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Identifying the difference in bacterial composition and life history strategies between high versus low nutrient soils

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ABSTRACT

In this study, we aim to identify bacterial composition in soils treated with and without wood chips, as well as high levels and low levels of nitrogen. In order to do this, we used both culture dependent methods including plating, observing, and GenIII plates; as well as culture independent methods which include microbiome sequencing and EcoPlates. By doing this, we concluded that the bacterial communities differ between unamended and wood-chipped treated soils. Multiple differences were identified: rate of carbon consumption, bacterial diversity, rRNA gene copy number, and colony morphologies. Identifying the bacteria in wood-chipped soils will allow us to understand their effect on biogeochemical cycles, plant pathogens, and relationship with the trees (symbiotic/pathogen). Additionally, understanding the effect lower nitrogen levels have on the bacterial composition of soils can lead to reduction of synthetic fertilizer use, therefore lessening the environmental impact of nitrogen.

INTRODUCTION

Although there are many bacteria in soil (1,2) a large number of them are unculturable (3,4). Known as the “Great Plate Count Anomaly” (5), if a sample is observed directly from the soil using microscopy, it will have more cells than a sample grown on a petri plate in terms of colony forming units (CFUs) (6). Thus this causes the “Great Plate Count Anomaly” due to the discrepancy in direct cell counts of bacteria in comparison to counts based on culturing bacteria. This causes us to not be able to isolate and identify a large number of bacteria from soils; however, the culturable ones can still be compared.

Although a lot of bacterial identification is done through culture-dependent methods like the use of isolation and sequencing of specific genes or complete genomes and GenIII plates, culture-independent methods, like microbiome sequencing, are better in some aspects. Without underestimating the bacteria from culturing, culture-independent methods are able to more thoroughly characterize bacterial communities in soils. In this study, we specifically look at alpha diversity, the diversity in a specific sample, beta diversity, the diversity between two samples, and rRNA copy number, the amount of rRNA operons in a microbe.

Bacteria are often classified on a spectrum from copiotrophic to oligotrophic (7,8). Copiotrophic bacteria are adapted to high nutrient availability and are fast growing bacteria that have preferences for particular types of soil organic C (9). Specifically, they prefer glycine and sucrose (10). They exhibit higher growth rates when resources are abundant, therefore are considered r selected, as well as possessing higher rRNA copy numbers. This allows them to grow rapidly and quickly react to beneficial environmental changes (11). Oligotrophic bacteria are adapted to low nutrient availability, in addition to being slow growing bacteria that outcompete copiotrophs in lower nutrient availability due to having higher substrate affinities (9). Additionally, they tend to contain lower rRNA copy numbers because it leads to a selective advantage at low growth rates due to not wasting energy producing unnecessary rRNA (11).

A new agricultural management practice, referred to as whole orchard recycling (WOR), has brought many promising aspects. WOR is a multistep process where older trees are pushed over, grounded into wood chips, and incorporated into the top 12 inches of soil. Wood-chipping is promising mainly because of the environmental benefits it can have. Not only is it a carbon sequestering method, but can also lead to less nitrate and nitrous oxide emissions because of the excess carbon present in the soil causing the bacteria to hold onto the nitrogen. Some other benefits of WOR are that it: builds soil organic matter, improves nutrient retention in soil, and improves soil moisture (12). However, nothing is known about the effect this process has on the microbial diversity of soil.

The question we are trying to answer is: what are the differences between bacterial composition and their roles in the soil in wood-chipped versus conventionally managed soils? We are also trying to identify how the amount of nitrogen present in the soil affects the microbial communities. More specifically, for our culture dependent portion, our aim is to identify bacteria and their life history strategies through growing and culturing strains of microbes from the soil samples. For our culture independent portion, our aim is to use DNA sequencing and EcoPlate identification to characterize microbial community composition and infer their life history strategies by predicting rRNA copy number. My hypothesis is that under the high nutrient availability soils, wood-chipping and high nitrogen, there will be more copiotrophic bacteria isolated. I also hypothesize, from our culture independent portion, that the more copiotrophic bacteria will be present in the wood-chipped and high nitrogen soils.

MATERIALS AND METHODS

Culture dependent

Soil gathering and treatment

Soil samples were collected from block 92 at Kearney Agricultural and Research Extension center right after fertigation 15.0cm deep using a 5.0cm soil corer. Figure 1 indicates how each row of trees were treated. We had 12 different soil samples with a mix of wood-chipping, high nitrogen levels, low nitrogen levels, and unamended. Six of the twelve had high nitrogen, which is what the farmers currently use. The other six had low nitrogen, which is what we predicted to be the lowest amount of nitrogen needed by the orchards. Three of each of the nitrogen levels had a wood-chip, and an unamended treatment. See figure 2 for all treatments of samples.

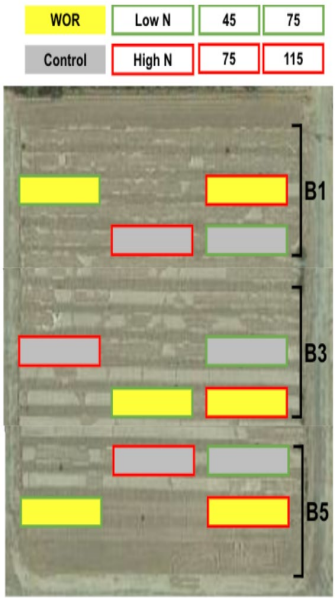

Figure 1 - Shows all the three blocks where trees were treated(yellow) or untreated(gray). The outline of the box, green(low), and red(high) show the amount of nitrogen that the row received.

Soil Sample ID	Block	Treatment	Nitrogen Treatment	Date Sampled
1460	1	Wood Chip	High nitrogen	6/1/2022
1461	1	Wood Chip	Low nitrogen	6/1/2022
1463	1	Control	High nitrogen	6/1/2022
1464	1	Control	Low nitrogen	6/1/2022
1466	3	Wood Chip	High nitrogen	6/1/2022
1467	3	Wood Chip	Low nitrogen	6/1/2022
1469	3	Control	High nitrogen	6/1/2022
1470	3	Control	Low nitrogen	6/1/2022
1472	5	Wood Chip	High nitrogen	6/1/2022
1473	5	Wood Chip	Low nitrogen	6/1/2022
1475	5	Control	High nitrogen	6/1/2022
1476	5	Control	Low nitrogen	6/1/2022

Figure 2 - Data table of samples and treatments received.

Media preparation

In order to identify both copiotrophic and oligotrophic bacteria, we prepared media with different nutrient availability using R2A. R2A contained: 0.5g of yeast extract, proteose peptone No. 3, casamino acids, dextrose, and soluble starch, 0.3g of: sodium pyruvate, dipotassium phosphate, 0.05g of magnesium sulfate, and 15.0g of agar. We made plates at 100%, 50%, 10% and 1% strength, adding extra agar to reach 15g L^{-1} to the 50%, 10%, and 1% media. To prevent fungal growth, all media were amended with 50 ug/mL cycloheximide. After preparation, plates were stored at 4˚C until used for inoculation of soil suspensions or plating of bacterial isolates.

Sonication, serial dilution, and inoculation

A soil suspension was prepared by adding 5 grams of soil to 45 mL of a 0.85% NaCl solution. The samples were then mixed with an orbital shaker for 2 hours at 200 rpm, then sonicated for 3 minutes at 300 W. The 12 samples were then serial diluted to 10^{-10}, however for the inoculation, we used only 10^{-3}, 10^{-5}, 10^{-8}, and 10^{-10} dilutions. During the inoculation, we pipetted 100 µl onto each plate of respective dilution level, and spread the liquid onto the whole plate. Plates were incubated at room temperature (25˚C) and monitored for growth as described below.

Growth monitoring and further isolation

Bacterial colonies were monitored daily for growth. When a plate colony had multiple colonies, we refrigerated them to inhibit further growth, and later isolated specific colonies. For strain isolation, we used a 1 µL loop, extracted some cells of a specific colony, and plated it by itself on another plate. We did this for every colony with different morphologies per each sample. For each isolation of a colony from the original plate, it was further isolated 1-2 times in order to be certain we had arrived at a pure culture for the identification using GenIII plates and DNA extraction. In order to correctly identify each strains, we named them on the formula #mAPP#D#C#. #m is the nutrient availability of the plate. 1m is 100%, 2m is 50%, 3m is 10%, and 4m is 1% R2A strength. APP# is the soil ID number seen in figure 2.2, like 1460. D# is the dilution factor, like 10^{-3}. C# is the colony number from the original plates

before isolation. Additionally, software ImageJ colony counter was used to count the colonies in all nutrient availabilities for 10^{-3} dilution levels.

GenIII plate setup

GenIII plates are 96 well plates with a variety of carbon sources in each well. GenIII plates are used in order to identify bacteria from a specific isolate based on the carbon sources they use. After getting pure cultures from earlier stages, we mixed bacteria with an inoculating fluid, IFA, which we then tested in a turbidimeter aiming for a 95% transmittance from the original 100%, which means bacteria blocked 5% of the wavelengths transmitted. Once enough bacteria was in the solution, we pipetted it onto the GenIII plates and incubated them at 28˚C for 36 hours. The individual bacteria strains consumed the carbon sources and changed the color of the solution because of the tetrazolium dye. The change in color was measured by the OmniLog and correlated to the consumption of carbon. Statistical language R was used to interpret the consumption rates.

DNA extraction of colonies and qPCR

We took 16 pure cultures isolated in earlier steps and extracted their DNA with a PureLink kit. Figure 3 shows which isolates we used in the DNA extractions. DNA was extracted by lysing the bacterial cells with proteinase K, passing those cells through a spin column in which DNA gets binded to, flushing out other molecules that are mixed in with the DNA, and then removing the DNA from the spin columns. Once we obtained our DNA, we performed qPCR in order to quantify the 16S rRNA gene using primers 338F and 518R at a concentration of 0.5 µM. We also performed qPCR on the rpoB gene with the same bacterial isolates using primers Univ_rpoB_F_deg and Univ_rpoB_R_deg at a concentration of 0.8 µM. In order to make sure all initial DNA amounts were similar, we used a nanodrop to measure the concentration, ng/µL, of our genomic DNA, then diluted the DNA with Qiagen elution buffer to equal proportions.

DNA Plate label	Isolate identity
#1	1M1472D3C1
#2	1M1473D3C3
#3	2M1476D5C2
#4	1M1463D3C3
#5	3M1473D3C1
#6	2M1460D5C1
#7	2M1470D5C4
#8	1M1473D3C1
#9	3M1461D3C3
#10	1M1461D3C3
#11	1M1467D5C4
#12	2M1475D5C1
#13	2M1467D5C5
#14	2M1464D5C1
#15	1M1470D5C2
#16	1M1464D5C7

Figure 3 - Data table showing the soil samples we decided to extract for DNA.

Culture independent

Microbiome sequence analysis

In addition to culturing, we also analyzed microbiome sequencing data sets from the samples. These data sets were generated by DNA extraction and amplification of the 16S rRNA gene v4 region using primers 515F and 806R. Samples were pooled to create a library that was sequenced using illumina MiSeq. Amplicon sequence variants (ASVs) were called via dada2 and the taxonomy of ASVs assigned using the Silva version 138 database. This is where we picked up and coded in R. We used the main package "phyloseq", but also an array of smaller packages like: HillR, vegan, ggplot2, tidyverse, rstatix, ggpubr, and tidyr. All of these packages were used to analyze and graph sequence data, from 2020 and 2021. We specifically looked at alpha diversity, beta diversity, and rRNA copy number.

Eco plate set up: community level physiological profiling

EcoPlates are 96 well plates with different carbon sources every 4 columns, as opposed to every well in genIII plates. This allows us to look at carbon utilization based off of the whole community, or soil sample in our case. To set this up, each soil sample was mixed with 0.85% NaCl in order to make an initial 10^{-1} dilution. The 10^{-1} dilutions were put in an orbital shaker for 2 hours at 200 rpm, rested for 30 minutes to let the solids fall to the bottom, and pipetted into another 0.85% NaCl solution to form a 10^{-2} dilution. The 10^{-2} dilution was then pipetted into an eco plate. Each soil sample received its own ecoplate. Once all plates were full, they were incubated in the OmniLog, at 28˚C and observed for 168 hours in 15 minute intervals. When the carbon sources are consumed, the solution changes into a purple color, and the OmniLog records the change in color to quantify the amount of carbon consumed.

RESULTS

Culture dependent

Plate growth

The different treatments, dilutions, and nutrient availability plates had differentiable growth. Plates on the higher end of the nutrient availability, 100% and 50%, but at the same dilution level, 10^{-3}, had faster initial growth and more colonies, than those in the lower end of the nutrient availability 10%, 1%. These results are exemplified in the comparison between figure 4 and 5. The figures show the same soil sample (unamended, high nitrogen), dilution, but different nutrient availability taken 1 day into the growing phase.

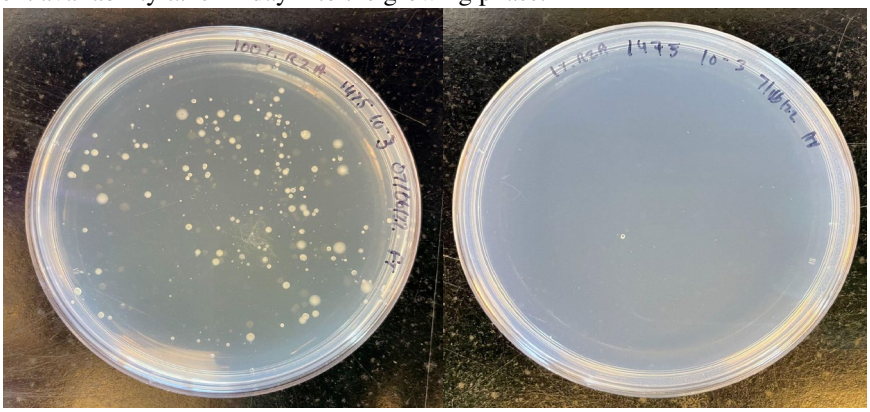

Figure 4(left) - 100% R2A 1475(unamended, high nitrogen) 10^{-3} dilution. The little white/yellow circles are bacterial colonies. Figure 5(right) - 1% R2A 1475(unamended, high nitrogen) 10^{-3} dilution. There is no growth. The little white dot in the middle is an air bubble.

In addition, plates which were further diluted had less colonies and slower growth. Seen in the comparison between figure 6 and 7, both plates have the same nutrient availability, same soil sample (control and high nitrogen), but different dilutions.

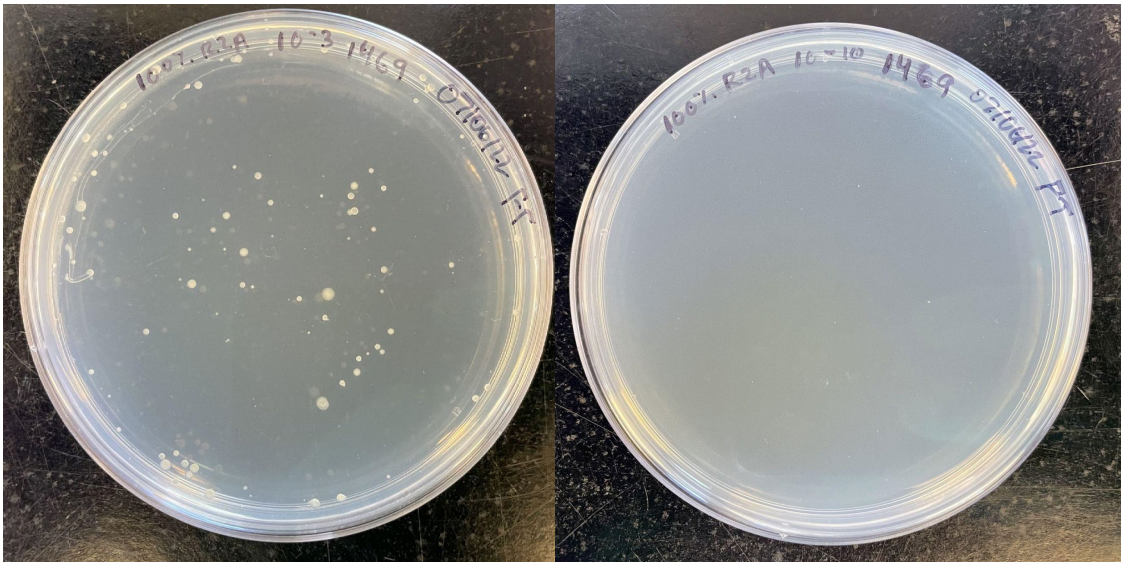

Figure 6(left) - 100% R2A 1469(control, high nitrogen) 10^{-3}. The little white/yellow circles are bacterial colonies.
Figure 7(right) - 100% R2A 1469(control, high nitrogen) 10^{-10}. There is no growth

Furthermore, when growth did occur in the lower nutrient availability media the colony morphologies were different. Under higher nutrient availability, the colonies tended to be a yellow/white mix, but in the lower nutrient availability, the colonies were white/transparent. Also, the colonies in smaller nutrient availability were smaller. Finally, in the higher nutrient availability we observed a greater variety of colony morphologies. This is evident in the comparison between figure 8 and 9, both plates are the same soil sample(wood-chip, high nitrogen) and dilution, but different nutrient availability.

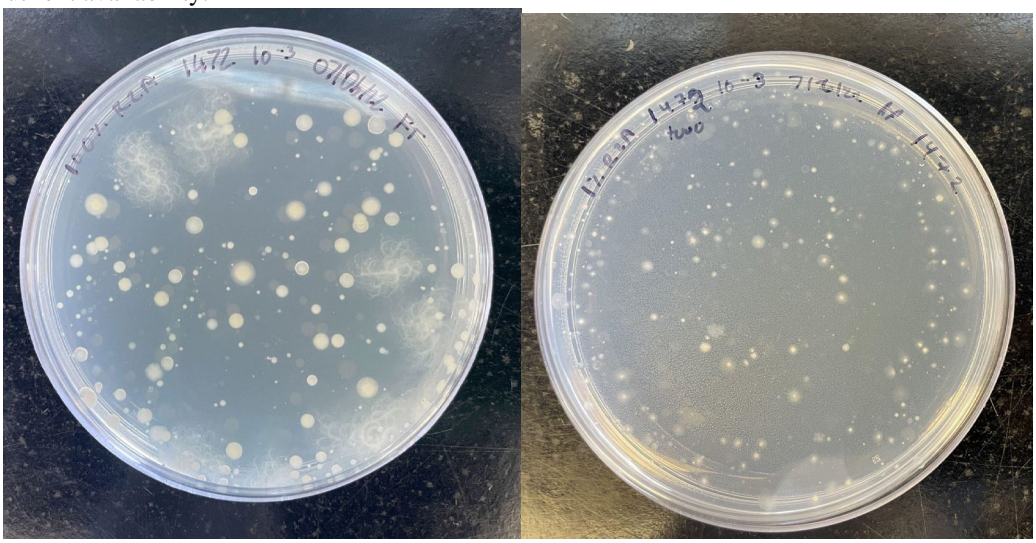

Figure 8(left) - 100% R2A 1472(wood-chip, high nitrogen) 10^{-3}. There are multiple colonies growing of different morphologies and sizes. Some are the white/yellow dots, but the white/yellow clouds are another different morphology. Colonies are of varying sizes
Figure 9(right) - 1% R2A 1472(wood-chip, high nitrogen) 10^{-3}. Multiple colonies growing, but of similar morphologies. They are all white/transparent and much smaller in size.

Lastly, lower nutrient availability plates had more growth after a longer period of time. Colony counter data, seen in figure 10, shows the colony forming units per gram of soil for each treatment in different nutrient strength media. CFUs were calculated by converting colonies observed to CFU per mL, then multiplying it by the dilution level, 10^{-1}, to convert it to grams. For the 1% plates, the colonies were counted 2 weeks after the 100%, 50%, and 10% in order to allow the oligotrophic bacteria to grow.

CFUs per g of soil in different nutrient strength media.

Media	Main Treatment	Fertilization Treatment	CFU per g soil ± SD
100% R2A	Control	High Nitrogen	1.04E+07 ± 1.20E+05
100% R2A	Control	Low Nitrogen	6.77E+06 ± 2.94E+05
100% R2A	Wood Chip	High Nitrogen	9.23E+06 ± 2.67E+05
100% R2A	Wood Chip	Low Nitrogen	9.00E+06 ± 7.19E+05
50% R2A	Control	High Nitrogen	8.67E+06 ± 3.35E+05
50% R2A	Control	Low Nitrogen	8.43E+06 ± 3.62E+05
50% R2A	Wood Chip	High Nitrogen	8.97E+06 ± 2.73E+05
50% R2A	Wood Chip	Low Nitrogen	8.73E+06 ± 2.28E+05
10% R2A	Control	High Nitrogen	5.00E+06 ± 1.90E+05
10% R2A	Control	Low Nitrogen	4.43E+06 ± 1.54E+05
10% R2A	Wood Chip	High Nitrogen	6.33E+06 ± 1.50E+05
10% R2A	Wood Chip	Low Nitrogen	4.93E+06 ± 3.45E+05
1%R2A	Control	High Nitrogen	1.43E+07 ± 9.54E+04
1%R2A	Control	Low Nitrogen	1.23E+07 ± 1.02E+06
1%R2A	Wood Chip	High Nitrogen	1.84E+07 ± 2.71E+05
1%R2A	Wood Chip	Low Nitrogen	1.36E+07 ± 2.49E+05

Figure 10 - Data table showing the CFUs per g of soil in different nutrient strength media. Also includes treatment for the different colony amounts.

qPCR results for 16S rRNA gene and rpoB gene

Once the Ct values from the qPCR targeting the 16S rRNA gene (potentially multiple copies) values were calculated, they were subtracted from the Ct values from the qPCR targeting the rpoB gene (single copy gene). Since three wells were ran for each sample for each gene, we took the mean of each subtraction and tablified it into figure 11, which shows the 16 different isolated strains and the Ct difference between the 16S rRNA gene and the rpoB gene. The higher the value for Ct difference, the more rRNA gene copies they have compared to the single copy of rpoB.

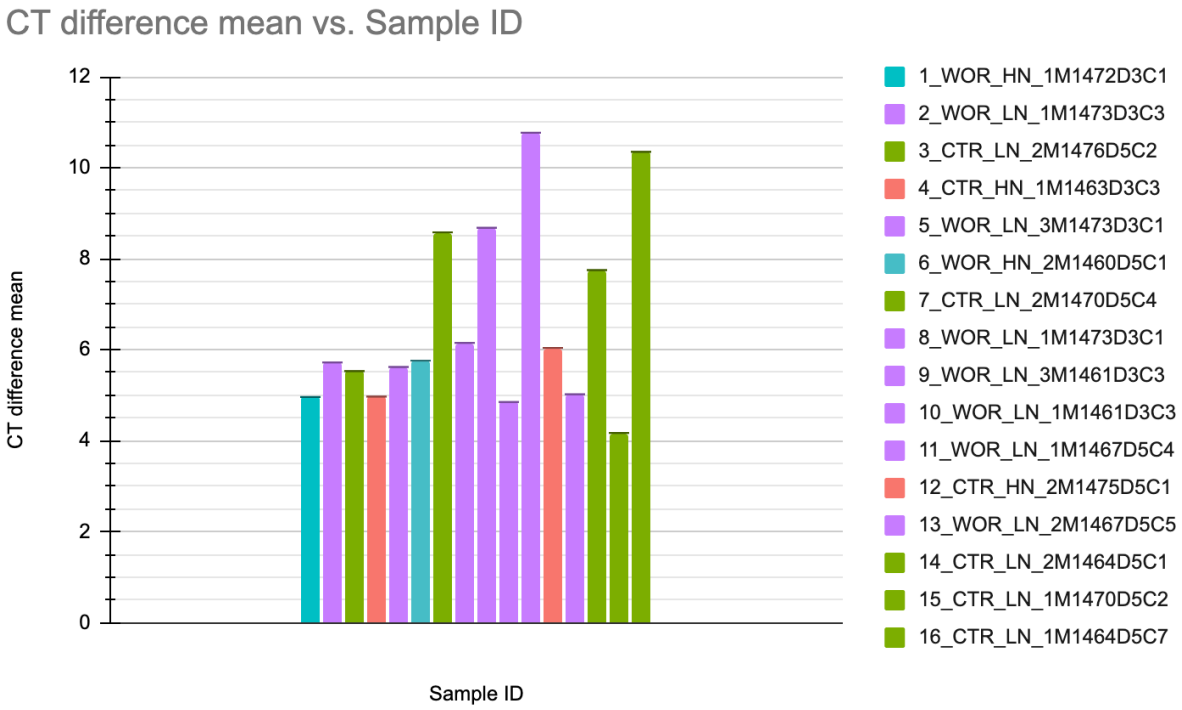

Figure 11 - This graph shows the ct difference mean for each sample ran in the qPCR. The different colors of bars correlate to different treatments. Green correlates to control, low nitrogen soil treatments. Salmon correlates to control, high nitrogen soil treatments. Purple correlates to wood-chipped, low nitrogen soil treatments. Turquoise correlates to wood-chipped, high nitrogen soil treatments. The higher the CT difference is, the farther it was from the ct value of the single copy rpoB gene; therefore, the more 16S rRNA copy numbers it have. This data shows comparatively how many more copies, than one, does a bacteria have for the 16S rRNA gene.

GenIII plate identification

Of the 16 isolates observed in the OmniLog, only four were able to be identified. Results were tablified into figure 12, which shows the different strains we isolated in the OmniLog as well as the treatment these strains received. It also shows the OmniLog species identifications, the morphology of the bacteria, and its rRNA count. 11 of the other isolates were unable to be identified due to the OmniLog database not recognizing its carbon consumption. This is because they are most likely novel taxa that need to be sequenced and identified. The last result was a false positive, likely due to an over inoculation of the sample causing too much carbon consumption in the wells; however, it was most likely a Paenibacillus species. Finally, by cross referencing species to the rrn database, we were able to acquire the average rRNA copy numbers for each bacteria identified.

Species Identified in OmniLog

Strain	Species ID	Organism Type	Treatment	rRNA copy number
2M1470D5C4	Paenibacillus wynnii	GP-Rod-SB	CON + LN	10
2M1467D5C5	Leuconostoc gelidum ss gelidum (26C)	GP-Coccus	WOR + LN	4
1M1467D5C4	Massilia aerilata	GN-Nent	WOR + LN	9
1M1472D5C3	Paenibacillus lautus	GP-Rod-SB	WOR + HN	10

Figure 12 - Data table of bacteria species identified in Omnilog. Organism type describes the type of organism; GP means gram positive, GN means gram negative, Rod and Coccus are the shape of the bacteria, Nent means non-enteric, and SB means spore-forming bacillus. The treatments from which the strains come from are also included in the data table. The last column indicates the average rRNA operon copy

Culture independent

Microbiome analysis

The alpha diversity analyzed through R was graphed onto a box plot seen in figure 13. The higher the number on the Y-axis, the higher the diversity in terms of that specific index. The 4 measures of alpha diversity (ASV richness, Shannon index, inverse Simpson index, and Simpson Evenness index) all have overlapping error bars; therefore, diversity tends to be similar between wood-chipped and control soils.

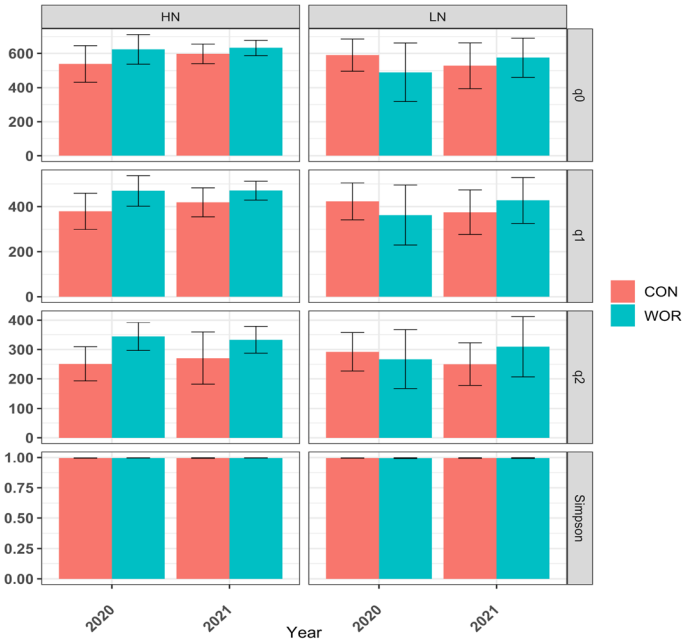

Figure 13 - X-axis shows the different years in which the soil samples were harvested. Y-axis shows the diversity of the sample. The graphs show how diverse the soil samples were in wood-chipped vs. control soil, as well as high and low nitrogen levels. The sub-sections q0, q1, q2, and Simpson are different measures of alpha diversity. q0 is the ASV richness. q1 is the Shannon index. q2 is the inverse Simpson index. Simpson is an evenness index. Although the wood-chipped soils mostly had more average diversity, since the error bars overlap, there is not a large enough statistical difference to make any observations.

Beta diversity was also analyzed through R and graphed into a coordinate system seen in figure 14. The closer two points are to one another, the more similar they are. The grouping of wood-chipped compared to unamended samples on both principal coordinates 1 and 2 identifies a difference in the biodiversity between those samples. For wood-chipped samples, there was a difference in grouping for 2020 and 2021 samples; however, nitrogen concentration had no effect on the grouping. For unamended soil samples, neither year nor nitrogen concentration had an effect on the grouping of samples.

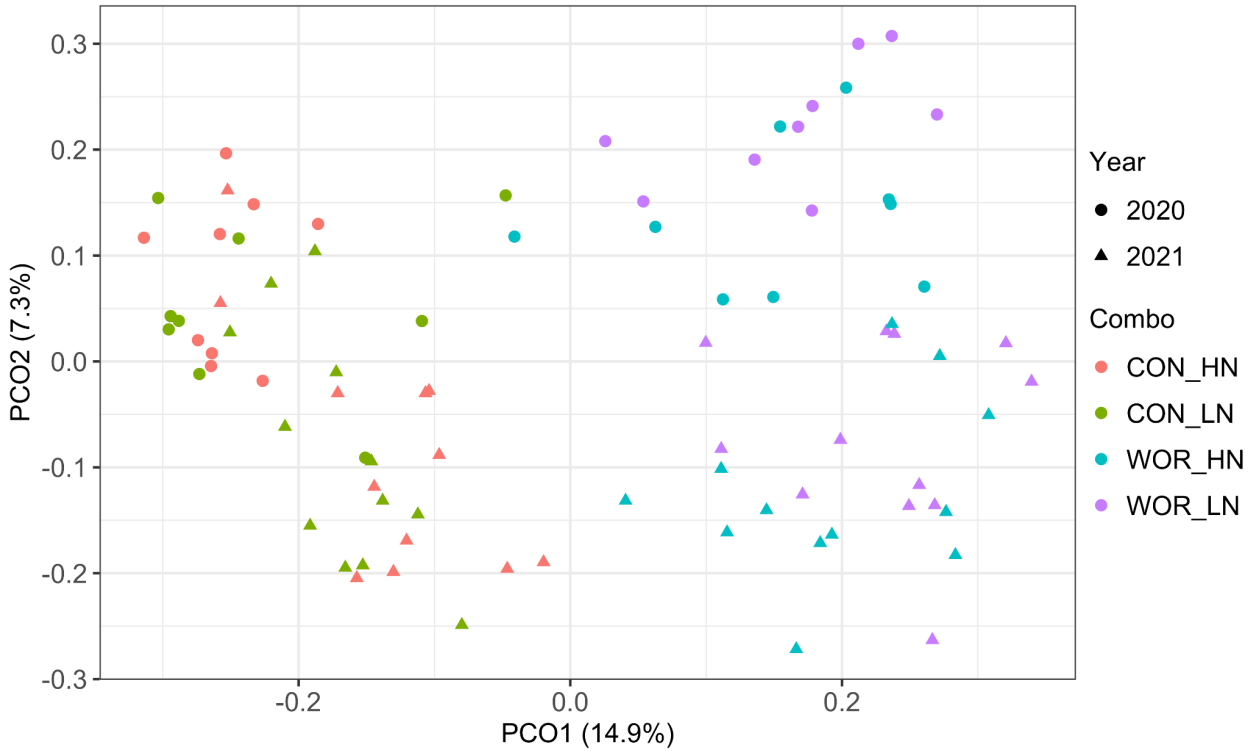

Figure 14 - X-axis shows the placement of the sample on principal coordinate analysis 1 in which 14.9% of variation is explained. Y-axis shows placement of the sample on principal coordinate analysis 2 in which 7.3% of variation is explained. Based on the legend, samples were grouped differently based on the treatment they received, wood-chip and unamended, and the year they were taken.

The final analysis through R was for rRNA copy number. We graphed the rRNA operons for each microbe sequenced from taxa levels Phylum to Genus. The clearest two graphs are rRNA copy number in the Phylum level for microbes in the soil, figure 15, and in the Order level, figure 16.

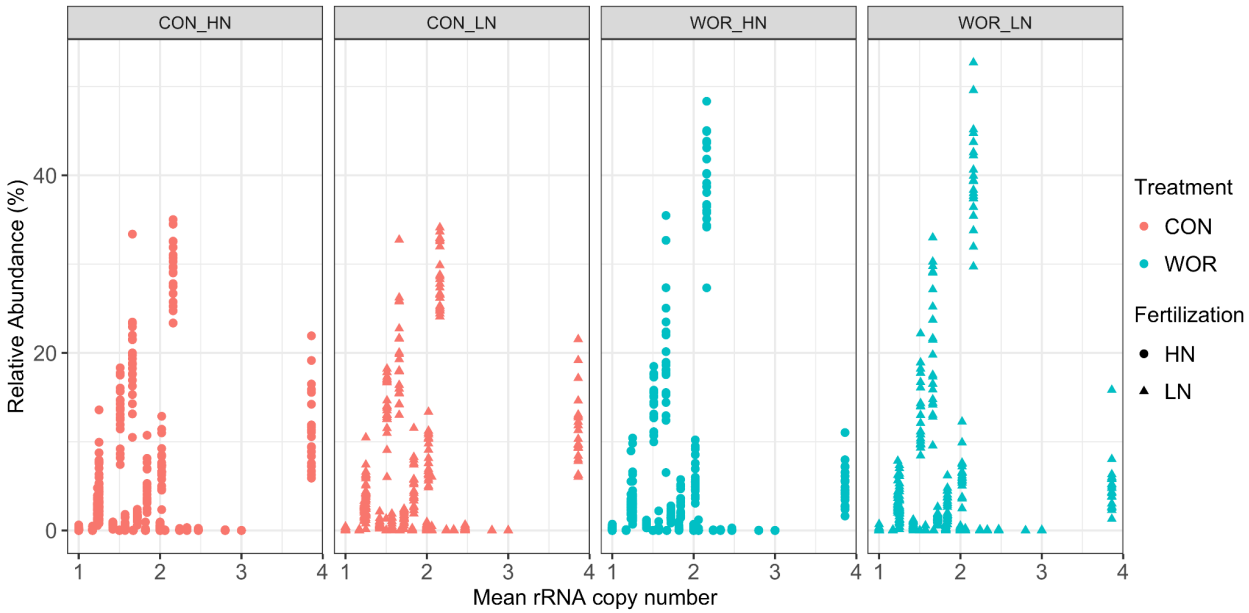

Figure 15 - Phylum taxa level graph for mean rRNA copy number, X-axis. Y-axis is the relative abundance percent of each taxa in the sample. Wood-chipped samples had more abundance around 2 rRNA copy numbers, while control samples had less relative abundance around 2 rRNA copy number, but more in the higher range, around 4.

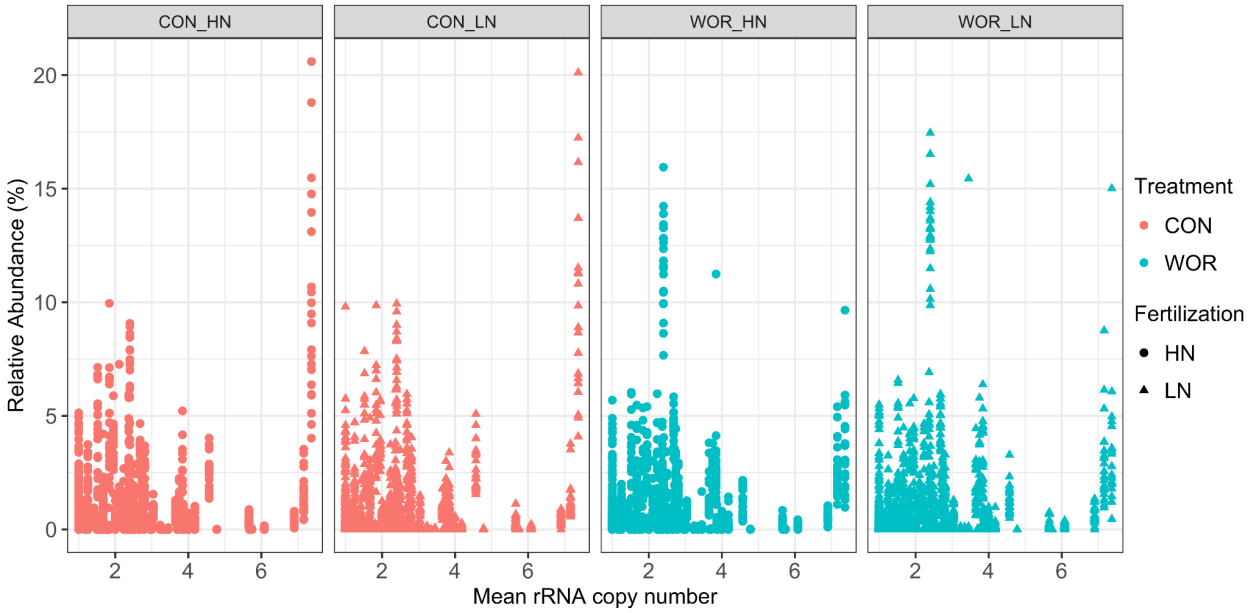

Figure 16 - Order taxa level graph for mean rRNA copy number, X-axis. Y-axis is the relative abundance percent of each taxa in the sample. Once again, wood-chipped samples had the majority of its clustering around 2 rRNA copy number, with a little peak around 8; however, the control samples had less of a peak at 2, but a far greater abundance around 8 rRNA copy numbers.

EcoPlate identification

Bacteria in EcoPlates grown in the OmniLog consumed multiple carbon sources from each well. The large multitude of carbon sources were consumed at a variety of rates by the bacteria from soil samples (figure 17).

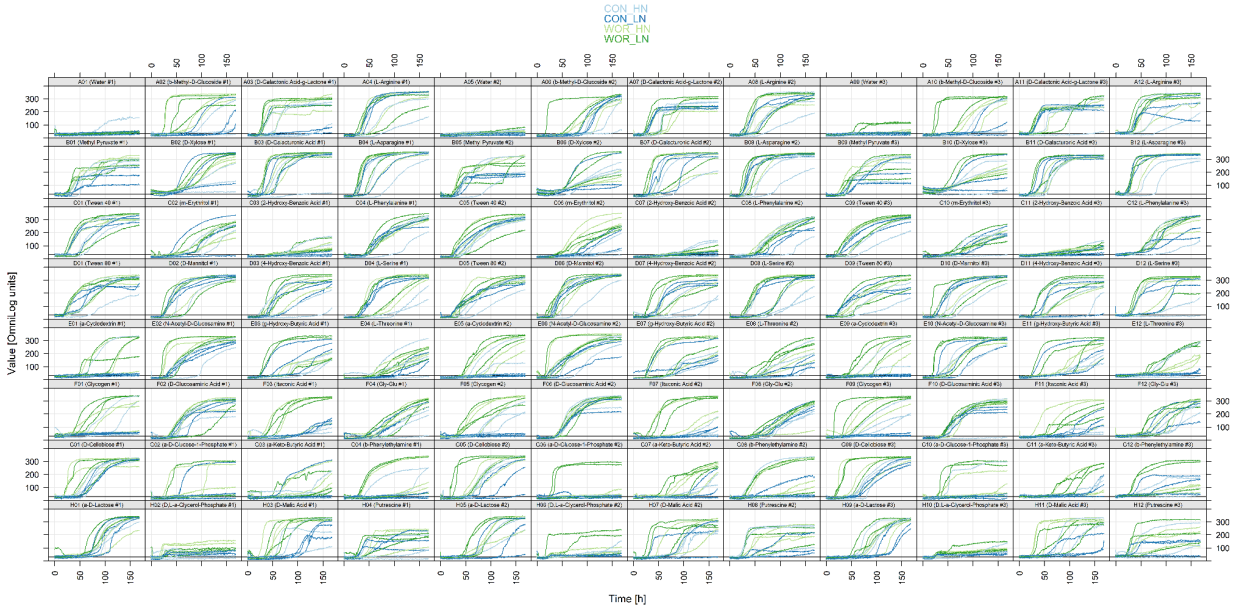

Figure 17 - Combination of graphs from each well in the EcoPlate. Shows the consumption of the carbon source over time for all the different strains of bacteria.

Two examples, seen in figure 18 and 19, show bacteria from wood-chipped samples consuming a carbon sample that the controls could not or not as efficiently. Similar trends were seen throughout every sample. Wood-chipped bacteria always consume the Carbon source at the same rate as unamended bacteria, or faster.

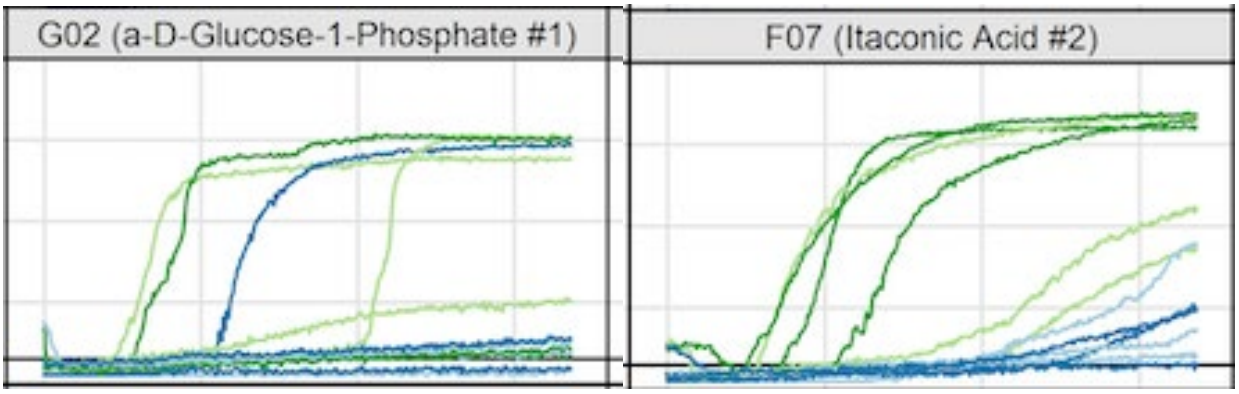

Figure 18(left) - Graph shows a-D-Glucose-1-Phosphate consumption over time. X-axis shows time, each vertical tick is 50 hours. Y-axis shows Glucose consumption based on the tetrazolium dye turning purple. The green lines are bacteria from wood-chipped soils, more specifically, dark green is low nitrogen and light green is high nitrogen. The blue lines are bacteria from unamended soils, more specifically, dark blue is low nitrogen and light blue is low nitrogen. The bacteria from wood-chip samples were the only ones that consumed the carbon sample and they did so quickly, in under 50 hours, before plateauing from lack of nutrients

Figure 19(right) - This graph shows itaconic acid consumption over time. X-axis shows time, each vertical tick is 50 hours. Y-axis shows itaconic acid consumption based on the tetrazolium dye turning purple. The green lines are bacteria from wood-chipped soils; dark green is low nitrogen and light green is high nitrogen. The blue lines are bacteria from unamended soils; dark blue is low nitrogen and light blue is low nitrogen. The bacteria from wood-chipped samples were once again able to consume a carbon source unamended soils were not able to.

Although compound cellobiose, closely related to cellulose introduced in wood-chipping, was consumed by all bacteria, wood-chipped bacteria were more efficient at consuming it. Figure 20 exemplifies D-Cellobiose consumption by bacteria from wood-chipped and unamended soils.

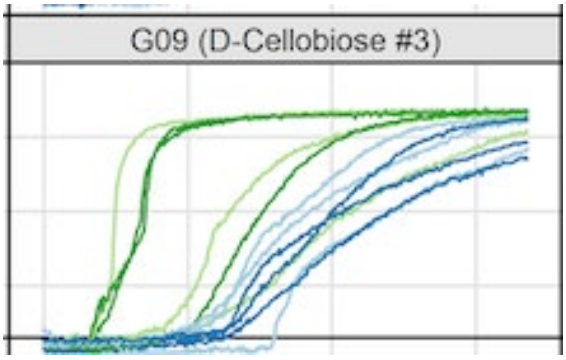

Figure 20 - This graph shows D-Cellobiose consumption over time. X-axis shows time, each vertical tick is 50 hours. Y-axis shows D-cellobiose consumption based on the tetrazolium dye turning purple. The green lines are bacteria from wood-chipped soils; dark green is low nitrogen and light green is high nitrogen. The blue lines are bacteria from unamended soils; dark blue is low nitrogen and light blue is low nitrogen. The bacteria from wood-chipped samples consumed the carbon source faster than bacteria from unamended soil.

Finally, the principal coordinate analysis of the EcoPlate data, figure 21, shows different groupings of soil treatments based on carbon consumption. Control low nitrogen soil samples grouped together, as well as wood-chipped samples in general.

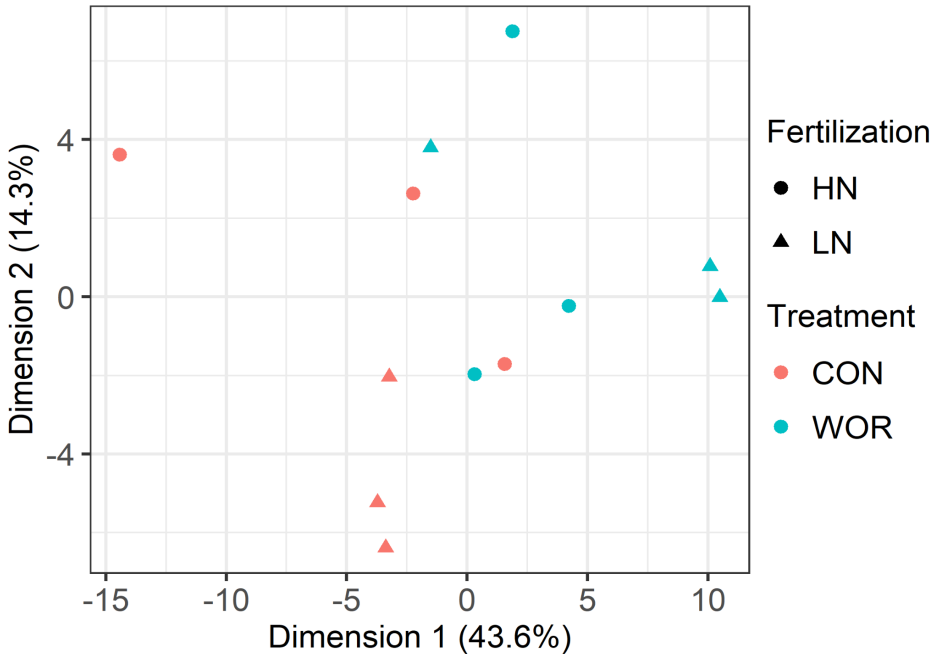

Figure 21 - Principal coordinate analysis of carbon consumption from EcoPlate data. The two axes are different dimensions the data was graphed on. 43.6% of the variation is explained in dimension 1. 14.3% of the variation is explained in dimension 2. Circles represent high nitrogen samples, while triangles represent low nitrogen samples. Salmon represents control samples, while turquoise represents wood-chipped samples. Samples are grouped differently based on the similarity to each other in terms of carbon consumption.

DISCUSSION

qPCR results for 16S rRNA gene and rpoB gene show higher rRNA copy numbers for control soils

The gene rpoB is used as it is a known single copy gene. Since the 16S rRNA gene copy numbers are unknown for the bacteria we cultured, we assumed that a lower Ct value for 16S rRNA gene in comparison to rpoB would indicate an isolate with multiple copies of rRNA genes. Isolates from control soils had higher mean CT differences, thus indicating their copy numbers are higher. We infer that control groups are more copiotrophic because high rRNA copy numbers are a key trait of copiotrophic bacteria (11). Nonetheless, we cannot conclude wood-chipped samples are oligotrophic, as mean CT differences were in the middle range, indicating they had a median rRNA copy number.

Higher nutrient density plates result in faster growing copiotrophic bacteria

Bacteria that quickly grew in plates were the ones isolated and used for GenIII identification due to time constraints. The growth occurred mainly in 100% R2A plates, seen in the pictures earlier. We streaked those plates for isolation 2.5 weeks after their initial inoculation. This explains the fact that the GenIII plates identified bacteria with higher copy numbers, thus most likely copiotrophic bacteria. Because GenIII plates were only able to identify 4 bacteria, the sample size is not large enough to make any inferences.

Lower nutrient density result in slower growing oligotrophic bacteria

The lower nutrient density plates grew slower, seen in the initial plate pictures, but when looked at after a month, their colony numbers surpassed the original copiotrophic plates. By using the colony counter data, we can conclude that lower nutrient density plates cause more oligotrophic bacteria to grow, but only after a longer period of time. This correlates exactly with past research indicating that oligotrophic bacteria take a longer time to grow and form colonies(8).

Microbiome analysis shows diversity between samples, but overall population diversity is similar

Because of the overlapping error bars in the alpha diversity graph, there is no difference in overall diversity of samples; however, the different groupings of soil treatments seen when looking at the principal coordinate analysis ordination of the Bray-Curtis index indicates a difference between samples. From this, we can conclude that there are differences in the diversity of bacterial species between wood-chipped and unamended soil samples, but the overall taxa diversity of the samples are the same.

Bacteria from wood-chipped soils are oligotrophic

When looking at the results of the rRNA copy number analysis, we identify that control soil samples have higher microbial rRNA copy numbers. Since this is a trait of copiotrophic bacteria (11), it can be assumed there are more copiotrophic bacteria in regular soil samples compared to wood-chipped ones. This goes against my hypothesis of more copiotrophic bacteria being in wood-chipped soil due to the higher nutrient levels, instead proving that wood-chipped soils seem to have more oligotrophic qualities. Although we are not exactly sure for the reason behind this, we believe that the addition of rare carbons – from wood-chips – creates a large niche for oligotrophic bacteria to thrive in; thus when looking at rRNA copy number, we see lower copy number in wood-chipped soil, indicating more oligotrophic bacteria.

Wood-chipping increases diversity of bacterial carbon consumption

All the different rates of consumptions for the carbon sources show there must be different bacteria in the wood-chipped, unamended, low nitrogen, and high nitrogen samples. Not only is this seen through the separate groupings of control and wood-chipped soils, in the principal coordinate analysis, but also when specifically looking at glucose and itaconic acid consumption, wood-chipped soil bacteria consumed them, while the unamended samples rarely consumed them and when they did, it was at slower rates. Because this trend is seen in all carbon sources observed, we believe that this difference is due to selection. Bacteria that can break down the broad range of carbon sources are selected over ones that cannot, thus changing the bacterial composition of the soils compared to unamended ones when wood-chips are integrated into the soil.

Additionally, the adaptation of the microbial community, seen in wood-chipped soil to carbon source cellobiose, is another indicator of the direct impact that wood-chipping has on bacterial composition. Bacteria that could better fit the niche of cellulose breakdown were selected over the broader nutrient consuming bacteria in unamended soils.

Lower nitrogen levels have no negative impact on microbial composition

One of the purposes of this study was to identify if lowering nitrogen concentrations would have negative effects on the bacterial composition of soils. Although past research has pointed towards lower nitrogen stinting plant growth (13), we found no indication that nitrogen lowered the diversity of soil microbiomes. The microbiome of soil is key to plant growth (14); therefore, the no effect of diversity, seen in alpha diversity analysis, of experimentally low nitrogen levels indicates we can lower nitrogen levels in soils. This will lead to less negative environmental impact of overapplication of synthetic fertilizers.

Conclusion

From all of this data, we conclude that there are multiple differences in the bacterial composition of wood-chipped and unamended soil samples. The first is that bacteria from wood-chipped soils lean more towards the oligotrophic side of the spectrum, making my original hypothesis incorrect. They also consume more carbon sources than unamended soil bacteria due to a different species diversity.

Additionally, less nitrogen can be used in soils. Throughout this study, lower nitrogen and high nitrogen samples were used, and no harmful impact on microbial communities in terms of diversity or nutrient consumption were found; therefore, we can lower our use of synthetic fertilizers without any drawbacks. Not only are there no drawbacks, but we will reduce the negative impact of over-applying fertilizers. It will lower the release of harmful greenhouse gas, nitrous oxide, from bacteria, as well as reduce nitrate leaking into groundwater through percolation (15).

The overall application of this study is that understanding how microorganisms affect nutrient cycling will allow us to better develop nutrient management. Understanding bacterial effects on biogeochemical cycles, like the nitrogen and carbon cycles, is just the first step in understanding how to best supplement these bacteria in order to have the best yields in all agriculture.

Further research

Future research done from this study can be on why the bacterial species that arise from wood-chipping soils are oligotrophic. Our study suggests that wood-chipping leads to more oligotrophic bacteria, but being able to understand why would help in understanding our effects on the bacterial microbiome and better manage the nutrients in the soil.

Furthermore, more research can be done in identifying the species of bacteria in wood-chipped soils. As our OmniLog testing only identified four species, having a larger understanding of the culturable bacterial species would help in analyzing their specific effects on the soil microbiome of wood-chipped and unamended soils.

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