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Biogeochemistry of a Saline, Alkaline, Terminal Lake Ecosystem in Transition; Walker Lake, Nevada

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BIOGEOCHEMISTRY OF A SALINE, ALKALINE, TERMINAL LAKE ECOSYSTEM IN TRANSITION; WALKER LAKE, NEVADA, USA

By

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ABSTRACT

BIOGEOCHEMISTRY OF A SALINE, ALKALINE, TERMINAL LAKE ECOSYSTEM IN TRANSITION; WALKER LAKE, NEVADA, USA

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Walker Lake is a saline, alkaline, terminal lake ecosystem located in west-central Nevada. For over one hundred years, anthropogenic streamflow diversions within the Walker River Basin have ultimately led to little or no water reaching Walker Lake, the basin's terminus for water flow. These diversions have resulted in a >46 meter decrease in the lake surface altitude and increases in salinity and dissolved salt constituents that have caused the elimination of native fish species. This study examines how the lack of freshwater inflow has altered the physical, chemical, and microbiological structure of Walker Lake during the lake's ongoing desiccation.

Between 2007 and 2015, water and sediment samples were collected from a central lake location of Walker Lake, coinciding with the historical timing of late summer thermal stratification. Physical parameters and chemical constituent measurements show Walker Lake to have shifted from a monomictic to a polymictic system sometime after 2008, with salinity increasing conservatively to values over 21 g L^{-1} in 2015. Illumina sequencing of the V4 region of the 16S rRNA gene was completed on all environmental filter and sediment samples to

observe trends and changes in the microbial populations of the water column and sediment as a result of the changing lake dynamics. Over time, distinct differences in overall community composition and diversity were observed between sampling dates. The sediment communities were found to be highly dissimilar from the overlying pelagic microbial communities and showed more similarity to microbial communities from anoxic hypolimnion water samples from the 2008 sampling event when lake stratification was observed.

The anthropogenic and climatic factors that Walker Lake has faced over the past century have dramatically altered the ecosystem. This study aims to contribute to the overall understanding of the Walker River Basin and to other terminal lake basins throughout the world. By examining the microbial communities of Walker Lake and documenting the limnological shift of this transitioning ecosystem, we gain insights into the physiological aspects of Walker Lake and possible ways to manage and restore this unique environment to the thriving ecosystem it once was.

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CHAPTER ONE

INTRODUCTION

Salinity and Alkalinity in Aqueous Environments

Approximately seven-tenths of the Earth's surface is covered by water, with oceanic regions holding about 96.1% of Earth's total volume of water (Sigee, 2005). Of the remaining 3.9% of global water, only about 0.04% is distributed throughout the Earth within freshwater lakes, saline lakes, soils, rivers, and streams (Sigee, 2005). This comparatively small volume of useable surface water and its unequal distribution over Earth's surface make freshwater systems a valuable resource for organismal habitat usage and for human consumption, and also making these environments the center of much economic, social, and scientific attention. Even though the approximate volume of water globally found within saline lakes (104,000 km^3) is almost equal to that within freshwater lakes (126,000 km^3), saline lake ecosystems remain understudied compared to freshwater systems and oceans (MBES Committee, 1987, Wetzel, 2001).

The ionic contents within Earth's aqueous environments exist in wide gradients. For example, freshwater environments typically contain concentrations of total dissolved solids (TDS) less than 500 mg L^{-1} (Kalff, 2002), while the average TDS of ocean environments is 3,500 mg L^{-1} (Wetzel, 2001). TDS is a metric used to explain salinity concentrations and is defined as all of the dissolved ionic constituents (i.e. Na^+, Cl^-, SO_4^{2-}) contained in a volume of liquid (Sigee, 2005). Salinity can also be measured in terms of the electrical current that passes between two electrodes in water, typically expressed in microSiemens (µS) cm^{-1}, with greater concentrations of dissolved salts yielding a greater electrical conductivity (Kalff, 2002). Saline waters are

classically defined as water bodies containing TDS concentrations ≥3,000 mg L^{-1} (Williams, 1981). Saline water bodies can be further subdivided into three categories according to their salinity concentrations: hyposaline (3,000-20,000 mg L^{-1}; ~5,500-30,000 µS cm^{-1}), mesosaline (20,000-50,000 mg L^{-1}; 30,000-70,000 µS cm^{-1}), and hypersaline (>50,000 mg L^{-1}; >70,000 µS cm^{-1}) (Hammer, 1986, Kalff, 2002).

Alkalinity is another important component used to describe aqueous systems. It is described in pH units (*puissance d'hydrogène*, or "strength of hydrogen") which range from <1 to 14 (Kalff, 2002) and explains the number of hydrogen ions (H^+) and hydroxide ions (OH^-) in a volume of water (Starr, *et al.*, 2015). Alkaline water bodies have pH levels greater than 7 and account for more than 80% of all inland water bodies, by volume (Grant, 2006). Aqueous pH levels are naturally controlled by sodium carbonate/bicarbonate-buffered systems (Sorokin, *et al.*, 2014). Soda lakes contain high concentrations of sodium carbonate (or other carbonate complexes) and low concentrations of calcium and magnesium (Jones, *et al.*, 1998, Grant, 2006). This imbalance of HCO_3^-/CO_3^{2-} ions to Ca^{2+}/Mg^{2+} ions is a factor of regional topography. Excessive evaporation causes the saturation of calcium and magnesium cations in the aqueous system, resulting in their precipitation out of solution as insoluble carbonate compounds (Jones, *et al.*, 1998). In lakes where calcium-rich groundwater seeps into alkaline water bodies, calcium ions form complexes with carbonate and precipitate in localized formations called "tufa" columns (Grant, 2006). As a result of the generally low concentrations of calcium and magnesium found in alkaline, saline lakes, the ionic composition of these water bodies tend to be enriched with sodium, chloride, and sulfate (Kalff, 2002).

While salinity and alkalinity are useful metrics to broadly categorize different saline and soda lakes, each saline-alkaline lake ecosystem is unique and has a collection of conditions and

features that are necessary for their existence. One of these unique conditions is the presence of saline-alkaline lakes within closed basins. Surface water drainage within a topographically closed, or endorheic, basin collects to form a terminal lake. Lakes within these types of terminal drainage basins retain inflowing waters and only allow outflow of water to occur through evaporation from the lake surface (Wetzel, 2001). Terminal lakes often contain saline and alkaline waters due to the retention and concentration of soluble salts as water evaporates from the lake. The increase in the concentrations of dissolved salt constituents in saline-alkaline terminal lakes can also be a climatic factor of the region experiencing more evaporation than precipitation, therefore these types of lakes tend to occur in more arid, dry climatic regions of the world (Cole, 1968). These unique conditions of terminal saline-alkaline lakes help to define and outline the overall characteristics and functionality of each individual ecosystem.

Biological Diversity in Aquatic Ecosystems and Response to Environmental Fluctuations

The diversity of organisms living in any body of water is determined by many factors (e.g. regional climate, sunlight exposure), but salinity and alkalinity are often key driving factors that can limit species diversity within an aquatic environment. Often the assemblage of environmental physiological demands placed upon aquatic organisms living in freshwater or saline water systems are so different from each other that species are typically adapted to tolerate or live within a certain salinity range (Sigee, 2005). Saline waters ($\geq$3,000 mg L^{-1}) often have an ionic content that is different than the ionic content found within cells. This places varying degrees of osmotic pressure and stress onto cells that can cause less internal free water to be present within cells that is necessary to sustain vital cellular activities, which can inhibit or cease

the growth and development of species within the aqueous environment (MBES Committee, 1987). Increasing salt concentrations, like those occurring in Walker Lake, can adversely shift the physiological demands on aquatic organisms, and those that are not able to tolerate or adapt to the higher salinities will either cease to exist in that environment or will persist at decreased growth rates and/or metabolic levels (Sigee, 2005).

Alkalinity is another feature that can limit species diversity in an environment. Most freshwater habitats exhibit relatively neutral pH levels (pH 6-8). Environments have wide ranges of pH values, but throughout most of them, the microorganisms are able to maintain a relatively neutral internal pH level using a variety of buffering mechanisms (Sigee, 2005). Walker Lake has historically shown relatively high pH levels (>9 pH). Microorganisms that optimally grow at pH levels greater than 9.0 are considered alkaliphilic and can include a wide variety of species (Madigan, *et al.*, 2009). Soda/alkaline lakes in tropical regions often have the highest levels of bacterial primary productivity globally (over 10 g C cm^{-2} day^{-1}) due to the large supply of CO_2, high ambient temperatures, and high daily light intensity (global mean lake and stream primary production ~0.6 g C cm^{-2} day^{-1}; Jones, *et al.*, 1998). These high productivity rates in soda lake systems can create a permanent or seasonal bloom of phototrophic microorganisms, visibly changing the color of the lake to shades of green or red (Jones, *et al.*, 1998). The combination of elevated primary productivity, high pH values, and evaporative concentration of dissolved constituents leads to high levels of dissolved organic carbon (DOC) in the water column of many soda lakes (Domagalski, *et al.*, 1989).

Increases in solar radiation and warming of waters often lead to springtime blooms of diatom and algal populations. Within a stratified lake system, which Walker Lake begins forming in late spring/early summer, the rapid increases in phytoplankton populations within the

warm epilimnion quickly deplete nutrients within this layer (such as phosphorous, nitrogen, and silica), thus limiting future growth. Algal and diatom blooms also increase photosynthetic activity and oxygen production and lead to supersaturation of dissolved oxygen in surface waters (Sigee, 2005). With the breakdown of stratification during the fall turn over event, the nutrient-rich hypolimnion replenishes the entire water column with nutrients, leading to a late fall algal and diatom bloom.

Some of the changes in the physical and chemical structure of an aqueous environment are normal transient conditions resulting from environmental seasonal cycles, including fluctuations in dissolved oxygen and nutrient availability. Changes in concentrations of dissolved oxygen, both increases and decreases, can have severe impacts on the populations of aquatic organisms. Within Walker Lake, higher oxygen demands in the hypolimnion by heterotrophic microorganisms during summer thermal stratification often leads to the hypolimnion becoming anoxic (Sigee, 2005), which places an environmental stressor on organisms that exhibit aerobic metabolisms. Anoxic niches are often found in close proximity to oxygenated niches, which facilitates the biogeochemical cycling of some elements, such as carbon, nitrogen, and sulfur.

Hydrogeology of the Great Basin and Walker River Basin

This study examines the current limnological and microbial characteristics of a terminal, saline-alkaline lake ecosystem, Walker Lake, Nevada, USA. Walker Lake is within the Walker River Basin, which covers approximately 10,200 km^2 of land on the Nevada-California border (Adams, 2007, Lopes & Allander, 2009). All surface water within this area drains toward the lowest point in the basin, Walker Lake. The Walker River is the principal tributary within the

Walker River drainage basin and originates in the southeastern Sierra Nevada Mountains as separate East and West Walker River forks that flow north and merge together in Mason Valley (Beutel, *et al.*, 2001, Adams, 2007). The Walker River flows towards Walker Lake, which is confined by the Wassuk Range to the west and the Gillis Range to the east (Lopes & Allander, 2009). As mentioned earlier, Walker Lake is defined as a terminal lake in that there is no outflow for retained water except for evaporation, which consequently causes the accumulation of dissolved ionic constituents that remain in the lake. Walker Lake represents the lowest region of the Walker River Basin, with an altitude of 1,173 m at the deepest part of the lake (Lopes & Smith, 2007).

The Walker River Basin is part of the larger Great Basin, which is a 518,000 km^2 area of the United States that encompasses most of Nevada and Utah, and regions of Oregon, Idaho, Wyoming, and California, and has no surface-water drainage to the ocean (Lopes & Allander, 2009). The Great Basin is geologically framed by the Wasatch Mountains to the east, the Sierra Nevada to the west, and the Snake River Plain to the north (National Park Service, 2016). As a result of the geology of this area, much of the Great Basin is classified as arid or semi-arid and exhibits high regional variability in annual mean temperatures and precipitation (Hammer, 1986). Given the Great Basin's internal drainage and aridity characteristics, all water that falls within this large area either evaporates, seeps underground, or flows towards lakes or playas (Lopes & Allander, 2009).

Climate of the Walker River Basin

Temperature and precipitation normals (the average value over a thirty year period) vary greatly across the Walker River Basin. Temperature variations throughout the basin are strongly influenced by land-surface elevation, as seen by the minimum and maximum temperature normals in low-elevations near Walker Lake (~1286 m above sea level) ranging from -3.8 to 35°C, respectively, to higher elevation areas of the basin near Bodie, California (2551 m above sea level) ranging from -14.4 to 25°C, respectively (Lopes & Allander, 2009). Daily temperatures at Walker Lake range from -10°C to 12°C in January and between 10°C and 38°C in July (Cooper & Koch, 1984).

Like regional temperatures, precipitation is also influenced by elevation and latitude, but also by prevailing regional weather patterns. Cold fronts bring broad, low-intensity storms in the fall through spring seasons, which account for about 75% of the annual precipitation in the Walker River Basin (most of which is as snowmelt) (Lopes & Allander, 2009). Monsoonal moisture is brought up to the Walker River Basin from the southwest during the summer as high-intensity, localized storms, which account for about 25% of the basin's annual precipitation (Lopes & Allander, 2009). Estimated precipitation normals in the Walker River Basin are not equally distributed and range from 10 cm at Walker Lake to 142 cm along the Sierra Nevada (Lopes & Medina, 2007). These unequal precipitation distributions within the Walker River Basin can be attributed to continentality, or more commonly known as the "rain shadow effect", which results from the Sierra Nevada mountain range shielding areas east of the range from moist oceanic winds (Houghton, *et al.*, 1975). As the Pacific winds rise up the western slopes of the Sierra Nevada, they cool and condense and lose much of their moisture. The winds continue

east of the Sierra Nevada range as drier winds that yield little or moderate precipitation over the Walker River Basin region (Lopes & Allander, 2009).

The average annual temperature within Nevada climate divisions 1 and 3 (the Walker River Basin falls on the boundary between these divisions) both have an increasing trend of 0.2˚F per decade between 1895 and 2015 (http://www.ncdc.noaa.gov/cag/), however, the average annual precipitation trend between 1895 and 2015 in these divisions is either increasing slightly (0.04" per decade in climate division 1) or not at all (climate division 3). Years of normal or above average rainfall allow for sufficient amounts of water to reach Walker Lake to maintain salinity levels and lake surface elevations. Years that bring less precipitation, however, result in less water that reaches the lake, and in some years no water at all. The increasing temperature trend seen in Nevada does not seem to have a bearing on the trends in precipitation, however the water usage and water demand needed to maintain agricultural yields continue to increase with the increasing annual temperatures.

Paleohydrology

Given the generally lower amounts of precipitation that the Walker River Basin (and the larger Great Basin) experiences, the consistent presence of water within terminal drainage basins seems improbable and unsustainable. However, other perennial lakes prevail in the Great Basin and have extensive climatic and hydrologic histories that have helped shape and define their current states. A few other well-known terminal, saline lakes that are present within the Great Basin are Great Salt, Pyramid, and Mono lakes. These lakes, as well as Walker Lake and numerous other smaller basin lakes, are remnant lakes of larger freshwater glacial lakes that

arose late in the Pleistocene epoch, which spanned 2.6 million to 11,700 years before present (yr B.P.) (Benson & Mifflin, 1986, Domagalski, *et al.*, 1989). Within the Great Basin region of North America, this time period was categorized by dramatic shifts in regional climate as drier, warmer air from the south met cooler air from the north, resulting in the median region experiencing more precipitation than evaporation (Cohen, 2003). The excess precipitation filled geologic basins, which spilled over and caused mass pooling of waters to form the two major pluvial water bodies of the Great Basin Pleistocene, Lake Lahontan and Lake Bonneville (Russell, 1895; Figure A.1). The approximate surface area of Lake Bonneville at its peak level, which once covered much of present-day northwestern Utah, was 51,560 km^2, about eight times larger than that of current Great Salt Lake (Galat, *et al.*, 1981, Hostetler, *et al.*, 1994). Lake Lahontan, the smaller of these two major paleolakes, was approximately 22,300 km^2 at its peak level and extended across present-day western Nevada. It encompassed seven subbasins, including the southernmost Walker Lake subbasin, which are separated by sills (horizontal sheets of intruding rock) of varying elevations (Benson & Mifflin, 1986, Benson, *et al.*, 1992). The highest sill within the Lake Lahontan system is the Adrian Valley Sill (1308 m above sea level), which separates the Walker Lake subbasin from the other Lake Lahontan subbasins (Benson, 1991). Decreases in Lake Lahontan's water level below 1308 m would isolate the Walker Lake subbasin from the rest of the Lahontan water body, making it an independent system from the rest of the subbasins until water levels rose above the Adrian Valley sill level (Benson & Thompson, 1987, Benson, 1991). As a result of this independent system based on basin topology, each separated water body is then regulated as a function of subregional hydrology (Benson & Mifflin, 1986). Walker Lake has experienced periods of desiccation before and after the timing of the last highstand (peak lake level) of Lake Lahontan when all subbasins were

connected, which occurred between 14,500 and ~12,500 yr B.P. (Benson & Thompson, 1987, Benson, 1991).

At the close of the Pleistocene epoch and beginning of the current Holocene epoch (~11,700 yr B.P.), global and regional climate shifts occurred as glacier ice sheets receded north, resulting in less precipitation and more evaporation (Hostetler, 1991, Kernan, *et al.*, 2010). During the early Holocene epoch, Walker Lake was likely a shallow (~1 m), ephemeral playa that experienced periods of full desiccation (Lopes & Allander, 2009). Periods of basin desiccation and refilling are not entirely due to the shifting climate changes, as the Walker River that feeds into Walker Lake gradually changed course and discharged into the Carson River Basin (14,000 to 12,500 yr B.P.) and meandered back into the Walker River Basin approximately 4,700 years ago (Benson & Thompson, 1987, Bradbury, *et al.*, 1989, Lopes & Allander, 2009). The most recent desiccation event of Walker Lake (2,800 to 2,000 yr B.P.) was likely a combined effect of Walker River meandering, long-term regional drought, and increased evaporative rates (Benson & Thompson, 1987, Bradbury, *et al.*, 1989). Since this last desiccation event, Walker Lake rapidly refilled and maintained a perennial lake system, with its water level having fluctuated several times by about 52 meters (Lopes & Allander, 2009). The Holocene period also sets itself aside from all other geological time periods with the rise of agriculture and industrial practices, which has caused drastic changes in the land cover and pollution of both terrestrial and aquatic ecosystems (Kernan, *et al.*, 2010).The introduction of agriculture to the Walker River Basin in the late 1800s introduced rapid surface level declines of Walker Lake, which is currently at its lowest level since refilling.

Recent Lake-Level Fluctuations and Impacts

Agriculture first became prevalent in the Walker River Basin in Mason Valley in the 1860s and quickly required extensive systems of irrigation ditches that diverted the Walker River to provide water to the expanding agricultural region within the entire basin (Collopy & Thomas, 2009). Greater water resources needed to be secured as more people began settling within regions of the Walker River Basin, which led to the developments of the Topaz Lake Reservoir along the West fork of the Walker River in 1921 and of the Bridgeport Reservoir on the East fork in 1923 (Lopes & Allander, 2009).The Walker River Paiute Reservation, which encompasses the northeastern part of the Walker River Basin at the northern end of Walker Lake, contains the Weber Reservoir (built in 1934), used by the Paiute Tribe for fishing, recreation, and storing irrigation water on the Reservation (Lopes & Allander, 2009). These reservoirs and irrigation systems (as well as numerous other streamflow diversions) along the length of the Walker River have sequentially reduced the amount of streamflow that reaches the basin terminus, Walker Lake, and have ultimately led to the greater than 46 m decline in the surface level of the lake since 1882 (Russell, 1885). The lack of freshwater inflow into the lake system that has resulted in the drastic lake-level declines has an inverse correlation with the concentration of total dissolved solids, which is currently 8.4 times more concentrated than the first documented measurement in 1882 (Russell, 1885; Figure A.2).

The first limnological study of Walker Lake was conducted by Israel Russell (1885) in which he observed the size of Walker Lake to be 41 km long and 7 km wide, with an approximate surface area of 246 km^2. Russell also noted a maximum lake depth of 68.6 m and TDS concentrations of 2,500 mg L^{-1}. Cooper and Koch (1984) showed an 18 m decline in Walker Lake's surface elevation between 1937 and 1975, with a correlated increase in TDS

concentrations from 5,650 mg L^{-1} to 10,650 mg L^{-1}, respectively. The 2015 maximum depth of Walker Lake was 17.1 m (approximately 1190 m above sea level), half of the recorded maximum depth 30 years ago (Domagalski, *et al.*, 1989), and TDS concentrations were 21,400 mg L^{-1}.

The typical order of ionic salts in freshwater (in decreasing concentration) for the cations is Ca^{2+}, Na^{+}, Mg^{+}, and K^{+}, and for the anions is HCO_3^{-}, SO_4^{2-}, and Cl^{-} (Sigee, 2005). The 2015 ionic composition of Walker Lake was dominated by Na (30%), SO_4^{2-} (29.7%), Cl^{-} (20%) and HCO_3^{-}/CO_3^{2-} (17.9%); the order of cation abundance was $Na^{+} > K^{+} > Mg^{2+} > Ca^{2+}$ and the order of anion abundance was $SO_4^{2-} > Cl^{-} > HCO_3^{-} > CO_3^{2-}$. While the concentrations of salt constituents has increased dramatically with the declining lake level, the overall composition of Walker Lake water in 2015 was nearly identical to that determined by Cooper and Koch (1984), with the only difference being that sulfate had overtaken chloride as the second most abundant ionic constituent.

Walker Lake has historically exhibited a monomictic mixing regime in which a period of summer thermal stratification from May through November is followed by a complete turnover of the lake water in November, which persists throughout winter and spring (Cooper & Koch, 1984, Domagalski, *et al.*, 1989, Beutel, *et al.*, 2001). Summertime stratification in Walker Lake forms as the surface water temperatures of the lake start to increase in spring, gradually forming a distinct layer of warmer, lighter water (epilimnion) that lies above the cooler, denser water at the bottom of the lake (hypolimnion). The epilimnion interacts with the atmosphere and is mixed by wind and is usually where most primary production in the lake occurs. The hypolimnion does not interact with the atmosphere or mix with the waters of the epilimnion, and utilizes organic matter that sinks from the epilimnion (Kalff, 2002). In some lake systems, oxygen concentrations

become depleted in the hypolimnion throughout summer thermal stratification by the action of microorganisms, and the advent of the fall turnover event temporarily lowers the dissolved oxygen content of the entire water column with the mixing of anoxic hypolimnetic waters (Cooper & Koch, 1984). Between these two distinct layers that form during thermal stratification is a metalimnion layer (also can be referred to as a thermo-, oxy-, or chemocline if the respective temperature, dissolved oxygen, or salinity values change between the epi- and hypolimnion) where physical and chemical parameters rapidly change between the epi- and hypolimnion layers. The metalimnion places restrictions on how nutrients and chemicals circulate in the lake system, therefore making stratification an important process for the macro- and microbiota of the pelagic and benthic environments (Kalff, 2002).

The mixing resistance exhibited in a thermally stratified lake weakens as cooler autumn temperatures begin to cool the surface waters of the epilimnion. As the temperature of the epilimnion lowers to a temperature near that of the hypolimnion, the density barrier between the layers weakens until the entire lake volume mixes from top to bottom and becomes isothermal (Kalff, 2002). In warm monomictic systems such as Walker Lake, this isothermal water column remains isothermal and mixes throughout winter until stratification occurs again in springtime. The last stratified profile observed by our research group within Walker Lake was in 2008, when the lake had a maximum depth of 23 m with the thermocline ranging from 17.5 m to 19 m.

Walker Lake was one of the few saline lakes to have supported a fishery and contained four endemic fish species: Lahontan cutthroat trout (LCT) (*Oncorhynchus clarkii henshawi*), Tui chub minnows (*Gila bicolor*), Lahontan redside shiner (*Richardsonius egregious*), and Tahoe sucker (*Catostomus tahoensis*). Generally speaking, fish are quite sensitive to habitat changes and fluctuations in the physical and chemical composition of the water. The construction of the

Weber Reservoir along the Walker River in 1934 hindered obligatory stream spawning by LCT, leading to the extirpation of the Walker Lake LCT strain and to the subsequent hatchery and stocking programs for non-Walker Lake strains of LCT (Cooper & Koch, 1984, Beutel, *et al.*, 2001). Up until 2008, LCT were stocked in the lake, but it was observed that stocked trout were not able to withstand the conditions of the lake without intense acclimation under similar lake conditions in the hatchery prior to lake introduction (Bigelow, *et al.*, 2010). By this time, TDS concentrations had reached >14,000 mg L^{-1}, causing detrimental health effects and major declines of the Tui chub population, the major food source of LCT (Wright, 2009). A study conducted on Pyramid Lake strain of LCT found that LCT experienced mortality between TDS concentrations of 12,000 mg L^{-1} and 13,800 mg L^{-1} (Taylor, 1972). A combination of factors including disappearing food source, toxic concentrations of TDS that inhibit growth and reproduction, and inhabitable waters during summertime anoxia (Cooper & Koch, 1984, Beutel, *et al.*, 2001, Wright, 2009) led to environmental pressures on LCT that could not be overcome under recent ecosystem trends and conditions.

Comparison of Walker Lake to Other Saline Lakes

Saline lakes are common throughout the world and are present on every global continent (Hammer, 1986). Among these lakes there is a wide variety of salinities and chemical constituents as a result of the broad climatic and geologic settings in which these lakes occur (Eugster & Hardie, 1978). Within the U.S. Great Basin, two other terminal lake systems, Pyramid and Mono lakes, are regionally close to Walker Lake and can be used as key comparisons in discussing ecosystem similarities.

Pyramid Lake is a hyposaline, monomictic terminal lake ecosystem located approximately 169 km NW of Walker Lake in Nevada. Pyramid Lake is also a remnant terminal lake of Pleistocene pluvial Lake Lahontan, although Pyramid Lake is not thought to have desiccated since the last highstand of Lake Lahontan 14,500 yr B.P. like Walker Lake has (Benson & Thompson, 1987). When the lake level of Lake Lahontan was above the highest interbasin point, the Adrian Valley sill at 1308 m, the Walker Basin connected with the other subbasins (including Pyramid) and formed one united body of water.

Like Walker Lake, Pyramid Lake has also been detrimentally affected by anthropogenic diversions of streamflow. In 1905, Derby Dam was built on the Truckee River upstream of Pyramid Lake, and the use of diverted water from the Truckee River caused the consequent decline of the lake level of Pyramid Lake by 23 m between 1905 and 1979 and the complete desiccation of adjacent Lake Winnemucca by 1939 (Galat, *et al.*, 1981, Thompson, *et al.*, 1986, Yang, *et al.*, 2003). The anthropogenic decline of the lake level of Pyramid Lake caused a correlating increase in total dissolved solid concentrations from 3,800 mg L^{-1} in 1933 to 5,300 mg L^{-1} in 1979 (Horne & Galat, 1985). Aside from the TDS concentration being far less than that of Walker Lake, Pyramid Lake shares a similar aqueous ionic composition to Walker Lake, with the exception that the concentration of carbonates exceeds that of sulfate (Galat, *et al.*, 1981, Cooper & Koch, 1984).

Mono Lake is an alkaline (pH 9.8) , hypersaline (TDS=88 g L^{-1} in 2015; Mono Lake Committee, 2016), terminal lake located approximately 80 km SSW of Walker Lake in California at the eastern base of the Sierra Nevada range (MBES Committee, 1987, Domagalski, *et al.*, 1989). Mono Lake is a remnant lake of the larger Pleistocene pluvial Lake Russell (Domagalski, *et al.*, 1989). Like Walker Lake, Mono Lake has also been detrimentally affected

by anthropogenic diversions of streamflow. Starting in 1941, water from streams that lead towards Mono Lake was diverted for municipal use in Los Angeles, causing the lake-level of Mono Lake to decline by 12 meters and the TDS concentration to reach its highest recorded value of 97 g L^{-1} by 1982 (Mono Lake Committee, 2016). Prior to streamflow diversions, Mono Lake's salinity was 48 g L^{-1}. Several legal battles since then have resulted in the ruling that the state of California must take action to preserve and protect the declining ecosystem of Mono Lake by reallocating water rights to allow more water to flow into the lake to maintain lake inflow to a maximum salinity of 85 g L^{-1} (MBES Committee, 1987, Mono Lake Committee, 2016).

The high salinity of Mono Lake (about 2.5 times greater than the salinity of the Pacific Ocean) has prevented fish from being able to live in the lake (MBES Committee, 1987). This lack of predators in Mono Lake allows for large populations of brine shrimp and brine flies to thrive, which in turn provides an ample and vital food source for migratory birds (Cooper, *et al.*, 1984). Even though Mono Lake's salinity is much greater than that of Walker Lake, the two systems do share similar aqueous chemistry composition and contain multiple ion dominance, with relatively equal proportions of chloride, sulfate, and carbonates (Cooper & Koch, 1984).

Mono Lake also has historically exhibited a monomictic mixing regime, but particularly wet years in the early 1980s brought large inflows of fresh water to Mono Lake, raising the lake level, and led to long term meromixis (fresh water forms a distinct layer above water with higher salinity). This chemical stratification persisted through two drought years until 1988. At that point, evaporation from the lake surface concentrated the TDS of the top surface layer (mixolimnion) to a similar salinity as the bottom lake layer (monimolimnion), thus reducing the

salinity gradient of the chemical stratification to the point where the entire lake could intermix and return to normal monomictic mixing regime (Domagalski, *et al.*, 1989).

Methods Used to Address Microbial Diversity

Within the first years of this project (2007-2008) the molecular-based technique of generating amplicon clone libraries that targeted the 16S ribosomal RNA (rRNA) gene region was used to explore microbial diversity within Walker Lake. This conserved gene is present in all known prokaryotic organisms and can thus be used as a genetic marker to identify and determine phylogenetic relatedness of different organisms (Breakwell, *et al.*, 2014). Broadly, the amplicon clone library technique involves extracting genomic DNA from environmental samples, amplifying the amount of DNA with Polymerase Chain Reaction (PCR) using specific primers that target the 16S rRNA gene, inserting the resulting product into plasmid vectors, transforming the vectors into host cells to generate many copies of a single sequence (*Escherichia coli* cells are commonly used). The gene insert is then sequenced to obtain the near full length 16S rRNA gene sequence of successfully cloned species (Vieites, *et al.*, 2010). This technique has been widely used as a molecular tool in understanding and describing the microbial diversity of an environment and has provided a reliable method in identifying species without relying on cultivation efforts or morphologic classifications.

The use of amplicon clone libraries to analyze community structure is limited and biased, however, by the nature of the protocol itself. Clone libraries rarely give a comprehensive and even representation of the complex diversity within an ecosystem, with the most abundant organisms often dominating the library and the less abundant or rare organisms often not appearing (Vieites, *et al.*, 2010). The generation of clone libraries does provide a general idea of

the overall structure of a microbial community and also provides near full length sequences of the 16S rRNA gene (~1500 base pairs), which is useful in comparing different organisms phylogenetically. However, given the limitations of this technique, a more encompassing approach to determine environmental species diversity, next-generation sequencing (NGS), was later introduced to this research (with my matriculation into the graduate program) to provide a deeper assessment of the microbial diversity. NGS methods yield substantially greater amounts of sequence data than classic sequencing methods and allow for the comparison of sequences from nearly all organisms present in an environment. Also, with NGS methods, rare organisms within an environment are detected and counted as part of the community structure, whereas in more classic cultivation or molecular based techniques, these organisms would have been lost among the more abundant organisms.

The NGS platform chosen to use in this research was bridge amplification sequencing (also referred to as Solexa or Illumina sequencing) using the fourth hypervariable (V4) region of the 16S rRNA gene. The 16S rRNA gene contains highly conserved sequence interrupted by nine regions of hypervariability dispersed throughout the length of the gene that exhibit considerable sequence diversity among prokaryotic species (Breakwell, *et al.*, 2014). Due to the hypervariability of these regions of the 16S rRNA gene, they can be targeted during sequencing and used to distinguish closely related organisms. While no single hypervariable region of the 16S rRNA gene can be used to differentiate all prokaryotic microorganisms, the V4 hypervariable region was chosen to elucidate microbial diversity in this study because it comprises approximately 250 base pairs of the 16S rRNA gene (~15%) and is flanked by highly conserved regions of the gene (Figure A.3). The V4 hypervariable region is commonly used to explore microbial diversity and has been used in large-scale research projects such as the Human

Microbiome Project (HMP Consortium, 2012) and the Earth Microbiome Project (Gilbert, *et al.*, 2014).

Broadly, with the Illumina MiSeq sequencing platform, amplified template DNA is ligated to short adaptor sequences, which anneal to complementary oligonucleotide sequences attached to a glass flow cell. Deoxynucleotides are passed through the flow cell to be incorporated into the complementary DNA strand, as well as the adaptor sequence at the unbound end. This complementary strand bends to anneal the adaptor sequence of the unbound end to a neighboring free oligonucleotide on the flow cell to repeat the synthesis process. This amplification process is repeated many times to form a cluster on the flow cell of thousands of copies of the template DNA (Figure A.4; Dale, *et al.*, 2012, Bragg & Tyson, 2014). Each cluster on the flow cell (a flow cell can hold up to 50 million clusters) is then sequenced by incorporating individual deoxynucleotides with bound fluorophores into the growing DNA strand and releasing a detectable fluorescent signal upon excitation by a laser that is recorded by a computer, thus building the sequence of the template DNA base by base (Dale, *et al.*, 2012).

CHAPTER TWO

LIMNOLOGICAL CHARACTERIZATION AND MICROBIAL BIOGEOCHEMISTRY OF A TERMINAL LAKE ECOSYSTEM; WALKER LAKE, NEVADA

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Graduate Student's Involvement and General Background

The research detailed in this chapter was conducted using samples collected by The Desert Research Institute (DRI) as part of the Walker Basin Project in 2007 and 2008, prior to my matriculation into the Master's program at University of Nevada, Las Vegas and prior to my employment and research at DRI, Las Vegas. Much of this research was conducted by previous DRI post-docs, Drs. James C. Bruckner and especially Jen C. Fisher. Graduate student Katherine Willever contributed to the following areas for this research project: initiating and facilitating Illumina sequencing of DNA samples from 2007 and 2008, generation and interpretation of resulting data, and the rewriting and editing of the subsequent manuscript.

This research characterizes the limnological properties and microbial diversity of Walker Lake in 2007 and 2008 and its relation to annual lake stratification by utilizing a variety of cultivation-based and molecular-based microbial diversity assessments. Initially, this project relied solely upon the molecular-based technique of generating amplicon clone libraries that targeted the 16S ribosomal RNA (rRNA) gene region. The use of amplicon clone libraries has been widely used as a molecular tool in understanding and describing the microbial diversity of an environment and has provided a reliable method in identifying species without relying on cultivation efforts or morphologic classifications. However, given the limitations of this technique, a more encompassing approach to determine environmental species diversity, next-generation sequencing (NGS), was later introduced to this research (with my matriculation into the graduate program) to provide a deeper assessment of the microbial diversity. NGS methods yield substantially greater amounts of sequence data than classic sequencing methods and allow for the comparison of sequences from nearly all organisms present in an environment. Also, with NGS methods, uncultivable and rare organisms within an environment are detected and counted

as part of the community structure, whereas in older cultivation or molecular based techniques, these organisms would have been lost among the more abundant organisms.

Using NGS technology with the Walker Lake data that encompasses the timeframe of my Master's thesis research (2013-2015) allowed me to delve deeper into the microbial diversity to gain a more comprehensive understanding of the pelagic microbial communities present at the time of sampling. Initiating and processing the Illumina sequence data from Walker Lake samples from 2007 and 2008 also provided a comparable dataset to examine microbial changes occurring in Walker Lake over a greater period of time and to describe the effects of summer thermal stratification in Walker Lake on the microbial communities at a deeper level than more traditional diversity analyses can accomplish.

Abstract

Walker Lake (Nevada, USA) has been impacted by anthropogenic water diversion over the past century, resulting in a >75% reduction in volume and a >5-fold increase in salinity. Decreased volume has also led to higher water temperatures and changes in chemical composition, both of which can be deleterious to native fish species, including the threatened Lahontan cutthroat trout. The evaporative concentration of solutes alters the structure of microbial populations occupying various zones of the lake and leads to changes in biogeochemical cycling within Walker Lake. We sampled Walker Lake during mixed and stratified conditions in 2007 and 2008. Physical and chemical analyses from aqueous samples showed an 11.7% increase in salinity and a correlated conservative increase in most major ion constituents between 10.7-14.5%. Dissolved phosphorous was extremely high (0.55-0.86 mg L^{-1}), while nitrogen was severely limited (at or below limit of detection), with the only readily detectable form of nitrogen being ammonia in the suboxic regions of the lake (0.11-0.47 mg-N L^{-1}).

Microbiological community surveys were conducted using both 16S rRNA gene clone libraries and Illumina MiSeq platform sequencing (V4 region) and cultivation-based approaches targeting different microbial physiotypes, revealing diverse alkaliphilic communities spatially constrained by lake stratification in 2008, but a fairly homogeneous community during mixis in 2007. Nearly 2,000 OTUs (97% identity) were identified, spread across 71 phyla, most of which were less than 0.5% abundant. Dominant phyla included Actinobacteria, Bacteroidetes, Cyanobacteria, Firmicutes, Tenericutes and Proteobacteria.

Several lines of evidence were consistent with metal reduction-oxidation cycling (arsenic, iron), a tightly coupled hypolimnetic sulfur cycle, and anoxygenic photosynthesis. These processes

may contribute to the removal of both sulfide and ammonia from the hypolimnion, as concentrations of both of these constituents were lower in 2008 than observed historically. Collectively, these results suggest that Walker Lake has a dynamic and diverse microbial community that is sensitive to environmental changes resulting from anthropogenic lake decline. Rapid changes in lake depth and volume that influence the seasonal mixing regimes may therefore impact the community members present, further augmenting the biogeochemical changes in the lake.

Introduction

Terminal lakes occur in endorheic drainage basins in which there is no outflow for water except for evaporation. As a result, these lakes tend to have high salinities from the accumulation of solutes and elevated pH levels. These 'soda' lakes typically have many or all of the following biogeochemical features: nitrogen limitation (Cooper & Koch, 1984), high total and dissolved organic carbon (TOC/DOC) concentrations (Humayoun, *et al.*, 2003, Jørgensen, *et al.*, 2003), sulfide and ammonia production in the seasonally stratified (or permanently meromictic) anoxic bottom water (Beutel, *et al.*, 2001, Dimitriu, *et al.*, 2008), and diverse routes for anaerobic respiration (Hollibaugh, *et al.*, 2005, Grant, 2006). The importance of microorganisms in the recycling of nutrients and food web dynamics is well established for freshwater and ocean environments (Fenchel, 2008), but remains less understood in soda lakes where nitrogen is severely limited, phosphorous is unusually high, and high concentrations of microbial biomass and DOC are found together (Humayoun, *et al.*, 2003).

Walker Lake, an alkaline (pH ~9.4), moderately saline terminal lake located in west-central Nevada, is a remnant lake of the vast Pleistocene Lake Lahontan (Adams & Wesnousky, 1998). Although the surface water elevation of Walker Lake has declined over the centuries (Benson, *et al.*, 1991), desiccation has accelerated over the past 150 years due to agricultural diversions of the lake's primary water source, the Walker River (Lopes & Allander, 2009), causing the concomitant increase in total dissolved solids (TDS) from ~2500 mg L^{-1} in 1882 to greater than 19,000 mg L^{-1} in 2008 (Collopy & Thomas, 2009). The reduced inflow and increased evaporative losses have reduced the lake volume by over 75% since 1882 (from 11.1 km^3 to less than 2.5 km^3; Beutel, *et al.*, 2001). As of 2008, Walker Lake was one of very few desert terminal lakes in the United States to have supported fish populations (Collopy & Thomas, 2009). The loss of cool, oxygenated hypolimnetic waters, along with the elevated TDS concentrations, have consequently caused the elimination of the lake's native fish populations, including those of the Tui chub (*Gila bicolor*), Tahoe sucker (*Catostomus tahoensis*), Lahontan redside shiner (*Richardsonius egregious*), and the threatened Lahontan cutthroat trout (*Oncorhynchus clarkii henshawi*) (Collopy & Thomas, 2009).

The desiccation of Walker Lake has also reduced the hypolimnetic volume during summer thermal stratification, resulting in rapid anoxia within the hypolimnion due to the biological activity working to meet the oxygen demand (Beutel, 2001). Walker Lake exhibits a monomictic mixing regime in which summer thermal stratification begins to form in May and lake turn over occurs in October or November. Historically, the anoxic conditions that occur after Walker Lake thermally stratifies have reportedly been concurrent with elevated hypolimnetic hydrogen sulfide and ammonia concentrations (Beutel, 2001). The physical and chemical changes occurring in Walker Lake are intimately linked with planktonic

microorganisms, and are ultimately the driving force behind microbial diversity and activity. Although various studies have documented the gradual shift in the limnological and ecological status of Walker Lake (Cooper, 1985, Beutel, *et al.*, 2001), to date, no substantial study of its bacterial communities has been performed. This study characterizes a number of aspects of the baseline microbial ecology of monomictic Walker Lake using a combination of cultivation-based methods and molecular techniques to assess microbial community structure. Cultivation-based approaches to microbial diversity provide quantifiable assessments of potential microbial function and different physiotypes, while molecular-based analyses explore the phylogenetic diversity of microbial communities without cultivation bias. These results, coupled with the characterization of Walker Lake's physical and chemical variables, reveal a great deal about the functional potential of this ecosystem, in particular the presence of metal reduction-oxidation cycling (arsenic, iron), a tightly coupled hypolimnetic sulfur cycle, and anoxygenic photosynthesis. If unabated, the ongoing anthropogenic desiccation of Walker Lake will lead to changes in the limnological and microbial community structures, causing consequential shifts in the biogeochemical cycles.

Materials and Methods

Sample Collection and Processing. Past bathymetric studies of Walker Lake (Lopes & Smith, 2007) revealed relatively steep western and eastern shores with a broad, flat bottom, with the deepest spot occupying a slight depression near mid-lake (38˚42'00.0"N, 118˚43'17.9"W; Figure 2.1). Samples collected at this mid-lake location on three dates (May 22nd, 2007, October 3rd, 2007, and September 18th, 2008) coincided with lake conditions during periods of mixis (2007) and thermal stratification (2008). Water column parameters were measured *in situ* using a

Yellow Springs Instruments (YSI, Yellow Springs, OH) multi-parameter water quality sonde (YSI 6600) equipped with temperature, dissolved oxygen (DO), pH, specific conductivity, oxidation-reduction potential (ORP), chlorophyll-*a* fluorescence, and phycocyanin fluorescence sensors. A LI COR 2π photosynthetically active radiation (PAR) sensor deployed through the water column measured downwelling irradiance (LI-COR Biosciences, Lincoln, NE). A peristaltic pump (Masterflex E/S Portable Sampler, Cole-Parmer) and sterile platinum-cured silicone tubing (Masterflex 96420-24, Saint-Gobain Performance Plastics Corporation) with a 178 µm stainless steel pipe line strainer (ASCO Valve Inc., PKG86042) placed inline to remove larger debris were used to collect water from defined depths based on real-time profile data from the sonde. Depths chosen corresponded with physically distinct zones in the lake (surface mixed layer/epilimnion, thermocline/metalimnion, and the hypolimnion/bottom water) as determined by temperature and dissolved oxygen readings.

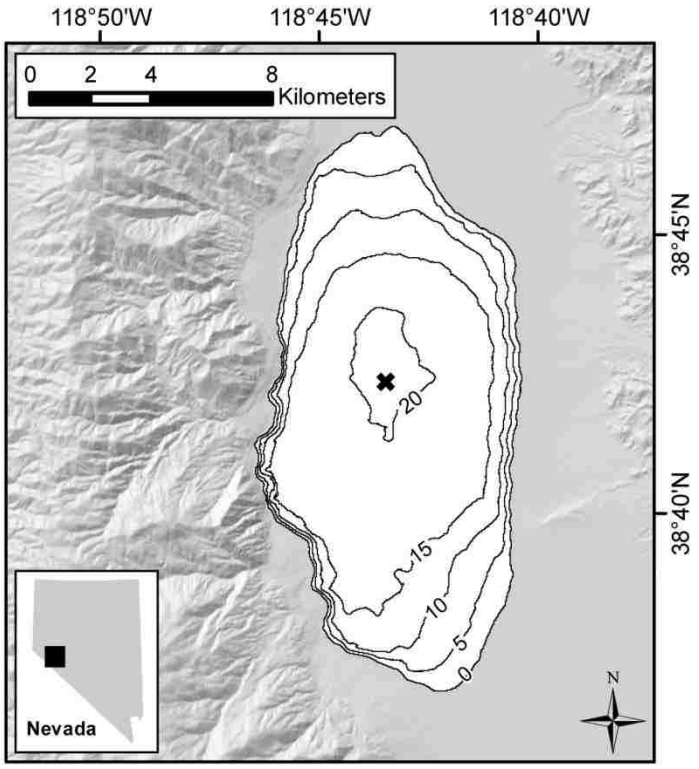


Figure 2.1. Walker Lake is located in west-central Nevada, near the Nevada/California border. Samples were collected at multiple depths from a central lake station, indicated by (x) on the map. Lake bathymetry is given in five-meter increments.

Chlorophyll-*a*, major ion, nutrient, and trace element samples, collected concurrently using a General Oceanics 5 Liter Niskin bottle, were not pre-screened through a 178 µm stainless steel strainer (ASCO Valve Inc., PKG86042) prior to filtration and analysis. Major ion and nutrient samples were collected in sterile two-liter Nalgene HDPE bottles, triple rinsed with sample water prior to filling, and filtered prior to analysis by the Desert Research Institute (DRI) Analytical Chemistry Laboratory. Trace element samples were filtered in the field through 0.45-µm polyethersulfone groundwater cartridge filters (Millipore, Darmstadt, Germany, GWSC04501) pre-rinsed with sterile deionized water and were collected and acidified in the field in 500-mL LDPE bottles that had been soaked for at least two weeks in 5% Sea Star

Instrument Quality HNO_3 and rinsed with sterile deionized water prior to sample collection. Chlorophyll-*a* samples were collected into 1000-mL amber Nalgene HDPE bottles and stored in the dark on ice until transfer to the laboratory (~4 hour hold time), where they were filtered onto 25 mm Whatman GF/F glass microfiber filters and stored at -80°C until analyzed. Major ion, nutrient, and trace element samples were stored in the dark on ice until transport to the DRI Analytical Chemistry Laboratory (U.S. Environmental Protection Agency (EPA) certified); where samples were refrigerated in the dark at 4°C until analysis.

Samples for cultivation were collected via 22 G needles from the flowing sample line into 140-mL N_2-flushed, evacuated serum vials sealed with butyl rubber stoppers and crimped with aluminum caps. Flow cytometry samples were fixed in the field with glutaraldehyde (2.5% v/v) in 50-mL polypropylene tubes. Samples for DNA analysis were collected using autoclaved 45 mm, 0.22 μm polysulfone filters (Supor, Pall Corporation; Port Washington, NY) connected directly to the flowing line and stored on dry ice until transfer to a -80°C freezer upon return to the laboratory. Sediment samples were obtained by transferring intact sediment collected with a 6" x 6" Petite PONAR dredge (Model 1728-G30, Wildco, Yulee, FL) to a plastic dish pan. Sediment samples for cultivation were collected by manually scooping dredge material into sterile 50 mL polypropylene centrifuge tubes and temporarily stored on ice and used for inoculations within 24 hours.

Samples for measurement of total arsenic were collected from the 0.22 μm unfiltered sample line and preserved in the field with 1% Sea Star Instrument Grade HNO_3. Filtered samples for measurement of arsenic/thioarsenate speciation were immediately frozen on dry ice. Preloaded 60-mL syringes containing 10 mL 3M $CdCl_2$ were filled off the unfiltered flowing line for sulfur isotope analysis of sulfate and sulfide and stored at 4˚C.

Pigment analysis. Chlorophyll-*a* pigment concentrations were determined for discrete samples in the laboratory using a fluorometric method (Welschmeyer, 1994) on a Turner Designs model 10AU Fluorometer. This method was calibrated with commercially available standards (*Anacystis nidulans* chlorophyll-*a*; Sigma Corp.; St. Louis, MO). The fluorometric chlorophyll-*a* concentrations were compared against a spectrophotometric method (Parsons, *et al.*, 1984).

Aqueous chemistry. Major ion and nutrient samples, as appropriate, were filtered in the laboratory through 0.45 μm filters prior to analysis by the DRI Water Laboratory (Reno, NV). Samples were analyzed using appropriate EPA drinking water and waste water procedures. Due to the high salinity of Walker Lake, some water quality constituents (total phosphorus, orthophosphate, nitrate and ammonia) were analyzed by methods developed as part of the EPA Clean Lakes Grant Program for Nevada lakes (Solórzano, 1969, Liddicoat, *et al.*, 1975, Jones, 1984, Reuter & Goldman, 1990). Trace element samples were analyzed by inductively coupled plasma mass spectrometry (ICP-MS; Thermo Finnigan Element2) in the DRI Trace Chemistry Laboratory (Reno, NV).

Samples for arsenic speciation (arsenate, arsenite, mono-, di- and tri-thioarsenate) were thawed in a glove-box atmosphere of 95% nitrogen and 5% hydrogen to prevent oxidation and analyzed according to a previously developed method (Planer-Friedrich, *et al.*, 2007, Wallschläger & Stadey, 2007). Briefly, separation was achieved by ion chromatography on a Dionex ICS-3000 SP, with an AG16/AS16 IonPac column and an alkaline eluent with a gradient of 20-100 mM NaOH. Species were quantified by ICP-MS on a Thermo Fisher XSeries2 with oxygen as the reaction gas to avoid $ArCl^+$ interferences at m/z 75 and detect arsenic as AsO^+ at m/z 91. Total arsenic was measured on the same ICP-MS in a separate run.

Sulfur isotopes were determined for the aqueous sulfate and sulfide species (Indiana University, Bloomington, IN) by using a slightly modified version of previously published methods (Brüchert & Pratt, 1996, Lefticariu, *et al.*, 2006). Briefly, sulfate was precipitated with $Ba(NO_3)_2$, and sulfide was precipitated during collection in the field with $CdCl_2$. Precipitates were dried and analyzed for sulfur isotopes with a Finnigan MAT 252 mass spectrometer. Data are presented in delta notation relative to standard material (Vienna Canyon Diablo Troilite (V-CDT) scale).

Cell counts. Total and autofluorescent cell counts were performed by flow cytometry (MicroPRO, Advanced Analytical Technology Inc.). Samples were pre-filtered in the laboratory with a 40μm strainer to prevent debris or large cells from blocking the sample port and adjusted to a neutral pH with phosphate buffer solution (FACSMicroCount, BD, Sparks, MD). Samples for total biomass counts were stained with a proprietary nucleic acid stain (BRAG3, Advanced Analytical Technology Inc.) for 5 minutes prior to counting; samples for autofluorescent counts were not stained. Cells were counted by laser excitation at 653 nm using standard manufacturer protocols.

Cultivation. Media formulations were developed based upon published descriptions of Walker Lake solute chemistry (Beutel, 2001, Beutel, *et al.*, 2001). Walker Lake Basal Salts medium (WLBS; defined as, per liter: 4.9 g NaCl, 4.0 g Na_2SO_4, 1.25 g Na_2CO_3, 1.25 g $NaHCO_3$, 1.07 g $MgCl_2 \cdot 6H_2O$, and 0.4 g KCl, adjusted to pH 9.3 with NaOH) was used as an isotonic base for physiotype-specific media including: aerobic heterotrophs (R2 broth), nitrate-reducing microorganisms (Nitrate Broth, Difco), fermentative microbes (peptone-tryptone-yeast extract-glucose "PTYG" medium; DSMZ Medium 914), sulfate-reducing microorganisms (Postgate's medium), iron-reducing microorganisms (WLBS supplemented with R2A and 5 mM Fe(III)-

NTA or 5 mM Fe(III)-citrate), and arsenate-reducing and arsenite-oxidizing microorganisms (5 mM As(V) as sodium arsenate and 1 mM As(III) as sodium arsenite, respectively). Aerobic incubations were set up in 18 x 150 mm borosilicate slip cap culture tubes (catalog no. 47729-580, VWR, Radnor, PA) and anaerobic incubations were set up in 18x150 mm Balch-type tubes (catalog no. 2048-00150, Bellco Glass Inc., Vineland, NJ) using N_2-sparged anaerobic media. Liquid media were inoculated and diluted to extinction to estimate, to an order of magnitude, the concentration of specific cultivable physiotypes. Prior to serial dilutions, sediment was diluted to a 50:50 slurry with anaerobic WLBS within an anaerobic chamber. All incubations were performed at room temperature (~22 °C).

Arsenite Oxidation/Arsenate Reduction Assays. Colorimetric assays were conducted to quantify microorganisms capable of arsenite oxidation under aerobic conditions or arsenate reduction under anaerobic conditions. Aliquots of inoculated sample cultures (200 µL) were added to 96-well microtiter plates. For arsenite oxidation assays, 20 µL of 30 mM potassium permanganate was added to the culture aliquot (Salmassi, *et al.*, 2002). This yielded a bright pink color in the presence of arsenate, confirming that arsenite had been oxidized. Wells containing only arsenite turned orange, indicating no oxidation activity. Arsenate reduction sample cultures (200 µL aliquots) were scored based on the production of orpiment (yellow precipitate) following the addition of 20 µL each of 1 M hydrochloric acid and 0.1 M sodium sulfide if arsenate had been reduced to arsenite (Kuai, *et al.*, 2001).

Clone library generation. Commercially available kits (UltraClean Soil DNA Isolation Kits, MoBio Laboratories Inc., Carlsbad, CA) were used to extract nucleic acids from the 0.22 µm filters from five selected depths (surface and bottom water samples from October 2007 and surface and hypolimnetic water samples from September 2008 samples) using manufacturer's

protocols amended to include three initial freeze/thaw cycles (-80°C/65°C; 20 minutes each). Universal bacterial primers (27bF/1492uR; Lane, 1991) were used to generate 16S rRNA gene amplicons from the selected depths according to standard PCR reaction conditions: an initial five minute denaturing step at 95°C preceded 35 cycles of 95°C (30 sec), 50°C (30 sec), and 72°C (90 sec) and was followed by a final five minute extension at 72°C. PCR products were purified (UltraClean GelSpin DNA Purification Kit, MO BIO Laboratories Inc., Carlsbad, CA) and subjected to molecular cloning methodology using TOPO-TA kits (Invitrogen, Carlsbad, CA) according to the manufacturer's protocols. Selected clones were sequenced with forward and reverse primers to generate nearly full length 16S rRNA gene sequences (Functional BioSciences, Madison, WI).

Next-generation sequencing. Extracted DNA was sequenced using Illumina MiSeq platform technology for 16S rRNA gene sequencing (Micro-Seq Enterprises, Las Vegas, NV). Amplification and sequencing of the V4 region of the 16S rRNA gene was performed essentially as described in Kozich, *et al.* (2013), with the following modifications: forward primer 515F was modified to contain a C or T at the 4th position from the 5' end (5' GTGYCAGCMGCCGCGGTAA) to expand coverage of Archaeal lineages (Hou, *et al.*, 2013), and a corresponding modification was made to the Read 1 sequencing primer; 5 Prime HotMasterMix DNA polymerase was used (5 Prime Inc., Gaithersburg, MD, USA); 33 cycles were used in PCR.

Raw Illumina sequence data were merged using the *make.contigs* command in mothur v.1.36.0 (Schloss, *et al.*, 2009). Chimeric sequences were identified using *uchime* and removed from the final pool of merged sequences. High quality sequence data were clustered into operational taxonomic units (OTUs) using the Minimum Entropy Decomposition (MED)

algorithm v.1.0 (Eren, *et al.*, 2015), setting the parameters –M and –V equal to 6 and 2, respectively. The –M parameter removes low abundance sequences (<0.001% of the total number of sequences) and the –V parameter sets the maximum number of variable nucleotides within an OTU sequence. MED does not use a set similarity threshold (e.g. 97%) for establishing OTUs, but instead uses similarity of high entropy nucleotide positions to group sequences with greater taxonomic homogeneity. The global alignment sequence taxonomy (GAST) pipeline in VAMPS (vamps.mbl.edu) provided taxonomic assignments for MED OTUs (Huse, *et al.*, 2014).

Community analyses. Consensus sequences from the clone libraries were aligned and matched with nearest cultivated neighbors using Sequencher 4.8 (Gene Codes), and checked for chimeras using Greengenes (DeSantis, *et al.*, 2006). A total of 365 nearly full-length (~1600 base pairs) clone sequences remained after exclusion of putative chimeras. Alignments were refined and phylogenetic relationships determined using MEGA5 (Tamura, *et al.*, 2011). Neighbor-joining trees with 1000 bootstrap replications were constructed based on the Jukes-Cantor model for estimating evolutionary distances. Clone sequences were deposited into GenBank under the accession numbers KC358195 - KC358337.

Further microbial community analyses were conducted with the Illumina 16S rRNA gene V4 dataset using QIIME v 1.9.0 (Caporaso, *et al.*, 2010). The OTU table resulting from MED analysis was used to construct a phylogenetic tree of all OTUs. Species richness, Chao 1 estimated species richness, Shannon, Simpson, and Faith diversity indices were calculated based on 100 rarefied OTU tables of 10,000 randomly selected sequences per sample (Shannon & Weaver, 1949, Simpson, 1949, Chao, 1984, Faith, 1992) to assess community diversity. UniFrac analyses (Lozupone, *et al.*, 2011) were invoked to calculate pairwise distances between

microbial communities, based on 100 rarefactions of 10,000 randomly selected sequences per sample.

All remaining statistical analyses of the Illumina MiSeq 16S rRNA gene V4 dataset were conducted using RStudio (R Core Team, 2014). Principal component analysis (PCA) ordinations of weighted and unweighted UniFrac distances were constructed using the vegan package to evaluate community similarities (Oksanen, *et al.*, 2015). A heatmap was constructed to visualize all OTUs with ≥1% relative abundance in at least one sample, and samples were hierarchically clustered based on computed pairwise Bray-Curtis distances, using the Heatplus, RColorBrewer, and vegan packages (Ploner, 2012, Neuwirth, 2014, Oksanen, *et al.*, 2015). Clustering of microbial communities was evaluated by construction of unweighted pair group method with arithmetic mean (UPGMA) based on distance matrices produced for both weighted and unweighted UniFrac distances. Node support values were calculated from 100 jackknifed subsamples of 10,000 sequences per sample in each subsample. Bootstrapped UPGMA clustering trees were visualized with FigTree v1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/). Lastly, Similarity Percentage (SIMPER) calculations were conducted to identify differentially abundant OTUs between the hypolimnion and epilimnion samples from 2008, using the vegan package.

Results

Water column profile. Water samples were collected at the deepest point in Walker Lake, during spring and autumnal mixing in 2007 and during summer thermal stratification in 2008. This provided the opportunity to survey full depth profiles of limnological characteristics under

two distinct conditions (Figure 2.2, Table B.1). The maximum lake depth was 24 m in 2007 and had declined to 22.9 m in 2008. The October 2007 sampling occurred immediately after, or possibly during, the autumnal lake turn over event, and was characterized by a rapid breakdown of summer thermal stratification resulting in a nearly complete mixing of the lake (Figure 2.2a). A sharp decrease in temperature and dissolved oxygen observed in October 2007 below 22 meters indicated the presence of a thin, putative hypolimnetic remnant of thermal stratification prior to full entrainment into the water column.

Water column profiles collected in September 2008 were characterized by a sharp thermocline and oxycline between 17.5 m and 19 m, with anoxic waters below 19 m (Figure 2.2b). Dissolved oxygen concentrations were constant and below the saturation value of ~8.5 mg L^{-1} in the epilimnion (~6.5 mg L^{-1}), but declined rapidly within the metalimnion and dropped to near zero in the hypolimnion. Measured oxidation-reduction potential (ORP) was positive and fairly constant (~50 mV) throughout the epilimnion and then declined rapidly to values of -300 mV or lower below the metalimnion. Salinity decreased slightly below the thermocline, consistent with water loss by evaporation in the epilimnion following the onset of stratification. The pH increased over the course of this study, and within the September 2008 samples, pH increased with depth throughout the water column, ranging from 9.41 at the surface to 9.64 at the deepest depth.

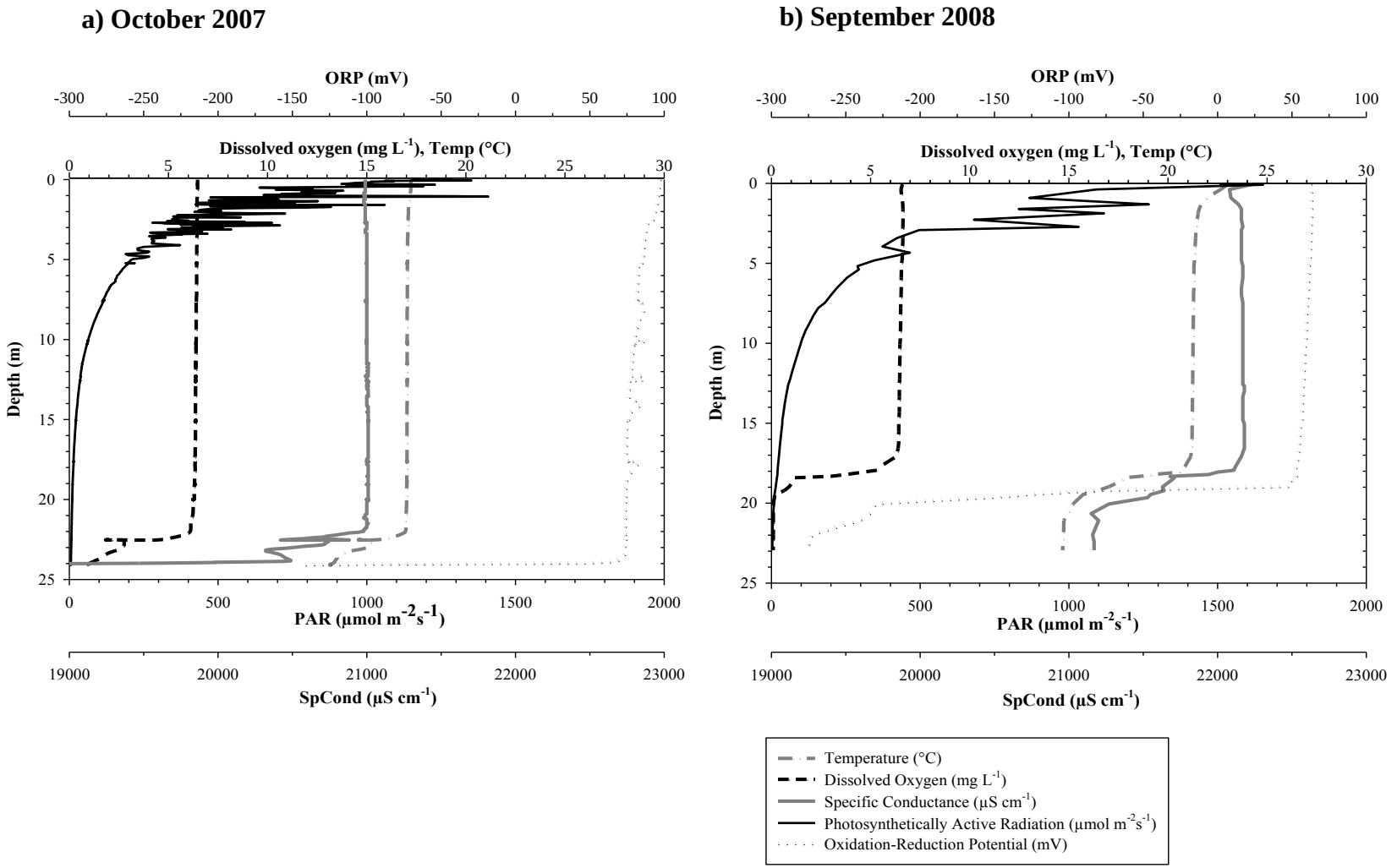


Figure 2.2. Physical *in situ* water column profiles from (a) October 2007 and (b) September 2008. Dissolved oxygen, salinity, temperature, photosynthetically active radiation (PAR), and oxidation/reduction potential (ORP) were measured. A thermocline/oxycline was present between approximately 17.5 m and 19 m depth in 2008, and a putative hypolimnetic remnant is present in 2007 below 22 m.

Pigment fluorescence measurements (phycocyanin, phycoerythrin, and chlorophyll-*a*) taken during the 2008 sonde cast are shown in Figure 2.3, along with total cell counts and autofluorescent cell counts. Phycoerythrin fluorescence was <1 relative fluorescence unit (RFU) in the epilimnion, but increased rapidly below 18 m to nearly 12 RFU at 22 m, higher than any other observed pigment. Discrete fluorometric chlorophyll-*a* measurements matched the *in situ* measurement and corresponded with the related increasing trend in autofluorescent cell counts and relative phycocyanin fluorescence. Autofluorescent cells comprised ~10% of the total number of cells throughout the water column. Both total cell counts and autofluorescent cell counts increased with depth and were nearly an order of magnitude higher in both measurements at 22 m (~10^5 autofluorescent cells per mL; ~10^6 total cells per mL) when compared to the surface (~10^4 autofluorescent cells per mL, ~10^5 total cells per mL).

Aqueous chemistry. Nutrient, major ion, and trace element measurements were determined for all discrete sampled depths in 2008 (0, 5, 10, 17.5, 18, 19, and 22 m). Values for all measured constituents from October and May 2007 were relatively uniform throughout the water column, therefore values from just the top, middle, and bottom waters of 2007 are displayed in Tables 2.1 and 2.2 (full nutrient dataset provided in Table B.2). Almost no nitrate or nitrite was detected at any depth for any of the samples. In September 2008, ammonia was highest at 22 m (0.47 mg L^{-1}), decreased in the thermocline, and was nearly absent (0.02 mg L^{-1}) in the epilimnion. Total and dissolved phosphorus (TP and DP) concentrations were nearly equal to each other and uniform throughout the water column of all sampling dates (range 0.55-0.86 mg L^{-1}) and were higher than the combined inorganic nitrogen concentrations at all depths. High total and dissolved organic carbon (TOC and DOC; 40-43 mg L^{-1} for both measurements in all samples) showed no pattern with depth or year.

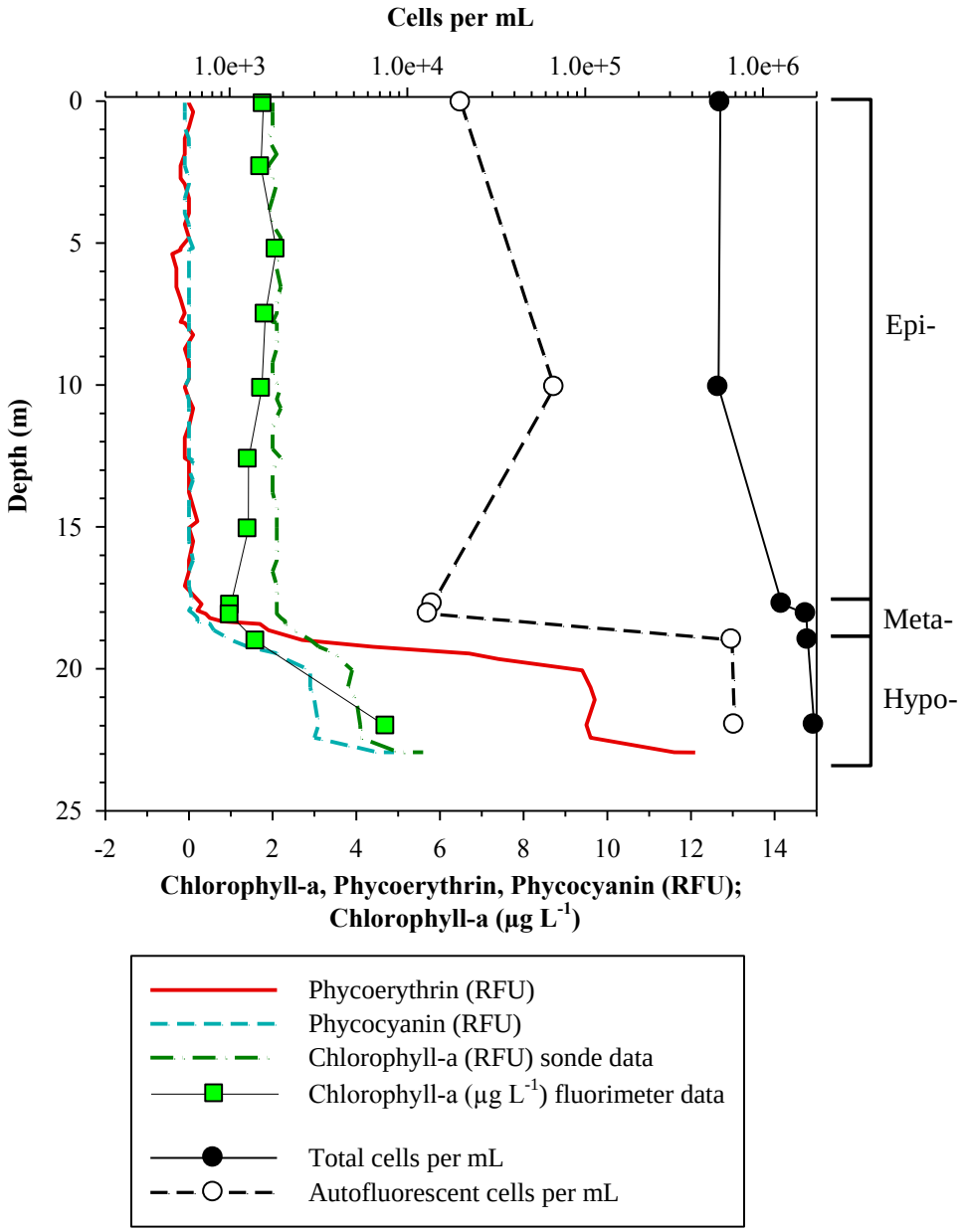


Figure 2.3. September 2008 *in situ* pigment measurements and cell counts. Phycoerythrin (solid red), phycocyanin (dashed blue), and chlorophyll-*a* (dashed green) measurements all reported in relative fluorescence units (RFU). Discrete chlorophyll-*a* fluorimeter measurements (µg/L) are denoted by green squares. Total cell biomass and autofluorescent cell counts (cells per mL) are represented on the top log-scale axis using black (●) and white (○) circles respectively. Regions of the epi-, meta- and hypo- limnia are indicated on the right.

Table 2.1. Carbon, phosphorous, and nitrogen compound measurements from top, middle, and bottom waters of mixing dates (May and October) and all water samples from stratified waters (September). DOC=dissolved organic carbon, DP=dissolved phosphorous. Measurements for total organic carbon and phosphorous were nearly identical to dissolved counterparts and were not included in this table.

Date	Depth (m)	DOC mg L^{-1}	DP mg L^{-1}	$O\text{-}PO_4$ as P; mg L^{-1}	NH_3^+ as N; mg L^{-1}	NO_2^- as N; mg L^{-1}	NO_3^- as N; mg L^{-1}
May 2007	**0**	40.2	0.84	0.69	0.01	<0.01	<0.01
	10	40.4	0.83	0.70	<0.01	<0.01	<0.01
	20	40.5	0.86	0.74	0.06	<0.01	0.02
Oct. 2007	**0**	43.0	0.62	0.56	0.04	<0.01	<0.01
	10	42.3	0.63	0.68	0.05	<0.01	0.02
	22.5	42.7	0.62	0.58	0.08	<0.01	<0.01
Sept. 2008	**0**	42.4	0.55	0.48	0.02	0.01	<0.01
	10	40.9	0.57	0.50	0.02	<0.01	<0.01
	17.5	42.1	0.58	0.51	0.11	0.01	<0.01
	18	41.9	0.60	0.51	0.20	<0.01	<0.01
	19	41.5	0.61	0.53	0.24	<0.01	<0.01
	22	40.6	0.64	0.57	0.47	0.02	0.02

Table 2.2. Ionic constituent measurements from top, middle, and bottom waters of mixing dates (May and October) and all water samples from stratified waters (September).

Date	Depth (m)	Na^{+} mg L^{-1}	HCO_3^{-} mg L^{-1}	CO_3^{2-} mg L^{-1}	Cl^{-} mg L^{-1}	SO_4^{2-} mg L^{-1}	K^{+} mg L^{-1}	Ca^{2+} mg L^{-1}	Mg^{2+} mg L^{-1}	Fe µg L^{-1}	Mn µg L^{-1}	As µg L^{-1}
May 2007	**0**	5070	2420	1010	3460	3580	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	10	4930	2400	1010	3490	3550	256	10.4	177	n.d.	n.d.	n.d.
	20	4900	2400	1010	3500	3560	250	10	187	n.d.	n.d.	n.d.
Oct. 2007	**0**	5110	2400	1120	3730	3700	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	10	5230	2440	1130	3710	3720	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	22.5	5160	2460	1130	3750	3730	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Sept. 2008	**0**	5610	2560	1170	3910	3970	294	9.07	206	35	6	1438
	10	5620	2540	1170	3890	3980	296	9.02	206	40	6	1421
	17.5	5560	2550	1160	3910	3960	290	8.82	205	30	9	1463
	18	5610	2560	1150	3820	3950	289	8.67	203	24	11	1296
	19	5540	2540	1130	3880	3930	286	8.40	203	22	15	1300
	22	5420	2540	1110	3720	3890	283	8.03	198	17	23	1408

n.d.= not determined

Measured major ions increased over the course of this study, with the exception of calcium (Table 2.2). Between May 2007 and September 2008, most measured ionic constituents increased by 10.7%-14.5%. Bicarbonate measurements increased by just 5.89%, while calcium measurements decreased by 15.02%. Total iron and manganese values measured in 2008 displayed opposing profiles with Fe concentrations decreasing from surface (35 μg L^{-1}) to bottom waters (17 μg L^{-1}) while Mn concentrations were lower at the surface (6 μg L^{-1}) and increased with depth (23 μg L^{-1} at 22 m). Total arsenic concentrations measured in 2008 ranged from 1296-1463 μg L^{-1} throughout the water column and showed no clear trend in regards to lake stratification.

Arsenic speciation. In addition to the total arsenic measurements, arsenic chemical species (arsenate, arsenite, monothioarsenate, dithioarsenate, and trithioarsenate) were determined for the 0, 17.5, 19, and 22 m depth samples from September 2008 (Figure 2.4). Arsenate was the only form of arsenic detected in the 0 and 17.5 m samples. A small amount of arsenite (<10% of total As) was detected in the 19 and 22 m samples, and thioarsenates were detected only in the deepest sample (22 m), where they accounted for >60% of the total As. The thioarsenate fraction was composed of approximately equal amounts of mono- and di-thioarsenate (~261 μg L^{-1}), with trace amounts of trithioarsenate (~19 μg L^{-1}).

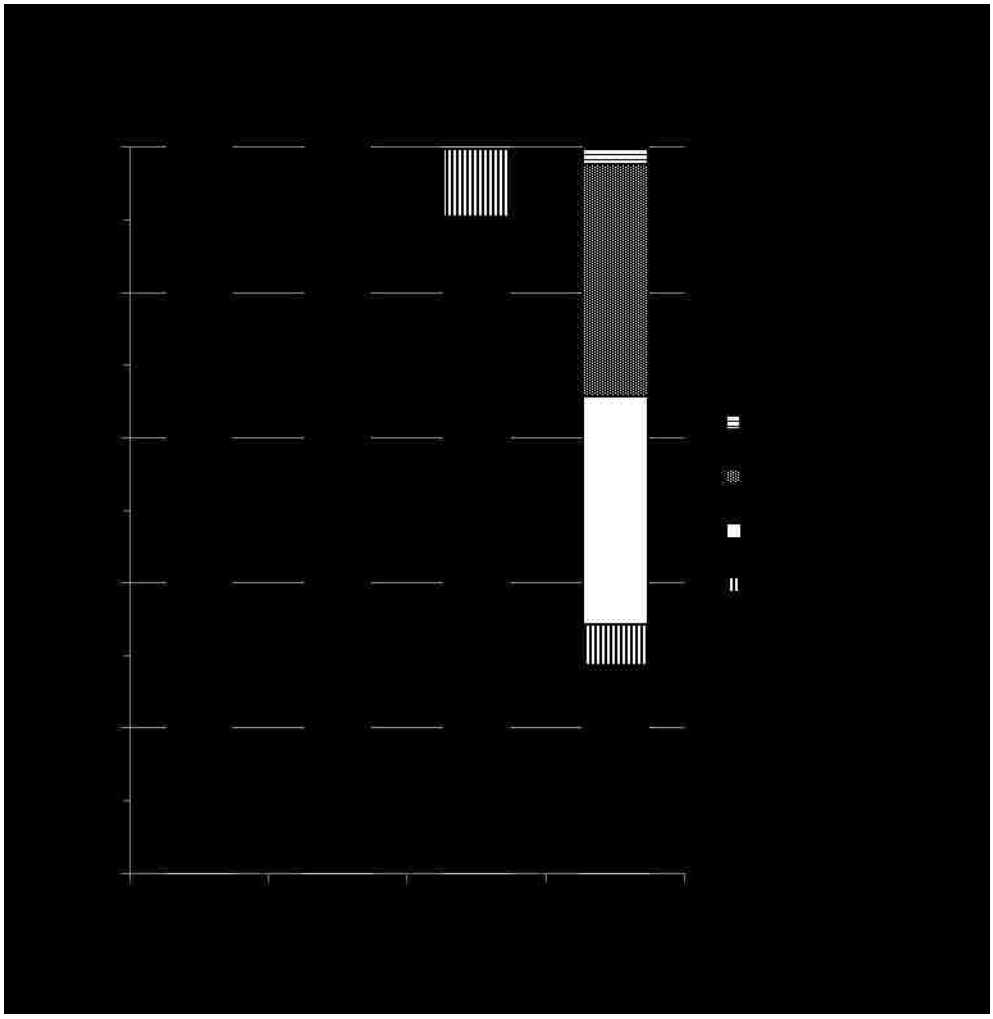


Figure 2.4. Arsenic speciation as a percentage of total arsenic for September 2008. Total amount of all arsenic species is listed at the top of each column. Arsenate was the only form of arsenic present in the 0 and 17.5 m samples. A small amount of arsenite appeared at 19 m. Thioarsenates, composed mainly of mono- and dithioarsenate, with small amounts of trithioarsenate, made up over 60% of the total arsenic at 22 m.

Sulfur chemistry. Sulfate concentrations were relatively uniform throughout the stratified September 2008 water column (3950 ± 31 mg L^{-1}; Table 2.3), decreasing slightly with depth in a conservative manner that paralleled the decrease in salinity. Sulfide concentrations were below detection limits using a colorimetric method onsite (Water Quality Analysis kit, LaMotte Company) and in the laboratory (Cline & Richards, 1969; data not shown), and only a trace amount was recovered during isotope analysis by precipitation with $AgNO_3$. However, a slight sulfidic odor was noted during collection of the 22 m water sample and sediment samples. Stable isotopic fractionation ($\delta^{34}S\text{-}SO_4$) of sulfate was consistent across the depth profile at ~7 ‰. However, $\delta^{34}S\text{-}H_2S$, measurable only in the 22 m sample, was highly depleted (-32.9 ‰).

Table 2.3. Sulfate concentrations and sulfur isotope measurements for September 2008. Depths are segregated by stratification zones (epi-, meta-, and hypolimnion).

Depth (m)	Sulfate (mg L^{-1})	$\delta^{34}S\text{-}SO_4$	$\delta^{34}S\text{-}H_2S$
0	3970	6.9	-[a]
10	3970	7.0	-
17.5	3980	6.9	-
18	3950	7.0	-
19	3930	7.1	-
22	3890	6.9	-32.9

[a] none detected

Enumeration of microbial physiotypes. Cultivation-based analysis of the microbial community from September 2008 using media designed for specific physiotypes revealed a wide diversity of

metabolic capabilities (Table 2.4). Physiotypes were distributed concurrent with thermal stratification in the 2008 samples (e.g., anaerobic metabolisms were confined to the anoxic hypolimnion), whereas they were found throughout the water column in October 2007 (data not shown, analysis not conducted for May 2007 samples), further suggesting that the lake had just mixed or was mixing during the fall 2007 sampling. Estimations of aerobic heterotrophs were rather uniform between surface waters, bottom waters, and sediments, and accounted for ~1-10% of the total number of cells (10^4-10^5 cells mL^{-1}; 10^6 cells g^{-1} in sediments) measured by flow cytometry. As expected, fermentative microorganisms were more prevalent in the anoxic hypolimnion and sediments (10^5 cells mL^{-1}) compared to the surface (10^1 cells mL^{-1}). Nitrate reducers were present at all depths, although in very low numbers at 0 m and 10 m depths (<10 cells mL^{-1}). Colorimetric assays showed nitrate-reducing cultures produced nitrite and possibly ammonia (data not shown). Sulfur-, sulfate-, Fe(III)-, and arsenate- reducing microorganisms were absent from the surface water, but appeared at 10 m or 17.5 m and increased by an additional 2-3 orders of magnitude in the metalimnion and hypolimnion, with the highest abundances present in the sediment cultures. Arsenite-oxidizing organisms were present at all depths in the water and sediment at between 10^2 and 10^4 cells mL^{-1}. Conversely, arsenate reducers were present (between 10^1 and 10^4 cells mL^{-1}) only at 17.5 m and below. Arsenic-tolerant organisms (those capable of growth in the presence of high arsenic concentrations (1 mM arsenite or 5 mM arsenate), but unable to change the redox state of arsenic) were found in numbers comparable to those of aerobic heterotrophs throughout the water column and sediments.

Table 2.4. Cultivation of targeted physiotypes from September 2008 Walker Lake samples. Cell numbers per mL are given as an order of magnitude based on serial dilution growth.

Depth (m)	Aerobic Heterotrophs	Fermenters	Nitrate Reducers	Sulfate Reducers	Sulfur Reducers	Iron (Fe-NTA) Reducers	Iron (Fe-citrate) Reducers	Arsenate Reducers	Arsenite Oxidizers
0	10^5	10^1	10^1	0	0	0	0	n.d.[a] (10^3)[b]	10^{2a} (10^5)[b]
10	10^5	10^4	10^1	0	0	0	10^1	n.d.(10^3)	10^3 (10^4)
17.5	10^4	10^4	10^2	10^1	10^2	10^1	10^1	10^2(10^3)	10^2 (10^7)
18	10^4	10^5	10^3	10^1	10^2	10^2	10^1	10^3 (10^4)	10^2 (10^5)
19	10^4	10^5	10^3	10^1	10^2	10^3	10^2	10^1 (10^4)	10^3 (10^5)
22	10^4	10^5	10^4	10^2	10^4	10^4	10^5	10^4 (10^4)	10^3 (10^4)
SS	10^6	10^5	10^4	10^3	10^4	10^3	10^4	10^4 (10^5)	10^4 (10^6)
DS	10^6	10^5	10^4	10^4	10^4	10^3	10^4	10^3 (10^5)	10^4 (10^5)

SS= surface sediment (0-2 cm); DS= deeper sediment (2+ cm); n.d.= not determined

[a] Positive for arsenic metabolism

[b] Positive for growth in the presence of 5 mM As(V) or 1 mM As(III)

16S rRNA gene V4 region Illumina phylogenetic analysis. MED analysis of the Illumina MiSeq 16S rRNA gene V4 region sequence data revealed 513,965 quality-filtered sequence reads and 1,976 OTUs across 71 phyla among the 21 discrete samples from May 2007, October 2007 and September 2008. Fifteen phyla showed ≥0.5% abundance in any one of the 21 samples and are shown in Figure 2.5 (class-level for Proteobacteria) for the top, middle, and bottom water samples from each collection date (abundances for all samples shown in Figure B.1).

Overall, the microbial communities observed in Walker Lake during any sampling time or at any depth were dominated by the phyla Actinobacteria, Bacteroidetes, Cyanobacteria, Firmicutes, Tenericutes and Proteobacteria. Observed OTU richness increased with depth in all sampling events (alpha diversity indices shown in Table B.3). On average, Actinobacteria were highly abundant in all mixing and epilimnetic water samples (mean 18.74 ± 0.55% standard error of the mean (SEM)), but decreased to an average of 7.49 ± 2.05% SEM in hypolimnetic samples (19 m and 22 m in 2008). Members of the Firmicutes phylum were present in low abundance in all oxygenated samples (mean 0.31 ± 0.05% SEM) and increased in the hypolimnion in September 2008 (mean 5.52 ± 2.43% SEM).

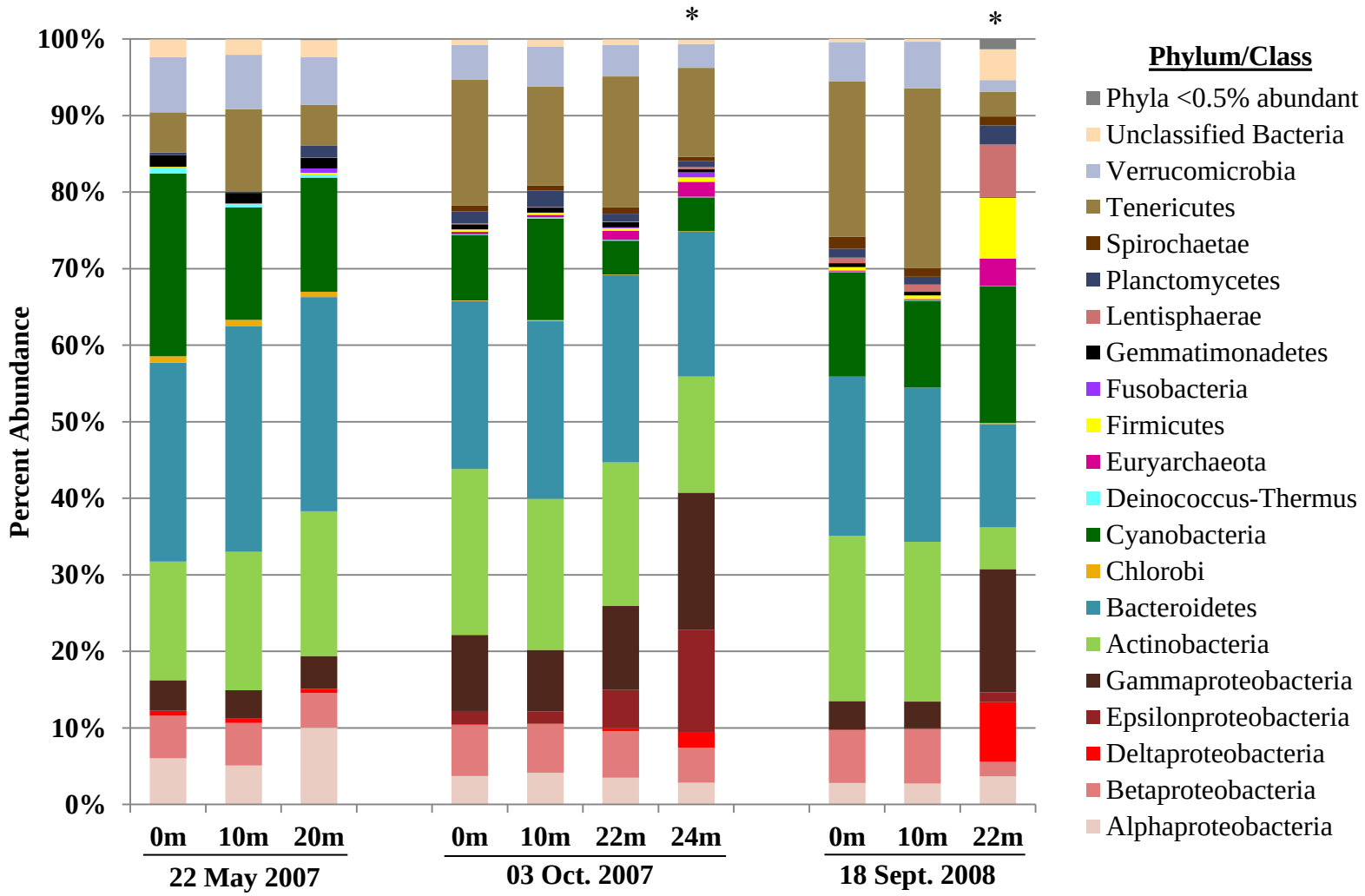


Figure 2.5. Relative abundance of bacterial phyla/classes (≥0.5% abundant) that make up the microbial communities of the surface, middle, and bottom water samples of each collection date. (*) symbol above column indicates suboxic water conditions at the time of sampling.

Tenericutes were present in lower abundances in May 2007 and September 2008 hypolimnetic samples (mean 6.37 ± 0.83% SEM and 4.32 ± 1.09% SEM, respectively) than in all of the October 2007 samples (mean 16.48 ± 1.25% SEM) and the epi- and meta-limnetic samples of September 2008 (mean 18.34 ± 2.16% SEM). One unclassified OTU within the Mollicutes class (OTU 8685) was the second most abundant organism in the entire Illumina DNA sequence dataset, making up 6.73% of the 513,965 sequence reads from all 21 samples. The sequence of OTU 8685 matched identically to several clone sequences from the October 2007 and September 2008 clone libraries, although the database used to phylogenetically define the clones loosely classified (81% confidence threshold) this microorganism as a member of the Firmicutes, a sister clade of the Tenericutes.

Among the Proteobacteria, α- and β-Proteobacteria had consistent abundances across all samples (mean 4.69 ± 0.38% SEM and 5.66 ± 0.30% SEM, respectively). Deltaproteobacteria were in low abundances in all oxygenated samples (mean 0.49 ± 0.11% SEM) and increased in the hypolimnion of 2008 (mean 5.99 ± 1.81% SEM). Epsilonproteobacteria were present in low abundances in all of the May 2007 and September 2008 samples (mean 0.25 ± 0.12% SEM) and were present in higher abundances in October 2007 (overall mean 3.53 ± 1.74% SEM), with the highest abundance occurring in the 24 m sample (13.47%). Gammaproteobacteria had relatively even abundances in fully oxygenated samples (mean 5.59 ± 0.64% SEM), but were more abundant in suboxic samples (bottom water sample of October 2007 and meta- and hypolimnion regions of September 2008; mean 16.13 ± 1.45% SEM).

Cyanobacteria were generally more abundant in May 2007 (mean 19.89 ± 1.37% SEM) and September 2008 (mean 13.92 ± 1.18% SEM) samples than in October 2007 (mean 7.08 ± 1.30% SEM). One unclassified OTU within the Cyanobacteria phylum (OTU 7356) was the

most abundant organism in the entire Illumina DNA sequence dataset, making up 9.93% of the 513,965 sequence reads from all 21 samples. The sequence of OTU 7356 matched identically to several Cyanobacteria clone sequences from the October 2007 and September 2008 clone libraries, all of which were identified as species of *Synechococcus* (GpIIa). OTU 7356 had high, but patchy distribution in mixed May 2007 water column samples, accounting for 13.4-22.4% of the sequence data of each sampled depth and showing no trend with depth. In September 2008, however, OTU 7356 showed low abundances in the epilimnion (0.8-1.1%) and increased in suboxic and anoxic regions, reaching a maximum abundance at the lake bottom (22 m; 16.5%).

Principal component analysis (PCA) ordination of all samples using abundance-weighted UniFrac distances (Figure 2.6a) shows 89.2% of the variance in the data explained by the first two components. Clustering of the samples on the PCA ordination using the weighted UniFrac UPGMA tree (Figure 2.6b) shows all of the May 2007 samples grouping together with 100% bootstrap support. The 2008 epilimnetic samples (0 m and 10 m) grouped together with all of the October 2007 samples with 100% bootstrap support, with the exception of the October 2007 24 meter sample, which formed an individual group (Figure 2.6a). The metalimnetic samples (17 m and 18 m) and the hypolimnetic samples (19 m and 22 m) from September 2008 formed two distinct clusters. PCA ordination using unweighted UniFrac distances showed a very similar ordination of the samples (Figure B.2a) to the weighted PCA, with 77.5% of the data variance explained by the first two components. However, clustering of the samples using the unweighted UniFrac UPGMA tree (Figure B.2b) shows all samples from May 2007 and October 2007 grouping together with the epi- and meta-limnetic samples from September 2008 (100% bootstrap support), with only the anoxic hypolimnetic samples (19 m and 22 m) forming a distinct cluster.

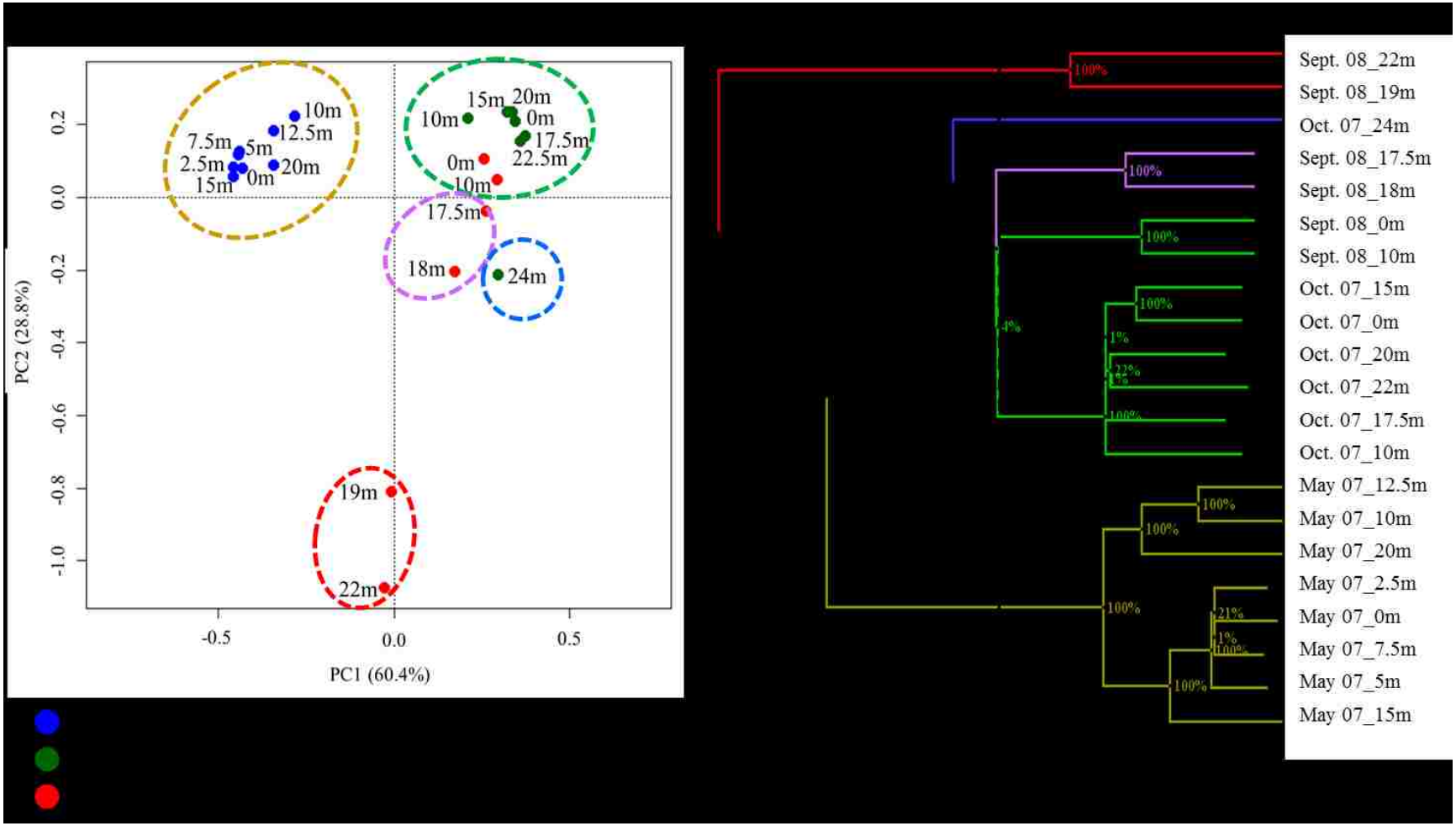


Figure 2.6. Microbial community separation by principal component analysis using weighted UniFrac distances of Illumina V4 16S rRNA gene dataset. Each dot represents a complete Illumina sequence dataset of the corresponding sampled depth in the water column from May 2007 (blue), October 2007 (green), and September 2008 (red). Colored ellipses drawn around groupings correspond with clade separation based on bootstrap support values from weighted UniFrac UPGMA tree (b)**.**

A colored gradient heatmap generated from all 21 water column samples between 2007 and 2008 (Figure 2.7) shows the 56 OTUs that contribute ≥1% to the Illumina sequence dataset in at least one sampled water column depth and their relative abundances within each sample (OTU identities given in Table B.4). There were 20 OTUs that individually accounted for ≥1% abundance of all sequences in the entire Illumina dataset (denoted in Figure 2.7 by * next to OTU number). All May 2007 samples shared similar patterns in the relative abundances of these 56 OTUs. October 2007 samples also showed similar patterns of the relative abundances, except for the 24 m sample. The OTU abundance profile for the September 2008 epilimnion samples showed similar patterns to the October 2007 samples, while the meta- and hypolimnion samples exhibited distinct OTU abundance profiles.

Similarity percentage (SIMPER) analysis of September 2008 sequence data (Table 2.5) shows the top ten OTUs that account for 44.48% of the dissimilarity observed between the microbial communities of the epilimnion and the hypolimnion (19 m and 22 m samples). The two OTUs (OTUs 7356 and 7360) that contributed the most to the community dissimilarity (18.59%) were both *Synechococcus* species. While both of these OTUs had similar mean abundances in the epilimnion (5.3%), their mean abundances varied greatly in the hypolimnion (OTU 7360 mean 0.23%; OTU 7356 mean 16.12%). An unidentified Mollicutes OTU, the second most abundant OTU in the entire Illumina dataset (OTU 8685), accounted for 5.74% of the dissimilarity and was present in higher abundance in the epilimnion (mean 10.92%) than the hypolimnion (2.30%). Several OTUs belonging to the γ-Proteobacteria (OTU 8780, *Thioalkalispira*; OTU 7214, *Thiomicrospira*; OTU 3945, *Thiocapsa*) collectively contributed 11.63% to the community dissimilarity and were more abundant in the anoxic hypolimnion than the epilimnion.

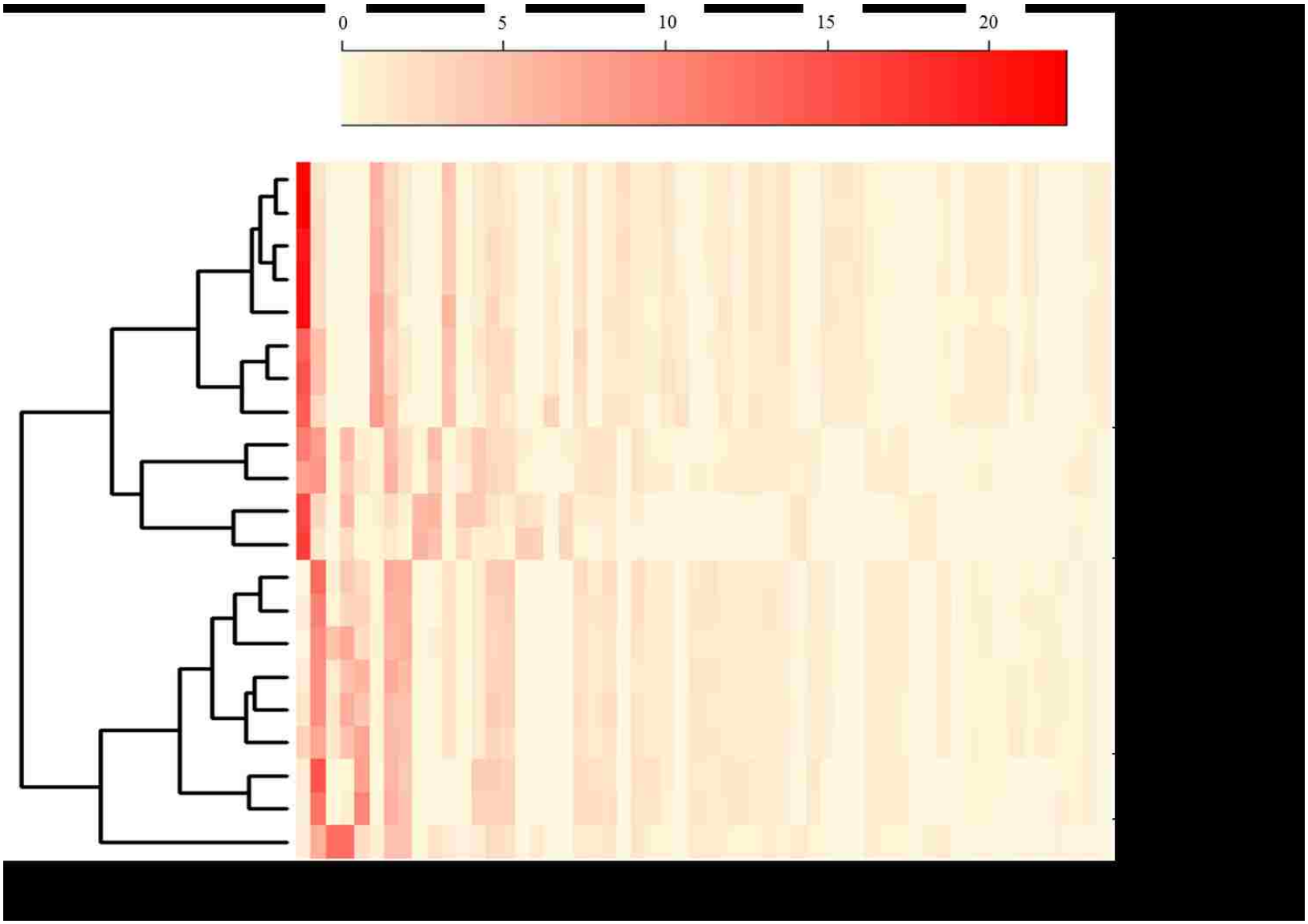
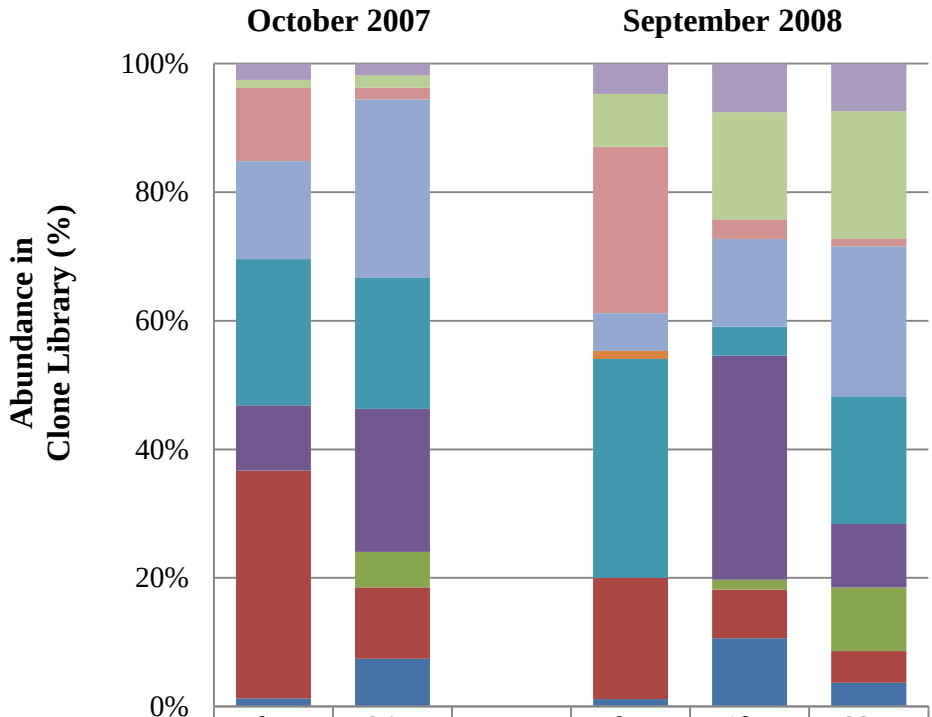


Figure 2.7. Heatmap depicting the top OTUs that contribute ≥1% to the Illumina sequence dataset in at least one sample. (*) symbol next to OTU number indicates that OTU accounted for ≥1% abundance of all sequences in the entire Illumina library from all 21 samples.

Table 2.5. SIMPER analysis results of Illumina sequence data showing the top ten organisms that contribute to the dissimilarity between microbial communities of the epilimnion and hypolimnion (19 m and 22 m samples) observed in Walker Lake in 2008.

OTU	Phylum	Genus	Percent Contribution	Mean abundance in 2008 epilimnion (%)	Mean abundance in 2008 hypolimnion (%)
7356	Cyanobacteria	*Synechococcus* sp.	11.76	5.26	16.12
7360	Cyanobacteria	*Synechococcus* sp.	6.83	5.30	0.23
8685	Tenericutes	Unidentified Mollicutes	5.74	10.92	2.30
8780	Proteobacteria	*Thioalkalispira* sp.	4.92	2.34	5.42
7214	Proteobacteria	*Thiomicrospira* sp.	3.77	2.61	3.93
3945	Proteobacteria	*Thiocapsa* sp.	2.94	0.21	5.71
4393	Proteobacteria	Unidentified Rhodobacteraceae	2.35	0.73	3.42
7840	Bacteroidetes	*Owenweeksia* sp.	2.24	3.42	0.61
7810	Actinobacteria	*Nitriliruptor* sp.	2.20	5.95	1.74
7852	Bacteroidetes	Unidentified Sphingobacteriales	1.73	0.36	2.93

16S rRNA gene clone library phylogenetic analysis. The generation of five clone libraries from the selected depths (surface and bottom water samples from October 2007 and surface and hypolimnetic water samples from September 2008 samples) yielded 365 individual 16S rRNA gene clones. The distribution of individual clones among phyla between the 2007 and 2008 libraries, and among the 0 m, 19 m and 22 m samples within 2008, showed a diverse bacterial community containing strong representation from the following phyla: Proteobacteria (α-, β-, γ-, and δ-classes), Firmicutes, Bacteroidetes, Actinobacteria, Cyanobacteria, and Spirochaetes, as well as two deeply branching OTUs that most closely grouped with the Tenericutes and the candidate phylum BRC1 (Figure 2.8). All five clone libraries shared many of the same phyla; however, the individual OTUs within the phyla were often unique for certain depths or grouped in clades by depth (Figure B.3a-c).



	0m	24m		0m	19m	22m
Unclassified	2	1		4	5	6
Cyanobacteria	1	1		7	11	16
Actinobacteria	9	1		22	2	1
Bacteroidetes	12	15		5	9	19
Spirochaetes	0	0		1	0	0
Firmicutes	18	11		29	3	16
Gammaproteobacteria	8	12		0	23	8
Deltaproteobacteria	0	3		0	1	8
Betaproteobacteria	28	6		16	5	4
Alphaproteobacteria	1	4		1	7	3

Figure 2.8. Distribution of bacterial phyla based on number of clone representatives from the five constructed clone libraries from October 2007 and September 2008.

Discussion

October and May 2007 sampling events coincided with periods of lake mixing and full summer thermal stratification was observed during September 2008. During the sampling event in October 2007, the lake had already turned over (or was in the process of mixing); whereas in the past, lake mixing did not occur until November (Cooper & Koch, 1984, Beutel, *et al.*, 2001). This is likely caused by the decreased mean and maximum depths of the lake, which creates a less stable temperature gradient during stratification that requires less energy to mix completely. Notably, the mixing depth of the epilimnion has not changed despite changes in overall lake depth; the thermocline/oxycline still begins near 17 m even though there was a nearly 10 m decrease in the maximum depth between the 1970s and the present (Cooper & Koch, 1984).

Aqueous chemistry and nutrients. Within the stratified water column observed in September 2008, higher concentrations of dissolved analytes were present in the epilimnion; conductivity measurements show a 16.6% difference between the epilimnion and hypolimnion salinities. The appearance of this inverse chemocline is a result of evaporative losses from the epilimnion during summer thermal stratification, possibly contributing to a partial destabilization of thermal structure. Between May 2007 and September 2008, a conservative ionic constituent would be expected to increase by 11.7%, based on conductivity measurements. Over the course of this study, most measured ionic constituents conformed to this expected increase, ranging from 10.7%-14.5% increases, including Na^+, CO_3^{2-}, Cl^-, SO_4^{2-}, K^+, and Mg^{2+}. Conversely, a number of analytes were controlled in some manner other than simple evaporation; HCO_3^-measurements increased by just 5.9%, while Ca^{2+} measurements decreased by 15.0%. CO_3^{2-} concentrations showed a 13.6% increase between May 2007 and September 2008, ~2% more than can be explained by evaporative concentration of solutes. Conversely, HCO_3^- increases were half of the

expected evaporative increase. These shifts in the balance of CO_3^{2-}/HCO_3^- could explain the slight increase in the pH observed between May 2007 and September 2008.

Walker Lake, typical of other soda lakes, exhibits high levels of phosphorous and severe nitrogen limitation. The ratio of total inorganic nitrogen to orthophosphate in mixing/epilimnetic waters of Walker Lake averaged 0.35:1, and the hypolimnetic waters averaged 1.22:1. A balance of nutrients is typically indicated by a N:P ratio of 16:1 (Sigee, 2005), therefore showing that Walker Lake is severely nitrogen limited. Previous nutrient analysis at Walker Lake (Beutel, *et al.*, 2001) noted total phosphorus measurements ranging from 0.64 to 0.88 mg-P L^{-1} and nitrate concentrations commonly less than 50 µg-N L^{-1}, though nitrate initially accumulated in the hypolimnion, then decreased after the onset of anoxia. Historical hypolimnetic ammonia concentrations in Walker Lake (0.70-0.80 mg NH_3-N L^{-1}; Beutel, 2001) were much higher than those observed during the 2007 and 2008 samplings. In this study, nitrate and nitrite were both at the limit of detection, and most of the detected inorganic nitrogen was in the form of ammonia (<0.01-0.08 mg L^{-1} in epilimnion; 0.11-0.47 mg L^{-1} in hypolimnion). Nitrogen limitation is a common feature of lakes in semi-arid environments as a result of the regional low annual precipitation creating low atmospheric nitrogen deposition (Hammer, 1986, Beutel, *et al.*, 2001). Brief, intense periods of rain, in conjunction with sparse vegetation, cause high levels of phosphorous loading into water bodies.

Dissolved organic carbon (DOC) concentrations were very high and ranged from 40.2-43.0 mg L^{-1}, indicating a possible eutrophication of the Walker Lake system (in conjunction with the severe nutrient imbalance). However, the combination of elevated primary productivity, high pH values, and evaporative concentration of dissolved constituents lead to high levels of

dissolved organic carbon (DOC) in the water column of many soda lakes (Domagalski, *et al.*, 1989).

Bacterial community structure. The basal medium used to cultivate microorganisms from Walker Lake was designed to match the aqueous chemical composition of lake water (ionic constituent concentrations and pH). All physiotypes tested grew at many or all depths, consistent with a community of alkaliphilic microorganisms. Many of the closest cultured relatives to sequences from the 16S rRNA gene clone libraries were also from alkaline, saline environments (Supplementary Figure 2.3). Cultivable microorganisms, capable of both aerobic heterotrophy and anaerobic growth on a variety of terminal electron acceptors or by fermentation, were present throughout the water column and sediments. Several physiotypes were present in relatively high numbers (up to 10^5 cells mL^{-1}; aerobic heterotrophs, fermenters, Fe-citrate reducers, and arsenite-tolerant organisms). These relatively high proportions of cultivable microorganisms may be significant since typically less than 1% of environmental microorganisms are thought to be cultivable (Amann, *et al.*, 1995). The presence of aerobic microorganisms in the anoxic regions and fermentative microorganisms and nitrate reducers in oxic regions suggests a metabolically flexible community that can adapt to seasonal variations in lake conditions (aerobic or anaerobic).

The high numbers of microorganisms involved in metal(loid) and sulfur cycling, especially Fe(III) reducers and arsenic oxidizers and reducers in the lake's oxycline and hypolimnion (up to 10^5 cells mL^{-1}) , are potentially important to the ecological function of this lake. Total iron values measured in 2008 displayed concentrations decreasing from surface (35 $\mu g\ L^{-1}$) to bottom waters (17 $\mu g\ L^{-1}$). The failure of soluble iron to accumulate in the hypolimnion suggests a possible sink. Sequences of bacterial phylotypes from clone libraries

included a variety of Proteobacteria represented by genera with cultured sulfate-, arsenate-, and Fe(III)-reducing representatives (δ-Proteobacteria) as well as S^0/thiosulfate oxidizers (α-Proteobacteria) and arsenite oxidizers (β-Proteobacteria—*Hydrogenophaga*, *Burkholderia*; Heinrich-Salmeron, *et al.*, 2011). As this is the first study of the bacterial communities in Walker Lake, it is unclear whether the presence of these organisms as dominant taxa is a new feature of the lake or if they have been active in the past.

Past surveys have revealed high microbial diversity within soda lakes (Rees, *et al.*, 2004, Dong, *et al.*, 2006, Mesbah, *et al.*, 2007, Klepac-Ceraj, *et al.*, 2012), including nearby Mono Lake, California (Hollibaugh, *et al.*, 2001, Humayoun, *et al.*, 2003). Illumina sequencing revealed nearly 2,000 unique OTUs within our sample set, distributed across 71 phyla, defining a phylogenetically and metabolically rich and diverse population comparable to studies of similar lake systems utilizing similar techniques (Andrei, *et al.*, 2015, Vavourakis, *et al.*, 2016). In addition to the sequences with closely related cultured representatives, or phylogenetic affiliation to well-characterized functional groups, many OTUs had no affiliation to a known isolate at the genus or even order level. Several OTUs could not be assigned with confidence at the phylum or class level.

Principal component analysis (PCA) ordination of the Illumina MiSeq sequence data using weighted UniFrac distances showed grouping of samples primarily by date, suggesting a temporal disparity among the microbial communities. Suboxic (October 2007 24 m sample and metalimnion samples from September 2008) and anoxic samples (19 m and 22 m from September 2008) formed distinct clusters, indicating strong differences in microbial community composition driven by the presence of dissolved oxygen and ,to a lesser extent, its concentration. Fully oxygenated and suboxic samples were more similar to each other than they were to the

completely anoxic samples. This trend was also supported by the PCA ordination using unweighted UniFrac distances (forms relationships between samples based on OTU presence/absence without taking abundances into consideration), which grouped all samples together with 100% bootstrap support except for the anoxic hypolimnetic samples from September 2008 (Figure B.2). This suggests that the microbial communities of the anoxic hypolimnion were truly distinct from all other samples, and that the overall microbial community of the Walker Lake water column became more uniform in the presence of dissolved oxygen.

Arsenic and sulfur biogeochemical cycling. The presence of oxidized arsenic (arsenate) in the surface waters and reduced species (arsenite and thioarsenates) below the oxycline (Table 2.4) suggests an active arsenate-reducing microbial community in the deeper lake water, and arsenite oxidizers in the epilimnion. Serial dilution enrichments confirmed the arsenite-oxidizing and arsenate-reducing capabilities of the microbial community and were consistent with the presence of a complete and microbially-driven arsenic cycle similar to that found in Mono Lake, California (Hollibaugh, *et al.*, 2005).

Sequences from the 16S rRNA gene libraries shared identity with those of cultured metalloid reducers (e.g., arsenate-reducing strain MLMS-1 and the selenate-reducing *Pelobacter seleniigenes*; Figure B.3b; Hoeft, *et al.*, 2004, Narasingarao & Häggblom, 2007). Arsenite oxidizers were present in all enrichment cultures, both water and sediments (10^2-10^4 cells mL^{-1}), and 2.3% of all sequence reads in the Illumina V4 16S rRNA gene dataset identify as species within the genus *Hydrogenophaga*, many members of which are known to be able to oxidize arsenite (Heinrich-Salmeron, *et al.*, 2011). Furthermore, almost all microorganisms capable of growth in culture media could tolerate elevated arsenic concentrations (1 mM arsenite or 5 mM arsenate, more than two orders of magnitude greater than *in situ* arsenic concentrations), even if

they could not oxidize or reduce arsenic oxyanions. Diverse modes of arsenic metabolism in soda lakes have been described extensively (Oremland, *et al.*, 2004, Oremland, *et al.*, 2005, Kulp, *et al.*, 2007) and future research at Walker Lake may reveal further similarities to other Lahontan-remnant lakes (e.g., Mono, Pyramid). The presence of thioarsenate species, observed only at 22 m depth, has been shown to be a common feature of arsenic-rich alkaline lakes (Hollibaugh, *et al.*, 2005, Fisher & Hollibaugh, 2008) and geothermal environments such as Yellowstone National Park alkaline hot springs (Cornelia & Britta, 2012). Thioarsenates likely form abiotically via thioarsenites from arsenite and sulfide (Suess, *et al.*, 2009, Planer-Friedrich, *et al.*, 2010) or from the reaction of arsenite with elemental sulfur (S^0) such as in polysulfides (Stauder, *et al.*, 2005).

Despite its biological activity, bulk sulfate concentrations increased conservatively (10.7%). Sulfate concentrations were very high (nearly 4000 mg L^{-1}), thus sulfate consumed as a terminal electron acceptor would not significantly affect the total sulfate concentration. The presence of both dithioarsenate and trithioarsenate suggests that sulfide/polysulfide was produced in the bottom waters or diffused from sediments in amounts sufficient to allow for the formation of thioarsenates measured in the 22 m anoxic hypolimnion sample (258, 264, and 19 µg L^{-1} of mono-, di-, and trithioarsenate, respectively). This evidence supports *in situ* microbial sulfate or S^0 reduction despite the absence of readily detectable sulfide.

The presence of sulfate reducers (10^1-10^4 cells mL^{-1}) in culture enrichments and S-isotopic evidence are other indicators of sulfide production. The difference in the $\delta^{34}S$ of the S^{2-} component (found only at 22 m) versus the $\delta^{34}S$ of the sulfate throughout the water column was nearly 40‰, a difference typical of the biological fractionation that occurs during microbial dissimilatory sulfate reduction (Habicht & Canfield, 2001). A tightly coupled sulfur cycle is

created through the rapid scavenging of sulfide produced by sulfate reducers through the precipitation of iron sulfides, formation of thioarsenates, or oxidation by chemo/photoautotrophs. Sulfate reduction is one of the most common processes in anoxic waters and sediments of soda lakes (Foti, *et al.*, 2006). Historic hypolimnetic sulfide concentrations in Walker Lake (12-18 mg-S^{2-} L^{-1}; Beutel, 2001) were significantly higher than those observed in 2007 and 2008 (barely any detectable S^{2-}), indicating a change in one or more of the sinks for the sulfide produced in the hypolimnion and sediments. For example, with the decline in lake level and hypolimnetic volume, a greater proportion of the hypolimnion will have been within the photic zone in 2008 compared to prior years, increasing habitable space for sulfide-utilizing anoxygenic phototrophs.

The formation of thioarsenates represents another possible sulfide sink. Precipitation of iron sulfides from biologically reduced iron is another (Nealson & Saffarini, 1994), which would also help explain the decrease in total iron concentrations below the oxycline. Chemoautotrophic sulfide/sulfur/thiosulfate oxidation is a widespread process in soda lakes and constitutes another probable sulfide sink (Sorokin & Kuenen, 2005, Grant, 2006, Klepac-Ceraj, *et al.*, 2012); however, no biological assays for sulfur oxidation were performed to confirm this activity. Clone library sequence data suggest that a significant population of these relevant microorganisms is present both at 19 m and 22 m. Phylogeny does not always correlate with biogeochemical activity (e.g., arsenate reducers are found in nearly all bacterial phyla and several archaeal classes; Oremland & Stolz, 2005); but several groups, particularly those involved in sulfur metabolism, form functional clades. These include the S^0- and sulfate-reducing δ-Proteobacteria and sulfur-oxidizing γ-Proteobacteria (Grant, 2006). Within the September 2008 hypolimnetic samples, nearly 35% of 16S rRNA gene sequences from the 19 m clone library (10% from 22 m clone library) and 12.52% of 19 m and 22 m samples from the Illumina dataset were highly

similar to known sulfur-oxidizing genera. These include *Thiocapsa* (anoxygenic phototrophs that grow at low light levels), *Thioalkalispira*, and *Thioalkalimicrobium* (sulfide, sulfur, and thiosulfate oxidizers that grow under microaerophilic or anaerobic conditions (Figure B.3a; Sorokin, *et al.*, 2002, Sorokin & Kuenen, 2005, Kompantseva, *et al.*, 2010)).

***Synechococcus* bloom in hypolimnion.** The study of alkaline, saline lakes has shown them to be unique microbial habitats and highly productive ecosystems where primary production is often driven by cyanobacteria as opposed to eukaryotic algae (Joye, *et al.*, 1999, Grant, 2006). Increases in total cell numbers within the hypolimnion (19 m and 22 m; September 2008) was paralleled by an increase in autofluorescent cells as well as both chlorophyll-*a* and phycocyanin pigments (Figure 2.3). The increase in total and autofluorescent cell numbers below the oxycline in the 2008 sample set may be a result of anoxygenic cyanobacterial bloom previously or decreased predation by zooplankton (Humayoun, *et al.*, 2003). Also, the membrane filters used for DNA collection turned a bright pink color (characteristic of red pigments) following filtration of hypolimnetic water. This evidence, in combination with microscopic observations of a dominance of fluorescent coccoid cells under a rhodamine filter (data not shown) and 16S rRNA gene sequences, points to a cyanobacterial bloom dominated by *Synechococcus* species and an unclassified cyanobacterium (OTU 7356). Two closely related, but distinct, lineages segregated by depth (0 m versus 19 and 22 m) were present in16S rRNA gene libraries (Figure B.3b).

Synechococcus species were previously observed (Cooper & Koch, 1984, Collopy & Thomas, 2009), but were not considered a major component of the phototrophic community because of their relatively low biovolume, when considered in light of larger forms, such as diatoms and *Nodularia* species. Numerically, however, *Synechococcus* species are prominent members of the prokaryotic community, comprising 18-20% of the hypolimnetic population (19

m, 22m) as a proportion of sequences in 16S rRNA gene clone libraries and 2.71% of all sequence reads in the Illumina dataset. The most abundant OTU in the Illumina dataset (OTU 7356) was unidentified at the class level (Cyanobacteria) within VAMPS (Huse, *et al.*, 2014). The sequence of this unknown OTU, however, has a 100% identity match to two unique Walker Lake clones (22m_03H and 19m_01A, Figure B.3b), which have been identified as *Synechococcus* species. The Walker Lake *Synechococcus*-like clone sequences grouped closely (97% identity) with an isolate from Mono Lake, California that contained high levels of phycoerythrin and became dark red in color under low light conditions (Budinoff & Hollibaugh, 2007). Conversely, cyanobacteria were nearly absent from the 2007 clone libraries, which represented the lake under mixed conditions, suggesting that summer thermal stratification may be a driver of a hypolimnetic cyanobacterial bloom.

The presence of cyanobacteria in the anoxic bottom waters may be associated with several biogeochemical cycles. These organisms may be stimulated by the ammonia that is present in high concentrations in the hypolimnion, rather than fixing N_2, thereby effectively intercepting the internal nitrogen loading to the lake. The anoxygenic cyanobacteria may also serve as a mechanism for the removal of sulfide, which can be toxic to fish populations. Sulfide-driven anoxygenic photosynthetic cyanobacteria have been documented in other aquatic systems (Cohen, *et al.*, 1975, Klatt, *et al.*, 2015). It is unclear whether the observed bloom is a new biological feature in the lake driven by changes in lake level, euphotic zone, and water chemistry, or whether it will be a persistent phenomenon in years to come. However, the potential controls on nutrient cycling affected by these organisms may be significant.

The nitrogen-fixing filamentous cyanobacterium *Nodularia spumigena* has been the dominant phytoplankton in Walker Lake in all published studies, accounting for up to 97% of the

planktonic algal community, with populations in the surface waters sometimes exceeding 3×10^4 units (filaments) mL^{-1} during spring blooms (Cooper & Koch, 1984, Beutel, *et al.*, 2001, Collopy & Thomas, 2009). Thus, the failure to detect *Nodularia* species in 16S rRNA gene clone libraries or by direct microscopic examination stands in contrast to the earlier paradigm. Within the Illumina dataset, only 3 OTUs identified as members of *Nodularia*, however, their presence only accumulated to 0.025% of all sequence reads from the 21water column filter samples.

The significance of this finding is unclear, however, it is likely that any *Nodularia* species, which typically form long filaments (mean filament length 307 ± 273 µm; Karlson, *et al.*, 2012), present at the time of sampling were likely excluded from DNA filters by the use of a 178-µm pre-filter in the sampling apparatus. The use of the pre-filter was intended to exclude zooplankton, or more specifically, their excreted gut microbiota that might otherwise overprint the planktonic bacterial community. An unintentional consequence of pre-filtration may have been an overrepresentation of microscopic (1.5-3 µm) unicellular cyanobacteria related to *Synechococcus* species in assessing the photosynthetic community.

Conclusions

The ecology of Walker Lake has previously been described in terms of its fishery potential (Marioni, *et al.*, 2005) and through the characterization of its algal and zooplankton communities (Cooper & Koch, 1984, Beutel, *et al.*, 2001). This study served as a baseline survey of major bacterial taxonomic and functional groups, potentially important biogeochemical processes, and notable genera that took part in these processes in the final years of Walker Lake's ability to support endemic fish populations. Walker Lake contains a diverse alkaliphilic

microbial community, the activity of which, coupled with the shifting aqueous chemical parameters, affects lake habitability for fish. It is evident that a better understanding of microbial communities and processes that contribute to the overall ecological function of the lake will be critical for developing sound management strategies.

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CHAPTER THREE

SHIFTING MICROBIAL BIOGEOCHEMISTRY AND LIMNOLOGY OF A TRANSITIONING TERMINAL LAKE ECOSYSTEM; WALKER LAKE, NEVADA, USA

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Graduate Student's Involvement and General Background

The research detailed in this chapter was conducted between 2008 and 2015 by me, other lab members, and collaborators. This research structured my graduate thesis work during my tenure as a Master's student at the University of Nevada, Las Vegas and while employed and conducting research at The Desert Research Institute. I contributed to the following areas of this research project: organization of field sampling events, sample collection, curation and dissemination of samples for analysis, enrichment culturing of microbial physiotypes, extraction of DNA from environmental samples for Illumina sequencing, organization, analysis, and interpretation of collected data, and writing of the manuscript. The data presented here were collected from Walker Lake on several discrete sampling trips conducted between 2013 and 2015. In some comparative cases, data from September 2008 (the only date on which our lab sampled Walker Lake during summer thermal stratification) is included to highlight the differences and changes occurring in Walker Lake biogeochemistry as a result of loss of stratification.

Abstract

Walker Lake is a saline, alkaline, terminal lake ecosystem located in west-central Nevada. Anthropogenic streamflow diversions within the Walker River Basin have accelerated the long term lake desiccation, resulting in a >46 meter decrease in the lake surface altitude since 1882. To examine the effects that this decline in lake level has had on the limnological and microbial community structures, water and sediment samples were collected from a central lake location of Walker Lake with particular emphasis on late summer, which historically coincided with maximal thermal stratification. During the course of this study (2008-2015), the lake level declined by 5.8 m and physical parameters show Walker Lake to have shifted from a monomictic to a polymictic system sometime after 2008. Conservative and non-conservative increases in major ionic constituents (e.g., Na^+, SO_4^{2-}) corresponded with an overall 50.3% increase in salinity, from ~14.3 g L^{-1} in 2008 to ~21.4 g L^{-1} in 2015.

Cultivation-based microbial assessments revealed that physiotype distributions were at least partially controlled by environmental reduction-oxidation values, with aerobic heterotrophs being abundant throughout the water column, but fermentative microorganisms, nitrate-, iron-, manganese-, and sulfate-reducing microorganisms showing preference for anaerobic zones, especially in the sediment. Illumina sequencing of the V4 region of the 16S rRNA gene showed distinct differences in microbial community composition over time and sample type. The sediment communities were distinct from the overlying pelagic microbial communities and showed more similarity to microbial communities from the anoxic hypolimnion from the 2008 sampling event when summer thermal stratification was observed. The most abundant groups of microorganisms in the water column were Cyanobacteria (in particular *Synechococcus* species, mean abundance 10.5%) and Nitriliruptoraceae (mean abundance of 11.9%). Collectively, this

project records a wide range of baseline biogeochemical and microbiological responses to desiccation-induced physical shifts which occurred over a critical period of transition at Walker Lake.

Introduction

Terminal lakes occur in endorheic drainage basins, typically in semi-arid climates, and have no outlet for inflowing water other than evaporation (Cole, 1968, Wetzel, 2001). Walker Lake is a moderately saline, alkaline terminal lake located in west-central Nevada within the U.S. Great Basin and is a remnant lake of larger pluvial Lake Lahontan, which arose during the Pleistocene Epoch (2.6 million to 11,700 years before present (yrs B.P.); Benson & Mifflin, 1986, Domagalski, *et al.*, 1989). Agriculture became prevalent throughout the Walker River Basin in the 1860s and much streamflow from the Walker River, the primary tributary of Walker Lake, is appropriated for agricultural use, particularly during low water years (Collopy & Thomas, 2009). These long-term water diversions along the length of the Walker River have reduced the amount of streamflow that reaches Walker Lake, with little or no water inflow in some years of low precipitation (Adams, 2007). Ultimately, the anthropogenic influences within the Walker River Basin have led to >46 meter decline of the surface level of Walker Lake since 1882 (Russell, 1885).

The lack of freshwater inflow into Walker Lake causing the lake-level decline is inversely correlated with the total dissolved solids (TDS) concentration, which is currently 8.4 times more concentrated than the first documented measurement in 1882 (Russell, 1885). The loss of water through evaporation leaves dissolved chemical constituents in the water,

concentrating dissolved components. The first limnological study of Walker Lake was conducted by Russell (1885), in which the observed maximum lake depth of 68.6 m and TDS concentrations of 2,500 mg L^{-1} were described. Cooper and Koch (1984) showed an 18 m decline in Walker Lake's surface elevation between 1937 and 1975, with a correlated increase in TDS concentrations of 5,650 mg L^{-1} to 10,650 mg L^{-1}, respectively. The current maximum depth of Walker Lake is 17.1 m, half of the recorded maximum depth just 30 years ago (Domagalski, *et al.*, 1989), and TDS concentrations are 21,400 mg L^{-1}.

Walker Lake was one of few terminal lakes worldwide to have supported a fishery and contained four endemic fish species: Lahontan cutthroat trout (LCT) (*Oncorhynchus clarkii henshawi*), Tui chub minnow (*Gila bicolor*), Lahontan redside shiner (*Richardsonius egregius*), and Tahoe sucker (*Catostomus tahoensis*). A combination of factors stemming from the anthropogenic and climatic influences on Walker Lake, including disappearing food sources, toxic TDS concentrations that inhibit growth and reproduction, and inhabitable waters during summertime anoxia (Cooper & Koch, 1984, Beutel, *et al.*, 2001, Wright, 2009), led to lethal environmental pressures on endemic fish species that could not be overcome under the current lake conditions.

Walker Lake has been historically identified as a monomictic lake system, exhibiting summer thermal stratification from May to November, followed by autumnal mixing and water column circulation throughout winter. Walker Lake has shown periods of meromixis in some particularly wet seasons in which the incoming fresh water layer does not intermix with the more saline water present (Beutel, *et al.*, 2001). Between 2013 and 2015, summer thermal stratification was not detected in Walker Lake, likely due to the lake currently being too shallow to hold stratification. This shift to a polymictic lake system (continuous mixing throughout the year) and

the ongoing desiccation of Walker Lake alters the physical and chemical composition of the lake, also changing the biogeochemical cycling and microbiological community structure.

The anthropogenic and climatic factors that Walker Lake has experienced over the past century have dramatically altered the ecosystem. This study describes the changes that have occurred within the Walker Lake system as a result of the long-term desiccation and also examines the resulting shift in the limnological and microbiological community patterns. By examining the microbial communities of Walker Lake and documenting the limnological shift of this transitioning ecosystem, we gain insights into the physiological aspects of Walker Lake (e.g., loss of hypolimnetic ammonia and sulfide accumulation, shifts in biogeochemical cycles resulting from changing microbial populations) and possible ways to manage and restore this unique environment to the thriving ecosystem it once was.

Materials and Methods

Field site description. Walker Lake is located in west-central Nevada, immediately north of the town of Hawthorne. Previous bathymetric studies of Walker Lake revealed relatively steep western and eastern shores with a broad, flat lake bottom, with the deepest spot occupying a slight depression mid-lake (Lopes & Smith, 2007). Currently, the lake is ~8.4 km wide and ~18.1 km long (Figure 3.1). Water column and sediment samples were collected from this deepest, mid-lake location (38˚42’00.0”N, 118˚43’17.9”W). Four sampling events occurred between 2013 and 2015 (September 6th, 2013; November 2nd, 2013; September 4th, 2014; and September 8th, 2015). The earliest sampling date acted as an initial reconnaissance trip, whereas full suites of samples were obtained on the three later dates. To broaden the temporal perspective of this

study, microbiological and limnological data obtained from a September 2008 sampling event by the same research group are included to compare current lake conditions to those from a time when the lake was deeper and exhibited monomictic behavior (Collopy & Thomas, 2009).

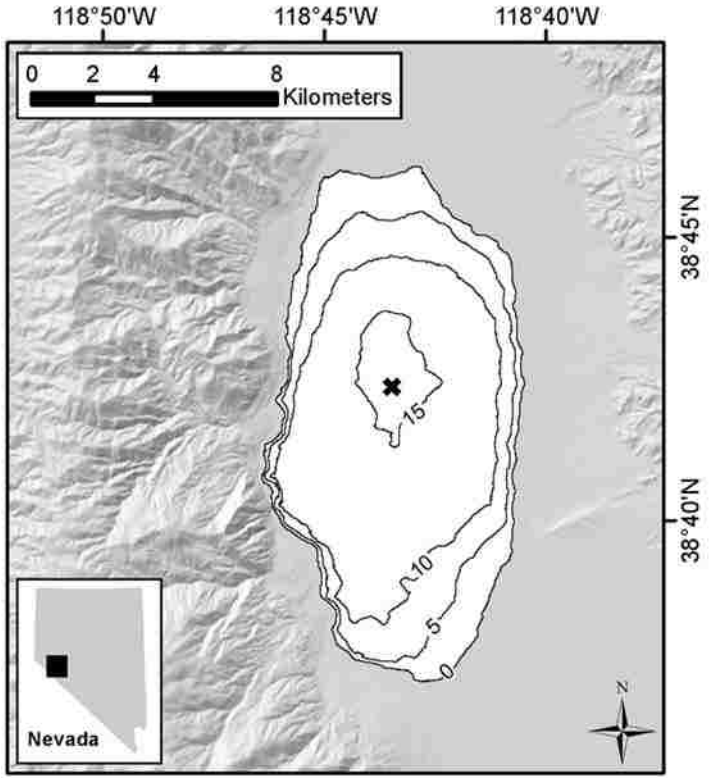

Figure 3.1. Walker Lake is located in west-central Nevada, near the Nevada/California border. Samples were collected at multiple depths from a central lake station, indicated by (x) on the map. The bathymetry of the lake is given in five-meter increments.

Physical measurements and sampling. Physical parameters of the water column were measured using a 6920-V2 multi-parameter sonde (Yellow Springs Instruments, Yellow Springs, OH). Measured parameters include temperature, depth, pH, dissolved oxygen (DO), total

dissolved solids (TDS), conductivity, and oxidation-reduction potential (ORP). Photosynthetically active radiation (PAR) was measured using a LI-COR 4π sensor (Li-193) and L-1400 data logger (LI-COR Biosciences, Lincoln, NE). Water samples were collected from defined depths using peristaltic pumps (GeoPump II, Geotech, Denver, CO and Masterflex E/S, Cole Parmer, Vernon Hills, IL) with autoclaved, platinum-cured silicone tubing (Masterflex LS-14) attached to and deployed with the sonde. The depth sensor on the sonde maintained precise control of *in situ* water collection from discrete depths. Approximately two liters of water were passed through the tubing lines between sample collections from each depth to ensure all water from the previous sampled depth had been sufficiently flushed out.

Each peristaltic pump tubing line had a 178 µm stainless steel pipe line strainer (ASCO Valve Inc., PKG86042) placed in-line upstream of the sampling port to remove larger debris and zooplankton (and their associated gut microflora) from the pelagic microbial community. To examine the portion of the biological community being removed by the pre-filters, plankton tows were conducted using a 5-inch diameter 80 µm mesh plankton net sampler (LaMotte Company, Chestertown, MD), deployed in a single vertical tow from the bottom to surface with a calibrated tape reel and concentrated into a 60 mL glass collection vial. Collected tow was passed through a 0.22 µm polyethersulfone filter unit (Sterivex-GP, Millipore, Darmstadt, Germany) for DNA analysis and frozen on dry ice in the field until being stored at -80˚C.

Water samples were collected as either "unfiltered" (e.g., passed only through the 178 µm pre-filter) or filtered. Filtered samples were collected as filtrate from a 0.22 µm polyethersulfone filter unit (Sterivex-GP, Millipore, Darmstadt, Germany). Unfiltered sampled included those for total organic carbon (TOC), alkalinity measurements, and cultivation. Samples for cultivation assays were collected from the unfiltered flowing line via 22G hypodermic

needles into sterile, N_2-flushed, pre-evacuated serum bottles, crimp sealed with blue butyl rubber stoppers, and stored on ice in the dark until transport to the laboratory. Filters for microbial DNA extraction were collected using 0.22 µm polyethersulfone filter units (Sterivex-GP, Millipore, Darmstadt, Germany), connected directly to the flowing line, with approximately 2-4 liters of water having passed through each filter. Water samples for dissolved aqueous chemistry analysis (dissolved organic carbon (DOC), dissolved nitrogen and phosphorous compounds, cations and anions, dissolved metals, and trace elements) were taken from the 0.22 µm polyethersulfone filter effluent, collected after ~ 1 L of water had passed through the filters to ensure that any chemical residues from the filters had been flushed away. Water samples were shipped within 24 hours of collection for chemical analysis to either the Desert Research Institute Water Analysis Laboratory (DRI, Reno, NV) or ACZ Laboratories, Inc. (Steamboat Springs, CO; US EPA SW-846, APHA, *et al.*, 1915, US EPA, 1983, US EPA, 1993, US EPA, 1994).

Sediment samples were obtained by transferring intact sediment collected with a 6" x 6" Petite PONAR dredge (Model 1728-G30, Wildco, Yulee, FL) to a plastic dish pan. Sediment samples, divided into upper and lower subsamples, were collected by manually scooping dredge material into sterile 50 mL polypropylene centrifuge tubes. The upper subsamples were loose sediment of a light pink or green color that represented the top 0-2 centimeters; whereas, the deeper sediments were darker brown-black in color with a noticeable sulfide smell and represented most of the volume of the dredged samples (2+ cm). During the 2015 sampling event, sediment samples were collected in a transect line from the central lake position to near-shore position at intervals of 5 meters of water depth to examine uniformity of sediment microbial communities at different points and depths within the lake. Sediment samples for

microbial cultivation were temporarily stored on ice and refrigerated thereafter. Samples for DNA extraction were frozen on dry ice in the field and then stored at -80^{o}C thereafter.

Microbial cultivation. Dilution cultivation for specific microbial physiotypes of expected relevance were conducted in a basal liquid medium, adapted to reflect today's higher salinity, from a previously-developed synthetic Walker Lake basal salts (WLBS) formulation (Beutel, 2001, Beutel, *et al.*, 2001), containing per liter of deionized water: 1.0 g $MgSO_4 \cdot 7H_2O$, 1.04 g $MgCl_2 \cdot 6H_2O$, 0.0735 g $CaCl_2 \cdot 2H_2O$, 0.197 g K_2HPO_4, 0.121 g NH_4Cl, 0.6 g KCl, 7.3 g NaCl, 4.68 g Na_2SO_4, 2.385 g $NaHCO_3$, 1.073 g Na_2CO_3, 1 mL Trace Mineral Supplement (ATCC), 1 mL Vitamin Supplement (ATCC). These basal salts were adjusted to pH 9.4 with NaOH and subsequently used to prepare duplicate serial dilutions from collected water and sediment samples to determine relative microbial abundances. Sediment was diluted 1:1 with sterile WLBS prior to serial dilutions. Dilutions were performed by inoculating 9 mL of synthetic WLBS medium with 1 mL of Walker Lake water or sediment slurry and serially diluting one milliliter throughout the remaining tubes until a dilution of 10^{-9} was reached. All cultivation enrichments were performed at room temperature (~22 ^{o}C) and were inoculated within 48 hours of sample collection.

Cultivation tubes containing WLBS medium were supplemented with a range of energy substrates, targeting particular microbial physiotypes. Aerobic heterotroph cultivations were supplemented with 1.6 g L^{-1} of 1X R2 broth (Atlas, 2004). Nitrate reduction cultivations contained 10 mM KNO_3 and 5 mM each of sodium formate, lactate, and acetate (FLA) and were conducted in screw-cap tubes incubated in an anaerobic chamber (Coy, Grass Lake, MI) under an atmosphere of N_2:CO_2:H_2 (10% H_2, the balance in N_2:CO_2 80:20). Sulfate reduction enrichments were cultivated in Postgate's Medium B for Sulfate Reducers (Atlas, 2004),

modified to include WLBS and 5 mM FLA, adjusted to pH 9.4 with NaOH, and were conducted anaerobically in Balch pressure tubes (Bellco Glass, Vineland, NJ) with a N_2 headspace. Fermentative microorganisms were cultivated anaerobically in Balch pressure tubes in PTYG medium (Atlas, 2004) with WLBS and a N_2 headspace . Cultivations for metal reducers were conducted in WLBS, with the omission of sulfate and 5 mM FLA, inoculated in screw-cap tubes incubated within an anaerobic chamber under N_2:CO_2:H_2. To identify the best performing iron-oxide formulation at Walker Lake's high pH, three different preparations were tested: hydrous ferric oxide (HFO; Fredrickson, *et al.*, 1998, Fredrickson, *et al.*, 2000), iron (III) citrate, and ferric nitrilotriacetate (Fe NTA; Kostka & Nealson, 1998). Additional metal-reduction assays were performed using manganese (IV) oxide (Kostka & Nealson, 1998). HFO reduction cultivations contained 2.2 mM HFO and iron (III) citrate and Fe NTA reduction cultivations both contained 2.5 mM of iron oxide, respectively. Manganese (IV) oxide reduction cultivations contained ~4.1 mM MnO_2. Growth of all culture enrichments was assessed on plates as countable colonies or in liquid by turbidity, direct microscopic counts, and/or substrate color change.

16S rRNA gene V4 region Illumina sequencing and data processing. Filter membranes were manually excised from the Sterivex-GP filter cartridge units and genomic DNA was extracted using the Power Soil DNA Isolation Kit per the manufacturer's instructions (MoBio Laboratories, Inc., Carlsbad, CA). Extracted DNA was submitted for Illumina sequencing of the V4 region of the 16S rRNA gene (Micro-Seq, Las Vegas, NV; MR DNA, Shallowater, TX; Westcore DNA Core Facility, Black Hills State University (BHSU), Spearfish, SD) using a degenerate primer set, F515 (5' GTG YCA GCM GCC GCG GTA A 3') and R806 (5' GGA CTA CHV GGG TWT CTA AT 3'), to allow for better coverage of archaeal lineages (Hou, *et*

al., 2013). Forward and reverse reads from 2008 and 2013 samples (Micro-Seq, Las Vegas, NV) were sequenced with Illumina MiSeq sequencing platform 2x150bp and were merged with a minimum sequence overlap of 30 nucleotides. Samples from 2014 were sequenced with Illumina MiSeq 2x300bp (MR DNA, Shallowater, TX). Forward reads were truncated to 257 bp to remove primer region and adaptor sequence. Forward and reverse reads from 2015 (Westcore DNA Core Facility, BHSU, Spearfish, SD) were sequenced with Illumina MiSeq 2x250 bp and were merged with a minimum sequence overlap of 200 nucleotides.

All sequence reads were demultiplexed, and quality filtered using QIIME 1.9.1 (Caporaso, *et al.*, 2010) and concatenated into a single file. The Greengenes 13_8 reference sequence database (McDonald, *et al.*, 2012) was used to identify chimeric sequences (identify_chimeric_seqs.py command) with the usearch61 algorithm (Edgar, 2010). Operational taxonomic units (OTUs) were formed based on 97% sequence similarity and taxonomy assignments were made with a subsampled open-reference OTU-picking strategy using the usearch61 and uclust algorithms (Rideout, *et al.*, 2014) , respectively, against the Greengenes 13_8 database. A phylogenetic neighbor-joining tree (Price, *et al.*, 2010) was generated based on PyNAST-aligned OTU sequences (Caporaso, *et al.*, 2010) and used for alpha and beta diversity metrics. Lastly, to account for differences in sequencing depth, the OTU table was rarefied to a depth of 10,000 sequences per sample. This rarefied OTU table was used for all subsequent analyses, except for the generation of the OTU heatmaps. UniFrac analyses (Lozupone, *et al.*, 2011) were invoked to calculate pairwise distances between microbial communities, based on 100 rarefactions of 10,000 randomly selected sequences per sample.

Microbial community statistical analyses. Statistical analyses of the Illumina MiSeq 16S rRNA gene V4 dataset were conducted using RStudio (R Core Team, 2014). Analysis of

Similarity (ANOSIM) tests were conducted with the vegan package (Oksanen, *et al.*, 2015) using oxygenated water column samples collected between 2008 and 2015 to determine whether the microbial community composition (unweighted UniFrac distance matrix) or the microbial community structure (weighted UniFrac distance matrix) were significantly different when grouped by year and sequencing laboratory. Labels were permuted 999 times to calculate a p-value. Principal component analysis (PCA) ordinations of weighted and unweighted UniFrac distances were constructed using the vegan package to evaluate community similarities (Oksanen, *et al.*, 2015). A heatmap was constructed from the full OTU table to visualize all OTUs with ≥1% relative abundance in at least one sample, and samples were hierarchically clustered based on computed pairwise Bray-Curtis distances, using the Heatplus, RColorBrewer, and vegan packages (Ploner, 2012, Neuwirth, 2014, Oksanen, *et al.*, 2015). A Venn diagram was made from the rarefied OTU table to show the OTUs shared between water column samples from 2008 through 2015. An OTU was counted in the Venn diagram if it was present in at least one of the sampled depths per year.

Unweighted pair group method with arithmetic mean (UPGMA) clustering of microbial communities was conducted based on the calculated weighted and unweighted UniFrac distance matrices. Node support values were calculated from 100 jackknifed subsamples of 10,000 sequences per sample in each subsample. Bootstrapped UPGMA cluster trees were generated in FigTree v1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/). Pairwise Spearman correlation was used to compare various aqueous chemical and physical parameters and a correlation plot was generated in R using the corrplot package (Wei, 2013). Environmental physical and chemical data was then z-score transformed and Euclidean distances were computed for each environmental variable in the ade4 R package (Dray & Dufour, 2007). Mantel tests were

performed (9,999 permutations) to correlate microbial community structure (weighted UniFrac distance matrix) with the Euclidean distance matrices of each z-score transformed environmental variable.

Results

Physical lake structure. Over the course of this study, maximum depth decreased from 22.9 meters in 2008 to 17.1 meters in 2015 (~1190 m above sea level), which corresponded with a 50.3% increase in conductivity between 2008 and 2015. A distinct stratified profile was present in September 2008, with the metalimnion occurring at 17.5 m and the hypolimnion below 19 m (Figure 3.2a, Table C.1). Average temperature, oxidation-reduction potential (ORP), and dissolved oxygen measurements were consistent in the epilimnion (22.1°C, 61 mV, and 6.55 mg L^{-1}, respectively) and rapidly dropped below 17.5 m to the lowest values measured at the lake bottom (14.74°C, -272.5 mV, and 0.1 mg L^{-1}, respectively). Salinity measurements (TDS and conductivity) were higher in the epilimnion, while pH values were higher in the hypolimnion. Photosynthetically active radiation (PAR) values gradually decreased with depth to low, but measurable, values at the sediment-water interface (3.6 µmol $m^{-2}s^{-1}$ at 22 m).

No evidence for lake stratification was obtained during any of the four sampling events between 2013 and 2015; rather, all *in situ* sonde measurements were uniform throughout the water column (Figure 3.2 b-d, Table C.1; data from September 2013 not shown). Temperature values reflected typical seasonal variation, with uniform temperatures of 14 °C in November of 2013 and 22 – 23 °C in September of 2014 and 2015. Calculated TDS values were equivalent for all depths within sampling years but increased steadily over the monitoring period, from ~14.3 g

L^{-1} in 2008 to ~21.4 g L^{-1} in 2015. Also, consistent with active mixing, dissolved oxygen profile data were uniform from surface to lake bottom in each profile, averaging 8.76 mg L^{-1} in November of 2013 and 6.82-7.17 mg L^{-1} in both September profiles. ORP measurements were slightly positive in all sampling events between 2014 and 2015, but showed some tendency to drift, mostly likely due to instrument calibration effects (2013 ORP measurements not shown). The sharp downward deflection of ORP at the very bottom of the 2014 and 2015 profiles (Figure 3.2b and c) is indicative of the sonde entering anoxic sediments, confirming full mixing to the lake bottom. pH remained consistent at 9.3-9.4 for all depths and throughout the course of this study. PAR measurements from the September 2014 and 2015 profiles showed PAR values gradually decreased with depth to low but measurable values at the sediment-water interface (18.2 µmol $m^{-2}s^{-1}$ at 17.5 m in 2014, 43.3 µmol $m^{-2}s^{-1}$ at 16.5 m in 2015).

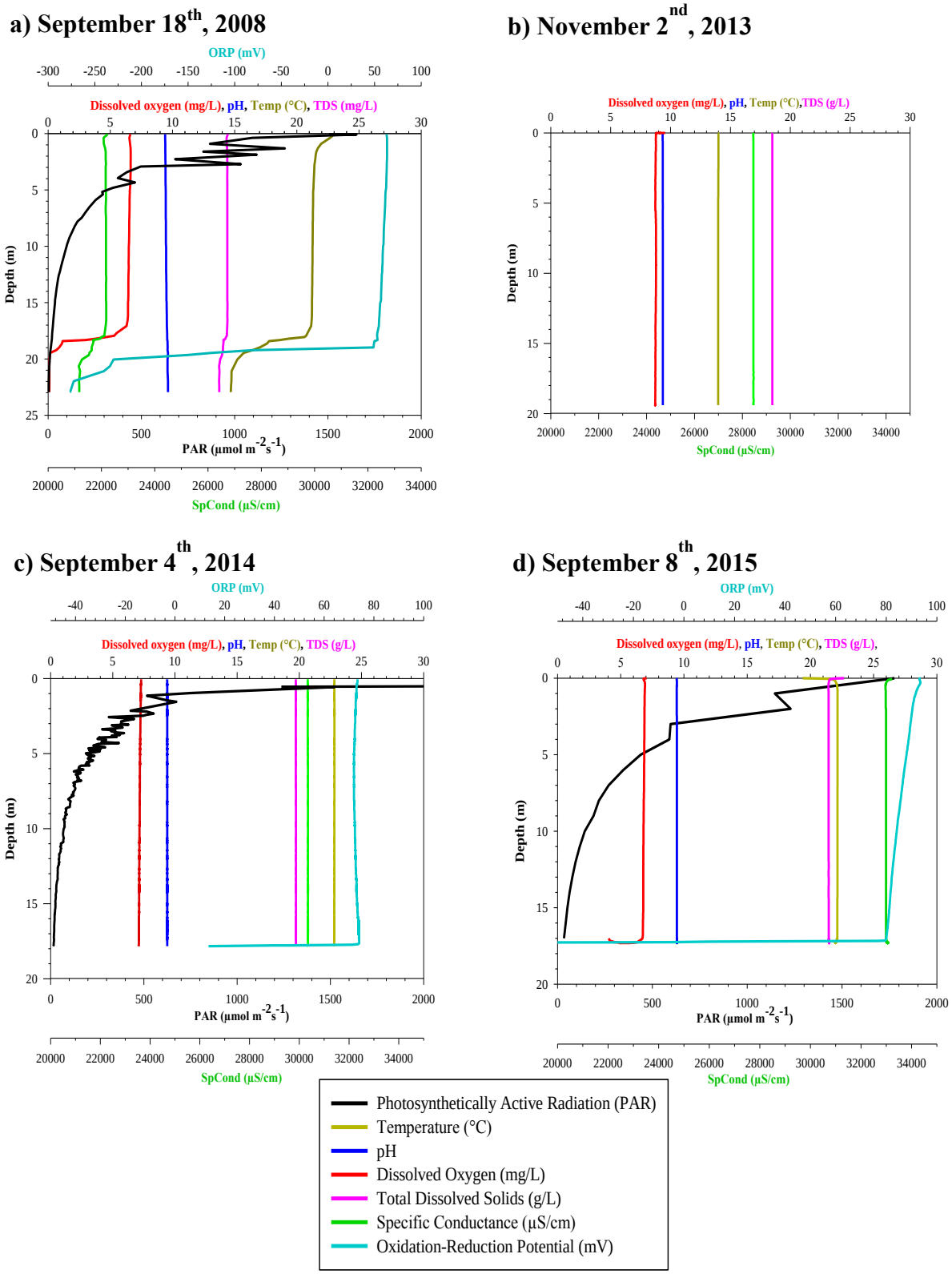

Figure 3.2. Physical *in situ* parameters of Walker Lake from different sampling dates between 2008 and 2015 (a-d). Note increase in specific conductance over time and decrease in lake depth.

Aqueous chemistry. The 2015 ionic composition was dominated by Na^+ (30%), SO_4^{2-} (29.7%), Cl^- (20%) and HCO_3^-/CO_3^{2-} (17.9%); the order of measured cation abundance was $Na^+ > K^+ > Mg^{2+} > Ca^{2+}$ and the order of measured anion abundance was $SO_4^{2-} > Cl^- > HCO_3^- > CO_3^{2-}$ (Table 3.1). The percent increase between 2008 and 2015 of Cl^- (47.5%), Na^+ (49.5%), and K^+ (45.5%) were the only major ionic constituents that increased conservatively with the 50.3% increase seen in conductivity over this time period. Other aqueous analytes were controlled in some manner other than evaporation. HCO_3^- , Ca^{2+}, and Mg^{2+} exhibited smaller increases of 8.7%, 8.0%, and 5.1%, respectively. SO_4^{2-} and CO_3^{2-} concentrations increased more than the predicted 50.3% from evaporative concentration of solutes and showed percent increases of 95.6% and 74.9%, respectively.

The average molar ratio of inorganic nitrogen to phosphorous (N:P) in Walker Lake between 2013 and 2015 was 0.30:1. Nitrate and nitrite concentrations were extremely low over the course of the study, remaining at or below the 0.01 mg L^{-1} detection limit offered by the contract laboratory used for most dates (ACZ) and for most depths. To obtain better sensitivity, nitrate and nitrite were measured for the 2015 samples by the DRI Water Laboratory and ranged from 0.010 to 0.017 mg L^{-1}. While ammonia concentrations were sporadic, it was the most abundant form of bioavailable inorganic nitrogen, with values ranging from below the 0.01 mg L^{-1} limit of detection (ACZ) in 2013 and 2014 to 0.038 mg L^{-1} in 2015. The single highest value by far was 0.47 mg L^{-1}, obtained in the 2008 anoxic hypolimnion, an enrichment of twenty-three fold over the 0.02 mg L^{-1} values that were present in the epilimnion on that date. Soluble phosphorous remained high, but appeared to decline somewhat with time, from ~0.55 mg L^{-1} in 2008 to ~0.35 mg L^{-1} in 2015. The highest phosphorous value was obtained from the 22 m hypolimnetic sample in 2008 (0.64 mg L^{-1}). Dissolved and total organic carbon (D/TOC) values

were approximately equal to one another and more than doubled between 2008 and 2013 (from about 40 mg L^{-1} to 90 mg L^{-1}); however, DOC and TOC declined to values of ~65 mg L^{-1} in 2014 and ~60 mg L^{-1} in 2015. Trace element analysis (Table C.2) showed high levels of arsenic and boron in 2014 and 2015 (average 1.7 mg L^{-1} As and 48 mg L^{-1} B).

Table 3.1. Aqueous chemistry measurements.

Date	Depth (m)	HCO_3^- mg/L	CO_3^- mg/L	Cl^- mg/L	SO_4^{2-} mg/L	Na^+ mg/L	K^+ mg/L	Ca^{2+} mg/L	As^+ mg/L	Mg^{2+} mg/L	NO_3^- mg-N/L	NO_2^- mg-N/L	NH_3^+ mg-N/L	Soluble P mg-P/L
2008 Sept.	0	2560	1170	3910	3970	5610	294	9.07	1.44	206	<0.01	0.01	0.02	0.55
	10	2540	1170	3890	3980	5620	296	9.02	1.42	206	<0.01	<0.01	0.02	0.57
	22	2540	1110	3720	3890	5420	283	8.03	1.41	198	0.02	0.02	0.47	0.64
2013 Nov.	0	3102	1601	5240	5140	7450	380	9.09	n.d.	176	<0.01	<0.01	<0.01	0.49
	10	3090	1600	5200	5130	7540	385	9.14	n.d.	170	0.01	<0.01	<0.01	0.49
	19	2880	1490	5200	5120	7470	379	9.14	n.d.	168	<0.01	<0.01	<0.01	0.49
2014 Sept.	0	2647	1956	5590	5490	7410	381	9	1.78	204	<0.02	<0.01	<0.05	0.44
	5	2696	1974	5510	5400	7750	393	9.2	n.d.	206	<0.02	<0.01	<0.05	0.44
	10	2684	1938	5530	5470	7670	395	8.8	n.d.	203	<0.02	<0.01	<0.05	0.45
	15	1696	2664	4990	5130	7680	385	9	1.76	211	<0.02	<0.01	<0.05	0.45
	17.5	2647	1956	5530	5430	7600	387	9.1	n.d.	203	<0.02	<0.01	<0.05	0.45
2015 Sept.	0	2770	1878	5460	7560	8360	418	9	1.7	211	0.017	0.01	0.037	0.35
	10	2782	2064	5410	8040	8470	428	9.2	1.7	217	0.012	<0.01	0.037	0.34
	16.5	2757	2082	6190	7560	8100	420	10	1.6	212	0.010	<0.01	0.038	0.34

n.d.= not determined

Cultivation-based microbial assessments. To determine the distribution of cultivable microorganisms belonging to major metabolic physiotypes, targeted cultivations were performed immediately after the sampling events of 2008, 2013, and 2014 (Table 3.2). Water column depths below 17.5 m were anoxic in 2008. Aerobic heterotrophs were uniformly abundant throughout the water column in each of the three years where cultivations were conducted and were detected in higher abundances (up to three orders of magnitude, 10^4 vs 10^7 cells mL^{-1}) in 2013 and 2014. Sediment abundances were even higher (up to 10^9 cells mL^{-1}). Fermentative microorganisms had a patchy distribution throughout the water column and sediment in all three years (ranging from 10^1 to 10^9 cells mL^{-1}) and were present in high abundance, even in oxygenated waters.

Nitrate-reducing microorganisms were not abundant in the oxygenated water column, but increased in abundance in the anoxic hypolimnion in 2008 (10^3 to 10^4 cells mL^{-1}) and in all three years were most abundant in the sediment (10^3 to 10^7 cells mL^{-1}). Similar patterns were seen for sulfate-reducing bacteria (SRBs), with near complete absence in oxygenated regions of the water column ($0 – 10^1$ cells mL^{-1}) and relatively low abundance ($10^1 – 10^2$ cells mL^{-1}) in the anoxic hypolimnion. The highest abundances of SRBs were present in the sediment samples (10^3 to 10^6 cells mL^{-1}) in all three years.

For the most part, iron- and manganese-reducing microorganisms were present in low abundances in the oxygenated water column (0 to 10^2 cells mL^{-1}), with the exception of the iron citrate-reducing microorganisms, which had relatively high abundances in the water column in 2014 (10^3 to 10^4 cells mL^{-1}). Hydrous ferrous oxide (HFO) was tested as a potential terminal electron acceptor in 2013 and 2014, and while almost none were detected in the water column samples, reasonable abundances were detected in the sediment ($10^3 – 10^6$ cells mL^{-1}). Likewise,

manganese oxide reducers were not detected in the water column. Overall, the highest abundances of iron- and manganese-reducing microorganisms were obtained in the sediment samples (10^3 to 10^7 cells mL^{-1}).

Table 3.2. Quantitative cultivation of targeted microbial physiotypes. Note: different water column depths were sampled for each sampling date. Depths that were not analyzed in a given year are indicated by (--) and changes in the lake depth are reflected as empty cells. Light blue table cells indicate 2008 epilimnetic water samples, darker blue cells indicate 2008 hypolimnetic water samples, yellow cells indicate 2013 sampling, and red cells indicate 2014 sampling.

Depth (m)	Aerobic Heterotrophs			Fermentative Organisms			NO_3^- Reducers			SO_4^{2-} Reducers			Iron (FeCitrate) Reducers			Iron (FeNTA) Reducers			Iron HFO Reducers			Manganese Oxide Reducers		
0	10^5	10^7	10^7	10^1	10^7	10^5	10^1	10^1	0	0	10^1	0	0	10^1	10^4	0	10^1	10^1	n.d.	0	10^1	n.d.	0	0
5	--	--	10^6	--	--	10^5	--	--	0	--	--	0	--	--	10^4	--	--	10^1	n.d.	--	0	n.d.	--	0
10	10^5	10^7	10^7	10^4	10^9	10^5	10^1	10^1	0	0	0	0	10^1	10^1	10^4	0	10^1	10^2	n.d.	0	0	n.d.	0	0
15	--	--	10^6	--	--	10^5	--	--	0	--	--	0	--	--	10^3	--	--	10^2	n.d.	--	0	n.d.	--	0
17.5	10^4	--	10^7	10^4	--	10^5	10^2	--	10^1	10^1	--	0	10^1	--	10^4	10^1	--	10^1	n.d.	--	0	n.d.	--	0
18	10^4	--		10^5	--		10^3	--		10^1	--		10^1	--		10^2	--		n.d.	--		n.d.	--	
19	10^4	10^7		10^5	10^9		10^3	10^1		10^1	10^1		10^2	10^1		10^3	10^1		n.d.	0		n.d.	0	
22	10^4			10^5			10^4			10^2			10^5			10^4			n.d.			n.d.		
SS	10^6	10^8	10^9	10^5	10^7	10^7	10^4	10^3	10^7	10^3	10^6	10^3	10^4	10^5	10^7	10^3	10^5	10^7	n.d.	10^5	10^4	n.d.	10^3	10^4
DS	10^5	10^7	10^7	10^5	10^7	10^5	10^4	10^5	10^7	10^4	10^5	10^4	10^5	10^5	10^7	10^4	10^6	10^5	n.d.	10^5	10^3	n.d.	10^3	10^3
Year	**'08**	**'13**	**'14**	**'08**	**'13**	**'14**	**'08**	**'13**	**'14**	**'08**	**'13**	**'14**	**'08**	**'13**	**'14**	**'08**	**'13**	**'14**	**'08**	**'13**	**'14**	**'08**	**'13**	**'14**

SS= surface sediment (0-2 cm); DS= deeper sediment (2+ cm)
n.d.= not determined

16S rRNA gene V4 region Illumina phylogenetic analysis. Illumina libraries targeting the V4 region of the 16S rRNA gene were performed on all water column depths, surface sediments, and deeper sediments for all of the sampling dates between 2008 and 2015. Three different sequencing facilities were used for portions of this dataset. Analysis of similarity (ANOSIM) showed a large proportion of the variance among microbial community structures from 2008-2015 oxygenated water column samples was explained more strongly by the year the samples were taken (unweighted: R_{ANOSIM}=0.8883, p=0.001; weighted: R_{ANOSIM}=0.8171, p=0.001), rather than the sequencing facility used. Thus, although much of the data variance of these samples could also be explained by the sequencing facility used (unweighted: R_{ANOSIM}=0.7655, p=0.001; weighted: R_{ANOSIM}=0.8310, p=0.001), the year the sampling events took place exhibited stronger unweighted ANOSIM results.

Principal component analysis (PCA) ordination of all water column and sediment samples from 2008 to 2015 (Figure 3.3a) showed 85.3% of the data variance explained by the two axes, with clustering occurring based primarily upon sample type (i.e. water column, surface sediment, deeper sediment). Clustering of the PCA ordination using the bootstrap support values of the weighted UniFrac UPGMA tree (Figure 3.3b) showed the samples from the surface sediments (0-2 cm) and the deeper sediments (2+ cm) grouping into two distinct clades. The anoxic water column samples from the 2008 hypolimnion (19 m and 22 m) formed a distinct group; as did the 0, 10, and 15 m samples from 2014. All of the other water column samples grouped together.

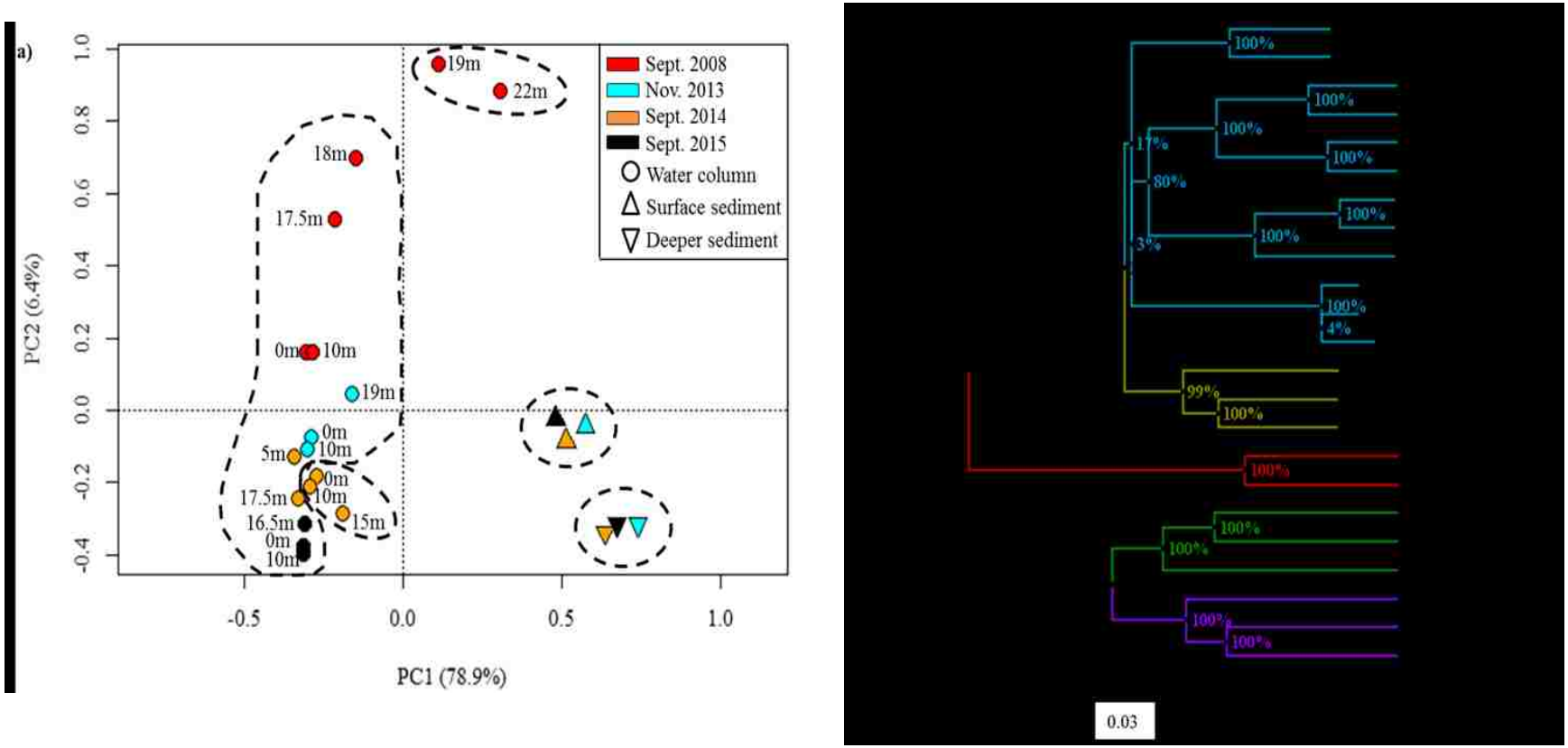

Figure 3.3. Microbial community differences by principal component analysis (PCA) ordination (a) using weighted UniFrac distances of all water column and sediment samples from September 2008 (red), November 2013 (blue), September 2014 (orange) and September 2015 (black). Each shape represents a complete Illumina sequence dataset of the corresponding depth/sample. Circles (o) indicate samples from the water column, upright triangles (Δ) indicate sediment samples from the surface of the lake bottom (0-2 cm), and upside down triangles (∇) indicate sediment samples below the surface sediment layer (2+ cm). Ellipses drawn groupings correspond with clade separation based on 100% bootstrap support values from weighted UniFrac UPGMA tree (b).

A Venn diagram made from the rarefied OTU table shows the OTUs that are shared between water column samples from 2008 through 2015 (Figure 3.4). Of the 9,298 detected OTUs from the water column samples between 2008 and 2015, 125 OTUs were present in at least one sampled depth in all of the four project years. Of these shared OTUs, 18 OTUs appeared in every water column sample from all four years, indicating a core microbiome community that spatiotemporally persists within Walker Lake (Table C.3). Core OTUs included: Actinomycetales, Nitriliruptoraceae, Cyclobacteriaceae, Cryomorphaceae, Flavobacteriaceae, Sphingobacteriales, S*ynechococcus*, Rhodocyclaceae, Mollicutes, Puniceicoccaceae, and *Luteolibacter*.

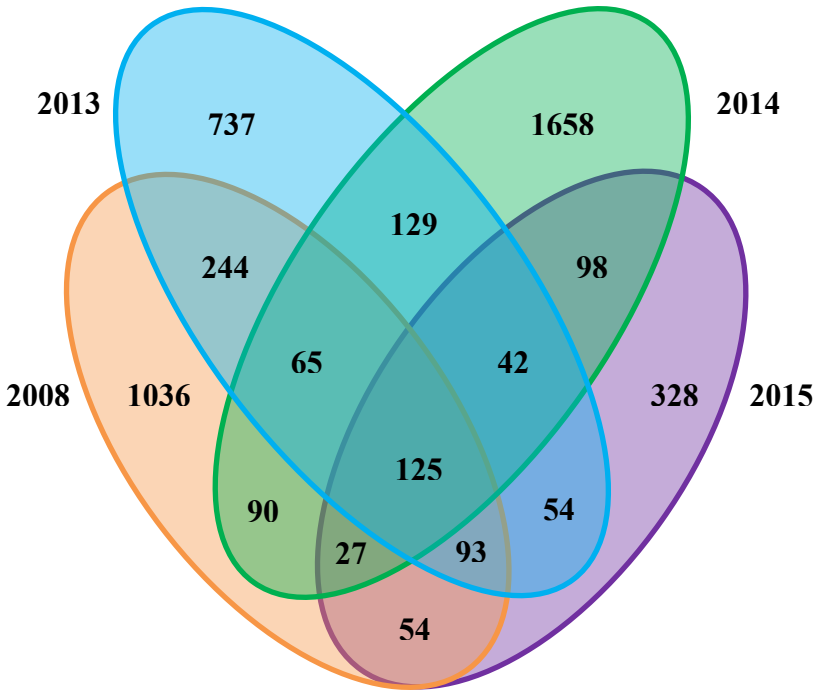

Figure 3.4. Venn diagram showing shared OTUs between water column samples from 2008 (orange), 2013 (blue), 2014 (green), and 2015 (purple). OTUs were counted if sequence appeared at least once per year. 10,000 sequences were randomly selected per sample. Of the 125 OTUs that are present at least once in any of the sample depths of each year, 18 OTUs appeared in every sample in all years, indicating a core microbiome.

A heatmap generated with a subset of water column samples from 2013-2015 shows 62 OTUs that have greater than 1% abundance in any sample (Figure 3.5, OTU identities listed in Table C.4). The colored profile pattern of the abundances of these OTUs from each sample show groupings based on sample date, with no significant trend occurring with sample depth. Twelve of the 18 core water column OTUs were present in greater than 1% abundance in the water column samples. The most abundant OTU within the water column samples between 2008 and 2015 was OTU 26698, a *Synechococcus* species (mean 7.10 ± 1.39% standard error of the mean (SEM)) with the highest abundances observed in September 2014 samples (mean 13.81 ±2.75% SEM). 388 OTUs identified as *Synechococcus* species, accounting for a mean abundance of 10.48 ± 0.02% SEM for all the water column microbial communities between 2008 and 2015. The second most abundant OTU within the water column samples was OTU 71, a member of the genus *Nitriliruptor* (mean 5.60 ± 0.97% SEM), with the highest abundances observed in September 2015 samples (mean 10.38 ± 0.27% SEM). There were 617 OTUs that fell within the Nitriliruptoraceae, accounting for a mean abundance of 11.91 ± 1.14% SEM in all of the water column microbial communities between 2008 and 2015.

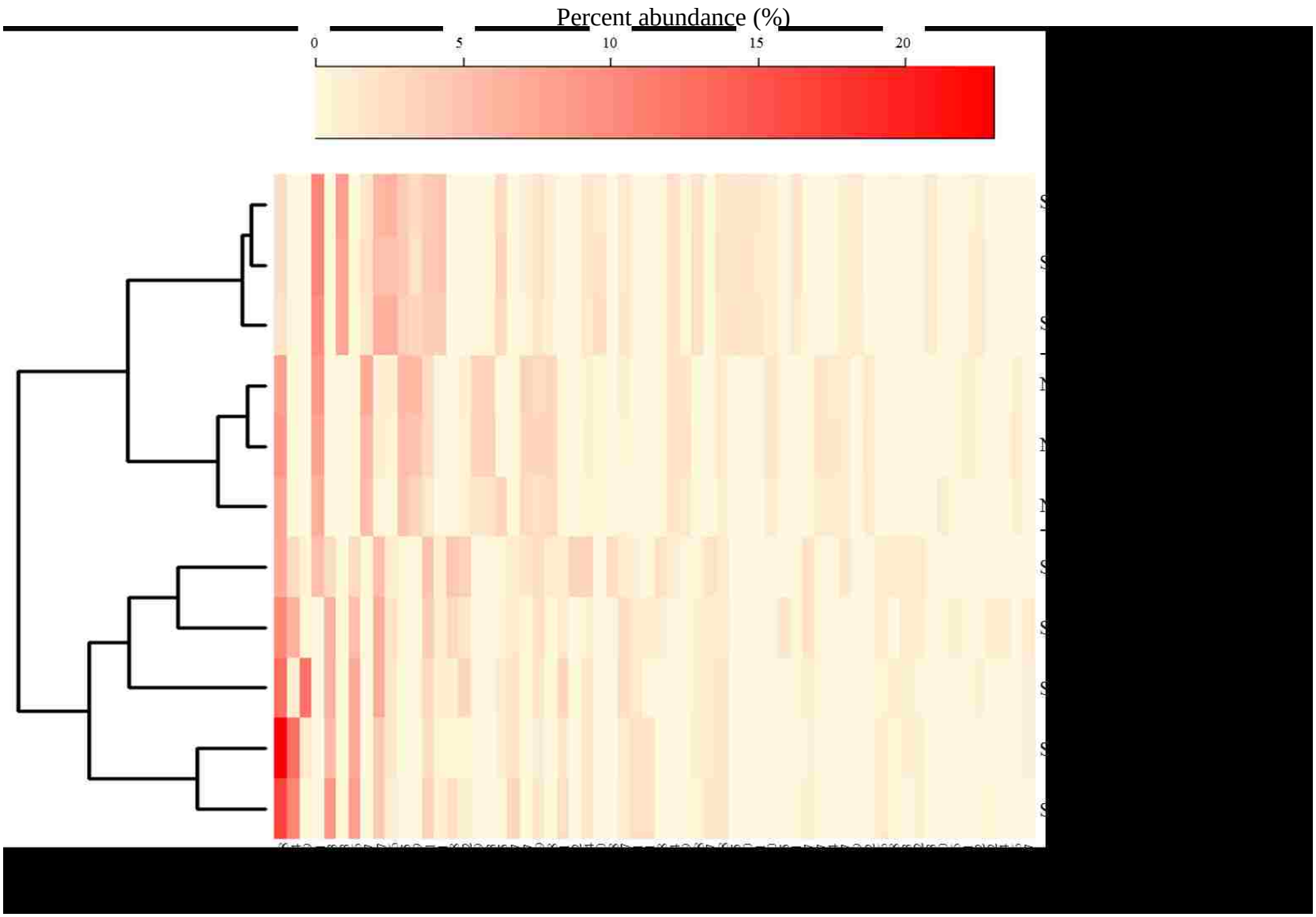

Figure 3.5. Heatmap depicting OTUs with abundances >1% in water column samples. OTUs with a (*) in front are part of the 2008-2015 core microbiome of Walker Lake water column community. Identities of listed OTUs is given in Table C.4.

A heatmap generated with the sediment samples from 2013-2015 showed the 58 OTUs that had >1% abundance in any sample from the sediment (Figure 3.6, OTU identities listed in Table C.5). The colored profile pattern of the abundances of these OTUs showed no discernible trends following year or sediment depth. The most abundant OTUs within the sediment samples were OTU 762, a species of *Thioalkalivibrio* (mean 2.06 ± 0.83% SEM) and OTU 758, an unclassified genus within the family ML1228J-1, in the order Natranaerobiales (mean 1.92 ± 0.85% SEM). Only one OTU out of the 18 core water column OTUs was present in >1% abundance in the sediment samples (OTU 1615, an unclassified genus within the family Flavobacteriaceae; mean 0.35 ± 0.20% SEM in all sediment samples).

To better evaluate the effect that 178µm pre-filtration may have had on assessments of planktonic community structure, plankton tows were completed and analyzed in 2014 and 2015 and compared against the water column samples of the same years. Since the DNA extracted from plankton tows contains the associated gut microbiota of zooplankton as well as larger planktonic bacteria, broad comparisons were difficult to discern (data not shown). However, there were 127 OTUs that were present in all the plankton tow samples that were not present in any of the water column samples (or were in low abundances; less than 50 sequence reads in any water column sample) from the same years. Most notable was the presence of *Nodularia* species in the plankton tow samples (0.32-1.51% abundant), which were nearly completely absent in the sequence dataset from the 2008-2015 water samples that passed through the 178 µm pre-filter.

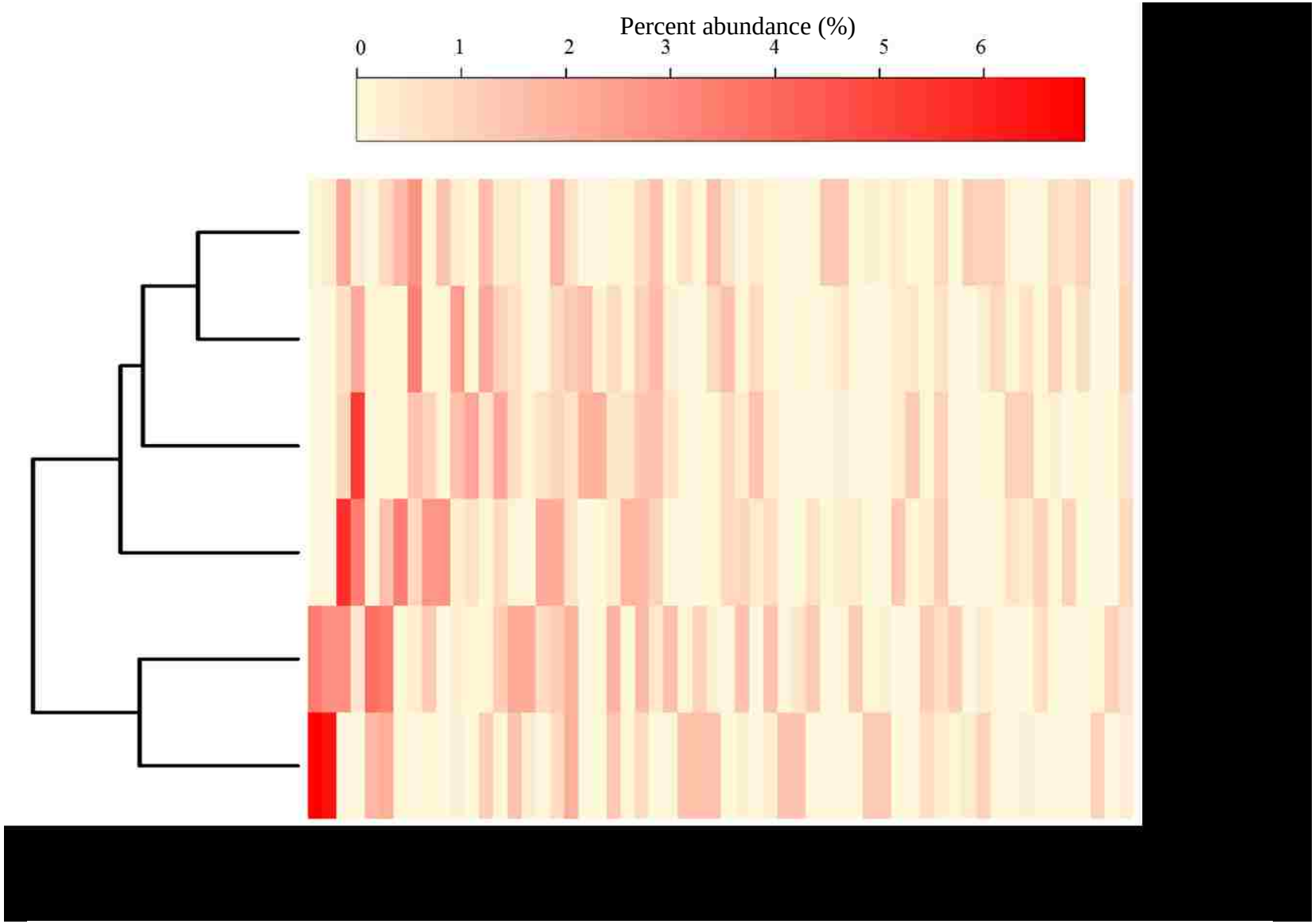

Figure 3.6. Heatmap depicting OTUs with abundances >1% in surface sediment (0-2 cm) and deeper sediment (2+ cm) samples. OTUs with a (*) in front are part of the 2008-2015 core microbiome of Walker Lake water column community. Identities of listed OTUs is given in Table C.5.

Percent relative abundance of microbial communities (Figure 3.7) at the phylum level (class level for Proteobacteria) from Illumina 16S rRNA gene libraries showed the water column communities of 2013-2015 were dominated by Actinobacteria (mean 28.92 ± 0.99% SEM) and Bacteroidetes (mean 19.74 ± 1.60% SEM). There were also high proportions of Betaproteobacteria (mean 11.90 ± 0.80% SEM), Cyanobacteria (mean 11.17 ± 1.66% SEM), and Tenericutes (mean 10.79 ± 1.42% SEM).The water column communities from 2013 through 2015 were more similar to each other than to the sediment microbial communities (weighted R_{ANOSIM}=1, p=0.001, 999 permutations).

In comparison to the pelagic microbial communities, the sediments exhibited higher levels of diversity (Table C.6). Sediments generally contained higher abundances of Deltaproteobacteria (mean 11.17 ± 0.75% SEM), Gammaproteobacteria (mean 12.88 ± 1.93% SEM), Firmicutes (mean 13.34 ± 2.06% SEM), Chloroflexi (mean 7.10 ± 1.79% SEM), Gemmatimonadetes (mean 3.09 ± 0.25% SEM), Planctomycetes (mean 3.02 ± 0.36% SEM), and Thermi (mean 2.92 ± 1.37% SEM), while exhibiting lower abundances of Betaproteobacteria (mean 2.74 ± 0.46% SEM), Actinobacteria (mean 1.80 ± 0.21% SEM), Cyanobacteria (mean 2.93 ± 0.73% SEM), and Tenericutes (mean 0.41 ± 0.07% SEM). Archaeal phyla, absent from the water column communities, were present only in the sediment samples in low abundances. There were also greater abundances of OTUs unclassified at the phylum level (mean 6.34 ± 0.35% SEM) and phyla that were less than 0.5% abundant.

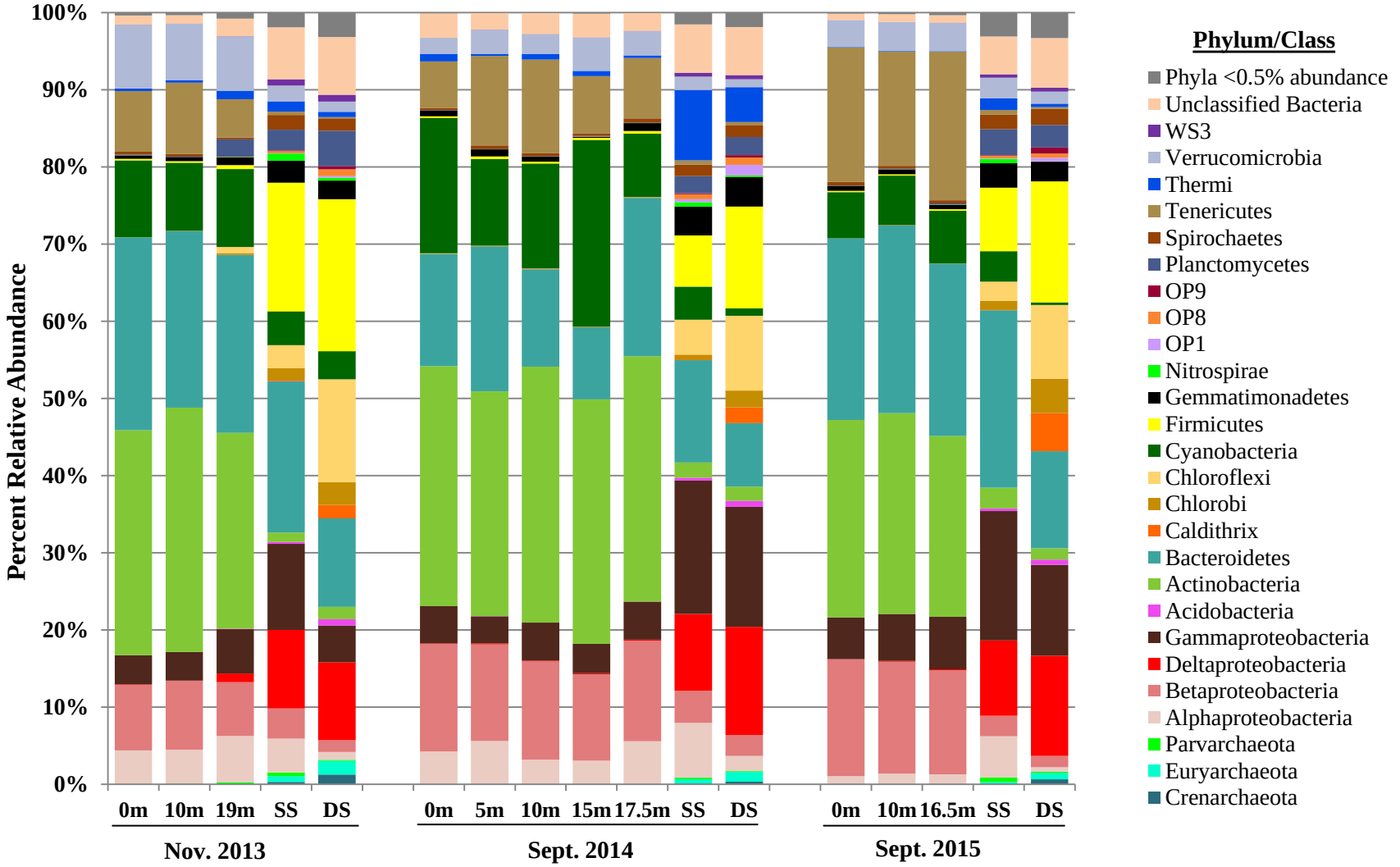

Figure 3.7. Percent relative abundance of microbial communities at the phylum level (class level for Proteobacteria) from Illumina 16S rRNA gene libraries. Presented distributions are from the water column, surface sediment (SS), and deeper sediment (DS). Phyla that contributed less than 0.5% to the overall community structure were grouped into a single category.

Samples from anoxic hypolimnion of 2008 showed greater similarity of associated microbial communities to the surface sediments of 2013 through 2015 than they did to the overlying pelagic communities of the epilimnion from the same year (R_{ANOSIM}= 1, p=0.003, 999 permutations) (Figure 3.8). The epilimnetic samples showed higher abundances of Betaproteobacteria (mean 6.52 ± 0.39% SEM), Actinobacteria (mean 20.07 ± 0.79% SEM), and Tenericutes (mean 17.83 ± 2.19% SEM) compared to the hypolimnion and sediments. The hypolimnion and sediments exhibited higher abundances of Gammaproteobacteria (mean 14.01 ± 1.65% SEM), Chloroflexi (mean 5.39 ± 1.73% SEM), Firmicutes (mean 11.51 ± 1.99% SEM), and Planctomycetes (mean 2.70 ± 0.35% SEM) than the epilimnion.

Sediment transect sequence data from 2015 shows that the microbial community structures of sediments taken at different points of the lake were quite similar to each other (R_{ANOSIM}=1, p=0.1, 720 permutations) (Figure 3.9). At the deepest point in the lake (17 m in 2015), the deeper sediment sample was most dissimilar from its overlaying surface sediment than the shallower transect sediment samples were to their surficial counterparts.

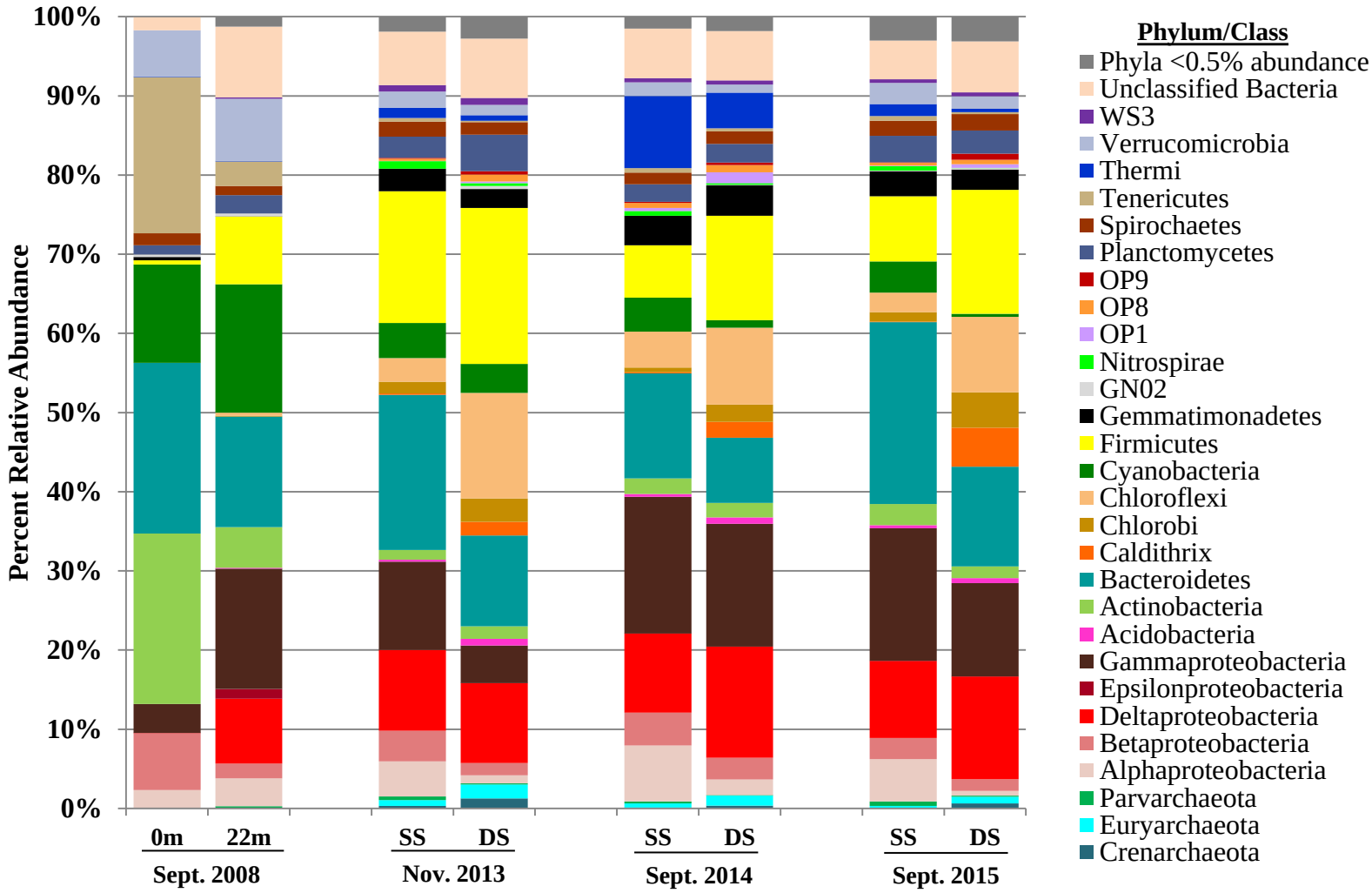

Figure 3.8. Comparison of microbial communities from anoxic sediments and hypolimnion at phylum level (class level for Proteobacteria) from Illumina 16S rRNA gene libraries. Surface (SS) and deeper sediment (DS) samples from 2013 and 2014 are compared to the epi- and hypo- limnetic waters observed in 2008 to show that the microbial community structure in anoxic regions (e.g. hypolimnion and sediments) are more similar to each other than oxygenated epilimnetic waters.

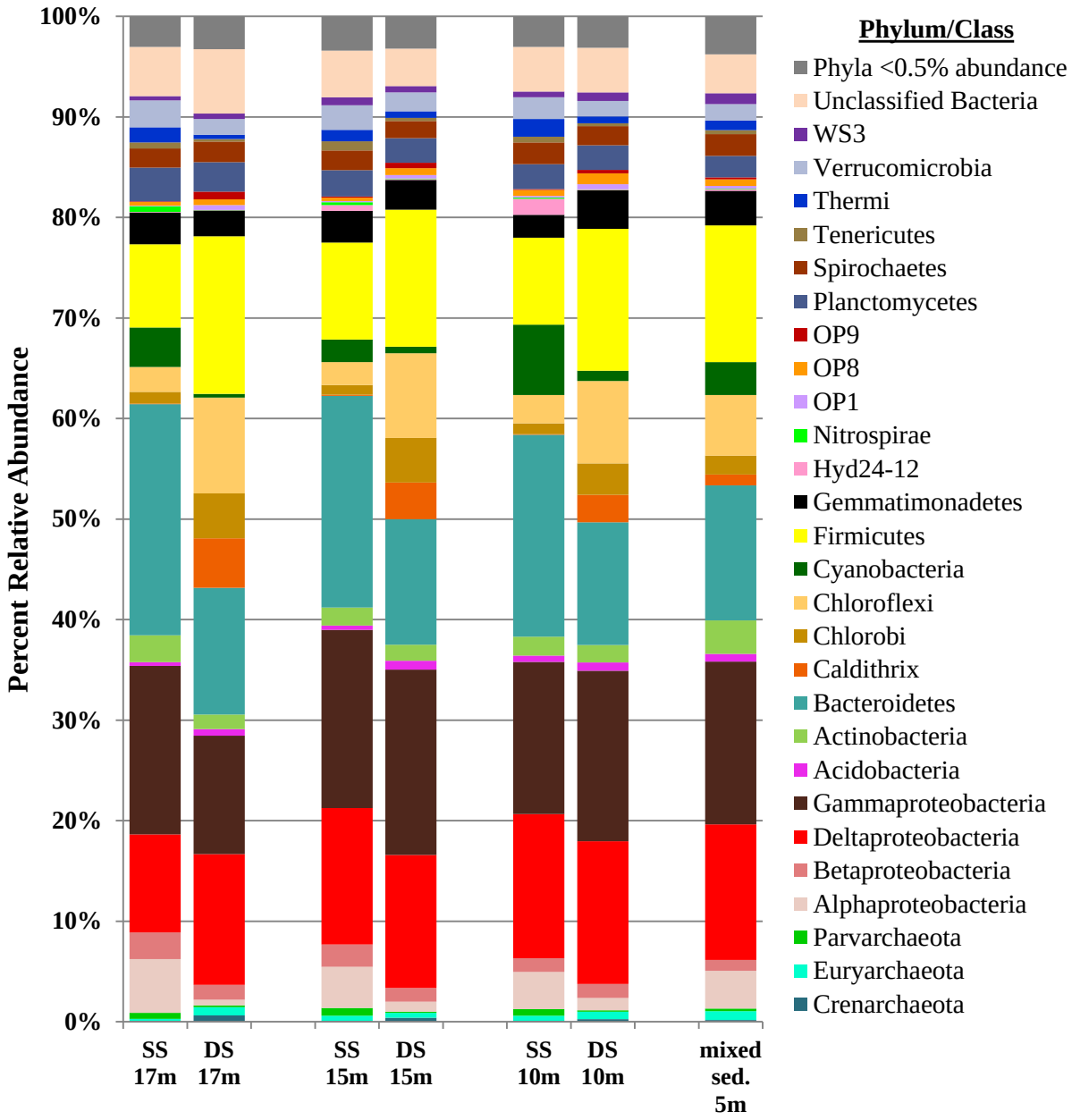

Figure 3.9. Microbial community structure of transect of sediments taken from central lake station to near-shore from Illumina 16S rRNA gene libraries. Presented distributions are from the surface sediment (SS) and deeper sediment (DS) taken from central lake location (17 m) and then at regular intervals going towards shore (15 m, 10 m, and 5 m water depth). Sediment at 5 meters was sandy and did not contain distinct formed layers. Phyla that contributed less than 0.5% to the overall community structure were grouped into a single category.

Mantel test of the microbial communities and the various chemical and physical parameters of the water column samples between 2013 and 2015 (Figure 3.10, Table C.7) showed TDS/conductivity correlated most strongly with microbial community composition (Mantel's $r > 0.50$, $p < 0.001$). Other significant drivers of observed community differences were dissolved oxygen, temperature, DOC, and magnesium (Mantel's $r > 0.50$, $p = 0.001 < x < 0.01$).

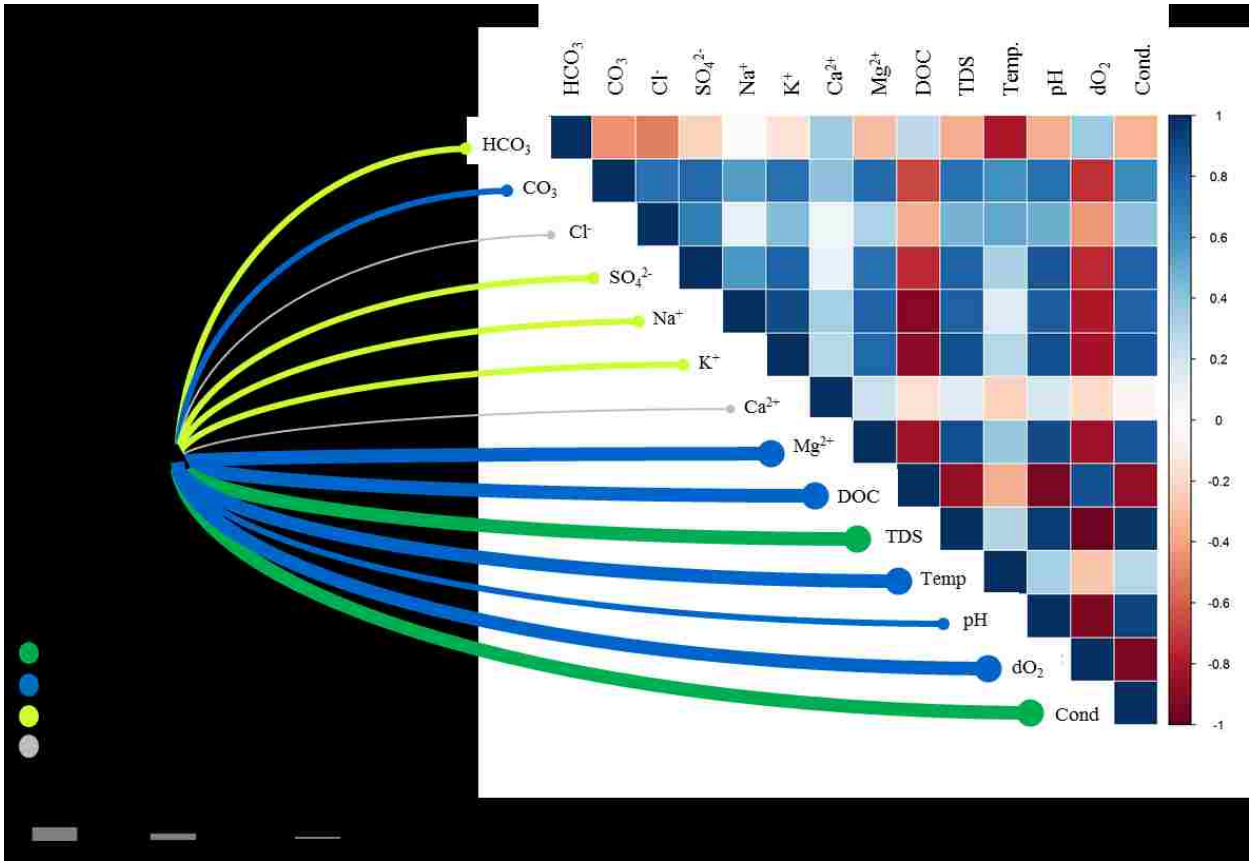

Figure 3.10. Pairwise comparison of environmental factors with color gradient denoting Spearman's correlation coefficients. Taxonomic composition (based on an OTU table rarefied to 10,000 sequences per sample) was related to each environmental factor using Mantel tests. Edge width corresponds to the Mantel's r statistic for each corresponding distance correlations. Edge color denotes statistical significance of the correlation, based on 9,999 permutations.

Discussion

Limnological Aspects. Walker Lake had a maximum depth of 22.9 m in 2008, a 45.7 m decline from the 68.6 m recorded in 1882 (Russell, 1885). At the end of this study, the maximum depth of Walker Lake had declined an additional 5.8 m to a depth of 17.1 m (~1190 m above sea level), half of the maximum depth (35 m) recorded 30 years ago (Domagalski, *et al.*, 1989). At the beginning of this study (2008), and for at least the preceding three decades, Walker Lake exhibited a monomictic mixing regime, with an anoxic hypolimnion and thermocline reliably appearing annually at ~17.5 m ± 2 m (Cooper & Koch, 1984, Beutel, *et al.*, 2001, Collopy & Thomas, 2009). Between 2013 and 2015, however, uniformly warm temperatures (22-23°C) were observed throughout the water column during September sampling events that were consistent with reported historic epilimnetic temperature maxima (Beutel, *et al.*, 2001, Collopy & Thomas, 2009). Additionally, measurements of dissolved oxygen, oxidation-reduction potential (ORP), pH, and conductivity (Figure 3.2) throughout the water column failed to deviate from surface values, indicating that the annual formation of an anoxic hypolimnion was no longer occurring. This was likely a reflection of declining lake depths being unable to stabilize sufficient hypolimnetic volume to preserve thermal stratification throughout the summer. Beutel, *et al.* (2001) predicted many of the changes that have already happened or are beginning to manifest in the data presented in this work:

> *"In the absence of any management strategies to stabilize elevation, water balance calculations project that in 20 years Walker Lake will be too saline to support Tui chub and stocked Lahontan cutthroat trout. In another 35 years the lake will reach hydrologic steady state with inflow equaling evaporative losses. At*

equilibrium the lake will have a volume of 1.0 km^3, one-third of its current volume (2.7 km^3), and one tenth of its volume prior to anthropogenic desiccation (11.1 km^3). The maximum depth of the lake will have dropped from 70 m in 1882 to 15 m, and its mixing regime will likely shift from monomictic to polymictic. The salinity of the lake will increase to 34 g L^{-1}, resulting in a decrease in species diversity of phytoplankton and zooplankton."

Several of these changes have already occurred within Walker Lake. Sometime during the course of this study (~2010), both the Tui chub and Lahontan cutthroat trout have died out as a result of the elevated salinity (Wright, 2009). Salinity, at 21 g L^{-1} at the end of our study in 2015, is on track to reach the 34 g L^{-1} predicted by Beutel, *et al.* (2001). While we have no evidence that the lake has reached a hydrologic steady state, the prediction of a shift from monomictic to polymictic is documented in the work presented here.

Since 1882, the reduced freshwater inflow from the Walker River has caused an accumulation of dissolved salt constituents in the lake, which are at their highest level in recorded history. Although lake level declines have been gradually occurring as a result of natural climatic shifts (Adams, 2007), the decline has been accelerated over the past ~130 years due to the introduction of anthropogenic influences on the drainage basin in the 19th century (Collopy & Thomas, 2009). The measured concentrations of total dissolved solids (TDS) and conductivity from 2008 (14.3 g L^{-1}) classified Walker Lake as a hyposaline lake (3-20 g L^{-1}; ~5,500-30,000 µS cm^{-1}), whereas in 2015, these concentrations had become sufficiently elevated (21.4 g L^{-1}) to support reclassification as mesosaline (20-50 g L^{-1}; 30,000-70,000 µS cm^{-1}; Hammer, 1986, Kalff, 2002). While the salinity has increased dramatically, the ionic

composition of the water has remained largely unchanged. The ionic balance in inland saline lakes is typically dominated by sodium chloride, however, sulfate and carbonate can also be dominant constituents as a result of weathering of regional rock types or influence from alkaline soil or irrigation runoff (Clarke, 1924). The overall composition of Walker Lake water (w/w) at the beginning of this study was similar to that determined by Cooper and Koch (1984) ($Na^+ > Cl^- \geq SO_4^{2-} > HCO_3^-/CO_3^{2-}$), with the exception that by 2015, sulfate had overtaken chloride as the second most abundant anion. The ionic composition of Walker Lake exhibits a multiple ion dominance shared by Na^+, SO_4^{2-}, and Cl^- (with lesser, but significant contribution by HCO_3^-/CO_3^{2-}), which is a common feature among saline lakes (Hammer, 1986). Aqueous systems with deficient calcium levels from the formation of calcium precipitates often lead to waters with multiple ion dominance containing relatively equal proportions of alkaline carbonates, sulfates, and chlorides (Clarke, 1924).

Over the course of this study, HCO_3^- concentrations varied, but ultimately increased by 8.7%, while CO_3^{2-} concentrations increased by 74.9%, suggesting the presence of a partial sink. There are a number of possible sinks for the inorganic carbon complex. For example, calcium carbonate tufa formations are known to occur in Walker Lake (Berelson, *et al.*, 2009). Marl deposits or dolomite ($CaMg(CO_3)_2$) are also a possibility and could also explain the consistency in the concentrations of Ca^{2+} and Mg^{2+}. DOC and TOC values nearly doubled between 2008 and 2013 (from ~40 mg L^{-1} to ~90 mg L^{-1}); however, both declined to values of ~65 mg L^{-1} in 2014 and ~60 mg L^{-1} in 2015. These extraordinarily high concentrations of total and dissolved organic carbon should give rise to organic acids, accumulating to give the lake a lower pH (Kernan, *et al.*, 2010). The high pH of 9.34, however, suggests a strong buffering capacity by the lake's carbonates and bicarbonates or possibly that the high pH contributes to the hydrolysis of algal

debris, yielding more D/TOC (Domagalski, *et al.*, 1989). The high DOC and TOC values from 2013 may also reflect a residual organic carbon spike from fish and possibly plankton populations that died off in years prior due to elevated salinity.

Walker Lake, typical of other soda lakes, is characterized by high concentrations of phosphorous and severe nitrogen limitation (NO_3^-/NO_2^- generally near the 0.01 mg-N L^{-1} detection limit). Soluble phosphorous remained high, but appeared to decline somewhat over the course of the study, from ~0.55 mg L^{-1} in 2008 to ~0.35 mg L^{-1} in 2015. As might be expected, the highest phosphorous value was obtained from the one hypolimnetic sample from the 22 m depth in 2008 (0.64 mg L^{-1}).The average molar ratio of inorganic nitrogen to phosphorous (N:P) in Walker Lake between 2013 and 2015 was 0.30:1. A balance of nutrients is typically indicated by a N:P ratio of 16:1 (Sigee, 2005), therefore indicating that Walker Lake is severely nitrogen limited. Conversely, the high hypolimnetic ammonium values from 2008 (0.11-0.47 mg L^{-1}) are consistent with the general paradigm for lake function from prior work (Beutel, *et al.*, 2001, Collopy & Thomas, 2009) and serve as evidence of internal loading as the primary controlling mechanism for delivery of bioavailable inorganic nitrogen to the food web under conditions prior to 2013. With the loss of the lake's hypolimnion, the corresponding loss of this substantial source-reservoir of bioavailable inorganic nitrogen probably represents the greatest measurable change in the lake. Thus, we hypothesize that with the loss of the hypolimnion due to declining water levels, the large pulse of internally-loaded ammonium that may have previously controlled productivity as recently as 2008 (Beutel, 2001, Humayoun, *et al.*, 2003) in the future may be replaced by nitrogen fixation by cyanobacteria (such as the *Nodularia* spp. blooms that occur in Pyramid Lake (Galat, *et al.*, 1990).

Although dissolved organic nitrogen was not measured for this project, the unexpectedly high abundance of OTUs that identify within the family Nitriliruptoraceae (617 OTUs) in the 16S rRNA gene Illumina dataset suggests that there may be a large population of putative nitrile-degrading microorganisms within Walker Lake. This physiology could help explain the ability of this ecosystem to function in spite of severe inorganic nitrogen limitation. Future work focusing on the amount of nitrogen bound as simple cyanides (e.g. CN^-, NaCN), organic nitrile compounds, or metal-cyanide complexes, and the potential of certain microorganisms to utilize nitrogen from these sources, may represent an important area of future research.

Cultivation-based assessment of functional diversity. Cultivation efforts from September 2008 samples showed generally higher abundances of microorganisms in the suboxic/anoxic samples in each of the different physiotypes. Aerobic heterotrophs were uniformly abundant in September 2008 cultivation, which may be explained by a large presence of facultatively anaerobic microorganisms in the water column. For example, many species within the Gammaproteobacteria (which were 15.21% abundant in the 22 m hypolimnion sample) are facultatively anaerobic. Aerobic heterotrophs were detected in higher abundances (up to three orders of magnitude, 10^4 vs 10^7 cells mL^{-1}) in 2013 and 2014, which may be due to increased heterotrophy of high organic carbon content.

The high numbers and patchy distribution of fermentative microorganisms, even in the mixed, oxygenated water column, could be explained in a number of ways, including, for example, an association of fecal material from the lake's abundant zooplankton. Microbial fermentation is an anaerobic process, but can be mediated by either obligate or facultative anaerobes. The very high numbers of fermentative microorganisms noted in 2013 (up to 10^9 cells mL^{-1}) may reflect an early stage in the diagenesis of decaying matter following collapse of the

fishery. Sulfate-reducing microorganisms are obligate anaerobes, so their relatively high numbers in sediment and low numbers in the oxygenated water column are consistent with known lake conditions. Their near complete absence in the water column in 2013 and 2014 is consistent with the lack of an anoxic hypolimnion.

Nitrate-reducing microorganisms are facultative anaerobes that utilize nitrate as an alternate respiratory electron acceptor in the absence of dissolved oxygen. This group can include denitrifying organisms or microorganisms that perform dissimilatory nitrate reduction to ammonia. In either case, the enumeration of such organisms is consistent with higher abundances from the anoxic hypolimnion from 2008 and the relative scarcity in the water column in subsequent years, when the lake was mixing. Likewise, the relative abundance of nitrate reducers in the sediment ($10^3 – 10^7$ cells mL^{-1}) is consistent with expectations that the sediment will contain a discrete nitrate reduction zone, especially under an oxygenated water column (MacGregor, *et al.*, 2001).

Iron and manganese reducers can be either facultative or obligate anaerobes. These groups were present mostly in the lake's anaerobic zones (19 m and 22 m water samples from 2008 and all sediment samples). The ecology of metal-reducing bacteria is a developing field and relatively little is known about the diversity and abundance of iron reducers in alkaline lakes (Ye, *et al.*, 2004, Zavarzina, *et al.*, 2006). The higher numbers of putative iron reducers in the 2014 water column (10^4 cells mL^{-1}) were only found in the ferric citrate enrichments and most likely reflected the presence of microorganisms able to ferment citrate rather than grow by dissimilatory iron reduction. Manganese reducers were rare in all of the water column samples, but were not tested in the anoxic hypolimnion from 2008; therefore, it is unknown if a water column manganese reduction-oxidation cycle may occur under stratified conditions, as has been

noted in other stratified lakes (Aguilar & Nealson, 1994, Aguilar & Nealson, 1998). Conversely, the data clearly show that manganese reducers were fairly abundant in the lake sediments (2013 – 2014). Thus, it seems likely that this group has a function in the Walker Lake system. Metal reducers (especially manganese reducers) can be abundant and ecologically important in alkaline lakes (Aguilar & Nealson, 1994) where they serve as an important means for the mineralization of organic carbon.

Molecular-based assessment of microbial diversity. While the water column and sediment samples of 2013 through 2015 possessed very different bacterial communities, the sediment samples were relatively similar between the three years. Overall, the most abundant phylum of microorganisms detected in all of the water column samples between 2008 and 2015 was the Actinobacteria (mean 24.30 ± 1.92% SEM). Conversely, Actinobacteria had an average abundance of 1.80 ± 0.21% SEM across all of the sediment samples. A large portion of the Actinobacteria in all of the water column samples belonged to a single OTU (OTU 71, a *Nitriliruptor* species), which was the second most abundant OTU of all water column microbial communities. Members of the Nitriliruptoraceae family are known as alkaliphilic, moderately salt-tolerant aerobic heterotrophs from soda lakes (Sorokin, *et al.*, 2009). They are capable of degrading organic nitrile compounds, which may be a key component to the severe nitrogen limitation within Walker Lake.

The next most prominent phylum of microorganisms in both the water and sediment samples was the Bacteroidetes. Patterns of abundance at the class level, however, were distinct between water and sediment samples, indicating that different Bacteroidetes were present in each of these distinct habitats. For example, Flavobacteria, with a cumulative average abundance of 10.97% across all four years were a dominant Bacteroidetes group in water column samples, but

represented only an average of 0.75% of bacteria in the sediments. Meanwhile, members of the Bacteroidetes class Bacteroidia were present in the water column at an average abundance of 1.18%, but represented an average of 8.93% of the sediment communities.

Members of the phylum Chloroflexi are common in aquatic habitats, yet this group represented an average of only 0.097% bacteria in water column samples between 2008 and 2015. The Chloroflexi were, however, relatively abundant in the sediment samples. In particular, the class Anaerolineae was associated with sediment, especially the deep sediments, where they comprised an average of 7.88% of all microorganisms detected. Members of the Anaerolineae are known to be strict anaerobes, found in a variety of environments, and are multicellular filamentous organisms (Yamada*, et al.*, 2006).

Although the presence of Cyanobacteria across water samples was variable (5.96 – 24.23% of all bacteria), this phylum represents one of the more abundant microbial physiotypes present. Even though Cyanobacteria were most abundant in the water column, they also represented about 4.21% of bacteria detected in the surficial sediment samples. In this study, members of the class Synechococcophycideae, specifically *Synechococcus* spp., represented by far the most abundant cyanobacterial lineages detected. The most abundant OTU in the entire 16S rRNA gene Illumina sequencing dataset, OTU 26698, identified as *Synechococcus*. This genus comprised an average of 10.48% of all of the water column samples between 2008 and 2015 and was highly abundant in the anoxic hypolimnion sampled in 2008 (mean 15.62%). *Synechococcus* spp. remains an integral part of the Walker Lake microbial community even under polymictic conditions. While the ecological role of *Synechococcus* remains to be determined in Walker Lake, members of this genus are among the most abundant and ubiquitous

members of photoautotrophic picoplankton in freshwater and marine environments (Waterbury, *et al.*, 1979, Scanlan & West, 2002).

Members of the class Clostridia were rare in oxygenated water column samples between 2008 and 2015 (mean 0.12% of all bacteria), but were abundant in the anoxic hypolimnion (mean 5.86%) and sediments (mean 13.26%). The most abundant of these was an unclassified genus within the family ML1228J-1, which is part of the Natranaerobiales order (mean 9.59% in sediment samples). Members of the Natranaerobiales are known to be strict haloakaliphilic anaerobes (Mesbah, *et al.*, 2007), characteristics consistent with Walker Lake conditions. ML1228J-1 is an uncharacterized family that originated from uncultured bacteria from the anoxic hypolimnion of Mono Lake, California (Humayoun, *et al.*, 2003, Glaring, *et al.*, 2015).

Two classes within the Proteobacteria phylum, Delta- and Gammaproteobacteria, were generally present in lower abundance in the oxygenated water column samples between 2008 and 2015 (respective means of Delta- and Gammaproteobacteria in all years: 0.27% and 5.84%) than in the anoxic hypolimnion (respective means 6.28% and 17.42%) and sediments (respective means 11.17% and 12.88%). The physiological diversity of Gammaproteobacteria is extremely high, including aerobic metabolisms, however, the group is also know for a wide variety of anaerobic metabolisms, including metal and sulfur reduction. Deltaproteobacteria are less physiologically diverse and best known for anaerobic metal and especially sulfate reduction. Strong representation from Delta- and Gammaproteobacteria was observed in the sediments, in particular from *Desulfosarcina*, *Desulfonatronovibrio*, *Thioalkalivibrio,* and an unclassified genus within the Piscirickettsiaceae family. *Desulfonatronovibrio* are anaerobic, extremely alkaliphilic microorganisms that reduce sulfate or other oxidized sulfur compounds to sulfide and are dependent on sodium and carbonates for growth (Garrity, *et al.*, 2005). *Desulfosarcina* are

also anaerobic organisms that reduce sulfur compounds to sulfide, but can also utilize fermentative metabolisms and are not extremely alkaliphilic like *Desulfonatronovibrio* (Garrity, *et al.*, 2005). Members within the genus *Thioalkalivibrio* are obligate chemolithoautotrophs that oxidize sulfide, thiosulfate, sulfur, and tetrathionate (Brenner, *et al.*, 2005) and are commonly found in alkaline lake water and sediments. The family Piscirickettsiaceae consists of aerobic members isolated from marine/brackish environments (Brenner, *et al.*, 2005).

Members of the phylum Tenericutes were abundant in all water column samples (mean 11.66%), but were relatively rare in sediment (mean 0.41%). Almost all of the Tenericutes of the water column samples belonged to an unclassified order within the class Mollicutes. Since this taxon is unclassified, not much is known concerning its role in the Walker Lake environment. Key characteristics of the Mollicutes, however, include small cell sizes that lack cell walls and are bounded only by a plasma membrane due to the inability to synthesize peptidoglycan. Most Mollicutes species are facultative anaerobes, but some are obligate anaerobes and all known examples are commensals or parasites (Krieg, *et al.*, 2010).

In general, sediment samples contained a higher proportion of classes that contributed less than 0.5% to the total percent abundance for a given microbial community. This helps confirm that the sediment contains a much more diverse set of microorganisms that persist in lower abundances than what is found in the water column. In this manner, the sediment may serve as a repository of microbial diversity, enabling the establishment of individual lineages suited to specific conditions under changing environmental conditions.

Plankton tows were completed and analyzed in 2014 and 2015 and compared against the water column samples of the same years to better constrain the effect that field pre-filtration (178

micron) may have had on assessments of planktonic community structure. Since DNA extracted from plankton tows contains the associated gut microbiota of zooplankton as well as larger planktonic bacteria, broad comparisons were difficult to discern. *Nodularia* species are shown to be the dominant phytoplankton in Walker Lake (Cooper & Koch, 1984, Beutel, *et al.*, 2001), but were nearly absent in the sequence dataset from the 2008-2015 water samples that passed through the 178 µm pre-filter. Their presence in the 2014 and 2015 plankton tow samples (0.32-1.51%) showed that this cyanobacterial genus was being excluded from our dataset, but that they were not dominant phytoplankton in the Walker Lake system at the time of sampling. Large microorganisms or those associated with large phyto- and zooplankton are likely important in Walker Lake, however, the extremely patchy distribution of microorganisms among the plankton tows and the inability to discern gut and pelagic bacteria indicates quite clearly that these larger organisms should be considered separately.

Changes in lake ecology. The variation in the microbial community structure between the water column samples and the sediment samples seems to be determined by the presence of dissolved oxygen. Dissolved oxygen is a key driving factor in aquatic biodiversity, especially evident in anoxic hypolimnetic regions or aquatic sediments where the microbial communities largely consist of anaerobic or facultatively anaerobic physiologies. Dissolved oxygen within sediment decreases quickly with depth (Sigee, 2005), as such the surface and deeper sediment layers within Walker Lake contained different microbial communities. The PCA ordination and weighted UniFrac UPGMA tree of all water column and sediment samples showed clear separation in the clustering of samples (with the exception of September 2014, Figure 3.3) by location in the lake (i.e. oxygenated water, hypolimnetic water, sediment) and subsequently by the concentration of dissolved oxygen in each distinct zone. The microbial communities of the

anoxic water samples from 2008 (19 m and 22 m) showed greater similarities to sediment communities than to all other water column samples between 2008 and 2015 (Figure 3.3b).

Relation of the taxonomic composition to environmental parameters between 2013 and 2015 by Mantel test (Figure 3.10) revealed that microbial communities correlated strongest with conductivity/TDS concentrations, which reflects the influence of the increasing solute load from ongoing lake desiccation on the biological system. As Walker Lake continues to desiccate and the salinity increases to historic highs, biodiversity and functioning physiologies may decline as species that are unable to tolerate the higher salinities will no longer be part of the microbial community (Sigee, 2005), in turn affecting the biogeochemical cycles in the lake. Many zooplankton species have become extinct from the Walker Lake ecosystem (Beutel, *et al.*, 2001) and endemic fish populations have been completed eliminated as a response to anthropogenic desiccation.

With the lack of evidence throughout this study supporting the occurrence of lake stratification since at least 2013, it seems likely that Walker Lake has become too shallow to hold summer thermal stratification. This shift from a monomictic to a polymictic system eliminates the development of anoxic conditions in a present hypolimnion, augmenting the microorganisms that persist in the water column. This study documented the shift of an ecosystem and the changes that result on the microbial level. While recent microbial studies on Walker Lake from this research group were conducted while the lake still exhibited thermal stratification and subsequent anoxia, full bacterial community examinations have not been done prior to the elevation of salinity to concentrations that are toxic to fish and that alter the diversity of sensitive microbial populations. However, future studies can further examine the effects of increasing salinity on the Walker Lake ecosystem.

The lack of freshwater inflow into the lake system has dramatically altered the functionality of the lake. The return of consistent inflow to the lake from the Walker River through the purchase of water rights within the Walker River Basin and other management options would alleviate some of the pressures placed upon micro- and macrobiota. However, the Great Basin continues to experience less annual precipitation, leading to the higher demand of available water resources by humans and nature alike, and causing further demise and the ultimate loss of the Walker Lake ecosystem.

Acknowledgements

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CHAPTER FOUR

DISCUSSION OF THESIS

Humans often attempt to illustrate the importance of an environment or ecosystem by trying to place a value on it, measurable by the resources gained from the ecosystem that are beneficial to humans (Kernan, *et al.*, 2010). Over the course of modern human history, the revolution of new techniques to use and divert water to meet exponentially growing human demand has left very few aqueous ecosystems unaltered by the influences of industry and agriculture (Kalff, 2002). Anthropogenic activities impact the environment in increasingly detrimental ways, from the use of toxic fertilizers and pesticides that harm the natural flora and fauna to the deforestation and water diversions that remove and eliminate them. These types of problems humans have placed upon Earth's environments are self-inflicted and have caused irreparable damage that leaves humans searching for management options and restoration efforts to preserve the precious resources we have left.

The Walker River Basin has endured over a century of stress in the form of land use changes and stream flow diversions, compounded with the impacts of climate change on the region. These types of pressures exerted on aquatic environments create water level imbalances within the ecosystem and can ultimately result in the complete loss of water bodies (Kernan, *et al.*, 2010). The concentration of dissolved constituents over time within terminal lakes results in many of these systems having difficulties supporting freshwater aquatic life. The extinction of several zooplankton species (*Ceriodaphnia quadrangula* and *Acanthocyclops vernalis* (Beutel, *et al*., 2001)) and the loss of all endemic fish species within the Walker Lake system has caught the

attention of ecological management groups, such as the U.S. Fish and Wildlife Service, to seek out ways to preserve and restore this ecosystem. In order to effectively restore aquatic ecosystems, attention needs to be turned to the reference conditions of the system, such as how the current conditions differ from the natural conditions (e.g., Walker River Basin before human disturbance and activity), identifying what factors have changed, and the driving forces behind these observed changes (Kernan, *et al.*, 2010).

The average annual temperature within Nevada climate divisions 1 and 3 (the Walker River Basin falls on the boundary of these divisions) both have an increasing trend of 0.2˚F per decade between 1895 and 2015 (http://www.ncdc.noaa.gov/cag/), however, the average annual precipitation trend between 1895 and 2015 in these divisions is either increasing slightly (0.04" per decade in climate division 1) or not at all (climate division 3).While in years of normal or above average rainfall the amount of water that reaches Walker Lake is sufficient to maintain salinity levels and lake surface elevations, years that bring less precipitation result in less water that reaches the lake. The increasing temperature trend in climate divisions 1 and 3 of Nevada does not seem to have a bearing on the trends in precipitation, however the water usage and water demand needed to maintain agricultural yields continue to increase with increasing annual temperatures.

While water bodies within terminal drainage basins like the Walker River Basin are often susceptible to damaging increases in salinity due to the nature of the collection of aqueous dissolved salt constituents (Hammer, 1986), maintaining a lower concentration of salinity can provide a stable environment that is not harmful to plant, animal, and certain microbial life. This practice of salinity control has been attempted at Mono Lake, California. TDS concentrations within Mono Lake reached the highest recorded value of 97 g L^{-1} by 1982 due to anthropogenic

diversions of streamflow to the city of Los Angeles (Mono Lake Committee, 2016). Prior to streamflow diversions, Mono Lake's salinity was 48 g L^{-1}. Several legal battles since then have resulted in the ruling that the State of California must take action to preserve and protect the declining ecosystem of Mono Lake by reallocating water rights to maintain lake inflow to a maximum salinity of 85 g L^{-1} (MBES Committee, 1987, Mono Lake Committee, 2016). Even though this set maximum salinity is too high to allow for the survival of fish species, it has allowed populations of brine shrimp and brine flies to thrive, which in turn provides an ample and vital food source for migratory birds (Cooper, *et al.*, 1984, MBES Committee, 1987).

The water diverted from the Walker River is used in urban and agricultural systems within the Walker River Basin. The water allocations that dictate how much streamflow must reach certain points of the Walker River for different regions have left very little water to flow into Walker Lake, the river's terminus. In order to stabilize lake level conditions and to protect salinity-sensitive biota, the amount of water entering the system needs to be equal to that which is lost to evaporation (Beutel, 2001). While efforts are being made to purchase water rights along the Walker River to allow more water to flow into Walker Lake, greater efforts will need to be made to restore the fish populations into the system. A study conducted on the Pyramid Lake strain of Lahontan cutthroat trout observed mortality between TDS concentrations of 12,000 mg L^{-1} and 13,800 mg L^{-1} (Taylor, 1972). While Lahontan cutthroat trout have been shown to tolerate tightly controlled acclimation to Walker Lake water (~17,000 mg L^{-1}), long term survival was not observed (Bigelow, *et al.*, 2010).

The research described in this thesis provides a benchmark of limnological and microbial community characteristics from a period when Walker Lake was still able to host a thriving fishery and exhibited annual thermal stratification. We documented the shift from a monomictic

to a polymictic system as a result of anthropogenic influences and showed that the increasing concentrations of salinity are directly correlated to the taxonomic relationships of the microbial communities. The lack of freshwater inflow and the subsequent loss of an anoxic hypolimnion during summer stratification alters the microbial populations and the associated biogeochemical cycles that occur within the lake. The return of consistent streamflow to the lake from the Walker River through the purchase of water rights within the Walker River Basin and other management options would alleviate some of the pressures placed upon micro- and macrobiota. However, the U.S. Great Basin continues to experience a lower level of annual precipitation, while the demand of available water resources by both humans and nature increases, causing further demise and the ultimate loss of the Walker Lake ecosystem.

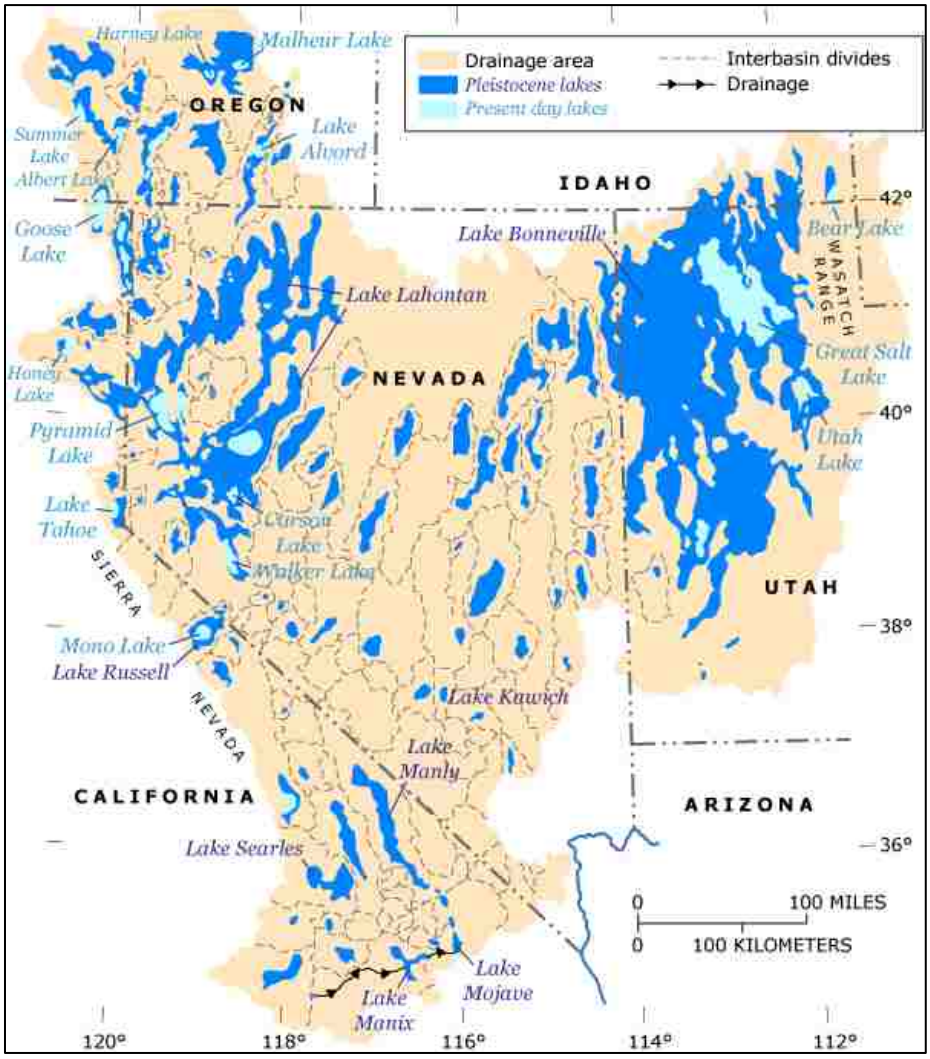

Figure A.1. Present-day and Pleistocene lakes of the U.S. Great Basin drainage area. Darker blue areas are late Pleistocene pluvial lakes, lighter blue areas are present day lakes. Image source: http://serc.carleton.edu/details/images/51472.html

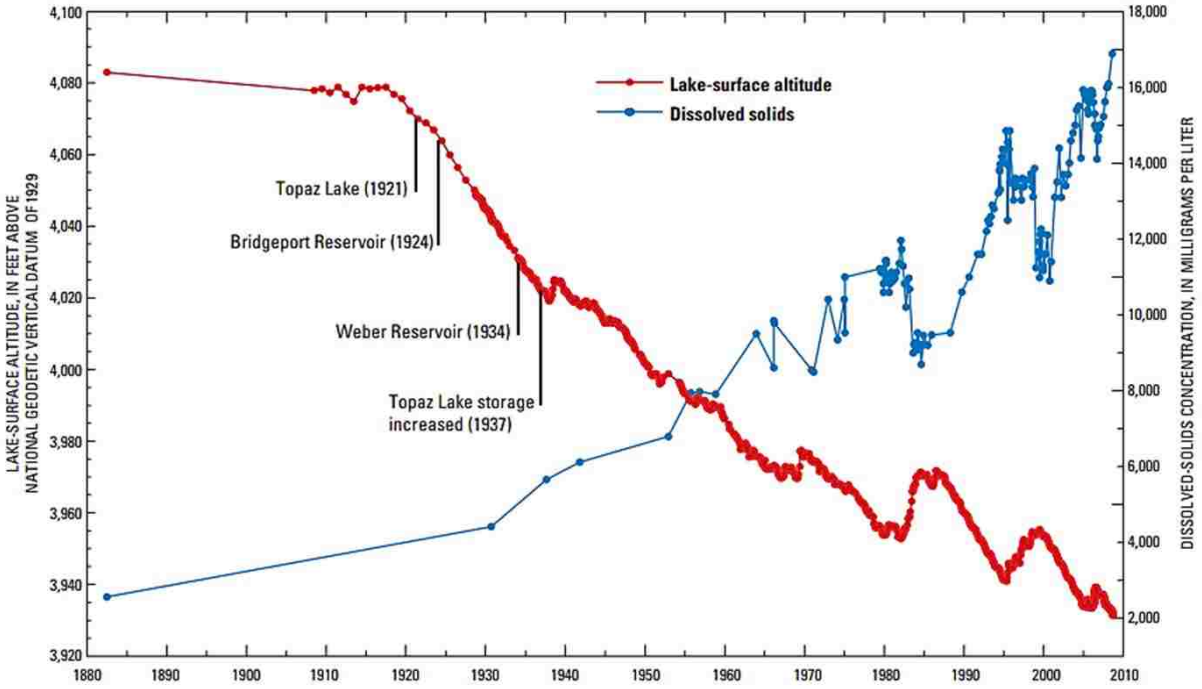

Figure A.2. Inverse relationship between Walker Lake surface altitude (red) and total dissolved solids concentrations (blue). Image source: Lopes and Allander, 2009.

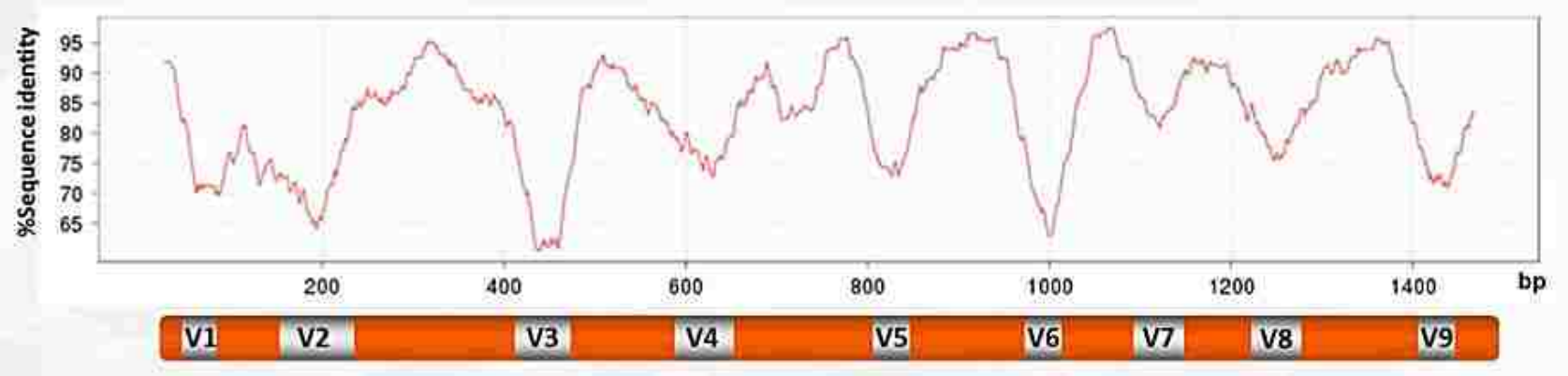

Figure A.3. Schematic overview of the prokaryotic 16S rRNA gene. The nine hypervariable regions (V1-V9) are denoted in gray, with conserved regions in orange. Image source:(Wahl, *et al.*, 2014Wahl, *et al.*, 2014)

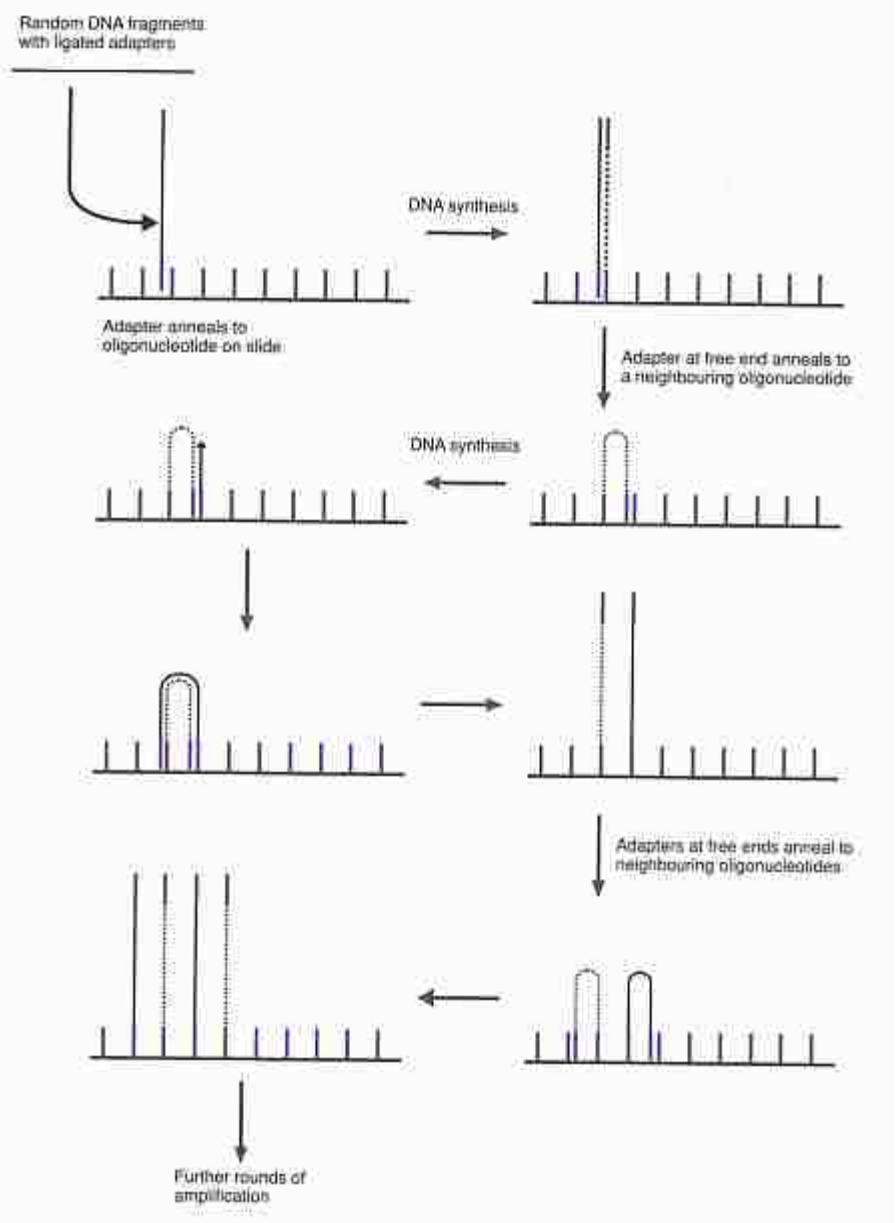

Figure A.4. Schematic overview of Illumina bridge amplification. Image source: (Dale, *et al.*, 2012).

APPENDIX B: SUPPLEMENTAL MATERIAL FOR CHAPTER TWO

Table B.1. Physical lake parameters from sonde casts from top, middle, and bottom waters of mixing dates (May and October) and all water samples from stratified waters (September). dO_2= dissolved oxygen; TDS=total dissolved solids; ORP=oxidation-reduction potential; PAR=photosynthetically active radiation.

Date	Depth (m)	Temp. °C	pH	dO_2 mg L^{-1}	TDS g L^{-1}	ORP (mV)	PAR µmol m^{-2} s^{-1}	Conductivity µS cm^{-1}
May 2007	**0**	n.d.	n.d.	n.d.	15.1	n.d.	n.d.	19670
	10	n.d.	9.37	n.d.	15.0	n.d.	n.d.	19670
	20	n.d.	9.38	n.d.	15.0	n.d.	n.d.	19600
Oct. 2007	**0**	17.2	9.16	6.46	13.64	96.5	895.2	20990
	10	17.1	9.27	6.39	13.7	86.5	63.2	21010
	22.5	15.63	9.46	4.69	13.29	74.8	7.1	20450
	24	13.31	9.46	1.0	12.52	44.8	5.2	19470
Sept. 2008	**0**	22.9	9.41	6.62	14.45	62.9	1507	22230
	10	21.3	9.49	6.48	14.41	59.1	97.9	22170
	17.5	20.85	9.57	5.49	14.38	52.9	21.7	22120
	18	20.61	9.57	4.66	14.3	52.9	20.6	22000
	19	17.02	9.61	0.89	14.06	48.8	14.6	21630
	22	14.74	9.64	0.1	13.75	-272.5	3.6	21600

n.d.= not determined

Table B.2. Carbon, phosphorous, and nitrogen compound measurements from all collected water samples. T/DOC=total/dissolved organic carbon, T/DP=total/dissolved phosphorous. Shaded samples were previously shown in Table 2.1.

Date	Depth (m)	TOC mg L^{-1}	DOC mg L^{-1}	TP mg L^{-1}	DP mg L^{-1}	$O\text{-}PO_4$ as P; mg L^{-1}	NH_3 as N; mg L^{-1}	NO_2^- as N; mg L^{-1}	NO_3^- as N; mg L^{-1}
May 2007	0	n.d.	40.2	0.84	0.84	0.69	0.01	<0.01	<0.01
	2.5	n.d.	40.3	0.84	0.84	0.72	<0.01	<0.01	<0.01
	5	n.d.	40.3	0.84	0.84	0.70	<0.01	<0.01	<0.01
	7.5	n.d.	40.3	0.84	0.84	0.70	<0.01	<0.01	<0.01
	10	n.d.	40.4	0.83	0.83	0.70	<0.01	<0.01	<0.01
	12.5	n.d.	40.4	0.84	0.84	0.71	<0.01	<0.01	<0.01
	15	n.d.	40.4	0.84	0.84	0.71	<0.01	<0.01	<0.01
	20	n.d.	40.5	0.86	0.86	0.74	0.06	<0.01	0.02
Oct. 2007	0	n.d.	43	0.64	0.62	0.56	0.04	<0.01	<0.01
	10	n.d.	42.3	0.63	0.63	0.68	0.05	<0.01	0.02
	15	n.d.	42.6	0.64	0.63	0.57	0.05	<0.01	0.03
	17.5	n.d.	42.2	0.63	0.63	0.57	0.05	<0.01	<0.01
	20	n.d.	42.6	0.64	0.63	0.57	0.05	<0.01	<0.01
	22.5	n.d.	42.7	0.66	0.62	0.58	0.08	<0.01	<0.01
Sept. 2008	0	43.2	42.4	0.55	0.55	0.48	0.02	0.01	<0.01
	10	42.2	40.9	0.57	0.57	0.50	0.02	<0.01	<0.01
	17.5	42.7	42.1	0.58	0.58	0.50	0.11	0.01	<0.01
	18	42.3	41.9	0.59	0.60	0.51	0.20	<0.01	<0.01
	19	41.4	41.5	0.61	0.61	0.53	0.24	<0.01	<0.01
	22	38.1	40.6	0.66	0.64	0.57	0.47	0.02	0.02

Table B.3. Alpha diversity indices for Walker Lake water column samples from Illumina sequence dataset between 2007 and 2008.

Date	Depth (m)	Observed OTUs	Chao1	Faith Index	Shannon Index	Simpson Index
May 2007	0	478.55 ± 10.97	940.78 ± 91.32	39.06 ± 1.13	6.01 ± 0.02	0.9499 ± 0.0011
	2.5	472.52 ± 10.80	913.60 ± 74.68	38.83 ± 1.15	6.00 ± 0.02	0.9511 ± 0.0010
	5	480.80 ± 12.18	987.20 ± 80.41	39.57 ± 1.30	6.04 ± 0.02	0.9565 ± 0.0009
	7.5	495.39 ± 13.32	1016.10 ± 96.33	41.48 ± 1.26	6.06 ± 0.03	0.9543 ± 0.0011
	10	489.57 ± 9.98	1066.62 ± 96.56	43.23 ± 1.07	6.18 ± 0.02	0.9678 ± 0.0006
	12.5	566.81 ± 12.82	1224.19 ± 103.84	49.71 ± 1.41	6.24 ± 0.02	0.9660 ± 0.0006
	15	501.65 ± 10.66	946.80 ± 73.09	42.25 ± 0.98	5.91 ± 0.03	0.9495 ± 0.0011
	20	623.31 ± 10.92	1277.39 ± 96.32	56.17 ± 1.10	6.34 ± 0.02	0.9665 ± 0.0005
Oct. 2007	0	475.70 ± 11.59	942.35 ± 96.66	44.73 ± 1.04	6.17 ± 0.02	0.9691 ± 0.0005
	10	493.25 ± 12.42	990.25 ± 92.34	48.37 ± 1.30	6.24 ± 0.02	0.9716 ± 0.0004
	15	502.29 ± 10.57	1076.65 ± 99.52	48.89 ± 1.16	6.10 ± 0.02	0.9661 ± 0.0005
	17.5	458.01 ± 11.29	912.33 ± 84.90	47.62 ± 1.36	5.96 ± 0.02	0.9623 ± 0.0006
	20	527.09 ± 12.65	1056.69 ± 101.37	52.04 ± 1.38	6.22 ± 0.02	0.9690 ± 0.0006
	22.5	565.09 ± 13.72	1116.64 ± 97.39	57.42 ± 1.42	6.20 ± 0.02	0.9675 ± 0.0006
	24	615.72 ± 14.11	1293.11 ± 114.45	63.34 ± 1.45	5.97 ± 0.03	0.9522 ± 0.0009
Sept. 2008	0	477.03 ± 13.07	944.44 ± 81.52	42.79 ± 1.16	5.88 ± 0.02	0.9585 ± 0.0006
	10	400.38 ± 11.92	738.49 ± 79.36	38.24 ± 1.04	5.74 ± 0.02	0.9550 ± 0.0008
	17.5	595.57 ± 13.24	1192.24 ± 96.81	57.18 ± 1.42	6.37 ± 0.02	0.9707 ± 0.0005
	18	633.91 ± 14.18	1278.89 ± 100.19	59.86 ± 1.37	6.26 ± 0.02	0.9659 ± 0.0005
	19	687.37 ± 12.87	1200.82 ± 74.44	68.41 ± 1.38	6.41 ± 0.03	0.9646 ± 0.0007
	22	792.75 ± 14.28	1307.90 ± 76.00	78.88 ± 1.51	6.80 ± 0.03	0.9674 ± 0.0007

Table B.4. Ordered list of OTUs from heatmap (Figure 2.7) depicting OTUs present in water column samples between May 2007 and September 2008 that have ≥1% relative abundance in any one sample. A (*) denotes that the OTU accounts for ≥1% of all sequences in the entire Illumina dataset.

OTU#	TAXONOMY
* 7356	Bacteria;Cyanobacteria
* 8685	Bacteria;Tenericutes;Mollicutes
* 8442	Bacteria;Proteobacteria;Epsilonproteobacteria;Campylobacterales;Helicobacteraceae;Sulfurovum
* 7214	Bacteria;Proteobacteria;Gammaproteobacteria;Thiotrichales;Piscirickettsiaceae;Thiomicrospira
* 7360	Bacteria;Cyanobacteria;empty_class;Chroococcales;empty_family;Synechococcus
* 8852	Bacteria;Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae;empty_genus;Flavobacteriaceae bacterium KHS1
* 7810	Bacteria;Actinobacteria;Nitriliruptoria;Nitriliruptorales;Nitriliruptoraceae;Nitriliruptor
* 7840	Bacteria;Bacteroidetes;Flavobacteriia;Flavobacteriales;Cryomorphaceae;Owenweeksia
3945	Bacteria;Proteobacteria;Gammaproteobacteria;Chromatiales;Chromatiaceae;Thiocapsa
* 8780	Bacteria;Proteobacteria;Gammaproteobacteria;Chromatiales;Ectothiorhodospiraceae;Thioalkalispira
* 5508	Bacteria;Bacteroidetes;Flavobacteriia;Flavobacteriales
4393	Bacteria;Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae
* 8592	Bacteria;Bacteroidetes;Flavobacteriia;Flavobacteriales;Cryomorphaceae;Wandonia
* 8939	Bacteria;Actinobacteria;Actinobacteria;Micrococcales;Microbacteriaceae
* 8561	Bacteria;Tenericutes;Mollicutes
7852	Bacteria;Bacteroidetes;Sphingobacteriia;Sphingobacteriales
4682	Bacteria;Proteobacteria;Deltaproteobacteria;Desulfuromonadales;Desulfuromonadaceae;Desulfuromusa
4671	Bacteria;Proteobacteria;Alphaproteobacteria;Rickettsiales
7327	Bacteria
* 6125	Bacteria;Actinobacteria;Actinobacteria;Micrococcales;Microbacteriaceae
8155	Bacteria;Bacteroidetes;Sphingobacteriia;Sphingobacteriales

Table B.4 (continued). Ordered list of OTUs from heatmap (Figure 2.7) depicting OTUs present in water column samples between May 2007 and September 2008 that have ≥1% relative abundance in any one sample. A (*) denotes that the OTU accounts for ≥1% of all sequences in the entire Illumina dataset.

* 4618	Bacteria;Actinobacteria;Actinobacteria;Frankiales;Sporichthyaceae
7505	Bacteria
* 5457	Bacteria;Proteobacteria;Betaproteobacteria;Burkholderiales;Comamonadaceae;Hydrogenophaga
8131	Bacteria;Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae
* 7387	Bacteria;Bacteroidetes;Cytophagia
4323	Bacteria;Proteobacteria;Alphaproteobacteria;Rickettsiales
8508	Bacteria;Bacteroidetes;Sphingobacteriia;Sphingobacteriales;Saprospiraceae
5257	Bacteria;Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae
* 8162	Bacteria;Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales
7130	Bacteria;Proteobacteria;Betaproteobacteria;Methylophilales;Methylophilaceae
* 1345	Bacteria;Bacteroidetes;Cytophagia
5701	Bacteria;Bacteroidetes;Cytophagia;Cytophagales;Cyclobacteriaceae;Cecembia;lonarensis
* 7101	Bacteria;Proteobacteria;Betaproteobacteria;Burkholderiales;Alcaligenaceae
3679	Archaea;Euryarchaeota;Halobacteria;Halobacteriales
6883	Bacteria;Tenericutes;Mollicutes
7984	Bacteria;Bacteroidetes;Flavobacteriia;Flavobacteriales;Flavobacteriaceae
7707	Bacteria;Actinobacteria;Acidimicrobiia;Acidimicrobiales
6456	Bacteria;Verrucomicrobia;Spartobacteria;empty_order;empty_family;Chthoniobacter
5427	Bacteria;Proteobacteria;Betaproteobacteria;Burkholderiales;Comamonadaceae;Hydrogenophaga
8510	Bacteria;Bacteroidetes;Cytophagia;Cytophagales;Cyclobacteriaceae
8381	Bacteria;Spirochaetae;Spirochaetes;Spirochaetales;Spirochaetaceae;Spirochaeta

Table B.4 (continued). Ordered list of OTUs from heatmap (Figure 2.7) depicting OTUs present in water column samples between May 2007 and September 2008 that have ≥1% relative abundance in any one sample. A (*) denotes that the OTU accounts for ≥1% of all sequences in the entire Illumina dataset.

6169	Bacteria;Lentisphaerae
5988	Bacteria;Proteobacteria;Deltaproteobacteria;Desulfobacterales;Desulfobulbaceae;Desulfurivibrio
7722	Bacteria;Bacteroidetes;Sphingobacteriia;Sphingobacteriales
8049	Bacteria;Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Rhodopirellula
7250	Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;Pseudospirillum
4866	Bacteria;Gemmatimonadetes;Gemmatimonadetes
7045	Bacteria;Verrucomicrobia;Opitutae
4082	Bacteria;Cyanobacteria;empty_class;Chroococcales;empty_family;Synechococcus;TAGS
8007	Bacteria;Bacteroidetes;Cytophagia;Cytophagales;Cyclobacteriaceae;Belliella
6604	Bacteria;Proteobacteria;Alphaproteobacteria;Rickettsiales;Anaplasmataceae
7021	Bacteria;Tenericutes;Mollicutes;Acholeplasmatales;Acholeplasmataceae;Acholeplasma
8908	Bacteria;Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Rhodopirellula
8313	Bacteria;Actinobacteria;Actinobacteria;Micrococcales;Microbacteriaceae
4725	Bacteria;Proteobacteria;Gammaproteobacteria;Alteromonadales;Idiomarinaceae;Aliidiomarina

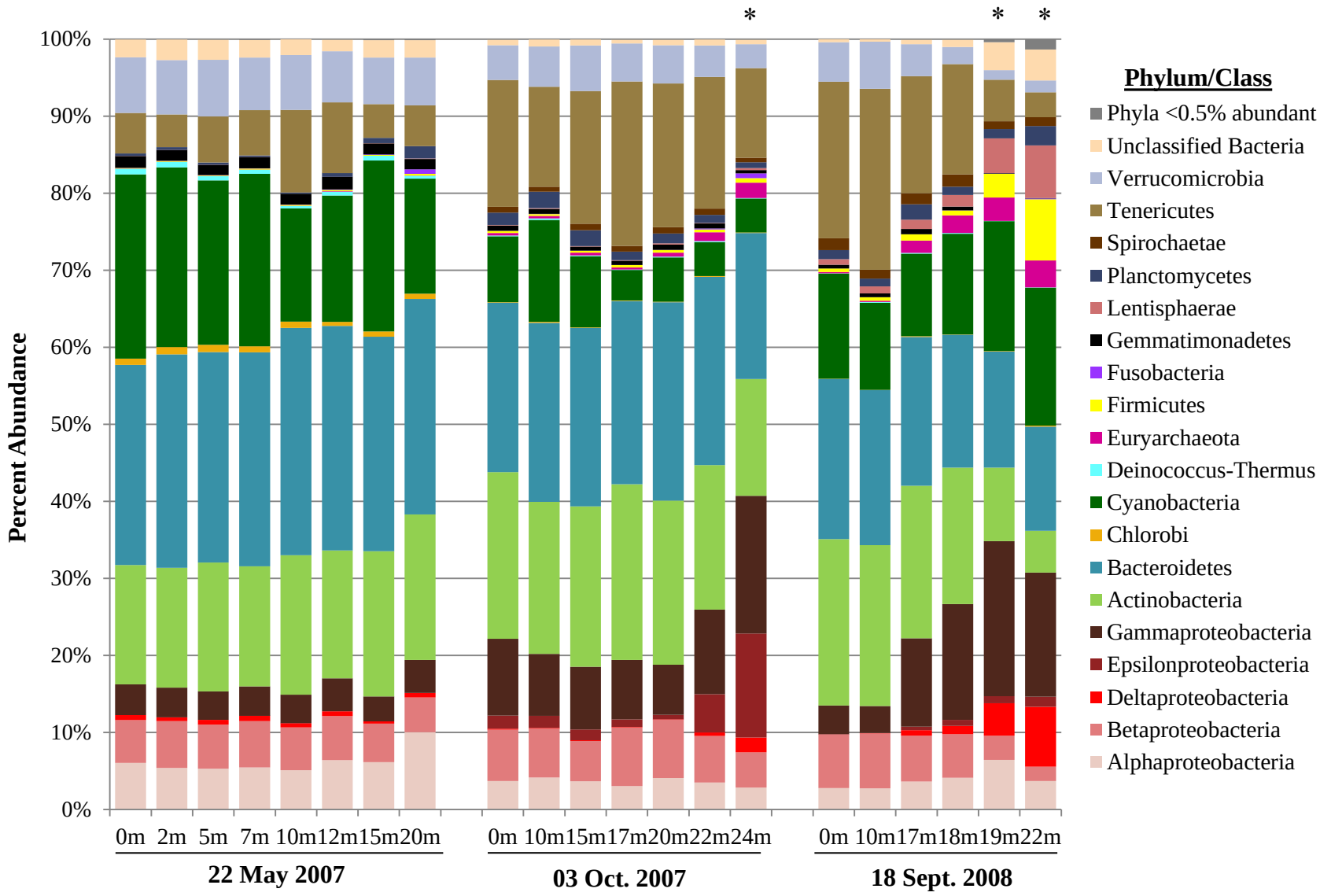

Figure B.1. Relative abundance of bacterial phyla/classes (≥0.5% abundant) that make up the microbial communities of all collected water samples of each collection date. (*) above column indicates a suboxic water condition at the time of sampling.

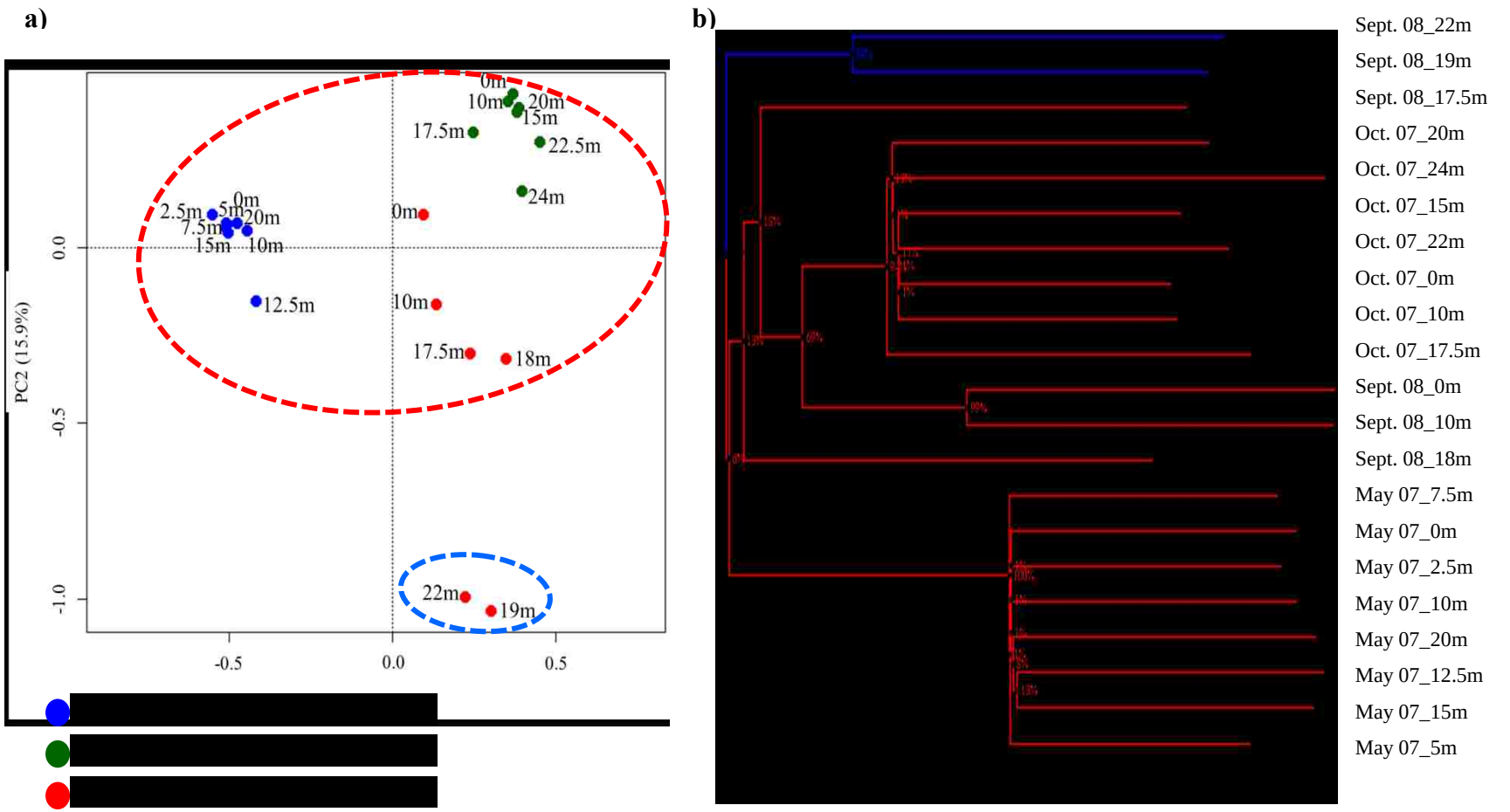

Figure B.2. Microbial community separation by principal component analysis (a) using unweighted UniFrac distances of Illumina V4 16S rRNA gene dataset. Each dot represents a complete Illumina sequence dataset of the corresponding sampled depth in the water column from May 2007 (blue), October 2007 (green), and September 2008 (red). Colored ellipses drawn around groupings correspond with clade separation based on bootstrap support values from unweighted UniFrac UPGMA tree (b).

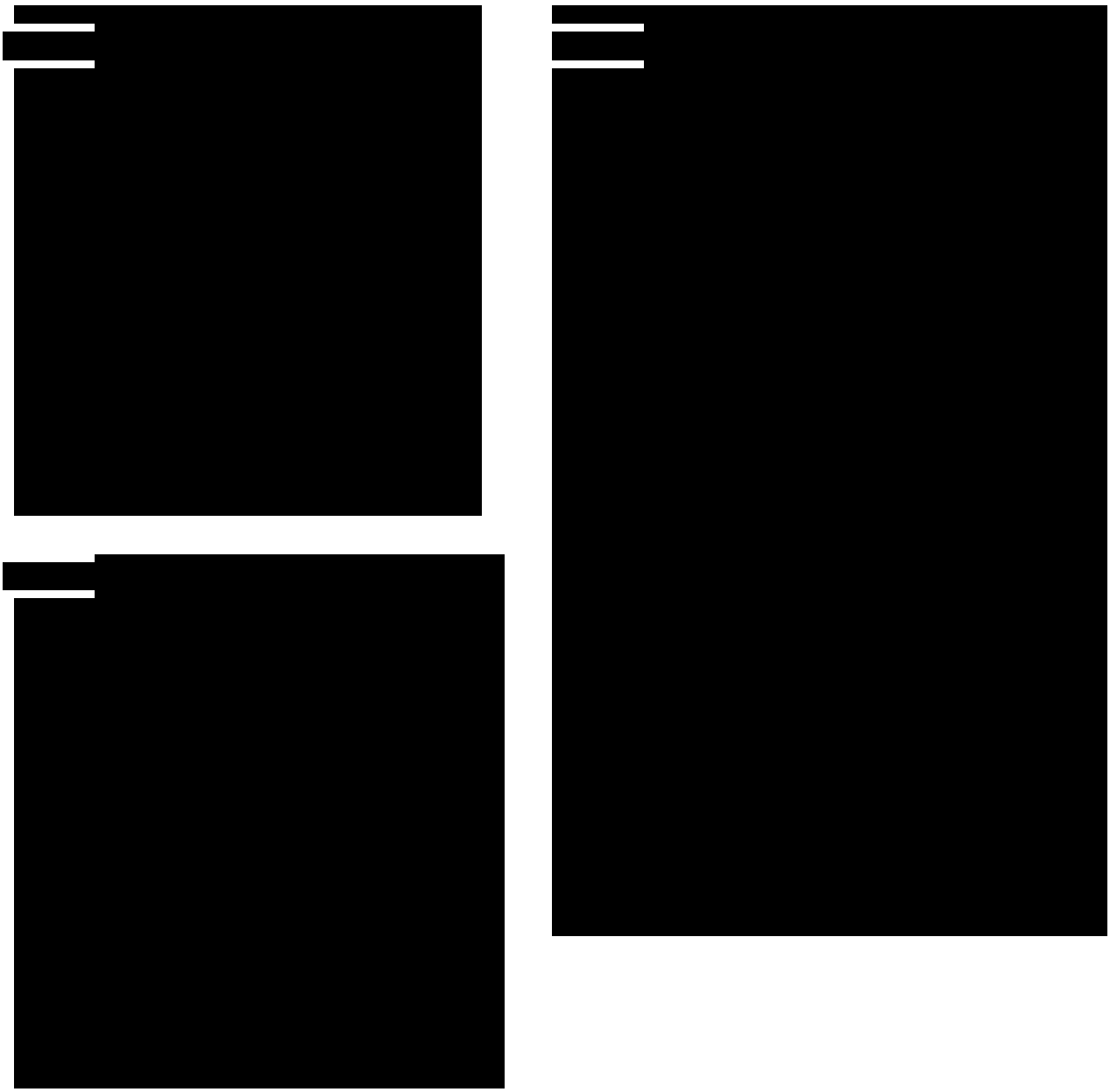

Figure B.3. Evolutionary relationships of taxa from clone libraries. The evolutionary history was inferred using the neighbor-joining method. The bootstrap consensus tree inferred from 1000 replicates represents the evolutionary history of the taxa analyzed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Jukes-Cantor method and are in the units of the number of base substitutions per site. a) Gamma-Proteobacteria; b) Cyanobacteria ; c) Delta-Proteobacteria

APPENDIX C: SUPPLEMENTAL MATERIAL FOR CHAPTER THREE

Table C.1. Physical parameters measured *in situ* using multiparameter sonde. dO_2=dissolved oxygen; ORP=oxidation/reduction potential; Sp. Cond.=specific conductance; PAR=photosynthetically active radiation

Date	Depth (m)	Temp. (°C)	pH	dO_2 (mg L^{-1})	ORP (mV)	Sp. Cond. (µS/cm)	PAR (µmol/m^2 s)
Nov. 2013	**0**	14.0	9.34	8.78	-39.5	28470	nd
	10	13.9	9.36	8.79	-36.8	28460	nd
	19	13.9	9.36	8.72	-34.4	28460	nd
Sept. 2014	**0**	22.82	9.38	7.28	73.5	30340	3732
	5	22.83	9.38	7.20	72.0	30340	193.5
	10	22.82	9.38	7.15	72.4	30350	70.18
	15	22.81	9.38	7.12	73.3	30350	25.37
	17.5	22.81	9.38	7.10	71.3	30350	18.18
Sept. 2015	**0**	22.02	9.44	6.92	93.3	33010	1776.5
	10	22.14	9.44	6.80	84.1	32990	143.65
	17	22.13	9.44	6.75	80.6	32990	43.3

nd= not determined

Note: Negative ORP seen in 2013 was likely an instrument artifact

Table C.2. Trace element concentrations for 2014 and 2015 water samples. Note high concentrations of As (drinking water MCL = 10 μg/L), U, and B.

Metal Parameter (mg L^{-1})	September 2014		September 2015		
	0m	15m	0m	10m	16.5m
Aluminum	0.013	0.014	0.007	0.008	0.008
Antimony	0.013	0.013	0.0171	0.0164	0.0163
Arsenic	1.78	1.76	1.7	1.7	1.6
Barium	0.159	0.167	0.1655	0.1533	0.1547
Beryllium	*	*	*	*	*
Boron	41	43	52	52	51
Cadmium	*	*	0.0006	0.0003	0.0004
Cesium	0.001	*	0.0003	*	*
Chromium	*	*	*	*	0.0009
Cobalt	0.0005	0.0005	0.0003	0.00036	0.00039
Copper	*	*	*	*	0.0125
Lead	*	*	*	*	*
Manganese	*	*	0.0049	0.006	0.006
Molybdenum	0.609	0.653	*	*	*
Nickel	*	*	*	*	*
Selenium	*	*	0.0023	0.0042	0.0065
Silver	*	*	0.00009	0.00009	0.0001
Tellurium	*	*	*	0.001	0.001
Thallium	*	*	*	*	*
Thorium	*	*	*	*	*
Tin	*	*	*	*	*
Uranium	0.360	0.3785	0.4	0.3	0.3
Vanadium	0.005	0.005	0.0045	0.0043	0.0042
Zinc	*	*	0.006	0.006	0.006

*Below detection limit.

Table C.3. Taxonomy and abundance of the eighteen 'core' OTUs present in every water column sample between 2008 and 2015.

OTU ID#	Average Abundance and SEM (%)	Taxonomy
27202	0.69 ± 0.26	k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__; g__; s__
26908	1.11 ± 0.14	k__Bacteria; p__Actinobacteria; c__Nitriliruptoria; o__Nitriliruptorales; f__Nitriliruptoraceae; g__; s__
27015	0.39 ± 0.06	k__Bacteria; p__Actinobacteria; c__Nitriliruptoria; o__Nitriliruptorales; f__Nitriliruptoraceae; g__; s__
27204	0.93 ± 0.19	k__Bacteria; p__Actinobacteria; c__Nitriliruptoria; o__Nitriliruptorales; f__Nitriliruptoraceae; g__; s__
26965	0.09 ± 0.02	k__Bacteria; p__Bacteroidetes; c__Cytophagia; o__Cytophagales; f__Cyclobacteriaceae; g__; s__
26699	2.25 ± 0.30	k__Bacteria; p__Bacteroidetes; c__Flavobacteriia; o__Flavobacteriales; f__Cryomorphaceae; g__; s__
26801	3.43 ± 0.28	k__Bacteria; p__Bacteroidetes; c__Flavobacteriia; o__Flavobacteriales; f__Cryomorphaceae; g__; s__
27164	0.09 ± 0.02	k__Bacteria; p__Bacteroidetes; c__Flavobacteriia; o__Flavobacteriales; f__Cryomorphaceae; g__; s__
1615	1.02 ± 0.30	k__Bacteria; p__Bacteroidetes; c__Flavobacteriia; o__Flavobacteriales; f__Flavobacteriaceae; g__; s__
26765	0.14 ± 0.02	k__Bacteria; p__Bacteroidetes; c__Flavobacteriia; o__Flavobacteriales; f__Flavobacteriaceae; g__; s__
27221	0.77 ± 0.22	k__Bacteria; p__Bacteroidetes; c__Sphingobacteriia; o__Sphingobacteriales; f__; g__; s__
26698	7.10 ± 1.51	k__Bacteria; p__Cyanobacteria; c__Synechococcophycideae; o__Synechococcales; f__Synechococcaceae; g__Synechococcus; s__
26761	0.19 ± 0.06	k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Rhodocyclales; f__Rhodocyclaceae; g__; s__
26947	1.15 ± 0.15	k__Bacteria; p__Tenericutes; c__Mollicutes; o__; f__; g__; s__
26876	1.91 ± 0.50	k__Bacteria; p__Tenericutes; c__Mollicutes; o__; f__; g__; s__
26867	3.22 ± 0.62	k__Bacteria; p__Tenericutes; c__Mollicutes; o__; f__; g__; s__
26748	0.60 ± 0.18	k__Bacteria; p__Verrucomicrobia; c__Opitutae; o__Puniceicoccales; f__Puniceicoccaceae; g__; s__
27102	0.32 ± 0.10	k__Bacteria; p__Verrucomicrobia; c__Verrucomicrobiae; o__Verrucomicrobiales; f__Verrucomicrobiaceae; g__Luteolibacter; s__

Table C.4. List of OTU taxonomic assignments from water column heatmap (Figure 3.5).

OTU ID #	Taxonomy
26698	k__Bacteria; p__Cyanobacteria; c__Synechococcophycideae; o__Synechococcales; f__Synechococcaceae; g__Synechococcus; s__
26824	k__Bacteria; p__Actinobacteria; c__Nitriliruptoria; o__Nitriliruptorales; f__Nitriliruptoraceae; g__; s__
26739	k__Bacteria; p__Actinobacteria; c__Nitriliruptoria; o__Nitriliruptorales; f__Nitriliruptoraceae; g__; s__
71	k__Bacteria; p__Actinobacteria; c__Nitriliruptoria; o__Nitriliruptorales; f__Nitriliruptoraceae; g__Nitriliruptor
26733	k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Microbacteriaceae; g__; s__
2143	k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Burkholderiales; f__Comamonadaceae
27006	k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Burkholderiales; f__Comamonadaceae
387	k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__; g__; s__
26867	k__Bacteria; p__Tenericutes; c__Mollicutes; o__; f__; g__; s__
26876	k__Bacteria; p__Tenericutes; c__Mollicutes; o__; f__; g__; s__
1175	k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Microbacteriaceae; g__; s__
909	k__Bacteria; p__Tenericutes; c__Mollicutes; o__; f__; g__; s__
26801	k__Bacteria; p__Bacteroidetes; c__Flavobacteriia; o__Flavobacteriales; f__Cryomorphaceae; g__; s__
26711	k__Bacteria; p__Bacteroidetes; c__Flavobacteriia; o__Flavobacteriales; f__Flavobacteriaceae; g__; s__
26918	k__Bacteria; p__Bacteroidetes; c__Flavobacteriia; o__Flavobacteriales; f__Flavobacteriaceae; g__; s__
27202	k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__; g__; s__
429	k__Bacteria; p__Bacteroidetes; c__Flavobacteriia; o__Flavobacteriales; f__Flavobacteriaceae; g__; s__
53	k__Bacteria; p__Bacteroidetes; c__[Saprospirae]; o__[Saprospirales]; f__; g__; s__
1615	k__Bacteria; p__Bacteroidetes; c__Flavobacteriia; o__Flavobacteriales; f__Flavobacteriaceae; g__; s__
27017	k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Burkholderiales; f__Alcaligenaceae; g__; s__
1257	k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Burkholderiales; f__Comamonadaceae
26699	k__Bacteria; p__Bacteroidetes; c__Flavobacteriia; o__Flavobacteriales; f__Cryomorphaceae; g__; s__
748	k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Burkholderiales; f__Alcaligenaceae; g__; s__
26741	k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Microbacteriaceae; g__Candidatus Aquiluna; s__rubra
72	k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Microbacteriaceae; g__; s__
27204	k__Bacteria; p__Actinobacteria; c__Nitriliruptoria; o__Nitriliruptorales; f__Nitriliruptoraceae; g__; s__

Table C.4 (continued). List of OTU taxonomic assignments from water column heatmap (Figure 3.5).

2360	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Vibrionales; f__Vibrionaceae; g__Vibrio; s__metschnikovii
948	k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Burkholderiales; f__Comamonadaceae; g__Hydrogenophaga; s__
26947	k__Bacteria; p__Tenericutes; c__Mollicutes; o__; f__; g__; s__
27101	Unassigned
27131	k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__; g__; s__
108	k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Microbacteriaceae; g__; s__
1874	k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Microbacteriaceae; g__Candidatus Aquiluna; s__rubra
1899	k__Bacteria; p__Verrucomicrobia; c__Verrucomicrobiae; o__Verrucomicrobiales; f__Verrucomicrobiaceae; g__Luteolibacter; s__
26748	k__Bacteria; p__Verrucomicrobia; c__Opitutae; o__Puniceicoccales; f__Puniceicoccaceae; g__; s__
27007	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Vibrionales; f__Vibrionaceae; g__Vibrio; s__metschnikovii
26908	k__Bacteria; p__Actinobacteria; c__Nitriliruptoria; o__Nitriliruptorales; f__Nitriliruptoraceae; g__; s__
1785	k__Bacteria; p__Cyanobacteria; c__Chloroplast; o__Chlorophyta; f__; g__; s__
26940	k__Bacteria; p__Bacteroidetes; c__Sphingobacteriia; o__Sphingobacteriales; f__NS11-12; g__; s__
161	k__Bacteria; p__Cyanobacteria; c__Chloroplast; o__Cryptophyta; f__; g__; s__
2290	k__Bacteria; p__Bacteroidetes; c__Cytophagia; o__Cytophagales; f__Cyclobacteriaceae; g__; s__
26745	k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Microbacteriaceae; g__; s__
1801	k__Bacteria; p__Bacteroidetes; c__[Rhodothermi]; o__[Rhodothermales]; f__[Balneolaceae]; g__KSA1; s__
26767	k__Bacteria; p__Bacteroidetes; c__Cytophagia; o__Cytophagales; f__Cyclobacteriaceae; g__; s__
1977	k__Bacteria; p__Bacteroidetes; c__Flavobacteriia; o__Flavobacteriales; f__Cryomorphaceae; g__; s__
2234	k__Bacteria; p__Verrucomicrobia; c__Verrucomicrobiae; o__Verrucomicrobiales; f__Verrucomicrobiaceae; g__Luteolibacter; s__
927	k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Microbacteriaceae; g__; s__
1699	k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Burkholderiales; f__Comamonadaceae; g__Hydrogenophaga; s__
1812	k__Bacteria; p__Verrucomicrobia; c__Verrucomicrobiae; o__Verrucomicrobiales; f__Verrucomicrobiaceae; g__Luteolibacter; s__
27206	k__Bacteria; p__Bacteroidetes; c__[Rhodothermi]; o__[Rhodothermales]; f__[Balneolaceae]; g__KSA1; s__
26738	k__Bacteria; p__Bacteroidetes; c__[Rhodothermi]; o__[Rhodothermales]; f__[Balneolaceae]; g__; s__
27203	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rickettsiales; f__Pelagibacteraceae; g__; s__
27102	k__Bacteria; p__Verrucomicrobia; c__Verrucomicrobiae; o__Verrucomicrobiales; f__Verrucomicrobiaceae; g__Luteolibacter; s__
343	k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Methylophilales; f__Methylophilaceae; g__; s__

Table C.4 (continued). List of OTU taxonomic assignments from water column heatmap (Figure 3.5).

330	k__Bacteria; p__Cyanobacteria; c__Synechococcophycideae; o__Synechococcales; f__Synechococcaceae; g__Synechococcus; s__
26746	k__Bacteria; p__Bacteroidetes; c__[Rhodothermi]; o__[Rhodothermales]; f__[Balneolaceae]; g__; s__
1031	k__Bacteria; p__Bacteroidetes; c__Cytophagia; o__Cytophagales; f__Cyclobacteriaceae
432	k__Bacteria; p__Bacteroidetes; c__Sphingobacteriia; o__Sphingobacteriales; f__NS11-12; g__; s__
26742	k__Bacteria; p__Bacteroidetes; c__Sphingobacteriia; o__Sphingobacteriales; f__NS11-12; g__; s__
7284	k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Burkholderiales; f__Alcaligenaceae; g__; s__
1096	k__Bacteria; p__Actinobacteria; c__Acidimicrobiia; o__Acidimicrobiales; f__; g__; s__
26747	k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Methylophilales; f__Methylophilaceae; g__; s__

Table C.5. List of OTU taxonomic assignments from sediment heatmap (Figure 3.6).

OTU ID #	Taxonomy
26743	k__Bacteria; p__[Thermi]; c__Deinococci; o__Deinococcales; f__Trueperaceae; g__B-42; s__
26938	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Thiotrichales; f__Piscirickettsiaceae; g__; s__
762	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Chromatiales; f__Ectothiorhodospiraceae; g__Thioalkalivibrio; s__
758	k__Bacteria; p__Firmicutes; c__Clostridia; o__Natranaerobiales; f__ML1228J-1; g__; s__
26701	k__Bacteria; p__Proteobacteria; c__Deltaproteobacteria; o__Desulfovibrionales; f__Desulfohalobiaceae; g__Desulfonatronovibrio; s__
26759	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Chromatiales; f__Ectothiorhodospiraceae; g__Thioalkalivibrio; s__
541	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__; g__; s__
1537	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Thiotrichales; f__Piscirickettsiaceae; g__; s__
26897	k__Bacteria; p__Caldithrix; c__Caldithrixae; o__Caldithrixales; f__Caldithrixaceae; g__LCP-26; s__
21	k__Bacteria; p__Proteobacteria; c__Deltaproteobacteria; o__Desulfovibrionales; f__Desulfohalobiaceae; g__Desulfonatronovibrio; s__
26831	k__Bacteria; p__Firmicutes; c__Clostridia; o__Natranaerobiales; f__ML1228J-1; g__; s__
26855	k__Bacteria; p__Chloroflexi; c__Anaerolineae; o__GCA004; f__; g__; s__
330	k__Bacteria; p__Cyanobacteria; c__Synechococcophycideae; o__Synechococcales; f__Synechococcaceae; g__Synechococcus; s__
747	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__; g__; s__
26796	k__Bacteria; p__Proteobacteria; c__Deltaproteobacteria; o__Desulfobacterales; f__Desulfobacteraceae; g__Desulfosarcina; s__
26702	k__Bacteria; p__Chloroflexi; c__Anaerolineae; o__Anaerolineales; f__Anaerolinaceae; g__T78; s__
26790	k__Bacteria; p__[Caldithrix]; c__KSB1; o__MSB-5B5; f__; g__; s__
26906	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__ML635J-40; g__; s__
26956	k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Rhodocyclales; f__Rhodocyclaceae
994	k__Bacteria; p__Proteobacteria; c__Deltaproteobacteria; o__Desulfovibrionales; f__Desulfohalobiaceae; g__Desulfonatronovibrio; s__
1092	k__Bacteria; p__Chloroflexi; c__Anaerolineae; o__Anaerolineales; f__Anaerolinaceae; g__T78; s__
27226	Unassigned
26823	k__Bacteria; p__Chlorobi; c__Ignavibacteria; o__Ignavibacteriales; f__Ignavibacteriaceae; g__; s__
27224	k__Bacteria; p__Gemmatimonadetes; c__Gemm-2; o__; f__; g__; s__
439	k__Bacteria; p__Bacteroidetes; c__Cytophagia; o__Cytophagales; f__Cyclobacteriaceae; g__; s__
26725	k__Bacteria; p__Firmicutes; c__Clostridia; o__Natranaerobiales; f__ML1228J-1; g__; s__
26881	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rhodobacterales; f__Rhodobacteraceae; g__; s__

Table C.5 (continued). List of OTU taxonomic assignments from sediment heatmap (Figure 3.6).

26727	k__Bacteria; p__Gemmatimonadetes; c__Gemm-2; o__; f__; g__; s__
26877	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__; f__; g__; s__
1867	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__ML635J-40; g__; s__
27227	k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__; g__; s__
1793	k__Bacteria; p__Proteobacteria; c__Deltaproteobacteria; o__Desulfobacterales; f__Desulfobacteraceae; g__Desulfosarcina; s__
26811	k__Bacteria; p__Firmicutes; c__Clostridia; o__Natranaerobiales; f__ML1228J-1; g__; s__
26804	k__Bacteria; p__Cyanobacteria; c__Synechococcophycideae; o__Synechococcales; f__Synechococcaceae; g__Synechococcus; s__
26929	k__Bacteria; p__Bacteroidetes; c__Cytophagia; o__Cytophagales; f__Cyclobacteriaceae; g__; s__
27228	k__Bacteria; p__Chloroflexi; c__Dehalococcoidetes; o__GIF9; f__; g__; s__
1015	k__Bacteria; p__Cyanobacteria; c__Oscillatoriophycideae; o__Chroococcales; f__Xenococcaceae; g__; s__
2015	k__Bacteria; p__Gemmatimonadetes; c__Gemm-2; o__; f__; g__; s__
27238	k__Bacteria; p__Chlorobi; c__Ignavibacteria; o__Ignavibacteriales; f__Ignavibacteriaceae; g__; s__
26879	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rhodobacterales; f__Rhodobacteraceae; g__Anaerospora; s__
26928	k__Bacteria; p__[Thermi]; c__Deinococci; o__Deinococcales; f__Trueperaceae; g__B-42; s__
1309	k__Bacteria; p__Proteobacteria; c__Deltaproteobacteria; o__Desulfobacterales; f__Desulfobulbaceae; g__; s__
1579	k__Bacteria; p__Cyanobacteria; c__Synechococcophycideae; o__Synechococcales; f__Synechococcaceae; g__Synechococcus; s__
26714	k__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__ML635J-40; g__; s__
27229	k__Bacteria; p__Firmicutes; c__Clostridia; o__Natranaerobiales; f__ML1228J-1; g__; s__
27236	k__Bacteria; p__OP1; c__OPB14; o__; f__; g__; s__
27173	k__Bacteria; p__Bacteroidetes; c__[Saprospirae]; o__[Saprospirales]; f__Saprospiraceae; g__; s__
26788	k__Bacteria; p__Proteobacteria; c__Deltaproteobacteria; o__Desulfuromonadales; f__Desulfuromonadaceae; g__; s__
1615	k__Bacteria; p__Bacteroidetes; c__Flavobacteriia; o__Flavobacteriales; f__Flavobacteriaceae; g__; s__
1113	k__Bacteria; p__Chloroflexi; c__Anaerolineae; o__GCA004; f__; g__; s__
26863	k__Bacteria; p__Firmicutes; c__Clostridia; o__Natranaerobiales; f__ML1228J-1; g__; s__
26775	k__Bacteria; p__Chloroflexi; c__Dehalococcoidetes; o__; f__; g__; s__
401	k__Bacteria; p__Proteobacteria; c__Deltaproteobacteria; o__Desulfuromonadales; f__Desulfuromonadaceae; g__; s__
2011	k__Bacteria; p__Chlorobi; c__BSV26; o__; f__; g__; s__
1801	k__Bacteria; p__Bacteroidetes; c__[Rhodothermi]; o__[Rhodothermales]; f__[Balneolaceae]; g__KSA1; s__
25448	k__Bacteria; p__Bacteroidetes; c__[Rhodothermi]; o__[Rhodothermales]; f__[Balneolaceae]; g__; s__

Table C.5 (continued). List of OTU taxonomic assignments from sediment heatmap (Figure 3.6).

26715	k__Bacteria; p__Chloroflexi; c__Anaerolineae; o__GCA004; f__; g__; s__
26779	k__Bacteria; p__Firmicutes; c__Clostridia; o__Natranaerobiales; f__ML1228J-1; g__; s__

Table C.6. Alpha diversity indices for Walker Lake water column and sediment samples between 2008 and 2015. Mean ± SEM for each index.

Date	Depth (m)	Observed OTUs	Chao1	Faith Index	Shannon Index	Simpson Index
Sept. 2008	0	477.03 ± 13.07	944.44 ± 81.52	42.79 ± 1.16	5.88 ± 0.02	0.9585 ± 0.0006
	10	400.38 ± 11.92	738.49 ± 79.36	38.24 ± 1.04	5.74 ± 0.02	0.9550 ± 0.0008
	17.5	595.57 ± 13.24	1192.24 ± 96.81	57.18 ± 1.42	6.37 ± 0.02	0.9707 ± 0.0005
	18	633.91 ± 14.18	1278.89 ± 100.19	59.86 ± 1.37	6.26 ± 0.02	0.9659 ± 0.0005
	19	687.37 ± 12.87	1200.82 ± 74.44	68.41 ± 1.38	6.41 ± 0.03	0.9646 ± 0.0007
	22	792.75 ± 14.28	1307.90 ± 76.00	78.88 ± 1.51	6.80 ± 0.03	0.9674 ± 0.0007
Nov. 2013	0	583.63 ± 14.76	1380.64 ± 123.48	51.45 ± 1.23	6.12 ± 0.02	0.9679 ± 0.0005
	10	554.78 ± 13.30	1273.22 ± 95.41	49.02 ± 1.19	6.05 ± 0.02	0.9657 ± 0.0005
	19	1036.39 ± 17.08	2071.74 ± 111.60	87.58 ± 1.44	7.17 ± 0.02	0.9777 ± 0.0004
	SS	2222.39 ± 21.35	4765.98 ± 173.49	152.35 ± 1.70	9.13 ± 0.02	0.9940 ± 0.0001
	DS	1948.29 ± 20.53	4230.26 ± 153.98	145.54 ± 1.55	8.76 ± 0.02	0.9919 ± 0.0002
Sept. 2014	0	592.74 ± 15.14	1746.28 ± 195.73	53.15 ± 1.57	5.44 ± 0.02	0.9396 ± 0.0009
	5	777.35 ± 16.69	1778.32 ± 155.42	61.64 ± 1.81	6.36 ± 0.02	0.9669 ± 0.0005
	10	654.48 ± 16.12	1722.99 ± 138.24	55.87 ± 1.55	5.69 ± 0.03	0.9487 ± 0.0010
	15	444.30 ± 10.88	1082.80 ± 109.61	50.57 ± 1.36	5.18 ± 0.02	0.9168 ± 0.0013
	17.5	775.51 ± 18.43	1882.64 ± 167.44	59.13 ± 1.76	6.51 ± 0.02	0.9756 ± 0.0004
	SS	1940.74 ± 29.81	4659.59 ± 219.36	141.90 ± 2.18	8.46 ± 0.03	0.9865 ± 0.0004
	DS	1686.02 ± 23.30	3776.00 ± 200.80	124.51 ± 1.96	8.25 ± 0.03	0.9893 ± 0.0002
Sept. 2015	0	415.84 ± 12.06	939.33 ± 93.12	39.71 ± 1.34	5.74 ± 0.02	0.9618 ± 0.0007
	10	448.64 ± 13.09	1074.12 ± 119.76	42.72 ± 1.32	5.87 ± 0.02	0.9645 ± 0.0005
	16.5	474.24 ± 13.85	1056.02 ± 111.62	46.99 ± 1.49	5.87 ± 0.02	0.9642 ± 0.0007
	SS	1630.82 ± 23.32	3072.58 ± 141.52	135.37 ± 2.07	8.82 ± 0.02	0.9943 ± 0.0001
	DS	1262.77 ± 20.73	2437.43 ± 134.26	108.14 ± 1.95	8.03 ± 0.03	0.9885 ± 0.0003

Table C.7. Mantel-r statistics and p-values for Mantel tests performed to relate environmental factors with taxonomic composition. P-values based on 9,999 permutations.

	Weighted	
Parameter	Mantel's r	p-value
HCO_3^-	0.3769766	0.0169
CO_3^-	0.454915	0.0045
Cl^-	0.2395541	0.1085
SO_4^{2-}	0.3468378	0.0202
Na^+	0.2915361	0.043
K^+	0.3488629	0.0196
Ca^{2+}	0.004139964	0.3279
Mg^{2+}	0.5210108	0.0021
DOC	0.501072	0.0035
TDS	0.576717	0.0008
Temp.	0.5082545	0.0029
pH	0.4683414	0.0054
dO_2	0.5259752	0.0023
Conductivity	0.5770996	0.0006

REFERENCES

Adams KD (2007) Late holocene sedimentary environments and lake-level fluctuations at Walker Lake, Nevada, USA. *Geological Society of America Bulletin* **119**: 126-139.

Adams KD & Wesnousky SG (1998) Shoreline processes and the age of the Lake Lahontan highstand in the Jessup embayment, Nevada. *Geological Society of America Bulletin* **110**: 1318-1332.

Aguilar C & Nealson KH (1994) Manganese reduction in Oneida Lake, New York: Estimates of spatial and temporal manganese flux. *Canadian Journal of Fisheries and Aquatic Sciences* **51**: 185-196.

Aguilar C & Nealson KH (1998) Biogeochemical cycling of manganese in Oneida Lake, New York: whole lake studies of manganese. *Journal of Great Lakes Research* **24**: 93-104.

Amann RI, Ludwig W & Schleifer KH (1995) Phylogenetic identification and in-situ detection of individual microbial cells without cultivation. *Microbiological Reviews* **59**: 143-169.

Andrei A-S, Robeson MS, Baricz A, Coman C, Muntean V, Ionescu A, Etiope G, Alexe M, Sicora CI, & Podar M (2015) Contrasting taxonomic stratification of microbial communities in two hypersaline meromictic lakes. *ISME Journal* **9**: 2642-2656.

American Public Health Association (APHA), American Water Works Association (AWWA), & Water Environment Federation (WEF) (1915) *Standard methods for the examination of water and wastewater*.

Atlas RM (2004) *Handbook of Microbiological Media*. CRC Press LLC, Boca Raton.

Benson L (1991) Timing of the last highstand of Lake Lahontan. *Journal of Paleolimnology* **5**: 115-126.

Benson L & Thompson R (1987) Lake-level variation in the Lahontan Basin for the past 50,000 years. *Quaternary Research* **28**: 69-85.

Benson L, Currey D, Lao Y & Hostetler S (1992) Lake-size variations in the Lahontan and Bonneville basins between 13,000 and 9000 14 C yr BP. *Palaeogeography, Palaeoclimatology, Palaeoecology* **95**: 19-32.

Benson LV & Mifflin MD (1986) Reconnaissance bathymetry of basins occupied by Pleistocene Lake Lahontan, Nevada and California.

Benson LV, Meyers PA & Spencer R (1991) Change in the size of Walker Lake during the past 5000 years. *Palaeogeography, Palaeoclimatology, Palaeoecology* **81**: 189-214.

Berelson W, Corsetti F, Johnson B, Vo T & Der C (2009) Carbonate-associated sulfate as a proxy for lake level fluctuations: a proof of concept for Walker Lake, Nevada. *Journal of Paleolimnology* **42**: 25-36.

Beutel MW (2001) Oxygen consumption and ammonia accumulation in the hypolimnion of Walker Lake, Nevada. *Hydrobiologia* **466**: 107-117.

Beutel MW, Horne AJ, Roth JC & Barratt NJ (2001) Limnological effects of anthropogenic desiccation of a large, saline lake, Walker Lake, Nevada. *Hydrobiologia* **466**: 91-105.

Bigelow JP, Rauw WM & Gomez-Raya L (2010) Acclimation improves short-term survival of hatchery Lahontan cutthroat trout in water from saline, alkaline Walker Lake, Nevada. *Journal of Fish and Wildlife Management* **1**: 86-92.

Bradbury JP, Forester R & Thompson R (1989) Late Quaternary paleolimnology of Walker Lake, Nevada. *Paleolimnology and the Reconstruction of Ancient Environments: Paleolimnology Proceedings of the XII INQUA Congress,* (Davis RB, ed.), pp. 39-57. Springer Netherlands, Dordecht.

Bragg L & Tyson GW (2014) Metagenomics using next-generation sequencing. *Environmental Microbiology: Methods and Protocols* 183-201.

Breakwell K, Tetu SG & Elbourne LD (2014) Next generation barcode tagged sequencing for monitoring microbial community dynamics. *Environmental Microbiology: Methods and Protocols* 101-110.

Brenner D, Krieg N, Staley J, Garrity G, Boone D, De Vos P & Garrity G (2005) The proteobacteria, part B, the gammaproteobacteria. *Bergey's Manual of Systematic Bacteriology* **2**.

Brüchert V & Pratt LM (1996) Contemporaneous early diagenetic formation of organic and inorganic sulfur in estuarine sediments from St. Andrew Bay, Florida, USA. *Geochimica et Cosmochimica Acta* **60**: 2325-2332.

Budinoff CR & Hollibaugh JT (2007) Ecophysiology of a Mono Lake picocyanobacterium. *Limnology and Oceanography* **52**: 2484-2496.

Caporaso JG, Bittinger K, Bushman FD, DeSantis TZ, Andersen GL & Knight R (2010) PyNAST: a flexible tool for aligning sequences to a template alignment. *Bioinformatics* **26**: 266-267.

Caporaso JG, Kuczynski J, Stombaugh J, *et al.* (2010) QIIME allows analysis of high-throughput community sequencing data. *Nature methods* **7**: 335-336.

Chao A (1984) Nonparametric estimation of the number of classes in a population. *Scandinavian Journal of Statistics* 265-270.

Clarke FW (1924) *The composition of the river and lake waters of the United States*. US Government Printing Office.

Cline JD & Richards FA (1969) Oxygenation of hydrogen sulfide in seawater at constant salinity, temperature and pH. *Environmental Science & Technology* **3**: 838-843.

Cohen AS (2003) *Paleolimnology: the history and evolution of lake systems*. Oxford University Press, USA.

Cohen Y, Padan E & Shilo M (1975) Facultative anoxygenic photosynthesis in the cyanobacterium *Oscillatoria limnetica*. *Journal of Bacteriology* **123**: 855-861.

Cole GA (1968) Desert Limnology. *Desert Biology*, Vol. 1 (Brown GW, ed..), pp. 423-486. Academic Press, New York.

Collopy MW & Thomas JM (2009) Restoration of a desert lake in an agriculturally dominated watershed: the Walker Lake Basin. *Bureau of Reclamation Report*.

Cooper JJ (1985) Age, growth, and food habits of tui chub, Gila bicolor, in Walker Lake, Nevada. *The Great Basin Naturalist* 784-788.

Cooper JJ & Koch DL (1984) Limnology of a desertic terminal lake, Walker Lake, Nevada, USA. *Hydrobiologia* **118**: 275-292.

Cooper SD, Winkler DW & Lenz PH (1984) The effect of grebe predation on a brine shrimp population. *The Journal of Animal Ecology* 51-64.

Cornelia Hr & Britta P-F (2012) Thioarsenate transformation by filamentous microbial mats thriving in an alkaline, sulfidic hot spring. *Environmental science & technology* **46**: 4348-4356.

Dale JW, Von Schantz M & Plant N (2012) *From genes to genomes: concepts and applications of DNA technology*. John Wiley & Sons.

DeSantis TZ, Hugenholtz P, Larsen N, *et al.* (2006) Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Applied and environmental microbiology* **72**: 5069-5072.

Dimitriu PA, Pinkart HC, Peyton BM & Mormile MR (2008) Spatial and Temporal Patterns in the Microbial Diversity of a Meromictic Soda Lake in Washington State. *Applied and Environmental Microbiology* **74**: 4877-4888.

Domagalski JL, Orem WH & Eugster HP (1989) Organic geochemistry and brine composition in Great Salt, Mono, and Walker Lakes. *Geochimica et Cosmochimica Acta* **53**: 2857-2872.

Dong HL, Zhang GX, Jiang HC, Yu BS, Chapman LR, Lucas CR & Fields MW (2006) Microbial diversity in sediments of saline Qinghai Lake, China: Linking geochemical controls to microbial ecology. *Microbial Ecology* **51**: 65-82.

Dray S & Dufour A-B (2007) The ade4 package: implementing the duality diagram for ecologists. *Journal of statistical software* **22**: 1-20.

Edgar RC (2010) Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* **26**: 2460-2461.

Eren AM, Morrison HG, Lescault PJ, Reveillaud J, Vineis JH & Sogin ML (2015) Minimum entropy decomposition: Unsupervised oligotyping for sensitive partitioning of high-throughput marker gene sequences. *The ISME journal* **9**: 968-979.

Eugster HP & Hardie LA (1978) Saline lakes. *Lakes* (Lerman A, ed.) pp. 237-293. Springer, New York, NY.

Faith DP (1992) Conservation evaluation and phylogenetic diversity. *Biological conservation* **61**: 1-10.

Fenchel T (2008) The microbial loop – 25 years later. *Journal of Experimental Marine Biology and Ecology* **366**: 99-103.

Fisher JC & Hollibaugh JT (2008) Selenate-dependent anaerobic arsenite oxidation by a bacterium from Mono Lake, California. *Applied and environmental microbiology* **74**: 2588-2594.

Foti M, Ma S, Sorokin DY, Rademaker JLW, Kuenen JG & Muyzer G (2006) Genetic diversity and biogeography of haloalkaliphilic sulphur-oxidizing bacteria belonging to the genus *Thioalkalivibrio*. *Fems Microbiology Ecology* **56**: 95-101.

Fredrickson JK, Kostandarithes HM, Li S, Plymale AE & Daly MJ (2000) Reduction of Fe (III), Cr (VI), U (VI), and Tc (VII) by *Deinococcus radiodurans* R1. *Applied and Environmental Microbiology* **66**: 2006-2011.

Fredrickson JK, Zachara JM, Kennedy DW, Dong H, Onstott TC, Hinman NW & Li S-M (1998) Biogenic iron mineralization accompanying the dissimilatory reduction of hydrous ferric oxide by a groundwater bacterium. *Geochimica et Cosmochimica Acta* **62**: 3239-3257.

Galat D, Lider E, Vigg S & Robertson S (1981) Limnology of a large, deep, North American terminal lake, Pyramid Lake, Nevada, USA. *Salt Lakes*,(Lerman A, ed.) pp. 281-317. Springer, New York.

Galat DL, Verdin JP & Sims LL (1990) Large-scale patterns of *Nodularia spumigena* blooms in Pyramid Lake, Nevada, determined from Landsat imagery: 1972–1986. *Hydrobiologia* **197**: 147-164.

Garrity G, Brenner D, Krieg N & Staley J (2005) *Bergey's Manual of Systematic Bacteriology, Volume Two: The Proteobacteria, Part C: The Alpha-, Beta-, Delta-, and Epsilonproteobacteria.* Springer, New York.

Gilbert JA, Jansson JK & Knight R (2014) The Earth Microbiome project: successes and aspirations. *BMC biology* **12**: 69.

Glaring MA, Vester JK, Lylloff JE, Al-Soud WA, Sørensen SJ & Stougaard P (2015) Microbial Diversity in a Permanently Cold and Alkaline Environment in Greenland. *PloS one* **10**.

Grant W (2006) Alkaline environments and biodiversity. *Extremophiles.* Encyclopedia of Life Support Systems (EOLSS) Publishers, Oxford, UK.

Habicht KS & Canfield DE (2001) Isotope fractionation by sulfate-reducing natural populations and the isotopic composition of sulfide in marine sediments. *Geology* **29**: 555-558.

Hammer UT (1986) *Saline lake ecosystems of the world*. Dr W. Junk Publishers, Dordrecht, The Netherlands.

Heinrich-Salmeron A, Cordi A, Brochier-Armanet C, Halter D, Pagnout C, Abbaszadeh-Fard E, Montaut D, Seby F, Bertin PN, Bauda P & Arsene-Ploetze F (2011) Unsuspected diversity of arsenite-oxidizing bacteria revealed by a widespread distribution of the aoxB gene in prokaryotes. *Applied and environmental microbiology* **77**: 4685-4692.

Hoeft SE, Kulp TR, Stolz JF, Hollibaugh JT & Oremland RS (2004) Dissimilatory arsenate reduction with sulfide as electron donor: experiments with mono lake water and isolation of strain MLMS-1, a chemoautotrophic arsenate respirer. *Applied and environmental microbiology* **70**: 2741-2747.

Hollibaugh J, Carini S, Gürleyük H, Jellison R, Joye SB, LeCleir G, Meile C, Vasquez L & Wallschläger D (2005) Distribution of arsenic species in alkaline, hypersaline, Mono Lake, California and response to seasonal stratification and anoxia. *Geochimica Et Cosmochimica Acta* **69**: 1925-1937.

Hollibaugh JT, Wong PS, Bano N, Pak SK, Prager EM & Orrego C (2001) Stratification of microbial assemblages in Mono Lake, California, and response to a mixing event. *Hydrobiologia* **466**: 45-60.

Horne A & Galat D (1985) Nitrogen fixation in an oligotrophic, saline desert lake: Pyramid Lake, Nevada. *Limnology and oceanography* **30**: 1229-1239.

Hostetler S (1991) Simulation of lake ice and its effect on the late-Pleistocene evaporation rate of Lake Lahontan. *Climate Dynamics* **6**: 43-48.

Hostetler S, Giorgi F, Bates G & Bartlein P (1994) Lake-atmosphere feedbacks associated with Paleolakes Bonneville and Lahontan. *Science* **263**: 665-668.

Hou W, Wang S, Dong H, *et al.* (2013) A comprehensive census of microbial diversity in hot springs of Tengchong, Yunnan Province China using 16S rRNA gene pyrosequencing. *PloS one* **8**: e53350.

Houghton JG, Sakamoto CM & Gifford RO (1975) *Nevada's weather and climate*. NV Bureau of Mines & Geology.

Human Microbiome Project (HMP) Consortium (2012) Structure, function and diversity of the healthy human microbiome. *Nature* **486**: 207-214.

Humayoun SB, Bano N & Hollibaugh JT (2003) Depth distribution of microbial diversity in Mono Lake, a meromictic soda lake in California. *Applied and Environmental Microbiology* **69**: 1030-1042.

Huse SM, Welch DBM, Voorhis A, Shipunova A, Morrison HG, Eren AM & Sogin ML (2014) VAMPS: a website for visualization and analysis of microbial population structures. *BMC bioinformatics* **15**: 1.

Jones BE, Grant WD, Duckworth AW & Owenson GG (1998) Microbial diversity of soda lakes. *Extremophiles* **2**: 191-200.

Jones MN (1984) Nitrate reduction by shaking with cadmium: alternative to cadmium columns. *Water Research* **18**: 643-646.

Jørgensen NOG, Stepanaukas R, Pedersen A-GU, Hansen M & Nybroe O (2003) Occurrence and degradation of peptidoglycan in aquatic environments. *FEMS Microbiology Ecology* **46**: 269-280.

Joye SB, Connell TL, Miller LG, Oremland RS & Jellison RS (1999) Oxidation of ammonia and methane in an alkaline, saline lake. *Limnology and oceanography* **44**: 178-188.

Kalff J (2002) *Limnology: inland water ecosystems*. Prentice Hall New Jersey.

Karlson AM, Nascimento FJ, Suikkanen S & Elmgren R (2012) Benthic fauna affects recruitment from sediments of the harmful cyanobacterium *Nodularia spumigena*. *Harmful Algae* **20**: 126-131.

Kernan MR, Battarbee RW & Moss B (2010) *Climate change impacts on freshwater ecosystems*. Wiley Online Library.

Klatt JM, Al-Najjar MA, Yilmaz P, Lavik G, de Beer D & Polerecky L (2015) Anoxygenic photosynthesis controls oxygenic photosynthesis in a cyanobacterium from a sulfidic spring. *Applied and environmental microbiology* **81**: 2025-2031.

Klepac-Ceraj V, Hayes CA, Gilhooly WP, Lyons TW, Kolter R & Pearson A (2012) Microbial diversity under extreme euxinia: Mahoney Lake, Canada. *Geobiology* **10**: 223-235.

Kompantseva EI, Komova AV & Sorokin DY (2010) Communities of anoxygenic phototrophic bacteria in the saline soda lakes of the Kulunda steppe (Altai Krai). *Microbiology* **79**: 89-95.

Kostka J & Nealson K (1998) Isolation, cultivation and characterization of iron-and manganese-reducing bacteria. *Techniques in Microbial Ecology* pp. 58-78.

Kozich JJ, Westcott SL, Baxter NT, Highlander SK & Schloss PD (2013) Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. *Applied and environmental microbiology* **79**: 5112-5120.

Krieg NR, Ludwig W, Whitman W, Hedlund BP, Paster BJ, Staley JT, Ward N, Brown D & Parte A (eds.) (2010) *Bergey's manual of systematic bacteriology: Vol. 4, The bacteroidetes, spirochaetes, tenericutes (mollicutes), acidobacteria, fibrobacteres, fusobacteria, dictyoglomi, gemmatimonadetes, lentisphaerae, verrucomicrobia, chlamydiae, and planctomycetes*. Springer-Verlag, New York.

Kuai L, Nair AA & Polz MF (2001) Rapid and simple method for the most-probable-number estimation of arsenic-reducing bacteria. *Applied and environmental microbiology* **67**: 3168-3173.

Kulp T, Han S, Saltikov C, Lanoil B, Zargar K & Oremland R (2007) Effects of imposed salinity gradients on dissimilatory arsenate reduction, sulfate reduction, and other microbial processes in sediments from two California soda lakes. *Applied and environmental microbiology* **73**: 5130-5137.

Lane D (1991) 16S/23S rRNA sequencing. Vol. 5 (M. Goodfellow & Stackebrandt E, eds.), pp. 125-175. Wiley, Chichester, UK.

Lefticariu L, Pratt LM & Ripley EM (2006) Mineralogic and sulfur isotopic effects accompanying oxidation of pyrite in millimolar solutions of hydrogen peroxide at temperatures from 4 to 150 C. *Geochimica et Cosmochimica Acta* **70**: 4889-4905.

Liddicoat M, Tibbits I & Butler E (1975) The determination of ammonia in seawater. *Limnology and Oceanography* **20**: 131-132.

Lopes TJ & Medina RL (2007) Precipitation zones of west-central Nevada. *Journal of the Nevada Water Resources Association* **4**: 1-18.

Lopes TJ & Smith JL (2007) *Bathymetry of Walker Lake, West-Central Nevada*. US Department of the Interior, US Geological Survey.

Lopes TJ & Allander KK (2009) *Water budgets of the Walker River Basin and Walker Lake, California and Nevada*. US Geological Survey.

Lopes TJ & Allander KK (2009) *Hydrologic setting and conceptual hydrologic model of the Walker River Basin, west-central Nevada*. US Geological Survey.

Lozupone C, Lladser ME, Knights D, Stombaugh J & Knight R (2011) UniFrac: an effective distance metric for microbial community comparison. *The ISME journal* **5**: 169.

MacGregor BJ, Moser DP, Baker BJ, Alm EW, Maurer M, Nealson KH & Stahl DA (2001) Seasonal and spatial variability in Lake Michigan sediment small-subunit rRNA concentrations. *Applied and Environmental Microbiology* **67**: 3908-3922.

Madigan MT, Martinko JM, Dunlap PV & Clark DP (2009) *Brock Biology of Microorganisms* Benjamin Cummings.

Marioni NK, Tracy CR & Zimmerman LC (2005) Effects of declining lake levels on fish populations: Lahontan cutthroat trout and tui chub in Walker Lake, NV. *Integrative and Comparative Biology* **45**: 1037-1037.

McDonald D, Price MN, Goodrich J, Nawrocki EP, DeSantis TZ, Probst A, Andersen GL, Knight R & Hugenholtz P (2012) An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. *The ISME Journal* **6**: 610-618.

Mesbah NM, Abou-El-Ela SH & Wiegel J (2007) Novel and unexpected prokaryotic diversity in water and sediments of the alkaline, hypersaline lakes of the Wadi An Natrun, Egypt. *Microbial Ecology* **54**: 598-617.

Mesbah NM, Hedrick DB, Peacock AD, Rohde M & Wiegel J (2007) *Natranaerobius thermophilus* gen. nov., sp. nov., a halophilic, alkalithermophilic bacterium from soda lakes of the Wadi An Natrun, Egypt, and proposal of Natranaerobiaceae fam. nov. and Natranaerobiales ord. nov. *International journal of systematic and evolutionary microbiology* **57**: 2507-2512.

Mono Basin Ecosystem Study (MBES) Committee (1987) *The Mono Basin Ecosystem: Effects of Changing Lake Level*. National Academies Press.

Mono Lake Committee (2016) www.monolake.org. Accessed: January 24, 2016.

Narasingarao P & Häggblom MM (2007) *Pelobacter seleniigenes* sp. nov., a selenate-respiring bacterium. *International journal of systematic and evolutionary microbiology* **57**: 1937-1942.

National Park Service US Department of the Interior (2016) The Great Basin. https://www.nps.gov/grba/planyourvisit/the-great-basin.htm. Accessed: February 7, 2016.

Nealson KH & Saffarini D (1994) Iron and manganese in anaerobic respiration - environmental significance, physiology, and regulation. *Annual Review of Microbiology* **48**: 311-343.

Neuwirth E (2014) RColorBrewer: ColorBrewer palettes. *R package version 1.1-2*.

Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH & Wagner H (2015) vegan: Community Ecology Package. R package version 2.0-10.

Oremland RS & Stolz JF (2005) Arsenic, microbes and contaminated aquifers. *Trends in Microbiology* **13**: 45-49.

Oremland RS, Stolz JF & Hollibaugh JT (2004) The microbial arsenic cycle in Mono Lake, California. *FEMS Microbiology Ecology* **48**: 15-27.

Oremland RS, Kulp TR, Blum JS, Hoeft SE, Baesman S, Miller LG & Stolz JF (2005) A microbial arsenic cycle in a salt-saturated, extreme environment. *Science* **308**: 1305-1308.

Parsons T, Maita Y & Lalli C (1984) A Manual of Chemical and Biological Methods for Seawater Analyses. Pergamon Press, Oxford.

Planer-Friedrich B, Suess E, Scheinost AC & Wallschläger D (2010) Arsenic speciation in sulfidic waters: reconciling contradictory spectroscopic and chromatographic evidence. *Analytical chemistry* **82**: 10228-10235.

Planer-Friedrich B, London J, McCleskey RB, Nordstrom DK & Wallschläger D (2007) Thioarsenates in geothermal waters of Yellowstone National Park: determination, preservation, and geochemical importance. *Environmental science & technology* **41**: 5245-5251.

Ploner A (2012) Heatplus: Heatmaps with row and/or column covariates and colored clusters. *R package version* **2**.

Price MN, Dehal PS & Arkin AP (2010) FastTree 2–approximately maximum-likelihood trees for large alignments. *PloS one* **5**: e9490.

R Core Team (2014) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/.

Rees HC, Grant WD, Jones BE & Heaphy S (2004) Diversity of Kenyan soda lake alkaliphiles assessed by molecular methods. *Extremophiles* **8**: 63-71.

Reuter J & Goldman C (1990) Water quality of selected Nevada Lakes with special emphasis on investigating the impacts of nutrient loading on phytoplankton growth in Pyramid Lake. *Submitted to U.S. EPA Region 9 and U.S. EPA Clean Lakes Office*.

Rideout JR, He Y, Navas-Molina JA, Walters WA, Ursell LK, Gibbons SM, Chase J, McDonald D, Gonzalez A & Robbins-Pianka A (2014) Subsampled open-reference clustering creates consistent, comprehensive OTU definitions and scales to billions of sequences. *PeerJ* **2**: e545.

Russell IC (1885) *Geological history of Lake Lahontan: a Quaternary lake of northwestern Nevada*. US Government Printing Office.

Russell IC (1895) *Present of Extinct Lakes of Nevada*. American Book Company.

Salmassi TM, Venkateswaren K, Satomi M, Nealson KH, Newman DK & Hering JG (2002) Oxidation of arsenite by *Agrobacterium albertimagni*, AOL15, sp. nov., isolated from Hot Creek, California. *Geomicrobiology Journal* **19**: 53-66.

Scanlan DJ & West NJ (2002) Molecular ecology of the marine cyanobacterial genera *Prochlorococcus* and *Synechococcus*. *FEMS Microbiology Ecology* **40**: 1-12.

Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ, Sahl JW, Stres B, Thallinger GG, Van Horn DJ & Weber CF (2009) Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and Environment Microbiology* **75**: 7537-7541.

Shannon CE & Weaver W (1949) The mathematical theory of communication. University of Illinois Press, Urbana, IL.

Sigee DC (2005) Freshwater Microbiology: Biodiversity and Dynamic Interactions of Microorganisms in the Aquatic Environment. Wiley, Chichester, UK.

Simpson EH (1949) Measurement of diversity. *Nature* **163**: 688.

Solórzano L (1969) Determination of ammonium in natural waters by the phenolhypochlorite method. *Limnology and Oceanography* **14**: 799-801.

Sorokin DY & Kuenen JG (2005) Haloalkaliphilic sulfur-oxidizing bacteria in soda lakes. *Fems Microbiology Reviews* **29**: 685-702.

Sorokin DY & Kuenen JG (2005) Chemolithotrophic halo alkaliphiles from soda lakes. *Fems Microbiology Ecology* **52**: 287-295.

Sorokin DY, van Pelt S, Tourova TP & Evtushenko LI (2009) *Nitriliruptor alkaliphilus* gen. nov., sp. nov., a deep-lineage haloalkaliphilic actinobacterium from soda lakes capable of growth on aliphatic nitriles, and proposal of Nitriliruptoraceae fam. nov. and Nitriliruptorales ord. nov. *International Journal of Systematic and Evolutionary Microbiology* **59**: 248-253.

Sorokin DY, Gorlenko VM, Tourova TP, Tsapin A, Nealson KH & Kuenen GJ (2002) *Thioalkalimicrobium cyclicum* sp nov and *Thioalkalivibrio jannaschii* sp nov., novel species of haloalkaliphilic, obligately chemolithoautotrophic sulfur-oxidizing bacteria from hypersaline alkaline Mono Lake (California). *International Journal of Systematic and Evolutionary Microbiology* **52**: 913-920.

Sorokin DY, Berben T, Melton ED, Overmars L, Vavourakis CD & Muyzer G (2014) Microbial diversity and biogeochemical cycling in soda lakes. *Extremophiles* **18**: 791-809.

Starr C, Taggart R, Evers C & Starr L (2015) *Biology: The unity and diversity of life*. Nelson Education.

Stauder S, Raue B & Sacher F (2005) Thioarsenates in sulfidic waters. *Environmental science & technology* **39**: 5933-5939.

Suess E, Scheinost AC, Bostick BC, Merkel BJ, Wallschlaeger D & Planer-Friedrich B (2009) Discrimination of thioarsenites and thioarsenates by X-ray absorption spectroscopy. *Analytical chemistry* **81**: 8318-8326.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M & Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular biology and evolution* **28**: 2731-2739.

Taylor REL (1972) Effects of increasing salinity on the Pyramid Lake fishery. *Max C Fleischmann Coll Agric R*.

Thompson RS, Benson L & Hattor EM (1986) A revised chronology for the last Pleistocene lake cycle in the central Lahontan basin. *Quaternary Research* **25**: 1-9.

US EPA (1983) Methods for Chemical Analysis of Water and Wastes. *EPA 600/4-83-020*.

US EPA (1993) Methods for the Determination of Inorganic Substances in Environmental Samples. *EPA 600/R-93-100*.

US EPA (1994) Methods for the Determination of Metals in Environmental Samples-Supplement I. *EPA 600/R-94-111*.

US EPA SW-846. U Test Methods for Evaluating Solid Waste. *EPA SW-846*.

Vavourakis CD, Ghai R, Rodriguez-Valera F, Sorokin DY, Tringe SG, Hugenholtz P & Muyzer G (2016) Metagenomic Insights into the Uncultured Diversity and Physiology of Microbes in Four Hypersaline Soda Lake Brines. *Frontiers in Microbiology* **7**: 211.

Vieites JM, Guazzaroni M-E, Beloqui A, Golyshin PN & Ferrer M (2010) Molecular methods to study complex microbial communities. *Metagenomics: Methods and Protocols* 1-37.

Wahl B, Ernst F, Kumar Y, Müller B, Stangier K & Paprotka T (2014) Defining the Microbial Composition of Environmental Samples Using Next Generation Sequencing. *GATC Biotech AG*.

Wallschläger D & Stadey CJ (2007) Determination of (oxy) thioarsenates in sulfidic waters. *Analytical chemistry* **79**: 3873-3880.

Waterbury JB, Watson SW, Guillard RR & Brand LE (1979) Widespread occurrence of a unicellular, marine, planktonic, cyanobacterium. *Nature* **277**: 293-294.

Wei T (2013) Corrplot: Visualization of a Correlation Matrix. *R package version 0.73*.

Welschmeyer NA (1994) Fluorometric analysis of chlorophyll a in the presence of chlorophyll b and pheopigments. *Limnology and Oceanography* **39**: 1985-1992.

Wetzel RG (2001) *Limnology: Lake and River Ecosystems*. Academic Press, San Diego.

Williams W (ed.) (1981) Inland salt lakes: An introduction. *Salt Lakes: Proceedings of the International Symposium on Athalassic (Inland) Salt Lakes*. pp. 1-14. Springer: Netherlands.

Wright KA (2009) Effects of increasing total dissolved solids on the Walker Lake, Nevada, fishery. *Natural Resources and Environmental Issues* **15**.

Yamada T, Sekiguchi Y, Hanada S, Imachi H, Ohashi A, Harada H & Kamagata Y (2006) *Anaerolinea thermolimosa* sp. nov., *Levilinea saccharolytica* gen. nov., sp. nov. and *Leptolinea tardivitalis* gen. nov., sp. nov., novel filamentous anaerobes, and description of the new classes Anaerolineae classis nov. and Caldilineae classis nov. in the bacterial phylum Chloroflexi. *International journal of systematic and evolutionary microbiology* **56**: 1331-1340.

Yang Z, Graham E & Lyons W (2003) Geochemistry of Pyramid Lake sediments: influence of anthropogenic activities and climatic variations within the basin. *Environmental Geology* **43**: 688-697.

Ye Q, Roh Y, Carroll SL, Blair B, Zhou JZ, Zhang CL & Fields MW (2004) Alkaline anaerobic respiration: Isolation and characterization of a novel alkaliphilic and metal-reducing bacterium. *Applied and Environmental Microbiology* **70**: 5595-5602.

Zavarzina D, Kolganova T, Boulygina E, Kostrikina N, Tourova T & Zavarzin G (2006) *Geoalkalibacter ferrihydriticus* gen. nov. sp. nov., the first alkaliphilic representative of the family Geobacteraceae, isolated from a soda lake. *Microbiology* **75**: 673-682.

CURRICULUM VITAE

Katherine L. Willever

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EDUCATION

University of Nevada, Las Vegas August 2016
M.S., Biological Sciences—Concentration in Microbiology

York College of Pennsylvania May 2012
B.S., Biological Sciences—Minor in Mathematics

RESEARCH EXPERIENCE

Desert Research Institute August 2012 — August 2016
Graduate Research Assistant

- Master's Thesis title: "Biogeochemistry of a saline, alkaline, terminal lake ecosystem in transition; Walker Lake, Nevada, USA"—Principal Investigator: Dr. Duane P. Moser
- This study analyzed and documented microbial and limnological shifts within a terminal lake over a period of critical change resulting from anthropogenic and climatic factors
- Cultured various microbial physiotypes from environmental samples, extracted and analyzed DNA sequences, organized and executed field sampling expeditions, maintained laboratory equipment

Rutgers University of New Jersey, Camden May 2010 — August 2010
NSF Research Experiences for Undergraduates (REU)—Research Assistant

- Participated in an intense 10-week summer program learning laboratory techniques, hypothesis-driven experimental design, and DNA sequence data analysis
- Collected leech specimens from lakes and ponds and conducted morphologic and phylogenetic identification and facilitated in the discovery of new leech species

TEACHING EXPERIENCE

University of Nevada, Las Vegas May 2015—July 2015
Microbiological Laboratory Teaching Assistant

- Instructed undergraduate microbiology laboratory section and supervised laboratory experiments
- Generated lecture materials, graded exams and quizzes, and recorded students' course grades

York College of Pennsylvania August 2009—May 2012
Mathematics Tutor in the Center for Teaching and Learning

- Tutored college students in variety of mathematic subjects, including, but not limited to: Calculus I & II, Statistics, Algebra, and Critical Thinking and Problem Solving
- Adapted to individual needs of students to help them develop and strengthen their math skills

RELEVANT SKILLS

- Aerobic and anaerobic microbial cell isolation, culturing techniques, and microscopy
- Extraction, amplification, and analysis of microbial DNA sequences, Illumina DNA sequencing of 16S rRNA gene
- Extensive field work and sensitive sample collection from aquatic and desert environments
- Enthusiastic and motivated person who works efficiently as part of a team and individually

PRESENTATIONS AND POSTERS

Willever, K.L. 2015. Microbial biogeochemistry and limnology of a transitioning terminal lake ecosystem; Walker Lake, NV. Graduate Research Colloquium, University of Nevada, Las Vegas, NV. Oral presentation, October 2015.

Willever, K.L., Khan, M., Hamilton-Brehm, S.D., Campbell, J.H., Sackett, J.D., Moser, D.P. 2014. Microbial biogeochemistry of a transitioning terminal lake ecosystem; Walker Lake, NV. 99th Ecological Society of America, Annual Meeting, Sacramento, CA. Oral presentation, OOS 49-2. Poster, PS 50-61.

Willever, K.L. 2013. Microbial biogeochemistry and limnology of Walker Lake, NV. Graduate Research Colloquium, University of Nevada, Las Vegas, NV. Oral presentation, September 2013.

PUBLICATIONS

Fisher, J.C., **Willever, K.L.**, Bruckner, J.C., Sackett, J.D., Pratt, L.M., Planer-Friedrich, B., Campbell, J.H., Hershey, R.L., Memmott, J., Fritsen, C.H., Dodsworth, J.A., Moser, D.P. 2016. Limnological characterization and microbial biogeochemistry of a terminal lake ecosystem; Walker Lake, Nevada. (*in preparation*)

Willever, K.L., Sackett, J.D., Fisher, J.C., Moser, D.P. 2016. Shifting microbial biogeochemistry and limnology of a transitioning terminal lake ecosystem; Walker Lake, Nevada. (*in preparation*)

Willever, K.L. 2016. Biogeochemistry of a saline, alkaline, terminal lake ecosystem in transition; Walker Lake, Nevada, USA. Master's Thesis from University of Nevada, Las Vegas. (*in preparation*)

PROFESSIONAL ASSOCIATIONS AND VOLUNTEER ACTIVITIES

- Ecological Society of America (ESA) (2013—Present)
- American Society for Microbiology (ASM) (2013—Present)
- Vice President of UNLV ASM Student Chapter (2013—2015)
- Externship with Pennsylvania Dept. of Conservation and Natural Resources (DCNR) Bureau of Forestry (June 2011—August 2011)