UNIVERSITY OF CALIFORNIA, MERCED

Diversity and Distribution of Archaea and Bacteria in Marine Lakes, Palau

A Thesis submitted in partial satisfaction of the requirements for the degree Master of Science

in

Environmental Systems

by

Matthew Meyerhof

Committee in Charge:

Professor Michael Beman, Chair
Professor Michael Dawson
Professor Peggy O'Day

2011

The Thesis of Matthew Meyerhof is approved and it is acceptable in quality and form for publication on microfilm and electronically:

Chair

University of California, Merced

2011

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGEMENTS

I would like to acknowledge Professor Michael Beman for his advising, mentoring, and support during graduate school as well as being the chair of my committee. During these last two years, his guidance and assistance has been invaluable to my success in the Master's program.

I would also like to acknowledge Professor Michael Dawson for introducing me to Palau and assisting me and Professor Michael Beman in the field during our research trip in 2010. His extensive knowledge about Palau helped make the research presented below possible.

I would also like to acknowledge Professor Peggy O'Day for teaching me the fundamentals of environmental chemistry – knowledge that greatly helped me understand the chemical components of Palau.

Lastly, I would like to extend a special thank you to Pat and Lori Colin of the Coral Reef Research Foundation (CRRF) for providing laboratory space, logistical support, and access to field sites; Emilio, Gerda, and Matt (CRRF), Sharon Patris (UC Merced), and Herwig Stibor (University of Brest) for their invaluable help in the field; Susan Alford, Daniel Santillano, Rodney Severson, Jesse Wilson, and Lolo Cardenas for their assistance with laboratory analyses; and, finally, the UC Merced Graduate Research Council for providing funds to support this research.

ABSTRACT OF THE THESIS

Diversity and Distribution of Archaea and Bacteria in Marine Lakes, Palau

by

Matthew Meyerhof

Masters of Science in Environmental Systems

University of California, Merced, 2011

Professor Michael Beman, Chair

Archaea and bacteria affect the chemistry, biology, and ecology of ecosystems around the world. With anthropogenic activities altering terrestrial and aquatic systems (e.g. fertilizer runoff changing the chemistry of coastal marine waters), knowledge about the tolerances of microbes to changing environmental conditions is critical. While having been extensively studied in saline and marine systems (e.g. oxygen minimum zones, the Black Sea, and Mono Lake), microbes are not well understood in marine lakes, especially those located in Palau. The marine lakes of Palau are ideal for microbial ecology because these unique habitats act like oceanic mesocosms – making studies of microbes found in other saline systems (e.g. the ocean) much more feasible. Using Automated Ribosomal Intergenic Spacer Analysis (ARISA) and a next generation

sequencing technique, 454 pyrosequencing, the richness, diversity, and composition of microbial communities in the marine lakes was analyzed. Lake bacterial groups showed a strongly supported dissimilarity (100% for ~11% similarity) between those from all but one low oxygen (<5 μM) depth and those from all but two higher oxygen (>10 μM) depths, and bacterial community similarities decreased with increasing depth (decreasing oxygen) in the meromictic lakes. While some bacterial groups were ubiquitous among the lakes, ones that participate in biogeochemical cycling of carbon, nitrogen, and sulfur were found in the low oxygen (<5 μM) waters of the meromictic lakes. This research helps show the significance of marine lakes to microbial ecology as well as to understanding other saline systems.

1 Introduction: Microbes, marine ecosystems, and saline lakes

Microorganisms are Earth's unseen majority. Their sheer abundance, vast diversity, and large range of metabolic functions allow them to survive in any location on the globe (reviewed in Azam and Worden 2004). Microbes have been found to affect ecosystems chemically, biologically, and ecologically – altering the flow of vital elements through natural systems, and controlling the stability of food webs (Falkowski et al. 2008, Fuhrman 2009). In recent times, we have seen our dynamic planet change due to anthropogenic alteration of ecosystems (e.g. coral reefs, Hughes et al. 2003), biogeochemical cycles (e.g. phosphorus, Bennett et al. 2001), and entire oceans (reviewed in Halpern et al. 2008). Information about how microbes will respond to changing environmental conditions (e.g. ocean acidification, Beman et al. 2011; Joint et al. 2011) is therefore critical. The purpose of my research is to compare microbial communities within and among marine lakes in Palau using two DNA sequencing techniques and understand how environmental components correspond to these microbial communities. Marine lakes are ideal locations for uncovering this information about microbes because they act as oceanic mesocosms that can be studied more easily than the open ocean. I provide background into oxygen minimum zones (OMZs), the Black Sea, and Mono Lake below as a prelude to the main chapter of my thesis. The purpose of this chapter is to summarize what is currently known about these three well-studied systems as a means to introduce a comparable, less-studied environment – the marine lakes of

Palau – that can help us improve our understanding of microbes and their tolerances to dynamic chemical conditions in aquatic ecosystems, including the ocean.

1.1 Background – Marine Ecosystems

The oceans and other marine ecosystems vary greatly in physical and geochemical structure; these two factors subsequently shape the biota of the local ecosystems, as well as ecosystem processes. For example, Beman et al. (2008) linked the concentrations of different nitrogen species with abundances of microbial participants in ammonia oxidation throughout the water columns of two marine basins in the Gulf of California, and found that gene copies for archaeal ammonia oxidation were very highly correlated with ammonium oxidation rates, suggesting archaeal involvement in oceanic nitrification. Lin et al. (2006) demonstrated differences in microbial distributions with oxygen between and throughout the Black Sea and Cariaco Basin. Furthermore, Fry et al. (1991) analyzed the chemical differences between these marine systems while Kessler et al. (2006) discussed how these environments differ in their physical structures. Additionally, the fine-scale environments within marine systems can be drastically different from each other even though they may only be centimeters apart (e.g. Owen 1989 and Steinbuck et al. 2010). Future global changes to biogeochemical cycles may also be mitigated by microbes (Canfield et al. 2010). Focusing studies on the largest ecosystem in the world – the ocean – and the most dominant organisms on Earth – microbes – will therefore enhance our understanding of future conditions on this ever-changing planet. In turn, the physical, chemical, and biological structures of particular

marine esosystems – such as oxygen minimum zones, saline lakes, and other marine basins – can be studied to understand the complex dynamics of the ocean.

1.1.1 Oxygen Minimum Zones

Chemical gradients characterize the interface between oxygenic and anoxygenic waters in marine and freshwater habitats (reviewed in Overmann et al. 1999). While some large organisms avoid areas of oxygen deficiency (or oxygen minimum zones), lending them the name 'dead zones,' microbial communities flourish in these pockets of the water column (Levin 2003). Oxygen minimum zones (OMZs) are found in the highly productive, upwelling regions located along western coastlines and in other marine systems (e.g. the Black Sea, Murray et al. 1989), and they have drawn attention because they are expanding (Diaz and Rosenberg 2008, Fuenzalida et al. 2008, Stramma et al. 2008). While OMZs are known to fluctuate in size regularly over geological time (Levin 2003), they have expanded exponentially since the 1960s (Diaz and Rosenberg 2008) – raising much concern about the survival of the macrobiota in the surrounding water (Levin 2003).

One major cause of deoxygenation in the ocean is climate change, but the two proximate causes include (1) gas solubility decreasing with increasing temperature according to Henry's Law and (2) warmer temperatures increasing stratification of the upper ocean and preventing circulation of oxygen to deeper depths (Keeling and Garcia 2002). Oxygen concentrations are higher in the upper and deeper waters of the ocean due to strong ventilation and lower rates of utilization, respectively (Keeling et al. 2010). Models predict that movement of oxygen to the ocean's deep waters will be affected more than utilization of oxygen in the deep waters – therefore, the ocean interior will

exhibit a decrease of oxygen over time as long as stratification of the ocean increases from sea surface temperatures increasing (Keeling and Garcia 2002, Keeling et al. 2010). Some evidence for this already exists (Bograd et al. 2008, Chan et al. 2008, Stramma et al. 2008, Barth et al. 2009). Nutrient transport and vertical mixing of inorganic compounds will also slow as stratification strengthens (Keeling et al. 2010). Moreover, oxygen depletion in the ocean would cause nitrate (and sulfate if conditions persist) to become energy sources in the system – biogeochemical cycles will therefore be changed as oxygen concentrations decrease at depth (Canfield et al. 2010, Keeling et al. 2010, Deutsch et al. 2011).

Before oxygen entered the atmosphere and was utilized in the ocean, electron acceptors and donors containing nitrogen, sulfur, and iron were substrates for metabolism in the ancient ocean (> 2.8-2.2 billion years ago; Crowe et al. 2008, Sleep and Bird 2008, Godfrey and Falkowski 2009, Johnston et al. 2009, Koehler et al. 2010). Anoxygenic photosynthesis predated oxygenic photosynthesis as the oxidation of sulfide and ferrous iron/ferrous oxide (Crowe et al. 2008, Falkowski et al. 2008, Sleep and Bird 2008). As oxygen decreases, denitrification (conversion of nitrate to dinitrogen gas) begins to dominate – interfering with the balance between nitrogen fixation and denitrification and leading to nitrogen loss from the ocean (reviewed in Bazylinski and Blakemore 1983, Gruber and Galloway 2008). As oxygen and nitrogen decrease in the ocean, certain anoxygenic photoautotrophs (including physiologically versatile cyanobacteria) can switch from oxygenic to anoxygenic photosynthesis (reviewed in Johnston et al. 2009). As Crowe et al. (2008) show, some aquatic systems (including the Black Sea, discussed

below) are natural environments that are representative of how the ocean was in the past and what portions of it may become as deoxygenation progresses.

As OMZs expand, they greatly limit the area in which organisms that require oxygen can persist – eventually changing the dynamics of marine food webs. Furthermore, the microbial communities of the surrounding water begin to change as oxygen becomes depleted due to physical processes (e.g. more intense upwelling) and respiration by aerobic organisms (Fuenzalida et al. 2008, Deutsch et al. 2011). Shifts in oxygen concentrations may modify the diversity of microbial communities and will regulate what chemical processes take place. Oxygen gets replaced by nitrate as the preferred compound for respiration – this reduction of nitrate (denitrification or anammox) can only occur in suboxic and anoxic conditions, representing an example of how microbial communities will change as oxygen concentrations decrease in and around OMZs (Stramma et al. 2008, Zehr 2009, Deutsch et al. 2011).

1.1.2 Saline Systems

By determining how microbial community composition and geochemical conditions change with depth in well-oxygenated and stratified systems, marine lakes can potentially provide us with an understanding of how microbial communities associated with OMZs will change as OMZs continue to expand. Previous studies have examined microbial diversity and activity in a variety of low-oxygen aquatic ecosystems, such as the Cariaco Basin (Taylor et al. 2006), Gulfo Dulce (Ferdelman et al. 2006), Lake Kivu (Sarmento et al. 2008), Lake Nakuru (Ballot et al. 2004), Lake Natron (Yakimov et al. 2001), Lake Magadi (Oren 2002), Lake Assal (Brisou et al. 1974), Lake Kaiike (Koizumi et al. 2004), Solar Lake (Cytryn et al. 2000), The Great Salt Lake (Weimer et al. 2009),

and the Dead Sea (Bodaker et al. 2009). By far, the best-studied systems have similar chemical structures and microbial communities to those of the meromictic marine lakes in Palau: the Black Sea and Mono Lake. Below I review the extensive studies that have examined microbial ecology and biogeochemistry in the Black Sea and Mono Lake.

1.1.2.1 Black Sea

The Black Sea is the largest permanently stratified body of water in the world (Coolen et al. 2007, Lam et al. 2007, Oakley et al. 2007). It is 2200 m deep and has an upper, oxic layer and a lower, sulfidic layer with a suboxic zone intermediate between them at around 50-120 m depth (Coolen et al. 2007, Lam et al. 2007, McCarthy et al. 2007, Oakley et al. 2007). Temperature is highest at the surface (12.2°C) and decreases with depth to about 25 m, where it stays between 7 and 8.5°C throughout the rest of the water column (McCarthy et al. 2007). The bottom of the euphotic zone is located between 20 and 35 m depth (McCarthy et al. 2007). The suboxic layer, or oxygen minimum zone (OMZ), is between 20 and 30 m in thickness, varies in depth and width throughout the basin, and contains low concentrations of both oxygen and sulfide (Coolen et al. 2007). This formation of a permanent pycnocline causes an inhibition of the transportation of dissolved compounds between the deep waters and the surface layer (McCarthy et al. 2007, Oakley et al. 2007). The Black Sea has a very restricted water channel that connects it to the ocean. A surface outflow of brackish (18-20 ppt) water leaves the basin through the Turkish Straits, which flow into the Aegean and Mediterranean seas; denser Mediterranean Sea water (36-38 ppt) flows into the Black Sea via the same outflow channel and sinks, maintaining the intermediate and deep layers at a

constantly higher salinity (approximately 22 ppt) than the surface waters (McCarthy et al. 2007).

The chemistry of the Black Sea varies with depth and influences microbial processes in the basin (e.g. nitrogen cycling processes, nitrification and denitrification). Oxygen is highest at the surface (200 – 325 μM) and decreases with depth until it reaches 0 μM at the beginning of the sulfide layer (Lam et al. 2007, McCarthy et al. 2007). However, Lam et al. (2007) reported a spike in oxygen at 20 m depth that was almost double the surface concentration at the time. Also, nitrite varies over time throughout the water column: sometimes, it has a major spike in or above the OMZ (Coolen et al. 2007); other times, it has minor, if any, peaks in the water column (Lam et al. 2007, McCarthy et al. 2007). Nitrate tends to have one major peak either in or directly above the OMZ. Ammonium is undetectable in the oxic layer (Lam et al. 2007), and ammonium and sulfide increase with depth below the OMZ to concentrations above 6 μM (McCarthy et al. 2007).

Nitrification and denitrification have been observed in detail in the Black Sea. Nitrification is assumed to occur at the intermediate depths above the OMZ due to the consistent nitrite and nitrate maxima (McCarthy et al. 2007). Additionally, ammonia oxidation was reported to be highest at 40 m (McCarthy et al. 2007). Heterotrophic denitrification has been observed through the appearance of a secondary nitrite peak that seems to be coupled to nitrate reduction (Oakley et al. 2007). Denitrification causes the nitrate levels to decrease with depth below the OMZ (McCarthy et al. 2007). The community of denitrifying microbes appears to be low in species richness – with only a few dominating sequence types – which may be because of the limited supply of nitrite

and nitrate as terminal electron acceptors for denitrifying microbes (Oakley et al. 2007). Another possibility is that only a few species are able to tolerate the fairly consistent, intermediate salinity of the deeper waters (the bottom layer is not as saline as the ocean but not as fresh as a lake).

Microbial activities are not limited to only nitrification and denitrification in the Black Sea (Wakeham et al. 2007). Aerobic bacterial oxidation of methane and sulfide has been observed to occur at the chemocline (Wakeham et al. 2007, Pape et al., 2008). Bacterial sulfate reduction and anaerobic archaeal methane oxidation have been found to exist in the bottom, anoxic layer of the Black Sea (Wakeham et al. 2007), and anaerobic ammonia oxidation (anammox) occurs in the suboxic waters of the Black Sea (Lam et al. 2007). Anammox may account for between 10 and 15% of the dinitrogen gas production in the Black Sea (Kuypers et al. 2003).

1.1.2.2 Mono Lake

Mono Lake is not marine, but is an alkaline (pH 9.8), hypersaline, seasonally meromictic basin that has no connection to the ocean and is located in eastern California near the Sierra Nevada Mountains (Joye et al. 1999, Ward et al. 2000, Humayoun et al. 2003, Carini and Joye 2008). Stratification is caused by the saline lake water and in-flowing freshwater creating separate layers, and by the lack of mixing events – which are more common in estuaries and the ocean (Humayoun et al. 2003). This creates an oxic-anoxic interface that ranges between 12 and 17 m depth throughout the year (Ward et al. 2000). Additionally, surface temperature is highest in the summer (21.5°C) and lowest during the winter (6°C) (Joye et al. 1999). Temperature decreases with depth to 3.5°C in the summer and 1.5°C in the winter (lowest temperature reported at 25 m by Joye et al.

1999). The thermocline is steep between 9 and 24 m, and the chemocline exists between 23 and 26 m in the anoxic layer (Humayoun et al. 2003).

Mono Lake has oxygen concentrations higher at the surface during the spring (May, 7 mg O_2 L^{-1}) than the winter (November, 3.5 mg O_2 L^{-1}) or summer (August, 4 mg O_2 L^{-1}; Carini and Joye 2008), but always decreases with depth to 0 μM at no deeper than 25 m (Joye et al. 1999). Furthermore, ammonium concentrations are low (0 – 2 μM) in the oxic layer (Joye et al. 1999, Carini and Joye 2008), and ammonium and sulfide increase with depth starting at 17 – 20 m and reach their maxima at 35 m depth (Joye et al. 1999, Humayoun et al. 2003, Carini and Joye 2008). Nitrate concentrations are highest between ~13 and 14 m depth (~350-490 nmol L^{-1}), and nitrous oxide are highest between 12 and 14 m depth (~30-40 nmol L^{-1}; Carini and Joye 2008).

Nitrification appears to be more prevalent than denitrification in Mono Lake. Ammonia oxidation seems to be very important in Mono Lake (Joye et al. 1999), and light and oxygen concentrations do not appear to obstruct ammonia oxidation in the euphotic zone, as it dominates the surface waters and the oxycline (Joye et al. 1999). The relative distribution of ammonia and ammonium due to the fairly high pH of the water favors ammonia oxidation (Joye et al. 1999). Also, the high temperatures of the euphotic zone may stimulate an increase in the activity of ammonia oxidation. The rate of ammonia oxidation maximizes at 13 – 14 m depth (Carini and Joye 2008), and ammonia-oxidizing bacteria (AOB) found in Mono Lake include β-proteobacteria that can tolerate the high pH and high salinity of the system (Ward et al. 2000, Carini and Joye 2008). Other bacteria that are common in Mono Lake include Actinobacteria, Flexibacter-Bacteroides, Bacillus, and Clostridium (Humayoun et al. 2003). Even though archaeal

amoA genes were not detected in Mono Lake, there seems to be potential for archaea to exist in the basin (Carini and Joye 2008). Moreover, there appears to be a higher diversity of microbes in the anoxic layer compared to the oxic layer, possibly due to there being a higher availability of niches in the bottom waters than the surface waters (Humayoun et al. 2003). According to Jellison et al. (1993), denitrification in Mono Lake is probably low due to the low concentrations of nitrite and nitrate.

Similar to the Black Sea, other microbial activities have been reported for Mono Lake. High methane oxidation rates are caused by type I and II methanotrophs (Lin et al. 2005). Type I methanotrophs are the most abundant and include *Methylobacter*, *Methylomicrobium*, and *Methylothermus*, whereas Type II consists of *Methylocystis* (Lin et al. 2005). Both aerobic and anaerobic methane oxidation seems to occur in Mono Lake (Lin et al. 2005, McGenity 2010).

The importance of studying other saline ecosystems like seas and saline lakes is that they provide smaller scale versions of the ocean. While these natural mesocosms may be different from one another, there are similarities (e.g. microbial activities) that can be observed. Finding similarities and differences among saline systems (e.g. seas and saline lakes) is like comparing and contrasting various regions of the ocean to one another; Palau's marine lakes offer a variety of geochemical conditions, many replicates, and, because they contain seawater and ocean-derived organisms, they may accurately reflect conditions in the ocean. Therefore, understanding the characteristics of marine lakes in Palau can be useful for comparing the chemistry and biology of this habitat to other saline systems – and, in turn, for gaining more knowledge about how the diverse habitats of the ocean may operate.

1.2 Microbial Ecology of Marine Lakes, Palau

Palau is a group of islands located in the Indo-Pacific that contain ~55 marine lakes (Hamner et al. 1982, Hamner and Hamner 1998). As sea level rose following the last glaciation (~19000 to 20000 years ago), seawater flooded depressions within the islands via tunnels and cracks in the karst structures (Dawson 2006). Over time, these depressions became the marine lakes, which have either holomictic or meromictic features, or a combination of holomictic, meromictic, and/or ocean characteristics (Hamner et al. 1982, Hamner and Hamner 1998). Also, the marine lakes are each their own unique marine environments, ranging in physical features, chemical conditions, and biological diversity (Muscatine and Marian 1982, Landing et al. 1991, Venkateswaran et al. 1993, Hamner and Hamner 1998, Dawson et al. 2001, Dawson and Hamner 2003, Dawson and Hamner 2005). While macroorganisms that inhabit the lakes have been studied (e.g. jellyfish, Dawson et al. 2001, Dawson and Hamner 2003, Dawson and Hamner 2005), the microorganisms that populate the water column have not received much attention (Venkateswaran et al. 1993). Chemical conditions found in Palauan meromictic lakes (e.g. Jellyfish Lake, Landing et al. 1991), which resemble those found in other saline systems like the Black Sea and Mono Lake, are ideal locations for understanding how microbes can tolerate chemical gradients. Comparison of meromictic lakes with holomictic lakes can provide information about how microbial communities and geochemistry vary from upper, well-oxygenated open ocean environments to oxygen-deficient (OMZ) ecosystems. Studying the dynamics of microbial communities and geochemistry in these oceanic mesocosms will make understanding the present and

potentially future conditions of the world's largest saline system – the ocean – much more feasible.

Using these lakes as model systems for microbial ecology research, my thesis seeks to answer the following questions in Chapter 2:

1) What are the most common microbes in the Palauan marine lakes?
2) What microbes are common among lakes?
3) Which microbes differ among lakes?
4) How does the microbial diversity of marine lakes compare to that of other saline systems?

I hypothesized that microbial community composition was predicted to vary among the marine lakes, with some groups being found in all of them (e.g. the SAR11 clade, which is ubiquitous throughout the ocean, Morris et al. 2002). Any major variations in community composition among lakes could be due to differences in chemical conditions – for instance, meromictic lakes would probably have microbes in high abundances, such as in the anoxic waters, that are rare in holomictic lakes because of dramatic differences in their chemistries. Furthermore, with the observed chemical similarities between the meromictic lakes of Palau and the three aforementioned systems (OMZs, the Black Sea, and Mono Lake), microbes found in these three environments are likely to be found in the meromictic lakes of Palau, and possibly in the holomictic ones as well.

2 Pyrosequencing and fingerprinting microbial diversity in holomictic and meromictic 'marine lakes' of Palau

2.1 Abstract

Chemical gradients occur in aquatic systems where oxygen is depleted through microbial activity, such as in oceanic oxygen minimum zones (OMZs). Recent observations show that these regions are expanding and intensifying, reducing potential habitat availability for aerobic macroorganisms, but also altering microbial community composition and activity. Marine lakes are ideal locations in which to examine microbial responses to environmental variability and change, because they offer a spectrum of environmental conditions – from well-mixed holomictic lakes to stratified meromictic lakes – and I sampled 12 such lakes in the Republic of Palau. Samples were analyzed using Automated Ribosomal Intergenic Spacer Analysis (ARISA) and next-generation pyrosequencing of the 16S rRNA gene, and ARISA results demonstrated the breadth of similarities among bacterial communities, ranging from 0.20% to 90.3%. Lake samples showed a strong division between bacterial communities from low oxygen (<5 μM) depths and higher oxygen (>10 μM) depths, and bacterial community similarities declined with increasing depth (decreasing oxygen) in the meromictic lakes. Many samples from oceanic sites, holomictic lakes, Ongael Lake, and T Lake were less similar based on pyrosequencing, suggesting that rare members of the communities differ more

from each other than do the abundant members. I also found that microbial richness and diversity varied according to the environmental conditions of each oceanic site and marine lake. Community composition varied among the sampling locations and samples: while microbial communities were dominated by Cyanobateria and SAR11 bacteria in the holomictic lakes, bacterial groups (such as δ-proteobacteria and members of Planctomycetes) known to participate in the biogeochemical cycling of carbon, nitrogen, and sulfur were found in the low oxygen (<5 μM) waters of the meromictic lakes. My results offer new insight into microbial community composition and diversity in marine lakes, and demonstrate how microbial community composition changes as oxygen concentration decreases in marine environments – a natural depiction of how further ocean deoxygenation may alter microbial communities and biogeochemical cycles in the ocean, possibly with rare members of the community becoming dominant as conditions change. Examples of how the microbial communities and water chemistry could change during ocean deoxygenation are seen at depth in the meromictic lakes – increases in anammox and sulfate-reducing bacterial presence as well as increases in ammonia and phosphate – suggesting that the marine lakes of Palau can help us improve our understanding of analogous environments in the ocean.

2.2 Introduction

Chemical gradients characterize the interface between oxygenic and anoxygenic waters in saline and freshwater habitats (reviewed in Overmann et al. 1999). While large organisms avoid oxygen deficient regions – lending them the name ‘dead zones’ – microorganisms ultimately generate these conditions via consumption of dissolved

oxygen (Breitburg et al. 2010), and diverse microbial communities flourish in these extreme pockets of the water column (Levin 2003, Stevens and Ulloa 2008, Stewart et al. 2011). Oxygen deficiency occurs in many aquatic habitats, including stratified freshwater lakes (Bosshard et al. 2000), saline lakes (Landing et al. 1991, Hamner and Hamner 1998, Cytryn et al. 2000, Koizumi et al. 2004, Sarmento et al. 2006, Carini and Joye 2008), coastal fjords and basins (Ferdelman et al. 2006, Zaikova et al. 2010), and large basins with restricted circulation, such as the Black Sea (Murray et al. 1989) and Cariaco Basin (Scranton et al. 2006). Open ocean oxygen minimum zones (OMZs) are also found beneath productive upwelling zones located along western coastlines and the equator (eastern tropical North Atlantic, central equatorial Atlantic, eastern tropical South Atlantic, eastern equatorial Pacific Ocean, central equatorial Pacific Ocean, and the eastern equatorial Indian Ocean; Stramma et al. 2008, Keeling et al. 2010), and play an important role in global biogeochemical cycles of carbon and nitrogen (Lam and Kuypers 2011).

However, current OMZs are now expanding and intensifying as a consequence of anthropogenic climate change (Diaz and Rosenberg 2008, Fuenzalida et al. 2008, Stramma et al. 2008), while new OMZs appear to be emerging (e.g., off the coast of Oregon; Chan et al. 2008). These changes in ocean chemistry reduce potential habitat area for organisms that require oxygen – probably altering marine food webs (Levin 2003) – but they also affect microbial activity and community composition. As oxygen is depleted, alternative electron acceptors such nitrate, manganese, and sulfate are used by microorganisms (Lam and Kuypers 2011); anaerobic microbial activity subsequently alters local and global biogeochemistry through conversion of dissolved nitrogen to

gaseous forms such as the ozone-depleting greenhouse gas nitrous oxide (Dore et al. 1998, Naqvi et al. 2000, Ravishankara et al. 2009), or through production of toxic reduced sulfur compounds such as hydrogen sulfide (Lavik et al. 2009, Breitburg et al. 2010, Canfield et al. 2010).

Despite their importance, these microbial processes remain little understood in marine ecosystems. Whether denitrification or anaerobic ammonium oxidation (anammox) dominates nitrogen loss in OMZs is actively debated (Lam et al. 2009, Ward et al. 2009, Zehr 2009), while a recent study in the OMZ off Chile uncovered a 'cryptic sulfur cycle' where sulfate was reduced to sulfide and subsequently rapidly oxidized before it could accumulate to detectable levels (Canfield et al. 2010). Understanding these processes will be critical for predicting future biogeochemical consequences of progressive ocean deoxygenation (Keeling et al. 2010), yet comparative studies of low oxygen systems are rare. Lin et al. (2006) compared microbial abundance and community composition of the Black Sea and the Cariaco Basin and found significant differences among their communities despite equivalent geochemical conditions. β-proteobacteria were abundant throughout the water column of the Cariaco Basin, for instance, but were undetectable in the Black Sea. Ward et al. (2009) compared denitrification and anammox rates, and abundances of organisms involved in these processes, in the OMZs of the Arabian Sea and eastern tropical South Pacific Ocean (ETSP), finding that denitrification dominates N loss from the Arabian Sea but not the ETSP. These studies indicate that microbial community composition and activity in low-oxygen waters among systems deserves additional study in the context of ocean

deoxygenation. I used a next-generation pyrosequencing technique to comprehensively analyze such differences in the marine lakes of Palau (discussed below).

Marine lakes offer a natural framework for comparing microbial community ecology along a continuum of deoxygenation. In Palau, there are approximately 55 marine lakes: fully-mixed, ocean-like, holomictic lakes; highly-stratified meromictic lakes; and a group of lakes (such as Ongael Lake and T Lake) that do not fall into either of these two categories (accurate classification of these lakes is currently being developed; Dawson unpublished). Many meromictic and holomictic lakes are present in Palau, creating a continuum of replicated natural experiments (Hamner and Hamner 1998, Dawson and Hamner 2009). Significantly, oxygen decreases with depth in the meromictic lakes to low – and sometimes zero – oxygen concentrations, creating compressed natural gradients ideal for microbial ecology studies (Landing et al. 1991, Venkateswaran et al. 1993, Hamner and Hamner 1998, Dawson and Hamner 2009). Hamner and Hamner (1998) provide an equation for determining the depth of the mixolimnion (regression of depth of the mixolimnion versus maximum depth of the lake: $y = 0.88x – 7.71$, $r^2 = 0.933$). These compressed gradients (meters to tens of meters) can be studied from small boats, making them tractable systems in which to study processes that otherwise occur on scales of hundreds to thousands of meters in the open ocean.

The marine lakes of Palau formed after the last glacial maximum (Dawson 2006). Melting ice sheets raised global sea level >100 m after the last glaciation about 19000-20000 years ago, inundating hundreds of low-lying karstic basins throughout the Indo-Pacific and forming marine lakes – bodies of seawater entirely surrounded by land. The

lakes were inoculated with marine life and isolated to varying degrees for the next 15,000-6,000 years.

Given strong gradients in light, temperature, organic matter, nutrients, and oxygen concentrations with depth in the ocean, observations of the distance-decay pattern are not surprising, and I expect to observe similar distance-decay in marine lakes of Palau. In particular, I expect that the differences in geochemical properties found in meromictic lakes would lead to little compositional overlap among bacterial communities present at different depths. Such patterns have been observed in other stratified, oxygen-deficient marine and saline systems, such as the Black Sea, where most OTUs were found at more than one sampled depth, only two OTUs were detected in every sample collected, and most were confined to anoxic, suboxic, or oxic layers (Vetriani et al. 2003). The Cariaco Basin also shows three distinct layers with distinct microbial communities (Lin et al. 2006, Taylor et al. 2006). Furthermore, Madrid et al. (2001) found that microbial communities present in anoxic waters in the Cariaco Basin resemble those found in other anaerobic habitats. Similar to these marine systems, Mono Lake – a saline, soda lake found in the Eastern Sierra Nevada mountains of California (Jellison and Melack 1993) – has a unique microbial community that inhabits the interface between the oxic and anoxic waters (Humayoun et al. 2003). While Mono Lake undergoes periodic mixing, it exhibits variation in bacterial community composition among different regions of the water column during stratification (Hollibaugh et al. 2001, Humayoun et al. 2003). Adding the Palau's meromictic marine lakes to the comparison, all four systems show substantial variation in bacterial community composition along geochemical gradients – which are proximally generated by the interplay between microbial activity (e.g., consumption of

oxygen, production of hydrogen sulfide) and the physical limnology and oceanography of these lakes and basins. Because previous studies have observed increased richness and evenness with depth in oceanic OMZs (Stevens and Ulloa 2008), I hypothesized that meromictic lakes may display this pattern; conversely, diversity may be highest in surface waters of holomictic lakes, where productivity is greatest and where there are inputs of substrates from land. I also reasoned that bacterial communities present at the oxycline in meromictic lakes commonly experience changes in geochemical conditions that will affect diversity. In this study, I examined microbial diversity and community composition with water chemistry in 12 of the marine lakes in Palau (Figure 2.1).

2.3 Materials and Methods

2.3.1 Study Site

Enclosed by vegetation-covered karst, the marine lakes of Palau have limited connections to the ocean via tunnels and/or fissures in the karst (Hamner and Hauri 1981, Hamner et al. 1982, Hamner and Hamner 1998); heavy precipitation (20-40 cm/month) is another characteristic affecting these isolated tropical ecosystems (Hamner and Hamner 1998). Among lakes, the degree of connectivity to the ocean has led to a range of lake types – well-mixed (holomictic) bodies of water, stratified (meromictic) lakes, where a distinct chemocline separates the less saline, oxygenated mixolimnion from the anoxic, hydrogen sulfide-containing monimolimnion, and a group of unclassified lakes that do not fall into these two categories (Hamner and Hamner 1998, Dawson unpublished). Sampled holomictic lakes included Heliofungia Lake (HLO), Mekeald Lake (MLN), Ngchas Lake (ULN), Ngeruktabel Lake (NLN), and Flatworm Lake (FLK) (Figure 2.1).

Meromictic lakes consisted of Ongeim'l Tketau Lake (OTM, also known as Jellyfish Lake), Spooky Lake (SLM), Ngermeuangel Lake (NLK, also known as Big Jellyfish Lake), Clear Lake (CLM), and Goby Lake (GLK) (Figure 2.1). Lakes from the unclassified group include Ongael Lake (OLO) and T Lake (TLN) (Figure 2.1). For purposes of comparison, I also sampled four oceanic sites that surround the islands: an open ocean site on the northeastern side of the islands called Short Drop Off (SDO), the German Channel (GC), a lagoonal site (LAG1), and Malakal Harbor (MH) (Figure 2.1). Table 2.3 provides some physical features of the marine lakes. The microbial ecology of the marine lakes is largely unexplored (Venkateswaran et al. 1993, Dawson and Hamner 2008) – despite the variable geochemistry of these lakes (Table 2.4).

2.3.2 Sample Collection

Twelve marine lakes and four oceanic sites were sampled in Palau in August and September 2010 (Figure 2.1). Samples were collected from a small motor boat in the ocean and an inflatable pontoon in the lakes (except an inflatable raft was used in Spooky Lake to avoid possible contact with crocodiles) using a horizontal, 2.5 L GoFlo bottle (General Oceanics Model 1010C Series Convertible Water Sampler, Miami, FL, USA). Dissolved oxygen, temperature, and pH were measured at meter intervals using a HydroLab DS5 (Hach Company, Loveland, CO, USA; accuracy of each sensor in Table 2.1). The dissolved oxygen (Hach LDO) sensor measures oxygen concentrations by shining blue light from an LED on a luminescent material on the sensor cap, and detecting red light with a photodiode that is released from the excited luminescent chemical as the material relaxes (oxygen concentration and amount of red light released are inversely proportional). The time the chemical takes to return to a relaxed state is

also measured, which is also inversely proportional to oxygen concentration. A red LED of known intensity, which acts as an internal standard for reference purposes, flashes between flashes from the blue LED. The temperature sensor is a 30k ohm variable resistance thermistor. For the pH sensor, a salt bridge is formed between a KCl impregnated glass bulb that is permeable to hydrogen ions and a reference electrode filled with 3M KCl that has a porous Teflon junction; and a potential is measured. Using lake information from other studies (e.g. Hamner and Hamner 1998) and on-site dissolved oxygen measurements to determine the depth of the oxycline, samples in the meromictic lakes were gathered throughout the water column at different oxygen concentrations, chosen with reference to the depth of the oxycline, such that at least one sample was collected above, at least one within, and at least one below the oxycline. Sampling depths were spaced every 2-5 m, or 10 m, through the water columns of the holomictic lakes, OLO, TLN, and the oceanic sites because oxygen concentrations were consistently high (>55 µM O_2) throughout the water columns of these sites. GoFlo bottle casts were transferred to 1 L polycarbonate bottles, and stored in the dark during ~1-2 hours transportation back to the Coral Reef Research Foundation laboratory in Koror, Palau. Also, I collected samples in two different locations in OTM, SLM, and TLN for comparison, and in one location in OTM on three consecutive days for comparison.

Water samples were filtered using a peristaltic pump and 0.22 µm Durapore PVDF hydrophilic filters (Millipore, Billerica, MA, USA). Filters were immediately stored in 800 µL Sucrose-Tris-EDTA (STE) lysis buffer (750 mM sucrose, 20 mM EDTA, 400 mM NaCl, and 50 mM Tris) in 2 mL Lysing Matrix E tubes (MP Biomedicals, Solon, OH, USA), and frozen at -20°C. (Dry ice, liquid nitrogen, and -

80°C freezers are not available in the Republic of Palau.) Nutrient samples (see below) and filter tubes were transported in coolers via commercial airlines. Filter tubes were placed in a residential freezer upon arrival to either Los Angeles or San Jose, California, only being out of a freezer but still kept cold for ~24 hours. Filter tubes were transported on ice from Los Angeles or San Jose to the University of California, Merced, where they were stored in a -20°C freezer. Nutrient samples were kept in their cooler until their arrival to the University of California, Merced, where they were also stored in a -20°C freezer.

2.3.3 Nutrient Measurements

During sample filtration, 50 mL of filtrate was collected in HDPE bottles for subsequent nutrient analysis at the UCSB Marine Analytical Laboratory (http://www.msi.ucsb.edu/services/analytical-lab). Samples were analyzed for ammonia (UCSB MAL analytical method for ammonia, see below; Diamond and Huberty 1996), nitrite (EPA Method 353.2, Schroeder 1997), nitrite+nitrate (EPA Method 353.2, Diamond 1997), and phosphate (EPA Method 365.1, Huberty and Diamond 1998), on a Lachat QuikChem 8000 Flow Injection Analyzer (Hach Company, Loveland, CO, USA). Flow injection analysis is a technique used for automating wet chemical analysis that generally occurs in a three-step process: sampling, processing, and detection. During sampling, the instrument injects a known volume of the sample into the flowing carrier stream. Analytes in the sample are then transformed (processed) into species that can be measured by the instrument's detectors. During the last step, each analyte (or its derivative) causes a signal peak, which is used to quantify the concentration of the measured compound. Samples containing sulfide were not analyzed for nitrite+nitrate as

sulfide damages the cadmium reduction column. For ammonia analysis, the sample was injected into a flowing carrier stream by means of an injection valve, and was then merged with an alkaline solution stream. The ammonia generated was then separated from the matrix by diffusing through a hydrophobic, gas-permeable membrane into a recipient stream carrying a pH indicator. The indicator solution changed color, due to the resulting pH increase. The increase in absorbance at 570 nm is proportional to the ammonia concentration. For all analyses, a mid-range check standard bracketed every 20 samples to verify the accuracy of the measurements, and samples that were detected outside of the standards' range were diluted 1:10 and reanalyzed. Detection limits were 0.10 µM for phosphate, 0.10 µM for nitrite, 0.20 µM nitrite+nitrate, and 0.10 µM for ammonium. Accuracy and precision for the measurement of each nutrient is given in Table 2.1.

2.3.4 DNA extraction and quantification

DNA was extracted following Beman et al. (2008). 100µL 10% sodium dodecyl sulfate (SDS) was added to tubes containing STE buffer and filters, samples were bead-beat for 2 minutes (BioSpec Products, Inc., Bartlesville, OK, USA), and incubated for 3 minutes on a dry heat block at 99°C. Following transfer of sample solutions to 1.5 mL LoBind Microcentrifuge tubes (Eppendorf, Hauppauge, NY, USA), 50µL proteinase K (20mg mL^{-1}; Qiagen, Inc., Valencia, CA, USA) was added, and tubes were incubated at 55°C for 3 hours. Lysates were purified using a DNeasy Blood and Tissue Kit according to the manufacturer's protocol (Qiagen, Inc., Valencia, CA, USA). DNA concentrations were measured using the Quant-iT PicoGreen dsDNA Assay Kit and the manufacturer's protocol (Invitrogen Corporation, Carlsbad, CA, USA), which included using standard

total DNA concentrations to quantify the sample total DNA concentrations, on a Stratagene MX 3005P (Agilent Technologies, Inc., Santa Clara, CA, USA).

2.3.5 Automated Ribosomal Intergenic Spacer Analysis (ARISA)

ARISA was used to examine bacterial diversity. For each sample, 6 ng of total DNA were amplified in triplicate (i.e. 6 ng of total DNA was placed in each of three wells in a 96-well plate, therefore 18 ng of total DNA per sample were amplified) using the bacterial-specific primers 16S-1392F (5'-GYACACACCGCCCGT-3'; Fisher and Triplett 1999) and Hex-labeled 23S-125R^{HEX} (5'-GGGTTBCCCCATTCRG-3'; Fisher and Triplett 1999) at a concentration of 400 nM, 12.5 µL Premix F (Epicentre Biotechnologies, Madison, WI, USA), 2 mM $MgCl_2$, 1.25 units AmpliTaq polymerase (Life Technologies Corporation, Carlsbad, CA, USA), and 40 ng μL^{-1} BSA (Life Technologies Corporation, Carlsbad, CA, USA) following Brown et al. (2005). Jones et al. (2007) note that while primer design can affect the total number of OTUs recovered via ARISA (e.g., see Cardinale et al. 2004), this does not affect comparisons among samples, which was the main objective of my study, because all samples were amplified using the same primers. Cycling conditions were 3 minutes at 94°C; 30 cycles of 30 seconds at 94°C, 30 seconds at 56°C, and 90 seconds at 72°C; and 7 minutes at 72°C. Triplicate PCR reactions were pooled to minimize PCR bias; the pooled samples were then cleaned and concentrated (Zymo Research, Irvine, CA, USA) and requantified using PicoGreen. Pooled samples were normalized to 50 ng total DNA and added to 96-well plates with MapMarker (BioVentures, Inc., Murfreesboro, TN, USA), Tracking Dye (BioVentures, Inc., Murfreesboro, TN, USA), and Hi-Di Formamide (Life Technologies Corporation, Carlsbad, CA, USA). Samples were analyzed using capillary

electrophoresis separation on an Applied Biosystems 3130xl Genetic Analyzer (Life Technologies Corporation, Carlsbad, CA, USA) following Brown et al. 2005.

ARISA data were processed and analyzed using GeneMapper (Life Technologies Corporation, Carlsbad, CA, USA), R (http://www.R-project.org), and PAST (http://folk.uio.no/ohammer/past/, University of Oslo, Norway). GeneMapper was used to check that the MapMarker was properly detected and MapMarker standard lengths used to determine DNA fragment lengths were 50, 75, 100, 125, 150, 200, 250, 300, 350, 400, 450, 475, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, and 1000 base pairs (bp). Samples in which MapMarker was not detected were rerun through the ARISA protocol. I used R to process DNA fragment data using customized scripts (Ramette 2009): fragments were binned (smallest band size = 100, largest band size = 1000, minimum relative fluorescence intensity (RFI) cutoff = 0.09, window size = 2, shift size = 0.1; Ramette 2009) and used in downstream analyses. Analyses of the data were performed in R and PAST. R was used to calculate ANOVA and Bray-Curtis similarity (Bray and Curtis 1957), whereas PAST was used for cluster analysis and bootstrapping.

2.3.6 454 pyrosequencing

Samples for each lake and oceanic site were chosen based on ARISA-based Bray-Curtis similarities and the water chemistry of the depth at which the sample was collected. Three samples were selected from each site (except only two were chosen from OLO and FLK because they are two shallow, <5 m in depth, lakes in which only two water samples were gathered originally.) These samples not only contain bacterial communities that are highly dissimilar to one another (comparisons among these samples vs. comparisons among another combination of samples from the same site) but also

represent the chemical variability found throughout the water column of each site. The selected samples and their similarities based on ARISA are shown in Table 2.2.

DNA samples were sequenced using Titanium chemistry on the Roche 454 GS FLX platform at Research and Testing Laboratories (http://researchandtesting.com/). I used the primers 926F (5′-AAACTYAAAKGAATTGACGG-3′) and 1392R (5'-ACGGGCGGTGTGTRC-3') – which are effective universal bacteria and archaea primers that do not inflate richness estimates (Engelbrektson et al. 2010) – to amplify a portion of the 16S rRNA gene. Relevant Roche linkers were attached to primers, as were 8-base 'barcodes' (Hamady et al. 2008) used to sort the 46 individual samples. Sequences were quality-screened using the approach of Huse et al. (2007; see also Huber et al. 2007, Brown et al. 2009, Galand et al. 2009, Lauber et al. 2009, Brazelton et al. 2010). In brief, I used the program mothur (http://www.mothur.org; Schloss et al. 2009) to discard sequences: > ±100 bp from the median sequence length, containing any ambiguous bases, containing homopolymers >8 bp, of <25 average quality score, and that did not exactly match the forward primer and barcode sequence. I did not screen sequences based on the reverse primer, as some of the reads are high quality sequences that did not extend to the reverse primer. In total, 120,732 sequences (13.9%) did not meet quality control criteria and were excluded from subsequent analyses. A total of 581,564 16S rRNA sequences were recovered from 46 DNA samples drawn from 12 lakes and four ocean sites, ranging from 6,564-28,241 sequences per sample; median read length was 460 bp. The largest and smallest pyrosequencing libraries were both from HLO, at 15 m depth (6,564) and 10 m depth (28,241).

Bacterial and archaeal 16S rRNA sequences were aligned to the greengenes alignment (DeSantis et al. 2006) in mothur (Schloss et al. 2009); I elected to use greengenes as it is a universal alignment that includes both archaea and bacteria. The alignment was optimized to the start position and I discarded sequences starting after the position that 95% of the sequences start; the alignment was then manually curated to remove sequences clearly out of alignment (n = 25). Kunin et al. (2010) raised the issue of pyrosequencing errors inflating diversity estimates, and Huse et al. (2010) proposed a pseudo-single linkage 'preclustering' algorithm as a means of reducing these errors; I used this approach as implemented in mothur to remove sequences that are likely due to pyrosequencing errors. I found that increased sequencing effort led to increasing estimates of total richness (r^2 = 0.29-0.42) and observed richness (r^2 = 0.60), and so for comparisons among samples, I renormalized to a common library size of 6500 sequences. A 97% sequence identity across a 460 bp segment of 16S rRNA gene was used to define operational taxonomic units (OTUs), and to explore the richness and diversity of the samples based on the number of OTUs observed, the ACE richness estimator (Chao et al. 1993), the Chao1 richness estimator (Chao 1984), the inverse of the Simpson diversity estimator (Simpson 1949), and the Shannon index (Shannon 1948). Beta diversity (Bray-Curtis similarity) calculations were performed for each site and compared to those of ARISA. Community composition of individual samples was determined using the Ribosomal Database Project Classifier (http://pyro.cme.msu.edu/; Wang et al. 2007) using default parameters.

2.4 Results

2.4.1 Variability in bacterial community composition across samples

ARISA-based Bray-Curtis similarity ranged from 90.3% (for SDO samples at 15 m and 25 m depths) to as low as 0.20% for a sample collected at 1 m in T Lake (TLN) compared with a sample collected at 3 m in Spooky Lake (SLM). Samples from two locations in the same lake were highly similar: in TLN, the highest similarity was observed for 7 m samples (80.9% similar) and the lowest similarity was between samples collected at 3 m, which were 63.6% similar to each other (Figures 2.3 and 2.9). In SLM, however, similarities among samples collected from different basins ranged from 77.4% among 0 m samples to 42.9% among 2 m samples (Figures 2.3 and 2.9). This may reflect variability in community composition along the same depth horizon in different locations within a lake, as in OTM, I observed similarities of up to 72.9% at 10 m and as low as 31.6% at 5 m when comparing communities at the same depths in different basins. Pseudo-replicates – water samples from the same GoFlo bottle cast that were filtered and processed separately – ranged in similarity from 39.1% to 92.1% (n = 36, Figures 2.3 and 2.9), suggesting that variability in community composition can occur within a single bottle cast.

Boostrapping was performed on the ARISA data of the samples from the 12 lakes and four oceanic sites (Figure 2.2). There was complete support (100% of the replicates) of the ~11% similarity between the group of 28 lake samples, which all but pseudo-replicate samples from 2 m depth in SLM1 were gathered from depths containing <5 μM of oxygen, and the group of the other sites' samples, which all but the 12 m depth

samples from the two basins in OTM (OTM1 and OTM2) were collected from depths containing >10 µM of oxygen. There was some support (10% of the replicates) for the ~19% similarity among the bacterial communities of the above group of 28 lake samples. Some support (9% of the replicates) was found for the ~17% similarity between the group of SLM samples gathered from depths containing >10 µM of oxygen and the group of other lake and oceanic samples collected from depths containing >10 µM of oxygen (or <5 µM of oxygen in the cases of the 12 m depth samples from the two basins in OTM. Also, there was some support (10% of the replicates) for the ~22% similarity between the group of oceanic samples taken from depths with >150 µM of oxygen and the group of lake samples (excluding SLM samples) gathered from depths with >10 µM of oxygen (or <5 µM of oxygen for the 12 m depth samples from OTM1 and OTM2). No support (0% of the replicates) was found for the ~25% similarity of the lake samples (except from SLM) from depths with >10 µM of oxygen or <5 µM of oxygen for the 12 m depth samples from the two basins in OTM. There was strong support (58% of the replicates) for the ~45% similarity among the oceanic samples, which were collected from depths with >150 µM of oxygen. Temperature and pH varied across the sampled environments. Lake depths with <5 µM of oxygen had variable nitrite concentrations, whereas lake depths with >10 µM of oxygen had <0.3 µM of nitrite. Nitrate concentrations were variable across all of the sampled environments in which nitrate was measured. Most of the sampled depths containing <5 µM of oxygen (25 of 28) also had concentrations of >5 µM of ammonia.

2.4.2 Dissimilarity in community composition with depth based on ARISA

The data demonstrate distance-decay patterns, as the lowest Bray-Curtis similarities in meromictic lakes were 4.99% in CLM, 8.74% in GLK, 4.59% in NLK, 3.44% in OTM, and just 0.52% in SLM (Figures 2.3 and 2.9). In contrast, the lowest similarities in holomictic lakes were 58.1% in FLK, 78.1% in HLO, 45.1% in MLN, 64.2% in NLN, and 63.3% in ULN (Figures 2.3 and 2.9). The other lakes OLO and TLN fell more toward the holomictic lakes than the meromictic lakes and were 53.8% and 46.1% in lowest similarity (Figures 2.3 and 2.9).

I observed declines in bacterial community similarity with increasing depth in CLM, GLK, NLK, OTM, and SLM, the five sampled meromictic lakes (Figure 2.3). Similarities among depths within each of the other sampled lakes (FLK, HLO, MLN, NLN, OLO, TLN, and ULN) were consistently fairly high (> 45%), therefore, no patterns appear among the similarities of the bacterial communities within these lakes (Figure 2.3).

2.4.3 Microbial diversity of marine lakes based on pyrosequencing

Across the sampled lakes, observed richness varied from 700 to 1,809 OTUs. A similar range of richness values was found in the holomictic lakes (801-1,809 OTUs), the meromictic lakes (727-1,561), and the remaining lakes of TLN and OLO (700-1,137) (Figure 2.4). Overall the highest observed richness was found at 30 m depth in German Channel (1,858 OTUs). The ACE and Chao1 richness estimators displayed a wider range of values, with ACE presenting higher values than Chao1: ACE ranged from 1,564-6,085

OTUs and Chao1 ranged from 1,376-3,624 OTUs, also with no distinct differences among lakes types. For marine sites, estimated richness fell within similar bounds.

The sampled lakes and oceanic sites did not show categorical differences in microbial diversity, but at least two possible trends in richness and diversity (including evenness) may occur when compared to certain environmental components within specific lakes and the oceanic sites: (1) an increase with the component, or (2) a decrease with the component. Different patterns may also occur for observed vs. estimated richness, and richness vs. evenness.

Each environmental component appeared to affect richness (both observed and estimated) and diversity in different ways within each sampled site (comparing Figures 2.4 and 2.5). With increasing oxygen concentration, observed richness increased in FLK, OLO, and ULN and decreased in NLK, SLM, NLN, and SDO. Observed richness increased in SLM, FLK, OLO, and ULN, and decreased in NLN and SDO, with increasing temperature. Increasing observed richness occurred in FLK, NLN, and OLO with increasing pH, whereas observed richness decreased in GLK, NLK, and SLM. Observed richness increased in NLN with increasing nitrite, but it decreased in SLM, OLO, and GC with increasing nitrate. With increasing ammonia, observed richness increased in NLK, HLO, OLO, and LAG1 and decreased in TLN. Also, observed richness increased in CLM with increasing phosphate.

ACE and Chao1 seemed to show similar trends with each environmental component in certain sites but not others (comparing Figures 2.4 and 2.5). ACE increased in FLK, MLN, OLO, and TLN, but decreased in NLK, with increasing oxygen; the same trends were seen in FLK, MLN, OLO, and NLK for Chao1. While temperature

increased, ACE increased in FLK, MLN, OLO, and TLN, which also occurred for these lakes with Chao1 except in TLN. ACE increased in FLK, MLN, OLO, and TLN, whereas it decreased in GLK and NLK, with increasing pH; Chao1 had the same patterns in these lakes except in TLN. No trends were observed for estimated richness with nitrite. While ACE increased in MH with increasing nitrate, it decreased in NLN, OLO, GC, and LAG1; Chao1 followed the same trends for these sites. ACE increased in NLK, OLO, and MH and decreased in NLN with increasing ammonia, which also occurred for Chao1 in these sites. ACE and Chao1 increased in CLM with increasing phosphate.

The Shannon index and the inverse of the Simpson diversity estimator also showed similar trends with certain environmental components in some sites but not others (comparing Figures 2.4 and 2.5). The Shannon index increased in FLK, OLO, and ULN, whereas it decreased in NLK, OTM, SLM, NLN, and SDO, with increasing oxygen; the inverse of the Simpson estimator showed the same patterns in FLK, OLO, and SDO. With increasing temperature, the Shannon index increased in SLM, FLK, OLO, and ULN, but it decreased in OTM, NLN, and SDO; the inverse of the Simpson estimator showed the same trends in FLK, OLO, and SDO. While the Shannon index increased in FLK, NLN, and OLO with increasing pH, it decreased in GLK, NLK, OTM, and SLM; the same occurred for the inverse of the Simpson estimator in FLK, OLO, and GLK. The Shannon index increased with increasing nitrite in NLN, while the inverse of the Simpson estimator had no pattern for nitrite in NLN. With increasing nitrate, the Shannon index decreased in SLM, OLO, and GC, which was the same for the inverse of the Simpson estimator in OLO and GC. The Shannon index increased in NLK, OTM, HLO, and OLO, but decreased in TLN, with increasing ammonia; the inverse of the

Simpson estimator showed the same trends in OLO and TLN. Lastly, the Shannon index and the inverse of the Simpson estimator increased with increasing phosphate in CLM.

2.4.4 Microbial community composition in marine lakes

Pyrosequencing and ARISA estimates of community similarity were correlated (r^2 = 0.386, $p<0.00001$, Figure 2.6). (While I analyzed both bacteria and archaea using 454 pyrosequencing, archaea probably had a minimal effect on community similarity because communities were composed of no more than 0.3% of archaea.) The direct comparison among techniques was stronger when considering lakes alone (r^2 = 0.473, $p<0.0001$), and stronger still for meromictic lakes alone (r^2 = 0.663, $p<0.001$). Many comparisons display higher similarity for ARISA than for 454. This was seen more for the comparisons within the holomictic lakes, OLO, TLN, and the oceanic sites (76% of these comparisons had a difference between the ARISA and 454 similarities that was greater than 0.1, while 52% had a difference that was above 0.25) than for the comparisons within the meromictic lakes (0% of these comparisons had a difference between the ARISA and 454 similarities that was greater than 0.1).

A few samples were more similar based on the comparison of pyrosequencing libraries, and this was most evident among the Ongael samples from 1 and 3 m, and the Malakal Harbor 25 m sample compared with the 5 and 10 m samples. Both the OLO 3 m sample and MH 25 m sample were dominated by γ-proteobacteria (77% and 59% of the libraries, respectively), with the OLO 3 m library containing 67% *Vibrio* and the MH 25 m library consisting of 30% *Oceanospirillum* (Figure 2.7).

Cyanobacteria – and particularly the abundant marine groups *Prochlorococcus* and *Synechococcus* (Partensky and Garczarek 2010) – were detected in every sample;

they were abundant throughout the water column of the holomictic lakes and generally decreased with depth in the meromictic lakes (Figure 2.7). The α-proteobacteria, and specifically the SAR11 (or *Pelagibacter*) clade, were prevalent throughout the holomictic lakes and were particularly abundant in HLO and MLN, where they numbered up to 24% of 16S rRNA sequences. SAR11 constituted up to 43% of sequences collected at SDO and in the German Channel (GC). SAR11 bacteria were also found at high levels in the meromicitc lakes NLK (31% at 10 m, where oxygen reached 87.8 ± 0.3 µM) and OTM (14% at 10 m, where oxygen was 26.9 ± 0.3 µM). Actinobacteria and Flavobacteria were also common in both holomictic (up to 14.5% and 13.3%, respectively) and meromictic (up to 8.7% and 3.6%, respectively) lakes.

δ-proteobacteria were abundant in the anoxic waters of all of the meromictic lakes, mediated by the dominant 16S rRNA sequences from the sulfate-reducing bacteria (SRB) *Desulfobacterales* (Figure 2.7). Common within and above anoxic waters (16-64% of 16S rRNA sequences in these libraries) were Chlorobi that utilize reduced sulfur during anoxygenic photosynthesis. Other classes of bacteria abundant in anoxic and suboxic waters of the meromictic lakes included the ε-proteobacteria (e.g., at 14 m in OTM with 0.9 µM ± 0.3 of oxygen, 5 m in SLM with 2.0 µM ± 0.3 of oxygen, and 10 m in GLK with 1.6 µM ± 0.3 of oxygen), Sphingobacteria (e.g., 10 m in OTM with 26.9 µM ± 0.3 of oxygen, 1 m in SLM with 32.2 µM ± 0.3 of oxygen), Chloroflexi (specifically *Anaerolineae* in all anoxic samples), and the Planctomycetes (also present in all anoxic samples).

The β-proteobacteria were present in all samples, but formed at most 2.12% of libraries. Of the β-proteobacterial groups, the methylotrophs were most abundant, and

typically within shallow samples from meromictic lakes. They formed 1.11% of the 5 m CLM library, 2.02% of the 0 m SLM library, and 2.12% of the 5 m OTM library.

16S rRNA sequences from γ-proteobacteria were detected in all samples from all lakes and contributed ~15% of all sequences recovered. Both the OLO 3 m sample and MH 25 m sample were dominated by γ-proteobacteria (77% and 59% of the libraries, respectively), with the OLO 3 m library containing 67% *Vibrio* and the MH 25 m library consisting of 30% *Oceanospirillum* (Figure 2.7)., but there was also significant variation in the Pseudomonads, Alteromonads, and Thiotrichales, which include many known anaerobes. The Chromatiales (purple sulfur bacteria) were also variable among the meromictic lakes, and were most abundant at 20 m in NLK (19%) and 14 m in OTM (5%).

Many γ-proteobacterial sequences were unclassified (e.g., 58% of the SLM 0 m library and 55% of the TLN 7 m library). Along these lines, up to 59% of 16S rRNA sequences could not be classified in the 1 m TLN sample, and samples from the holomictic lakes HLO (10 and 15m), MLN (20 m), and NLN (5 and 10 m) contained ca. 40% unclassified 16S rRNA sequences (Figure 2.8).

2.4.5 Microbial community composition correlated with water chemistry

Some bacterial groups showed correlations with some of the environmental components (comparing Figures 2.5 and 2.7). When comparing all of the pyrosequencing samples with their chemical measurements, δ-proteobacteria and Chloroflexi positively correlated with ammonia concentrations ($r^2 = 0.933$, $p<0.00001$

and $r^2 = 0.842$, $p<0.00001$, respectively), Flavobacteria positively and Chloroflexi inversely correlated with pH ($r^2 = 0.511$, $p<0.00001$ and $r^2 = 0.503$, $p<0.00001$, respectively), and Flavobacteria positively correlated with oxygen concentrations ($r^2 = 0.532$, $p<0.00001$). When looking at only the lake samples, almost all of the previous correlations occurred. δ-proteobacteria and Chloroflexi correlated positively with ammonia concentrations ($r^2 = 0.929$, $p<0.00001$ and $r^2 = 0.830$, $p<0.00001$, respectively) and negatively with pH ($r^2 = 0.512$, $p<0.00001$ and $r^2 = 0.520$, $p<0.00001$, respectively), and Flavobacteria positively correlated with oxygen concentrations ($r^2 = 0.504$, $p<0.00001$). The meromictic lake samples had more correlations with their chemical measurements than the holomictic lake samples, OLO samples, and TLN samples grouped together. In the meromictic lakes, α-proteobacteria correlated positively, and δ-proteobacteria and Chloroflexi negatively, with pH (0.525, $p<0.01$; 0.545, $p<0.01$; and 0.529, $p<0.01$, respectively). β-proteobacteria positively correlated with nitrate concentrations (0.502, $p<0.01$), and Flavobacteria positively correlated with oxygen concentrations (0.600, $p<0.001$). δ-proteobacteria and Chloroflexi positively correlated with ammonia concentrations (0.915, $p<0.00001$ and 0.796, $p<0.00001$, respectively). δ-proteobacteria, Chloroflexi, and Planctomycetes positively correlated with phosphate concentrations (0.855, $p<0.0001$; 0.696, $p<0.001$; and 0.546, $p<0.01$). In the holomictic lakes, including OLO and TLN, β-proteobacteria positively correlated with nitrate concentrations (0.638, $p<0.01$), and Chlorobi positively correlated with ammonia concentrations (0.620, $p<0.01$).

2.5 Discussion

Decay in community similarity with increasing distance is a commonly observed property of ecological communities (e.g. Cho and Tiedje 2000, Oda et al. 2003, Whitaker et al. 2003, Green et al. 2004,), and has recently been reported for marine microbial communities (DeLong et al. 2006, Hewson et al. 2006). Community similarities, which decrease with increasing depth (decreasing oxygen) in the meromictic lakes and stay consistently fairly high (> 45%) throughout the other lakes, appear to vary in relation to changes in water chemistry (Figure 2.2). According to bootstrapping and cluster analysis of the ARISA samples, bacterial communities in the marine lakes and oceanic sites are highly dissimilar when oxygen concentrations fall below 5 μM. Pyrosequencing allowed for further investigation of the microbial communities of the marine lakes and oceanic sites, and more insight as to how the richness, diversity, and composition of these communities relate to the environmental conditions in which they inhabit.

454 pyrosequencing revealed that most samples from marine lakes and oceanic sites near Palau host thousands of microbial OTUs, and for non-normalized libraries, these estimates reach 22,509 OTUs. Because samples were selected based on ARISA fingerprint data without information on their phylogenetic composition, their community composition may strongly influence observed and estimated richness and diversity – e.g., they may be dominated by Chlorobi (green sulfur bacteria; see below). Similarities and differences in beta diversity between ARISA and pyrosequencing for the marine lakes and oceanic sites were observed. These observations are readily explained by the ability of pyrosequencing to detect the 'rare biosphere' (Sogin et al. 2006), which can vary

significantly in composition throughout the sea (Galand et al. 2009). My data indicate that, with the increased sensitivity of detection provided by pyrosequencing, many samples from marine sites and holomictic lakes become less similar – i.e., the slope of the relationship suggests that samples sharing 60% similarity based on ARISA would be 45% similar based on pyrosequencing. Put another way, the rare members of communities separated by just a few meters differ much more from one another than the abundant members.

A few samples were more similar based on the comparison of pyrosequencing libraries, and this was most evident among the Ongael samples from 1 and 3 m, and the Malakal Harbor 25 m sample compared with the 5 and 10 m samples. Both the OLO 3 m sample and MH 25 m sample were dominated by γ-proteobacteria (77% and 59% of the libraries, respectively), with the OLO 3 m library containing 67% *Vibrio* and the MH 25 m library consisting of 30% *Oceanospirillum* (Figure 2.7). Samples dominated by particular groups may possess a less distinctive rare biosphere, or that it may be more difficult to detect these rare members. While *Vibrio* are common though not abundant in the ocean (and are common pathogens), they dominate the 3 m community in the 4 m deep Ongael Lake. Some members of *Oceanospirillum* are known hydrocarbon degraders (Hedlund et al. 1999, Valentine et al. 2010), and their presence at depth within Malakal Harbor may be consistent with this role.

In both the ARISA and pyrosequencing data, similarity among the bacterial communities in the lakes was always – even for disparate samples of distinct geochemistry – greater than zero. This indicates that some bacterial groups are found throughout the lakes, and I found that many of these groups are also common marine

bacteria. HLO and MLN are the most oligotrophic lakes, with wide tunnels and marine fauna – such as corals (Fabricius et al. 2004) – that are present in the surrounding sea. Hence the high abundance of SAR11 in these lakes is consistent with their ubiquity in the ocean (Morris et al. 2002).

While microbial community compositions were consistent throughout the holomictic lakes, I observed substantial differences in community composition in the meromictic lakes. This is not surprising given the known geochemical gradients present in these lakes – which are proximally generated by microbes following physical stratification. For example, δ-proteobacteria were abundant in the anoxic waters of all of the meromictic lakes, mediated by the dominant 16S rRNA sequences from the sulfate-reducing bacteria (SRB) *Desulfobacterales* (Figure 2.7), and is obviously consistent with the accumulation of hydrogen sulfide at these depths. Many of the bacterial groups found in the suboxic and anoxic waters of the meromictic lakes contain known anaerobes that play an important role in biogeochemical cycling of carbon, nitrogen, and sulfur. Anammox bacteria are members of the Planctomycetes, for instance, and were present in highest numbers in the deep waters of the lakes (data not shown).

Although Archaea increased in proportion with depth, they formed at most only a fraction of 16S rRNA sequences and no known methanogens were detected – this suggests that methane is not produced within the lakes' water columns, but is likely produced within the sediments and diffuses upward, where it may be oxidized by microorganisms. In Mono Lake, for example, methane (and ammonia) concentrations increase with depth (most apparently in the anoxic layer), and methane oxidation occurs throughout the water column (Joye et al. 1999, Carini et al. 2005).

The Chromatiales (purple sulfur bacteria) were also variable among the meromictic lakes, and were most abundant at 20 m in NLK (19%) and 14 m in OTM (5%; Venkateswaran et al. (1993) found ~4% at the same depth in OTM). Pyrosequencing data suggest that of the phototrophic sulfur bacteria, Chlorobi are more widespread and abundant than Chromatiales in the meromictic marine lakes of Palau; however the Chromatiales are known to form dense 'plates' in OTM and are likely present in high abundance at discrete depths in other meromictic lakes (Hamner et al. 1982, Venkateswaran et al. 1993, Hamner and Hamner 1998).

Many γ-proteobacterial sequences were unclassified and likely represent environmentally-relevant groups that are not included in the RDP database. This includes the SAR86 group, which is common in the ocean (Eilers et al. 2000), and SUP05 and related bacteria, which oxidize reduced sulfur compounds using nitrate and are prevalent in open ocean OMZs (Lavik et al. 2009, Walsh et al. 2009, Canfield et al. 2010, Zaikova et al. 2010). Based on the similar geochemistry and microbial communities present in meromictic marine lakes of Palau, I hypothesize that these groups are present in high abundance in the lakes, and this will be determined through additional analysis. Also, based on the high percentages of unclassified sequences in TLN, HLO, MLN, and NLN, I conclude that microorganisms, which are not well-represented in current databases, are present in the marine lakes of Palau.

2.6 Conclusions

Expansion of oceanic OMZs will change the structure and function of marine systems (reviewed in Stramma et al. 2010). A shift from oxic to anoxic conditions causes

a shift in energy flow from higher-level species to microbes, eventually leading to the utilization of compounds such as sulfide when oxygen levels are very low (Diaz and Rosenberg 2008). In the absence of oxygen, different bacterial groups persist and, in turn, dominate communities depending on the availability of substrates. For example, Castro-González et al. (2005) found that oxygen, nitrite, and nitrate gradients affect the community composition of denitrifiers in the eastern South Pacific OMZ, while microbial communities appear to vary with depth based on the water chemistry of the Black Sea (Wakeham et al. 2007). Previous studies have demonstrated that distinct layering of microbial communities develops in anoxic saline and marine ecosystems, such as the Black Sea (Lin et al. 2006, Wakeham et al. 2007), Cariaco Basin (Madrid et al. 2001, Lin et al. 2006, Taylor et al. 2006), and Mono Lake (Hollibaugh et al. 2001, Humayoun et al. 2003). In Palau's marine lakes, similar layering is evident in meromictic lakes (Hamner et al. 1982, Venkateswaran et al. 1993, Hamner and Hamner 1998), and microbial communities present in these lakes resemble those found in comparable environments (e.g., in the presence of known anaerobes such as anammox bacteria, SRBs, and phototrophic sulfur bacteria).

The marine lakes available in Palau – and analysis of these lakes using multiple molecular approaches – provide a naturally evolved, comparative framework for studying the effects of oxygen depletion on microbial communities. While the highest dissimilarities among bacterial communities may seem to be governed by oxygen concentrations (Figure 2.2), especially observed with similarities decreasing with increasing depth (decreasing oxygen) in the meromictic lakes (Figure 2.3), the effects of the other environmental conditions of the lakes and oceanic sites cannot be ruled out

when determining what factor(s) shape(s) microbial communities in the marine lakes and oceanic sites. For example, ammonia and phosphate concentrations increase, while oxygen concentrations decrease, with depth in the meromictic lakes – suggesting that the availability of compounds, other than oxygen, could determine the diversity and composition of microbial communities in the lakes and oceanic sites, and potentially other saline systems. Furthermore, comparison of richness and diversity (Figure 2.4), and community composition (Figure 2.7), with environmental conditions (Figure 2.5) demonstrates how the environment affects each site's microbial communities differently – therefore, there is probably more than one environmental component dictating the richness, diversity, and composition of the microbial communities in the lakes and oceanic sites. Additionally, I found that holomictic lakes are dominated by common marine bacterial groups such as SAR11, Cyanobacteria, and Flavobacteria (Figure 2.7). Also, with increasing deoxygenation with depth and across lakes, availability of new carbon, nitrogen, and sulfur substrates is correlated with the development of diverse microbial communities; such communities contain many individual OTUs, have high evenness, and contain a wide range of phylogenetic diversity. Yet holomicitic lakes contain significant uncharacterized microbial diversity (Figure 2.8), including a rare biosphere that appears to differ markedly among lakes. Members of this rare community may act as a 'seed bank' (Pedros-Alio 2006, Brazelton et al. 2010) and become more abundant and biogeochemically-significant as conditions change. How this rare biosphere varies among lakes, within lakes, and in relation to abundant community members, is the subject of ongoing investigation.

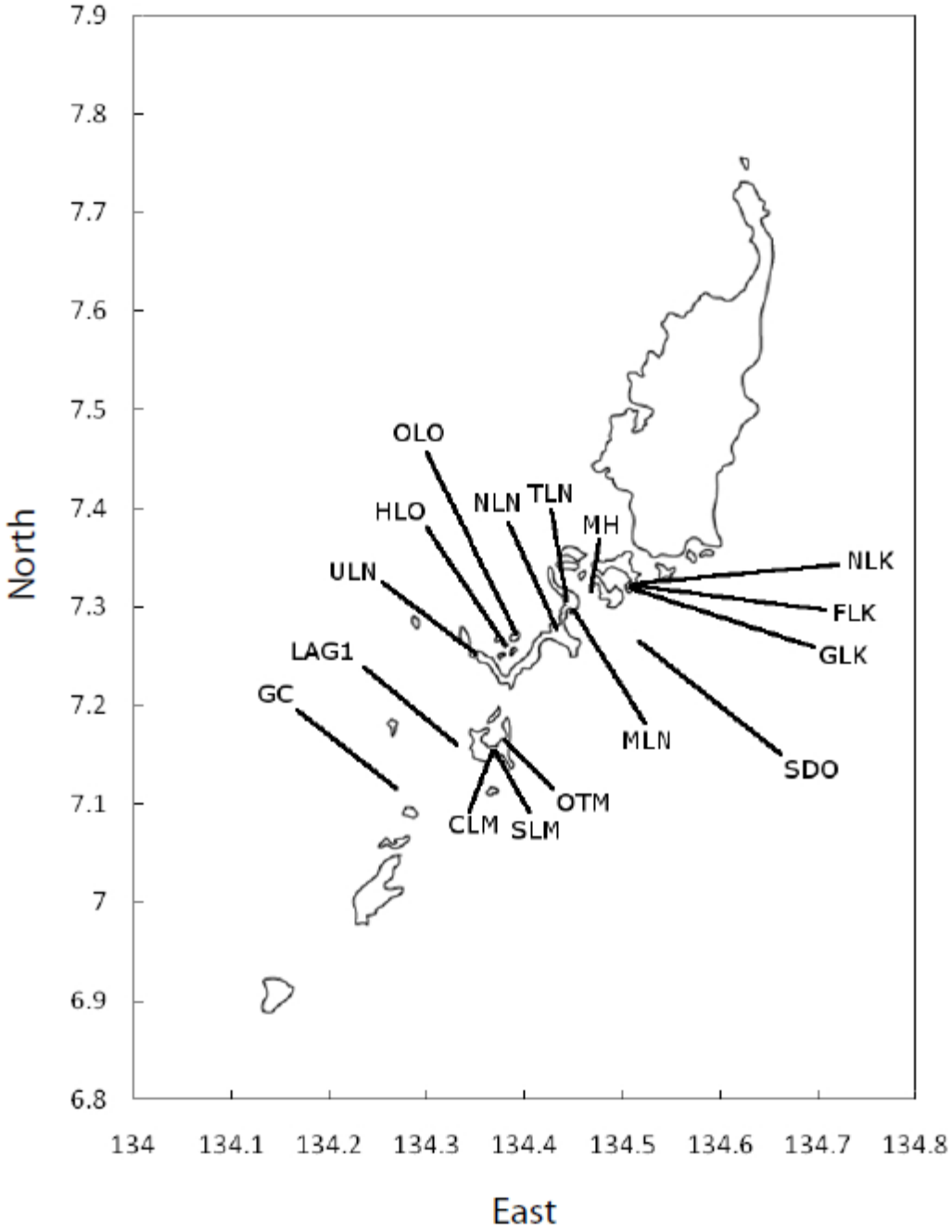
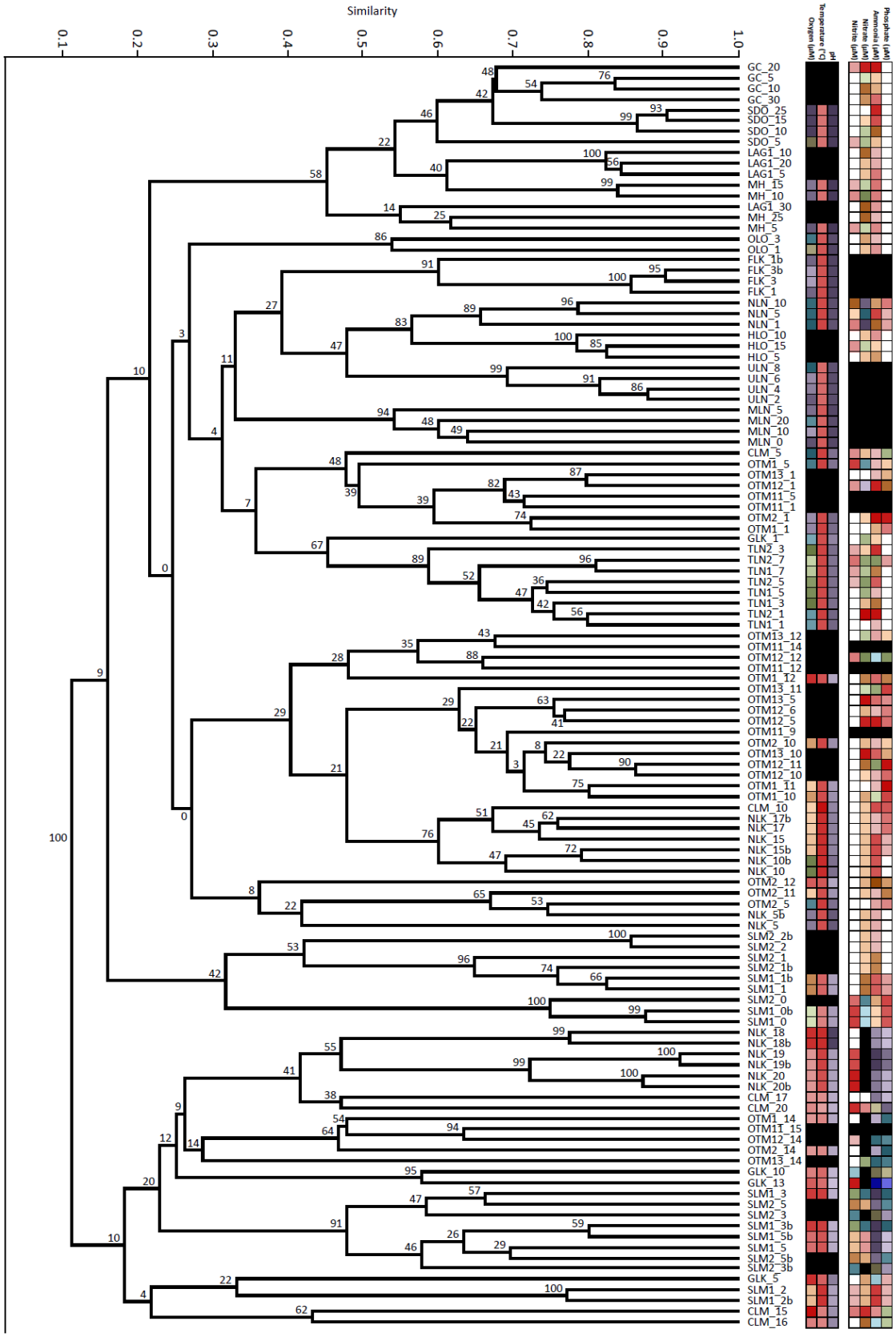


Figure 2.1. Map of sampling locations in Palau. Sites designated by their 2- or 3-lettered code as follows: holomictic lakes – Heliofungia Lake (HLO), Mekeald Lake (MLN), Ngchas Lake (ULN), Ngeruktabel Lake (NLN), and Flatworm Lake (FLK); meromictic lakes – Ongeim'l Tketau Lake (OTM, also known as Jellyfish Lake), Spooky Lake (SLM), Ngermeuangel Lake (NLK, also known as Big Jellyfish Lake), Clear Lake (CLM), and Goby Lake (GLK); unclassified lakes – Ongael Lake (OLO) and T Lake (TLN); and ocean locations – Short Drop Off (SDO), German Channel (GC), a lagoonal site (LAG1), and Malakal Harbor (MH).

Figure 2.2. Cluster analysis of ARISA data, Continued. Vertical axis represents Bray-Curtis similarity, and the number at each node refers to the percentage of replicates where that node is still supported based on bootstrapping. Sample names are written as ‘site code’_‘depth’. Environmental components of the depths at which the samples were gathered are listed next to the sample names based on a color code, which is provided on the page after the dendrogram. While squares represent to nutrient concentrations that were not detectable, and black squares refer to environmental components that were not measured (see Materials and Methods).


Similarity
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
Oxygen (uM)
Temperature (°C)
pH
Nitrite (uM)
Nitrate (uM)
Ammonia (uM)
Phosphate (uM)
GC_20
GC_5
GC_10
GC_30
SDO_25
SDO_15
SDO_10
SDO_5
LAG1_10
LAG1_20
LAG1_5
MH_15
MH_10
LAG1_30
MH_25
MH_5
OLO_3
OLO_1
FLK_1b
FLK_3b
FLK_3
FLK_1
NLN_10
NLN_5
NLN_1
HLO_10
HLO_15
HLO_5
ULN_8
ULN_6
ULN_4
ULN_2
MLN_5
MLN_20
MLN_10
MLN_0
CLM_5
OTM1_5
OTM13_1
OTM12_1
OTM11_5
OTM11_1
OTM2_1
OTM1_1
GLK_1
TLN2_3
TLN2_7
TLN1_7
TLN2_5
TLN1_5
TLN1_3
TLN2_1
TLN1_1
OTM13_12
OTM11_14
OTM12_12
OTM11_12
OTM1_12
OTM13_11
OTM13_5
OTM12_6
OTM12_5
OTM11_9
OTM2_10
OTM13_10
OTM12_11
OTM12_10
OTM1_11
OTM1_10
CLM_10
NLK_17b
NLK_17
NLK_15
NLK_15b
NLK_10b
NLK_10
OTM2_12
OTM2_11
OTM2_5
NLK_5b
NLK_5
SLM2_2b
SLM2_2
SLM2_1
SLM2_1b
SLM1_1b
SLM1_1
SLM2_0
SLM1_0b
SLM1_0
NLK_18
NLK_18b
NLK_19
NLK_19b
NLK_20
NLK_20b
CLM_17
CLM_20
OTM1_14
OTM11_15
OTM12_14
OTM2_14
OTM13_14
GLK_10
GLK_13
SLM1_3
SLM2_5
SLM2_3
SLM1_3b
SLM1_5b
SLM1_5
SLM2_5b
SLM2_3b
GLK_5
SLM1_2
SLM1_2b
CLM_15
CLM_16

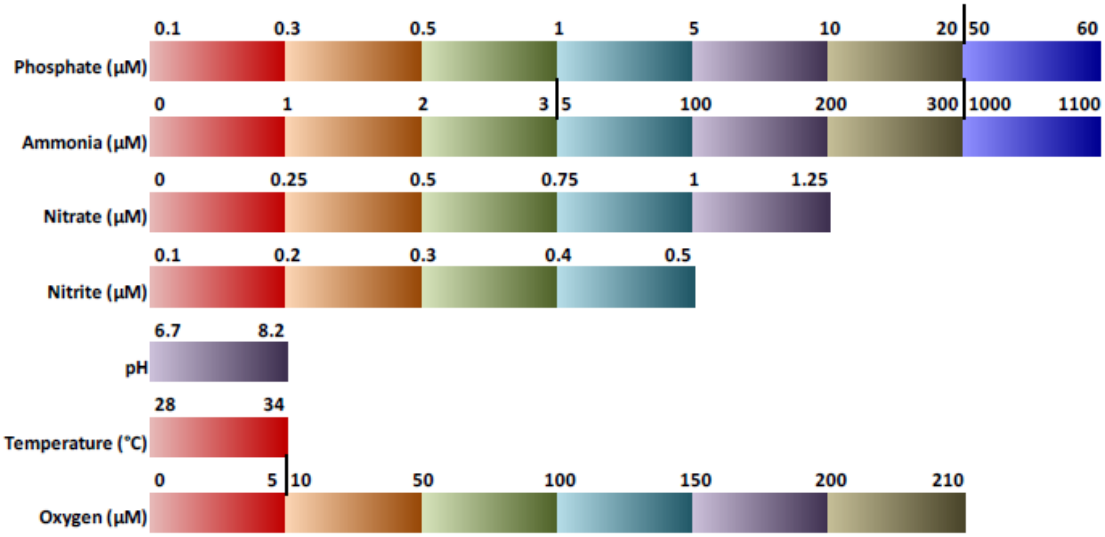
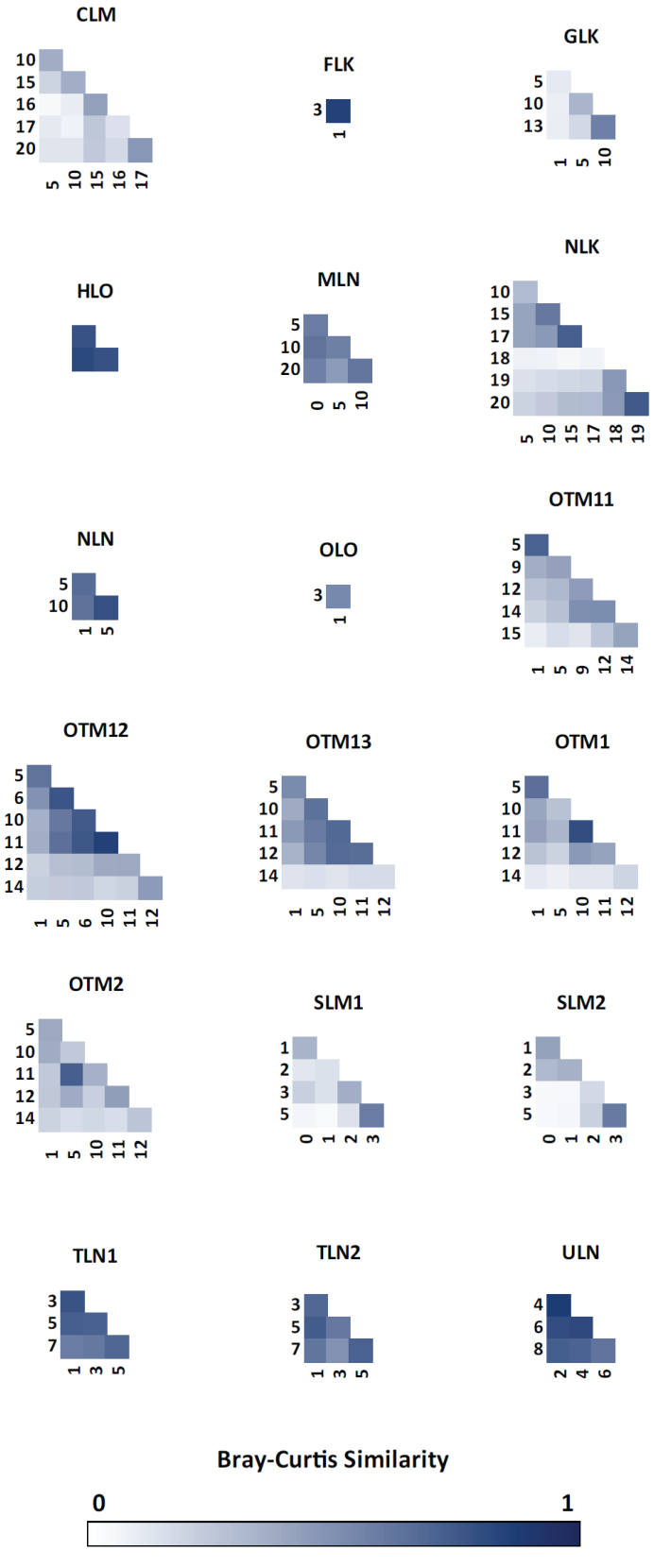

Phosphate (µM)
0.1
0.3
0.5
1
5
10
20
50
60
Ammonia (µM)
0
1
2
3
5
100
200
300
1000
1100
Nitrate (µM)
0
0.25
0.5
0.75
1
1.25
Nitrite (µM)
0.1
0.2
0.3
0.4
0.5
pH
6.7
8.2
Temperature (°C)
28
34
Oxygen (µM)
0
5
10
50
100
150
200
210

Figure 2.3. Bray-Curtis similarity comparison within lakes. Lakes are listed above their comparisons. A ‘1’ or ‘2’ next to the site code of a lake refers different locations sampled within the lake. OTM11, OTM12, and OTM13 represent three separate days of sampling (August 11, 12, and 13) in OTM. Sampled depths listed along the left and bottom of each lake’s comparisons. The color gradient at the bottom represents the Bray-Curtis similarities between two depths within a lake.


CLM
10
15
16
17
20
5 10 15 16 17
FLK
3
1
GLK
5
10
13
1 5 10
HLO
MLN
5
10
20
0 5 10
NLK
10
15
17
18
19
20
5 10 15 17 18 19
NLN
5
10
1 5
OLO
3
1
OTM11
5
9
12
14
15
1 5 9 12 14
OTM12
5
6
10
11
12
14
1 5 6 10 11 12
OTM13
5
10
11
12
14
1 5 10 11 12
OTM1
5
10
11
12
14
1 5 10 11 12
OTM2
5
10
11
12
14
1 5 10 11 12
SLM1
1
2
3
5
0 1 2 3
SLM2
1
2
3
5
0 1 2 3
TLN1
3
5
7
1 3 5
TLN2
3
5
7
1 3 5
ULN
4
6
8
2 4 6
Bray-Curtis Similarity
0
1

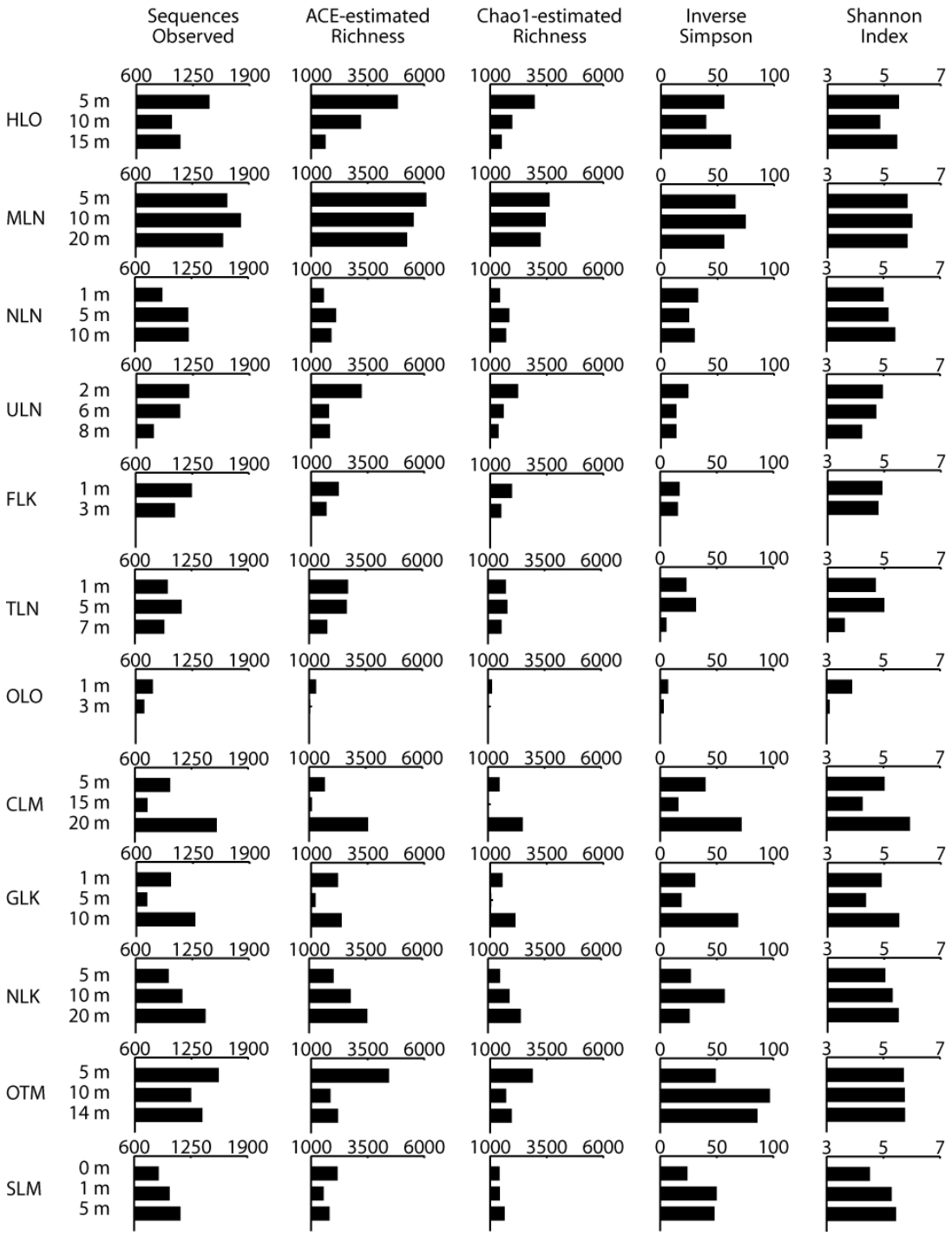
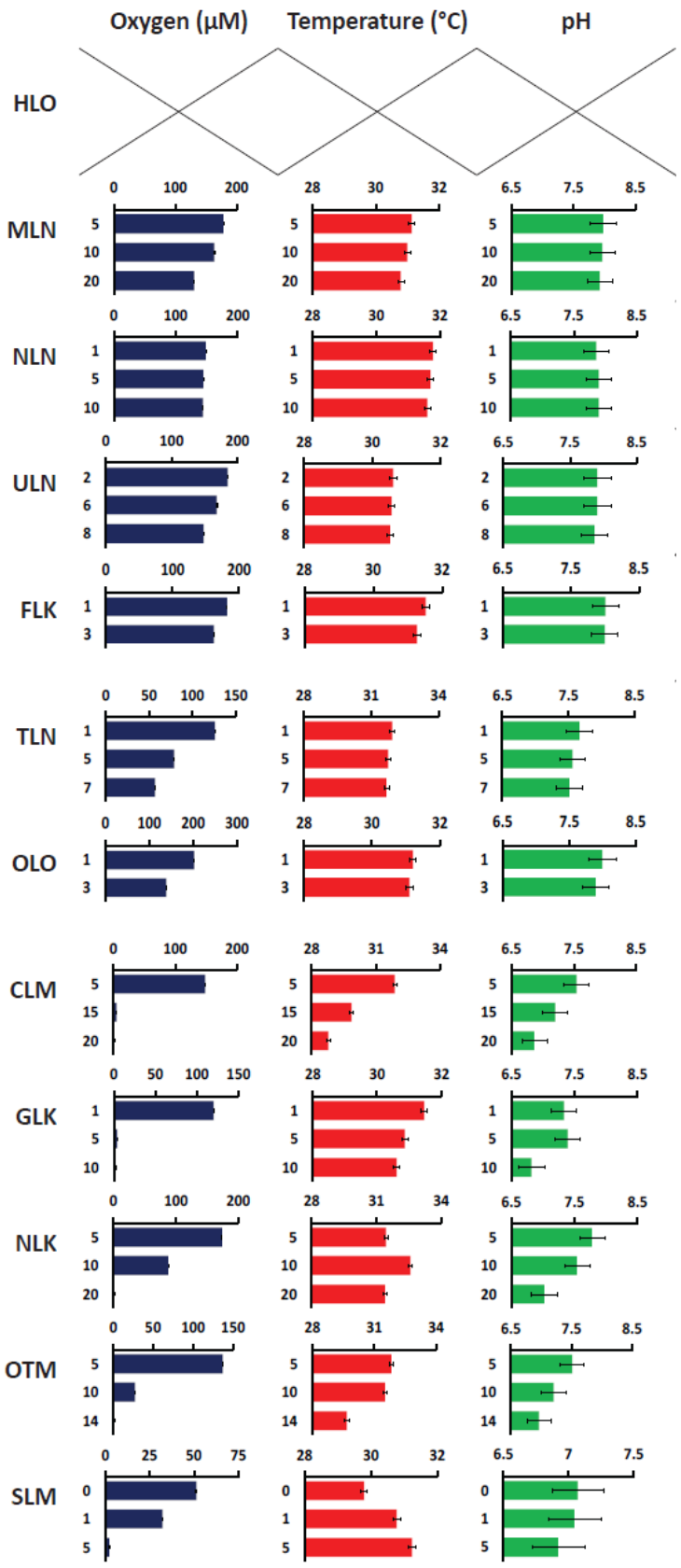
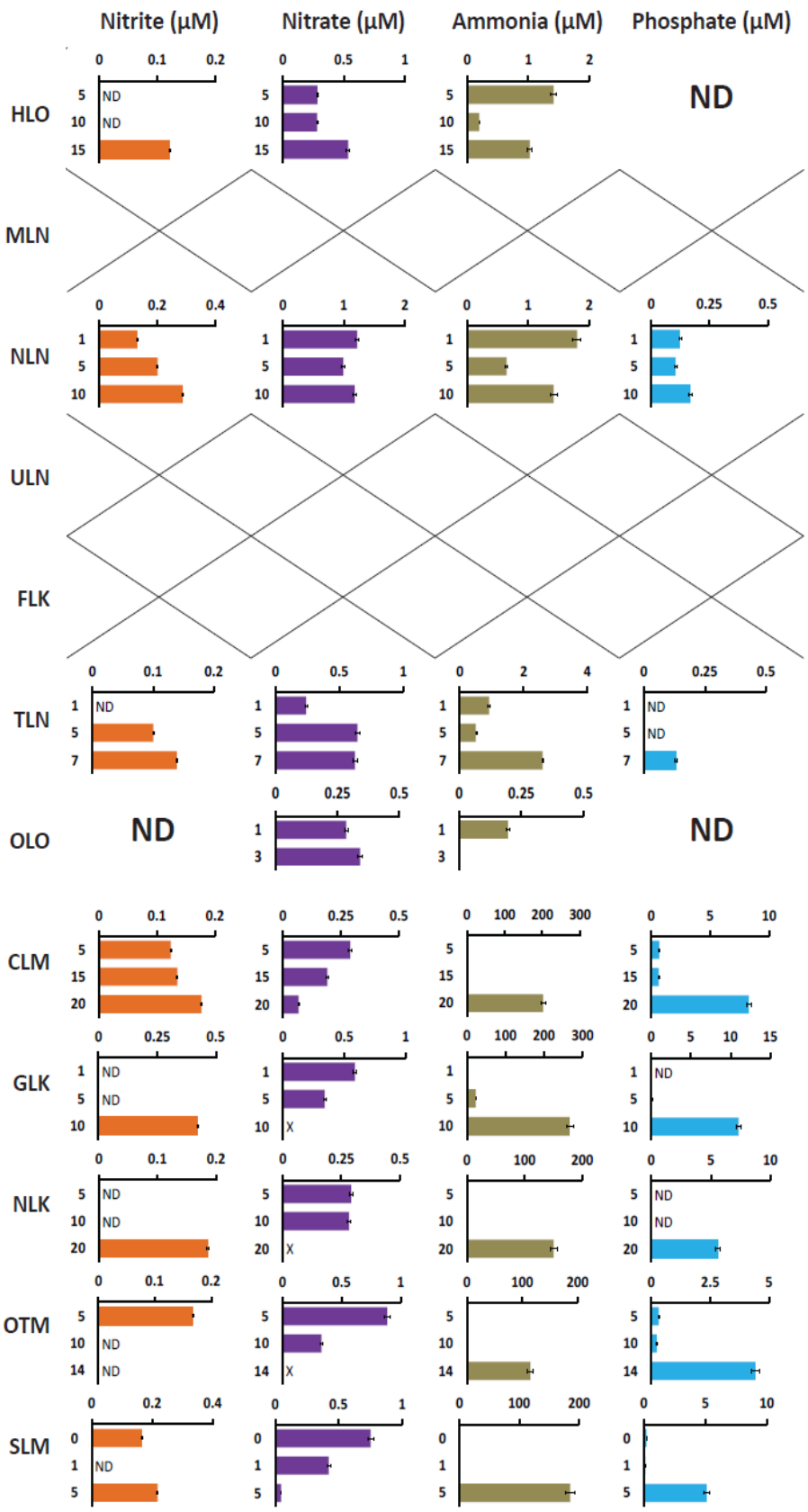


Figure 2.4. Alpha diversity of samples based on pyrosequencing data. Samples were normalized to a common library size of 6,500 sequences, and all calculations were performed at 97% sequence identity. Lake names and the depths samples are shown at left; from left to right, the graphs show numbers of observed sequences, ACE-estimated richness, Chao1-estimate richness, the inverse of the Simpson estimator, and the Shannon Index.

Figure 2.5. Environmental components of pyrosequencing lake samples, Continued. Environmental components are listed along the top, and 3-letter lake codes are listed on the left. The vertical axes represent concentrations (oxygen and nutrients), degrees (temperature), and units (pH), and the horizontal axes have the sampled depths for each lake. Error bars represent accuracy of each measurement (Table 2.1). X's refer to unmeasured components for either a specific depth or a whole lake, and ND pertains to measurements that were not detectable for either a specific depth or a whole lake.


Oxygen (µM)
Temperature (°C)
pH
HLO
MLN
NLN
ULN
FLK
TLN
OLO
CLM
GLK
NLK
OTM
SLM


Nitrite (µM)
Nitrate (µM)
Ammonia (µM)
Phosphate (µM)
HLO
ND
ND
MLN
NLN
ULN
FLK
TLN
ND
ND
ND
OLO
ND
ND
CLM
GLK
ND
ND
X
NLK
ND
ND
X
OTM
ND
ND
X
SLM
ND

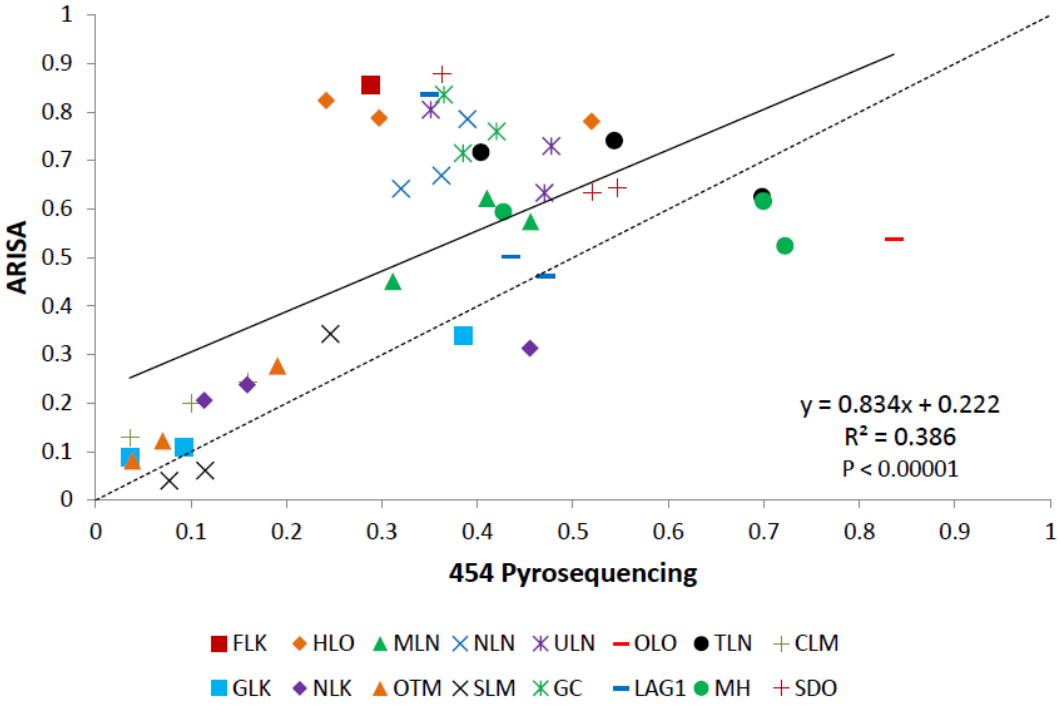
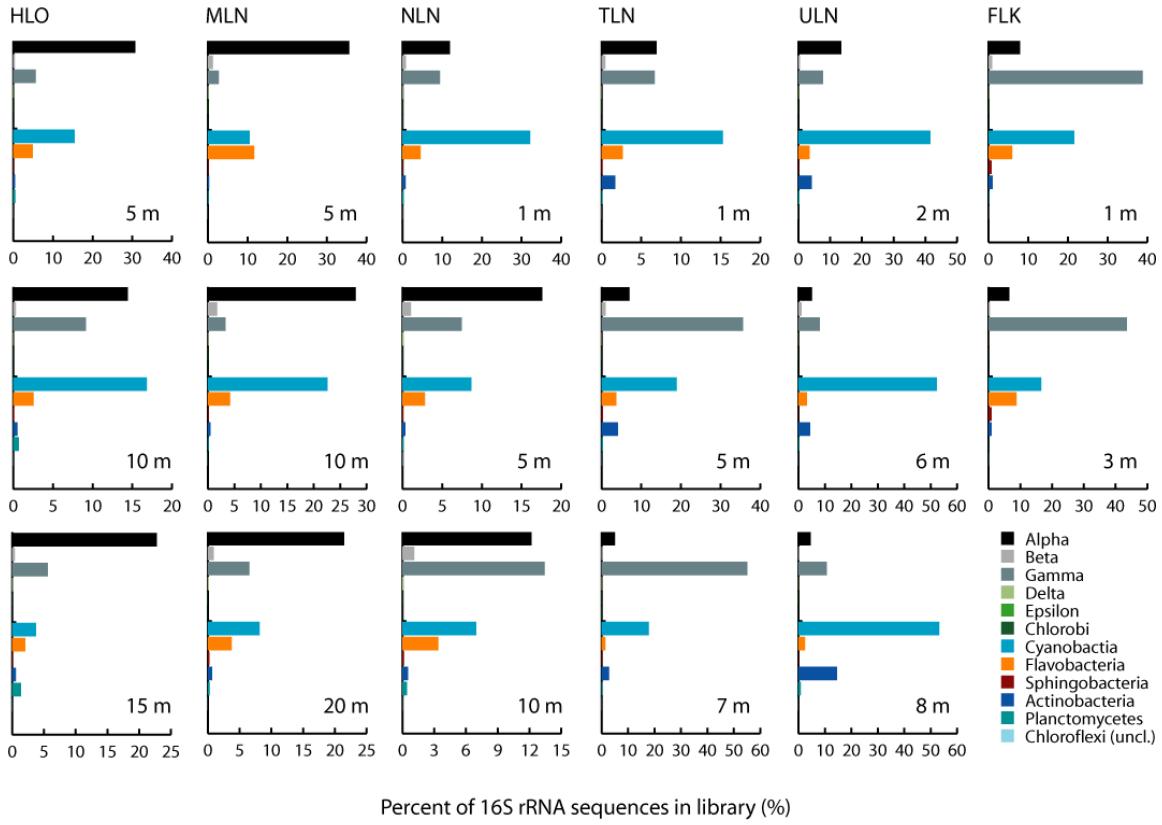
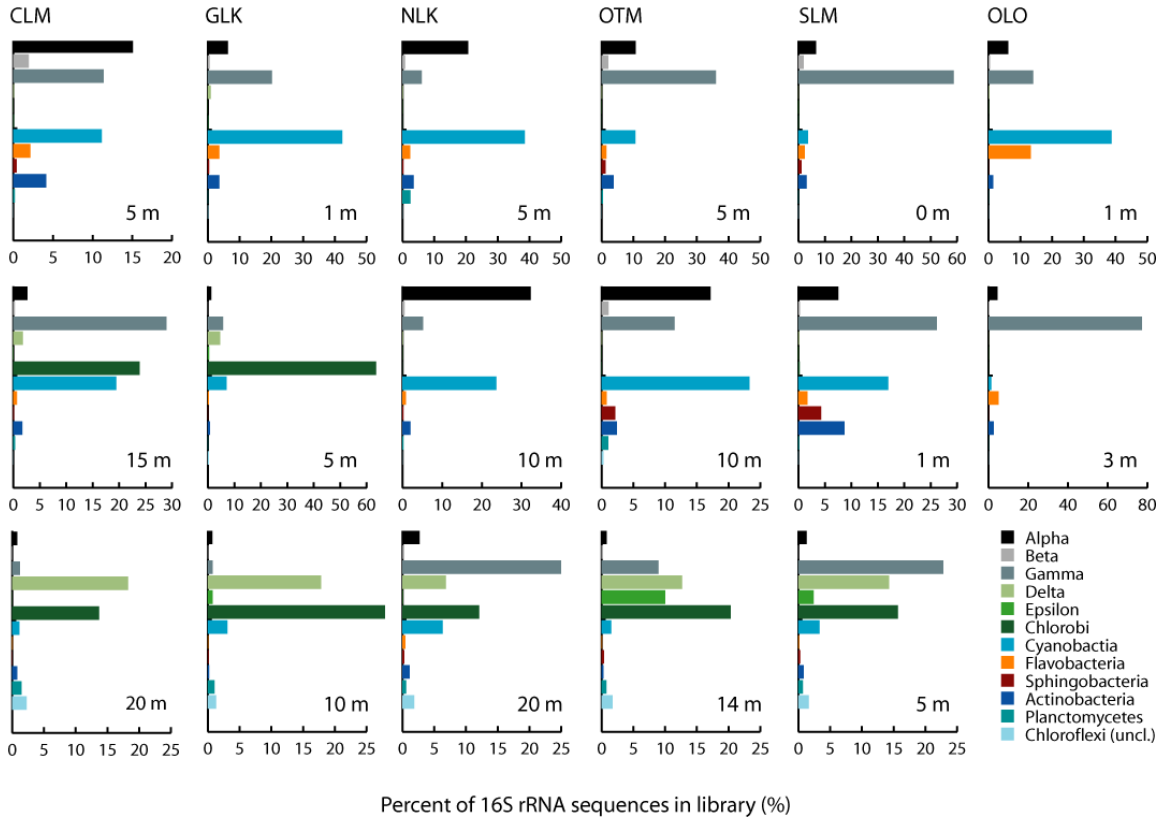


Figure 2.6. Comparison of Bray-Curtis similarity values retrieved from pyrosequencing and ARISA. Axes represent Bray-Curtis similarity values for each method, and samples are color-coded based on the location sampled. The solid line refers to the best fit line of all of the samples (including its equation, R^2 value, and p-value) and a dotted 1:1 line is shown for reference.

Figure 2.7. Community composition of pyrosequencing libraries. Lake names are shown across the top of the figure, and the depth of sampling is indicated in the lower right corner of each graph. Graphs depict all classes of bacteria that comprise more than 1% of any library. Where values do not sum to 100%, this represents both small contributions from other groups, and the presence of bacteria and archaea that could not be classified into phyla (note differences in scales between axes). The Chloroflexi numbered more than 1% of several libraries but could not be assigned to a particular class; data for the phylum are shown.


HLO
MLN
NLN
TLN
ULN
FLK
5 m
5 m
1 m
1 m
2 m
1 m
10 m
10 m
5 m
5 m
6 m
3 m
15 m
20 m
10 m
7 m
8 m
Alpha
Beta
Gamma
Delta
Epsilon
Chlorobi
Cyanobactia
Flavobacteria
Sphingobacteria
Actinobacteria
Planctomycetes
Chloroflexi (uncl.)
Percent of 16S rRNA sequences in library (%)


CLM
GLK
NLK
OTM
SLM
OLO
5 m
1 m
5 m
5 m
0 m
1 m
15 m
5 m
10 m
10 m
1 m
3 m
20 m
10 m
20 m
14 m
5 m
Alpha
Beta
Gamma
Delta
Epsilon
Chlorobi
Cyanobactia
Flavobacteria
Sphingobacteria
Actinobacteria
Planctomycetes
Chloroflexi (uncl.)
Percent of 16S rRNA sequences in library (%)

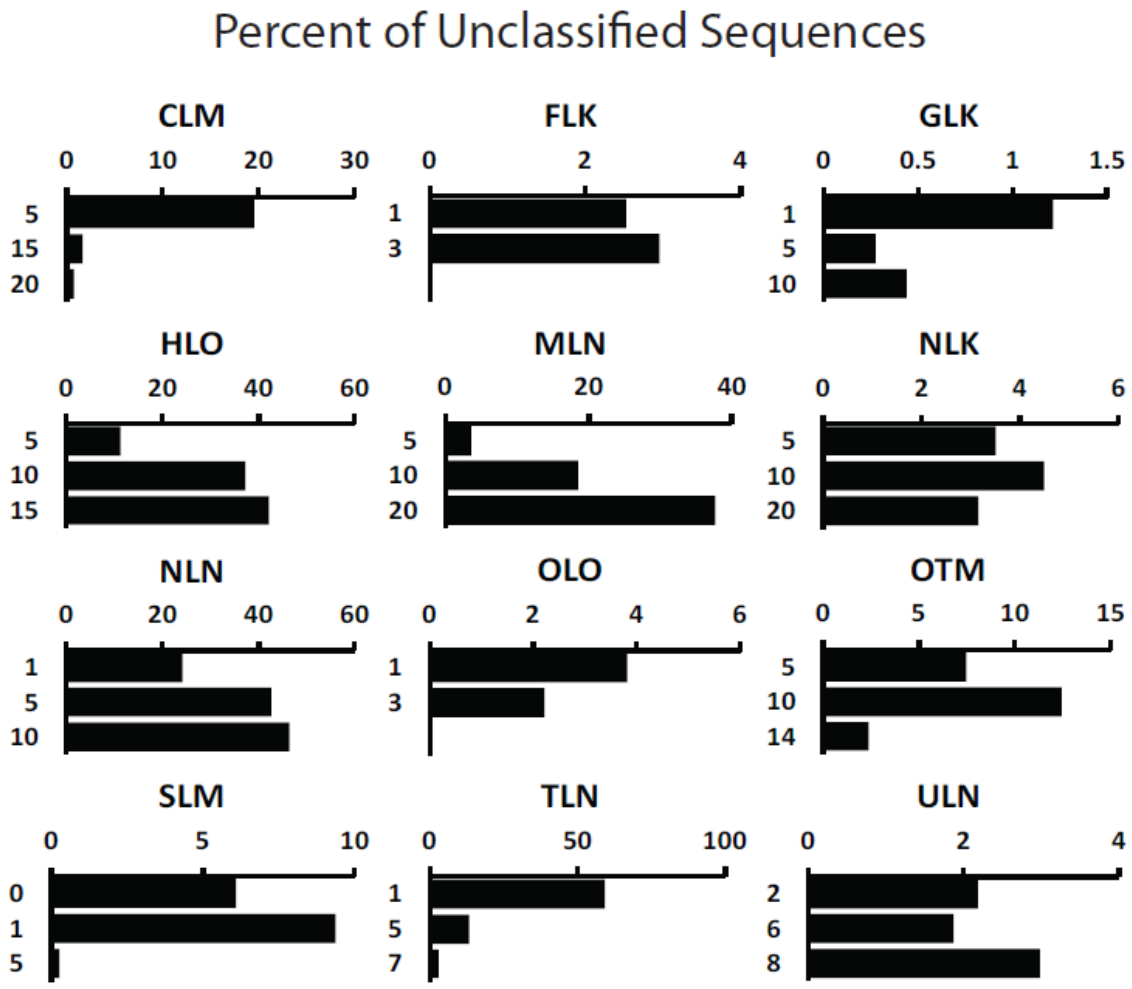
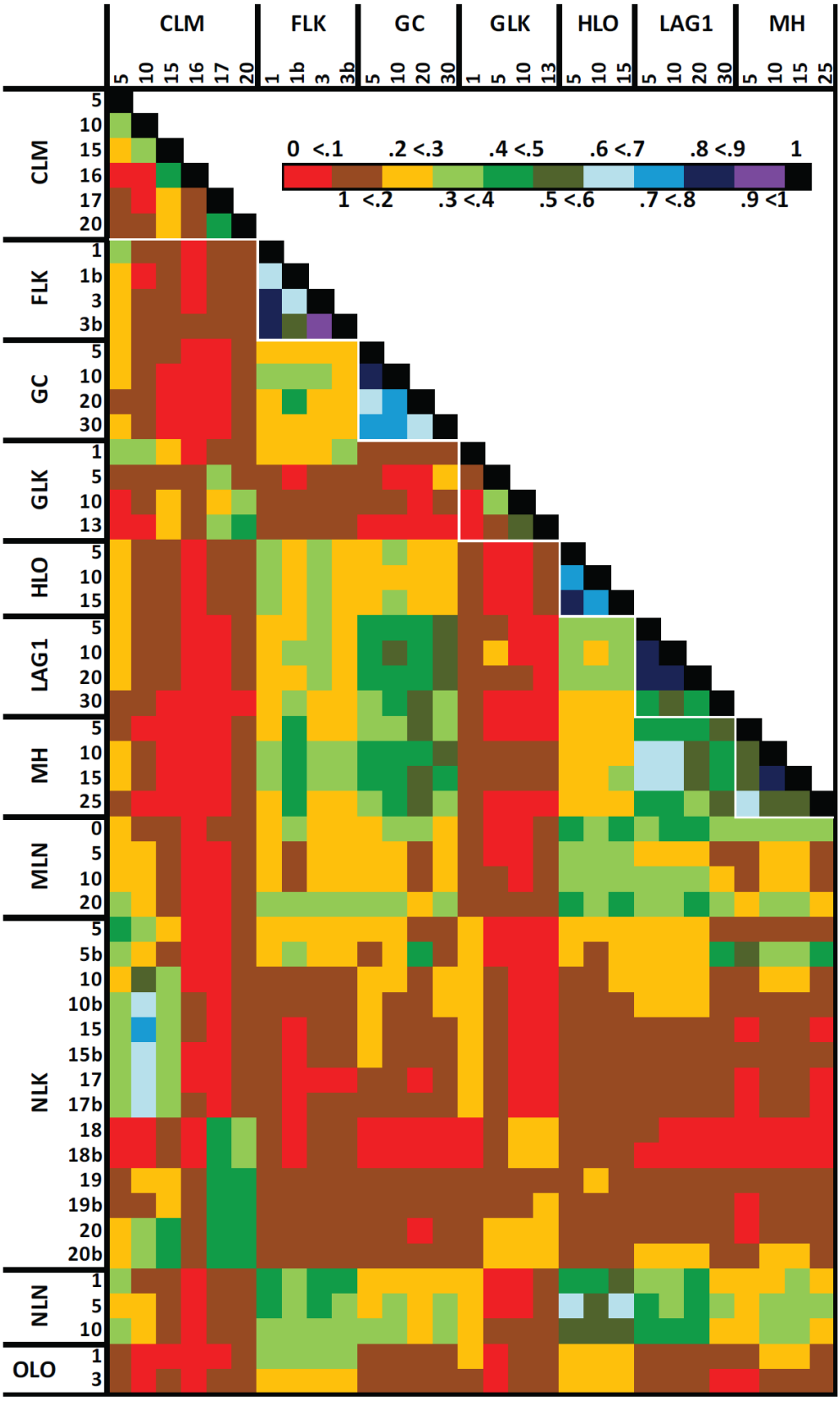
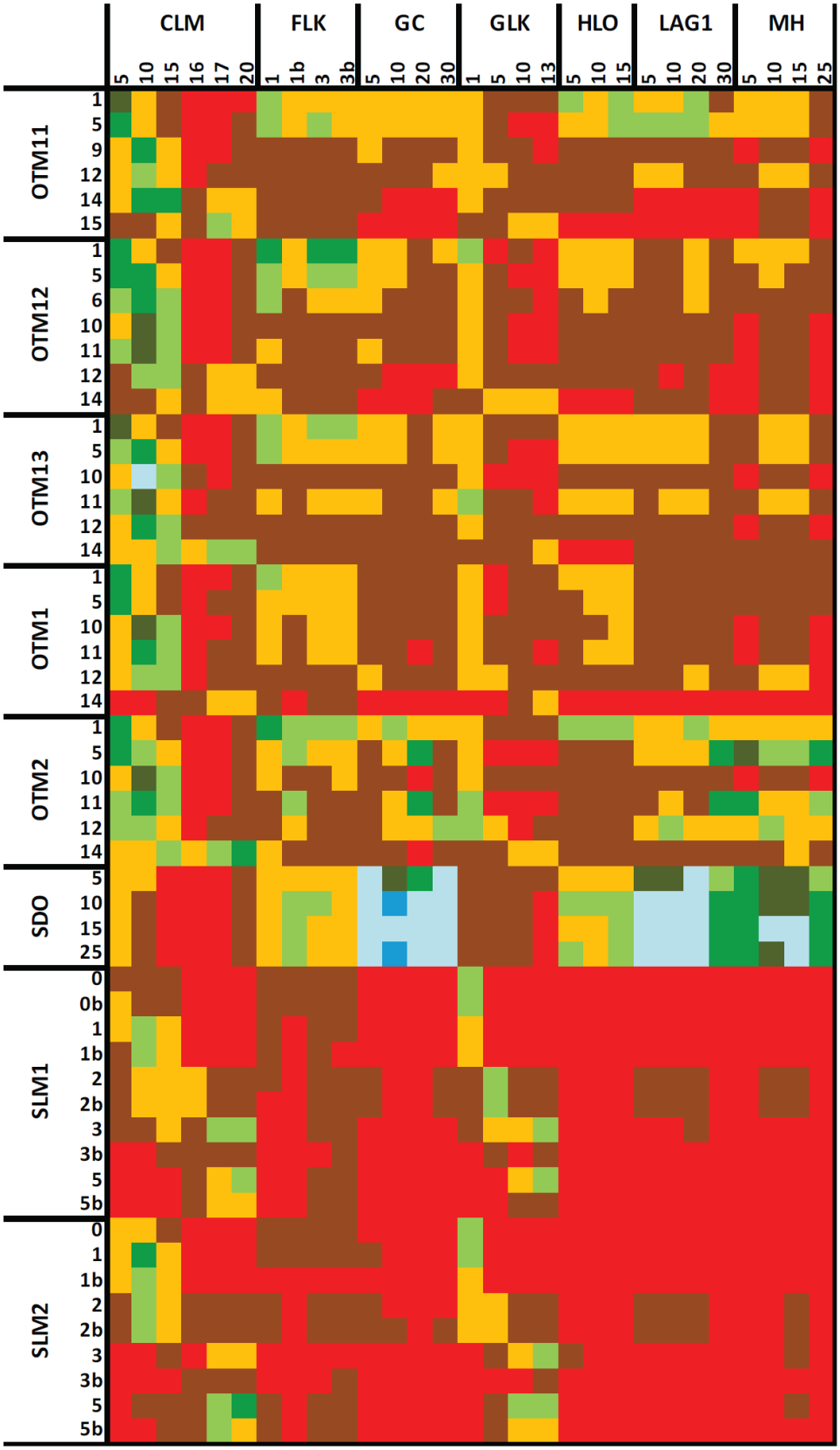
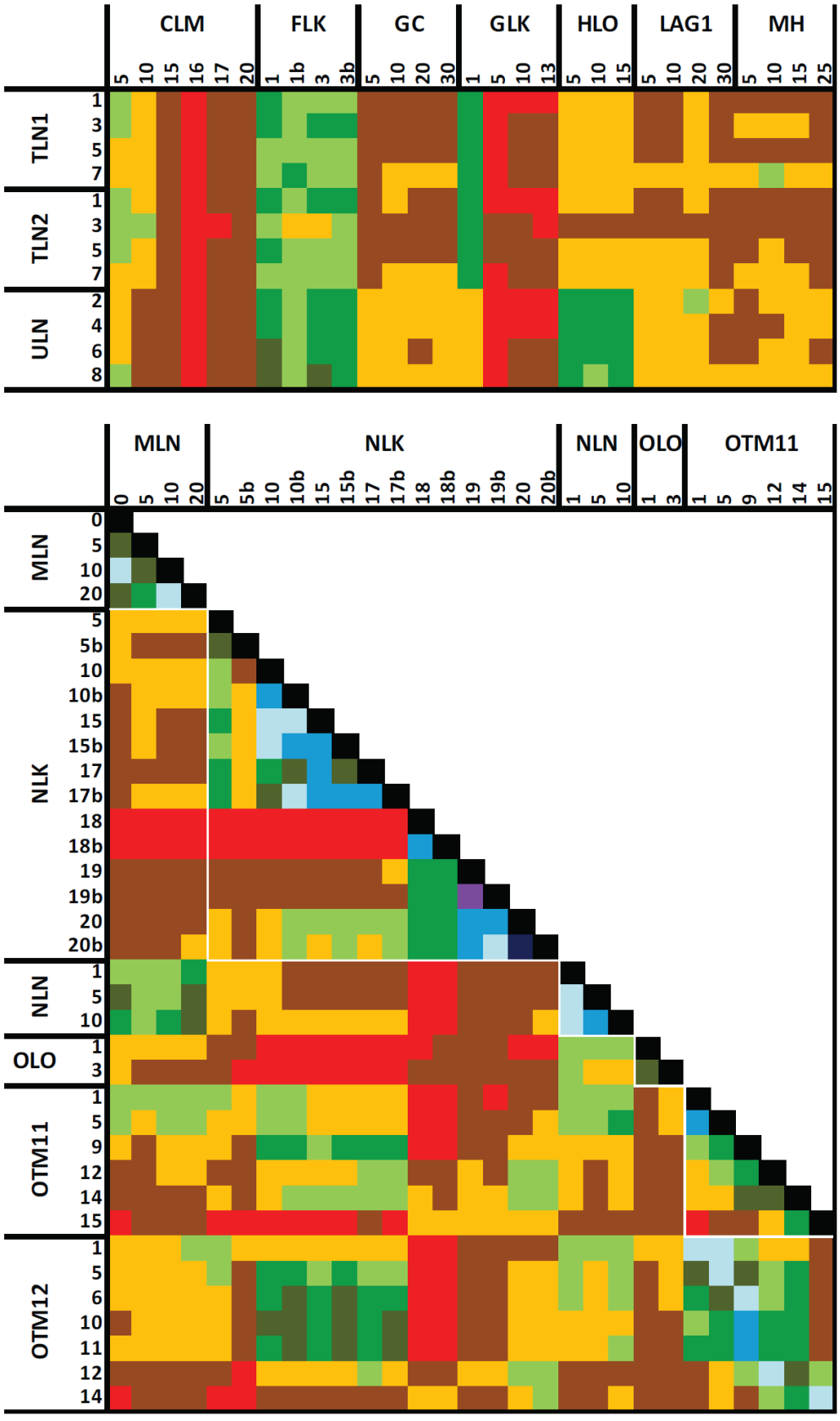


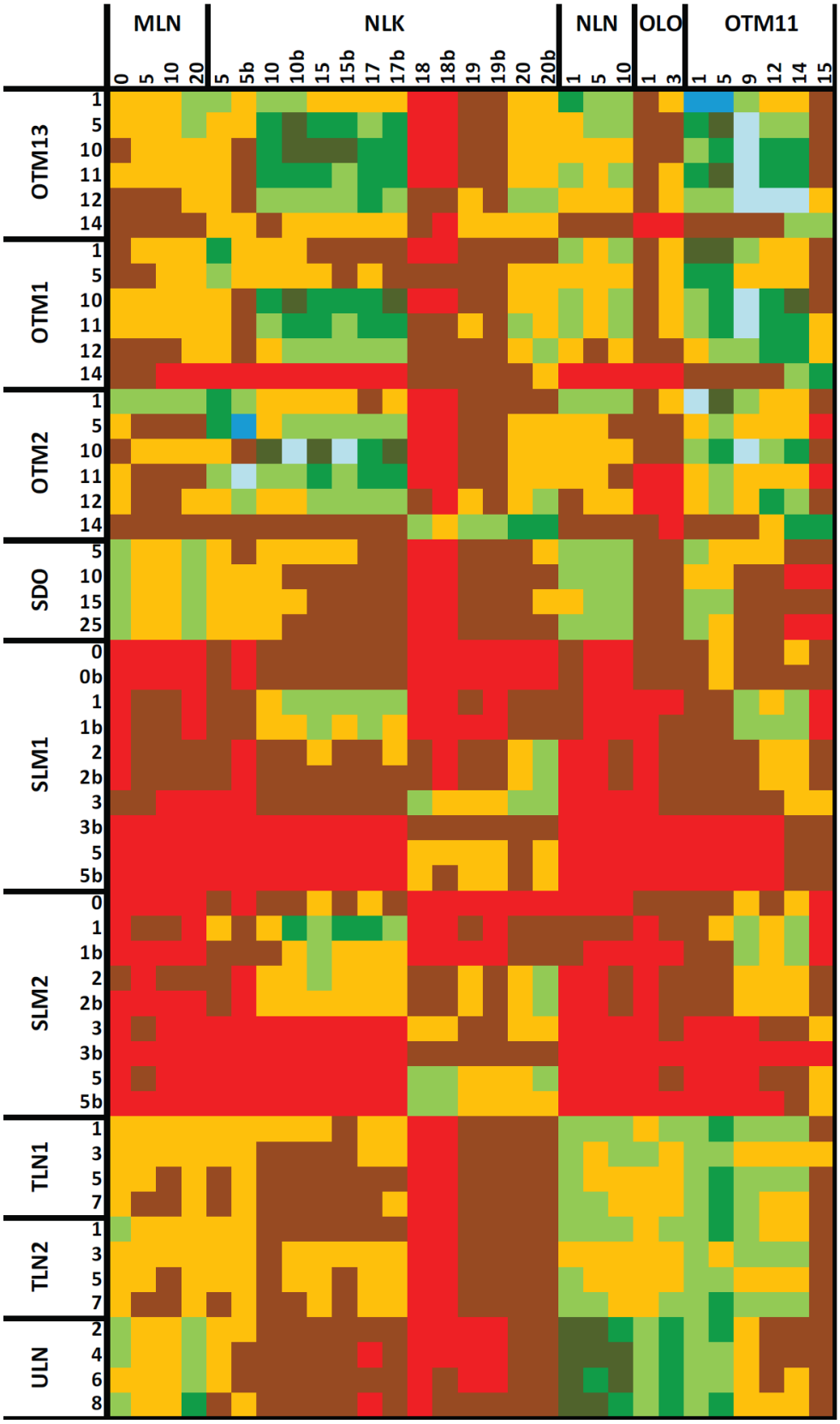
Figure 2.8. Percent of unclassified sequences. Lakes are listed above their graphs. Axes represent percent of unclassified sequences based on pyrosequencing (horizontal) and sampled depths (vertical).

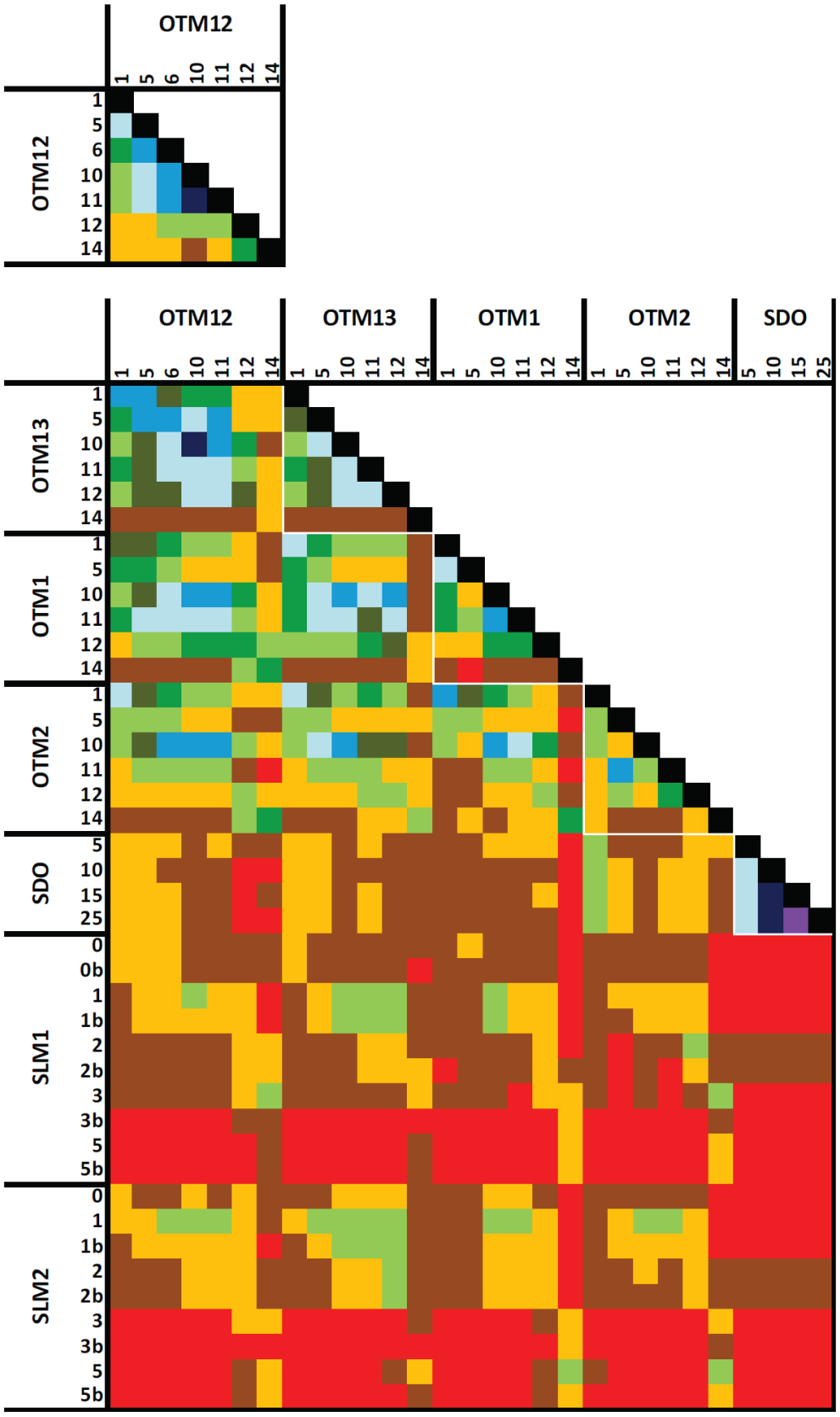
Figure 2.9. ARISA Bray-Curtis similarity values across all samples, Continued. Sampled locations and depths are listed on top and left sides of matrix. A ‘1’ or ‘2’ next to the site code of a lake refers to different locations sampled within the lake. OTM11, OTM12, and OTM13 represent three separate days of sampling (August 11, 12, and 13) in OTM. Samples with ‘b’ are pseudo-replicates. Similarity values follow the color code shown on the first part of the matrix.


CLM
FLK
GC
GLK
HLO
LAG1
MH
5 10 15 16 17 20
1 1b 3 3b
5 10 20 30
1 5 10 13
5 10 15
5 10 20 30
5 10 15 25
0 5 10 20
5 5b 10 10b 15 15b 17 17b 18 18b 19 19b 20 20b
1 5 10
1 3
MLN
NLK
NLN
OLO
0 <.1
.2 <.3
.4 <.5
.6 <.7
.8 <.9
1
1 <.2
.3 <.4
.5 <.6
.7 <.8
.9 <1


CLM
5
10
15
16
17
20
FLK
1
1b
3
3b
GC
5
10
20
30
GLK
1
5
10
13
HLO
5
10
15
LAG1
5
10
20
30
MH
5
10
15
25
OTM11
1
5
9
12
14
15
OTM12
1
5
6
10
11
12
14
OTM13
1
5
10
11
12
14
OTM1
1
5
10
11
12
14
OTM2
1
5
10
11
12
14
SDO
5
10
15
25
SLM1
0
0b
1
1b
2
2b
3
3b
5
5b
SLM2
0
1
1b
2
2b
3
3b
5
5b


CLM
FLK
GC
GLK
HLO
LAG1
MH
TLN1
TLN2
ULN
MLN
NLK
NLN
OLO
OTM11
OTM12


MLN
0
5
10
20
NLK
5
5b
10
10b
15
15b
17
17b
18
18b
19
19b
20
20b
NLN
1
5
10
OLO
1
3
OTM11
1
5
9
12
14
15
OTM13
1
5
10
11
12
14
OTM1
1
5
10
11
12
14
OTM2
1
5
10
11
12
14
SDO
5
10
15
25
SLM1
0
0b
1
1b
2
2b
3
3b
5
5b
SLM2
0
1
1b
2
2b
3
3b
5
5b
TLN1
1
3
5
7
TLN2
1
3
5
7
ULN
2
4
6
8


OTM12
1
5
6
10
11
12
14
OTM12
OTM13
OTM1
OTM2
SDO
1
5
10
11
12
14
15
25
OTM13
OTM1
OTM2
SDO
SLM1
SLM2
0
0b
1b
2
2b
3
3b
5b

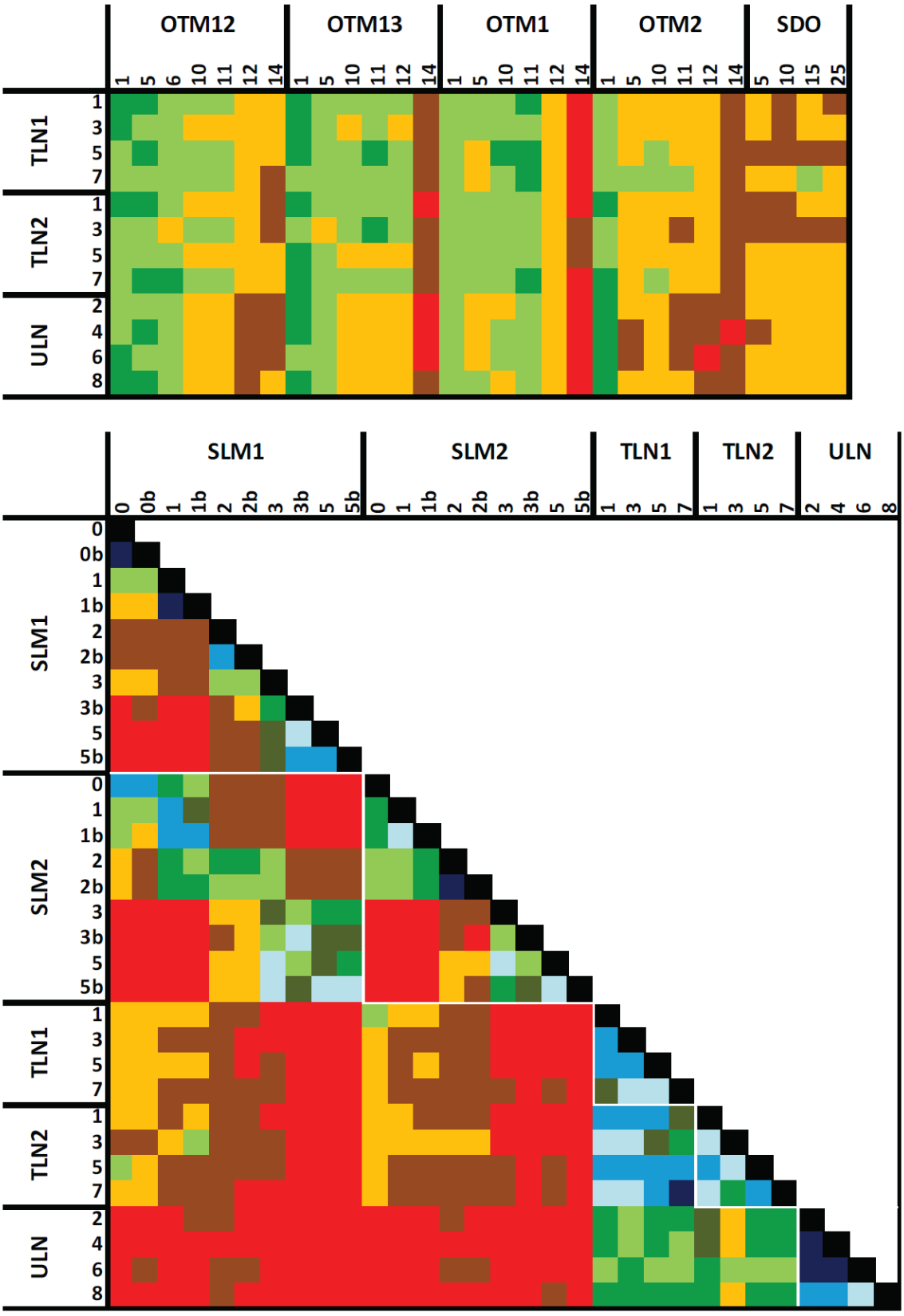

OTM12
OTM13
OTM1
OTM2
SDO
TLN1
TLN2
ULN
SLM1
SLM2
TLN1
TLN2
ULN

Table 2.1. Precision and accuracies of environmental component measurements. A) Accuracy of oxygen, temperature, and pH measurements (± value). B) Precision of nutrient measurements (± standard deviation). C) Accuracy of nutrient measurements (± % error). The percent error pertaining to the check standard concentration that was directly higher than the measured concentration was used to calculate the accuracy of that particular measurement.

A		
Accuracy		
Oxygen (µM)	**Temperature (°C)**	**pH**
0.3125	0.1	0.2

B			
Precision (Standard Deviation, µM)			
Phosphate	**Nitrite**	**Nitrite+Nitrate**	**Ammonia**
0.0806	0.0270	0.1448	0.0416

C				
	Micromolar			
	Phosphate	**Nitrite**	**Nitrite+Nitrate**	**Ammonia**
Check Standard 6	4	2	6	4
Check Standard 7	8			8
Check Standard 8	20			20
Check Standard 9	40			40
Check Standard 10	100			100
Check Standard 11				200
	Accuracy (% Error)			
	Phosphate	**Nitrite**	**Nitrite+Nitrate**	**Ammonia**
Check Standard 6	4.4583	0.8620	2.6641	3.5649
Check Standard 7	3.8994			1.0494
Check Standard 8	2.3769			3.2872
Check Standard 9	0.5024			3.3486
Check Standard 10	0.9622			4.7937
Check Standard 11				4.3689

Table 2.2. ARISA Bray-Curtis similarity values for 454 samples. Sampling locations ('site code'_'depth') are listed at left. Similarity values, which are listed in decimal form, pertain to comparisons within sites for samples selected for pyrosequencing.

Sampling Location	Similarity of upper and lower sample	Similarity of upper and middle sample	Similarity of lower and middle sample
CLM	0.129	0.200	0.244
FLK	0.856	-	-
GC	0.715	0.836	0.760
GLK	0.087	0.110	0.339
HLO	0.824	0.781	0.788
LAG1	0.462	0.835	0.502
MH	0.616	0.594	0.524
MLN	0.451	0.574	0.622
NLK	0.206	0.313	0.238
NLN	0.642	0.669	0.786
OLO	0.538	-	-
SDO	0.643	0.635	0.878
OTM	0.081	0.277	0.122
SLM	0.061	0.342	0.040
TLN	0.626	0.741	0.717
ULN	0.730	0.805	0.633

Table 2.3. Key properties of sampled marine lakes in Palau (Colin 2009, Michael Dawson, personal communication, this study). Lakes listed as follows: Flatworm Lake (FLK), Heliofungia Lake (HLO), Mekeald Lake (MLN), Ngeruktabel Lake (NLN), Ngchas Lake (ULN), Ongael Lake (OLO), T Lake (TLN), Clear Lake (CLM), Goby Lake (GLK), Ngermeuangel Lake (NLK), Ongeim'l Tketau Lake (OTM), and Spooky Lake (SLM).

Lake	**Latitude**	**Longitude**	**Chemocline Depth (m)**	**Maximum Depth (m)**	**Distance Overland (m)**	**Area (m^2)**
FLK	7.3214	134.5059		4		5000
HLO	7.2518	134.3736		25		19000
MLN	7.2980	134.4485		27	70	20300
NLN	7.2781	134.4325		14	50	27000
ULN	7.2532	134.3450		11	70	22000
OLO	7.2573	134.3825		4	150	9000
TLN	7.3076	134.4420		8	100	6900
CLM	7.1532	134.3594	16	30	340	39000
GLK	7.3152	134.5022	5	15	135	21000
NLK	7.3229	134.5086	18	38	210	43000
OTM	7.1611	134.3770	12	30	150	50000
SLM	7.1526	134.3634	2	14	510	11000

Table 2.4. Environmental components of sampled locations, Continued. Sites listed according to their 2- or 3-letter code. A ‘1’ or ‘2’ next to the site code of a lake refers different locations sampled within the lake. OTM11, OTM12, and OTM13 represent three separate days of sampling (August 11, 12, and 13) in OTM. Nutrient measurements below detection limits are listed as ‘<0.10,’ or ND for nitrate (which means nitrite+nitrate concentration was <0.20). Where sulfide presence prevented nitrite+nitrate measurements (see Materials and Methods), or measurements were not made for a particular site, cells are left blank.

Site	Depth (m)	Oxygen (µM)	Temperature (°C)	pH	Nitrite (µM)	Nitrate (µM)	Ammonia (µM)	Phosphate (µM)
CLM	5	148.13	31.89	7.54	0.12	0.29	0.00	0.68
CLM	10	11.25	33.51	7.31	<0.10	0.28	0.58	0.21
CLM	15	4.69	29.86	7.20	0.13	0.19	0.23	0.64
CLM	16	1.56	29.73	7.28	<0.10	0.44	6.00	0.61
CLM	17	0.94	29.39	6.91	<0.10	ND	151.05	5.30
CLM	20	1.25	28.78	6.87	0.18	0.07	202.28	8.25
FLK	1	181.56	31.52	8.01				
FLK	3	162.03	31.27	7.99				
GC	5				<0.10	0.50	1.06	<0.10
GC	10				<0.10	0.44	1.28	<0.10
GC	20				0.11	0.21	0.88	<0.10
GC	30				<0.10	0.37	0.42	<0.10
GLK	1	120.31	31.46	7.33	<0.10	0.58	1.05	<0.10
GLK	5	3.75	30.86	7.39	<0.10	0.34	23.06	0.11
GLK	10	1.56	30.61	6.82	0.42		266.46	10.98
GLK	13	2.50	30.44	6.73	0.19		1096.33	52.79
HLO	5				<0.10	0.28	1.41	<0.10
HLO	10				<0.10	0.28	0.20	<0.10
HLO	15				0.12	0.53	1.02	<0.10
LAG1	5				<0.10	0.28	0.35	<0.10
LAG1	10				<0.10	0.45	0.03	<0.10
LAG1	20				<0.10	0.29	0.06	<0.10
LAG1	30				<0.10	0.47	0.18	<0.10
MH	5	186.25	30.41	8.12	0.12	0.53	0.26	<0.10
MH	10	180.63	30.37	8.11	0.12	0.68	0.32	<0.10
MH	15	175.94	30.32	8.11	0.10	0.54	0.37	<0.10
MH	25				<0.10	0.45	0.00	<0.10
MLN	0	188.44	31.04	7.97				
MLN	5	178.44	31.13	7.98				
MLN	10	163.59	31.00	7.96				
MLN	20	130.63	30.79	7.92				
NLK	5	173.75	31.44	7.78	<0.10	0.29	0.06	<0.10
NLK	10	87.81	32.58	7.54	<0.10	0.28	0.55	<0.10
NLK	15	15.94	32.47	7.35	<0.10	0.29	0.60	0.11
NLK	17	12.50	32.45	7.36	<0.10	0.27	0.00	0.18
NLK	18	3.75	32.54	8.03	<0.10		134.57	5.15
NLK	19	0.94	31.86	7.07	0.16		193.44	7.85
NLK	20	0.94	31.41	7.02	0.19		149.84	5.58

Site	Depth (m)	Oxygen (µM)	Temperature (°C)	pH	Nitrite (µM)	Nitrate (µM)	Ammonia (µM)	Phosphate (µM)
NLN	1	148.44	31.77	7.85	0.13	1.22	1.80	0.12
NLN	5	144.53	31.69	7.89	0.20	0.99	0.65	0.10
NLN	10	143.59	31.60	7.90	0.29	1.17	1.42	0.17
OLO	1	202.66	31.21	7.99	<0.10	0.29	0.19	<0.10
OLO	3	138.28	31.12	7.89	<0.10	0.34	0.00	<0.10
OTM1	1	170.00	31.87	7.58	<0.10	ND	1.27	0.17
OTM1	5	137.19	31.84	7.51	0.17	0.89	0.00	0.31
OTM1	10	26.88	31.52	7.20	<0.10	0.33	2.07	0.23
OTM1	11	12.81	31.47	7.10	<0.10	ND	0.00	0.29
OTM1	12	3.75	31.31	6.98	<0.10	0.40	0.43	0.42
OTM1	14	0.94	29.67	6.96	<0.10		114.85	4.42
OTM11	1							
OTM11	5							
OTM11	9							
OTM11	12							
OTM11	14							
OTM11	15							
OTM12	1				0.12	1.02	0.84	0.46
OTM12	5				<0.10	0.21	0.86	0.18
OTM12	6				<0.10	0.31	0.00	0.16
OTM12	10				<0.10	0.25	0.02	0.19
OTM12	11				<0.10	0.43	2.55	0.28
OTM12	12				0.13	0.67	7.58	0.80
OTM12	14				0.10		86.80	3.59
OTM13	1				<0.10	ND	0.00	0.35
OTM13	5				<0.10	0.23	0.43	0.15
OTM13	10				<0.10	0.23	0.48	0.36
OTM13	11				<0.10	0.52	2.45	0.23
OTM13	12				<0.10	0.55	0.11	0.31
OTM13	14				<0.10	0.61	90.73	3.94
OTM2	1	167.81	31.60	7.62	<0.10	0.26	0.94	0.27
OTM2	5	133.44	32.04	7.54	<0.10	ND	0.11	0.15
OTM2	10	25.63	31.70	7.22	<0.10	0.30	0.00	0.31
OTM2	11	12.50	31.47	7.17	<0.10	0.28	0.00	0.42
OTM2	12	2.50	31.17	6.97	<0.10	0.31	2.00	0.39
OTM2	14	0.94	29.60	6.97	<0.10		120.31	4.89
SDO	5	206.56	30.30	8.08	0.11	0.57	1.15	<0.10
SDO	10	195.63	30.27	8.11	<0.10	0.55	1.82	<0.10

Site	Depth (m)	Oxygen (µM)	Temperature (°C)	pH	Nitrite (µM)	Nitrate (µM)	Ammonia (µM)	Phosphate (µM)
SDO	15	194.69	30.26	8.11	<0.10	0.25	0.58	<0.10
SDO	25	193.44	30.26	8.11	<0.10	ND	0.81	<0.10
SLM1	0	51.09	29.80	7.07	0.17	0.75	1.01	0.21
SLM1	1	32.19	30.77	7.05	<0.10	0.42	0.50	0.13
SLM1	2	17.50	32.32	7.04	0.10	0.31	0.69	0.11
SLM1	3	3.44	32.01	6.86	0.35	0.95	194.11	4.70
SLM1	5	2.03	31.23	6.92	0.22	0.05	185.11	5.08
SLM2	0				0.14	0.91	1.31	0.23
SLM2	1				<0.10	0.27	1.58	<0.10
SLM2	2				<0.10	0.28	0.00	<0.10
SLM2	3				0.47		275.46	6.52
SLM2	5				0.26	0.33	160.93	3.50
TLN1	1	125.00	31.51	7.65	<0.10	ND	0.00	<0.10
TLN1	3	90.63	31.56	7.56	<0.10	0.30	1.69	<0.10
TLN1	5	83.75	31.60	7.57	<0.10	0.60	0.00	<0.10
TLN1	7	60.63	31.62	7.53	0.11	0.56	1.62	<0.10
TLN2	1	125.94	31.94	7.66	<0.10	0.24	0.91	<0.10
TLN2	3	90.63	31.80	7.58	0.11	0.27	0.75	<0.10
TLN2	5	78.75	31.75	7.56	0.10	0.64	0.50	<0.10
TLN2	7	56.88	31.69	7.51	0.14	0.62	2.59	0.13
ULN	2	185.00	30.61	7.91				
ULN	4	172.34	30.54	7.89				
ULN	6	168.75	30.57	7.91				
ULN	8	148.44	30.52	7.86				

References

Azam F and Worden AZ. 2004. Oceanography. microbes, molecules, and marine ecosystems. Science 303(5664):1622-4.

Ballot A, Krienitz L, Kotut K, Wiegand C, Metcalf JS, Codd GA, Pfligmacher S. 2004. Cyanobacteria and cyanobacterial toxins in three alkaline Rift Valley lakes of Kenya – Lakes Bogoria, Nakuru and Elmenteita. Journal of Plankton Research 26(8):925-35.

Barth JA, Shearman RK, Chan F, Pierce SD, Erofeev AY, Brodersen J, Levine MD, Page-Albims K, Risien C, Rubiano-Gomez L, Waldorf BW. 2009. An expanding observatory to monitor hypoxia in the Northern California Current System.

Bazylinski DA and Blakemore RP. 1983. Denitrification and assimilatory nitrate reduction in aquaspirillum magnetotacticum. Appl Environ Microbiol 46(5):1118-24.

Beman JM, Chow CE, King AL, Feng Y, Fuhrman JA, Andersson A, Bates NR, Popp BN, Hutchins DA. 2011. Global declines in oceanic nitrification rates as a consequence of ocean acidification. Proc Natl Acad Sci U S A 108(1):208-13.

Beman JM, Popp BN, Francis CA. 2008. Molecular and biogeochemical evidence for ammonia oxidation by marine crenarchaeota in the gulf of california. ISME J 2(4):429-41.

Bennett EM, Carpenter SR, Caraco NF. 2001. Human impact on erodable phosphorus and eutrophication: A global perspective. BioScience 51(3):227-34.

Bodaker I, Beja O, Sharon I, Feingersch R, Rosenberg M, Oren A, Hindiyeh MY, Malkawi HI. 2009. Archaeal diversity in the Dead Sea: Microbial survival under increasingly harsh conditions. Natural Resources and Environmental Issues 15(25):137-43.

Bograd SJ, Castro CG, Lorenzo ED, Palacios DM, Bailey H, Gilly W, Chavez FP. 2008. Oxygen declines and the shoaling of the hypoxic boundary in the California Current. Geophysical Research Letters 35:1-6.

Bosshard PP, Stettler R, Bachofen R. 2000. Seasonal and spatial community dynamics in the meromictic lake cadagno. Arch Microbiol 174(3):168-74.

Bray JR and Curtis JT. 1957. An ordination of the upland forest communities of Southern Wisconsin. Ecological Monographs 27(4):325-49.

Brazelton WJ, Ludwig KA, Sogin ML, Andreishcheva EN, Kelley DS, Shen CC, Edwards RL, Baross JA. 2010. Archaea and bacteria with surprising microdiversity show shifts in dominance over 1,000-year time scales in hydrothermal chimneys. Proc Natl Acad Sci U S A 107(4):1612-7.

Breitburg DL, Crump BC, Dabiri JO, Gallegos CL. 2010. Ecosystem engineers in the pelagic realm: Alteration of habitat by species ranging from microbes to jellyfish. Integr Comp Biol 50(2):188-200.

Brisou J, Courtois D, Denis F. 1974. Microbiological study of a hypersaline lake in french somaliland. Appl Microbiol 27(5):819-22.

Brown MV, Philip GK, Bunge JA, Smith MC, Bissett A, Lauro FM, Fuhrman JA, Donachie SP. 2009. Microbial community structure in the north pacific ocean. ISME J 3(12):1374-86.

Brown MV, Schwalbach MS, Hewson I, Fuhrman JA. 2005. Coupling 16S-ITS rDNA clone libraries and automated ribosomal intergenic spacer analysis to show marine microbial diversity: Development and application to a time series. Environ Microbiol 7(9):1466-79.

Canfield DE, Stewart FJ, Thamdrup B, De Brabandere L, Dalsgaard T, Delong EF, Revsbech NP, Ulloa O. 2010. A cryptic sulfur cycle in oxygen-minimum-zone waters off the chilean coast. Science 330(6009):1375-8.

Cardinale M, Brusetti L, Quatrini P, Borin S, Puglia AM, Rizzi A, Zanardini E, Sorlini C, Corselli C, Daffonchio D. 2004. Comparison of different primer sets for use in automated ribosomal intergenic spacer analysis of complex bacterial communities. Appl Environ Microbiol 70(10):6147-56.

Carini SA and Joye SB. 2008. Nitrification in mono lake, california: Activity and community composition during contrasting hydrological regimes. Limnol Oceanogr 53(6):2546-57.

Carini S, Bano N, LeCleir G, Joye SB. 2005. Aerobic methane oxidation and methanotroph community composition during seasonal stratification in mono lake, california (USA). Environ Microbiol 7(8):1127-38.

Castro-Gonzalez M, Braker G, Farias L, Ulloa O. 2005. Communities of nirS-type denitrifiers in the water column of the oxygen minimum zone in the eastern South Pacific. Environmental Microbiology 7(9):1298–1306.

Chan F, Barth JA, Lubchenco J, Kirincich A, Weeks H, Peterson WT, Menge BA. 2008. Emergence of anoxia in the california current large marine ecosystem. Science 319(5865):920.

Chao A. 1984. Nonparametric estimation of the number of classes in a population. Scandinavian Journal of Statistics 11(4):265-70.

Chao A, Ma MC, Yang MCK. 1993. Stopping rules and estimation for recapture debugging with unequal failure rates. Biometrika 80(1):193-201.

Cho JC and Tiedje JM. 2000. Biogeography and degree of endemicity of fluorescent pseudomonas strains in soil. Appl Environ Microbiol 66(12):5448-56.

Colin PL. 2009. Marine environments of Palau. Edition ed. Koror: Coral Reef Research Foundation.

Coolen MJ, Abbas B, van Bleijswijk J, Hopmans EC, Kuypers MM, Wakeham SG, Sinninghe Damste JS. 2007. Putative ammonia-oxidizing crenarchaeota in suboxic waters of the black sea: A basin-wide ecological study using 16S ribosomal and functional genes and membrane lipids. Environ Microbiol 9(4):1001-16.

Crowe SA, Jones C, Katsev S, Magen C, O'Neill AH, Sturm A, Canfield DE, Haffner GD, Mucci A, Sundby B, Fowle DA. 2008. Photoferrotrophs thrive in an archean ocean analogue. Proc Natl Acad Sci U S A 105(41):15938-43.

Cytryn E, Minz D, Oremland RS, Cohen Y. 2000. Distribution and diversity of archaea corresponding to the limnological cycle of a hypersaline stratified lake (solar lake, sinai, egypt). Appl Environ Microbiol 66(8):3269-76.

Dawson MN. 2006. Island evolution in marine lakes. JMBA Global Marine Environment 3:26-29.

Dawson MN and Hamner WM. 2005. Rapid evolutionary radiation of marine zooplankton in peripheral environments. Proc Natl Acad Sci U S A 102(26):9235-40.

Dawson MN and Hamner WM. 2008. A biophysical perspective on dispersal and the geography of evolution in marine and terrestrial systems. J R Soc Interface 5(19):135-50.

Dawson MN and Hamner WM. 2003. Geographic variation and behavioral evolution in marine plankton: the case of Mastigias (Scyphozoa, Rhizostomeae). Marine Biology 43:1161–74.

Dawson MN and Hamner WM. 2009. A character-based analysis of the evolution of jellyfish blooms: adaptation and exaptation. Hydrobiologia 616:193–215.

Dawson MN, Martin LE, Penland LK. 2001. Jellyfish swarms, tourists, and the Christ-child. Hydrobiologia 451:131–44.

DeLong EF, Preston CM, Mincer T, Rich V, Hallam SJ, Frigaard NU, Martinez A, Sullivan MB, Edwards R, Brito BR, Chisholm SW, Karl DM. 2006. Community genomics among stratified microbial assemblages in the ocean's interior. Science 311(5760):496-503.

DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K, Huber T, Dalevi D, Hu P, Andersen GL. 2006. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl Environ Microbiol 72(7):5069-72.

Deutsch C, Brix H, Ito T, Frenzel H, Thompson L. 2011. Climate-forced variability of ocean hypoxia. Science 333(6040):336-9.

Diamond DH. 1997. Determination of nitrate in brackish or seawater by flow injection analysis. QuikChem Method 31-107-04-1-A for Lachat Instruments. Milwaukee, WI, USA.

Diamond D and Huberty A. 1996. Determination of ammonia by flow injection analysis. QuikChem Method 31-107-06-5-A. Lachat Instruments, Milwaukee, WI.

Diaz RJ and Rosenberg R. 2008. Spreading dead zones and consequences for marine ecosystems. Science 321(5891):926-9.

Dore JE, Popp BN, Karl DM, Sansone FJ. 1998. Alarge source of atmospheric nitrous oxide from subtropical North Pacific surfacewaters. Nature 396:63-6.

Eilers H, Pernthaler J, Glockner FO, Amann R. 2000. Culturability and in situ abundance of pelagic bacteria from the north sea. Appl Environ Microbiol 66(7):3044-51.

Engelbrektson A, Kunin V, Wrighton KC, Zvenigorodsky N, Chen F, Ochman H, Hugenholtz P. 2010. Experimental factors affecting PCR-based estimates of microbial species richness and evenness. ISME J 4(5):642-7.

Fabricius KE, Mieog JC, Colin PL, Idip D, van Oppen MJ. 2004. Identity and diversity of coral endosymbionts (zooxanthellae) from three palauan reefs with contrasting bleaching, temperature and shading histories. Mol Ecol 13(8):2445-58.

Falkowski PG, Fenchel T, Delong EF. 2008. The microbial engines that drive earth's biogeochemical cycles. Science 320(5879):1034-9.

Ferdelman TG, Thamdrup B, Canfield DE, Glud RN, Kuever J, Lillebaek R, Ramsing NB, Wawer C. 2006. Biogeochemical controls on the oxygen, nitrogen and sulfur distributions in the water column of Golfo Dulce: an anoxic basin on the Pacific coast of Costa Rica revisited. Rev. Biol. Trop. 54:171-91.

Fisher MM and Triplett EW. 1999. Automated approach for ribosomal intergenic spacer analysis of microbial diversity and its application to freshwater bacterial communities. Appl Environ Microbiol 65(10):4630-6.

Fry B, Jannasch HW, Molyneaux SJ, Wirsen CO, Muramoto JA, King S. 1991. Stable isotope studies of the carbon, nitrogen and sulfur cycles in the Black Sea and the Cariaco Trench. Deep-Sea Research 38:S1003-19.

Fuenzalida R, Schneider W, Garces-Vargas J, Bravo L, Lange C. 2009. Vertical and horizontal extension of the oxygen minimum zone in the eastern South Pacific Ocean. Deep-Sea Research II 56:992–1003.

Fuhrman JA. 2009. Microbial community structure and its functional implications. Nature 459(7244):193-9.

Galand PE, Casamayor EO, Kirchman DL, Lovejoy C. 2009. Ecology of the rare microbial biosphere of the arctic ocean. Proc Natl Acad Sci U S A 106(52):22427-32.

Godfrey LV and Falkowski PG. 2009. The cycling and redox state of nitrogen in the Archean ocean. Nature Geosciences 2:725–9.

Green JL, Holmes AJ, Westoby M, Oliver I, Briscoe D, Dangerfield M, Gillings M, Beattie AJ. 2004. Spatial scaling of microbial eukaryote diversity. Nature 432(7018):747-50.

Gruber N and Galloway JN. 2008. An earth-system perspective of the global nitrogen cycle. Nature 451(7176):293-6.

Halpern BS, Walbridge S, Selkoe KA, Kappel CV, Micheli F, D'Agrosa C, Bruno JF, Casey KS, Ebert C, Fox HE, Fujita R, Heinemann D, Lenihan HS, Madin EMP, Perry MT, Selig ER, Spalding M, Steneck R, Watson R. 2008. A global map of human impact on marine ecosystems. Science 319(5865):948-52.

Hamady M, Walker JJ, Harris JK, Gold NJ, Knight R. 2008. Error-correcting barcoded primers for pyrosequencing hundreds of samples in multiplex. Nat Methods 5(3):235-7.

Hamner WM and Hamner PP. 1998. Stratified marine lakes of Palau (Western Caroline Islands). Physical Geography 19(3):175-220.

Hamner WM and Hauri IR. 1981. Long-distance horizontal migrations of zooplankton (Scyphomedusae: Mastigias). Limnology and Oceanography 26(3):414-23.

Hamner WM, Gilmer RW, Hamner PP. 1982. The physical, chemical, and biological characteristics of a stratified, saline, sulfide lake in Palau. Limnology and Oceanography 27(5):896-909.

Hedlund BP, Geiselbrecht AD, Bair TJ, Staley JT. 1999. Polycyclic aromatic hydrocarbon degradation by a new marine bacterium, neptunomonas naphthovorans gen. nov., sp. nov. Appl Environ Microbiol 65(1):251-9.

Hewson I, Steele JA, Capone DG, Fuhrman JA. 2006. Remarkable heterogeneity in meso- and bathypelagic bacterioplankton assemblage composition. Limnology and Oceanography 51(3):1274-83.

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.

Huber JA, Mark Welch DB, Morrison HG, Huse SM, Neal PR, Butterfield DA, Sogin ML. 2007. Microbial population structures in the deep marine biosphere. Science 318(5847):97-100.

Huberty A and Diamond D. 1998. Determination of phosphorus by flow injection analysis colorimetry. QuikChem Method 31-115-01-3-A for Lachat Instruments. Milwaukee, WI.

Hughes TP, Baird AH, Bellwood DR, Card M, Connolly SR, Folke C, Grosberg R, Hoegh-Guldberg O, Jackson JB, Kleypas J, Lough JM, Marshall P, Nystrom M, Palumbi SR, Pandolfi JM, Rosen B, Roughgarden J. 2003. Climate change, human impacts, and the resilience of coral reefs. Science 301(5635):929-33.

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(2):1030-42.

Huse SM, Huber JA, Morrison HG, Sogin ML, Welch DM. 2007. Accuracy and quality of massively parallel DNA pyrosequencing. Genome Biol 8(7):R143.

Huse SM, Welch DM, Morrison HG, Sogin ML. 2010. Ironing out the wrinkles in the rare biosphere through improved OTU clustering. Environ Microbiol 12(7):1889-98.

Jellison R and Melack JM. 1993. Meromixis in hypersaline Mono Lake, California. 1. Stratification and vertical mixing during the onset, persistence, and breakdown of meromixis. Limnology and Oceanography 38(5):1008-19.

Jellison R, Miller LG, Melack JM, Dana GL. 1993. Meromixis in hypersaline Mono Lake, California. 2. Nitrogen fluxes. Limnology and Oceanography 38(5):1020-39.

Johnston DT, Wolfe-Simon F, Pearson A, Knoll AH. 2009. Anoxygenic photosynthesis modulated proterozoic oxygen and sustained earth's middle age. Proc Natl Acad Sci U S A 106(40):16925-9.

Joint I, Doney SC, Karl DM. 2011. Will ocean acidification affect marine microbes? ISME J 5(1):1-7.

Jones SE, Shade AL, McMahon KD, Kent AD. 2007. Comparison of primer sets for use in automated ribosomal intergenic spacer analysis of aquatic bacterial communities: An ecological perspective. Appl Environ Microbiol 73(2):659-62.

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(1):178-88.

Keeling RF and Garcia HE. 2002. The change in oceanic O2 inventory associated with recent global warming. Proc Natl Acad Sci U S A 99(12):7848–53.

Keeling RF, Kortzinger A, Gruber N. 2010. Ocean deoxygenation in a warming world. Annu. Rev. Mar. Sci. 2:199–229.

Kessler JD, Reeburgh WS, Tyler Sc. 2006. Controls on methane concentration and stable isotope (δ2H-CH4 and δ13C-CH4) distributions in the water columns of the Black Sea and Cariaco Basin. Global Biogeochemical Cycles 20:1-13.

Koehler I, Konhauser K, Kappler A. 2010. Chapter 14: Role of microorganisms in banded iron formations. In: Geomicrobiology: Molecular and Environmental Perspective. Barton LL, Mandl M, Loy A, editors. Edition ed. Berlin: Springer Netherlands. 309 p.

Koizumi Y, Kojima H, Fukui M. 2004. Dominant microbial composition and its vertical distribution in saline meromictic lake kaiike (japan) as revealed by quantitative oligonucleotide probe membrane hybridization. Appl Environ Microbiol 70(8):4930-40.

Kunin V, Engelbrektson A, Ochman H, Hugenholtz P. 2010. Wrinkles in the rare biosphere: Pyrosequencing errors can lead to artificial inflation of diversity estimates. Environ Microbiol 12(1):118-23.

Kuypers MM, Sliekers AO, Lavik G, Schmid M, Jorgensen BB, Kuenen JG, Sinninghe Damste JS, Strous M, Jetten MS. 2003. Anaerobic ammonium oxidation by anammox bacteria in the black sea. Nature 422(6932):608-11.

Lam P and Kuypers MM. 2011. Microbial nitrogen cycling processes in oxygen minimum zones. Ann Rev Mar Sci 3:317-45.

Lam P, Jensen MM, Lavik G, McGinnis DF, Muller B, Schubert CJ, Amann R, Thamdrup B, Kuypers MMM. 2007. Linking crenarchaeal and bacterial nitrification to anammox in the black sea. Proceedings of the National Academy of Sciences of the United States of America 104(17):7104-9.

Lam P, Lavik G, Jensen MM, van de Vossenberg J, Schmid M, Woebken D, Gutierrez D, Amann R, Jetten MS, Kuypers MM. 2009. Revising the nitrogen cycle in the peruvian oxygen minimum zone. Proc Natl Acad Sci U S A 106(12):4752-7.

Landing WM, Burnett WC, Lyons WB, Orem WH. 1991. Nutrient cycling and the biogeochemistry of manganese, iron, and zinc in Jellyfish Lake, Palau. Limnology and Oceanography 36(3):515-25.

Lauber CL, Hamady M, Knight R, Fierer N. 2009. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. Appl Environ Microbiol 75(15):5111-20.

Lavik G, Stuhrmann T, Bruchert V, Van der Plas A, Mohrholz V, Lam P, Mussmann M, Fuchs BM, Amann R, Lass U, Kuypers MMM. 2009. Detoxification of sulphidic african shelf waters by blooming chemolithotrophs. Nature 457(7229):581-4.

Levin LA. 2003. Oxygen minimum zone benthos: adaptation and community response to hypoxia. Oceanography and Marine Biology: an Annual Review 41:1–45.

Lin JL, Joye SB, Scholten JC, Schafer H, McDonald IR, Murrell JC. 2005. Analysis of methane monooxygenase genes in mono lake suggests that increased methane oxidation activity may correlate with a change in methanotroph community structure. Appl Environ Microbiol 71(10):6458-62.

Lin X, Wakeham SG, Putnam IF, Astor YM, Scranton MI, Chistoserdov AY, Taylor GT. 2006. Comparison of vertical distributions of prokaryotic assemblages in the anoxic cariaco basin and black sea by use of fluorescence in situ hybridization. Appl Environ Microbiol 72(4):2679-90.

Madrid VM, Taylor GT, Scranton MI, Chistoserdov AY. 2001. Phylogenetic diversity of bacterial and archaeal communities in the anoxic zone of the cariaco basin. Appl Environ Microbiol 67(4):1663-74.

McCarthy JJ, Yilmaz A, Coban-Yildiz Y, Nevins JL. 2007. Nitrogen cycling in the offshore waters of the black sea. Estuarine Coastal and Shelf Science 74(3):493-514.

McGenity TJ. 2010. Methanogens and methanogenesis in hypersaline environments. In: Handbook of Hydrocarbon and Lipid Microbiology. Timmis KN, editor. Edition ed. Berlin: Springer-Verlag. 665 p.

Morris RM, Rappe MS, Connon SA, Vergin KL, Siebold WA, Carlson CA, Giovannoni SJ. 2002. SAR11 clade dominates ocean surface bacterioplankton communities. Nature 420(6917):806-10.

Murray JW, Jannasch HW, Honjo S, Anderson RF, Reeburgh WS, Top Z, Friederich GE, Codispoti LA, Izdar E. 1989. Unexpected changes in the oxic/anoxic interface in the Black Sea. Nature 338:411-3.

Muscatine L and Marian RE. 1982. Dissolved Inorganic Nitrogen Flux in Symbiotic and Nonsymbiotic Medusae. Limnology and Oceanography 27(5):910-7.

Naqvi SW, Jayakumar DA, Narvekar PV, Naik H, Sarma VV, D'Souza W, Joseph S, George MD. 2000. Increased marine production of N2O due to intensifying anoxia on the indian continental shelf. Nature 408(6810):346-9.

Oakley BB, Francis CA, Roberts KJ, Fuchsman CA, Srinivasan S, Staley JT. 2007. Analysis of nitrite reductase (nirK and nirS) genes and cultivation reveal depauperate community of denitrifying bacteria in the black sea suboxic zone. Environmental Microbiology 9(1):118-30.

Oda Y, Star B, Huisman LA, Gottschal JC, Forney LJ. 2003. Biogeography of the purple nonsulfur bacterium rhodopseudomonas palustris. Appl Environ Microbiol 69(9):5186-91.

Oren A. 2002. Molecular ecology of extremely halophilic archaea and bacteria. FEMS Microbiol Ecol 39(1):1-7.

Overmann J, Coolen MJ, Tuschak C. 1999. Specific detection of different phylogenetic groups of chemocline bacteria based on PCR and denaturing gradient gel electrophoresis of 16S rRNA gene fragments. Arch Microbiol 172(2):83-94.

Owen RW. 1989. Microscale and finescale variations of small plankton in coastal and pelagic environments. Journal of Marine Research 47:197-240.

Pape T, Blumenberg M, Seifert R, Bohrmann G, Michaelis W. 2008. Marine methane biogeochemisty of the Black Sea: a review. In: Links Between Geological Processes, Microbial Activities & Evolution of Life. Dilek Y, Furnes H, Muehlenbachs K, editors. Edition ed. Springer Science + Business Media B.V. 281 p.

Partensky F and Garczarek L. 2010. Prochlorococcus: Advantages and limits of minimalism. Ann Rev Mar Sci 2:305-31.

Pedros-Alio C. 2006. Marine microbial diversity: Can it be determined? Trends Microbiol 14(6):257-63.

Ramette A. 2009. Quantitative community fingerprinting methods for estimating the abundance of operational taxonomic units in natural microbial communities. Appl Environ Microbiol 75(8):2495-505.

Ravishankara AR, Daniel JS, Portmann RW. 2009. Nitrous oxide (N2O): The dominant ozone-depleting substance emitted in the 21st century. Science 326(5949):123-5.

Sarmento H, Isumbisho M, Descy JP. 2006. Phytoplankton ecology of Lake Kivu (eastern Africa). Journal of Plankton Research 28(9):815-29.

Sarmento H, Unrein F, Isumbisho M, Stenuite S, Gasol JM, Descy JP. 2008. Abundance and distribution of picoplankton in tropical, oligotrophic Lake Kivu, eastern Africa. Freshwater Biology 53:756–71.

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 Environmental Microbiology 75(23):7537–41.

Schroeder S. 1997. Determination of nitrite in brackish or seawater by flow injection analysis. QuikChem Method 31-107-05-1-A for Lachat Instruments. Milwaukee, WI: Lachat Instruments.

Scranton MI, McIntyre M, Taylor GT, Muller-Karger F, Fanning K, Astor Y. 2006. Temporal variability in the nutrient chemistry of the Cariaco Basin. In: Past and Present Water Column Anoxia. Neretin LN, editor. Edition ed. Dordrecht: Springer. 139 p.

Shannon CE. 1948. A mathematical theory of communication. Bell System Tech. J. 27:379-423, 623-656.

Simpson EH. 1949. Measurement of diversity. Nature 163:688.

Sleep NH and Bird DK. 2008. Evolutionary ecology during the rise of dioxygen in the earth's atmosphere. Philos Trans R Soc Lond B Biol Sci 363(1504):2651-64.

Sogin ML, Morrison HG, Huber JA, Mark Welch D, Huse SM, Neal PR, Arrieta JM, Herndl GJ. 2006. Microbial diversity in the deep sea and the underexplored "rare biosphere". Proc Natl Acad Sci U S A 103(32):12115-20.

Steinbuck JV, Genin A, Monismith SG, Koseff JR, Holzman R, Labiosa RG. 2010. Turbulent mixing in fine-scale phytoplankton layers: Observations and inferences of layer dynamics. Continental Shelf Research 30:442–55.

Stevens H and Ulloa O. 2008. Bacterial diversity in the oxygen minimum zone of the eastern tropical south pacific. Environ Microbiol 10(5):1244-59.

Stewart FJ, Ulloa O, DeLong EF. 2011. Microbial metatranscriptomics in a permanent marine oxygen minimum zone. Environ Microbiol, in press.

Stramma L, Johnson GC, Sprintall J, Mohrholz V. 2008. Expanding oxygen-minimum zones in the tropical oceans. Science 320(5876):655-8.

Stramma L, Schmidtko S, Levin LA, Johnson GC. 2010. Ocean oxygen minima expansions and their biological impacts. Deep-Sea Research Part I-Oceanographic Research Papers 57:587–95.

Taylor GT, Iabichella-Armas M, Varela R, Muller-Karger F, Lin X, Scranton MI. 2006. Microbial ecology of the Cariaco Basin's redoxcline: the U.S.-Venezuela CARIACO time series program. In: Past and Present Water Column Anoxia. Neretin LN, editor. Edition ed. Dordrecht: Springer. 473 p.

Valentine DL, Kessler JD, Redmond MC, Mendes SD, Heintz MB, Farwell C, Hu L, Kinnaman FS, Yvon-Lewis S, Du M, Chan EW, Tigreros FG, Villanueva CJ. 2010. Propane respiration jump-starts microbial response to a deep oil spill. Science 330(6001):208-11.

Venkateswaran K, Shimada A, Maruyama A, Higashihara T, Sakou H, Maruyama T. 1993. Microbial characteristics of Palau Jellyfish Lake. Can. J. Microbiol. 39:506-12.

Vetriani C, Tran HV, Kerkhof LJ. 2003. Fingerprinting microbial assemblages from the oxic/anoxic chemocline of the black sea. Appl Environ Microbiol 69(11):6481-8.

Wakeham SG, Amann R, Freeman KH, Hopmans EC, Jorgensen BB, Putnam IF, Schouten S, Damste JSS, Talbot HM, Woebken D. 2007. Microbial ecology of the stratified water column of the Black Sea as revealed by a comprehensive biomarker study. Organic Geochemistry 38:2070–97.

Walsh DA, Zaikova E, Howes CG, Song YC, Wright JJ, Tringe SG, Tortell PD, Hallam SJ. 2009. Metagenome of a versatile chemolithoautotroph from expanding oceanic dead zones. Science 326(5952):578-82.

Wang Q, Garrity GM, Tiedje JM, Cole JR. 2007. Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol. 73(16):5261-7.

Ward BB, Devol AH, Rich JJ, Chang BX, Bulow SE, Naik H, Pratihary A, Jayakumar A. 2009. Denitrification as the dominant nitrogen loss process in the arabian sea. Nature 461(7260):78-81.

Ward BB, Martino DP, Diaz MC, Joye SB. 2000. Analysis of ammonia-oxidizing bacteria from hypersaline mono lake, california, on the basis of 16S rRNA sequences. Applied and Environmental Microbiology 66(7):2873-81.

Weimer BC, Rompato G, Parnell J, Gann R, Ganesan B, Navas C, Gonzalez M, Clavel M, Albee-Scott S. 2009. Microbial biodiversity of Great Salt Lake, Utah. Natural Resources and Environmental Issues 15(1):15-22.

Whitaker RJ, Grogan DW, Taylor JW. 2003. Geographic barriers isolate endemic populations of hyperthermophilic archaea. Science 301(5635):976-8.

Yakimov MM, Giuliano L, Chernikova TN, Gentile G, Abraham WR, Lunsdorf H, Timmis KN, Golyshin PN. 2001. Alcalilimnicola halodurans gen. nov., sp. nov., an alkaliphilic, moderately halophilic and extremely halotolerant bacterium, isolated from sediments of soda-depositing lake natron, east africa rift valley. Int J Syst Evol Microbiol 51(Pt 6):2133-43.

Zaikova E, Walsh DA, Stilwell CP, Mohn WW, Tortell PD, Hallam SJ. 2010. Microbial community dynamics in a seasonally anoxic fjord: Saanich Inlet, British Columbia. Environmental Microbiology 12(1):172–91.

Zehr JP. 2009. New twist on nitrogen cycling in oceanic oxygen minimum zones. Proc Natl Acad Sci U S A 106(12):4575-6.