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Honors Thesis

An Honors Thesis Titled

Analysis of Soil Bacterial and Fungal Biomass and Determination of Soil Microbial Activity in Four Landscapes on the Salisbury University Campus

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by

Hayley Schindler

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Signatures of Honors Thesis Committee

Mentor: Dr. Elizabeth Emmert		
Reader 1: Dr. John Lee		
Reader 2: Dr. LeAnne Wood		
Director:		
	Signature	Print

Abstract

Though they are small, microbial communities in the soil play a large role in global climate change through carbon sequestration. Prior research has indicated that atmospheric changes in carbon dioxide concentrations directly impact soil microbial communities. The microbial responses, however, can be positive or negative, and so far, the magnitude and direction of these responses is uncertain (Castro et al., 2010). My goal is to sample the four different landscapes located around Salisbury University's campus: lawn, savannah, garden, and forest. I will analyze their contents by means of phospholipid fatty acid (PLFA) analysis, which determines the bacterial and fungal biomass, and also measure the microbial activity via soil enzymes like β-glucosidase, an enzyme that degrades cellulose. With this information, it will be possible to see the bacterial and fungal composition of the soils and also how active the microbes are, paving the way to determining what soils around the campus are more likely to sequester carbon and which are more likely to emit carbon. This study is part of a larger project that aims to discover the role of Salisbury University's Arboretum in carbon sequestration and ultimately its role in global climate change.

Introduction

Greenhouse gas emissions are a leading cause of global climate change, specifically global warming, and one of the primary gases involved is carbon dioxide (CO_2). Currently, ground soils contain about 2,000 Pg of organic carbon, which is twice the amount of carbon in the atmosphere (Singh et al., 2010). Thus, soil microbial communities, or communities of bacteria and fungi that live together, have a great responsibility in the cycling of carbon. However, their activities are regulated by biotic and abiotic factors like quality of litter (i.e.,

leaves, plant debris, and other materials covering the soil surface), changes in atmospheric temperature, and moisture content (Castro et al., 2010). These factors can affect greenhouse gas fluctuations in the atmosphere by altering the existing microbial community structure and/or by modifying the physiology of existing populations (Singh et al., 2010).

Increased temperature has been directly linked to increased soil respiration, which limits the amount of carbon sequestered in the soil, mainly due to increases in microbial activity. Different microbial groups have distinct optimal temperature ranges for both growth and activity, so increased temperatures can affect the composition of a community, which in some cases can result in the release of soil organic carbon. Soil carbon includes both inorganic carbon as carbonate minerals, and organic carbon as soil organic matter. Organic carbon comes from both living and dead biomass, such as worms in the soil or decaying plants. It has been noted that fungi assimilate more carbon than bacteria, so a microbial community dominated by fungi will have lower respiration rates, leading to more sequestered carbon (Singh et al., 2010). Nevertheless, the estimation of carbon loss and storage by microbial communities due to climate change is still ambiguous. Thus, by gaining information on soil microbial communities, it is possible to determine the relationship between the microbial community composition and soil carbon composition and use this evidence to better predict how soils will influence greenhouse gas emissions in other ecosystems.

An Arboretum is a garden devoted to multiple types of trees, and Salisbury University has a nationally ranked Arboretum. The Salisbury Arboretum Project, a vast project involving multiple scientific disciplines, aims to determine Salisbury University's role in global warming by quantifying the ecosystem services provided by trees and soil as they relate to carbon sequestration. To do this, the arboretum was divided into four landscape categories: lawn—an

area of grasses, savannah—a mixed woodland and grassland landscape, garden—an area of rich soil with multiple plants, like flowers and bushes, and forest—an area covered with trees. Each of these sites had soil samples taken and analyzed. In the current study, phospholipid fatty acid analysis, or PLFA analysis, and tests for β-glucosidase will be performed. PLFA analysis is useful in identifying microbial community groups because bacteria and fungi can be represented by extraction of signature lipid biomarkers from the cell membranes and walls of the microorganisms (Lucas et al., 2007). β-glucosidase is an enzyme that breaks down cellulose, and its activity reflects the soil's microbial activity which is related to the amount of carbon in the soil. This enzyme is important to study because the degradation of cellulose produces reduced forms of carbon, which is the main energy source for microbial growth and activity (Sarathchandra & Perrott, 1983).

Methods

Soil Sample Collection:

Soil samples from four different landscapes on the Salisbury University campus were collected, consisting of lawn, savannah, garden, and forest. Four subplots were created within each landscape, and five soil cores from each of the four subplots within a landscape were collected. The soil cores were collected using a coring tube to the depth of 7.5 cm and the five samples from each subplot were combined and homogenized, or mixed thoroughly, within a Ziploc bag. The contents from each bag were sieved to 2 mm to remove organic debris before further analysis. From each soil bag, ~4-5 grams (g) of soil were saved for β-glucosidase analysis, and thick blue cap tubes were ¾ full with each soil for PLFA analysis (~40-45g of soil in each tube).

β-glucosidase Activity:

To determine the microbial activity of the soil via enzymes, β-glucosidase enzymatic activity was measured. The method for this procedure followed the methods presented in Allison and Jastrow (2006). One gram of soil sample was combined with 60 mL Tris buffer (50 mM, pH 7) and placed in a shaker at room temperature for 5 minutes. In a centrifuge tube, 2 mL of the homogenate was combined with 2 mL of substrate (5 mM pNP-β-D-glucopyranoside in 50 mM Tris buffer) and shaken for 2 hours at room temperature. After centrifugation at 2500 x g for 2 minutes (may have to be repeated if soil hasn't settled), the supernatants (variants of light yellow in color) were decanted and the absorbance of p-nitrophenol in the supernatant was measured at 410 nm on a spectrophotometer. From this, the equation from the pNP standard curve was used to calculate concentration of β-glucosidase in each sample. See Calculations section for how to create the standard curve. This concentration was then converted to an actual amount with units expressed as $\frac{\text{umol product}*\text{hr}}{\text{fraction dry weight}}$. See Calculations section for data conversions.

In order to get the final β-glucosidase value, dry weights of the soil samples needed to be determined. To do this, an aluminum pan was filled to the brim with wet soil (~27-30g of soil per pan). The pans were then placed in an oven at approximately 110℃, and the weight of the soil was measured for at least two consecutive days, or until the weight stops changing (at this point, all the water is dried out). From there, the amount of weight lost was used to convert the 1 gram wet samples into dry weights. See Calculations section for data conversion.

Phospholipid Fatty Acid (PLFA) Analysis:

To determine the bacterial and fungal biomass in the soil, a phospholipid fatty acid (PLFA) analysis was performed. The methods for this procedure followed the procedures of Ng et al. (2014) and the assistance of Salisbury University professors, such as Dr. Eugene Williams, Biology, and Dr. Katherine Miller, Chemistry. Lipids were extracted from 4g of soil using a 15.6 mL citrate buffer (0.15 M, pH 4): chloroform: methanol (0.8:1:2 v/v/v). See Solution Recipe section. The samples were then shaken for 1 hour at room temperature. Large glass tubes with screw cap lids were used for this (figure 2). After shaking, the mixture was transferred to small glass tubes with screw cap lids (figure 2) so that they fit into the large centrifuge. The tube was then centrifuged at 1900 x g for 10 minutes. The supernatant (yellow in color) was transferred to medium sized tubes (figure 2) and 11.7 mL of citrate buffer: chloroform: methanol (0.9:1:2 v/v/v) mixture was added to the soil pellet. The tube was shaken again at room temperature, followed by centrifugation at 1900 x g for 10 minutes. The supernatants were pooled and an additional 11.3 mL of citrate buffer: chloroform: methanol (0.9:1:2 v/v/v) mixture was added to the pool. The pooled samples were left overnight for phase separation.

The aqueous layer was removed and discarded; the organic layer (bottom) was kept. To do so, it is easiest to use a pipette and remove the bottom layer, placing the bottom layer into a new tube. This avoids leaving any of the aqueous layer behind with the organic layer. The organic layer was evaporated under nitrogen gas, N_2, in Dr. William's lab hood. The samples take about 30-60 minutes to evaporate, depending on the size. Once evaporated, the samples were re-dissolved in 2 mL chloroform and transferred to a lipid extraction cartridge (silica acid column—see figure 3). To set up the column, silica gel powder (dehydrated in oven

overnight at 110℃) is poured into a column about 2 inches high, or high enough so that the sample can fit within the gel limits when poured. The column is then wet with chloroform and run until it almost hits the top of the gel. The sample is then added, making sure the column never dries out. 2 mL of chloroform containing the lipids is added, followed by 2 aliquots of 5 mL acetone. This extracts the glyco and neutral lipids which were then discarded. The phospholipids were extracted with 5 mL methanol and this layer was kept. The phospholipid sample was then evaporated under N_2. See figure 4 for how the column looks when each reagent is added.

To transform the ester linked fatty acids into fatty acid methyl esters, the phospholipid fraction was incubated at 37 C for 20 minutes (oven in HS 252) with 1 mL 1:1 mixture methanol and toluene, and 1 mL of methanolic KOH (0.2 M) was added. See Solution Recipes section for how to make methanolic KOH. The sample was neutralized with 0.3 mL acetic acid (1 M) and 2 mL of ultra-pure water. See Recipes section. Two extraction were then carried out with a mixture of 2 mL hexane: chloroform (4:1 v/v) and the organic phases (top layer) combined. This layer was evaporated under N2, and re-suspended in 200 uL hexane including methyl decanoate (see Recipe section) until the sample can be analyzed by gas chromatography (GC). The GC machine is located on third floor of Henson in Chemistry department.

To load samples into the GC, 200 uL of hexane containing .005mg/mL methyl decanoate was added to each soil sample. Using a needle syringe, 1 uL of the sample was injected into the GC machine. It was made sure that there were no bubbles present in the syringe before injection, and that the start button was hit on the GC as soon as the solution is injected, as timing is important for peak analysis. The solution took about 30 minutes to be analyzed. The syringe was then rinsed with hexane between each new sample loading.

Statistical Analysis:

Standard error values were determined using the standard deviations from the average β-glucosidase values for each landscape. See Calculations section for conversions. A one way ANOVA Tukey pairwise comparison test was performed on the β-glucosidase values to determine statistical significance between numbers (figure 1).

Data Calculations

1. Creating the β-glucosidase Standard Curve

A 1 mM stock solution of the desired product was created by dissolving 0.007g substrate, pNP-β-D-glucopyranoside, in 50 mL Tris buffer.

139.11 g/L = 1 M so 139.11 mg/L = 1mM

0.050 L * (139.11 mg/L) = 6.96 mg = 0.007 g substrate in 50 mL Tris = 1 mM

However, the concentrations that resulted from this were too concentrated to be read on the spectrophotometer, so I diluted the solutions further.

1 mL of the 1 mM solution was added to 9 mL of buffer to create a 10 mL solution of 0.1 mM stock.

To create separate concentration standards, specific amounts of 0.1 mM stock were combined with Tris buffer to create a 5 mL total solution.

Amounts of Stock and Buffer and Resulting Absorbance that went into making the standard curve.

0.1 mM Stock (mL)	Tris Buffer (mL)	Concentration* (mM)	Absorbances (410 nm)
0.5	4.5	0.01	0.096
1	4	0.02	0.206
1.5	3.5	0.03	0.308
2	3	0.04	0.386
3	2	0.06	0.576
3.5	1.5	0.07	0.673
4	1	0.08	0.77
4.5	0.5	0.09	0.9

*Concentrations determined using C1V1=C2V2 equation

C1V1 = C2V2 example calculation:

(0.1 mM)*(0.5 mL) = C2(5 mL) → C2 = 0.01 mM

These standards were measured on a spectrophotometer and their absorbance's recorded.

Sample calculation for one of the standards:

Absorbance of Lawn sample #1 = 0.051

Standard curve equation: y = 9.7294 x + 0.003 where y = absorbance and x = concentration in mM

0.051 = 9.7294 x + 0.003

x = 0.0049 mM product (β-glucosidase)

See below for how to convert this mM value to umol

2. Sample calculation for determining umol β–glucosidase:

$$0.005 \text{ mM product} = \frac{0.005 \text{ mmol}}{1 \text{ L}} * \frac{1 \text{ mol}}{1000 \text{ mmol}} * \frac{1 \text{ umol}}{1 * 10^{-6} \text{mol}} * 4 \text{ mL total volume}$$
$$= 0.02 \text{ umol product}$$

This calculation was done for each of the 16 samples (see table 1).

3. Sample calculation for converting dry weights:

(Dry weight/wet weight)*100 = x%

(27.27g/31.536 g)*100 = 86%

Now use this 86% to convert your 1 gram sample…

1.067g * 0.86 = 0.917g dry weight soil

This conversion was done for each of the 16 samples (see table 2).

4. Sample calculation for final β-glucosidase value (umol product/dry weight/hr)

$$\frac{0.02 \text{ umol product}}{0.917 \text{ g dry weight}} * 2 \text{ hours} = 0.043 \text{ umol β-glucosidase}$$

This calculation was done for each of the 16 samples (see table 3).

5. Sample calculation for standard error bars for Lawn:

- In excel, "=STDEV" and select β-glucosidase values for lawn.
- Standard error = St. dev/square root of sample size (sample size = 4 because 4 samples from each landscape)
- Standard error = 0.016/(√4) = 0.008

This calculation was done for each of the landscapes (table 4).

Solution Recipes for β-glucosidase Assay:

1. 50 mM Tris Buffer

$50\ mM * \frac{1\ M}{1000\ mM} = 0.05\ mol/L$

$\frac{0.05\ mol}{1\ L} * \frac{121.4\ g\ Tris}{1\ mol}$ = 6.057g Tris/1 L water

add hydrochloric acid until pH 7 is reached

2. 5 mM substrate (5 mM pNP-β-D-glucopyranoside) in 50 mM Tris buffer

$5\ mM\ substrate * \frac{1\ M}{1000\ mM} * \frac{301.25\ mg\ substrate}{1\ mol}$ = 1506.25 mg/ 1 L substrate

1506 mgL * 0.01 L = 15.06 mg = 0.0156g substrate in 10 mL Tris buffer

Solution Recipes for PLFA Analysis:

1. Citrate Buffer (0.15 M, pH 4)

$\frac{0.15\ mol}{1\ L} * \frac{294.1\ g\ citrate}{1\ mol}$ = 44.115 g/L citrate

$\frac{44.15\ g}{1000\ mL} = \frac{x}{200\ mL}$ → x = 8.83g citrate/ 200 mL water

2. Sample calculation for making a mixture: 15.6 mL citrate buffer: chloroform: methanol (0.8:1:2 v/v/v)

- Total up all parts (0.8 + 1 + 2) = 3.8
- Divide 15.6 mL by 3.8 = 4.1 mL = 1 part = chloroform
- 2 parts = 8.2 mL = methanol
- 0.8 parts = 4.1 mL * 0.8 = 3.28 mL citrate buffer

3. Methanolic KOH (0.2 M)

$\frac{0.2\ mol}{1\ L} * \frac{56.11\ g\ KOH}{1\ mol} = \frac{11.2\ g}{1\ L} * \frac{1\ L}{1000\ mL}$ = 0.0112 g KOH/ mL methanol = 0.112g KOH/ 10 mL methanol

4. Acetic Acid (1 M)

Stock Acetic acid = 17.4 M → we want 1 M, so we want 16 parts water: 1 part acetic acid

To make a stock solution of 10 mL…

10 mL/17 = 1 part = 0.58 mL acetic acid

16 parts = 9.42 mL = water

Results

Table 1. Absorbance at 410 nm and concentration of β-glucosidase for soil samples.

Sample #	Absorbance	Concentration of B-gluc (mM)
1 L	0.051	0.004933501
2	0.04	0.003802907
3	0.07	0.006886344
4	0.08	0.007914157
5 S	0.088	0.008736407
6	0.14	0.014081033
7	0.14	0.014081033
8	0.19	0.019220096
9 G	0.061	0.005961313
10	0.069	0.006783563
11	0.07	0.006886344
12	0.06	0.005858532
13 F	0.05	0.004830719
14	0.09	0.00894197
15	0.052	0.005036282
16	0.075	0.007400251

*L, S, G, and F indicate the 4 samples from lawn, savannah, garden, and forest landscapes

Table 2. Amount of soil used and amount of dry weight per sample

Sample #	Amount of soil (g)	Dry Weight (g)
1	1.067	0.91762
2	1.030	0.8858
3	1.015	0.86275
4	1.089	0.93654
5	1.050	0.903
6	1.004	0.83332
7	1.068	0.89712
8	1.015	0.86275
9	1.092	0.80808
10	1.095	0.8103
11	1.079	0.82004
12	1.054	0.81158
13	1.002	0.83166
14	1.087	0.92395
15	1.036	0.89096
16	1.017	0.83394

Table 3. Amount of β-glucosidase activity per soil sample

Sample #	Amount of β-glucosidase (umol product/dry weight/hr)
1	0.043011273
2	0.034345511
3	0.063854831
4	0.067603366
5	0.077398956
6	0.135180077
7	0.125566548
8	0.178221694
9	0.059017059
10	0.06697335
11	0.067180572
12	0.057749396
13	0.046468213
14	0.077423841
15	0.045221171
16	0.070990726

Table 4. Standard deviation and standard error of β-glucosidase values for four landscape types

Landscape Type	Standard Deviation	Standard Error
Lawn	0.016086373	0.008043187
Savannah	0.041373869	0.020686934
Garden	0.005046648	0.002523324
Forest	0.016592234	0.008296117

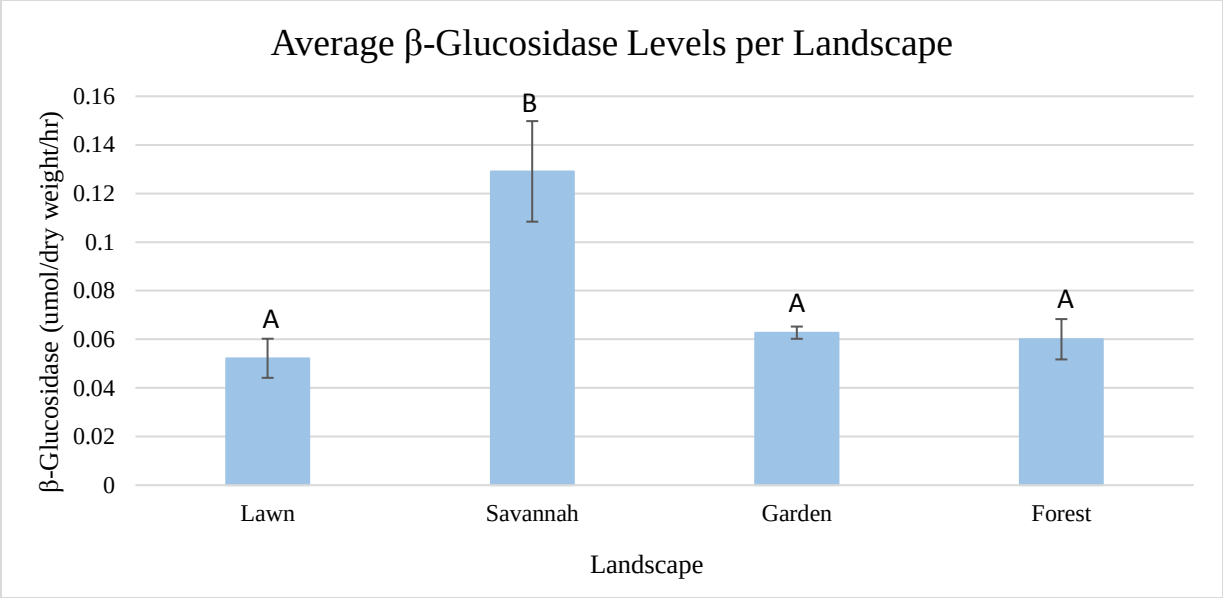


Figure 1. A one-way ANOVA and Tukey pairwise comparison test was run to determine any significant difference between landscapes. P value = 0.002. Different letters denote significant difference between values.

Figure 2. Large, medium, and small glass tubes with screw cap lids used for PLFA analysis. Large tubes taken from BIOL 210 and medium/small tubes from Dr. William's lab.

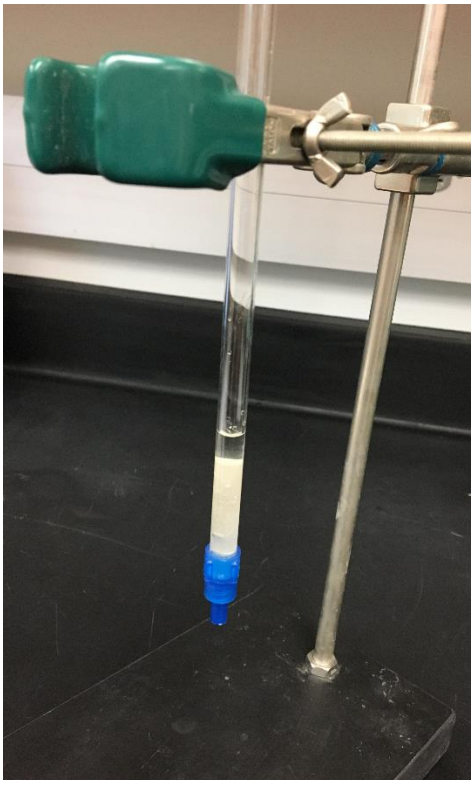


Figure 3. Silica Acid column set-up.

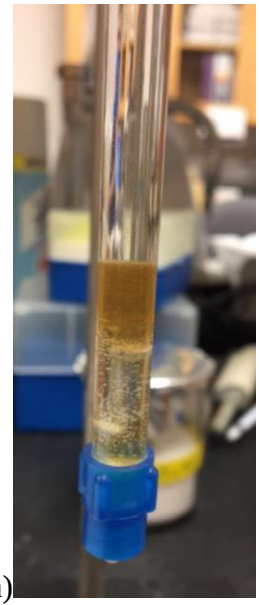
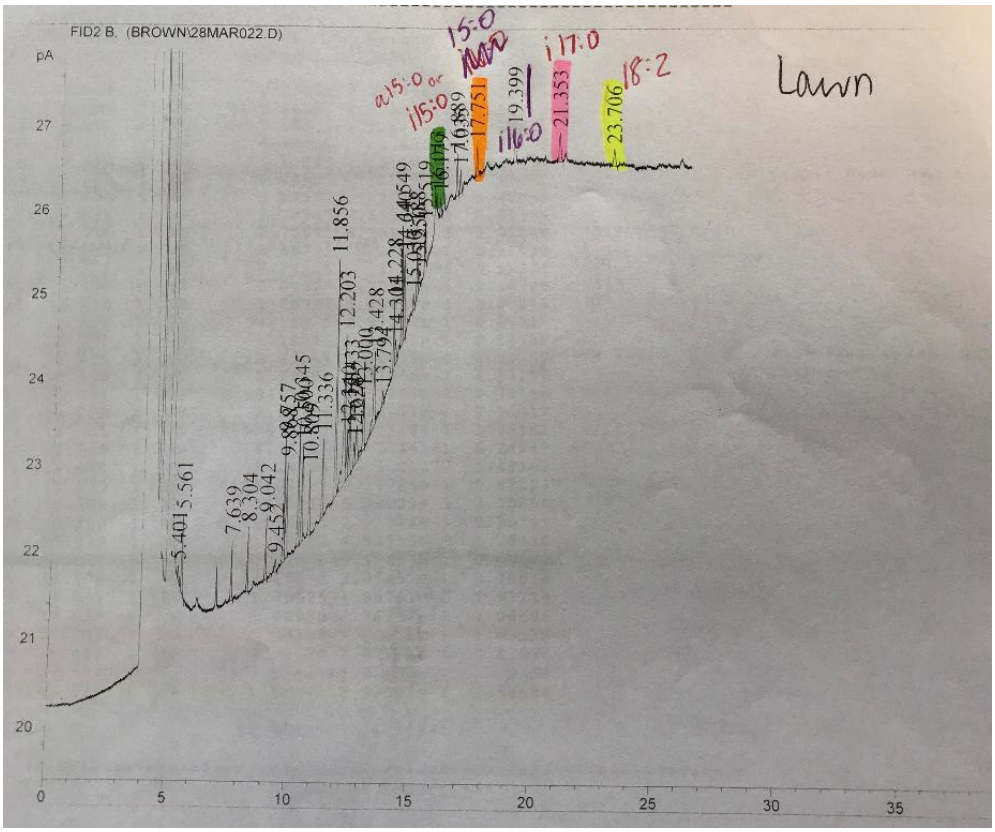
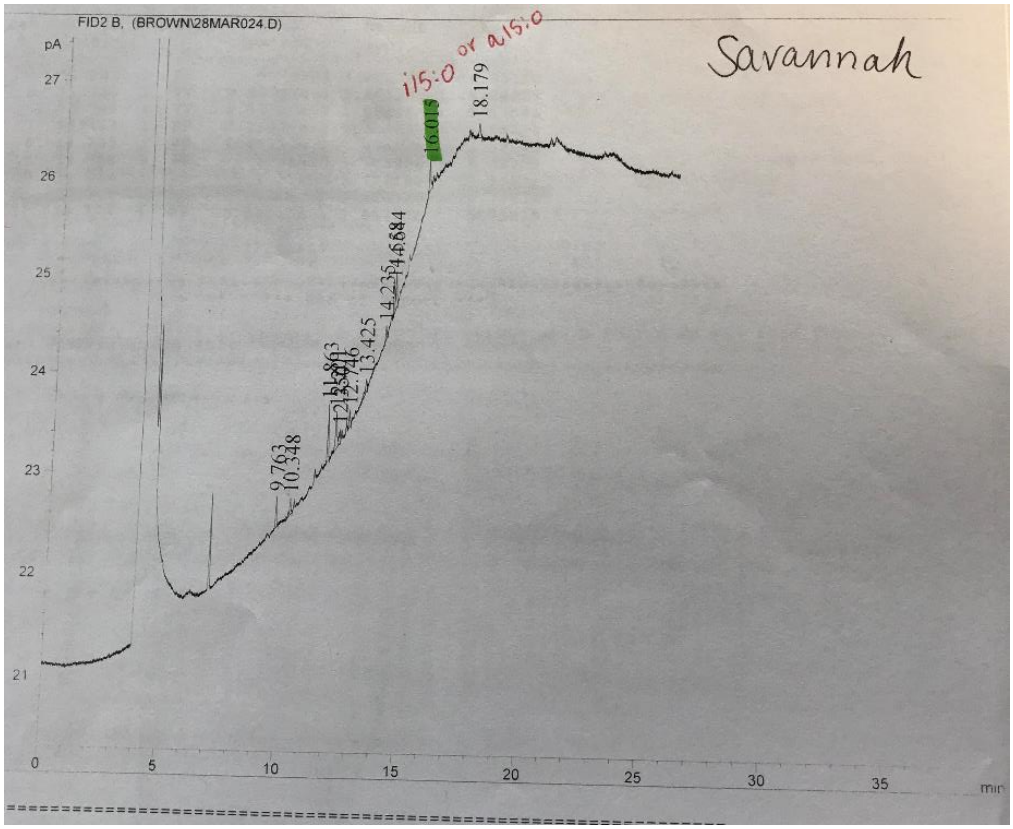
a)  b)  c) 

Figure 4. Silica column color change with addition of a) chloroform, b) acetone, c) methanol to extract different lipids.

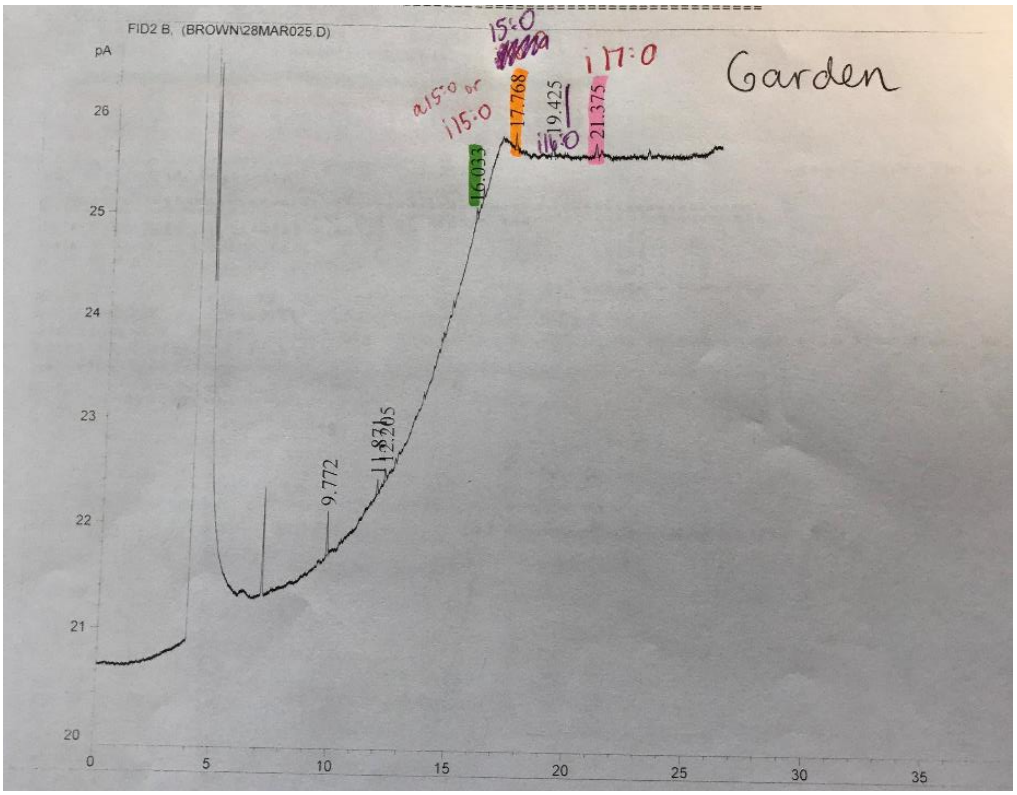
a)


FID2 B, (BROWN\28MAR022.D)
pA
Lawn
a15:0 or i15:0
i15:0
i16:0
i17:0
18:2
15.561
17.751
19.399
21.353
23.706
11.856
12.203
11.336
7.639
8.304
9.042
5.401
0
5
10
15
20
25
30
35

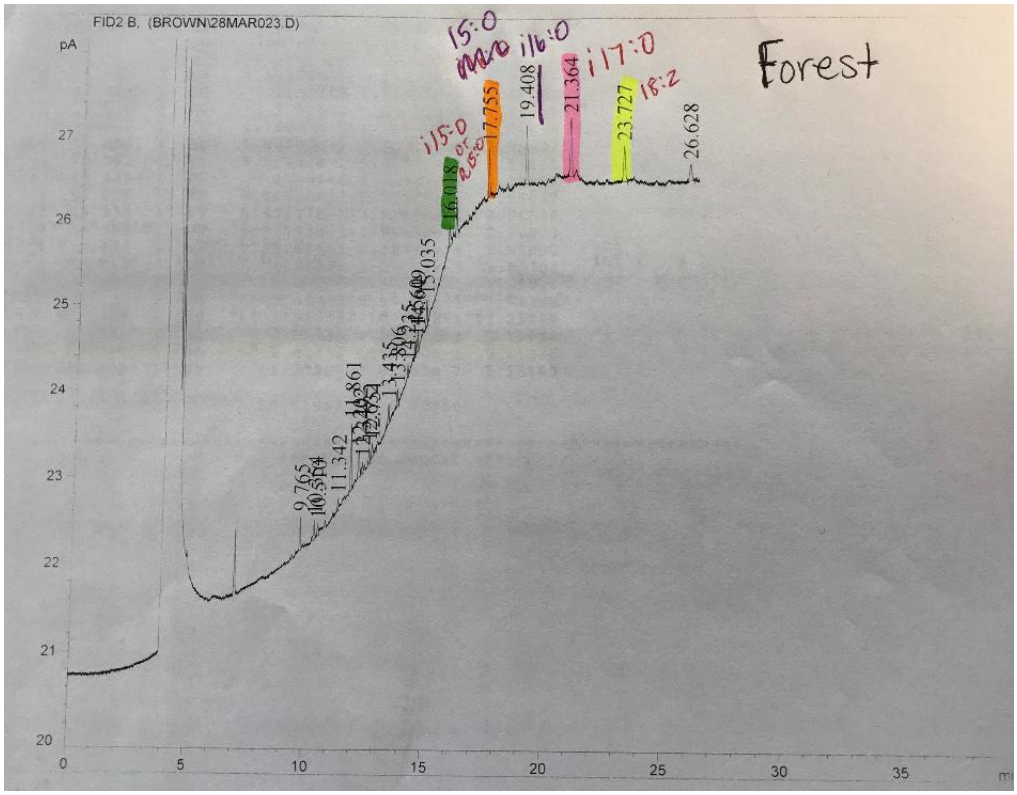
b)

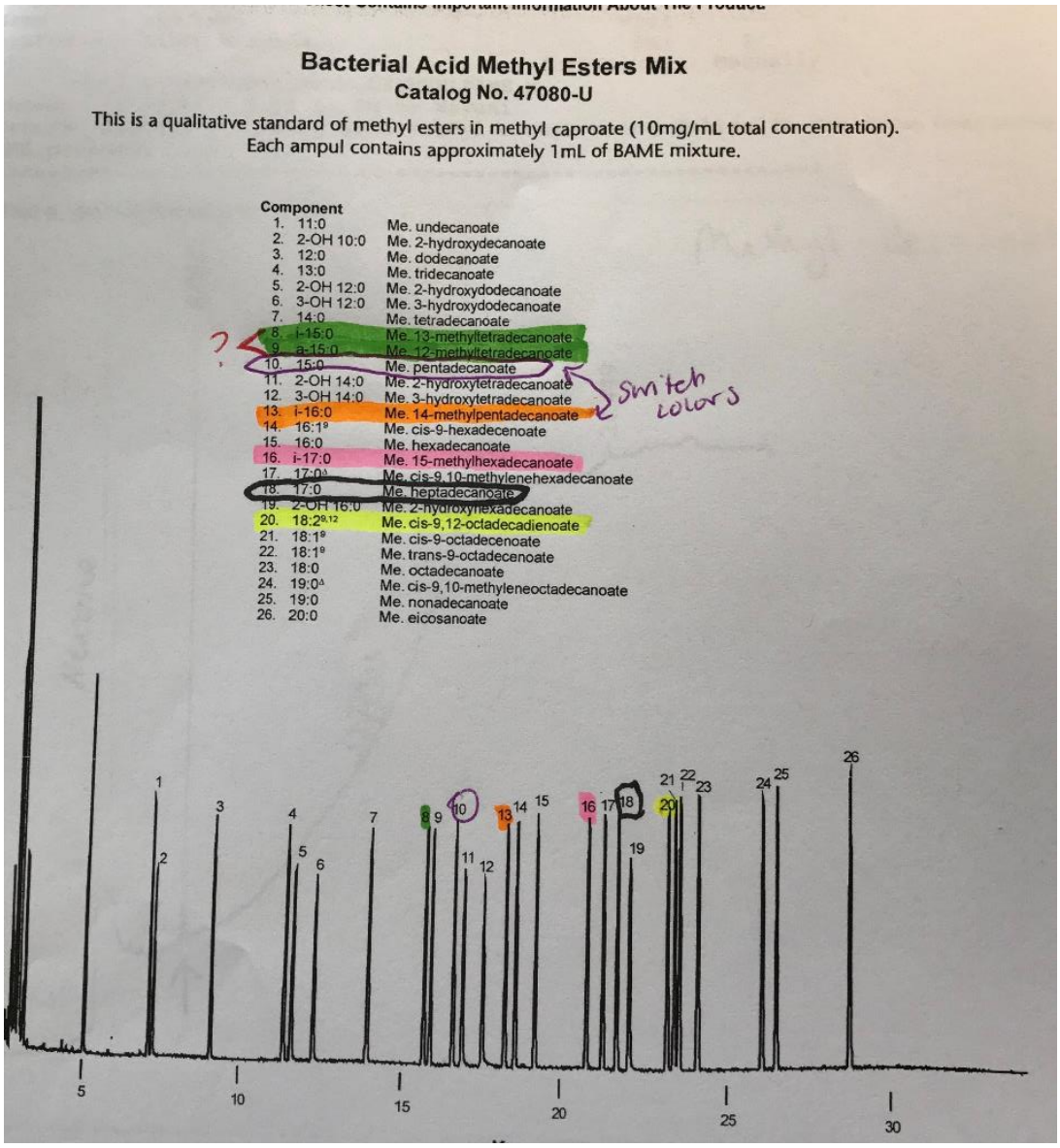

FID2 B, (BROWN\28MAR024.D)
pA
Savannah
i15:0 or a15:0
18.179
16.013
14.235
13.425
12.746
10.348
9.763
0
5
10
15
20
25
30
35
min

c)


FID2 B, (BROWN\28MAR025.D)
pA
15:0
i15:0 or a15:0
i16:0
i17:0
Garden
17.768
19.425
21.375
16.033
9.772
11.871
12.265
26
25
24
23
22
21
20
0
5
10
15
20
25
30
35

d)


FID2 B, (BROWN\28MAR023.D)
pA
15:0
i16:0
i17:0
18:2
Forest
i15:0 or a15:0
16.018
17.755
19.408
21.364
23.727
26.628
9.765
10.564
11.342
27
26
25
24
23
22
21
20
0
5
10
15
20
25
30
35



e)

Figure 5. Gas Chromatography results for each landscape sample. a) Lawn b) Savannah c) Garden d) Forest e) BAME standard

PLFA Analysis: GC Data

By comparing each landscape's GC results to BAME standards, the bacteria and fungi present in each soil sample was determined. By looking through scientific papers, the important lipids in identifying bacteria were determined to be i15:0, a15:0, i16:0, i17:0, 17:0, and for fungi, 18:2 w6, 9 (18:2 in BAME). The following bacteria and fungi identified in each soil type is identified in table 5. See figure 5 for GC analysis peaks.

Table 5. Bacteria/fungi presence in Landscape Types

Phospholipid Component	Bacteria Name	Soil Types in which Bacteria/fungi is present
i15:0	Me. 13-methyltetradecanoate	Lawn, Savannah, Forest, Garden
a15:0	Me. 12- methyltetradecanoate	Lawn, Savannah, Forest, Garden
15:0	Me. pentadecanoate	Lawn, Forest, Garden
i16:0	Me. 14-methylpentadecanoate	Lawn, Forest, Garden
i17:0	Me. 15-methylhexadecanoate	Lawn, Forest, Garden
17:0	Me. heptadecanoate	No presence determined
18:2 w6,9	Me. Cis-9,12-octadecadienoate	Lawn, Forest

Discussion

Based on the results from the β-glucosidase analysis, the Savannah landscape has much higher amounts of β-glucosidase, meaning the microbes in the Savannah are more active than in the other three landscapes. This makes sense, because the Savannah is frequently covered with leaf debris that is easily broken down. The more debris there is to break down, the more microbial activity is expected. Lawn, Garden, and Forest are all statistically similar to each other but significantly different than Savannah. This is surprising because these three landscapes are very different from one another, so one would think that their levels of β-glucosidase would be diverse. The reason behind their similar levels is unknown, but future PLFA analysis may guide us in the direction of why certain landscapes have more microbial activity.

The PLFA analysis will reveal the amount of bacteria and fungi in each landscape. This is very useful information because if one landscape is dominated by bacteria or fungi, it may give clues as to why a landscape does or does not have a lot of activity occurring. For example, if the Savannah landscape shows high levels of bacteria and low levels of fungi, then it may be assumed that bacteria play a much higher role in the production of β-glucosidase than that of fungi. Or, the Savannah may show equal amounts of bacteria and fungi while other landscapes show varying amounts, which could mean that the microbes create the most β-glucosidase when working together in equal numbers.

Higher levels of β-glucosidase indicate that there is more organic matter in that landscape for the microbes to break down, meaning there is more carbon being actively processed. With this information, it is possible to analyze the landscape compositions and try to determine which are contributing most to overall carbon sequestration at Salisbury

University, paving the way to developing methods to control or aid in the process of carbon processing.

After comparing the BAME standards to each landscape sample, it is clear that the Lawn, Forest, and Garden contain much more of a variety of bacteria than the Savannah, with Lawn and Forest containing the most. Fungi was only present in the Lawn and Forest. It was hard to assign i15:0 and a15:0 to a particular landscape, because there retention times are so close that either bacteria could be the bacteria present in the landscape. Because of this, both were included in table 5 as being present. However, all other notable bacteria and fungi were correlated with a peak in the GC data. From the information gathered, it seems that the Lawn and Forest landscapes on Salisbury University's campus most likely sequester the most carbon, therefore being the most useful soils in combatting the effects of global warming.

Although this study was specific to carbon sequestration on the Salisbury University campus, there is a "big picture" message to be taken from it. The more carbon that is emitted into the atmosphere only helps to increase the effects of global warming, posing threats to earth's ecosystems, cultures, and geopolitical stability (Erlandson, 2008). Global warming brings about the warming of oceans, melting of glaciers, and rising sea levels, ultimately destroying many marine ecosystems. Billions of dollars are being spent annually in an attempt to predict the effects of global warming on earth's ecosystems and ultimately human communities (Erlandson, 2008). However, an important ecosystem that hints at the prevalence of global warming is in the soil: microbial communities. Microbes are very active with the cycling of carbon, influencing the effects of global warming. In a study performed by Contosta et al. (2015), biomass shifts and shifts in microbial community composition have occurred after a twelve year warming study. Although this shift may not be major, a changing ecosystem

that forms the basis of life can alter many other systems on earth. Studying their activities, cycling of matter, and how they may be changing, even in a small space like the Salisbury campus, can give a look at the detrimental effects of global warming and aid in ways to combat it.

References

Allison, D. S., & Jastrow, D. J. (2006). Activities of extracellular enzymes in physically isolated fractions of restored grassland soils. *Soil Biology and Biochemistry*, 38, 3245-3256.

Castro, H. F., Classen, A. T., Austin, E. E., Norby, R. J., & Schadt, C. W. (2010). Soil Microbial Community Responses to Multiple Experimental Climate Change Drivers. *Applied and Environmental Microbiology*, 76, 999-1007.

Contosta, A. R., Frey, S. D., & Cooper, A. B. (September 01, 2015). Soil microbial communities vary as much over time as with chronic warming and nitrogen additions. *Soil Biology and Biochemistry*, 88, 19-24.

Erlandson, J. M. (July 01, 2008). Racing a Rising Tide: Global Warming, Rising Seas, and the Erosion of Human History. *Journal of Island & Coastal Archaeology*, 3, 2.)

Lucas, R. W., Casper, B. B., Jacks, J. K., & Balser, T. C. (2007). Soil microbial communities and extracellular enzyme activity in the New Jersey Pinelands. *Soil Biology and Biochemistry*, 39, 2508-2519.

Ng, E., Rose, M.T., Schefe, C.R., Wilkinson, K., Smernik, R.J., & Cavagnaro, T.R., (2014). Does the chemical nature of soil carbon drive the structure and functioning of soil microbial communities? *Soil Biology and Biochemistry*, 70, 54-61.

Sarathchandra, S. U., & Perrott, K. W. (1983). Assay of β-Glucosidase Activity in Soils. *Soil Science*, 138, 15-19.

Singh, B. K., Bardgett, R. D., Smith, P., & Reay, D. S. (2010). Microorganisms and Climate Change: terrestrial feedbacks and mitigation options. *Nature Reviews Microbiology*, 8, 779-790.