MICROBIAL ABUNDANCE ACROSS GOYDER'S LINE: A COMPARATIVE SOIL BACTERIAL ABUNDANCE VARIATION STUDY TO MITIGATE DROUGHT CONDITIONS

By

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ABSTRACT

The Goyder's Line is a significant demarcation line in South Australia. This surveyed line is a vital and historic terrestrial marker separating regions with an average annual rainfall of 250 mm. There is a clear research gap presented with a lack of knowledge on microbial population abundance and the environmental factors such as rainfall which contribute to changes in microbial abundances within the region. This study observed bacterial abundances in ten sites north and south of the Goyder's line. The abundance of bacterial populations was calculated using flow cytometric analysis, and environmental factors that influence the abundance of bacteria, such as pH, were also measured. The abundance values and other factors were visualized using Pearson's Correlation and Independent Samples T-test. Mean bacterial abundance was significant in the south ($p=0.040$), with a greater bacterial abundance of $M= 6.0x\ 10^6 \pm 7.5\ x10^5$ cells ml^{-1} compared to the north which was $M= 3.7\ x\ 10^6 \pm 4.9\ x10^5$ cells ml^{-1}. Although not significant ($p=0.452$), The ridge had a mean bacterial abundance $M = 3.6x10^6 \pm 3.9x\ 10^5$ cells ml^{-1} compared to the furrows, which were ($M = 4.4x10^6 \pm 8.6x10^5$cells ml^{-1}. Virus-like particle abundances were not measured as it was harder to differentiate them from the noise controls. The Pearson correlation of pH to the abundance of bacteria was (r) = - 0.365 and a significance value of ($p= 0.299$) which made it statistically insignificant, Isolated observations indicated that in some samples lower bacterial abundance was present in higher pH values as well as agricultural practices such as animal grazing between crop rotations also promoted bacterial abundance Because these observations could not be statistically proven further research should be done to obtain a clear idea on this matter. Furthermore, several environmental conditions such as salinity, availability of Phosphorus, Potassium, Nitrogen, and soil organic carbon were identified that influenced the abundance of bacteria that were not tested in this study. The main conclusion of the study is that although rainfall had a significant influence on soil microbial abundance furrows which were assumed to have higher levels moisture of did not have a significant influence. The future direction of this study is to identify the taxa in the soils that contributed to the abundance and identify bacteria that contain drought-resistant genes or produce

substances such as extracellular polysaccharides (EPS).

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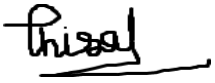
Signed.....Fransiscu Lamhewage Thisal Dewin Jayawardena..... 

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1. INTRODUCTION

1.1 Overview of Goyder's Line

The Goyder's Line is an important separation track in South Australia. This surveyed line is a vital and historic terrestrial marker separating regions with an average annual rainfall of 250mm. While the north of the line has less average annual rainfall and is used for animal grazing, the south receives more and is considered suitable for maintaining crops (Bren, 2023). The boundary line is an important component of South Australian history, which affected farming methods, land use, and the settlements of South Australians. This line was recognized in 1865 (Tozer et al., 2014). However, because of the changing climate conditions and stronger El Niño Southern Oscillation, Indian Ocean sea-surface temperature, Southern Annular Mode, and the subtropical ridge intensity, the line is moving south (Nidumolu et al., 2012; Tozer et al., 2014). Furthermore, as the line is migrating, the microbial diversity in the soil is also affected. Since the soil microbiome is responsible for many functions of plant growth, fluctuations in the climate would hinder it directly and indirectly (Tozer et al., 2014). As this is the case, active monitoring and forecasting models of the gradient should be developed so that agricultural practices can be changed accordingly. If not, the region's harvest would be low, and the amount of fertilizer and plant care used to grow crops would be unproductive.

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Figure 1 - The current status of Goyder's line states the volatility of the previously surveyed margins. The black line shows the surveyed Goyder's line. The 220mm rainfall in Isohyet regions under El Nino and STRI positive is shown as the red line which is south of the Goyder's line and La Nina STRI Negative conditions are shown as the blue line which is drawn north of the Goyder's line (Tozer et al., 2014).

1.2 Soil Microbiomes

Soil microbiomes are extensively diverse and complex ecosystems that play a vital role in maintaining the functions of terrestrial environments (Islam et al., 2020). So, knowledge of the diversity and abundance of these soil microbes must be obtained to comprehend the effects of environmental conditions on these microbiomes (Maron et al., 2018). The microbiome of the soil plays a significant role in plant health. It maintains the fertility level of the soil, as well as recycling the nutrients the plants use for their growth. The microbes also preserve the structure of the soil and contribute to decomposing organic matter in the soil, which is made into simple compounds that the plants can absorb (Paul, 2016). The soil near the gradient comprises calcareous soil with more significant amounts of calcium

carbonate. This kind of soil is commonly found in arid and semi-arid subtropics and has a unique composition (Wahba et al., 2019). The soil microbiome is affected by many environmental conditions, such as temperature, pH, and soil moisture content. These environmental factors and the unique soil composition have created distinct microbial communities across the region, influencing soil health and agricultural productivity.

1.3 Aims of the Study

As the studies on the distinct microbial abundance across Goyder's Line are limited and a significant knowledge gap was found in the literature, this significantly limits the ability to manage the challenges affecting agricultural productivity across Goyder's. This study hopes to find whether there is a higher level of microbial abundance, which is statistically significant, present in the south compared to the north. The second hypothesis that will be tested inthis study is whether furrows contain more bacterial abundance than ridges because they contain more water. Also, the correlation between pH and bacterial abundance is tested to find whether it promotes or inhibits bacterial growth. Another objective of this study is to create a simple, cost-effective, and standardized method that extracts microbes from calcareous soil samples, which can be used in flow cytometry.

1.4 Background

1.4.1 Importance of Soil Microbiome

The soil microbiome consists of all the primary microbial particles, such as bacteria, viruses, fungi, protozoa, and archaea. It is essential for maintaining soil health and productivity. (Fierer, 2017). Given the water scarcity in regions with low precipitation, the soil microbiome enhances soil potency and nutrient cycling, enabling plants to thrive despite challenging

conditions. Microorganisms have a more significant role in decomposing organic matter, releasing vital nutrients for plants to absorb. Some microbes, such as mycorrhiza fungi, form symbiotic relationships with plants, spreading the plants' roots to improve water and nutrient absorption. This is especially crucial in arid areas such as near Goyder's line, where efficient water usage is vital (Fierer, 2017).

Moreover, the microbiome improves soil structure and water retention, critical for conserving water in dry environments. Microorganisms produce materials such as extracellular polymeric substances (EPS) that bind soil particles into collections, enhancing the structure of the soil and reducing erosion. They also help to create channels and pores in the soil, improving water infiltration and storage (Costa et al., 2018; Fierer, 2017). Thus, the microbiome supports soil fertility, water retention, and plant growth in low-rainfall areas. Understanding and managing the soil microbiome in regions across the gradient can lead to sustainable agricultural practices and reduce fertilizer use. Nurturing the diversity and activity of beneficial microbes can enhance soil fertility and water retention, increasingplant resilience to drought and contributing to the long-term sustainability of the ecosystem (Bissett et al., 2016; Fierer, 2017).

The Baas-Becking hypothesis, introduced by LGM Baas-Becking in 1934, states that "everything is everywhere, but the environment selects." (Hazard et al., 2013). In this context, microorganisms such as mycorrhizal fungi have a diverse distribution, but their abundance and diversity are shaped by environmental factors such as temperature, pH, and soil moisture content. As mentioned before, mycorrhizal fungi form symbiotic relationships with plant roots, providing nutrients to plants in exchange for nutrients. (Hazard et al., 2013) Accordingto the Baas-Becking hypothesis, these fungi can be potentially found in all environments.

Still, their presence and population are influenced by temperature, pH, soil moisture content, and plant community composition. Different species of mycorrhizal fungi are adapted to

specific conditions and have variable environmental preferences. The hypothesis implies that ecological conditions select certain microorganisms, so those conditions determinethe presence and abundance of the particular microbes in a given environment. Factors like land use intensity, agricultural practices, soil type, and rainfall can affect the composition and diversity of microbial communities. Understanding the factors that influence the distribution and diversity of microbes is crucial for comprehending their ecological roles and their potential to enhance plant growth and ecosystem functioning (Hazard et al., 2013). The Baas-Becking hypothesis offers a framework for understanding the relationship between microbial communities and their environment, and further study in the region near the Goyder's line is vital to understanding environmental factors in shaping microbial distribution and diversity.

Niche theory is an essential concept in ecology, which describes the environmental conditions necessary for an organism's survival and reproduction and its interactions with other organisms. While it has been extensively studied for larger plants and animals, understanding microbial niches, especially those of arbuscular mycorrhizal (AM) fungi, has been limited. AM fungi, which form symbiotic relationships with up to 72% of plant species, receive carbon from plants in exchange for mineral nutrients from the soil. Ground-breaking research by Davison et al. in 2021 has provided the first comprehensive insight into the niches of AM fungi at a guild level. The study found that the global distribution of most AM fungal taxa is primarily influenced by mean annual temperature and soil pH. These findings align with the physiological traits of AM fungi, as soil pH indicates nutrient availability and temperature affects metabolic rates. The study also highlights the importance of biotic interactions, such as host relationships, in understanding the niches of these symbionts. The results support the idea that a single driver, such as climate, can influence the distribution of organisms across the tree of life (Kivlin et al., 2021; Malard & Guisan, 2023).

Knowledge of AM fungal environmental tolerances can be applied in agricultural fields and habitat restoration, allowing for selecting eco-region-specific consortia of AM fungi.

Ecological niche models can also predict suitable habitats for AM fungi and other microorganisms, considering biological interactions and future climate change scenarios.

Further research emphasizes the challenges in studying microbial niches and proposes a metabolic niche framework to understand habitat preferences, metabolic plasticity, niche shifts, and microbial invasions. By integrating multi-omics approaches, researchers can define the fundamental and realized metabolic niches of microorganisms, including AM fungi, within the environmental space. This framework allows for the study of metabolic plasticity, differentiation between specialist and generalist phylotypes, assessment of the impacts of microbial invasions, and improvement of cultivation techniques. Combining niche theory with studying AM fungi and other microorganisms provides valuable insights into microbial ecology and opens new avenues for research in this field (Kivlin et al., 2021; Malard & Guisan, 2023).

Neutral theory in microbial ecology diversity theorises that all microbial species are equivalent on a per-capita basis, predicting a balance between the random extinction of species and the emergence of new species through immigration or speciation. According to this theory, coexisting species are selected for similar environmental conditions, implying that they share the same general niche or adopt the broadest possible niche, which suggests functional equivalence and redundancy within a microbial community. However, the neutral theory does not account for niche specialisation and interspecific competition, making it controversial and debated as it does not explain the difference observed in natural ecological communities. Experimental evidence often contradicts the idea that identical assumptions apply across habitats (Saleem et al., 2015).

Despite its limitations, neutral theory offers some valuable insights. It controversially predicts functional equivalence, which might be relevant in the vast and complex microbial world when considered alongside other niche factors. For instance, the distribution pattern of ammonia-oxidizing bacteria in wastewater aligns with neutral theory predictions. Host-associated microbes have alternative environmental reservoirs that can disperse efficiently among hosts, displaying a relatively neutral population structure. Similarly, a near-neutral population structure is seen in various pathogenic and non-pathogenic microbes due to asymptomatic carriage, overlapping micro-epidemics, generalist behaviour, or dominance in specific habitats (Saleem et al., 2015).

Ultimately, the patterns of microbial ecological communities are shaped by an interplay of environmental factors (niche theory) and neutral processes. This integrated perspective helps predict and understand the dynamics within microbial communities.

1.4.2 Current Research Trends

High-throughput sequencing, commonly known as next-generation sequencing (NGS), revolutionized genomics by enabling the rapid and cost-effective sequencing of large volumes of DNA. This advancement paved the way for the development of nanopore technology, which has gained prominence in various fields, including soil microbiology. Nanopore technology operates by embedding nano-scale holes in a thin membrane and measuring electrochemical signals as charged biological molecules, such as DNA, pass through these pores. In DNA sequencing, the DNA molecules traverse the nanopore, and the changes in electrical current are used to determine the sequence of DNA bases in real-time (Lin et al., 2021).

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Carl Woese transformed taxonomy identification by using 16S rRNA gene sequencing to categorize organisms, revealing the Archaea domain (Luehrsen., 2014) then scientist Norman Pace expanded this method to environmental microbiology, which enabled the identification of diverse microbial communities in the soil without culturing. This has transformed the knowledge of evolutionary relationships. (Zhulin.,2016)

Additionally, the cost of nanopore sequencing, though decreasing, still requires substantial investment in equipment and consumables. Furthermore, analysing and interpreting data from nanopore sequencing can be computationally demanding and require specialised bioinformatics expertise (Lin et al., 2021).

Flow cytometry is a technique used in microbiology and clinical settings to analyze individual cells rapidly. It can be used in microbial communities to observe microbes and measure their distribution within the microbiome. Flow cytometry uses lasers to measure cell characteristics such as size and granularity through techniques that use light scattering and fluorescence from the biomarkers embedded in the cells. This enables an understanding of microbial diversity, abundance, and physiological state of the microbes (Props et al., 2016).

Soil microbiomes exhibit significant physiological variability even over short terrestrial distances. However, not all microbial populations in the soil are active during observation time. Some remain dormant and are difficult to culture and study. To understand soil microbial ecology, accurately quantifying microbial counts is essential.

Recent studies have focused on molecular techniques such as 16S rRNA genetic sequencing to observe microbial community structure and diverse taxonomy. However, flow cytometry is a more effective method for classifying and calculating viable bacteria, demonstrating that it is much more effective than traditional culturing or microscopy methods. (Ou., et al.,

2017) Flow cytometry combined with specific dyes such as SYBR Green permits the rapid and reliable counting of total and active cells. Also, it is significantly more time-efficient (Priyadarsini et al., 2023).

Using another metric such as ATP assay would have been better in obtaining a much clearer picture of the microbial activity of the soil. This is because the ATP assays yielded significantly higher average bacterial abundances than Flow cytometry in all samples apart from soil samples collected from stream sediments. (Frossard., et al. 2016) Also, the cell counts calculated from the ATP assay showed a higher variation than the cell numbers obtained by flow cytometry. This is due to, the presence of inactive cells although reduced, ATP is used to maintain metabolism so, a portion of the biomass detected by the ATP assay could be accounted for by dormant bacterial cells. (Frossard., et al. 2016) Thus the true activity of the bacteria within the soil can be found.

Separating active and dormant cells is essential for understanding their physiological roles. Flow cytometry has shown promising results in distinguishing viable but non-culturable microorganisms, providing a broader scope of soil microbial ecosystems. Although flow cytometry has many advantages, it has some limitations in characterizing microbes. As the soil types differ, obtaining results under flow cytometry involves effective dispersion, separation, and microbial purification from complex soil environments. (Frossard., et al. 2016) As with other methods of dispersion, a standard protocol is needed for each type. Although this is the case, there is a shortage of standardized and validated procedures for soil bacterial extraction,which has caused a significant obstacle to widespread adaptation (Priyadarsini et al., 2023).

1.4.3 The Microbial Influence on Plants in Drought Conditions.

Plants face many abiotic stresses in arid regions under changing climate conditions, such as salinity, drought, alkalisation and acidification, and temperature stress. The microbes in the soil can alleviate that stress on the plant (Phour & Sindhu, 2022). As this is the case, it is vital

to focus on recent studies to identify these microbes and investigate their function.

Studies show that growth-promoting bacteria help mitigate these stresses. The phosphate The phosphate solubilizing bacteria (PSB) comprised of *Bacillus* sp. and *Pseudomonas* sp. develop root functions and increase water absorption. Potassium solubilizing bacteria (KSB) comprised of *Thiobacillus* sp increases photosynthesis rate and regulates it to help mitigate drought stress. Nitrogen-fixing bacteria (NSB), which was comprised of *Azospirillum* sp., Bacillus sp., and *Azotobacter* sp., increases plant growth parameters such as total plant height, stem and leaf dry weights, and leaf area so that the plant can grow at an increased speed in periods of complementary environmental conditions. However, significant results could be observed when a combined inoculum comprising all three growth-promoting bacteria (Phour & Sindhu, 2022; Taghizadeh et al., 2023).

Salinity stress occurs when more ions, such as K+, Ca2+, and Na+, are in the groundwater. This raises the osmotic potential, and plant death occurs through plasmolysis. Under drought conditions, this occurrence rises (Ayangbenro & Babalola, 2021). EPS-producing microbes would be a solution for this. Some EPS-producing genera, such as *Bacillus, Pseudomonas*, and *Rhizobium*, produce rhizosheaths and hydrophilic biofilms to manage salinity stress. (Phour & Sindhu, 2022) EPS produced by other microbes such as *Bacillus sp., Microbacterium sp., Paenibacillus sp., and Enterobacter sp.* tends to bind to the cations above K+, Ca2+, and Na+ directly, easing salt stress in plants such as wheat (Ayangbenro & Babalola, 2021).

Microorganisms play an important role in helping plants manage temperature stress in extreme heat and cold environments. Pseudomonas strains express stress sigma factors such as AlgU and, RpoH (σ32) which are involved in the response to cell wall stress and heat shock. Extreme temperatures and low soil moisture leadto plants producing increased amounts of ethylene; this causes an overall reduction and abnormal plant growth, especially limiting the growth of roots and shoots. In some cases, it also causes abscission and early

senescence (Phour & Sindhu, 2022). Aminocyclopropane carboxylic acid, the ethylene precursor, is metabolized by the ACC deaminase enzyme and reduces ethylene concentration. This enzyme can be found in soil microbial taxa such as *Burkholderia phytofirmans, Variovorax paradoxus 5C-2, Enterobacter spp., Achromobacterpiechaudii, and Microbacterium sp.* (Phour & Sindhu, 2022) G16 increases plant growth, stem height, and shoot and root biomass. In colder temperatures, soil bacteria, such as *Erwinia herbicola* and *Pseudomonas syringae*, which have ice-nucleating ability, protect plants from frost injury insub-zero temperatures. It should be noted that Burkholderia phytofirmans also influence grape plants in colder conditions (Phour & Sindhu, 2022).

It should be noted that arbuscular mycorrhizal fungi (AMF) also play an important role in drought, salinity, and temperature stress management in plants. Under low moisture (drought) conditions, it increases the area in which the root system spreads and increases plant osmotic potential by enhancing proline & sugar production, which reduces dehydration of the plant. It also enhances the increase of chlorophyll amount in leaves and secrete nitrogen and phosphorus metabolic enzymes. In high salinity conditions, they increase root and shoot biomass and improve the production of antioxidant enzymes so oxidative damage is minimized. In extreme temperatures, it was observed that there was an increase in leaf length, height of the plant, number of leaves, and higher nutrient intake in plants inoculated with arbuscular mycorrhizal fungi, which led to a higher grain count (Begum et al., 2019). Asmost of the locations near the Goyder's line grow wheat (Triticum aestivum), it was taken as the host species, and some of the arbuscular mycorrhizal fungi that inoculate it are *Glomus mosseae, Glomus fasciculatum, Gigaspora decipiens, Rhizophagus irregularis, Funneliformis mosseae, Funneliformis geosporum, Claroideoglomus claroideum* (Begum et al., 2019).

1.5 Debates and Controversies

Though studies on the diversity of soil microbiomes have uncovered invaluable knowledge on the structure and function of microbial environments, they remain a subject of many

debates and controversies. This is because of the subject's complex composition and the various methodologies used to obtain results. The microbial ecosystem is also vast, so the scientific community has an ongoing debate on the validity of methods, results, and ethical dilemmas.

While molecular techniques such as 16S rRNA gene sequencing and nanopore have transformed our knowledge of microbial communities, they are praised for being accurate and providing much insight into microbial diversity estimations. They are not without their limitations. Because preconceptions made during DNA extraction, amplification, and sequencing can also introduce contaminants, it is under debate that procedures frequently result in an incomplete picture or an inaccurate one (Ambardar et al., 2016). For instance, differences in cell wall characteristics, which lead to poor DNA extraction efficiency, may result in the underrepresentation or neglect of some microbial taxa (Magi et al., 2018). Furthermore, some sequencing, such as nanopore systems, provides long read lengths, which makes it harder to differentiate between closely related species. This leads to incorrect identification, which raises questions about the validity of the results (Ambardar et al., 2016). So, the debate continues about whether high-throughput sequencing methods can give an accurate and complete picture of the respective microbial community.

Secondly, a controversial topic is the interpretation of data obtained from microbial diversity concerning ecosystem function, structure, and overall impact on the surrounding environment. The scientific community acknowledges that various microbial communities positively influence soil health and plant growth. However, the precise processes of most of these functions are still unknown (Delgado-Baquerizo et al., 2016). Some scientists say functional redundancy is one of the most argued about. This states that some microbes have the same function in the ecosystem, so they can be interchanged with other microbes in related taxa or completely different species. However, some debate that although the function

is similar, its effect could not be comparable because it might not use the same pathway to achieve its goal (Delgado-Baquerizo et al., 2016).

The third debated topic that needs to be addressed is the use of synthetic soil microbial communities in agricultural practices and research. Synthetic soil microbial communities are obtained from a particular environment, cultured, and introduced to another environment as soil microbe colonies or used as biofertilizers in a larger agricultural area (Shayanthan et al., 2022). This has research as well as ethical implications because if the microbes are studied in a controlled environment, the complete magnitude of the effect it has on the plant could not be understood clearly, because in situ environments, many components, as well as other microbes, interact with the introduced environment. The ethical conundrum is that if a set of new microbes is introduced to a larger agricultural land as fertilizer, it will cause harm to the native microbes, animals, and plants. Also, there is a chance that synthetic soil's microbial function will make another microbe pathogenic (De Roy et al., 2014).

The final debated topic is whether either niche or neutral theories drive the soil microbial community. This determines the fundamental instruments that drive its assembly and biodiversity. Niche theory states that the structure of a particular microbiome is molded by the environmental conditions it's under and the interactions it has within it as well as with other species. In this theory, predation, competition, and mutualism play important roles in the abundance and diversity of the microbial community (Kivlin et al., 2021; Malard & Guisan, 2023). The neutral theory proposes that the composition of a particular microbial community is driven as a random probability distribution or pattern and driven by births, deaths, and speciation events that happen randomly. The variability of two microbial communities living under similar conditions is a cause of the random creation of new species and the death of a species, which happens randomly over time without the influence of abiotic factors or interacting species (Saleem et al., 2015). Although both theories have valuable insights, neither can completely explain the diversity or abundance of a microbial

community.

1.6 Research Gaps

One of the prominent limitations identified was that in characterizing microbes with flow cytometry, it uses effective dispersion in solutions, separating, and microbial purification from complex soil environments. In the calcareous soil near the Goyder's line, this has not been done previously, so there are no standard methods for dispersion, solutions that need to be used, or standard protocols to be found for this type of soil. This hinders the timely execution of experiments of this kind in the future, so creating a standardized and validated procedure for soil bacterial extraction from calcareous soil would be helpful in a significant change for widespread adaptation (Priyadarsini et al., 2023).

The second research gap identified was whether niche theory, neutral theory, or both drive the soil microbial community near Goyder's line. It would determine whether the microbial community abundance and diversity change rapidly due to differences in environmental conditions, such as sudden drought conditions and moisture content driving its assembly and biodiversity (Kivlin et al., 2021; Malard & Guisan, 2023). currently, available research tends to quantify the influence of niche and neutral processes on microbial community diversity separately. However, understanding their comparative contribution would provide a complete understanding, proving if synthetic soil microbial communities could or should be introduced to the soil to improve its health (Delgado-Baquerizo et al., 2018; Shayanthan et al., 2022).

Third, the identified research gap is that several agricultural research studies have been done in South Australia and near Goyder's line region. But most of them are focused on crops, livestock rotations, and weather. However, there is a significant gap in soil microbial studies near Goyder's line, and studies that use advanced techniques, such as flow cytometry, in thisregion, could not be found. This project will use state-of-the-art techniques such as Flow

Cytometry to understand the microbial landscape in the region (Delgado-Baquerizo et al.,2018; Xue et al., 2018).

Goyder's Line demarcates a boundary between arid and semi-arid climates, leading to a significant environmental rainfall gradient. There is a need to understand how this gradient influences the microbial communities. There is a research gap in this area which was not previously filled. So, a major component of this study is to analyze soil physicochemical properties and soil microbial abundance to determine how environmental factors shape microbial communities (Tozer et al., 2014). Given the region's agricultural significance, the project will investigate how conventional and sustainable farming practices affect microbial diversity and function, providing insights that could informbetter land management strategies.

2 MATERIALS AND METHODS

2.1 Sample locations

Table 1- This table shows the collected samples' locations and the GPS coordinatesfor each location's latitude and longitude, respectively. The farm to which each site belongs is shownin the 3rd column. A description of the sample site and the kind of plant grown in the field.

Sample	**GPS Location**	**Farm**	**Description (Ridge/Furrow /Mixed)**	**Plant/ Grazing Land**	**North/ South of the Line**
Sample 1	-32.80598, 138.40051	Joe and JessKoch farm	Ridge	wheat	North
Sample 2	-32.80598, 138.40051	Joe and JessKoch farm	Furrow	wheat	North
Sample 3	-32.87754, 138.37299	Matt and AliceNottle Farm	Ridge	wheat	North
Sample 4	-32.87754, 138.37299	Matt and AliceNottle Farm	Furrow	wheat	North
Sample 5	-33.23839, 138.52032	Peter and Mel Kitschke	Ridge	wheat	South
Sample 6	-33.23839, 138.52034	Peter and Mel Kitschke	Furrow	wheat	South
Sample 7	-33.3045, 138.61021	Luke and ScottClark	Mixed(soil pit) 1.5m deep	canola	South
Sample 8	-33.3045, 138.61021	Luke and ScottClark	Mixed soil	canola	South
Sample 9	-33.26020, 138.532905	Peter and Mel Kitschke	Topsoil	Animal grazing	South
Sample 10	-33.30336, 138.61178	Luke and ScottClark	Topsoil	canola	South

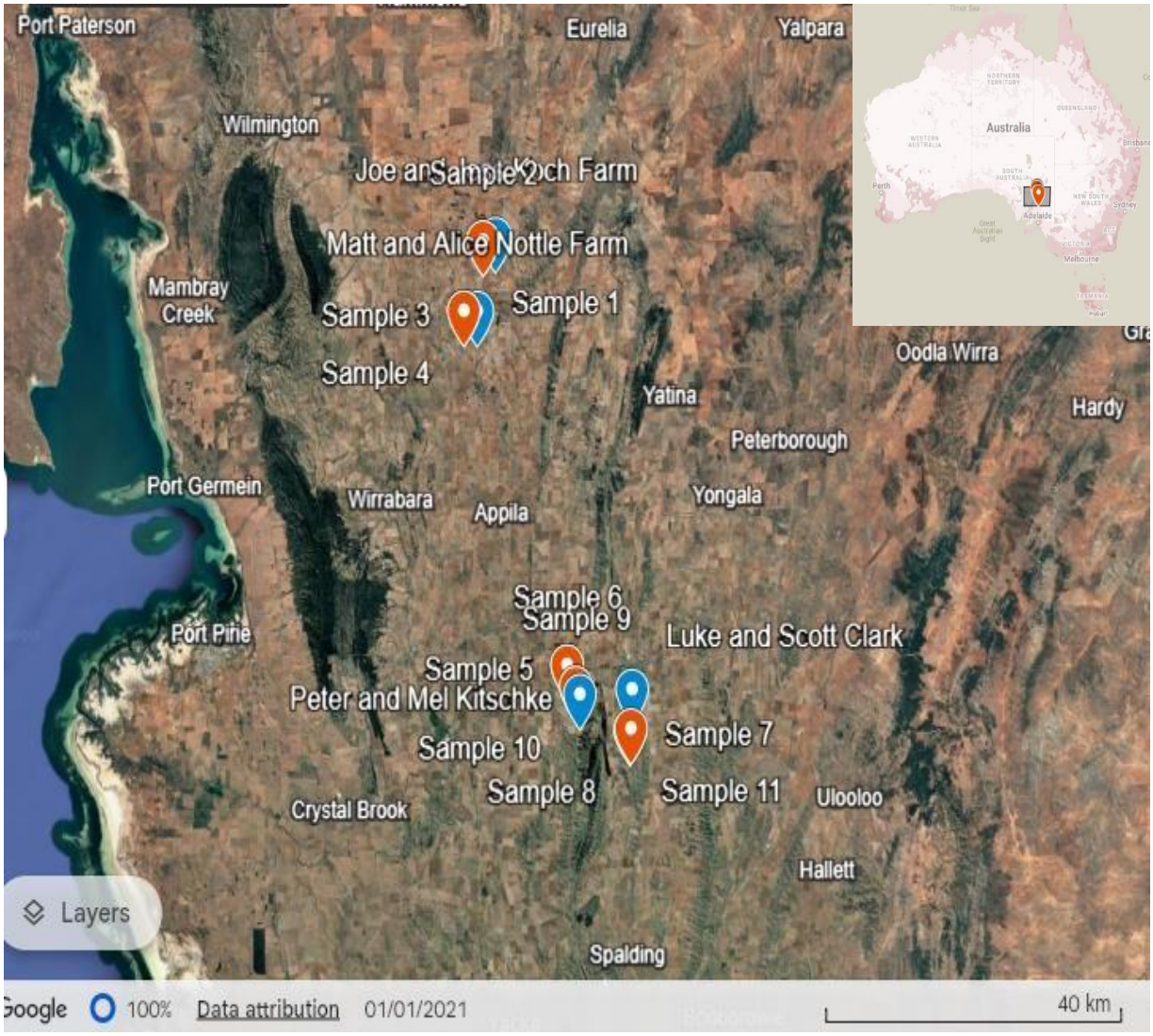

Figure 2-Sampling locations and farms in South Australia. The blue color tags correspond to the farm names, while the red ones represent sample coordinates. A total of 10 samples are shown, and some share the same GPS coordinates. The scale is shown as 1cm on the map is equivalent to 40km on the ground. Obtained from https://www.google.com.au/earth/

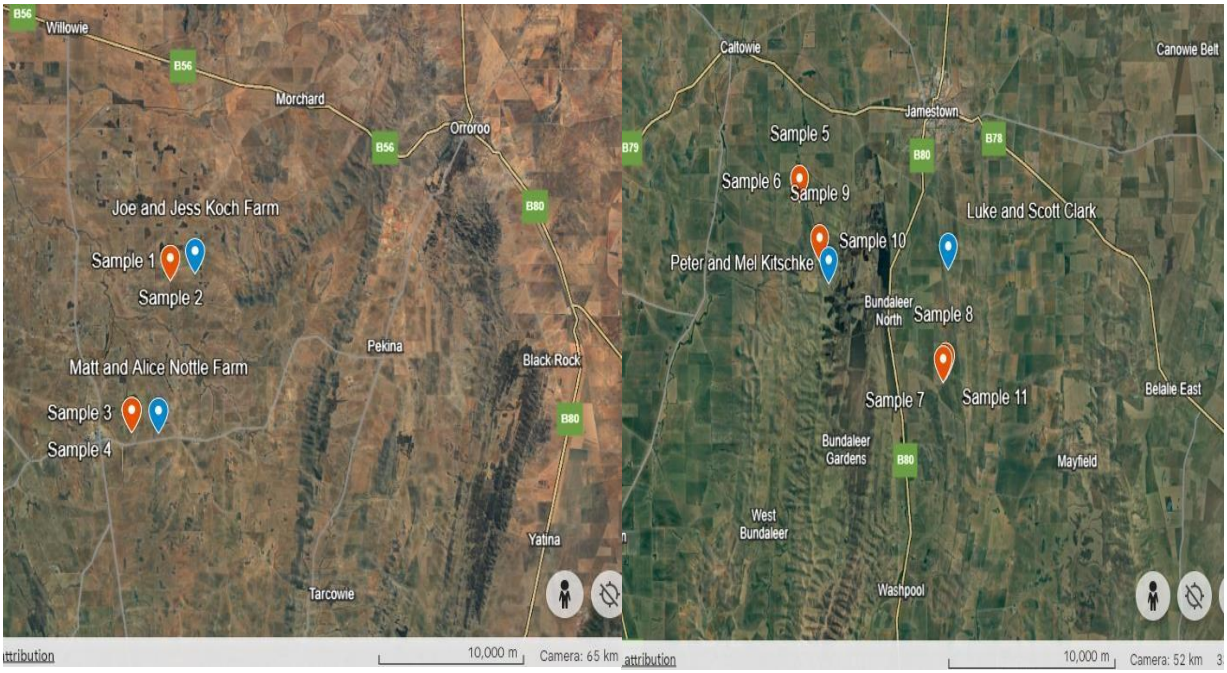

Figure 3- This figure shows the relative terrain north of the Goyders line, shown on the left, and the terrain south of the line, shown on the right. The blue colour tags correspond to the farm names, while the red colour tags represent sample coordinates. A total of10 samples are shown, while some share the same GPS coordinates. The scale is shown as 1cm on the map is equivalent to 10km on the ground. Obtained from https://www.google.com.au/earth/

This study was conducted in South Australia, near Goyder's Line, which included four farms, mainly from Jamestown and Boolaroo. According to Figures 2 and 3, some of the locations were north of the line, and others were in the south. In Figure 3, the differences in the terrain can be seen: the south is greener, while the north is a combination ofbrown and green patches, which shows a reduction in crop paddocks. As shown in Table 1, the GPS coordinates for each site in which the samples were taken are shown. Matt & Alice Nottle farm and the Joe & Jess Koch farm were located north of the line, while the Peter and Mel Kitschke and Luke & Scott Clark farm were located south of the line. Soil samples were collected from each site on March 21st, 2024, after the harvesting season. The area was comparatively dry as it was near the end of summer, and thetemperature ranged from 20°C to 30°C. A total of 10 samples were taken, with some

collected from ridges and others from furrows. Samples 7 and 8 were taken from mixed soil pits which the farmers dug for soil testing. Sample 7 was collected from a pit that was dug 1.5m deep, and the pit was filled with mixed soil, which was taken out of it, whilesample 8 was taken from a pit of mixed soil, but topsoil was taken. The plant variation at each location is also detailed in Table 1. Most of the samples were taken from wheat farms, apart from samples 7,8 , and 10 which were from canola fields, and sample 9 was the only sample from animal grazing land. This variation is because the farmers rotate crops between planting cycles, and some paddocks are often left as livestock grazing areas.

The rationale for this site and sample selection is the focus on paddocks that had prominent characteristics of calcareous soil, such as the clay soil, which had calcium carbonate particles visible in the soil. Sample collection of soil from different variations of crops and agricultural practices, such as animal grazing, was taken to observe whether it had a significant impact on microbial abundance. However, the reason for the lower amount of samples was because the samples were collected within one day, and there was a larger area to be covered.

2.2 Sample Storage

Soil samples were collected in sterile 50ml sampling tubes, and large quantities of soil were collected in sterile zip-lock bags. In order to account for heterogeneity in fields a couple of soil samples were taken from each of the respective sites and mixed together in the sterile zip-lock bags. This mixed soil was then transferred to the 50mL sampling tubes. In the laboratory, the sampling tubes were kept at -20 °C (Rubin et al., 2013), and the zip-lock bags were kept at room temperature for 30 days and then transferred to the freezer. This was done to avoid the risk of microbial cell death (Rubinet al., 2013) when freezing and minimize the effect on the flow cytometer readings.

2.3 Microbial Extraction from Soil.

To extract microbes from the soil, 0.1g of the soil samples were collected and measured using an electronic balance, and they were added to a sampling tube with a spatula, which had been sterilised with 70% ethanol. (Khalili et al., 2019)A stock solution of 5ml of a solution containing 0.025 M sodium tetraphosphate, 0.5% Tween80, and 0.85% sodium chloride wasthen added to the tube.(Khalili et al., 2019) The tube was shaken and vortexed for one minute. After vortexing, the sample tubes were placed in a rack for five minutes to allow the solution to settle. From the soil suspension that settled at the bottom, 1 ml was transferred to a sterile microcentrifuge tube. This tube was then centrifuged for 90 seconds at 1500 rpm andkept in a tube rack to process the next steps. The original centrifugation step was taken from (Khalili et al., 2019) but the speed and duration were changed, as this method did not use a Nycodenz gradient, to create a cost-effective and simplified method.

2.4 Flow Cytometry Protocol

To prepare samples for flow cytometry, 20 µl of 25% glutaraldehyde was first pipetted into sterile cryo-vials. Then, 980 µl of the previously collected soil solution was added, and the mixture was inverted to mix it thoroughly. The cryo-vials were then placed on ice for 15 minutes. After this, they were dipped into liquid nitrogen until the bubbles dissipated for 10 minutes. (Dann et al., 2014) On the day of flow cytometry analysis, the cryo-vials were removed from the freezer and allowed to thaw at room temperature. While the samples were thawing, the SYBR Green solution was also placed in the fume hood to thaw, while kept in the dark. Three replicates were prepared to account for noise; these were the experiment's controls. Tris-EDTA buffer solution was filtered through a 0.2 µm syringe and sterilized under UV light for 15 minutes. This process was also applied to the milli-q wat

Tetrasodium pyrophosphate is a soil dispersant agent that breaks up soil aggregates and releases the microbes embedded in between soil particles. This dispersal effect works particularly well in dense soils (Dascalu et al., 2024). Tween 80 is a non-ionic surfactant. Which reduces surface tension and helps microbial extraction by loosening the interactions between cells and the soil surface (Cheng et al., 2017). , and 50 ml of both the buffer and milli-q water were collected. (Dann et al., 2014)

A volume of 12.5 µl of SYBR Green was added to the samples inside the fume hood. Three replicates of each dilution were also prepared using a 5 mL stock solution, which contained 0.5% Tween80, 0.025 M sodium pyrophosphate, and 0.85% sodium chloride. These solutions along with SYBR and TE buffer were used in noise samples. This process was conducted in the dark to avoid auto fluorescence from the SYBR Green during flow cytometry readings. All tubes were vortexed for 30 seconds and covered with aluminium foil to protect them fromlight. After that, the samples were incubated in a water bath at 80°C for 10 minutes. (Patersonet al., 2013) During the incubation, 100 µl of bead stock was mixed with 900 µl of filtered milli-q water to prepare a bead stock solution. The flow cytometry tubes were also wrapped in aluminium foil to maintain the light interruption. After labeling the sample tubes, three dilutions were prepared: 1:500, 1:1000, and 1:2000. For the 1:500 dilution, 0.1 microliters of the sample were mixed with 499 µl of TE buffer. For the 1:1000 dilution, 0.5 µl of the samplewas combined with 499.5 µl of TE buffer. (Paterson et al., 2013) This process was repeated for the remaining samples. Before analysis in the flow cytometry lab, 10 µl of beads were added to each sample, vortexed for 30 seconds, and then analyzed using the cytoflex flow cytometer. It was measured under a medium flow rate. The recording of data started after 10 seconds and the measurements were taken for 2 minutes. This was consistent in all samples.

2.5 FlowJo Analysis

The cytograms were created with the help of Flowjo 10.8r1. The readings of the cytoflex flow cytometer were exported as flow cytometry standard files.The side scatter (SSC) function was used, and this is a component that is used to differentiate between viral and bacterial populations. (Dann et al., 2014) Side scatter indicatescell complexity, along with SYBR Green fluorescence, which measures nucleic acid content. Populations of bacteria, virus-like particles, debris, and beads were gated accordingly. The counts for these four components were then compiled and exported into an Excel file for analysis. (Dann et al., 2014)

Gating is done to isolate the cells of interest according to forward scatter and side scatter. Larger and more complex cells have higher values for both of these parameters. Identifying the size and makeup of the interested cells with the help of previous studies would be valuable in order to isolate the population of cells accordingly. This would help identify bacteria, virus-like particles, and fungi. (Khalil., et al. 2016) The fluorescence readings identified as debris in this study are the larger particles shown in the cytogram with a higher intensity and variation. This made it harder to categorize as a bacterial population, virus-like particles or fungi and was assumed to be portions of broken-down plant root DNA, animal DNA etc.

2.6 Bacterial And Virus-like Particle Abundance Calculation

Fluorescent 1 µm beads used in each flow session were added to the tubes containing 1 mL of samples. The abundance of bacteria, virus-like particles (VLP), debris, and beads was calculated using the following formula.

Events in the mL-1 of solution

= Dilution (Total count of each particle - Mean noise count of the respective particle)

The volume of the sample analyzed

2.7 Method optimisation for Calcareous soil

A new method needed to be used because there wasn't a standardised protocol for extracting microbes from the Calcareous soil present. Solution selection was essential to observe the effect. Three combinations, including 0.85% NaCl, Tetrasodium Pyrohosphateat 0.025 M concentration (pH 8), and 0.5% Tween 80 by v/v, were used. (Khalili et al., 2019)

1) 0.85% NaCl, Tetrasodium Pyrophosphate at 0.025 M concentration (pH 8)

2) 0.85% NaCl and 0.5% Tween 80 by (v/v)

3) 0.85% NaCl, Tetrasodium Pyrophosphate at 0.025 M concentration (pH 8), and 0.5% Tween 80 by (v/v)

All of these solution combinations accounted for 5 ml and were mixed with 0.1g of soil. The mixtures were analyzed using a Cytoflex flow cytometry machine. Dilutions and triplicates of noise and samples in 1:1000 were done following the procedure described above in Section 2.4.

After that, the effect of centrifugation was examined, as the previous experiments did not show much of the bacterial populations. As per section 2.4 above, after vortexing with 0.1g of soil and 5ml containing 0.85% NaCl, Tetrasodium Pyrophosphateat 0.025 M concentration (pH 8), and 0.5% Tween 80 by (v/v) and after sitting for 5 minutes, the supernatant was

collected and distributed between two sterile microcentrifuge tubes. (Khalili et al., 2019) Each tube contains 1.5 ml of supernatant. Then, one tube was centrifugedat 1500 rpm for 90 seconds, while the other was centrifuged at 1500 rpm for 5 minutes. After centrifugation, both samples were analyzed using flow cytometry, as mentioned in

section 2.4 above with noise controls containing 0.85% NaCl, Tetrasodium Pyrophosphate at 0.025 M concentration (pH 8), and 0.5% Tween 80 by (v/v) and Tris EDTA buffer. Dilutions and triplicates of noise and samples in 1:500 and 1:1000 were done.

2.8 Soil DNA extraction

DNA extraction was done on all 10 samples shown in Table 1 above. From each site, soil was taken in sterile containers. These samples were subjected to DNA extraction using the FastDNA® 50ml spin Kit for soil. First, 10 g of the soil samples were measured using an electronic balance. The spatula and plastic container were sterilised using an 80% ethanol solution. (Kaushal et al., 2021) The measuring container was changed for each of the samples. The soil was added to the 50ml Garnet Lysing Matrix Tubes in the DNA extraction kit. After that, 15 ml of sodium phosphate buffer was added to each tube. Then, the pre-lysing solution bottle was shaken, and 5 ml was added to each tube. The tubes containing soil solutions were shaken and vortexed for 10 to 15 seconds to mix the contents. (Kaushal et al., 2021) Then, the tubes were placed in arocker for 5 minutes at room temperature. Afterward, the tubes were taken out and centrifuged under 3500 x g for 10 minutes at room temperature. The supernatant was decanted and discarded to remove the wooded soil particles and other impurities. After that, 9.8ml of sodium phosphate buffer was added to each tube. Then 1.2ml of the MT Buffer (lysing solution) was added to each tube, shaken vigorously, and vortexed for 10 to15 seconds to mix the contents (Kaushal et al., 2021). The vortex was used to homogenize the samples for 2 minutes as there was no Fastprep®24 device. Afterward, the tubes were taken out and centrifuged under 3500 x g for 5 minutes at room temperature. After this step, the supernatant was transferred into the clean, sterile 50ml Collection Tubes.

The 50ml collection tubes were filled with 2.5ml of protein precipitation solution, which was added and shaken vigorously to mix the contents. As the next step, the tubes were incubated at 4 ℃ for 10 minutes and centrifuged again under 3500 x g for 5 minutes. After that, a 50 ml Spin Filter Tube was taken, and 10 ml of the binding matrix was added. Then, the supernatant was transferred to the Spin Filter Tube. Then, the tubes were shaken gently to resuspend the pellet and placed in the rocker for 5 minutes. The flow through was collected and discarded after centrifuging them at 3500 x g for 5 minutes. The samples were washed with 10ml of the guanidine thiocyanate wash buffer. The tubes were flicked and shaken gently to resuspend the pellet again. The flow through was collected and discarded after centrifuging them at 3500 x g for 5 minutes. After that, 6ml of the SEWS-M solution containing ethanol was added to the tubes and shaken gently. The flow-through was collected and discarded after centrifuging them at 3500 x g for 5 minutes. This step was repeated to dry the samples (Kaushal et al., 2021). After that, the spin filter was transferred to a new 50ml collection tube. After that, 5 ml of the TES solution was added to the filter's centre and centrifuged at 3500 x g for 5 minutes. Finally, the spin filter was discarded, and the DNA extract was frozen in the refrigerator. It was stored for a longer period, at -20°. (Díaz-Torres et al., 2021)

2.9 Polymerase chain reaction (PCR)

First, 4 solutions were added to the autoclaved sterile PCR tubes. This contained 20 µlof nuclease-free water, 4 µl of the sample DNA, 1 µl of Rapid Barcode Primer(RLB01), and LongAmp Taq 2x master mix. (Tyler et al., 2018)These were mixed gently and spun on a microfuge. A negative control containing no DNA and a positive control containing 1-5ng template DNA was also prepared. Each of the 10 DNA samples was loaded into the PCR machine.

Then the PCR equipment was programmed according to the following parameters. The first

cycle was the initial denaturation which was done at 95°C for 3 minutes. This was done in only one cycle, then 24 cycles of denaturation at 95°C for 15 seconds, Annealing 56°Cfor 15 seconds, extension at 65°C for 6 minutes with the final extension being 65°C for 6 minutes and 4°C indefinite was programmed and run (Tyler et al., 2018). After that was done 4 µl of EDTA was added to each tube. Then, they were incubated at room temperature.One µl of each barcoded sample was then measured through the Qubit fluorometer.

2.10 pH Measurement of Samples

To measure the pH of the 10 samples. 10g air-dried soil was measured using an analytical balance. After that, 50ml of sterile milli-q water was also measured using a measuring cylinder. The soil and water were mixed in a ratio of 1:5 (w/v) in a clean beaker. (Cho et al., 2016) The contents were gently mixed with a glass rod for 1 minute. Then, the readings were taken 3 times independently from each sample using the OHAUS starter 2100pH meter. Before the readings were taken, the pH meter was calibrated at two points using ACR standard buffer solutions at pH 7 and 10.

2.11 Statistical Analysis

In this study, the statistical analysis was done using IBM® SPSS ® version 22 Software. The Pearson correlation test, as well as the independent samples t-test, was performed. The Pearson correlation test was done to observe the relationship between environmental variables such as pH and the averages of the bacterial count among the samples (Zhang et al., 2019). Then, the independent variable t-test was performed to observe whether there was a mean difference in the averages of bacterial count between the north and south of Goyder's line. This was again performed to observe whether the mean difference in bacterial abundance was significant between ridges and furrows in wheat farms. (Yang et al., 2018) When performing the Pearsoncorrelation analysis between pH and bacterial count, it was

assumed that there was a linear relationship between each of these components, and the data had a normal distribution. First, the data entry was done using SPSS software. Average pH values, as well as average bacterialcount, were entered. Then, the analysis, correlation, and bivariate steps were followed. The significance was chosen as two tails. After that, the correlation coefficient obtained was checked to see whether it was closer to -1 or +1 to determine whether it had a positive or a negative correlation, and the strength was determined as strong, moderate, or weak. Also, the statistical significance was concluded when the significance value was less than 0.05, meaning there is statistical significance (Xu et al., 2022).

Secondly, the mean difference between bacterial abundances between the north and south of the Goyder's line , and between the ridges and furrows in farms is calculated. First, the data set was divided into two groups. The independent variables were the ridge or furrow in the first test and the direction north and south in the second test. The first test used the average bacterial count from samples 1 to 6, taken specifically from ridges and furrows. The second test used all 10 samples and was categorized as the first four samples from the north and the rest from the south. The averages of bacterial counts were assumed to be of normal distribution and continuous data. (Ozgoz et al., 2013) After defining the variables, the calculation was performed in steps: analyze, compare, and an Independent Sample T Test was selected. After that, the independent and dependent variables were selected, and the groups were defined as the independent variable. Finally, the test was performed. The outcome of thedata was interpreted using the significance level of ($p<0.05$). The null hypothesis for this experiment was that there wasn't a mean difference in average bacterial abundance between the two independent groups. The alternate hypothesis was that there is a mean difference present between the two independent groups. (Ozgoz et al., 2013)

3 RESULTS

3.1 Microbial Extraction of Calcareous Soil

3.1.1 Effect of Solutions

Flow cytometry analysis was done on the collected samples according to section 2.7, included in the method section above. Three combinations of the solutions, Tween80, Tetrasodium pyrophosphate, and Sodium chloride solutions, were used. This was done to clearly show which solutions or combinations extract more microbes from this specific soil, as there was no standardised method for extracting microbes from this specific soil. Soils from sample 1 mentioned in Table 1 were used as a test sample for this experiment and a dilution of 1:1000 was made. The three combinations were 1) 0.85% NaCl, Tetrasodium Pyrophosphate at 0.025 M concentration (pH 8), 0.85% NaCl and 0.5% Tween 80 by (v/v) and 0.85% NaCl, Tetrasodium Pyrophosphate at 0.025 M concentration (pH 8), and 0.5% Tween 80 by (v/v). The noise readings were subtracted from the sample readings to minimize noise interference. The following data table was made after calculating the results obtained by Flowjo 10.8r.1, shown in the appendices section, and using the equation in section 2.6.

The debris was quantified as per section 2.5 by gating the higher and more intense fluorescence measurements which indicate a larger size that cannot be categorized as bacteria or virus-like particles.

Table 2- The Bacteria, Virus-like particles(VLP), and Debris counts that were present in 1ml of extracted soil solutions which used the three solution combinations Tetrasodium pyrophosphate, Tween80, and Sodium Chloride

Sample	Bacteria | Count (mL^{-1})	VLP | Count(mL^{-1})	Debris | Count (mL^{-1})
TSP 0.025M(pH 8)+0.85%NaCl	$4.2x\ 10^5$	$6.3x\ 10^3$	$4.3x\ 10^6$
0.5%Tween80+0.85%NaCl	$1.5x\ 10^5$	$2.9x\ 10^4$	$1.7x10^7$
TSP 0.025M(pH 8) **0.5%Tween80+0.85%NaCl**	$2.3\ x\ 10^5$	$1.0\ x\ 10^5$	$1.9\ x\ 10^8$

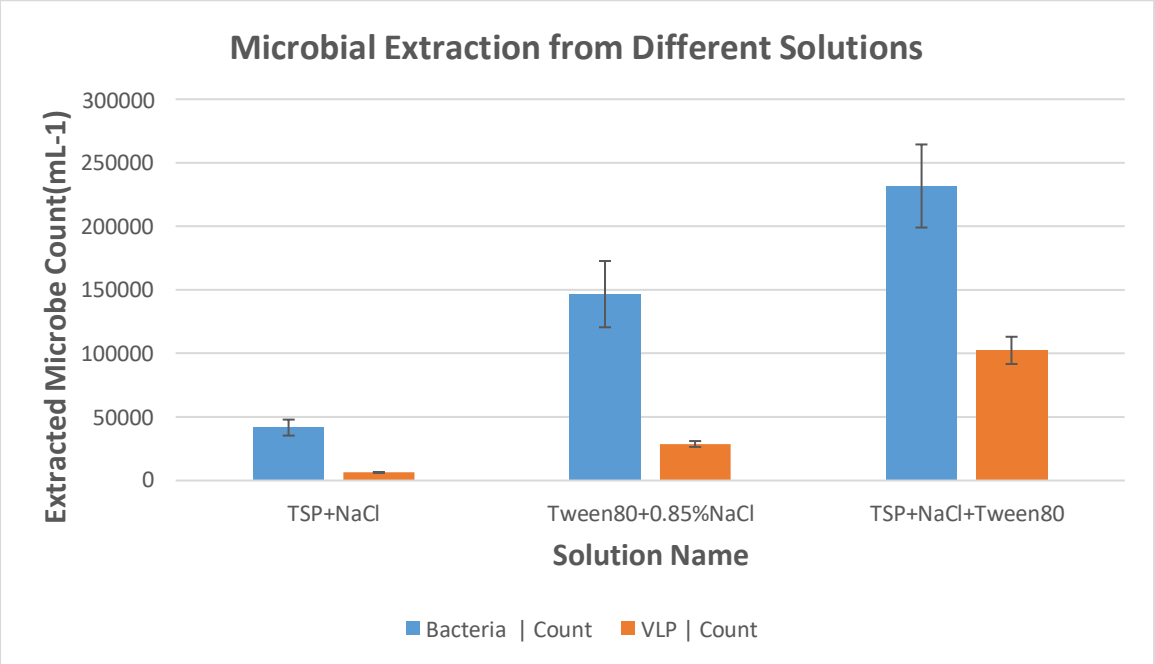

Figure 4- The Bacteria, Virus-like particles (VLP) present in 1ml of extracted according to eachsolution combination: combinations of Tetrasodium Pyrophosphate, Tween80, and Sodium Chloride. The error bars were calculated by dividing the square root of the samples measured from the standard deviation.

The above Figure 4 shows the microbial extraction of each solution combination. Solution combination 1 which includes 0.85% NaCl, and Tetrasodium Pyrophosphate at 0.025 M concentration (pH 8) shows the least number of bacteria and Virus-like particle extraction, The second combination 0.85% NaCl and 0.5% Tween 80 extracts more microbes than the First combination. Most microbial extractions were done with the combination of Tetrasodium Pyrophosphate at 0.025 M concentration (pH 8), 0.5% Tween 80 by (v/v) and 0.85% NaCl. It extracts around 2.3×10^5 bacteria and around 1.0×10^5 Virus-like particles. As 0.85% NaCl is equal in all the solutions, the main extraction effect comes from Tetrasodium Pyrophosphate at 0.025 M and 0.5% Tween 80. The only downside is that the amount of debris also increases with a higher amount of microbial extraction as seen in Table 2.

3.1.2 Effect of Centrifugation

In this section, the effect of centrifugation in extracting soil microbes from the soil was observed. According to section 2.7 above, the flow cytometric analysis was done. The combination of 0.85% NaCl, Tetrasodium Pyrophosphate at 0.025 M (pH 8) and 0.5% Tween 80 by (v/v) was used for this experiment. Soil sample 1 mentioned in Table 1 was used as a test sample for this experiment and dilutions of 1:500 and 1:1000 were observed. The difference in these samples were that after 1.5 ml of supernatant was collected one set was centrifuged at 1500 rpm for 90 seconds, while the other was centrifuged at 1500 rpm for 5 minutes. The noise readings were subtracted from the sample readings to minimize noise interference. The following data table was made after calculating the results obtained by Flowjo 10.8r.1 using the equation in section 2.6 which is shown in the appendices section.

Table 3- The Bacteria, Virus-like particles(VLP), and Debris counts that were present in 1ml of extracted soil solutions which were centrifuged at 1500rpm for 90 seconds and 5 minutes. Each dilution was multiplied from the dilution factor to obtain the count in 1mL

Sample	Bacteria | Count	VLP | Count	Debris | Count
Centrifuged at 1500 rpm for 5 minutes(1:500)	1.4×10^5	-1.2×10^4	3.6×10^5
Centrifuged at 1500 rpm for 5 minutes(1:1000)	1.5×10^5	1.1×10^4	4.3×10^5
Centrifuged at 1500 rpm for 90 seconds(1:500)	9.4×10^5	-2.2×10^4	5.7×10^7
Centrifuged at 1500 rpm for 90 seconds(1:1000)	9.2×10^5	5.7×10^4	5.1×10^7

As per the values in Table 3 above some of the Virus-like particles(VLP), values are minus values. This is done by this is illogical as a count of Virus-like particles could not be negative. The reason for this figure is that the amount of virus-like particles is much closer to the sample values. And when it is multiplied by the dilution factor a large number can be seen. This error is smaller than the standard error so analyzing the virus-like particles in this particular experiment would not produce significant results. So the focus is shifted to the bacterial population**.** The reason behind the bacterial counts in dilutions 1:1000 and 1:500 producing relatively similar numbers was that the dilutions were done independently from each other and from the initial data the obtained counts were multiplied by the dilution factor. So the final values are relatively similar.

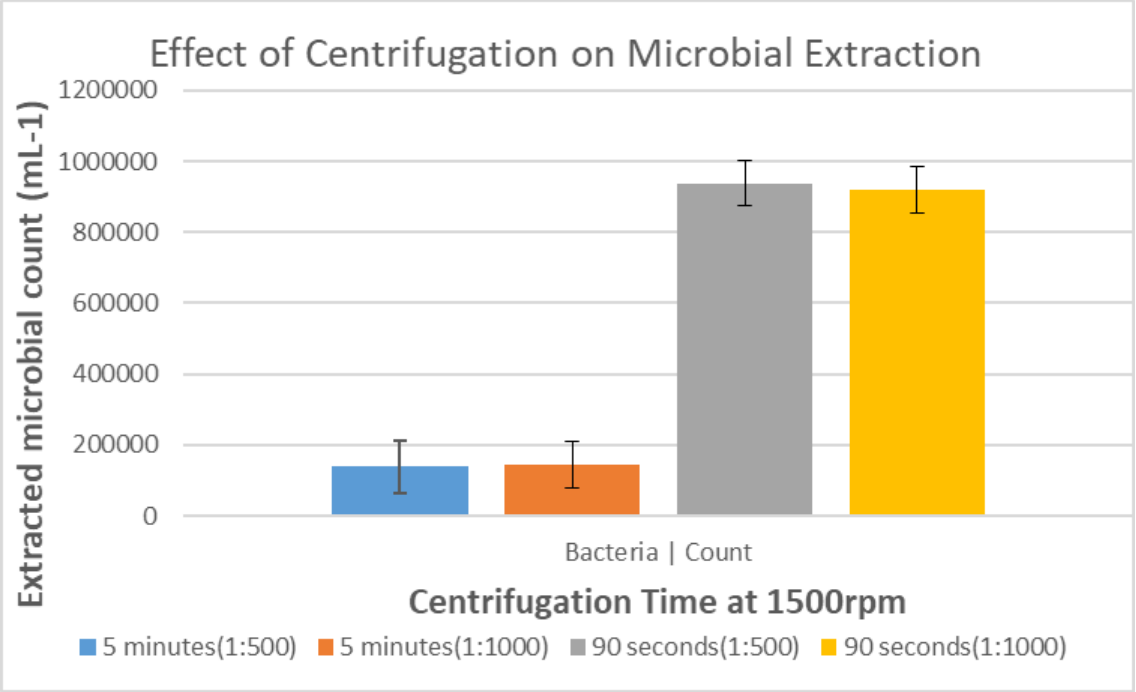

Figure 5-The Bacteria, present in 1ml of extracted according to Centrifugation times of 90 seconds and 5 minutes under 1500rpm. These were derived by multiplying the dilution from the dilution factor 1:1000 and 1:500

The above Figure 5 shows the microbial extraction out of the centrifugation at times 90 seconds and 5 minutes under 1500rpm. The 5-minute centrifugation time shows the least amount of bacteria extraction, Although the centrifugation time of 90 seconds at the speed of 1500rpm shows the most extraction. It should be noted that the dilution factors of 500 and 1000 add up to almost equal figures which means that there was less contamination or interference. One of the upsides is that the amount of debris decreases when the samples undergo centrifugation for a longer time so it won't negatively impact the microbial extraction results as seen in Table 3.

It can be observed that the combined solution method produced the most cells, but also the most debris. This can be seen as a trade-off in cells vs. debris. Suggesting that the longer centrifuge speed be used, or just the lysis solution that produces the least debris combined with a shorter spin time. However, the objective of this method was not to obtain the least amount of debris. It was to extract a larger amount of cells while keeping the debris interference at a minimum. In order to obtain a more optimized method a series of

centrifugations should be done to find the point with the most cells and least debris which exceeding this limit impacts the accuracy of the results.

3.1.3 Flow cytometry results of the Samples

Table 4- This Table shows the multiplication of all dilution ratios and average bacterial abundance of all 10 samples from soil sites, present in 1ml of extracted solutions from each site, and was analyzed by flow cytometry. The average was calculated by obtaining the average of all 1:500, 1:1000, and 1:2000 which was multiplied by the dilution factor.

Sample Name	1:500 Ratio	1:1000 Ratio	1:2000 Ratio	Average Bacterial Count
Sample 1	$1.3x10^6$	$2.3 x10^6$	$5.3 x10^6$	$3.0 x10^6$
Sample 2	$1.0 x10^6$	$5.4 x10^4$	$1.3 x10^7$	$4.7 x10^6$
Sample 3	$1.0 x10^6$	$3.6 x10^4$	$1.2 x10^7$	$4.3 x10^6$
Sample 4	$1.4 x10^6$	$8.2 x10^4$	$6.7 x10^6$	$2.7 x10^6$
Sample 5	$2.8 x10^6$	$4.7 x10^6$	$2.7 x10^6$	$3.4 x10^6$
Sample 6	$3.1 x10^6$	$4.7 x10^6$	$9.1 x10^6$	$5.7 x10^6$
Sample 7	$1.4 x10^6$	$4.8 x10^6$	$7.3 x10^6$	$4.5 x10^6$
Sample 8	$4.6 x10^6$	$2.4 x10^6$	$1.4 x10^7$	$7.1 x10^6$
Sample 9	$4.9 x10^6$	$7.5 x10^6$	$1.3 x10^7$	$8.6 x10^6$
Sample 10	$3.8 x10^6$	$5.1 x10^6$	$9.4 x10^6$	$6.1 x10^6$

In this section, all 10 samples that were collected near the Goyder's line were subjected to microbial extraction from the soil, and the flow cytometry readings were observed according to section 2.7 above, In this test the method which proved to extract the most amount

microbes, while reducing debris was used. This was the combination of 0.85% NaCl, Tetrasodium Pyrophosphate at 0.025 M (pH 8), and 0.5% Tween 80 by (v/v) was used. And the centrifugation time of 90 seconds at 1500rpm was used. Dilutions of 1:500,1:1000 and

1: 2000 with triplicates were observed which accounted for 99 readings including noise controls. These readings can be observed in the appendices section for future research. Table 4 above was created using the averages of triplicates and accounting for noise interference and after calculating the results obtained by Flowjo 10.8r.1 using the equation in section 2.6. Then the averages of each of all three dilution factors were taken and the average values of all 10 samples were taken as there were differences in dilution factors which can be seen in Figure 6 below.

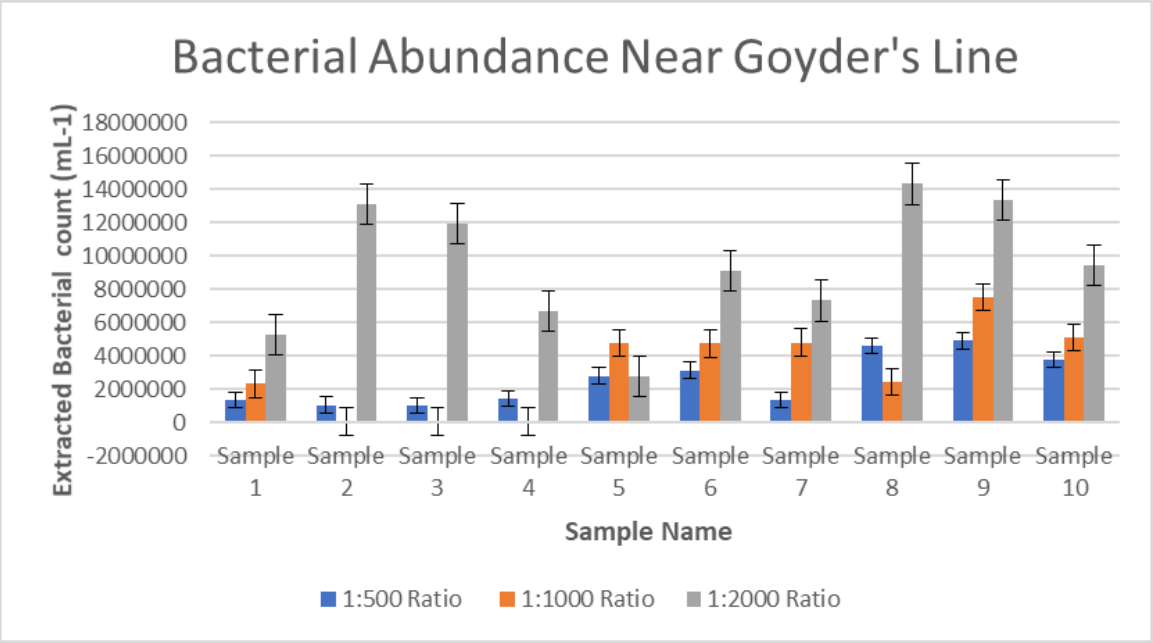

Figure 6- This graph shows the bacterial abundance of all 10 samples of Bacteria, present in 1ml of extracted solutions from each site and analyzed in triplicate of dilutions of 1:500, 1:1000, and 1:2000. These values were calculated by multiplying the dilution from the dilution factor.

The graph which can be seen in Figure 6 above shows the bacterial abundance near Goyder's line which is cumulative of 10 samples using dilutions of 1:500, 1:1000, and 1:2000. The y-axis shows the bacterial count per milliliter (mL^{-1}) which ranges from 0 to 30,000,000 and the x-axis is showing the number of the samples. Each segment of color depicts the contribution each dilution factor makes toward the overall bar. As an example, the 1:500 ratio

shown with the blue color segment. In the graph, the contribution of 1:500 is rather low compared to the contribution of 1:2000. Although under normal conditions these values should be slightly similar to each other with a slight margin of error. But this is not the case presented here. So some of the samples that showed more differences than average were repeated under the same conditions in section 3.3 below. The graph shows a wide variation of abundance in bacteria across each of the sites. Sample 9 shows a much higher bacterial count compared to others. This was evident in all 3 dilution factors as well. This indicates that a higher amount of bacterial presence is found in this area. In contrast to sample 9, samples 1,3, and 5 show a much smaller amount of bacterial abundance level. These samples should be closely inspected in order to find the factors that drive them.

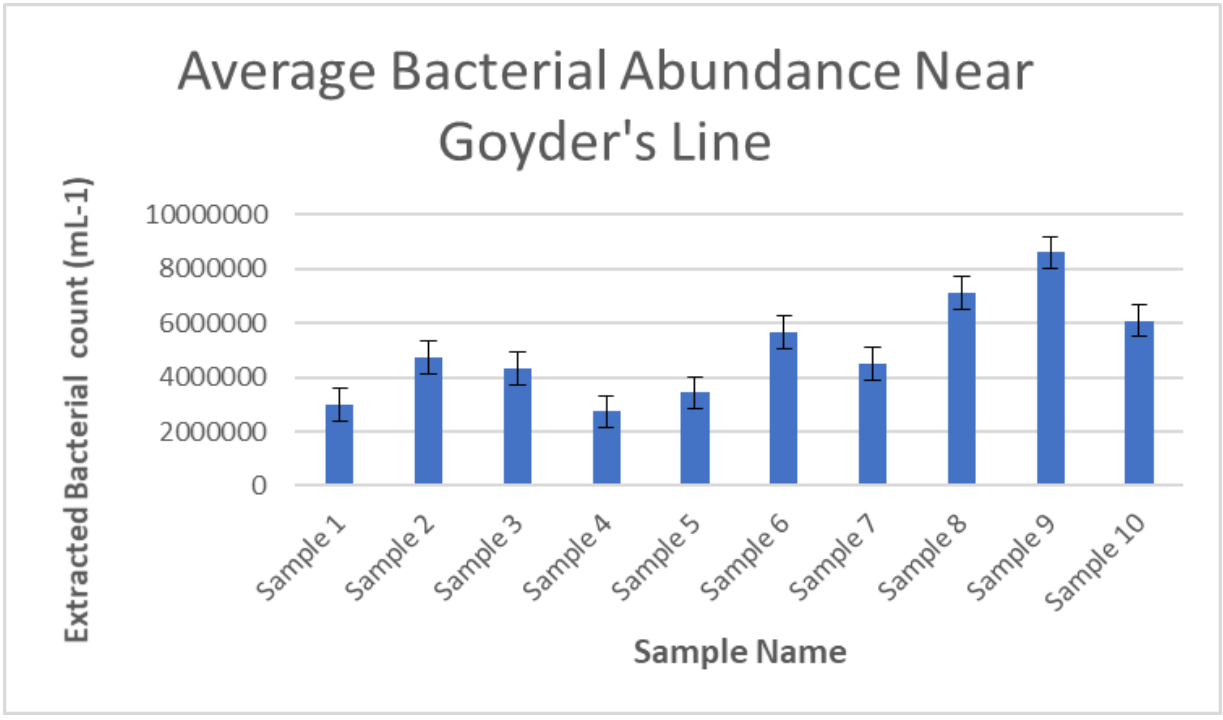

Figure 7 - This graph shows the average bacterial abundance of all 10 samples from soil sites, present in 1ml of extracted solutions from each site and analyzed. The X-axis shows the sample names ranging from 1 to 10 and the y-axis depicts the Extracted Bacterial count (mL^{-1})

So, this graph (Figure 7) shows the average bacterial abundance near Goyder's Line, This graph is made using the data values by an average of all three dilution factors which was obtained from Table 4. The x-axis shows the sample names, while the y-axis shows the

extracted bacterial count per milliliter. When comparing with Figure 6, it can be observed that the highest value is also shown in sample 9 in this graph as well, while the same lowest value is repeated in samples 1, 4, and 5. The reason for these changes in variation of bacterial abundance might be caused by environmental factors such as pH, which will be tested in section 3.4 below. In a comparison of the bacterial count in each sample with the description of the sample site which is present in Table 1, it should be noted that sample 9 had the highest bacterial count value, the difference between it and other samples is that it is the only sample which is taken from an animal grazing land. When finding a pattern for other samples, it can be seen that in Samples 1, 2, 3, 4, 5, and 6, the ridges show less bacterial abundance compared to furrows, changing only in samples 3 and 4.

3.2 Repetition of Samples

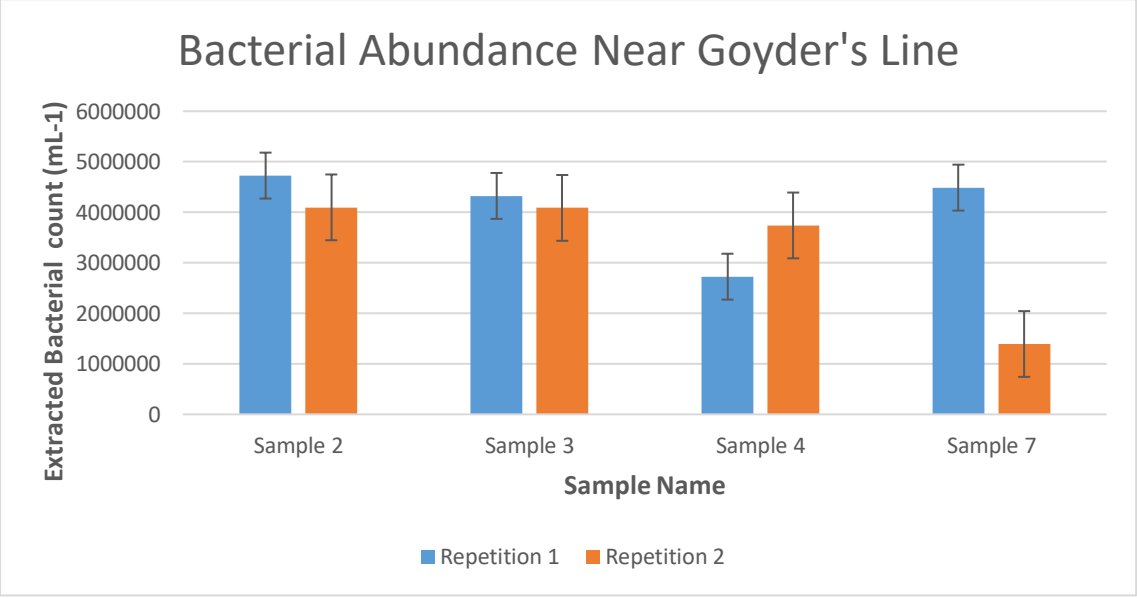

Figure 8- This graph shows the average bacterial abundance comparison between 4 sample soils, Samples 2,3,4, and 7 were observed. The average was calculated by obtaining the average of all 1:500, 1:1000, and 1:2000 multiplied by the dilution factor. The blue color shows the values from Figure 7. The orange color shows the averages of the second experiment.

Figure 8 above shows the comparison of bacterial abundance between sample repetitions, which means that in Figure 6, it showed that in samples 2, 3, 4, and 7, the contribution of

dilution 1:500 and 1:2000 varied significantly. In this experiment, the variation is observed so that an understanding can be observed whether it was caused by contaminants or it is the structure of the soil. The x-axis shows the sample names, which are divided between repetition 1 which was obtained from the values in Figure 7, and repetition 2 in which the average bacterial abundance counts were obtained by doing a separate experiment that involved the same four samples as well as the same methodology, which is present in the appendix, It can be observed that there is a notable variation in sample 7 and a slight difference in sample 4. However, samples 2 and 3 display a very limited difference between repetition 1 and repetition 2, so it can be assumed that the variations between samples 2 and 3 experiments were not significant. Although this is the case, it can also be assumed that something is interfering with the results in sample 7, which needs to be investigated further.

This comparison is valuable because it proves the reliability of the experiments and the experiment methods across soil samples. The difference between the repetitions could suggest that there are differences in the errors caused in handling samples or the problem lies with the soil itself, whether it's interference with debris or there is a microbial distribution difference within the soil. So, in order to increase the significance of the experiments, more repetitions of the same experiment should be done to minimize errors.

3.3 pH variance of the Samples

Table 5- This table shows pH readings obtained from all 10 samples. Three independent readings were taken after dissolving 10g of soil in 50 ml of milli-q water. The average was calculated from all three readings of the respective sample.

Sample Name / pH	Reading 1	Reading 2	Reading 3	Average
Sample 1	7.23	7.21	7.24	7.2
Sample 2	7.58	7.61	7.57	7.6
Sample 3	5.72	5.7	5.71	5.7
Sample 4	6.05	6.06	6.06	6.1
Sample 5	6.88	6.86	6.87	6.9
Sample 6	6.15	6.16	6.14	6.2
Sample 7	8.31	8.35	8.34	8.3
Sample 8	5.48	5.47	5.47	5.5
Sample 9	6.05	6.06	6.04	6.1
Sample 10	6.72	6.7	6.7	6.7

Correlations

		Bacterial_Count	Average_pH
Bacterial_Count	Pearson Correlation	1	-.365
	Sig. (2-tailed)		.299
	N	10	10
Average_pH	Pearson Correlation	-.365	1
	Sig. (2-tailed)	.299	
	N	10	10

Figure 9- Pearson correlation obtained from SPSS software on the correlation between Average Bacterial Count and Average This was done on all 10 samples. The correlation was a --0.035 and the significance is 0.299

Table 5 above was created using the pH readings that were done on all 10 samples of soil. After taking three independent readings from each soil solution, 10g of soil was dissolved per 50 mL of Milli-Q water. Then, the average of all three readings was taken andrecorded. When observing the average readings, sample 7 had the highest pH value among all samples, which was a value of 8.33, and sample 8 had the lowest pH value, which was 5.47. Others were around neutral, the pH values of 6 and 7, except sample 3, which had a value of 5.7. It should be noted that samples 7 and 8 are from the same farm closer to each other. Both were mixed soils from around the area. The only difference was that sample 7 was from a soil pit dug to 1.5m down and filled with the same soil.

Figure 9 shows the correlation between the bacterial counts of each of the 10 samples and the average pH of 10 samples. This was calculated using a Pearson correlation test. The results obtained from that test concluded that it had a Pearson correlation (r) = - 0.365 and a significance of 0.299. This indicates a negative correlation between the average bacterial count and the average pH value. This means that when the pH increases, the bacterial count decreases slightly. However, this is the case as the significance value(p) is higher than 0.05 ($p > 0.05$) is considered as not statistically significant. This means there is no sufficient evidence of a relationship between bacterial count and pH in this context.To obtain a larger significance value, the number of samples needs to increase.

3.4 Concentration of DNA of the Samples

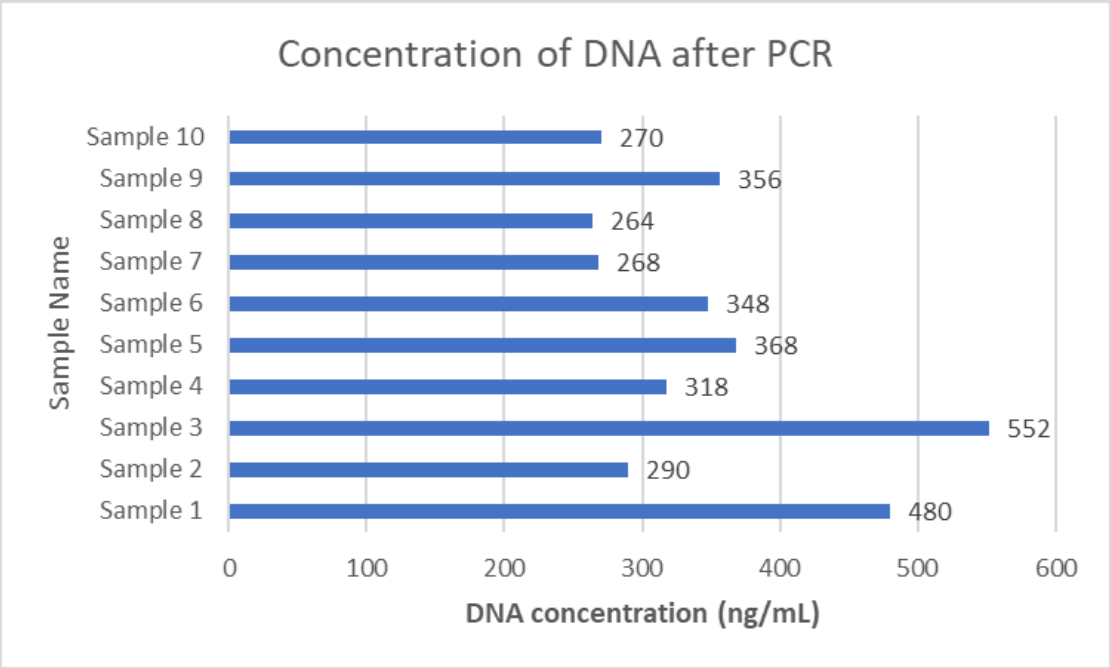

Figure 10- Concentrations of DNA measured on 10 sample sites across Goyder's line. Obtained from Qubit fluorometer after Polymerase chain reaction. Each bar represents the respective concentration in (ng/mL)

Figure 10 above shows the concentrations of extracted DNA that have undergone a polymerase chain reaction. These values were obtained from the qubit fluorometer. In each of the 10 samples, there is a variation in concentration. Sample 3 has a higher concentration of DNA compared to others, which is around 552ng/mL. The sample with the least concentration was sample 8, which was around 268ng/mL. These values do not coincide with the average bacterialcount values in Figure 7 for each sample. As this is the case, there should be another explanation for the variation in value. The DNA concentrations should be much higher than the present values after the polymerase chain reaction (PCR) to obtain a satisfactory sequence reading from the Minion sequence machine. The faults resulting in these concentration numbers should be addressed to do that. There was limited time for the project, so the sequencing step was not done. However, sequencing of these samples would be vital to find the species of bacteria and other microbes that live in this specific soil.

To achieve that, it is vital to identify the errors that would have happened. The errors could have been due to faults in the DNA extraction process, contamination of the DNA that halted the polymerase chain reaction, or errors made when following the polymerase chain reaction protocol. These issues are addressed inthe discussion section.

3.5 Bacterial Abundance Between Ridge and Furrow

Group Statistics

	Ridge_Furrow	N	Mean	Std. Deviation	Std. Error Mean
Bacterial_Abundence	Ridge	3	3578666.6667	681969.72309	393735.40320
	Furrow	3	4370111.1113	1499083.77651	865496.42191

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means
		F	Sig.	t
Bacterial_Abundence	Equal variances assumed	2.007	.230	-.832
	Equal variances not assumed			-.832

		t-test for Equality of Means		
		df	Sig. (2-tailed)	Mean Difference
Bacterial_Abundence	Equal variances assumed	4	.452	-791444.44467
	Equal variances not assumed	2.794	.470	-791444.44467

		t-test for Equality of Means		
		Std. Error Difference	95% Confidence Interval of the Difference	
			Lower	Upper
Bacterial_Abundence	Equal variances assumed	950847.84488	-3431421.28931	1848532.39997
	Equal variances not assumed	950847.84488	-3947806.16781	2364917.27847

Figure 11- The Independent Sample T-test measured the variance between the bacterial abundance of the Ridges and Furrows. Samples from 1 to 6 were observed.

As shown in Figure 11 above, an independent samples t-test was conducted to observe the variation between bacterial abundance across samples obtained from ridges and furrows of

the wheat farms, the results did not show a statistically significant difference between ridges and furrows. In the ridge, it had a mean bacterial abundance M = $3.6x10^6 \pm 3.9x\ 10^5$ cells ml^{-1} and in the furrows the mean value was about M = $4.4x10^6 \pm 8.6x10^5$cells ml^{-1}. After that, it showed that Levene's test for equality of variances was 0.230, which is not significant as it exceeded the average considered value of 0.05, thus confirming the null hypothesis assumption that equal variances were met. Also, in the case of the t-test, the equality of means was at 0.452, which is not a statistically significantvariable. It has a higher value than the average p-value of 0.05 and shows a mean difference of $-7.9 \times 10^5 \pm 9.5 \times 10^5$ cells ml^{-1} Because of this, it can be assumed that there is no significant difference in bacterial abundance between the ridges and furrows of the wheat farms. From all these data, there is not a statistically significant variation in bacterial abundance between the ridges and furrows. It also paves the way for the assumption that higher amounts of water accumulated in furrows do not significantly influence the abundance of bacteria in the ridges. Although this is openfor interpretation as some limitations affect this conclusion, which will be elaborated in the discussion section.

3.6 Bacterial Abundance Between North and South

Group Statistics

	Direction	N	Mean	Std. Deviation	Std. Error Mean
Bacterial_Abundence	North	4	3689861.1113	983759.67775	491879.83887
	South	6	5892833.3333	1837927.20750	750330.64046

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means
		F	Sig.	t
Bacterial_Abundence	Equal variances assumed	.944	.360	-2.170
	Equal variances not assumed			-2.455

		t-test for Equality of Means		
		df	Sig. (2-tailed)	Mean Difference
Bacterial_Abundence	Equal variances assumed	8	.062	-2202972.22208
	Equal variances not assumed	7.815	.040	-2202972.22208

		t-test for Equality of Means		
		Std. Error Difference	95% Confidence Interval of the Difference	
			Lower	Upper
Bacterial_Abundence	Equal variances assumed	1015331.21164	-4544330.19472	138385.75055
	Equal variances not assumed	897185.51365	-4280427.51417	-125516.93000

Figure 12- The Independent Sample T-test measured the mean-variance between the bacterial abundance between samples taken from North of the Goyder's line and thesouth. All 10 samples were considered.

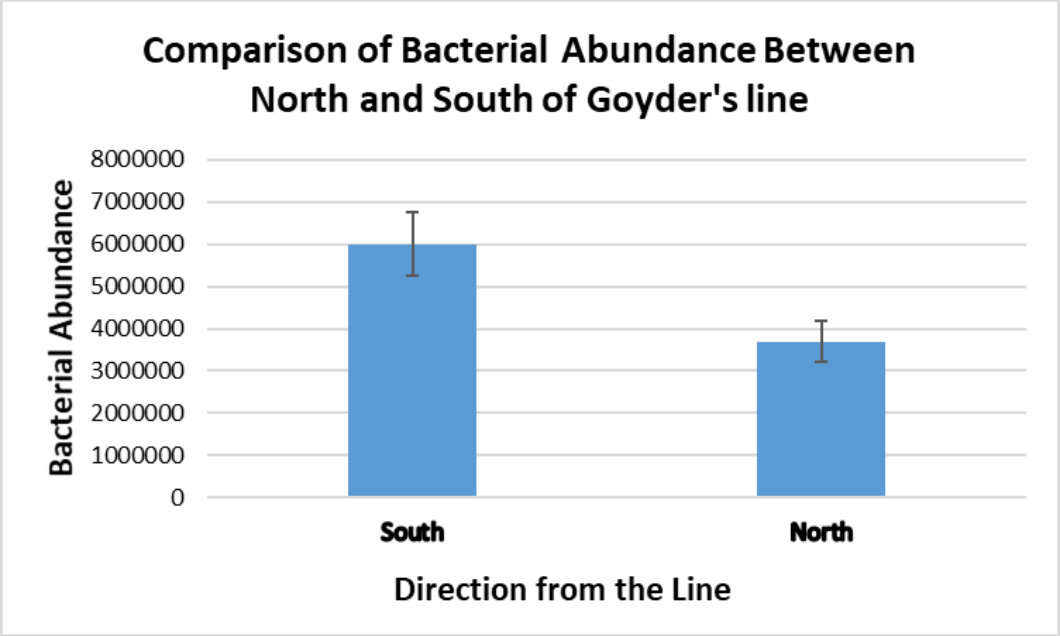

Figure 12a- Comparison of Bacterial Abundance Between the North and South of Goyder's line. The average bacterial abundance was taken from 10 samples. The standard error of the mean was obtained from the independent samples t-test values.

As shown in Figures 12 and 12a above, an independent samples t-test was conducted to observe the variation between bacterial abundance across samples from north and south of the Goyder's line; the results showed a significant difference between the two directions. In the south, it had a greater bacterial abundance of $6.0 \times 10^6 \pm 7.5 \times 10^5$ cells ml^{-1} compared to the north which was about $3.7 \times 10^6 \pm 4.9 \times 10^5$ cells ml^{-1}. After that, it showed that Levene's test for equality of variances was 0.360, which is not a significant figure as it exceeded the average considered value of 0.05, thus confirming that the null hypothesis assumption, which is that equal variances were met. However in the case of the t-test for equality of means was at 0.040, which is a statistically significant variable compared to the average value of 0.05 and shows a mean difference of $-2.2 \times 10^6 \pm 9.0 \times 10^5$ cells ml^{-1} Because of this, there is a significant difference in bacterial abundance between the North and South of the Goyder's line. From all these data, there is a statistically significant variation in bacterial abundance between the north and the south. The higher abundance of bacteria in the south of the Goyder's line might be influenced by the higher amount of rainfall affecting the region.

4 DISCUSSION

4.1 Optimising Method for Microbial Extraction

From the results obtained from sections 3.1 and 3.2, it can be observed that the best method to extract microbes was to use the combination of reagents, which included Tetrasodium Pyrophosphate at 0.025 M concentration (pH 8), 0.5% Tween 80 by (v/v), and 0.85% NaCl. Furthermore, the centrifugation time should be kept at a minimum. If the centrifugation is high, the risk of reducing microbial extraction increases (Peterson et al., 2012). But if centrifugation isn't done, the amount of debris that interferes with the data increases. The reason behind the increased microbial extraction is due to the unique properties of the chemicals that were used. Tetrasodium pyrophosphate has the property of being a soil dispersant agent. This is the process of breaking up soil aggregates, thus releasing the microbes embedded in between soil particles. This dispersal effect works particularly well in dense soils such as the calcareous soil (Dascalu et al., 2024). Tween 80

acts as a non-ionic surfactant. This property reduces surface tension and helps microbial extraction by loosening the interactions between cells and the soil surface. Tween 80 works significantly well in extracting bacteria that bind strongly to the soil particles (Cheng et al., 2017). Together, both of these components create a media that helps the microbes suspend soil solution separated from a large amount of debris.

The function of NaCl in this solution is that it makes the environment that maintains a stable osmotic pressure. This environment preserves the structural integrity of the cells, such as bacteria, by reducing the effect of osmosis. (Chowdhury et al., 2011) The NaCl solution also helps prevent the re-binding of the cells to the soil particles, making a more efficient cell extraction process. Finally, when it comes to centrifugation, a higher speed or time would cause many issues. Excessive centrifugation would cause cell lysis in some of the cells extracted into the solution. The cell walls and membranes of microbes wouldbreak due to the stress. (Peterson et al., 2012) Also, reducing the centrifugation time and speed would stop cells from clumping together and sinking to the bottom with the soil particles and lessen the amount of DNA shearing in particles, which interferes with flow cytometry identification as SyBr green reagent binds to DNA.

4.2 Bacterial Extraction of Samples.

In observations of sections 3.3 and 3.4, it is apparent that there is a variation between the samples in terms of average extracted bacterial count. The highest recorded sample was sample 9. When figuring out which should be noted, the difference between it and other samples is that sample 9 was the only sample taken from animal grazing land.

In the case of animal grazing lands, the increase in microbial abundance can be attributed to the higher levels of manure and urine from animal excretions, which add important nutrients such as nitrogen and phosphorus to the soil. These nutrients promote microbial growth as they break down the organic matter present in the soil (Yang et al., 2019). Animals that graze on the land compact and aerate the soil through constant activities such as walking. The decomposition done by microbes within the topsoil increases when the soil is aerated, thus increasing the population abundance in microbes (Zhao et al., 2017). Another result of grazing is that the pressure caused by it increases plant root exudates into the soil. These exudates include metabolites such as amino acids, saccharides, phenolics, and flavonoids, which are nutrients for bacteria (Vives-Peris et al., 2020).

The second important finding is that there is a significant variation observed in the average extracted bacterial count of sample 7. In the interpretation of the sample 7 results, the unique characteristics of the soil in which it was collected should be considered. Sample 7 contained mixed soil from a soil pit which was dug to a depth of 1.5 m. It should also be noted that in the region there is a calcium carbonate layer deposited. (Bolan et al., 2023) The presence of this calcium carbonate ($CaCO_3$) layer is the most probable cause which influences the microbial count of this sample. As it is a soil mixture, there should be calcium carbonate particles dispersed throughout the mixture under different concentrations, making the soil heterogeneous. (Bolan et al., 2023) Which creates differences in flow cytometry readings when the experiment is repeated.

Calcium carbonate raises the pH of the soil when it's present, so this can promote or inhibit the presence of different species of microbes according to their pH tolerance (Bolan et al., 2023). Most of the microbes present in the soil thrive under neutral or slightly acidic conditions. Because of the presence of calcium carbonate, the soil becomes more basic. It

will inhibit the growth of the acidophilic microbes. The abundance of microbes would also decrease because of the reduction in nutrient and organic matter present in deeper soil layers.

4.3 Relationship between pH and Microbial abundance.

The results obtained from the Pearson correlation test in section 3.4, figure 9 concluded that it had a Pearson correlation (r) = -0.365 and a significance of 0.299. This indicated that the correlation between the average bacterial count and the average pH value is had no significance The meaning of this is that the increase in pH value, did not have an effect on the bacterial count according to this study. This is because the significance value(p) is higher than 0.05, it is not statistically significant. Meaning there is not sufficient evidence that there is a relationship between bacterial count and pH. The relationship between microbial abundance and pH, especially when it comes to the bacterial count, has been a focus of soil science for many decades (Siciliano et al., 2014). This is because of pH on the cycling of nutrients and maintaining soil health. The soil pH influences the enzyme activity and nutrient availability of the soil, thus impacting the composition of the microbial communities (Neina., 2019). Generally, microbial abundance is higher in neutral pH levels, such as pH 6 to 7, where the soil functions such as organic matter decomposition, fixation of nitrogen, and cycling of carbon are higher. However, in extreme pH conditions, such as acidic or basic, they tend to reduce microbial abundance. (Lin et al., 2024)

Some bacterial communities do show increases in extreme pH conditions, too. While higher bacterial abundances are shown in neural conditions, species that belong to Acidophilic bacteria tend to show an increase in acidic conditions (Sharma et al., 2012). Some species within the phylum of Actinobacteria live in slightly basic conditions. This indicates that pH serves as a condition for determining the population densities of bacteria.

When it comes to the Pearson correlation test shows that the sample size was low.

Thus, resulting in a significance value of 0.299 indicates that the experiment's sample size was too low to obtain a statistically significant result. If the sample size was increased in future studies, it should enhance the power of the study to obtain a meaningful relationship pattern between the two components. Previous studies on pH and microbial abundance show a bell-shaped graph where the highest levels are shown in neutral pH areas and the lowest levels in extreme pH conditions (Lin et al., 2024). The currentexperiment not being able to reach a statistically significant figure, may have been a result of other variables influencing the abundance of bacteria such as soil moisture levels. Finally, the relationship between soil pH and the abundance of bacteria is influenced by multiple environmental and biological factors. Thus, focusing on a more comprehensive study of all those factors would result in a much clearer understanding of this relationship.

If the pH and abundance were transformed, this could have changed the significance because pH is a logarithmic scale and each unit of pH increase or decrease would be a tenfold change in hydrogen concentration. As microbial abundance is linear in nature, doing a Pearson test would introduce issues for the test as both variables are not similar in nature. Thus transforming it would potentially lead to significant results. (Springer., 2014).

This difference between the natures of the variables could have also been mitigated if a non-parametric test such as Spearman’s rank test was used because it is robust to outliers and works well with non-linear or non-normal distributions and gives a more accurate observation rather than the Pearson’s test which is based on an assumption of linear and normal distribution. (Rebekić et al.2015)

4.4 Limitations in DNA Extraction and Polymerase Chain Reaction.

After extracting DNA from all 10 samples and after the Polymerase Chain Reaction(PCR), concentration was measured. Sample 3 had the highest concentration of DNA compared to the others, around 552ng/mL. The sample that had the least concentration was sample 8, around 268ng/mL. Although both concentrations were not enough to do MinIon sequencing, the faults are analyzed here. It is also possible that as the amount of bacteria was not enough in order to obtain a sufficient amount of DNA.

Because soil is variable and complex, extracting DNA from it presents several challenges. These can interfere with the quantity and quality of the DNA obtained. Soil contains different components apart from microbes, such as organic components, minerals, and humic acids. Humic acids pose a major challenge in extracting DNA(Wnuk et al., 2020). These organic compounds are extracted along with DNA in the extraction process. They affect the yield and purity of the DNA. These acids also affect the amplification process of the polymerase chain reaction because they bind to the DNA and reduce the number of templates available for amplification, making it harder to obtain a higher concentration of DNA suitable for sequencing even after PCR. Contaminants in the soil can also affect the extraction process by binding to the DNA (Técher et al., 2010).

Furthermore, errors and faults in the DNA extraction protocol also affect the concentration of DNA. The extraction process often uses harsh chemicals and reagents, so if the amounts and concentrations are not properly applied, it can lead to DNA shearing. Also, incorrect times or speeds of centrifugation, contamination of the DNA samples, and incorrect pipetting techniques could lead to reduced final concentrations. Even if the DNA is extracted correctly, if the DNA is diluted or degraded, the PCR amplification would be impacted negatively (Alaeddini, 2012).

Problems caused when running the PCR cycles also affect the reduction of the final DNA concentration. One of them is the competition among the DNA sequences. If the primers bind to different DNA sequence templates non-specifically because there is a large number of DNA sequences present in the solution, the concentration of the target DNA would be reduced (Rodríguez et al., 2015). Also, insufficient PCR cycles, errors made in inputting annealing times, and faults in the PCR equipment would reduce the DNA concentration.

Finally, the low initial extraction of DNA yield, inhibitors such as humic acids, DNA degradation, and nonspecific amplification would create insufficient DNA concentration even after PCR. To overcome these challenges the DNA extraction protocol should be optimized by adding DNA purification steps, further concentration of DNA by ethanol precipitation, use of inhabitant-resistant polymerases, and selecting specific primers (Rodríguez et al., 2015).

4.5 Bacterial Abundance Between Ridge and Furrow.

According to Figure 11 in section 3.6 above, the data depicts no statisticallysignificant variance in the mean of bacterial abundances between the ridge and the furrow. The ridge had a mean bacterial abundance of $3.6x10^6 \pm 3.9x\ 10^5$ cells ml^{-1} The mean value observed from the furrows was $4.4x10^6 \pm 8.6x10^5$cells ml^{-1}. Although this was the case, Levene's test showed a value of 0.230, which was not significant as it exceeded 0.05, thus confirming the null hypothesis assumption, which is that equal variances were met. Similarly,the t-test for equality of means was at 0.452, which is not a statistically significant variable.

This indicates no significant difference in bacterial abundance between the two areas. The reason behind the decreased statistical significance may have several underlying explanations. The higher p values indicate that the differences in the mean of bacterial

abundance were due to random variations occurring between the areas rather than a clear environmental factor influencing the bacterial abundance counts. Soil is a complex structure that has several components, such as pH, organic matter, and nutrient availability. A small fluctuation of these components could be the reason for an increase or decrease in bacterial abundance. (Serna-Chavez et al., 2013)

Another reason for not achieving a statistically significant figure might be connected with the soil's moisture distribution. Though it is expected that when wheat crops are farmed the furrows contain runoff water while the ridges remain comparatively dry; when the soil samples were collected after harvest season and the fields remained dry for a while, the expected differences in moisture content were not present. Thus bacteria colonies present in both ridges and furrows might have been dormant or reduced in number for the period. (Lebreet al., 2017)

Furthermore, the standard deviations of both ridge and furrow in terms of bacterial counts were relatively high, indicating an increased variability of the bacterial count within the respective site. Thus, reducing the sensitivity of the statistical test in terms of detecting a meaningful difference. Another possibility is that the sample size of this experiment was insufficient to observe a significant difference. So, it is recommended that in further studies the sample size and amount of sites that need to be tested should be increased to detect statistically significant differences. (Wolf et al., 2013) In stating the limitations of this study, it should be noted that the bacterial abundance values of ridges and furrows were taken from the sites both north and south of the line rather than calculated separately. This was to increase the sample size and generalise the observed result to both regions. However,this might have introduced other variables to the experiment, which reduced the significance of the differences in bacterial abundance.

4.6 Bacterial Abundance Between North and South.

According to Figure 12 in section 3.7 above, the data shows a statistically significant variance in the mean of bacterial abundances between the northern and southern regions of an area around Goyder's line. The south had a mean bacterial abundance of $6.0x\ 10^6 \pm 7.5\ x10^5$ cells ml^{-1} the mean value observed in the north was $3.7\ x\ 10^6 \pm 4.9\ x10^5$ cells ml^{-1} Levene's test showed a value of 0.360 was not significant as it exceeded the average considered value of 0.05, thus confirming the null hypothesis assumption that equal variances were met. However, the t-test for equality of means was at 0.040, which is statistically significant. This indicated that there is a statistically significant difference in bacterial abundance between the North and South of the Goyder's line.

The first possible reason that there is a significant difference in bacterial abundance would be the climatic variation between the two regions. The main demarcation of the Goyder's line is that the south of the line receives higher averages of annual rainfall levels while it is significantly low in the north. As bacteria thrive in environments with higher levelsof moisture, it would have promoted higher levels of growth in the south, indicating the availability of water directly coincides with bacterial abundance. (Bachar et al., 2010)

However, the moisture content might not be the only factor that causes this significant statistical difference. As the soil composition also directly influences the abundance of bacterial populations, if the soil has a more favorable structure in the south, it will also promote growth. Vegetation is one of the factors influencing the change in soil structure, which reduces soil erosion and degradation. Thus, higher vegetation levels indirectly promote bacterial abundance. (Zhang et al., 2014) Also, the presence of compounds such as extracellular polysaccharides would create a healthy soil structure. These parameters

would then act as positive feedback, which promotes more bacterial growth.

The limitations of this experiment are that the sample size was low and that some of the samples, which included different crops or grazing areas, would impact the results obtained. Further research is advised, focusing on reducing limitations and finding which bacterial populations are dominant in each area.

4.7 Conclusion and Future Directions

In conclusion, studying Goyder's Line and its associated soil bacterial abundances and the factors influencing the growth showed that it is essential for mitigating drought and ensuring sustainable agricultural practices in South Australia. One of the main findings of this study was that there is a higher level of microbial abundance present in the south than in the north, which had statistical significance. However, the other factor that was tested by hypothesising that furrows contain more bacterial abundance compared to ridges was not proven with a statistical significance. Also, there was a weak to moderate correlation betweenpH and bacterial abundance, which was proven to be statistically insignificant. However, therewere isolated findings with lower bacterial abundances in higher pH levels. Agricultural practices such as animal grazing in between crop rotations were shown to promote bacterial abundance but should be further investigated to obtain statistically significant results.

By advancing the understanding of microbial diversity and resilience, this research will contribute to the long-term sustainability of agricultural ecosystems and people's livelihoods in South Australia. The future direction of this study should be to observe the taxonomies present in the soils that contributed to the abundance and epigenetic study to identify bacteria that contain drought-resistant genes or produce substances such as Extracellular Polysaccharide (EPS).

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6 APPENDICES

Table 6- Counts of Beads, Bacteria, Virus-like Particles and Debris. Obtained from 1:1000 dilution from solution combinations of 0.5%Tween 80, 0.025M TSP+ 0.85% NaCl and SYBR-1 green dye.

Sample	Beads | Count	Bacteria | Count	VLP | Count	Debris | Count
N1rep1-sybr+TSP+0.85%NaCl dil.1000	959	53	36	1174
N1rep2-sybr+TSP+0.85%NaCl dil.1000	786	23	53	765
N1rep3-sybr+TSP+0.85%NaCl dil.1000	839	36	54	652
N2rep1-sybr+Tween80+0.85%NaCl dil.1000	1077	27	23	7901
N2rep2-sybr+Tween80+0.85%NaCl dil.1000	1025	29	35	16575
N2rep3-sybr+Tween80+0.85%NaCl dil.1000	966	32	16	15102
N3rep1-sybr+TSP+Tween80+0.85%NaCl dil.1000	973	22	19	7536
N3rep2-sybr+TSP+Tween80+0.85%NaCl dil.1000	974	40	25	5960
N3rep3-sybr+TSP+Tween80+0.85%NaCl dil.1000	1002	27	37	6620
Sample1rep1-sybr+TSP+0.85%NaCl dil.1000	946	64	58	3770
Sample1rep2+sybr+TSP+0.85%NaCl dil.1000	1016	109	42	6375
Sample1rep3+sybr+TSP+0.85%NaCl dil.1000	572	64	62	5253
Sample2rep1+sybr+Tween80+0.85%NaCl dil.1000	1125	226	66	35571
Sample2rep2+sybr+Tween80+0.85%NaCl dil.1000	1064	171	55	28667
Sample2rep3+sybr+Tween80+0.85%NaCl dil.1000	983	131	39	25465
Sample3rep1-sybr+TSP+Tween80+0.85%NaCl dil.1000	1070	331	168	35154
Sample3rep2-sybr+TSP+Tween80+0.85%NaCl dil.1000	993	186	102	17331
Sample3rep3-sybr+TSP+Tween80+0.85%NaCl dil.1000	1155	267	118	24624
Mean	919	102	56	13583
SD	265	95.6	38.6	11741

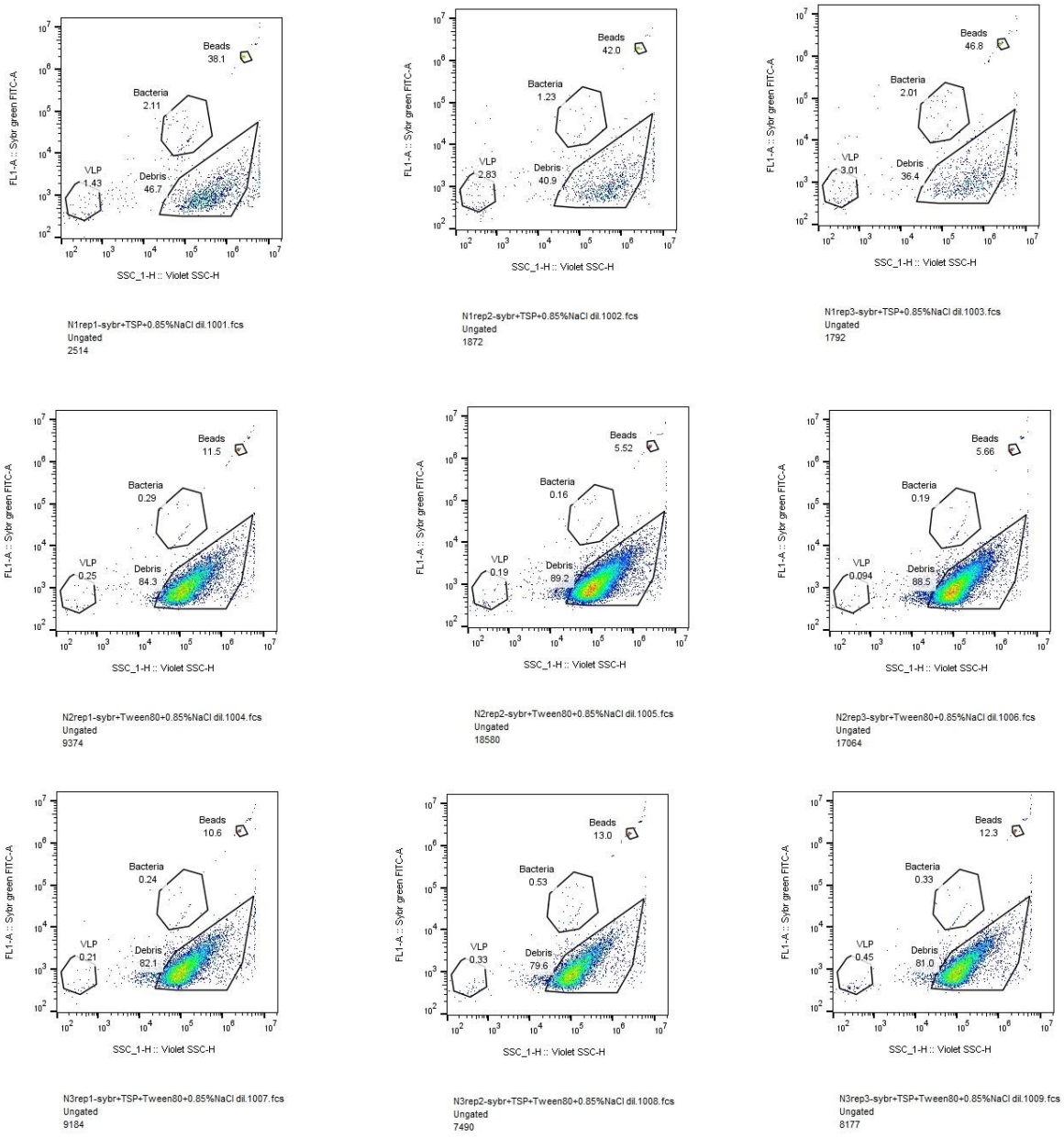

Figure 13- Gated cytograms created Using Flowjo 10.08r1. The bacterial and viral groups were distinguished by SYBR-I Green fluorescence and side scatter. Obtained from 1:1000 dilution from solution combinations of 0.5%Tween 80, 0.025MTSP+ 0.85% NaCl and SYBR-1 Green dye.

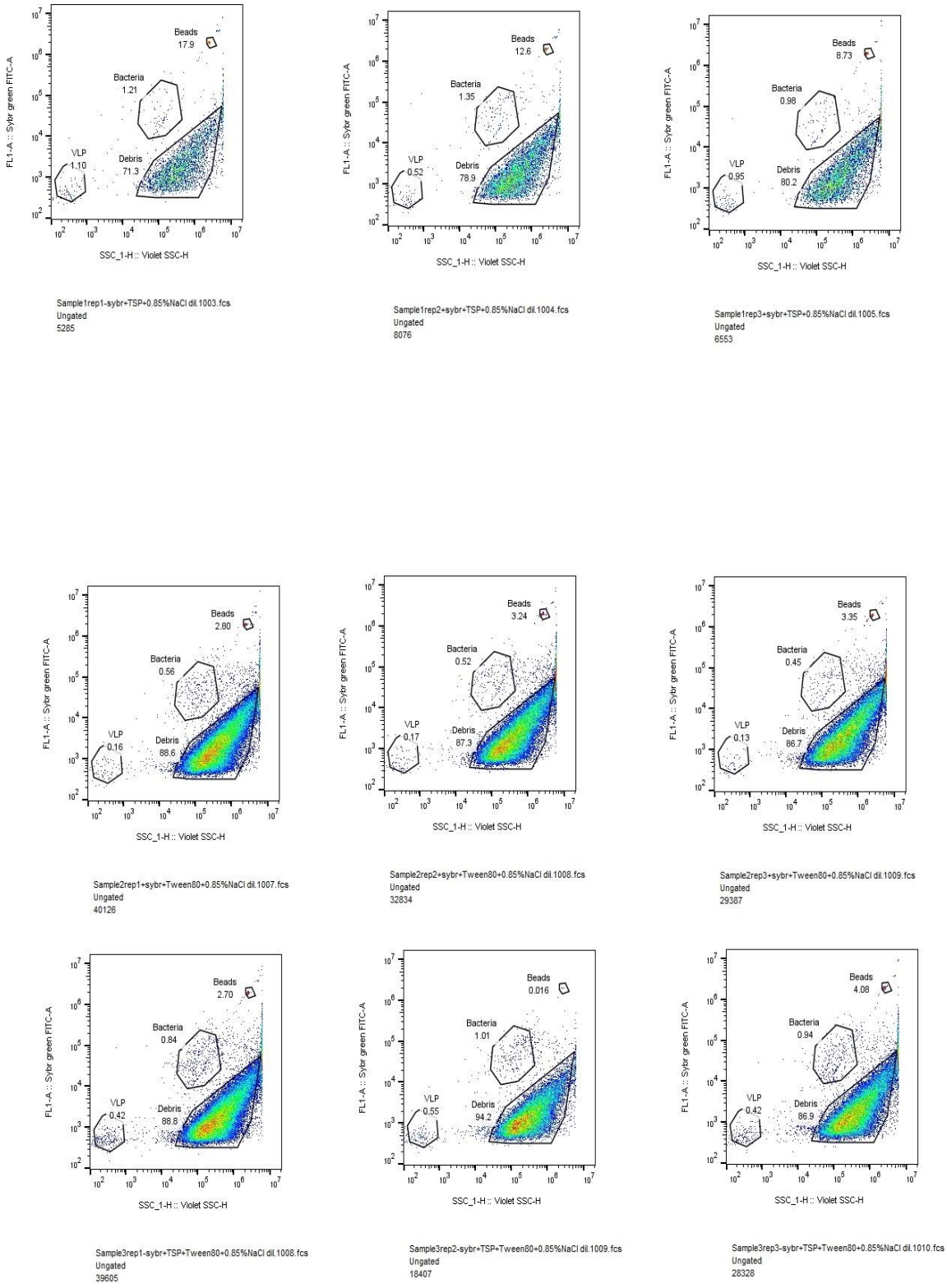

Figure 14- Gated cytograms created Using Flowjo 10.08r1. The bacterial and viral groups were distinguished by SYBR-I Green fluorescence and side scatter. Obtained from 1:1000 dilution from solution combinations of 0.5%Tween 80, 0.025MTSP+ 0.85% NaCl and SYBR-1 Green dye.

Table 7- Counts of Beads, Bacteria, Virus-like Particles and Debris. Obtained from 1:1000 dilution from solution combinations of 0.5%Tween 80, 0.025M TSP+ 0.85% NaCl and SYBR-1 green dye. Centrifuged under 5minutes and 90 seconds at 1500rpm.

	Beads | Count	Bacteria | Count	VLP | Count	Debris | Count
N1rep1dil500.fcs	124	163	124	365
N1rep2dil500.fcs	53	82	79	327
N1rep3dil500.fcs	54	44	31	246
N2rep1dil1000.fcs	40	64	46	270
N2rep2dil1000.fcs	32	50	30	248
N2rep3dil1000.fcs	33	30	18	144
Sample1centry+rep1dil500+5min	247	374	66	910
Sample1centry+rep1dil1000+5min	48	133	26	499
Sample1centry+rep2dil500+5min	60	399	54	1287
Sample1centry+rep2dil1000+5min	53	132	33	472
Sample1centry+rep3dil1000+5min	71	316	68	973
Sample1centry+rep3dil500+5min	73	346	45	927
Sample1centry+rep1dil500+90sec	97	1857	41	110158
Sample1centry+rep1dil1000+90sec	45	1092	47	73886
Sample1centry+rep2dil500+90sec	49	2078	29	114911
Sample1centry+rep2dil1000+90sec	44	216	24	12566
Sample1centry+rep3dil500+90sec	64	1985	30	103687
Sample1centry+rep3dil1000+90sec	51	1592	40	67297
Mean	68.8	608	46.2	27176
SD	49.8	744	25.5	44024

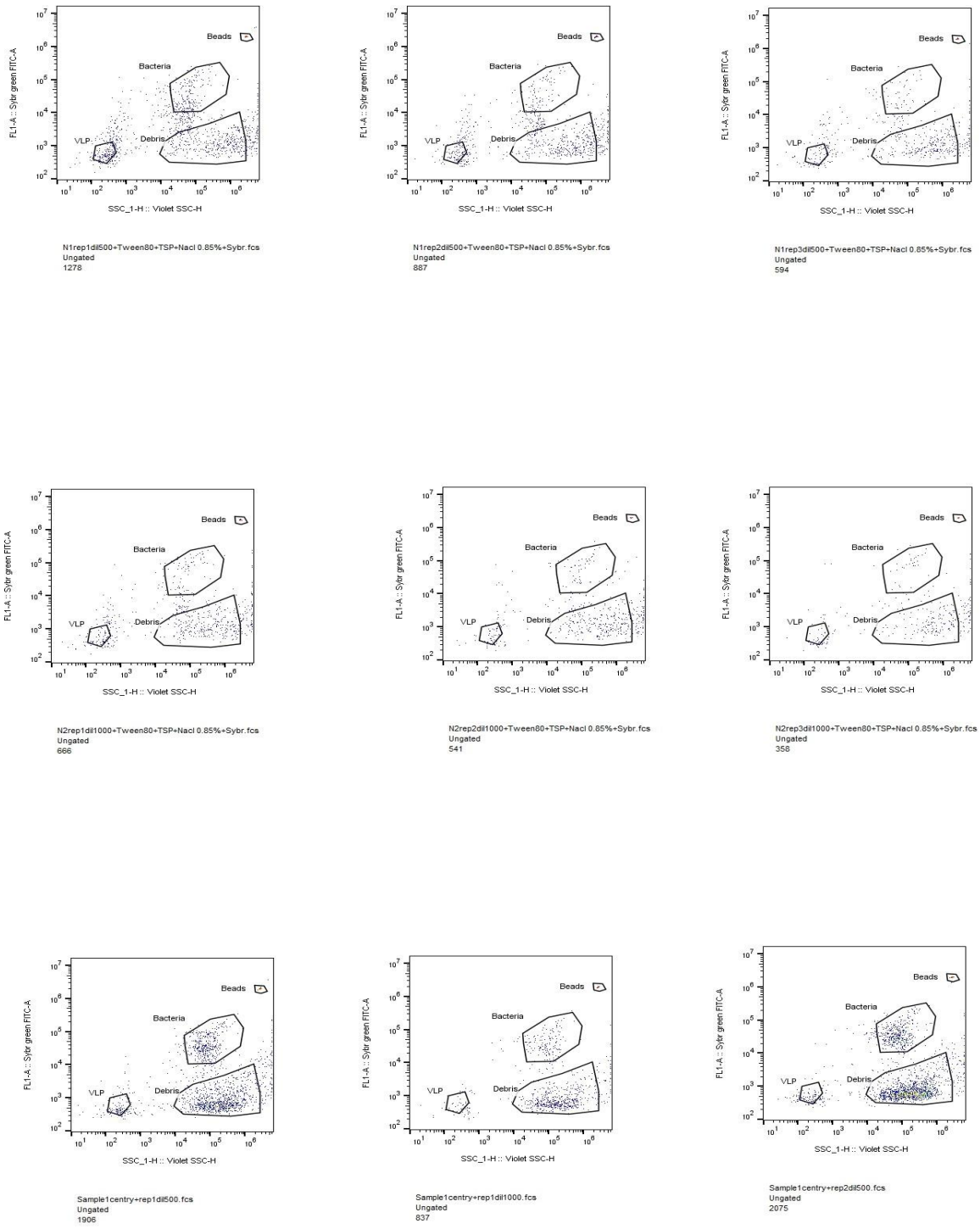

Figure 15- Gated cytograms created using Flowjo 10.08r1. The bacterial and viral groups were distinguished by SYBR-I Green fluorescence and side scatter. Obtained from 1:500 and 1:1000 dilution from solution combinations of 0.5%Tween 80, 0.025MTSP+ 0.85% NaCl, and SYBR-1 Green dye differentiating between Centrifugation Time.

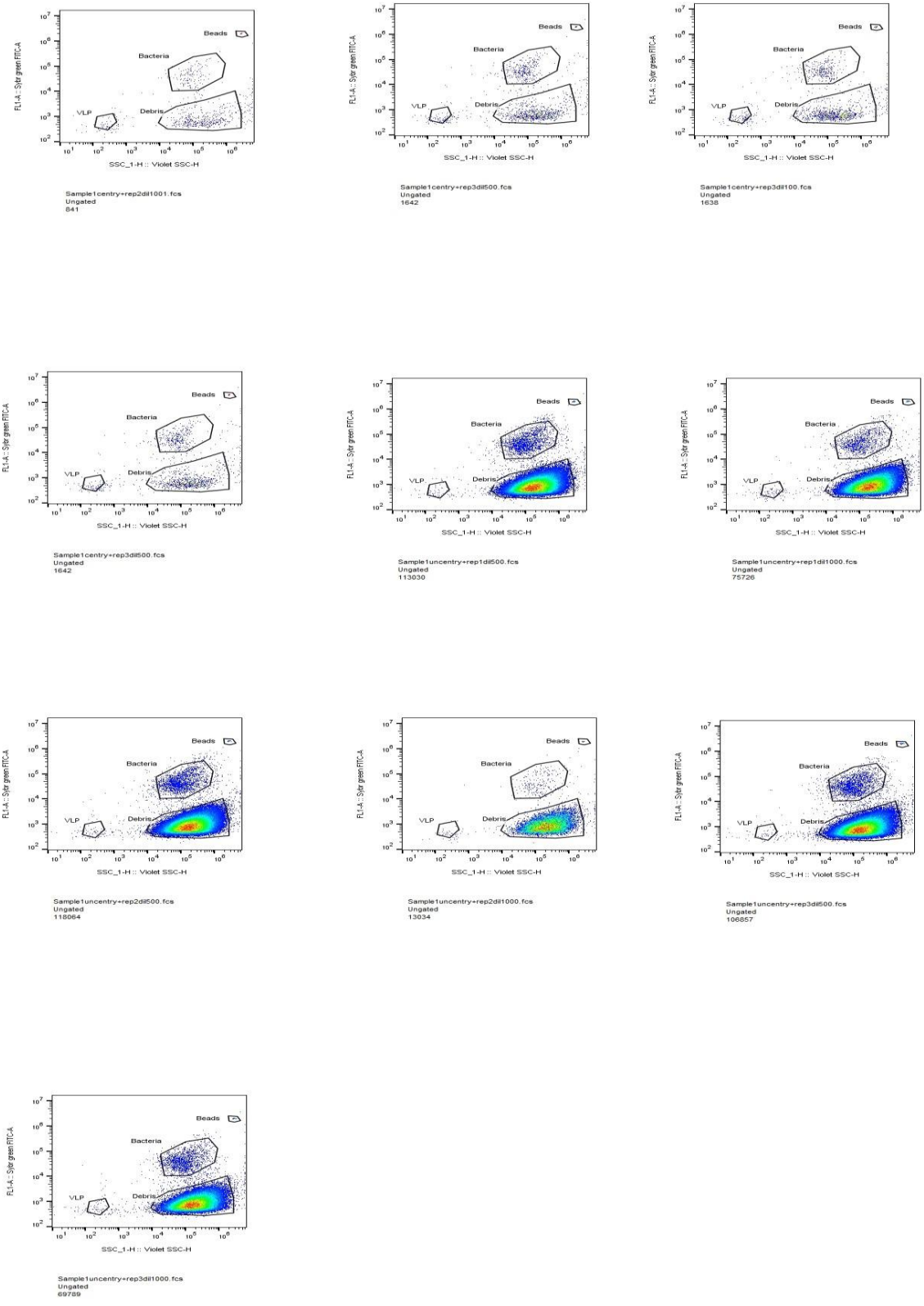

Figure 16-Gated cytograms created Using Flowjo 10.08r1. The bacterial and viral groups were distinguished by SYBR-I Green fluorescence and side scatter. Obtained from 1:500 and 1:1000 dilution from solution combinations of 0.5%Tween 80, 0.025M TSP+ 0.85% NaCl, and SYBR-1 green dye differentiating between Centrifugation Time

Table 8- Counts of Beads, Bacteria, Virus-like Particles and Debris in all 10 samples. Obtained from 1:500 and 1:1000 and 1:2000 dilutions and triplications from solution combinations of 0.5%Tween 80, 0.025M TSP+ 0.85% NaCl, and SYBR-1 green dye.

	Beads | Count	Bacteria | Count	VLP | Count	Debris | Count
N1rep1dilution 1-500 Tween80+TSP+Sybr2.fcs	970	37	98	715
N1rep2 dilution 1-500 Tween80+TSP+Sybr3.fcs	1031	47	90	660
N1rep3 dilution 1-500 Tween80+TSP+Sybr.fcs	930	31	52	526
N2rep1dilution 1-1000 Tween80+TSP+Sybr3.fcs	936	42	35	559
N2rep2dilution 1-1000 Tween80+TSP+Sybr4.fcs	945	35	26	486
N2rep3dilution 1-1000 Tween80+TSP+Sybr5.fcs	1037	35	34	500
N3rep1dilution 1-2000 Tween80+TSP+Sybr4.fcs	901	36	15	295
N3rep2dilution 1-2000 Tween80+TSP+Sybr5.fcs	984	34	15	267
N3rep3dilution 1-2000 Tween80+TSP+Sybr6.fcs	932	19	14	262
S1 rep1dilution 1-500 Tween80+TSP+Sybr7.fcs	946	2737	34	114606
S1 rep1dilution 1-1000 Tween80+TSP+Sybr1.fcs	1037	2437	56	134756
S1 rep1dilution 1-2000 Tween80+TSP+Sybr2.fcs	987	2666	51	100502
S1 rep2dilution 1-500 Tween80+TSP+Sybr8.fcs	963	2806	33	113096
S1 rep2dilution 1-1000 Tween80+TSP+Sybr.fcs	986	2328	60	137685
S1 rep2dilution 1-2000 Tween80+TSP+Sybr3.fcs	935	2703	50	101043
S1 rep3dilution 1-500 Tween80+TSP+Sybr.fcs	1011	2761	36	114054
S1 rep3dilution 1-1000 Tween80+TSP+Sybr1.fcs	927	2459	76	142427
S1 rep3dilution 1-2000 Tween80+TSP+Sybr4.fcs	961	2722	32	99524
S2 rep1dilution 1-500 Tween80+TSP+Sybr3.fcs	955	2859	228	907000
S2 rep1dilution 1-1000 Tween80+TSP+Sybr6.fcs	947	141	38	229428
S2 rep1dilution 1-2000 Tween80+TSP+Sybr9.fcs	945	6583	146	805000
S2 rep2dilution 1-500 Tween80+TSP+Sybr4.fcs	992	1880	198	860000
S2 rep2dilution 1-1000 Tween80+TSP+Sybr7.fcs	970	127	43	230177
S2 rep2dilution 1-2000 Tween80+TSP+Sybr10.fcs	948	6630	127	800000
S2 rep3dilution 1-500 Tween80+TSP+Sybr5.fcs	992	1768	176	851000
S2 rep3dilution 1-1000 Tween80+TSP+Sybr8.fcs	962	117	43	224444
S2 rep3dilution 1-2000 Tween80+TSP+Sybr11.fcs	973	6583	126	794000
S3 rep1dilution 1-500 Tween80+TSP+Sybr12.fcs	1037	2738	33	162657
S3 rep1dilution 1-1000 Tween80+TSP+Sybr15.fcs	973	87	4	6620

S3 rep1dilution 1-2000 Tween80+TSP+Sybr18.fcs	957	4828	61	193628
S3 rep2dilution 1-500 Tween80+TSP+Sybr13.fcs	981	2748	27	162421
S3 rep2dilution 1-1000 Tween80+TSP+Sybr16.fcs	1022	70	2	6567
S3 rep2dilution 1-2000 Tween80+TSP+Sybr19.fcs	978	4970	47	191541
S3 rep3dilution 1-500 Tween80+TSP+Sybr14.fcs	986	2672	25	162535
S3 rep3dilution 1-1000 Tween80+TSP+Sybr17.fcs	993	71	3	6670
S3 rep3dilution 1-2000 Tween80+TSP+Sybr20.fcs	974	4932	45	190737
S4 rep1dilution 1-500 Tween80+TSP+Sybr21.fcs	998	2998	23	170066
S4 rep1dilution 1-1000 Tween80+TSP+Sybr24.fcs	999	131	6	9380
S4 rep1dilution 1-2000 Tween80+TSP+Sybr.fcs	972	3498	32	92972
S4 rep2dilution 1-500 Tween80+TSP+Sybr22.fcs	916	2955	24	170352
S4 rep2dilution 1-1000 Tween80+TSP+Sybr25.fcs	1058	154	7	18483
S4 rep2dilution 1-2000 Tween80+TSP+Sybr.fcs	934	3250	23	91094
S4 rep3dilution 1-500 Tween80+TSP+Sybr23.fcs	941	2884	28	172021
S4 rep3dilution 1-1000 Tween80+TSP+Sybr26.fcs	1059	185	5	25865
S4 rep3dilution 1-2000 Tween80+TSP+Sybr.fcs	1048	3418	25	90611
S5 rep1dilution 1-500 Tween80+TSP+Sybr.fcs	940	5663	232	84762
S5 rep1dilution 1-1000 Tween80+TSP+Sybr.fcs	1039	4767	213	70363
S5 rep1dilution 1-2000 Tween80+TSP+Sybr.fcs	985	1398	362	26097
S5 rep2dilution 1-500 Tween80+TSP+Sybr.fcs	957	5607	244	85516
S5 rep2dilution 1-1000 Tween80+TSP+Sybr2.fcs	1008	4867	221	70726
S5 rep2dilution 1-2000 Tween80+TSP+Sybr.fcs	985	1475	290	26534
S5 rep3dilution 1-500 Tween80+TSP+Sybr1.fcs	1007	5738	228	85761
S5 rep3dilution 1-1000 Tween80+TSP+Sybr.fcs	975	4839	241	71021
S5 rep3dilution 1-2000 Tween80+TSP+Sybr1.fcs	971	1411	273	25903
S6 rep1dilution 1-500 Tween80+TSP+Sybr2.fcs	959	6917	51	196205
S6 rep1dilution 1-1000 Tween80+TSP+Sybr5.fcs	979	4819	50	92027
S6 rep1dilution 1-2000 Tween80+TSP+Sybr8.fcs	927	4621	32	115292
S6 rep2dilution 1-500 Tween80+TSP+Sybr3.fcs	968	6098	76	182481
S6 rep2dilution 1-1000 Tween80+TSP+Sybr6.fcs	1020	4857	49	91509
S6 rep2dilution 1-2000 Tween80+TSP+Sybr9.fcs	973	4571	36	114526
S6 rep3dilution 1-500 Tween80+TSP+Sybr4.fcs	997	6015	51	179815
S6 rep3dilution 1-1000 Tween80+TSP+Sybr7.fcs	1042	4770	38	90274
S6 rep3dilution 1-2000 Tween80+TSP+Sybr10.fcs	996	4641	32	112588
S7 rep1dilution 1-500 Tween80+TSP+Sybr11.fcs	983	2883	228	534000

S7 rep1dilution 1-1000 Tween80+TSP+Sybr14.fcs	979	4865	197	942000
S7 rep1dilution 1-2000 Tween80+TSP+Sybr17.fcs	952	3582	252	539000
S7 rep2dilution 1-500 Tween80+TSP+Sybr12.fcs	985	2738	233	524000
S7 rep2dilution 1-1000 Tween80+TSP+Sybr15.fcs	899	4753	174	943000
S7 rep2dilution 1-2000 Tween80+TSP+Sybr18.fcs	973	3793	264	539000
S7 rep3dilution 1-500 Tween80+TSP+Sybr13.fcs	998	2780	187	521000
S7 rep3dilution 1-1000 Tween80+TSP+Sybr16.fcs	952	5000	191	942000
S7 rep3dilution 1-2000 Tween80+TSP+Sybr19.fcs	912	3759	219	541000
S8 rep1dilution 1-500 Tween80+TSP+Sybr20.fcs	1026	9338	26	98831
S8 rep1dilution 1-1000 Tween80+TSP+Sybr23.fcs	977	2588	13	67845
S8 rep1dilution 1-2000 Tween80+TSP+Sybr26.fcs	1004	7266	36	72479
S8 rep2dilution 1-500 Tween80+TSP+Sybr21.fcs	961	9396	32	98503
S8 rep2dilution 1-1000 Tween80+TSP+Sybr24.fcs	996	2517	13	67976
S8 rep2dilution 1-2000 Tween80+TSP+Sybr27.fcs	982	7161	41	72204
S8 rep3dilution 1-500 Tween80+TSP+Sybr22.fcs	972	9052	33	98338
S8 rep3dilution 1-1000 Tween80+TSP+Sybr25.fcs	988	2383	15	67738
S8 rep3dilution 1-2000 Tween80+TSP+Sybr28.fcs	910	7209	42	71790
S9 rep1dilution 1-500 Tween80+TSP+Sybr29.fcs	945	9869	78	183917
S9 rep1dilution 1-1000 Tween80+TSP+Sybr32.fcs	1008	7578	51	114168
S9 rep1dilution 1-2000 Tween80+TSP+Sybr35.fcs	958	12978	161	266703
S9 rep2dilution 1-500 Tween80+TSP+Sybr30.fcs	991	9874	71	181689
S9 rep2dilution 1-1000 Tween80+TSP+Sybr33.fcs	1004	7634	73	114347
S9 rep2dilution 1-2000 Tween80+TSP+Sybr36.fcs	964	13472	157	269953
S9 rep3dilution 1-500 Tween80+TSP+Sybr31.fcs	1008	9834	73	181426
S9 rep3dilution 1-1000 Tween80+TSP+Sybr34.fcs	1072	7527	77	113591
S9 rep3dilution 1-2000 Tween80+TSP+Sybr37.fcs	971	13850	139	273375
S10 rep1dilution 1-500 Tween80+TSP+Sybr38.fcs	983	7677	71	138860
S10 rep1dilution 1-1000 Tween80+TSP+Sybr41.fcs	978	5184	22	129256
S10 rep1dilution 1-2000 Tween80+TSP+Sybr44.fcs	933	4758	66	91002

S10 rep2dilution 1-500 Tween80+TSP+Sybr39.fcs	965	7572	69	135378
S10 rep2dilution 1-1000 Tween80+TSP+Sybr42.fcs	1051	5223	26	128753
S10 rep2dilution 1-2000 Tween80+TSP+Sybr45.fcs	965	4804	90	91029
S10 rep3dilution 1-500 Tween80+TSP+Sybr40.fcs	1028	7579	70	136617
S10 rep3dilution 1-1000 Tween80+TSP+Sybr43.fcs	921	5097	30	128646
S10 rep3dilution 1-2000 Tween80+TSP+Sybr46.fcs	986	4712	100	90907
Mean	978	4167	87.8	201202
SD	36.6	3140	83.4	245500

Table 9- Counts of Beads, Bacteria, Virus-like Particles and Debris in repeated samples 2,3,4 and 7. Obtained from 1:500 and 1:1000 and 1:2000 dilutions from solution combinations of 0.5%Tween 80, 0.025M TSP+ 0.85% NaCl, and SYBR-1 greendye.

	Beads | Count	**Bacteria | Count**	**VLP | Count**	**Debris | Count**
N1rep1dil1-500.fcs	855	386	17	377
N1rep2dil1-500.fcs	860	206	13	343
N1rep3dil1-500.fcs	892	154	33	268
N2rep1dil1-1000.fcs	2537	203	27	138
N2rep2dil1-1000.fcs	2498	278	59	136
N2rep3dil1-1000.fcs	2596	251	68	105
N3rep1dil1-2000.fcs	2630	206	20	339
N3rep2dil1-2000.fcs	2708	118	10	319
N3rep3dil1-2000.fcs	2672	143	30	310
Sample2rep1dil1-500.fcs	1162	2825	285	925000
Sample2rep1dil1-1000.fcs	1393	2046	263	933000
Sample2rep1dil1-2000.fcs	2651	5199	37	655000
Sample2rep2dil1-500.fcs	1253	1212	261	930000
Sample2rep2dil1-1000.fcs	825	1719	44	693000
Sample2rep3dil1-500.fcs	1262	1175	236	933000
Sample2rep3dil1-1000.fcs	827	1557	32	673000
Sample2rep3dil1-2000.fcs	2496	5130	34	633000
Sample3rep1dil1-500.fcs	2238	2637	124	472000
Sample3rep1dil1-1000.fcs	2316	3091	57	306685
Sample3rep1dil1-2000.fcs	2730	4316	32	266880
Sample3rep2dil1-500.fcs	2156	2563	112	472000
Sample3rep2dil1-1000.fcs	2194	3067	64	300713
Sample3rep2dil1-2000.fcs	2741	4339	45	268168
Sample3rep3dil1-500.fcs	2230	2572	121	469000
Sample3rep3dil1-1000.fcs	2234	3009	55	313976
Sample3rep3dil1-2000.fcs	2678	4228	33	264830
Sample4rep1dil1-500.fcs	2673	861	113	442000
Sample4rep1dil1-1000.fcs	2655	4514	25	137847
Sample4rep1dil1-2000.fcs	2827	3541	24	153316

Sample4rep2dil1-500.fcs	2699	834	94	436000
Sample4rep2dil1-1000.fcs	2764	4381	29	135387
Sample4rep2dil1-2000.fcs	2762	3505	18	152451
Sample4rep3dil1-500.fcs	2625	852	106	434000
Sample4rep3dil1-1000.fcs	2706	4361	20	135390
Sample4rep3dil1-2000.fcs	2695	3537	23	150419
Sample7rep1dil1-500.fcs	2640	968	135	432000
Sample7rep1dil1-1000.fcs	2605	1450	99	266160
Sample7rep1dil1-2000.fcs	2668	1452	112	262234
Sample7rep2dil1-500.fcs	2733	895	150	432000
Sample7rep2dil1-1000.fcs	2719	1422	77	261878
Sample7rep2dil1-2000.fcs	2693	1525	91	262246
Sample7rep3dil1-500.fcs	2686	886	145	429000
Sample7rep3dil1-1000.fcs	2683	1338	90	263849
Sample7rep3dil1-2000.fcs	2755	1515	83	261923
Mean	2278	2056	80.6	330902
SD	665	1562	70.2	274487

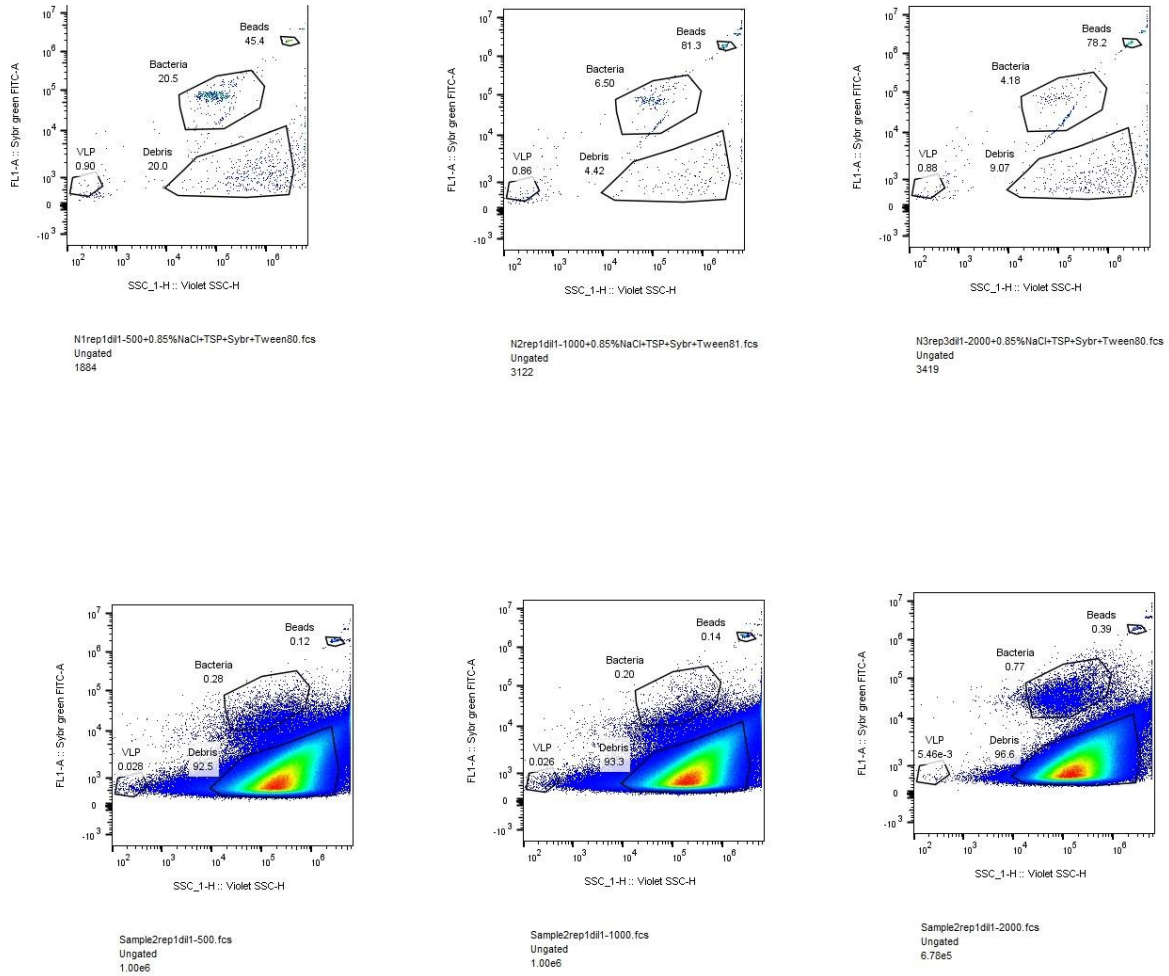

Figure 17-Gated cytograms created Using Flowjo 10.08r1. The bacterial and viral groups were distinguished by SYBR-I Green fluorescence and side scatter. Virus-like Particles and Debris in repeated samples 2,3,4 and 7. Obtained from 1:500 and 1:1000 and 1:2000 dilutions from solution combinations of 0.5%Tween 80, 0.025M TSP+ 0.85% NaCl, and SYBR-1 green dye

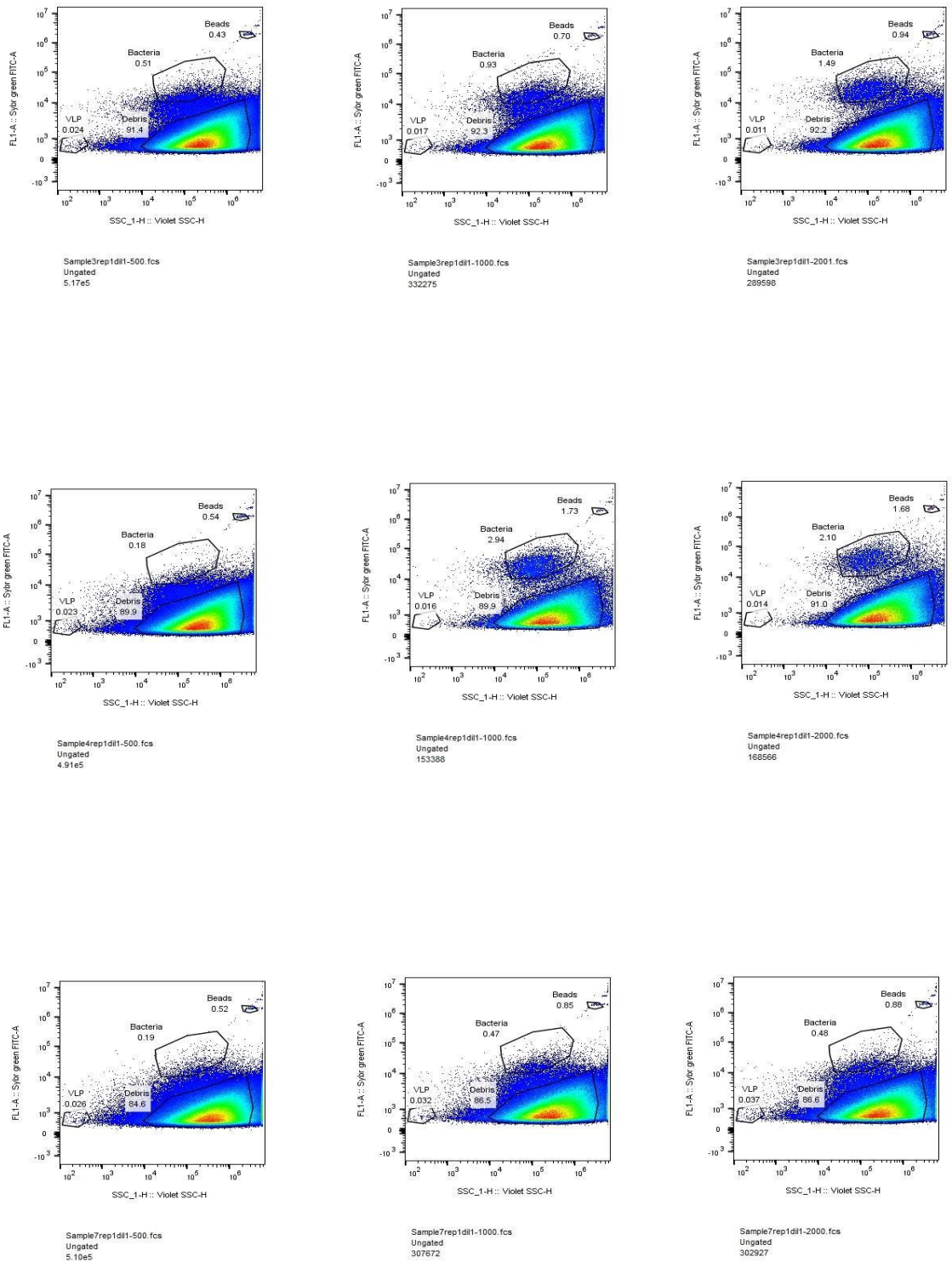

Figure 18- Gated cytograms created Using Flowjo 10.08r1. The bacterial and viral groups were distinguished by SYBR-I Green fluorescence and side scatter. Virus-like Particles and Debris in repeated samples 2,3,4 and 7. Obtained from 1:500 and 1:1000 and 1:2000 dilutions from solution combinations of 0.5%Tween 80, 0.025M TSP+ 0.85% NaCl, and SYBR-1 green dye