about the experiment. For future experiments, it would be beneficial to track the pH content. Towards the end of sampling, the pH content of each soil sample (control, urea, fertilizer) was tested by adding one gram of soil and 2 (mL.) of water in small tubes. This practice could have achieved better microbial biomass for each of the samples. A nutrient analysis also could have been done for the nitrogen, phosphorus, and potassium (N, P, K) could have also

provided a better understanding of how to take care of the samples. We also could conduct a baseline sample to determine the soil samples makeup while potted.

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