THESIS

ENHANCING OLEOPHILIC BIOBARRIERS FOR NON-TIDAL SEDIMENTS IMPACTED WITH PETROLEUM HYDROCARBON

Submitted by

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ABSTRACT

ENHANCING OLEOPHILIC BIOBARRIERS FOR NON-TIDAL SEDIMENTS IMPACTED WITH PETROLEUM HYDROCARBON

The objective of this study is to develop tools to prevent petroleum hydrocarbons trapped in non-tidal sediments from causing detrimental effects such as sheens. Oleophilic biobarriers (OBBs) provide a robust, low-cost solution for managing petroleum hydrocarbon contamination at groundwater-surface water interfaces in tidal zones but are untested in non-tidal zones. This study evaluates enhanced OBB remedies for petroleum hydrocarbon contamination in non-tidal zones by incorporating amendments within the OBB. The amended OBB is intended to serve as an engineered bioremediation tool to enhance microbial growth and degradation of petroleum hydrocarbons by supplying the system with a resource of electron donors and nutrients while simultaneously mitigating petroleum hydrocarbon releases to surface water.

Complementary laboratory and field studies were conducted to test non-tidal OBBs (NOBBs) with six amendment types: (1) hematite (H), (2) greensand (GS), (3) greensand + hematite (GS+H), (4) gypsum (GYP), (5) hematite + greensand + gypsum (ALL), and (6) blank (B). The laboratory study was constructed as a series of chemostats using sediment and water samples from the field site. This study observed the productivity of petroleum hydrocarbon degradation through biweekly headwater extractions analyzing alkalinity, dissolved inorganic carbon (DIC), and pH as well as continuously monitored oxidation reduction potential (ORP). Results from these tests indicated that the GYP amendment was most effective in degrading petroleum hydrocarbons while the B and ALL amendments were least effective. However, all systems exhibited increased effluent DIC characteristic of enhanced petroleum hydrocarbon degradation. The field study was constructed as a series of OBB disks deployed atop petroleum hydrocarbon impacted sediments in a non-tidal setting. Results from the laboratory and field study illustrated abundant microbial growth after six months. The NOBBs with the top three highest numbers of microbial abundance were found in the field (F): F-GS+H, F-B, and F-GS. The overall results of both lab and field studies suggest that NOBBs, whether amended or not, provide effective media for petroleum hydrocarbon-degrading microorganisms.

This study illustrates the promise of the non-tidal OBB as a bioreactive barrier for petroleum hydrocarbon impacted sediments. Further study is needed to evaluate the rate of petroleum hydrocarbon degradation in a non-tidal OBB relative to the rate of loading.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1. INTRODUCTION

Petroleum hydrocarbons enter aquatic and terrestrial environments and cause impacts to biological, geochemical, and physical processes occurring between soils, microorganisms, plants, animals, and humans within an ecosystem. Petroleum hydrocarbon contaminants can readily spread across ecosystems and cause detrimental long-term and short-term effects.

However, slowly, sediment can naturally attenuate petroleum hydrocarbons through biological processes involving native microorganisms that degrade organic contaminants (He, 2017). This study investigates how naturally occurring microbial degradation mechanisms can be enhanced through bioremediation techniques while simultaneously preventing oil sheening.

Since 2010, Colorado State University (CSU) has been exploring the topic of hydrocarbon sheens in surface water bodies adjacent to petroleum activities. Oleophilic bio-barriers (OBBs) (US Patent 10,112,854 B2) were developed through a combination of applied fieldwork and field-focused lab work at CSU (Chalfant, 2015; Campbell, 2017; Tochko, 2018; Bojan, 2019; Hogan, 2020). A schematic of a tidal OBB is shown in Figure 1. An OBB is comprised of a rigid geonet sandwiched between outer layers of nonwoven geotextile and associated adjacent layers as shown in Figure 1. OBBs served as a robust, low-cost solution for sustainable management of petroleum, including sheens, at groundwater-surface water interfaces (GSIs) in tidal zones.

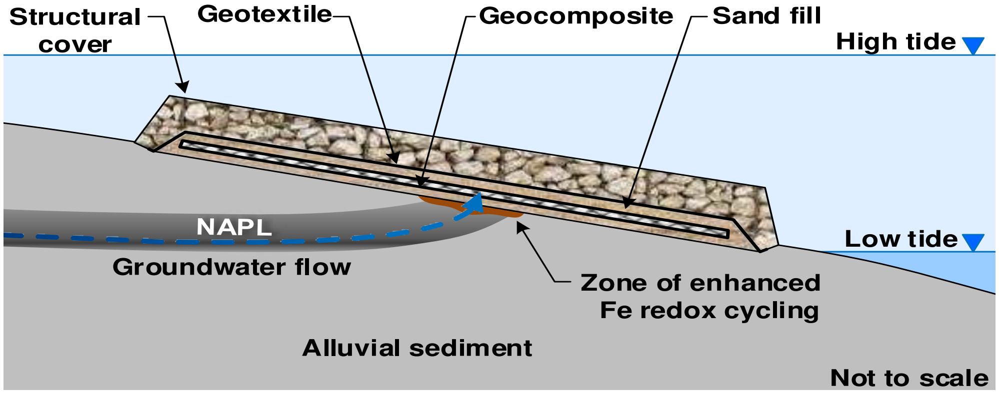

Figure 1. Conceptual schematic of a tidal OBB

In tidal settings, OBBs use diurnal water level fluctuations to sustain natural delivery of atmospheric oxygen and oxygenated surface water to facilitate the predominantly aerobic degradation of hydrocarbons sorbed to the OBB. The oleophilic geocomposite of an OBB has a non-aqueous phase liquid (NAPL) capacity of approximately 3 L/m2 (depending on the weight of the nonwoven geotextiles used; Chalfant, 2015). OBBs enhance the maximum NAPL stored at the GSI, thereby limiting petroleum hydrocarbon release to the surface water.

The tidal OBB has served as a successful tool for preventing oil sheening (Chalfant, 2015) and promoting microbial growth (Tochko, 2018) in aerobic settings (i.e., at tidal GSIs). However, the effectiveness of OBBs in non-tidal settings where the absence of regular natural water level fluctuations and lack of aeration has been unknown. Petroleum hydrocarbon impacts to sediments in non-tidal settings has led to an interest in deploying OBBs in non-tidal settings.

This study aims to enhance OBB remedies for petroleum hydrocarbons at non-tidal GSIs using the addition of amendments within the OBB's geocomposite layer. Amendments, such as electron acceptors and nutrients, were added within the geonet of each OBB to enhance the microbial growth and degradation of petroleum hydrocarbon. Amended OBBs were deployed at a petroleum hydrocarbon impacted non-tidal field site and in laboratory chemostat jars to evaluate the effectiveness of an OBB to serve as a petroleum hydrocarbon bioreactor in non-tidal settings.

Petroleum hydrocarbons in non-tidal, low-energy environments and many groundwaters typically requires long durations for natural attenuation due to the anaerobic nature of these systems and the deficiency of soluble electron acceptors, such as dissolved oxygen (DO), nitrate, or sulfate (Kolhatkar, 2017). The primary purpose of amended OBBs in the non-tidal setting is to overcome environmental deficiencies through biostimulation. In biostimulation, added nutrients or other growth-limiting compounds stimulate indigenous petroleum hydrocarbon-degrading microorganisms (Das, 2011). The NOBB was modified to contain electron acceptor and nutrient additives fixed within the interior geonet and sandwiched between geotextiles. These additives, referred to as amendments, serve to improve the effectiveness of NOBB as an engineered bioremediation tool for non-tidal anaerobic settings.

The primary expectation of the amendments is to create a more suitable environment for bioremediation and bioaugmentation under non-tidal, anaerobic conditions. The non-tidal OBB (NOBB) amendments in this study were chosen to biostimulate the growth of existing microbial communities by enhanced biochemical conditions. No longer lacking the essential conditions to thrive, microbial reactions are expected to accelerate through the additional supply of limiting nutrients and electron acceptors (He, 2017).

Another expectation of the NOBB amendments is to serve as a remediation solution that requires minimal operation and maintenance efforts to maintain functionality. Since OBBs are meant to last at a site for many years, the amendments must be capable of withstanding similar durations without depleting of additives. Slow-release amendments were chosen to provide amendment longevity. The amendments added to the NOBBs in this study include (1) hematite (H), (2) greensand (GS), (3) gypsum (GYP), (4) hematite + greensand (GS+H), and (5) hematite + greensand + gypsum (ALL). Demonstrating the persistent longevity of the NOBB amendments and subsequent ability to enhance the degradation of hydrocarbons is critical to gaining regulatory acceptance of effective-low cost OBB remedies.

Building on previous CSU sensor research, oxidation-reduction potential (ORP) data (defined herein as electrical potential relative to an Ag/AgCl reference electrode, i.e., $E_{Ag/AgCl}$) was continuously collected within a subset of laboratory chemostats both inside the OBB and in the underlying sediment. This data was automatically uploaded to a secure website so that experiments could be continuously monitored online. The ORP results were used as a tool for monitoring and verifying the redox condition within the laboratory chemostats.

Throughout the laboratory study, headwater effluent from the chemostat jars was collected and analyzed for pH and alkalinity. At the conclusion of the study, half of the amended NOBBs were retrieved from the field and disassembled from the laboratory chemostat to perform microbial analysis and assess the condition of the remaining amendment inside the NOBBs.

1.1 Objectives

This research serves to advance OBBs in non-tidal settings. The objectives of this study were:

1. Verify the feasibility of amended OBBs in the non-tidal settings using simultaneous field and laboratory studies;
2. Continuously monitor the degradation of petroleum hydrocarbons in the laboratory chemostat study using biweekly extracted chemostat headwater analyses and an ORP IoT monitoring system;
3. Observe additive loss from NOBBs;
4. Quantify microbial growth on NOBBs, and assess the potential functionality of a NOBB as a bioreactor for petroleum hydrocarbons.

1.2 Organization

The organization of this thesis is as follows. In *Chapter 2: Problem Statement*, the problem of petroleum hydrocarbons in aquatic and terrestrial ecosystems is presented. A description of the amendments chosen for this study is also provided. In *Chapter 3: Field Study*, the methods for implementing and analyzing NOBBs in the field site is outlined. In *Chapter 4: Laboratory Chemostat Studies*, the proof-of-concept laboratory demonstration is described. In *Chapter 5: Results and Discussion*, the analytical results are presented and discussed to provide a basis for the design and implementation of future NOBBs. In *Chapter 6: Summary and Conclusions*, conclusions of the study are presented and an outlook of future continuation of work is presented. Supplementary material is provided in the appendices.

2. PROBLEM STATEMENT AND BACKGROUND

The purpose of this chapter is to establish the importance and relevance of this study and provide background on concepts relevant to this study. First, the characteristics of petroleum hydrocarbon compounds are outlined. Second, the complexities of petroleum hydrocarbon contamination are reviewed. This discussion includes contamination in soil and sheening contamination on surface waters. Third, bioremediation tools for petroleum hydrocarbon contamination are discussed. Fourth, the usage of OBBs as a bioremediation tool is presented. Fifth, the motivation for adding amendments to the NOBBs is discussed, and background on potential amendments is provided.

2.1 Definition of Petroleum Hydrocarbons

The term “petroleum hydrocarbons” refers to a complex range of substances including crude oil, diesel, gasoline, heavy oil, and kerosene (Ahmed, 2018). Petroleum hydrocarbons can be further divided into four categories based on chemical structure: (1) saturates, (2) aromatics, (3) asphaltenes, and (4) resins (Truskewycz, 2019). Depending on the extraction source and level of refining, the composition of petroleum hydrocarbons can be complex.

Crude oil, for example, is a complex mixture of petroleum hydrocarbons with carbon numbers ranging from C_3 to C_{45+} (American Petroleum Institute, 2001). Crude oil contains varying ranges of carbon (83-87%) and hydrogen (10-14%), as well as smaller amounts of nitrogen, oxygen, sulfur, and metals (Ahmed, 2018). Crude oil is mainly short-chain hydrocarbons composed of paraffins, naphthalenes, and aromatics as well as saturates, aromatics, and olefins. Crude oil also consists of a smaller portion of non-hydrocarbon compounds, such as naphthenic acids, phenols, thiol, heterocyclic nitrogen, sulphur compounds, and metalloprophyrins and asphaltenes (Ahmed, 2018).

Gasoline is a type of petroleum hydrocarbon that is refined or extracted from crude oil by fractional distillation. Gasoline is a generic term used to describe volatile petroleum fuels primarily used in internal combustion engines. Gasoline hydrocarbons range from carbon numbers between C_4 to C_{12} and are a composite of about 50% aromatics, 35% iso-alkanes as alkanes, alkenes, and cycloalkanes (Ahmed, 2018). Gasoline is also referred to as gasoline range organics (GROs) (Todd et al., 1999).

Diesel fuel, kerosene, and jet fuel are types of middle distillates from crude oil separated by fractional distillation. Diesel fuel contains a carbon number between C_{11} and C_{25} and includes approximately 64% aliphatic hydrocarbons, 1-2% olefinic hydrocarbons, and 35% aromatic hydrocarbons (Ahmed, 2018). Kerosene is a liquid mixture of distillation produced chemicals that contains hydrocarbons between C_{11} to C_{12} (Ahmed, 2018). Diesel is also referred to as diesel range organics (DROs) (Todd et al., 1999).

Lastly, heavy oil is the dense residue of crude oil distillation having a weight composition of about 88% carbon, 10% hydrogen, 1% sulfur, 0.5% water, and 0.1% ash. Heavy oil has carbon numbers ranging from primarily C_{20} to C_{50+} (American Petroleum Institute, 2012). Heavy oil is known for containing dispersed solid or semi-solid particles, such as asphaltenes, minerals, metallic particles, heavy metal contaminants, and other leftover components to the refinery process (Ahmed, 2018; Meyer, 2003). The low low hydrogen to carbon ratio of heavy oil causes heavy oil to be highly viscous and not readily biodegradable (Ahmed, 2018; Meyer, 2003).

When petroleum hydrocarbon substances enter the environment as contamination, their exact compositions are typically unknown. Therefore, petroleum hydrocarbons are commonly addressed as NAPLs due to characteristic immiscibility in water. Light non-aqueous phase liquids (LNAPLs) and dense non-aqueous phase liquids (DNAPLs) refer to NAPLS with respectively lower and greater densities in comparison to water (Newell, 1995). Since gasoline and jet fuel have densities lower than water (0.72-0.76 g/cm^3 and about 0.75 g/cm^3, respectively), these petroleum hydrocarbon products are considered LNAPLs (Newell, 1995). Examples of DNAPLs include coal tar and heavy oil (The Interstate Technology and Regulatory Council, 2003).

2.2 Complexity of Petroleum Hydrocarbon Transformations in the Environment

Petroleum hydrocarbon contamination is a substantial environmental issue due to the inherent complexity of compounds and the subsequent complexity of interactions within the environment.

Depending on the composition, molecular weight, size, chemical structure, functionalities, and other properties, petroleum hydrocarbon compounds interact differently in the environment. When petroleum hydrocarbons enter the environment during release events, they prompt a diverse range of impacts, interactions, and fates due to the unique characteristics of the substances. This makes remediation inherently complex and challenging.

The fate of petroleum hydrocarbons upon entering the environment predominantly depends on two key factors: (1) the composition and (2) environmental setting. The type of petroleum hydrocarbon contamination addressed in this study is such that (1) the contaminant composition is composed mainly of heavy oil and (2) the contamination setting is a low-energy non-tidal environment with minimal weather impacts.

The site used in this study is a non-tidal freshwater lagoon. The petroleum hydrocarbons investigated in this study (described in greater detail subsequently) have undergone prolonged volatilization and weathering impacts. Specifically, lighter petroleum hydrocarbon substances, such as light crudes and gasoline, as well as lighter aromatics, such as ethylbenzene (BTEX), and other simple ringed, shorter chain compounds have likely already volatilized, evaporated, or dispersed to the atmosphere. The solubility and readiness of dissolution of the petroleum hydrocarbons used in this study are likely to be low due to the higher chain lengths and the larger number of aromatic rings of the substances (Truskewycz, 2019).

Secondly, the location of the field site yields a moderate climate with warmer, temperate summers and mild winters. During warmer conditions, heavier-weight petroleum hydrocarbons are capable of volatilizing (Truskewycz, 2019). However, during cooler conditions, the capacity of biological weathering through microbial catabolism of the petroleum hydrocarbons is reduced. Therefore, natural degradation occurs more slowly during cooler conditions. The field site is a non-tidal former shipping channel that is now isolated from tidal fluctuations.

2.2.1 Petroleum Hydrocarbon Contamination in Soil

Petroleum hydrocarbons possess an affinity to sorb onto soil particles due to mutual hydrophobicity. The sorption and desorption kinetics of petroleum hydrocarbons are also influenced by soil structure and composition. Processes by which petroleum hydrocarbons sorb onto soil particles include partitioning of petroleum hydrocarbons into soil organic matter (OM), diffusion into nanoparticles, or attachment and formation of strong bonds with sites on soil OM (Truskewycz, 2019). Increased OM, clay content, and hydrocarbon hydrophobicity lead to an increase in hydrocarbon sorption to soil. An increase of hydrocarbon sorption to soil decreases water solubility and accessibility to microorganisms (Truskewycz, 2019). Desorption processes can be improved upon by increasing sand content, temperatures, and interactions between the petroleum hydrocarbons and secreted plant and microorganism bioactive compounds (Truskewycz, 2019).

Petroleum hydrocarbon products deteriorate biochemical and physicochemical soil properties. Examples of soil property changes that petroleum hydrocarbon contaminants yield include deficits of water and oxygen as well as shortages of available nitrogen and phosphorus. The availability of essential nutrients is limited due to the detrimental influence petroleum hydrocarbon contaminants have on soil structures. Soil enzyme activities are adversely affected by petroleum hydrocarbon contamination. Thereby, important biotic processes responsible for soil biochemical reactions, such as those promoting plant growth, are prevented (Ahmed, 2018).

2.2.2 Sheening of Petroleum Hydrocarbons on Water Surfaces

In the United States, due to the Clean Water Act of 1972, the discharge of oil to surface water is regulated such that sheens must be reported to the federal government. Sheens are a thin layer (0.1-100 micron) of LNAPL on the water surface (Chalfant, 2015). Sheens may be liquid, waxy, semi-solid, or solid iridescent films. Figure 2 shows a petroleum hydrocarbon sheen from the field site in this study.

Figure 2. Petroleum hydrocarbon sheen from field site

Processes including ebullition (Figure 3), erosion (Figure 4), and episodic seeps (Figure 5) cause oil trapped in the sediment to rise from the bottom of the GSI to the surface water. Oils trapped in sediments are often LNAPLs that have accumulated sediments such that the oil-particulate aggregate (OPA) or tar balls becomes denser than water and sinks. Upland sources can also cause oil to get trapped in sediments. Depending on the sheening pathway, releases of trapped oil in sediment may occur chronically, periodically, or sporadically.

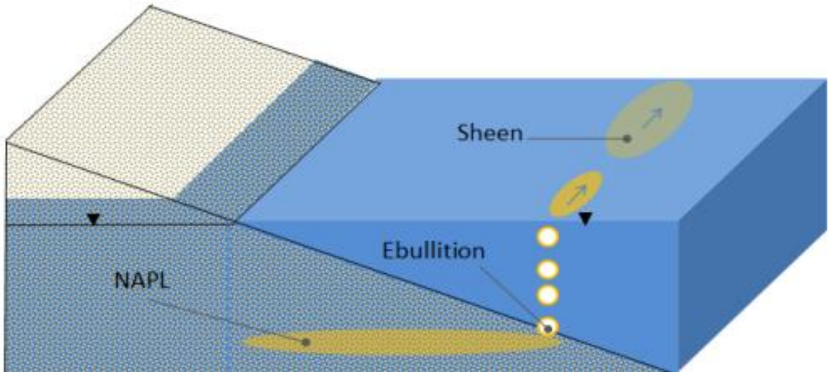

Figure 3. Illustration of sheening pathway through ebullition (Chalfant, 2015)

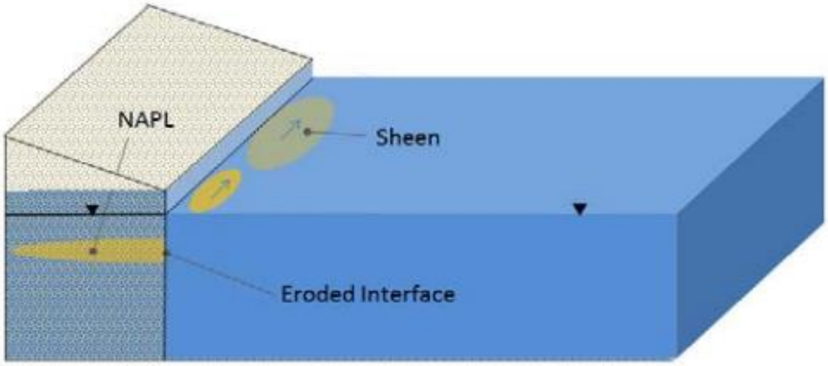

Figure 4. Illustration of sheening pathway through erosion (Chalfant, 2015)

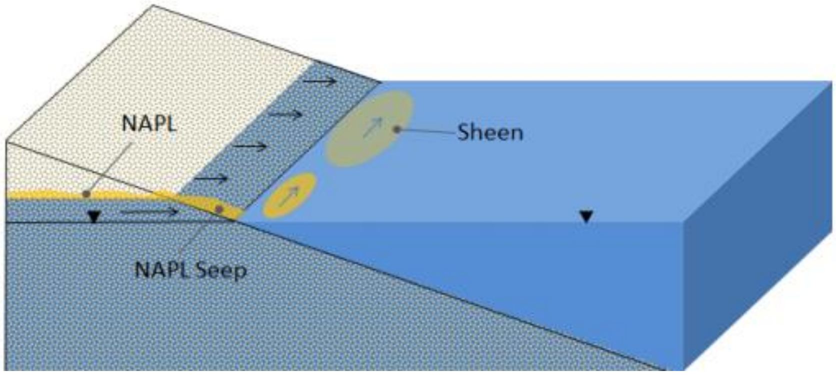

Figure 5. Illustration of sheening pathway through a seep (Chalfant, 2015)

This study specifically addresses the pathway of ebullition (although NOBBs also serve the purpose of preventing sediment erosion). During ebullition, gas bubbles generated in sediments carry oil to the surface of the water column as an intermediate wetting fluid. These bubbles are often generated as anaerobic degradation byproducts of NAPL, such as carbon dioxide (CO_2) and methane (CH_4) (Chalfant, 2015; Hogan, 2020). Typically, sheening from ebullition occurs periodically. Without a barrier at the sediment surface, these bubbles which transport oil as an intermediate wetting fluid between the gas and water phases burst at the water surface, and the oil forms a surface water sheen.

In addition to being a regulatory violation, sheens interfere with recreation, can cause natural resource damage, and may be hazardous and toxic material. Common sheen remedies can be costly and often lack the robustness to withstand long-term site conditions and multiphase (gas and NAPL) loading.

2.3 Bioremediation of Petroleum Hydrocarbon

Contamination by petroleum hydrocarbons will substantially change the composition of microbial communities in situ (Truskewycz, 2019). Microbial populations adversely impacted by petroleum hydrocarbons will decrease. Conversely, specific microorganisms capable of degrading petroleum hydrocarbons will persist until degradation is complete. To a certain degree, a site impacted with petroleum hydrocarbons can naturally attenuate through slow biological processes involving native microorganisms that evolve to degrade the organic contaminants without additional inputs (Truskewycz, 2019). This study involves how non-tidal microbial degradation mechanisms can be improved.

Bioremediation is defined as any activity encouraging the natural process of petroleum hydrocarbon degradation through microorganisms. Microorganisms behave as biocatalysts by biochemically consuming the contaminants and converting them into other substances. More specifically, microbial communities ultimately transform petroleum hydrocarbons, into products including CO_2 and CH_4 (Koshlaf and Ball, 2017). Bioremediation processes are limited by slow microbial biodegradation rates, consequently hindering research advances (Abatenh, 2017).

This study advances engineered bioremediation. In engineered bioremediation, a system is engineered to supply added nutrients and electron acceptors to increase the growth and activity of contaminant-degrading microorganisms (National Research Council, 1993). In this study, these added materials are called NOBB amendments.

2.4 OBBs as a Bioremediation Tool

An OBB is a technology developed and patented US Patent 10,112,854 B2) by Colorado State University and Chevron ETC as a method for managing petroleum hydrocarbons at tidal GSIs (Zimbron, 2016). The original goal of the OBB was to prevent surface water sheening from upward migration of petroleum hydrocarbons generally flowing atop the water tables from upland sources (Chalfant, 2015). Built off concepts from permeable reactive barriers (PRBs) and other capping materials, the OBB provides a structural cover at the GSI to sorb petroleum hydrocarbons preventing sheening while simultaneously allowing upward migrating gas bubbles to pass, and harboring microbes to foster biodegradation. With the OBB positioned on the ground surface at the GSI, the upward and downward flow of water and gases is maintained across the OBB via tidal pumping.

OBBs provide an inexpensive and effective sheening remedy and bioremediation pathway for petroleum hydrocarbon contamination. With an enhanced storage capacity, the time during which NAPL is present in the OBB also increases. Since biodegradation is time-dependent, this capability improves the depletion of NAPL through biological degradation (Chalfant, 2015). OBBs serve as a reactive barrier passively retaining NAPL and harboring robust microbial communities, thus promoting bioremediation. Ultimately, bioremediation depends on environmental conditions, hydrocarbon properties, and microbial communities present. While the OBB has proven suitable for tidal aerobic petroleum hydrocarbon degradation, the effectiveness of bioremediation in non-tidal anaerobic settings was unknown and is explored in this study.

Bubbles rising upward via ebullition can pass through the vertically transmissive rigid inner geonet, but any NAPL is sorbed to the OBB. While the geonet and the geotextile of the OBB (Figure 6) are both oleophilic, the outer nonwoven geotextiles are the primary NAPL-sorbing material due to their larger surface area. In addition to sorbing petroleum hydrocarbons, the geotextile effectively limits the intrusion of sediment particles into the geonet core, thereby mitigating erosion and minimizing sediment fouling. A conceptual diagram of a NOBB preventing ebullition-transported oil sheening in a non-tidal field setting is shown in Figure 7.

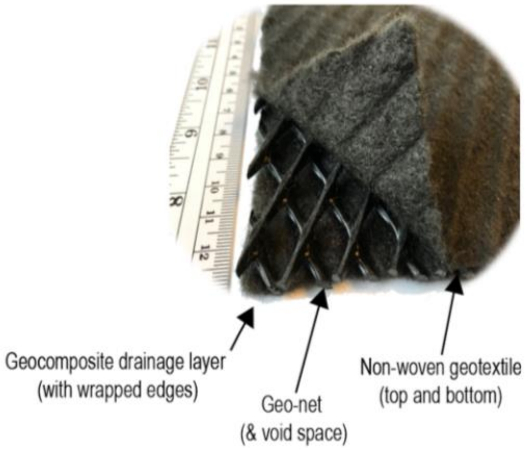

Figure 6. Illustration of an unamended geocomposite used in an OBB

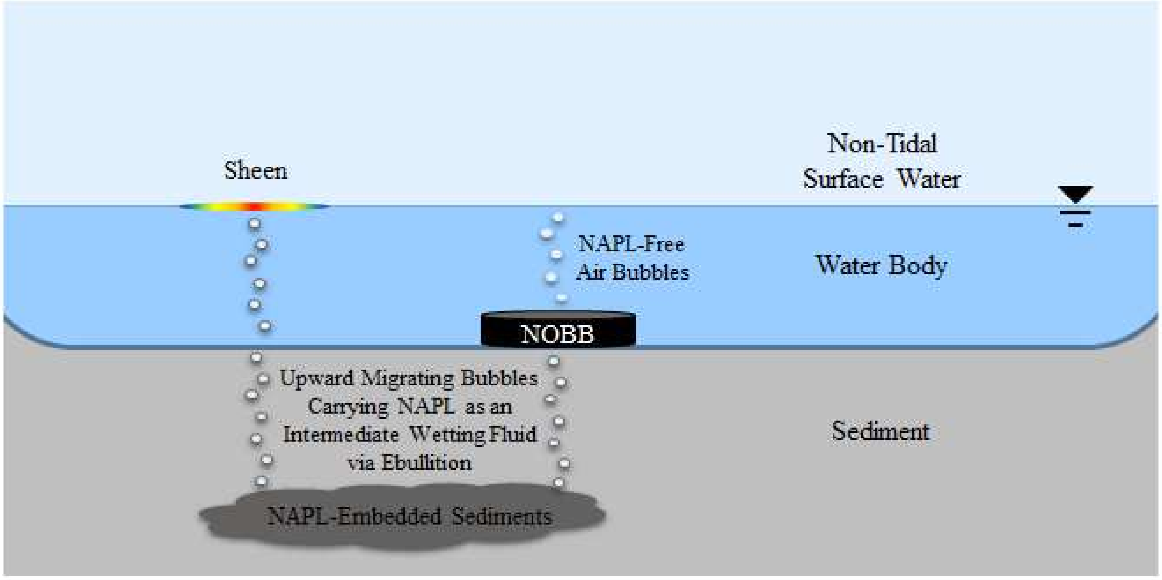

Figure 7. NOBB conceptual diagram

2.5 Amendments to Enhance Anaerobic Petroleum Hydrocarbon Degradation

This section provides a background on the amendments added to the interior of the NOBBs to potentially enhance biodegradation of petroleum hydrocarbons. Amendments include (1) hematite, (2), greensand, and (3) gypsum.

2.5.1 Hematite

Hematite, also known as ferric iron oxide (Fe_2O_3), provides a source of ferric iron ions (Fe^{3+}) and serves as a biostimulant for iron-reducing bacteria. Fe^{3+} is a soluble inorganic terminal electron acceptor that can be consumed during anaerobic respiration, or the process by which electron acceptors other than oxygen are used by organisms to perform metabolic reactions and processes in cells.

Since Fe^{3+} has a lower reduction potential (+100 mV to -100 mV) than oxygen (+600 mV to +400 mV), less energy is gained during anaerobic iron reduction in comparison to aerobic oxygen reduction (van Hullebusch, 2005). Fe^{3+} is the oxidized form of the redox couple Fe^{3+}/Fe^{2+} and Fe^{2+} is the reduced form. In this way, Fe^{2+} can be oxidized into Fe^{3+} by microorganisms with either O_2 or nitrate electron acceptors to gain energy (Zhang, 2009). Iron-reducing and oxidizing microorganisms geochemically cycle iron through biological reduction and oxidation of iron in contaminated environments (Zhang, 2009). Specifically, ferric iron-reducing microorganisms are capable of consuming petroleum hydrocarbons, such as those found in petroleum hydrocarbon-contaminated environments, making hematite a valuable addition for bioremediation pathways (LibreTexts, 2021). Many iron-reducing bacteria are also capable of anaerobically consuming other inorganic ions, such as sulfate. Furthermore, iron is known as a micronutrient for microorganisms (van Hullebusch, 2005).

2.5.2 Greensand

Greensand is a naturally occurring mixture of sand and Fe-rich clay minerals found in marine sedimentary deposits and ancient ocean floors. Glauconite is the primary component of greensand and is a blue-green siliceous mineral abundant in iron, potassium, and magnesium and containing approximately 30 other trace minerals (Grant, 2021). Greensand is also natural source of potash, which refers to a group of minerals containing potassium (K), a nutrient required for cellular metabolism (Shreve, 1921; He, 2017). Ultimately, greensand offers a slow, gentle release of nutrients and minerals. These additives are critical for successful petroleum hydrocarbon bioremediation because they help stimulate and maintain microbial populations (Das, 2011; He, 2017).

2.5.3 Gypsum

Calcium sulfate-dihydrate ($CaSO_4$-$2H_2O$), also known as gypsum, is hypothesized to improve the biodegradation of petroleum hydrocarbon contamination in groundwater settings by serving as a limiting electron acceptor (Joutey, 2013). As gypsum gradually dissolves, sulfate (SO_4^{2-}) is released (Buscheck, 2019). Although the inorganic SO_4^{2-} molecule has a lower reduction potential (-100 mV to -200 mV) than oxygen and iron, this electron acceptor provides a valuable pathway for anaerobic respiration and accelerating reactions through the process of sulfate reduction (LibreTexts, 2021; van Hullebusch, 2005).

In sulfate reduction, sulfate serves as a terminal electron acceptor and is consumed by the cells of a microorganism to perform anaerobic respiration. Sulfate-reducing conditions have been observed with groundwater plumes of petroleum hydrocarbon contamination (Kolhatkar, 2017). Microorganisms that consume sulfate, also known as sulfate-reducing bacteria, are commonly found in bioremediation of contaminated, oxygen-depleted environments with abundant supplies of organic material. Sulfate-reducing bacteria may also participate in methane oxidation through the following pathway: $CH_4 + SO_4^{2-} \rightarrow HCO_3^- + HS^- + H_2O$. These microorganisms are considerably robust with a lineage tracing over 3.5 billion years (LibreTexts, 2021).

Since sulfate depletion in groundwater limits the natural attenuation of petroleum hydrocarbon contamination via biodegradation, gypsum was evaluated in this study as a cost-effective way to deliver sulfate and accelerate bioremediation reactions (Kolhatkar, 2017). Additionally, since gypsum dissolves in water as Ca^{2+} and SO_4^{2-}, this amendment provides essential calcium and sulfur for microbial growth. Ultimately, gypsum satisfies the first step in remediation by promoting a more suitable environment for biological activity.

2.5.4 Combination of Hematite, Greensand, and Gypsum

The chosen amendments (hematite, greensand, and gypsum) were methodically combined to form two additional amendment options: (1) hematite + greensand (GS+H) and (2) hematite + greensand + gypsum (ALL). Since low nutrient concentrations may decrease or limit microbial activity, greensand was included in both of these amendment options as a source of gently releasing minerals and nutrients. The GS+H and ALL amendments were advantageously designed to contain both a nutrient source and an electron acceptor source. The combination of slow-releasing greensand with the relatively more rapidly degrading hematite and gypsum sustains reactions and addresses the issue of providing a longer-term solution while minimizing maintenance costs (He, 2017). While an excess of nutrients may retard the bioremediation process or worsen the remediation process, this impact is not expected due to the low loading rate and severe deficiency of nutrients (Das, 2011; Hodges and Simmers, 1997).

3. FIELD STUDY

Six different amendment types were evaluated in this study: (1) hematite (H), (2) greensand (GS), (3) greensand + hematite (GS+H), (4) gypsum (GYP), (5) hematite + gypsum + greensand (ALL), and (6) blank (B). Field study outputs include analyses of the retrieved NOBBs for (1) quantification and characterization of microbial communities and (2) assessment of amendment retention in NOBBs. The value of this work is to provide stakeholders with multiple lines of evidence that amended NOBBs sorb and retain petroleum hydrocarbons, facilitate biodegradation, and serve to prevent oil sheening in anaerobic, non-tidal conditions.

3.1 Field Study Objectives

The primary objective of the field study is to evaluate the concept of amended OBBs in non-tidal environmental settings contaminated with petroleum hydrocarbons as an in situ bioreactive barrier. The primary outputs from the field study are the analysis of the retrieved OBBs for microbial communities and qualitative assessment of amendment retention.

3.2 Site Description

The field site explored in this study is a former man-made channel located in a warm, temperate climate. While the channel was constructed over 100 years ago, the channel is now isolated from adjacent surface water and groundwater bodies via dam, groundwater cutoff walls, and hydraulic containment. Today, the size of the isolated channel is approximately 1150 m long and 120 m wide with an average water level fluctuation of between 0.9 m seasonally.

The channel serves as an active treatment for process wastewater stream effluent as well as an industrial process wastewater and stormwater flow pathway. While the channel previously served as the primary aeration basin for the process wastewater stream, the channel now functions complementarity to a new bioreactor system and continues to receive new contaminant inputs.

The channel was constructed by dredging. Over time the channel bottom accumulated soft, fine-grained sediments. Much of these sediments are impacted with embedded petroleum hydrocarbons. The upper sediment layer is approximately 0.5 m thick and has a low-density, heavy liquid consistency.

3.3 Field Study Methods

Three primary field tasks were completed as part of this study. First, the NOBBs were prepared. Second, the NOBBs were deployed atop anaerobic oily sediment at the non-tidal field site. Lastly, after approximately six months, half of the deployed NOBBs were retrieved from the field site and returned to the laboratory for analysis.

3.3.1 Preparation of the NOBBs

The preparation of the OBBs was performed in two parts. First, the OBBs were filled with designated amendments and securely sealed in the laboratory. Second, the NOBBs were loaded with an emulsion of petroleum hydrocarbon and air at the field site.

3.3.1.1 Filling the Field NOBBs with Amendments

The Engineering Research Center (ERC) Machine Shop at CSU assisted in cutting out 42 circles of 15.24 cm in diameter of the geocomposite drainage layer material (GDL) with a 0.9525 cm hole in each center. The GDL material is Tendrain II-1010 from Syntec Corp. The GDL consists of a rigid 3D open-latticed geonet core made of high-density polyethylene (HDPE) thermally fused between two layers of 0.34 kg/m^2 nonwoven polypropylene geotextile.

The average mass of the un-amended geocomposite material was approximately 49.4 grams. The top layer of oleophilic geotextile fabric was removed so that the amendment could be input. Each amendment type was used to fill seven NOBBs for a total of 42 amended NOBBs. Since only 36 amended OBBs would be deployed into the field, an extra NOBB of each amendment type was retained.

Alpha Chemicals Red Iron Oxide (Fe_2O_3) was chosen as the source of the iron oxide amendment for this study. The product originated from a natural source and contained a typical analysis of 82% Fe_2O_3, 55% Fe, 8.0% SiO_2, 2.9% Al2O3, 1.1% MgO, and 0.4% Mn (Alpha Chemicals). The average particle size of this iron oxide product was 30 microns (about 500 mesh) with a specific gravity of 5.24 (Pestell Minerals & Ingredients, 2012). Since Red Iron Oxide is dominated by hematite (Fe_2O_3), this product is referred to as "hematite" for convenience.

Down to Earth All Natural Fertilizers All Natural Greensand was chosen as a source of greensand amendment for this study. This product is primarily comprised of the mineral glauconite, an iron potassium silicate with the formula $(K,Na)(Fe^{3+},Al,Mg)_2(Si,Al)_4O_{10}(OH)_2$. This product consists of sand-like green particles with a specific gravity of 2.65 (Down to Earth Distributors, Inc., 2007).

Encap® Fast Acting™ Gypsum was chosen for the gypsum amendment in a spherical prill form. The active ingredient of the gypsum used, 1.5% anionic polyacrylamide copolymer, aids in solid-liquid separation via polymer bridging. Mined gypsum comprises 92% of the product of which 20% is calcium and 16% is sulfur. Lignosulfonates serve as a binding agent and comprise 2% of the product. Other inert ingredients (accessory minerals) contribute 4.5% of the product.

The NOBBs were filled by carefully pouring the amendments on top of the geonet and bottom geocomposite until the maximum capacity was reached. Due to the density and shape of the amendments, the total mass added to the OBBs differed. The average mass of each amendment type per estimated area of 181.7 cm^2 is provided in Table 1. As shown in the table, NOBBs filled with greensand (GS, GS+H, and ALL) contained the greater mass/area of amendment due to the greater density of the mixture compared to hematite (H) and gypsum (GYP) amendments.

Table 1. Mass per area of amendments added to OBB for field study

#	H (kg/m^2)	GS (kg/m^2)	GS+H (kg/m^2)	GYP (kg/m^2)	ALL (kg/m^2)	B (kg/m^2)
1	2.75	3.86	4.09	2.59	3.73	0.00
2	2.70	2.99	4.66	2.62	3.59	0.00
3	2.74	3.44	3.66	2.80	3.53	0.00
4	2.67	3.15	3.12	2.75	3.63	0.00
5	2.51	3.69	3.26	2.56	3.99	0.00
6	2.74	4.24	4.12	2.72	4.03	0.00
7	2.96	4.20	3.84	2.51	3.44	0.00
Avg	2.72	3.66	3.82	2.65	3.70	0.00

An image of a completely assembled field NOBBs set on top of a container to dry is shown in Figure 8. Water was sprayed using a spray bottle to gently adhere the added amendment to the geonet. Once dry, Loctite E-120HP Hysol Epoxy Adhesive was used to glue the top geotextile fabric material back onto the geonet. After the top layer of geotextile was securely fastened to the geonet, Permatex Ultra Black Gasket Maker Oil-Resistant glue was applied along the circumference of the NOBB. A thin layer of nonwoven geotextile was placed on top of the wet glue to ensure the sealed edges of the NOBB and prevent horizontal loss of additives from occurring.

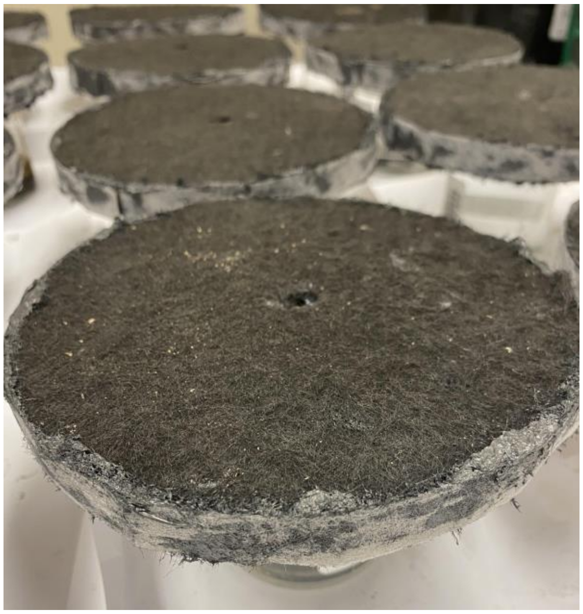

Figure 8. Completely assembled NOBBs for field study drying after gluing

3.3.1.2 Preloading the Field NOBBs with the Petroleum Hydrocarbons

The NOBBs were preloaded with an emulsion made from petroleum hydrocarbon product floating on the surface of the field site channel and air. The emulsion was created by vigorously shaking the components in a closed jar until homogeneous. The purpose of preloading the NOBBs with the petroleum hydrocarbons is to expedite the systems' exposure to the petroleum hydrocarbons by providing a uniform initial source of petroleum hydrocarbons. The pre-loaded petroleum hydrocarbons sorbed to the nonwoven geotextiles, such that excess petroleum hydrocarbon was not released to the environment. Any undegraded petroleum hydrocarbon was retrieved at the termination of the experiment.

Images taken during and after the preloading process are provided in Figure 9. Figure 10 illustrates a schematic of the fully assembled field site NOBB. Before each loading, a 2 L plastic bottle was vigorously shaken 60 times until the emulsion was well mixed (about 30 seconds). Then, 40 mL of the emulsion was poured from the bottle and dispensed into a circular aluminum pan approximately the diameter of the NOBB. The assembled NOBBs were individually placed onto the pan so that the emulsion could be absorbed on the NOBB's bottom geotextile fabric as uniformly as possible. The OBB was rubbed along the pan to ensure that nearly all the emulsion was absorbed.

Finally, 18-8 stainless steel, 0.9525 cm thread 20.3 cm long thread metal rods were screwed into the center hole for each NOBB. The rods served to anchor the disk to the sediment bed. Since the seven extra NOBBs would not be deployed in the field, these samples did not have rods screwed into them. Additional photos are provided in *Appendix Aa — Field Study Images*.

Figure 9. Images of a preloaded field site NOBB with metal rods screwed in center hole

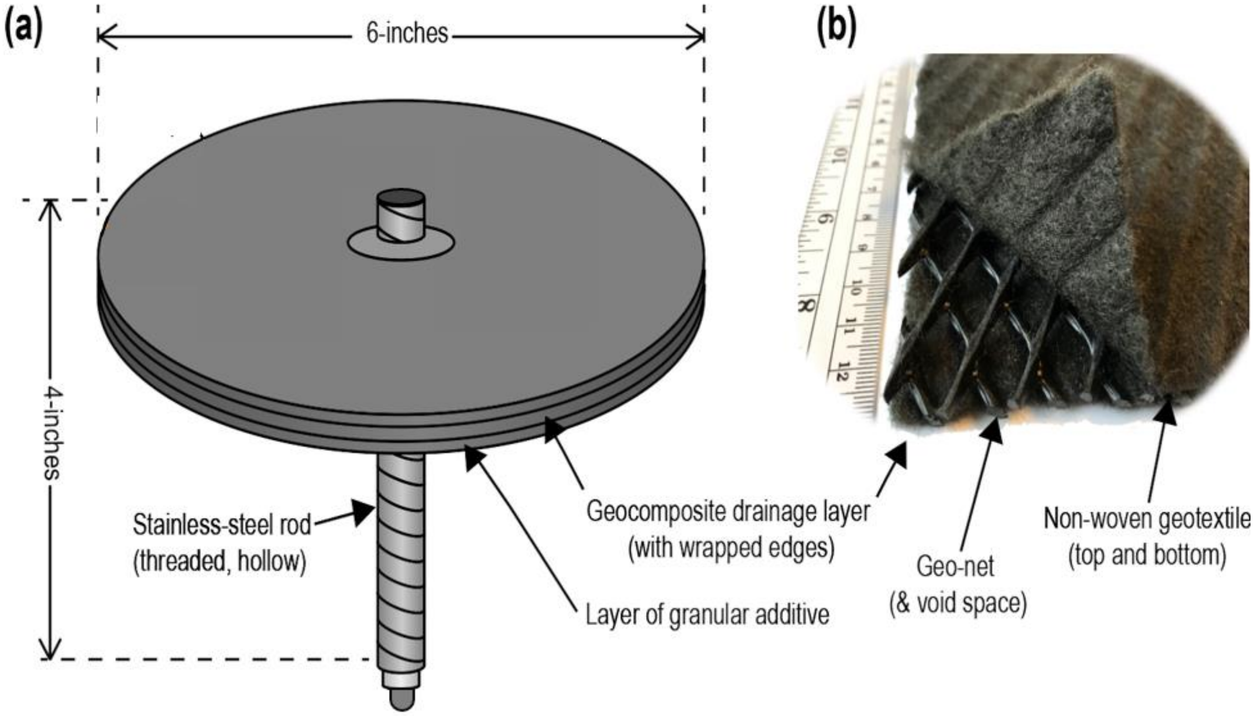

Figure 10. (a) Schematic of fully assembled field site NOBB with (b) an image of the GDL material

3.3.2 Deploy OBBs in Field

An image of a fully assembled field site OBB with a deployment cord (red) is provided in Figure 11. Thirty-six non-tidal NOBB disks were deployed at the field site using a deployment cord. A cord was tied to the top of each metal rod and secured with a screwed bolt.

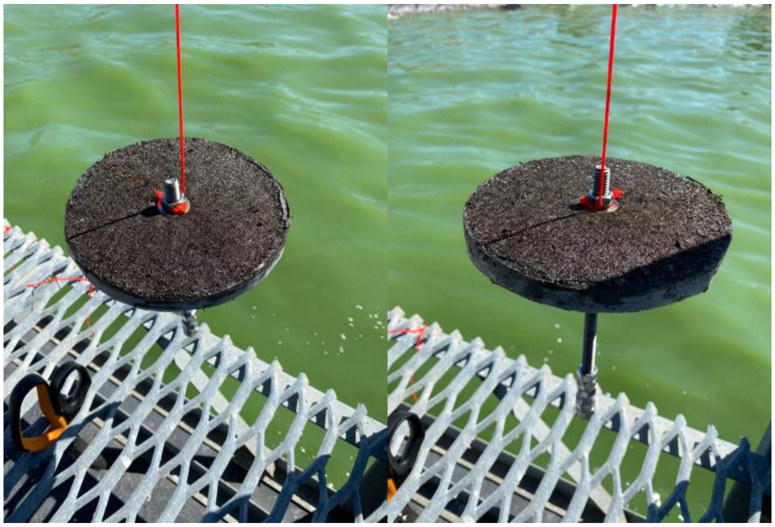

Figure 11. Fully assembled NOBB prepared with a cord to be deployed

The NOBBs were deployed from a floating dock platform approximately 7 m from the channel shore. The NOBBs were deployed approximately 30-cm apart and submerged under at least 60 cm of water. The NOBBs were slowly lowered into the water via their attached cord until slack became apparent. Their cords were then secured to the platform and labeled (Figure 12).

Figure 12. Multiple views of deployed field NOBBs tied to the end of the loading dock

3.4 Material Collection

Materials were collected from the field site on two occasions. The initial material collection consisted of sediment, water, and oil for laboratory testing (described in the next section). The second material collection retrieved half of the deployed NOBBs for analysis. A final material collection of the OBBs is anticipated for future work.

3.4.1 Initial Material Collection of Sediment and Water

The initial material collection was performed during the deployment visit to the field site in September 2020 in prerequisite to beginning the subsequent laboratory analysis. Approximately 75 L of oily sediments and approximately 150 L of water were collected for companion laboratory testing and experimentation. To collect oily sediments, a 4 L steel bucket was attached to the end of a telescoping fiberglass/aluminum pole. The bucket was then dragged through soft sediments at locations with a minimum of 2-feet of water above the sediments. The full bucket was then lifted, pulled to shore, and used to carefully fill four 19 L (5-gallon) buckets while avoiding agitation and aeration of the sediment. After filling, sediment in the buckets was allowed to settle for about 15 minutes to separate free water and decanted the liquid back into the channel by pouring. Additional sediment was then added until each bucket was full. Similarly, the canal water was collected using a 4 L stainless steel bucket on a pole by lowering into the channel surface and pouring into eight 19 L (5-gallon) buckets. Once filled, buckets were sealed, containerized, shipped via truck, and stored at room temperature in CSU's laboratory. The field sediment samples were used to fill the laboratory chemostat jars, and the field water samples were used to perform biweekly laboratory analyses described *Chapter 4: Laboratory Chemostat Study*.

3.4.2 Final Material Collection of Field NOBBs

Approximately six months after the deployment of the field NOBBs, half of the disks were retrieved from the field site and returned to CSU. Specifically, three OBBs of each amendment type were retrieved (i.e., H4, H5, H6, GS4, GS5, GS6, GS+H4, GS+H5, GS+H6, GYP4, GYP5, GYP6, ALL4, ALL5, ALL6, B4, B5, and B6). Following the reverse of the installation procedure, the NOBBs cords were untied from the end of the floating dock and slowly lifted from the canal water so that the NOBBs were lifted without scraping across the oily sediments. Then, the retrieved field NOBBs were wrapped in aluminum foil, double-bagged, placed in a cooler on dry ice, and shipped back to CSU. The bags were sealed with minimum to no headspace. Once at CSU, NOBBs were stored in a cryogenic freezer at -80°C until used for biological analysis.

3.5 Field Site NOBB Analysis

The retrieved NOBBs were analyzed for (1) microbial communities and (2) amendment retention. Both of these analyses were performed in tandem with the corresponding laboratory NOBB analysis.

3.5.1. Microbial Analysis

The retrieved field NOBBs were removed from the cryogenic freezer and prepared for subsequent analysis. Since the threaded center rods were not removed from the field NOBB before shipping on dry ice, the frozen rods were clipped with a bolt cutter. Thereafter, pie-shaped slices were cut from the NOBBs to retrieve geocomposite samples of the NOBBs. Three geocomposite samples for each amendment type were combined to total an approximate mass of 5 g. These samples were then briefly stored in a freezer until analyzed.

Microbial analysis was completed following the methods described in Irianni-Renno et al. (2016), Tochko (2018), and Irianni-Renno et al. (2018). The results from the microbial analysis are discussed in *Chapter 5: Results and Discussion*. Additional results are found in *Appendices C-F*.

3.5.2. Amendment Retention Analysis

After collecting samples for the microbial analysis, the NOBBs were dried for 7 days at 30^{o}C until the mass of all disks stopped changing over a 24-hr period. The mass of each dried sample was recorded as well as an estimated area of the cut NOBB slice. An image of the dried and cut field NOBBs is provided in Figure 13. *Section 5.6: Overall Amendment Retention* provides an analysis of the field NOBB amendment retention.

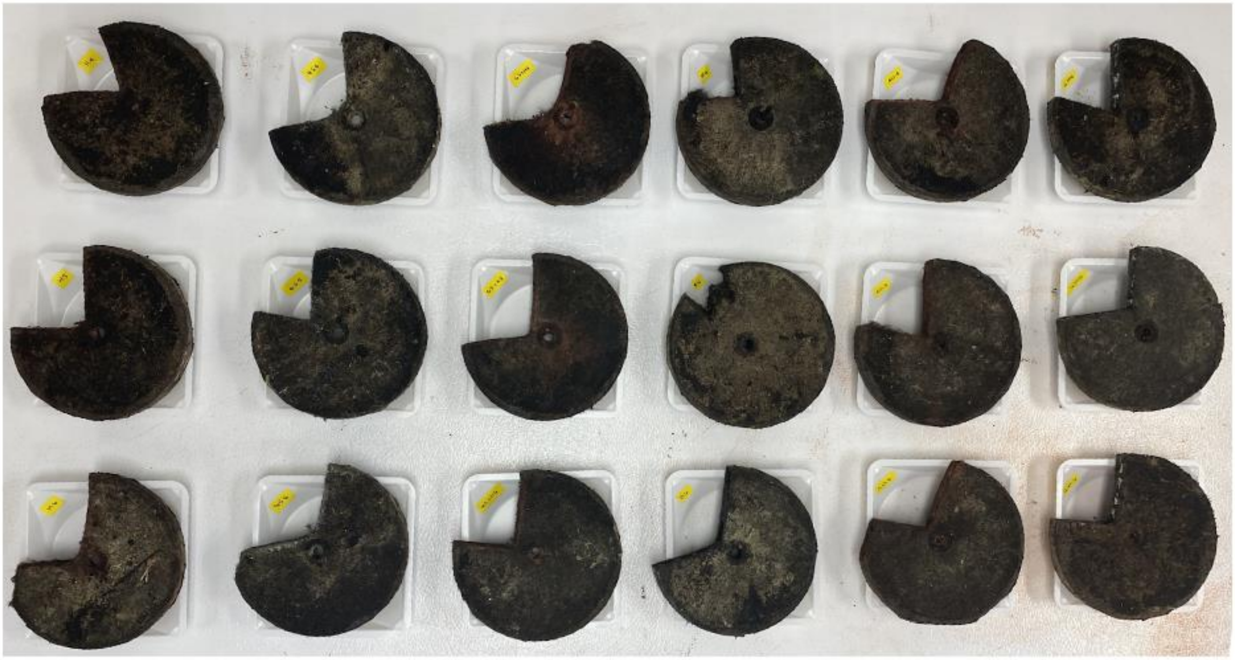

Figure 13. NOBBs extracted from the field site, partially dissected, then dried at 30oC

4. LABORATORY CHEMOSTAT STUDY

This chapter describes the laboratory chemostat study. First, the objectives of the laboratory chemostat study are provided. Second, the methods for preparing the laboratory chemostats are discussed. Third, the chemostat headwater data collection and analysis are presented, including (1) alkalinity analysis, (2) dissolved inorganic carbon (DIC) analysis, and (3) gas chromatograph equipped with a flame ionization detector (GC-FID) methane concentration analysis. Fourth, the ORP data analysis is detailed. Fifth, the analyses performed on the laboratory chemostat NOBBs are described.

4.1 Objectives

The primary objective of the laboratory chemostat study is to establish multiple lines of evidence supporting the usage of amended OBBs in non-tidal, anaerobic systems to deplete petroleum hydrocarbon contaminants and promote the growth of microbial communities. Outputs of the laboratory chemostat study include (1) real-time, continuous ORP data via Internet of Things (IoT) technology (Hogan, 2020), (2) biweekly chemostat headwater analysis, (3) quantification and analysis of microbial communities on NOBBs, and (4) qualitative assessment of amendment retention in NOBBs.

4.2 Methods

To emulate field site conditions, contaminated sediment and reservoir water were collected from the field site (*Section 3.4.1: Initial Material Collection of Sediment and Water*) and used to assemble the laboratory chemostats. The chemostats serve as long-term bioreactors to observe chemical and biological changes through batch analyses by pumping in fresh reservoir water and displacing headspace water. Biweekly flushing of the chemostat headwater imitated the natural removal of reaction byproducts via mixing of the surface water.

Thirty-six chemostats were assembled consisting of six duplicates of the six different amendment types as described in the field study. First, the amended NOBBs were prepared. Then, the chemostat jars were assembled. Finally, the piping system was installed to connect all the chemostat jars to an enclosed reservoir bucket containing the contaminated channel water from the field site.

4.2.1 Preparation of the NOBBs

Preparation of the laboratory NOBBs mirrors that of the field study with some notable differences. First, the NOBBs were filled with their designated amendments and securely sealed in the laboratory. The additional layer of nonwoven geotextile material described in *Section 3.3.1.1: Filling the Field NOBBs with Amendments* was not applied to the laboratory NOBBs. Second, the NOBBs were pre-loaded in the lab with the emulsion and immediately inserted into respective chemostat jars.

4.2.1.1 Filling the Laboratory NOBBs with Amendments

The ERC Machine Shop at CSU cut 42 7.6 cm diameter circles of the GDL material used in the field study. The average mass of the unamended laboratory NOBB material was approximately 11.7 g. The same procedure and amendments were used to fill the laboratory NOBBs with amendments. Table 2 provides the average mass of each amendment additive per estimated area of 45.3 cm^2.

Table 2. Mass of amendments added to OBB per area of OBB for field study

#	H (kg/m^2)	GS (kg/m^2)	GS+H (kg/m^2)	GYP (kg/m^2)	ALL (kg/m^2)	B (kg/m^2)
1	3.71	4.12	5.11	3.30	4.85	0.00
2	3.63	4.16	5.23	3.69	4.62	0.00
3	3.47	3.78	5.35	2.92	5.69	0.00
4	4.15	5.13	5.20	2.65	5.12	0.00
5	4.38	4.03	4.34	2.75	4.54	0.00
6	4.05	4.23	5.43	2.96	4.72	0.00
7	3.88	5.26	5.31	2.81	4.47	0.00
Average	3.90	4.39	5.14	3.01	4.86	0.00

The laboratory NOBBs were assembled following the methodology described in the field study. Figures 14-18 provide snapshots of each amendment type after spraying the amendments with water to adhere to the geonet. Figure 19 shows the Loctite E-120HP Hysol Epoxy Adhesive glue placed on top of the geonet to securely glue the geotextile on top. Unlike the field OBBs, the edges of the laboratory OBBs were not sealed with a thin layer of nonwoven geotextile material since the edges would be surrounded by glass in the chemostat jars.

Figure 14. H-amended NOBB

Figure 16. GS-amended NOBB

Figure 15. GS+H-amended NOBB

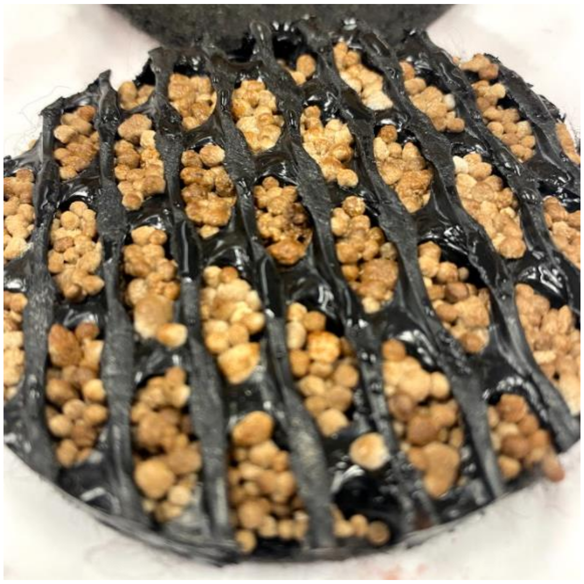

Figure 17. GYP-amended NOBB

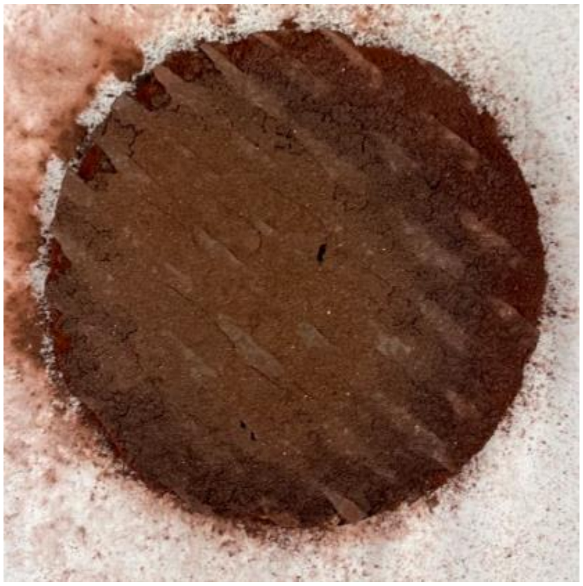

Figure 18. ALL-amended lab NOBB

Figure 19. GYP-amended NOBB prepared to glue the top geotextile back onto the geonet

4.2.1.2 Preloading the Laboratory NOBBs with Petroleum Hydrocarbon

The laboratory NOBBs were preloaded following the procedure described in the field study. 10 mL of the emulsion was pipetted onto weighing boats the diameter of the 7.6 cm diameter laboratory OBBs. Immediately after, the assembled NOBBs were individually placed into the pan to sorb the emulsion onto the NOBB's bottom geotextile fabric. Amended NOBBs were observed to more readily sorb the emulsion than the blank NOBBs.

4.2.2 Assembly of the Chemostats

Cole-Parmer Essentials Precleaned Clear Glass 500 mL (17 oz) Sample Jars were used to contain the individual chemostats for the laboratory studies. The jars were filled with approximately 235 mL of contaminated field sediment. Immediately after preloading each NOBB with the contaminant, the preloaded side of an NOBB was carefully placed inside a jar resting on top of the sediment.

The remaining volume of each jar was filled with channel water from the field site. Leakproof PTFE-lined disc friction fitted lids were screwed onto the jars and closed tightly to avoid oxygenating the system. Two adjacent entry points were installed on the lids to be connected to the piping system described in the following section. Finally, a rigid black geomembrane plastic covering was placed over each chemostat jar to protect the system from light.

4.2.3 Description of the Piping System

The piping system was designed to transport influent water from the 18.9-L (5-gallon) reservoir bucket to each of the 36 chemostat jars through one of the entry points on the lid while simultaneously expelling the existing headwater from the jars through the adjacent entry point. Swagelok's Stainless Steel Bulkhead Fittings were secured at these entry points to access the piping system. Swagelok's Stainless Steel Plug for 3.175 mm diameter tubing connected these entry points to the piping system. The tubing used in this system was Dayco Imperial Poly-Flo Polyethylene Tubing 22-PE-⅛-NSF containing an inner diameter of 3.175 mm and an outer diameter of 6.35 mm. Compression ferrules were used to secure the tubing to the fittings inside the plugs. A plug and a compression ferrule secured every tubing connection in this piping system. An image of an assembled lid is shown in Figure 20.

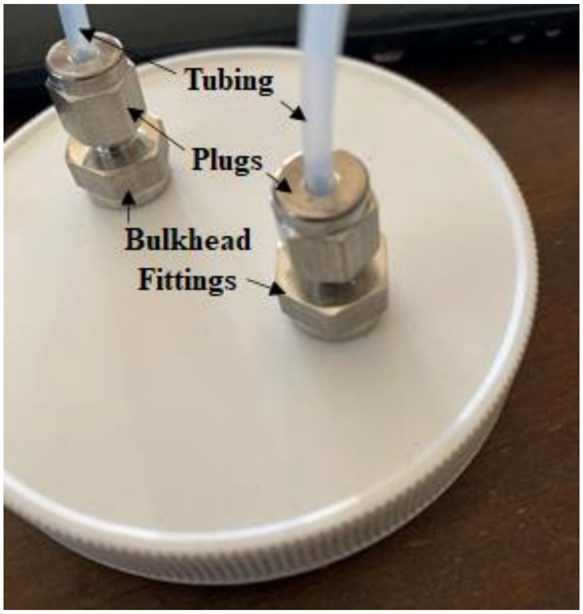

Figure 20. Image of assembled lid for chemostat jar

The first entry point on each lid, also referred to as the inflow, was connected to one of the five tubing sections leading from the reservoir water bucket (Figure 21). These sections improved the efficiency of the headwater extraction process by allowing up to five chemostat jars or one jar per section to be flushed simultaneously. The section closest to the reservoir water contained the chemostats that were analyzed with the ORP sensors (H4, H5, H6, GS6, GYP6, GS+H6, ALL6).

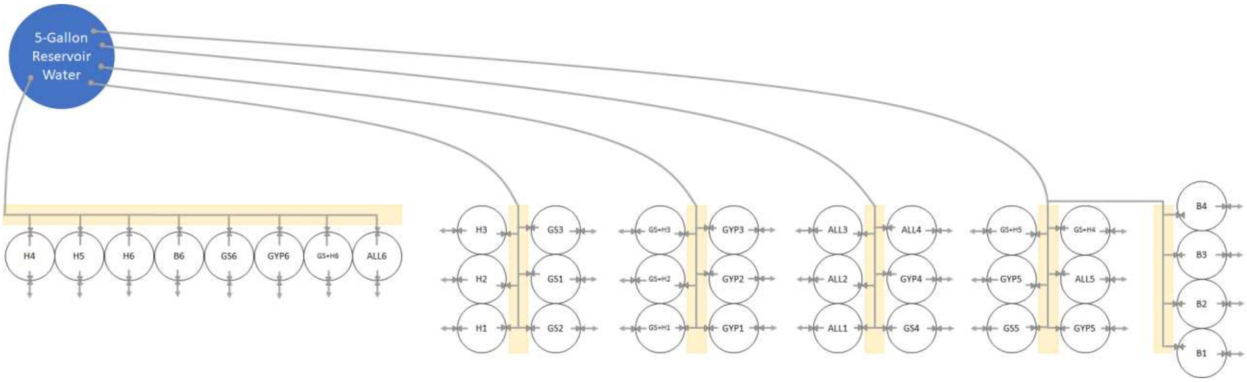

Figure 21. Overview schematic of piping system in the laboratory chemostat study

Figure 22 illustrates a single laboratory chemostat jar without (a) and with (b) the geomembrane plastic covering. Valves were installed onto each entry point tubing to control (1) the inflow of reservoir water and (2) the outflow of extracted chemostat headwater. Swagelok's Stainless Steel 15.875 mm Ball and Quarter-Turn Plug Valves containing One-Piece Instrumentation Ball Valves and 2-Way Straight Pattern were used to control the chemostat inflows and outflows. The connection size for these values matched the 3.175 mm diameter of the tubing. The plugs and compression ferrules were used to connect these valves to the tubing.

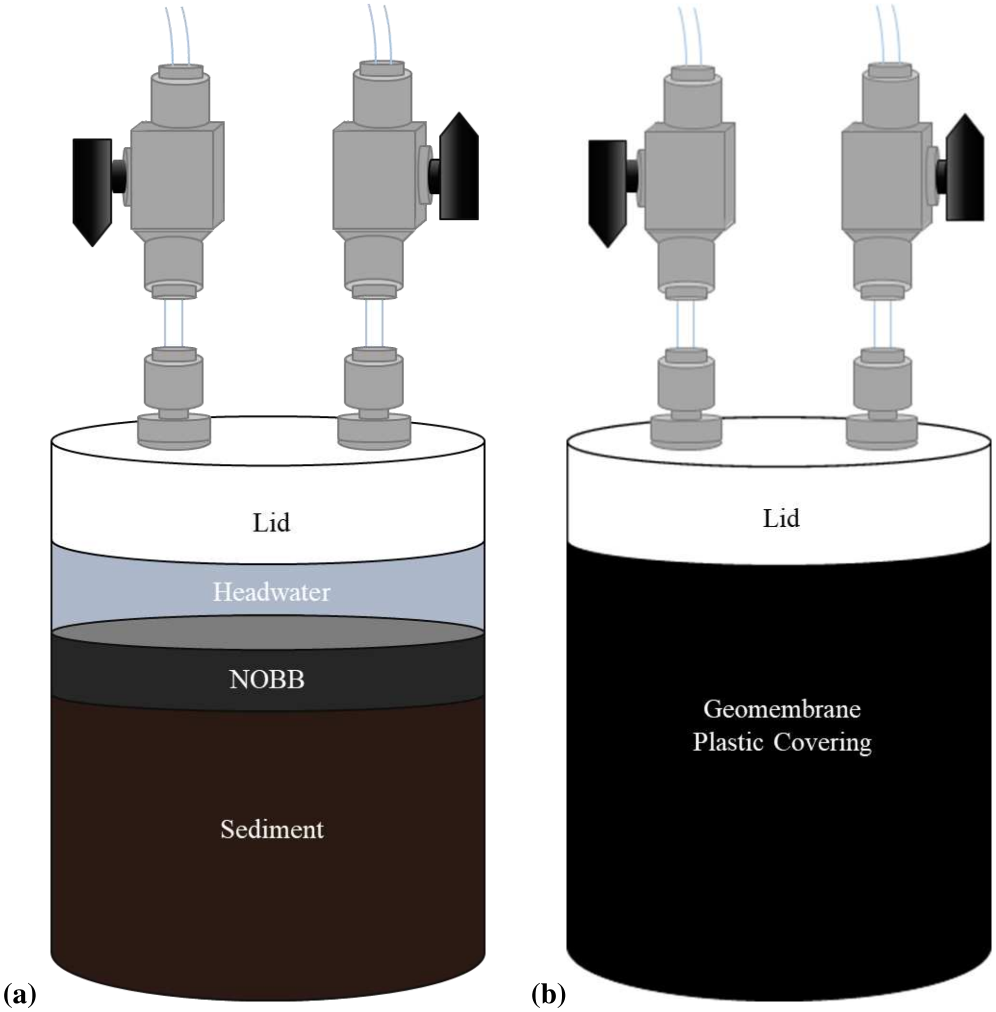

Figure 22. Schematic of a single chemostat jar (a) without and (b) with geomembrane plastic covering such that the jars are comprised of sediment, an NOBB, and headspace water (bottom to top)

To extract the headwater in a chemostat jar, both inflow and outflow valves were opened. The inflow water from the reservoir container displaced the existing water in the jar displacing the chemostat headwater through the outflow tubing to be collected into a glass container.

4.3 Headwater Collection and Analysis

Approximately 100 mL of headwater was extracted from each of the 36 chemostat jars on a biweekly basis. The extracted chemostat headwater samples were then used to perform the following analyses: (1) alkalinity, (2) DIC, and (3) headspace GC-FID. If the headwater analyses could not be performed immediately, the samples were refrigerated in glass jars for up to two weeks to preserve for later analysis.

4.3.1 Headwater Alkalinity

The alkalinity analysis on the extracted chemostat headwater was conducted using Hach's TNT 870 Total Alkalinity. The test kit procedures specified were followed except for the specified measuring device. Instead of using Hach's recommended spectrophotometer instrument options, the Genesys 10uV Thermo Scientific spectrophotometer was used to measure the absorbance with Einmal-Küvetten 1.5 mL Semi-Micro Disposable Cuvettes. An alkalinity calibration curve (Figure 23) was created using Sigma-Aldrich Alkalinity Calibration Standard. The absorbance data from the samples were converted into alkalinity using the generated calibration curve.

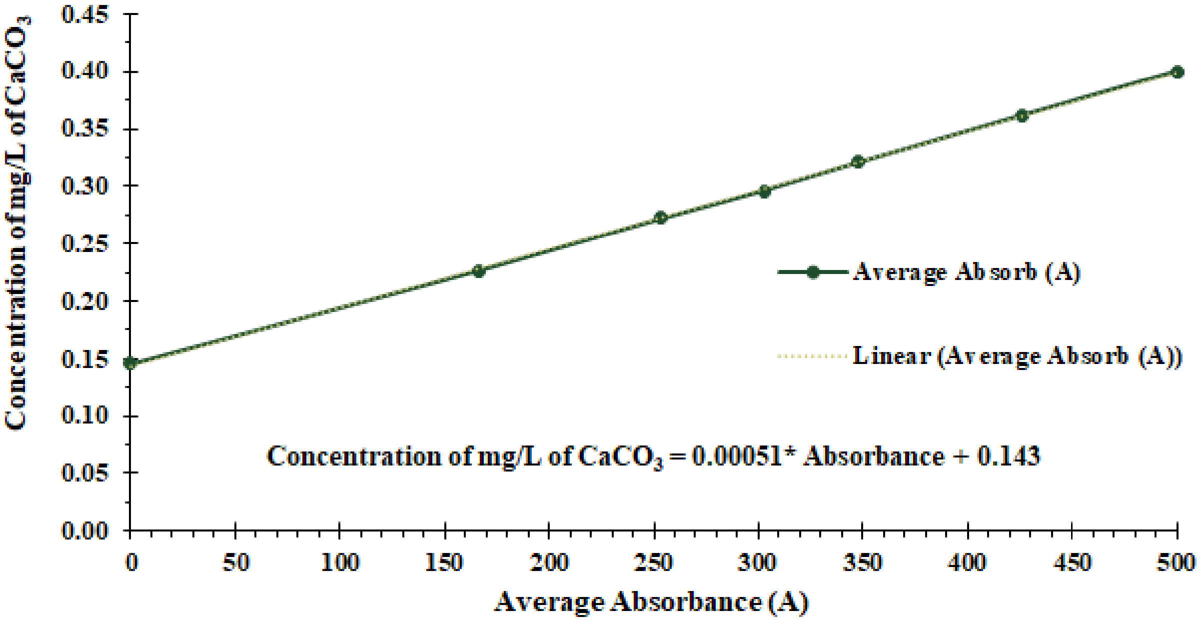

Figure 23. Alkalinity calibration curve

4.3.2 DIC Analysis

DIC results were estimated using alkalinity and pH data from biweekly sampling. DIC, also referred to as total CO_2 or total carbon (C_T), is the sum of dissolved CO_2, H_2CO_3, HCO_3^-, and CO_3^{2-} (DIC = $C_T = [CO_2aq] + [H_2CO_3] + [HCO_3^-] + [CO_3^{2-}]$) (Wei et al., 2018). The fraction of each carbonate species of the total DIC (α) was calculated as a function of pH. PEHANON® pH 6.0-8.1 Indicator Strips used to measure pH on February 23, 2021. The apparent solubility and acidity (dissociation) constants of carbonic acid (K) were determined as $K_1 = 4.188*10^7$ and $K_2 = 0.430*10^{10}$ for 20°C and 0% salinity (S). The following equations were solved to convert alkalinity to DIC (Emerson and Hedges, 2008).

$$DIC = C_T = \frac{alk}{\alpha_1 + 2\alpha_2}$$

$$\alpha_1 = \frac{C_T}{HCO_3^-}$$

$$\alpha_1 = \frac{[H^+] * K_1}{[H^+]^2 + [H +] * K_1 + K_1 * K_2}$$

$$\alpha_2 = \frac{C_T}{CO_3^{2-}}$$

$$\alpha_2 = \frac{K_1 * K_2}{[H^+]^2 + [H +] * K_1 + K_1 * K_2}$$

4.3.3 GC-FID Methane Concentration Analysis

A Tekmar 7000 Headspace Autosampler Gas Chromatograph equipped with a Flame Ionization Detector (GC-FID) was used to measure the concentration of dissolved methane (CH_4) in the extracted chemostat headwater samples following methods from Campbell (2017). After collecting extractions on February 1st and February 17th, 2021, approximately 5 mL of the headwater was immediately transferred into 20 mL Restek Headspace Vials. A crimper was used to seal 20 mm Preassembled Aluminum Seals with Septa.

The CH_4 peaks generated using the GC-FID were divided by the calibration number (32,324,801) to generate the µmoles of methane in each sample. The moles were divided by the sample mass of extracted chemostat headwater to generate the concentration of methane in the samples (µmoles/mL). The average results are discussed in *Section 5.3: Laboratory GS-FID Methane Results* and the entire list of results is provided in *Appendix B — Laboratory Test Results*.

4.4 ORP Analysis

ORP sensors connected to an IoT monitoring system (Hogan, 2020) were used to measure real-time ORP data. Sets of two ORP sensors were installed in eight of the 36 chemostat jars (H4, H5, H6, GS6, GYP6, GS+H6, ALL6, and B6). Each jar had one sensor securely glued inside the NOBB between the upper geotextile and the geonet. The second sensor was positioned below the OBB at the sediment surface.

An image of the ORP IoT system is shown in Figure 24. The image shows the 16 red and blue ORP sensor cords clasped to the green connector and the orange Ag-AgCl reference electrode attached to the adjacent beige connector.

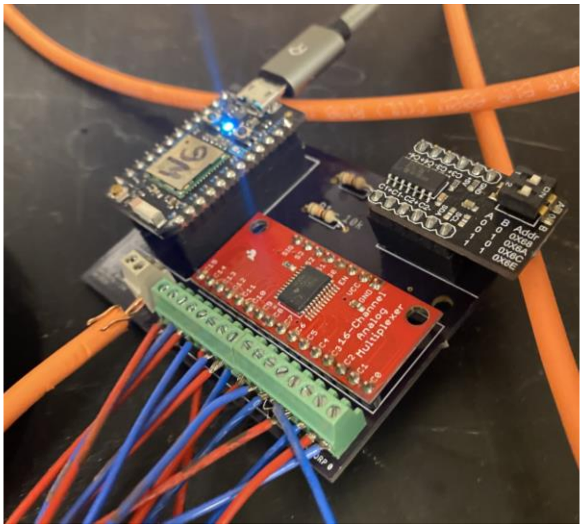

Figure 24. ORP IoT data acquisition system

4.5 Laboratory NOBB Analysis

After approximately 6 months, two laboratory chemostats per amendment type were removed from the piping system and disassembled for analysis. The laboratory NOBBs were analyzed for (1) microbial communities and (2) amendment retention. Images from this deconstruction are provided in *Appendix Aa — Laboratory Study Images*.

4.5.1 Microbial OBB Analysis

Immediately after deconstructing the NOBBs from the laboratory piping system, a piece of each top geotextile material was cut from the decommissioned lab NOBBs for microbial analysis. The two geocomposite samples of each sample type were combined to sum an approximate mass of 5 g. These samples were briefly stored in a freezer with the field site NOBBs until analyzed. The microbial analysis was completed following the methods described in Irianni-Renno et al. (2016), Tocko (2018), and Irianni-Renno et al. (2018). The results from the microbial analysis are discussed in *Section 5.5*.

4.5.2 Amendment Retention

After collecting samples for the microbial analysis, the laboratory NOBBs were dried with the field NOBBs for 7 days at 30ºC until the mass of all disks stopped changing over a 24-hr period. The mass of each dried sample was taken as well as an estimate for the missing geotextile area of the cut lab NOBB. An image of the dried lab NOBB samples is provided in Figure 25. *Section 5.6* provides an analysis of the laboratory NOBB amendment retention.

Figure 25. Two of each amendment type NOBBs extracted from the laboratory chemostats, partially dissected, then dried at 30ºC

5. RESULTS AND DISCUSSION

This chapter presents results from the field and laboratory studies. First, the laboratory results are presented in the following order: (1) alkalinity, (2) DIC, (3) methane concentration, and (4) ORP. Then, the microbial analysis results are discussed for both field and laboratory studies. Thereafter, amendment retention in field and laboratory NOBBs is examined. Lastly, additional observations are provided.

5.1 Laboratory Alkalinity Results

The primary purpose of collecting laboratory alkalinity data was to calculate DIC. The alkalinity data supplies a line of evidence for petroleum hydrocarbon degradation through the DIC results. Table 3 provides a summary of the average alkalinity and standard deviation for each amendment type from February 17th to April 26th, 2021. Figure 26 illustrates a box and whiskers plot of the average alkalinity results for each amendment type. Figure 27 illustrates a box and whiskers plot of the overall alkalinity results for each sample. *Appendix B — Laboratory Test Results* provides a complete list of each sample alkalinity results and respective average values.

Table 3. Summary of the average alkalinity and standard deviation for hematite (H), greensand (GS), greensand + hematite (GS+H), gypsum (GYP), hematite + greensand + gypsum (ALL), and blank (B) headwater samples from laboratory chemostats as well as the reservoir influent (R)

Amendment Type	Average Alkalinity (mg/L as $CaCO_3$)	Standard Deviation (mg/L as $CaCO_3$)
H	700.326	17.228
GS	697.130	32.671
GS+H	684.116	15.364
GYP	724.152	66.763
ALL	679.305	31.929
B	672.048	61.243
R	564.498	18.494

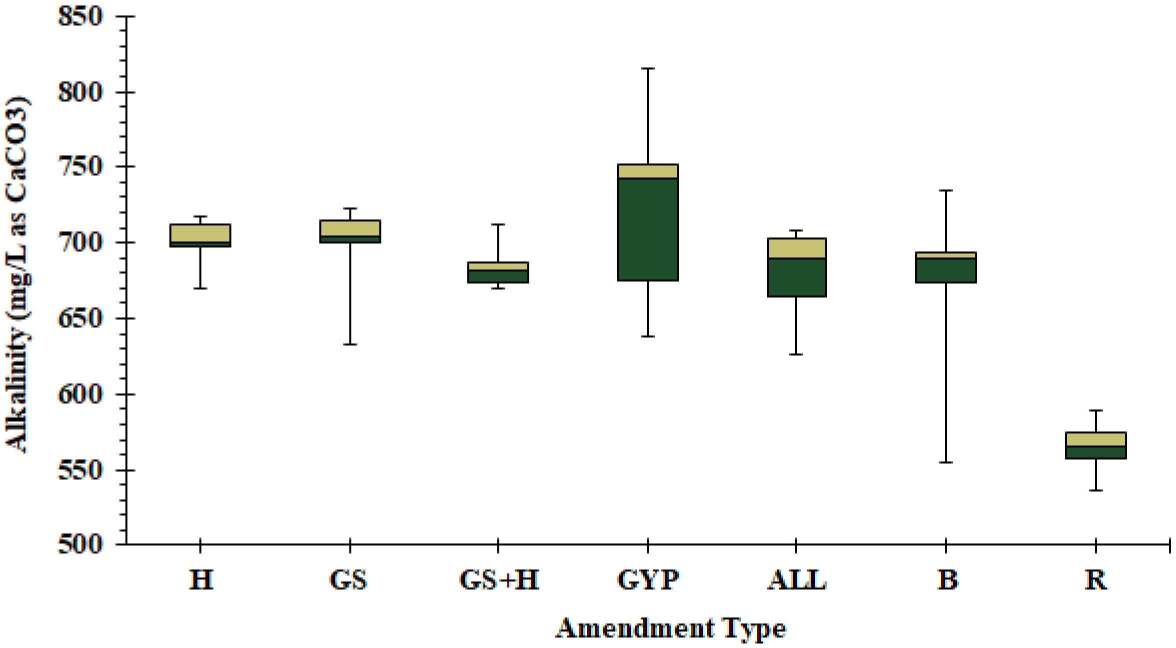

Figure 26. Average alkalinity (mg/L as CaCO3) results for each amendment type and the reservoir influent from February 17th to April 26th, 2021

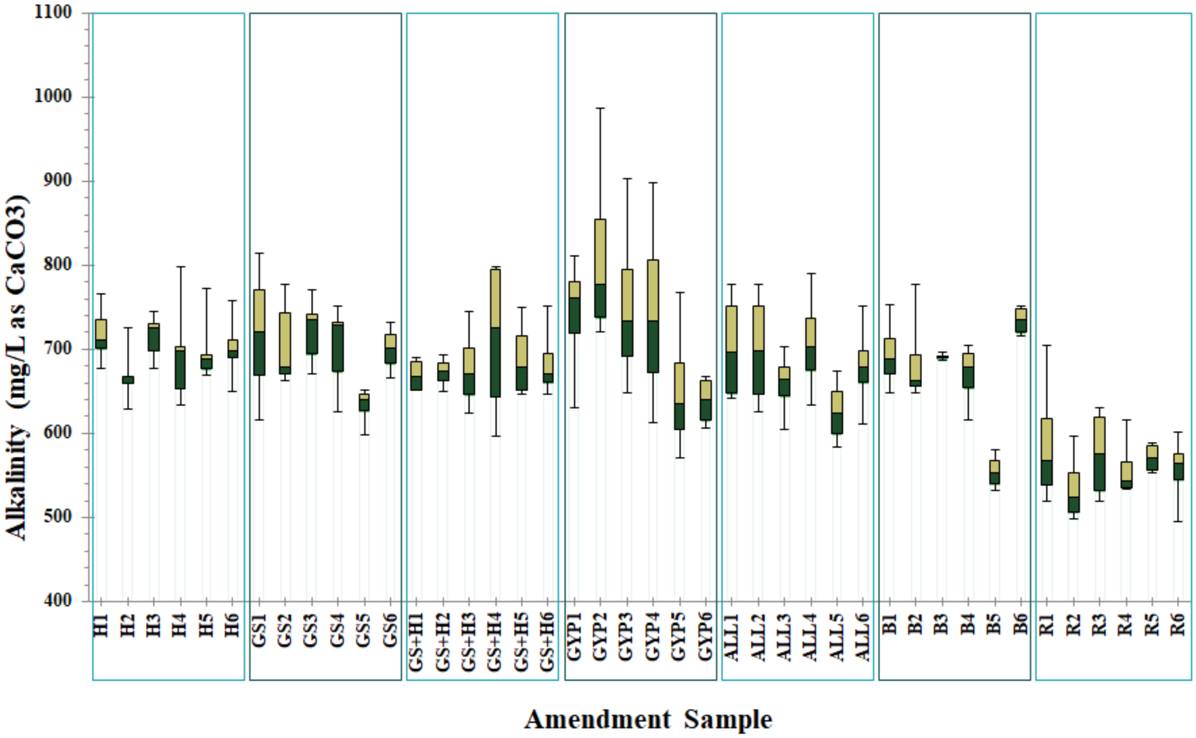

Figure 27. Alkalinity results for each sample from February 17th to April 26th, 2021

Alkalinity is an indicator of the buffering capacity of water. The high alkalinity levels present in these systems indicate that the water can buffer acid, thus preventing biologically harmful pH changes.

However, since the alkalinity levels are greater than 150 mg/L as $CaCO_3$, the headwaters may contribute to scaling on the NOBB surfaces (Mechenich and Andrews, 2004).

5.2 Laboratory DIC Results

DIC was analyzed based on alkalinity and pH values to evaluate how well the system is degrading petroleum hydrocarbons. DIC is generated during biodegradation (Wei et al., 2018). An increase in DIC concentration indicates a general decrease in petroleum hydrocarbon concentration. Table 4 summarizes the average pH, average DIC, and standard deviation for each amendment type over a 68-day experimental period from February 17th to April 26th, 2021. The average DIC results are illustrated as a box and whiskers plot in Figure 28. Figure 29 illustrates a box and whiskers plot of the overall DIC results for each amendment sample. *Appendix B — Laboratory Test Results* provides a complete list of each sample's DIC results and respective average values.

Table 4. Summary of the average pH, average DIC, and standard deviation (Stand. Dev.) for each amendment type and the reservoir influent over the 68-day experimental period from February 17th to April 26th, 2021

Amendment Type	pH	Average DIC (mg-C/L)	Stand. Dev. (mg-C/L)
H	7.65	353.311	9.1376
GS	7.65	360.894	20.2544
GS+H	7.55	345.727	18.6826
GYP	7.70	363.992	35.2095
ALL	7.55	339.204	26.6597
B	7.60	339.775	9.6685
R	7.45	297.048	20.5550

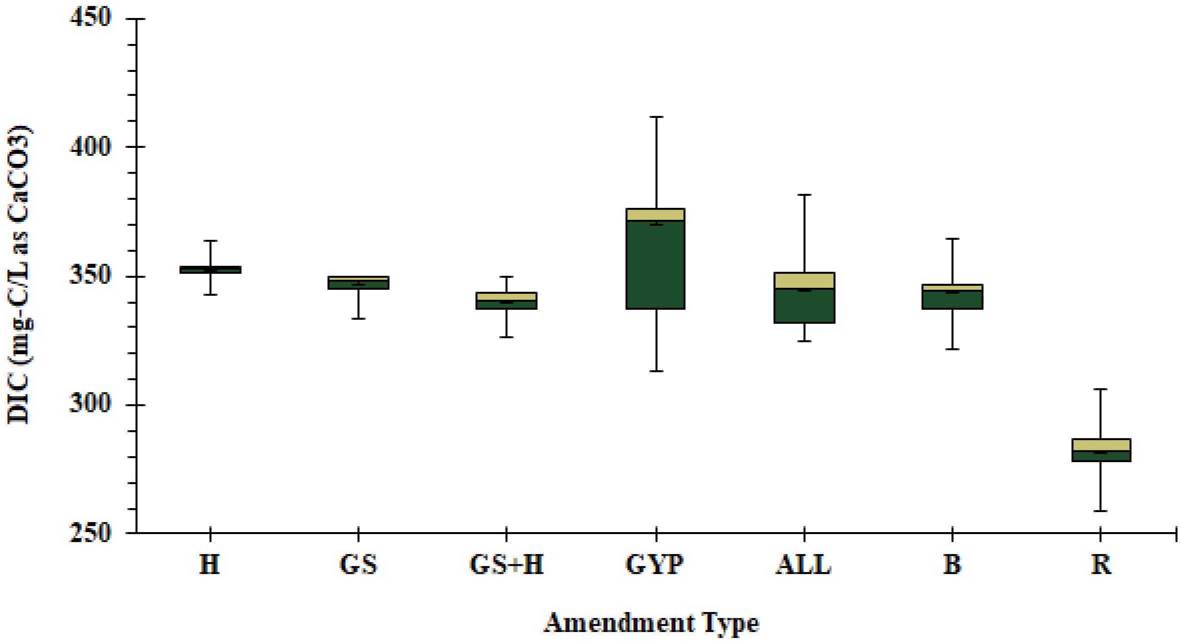

Figure 28. Average DIC results for each amendment type and the reservoir influent from February 17th to April 26th, 2021

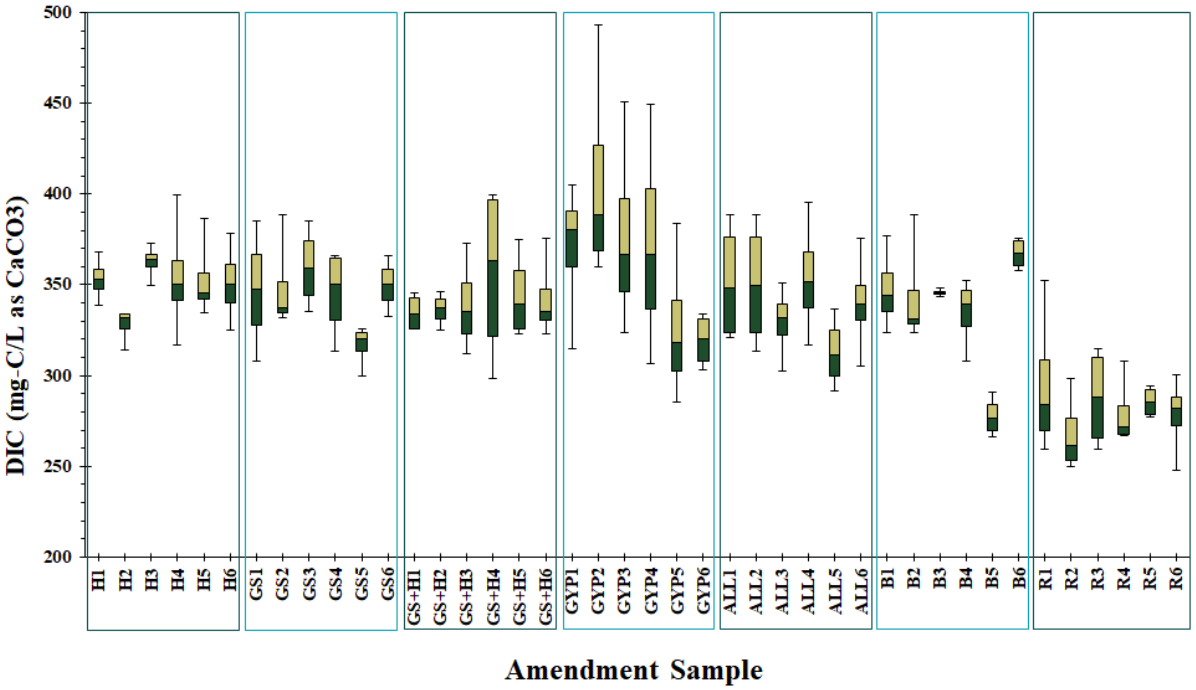

Figure 29. DIC results for each sample and reservoir influent samples from February 17th to April 26th, 2021

As illustrated in Figures 28 and 29, the reservoir water has the lowest average concentration of DIC. Headwater from the unamended (B) NOBB chemostat jars yield higher average DIC concentrations than the reservoir water. This finding suggests that even unamended NOBBs are supportive facilitators of bioremediation. The DIC results from the B-amended NOBBs were comparable to that of the ALL-amended NOBBs. This suggests that the mixture of H, GS, and GYP used in the ALL-amended NOBBs was not more advantageous in degrading the contaminant than the unamended NOBBs.

The highest concentrations of DIC are found in headwaters extracted from chemostats with only one type of amendment (H-, GS-, GYP-amended NOBBs). Since the H-, GS-, and GYP-amended systems outperformed the GS+H and ALL systems regarding DIC concentrations, the GS+H and ALL amendments are not as promising as the single-type amendments. The combination of GS+H was only slightly more successful than the ALL amendments. Although this may change over time as resources become more depleted and microbial communities develop accordingly.

GYP-amended NOBB chemostats yielded the greatest average concentration of DIC. This result is expected since increased sulfate concentrations are known to enhance sulfate reduction through sulfate-reducing bacteria. Consequently, sulfate reduction causes petroleum hydrocarbon concentrations to decrease and DIC concentrations to increase. Therefore, NOBBs containing GYP are expected to have the lowest concentration of petroleum hydrocarbons.

GS-amended chemostats closely resembled the DIC results from the GYP-amended chemostats. This finding indicates that the microorganisms in these environments were lacking nutrients, minerals, or other conditions that GS was able to supply. Future work should evaluate DIC concentrations in NOBB systems amended with greensand and gypsum. This evaluation could provide further evidence that nutrients and electron acceptors are both crucial for biodegradation.

5.3 Laboratory GC-FID Methane Concentration Results

Headwater chemostat extractions performed on February 1st and February 17th, 2021 were evaluated for methane concentrations (µmoles/mL) with a GC-FID. Table 5 presents corresponding methane concentrations for each laboratory amendment treatment. These results are illustrated in Figure 30. A complete list of methane concentrations, peak areas, and other data related to this analysis is provided in *Appendix B — Laboratory Test Results* for each sample.

Table 5. Methane concentration in headspace sample using GC-FID on February 1st and February 17th, 2021

Amendment Type	Average CH_4 (µmoles/mL)	CH_4 on Feb 1, 2021 (µmoles/mL)	CH_4 on Feb 17, 2021 (µmoles/mL)
H	0.05745	0.06437	0.05054
GS	0.08883	0.10314	0.07453
GS+H	0.04998	0.06149	0.03848
GYP	0.00599	0.00663	0.00535
ALL	0.00144	0.00120	0.00168
B	0.00275	0.00189	0.00360
R	0.00075	0.00000	0.00149

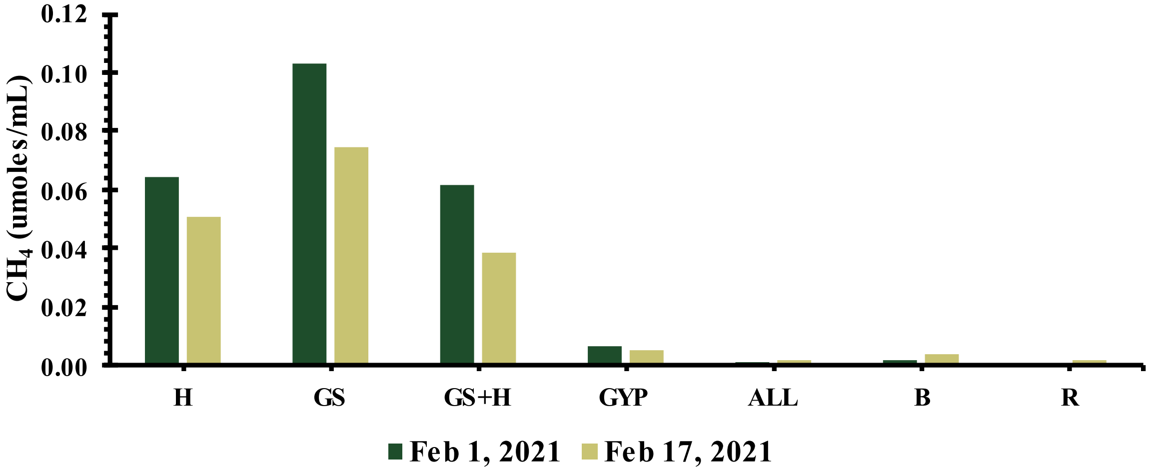

Figure 30. Average methane concentration (µmoles/mL) for each laboratory amendment treatment on February 1, 2021 and February 17, 2021 using GC-FID

The results of methane measurements indicate that GS, H, and GS+H systems produced the greatest methane concentrations on average (0.08883 µmoles/mL, 0.05745 µmoles/mL, and 0.04998 µmoles/mL, respectively). The higher methane concentrations in these systems suggest that they are more methanogenic than the GYP, ALL, B, and R systems. The GYP, B, ALL, and R systems yielded the lowest methane concentrations on average (0.00599 µmoles/mL, 0.00275 µmoles/mL, 0.00144 µmoles/mL, and 0.00075 µmoles/mL, respectively.) An alternative theory to explain the different methane concentration results is that preexisting methane bubbles were trapped in the underlying sediments, and through the process of ebullition, a major driving transport mechanism responsible for sheening, these bubbles rose through the semipermeable NOBB material and entered the headwater. Further study is needed to make inferences from the methane data.

5.4 Laboratory ORP Sensor Results

Figure 31 presents the results of in-NOBB ORP sensors over the period February 17 to April 26, 2021. Governing redox couples reported by Sale et al. (2021) are included in Figure 31. The ORP sensor results illustrate that all NOBB chemostats were anaerobic, but not poised at a specific redox couple associated with different amendments. Different amendments do not appear to have yielded substantially different measured ORP relative to the unamended NOBB (blank). ORP exhibit an interim value below that of iron reduction [$Fe(OH)_3 \leftrightarrow Fe_3O_4$] but above sulfate reduction ($SO_4^{2} \leftrightarrow FeS_2$). ORP values in this range have been observed at other sites undergoing natural attenuation of petroleum hydrocarbons (Sale et al. 2021). The interpretation of ORP values in this range warrants further study.

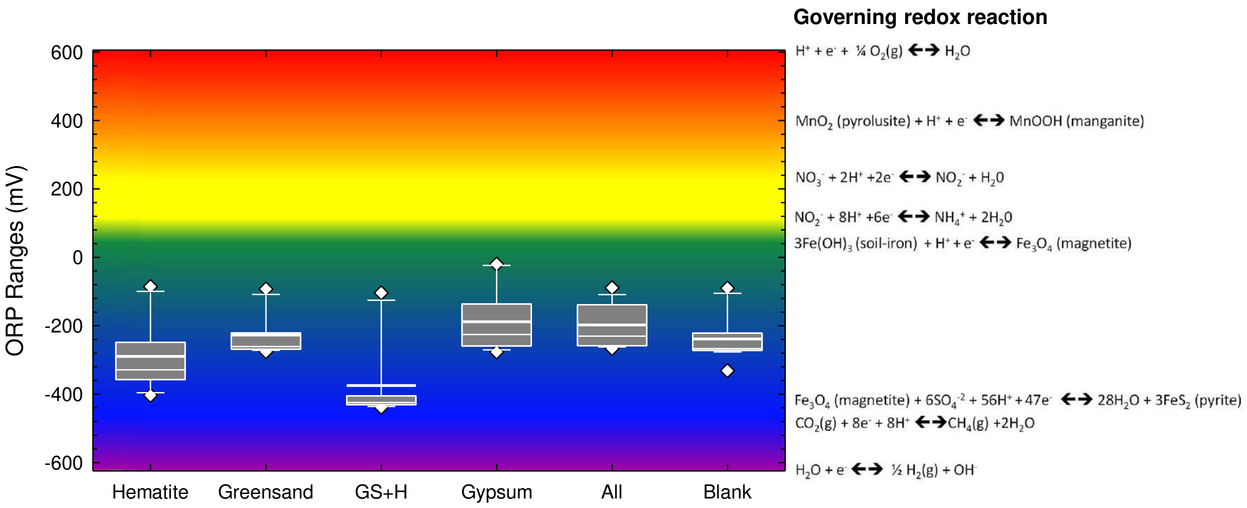

Figure 31. Non-tidal OBB ORP sensor results from chemostats over the period February 17 to April 26, 2021. Redox couples from Sale et al. (2021) included to aid interpretation.

Measured ORP were generally constant during the experiments, and were similar for sensors within the NOBBs, and at the sediment-NOBB interface.

5.5 Overall Microbial Analysis Results

Microbial analysis results were analyzed using four different approaches. *Section 5.5.1* describes how the number of 16s transcripts was used as a tool for estimating the activity of archaea, bacteria, sulfate reducers, methyl reductase, and aerobic alkane dgraders. *Section 5.5.2* outlines microbial community data analysis based on kingdoms. *Section 5.5.3* describes how scanning the relative amount of top 30 organisms is a quick and effective tool for determining microbial features present. *Section 5.5.4* describes a more extensive sorting of the microbial community data and provides a comprehensive analysis based on electron acceptor and donor types.

5.5.1. 16s Results

The number of 16s ribosomal ribonucleic acid (rRNA) gene copies in each NOBB amendment type were analyzed using qualitative polymerase chain reaction (qPCR) as described in Tochko (2018). 16s rRNA analysis targets organism types to produce a relative estimate of activity rather than an absolute quantity. Unfortunately, the methods used in this analysis were tailored to an alternative microbial system containing predominantly methanogenic microbes growing over a longer duration, the results did not provide an accurate indicator of the microorganisms present in this study when compared to other analyses performed (microbial community characterization). The results from the 16s analysis are found in *Appendix C — Microbial 16s Analysis*.

5.5.2. Microbial Taxonomy Results

The microbial community data were analyzed in terms of taxonomy. At the Kingdom Level, over 99% of microorganisms for each amendment type are a type of bacteria. The remaining less than 1% of microorganisms were classified as archaea, eukaryota, or unassigned. These results are shown in *Appendix D — Microbial Taxonomy Analysis*.

5.5.3. Microbial Features Results

Microbial community data were analyzed for unique features, or unique genetic sequences. These features correspond to a selection of the top 30 microorganisms based on a particular taxonomy level. Each chosen microorganism is classified as a feature. Different features may match up to the same microorganisms, and the same microorganism may be selected to satisfy multiple (different) features. This approach is a quick and effective method for scanning large microbial community data based on refined features. In this analysis, the same top 30 microorganisms are compared for all OBB sample types such that a relative abundance is generated. A description of each feature and the results from this analysis are provided in *Appendix E — Microbial Features*.

5.5.4. Microbial Categorization Results

The microbial community data were sorted at the genus level following the format provided in Tochko (2018). Archaea were identified as methanogens. Bacteria were categorized based on the following putative electron acceptor and donor types: aerobic, iron oxidizers, nitrate reducers, iron reducers, sulfate reducers, fermenters, and other. Categories for broadly classified archaea and bacteria were generated for organisms higher than the genus level without an assigned putative electron acceptor or donor.

Results from the microbial categorization are based on the microbial number of reads, or the quantity of microorganisms. Figure 32 illustrates a 1:1 comparison of the microbial number of reads per like function for each amendment type in relation to the laboratory versus field OBBs. These findings indicate that the results from the laboratory and field studies differed such that H, GS+H, ALL, and B amendment types were higher in the laboratory while GS and GYP were higher in the field.

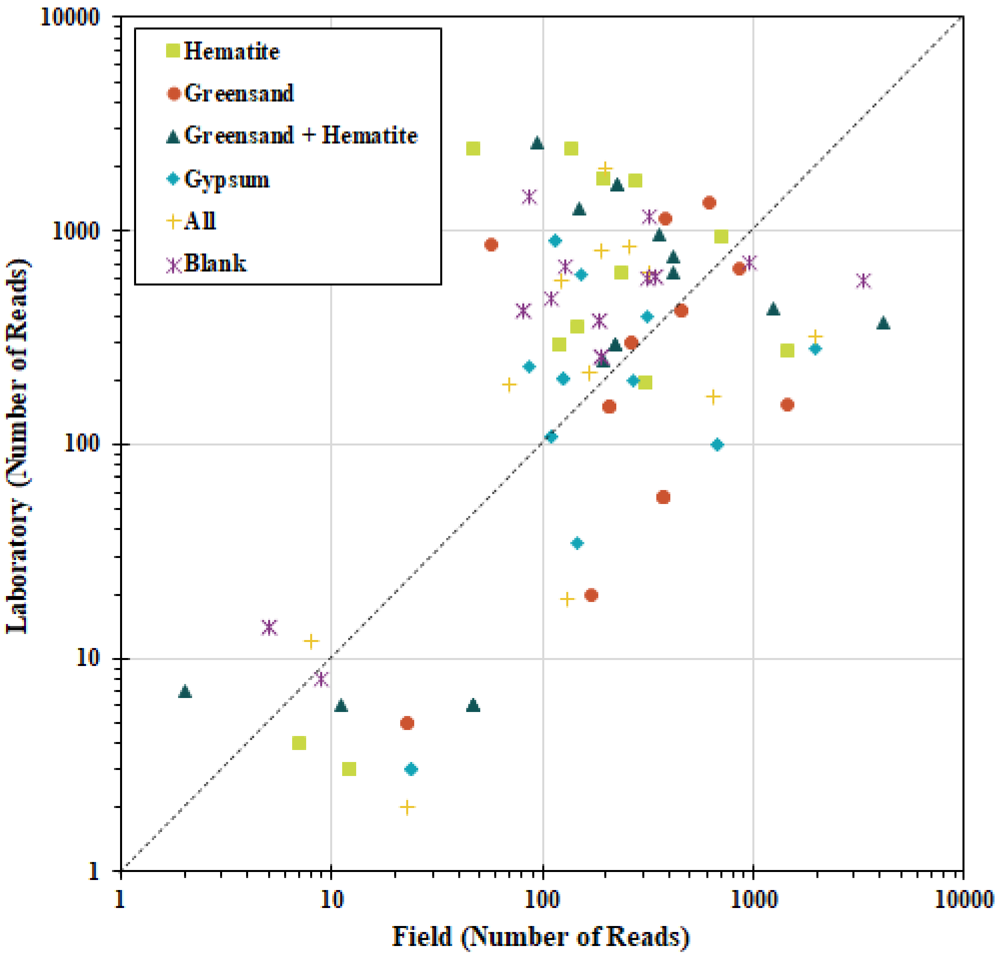

Figure 32. 1:1 comparison of number of functions in laboratory and field OBBs for like functions per amendment type

Appendix F — Microbial Characterization outlines the microbial results based on the following categories: aerobic, iron oxidizers, methane oxidizers, nitrate reducers, iron reducers, sulfate reducers, fermenters, broadly classified, and other (Tochko, 2018). Results from the microbial characterization indicate a diverse consortium of degraders present on all NOBBs. The functions observed do not specifically correlate to the amendment types. This is likely due to the predominant characteristics of the sediment.

5.5.5 Discussion of Petroleum Hydrocarbon-Degrading Microorganisms

This study illustrates that bacteria can degrade the complex combination of petroleum hydrocarbon contaminants that were present on NOBBs within the 6-month testing period. The rates at which the petroleum hydrocarbon contaminants, such as crude oil and diesel, are removed vary. Table 6 compares biodegradation results of various petroleum hydrocarbon-degrading microorganisms with the percentage of total reads of those microorganisms found on the NOBBs in this study (Ahmed, 2018).

Table 6. Comparison of known petroleum hydrocarbon-degrading microorganisms with microorganisms found in NOBBs

Degradation	Microorganism	NOBB	Perc. of Total Reads
Marine environment degradation of petroleum hydrocarbons (Sutiknowati, 2007)	*Alcanivorax (g)*	F-GS F-ALL	0.0940% 0.0428%
Degradation of petroleum hydrocarbons in marine sediments of the Niger Delta (Chikere, 2011)	*Staphylococcus (g)*	L-H	0.0150%
	Flavobacterium (g)	L-GYP F-H F-GS F-GS+H F-GYP F-ALL F-B	0.0195% 0.1142% 0.1583% 0.0702% 0.1301% 0.0428% 0.0435%
	Pseudomonas (g)	L-H L-GS L-GS+H L-GYP L-ALL L-B F-H F-GS F-GS+H F-GYP F-ALL F-B	8.1896% 0.8000% 4.2567% 0.2407% 1.7618% 0.1642% 0.0571% 0.1731% 0.1737% 0.1236% 0.0214% 0.1160%
	Acinetobacter (g)	L-ALL	0.0488%
Achieved maximum crude oil biodegradation of 78% (Rahman, 2002)	*Corynebacterium (s)*	L-B	2.5778%
	Flavobacterium (g)	See above	
	Pseudomonas (g)	See above	
Removed 52.1% crude oil (Zhao, 2011)	*Roseomonas (g)*	L-GS+H L-B F-H F-GS F-GS+H	0.0221% 0.0608% 0.0163% 0.0445% 0.1036%

Degradation	Microorganism	NOBB	Perc. of Total Reads
		F-GYP F-ALL F-B	0.0716% 0.0928% 0.1304%
Achieved maximum diesel degradation of 55% (Binsadiq, 2014)	*Acinetobacter (g)*	See above	
Achieved maximum diesel degradation of 49.93% in 20 days (Panda, 2013)	*Pseudomonas (g)*	See above	

5.6 Overall Amendment Retention

A visual assessment of the NOBB amendment retention was performed by peeling off the top geocomposite drainage layer and revealing the amendment-filled geonet. Given that each NOBB was filled to capacity with amendment at the start of the study, a qualitative comparison was made for the amendment retention between amendment types. An accurate quantitative analysis was unable to be achieved since exact mass measurements of the original NOBBs and the partially dissected post-experimental NOBBs were not precisely determined. For example, some amendment fell out of the NOBBs during the assembly process.

An image of each dried NOBB per amendment types hematite (H), greensand (GS), greensand + hematite (GS+H), gypsum (GYP), and hematite + greensand + gypsum (ALL) are provided in Figures 33-37, respectively. Additional images of this analysis are provided in *Appendix A — Images*.

Figure 33. Hematite amendments from field (H4, H5, H6) and lab (H1, H2) (left to right)

Figure 34. Greensand amendments from field (GS4, GS5, GS6) and lab (GS1, GS2) (left to right)

Figure 35. Greensand + hematite amendments from field (GS+H4, GS+H5, GS+H6) and lab (GS+H1, GS+H2) (left to right)

Figure 36. Gypsum amendments from field (GYP4, GYP5, GYP6) and lab (GYP1, GYP2) (left to right)

Figure 37. ALL amendments from field (ALL4, ALL5, ALL6) and lab (ALL1, ALL2) (left to right)

The GS-amended NOBBs visually retained the highest amount of amendment while the most depleted NOBBs were filled with GYP. The GS-amended NOBBs retained the least amount of amendment fixed to the upper geotextile while the ALL-amended NOBBs retained the most. The following order indicates the highest to lowest visually assessed amendment retention: GS > GS+H > ALL > H > GYP.

Since the field NOBBs were deployed a few weeks before the laboratory NOBBs were deployed in the chemostat jars, the laboratory NOBBs typically retained more amendment per amendment type in comparison to the field NOBBs. Generally, amendment retention results per amendment type were similar for the field and lab. However, the biggest difference in amendment retention between field and lab NOBBs was due to the rod hole in the center of the field NOBBs. As seen in images of H4, GS+H4, and GS+H6, for example, the center holes of the field NOBBs appear to be a zone of amendment depletion.

5.7 GC-FID TPH OBB Analysis

An analysis of total petroleum hydrocarbons (TPH) sorbed to the OBB during preloading was performed on the GC-FID following the methods provided by Bojan (2019) and Tochko (2018). Unfortunately, these methods proved ineffective for the petroleum hydrocarbons tested in this study so the chromatogram did not produce TPH results. The lack of results using these methods is likely due to the difficulty of extracting TPH from the GDL material. Additional method development is required to resolve this issue.

5.8 Additional Observations

Laboratory headwater samples collected from GYP chemostats began showing sheen-looking biofilms about 6 weeks into experimentation. Images taken on April 12, 2021 (Figure 38) provide snapshots of the biofilm sheens floating on top of extracted GYP1 and GYP2 chemostat headwater samples. Furthermore, when the GYP1 chemostat was deconstructed two weeks later to perform the end-of-use NOBB analysis, the headwater atop of the NOBBs revealed a similar film (Figure 39).

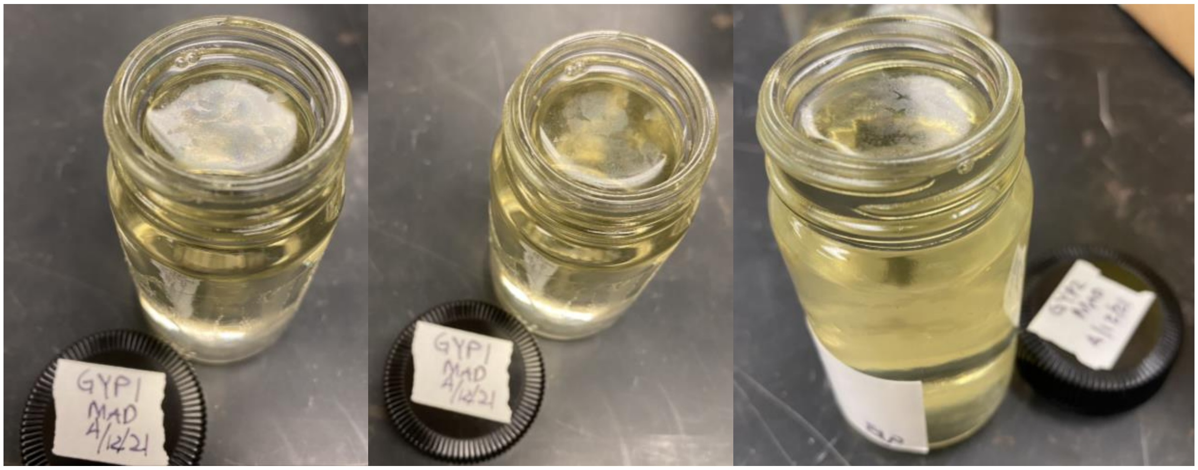

Figure 38. Biofilm sheens floating on top of extracted GYP1 (left and middle) and GYP2 (right) extracted chemostat headwater samples from April 12, 2021

Figure 39. Opened laboratory chemostat jar for GYP1 after completing the duration of its experimentation from April 26, 2021

Biofilms are typically formed by a diverse group of microorganisms that act as structurally organized communities (Guo, 2020). Since the composition of the discussed biofilms was not analyzed, the precise microbial contributions are unknown. However, these gypsum-induced biofilm sheens are believed to be caused by sulfate-reducing bacteria, such as *Desulfovibrionales (f)* (Heggendorn, 2018). Sulfate-reducing bacteria are known to produce biofilms under conditions of anoxic sulfate-rich environments often with iron present (Enning, 2014; Asaulenko, 2004). Since the ALL amendment type exposed the system to an excess in iron via hematite, in this case, the excess supply of iron is believed to have regulated biofilm formation (Guo, 2020).

5.9 Limitations of the Amended OBB

This section discusses the limitations of the amended NOBBs. While OBBs provide a cost-effective solution to the environmental issue of petroleum hydrocarbon contamination, two main limitations persist for the application of amended OBBs in non-tidal settings: (1) amendment longevity and (2) undetermined degradation byproducts and intermediate compounds in response to added amendments. First, demonstrating the persistent longevity of the NOBB's amendments and subsequent ability to enhance the degradation of hydrocarbons is critical to gaining regulatory acceptance of effective-low cost OBB remedies in non-tidal settings. Since OBBs are meant to last at a site for many years, the amendments must be capable of withstanding similar durations without depletion. Second, the added amendments are designed to create alternative degradation pathways suitable in the anaerobic setting. Consequently, undetermined byproducts and intermediate compounds are likely to be generated. These substances may be readily soluble in surface water. Due to the complexity of the contaminants, the subsequent degradation pathways are likely to also be complex.

The efficiency of amendment delivery is also uncertain. Specifically, microbial communities may consume the amendment for non-specific demands excluding bioremediation, such as cellular growth and other life processes (He, 2017). This makes calculations associated with the NOBB amendments complex and uncertain. Specifically, the exact stoichiometric reactions, electron acceptor flux, and use efficiency are beyond the scope of this study.

Another limitation of the amended NOBB is that the effectiveness of amendment delivery into contaminated sediments relies on inward diffusion through fine grained soils. As designed, the amended NOBB offers minimal mixing of nutrient and electron acceptor-rich areas potentially limiting availability to microbial communities. Low-energy environments, such as the field site used in this research, also supply minimal mixing rates.

Other co-contaminants, secondary water quality issues, and undesirable changes in the geochemical conditions may lead to incomplete degradation, unfavorable byproducts, toxic intermediate compounds, and mobilization of other contaminants. With a finite oleophilic loading capacity, the OBB is limited by its finite and uncertain lifespan (Chalfant, 2015). However, given slow loading via episodic ebullition and persistent biodegradation in non-tidal (sediment cap) settings, the loading capacity of OBBs may be sufficient in these settings.

6. SUMMARY AND CONCLUSIONS

Oleophilic biobarriers (OBBs) were explored in a non-tidal setting through complementary laboratory and field studies. Amendments were fixed within the geocomposite drainage layer (GDL) to produce an engineered bioremediation approach of adding electron acceptors and nutrients to an oleophilic sediment cap. Six different amendments types were explored: (1) hematite (H), (2) greensand (GS), (3) greensand + hematite (GS+H), (4) gypsum (GYP), (5) hematite + greensand + gypsum (ALL), and (6) blank (B). Amendment-filled GDLs were termed non-tidal OBBs (NOBBs).

In the laboratory study, a collection of 36 chemostats (six of each amendment type) was tested. Each 500 mL jar was filled with approximately 243 mL of field site sediment, covered with a 7.62-cm diameter NOBB disk, filled to volume with the site water (such that no headspace was presented), and contained by a lid connected to a piping system. Sets of two oxidation reduction potential (ORP) sensors were installed in eight of the chemostats: H4, H5, H6, GS6, GS+H6, GYP6, ALL6, B6. One sensor was installed inside the NOBB and the other sensor was placed at the sediment surface. These sensors were connected to an Internet of Things (IoT) monitoring system to measure real-time ORP data and characterize the oxidation-reduction (redox) conditions of the systems. A biweekly chemostat headwater collection was performed on each chemostat to analyze alkalinity, pH, DIC, and methane concentration. After 6 months, two chemostats of each amendment type were deconstructed for subsequent microbial analysis.

Six amended NOBBs of each amendment type (36 total) were deployed at the sediment surface of a petroleum hydrocarbons impacted field site. After six months, half of the NOBBs per amendment type were retrieved for subsequent microbial analysis. Results from the microbial analysis indicate that field NOBBs (F) typically yielded higher total reads in comparison to laboratory NOBBs (L). The highest of which were F-GS+H (29,929), F-B (25,304), F-GS (20,219), and L-H (20,001). The lowest recorded number of microbial reads were F-GYP (15,367), F-ALL (14,012), L-GS (13,875), and F-H (12,262). These results suggest that microbial communities present in laboratory and field NOBBs developed, grew, and functioned differently. Therefore, while the laboratory study was a useful tool in verifying multiple lines of evidence for the development of an oleophilic bioreactive barrier, the laboratory results are not an ideal indicator of how NOBBs operate in the field setting.

Dissolved inorganic carbon (DIC) results from the laboratory study indicate that GYP and GS amendments were most effective at degrading organic matter in the chemostats with their higher average DIC concentrations of 364.0 mg-C/L and 361.9 mg-C/L, respectively. In contrast, the NOBB systems amended with GS+H (345.7 mg-C/L), ALL (339.2 mg-C/L) and B (339.8 mg-C/L) yielded the lowest DIC values. These results suggest that combining amendments was not more effective at facilitating degradation in comparison to applying an unamended NOBB.

Loss of amendments from NOBBs via dissolution was observed in retrieved laboratory and field NOBBs. Results from amendment retention analysis suggests that greensand is the most durable amendment used in this while gypsum is the least durable

6.2 Future Work

This section discussed recommendations for future work to enhance NOBBs. Ultimately, future research needs to address more comprehensively what must be satisfied to implement amended OBBs in non-tidal settings.

Delays related to COVID-19 prevented the field samples from being deployed within the originally planned time frame of early March for testing over the hotter and more biologically active summer season; the field portion of this research was delayed until mid-September, the field samples were subjected to the colder temperatures of the winter season. Consequently, the microbial analysis reported in this research may have been impacted. Since temperature is a prominent factor influencing microbial growth, future short-term research projects should be conducted during the warmer months when microbial communities are more active. Fortunately, half of the NOBB field samples are still remaining at the field site for collection after the summer (retrieved approximately 12 months after their installation). Future work must be conducted to analyze the remaining NOBB field samples and should be informed by the results of microbial analysis in this study. Tools for analyzing microbial communities should be tailored for the system of study. While current microbial analysis systems work at an initial analytical level, a more refined methodology for quantifying and monitoring progress will need to be tailored beyond the initial analysis provided in this study.

Future work with amended OBBs should include a holistic understanding of the site conditions. This will make the application of the OBB amendments more aligned with specific application goals. Since biodegradation can be inhibited by excessive nutrient concentrations, the inhibiting nutrient concentration specific to the site conditions needs to be determined before selecting appropriate amendments.

A new form of gypsum should be explored for future work with amended OBBs. While the gypsum chosen in this research, Encap® Fast Acting™ Gypsum, provides many advantageous features and is widely used in agricultural settings, the gypsum appears to have rapidly dissolved and may not provide long-term performance.

A customized GS-FID method should be developed to analyze the TPH on NOBBs when heavy oils are present. Since the methods from Bojan (2019) and Tockho (2018) did not produce results, further work is required to refine the proper extraction time, solvent, and methods to produce results on the chromatogram. While dichloromethane is known to dissolve the OBB materials, this solvent could be used for TPH extraction analysis if properly accounted for through control testing.

Research to enhance the NAPL capacity should be conducted in future work. Multiple layers of oleophilic material and potentially other sorptive materials would help sites also containing non-HPC NAPLs. Additional layers may support OBB application where dissolved-phase loading to surface water is a concern (Tochko, 2018). Discovering a method to quantify the contaminants on the NOBBs would support this objective. This research should optimize a balance of permeability, capacity, and cost-effectiveness.

Lastly, the ultimate test of a NOBB is the ability to prevent sheening. Therefore, given the promising preliminary results presented in this study, pilot-scale deployment of a NOBB is recommended. Key attributes of a site for pilot-scale testing include:

- sparse oil in non-tidal sediment causing episodic ebullition sheening;

- site specific business case for advancing NOBBs;
- site access;
- and potential and appetite for technology trial.

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APPENDIX A — IMAGES

Appendix Aa — Field Study Images

Figure Aa1. Fully assembled NOBBs for field study were placed on top of jars to dry after gluing without adhering to unwanted surfaces (progressive close-up from top to bottom).

Figure Aa2. Stainless steel rods used in field study to anchor NOBBs into GSI. These rods are screwed into the center hole of the 6” NOBBs used for the field site.

Figure Aa3. Preparation for loading field site NOBBs with contaminant emulsion. The disk is laying in an aluminum pan of about equal diameter. The emulsion will be ejected onto the pan for the disk to absorb as uniformly as possible. The adjacent stainless-steel rod will be screwed into the center of the loaded disk and prepared for deployment into the field.

Figure Aa4. Chevron company employee preparing to deploy the NOBBs into the field site via their umbilical cords.

Figure Aa5. View of dock at field site from which NOBBs were deployed

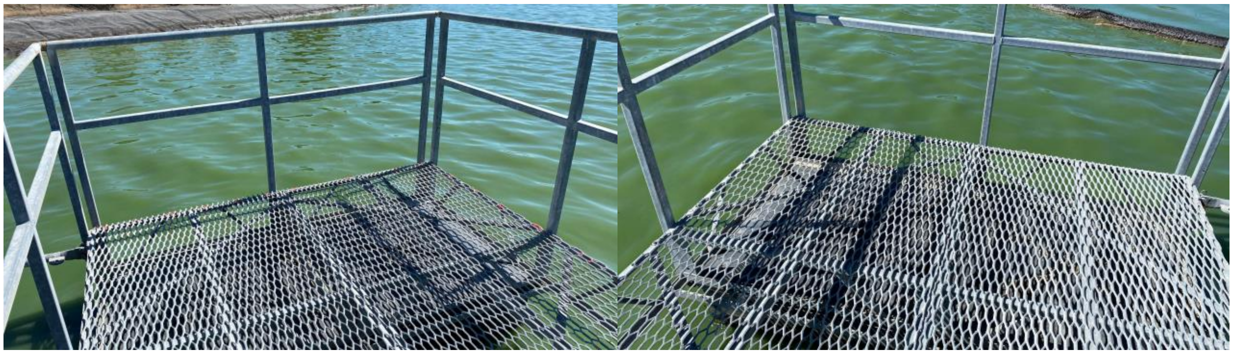

Figure Aa6. End of dock at field site from which NOBBs were deployed

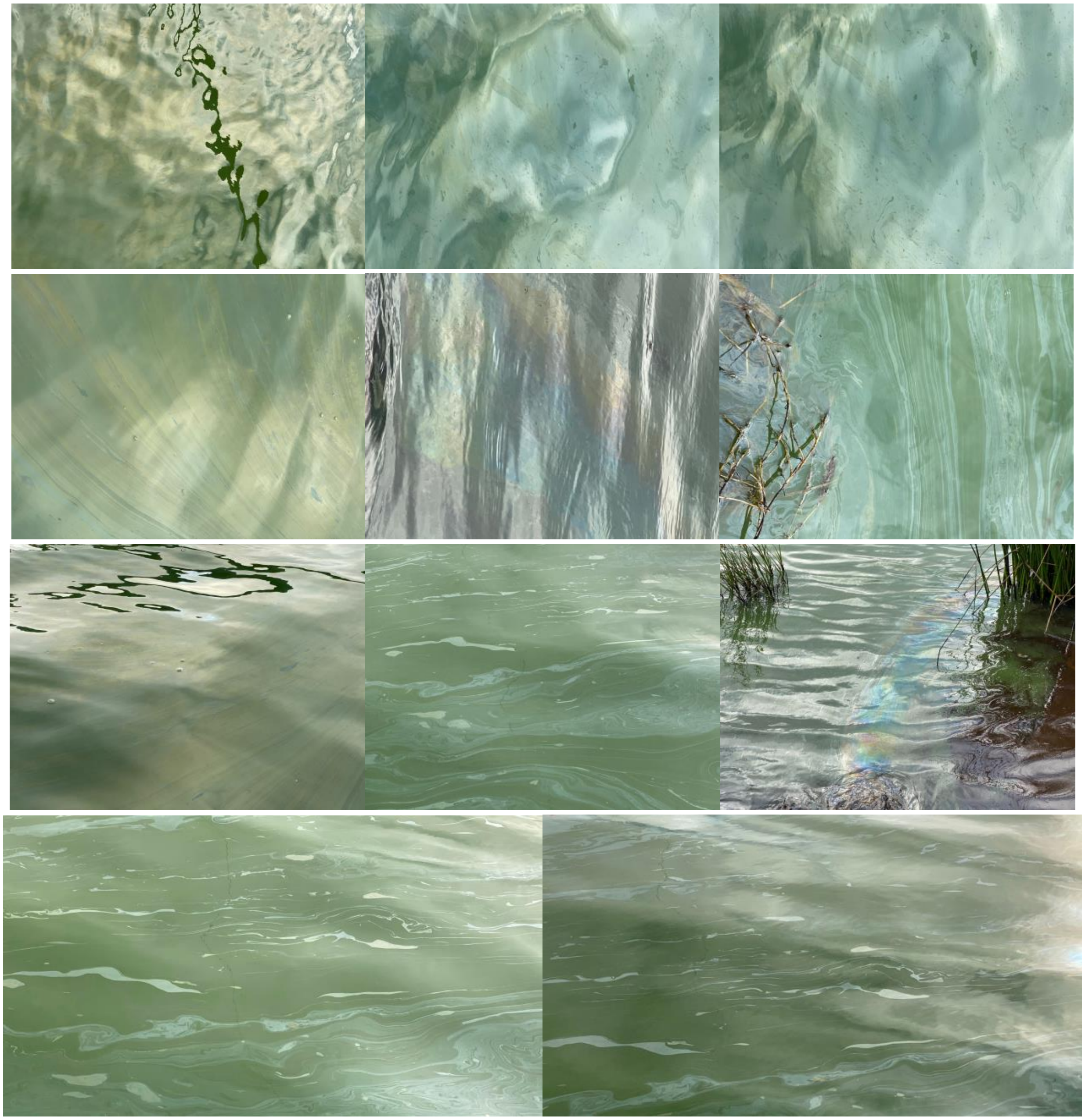

Figure Aa7. Sheening at field site

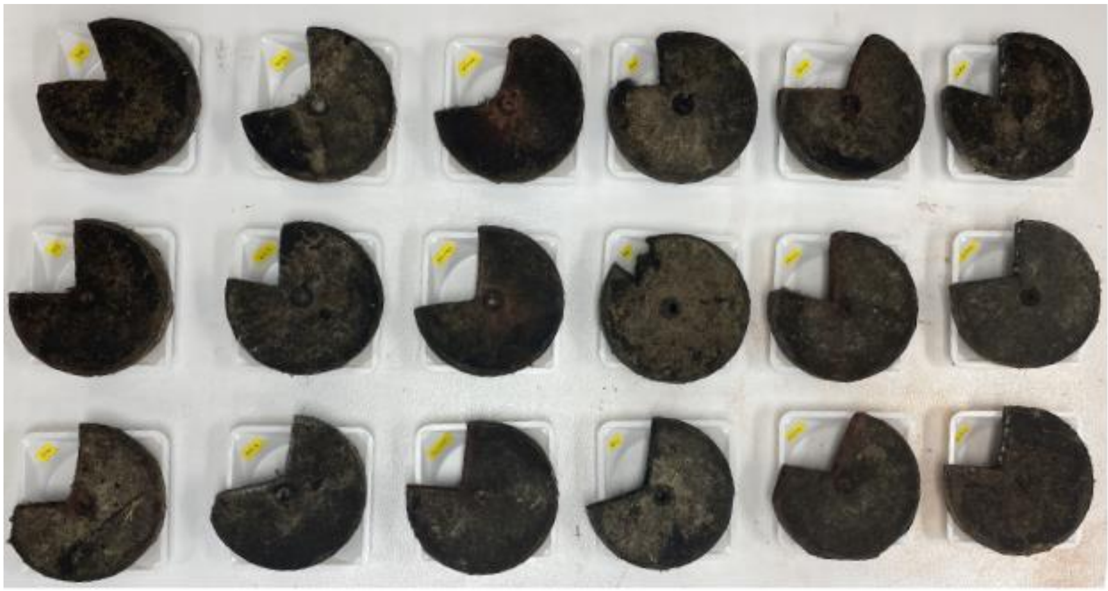

Figure Aa8. NOBBs extracted from the field site, partially dissected, then dried at 30°C. These samples comprise half of the total samples deployed in the field.

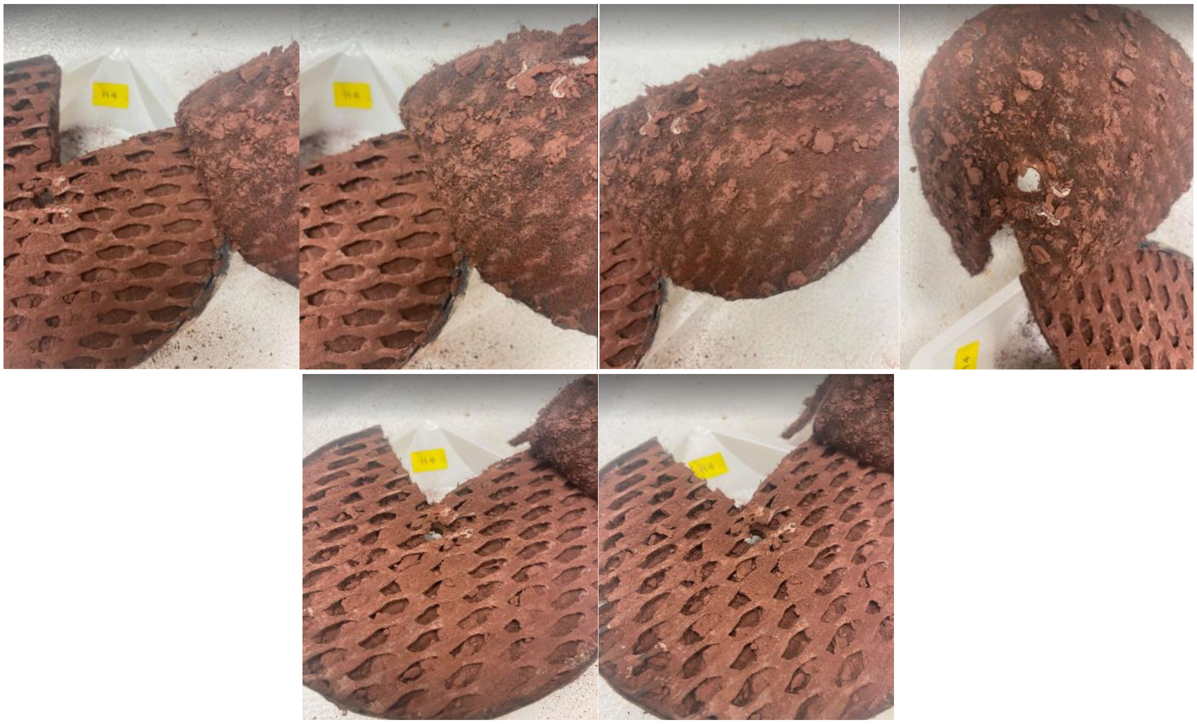

Figure Aa9. Field H4 NOBB partially dissected and dried

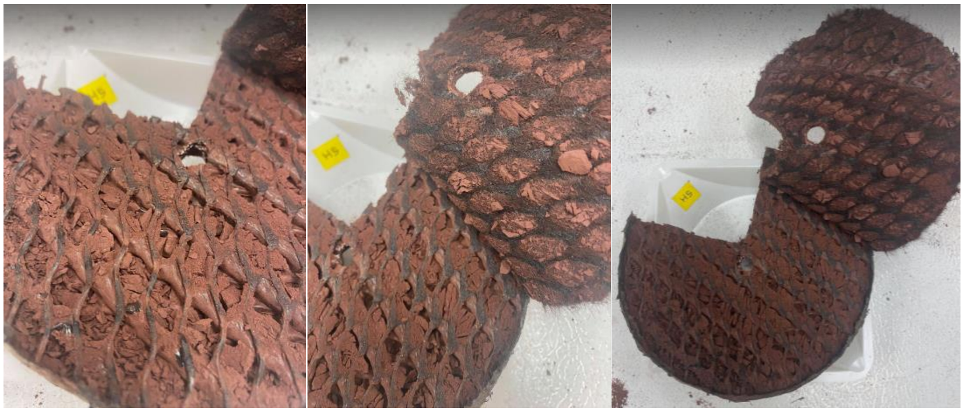

Figure Aa10. Field H5 NOBB partially dissected and dried

Figure Aa11. Field H6 NOBB partially dissected and dried

Figure Aa12. Field GS4 NOBB partially dissected and dried

Figure Aa13. Field GS5 NOBB partially dissected and dried

Figure Aa14. Field GS6 NOBB partially dissected and dried

Figure Aa15. Field GS+H4 NOBB partially dissected and dried

Figure Aa16. Field GS+H5 NOBB partially dissected and dried

Figure Aa17. Field GS+H6 NOBB partially dissected and dried

Figure Aa18. Field GS4 NOBB partially dissected and dried

Figure Aa19. Field GS5 NOBB partially dissected and dried

Figure Aa20. Field GS6 NOBB partially dissected and dried

Figure Aa21. Field ALL4 NOBB partially dissected and dried

Figure Aa22. Field ALL5 NOBB partially dissected and dried

Figure Aa23. Field ALL6 NOBB partially dissected and dried

Appendix Ab — Laboratory Study Images

Figure Ab1. Top view of two separate laboratory hematite-amended NOBB during assembly. A spray bottle was just used to adhere the hematite powder to the geonet and bottom geotextile prior to sealing the top geotextile back onto the geonet.

Figure Ab2. Top view of three separate laboratory greensand-amended NOBB during assembly. A spray bottle was just used to adhere the greensand powder to the geonet and bottom geotextile prior to sealing the top geotextile back onto the geonet.

Figure Ab3. Top view (first and second images) and side view (third image) of laboratory GS+H NOBBs during assembly. A spray bottle was just used to adhere the powdered to the geonet and bottom geotextile prior to sealing the top geotextile back onto the geonet.

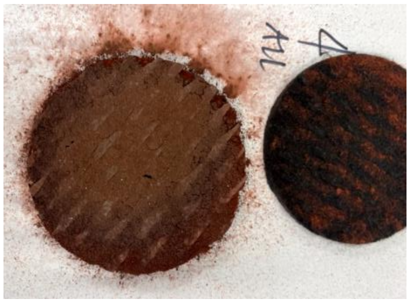

Figure Ab4. Top view of laboratory ALL-amended NOBB during assembly. A spray bottle was just used to adhere the amendments to the geonet and bottom geotextile prior to sealing the top geotextile back onto the geonet.

Figure Ab5. Top view of a laboratory gypsum-amended NOBB during assembly. A spray bottle was just used to adhere the gypsum pellets to the geonet and bottom geotextile prior to sealing the top geotextile back onto the geonet.

Figure Ab6. Top view of a laboratory gypsum-amended NOBB during assembly. Loctite E-120HP Hysol Epoxy Adhesive is placed on top of the geonet and the geotextile is prepared to be securely glued on top to completely reassemble to NOBB.

Figure Ab7. Preparation of NOBBs for laboratory chemostat. The geonets were recently glued with Loctite E-120HP Hysol Epoxy Adhesive and respective geotextiles were placed on top. The glass jars were used to securely adhere the pieces back together and complete the NOBB assembly.

Figure Ab8. Close-up view of assembled lid used in the laboratory chemostat experiments.

Figure Ab9. Custom-built syringe used to suction chemostat headwater in laboratory piping system from the outflow tubing and stimulate a siphon effect when flow slowed or stopped. To siphon the outflow tube was stuck inside the tip of the syringe and the cap was pulled to pull water inward.

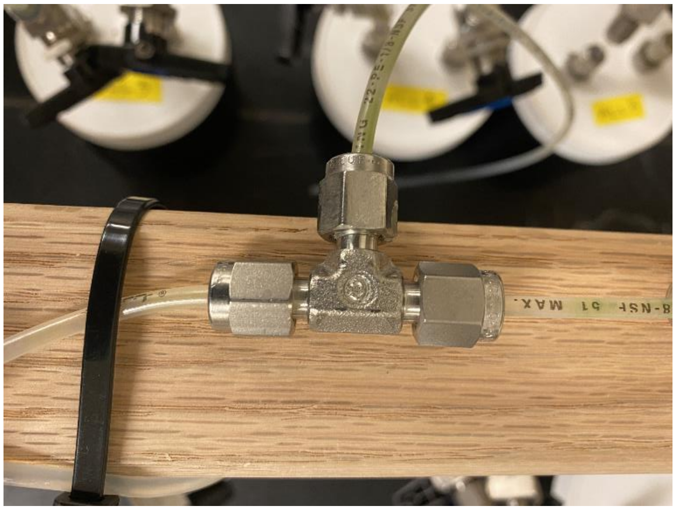

Figure Ab10. Three-headed tubing connection along wooden platform leading from the reservoir water to a chemostat jar. Zip Ties secured the tubing along the top of the wooden platforms. Swagelok 316 Stainless Steel Compression Tees connected the system of inflow tubes into one of the five sections. The plugs were used to connect the tee to the tubing, and compression ferrules were used to secure the seal.

Figure Ab11. Outside image of laboratory chemostat jar ALL1 before covering with black plastic wrapping.

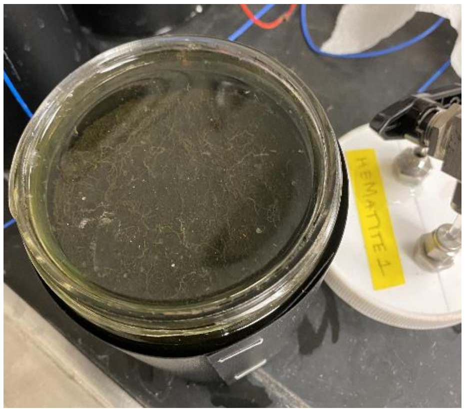

Figure Ab12. Top view of opened H1 laboratory chemostat jar after completing the duration of its experimentation.

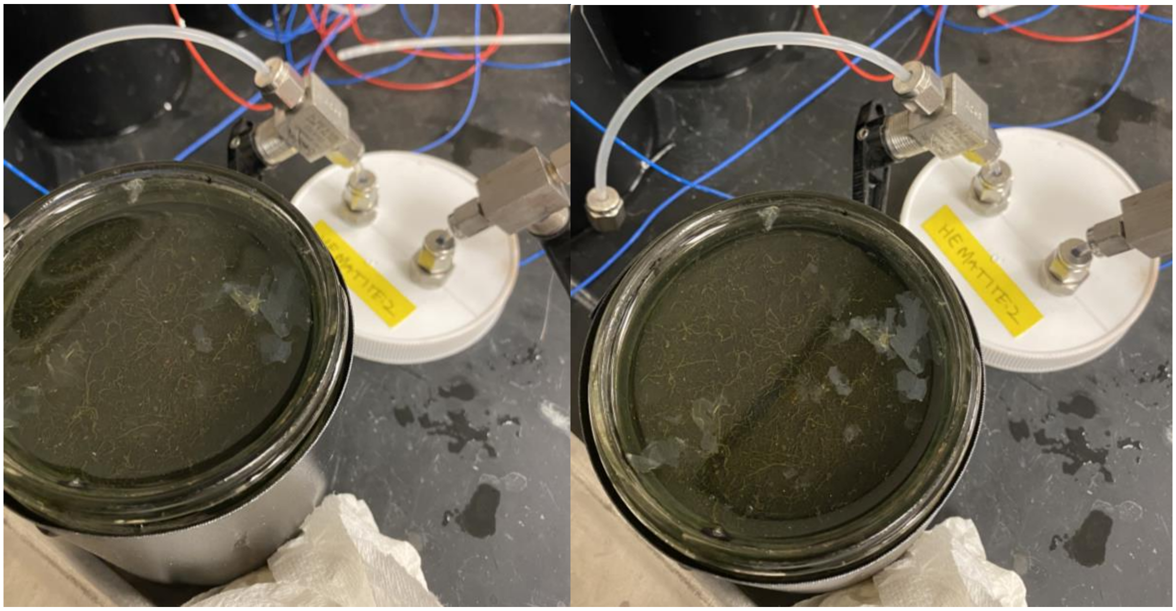

Figure Ab13. Top view of opened H2 laboratory chemostat jar after completing the duration of its experimentation.

Figure Ab14. Top view of opened GS1 laboratory chemostat jar after completing the duration of its experimentation.

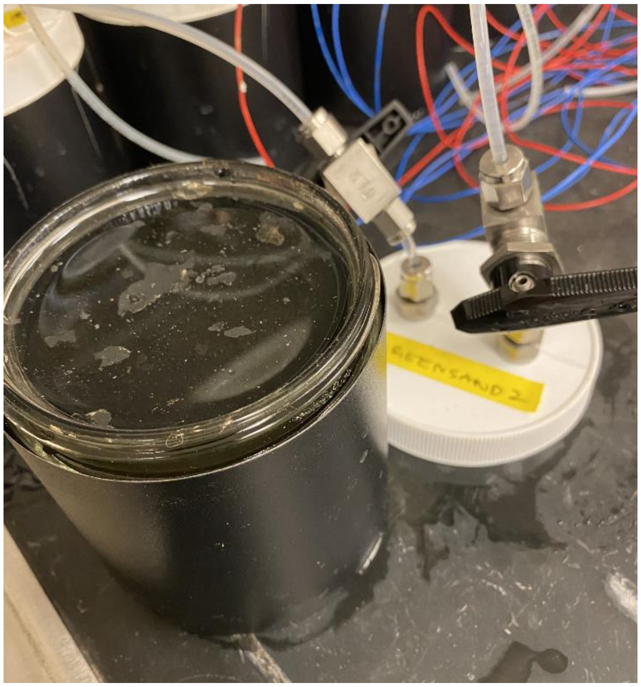

Figure Ab15. Top view of opened GS2 laboratory chemostat jar after completing the duration of its experimentation.

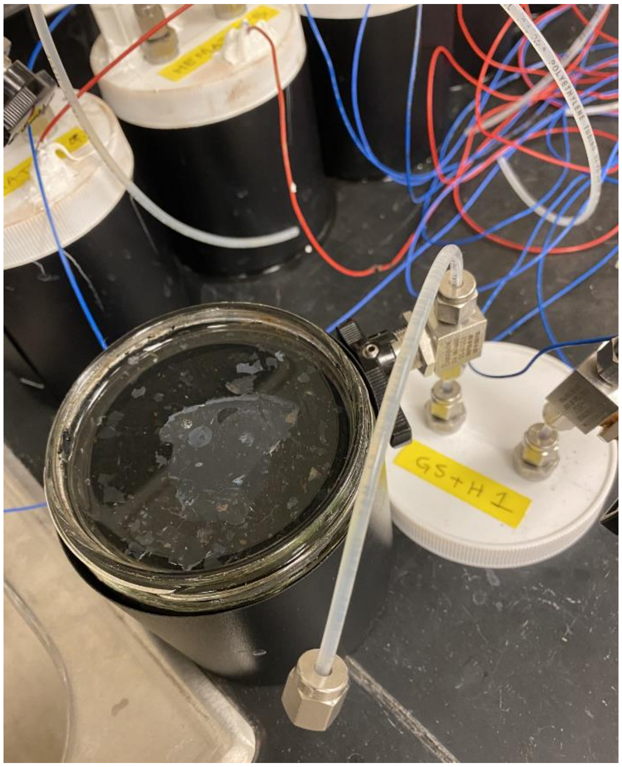

Figure Ab16. Top view of opened GS+H1 laboratory chemostat jar after completing the duration of its experimentation.

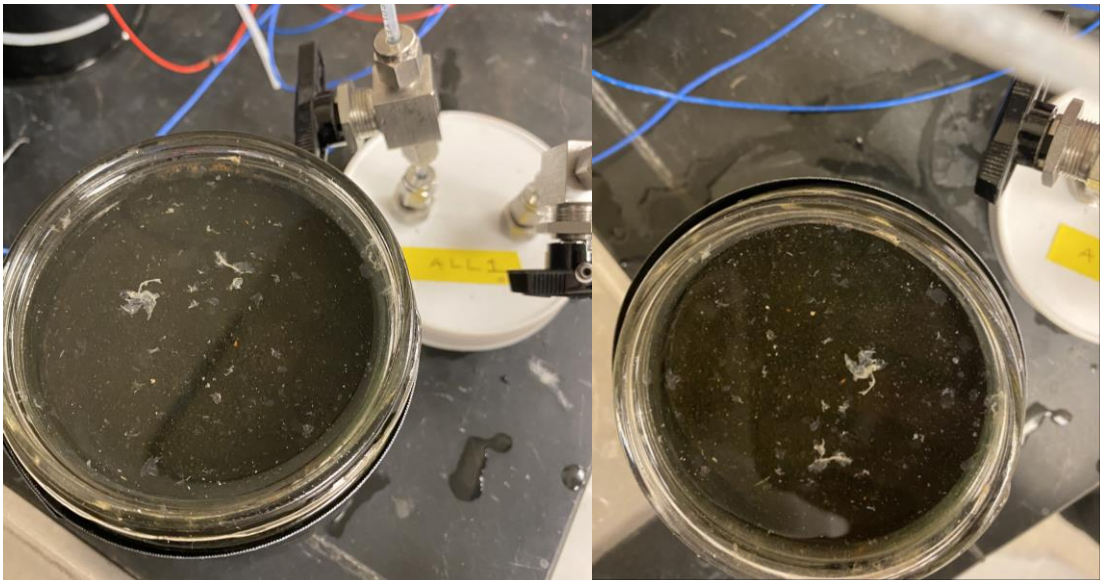

Figure Ab17. Top view of opened ALL1 laboratory chemostat jar after completing the duration of its experimentation.

Figure Ab18. Top view of opened ALL2 laboratory chemostat jar after completing the duration of its experimentation.

Figure Ab19. Top view of contaminated sediment in chemostat jar after completing the duration of its experimentation. Image is taken after opening the lid, draining chemostat headwater, removing NOBB, and exposing contaminated sediment.

Figure Ab20. Top view of NOBBs removed from chemostat jar after completing the duration of its experimentation.

Figure Ab21. NOBBs extracted from the laboratory, partially dissected, then dried at 30ºC. These samples comprise 1/3 of the total samples from the laboratory.

Figure Ab22. Laboratory H1 NOBBs partially dissected and dried.

Figure Ab23. Laboratory H2 NOBBs partially dissected and dried.

Figure Ab24. Laboratory GS1 NOBBs partially dissected and dried.

Figure Ab25. Laboratory GS2 NOBBs partially dissected and dried.

Figure Ab26. Laboratory GS+H1 NOBBs partially dissected and dried

Figure Ab27. Laboratory GS+H2 NOBBs partially dissected and dried

Figure Ab28. Laboratory GYP1 NOBBs partially dissected and dried

Figure Ab29. Laboratory GYP2 NOBBs partially dissected and dried

Figure Ab30. Laboratory ALL1 NOBBs partially dissected and dried

Figure Ab31. Laboratory ALL2 NOBBs partially dissected and dried

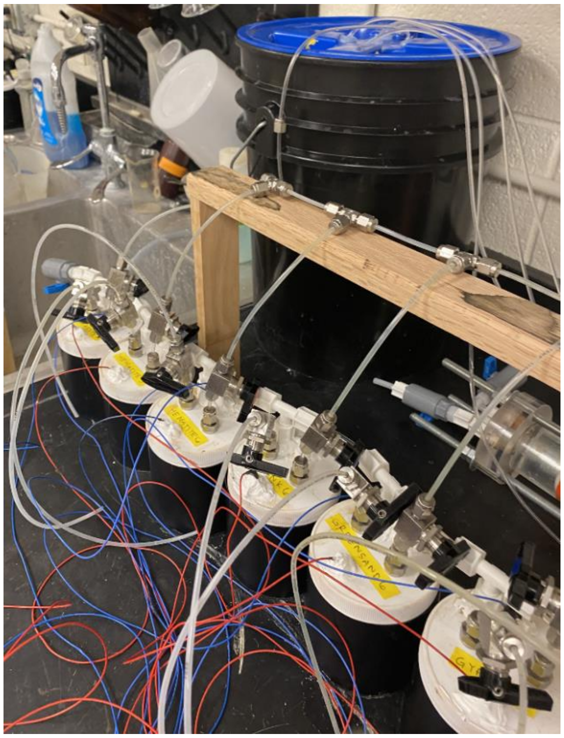

Figure Ab32. Laboratory setup featuring reservoir bucket in black with tubing coming out of top blue lid and chemostat with ORP sensors (top to bottom)

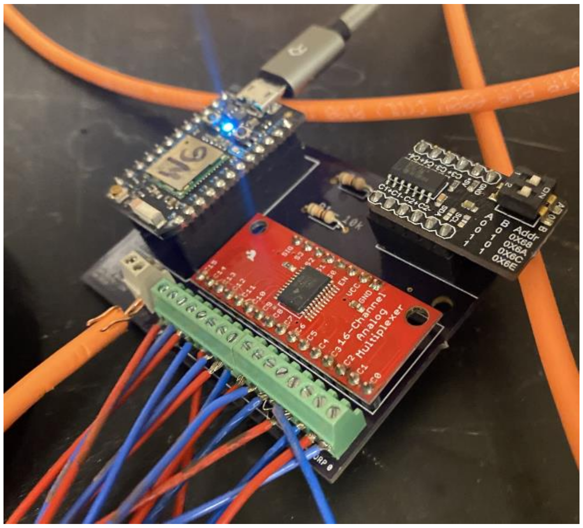

Figure Ab33. IoT system made by Wes Hogan (2020) featuring pH sensor in orange and 16 ORP sensors (left to right)

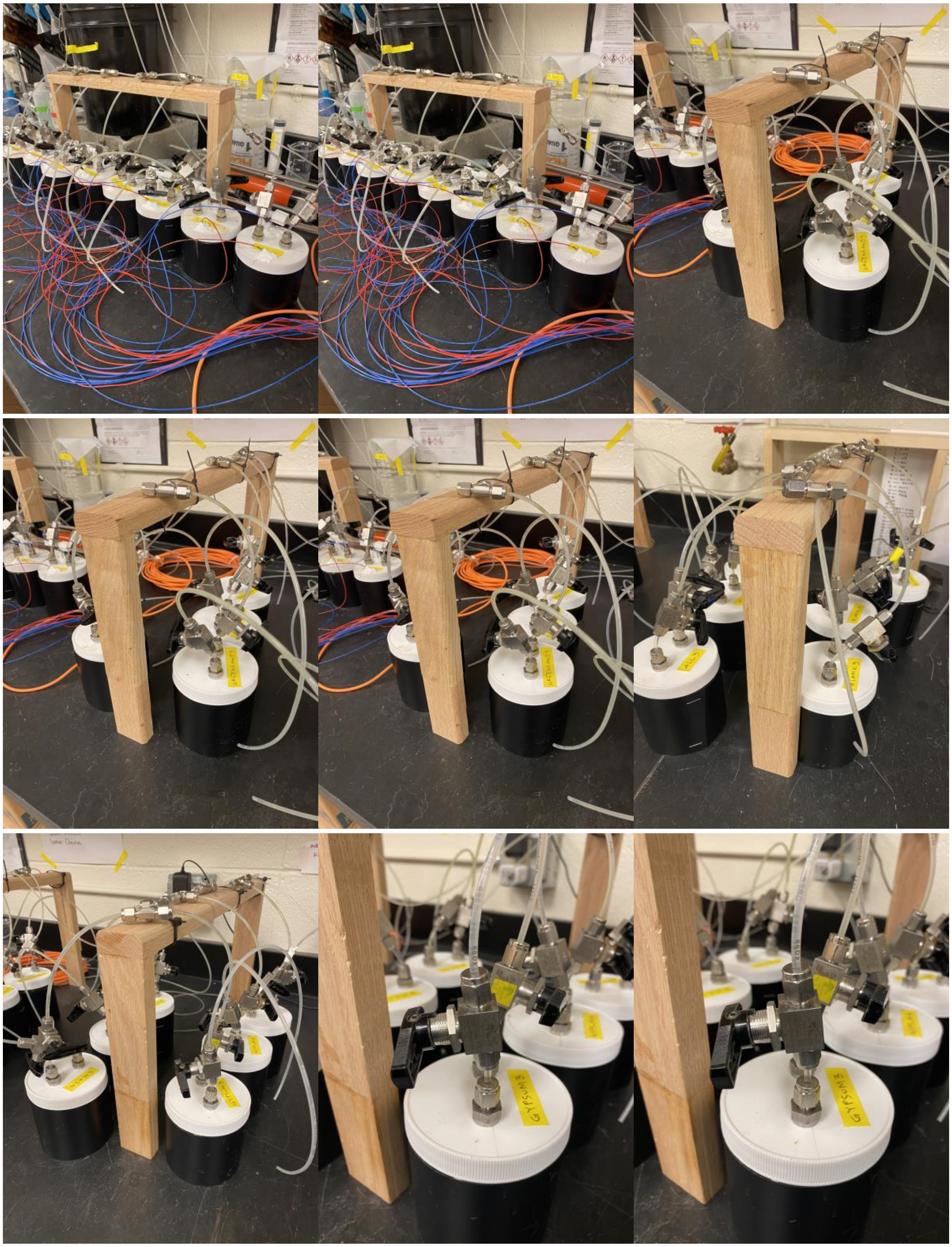

Figure Ab34. New laboratory chemostat setup after reducing from 36 to 24 at end of study.

Figure Ab35. Lid of 5-gallon reservoir bucket with five sealed tube entry points

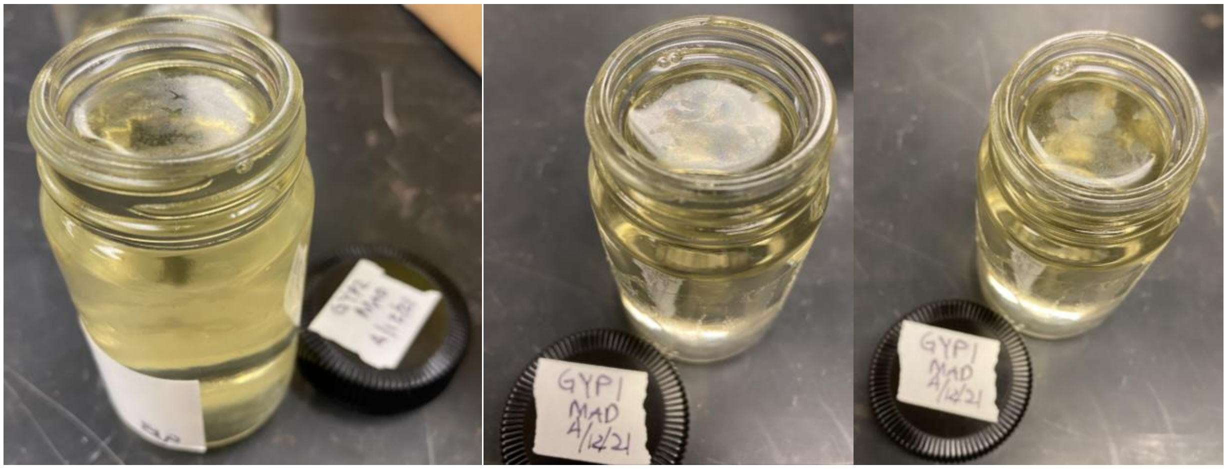

Figure Ab36. Biofilm sheen floating on top of extracted GYP1 (top) and GYP2 (bottom) extracted chemostat headwater samples from April 12, 2021

Figure Ab37. Samples prepared in Falcon test tubes from 12 laboratory NOBB chemostat and 18 field NOBBs for microbial analysis. A portion of the top geotextile was cut from each retrieved NOBBs amendment type and were combined into Falcon test tubes with an appropriate mass of 5 g. Thereafter, the samples were analyzed by Maria Irianno-Renno and Jorge Rico

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APPENDIX B — LABORATORY TEST RESULTS

Table Ba. Summary alkalinity results from February 17th to April 26th, 2021

Amendment Type	**Minimum (mg/L as $CaCO_3$)**	**Median (mg/L as $CaCO_3$)**	**Maximum (mg/L as $CaCO_3$)**	**Average (mg/L as $CaCO_3$)**	**Stand. Dev. (mg/L as $CaCO_3$)**
H	628.2	698.6	798.4	700.5	41.2
GS	598.8	676.1	776.9	689.0	49.7
GS+H	596.9	672.2	798.4	684.1	50.5
GYP	571.4	713.3	986.3	724.2	105.2
ALL	583.2	672.2	790.6	679.3	59.7
B	532.3	688.8	776.9	672.0	65.5
R	495.1	555.8	704.5	564.5	48.3

Table Bb. Complete list of alkalinity results from February 17th to April 26th, 2021

Sample	**Average (mg/L as $CaCO_3$)**	**2/17/21 (mg/L as $CaCO_3$)**	**3/1/21 (mg/L as $CaCO_3$)**	**4/1/21 (mg/L as $CaCO_3$)**	**4/26/21 (mg/L as $CaCO_3$)**
H1	717.8	735.8	710.4	700.6	677.1
H2	669.7	667.3	659.5	628.2	667.3
H3	715.5	745.6	729.9	726.0	698.6
H4	697.5	698.6	702.5	798.4	634.1
H5	700.2	692.8	688.8	773.0	669.3
H6	701.4	757.3	690.8	649.7	710.4
GS1	718.2	771.0	720.2	669.3	616.4
GS2	706.8	776.9	679.1	663.4	671.2
GS3	722.9	771.0	741.7	671.2	694.7
GS4	702.2	731.9	728.0	673.2	626.2
GS5	633.1	651.7	645.8	636.0	598.8
GS6	699.6	712.3	731.9	688.8	665.4
GS+H1	669.3	690.8	683.0	651.7	651.7
GS+H2	672.7	681.0	649.7	667.3	692.8

Sample	Average (mg/L as $CaCO_3$)	2/17/21 (mg/L as $CaCO_3$)	3/1/21 (mg/L as $CaCO_3$)	4/1/21 (mg/L as $CaCO_3$)	4/26/21 (mg/L as $CaCO_3$)
GS+H3	677.6	686.9	624.3	745.6	653.6
GS+H4	711.8	798.4	659.5	792.6	596.9
GS+H5	688.4	749.5	645.8	704.5	653.6
GS+H6	684.9	751.5	677.1	665.4	645.8
GYP1	740.2	810.2	771.0	749.5	630.1
GYP2	815.1	810.2	743.6	986.3	720.2
GYP3	753.9	706.5	759.3	902.2	647.7
GYP4	744.6	775.0	692.8	898.2	612.5
GYP5	652.6	767.1	571.4	655.6	616.4
GYP6	638.5	667.3	661.4	618.4	606.7
ALL1	703.0	743.6	649.7	776.9	641.9
ALL2	700.1	743.6	653.6	776.9	626.2
ALL3	659.0	702.5	657.5	671.2	604.7
ALL4	707.9	718.2	688.8	790.6	634.1
ALL5	625.7	641.9	583.2	673.2	604.7
ALL6	680.0	677.1	681.0	751.5	610.6
B1	694.7	698.6	753.4	679.1	647.7
B2	687.4	647.7	776.9	665.4	659.5
B3	691.3	686.9	696.7	690.8	690.8
B4	669.8	616.4	690.8	667.3	704.5
B5	554.8	581.2	532.3	563.6	542.1
B6	734.3	722.1	751.5	747.6	716.2
R1	589.5	704.5	589.0	518.6	546.0
R2	535.7	596.9	499.0	508.8	538.2
R3	575.3	630.1	616.4	518.6	536.2

Sample	Average (mg/L as $CaCO_3$)	2/17/21 (mg/L as $CaCO_3$)	3/1/21 (mg/L as $CaCO_3$)	4/1/21 (mg/L as $CaCO_3$)	4/26/21 (mg/L as $CaCO_3$)
R4	559.2	616.4	536.2	534.2	549.9
R5	570.9	583.2	589.0	553.8	557.7
R6	556.3	600.8	567.5	561.6	495.1

Table Bc. Summary DIC results from February 17th to April 26th, 2021

Amendment Type	Minimum (mg-C/L)	Median (mg-C/L)	Maximum (mg-C/L)	Average (mg-C/L)	Stand. Dev. (mg-C/L)
H	314	349	399	350	21
GS	299	338	388	345	25
GS+H	298	336	399	343	24
GYP	286	357	493	362	53
ALL	292	336	395	340	30
B	266	344	388	336	33
R	248	278	352	282	24

Table Bd. Complete list of DIC results from February 17th to April 26th, 2021

Sample	Average (mg-C/L)	2/17/21 (mg-C/L)	3/1/21 (mg-C/L)	4/1/21 (mg-C/L)	4/26/21 (mg-C/L)
H1	368	355	350	339	353
H2	334	330	314	334	328
H3	373	365	363	349	363
H4	349	351	399	317	354
H5	346	344	386	335	353
H6	379	345	325	355	351
GS1	386	360	335	308	347
GS2	388	340	332	336	349
GS3	386	371	336	347	360
GS4	366	364	337	313	345

Sample	Average (mg-C/L)	2/17/21 (mg-C/L)	3/1/21 (mg-C/L)	4/1/21 (mg-C/L)	4/26/21 (mg-C/L)
GS5	326	323	318	299	317
GS6	356	366	344	333	350
GS+H1	345	341	326	326	335
GS+H2	341	325	334	346	336
GS+H3	343	312	373	327	339
GS+H4	399	330	396	298	356
GS+H5	375	323	352	327	344
GS+H6	376	339	333	323	342
GYP1	405	386	375	315	370
GYP2	405	372	493	360	408
GYP3	353	380	451	324	377
GYP4	387	346	449	306	372
GYP5	384	286	328	308	326
GYP6	334	331	309	303	319
ALL1	372	325	388	321	352
ALL2	372	327	388	313	350
ALL3	351	329	336	302	330
ALL4	359	344	395	317	354
ALL5	321	292	337	302	313
ALL6	339	341	376	305	340
B1	349	377	340	324	347
B2	324	388	333	330	344
B3	343	348	345	345	346
B4	308	345	334	352	335
B5	291	266	282	271	277
B6	361	376	374	358	367

Sample	Average (mg-C/L)	2/17/21 (mg-C/L)	3/1/21 (mg-C/L)	4/1/21 (mg-C/L)	4/26/21 (mg-C/L)
R1	352	295	259	273	295
R2	298	250	254	269	268
R3	315	308	259	268	288
R4	308	268	267	275	280
R5	292	295	277	279	285
R6	300	284	281	248	278

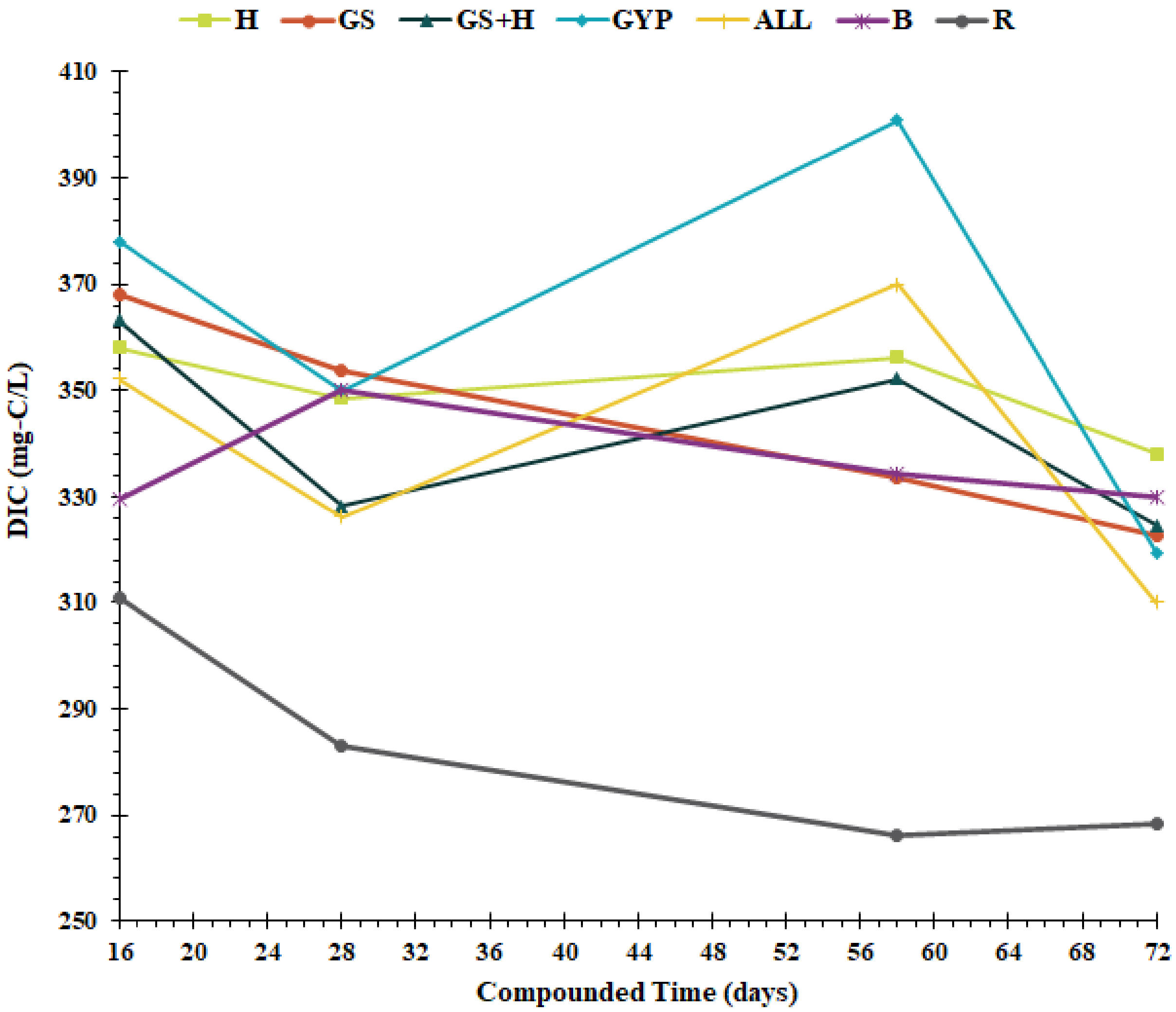

Figure Ba. Average DIC (mg-C/L) results per amendment type vs compounded time (days) during the laboratory chemostat study

Table Be. Summary pH results from February 23, 2021

Amendment Type	Sample	pH	Average pH	[H+]
Hematite	**H1**	7.7	7.65	$2.24*10^{-8}$
	H3	7.6		
Greensand	**GS1**	7.7	7.65	$2.24*10^{-8}$
	GS3	7.6		
Greensand + Hematite	**GS+H1**	7.6	7.55	$2.82*10^{-8}$
	GS+H3	7.5		
Gypsum	**GYP1**	7.8	7.70	$2.00*10^{-8}$
	GYP3	7.6		
All	**ALL1**	7.7	7.55	$2.82*10^{-8}$
	ALL3	7.4		
Blank	**B1**	7.7	7.60	$2.51*10^{-8}$
	B3	7.5		
Reservoir Water	**R1**	7.4	7.45	$3.55*10^{-8}$
	R3	7.5		

Table Bf. Complete list of data required to calculate methane concentration in extracted headspace water samples on February 1, 2021 and February 17, 2021 using GSMS and a calibration number of 32324801

Sample	February 1, 2021				February 17, 2021			
	Water Mass (g)	Peak Area	µmoles of CH_4	µmoles/ mL	Water Mass (g)	Peak Area	µmoles of CH_4	µmoles/ mL
H1	5.13	$1.25*10^{7}$	0.38570	0.07519	5.54	$9.23*10^{6}$	0.28553	0.05154
H2	4.97	$1.15*10^{7}$	0.35706	0.07184	5.58	$1.50*10^{6}$	0.04640	0.00831
H3	5.05	$2.03*10^{7}$	0.62853	0.12446	4.88	$1.75*10^{7}$	0.54008	0.11067
H4	5.09	$6.67*10^{5}$	0.02062	0.00405	5.26	$4.50*10^{6}$	0.13935	0.02649
H5	4.95	$3.79*10^{6}$	0.11720	0.02368	5.22	$2.95*10^{6}$	0.09126	0.01748

Sample	February 1, 2021				February 17, 2021			
	Water Mass (g)	Peak Area	µmoles of CH_4	µmoles/ mL	Water Mass (g)	Peak Area	µmoles of CH_4	µmoles/ mL
H6	5.06	$1.42*10^{7}$	0.44011	0.08698	5.05	$1.45*10^{7}$	0.44822	0.08876
GS1	4.94	$1.69*10^{7}$	0.52397	0.10607	4.87	$1.09*10^{7}$	0.33731	0.06926
GS2	4.93	$1.37*10^{7}$	0.42513	0.08623	5.46	$1.35*10^{7}$	0.41900	0.07674
GS3	4.9	$2.47*10^{7}$	0.76406	0.15593	5.03	$1.82*10^{7}$	0.56248	0.11182
GS4	5.35	$1.56*10^{7}$	0.48170	0.09004	5.53	$4.06*10^{6}$	0.12549	0.02269
GS5	5.39	$5.26*10^{5}$	0.01628	0.00302	4.97	N/A	0.00000	0.00000
GS6	5.22	$3.00*10^{7}$	0.92689	0.17756	5.27	$2.84*10^{7}$	0.87814	0.16663
GS+H1	5.04	$8.42*10^{6}$	0.26042	0.05167	5.04	$9.20*10^{6}$	0.28454	0.05646
GS+H2	5.11	$6.02*10^{6}$	0.18611	0.03642	5.26	$1.42*10^{6}$	0.04386	0.00834
GS+H3	4.97	$6.06*10^{6}$	0.18759	0.03774	5.00	$2.24*10^{5}$	0.00694	0.00139
GS+H4	5.34	$5.73*10^{6}$	0.17727	0.03320	5.12	$3.14*10^{6}$	0.09707	0.01896
GS+H5	4.96	$5.20*10^{5}$	0.01608	0.00324	4.98	$5.22*10^{5}$	0.01616	0.00325
GS+H6	5.11	$3.41*10^{7}$	1.05596	0.20664	5.20	$2.40*10^{7}$	0.74099	0.14250
GYP1	4.96	$3.19*10^{5}$	0.00988	0.00199	5.34	$1.73*10^{6}$	0.05341	0.01000
GYP2	5.3	$6.93*10^{5}$	0.02144	0.00405	5.40	$2.30*10^{6}$	0.07100	0.01315
GYP3	5.1	$1.08*10^{6}$	0.03339	0.00655	5.19	$2.45*10^{5}$	0.00757	0.00146
GYP4	5.19	$1.92*10^{6}$	0.05950	0.01147	4.99	$5.86*10^{5}$	0.01814	0.00363
GYP5	5.28	$3.90*10^{5}$	0.01206	0.00228	4.92	$3.16*10^{5}$	0.00979	0.00199
GYP6	5.05	$2.19*10^{6}$	0.06784	0.01343	5.03	$3.03*10^{5}$	0.00939	0.00187
ALL1	5.25	$5.64*10^{5}$	0.01743	0.00332	5.15	$4.03*10^{5}$	0.01247	0.00242
ALL2	4.9	$3.63*10^{5}$	0.01122	0.00229	5.19	$2.87*10^{5}$	0.00887	0.00171
ALL3	5.06	N/A	0.00000	0.00000	5.11	$3.07*10^{5}$	0.00950	0.00186
ALL4	5.09	$2.65*10^{5}$	0.00821	0.00161	5.08	$4.03*10^{5}$	0.01247	0.00246
ALL5	5.38	N/A	0.00000	0.00000	4.94	N/A	0.00000	0.00000
ALL6	5.2	N/A	0.00000	0.00000	4.90	$2.56*10^{5}$	0.00792	0.00162

Sample	February 1, 2021				February 17, 2021			
	Water Mass (g)	Peak Area	µmoles of CH_4	µmoles/ mL	Water Mass (g)	Peak Area	µmoles of CH_4	µmoles/ mL
B1	5	$2.01*10^5$	0.00623	0.00125	4.90	$3.87*10^5$	0.01198	0.00244
B2	5.29	$7.59*10^5$	0.02348	0.00444	5.28	$6.01*10^5$	0.01860	0.00352
B3	5.11	$2.88*10^5$	0.00892	0.00175	4.94	$6.96*10^5$	0.02152	0.00436
B4	5.21	N/A	0.00000	0.00000	5.02	$4.29*10^5$	0.01327	0.00264
B5	5.28	N/A	0.00000	0.00000	5.29	N/A	0.00000	0.00000
B6	5.03	$6.38*10^5$	0.01974	0.00392	5.19	$1.45*10^6$	0.04482	0.00864
R1	5.24	N/A	0.00000	0.00000	4.83	$2.33*10^5$	0.00722	0.00150
R2	N/A	N/A	N/A	N/A	5.45	$2.62*10^5$	0.00810	0.00149

APPENDIX C — MICROBIAL 16S ANALYSIS

The marker for 16sA indicates the number of archaeal (A) transcripts found on the NOBB geocomposite. Except for L-H and F-B whose microbial analysis revealed no archaeal transcripts, typical archaeal levels were on the order of 10^4 to 10^7 16s transcripts/g samples (Figure Ca). In comparison, the typical range of bacterial 16s transcripts (16sB) were on the order of 10^5 to 10^{14}. 16sB results were not found for L-H, F-H, F-GYP, and F-ALL (Figure Cb). A comparison of 16sA and 16sB transcripts for each amendment type is shown in Figure Cc.

DSRA is a marker for sulfate reducers. The DSRA maker was found in the order of 10^4 to 10^5 for all samples except the laboratory hematite (L-H) and field blank (F-B). MCRA is for methyl reductase, an enzyme that is used to produce methane. The MCRA marker was only found in the field blank (F-B) at an order of 10^5. The limited MCRA results are expected due to the brief deployment duration. No makers for aerobic alkane degradation (ALKB) were detected in the field and laboratory samples. These results are shown in Table Ca and illustrated in Figures Cd-Ce.

Table Ca. qPCR and 16s results for field and laboratory studies

Treatment	**16sA Transcript Pts/g**	**16sB Transcript Pts/g**	**DSRA Transcript Pts/g**	**MCRA Transcript Pts/g**	**ALKB Pts/g**
L-H	0	0	0	0	0
F-H	$9.15*10^{\backslash 6}$	0	$7.56*10^{4}$	0	0
L-GS	$7.06*10^{7}$	$2.07*10^{13}$	$2.66*10^{5}$	0	0
F-GS	$7.90*10^{5}$	$1.93*10^{9}$	$4.60*10^{3}$	0	0
L-GS+H	$6.25*10^{7}$	$6.80*10^{13}$	$2.02*10^{5}$	0	0
F-GS+H	$3.81*10^{6}$	$6.38*10^{5}$	$7.72*10^{4}$	0	0
L-GYP	$2.18*10^{7}$	$6.67*10^{10}$	$2.09*10^{5}$	0	0
F-GYP	$1.29*10^{6}$	0	0	0	0
L-ALL	$8.83*10^{7}$	$3.17*10^{10}$	$7.79*10^{5}$	0	0
F-ALL	$3.07*10^{4}$	0	0	0	0
L-B	$4.10*10^{7}$	$1.46*10^{14}$	$5.45*10^{5}$	0	0

Treatment	16sA Transcript Pts/g	16sB Transcript Pts/g	DSRA Transcript Pts/g	MCRA Transcript Pts/g	ALKB Pts/g
F-B	0	$6.66*10^9$	0	$3.46*10^5$	0

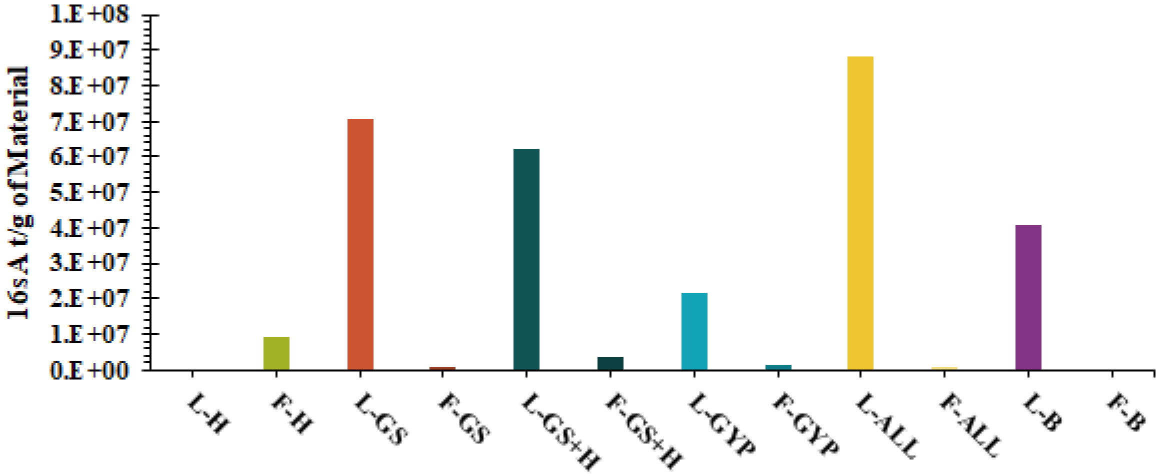

Figure Ca. 16sA t/g of material for laboratory (L) and field (F) site NOBB samples

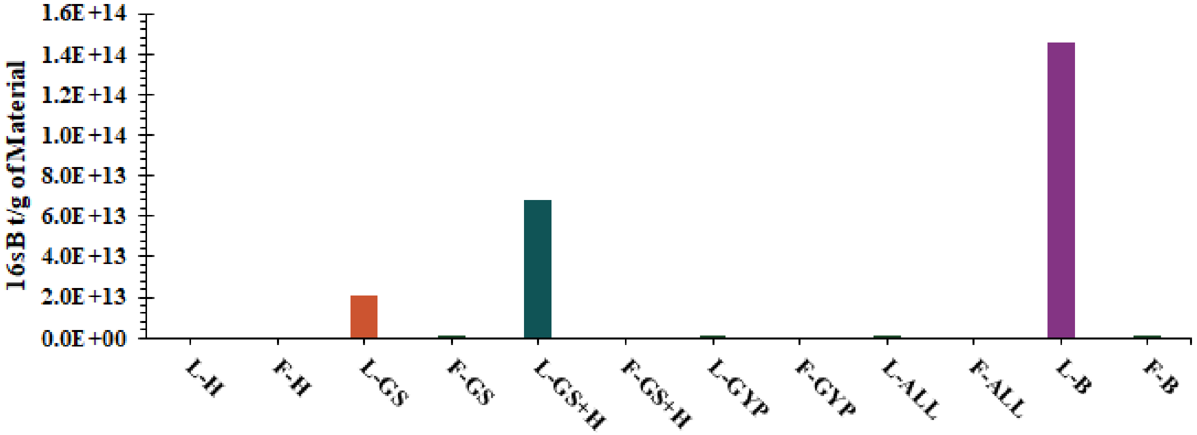

Figure Cb. 16sB t/g of material for laboratory (L) and field (F) site NOBB samples

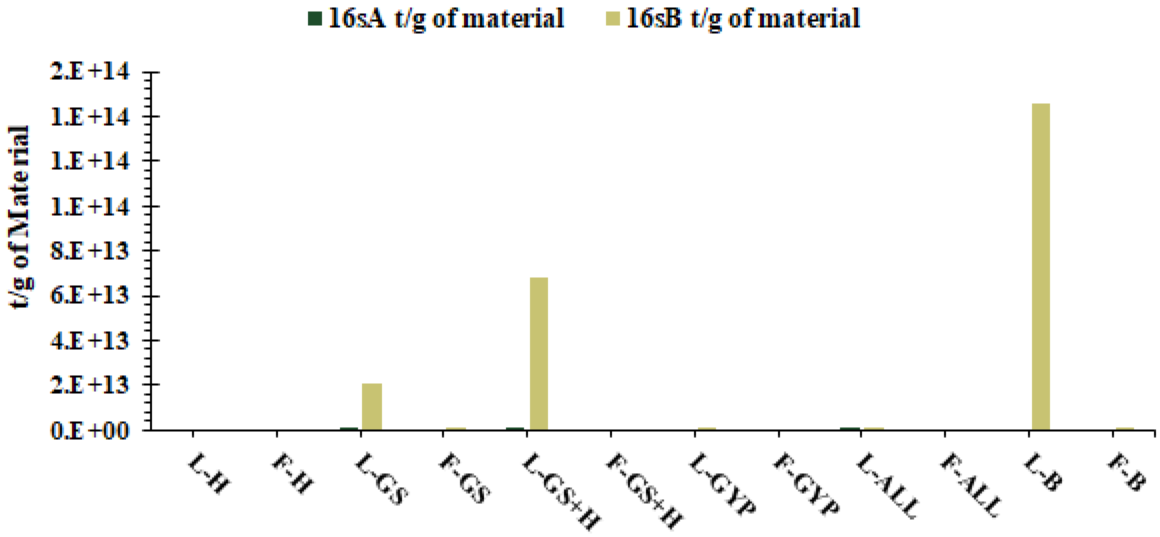

Figure Cc. Comparison of 16sA and 16sB t/g of material for laboratory (L) and field (F) site NOBB samples

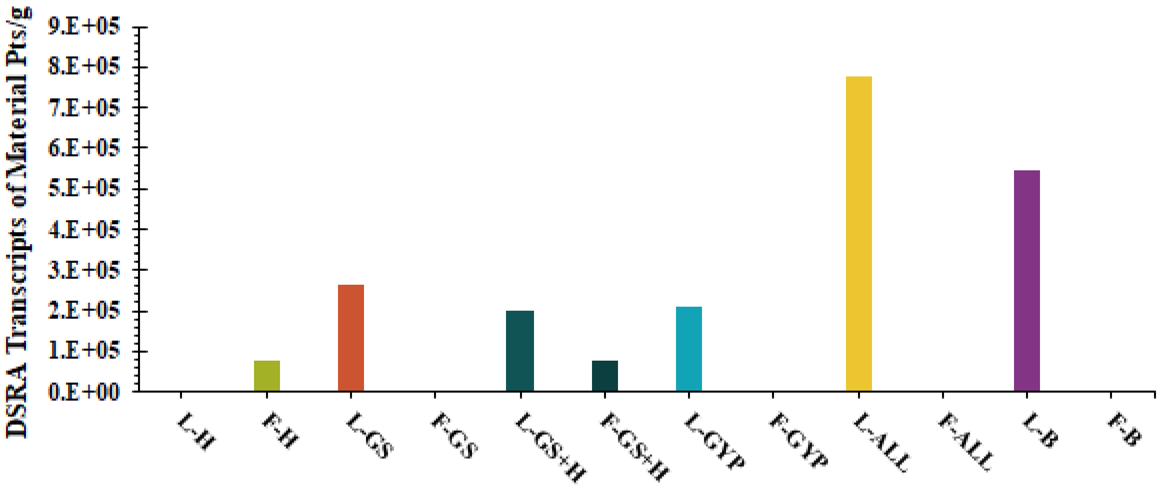

Figure Cd. DSRA transcripts of material pts/g for laboratory (L) and field (F) site NOBB samples

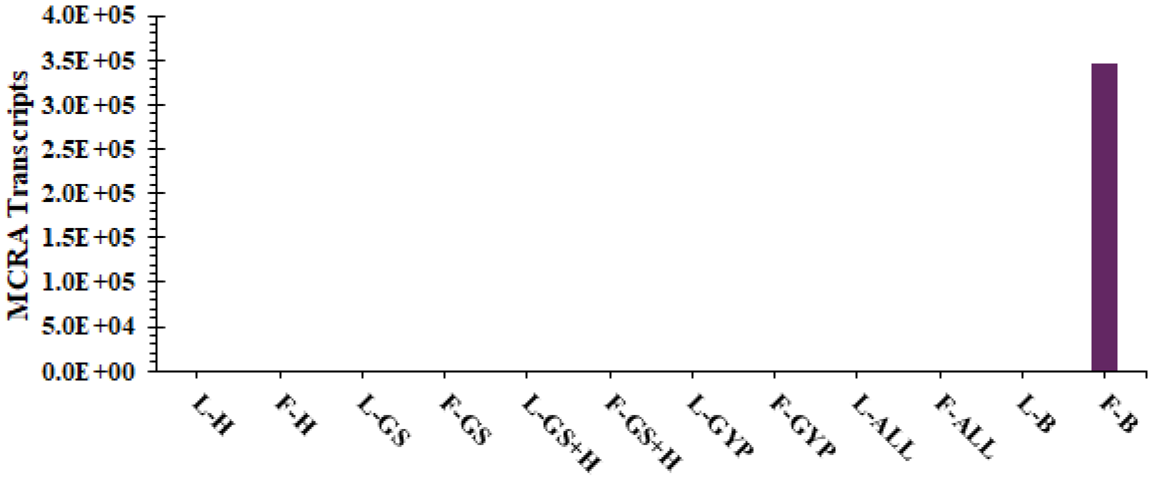

Figure Ce. MCRA transcripts for laboratory (L) and field (F) site NOBB samples

APPENDIX D — MICROBIAL TAXONOMY ANALYSIS

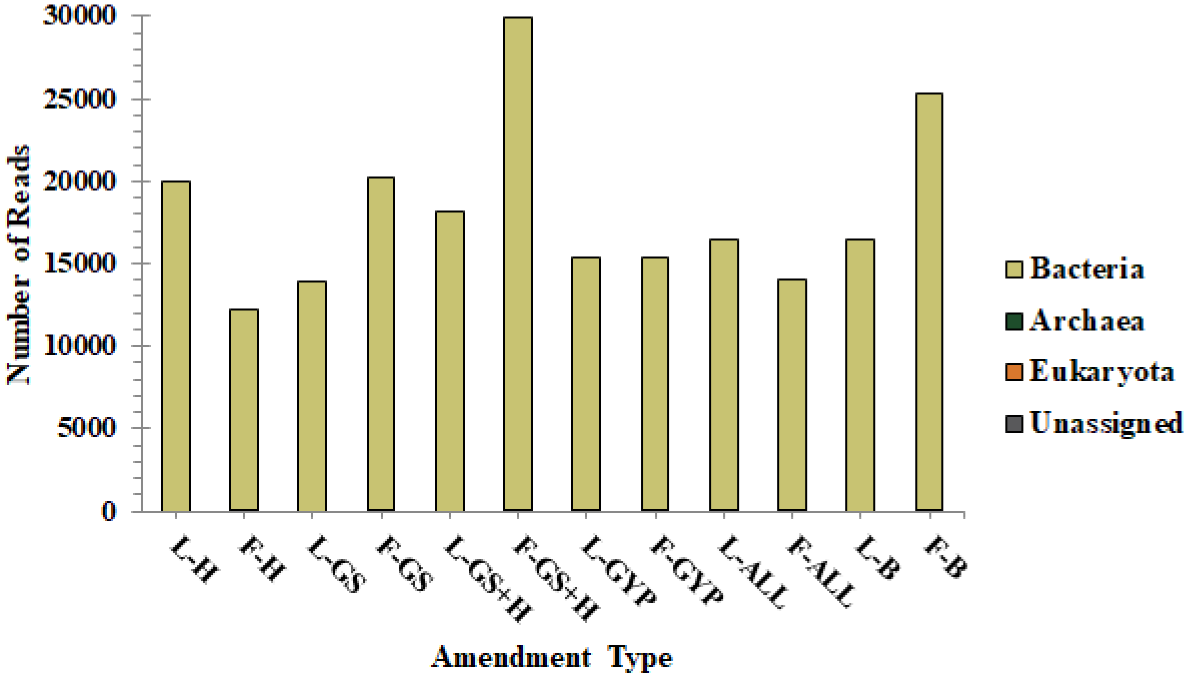

Figure Da. Taxonomy of microbial communities at Level 1 (Kingdom)

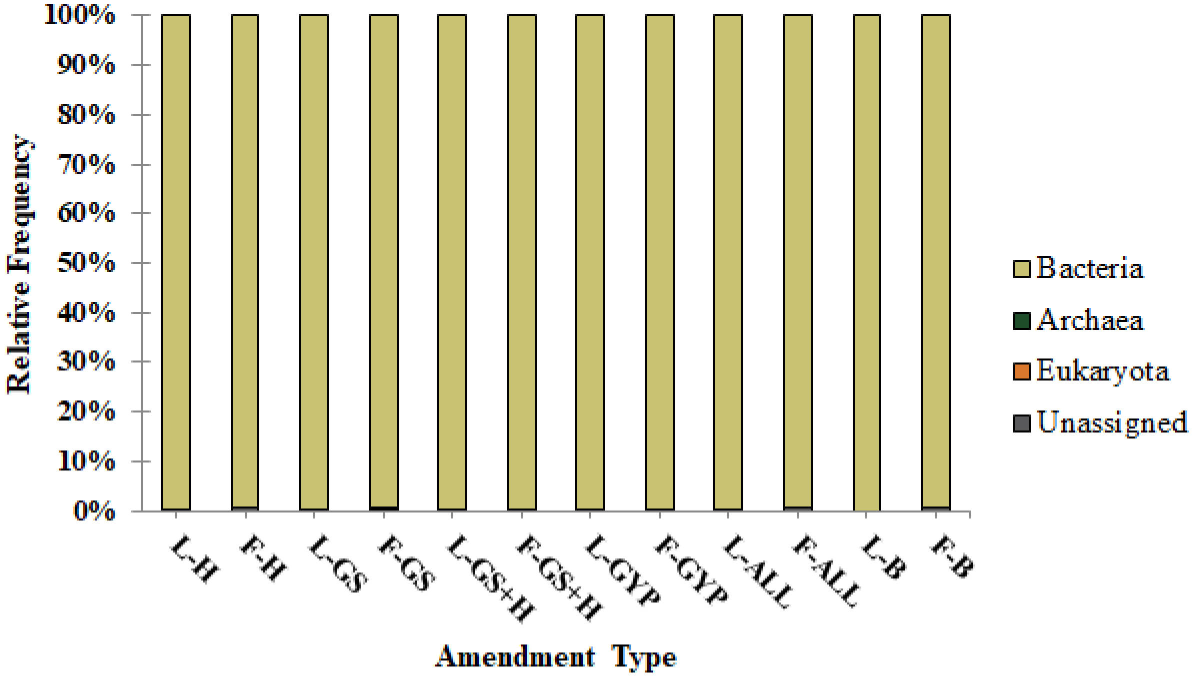

Figure Db. Relative frequency of taxonomy of microbial communities at Level 1 (Kingdom)

Table Da. Microbial analysis results at Level 1 (Kingdom) as microbial number of reads (Count) and relative frequency (RF)

Amendment Type	Bacteria		Archaea		Eukaryota		Unassigned		Total Count
	Count	**RF**	**Count**	**RF**	**Count**	**RF**	**Count**	**RF**	
L-H	19986	99.93%	15	0.07%	0	0.00%	0	0.00%	20001
L-GS	13864	99.92%	11	0.08%	0	0.00%	0	0.00%	13875
L-GS+H	18122	99.92%	12	0.07%	0	0.00%	2	0.01%	18136
L-GYP	15331	99.73%	41	0.27%	0	0.00%	0	0.00%	15372
L-ALL	16328	99.54%	73	0.45%	0	0.00%	3	0.02%	16404
L-B	16440	99.95%	8	0.05%	0	0.00%	0	0.00%	16448
F-H	12183	99.36%	0	0.00%	0	0.00%	79	0.64%	12262
F-GS	20120	99.51%	7	0.03%	0	0.00%	92	0.46%	20219
F-GS+H	29828	99.66%	3	0.01%	0	0.00%	98	0.33%	29929
F-GYP	15325	99.73%	3	0.02%	0	0.00%	39	0.25%	15367
F-ALL	13940	99.49%	6	0.04%	0	0.00%	66	0.47%	14012
F-B	25180	99.51%	0	0.00%	5	0.02%	119	0.47%	25304

APPENDIX E — MICROBIAL FEATURES

Table Ea. Microbial features

#	Feature	Description
1	Methanobacterium (g) [1]	Methane-producing bacteria (methanogen)
2	Smithella (g) [2,3]	Anaerobic, synanthropic, propionate-oxidizing, methanogenic alkane-degrading bacteria; fermenting bacteria
3	Leptolinea (g) [4]	Fermenting Bacteria
4	Desulfoprunum (g) [5]	Anaerobic, sulfate-reducing bacteria
5	Cryptanaerobacter (g) [6]	Anaerobic, methanogenic bacteria
6	Desulfurivibrio (g) [7,8]	Sulfate-reducing bacteria
7	Bacterium Enrichment (s)	
8	Planktothrix agardhii (s) [9]	Planktonic Cyanobacteria
9	Methanocella (g) [10]	Anaerobic Methanogen
10	KCM-B-112 (g) [11]	Proteobacteria
11	Desulfosarcinaceae (f) [12]	Sulfate-reducing prokaryote
12	Methylophilaceae (g) [13]	Aerobic respiratory metabolism, oxidize methanol
13	Anaerolineaceae (f) [14]	Fermenting Bacteria
14	Methanosaeta (g) [15]	Anaerobic Methanogen

#	Feature	Description
15	KCM-B-112 (g) [11]	Proteobacteria
16	Anaerolineaceae (f) [14]	Fermenting Bacteria
17	Leptolinea (g) [16]	Anaerobic Fermenting Bacteria
18	Methanosaeta (g) [15]	Methanogenic Archaea
19	Incertae Sedis (g) [17]	
20	Candidatus Woesebacteria (g)	
21	Acaryochloris MBIC11017 (g) [18]	Cyanobacterium
22	Chlorobium (g) [19,20]	Aerobic, Iron-Oxidizing Bacteria
23	Sulfuritalea (g) [21]	Aerobic, Nitrate-Reducing Bacteria
24	Desulfococcaceae (f) [22]	Anaerobic Chemoorganoheterotrophic
25	Methanomicrobiales (f)	Methanogenic Archaea
27	Anaerolineaceae (f) [14]	Fermenting Bacteria
28	Incertae Sedis (g) [20]	
30	Lutispora (g) [23]	Anaerobic Methanogenic Bacteria
31	Run-SP154 (g) [24]	Proteobacteria
32	Desulfocapsaceae (f) [25]	Anaerobe with respiratory, fermentative, or sulfur metabolism

#	Feature	Description
Other	Other	

Table Eb. Relative abundance of microbial taxonomy features for each NOBB sample type

#	L-H	F-H	L-GS	F-GS	L-GS+H	F-GS+H	L-GYP	F-GYP	L-ALL	F-ALL	L-B	F-B
1	0.0209	0	0.0226	0	0	0	0	0	0	0.0302	0	0
2	0	0	0	0	0	0.1163	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0.0981	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0.1279	0	0	0	0	0	0	0	0	0
7	5.8121	0	19.6087	0	0.2739	0.0986	53.9816	0	17.4834	0	0.9545	0
8	0.1257	6.5367	0.0301	14.0436	0.1107	12.4890	0	2.4684	0	1.7286	0.4676	12.7453
9	0	0	0	0	0	0	0	0	0	0	0	0
10	0.5707	2.6479	0.5192	3.1341	3.1465	3.5300	0.1919	8.7795	0.4390	6.7784	11.3645	5.4563
11	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0
15	0.2985	0.9525	0	2.0599	0.7575	3.1848	0	10.5778	0	7.1332	4.2601	4.7826
16	0	0	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	0

#	L-H	F-H	L-GS	F-GS	L-GS+H	F-GS+H	L-GYP	F-GYP	L-ALL	F-ALL	L-B	F-B
19	2.8746	6.2833	4.3115	2.7117	2.9425	1.4550	1.302083	1.2991	3.6671	1.2455	3.0109	1.2762
20	0	0	0	0	0	0	0	0	0	0	0	0
21	0.3718	0.0524	0	0.2607	0	7.5885	0	4.1846	0	4.7856	0	4.2387
22	12.0955	0	5.7938	0	1.2236	0	1.3295	0	0.9813	0	3.9462	0
23	0	0	0	0	0	0	0	0	0	0	0	0
24	2.7123	3.8801	1.6178	2.3571	1.7131	1.0393	0.3358	0.8410	1.7238	1.1851	1.9411	0.8661
25	0	0	0	0	0	0	0	0	0	0	0	0
27	0	0	0	0	0	0	0	0	0	0	0	0
28	1.8169	2.5605	3.3333	1.2933	2.4240	0.5566	1.1856	0.6564	2.8214	0.6944	2.7995	0.5147
30	0	0	0	0	0	0	0	0	0	0	0	0
31	0.1152	0.7166	1.3318	3.3271	1.8821	1.2929	0.5277	1.8051	1.1363	2.2418	0.7880	1.4352
32	0.6755	3.3732	0.6321	2.8942	0.5943	1.4092	0.0000	1.9077	0.5100	2.9363	0.4228	1.4645
Other	72.5102	72.9966	62.6712	67.9182	84.9318	67.2397	41.1458	67.4803	71.2377	71.1428	70.0448	67.2204

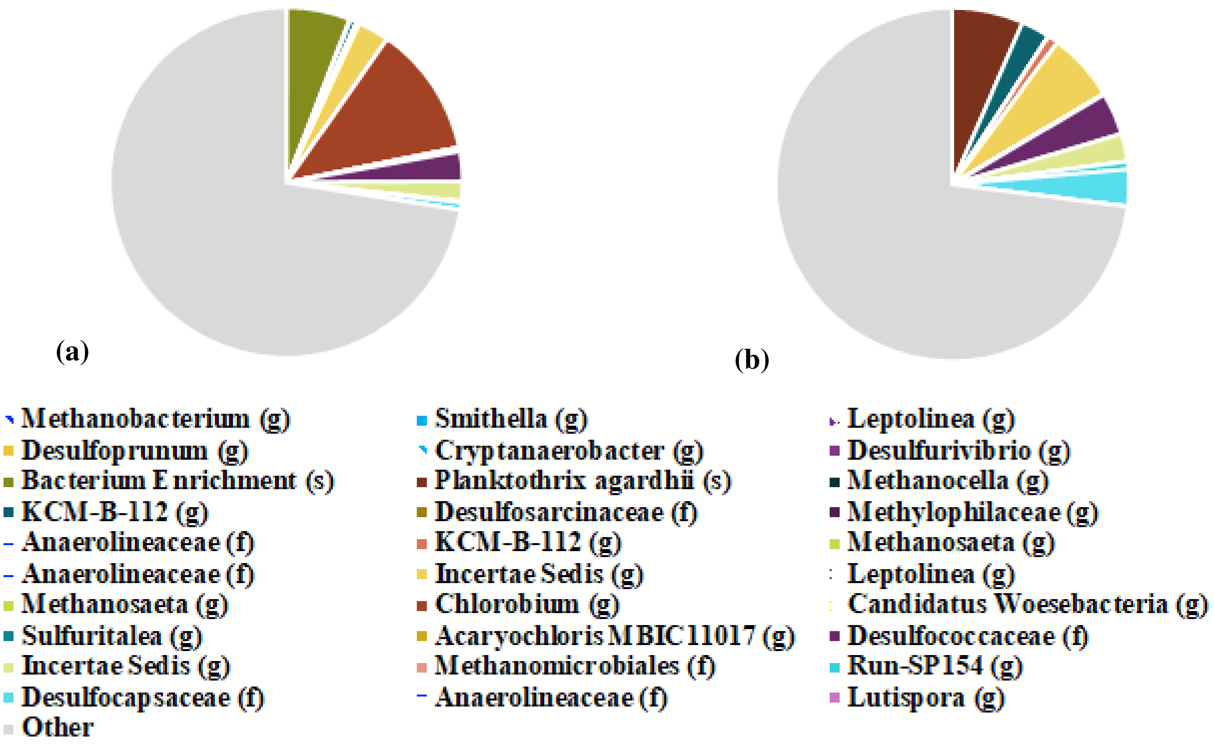

Figure Ea. Pie chart of relative abundance of taxonomy features in (a) L-H and (b) F-H

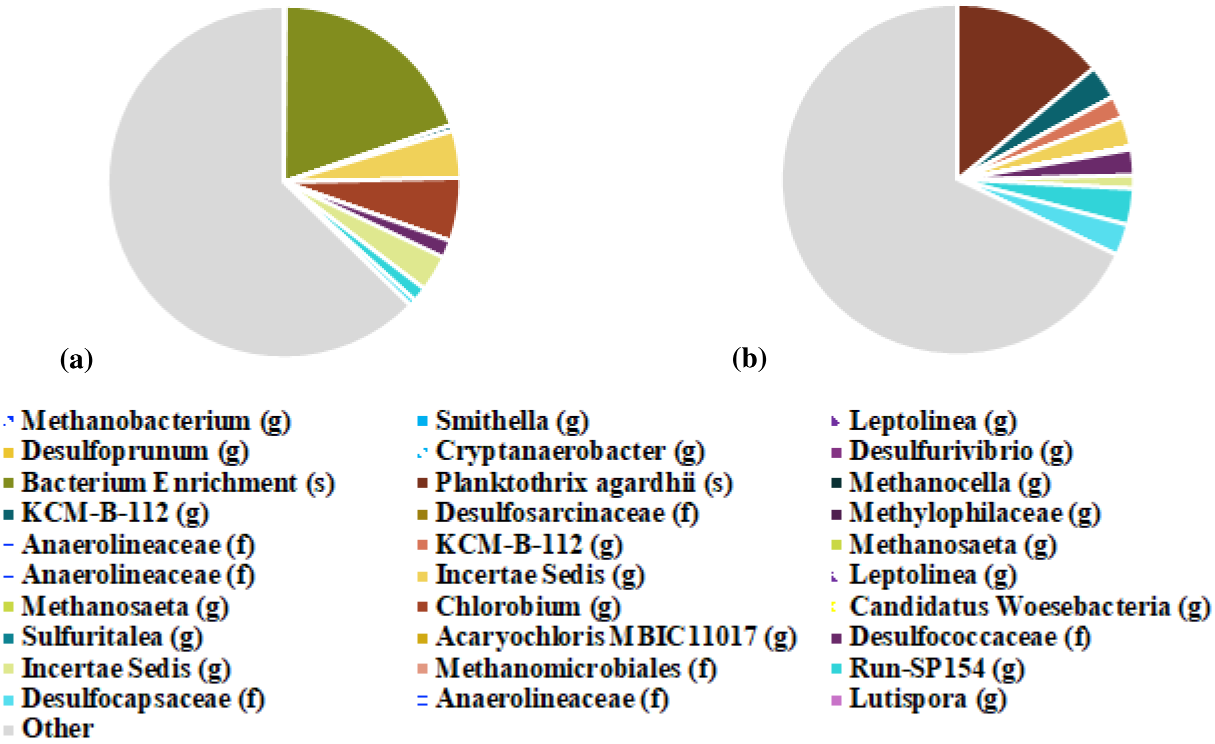

Figure Eb. Pie chart of relative abundance of taxonomy features in (a) L-GS and (b) F-GS

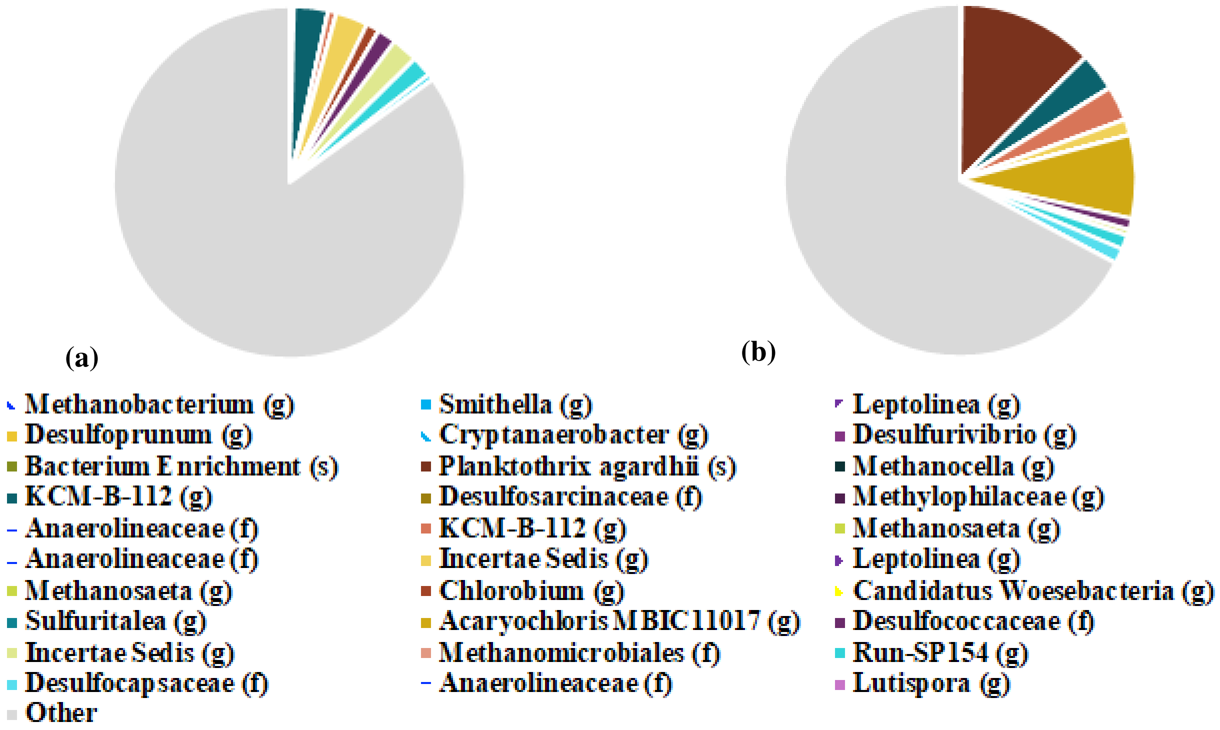

Figure Ec. Pie chart of relative abundance of taxonomy features in (a) L-GS+H and (b) F-GS+H

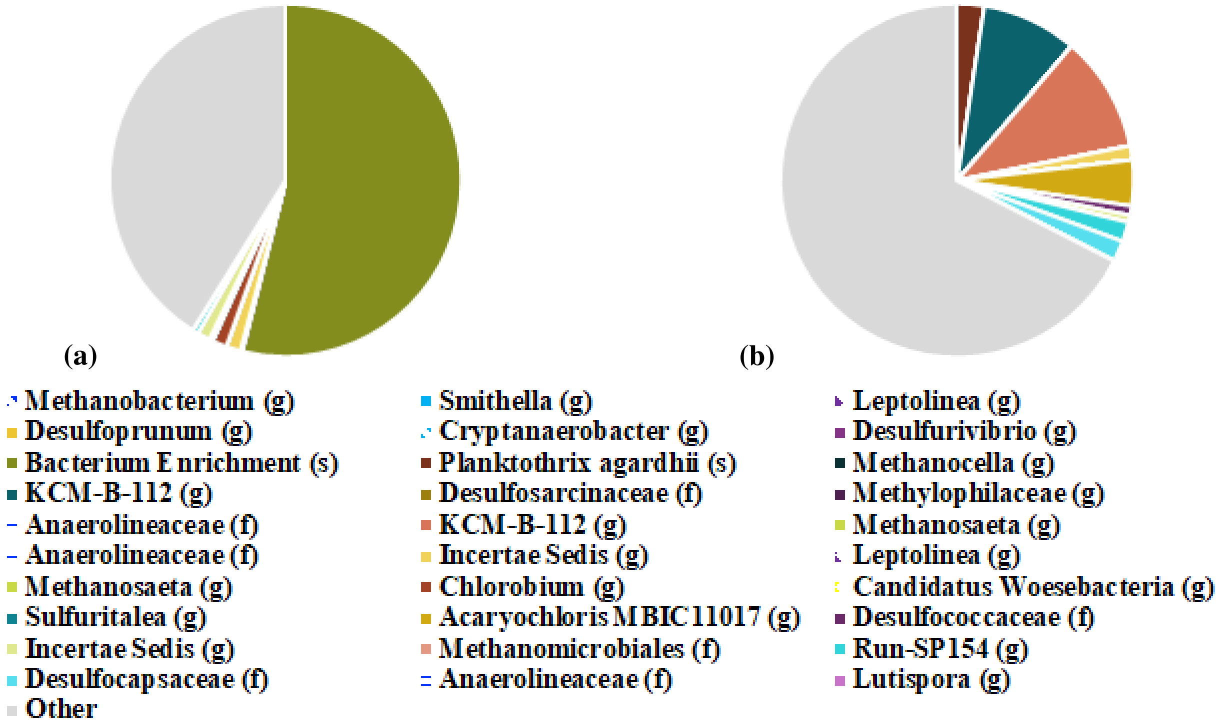

Figure Ed. Pie chart of relative abundance of taxonomy features in (a) L-GYP and (b) F-GYP

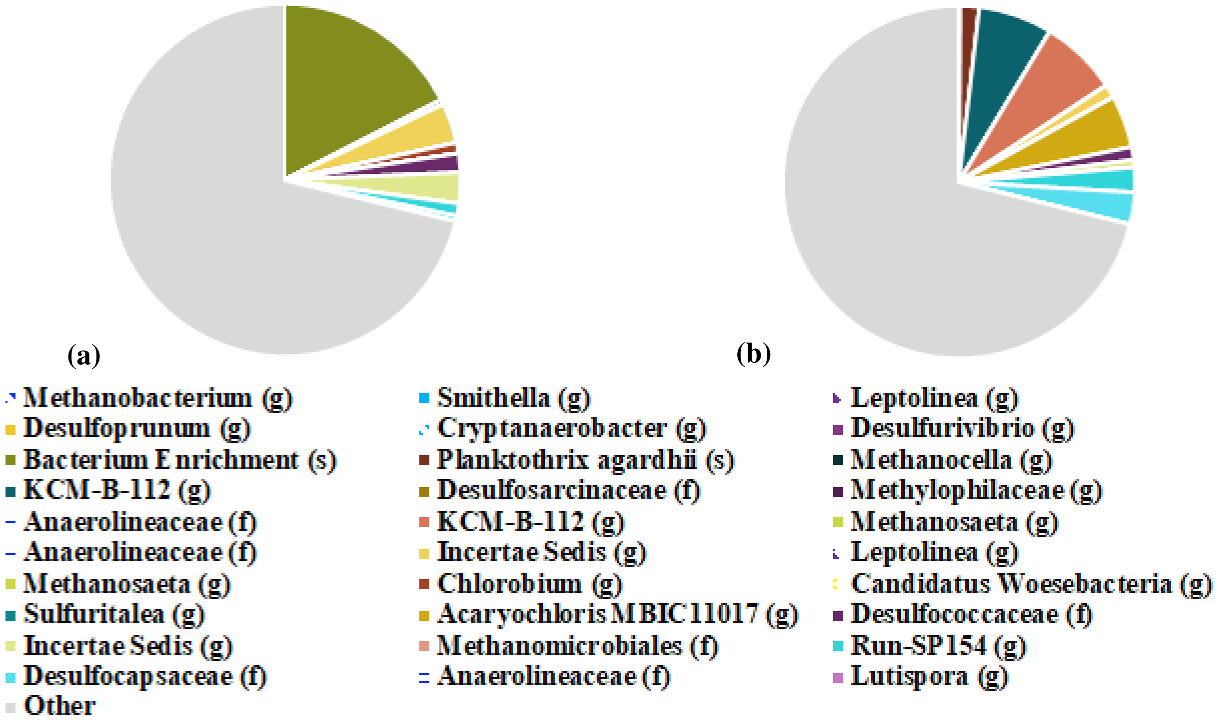

Figure Ee. Pie chart of relative abundance of taxonomy features in (a) L-ALL and (b) F-ALL

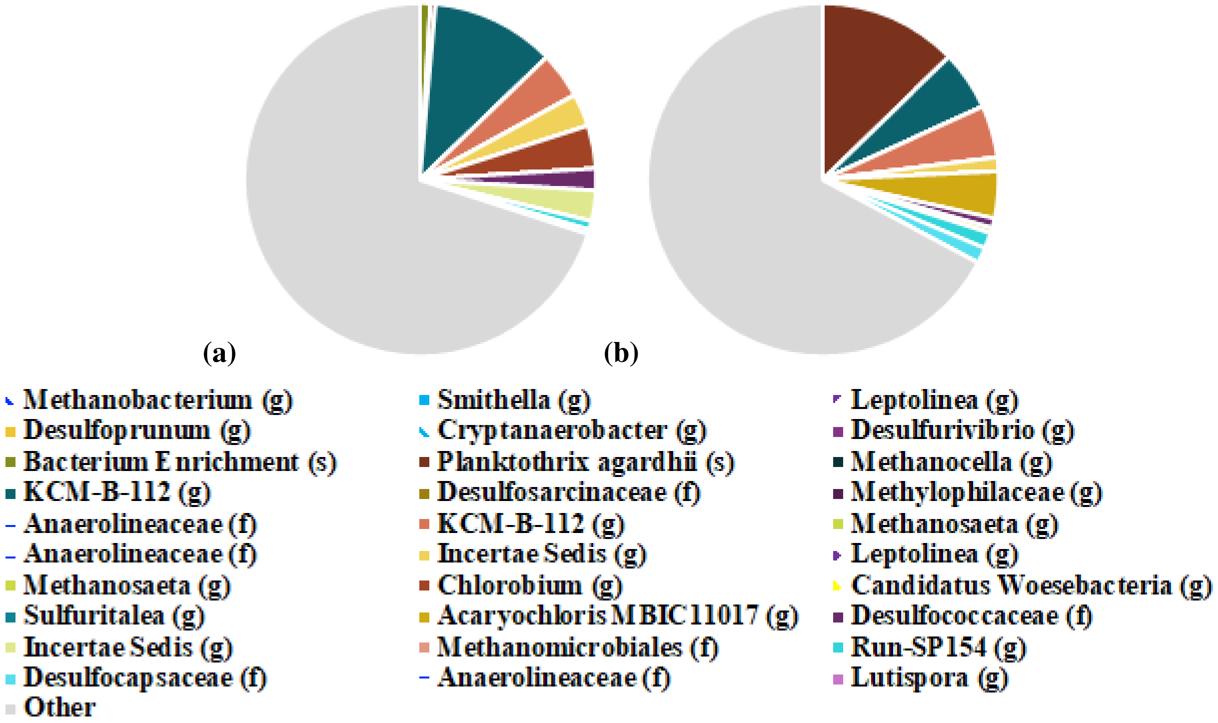

Figure Ef. Pie chart of relative abundance of taxonomy features in (a) L-B and (b) F-B

Appendix Ea – References

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APPENDIX F – MICROBIAL CATEGORIZATION

F1. Overall Results

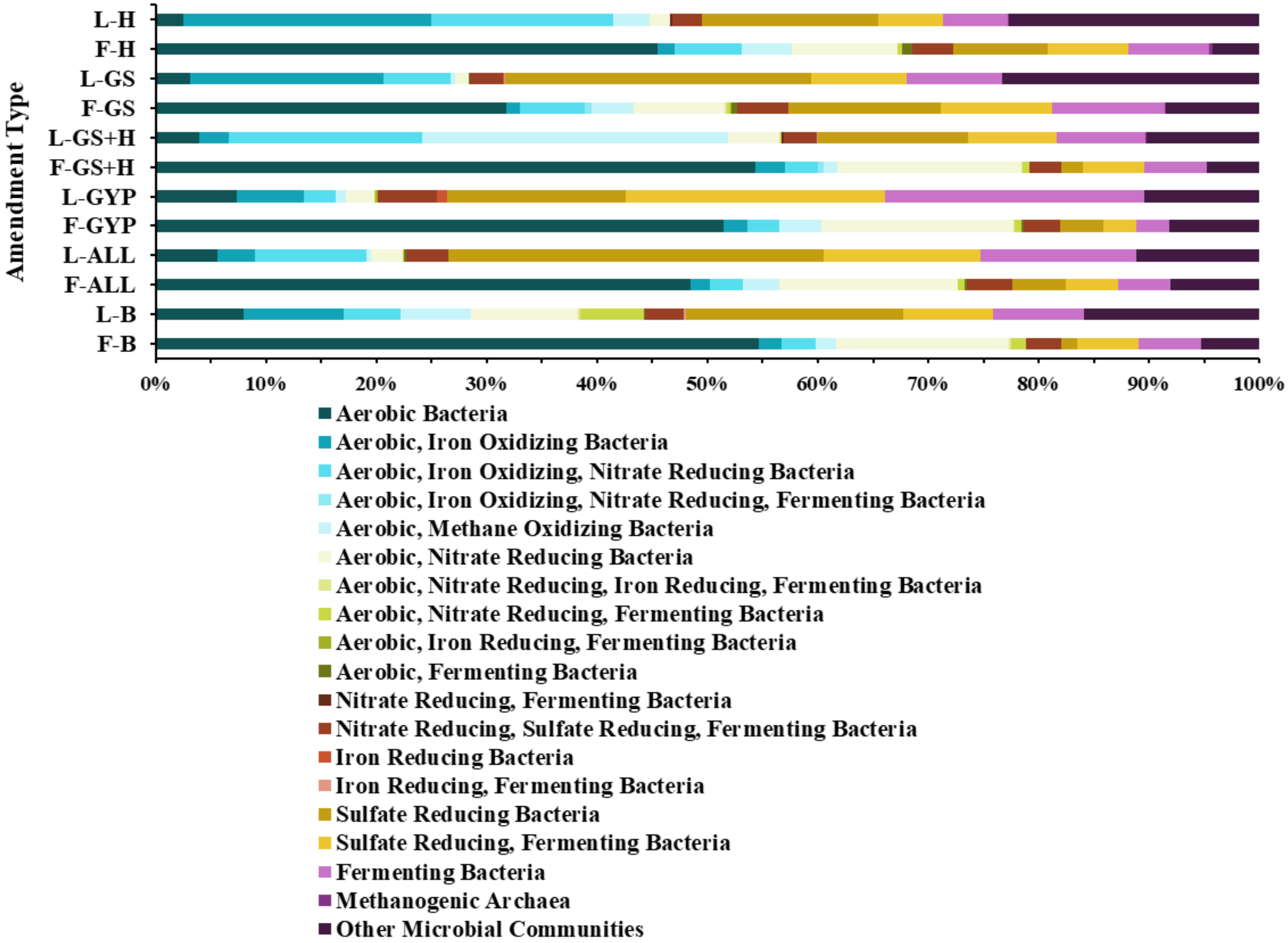

Figure F1a. Summary graph of relative abundance of microbial communities

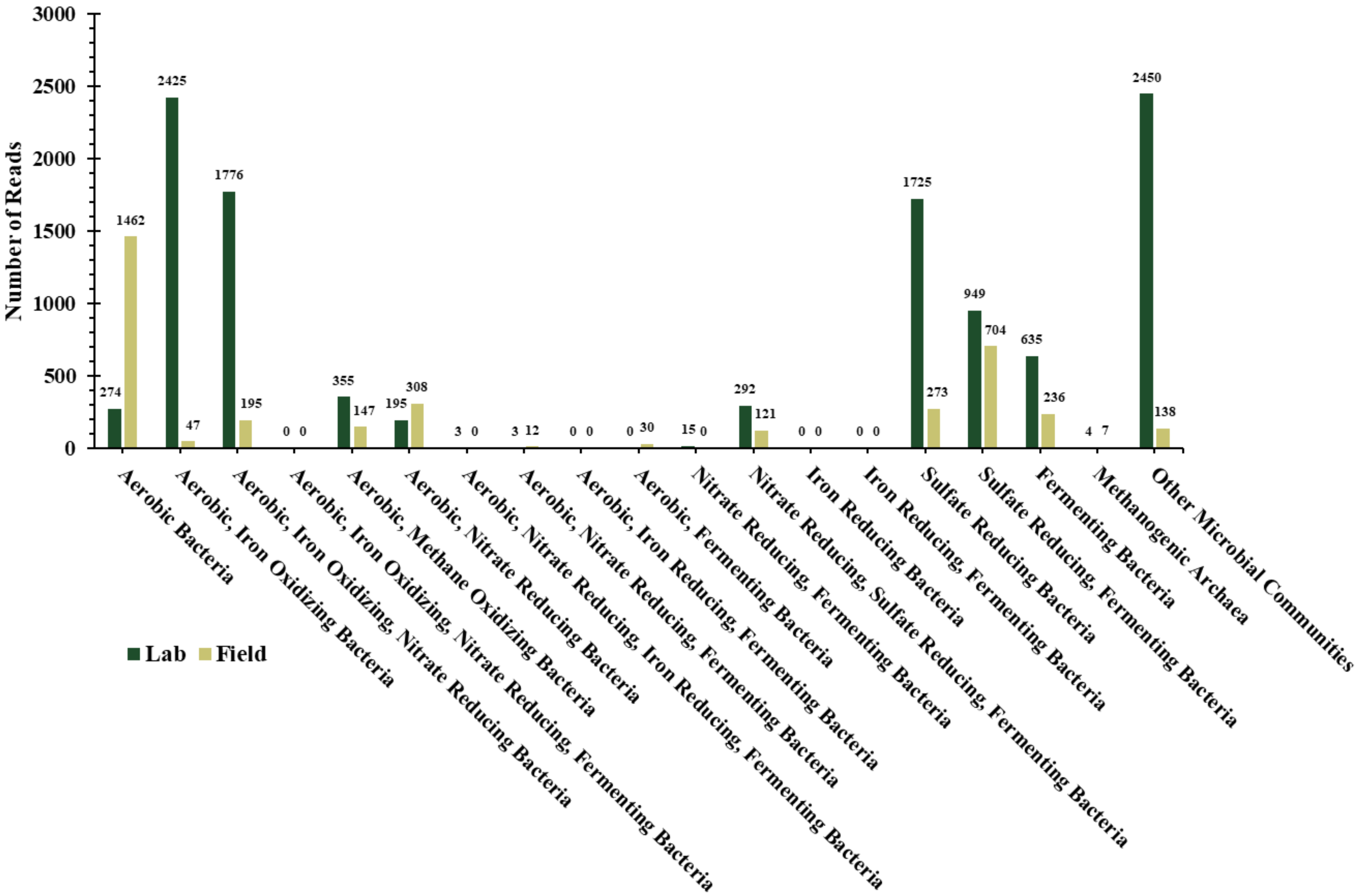

Figure F1b. Summary graph comparing number of reads for *hematite* laboratory (green) and field (gold) results per category of microbial communities

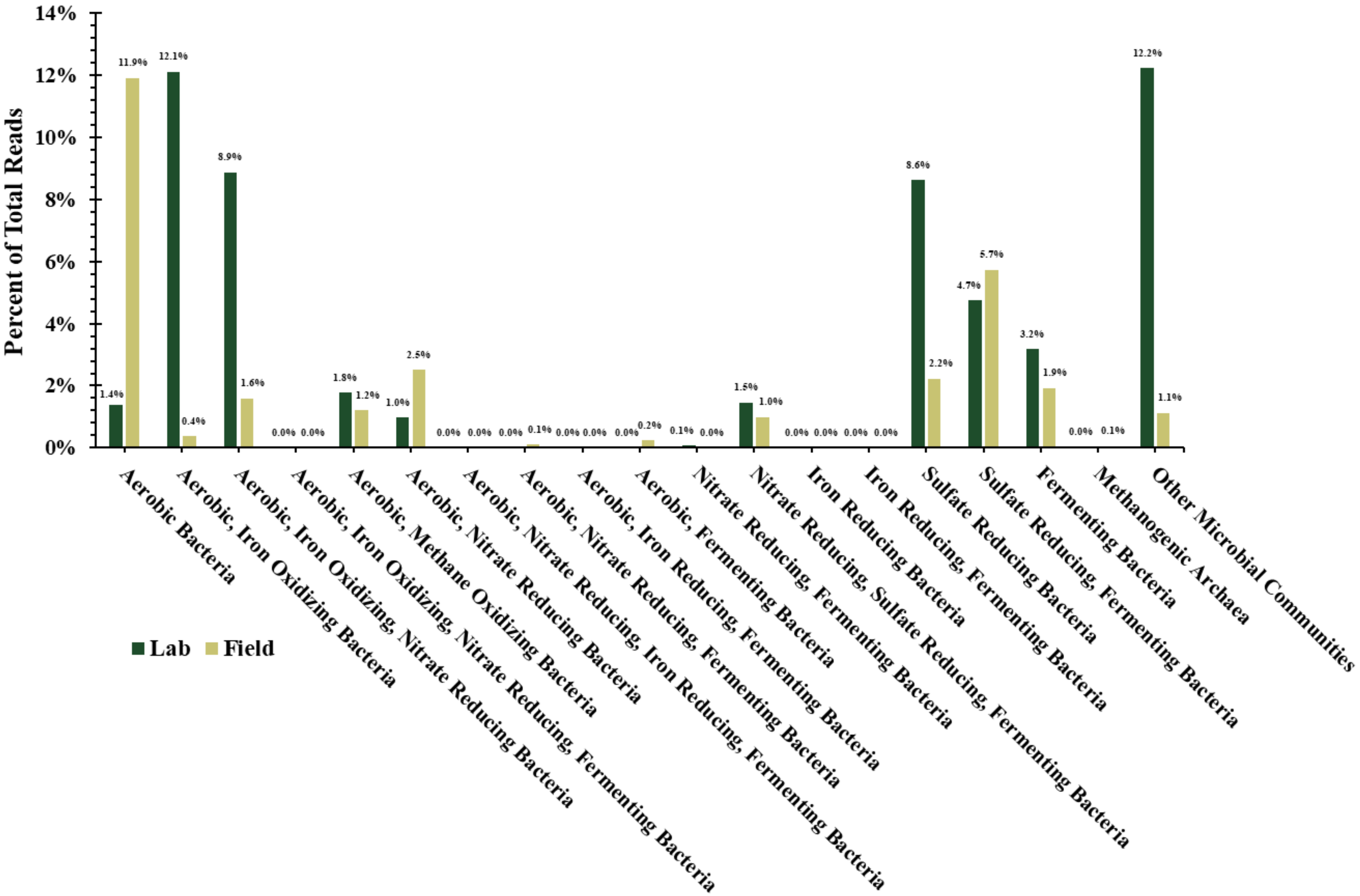

Figure F1c. Summary graph comparing percentage of total number of reads for *hematite* laboratory (green) and field (gold) results per category of microbial communities

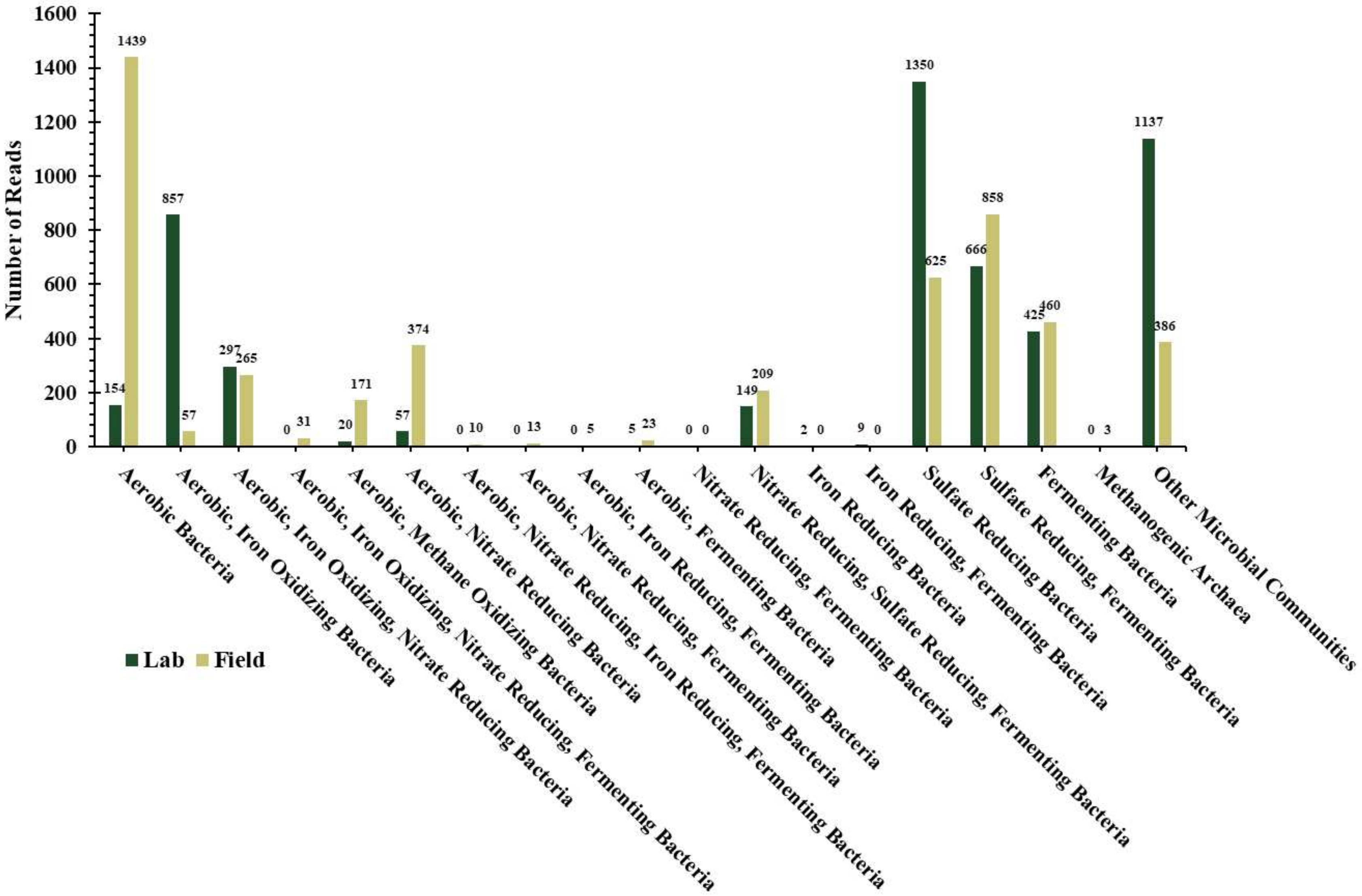

Figure F1d. Summary graph comparing number of reads for *greensand* laboratory (green) and field (gold) results per category of microbial communities

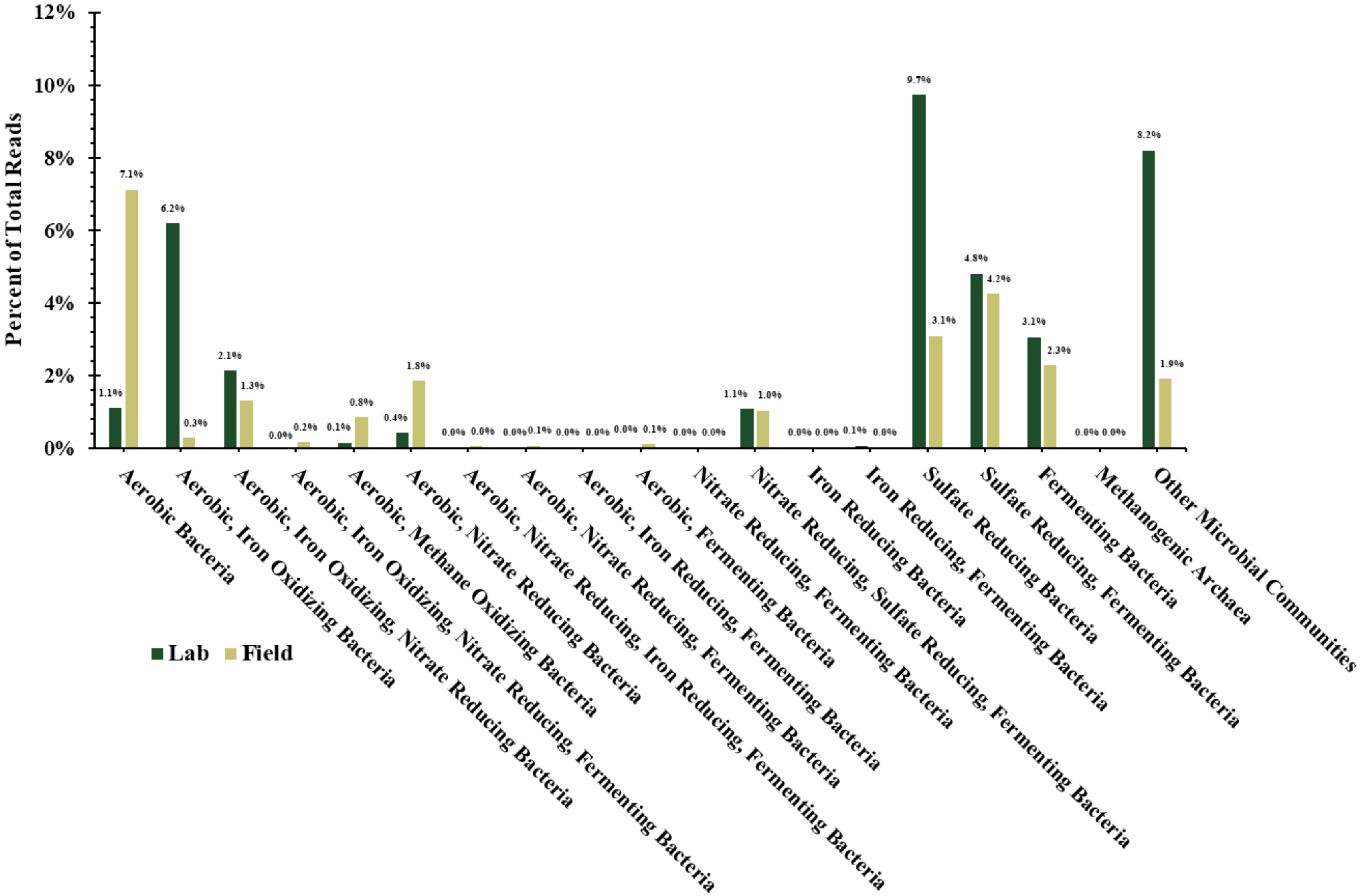

Figure F1e. Summary graph comparing percentage of total number of reads for *greensand* laboratory (green) and field (gold) results per category of microbial communities

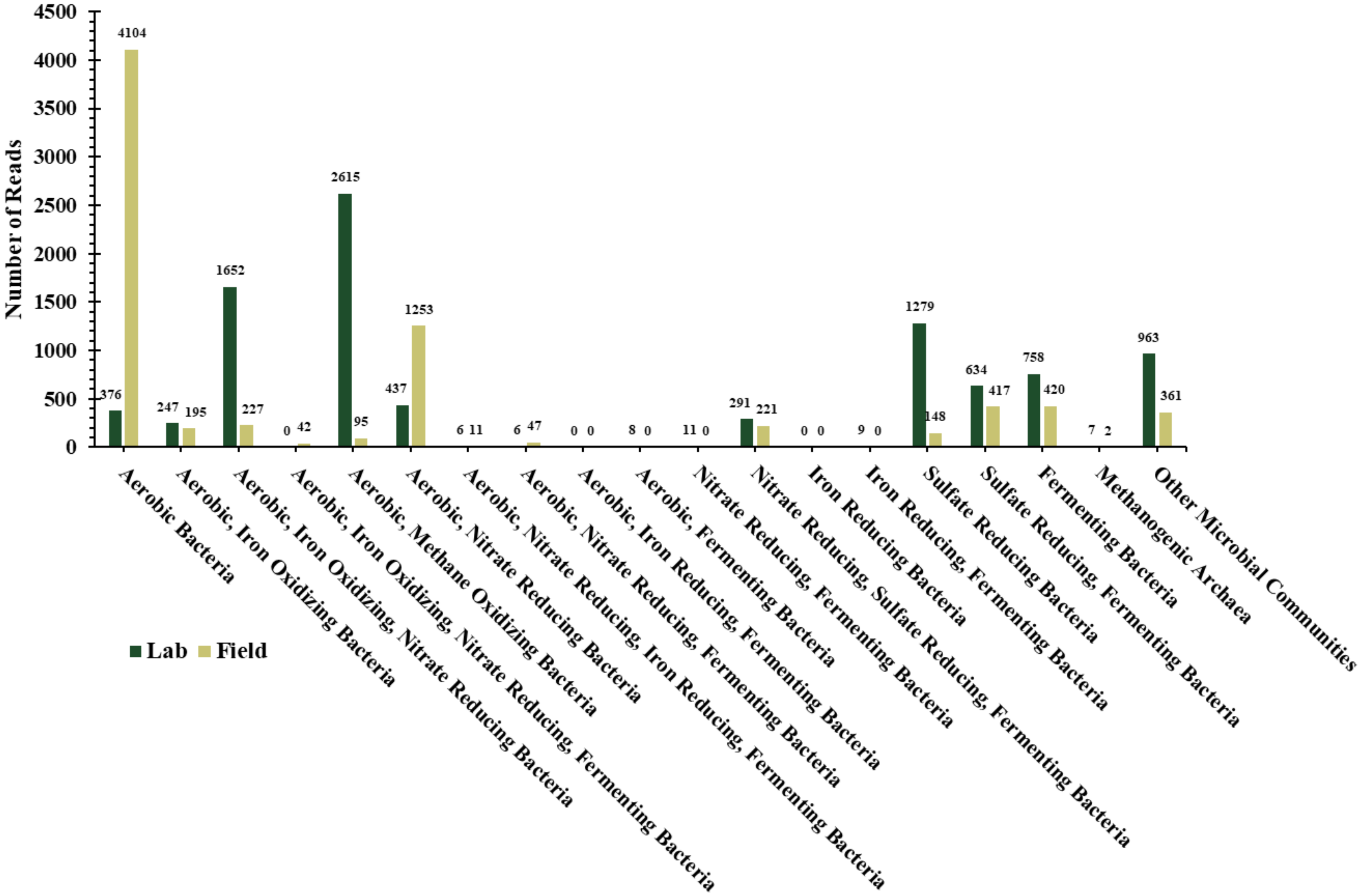

Figure F1f. Summary graph comparing number of reads for *greensand and hematite* laboratory (green) and field (gold) results per category of microbial communities

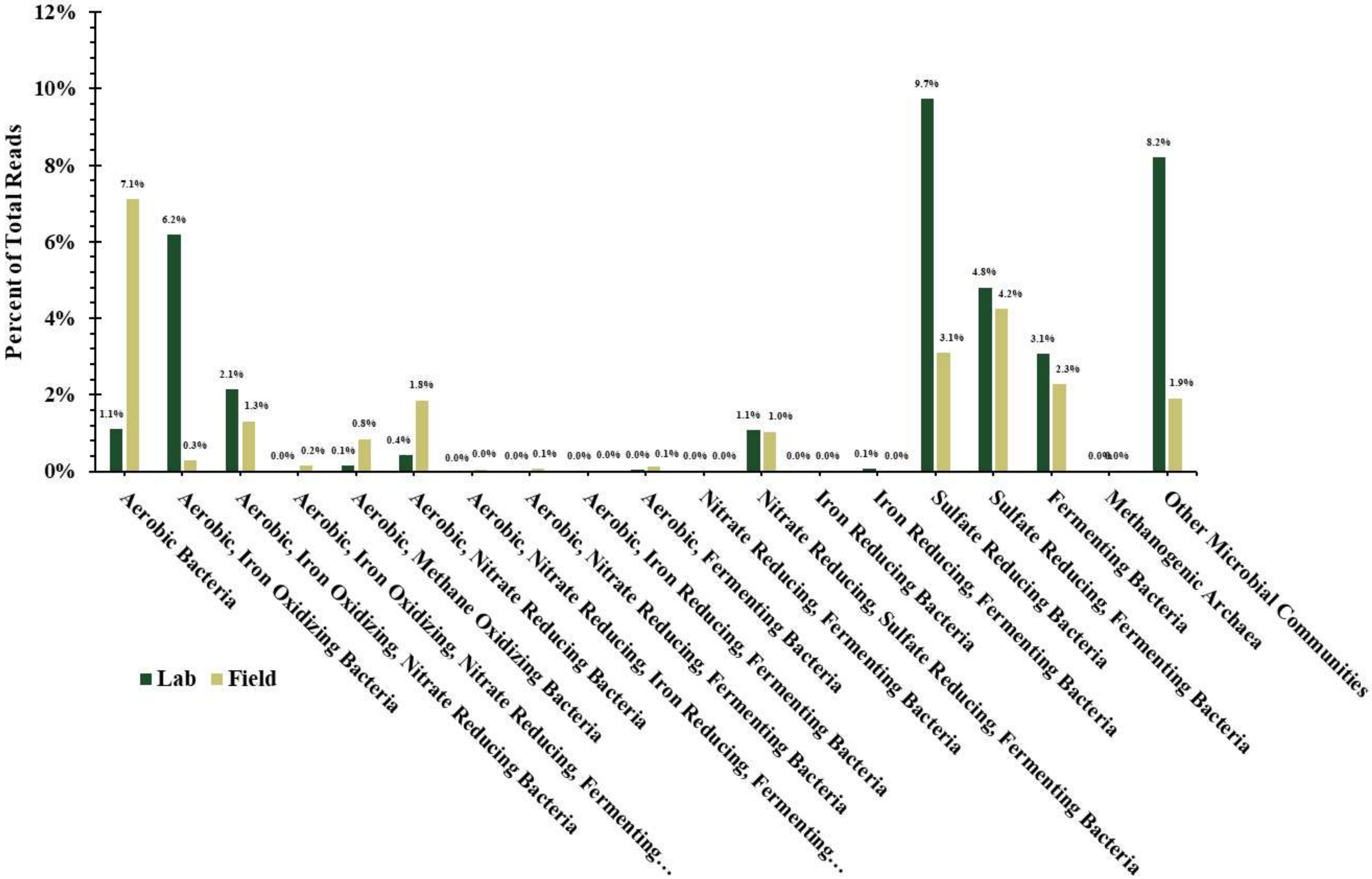

Figure F1e. Summary graph comparing percentage of total number of reads for *greensand and hematite* laboratory (green) and field (gold) results per category of microbial communities

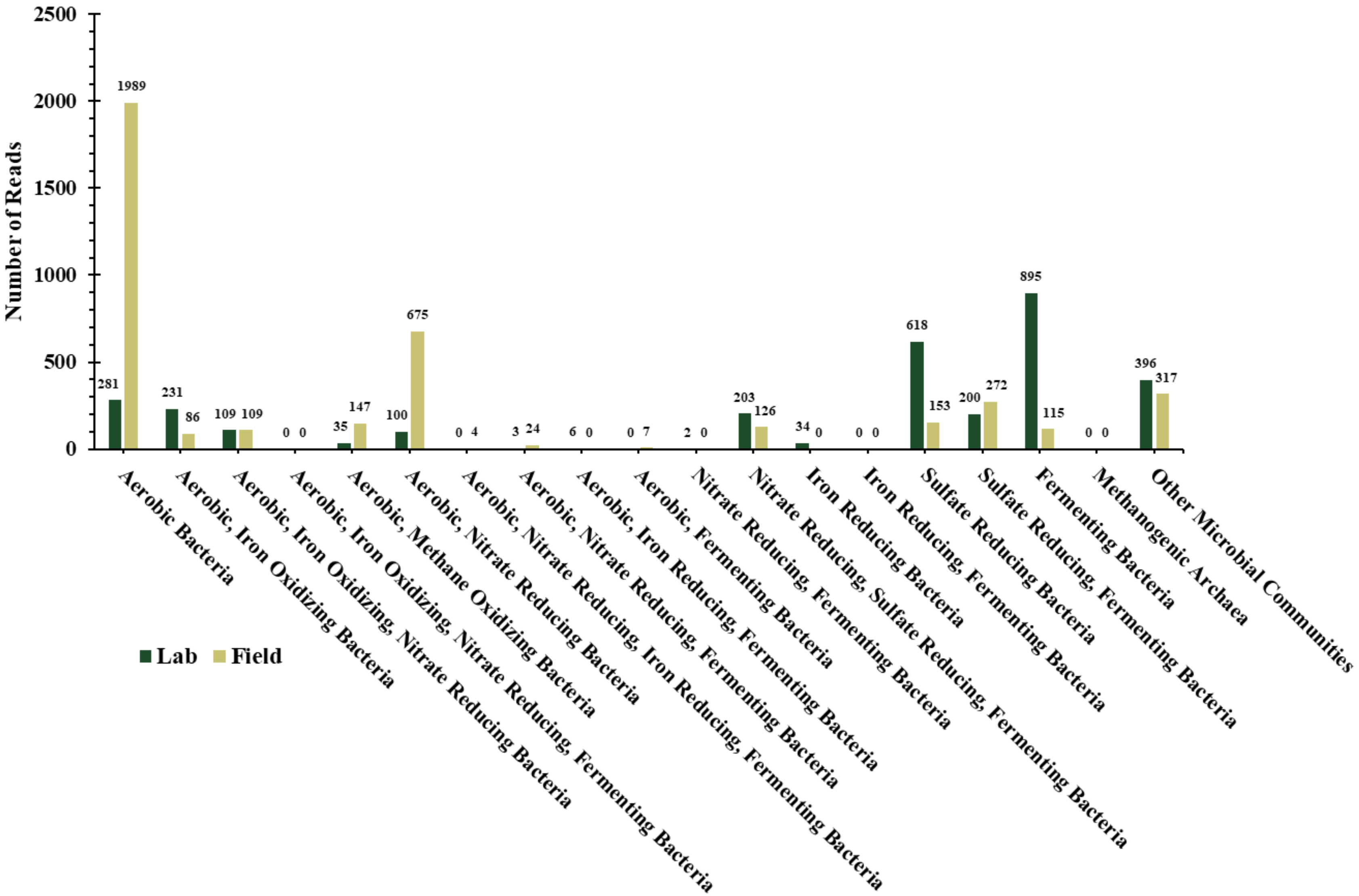

Figure F1h. Summary graph comparing number of reads for *gypsum* laboratory (green) and field (gold) results per category of microbial communities

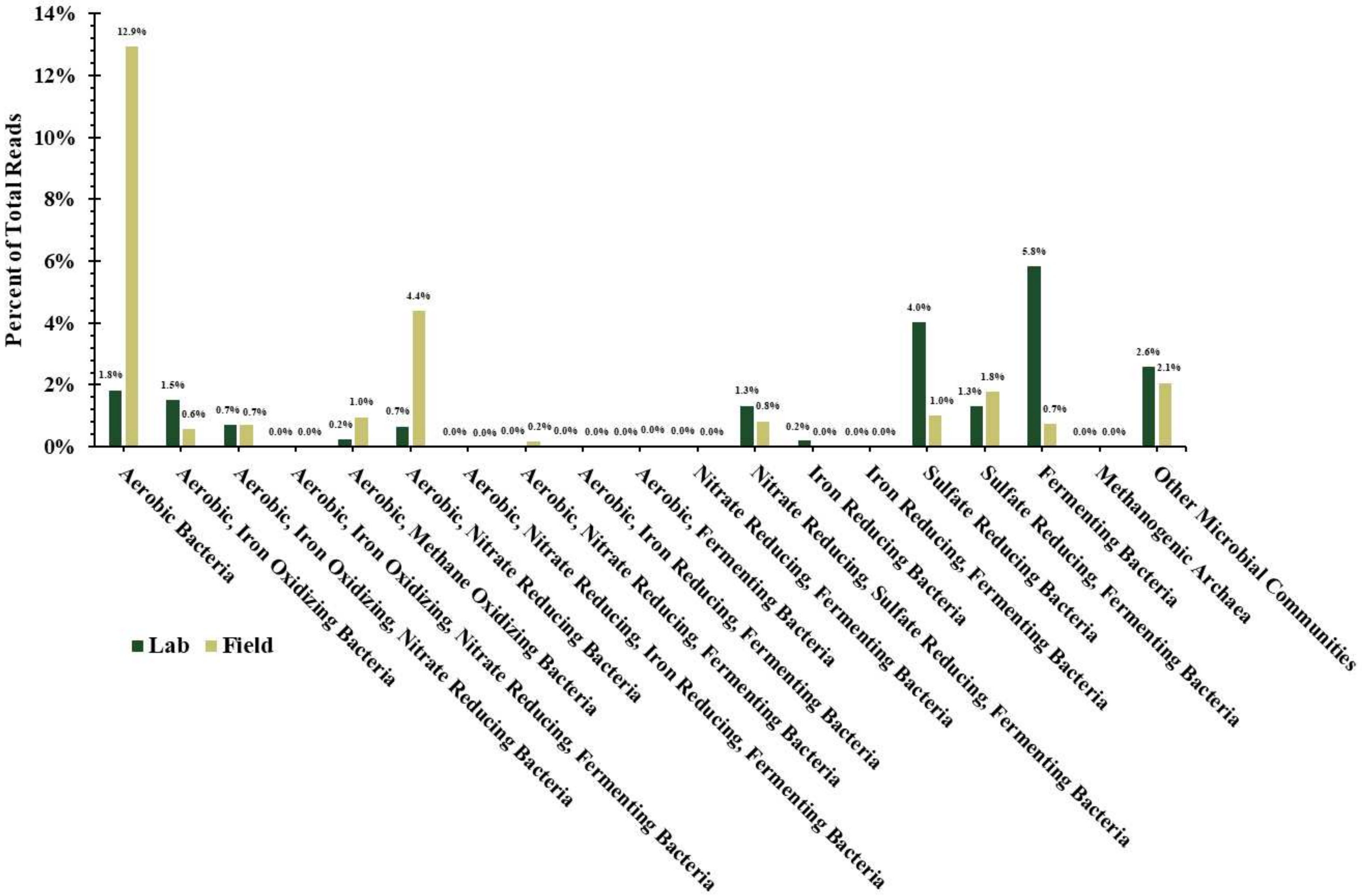

Figure F1i. Summary graph comparing percentage of total number of reads for *gypsum* laboratory (green) and field (gold) results per category of microbial communities

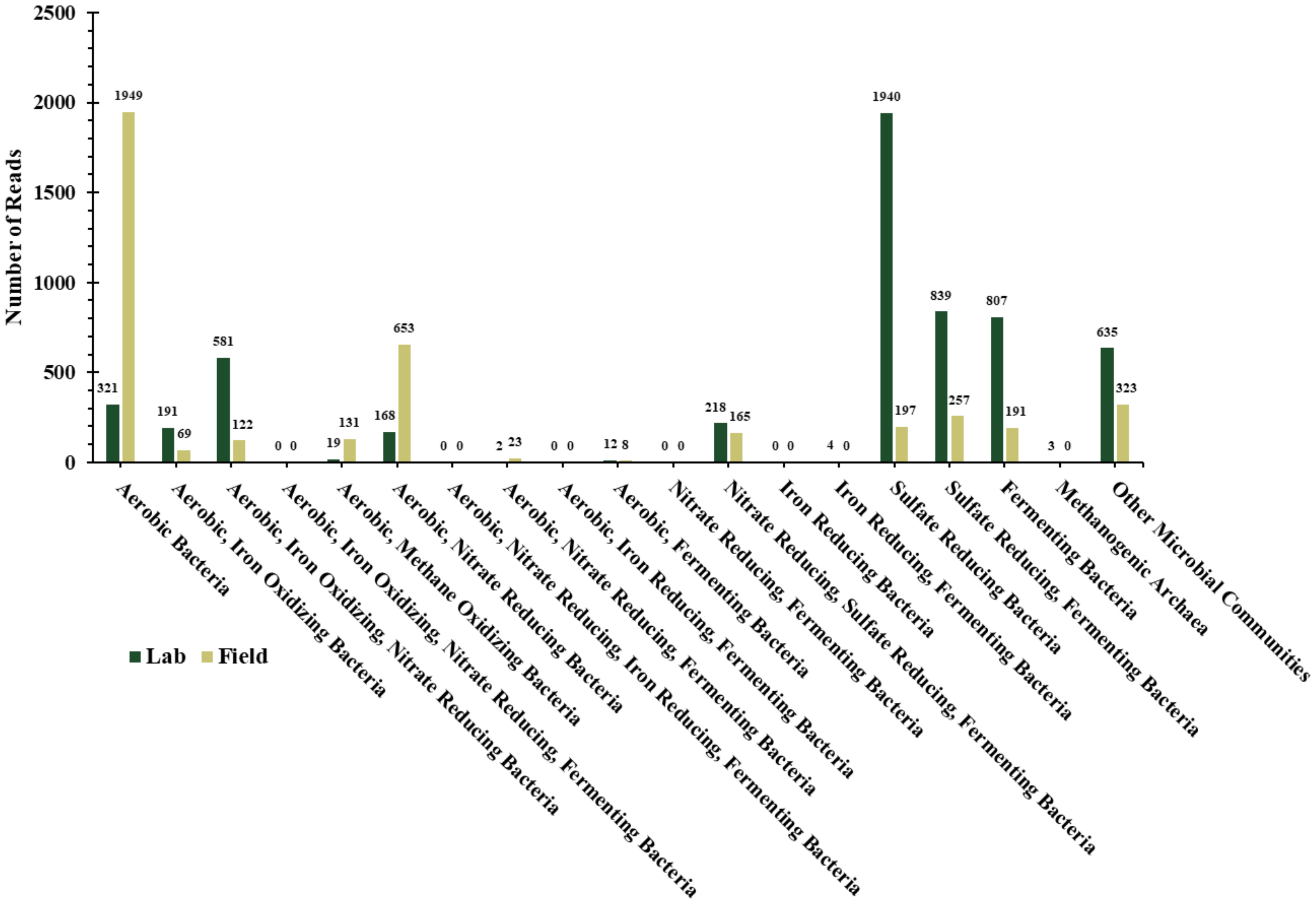

Figure F1j. Summary graph comparing number of reads for *all* laboratory (green) and field (gold) results per category of microbial communities

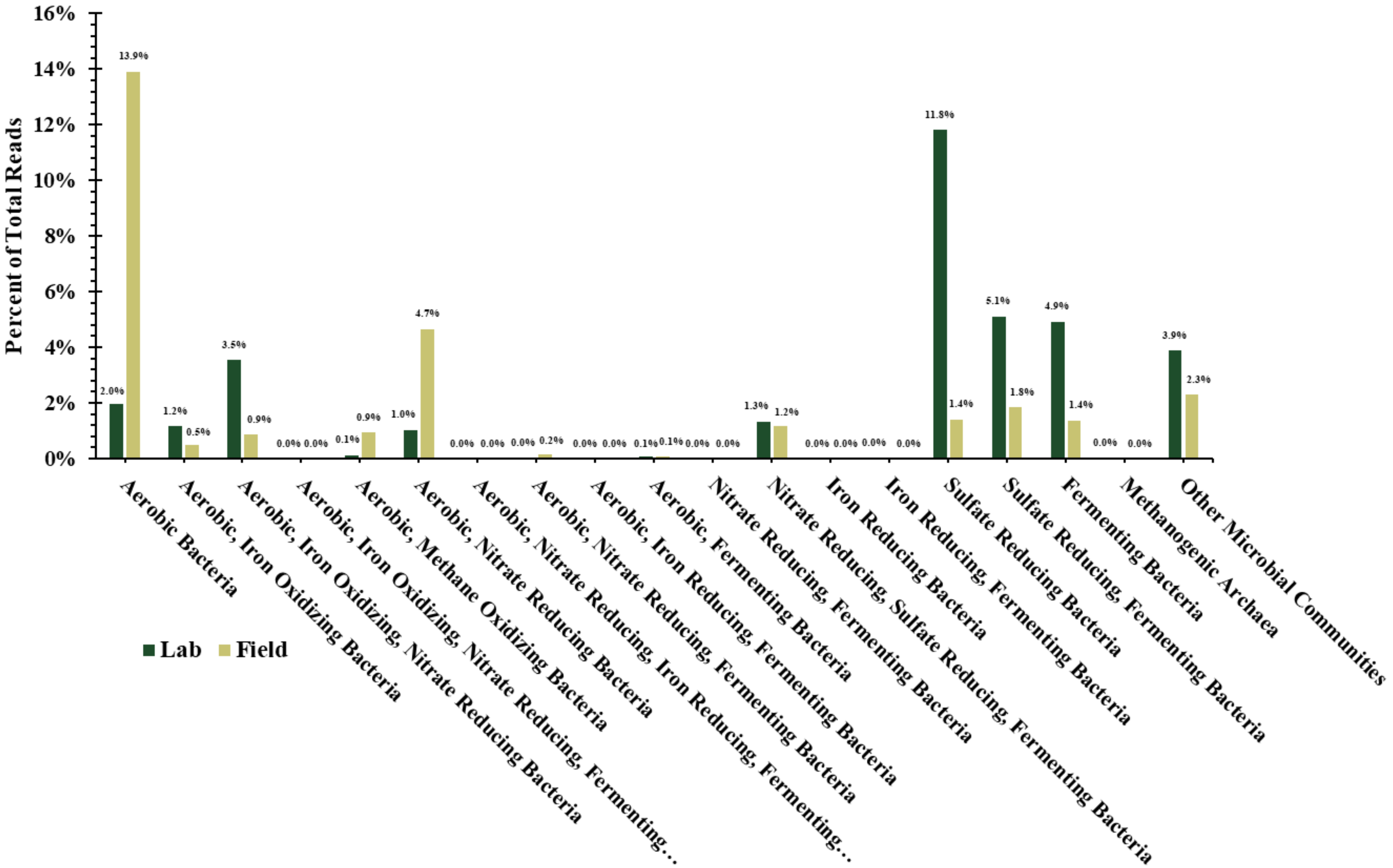

Figure F1k. Summary graph comparing percentage of total number of reads for *all* laboratory (green) and field (gold) results per category of microbial communities

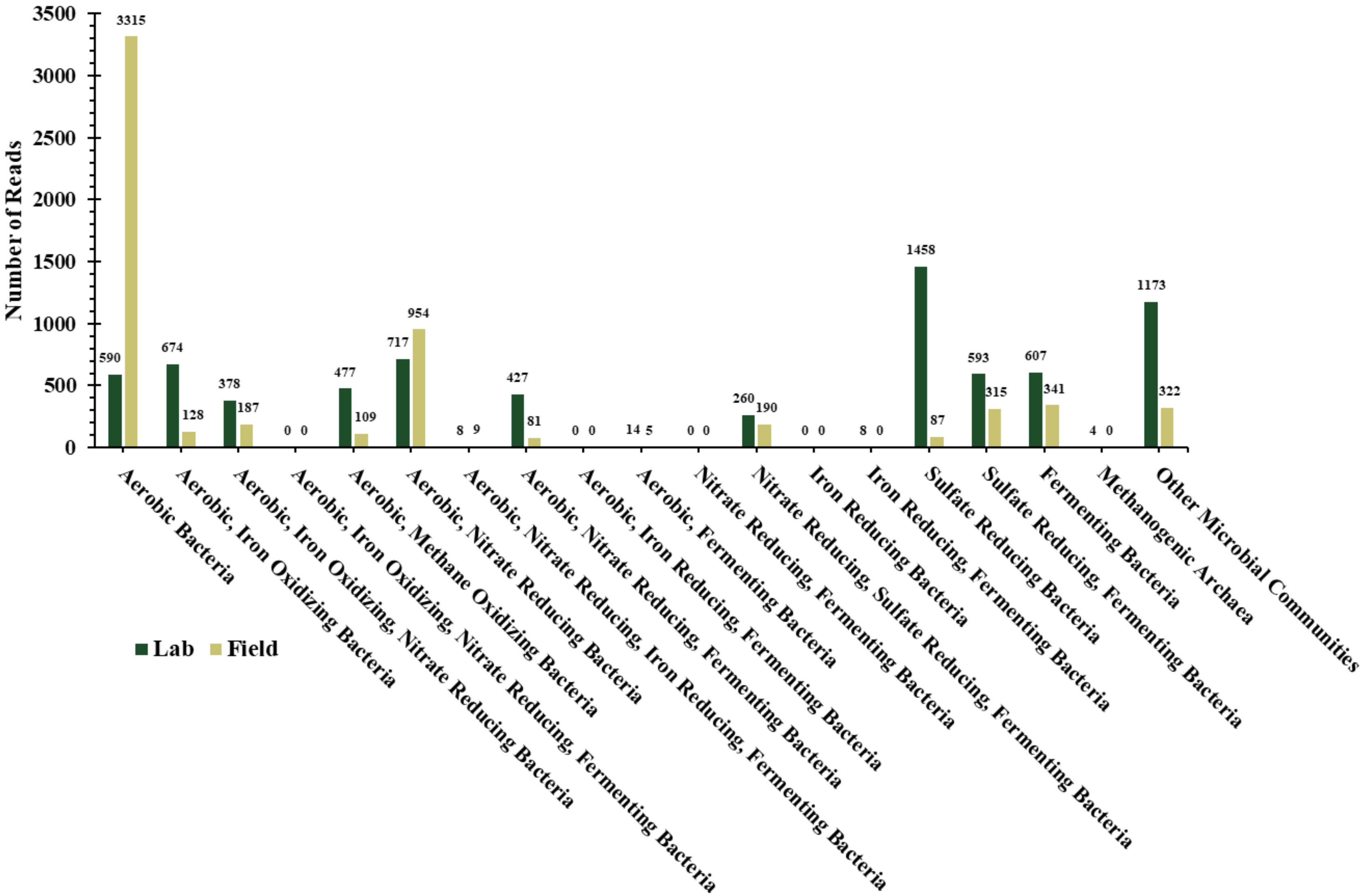

Figure F1l. Summary graph comparing number of reads for *blank* laboratory (green) and field (gold) results per category of microbial communities

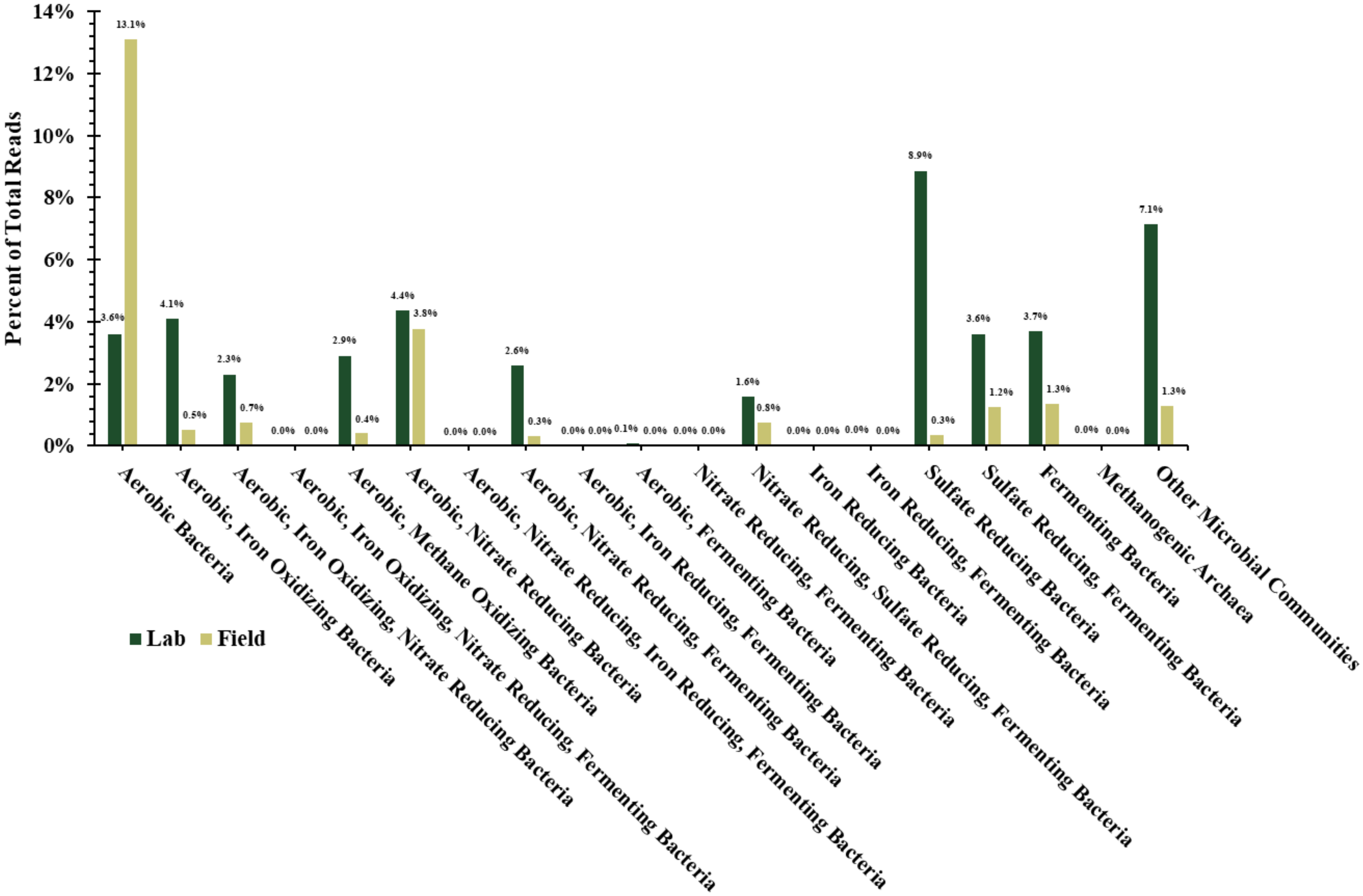

Figure F1m. Summary graph comparing percentage of total number of reads for *blank* laboratory (green) and field (gold) results per category of microbial communities

Table F1a. Overall results from microbial categorization with number of counts and relative frequency

Amendment	L-H	F-H	L-GS	F-GS	L-GS+H	F-GS+H	L-GYP	F-GYP	L-ALL	F-ALL	L-B	F-B
Aerobic Bacteria	274	1462	154	1439	376	4104	281	1989	321	1949	590	3315
	2.47%	39.73	3.00%	29.19%	4.04%	54.41%	9.03%	49.43%	5.59%	47.68%	7.99%	54.86%
Aerobic, Iron Oxidizing Bacteria	2425	47	857	57	247	195	231	86	191	69	674	128
	21.84%	1.28%	16.71%	1.16%	2.66%	2.59%	7.42%	2.14%	3.33%	1.69%	9.12%	2.12%
Aerobic, Iron Oxidizing, Nitrate Reducing Bacteria	1776	195	297	265	1652	227	109	109	581	122	378	187
	16.00%	5.30%	5.79%	5.38%	17.77%	3.01%	3.50%	2.71%	10.12%	2.98%	5.12%	3.09%
Aerobic, Iron Oxidizing, Nitrate Reducing,	0	0	0	31	0	42	0	0	0	0	0	0
	0.00%	0.00%	0.00%	0.63%	0.00%	0.56%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Aerobic, Methane Oxidizing Bacteria	355	147	20	171	2615	95	35	147	19	131	477	109
	3.20%	3.99%	0.39%	3.47%	28.12%	1.26%	1.12%	3.65%	0.33%	3.20%	6.46%	1.80%
Aerobic, Nitrate Reducing Bacteria	195	308	57	374	437	1253	100	675	168	653	717	954
	1.76%	8.37%	1.11%	7.59%	4.70%	16.61%	3.21%	16.77%	2.93%	15.97%	9.70%	15.79%
Aerobic, Nitrate Reducing, Iron Reducing,	3	0	0	10	6	11	0	4	0	0	8	9
	0.03%	0.00%	0.00%	0.20%	0.06%	0.15%	0.00%	0.10%	0.00%	0.00%	0.11%	0.15%
Aerobic, Nitrate Reducing, Fermenting Bacteria	3	12	0	13	6	47	3	24	2	23	427	81
	0.03%	0.33%	0.00%	0.26%	0.06%	0.62%	0.10%	0.60%	0.03%	0.56%	5.78%	1.34%
Aerobic, Iron Reducing, Fermenting Bacteria	0	0	0	5	0	0	6	0	0	0	0	0
	0.00%	0.00%	0.00%	0.10%	0.00%	0.00%	0.19%	0.00%	0.00%	0.00%	0.00%	0.00%
Aerobic, Fermenting Bacteria	0	30	5	23	8	0	0	7	12	8	14	5
	0.00%	0.82%	0.10%	0.47%	0.09%	0.00%	0.00%	0.17%	0.21%	0.20%	0.19%	0.08%
Nitrate Reducing, Fermenting Bacteria	15	0	0	0	11	0	2	0	0	0	0	0
	0.14%	0.00%	0.00%	0.00%	0.12%	0.00%	0.06%	0.00%	0.00%	0.00%	0.00%	0.00%
Nitrate Reducing, Sulfate Reducing, Fermenting Bacteria	292	121	149	209	291	221	203	126	218	165	260	190
	2.63%	3.29%	2.91%	4.24%	3.13%	2.93%	6.52%	3.13%	3.80%	4.04%	3.52%	3.14%
Iron Reducing Bacteria	0	0	2	0	0	0	34	0	0	0	0	0
	0.00%	0.00%	0.04%	0.00%	0.00%	0.00%	1.09%	0.00%	0.00%	0.00%	0.00%	0.00%
Iron Reducing, Fermenting Bacteria	0	0	9	0	9	0	0	0	4	0	8	0
	0.00%	0.00%	0.18%	0.00%	0.10%	0.00%	0.00%	0.00%	0.07%	0.00%	0.11%	0.00%
Sulfate Reducing Bacteria	1725	273	1350	625	1279	148	618	153	1940	197	1458	87
	15.54%	7.42%	26.33%	12.68%	13.75%	1.96%	19.85%	3.80%	33.80%	4.82%	19.73	1.44%
Sulfate Reducing, Fermenting Bacteria	949	704	666	858	634	417	200	272	839	257	593	315
	8.55%	19.13	12.99%	17.41%	6.82%	5.53%	6.42%	6.76%	14.62%	6.29%	8.03%	5.21%
Fermenting Bacteria	635	236	425	460	758	420	895	115	807	191	607	341
	5.72%	6.41%	8.29%	9.33%	8.15%	5.57%	28.75%	2.86%	14.06%	4.67%	8.22%	5.64%
Methanogenic Archaea	4	7	0	3	7	2	0	0	3	0	4	0
	0.04%	0.19%	0.00%	0.06%	0.08%	0.03%	0.00%	0.00%	0.05%	0.00%	0.05%	0.00%
Other Microbial Communities	2450	138	1137	386	963	361	396	317	635	323	1173	322
	22.07%	3.75%	22.17%	7.83%	10.36%	4.79%	12.72%	7.88%	11.06%	7.90%	15.88	5.33%
Sum	11101	3680	5128	4929	9299	7543	3113	4024	5740	4088	7388	6043
Portion of Total	55.5%	26.5%	28.3%	32.1%	56.7%	45.9%	25.4%	19.9%	19.2%	26.6%	52.7%	23.9%

F2. Aerobic Bacteria

Table F2a. Summary results for *Aerobic Bacteria*

Amendment	No. of Reads	Perc. of Total Reads
L-H	274	1.3699%
F-H	1462	11.9230%
L-GS	154	1.1099%
F-GS	1439	7.1171%
L-GS+H	376	2.0732%
F-GS+H	4104	13.7125%
L-GYP	281	1.8280%
F-GYP	1989	12.9433%
L-ALL	321	1.9568%
F-ALL	1949	13.9095%
L-B	590	3.5871%
F-B	3315	13.1007%

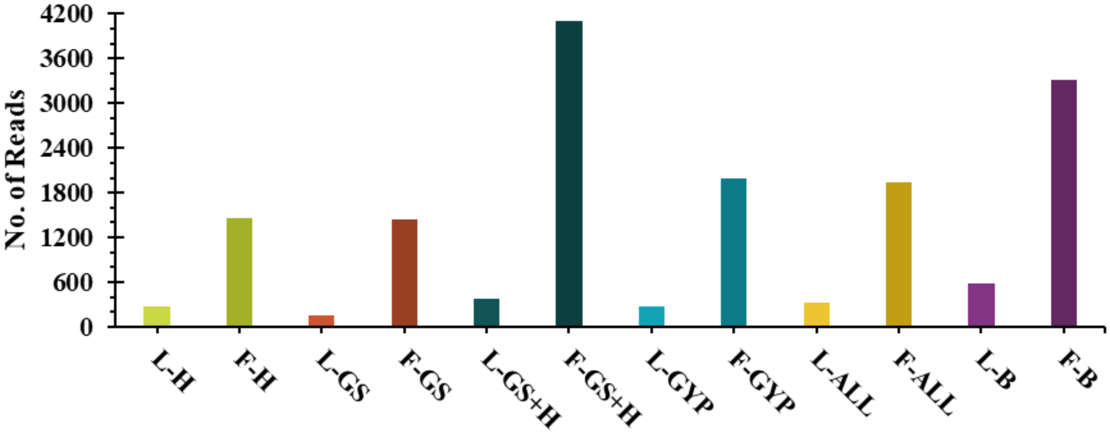

Figure F2a. Summary graph of number of reads for each amendment type in the category of *Aerobic Bacteria*

Table F2b. Classification of *Aerobic Bacteria*

Aerobic Bacteria	**Amendment**	**No. of Reads**	**Perc. of Total Reads**	**Classification**
Acetobacteraceae (f) [1]	F-GS+H	31	0.1036%	Roseomonas (g)
	F-B	31	0.1225%	Roseomonas (g)
	L-B	10	0.0608%	Roseomonas (g)
	F-ALL	10	0.0714%	Roseomonas (g)
	F-GS	9	0.0445%	Roseomonas (g) metagenome (s)
	F-GS+H	7	0.0234%	-
	F-GYP	7	0.0456%	Roseomonas (g)
	F-B	4	0.0158%	-
	L-GS+H	4	0.0221%	Roseomonas (g)
	F-GYP	4	0.0260%	Roseomonas (g) metagenome (s)
	F-ALL	4	0.0285%	uncultered (g)
	F-ALL	3	0.0214%	Roseomonas (g) metagenome (s)
	F-B	2	0.0079%	Roseomonas (g) metagenome (s)
	F-H	2	0.0163%	Roseomonas (g) Roseomonas lacus (s)
	F-GS	2	0.0099%	uncultured (g)
Acinetobacter (g) [2]	L-ALL	8	0.0488%	-
Agitococcus lubricus group (g) [3]	F-B	5	0.0198%	-
Aminobacter (g) [4]	F-ALL	20	0.1427%	-
	L-GYP	13	0.0846%	Acidaminobacter hydrogenoformans (s)
	L-ALL	2	0.0122%	Acidaminobacter hydrogenoformans (s)
	L-B	2	0.0122%	Acidaminobacter hydrogenoformans (s)
Aquicella (g) [5]	L-GS+H	2	0.0110%	-
Aquimonas (g) [6]	F-B	48	0.1897%	uncultured bacterium (s)
	F-GS+H	29	0.0969%	uncultured bacterium (s)
	L-GYP	9	0.0585%	uncultured bacterium (s)
Armatimonadetes Bacterium (s) [7]	F-B	2	0.0079%	-

Aerobic Bacteria	Amendment	No. of Reads	Perc. of Total Reads	Classification
Armatimonadetes Uncultured (s) [8]	L-B	4	0.0243%	-
Bauldia (g) [9]	F-GS+H	51	0.1704%	-
	F-B	49	0.1936%	-
	F-GS	42	0.2077%	-
	F-ALL	28	0.1998%	-
	L-ALL	12	0.0732%	-
	L-B	12	0.0730%	-
Bdellovibrionales (o) [10]	F-GS+H	7	0.0234%	Bdellovibrionaceae (f) OM27 clade (g) uncultured bacterium (s)
	L-ALL	6	0.0366%	Bdellovibrionaceae (f) OM27 clade (g) uncultured bacterium (s)
	F-GS+H	4	0.0134%	Bdellovibrionaceae (f) OM27 clade (g)
	F-B	4	0.0158%	Bdellovibrionaceae (f) OM27 clade (g) uncultured_bacterium (s)
	F-GS	3	0.0148%	Bdellovibrionaceae (f) Bdellovibrio (g) uncultured bacterium (s)
	F-H	2	0.0163%	Bdellovibrionaceae (f) Bdellovibrio (g) uncultured bacterium (s)
	F-GS+H	2	0.0067%	Bdellovibrionaceae (f) Bdellovibrio (g) uncultured bacterium (s)
Beijerinckiaceae (f) [11]	F-GS+H	18	0.0601%	-
	F-B	7	0.0277%	-
	F-H	6	0.0489%	-
	F-GYP	5	0.0325%	Bosea (g)
	F-H	5	0.0408%	alphaI cluster (g) metagenome (s)
	L-GYP	4	0.0260%	-
Blastocatellia (c) [12]	F-GS+H	127	0.4243%	Blastocatellales (o) Blastocatellaceae (f) uncultured (g) Acidobacteria bacterium (s)
	F-B	118	0.4663%	Blastocatellales (o) Blastocatellaceae (f) uncultured (g) Acidobacteria bacterium (s)

Aerobic Bacteria	**Amendment**	**No. of Reads**	**Perc. of Total Reads**	**Classification**
Blastocatellia (c) [12]	F-GYP	62	0.4035%	Blastocatellales (o) Blastocatellaceae (f) uncultured (g) Acidobacteria bacterium (s)
	F-ALL	40	0.2855%	Blastocatellales (o) Blastocatellaceae (f) uncultured (g) Acidobacteria bacterium (s)
	F-GS	31	0.1533%	Blastocatellales (o) Blastocatellaceae (f) uncultured (g) Acidobacteria bacterium (s)
	F-B	29	0.1146%	Blastocatellales (o) Blastocatellaceae (f) uncultured (g)
	F-H	27	0.2202%	Blastocatellales (o) Blastocatellaceae (f) uncultured (g) Acidobacteria bacterium (s)
	F-GS+H	23	0.0768%	Blastocatellales (o) Blastocatellaceae (f) JGI 0001001-H03 (g)
	F-GS+H	19	0.0635%	Blastocatellales (o) Blastocatellaceae (f) uncultured (g)
	F-GYP	10	0.0651%	Blastocatellales (o) Blastocatellaceae (f) JGI 0001001-H03 (g)
	F-B	10	0.0395%	Blastocatellales (o) Blastocatellaceae (f) JGI 0001001-H03 (g)
	F-GYP	8	0.0521%	Blastocatellales (o) Blastocatellaceae (f) uncultured (g)
	F-H	5	0.0408%	Blastocatellales (o) Blastocatellaceae (f) uncultured (g)
	L-H	5	0.0250%	Blastocatellales (o) Blastocatellaceae (f) uncultured (g) Acidobacteria bacterium (s)
Brevundimonas (g) [13]	F-GS+H	24	0.0802%	-
	F-B	14	0.0553%	-
	F-GYP	8	0.0521%	-
	F-ALL	8	0.0571%	-
Bryobacter (g) [14] Bryobacter (g) [14]	F-GS+H	4	0.0134%	uncultured prokaryote (s)
	F-GYP	3	0.0195%	uncultured prokaryote (s)
Burkholderiaceae (f) [15]	L-B	114	0.6931%	Lautropia (g) uncultured beta (s)

Aerobic Bacteria	Amendment	No. of Reads	Perc. of Total Reads	Classification
	F-B	78	0.3083%	Lautropia (g) uncultured beta (s)
	L-GS+H	59	0.3253%	Lautropia (g) uncultured beta (s)
	F-GS+H	53	0.1771%	Lautropia (g) uncultured beta (s)
	F-GS	51	0.2522%	Lautropia (g) metagenome (s)
	F-H	45	0.3670%	Lautropia (g) uncultured beta (s)
	F-GS+H	44	0.1470%	F-GS+H: Lautropia (g) metagenome (s)
	F-GYP	40	0.2603%	Lautropia (g) uncultured beta (s)
	F-ALL	33	0.2355%	Lautropia (g) uncultured beta (s)
	F-GYP	26	0.1692%	Lautropia (g) metagenome (s)
	L-B	25	0.1520%	Lautropia (g)
	F-ALL	24	0.1713%	Lautropia (g) metagenome (s)
	F-GS+H	20	0.0668%	Limnobacter (g)
	L-B	16	0.0973%	L-B: Limnobacter (g)
	F-B	12	0.0474%	Lautropia (g) metagenome (s)
	F-GYP	12	0.0781%	Lautropia (g) uncultured beta (s)
	F-GS	7	0.0346%	Limnobacter (g)
	F-GYP	7	0.0456%	Limnobacter (g)
	F-GS	2	0.0099%	Lautropia (g)
Candidatus Berkiella (g) [16]	F-GYP	12	0.0781%	uncultured bacterium (s)
	F-GS+H	9	0.0301%	-
	F-B	7	0.0277%	uncultured bacterium (s)
Candidatus Nitrotoga (g) [17] Candidatus Nitrotoga (g) [17]	F-GS	35	0.1731%	uncultured beta (s)
	F-GS+H	26	0.0869%	F-GS+H: uncultured beta (s)
	F-ALL	25	0.1784%	uncultured beta (s)
	F-H	22	0.1794%	uncultured beta (s)
	F-GYP	14	0.0911%	uncultured beta (s)
Caulobacteraceae (f) [18]	L-GYP	55	0.3578%	Caulobacter (g)
	F-GS+H	24	0.0802%	Brevundimonas (g)

Aerobic Bacteria	Amendment	No. of Reads	Perc. of Total Reads	Classification
	F-B	14	0.0553%	Brevundimonas (g)
	F-GYP	8	0.0521%	Brevundimonas (g)
	F-ALL	8	0.0571%	Brevundimonas (g)
	L-ALL	4	0.0244%	Caulobacter (g)
Chryseolinea (g) [19]	F-GS+H	21	0.0702%	uncultured Flammeovirgaceae (s)
	F-ALL	16	0.1142%	uncultured Flammeovirgaceae (s)
	F-H	11	0.0897%	uncultured Flammeovirgaceae (s)
	F-B	11	0.0435%	uncultured Flammeovirgaceae (s)
	F-GYP	10	0.0651%	uncultured Flammeovirgaceae (s)
	F-GS	3	0.0148%	uncultured Flammeovirgaceae (s)
Coxiella (g) [20]	L-H	32	0.1600%	-
	F-GS+H	14	0.0468%	uncultured bacterium (s)
	F-B	12	0.0474%	uncultured bacterium (s)
	F-B	6	0.0237%	-
	L-GS	5	0.0360%	-
	L-H	4	0.0200%	uncultured bacterium (s)
	F-GYP	3	0.0195%	uncultured bacterium (s)
	L-GYP	2	0.0130%	-
	L-GYP	2	0.0130%	uncultured bacterium (s)
Defluviicoccus (g) [21]	L-GS	9	0.0649%	metagenome (s)
	L-GYP	6	0.0390%	metagenome (s)
	F-GS+H	5	0.0167%	-
	L-GS+H	5	0.0276%	metagenome (s)
	F-B	5	0.0198%	uncultured bacterium (s)
	L-B	3	0.0182%	-
	F-GS	3	0.0148%	uncultured Alphaproteobacteria (s)
Defluviicoccus (g) [21]	F-GYP	2	0.0130%	metagenome (s)
	F-B	2	0.0079%	metagenome (s)

Aerobic Bacteria	Amendment	No. of Reads	Perc. of Total Reads	Classification
Dokdonella (g) [22]	F-GS+H	4	0.0134%	-
	F-ALL	2	0.0143%	-
Dongia (g) [23]	F-GS+H	19	0.0635%	metagenome (s)
	F-H	14	0.1142%	metagenome (s)
	F-B	14	0.0553%	metagenome (s)
	F-ALL	12	0.0856%	metagenome (s)
	F-GS	8	0.0396%	metagenome (s)
	F-GYP	7	0.0456%	metagenome (s)
	L-GYP	3	0.0195%	metagenome (s)
Ferruginibacter (g) [24]	F-GYP	3	0.0195%	-
	F-ALL	3	0.0214%	-
Frankia (o) [25]	F-GS+H	4	0.0134%	Frankiales (f) Frankiales (g) uncultured bacterium (s)
Gemmatales (o) [26]	F-GS+H	35	0.1169%	Gemmataceae (f) uncultured (g) metagenome (s)
	F-B	31	0.1225%	Gemmataceae (f) uncultured (g) metagenome (s)
	F-GS+H	20	0.0668%	Gemmataceae (f) Zavarzinella (g)
	F-ALL	19	0.1356%	Gemmataceae (f) uncultured (g) metagenome (s)
	F-GYP	16	0.1041%	Gemmataceae (f) uncultured (g) metagenome (s)
	F-B	15	0.0593%	Gemmataceae (f) uncultured (g) uncultured_bacterium (s)
	F-B	10	0.0395%	Gemmataceae (f) Zavarzinella (g)
	F-GYP	6	0.0390%	Gemmataceae (f) Zavarzinella (g)
	F-GS+H	6	0.0200%	Gemmataceae (f) uncultured (g) uncultured_bacterium (s)
	F-GYP	4	0.0260%	Gemmataceae (f) Fimbriiglobus (g)
Gemmatales (o) [26]	F-B	4	0.0158%	Gemmataceae (f) Gemmata (g) metagenome (s)

Aerobic Bacteria	Amendment	No. of Reads	Perc. of Total Reads	Classification
	F-GS	4	0.0198%	Gemmataceae (f) uncultured (g) uncultured_bacterium (s)
	F-GS+H	3	0.0100%	Gemmataceae (f) Fimbriiglobus (g)
	F-GYP	3	0.0195%	Gemmataceae (f) Gemmata (g) metagenome (s)
	F-GYP	3	0.0195%	Gemmataceae (f) uncultured (g) uncultured_bacterium (s)
	F-H	2	0.0163%	Gemmataceae (f) Zavarzinella (g)
	F-GS	2	0.0099%	Gemmataceae (f) Zavarzinella (g)
Gemmatimonadales (o) [27,28,29]	F-GYP	24	0.1562%	Gemmatimonadaceae (f) uncultured (g)
	F-ALL	18	0.1285%	Gemmatimonadaceae (f) uncultured (g)
	F-GS	11	0.0544%	Gemmatimonadaceae (f) uncultured (g)
	F-GYP	9	0.0586%	Gemmatimonadaceae (f) Gemmatimonas (g) uncultured Gemmatimonas (s)
	F-B	8	0.0316%	Gemmatimonadaceae (f) uncultured (g)
	F-B	8	0.0316%	Gemmatimonadaceae (F) uncultured (g) metagenome (s)
	F-GYP	7	0.0456%	Gemmatimonadaceae (F) uncultured (g) metagenome (s)
	F-GS+H	6	0.0200%	Gemmatimonadaceae (f) uncultured (g)
	F-ALL	6	0.0428%	Gemmatimonadaceae (F) uncultured (g) metagenome (s)
Gemmatimonadales (o) [27,28]	F-GS+H	3	0.0100%	Gemmatimonadaceae (F) uncultured (g) metagenome (s)
	F-GYP	2	0.0130%	Gemmatimonadaceae (f) uncultured (g) Gemmatimonadetes bacterium (s)
Gemmatimonadetes Uncultured (s) [27,28]	F-ALL	5	0.0357%	-
Gemmatimonadetes (c) [27,28,29]	F-GYP	24	0.1562%	Gemmatimonadales (o) Gemmatimonadaceae (f) uncultured (g)
	F-ALL	18	0.1285%	Gemmatimonadales (o) Gemmatimonadaceae (f) uncultured (g)
	F-GS	11	0.0544%	Gemmatimonadales (o) Gemmatimonadaceae (f) uncultured (g)

Aerobic Bacteria	Amendment	No. of Reads	Perc. of Total Reads	Classification
	F-GYP	9	0.0586%	Gemmatimonadales (o) Gemmatimonadaceae (f) Gemmatimonas (g) uncultured Gemmatimonas (s)
	F-B	8	0.0316%	Gemmatimonadales (o) Gemmatimonadaceae (f) uncultured (g)
	F-B	8	0.0316%	Gemmatimonadales (o) Gemmatimonadaceae (f) uncultured (g) metagenome (s)
	F-GYP	7	0.0456%	Gemmatimonadales (o) Gemmatimonadaceae (f) uncultured (g) metagenome (s)
	F-GS+H	6	0.0200%	Gemmatimonadales (o) Gemmatimonadaceae (f) uncultured (g)
	F-ALL	6	0.0428%	Gemmatimonadales (o) Gemmatimonadaceae (f) uncultured (g) metagenome (s)
	F-GS+H	3	0.0100%	Gemmatimonadales (o) Gemmatimonadaceae (f) uncultured (g) metagenome (s)
	F-GYP	2	0.0130%	Gemmatimonadales(o) Gemmatimonadaceae (f) uncultured (g) Gemmatimonadetes bacterium (s)
Gemmatimonas (g) [27,28,29]	F-GYP	9	0.0586%	uncultured Gemmatimonas (s)
Haliangium (g) [30]	F-GS+H	12	0.0401%	uncultured bacterium (s)
	F-GYP	5	0.0325%	uncultured bacterium (s)
	F-B	5	0.0198%	uncultured bacterium (s)
	F-ALL	3	0.0214%	-
	F-GS	13	0.0643%	-
	F-B	13	0.0514%	-
	L-H	20	0.1000%	Hyphomicrobium (g)
	L-GS	21	0.1514%	Hyphomicrobium (g)
	L-GYP	27	0.1756%	Hyphomicrobium (g)
	L-ALL	45	0.2743%	Hyphomicrobium (g)
	L-B	13	0.0790%	Hyphomicrobium (g)

Aerobic Bacteria	Amendment	No. of Reads	Perc. of Total Reads	Classification
Hyphomicrobiaceae (f) [31,32]	F-H	102	0.8318%	Hyphomicrobium (g)
	F-GS	100	0.4946%	Hyphomicrobium (g)
	F-GS+H	322	1.0759%	Hyphomicrobium (g)
	F-GYP	89	0.5792%	Hyphomicrobium (g)
	F-ALL	92	0.6566%	Hyphomicrobium (g)
	F-B	203	0.8022%	Hyphomicrobium (g)
	F-H	9	0.0734%	Hyphomicrobium (g) metagenome (s)
Hyphomicrobiaceae (f) [31,32]	F-GS	41	0.2028%	Pedomicrobium (g)
	F-GS+H	91	0.3041%	Pedomicrobium (g)
	F-GYP	32	0.2082%	Pedomicrobium (g)
	F-ALL	28	0.1998%	Pedomicrobium (g)
	F-B	55	0.2174%	Pedomicrobium (g)
	L-GYP	8	0.0520%	Pedomicrobium (g) metagenome (s)
	F-H	32	0.2610%	Pedomicrobium (g) uncultured bacterium (s)
	F-GS+H	36	0.1203%	Pedomicrobium (g) uncultured bacterium (s)
	F-GYP	28	0.1822%	Pedomicrobium (g) uncultured bacterium (s)
	F-ALL	29	0.2070%	Pedomicrobium (g) uncultured bacterium (s)
	F-B	41	0.1620%	Pedomicrobium (g) uncultured bacterium (s)
	F-H	5	0.0408%	uncultured (g) metagenome (s)
	F-GS	7	0.0346%	uncultured (g) metagenome (s)
	F-GS+H	25	0.0835%	uncultured (g) metagenome (s)
	F-GYP	10	0.0651%	uncultured (g) metagenome (s)
	F-ALL	13	0.0928%	uncultured (g) metagenome (s)
Ilumatobacteraceae (f) [33]	F-ALL	28	0.1998%	-
	F-GS+H	25	0.0835%	-

Aerobic Bacteria	**Amendment**	**No. of Reads**	**Perc. of Total Reads**	**Classification**
	F-B	22	0.0869%	-
	F-GS+H	22	0.0735%	-
	F-GYP	21	0.1367%	-
	F-GS	16	0.0791%	-
	F-GS	16	0.0791%	CL500-29 marine group (g)
	F-GS	16	0.0791%	CL500-29 marine group (g) metagenome (s)
	F-GYP	15	0.0976%	CL500-29 marine group (g) metagenome (s)
	F-H	14	0.1142%	CL500-29 marine group (g) metagenome (s)
	F-B	14	0.0553%	CL500-29 marine group (g) metagenome (s)
	F-ALL	13	0.0928%	Ilumatobacter (g)
	F-ALL	8	0.0571%	Ilumatobacter (g)
	F-GS+H	7	0.0234%	Ilumatobacter (g)
	F-H	2	0.0163%	Ilumatobacter (g)
Labrys (g) [34] Labrys (g) [34]	F-B	16	0.0632%	metagenome (s)
	F-GS+H	12	0.0401%	metagenome (s)
	F-GS	9	0.0445%	metagenome (s)
	F-H	6	0.0489%	metagenome (s)
Lautropia (g) [35]	L-B	114	0.6931%	uncultured beta (s)
	F-B	78	0.3083%	uncultured beta (s)
	L-GS+H	59	0.3253%	uncultured beta (s)
	F-GS+H	53	0.1771%	uncultured beta (s)
	F-GS	51	0.2522%	metagenome (s)
	F-H	45	0.3670%	uncultured beta (s)
	F-GS+H	44	0.1470%	metagenome (s)
	F-GYP	40	0.2603%	uncultured beta (s)
	F-ALL	33	0.2355%	uncultured beta (s)

Aerobic Bacteria	Amendment	No. of Reads	Perc. of Total Reads	Classification
	F-GYP	26	0.1692%	metagenome (s)
	L-B	25	0.1520%	-
	F-ALL	24	0.1713%	F-ALL: metagenome (s)
	F-B	12	0.0474%	metagenome (s)
	L-GYP	12	0.0781%	uncultured beta (s)
	F-GS	2	0.0099%	-
Legionella (g) [36]	F-ALL	21	0.1499%	-
	F-B	20	0.0790%	Legionella lytica (s)
	F-GS+H	17	0.0568%	-
	F-GS	12	0.0594%	-
	F-GS+H	12	0.0401%	Legionella lytica (s)
	F-ALL	12	0.0856%	uncultured bacterium (s)
	L-B	11	0.0669%	uncultured bacterium (s)
	L-B	7	0.0426%	-
	L-GS+H	7	0.0386%	uncultured bacterium (s)
	F-H	7	0.0571%	uncultured bacterium (s)
	F-GS	6	0.0297%	F-GS: uncultured bacterium (s)
	F-B	6	0.0237%	uncultured bacterium (s)
	L-H	5	0.0250%	uncultured bacterium (s)
Legionella (g) [36]	F-GS+H	5	0.0167%	uncultured bacterium (s)
	L-GYP	2	0.0130%	-
	F-GYP	2	0.0130%	uncultured bacterium (s)
	L-ALL	76	0.4633%	Leptospiraceae (f) RBG-16-49-21 (g)
	L-H	49	0.2450%	Leptospiraceae (f) RBG-16-49-21 (g) uncultured bacterium (s)
	L-H	39	0.1950%	Leptospiraceae (f) RBG-16-49-21 (g)
	L-GYP	34	0.2212%	Leptospiraceae (f) RBG-16-49-21 (g)
	L-ALL	30	0.1829%	Leptospiraceae (f) RBG-16-49-21 (g) uncultured bacterium (s)

Aerobic Bacteria	Amendment	No. of Reads	Perc. of Total Reads	Classification
Leptospira (o) [37]	L-GS	27	0.1946%	Leptospiraceae (f) RBG-16-49-21 (g) uncultured bacterium (s)
	L-B	25	0.1520%	Leptospiraceae (f) RBG-16-49-21 (g) uncultured bacterium (s)
	L-GS+H	21	0.1158%	Leptospiraceae (f) RBG-16-49-21 (g) uncultured bacterium (s)
	L-GS+H	17	0.0937%	Leptospiraceae (f) RBG-16-49-21 (g)
	L-B	13	0.0790%	Leptospiraceae (f) RBG-16-49-21 (g)
	L-GYP	11	0.0716%	Leptospiraceae (f) RBG-16-49-21 (g) uncultured bacterium (s)
	L-H	11	0.0550%	Leptospiraceae (f) RBG-16-49-21(g) uncultured organism (s)
	F-GS+H	9	0.0301%	Leptospiraceae (f) RBG-16-49-21 (g) uncultured Leptospira (s)
	F-B	9	0.0356%	Leptospiraceae (f) RBG-16-49-21 (g) uncultured Leptospira (s)
	F-H	8	0.0652%	Leptospiraceae (f) RBG-16-49-21 (g) uncultured Leptospira (s)
	L-GS	7	0.0505%	Leptospiraceae (f) RBG-16-49-21 (g)
	F-GYP	7	0.0456%	Leptospiraceae (f) RBG-16-49-21 (g)
Leptospira (o) [37]	F-GYP	7	0.0456%	Leptospiraceae (f) Turneriella (g) metagenome (s)
	F-GS	5	0.0247%	Leptospiraceae (f) RBG-16-49-21 (g) uncultured Leptospira (s)
	F-H	4	0.0326%	F-H: Leptospiraceae (f) RBG-16-49-21 (g) metagenome (s)
	F-ALL	3	0.0214%	Leptospiraceae (f) RBG-16-49-21 (g)
	F-GS+H	3	0.0100%	Leptospiraceae (f) RBG-16-49-21 (g) metagenome (s)
	F-ALL	3	0.0214%	Leptospiraceae (f) Turneriella (g) metagenome (s)
	F-GS+H	2	0.0067%	Leptospiraceae (f) Turneriella (g) metagenome (s)
Mesorhizobium (g) [38]	F-GS+H	214	0.7150%	-

Aerobic Bacteria	Amendment	No. of Reads	Perc. of Total Reads	Classification
	F-B	199	0.7864%	-
	F-ALL	95	0.6780%	-
	F-GYP	70	0.4555%	-
	F-H	55	0.4485%	-
	F-GS	30	0.1484%	-
Moraxellaceae (f) [39]	F-H	21	0.1713%	Alkanindiges (g)
	F-ALL	12	0.0856%	Alkanindiges (g)
	F-GYP	10	0.0651%	Alkanindiges (g)
	L-ALL	8	0.0488%	Acinetobacter (g)
	L-GS+H	6	0.0331%	uncultured (g)
	F-B	5	0.0198%	Psychrobacter (g)
	F-B	5	0.0198%	[Agitococcus] lubricus_group (g)
	F-GS	4	0.0198%	Alkanindiges (g)
Mycobacterium (g) [40]	F-B	21	0.0830%	-
	F-GS+H	14	0.0468%	-
	F-GYP	14	0.0911%	-
	F-ALL	11	0.0785%	-
	L-GS+H	6	0.0331%	-
	F-GS	6	0.0297%	-
	L-ALL	5	0.0305%	-
	L-GYP	4	0.0260%	-
Myxococcales bacterium (s) [41]	F-H	4	0.0326%	Myxococcales_bacterium
Myxococcales (o) [41]	F-GS+H	3	0.0100%	Myxococcaceae (f) P3OB-42 (g)
	F-GS	3	0.0148%	Myxococcaceae (f) P3OB-42 (g)
	F-B	2	0.0079%	Myxococcaceae (f) P3OB-42 (g) uncultured delta (s)
	L-ALL	2	0.0122%	Myxococcaceae (f) P3OB-42 (g) uncultured organism (s)

Aerobic Bacteria	Amendment	No. of Reads	Perc. of Total Reads	Classification
Nannocystaceae (f) [42]	F-GYP	117	0.7614%	Nannocystis (g) metagenome (s)
	F-ALL	84	0.5995%	Nannocystis (g) metagenome (s)
	F-GS+H	81	0.2706%	Nannocystis (g) metagenome (s)
	F-H	70	0.5709%	Nannocystis (g) metagenome (s)
	F-B	65	0.2569%	Nannocystis (g) metagenome (s)
	F-GS	45	0.2226%	Nannocystis (g) metagenome (s)
	F-GS+H	17	0.0568%	Nannocystis (g) uncultured bacterium (s)
	F-B	17	0.0672%	uncultured (g) uncultured delta (s)
	F-B	15	0.0593%	Nannocystis (g) uncultured bacterium (s)
	F-GS+H	13	0.0434%	uncultured (g) uncultured delta (s)
	L-GYP	8	0.0520%	Nannocystis (g) metagenome (s)
	F-GYP	6	0.0390%	uncultured (g) uncultured delta (s)
	L-GS	5	0.0360%	Nannocystis (g) metagenome (s)
Nannocystaceae (f) [42]	F-ALL	5	0.0357%	uncultured (g) uncultured delta (s)
	L-B	4	0.0243%	Nannocystis (g) metagenome (s)
	L-ALL	3	0.0183%	Nannocystis (g) metagenome (s)
Nannocystis (g) [42]	F-GYP	117	0.7614%	metagenome (s)
	F-ALL	84	0.5995%	metagenome (s)
	F-GS+H	81	0.2706%	metagenome (s)
	F-H	70	0.5709%	metagenome (s)
	F-B	65	0.2569%	metagenome (s)
	F-GS	45	0.2226%	metagenome (s)
	F-GS+H	17	0.0568%	metagenome (s)
	F-B	15	0.0593%	uncultured bacterium (s)
	L-GYP	8	0.0520%	metagenome (s)
	L-GS	5	0.0360%	metagenome (s)
	L-B	4	0.0243%	metagenome (s)
	L-ALL	3	0.0183%	metagenome (s)

Aerobic Bacteria	Amendment	No. of Reads	Perc. of Total Reads	Classification
Nitrosomonadaceae (f) [43]	L-B	42	0.2554%	Nitrosomonas (g)
	L-GS+H	41	0.2261%	Nitrosomonas (g)
	F-H	31	0.2528%	Nitrosomonas (g)
	F-GS+H	26	0.0869%	Nitrosomonas (g) Nitrosomonas nitrosa (s)
	F-ALL	25	0.1784%	Nitrosomonas (g)
	F-GS	24	0.1187%	Nitrosomonas (g)
	F-GS+H	24	0.0802%	mle1-7 (g)
	F-B	17	0.0672%	Nitrosomonas (g)
	F-B	17	0.0672%	mle1-7 (g)
	F-GYP	15	0.0976%	Nitrosomonas (g) Nitrosomonas nitrosa (s)
	F-GS	13	0.0643%	Nitrosomonas (g) Nitrosomonas nitrosa (s)
	F-B	13	0.0514%	Nitrosomonas (g) Nitrosomonas nitrosa (s)
	L-H	11	0.0550%	Nitrosomonas (g)
	F-GS+H	11	0.0368%	Nitrosomonas (g)
Nitrosomonadaceae (f) [43]	F-GYP	10	0.0651%	Nitrosomonas (g)
	L-GS	7	0.0505%	Nitrosomonas (g)
	F-ALL	7	0.0500%	mle1-7 (g)
	F-GS+H	3	0.0100%	MND1 (g)
	F-H	3	0.0245%	mle1-7 (g)
	F-GS+H	35	0.1169%	Oikopleura (g)
	F-B	29	0.1146%	Oikopleura (g)
	L-GS+H	29	0.1599%	Pedosphaeraceae (g)
	L-ALL	23	0.1402%	Pedosphaeraceae (g)
	L-GS	21	0.1514%	Pedosphaeraceae (g)
	L-H	13	0.0650%	Pedosphaeraceae (g)
	L-B	11	0.0669%	L-B: Pedosphaeraceae (g)

Aerobic Bacteria	Amendment	No. of Reads	Perc. of Total Reads	Classification
Pedosphaeraceae (f) [44]	F-B	11	0.0435%	Pedosphaeraceae (g) uncultured bacterium (s)
	F-ALL	10	0.0714%	F-ALL: Oikopleura (g)
	L-H	10	0.0500%	L-H: Pedosphaera (g)
	F-GYP	9	0.0586%	F-GYP: Oikopleura (g)
	F-B	9	0.0356%	SH3-11 (g) uncultured verrucomicrobium (s)
	L-GS+H	8	0.0441%	Pedosphaeraceae (g) metagenome (s)
	L-H	7	0.0350%	-
	F-GYP	7	0.0456%	Pedosphaeraceae (g) uncultured bacterium (s)
	F-GS+H	7	0.0234%	SH3-11 (g) uncultured verrucomicrobium (s)
	F-GS+H	6	0.0200%	Pedosphaeraceae (g)
	L-B	6	0.0365%	Pedosphaeraceae (g) metagenome (s)
	F-H	6	0.0489%	SH3-11 (g) uncultured verrucomicrobium (s)
	F-ALL	6	0.0428%	SH3-11 (g) uncultured verrucomicrobium (s)
Pedosphaeraceae (f) [44]	L-GYP	5	0.0325%	Pedosphaeraceae (g)
	L-GS+H	5	0.0276%	Pedosphaeraceae (g) uncultured bacterium (s)
	L-GS+H	4	0.0221%	-
	L-ALL	4	0.0244%	-
	F-B	4	0.0158%	Oikopleura (g) metagenome (s)
	F-GS+H	3	0.0100%	-
	F-GYP	3	0.0195%	Oikopleura (g) metagenome (s)
	L-GS	3	0.0216%	Pedosphaeraceae (g) metagenome (s)
	F-GYP	3	0.0195%	SH3-11 (g) uncultured verrucomicrobium (s)
	L-GS	2	0.0144%	-

Aerobic Bacteria	Amendment	No. of Reads	Perc. of Total Reads	Classification
	F-GYP	2	0.0130%	-
	F-ALL	2	0.0143%	-
Peredibacter (g) [45]	F-H	14	0.1142%	microbial mat (s)
	F-GS+H	10	0.0334%	microbial mat (s)
	L-GS	8	0.0577%	Peredibacter starrii (s)
	F-GS	4	0.0198%	metagenome (s)
	F-ALL	3	0.0214%	microbial mat (s)
Phenylobacterium (g) [46]	F-B	29	0.1146%	-
	F-GS+H	19	0.0635%	-
	F-GYP	19	0.1236%	-
	L-GYP	12	0.0781%	-
	L-B	12	0.0730%	-
Phenylobacterium (g) [46]	L-ALL	6	0.0366%	-
	L-GS+H	5	0.0276%	-
Pirellula (f) [47]	F-B	125	0.4940%	Planctomycetes bacterium (s)
	F-GS+H	105	0.3508%	uncultured organism (s)
	F-B	90	0.3557%	uncultured organism (s)
	F-B	73	0.2885%	-
	F-GS+H	70	0.2339%	Planctomycetes bacterium (s)
	F-GS+H	63	0.2105%	-
	F-GYP	47	0.3059%	uncultured organism (s)
	F-GYP	46	0.2993%	Planctomycetes bacterium (s)
	F-GS+H	39	0.1303%	uncultured bacterium (s)
	F-B	39	0.1541%	uncultured bacterium (s)
	F-GYP	33	0.2147%	-
	F-GS+H	26	0.0869%	uncultured Pirellula (s)
	F-GS	25	0.1236%	-
	F-GS	25	0.1236%	Planctomycetes bacterium (s)

Aerobic Bacteria	Amendment	No. of Reads	Perc. of Total Reads	Classification
Pirellula (f) [47]	F-GS	25	0.1236%	uncultured organism (s)
	F-ALL	25	0.1784%	uncultured organism (s)
	F-ALL	23	0.1641%	-
	F-ALL	23	0.1641%	Planctomycetes bacterium (s)
	F-GS+H	23	0.0768%	bacterium enrichment (s)
	F-B	23	0.0909%	metagenome (s)
	F-GYP	19	0.1236%	uncultured bacterium (s)
	F-ALL	17	0.1213%	uncultured bacterium (s)
	L-GS+H	15	0.0827%	uncultured bacterium (s)
	F-H	14	0.1142%	Planctomycetes bacterium (s)
	F-B	13	0.0514%	uncultured Pirellula (s)
	F-B	13	0.0514%	bacterium enrichment (s)
	F-GYP	10	0.0651%	uncultured Pirellula (s)
	F-H	9	0.0734%	uncultured bacterium (s)
	F-GYP	8	0.0521%	Pirellula sp. (s)
	F-GS+H	6	0.0200%	Pirellula staleyi (s)
	F-GYP	6	0.0390%	metagenome (s)
	L-B	5	0.0304%	uncultured bacterium (s)
	F-ALL	4	0.0285%	uncultured Pirellula (s)
	F-H	4	0.0326%	bacterium enrichment (s)
	L-GS	3	0.0216%	uncultured bacterium (s)
	F-GS	2	0.0099%	bacterium enrichment (s)
Polyangiaceae Uncultured (s) [48]	L-GS+H	12	0.0662%	-
	L-H	6	0.0300%	-
	L-GS	6	0.0432%	-
	L-B	5	0.0304%	-
	F-GS+H	24	0.0802%	uncultured (g) metagenome (s)
	F-B	19	0.0751%	uncultured (g) metagenome (s)

Aerobic Bacteria	Amendment	No. of Reads	Perc. of Total Reads	Classification
Polyangiaceae (f) [48]	F-B	10	0.0395%	Pajaroellobacter (g) uncultured delta (s)
	F-GS+H	9	0.0301%	Pajaroellobacter (g)
	F-GYP	9	0.0586%	uncultured (g) metagenome (s)
	F-ALL	8	0.0571%	uncultured (g) metagenome (s)
	F-B	6	0.0237%	Pajaroellobacter (g) uncultured Polyangiaceae (s)
	F-H	5	0.0408%	uncultured (g) metagenome (s)
	L-H	4	0.0200%	uncultured (g) metagenome (s)
Polyangiaceae (f) [48]	F-GS	4	0.0198%	uncultured (g) metagenome (s)
Porphyrobacter (g) [49]	F-ALL	105	0.7494%	-
	F-GS+H	93	0.3107%	-
	F-GS	72	0.3561%	-
	F-GYP	71	0.4620%	-
	F-B	48	0.1897%	-
	F-H	36	0.2936%	-
	L-B	13	0.0790%	-
	L-GS+H	5	0.0276%	-
Pseudoxanthobacter (g) [50,51]	F-GS+H	31	0.1036%	uncultured Alphaproteobacteria (s)
	F-GS	21	0.1039%	uncultured Alphaproteobacteria (s)
	F-B	21	0.0830%	uncultured Alphaproteobacteria (s)
Pseudoxanthomonas (g) [52]	F-GYP	23	0.1497%	-
	F-ALL	22	0.1570%	-
	F-GS+H	5	0.0167%	-
	F-B	5	0.0198%	-
	L-GYP	4	0.0260%	Pseudoxanthomonas mexicana (s)
Psychrobacter (g) [53]	F-B	5	0.0198%	-
	F-GS+H	214	0.7150%	Mesorhizobium (g)
	F-B	199	0.7864%	Mesorhizobium (g)

Aerobic Bacteria	Amendment	No. of Reads	Perc. of Total Reads	Classification
Rhizobiaceae (f) [54]	F-GS+H	189	0.6315%	Hoeflea (g)
	F-B	113	0.4466%	Hoeflea (g)
	F-ALL	95	0.6780%	Mesorhizobium (g)
	F-GYP	70	0.4555%	Mesorhizobium (g)
	F-GS+H	67	0.2239%	-
	F-H	55	0.4485%	Mesorhizobium (g)
	F-ALL	54	0.3854%	Hoeflea (g)
	F-GYP	53	0.3449%	Hoeflea (g)
	F-B	38	0.1502%	-
	F-GS	32	0.1583%	Hoeflea (g)
	F-H	30	0.2447%	Hoeflea (g)
	F-GS	30	0.1484%	Mesorhizobium (g)
Rhizobiaceae (f) [54]	F-GS	22	0.1088%	Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium (g)
	F-GYP	19	0.1236%	-
	F-ALL	19	0.1356%	-
	L-ALL	19	0.1158%	Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium (g)
	F-H	13	0.1060%	-
	F-GS	12	0.0594%	-
	F-H	12	0.0979%	Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium (g)
Saprospiraceae (f) [55]	F-H	449	3.6617%	uncultured (g) uncultured bacterium (s)
	F-GS+H	391	1.3064%	uncultured (g) uncultured bacterium (s)
	F-B	235	0.9287%	uncultured (g) uncultured bacterium (s)
	F-GS	231	1.1425%	uncultured (g) uncultured bacterium (s)
	F-GS+H	187	0.6248%	uncultured (s)
	F-B	159	0.6284%	uncultured (s)
	F-ALL	151	1.0776%	uncultured (g) uncultured bacterium (s)

Aerobic Bacteria	Amendment	No. of Reads	Perc. of Total Reads	Classification
Saprospiraceae (f) [55]	F-GYP	146	0.9501%	uncultured (g) uncultured bacterium (s)
	F-ALL	78	0.5567%	uncultured (s)
	F-GYP	75	0.4881%	uncultured (s)
	F-GS+H	52	0.1737%	Lewinella (g) uncultured Saprospiraceae (s)
	F-H	39	0.3181%	uncultured (s)
	F-GS	30	0.1484%	uncultured (s)
	F-B	29	0.1146%	Lewinella (g) uncultured Saprospiraceae (s)
	F-GYP	24	0.1562%	Lewinella (g) uncultured Saprospiraceae (s)
	F-ALL	22	0.1570%	Lewinella (g) uncultured Saprospiraceae (s)
	F-GS	19	0.0940%	Lewinella (g) uncultured Saprospiraceae (s)
	F-GS+H	18	0.0601%	uncultured (g) metagenome (s)
	F-H	17	0.1386%	uncultured bacterium (s)
	F-B	15	0.0593%	uncultured (g) metagenome (s)
	L-H	15	0.0750%	uncultured (g) uncultured bacterium (s)
	F-GS+H	13	0.0434%	Phaeodactylibacter (g) metagenome (s)
	F-GS	12	0.0594%	Phaeodactylibacter (g) metagenome (s)
	F-GYP	11	0.0716%	uncultured (g) metagenome (s)
	F-H	10	0.0816%	uncultured (g) metagenome (s)
	F-GS+H	9	0.0301%	Lewinella (g) uncultured bacterium (s)
	F-GS	9	0.0445%	uncultured bacterium (s)
	F-GS+H	9	0.0301%	uncultured bacterium (s)
	F-B	9	0.0356%	uncultured bacterium (s)
	F-ALL	8	0.0571%	uncultured bacterium (s)
	F-ALL	8	0.0571%	uncultured (g) metagenome (s)
	F-ALL	7	0.0500%	Phaeodactylibacter (g) metagenome (s)

Aerobic Bacteria	**Amendment**	**No. of Reads**	**Perc. of Total Reads**	**Classification**
Saprospiraceae (f) [55]	F-GS+H	7	0.0234%	uncultured (g) uncultured Saprospiraceae (s)
	L-B	7	0.0426%	uncultured (g) uncultured bacterium (s)
	F-H	6	0.0489%	-
	F-GS	6	0.0297%	-
	F-GYP	6	0.0390%	Phaeodactylibacter (g) metagenome (s)
	F-GS+H	5	0.0167%	Phaeodactylibacter (g)
	F-B	4	0.0158%	Lewinella (g) uncultured bacterium (s)
	F-GS	4	0.0198%	uncultured (g) Tetraselmis sp. (s)
	F-H	3	0.0245%	Lewinella (g) uncultured Saprospiraceae (s)
	F-ALL	3	0.0214%	uncultured (g) Tetraselmis sp. (s)
	L-H	2	0.0100%	Lewinella (g) uncultured Saprospiraceae (s)
Solimonas Uncultured (s) [56,57]	F-GS+H	3	0.0100%	-
Solirubrobacterales (o) [57,58]	L-GS+H	32	0.1764%	L-GS+H: 67-14 (f) 67-14 (g)
	F-B	32	0.1265%	F-B: 67-14 (f) 67-14 (g) olirubrobacterales bacterium (s)
	F-ALL	29	0.2070%	67-14 (f) 67-14 (g) olirubrobacterales bacterium (s)
	F-GS+H	25	0.0835%	67-14 (f) 67-14 (g) olirubrobacterales bacterium (s)
	F-H	19	0.1550%	67-14 (f) 67-14 (g) olirubrobacterales bacterium (s)
	L-GS+H	18	0.0993%	67-14 (f) 67-14 (g) uncultured bacterium (s)
	L-H	17	0.0850%	L-H: 67-14 (f) 67-14 (g)
	F-GS+H	15	0.0501%	67-14 (f) 67-14 (g) uncultured bacterium (s)
	F-GYP	13	0.0846%	67-14 (f) 67-14 (g) uncultured bacterium (s)
	L-GS	12	0.0865%	L-GS: 67-14 (f) 67-14 (g)

Aerobic Bacteria	Amendment	No. of Reads	Perc. of Total Reads	Classification
Solirubrobacterales (o) [57,58]	F-B	11	0.0435%	F-B: 67-14 (f) 67-14 (g)
	L-ALL	11	0.0671%	67-14 (f) 67-14 (g) uncultured bacterium (s)
	L-B	10	0.0608%	67-14 (f) 67-14 (g)
	L-GYP	10	0.0651%	67-14 (f) 67-14 (g) uncultured bacterium (s)
	L-ALL	9	0.0549%	67-14 (f) 67-14 (g)
	L-GS	9	0.0649%	67-14 (f) 67-14 (g) uncultured bacterium (s)
	F-B	9	0.0356%	67-14 (f) 67-14 (g) uncultured bacterium (s)
	F-GS	8	0.0396%	67-14 (f) 67-14 (g) uncultured bacterium (s)
	F-ALL	7	0.0500%	67-14 (f) 67-14 (g) uncultured bacterium (s)
	F-GYP	5	0.0325%	67-14 (f) 67-14 (g) olirubrobacterales bacterium (s)
	L-GS+H	4	0.0221%	Solirubrobacteraceae (f)
	L-GS	2	0.0144%	67-14 (f) 67-14 (g) uncultured_soil (s)
Sphingomonadaceae (f) [59]	F-ALL	105	0.7494%	Porphyrobacter (g)
	F-GS+H	93	0.3107%	Porphyrobacter (g)
	F-GS	72	0.3561%	Porphyrobacter (g)
	F-GYP	71	0.4620%	Porphyrobacter (g)
	F-B	48	0.1897%	Porphyrobacter (g)
	F-B	48	0.1897%	uncultured (g)
	F-B	44	0.1739%	Sphingopyxis (g)
	F-GS+H	39	0.1303%	uncultured (g)
	F-H	36	0.2936%	Porphyrobacter (g)
	F-GS+H	33	0.1103%	Sphingopyxis (g)
	F-GS+H	31	0.1036%	Novosphingobium (g)
	F-ALL	29	0.2070%	Sphingopyxis (g)

Aerobic Bacteria	Amendment	No. of Reads	Perc. of Total Reads	Classification
Sphingomonadaceae (f) [59]	F-B	28	0.1107%	Sphingosinicella (g)
	F-H	28	0.2283%	uncultured (g)
	F-GYP	26	0.1692%	uncultured (g)
	F-B	25	0.0988%	Porphyrobacter (g)
	F-GS+H	23	0.0768%	Sandaracinobacter (g) uncultured bacterium (s)
	F-GYP	23	0.1497%	Sphingopyxis (g)
	F-GS+H	20	0.0668%	Sphingosinicella (g)
	F-ALL	19	0.1356%	uncultured (g)
	F-ALL	17	0.1213%	Novosphingobium (g)
	F-GS	16	0.0791%	uncultured (g)
	L-GYP	15	0.0976%	Altererythrobacter (g)
	L-ALL	15	0.0914%	Altererythrobacter (g)
	F-GS+H	15	0.0501%	Qipengyuania (g)
	F-B	14	0.0553%	Qipengyuania (g)
	F-ALL	14	0.0999%	Sphingosinicella (g)
	L-B	13	0.0790%	Porphyrobacter (g)
	F-ALL	12	0.0856%	Sandaracinobacter (g) uncultured bacterium (s)
	F-GYP	11	0.0716%	Sandaracinobacter (g) uncultured bacterium (s)
	L-B	9	0.0547%	Sphingosinicella (g)
	F-GYP	7	0.0456%	Sphingosinicella (g)
	L-GS+H	5	0.0276%	Porphyrobacter (g)
	L-GYP	5	0.0325%	Sphingosinicella (g)
	F-H	5	0.0408%	Sphingosinicella (g)
	F-H	4	0.0326%	Sandaracinobacter (g) uncultured bacterium (s)
	F-GS	4	0.0198%	Sphingopyxis (g)
	L-GYP	3	0.0195%	DSSF69 (g) uncultured bacterium (s)

Aerobic Bacteria	Amendment	No. of Reads	Perc. of Total Reads	Classification
	F-GS+H	2	0.0067%	-
	F-B	2	0.0079%	-
Stella (g) [60]	F-GS	10	0.0495%	metagenome (s)
	F-ALL	4	0.0285%	-
	L-ALL	2	0.0122%	metagenome (s)
Vampirovibrio (g) [61]	L-H	2	0.0100%	-
Xanthobacteraceae (f) [62,63]	L-B	50	0.3040%	-
	F-GS+H	31	0.1036%	Pseudoxanthobacter (g) uncultured Alphaproteobacteria (s)
	L-ALL	28	0.1707%	Xanthobacter (g)
	F-GS	21	0.1039%	Pseudoxanthobacter (g) uncultured Alphaproteobacteria (s)
	F-B	21	0.0830%	Pseudoxanthobacter (g) uncultured Alphaproteobacteria (s)
	L-GYP	15	0.0976%	Xanthobacter (g)
	F-B	14	0.0553%	-
	F-ALL	14	0.0999%	Pseudorhodoplanes (g)
	F-GYP	12	0.0781%	-
	F-GS	11	0.0544%	-
	L-H	9	0.0450%	Xanthobacter (g)
	L-H	7	0.0350%	Bradyrhizobium (g)
	L-GS+H	7	0.0386%	Bradyrhizobium (g)
	L-B	5	0.0304%	Xanthobacter (g)
	L-GS	2	0.0144%	Pseudorhodoplanes (g)
Xanthomonadaceae (f) [62,63]	F-GS+H	142	0.4745%	Arenimonas (g) uncultured gamma (s)
	F-B	105	0.4150%	Arenimonas (g) uncultured gamma (s)
	F-GYP	56	0.3644%	Arenimonas (g) uncultured gamma (s)
	F-ALL	33	0.2355%	Arenimonas (g) uncultured gamma (s)
	F-GYP	23	0.1497%	Pseudoxanthomonas (g)

Aerobic Bacteria	**Amendment**	**No. of Reads**	**Perc. of Total Reads**	**Classification**
Xanthomonadaceae (f) [62,63]	F-GS	22	0.1088%	Arenimonas (g) uncultured gamma (s)
	F-ALL	22	0.1570%	Pseudoxanthomonas (g)
	F-H	17	0.1386%	Arenimonas (g) uncultured gamma (s)
	F-ALL	10	0.0714%	F-ALL:
	L-H	6	0.0300%	Arenimonas (g) uncultured gamma (s)
	F-GS+H	5	0.0167%	Pseudoxanthomonas (g)
	F-B	5	0.0198%	Pseudoxanthomonas (g)
	L-GYP	4	0.0260%	L-GYP: Pseudoxanthomonas (g) Pseudoxanthomonas mexicana (s)
Zavarzinella (g) [64]	F-GS+H	20	0.0668%	Pseudoxanthomonas (g)
	F-B	10	0.0395%	-
	F-GYP	6	0.0390%	-
	F-H	2	0.0163%	-
	F-GS	2	0.0099%	-

F3. Aerobic, Iron Oxidizing Bacteria

Table F3a. Summary results for *Aerobic, Iron Oxidizing Bacteria*

Amendment	No. of Reads	Perc. of Total Reads
L-H	2425	12.1244%
F-H	47	0.3833%
L-GS	857	6.1766%
F-GS	57	0.2819%
L-GS+H	247	1.3619%
F-GS+H	195	0.6515%
L-GYP	231	1.5027%
F-GYP	86	0.5596%
L-ALL	191	1.1644%
F-ALL	69	0.4924%
L-B	674	4.0978%
F-B	128	0.5058%

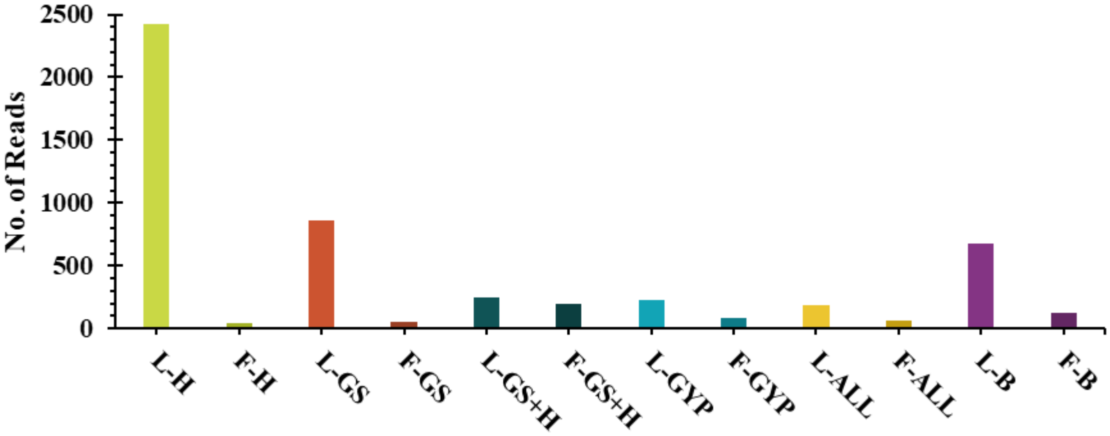

Figure F3a. Summary graph of number of reads for each amendment type in the category of *Aerobic, Iron Oxidizing Bacteria*

Table F3b. Classification of *Aerobic, Iron Oxidizing Bacteria*

Aerobic, Iron Oxidizing Bacteria	**Amendment**	**No. of Reads**	**Perc. of Total Reads**	**Classification**
Arenimonas (g) [65]	F-GS+H	142	0.4745%	uncultured gamma (s)
	F-B	105	0.4150%	uncultured gamma (s)
	F-GYP	56	0.3644%	uncultured gamma (s)
	F-ALL	33	0.2355%	uncultured gamma (s)
	F-GS	22	0.1088%	uncultured gamma (s)
	F-H	17	0.1386%	uncultured gamma (s)
	L-H	6	0.0300%	uncultured gamma (s)
Gallionellaceae (f) [66]	F-GS	35	0.1731%	Candidatus Nitrotoga (g) uncultured beta (s)
	F-GS+H	26	0.0869%	Candidatus Nitrotoga (g) uncultured beta (s)
	F-ALL	25	0.1784%	Candidatus Nitrotoga (g) uncultured beta (s)
	F-H	22	0.1794%	Candidatus Nitrotoga (g) uncultured beta (s)
	F-GYP	14	0.0911%	Candidatus Nitrotoga (g) uncultured beta (s)
Leptothrix (g) [67]	L-B	30	0.1824%	-
Ferritrophicum (g) [68]	L-GS+H	8	0.0441%	-
Rhodomicrobium (g) [69]	L-H	91	0.4550%	uncultured bacterium (s)
	L-GS	87	0.6270%	uncultured bacterium (s)
	L-GS+H	29	0.1599%	uncultured bacterium (s)
	F-GS+H	27	0.0902%	uncultured Alphaproteobacteria (s)
	F-B	23	0.0909%	uncultured Alphaproteobacteria (s)
	F-GYP	16	0.1041%	uncultured Alphaproteobacteria (s)
	F-ALL	11	0.0785%	uncultured Alphaproteobacteria (s)
	L-GYP	9	0.0585%	uncultured bacterium (s)
	F-H	8	0.0652%	uncultured Alphaproteobacteria (s)
	L-ALL	3	0.0183%	uncultured bacterium (s)

Aerobic, Iron Oxidizing Bacteria	Amendment	No. of Reads	Perc. of Total Reads	Classification
Chlorobium (g) [70,71]	L-H	2328	11.6394 %	-
	L-GS	770	5.5495%	-
	L-B	644	3.9154%	-
Chlorobium (g) [70,71]	L-GYP	222	1.4442%	-
	L-GS+H	210	1.1579%	-
	L-ALL	188	1.1461%	-

F4. Aerobic, Iron Oxidizing, Nitrate Reducing Bacteria

Table F4a. Summary results for *Aerobic, Iron Oxidizing, Nitrate Reducing Bacteria*

Amendment	No. of Read	Perc. of Total
L-H	1776	8.8796%
F-H	195	1.5903%
L-GS	297	2.1405%
F-GS	265	1.3106%
L-GS+H	1652	9.1090%
F-GS+H	227	0.7585%
L-GYP	109	0.7091%
F-GYP	109	0.7093%
L-ALL	581	3.5418%
F-ALL	122	0.8707%
L-B	378	2.2982%
F-B	187	0.7390%

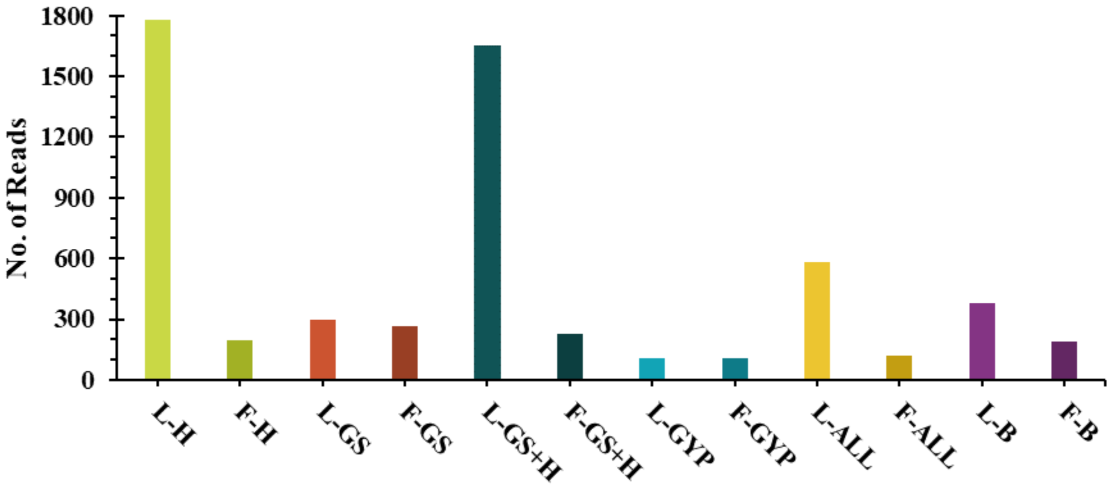

Figure F4a. Summary graph of number of reads for each amendment type in the category of *Aerobic, Iron Oxidizing, Nitrate Reducing Bacteria*

Table F4b. Classification of *Aerobic, Iron Oxidizing, Nitrate Reducing Bacteria*

Aerobic, Iron Oxidizing, Nitrate Reducing Bacteria	Amendment	No. of Reads	Perc. of Total	Classification
Pedomicrobium (g) [72]	F-GS+H	91	0.3041%	-
	F-B	55	0.2174%	-
	F-GS	41	0.2028%	-
	F-B	41	0.1620%	uncultured bacterium (s)
	F-GS+H	36	0.1203%	uncultured bacterium (s)
	F-GYP	32	0.2082%	-
	F-H	32	0.2610%	uncultured bacterium (s)
	F-ALL	29	0.2070%	uncultured bacterium (s)
	F-ALL	28	0.1998%	-
	F-GYP	28	0.1822%	uncultured bacterium (s)
	L-GYP	8	0.0520%	metagenome (s)
Pseudomonas (g) [73,74]	L-H	1638	8.1896%	-
	L-GS+H	772	4.2567%	-
	L-ALL	289	1.7618%	-
	L-GS	111	0.8000%	-

Aerobic, Iron Oxidizing, Nitrate Reducing Bacteria	Amendment	No. of Reads	Perc. of Total	Classification
Pseudomonas (g) [73,74]	F-GS+H	50	0.1671%	-
	L-GYP	37	0.2407%	-
	F-GS	35	0.1731%	Pseudomonas anguilliseptica (s)
	L-B	27	0.1642%	-
	F-B	23	0.0909%	-
	F-GYP	19	0.1236%	-
	F-B	19	0.0751%	Pseudomonas anguilliseptica (s)
	F-H	7	0.0571%	-
	F-ALL	3	0.0214%	-
	F-GS+H	2	0.0067%	Pseudomonas flexibilis (s)
Thiobacillus (g) [75,76]	L-GS+H	880	4.8522%	-
	L-B	351	2.1340%	-
	L-ALL	292	1.7801%	-
	F-GS	189	0.9348%	-
	L-GS	186	1.3405%	-
	F-H	156	1.2722%	-
	L-H	138	0.6900%	-
	L-GYP	64	0.4163%	-
	F-ALL	62	0.4425%	-
	F-B	49	0.1936%	-
	F-GS+H	48	0.1604%	-
	F-GYP	30	0.1952%	-

F5. Aerobic, Iron Oxidizing, Nitrate Reducing, Fermenting Bacteria

Table F5a. Summary results for *Aerobic, Iron Oxidizing, Nitrate Reducing, Fermenting Bacteria*

Amendment	No. of Reads	Perc. of Total Reads
L-H	0	0.0000%
F-H	0	0.0000%
L-GS	0	0.0000%
F-GS	31	0.1533%
L-GS+H	0	0.0000%
F-GS+H	42	0.1403%
L-GYP	0	0.0000%
F-GYP	0	0.0000%
L-ALL	0	0.0000%
F-ALL	0	0.0000%
L-B	0	0.0000%
F-B	0	0.0000%

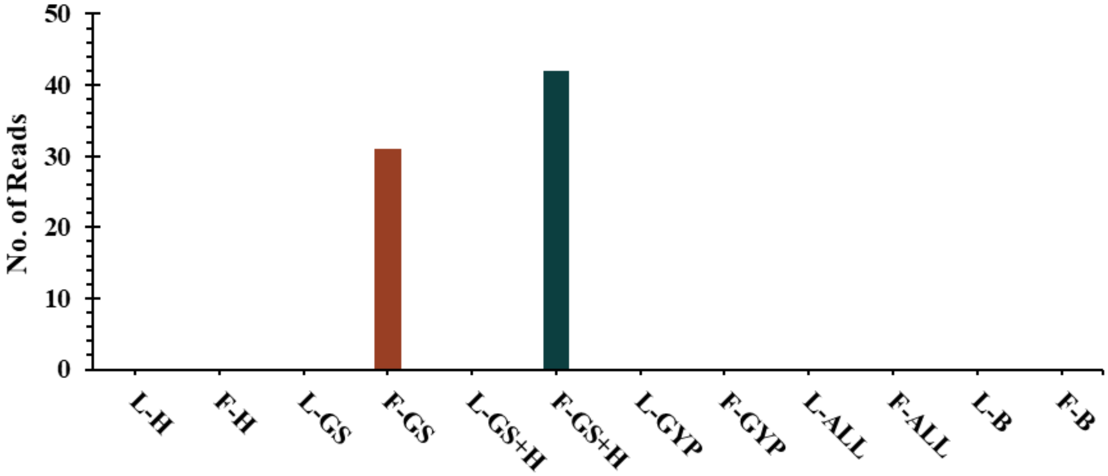

Figure F5a. Summary graph of number of reads for each amendment type in the category of *Aerobic, Iron Oxidizing, Nitrate Reducing, Fermenting Bacteria*

Table F5b. Classification of *Aerobic, Iron Oxidizing, Nitrate Reducing, Fermenting Bacteria*

Aerobic, Iron Oxidizing, Nitrate Reducing, Fermenting Bacteria	Amendment	No. of Reads	Perc. Of Total Reads	Classification
Rhodobacter (g) [77]	F-GS+H	42	0.1403%	-
	F-GS	31	0.1533%	-

F6. Aerobic, Methane Oxidizing Bacteria

Table F6a. Summary results for *Aerobic, Methane Oxidizing Bacteria*

Amendment	No. of Reads	Perc. of Total Reads
L-H	355	1.7749%
F-H	147	1.1988%
L-GS	20	0.1441%
F-GS	171	0.8457%
L-GS+H	2615	14.4188%
F-GS+H	95	0.3174%
L-GYP	35	0.2277%
F-GYP	147	0.9566%
L-ALL	19	0.1158%
F-ALL	131	0.9349%
L-B	477	2.9000%
F-B	109	0.4308%

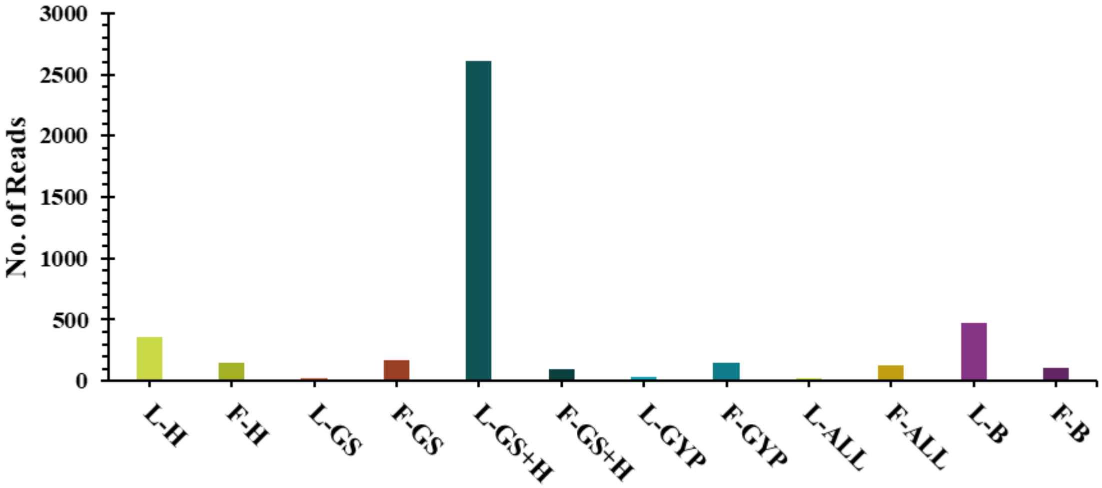

Figure F6a. Summary graph of number of reads for each amendment type in the category of *Aerobic, Methane Oxidizing Bacteria*

Table F6b. Classification of *Aerobic, Methane Oxidizing Bacteria*

Aerobic, Methane Oxidizing Bacteria	Amendment	No. of Reads	Perc. of Total Reads	Classification
Methylobacter (g) [78]	L-GS+H	383	2.1118%	-
	L-H	87	0.4350%	-
	L-B	86	0.5229%	-
	F-GYP	28	0.1822%	-
	F-ALL	26	0.1856%	-
	F-H	19	0.1550%	-
	F-GS	15	0.0742%	-
	L-GS	10	0.0721%	-
Methylococcales (o) [79,81]	L-GS+H	1743	9.6107%	Methylomonadaceae (f) Methylomonas (g)
	L-GS+H	383	2.1118%	Methylomonadaceae (f) Methylobacter (g)
	L-H	127	0.6350%	Methylomonadaceae (f) Methylomonas (g)
	L-B	114	0.6931%	Methylomonadaceae (f) Methylomicrobium (g)
	L-H	87	0.4350%	Methylomonadaceae (f) Methylobacter (g)
	L-B	86	0.5229%	Methylomonadaceae (f) Methylobacter (g)
	F-GS	71	0.3512%	Methylomonadaceae (f) Marine Methylotrophic Group 2 (g)
	L-B	45	0.2736%	Methylomonadaceae (f) Methylomonas (g)
	F-ALL	37	0.2641%	Methylomonadaceae (f) Marine Methylotrophic Group 2 (g)
	L-GS+H	36	0.1985%	Methylomonadaceae (f) Methylomicrobium (g)
	F-GS	35	0.1731%	Methylococcaceae (f) Methyloparacoccus (g) uncultured

Aerobic, Methane Oxidizing Bacteria	**Amendment**	**No. of Reads**	**Perc. of Total Reads**	**Classification**
Methylococcales (o) [79,81]				bacterium (s)
	F-GYP	35	0.2278%	Methylococcaceae (f) Methyloparacoccus (g) uncultured bacterium (s)
	L-GYP	35	0.2277%	Methylomonadaceae (f) Methylomonas (g)
	F-B	33	0.1304%	Methylococcaceae (f) Methyloparacoccus (g) uncultured bacterium (s)
	F-GS+H	33	0.1103%	Methylomonadaceae (f) Marine Methylotrophic Group 2 (g)
	F-GS+H	31	0.1036%	Methylococcaceae (f) Methyloparacoccus (g) uncultured bacterium (s)
	F-B	29	0.1146%	Methylomonadaceae (f) Marine Methylotrophic Group 2 (g)
	F-GYP	28	0.1822%	Methylomonadaceae (f) Methylobacter (g)
	F-H	27	0.2202%	Methylomonadaceae (f) Marine Methylotrophic Group 2 (g)
	F-ALL	26	0.1856%	Methylomonadaceae (f) Methylobacter (g)
	F-H	24	0.1957%	Methylococcaceae (f) Methyloparacoccus (g) uncultured bacterium (s)
	F-ALL	21	0.1499%	Methylococcaceae (f) Methyloparacoccus (g) uncultured bacterium (s)
	F-GYP	21	0.1367%	Methylomonadaceae (f) Marine Methylotrophic Group 2 (g)
	F-H	19	0.1550%	Methylomonadaceae (f) Methylobacter (g)
Methylococcales (o) [79,81]	L-H	18	0.0900%	Methylococcaceae (f) Methyloparacoccus (g) uncultured bacterium (s)

Aerobic, Methane Oxidizing Bacteria	**Amendment**	**No. of Reads**	**Perc. of Total Reads**	**Classification**
	L-H	18	0.0900%	Methylomonadaceae (f) Methylomicrobium (g)
	L-GS+H	17	0.0937%	Methylococcaceae (f) Methyloparacoccus (g) uncultured bacterium (s)
	F-H	17	0.1386%	Methylomonadaceae (f) Methylomicrobium (g)
	L-B	16	0.0973%	Methylococcaceae (f) Methyloparacoccus (g) uncultured bacterium (s)
	F-GS	15	0.0742%	Methylomonadaceae (f) Methylobacter (g)
	L-GS	10	0.0721%	Methylomonadaceae (f) Methylobacter (g)
	F-B	7	0.0277%	Methylomonadaceae (f) Methylomicrobium (g)
	L-ALL	7	0.0427%	Methylomonadaceae (f) Methylomonas (g)
	L-ALL	6	0.0366%	Methylococcaceae (f) Methyloparacoccus (g) uncultured bacterium (s)
Methylomicrobium (g) [80]	L-B	114	0.6931%	-
	L-GS+H	36	0.1985%	-
	L-H	18	0.0900%	-
	F-H	17	0.1386%	-
	F-B	7	0.0277%	-
Methyloparacoccus (g) [81]	F-GS	35	0.1731%	uncultured bacterium (s)
	F-GYP	35	0.2278%	uncultured bacterium (s)
	F-B	33	0.1304%	uncultured bacterium (s)
	F-GS+H	31	0.1036%	uncultured bacterium (s)
	F-H	24	0.1957%	uncultured bacterium (s)

Aerobic, Methane Oxidizing Bacteria	Amendment	No. of Reads	Perc. of Total Reads	Classification
	F-ALL	21	0.1499%	uncultured bacterium (s)
	L-GS+H	17	0.0937%	uncultured bacterium (s)
	L-B	16	0.0973%	uncultured bacterium (s)
	L-ALL	6	0.0366%	uncultured bacterium (s)

F7. Aerobic, Nitrate Reducing Bacteria

Table F7a. Summary results for *Aerobic, Nitrate Reducing Bacteria*

Amendment	No. of Reads	Perc. of Total Reads
L-H	195	0.9750%
F-H	308	2.5118%
L-GS	57	0.4108%
F-GS	374	1.8497%
L-GS+H	437	2.4096%
F-GS+H	1253	4.1866%
L-GYP	100	0.6505%
F-GYP	675	4.3925%
L-ALL	168	1.0241%
F-ALL	653	4.6603%
L-B	717	4.3592%
F-B	954	3.7702%

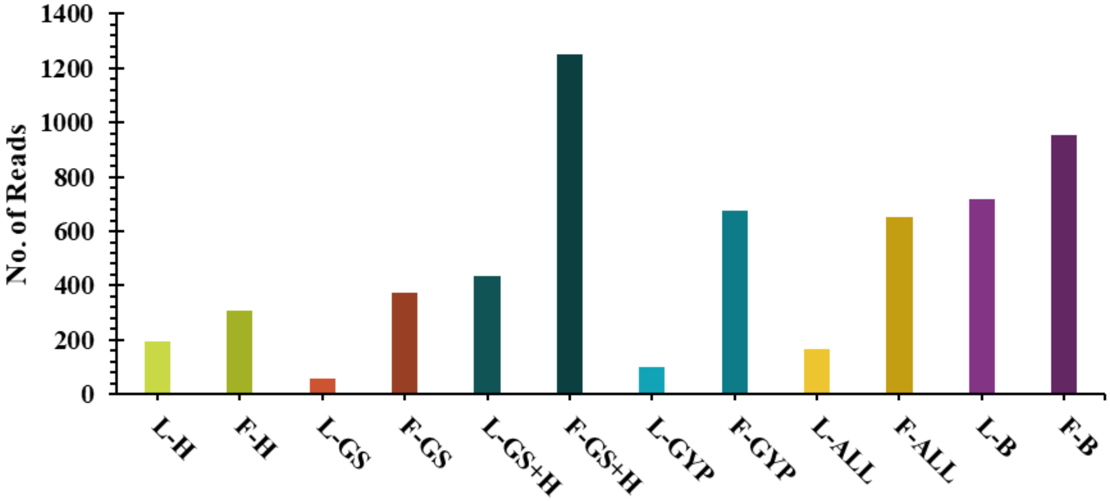

Figure F7a. Summary graph of number of reads for each amendment type in the category of *Aerobic, Nitrate Reducing Bacteria*

Table F7b. Classification of *Aerobic, Nitrate Reducing Bacteria*

Aerobic, Nitrate Reducing Bacteria	Amendment	No. of Reads	Perc. of Total Reads	Classification
Alcanivoracaceae (f) [82,83]	F-GS	19	0.0940%	Alcanivorax (g) uncultured gamma (s)
	F-GS+H	8	0.0267%	Ketobacter (g) uncultured soil (s)
	F-GS	7	0.0346%	Ketobacter (g) uncultured bacterium (s)
	F-ALL	6	0.0428%	Alcanivorax (g) uncultured gamma (s)
	F-B	6	0.0237%	Ketobacter (g) uncultured bacterium (s)
	F-H	4	0.0326%	Ketobacter (g) uncultured bacterium (s)
Azoarcus (g) [84]	F-ALL	42	0.2997%	-
	F-B	33	0.1304%	-
	F-GS+H	31	0.1036%	-
Beijerinckiaceae (f) [11,85]	F-GS+H	18	0.0601%	-
	F-B	7	0.0277%	-
	F-H	6	0.0489%	-
	F-GYP	5	0.0325%	Bosea (g)
	F-H	5	0.0408%	alphaI cluster (g) metagenome (s)
	L-GYP	4	0.0260%	-
Bradyrhizobium (g) [86]	L-H	7	0.0350%	-
	L-GS+H	7	0.0386%	-
Candidatus Competibacter (g) [87,88]	F-GS	93	0.4600%	uncultured gamma (s)
	F-B	81	0.3201%	uncultured gamma (s)
	F-GYP	46	0.2993%	uncultured gamma (s)
	F-ALL	38	0.2712%	uncultured gamma (s)
	F-GS+H	32	0.1069%	uncultured gamma (s)
	F-H	25	0.2039%	uncultured gamma (s)
	F-B	15	0.0593%	uncultured Candidatus (s)
Flavobacterium (g)	F-GS	32	0.1583%	-

Aerobic, Nitrate Reducing Bacteria	**Amendment**	**No. of Reads**	**Perc. of Total Reads**	**Classification**
[89] Flavobacterium (g) [89]	F-GS+H	21	0.0702%	-
	F-GYP	20	0.1301%	-
	F-H	14	0.1142%	-
	F-B	11	0.0435%	-
	F-ALL	6	0.0428%	-
	L-GYP	3	0.0195%	-
	F-GS+H	2	0.0067%	Flavobacterium orientale (s)
Halieaceae (f) [90,91,92]	F-GS+H	60		-
	F-GYP	44		-
	F-B	41		-
	F-GS	31		-
	F-GS+H	30		OM60(NOR5) clade (g)
	F-ALL	27		-
	F-H	14		-
	F-B	13		OM60(NOR5) clade (g)
	F-ALL	11		OM60(NOR5) clade (g)
	F-H	10		OM60(NOR5) clade (g)
	L-ALL	5		-
	L-H	3		-
Halothiobacillus (g) [93]	L-GYP	15	0.0976%	uncultured bacterium (s)
	L-GS+H	6	0.0331%	-
	L-GS	4	0.0288%	uncultured bacterium (s)
Hydrogenophaga (g) [94]	F-GS+H	328	1.0959%	-
	F-B	242	0.9564%	-
	F-ALL	185	1.3203%	-
	F-GYP	123	0.8004%	-

Aerobic, Nitrate Reducing Bacteria	**Amendment**	**No. of Reads**	**Perc. of Total Reads**	**Classification**
Hydrogenophaga (g) [94]	L-B	116	0.7053%	-
	F-H	96	0.7829%	-
	L-ALL	87	0.5304%	-
	F-GS	63	0.3116%	-
	L-GS+H	51	0.2812%	-
	L-GS	30	0.2162%	-
	L-H	21	0.1050%	-
	L-GYP	13	0.0846%	uncultured Hydrogenophaga (s)
Hyphomicrobium (g) [95]	F-GS+H	322	1.0759%	-
	F-B	203	0.8022%	-
	F-H	102	0.8318%	-
	F-GS	100	0.4946%	-
	F-ALL	92	0.6566%	-
	F-GYP	89	0.5792%	-
	L-ALL	45	0.2743%	-
	L-GYP	27	0.1756%	-
	L-GS	21	0.1514%	-
	L-H	20	0.1000%	-
	L-B	13	0.0790%	-
	F-H	9	0.0734%	metagenome (s)
Iamia (g) [96]	F-GYP	102	0.6638%	-
	F-ALL	56	0.3997%	-
	F-B	40	0.1581%	-
	F-GS+H	33	0.1103%	-
	F-GS+H	31	0.1036%	-
	F-H	7	0.0571%	-

Aerobic, Nitrate Reducing Bacteria	**Amendment**	**No. of Reads**	**Perc. of Total Reads**	**Classification**
Iamia (g) [96]	F-ALL	2	0.0143%	uncultured beta (s)
Magnetospirillum (g) [97]	F-B	3	0.0119%	uncultured bacterium (s)
Nitrospira (g) [98,99]	F-GS+H	50	0.1671%	Nitrospira marina (s)
	F-GYP	28	0.1822%	Nitrospira marina (s)
	F-B	28	0.1107%	Nitrospira marina (s)
	F-ALL	18	0.1285%	Nitrospira marina (s)
	F-GS+H	17	0.0568%	uncultured Nitrospirae (s)
	F-B	15	0.0593%	uncultured Nitrospirae (s)
	F-GYP	7	0.0456%	uncultured Nitrospirae (s)
	L-B	5	0.0304%	-
	F-B	3	0.0119%	-
	F-H	3	0.0245%	Nitrospira marina (s)
	L-H	2	0.0100%	Nitrospira marina (s)
Novosphingobium (g) [100]	F-GS+H	31	0.1036%	-
	F-B	25	0.0988%	-
	F-ALL	17	0.1213%	-
Oceanibaculum (g) [101,102,103]	L-GS+H	2	0.0110%	-
	F-B	2	0.0079%	-
Paracoccus (g) [104]	F-GYP	132	0.8590%	-
	F-ALL	104	0.7422%	-
	F-GS+H	91	0.3041%	-
	F-B	60	0.2371%	-
Pseudohongiella (g) [105,106,107]	F-GS	5	0.0247%	uncultured gamma (s)
Reyranella (g) [108,109]	L-B	21	0.1277%	-

Aerobic, Nitrate Reducing Bacteria	**Amendment**	**No. of Reads**	**Perc. of Total Reads**	**Classification**
	L-GS+H	6	0.0331%	-
	L-GS	2	0.0144%	-
Rhizobacter (g) [110]	F-GS+H	87	0.2907%	-
	F-B	59	0.2332%	-
	F-GYP	57	0.3709%	-
	F-ALL	36	0.2569%	-
Roseomonas (g) [111]	F-GS+H	31	0.1036%	-
	F-B	31	0.1225%	-
	L-B	10	0.0608%	-
	F-ALL	10	0.0714%	-
	F-GS	9	0.0445%	metagenome (s)
	F-GYP	7	0.0456%	-
	L-GS+H	4	0.0221%	-
	F-GYP	4	0.0260%	metagenome (s)
	F-ALL	3	0.0214%	metagenome (s)
	F-H	2	0.0163%	Roseomonas lacus (s)
	F-B	2	0.0079%	metagenome (s)
Sulfuritalea (g) [112]	L-B	471	2.8636%	-
	L-GS+H	264	1.4557%	-
	L-H	122	0.6100%	-
Terrimonas (g) [113]	F-GS+H	14	0.0468%	-
	F-GYP	11	0.0716%	-
	F-B	5	0.0198%	-
Thauera (g) [114]	L-GS+H	76	0.4191%	-
	L-B	34	0.2067%	-
	F-B	29	0.1146%	-

Aerobic, Nitrate Reducing Bacteria	**Amendment**	**No. of Reads**	**Perc. of Total Reads**	**Classification**
Thauera (g) [114]	L-GYP	22	0.1431%	-
	L-H	20	0.1000%	-
	F-GS+H	16	0.0535%	-
	F-GS	15	0.0742%	-
	F-H	11	0.0897%	-
Thiomonas (g) [115]	L-B	47	0.2857%	Thiomonas sp. (s)
	L-ALL	31	0.1890%	Thiomonas sp. (s)
	L-GS+H	21	0.1158%	-
	L-GYP	16	0.1041%	Thiomonas sp. (s)

F8. Aerobic, Nitrate Reducing, Iron Reducing, Fermenting Bacteria

Table F8a. Summary results for Aerobic, Nitrate Reducing, Iron Reducing, Fermenting Bacteria

Amendment	No. of Reads	Perc. of Total Reads
L-H	3	0.0150%
F-H	0	0.0000%
L-GS	0	0.0000%
F-GS	10	0.0495%
L-GS+H	6	0.0331%
F-GS+H	11	0.0368%
L-GYP	0	0.0000%
F-GYP	4	0.0260%
L-ALL	0	0.0000%
F-ALL	0	0.0000%
L-B	8	0.0486%
F-B	9	0.0356%

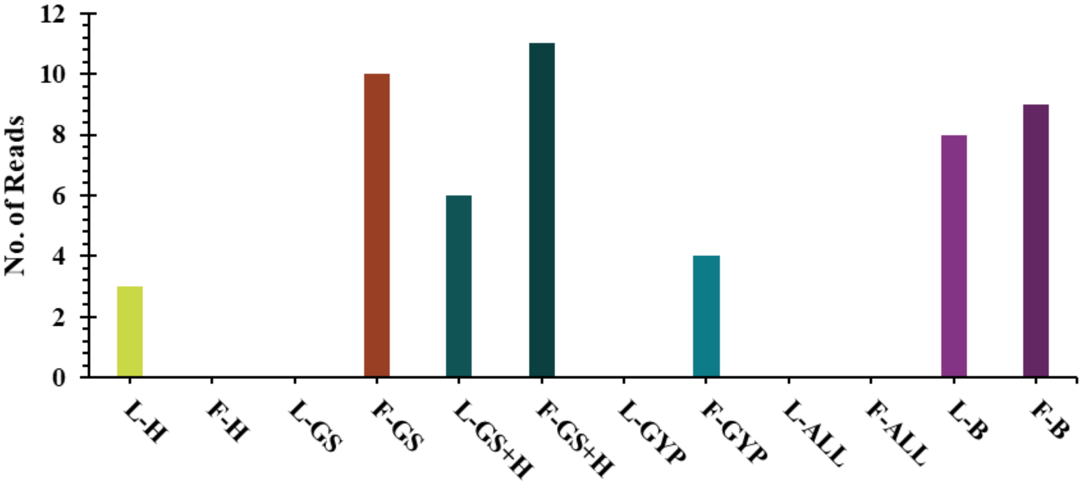

Figure F8a. Summary graph of number of reads for each amendment type in the category of *Aerobic, Nitrate Reducing, Iron Reducing, Fermenting Bacteria*

Table F8b. Classification of *Aerobic, Nitrate Reducing, Iron Reducing, Fermenting Bacteria*

Aerobic, Nitrate Reducing, Iron Reducing, Fermenting Bacteria	Amendment	No. of Reads	Perc. of Total Reads	Classification
Paludibaculum (g) [116]	F-B	9	0.0356%	uncultured bacterium (s)
	L-B	8	0.0486%	uncultured bacterium (s)
	F-GS+H	7	0.0234%	uncultured bacterium (s)
	L-GS+H	6	0.0331%	-
	F-GS	6	0.0297%	uncultured bacterium (s)
	F-GS	4	0.0198%	metagenome (s)
	F-GS+H	4	0.0134%	metagenome (s)
	F-GYP	4	0.0260%	uncultured bacterium (s)
	L-H	3	0.0150%	uncultured bacterium (s)

F9. Aerobic, Nitrate Reducing, Fermenting Bacteria

Table F9a. Summary results for *Aerobic, Nitrate Reducing, Fermenting Bacteria*

Amendment	No. of Reads	Perc. of Total Reads
L-H	3	0.0150%
F-H	12	0.0979%
L-GS	0	0.0000%
F-GS	13	0.0643%
L-GS+H	6	0.0331%
F-GS+H	47	0.1570%
L-GYP	3	0.0195%
F-GYP	24	0.1562%
L-ALL	2	0.0122%
F-ALL	23	0.1641%
L-B	427	2.5961%
F-B	81	0.3201%

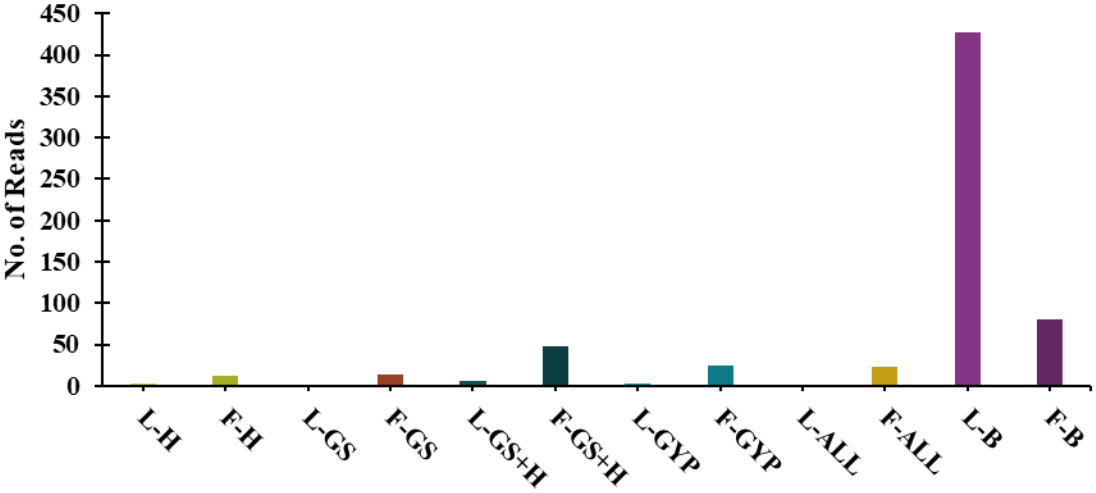

Figure F9a. Summary graph of number of reads for each amendment type in the category of *Aerobic, Nitrate Reducing, Fermenting Bacteria*

Table F9b. Classification of *Aerobic, Nitrate Reducing, Fermenting Bacteria*

Aerobic, Nitrate Reducing, Fermenting Bacteria	Amendment	No. of Reads	Perc. of Total Reads	Classification
Corynebacterium (g) [117]	L-B	424	2.5778%	-
Phycisphaeraceae (f) [118,119]	F-B	56	0.2213%	SM1A02 (g) uncultured bacterium (s)
	F-GS+H	40	0.1336%	SM1A02 (g) uncultured bacterium (s)
	F-GYP	22	0.1432%	SM1A02 (g) uncultured bacterium (s)
	F-ALL	20	0.1427%	SM1A02 (g) uncultured bacterium (s)
	F-B	11	0.0435%	CL500-3 (g) uncultured marine (s)
	F-H	9	0.0734%	CL500-3 (g) uncultured marine (s)
	F-GS	8	0.0396%	CL500-3 (g) uncultured marine (s)
	F-GS+H	7	0.0234%	CL500-3 (g) uncultured marine (s)
	L-GS+H	6	0.0331%	SM1A02 (g) uncultured bacterium (s)
	F-GS	5	0.0247%	SM1A02 (g) uncultured bacterium (s)
	L-GYP	3	0.0195%	CL500-3 (g) uncultured marine (s)
	F-B	3	0.0119%	CL500-3 (g) uncultured planctomycete (s)
	F-B	3	0.0119%	SM1A02 (g)
	F-ALL	3	0.0214%	SM1A02 (g) uncultured Planctomycetales (s)
	F-B	3	0.0119%	SM1A02 (g) uncultured Planctomycetales (s)
	L-B	3	0.0182%	SM1A02 (g) uncultured bacterium (s)

Phycisphaeraceae (f) [118,119]	F-H	3	0.0245%	SM1A02 (g) uncultured bacterium (s)
	F-B	3	0.0119%	uncultured (g) uncultured bacterium (s)
	F-GYP	2	0.0130%	CL500-3 (g) uncultured bacterium (s)
	F-B	2	0.0079%	CL500-3 (g) uncultured bacterium (s)
	L-ALL	2	0.0122%	uncultured (g) uncultured planctomycete (s)
Staphylococcus (g) [120]	L-H	3	0.0150%	-

F10. Aerobic, Iron Reducing, Fermenting Bacteria

Table F10a. Summary results for *Aerobic, Iron Reducing, Fermenting Bacteria*

Amendment	No. of Reads	Perc. of Total Reads
L-H	0	0.0000%
F-H	0	0.0000%
L-GS	0	0.0000%
F-GS	5	0.0247%
L-GS+H	0	0.0000%
F-GS+H	0	0.0000%
L-GYP	6	0.0390%
F-GYP	0	0.0000%
L-ALL	0	0.0000%
F-ALL	0	0.0000%
L-B	0	0.0000%
F-B	0	0.0000%

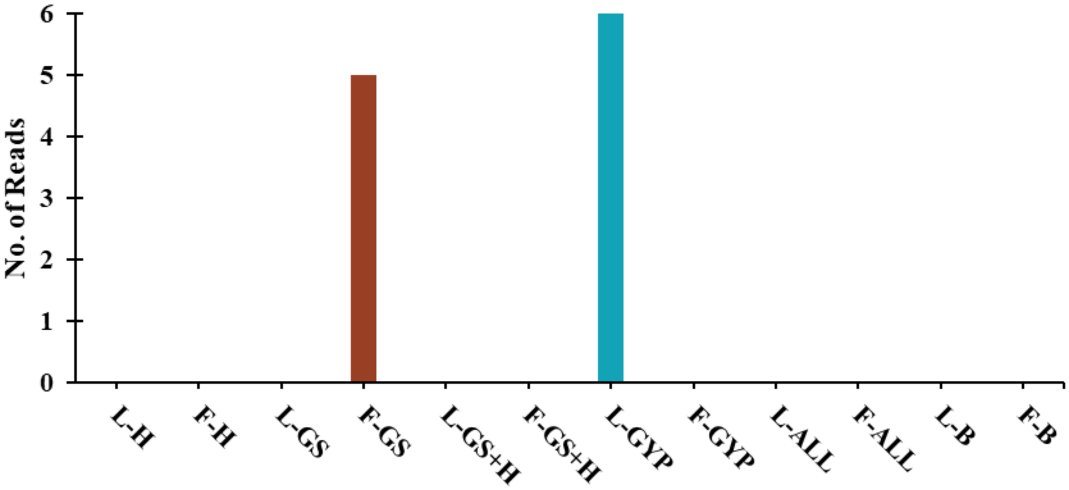

Figure F10a. Summary graph of number of reads for each amendment type in the category of *Aerobic, Iron Reducing, Fermenting Bacteria*

Table F10b. Classification of *Aerobic, Iron Reducing, Fermenting Bacteria*

Aerobic, Iron Reducing, Fermenting Bacteria	**Amendment**	**No. of Reads**	**Perc. of Total Reads**	**Classification**
Sulfurospirillum (g) [121]	L-GYP	6	0.0390%	Sulfurospirillum deleyianum (s)
	F-GS	5	0.0247%	uncultured bacterium (s)

F11. Aerobic, Fermenting Bacteria

Table F11a. Summary results for *Aerobic, Fermenting Bacteria*

Amendment	No. of Reads	Perc. of Total Reads
L-H	0	0.0000%
F-H	30	0.2447%
L-GS	5	0.0360%
F-GS	23	0.1138%
L-GS+H	8	0.0441%
F-GS+H	0	0.0000%
L-GYP	0	0.0000%
F-GYP	7	0.0456%
L-ALL	12	0.0732%
F-ALL	8	0.0571%
L-B	14	0.0851%
F-B	5	0.0198%

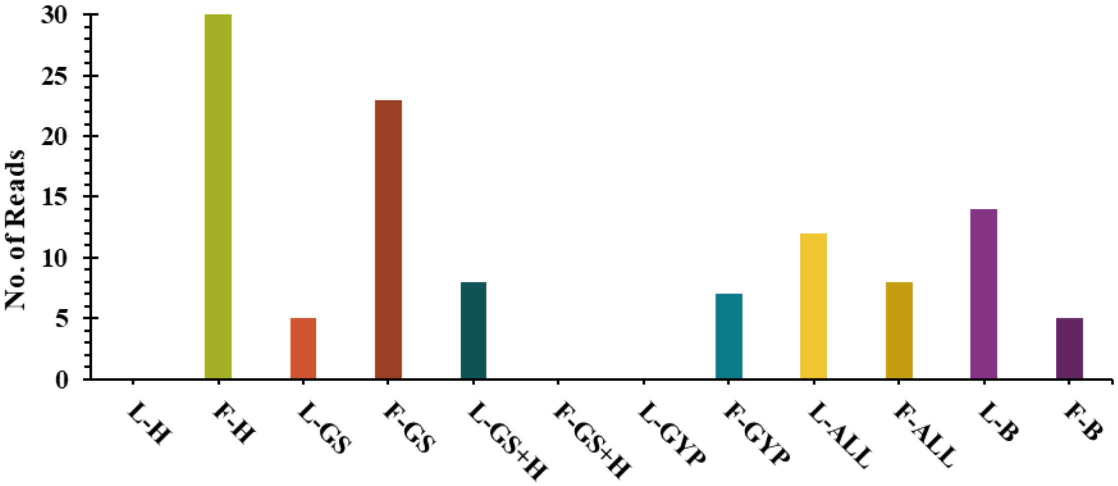

Figure F11a. Summary graph of the number of reads for each amendment type in the classification of *Aerobic, Fermenting Bacteria*

Table F11b. Classification of *Aerobic, Fermenting Bacteria*

Aerobic, Fermenting Bacteria	Amendment	No. of Reads	Perc. of Total Reads	Classification
Ignavibacterium (g) [122]	F-H	30	0.2447%	uncultured bacterium (s)
	F-GS	23	0.1138%	uncultured bacterium (s)
	L-B	14	0.0851%	uncultured bacterium (s)
	L-ALL	12	0.0732%	uncultured bacterium (s)
	L-GS+H	8	0.0441%	uncultured bacterium (s)
	F-ALL	8	0.0571%	uncultured bacterium (s)
	F-GYP	7	0.0456%	uncultured bacterium (s)
	L-GS	5	0.0360%	uncultured bacterium (s)
	F-B	3	0.0119%	uncultured bacterium (s)
	F-B	2	0.0079%	-

F12. Nitrate Reducing, Fermenting Bacteria

Table F12a. Summary results for *Nitrate Reducing, Fermenting Bacteria*

Amendment	No. of Reads	Perc. of Total Reads
L-H	15	0.0750%
F-H	0	0.0000%
L-GS	0	0.0000%
F-GS	0	0.0000%
L-GS+H	11	0.0607%
F-GS+H	0	0.0000%
L-GYP	2	0.0130%
F-GYP	0	0.0000%
L-ALL	0	0.0000%
F-ALL	0	0.0000%
L-B	0	0.0000%
F-B	0	0.0000%

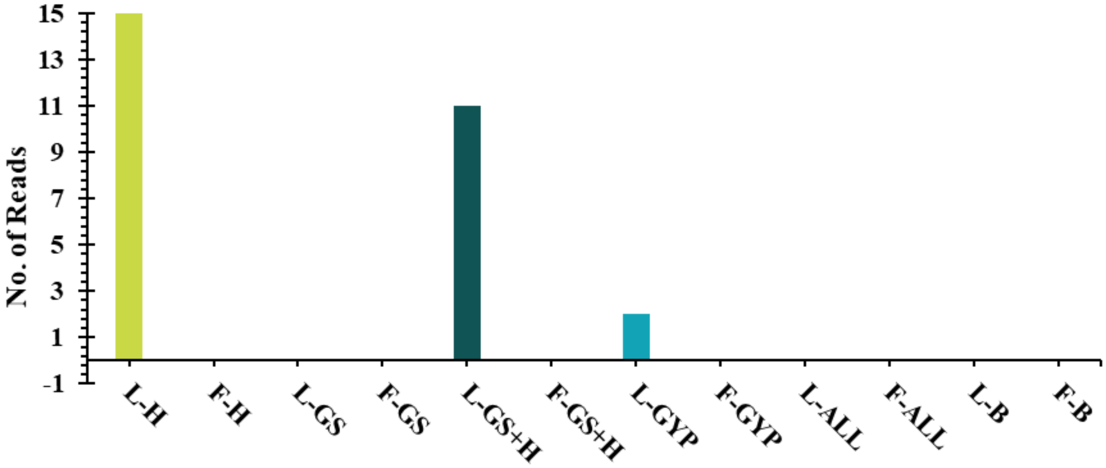

Figure F12a. Summary graph of the number of reads for each amendment type in the classification of *Nitrate Reducing, Fermenting Bacteria*

Table F12b. Classification of *Nitrate Reducing, Fermenting Bacteria*

Nitrate Reducing, Fermenting Bacteria	Amendment	No. of Reads	Perc. of Total Reads	Classification
Caldithrix (g) [123,124]	L-H	15	0.0750%	uncultured bacterium (s)
	L-GS+H	11	0.0607%	uncultured bacterium (s)
	L-GYP	2	0.0130%	uncultured bacterium (s)

F13. Nitrate Reducing, Sulfate Reducing, Fermenting Bacteria

Table F13a. Summary results for *Nitrate Reducing, Sulfate Reducing, Fermenting Bacteria*

Amendment	No. of Reads	Perc. of Total Reads
L-H	292	1.4599%
F-H	121	0.9868%
L-GS	149	1.0739%
F-GS	209	1.0337%
L-GS+H	291	1.6045%
F-GS+H	221	0.7384%
L-GYP	203	1.3206%
F-GYP	126	0.8199%
L-ALL	218	1.3289%
F-ALL	165	1.1776%
L-B	260	1.5807%
F-B	190	0.7509%

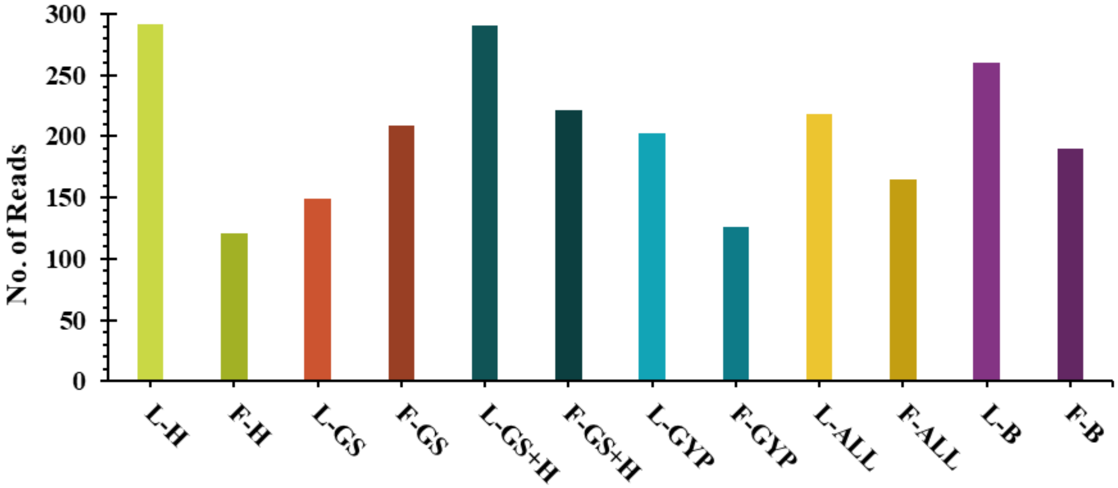

Figure F13a. Summary graph of the number of reads for each amendment type in the classification of *Nitrate Reducing, Sulfate Reducing, Fermenting Bacteria*

Table F13b. Classification of *Nitrate Reducing, Sulfate Reducing, Fermenting Bacteria*

Nitrate Reducing, Sulfate Reducing, Fermenting Bacteria	Amendment	No. of Reads	Perc. of Total Reads	Classification
Desulfomonile (g) [125]	L-H	284	1.4199%	uncultured bacterium (s)
	L-GS+H	270	1.4888%	uncultured bacterium (s)
	L-B	233	1.4166%	uncultured bacterium (s)
	F-GS+H	221	0.7384%	uncultured bacterium (s)
	L-ALL	209	1.2741%	uncultured bacterium (s)
	L-GYP	203	1.3206%	uncultured bacterium (s)
	F-GS	198	0.9793%	uncultured bacterium (s)
	F-B	190	0.7509%	uncultured bacterium (s)
	F-ALL	165	1.1776%	uncultured bacterium (s)
	L-GS	149	1.0739%	uncultured bacterium (s)
	F-GYP	126	0.8199%	uncultured bacterium (s)
	F-H	121	0.9868%	uncultured bacterium (s)
	L-B	27	0.1642%	-
	L-GS+H	21	0.1158%	-
	F-GS	11	0.0544%	-
	L-ALL	9	0.0549%	-
	L-H	8	0.0400%	-

F14. Iron Reducing Bacteria

Table F14a. Summary results for *Iron Reducing Bacteria*

Amendment	No. of Read	Perc. of Total Reads
L-H	0	0.0000%
F-H	0	0.0000%
L-GS	2	0.0144%
F-GS	0	0.0000%
L-GS+H	0	0.0000%
F-GS+H	0	0.0000%
L-GYP	34	0.2212%
F-GYP	0	0.0000%
L-ALL	0	0.0000%
F-ALL	0	0.0000%
L-B	0	0.0000%
F-B	0	0.0000%

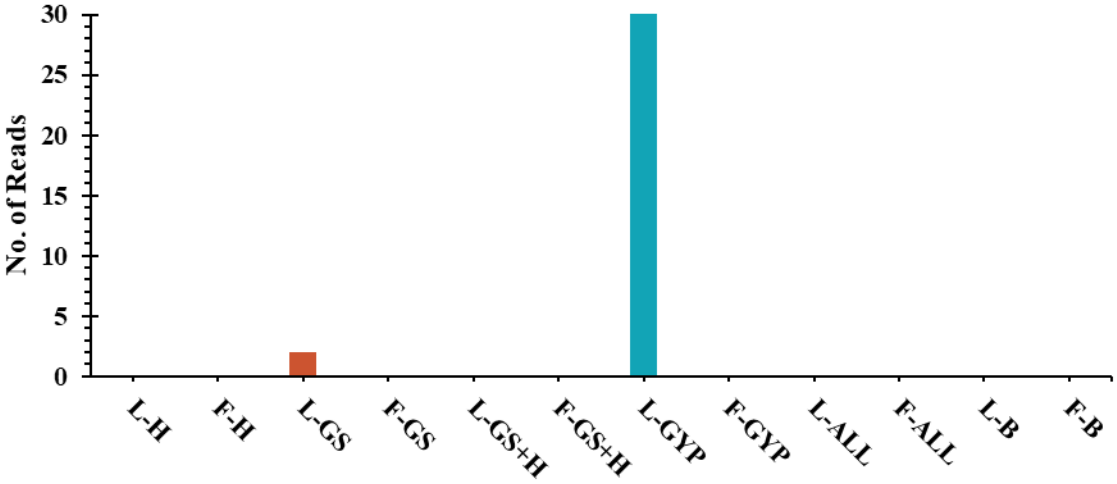

Figure F14a. Summary graph of the number of reads for each amendment type in the classification of *Iron Reducing Bacteria*

Table F14b. Classification of *Iron Reducing Bacteria*

Iron Reducing Bacteria	**Amendment**	**No. of Reads**	**Perc. of Total Reads**	**Classification**
Desulfuromonas (g) [126,127]	L-GYP	34	0.2212%	Desulfuromonas thiophila (s)
Geobacter Uncultured (s) [128]	L-GS	2	0.0144%	-

F15. Iron Reducing, Fermenting Bacteria

Table F15a. Summary results for *Iron Reducing, Fermenting Bacteria*

Amendment	No. of Reads	Perc. of Total Reads
L-H	0	0.0000%
F-H	0	0.0000%
L-GS	9	0.0649%
F-GS	0	0.0000%
L-GS+H	9	0.0496%
F-GS+H	0	0.0000%
L-GYP	0	0.0000%
F-GYP	0	0.0000%
L-ALL	4	0.0244%
F-ALL	0	0.0000%
L-B	8	0.0486%
F-B	0	0.0000%

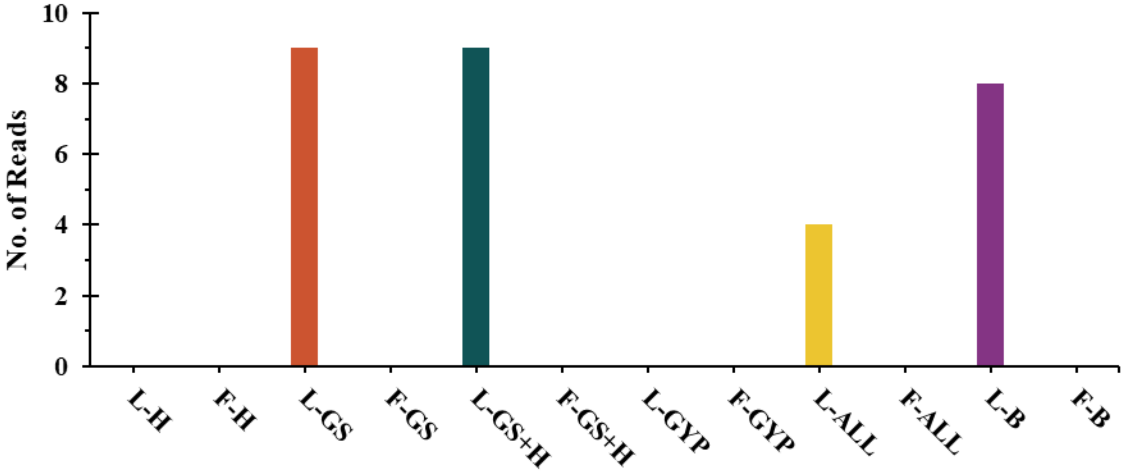

Figure F15a. Summary graph of the number of reads for each amendment type in the classification of *Iron Reducing, Fermenting Bacteria*

Table F15b. Classification of *Iron Reducing, Fermenting Bacteria*

Iron Reducing, Fermenting Bacteria	**Amendment**	**No. of Reads**	**Perc. of Total Reads**	**Classification**
Thermoanaerobaculum (g) [129]	L-GS	9	0.0649%	uncultured bacterium (s)
	L-GS+H	9	0.0496%	uncultured bacterium (s)
	L-B	8	0.0486%	uncultured bacterium (s)
	L-ALL	4	0.0244%	uncultured bacterium (s)

F16. Sulfate Reducing Bacteria

Table F16a. Summary results for *Sulfate Reducing Bacteria*

Amendment	No. of Reads	Perc. of Total Reads
L-H	1725	8.6246%
F-H	273	2.2264%
L-GS	1350	9.7297%
F-GS	625	3.0912%
L-GS+H	1279	7.0523%
F-GS+H	148	0.4945%
L-GYP	618	4.0203%
F-GYP	153	0.9956%
L-ALL	1940	11.8264%
F-ALL	197	1.4059%
L-B	1458	8.8643%
F-B	87	0.3438%

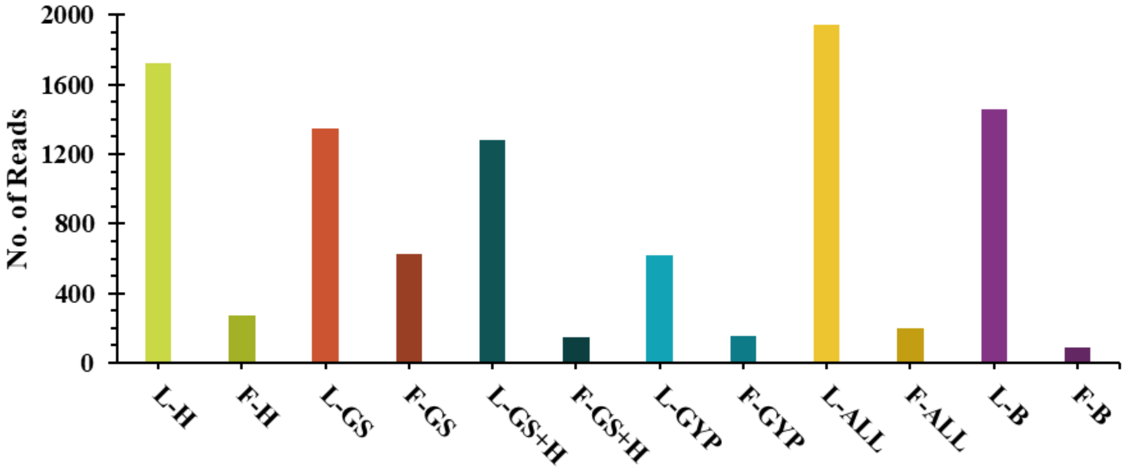

Figure F16a. Summary graph of the number of reads for each amendment type in the classification of *Sulfate Reducing Bacteria*

Table F16b. Classification of *Sulfate Reducing Bacteria*

Sulfate Reducing Bacteria	**Amendment**	**No. of Reads**	**Perc. of Total Reads**	**Classification**
Desulfatiglans (g) [130]	L-H	1090	5.4497%	-
	L-ALL	969	5.9071%	-
	L-B	913	5.5508%	-
	L-GS+H	859	4.7364%	-
	L-GS	719	5.1820%	-
	L-GYP	363	2.3614%	-
	F-GS	207	1.0238%	-
	F-GS	206	1.0188%	uncultured organism (s)
	L-H	122	0.6100%	uncultured organism (s)
	F-H	120	0.9786%	-
	F-H	114	0.9297%	uncultured organism (s)
	L-ALL	96	0.5852%	uncultured organism (s)
	L-GS+H	88	0.4852%	uncultured organism (s)
	F-GS	87	0.4303%	uncultured bacterium (s)
	L-B	86	0.5229%	uncultured organism (s)
	F-ALL	82	0.5852%	uncultured organism (s)
	L-H	78	0.3900%	uncultured bacterium (s)
	L-GS	68	0.4901%	uncultured organism (s)
	F-ALL	66	0.4710%	-
	L-ALL	57	0.3475%	uncultured bacterium (s)
	F-GS+H	48	0.1604%	uncultured organism (s)
	F-GYP	47	0.3059%	uncultured organism (s)
	L-GS	46	0.3315%	uncultured Desulfobacteraceae (s)
	L-GS+H	44	0.2426%	uncultured Desulfobacteraceae (s)
	F-GYP	44	0.2863%	-

Sulfate Reducing Bacteria	Amendment	No. of Reads	Perc. of Total Reads	Classification
Desulfatiglans (g) [130]	F-B	41	0.1620%	uncultured organism (s)
	L-GS	41	0.2955%	uncultured bacterium (s)
	L-GYP	41	0.2667%	uncultured bacterium (s)
	L-B	41	0.2493%	uncultured bacterium (s)
	L-GYP	40	0.2602%	uncultured organism (s)
	L-GS+H	39	0.2150%	uncultured bacterium (s)
	L-ALL	35	0.2134%	uncultured sulfate-reducing (s)
	L-H	33	0.1650%	uncultured Desulfobacteraceae (s)
	L-GYP	31	0.2017%	uncultured sulfate-reducing (s)
	L-GS	30	0.2162%	uncultured sulfate-reducing (s)
	F-H	29	0.2365%	uncultured bacterium (s)
	L-H	28	0.1400%	uncultured sulfate-reducing (s)
	L-B	28	0.1702%	uncultured Desulfobacteraceae (s)
	F-GS+H	26	0.0869%	uncultured bacterium (s)
	L-ALL	23	0.1402%	uncultured Desulfobacteraceae (s)
	F-GS+H	22	0.0735%	-
	F-B	21	0.0830%	uncultured bacterium (s)
	F-ALL	20	0.1427%	uncultured bacterium (s)
	F-GYP	19	0.1236%	uncultured bacterium (s)
	L-GS+H	17	0.0937%	uncultured sulfate-reducing (s)
	F-GS+H	16	0.0535%	uncultured prokaryote (s)
	F-B	16	0.0632%	-
	L-H	2	0.0100%	Desulfobacteraceae bacterium (s)
Desulfobacca (g) [131]	L-B	91	0.5533%	uncultured delta (s)
	L-GS+H	88	0.4852%	uncultured delta (s)
	L-ALL	65	0.3962%	uncultured delta (s)

Sulfate Reducing Bacteria	**Amendment**	**No. of Reads**	**Perc. of Total Reads**	**Classification**
Desulfobacca (g) [131]	L-GS	56	0.4036%	uncultured delta (s)
	L-ALL	48	0.2926%	uncultured bacterium (s)
	L-GS	44	0.3171%	uncultured bacterium (s)
	L-GS+H	42	0.2316%	uncultured bacterium (s)
	L-B	31	0.1885%	uncultured bacterium (s)
	L-GYP	24	0.1561%	uncultured delta (s)
	L-H	15	0.0750%	uncultured bacterium (s)
	L-H	13	0.0650%	uncultured delta (s)
	L-GYP	12	0.0781%	uncultured bacterium (s)
	F-H	10	0.0816%	uncultured bacterium (s)
	F-GS	9	0.0445%	uncultured delta (s)
	F-GS	7	0.0346%	uncultured bacterium (s)
	F-GS+H	5	0.0167%	uncultured bacterium (s)
	F-GS	2	0.0099%	uncultured prokaryote (s)
Desulfocapsa (g) [132]	L-ALL	620	3.7796%	-
	L-GS	342	2.4649%	-
	L-H	341	1.7049%	-
	L-B	256	1.5564%	-
	L-GYP	107	0.6961%	-
	L-GS+H	85	0.4687%	-
Desulfonatronum (g) [133]	F-GS	64	0.3165%	-
	F-GYP	22	0.1432%	-
	F-GS+H	20	0.0668%	-
	F-GS	17	0.0841%	uncultured bacterium (s)
	F-ALL	10	0.0714%	-
	F-B	9	0.0356%	uncultured bacterium (s)

Sulfate Reducing Bacteria	**Amendment**	**No. of Reads**	**Perc. of Total Reads**	**Classification**
Desulfonatronum (g) [133]	F-GYP	8	0.0521%	uncultured bacterium (s)
Desulforhabdus (g) [134]	F-ALL	3	0.0214%	Candidatus Desulfonatronobulbus (s)
Syntrophobacteraceae (f) [134,135]	F-GS	26	0.1286%	-
	L-ALL	20	0.1219%	-
	F-ALL	13	0.0928%	-
	L-B	12	0.0730%	-
	F-GS+H	11	0.0368%	-
	F-GYP	11	0.0716%	-
	L-GS+H	11	0.0607%	uncultured (g) uncultured bacterium (s)
	L-ALL	7	0.0427%	uncultured (g) uncultured delta (s)
	L-GS+H	6	0.0331%	uncultured (g) uncultured delta (s)
	L-GS	4	0.0288%	uncultured (g) uncultured delta (s)
	L-H	3	0.0150%	-
	F-ALL	3	0.0214%	Desulforhabdus (g) Candidatus Desulfonatronobulbus (s)
	F-GYP	2	0.0130%	uncultured (g) uncultured delta (s)

F17. Sulfate Reducing, Fermenting Bacteria

Table F17a. Summary results for *Sulfate Reducing, Fermenting Bacteria*

Amendment	No. of Reads	Perc. of Total Reads
L-H	949	4.7448%
F-H	704	5.7413%
L-GS	666	4.8000%
F-GS	858	4.2435%
L-GS+H	634	3.4958%
F-GS+H	417	1.3933%
L-GYP	200	1.3011%
F-GYP	272	1.7700%
L-ALL	839	5.1146%
F-ALL	257	1.8341%
L-B	593	3.6053%
F-B	315	1.2449%

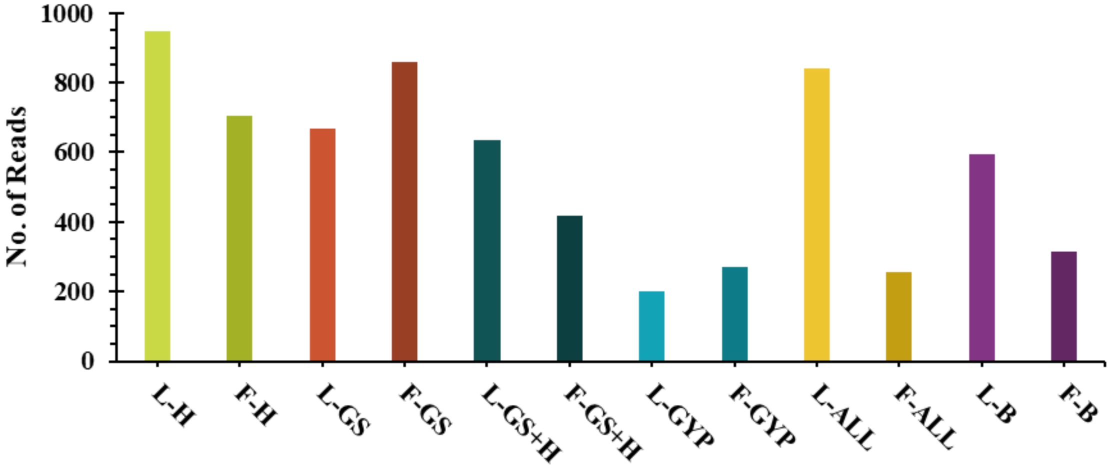

Figure F17a. Summary graph of the number of reads for each amendment type in the classification of *Sulfate Reducing, Fermenting Bacteria*

Table F17b. Classification of *Sulfate Reducing, Fermenting Bacteria*

Sulfate Reducing, Fermenting Bacteria	Amendment	No. of Reads	Perc. of Total Reads	Classification
Desulfococcus (g) [136]	L-ALL	68	0.4145%	-
	L-ALL	65	0.3962%	Desulfococcus biacutus (s)
	L-B	46	0.2797%	-
	L-H	36	0.1800%	-
	F-GS+H	33	0.1103%	-
	L-GS	32	0.2306%	-
	F-B	31	0.1225%	-
	F-H	30	0.2447%	-
	L-GS+H	28	0.1544%	Desulfococcus biacutus (s)
	L-GYP	28	0.1821%	Desulfococcus biacutus (s)
	F-H	20	0.1631%	Desulfococcus biacutus (s)
	F-ALL	17	0.1213%	-
Desulfobacteraceae (f) [137]	L-ALL	249	1.5179%	Sva0081 sediment group (g) uncultured Desulfobacteraceae (s)
	L-GS	244	1.7586%	Sva0081 sediment group (g) uncultured Desulfobacteraceae (s)
	L-GS+H	193	1.3910%	Sva0081 sediment group (g) uncultured Desulfobacteraceae (s)
	L-B	187	1.1369%	Sva0081 sediment group (g) uncultured Desulfobacteraceae (s)
	L-H	146	0.7300%	Sva0081 sediment group (g) uncultured Desulfobacteraceae (s)
	F-H	121	0.9868%	Sva0081 sediment group (g) uncultured Desulfobacteraceae (s)
	F-GS	113	0.5589%	-
	F-GS	111	0.8000%	uncultured (g) uncultured bacterium (s)
	L-H	97	0.4850%	uncultured (g) uncultured bacterium (s)
	L-GS+H	95	0.5238%	uncultured (g) uncultured bacterium (s)

Sulfate Reducing, Fermenting Bacteria	**Amendment**	**No. of Reads**	**Perc. of Total Reads**	**Classification**
Desulfobacteraceae (f) [137]	F-GS	95	0.4699%	Sva0081 sediment group (g) uncultured Desulfobacteraceae (s)
	F-H	89	0.7258%	uncultured (g) uncultured bacterium (s)
	L-ALL	76	0.4633%	uncultured (g) uncultured bacterium (s)
	F-GS+H	76	0.2539%	Sva0081 sediment group (g) uncultured Desulfobacteraceae (s)
	L-B	65	0.3952%	uncultured (g) uncultured Desulfobacteraceae (s)
	L-H	63	0.3150%	uncultured (g)
	L-H	58	0.2900%	SEEP-SRB1 (g) Desulfobacteraceae bacterium (s)
	L-B	53	0.3222%	uncultured (g) uncultured bacterium (s)
	F-H	50	0.4078%	-
	L-GS	48	0.3459%	uncultured (g) uncultured bacterium (s)
	L-ALL	48	0.2926%	uncultured (g) uncultured Desulfobacteraceae (s)
	L-GS	46	0.3315%	Desulfatiglans (g) uncultured Desulfobacteraceae (s)
	F-B	45	0.1778%	Sva0081 sediment group (g) uncultured Desulfobacteraceae (s)
	L-GS+H	44	0.2426%	Desulfatiglans (g) uncultured Desulfobacteraceae (s)
	L-GS	44	0.3171%	Desulfobacter (g)
	L-GS+H	43	0.2371%	SEEP-SRB1 (g) Desulfobacteraceae bacterium (s)
	L-GYP	43	0.2797%	Sva0081 sediment group (g) uncultured Desulfobacteraceae (s)
	F-ALL	43	0.3069%	Sva0081 sediment group (g) uncultured Desulfobacteraceae (s)
	F-GS+H	42	0.1403%	-
	F-B	39	0.2811%	uncultured (g) uncultured bacterium (s)

Sulfate Reducing, Fermenting Bacteria	Amendment	No. of Reads	Perc. of Total Reads	Classification
Desulfobacteraceae (f) [123]	F-GS	39	0.1929%	Desulfatirhabdium (g) uncultured Desulfobacteraceae (s)
	L-GYP	38	0.2472%	Desulfobacter (g)
	L-H	33	0.1650%	Desulfatiglans (g) uncultured Desulfobacteraceae (s)
	F-GYP	33	0.2147%	-
	F-GYP	32	0.2082%	uncultured (g) uncultured bacterium (s)
	L-ALL	32	0.1951%	SEEP-SRB1 (g) Desulfobacteraceae bacterium (s)
	F-GYP	32	0.2306%	Sva0081 sediment group (g) uncultured Desulfobacteraceae (s)
	L-H	31	0.1550%	Desulfatirhabdium (g) uncultured Desulfobacteraceae (s)
	F-H	31	0.2528%	Desulfatirhabdium (g) uncultured Desulfobacteraceae (s)
	L-GS+H	30	0.1654%	Desulfobacterium (g) uncultured delta (s)
	L-B	30	0.1824%	Desulfobacterium (g) uncultured delta (s)
	L-GS+H	29	0.1599%	-
	L-ALL	29	0.1768%	Desulfatirhabdium (g) uncultured Desulfobacteraceae (s)
	L-B	28	0.1702%	Desulfatiglans (g) uncultured Desulfobacteraceae (s)
	L-ALL	26	0.1585%	-
	F-ALL	25	0.1784%	-
	L-GS	25	0.1802%	uncultured (g)
	F-GS	24	0.1187%	Desulfobacter (g)
	F-ALL	24	0.1713%	uncultured (g) uncultured bacterium (s)
	L-GS	24	0.1730%	SEEP-SRB1 (g) Desulfobacteraceae bacterium (s)

Sulfate Reducing, Fermenting Bacteria	Amendment	No. of Reads	Perc. of Total Reads	Classification
Desulfobacteraceae (f) [137]	L-H	24	0.1200%	uncultured (g) uncultured Desulfobacteraceae (s)
	L-ALL	23	0.1658%	Desulfatiglans (g) uncultured Desulfobacteraceae (s)
	L-H	23	0.1150%	-
	L-GS	23	0.1658%	Desulfatirhabdium (g) uncultured Desulfobacteraceae (s)
	L-H	22	0.1100%	Desulfobacterium (g) uncultured delta (s)
	L-B	21	0.1277%	-
	F-B	21	0.0830%	-
	F-H	21	0.1713%	Desulfobacterium (g)
	L-B	21	0.1277%	SEEP-SRB1 (g) Desulfobacteraceae bacterium (s)
	L-B	20	0.1216%	Desulfatirhabdium (g) uncultured Desulfobacteraceae (s)
	F-GS+H	19	0.0635%	Desulfatirhabdium (g) uncultured Desulfobacteraceae (s)
	F-ALL	18	0.1285%	Desulfatirhabdium (g) uncultured Desulfobacteraceae (s)
	L-GS+H	16	0.0882%	Desulfatirhabdium (g) uncultured Desulfobacteraceae (s)
	L-B	15	0.0912%	Desulfobacter (g)
	F-GS	14	0.0692%	Desulfobacterium (g)
	F-GYP	14	0.0911%	Desulfatirhabdium (g) uncultured Desulfobacteraceae (s)
	L-H	14	0.0700%	SEEP-SRB1 (g) uncultured Desulfobacteraceae (s)
	L-GS+H	13	0.0717%	Desulfobacter (g)
	L-GS	12	0.0865%	-
	L-ALL	12	0.0732%	Desulfobacterium (g)

Sulfate Reducing, Fermenting Bacteria	**Amendment**	**No. of Reads**	**Perc. of Total Reads**	**Classification**
Desulfobacteraceae (f) [137]	F-H	11	0.0897%	Desulfobacter (g)
	F-ALL	10	0.0714%	uncultured (g) uncultured delta (s)
	F-GS+H	9	0.0301%	uncultured (g) uncultured delta (s)
	L-H	8	0.0400%	Desulfobacter (g)
	F-GYP	7	0.0456%	Desulfobacter (g)
	F-GYP	4	0.0260%	Desulfobacterium (g)
	F-GS	3	0.0216%	uncultured (g) uncultured delta (s)
	L-H	2	0.0100%	Desulfatiglans (g) Desulfobacteraceae bacterium (s)
Desulfosarcina (g) [138]	L-H	368	1.8399%	-
	F-H	259	2.1122%	-
	F-GS	240	1.1870%	-
	L-ALL	171	1.0424%	-
	F-GS+H	159	0.5313%	-
	L-GS	148	1.0667%	-
	F-B	120	0.4742%	-
	L-GS+H	103	0.5679%	-
	L-B	95	0.5776%	-
	F-GYP	78	0.5076%	-
	F-ALL	70	0.4996%	-
	F-GS	34	0.1682%	uncultured bacterium (s)
	F-H	30	0.2447%	uncultured bacterium (s)
	L-GS+H	23	0.1268%	uncultured bacterium (s)
	L-H	21	0.1050%	uncultured bacterium (s)
	L-GS	16	0.1153%	uncultured bacterium (s)
	L-ALL	13	0.0792%	uncultured bacterium (s)

Sulfate Reducing, Fermenting Bacteria	**Amendment**	**No. of Reads**	**Perc. of Total Reads**	**Classification**
	F-B	11	0.0435%	uncultured bacterium (s)
Desulfovibrio (g) [139]	F-GS	7	0.0346%	-
	F-GS+H	4	0.0134%	-
Desulfovibrio (g) [139]	F-GYP	3	0.0195%	-
Desulfovibrionales (o) [140]	L-GYP	64	0.4163%	Desulfomicrobiaceae (f) Desulfomicrobium (g)
	F-GS	64	0.3165%	Desulfomicrobiaceae (f) Desulfomicrobium (g)
	F-GS	64	0.3165%	Desulfonatronaceae (f) Desulfonatronum (g)
	F-H	42	0.3425%	Desulfomicrobiaceae (f) Desulfomicrobium (g)
	F-GS+H	40	0.1336%	Desulfomicrobiaceae (f) Desulfomicrobium (g)
	F-B	39	0.1541%	Desulfomicrobiaceae (f) Desulfomicrobium (g) Desulfomicrobium sp. (s)
	L-GYP	27	0.1756%	Desulfomicrobiaceae (f) Desulfomicrobium (g) uncultured bacterium (s)
	F-ALL	24	0.1713%	Desulfomicrobiaceae (f) Desulfomicrobium (g)
	F-GYP	23	0.1497%	Desulfomicrobiaceae (f) Desulfomicrobium (g)
	F-GYP	22	0.1432%	Desulfonatronaceae (f) Desulfonatronum (g)
	F-GS+H	20	0.1441%	Desulfonatronaceae (f) Desulfonatronum (g)
	F-GS	17	0.0841%	Desulfonatronaceae (f) Desulfonatronum (g) uncultured bacterium (s)
	F-ALL	10	0.0714%	Desulfonatronaceae (f) Desulfonatronum (g)

Sulfate Reducing, Fermenting Bacteria	**Amendment**	**No. of Reads**	**Perc. of Total Reads**	**Classification**
Desulfovibrionales (o) [140]	F-B	9	0.0356%	Desulfonatronaceae (f) Desulfonatronum (g) uncultured bacterium (s)
	F-GYP	8	0.0521%	Desulfonatronaceae (f) Desulfonatronum (g) uncultured bacterium (s)
	F-GS	7	0.0346%	Desulfovibrionaceae (f) Desulfovibrio (g)
	F-GS+H	4	0.0134%	Desulfovibrionaceae (f) Desulfovibrio (g)
	F-GYP	3	0.0195%	Desulfovibrionaceae (f) Desulfovibrio (g)
Syntrophobacteraceae (f) [134,135]	F-GS	26	0.1286%	-
	L-ALL	20	0.1219%	-
	F-ALL	13	0.0928%	-
	L-B	12	0.0730%	-
	F-GS+H	11	0.0368%	-
	F-GYP	11	0.0716%	-
	L-GS+H	11	0.0607%	uncultured (g) uncultured bacterium (s)
	L-ALL	7	0.0427%	uncultured (g) uncultured delta (s)
	L-GS+H	6	0.0331%	uncultured (g) uncultured delta (s)
	L-GS	4	0.0288%	uncultured (g) uncultured delta (s)
	L-H	3	0.0150%	-
	F-ALL	3	0.0214%	Desulforhabdus (g) Candidatus Desulfonatronobulbus (s)
	F-GYP	2	0.0130%	uncultured (g) uncultured delta (s)

F18. Fermenting Bacteria

Table F18a. Summary results for *Fermenting Bacteria*

Amendment	No. of Reads	Perc. of Total Reads
L-H	635	3.1748%
F-H	236	1.9246%
L-GS	425	3.0631%
F-GS	460	2.2751%
L-GS+H	758	4.1795%
F-GS+H	420	1.4033%
L-GYP	895	5.8223%
F-GYP	115	0.7484%
L-ALL	807	4.9195%
F-ALL	191	1.3631%
L-B	607	3.6904%
F-B	341	1.3476%

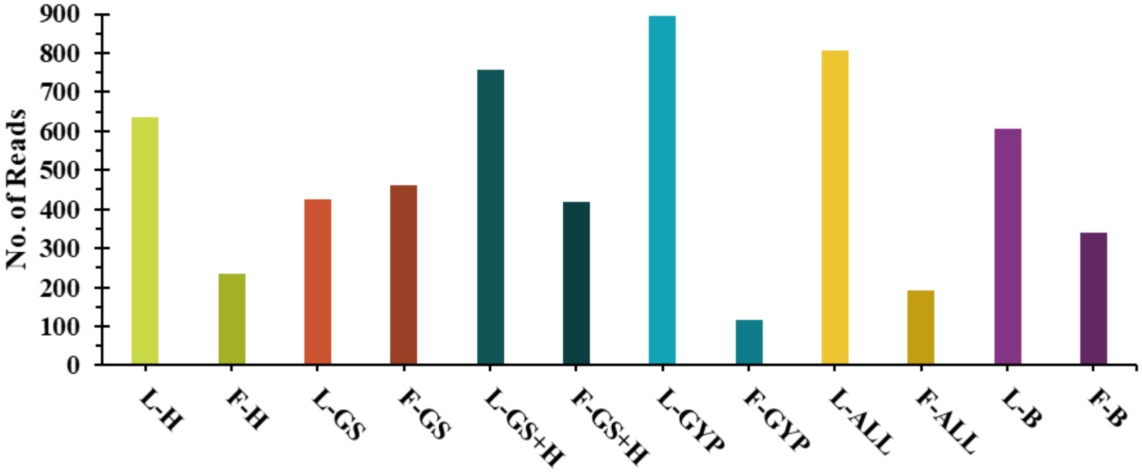

Figure F18a. Summary graph of the number of reads for each amendment type in the classification of *Fermenting Bacteria*

Table F18b. Classification of *Fermenting Bacteria*

Fermenting Bacteria	Amendment	No. of Reads	Perc. of Total Reads	Classification
Anaerolineae (c) [141]	L-GS+H	320	1.7644%	Anaerolineales (o) Anaerolineaceae (f) uncultured (g)
	L-ALL	236	1.4387%	Anaerolineales (o) Anaerolineaceae (f) uncultured (g)
	L-H	210	1.0499%	Anaerolineales (o) Anaerolineaceae (f)
	L-B	169	1.0275%	Anaerolineales (o) Anaerolineaceae (f) uncultured (g)
	L-H	135	0.6750%	Anaerolineales (o) Anaerolineaceae (f) uncultured (g)
	L-ALL	129	0.7864%	Anaerolineales (o) Anaerolineaceae (f)
	L-GS+H	124	0.6837%	Anaerolineales (o) Anaerolineaceae (f)
	L-GYP	99	0.6440%	Anaerolineales (o) Anaerolineaceae (f)
	L-GS	98	0.7063%	Anaerolineales (o) Anaerolineaceae (f) uncultured (g)
	L-GS	92	0.6631%	Anaerolineales (o) Anaerolineaceae (f)
	L-B	89	0.5411%	Anaerolineales (o) Anaerolineaceae (f)
	F-H	86	0.7014%	Anaerolineales (o) Anaerolineaceae (f)
	L-ALL	85	0.5182%	Anaerolineales (o) Anaerolineaceae (f) uncultured (g) uncultured Chloroflexi (s)
	L-GS+H	80	0.4411%	Anaerolineales (o) Anaerolineaceae (f) uncultured (g) uncultured Chloroflexi (s)
	L-H	73	0.3650%	Anaerolineales (o) Anaerolineaceae (f) uncultured (g) uncultured Chloroflexi (s)
	L-GYP	69	0.4489%	Anaerolineales (o) Anaerolineaceae (f) uncultured (g)
	L-GYP	65	0.4228%	Anaerolineales (o) Anaerolineaceae (f) uncultured (g) uncultured Chloroflexi (s)

Fermenting Bacteria	Amendment	No. of Reads	Perc. of Total Reads	Classification
Anaerolineae (c) [141]	L-GYP	48	0.3123%	Anaerolineales (o)Anaerolineaceae (f) uncultured (g) uncultured Longilinea (s)
	F-GS	47	0.2325%	Anaerolineales (o) Anaerolineaceae (f)
	L-GYP	43	0.2797%	Anaerolineales (o) Anaerolineaceae (f) Longilinea (g) uncultured Chloroflexi (s)
	L-GYP	43	0.2797%	Anaerolineales (o) Anaerolineaceae (f) Ornatilinea (g) uncultured Chloroflexi (s)
	F-GS+H	39	0.1303%	Caldilineales (o) Caldilineaceae (f) uncultured (g) uncultured bacterium (s)
	F-B	38	0.1502%	Caldilineales (o) Caldilineaceae (f) uncultured (g) uncultured bacterium (s)
	F-GS	36	0.1781%	Anaerolineales (o) Anaerolineaceae (f) uncultured (g) metagenome (s)
	F-B	34	0.1344%	Anaerolineales (o) Anaerolineaceae (f)
	L-GS	34	0.2450%	Anaerolineales (o) Anaerolineaceae (f) uncultured (g) uncultured Chloroflexi (s)
	L-ALL	34	0.2073%	uncultured (o) uncultured (f) uncultured (g) uncultured bacterium (s)
	F-GS+H	32	0.1069%	Anaerolineales (o) Anaerolineaceae (f)
	L-B	31	0.1885%	Anaerolineales (o) Anaerolineaceae (f) Longilinea (g) uncultured Chloroflexi (s)
	L-GS+H	31	0.1709%	uncultured (o) uncultured (f) uncultured (g) uncultured bacterium (s)
	L-ALL	27	0.1646%	-
	F-B	24	0.0948%	Ardenticatenales(o) uncultured (f) uncultured (g)
	F-GS	23	0.1138%	Anaerolineales (o) Anaerolineaceae (f) Ornatilinea (g) uncultured Chloroflexi (s)

Fermenting Bacteria	Amendment	No. of Reads	Perc. of Total Reads	Classification
Anaerolineae (c) [141]	F-GYP	22	0.1432%	Ardenticatenales(o) uncultured (f) uncultured (g)
	L-B	21	0.1277%	Anaerolineales (o) Anaerolineaceae (f) Anaerolineaceae UCG-001 (g) uncultured Chloroflexi (s)
	F-GS+H	20	0.0668%	Ardenticatenales(o) uncultured (f) uncultured (g)
	L-GYP	20	0.1301%	uncultured (o) uncultured (f) uncultured (g) uncultured bacterium (s)
	L-B	19	0.1155%	Anaerolineales (o) Anaerolineaceae (f) uncultured (g) bacterium (s)
	L-B	19	0.1155%	Anaerolineales (o) Anaerolineaceae (f) uncultured (g) uncultured Chloroflexi (s)
	F-H	18	0.1468%	Anaerolineales (o) Anaerolineaceae (f) uncultured (g)
	L-H	18	0.0900%	Anaerolineales (o) Anaerolineaceae (f) uncultured (g) uncultured prokaryote (s)
	F-GS	17	0.0841%	Anaerolineales (o) Anaerolineaceae (f) Anaerolineaceae UCG-001 (g) uncultured Chloroflexi (s)
	L-H	17	0.0850%	Anaerolineales (o) Anaerolineaceae (f) Longilinea (g) uncultured Chloroflexi (s)
	F-ALL	17	0.1213%	Anaerolineales (o) Anaerolineaceae (f) Ornatilinea (g) uncultured Chloroflexi (s)
	L-GS+H	16	0.0882%	-
	F-ALL	16	0.1142%	Anaerolineales (o) Anaerolineaceae (f)
	L-H	16	0.0800%	Anaerolineales (o) Anaerolineaceae (f) Anaerolineaceae UCG-001 (g) uncultured Chloroflexi (s)
	L-ALL	16	0.0975%	Anaerolineales (o) Anaerolineaceae (f) Anaerolineaceae UCG-001 (g) uncultured Chloroflexi (s)

Fermenting Bacteria	Amendment	No. of Reads	Perc. of Total Reads	Classification
Anaerolineae (c) [141]	L-GYP	16	0.1041%	Anaerolineales (o) Anaerolineaceae (f) Leptolinea (g) uncultured bacterium (s)
	L-GS	15	0.1081%	ADurb.Bin180 (o) ADurb.Bin180 (f) ADurb.Bin180 (g)
	L-ALL	15	0.0914%	Anaerolineales (o) Anaerolineaceae (f) Longilinea (g) uncultured Chloroflexi (s)
	F-ALL	15	0.1071%	Ardenticatenales(o) uncultured (f) uncultured (g)
	F-H	15	0.1223%	Caldilineales (o) Caldilineaceae (f) uncultured (g) uncultured bacterium (s)
	F-GYP	15	0.0976%	Caldilineales (o) Caldilineaceae (f) uncultured (g) uncultured bacterium (s)
	L-GS	15	0.1081%	uncultured (o) uncultured (f) uncultured (g) uncultured bacterium (s)
	L-B	14	0.0851%	ADurb.Bin180 (o) ADurb.Bin180 (f) ADurb.Bin180 (g)
	L-GS+H	14	0.0772%	Anaerolineales (o) Anaerolineaceae (f) Longilinea (g) uncultured Chloroflexi (s)
	L-GS+H	14	0.0772%	SBR1031 (o) SBR1031 (f) SBR1031 (g)
	L-GS+H	14	0.0772%	SBR1031 (o) BR1031 (f) SBR1031 (g) uncultured bacterium (s)
	L-B	14	0.0851%	uncultured (o) uncultured (f) uncultured (g) uncultured bacterium (s)
	F-ALL	13	0.0928%	Anaerolineales (o) Anaerolineaceae (f) Leptolinea (g)
	L-B	13	0.0790%	Anaerolineales (o) Anaerolineaceae (f) Ornatilinea (g) uncultured Chloroflexi (s)
	F-GS+H	13	0.0434%	Anaerolineales (o) Anaerolineaceae (f) uncultured (g) metagenome (s)
	L-GS	13	0.0937%	Anaerolineales (o)Anaerolineaceae (f) uncultured (g) uncultured Longilinea (s)

Fermenting Bacteria	Amendment	No. of Reads	Perc. of Total Reads	Classification
Anaerolineae (c) [141]	L-B	13	0.0790%	Anaerolineales (o)Anaerolineaceae (f) uncultured (g) uncultured Longilinea (s)
	F-GS	13	0.0643%	Ardenticatenales(o) uncultured (f) uncultured (g)
	L-B	13	0.0790%	SBR1031 (o) SBR1031 (f) SBR1031 (g)
	L-GS	13	0.0937%	SBR1031 (o) BR1031 (f) SBR1031 (g) uncultured bacterium (s)
	L-B	13	0.0790%	SBR1031 (o) BR1031 (f) SBR1031 (g) uncultured bacterium (s)
	L-H	12	0.0600%	ADurb.Bin180 (o) ADurb.Bin180 (f) ADurb.Bin180 (g)
	L-H	12	0.0600%	Anaerolineales (o) Anaerolineaceae (f) Leptolinea (g)
	L-GS+H	12	0.0662%	Anaerolineales (o) Anaerolineaceae (f) Leptolinea (g)
	F-ALL	12	0.0856%	Caldilineales (o) Caldilineaceae (f) uncultured (g) uncultured bacterium (s)
	L-ALL	12	0.0732%	SBR1031 (o) BR1031 (f) SBR1031 (g) uncultured bacterium (s)
	F-GS+H	12	0.0401%	SBR1031 (o) BR1031 (f) SBR1031 (g) uncultured bacterium (s)
	L-B	12	0.0730%	SJA-15 (o) SJA-15 (f) SJA-15 (g) uncultured bacterium (s)
	F-GYP	11	0.0716%	Anaerolineales (o) Anaerolineaceae (f)
	L-GS	11	0.0793%	Anaerolineales (o) Anaerolineaceae (f) Anaerolineaceae UCG-001 (g) uncultured Chloroflexi (s)
	F-B	11	0.0435%	Anaerolineales (o) Anaerolineaceae (f) Anaerolineaceae UCG-001 (g) uncultured Chloroflexi (s)
	L-GS	11	0.0793%	Anaerolineales (o) Anaerolineaceae (f) Ornatilinea (g) uncultured Chloroflexi (s)

Fermenting Bacteria	Amendment	No. of Reads	Perc. of Total Reads	Classification
Anaerolineae (c) [141]	F-H	11	0.0897%	Anaerolineales (o) Anaerolineaceae (f) Ornatilinea (g) uncultured Chloroflexi (s)
	F-GS	11	0.0544%	Caldilineales (o) Caldilineaceae (f) uncultured (g) uncultured bacterium (s)
	F-B	11	0.0435%	SBR1031 (o) BR1031 (f) SBR1031 (g) uncultured bacterium (s)
	F-B	10	0.0395%	Anaerolineales (o) Anaerolineaceae (f) Ornatilinea (g) uncultured Chloroflexi (s)
	F-ALL	10	0.0714%	Anaerolineales (o) Anaerolineaceae (f) uncultured (g) metagenome (s)
	L-B	10	0.0608%	Ardenticatenales(o) uncultured (f) uncultured (g)
	F-GS	10	0.0495%	SBR1031 (o) A4b (f) A4b (g) uncultured organism (s)
	L-GS	9	0.0649%	-
	L-B	9	0.0547%	-
	L-GS+H	9	0.0496%	ADurb.Bin180 (o) ADurb.Bin180 (f) ADurb.Bin180 (g)
	F-H	9	0.0734%	Anaerolineales (o) Anaerolineaceae (f) Longilinea (g) uncultured Chloroflexi (s)
	F-H	9	0.0734%	Anaerolineales (o)Anaerolineaceae (f) uncultured (g) uncultured Longilinea (s)
	L-GS	9	0.0649%	RBG-13-54-9 (o) RBG-13-54-9 (f) RBG-13-54-9 (f) uncultured bacterium (s)
	L-H	9	0.0450%	SBR1031 (o) BR1031 (f) SBR1031 (g) uncultured bacterium (s)
	L-GS+H	8	0.0441%	Anaerolineales (o) Anaerolineaceae (f) Ornatilinea (g) uncultured bacterium (s)
	L-ALL	8	0.0488%	Anaerolineales (o) Anaerolineaceae (f) Ornatilinea (g) uncultured bacterium (s)

Fermenting Bacteria	Amendment	No. of Reads	Perc. of Total Reads	Classification
Anaerolineae (c) [141]	L-GS+H	8	0.0441%	MSB-5E12 (o) MSB-5E12 (f) MSB-5E12 (g) uncultured bacterium (s)
	L-GS	8	0.0577%	SJA-15 (o) SJA-15 (f) SJA-15 (g) uncultured bacterium (s)
	L-GYP	7	0.0455%	Anaerolineales (o) Anaerolineaceae (f) ADurb.Bin120 (f) uncultured organism (s)
	L-ALL	7	0.0427%	MSB-5E12 (o) MSB-5E12 (f) MSB-5E12 (g) uncultured bacterium (s)
	F-B	7	0.0277%	SBR1031 (o) A4b (f) A4b (g) uncultured gamma (s)
	L-GYP	7	0.0455%	uncultured (o)uncultured (f) uncultured (g) metagenome (s)
	L-H	6	0.0300%	-
	F-H	6	0.0489%	ADurb.Bin180 (o) ADurb.Bin180 (f) ADurb.Bin180 (g)
	L-B	6	0.0365%	Anaerolineales (o) Anaerolineaceae (f) Ornatilinea (g) uncultured bacterium (s)
	F-B	6	0.0237%	Anaerolineales (o) Anaerolineaceae (f) Ornatilinea (g) uncultured bacterium (s)
	F-GS	6	0.0297%	Anaerolineales (o)Anaerolineaceae (f) uncultured (g) uncultured Longilinea (s)
	L-H	6	0.0300%	Anaerolineales (o) Anaerolineaceae (f) uncultured (g) uncultured organism (s)
	L-GYP	6	0.0390%	MSB-5E12 (o) MSB-5E12 (f) MSB-5E12 (g) uncultured bacterium (s)
	L-GS+H	6	0.0331%	SBR1031 (o) A4b (f) A4b (g) uncultured Anaerolineae (s)
	L-GS	5	0.0360%	ADurb.Bin180 (o) ADurb.Bin180 (f) ADurb.Bin180 (g) uncultured bacterium (s)
	F-GYP	5	0.0325%	Anaerolineales (o) Anaerolineaceae (f) Ornatilinea (g) uncultured Chloroflexi (s)

Fermenting Bacteria	Amendment	No. of Reads	Perc. of Total Reads	Classification
	L-H	5	0.0250%	Anaerolineales (o) Anaerolineaceae (f) Ornatilinea (g) uncultured bacterium (s)
	F-GS+H	5	0.0167%	Anaerolineales (o) Anaerolineaceae (f) Ornatilinea (g) uncultured bacterium (s)
	F-GS+H	5	0.0167%	Anaerolineales (o) Anaerolineaceae (f) uncultured (g) bacterium (s)
	F-B	5	0.0198%	Anaerolineales (o)Anaerolineaceae (f) uncultured (g) uncultured Longilinea (s)
	L-B	5	0.0304%	Anaerolineales (o) Anaerolineaceae (f) uncultured (g) uncultured prokaryote (s)
	L-GS+H	5	0.0276%	RBG-13-54-9 (o) RBG-13-54-9 (f) RBG-13-54-9 (f) uncultured bacterium (s)
	F-GS+H	5	0.0167%	SBR1031 (o) A4b (f) A4b (g) uncultured bacterium (s)
	F-GYP	5	0.0325%	SBR1031 (o) A4b (f) A4b (g) uncultured bacterium (s)
	F-ALL	5	0.0357%	SBR1031 (o) A4b (f) A4b (g) uncultured organism (s)
	L-GS+H	5	0.0276%	SBR1031 (o) SBR1031 (f) SBR1031 (g) metagenome (s)
	F-GS	5	0.0247%	SBR1031 (o) BR1031 (f) SBR1031 (g) uncultured bacterium (s)
	F-GYP	4	0.0260%	Anaerolineales (o) Anaerolineaceae (f) Ornatilinea (g) uncultured bacterium (s)
	F-ALL	4	0.0285%	Anaerolineales (o) Anaerolineaceae (f) uncultured (g) bacterium (s)
	L-H	4	0.0200%	SBR1031 (o) A4b (f) A4b (g) uncultured organism (s)
	F-GYP	4	0.0260%	SBR1031 (o) BR1031 (f) SBR1031 (g) uncultured bacterium (s)
	L-GYP	4	0.0260%	SJA-15 (o) SJA-15 (f) SJA-15 (g) uncultured soil (s)

Fermenting Bacteria	Amendment	No. of Reads	Perc. of Total Reads	Classification
Anaerolineae (c) [141]	L-ALL	4	0.0244%	SJA-15 (o) SJA-15 (f) SJA-15 (g) uncultured soil (s)
	L-B	4	0.0243%	uncultured (o)uncultured (f) uncultured (g) metagenome (s)
	F-GS	4	0.0198%	uncultured (o)uncultured (f) uncultured (g) metagenome (s)
	L-H	4	0.0200%	uncultured (o) uncultured (f) uncultured (g) uncultured organism (s)
	F-GS+H	4	0.0134%	uncultured (o) uncultured (f) uncultured (g) uncultured organism (s)
	L-ALL	3	0.0183%	ADurb.Bin180 (o) ADurb.Bin180 (f) ADurb.Bin180 (g) uncultured bacterium (s)
	F-H	3	0.0245%	Anaerolineales (o) Anaerolineaceae (f) Anaerolineaceae UCG-001 (g) uncultured Chloroflexi (s)
	F-GS+H	3	0.0100%	Anaerolineales (o) Anaerolineaceae (f) Anaerolineaceae UCG-001 (g) uncultured Chloroflexi (s)
	F-ALL	3	0.0214%	Anaerolineales (o) Anaerolineaceae (f) Anaerolineaceae UCG-001 (g) uncultured Chloroflexi (s)
	L-GS+H	3	0.0165%	Anaerolineales (o) Anaerolineaceae (f) uncultured (g) uncultured organism (s)
Anaerolineae (c) [141]	L-GS	3	0.0216%	Caldilineales (o) Caldilineaceae (f) uncultured (g) uncultured bacterium (s)
	F-ALL	3	0.0214%	SBR1031 (o) A4b (f) A4b (g) uncultured bacterium (s)
	L-ALL	3	0.0183%	SBR1031 (o) SBR1031 (f) SBR1031 (g)
	F-ALL	3	0.0214%	SBR1031 (o) BR1031 (f) SBR1031 (g) uncultured bacterium (s)
	L-H	3	0.0150%	SBR1031 (o) SBR1031 (f) SBR1031 (g) uncultured organism (s)
	L-GS+H	3	0.0165%	SBR1031 (o) SBR1031 (f) SBR1031 (g)

Fermenting Bacteria	Amendment	No. of Reads	Perc. of Total Reads	Classification
				uncultured organism (s)
	L-ALL	3	0.0183%	SJA-15 (o) SJA-15 (f) SJA-15 (g) metagenome (s)
	F-GS+H	3	0.0100%	SJA-15 (o) SJA-15 (f) SJA-15 (g) uncultured bacterium (s)
	L-GS+H	3	0.0165%	uncultured (o) uncultured (f) uncultured (g) uncultured organism (s)
	F-GS+H	2	0.0067%	ADurb.Bin180 (o) ADurb.Bin180 (f) ADurb.Bin180 (g)
	F-H	2	0.0163%	ADurb.Bin180 (o) ADurb.Bin180 (f) ADurb.Bin180 (g) uncultured bacterium (s)
	F-H	2	0.0163%	Anaerolineales (o) Anaerolineaceae (f) Anaerolinea (g) uncultured soil (s)
	L-GS+H	2	0.0110%	Anaerolineales (o) Anaerolineaceae (f)Levilinea (g) Levilinea saccharolytica (s)
	L-B	2	0.0122%	Anaerolineales (o) Anaerolineaceae (f) Longilinea (g) uncultured bacterium (s)
	F-GS	2	0.0099%	Anaerolineales (o) Anaerolineaceae (f) uncultured (g)
	F-B	2	0.0079%	Anaerolineales (o) Anaerolineaceae (f) uncultured (g)
	F-H	2	0.0163%	Anaerolineales (o) Anaerolineaceae (f) uncultured (g) bacterium (s)
	F-GYP	2	0.0130%	Anaerolineales (o) Anaerolineaceae (f) uncultured (g) bacterium (s)
	L-GS+H	2	0.0110%	Ardenticatenales (o) uncultured (f) uncultured (g) uncultured bacterium (s)
	L-ALL	2	0.0122%	Ardenticatenales (o) uncultured (f) uncultured (g) uncultured organism (s)
	F-GS+H	2	0.0067%	Caldilineales (o) Caldilineaceae (f)
	L-GYP	2	0.0130%	Caldilineales (o) Caldilineaceae (f)

Fermenting Bacteria	Amendment	No. of Reads	Perc. of Total Reads	Classification
				uncultured (g) uncultured bacterium (s)
	F-H	2	0.0163%	SBR1031 (o) A4b (f) A4b (g)
	L-B	2	0.0122%	SBR1031 (o) A4b (f) A4b (g) uncultured Anaerolineae (s)
	L-GS	2	0.0144%	SBR1031 (o) SBR1031 (f) SBR1031 (g) toluene-degrading methanogenic (s)
	L-GYP	2	0.0130%	SBR1031 (o) SBR1031 (f) SBR1031 (g) toluene-degrading methanogenic (s)
	L-GYP	2	0.0130%	SBR1031 (o) BR1031 (f) SBR1031 (g) uncultured bacterium (s)
Cellulosilyticum (g) [142]	L-GS	3	0.0216%	uncultured bacterium (s)
Cellulosilyticum Uncultured (s) [142]	L-ALL	5	0.0305%	-
Chitinivibrionia (c) [143]	L-H	8	0.0400%	uncultured (o) uncultured (f) uncultured (g) uncultured bacterium (s)
	L-B	4	0.0243%	uncultured (o) uncultured (f) uncultured (g) uncultured bacterium (s)
Clostridium (g) [144]	F-GS	144	0.7122%	Clostridium sensu stricto 5 (g) Clostridium bovipellis (s)
	L-GYP	36	0.2342%	Clostridium sensu stricto 1 (g)
	L-ALL	35	0.2134%	Clostridium sensu stricto 13 (g)
	L-GS+H	15	0.0827%	Clostridium sensu stricto 13 (g)
	L-H	13	0.0650%	Clostridium sensu stricto 13 (g)
	L-B	13	0.0790%	Clostridium sensu stricto 13 (g)
	F-B	12	0.0474%	Clostridium sensu stricto 5 (g) Clostridium bovipellis (s)
	L-GYP	11	0.0716%	Clostridium sensu stricto 8 (g) uncultured bacterium (s)
	F-GS+H	9	0.0301%	Clostridium sensu stricto 5 (g) Clostridium bovipellis (s)

Fermenting Bacteria	**Amendment**	**No. of Reads**	**Perc. of Total Reads**	**Classification**
Clostridium (g) [144]	L-ALL	8	0.0488%	Clostridium sensu stricto 8 (g) uncultured bacterium (s)
	L-ALL	7	0.0427%	Clostridium sensu stricto 13 (g)
	F-GS	4	0.0198%	Clostridium sensu stricto 1 (g)
	F-GYP	3	0.0195%	Clostridium sensu stricto 3 (g) Clostridiaceae bacterium (s)
	L-GS	2	0.0144%	Clostridium sensu stricto 1 (g) Clostridium gasigenes (s)
	F-H	2	0.0163%	Clostridium sensu stricto 12 (g) Clostridium drakei (s)
Coriobacteriaceae Uncultured (s) [145.146]	L-GS+H	8	0.0441%	-
	L-GYP	8	0.0520%	-
	L-ALL	8	0.0488%	-
	L-B	6	0.0365%	-
Elusimicrobia (c) [147]	L-GYP	17	0.1106%	Lineage IV (o) Lineage IV (f) Lineage IV (g) uncultured bacterium (s)
	L-GS	2	0.0144%	Lineage IV (o) Lineage IV (f) Lineage IV (g) uncultured bacterium (s)
Eubacteriaceae (f) [148]	L-GYP	95	0.6180%	Acetobacterium (g)
	L-GYP	27	0.1756%	Acetobacterium (g) uncultured Firmicutes (s)
	L-ALL	11	0.0671%	Acetobacterium (g)
	L-ALL	11	0.0671%	Acetobacterium (g) uncultured Firmicutes (s)
	L-B	10	0.0608%	Acetobacterium (g)
	L-H	7	0.0350%	Acetobacterium (g)
Holophaga (s) [149]	F-ALL	7	0.0500%	-
	F-B	3	0.0119%	-
	L-GS+H	24	0.1323%	Latescibacterales (o) Latescibacteraceae (f) Candidatus Latescibacter (g)

Fermenting Bacteria	**Amendment**	**No. of Reads**	**Perc. of Total Reads**	**Classification**
Latescibacteria (c) [150]	L-ALL	18	0.1097%	Latescibacterales (o) Latescibacteraceae (f) Candidatus Latescibacter (g) uncultured bacterium (s)
	L-GS	11	0.0793%	Latescibacterales (o) Latescibacteraceae (f) Candidatus Latescibacter (g)
	L-B	11	0.0669%	Latescibacterales (o) Latescibacteraceae (f) Candidatus Latescibacter (g) uncultured bacterium (s)
	L-ALL	10	0.0610%	Latescibacterales (o) Latescibacteraceae (f)
	L-ALL	10	0.0610%	Latescibacterales (o) Latescibacteraceae (f) Latescibacteraceae (g) uncultured organism (s)
	L-GS	8	0.0577%	Latescibacterales (o) Latescibacteraceae (f) Candidatus Latescibacter (g) metagenome (s)
	L-GS	7	0.0505%	Latescibacterales (o) Latescibacteraceae (f) Latescibacteraceae (g) uncultured bacterium (s)
Latescibacteria (c) [150]	L-ALL	7	0.0427%	Latescibacterales (o) Latescibacteraceae (f) Latescibacteraceae (g) uncultured bacterium (s)
	L-B	7	0.0426%	Latescibacterales (o) Latescibacteraceae (f) Latescibacteraceae (g) uncultured bacterium (s)
	L-GYP	4	0.0260%	Latescibacterales (o) Latescibacteraceae (f)
	L-GYP	4	0.0260%	Latescibacterales (o) Latescibacteraceae (f) Candidatus Latescibacter (g) uncultured bacterium (s)
	L-GS+H	4	0.0221%	Latescibacterales (o) Latescibacteraceae (f) Latescibacteraceae (g) uncultured bacterium (s)
	L-ALL	3	0.0183%	Latescibacterales (o) Latescibacteraceae (f) Candidatus Latescibacter (g) uncultured organism (s)

Fermenting Bacteria	Amendment	No. of Reads	Perc. of Total Reads	Classification
	L-GYP	2	0.0130%	Latescibacterales (o) Latescibacteraceae (f) Candidatus Latescibacter (g)
	L-GYP	2	0.0130%	Latescibacterales (o) Latescibacteraceae (f) Latescibacteraceae (g) uncultured bacterium (s)
Leptolinea (g) [151]	L-GYP	16	0.1041%	uncultured bacterium (s)
	F-ALL	13	0.0928%	-
	L-H	12	0.0600%	-
	L-GS+H	12	0.0662%	-
Levilinea (g) [152]	L-GS+H	2	0.0110%	Levilinea saccharolytica (s)
Longilinea (g) [153]	L-GYP	48	0.3123%	uncultured Longilinea (s)
	L-GYP	43	0.2797%	uncultured Chloroflexi (s)
	L-B	31	0.1885%	uncultured Chloroflexi (s)
	L-H	17	0.0850%	uncultured Chloroflexi (s)
	L-ALL	15	0.0914%	uncultured Chloroflexi (s)
	L-GS+H	14	0.0772%	uncultured Chloroflexi (s)
	L-GS	13	0.0937%	uncultured Longilinea (s)
	L-B	13	0.0790%	uncultured Longilinea (s)
	F-H	9	0.0734%	uncultured Chloroflexi (s)
	F-GS	9	0.0445%	uncultured Longilinea (s)
	F-B	5	0.0198%	uncultured Longilinea (s)
Longilinea (g) [153]	L-B	2	0.0122%	uncultured bacterium (s)
Romboutsia (g) [154,155]	L-GS	2	0.0144%	-
Smithella (g) [156]	L-GS	34	0.2450%	-
	F-GS+H	33	0.1103%	-
	L-B	29	0.1763%	-

Fermenting Bacteria	Amendment	No. of Reads	Perc. of Total Reads	Classification
	L-ALL	26	0.1585%	-
	L-H	24	0.1200%	-
	F-GS	20	0.0989%	-
Sporobacter (g) [157]	L-ALL	11	0.0671%	uncultured bacterium (s)
Syntrophus (g) [158]	L-GYP	117	0.7611%	-
	L-ALL	38	0.2317%	-
	L-GYP	25	0.1626%	uncultured prokaryote (s)
	L-H	24	0.1200%	-
Terrimicrobium (g) [159]	F-GS+H	126	0.4210%	uncultured bacterium (s)
	F-GS+H	107	0.3575%	-
	F-B	92	0.3636%	uncultured bacterium (s)
	F-B	81	0.3201%	-
	F-GS	76	0.3759%	uncultured bacterium (s)
	F-H	47	0.3833%	uncultured bacterium (s)
	F-ALL	45	0.3212%	uncultured bacterium (s)
	F-GYP	28	0.1822%	uncultured bacterium (s)
	F-GS	27	0.1335%	-
	F-ALL	23	0.1641%	-
	F-GYP	16	0.1041%	-
	F-H	13	0.1060%	-
Terrimicrobium (g) [159]	L-GS	5	0.0360%	uncultured bacterium (s)
	L-B	3	0.0182%	uncultured bacterium (s)
	L-GYP	2	0.0130%	uncultured bacterium (s)
Treponema (g) [160,161]	F-GS	6	0.0297%	uncultured prokaryore (s)
	L-GYP	3	0.0195%	uncultured prokaryore (s)
	L-GYP	2	0.0130%	uncultured Spirochaetaceae (s)

Fermenting Bacteria	Amendment	No. of Reads	Perc. of Total Reads	Classification
	F-ALL	2	0.0143%	uncultured Spirochaetaceae (s)

F19. Methanogenic Archaea

Table F19a. Summary results for *Methanogenic Archaea*

Amendment	**No. of Reads**	**Perc. of Total Reads**
L-H	4	0.0200%
L-GS	7	0.0505%
L-GS+H	0	0.0000%
L-GYP	3	0.0195%
L-ALL	7	0.0427%
L-B	2	0.0122%
F-H	0	0.0000%
F-GS	0	0.0000%
F-GS+H	3	0.0100%
F-GYP	0	0.0000%
F-ALL	4	0.0285%
F-B	0	0.0000%

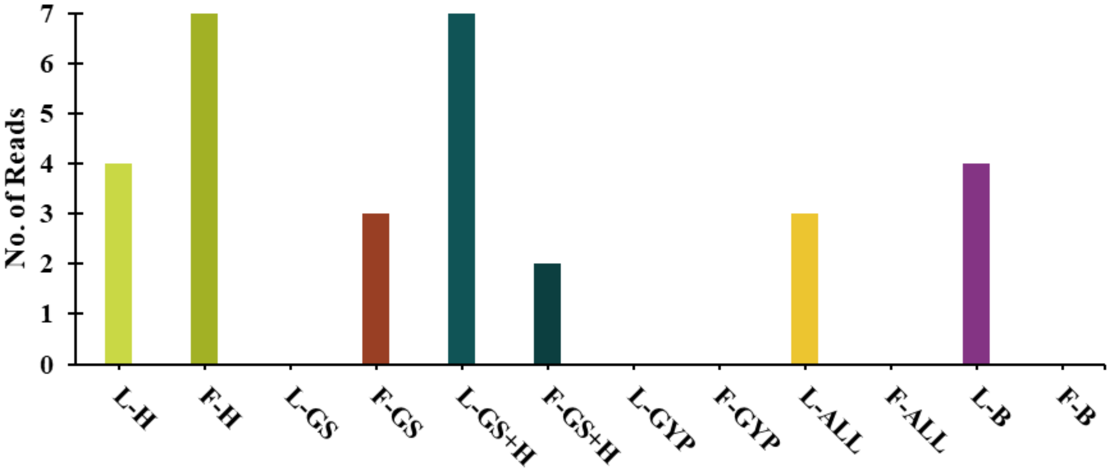

Figure F19a. Summary graph of the number of reads for each amendment type in the classification of *Methanogenic Archaea*

Table F19b. Classification of *Methanogenic Archaea*

Methanogenic Archaea	Amendment	No. of Reads	Perc. of Total Reads	Classification
Methanobacteriaceae (f) [162]	F-GS	7	0.0346%	Methanobacterium (g)
	L-H	4	0.0200%	Methanobacterium (g)
	F-ALL	4	0.0285%	Methanobacterium (g)
	L-GS	3	0.0216%	Methanobacterium (g)
	F-GS+H	3	0.0100%	Methanobacterium (g)
Methanomicrobia (c) [163,164]	L-GS	2	0.0144%	Methanomicrobiales (o) Methanocorpusculaceae (f) Methanocorpusculum (g) uncultured archaeon (s)
Methanomicrobiales (o) [163,164]	L-GS	2	0.0144%	Methanocorpusculaceae (f) Methanocorpusculum (g) uncultured archaeon (s)
Methanosaeta (g) [165]	L-ALL	7	0.0427%	Methanothrix sp. (s)
	L-GYP	3	0.0195%	Methanothrix sp. (s)
	L-B	2	0.0122%	metagenome (s)

F20. Other Microbial Communities

Table F20a. Summary results for *Other Microbial Communities*

Amendment	No. of Reads	Perc. of Total Reads
L-H	2450	12.2494%
F-H	138	1.1254%
L-GS	1137	8.1946%
F-GS	386	1.9091%
L-GS+H	963	5.3099%
F-GS+H	361	1.2062%
L-GYP	396	2.5761%
F-GYP	317	2.0629%
L-ALL	635	3.8710%
F-ALL	323	2.3052%
L-B	1173	7.1316%
F-B	322	1.2725%

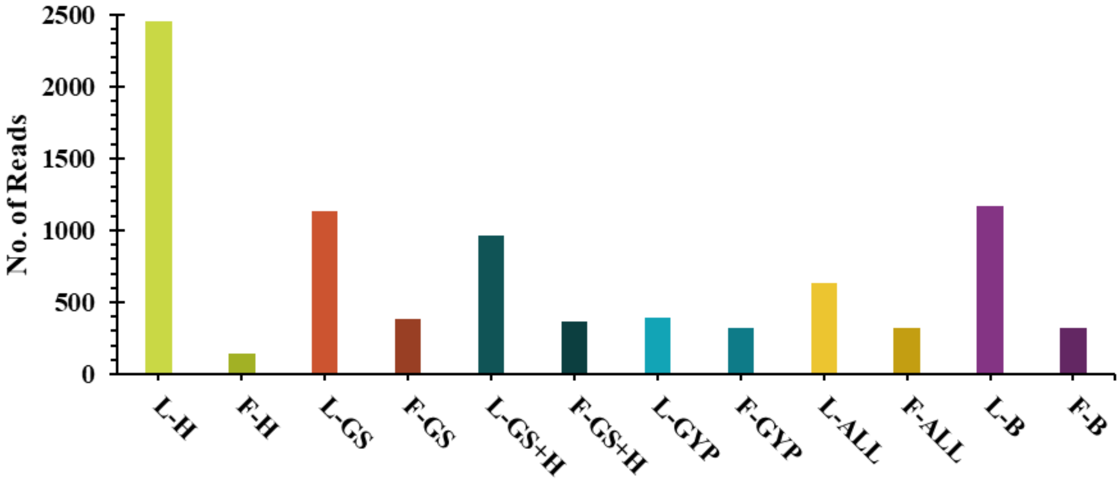

Figure F20a. Summary graph of the number of reads for each amendment type in the classification of *Other Microbial Communities*

Table F20b. Classification of *Other Microbial Communities*

Other	Type	Amendment	No. of Reads	Perc. of Total Reads	Classification
Caldisericia (c) [166]	Non-sulfate sulfur compounds	F-GS	41	0.2028%	Caldisericales (o) WCHB1-02 (f) WCHB1-02 (g) uncultured Firmicutes (s)
		L-H	32	0.1600%	Caldisericales (o) WCHB1-02 (f) WCHB1-02 (g) uncultured Firmicutes (s)
		F-H	20	0.1631%	Caldisericales (o) WCHB1-02 (f) WCHB1-02 (g) uncultured Firmicutes (s)
		L-GS+H	14	0.0772%	Caldisericales (o) WCHB1-02 (f) WCHB1-02 (g) uncultured Firmicutes (s)
		F-GS+H	14	0.0468%	Caldisericales (o) WCHB1-02 (f) WCHB1-02 (g) uncultured Firmicutes (s)
		F-B	13	0.0514%	Caldisericales (o) WCHB1-02 (f) WCHB1-02 (g) uncultured Firmicutes (s)
		L-GS	10	0.0721%	Caldisericales (o) WCHB1-02 (f) WCHB1-02 (g) uncultured Firmicutes (s)
		L-GS	6	0.0432%	Caldisericales (o) WCHB1-02 (f) WCHB1-02 (g) uncultured bacterium (s)
		F-GS	5	0.0247%	Caldisericales (o) WCHB1-02 (f) WCHB1-02 (g) uncultured bacterium (s)
		L-H	4	0.0200%	Caldisericales (o) WCHB1-02 (f) WCHB1-02 (g) uncultured bacterium (s)

Other	Type	Amendment	No. of Reads	Perc. of Total Reads	Classification
Caldisericia (c) [166]	Non-sulfate sulfur compounds	F-GYP	3	0.0195%	Caldisericales (o) WCHB1-02 (f) WCHB1-02 (g) uncultured Firmicutes (s)
		L-GS+H	3	0.0165%	Caldisericales (o) WCHB1-02 (f) WCHB1-02 (g) uncultured bacterium (s)
		L-GYP	2	0.0130%	Caldisericales (o) WCHB1-02 (f) WCHB1-02 (g) uncultured Firmicutes (s)
Candidatus Omnitrophus (g) [167]	Non-sulfate sulfur compounds	L-H	43	0.2150%	uncultured bacterium (s)
		L-GS+H	14	0.0772%	uncultured bacterium (s)
		L-GS	11	0.0793%	uncultured bacterium (s)
		L-GS	8	0.0577%	Candidatus Omnitrophus (s)
		L-GS+H	8	0.0441%	uncultured prokaryote (s)
		L-ALL	7	0.0427%	uncultured bacterium (s)
		L-GYP	5	0.0325%	uncultured bacterium (s)
		L-B	5	0.0304%	uncultured bacterium (s)
		F-GS+H	5	0.0167%	uncultured bacterium (s)
		L-GS	5	0.0360%	uncultured prokaryote (s)
		L-GS+H	3	0.0165%	uncultured organism (s)
		F-H	2	0.0163%	-
		F-H	0	0.0000%	uncultured bacterium (s)
		F-GS	0	0.0000%	uncultured bacterium (s)
Chlorobia (g) [168]	Phototroph	L-H	2328	11.6394 %	Chlorobiales (o) Chlorobiaceae (f) Chlorobium (g)
		L-GS	770	5.5495%	Chlorobiales (o) Chlorobiaceae (f) Chlorobium (g)

Other	Type	Amendment	No. of Reads	Perc. of Total Reads	Classification
Chlorobia (g) [168]	Phototroph	L-B	644	3.9154%	Chlorobiales (o) Chlorobiaceae (f) Chlorobium (g)
		L-GYP	222	1.4442%	Chlorobiales (o) Chlorobiaceae (f) Chlorobium (g)
		L-GS+H	210	1.1579%	Chlorobiales (o) Chlorobiaceae (f) Chlorobium (g)
		L-ALL	188	1.1461%	Chlorobiales (o) Chlorobiaceae (f) Chlorobium (g)
Chromatiaceae (f) [169]	Phototroph	L-GS+H	395	2.1780%	Thiolamprovum (g) Chromatiaceae bacterium (s)
		L-B	369	2.2434%	Thiolamprovum (g) Chromatiaceae bacterium (s)
		F-GS	305	1.5085%	Thiocapsa (g) Thiocapsa sp. (s)
		L-ALL	228	1.3899%	Thiolamprovum (g) Chromatiaceae bacterium (s)
		F-ALL	187	1.3346%	Thiocapsa (g) Thiocapsa sp. (s)
		L-GS+H	180	0.9925%	Thiocapsa (g) Thiocapsa sp. (s)
		L-GS	179	1.2901%	Thiolamprovum (g) Chromatiaceae bacterium (s)
		F-GYP	173	1.1258%	Thiocapsa (g) Thiocapsa sp. (s)
		F-GS+H	172	0.5747%	Thiocapsa (g) Thiocapsa sp. (s)
		F-B	153	0.6046%	Thiocapsa (g) Thiocapsa sp. (s)
		L-ALL	133	0.8108%	Thiocapsa (g) Thiocapsa sp. (s)
		F-GS+H	130	0.4344%	-
		L-B	99	0.6019%	Thiocapsa (g) Thiocapsa sp. (s)
		L-GS	81	0.5838%	Thiocapsa (g) Thiocapsa sp. (s)
		F-B	77	0.3043%	-

Other	Type	Amendment	No. of Reads	Perc. of Total Reads	Classification
Chromatiaceae (f) [169]	Phototroph	L-GYP	76	0.4944%	Thiolamprovum (g) Chromatiaceae bacterium (s)
		F-H	72	0.5872%	Thiocapsa (g) Thiocapsa sp. (s)
		L-GS+H	64	0.3529%	-
		F-ALL	58	0.4139%	-
		F-GYP	57	0.3709%	-
		L-GS+H	49	0.2702%	Thiocapsa (g)
		L-GYP	49	0.3188%	Thiocapsa (g) Thiocapsa sp. (s)
		F-H	37	0.3017%	-
		L-H	36	0.1800%	Thiolamprovum (g) Chromatiaceae bacterium (s)
		F-GS	35	0.1731%	-
		L-ALL	34	0.2073%	Thiocapsa (g)
		F-GYP	33	0.2147%	Thiolamprovum (g) Chromatiaceae bacterium (s)
		F-GS+H	31	0.1036%	Thiolamprovum (g) Chromatiaceae bacterium (s)
		L-ALL	30	0.1829%	-
		F-B	29	0.1146%	Thiocapsa (g)
		F-ALL	28	0.1998%	Thiolamprovum (g) Chromatiaceae bacterium (s)
		F-GYP	27	0.1757%	Thiocapsa (g)
		L-GYP	24	0.1561%	Thiocapsa (g)
		F-ALL	23	0.1641%	Thiocapsa (g)
		L-B	22	0.1338%	Thiocapsa (g)

Other	**Type**	**Amendment**	**No. of Reads**	**Perc. of Total Reads**	**Classification**
Chromatiaceae (f) [169]	Phototroph	F-B	21	0.0830%	Thiolamprovum (g) Chromatiaceae bacterium (s)
		L-GS	11	0.0793%	-
		F-ALL	9	0.0642%	Allochromatium (g)
		F-B	9	0.0356%	Allochromatium (g)
		L-B	8	0.0486%	Allochromatium (g)
		L-GYP	7	0.0455%	Allochromatium (g)
		F-H	7	0.0571%	Allochromatium (g)
		L-GS	6	0.0432%	Thiocystis (g) uncultured bacterium (s)
		L-GS+H	5	0.0276%	Allochromatium (g)
Dehalococcoidia (c) [170]	Reductive dehalogenation	F-GYP	24	0.1562%	S085 (o) S085 (f) S085 (g) metagenome (s)
		F-ALL	18	0.1285%	S085 (o) S085 (f) S085 (g) metagenome (s)
		F-B	17	0.0672%	S085 (o) S085 (f) S085 (g) metagenome (s)
		L-GS	12	0.0865%	MSBL5 (o) MSBL5 (f) MSBL5 (g) uncultured bacterium (s)
		L-GYP	8	0.0520%	GIF9 (o) AB-539-J10 (f) AB-539-J10 (g)
		L-GS+H	8	0.0441%	MSBL5 (o) MSBL5 (f) MSBL5 (g) uncultured bacterium (s)
		L-ALL	7	0.0427%	MSBL5 (o) MSBL5 (f) MSBL5 (g) uncultured bacterium (s)
		L-GS	4	0.0288%	GIF3 (o) GIF3 (f) GIF3 (g) uncultured bacterium (s)
		L-GS+H	4	0.0221%	GIF3 (o) GIF3 (f) GIF3 (g)

Other	Type	Amendment	No. of Reads	Perc. of Total Reads	Classification
Dehalococcoidia (c) [170]	Reductive dehalogenation				uncultured organism (s)
		L-ALL	4	0.0244%	GIF3 (o) GIF3 (f) GIF3 (g) uncultured organism (s)
		L-ALL	4	0.0244%	GIF9 (o) AB-539-J10 (f) AB-539-J10 (g) uncultured organism (s)
		L-GS	4	0.0288%	GIF9 (o) AB-539-J10 (f) SCGC-AB-539-J10 (g) uncultured bacterium (s)
		L-GS+H	4	0.0221%	GIF9 (o) AB-539-J10 (f) SCGC-AB-539-J10 (g) uncultured bacterium (s)
		L-B	4	0.0243%	MSBL5 (o) MSBL5 (f) MSBL5 (g) uncultured bacterium (s)
		L-GS	3	0.0216%	GIF3 (o) GIF3 (f) GIF (g)
		L-H	3	0.0150%	GIF9 (o) GIF9 (f) GIF9 (g) uncultured bacterium (s)
		L-B	3	0.0182%	GIF9 (o) GIF9 (f) GIF9 (g) uncultured Chloroflexi (s)
		L-GYP	3	0.0195%	MSBL5 (o) MSBL5 (f) MSBL5 (g) uncultured bacterium (s)
		L-GS	2	0.0144%	FW22 (o) FW22 (f) FW22 (g) uncultured bacterium (s)
		L-GS+H	2	0.0110%	GIF3 (o) GIF3 (f) GIF3 (g) uncultured bacterium (s)
		L-H	2	0.0100%	GIF9 (o) AB-539-J10 (f) SCGC-AB-539-J10 (g) uncultured bacterium (s)
		L-B	2	0.0122%	GIF9 (o) GIF9 (f) GIF9 (g) uncultured Chloroflexi (s)

Other	Type	Amendment	No. of Reads	Perc. of Total Reads	Classification
Dehalococcoidia (c) [170]	Reductive dehalogenation	L-H	2	0.0100%	MSBL5 (o) MSBL5 (f) MSBL5 (g) uncultured bacterium (s)
		L-GS L-GS	2 2	0.0144% 0.0144%	SAR202 clade (o) SAR202 clade (f) SAR202 clade (g) uncultured marine (s)
Desulfurivibrio (g) [171]	Non-sulfate sulfur compounds	L-GS	17	0.1225%	-
		L-B	17	0.1034%	uncultured Desulfobacterales (s)
Micavibrio (g) [172]	Parasite	F-B	3	0.0119%	Micavibrio sp. (s)
Rickettsia (g) [173]	Parasite	F-GS+H	9	0.0301%	-
Thiocystis (g) [174]	Phototroph	L-GS	6	0.0432%	uncultured bacterium (s)

F21. Cyanobacteria

- Chroococcidiopsis (g) [175]
- Cyanobacteria (p) [176]
- Leptolyngbya (g) [177]
- Microcystis (g) [178]
- Nostoc (g) [179]
- Pleurocapsa (g) [180]
- Stanieria (s) [181]
- Tolypothrix (g) [182]

F22. Canidate Division

- Zixibacteria (p) [183]

F23. Broadly Classified Bacteria

- Acidimicrobiales Uncultured (s) [184]
- Acidimicrobiia (c) [185]
- Acidobacteria (c) [186]
- Acidobacteriaceae Uncultured (s) [187]
- Actinobacteria (p) [188]
- Alphaproteobacteria (c) [189]
- Bacteroidetes (f) [190]
- Burkholderiales (o) [191]
- Caldilineaceae (f) [192]
- Chitinophagaceae (f) [193]
- Chloroflexi (p) [194]
- Clostridia (c) [195]
- Desulfuromonadales Uncultured (s) [196]
- Firmicutes (p) [197]
- Frankiales (o) [198]
- Gammaproteobacteria (c) [199]
- Holophagae (c) [200]
- Hydrogenedentes (p) [201]
- Iamiaceae (f) [202]
- Ignavibacteriales (o) [203]
- Microgenomates Uncultured (s) [204]
- Mycobacteriaceae (f) [205]
- Nitrospiraceae (f) [206]
- Nitrospirae Uncultured (s) [207]
- Nitrospirales (o) [208]
- Omnitrophica (s) [209]
- Phycisphaeraceae (f) [210]
- Planctomycetaceae Uncultured (s) [211]
- Planctomycetales (o) [212]
- Proteobacteria (p) [213]
- Rhizobiales (o) [214]
- Rhodobacterales (o) [215]
- Rhodocyclaceae (f) [216]
- Rhodospirillaceae Uncultured (s) [217]
- Rhodospirillales (o) [218]
- Sphingobacteriales (o) [219]
- Spirochaetales (o) [220]
- Spirochaetia (c) [221]
- Syntrophaceae (f) [222]
- Syntrophobacterales (o) [223]
- Thermomicrobiales (o) [224]
- Unclassified Bacteria
- Verrucomicrobiae (c) [225]

F24. Broadly Classified Archaea:

- Crenarchaeota (p) [226]
- Euryarchaeota (p) [227]
- Thermoplasmata (c) [228]
- Unclassified Arachaea

F25. Unpresented Microbial Categories

The following microbial community categories were not found:

- Aerobic, Iron Oxidizing, Iron Reducing Bacteria
- Aerobic, Nitrate Reducing, Iron Reducing Bacteria
- Aerobic, Iron Reducing Bacteria
- Nitrate Reducing
- Nitrate Reducing, Iron Reducing Bacteria
- Nitrate Reducing, Iron Reducing, Fermenting Bacteria
- Nitrite Reducing, Methane Oxidizing Bacteria
- Iron Reducing, Sulfate Reducing Bacteria
- Iron Reducing, Sulfate Reducing, Fermenting Bacteria
- Ammonia Oxidizing Archaea
- Methane Oxidizing, Nitrate Reducing Archaea
- Fermenting Archaea
- Eukaryotes

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