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With rising concentrations of CO_2 in the Earth's atmosphere causing concern about climate change, many solutions are being presented to decrease emissions. One of the proposed solutions is to sequester excess CO_2 in geological formations such as basalt. The deep subsurface is known to harbor much of the microbial biomass on earth and questions abound as to how this deep life is going to respond to the injection of CO_2. Many studies have used model microorganisms to demonstrate the ability of microbes to aid in the safe, permanent sequestration of CO_2 in the subsurface. The objective of this research is to characterize the microbial community present in the basalts at the Wallula pilot carbon sequestration well prior to the injection of CO_2 and then perform laboratory studies to determine how the native microbial community will respond to carbon sequestration conditions. Six samples were collected from the Wallula pilot well prior to the injection of CO_2 into the system. The microorganisms in these samples were characterized by pyrosequencing of 16S rRNA genes, revealing a community dominated by the Proteobacteria, Firmicutes, and Actinobacteria. The organisms detected were

related to microbes known to metabolize hydrogen, sulfur, and single carbon compounds. These microorganisms may be stimulated in formations located at the fringe of the pool of injected CO_2. Laboratory studies revealed that the native microbial community suffered a two order of magnitude loss of population upon exposure to CO_2 under carbon sequestration conditions. The community also shifted from being dominated by Proteobacteria prior to CO_2 exposure to being dominated by Firmicutes after exposure. Specifically, the genus *Alkaliphilus*, which was previously undetected, appeared after CO_2 exposure and became dominant. The dominance of *Alkaliphilus*, along with other rare organisms which did not compose a majority of the population prior to the introduction of CO_2 to the system, indicates that members of the rare biosphere may be better adapted to changing environmental conditions specific to CO_2 sequestration than other indigenous cells. Thus, the rare biosphere should be examined closely as part of any environmental study, as these minority microorganisms may be the first indication of perturbation or impact.

Microbiology of Basalts Targeted for Deep Geological Carbon Sequestration: Field Observations and Laboratory Experiments

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Heather J. Lavalleur, Author

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1. INTRODUCTION

Carbon dioxide in the atmosphere and earth system changes

Over the last decade there has been increasing concern and interest in the role of carbon dioxide levels in climate change. Such interest has led to an investigation of methods that could potentially be used to decrease the concentration of CO_2 in the atmosphere either by capturing CO_2 from source emissions or by removing the greenhouse gas directly from the atmosphere. CO_2 is not the only gas that contributes to the greenhouse effect; however, it is a major player due to severe concentration increases that result from anthropogenic outputs such as fossil fuel burning. Since the industrial revolution concentrations of CO_2 in the atmosphere have risen from 280 ppm to 380 ppm (Lal 2008). Continuing at the current rate of 2 ppm a year (Benson & Surles 2006), the concentration of CO_2 in the atmosphere will increase to 450 ppm by mid-century (Keith 2009), the concentration at which the earth's temperature is expected to rise by 2°C.

A rise of 2°C in the surface of the earth due to increased CO_2 concentrations will lead to potentially severe environmental consequences. The most well understood and well modeled environmental changes associated with increased CO_2 concentrations (and thus warmer temperatures) are those changes occurring in the world's oceans. Of

particular concern is ocean acidification, from which many other problems stem (Benson & Surles 2006). Ocean acidification leads to a rise of the carbonate compensation depth (CCD), the dissolution of carbonate lifeforms, and ultimately the death of marine life (Feely et al. 2004; Sabine et al. 2004). Warming of the oceans also leads to stratification of the oceans, which alters ocean currents and ultimately affects weather patterns (Hegerl & Bindoff 2005; Sabine et al. 2004). Lastly, warming of the oceans results in changes in global sea level (Benson & Surles 2006). Models predict that by 2100 sea level will rise by one meter (Overpeck & Weiss 2009). Potentially a seven meter rise in sea level could occur, plunging the coastline of every continent beneath the ocean.

Thus severe environmental problems involving the earth's oceans are associated with the unprecedented rapid rise of CO_2 levels in the earth's atmosphere. One proposed solution to the problem of increased concentrations of CO_2 emitted into the atmosphere is to capture the CO_2 from point sources and store the CO_2. Various methods are available to capture and store the carbon and these options are discussed in detail in the next section.

Carbon capture and storage

Three methods exist for capturing CO_2 from sources of emission: postcombustion capture, precombustion capture, and oxygen combustion capture (Benson & Surles 2006). Postcombustion capture involves the

removal of CO_2 from flue gas generated at a power plant. In precombustion capture, CO_2 is removed from the reaction before it undergoes combustion. Using this method of carbon capture, a syngas is created which can be used to generate electricity. The last method of carbon capture is oxygen combustion. The process of oxygen combustion uses pure oxygen, not air, for the combustion of fossil fuels and thus produces cleaner emissions, making the CO_2 easy to capture from emissions.

Once the CO_2 is captured it must be stored to prevent it from contributing to the increasing levels of CO_2 in the atmosphere. Numerous options are available for storage. CO_2 is naturally stored in the terrestrial biosphere (Benson & Surles 2006). From the years 1800-1994, an estimated 244 ± 20 gigatons (Gt) of carbon have anthropogenically been emitted into the atmosphere, with an additional 100-180 Gt carbon released into the atmosphere due to land changes (Sabine et al. 2004). Two-thirds of these emissions have remained in the atmosphere (165 ± 4 Gt carbon), while an estimated 61-141 Gt carbon have been taken up by the terrestrial biosphere (Sabine et al. 2004).

Oceans also act as a natural reservoir as CO_2 from the atmosphere equilibrates with the surface waters of the ocean (Sabine et al. 2004). Without this natural uptake into the ocean, the concentration of CO_2 in the atmosphere would be 55 ppm higher than it is today (Sabine et al. 2004). CO_2 can also be

stored in the oceans via the stimulation of phytoplankton photosynthesis, sequestering the carbon in biomass (Lal 2008).

Storage of CO_2 in saline formations and sedimentary basins has been investigated in detail. In many cases, sedimentary rock layers or formations are seen as highly favorable for storage, as the formations are porous, already containing plenty of space to store oil, water, and natural gas (Benson & Surles 2006). It is estimated that the terrestrial subsurface, which includes such formations in sedimentary basins, can store up to 14220 Gt of CO_2 (Orr 2009). It is estimated that stored CO_2 in saline formations and sedimentary basins leaks at a low rate (Schrag 2007). Indeed, oil, water, and natural gas have been contained in sedimentary basins for millennia. However, the capacity of old oil basins is limited and not of sufficient size to sequester the 1 trillion tons of CO_2 that must be stored over the next several hundred years (Lackner 2003; Schrag 2007). In addition to limited storage space, the storage lifetime of CO_2 in sedimentary basins is uncertain, as is the seismic stability of the systems. Another unknown is the potential migration of injected CO_2 in sedimentary basins (Lackner 2003). Thus, while storage in sedimentary basins holds promise, more research into the uncertainties of storage lifetime and storage security must be done.

Large igneous provinces (LIPS) consisting of mafic rocks such as basalts are another storage option for CO_2. Basalts are favorable for storage of CO_2 as the rock contains more of the minerals that are useful for the

geochemical trapping of CO_2 than sedimentary rocks (Orr 2009). Geochemical trapping occurs via the reaction of CO_2 with the host rock of geological reservoirs to form new stable minerals that contain CO_2 as carbonate. The geochemical trapping of CO_2 solves the problems of storage lifetime and security associated with sedimentary basins, as the geochemical trapping of CO_2 may permanently store CO_2 in mineral form. Basalts will be discussed in detail in a later section.

As shown above, numerous options exist for the capture and storage of CO_2. Several major CO_2 storage projects are currently in operation and four of these provide a proof of concept for storing CO_2 in sedimentary basins and saline formations. The Sleipner project, located in the North Sea near Norway, extracts natural gas from below the sea floor and sequesters 1 megaton (Mt) of CO_2 per year into deep subsea brines and sandstone formations (Benson & Surles 2006). Also utilizing depleted natural gas fields for storage is the In Salah project located in Algeria, which also sequesters around 1 Mt of CO_2 per year (Benson & Surles 2006). An enhanced oil recovery (EOR) project located in Saskatchewan, Canada transports CO_2 from a power plant located in North Dakota to the Weyburn oil fields where the CO_2 is pumped down into oil containing reservoirs to recover more oil from the formations (Benson & Surles 2006). Lastly, another EOR project in west Texas injects 30 Mt of CO_2 into oil reservoirs (Orr 2009). While the above

projects aide in the reduction of CO_2 emissions, their efforts must be combined with others so as to halt the immanent rise in the earth's temperature.

In order to reduce the level of CO_2 in the atmosphere to pre-industrial numbers, CO_2 needs to be sequestered at a rate of 10 Gt of CO_2 per year for approximately 50 years (Schrag 2007). Pacala and Socolow estimated that approximately 3500 Sleipners (3.5 Gt of CO_2 per year sequestered), in addition to other offsets, are needed to combat rising levels of CO_2 emissions (Pacala & Socolow 2004). The call for more CCS projects has been answered, and many CCS projects are in the pilot/planning stage in the United States, Canada, and Europe, with two EOR projects in the pilot stage in China. India currently has no plans to build CCS infrastructure (Scottish Carbon Capture and Storage 2011). As far as sedimentary basins are concerned, the knowledge, expertise, and infrastructure exist to make CCS a reality, solving the problem of increasing CO_2 concentrations in the atmosphere.

Geological carbon sequestration

General geological carbon sequestration. When CO_2 is injected into geologic media as part of a CCS project, it is injected in its supercritical form. Carbon dioxide turns supercritical at temperature and pressure conditions more than 31.1°C and 73.8 bar (Leitner 2000). Supercritical CO_2 also has strong solvent capabilities, has a high rate of diffusion, and has a low viscosity (Leitner 2000). Two mechanisms exist for trapping CO_2 in the subsurface: physical trapping and geochemical trapping. In physical trapping, low-permeability caprocks or

stratigraphic seals act as a barrier to the upwards migration of CO_2 towards the surface (Gunter et al. 2004). Geochemical trapping involves the long-term chemical reaction of host rock minerals with CO_2 saturated formation water to form stable minerals such as carbonates, thus storing the injected CO_2 in solid mineral form (Gunter et al. 2004). The following reactions generally characterize geochemical trapping in magnesium and calcium bearing rocks:

$$CO_2(g) \rightleftharpoons CO_2(aq)$$

$$CO_2(aq) + H_2O \rightleftharpoons HCO_3^- + H^+$$

$$Mg_2SiO_4(olivine) + 4H^+ \rightleftharpoons 2Mg^+ + 2H_2O + SiO_2$$

or

$$CaAl_2Si_2O_8(plagioclase) + 2H^+ + H_2O \rightleftharpoons Ca^{2+} + Al_2Si_2O_5(OH)_4$$

$$(Ca, Mg)^{2+} + HCO_3^- \rightleftharpoons (Ca, Mg)CO_3 + H^+$$

Geochemical trapping of CO_2 has been well documented in basalt, a type of mafic volcanic rock particularly suited for carbon sequestration due to the rocks' mineralization potential because it contains high contents of plagioclase, pyroxene, and olivine (Matter et al. 2007; McGrail et al. 2006; Schaef & McGrail 2009).

Geological carbon sequestration in basalts. Basalt may be ideal for storing CO_2 in CCS projects due to its availability, storage capacity, porosity and permeability, and, as mentioned above, geochemical trapping ability. Approximately 42% of the earths' surface is composed of mafic, magmatic rock such as basalt (Best 1995). Mafic rocks, like basalt, consist mostly of magnesium rich silicate minerals and other minerals containing iron. Basalt is

defined as containing approximately 50 wt% silica, and being composed of varying percentages of the basic minerals plagioclase, pyroxene, and olivine (Best 1995). Basalt is most commonly composed of plagioclase, and then to a lesser degree, pyroxene and olivine, as well as volcanic glass and spinel (J. Dilles, personal communication). Table 1.1 details the mineralogy of basalt, demonstrating that the minerals plagioclase, pyroxene, and olivine can be reactive with fluid to produce cations such as iron, calcium, and magnesium, which are essential in geochemical trapping. Indeed, basalts generally contain up to 25 wt% of iron, calcium, and magnesium as silicates and oxides (McGrail et al. 2006).

The extent of basalt is vast. Basalts are the major rocks of Large Igneous Provinces (LIPs), which cover thousands to millions of square kilometers of continental and oceanic crust (Saunders 2005). One of the best studied and characterized LIPs is the Columbia River Basalt Group (CRBG), which formed from fissure volcanic eruptions 17-6 Ma. The CRBG consists of 300 individual lava flows (Figure 1.1) and covers 164,000 square kilometers of land in Washington state, Oregon, and Idaho (Figure 1.2) (McGrail et al. 2006). While well studied, the CRBG is not the most extensive LIP; for comparison, the Deccan Volcanic Province in India covers 500,000 square kilometers of land (Tiwari et al. 2001).

Even though the CRBG is one of the smaller LIPs, the basalt formations are estimated to have the capacity to store up to 100 Gt of CO_2 (McGrail et al.

2006). The US emits CO_2 at a rate of 1.7 Gt of carbon per year; thus the CRBG alone could store over half a century's worth of CO_2 emissions (Goldberg et al. 2008). The large storage capacity of basalt is due in part to the porosity and permeability of basalt rock. Permeability can be implied through low values of hydraulic conductivity, which is a measure of the ease of flow of a fluid through a porous medium. The porosity of more permeable basalt is 5-35%, with a hydraulic conductivity of $4 \times 10^{-7} – 2 \times 10^{-2}$ m/s (Schwartz & Zhang 2003). The permeability of basalt is largely due to the interconnected pore spaces in basalt, as well as fractures and joints (McGrail et al. 2006). Thus basalts contain the necessary porosity and permeability to safely inject CO_2 at reasonable rates into geologic reservoirs for permanent storage (Matter & Kelemen 2009).

While basalts have some highly permeable sections, some impermeable basalt layers blanket the permeable zones into which $scCO_2$ will be injected, providing a physical mechanism for slowing the migration of the reacted CO_2 (McGrail et al. 2006). These basalt layers are composed of the interior portion of a basalt flow (entablature) and are termed cap rocks (McGrail et al. 2006) The entablature portion of a basalt flow has a much lower hydraulic conductivity than more permeable basalt, thus only allowing very slow vertical migration of the injected $scCO_2$ through interconnected pore spaces in basalt (McGrail et al. 2006).

Within the pore spaces of basalt, water-rock reactions occur which geochemically trap CO_2 in the deep subsurface. The olivine, pyroxene, and plagioclase in basalt offer the greatest geochemical trapping capacity (Matter & Kelemen 2009). As $scCO_2$ mixes with the formation water of the geologic reservoir, CO_2 turns into an aqueous state and forms bicarbonate:

$$CO_2(g) \rightleftharpoons CO_2(aq)$$

$$CO_2(aq) + H_2O \rightleftharpoons HCO_3^- + H^+$$

The acidic solution then acts on the minerals, such as plagioclase demonstrated below, releasing cations which can then react with the bicarbonate to form carbonates, whose formation are favored in basalt water-rock reactions (Goldberg et al. 2008; Matter et al. 2007; Schaef & McGrail 2009):

$$Na_x(Ca,Mg,Fe)_ySi_3AlO_8 + (x+2y+3)H^+ + 4H_2O \rightarrow xNa^+ + y(Ca,Mg,Fe)^{2+} + 3H_4SiO_4(aq) + Al^{3+}$$

$$(Ca,Mg,Fe)^{2+} + HCO_3^- \rightleftharpoons (Ca,Mg,Fe)CO_3 + H^+$$

Such water-rock reactions in basalt are mediated by environmental parameters such as pH and temperature. In general, dissolution rates of basalt decrease with increasing (basic) pH values . Unless the excess hydrogen ions are consumed, minor carbonate formation will not occur (Matter & Kelemen 2009). Temperature also plays a role in water-rock reactions. As temperature decreases, reaction rates decrease, so the longer a system will take to reach steady state (Schaef & McGrail 2009). However, under the right conditions, precipitation of carbonates can occur quickly (Matter et al. 2007).

In a field-scale test in a hydraulically isolated basalt aquifer, Matter and Takahashi injected CO_2 to discover that all of the CO_2 was stored in mineral form hours after injection (Matter et al. 2007), however, precipitation of carbonate depends on the reaction rate and the ratio of aqueous CO_2 to rock. Such geochemical trapping ability may meet the CCS goals of permanence, safety, and long-term storage (Matter & Kelemen 2009).

The Wallula pilot project

Within the CRBG a pilot CCS well has been drilled in order to investigate the feasibility of sequestering CO_2 in basalt formations. The Wallula pilot well is located in eastern Washington State on site at the Boise Paper Mill near the town of Wallula, Washington (+46° 6' 17.96", -118° 54' 56.96") (Figure 1.2). The well is nearly vertical and passes through three CRBG formations, each consisting of many individual lava flows: Saddle Mountain, Wanapum, and Grand Ronde (Figure 1.3). The Saddle Mountain formation is the most recent basalt that exists at the site of the Wallula pilot well. The Saddle Mountain formation resulted from basalt eruptions between 13.5 and 6 Ma (Johnson et al. 1993) and covers the depth interval of 13.4-322.5 meters below land surface (mbls) at the Wallula pilot well. The Wanapum formation erupted over a period of 1 Ma between 15.6 and 14.5 Ma and occurs from 322.5-531.6 mbls (Johnson et al. 1993). The deepest and oldest formation at the Wallula pilot well is the Grande Ronde. Basalt in the Grande Ronde formation is between 17 and 15.6 Ma and begins at 531.6 mbls

(Johnson et al. 1993). The Wallula pilot well ends in the Grande Ronde at a depth of 1252.7 mbls. The target CO_2 reservoir is contained in the Grande Ronde formation in the Slack Canyon and Ortley members between 827.8-886.9 mbls (Figure A.1). Two upper seals and one lower seal with low permeability provide a physical mechanism to contain the injected $scCO_2$ in the formation (McGrail et al. 2009). Currently $scCO_2$ has not been injected into the well due to the discovery of a permeable vertical structure near the injection site.

Care was taken in drilling the Wallula pilot well in order to provide pristine chemical and microbiological samples prior to $scCO_2$ injection. Samples were collected as the borehole was advanced, using a progressive *drill-and-test characterization* method. As the drill moved deeper through the basalt, an identified aquifer was packed off using inflatable packers to isolate the depth interval of interest. Chemical and microbiological samples were acquired at each isolated test zone (Figure 1.3). The packed intervals are rather long vertically; thus, the chemistry and microbiology is integrated over a long distance. Another method utilized to mitigate possible contamination is the use of an under-balanced drilling technique in which drilling fluid was not contaminating the surrounding environment. Lastly, three or more well volumes of water were purged before samples were collected. These three techniques ensure that the chemistry and microbiology samples acquired are representative of the formations from which they were acquired. The

geochemistry of the CRBG has been well characterized, however, much less is known about the microbiology of the system; particularly how the native microorganisms will react to CO_2 injection as part of a CCS project.

Microbiology of the subsurface

General microbiology of the subsurface. Microorganisms are ubiquitous on planet Earth and have adapted to life in the most extreme environments such as acidic hot springs, deep sea hydrothermal vents, the deep oceanic crust, and the deep subsurface (c.f. Fredrickson & Balkwill 2006; Mason et al. 2010). Living under extreme conditions, such as those found in the deep subsurface, present microorganisms with a unique set of challenges not encountered on the surface of Earth. Lack of sunlight, high temperatures, and limited access to nutrients define life in the deep subsurface (Pedersen 2000). In addition to the above factors, the deep subsurface has a large solid surface-area-to-water-volume ratio (and, many cells prefer to be attached to mineral surfaces) (Pedersen 2000). Due to the large solid surface-area-to-water-volume ratio, diffusion of nutrients occurs slowly through the subsurface and also limits life and how fast microorganisms can reproduce (Krumholz 2000). Despite the above challenges, microorganisms have managed to survive in the deep subsurface.

Microorganisms can be found to a total depth of 4 kilometers below land surface, which is the point at which a temperature of 125°C reaches the limits of known life (Whitman et al. 1998). Due to the correlation between

depth and temperature, cell numbers generally decline as depth increases (Onstott 2005; Parkes et al. 1994; Wang et al. 2008). The total number of microorganisms in the subsurface is estimated to be equal to 3.8-6 x 10^{30} cells, with the terrestrial subsurface yielding 0.25-2.5 x 10^{30} cells (Whitman et al. 1998). However, 97% of the total number of microorganisms in the subsurface occur at depths less than 600 mbls (Whitman et al. 1998). Thus the deep subsurface contains far fewer cells than the shallow subsurface. The estimated total number of microorganisms in the deep subsurface is 0.13 x 10^{30} cells (Whitman et al. 1998). Common concentrations of microorganisms in the terrestrial subsurface range from a low 10^{3} cells per mL of groundwater or g of sediment to 10^{8} cells per mL of groundwater or g of sediment (Pedersen 2000). While cell numbers provide insight into microbial abundance and demonstrate microbial ability to survive the harsh conditions of the subsurface, the numbers do not show who the most abundant organisms are or which metabolisms are the most successful for survival in the deep subsurface.

Numerous reviews and studies in recent years have explored the microbial diversity of the deep subsurface (Basso et al. 2009; Boyd et al. 2007; Chandler et al. 1998; Fredrickson & Balkwill 2006; Krumholz 2000; Lovely & Chapelle 1995; MacLean et al. 2007; Pedersen 2000; Zhang et al. 2005). Members of the *Proteobacteria*, *Firmicutes*, and *Actinobacteria* often comprise the majority of subsurface communities (Basso et al. 2009; Boyd et al. 2007;

Fredrickson & Balkwill 2006; MacLean et al. 2007; Zhang et al. 2005). Other bacteria that appear in the subsurface include members of *Cytophaga*, *Flexibacter*, *Bacteriodes*, *Planctomycetales*, *Spirochaetales*, and *Nitrospirae* (Fredrickson & Balkwill 2006; MacLean et al. 2007; Zhang et al. 2005). Fewer studies exist that investigate the microbial metabolisms most prevalent in the subsurface and most studies infer metabolism based on microbial phylogeny. However, a trend can be delineated, showing that subsurface conditions support particular microbial metabolisms. Sulfate reducing bacteria (SRB), acetogens, methanogens, iron reducing bacteria, nitrate reducing bacteria, and manganese reducing bacteria are all common in the subsurface environment, showing a general preference for oxidants other than oxygen (Basso et al. 2009; Fredrickson & Balkwill 2006; Krumholz 2000; Lovely & Chapelle 1995; MacLean et al. 2007; Pedersen 2000). Deep igneous rock aquifers, such as those being investigated in the Wallula pilot well in the CRBG, support autotrophic, hydrogen-dependent microbial communities which are unique to basalts (Fredrickson & Balkwill 2006; Krumholz 2000; Pedersen 2000).

Microbiology of basalts. Basalts are dominated by members of the *Proteobacteria* (Lehman et al. 2004; MacLean et al. 2007; Mason et al. 2008). Also common are members of the phyla *Firmicutes*, *Actinobacteria*, *Cytophaga*, *Flexibacter*, *Bacteroides*, *Spirochaetes*, and *Nitrospirae* (Lehman et al. 2004; MacLean et al. 2007; Mason et al. 2008). Genera that are

frequently observed in basalts are *Pseudomonas*, *Burkholderia*, *Brevundimonas*, *Acidovorax*, *Hydrogenophaga*, *Xanthobacter*, *Alcaligenes*, *Aurobacterium*, *Flavobacterium*, *Rhodococcus*, *Rhodobacter*, *Nocardia*, *Paenibacillus*, and *Micrococcus* (Lehman et al. 2004). Knowing which organisms reside in basalt provides some insight into common metabolisms of microorganisms in basalt. Methanotrophs appear to be abundant in the Snake River Plain Aquifer in Idaho (Erwin et al. 2005; Newby et al. 2004). Marine basalts may harbor unique ecotypes specific to basalts and contain genes for the following metabolisms: sulfate reduction, iron reduction, carbon fixation, methane production and oxidation, nitrogen fixation, ammonium oxidation, and nitrate and nitrite reduction (Mason et al. 2008). Continental basalts from a South African gold mine harbor sulfate reducing bacteria (SRB) and iron reducing bacteria (MacLean et al. 2007). Thus organisms utilizing single-carbon compounds (such as methane and carbon dioxide), sulfate, and iron define the geomicrobiology of general basalts.

The geomicrobiology of the CRBG, where the Wallula pilot well is located, has been characterized several times in the past. Microorganisms present in the alkaline formation water of the CRBG mirror those found in other basalts. Bacteria are more abundant than archaea (Fry et al. 1997) and common metabolisms include CO_2 reduction, methanogenesis, sulfate reduction, and iron reduction (Stevens et al. 1993). Unique to the CRBG and other mafic or ultramafic igneous rocks capable of the abiotic generation of

hydrogen are communities of microorganisms known as Subsurface Lithoautotrophic Microbial Ecosystems (SLiMEs) (Chapelle et al. 2002; Stevens & McKinley 1995; Takai et al. 2004). A SLiME is composed of a community of microorganisms which exists without the influence of sunlight; microorganisms in the community support themselves from inorganic electron donors, CO_2 as an electron acceptor, and geologically produced hydrogen. The SLiME hypothesis originated in the CRBG, where a microbial community consisting mainly of autotrophs, with few heterotrophs, existed (Stevens & McKinley 1995). This SLiME community was composed of acetogens, methanogens, and SRBs (Stevens & McKinley 1995). A similar hypothesis was developed in granitic aquifers, where a community of microorganisms largely consisting of acetogens, methanogens, iron reducers, and SRBs exists due to the presence of geologically produced hydrogen (Pedersen 1999). Thus the presence of geochemically produced hydrogen from water-rock interactions is key to sustaining microbial communities deep in the subsurface, where products from photosynthesis are non-existent or occur in low abundance. The SLiME hypothesized to exist in the CRBG was later challenged (Anderson et al. 1998); however, others recently claimed to have discovered SLiMEs in the Snake River Plain Aquifer (Chapelle et al. 2002) and in a deep sea hydrothermal field (Takai et al. 2004). Unique communities in the CRBG, such as SLiMEs, will be affected by the injection of $scCO_2$ as part of a CCS project.

Microbiology associated with supercritical CO_2

Microbial diversity has been studied in conjunction with CO_2, both in the ocean and on the land, and such studies provide insight into the events that may occur in the Wallula pilot well once $scCO_2$ is injected. One study investigated the biogeochemistry and microbial diversity of a microbial population associated with a natural $scCO_2$ lake near the Yonaguni Knoll IV hydrothermal field (Chiba et al. 2006). ANME-2 group archaea and members of the *Deltaproteobacteria* were the most abundant phylotypes identified in the liquid CO_2/CO_2-hydrate-bearing marine sediments (Chiba et al. 2006). Minor phylotypes included the archaeal Marine Benthic Group-D, *Gammaproteobacteria*, and *Epsilonprotebacteria* (Chiba et al. 2006). Identified microorganisms were closely related to known methanogens, SRBs, and sulfur- and or hydrogen-oxidizing chemolithoautotrphs (Chiba et al. 2006). Most notably, microbial numbers decreased by two orders of magnitude as the liquid CO_2/CO_2 hydrate interface was approached (Chiba et al. 2006), demonstrating the decline in microbial abundance near $scCO_2$. A study conducted in NE Slovenia near a natural CO_2 spring, or mofette, also saw a decline in the microbial population as areas of higher concentrations of CO_2 were approached (Videmsek et al. 2009). These two studies show that microorganisms may be challenged to adapt to high concentrations of CO_2. While few microorganisms may be able to adapt to high concentrations of

CO_2, the studies prove that some microorganisms can survive exposure to high levels of CO_2.

In addition to the natural systems mentioned above, artificial systems in the form of gaseous CO_2 injections into the subsurface exist and provide additional support that some microorganisms are able to adapt to high concentrations of CO_2. A pilot CCS project carried out in a siltstone/sandstone saline aquifer examined the microbial community using fluorescent *in situ* hybridization (Morozova et al. 2010). The microbial community was enumerated and characterized before, during, and after gaseous CO_2 injection into the target aquifer. Cell counts decreased significantly after CO_2 was injected, followed by a recovery to pre-injection cell abundance after 5 months of exposure to gaseous CO_2 (Morozova et al. 2010). After the CO_2 was injected into the system the SRB population declined until it was no longer detected (Morozova et al. 2010). During the decline of the SRB population, the archaeal population increased (Morozova et al. 2010), indicating that archaea may be able to adapt more readily to the more acidic conditions after CO_2 injection into an aquifer. After a five month period of exposure to CO_2, the SRB population returned in numbers greater than were seen prior to CO_2 injection, with archaea becoming undetectable (Morozova et al. 2010). Thus the injection of CO_2 into the subsurface may heavily influence the native microbial community, causing the extinction of some members, all the while

favoring the survival of others more adapted to low pH environments which will prevail after CO_2 injection.

Microbial mediated carbon sequestration

Those organisms able to survive and thrive after the injection of $scCO_2$ into the environment may be able to utilize the abundant CO_2 in the subsurface. Many microorganisms use CO_2 for growth and for energy purposes (Table 1.2). Autotrophs are organisms that are able to fix their own carbon, derived from CO_2, for growth (Madigan & Martinko 2006). A photoautotroph is a microorganism which utilizes sunlight as its sole source of energy and CO_2 as its sole source of carbon, while a chemoautotroph utilizes inorganic compounds as its sole source of energy and CO_2 as its sole carbon source (Madigan & Martinko 2006). Microorganisms living in the subsurface are most often classified as chemolithoautotrophs, which means these microorganisms obtain their energy through the oxidation of inorganic compounds and they utilize CO_2 as their sole source of carbon (Madigan & Martinko 2006).

In addition to using CO_2 for the production of energy and for fixing CO_2 into microbial biomass, cells can also aid in the sequestration of CO_2 by acting as nucleation sites for the formation of carbonate crystals (Dupraz et al. 2009). Experiments conducted with *Sporosarcina pasteurii*, an organism that aggressively precipitates calcium carbonate through urea hydrolysis, show that microbial cells act as nucleation sites on which carbonate can form;

specifically, calcite is molecularly linked to the cell walls of the bacteria (Dupraz et al. 2009). Thus the presence of microbial cells can enhance the precipitation of calcite.

Microorganisms also aid in the sequestration of CO_2 by forming biofilms (Cunningham et al. 2009; Mitchell et al. 2009). A biofilm is an attached community of microorganisms encapsulated in an adhesive material, usually composed of a polysaccharide matrix (Madigan & Martinko 2006). Experiments conducted at Montana State University provide evidence that microbial communities growing in biofilms can alter the permeability of porous media, slowing or preventing the migration of contaminants and CO_2. In one experiment, biofilms of *Shewanella frigidimarina* were shown to reduce the permeability of sandstone cores, thus sealing pore spaces through which material can otherwise move (Cunningham et al. 2009). The main instrument involved in the reduction of core permeability was the precipitation of calcite, which was aided by the presence of the microorganisms. Organisms that are able to hydrolyze urea added to a system are excellent at manipulating the pH of their environments, creating a more favorable solution chemistry (increased pH) for the precipitation of calcite (Fujita et al. 2008). In another set of experiments conducted by Mitchell et al, *S. frigidimarina* was once again grown in biofilms and shown to reduce core permeability in the presence of $scCO_2$ (Mitchell et al. 2009). While the numbers of *S. frigidimarina* decreased by 3 orders of magnitude over the course of the experiment, the numbers of

native microorganisms increased, demonstrating that native microorganisms from geological formations are more resilient to $scCO_2$ (Mitchell et al. 2009).

Calcite precipitation, and thus the subsequent storage of CO_2, occurs more readily in a basic environment. In addition to organisms increasing the pH of their environment via urea hydrolysis, microorganisms can also increase the pH through the reduction of iron compounds (Onstott 2005). Iron reducing microorganisms are of a particular interest as they have previously been detected in the CRBG (Stevens et al. 1993) and thus may be involved in the microbial enhanced sequestration of CO_2 in the Wallula pilot well. The reduction of Fe (III) will be more favorable in the more acidic conditions existing after the injection of $scCO_2$, thus Fe-reducers are likely to be stimulated (Onstott 2005). An active Fe-reducing community will aid in increasing the pH of the system (Onstott 2005), which ultimately favors calcite precipitation and thus the microbiologically enhanced sequestration of CO_2. Onstott's studies provide insight, however, the studies undergone were models/simulations; thus laboratory carbon sequestration simulations with native microorganisms are needed to understand how a microbial community will respond to CO_2 injection in geological reservoirs.

Summary

Geological carbon sequestration in basalts is a promising solution to the problem of increasing anthropogenic inputs of CO_2 into the earths' atmosphere. Basalts have been shown to provide all the necessary geological

and geochemical conditions to safely store CO_2, as the mafic rock possesses both physical trapping and geochemical trapping abilities. Microorganisms are numerous in the subsurface and basalts may harbor unique communities of microorganisms capable of living without the influence of photosynthesis. Injection of supercritical CO_2 into the subsurface affects the abundance and diversity of the community of microorganisms native to a CCS project site. However, many microorganisms are able to utilize the injected CO_2 and may possibly aid in the sequestration of CO_2. The Wallula pilot project in eastern Washington State allows us to investigate the effect of the injection of supercritical CO_2 on the *in situ* microbial community in the CRBG.

To understand how the microbial community in the Wallula pilot well changes in response to $scCO_2$ injection, a baseline community must first be established. Chapter 2 details the microbial community characterization of the Wallula pilot well prior to the injection of $scCO_2$. Cell concentration was determined via quantitative polymerase chain reaction (qPCR). The diversity of the microbial community present at each depth interval sampled was illuminated using pyrosequencing. Results of the microbial characterization of the Wallula pilot well are presented in Chapter 2.

Laboratory experiments simulating the injection of $scCO_2$ into CRBG formation water and basalt were performed in order to gain knowledge of how the natural microbial communities in the Wallula pilot well will respond to the injection of $scCO_2$. Our objective was to determine the effect of $scCO_2$

injection on CRB, CRBG formation water, and the natural microbial communities in CRB formation water. In order to accomplish this objective we utilized chemical, molecular, and visual techniques to analyze chemical, microbiological, and geological samples. Results of these studies are found in Chapter 3.

The two studies presented in this thesis demonstrate the ability of native basalt microorganisms to survive geological carbon sequestration experiments. The capability of native microorganisms in basalts to survive and participate in geological carbon sequestration has not been shown before, and such findings should be of interest to the microbiological community as well as the geological and engineering community, as microorganisms play an important role in the geological sequestration of carbon dioxide.

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Table 1.1. Mineralogy of basalt. Basalt is commonly contains about 50 wt% silica, with varying proportions of plagioclase, pyroxene, and olivine making up the rock mineralogy. Note the presence of the elements calcium, magnesium, and iron which are important for fueling precipitation reactions that are likely to be important during $scCO_2$ sequestration (data are adapted from Best 1995).

Mineral	Formula	Percent Composition (Volume %)
Silica (Quartz)	SiO_2	45-52 (weight %)
Plagioclase	$(Na, Ca)(Al, Si)_4O_8$	50-100
Ortho-pyroxene	$(Mg, Fe)SiO_3$	0-70
Clino-pyroxene	$(Ca, Na)(Mg, Fe, Al, Cr, Ti)(Si, Al)_2O_6$	0-70
Olivine	$(Mg, Fe)_2SiO_4$	40-80

Table 1.2. Microbial carbon metabolisms. Many microorganisms can autotrophically fix CO_2. Fixation of CO_2 via the Calvin-Benson cycle is the most common form of incorporating CO_2 into microbial biomass. Carbon fixation also occurs via the reductive tricarboxylic acid cycle and the 3-hydroxypropionate cycle. Other cycles/pathways involving CO_2 can be used for energy metabolism, such as the acetyl-CoA pathway or methanogenesis (revised from Madigan and Martinko 2006).

Cycle/Pathway	Reaction/Pathway
Chemoautotrophic assimilation of CO_2 via the Calvin-Benson Cycle	$6CO_2 + 12NADPH + 18ATP \rightarrow C_6H_{12}O_6(PO_3H_2) + 12NADP^+ + 9ADP + 17P_i$
Autotrophic fixation of CO_2 via the reductive tricarboxylic acid cycle	$3CO_2 + 12H + 5ATP \rightarrow triose - P$
Carbon fixation in the dark via the 3-hydroxypropionate cycle	$2CO_2 + 6H + 3ATP \rightarrow glyoxylate$
Acetogenesis via the acetyl-CoA pathway	$4H_2 + 2CO_2 \rightarrow CH_3CO_2H + 2H_2O$
Methanogenesis	$CO_2 + 8H_2 \rightarrow CH_4 + 2H_2O$

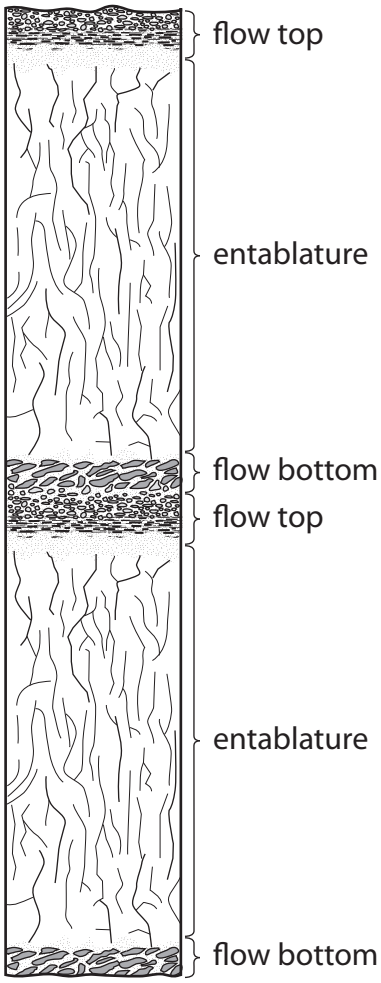


Figure 1.1. Anatomy of a basalt flow. Flow tops and flow bottoms are broken rubble zones containing ample porosity and permeability to store CO_2. The entablature is less porous and less permeable and may act as a seal between the different basalt flows (modified from McGrail et al 2006).

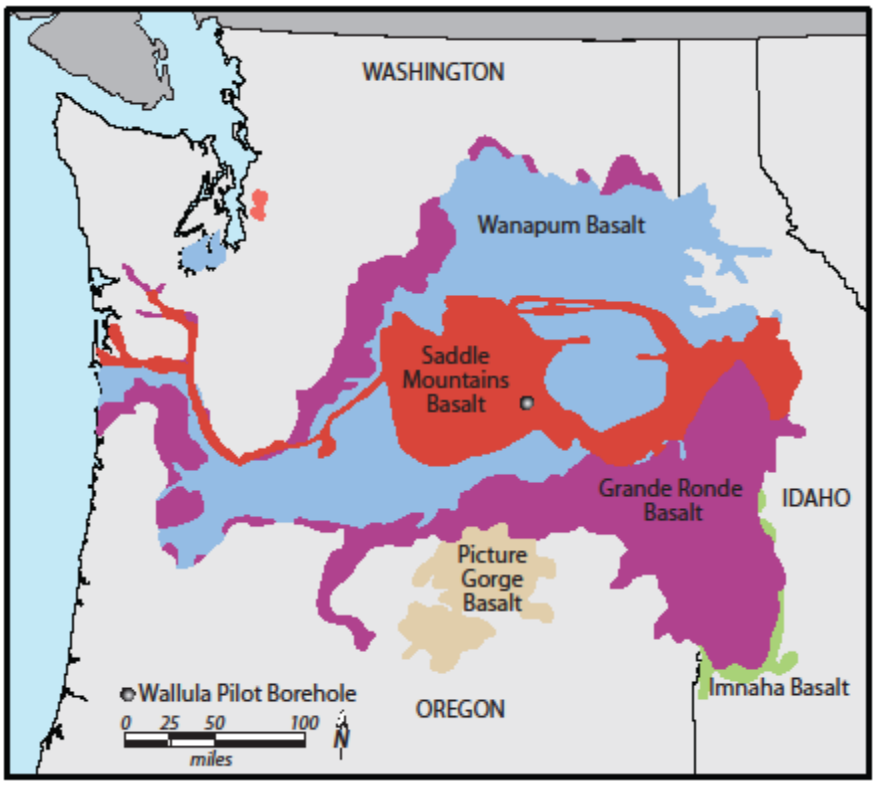
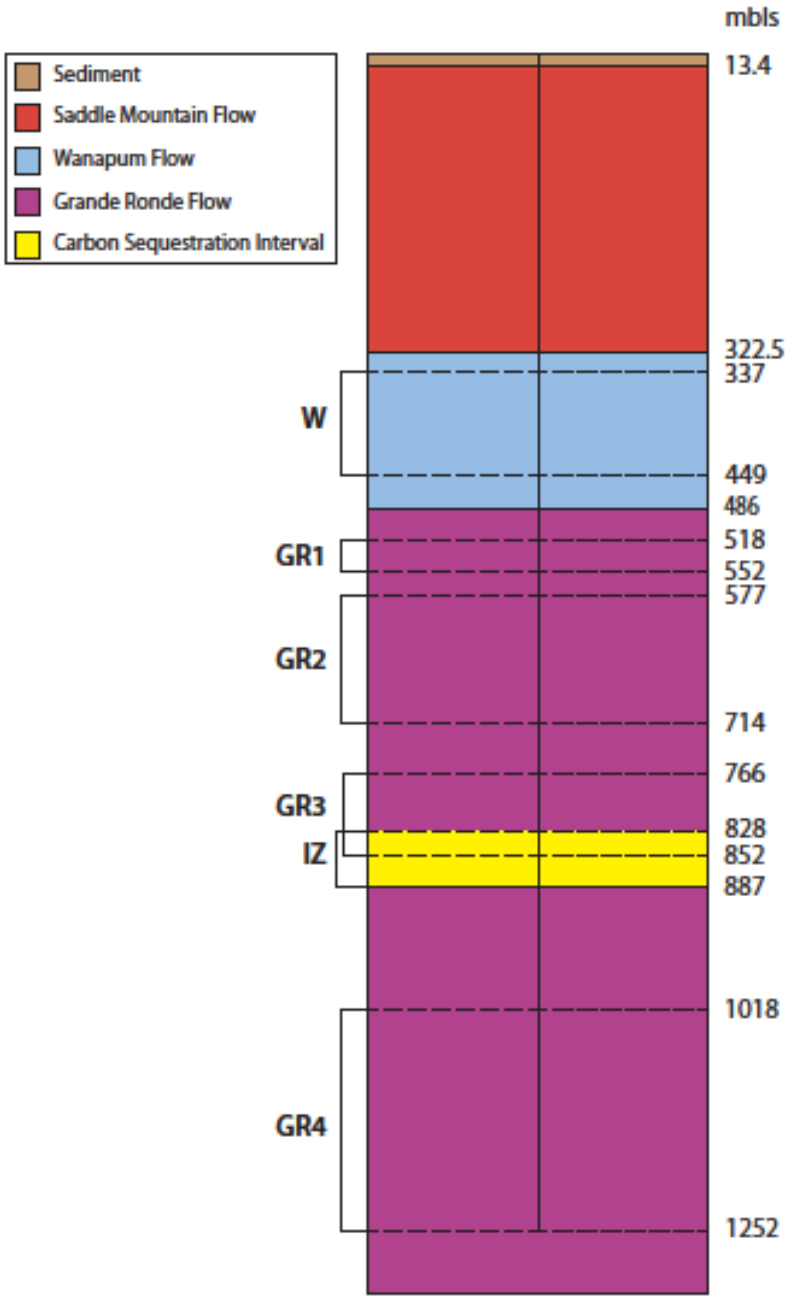


Figure 1.2. Map showing the aerial extent of the CRBG. The CRBG covers extensive portions of the states of Washington, Oregon, and Idaho. Several large basalt flows comprise the majority of the CRBG: the Saddle Mountain basalts, the Wanapum basalts, and the Grande Ronde basalts (modified from McGrail et al 2009).

Figure 1.3. Wallula pilot well stratigraphy showing penetration through three formations. The well terminates in the Ortley member of the Grande Ronde flow at a depth of 887 mbls, where $scCO_2$ is slated for injection. Total depth of the well prior to completion was 1252 mbls. Dashed lines indicate the sampling depth intervals from which water was collected for hydrochemical and microbiological analysis. For example, sample W was collected from the shallowest depth interval in the Wanapum formation over a depth interval of 337-449 mbls. The samples GR1, GR2, GR3, and GR4 were collected as the well was drilled deeper into the Grand Ronde formation over the respective depth intervals indicated in the figure. Sample IZ was collected from the future injection zone of the Wallula pilot well and its depth interval overlaps with that of sample GR3.


Sediment
Saddle Mountain Flow
Wanapum Flow
Grande Ronde Flow
Carbon Sequestration Interval
mbls
13.4
322.5
337
W
449
486
GR1
518
552
577
GR2
714
766
GR3
828
IZ
852
887
1018
GR4
1252

2. MICROBIAL CHARACTERIZATION OF BASALT FORMATION WATERS TARGETED FOR GEOLOGICAL CARBON SEQUESTRATION

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Abstract

Geological carbon sequestration in basalts is a promising solution to mitigate carbon emissions into the Earth's atmosphere. The Wallula pilot well in eastern Washington State provides an opportunity to investigate how native microbial communities in basalts are affected by the injection of supercritical carbon dioxide into deep, alkaline formation waters of the Columbia River Basalt Group. Our objective was to characterize the microbial communities at five depth intervals in the Wallula pilot well prior to CO_2 injection in order to establish a baseline community for comparison after the CO_2 is injected. Microbial communities were examined using quantitative polymerase chain reaction to enumerate bacterial cells and 454 pyrosequencing to compare and contrast the diversity of the native microbial communities. The deepest depth sampled contained the greatest amount of bacterial biomass, as well as the highest bacterial diversity. The shallowest depth sampled harbored the greatest archaeal diversity. Pyrosequencing revealed the well to be dominated by the Proteobacteria, Firmicutes, and Actinobacteria, with microorganisms related to hydrogen oxidizers, methylotrophs, methanotrophs, iron reducers, sulfur oxidizers, acetogens, and methanogens. Thus the Wallula pilot well is composed of a unique microbial community dependent on hydrogen and single-carbon compounds.

Introduction

Geologic carbon sequestration is considered a partial answer to the problem of increasing atmospheric concentrations of CO_2 which contribute to global climate change (Pacala & Socolow 2004). Geologic carbon sequestration consists of pumping supercritical CO_2 ($scCO_2$) into suitable saline reservoirs, into the deep ocean crust, or into reactive rock formations (Bickle 2009). The $scCO_2$ remains in place in a storage formation due to physical and geochemical trapping mechanisms. Physical trapping involves low-permeability cap rocks or stratrigraphic seals which act as a physical barrier to the upward migration of $scCO_2$ (Gunter et al. 2004). Geochemical trapping involves storage of CO_2 in mineral form via long-term chemical reactions between CO_2 saturated formation water and the host rock (Gunter et al. 2004). Among the several options available for geological carbon sequestration, basalts are one candidate.

Basalts are being investigated as potential geologic storage units for CO_2 due to the extent, storage capacity, porosity, permeability, and geochemical trapping ability of the igneous rock. The Columbia River Basalt Group (CRBG), where a pilot carbon capture and storage (CCS) well is located, extends over 164,000 km^2 and has a volume of 174,000 km^3 (McGrail et al. 2006). The storage capacity of basalt is largely due to the porosity and permeability of basalt which results from the interconnected pore spaces of the rock (McGrail et al. 2006). Geochemical trapping is more favorable in mafic

and ultramafic rocks like basalt, as basalt contains more reactive mineral species necessary for geochemical trapping (Orr 2009). Over time, as the injected $scCO_2$ mixes with the formation water of the geological reservoir in which it is stored, bicarbonate forms:

$$CO_2(g) \rightleftharpoons CO_2(aq)$$

$$CO_2(aq) + H_2O \rightleftharpoons HCO_3^- + H^+$$

The resulting acidic solution acts on the minerals present in basalt, such as plagioclase, releasing cations which then react with the bicarbonate to form carbonates:

$$Na_x(Ca, Mg, Fe)_y Si_3 AlO_8 + (x + 2y + 3)H^+ + 4H_2O$$

$$\rightarrow xNa^+ + y(Ca, Mg, Fe)^{2+} + 3H_4SiO_4(aq) + Al^{3+}$$

$$(Ca, Mg, Fe)^{2+} + HCO_3^- \rightleftharpoons (Ca, Mg, Fe)CO_3 + H^+$$

The formation of carbonates in basalt is more likely than in other geologic reservoirs (Goldberg et al. 2008; Matter et al. 2007; Schaef & McGrail 2009).

Given our knowledge that microorganisms exist in the subsurface, examinations of the geochemistry of CCS should include biologically active microbial communities. Several past studies have revealed the microbiology of the CRBG. Stevens et al used enrichment cultures to examine the presence of specific functional groups hypothesized to exist based on the geochemistry of the two aquifers being studied (Stevens et al. 1993). Both the planktonic and the attached communities were examined in the Priest Rapids

formation and the Grande Ronde formation of the CRBG. Acridine orange direct counts revealed that the Priest Rapids formation contained fewer microbes than the deeper Grande Ronde formation (Stevens et al. 1993). Iron (III) reducing enrichments and sulfate reducing enrichments produced the most biomass from the Grand Ronde, while methanogenic enrichments produced the most biomass from Priest Rapids (Stevens et al. 1993). Later, Stevens and McKinley conducted a survey of the metabolic capabilities of organisms from eight different aquifers in the CRGB and found that Fe (III) reducers were present at low numbers, fermentors were common, and acetogens and methanogens were ubiquitous (Stevens & McKinley 1995). Methanogen numbers were found to be low in high sulfate containing waters where sulfate reducers were present in high numbers (Stevens & McKinley 1995). Lastly, Fry et al characterized the microbial community present in two CRBG aquifers using taxon-specific oligonucleotide probes and a select clone library (Fry et al. 1997). Bacteria were found to be more abundant than archaea, while a small clone library (20 clones) made using primers specific for the deltaproteobacteria showed that 17 of the 20 clones were related to *Desulfovibrio* sp. strain PT-2 and *D. longreachii* (Fry et al. 1997).

These prior studies provide a glimpse into the microbial diversity of the CRBG at locations distant from the Wallula pilot well; however, they lack site-specific diversity data required in order to better understand how the microbial community will respond to the excess of carbon and the harsh geochemistry

associated with $scCO_2$ injection. Accordingly, our objective was to characterize the microbial communities present at five depths in the Wallula pilot well deeply and broadly in order to determine differences in the communities at each depth interval sampled. To accomplish this we enumerated bacterial cell numbers using qPCR and characterized the diversity of the microbial community using pyrosequencing.

Materials and Methods

Site description and contamination considerations. The Wallula Pilot well is located in Eastern Washington state on site at the Boise Paper Mill near the town of Wallula, Washington at +46° 6' 17.96", -118° 54' 56.96" (Figure 2.1a). The CRBG at the Wallula pilot well has been extensively characterized previously (McGrail et al. 2009) and information from the report is summarized below. The well was drilled vertically and intersects three CRBG formations: Saddle Mountain (13.4-322.5 mbls; 13.5-6 Ma), Wanapum (322.5-531.6 mbls; 15.6-14.5 Ma), and Grande Ronde (531.6-1252.7 mbls; 17-15.6 Ma) (Figure 2.1b). The proposed CO_2 injection zone is contained within the Grande Ronde formation in the Slack Canyon and Ortley members at 827.8-886.9 mbls (Figure A.1). Two upper seals and one lower seal expand the depth interval of CO_2 injection from 746.8 mbls-899.2 mbls (McGrail et al. 2009).

Hydrochemical and microbiological samples were collected from five depth intervals in the Wanapum and Grande Ronde formations by using a packer pump (Figure 2.1b). GR3 and IZ were most closely aligned with the

planned CO_2 injection zone. Samples W, GR1, GR2, GR3, and GR 4 were collected during the drilling of the well as the borehole was advanced using a progressive *drill-and-test* technique. As the drill moved deeper through the basalt, aquifers of interest were packed off using inflatable packers to isolate the aquifer of interest. An under-balanced drilling technique minimized the entry of drilling fluid into the formation. Instead, water entering from the rock into the borehole acted as the drilling fluid. Formation water samples were collected at the end of extensive pump tests, yielding water that should be more representative of the native conditions. Sample IZ was collected approximately a year after the borehole had been drilled after an extensive pump test.

Hydrochemistry analysis. Chemical analysis of formation water was performed by Pacific Northwest National Laboratory as previously described (McGrail et al. 2009). Samples were collected at the end of pumping periods and were delivered to the receiving laboratory, General Engineering Laboratories (Charleston, SC), as soon as possible. Formation water samples were analyzed for major anions, cations, and trace metals.

Microbiology sampling. On a given sampling date, formation water from the well was collected into sterile 20 L carboys (Nalgene, Rochester, NY) and varying amounts of water were pumped using a Masterflex® l/P® peristaltic pump with Masterflex® peroxide-cured silicone tubing, l/P® 73 (Cole-Parmer, Vernon Hills, IL) through a tripod filter holder onto a sterile 142 mm, 0.22 µm

PVDF Durapore® filter membrane (Millipore, Billerica, MA). Filtration occurred in the field within 1-2 hrs of water collection. Samples W, GR1, GR2, GR3, and GR 4 were acquired using a sterile acrylic in-line filter holder (Geotech, Denver, CO). Sample IZ was acquired using a sterile aluminum in-line filter holder (Geotech, Denver, CO). After filtration, filters were aseptically placed in a VWR® sterile sample bag (VWR International, Radnor, PA) and transported back to OSU on dry ice, then stored at -80°C until DNA extraction.

Nucleic acid extraction. Total microbial DNA was extracted from filters in duplicate using a PowerMax Soil DNA Isolation kit (Mo Bio Laboratories, Carlsbad, CA) in duplicate following the manufacturer's protocol with modifications as follows. Crushed filters in 50 mL falcon centrifuge tubes were heated at 65°C for 10 min, then subjected to bead-beating for 8 min. Extracted DNA was concentrated using Amicon Ultra centrifugal units (Millipore, Billerica, MA) and duplicate samples were pooled. DNA concentration was measured using a Qubit® (Invitrogen, Inc., Carlsbad, CA) according to the manufacturer's recommendations.

Bacterial quantification. To estimate copy numbers of bacterial 16s rRNA genes, quantitative PCR (qPCR) was performed with Power SYBR Green PCR Master Mix on an ABI PRISM® 7500 FAST Sequence Detection System following the manufacturer's protocol, with modifications. Each 20 µL reaction, performed in triplicate, consisted of 1X SYBR® Green PCR Master Mix (Applied Biosystems, Carlsbad, CA), 900 nM 27F primer (Eurofins MWG

Operon, Huntsville, AL) (DeLong 1992), and 50 nM 338R primer (Amann et al. 1990) (Eurofins MWG Operon, Huntsville, AL). Initial denaturation occurred at 95°C for 1min. Cycles of denaturation at 95°C for 15 s, annealing at 60°C for 1 min, and extension/data collection at 81°C for 1 min occurred 40 times. Disassociation occurred according to the manufacturer's suggestions.

Pyrosequencing. Pyrosequencing of extracted and pooled DNA was performed at the Marine Biological Laboratory (Woods Hole, MA) using the Roche Titanium platform (454 Life Sciences, Branford, CT) as previously described (German et al. 2010). PCR amplicons of the bacterial and archaeal V4-V6 hypervariable rRNA region were generated (German et al. 2010). Sequences were deposited in the VAMPS database (https://vamps.mbl.edu).

Data analysis. Pyrosequence data was processed using the QIIME pipeline (http://qiime.sourceforge.net/), which incorporates Unifrac (Lozupone & Knight 2005) to allow statistical analyses of smaller sample sizes. Sequences underwent a rigorous quality control process following standard practice and the algorithms incorporated into QIIME prior to operational taxonomic unit (OTU) picking. Sample barcode and primer sequences were trimmed from the proximal and distal ends of the reads. Reads which did not match the reverse primer sequence and reads which could not be assigned to a sample were discarded. Any read shorter than 50 nucleotides or containing one or more ambiguities was removed, as well as reads which could not be assigned to a domain. Sequences were assigned taxonomy at 97% similarity using the RDP

classifier. Analysis of the alpha (microbial diversity at the local scale) and beta (microbial diversity among the different samples) diversity of the microbial communities was carried out using QIIME (Caporaso et al. 2010).

Results

Hydrochemistry. Each depth interval sampled was of similar hydrochemical character and was described as generally dilute, sodium-bicarbonate chemical water-type (McGrail et al. 2009). Formation water from each depth interval exhibited elevated pH (9.0-9.7), fluoride, bicarbonate, and sodium (McGrail et al. 2009) (Table 2.1).

Microbiological quantification. The quantity of DNA extracted from the filters ranged from 0.001-0.171 ng per µL. Bacterial cell numbers, as determined by qPCR, demonstrated that the Wallula pilot well formation waters contained a range of abundance of bacteria, with cell concentrations ranging from 3.3 ± 0.2 x 10^3 cells mL^{-1} of formation water to 4 ± 2 x 10^8 cells mL^{-1} of formation water (Table 2.1). The deepest depth interval sampled (1018-1252 mbls) harbored the greatest amount of microbial biomass. Cell concentrations at the depth of CO_2 injection (766-886.9 mbls) changed over time, from 3.3 ± 0.2 x 10^3 cells mL^{-1} formation water during the first sampling to 2 ± 1 x 10^7 cells mL^{-1} formation water after the well was developed.

Microbiological alpha diversity. After the quality control process described in the methods, 70610 reads were classified for the bacteria, with 23 reads

unclassified at the phylum level. For the archaea, 6941 reads were classified, with 4917 matching to bacteria and the majority of the archaeal reads (14755) remaining unclassified at the domain level.

Rarefaction analysis of the bacterial community showed GR1 and GR3 to be relatively well sampled, as indicated by the slight leveling in the curve, whereas GR4 was not sampled as well, as shown by the steeper nature of the curve (Figure 2.2).

Dominant (i.e., greater than 30% of the identified taxa) bacterial phyla found were Proteobacteria, Firmicutes, and Actinobacteria (Figure 2.3). Proteobacteria contributed to greater than 50% of the taxa of the bacterial community of the first four sampling depths of 337-449 mbls, 518-552 mbls, 557-714 mbls, and 766-852 mbls, making up 98.1%, 98.5%, 95.1%, and 88.9% of the community, respectively. The second sampling of the depth interval of 827.8-886.9 mbls saw a shift in the bacterial community, with Proteobacteria composing 62.2% of the taxa and Actinobacteria composing 30.1% of the taxa. The deepest depth interval was dominated by Firmicutes at 56.8% of the bacterial taxa, with the Proteobacteria comprising only 30.7% of the community. Other identified bacterial phyla forming less than 30% of the population included Bacteroidetes, Chloroflexi, Cyanobacteria, Fusobacteria, Spirochaetes, TM7, and Verrucomicrobia (Figure 2.3). The alpha diversity of each depth interval is detailed below for taxa composing greater than 5% of the reads. See appendix A for additional alpha diversity considerations.

The shallowest sample (W (337-449 mbls)) was composed of mainly beta- and gammaproteobacteria. *Thiovirga* contributed to 45.1% of the taxa, while *Acinetobacter* and *Aquabacterium* composed 15.5% and 11.6% of the identified taxa, respectively. *Hydrogenophaga* comprised 6.0% of the number of taxa from Sample W.

Although all of the other samples were obtained from the water collected in the Grande Ronde flow unit, substantial differences were observed in community characteristics. Sample GR1 (518-552 mbls) was mainly composed of alpha-, beta-, delta-, and gamma-proteobacteria. The genera *Methylotenera* and *Hydrogenophaga* represented 31.4% and 23.7% of the taxa obtained from GR1. *Methylomonas*, *Aquabacterium*, *Desulfromonas*, and unclassified Comamonadaceae composed 7.9%, 7.9%, 5.6%, and 5.5% of the identified taxa, respectively.

Sample GR2 (577-714 mbls) was composed of mainly alpha-, beta-, delta-, and gamma-proteobacteria, as well as Firmicutes. *Hydrogenophaga*, *Methylotenera*, *Desulfuromonas*, and *Croceicoccus* composed 20.1%, 13.6%, 10.1%, and 10.1% of the taxa from Sample 3, respectively. *Methylomonas* and unclassified Comamonadaceae made up 9.6% and 9.3% of the identified taxa from GR2, respectively.

Sample GR3 (766-852 mbls) was mainly composed of alpha-, beta-, delta-, and gamma-proteobacteria, as well as a members of the Clostridia and the Actinobacteria. The genera *Hydrogenophaga*, *Methylotenera*, and

Desulfuromonas contributed to 33.0%, 10.6%, and 9.8% of the taxa from GR3, respectively. Unclassified Comamonadaceae and *Croceicoccus* made up 8.8% and 6.5% of GR3, respectively.

Sample GR4 (1018-1252 mbls) was mainly composed of alpha- and beta-proteobacteria, as well as Clostridia, Erysipelotrichales, Bacterodia, Chloroflexi, and Verrucomicrobia. Unclassified Veillonellaceae, unclassified Clostridiales, and *Symbiobacterium* made up 17.3%, 15.3%, and 14.0% of the bacterial taxa from GR4, respectively. The genera *Holdemania* composed 5.4% of the number of taxa from GR4, respectively.

Sample IZ (827.8-886.9 mbls) was mainly composed of alpha-, beta-, and gamma-proteobacteria, as well as Actinobacteria, Bacteroidetes, and Clostridia. The genera *Malikia* and *Olsenella* composed 46.4% and 27.2% of the bacterial taxa for IZ, respectively.

Rarefaction analysis showed that the archaea were poorly sampled relative to the bacteria (Figure 2.2). The dominant (i.e., greater than 50% of the identified taxa) archaeal phylum was Crenarchaeota across most of the depths sampled (Figure 2.3). Samples W, GR2, GR3, and GR4 contained the greatest amount of Crenarchaeota, making up 68.8%, 57.9%, 63.0%, and 65.0% of the archaeal phyla from these depths, respectively. All Crenarchaeota fall into the archaeal class Thermoprotei, which then is further broken down to four different orders: Acidobales, Desulfurococcales, Thermoproteales, and others, with the majority (between 25% and 56% of the

OTU's) from each depth interval being in the "other" category. Euryarchaeota composed 70.5% of the population of sample GR1 and 58.1% of the population of sample IZ.

Sample W (337-449 mbls) was mainly composed of the Crenarcheaotal genus *Thermoprotei*, which contributed to 53.7% of the taxa. Unclassified Euryarchaeota, unclassified Archaea, *Methermicrococcus*, and *Thermofilium* composed 9.8%, 7.6%, 6.6%, and 6.4% of the archeal taxa identified from W.

The majority of sample GR1 (518-552 mbls) was composed of *Methermicoccus*, which made up 57.4% of the taxa. The unclassified Thermoprotei and Methanosarcinales comprised 27.9% and11.5% of GR1, respectively.

Sample GR2 (577-714 mbls) mainly contained unclassified Thermoprotei and unclassified Archaea at 45.4%, and 31.1%, respectively. *Thermofilium* and *Thermogymnomonas* composed 6.0% each of the identified archaeal taxa from GR2.

Sample GR3 (766-852 mbls) was made up of mainly three identified OTU's: the unclassified Thermoprotei at 55.6%, the unclassified Archaea at 37.0%, and the unclassified Desulfurococcales at 7.4%.

Sample GR4 (1018-1252 mbls) mainly consisted of unclassified Thermoprotei, at 53.5% of the indentified taxa. Unclassified Archaea,

unclassified Desulfurococcales, and unclassified Euryarchaeota made up 19.9%, 9.3%, and 8.4% of the taxa from GR4, respectively.

Sample IZ (827.8-886.9 mbls) mainly contained *Methanobacterium* at 34.2% of the taxa and unclassified Thermoprotei at 30.7% of the taxa. Unclassified Euryarchaeota composed another 12.7% of the taxa from IZ. *Methermicoccus* and unclassified Archaea composed 8.6% and 8.0% of the number of identified taxa from IZ, respectively.

Bacterial beta diversity. The most bacterially diverse formation was the deepest (1018-1252 mbls) with 86 bacterial phylotypes recognized. Twelve bacterial phylotypes appear in all six of the samples: unclassified Clostridiales, *Phenylobacterium*, *Rhodobacter*, *Croceicoccus*, unclassified Erythrobacteraceae, *Hydrogenophaga*, *Hylemonella*, unclassified Comamonadaceae, unclassified Burkholderiales, *Methylotenera*, *Desulfuromonas*, and *Methylomonas*. Seven bacterial phylotypes were unique to W, three bacterial phylotypes were unique to GR1, eight bacterial phylotypes were unique to GR2, nineteen phylotypes were identified as unique to GR3, GR4 contained twenty-six unique phylotypes, and IZ was composed of twelve unique bacterial phylotypes (Table 2.2). At the 3% difference level, a UPGMA tree calculated from weighted UniFrac distances (Figure 2.4a) showed samples GR1, GR2, and GR3 grouped together, indicating similarity between these sampling depths. Samples W and GR4 did not group closely

with any of the other samples. Sample IZ was closely linked to GR1, GR2, and GR3.

Archaeal beta diversity. Sample W contained the most diverse archaeal community, consisting of 15 identified OTU's. Two archaeal phylotypes appeared in all six samples: the unclassified Desulfurococcales and the unclassified Thermoprotei. Samples GR1, GR2, and GR3 contained no archaeal phylotypes unique to their respective depth intervals. Sample W contained three unique phylotypes: the unclassified Archaeoglobaceae at 0.20%, the genus *Methanopyrus* at 0.10%, and the unclassified Thermoplasmatales at 0.10%. Sample GR4 contained one phylotype unique to that particular depth interval: the genus *Methanosaeta* at 0.90%. Sample IZ also was composed of one unique phylotype: the genus *Methanobacterium* at 34.20%. At the 3% difference level a UPGMA tree calculated from weighted UniFrac distances (Figure 2.4b) showed samples W and GR2 together, with sample GR3 completing the group which indicated similarity among the samples. Samples GR1 and IZ are related to each other, while sample GR4 groups with no other sample.

Discussion

Geological carbon sequestration is being considered as a viable option for reducing concentrations of CO_2 emitted into the atmosphere. Microbial communities may play a pivotal role in meeting the CCS goals of permanence, safety, and long-term storage (Bénézeth et al. 2009) of CO_2 injected into

geologic material. This study provides an investigation of the microbiology of a CCS pilot well in basalt prior to CO_2 injection and establishes a baseline microbial community to which samples acquired after the injection of CO_2 can be compared.

Microbiological quantification. The Wallula pilot well is located in the CGRB and cell concentrations would be expected to follow the general trend of decreasing cell concentrations as depth increases (Onstott et al. 1999; Parkes et al. 1994). However, this was not the case for the Wallula pilot well. The deepest depth harbored the greatest amount of bacterial biomass, at $4 \pm 2 \times 10^8$ cells mL^{-1}, while the shallowest depth contained $8 \pm 2 \times 10^5$ cells mL^{-1}. A previous study of the microbiology of the CRBG also reports higher cell numbers for the deeper Grande Ronde formation than for the shallower Priest Rapids member in the Wanapum formation (Stevens et al. 1993). Using the acridine orange direct count method, they found the deeper Grande Ronde formation water contained $7.6 \pm 1.4 \times 10^5$ cells mL^{-1} and the shallower Priest Rapids aquifer held $3.6 \pm 1.4 \times 10^3$ cells mL^{-1}. The difference between the two studies may be due to the different location of the wells in the CGRB, the way in which samples were collected, or how the microorganisms were enumerated (AODC versus qPCR). The qPCR approach used to analyze samples from the Wallula pilot well could have over-estimated the number of microorganisms due to an inaccurate plasmid standard (Hou et al. 2010). However, qPCR is most likely to underestimate cell abundance due to less

than 100% DNA extraction efficiency or PCR inhibitors (Zhang & Fang 2006). We attempted to minimize these factors by using an optimized DNA extraction and qPCR reaction.

Despite the differences observed between the two wells in the CGRB, data shows that the deeper Grande Ronde formation sustains a larger microbial population than the shallower surface formations. Others have observed such a deviation in different subsurface environments. For example, higher cell concentrations have been observed below the seafloor at the sulfate-methane transition zone (D'Hondt et al. 2004). Higher cell counts have also been observed at greater depths in the Fennoscandian shield (Hallbeck & Pedersen 2008). A proposed reason for this deviation from the normal decrease in cell abundance with depth is a correlation between organic carbon content and cell density (O'Connell et al. 2003; Parkes et al. 2005). Studies of trichloroethylene (TCE) contaminated wells in the basaltic Snake River Plain aquifer found higher cell numbers in waters containing elevated concentrations of dissolved organic carbon (O'Connell et al. 2003). In the marine subsurface Parkes et al confirmed a greater abundance of microorganisms in the presence of higher percentages of organic carbon (Parkes et al. 2005). Thus the reason for a greater population of bacteria in the deeper Grande Ronde formation may be the higher concentrations of organic carbon measured at depth, for both this study as well as the 1993 study (Stevens et al. 1993).

Microbiological alpha diversity. Results show that the Wallula pilot well is dominated by Proteobacteria, Firmicutes, and Actinobacteria for the bacteria and Crenarchaeota for the archaea. While it may be tempting to compare our results to those who have studied the CRBG previously, the previous studies did not use molecular techniques to examine the whole community, thus no whole community taxa specific data is available for comparison. Fry et al did construct a specific desulfovibrio/metal-reducing group clone library, which demonstrated the dominance of desulfovibrios over other metal-reducing bacteria (Fry et al. 1997). At the Wallula pilot well, *Desulfovibrio* was identified only in sample IZ and contributed to less than 0.10% of the reads. Thus insufficient data from the CRBG is available for comparison. However, both terrestrial and marine basalts have been extensively characterized in other regions and can provide a base for comparison. Proteobacteria are abundant in the subsurface and comprise the majority of identified taxa in basalts (Lehman et al. 2004; MacLean et al. 2007; Mason et al. 2008). Firmicutes are not unusual to find in the deep subsurface (Briggs et al. 2012; Lin et al. 2006; Morozova et al. 2010; Rastogi et al. 2009), as members of this phylum can form endospores, allowing them to survive under harsh conditions over geologic time scales, as is the case with *Bacillus* type 2-9-3 (Vreeland et al. 2000). The greater abundance of Crenarchaeota was surprising, as this phylum is traditionally associated with hyperthermophiles and cold-dwelling archaea. But Crenarchaeota were found to compose the majority of the

archaeal population in TCE contaminated wells in the basaltic Snake River Plain Aquifer, where12 of the 22 clones relating to the archaea matched with non-thermophilic Crenarchaeota (O'Connell et al. 2003).

Metabolisms of the microorganisms most closely related to the taxa that we detected may be inferred, but not confirmed by our study. However, the related metabolisms provide a useful base for comparison to what types of microorganisms have been found in the CGRB and other basalts before. Methanotrophs have been previously detected in the Snake River Plain Aquifer, a basaltic aquifer system located in Idaho (Erwin et al. 2005; Newby et al. 2004). Enrichment studies from the CRBG reveal the presence of iron reducers, sulfate reducers, methanogens, and acetogens (Stevens et al. 1993; Stevens & McKinley 1995). Hydrogen oxidizers, methylotrophs, sulfur reducers, methanotrophs, acetogens, and methanogens are all implied metabolisms indicated by our results and show a dependence on hydrogen, single carbon compounds such as methane, and sulfur; key components to the deep biosphere that sustain deep life. Our results do not show a great number of iron reducers or any sulfate reducers, which made up the majority of the population of the CGRB in enrichment studies performed by Stevens et al (Stevens et al. 1993). The discrepancy may be due to the fact that the iron reducers and sulfate reducers appear dominant in culture studies if they are easier to culture than other subsurface dwelling microorganisms. Microbes related to the genus *Hydrogenophaga*, a known hydrogen-oxidizing bacterium,

were abundant and/or present in each of the depth intervals sampled, perhaps providing evidence of the importance of hydrogen in the dark, deep subsurface (Pedersen 1999; Stevens & McKinley 1995).

Microbiological beta diversity. Results also show that for the bacterial community, Sample GR4 contained the highest diversity**.** For the archaeal community, Sample W had the highest diversity**.** Even though the groundwater sampled at all of the depth intervals was classified as being typical of CGRB formation water, some differences are apparent (see Table 2.1)**.** For example, the deepest depth had higher values for dissolved organic carbon, iron, and sulfate than the other depths sampled, while the shallowest depth had the lowest values, with dissolved organic carbon and sulfate values below the detection limit. Such conditions at the shallowest depth favor the archaeal methanogens**.** Thus the reason for the higher bacterial diversity at the deepest depth may be related to the availability of nutrients (Torsvik et al. 2002). A study of an ancient (15.8-25.0 Ma), deep (2.8 km) fractured basalt aquifer in a South African gold mine also found nutrient concentrations to be higher at depth than the shallower subsurface above, suggesting an energy rich deep biosphere (Lin et al. 2006).

Bacterial beta diversity analyses indicated that the microbial community sampled at the proposed depth of carbon sequestration changed over time. While these two samples, GR3 and IZ, did not cover the exact same depth interval, the depth intervals that were sampled overlapped spatially with each

other, and therefore might be expected to sample the same microbial community. The community could have changed over time simply due to the fact that examination of the deep subsurface (i.e., drilling, pumping, well development) quite likely alters the hydrological and hydrochemical environment (Hirsch et al. 1995). Indeed, an investigation of the basaltic Snake River Plain Aquifer found a greater biomass of microorganisms in wells incubated with dialysis chambers compared to wells sampled with cores and filtered groundwater (Lehman et al. 2004). Thus, by studying a well and introducing non-native components, we alter the natural microbial community present in the well.

Implications for Carbon Sequestration. The injection of $scCO_2$ into the Wallula pilot well will most certainly initially alter the microbial community present at the depth of proposed carbon sequestration. After a period of time, the microbial community may adapt to the changes and return to its pre-injection composition (Morozova et al. 2010; Wandrey et al. 2011). An initial increase in the population of autotrophic microorganisms is expected to occur with the addition of CO_2 to the system. The methanogens already present, such as *Methermicoccus* and *Methanobacterium*, may dominate the microbial community for a period of time after the injection of CO_2 into the system, as was seen in a carbon sequestration study performed in a saline aquifer where the microbial community was monitored using fluorescence *in situ* hybridization (Morozova et al. 2010). Microorganisms not previously detected

in the system or part of the rare biosphere may become more abundant after the injection of $scCO_2$ (Gulliver & Gregory 2011; Lavalleur et al. unpublished results). For example, the genus *Marinobacter* became prevalent in saline formation water exposed to varying levels of CO_2 partial pressures in laboratory experiments simulating the Arbuckle aquifer (Gulliver & Gregory 2011). Although our results suggest that the Fe (III) reducing microbial community is not prevalent in the Wallula pilot well, microbial Fe (III) reduction may be stimulated by the injection of CO_2 into the groundwater, as modeled by Onstott (Onstott 2005). An increase in iron reduction is expected to lead to an eventual increase in the pH of the system (Onstott 2005), which aids in the precipitation of carbonates and thus the storage of the injected CO_2 in mineral form.

In summary, the Wallula pilot well has provided a unique opportunity to examine the microbial diversity of basalts prior to geological carbon sequestration. Distinct communities were found at each depth sampled, including a zone that is targeted for CO_2 injection. Future studies will involve the sampling of the microbial communities after the injection of CO_2 to examine how the native microorganisms respond to the presence of excess carbon. The addition of CO_2 to the system may stimulate the autotrophic population present in the system, particularly the methanogens (Morozova et al. 2010), resulting in the conversion of CO_2 to methane.

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Table 2.1. Select hydrochemical values important in microbiology and cell counts from the depth intervals sampled in the Wallula pilot well.

Sample	Depth Interval (mbls)	Sample Date	Volume Of Water Filtered (L)	DOC (mg L^{-1})	Fe^{2+} (µg L^{-1})	NO_3^- (mg L^{-1})	NO_2^- (mg L^{-1})	pH	SO_4^{2+} (mg L^{-1})	Specific Conductivity (µmhos cm^{-1})	Temp (°C)	Cell Counts (cells mL^{-1})[c]
W	337-449	2/13/09	10	0[a]	174	0[a]	0[a]	9.1	0[a]	314	19.9-22.9	$8 \pm 2 \times 10^5$
GR1	518-552	2/20/09	3	1.01	438	0[a]	0[a]	8.99	0.363	320	24.7-25.7	$4.8 \pm 0.7 \times 10^3$
GR2	577-714	3/1/09	2.15	0.672	223	0[a]	0[a]	8.95	0.445	320	26.3-30	$9 \pm 9 \times 10^5$
GR3	766-852	3/9/09	3	N/A[b]	411	0[a]	0.035	9.51	0.199	376	34.7[d]	$3.3 \pm 0.2 \times 10^3$
GR4	1018-1252	4/13/09	0.63	1.87	764	0.084	0[a]	9.22	1.11	529	38.1-44.4	$4 \pm 2 \times 10^8$
IZ	828-887	3/4/11	5	0.665	583	0[a]	0[a]	10.1	0.96	451	34.7[d]	$2 \pm 1 \times 10^7$

[a] 0 value indicative of below detection limit
[b] Data not available/not reported
[c] Average cell concentration as determined by triplicate qPCR reactions of each sample; ± standard deviation
[d] Temperature calculated using a geothermal gradient of -0.147°F ft^{-1} based on observed measurements within the Wallula pilot well (McGrail et al, 2009)

Table 2.2. Bacterial beta diversity of the Wallula pilot well demonstrating unique OTUs found at each depth. Identified OTUs at the 3% difference level compose less than 3% of the reads from each depth interval

Table 2.2

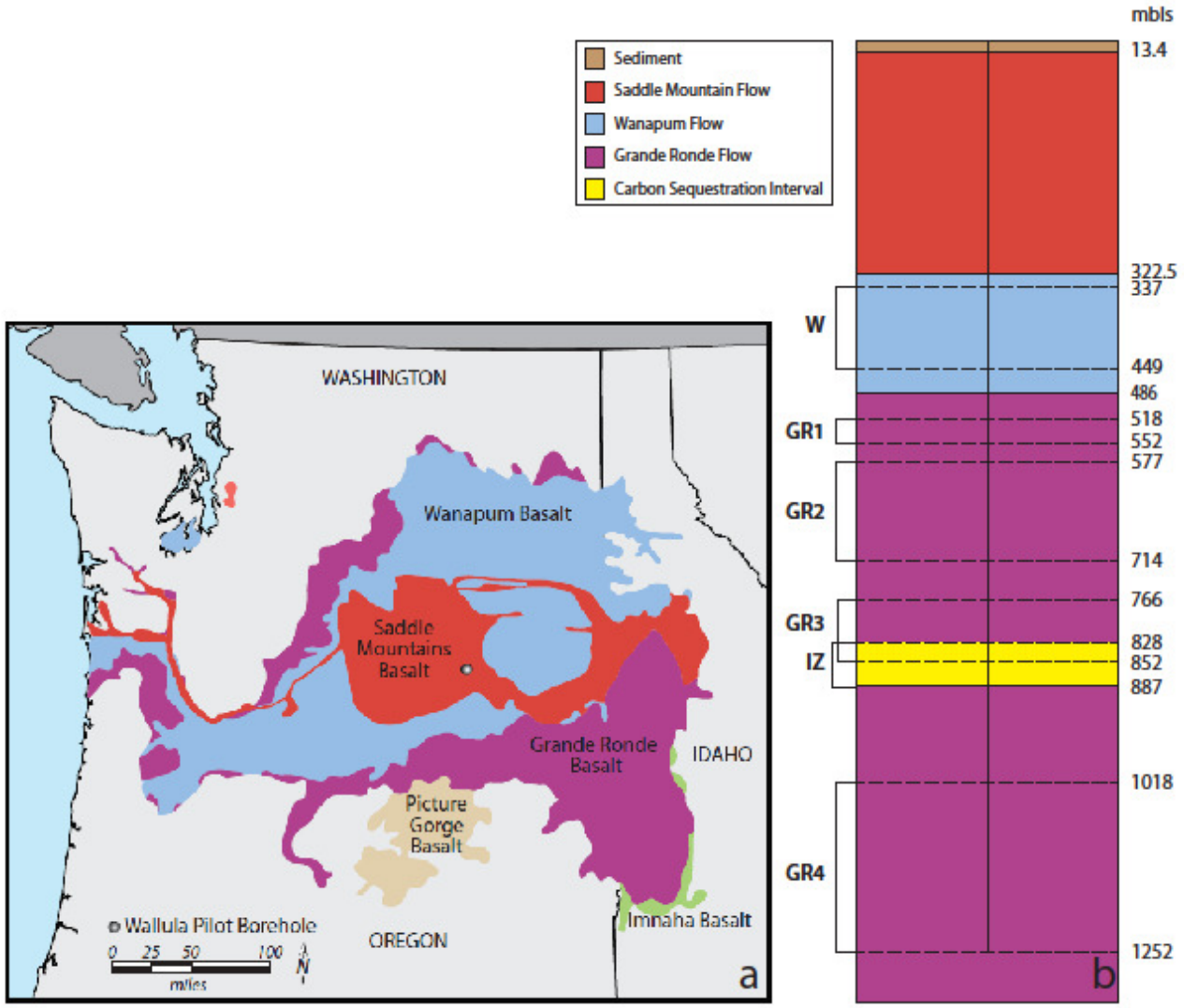
W	GR1	GR2	GR3	GR4	IZ
unclassified Oxalobacteraceae	unclassified Ilumatobacter	unclassified Streptophyta	unclassified Bradyrhizobiaceae	unclassified Deltaproteobacteria	unclassified Thermoanaerobacteraceae
Anaerosinus	unclassified Actinomycetales	unclassified Methylophilaceae	unclassified Enterobacteriaceae	unclassified Marinilabiaceae	unclassified Flavobacteriales
Pseudorhodoferax	*Tumebacillus*	unclassified Spirochaetales	*Corynebacterium*	unclassified Bacteroidales	unclassified TM7 genera incertae sedis
Herminiimonas		unclassified Spirochaetaceae	*Blastococcus*	unclassified Porphyromonadaceae	unclassified Xanthomonadaceae
Yonghaparkia		*Pseudacidovorax*	*Rothia*	unclassified Clostridiaceae	unclassified Actinobacteria
Alishewanella		*Caenimonas*	*Prevotella*	unclassified Anaeroliineaceae	*Erythromicrobium*
Perlucidibaca		*Delftia*	*Granulicatella*	unclassified incertae Sedis XI	*Porphyrobacter*
		Haematobacter	*Lactobacillus*	unclassified Erysipelotrichaceae	*Tepidicella*
			Lactococcus	unclassified Firmicutes	*Microbacterium*
			Streptococcus	unclassified Rhizobiales	*Silanimonas*
			Fusobacterium	*Oxobacter*	*Stenotrophomonas*
			Leptotrichia	*Geosporobacter*	*Desulfitibacter*
			Actinomyces	*Anaerovorax*	
			Klebsiella	*Dehalobacter*	
			Mobiluncus	*Syntrophothermus*	
			Pantoea	*Dendrosporobacter*	
			Serratia	*Bulleidia*	

Table 2.2 (Continued)

W	GR1	GR2	GR3	GR4	IZ
			Cobetia *Xanthomonas*	*Paludibacter* *Algoriphagus* *Blastobacter* *Belliella* *Catellibacterium* *Paracraurococcus* *Altererythrobacter* *Anerophaga* *Methyloversatilis*	

.

Figure 2.1. (a) Map of the Wallula pilot well and (b) schematic of the well. Depth intervals indicated on the well schematic correspond to the respective samples. Samples were integrated over large distances.


Sediment
Saddle Mountain Flow
Wanapum Flow
Grande Ronde Flow
Carbon Sequestration Interval
WASHINGTON
Wanapum Basalt
Saddle Mountains Basalt
Grande Ronde Basalt
IDAHO
Picture Gorge Basalt
Wallula Pilot Borehole
OREGON
Imnaha Basalt
0 25 50 100
miles
N
a
mbls
13.4
322.5
337
W
449
486
GR1
518
552
GR2
577
714
GR3
766
828
IZ
852
887
GR4
1018
1252
b

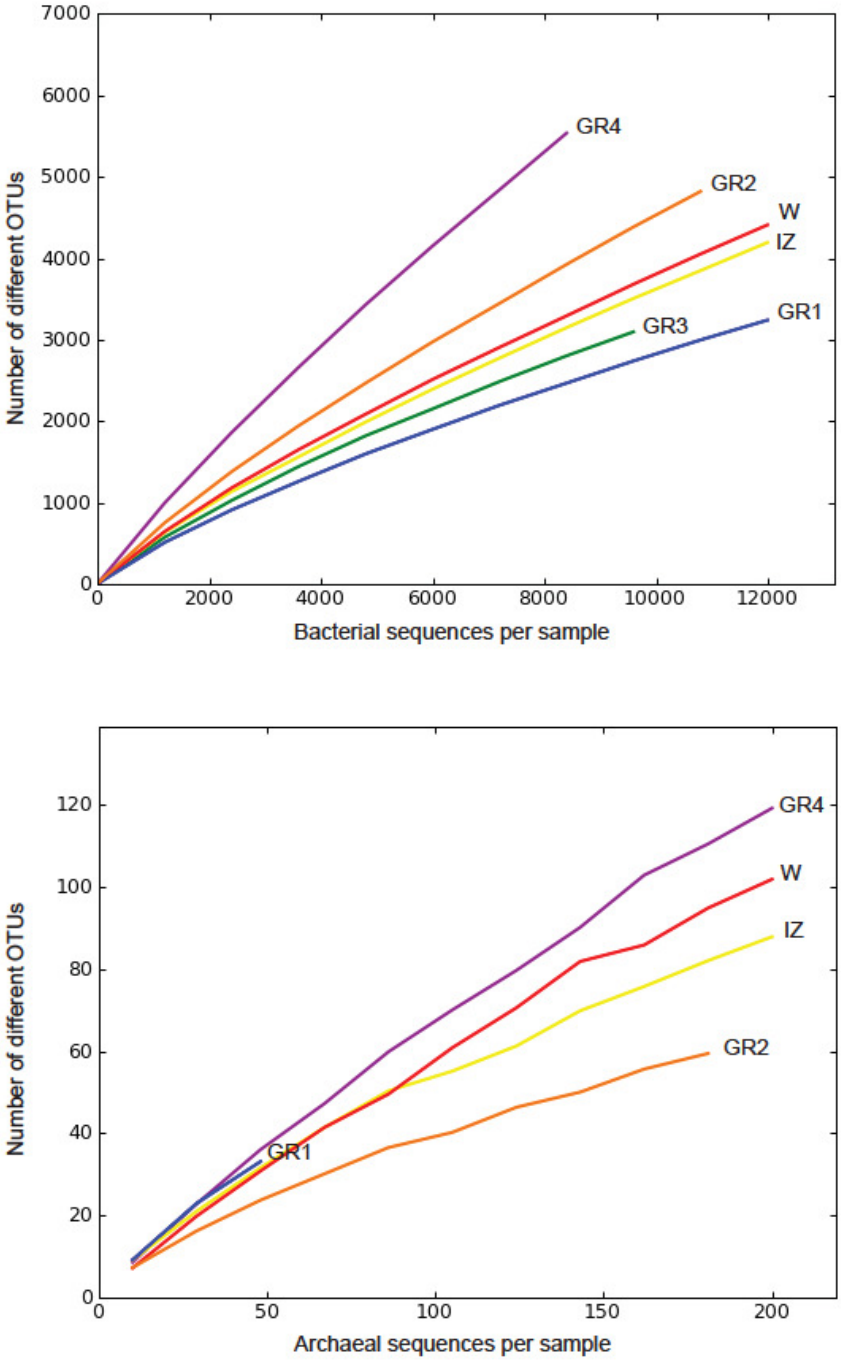
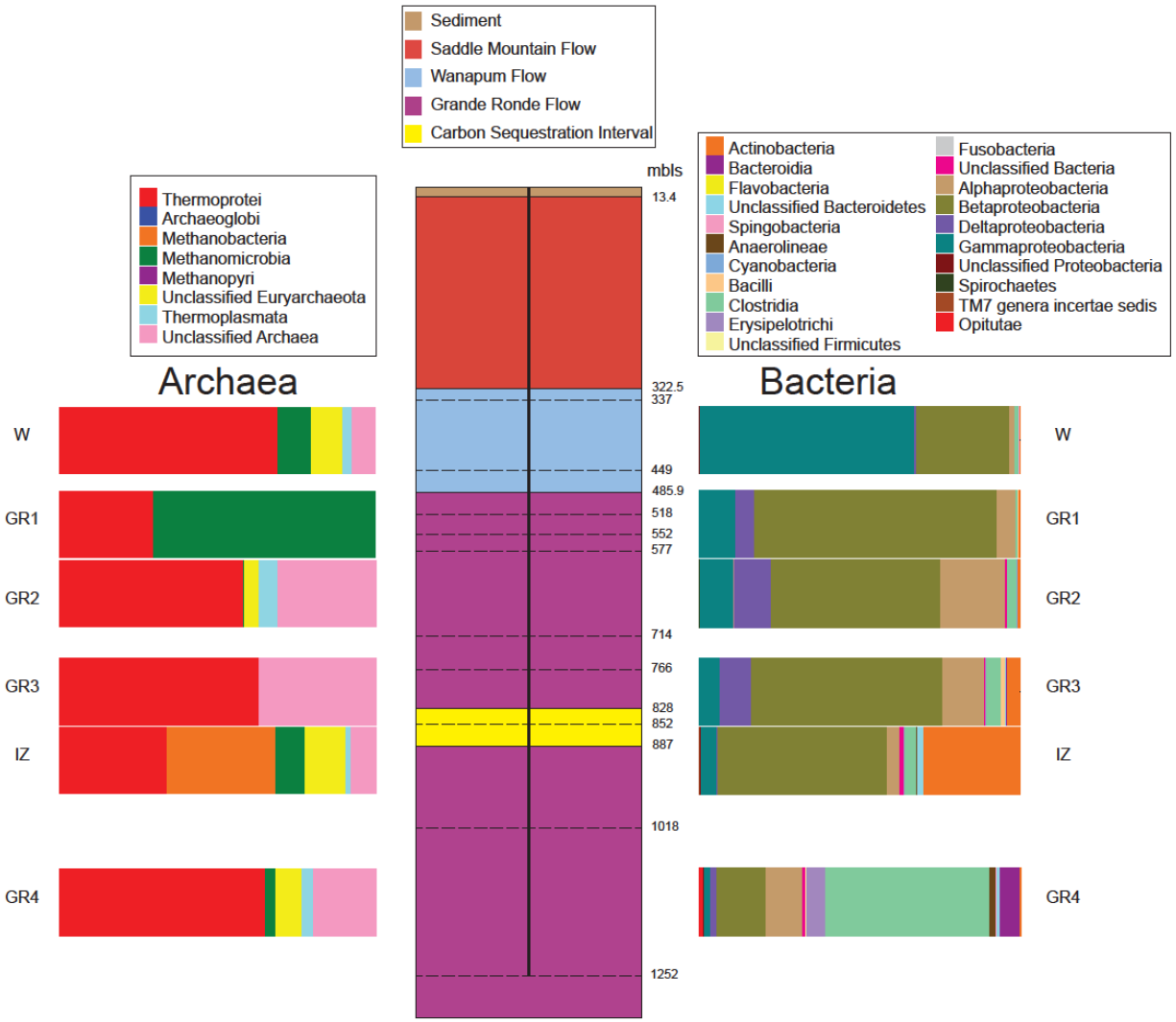


Figure 2.2. Rarefaction curves at the 3% difference level for each sample for the bacteria and the archaea. Sample GR3 for the archaea does not appear due to too few sequences.

Figure 2.3. Taxonomic composition of each sample at the class level. Only those classes that comprised greater than 0.10% of the number of taxa are shown. Bacterial reads (70610) outnumbered those of the archaea (6941).


Sediment
Saddle Mountain Flow
Wanapum Flow
Grande Ronde Flow
Carbon Sequestration Interval
mbls
13.4
322.5
337
449
485.9
518
552
577
714
766
828
852
887
1018
1252
Thermoprotei
Archaeoglobi
Methanobacteria
Methanomicrobia
Methanopyri
Unclassified Euryarchaeota
Thermoplasmata
Unclassified Archaea
Archaea
W
GR1
GR2
GR3
IZ
GR4
Actinobacteria
Bacteroidia
Flavobacteria
Unclassified Bacteroidetes
Spingobacteria
Anaerolineae
Cyanobacteria
Bacilli
Clostridia
Erysipelotrichi
Unclassified Firmicutes
Fusobacteria
Unclassified Bacteria
Alphaproteobacteria
Betaproteobacteria
Deltaproteobacteria
Gammaproteobacteria
Unclassified Proteobacteria
Spirochaetes
TM7 genera incertae sedis
Opitutae
Bacteria
W
GR1
GR2
GR3
IZ
GR4

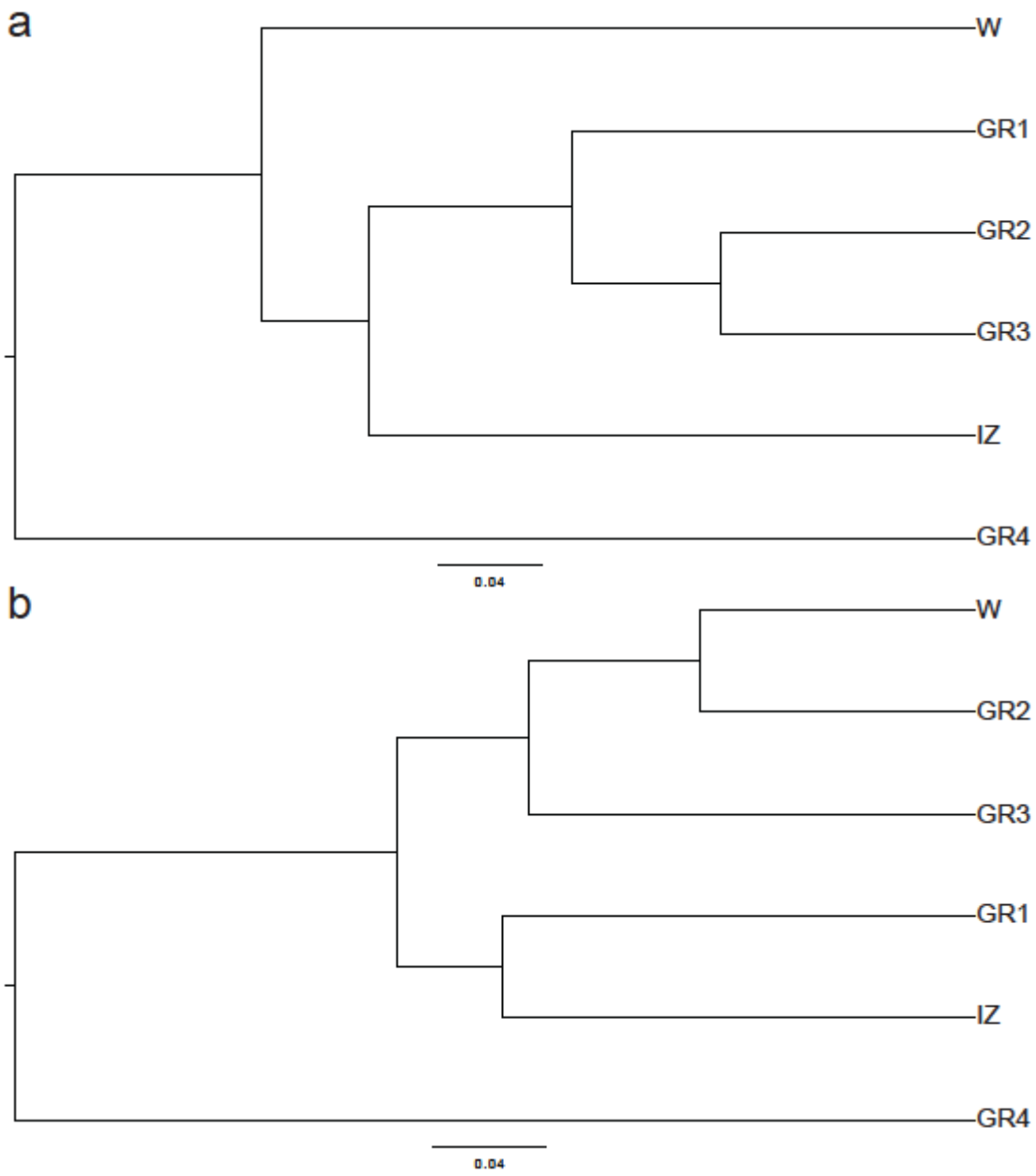


Figure 2.4. UPGMA tree calculated from Unifrac distances for the (a) bacterial and (b) archaeal communities.

3. CHANGES IN NATURAL MICROBIAL COMMUNITIES EXPOSED TO SUPERCRITICAL CARBON DIOXIE IN BASALTS

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For Submission to the International Journal of Greenhouse Gas Control

Abstract

Microorganisms are numerous in the subsurface and may play a pivotal role in the geologic sequestration of CO_2. The Wallula pilot well in Eastern Washington state provides the opportunity to investigate how a native microbial community will respond to the injection of supercritical CO_2 ($scCO_2$) into basalt. Formation water from the depth of planned CO_2 sequestration was used to inoculate basalt cores contained in pressure vessels maintained at the temperature (35°C) and pressure (82.7 bar) of the sequestration interval. Carbon dioxide was injected into the system and cores were exposed to $scCO_2$ for as long as 146 days. Petrography and scanning electron microscopy (SEM) revealed the presence of thick ferron-magnesium carbonate lining the vugs in basalts exposed to $scCO_2$ and microorganisms. SEM analysis also demonstrated the presence of biofilms within the fissures of the basalt core. Molecular analyses showed that microbes survived the $scCO_2$ exposure and that cell numbers slowly increased over time after the initial exposure to $scCO_2$. Microbial community analysis revealed a shift in the community from being initially dominated by Proteobacteria to being dominated by Firmicutes, particularly the genus *Alkaliphilus*. These results indicate that microbes may assist in sealing fractures in geologic media and suggest the importance of monitoring the microbial community in formation waters exposed to CO_2 during the geological sequestration of carbon in the subsurface.

Introduction

Geological carbon sequestration is one proposed solution to combat rising temperatures due to increased concentrations of CO_2 in the earth's atmosphere. Geological carbon sequestration involves the pumping of supercritical CO_2 ($scCO_2$) into suitable reservoirs contained in geologic strata (Bickle 2009; Peter McGrail et al. 2006) at depths sufficient to attain temperatures and pressures that will keep the CO_2 in a supercritical state. The $scCO_2$ remains sequestered in the chosen reservoir due to the physical and geochemical trapping of the injected $scCO_2$. Physical trapping consists of low-permeability cap rocks or stratigraphic seals which impede the upward migration of the injected $scCO_2$ (Gunter et al. 2004). Geochemical trapping involves the storage of CO_2 in mineral form due to long-term water-rock-$scCO_2$ chemical reactions (Gunter et al. 2004).

One candidate geologic media considered for geological carbon sequestration is basalt (Rosenbauer et al. 2005; Schaef et al. 2009; Schaef & McGrail 2009) because it is estimated to provide the storage capacity, porosity, permeability, and geochemical trapping ability to safely sequester CO_2 for geologic time periods (McGrail et al. 2006). The high permeability of basalt is largely due to interconnected pore spaces (McGrail et al. 2006) that act as reaction chambers for the geochemical trapping of CO_2, a process that is favored due to the abundance of reactive minerals in basalts (Goldberg et al. 2008; Matter et al. 2007; Schaef & McGrail 2009). Upon injection, $scCO_2$

dissolves into the formation water of the sequestration reservoir, forming carbonic acid which then dissociates into aqueous carbonate:

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \quad (1)$$

$$HCO_3^- + H^+ \leftrightarrow 2H^+ + CO_3^{2-} \quad (2)$$

This acidic solution weathers the minerals in basalt, such as the augite demonstrated below, releasing cations into solution, which then react with the aqueous carbonate ion to precipitate crystalline carbonate species such as ferron magnesite or magnesian calcite:

$$Ca_4(Mg_3Fe)Si_8O_{24} + H^+_{(acid)} + H_2O \rightarrow (Ca,Mg,Fe)^{2+} + 3H_4SiO_4\ (aq) \quad (3)$$

$$(Ca,Mg,Fe)^{2+} + HCO_3^- \rightarrow (Ca, Mg, Fe)CO_3 + H^+ \quad (4)$$

$$Mg_2SiO_4 + 2CO_2 \rightarrow 2MgCO_3 + SiO_2 + H_2O \quad (5)$$

$$3Mg_2SiO_4 + 2SiO_2 + 4H_2O \rightarrow 2Mg_3[Si_2O_5(OH)_4] \quad (6)$$

$$3Fe_2SiO_4 + 2H_2O \rightarrow 2Fe_3O_4 + 3SiO_2 + 2H_2 \quad (7)$$

Reactions such as those described between CO_2, basalt, and the groundwater will co-occur with native microorganisms in the formation water and thus become biogeochemical reactions. Autotrophic microorganisms, such as methanogens and acetogens, can utilize CO_2 directly as a carbon source (c.f. Pedersen 2000). Organisms stimulated by the injected CO_2 can form biofilms in cracks and fractures in the rock, thus sealing pore space and preventing the migration of CO_2 (Cunningham et al. 2009; Mitchell et al. 2009). Microorganisms can also act as nucleation sites for the formation of carbonate species (Dupraz et al. 2009). Lastly, through normal metabolic activities microorganisms can increase the pH of a microenvironment thereby aiding in

the precipitation of carbonates (Onstott 2005). For example, the activity of Fe(III) reducing microorganisms increases the pH of the environment, enabling the precipitation of carbonates and the storage of CO_2 in mineral form (Onstott 2005).

Currently, little is known about how a native microbial community will respond to $scCO_2$. Laboratory studies demonstrate that some microorganisms survive exposure to $scCO_2$ and reduce the permeability of sandstone cores, even though the number of cells decreases by two orders of magnitude over the course of the experiment (Cunningham et al. 2009; Mitchell et al. 2009). Observations from a deep geological injection of CO_2 highlight the ability of some microorganisms in sandstone saline aquifers to survive exposure to CO_2 (Morozova et al. 2010). Using fluorescence *in situ* hybridization (FISH), the sulfate reducing and methanogen communities were tracked for a five month CO_2 exposure period, with the sulfate reducers becoming undetectable during the course of the experiment while methanogens flourished. These investigations illuminate some aspects of how microbial communities respond to $scCO_2$ injection; however, target formations differ substantially from each other and it can be difficult to extrapolate between geological media to infer common occurrences.

Our objectives were to determine the effect of $scCO_2$ injection on Columbia River Basalt Group (CRBG) cores, formation water, and microbial communities native to the CRB formation water, and also to determine the role

microbial communities play in the precipitation of carbonates. To accomplish this we used a variety of geochemical and molecular techniques to analyze the effects of $scCO_2$ injection on the basalts and on the native microbial community.

Materials and Methods

Site description and sampling. The site of the Wallula pilot well has been described previously (Lavalleur et al. in preparation; McGrail et al. 2009). Natural formation water was collected on 4 March 2011 from the completed well at the proposed depth of CO_2 injection (829-887 meters below land surface (mbls)) after an extensive pump test in order to minimize contamination and collect water more representative of the formation. Collection of geochemical samples was performed by Pacific Northwest National Laboratory as previously described (McGrail et al. 2009). Formation water from the well was directly collected into a sterile 20 L carboy (Nalgene, Rochester, NY) and immediately transported back to the laboratory at ambient temperature, then stored at 4°C overnight until added to the pressure vessels. Three aliquots of approximately 0.5 L were dispensed from the carboy into 1 L glass bottles, then autoclaved for 1 h at 121°C and 1.03 bar in order to sterilize the formation water for use in the control pressure vessels.

Three cores from well DDH-3, designated Core A, B, and C, came from 686.7, 696.8, and 722.4 mbls, respectively, were used for incubation experiments performed at the National Energy Technology Laboratory in

Albany, Oregon. The cores came from a vesicular basalt flowtop zone in the Grande Ronde formation and were 4.6-4.7 cm in diameter. Each core was halved crosswise, producing 6 cores (A-1, A-2, B-1, B-2, C-1, C-2) ranging from 12.76 cm to 15.75 cm in length. Cores were wrapped in aluminum foil, then sterilized via gamma irradiation with a dose of 4 kGy using a cobalt-60 source at the Oregon State University Radiation Center (Corvallis, OR).

Experimental design and sampling procedure. Six 2 L, 316 stainless steel Parr Pressure Reactors (Figure 3.1), four designated to receive CO_2 and two designated to receive Ar, were cleaned and sterilized prior to the addition of the sterilized cores and CRBG formation water. The pressure vessel, cap, and Teflon liners (Parr Instrument Co., Moline, IL) were immersed in a 10% bleach bath then rinsed three times with sterile nanopure water. The pressure vessels were assembled (Figure 3.2) and then sterilized (60 min at 121°C and 1.03 bar). After sterilization, pressure vessels were cooled to ambient conditions. Each of the vessels then received a core and either natural or sterilized formation water in order to determine the effect of $scCO_2$ as well as the presence of microbes on the basalts during extended incubations (Table 3.1). Three pressure vessels were designated as controls, and three pressure vessels were designated as experimental. Controls revealed how microorganisms may affect the precipitation of carbonates, showed how microorganisms were affected by the $scCO_2$, and demonstrated overall system

sterility. Experimental pressure vessels were exposed to $scCO_2$ to evaluate how the microbial community changed over time.

After receiving the designated core and amount and type of formation water the pressure vessels were brought up to reservoir sequestration conditions (35°C and 82.7 bar). All pressure vessels were then purged with 99.999% Ar long enough to pass through three head space volume exchanges (Table 3.1). All pressure vessels remained under Ar gas (used to create an anoxic atmosphere) for 2 weeks in order to allow colonization of the cores by the microorganisms in the natural formation water (Stevens et al, 1993). After the incubation period, the designated pressure vessels (Table 3.1) were injected with 99.99% $scCO_2$ to displace the Ar. Temperature and pressure were monitored for each pressure vessel during the experiment until each pressure vessel was sacrificed at the end of its respective time period and slowly returned to atmospheric temperature and pressure (Table 3.1).

Cores were removed with sterile crucible tongs and placed on sterile aluminum foil. The core was then placed in two VWR® sterile sample bags (VWR International, Radnor, PA) and then samples for DNA extraction, cell growth, and cell preservation experiments were cut using a manual core splitter. The blades of the core cutter were wrapped in aluminum foil and sterilized by autoclaving for 20 min at 121°C and 1.03 bar prior to use, and sterilized between each core sampling by immersing in a 10% bleach solution then rinsing twice with sterilized nanopure water. Core samples for DNA

extraction were placed in a VWR® sterile sample bag (VWR International, Radnor, PA), then on dry ice and eventually (within 3 h) stored at -80°C until processed. Core samples for cell growth experiments were placed in 125 mL glass bottles with septa screw-caps along with 50 mL of the respective incubated formation water. Bottles were placed on ice for transport back to the laboratory where they were first purged for 20 min with nitrogen before storage at 4°C until analysis. Core samples for cell preservation were placed in 50 mL Falcon tubes containing 4 mL of 38% formaldehyde (Mallinckrodt Baker, Inc., Phillipsburg, NJ) and 41 mL of groundwater, for a 3.4% formaldehyde solution. The falcon tubes were placed on ice for transport back to the laboratory, where they were stored at 4°C until analysis by ESEM.

Geochemistry. Chemical analysis of the initial natural formation water was performed by Pacific Northwest National Laboratory as previously described (Lavalleur et al. in preparation; McGrail et al. 2009). Post-experiment solution samples were analyzed by ALS Laboratory Group, Environmental Division (Ft. Collins, CO) following EPA methods published on their website (http://www.alsglobal.com/). Metals were analyzed by inductively coupled plasma emission spectroscopy, following preparation by EPA methods SW3005A or SW3010A. Inorganic anions (Cl, NO_2, NO_3, SO_4, PO_4) were determined by ion chromatography following EPA method 300.0. Bicarbonate, carbonate, and total alkalinity were determined by titration by EPA method

310.1. Dissolved organic carbon and conductivity were determined by EPA methods 415.1 and 120.1, respectively.

Using multiple iterations to demonstrate kinetically-favored phases, CHIM-XPT was used to geochemically predict the pH and mineral precipitation expected after injection of $scCO_2$ into the initial natural formation water (Reed & Spycher 1984; Reed 1998). This method computes the distribution of aqueous species and gases using equilibrium constants at selected temperature and pressure from a thermodynamic database SOLTHERM. Aqueous activity coefficients are determined by using the extended Debye-Hückel equation and gas fugacity coefficients as modified by Tanger & Helgeson (Tanger & Helgeson 1988). Basaltic rock (100 g) was slowly titrated into the equilibrated Wallula solution chemistry with a mass balance of H^+ to predict solution system equilibrium. Pure (100%) CO_2 was then added into the system to produce a saturation pressure that is equal to the estimated downhole conditions in the Wallula pilot well.

Nucleic acid extraction, amplification, and quantification. Total DNA was extracted from 10 g (wet weight) of each incubated core sample using a PowerMax Soil DNA Isolation kit (Mo Bio Laboratories, Carlsbad, CA) in duplicate, along with a negative control containing only reagents, following the manufacturer's protocol with modifications. Approximately 20 g (wet weight) of incubated basalt sample was removed aseptically from the frozen sub core sample and crushed to a sandy consistency using a sterilized steel mortar and

pestle. Ten grams of each crushed basalt sample was placed into a 50 mL Falcon tube and then the tubes were heated to 65°C for 10 min according to the manufacturer's recommendations. Samples then underwent bead-beating for 10 min. Extracted DNA was concentrated using Amicon Ultra centrifugal units (Millipore, Billerica, MA) and the DNA concentration was measured using a Qubit® (Invitrogen, Inc., Carlsbad, CA) according to the manufacturer's recommendations.

Two µL of each concentrated DNA extract was amplified by polymerase chain reaction (PCR) using a Veriti Thermocycler (Applied Biosystems, Inc., Foster City, CA). Each 20 µL PCR reaction contained 0.0625 U AmpliTaq Gold® LD polymerase (Applied Biosystems, Inc., Foster City, CA), 1X PCR buffer, 4 mM $MgCl_2$, 800 µM dNTP's (Applies Biosystems, Inc., Warrington, UK), and 0.5 µM of each primer (Eurofins MWG Operon, Huntsville, AL). Bacterial 16S rDNA was amplified using primers 27F (DeLong 1992) and 926R (Muyzer et al. 1995). Archaeal 16S rDNA was amplified using primers 181F (5'-TAGGATGGATCTGCGGCCA-3') and 1392R (5'-CCCCTGCGAACCTAGATT-3'). Initial denaturation was performed at 95°C for 5 min, followed by 34 cycles of denaturation at 95°C for 40 s, annealing at 50°C for 40s, and elongation at 72°C for 1 min. The final elongation step occurred at 72°C for 10 min. Amplicons were then stained and visualized on a 1.2% E-gel containing ethidium bromide (Invitrogen, Inc., Carlsbad, CA).

To estimate copy numbers of bacterial 16s rRNA genes, quantitative PCR (qPCR) was performed with Power SYBR Green PCR Master Mix on an ABI PRISM® 7500 FAST Sequence Detection System following the manufacturer's protocol, with modifications. Each 20 µL reaction, performed in triplicate, consisted of 1X SYBR® Green PCR Master Mix (Applied Biosystems, Carlsbad, CA), 900 nM 27F (DeLong 1992), and 50 nM 338R (Amann et al. 1995) (Eurofins MWG Operon). Initial denaturation occurred at 95°C for 1 min and was followed by 40 cycles of denaturation at 95°C for 15 s, annealing at 60°C for 1 min, and extension/data collection at 81°C for 1 min. Disassociation occurred according to the manufacturer's directions. qPCR products were then stained and visualized on a 0.8% E-gel containing ethidium bromide (Invitrogen, Inc.).

Pyrosequencing and data analysis. Pyrosequencing of extracted and pooled DNA was performed at the Oregon State University Center for Genome Research and Biocomputing using the Roche 454 GS Junior platform. PCR amplicons of the bacterial V4-V6 hypervariable rRNA region were prepared in triplicate using the primers listed in Table B.1 following the AmpliTaq Gold® protocol with minor modifications. Amplification of bacterial DNA amplicons utilized an annealing temperature of 52°C, an elongation time of 45 s, and a final elongation step of 5 min. Prior to submission, pooled triplicate reactions were cleaned using the QIAquick® PCR Purification Kit (Qiagen, Inc., Valencia, CA).

Pyrosequencing data was processed using the Qiime pipeline (http://qiime.sourceforge.net/), which incorporates Unifrac (Lozupone & Knight 2005) to allow statistical analyses of smaller sample sizes. Sequences underwent a rigorous quality control process to ensure the integrity of the data, as is standard practice. Sample barcodes and primers were trimmed from the proximal and distal ends of the reads. Reads that did not match the forward or reverse primer sequence and reads that could not be assigned to a sample were discarded (i.e. reads were discarded when a match could not be made to a barcode). Reads shorter than 50 nucleotides or containing one or more ambiguities were removed. Sequences were assigned taxonomy using the RDP classifier at 97% similarity and any reads unable to be classified at the domain level were removed.

SEM and ESEM. For petrographic analyses, a sterile saw was used to cut a middle section from basalt cores and bookends were made into 1) thin sections immediately filled with epoxy for light microscopy and 2) pieces mounted in epoxy and coated in Pd for analysis with a FEI Inspect F Scanning Electron Microscope (SEM) to obtain backscattered electron images (BSE) and Energy Dispersive Spectroscopy (EDS) data, optimized with Fe (pyrite) and Cu standards for quantification. Pre-experimental basalt samples from the same cores have previously been studied microscopically by Verba et al., in preparation.

A FEI Quanta 200 Environmental SEM in low vacuum water vapor mode at University of Oregon was used to examine vesicles in samples retained in formaldehyde to preserve microbial cells. Basalt was held for 1 h at room temperature in a desiccator in the presence of ethanol to dehydrate water prior to examination with the ESEM.

Petrography. Eight 30-micron thick thin sections were examined at the National Energy Technology Laboratory in Albany, Oregon using an Olympus BX41 petrographic microscope with both transmitted and reflected light. High-resolution images were captured by the attached 12MP Leica DFC 500 camera and Leica Application Suite 8.0 software. Adobe Photoshop CS5 and Image-Pro Plus 7.0 were used to analyze the images for alteration measurements and comparison between samples. The three pre-experiment samples were impregnated with blue epoxy to better identify pore space, and the post-experiment samples were impregnated with clear epoxy. Minerals were identified based on optical properties when possible, but many phases were too fine-grained to visually identify. SEM and XRD results were used in concert with the petrography to identify unknown phases.

XRD. Due to instrument availability and differing available sample types, several methods were used to prepare samples for XRD analysis at the National Energy Technology Laboratory in Albany, Oregon. The three pre-experiment, unaltered basalts from Cores A, B, and C were analyzed using a Rigaku Ultima-III X-ray diffractometer using Cu K-alpha radiation, and two

samples were analyzed using an InXitu Terra X-ray diffractometer using Co K-alpha radiation. The samples analyzed with the Rigaku XRD included representative basalt groundmass and a blue-green secondary mineral which were run as solid samples due to a coating of epoxy on one side. A third small sample of a blue-green secondary mineral was isolated and also run on the Rigaku. Two epoxy-free samples of the representative basalt were ground to powder in a mortar and pestle and analyzed on the InXitu XRD.

Both bulk sample and specific vug-filling minerals of interest were analyzed from the post-experiment samples. All of these were analyzed as powders on the Rigaku XRD. An ethylene glycol test was done on one post-experiment sample, core A-1 from AC 6, to confirm the presence of smectite (montmorillonite) as the primary clay mineral in the sample. A subsample of the prepared XRD powder was separated via gravity settling to obtain a sample with particles less than 5 μm. The resulting clay slurry was dried on a glass slide in a low-temperature oven (~50°C) and allowed to cool. XRD scans were conducted after drying and after vapor glycolation treatment.

JADE 9 software and the International Centre for Diffraction Data PDF4+ database were used for mineral identification. Major (>25%), minor (~5%-~25%), and trace (<5%) designations were roughly estimated based on peak intensity, number of peaks, and scattering factors.

Results

Geochemistry. Prior to incubation in the pressure vessels the CRBG formation water was characterized as a dilute, sodium-bicarbonate water type (McGrail et al. 2009). The natural formation water exhibited an elevated, alkaline pH of 10.1 (Table 3.2). The influence of pH on the microbial community of the Wallula pilot well is of interest as the increase in acidity of the natural formation water is expected to decrease the diversity of the native microbial community. Using a P_{CO2}= 10 MPa and a temperature of 35^{o}C, CHIM-XPT determined the final pH to be 3.9. Additional results from the model are reported in appendix B.

Laboratory incubation experiments with $scCO_2$ produced a strong increase in bicarbonate, sulfate, calcium, magnesium, potassium, sodium, total alkalinity, and specific conductivity over time (Table 3.2). A strong increase in iron concentration was seen at 146 days under both $scCO_2$ and Ar in natural formation water containing microorganisms. Samples held under an Ar atmosphere showed increased concentrations of sulfate, calcium, sodium, and specific conductivity; however, the response was not as strong as that seen in the $scCO_2$ samples. Bicarbonate and total alkalinity remained below detection limit in the samples incubated with Ar.

Microbial DNA extraction, concentration, and characterization. The quantity of DNA extracted from the basalts ranged from 0.01 ng μL^{-1} to 0.32 ng μL^{-1}. Blank DNA extractions performed alongside each pressure vessel DNA

extraction also yielded DNA, except for sample B7 (negative control sample extracted alongside the basalt sample from AC 7) (Table B.1), which correlated to the basalt sample from AC 7 (the pressure vessel simulating the natural aquifer).

The natural formation water collected from the Wallula pilot well contained an average of $2 \pm 1 \times 10^7$ cells mL^{-1}. After 55 days of exposure to $scCO_2$ cell numbers had dropped two orders of magnitude to $3 \pm 4 \times 10^5$ cells g^{-1} of basalt (Table 3.2). Cell concentration rose to $4.4 \pm 0.7 \times 10^5$ cells g^{-1} of basalt after 146 days of incubation with $scCO_2$. The control pressure vessel designed to simulate the natural aquifer environment harbored the highest cell population at $5 \pm 1 \times 10^7$ cells g^{-1} of basalt. During the course of the experiment pressure vessels containing sterilized formation water became contaminated due to the transfer of water between vessels.

Pyrosequencing of the natural formation water from the Wallula pilot well provided an in depth characterization of the unattached microbial community used to inoculate the pressure vessels and results are detailed in Lavalleur et al (in preparation). Proteobacteria composed 62.2% of the identified bacterial taxa, while Actinobacteria made up 30.1% of the identified taxa used to inoculate the pressure vessels. Minor phyla present were Bacteroidetes, Chloroflexi, Firmicutes, TM7, Verrucomicrobia, and unclassified bacteria (Figure 3.3). The genera *Malikia* and *Olsenella* comprised 46.4% and 27.2% of the bacterial taxa identified from the inoculum, respectively.

Euryarchaeota dominated the archaeal population, making up 58.1% of the reads from the native microbial community used to inoculate the basalt cores.

After incubation with $scCO_2$, amplification of bacterial and archaeal DNA revealed the presence of bacteria and the absence of archaea in all extracted basalt samples. Pyrosequencing yielded a total of 90990 reads from the bacteria, with 47249 reads originating from the six pressure vessels. The rest of the reads came from the blank DNA extractions performed alongside each pressure vessel DNA extraction. Reads unable to be classified at the phyla level totaled 36, with 25 reads corresponding to the pressure vessel extractions.

Changes were easily observed between the control pressure vessels and the experimental pressure vessels despite the fact that the control pressure vessels did not maintain sterility during the entire course of the experiment. Autoclave 7, designed to simulate the downhole conditions of the Wallula pilot well, was dominated by the Proteobacteria, followed by the Firmicutes and Actinobacteria (Figure 3.3). Approximately 17.6% of the reads from Autoclave 7 were related to the genus *Malikia* and another 13.3% of the reads corresponded to the genera *Denitratisoma* and *Olsenella*. 12.2% of the reads belonged to *Dethiobacter.* The genera *Malikia* and *Olsenella* were also the top genera found in the natural formation water used to inoculate the basalt samples in the pressure vessels, thus the natural simulated aquifer somewhat reflects the natural formation water. *Dethiobacter* was present in

the natural formation water at 1.0% of the reads; however, *Denitratisoma* was unable to be detected in the natural formation water, indicating that changes occurred between the collection of the water and the end of the experiment.

Autoclave 8, which was designed to serve as a sterile control, did not remain sterile mainly due to the transfer of water between Autoclave 7 and Autoclave 8 or due to an incomplete sterilization of the formation water. At the end of the experiment Autoclave 8 contained mostly Actinobacteria and Proteobacteria (Figure 3.3), with the genus *Olsenella* comprising 50.0% of the reads. The blank DNA extraction done in concert with the DNA extraction from Autoclave 8 mainly contained Firmicutes and Proteobacteria.

Autoclave 6 served the purpose of a sterile control exposed to $scCO_2$. Again, the water did not remain sterile during the course of the entire experiment. Autoclave 6 was dominated by the Proteobacteria (Figure 3.3), being almost entirely composed of the genera *Escherichia/Shigella* at 99.8% of the reads. The blank DNA extraction performed alongside the basalt DNA extraction was also mainly composed of *Escherichia/Shigella*, suggesting that Autoclave 6 may have remained sterile (except for the transfer of formation water between autoclaves) during the experiment and the contamination is due to the DNA extraction process.

Autoclave 3 was terminated at 55 days post $scCO_2$ injection and provided the first insight into how the microbial community responded to the abundance of carbon and acidic conditions prevalent following the injection of

CO_2. The microbial community was dominated by the Proteobacteria (Figure 3.3) and was mainly composed of the genera *Escherichia/Shigella,* once again suggesting contamination during the DNA extraction process. The blank DNA extraction done with the matching sample consisted of 21.4% *Tumebacillus*, and 7.9% *Maraxella*.

After 106 days of exposure to $scCO_2$ the microbial community in Autoclave 4 shifted, and was mainly composed of Firmicutes and Proteobacteria (Figure 3.3). The most dominant genus present was *Alkaliphilus*, at 54.7%. The blank DNA extraction performed with the basalt sample was also dominated by the Firmicutes, with the genus *Tumebacillus* composing 43.6% of the reads from the blank DNA extraction.

The microbial community remained mostly composed of Firmicutes and Proteobacteria after 146 days of exposure to $scCO_2$ (Figure 3.3). The main genus was *Alkaliphilus*, which composed 72.7% of the reads from Autoclave 5. The blank extraction done with the basalt sample from Autoclave 5 produced reads corresponding solely to the genera *Alkaliphilus* and *Geosporobacter*, which are the top two genera extracted from the basalt sample.

Mineralogy and petrography. The three initial basalt samples were composed primarily of fine-grained sodic plagioclase feldspar ($(Na,Ca)(Si,Al)_4O_8$). Clinopyroxene, identified as augite ($(Ca,Mg,Fe,Al)_2(Si,Al)_2)_6$) by XRD, was common in the groundmass, and olivine phenocrysts were identified petrographically but could not be identified by XRD. Smectite clay, most likely

montmorillonite ($Ca_{0.2}(Al,Mg)_2Si_4O_{10}(10)_2 \cdot H_2O$), was also identified in trace to minor amounts in the XRD analyses. Zeolites, primarily heulandite ($(Ca,Na)_9Al_9Si_{27}O_{72} \cdot 24H_2O$), occurred in all basalt samples as secondary minerals lining or filling vesicles. Preliminary petrographic analysis of each core can be found in appendix B, as well as results from the cores exposed to Ar gas.

No significant differences were observed in the post-experiment XRD analyses; however, petrographic analyses indicated major alteration of the basalts due to $scCO_2$ exposure. After 106 days in $scCO_2$ and natural formation water, a thin (40-100 μm) fine-grained bright yellow-orange and red-orange presumed iron-rich alteration was common in the groundmass of sample C-1 (Figure 3.4a-c). The alteration here and in the other samples was too fine-grained to identify under the petrographic microscope, but was identified as likely carbonate by SEM-EDS mapping.

The two samples that were exposed to $scCO_2$ for 146 days underwent more dramatic changes. Both samples (A-1 and A-2) showed strikingly orange presumed iron-rich alteration of mineral grains and the basalt groundmass as well as significantly altered vugs (Figure 3.4d-h). This is identified by SEM-EDS as iron-carbonate and calcite, though the lack of carbonate phases or iron-rich phases identified by XRD suggests that some of the visual alteration may also be amorphous. The carbonate was generally thicker in sample A-2, from the natural formation water, than sample A-1, in

the sterile formation water (rough averages are 287 μm vs. 225 μm, respectively). One striking feature observed was black films lining vugs in A-1 and A-2 (Figure 3.4g). This material was found to be poorly crystalline and contained a high proportion of clay (identified by an ethylene glycol test) as well as plagioclase, pyroxene, and zeolites from the basalt. SEM analysis of these films is discussed below.

Basalt samples incubated with natural formation water and $scCO_2$ contained very similar features to those seen in $scCO_2$ exposure with sterile formation water. However, the basalt cores incubated with natural formation water contained barely visible, macroscopic black coverings in vugs and fractures. SEM-EDS analysis concluded this covering to be biofilm, as it contained more than 74 wt% carbon; the remainder of spectra resulted from the electron beam reflecting the minerals below the biofilm. Detailed analysis of the basalt samples under SEM revealed the presence of spherical and rod shaped microbes in thin biofilms, fractures and vesicle edges. Sample C-1 (106 days, $scCO_2$ + natural formation water) confirmed the presence of spherical microbes 0.3-0.85 μm in size as seen in Figure 3.5a. Sample A-2 (146 days, $sCCO_2$ + natural formation water) had many microfractures with biofilm attached that contained likely spherical and rod shaped microbes, 0.3-1.5 μm in size, seen in Figure 3.5b,c. This sample also contained many regions where the zeolites had cilia-like arms in which the microbes nested.

Shallow fractures <50 μm had biofilm stretching across, specifically in regions where the zeolites attached to the basalt began to fragment.

Sample C-2 (Ar + natural formation water) had the highest microbial population, reflecting the anoxic conditions without the influence of CO_2 injection. The initial solution contained 100 mg/L of sulfate and the final solution had 64 mg/L sulfate as well as a supersaturation of iron (49 mg/L). Framboidal pyrite (FeS_2) 10 μm in size formed in altered zeolite lining vesicles (Figure 3.6). These tiny cubic (0.05-1 μm) precipitates were surrounded by a carbon rich biofilm and suggest a direct microbial impact. These framboids are characterized by heterogeneous concentrations of a 3-5 wt% carbonate coating and 80-90 wt% of FeS_2, as determined by EDS. This sample also displayed an alteration that differed from the other samples. The edges of the sample, up to 100 μm thick, appeared to be enriched in iron and magnesium where FeS_2 was absent, as determined by EDS. Additionally, this sample contained dense microbial features in the epoxy surrounding open pore spaces, fractures and vesicles.

Discussion

Storage of CO_2 in geological reservoirs plays a vital role in the reduction of carbon emissions to the atmosphere and the abiotic geochemical trapping ability of basalts provides a more permanent storage solution for CO_2 than other geologic strata. Native microbial communities may also aide in the storage of CO_2 in geologic strata and thus play a pivotal role in meeting the

carbon capture and storage goals of permanence, safety, and long-term storage of CO_2 in geologic media (Bénézeth et al. 2009; Onstott 2005). This study examines the effect of $scCO_2$ injection on CRB and a native CRB microbial community and provides insight into how the *in situ* microbial community may be involved in processes related to the sequestration of CO_2 at the Wallula pilot well.

Microbial characterization and changes due to $scCO_2$ injection. The microbial community suffered a two order of magnitude loss in biomass upon the injection of $scCO_2$, but over time the biomass increased. An *in situ* study conducted in a sandstone basin reported a sharp decline in cell numbers after introduction of CO_2 to the system with biomass recovery by 5 months later (Morozova et al. 2010). A decrease in cell numbers can be attributed to the fact that $scCO_2$ is a strong solvent used for sterilization processes. Due to its low viscosity, $scCO_2$ easily penetrates cell walls and denatures membrane proteins (Booth et al. 2002; Dillow et al. 1999). The pH inside the cell is then lowered, damaging DNA and leading to cell death (Booth et al. 2002).

Still, some microorganisms can survive exposure to $scCO_2$, particularly those congregated in biofilms (Mitchell et al. 2008, 2009). Numbers of *Bacillus mojavensis* in planktonic form decreased strongly when exposed to $scCO_2$, whereas the numbers of these cells in biofilm form decreased slightly, suggesting microorganisms capable of forming biofilms are more resistive to the corrosive effects of $scCO_2$ (Mitchell et al. 2008). Similarly, in a high

pressure, moderate temperature flow-through system containing a Berea sandstone core and $scCO_2$, a *Shewanella fridgidimarina* biofilm formed (Mitchell et al. 2009).

The native microbial community obtained in groundwater from the Wallula pilot well survived the injection of $scCO_2$; however, the attached community did not remain completely intact. After CO_2 exposure archaea could not be detected by amplification of the 16S rRNA gene specific for archaea. This agrees with previous findings that archaea (specifically methanogens) increase upon CO_2 injection, then became undetectable after the 5 month exposure to CO_2 (Morozova et al. 2010). The attached bacterial community also underwent dramatic changes, some of which may be due to the removal of the community from the subsurface and artificial conditions intending to simulate the natural conditions found at the Wallula pilot well (Hirsch et al. 1995) or due to the difference in the way the samples were collected and the DNA was extracted (formation water samples versus basalt samples and the more rigorous technique used to extract DNA from the basalt). Such differences in DNA extraction technique are known to create biases in the amount of DNA extracted from samples (Miller et al. 1999). The initial unattached microbial community used to inoculate the basalt cores mostly consisted of the genera *Malikia* and *Olsenella*. The naturally simulated pressure vessel's main component was also *Malikia*; however, the next major contributing genus to the diversity in the pressure vessel was *Denitratisoma*,

which was a rare (<0.10% of the reads) member of the Wallula pilot well. This phenomenon of previously undetected and/or rare members of a microbial community becoming dominant after exposure to $scCO_2$ has been reported previously (Gulliver & Gregory 2011).

The experimental pressure vessel results cannot be compared to the natural formation water used to inoculate the basalts, as the laboratory conditions altered the microbial community. The 56-day incubation with $scCO_2$ consisted almost entirely of the genus *Escherichia/Shigella*, whose presence points to outside contamination (Welch) and thus these results must be omitted from the analysis. However, the vessel opened after 106 days of exposure to $scCO_2$ was not compromised and yielded a microbial community consisting mainly of *Alkaliphilus*, *Pseudomonas*, *Bacillus*, and a smaller amount of *Geosporobacter*. *Alkaliphilus* and *Bacillus* were not detected in the initial inoculum; however, *Alkaliphilus*, *Pseudomonas*, *Bacillus*, and *Geosporobacter* were present in the naturally simulated pressure vessel, although to a much lesser extent. The sample exposed to $scCO_2$ for 146 days was composed of an even greater amount of *Alkaliphilus* and *Geosporobacter*.

The reason for the dominance of *Alkaliphilus* is not clear, as the genus is known to be extremely alkaliphilic and thus would not be prone to survive in the acidic conditions that prevailed after the injection of $scCO_2$ into the system. However, *Alkaliphilus* is a spore former and as such may be able to survive a lower pH by remaining dormant until more favorable conditions return. Other

studies that have investigated the affect of $scCO_2$ on microbial populations found that sporeforming bacteria were more resistant to the sterilizing nature of $scCO_2$ than non-sporeformers (Dillow et al. 1999; Enomoto et al. 1997; Werner & Hotchkiss 2006). For example, a temperature of 60°C at 205 bar was needed to completely inactivate spores of *Bacillus cereus* (Dillow et al. 1999), a temperature and pressure much greater than that encountered in this study. These data suggest that spore forming bacteria from the phylum Firmicutes may survive proximal to the injection of $scCO_2$ in the Wallula pilot well and would be the first to reproduce in the formation water once conditions become more favorable after a period of time.

Mineral precipitation and the role of microorganisms. The storage of CO_2 in mineral form is a key factor to the permanent geological sequestration of CO_2 in basalts. In this study, dissolution and precipitation of minerals in the basalt occurred as expected and as previously modeled when the rock was exposed to $scCO_2$ (McGrail et al. 2006). Minerals initially dissolved from the basalt groundmass, increasing the amount of iron in solution (see Table 3.2). Despite the increase in iron, iron carbonates were not expected to form and precipitation of these minerals was not noted in prior studies (McGrail et al. 2006). Nevertheless, SEM-EDS mapping confirmed the presence of iron and magnesium carbonates and oxides or sulfides, especially in the presence of microbial-containing formation water. Iron reducing microorganisms can aid in the precipitation of iron carbonates, and thus the geochemical trapping of

injected CO_2, as the energy available for the reduction of iron increases with the introduction of CO_2 to the system (Kirk 2011; Onstott 2005). Indeed, thicker iron containing precipitates were seen in the basalts exposed to the natural microbe-containing formation water as compared to the basalts exposed to the sterilized formation water. However, microorganisms related to iron-reducers were not detected in our system and furthermore, iron was in an oxidized state and not present in the form expected. Enrichments were attempted to grow iron-reducers (see appendix B) but were unsuccessful/inconclusive (data not shown). Thus the thicker precipitates are not likely due to microbial activity.

The physical presence of microorganisms can also support the precipitation of minerals such as calcite. Experiments with *Sporosarcina pasteurii*, a model bacterium often used to enhance the precipitation of calcite, to determine the role of microorganisms in the geological sequestration of CO_2 show that microorganisms can act as nucleation sites for the precipitation of minerals (Dupraz et al. 2009). The precipitation of calcite can also be encouraged by the presence of ureolytic microorganisms, whereby the action of splitting urea increases the pH of the system. This favors the precipitation of calcite and the incorporation of the injected CO_2 into the mineral precipitate (Mitchell et al. 2010).

In addition to the observed precipitation of carbonates, the deposition of pyrite on the basalt core was seen in the pressure vessel simulating the

natural environment (see Figure 3.6). Pyrite may form in conjunction with microorganisms and it is hypothesized that the cells themselves are involved in the production of the mineral (Folk 2005). Sulfate reducing bacteria aid in the precipitation of pyrite by providing H_2S to undergo reaction with FeS to produce pyrite (Bottrell et al. 1995; Drobner et al. 1990). The pyrite that appeared in the pressure vessel that simulated the natural aquifer could be due to the presence of sulfate reducing bacteria in the formation water; however, a more detailed study would need to be performed to determine if they are present in the formation water, as enrichments and pyrosequencing could not confirm their signature.

Conclusions

In summary, pressure vessel simulation experiments gave valuable insight into the effects of $scCO_2$ on basalts and how the presence of microorganisms enhance the geological sequestration of CO_2. Native microorganisms of the CRBG at the exact location of future $scCO_2$ injection in the Wallula pilot well were able to survive $scCO_2$ injection. Although the community was strongly altered (i.e. no archaea were detected after the natural formation water was incubated with $scCO_2$ and the community domination shifted from Proteobacteria to Firmicutes) those microorganisms capable of surviving began to recover, and in the process appear to have aided in the sequestration of CO_2 by influencing the formation of carbonate minerals. Thus microorganisms should be considered as part of any

geological sequestration endeavor, as a community shift such as the one observed in this study may be used to trace the movement of CO_2 in groundwater exposed to geological carbon sequestration endeavors.

Future studies should include modifications to the pressure vessel system to prevent water transfer between pressure vessels. Also, the use of a computed tomography scanner to show changes in the porosity of cores subjected to $scCO_2$ may permit determinations of whether the presence of microorganisms influences the pore structure or secondary mineralization of the basalt. Key data related to the biogeochemical cycling of carbon in basalts exposed to $scCO_2$ may be derived by determining the activity of surviving microorganisms, particularly the activity of microbes able to incorporate CO_2 in such subsurface simulations.

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Table 3.1. Pressure vessel designations and descriptions. Natural water contained microorganisms from the Wallula pilot well. Water amount indicates the volume of water added to each pressure vessel to yield a 5:1 water-to-rock ratio. Core mass is the weight of the core prior to microbiological and mineralogical sampling. Purge time indicates how long Ar gas was bubbled through the formation water to create an anaerobic atmosphere. Time shows the period over which the respective cores were exposed to either Ar or $scCO_2$.

Autoclave Name	Autoclave Type	Water Type	Water Amount (mL)	Name of Core	Core Mass (g)	Exposure	Purge Time (s)	Time (days)
AC 3	Experimental	Natural	513	B-1	553.51	$scCO_2$	125	55
AC 4	Experimental	Natural	340	C-1	367.36	$scCO_2$	143	106
AC 5	Experimental	Natural	408	A-2	440.64	$scCO_2$	136	146
AC 6	Control	Sterile	370	A-1	399.55	$scCO_2$	140	146
AC 7	Control	Natural	372	C-2	401.65	Ar	140	146
AC 8	Control	Sterile	540	B-2	582.86	Ar	122	146

Table 3.2. Select geochemical and cell count values for the initial formation water (natural) added to the pressure vessels and for each pressure vessel following incubation.

Autoclave Name	Treatment	Anions (mg L^{-1})	Ion Chromatography (mg L^{-1})		Cations (mg L^{-1})				Total Alkalinity as $CaCO_3$	Specific Conductivity (umhos/cm)	pH[a]	Cell Counts (cells mL^{-1})[b]
		HCO_3^- as $CaCO_3$	Sulfate	Ca	Fe	Mg	Si	S				
	Natural (0 days)	0.725	0.96	1.86	0.583	0.145	97.9	n/a	169	451	10.1	$2 \pm 1 \times 10^7$
AC 3	$scCO_2$ + Natural (55 days)	2100	100	200	6.5	47	50	37	2100	4280	6.01	$3 \pm 4 \times 10^5$
AC 4	$scCO_2$ + Natural (106 days)	2600	130	260	2.1	63	54	45	2600	5025	6.2	$4 \pm 1 \times 10^5$
AC 5	$scCO_2$ + Natural (146 days)	3000	130	340	67	82	54	51	3000	3990	6.1	$4.4 \pm 0.7 \times 10^5$
AC 6	$scCO_2$ + Sterile (146 days)	2700	120	250	8.5	76	51	46	2700	3880	6.2	$3 \pm 3 \times 10^7$
AC 7	Ar + Natural (146 days)	0[c]	64	14	49	7.1	38	23	0[c]	688	9.57	$5 \pm 1 \times 10^7$
AC 8	Ar + Sterile (146 days)	0[c]	92	11	2.6	0[c]	30	32	0[c]	1008	9.31	$8 \pm 1 \times 10^5$

[a] pH as recorded directly from the pressure vessel upon opening.

[b] Average cell concentration as determined by triplicate qPCR reactions of each sample; ± standard deviation.

[c] Zero value indicates measurement was below the detection limit.

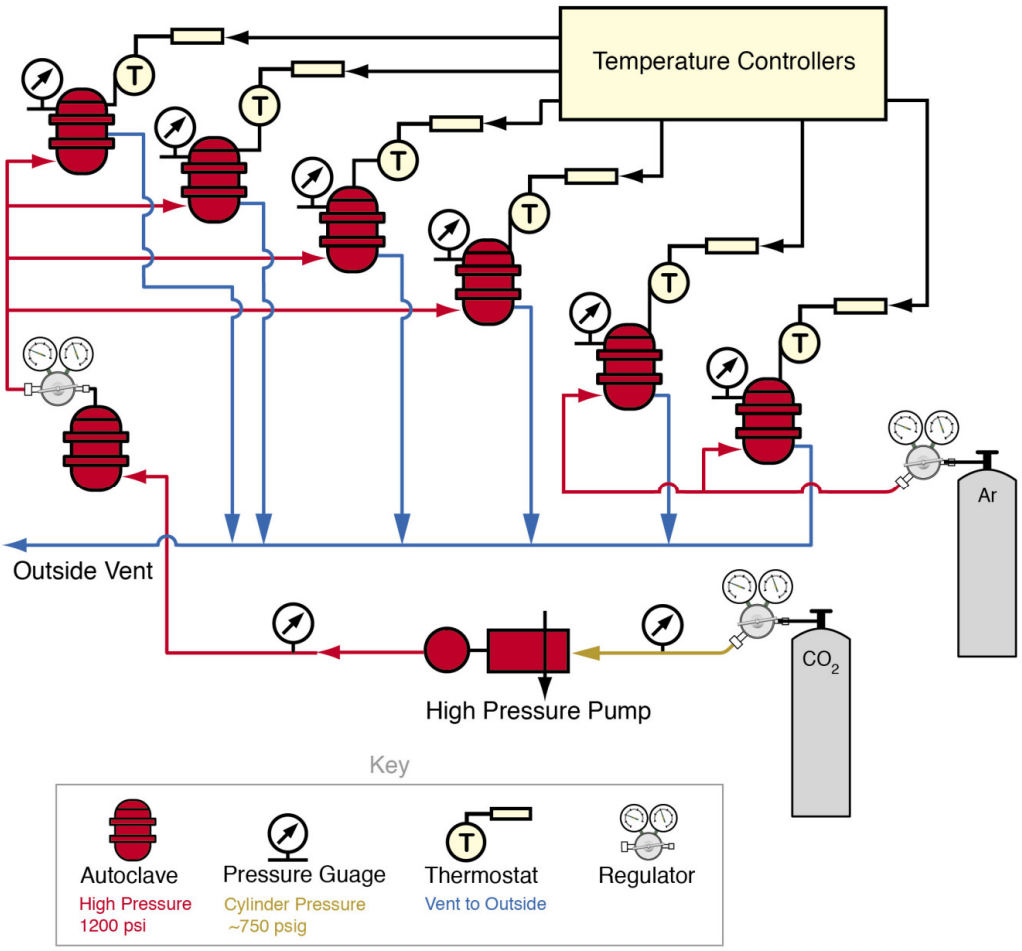


Figure 3.1. Schematic of pressure vessel system. Argon pressure vessels were isolated from scCO2 pressure vessels. Each pressure vessel was equipped with a thermocouple that measured and recorded the temperature and pressure during the experiment, as well as an analog pressure gauge, which was recorded daily.

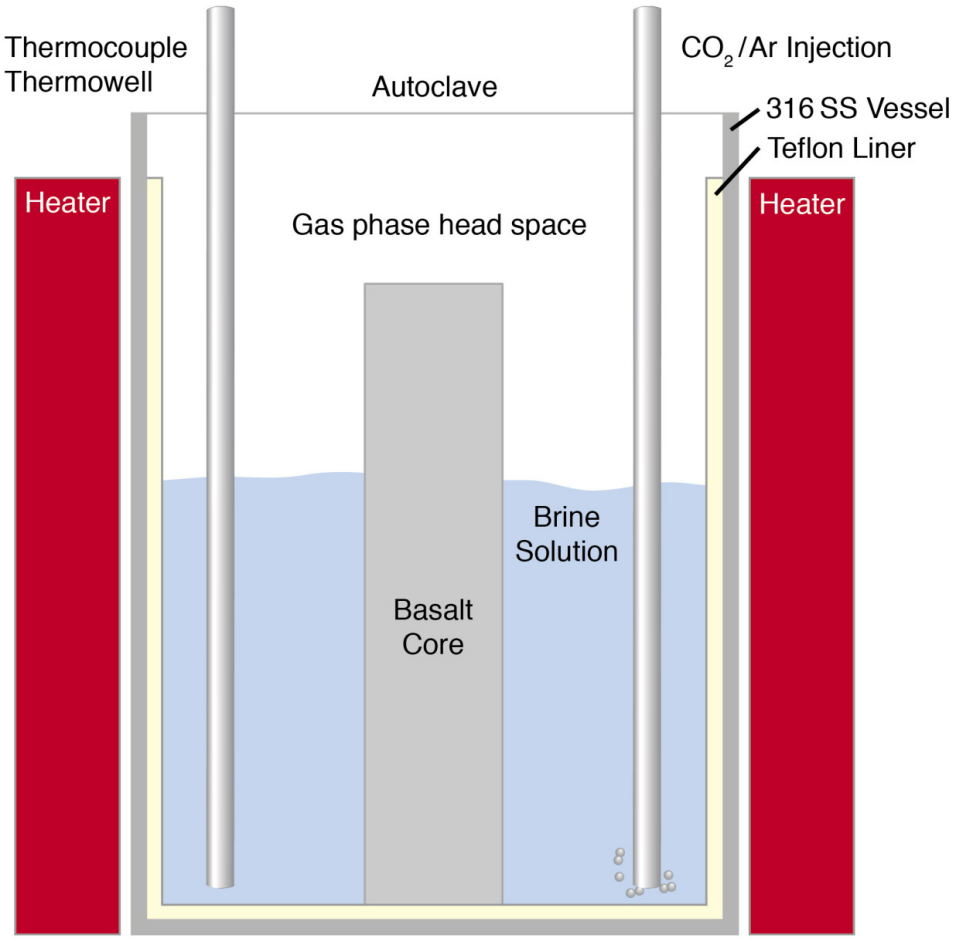


Figure 3.2. Diagram of a single pressure vessel demonstrating the assembly of the pressure vessel and the placement of the basalt core. Each core was halfway submerged in the solution.

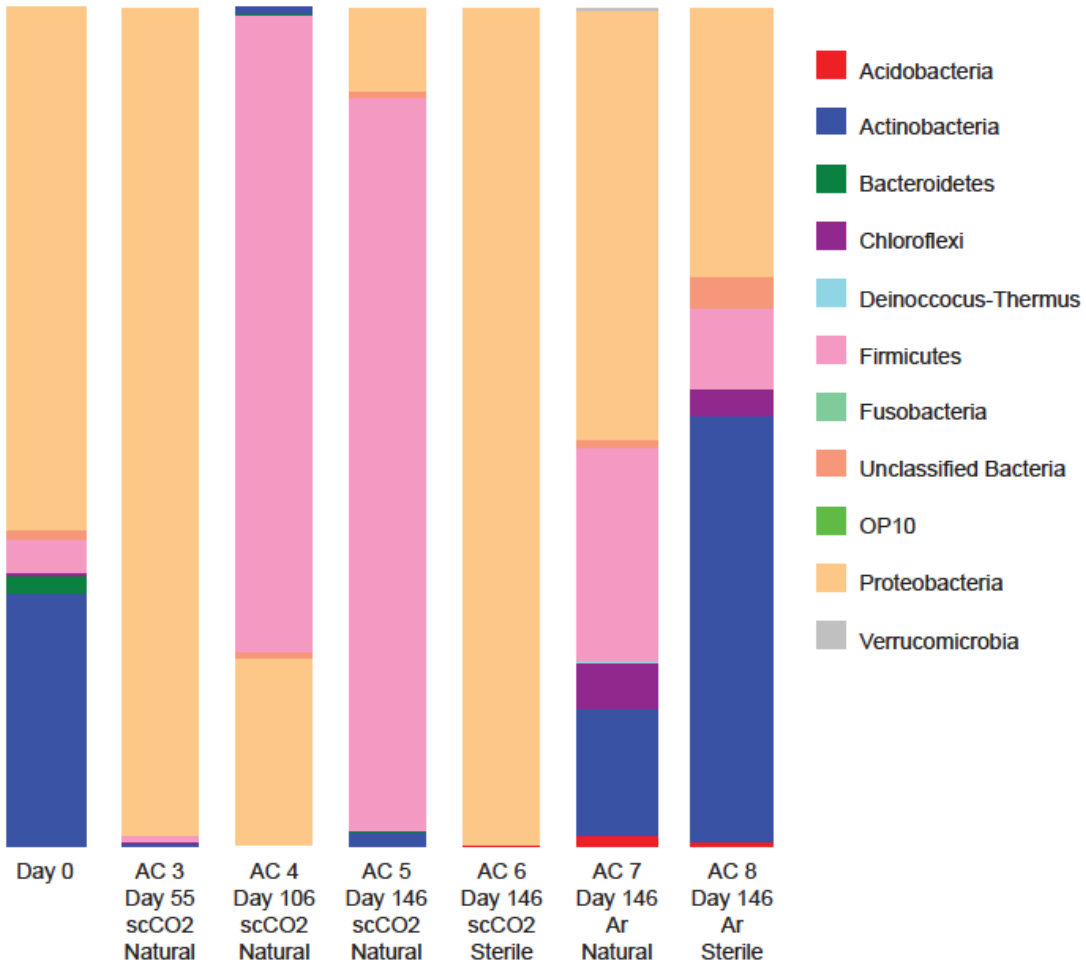
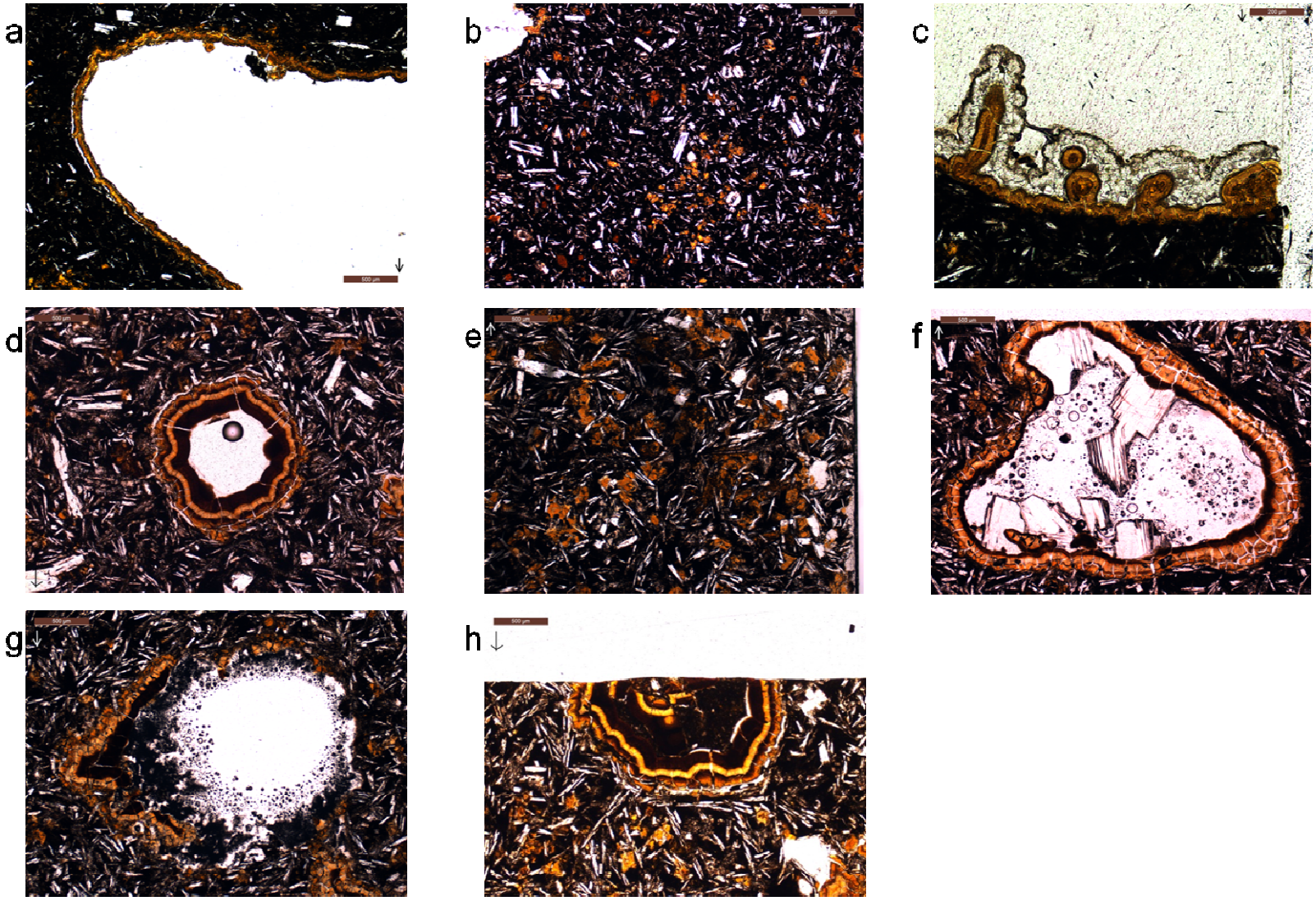


Figure 3.3. Bacterial community composition in respective autoclaves following exposure to $scCO_2$.

Figure 3.4. Photomicrographs of post-experiment samples exposed to $scCO_2$. (a) Iron hydroxide (yellow-orange) after 106 days (core C-1) (b) Iron-rich carbonate (yellow-orange) in the groundmass with abundant plagioclase after 106 days (c) Zeolites (white in vug) and iron hydroxide after 106 days (d) Iron-rich carbonate (brown-orange) filling a vug after 146 days in natural formation water (e) Carbonate (orange) in the groundmass after 146 days in sterile formation water (f) Zeolite (white in vug) degradation with iron-rich carbonate (brown-orange) after 146 days in natural formation water (g) Iron-rich carbonate (brown-orange) and degraded zeolites/partial infilling in a vug after 146 days in sterile formation water (h) Filled vug with iron-rich carbonate (brown-orange), 146 days in natural formation water


a
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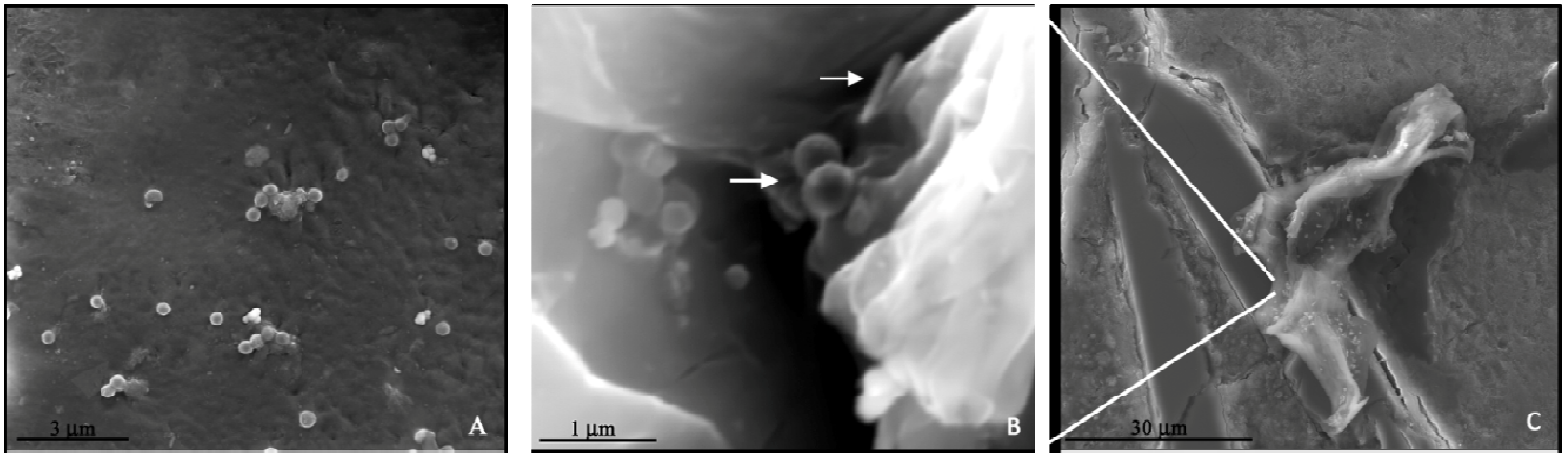

Figure 3.5. SEM micrographs depicting microorganisms in basalts. (a) Sample C-1 showing presumptive microbial cells on dense biofilm after 106 days of exposure to $scCO_2$; (b) Close up of (c) Sample A-2 exposed to 146 days of $scCO_2$ containing both spherical and rod shaped objects that are consistent with the size and shape of microbial cells (indicated by arrows) and embedded in an apparent biofilm; (c) Apparent biofilm attached to a plagioclase grain.

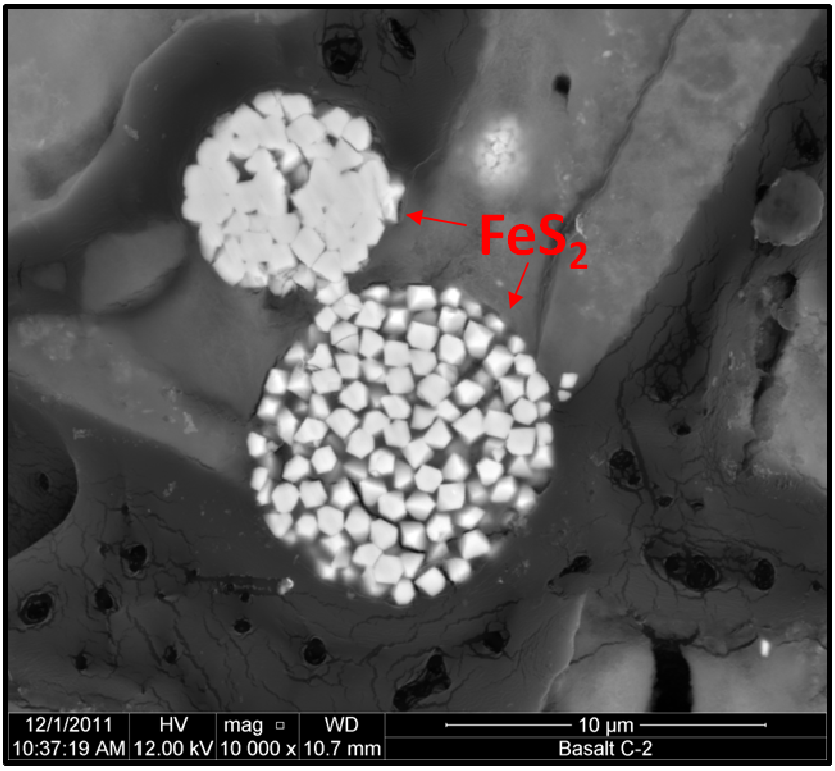

Figure 3.6. SEM BSE image of framboidal pyrite surrounded by carbon-rich material presumed to be biofilm (dark) attached to exterior of basalt vesicle.

4. CONCLUSION

This thesis contributes to the growing field of the microbiology of geological carbon sequestration. The objective of this work was to describe the microbial community of the Wallula pilot geological carbon sequestration well and to determine how the microbial community may be affected by the injection of $scCO_2$ into deep subsurface basalts using a model system in the laboratory. In order to accomplish this goal formation water samples, representative of their respective formations, were obtained from the Wallula pilot well. The five different samples described in Chapter 2 were obtained using three different methods aimed at minimizing contamination during collection of representative samples. The progressive drill-and-test method utilized packer technology to isolate aquifers of interest from surrounding formations. Use of an underbalanced drilling technique kept drilling mud from contaminating the deep subsurface, as the formation water itself acted as lubricant for the drill bit. Lastly, hydrochemical and microbiological samples were collected at the end of extensive pump tests. Thus the water collected for microbiological analysis was representative of the formation from which the water was obtained. The quality of the sample analysis was also assured using spiked positive and negative controls with each DNA extraction and PCR performed. In addition, each microbiological method used in this research was optimized using a spiked positive control designed to mimic the actual samples from each study.

Chapter 2 covers the microbial characterization of the Wallula pilot well prior to the injection of $scCO_2$. This characterization provides baseline diversity data for the microbial community to which future samples collected after the injection of $scCO_2$ can be compared. Bacterial biomass from these six samples as determined by qPCR ranged from $3.3 \pm 0.2 \times 10^3$ cells mL^{-1} of formation water to $4 \pm 2 \times 10^8$ cells mL^{-1} of formation water, with the deepest depth harboring the greatest concentration of bacterial biomass. Pyrosequencing of the microbial community revealed that the Wallula pilot well is dominated by the bacterial phyla Proteobacteria, Firmicutes, and Actinobacteria and by the archaeal phylum Crenarchaeota. Identified members of these phyla are related to hydrogen oxidizers, methylotrophs, sulfur reducers, methanotrophs, acetogens, and methanogens. Comparing the identified microbial community to that of models for a mafic system like the CRBG (Onstott 2005) and a previous pilot scale study in a different subsurface setting (Morozova et al. 2010) we project that the acetogens and methanogens could increase upon the injection of $scCO_2$ into the Wallula pilot well.

Chapter 3 reports the results from a study designed to simulate the injection of $scCO_2$ into the Wallula pilot well. The goal of this experiment was to determine how the microbial community would change in response to the presence of $scCO_2$. After 55 days of exposure to $scCO_2$ the concentration of bacteria in the pressure vessel dropped from $2 \pm 1 \times 10^7$ cells mL^{-1} to $3 \pm 4 \times$

10^5 cells g^{-1} of basalt, indicating that the bacterial community may be altered but is able to survive in close proximity to $scCO_2$. However, the archaeal community was unable to be detected via amplification of 16S rRNA genes after CO_2 exposure. Pyrosequencing of the community before and after CO_2 exposure showed a shift from an initial community dominated by Proteobacteria to a community mainly composed of Firmicutes; specifically the genus *Alkaliphilus*. This result suggests that microorganisms capable of surviving in a dormant, resistant state may form the majority of a microbial population exposed to the geological sequestration of carbon dioxide (Enomoto et al. 1997; Werner & Hotchkiss 2006).

By combining data from Chapters 2 and 3, a before/after exposure to $scCO_2$ microbial community can be inferred. Most notably, archaea could not be detected after contact with $scCO_2$. Archaea may have been unable to be detected from the basalts as they often require more stringent DNA extraction techniques than bacteria. So too, the archaea may have perished during transport from the field site to the laboratory upon exposure to oxic conditions, as many archaea are known to be strict anaerobes. However, archaea were observed to disappear after increasing in abundance in a saline aquifer exposed to CO_2 (Morozova et al. 2010). Thus, it is possible that the disappearance of the archaea is due to the presence of $scCO_2$ in our system as well.

Changes were also observed in the bacteria. The initial community, dominated by Proteobacteria, was replaced by one dominated by Firmicutes. Interestingly, the two genera that became most dominant over the course of the experiment were either not detected in the initial community (*Alkaliphilus*) or present at a low percentage of the identified taxa (*Geosporobacter*). In addition to these two genera, others also rose in abundance. *Bacillus* was not detected in the inoculating formation water, but was present to a great extent after 106 days of exposure to $scCO_2$. *Pseudomonas* and *Methylibium* were also rare in the initial formation water, but grew in abundance over time. The dominance of an organism that was not initially present in the inoculating formation water was also reported for a study performed on saline water collected from another prospective site for geological sequestration of carbon (Gulliver & Gregory 2011). These results indicate the important role of the rare biosphere in microbial communities exposed to anthropogenic changes in the environment. These rare members may not appear to play a significant role in the natural environment; however, their ability to withstand exposure to $scCO_2$ and increase in abundance subsequent to that exposure suggests their nominal lifestyle is especially suited for survival under harsh conditions. Thus, the rare biosphere becomes essential in the aftermath of the geological sequestration of CO_2 and should be examined in detail at the Wallula pilot well prior to the injection of $scCO_2$ into the basalt.

Several problems occurred during our laboratory study to examine the changes that occurred in the microbial community when exposed to $scCO_2$ in basalts. Significant formation water transfer from all of the pressure vessels running longer than two months was encountered, leaving little formation water in some of the pressure vessels and double the amount of formation water in others. Transfer of formation water resulted in the sterile pressure vessels becoming contaminated so that sharp conclusions cannot be drawn with regards to the presence/absence of microorganisms affecting the precipitation of carbonates on the basalts. We were unable to collect all of the desired samples due to the limited amount of formation water in the pressure vessels and thus the full analysis could not be accomplished on all samples. In order to remedy the situation for future experiments, holes have been drilled in the gas injection tubes to prevent the siphoning of formation water, thus isolating each pressure vessel.

Contamination was also observed in some of the DNA extracts from the basalts. Cores from AC 3 and AC 6 were particularly affected by the contamination of the genera *Escherichia/Shigella*. Such contamination could have occurred during several analyses. First, the use of 40 kGy may not have been sufficient for complete sterilization of the basalt cores, although it was double the amount reported in the literature for the sterilization of sediments. However, it is unlikely that the contamination occurred at this point in the process, as the basalt cores were not heavily exposed to microorganisms

while in storage. Future experiments will investigate the amount of radiation needed to completely sterilize basalt cores spiked with microorganisms. The next step where contamination may have occurred is during the DNA extraction process. The steel mortar and pestle had previously been used during the extraction of DNA from *Escherichia coli* spiked basalt samples. The mortar and pestle was sterilized via autoclaving after each DNA extraction; however, the treatment may not have been adequate for the removal of the *E. coli* culture. Future studies will investigate the best means for the sterilization of the instrument. Lastly, contamination could have occurred during the DNA extraction procedure as the reagents could have been contaminated during previous use. This seems unlikely as several new kits were used throughout the course of the experiment to extract DNA. Thus, future investigations will incorporate rigorous quality control methods to reduce the source of contamination.

Opportunities abound for future studies of the effects of $scCO_2$ on microbial populations in basalts targeted for geological carbon sequestration. Pyrosequencing of samples collected after the injection of $scCO_2$ into the CRBG will aid in understanding how the microbial community responds to $scCO_2$ and how it may be contributing to or interfering with the geological sequestration of carbon. In addition to determining the microbial response to $scCO_2$, close monitoring of the microbial community via pyrosequencing could also serve as an indicator of CO_2 movement in the aquifer.

One of the drawbacks of this research is that only the formation water, containing the unattached microbial community, was studied. It is widely accepted that the attached community is more representative of a given formation (Alfreider et al. 1997; Lehman et al. 2001, 2004). Therefore future studies of the Wallula pilot well should include the examination of the attached community, as biofilms have been shown to better survive exposure to $scCO_2$ (Mitchell et al. 2008; Mitchell et al. 2009). A system such as FLOCS (Orcutt et al. 2009) could be deployed in the Wallula pilot well containing whole basalt cores or crushed basalt for a few months prior to the injection of $scCO_2$ in order to perform *in situ* sampling of the microbial community prior to, during, and after the injection phase of the project. Changes in the microbial community could be determined using pyrosequencing and changes in functional genes, and thus microbial activity, could be monitored using qPCR.

The activity and viability of the attached microorganisms exposed to carbon sequestration conditions needs to be investigated. In addition to the *in situ* study described above, the pressure vessel system could also be utilized to study changes in functional genes related to carbon metabolism (Table 1.2), as well as examine the incorporation of injected labeled $sc^{13}CO_2$ into biomass by the microbial community. Other studies of interest include exposing the native microbial community to various concentrations of $scCO_2$ to determine how the community will respond to the injected $scCO_2$ plume along a gradient and subjecting the native microbial community directly to $scCO_2$ via a flow-

through reactor system instead of the static system used in this research in order to see how the microbial community is affected by direct contact with $scCO_2$.

In conclusion, this work adds a native microbial perspective to previous studies investigating the microbiology of geological carbon sequestration. Much research has been done using model microorganisms which readily form biofilms (Dupraz, Parmentier, et al. 2009; Dupraz, Menez, et al. 2009; Mitchell et al. 2008; Mitchell et al. 2009; Mitchell et al. 2010). These studies provided valuable insight into how microorganisms aid in the precipitation of carbonates and how the formation of biofilms acts to seal pores and reduce permeability of sandstone cores, thus demonstrating how microorganisms may aid in the geological storage of CO_2. More studies including the use of native and/or mixed populations of microorganisms will elucidate how the microbial community will affect the permanent storage of carbon in the deep geologic subsurface.

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6. APPENDICES

Appendix A: Chapter 2 Supplemental Materials

Additional alpha diversity considerations. Those taxa occurring as less than five percent of the identified OTU's from each sample are indicated in the following section.

Unclassified Comamonadaceae comprised 3.9% of the reads from Sample W. Unclassified Burkholderiales, *Simplicispira*, *Azomonas*, unclassified Pseudomonadaceae, unclassified Burkholderiales incertae sedis, and *Pseudomonas* all made up between 2% and 1% of the taxa from Sample W.

Denitratisoma and *Croceicoccus* composed 2.8% and 2.5% of the taxa from Sample GR1, respectively. *Simplicispira*, *Novosphingobium*, and *Azomonas* contained 1.4%, 1.0%, and 1.0% of the number of taxa from Sample GR1, respectively.

Novosphingobium made up 4.3% of the number of taxa from Sample GR2. The genera *Rhodobacter*, *Aquabacterium*, and *Symbiobacterium* comprised 2.5%, 2.4%, and 2.2% of the number of taxa from Sample GR2, respectively. Unclassified Erythrobacteraceae, *Denitratisoma*, unclassified Burkholderiales, *Acidovorax*, and *Methylophilus* composed 1.2%, 1.1%, 1.1%, 1.0%, and 1.0% of the taxa from Sample GR2, respectively.

Symbiobacterium composed 3.6% of the number of taxa from Sample GR3. The genera *Denitratisoma*, *Rhodobacter*, and *Propionibacterium*

comprised 2.9%, 2.6%, and 2.0% of the taxa from Sample GR3, respectively. The genera *Pseudomonas*, *Novosphingobium*, *Methylomonas*, *Olsenella*, and *Acidovorax* composed 1.9%, 1.7%, 1.4%, 1.1%, and 1.0% of the number of taxa from Sample GR3, respectively.

Croceicoccus, *Hydrogenophaga*, and *Methylotenera* comprised 4.6%, 3.9%, and 3.9% of the number of taxa from Sample GR4, respectively. Unclassified Porphyromonadaceae, *Paudibacter*, unclassified Comamonadaceae, and *Bellilinea* comprised 2.5%, 2.1%, 1.7%, and 1.6% of the taxa from Sample GR4, respectively. *Optitutus*, unclassified Rhodobacteraceae, *Rhodobacter*, unclassified bacteria, and *Denitratisoma* made up 1.4%, 1.2%, 1.1%, 1.1%, and 1.0% of the number of taxa from Sample GR4, respectively.

Unclassified Comamonadaceae and unclassified Coriobacteriaceae made up 3.3% and 2.1% of the taxa from Sample IZ, respectively. *Croceicoccus*, unclassified Bacteroidetes, and *Hydrogenophaga* comprised 1.7%, 1.7%, and 1.6% of the number of taxa from Sample IZ, respectively. *Silanimonas*, unclassified Clostridiales, unclassified Bacteria, *Dethiobacter*, and *Stenotrophomonas* encompassed 1.3%, 1.2%, 1.2%, 1.0%, and 1.0% of the number of taxa from Sample IZ, respectively.

Unclassified Desulfurococcales composed 4.2% of the archaeal taxa from Sample W, respectively. Unclassified Methanosarcinales, *Thermogymnomonas*, unclassified Desulfurococcacea, unclassified

Thermoproteales, and unclassified Methanomicrobia made up 2.9%, 2.8%, 2.4%, 1.6%, and 1.0% of the taxa from Sample W, respectively.

The unclassified Desulfurococcales and Methanomicrobia comprised 1.6% each of the identified archaeal taxa from Sample GR1.

Unclassified Euryarchaeota, unclassified Desulfurococcacea, and unclassified Desulfurococcales made up 4.4%, 3.8%, and 1.6% of the number of taxa from Sample GR2, respectively.

Unclassified Desulfurococcales and *Thermogymnomonas* each made up 1.8% of the number of taxa from Sample IZ, respectively.

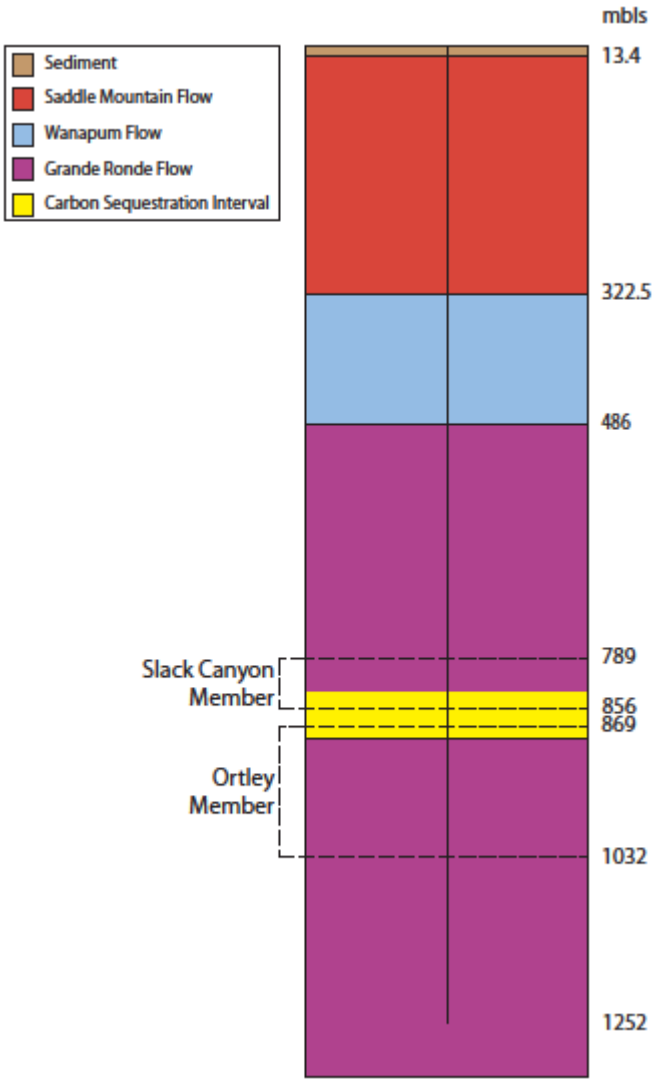

Figure A.1. Wallula pilot well cross section showing the location of the Slack Canyon and Ortley members. These members form the interflow zone into which $scCO_2$ can be injected, as well as the cap rocks above and below the injection zone (indicated in yellow). Dashed lines represent the depth interval covered by the Slack Canyon and Ortley members, respectively.

Appendix B: Chapter 3 Supplemental Materials

Materials and Methods

Cell enrichment. Enrichment cultures of sulfate reducers and iron reducers were attempted from basalt sub cores obtained from the pressure vessels in order to isolate a model organism for future studies. Culture media for microbial enrichments were prepared anaerobically in Hungate tubes fitted with screw-caps with septa. For sulfate-reducing enrichments, two different media were prepared: Postgate's medium B and Postgate's medium C (Hines et al, 2007). *Desulfovibrio vulgaris* (ATCC 29579) was used as a positive control culture for sulfate reduction. For iron-reducing enrichments, three different media were prepared: M and N (Basso et al, 2009) and JF-5 (Magnusson et al, 2010). *Shewanella oneidensis* (courtesy of Gabe Iltis) was used as a positive control culture for iron reduction.

5 mL of each media was injected into Hungate tubes filled with N_2 and all tubes were then autoclaved for 15 min at 121°C and 1.03 bar. Samples for growth were prepared from each pressure vessel by vortexing the 125 mL glass bottles containing the incubated formation water and basalt sample for 15 min. 1 mL of the formation water-basalt solution was then injected into each media, respectively, inside an anaerobic chamber. Tubes were placed on a gently shaking platform at 30°C for 5 months and monitored for signs of growth.

Results

Geochemistry. At downhole pressure and temperature the model CHIM-XPT demonstrated that minor concentrations of siderite are predicted to precipitate, whereas both gypsum ($CaSO_4 \cdot 2H_2O$) and ankerite ($Ca(Fe, Mg, Mn)(CO_3)_2$) are slightly undersaturated in solution and have potential to precipitate as aqueous species are dissolved from the basalt.

Mineralogy and petrography. Prior to the experiments in cores A and C zeolites generally occurred as unidentifiably small to 0.5 mm tabular crystals (Figure B.1a,c), and the zeolite linings in the vugs ranged from <100 μm thick to more than 250 μm thick. In core B zeolites occurred as both unidentifiably fine-grained vesicle-linings as well as well-formed radial aggregates of acicular crystals (Figure B.1b). Celadonite ($K(Mg,Fe,Al)_2(Si,Al)_4O_{10}(OH)_2$) occured with the heulandite in core B (Figure B.1b,e). Quartz, cristobalite, and tridymite, all polymorphs of SiO_2, were also present filling vesicles in the basalt, particularly in core C. Ultra-fine-grained iron hydroxide crystals too small to identify under the petrographic microscope were confirmed by SEM and formed a brown-orange lining around vugs and micro-vugs in all three basalt cores (Figure B.1d,f). From SEM identification of iron and titanium, fine grained opaque minerals were likely oxides such as magnetite (Fe_3O_4) or ilmenite ($FeTiO_3$).

Dissolution of plagioclase was evident around vesicles in cores A-1 and A-2 after 146 days of exposure to $scCO_2$. Many vesicles in these samples

were filled in by fragments of plagioclase, augite, and smectite, likely weathered from the initial basalt. Carbonate and very-fine-grained oxides or sulfides were also identified under the microscope (Figure 3.4h). The oxides or sulfides were also seen within the carbonate alteration around vugs. Vugs within the core showed alteration similar to the vugs on the edges of the core. Both samples showed significant degradation of zeolites compared to the initial sample. Bubbles and mineral fragments within vugs may be indications of zeolite degradation (Figure 3.4f,g).

Sample C-2 was exposed to argon gas (Ar) for 146 days in natural formation water, and sample B-2 was exposed to Ar for 146 days in sterile formation water. Again, no significant differences from the initial samples were observed in the XRD results from the post-experiment samples, but petrographic analysis indicated a number of changes. Sample C-2 differs from the other samples analyzed in that there were two distinct types of alteration visible. Some vesicles were lined with what is most likely iron hydroxide, which is dark brown-orange, highly fractured, difficult to distinguish from the glass in plane polarized transmitted light, and roughly 25 μm thick (Figure B.2a,c). This may be remnant iron hydroxide as seen in the initial sample, which typically dissolves when carbonic acid reacts with the basalt. Other vugs showed thick, bright orange alteration which was generally 50-100 μm thick, and comparable to the carbonation in the $scCO_2$ treated samples (Figure B.2b,c). The thicker, bright orange alteration was rare compared to the

thinner, dark orange alteration. Zeolites were present, but generally formed thinner layers than those in the initial sample, and in some places showed evidence of degradation (Figure B.2a,c).

Post-exposure to Ar, sample B-2 contained celadonite as well as radial, acicular zeolites; however, much of the celadonite was altered to an iron rich mineral. This iron-rich phase also occurred in the basalt groundmass as plagioclase, pyroxene, and olivine reacted. There was no significant indication noted of destruction of zeolites during the experiment as seen in the CO_2 exposed samples. The B-2 sample appeared visually similar to the initial sample. Some very fine-grained alteration partially filled vugs and occurred around zeolites in B-2.

Vugs in both Ar samples (B-2 and C-2) contained or were partially filled by fine-grained detritus, including mineral fragments and material too small to be identified. Very fine-grained opaque minerals, likely oxides or sulfides, were seen in reflected light, and could either be remnant from the initial basalt or newly formed. In C-2, this may be the pyrite identified by SEM.

Table B.1. Pyrosequencing primers, barcodes, and adapters used to distinguish each sample from one another. The number of reads obtained from each sample is also listed. Each sample was extracted in a batch with a negative control that was also sequenced due to contamination issues. Negative controls are indicated by a "B" designation. Samples were sequenced in the reverse direction, using primer 1046R.

Sample	Adapter	Barcode	Primer	Number of Reads
B3	CCATCTCATCCCTGCGTGTCTCCGACTCAG	GATCT	CGACRRCCATGCANCACCT	7024
A3	CCATCTCATCCCTGCGTGTCTCCGACTCAG	ATCAG	CGACRRCCATGCANCACCT	6195
B4	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CACAC	CGACRRCCATGCANCACCT	6016
A4	CCATCTCATCCCTGCGTGTCTCCGACTCAG	ACAGA	CGACRRCCATGCANCACCT	7469
B5	CCATCTCATCCCTGCGTGTCTCCGACTCAG	ACATG	CGACRRCCATGCANCACCT	2
A5	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CAGAG	CGACRRCCATGCANCACCT	8458
B6	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CGCAG	CGACRRCCATGCANCACCT	10622
A6	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CTGTG	CGACRRCCATGCANCACCT	8762
B7	CCATCTCATCCCTGCGTGTCTCCGACTCAG	GTGAG	CGACRRCCATGCANCACCT	1
A7	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TCATG	CGACRRCCATGCANCACCT	7367
B8	CCATCTCATCCCTGCGTGTCTCCGACTCAG	AGCAT	CGACRRCCATGCANCACCT	6383
A8	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CAGCT	CGACRRCCATGCANCACCT	9033
Bac518F	CCTATCCCCTGTGTGCCTTGGCAGTCTCAG		CCAGCAGCYGCGGTAAN	

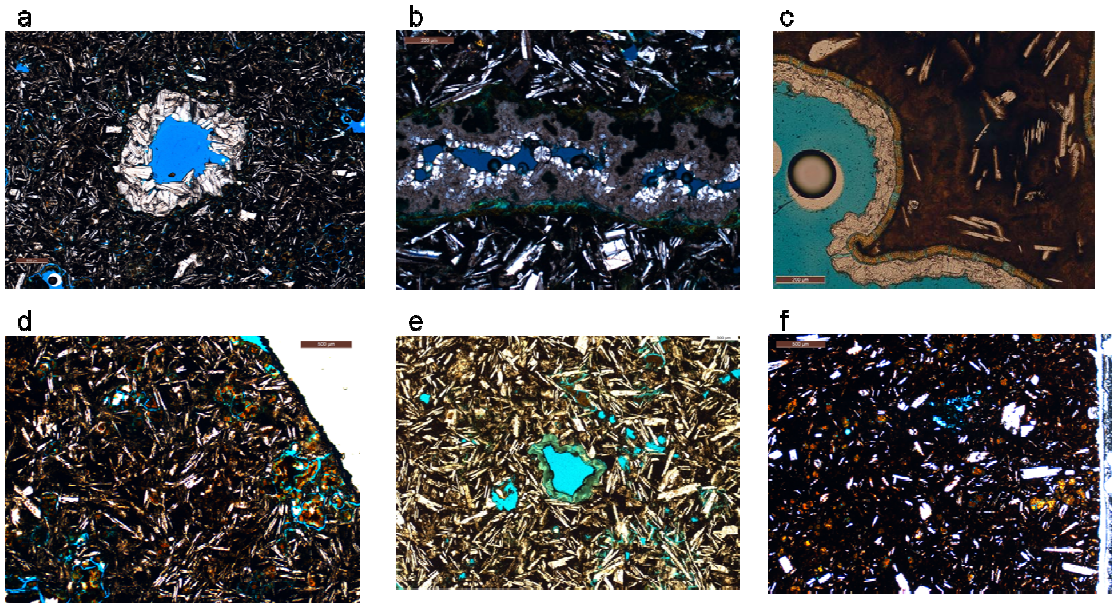

Figure B.1. Photomicrographs of initial (pre-experiment) basalt cores. Scales differ. (a) Zeolites (white in vug) and groundmass with abundant plagioclase from core A (b) Zeolites (white and gray in center) from core B with celadonite (green)(cross-polarized light) (c) Zeolites (white in vug) and iron hydroxide (yellow-orange) from core C (d) Iron hydroxide (yellow-orange) in cracks and micro-vugs from core A (e) Celadonite (green) in a vesicle from core B (f) Iron hydroxide (yellow-orange) in micro-vugs from core C.

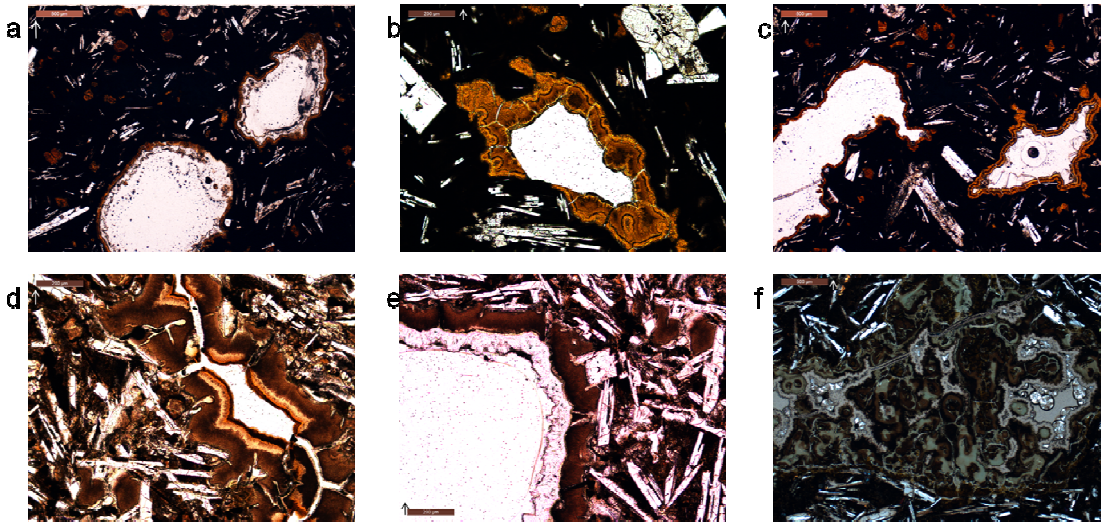

Figure B.2. Post-experiment samples exposed to argon. Scales differ. (a-c) 146 days in natural formation water (d-f) 146 days in sterile formation water (a) Iron hydroxide (red-brown) and zeolites (white in vug) lining vugs in plagioclase-rich groundmass (b) Carbonate (orange) altering a vug with plagioclase and pyroxene in the groundmass (c) Two types of alteration around vugs; iron hydroxide (red-brown, left vug) and carbonate (orange, right vug) with plagioclase and pyroxene in the groundmass (d) Carbonation (brown) around a vug in plagioclase-rich groundmass (e) Carbonate (brown) and zeolites (white in vug) lining a vug in plagioclase-rich groundmass (f) Brown alteration of celadonite (green) with zeolites (grey, round white) in plagioclase-rich groundmass (crossed-polarized light).

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