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Microbial Repopulation Following In Situ STAR Remediation

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Abstract

In situ STAR (Self-sustaining Treatment for Active Remediation) is an emerging remediation technology which uses smouldering combustion to destroy non-aqueous phase liquid (NAPL) contamination in the subsurface. Since STAR smouldering travels through contaminated soils slowly (~0.5 to 5 m/day) and subjects them to high temperatures (400–1000°C), it is expected that this technology will thoroughly dry and sterilize the zones which it treats. Further, soils surrounding the treatment zone which are not smouldered will be heated, although not smouldered, by virtue of their proximity to STAR, impacting microbial communities within them. Therefore, the objectives of this work are to quantify the microbial repopulation of the STAR treated zone, and observe heating effects on microorganisms living in surrounding soils. STAR is currently being applied as a full scale, in situ remedy for coal tar beneath a former creosol manufacturing facility in New Jersey, USA. This study analyzed soil cores taken at regular intervals following STAR treatment, allowing time for groundwater to re-infiltrate and for microbial populations to potentially reestablish. Treated soil, as well as untreated soil above the treatment zone and groundwater were analyzed for bacteria abundance and microbial diversity. Results demonstrate bacterial repopulation over a six-month period to ~10^7 gene copies/g of soil in the treated zone, and variable bacterial concentrations within untreated soils adjacent to and ~1m above the treated zone. In general, long term microbial abundance was largely dependent on the amount of organic matter present in the soil following STAR. To examine microbial transport and repopulation of STAR treated soils in more detail, and to consider the effects of bio-stimulating amendments, a bench top column study using site soil and artificial groundwater was set up to determine the rate at which STAR-treated soil is repopulated with naturally occurring microorganisms in the presence and absence of lactate and elevated sulfate concentrations. Results demonstrated that this amendment scheme increased the carrying capacity of the STAR treated soil and shifted the microbial community to promote sulfate reducing bacteria. Overall, the work illustrates that microbial populations in STAR treated soil do recover via groundwater infiltration but robust communities will take time to naturally establish.

Keywords

Microbial repopulation, bio-transport, microbial community structure, biostimulation, field investigation, column study, qPCR, Illumina, smouldering remediation, STAR

Co-Authorship Statement

This thesis was written by the candidate in accordance with the guidelines and regulations as stipulated by the Faculty of Graduate Studies at the University of Western Ontario. The candidate conducted research design, data collection and data analysis for both the laboratory experiments and field study under the guidance of Dr. Jason Gerhard and Dr. Elizabeth Edwards. The co-authorship of Chapter 3 is as follows:

Chapter 3: Microbial Abundance and Community Structure Before and After In Situ STAR Remediation.

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G. Overbeeke	Design and implementation of field study and laboratory experiments. Performed all data interpretation and wrote chapter drafts
J. Gerhard	Initiated research topic, supervised field study and laboratory experiments, assisted in design and data interpretation in both the

	field study and laboratory experiments, and reviewed/revised draft chapters
E. Edwards	Supervised field study and laboratory experiments, assisted in design and data interpretation for the field study and laboratory experiments, and reviewed/revised draft chapters
L. Lomheim	Provided training and troubleshooting for DNA extraction and qPCR
I. Yang	Performed Illumina data manipulation and visualization via QIIME
L. Kinsman	Provided experimental data on the sterilizing effect of STAR using bacterial plating methods

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List of Abbreviations

NAPL……Non-Aqueous Phase Liquid

DNAPL……..Dense Non-Aqueous Phase Liquid

PCB……Poly-chlorinated biphenyls

STAR…….Self-sustaining Treatment for Active Remediation

qPCR……Quantitative polymerase chain reaction

COC……..Contaminant of concern

MGP…….Manufactured gas plant

ISTD……..In situ thermal desorption

ERH……Electrical resistance heating

SEE……..……Steam enhanced extraction

ROI……..Radius of influence

bgs……Below ground surface

VOC……...Volatile organic compounds

EPS…….……..…Extracellular polymeric substances

IP…….Ignition point

ORP……....……Oxidation reduction potential

DO……Dissolved oxygen

AGW…….Artificial groundwater

EtOH……Ethanol

UV……Ultraviolet

RPM……..Revolutions per minute

EC……Electroconductivity

ID……Inner diameter

PTFE…….Polytetrafluoroethylene

PV……..Pore volume

LOQ……...Limit of quantification

QA/QC……Quality Assurance/Quality Control

CFU……Colony forming unit

DI……Deionized

NMDS…….Non-metric multidimensional scaling

1 Introduction

1.1 Background

Non-aqueous phase liquids (NAPLs), a class of hazardous and typically carcinogenic organic contaminants, have been introduced into the subsurface at thousands of sites across the globe (Pankow & Cherry, 1996). Many NAPLs, such as coal tar, creosote, chlorinated solvents and polychlorinated biphenyls (PCBs), are highly recalcitrant, having the potential to persist for thousands of years within the subsurface (Gerhard et al., 2007; Pape et al., 2015). Further, such NAPLs are typically denser than water (DNAPLs) and exhibit low solubility, allowing them to migrate deep into the subsurface, pooling on layers of low permeability soils below the water table (Gerhard et al., 2007; Kueper & Davis, 2009). Current remedial strategies for such DNAPLs includes excavation, encapsulation, in situ thermal desorption, and in situ chemical oxidation (Scholes et al., 2015).

In situ STAR (Self-sustaining Treatment for Active Remediation) is an alternative remediation approach which uses smouldering combustion as a mechanism to eradicate pools of DNAPL from the subsurface. Remediation of free phase coal tar at a former industrial site by STAR demonstrated a 97.3 – 99.3% reduction in contamination, highlighting the effectiveness of STAR as a tool for aggressive removal of NAPL (Scholes et al., 2015). Due to its high temperatures, STAR remediation not only removes organics from the soil, but also likely sterilizes the soil (Pape et al., 2015). Microbes within the subsurface perform a variety of services for subsurface and above ground ecosystems. Moreover, they are known to play a role in biodegradation and natural attenuation of contaminants. Therefore, it is important to understand the influence of STAR on subsurface

microbial populations. While one study has examined microbial growth in microcosms containing STAR-treated soil,, the influence of STAR on microbial populations has never before been studied in situ or in ex situ systems with flowing groundwater.

1.2 Research Objectives

The overall scope of this research was to provide an understanding of microbial dynamics in STAR impacted soils, and may be broken down into 2 main objectives. The first objective was to quantify microbial abundance and community structure within and bordering an in-situ STAR treatment zone. This was accomplished by means of a field study that analyzed DNA from soil cores taken before, and up to six months after, STAR was applied. This was repeated at two treatment locations within the context of a full STAR site remedy being applied in the coal tar contaminated soil beneath a former chemical manufacturing facility. The second objective was to elucidate the processes that support the transport and growth of microbes in STAR-treated zones. This objective was met by performing a laboratory-based column study using soils from the field site. The study was designed to evaluate whether groundwater alone would facilitate the transport of microbes from contaminated, up-gradient soil to treated, down-gradient soil. This experiment was repeated with the groundwater amended with sulfate and lactate as biostimulating compounds. This explored the additional questions of whether biostimulation enhances microbial repopulation and how it affects the resulting microbial community.

1.3 Thesis Outline

This thesis is written as an "Integrated Article". A summary of the chapters is given below:

Chapter 1: Topic introduction and research objectives

Chapter 2: Provides a review of the relevant literature. Specific topics include DNAPL contaminated sites and their remediation, microbial groundwater transport and subsurface colonization, and the fate of microorganisms within soils heated to various degrees by surficial fires or thermal remediation.

Chapter 3: Describes the materials used, setup, sampling procedures, and data analysis for the field and column studies. Results for each objective are presented and discussed, providing insight into microbial dynamics before and after STAR treatment.

Chapter 4: Summarizes conclusions for each objective and provides recommendations for future work

Appendices A-D contain supplementary information for Chapter 3:

Appendix A: Contains raw data from the field study (i.e., data relevant to Objective 1)

Appendix B: Contains raw data from the column experiments (i.e., data relevant to Objective 2)

Appendix C: Contains images at various stages of the column experiments

Appendix D: Contains tables and figures of statistics associated with qPCR and Illumina data analysis

1.4 References

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2 Background Literature

2.1 Introduction

This review begins by describing typical features of coal tar contaminated sites and the microbial life potentially found within them. It then presents the available thermal treatment methods for such sites, focusing in particular on the STAR technology. The impacts of heating on soil are discussed, which leads to a review of microbial repopulation in thermally impacted soils. No studies exist to date which measure in situ colonization of STAR treated zones or address the ability of groundwater to transport microbes in STAR-treated soils. Therefore, this review surveys the literature within a range of related fields. This includes those studies that quantify microbial repopulation following other methods of thermal remediation, although it is noted that these typically use lower temperatures than those applied in STAR. Also relevant are studies discussing microbial repopulation in surface soils following brush and forest fires. Finally, this review also summarizes the main mechanisms of bacterial transport, retardation, sorption, and colony formation within the subsurface.

2.2 Coal Tar Sites

2.2.1 Site Characterization

NAPL contamination within the subsurface arises through accidental and premeditated releases during a variety of industrial processes, including chemical manufacturing, industrial liquid storage and transport, and industrial waste storage and disposal (Pankow & Cherry, 1996). NAPLs such as coal tars, creosotes, chlorinated solvents, PCBs are denser than water (DNAPLs), and thus percolate down through the subsurface, pooling on soils

with low permeability, and leaving a trail of residual DNAPL blobs and ganglia above them (Fig 2.1). Compounds of concern (COC) within the DNAPL pool and residuals partition into the groundwater and vadose zone, bringing them into direct contact with humans and the surrounding ecosystem (Fig 2.1). Coal tar, the DNAPL considered in this thesis, is a byproduct of historical manufactured gas plant (MGP) operations, and contains a mixture of aliphatic and aromatic compounds (Scholes et al., 2015). Although coal tar contains a plethora of compounds, major COCs which exist in significant proportions include naphthalene, phenanthrene, benzo[a]pyrene, and BTEX (benzene, toluene, ethylbenzene, xylene) compounds (Kueper & Davis, 2009).

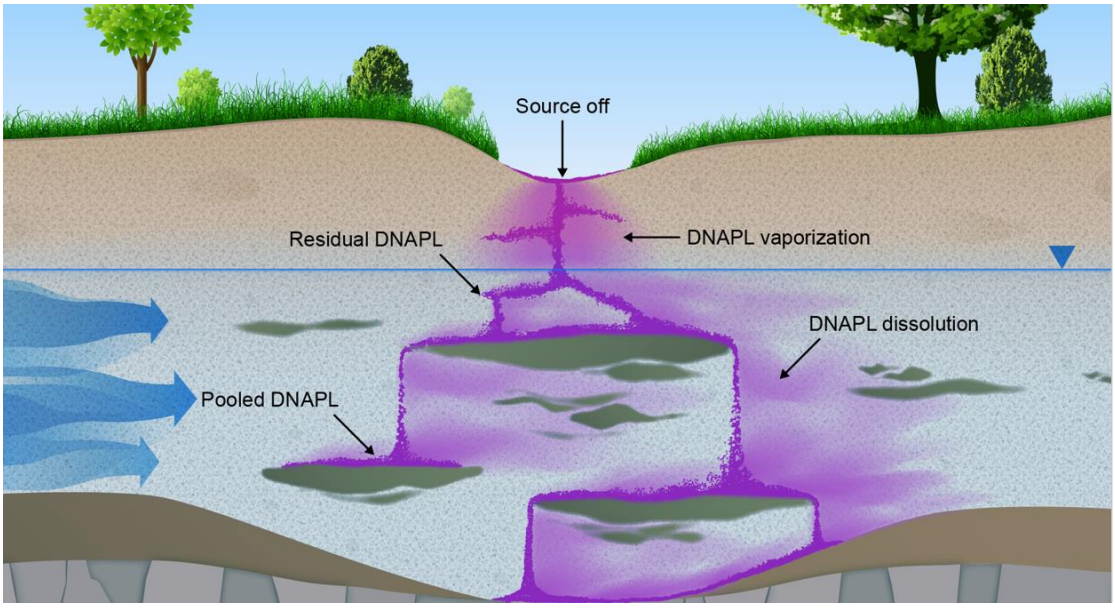

Figure 2.1: DNAPL contamination in the subsurface. *Image taken from Kueper et al., (2013)*

2.2.2 Microbial Characterization

Few studies describe abundances of microbial taxa in sites contaminated specifically with coal tar. However, in most DNAPL contaminated soils, microbes are distributed in relatively high concentrations within the aqueous phase adjacent to the DNAPL, and are inhibited from growth within the DNAPL itself (Capiro et al., 2015; Mueller et al., 1989; Philips et al., 2012). Although coal tar NAPL is recalcitrant, various microorganisms have demonstrated the ability to degrade coal tar components in the aqueous phase (Bakermans et al., 2002; Liou et al., 2008; Nyyssonen et al., 2009). Since coal tar and creosote are a mixture of various hydrocarbon contaminants, microbial communities at coal tar contaminated sites often show large proportions of hydrocarbon-degrading bacteria (Ding et al., 2013; Lors et al., 2010). Based on published reports, the most important hydrocarbon-degrading bacterial genera in contaminated soil environments include *Achromobacter*, *Acinetobacter*, *Alcaligenes*, *Arthrobacter*, *Bacillus*, *Burkholderia*, *Collimonas*, *Corynebacterium*, *Dietzia*, *Flavobacterium*, *Gordonia*, *Micrococcus*, *Mycobacterium*, *Nocardia*, *Nocardioides*, *Pseudomonas*, *Ralstonia*, *Rhodococcus*, *Sphingomonas* and *Variovorax* (Chikere et al., 2011).

2.3 STAR Remediation of Coal Tar & Creosote Sites

Due to the physical and chemical properties of coal tar, remediation is very difficult and economically demanding. Coal tar is an “intermediate” DNAPL, being only slightly denser than water, which causes it to have greater lateral distribution and thus increases the total volume of the subsurface needing to be treated relative to other denser DNAPLs (Gerhard et al., 2007). The high viscosity of coal tar (20-100 centipoise) makes it difficult to move,

limiting pumping extraction technologies (Scholes et al., 2015). Further, by virtue of the complex and recalcitrant nature of the compounds within it, coal tar exhibits a resistance to volatility based remedial strategies and bioremediation (Scholes et al., 2015). Thermal remediation technologies such as in situ thermal desorption (ISTD), electrical resistance heating (ERH), steam enhanced extraction (SEE) and STAR have shown the ability to remediate coal tar. While SEE and ERH typically operate at maximum temperatures of 100^{o}C, ISTD may be applied at temperatures of 400^{o}C and greater for months at a time, and STAR operates in temperature ranges of 400^{o}C to 1200^{o}C (Baker et al., 2016; Heron et al., 2005; McGowan et al., 1996; Pironi et al., 2011; Scholes et al., 2015; Switzer et al., 2009).

STAR has recently been applied to remediate pools of highly recalcitrant NAPLs such as coal tar. STAR operates using smoldering combustion, an exothermic oxidation reaction of a solid or liquid fuel which occurs on the fuel surface (Pironi et al., 2011; Switzer et al., 2009). Maximum temperatures within the STAR smoldering reaction typically range from 400-1200^{o}C, depending on contamination type (Pironi et al., 2011; Switzer et al., 2009). The smoldering combustion associated with STAR is self-sustaining, as the porous medium (here, the soil) absorbs energy from the reaction and re-emits it back into the un-burnt fuel (Howell et al., 1996; Switzer et al., 2009). This self-sustaining helps to make STAR an energy conserving and therefore cost efficient option for coal tar remediation (Pironi et al., 2011; Switzer et al., 2009).

In situ STAR treatment is delivered by pumping air down a well past an in-line heater into a contamination source zone (Fig 2.2). The hot air dries and heats the soil around the well screen until temperatures conducive to smoldering are reached (Scholes et al., 2015). Once

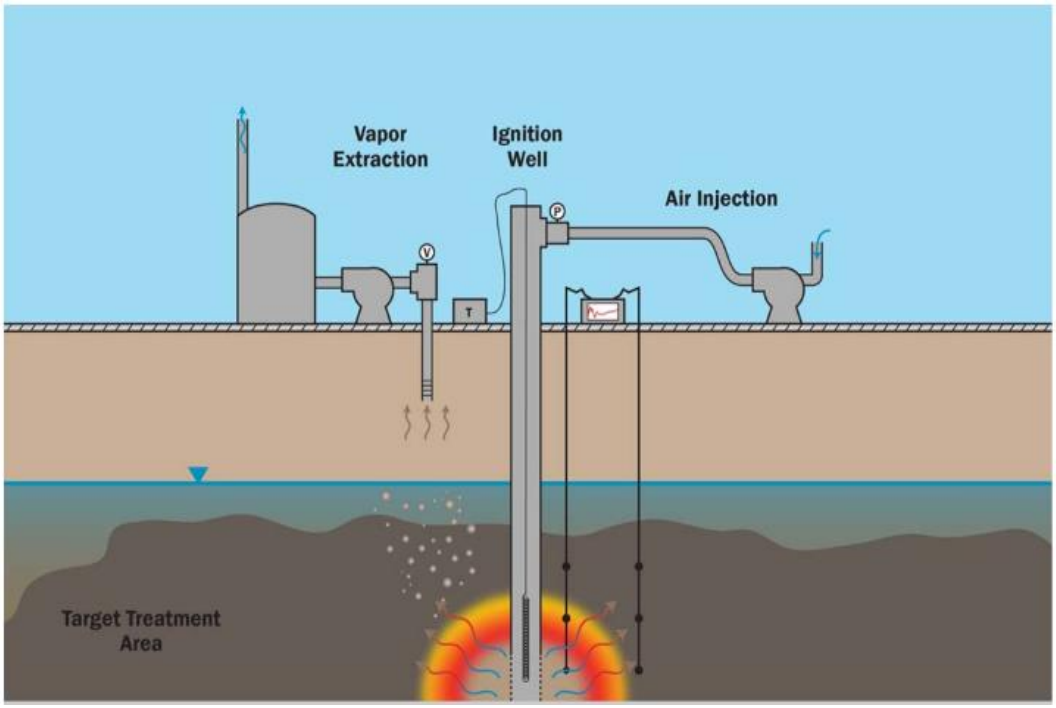

Figure 2.2: In situ application of STAR. *Image modified from Scholes et al. (2015).*

smoldering begins, the reaction is self-sustaining, and the heater is turned off. The self-sustaining reaction relies only on continued injection of air. Vapors produced by the reaction are removed via soil vapor extraction units (Scholes et al., 2015). The radius of influence (ROI) around each ignition well during in situ STAR is influenced by delivery of air to the reaction as it propagates outwards from the ignition point and through the treatment zone. The feasibility of in situ STAR was recently demonstrated by a pilot test in a coal tar contaminated aquifer roughly 7.9 m below ground surface (bgs). During this

test, a treatment zone up to 1.9 m thick with an ROI of 3.7 m was achieved (Scholes et al., 2015). Comparison of pre- and post- STAR sampling from multiple locations within the targeted treatment area confirmed that 98.6% of aromatics, 99.7% of aliphatics, and 100% VOC/BTEX compounds were removed by STAR (Scholes et al., 2015). A similar test in a shallower aquifer (~3m bgs) followed the same trend, with overall coal tar removal of 99.72% (Scholes et al., 2015).

2.4 Effects of Heating on Soil

During heating events in the subsurface, temperature induced changes occur to the biological and physicochemical properties of the soil (Diazravina et al., 1992; Whitmore, 1984). It is generally understood that maximum temperatures attained during thermal remediation (i.e., 100^{o}C or greater) will be deleterious to the immediate well-being of typical soil microbial communities, with microbial die-off proportional to the severity of heating (Dettmer, 2002; Neary et al., 1999; Pape et al., 2015; Richardson et al., 2002). It is also known that soil structure, porosity, pH, and water storage are altered during severe heating, often accompanied by a loss of organic matter and nutrients (Pape et al., 2015; Powers et al., 1990). Indeed, it has been shown that at temperatures greater than 500^{o}C, losses of organic matter and nitrogen in the soil are nearly complete, and clay particles begin to break down and aggregate, altering soil structure and making it difficult to retain valuable nutrients (Pape et al., 2015; Weast, 1988). As temperatures rise, more nutrients are lost. Nutrient compounds containing potassium volatilize at 760^{o}C, containing phosphorus at 774^{o}C, sulfur at 800^{o}C, sodium at 880^{o}C and magnesium at 1107^{o}C (Weast, 1988). Further, soils may become water repellant after exposure to elevated temperatures

(Debano, 1981). This phenomenon is initiated at temperatures above 176^{o}C and is terminated at temperatures greater than 288^{o}C due to formation and destruction of hydrophobic materials at each respective temperature (Debano, 1981; DeBano et al., 1977; Debano et al., 1976). Hydrophobic layers often adsorb large amounts of organic matter, allowing them to support small, high growth rate microbial communities shortly after (Pietikainen et al., 2000). It is likely that the high temperatures associated with STAR will thus remove most of the hydrophobic material from the treatment zone. However, periphery temperatures surrounding the treatment zone may fall within this range, influencing microbial communities within them.

2.5 Microbial Repopulation of Heated Soil

2.5.1 Soil Repopulation Following Surface Fires

The overall effects of heat transfer from fires into subsurface ecosystems are complex, with impacts on subsurface physical, chemical, and microbial processes (Neary et al., 1999). These impacts may cause either positive or deleterious effects on the subsurface, depending on the severity of the fire (Neary et al., 1999). The maximum soil temperature during an above ground fire varies with fuel type, and can range from 200^{o}C-700^{o}C (Fig 2.3), with instantaneous "flash" temperatures greater than 1,500^{o}C (Dunn & Debano, 1977; Rundel, 1983). In severe fires, microbes up to 50 mm below the ground surface may be killed, while mild fires often have no deleterious effects (DeBano et al., 1977).

Post–fire subsurface microbial abundances reported in the literature are highly variable. In one study, microbial concentrations were significantly increased as early as 1 month after a fire (Vazquez et al., 1993). On the other hand, another study showed decreases in

microbial biomass of up to 50% following a wildfire, with little recovery over a 2-year

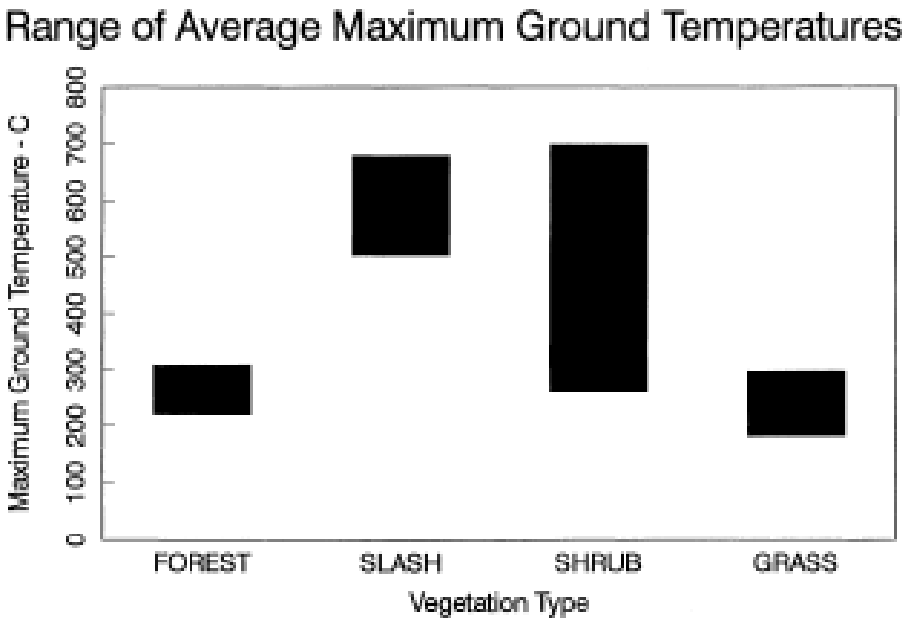

Figure 2.3: Maximum ground temperatures during a fire as a function of vegetation cover. *Reprinted with permission byRundel (1983).*

period (Prieto-Fernandez et al., 1998). The explanation for this apparent discrepancy is that microbial life in the subsurface is more negatively impacted with increasing severity of heating (Neary et al., 1999). Soil alterations (i.e., loss of organics, nutrients, etc.) caused by sustained high temperatures stunt microbial repopulation, while shorter exposures and lower temperatures may actually alter soil chemistry in such a way as to spur microbial growth during repopulation (Acea & Carballas, 1996; Ahlgren & Ahlgren, 1960; DeBano et al., 1977; Hossain et al., 1995; Mabuhay et al., 2003; Vazquez et al., 1993; Whitmore, 1984). The species of microorganisms that emerge dominant in the shallow subsurface following a severe surface fire differs between studies, and this is likely because of the

variability in the physicochemical properties of the post-burn soils, as well as the resilience of different microbial species to heating (Acea & Carballas, 1996).

2.5.2 Microbial Repopulation Following Thermal Remediation

Thermal remediation, the heating of the subsurface via hot air or steam injection, electrical resistance, radio wave heating, thermal conduction, or smoldering (i.e., STAR), is a remediation type that targets NAPLs within the subsurface. Typical temperature values during thermal treatment range from ~100^{o}C during steam injection to ~1200^{o}C during STAR treatment (Dettmer, 2002; Switzer et al., 2009). Though repopulation following thermal remediation is not often studied, the studies that have been done agree that thermal remediation technologies have a significant impact on the microbial communities within and above the targeted treatment zone.

Contaminated soils subjected to treatments which operate at higher temperatures, (i.e., STAR, radio wave heating and thermal conduction) experience heavy die off of microbial life during remediation (Dettmer, 2002; Pape et al., 2015). In a study by Pape et al. (2015), it was found that microbial repopulation over an 8-week period after STAR or after exposure to temperatures of 500^{o}C or greater was 2-3 orders of magnitude lower than an unheated control soil. However, the same soil type, if heated to temperatures below 500^{o}C, exhibited rapid recovery to similar bacterial concentrations seen in the control. In this study, differences in success of repopulation was attributed to the loss of organics and nutrients caused by temperatures at or above 500^{o}C (Pape et al., 2015). Amendments of organic matter and specific nutrients were recommended by the authors in order to facilitate complete recolonization, a strategy which still requires further study (Pape et al., 2015). In

shallow soils which have been thermally treated, nutrient amendments may be coupled with the addition of certain plants and microorganisms to initiate a semi-natural succession (Bradshaw, 1997).

Surrounding the treatment zone are fringe soils. The “fringe” is here defined as the zone surrounding the treatment zone, where a gradient exists between the treatment temperature and the ambient soil temperature. Because thermal treatments often target DNAPL pools specifically, fringe zones may also be contaminated with residual DNAPL which goes untreated, especially in the soils above the treatment zone, through which DNAPL has previously migrated. Thus, hydrocarbon degrading microorganisms may play a role in natural attenuation of DNAPL in these soils. Therefore, it is important to understand the impacts heating will have on the fringe zone microbial communities. Due to lower temperatures in fringe soil relative to the treatment zone, microbial populations in the fringe may be more abundant during treatment (Krauter et al., 1996). However, if temperatures in fringe soils are high enough to cause partial or complete sterilization, heat-generated release of organics and nutrients often cause repopulating microbes to exhibit a rapid and robust recovery, and occasionally even biostimulation after treatment is completed and temperatures return to ambient (Dettmer, 2002; Pape et al., 2015; Richardson et al., 2002). Indeed, it has been observed that moderate heating (i.e., <500 ^{o}C) may result in post treatment recovery to numbers equal to or even greater than the original pre-heated population (Dettmer, 2002; Fletcher et al., 2011; Pape et al., 2015; Richardson et al., 2002). Further, because fringe zones experience a temperature gradient between treatment and ambient conditions, it is likely that the optimal temperature for microbial growth will be obtained at some point within the fringe zone. Therefore, there is potential

for biostimulation of fringe soil during treatment. Further, different microbial community structures have been shown to thrive at different temperatures, so it is likely that fringe zone microbial communities will vary in structure as a function of soil temperature (Norris et al., 2002; Zogg et al., 1997).

2.6 Subsurface Microbial Transport

2.6.1 Transport Processes

Movement of microorganisms through the subsurface is primarily influenced by the Darcy velocity of the groundwater, the size of the microorganism, and the physical properties of the porous medium through which it travels (Camesano & Logan, 1998; Ginn et al., 2002). Advection is the primary mode of transportation of microorganisms in the groundwater. During advective transport, microorganisms undergo some mixing due to dispersion and Brownian motion (Corapcioglu & Haridas, 1984). At high Darcy velocities, both motile and non-motile microorganisms are subjected to this mixing effect (Camesano & Logan, 1998). However, at lower Darcy velocity, only non-motile microorganisms exhibit mechanical mixing, since motile microorganisms can overcome the force of the groundwater flow and swim in any direction through the pore space (Camesano & Logan, 1998). Mixing is also limited by the size of the microorganism. In general, microorganisms exhibit a dampened Brownian motion, limiting the mixing effect of microbes in the subsurface (Li et al., 1996). Further, by virtue of their size, microbial cells preferentially experience the higher pore water velocities associated with the center of the pore (de Marsily, 1986; Dodds, 1982). Repulsive interactions between soil grain and microorganism surface charges may also push the microorganisms away from the soil grains and into the

center of the pore (Bradford et al., 2014). As a result of reduced mixing and preferential high velocity exposure, some microorganisms have been reported to breakthrough before conservative tracers (Ginn et al., 2002). Indeed, numerous field and column studies have shown microbes transported at speeds as much as 70% greater than the mean pore water velocity (Enfield & Bengtsson, 1988; Hubbard et al., 2001; Li et al., 1996; Zhang et al., 2001).

Many microorganisms have the ability to move themselves through the subsurface, propelled by their flagellum or Cilia (Corapcioglu & Haridas, 1984). Swimming speeds can vary greatly for a cell over time, and there are differences in minimum and maximum speeds between different species (Armitage et al., 1999). For example, bacteria have a swimming speed range of 1-1000 μm/s documented in the literature (Fenchel & Thar, 2004; Marwan et al., 1991). At the lower end of this spectrum, the benefit of movement may be lost, since nutrients will likely diffuse faster than the bacterium travels (Mitchell & Kogure, 2006). At the higher end, maximum motor rotation and optimal length/number of flagellum is reached, making 1000 μm/s a physically constrained maximum speed (Mitchell & Kogure, 2006). Flagellar travel velocity is dependent on multiple factors, such as the length and number of flagellum, size, and shape of the microorganism, as well as environmental conditions (Armitage et al., 1999; Calladine, 1978; Mitchell & Kogure, 2006; Scharf, 2002). Migration in small pores is an issue especially effecting motile microbes since the flagella add extra length, making it difficult to navigate in tight spaces. Lui & Papadapoulos, (1995) found that *E. coli* in a 6 μm wide capillary could only swim in one direction, unable to turn due to their flagella. In a 3 μm wide capillary, geometric restriction not only precluded turning, but also passing alongside other microorganisms

(Liu et al., 1997). Indeed, evidence has been shown that pore radius selects for a microorganism's flagella length, number, and cell size (Chen et al., 1998; Mitchell & Kogure, 2006).

2.6.2 Retardation Processes: Straining

Straining refers to the entrapment of microorganisms in pore throats between soil grains which are too small to allow passage, and is exclusively a result of pore geometry (Corapcioglu & Haridas, 1984). Microbial removal by straining is considered to be an irreversible process, in that strained microorganisms do not reenter the pore water (Tufenkji, 2007). Silliman, 1995, found that straining of latex colloids which were in the size range of 2-90 μm in diameter by a glass bead porous medium was enhanced as heterogeneity increased. In his experiments, Silliman found that colloids deposited in the porous media had the highest probability to be found at contacts where the pore water moved from large to small pores (Silliman, 1995). Further, the more perpendicular the contacts were to the flow of the water, the more colloids were deposited (Silliman, 1995). Theoretically, a particle of any size may get wedged in a crevice between two soil grains in the subsurface (Corapcioglu & Haridas, 1984). Nevertheless, it is estimated that straining only has a significant effect on mass removal when the colloid passing through a pore has a diameter greater than 5% of the porous media grain diameter (Corapcioglu & Haridas, 1984; Harvey & Garabedian, 1992; Herzig et al., 1970; McDowellboyer et al., 1986). Once the particle diameter is less than 5% of the grain diameter, the predominant mechanism for attachment to the soil grains will be surface forces (Corapcioglu & Haridas, 1984). Therefore, the ratio of microbe to pore throat diameters will determine the role of microbial straining in a porous medium.

2.6.3 Retardation Processes: Sedimentation

Sedimentation, or gravitational deposition onto the grains of the porous media, may also occur when a microorganism travelling through pore space has a density greater than the pore water (Corapcioglu & Haridas, 1984). While the majority of microorganisms are so small that gravitational settling is negligible, some microorganisms may exhibit densities greater than water, in which case transport velocity will be reduced by sedimentation (Corapcioglu & Haridas, 1984; Reynolds et al., 1989; Bradford er al., 2014). Yao et al, 1971, showed that sedimentation only plays a significant role in increasing the efficiency of a collector (i.e., a sand grain which collects particles such as microorganisms from the pore water) if the particle is greater than 5 μm (Fig 2.4). Some microbes have

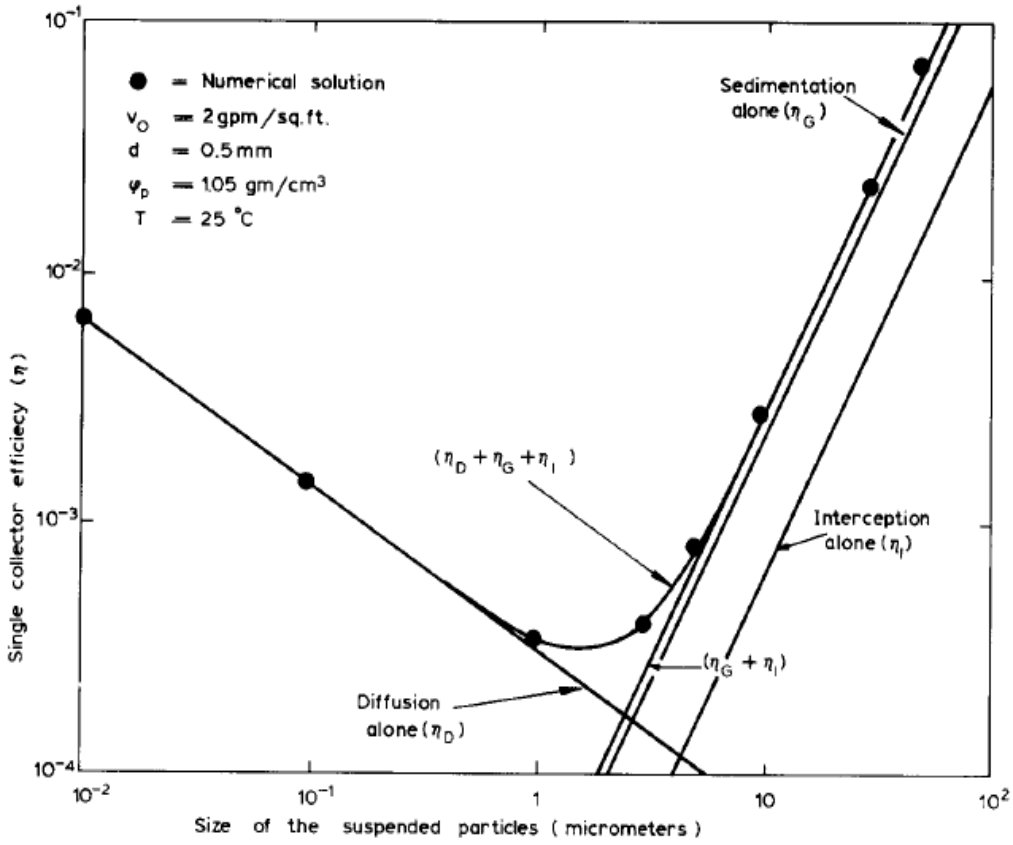

Figure 2.4: Collector efficiencies of various removal mechanisms as a function of particle size. *Reprinted with permission from Yao et al. copywright (1971) American Chemical Society.*

exhibited the ability to produce bio-gas, which in some way enhanced travel efficiency (Reynolds et al., 1989). In theory, gas production could increase the buoyancy effect for microorganisms, reducing sedimentation effects.

2.6.4 Retardation Processes: Interception

It is inevitable that even the most minute suspended particles will at some point be unable to follow the tortuous flow paths and collide with the porous media (Corapcioglu & Haridas, 1984). However, as seen in Fig 2.4, Interception of small particles is a very rare

event, making it negligible as a standalone phenomenon. Microbial cells, on the other hand, are much larger, and thus often intercept pore walls, forming clusters on the grain surface (Corapcioglu & Haridas, 1984). Clustering is often so extensive that it causes the effective grain size diameter of the soil grain to increase (Corapcioglu & Haridas, 1984). This increase leads to increased straining of smaller particles in the pore throat and filtration/sorption of particles to the bacterial cluster (Konhauser, 2007; Krone et al., 1958). These accumulations often grow to the point of instability, at which point large bunches of the cluster slough off and are transported down gradient (Corapcioglu & Haridas, 1984). The rate of sloughing is a function of the pore water flow rate and the size and density of the microbial cluster (Krone et al., 1958).

2.6.5 Retardation Processes: Adsorption

Rather than colliding with a grain surface via straining, sedimentation and interception, a microorganism may biochemically adsorb to it (Konhauser, 2007). There are three main mechanisms that determine the success or failure of complete microbial adsorption to a grain surface: the solution chemistry, substratum chemistry, and finally, bio-attachment (Konhauser, 2007). Generally, adsorption of microbes to a surface is reversible until the microbially-mediated bio-attachment is complete (Konhauser, 2007).

Reversible Attachment: Solution Chemistry

Initially, the adsorption between a microorganism and a soil grain is termed reversible adhesion (Konhauser, 2007). Reversible adhesion is an instantaneous attraction by long range forces holding the microbe at a distance of 1-10 nm from the grain surface, depending largely on the ionic strength of the solution (Konhauser, 2007). At this stage, the

microorganism can easily be pulled away from the surface by a rotation of its flagella or shear forces of the passing groundwater (Marshall et al., 1971). This initial and reversible attraction can be predictably modeled using DLVO (Derjaguin-Landau-Verwey-Overbeek) theory (Fig 2.5) (Konhauser, 2007). According to DLVO theory, surfaces have a tendency to minimize their total Gibbs free energy by satisfying their charges, and one way of doing this is via microbial adsorption (Absolom et al., 1983). Assuming that steric effects are negligible, the total Gibbs free energy (G_T, Fig 2.5) will be the difference of the van der Walls attractive energy (G_A, Fig 2.5) and the electrostatic repulsive energy (G_R, Fig 2.5) (Konhauser, 2007). At low ionic strength, the repulsive forces are greater than the attractive forces between the microorganism and the grain surface (Vanloosdrecht et al., 1989). This creates a positive spike in total Gibbs free energy between the microbe and the surface, making it thermodynamically unsatisfactory for the microorganism to approach any closer (Vanloosdrecht et al., 1989). As the ionic strength of the solution increases, the electrostatic repulsive energy decreases (Vanloosdrecht et al., 1989). This causes the attractive van der Walls energy to exceed electrostatic repulsive forces within the aqueous phase, removing the positive Gibbs free energy barrier, and creating a state of increasingly negative Gibbs free energy from the microbe to the grain surface (Stumm & Morgan, 1996;

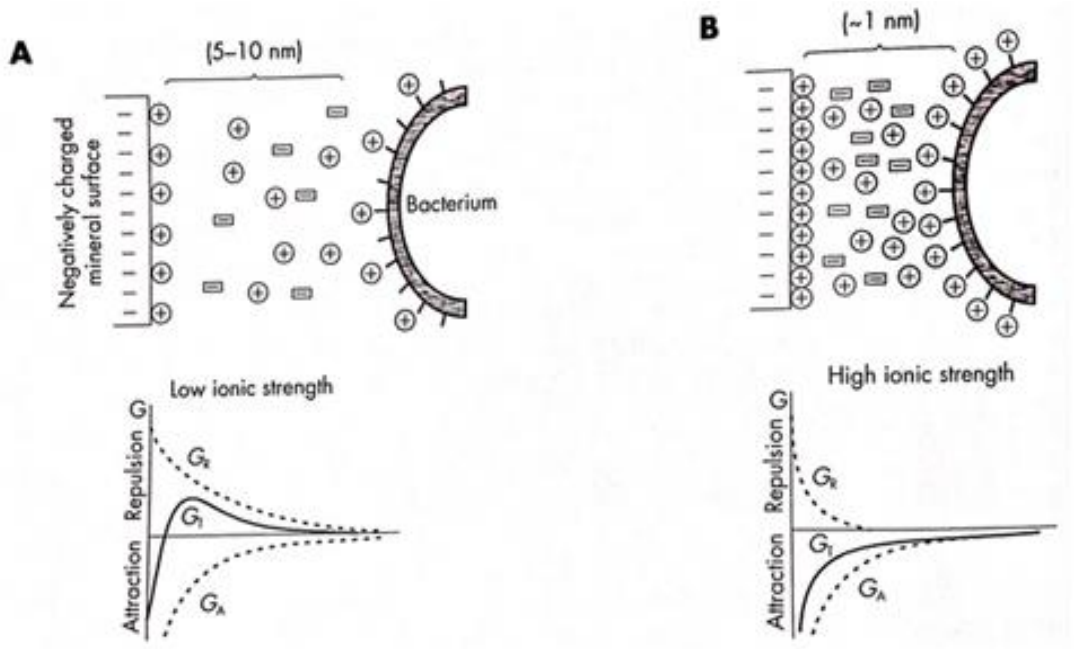

Figure 2.5: Reversible adhesion of a bacterium to a mineral surface on soil grain at low (A) and high (B) ionic strength. Where G_T is total Gibbs free energy, G_A is van der Waals attraction, and G_R is electrostatic repulsion (Konhauser, 2007).

Vanloosdrecht et al., 1989). Since Gibbs total energy is increasingly negative towards the grain surface, cation bridging in the solution acts to bring the microorganism closer to the surface (Stumm & Morgan, 1996). Both column and field experiments have backed up the theory of ionic strength dependent sorption, documenting an increased attachment efficiency with an increased ionic strength (Gannon et al., 1991; Scholl & Harvey, 1992; Tan et al., 1995).

Reversible Attachment: Substratum Chemistry

When considering initial adhesion, the chemical composition of the mineral and the microbial cell surfaces are also of importance. Depending on the chemistry of the mineral surfaces, their charge may be either positive or negative, whereas microbial cell surfaces are most often negative or neutral, and in some cases, positively charged (Konhauser, 2007). Thus, depending on the surface chemistry, there could be either attractive or repulsive electrostatic forces between the microorganism and the mineral surface (Konhauser, 2007).

Surface charges are controlled by the master variable pH. Microbial cell surfaces are teaming with ligands, which may become protonated or deprotonated at various pH levels (Konhauser, 2007). The most simplistic representation of reversible deprotonization takes the form of the following reaction (Konhauser, 2007):

$$R–AH \leftrightarrow R–A^- + H^+ \qquad Eq\ 2.1$$

Typical ligand types found on cell surfaces include hydroxyl (R–OH), carboxyl (R–C–OOH), sulfhydryl (R–S–H), phosphate ($P–O_4H_2$) and amino groups ($N–H_3$) (Konhauser, 2007). The distribution of protonated and deprotonated ligands can be quantified with the following equation (Konhauser, 2007):

$$K_a = \frac{[R - A^-][H^+]}{[R - AH]} \qquad Eq\ 2.2$$

where K_a is the acid dissociation constant for the reaction. As evidenced in Eq 2.2, when the pH increases, $[H^+]$ in solution decreases, causing an increase in $[R–A^-]$ and a decrease

in [R-AH]. This gives the cell surface an increasingly negative charge (Konhauser, 2007). On the other hand, as the pH lowers, [R–A^-] decreases, while [R-AH] increases, causing the cell surface to be neutrally or positively charged (Konhauser, 2007). The pH at which [R–A^-] and [R-AH] are equal is known as the pK_a, and is defined as the negative log of K_a (Konhauser, 2007). There are often multiple ligand types on a single cell surface each with its own pK_a (Fig 2.6-A) (Haas et al., 2001). This means that different ligands on a cell surface will dominate the change in surface charge at different pHs,

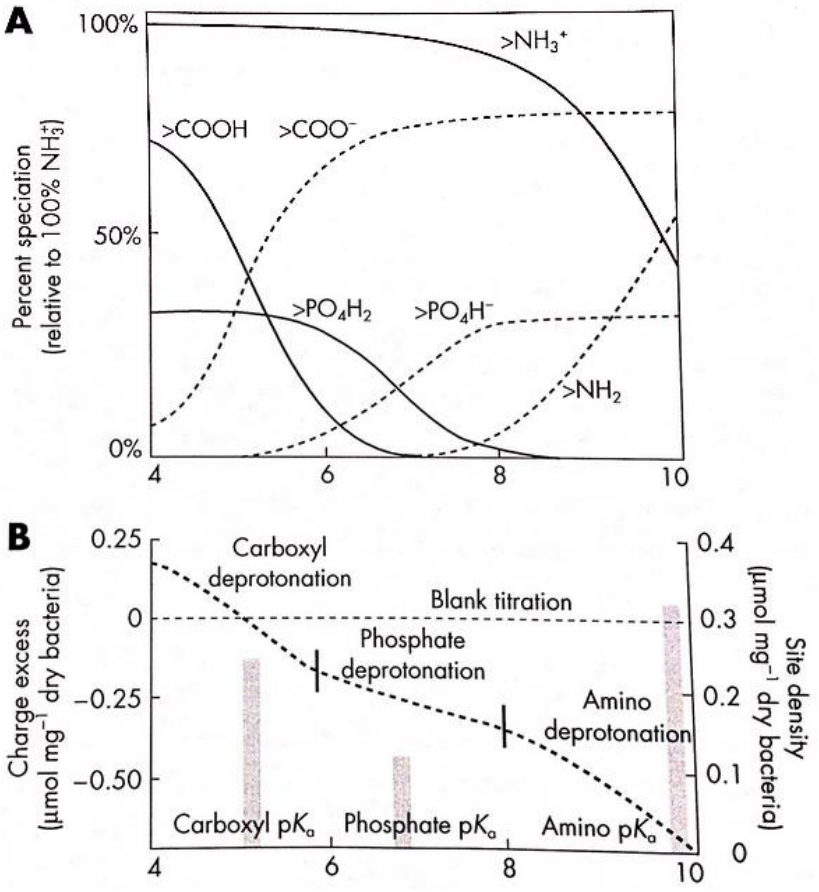

Figure 2.6: (A) Hypothetical speciation profile of major ligands associated with bacteria. (B) Hypothetical titration profile showing net surface charge with increasing pH. *Image modified from Konhauser (2007)*

typically resulting in a slowly increasingly negative charge as pH rises (Fig 2.6-B) (Haas et al., 2001). Depending on the growth conditions of the microorganism, the proportions of ligand types on its surface will often change, causing surface charges to vary at different stages in cellular growth (Fletcher, 1977; Gilbert et al., 1991). The pH also has an effect on the charge of the mineral surface being adhered to by the microorganism. In a study by Yee et al, 2000, *B. subtilis* would not adhere to a quartz surface at a neutral pH, since both

the bacterium and the mineral are negatively charged at pH's above 2.5. However, *B. subtilis* did adhere to corundum (a crystalline aluminum oxide) at neutral pH, since corundum is positively charged at pH values less than 9 (Yee et al., 2000). When the pH was driven past 9, both *B. subtilis* and the corundum were negatively charged, and repelled each other (Yee et al., 2000). The presence of organic and inorganic compounds sorbed to the mineral surface may also alter its charge (Mills et al., 1994). Quartz is often coated with iron hydroxide, giving it a positive charge at neutral pH (Mills et al., 1994). However, organic molecules may also sorb to the Fe-coated quartz, making the surface negative once again and repelling microorganisms (Sharma et al., 1985). On the other hand, hydrophobic adhesion of organic matter onto cell surfaces increases the microorganisms overall negative charge, and leads to an increased attraction to the Fe-quartz surface (Johnson & Logan, 1996).

Aside from electrostatic forces, hydrophobicity is another driver for microbial adsorption. In a study by Yee et al, 2000, it was shown that microbes adsorbed to corundum, even though their cell surfaces were neutrally charged and the corundum surface was positively charged. It was hypothesized that since the cell surface was hydrophobic, it was attracted to the nearest non-aqueous phase, the corundum, despite the lack of electrostatic attraction between them (Yee et al., 2000). pH is also the controlling variable for hydrophobic attraction to a mineral surface. As ligands protonate and deprotonate with changing pH, they become respectively hydrophobic or hydrophilic (Konhauser, 2007; Yee et al., 2000). Thus, microorganisms may transition from hydrophobic attraction → electrostatic repulsion → electrostatic attraction to a mineral surface as the pH rises (Konhauser, 2007). For example, a microbe at low pH is protonated and hydrophobic, so it attaches to a mineral

surface via hydrophobic attraction. As the pH rises, the microbe becomes increasingly deprotonated. This reduces hydrophobicity, causing the microbe to detach from the mineral surface. However, as pH continues to rise, the microbes surface becomes increasingly anionic, electrostatic attractive forces increase, and the microbe re-adsorbs to the site (Konhauser, 2007).

Irreversible Attachment & Biofilm Formation

If a reversibly attached microbe is not carried away by shear forces, and is in a desirable location, it will use biological means to more permanently adhere itself to the surface (Ginn et al., 2002; Jucker et al., 1998; Konhauser, 2007). Microorganisms have many unique ways to initiate a permanent attachment (O'Toole et al., 2000). Most microorganisms utilize extracellular polymeric substances (EPS) to affix themselves to a surface (Ginn et al., 2002; Konhauser, 2007). It has been shown in the literature that the initial state of reversible adhesion to a surface triggers genes that regulate EPS (Davies & Geesey, 1995). Other mechanisms include the use of pili, fibrils, holdfasts, lipopolysaccharides (LPS), etc., to adhere to the surface (Deweger et al., 1987; Doig et al., 1988; Konhauser, 2007; Makin & Beveridge, 1996). It is interesting to note that starving microorganisms (i.e., microorganisms repopulating an oligotrophic environment) are more likely to adhere permanently to a surface (Konhauser, 2007). When starving, microbes shrink in size, but produce more EPS, making them more adhesive (Konhauser, 2007). This is ecologically advantageous, as there are typically more organic and inorganic compounds that can be used for growth accumulated at solid-liquid interfaces (Dawson et al., 1981; Konhauser, 2007). Once adhered to the soil grain, microorganisms such as *P. aeruginosa* can travel using pili to push or pull themselves across the surface (Boyd & Chakrabarty, 1994; Wu &

Kaiser, 1995). Others use a twitching motility to facilitate surface travel (Semmler et al., 1999). Surface transport is utilized by some microbial species to initiate biofilm formation, as they congregate into large clusters (O'Toole & Kolter, 1998). These clusters may then travel as a unit across the surface, potentially to a more favorable location (O'Toole & Kolter, 1998).

Once attached to the surface, the microorganisms adapt to life in a biofilm (O'Toole et al., 2000; Probst et al., 2013). Primarily, production of EPS is increased dramatically (Konhauser, 2007). In fact, mature biofilms may be upwards of 90% composed of EPS (Christensen & Characklis, 1990). Aside from adhering the microbes to a surface (Fig 2.7), EPS provides several vital services. These services include: attracting and storing organics and inorganics, buffering environmental stressors such as temperature and pH, and allowing for mixed communities to live in close proximity (Decho, 2000). Microbes living in biofilms also exhibit increased antibiotic resistance, resistance to UV light, increased rates of genetic exchange, altered biodegradative capabilities, and increased secondary metabolite production (Annachhatre, 1996; Brazil et al., 1995; Gross & Logan, 1995; Karamanev et al., 1998; Marshall et al., 1971; Moller et al., 1998; Sarra et al., 1999; Wolfaardt et al., 1994; Zobell, 1943). Once a biofilm is formed, it increases straining and interception, and thus instigates its own growth (Little et al., 1997). In a column experiment by Morales et al, 2007, it was found that colloid transport through porous media was heavily influenced by the presence of biofilms. As the biofilm matured with time, colloid breakthrough concentrations decreased, until complete retention was observed by three weeks (Morales et al., 2007). After some time, microorganisms may slough off the biofilm, forming large pulses travelling through the pore water (G. O'Toole et al., 2000). Although

little is known of the regulatory pathways that result in the release of individual microbes from the biofilm, the overexpression of the enzyme alginate lysase has been correlated with increasing the rate of detachment from some biofilms (Boyd & Chakrabarty, 1994).

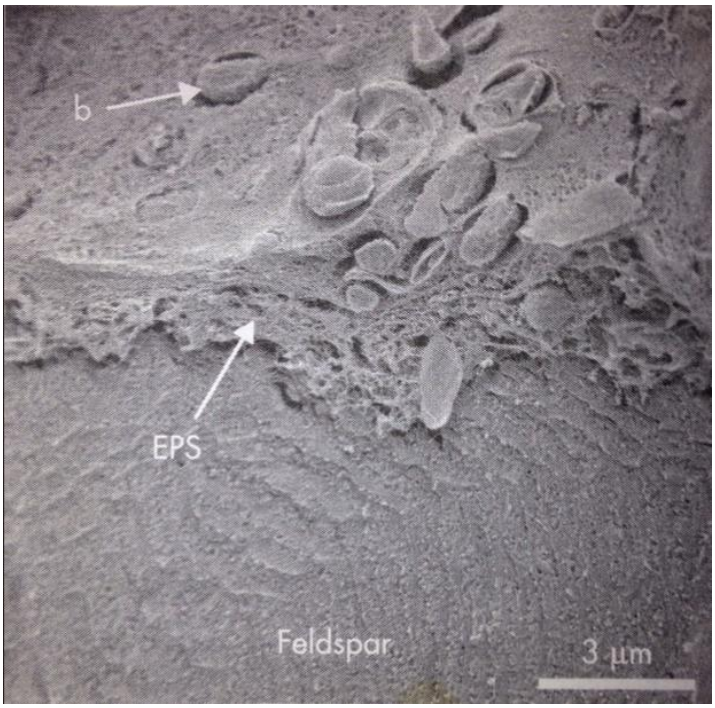

Figure 2.7: A Biofilm Formed on a Feldspar Grain. *Several bacteria (b) live on the surface, residing within the EPS (Konhauser, 2007).*

2.7 Summary

Little is known regarding the microorganisms in soils contaminated specifically with coal tar. However, the dominant microorganisms in the pore water within soils contaminated by similar hydrocarbons have been documented. NAPL thermal remediation technologies, such as STAR, operate at temperatures that can cause significant die-off of microbial life. Further, these technologies may alter the physical structure and chemistry of a soil during

treatment, making complete microbial repopulation challenging. There is a strong understanding regarding the general, various mechanisms of subsurface microbial transport and soil colonization. In addition, studies have explored the microbial repopulation of near-surface soil following brush and forest fires. However, little information exists regarding repopulation of treated and fringe zones following the in situ thermal remediation of coal tar and other recalcitrant NAPLs. This highlights a gap in knowledge regarding the long-term effects that STAR and other in situ thermal technologies have on subsurface microbiology. Understanding the effects of STAR on microbial communities will play an important role in the success of ecosystem recovery following remediation, reaching acceptable levels of soil health after remediation, and implementing microbially-mediated natural attenuation of contaminants in untreated fringe zones.

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3 Microbial Abundance and Community Structure in the Subsurface Before & After In Situ STAR

3.1 Introduction

Exposing soils to high temperatures (>100^{o}C) in situ is a common practice during thermal remediation of subsurface contaminants, resulting in partial or complete sterilization of microbial life within impacted soils (Krauter et al., 1996; Pape et al., 2015). Microbial communities within the subsurface provide a myriad of ecosystem services including nutrient and carbon cycling on a local and global scale, soil humus formation and enhancement of soil structure (Bordenave et al., 2013; Chapelle et al., 2002; Hubert et al., 2012; Kennedy & Smith, 1995; Simkus et al., 2016). Therefore, microbial repopulation following soil sterilization by thermal remediation is both desirable and advantageous. Further, since the soils directly above and downgradient from the zone targeted for thermal remediation may be involved with natural attenuation of residual free phase and dissolved contaminants, heat induced microbial die-off in these regions may slow or halt natural progression to complete remediation.

The success of microbial repopulation within a previously heat-treated volume of subsurface is largely dependent on the transport, retention, and growth of microorganisms within the thermally impacted soils. Many factors influence microbial transport, attachment, and growth within the subsurface, including nutrient and organic matter availability, various physical and chemical soil properties, and groundwater chemistry (Debano et al., 1976; Fletcher et al., 2011; Konhauser, 2007; Krauter et al., 1996; Pape et al., 2015; Richardson et al., 2002; Roland et al., 2008). These and other factors may be significantly altered by thermal remediation, depending on the magnitude and duration of heating experienced by the soil (Neary et al., 1999). It has been shown

that at temperatures <500 ^{o}C, soils retain their physical structure and chemical properties, while soils exposed to temperatures >500 ^{o}C tend to experience degradation and loss of organic matter and nutrients (Pape et al. 2015; Debano 1981; Weast 1988). Because of this, soils that are heated below this threshold will often experience a rapid and robust microbial repopulation event when cooled, at times even exceeding pre-heating microbial abundance, while soils heated above it are more likely to exhibit microbial repopulation to a fraction of original microbial abundances after cooling (Acea & Carballas, 1996; Prieto-Fernandez et al., 1998; Vazquez et al., 1993).

In situ STAR (Self-sustaining Treatment for Active Remediation) is a novel thermal remediation technology which utilizes smoldering combustion to remove targeted NAPL (Non – Aqueous Phase Liquid) pools from the subsurface (Scholes et al. 2015). During in situ STAR operation, groundwater is initially boiled away around an ignition well through the injection of hot air. Then, once temperatures in the dry, NAPL contaminated soil reach a critical temperature, the NAPL begins to smoulder. At this point, the heater in the ignition well is turned off, and ambient temperature air is continuously injected down the well. This propagates the smouldering front radially outwards from the well screen at a rate on the order of 0.5 m/day. Between the front and the well remains a zone of dry, NAPL-free soil. Temperatures during in situ STAR typically range between 400 and 1200^{o}C (Pironi et al., 2011; Scholes et al., 2015; Switzer et al., 2009). Considering that the temperatures as low as 170^{o}C for 1 hr are recommended for sterilization of surgical instruments (Rutala et al., 2008; Rutala & Weber, 2004), it is hypothesized that STAR temperatures are sufficient to sterilize the soil which it treats. Once STAR is complete and the air pressure is released from the injection well, groundwater imbibes the dry, treated zone. It is hypothesized that this groundwater could carry microorganisms which may repopulate the soil. However, given the potential loss of all organic matter and soil nutrients, it is possible that

microbial repopulation of such treated soils in the short term is challenging. Such hypotheses have motivated this work.

In a recent study by Pape et al. (2015), coal tar-contaminated soil was smouldered in the laboratory using a standard STAR bench-top column. These soils were then placed into microcosms and inoculated with microorganisms and a solution of compost, orange juice, and deionized water. After eight weeks of incubation at 27^{o}C, soils were analyzed for total bacteria. No additional organic matter, nutrients or bacteria were added during the eight weeks. The study found that bacterial concentrations in the STAR-treated soil were 2-3 orders of magnitude less than those in a non-contaminated, unheated control soil (i.e., on the order of 10^5 gene copies/g and 10^8 gene copies/g in the smouldered and control soils, respectively). After analysis of the soil chemistry and microbial characteristics, it was hypothesized that the removal of organic matter and nutrients from the soil following STAR resulted in the stunted microbial repopulation (Pape et al., 2015). It is important to note that this occurred despite the initial addition of organic matter solution, further suggesting that a single loading of organic matter is not sufficient to facilitate complete microbial recovery. However, microbial repopulation of STAR-treated soil in the field (i.e., in situ) has the advantage of continuous nutrient and organic matter delivery by the groundwater into the treated zone. Therefore, depending on the quantity and quality of organic matter and nutrients made available by the groundwater, microorganisms may be able to repopulate STAR-treated zones to greater concentrations than possible in a microcosm study.

The goal of this work is to better understand the fate of microorganisms in the context of in situ STAR. This includes the objectives of (1) quantifying the rate and extent of microbial repopulation of subsurface soils at a STAR-treated site, and (2) determining the in-situ processes

that support microbial transport and repopulation. To answer these objectives, an integrated set of field and laboratory studies were conducted. An 8-month field study was performed analyzing soil and groundwater obtained from a former industrial site contaminated with coal tar. Bacterial concentration and community structure were measured via quantitative Polymerase Chain Reaction (qPCR) and Illumina sequencing, respectively, on samples taken before and up to six months after in situ STAR was applied. Particular attention was paid to differences between subsurface zones that experienced smouldering and the adjacent zones that were not remediated but may have been heat-affected. In parallel, a two-month, laboratory, multi-column experiment was conducted that employed soils from the field site, with sterile groundwater flowing through coal tar-contaminated soil before flowing through STAR-treated soil. This experiment was repeated with amendments in the groundwater, exploring how biostimulation affects microbial repopulation. qPCR and Illumina sequencing were performed on the groundwater throughout the experiment as well as on the column soil afterwards. Taken as a whole, this work provides novel insights into the microbial communities present in a heavily coal tar-contaminated soil and how they respond within and adjacent to STAR-treated zones within the first few months after remediation.

3.2 Site Description

The site, an industrial area previously operated from the early 1900s until 1983, is located along the Passaic River in New Jersey, USA (Fig A1, Appendix A). The site is composed of two relatively permeable units: an upper fill unit and lower alluvium sand layer, separated by a confining clay “meadow mat” layer of variable thickness (Scholes et al., 2015). Both the deep sand and shallow fill layers are contaminated with coal tar DNAPL (Scholes et al., 2015). The

water table at the site is roughly 1m below ground surface (bgs) (Scholes et al., 2015). The average hydraulic conductivity in the fill and alluvium units are 6.8×10^{-4} and 1.4×10^{-4} m/s, respectively (Scholes et al., 2015)

In situ STAR was applied in the North-West corner of the site during November 1-5, 2015, in heavily contaminated soils directly under a former industrial waste lagoon. Six ignition wells, otherwise known as ignition points (IPs) were used to initiate smouldering in the alluvium sand over a depth interval of 6-9 m bgs. Two of these IPs, IP03 and IP06, located approximately 6.1 m apart from each other, were targeted for this study (Fig 3.1).

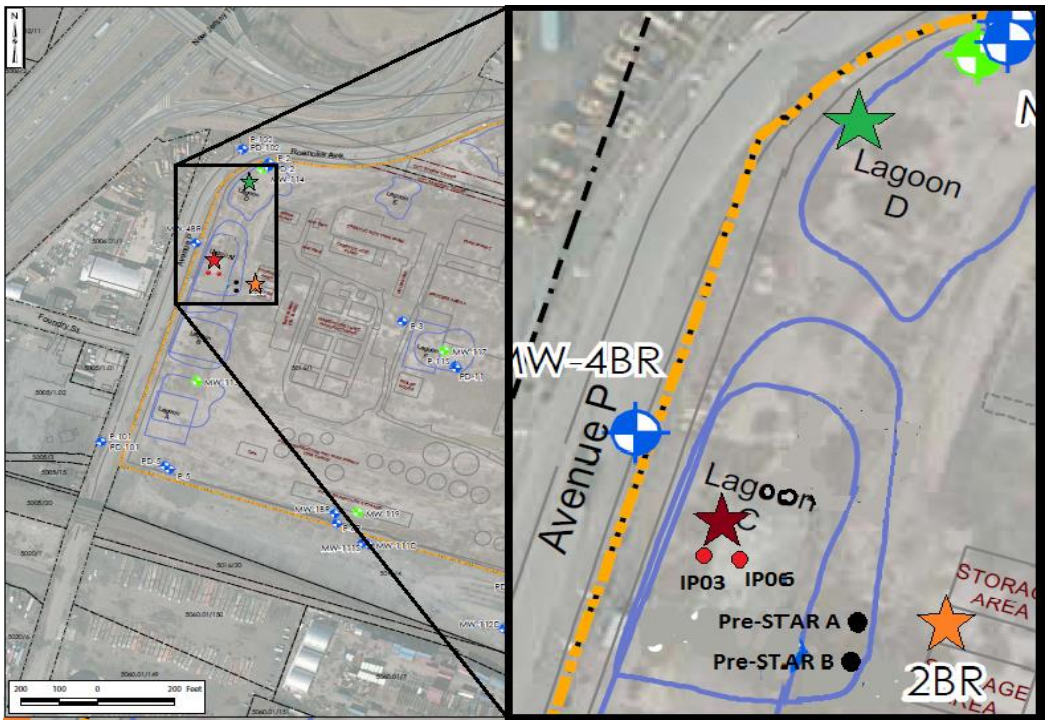


Figure 3.1: Soil coring and groundwater sampling locations. *Pre-STAR cores are denoted by black circles, while IP03 and IP06 are red circles. Stars indicate upgradient (green), study area (red) and downgradient (yellow) groundwater sampling events.*

3.3 Materials & Methodology

3.3.1 Soil Coring & Groundwater Sampling

Direct push soil coring was performed before and up to six months after STAR treatment (Table 3.1). Prior to STAR, cores were taken from contaminated soils under the same waste lagoon approximately 14 m South-East of IP03 and IP06 (Fig 3.1). Following STAR, coring was performed within a ~0.3 m radius around each of IP03 and IP06 (Fig A 2, Appendix A). Once taken out of the casing, the cores were logged, photographed, and cut via hacksaw into ~0.3 m sections. Soil from the pre-STAR cores in the depth interval of 7.9-8.2 m bgs was extracted, homogenized and sealed in sterile glass jars on site. Post-STAR core sections from 6.1-9.1 m bgs were kept intact and wrapped in saran wrap to minimize handling in the field. All core sections were labeled and packed into coolers with frozen gel packs, which were then shipped to the University of Western Ontario (UWO), London, Ontario, Canada, for further analysis. Note that, due to operational restrictions, no

Table 3.1: Timeline of Pre- and Post-STAR Coring Events.

Timeline	*Date*	*Days After STAR*	*Cores Taken*
Prior to STAR	Aug 25, 2015	-72	2
STAR Treatment	Nov 1-5, 2015	0	N/A
Post STAR T1	Dec 2, 2015	27	2
Post STAR T2	Jan 31, 2016	87	2
Post STAR T3	Mar 22, 2016	138	2
Post STAR T4	Apr 27, 2016	174	2

coring was permitted during or immediately after STAR (i.e., when the soil was dry prior to groundwater re-infiltration).

At UWO, pre- and post-STAR cores were stored in a cold room at 4 ^{o}C, and were subsampled within a maximum of 5 days following arrival. Post-STAR soil cores were visually delineated into three zones of interest: (a) untreated, (b) transition and (c) treated soil (Fig 3.2). There is a clear contrast between the red/brown colour of the treated soil and the black/grey of the untreated

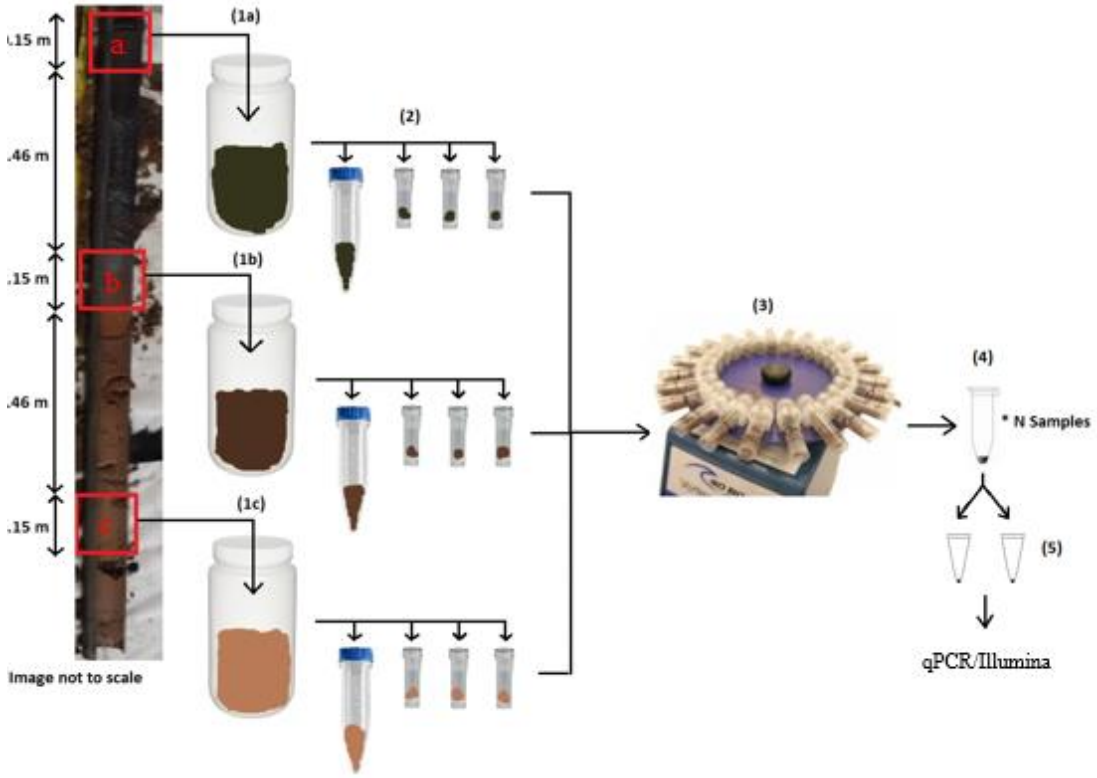


Figure 3.2: Work flow diagram for DNA analysis of (a) untreated, (b) transition and (c) treated soil zones in a typical core. *(1) Homogenization of each zone is followed by (2) subsampling into 0.25g and 10g aliquots, which undergo (3) DNA extraction, resulting in (4) a DNA sample for each subsample. These DNA samples are then (5) split into aliquots for downstream qPCR and Illumina sequencing.*

soil, with the transition zone being taken as the interval separating the two. All soil samples obtained for analysis were taken well within the respective zone (i.e., at least 0.5m from the zones upper and lower boundaries). Each sample taken was approximately 0.15 m subsection of core, which was then homogenized in previously autoclaved plastic bottles (Steps 1a-1c; Fig 3.2). The homogenization process was performed in an anaerobic glove bag. Once homogenized, triplicate subsamples weighing ~0.25 g were taken and placed into individual sterile tubes (Step 2; Fig 3.2). Because pre-STAR soil was homogenized in the jars they arrived in, subsamples were taken directly from the jars. All samples were then frozen at -20 ^{o}C until DNA extraction (Step 3; Fig 3.2). Following DNA extraction, aliquots of DNA from each sample replicate (Step 4; Fig 3.2) were analyzed for total bacteria via quantitative Polymerase Chain Reaction (qPCR) analysis and community structure was analyzed via Illumina sequencing (Step 5; Fig 3.2). Initially, all soil samples were 0.25g. However, it was found that the bacterial concentrations of some samples were near or below the qPCR limit of quantification (LOQ) (Appendix A, Table A4). Therefore, a 10g subsample was subsequently taken from each field sample (Step 2, Fig 3.2) and analyzed.

Groundwater samples were collected from IP06, as well as from locations upgradient and downgradient of the STAR treatment zone (Fig 3.1). All groundwater samples were collected during a single sampling event which took place ~7 months following STAR treatment. Due to their proximity to the edge of the site, upgradient samples were assumed to be characteristic of background groundwater entering the site. At each sampling location, duplicate samples of ~950 mL were collected into sterile plastic bottles via peristaltic pump, with dedicated tubing used for each sample. Samples were only taken after substantial well purging (i.e., 3 to 5 well volumes) or until each groundwater parameter changed by less than 10% over time during continuous

monitoring. Groundwater parameters of pH, temperature, Oxidization/Reduction Potential (ORP), Dissolved Oxygen (DO) and specific conductance were measured every 5 minutes during purging (Appendix A, Tables A1-A3). It is noted that a true “upgradient” groundwater sample would be directly West from the study area, but this was not possible. In this case, the “upgradient” sample was taken from the nearest practical location, which was slightly North of the treatment zone (Fig 3.1). Nevertheless, it is still considered representative of groundwater near the influent boundary of the site.

3.3.2 Field Study: Soil Structure, Water Content, and Organic Matter Characterization

All field samples (i.e., untreated, transition and treated soil, as well as groundwater) were gravimetrically analyzed for water and hydrocarbon content (BSI, 1995). Precise details regarding the procedure are described in method A1, Appendix A.

The grain size distribution of alluvium sand from the STAR-treated zone was characterized by a mechanical sieve. Grains passing through the no. 200 sieve were further analyzed via a hydrometer test, which was monitored over a two-day period.

3.3.3 Column Study: Experimental Setup

In both experiments, a dual flow through column set up was set up (Fig 3.3). The two columns were packed with site soil using one of the field study cores acquired adjacent to IP06. Column A was packed with the coal tar contaminated soil from the untreated zone, while Column B was packed with soil taken from the center of the STAR treated zone. This setup was intended to

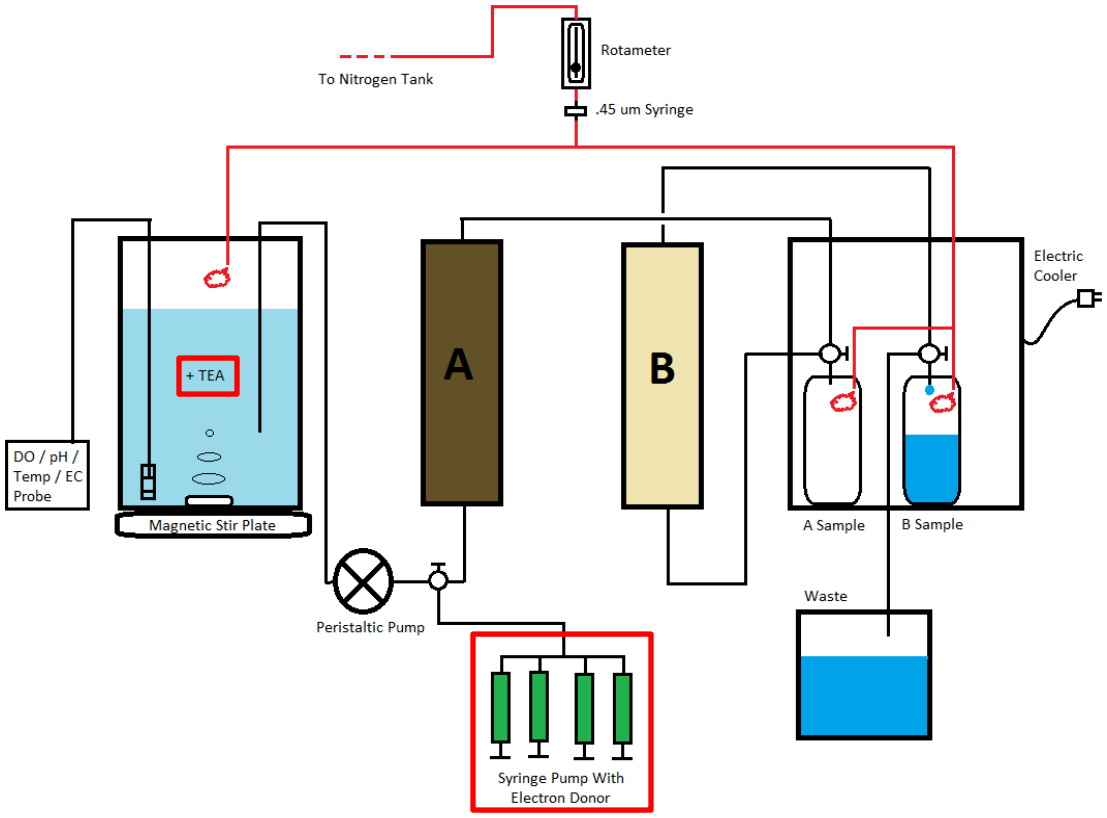


Figure 3.3: Experimental Setup for Column Studies 1 & 2. *Artificial groundwater flows from the anaerobic reservoir through the untreated (residually contaminated) soil (A) then through the STAR treated soil (B). Effluent samples from either column was acquired in an electric cooler. All nitrogen lines shown in red. Items boxed in red (Syringe pump with electron donor and addition of Terminal Electron Acceptor [+TEA] to the reservoir) only apply to Experiment 2, while all other items were identical in both experiments 1 & 2.*

mimic conditions on site where groundwater would flow through contaminated, up-gradient regions prior to entering the STAR-treated zone. It was hypothesized that any microorganisms entering the STAR-treated soil would need to enter the groundwater upgradient and be transported by advection.

To achieve this, sterile artificial groundwater (AGW) was pumped (Masterflex LS) from an anaerobic reservoir through the untreated soil in Column A and then through the STAR-treated soil of Column B. A flow rate of 0.30 mL/min, corresponding to a pore-water velocity of 0.042 cm/min, was chosen to match the average pore water velocity measured at the field site. Sampling valves for the effluent from either column were located in an electric cooler (Fig 3.3). Tracer tests were performed on Column B only in both experiments, since Column A was kept as pristine as possible until the experiment commenced. Precautions were taken to ensure the equipment and AGW were sterile All tubing, fittings, and the influent reservoir containing the AGW were autoclaved prior to experimental setup, while the glassware was baked at 220^{o}C for 24 hrs to ensure sterility. Plastic column parts which could not be heat treated were rinsed with 70% EtOH and allowed to air dry prior to assembly. As a further precaution, an Acrodisc syringe filter (0.45 um membrane) was attached to the N_2 feed line, to ensure no microbial life could enter the system through the N_2 gas feed. Each experiment was run for ~50 days.

Experiment 2 was set up identically, except that lactate and sulfate were added as electron donor and acceptor compounds, respectively (Fig 3.3). Sulfate was continuously supplied in the AGW, while lactate solution was administered five days per week into the tube feeding Column A (Fig 3.3). At the beginning of each week, two sterile 60 mL syringes were filled with the donor solution and placed on the syringe pump (Kd Scientific). Prior to drawing the donor solution into

the syringes, the solution and the syringes were left to equilibrate in an anaerobic box for 2 hr to remove the oxygen. The tubing between the syringe pump and the column influent line was primed by running the syringe pump until donor solution was seen to drip out of the end of the tubing. At this point, the tube was attached to a 3-way valve in the influent line of column A. The 3-way valve was then opened to allow the lactate pulse to enter the column, while flow from the AGW reservoir was shut off. The flow rate from the syringe pump was matched to the normal flow rate of the system to ensure continuity and evenness of flow. After ~20 mL of donor solution was injected, the syringe pump was shut down, and inflow from the AGW reservoir was resumed.

3.3.4 Column Study: Materials & Reagents

AGW used for experiments was modified from the recipe of Middeldorp et al. (1998). The AGW contained: $Na_2HPO_4 \cdot 2H_2O$, 0.20 mM; KH_2PO_4, 0.51 mM; Na_2SO_4, 0.06 mM, $NaHCO_3$, 1 mM; NH_4Cl, 0.93 mM; $MgCl_2$, 0.05 mM; $MnCl_2$, 0.02 mM; NaCl, 0.12; $CaCl_2$ 1 mM; demineralized and sterile water 30 L from a UV water dispenser (Barnstead EasyPure II). During the second experiment, concentrations of Na_2SO_4 were elevated to 0.76 mM. Upon mixing of the recipe, the entire carboy was autoclaved for 1 hr. Then, the carboy was purged with N_2 for ~1 hr while vigorously stirred (1000 RPM), after which time the purge was turned off and the headspace was kept under positive pressure with N_2 to ensure that the AGW was completely anaerobic throughout the experiments. The pH of the AGW was measured continuously (Orion 5-star probe, ThermoElectron Corp.), while dissolved oxygen (DO), temperature, and electroconductivity (EC) were measured continuously via a YSI Model 85 probe.

During Experiment 2, lactate was added 5 times per week throughout the experiment as 11.1 mmol/L of sodium lactate mixed with AGW. Lactate concentrations were chosen to be 1000 mg/L (11.1 mmol/L) based on a range of literature values (Abdelouas et al., 1998; Anid et al., 1993; Carrey et al., 2014; Davis et al., 2004; Ebihara & Bishop, 2002; Handley et al., 2012; Ikuma & Gunsch, 2013; Joner et al., 2002; Macur et al., 2001; Mosher et al., 2012; Nelson et al., 2012; Peyton, 1996; Tokunaga et al., 2003; Tront et al., 2008; Williams et al., 2005) (Table B 2, Appendix B). Lactate donor solution was prepared by dissolving a known mass of Na-Lactate into AGW, autoclaving for 30 min, and storing the cooled solution in a refrigerator. Sulfate and lactate concentrations in column B effluent were measured using Ion Chromatography (Dionex ICS-2100 equipped with an AS11 column).

Prior to both experiments, an autoclaved ~300 mg/L Br^- solution was used as a conservative tracer to examine the flow characteristics of Column B. Tracer effluent was collected in 4 mL fractions by a fraction collector (Spectra/Chrom CF-1) and analyzed via High Performance Liquid Chromatography (HPLC). Following the tracer test, 5 PVs of autoclaved deionized water and 1 PV of autoclaved AGW were pumped through the system.

3.3.5 Column Study: Column Preparation

The columns used were standard glass (Chromaflex) barrels with a 4.8 cm inner diameter (I.D.) and 15 cm length. Column barrel bottoms were sealed via PTFE o-rings attached to the PTFE end fittings, while the tops were fitted with Chromaflex flow adaptors, which enabled the columns to be filled with porous media to any desired height.

In both experiments, Column A was packed in three layers: (1) a 1cm layer of sterile coarse silica sand (2), a 10 cm layer of the contaminated, untreated soil from the site mixed with AGW as a

slurry, and (3), a final 1 cm sterile coarse grain sand cap. Columns were wet packed in a method modified from Oliviera et al. (1996) by pouring an initial small amount of sterile AGW into the base of the column, and adding 1cm layers of sand/AGW or soil/AGW slurry while stirring in the column with a sterile glass rod.

Column B was packed with ~250g of STAR-treated site soil in both experiments. A large beaker of STAR-treated soil from the site was placed in the oven at 220^{o}C for 24 hrs in order to dry and sterilize it, mimicking conditions immediately after treatment. Once cool, soil was dry packed by raining it directly from the oven treated beaker from a set height through a series of coarse sieves in a method similar to that described by Rad & Tumay (1987). STAR treated soil was capped on the effluent end of the column by a 1cm layer of sterile coarse silica sand. In both experiments porosity of the site soil in Column B was duplicated at 39.5 ± 0.2%. In both experiments, 1 Pore Volume (PV), accounting for the site soil and the coarse-grained sand, was calculated to be 82.30 ± 1.06 mL. Once packed, ~10 PVs of CO_2 gas were flushed through the column. This was followed by ~10 PVs of autoclaved anaerobic, demineralized water, dissolving any residual CO_2 remaining in the pores, resulting in complete saturation.

3.3.6 Column Study: Sampling Procedures

Effluent sampling timelines for both column studies were similar. During Experiment 1 (not amended), Column B effluent was sampled over a ~24 hr period every Monday and Friday, while Column A effluent samples were collected over ~6 hrs on Wednesdays for the first 4 weeks, and over ~10 hrs for the last 3 weeks. Sampling times were chosen in order to acquire a large enough effluent sample (i.e., >100mL), given the low flow rate (0.3 mL/min). Sample sizes needed to be large to collect enough biomass to measure potentially low microbial concentrations emerging

from the soil. Column B had a longer sampling time than Column A since it was possible that much of the influent bacteria might be filtered by the Column B soil, reducing effluent concentrations. Sample sizes from Column A were increased in Experiment 1 at later time in case bacterial depletion over time in the column caused lower effluent concentrations. In Experiment 2 (amended), sampling times were the same, except samples from Column A were kept at ~6 hrs for the duration of the experiment. Due to the sampling of Column A, there was no flow through Column B for ~5% of total column operation in both of the 50 day experiments.

All sample bottles were custom made by drilling three holes in the lid of a 700 mL autoclavable plastic bottle. A 20cm length of autoclavable tubing was inserted through one of the holes down into the bottom of the bottle. The cap and excess tubing on the outside of the bottle were then

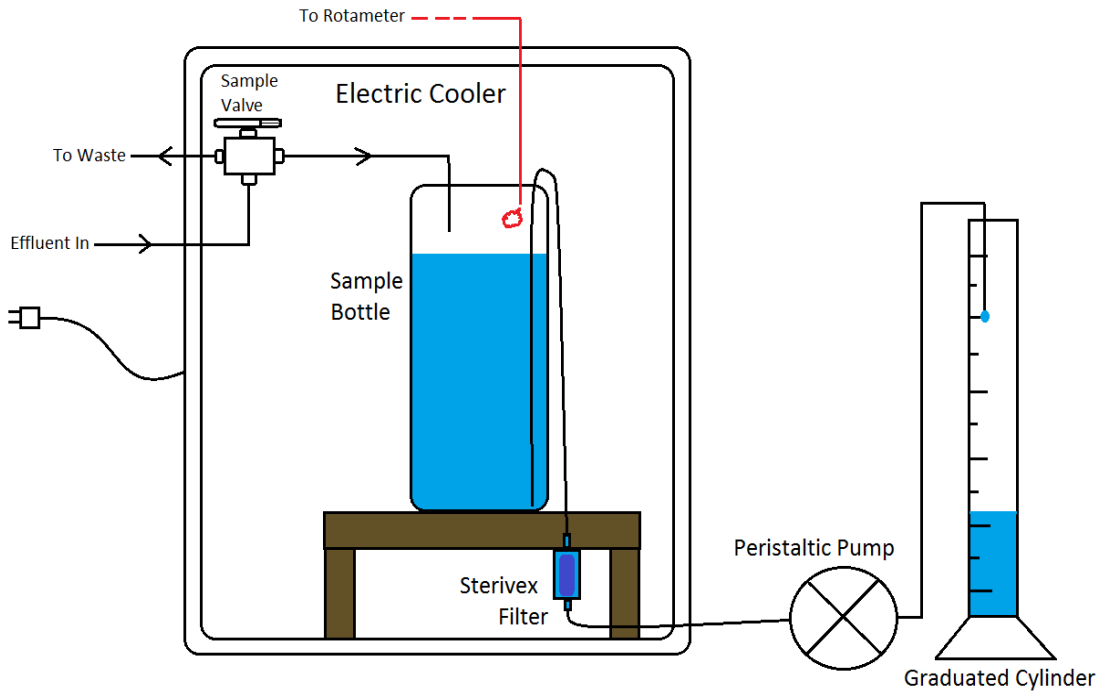


Figure 3.4: Sterivex filtration of biomass from column effluent sample. *Nitrogen (N_2) lines shown in red.*

covered with tin foil, autoclaved, and allowed to cool. Following autoclaving, all contact with the sample bottle was done using disinfected (70% EtOH) blue nitrile gloves. During a standard sampling event, the tin foil was removed and the bottle was placed in the electric cooler. A line from the effluent sampling valve was inserted into one of the remaining two holes and a N_2 line was fed into the other (Fig 3.4). Effluent sampling commenced ~10 min after N_2 flow began in order to ensure an anaerobic environment was present in the bottle during the sampling event. Once the allotted time for sampling was complete, the effluent sampling valve was shut off, diverting flow back to Column B (Column A sampling) or waste (Column B sampling) (Fig 3.4). A sterivex filter (0.45 um membrane) was then attached to the previously autoclaved tubing (Fig 3.4). The entirety of the sample was then passed through the Sterivex filter at a flow rate of ~2 mL/min using a peristaltic pump (Masterflex). Filtered volume was determined by collecting the filtered sample in a graduated cylinder (Fig 3.4) Samples continued to remain under N_2 stream until filtering completion to ensure anaerobic conditions were met. During Experiment 2, 3 mL aliquots were taken from the graduated cylinder to measure lactate and sulfate concentrations. Sample collection and sterivex filtering were performed within the electric cooler to ensure that the sample remained at low temperatures until it was completely filtered. Filters and effluent liquid samples were immediately transferred into a -20 ^{o}C freezer after acquisition.

In both experiments, soil samples representing pre-experimental conditions were acquired. From Column A, duplicate 0.25g samples and a single 10g sample were taken. From Column B, a 10g sample of the STAR treated soil was taken. All pre-experimental column samples were taken from the soil stocks used to pack the columns. For Column A samples, this entailed sampling from the beaker of soil slurry used to wet pack the column (0.25g samples) or from the soil prior to forming the slurry (10g samples). For Column B, this was accomplished by packing the oven

sterilized, STAR treated soil into 2 identical and sterile columns, one of which was the experimental column, and the other of which was used to take the 10g sample.

After the experiments were complete, soil from columns A and B were pushed out of the glass column barrels in 2 cm increments. Each subsection was then placed into individual sterile plastic bags and homogenized. Following homogenization, duplicate soil aliquots of ~0.25 g were subsampled into tough tubes placed in the freezer at -20^{o}C. Subsequently, fractions of each subsection from Column B were combined and homogenized, creating a single sample representing the whole column. A ~10g subsample of this was acquired and placed in the freezer at -20^{o}C. Sampling and homogenization of samples was performed in an anaerobic glove bag.

3.3.7 Field and Column Studies: DNA Extraction, Analysis & Statistics

DNA was extracted from sterivex filters, site soil and column soil samples using the PowerSoil® DNA Kit (MoBio Laboratories, Inc.) according to the manufacturer's instructions. Once extracted, DNA was eluted into sterile qPCR grade water and stored at -80 ^{o}C.

Total abundance of 16s rRNA in each DNA sample was measured by a quantitative Polymerase Chain Reaction (qPCR) thermocycler (BioRad). All qPCR setup was done in a laminar flow hood with pipettes dedicated to qPCR. The flow hood, pipettes and all other instruments involved in qPCR prep work were UV treated for ~1 hr prior to work. Each sample was run in duplicate, and each qPCR run contained blanks of the water used during DNA extraction.

100ng Aliquots of the extracted DNA samples were sent to the Genome Quebec and McGill University Innovation Centre. In Genome Quebec, the 16S rRNA gene library preparation was constructed using the primer sets 926fw-5'-AAACTYAAAKGAATWGRCGG and 1392rw-5'-

ACGGGCGGTGWGTRC to target both general bacteria and general archaea (Kunin et al., 2010). The amplified DNA products were subject to quality control. High throughput sequencing was conducted to the pooled samples (84 from this study, along with 60 samples from other research) using the Illumina MiSeq technology.

Demultiplexed (i.e., reads batched based on samples) sequence reads were provided by Genome Quebec. Analysis of the Illumina MiSeq sequencing data was performed using the open source software package QIIME version 1.9 (http://qiime.org/), which allowed the analysis of high throughput community sequencing data (Caporaso et al., 2010). Data Quality Assurance/Quality Checking (QA/QC) and analysis were performed by the Biozone research facility at the University of Toronto (Appendix D, Method D1).

3.3.8 Assessment of Soil Sterilization by STAR

Cores cannot be taken immediately after STAR in the field due to residual heat and pressure in the treated subsurface. The effect of STAR on bacterial concentrations immediately after treatment was evaluated by creating comparable STAR treated site soil in the laboratory using standard ex situ smouldering column experiments (i.e., Pironi et al., 2011). The treated soil was removed clean and dry within a few hours after the test. Here, agar plates were employed for observing growth of colony forming units (CFU). Pre-treatment site soil with 11% coal tar saturation and 21% coal tar saturation were also plated and analyzed for CFUs.

Treated and untreated soils were stored at room temperature at 15% moisture content in constant light for 24 hrs, after which time they were plated. To prepare the soil for plating, a stock solution of soil, deionized (DI) water and NaCl was created by mixing 1 g of soil with 9 mL of 0.85% NaCl solution. log order serial dilutions of the stock solution were created, and duplicate plates

were streaked with 0.1 mL of each dilution. All plates were then incubated for 24 hrs at room temperature to allow for CFU formation.

3.4 Results

3.4.1 Field Study: Impact of STAR on In Situ Hydrocarbon Concentrations

Within the targeted treatment zone, STAR was observed to reach temperatures of ~480^{o}C at thermocouples placed ~0.5m from each ignition point, indicating the in situ smouldering of coal tar (Fig A3, Appendix A). In both cases, smouldering temperatures were maintained for approximately 24 hours as the reaction propagated outwards from the ignition point and consumed the coal tar mass in its path. Figure 3.5 presents example soil cores taken adjacent to the two ignition points. It reveals an obvious contrast between the red (iron-oxidized) treated soil and the black untreated soil above. Such results are typical of in situ STAR treatment, which targets a specific treatment thickness in the subsurface (Scholes et al., 2015).

Pre-STAR soil cores (locations A and B, Fig 3.1) exhibited 13,000 mg/kg and 30,000 mg/kg of hydrocarbons respectively (Table A4, Appendix A); this is representative of intermediate to heavy coal tar DNAPL contamination and is typical of the areas targeted for treatment on site (Scholes et al., 2015). The STAR-treated zone exhibited hydrocarbon concentrations of 1,000 mg/kg (IP03) and 2,000 mg/kg (IP06) 27 days after STAR (i.e., at the first coring event). Over the next four months of the field study, hydrocarbon concentrations were consistently low in the STAR-treated zone, averaging 3,000±500 mg/kg (±Standard Error [SE]) (Calculated from values in Table A4, Appendix A). The slight increase is likely due to resaturation with contaminated groundwater from surrounding untreated zones. Groundwater samples within and downgradient

from the treated zone exhibited hydrocarbon concentrations of 4,000 mg/L and 2000 mg/L respectively, compared to up-gradient concentrations of 500 mg/L. In all soil cores, an untreated zone was identified (black/grey colour) as well as the transition zone between it and the STAR-treated zone. As illustrated in Fig. 3.5, some cores exhibited a sharp boundary between treated and untreated zones, while others exhibited a more gradual transition zone. The transition and untreated zones at both ignition locations exhibited average hydrocarbon concentrations of 6,000±1,000 mg/kg and 10,000±1,000 mg/kg, respectively (Calculated from values in Table A4, Appendix A). While nearly all transition zone soils had hydrocarbon concentrations under 10,000 mg/kg, the transition zone soils cored 138 days post-STAR at IP06 exhibited 15,000 mg/kg (Table A4, Appendix 4).

Figure 3.5: Soil cores revealing the contrast between the STAR-treated and untreated soil zones. *(Top) Core from IP06 obtained 138 days after STAR, exhibiting a sharp transition between treated and untreated zones (A); (Bottom) core from IP03 also obtained 138 days after STAR exhibits a more gradual transition between the two zones. The total length of core shown is ~1.5m, with depth below ground surface increasing to the right*

3.4.2 Field Study: qPCR Results

Comparison of 0.25g vs. 10g Soil Subsamples

In the majority of samples, bacterial concentrations were greater when estimated by DNA extracted from 10g of soil than from 0.25g of soil (Fig 3.6). Further, nearly all the 10g samples exhibit bacterial concentrations above their limit of quantification (LOQ), while many of the 0.25g samples have concentrations that are near or below their LOQ (Fig 3.6).

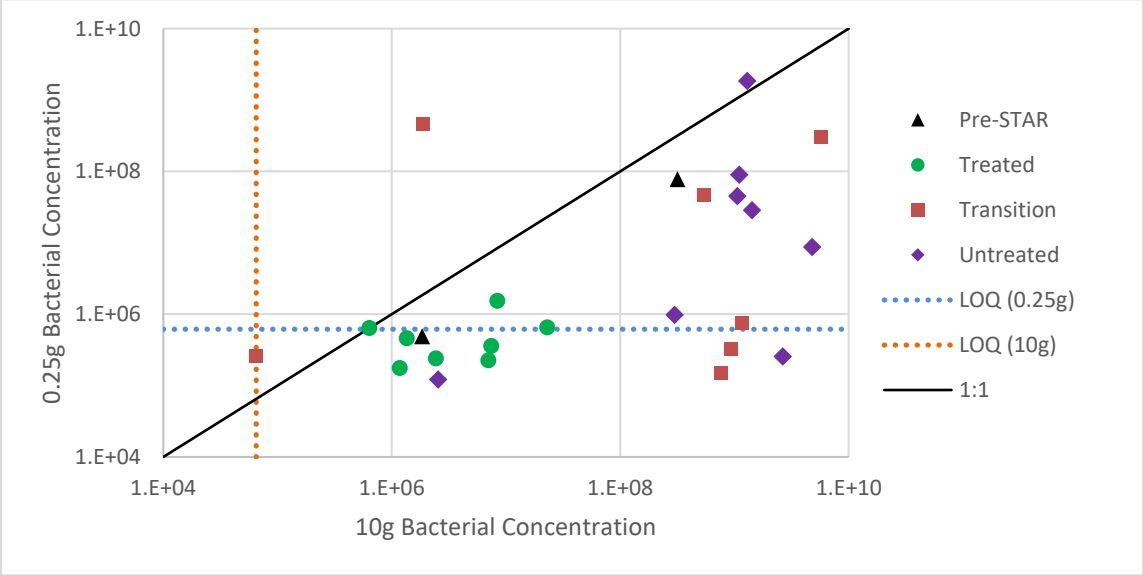


Figure 3.6: Pre- and Post-STAR soil bacterial concentrations from the field study as estimated by 0.25g vs 10g soil samples. A 1:1 ratio between 0.25g and 10g concentrations is delineated by the solid line, while the limit of quantification (LOQ) for 0.25g and 10g samples is shown by the dotted lines. *Samples skew to the right, indicating that 10g samples estimate higher bacterial concentrations than 0.25g samples of the same soil. Further, many of the 0.25g samples are below the LOQ, while nearly all the 10g samples exhibit concentrations far above the LOQ.*

Extracting DNA from 10g of soil enhances the accuracy of the bacterial concentration estimations by (a) removing much of the uncertainty associated with heterogeneity through acquisition of larger soil samples, (b) losing smaller proportions of DNA during extraction by virtue of larger handling volumes, and (c) allowing for extracted DNA to be concentrated up to 10 times if extracted DNA concentrations still remain to close to the LOQ. Therefore, bacterial concentrations estimated by the 10g samples are considered to be more accurate and are the focus of the data analysis and discussion for the rest of this study.

In Situ Bacterial Concentrations

In the Pre-STAR A and pre-STAR B soils, bacterial concentrations of 2×10^6 gene copies/g and 3×10^8 gene copies/g were observed, respectively (Fig 3.7). It is interesting to note that the bacterial concentrations in the pre-STAR soils were robust, although varying by several orders of magnitude between locations A and B. While few studies have quantified microbial concentrations at coal tar sites specifically, high concentrations of bacteria are often found adjacent to pure phase NAPL (Capiro et al., 2015; Mueller et al., 1989; Philips et al., 2012). Therefore, it is likely that similar conditions also exist at this site.

Immediately following STAR, soil in the treated zone is expected to be sterile. This was supported by the laboratory finding that 0 CFUs formed after 24hr incubation on agar plates streaked with STAR treated soil, compared to 2×10^7 CFUs formed on plates streaked with site soil exhibiting 11% and 21% coal tar saturation. Only 27 days following STAR at the field site, bacterial concentrations in the treated zone at IP03 and IP06 rebounded to 1×10^6 and 7×10^6 gene copies/g, respectively (Fig 3.7). After 174 days, bacterial concentrations in the treated zones

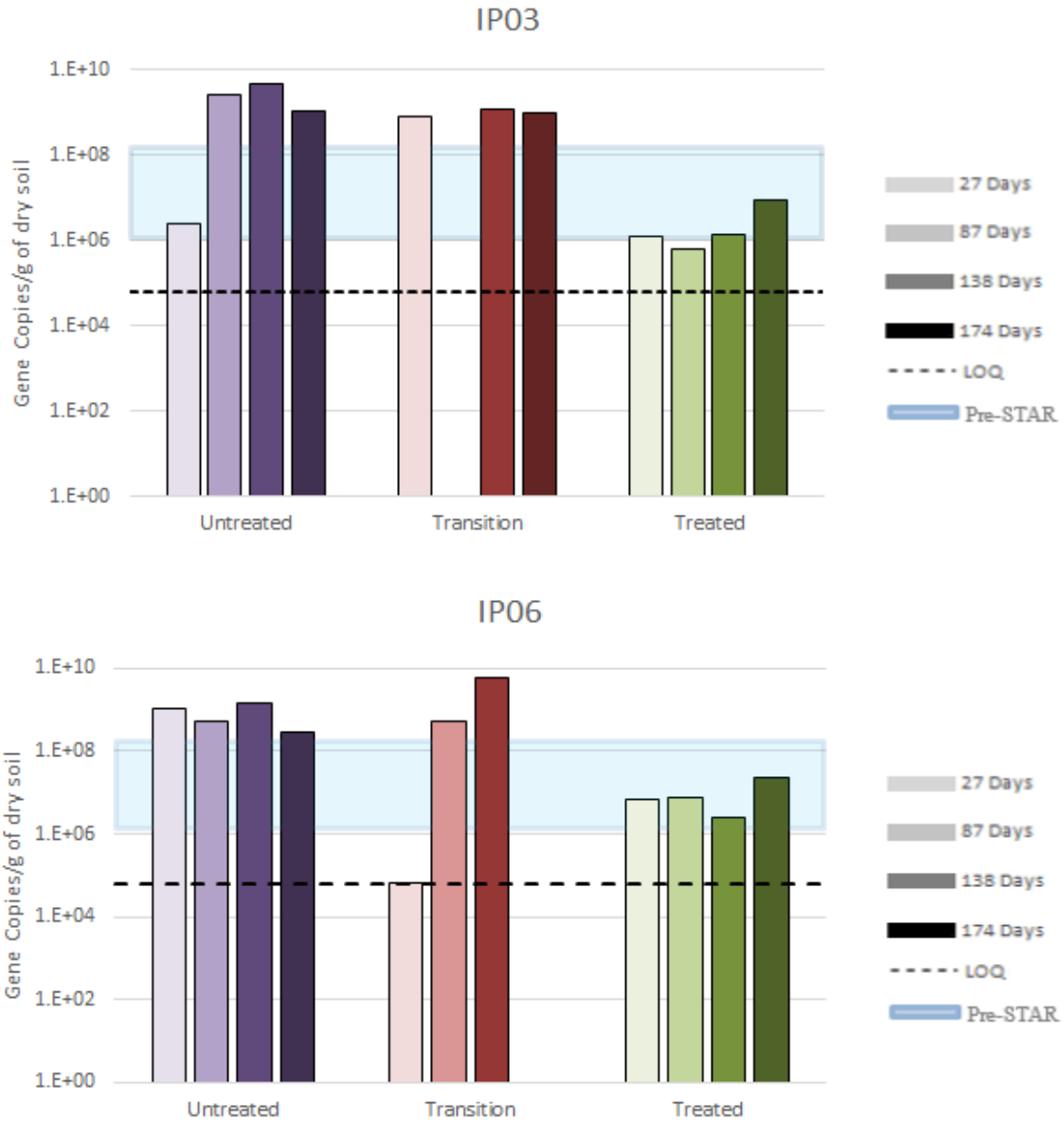


Figure 3.7: Bacterial concentrations (gene copies/g of wet soil) in untreated (purple), transition (red) and treated (green) zone soils at IP03 and IP06. *Darkening of the bars indicates progression of time following STAR. Bars represent bacterial concentrations within 10g samples. The Limit of Quantification (LOQ) is represented by a solid line. The range of Pre-STAR bacterial concentrations is shown in blue.*

and IP06, respectively) (Fig 3.7). These are within the range of bacterial concentrations found in the pre-STAR soils, albeit at the lower end of the range.

Bacterial concentrations in the untreated zones were predominantly high at $\sim 1 \times 10^9$ gene copies/g and remained relatively unchanged over time (Fig 3.7). The one exception was the first sample (27 days post-STAR) at IP03, which exhibited 3×10^6 gene copies/g. The transition zone samples exhibited different trends at the two locations. Consistently high bacterial concentrations at IP03 mirrored that of the untreated zones (Fig 3.7). However, at IP06, concentrations were below the LOQ at day 27 and rose consistently with time to 6×10^9 gene copies/g after 138 days (Fig 3.6). It is noted that the IP03 transition sample at 87 days and the IP06 transition sample at 174 days after STAR could not be acquired.

Representative groundwater samples were taken at the earliest opportunity, 207 days following STAR. The groundwater obtained up-gradient of the treatment area, and close to the site boundary (Fig 3.1), was tan in color and exhibited minimal odor. It contained 3×10^3 gene copies/mL (Appendix A, Fig A4). In contrast, the groundwater obtained from IP06 and downgradient from the treatment area was black and had a strong "coal tar" odor (Appendix A, Fig A6). The bacterial concentrations were 2×10^6 gene copies/mL in the treated zone at IP06 and 8×10^5 gene copies/mL downgradient.

The concentration of bacteria attached to the soil can be resolved from that in the pore water for a soil sample by:

$$C_{Grains} = \frac{gene\ copies_T\ - gene\ copies_{PW}}{M_T} \qquad Eq\ 3.1$$

where C_{Grains} is the concentration of bacteria attached to the soil grains in given sample (gene copies/g), $gene\ copies_T$ is the total amount of bacteria (where 1 gene copy ≈ 1 bacterium) in the sample, $gene\ copies_{PW}$ is the amount of bacteria within the pore water of a given sample and M_T is the total wet mass of the sample. Equations used to calculate $gene\ copies_T$ and $gene\ copies_{PW}$ may be found in Appendix A (Eqs A2 and A3). This assumes that the bacterial concentration in the pore water is constant over time.

The results of these calculations are displayed in Table 3.2. The table reveals that the percentage of bacteria attached to the soil ranged between 55% and effectively 100%. Moreover, it revealed that the average amount attached to the soil for the untreated and transition zone soils were 98% and 98%, respectively while the average for the treated zone soils was 85%. It is noted that the sample obtained from the IP03 transition zone at 27 days exhibited bacterial concentrations below the LOQ and thus the percent of bacteria attached to its soil could not be resolved from the groundwater concentration. There appears to be no trend with time in these percentages, although STAR-treated zones do exhibit an overall increase in the percentage of bacteria attached over time.

Table 3.2: Bacterial Concentrations In Pore Water & On Soil Grains

Days After STAR	Sample Location	Soil Zone	Total Bacteria/g of sample	Pore Water Bacteria/g of sample	Attached Bacteria\g of sample	% Attached Bacteria
-72	Pre-STAR A	Untreated	1.8E+06	2.2E+05	1.6E+06	88%
	Pre-STAR B	Untreated	3.2E+08	3.0E+05	3.2E+08	>99%
27	IP06	Untreated	1.1E+09	3.1E+05	1.1E+09	>99%
		Transition	6.4E+04	Unresolvable*		
		Treated	7.0E+06	3.3E+05	6.7E+06	95%
	IP03	Untreated	2.5E+06	3.2E+05	2.2E+06	87%
		Transition	7.7E+08	3.0E+05	7.7E+08	>99%
		Treated	1.2E+06	3.4E+05	8.3E+05	71%
87	IP06	Untreated	1.3E+09	3.1E+05	1.3E+09	>99%
		Transition	5.4E+08	3.3E+05	5.4E+08	>99%
		Treated	7.4E+06	2.6E+05	7.2E+06	97%
	IP03	Untreated	2.7E+09	2.7E+05	2.7E+09	>99%
		Transition	Sample Not Obtained - Poor Core Recovery			
		Treated	6.4E+05	2.9E+05	3.5E+05	54%
138	IP06	Untreated	1.4E+09	2.9E+05	1.4E+09	>99%
		Transition	5.7E+09	3.9E+05	5.7E+09	>99%
		Treated	2.4E+06	2.6E+05	2.2E+06	89%
	IP03	Untreated	4.8E+09	2.9E+05	4.8E+09	>99%
		Transition	1.2E+09	2.8E+05	1.2E+09	>99%
		Treated	1.4E+06	2.7E+05	1.1E+06	80%
174	IP06	Untreated	3.0E+08	2.6E+05	3.0E+08	>99%
		Transition	Sample Lost During DNA Extraction			
		Treated	2.3E+07	2.3E+05	2.3E+07	>99%
	IP03	Untreated	1.1E+09	2.7E+05	1.1E+09	>99%
		Transition	9.3E+08	2.7E+05	9.3E+08	>99%
		Treated	8.4E+06	2.8E+05	8.1E+06	97%

*Sample below LOQ

The logarithm of the bacterial concentrations attached on the soil grains at 174 days following STAR exhibited a positive linear relationship (R^2 = 0.6471) with the average hydrocarbon concentration in each zone (Fig 3.8). It is noted that bacterial concentrations at 138 days were used for the IP06 transition zone, since the sample at 174 days was unavailable. This figure

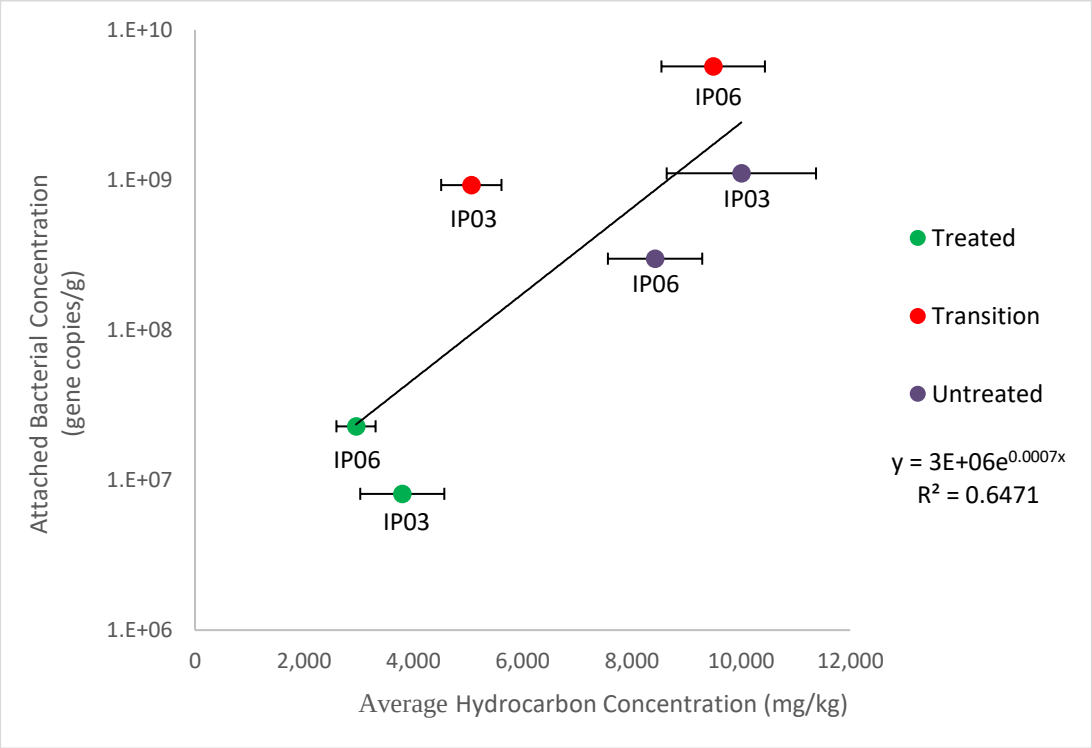


Figure 3.8: Bacterial concentrations attached to the soil at 174 days after STAR vs. average hydrocarbon content in treated (green), transition (red) and untreated (purple) zones. *Error bars represent standard error of hydrocarbon concentrations within triplicate samples. Note that the sample for the IP06 transition zone at 174 days was lost, and therefore the attached concentration at 138 days was used.*

reveals that there is a correlation between the average concentration of hydrocarbons in post-STAR soil and the abundance of bacteria which accumulate on the soil grains in a given time.

This agrees with previous findings be Pape et al., (2015), who found that the extent of microbial repopulation in smouldered and heated soils is largely dependent on the presence of organic matter.

3.4.3 Field Study: Amplicon Sequencing Results

Results from Illumina sequencing of field samples were visualized as heat maps at the class resolution (Fig 3.9). Heat maps in this context display the relative proportion of each class as a percentage of the entire microbial population. Percentages of all microbial genera within each class are also exhibited (Fig A9 & A10, Appendix A).

The microbial communities in all of the soil samples were primarily composed of *Proteobacteria* and, in particular, the class *gamma-proteobacteria* (Fig 3.9). Within this class, the genera *Psuedomonas* and *Acinetobacter* dominated, although the relative proportions of these two varied (Fig. A9, Appendix A). For example, while the Pre-STAR B soil was dominated by *Psuedomonas* (93%), the Pre-STAR A soil exhibited more *Acinetobacter* (25%) and also displayed a greater range of dominant microorganisms, including the *Proteobacteria* genera *Myxoccales* (7.6%) and the *Fermicutes* genera *Aerococcacaea* (19%) (Fig A9, Appendix A). Overall, *Proteobacteria* dominated both the pre-STAR samples (51% and 97% for A and B) and the STAR-treated soils (average of 75% and 77% over all times at IP03 and IP06) (Fig 3.8). Within the *Proteobacteria*, the class *Gamma-proteobacteria* dominated in pre- and post-STAR soil, although diversity widened to include higher proportions of other *proteobacteria* classes in

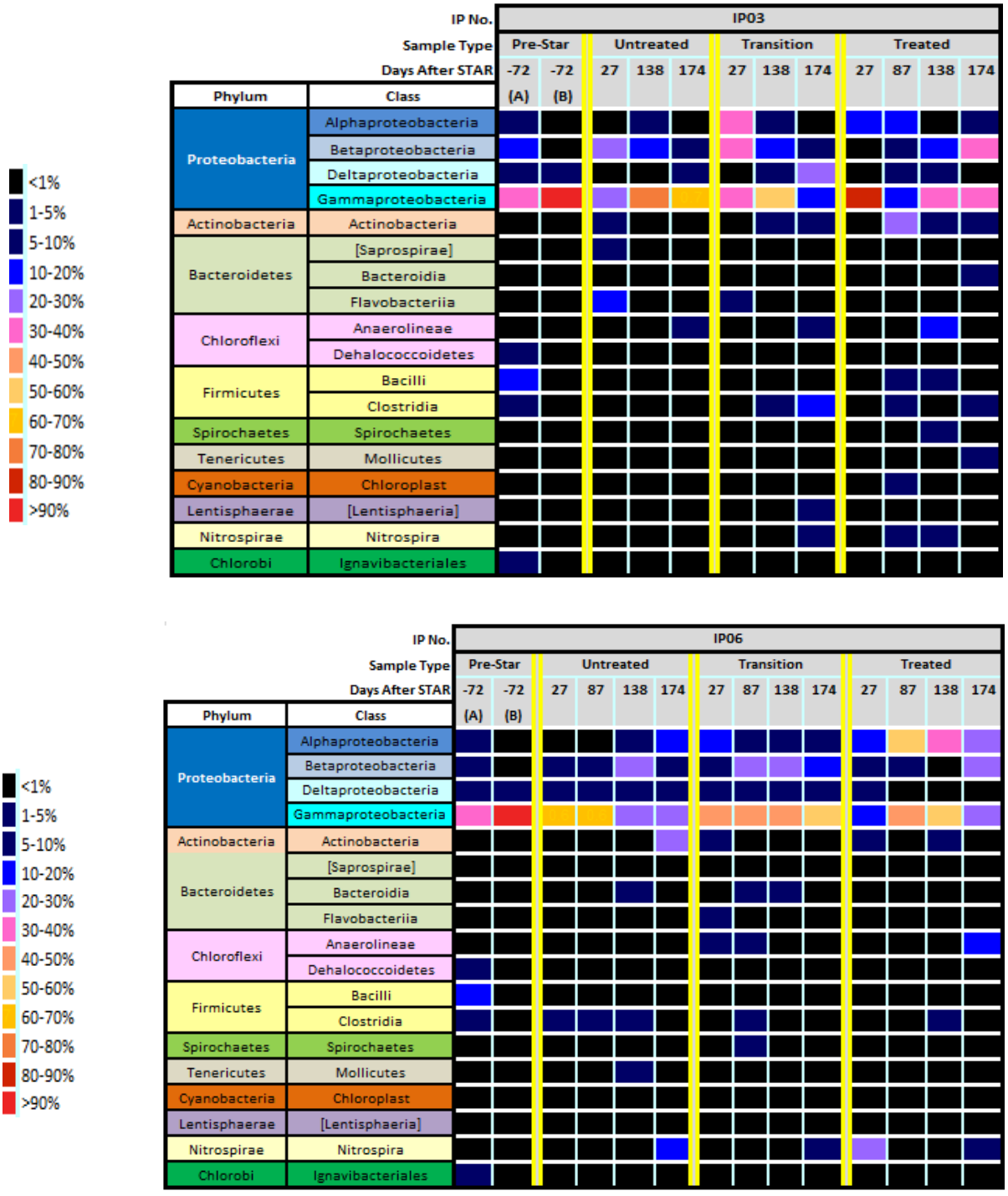


Figure 3.9: Heat maps showing percent values of microbial classes (OTU cutoff: 2%) present over time in Pre-STAR, Untreated, Transition and Treated soil zones. *Both Pre-STAR samples were taken at the same time (i.e., 72 days before STAR) and are therefore also classified as (A) and (B), in reference to the pre-STAR coring locations A and B.*

the treated soils than observed in the pre-STAR soils (Fig 3.9). The transition and untreated zones were particularly dominated by *Gamma-proteobacteria*, although other classes of *Proteobacteria* were also found in significant proportions (Fig 3.9). Minor genera (i.e., those present as less than 2% of the microbial population) were noted to increase over time in the transition and treated zones at IP03, but remained relatively consistent over time in all other soils (Fig A9 & A10, Appendix A). It is also interesting to note the presence of anaerobic and thermophilic genus *Thermoanaerobacterium* in the transition and untreated soils, suggesting the possibility of heat biostimulation in these zones (Fig A9 & A10, Appendix A).

Statistical differences in microbial community structure between all samples was shown on a NMDS plot, where increasing distance between samples on the plot indicates decreasing similarity of community structure (Fig 3.10). Clusters were circled on the plot to highlight particular regions with similar community structure, and arrows were used to reveal temporal changes in community structure. Nearly all post-STAR field samples were present initially (i.e., 27 days after STAR) in the upper right quadrant of the NMDS, and generally shift with time towards the lower left quadrant (Fig 3.10, arrows), suggesting similar patterns in community structure transitions within the different soil zones.

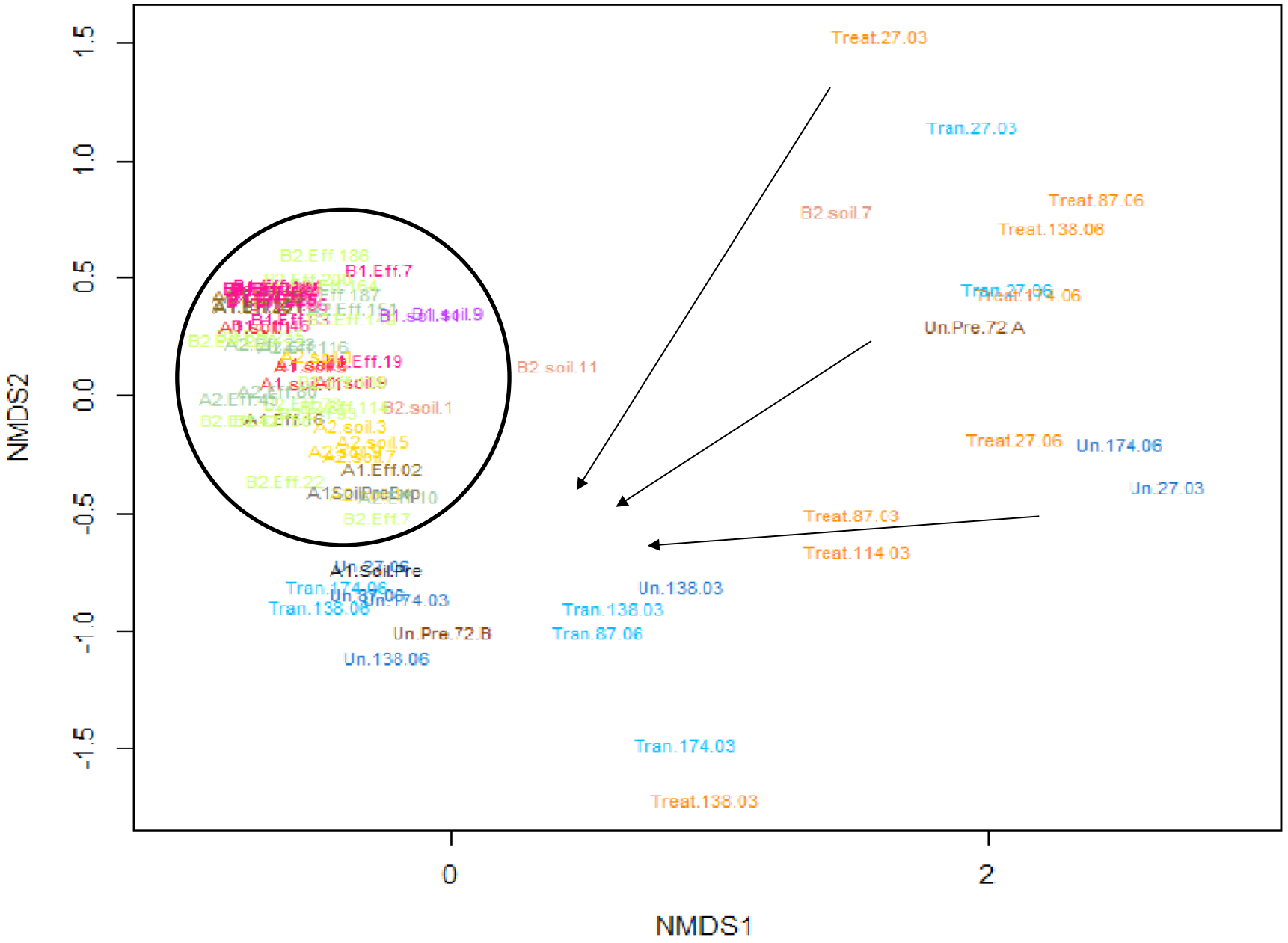

NMDS2
NMDS1
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
0
2
Treat.27.03
Tran.27.03
B2.soil.7
Treat.87.06
Treat.138.06
Tran.27.06
Treat.174.06
Un.Pre.72.A
B2.soil.11
Treat.27.06
Un.174.06
Un.27.03
Treat.87.03
Treat.114.03
Un.138.03
Tran.138.03
Tran.87.06
Un.Pre.72.B
Un.138.06
Tran.174.03
Treat.138.03
B2.Eff.186
B1.Eff.7
B1.soil.9
B2.Eff.116
A1.Eff.19
A1.soil.9
B2.soil.1
A1.Eff.6
A2.soil.3
A2.soil.5
A1.Eff.02
A1.SoilPre
B2.Eff.22
B2.Eff.7
Un.27.06
Tran.174.06
Un.174.03
Tran.138.06

Figure 3.10: NMDS visualization of all samples (field and column studies). *Greater distance between samples indicates less similarity of microbial community. Sample types are color coded and labeled. For field samples, Pre-STAR (dark brown), untreated (dark blue), transition (light blue), and treated (orange) soil zones are labeled in the following format: "soil zone . days after STAR . sample location" where sample locations include 03 (IP03), 06 (IP06), A (pre-STAR location A) and B (pre-STAR location B). Labeling of column samples has the format: "Column and Experiment . Sample type . Sample variable", where A1 would refer to column A, experiment 1, sample types include Eff (column effluent) and soil, and sample variables give the pore volumes passed prior to effluent sampling or a soil samples average distance from the influent port. Column study samples are color coded in the following format. Experiment 1: pre-experiment column A soil (grey), post-experiment column A soil (dark pink), column A effluent (light brown), column B soil (purple), column B effluent (light pink). Experiment 2: pre-experiment column A soil (black), post-experiment column A soil (yellow), column A effluent (dark green), column B soil (dark orange), column B effluent (light green). Column samples are shown to cluster (circled), while field samples change over time to become more similar in composition (arrows).*

3.4.4 Column Study: Tracer Test Results

Bromide tracer tests conducted on Column B (STAR-treated soil) in both Experiments 1 (not amended) and 2 (amended) exhibited similar step function breakthrough curves, with complete breakthrough of the tracer observed within 2 pore volumes (PV) of the onset of flow (Fig B3, Appendix B). The analytical solution (Eq. B1 – B6, Appendix B) for the 1-D advection dispersion equation was fit by minimizing the mean square error between the model and the breakthrough data. Based on the model fit, the dispersivity (α) of the packed soil in column B was determined to be 0.55 cm in Experiment 1 and 0.16 cm in Experiment 2. This difference indicates some variability in the physical structure of packed soil in Column B (including the

coarse grain sand cap). Overall, the differences in α are minor, revealing the similarity of Column B packing in both experiments.

3.4.5 Column Study: qPCR Results

Analysis of the column soil in Experiment 1 (not amended) and 2 (amended) revealed significant repopulation of Column B by bacteria from Column A. Column A exhibited bacterial concentrations of $1.2 \times 1\,0^{9}$ gene copies/g prior to experimentation (Fig 3.11). In both experiments, initial concentrations of bacteria in Column B were below the LOQ, confirming that the column was sterile prior to experimentation. Final bacterial concentrations exhibited in Column B were 8.6×10^{7} gene copies/g and 8.2×10^{8} gene copies/g in Experiments 1 and 2,

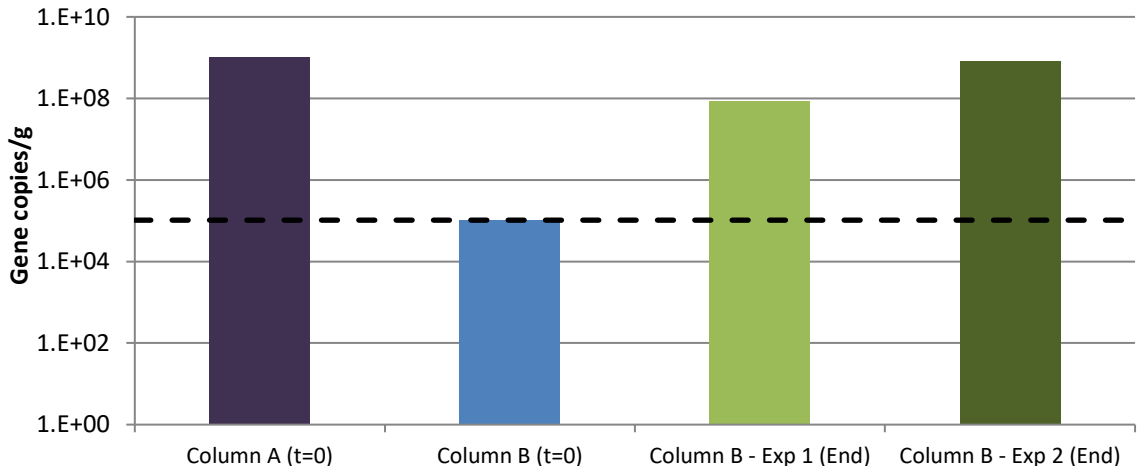


Figure 3.11: Bacterial concentrations in untreated soil used to pack Column A, and bacterial concentrations in Column B before (t=0) and at the end of Experiment 1 (not amended) and 2 (amended). *The limit of quantification (LOQ) is delineated by the dashed line.*

respectively (Fig 3.11). It is noted that net bacterial growth was assumed negligible, and thus all bacteria in Column B after both experiments was considered to be exclusively delivered by the groundwater flowing from Column A. In both experiments, bacterial concentrations in the

Column B influent were steadily supplied from Column A, highlighting the effectiveness of bacterial transport in groundwater (Fig 3.12). Also, influent concentrations of bacteria to Column B were consistently greater than or equal to the effluent concentrations coming from Column B, revealing that bacterial repopulation was occurring during the experiments (Fig 3.12).

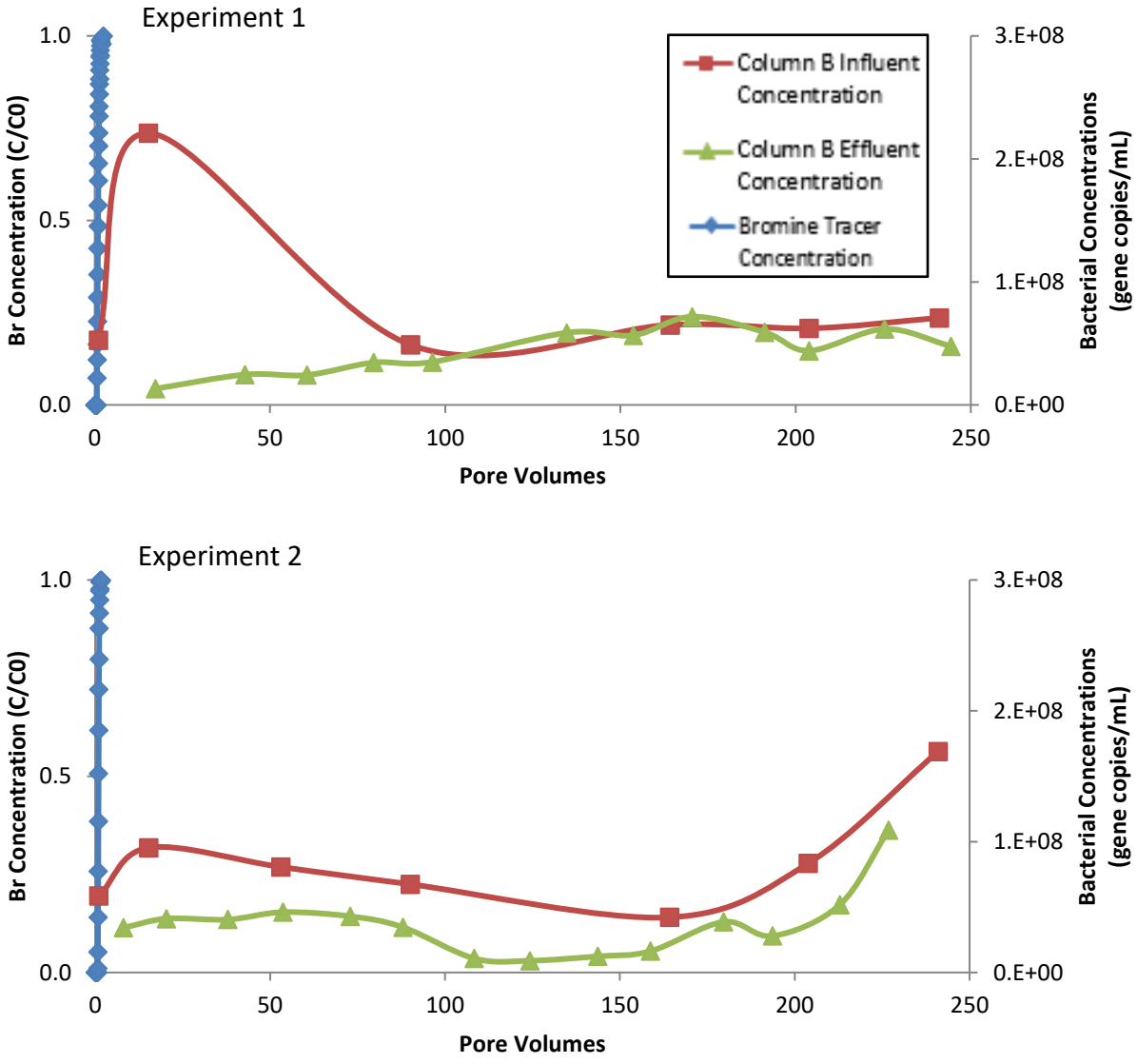


Figure 3.12: Column B influent (red) and effluent (green) bacterial concentrations and bromine (Br) breakthrough curve during Experiment 1 and 2.

The extent of bacterial repopulation within Column B was quantified using a gene copy "mass balance" approach and is summarized in Table 3.3. The expected number of total bacteria remaining in Column B at the end of each experiment was calculated by:

$$Total\ Expected\ Bacteria = Total\ Bacteria_{In} - Total\ Bacteria_{Out} \qquad Eq\ 3.2$$

Where $Total\ Bacteria_{In}$ represents the total number of bacteria introduced into Column B through the influent, and $Total\ Bacteria_{Out}$ is the total number of bacteria removed from Column B by the effluent. $Total\ Bacteria_{In}$ and $Total\ Bacteria_{Out}$ in each experiment were taken as the area under the breakthrough curve for Column B influent and effluent, respectively (Fig 3.12). The expected number of total bacteria remaining in column B calculated in this manner was compared to the actual number of total bacteria measured in Column B soil at the end of the experiment (where actual total bacteria = measured soil bacterial concentration × total mass of sand in Column B). Results show that: (a) in Experiment 2 (amended), measured total bacteria was similar to the abundance of expected total bacteria (9.1×10^{9} gene copies measured and 1.1×10^{10} gene copies expected) while (b) in Experiment 1 (not amended), measured total bacteria was much lower than the expected total bacteria (9.4×10^{8} gene copies measured and 1.1×10^{10} gene copies expected) (Table 3.3). These results suggest microbial die-off during repopulation of Column B, although much less die-off occurs in the presence of biostimulants during Experiment 2.

Table 3.3: Gene Copy Balance for Column B

Parameters	Experiment 1: Not Amended (gene copies)	Experiment 2: Amended (gene copies)
Total Influent Bacteria	2.1×10^{10}	1.8×10^{10}
Total Effluent Bacteria	1.0×10^{10}	7.4×10^{9}
Expected Total Bacteria in Column	1.1×10^{10}	1.1×10^{10}
Measured Starting Total Bacteria in Column	1.0×10^{5} *	1.0×10^{5} *
Measured Final Total Bacteria in Column	9.4×10^{8}	9.1×10^{9}

*Below LOQ

3.4.6 Column Study: Amendment Results

Analysis of lactate revealed minimal degradation of the organic substrate in Columns A and B (Appendix B, Fig B 2). This was attributed to the relatively high concentrations of organic contaminants present in column A (17,000 mg/kg), which would compete with the lactate as a potential electron donor. These contaminants were noted as a light yellowish-brown tinge in the effluent, and would accumulate on sterivex filters during liquid sampling of the columns. The filters routinely turned dark brown-grey after ~120mL of column A or ~450 mL of column B effluent, qualitatively highlighting the transport and retention of organic contaminants within column B.

On average, removal of sulfate was observed in columns A and B (Fig 3.13), indicating stimulation of sulfate reducing bacteria (SRB). In column A, effluent sulfate concentrations were reduced from ~0.073 g/L (i.e., equivalent to 0.108 g/L or 0.76 mM of Na_2SO_4) to 0.06±.001 g/L,

a decrease of ~18% (Fig 3.12). In column B, effluent concentrations were reduced further to 0.05±0.003 g/L, another decrease of ~18%[1], relative to the influent concentration. Further,

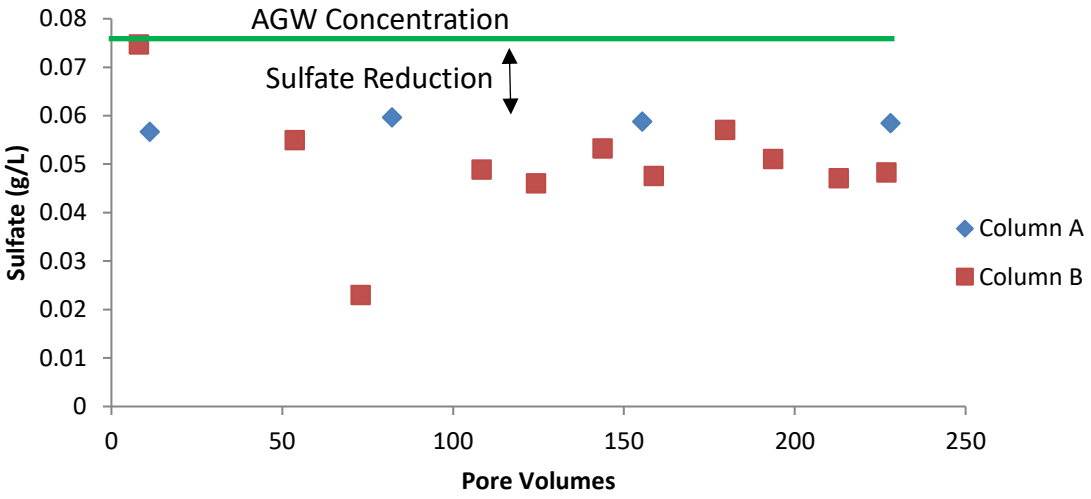


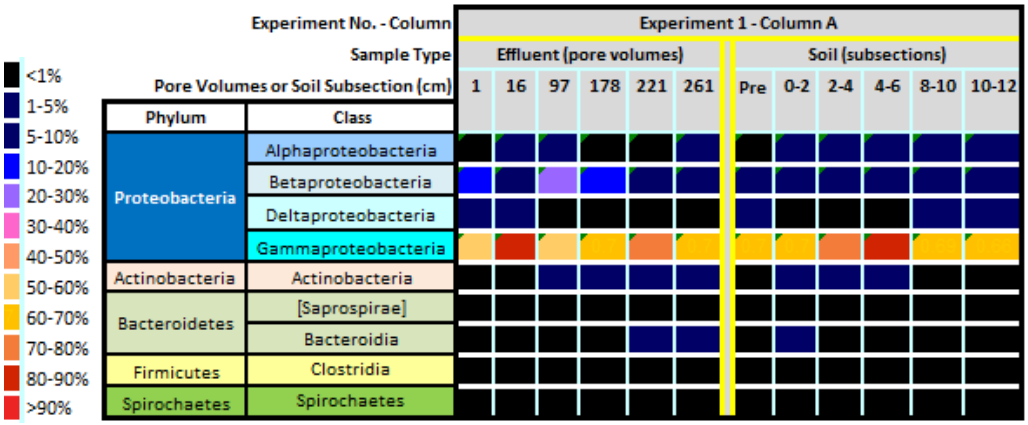
Figure 3.13: Sulfate Trends in Column A & Column B Effluent During Column Experiment 2. *Sulfate concentrations supplied in the AGW are denoted by the green line.*

Column B soil exhibited black veins of precipitate at the end of Experiment 2, which was not observed in Experiment 1, suggesting iron sulfide formation was occurring as a byproduct of sulfate reduction (Appendix B, Fig B5). These results suggest that sulfate is readily accepted as a TEA in both the untreated and STAR treated soils, and is likely the reason for the order of magnitude more total bacteria measured in Column B at the end of the Experiment 2.

[1] The percentage of 18% was generated disregarding the initial data point from the column B effluent, since it was taken before any column A effluent sample, and therefore the column A effluent sulfate concentration is unknown for that time.

3.4.7 Column Study: Amplicon Sequencing Results

In both experiments, it was shown that microbial taxa which were present in large proportions within Column A soil were (i) present in the Column A effluent and (ii) populated Column B to similar proportions (Fig 3.14). Although Experiment 2 (amended) showed greater microbial diversity, both experiments were dominated by the phylum *Proteobacteria*, in particular by the classes *Gamma-proteobacteria* and *Beta-proteobacteria* (Fig 3.14). This similarity causes column samples to cluster on the NMDS (Fig 3.10, cluster). In both experiments, the *Gamma-proteobacteria* were primarily composed by the genus *Psuedomonas* (on average, 69±3.3% and 32±2.7% of the entire microbial population throughout all samples in Experiment 1 and 2, respectively), while *Beta-proteobacteria* was dominated by the *Achromobacter* genus in Experiment 1 (5.1±0.01%) and by *Cupriavidus* (15±2.4%) in Experiment 2 (Appendix B, Fig B3-B6). It is noted that the increased diversity in Experiment 2 was skewed towards taxa with known ability to reduce sulfur compounds, including the *Pseudomonadaceae* family and the genus *Desulfovibrionaceae* (Fig B5 & B6, Appendix B).



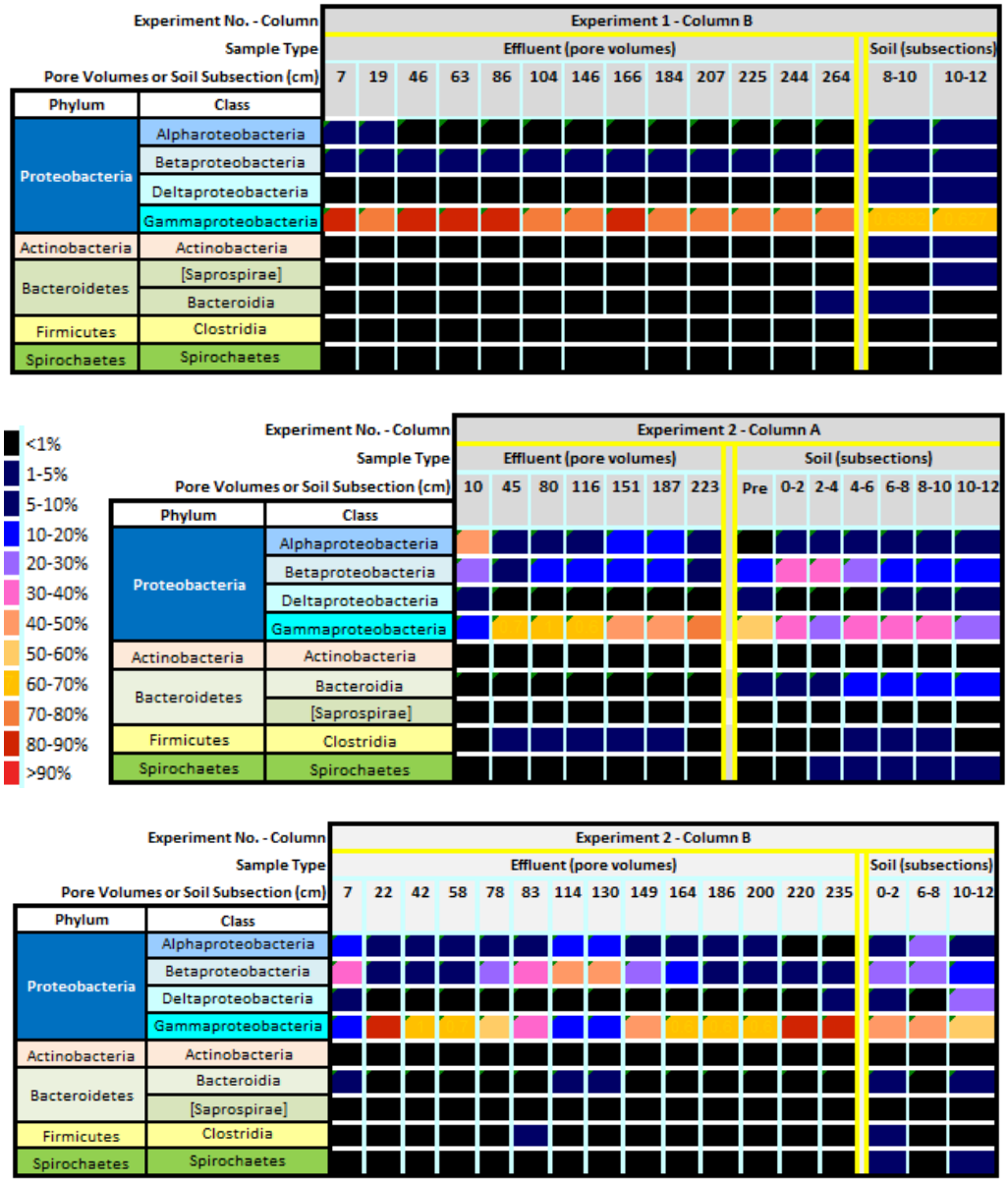


Figure 3.14: Heat maps showing percent values of microbial classes (OTU cutoff: 2%) present over time in the soil and effluent of Column A and B in Experiment 1 (not amended) and Experiment 2 (amended). *Effluent sampling times are given in units of pore volumes, and each soil subsection represents a depth interval in the column, with 0 cm being closest to the bottom of the column, near the inlet. It is noted that not all subsections of Column B soil could be presented due to insufficient DNA concentrations during Illumina sequencing.*

3.5 Discussion of Whole Study

First, it was discovered that heavily contaminated, coal tar DNAPL-occupied soils contained robust microbial communities on the order of 10^7-10^8 gene copies/g. Microorganisms in these communities reflect those often found at hydrocarbon-contaminated sites, and are likely surviving off the aqueous phase coal tar compounds and other organic matter in the soil, which is abundant. However, it is unlikely that these communities will achieve any significant removal of coal tar DNAPL in such heavily contaminated soils, for which reason coal tar sites are known to persist relatively unchanged for many decades. Because of this, large removals of coal tar mass through thermal treatments such as STAR are valuable for removing significant DNAPL mass. It is, however, possible that microbial communities could play a role in degrading remaining groundwater and soil contamination following aggressive, initial site treatment.

Integrating the laboratory and field results, it is understood that in situ STAR not only dried, but also sterilized and removed virtually all organic matter from the soil which it treated. Groundwater imbibing the treated zone carried a modest number of bacteria and organic matter, but continuous flow over time allowed for accumulation of bacteria and organics back onto the treated soil. It was found that organic matter played an important role in final bacterial concentrations attached to the soil. Despite the loading of organic matter onto STAR-treated soil by the groundwater, STAR-treated zones still had much less organic content than the surrounding soil. Nevertheless, treated soil repopulated to concentrations on the order of 10^6 gene copies/g after 27 days, and on the order of 10^7 gene copies/g after 174 days. If necessary, biostimulating amendments may be applied to potentially increase the microbial populations within the treated soil by an order of magnitude within a 50-day period. In general, microbial community structure

present in up-gradient soils was mirrored in the STAR-treated zone, which reveals conservative groundwater transport and non-selective colonization of microbial communities in STAR-treated soil.

Some bacterial depletion was noted in the untreated and transition zones at 27 days after STAR, but was followed by a rapid recovery to concentrations on the order of 10^9 gene copies/g. It was understood that the transition zone would likely experience heat effects, as it comprised the first 0.15m adjacent to the treatment zone. However, bacterial depletion in the untreated zone ~1m above the treatment zone suggests a possible increased range of heating impacts. This is likely due to steam and hot combustion gases rising upwards from the smouldering zone towards the vapor collection system at the surface.

Microbial diversity was also affected by STAR treatment. Prior to STAR, microbial communities in coal tar-contaminated soil displayed a relatively limited diversity of microbial life, likely due to the hostile environment they occupied. Following STAR, although similar microbial classes dominated the soil, there was a greater diversity of genera present. Biostimulation further diversified post-STAR soils, in particular selecting for those microorganisms which could best metabolize the amendments.

3.6 Conclusions

This study used a combined field and laboratory approach to evaluate the propensity of microbial repopulation in STAR-treated and STAR-impacted soils. Overall findings reveal that STAR not only cleans the targeted treatment zone, but also sterilizes it. Further, heat from STAR may influence microorganisms in the surrounding soil. Following STAR, microorganisms were transported via groundwater, resulting in a rapid colonization of STAR treated soils within 1

month, followed by a slow and continued growth thereafter. Negative impacts of STAR heating on microorganisms within soils surrounding the treatment zone were recovered from within 87 days. Biostimulation appears to have benefit but may not be necessary unless a more rapid repopulation is needed to meet specific project objectives.

It is noted that this study does contain various assumptions and limitations. One limitation was the inability to take cores during or immediately after STAR. Instead, soil plating on laboratory STAR-treated site soil was used to determine STAR's capacity to sterilize. A second limitation was the lack of multiple groundwater sampling events throughout the field study. Although column studies suggest relatively consistent groundwater concentrations over time, site conditions may have varied throughout the study. Another limiting factor was the inability to distinguish between coal tar constituents and natural organic compounds in the soil. The appearance of the soil (i.e., exuding black liquid and strong coal tar odor) suggests that coal tar makes up the majority of the hydrocarbon concentration. Nevertheless, none of these are critical to the main conclusions of the study. Long term monitoring of this and other sites treated by in situ STAR will be valuable in confirming the effective microbial recovery of STAR-treated soils.

3.7 References

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4 Conclusions and Recommendations

4.1 Conclusions

The effects of STAR on microorganisms in the treated and surrounding soils was evaluated in this thesis. Bacterial abundance and microbial community structures were examined in both soil and groundwater samples from a field and column study. The field investigation considered microbial concentration and community structure before and after STAR in treated, transition and untreated soil zones. To obtain a better understanding of groundwater microbial transport and colonization of STAR treated zones, a column experiment was performed using site soil and artificial groundwater. A second column experiment was also performed, identical to the first, except that biostimulating amendments were also added to investigate the effects of biostimulation on transport and repopulation.

Results from the field investigation suggest that:

- Significant bacterial concentrations exist in heavily contaminated, coal tar containing soils. These microbial communities are primarily composed of *Proteobacteria*, and include large percentages of genera associated with hydrocarbon degradation, such as *Pseudomonas* and *Acinetobacter*.

- Not only does STAR clean and dry the soil, but it also sterilizes it.

- STAR treated soil is rapidly colonized within the first 27 days following treatment, after which repopulation occurs more slowly.

- If bacterial concentrations in the untreated and transition zones are negatively impacted by STAR-induced heating, they quickly recover to concentrations on the order of 10^9 gene copies/g.

- The magnitude of bacterial concentrations accumulated on the soil is exponentially proportional to the average concentration of organic content within the same soil.

- Bacterial and organic matter abundance in the groundwater on site were much higher than those in the groundwater entering the site, indicating that on site contaminants may act as a food source for bacteria and thus enhance bacterial concentrations on site.

- All soils have an abundance of hydrocarbon degrading genera, suggesting that active biodegradation may be occurring on site both before and after STAR.

Results of the column experiments suggest:

- Groundwater flow acts as an effective transportation vector for microorganisms repopulating STAR-treated zones.

- Microbial community structure is similar in up-gradient and STAR-treated zones.

- Addition of biostimulants increases the total number of bacteria repopulating STAR-treated soil and increases the diversity of dominant genera in microbial communities, particularly selecting for microorganisms associated with sulfur reduction.

- Low uptake of lactate was noted, likely due to the overwhelming presence of organic contaminants, which could render lactate concentrations negligible.

4.2 Recommendations

While much work has been presented on microbial repopulation following STAR, the potential of microorganisms to degrade residual contamination within the post-STAR environment fell largely outside the scope of this study. Understanding this gap in knowledge would help inform the efficacy of natural attenuation used as a polishing step to achieve site remediation goals. It would be beneficial to study biodegradation in both the untreated and the treated zones, since the heavily contaminated groundwater likely re-introduces coal tar compounds to the treated soil.

It is also recommended that 10g soil samples be used for DNA extraction in order to enhance accuracy of bacterial concentrations measured by qPCR.

Finally, there is much future work which may be performed on the Illumina data. Analysis of microbial diversity in the field and column study samples has not yet been performed. Further, relationships between individual OTUs and sample metadata would provide insight into the variables which control community structure in soils at coal tar contaminated sites and in post-STAR soils.

Appendices

Appendix A. Field Study: Tables and Figures

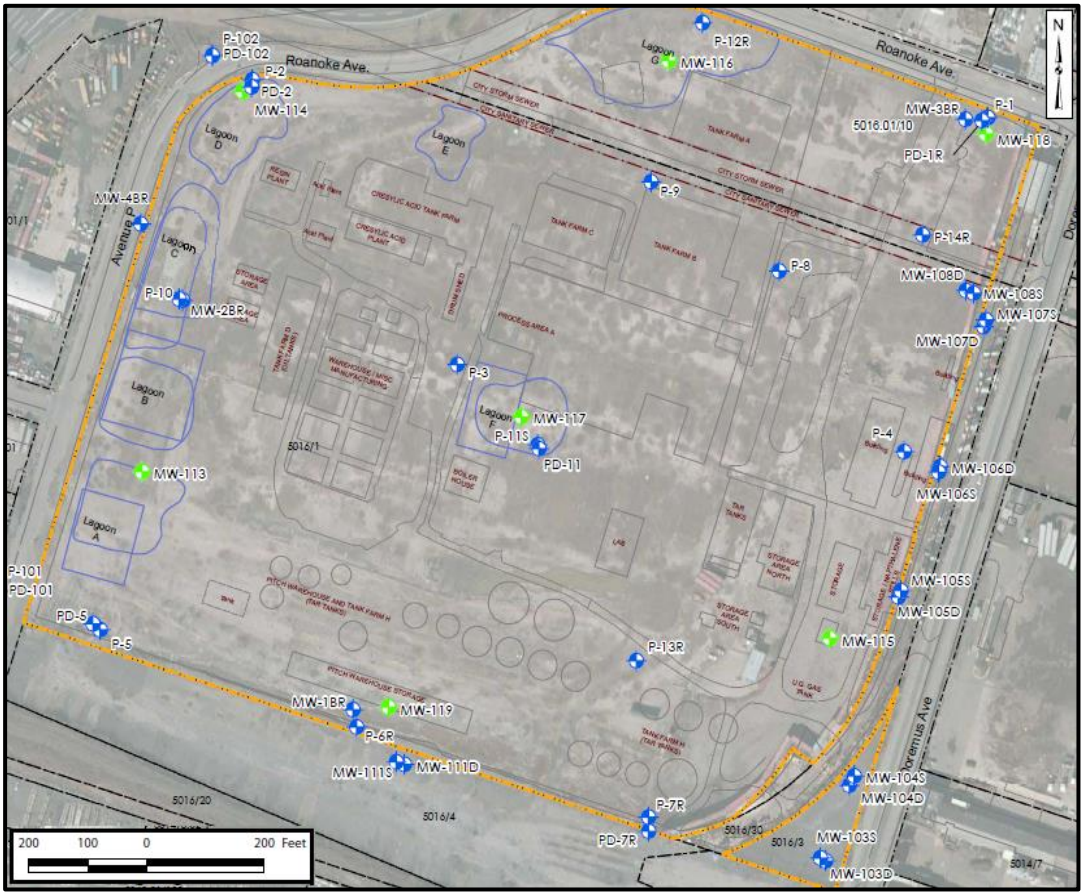

Figure A1: STAR treatment site in New Jersey, USA. *Areas outlined in blue represent historical locations of waste lagoons.*

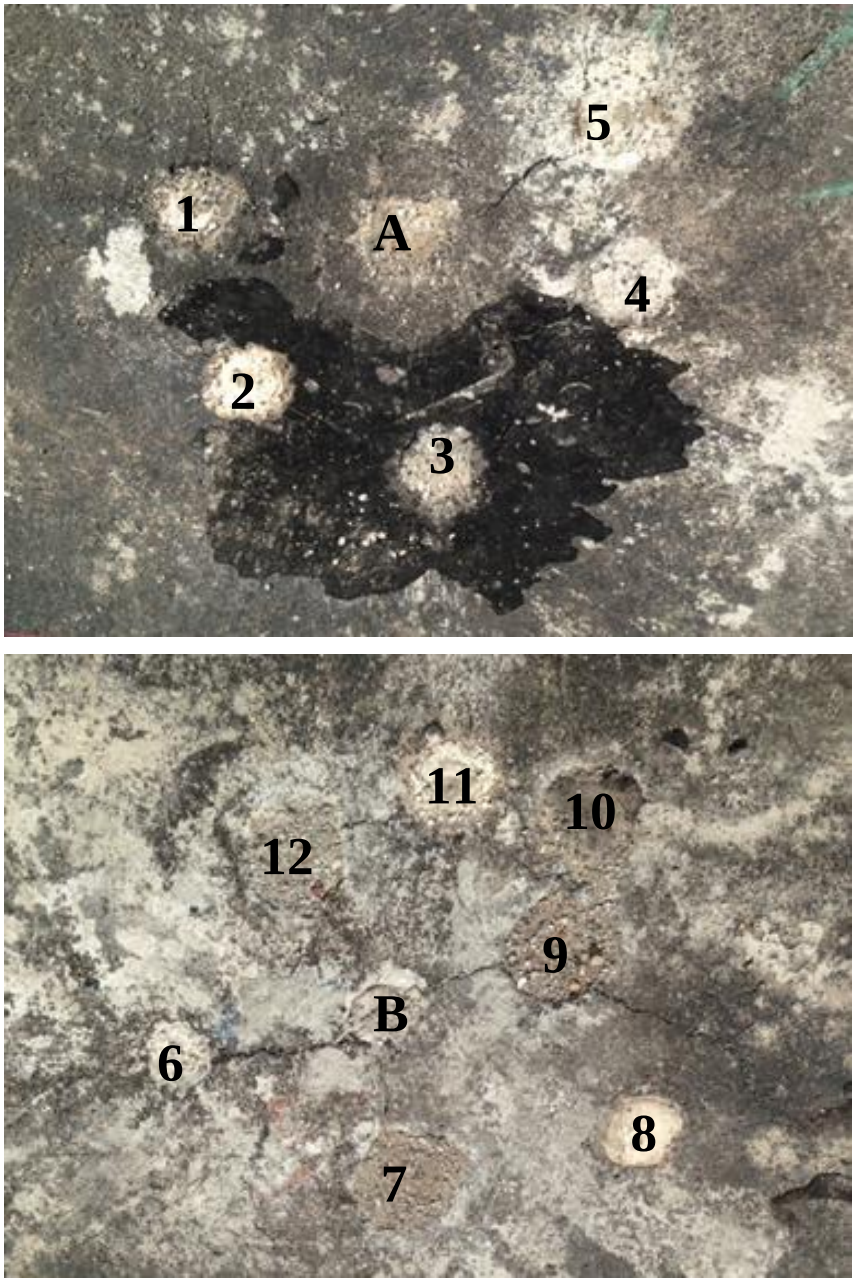

Figure A2: Grouted boreholes (1-12) radially surrounding IP03 (A) and IP06 (B) corresponding to post-STAR core sampling events and former thermocouple locations at each ignition point. *Grouted boreholes are ~5-8 cm in diameter.*

Table A1: STAR Treated Zone Groundwater Parameters During Well Purging

Purge Time (min)	Temperature (^{O}C)	pH	Specific Conductance (mS/cm)	DO (mg/L)	ORP (mV)
0	NA	NA	NA	NA	NA
5	28.29	6.86	4.786	0.67	-174.7
10	28.38	6.82	4.579	0.58	-174.1
15	28.39	6.79	4.6	0.47	-157
20	28.39	6.76	4.365	0.4	-135
25	28.32	6.75	4.295	0.35	-115
30	28.42	6.73	4.321	0.2	-114.5
35	28.34	6.72	4.245	0.21	-112.2
40	28.39	6.72	4.292	0.23	-104.3
45	28.39	6.72	4.134	0.23	-100.4

Table A2: Downgradient Groundwater Parameters During Well Purging

Purge Time (min)	Temperature (^{O}C)	pH	Specific Conductance (mS/cm)	DO (mg/L)	ORP (mV)
0	NA	NA	NA	NA	NA
5	19.47	9.18	5.421	0.06	-125.5
10	19.15	9.1	5.426	0.06	-112.9
15	18.94	9	5.423	0.03	-316.3
20	18.8	8.96	5.428	0.03	-180.7
25	18.6	8.96	5.416	0.04	-136.4
30	18.54	8.93	5.399	0.04	-138.9
35	18.39	8.92	5.39	0.05	-154.5
40	18.29	8.75	5.39	0.04	-252.6
45	18.22	8.31	5.338	0.06	-266.2
50	18.14	8.25	5.252	0.06	-267.1
55	18.08	8.24	5.199	0.06	-270.5

Table A3: Up Gradient Groundwater Parameters During Well Purging

Purge Time (min)	Temperature (^{O}C)	pH	Specific Conductance (mS/cm)	DO (mg/L)	ORP (mV)
0	NA	NA	NA	NA	NA
10	16.4	7.15	7.094	0.23	-153.3
15	16.37	7.15	6.983	0.2	-145.7
20	16.36	7.14	6.926	0.2	-137.1
25	16.38	7.16	6.858	0.19	-133.3
30	16.41	7.15	6.838	0.18	-130.4

Method A1: Gravimetric Analysis for Water and Hydrocarbon Content

In this procedure, triplicate subsamples of approximately 20g were taken from each homogenized sample (i.e., from sample bottles in Step 3 of Fig 3.2). Subsamples were weighed, oven dried for roughly 48 hr, weighed a second time, heated via muffle furnace at 550^{o}C for 6 hr, and weighed a third and final time. Water mass in each subsample was defined as the difference between the second and first measurements. Hydrocarbon mass in each subsample was defined as the difference between the final and second measurements.

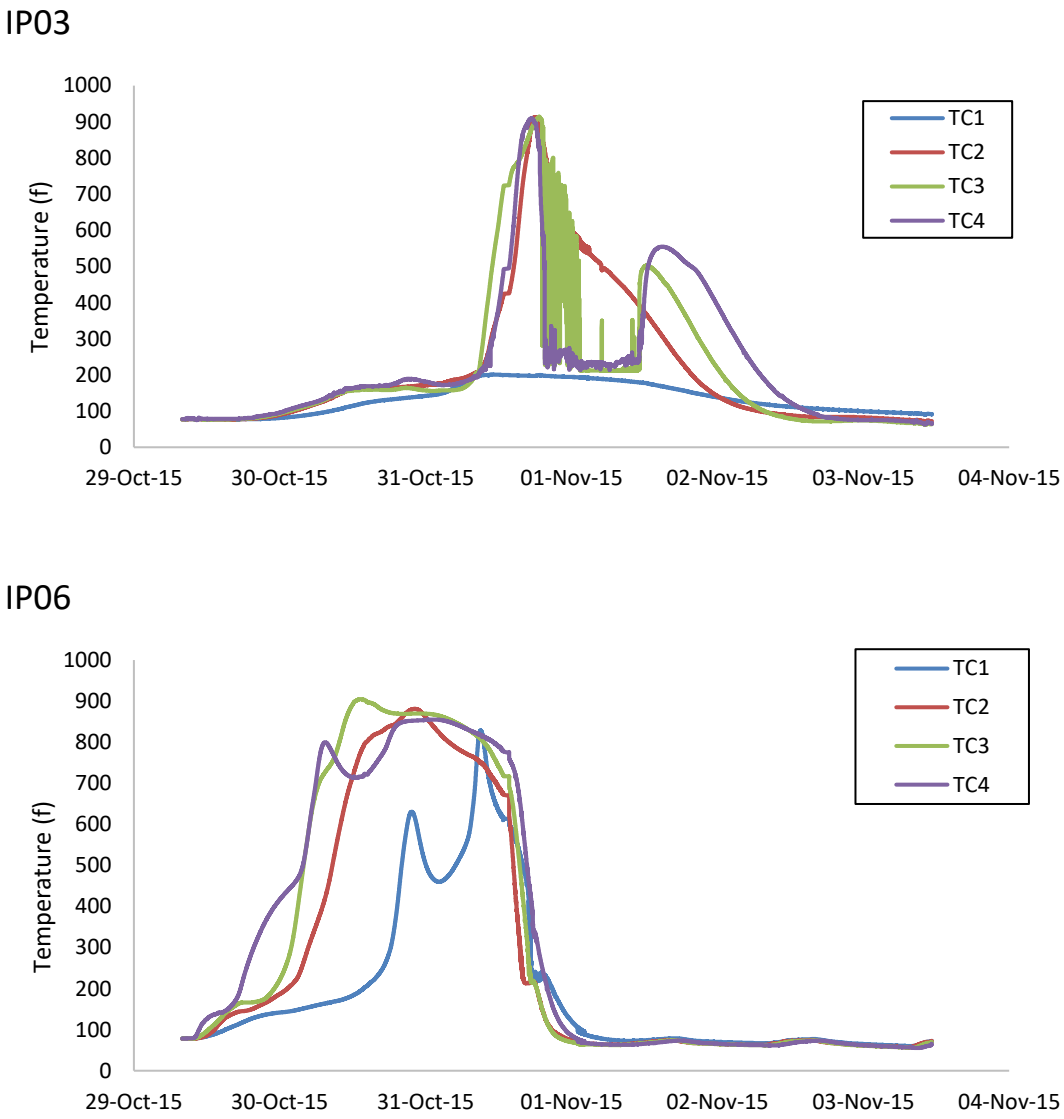

Figure A3: Thermocouple (TC) data for IP03 and IP06. *In both figures, TC4 is closest to the top of the treatment zone and TC1 is at the bottom, with 2 & 3 spaced in between.*

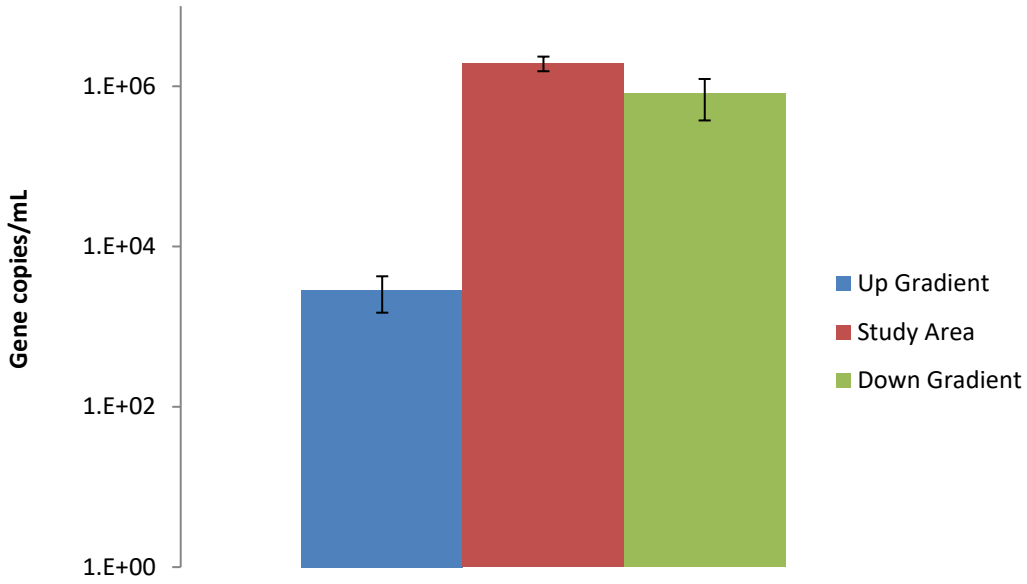

Figure A4: Groundwater bacterial concentrations on site.

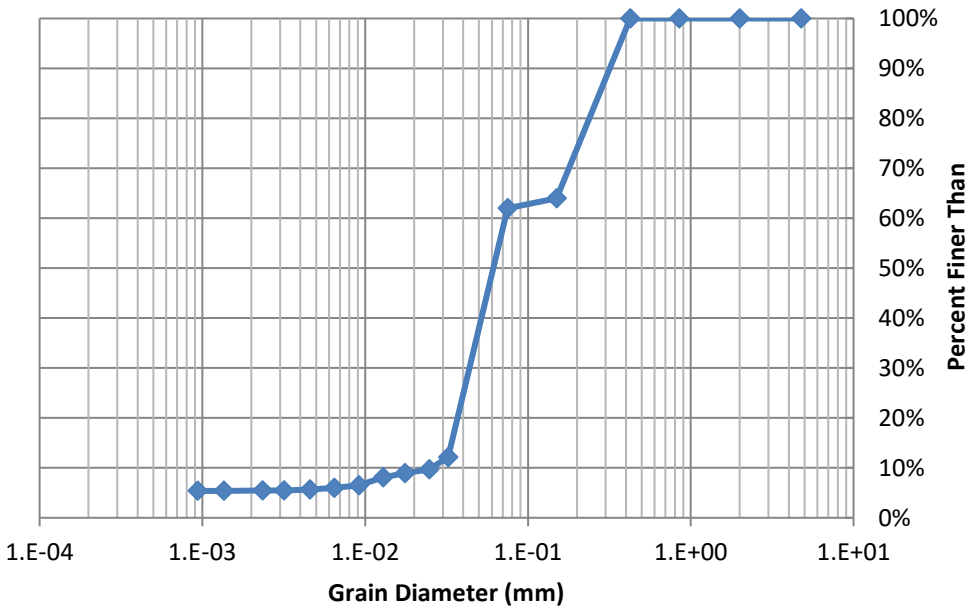

Figure A5: Grain size distribution in the alluvium soil unit.

Table A4: Amounts of gravimetrically determined water content (mL/g), and total, soil, and pore water hydrocarbon (HC) concentrations (mg/kg) in the field samples. *It is noted that total, pore water and soil hydrocarbon concentrations are all given per kg of wet sample, and are therefore directly comparable.* *Triplicate samples were taken from pre-STAR soil and untreated, transition and treated zones in post-STAR soil from IP03 & IP06. It is noted that the concentration of hydrocarbons in the pore water of each sample was calculated using duplicate measurements of the hydrocarbon concentration in the IP06 groundwater 207 days after STAR treatment.*

Days After STAR	*Location*	*Zone*	*Wet Sample Mass (g)*	*Dry Sample Mass (g)*	*Sample Water Content (mL/g)*	*Dry, HC Free Mass (g)*	*Total HC Concentration (mg/kg wet sample)*	*Pore Water HC Concentration (mg/kg wet sample)*	*Soil HC Concentration (mg/kg wet sample)*	*Average total HC concentration (mg/kg)*	*Average Pore Water HC Concentration (mg/kg)*	*Average Soil HC Concentration (mg/kg)*
-72	Pre-STAR A	untreated	25	22	110	*66	28,544	458	28,086	30,473	483	29,989
			21	19	114	*41	34,778	477	34,301			
			34	29	123	*76	28,096	515	27,581			
	Pre-STAR B		30	26	150	*78	15,417	625	14,792	12,753	651	12,102
			35	30	163	*83	12,164	679	11,485			
			21	18	156	*65	10,678	650	10,028			
27	IP06	untreated	29	24	162	24	9,904	678	9,226	9,762	669	9,093
			29	24	164	24	9,940	686	9,254			
			19	16	154	16	9,442	644	8,797			
	IP03		21	17	162	17	7,951	678	7,273	7,926	685	7,240
			28	23	166	23	7,871	691	7,180			
			24	20	164	20	7,955	686	7,269			
	IP06	transition	19	16	172	16	7,146	719	6,427	7,139	723	6,416
			24	20	176	20	7,123	733	6,389			

			21	17	172	17	7,150	717	6,433			
	IP03		26	22	155	21	6,022	647	5,375	5,985	652	5,334
			27	22	158	22	5,881	658	5,223			
			23	19	156	19	6,054	650	5,404			
	IP06	treated	28	23	172	23	1,663	719	944	1,729	713	1,015
			19	16	169	16	1,702	707	995			
			22	18	171	18	1,821	714	1,107			
	IP03		23	19	170	19	815	709	107	805	730	76
			21	17	181	17	762	756	5			
			18	15	173	15	839	724	115			
87	IP06	untreated	25	21	162	21	9,355	674	8,680	9,388	669	8,718
			20	17	160	17	9,318	666	8,652			
			30	25	160	25	9,490	667	8,823			
	IP03		19	17	148	42	14,248	616	13,632	14,186	623	13,562
			22	19	151	44	14,194	629	13,565			
			18	16	149	41	14,114	624	13,490			
	IP06	transition	24	20	156	20	8,172	653	7,519	7,807	712	7,096
			24	20	153	20	8,121	639	7,481			
			16	12	202	12	7,130	843	6,287			
	IP06	Treated	18	15	129	*39	1,888	540	1,348	1,894	550	1,344
			17	15	132	*41	1,862	552	1,310			
			17	15	134	*40	1,931	558	1,372			
	IP03		20	17	140	17	2,167	585	1,582	2,113	591	1,522
			17	14	138	14	2,089	578	1,511			

			22	19	146	19	2,084	610	1,474			
138	IP06	Untreated	25	22	150	22	4,988	627	4,361	4,931	629	4,302
			23	19	152	19	4,848	634	4,214			
			23	20	150	20	4,956	625	4,331			
	IP03		30	26	145	25	11,810	607	11,202	12,044	615	11,429
			27	23	153	23	13,306	638	12,669			
			27	23	144	23	11,016	599	10,417			
	IP06	transition	20	13	333	13	9,795	1,392	8,403	11,592	844	10,748
			24	21	138	20	13,351	575	12,777			
			20	17	135	17	11,630	564	11,066			
	IP03		19	16	141	Sub-Sample Lost				2,708	597	2,111
			17	15	140	15	2,679	585	2,094			
			18	16	146	16	2,737	608	2,129			
	IP06	Treated	26	23	136	23	6,170	568	5,602	5,021	559	4,461
			16	14	128	14	4,287	534	3,753			
			23	20	138	20	4,604	576	4,028			
	IP03		27	23	140	23	3,178	583	2,595	3,203	589	5,702
			25	22	142	21	12,466	591	11,875			
			19	17	142	17	3,228	592	2,636			
174	IP06	untreated	22	19	130	19	5,201	545	4,657	5,094	552	4,542
			17	15	131	15	5,236	545	4,691			
			18	16	135	16	4,843	565	4,278			
	IP03		18	16	139	16	13,711	579	13,131	13,953	587	13,366
			17	14	134	14	13,633	561	13,072			

			25	22	149	21	14,517	622	13,895			
	IP06	transition	22	19	139	19	3,805	581	3,224	3,764	594	3,170
			21	18	144	18	3,713	601	3,112			
			20	17	144	17	3,773	600	3,173			
	IP03		34	29	140	29	5,229	584	4,645	5,383	577	4,806
			28	24	141	24	5,333	591	4,742			
			24	21	133	21	5,586	556	5,031			
	IP06	Treated	20	18	117	18	2,893	488	2,405	2,795	487	2,308
			29	25	114	25	2,678	477	2,201			
			19	17	119	17	2,815	498	2,317			
	IP03		19	16	141	16	1,400	590	810	1,292	613	680
			21	18	148	18	1,242	618	624			
			25	21	151	21	1,236	630	606			

**larger soil subsamples were used to calculate HC content than those used to calculate water content*

Table A5: Compendium of qPCR Results for All Soil Core Samples. *Each post-STAR core includes triplicate 0.25g and single 10g samples of untreated, transition and treated zone soil from both IP06 & IP03. Samples in red have quantitation cycle (Cq) values greater than the reagent blanks and are thus below the qPCR limit of quantification. Total gene copies were determined by multiplying the extracted DNA concentration (gene copies/µL) by dilution and total extracted volumes.*

Days After STAR	Soil Type	Zone	Cq	Gene Copies/µL	Total Gene Copies	Wet Soil Mass (g)	Gene Copies/g Wet Soil	Average Gene Copies/g Wet Soil	Standard Deviation (Gene Copies/g)
-72	Pre-STAR A	Untreated	31.95	3.2E+02	1.6E+05	0.23	7.1E+05	4.4E+05	2.0E+05
			32.29	2.5E+02	1.3E+05	0.23	5.6E+05		
			31.31	5.0E+02	2.5E+05	0.46	5.5E+05		
			32.97	1.6E+02	8.0E+04	0.46	1.8E+05		
			32.73	1.9E+02	9.4E+04	0.25	3.7E+05		
			33.30	1.3E+02	6.4E+04	0.25	2.5E+05		
			17.45	9.2E+05	4.6E+09	14.57	3.1E+08	3.2E+08	NA
			17.43	9.3E+05	4.6E+09	14.57	3.2E+08		
-72	Pre-STAR B	Untreated	24.64	4.7E+04	2.3E+07	0.29	7.9E+07	6.7E+07	2.3E+07
			25.43	2.7E+04	1.4E+07	0.29	4.6E+07		
			24.16	6.5E+04	3.2E+07	0.42	7.7E+07		
			25.03	3.6E+04	1.8E+07	0.42	4.2E+07		
			25.20	3.2E+04	1.6E+07	0.29	5.6E+07		
			24.28	5.9E+04	3.0E+07	0.29	1.0E+08		

			26.06	3.7E+03	1.9E+07	10.01	1.9E+06	1.8E+06	NA
			26.09	3.6E+03	1.8E+07	10.01	1.8E+06		
27	IP06	Untreated	26.77	1.1E+04	5.5E+06	0.19	2.9E+07	4.5E+07	4.9E+07
			26.96	9.6E+03	4.8E+06	0.19	2.5E+07		
			25.28	3.0E+04	1.5E+07	0.28	5.4E+07		
			23.90	7.7E+04	3.9E+07	0.28	1.4E+08		
			27.77	5.5E+03	2.8E+06	0.34	8.1E+06		
			27.03	9.2E+03	4.6E+06	0.34	1.3E+07		
			15.81	2.6E+06	1.3E+10	12.53	1.0E+09	1.1E+09	NA
			15.76	2.7E+06	1.3E+10	12.53	1.1E+09		
27	IP06	Transition	33.12	1.4E+02	7.2E+04	0.25	2.9E+05	2.6E+05	1.4E+05
			32.87	1.7E+02	8.6E+04	0.25	3.4E+05		
			32.64	2.0E+02	1.0E+05	0.31	3.3E+05		
			32.32	2.5E+02	1.2E+05	0.31	4.1E+05		
			33.94	8.3E+01	4.2E+04	0.27	1.5E+05		
			36.14	1.9E+01	9.3E+03	0.27	3.4E+04		
			31.29	1.3E+02	6.6E+05	8.53	7.7E+04	6.4E+04	NA
			31.91	8.9E+01	4.4E+05	8.53	5.2E+04		
27	IP06	Treated	33.40	1.2E+02	6.0E+04	0.29	2.0E+05	2.3E+05	1.0E+05
			33.68	9.9E+01	5.0E+04	0.29	1.7E+05		
			33.35	1.2E+02	6.2E+04	0.24	2.6E+05		
			34.21	6.9E+01	3.4E+04	0.24	1.4E+05		
			32.20	2.7E+02	1.4E+05	0.33	4.1E+05		
			33.56	1.1E+02	5.4E+04	0.33	1.6E+05		
			20.13	9.3E+05	9.3E+07	12.31	7.6E+06	7.0E+06	NA
			20.40	8.0E+05	8.0E+07	12.31	6.5E+06		

27	IP03	Untreated	33.24	1.3E+02	6.7E+04	0.23	2.9E+05	1.2E+05	9.9E+04
			33.96	8.2E+01	4.1E+04	0.23	1.8E+05		
			35.74	2.4E+01	1.2E+04	0.25	4.8E+04		
			34.68	5.0E+01	2.5E+04	0.25	1.0E+05		
			36.17	1.8E+01	9.1E+03	0.22	4.2E+04		
			35.58	2.7E+01	1.4E+04	0.22	6.3E+04		
			25.96	4.0E+03	2.0E+07	8.83	2.2E+06	2.5E+06	NA
			25.59	5.0E+03	2.5E+07	8.83	2.8E+06		
27	IP03	Transition	33.80	9.1E+01	4.6E+04	0.22	2.1E+05	1.5E+05	3.7E+04
			34.12	7.3E+01	3.7E+04	0.22	1.6E+05		
			34.26	6.7E+01	3.3E+04	0.26	1.3E+05		
			34.73	4.8E+01	2.4E+04	0.26	9.5E+04		
			34.35	6.3E+01	3.1E+04	0.22	1.4E+05		
			34.39	6.1E+01	3.0E+04	0.22	1.4E+05		
			17.12	1.1E+06	5.6E+09	7.88	7.1E+08	7.7E+08	NA
			16.91	1.3E+06	6.5E+09	7.88	8.2E+08		
27	IP03	Treated	33.70	9.8E+01	4.9E+04	0.31	1.6E+05	1.8E+05	4.6E+04
			33.65	1.0E+02	5.0E+04	0.31	1.6E+05		
			34.07	7.6E+01	3.8E+04	0.23	1.7E+05		
			34.55	5.5E+01	2.7E+04	0.23	1.2E+05		
			33.35	1.2E+02	6.2E+04	0.24	2.5E+05		
			33.72	9.6E+01	4.8E+04	0.24	2.0E+05		
			24.06	9.3E+04	9.3E+06	8.14	1.1E+06	1.2E+06	NA
			23.98	9.8E+04	9.8E+06	8.14	1.2E+06		
87	IP06	Untreated	20.84	6.2E+05	3.1E+08	0.28	1.1E+09	1.8E+09	8.8E+08
			21.13	5.1E+05	2.5E+08	0.28	9.1E+08		

			19.14	2.0E+06	9.8E+08	0.34	2.9E+09		
			19.13	2.0E+06	9.9E+08	0.34	2.9E+09		
			19.99	1.1E+06	5.5E+08	0.33	1.7E+09		
			20.09	1.0E+06	5.2E+08	0.33	1.6E+09		
			16.15	2.1E+06	1.1E+10	8.30	1.3E+09	1.3E+09	NA
			16.09	2.2E+06	1.1E+10	8.30	1.3E+09		
87	IP06	Transition	24.29	5.9E+04	3.0E+07	0.18	1.6E+08	4.7E+07	5.7E+07
			26.58	1.2E+04	6.2E+06	0.18	3.4E+07		
			26.77	1.1E+04	5.5E+06	0.28	1.9E+07		
			27.47	6.8E+03	3.4E+06	0.28	1.2E+07		
			26.89	1.0E+04	5.0E+06	0.18	2.8E+07		
			26.89	1.0E+04	5.0E+06	0.18	2.8E+07		
			17.61	8.3E+05	4.1E+09	8.10	5.1E+08	5.4E+08	NA
			17.44	9.2E+05	4.6E+09	8.10	5.7E+08		
87	IP06	Treated	33.07	1.5E+02	7.5E+04	0.32	2.4E+05	3.6E+05	3.2E+05
			30.95	6.4E+02	3.2E+05	0.32	1.0E+06		
			33.28	1.3E+02	6.5E+04	0.20	3.2E+05		
			34.34	6.3E+01	3.1E+04	0.20	1.6E+05		
			33.47	1.1E+02	5.7E+04	0.25	2.3E+05		
			33.73	9.6E+01	4.8E+04	0.25	1.9E+05		
			20.50	7.5E+05	7.5E+07	9.85	7.6E+06	7.4E+06	NA
			20.59	7.1E+05	7.1E+07	9.85	7.2E+06		
87	IP03	Untreated	34.03	7.8E+01	3.9E+04	0.36	1.1E+05	2.6E+05	1.1E+05
			33.37	1.2E+02	6.1E+04	0.36	1.7E+05		
			32.21	2.7E+02	1.3E+05	0.31	4.3E+05		
			33.00	1.6E+02	7.9E+04	0.31	2.5E+05		

			32.41	2.3E+02	1.2E+05	0.37	3.2E+05		
			32.73	1.9E+02	9.4E+04	0.37	2.6E+05		
			14.97	4.5E+06	2.2E+10	8.62	2.6E+09	2.7E+09	NA
			14.90	4.7E+06	2.3E+10	8.62	2.7E+09		
87	IP03	Transition	Sample Lost During Coring Event						
87	IP03	Treated	33.11	1.5E+02	7.3E+04	0.19	3.9E+05	6.4E+05	5.4E+05
			34.40	6.1E+01	3.0E+04	0.19	1.6E+05		
			33.38	1.2E+02	6.1E+04	0.17	3.6E+05		
			33.76	9.4E+01	4.7E+04	0.17	2.7E+05		
			30.64	7.8E+02	3.9E+05	0.27	1.4E+06		
			30.86	6.7E+02	3.4E+05	0.27	1.2E+06		
			24.37	7.8E+04	7.8E+06	12.18	6.4E+05	6.4E+05	NA
			24.38	7.7E+04	7.7E+06	12.18	6.3E+05		
138	IP06	Untreated	32.00	4.9E+02	2.4E+05	0.21	1.1E+06	2.9E+07	4.3E+07
			32.14	4.5E+02	2.2E+05	0.21	1.0E+06		
			25.13	4.6E+04	2.3E+07	0.32	7.2E+07		
			24.75	6.0E+04	3.0E+07	0.32	9.3E+07		
			31.23	8.1E+02	4.1E+05	0.21	1.9E+06		
			31.63	6.2E+02	3.1E+05	0.21	1.5E+06		
			16.48	1.7E+06	8.5E+09	6.30	1.3E+09	1.4E+09	NA
			16.32	1.9E+06	9.4E+09	6.30	1.5E+09		

138	IP06	Transition	22.39	2.8E+05	1.4E+08	0.20	7.1E+08	3.0E+08	2.2E+08
			23.34	1.5E+05	7.6E+07	0.20	3.8E+08		
			24.35	7.7E+04	3.9E+07	0.18	2.1E+08		
			24.42	7.4E+04	3.7E+07	0.18	2.0E+08		
			24.44	7.3E+04	3.7E+07	0.23	1.6E+08		
			24.47	7.2E+04	3.6E+07	0.23	1.6E+08		
			14.14	7.6E+06	3.8E+10	6.74	5.6E+09	5.7E+09	NA
			14.10	7.8E+06	3.9E+10	6.74	5.8E+09		
138	IP06	Treated	33.97	1.3E+02	6.6E+04	0.20	3.3E+05	2.4E+05	7.4E+04
			34.19	1.1E+02	5.7E+04	0.20	2.8E+05		
			33.88	1.4E+02	7.0E+04	0.31	2.2E+05		
			34.38	1.0E+02	5.1E+04	0.31	1.6E+05		
			33.98	1.3E+02	6.6E+04	0.22	3.0E+05		
			35.04	6.5E+01	3.3E+04	0.22	1.5E+05		
			22.59	2.2E+05	2.2E+07	10.57	2.1E+06	2.4E+06	NA
			22.10	2.9E+05	2.9E+07	10.57	2.8E+06		
138	IP03	Untreated	NA	NA	NA	NA	NA	1.0E+07	7.5E+06
			27.94	7.2E+03	3.6E+06	0.18	2.0E+07		
			30.01	1.8E+03	9.1E+05	0.28	3.3E+06		
			30.25	1.6E+03	7.8E+05	0.28	2.8E+06		
			27.51	9.6E+03	4.8E+06	0.35	1.4E+07		
			27.75	8.1E+03	4.1E+06	0.35	1.2E+07		
			13.96	8.5E+06	4.2E+10	8.49	5.0E+09	4.8E+09	NA
			14.11	7.8E+06	3.9E+10	8.49	4.6E+09		
138	IP03	Transition	31.62	6.3E+02	3.1E+05	0.19	1.7E+06	7.5E+05	7.9E+05
			31.48	6.9E+02	3.4E+05	0.19	1.8E+06		

			33.12	2.3E+02	1.2E+05	0.22	5.2E+05		
			34.28	1.1E+02	5.4E+04	0.22	2.4E+05		
			34.89	7.2E+01	3.6E+04	0.26	1.4E+05		
			35.03	6.6E+01	3.3E+04	0.26	1.3E+05		
			16.38	1.8E+06	9.1E+09	7.75	1.2E+09	1.2E+09	NA
			16.39	1.8E+06	9.0E+09	7.75	1.2E+09		
138	IP03	Treated	33.31	2.0E+02	1.0E+05	0.22	4.7E+05	4.6E+05	1.9E+05
			33.13	2.3E+02	1.2E+05	0.22	5.3E+05		
			33.62	1.7E+02	8.4E+04	0.31	2.7E+05		
			33.63	1.7E+02	8.3E+04	0.31	2.7E+05		
			32.24	4.2E+02	2.1E+05	0.27	7.7E+05		
			33.08	2.4E+02	1.2E+05	0.27	4.4E+05		
			23.89	1.0E+05	1.0E+07	7.96	1.3E+06	1.4E+06	NA
			23.74	1.1E+05	1.1E+07	7.96	1.4E+06		
174	IP06	Untreated	32.08	4.6E+02	2.3E+05	0.29	8.0E+05	9.8E+05	3.6E+05
			32.53	3.4E+02	1.7E+05	0.29	5.9E+05		
			33.03	2.5E+02	1.2E+05	0.22	5.7E+05		
			31.78	5.6E+02	2.8E+05	0.22	1.3E+06		
			32.00	4.9E+02	2.4E+05	0.20	1.2E+06		
			31.88	5.3E+02	2.6E+05	0.20	1.3E+06		
			18.35	5.1E+05	2.6E+09	8.97	2.9E+08	3.0E+08	NA
			18.22	5.6E+05	2.8E+09	8.97	3.1E+08		
174	IP06	Transition	22.88	2.1E+05	1.0E+08	0.36	2.9E+08	4.6E+08	2.2E+08
			22.71	2.3E+05	1.1E+08	0.36	3.2E+08		
			21.98	3.7E+05	1.9E+08	0.27	6.9E+08		
			21.80	4.2E+05	2.1E+08	0.27	7.8E+08		

			22.90	2.0E+05	1.0E+08	0.29	3.5E+08		
			22.86	2.1E+05	1.0E+08	0.29	3.6E+08		
			25.65	4.8E+03	2.4E+07	12.96	1.9E+06	1.9E+06	NA
			25.61	5.0E+03	2.5E+07	12.96	1.9E+06		
174	IP06	Treated	32.01	4.8E+02	2.4E+05	0.27	8.9E+05	6.5E+05	3.1E+05
			32.09	4.6E+02	2.3E+05	0.27	8.5E+05		
			33.42	1.9E+02	9.5E+04	0.33	2.9E+05		
			33.62	1.7E+02	8.3E+04	0.33	2.6E+05		
			32.85	2.8E+02	1.4E+05	0.22	6.5E+05		
			32.25	4.1E+02	2.1E+05	0.22	9.6E+05		
			18.10	3.1E+06	3.1E+08	12.31	2.5E+07	2.3E+07	NA
			18.36	2.6E+06	2.6E+08	12.31	2.1E+07		
174	IP03	Untreated	23.98	9.9E+04	5.0E+07	0.17	2.9E+08	8.9E+07	1.3E+08
			24.41	7.5E+04	3.7E+07	0.17	2.2E+08		
			29.77	2.1E+03	1.1E+06	0.27	4.0E+06		
			30.16	1.7E+03	8.3E+05	0.27	3.1E+06		
			29.52	2.5E+03	1.3E+06	0.17	7.5E+06		
			29.69	2.3E+03	1.1E+06	0.17	6.7E+06		
			16.06	2.2E+06	1.1E+10	10.41	1.1E+09	1.1E+09	NA
			15.96	2.4E+06	1.2E+10	10.41	1.1E+09		
174	IP03	Transition	33.48	1.8E+02	9.2E+04	0.31	3.0E+05	3.2E+05	1.7E+05
			32.31	4.0E+02	2.0E+05	0.31	6.5E+05		
			33.43	1.9E+02	9.5E+04	0.30	3.1E+05		
			34.16	1.2E+02	5.8E+04	0.30	1.9E+05		
			34.15	1.2E+02	5.9E+04	0.35	1.7E+05		
			33.23	2.2E+02	1.1E+05	0.35	3.1E+05		

			16.10	2.2E+06	1.1E+10	11.65	9.3E+08	9.3E+08	NA
			16.12	2.1E+06	1.1E+10	11.65	9.2E+08		
174	IP03	Treated	35.58	4.6E+01	2.3E+04	0.40	5.8E+04	1.5E+06	1.9E+06
			35.55	4.6E+01	2.3E+04	0.40	5.9E+04		
			30.17	1.6E+03	8.2E+05	0.20	4.1E+06		
			30.30	1.5E+03	7.6E+05	0.20	3.8E+06		
			32.08	4.6E+02	2.3E+05	0.37	6.3E+05		
			32.24	4.2E+02	2.1E+05	0.37	5.7E+05		
			Replicate Lost During qPCR Preparation						
			20.06	9.7E+05	9.7E+07	11.60	8.4E+06	NA	NA
NA	NA	NA	33.26	NA					
			32.93						
NA	NA	NA	33.17						
			32.61						
NA	NA	NA	33.77						
			ND*						
NA	NA	NA	33.70						
			32.97						
NA	NA	NA	31.21						
			31.33						

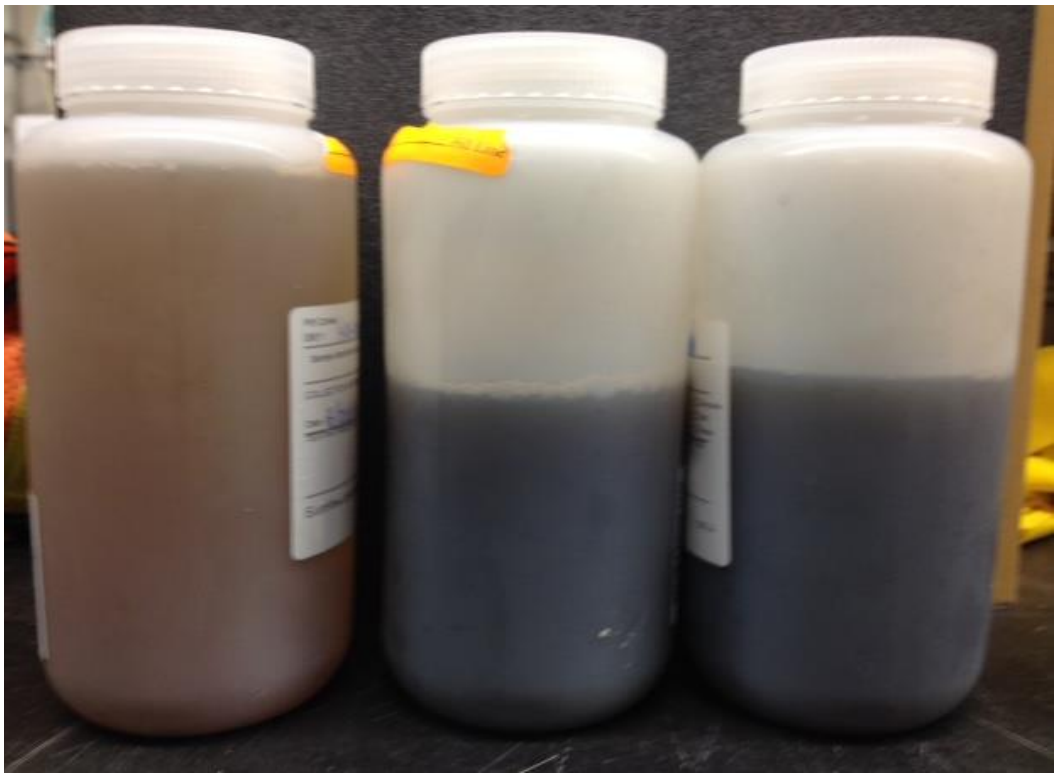

Figure A6: From left to right: up-gradient, downgradient and IP06 treated zone groundwater samples.

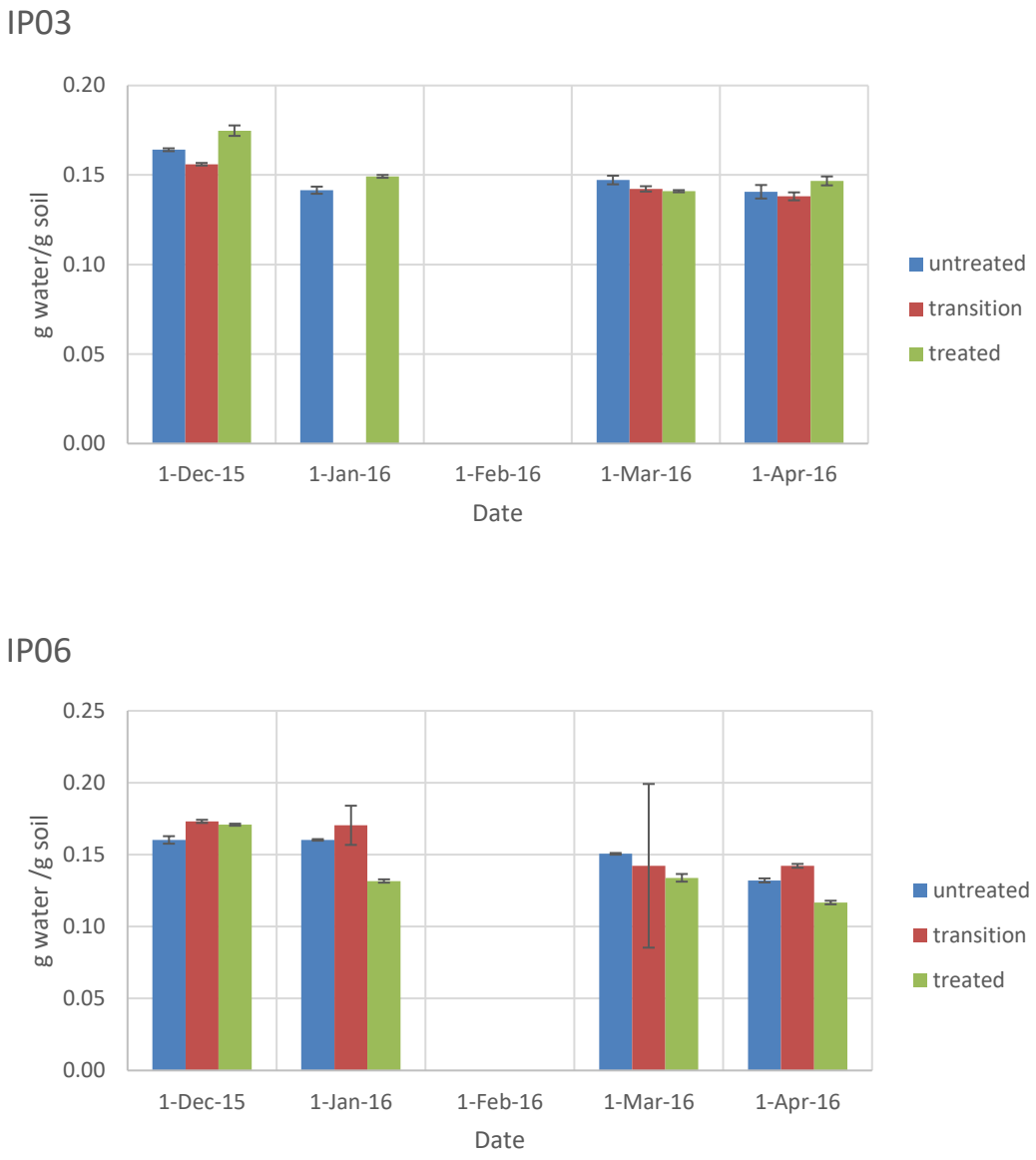

Figure A7: Water content at IP03 & IP06 following STAR treatment.

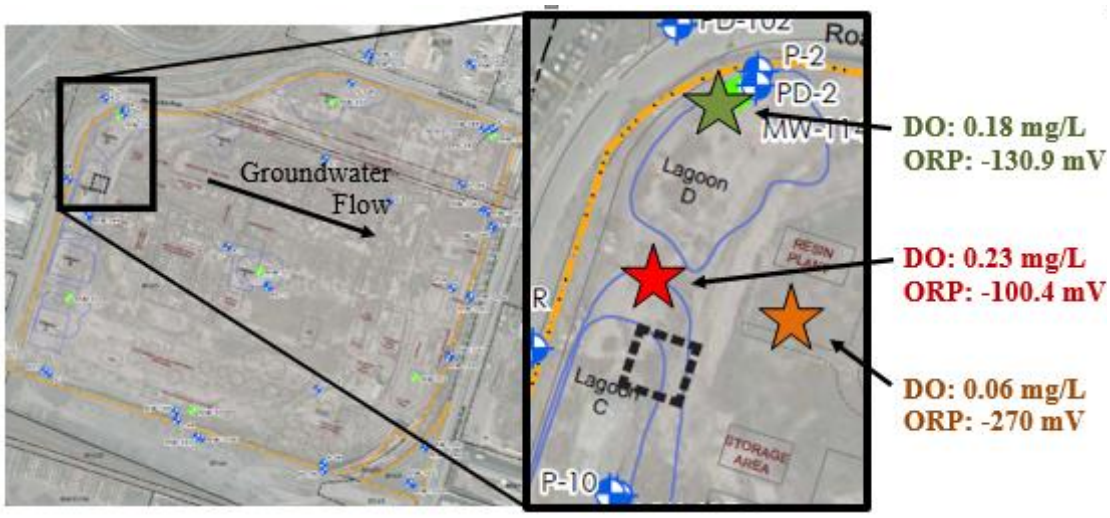

Figure A8: DO & ORP Measurements from: (1) the STAR treated zone (red), (2) an untreated, contaminated zone down-gradient from the STAR treated zone (orange), and (3) up gradient from the STAR treated zone, also in a contaminated, untreated soil (green).

Eq A1 and A2:

$$Gene\ copies_T = C_{Sample} * M_{Sample} \qquad Eq\ A1$$

$$Gene\ copies_{PW} = C_{GW} * M_{Sample} * \frac{M_{PW}}{M_{Sample}} * \rho_W \qquad Eq\ A2$$

Where C_{Sample} is the concentration of bacteria in the sample, M_{Sample} is the mass of the wet sample (g), C_{GW} is the bacteria concentration measured 7-months after STAR in the groundwater (gene copies/mL), assumed constant in all samples, $\frac{M_{PW}}{M_{Sample}}$ is the fraction of pore water mass to wet sample mass (g/g), and ρ_W is the density of water (constant; 1 g/mL).

Ignition Point No.			IP 6													
Sample Type			Pre-Star		Untreated				Transition				Treated			
Days After STAR			-72	-72	27	87	138	174	27	87	138	174	27	87	138	174
Phylum	Class	Genus (or the Lowest Identified Taxa Level)	(A)	(B)												
Proteobacteria	Alphaproteobacteria	Unclassified Caulobacteraceae (OTU4353264)	1.9%	0.0%	0.0%	0.0%	0.0%	14.3%	2.9%	1.7%	0.0%	0.8%	5.6%	14.8%	14.6%	9.3%
		Mycoplana	1.7%	0.0%	0.0%	0.0%	0.1%	0.1%	3.8%	0.0%	0.2%	0.0%	2.7%	10.8%	5.3%	4.9%
		Afipia	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	5.5%	0.0%
		Agrobacterium	0.0%	0.0%	0.0%	0.0%	1.9%	0.0%	0.0%	0.0%	1.5%	0.4%	0.0%	0.0%	0.0%	12.5%
		Azorhizobium	0.0%	0.0%	0.0%	0.2%	1.5%	0.0%	0.0%	2.2%	0.3%	0.2%	0.0%	0.0%	0.0%	0.0%
		Rhodobacter	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	8.9%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
		Unclassified Acetobacteraceae (OTU314477)	0.0%	0.0%	0.0%	0.0%	0.0%	4.9%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
		Unclassified Erythrobacteraceae (OTU916088)	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	3.9%	0.0%	0.0%	0.0%
		Sphingobium	0.0%	0.0%	0.1%	0.0%	1.1%	0.0%	0.0%	0.0%	2.0%	0.1%	0.0%	0.0%	0.0%	0.0%
		Sphingomonas	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	2.2%	1.1%	0.0%	0.0%	0.0%	25.1%	8.3%	0.0%
	Betaproteobacteria	Unclassified Comamonadaceae (OTU241827)	0.0%	0.0%	0.0%	1.3%	1.2%	0.0%	0.0%	18.5%	0.4%	0.2%	1.0%	0.0%	0.0%	0.0%
		Unclassified Comamonadaceae (OTU849642)	1.6%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	2.5%	0.0%	0.0%	11.0%
		Unclassified Comamonadaceae (OTU4417996)	0.0%	0.0%	0.4%	0.9%	0.0%	0.0%	0.0%	0.1%	0.1%	4.6%	0.0%	0.0%	0.0%	0.0%
		Delftia	2.1%	0.1%	0.0%	0.0%	0.0%	8.6%	6.5%	0.2%	0.0%	0.0%	3.5%	2.1%	0.0%	4.7%
		Unclassified Oxalobacteraceae (OTU4295348)	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	2.9%
		Ralstonia	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	2.6%	0.0%	0.0%	0.0%
		Unclassified Rhodocyclaceae (OTU645617)	0.0%	0.0%	2.4%	0.2%	7.0%	0.0%	0.0%	0.0%	1.4%	0.2%	0.0%	0.0%	0.0%	0.0%
		Unclassified Rhodocyclaceae (OTU4325659)	0.0%	0.0%	3.7%	0.3%	1.6%	0.0%	0.0%	0.0%	0.2%	0.1%	0.1%	0.0%	0.0%	0.0%
		Azovibrio	0.0%	0.0%	0.1%	0.4%	12.4%	0.0%	0.0%	3.4%	7.3%	2.4%	0.0%	0.0%	0.0%	8.0%
		Methylibium	4.3%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
		Thauera	0.0%	0.0%	0.0%	0.1%	0.6%	0.0%	0.0%	0.0%	8.4%	0.4%	0.0%	0.0%	0.0%	0.0%
		Uliginosibacterium	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	2.6%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
		Geobacter	0.0%	1.1%	3.5%	6.6%	8.1%	2.0%	0.0%	2.6%	3.1%	1.1%	0.0%	0.0%	0.0%	0.0%
		Myxococcales	7.6%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
		Unclassified 0319-6G20 (OTU3454566)	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	5.9%	0.0%	0.0%	0.0%
		Unclassified Syntrophobacteraceae (OTU1)	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	6.5%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
	Gammaproteobacteria	Marinobacter	0.0%	0.0%	3.2%	0.7%	1.0%	0.0%	0.0%	0.0%	2.1%	2.3%	0.0%	0.0%	0.0%	0.0%
		Unclassified Enterobacteriaceae (OTU4469420)	0.0%	0.1%	0.1%	0.1%	0.0%	11.1%	19.3%	1.7%	0.0%	0.0%	7.6%	31.8%	21.7%	8.0%
		Unclassified Enterobacteriaceae (OTU4300950)	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	3.7%	0.0%	0.0%	0.0%
		Trabulsiella	0.0%	0.0%	1.6%	2.9%	0.2%	0.0%	0.1%	1.7%	0.0%	0.0%	0.1%	0.1%	0.1%	0.1%
		Acinetobacter	25.3%	0.0%	0.3%	0.0%	0.1%	10.8%	13.0%	3.0%	0.0%	0.1%	2.2%	9.1%	32.9%	12.4%
		Pseudomonas	2.1%	94.0%	46.7%	55.5%	10.7%	0.0%	4.3%	22.5%	20.7%	42.2%	0.0%	0.0%	0.0%	5.5%
		Unclassified Xanthomonadaceae (OTU4345543)	0.0%	0.0%	2.1%	0.9%	6.2%	0.0%	0.0%	7.5%	10.0%	4.1%	0.0%	0.0%	0.0%	0.0%
		Unclassified Xanthomonadaceae (OTU4416707)	0.0%	0.0%	0.9%	0.3%	3.0%	0.0%	1.6%	3.7%	5.3%	2.2%	0.0%	0.0%	0.0%	0.0%
		Unclassified Pseudomonadaceae (OTU4434147)	0.0%	1.1%	6.5%	2.7%	2.0%	0.0%	0.0%	2.7%	1.3%	2.0%	0.0%	0.0%	0.0%	0.0%
		Sinobacteraceae	2.8%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%

Actinobacteria	Actinobacteria	Unclassified Actinomycetales (OTU458474)	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	6.1%	0.0%
		Unclassified Actinomycetales (OTU103288)	0.0%	0.0%	0.0%	0.0%	0.0%	5.9%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
		Actinomyces	0.0%	0.0%	0.2%	0.1%	0.2%	3.7%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%
		Brevibacterium	0.0%	0.0%	0.0%	0.0%	0.0%	11.4%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
		Microbacterium	0.0%	0.0%	0.0%	0.0%	0.0%	3.2%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
		Unclassified Nocardioidaceae (OTU324217)	0.0%	0.0%	0.0%	0.0%	0.0%	0.2%	0.0%	0.0%	0.0%	0.0%	3.8%	0.0%	0.0%	0.0%
		Propionibacterium	0.0%	0.0%	0.0%	0.0%	0.1%	0.4%	2.6%	0.0%	0.0%	0.0%	2.2%	0.0%	1.2%	0.0%
Bacteroidetes	Bacteroidia	Unclassified Porphyromonadaceae (OTU4475604)	0.0%	0.0%	0.1%	0.1%	0.1%	0.0%	0.0%	2.2%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%
		Paludibacter	0.0%	0.0%	0.0%	0.0%	4.9%	0.0%	0.0%	0.0%	2.5%	0.1%	0.0%	0.0%	0.0%	0.0%
	Flavobacteriia	Unclassified [Weeksellaceae] (OTU4437011)	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	3.3%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Chloroflexi	Anaerolineae	Unclassified Anaerolinaceae (OTU6)	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	8.9%
		Unclassified Anaerolinaceae (OTU4313874)	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	6.2%
		T78	0.0%	0.0%	0.5%	0.6%	0.3%	0.0%	6.5%	6.8%	0.1%	0.5%	0.0%	0.0%	0.0%	0.0%
	Dehalococcoidetes	Dehalococcoides	6.6%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Firmicutes	Bacilli	Aerococcaceae	19.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
	Clostridia	Unclassified Clostridiales (OTU26)	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
		Thermoanaerobacterium	0.0%	0.0%	2.1%	1.0%	0.5%	0.0%	0.0%	0.0%	0.1%	0.1%	0.0%	0.0%	0.0%	0.0%
		Peptococcaceae	2.3%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
		Unclassified Ruminococcaceae (OTU0)	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	4.2%	0.0%
Spirochaetes	Spirochaetes	Treponema	0.0%	0.0%	0.4%	0.9%	0.4%	0.0%	0.0%	5.6%	0.2%	0.7%	0.8%	0.0%	0.0%	0.0%
Nitrospirae	Nitrospira	GOUTA19	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
		Unclassified Bacteria (OTU4481422)	0.0%	0.0%	0.0%	0.0%	0.0%	7.3%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
		Unclassified (OTU173)	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	24.3%	0.0%	0.0%	0.0%
		Unclassified (OTU13)	0.4%	0.0%	0.0%	0.0%	0.0%	4.8%	0.0%	0.8%	0.0%	0.0%	0.0%	0.0%	0.0%	1.4%
		Unclassified (OTU115)	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	5.0%	0.0%	0.0%	0.0%	0.0%
Chlorobi	Ignavibacteriales	[Melioribacteraceae]	3.8%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Sum of Minor Genera (<2%)			22.1%	3.5%	24.8%	24.0%	34.5%	11.2%	16.0%	12.0%	32.4%	29.7%	27.4%	6.0%	0.0%	4.3%

Figure A9: Percentages of the microbial population made up by each genus (or lowest resolvable taxonomic unit) in pre-STAR and post-STAR Untreated, Transition and Treated soil at IP06. *Both Pre-STAR samples were taken at the same time (72 days before STAR) and are therefore also classified as (A) and (B), in reference to pre-STAR coring locations A and B. Genera present at greater than 2% in at least 1 sample are shown individually, while those below 2% across all samples are presented as a summed percentage.*

Ignition Point No.			IP 3										
Sample Type			Pre-Star		Untreated			Transition			Treated			
Days After STAR			-72	-72	27	138	174	27	138	174	27	87	138	174
Phylum	Class	Genus (or the Lowest Identified Taxa Level	(A)	(B)										
Proteobacteria	Alphaproteobacteria	Unclassified BD7-3 (OTU902489)	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	2.1%	0.0%	0.0%	0.0%
		Unclassified Caulobacteraceae (OTU4353264)	1.9%	0.0%	0.0%	0.2%	0.0%	2.9%	1.2%	2.4%	9.4%	4.7%	0.0%	3.0%
		Bradyrhizobium	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	2.9%	0.2%	0.0%
		Paracoccus	0.0%	0.0%	0.0%	0.0%	0.6%	0.0%	0.0%	0.0%	0.0%	2.8%	0.0%	0.0%
		Sphingomonas	0.0%	0.0%	0.0%	2.6%	0.0%	28.9%	0.0%	1.5%	3.5%	5.3%	0.7%	0.0%
	Betaproteobacteria	Unclassified Comamonadaceae (OTU241827)	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	10.8%
		Unclassified Comamonadaceae (OTU849642)	1.6%	0.0%	6.8%	4.6%	0.1%	0.0%	2.3%	0.4%	0.0%	4.1%	0.0%	0.5%
		Unclassified Comamonadaceae (OTU243487)	0.0%	0.0%	0.0%	0.0%	3.7%	0.0%	0.3%	0.0%	0.0%	0.2%	0.0%	0.0%
		Unclassified Comamonadaceae (OTU4481234)	0.0%	0.0%	0.0%	0.0%	2.6%	0.0%	0.3%	2.9%	0.0%	0.0%	2.3%	3.1%
		Unclassified Comamonadaceae (OTU3456231)	0.0%	0.0%	0.0%	2.2%	0.4%	0.0%	2.3%	1.2%	0.0%	0.0%	0.1%	0.2%
		Unclassified Comamonadaceae (OTU773739)	0.0%	0.0%	0.0%	2.0%	0.5%	0.0%	2.0%	1.0%	0.0%	0.0%	0.1%	0.2%
		Delftia	2.1%	0.1%	4.7%	0.4%	0.0%	25.5%	2.4%	0.0%	0.0%	2.1%	0.3%	11.1%
		Unclassified Oxalobacteraceae (OTU4295348)	0.0%	0.0%	10.5%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	2.6%
		Ralstonia	0.0%	0.0%	3.1%	0.0%	0.0%	4.9%	0.2%	0.0%	0.0%	1.1%	0.1%	0.0%
		Thiobacillus	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	3.8%	0.0%
		Unclassified Rhodocyclaceae (OTU645617)	0.0%	0.0%	0.0%	6.9%	0.1%	0.0%	4.2%	0.0%	0.0%	0.0%	0.0%	3.2%
		Unclassified Rhodocyclaceae (OTU4325659)	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	4.5%	0.7%	0.0%	0.0%	0.0%	0.0%
		Methylibium	4.3%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
		Thauera	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	12.3%	0.0%
	Deltaproteobacteria	Unclassified Desulfobulbaceae (OTU694505)	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	2.9%	0.0%
		Geobacter	0.0%	1.1%	0.0%	0.0%	1.1%	0.0%	5.2%	28.5%	0.0%	4.3%	0.0%	0.6%
		Myxococcales	7.6%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
	Gammaproteobacteria	Marinobacter	0.0%	0.0%	0.0%	1.7%	0.6%	0.0%	0.0%	2.2%	0.0%	0.0%	2.1%	0.0%
		Unclassified Enterobacteriaceae (OTU4469420)	0.0%	0.1%	14.4%	4.2%	0.2%	0.0%	2.3%	1.3%	8.1%	0.0%	2.0%	14.9%
		Unclassified Enterobacteriaceae (OTU4475936)	1.4%	0.0%	0.2%	7.6%	0.3%	0.0%	1.9%	0.0%	0.2%	0.0%	0.0%	0.2%
		Trabulsiella	0.0%	0.0%	0.1%	2.4%	1.2%	0.0%	8.4%	0.0%	0.0%	0.0%	0.0%	0.1%
		Acinetobacter	25.3%	0.0%	13.5%	2.1%	0.1%	23.7%	3.9%	3.1%	56.1%	6.2%	3.7%	13.4%
		Pseudomonas	2.1%	94.0%	0.0%	41.5%	38.3%	6.9%	37.0%	4.1%	17.4%	11.6%	24.4%	6.3%
		Unclassified Xanthomonadaceae (OTU4345543)	0.0%	0.0%	0.0%	3.6%	17.0%	0.0%	0.3%	0.0%	0.0%	0.0%	0.7%	0.0%
		Unclassified Xanthomonadaceae (OTU4416707)	0.0%	0.0%	0.0%	2.1%	8.8%	0.0%	0.2%	0.0%	0.0%	0.0%	0.4%	0.0%
		Unclassified Pseudomonadaceae (OTU4434147)	0.0%	1.1%	0.0%	4.9%	1.3%	0.0%	0.9%	0.0%	0.0%	0.0%	1.5%	1.3%
		Sinobacteraceae	2.8%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
		Unclassified Pseudomonadaceae (OTU4376926)	0.0%	0.0%	0.0%	2.3%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Actinobacteria	Actinobacteria	Corynebacterium	0.0%	0.0%	0.0%	0.0%	0.2%	0.0%	3.6%	1.5%	0.0%	13.1%	0.4%	0.5%
		Rothia	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.2%	0.0%	0.0%	4.1%	0.0%	0.0%
		Microlunatus	0.0%	0.0%	3.6%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
		Propionibacterium	0.0%	0.0%	5.9%	0.0%	0.1%	0.0%	0.4%	6.1%	0.0%	8.6%	2.9%	1.0%

Bacteroidetes	[Saprospirae]	Unclassified Saprospiraceae (OTU792729)	0.0%	0.0%	7.8%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
	Bacteroidia	Unclassified Porphyromonadaceae (OTU218650)	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	4.1%
		Chryseobacterium	0.0%	0.0%	0.0%	0.0%	0.0%	2.4%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
		Flavobacterium	0.0%	0.0%	15.5%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Chloroflexi	Anaerolineae	Unclassified Anaerolinaceae (OTU6)	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%
		Unclassified Anaerolinaceae (OTU4313874)	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	8.1%	0.0%
		SHD-231	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	2.9%	0.0%
		T78	0.0%	0.0%	0.0%	0.0%	1.6%	0.0%	0.8%	2.7%	0.0%	0.0%	5.2%	0.0%
	Dehalococcoidetes	Dehalococcoides	6.6%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Firmicutes	Bacilli	Staphylococcus	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	5.2%	0.0%	0.0%
		Aerococcaceae	19.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
		Streptococcus	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.4%	0.0%	0.0%	2.4%	1.2%	0.0%
	Clostridia	Unclassified Clostridiales (OTU26)	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	1.6%	3.6%	0.0%	0.0%	0.0%	0.0%
		Unclassified Clostridiales (OTU4425663)	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	2.4%	0.0%	0.0%
		Anaerococcus	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	4.3%	0.0%	0.0%
		Tissierella_Soehngenia	0.0%	0.1%	0.0%	0.0%	0.1%	0.0%	0.0%	2.9%	0.0%	0.0%	0.0%	4.6%
		Thermoanaerobacterium	0.0%	0.0%	0.0%	0.0%	0.3%	0.0%	0.0%	8.1%	0.0%	0.0%	0.0%	1.1%
		Desulfosporosinus	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	3.0%	0.0%	0.0%	0.0%	3.0%
		Peptococcaceae	2.3%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Tenericutes	Mollicutes	Acholeplasma	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	3.1%
Cyanobacteria	Chloroplast	Unclassified Streptophyta (OTU0)	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	3.8%	0.0%	0.0%
Lentisphaerae	[Lentisphaeria]	Unclassified Victivallaceae (OTU4429963)	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.7%	3.4%	0.0%	0.0%	0.0%	0.0%
Nitrospirae	Nitrospira	GOUTA19	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	3.3%	0.0%
		Unclassified (OTU13)	0.4%	0.0%	0.0%	0.0%	0.1%	0.0%	0.3%	1.2%	0.0%	5.7%	0.0%	0.0%
		Unclassified (OTU55)	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	2.4%	0.0%	0.0%
Chlorobi	Ignavibacteriales	[Melioribacteraceae]	3.8%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Sum of Minor Genera (<2%)			22.5%	3.2%	13.7%	8.6%	20.2%	4.7%	12.2%	18.1%	3.2%	2.8%	18.5%	11.2%

Figure A10: Percentage of the microbial population made up by each genus (or lowest resolvable taxonomic unit) in pre-STAR and post-STAR Untreated, Transition and Treated soil at IP03. *Both Pre-STAR samples were taken at the same time (72 days before STAR) and are therefore also classified as (A) and (B), in reference to pre-STAR coring locations A and B. Genera present at greater than 2% in at least 1 sample are shown individually, while those below 2% across all samples are presented as a summed percentage*

Appendix B. Column Study: Tables and Figures

Table B1: Ion Concentrations in Various Artificial Groundwater Recipes

*Ion**	*This Study*	*Middelorp et al.*	*Peters & Gauthier*	*Idaho National Laboratory*	*Qafoku et al.*	*Mailloux & Fuller*	*Husman et al.*
NH^{4+}	0.93	1.00	0.00	0.00	0.00	0.00	0.00
Cl^{-}	3.19	3.26	0.63	3.50	0.16	0.65	0.10
Mg^{2+}	0.05	0.05	0.24	0.91	0.08	0.41	0.14
Mn^{2+}	0.02	0.02	0.00	0.00	0.00	0.00	0.00
Ca^{2+}	1.00	1.00	0.67	1.75	2.77	0.59	0.07
Na^{+}	2.14	4.02	0.43	1.14	0.34	1.40	0.25
PO_4^{2-}	0.60	0.60	0.00	0.00	0.00	0.00	0.00
K^{+}	0.15	0.15	0.20	0.10	1.20	0.11	0.06
SO_4^{2-}	0.06	0.06	0.39	0.91	0.17	0.73	0.21
NO^{3-}	0.00	0.00	0.62	0.08	0.00	0.00	0.06
HCO_3^{-}	1.00	1.00	0.43	1.16	0.00	1.40	0.14

*For Experiment 2, the concentration of SO_4^{2-} was increased by an order of magnitude to 0.6 M.

Table B2: Biostimulation Column Experiments in The Literature

Paper	*Purpose of Experiment*	*Lactate (mg/L)*	*Other Carbon Source (mg/L)*	*SO4 (mg/L)*	*Residence Time (min)**
Tokunaga et al, 2003	Biostimulation to enhance chromium (IV) degradation	800 & 4000	Tryptic Soy Broth (800 & 4,000)	0.00	NA
Davis et al, 2004	Biostimulation to enhance RDX degradation	0	acetate, ethanol, soluble starch, and acetate (500 mg/L)	0.00	525.66
Tront et al, 2008	Measured microbial activity as a function of lactate concentration provided	450.4 - 3693.28	0	0.29	1213.33
Benchiekh -Latmani et al	Measured gene regulation during Cr(IV) reduction	2702.4	0	0.29	NA
Mosher et al, 2012	Community succession dynamics during amendment procedure	2702.4	0	14.41	2826.09
Joner et al, 2002	Simulation of rhizosphere nutritional requirements	0	Root Exudates at unknown concentration	0.00	NA
Anid et al, 1992	Biostimulation to degrade BTEX	0.00	BTEX 200mg/L plus trace (.03-.08%) organics naturally occurring in soil	0.10	794.21
Abdelouas et al, 1998	Biostimulation of UV(1V) degraders	0.00	Ethanol at unknown concentration	9.56	NA

Handley et al, 2012	Biostimulation using acetate	0.00	Acetate at 590.437 mg/L	960.56	NA
Ebihara et al, 2002	Biostimulation to degrade PAHs	0.00	Acetate at 43 mg/L pulsed for 1 hr every 4 hrs	2.78	263.93
Peyton, 1996	pulsed vs continuous donor injection	0.00	Acetate at 2000 mg/L for 0.5 hr every 12 hrs	0.00	189.71 - 569.13
Williams et al, 2005	Geophysical imaging of biostimulation	252.22	0.00	377.50	817.86
Ikuma & Gunsch, 2013	Biostimulation in column to degrade toluene	0.00	Glucose at 1000 mg/L either continuous or pulsed every 5 days	NA	1285.20
Nelson et al, 2012	Pulsed vs continuous biostimulation	0.00	Molasses at 10% (140,000 mg/L) pulsed in every 27 days for 3 days	NA	NA
Macur et al, 2001	Biostimulation effects on mobilization of As	90.08	90.08 mg/L Glucose	192.11	56853.00

Carry et al, 2014	Biostimulation to reduce high levels of Nitrate	0.00	18.02 mg of Glucose every 3 days as 1801.55 mg/L	0.10	NA

*Residence time refers to the duration of time after which one PV is passed through the column

Table B3: Parameters Measured in Artificial Groundwater Reservoir

Experiment	Temperature (^{O}C)	DO (mg/L)	pH
1 (not amended)	21.11 ± 0.07	0.009 ± 0.008	7.36 ± 0.04
2 (amended)	21.31 ± 0.89	0.006 ± 0.006	7.17 ± 0.05

* Where all values are averaged over time ±standard error

Eq. B1 – B2

$$\frac{C(x,t)}{C_0} = 0.5[\exp(A_1)\, erfc(A_2) + \exp(B_1)\, erfc(B_2)] \qquad Eq\ B1$$

$$A_1 = \frac{x}{2D^*}\left(v^* - \sqrt{v^{*2} + 4D^*\lambda^*}\right) \qquad Eq\ B2$$

$$A_2 = \frac{x - t\sqrt{v^{*2} + 4D^*\lambda^*}}{\sqrt{4D^*\lambda^*}} \qquad Eq\ B3$$

$$B_1 = \frac{x}{2D^*}\left(v^* + \sqrt{v^{*2} + 4D^*\lambda^*}\right) \qquad Eq\ B4$$

$$B_2 = \frac{x + t\sqrt{v^{*2} + 4D^*\lambda^*}}{\sqrt{4D^*\lambda^*}} \qquad Eq\ B5$$

$$D = \alpha v \qquad Eq\ B6$$

Where $\frac{C(x,t)}{C_0}$ is the normalized concentration of the compound of interest at a specific time and distance from the source, relative to the original concentration at the source, x is distance from the source, t is time, D^* is the dispersion coefficient divided by the retardation factor $\left(D^* = \frac{D}{R}\right)$ v^* is the porewater velocity divided by the retardation factor, and λ^* is the decay function, divided by the retardation factor. Because a conservative tracer was used, $\lambda^* = 0$, and there is no retardation ($D^* \equiv D$, etc). Thus, the only unknown in the equation is D, which can be further broken down to solve for the dispersivity (α) of the soil.

Table B4: Bacterial Concentrations in Depth Intervals of Column A Soil After Experiment 1 (not amended) As Determined by 0.25g Samples. *The cycle of quantitation (Cq) is the main output of qPCR and is to calculate the gene copies/µL of extracted DNA. Gene copy/µL values were dilution corrected, multiplied by the total volume of extracted DNA and divided by the wet soil mass to obtain the bacterial concentration in each sample.*

Distance from Influent Inlet (cm)	*Cq*	*Gene Copies/µL*	*Wet Soil Mass (g)*	*Bacterial Concentration (gene copies/g)*	*Average Bacterial Concentration (gene copies/g)*	*Standard Deviation (gene copies/g)*
0-2	19.26	7.4E+05	0.26	1.6E+09	1.6E+09	3.9E+08
	18.87	9.4E+05	0.26	2.1E+09		
	19.38	6.8E+05	0.31	1.3E+09		
	19.29	7.2E+05	0.31	1.3E+09		
2-4	22.91	7.1E+04	0.22	2.1E+08	5.8E+08	4.8E+08
	23.12	6.2E+04	0.22	1.8E+08		
	20.91	2.6E+05	0.22	7.2E+08		
	20.13	4.2E+05	0.22	1.2E+09		
4-6	21.13	2.2E+05	0.26	5.1E+08	4.4E+08	7.9E+07
	21.53	1.7E+05	0.26	3.9E+08		
	21.07	2.3E+05	0.39	3.5E+08		
	20.52	3.3E+05	0.39	5.0E+08		
6-8	21.19	2.1E+05	0.30	5.3E+08	2.8E+08	3.1E+08
	21.10	2.3E+05	0.30	5.6E+08		
	27.85	3.0E+03	0.30	7.6E+06		
	27.75	3.2E+03	0.30	8.0E+06		
8-10	20.48	3.4E+05	0.27	7.6E+08	4.8E+08	3.1E+08
	20.53	3.3E+05	0.27	7.3E+08		
	22.38	1.0E+05	0.29	2.0E+08		
	22.22	1.1E+05	0.29	2.3E+08		
10-12 (Coarse Sand Cap)	19.64	5.8E+05	0.27	1.2E+09	1.3E+09	4.6E+08
	20.60	3.1E+05	0.27	6.8E+08		

	19.31	7.1E+05	0.25	1.7E+09		
	19.40	6.7E+05	0.25	1.6E+09		

Table B5: Bacterial Concentrations in Depth Intervals of Column A Soil After Experiment 2 (amended) As Determined by 0.25g Samples. *The cycle of quantitation (Cq) is the main output of qPCR and is to calculate the gene copies/µL of extracted DNA. Gene copy/µL values were dilution corrected, multiplied by the total volume of extracted DNA and divided by the wet soil mass to obtain the bacterial concentration in each sample.*

Distance from Influent Inlet (cm)	*Cq*	*Gene Copies/µL*	*Wet Soil Mass (g)*	*Bacterial Concentration (gene copies/g)*	*Average Bacterial Concentration (gene copies/g)*	*Standard Deviation (gene copies/g)*
0-2	23.01	4.4E+05	0.31	8.6E+08	6.7E+08	3.9E+08
	22.56	5.8E+05	0.31	1.1E+09		
	25.92	7.6E+04	0.16	2.9E+08		
	25.39	1.0E+05	0.16	4.1E+08		
2-4	24.34	2.0E+05	0.21	5.9E+08	9.9E+08	2.7E+08
	23.35	3.6E+05	0.21	1.1E+09		
	22.84	4.9E+05	0.28	1.1E+09		
	22.70	5.4E+05	0.28	1.2E+09		
4-6	23.51	3.3E+05	0.22	9.2E+08	5.5E+08	3.3E+08
	23.91	2.6E+05	0.22	7.2E+08		
	25.12	1.2E+05	0.21	3.5E+08		
	25.97	7.3E+04	0.21	2.1E+08		
6-8	24.97	1.4E+05	0.28	2.9E+08	6.3E+08	3.4E+08
	24.46	1.8E+05	0.28	4.0E+08		
	23.46	3.4E+05	0.21	9.6E+08		
	23.61	3.1E+05	0.21	8.8E+08		

8-10	24.42	1.9E+05	0.25	4.6E+08	2.4E+08	2.2E+08
	24.64	1.6E+05	0.25	4.0E+08		
	27.58	2.8E+04	0.33	5.0E+07		
	27.68	2.6E+04	0.33	4.7E+07		
10-12 (Coarse Sand Cap)	24.69	1.6E+05	0.28	3.5E+08	4.4E+08	1.8E+08
	24.08	2.3E+05	0.28	5.1E+08		
	24.70	1.6E+05	0.41	2.4E+08		
	23.08	4.2E+05	0.41	6.4E+08		

Table B6: Bacterial Concentrations in Depth Intervals of Column B Soil After Experiment 1 (not amended) As Determined by 0.25g Samples. *The cycle of quantitation (Cq) is the main output of qPCR and is to calculate the gene copies/µL of extracted DNA. Gene copy/µL values were dilution corrected, multiplied by the total volume of extracted DNA and divided by the wet soil mass to obtain the bacterial concentration in each sample. Samples in red are below the limit of quantification.*

Distance from Influent Inlet (cm)	*Cq*	*Gene Copies/µL*	*Wet Soil Mass (g)*	*Corrected Concentration (gene copies/g)*	*Average Concentration (gene copies/g)*	*Standard Deviation (gene copies/g)*
0-2	26.26	8.4E+03	0.18	2.7E+07	1.4E+07	1.3E+07
	26.54	7.0E+03	0.18	2.2E+07		
	29.36	1.2E+03	0.34	2.0E+06		
	28.89	1.6E+03	0.34	2.6E+06		
2-4	29.96	7.9E+02	0.25	1.8E+06	1.7E+06	3.0E+05
	30.19	6.8E+02	0.25	1.6E+06		
	30.10	7.2E+02	0.21	2.0E+06		
	30.78	4.7E+02	0.21	1.3E+06		
4-6	31.05	3.9E+02	0.26	9.3E+05	9.3E+05	3.1E+05
	31.21	3.5E+02	0.26	8.4E+05		

	31.75	2.5E+02	0.26	6.1E+05		
	30.49	5.6E+02	0.26	1.4E+06		
6-8	31.56	2.8E+02	0.23	7.1E+05	1.3E+06	1.1E+06
	31.26	3.4E+02	0.23	8.6E+05		
	31.43	3.1E+02	0.24	7.5E+05		
	29.27	1.2E+03	0.24	3.0E+06		
8-11.3	29.05	1.4E+03	0.27	3.1E+06	4.3E+06	2.3E+06
	29.97	7.8E+02	0.27	1.7E+06		
	28.25	2.3E+03	0.27	5.1E+06		
	27.75	3.2E+03	0.27	7.0E+06		
11.3-12 (Coarse Sand Cap)	24.46	2.6E+04	0.30	5.1E+07	9.9E+07	4.6E+07
	23.70	4.3E+04	0.30	8.2E+07		
	22.58	8.8E+04	0.31	1.6E+08		
	23.25	5.7E+04	0.31	1.0E+08		

*Red Cq values represent samples below the limit of quantification (LOQ). LOQ was determined by the highest Cq value generated by a sample blank.

Table B7: Bacterial Concentrations in Depth Intervals of Column B Soil After Experiment 2 (amended) As Determined by 0.25g Samples. *The cycle of quantitation (Cq) is the main output of qPCR and is to calculate the gene copies/µL of extracted DNA. Gene copy/µL values were dilution corrected, multiplied by the total volume of extracted DNA and divided by the wet soil mass to obtain the bacterial concentration in each sample.*

Distance from Influent Inlet (cm)	*Cq**	*Gene Copies/µL*	*Wet Soil Mass (g)*	*Corrected Concentration (gene copies/g)*	*Average Concentration (gene copies/g)*	*Standard Deviation (gene copies/g)*
0-2	29.97	6.5E+03	0.22	1.8E+07	1.2E+07	3.8E+06
	30.68	4.2E+03	0.22	1.1E+07		
	30.01	6.3E+03	0.32	1.2E+07		

	30.56	4.5E+03	0.32	8.5E+06		
2-4	34.99	3.1E+02	0.19	9.6E+05	8.9E+05	2.7E+05
	34.60	3.9E+02	0.19	1.2E+06		
	33.97	5.7E+02	0.45	7.7E+05		
	34.41	4.4E+02	0.45	5.9E+05		
4-6	33.92	5.9E+02	0.53	6.7E+05	1.6E+06	1.3E+06
	35.06	3.0E+02	0.53	3.3E+05		
	32.62	1.3E+03	0.30	2.6E+06		
	32.50	1.4E+03	0.30	2.8E+06		
6-8	30.45	4.8E+03	0.18	1.6E+07	1.4E+07	1.7E+07
	29.05	1.1E+04	0.18	3.8E+07		
	34.53	4.1E+02	0.25	1.0E+06		
	34.77	3.5E+02	0.25	8.7E+05		
8-11	33.21	9.0E+02	0.29	1.8E+06	6.4E+06	5.2E+06
	33.12	9.6E+02	0.29	1.9E+06		
	30.05	6.2E+03	0.34	1.1E+07		
	29.98	6.4E+03	0.34	1.1E+07		
11-12 (Coarse Sand Cap)	30.03	6.2E+03	0.24	1.5E+07	1.2E+08	1.3E+08
	29.82	7.1E+03	0.24	1.7E+07		
	25.38	1.1E+05	0.23	2.7E+08		
	26.00	7.2E+04	0.23	1.8E+08		

*Red Cq values represent samples below the limit of quantification (LOQ). LOQ was determined by the highest Cq value generated by a sample blank.

Table B8: Column A Effluent Bacterial Concentrations During Experiment 1 (not amended). *The pore volumes (PV) represent the cumulative amount of effluent passed through the column at the time when sampling started. 1 PV is ~80 mL. The Cycle of Quantitation (Cq) is the main output of qPCR and is used as an input to calculate the gene copies/µL of extracted DNA. The 2 Cq values corresponding to each PV represent duplicate qPCR runs of single effluent samples. In order to calculate the Bacterial Concentration, SQ concentrations were dilution corrected, multiplied by the total volume of extracted DNA and divided by the volume of effluent sampled. Cumulative bacteria passed represents the total number of bacteria (assuming 1 bacterium = 1 gene copy) transported within the effluent until the current PV, and was calculated by trapezoidal integration of effluent curves.*

Pore Volumes	*Cq*	*Gene Copies/µL*	*Corrected Concentration (gene copies/mL)*	*Average Concentration (gene copies/mL)*	*Cumulative Bacteria Passed (gene copies)*
1.03	14.40	1.6E+07	7.1E+07	5.3E+07	2.7E+07
	15.56	7.8E+06	3.4E+07		
15.03	12.58	5.2E+07	2.3E+08	2.2E+08	2.0E+09
	12.68	4.9E+07	2.1E+08		
53.11	Sample Lost During DNA Extraction				
90.11	14.85	1.2E+07	5.9E+07	4.9E+07	1.2E+10
	15.49	8.2E+06	3.9E+07		
164.21	13.69	2.6E+07	6.9E+07	6.5E+07	1.6E+10
	13.87	2.3E+07	6.1E+07		
203.89	13.87	2.3E+07	6.2E+07	6.2E+07	1.9+10
	13.87	2.3E+07	6.2E+07		
241.03	13.67	2.6E+07	7.1E+07	7.1E+07	2.1E+10
	13.68	2.6E+07	7.1E+07		

Table B9: Column A Effluent Bacterial Concentrations During Experiment 2 (amended). *The pore volumes (PV) represent the cumulative amount of effluent passed through the column at the time when sampling started. 1 PV is ~80 mL. The Cycle of Quantitation (Cq) is the main output of qPCR and is used as an input to calculate the gene copies/µL of extracted DNA. The 2 Cq values corresponding to each PV represent duplicate qPCR runs of single effluent samples. In order to calculate the Bacterial Concentration, SQ concentrations were dilution corrected, multiplied by the total volume of extracted DNA and divided by the volume of effluent sampled. Cumulative bacteria passed represents the total number of bacteria (assuming 1 bacterium = 1 gene copy) transported within the effluent until the current PV, and was calculated by trapezoidal integration of effluent curves.*

Pore Volumes	*Cq*	*Gene Copies/µL*	*Corrected Concentration (gene copies/mL)*	*Average Concentration (gene copies/mL)*	*Cumulative Bacteria Passed (gene copies)*
1.03	18.07	*8.9E+06*	5.9E+07	5.8E+07	3.0E+07
	18.12	*8.6E+06*	5.8E+07		
15.30	16.75	*2.0E+07*	9.7E+07	9.5E+07	1.1E+09
	16.80	*1.9E+07*	9.4E+07		
53.11	16.66	*2.1E+07*	9.5E+07	8.1E+07	4.4E+09
	17.26	*1.5E+07*	6.6E+07		
90.11	17.30	*1.4E+07*	6.9E+07	6.8E+07	7.1E+09
	17.37	*1.4E+07*	6.6E+07		
164.21	17.94	*9.6E+06*	4.8E+07	4.2E+07	1.1E+10
	18.40	*7.3E+06*	3.6E+07		
203.89	16.94	*1.8E+07*	8.7E+07	8.4E+07	1.4E+10
	17.09	*1.6E+07*	8.0E+07		
241.03	15.90	*3.3E+07*	1.8E+08	1.7E+08	1.8E+10
	16.20	*2.8E+07*	1.5E+08		

Table B10: Column B Effluent Bacterial Concentrations During Experiment 1 (not amended). *The pore volumes (PV) represent the cumulative amount of effluent passed through the column at the time when sampling started. 1 PV is ~80 mL. The Cycle of Quantitation (Cq) is the main output of qPCR and is used as an input to calculate the gene copies/µL of extracted DNA. The 2 Cq values corresponding to each PV represent duplicate qPCR runs of single effluent samples. In order to calculate the Bacterial Concentration, SQ concentrations were dilution corrected, multiplied by the total volume of extracted DNA and divided by the volume of effluent sampled. Cumulative bacteria passed represents the total number of bacteria (assuming 1 bacterium = 1 gene copy) transported within the effluent until the current PV, and was calculated by trapezoidal integration of effluent curves.*

Pore Volumes	*Cq*	*Gene Copies/µL*	*Corrected Concentration (gene copies/mL)*	*Average Concentration (gene copies/mL)*	*Cumulative Bacteria Passed (gene copies)*
1.03	Sample Lost During DNA Extraction				
17.28	15.98	9.9E+06	1.3E+07	1.3E+07	1.2E+08
	15.83	1.1E+07	1.4E+07		
42.90	14.78	2.1E+07	2.3E+07	2.5E+07	5.92E+08
	14.58	2.4E+07	2.6E+07		
60.59	15.04	1.8E+07	2.4E+07	2.4E+07	1.02E+09
	15.03	1.8E+07	2.4E+07		
79.59	14.21	3.0E+07	3.5E+07	3.4E+07	1.58E+09
	14.25	2.9E+07	3.4E+07		
96.34	14.25	2.9E+07	3.3E+07	3.5E+07	2.16E+09
	14.11	3.2E+07	3.6E+07		
134.70	13.80	3.9E+07	5.4E+07	5.9E+07	3.96E+09
	13.54	4.6E+07	6.4E+07		
153.71	13.58	4.4E+07	4.8E+07	5.7E+07	5.06E+09
	13.12	5.9E+07	6.5E+07		
170.46	13.39	5.0E+07	5.7E+07	7.2E+07	6.16E+09
	12.74	7.5E+07	8.6E+07		
191.17	13.40	5.0E+07	5.6E+07	5.9E+07	7.56E+09
	13.24	5.5E+07	6.2E+07		
203.89	13.81	3.9E+07	4.4E+07	4.4E+07	8.22E+09
	13.78	3.9E+07	4.4E+07		
225.36	13.26	5.4E+07	6.1E+07	6.2E+07	9.3E+09
	13.22	5.6E+07	6.2E+07		
244.33	13.64	4.3E+07	4.8E+07	4.8E+07	1.0E+10

	13.67	4.2E+07	4.7E+07		

Table B11: Column B Effluent Bacterial Concentrations During Experiment 2 (amended). *The pore volumes (PV) represent the cumulative amount of effluent passed through the column at the time when sampling started. 1 PV is ~80 mL. The Cycle of Quantitation (Cq) is the main output of qPCR and is used as an input to calculate the gene copies/µL of extracted DNA. The 2 Cq values corresponding to each PV represent duplicate qPCR runs of single effluent samples. In order to calculate the Bacterial Concentration, SQ concentrations were dilution corrected, multiplied by the total volume of extracted DNA and divided by the volume of effluent sampled. Cumulative bacteria passed represents the total number of bacteria (assuming 1 bacterium = 1 gene copy) transported within the effluent until the current PV, and was calculated by trapezoidal integration of effluent curves.*

Pore Volume	*Cq*	*Gene Copies/µL*	*Corrected Concentration (gene copies/mL)*	*Average Concentration (gene copies/mL)*	*Cumulative Bacteria Passed (gene copies)*
8.08	16.33	2.6E+07	*3.3E+07*	*3.4E+07*	1.40E+08
	16.18	2.8E+07	*3.6E+07*		
20.38	16.28	2.6E+07	*3.9E+07*	*4.1E+07*	6.00E+08
	16.08	3.0E+07	*4.4E+07*		
37.97	16.13	2.9E+07	4.1E+07	*4.0E+07*	1.32E+09
	16.16	2.8E+07	4.0E+07		
53.72	16.14	2.9E+07	4.5E+07	*4.6E+07*	2.00E+09
	16.07	3.0E+07	4.7E+07		
72.94	15.89	3.3E+07	4.2E+07	*4.3E+07*	2.86E+09
	15.84	3.4E+07	4.4E+07		
88.03	16.02	3.1E+07	3.6E+07	*3.5E+07*	3.44E+09
	16.17	2.8E+07	3.3E+07		
108.37	16.17	9.7E+06	1.2E+07	*1.1E+07*	3.90E+09
	16.60	7.5E+06	9.3E+06		

124.26	16.45	8.2E+06	1.1E+07	*9.0E+06*	4.06E+09
	16.99	5.9E+06	7.5E+06		
143.73	Replicate Lost			*NA*	4.3E+09
	16.17	9.7E+06	1.2E+07		
158.80	15.95	1.1E+07	1.4E+07	*1.6E+07*	4.49E+09
	15.49	1.5E+07	1.9E+07		
179.69	14.06	3.6E+07	4.6E+07	*3.9E+07*	5.06E+09
	14.69	2.4E+07	3.1E+07		
193.72	14.34	3.0E+07	3.8E+07	*2.8E+07*	5.07E+09
	15.50	1.5E+07	1.8E+07		
212.92	13.71	4.4E+07	5.7E+07	*5.2E+07*	5.08E+09
	14.06	3.6E+07	4.6E+07		
233.86	12.15	1.2E+08	1.6E+08	*1.1E+08*	6.8E+09
	13.84	4.1E+07	5.7E+07		
234.65	*End of Experiment*				7.3E+09

Table B12: Gene Copy Balance Parameters and Calculations. *Parameters used for the balance include total gene copies in influent and effluent of column B, previously calculated in tables B8-B11, as well as total gene copies in column B soil as estimated using concentrations in 10g samples of column B soil.*

Parameters	*Calculations Used*
Total Influent Bacteria	Trapezoidal Integration: Exp 1 - bottom cell in Column 6, Table B8, Appendix B Exp 2 – bottom cell Column 6, Table B9, Appendix B
Total Effluent Bacteria	Trapezoidal Integration: Exp 1 – bottom cell in Column 6, Table B10, Appendix B Exp 2 – bottom cell iln column 6, Table B11, Appendix B
Expected Total Copies In Column	Total Influent - Total Effluent
Measured Total Copies In Column	Gene copies/g * total g of soil

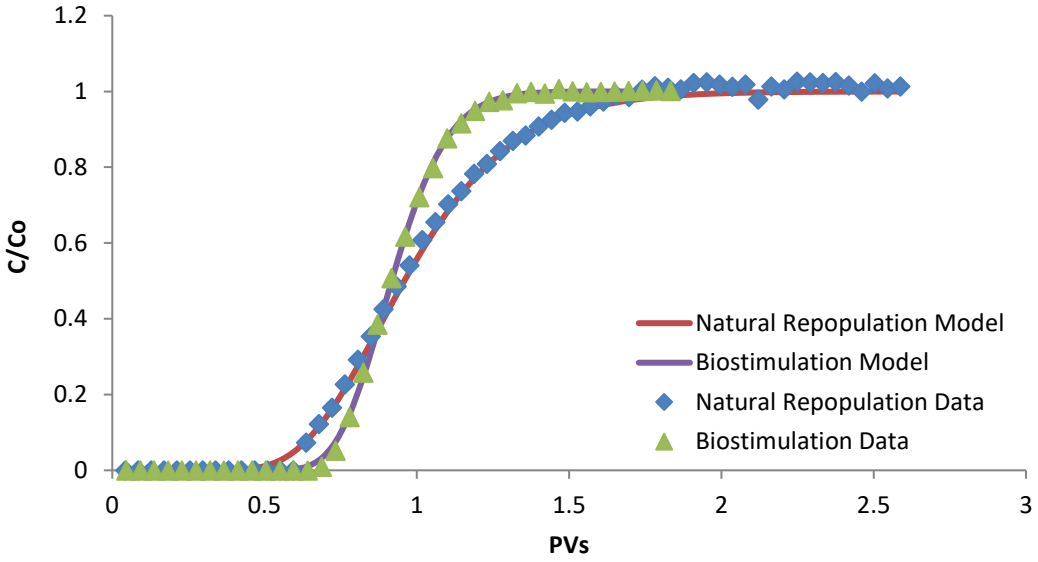

Figure B1: Conservative bromine tracer breakthrough curves and the corresponding advection dispersion equation solutions for Experiments 1 and 2.

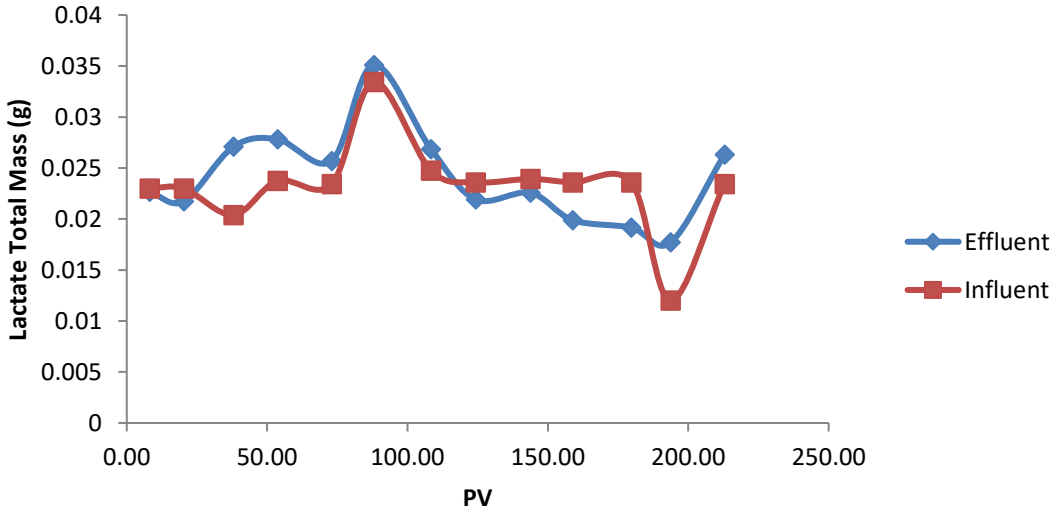

Figure B3: Column B influent vs. effluent lactate concentrations.

Experiment No. - Column			Experiment 1 - Column A											
Sample Type			Effluent						Soil					
Pore Volumes or Soil Subsection (cm)			1	16	97	178	221	261	Pre	0-2	2-4	4-6	8-10	10-12
Phylum	Class	Genus (or Lowest Indentified Taxa)												
Proteobacteria	Alphaproteobacteria	Sphingobium	0.1%	1.1%	0.5%	0.3%	0.2%	0.2%	0.3%	1.6%	1.2%	0.7%	1.0%	2.8%
		Sphingomonas	0.7%	1.3%	0.4%	0.2%	0.2%	0.1%	0.0%	0.3%	0.2%	0.1%	0.1%	0.3%
		Sphingopyxis	0.0%	0.0%	0.5%	0.4%	0.5%	0.8%	0.0%	1.1%	0.6%	0.3%	0.5%	0.3%
	Betaproteobacteria	Achromobacter	0.1%	0.4%	21.4%	12.9%	5.6%	9.3%	0.0%	7.8%	5.3%	2.9%	4.3%	3.5%
		UnclassifiedComamonadaceae (OTU243487)	5.5%	0.3%	0.0%	0.0%	0.0%	0.0%	1.6%	0.0%	0.0%	0.0%	0.3%	0.1%
		UnclassifiedComamonadaceae (OTU4481234)	4.3%	0.3%	0.1%	0.0%	0.0%	0.0%	1.2%	0.0%	0.0%	0.1%	0.1%	0.1%
		UnclassifiedComamonadaceae (OTU4417996)	2.2%	0.3%	0.1%	0.1%	0.1%	0.1%	0.8%	0.0%	0.1%	0.1%	0.2%	0.3%
		UnclassifiedComamonadaceae (OTU4453522)	2.1%	0.1%	0.0%	0.0%	0.0%	0.0%	0.6%	0.0%	0.0%	0.0%	0.1%	0.0%
	Deltaproteobacteria	Geobacter	6.6%	1.5%	0.1%	0.1%	0.1%	0.1%	3.3%	0.1%	0.7%	0.8%	1.5%	1.2%
	Gammaproteobacteria	Acinetobacter	2.6%	4.1%	0.7%	0.2%	0.2%	0.1%	0.1%	0.1%	0.2%	0.3%	1.0%	3.5%
		Pseudomonas	42.7%	76.8%	47.4%	61.0%	64.5%	61.9%	53.8%	61.2%	74.3%	78.2%	65.8%	59.0%
		UnclassifiedXanthomonadaceae (OTU4345543)	0.8%	0.2%	0.0%	0.0%	0.0%	0.0%	2.2%	0.0%	0.0%	0.0%	0.1%	0.5%
		Unclassified Pseudomonadaceae (OTU4434147)	5.7%	0.1%	0.3%	0.6%	0.6%	0.9%	7.3%	0.2%	0.3%	0.2%	0.2%	0.2%
		UnclassifiedPseudomonadaceae (OTU4376926)	0.6%	0.6%	1.7%	2.3%	3.3%	2.7%	0.9%	3.2%	1.2%	1.2%	0.8%	1.4%
		UnclassifiedPseudomonadaceae (OTU4312845)	1.6%	0.9%	0.9%	1.7%	2.5%	2.1%	2.7%	1.7%	0.4%	0.5%	0.3%	0.8%
		UnclassifiedPseudomonadaceae (OTU4453607)	0.3%	0.3%	0.7%	0.7%	0.9%	0.8%	0.4%	1.3%	0.8%	0.7%	0.5%	0.7%
		UnclassifiedPseudomonadaceae (OTU103411)	0.3%	2.3%	0.0%	0.0%	0.0%	0.0%	0.5%	0.0%	0.0%	0.0%	0.1%	0.1%
Actinobacteria	Actinobacteria	Rhodococcus	0.0%	0.1%	5.3%	1.6%	1.5%	1.1%	0.0%	1.4%	1.8%	1.4%	0.9%	0.4%
Bacteroidetes	[Saprospirae]	UnclassifiedChitinophagaceae (OTU0)	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
	Bacteroidia	UnclassifiedPorphyromonadaceae (OTU706795)	0.9%	0.0%	0.9%	0.8%	1.3%	1.4%	0.6%	1.2%	0.5%	0.3%	0.4%	0.4%
Sum of Minor Genera (<2%)			23.0%	9.1%	19.0%	16.9%	18.4%	18.5%	23.8%	18.8%	12.2%	12.2%	21.8%	24.5%

Figure B3: Percentage of the microbial population made up by each genus (or lowest resolvable taxonomic unit) in all effluent (pore volumes) and soil samples (subsections) from column A in experiment 1 (not amended). *Subsections of column soil increase in distance from the inlet at the base of the column (ie: subsection 0-2cm refers to a sample taken from a homogenized sample representing the first 2 cm adjacent to the inlet at the bottom of the column). Genera present at greater than 2% in at least 1 sample are shown individually, while those below 2% across all samples are presented as a summed percentage.*

Experiment No. - Column			Experiment 1 - Column B														
Sample Type			Effluent												Soil		
Pore Volumes or Soil Subsection (cm)			7	19	46	63	86	104	146	166	184	207	225	244	264	8-10	10-12
Phylum	Class	Genus (or Lowest Indentified Taxa)															
Proteobacteria	Alpharoteobacteria	Sphingobium	0.5%	0.9%	0.2%	0.3%	0.2%	0.1%	0.1%	0.0%	0.1%	0.0%	0.1%	0.0%	0.0%	0.0%	3.4%
		Sphingomonas	1.5%	2.0%	0.2%	0.3%	0.2%	0.1%	0.1%	0.0%	0.0%	0.0%	0.1%	0.0%	0.1%	0.0%	2.7%
		Sphingopyxis	0.3%	0.2%	0.0%	0.1%	0.2%	0.2%	0.2%	0.1%	0.2%	0.2%	0.2%	0.2%	0.2%	2.2%	0.3%
	Betaproteobacteria	Achromobacter	0.4%	0.4%	4.5%	5.9%	7.9%	9.7%	8.1%	6.6%	6.5%	6.0%	5.3%	6.3%	5.6%	8.7%	6.4%
		UnclassifiedComamonadaceae (OTU243487)	0.1%	0.9%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%
		UnclassifiedComamonadaceae (OTU4481234)	0.2%	1.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.3%	0.6%
		UnclassifiedComamonadaceae (OTU4417996)	0.4%	1.0%	0.1%	0.1%	0.0%	0.0%	0.1%	0.1%	0.1%	0.1%	0.1%	0.0%	0.1%	0.4%	2.0%
		UnclassifiedComamonadaceae (OTU4453522)	0.1%	0.4%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
	Deltaproteobacteria	Geobacter	0.5%	0.7%	0.5%	0.7%	0.7%	0.4%	0.5%	0.4%	0.6%	0.3%	0.5%	0.3%	0.4%	2.1%	2.9%
	Gammaproteobacteria	Acinetobacter	18.0%	2.4%	0.1%	0.2%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	3.3%	1.8%
		Pseudomonas	64.6%	73.9%	81.1%	77.9%	75.6%	73.1%	72.4%	73.6%	71.4%	72.3%	72.1%	72.1%	70.6%	59.6%	58.0%
		UnclassifiedXanthomonadaceae (OTU4345543)	0.1%	0.4%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.4%
		Unclassified Pseudomonadaceae (OTU4434147)	0.2%	0.4%	0.8%	0.9%	1.2%	1.3%	2.3%	2.3%	2.8%	2.2%	1.6%	0.7%	0.6%	0.0%	0.2%
		UnclassifiedPseudomonadaceae (OTU4376926)	0.4%	0.4%	1.7%	1.6%	1.5%	1.3%	1.5%	1.7%	1.6%	2.1%	2.2%	2.3%	2.5%	3.5%	1.3%
		UnclassifiedPseudomonadaceae (OTU4312845)	0.3%	0.7%	2.1%	1.7%	1.6%	1.5%	1.8%	2.1%	2.2%	2.7%	2.8%	3.0%	3.1%	0.0%	0.3%
		UnclassifiedPseudomonadaceae (OTU4453607)	0.1%	0.1%	0.3%	0.3%	0.2%	0.2%	0.2%	0.3%	0.2%	0.3%	0.4%	0.3%	0.4%	2.3%	0.7%
		UnclassifiedPseudomonadaceae (OTU103411)	0.0%	0.3%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Actinobacteria	Actinobacteria	Rhodococcus	0.0%	0.3%	0.1%	0.4%	0.3%	0.2%	0.2%	0.1%	0.1%	0.1%	0.3%	0.1%	0.3%	2.4%	2.4%
Bacteroidetes	[Saprospirae]	UnclassifiedChitinophagaceae (OTU0)	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	4.3%
	Bacteroidia	UnclassifiedPorphyromonadaceae (OTU706795)	0.1%	0.2%	0.2%	0.5%	0.5%	0.2%	0.3%	0.4%	0.4%	0.4%	0.6%	0.5%	1.2%	2.5%	0.8%
Sum of Minor Genera (<2%)			12.0%	13.5%	7.8%	9.1%	9.9%	11.6%	12.1%	12.1%	13.8%	13.2%	13.8%	14.0%	14.9%	12.6%	11.6%

Figure B4: Percentage of the microbial population made up by each genus (or lowest resolvable taxonomic unit) in all effluent (pore volumes) and soil samples (subsections) from column B in experiment 1 (not amended). *Subsections of column soil increase in distance from the inlet at the base of the column (ie: subsection 0-2cm refers to a sample taken from a homogenized sample representing the first 2 cm adjacent to the inlet at the bottom of the column). Genera present at greater than 2% in at least 1 sample are shown individually, while those below 2% across all samples are presented as a summed percentage.*

Experiment No. - Column			Experiment 2 - Column A												
Sample Type			Effluent							Soil						
Pore Volumes or Soil Subsection (cm)			10	45	80	116	151	187	223	Pre	1	3	5	7	9	11
Phylum	Class	Genus (or Lowest Indentified Taxa)														
Proteobacteria	Alphaproteobacteria	Sphingomonas	31.9%	0.4%	0.2%	0.4%	1.3%	1.5%	0.1%	0.0%	0.6%	0.6%	0.3%	0.1%	0.2%	0.2%
		Sphingobium	0.1%	0.9%	0.5%	0.3%	0.5%	1.3%	0.2%	0.1%	1.5%	0.6%	0.3%	0.7%	0.3%	0.3%
		Paracoccus	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.5%	0.0%	0.2%	0.1%	0.3%	0.3%	0.5%
		Methylobacterium	11.2%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%
		Mycoplana	0.0%	0.0%	0.0%	0.6%	4.4%	2.6%	0.1%	0.0%	0.1%	0.0%	0.0%	0.1%	0.0%	0.2%
		Unclassified Caulobacteraceae (OTU4353264)	0.0%	0.0%	0.0%	0.2%	0.9%	0.5%	0.0%	0.0%	0.1%	0.0%	0.1%	0.0%	0.0%	0.0%
		Unclassified Caulobacteraceae (OTU4466825)	0.9%	1.7%	2.2%	2.4%	6.1%	4.6%	0.9%	0.0%	7.2%	5.1%	3.7%	3.3%	2.0%	1.0%
	Betaproteobacteria	Azovibrio	2.3%	0.4%	1.1%	1.4%	1.7%	1.1%	0.4%	12.6%	1.0%	1.2%	0.7%	0.7%	1.9%	8.9%
		Ralstonia	0.0%	0.1%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.1%	0.0%
		Cupriavidus	19.3%	5.8%	7.9%	15.1%	11.0%	11.0%	4.6%	0.0%	33.1%	28.2%	20.0%	15.1%	9.9%	4.8%
		Delftia	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
		Unclassified Comamonadaceae (OTU4434564)	0.5%	0.5%	0.4%	0.4%	0.6%	0.3%	0.2%	0.0%	1.5%	2.5%	2.2%	1.3%	0.8%	0.9%
		Unclassified Comamonadaceae (OTU4357500)	0.1%	2.1%	1.5%	0.2%	0.0%	0.0%	0.4%	0.0%	1.1%	0.8%	0.8%	0.7%	0.5%	0.2%
	Deltaproteobacteria	Geobacter	1.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	3.1%	0.1%	0.7%	0.7%	1.7%	1.5%	1.9%
		Unclassified Desulfovibrionaceae (OTU3203828)	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.1%	0.1%	0.0%	0.1%
	Gammaproteobacteria	Unclassified Pseudomonadaceae (OTU4434147)	0.5%	0.0%	0.1%	0.0%	0.0%	0.0%	0.1%	1.0%	0.1%	0.1%	0.1%	0.1%	0.2%	0.3%
		Unclassified Pseudomonadaceae (OTU4376926)	0.0%	22.4%	17.1%	6.7%	0.4%	0.4%	18.1%	0.0%	6.7%	6.7%	4.5%	3.9%	4.9%	2.5%
		Unclassified Pseudomonadaceae (OTU4453607)	0.0%	17.0%	11.0%	3.1%	0.2%	0.2%	10.2%	0.0%	4.6%	4.3%	2.8%	2.4%	2.9%	1.6%
		Unclassified Pseudomonadaceae (OTU4312845)	0.1%	1.0%	2.2%	2.2%	0.2%	0.1%	4.2%	0.2%	0.5%	0.3%	0.3%	0.3%	0.6%	0.2%
		Pseudomonas	15.8%	27.9%	38.2%	49.2%	40.8%	38.4%	44.1%	55.0%	20.1%	17.0%	23.5%	26.9%	28.9%	25.0%
		Acinetobacter	0.0%	0.0%	0.0%	0.0%	0.7%	0.0%	0.0%	0.0%	0.1%	0.0%	0.2%	0.1%	0.0%	0.1%
		Unclassified Enterobacteriaceae (OTU4475936)	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.2%	0.0%	0.1%	0.3%	0.2%	0.2%	0.1%
		Unclassified Enterobacteriaceae (OTU4469420)	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%
		Unclassified Gammaproteobacteria (OTU4430952)	0.0%	0.0%	0.0%	0.1%	1.8%	1.7%	0.1%	0.0%	0.2%	0.1%	0.2%	0.1%	0.1%	0.1%
Bacteroidetes	Bacteroidia	Paludibacter	0.0%	0.0%	0.3%	0.2%	0.4%	0.2%	0.6%	0.1%	1.2%	3.9%	5.0%	4.9%	5.7%	4.0%
		Unclassified Porphyromonadaceae (OTU3506234)	0.1%	0.0%	0.1%	0.0%	0.1%	0.1%	0.2%	0.1%	0.8%	2.8%	3.7%	3.8%	3.4%	3.0%
		Unclassified Porphyromonadaceae (OTU4323661)	0.1%	0.0%	0.1%	0.0%	0.1%	0.1%	0.1%	0.7%	0.4%	2.0%	2.0%	2.1%	2.4%	2.3%
		Unclassified Porphyromonadaceae (OTU706795)	0.1%	0.0%	0.1%	0.1%	0.1%	0.1%	0.0%	0.5%	0.0%	0.4%	1.0%	1.0%	1.5%	2.1%
Firmicutes	Clostridia	Proteiniclasticum	0.0%	2.2%	2.5%	1.2%	3.3%	1.1%	0.2%	0.3%	0.3%	1.0%	1.2%	1.2%	1.4%	0.9%
Spirochaetes	Spirochaetes	Treponema	0.1%	0.1%	0.4%	0.1%	0.1%	0.1%	0.2%	0.4%	0.2%	1.7%	2.4%	3.1%	3.3%	4.1%
Unclassified (OTU3)			0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Sum of Minor Genera (<2%)			15.6%	17.4%	14.0%	16.0%	25.0%	34.6%	15.1%	24.8%	18.4%	19.6%	23.8%	25.7%	26.9%	34.5%

Figure B5: Percentage of the microbial population made up by each genus (or lowest resolvable taxonomic unit) in all effluent (pore volumes) and soil samples (subsections) from column A in experiment 2 (amended). *Subsections of column soil increase in distance from the inlet at the base of the column (ie: subsection 0-2cm refers to a sample taken from a homogenized sample representing the first 2 cm adjacent to the inlet at the bottom of the column). Genera present at greater than 2% in at least 1 sample are shown individually, while those below 2% across all samples are presented as a summed percentage.*

Experiment No. - Column			Experiment 2 - Column B															
Sample Type			Effluent													Soil		
Pore Volumes or Soil Subsection (cm)			7	22	42	58	78	83	114	130	149	164	186	200	220	235	1	7	11
Phylum	Class	Genus (or Lowest Indentified Taxa)																	
Proteobacteria	Alpha-proteobacteria	Sphingomonas	9.9%	2.3%	0.5%	0.4%	0.4%	0.9%	0.9%	1.0%	0.8%	0.3%	1.2%	0.3%	0.0%	0.1%	1.9%	5.8%	1.9%
		Sphingobium	0.3%	0.2%	0.5%	0.6%	0.2%	0.4%	0.8%	1.1%	0.3%	0.2%	0.7%	2.2%	0.1%	0.1%	0.3%	0.0%	0.0%
		Paracoccus	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	2.9%	0.0%
		Methylobacterium	0.8%	0.6%	0.1%	0.0%	0.0%	0.1%	0.1%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.4%	0.0%	0.0%
		Mycoplana	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.7%	1.3%	0.9%	0.9%	0.1%	0.1%	0.0%	0.0%	0.1%	0.2%	0.3%
		Unclassified Caulobacteraceae (OTU4353264)	0.0%	0.0%	0.0%	0.0%	0.1%	0.1%	0.3%	0.6%	0.7%	0.6%	1.2%	0.8%	0.0%	0.0%	0.2%	19.1%	2.0%
		Unclassified Caulobacteraceae (OTU4466825)	0.0%	0.4%	1.1%	2.6%	3.8%	5.4%	7.9%	10.3%	4.8%	4.5%	4.5%	5.7%	0.2%	0.2%	1.7%	0.2%	0.6%
	Beta-proteobacteria	Azovibrio	0.4%	0.6%	0.4%	0.5%	0.8%	0.7%	1.5%	1.2%	0.5%	0.3%	0.2%	0.8%	1.8%	2.5%	6.6%	0.0%	0.8%
		Ralstonia	0.1%	0.0%	0.0%	0.0%	0.1%	0.1%	0.2%	0.1%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	8.9%	0.0%
		Cupriavidus	36.8%	2.3%	2.8%	5.7%	20.3%	35.2%	46.7%	43.9%	26.2%	13.7%	4.9%	8.4%	1.2%	1.7%	11.9%	0.8%	8.8%
		Delftia	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	11.3%	0.4%
		Unclassified Comamonadaceae (OTU4434564)	1.9%	1.0%	0.2%	0.3%	0.4%	0.7%	1.3%	0.5%	0.5%	0.5%	0.4%	0.5%	0.1%	0.1%	4.6%	0.0%	0.8%
		Unclassified Comamonadaceae (OTU4357500)	0.0%	0.0%	4.1%	1.1%	0.3%	0.2%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.1%	0.8%	0.0%	0.0%
	Delta-proteobacteria	Geobacter	3.0%	0.1%	0.1%	0.1%	0.0%	0.1%	0.2%	0.6%	0.3%	0.1%	0.0%	0.0%	0.1%	0.3%	1.4%	0.0%	2.4%
		Unclassified Desulfovibrionaceae (OTU3203828)	0.0%	0.0%	0.0%	0.0%	0.0%	0.3%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.2%	0.8%	1.1%	0.0%	18.7%
	Gamma-proteobacteria	Unclassified Pseudomonadaceae (OTU4434147)	0.4%	48.5%	20.3%	15.2%	8.0%	5.8%	0.7%	0.3%	0.4%	0.3%	0.3%	0.9%	5.0%	4.6%	1.1%	0.0%	0.0%
		Unclassified Pseudomonadaceae (OTU4376926)	0.0%	0.0%	10.3%	12.1%	5.6%	4.5%	2.1%	2.4%	0.2%	0.2%	0.1%	0.3%	10.1%	7.1%	8.3%	1.1%	13.3%
		Unclassified Pseudomonadaceae (OTU4453607)	0.0%	0.0%	5.3%	7.2%	3.3%	2.5%	1.2%	1.1%	0.1%	0.0%	0.0%	0.1%	3.9%	2.8%	5.1%	0.7%	8.4%
		Unclassified Pseudomonadaceae (OTU4312845)	0.0%	0.2%	2.4%	1.7%	1.5%	0.7%	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%	4.1%	3.8%	0.1%	0.0%	0.1%
		Pseudomonas	17.2%	33.3%	30.3%	31.4%	34.9%	18.7%	9.9%	13.5%	36.4%	57.3%	51.5%	50.4%	63.6%	64.9%	32.4%	12.6%	18.0%
		Acinetobacter	0.0%	0.1%	0.1%	0.1%	0.0%	0.0%	0.0%	0.0%	0.4%	0.3%	3.9%	0.8%	0.0%	0.0%	0.7%	7.2%	2.3%
		Unclassified Enterobacteriaceae (OTU4475936)	0.2%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	15.3%	1.9%
		Unclassified Enterobacteriaceae (OTU4469420)	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	5.9%	7.3%
		Unclassified Gammaproteobacteria (OTU4430952)	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.6%	2.0%	5.9%	3.5%	8.1%	7.6%	0.0%	0.0%	1.0%	0.0%	0.0%
Bacteroidetes	Bacteroidia	Paludibacter	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.4%	0.3%	0.1%	0.0%	0.0%	0.1%	0.1%	0.1%	1.7%	0.0%	0.5%
		Unclassified Porphyromonadaceae (OTU3506234)	0.2%	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	0.1%	0.1%	0.0%	0.0%	0.0%	0.0%	0.1%	1.1%	0.0%	2.2%
		Unclassified Porphyromonadaceae (OTU4323661)	0.8%	0.0%	0.0%	0.0%	0.0%	0.1%	0.1%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.6%	0.0%	1.4%
		Unclassified Porphyromonadaceae (OTU706795)	0.4%	0.0%	0.0%	0.0%	0.1%	0.2%	0.4%	0.4%	0.1%	0.1%	0.1%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%
Firmicutes	Clostridia	Proteiniclasticum	0.0%	0.0%	0.1%	0.9%	0.9%	1.3%	0.7%	1.0%	0.3%	0.3%	0.1%	0.2%	0.1%	0.1%	2.7%	0.0%	0.2%
Spirochaetes	Spirochaetes	Treponema	0.5%	0.0%	0.0%	0.0%	0.1%	0.1%	0.3%	0.2%	0.1%	0.1%	0.1%	0.2%	0.1%	0.1%	1.3%	0.0%	1.3%
Unclassified (OTU3)			0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	2.9%	0.0%
Sum of Minor Genera (<2%)			26.9%	10.2%	21.4%	19.9%	19.2%	21.8%	22.5%	17.5%	20.5%	16.5%	22.2%	20.3%	9.3%	10.4%	12.8%	5.0%	6.8%

Figure B6: Percentage of the microbial population made up by each genus (or lowest resolvable taxonomic unit) in all effluent (pore volumes) and soil samples (subsections) from column B in experiment 2 (amended). *Subsections of column soil increase in distance from the inlet at the base of the column (ie: subsection 0-2cm refers to a sample taken from a homogenized sample representing the first 2 cm adjacent to the inlet at the bottom of the column). Genera present at greater than 2% in at least 1 sample are shown individually, while those below 2% across all samples are presented as a summed percentage.*

Appendix C. Column Study: Photos

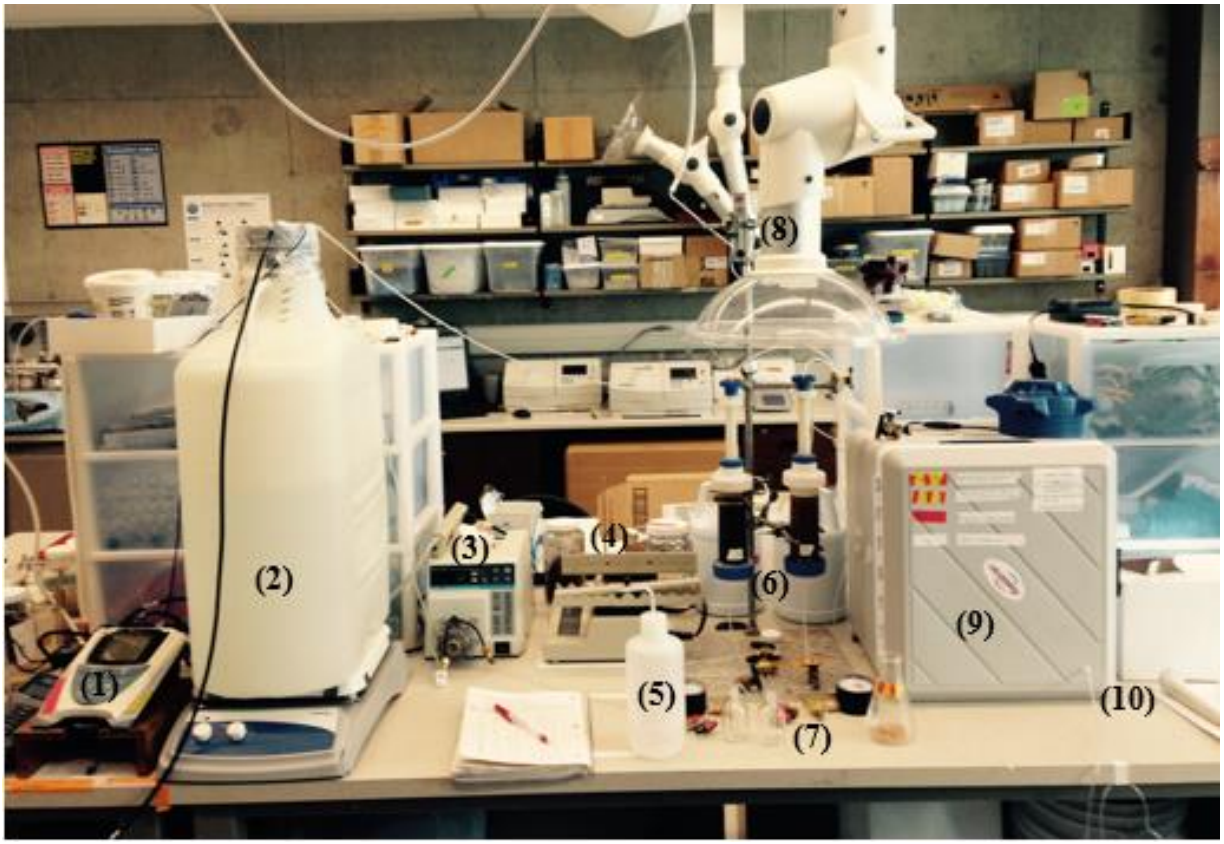

Figure C1: Overview of column setup prior to experimental commencement. *Proceeding from left to right, significant items include: (1) probes to measure artificial groundwater (AGW) parameters, (2) AGW reservoir, (3) peristaltic pump used to flow AGW (4) syringe pump, used only for delivering lactate pulses, (5) 70% EtOH used for disinfection of gloves prior to any interactions with the experimental setup, (6) columns A and B, directly following soil packing and prior to being wrapped in tin foil, (7) various valves used to direct flow from the peristaltic and syringe pumps to the appropriate columns, (8) rotameter used to control nitrogen gas flow rate, (9) electric cooler used for collecting samples and sterivex filtering, and (10) effluent line from sterivex filter to collection vessel.*

Figure C2: Columns A and B directly following packing.

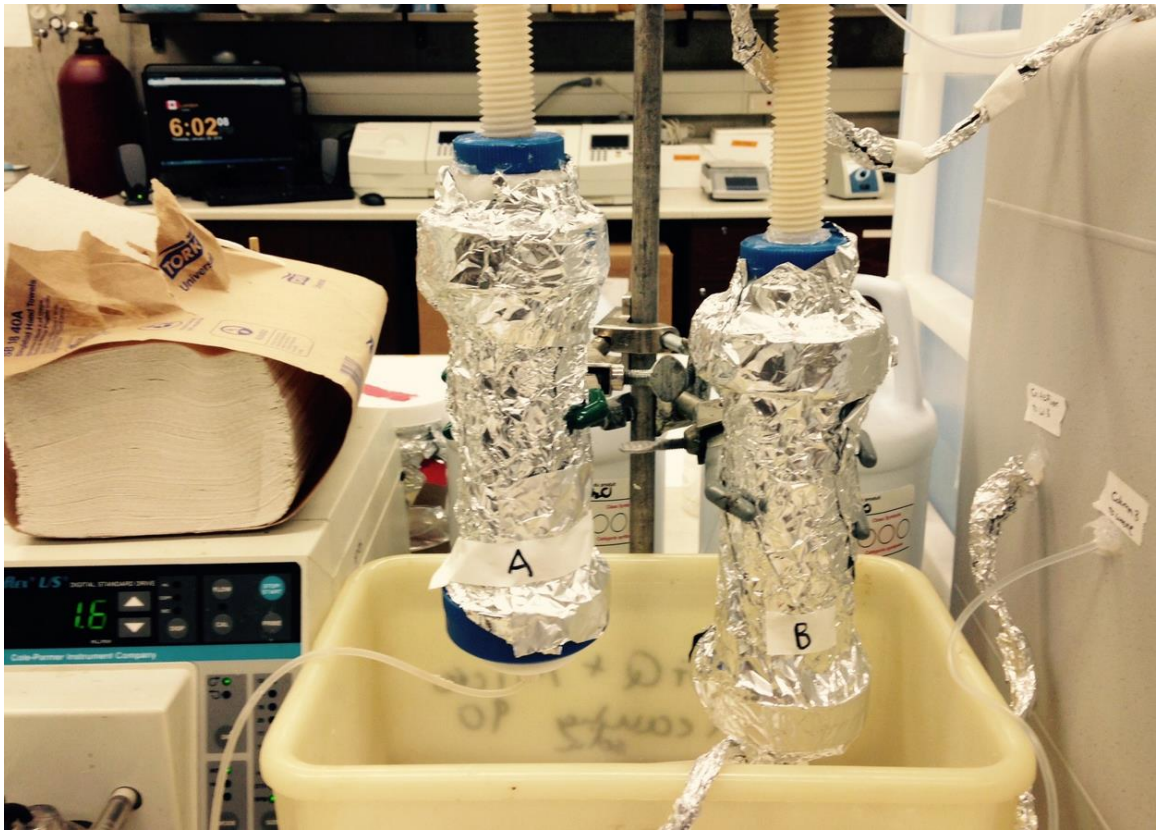

Figure C3: Columns A and B during experimental operation.

Figure C4: Formation of sulfide precipitate veins in Column B after Experiment 2 (amended).

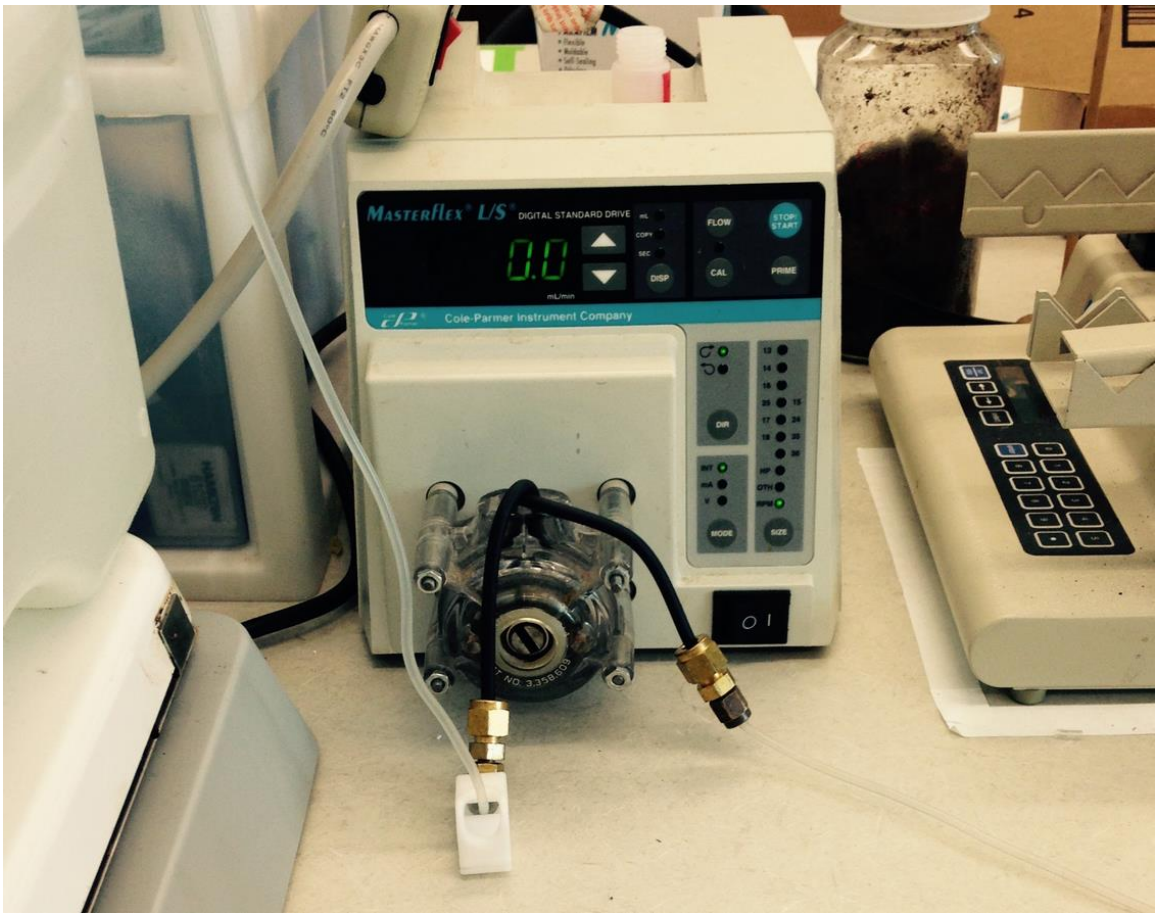

Figure C6: Peristaltic pump used to flow artificial groundwater.

Appendix D. DNA Statistics

Table C 1: Standard Curve Statistics from qPCR

Samples Quantified	Efficiency	R^2 Value
Column Exp A (Plate 1)	92%	0.984
Column Exp A (Plate 2)	90%	0.989
Column Exp B (Plate 1)	84%	0.982
Column Exp B (Plate 2)	87%	0.989
Field Study (0.25g soil) (Plate 1)	94%	0.997
Field Study (0.25g soil) (Plate 2)	97%	0.998
Groundwater	80%	0.999
Field Study (10g soil)	80%	0.999

Method D1: Illumina Data QA/QC and Analysis

Once the demultiplexed reads were sent from Genome Quebec, several steps were taken to analyze the data. First, the forward and reverse sequences for each sample were joined together using the following parameters: 1) minimum overlap size was 80 bp long; 2) maximum allowed percentage of differences within the joined region was eight; 3) joining method was fast join. The joined reads of individual samples then went through quality control and were merged into one single data file in the split library step, with a minimum phred quality threshold equal to twenty. The chimera sequences in the merged sequence file were identified and filtered using the USearch61 algorithm with the Ribosomal Database Project (RDP) 16s rRNA database as the reference. The chimera-free sequences were grouped into operational taxonomy units (OTUs) using the open-reference OTU picking approach with the USearch61 algorithm and the Greengene database (version 13_8) as the reference, and a representative sequence was picked for each OTU for downstream analysis (e.g. taxonomy classification and biom table construction). The summary of the taxonomic composition of each community was generated at different levels, from kingdom to OTU, in both biom and txt formats.

Based on the txt files at the genus level, the heatmaps of major organisms (i.e., >2% in the total population in each sample) were constructed in Excel (with the exception of unclassified organisms for which the percentage of specific OTUs were used instead).

Sample comparison and clustering was carried out using the nonmetric multi-dimensional scaling (NMDS) ordination numerical technique with the statistical software R (R-Core-Team, 2013). Initially, the Bray-Curtis dissimilarity scores were calculated by comparing the percentages of the major genera in a pair-wise manner for all samples, which were then transformed into rank orders. NMDS was applied to represent the original positions of communities (i.e., an 71-dimentional space due to 71 major genera) as closely as possible using a reduced number of dimensions (i.e., k) for easier yet accurate vitalization on a 2-D plot. The stress value and the Shepard plot were examined to ensure the appropriateness of the chosen k-value. The stress values were found to be 0.11 and 0.06 for k=2 and k=3 respectively, where the later indicated an excellent representation of the original data. The Shepard plot of k=3 also showed smaller scatter of samples than that generated with k=2 (Fig C 1, Appendix C). Therefore, k=3 was chosen for NMDS to compute the data. The sample names and the names of the genera were plotted on the NMDS plots to reflect the community differences and to visualize the affecting organisms.

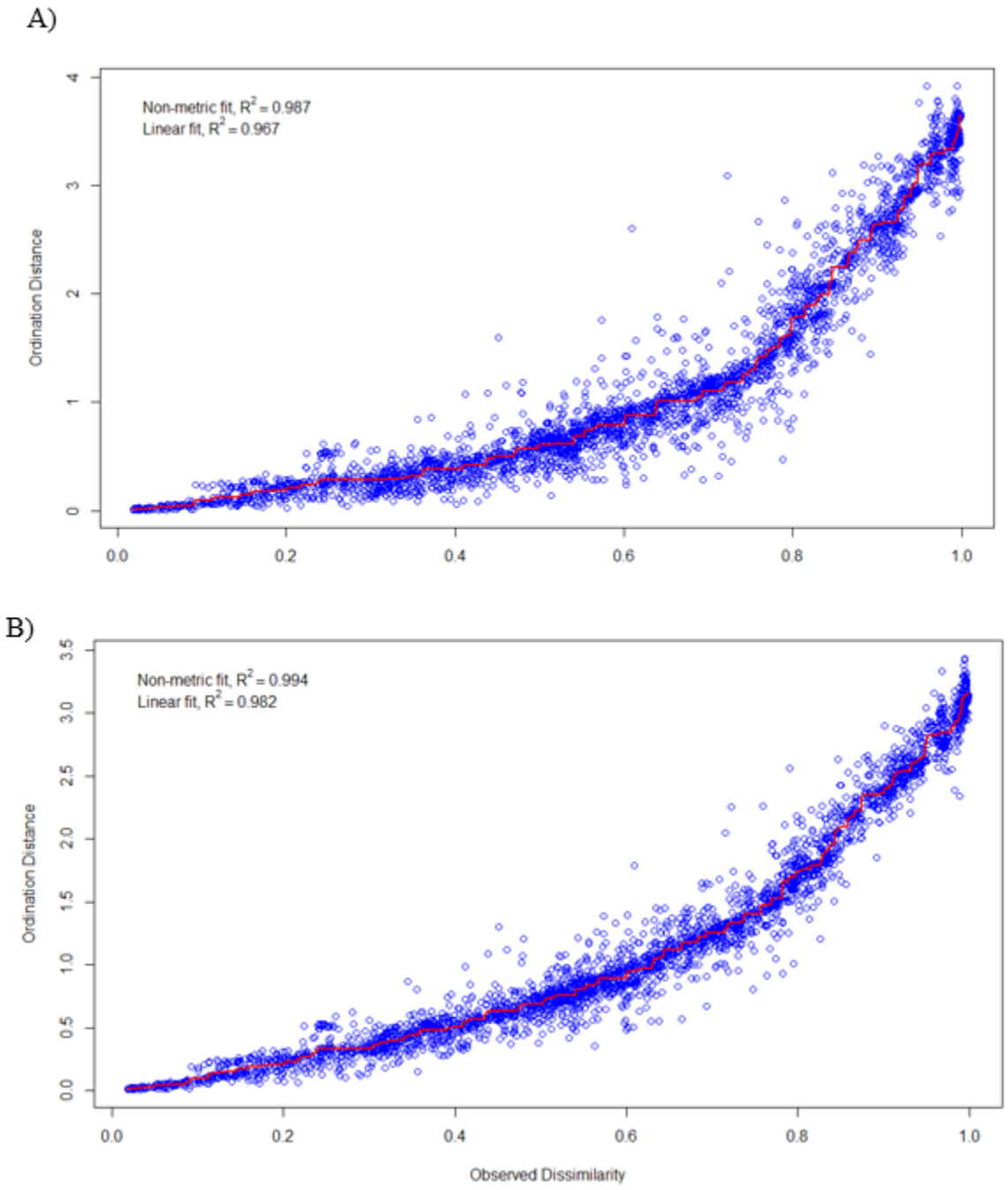

Figure C7: Shepard plots visualizing data scatter with k = 2 (A) and k = 3 (B)

Table C2: Statistics Regarding Forward and Reverse DNA Reads Generated by Illumina (Raw Reads), Combined Forward and Reverse Reads (After Joining), Combined Reads After Quality Control Check (QC) and After Chimera Check and OTU Selection.

Statistic	Raw Reads	Reads after Joining	Reads after QC	Reads after Chimera Check and OTU-picking
Average	67049	43071	41178	38339
Standard deviation	28422	18680	17893	16291
Maximum	100013	67503	64609	62038
Minimum	1859	881	844	843
Median	78944	50370	48071	43695

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Microbial Repopulation Following In Situ STAR Remediation
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Microbial Repopulation Following In Situ STAR Remediation
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