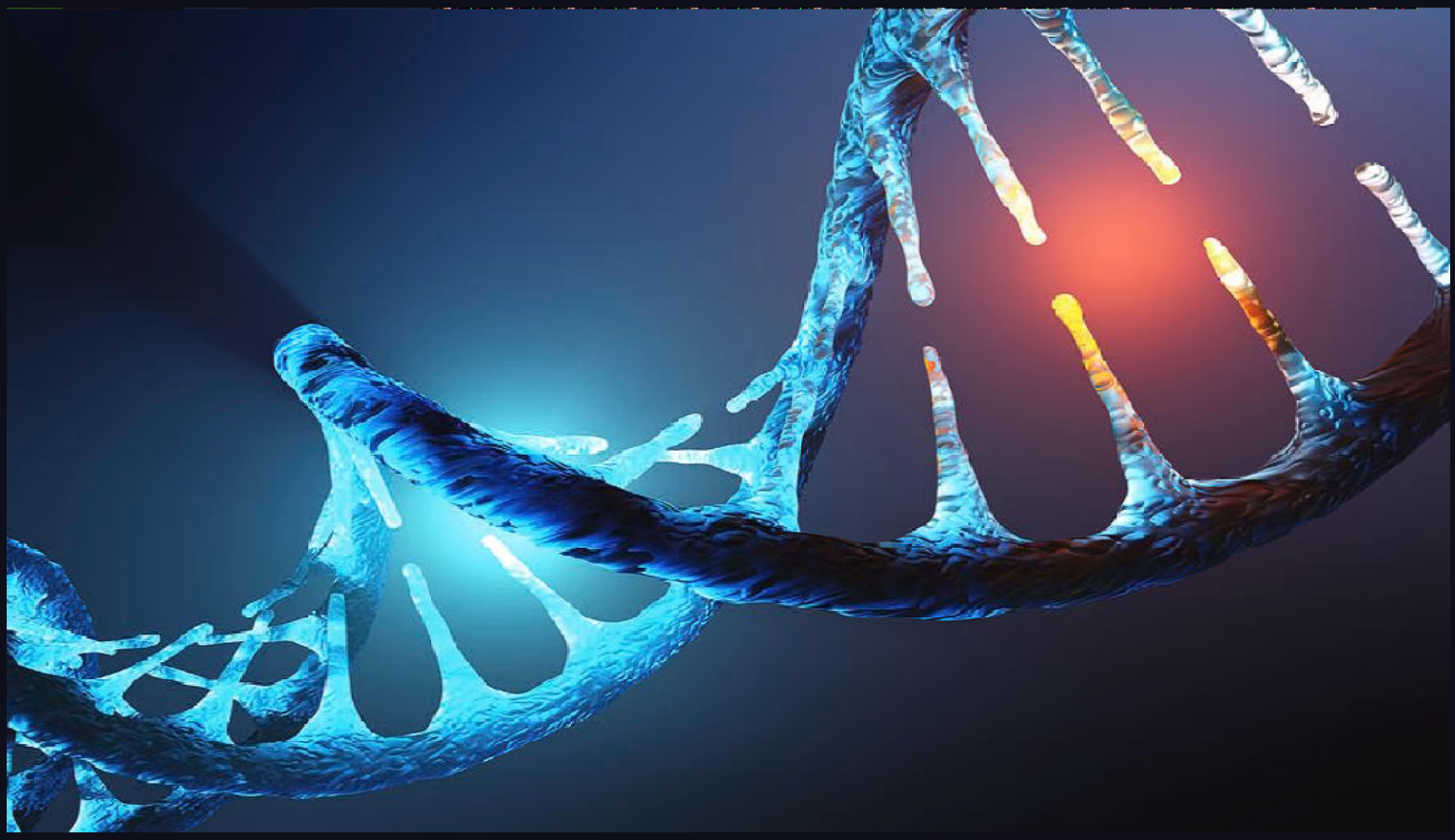


BioLogix

Genome Labs

Soil Analysis Report

Mt. Boucherie Estate Winery-Field Trial

Introduction

Bacteria make up the largest number of microorganisms in soil, as well as the largest biomass or weight. Therefore, bacteria account for approximately 99% of DNA found in soils. By doing direct soil DNA extractions, an approximation of the bacterial presence in any soil can be made, thus determining whether a soil is providing the best possible environment to the cultivated crops as well as promoting the best possible plant growth.

Why is Bacterial Life Important for Soils

Bacteria in soils are responsible for:

- Regulating nutrient availability,
- Stabilizing aggregate molecules,
- Carbon sequestration
- Degradation of pollutants
- Promotion of plant health and growth
- Cycling of trace gases such as nitrous oxide, nitric oxide, carbon monoxide, hydrogen and reduced sulfur species

There are 9 main phyla of bacteria found in soils with the most prominent being the following:

- **Proteobacteria:** are found in abundance in soils and are speculated to account for approximately 50% of the bacterial presence in soils. They are of great importance in soils because they have been found to be significantly responsible for cycling carbon, nitrogen, and sulfur.
- **Acidobacteria:** may account for approximately 13% of the bacterial presence in soils. They have been observed to promote plant growth and provide beneficial relationships among plants. It is also considered that this bacterial type is involved in regulation of various life cycles.
- **Actinobacteria:** are believed to be the third most abundant bacterial type in soils. This bacterial group is comprised of a broad range of hydrolytic enzymes; thus they are said to be of great importance to Eco-physiological processes such as plant residue decomposition and soil carbon sequestration.

Figure 1 (Fig.1) shows these, and all the other bacterial phyla commonly found in soils and their approximate populations.

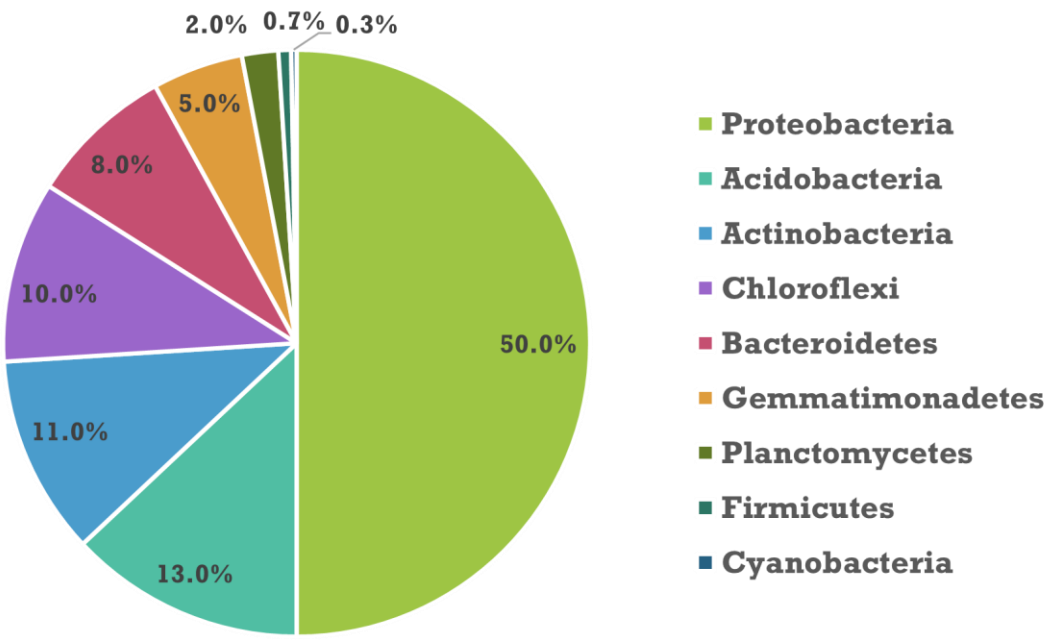


Figure 1. Approximate population percentages of the most common and prominent bacterial phyla found in agricultural soils according to scientific journals Reference.

Procedure

We performed a field trial at Mt. Boucherie Winery property located in Similkameen valley, British Columbia. Through conversations with Mr. Craig McCulloch and Mr. Jeff Hundertmark, a number of fields that have been presenting growth concerns were selected for testing.

From each selected plot, 9-12 samples of soil were taken 6 inches from the surface, as close to the stem as possible (environment known as the rhizosphere). The soil from each sample plot was placed in one bag and mixed thoroughly to obtain a fully representative sample. DNA was then extracted in our lab from each soil sample and used to determine the Total Life of Soil via DNA concentrations per 0.2grams of soil and bacterial cell population per 0.2 grams of soil.

The extracted DNA was then used for quantitative polymerase chain reaction (qPCR) tests. Approximately 50±1 ng of DNA was used as the standard quantity for each reaction. Primers for detecting the three major types of soil bacteria, Proteobacteria, Acidobacteria, and Actinobacteria (Fig. 1) were obtained from scientific literature and used to amplify each bacterial type using the SYBR fluorescence kit for qPCR. The raw data generated by the PCR instrument was analyzed and summarized in the results section. The raw data graphs are reported in the Appendix of this report.

Results

Total Life Analysis: Direct Soil DNA Extraction

A DNA extraction from each soil sample was done. Each sample was measured twice via Nano Drop and then averaged. The results are reported in Figure 2 (Fig. 2). All concentrations are reported in nanograms/microliter (ng/ul) of DNA in 0.2 grams of soil.

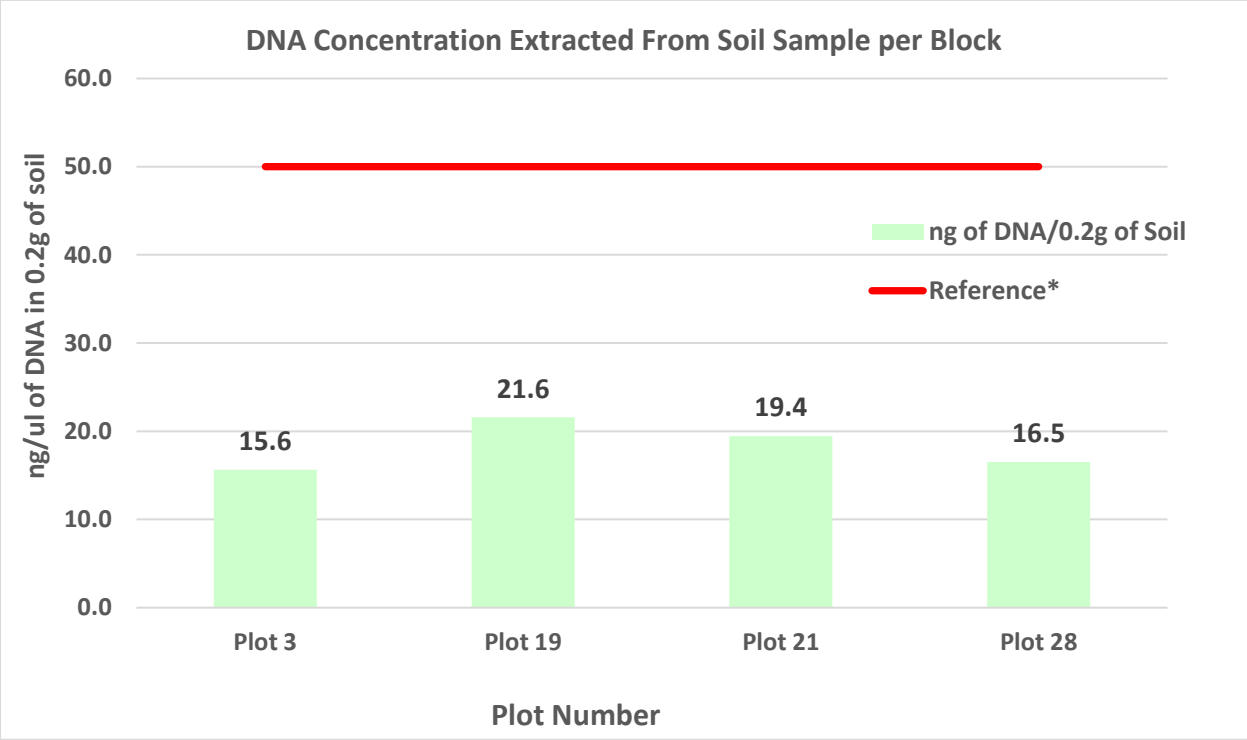


Figure 2. Mt. Boucherie Plots sampled.

*Please note that the reference value is a recommended parameter selected by BioLogix based on literature, experience, and observation and is subject to change. Once the entire property is tested a more representative value of the land may be applied.

Soil samples for Mt. Boucherie were taken in February therefore no observation of plant growth could be made for comparison with the DNA extraction results. We have set a threshold for healthy productive soil at 50ng/ul in 0.2gr of soil, indicated by the red line in Figure 2 (Fig. 2), based on literature, experience, and observations made across the Okanagan valley soils already tested. The values obtained for the Total Life analysis reported are for the most part, consistent with the information obtained from the staff at Mt. Boucherie with respect to the low yields obtained from the plots tested. It was indicated that for 2022 the yields for these plots were of 2.0, 2.5, 2.5, and 2.2 tons per acre for plots 3, 19, 21, and 28 respectively.

When the yield numbers are compared to the Total Life analysis results for Plots 3 and 28, it can be noted that the lower DNA values are consistent with the lower crop yield values obtained in

contrast to Plot 19 and Plot 21. When compared to Plot 3 and 28, Plot 19 and 21 had approximately 3 to 6 ng/ul of DNA more respectively. This difference in DNA concentration between Plots 3 and 28, and Plots 19 and 21, accounts for a difference in bacterial population of at least 59M bacterial cell/ul in 0.2 gr of soil (Figure 3).

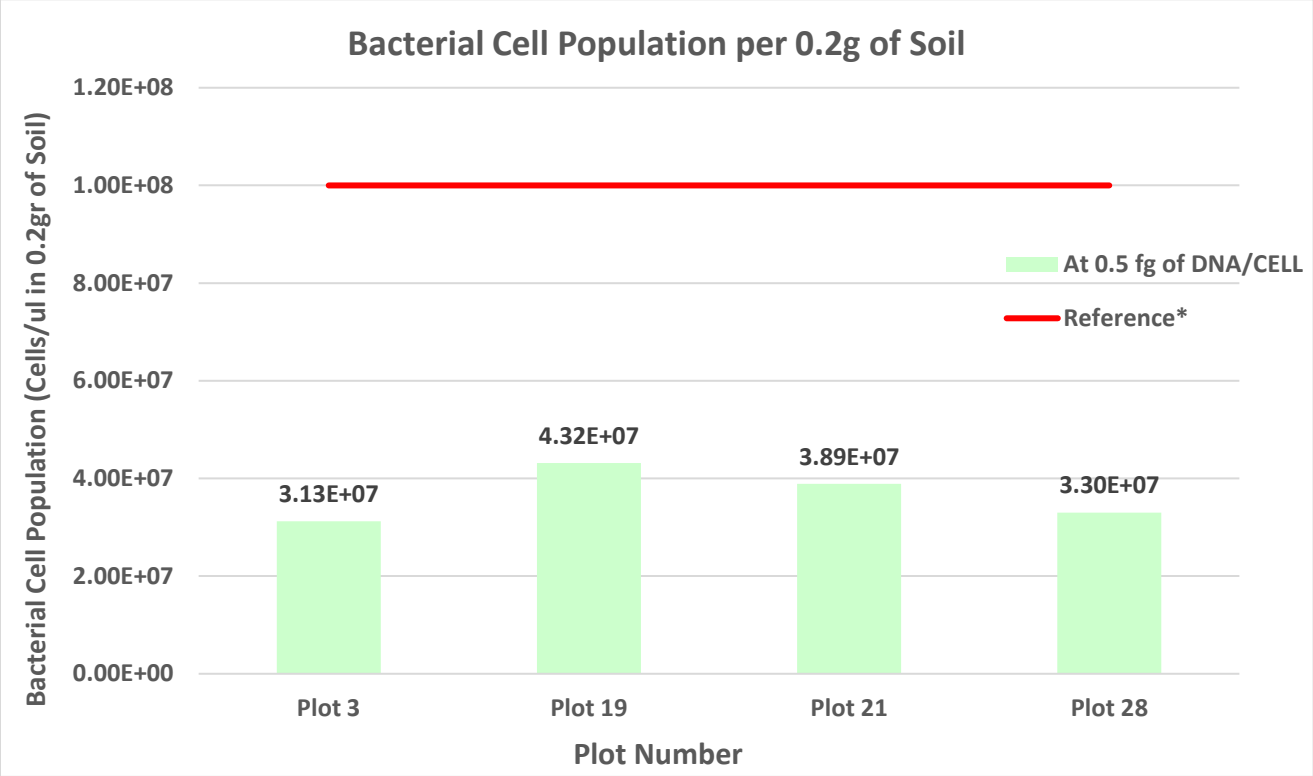


Figure 3. Mt. Boucherie Plots sampled.

*Please note that the reference value is a recommended parameter selected by BioLogix based on literature, experience, and observation and is subject to change. Once the entire property is tested a more representative value of the land may be applied.

Figure 3 (Fig. 3) illustrates the results from the DNA extraction procedures in terms of bacterial cell/ul presence in 0.2gr of soil. Ideally it would be preferred to have the bacterial cell population closer to 10^8 cells/ul per 0.2gr of soil. The threshold set for bacterial population is a simple conversion of the DNA value of 50ng/ul in 0.2gr of soil illustrated in Fig. 2 and is illustrated by the red line in Fig 3. As previously mentioned, Fig. 3 notes that Plot 19 and 21 are exhibiting higher bacterial cell concentrations than those obtained from Plot 3 and 28, which could be supporting higher crop yields in comparison.

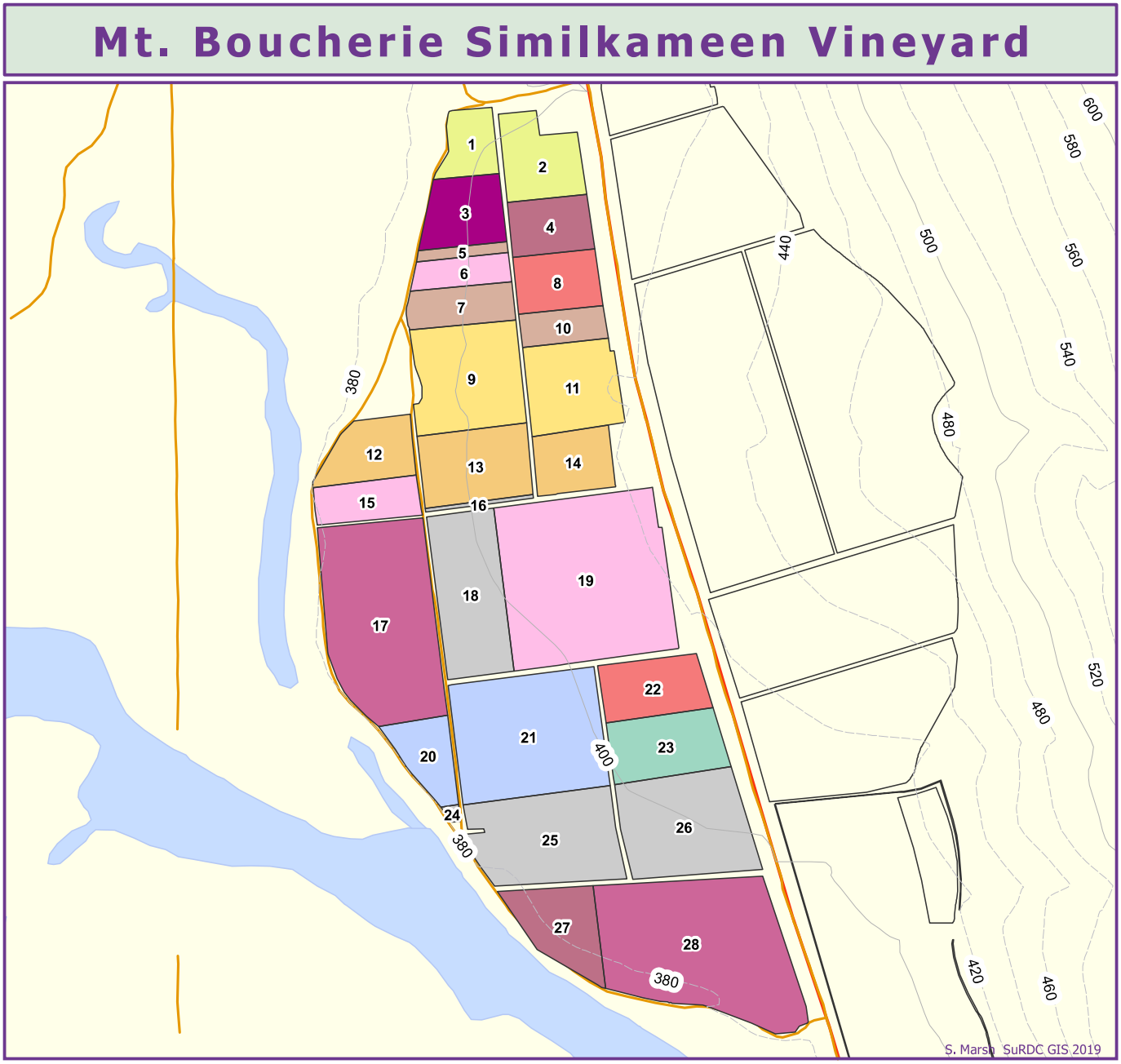
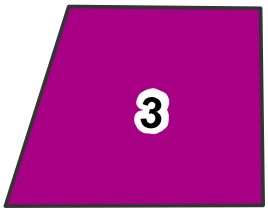


Figure 4. Mt. Boucherie Vineyard Map. Sampled and tested Plots are 3, 19, 21 and 28

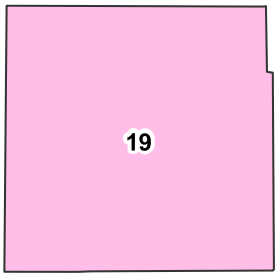
Information Breakdown by Field

Field No. 3



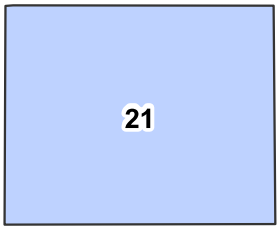
Acres: 1.68
Tons/acre: 2.00
Estimated Yield per Vine: 1.10kg
DNA Concentration: 15.6ng/ul in 0.2g of soil
Bacterial Cell Pop.: 3.13E+07 cells/ul in 0.2g of soil

Field No. 19



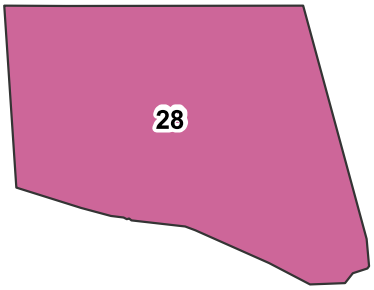
Acres: 8.43
Tons/acre: 2.50
Estimated Yield per Vine: 1.84kg
DNA Concentration: 21.6ng/ul in 0.2g of soil
Bacterial Cell Pop.: 4.82E+07 cells/ul in 0.2g of soil

Field No. 21



Acres: 5.57
Tons/acre: 2.50
Estimated Yield per Vine: 1.84kg
DNA Concentration: 19.4ng/ul in 0.2g of soil
Bacterial Cell Pop.: 3.89E+07 cells/ul in 0.2g of soil

Field No. 28



Acres: 7.57
Tons/acre: 2.20
Estimated Yield per Vine: 2.94kg
DNA Concentration: 16.5ng/ul in 0.2g of soil
Bacterial Cell Pop.: 2.72 E+07 cells/ul in 0.2g of soil

Total Life Analysis: Yield per Vine Comparison with Total Life Found per Field

The Plot dimensions and vine spacing data was provided by the Mt. Boucherie staff. This information was used to approximate the yield produced per vine. This data was then directly correlated to the DNA results from the Total Life analysis. The resulting graph is illustrated in Figure 5 (Fig. 5).

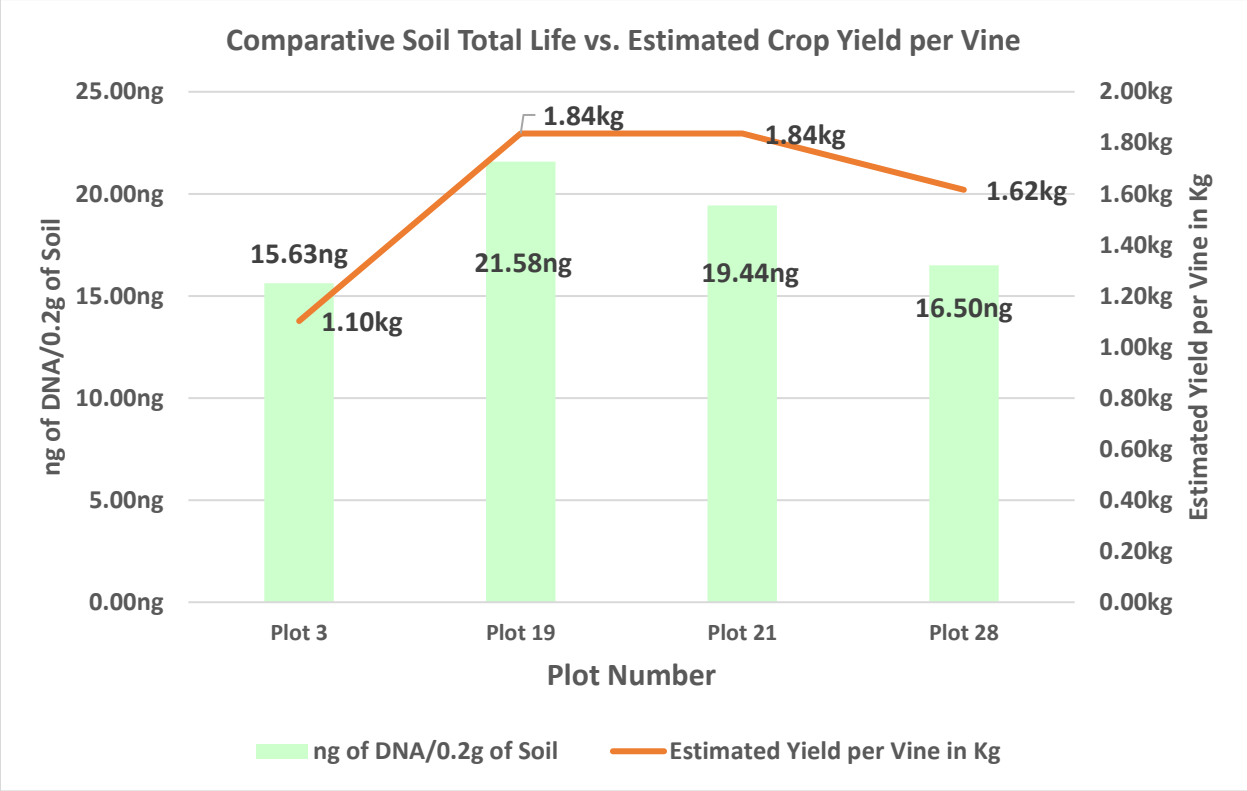


Figure 5. Total Life analysis DNA data summarized versus estimated crop yield per vine per Plot.

The data summarized in Fig 5. clearly shows a direct relationship between soil life and crop yield for all Plots tested. There is a clear trend that is being followed: the higher the life in the soil, the higher the approximate crop yield per vine.

Soil Bacterial Profile: Quantitative PCR Analysis

The DNA extractions were further analyzed via qPCR to determine a bacterial profile of the soil. The three most prominent soil bacterial types were selected for relative quantification. The raw data generated from the amplification of Proteobacteria, Acidobacteria, and Actinobacteria DNA, which are believed to make up 50, 13, and 11 percent of the bacterial biomass of soils respectively, are summarized in Figure 6 (Fig 6).

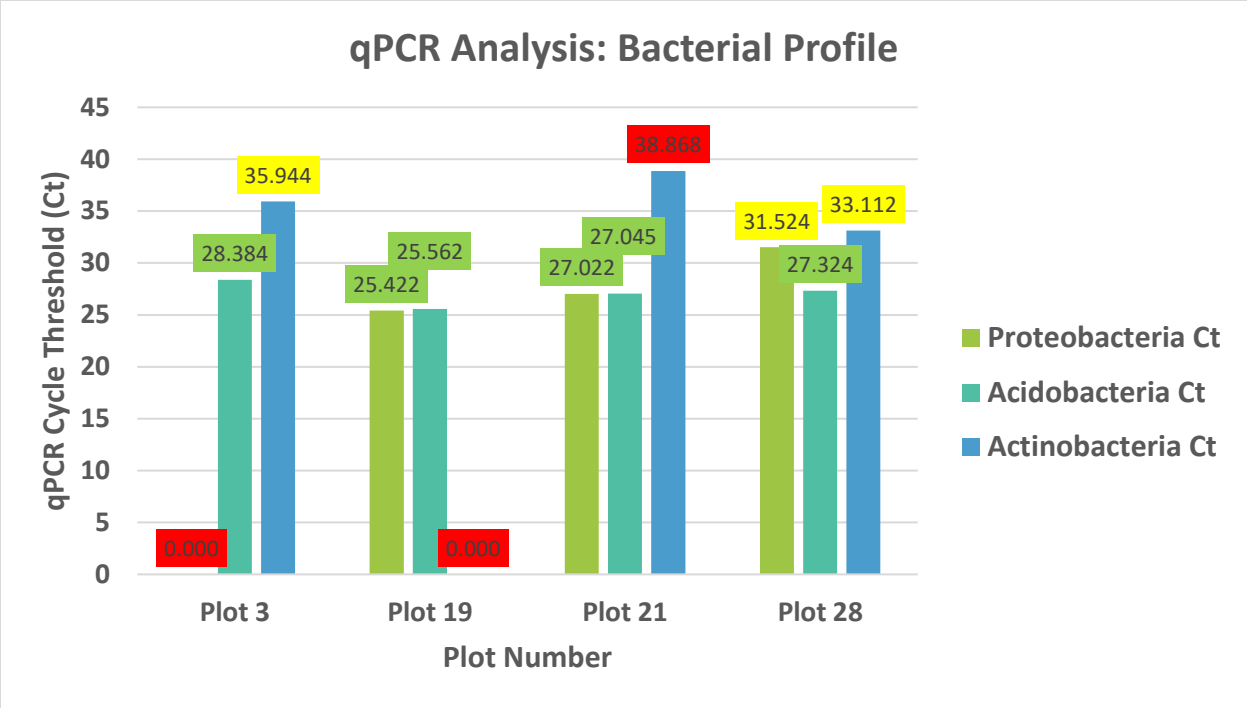


Figure 6. Mt. Boucherie Plots sampled. Green labels indicate the presence of abundant levels of target DNA, yellow labels indicate moderate levels and, red labels indicate low or not present. Cycle Threshold (Ct) is set automatically by the PCR instrument, and it indicates the number of DNA replication reaction cycles undergone before DNA is effectively detected.

The data in Fig. 6 is presented in terms of qPCR Cycle Threshold. This value represents the amount of DNA amplification cycles required for the instrument to detect a clear fluorescent signal. This value is inversely proportional to the amount of DNA copies originally present in the test sample. Values below 29 are good and indicative of abundant levels of DNA. Proteobacteria is expected to be the most abundant bacteria in soils and should therefore present the lowest Ct values and should be well below 29. Fig. 6 illustrates that the value for proteobacteria is trending much lower than expected for Plot 28. Furthermore, Proteobacteria, was not detected in Plot 3. Plot 19 is exhibiting good levels of Proteobacteria and slightly higher levels of acidobacteria than expected, and no actinobacteria. Plot 21 is following the same trend as Plot 21 with a minimal amount of Actinobacteria present. In both Plots 3 and 28 there is slightly higher than expected Acidobacteria. Finally, Actinobacteria appear to be present in the expected relative amounts for both Plots 3 and 28.

Conclusions

Given this is a field trial and the first time the soils have been tested, only observations can be made at this point in time. This year's testing will provide comparative values for future testing, at which point conclusions based on future agricultural practices will be made. Moreover, our observations are the following:

- Overall, the bacterial population is lower than the desired values as indicated by the Total Life analysis. However, Plots 19 and 21 are exhibiting considerably higher levels of bacterial life in the soil which may be supporting the higher crop yield obtained from these Plots as compared to Plots 3 and 28 (Fig 2 and 3).
- The qPCR results indicate that all three bacterial types are present in two of the fields tested (Plot 19 and 21, Fig 6), with proteobacteria and actinobacteria being present in the expected relative amount and with acidobacteria being slightly higher than expected. Furthermore, Proteobacteria was not detected in Plot 3, and only slightly detected in Plot 28, which is consistent with lower soil bacterial concentrations.
- Overall, the fields tested in the Mt. Boucherie Similkameen Vineyard follow the expected trend between bacterial cell population and crop yields as described by the graph in Fig 5. In general, higher DNA values correlate to improved cropped yields and is denoted by the trend observed among all the Plots tested.
- Given that the total life results for Plots 3 and 28 in Fig. 2 and 3 are lower than the suggested baseline, it is likely that the low DNA concentrations obtained are attributed to the low presence of Proteobacteria as indicated by the qPCR Ct values in Figure 6. Proteobacteria are the main bacterial type responsible for cycling carbon, nitrogen, and sulfur. It is our observation that the low presence of Proteobacteria in this soil could be resulting in nutrients not being readily available for plant intake, thus resulting in lower fruit yield.

Contact

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For more information about our company please visit our website: www.biologixlabs.com

Appendix: qPCR Raw Data

Figure 7. qPCR Proteobacteria Raw Data

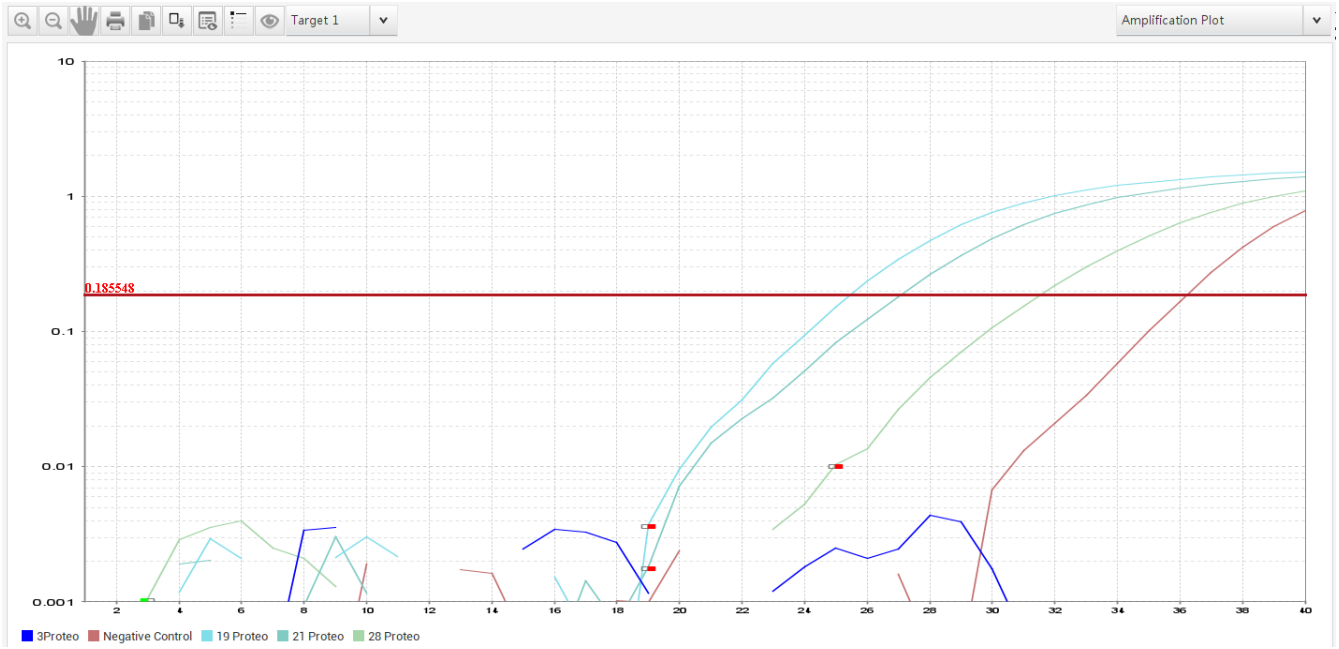

Figure 8. qPCR Acidobacteria Raw Data

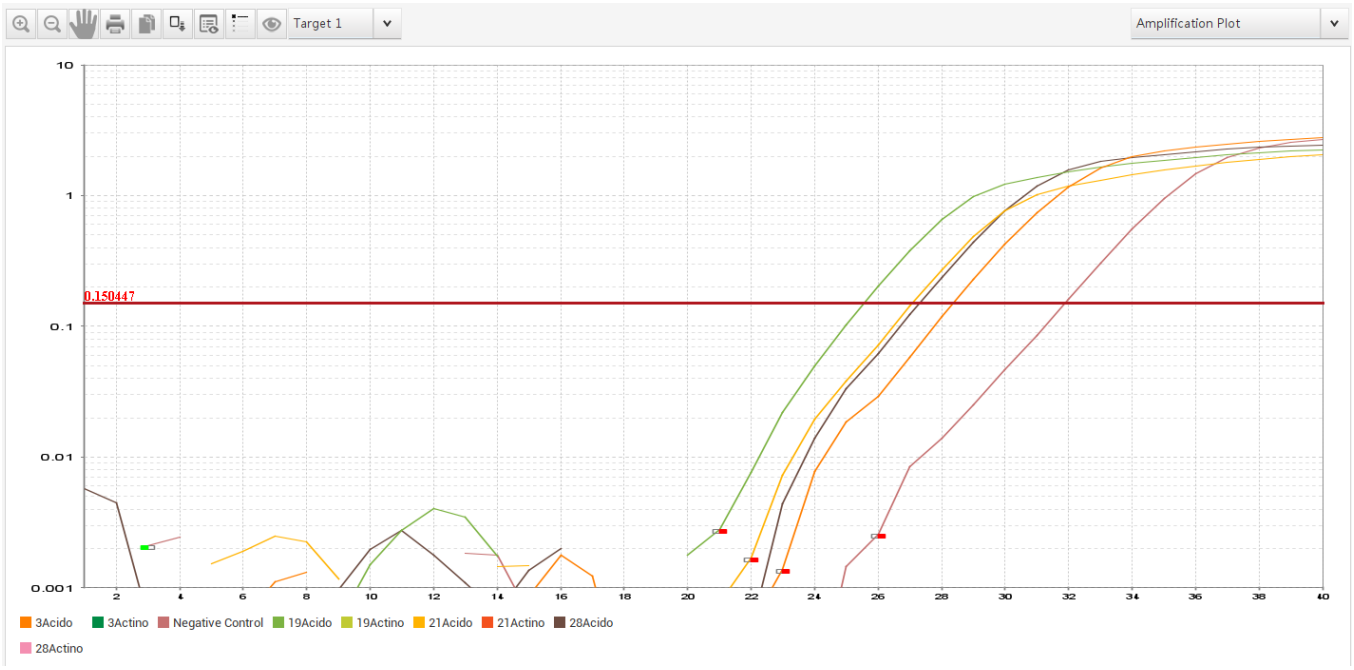

Figure 9. qPCR Actinobacteria Raw Data