DISSERTATION

INTRA AND INTER-ANNUAL PATTERNS IN LAKE YOJOA, HONDURAS: DISENTANGLING BIOGEOCHEMICAL DRIVERS OF A TROPICAL MONOMICTIC LAKE ECOSYSTEM

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ABSTRACT

INTRA AND INTER-ANNUAL PATTERNS IN LAKE YOJOA, HONDURAS: DISENTANGLING BIOGEOCHEMICAL DRIVERS OF A TROPICAL MONOMICTIC LAKE ECOSYSTEM

Since its inception, limnology has been disproportionately focused on temperate ecosystems, leaving tropical lakes underrepresented in the contemporary limnological literature. Beyond the relative paucity of published studies, knowledge of tropical lake ecology is limited by the inapplicability of various veins of temperate research to tropical lake ecosystems. This disconnect is largely due to higher annual temperatures and, in the case of monomictic lakes, the presence of a persistent, warm, anoxic hypolimnion during stratified months. These warm (> 20 °C) and anoxic waters define tropical lake biogeochemistry via anaerobic metabolisms operating without the thermal constraints observed at higher latitudes (with the exception of high elevation tropical lakes). This dissertation builds on recent advances in tropical limnology by providing new insight into the foundational processes underpinning the ecology of tropical monomictic lakes. The following chapters combine a literature review (Chapter 1) with an in-depth study of Lake Yojoa, Honduras (Chapters 2-5) to explore tropical lake biogeochemistry, with a particular focus on nitrogen.

Despite mounting evidence that phytoplankton in tropical lakes experience an array of limiting nutrients, references to low latitude lakes being predominantly nitrogen limited remain common in the literature. To assess the current understanding of nutrient limitation regimes in tropical lakes I performed a literature review to summarize observations of nitrogen (N), phosphorus (P), and colimitation in the collected studies. I also identified common drivers of tropical nutrient limitation regimes. I found that while N is a key limiting nutrient in some of the reviewed studies, it was not the dominant limiting nutrient in the majority of reviewed lakes. Rather, tropical lakes exhibited a diversity of nutrient limitation and there was often considerable heterogeneity of the dominant limiting nutrient within an individual lake. This

heterogeneity was driven largely by seasonal patterns of water column stratification and precipitation, land use and land cover (LULC), and the interaction of these characteristics with lake morphology.

Similar to many of the lakes identified in the literature review, Lake Yojoa experienced intra-annual nutrient limitation heterogeneity, being N and P colimited during the stratified months and P limited during the mixed water column phase. Over the last forty years (1980-2020), Lake Yojoa has seen increased watershed development as well as the introduction of a large net-pen Tilapia farm, resulting in a dramatic reduction in seasonal water clarity, increased trophic state, and altered nutrient dynamics, shifting Lake Yojoa from an oligotrophic (low productivity) to mesotrophic (moderate productivity) ecosystem. To assess the changes that have occurred in Lake Yojoa as well as putative drivers for those changes, I compared Secchi depth, dissolved inorganic nitrogen (DIN), and total phosphorus (TP) concentrations at continuous semi-monthly intervals for the three years between 1979-1983 and again at continuous 16-day intervals from 2018-2020. Between those two periods, I observed the loss of a clear water phase that previously occurred in the months when the water column was fully mixed. Seasonal peaks in DIN coincident with mixing suggest that an enhanced accumulation of ammonium in the hypolimnion during stratification, and release to the epilimnion with mixing maintained high algal abundance and subsequently low Secchi depth during what was previously the clear water phase.

In November of 2020, Hurricanes Eta and Iota made landfall near the Nicaragua-Honduras border, inundating the region with a large amount of late-season precipitation. To understand the impact of such storms on inland aquatic ecosystems, I compared two years of continuous monitoring in Lake Yojoa using one year prior to and one year following Hurricanes Eta and Iota. The storms resulted in an increase in Secchi depth and a decrease in algal abundance directly following the hurricanes and a lower-than-average accumulation of hypolimnetic nutrients from the onset of stratification until mixus the next November (2021). However, following annual water column turnover in 2021, epilimnetic nutrient concentrations returned to (and in some cases exceeded) pre-hurricane levels, presumably due to an internal release of ammonium previously stored in the sediments. Lake Yojoa's response suggests that trophic state was resilient to the disturbance imposed by the two hurricanes because of decades of nutrient enrichment and

super saturation of the lake's sediments. This indicates that ongoing eutrophication in Lake Yojoa will not likely be easily mitigated by decreasing allochthonous nutrient loading and may require years to decades to recover the trophic state observed in the early 1980's.

The work described above highlights the need to better define nutrient dynamics of warm anoxic hypolimnions, not just surface water nutrient concentrations, to more completely understand the mechanisms of environmental change in monomictic tropical lakes. To identify the dominant N transformation pathways that likely drive the observed dynamics, I combined geochemical data, thermophysical structure measurements and metatranscriptomic analysis of the water column microbiome. I sampled at two depths (1 and 16 m) in June 2021, when Lake Yojoa was stratified, and again in January 2022, when the water column was mixed. Within the dereplicated MAG database (n=552), 335 different lineages were identified. Microbial communities were significantly different across seasons and, in June, between depths. N metabolism gene expression was highest in the June hypolimnion. Across the three sampled locations, we found *nrfA* expression was upregulated relative to other N metabolism genes, suggesting that dissimilatory nitrate reduction to ammonium (DNRA) is a likely mechanism for inorganic N accumulation during stratification, in addition to the sediment nutrient release identified in Chapter 3.

Finally, to test the efficacy of using a remote sensing approach to expand the temporal resolution of this research, I adapted an existing machine learning model which uses remotely sensed variables to estimate Secchi depth and chlorophyll *a*. The model was trained on data from Chapters 2 and 3 using *in situ* observations from 2018 to 2020 and then used to estimate pelagic surface conditions in 2021. While the chlorophyll *a* model performed poorly, the Secchi depth model was comparable to other published remote sensing models and captured the clear water phase following Hurricanes Eta and Iota discussed in Chapter 3. In addition to exploring the opportunity of expanding the current temporal resolution of research efforts presented in this dissertation, this final chapter demonstrates how existing remote sensing models can be applied to new systems and developed using data from short term continuous monitoring efforts in order to create longer term data products, both to the benefit of future research, and local collaborators and stakeholders.

Though largely focused on a single ecosystem (Lake Yojoa), by layering multiple approaches, the research presented here leverages a unique historical dataset from an under-studied region (Central America) to generate new insight into foundational biogeochemistry of tropical lakes. By identifying changes in Lake Yojoa's intra-annual geochemical patterns which corresponded with observable inter-annual ecosystem-scale increases in primary productivity, we were able to hypothesize the primary mechanistic drivers. The natural experiment created by Hurricanes Eta and Iota provided further evidence for these initial hypotheses (which were ultimately tested by analyzing Lake Yojoa's microbial metatranscriptome) and confirmed that disturbance response is identifiable through remote monitoring of trophic state. Taken together, this work demonstrates the complex relationship between nutrient loading and physical structure in monomictic tropical lakes and illustrates the need to consider the critical role that the hypolimnion plays in governing whole ecosystem function. Whereas the deepest waters in lakes are often overlooked in ecosystems where low temperatures limit microbial metabolism and prevent rapid nutrient transformations, warm anoxic hypolimnions may prove to be the dominant driver of the overall characteristics of tropical lake ecosystems.

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DEDICATION

Dedicated to my dad, for instilling in me a love of water and teaching me that, like the salmon, I need the river to take me home. I'm so proud to be your daughter.

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INTRODUCTION

Central American lakes were essential to the success of pre-Columbian Mesoamerican societies (Luzzadder-Beach and Beach 2008; Luzzadder-Beach 2019; Waters et al. 2021). Today, Central American lakes continue to play a critical role in the livelihoods of local communities and global economies. However, the heightened demand for the ecosystem services they provide and environmental stressors related to global change are placing these vital ecosystems under increasing strain (Adrian et al. 2009; Winkler et al. 2013). This intersection of human dependence on ecosystem services and environmental stress due to global change is so particularly well illustrated in tropical lakes because of the abundance of local economies which have a heightened reliance on natural resources (Imbach et al. 2017). Furthermore, climate predictions for tropical regions are more uncertain than their higher latitude counterparts (Bender et al. 2010; Vichot-Llano et al. 2021). The uncertainty of future climate scenarios' impacts on freshwater resources at low latitudes is compounded by a historic imbalance between temperate and tropical research where the latter is largely underrepresented in the ecological literature (Ramírez et al. 2020; Nuñez et al. 2021). Temperate limnology cannot, in many instances, be applied to tropical lake ecosystems because of fundamental differences in annual temperature, precipitation regimes, and geologic origin, amongst other factors. Because of this, advances in our collective understanding of tropical limnology are necessary to sustain these societally critical resources for future generations.

Though temperate lake research dominates the modern zeitgeist of limnology, tropical research has a rich history dating back to the turn of the nineteenth century. Foundational work was conducted in Lake Tanganyika (Beadle 1981), Lake Malawi (Fullerborn 1900), several Sri Lankan lakes (Apstein 1910), and Lakes Amatitlán and Atitlán of Guatemala and Lakes Ilopango and Coatepeque in El Salvador (Juday 1908) in addition to countless efforts to understand tropical inland waters predating the documented work of European and North American scholars (Deevey 1957). Tropical limnology continued to grow through the first half of the twentieth century with the Cambridge Expedition to British Guiana (Carter 1934), the Sunda expedition in Indonesia (Rodhe 1974) and the Percy Sladen Expedition to Kenya (Jenkin 1932), among

others (Worthington 1929; Carter and Beadle 1930). From those foundational studies came a wide array of research topics and interests and a shift from largely descriptive studies to efforts to understand the mechanisms driving lake function (Deevey 1953; Lewis 1974; Talling 1969; Talling & Lemoalle, 1998; Tundisi et al. 1984; Viner 1977). Today, the field continues to expand and explore an ever-broadening array of questions across sub-disciplines. Recent advances in tropical limnology include a metagenomic survey of Lake Tanganyika (Tran et al. 2021a) that has provided insight into microbial metabolisms driving the largest (by volume) tropical lake in the world. Several paleolimnological studies have rigorously evaluated how tropical lakes responded to environmental stressors in the past (Moguel et al. 2021; Waters et al. 2021; Benito et al. 2022). Estimates of CO_2 flux are improving our understanding of the role that tropical lakes play in global carbon cycling (Ngochera and Bootsma 2020; Morana et al. 2022).

This dissertation seeks to further advance our understanding of tropical limnology with a particular focus on intra and inter-annual patterns in lake biogeochemistry. Understanding lake biogeochemistry, especially macronutrients nitrogen (N) and phosphorus (P), is particularly important given the interactive threat of climate change and cultural eutrophication in low latitude lakes and the potential threat of decreased water quality, and loss of associated ecosystem services to local communities. While micronutrients (e.g., iron, silicon, etc.) may limit primary productivity in aquatic ecosystems, phytoplankton growth is most often limited by N or P (or some combination of the two). As such, the first chapter is a qualitative analysis of published observations of nutrient limitation regimes in tropical inland lentic systems (inclusive lakes and reservoirs). The aims of the first chapter were to 1) quantify and summarize observations of N, P, and colimitation in the collected studies, 2) identify common drivers of tropical nutrient limitation regimes and 3) provide literature-informed recommendations for future research efforts into nutrient limitation of tropical lakes. This chapter highlights the complexity of biogeochemical interactions in tropical lakes and, in demonstrating the inadequacies of the N limitation paradigm, encourages a more critical evaluation of biogeochemistry in these warm, seasonally anoxic, freshwater ecosystems.

The remaining three chapters focus specifically on Lake Yojoa. Lake Yojoa, the largest natural lake in Honduras, has a long history of cultural and social significance to local populations. The watershed marks the southernmost regions of the Mayan empire, with pollen cores in Lake Yojoa suggesting the presence of agriculture beginning in ~3000 BCE (Rue et al. 2002). Notably, the iconography of ceramic vessels unearthed in Los Naranjos, located on the North shore of the lake, suggests that Lake Yojoa may have been the place of creation and origin for the Lencan people (Nielsen and Brady 2006). The precolonial name for Lake Yojoa was Lake Tauleve, from the Lencan *t'au* or *tauq* (house) and *lepa* or *leba* (jaguar), thus "house of the jaguar" (Nielsen and Brady 2006). Today, the Lake Yojoa watershed supports a population of nearly 80,000 people (Rivera 2003) and a diverse local economy (largely aquaculture, fishing, recreation and ecotourism).

Lake Yojoa offers an ideal case study for developing a greater understanding of nutrient biogeochemistry in monomictic tropical lakes. Like many lakes across the world, Lake Yojoa has entered an increased trophic state and experienced altered biogeochemical cycling due to local anthropogenic stressors interfaced with climate change. In the tropics, understanding the drivers of these changes can be difficult due to a lack of documented historic conditions or an absence of continuous monitoring that can distinguish between intra-annual variability and inter-annual directional change. However, documented historical conditions in Lake Yojoa (Vaux and Goldman 1984) paired with contemporary sample collection provided a unique opportunity to assess trophic state change and associated drivers in a historically data-poor region. In the second chapter of this dissertation, I identified intra and inter-annual changes in Lake Yojoa's trophic state and changes in the underlying biogeochemical patterns across forty years (1980-2020). This chapter illustrates how physical water column structure and nutrients interact in tropical monomictic lakes; a key phenomenon identified in the literature review. This work also demonstrates the need to more fully incorporate warm anoxic hypolimnions in monitoring plans to understand the function of tropical lake ecosystems.

In November 2020, Hurricanes Eta and Iota made landfall near the Nicaragua-Honduras border. Understanding hurricane disturbance in tropical lakes, such as Lake Yojoa, is particularly important because

increasing sea surface temperatures are predicted to alter the timing, intensity, and frequency of tropical storms (Saunders and Lea 2008; Knutson et al. 2010). Such storms pose a new and poorly described threat to inland aquatic ecosystems, with the potential to impact key ecosystem characteristics and compromise the ecosystem services they provide. The third chapter asks 1) what were the immediate effects of Hurricanes Eta and Iota on Lake Yojoa's trophic state? and 2) what were the putative biogeochemical drivers of any observed changes in trophic state? Lake Yojoa's response to hurricane disturbance further reinforces the importance of hypolimnetic and sediment associated biogeochemical cycling.

From my initial results in Chapters 2 and 3, I hypothesized that a few key biogeochemical pathways were likely responsible for the intra-annual nutrient patterns observed in Lake Yojoa. Most notably, I hypothesized that an upregulation of dissimilatory nitrate reduction to ammonium (DNRA), relative to N loss pathways (denitrification and, to a lesser extent, anaerobic ammonium oxidation, anammox) would explain the accumulation of hypolimnetic ammonium. To identify spatial and temporal variation in Lake Yojoa's biogeochemical pathways at a higher resolution, I sampled at two depths (1 and 16 m, above and below the oxycline) in June 2021, during the stratified season and again in January 2022, during the mixed water column season. In the fourth chapter, I characterized seasonal and spatial differences in dominant N transformation pathways by analyzing the genome resolved metatranscriptome. I then correlated changes in gene expression to differences in geochemical and thermophysical conditions to better understand the hypolimnetic ammonium accumulation and other geochemical patterns observed in Chapters 2 and 3.

Just as Chapter 2 provided the foundation for Chapters 3 and 4, my fifth and final chapter uses the data collected for, and the intra-annual patterns identified by Chapters 2 and 3 to assess the use of remote sensing for trophic state monitoring in Lake Yojoa. To conclude my dissertation work, I assessed the efficacy of using a machine learning algorithm to model Secchi depth and surface chlorophyll *a* concentration using remotely sensed variables. By adapting an existing model (Topp et al. 2021), I sought to expand the temporal resolution of available trophic state data while exploring ways in which remote monitoring tools remove barriers to data-driven management practices and increase environmental justice through data availability in historically data-poor regions.

Collectively, this dissertation provides critical insight into the biogeochemistry of tropical lakes by layering a literature review (Chapter 1), a rare historical dataset paired with an intentionally designed contemporary study (Chapter 2), a documented ecosystem scale response to an increasingly frequent natural disturbance (Chapter 3), an analysis of the metatranscriptome of Lake Yojoa's microbiome (Chapter 4), and the development of a model to predict trophic state using remotely sensed variables (Chapter 5). While significant research efforts are needed to close the gap between temperate and tropical limnology, bodies of work, such as the collection of studies presented here within, offer important insight into the ecology of warm, monomictic lakes. Combining a variety of tools and techniques to ask questions at multiple scales, both temporal and spatial, may prove to be the key to eliciting a greater understanding of how aquatic ecosystems are changing under persistent stressors, both local and global.

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CHAPTER 1: NITROGEN IS UNLIKELY TO CONSISTENTLY LIMIT PRIMARY PRODUCTIVITY IN MOST TROPICAL LAKES[1]

1.1 Introduction

Whereas phytoplankton growth in temperate lakes was long regarded as being predominantly phosphorus (P) limited (Schindler 1977), tropical lakes were, for many years, broadly thought of as being nitrogen (N) limited (Lewis 2002, hereafter referred to as the N limitation paradigm). The N limitation paradigm in tropical lakes gained traction, not only due to repeated observations of N limitation (largely in the African rift lakes), but also because P was thought to accumulate in low latitude lakes via evapo-concentration, making P limitation unlikely (Talling and Lemoalle 1998). The observed rates of denitrification in tropical lakes, which exceeded rates reported for temperate lakes, further reinforced expectations of N limitation (Lewis 1996). However, just as there have been identified inconsistencies in the assumption of P limitation among temperate lakes (Dzialowski et al. 2005; Scott et al. 2019, among others), the expectation of N limitation in tropical lakes has been refuted in the literature. Despite a lack of evidence for any relationship between latitude and dominant nutrient limitation (Huszar et al. 2006; Elser et al. 2007; Abell et al. 2012), recent and diverse studies continue to reference the N limitation paradigm, suggesting its sustained influence across different fields (e.g., Pantoja-Agreda and Otero-Morales 2017; Schabetsberger et al. 2017; Nankabirwa et al. 2019; Zieritz et al. 2019; Farrell et al. 2020; Goshu et al. 2020; da Silva et al. 2021; Sim et al. 2021; Tran et al. 2021, among others).

1.2 Methods

We collected studies of nutrient limitation in tropical lake ecosystems via a Web of Science search using pre-defined keywords (Table 1.1), producing 229 unique results. From the initial pool of 229 studies, we limited our review to studies which had measured phytoplankton macronutrient (i.e., N or P) limitation

[1] This chapter is *in revisions* at Ecosphere: Innovative Viewpoints with the following authors: Jemma Fadum and Ed Hall.

in tropical (between 23.5° N and 23.5° S) lakes or reservoirs (hereafter collectively referred to as lakes) using one or more of the following methods: stoichiometric inference, total nutrient concentration inference, nutrient addition bioassay, calculated N and P debt, turnover and uptake capacity using isotopically labeled compounds, alkaline phosphate activity assay, luxury uptake capacity or multiple regression analysis of chlorophyll *a* vs. total P and total N. In the reviewed literature, a suite of different ratios and nutrient concentration thresholds, derived from a range of different studies, were used to estimate nutrient limitation (Redfield 1958; Sakamoto 1966; Healey and Hendzel 1980; Smith 1982; Morris and Lewis 1988; Sas 1989; Reynolds 1999).

We excluded studies which only measured light limitation but included studies which considered both light and macronutrient limitation. We removed papers from further consideration if nutrient limitation was only measured with regards to a treatment or biomanipulation (e.g., species introduction) or if the focus was limited to a single species (e.g., *microcystis aeruginosa*). This resulted in 34 individual studies which, given the geographic extent and variety of ecosystems included, can be considered representative of a significant portion of tropical lakes (Fig. 1.1A). Among the 34 individual studies, eight lakes (Lake Zirahuén, the Alberca de Tacambaro, Cabiunas Reservoir, Serra da Mesa Reservoir, Lake Malawi, Lake Tanganyika, Valle de Bravo Reservoir, and Lake Titicaca) appeared in two different studies, resulting in a final set of 106 lakes, though all 114 reported limitation regimes are displayed in Fig. 1.1. To identify common drivers of documented nutrient limitation regimes, we collected summaries of explanations from each study and organized those explanations into three principal categories. Both empirically tested drivers and those theorized and supported by literature were accepted.

1.3 Results

Of the 114 limitation regimes we reviewed, only 49 systems were identified as being exclusively N limited (or ~43%). Of those 49 systems, 40 came from a single study of man-made lakes in a geographically isolated region of Rio Grande do Norte, Brazil (Brasil et al. 2016, Fig. 1.1A; Study 3). When we exclude that single study (Brasil et al. 2016) only 9 of the remaining 74 systems (<13%) concluded N limitation. Where N limitation was not observed (n=65), studies identified P limitation (n= 34), colimitation

(n=9), light limitation (n=3), heterogeneous limitation in time or space (n= 18) or did not identify any limitations to primary productivity (n=1, Fig. 1.1B).

We identified three principal categories of drivers of nutrient limitation in the studies we reviewed: 1) seasonality, 2) land use and land cover (LULC), and 3) lake morphology. Seasonality broadly refers to changes in nutrient limitation driven by seasonally reoccurring patterns in both water column thermo-physical structure as well as climate, namely annual patterns of precipitation. The LULC category includes both watershed LULC characteristics as well as industry and anthropogenic activities within the watershed that were reported to influence nutrient loading of the lake(s). Finally, lake morphology refers to depth as well as the spatial relationship between bathymetry and tributary inputs. Whereas within these categories, specific processes likely play a role in both temperate and tropical lakes, some processes, such as monsoonal rains and climate patterns (e.g., El Niño-Southern Oscillation, ENSO), are specific to tropical ecosystems.

1.4 Discussion

The studies we reviewed illustrate a diversity of nutrient limitation regimes among a broad geographic range of tropical lakes. Our analyses show that N limitation is not disproportionately dominant in low latitude aquatic ecosystems, as would be suggested by the N limitation paradigm, and is consistent with multiple studies of latitudinal gradients showing no increase in N limitation in low latitude lakes relative to temperate lakes (Huszar et al. 2006, Elser et al. 2007, Abell et al. 2012). There is a diversity of characteristics among tropical lakes (e.g., geologic origin, watershed characteristics, contemporary and legacy anthropogenic stressors, hydrology, etc.) that makes consistent N limitation unlikely across the majority of tropical lakes. In the following sections we explore and provide examples of each of the three principal identified drivers (seasonality, land use and land cover (LULC), and lake morphology) with regards to how each driver impacts nutrient limitation in tropical lakes. In the final section of the discussion, we identify limitations of this literature review and offer guidance for future studies on nutrient limitation in tropical lakes.

1.4.1 Seasonality

Whereas the tropics do not experience large intra-annual fluctuations in temperature, the seasons are commonly marked by pronounced differences in precipitation. This shift in seasonal precipitation may lead to temporally heterogeneous allochthonous nutrient loading, wherein terrestrial nutrients are transported from the landscape into the lake, or alternatively where run-off depleted in nutrients leads to nutrient dilution within the lake. Such intra-annual variation in nutrient limitation has been observed, for example, in Lake Zirahuén, Mexico, where N limitation during the dry season is replaced by P limitation during the rainy season due to differential nutrient inputs between seasons (Bernal-Brooks et al. 2016, Fig. 1.1A; Study 2).

Intra-annual patterns in water column stratification can also create temporal heterogeneity in nutrient limitation regimes by altering redox potential in the hypolimnion of lake ecosystems. Redox controlled nutrient fluxes include sediment nutrient release, and/or the addition or removal of nutrients from the bioavailable pool, such as the binding (oxic) or release (anoxic) of phosphate and iron (Fe). Other shifts following a change in redox include enhancement of nutrient removal pathways such as denitrification under anoxic conditions. Water column mixing can also initiate the release of sediment derived nutrients to the photic zone. Similar to Lake Zirahuén, Lake Bonilla, Costa Rica, is N limited during the dry season, and P limited during the rainy season (Villalobos 1997, Fig. 1.1A; Study 32). However, whereas Lake Zirahuén's nutrient limitation pattern is primarily driven by differences in nutrient delivery from seasonal precipitation, Lake Bonilla's N limitation is not only due to a seasonal decrease in watershed derived N but also by the abundant resuspension of sediment-derived P released during the mixing of the water column when the lake is not stratified. Conversely, Valle de Bravo Reservoir, Mexico, is N limited when the water column is stratified (Ramirez-Zierold et al. 2010, Fig. 1.1A; Study 23) before transitioning to a state of P limitation when the water column is mixed (Merino-Ibarra et al. 2008, Fig. 1.1A; Study 16). Depletion of reactive N in the water column during stratification, followed by subsequent introduction of hypolimnetic N during mixus that alleviates N limitation, has been documented in other tropical monomictic systems,

such as Lake Titicaca (Vincent et al. 1984; Wurtsbaugh et al. 1985, Fig. 1.1A; Studies 33 and 34, respectively) and Lake Yojoa (Fadum and Hall 2022).

These intra-annual patterns of nutrient limitation can be further altered by variations in weather specific to the tropics. For example, pronounced inter-annual climate patterns, such as ENSO may interact with the above intra-annual patterns of stratification, altering the timing, duration, or stability of stratification. This has been observed, in the Alberca de Tacámbaro, Mexico, where warm air temperatures associated with ENSO have been linked to higher N deficits (Caballero et al. 2016, Fig. 1.1A; Study 5) due to increased stability of stratification and decreased delivery of nutrients from the aphotic zone to the photic zone.

1.4.2 LULC

Land cover within the watershed is often the principal determinant of nutrient supply to all lake ecosystems with certain aspects of land cover being unique to the tropics. For example, low P inputs and subsequent P limitation in tropical lakes often stems from intensely weathered and therefore P deficient soils common to tropical ecosystems, such as in the Manton River Reservoir and Darwin River Reservoir of northern Australia (Townsend 2000, Fig. 1.1A; Study 30). A second mechanism that may promote P limitation is exemplified in the lakes of Cajas National Park, Ecuador, where the lakes are largely P limited due to the high P retention by páramo soils (Van Colen et al. 2017, Fig. 1.1A; Study 31). This P retention is exacerbated by high N fixation by microbial communities associated with páramo vegetation, further promoting P limitation by supplying lakes in the region with a disproportionately high N:P run-off (Van Colen et al. 2017).

In addition to the nutrient dynamics of natural land cover, anthropogenic activities within a watershed may have an antagonistic effect on the preexisting nutrient limitation conditions, creating both seasonal fluctuations and, in some cases, permanent shifts in nutrient limitation. In Lake Alchichica, Mexico, nutrient limitation fluctuates between N and P and colimitation due to P rich volcanic terrain, the weathering of which promotes N limitation, combined with N fertilizer application in the surrounding watershed, which increases N delivery to Lake Alchichica during the rainy season (Ramírez-Olvera et al.

2009, Fig. 1.1A; Study 24). This creates seasonal shifts in nutrient limitation dependent on run-off driven inputs from the watershed. Similarly, whereas Lake Zirahuén, Mexico, previously experienced persistent N and P colimitation (Bernal-Brooks et al. 2002, Fig. 1.1A; Study 1), the dominant nutrient limitation now fluctuates between either N limitation or P limitation due to the interaction of anthropogenic activities and seasonal precipitation (Bernal-Brooks et al. 2016, Fig. 1.1A; Study 2). Besides LULC within the watershed, anthropogenic activities beyond the watershed can also impact nutrient limitation, such as in Lake Jaisamand, India. Despite declining ratios of dissolved inorganic N to dissolved reactive P (DIN:DRP) in the contributing tributaries, within lake DIN:DRP continues to rise due to high levels of atmospheric N deposition originating from rapid urban-industrial growth that has occurred since 1997, resulting in more frequently observed and more severe P limitation (Pandey and Pandey 2013, Fig. 1.1A; Study 21).

1.4.3 Morphology

Lake morphology is the third principal driver of nutrient limitation that we identified, primarily due to its influence on stratification regimes. For example, shallow lakes do not maintain stable stratification, and thus are often polymictic, whereas deeper tropical lakes may have a monomictic or meromictic regime. The importance of lake morphology (and subsequent mixing regimes) to nutrient limitation is closely tied to changes in redox conditions, as described above. The relationship between morphology and nutrient limitation is well illustrated by floodplain (or várzea) lakes where the relative contributions of different nutrient sources (i.e., river water and the surrounding landscape, Forsberg et al. 1988) interact with the lake's morphology to determine dominant nutrient limitation. For example, while shallow várzea lakes, such as Laguna Bufeos, may be predominantly N limited in both the high and low water seasons (Rejas and Muylaert 2010, Fig. 1.1A; Study 26), deeper várzea lakes in the Central Amazon often experience seasonal variation in dominant macronutrient limitation (Melack and Forsberg 2001). Deep, seasonally stratified várzea lakes may experience enhanced denitrification during the high-water season. Conversely, stratification of the water column can also lead to sedimentation of P-rich particles from the photic zone, pushing the system towards P limitation (Melack and Forsberg 2001).

Beyond shifts in redox associated with stratification, the frequency of sediment resuspension in shallow, littoral zones is also tightly coupled with basin morphology and contributes to spatial heterogeneity of nutrient limitation within a lake. Resuspension events deliver sediment associated nutrients to the photic zone and promote light limitation due to increased turbidity. For example, in Lake Guiers in the lower Senegal River delta region, wind driven mixus and subsequent resuspension of sediment derived nutrients promotes frequent fluctuation between dominant nutrient limitations and changes in the intensity of nutrient limitation (Quiblier et al. 2008, Fig. 1.1A; Study 22). In Lake Muzahi, Rwanda, N limitation was observed in the deeper regions of the lake, while light limitation was observed in shallower regions due to frequent sediment resuspension (Mukankomeje et al. 1993, Fig. 1.1A; Study 17). Depth can also affect limitation when the combination of a deep mixed layer and a shallow euphotic zone interact to promote light limitation in the mixed layer, as seen in Lakes Shala and Chitu, Ethiopia (Ogato et al 2015, Ogato et al. 2016, Fig. 1.1A; Studies 18 and 19, respectively).

1.4.4 Challenges for future research

The most commonly used metrics for assessing nutrient limitation in the collected literature were stoichiometric and nutrient concentration inferences. However, such inferential tools have well documented limitations and inconsistencies. For example, while the Redfield ratio (Redfield 1958) is commonly applied to freshwater systems, the classic C:N:P of 106:16:1 is inconsistent with direct observations of dissolved and particulate stoichiometry in inland waters (Sterner et al. 2008), likely due to higher C and N content of allochthonously derived seston with greater variability than is found in the ocean (Hassett et al. 1997; They, Amado, and Cotner 2017). Beyond ratios of N and P, measurements of dissolved inorganic nitrogen (DIN) are indiscriminately paired with measurements of soluble reactive phosphorus (SRP) or total phosphorus (TP) (i.e. DIN:SRP and DIN:TP), which represent two very different aspects of the P cycle. Similarly, studies routinely pair total dissolved N, total N, ammonium (NH_4^+) or nitrate (NO_3^-) with some analyte of P, also producing results that are not directly comparable. Thus, the use of multiple ratios may lead to conflicting rather than harmonious interpretations of nutrient limitations. As such, inference-based approaches are best used in a complementary fashion (e.g., to improve spatial or temporal coverage for a

specific system) when paired with a more direct test of nutrient limitation, such as a nutrient addition bioassay, which were far less common in the studies we reviewed. However, nutrient enrichment bioassays carry their own possible limitations due to the dependency of luxury consumption on growth rate (Elrifi and Turpin 1985, Li et al. 2020) and inconsistencies with results of whole lake fertilization experiments in lakes where the depth of surface water mixing exceeds the euphotic zone (Carignan and Planas 1994). Therefore, nutrient limitation studies should carefully consider method selection and, when feasible, layer both empirical and inferential approaches.

One of the main challenges in the current analysis was the wide variety of methods used relative to the total number of studies collected, making it impossible to perform a proper meta-analysis consisting of response ratios, regression analyses, or other quantitative assessments. The number of studies included in the current analysis may have been limited by the inability of the chosen search terms to return all possible relevant studies and was also likely limited by the exclusion of studies not published in English, the inclusion of which would greatly improve this analysis (Konno et al. 2020).

Nutrient limitation regimes arise from the complex interaction of various drivers, which often produce spatially or temporally heterogeneous nutrient limitation patterns. As such, a single sample (either in space or time) is likely to lead to an incomplete understanding of lake nutrient limitation regimes. Further complicating the determination of dominant nutrient limitation regimes in lakes is variability of phytoplankton response to nutrient enrichment (Guildford et al. 2007, Fig. 1.1A; Study 14), ephemeral alterations to nutrient limitation regimes driven by tropical storm events (Corman et al. 2015, Fig. 1.1A; Study 9) and the possibility of untested though influential micronutrient limitations (e.g., Fe, Guildford et al. 2003, Fig. 1.1A; Study 13). Therefore, to the extent possible, a study should aim to directly assess nutrient limitation in a given system (across seasons and at multiple locations informed by system morphology) in place of applying overly broad paradigms of nutrient limitation to ecologically rich and geographically diverse regions of the globe (i.e., the tropics). Similarly, the observed heterogeneity may not be unique to tropical lakes, with seasonal variation in dominant nutrient limitation regime or limitation severity also occurring at higher latitudes (Morris and Lewis 1988, Levine and Whalen 2001, Paerl et al.

2015, Wang et al. 2019, among others). Heterogeneity of nutrient limitation outside of the tropics and subtropics may be underrepresented in the literature due to the historic underrepresentation, and logistical and methodological challenges of winter limnology (Powers and Hampton 2016, Block et al. 2019). Therefore, rather than relying on suggested paradigms, whether the N limitation paradigm for tropical lakes or the P limitation paradigm for temperate lakes, hypotheses may be improved by considering the ecosystem characteristics described above and elsewhere (Kosten et al. 2009).

1.5 Conclusion

The results of our analyses clearly demonstrate that tropical lakes experience a more diverse range of limitations than suggested by the N limitation paradigm. Of the papers we reviewed, the majority did not identify consistent, year-round N limitation. When we removed the single study that reported N limitation for 40 similar ecosystems (Brasil et al. 2016), less than 13% of the remaining 74 ecosystems were reportedly N limited, with two of those nine observations coming from the same ecosystem, Lake Titicaca. Of the 16 studies which included repeated measurements of the same lake, five of the eight lakes were classified differently across studies. This suggests that temporally heterogenous limitations may be common in tropical lakes and also highlights how study design can influence the interpretation of nutrient limitation. Each of the drivers discussed above may have unique impacts on a lake, given the intersectional relationship with additional drivers. We have identified no consistent combination of drivers that will formulaically produce a specific nutrient limitation regime uniformly across tropical lakes.

Assuming N limitation in tropical inland waters ranging from small tropical ponds (Sarnelle et al. 1998, Fig. 1.1A; Study 28) to hydrologically complex reservoirs (Rangel et al. 2012, Fig. 1.1A; Study 25) to the African rift lakes (Stenuite et al. 2007, Fig. 1.1A; Study 29) does not accurately describe the diversity of nutrient limitation regimes for the majority of tropical lakes. Paradigms, though helpful in constructing conceptual frameworks and formulating hypotheses, can also create limitations in inference by fostering inaccurate assumptions that are likely to limit discovery. Just as other established paradigms such as the Redfield ratio (Ptacnik et al. 2010, Geider and La Roche 2002, Sharoni and Halevy 2022) and the

archetypical two-stable state system of shallow lakes (Scheffer and van Nes 2007) have been revisited, so too must the N limitation paradigm for tropical lakes.

CHAPTER 1 TABLES AND FIGURES

Table 1.1. Search terms used in Web of Science literature in November 2020. Search results were combined and duplicates removed using the bibliometrix package (Aria and Cuccurullo 2017) in R (version 4.0.2).

Search ID	**Search terms**	**Results returned**
Search 1	(TS=(tropic* AND lake* AND *limit)) *AND* **LANGUAGE:** (English) *AND* **DOCUMENT TYPES:** (Article) Indexes=*SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, BKCI-S, BKCI-SSH, ESCI, CCR-EXPANDED, IC Timespan=1900-2020*	106
Search 2	(TS=(tropic* AND lake* AND nutrient limitation)) *AND* **LANGUAGE:** (English) *AND* **DOCUMENT TYPES:** (Article) Indexes=*SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, BKCI-S, BKCI-SSH, ESCI, CCR-EXPANDED, IC Timespan=1900-2020*	117
Search 3	(TS=(tropic* AND lake* AND nutrient deficien*)) *AND* **LANGUAGE:** (English) *AND* **DOCUMENT TYPES:** (Article) Indexes=*SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, BKCI-S, BKCI-SSH, ESCI, CCR-EXPANDED, IC Timespan=1900-2020*	19

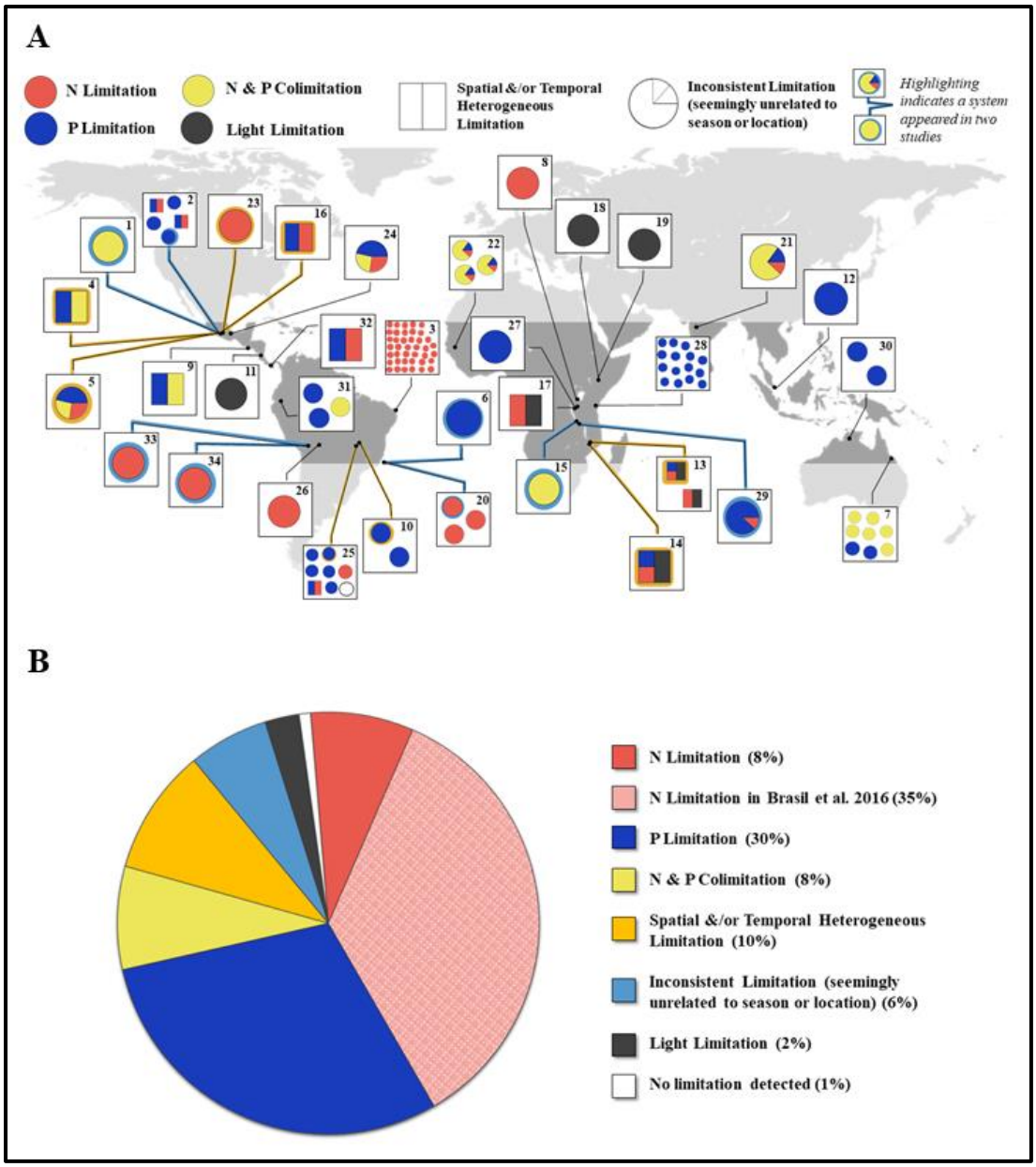

Figure 1.1 (A) Icons representing the primary findings of reviewed studies. White indicated unclear or no observed limitation. 1) Bernal Brooks et al. 2002, 2) Bernal Brooks et al. 2016, 3) Brasil et al. 2016, 4) Caballero and Vazquez 2020, 5) Caballero et al. 2016, 6) Carlsson et al. 2012, 7) Chang et al. 2014, 8) Cocquyt et al. 2010, 9) Corman et al. 2015, 10) de Magalhães et al. 2020, 11) Erikson et al. 1998, 12) Gin et al. 2011, 13) Guildford et al. 2003, 14) Guildford et al. 2007, 15) Järvinen et al. 1999, 16) Merino-Ibarra et al. 2008, 17) Mukankomeje et al. 1993, 18) Ogato et al. 2015, 19) Ogato et al. 2016, 20) Pålsson and Granéli 2004, 21) Pandey and Pandey 2013, 22) Quiblier et al. 2008, 23) Ramirez-Zierold et al. 2010, 24) Ramirez-Olvera et al. 2009, 25) Rangel et al. 2012, 26) Rejas and Muylaert 2010, 27) Sarmento et al. 2008 (via Sarmento et al. 2006), 28) Sarnelle et al. 1998, 29) Stenuite et al. 2007, 30) Townsend 2000, 31) Van Colen et al. 2017, 32) Villalobos 1997, 33) Vincent et al. 1984, 34) Wurtsbaugh et al. 1985. **(B)** Dominant nutrient limitation categories in reviewed literature.

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CHAPTER 2: THE INTERACTION OF PHYSICAL STRUCTURE AND NUTRIENT LOADING DRIVES ECOSYSTEM CHANGE IN A LARGE TROPICAL LAKE OVER 40 YEARS[2]

2.1 Introduction

It is increasingly evident that human activities (both local and global) are altering lake ecosystems around the world. The warming of surface waters due to climate change is altering the duration of stratification and water column stability (Pilla et al. 2020) as well as intensifying eutrophication (Moss et al. 2011). In some regions, increased nitrogen supply has shifted the macronutrient that limits primary productivity from nitrogen (N) to phosphorus (P) (Elser et al. 2009) and altered dissolved N:P stoichiometry of lakes (Isles et al. 2017). These impacts are often exacerbated by more local aspects of global change including changes in land use that can increase the loading of nutrients (Carpenter et al. 1998), metals (Yu et al. 2014), and organic pollutants (Lorgeoux et al. 2016) from the surrounding watershed. In-lake activities, such as aquaculture, are also altering key biogeochemical pathways (Christensen et al. 2000).

Understanding how these changes are affecting lakes in the tropics presents a challenge because of the paucity of monitoring efforts in many tropical regions (Bennun 2001; Riveros-Iregui et al. 2018). Without long-term datasets, it is difficult to distinguish between intra-annual variability, periodic or semi-periodic inter-annual variability (e.g., changes associated with El Niño-Southern Oscillation), and directional inter-annual changes. While studies of the African Great Lakes (Talling and Lemoalle 1998; Hecky 2000; Bootsma and Hecky 2003), Central American lakes such as Lake Atitlán (Corman et al. 2015; Weisman et al. 2018) and Brazilian reservoirs and floodplain lakes (Tundisi et al. 1993; Melack et al. 2020) among many others, have made considerable contributions to our understanding of tropical lake ecosystems, time series data remain far less common in tropical compared to temperate watersheds (Riveros-Iregui et al. 2018).

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Despite the critical role temperate lakes play in the understanding of aquatic ecosystem biogeochemistry, we cannot directly translate knowledge derived from the long-term monitoring of temperate lakes to tropical lakes because of differences in origin, basin morphology, hydrological drivers, climate, and seasonality that occur across latitudinal gradients. For example, temperature is unlikely to directly limit biogeochemical rate processes in tropical lake ecosystems (Talling and Lemoalle 1998). However, temperature does have an indirect impact on biogeochemical rate process in the tropics by influencing stratification and water column stability (MacIntyre and Melack 2009).

To assess changes in water transparency, nutrient dynamics, shift in trophic state, and potential drivers of these changes in a large monomictic tropical lake, we compared two intensive sampling campaigns in Lake Yojoa that took place forty years apart. The first campaign sampled Lake Yojoa at three locations semi-monthly from 1979 to 1983 (henceforth referred to as 'historic sampling', Vaux and Goldman 1984) and provides a rare opportunity to assess the historic conditions of a tropical lake. To create a comparative contemporary dataset, we sampled the lake at five pelagic stations (including three sites aligned with the three historic locations) every 16 days from 2018 to 2020. We also conducted bioassays during mixed (January) and stratified (June) conditions to assess the dominant limiting nutrient to algal productivity. We then compared our contemporary results to those from the historic period. The principal objectives of this study were to 1) identify changes in water transparency and macronutrient (N and P) concentrations over the past forty years and classify the corresponding shift in trophic state, 2) identify differences in inter and intra-annual patterns in these parameters and 3) evaluate the potential drivers of these observed changes to make the findings applicable to other monomictic tropical lake ecosystems.

2.2 Methods

2.2.1 Study site

Lake Yojoa, located in west central Honduras (Fig. 2.1), is the largest freshwater lake in the country (maximum depth = 27.3 m, mean depth= 14.6 m, Table S2.1) and the economic backbone of the surrounding region, supporting local livelihoods (e.g., fishing, agriculture, recreation, tourism, Studer 2007). Today, the Lake Yojoa watershed houses nine municipalities with an estimated combined population

of 74,624 (Rivera 2003) after seeing rapid population growth of approximately 68% in recent decades (House 2002). In addition to increasing anthropogenic activity in the watershed, industrial scale net-pen Tilapia production was introduced in 1997. As of 2016, Regal Springs Tilapia operated 106 Tilapia cages in Lake Yojoa (Monterey Bay Aquarium Seafood Watch 2017).

2.2.2 Sample collection

All historic data are from the El Cajon Project (Vaux and Goldman 1984). From September of 1979 to February of 1983, samples were collected semi-monthly at three pelagic stations. The most consistently sampled location from the historic sampling ("Index") aligns with contemporary station E, while "Station 2" aligns with contemporary station F, and "Station 1" is between stations R and P (Fig. 2.1). Briefly, samples from both the epilimnion and the hypolimnion were analyzed for total phosphorus (TP), ammonium (NH_4^+), and nitrate (NO_3^-) as described below. Additionally, on each sampling day, temperature, and dissolved oxygen (DO) profiles of the water column were collected and Secchi depth was also measured (Vaux and Goldman 1984). Full details of sampling and analyses methods are available in Vaux and Goldman (1984).

For the contemporary sampling campaign, in collaboration with the Asociación de Municipios del Lago de Yojoa y su Área de Influencia, AMUPROLAGO, we collected water samples and took Secchi depth measurements and DO and temperature profiles every 16 days from March 2018 to January 2020. In addition to the semi-monthly epilimnetic (surface water) sampling at five stations, in June of 2018 and 2019 and January of 2019 and 2020 we collected epilimnetic and hypolimnetic samples at each of the five regular stations (B, F, E, P and R) and 13 additional stations (18 sites in total, Fig. 2.1). For all sampling efforts, temperature and DO profiles were measured at two-meter intervals using a Hydrolab MS5 Multiparameter Sonde (OTT HydroMet, Loveland, CO). Epilimnetic samples were collected at one meter below the surface with an opaque Van Dorn water sampler and temporarily stored in opaque HDPE Nalgene bottles and placed in a cooler. Hypolimnetic samples were collected at all locations deep enough to be stratified during June sampling events. Samples were collected at a depth of two meters above the sediment (depths between 17-23 m) to ensure sampling occurred below the thermocline (~12 m) but above the sediment to avoid

contamination. Whole water samples were frozen for TP analysis while NH_4^+ and NO_3^- samples were first filtered through 25 mm glass microfiber filters, Grade GF/F via Polysulfone filter funnels and subsequently frozen. All samples were stored in 15 ml centrifuge tubes before being transported to Fort Collins, CO, USA while frozen. Analyses were performed at the EcoCore facility at Colorado State University, Fort Collins, CO, USA as described below. Contemporary precipitation data were collected for this study at the AMUPROLAGO office (Fig. 2.1) via HOBO U30 USB Weather Station Data Logger (Part # U30-NRC).

2.2.3 Nutrient enrichment bioassays

In order to directly address whether N or P were primarily limiting algal productivity, during January and June of 2019, forty-eight clear glass bottles (120 mL) were filled with lake water from station E after passing the water through an 80 μm mesh to remove grazers. In addition to the unamended control group, three treatment groups were prepared with twelve replicates each: +N (as NH_4SO_4, Sigma-Aldrich cat. no. A4418-100G), +P (as K_2PO_4, Sigma-Aldrich, cat. no. P3786-100G) and +NP. Concentrations of nutrient amendments were chosen to approximately double *in situ* concentrations of DIN and TP based on epilimnetic nutrient concentrations from March and June of 2018. N and P amendments in January 2019 were 6.92 μM N and 0.24 μM P, final concentration. In June 2019, N and P amendments were 3.15 μM N and 0.94 μM P, final concentration. We incubated each bottle in an outdoor water bath positioned near the lake as to expose bottles to ambient surface light conditions but buffer diel changes in air temperature. Between the start and end of the experiment bottles were agitated daily to minimize settling of algae. After three days, bottles were shaken gently but thoroughly to homogenize the bottle and algae from each bottle were collected on GF/F filters. Filters were folded, wrapped in aluminum foil, and immediately frozen for transport to Fort Collins, CO. Within three days of sampling, chlorophyll a (Chl-a) was measured on a 10-AU fluorometer (Turner Designs Part No. 10-AU-074, calibrated using Turner Designs liquid Chl-a standards) via extraction in 90% acetone with acidification to 0.003 N HCl with 0.1 N HCl (Arar 1997). Algal response for each treatment was recorded as percent increase from the mean algal concentration within the control treatment. We used pre-acidification values (Chl-a plus phaeophytin) for all controls and treatments to represent total changes in algal biomass.

2.2.4 Nutrient analyses

During the historic study, NH_4^+ was measured using the indophenol method (Solórzano 1969), NO_3^- was measured using the hydrazine reduction method (Kamphake et al. 1967), and TP was first digested to soluble reactive phosphorus (SRP) via acid hydrolysis (using H_2SO_4) then measured using the ascorbic acid-molybdate method (Murphy and Riley 1962). For the contemporary sampling campaign, TP was first digested to SRP via the Alkaline Potassium Persulfate method under sub-boiling (90 °C) temperature, then measured using a colorimetric ascorbic acid assay (EPA Method 365.3 in U.S. Environmental Protection Agency 1983) modified to be analyzed on a UV-STAR 96-well Microplate via Infinite M200 TECAN at 880 nm absorbance detection. NH_4^+ and NO_3^- were measured using a Flow Solution FS 3700 Automated Chemistry Analyzer (O.I. Analytical, College Station, TX). NH_4^+ was determined by an indophenol method (German Standard Methods, DIN in Society of German Chemists 1982). NO_3^- was determined via automated colorimetry (EPA Method 353.2 in O'Dell 1993) which relies on determination via diazotizing with sulfanilamide and coupling with N-(1-naphthyl)-ethylenediamine dihydrochloride and nitrate reduction to nitrite in a cadmium column prior to mixing with the color reagent. NH_4^+ and NO_3^- values are presented separately as well as combined and reported as dissolved inorganic nitrogen (DIN).

2.2.5 Calculations and statistical analyses

Data analyses were performed in R (version 4.0.2) using packages tidyverse (Wickham et al. 2019), ggplot2 (Wickham 2016), multcompView (Graves et al. 2019) and agricolae (de Mendiburu 2020) and base R. We calculated Schmidt stability index (SSI) values using rLakeAnalyzer (Winslow et al. 2019). We used one-way analysis of variance (ANOVA) to test for statistically significant differences for intra-annual and inter-annual comparisons of Secchi depth, DIN and TP. Treatment groups in the nutrient enrichment bioassay were compared using Tukey's HSD (honestly significant difference) test.

To make our results more comparable to other studies on changing lake trophic state, we calculated trophic state index (TSI) for the lake for each sampling campaign using Secchi depth according to Carlson (1977). We chose this method over similar metrics such as Toledo (1983), Salas and Martino (1991), Lamparelli (2004), or Cunha (2013) due to the reliability and consistency of Secchi depth compared to

other parameters, absence of Chl-a values from the historic campaign and concerns over under-estimation of trophic state of some models (Klippel et al. 2020). In Lake Yojoa, the majority of variance in Secchi depth can be explained by shifts in algal abundance, making Secchi depth an appropriate tool for estimating trophic state where historic Chl-a values are unavailable (Appendix A).

$$TSI(SD) = 10[6 - \frac{\ln(SD)}{\ln(2)}]$$

In addition to the nutrient enrichment bioassays, we compared the molar ratio of dissolved inorganic N to Total P (DIN:TP) in the epilimnion between the two sampling periods to use as a proxy for potential macronutrient limitation of primary productivity (Wetzel 2001; Bergström 2010). We used the ratio DIN:TP> 10 to suggest P limitation, 10>DIN:TP>5 to suggest co-limitation and DIN:TP <5 to suggest N limitation (Wetzel 2001).

2.3 Results

2.3.1 Stratification

To assess the mixing regime of contemporary Lake Yojoa, we analyzed DO profiles from sampling events across the two years of contemporary monitoring. For the years of our study, Lake Yojoa behaved principally as a monomictic system, with stratification beginning in February with the thermocline fully establishing at a depth of 12 m in April and water column mixing occurring again in November of each year (Fig. 2.2A). We did observe several partial mixing events during the stratified periods, but these were localized and/or intermittent and stratification was quickly reestablished (Fig. 2.2A). Temperature profiles between the two sampling periods differed, with increased temperature (mean change of 3.0 °C) in the contemporary relative to the historic period across all depths (Fig. 2.2B). Mean monthly SSI values indicated that water column stability was not significantly different between historic and contemporary sampling periods for nine of the eleven months we were able to compare (Fig. 2.2C).

2.3.2 Secchi depth and trophic state

We assessed changes in Lake Yojoa's trophic state between the two sampling periods (inter-annual) and between months within each sampling period (intra-annual) by comparing mean Secchi depths. Mean

annual Secchi depth and TSI from the historic period (mean Secchi depth = 7.3 ± standard deviation 2.1 m, and TSI = 31.1 ± 2.7) were significantly different (p<0.001, df = 107) than present day conditions (mean Secchi depth = 3.2 ± 1.0 m, and TSI = 43.3 ± 31.5).

The differences in annual mean transparency and trophic state between each sampling period were driven by changes in intra-annual dynamics. During the historic sampling period, Lake Yojoa experienced a clear water phase from August to March (mean Secchi depth = 8.25 ± 1.7 m and TSI = 29.8 ± 2.7) with annual minimum Secchi depth (mean Secchi depth = 5.0 ± 1.1 m and TSI = 37.0 ± 3.4) occurring between April and July (Fig. 2.3A). However, in the contemporary sampling, we observed no pronounced change in water clarity among seasons. Secchi depths for the months that previously had the minimum secchi depths (April to July) had, on average, 36% lower transparency (mean Secchi depth = 3.2 ± 1.0 m and TSI = 44.2 ± 4.8) in the contemporary sampling. Similarly, the months that previously had a clear water phase (August – March) were statistically indistinguishable (p=0.7, df= 87) from the months that had the annual minimum (mean Secchi depth = 3.2 ± 1.0 m and TSI= 43.7 ± 4.1), resulting in a complete loss of seasonality of water clarity between the two sampling periods.

2.3.3 Inorganic nitrogen and total phosphorus

To evaluate putative drivers of changes in the trophic state of Lake Yojoa we also assessed inter-annual and intra-annual changes in epilimnetic nutrient concentrations between contemporary and historic periods. During the historic period, DIN varied throughout the year but had no clear seasonality (mean DIN = 3.99 ± 2.61 μM, Fig. 2.3B). However, during contemporary periods, mean annual DIN and intra-annual variation in DIN were greater than that observed during the historic period (mean DIN= 12.28 ± 9.85 μM, Fig. 2.3B) with greater variance between contemporary and historic periods (DIN variance = 97.1 vs. 6.80, respectively). The increase in contemporary DIN was most pronounced directly following mixing in November (Fig. 2.2A and 2.3B). After mixing, epilimnetic concentrations of DIN remained significantly higher (p<0.001, df=65) than pre-mixing levels until the onset of stratification in April when epilimnetic DIN concentrations returned to pre-mixing levels (Fig. 2.3B).

In contrast to DIN, contemporary epilimnetic TP values did not have increased seasonality in the contemporary period compared to the historic period (Fig. 2.3C). Mean annual TP values increased between historic and contemporary periods (0.41 ± 0.67 μM and 0.72 ± 1.02 μM, respectively), but these differences were not significant (p=0.1, df = 117). There was a pronounced increase in epilimnetic TP in June compared to other months of the year that was significant in the historic period (p<0.05 for all months) but not significantly different from the other months (p> 0.5 for all months) in the contemporary sampling. We also observed a secondary peak in the contemporary data in November, coincident with mixing. Contemporary epilimnetic TP in November (2.20 ± 1.93 μM) was somewhat higher relative to the historic period (0.31 ± 0.29 μM), but this difference was only marginally significant (p=0.07, df=14, Fig. 2.3C).

We also compared differences in hypolimnetic concentrations of TP, NH_4^+ and NO_3^- between historic and contemporary periods during mixed (January) and stratified (June) sampling events to test if the increase in epilimnetic DIN and TP following November mixing was driven by changes in hypolimnetic N and P between sampling periods. During the historic period, there was no significant difference between epilimnetic or hypolimnetic concentrations of NO_3^- (p=0.504 df=9), TP (p= 0.884, df=9), or NH_4^+ (p= 0.298, df=9) when the lake was stratified (Table 1). In the contemporary sampling period, we observed no significant (p=0.67, df=5) difference in NO_3^- between the two strata (Table 1). However, in the contemporary sampling period, during stratification, the hypolimnion of Lake Yojoa was significantly enriched in NH_4^+ (p<0.001, df=5) and marginally enriched in TP (p= 0.078, df=7) relative to the epilimnion (Table 1), representing ~7.6 times more NH_4^+ and ~2.3 times more TP than was observed during stratification in the historic data (Table 1). The initial increase in epilimnetic DIN following mixing was driven by an abrupt increase in NH_4^+, that was quickly converted to NO_3^- (Fig. 2.4). NO_3^- remained the dominant DIN species in the epilimnion throughout the duration of the mixed water column phase (Fig. 2.4).

2.3.4 Nutrient limitation

To assess how these changes in accumulation of hypolimnetic DIN and release with mixing have altered the productivity of the lake, we evaluated nutrient limitation by comparing epilimnetic DIN:TP (by

atom) ratios between each sampling period and conducting a nutrient limitation assay. In the historic period, the ratio of DIN:TP in the epilimnion fluctuated between 1.9 and 65.6 (Fig. 2.5A). In contemporary sampling we observed a greater annual range of DIN:TP (1.0 to 564.3) in the photic zone of Lake Yojoa with a pronounced increase in DIN:TP directly following November mixing (Fig. 2.5B).

We then directly tested the current state of macronutrient limitation in Lake Yojoa with two nutrient enrichment bioassays, one conducted in January when the water column was mixed, and one conducted in June when the water column was stratified. In January, additions of N alone did not yield a significant increase in algal abundance relative to the control (p=0.52, df=32). However, algal abundance was significantly greater in the +P and +NP treatment groups than in both the control and +N treatments (p<0.001, df= 32, Fig. 2.5C). The +P and +NP groups were not significantly different from each other (p=0.18, df=32). In June, all treatments had a greater percent increase from the control than in January (Fig. 2.5D). However, only treatments that received both N and P (+NP) were significantly different from the control group (p=0.054, df=17).

2.3.5 Potential drivers

Whereas this study was not designed to quantify the relative contributions of various point and non-point sources of nutrients to Lake Yojoa, we were able to assess several putative drivers which were most likely to be responsible for the pronounced change in nutrient dynamics. To evaluate the sources of nutrients to contemporary Lake Yojoa we assessed intra-annual delivery of allochthonous (external) nutrients from the watershed using daily cumulative precipitation as a proxy for nutrient loading from the watershed. We compared daily cumulative precipitation in 2019 to epilimnetic DIN and TP concentrations in the pelagic zone of the lake (B, E, F P, and R) to assess the relationship between peak rainfall events and pelagic nutrient concentrations. We found no corresponding increase in epilimnetic nutrient concentrations concomitant with large precipitation events for any of the five principal sampling stations (Fig. 2.6).

We also evaluated the spatial distribution of contemporary hypolimnetic nutrient values during June 2018 and 2019 sampling campaigns. We hypothesized that if nutrient load from tributaries was a major contributor to hypolimnetic nutrient accumulation, hypolimnetic nutrient concentrations would be greatest

at sampling stations nearest tributaries (locations B, C, M, N, and O). For the June sampling campaigns, hypolimnetic NH_4^+ concentrations in Lake Yojoa ranged from 6.3 to 67.4 μM. However, mean hypolimnetic NH_4^+ concentrations at stations nearest the tributaries (locations B, C, M, N, and O mean = 39.03 ± 26.02 μM) were nearly 17% less than the total hypolimnetic mean (47.38 ± 18.22 μM, Fig. 2.7A, Table S3.2) whereas hypolimnetic NH_4^+ concentrations at stations in the deepest portion of the lake, Q, P, and R (55.79 ± 11.42 μM) were, on average, approximately 17% greater than the total hypolimnetic mean. In contrast, hypolimnetic NO_3^- concentrations were uniformly low (< 3 μM) at all stations of the lake (Fig. 2.7B, Table S3.2) suggesting that NH_4^+ is the dominant form of reactive N in the hypolimnion of Lake Yojoa. Hypolimnetic TP concentrations ranged from 0.6 μM to 3.2 μM. TP concentrations nearest the tributaries (mean= 1.39 ± 0.74 μM) were approximately 37% less than the total hypolimnetic mean (2.21 ± 1.04 μM) and stations in the center of the lake (Q, P and R) had approximately 63% greater TP concentrations (3.58 ± 0.21 μM) than the total hypolimnetic mean (Fig. 2.7C, Table S3.2). Secchi depth was statistically indistinguishable across regions (p>0.5) with total lake mean (2.79 ± 0.30 m), near-tributary mean (2.68 ± 0.32 m), and mean of stations Q, P and R (2.85 ± 0.30 m) all within the standard deviation of Secchi depth for the entire lake (Fig. 2.7D, Table S2.2).

2.4 Discussion

Comparison of historic and contemporary periods showed that over the past forty years Lake Yojoa has transitioned from an oligotrophic system with a distinct clear water phase (August to March) to a mesotrophic system with low water clarity and the absence of any pronounced seasonal differences in water clarity. This change in trophic state was accompanied by accumulation of hypolimnetic nutrients (NH_4^+ and to a lesser extent TP) during the stratified period and a release of nutrients to the epilimnion during mixing (usually in November). Delivery of NH_4^+ from the hypolimnion to the photic zone during the dry season maintained Lake Yojoa's mesotrophic state by alleviating previous N limitation (replacing phytoplankton N and P colimitation during the stratified water column months with P limitation, Fig. 2.5), allowing rapid uptake of P (also released from the hypolimnion) and preventing the development of the clear water phase that was present during the historic period. To explore drivers and consequences of these changes in the

context of contemporary Lake Yojoa, as well as a broader understanding of similar monomictic tropical lakes, in this discussion we focus on 1) potential sources of nutrients to Lake Yojoa, 2) implications for changing nutrient dynamics in other monomictic tropical lake systems, and 3) areas for further research.

2.4.1 Sources of nutrients to Lake Yojoa

The lake and its watershed maintain several livelihood generating activities including approximately 55 lakeside restaurants, agricultural activities (e.g., cultivation of yuca, sugar cane, coffee, and pineapple), subsistence fishing, ranching, and industrial aquaculture (Studer 2007). Transport of reactive nutrients derived from such activities within the watershed undoubtedly influence the biogeochemistry of Lake Yojoa. However, we did not see expected correlations between indicators of watershed loading and nutrient concentrations in the lake, as has been observed in other watersheds (Pandey and Verma 2004). Peak epilimnetic nutrient concentrations did not coincide with the rainy season when the majority of watershed discharge is delivered to the lake (Fig. 2.6). There are a few possible explanations for this observed asynchronicity. First, because the tributaries originate at higher elevations than the lake itself, the disconnect between epilimnetic nutrient concentrations and rain events could be explained by the subduction of cooler tributary waters and the formation of profile-bound density currents which could transport nutrients away from the littoral zone throughout the lake's hypolimnion, as has been observed in other systems (Talling and Lemoalle 1998). We hypothesized that increased hypolimnetic nutrient concentrations near the mouths of each tributary would suggest that loading from the watershed may be driving the accumulation of hypolimnetic nutrients. However, hypolimnetic sampling stations nearest the littoral zone near the mouths of tributaries were depleted rather than enriched in NH_4^+ and TP compared to sampling stations in the hypolimnion of the pelagic zone of the lake (Fig. 2.7A and C). Second, the asynchronicity between watershed loading (as estimated by precipitation) and epilimnetic nutrient concentrations may be explained by nutrient assimilation driven by co-limitation, as suggested by the June nutrient bioassays, leaving reactive nutrients available for only a short period. High rates of nutrient allocation to biomass, not only in phytoplankton but also in aquatic macrophytes has been previously observed in tropical ecosystems (Esteves 1982). However, Secchi depth was not significantly lower nearest

the tributaries as would be expected if reactive nutrients were driving spatial differences in algal abundance or if watershed inputs during the rainy season were primarily in the form of suspended sediments and particulate C and N, as observed in other tropical watersheds (McDowell and Asbury 1994). Therefore, we propose that the lack of spatial or temporal correlation between the watershed inputs and water column nutrient concentrations are consistent with the watershed not being the main driver of nutrient changes in Lake Yojoa.

Within the lake are two small "artisanal" net-pen Tilapia farms, one in the central region and another in the southern region of the lake. There is also a large "industrial" net-pen Tilapia operation in the north-central region of the lake (positioned nearest stations P, Q, and R, Fig. 2.1). Net-pen aquaculture cannot treat effluent, causing nutrient rich fish waste (both urea and fecal pellets) to directly mix with the surrounding ecosystem. In other lakes, net-pen aquaculture has been shown to increase the trophic state and contribute to sediment nutrient concentrations (Troell and Berg 1997). Using data shared by the operator of the net-pen aquaculture farm (Regal Springs) for 2011 and 2013, we estimated annual nutrient loading from the aquaculture operation was between 353 to 395 metric tonnes of N and 44 to 55 metric tonnes of P $year^{-1}$ (*pers. comm.* Regal Springs). In addition to the loading of dissolved nutrients, carbon (C) loading (particulate and dissolved) from the Tilapia farm may also be significant given the over 9000 tonnes of feed supplied to the Tilapia pens (based on 2011 and 2013 values), with loading estimates from previous studies on net-pen Tilapia farms ranging from 81 to 91% of C in feed ultimately transferring to the surrounding environment (Gondwe et al. 2011). These estimates suggest the industrial aquaculture operation in Lake Yojoa represents a significant source of C, N, and P to the lake and is a source that was not present during the historic sampling campaign. The importance of the nutrients derived from net-pen aquaculture to the observed changes in Lake Yojoa are also supported by our observations of enriched hypolimnetic NH_4^+ and TP at stations nearest the aquaculture operation (P, Q and R) relative to the other stations in the lake, including those nearest the tributary mouths (Fig. 2.7A, C). Despite achieving the highest standards of industry sustainability, including certifications from the Global Aquaculture Alliance/Best Aquaculture Practice (BAP) and the Aquaculture Stewardship Council (ASC), it is evident that the current aquaculture

operation is likely introducing unsustainable quantities of nutrients into Lake Yojoa that are altering the state of the ecosystem.

Another potential source of N loading to Lake Yojoa is atmospheric N deposition. While direct measurements of N deposition were unavailable for Lake Yojoa, estimates for the region range from 4.81 – 6.21 mmol –N m^{-2} $year^{-1}$ (Dentener 2006). Given the surface area of Lake Yojoa (88 km^2, Table S2.1), this would represent an annual deposition of between 4.23 x 10^5 and 5.46 x 10^5 mols per year, approximately two orders of magnitude less than inputs of reactive N derived from aquaculture.

2.4.2 Implications of ongoing change in Lake Yojoa

Lake Yojoa is a monomictic lake that mixes each November (Fig. 2.2A). In 1979-1983 stratification developed in late January or February with the hypolimnion becoming anoxic by the end of March and the thermocline rising to 13-15 m by the beginning of the rainy season (typically in May) and mixing occurring in November (Vaux and Goldman 1984). We observed the same timing of mixus and stratification in contemporary Lake Yojoa but with a slightly shallower thermocline (11-12m) (Fig. 2.2A). Therefore, the contemporary stratification conditions which allow for hypolimnetic nutrient accumulation existed in the 1980s, though in the 1980s both epilimnetic and hypolimnetic temperatures were slightly less (~ 3 °C) than what is observed today (Fig. 2.2B). In addition to the predicted seasonal mixing in November, during the contemporary sampling campaign, we also observed infrequent, ephemeral partial mixing events (i.e., only at some stations or not complete in depth with stratification quickly re-establishing) concurrent with large wind events and/or cold fronts (Fig. 2.2A). Similar partial mixing events during the stratified season were also noted in the historic data, as evidenced by anomalies in oxygen and temperature profiles (Vaux and Goldman 1984). However, these aseasonal mixing events have new ramifications under conditions of elevated levels of hypolimnetic nutrients in contemporary Lake Yojoa. Mixing events, during otherwise stratified months, introduce hypolimnetic nutrients to surface waters during peak annual temperatures. This pulse of nutrients can result in rapid increases in algal biomass (i.e., algal blooms). One such bloom occurred on June 9, 2020, and garnered national attention (La Tribuna, 2020). With increased intensity of weather events predicted with climate change (Knutson et al. 2010), these aseasonal mixing

events may become more frequent, with the potential for a concurrent increase in frequency of algal blooms during the months where the lake is normally stratified.

The accumulation of hypolimnetic NH_4^+ and TP and subsequent release into the photic zone during the dry season when watershed inputs are low is a relatively recent (<40 years) development in Lake Yojoa. However, we hypothesize that a similar pattern of nutrient delivery from the hypolimnion to the photic zone during the dry season may be driving changes in trophic state of other monomictic tropical lakes. For example, annual mixing in Lake Titicaca (Peru-Bolivia) introduced large quantities of nutrients to the euphotic zone, temporarily alleviating N and P limitations (Vincent et al. 1984a). Similarly, in Valle de Bravo Reservoir (Mexico), N limitation during stratification was replaced by P limitation following mixing due to an increase in the DIN:TP ratio (Merino-Ibarra et al. 2008). These findings in other monomictic tropical systems are consistent with the results from the nutrient bioassays and the seasonal shifts in DIN:TP we observed in this study (Fig. 2.5).

2.4.3 Other controls on lake N dynamics

To better understand the observed changes of hypolimnetic nutrient accumulation, we estimated changes in the rate of net hypolimnetic NH_4^+ and TP accumulation from January, when the water column was mixed, to July 1st, near peak stratification, between contemporary and historic periods. In the early 1980s, NH_4^+ accumulated in the hypolimnion at an estimated rate of 0.04 μM day^{-1}. Today, we estimate an accumulation rate of 0.33 μM day^{-1} over the same interval, a nearly tenfold increase in the rate of NH_4^+ accumulation in the hypolimnion between the historic and contemporary periods. We estimate that TP accumulated at a rate of 0.0005 μM day^{-1} in the historic period compared to 0.002 μM day^{-1} in the contemporary. Our calculations here suggest that this order of magnitude difference in N and P loading to the hypolimnion is likely primarily driven by the industrial aquaculture operation that was established in Lake Yojoa in 1997.

Given the warm, anoxic hypolimnion of Lake Yojoa, it is also likely that unique anaerobic microbial pathways may also contribute to the accumulation of NH_4^+ in the hypolimnion. Changes in N-cycling pathways that contribute (and remove) NH_4^+ may be an important aspect of the understanding how

hypolimnetic processes contribute to Lake Yojoa's changing trophic state. There are seven principal biogeochemical pathways that have the potential to influence the pool of NH_4^+ in of Lake Yojoa: N fixation, nitrification, uptake, mineralization, denitrification, anaerobic ammonium oxidation (anammox) and dissimilatory nitrate reduction to ammonium (DNRA). Of these, only uptake, mineralization, denitrification, anammox, and DNRA, are likely to be active in the aphotic, anoxic hypolimnion of Lake Yojoa. Thus, understanding controls and constraints on each of these pathways may provide further insight into the changing biogeochemistry of monomictic tropical lakes. For example, the large inputs of organic N from the fish farm as well as the warm temperatures present in the hypolimnion of Lake Yojoa likely result in accelerated rates of mineralization (Amado et al. 2013). In addition, concentrated organic matter loading below aquaculture operations has been shown to increase DNRA rates up to seven-fold of ambient conditions (Christensen et al. 2000) further exacerbating the accumulation of ammonium. Under conditions of low NO_3^-, as observed in Lake Yojoa's hypolimnion (Fig. 2.7B) and high concentrations of labile C, DNRA is favored over complete denitrification, the dominant loss pathway for reactive N in most lake ecosystems (Nizzoli et al. 2010). Given the large inputs of C that are likely associated with the industrial Tilapia operation in the deepest basin of the lake, conditions that favor DNRA are likely present in the hypolimnion of Lake Yojoa. Conditions that result in the selection for DNRA over denitrification would further promote the accumulation of NH_4^+, whereas historic conditions (e.g., lower autochthonous N and organic C inputs) may have favored denitrification and prevented the accumulation of hypolimnetic NH_4^+. Additional studies on controls over microbially mediated N transformation pathways in the warm, anoxic hypolimnions may be a key tool to better understand ecosystem scale change in tropical lakes.

2.5 Conclusion

The results from this study along with similar observations in other monomictic tropical lakes suggest that changes in hypolimnetic nutrient accumulation and release to the photic zone coincident with mixus are driving changes in the trophic state of monomictic tropical lake ecosystems (Vincent et al. 1984, Merino-Ibarra et al. 2008). In Lake Yojoa, the presence of large-scale aquaculture is likely driving the loading of N and P to the hypolimnion but in other lakes the source of N or P may be different. In

monomictic tropical lakes, the interaction of the physical structure of the water column and nutrient loading places hypolimnetic processes at the intersection of local drivers, such as nutrient loading from aquaculture, and global drivers, such as warming waters and increasing storm intensity associated with climate change (Knutson et al. 2010). Our results suggest that one key to improving the current understanding of tropical monomictic lake ecosystems may be defining the controls on hypolimnetic biogeochemical pathways. Comparisons between epilimnetic and hypolimnetic nutrient concentrations may therefore be an important indicator of trophic state threshold in monomictic lake ecosystems and provide a useful tool to assess the impact of contemporary uses and stressors on the overall trophic state of these societally important ecosystems.

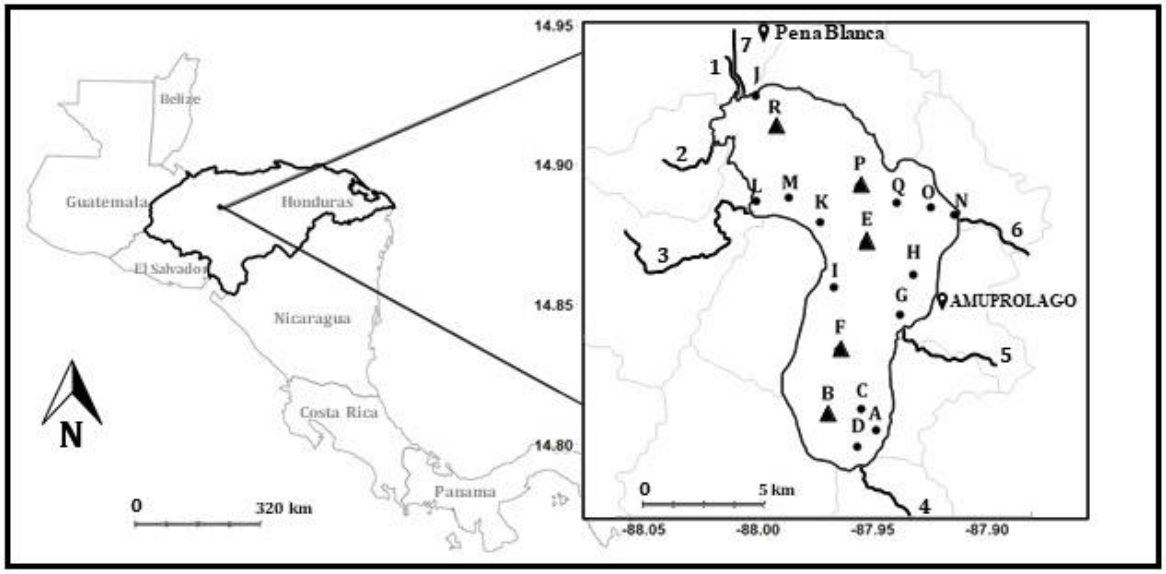

Figure 2.1. Contemporary sampling stations within Lake Yojoa for twice-annual (A-R) and semi-monthly (B, E, F, R and P) sampling. Principal tributaries 1) Rio Helado 2) Rio Balas 3) Rio Raices 4) Rio Varsovia 5) Rio Cacao 6) Rio Yure and outflow 7) Canal

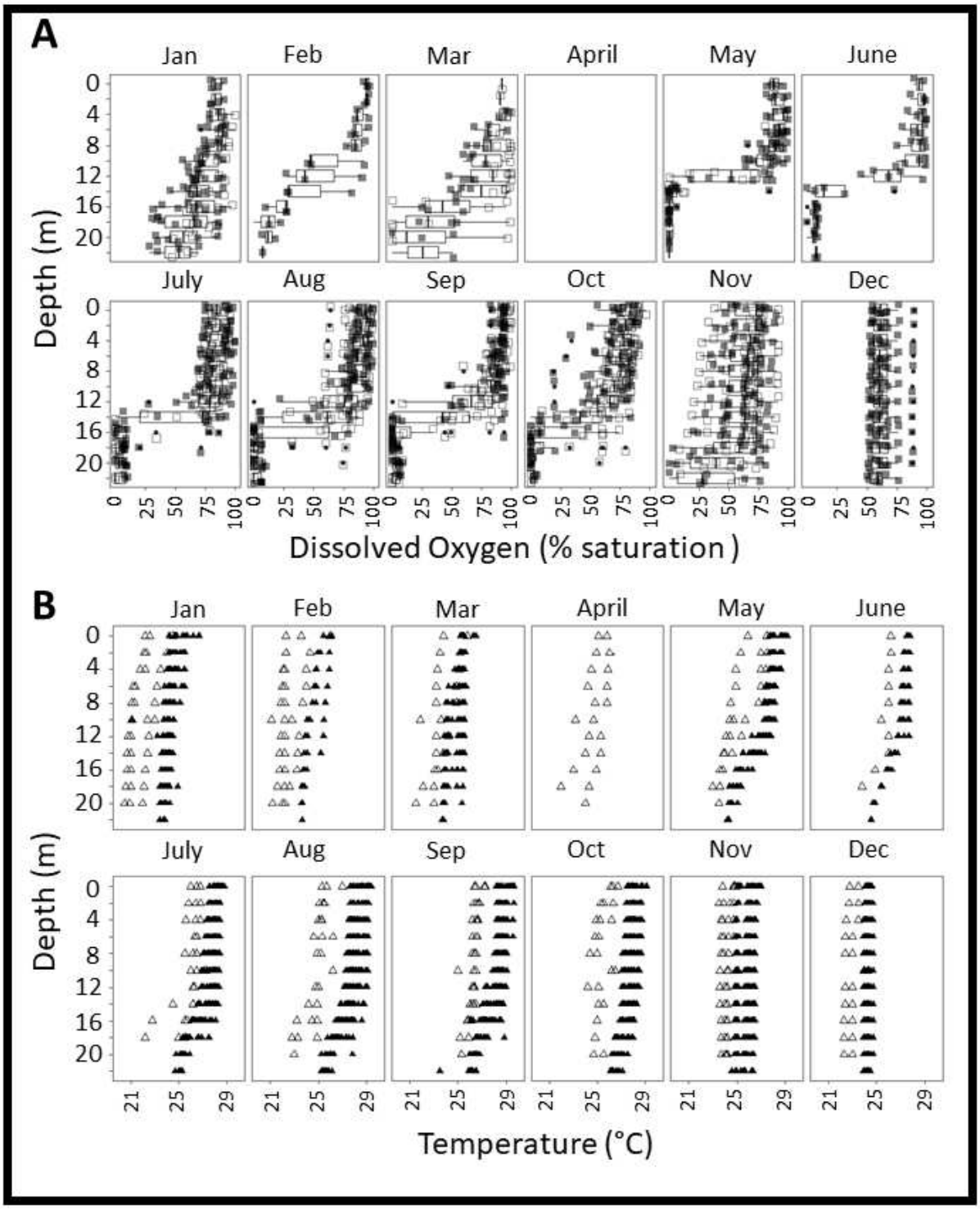

Figure 2.2 (A) Dissolved oxygen profiles for contemporary sampling for all months (1-12) in 2019 (open squares) and 2020 (filled squares) over two-year boxplots (min, interquartile range, and max). **(B)** Temperature profiles for each month of all years of contemporary (filled triangles) and historic (open triangles) sampling. Due to sonde malfunction and the COVID-19 pandemic, profile data is unavailable for April of both 2019 and 2020. **(C)** Monthly mean Schmidt Stability Index (SSI) values ± standard deviation.

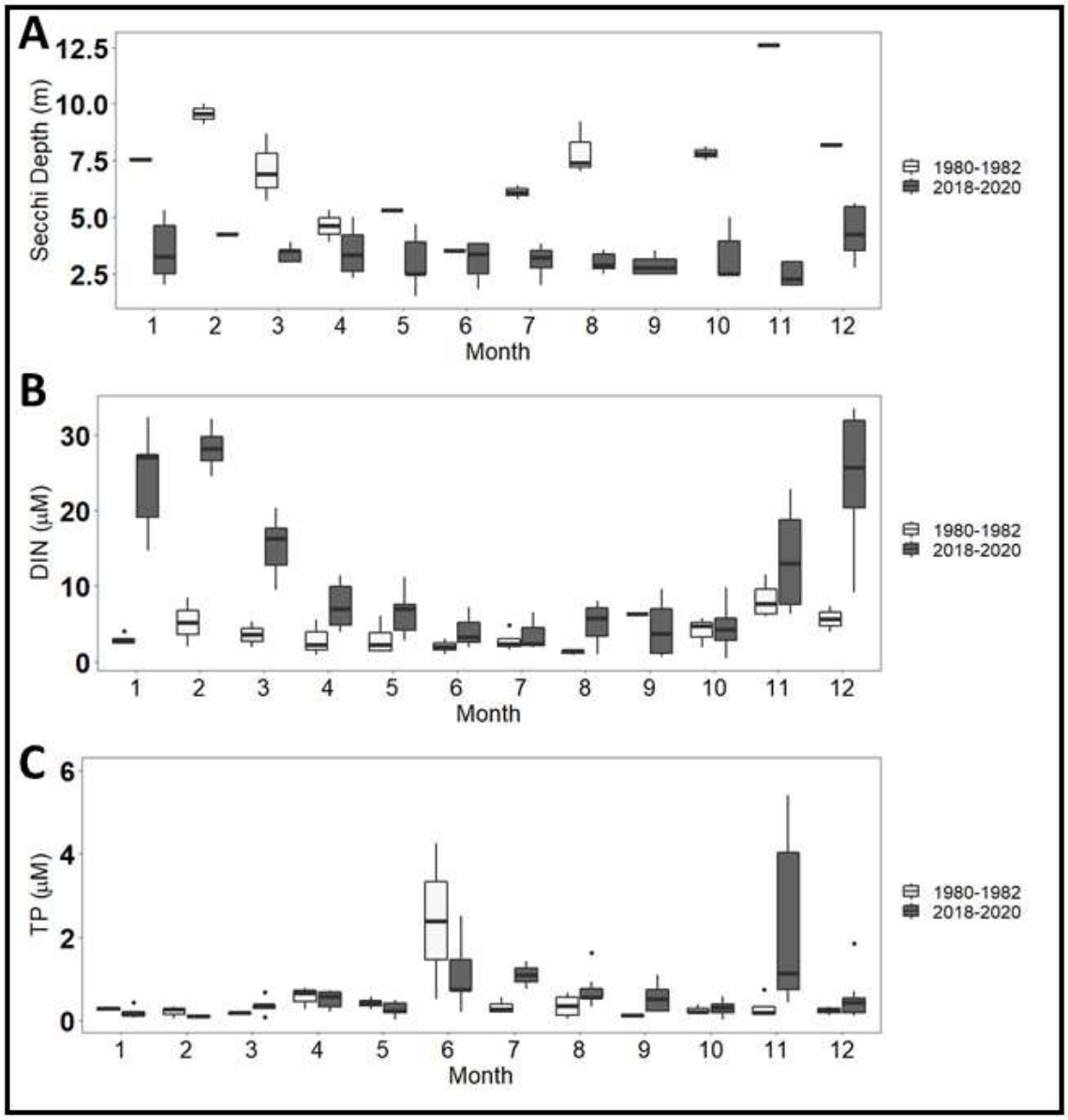

Figure 2.3 (A) Secchi depths **(B)** dissolved inorganic nitrogen (DIN) and **(C)** total phosphorus (TP) at station E. Samples collected from the epilimnion at a depth of 1 meter.

Table 2.1. Epilimnetic and hypolimnetic nutrient concentrations for each sampling campaign at station E (mean ± sd). Values are provided next to sampling depth.

	January (Mixed water column)			June and July (Stratified water column)		
	Total Phosphorus (µM)	**NH_4^+-N (µM)**	**NO_3^--N (µM)**	**Total Phosphorus (µM)**	**NH_4^+-N (µM)**	**NO_3^--N (µM)**
1980-1982	0.27 ± 0.04 (1 m)	1.36 ± 0.54 (1 m)	1.59 ± 0.79 (1 m)	1.01 ± 1.60 1.02 (1 m)	1.31 ± 1.51 (1 m)	1.19 ± 0.53 (1 m)
	0.42 ± 0.11 (>17 m)	3.86 ± 2.49 (>17 m)	3.75 ± 2.61 (>17 m)	0.88 ± 1.23 (>17 m)	7.27 ± 13.20 (>17 m)	1.49 ± 0.91 (>17 m)
2018-2020	0.18 ± 0.13 (1 m)	7.65 ± 7.88 (1 m)	16.54 ± 11.72 (1 m)	1.12 ± 0.75 (1 m)	2.69 ± 1.94 (1 m)	1.17 ± 0.75 (1 m)
	0.36 ± 0.07 (>17 m)	3.76 ± 6.51 (>17 m)	14.30 ± 9.72 (>17 m)	2.05 ± 0.31 (>17 m)	55.48 ± 10.70 (>17 m)	0.90 ± 0.56 (>17 m)

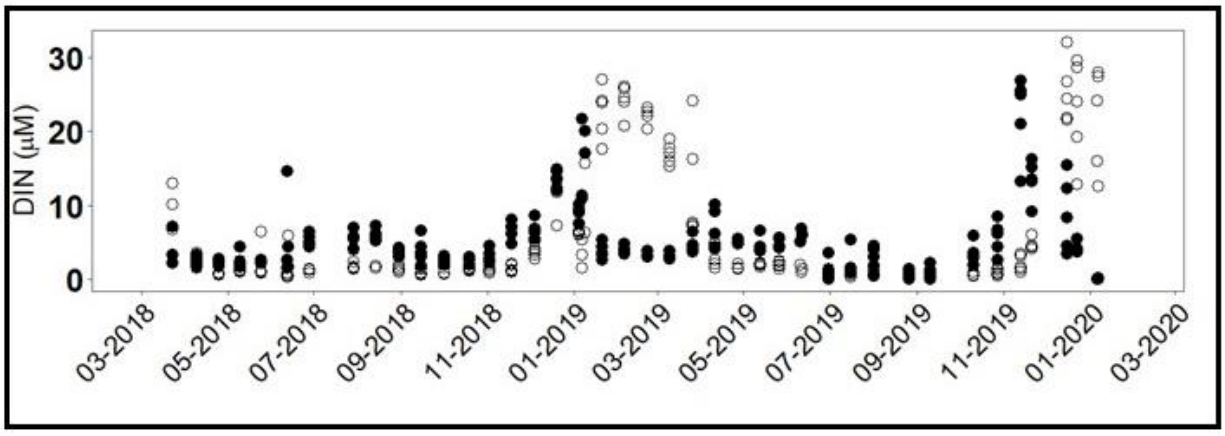

Figure 2.4. Epilimnetic ammonium (NH_4^+) (filled circles) and nitrate (NO_3^-) (open circles) at stations B, E, F, P and R.

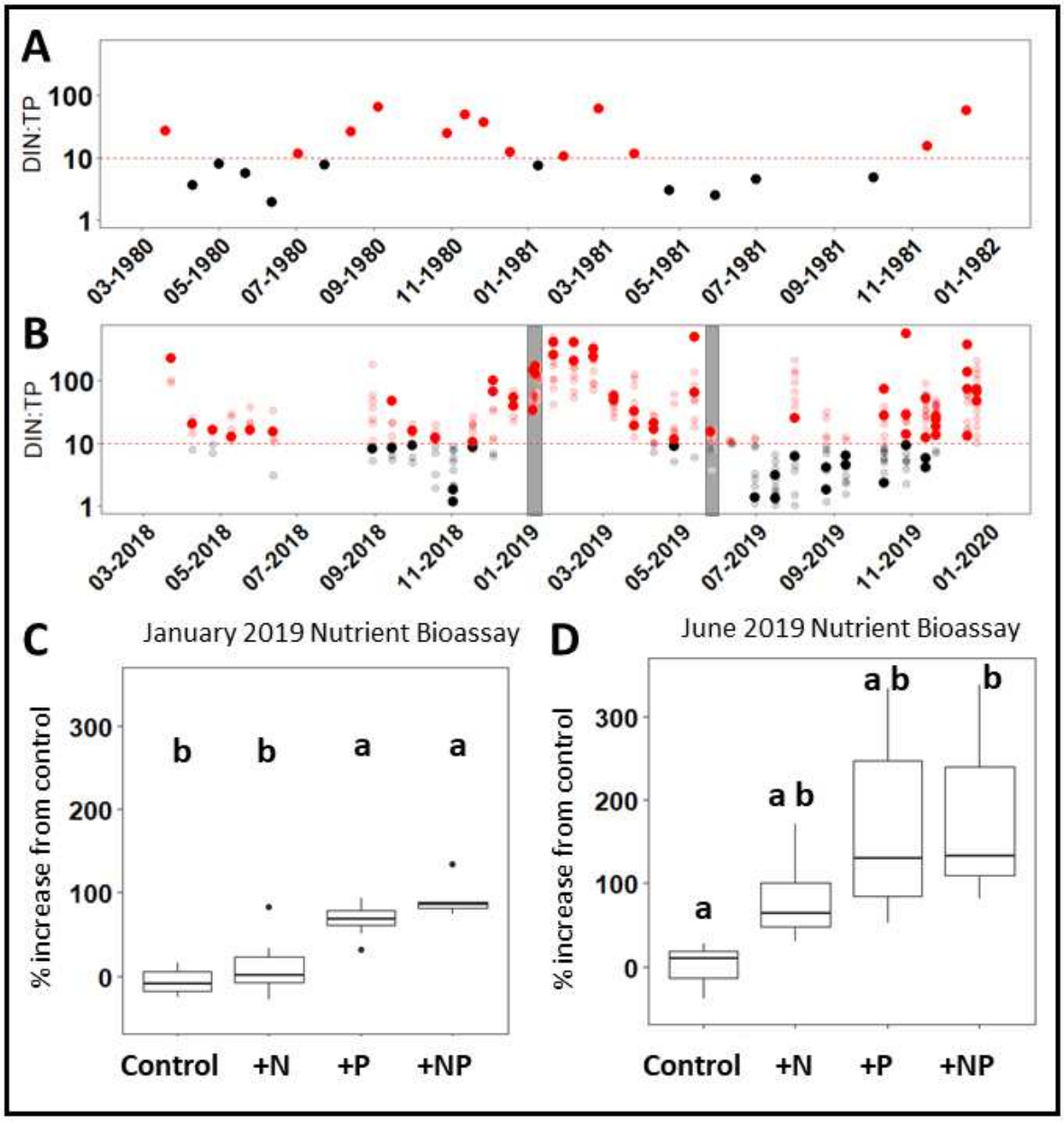

Figure 2.5. **(A)** Historic epilimnetic DIN:TP at location E **(B)** contemporary DIN:TP at all locations (B, F, R, P and E bolded). Red indicates likelihood of P limitation above the red dashed line at DIN:TP = 10. P limitation threshold suggested by Wetzel (2001). Grey bars indicate timing of nutrient enrichment bioassays **(C)** Nutrient enrichment bioassay from location E conducted January 2019. **(D)** Nutrient enrichment bioassay from location E conducted June 2019.

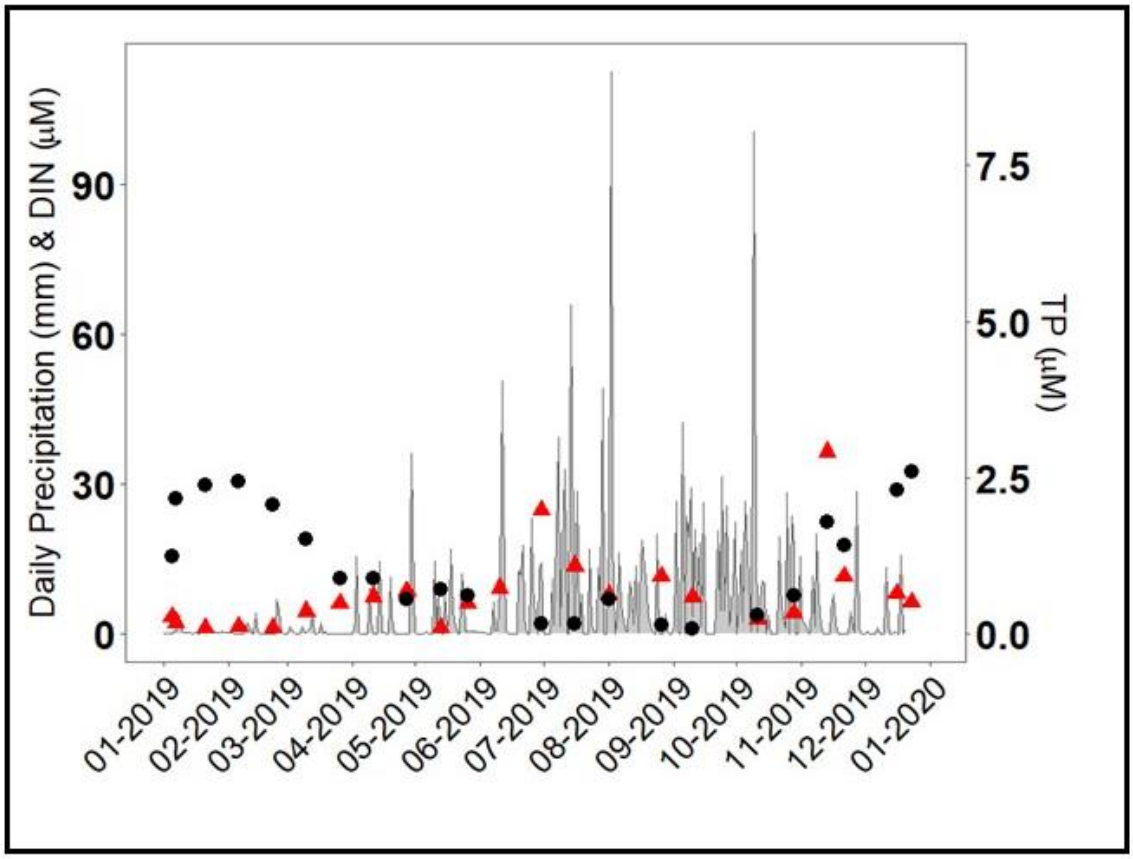

Figure 2.6. Daily precipitation (primary axis) and mean epilimnetic DIN (black circle) and TP (red triangles) at locations B, E, F, P and R (secondary axis).

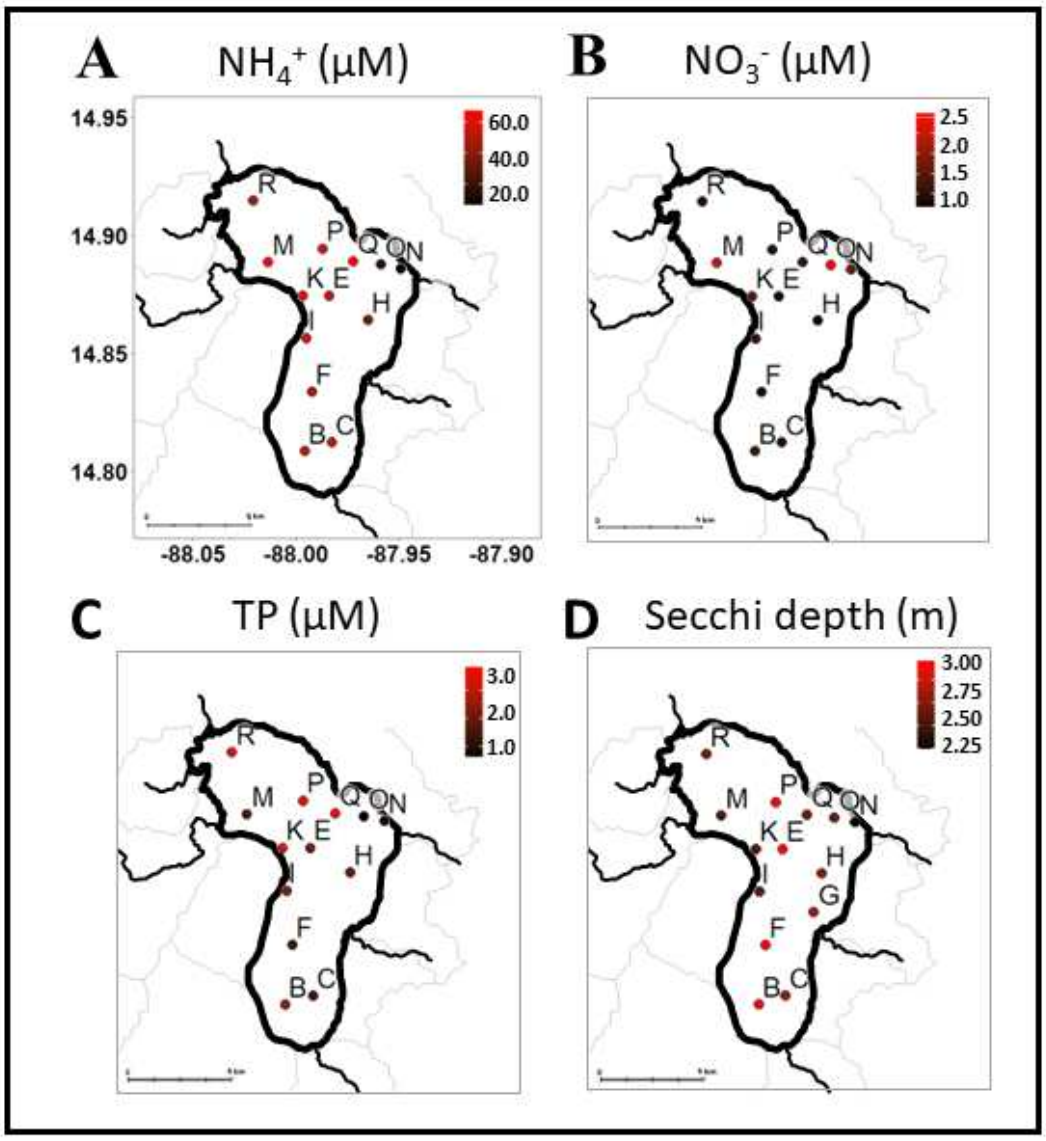

Figure 2.7 (A) Mean hypolimnetic NH_4^+ **(B)** NO_3^- **(C)** TP and **(D)** Secchi depth in June (mean 2018 and 2019) during contemporary sampling. Values provided in Table S2.2.

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CHAPTER 3: TROPHIC STATE RESILIENCE TO HURRICANE DISTURBANCE OF LAKE YOJOA, HONDURAS[3]

3.1 Introduction

Large lakes across the world are key resources for the persistence and subsistence of human and wildlife populations and have been for millennia; providing refugia for biodiversity, cultural significance to communities, and fuel for local economies (Sterner et al. 2020). However, local anthropogenic stressors and global changes, such as shifting climate patterns, threaten the ecosystem services supported by these lakes, and by extension, the communities who depend on them (Jenny et al. 2020). One such shifting climate pattern is the rise of extreme storm events, such as increasing hurricane frequency and intensity on account of rising sea surface temperatures (Knutson et al. 2010; Saunders and Lea 2008). Lakes and communities in Central America are particularly threatened by intensifying hurricane seasons due to tropical cyclone development in the Atlantic basin. In addition, Central American communities may be especially vulnerable to hurricane-driven shifts in ecosystem services due to a dependence of regional economies on agriculture and hydropower (Imbach et al. 2017). Regional climate projections suggest that Central America is likely to experience a drier future climate (Imbach et al. 2017; Vichot-Llano et al. 2021) with a higher frequency of more intense hurricanes over the coming decades (Bender et al. 2010). As such, the equitable and sustainable management of aquatic resources under future climate scenarios rests largely on maximizing resiliency through developing an understanding of how present weather events influence the ecology of impacted ecosystems.

The degree to which a particular tropical storm impacts an aquatic ecosystem depends on individual storm characteristics such as wind speed, amount of rainfall, and the specific path of the hurricane (Mallin and Corbett 2006). Potential impacts on lakes include altered thermal stability (Klug et al. 2012), increased

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suspended sediment concentrations and subsequent changes in light availability (Havens and Schelske 2011) as well as increased delivery of allochthonous materials including reactive nutrients that fuel primary productivity (Missimer, Thomas and Rosen 2021). These combined impacts can dramatically alter ecological function (Stockwell et al. 2020) particularly in shallow lakes (James et al. 2008; Havens et al. 2016). Though larger, inland lakes may be more resilient to the immediate impacts of large storm events than polymictic and coastal lakes, inland lakes are still subjected to extreme precipitation events related to hurricanes, tropical storms, and tropical depressions. These precipitation extremes are particularly relevant in tropical aquatic ecosystems due to the relationship between seasonal precipitation and the timing of watershed derived nutrient loading. The timing of precipitation facilitated nutrient additions interfaces with thermo-physical water column structure and stratification phenology to determine annual nutrient dynamics. Thus, hurricane driven alterations to these interactions may have measurable consequences to the trophic state of tropical lakes. Furthermore, determining if such alterations cause episodic limnological changes or else permanent shifts in trophic state is critical to evaluating ecological resiliency to storms.

In November 2020, Hurricanes Eta and Iota crossed over Lake Yojoa, Honduras during a period of continuous monitoring (2020-2022), providing an opportunity to measure whole ecosystem response to these anomalous, but increasingly frequent precipitation events. The Lake Yojoa watershed experienced its first late-season massive precipitation event of 2020 from November 3rd-5th when Hurricane Eta (Saffir-Simpson Hurricane Wind Scale: Category 4) made landfall near the Nicaragua-Honduras border before proceeding to dissipate to a tropical depression (Fig. 3.1) (Pasch et al. 2021). This initial storm event was followed only 12 days later by the second hurricane (Hurricane Iota) which made landfall in a nearly identical location in eastern Nicaragua on November 17th (Fig. 3.1) (Stewart 2021). Shortly before making landfall, Hurricane Iota was classified as a Category 5 storm making it the latest date a Category 5 hurricane had developed in the Atlantic basin since hurricane records began. Hurricane Iota weakened to a tropical depression on November 18th as it moved towards the Honduras-El Salvador border, however not before generating a second immense precipitation event that brought additional rainfall to Honduras's already saturated ecosystems, including Lake Yojoa. While exact precipitation values are unavailable in some

regions because the storms overwhelmed local rain gauges, best estimates are that Hurricanes Eta and Iota contributed up to 750 mm and 225 mm of rain, respectively, to the Lake Yojoa watershed (Pasch et al. 2021, Stewart 2021). The year prior (2019), the Lake Yojoa watershed received a cumulative 1893.42 mm of rainfall, typical for the area. Thus, within a few weeks Hurricanes Eta and Iota contributed half of the precipitation the watershed might otherwise expect to see in an entire year.

In recent decades, Lake Yojoa has transitioned from an oligotrophic to mesotrophic ecosystem with an annual mean water clarity (Secchi depth) of just over 3 meters (compared to over 7 meters in the 1980s) (Fadum and Hall 2022). One key mechanism for maintaining Lake Yojoa's recently elevated trophic state is intra-annual hypolimnetic nutrient accumulation during seasonal stratification (typically April to November) with release to the photic zone during annual mixing, eliminating what was previously a clear water phase (Fadum and Hall 2022). Therefore, changes in nutrient concentrations in both surface and deep waters is a critical metric for understanding trophic state development in this particular ecosystem. We hypothesized that Hurricanes Eta and Iota could have two possible effects on Lake Yojoa; either a nutrient loading effect, wherein a large amount of terrestrial nutrients would be transported to Lake Yojoa by surface run-off from the storms, or else the storms would have a diluting effect where the large quantity of nutrient-deplete runoff would decrease pelagic nutrient concentrations. To test for these hypothesized changes in Lake Yojoa following the hurricanes, we compared Secchi depth, and concentrations of chlorophyll a (Chl-a), NH_4^+, NO_3^-, total phosphorus (TP), dissolved organic carbon (DOC) and particulate carbon (C) and particulate nitrogen (N) in surface (1 m) and deep (16 m) water in 2020 (before the hurricanes) and 2021 (after the hurricanes) covering seasons when the water column was fully mixed and when the lake was stratified both before and after the impact of the hurricanes.

3.2 Methods

3.2.1 Lake Yojoa

Lake Yojoa is the largest natural freshwater lake in Honduras (~ 83 km2 surface area, 1.4 km3 volume, 27.3 m annual average max depth) and is a critical economic and water resource in the region. With an area of ~337 km2, the Lake Yojoa watershed supports three municipalities (Las Vegas, La Guama,

and Peña Blanca), two protected national parks (Parque Nacional Cerro Azul and Parque Nacional Montaña de Santa Bárbara) and is a hotspot for domestic tourism with a suite of hotels and restaurants that border the lake. The watershed sees persistently warm temperatures (annual average =23.4 C in 2019) with the warmest months corresponding with the monsoon season in which the majority of the precipitation (~85%) falls between June and October (based on 2019 measurements) (Fadum and Hall 2022). An industrial net-pen Tilapia aquaculture operation likely contributes large quantities of nutrients to the lake, with additional allochthonous nutrients being delivered during the monsoon season (Fadum and Hall 2022).

3.2.2 Sampling

We collected water for Chl-a and nutrient analysis (NH_4^+, NO_3^-, TP, DOC, and particulate C and N) from five stations (Fig. 3.1) at two depths (1 and 16 m) using an opaque Van Dorn water sampler every 16 days from January 2020 through December 2021 (excepting April 2020 due to COVID-19 restrictions). During each sampling event, Secchi depth was recorded. Temperature and dissolved oxygen (DO) profiles were measured at two-meter intervals using a Hydrolab MS5 Multiparameter Sonde (OTT HydroMet, Loveland, CO). Water collected from each depth was immediately placed in a cooler in opaque HDPE Nalgene bottles until we returned to the laboratory. Once in the laboratory whole water samples were frozen for TP analysis while NH_4^+, NO_3^-, and DOC samples were first filtered through 25 mm glass microfiber filters, Grade GF/F via Polysulfone filter funnels and subsequently frozen. Filters, kept for analysis of Chl-a and particulate C and N for surface and deep waters respectively, were folded in half, wrapped in aluminum foil, and immediately frozen. Water samples, stored in 15 ml centrifuge tubes, and filters and were transported to Fort Collins, CO, USA while frozen. Nutrient analyses were performed at the EcoCore facility at Colorado State University, Fort Collins, as described below.

In addition, we also collected surface sediments using a Eckman dredge in January 2020, prior to hurricane activity. Sediment samples were collected from 16 stations (Fig. 3.1). Sediments were collected throughout the lake including six open water stations and 10 stations nearer to tributaries that differed in land use inputs (e.g., mining, restaurants, protected areas). Sediment samples were freeze-dried and ground with a mortar and pestle prior to analysis.

3.2.3 Laboratory and statistical analyses

Lab analyses were consistent with previous research we have conducted for Lake Yojoa17. Briefly, Chl-a was measured on a 10-AU fluorometer (Turner Designs Part No. 10-AU-074, calibrated using Turner Designs liquid Chl-a standards) via extraction in 90% acetone with acidification to 0.003 N HCl with 0.1 N HCl (Arar 1997). TP was first digested to soluble reactive phosphorus (SRP) via the Alkaline Potassium Persulfate method under sub-boiling (90 □C) temperature, then measured using a colorimetric ascorbic acid assay (EPA Method 365.3) modified to be analyzed on a UV-STAR 96-well Microplate via Infinite M200 TECAN at 880 nm absorbance detection. NH_4^+ and NO_3^-, were measured using a Flow Solution FS 3700 Automated Chemistry Analyzer (O.I. Analytical, College Station, TX). DOC was measured on a Shimadzu TOC-L (Shimadzu Scientific Instruments, Inc). Particulate C and N were measured on a Velp 802 elemental analyzer.

Sedimentary organic C and N were measured using a Costech Elemental CHN analyzer and reported as percentages. Prior to analysis, sediment samples were fumigated in HCl vapors for 24 hours to remove carbonates. Sedimentary P was measured using an ICP analyzer following digestion with 15N HNO_3 in a heated block and following EPA method 6010B. P is reported as mg g^{-1} of sediment.

Statistical analyses were preformed, and figures were created in R (version 4.1.2) using packages tidyverse (Wickham et al. 2019), tidyr (Wickham and Girlich 2022), lubridate (Grolemund and Wickham 2011), and ggeasy (Carroll et al. 2022). Comparisons between pre-hurricane and post-hurricane parameters were evaluated using one-way analysis of variance (ANOVA). “Pre-hurricane” conditions refer to observations from January-December 2020 whereas “post-hurricane” conditions refer to observations from January-December 2021. To ensure, observations immediately following the hurricanes did not impact results, we also compared observations from January 2020 - October 2020 to those from November 2020 - December 2021. However, means between the two temporal groupings were not significantly changed, and statistical significance was unaffected by the choice of comparison grouping. Therefore, all comparisons were grouped by calendar year for simplicity and clarity.

3.3 Results

To understand the immediate impacts of Hurricanes Eta and Iota, we assessed trophic state before and after the hurricanes using Secchi depth and Chl-a concentrations and paired these observations with measurements of nutrient concentrations and thermo-physical structure. To unpack potential drivers of changing trophic state, we began by identifying the timing of water column mixus in 2020. The Lake Yojoa water column mixed between October 22nd and November 7th in 2020 (Fig. 3.2), consistent with observations from previous years (Fadum and Hall 2022). While we were unable to determine if the 2020 mixus was initiated by Hurricane Eta, as hurricanes are capable of mechanically mixing water columns (Imberger and Patterson 1989), the consistency of the timing of 2020 mixus with previous years suggest that observed changes in thermo-physical structure and nutrient dynamics in 2021 can be attributed to Hurricanes Eta and Iota and not premature water column destabilization or early hypolimnetic nutrient release.

For the two years on either side of the hurricanes (2020 vs. 2021 calendar years) we observed no significant difference ($p > 0.05$) in annual mean DO in surface (depth= 2 m) waters (annual mean ± st. dev, 2020= 6.49 ± 1.37 mgL^{-1}, 2021= 6.79 ± 1.86 mgL^{-1}, Table 3.1). While there was a significant difference in surface DO concentration for five months of the eleven months we compared (January, May, October, November, and December), there was no consistent directional change, with concentrations of DO in some months in 2021 being below 2020 values and others being above 2020 values (Table 3.1). Similarly, there was no significant difference ($p > 0.05$) in annual mean DO in deep (depth= 16 m) waters (annual mean ± st. dev, 2020= 2.34 ± 2.45 mgL^{-1}, 2021= 2.02 ± 2.19 mgL^{-1}, Table 3.1). DO in deep water was only significantly different in three months (February, June, and November) with no directional change.

These inconsequential changes in DO at 2 m and 16 m corresponded with the oxycline depth being unchanged the year following the hurricanes. In 2020, the fully developed oxycline was, on average, at 13.0 m compared to 13.7 m in 2021 ($p > 0.5$). Therefore, whereas there were clear differences in DO between years at certain depths and for certain months (Table 3.1), the lack of consistent directional change suggests that these differences could not be attributed to the storm events. In both 2020 and 2021, stratification driven

anoxia was fully established by May and water column mixus and reoxygenation of the full water column occurred in late October or early November (Fig. 3.2A).

Similar to DO, annual mean surface water temperature was not significantly different between pre (2020) and post (2021) hurricane years (annual mean ± st. dev, 2020= 26.71 ± 1.58 °C, 2021= 26.69 ± 1.59 °C, Table 3.1). While four months (January, July, October, and December) were significantly different in 2021 compared to 2020, as with DO, there was no consistent directional change in surface temperature among months that were different between years. Conversely, in 2021, deep water temperature was significantly cooler (p<0.001) in 2021 compared to 2020 (annual mean ± st. dev, 2020= 25.67 ± 1.28 °C, 2021= 24.94 ± 1.11 °C, Table 3.1). However, as with surface temperatures, the seven months that were significantly different in 2021 compared to 2020 (March, May-August, October, and December) were both significantly cooler and warmer than the previous year (Table 3.1, Fig. 3.2B).

Whereas thermo-physical structure appears to have only been minimally impacted, significant changes in Secchi depth and Chl-a were observed between 2021 and 2020. Annual mean Secchi depth was greater in the year following the hurricanes compared to the year prior to the hurricanes (annual mean ± st. dev, 2020= 2.84 ± 0.93 m, 2021= 3.14 ± 1.20 m, Table 3.1). This was concurrent with (and driven by) a decrease in Chl-a (measured at 1 m) in 2021 compared to 2020 (annual mean ± st. dev, 2020= 5.78 ± 3.90 μgL^{-1}, 2021= 3.51 ± 2.69 μgL^{-1}, Table 3.1). Monthly Secchi depth and Chl-a were significantly different in 8 months (January, February, July to September, and November for both parameters in addition to May and December for Secchi depth and June and October for Chl-a). This difference between years was most apparent in the months immediately following the hurricane when we observed a clear water phase characterized by increased Secchi depth and decreased algal abundance that had not been observed in Lake Yojoa for several years prior (Fig. 3.3). However, directional change was not always as anticipated such as in July and August where Secchi depth was slightly (though significantly, p<0.05) less in 2021 than in 2020 and Chl-a also decreased compared to 2020 levels (at p<0.001 and 0.01 for July and August respectively, Table 3.1).

Similar to observed differences in temperature in surface versus deep water, we observed a greater difference between 2020 and 2021 in deep compared to surface nutrient concentrations. Specifically, we observed significant decreases in the concentrations of three of four measured dissolved analytes (DOC, TP and NH_4^+, but not NO_3^-) at a depth of 16 m. Deep water DOC concentrations were significantly lower in all months of 2021 compared to 2020 (annual mean ± st. dev, 2020= 224.27 ± 75.52 µM, 2021= 206.50 ± 46.90 µM, Table 3.1). Similarly, we measured significant decreases in deep water TP (annual mean ± st. dev, 2020= 1.19 ± 1.21 µM, 2021= 0.70 ± 0.53 µM, Table 3.1) and NH_4^+ (annual mean ± st. dev, 2020= 42.90 ± 33.10 µM, 2021= 17.05 ± 11.97 µM, Table 3.1). Changes in nutrient accumulations at depth were particularly notable when the water column was stratified (Fig. 3.4B, 3.5B and 3.6B). This decrease in the dissolved hypolimnetic C and N pool was accompanied by a corresponding decrease in the particulate pool during stratified months with both particulate C and particulate N concentrations decreasing in the year following the hurricanes (Particulate C during stratified months; mean ± st. dev, 2020= 80.45 ± 25.95 µM, 2021= 69.00 ± 18.82 µM; Particulate N during stratified months; mean ± st. dev, 2020= 17.42 ± 5.09 µM, 2021= 13.27 ± 3.58 µM, Fig. S3.1).

TP in surface waters was also significantly less in 2021 compared to 2020 (annual mean ± st. dev, 2020= 0.72 ± 0.57 µM, 2021= 0.48 ± 0.37 µM, Table 3.1). In contrast, surface concentrations of NO_3^- and NH_4^+increased in 2021 relative to 2020. While the observed increase in NO_3^- was slight (annual mean ± st. dev, 2020= 4.20 ± 5.46 µM, 2021= 5.69 ± 6.86 µM, Table 3.1), we observed a much greater increase in NH_4^+ (annual mean ± st. dev, 2020= 3.67 ± 3.15 µM, 2021= 6.16 ± 7.84 µM, Table 3.1). The change in annual epilimnetic NH_4^+ was largely driven by water column concentrations following mixus the year following the hurricanes wherein NH_4^+concentrations in November and December 2021 significantly (p<0.001, Table 3.1) exceeded 2020 values (Fig 3.3A).

For the surface sediment samples taken in January the year preceding the November hurricanes (2020), nutrient values were consistently higher in the open water sites when compared to the values averaging all sites (Table 3.2). Organic C values show that the surface sediments of Lake Yojoa are high in organic matter (~7%) and a mixture of allochthonous and autochthonous-derived C sources (molar C:N

=14-15). N and P sediment concentrations suggest that substantial nutrient storage has occurred in the lake in modern times and molar N:P ratios show constant values ranging from 16 to 18 (Table 3.2).

3.4 Discussion

Extreme weather events, such as hurricanes, have been known to have wide and varying effects on lake ecosystems (Van Metro et al. 2006; James et al. 2008; Rodusky 2010). Here, we demonstrate that Hurricanes Eta and Iota had an ephemeral dilution effect on Lake Yojoa. While there was an initial decrease in Secchi depth and increase in Chl-a immediately following the storms (Fig. 3.2), this was likely due to the supply of abundant hypolimnetic nutrients to the photic zone directly following mixus, as we have previously documented (Fadum and Hall 2022) and not necessarily related to inputs from the hurricanes. Following this brief perennial algal bloom, Lake Yojoa experienced a clear water period with higher Secchi depth and lower Chl-a in January and February 2021 (two month mean ± st. dev, Secchi depth = 4.76 ± 0.97 m, Chl-a = 2.82 ± 1.59 μgL^{-1}, Fig. 3.3). This water column clearing was not observed in the year preceding the hurricane or in observations made in Lake Yojoa in previous years (Fadum and Hall 2022). As such, the anomalous clear phase suggests that the combination of the two storms had a nutrient diluting rather than a loading effect on Lake Yojoa. This dilution effect is further supported by the observed decrease in hypolimnetic water temperatures after the onset of stratification (Fig. 3.2), possibly due to the subduction of cooler contributing waters, and decreased hypolimnetic nutrient concentrations for three of the four measured dissolved analytes; NH_4^+, TP, and DOC but not NO_3^- (which was low in both years, Fig 3.4B, 3.5B and 3.6B). The decrease in the dissolved pool can be explained by decreases in hypolimnetic particulate nutrients in 2021 compared to 2020, resulting in reduced mineralization during the stratified season.

Despite lower nutrient concentrations in the hypolimnion preceding water column turnover in November 2021, concentrations of NH_4^+, TP and DOC in surface waters following water column mixus were equal to or in excess of observed values in November 2020. This suggests that the dissolved nutrient pool in the hypolimnion is not the only source of nutrients to the epilimnion directly following mixus. Instead, it is likely that Lake Yojoa's sediments are a more important contributor to internal nutrient cycling.

Observed sediment nutrient efflux rates vary substantially across systems (Austin and Lee 1973; Heinen and McManus 2004; Nowlin, Evarts and Vanni 2005; Li et al. 2008; Hou et al. 2021), but are often important contributors to whole-lake biogeochemistry such as in Lake Malawi where NH_4^+ efflux (0.44 mmol m− 2 d− 1) contributes up to 29% of total N inputs to the water column (Li et al. 2018). Furthermore, Lake Yojoa may experience even higher sediment nutrient efflux in regions impacted by aquaculture similar to what has been observed in Lake Kariba where sediments located near aquaculture cages have a 4-25 fold increase in NH_4^+ and PO_4^{-3} efflux (Troell and Berg 1997) compared to unimpacted sediments. Compared to Lake Kariba's aquaculture impacted sediments (2.8-4.4% C, 0.26-0.49% N), Lake Yojoa's sediment is enriched in C and N (1.19-11.56% C, 0.07- 1.10% N). In addition, despite Lake Yojoa's mesotrophic state, N and P in surface sediments were comparable to hypereutrophic lakes (Schelske et al. 2005; Wu et al. 2019; Waters et al. 2021) showing that a substantial amounts of both N and P could be internally loaded during mixing. For example, Lake Apopka, USA (with similar TP concentrations in surface sediments as Lake Yojoa, ~1.0 mg g^{-1}) is a shallow system known to maintain Chl-a values above 100 µg L^{-1} (compared < 10 µg L^{-1} in Lake Yojoa, Table 3.1) that are supported by sediment mixing and internal loading despite management of external nutrient inputs (Bachmann, Hoyer and Canfield 2001; Schelske et al. 2005). A system more comparable to Lake Yojoa in depth location, and origin, Lake Amatitlán, Guatemala (max depth = ~33) has a higher trophic state (Secchi depth= 0.8-1.0 m) but has similar surface sediment N (~0.63 % in Lake Amatitlán) and TP (1.17 mg g^{-1} in Lake Amatitlán) to Lake Yojoa (Waters et al. 2021).

Following the principles of Fick's first law (Paul et al. 2014), we can infer that compared to 2020, when overlying waters had a higher nutrient concentration, water column mixus in 2021 would have initiated a greater nutrient flux from Lake Yojoa's sediments due to a larger concentration gradient between the water column and the surface sediment pool. This may have resulted in an increased efflux of N and P from the sediments in 2021 compared to 2020 and previous years making up for the observed hypolimnetic water column deficit in 2021 compared to 2020 and resulting in comparable mixed water column N and P values between the two years.

Beyond assessing the impact of large, late-season precipitation events on an inland tropical lake, Hurricanes Eta and Iota presented a unique opportunity to understand the impact of decreased hypolimnetic nutrient concentrations under consistent stratification phenology in Lake Yojoa. Given Lake Yojoa's relatively short residence time (less than 1.5 years), and the timing of such anomalous precipitation events during expected annual turnover, the dilution event created by Hurricanes Eta and Iota offers a proxy for temporarily decreased nutrient loading. This natural experiment demonstrates that short-lived nutrient reduction may have only an ephemeral impact on trophic state. The recovery of epilimnetic nutrient concentrations following turnover, despite significantly lower dissolved nutrient concentrations in the hypolimnion in the preceding months, highlights the importance of other often unmeasured nutrient sources such as sediment flux from legacy nutrient inputs, annual autochthonous loading, and algal entrainment (Welch et al. 1992; Krivtsov, Sigee and Bellinger 2001; Carey and Rydin 2011; Hou et al. 2013; Henderson 2019). Our observations in Lake Yojoa are in line with a review of 35 case studies in which recovery of lakes spanning subtropical and temperate latitudes were monitored following nutrient loading reductions. Most lakes reached a new TP equilibrium in 5 to 10 years following nutrient reductions (Jeppesen et al. 2005). However, with some exceptions (Jensen et al. 1992), deep lakes saw an increase in total N concentrations in years following nutrient reduction, despite decreased N loading (Jeppesen et al. 2005). As such, long-term monitoring of Lake Yojoa is needed to understand the lasting impacts of a treatment (such as reduced nutrient loading). While likely not impossible, the single season benefit of nutrient dilution observed in Lake Yojoa shows that reversal of the ongoing eutrophication is likely to take years to recover to a trophic state similar to what had been observed in the early 1980's (Fadum and Hall 2022).

3.5 Conclusion

Hurricanes Eta and Iota had a diluting effect on the nutrient concentration and trophic state of Lake Yojoa. Despite the intensity and proximity Hurricanes Eta and Iota, the Lake Yojoa ecosystem was not permanently impacted by the hurricanes, at least in terms of trophic state. This suggests that Lake Yojoa may be resilient to future extreme weather events. Additional studies of other large, inland lakes will help to further identify which ecosystem characteristics, beyond sediment nutrients, provided Lake Yojoa's

resiliency to hurricane disturbance. Likewise, understanding similar disturbance response in other lake ecosystems with gradually increasing trophic states may provide insights into the potential effectiveness of nutrient reduction strategies. Ultimately, many lakes will, under future climate scenarios, bare the weight of greater storm intensity interfaced with cultural eutrophication. A mechanistic understanding of these ecological stressors may help to maintain the ecosystem services that local communities depend upon.

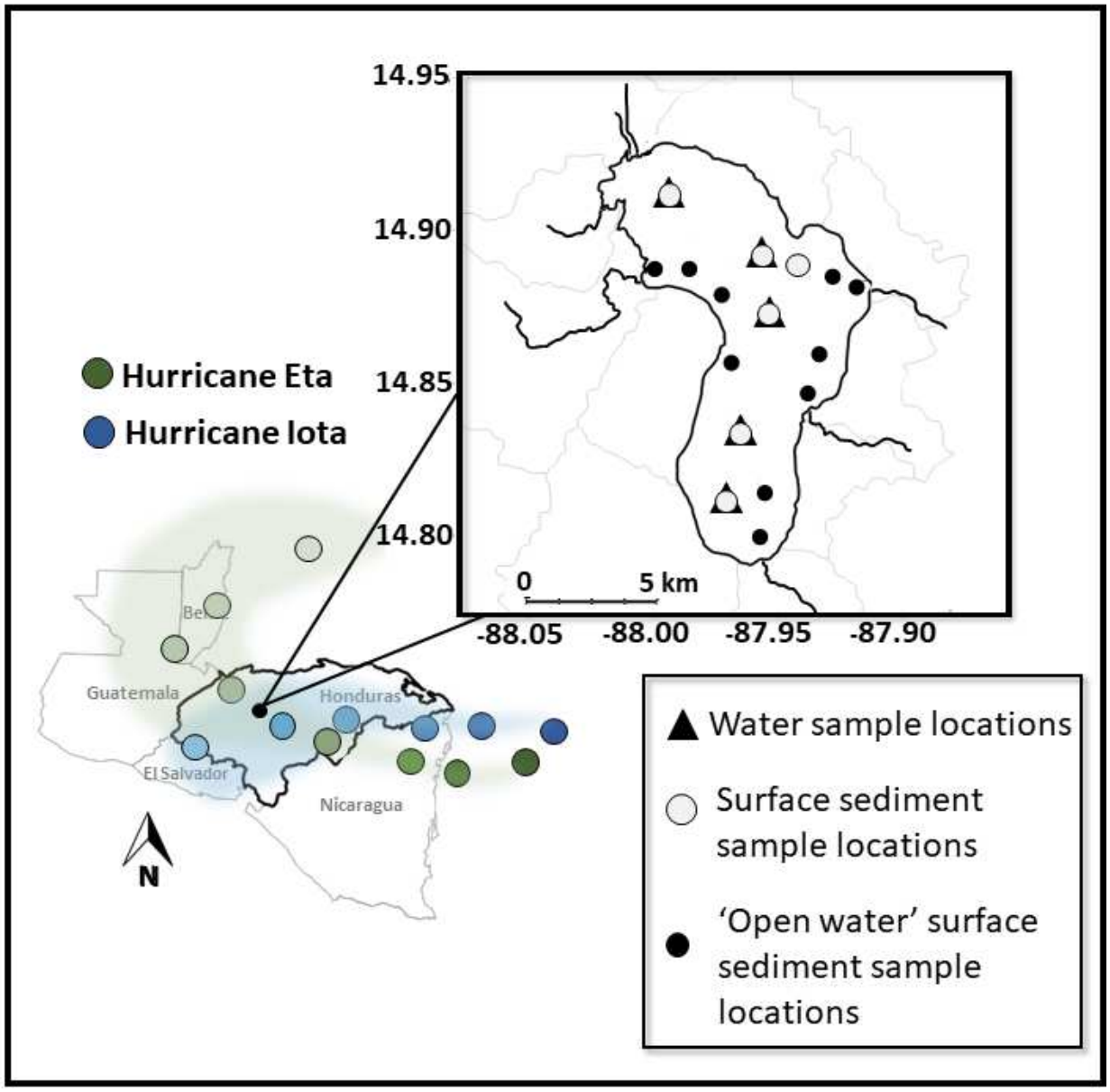

Figure 3.1. Sampling locations (water column and surface sediments) in Lake Yojoa and approximate paths of Hurricanes Eta and Iota

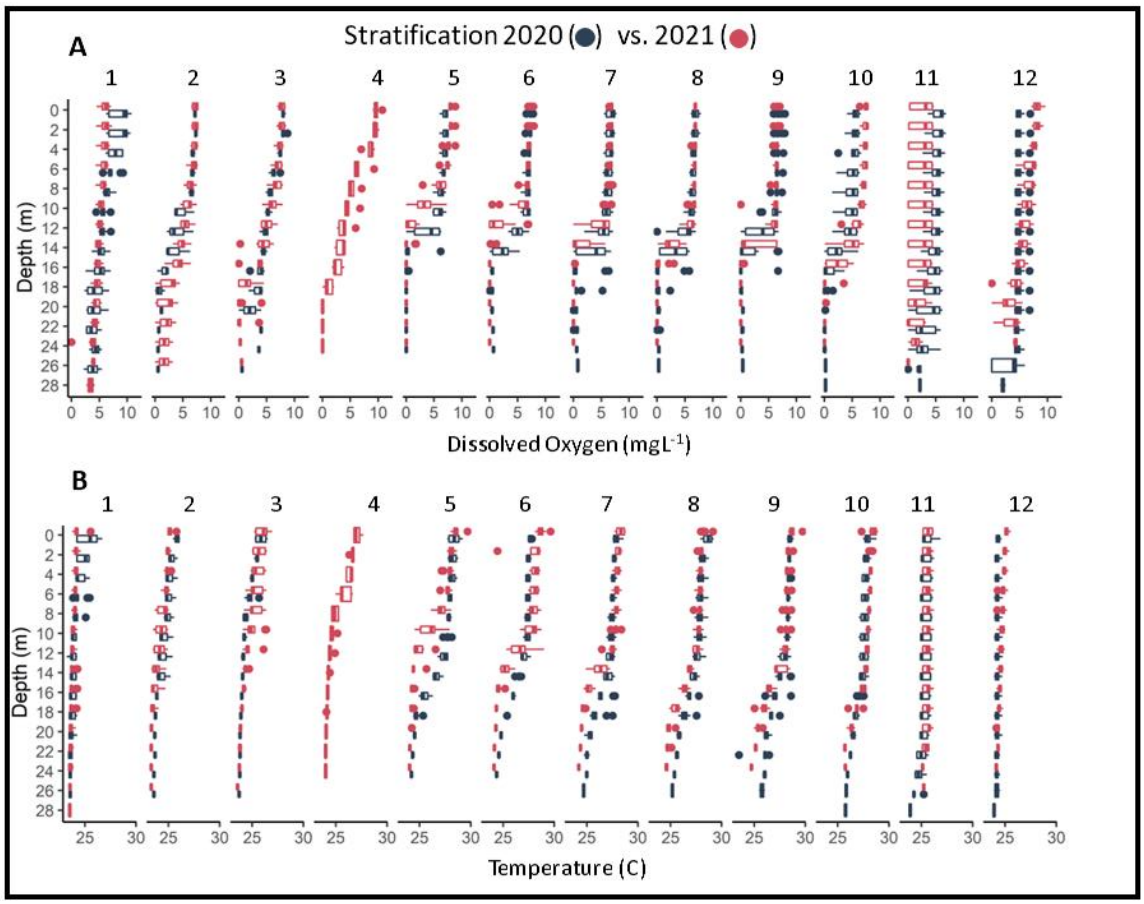

Figure 3.2. Profiles of monthly **(A)** dissolved oxygen and **(B)** temperature (minimum, maximum, IQR with outliers at 2 m intervals)

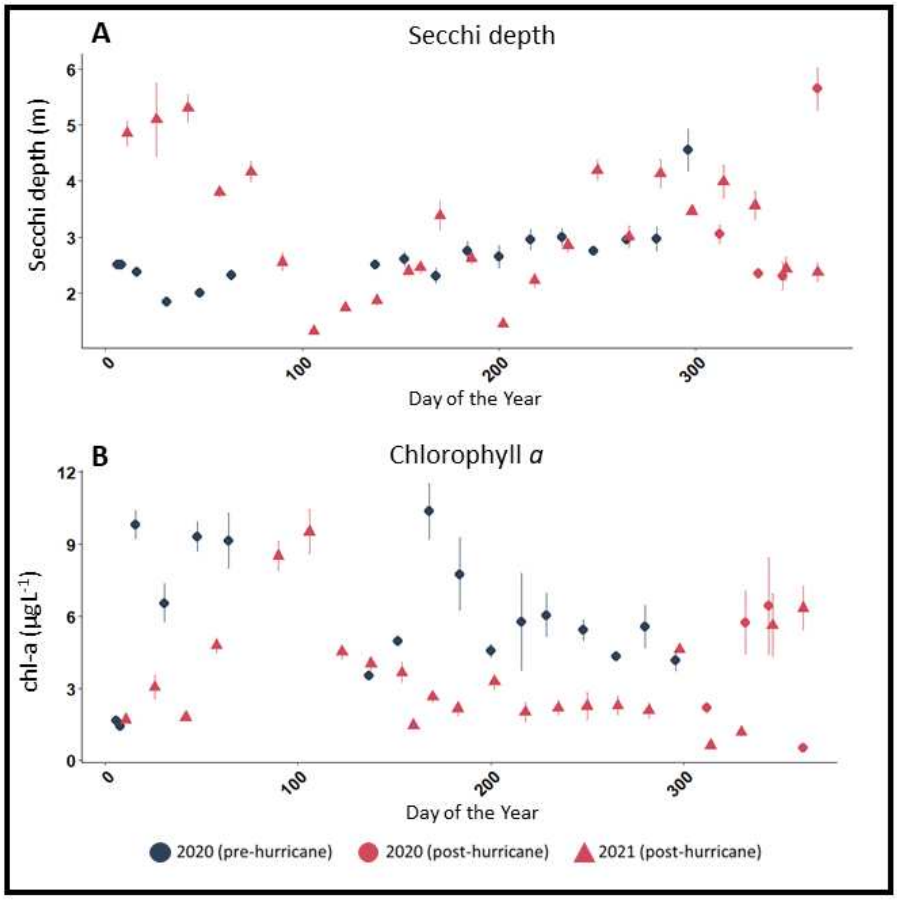

Figure 3.3 (A) Secchi depth and **(B)** Chl-a at five continuously monitored in-lake locations (mean ± st.).

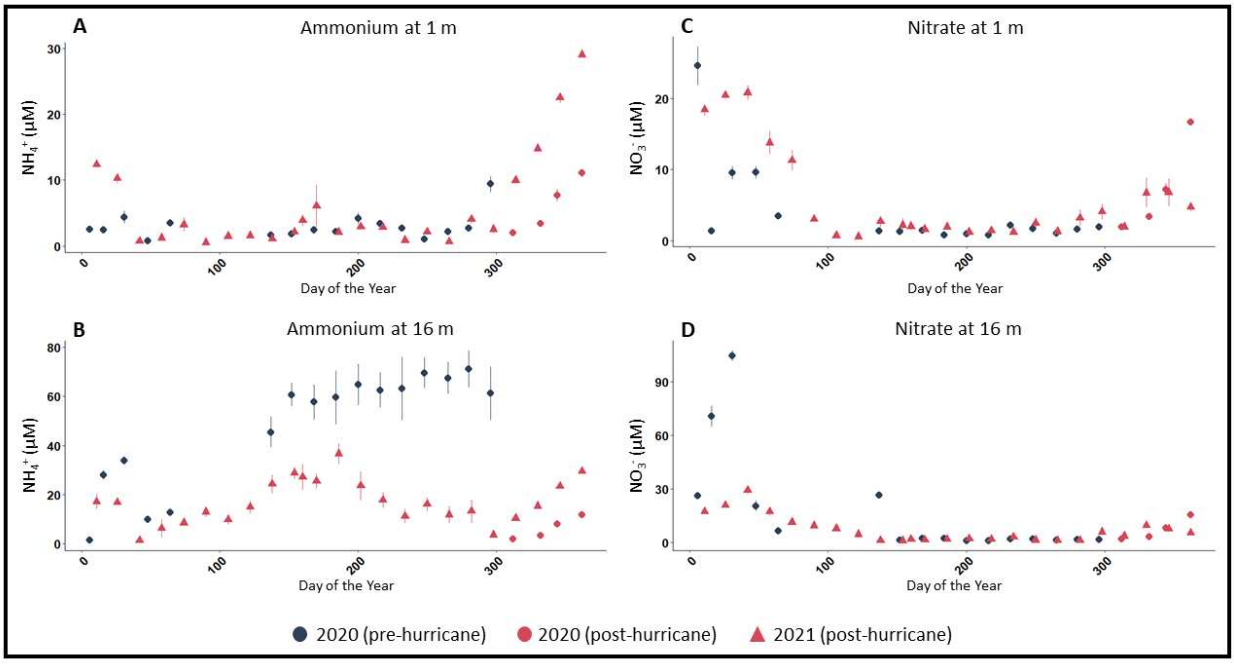

Figure 3.4 (A) Surface NH_4^+, **(B)** deep NH_4^+, **(C)** surface NO_3^-, and **(D)** deep NO_3^- at five continuously monitored in-lake locations (mean ± st.).

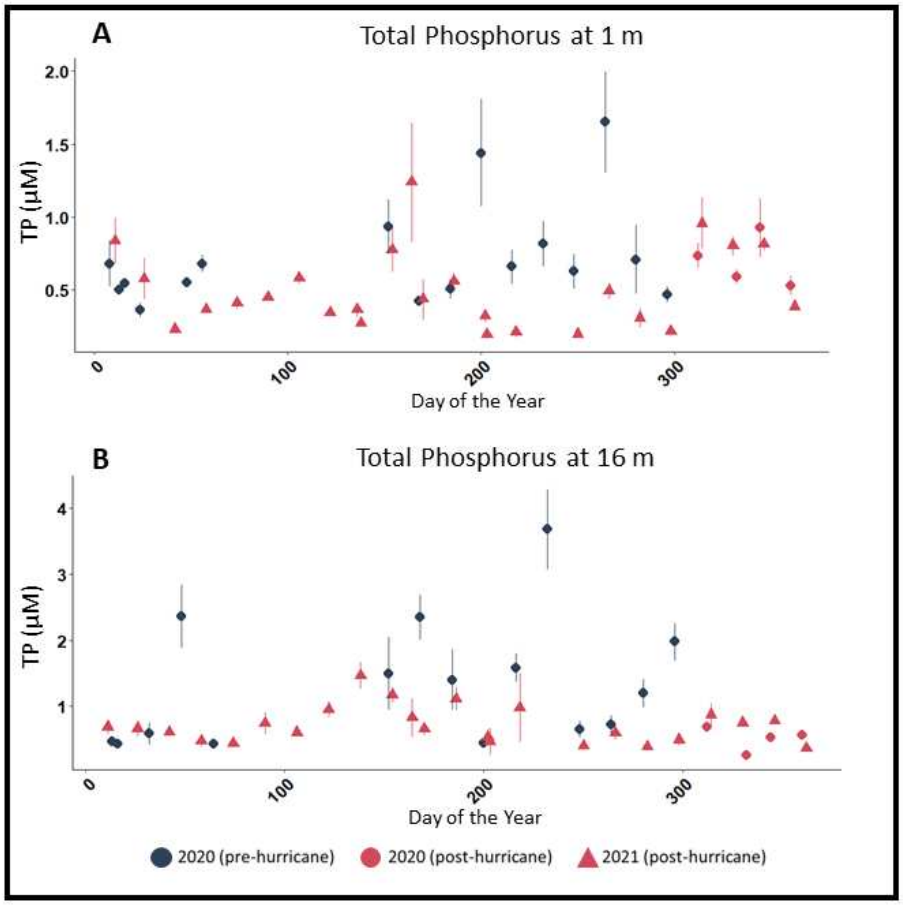

Figure 3.5 (A) Surface TP, and **(B)** deep TP at five continuously monitored in-lake locations (mean ± st.).

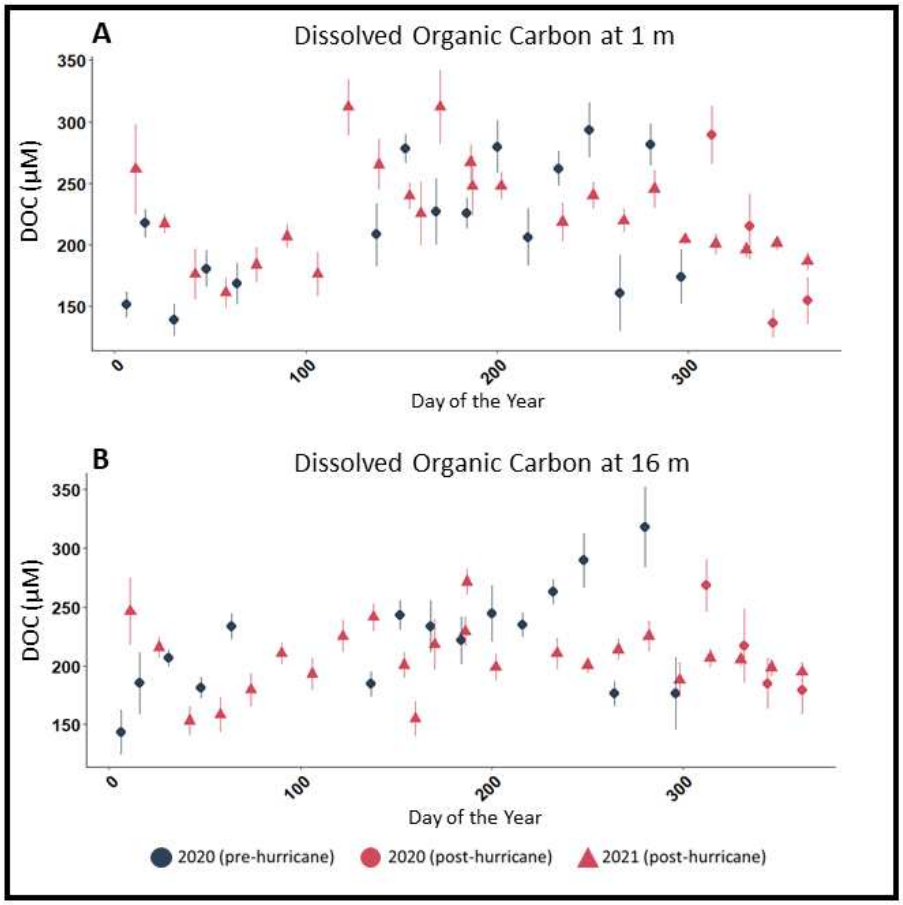

Figure 3.6 (A) Surface DOC, and **(B)** deep DOC at five continuously monitored in-lake locations (mean ± st.).

Table 3.1. Monthly and annual mean ± st. dev of thermo-physical structure and nutrient parameters.

		Stratified Period												
		January	February	March	April	May	June	July	August	September	October	November	December	**Annual mean**
Secchi depth (m)	2020	2.22 ± 0.32	2.00 ± 0.00	2.31 ± 0.31	NA	2.55 ± 0.19	2.30 ± 0.32	2.69 ± 0.41	2.97 ± 0.36	2.85 ± 0.17	3.75 ± 1.06	2.70 ± 0.46	3.97 ± 1.89	**2.84 ± 0.93**
	2021	4.97 ± 1.05 ***	4.55 ± 0.88 ***	3.35 ± 0.92	1.31 ± 0.13	1.79 ± 0.16 ***	2.78 ± 0.61	2.02 ± 0.64 *	2.53 ± 0.43 *	3.59 ± 0.74 **	3.79 ± 0.51	3.77 ± 0.62 ***	2.40 ± 0.41 *	**3.14 ± 1.20 ***
Chl-*a* (μgL^{-1})	2020	6.84 ± 3.63	9.30 ± 1.96	9.12 ± 3.56	NA	4.19 ± 0.93	10.35 ± 3.66	6.15 ± 3.73	5.9 ± 4.86	4.86 ± 1.17	4.85 ± 2.30	3.95 ± 3.44	3.47 ± 5.33	**5.78 ± 3.90**
	2021	2.36 ± 1.36 ***	3.27 ± 1.71 ***	8.49 ± 1.96	9.50 ± 2.91	4.27 ± 0.93	2.74 ± 1.25 ***	2.71 ± 1.08 ***	2.08 ± 1.13 **	2.26 ± 1.53 ***	3.33 ± 1.49 *	0.89 ± 0.51 ***	5.97 ± 3.49	**3.51 ± 2.69 ***
NH_4^+ at 1 m (μM)	2020	3.22 ± 2.20	0.72 ± 0.56	3.50 ± 1.39	NA	1.71 ± 1.31	2.40 ± 1.40	3.27 ± 2.18	3.00 ± 1.18	1.65 ± 1.01	5.85 ± 4.16	2.61 ± 0.90	9.35 ± 2.87	**3.67 ± 3.15**
	2021	11.33 ± 2.21 ***	0.98 ± 0.94	1.78 ± 2.48	1.44 ± 0.56	1.31 ± 1.10	3.95 ± 6.14	2.47 ± 1.02	2.35 ± 1.07	1.44 ± 1.24 *	3.46 ± 1.91 *	12.37 ± 2.88 ***	25.81 ± 3.80 ***	**6.16 ± 7.84 ***
NH_4^+ at 16 m (μM)	2020	21.04 ± 14.85	9.82 ± 3.39	12.83 ± 2.62	NA	55.57 ± 16.14	57.65 ± 21.82	61.99 ± 30.97	62.86 ± 32.03	68.43 ± 19.51	66.76 ± 26.57	2.61 ± 1.06	9.85 ± 2.97	**42.90 ± 33.10**
	2021	17.00 ± 6.52	4.00 ± 8.60	10.78 ± 5.63	9.90 ± 5.92	19.64 ± 10.97 ***	27.27 ± 9.19 ***	30.14 ± 16.67 ***	14.57 ± 9.67 ***	14.00 ± 9.72 ***	9.68 ± 11.96 ***	12.89 ± 3.01 ***	26.50 ± 3.65 ***	**17.05 ± 11.97 ***
NO_3^- at 1 m (μM)	2020	9.28 ± 9.14	9.58 ± 2.63	3.50 ± 1.39	NA	1.34 ± 0.35	1.47 ± 0.56	0.92 ± 0.46	1.51 ± 1.04	1.35 ± 0.49	1.78 ± 0.68	2.67 ± 0.92	11.92 ± 5.18	**4.20 ± 5.46**
	2021	19.39 ± 2.35 *	17.27 ± 5.48 ***	7.17 ± 5.17 *	0.75 ± 0.27	1.65 ± 1.6	1.94 ± 1.93	1.60 ± 0.75 **	1.33 ± 0.47	1.88 ± 1.75	3.65 ± 3.45 *	4.34 ± 5.19	5.76 ± 4.36 ***	**5.69 ± 6.86 ***
NO_3^- at 16 m (μM)	2020	67.19 ± 34.19	20.48 ± 6.89	6.47 ± 1.62	NA	9.75 ± 12.25	2.32 ± 1.77	1.67 ± 3.58	1.55 ± 1.14	1.66 ± 0.51	1.69 ± 0.62	2.61 ± 1.06	11.54 ± 4.65	**10.10 ± 21.27**
	2021	19.17 ± 3.05 ***	23.37 ± 9.25	10.43 ± 4.79	7.90 ± 2.70	2.98 ± 4.85 *	1.42 ± 0.81 *	2.09 ± 1.17	2.51 ± 1.89	1.26 ± 0.60 *	3.54 ± 3.82 *	6.69 ± 5.57 **	6.54 ± 4.11 **	**7.15 ± 8.13**
TP at 1 m (μM)	2020	0.48 ± 0.19	0.61 ± 0.15	NA	NA	0.93 ± 0.58	0.42 ± 0.08	0.97 ± 0.93	0.73 ± 0.42	1.13 ± 0.95	0.58 ± 0.49	0.66 ± 0.22	0.72 ± 0.50	**0.72 ± 0.57**
	2021	0.70 ± 0.47 *	0.29 ± 0.08 ***	0.43 ± 0.10	0.58 ± 0.13	0.32 ± 0.09 ***	0.75 ± 0.66	0.35 ± 0.21 **	0.21 ± 0.10 ***	0.34 ± 0.19 ***	0.26 ± 0.16 *	0.88 ± 0.41 *	0.60 ± 0.24	**0.48 ± 0.37 ***
TP at 16 m (μM)	2020	0.45 ± 0.12	1.47 ± 1.19	0.43 ± 0.09	NA	1.49 ± 1.73	2.35 ± 1.03	0.91 ± 1.10	2.63 ± 1.75	0.68 ± 0.39	1.58 ± 0.85	0.47 ± 0.26	0.54 ± 0.15	**1.19 ± 1.21**
	2021	0.67 ± 0.31 *	0.54 ± 0.22 **	0.59 ± 0.36	0.60 ± 0.20	1.20 ± 0.56	0.89 ± 0.47 ***	0.71 ± 0.56	0.97 ± 1.64 *	0.50 ± 0.26	0.44 ± 0.21 ***	0.81 ± 0.39 **	0.57 ± 0.26	**0.70 ± 0.53 ***
DOC at 1 m (μM)	2020	171.09 ± 50.25	180.47 ± 46.21	168.26 ± 52.36	NA	249.39 ± 61.24	226.90 ± 84.93	253.98 ± 60.37	234.07 ± 65.80	226.95 ± 106.94	230.45 ± 80.29	252.06 ± 85.66	145.21 ± 48.60	**214.14 ± 78.62**
	2021	239.20 ± 84.36	168.62 ± 52.32	195.16 ± 39.55	175.99 ± 56.49	288.38 ± 71.23	264.08 ± 77.37	253.67 ± 53.33	218.30 ± 45.75	229.84 ± 32.39	224.85 ± 39.88	198.31 ± 21.59	193.86 ± 19.94	**225.26 ± 63.60**
DOC at 16 m (μM)	2020	178.25 ± 47.61	181.25 ± 19.72	233.31 ± 24.86	NA	222.15 ± 42.92	233.22 ± 69.68	232.75 ± 69.43	249.28 ± 34.39	232.73 ± 79.67	247.13 ± 123.91	244.03 ± 84.57	181.99 ± 64.43	**224.27 ± 75.52**
	2021	230.90 ± 66.12 **	155.59 ± 41.68 **	194.25 ± 39.55 **	192.89 ± 44.22	233.04 ± 40.27 **	195.85 ± 52.05 **	232.96 ± 46.66 **	210.42 ± 40.76 **	207.05 ± 24.00 **	206.52 ± 45.87 **	205.97 ± 20.47 **	196.52 ± 22.26 **	**206.50 ± 46.90 **
Temperature at 2 m (C)	2020	24.84 ± 0.61	25.33 ± 0.50	25.44 ± 0.19	NA	28.22 ± 0.38	27.46 ± 0.22	27.66 ± 0.17	28.14 ± 0.37	28.42 ± 0.13	27.78 ± 0.28	25.40 ± 0.49	24.25 ± 0.23	**26.71 ± 1.58**
	2021	24.15 ± 0.24 **	24.94 ± 0.19	25.67 ± 0.63	26.60 ± 0.18	28.11 ± 0.29	27.97 ± 1.12	28.09 ± 0.26 ***	27.99 ± 0.19	28.35 ± 0.23	28.26 ± 0.10 ***	25.58 ± 0.43	25.01 ± 0.22 ***	**26.69 ± 1.59**
Temperature at 16 m (C)	2020	23.78 ± 0.31	23.87 ± 0.15	23.95 ± 0.09	NA	25.50 ± 0.48	26.00 ± 0.17	26.53 ± 0.60	26.94 ± 0.46	27.05 ± 0.67	27.13 ± 0.19	25.25 ± 0.46	24.25 ± 0.25	**25.67 ± 1.28**
	2021	23.76 ± 0.24	23.72 ± 0.51	24.19 ± 0.13 ***	24.19 ± 0.02	24.25 ± 0.09 ***	24.57 ± 0.27 ***	25.19 ± 0.38 ***	26.39 ± 0.35 **	26.51 ± 0.35	27.47 ± 0.29 *	25.54 ± 0.42	24.47 ± 0.18 *	**24.94 ± 1.11 ***
Dissolved Oxygen at 2 m (mgL^{-1})	2020	8.46 ± 1.84	7.22 ± 0.11	8.03 ± 0.48	NA	7.04 ± 0.59	7.10 ± 0.40	6.62 ± 0.78	6.80 ± 0.61	6.74 ± 0.56	5.47 ± 0.84	5.40 ± 1.18	5.00 ± 0.85	**6.49 ± 1.37**
	2021	6.00 ± 0.86 **	7.18 ± 0.51	7.66 ± 0.59	9.54 ± 0.49	8.04 ± 0.36 ***	7.31 ± 0.26	6.85 ± 0.19	6.83 ± 0.23	6.54 ± 0.32	7.30 ± 0.54 ***	2.34 ± 1.98 ***	8.17 ± 0.70 ***	**6.79 ± 1.86**
Dissolved Oxygen at 16 m (mgL^{-1})	2020	4.98 ± 1.52	1.56 ± 1.00	3.68 ± 1.17	NA	0.04 ± 0.14	0.38 ± 0.25	1.38 ± 2.44	1.10 ± 2.24	0.67 ± 2.12	0.95 ± 1.34	4.54 ± 1.49	4.93 ± 0.86	**2.34 ± 2.45**
	2021	4.73 ± 0.63	4.11 ± 1.31 *	3.16 ± 1.70	2.67 ± 0.94	0.00 ± 0.00	0.00 ± 0.00 ***	0.02 ± 0.07	0.58 ± 1.09	0.07 ± 0.20	2.39 ± 2.28	2.19 ± 1.97 **	4.98 ± 1.02	**2.02 ± 2.19**

Significant Differences (2020 vs. 2021): *** (0.001) ** (0.01) * (0.05)

Table 3.2. Mean ± st. dev of surface sediment samples collected from Lake Yojoa prior to hurricane impacts.

	Depth (m)	C (%)	N (%)	P (mg g^{-1})	C:N**	N:P**
Total*	17.3 ± 6.5	6.20 ± 3.2	0.58 ± 0.33	0.83 ± 0.4	14.1 ± 5.8	16.8 ± 8.9
Open Water	21.5 ± 3.0	7.85 ± 3.0	0.71 ± 0.34	1.05 ± 0.51	15.9 ± 8.8	18.8 ± 11.0

*Total samples include surface sediments collected near locations with various land use impacts.
**Nutrient ratios are molar ratios.

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CHAPTER 4: DOMINANT NITROGEN METABOLISMS OF A TROPICAL, MONOMICTIC LAKE IDENTIFIED USING GENOME RESOLVED METATRANSCRIPTOMICS[4]

4.1 Introduction

Inland waters respond to climate change in varied and complex ways (Williamson et al. 2009; Adrian et al. 2009; Goldman et al. 2012; Dokulil 2014; Gilarranza et al. 2022). As these ecosystems react on individual and regional levels, the cumulative impact of these changes could have global consequences due to the pivotal role that aquatic ecosystems play in global biogeochemical cycles (Tranvik et al. 2009; Bastviken et al. 2011; Panneer Selvam et al. 2014; Zheng et al. 2022). Furthermore, the rate at which individual ecosystems are changing in response to external stressors (whether global change or local impacts) often exceeds the pace at which we are establishing an understanding of current conditions, making it difficult to understand mechanisms of these changes (Williams et al. 2020).This knowledge lag is especially prevalent in low-latitude inland waters where a deficit of continuous data limits the possibility of distinguishing between intra-annual variability and inter-annual directional change (Bender et al. 2010; Ramírez et al. 2020; Vichot-Llano et al. 2021, Fadum and Hall, 2022).

Driving observable ecological changes are innumerable underlying microbially mediated biogeochemical processes (Kirchman 2018). While microbial membership alone is generally not a viable tool for assessing the microbial contribution to ecosystem function, with some exceptions (Abellán et al. 2006; Santiago-Rodriguez et al. 2012), assessing the emergent properties of the microbiome can help to better link microbial physiology to ecosystem response (Hall et al. 2018; Cordeiro et al. 2019; Premke et al. 2022). Multi-omics approaches (Hu et al. 2021) offer unparalleled resolution of the metabolic pathways that drive biogeochemical trends in aquatic ecosystems. This rapidly evolving suite of tools are critical in

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narrowing the knowledge gap of which microbial mechanisms drive differential responses of temperate and tropical aquatic ecosystems to global change (Nuñez et al. 2021).

Biogeochemical cycling in monomictic tropical lakes is distinct from that in their temperate counterparts, in part, due to high (>20 °C) temperatures of the anoxic hypolimnion during stratified months (Lewis 1996). These warm anoxic conditions dramatically alter ecosystem biogeochemistry via anaerobic metabolisms (Lewis 2010). Though recent work has gained insight into the microbial ecology of permanent anoxic zones in the open ocean (Ulloa et al. 2012; Babbin et al. 2017; Zakem et al. 2019, 2021), and in anoxic waters of temperate and arctic lakes (Cabello-Yeves et al. 2020; Cabrol et al. 2020; Vigneron et al. 2021), microbial mediated biogeochemistry of warm inland waters that sustain anoxia for all, or parts, of the year remain less well described (with some notable exceptions, e.g., Pajares et al. 2017, Tran et al. 2021).

To elicit a more complete understanding of the microbial drivers of tropical lake biogeochemistry, we analyzed the metatranscriptome of water column microbes in Lake Yojoa, Honduras. Lake Yojoa is a large monomictic tropical lake with documented trophic state increase most likely due to excess nutrient loading from net-pen aquaculture over the past 25 years (Fadum and Hall 2022). Notably, Lake Yojoa's mesotrophic state is, in part, maintained by the accumulation of ammonium (NH_4^+) in the hypolimnion during the stratified months of the year followed by release to the photic zone during mixing (Fadum and Hall 2022, Fadum, Waters and Hall *in review*). The accumulated NH_4^+ is converted to nitrate (NO_3^-) in the oxic, photic zone in a matter of weeks and consumption of NO_3^- by the phytoplankton maintains an elevated trophic state over the following months of the dry season, when watershed nutrient inputs are low. These months (typically November to March) previously had a clearer (~8 m Secchi depth) and more oligotrophic water column (Fadum and Hall 2022). This phenomena has also been documented in other monomictic tropical lakes such as Lake Titicaca, Peru-Bolivia (Vincent et al. 1984; Wurtsbaugh et al. 1985), Valle de Bravo Reservoir, Mexico (Merino-Ibarra et al. 2008) and Lake Manasbal, India (Yaseen and Bhat 2021). As such, here we explore the presence or absence of known microbial pathways which give rise to this pool of hypolimnetic inorganic nitrogen (N) and thus drive ecosystem scale observable changes.

Elemental fluxes, such as that of hypolimnetic nutrients, can be driven by changes in microbial membership which drive changes to community aggregated traits, or emergent properties of the microbiome that can determine how much the microbiome contributes to a given flux (Hall et al. 2018). Changes in microbial community properties ultimately define the microbial contribution to ecosystem processes, such as through known N metabolic pathways. Despite the complexity of microbial N metabolism and its relationship with other elemental cycles (Gruber and Galloway 2008; Wasmund et al. 2017; Kuypers et al. 2018b; Rodríguez-Ramos et al. 2022), few studies have used a multi-omics approach to disentangle ecosystem-scale N cycling in aquatic systems or to better understand specific N transformation pathways in tropical waters outside of marine systems (Turk et al. 2011; Beman et al. 2013; Jayakumar et al. 2017, among others). Therefore, we aim to further our understanding of biogeochemistry in tropical waters by identifying the microbial community properties associated with Lake Yojoa's dominant N metabolisms and associated nutrient fluxes using a genome resolved metatranscriptomic approach.

We hypothesize that, in addition to mineralization, dissimilatory nitrate reduction to ammonium (DNRA) is responsible for the observed accumulation of NH_4^+ in the anoxic hypolimnion during the stratified months. DNRA is a two-step transformation involving NO_3^- reduction to NO_2^- (respiratory) followed by NO_2^- reduction to NH_4^+ (chemolithotrophic/respiratory or fermentative- both facultative and obligate) without assimilation into microbial biomass. Based on thermodynamics, DNRA may be energetically preferrable to denitrification, the proposed but contended dominant inorganic N loss pathway in aquatic ecosystems (Burgin and Hamilton 2007), when electron donors (organic carbon or reduced inorganic compounds such as sulfide) are in greater supply relative to electron acceptors (NO_3^-) (Takai 2019). In addition to competition between DNRA and denitrification pathways, fermentive bacteria also compete for electron donors (organic carbon) and thereby alter the substrate pool also required by NO_3^- reducers (van den Berg et al. 2017).

Though sediment associated DNRA research (Smith et al. 2007; Decleyre et al. 2015; Yin et al. 2017, among others) outnumbers studies of DNRA in the water column, several studies have identified DNRA to be a significant NO_2^- reduction pathway in the water column. Such studies range from estuaries

(Dong et al. 2011), to lagoons (Broman et al. 2021), to marine ecosystems (Bonaglia et al. 2016; Pavlovska et al. 2021), with few comparable freshwater studies. However, it is important to balance understandings of tropical N microbial metabolism with comparable studies in freshwater ecosystems due to documented differences in metabolic function between marine and freshwater communities (Eiler et al. 2014). To address this knowledge gap created by the dominance of sediment associated DNRA studies and its confluence with the under-representation of tropical freshwater ecosystems in the literature, the following study sampled Lake Yojoa at two depths (1 m and 16 m) during both the stratified water column (June 2021) and mixed water column (January 2022) seasons at three distinct locations (Fig. 4.1A). The objective was to identify seasonal and spatial variation in gene expression of various NO_2^- reduction pathways (namely DNRA, denitrification, and anaerobic ammonium oxidation, anammox). Variation in gene expression was then considered in the context of seasonal changes in geochemical and thermophysical parameters to determine the depth discrete, seasonally variable importance of DNRA to ecosystem N metabolism and, more specifically, to Lake Yojoa's hypolimnetic NH_4^+ accumulation.

4.2 Methods

4.2.1 Lake Yojoa

Lake Yojoa (~ 83 km2 surface area, 1.4 km3 volume, 27.3 m annual average max depth) is located in the center of a ~337 km2 mixed land use/landcover watershed in West-Central Honduras. Located between Tegucigalpa and San Pedro Sula, Honduras's two largest cities, the watershed supports the local economy largely through ecotourism through visits to the lake and the two national parks (Parque Nacional Cerro Azul and Parque Nacional Montaña de Santa Bárbara) and Lake Yojoa. Additionally, the lake supports wild fisheries as well as one large and two much smaller industrial aquaculture operations. The watershed has persistently warm temperatures (annual average air temperatures above 20 ° C) with the warmest months corresponding with the monsoon season (June to October).

4.2.2 Field Sampling

In June of 2021 and January of 2022, we collected water for nutrient analysis (NH_4^+, NO_3^-, total phosphorus, dissolved organic carbon) from three stations (Fig. 4.1) at 1 and 16 m using an opaque Van

Dorn water sampler. Water was immediately placed in a cooler in opaque HDPE Nalgene bottles until we returned to the laboratory. At each station, temperature, dissolved oxygen (DO), and pH profiles were measured at two-meter intervals using a Hydrolab MS5 Multiparameter Sonde (OTT HydroMet, Loveland, CO). For DNA and RNA sample collection, 100-300 ml water samples were concentrated onto 0.22-µm pore size Sterivex filters (Sigma Aldrich Cat. # SVGP01050) using sterile 60 ml luer-locking syringes. Water was passed through filters until filter was at capacity. Filters were then quickly purged with air to remove excess water and 3 ml of RNALater (Sigma Aldrich Cat. # R0901500ML) was added. Sealed Sterivex filters were then placed in individual whirl-paks and placed in a cooler.

Once off the boat, whole water samples were frozen for TP analysis while NH_4^+, NO_3^- and DOC samples were first filtered through 25 mm glass microfiber filters, Grade GF/F via Polysulfone filter funnels and subsequently frozen. Water samples, stored in 15 ml centrifuge tubes, and filters were transported to Fort Collins, CO, USA while frozen. Nutrient analyses were performed at the EcoCore facility at Colorado State University, Fort Collins, as described below.

4.2.3 Laboratory geochemical analysis

Total phosphorus (TP) was first digested to soluble reactive phosphorus (SRP) via the Alkaline Potassium Persulfate method under sub-boiling (90 °C) temperature, then measured using a colorimetric ascorbic acid assay (EPA Method 365.3 in U.S. Environmental Protection Agency 1983) modified to be analyzed on a UV-STAR 96-well Microplate via Infinite M200 TECAN at 880 nm absorbance detection. NH_4^+ and NO_3^- were measured using a Flow Solution FS 3700 Automated Chemistry Analyzer (O.I. Analytical, College Station, TX). Dissolved organic carbon (DOC) was measured on a Shimadzu TOC-L (Shimadzu Scientific Instruments, Inc).

4.2.4 Extraction and sequencing

For each sampling trip (June 2021 and January 2022), one sample for each location (North, Central and South) and each depth (1 m and 16 m) was extracted for DNA (n =6 per sampling trip). RNA samples were sequenced at the same locations and depths in triplicate. DNA and RNA were coextracted using ZymoBIOMICS DNA/RNA Miniprep Kit (Zymo Research Cat. # R2002) coupled with RNA Clean &

Concentrator-5 (Zymo Research Cat. # R1013). Samples were eluted in 40 µL and stored at −20 °C until sequencing.

4.2.5 Metagenomic assembly, binning, and annotation

Genomic DNA was prepared for metagenomic sequencing using Plate-based DNA library preparation on the PerkinElmer Sciclone NGS robotic liquid handling system at the Joint Genome Institute. Briefly, one nanogram of DNA was fragmented and adapter ligated using the Nextera XT kit (Illumina) and unique 8bp dual-index adapters (IDT, custom design). The ligated DNA fragments were enriched with 12 cycles of PCR and purified using Coastal Genomics Ranger high throughput agarose gel electrophoresis size selection to 450-600bp. The prepared libraries were sequenced using Illumina NovaSeq sequencer following a 2x150nt indexed run recipe.

Resulting fastq files were assembled and binned using the GROWdb pipelines. Briefly, three assemblies were performed on each set of fastq files and binned separately: (1) Read trimming with sickle (v1.33), assembly with megahit (v1.2.9), and binning with metabat2 (2.12.1) (2) Read trimming with sickle (v1.33), random filtering to 25% of reads, assembly with idba-ud (1.1.0), and binning with metabat2 (2.12.1) (3) Bins derived from the JGI-IMG pipeline were downloaded. All resulting bins were assessed for quality using checkM (v1.1.2) and medium and high-quality MAGs with >50% completion and <10% contamination were retained.

For paired June 2021 and January 2022 metagenomes, subassemblies were also performed. Specifically trimmed reads from 12 samples were individually mapped to medium and high-quality MAGs derived from the three assembly types described above using bbmap (perfectmode=t). Unmapped reads for each sample were then assembled with idba-ud (1.1.0) and binned with metabat2 (2.12.1). These bins were also assessed for quality using checkM (v1.1.2) and MAGs with >50% completion and <10% contamination were retained in the database. The resulting 1,771 MAGs across all samples and assemblies were dereplicated at 99% identity using dRep (v2.6.2) to obtain the dereplicated Yojoa MAG database (n=552 MAGs). MAG taxonomy was assigned using GTDB-tk (v2.0.0) and annotated using DRAM (Shaffer et al. 2020).

4.2.6 Metatranscriptomic mapping and analysis

RNA was prepared from metatranscriptome sequencing according to JGI established protocols. Briefly, rRNA was removed from 10 ng of total RNA using Qiagen FastSelect probe sets for bacterial, yeast, and plant rRNA depletion (Qiagen) with RNA blocking oligo technology. The fragmented and rRNA-depleted RNA was reverse transcribed to create first strand cDNA using Illumina TruSeq Stranded mRNA Library prep kit (Illumina) followed by second strand cDNA synthesis which incorporates dUTP to quench the second strand during amplification. The double stranded cDNA fragments were then A-tailed and ligated to JGI dual indexed Y-adapters, followed with an enrichment of the library by 13 cycles of PCR. The prepared libraries were quantified using KAPA Biosystems' next-generation sequencing library qPCR kit and run on a Roche LightCycler 480 real-time PCR instrument. Sequencing of the flowcell was performed on the Illumina NovaSeq sequencer following a 2x150nt indexed run recipe.

Resulting fastq files were mapped via Bowtie2 (-D 10 -R 2 -N 1 -L 22 -i S,0,2.50) to the dereplicated Yojoa MAG database. Sam files were transformed to bam files using samtools, filtered to 97% id using reformat.sh and name sorted using sam tools. Transcripts were counted each gene with feature-counts. Counts were transformed to geTMM in R using edgeR package (Robinson et al. 2010).

4.2.7 Statistical analysis and figure generation

Statistical analysis and figure generation were performed primarily in R (version 4.1.2) using vegan (Oksanen 2022) and ggplot2 (Wickham 2016). Significant differences in geochemical and thermophysical structure data between depths and/or seasons were assessed using one-away analysis of variance (ANOVA). Circular dendrograms were created using RAWgraphs (Mauri et al. 2017). Test for taxonomic dissimilarity was performed using LEfSe (Segata et al. 2011).

4.3 Results

4.3.1 Water column structure and geochemistry

In June 2021, Lake Yojoa was stratified with the oxycline at ~12 m depth (with slight variation across the three sampling locations (Fig. 4.2A). In the oxic epilimnion, DO (mean ± st. dev, 6.71 ± 1.09 mgL^{-1}) significantly exceeded ($p< 0.001$) DO at the same depth in January 2022 (5.51 ± 0.83 mgL^{-1}, when

the water column was not stratified). In January, mean DO in the top half of the water column (0-12 m) did not differ from mean DO of the entire water column (5.37 ± 0.82 mgL^{-1}). Despite slight differences in DO concentrations across the three sampling locations (Fig. 4.2A), this evenly distributed DO across depths suggests complete water column mixing in January when Lake Yojoa was isothermal (24.36 ± 0.11 C, across all depths). Conversely, in June, mean temperature in the epilimnion (28.27 ± 0.62 C) was significantly higher (p<0.001) than January surface water temperatures. Temperature in the June hypolimnion (24.70 ± 0.66 C) mirrored January water column conditions (Fig. 4.2B).

In January, when the water column was mixed, there was little variation in measured geochemical parameters between sampling depths (Fig. 4.3A-D). Mean NH_4^+ was 38.01 ± 7.81 µM at 1 m and 39.86 ± 8.29 µM at 16 m. Mean NO_3^- was 15.45 ± 0.61 µM at 1 m and 15.50 ± 0.66 µM at 16 m. Mean DOC was 200.75 ± 17.67 µM at 1 m and 208.75 ± 18.94 µM at 16 m. Mean TP was 0.56 ± 0.08 µM at 1 m and 0.60 ± 0.21 µM at 16 m. There were also few significant differences among sampling locations at the same depth with the exception of NH_4^+, which was significantly (p<0.005) greater at the northern most sampling location at both depths (48.00 ± 0.56 µM at 1 m and 50.20 ± 0.14 µM at 16 m) than at the central (34.00 ± 1.55 µM at 1 m and 37.05 ± 1.34 µM at 16 m) or southern location (32.05 ± 0.07 µM at 1 m and 32.35 ± 0.07 µM at 16 m) (Fig. 4.3A).

In contrast, in June, when the water column was stratified, we observed significant differences in geochemical parameters between the epilimnetic and hypolimnetic strata (Fig. 4.3 E-H). Mean NH_4^+ across all three sampling locations was 3.85 ± 1.84 µM at 1 m and 27.25 ± 12.69 µM at 16 m. Mean NO_3^- was 2.04 ± 1.07 µM at 1 m and 1.79 ± 0.53 µM at 16 m. Mean DOC was 225.26 ± 63.47 µM at 1 m and 154.87 ± 36.40 µM at 16 m. Mean TP was 1.23 ± 0.98 µM at 1 m and 0.83 ± 0.69 µM at 16 m. Hypolimnetic concentrations of NH_4^+ were much greater than in the epilimnion at all three stations, particularly in the south (Fig. 4.3E). DOC concentrations were marginally higher in the epilimnion than in the hypolimnion (Fig. 4.3G). NO_3^- and TP were low both the hypolimnion and the epilimnion and not significantly different between strata (Fig. 4.3F and Fig. 4.3H).

4.3.2 Taxonomy and novelty

Our dereplicated MAG database (n=552) represented 335 different lineages, as defined by the Genome Taxonomy Database toolkit (GTDB-tk) (Chaumeil et al. 2020). Within the GTDB-tk defined lineages (76 classes across 37 phyla), many genomes represent novel taxonomies undefined at the family (4.5%), genus (21.1%), or species (86.5 %) level. Lineages with novel families often followed novel orders (n= 3) or alphanumerically identified orders (n=13). This abundance of novel taxonomy highlights the under-representation of tropical freshwater ecosystems in public genome databases and the degree to which tropical lake microbiomes are still poorly described (Aguilar et al. 2018).

The phyla *Proteobacteria* (n= 97), *Bacteroidota* (n=90), *Planctomycetota* (n=70), *Verrucomicrobiota* (n=66), and *Actinobacteriota* (n= 56) dominated the represented lineages, as may be expected in freshwater lake ecosystems (Newton et al. 2011). In June 2021, surface waters were dominated with *Lyngbya robusta* and *Microcystis wesenbergii*, two common cyanobacteria (Rejmánková et al. 2011; Komárek et al. 2013) as expected under conditions of N limitation (Fadum and Hall 2022). C*yanobacteriia* was the most abundant class during both seasons and across both depths. Interestingly, phyla more frequently identified in marine or saline environments, such as *Thermoproteota* (Ren and Wang 2022) and *Halobacteriota* were also identified. Additionally, taxonomies associated with gut microbiomes including *Fibrobacterota*, *Fusobacteriota*, and *Firmicutes*, were identified, possibly suggesting organic pollution from municipalities, ranching, and/or the industrial aquaculture operation located within the north basin of the lake. At the order level, January and June lineages were highly distinct (Fig. S4.1)

When considering only lineages containing the N cycling genes of interest (Table S4.1), only *Desulfomonilia* distinguishes the microbial community at 1 m compared to 16 m in January (Fig. 4.4). In June, lineages were much more distinct between depths. While only two orders (both from the phylum *Bdellovibrionota*) were unique to the epilimnion in June, >70% of the identified orders were present only in the hypolimnion in June, with all other identified lineages present at both 1 and 16 m (Fig. 4.4)

4.3.3. Seasonal differences in community membership

To assess seasonal differences in community membership, we compared MAG relative abundance across samples and identified consistent differences in membership between June and January (Fig. S4.2). While January membership was similar across locations and depths, June membership at 1 m was distinct from that at 16 m. The differences in microbiome membership between strata in June were likely due to the chemical differences between each strata, and principally, the anoxia at time of sampling (Fig. 4.2A). While hierarchical clustering revealed that epilimnetic membership in June was more similar to surface membership in January, relative to June hypolimnetic membership, the June surface water and January surface water microbiomes were distinct (Fig. S4.1). Differences between surface communities in January and June were undetectable at the phyla level, suggesting that diversity among seasons was structured by lower levels of classification (Fig. 4.5A). Conversely, depth discrete differences in membership in June were evident even at the phyla level (Fig. 4.5A).

Due to the dissimilarity of membership in January (both depths), June (epilimnion) and June (hypolimnion), differentiation among all dates and depths was not possible within a single two-dimensional ordination analysis. Therefore, we performed separate ordination analyses on January, June (epilimnion), and June (hypolimnion) samples (Fig. 4.5B). In January, we saw no difference in the microbiome at 1 vs. 16 m, as would be expected when the water column was mixed. However, the southern sampling point differed in membership from the other two sampling locations, confirming the results of hierarchical clustering (Fig. S4.2). Similarly, the June epilimnetic microbiome at the southern sampling point was distinct from the other two locations, which clustered together. In the June hypolimnion, the three sampling locations differed in the ordination analysis of microbiome membership, likely reflective of decreased physical mixing in the hypolimnion during stratification, when water at depth did not interact with the dominant wind patterns which mechanically mixed the epilimnion (Fig. 4.5B).

4.3.4 Dominant N metabolisms

As seen in the differences in the geochemical data and MAG relative abundance across seasons and depths, N metabolism gene expression in January was similar between depths and across locations (Fig.

4.6A). In all locations, at both 1 m and 16 m, NH_3 oxidation (*amo*) was upregulated along with genes associated with both NO_3^- and NO_2^- reduction (*narB*, *nasA*, *nirA, nirK, nirB, nirD, nrfA, nrfH, napA,* and *napB*) as well as N_2 fixation (*nifH, nifD,* and *nifK*). N_2O reductase (*nosZ*) was downregulated relative to expression of genes associated with prior steps in denitrification. Despite oxic conditions throughout the water column, several anaerobic pathways were also observed in January samples (Fig. 4.6D-E). Though genes were differentially expressed among depths and locations, there were no N metabolic genes whose expression was completely absent.

In June, there were clear differences in gene expression between the oxic epilimnion and the anoxic hypolimnion (Fig. 4.6C). In the hypolimnion, respiratory NO_2^- reductases were the most highly expressed of the genes of interest (Fig. 4.6G). Periplasmic nitrate reductase (*napA*) and hydroxylamine oxidase (*hao*) were the next most upregulated genes followed by cytochrome c-type protein (*napB*) (Fig. 4.6A). Both *napB* and *hao* expression was absent in the epilimnion in June. Periplasmic nitrate reductase followed by nitrate reductase 1 were the most upregulated genes in the epilimnion in June.

Respiratory NO_2^- reduction genes (Table S4.1) were upregulated across both seasons (at both depths in January and most highly expressed in the hypolimnion in June). Twenty lineages, in addition to lineages undefined at the order level, expressed respiratory NO_2^- reduction genes (*nirB*, *nirD*, *nrfA*, and *nrfH*) (Fig. S4.3). Order *Desulfomonilales* was the most abundant lineage followed by *Methylococcales*, *Anaerolineales* and an order of *Myxococcota* (*UBA796*). Looking only at lineages expressing *nrfA*, 11 of the initially identified 20 lineages (which were performing respiratory NO_2^- reduction) were likely performing DNRA (Fig. 4.7). Lineages from families *EnvOPS12*, *Anaerolineaceae* and *JAFGLY01* were the most abundant transcribers of *nrfA* with expression being significantly highest in *Anaerolineales Anaerolineaceae*.

4.4 Discussion

In the Lake Yojoa microbiome, membership and N metabolic gene expression is clearly driven by the monomictic stratification regime. While January sampling identified little difference between depths, as might be expected during the mixed water column phase, June sampling revealed distinct differences in

membership and gene expression between the epilimnion compared to the hypolimnion. To explore these seasonal and depth discrete patterns, the following discussion first explores the key results from analyses of the oxic water samples (January at both 1 and 16 m and June epilimnion) within the context of known ecosystem dynamics. The remainder of the discussion is then largely focused on the June hypolimnion with the goal of characterizing NO_3^- and NO_2^- reduction pathways and assessing the putative role of DNRA in N-cycling within Lake Yojoa.

While thermophysical structure, namely the presence or absence of oxygen, was the most dominant control on microbial processes, gene expression mirrored several other observed seasonal trends. For example, in January, both the epilimnion and the hypolimnion were enriched in *amo* (ammonia monooxygenase) relative to the water column in June. Ammonia monooxygenase catalyzes the first step in nitrification by oxidizing ammonia to hydroxylamine (NH_2OH) which is then oxidized to NO_2^-. The upregulation of *amo* in January is expected given oxidation of the water column and release of hypolimnetic NH_4^+ to the epilimnion following annual mixing (Fadum and Hall 2022). This pulse of NH_4^+ to the surface waters is then immediately followed by an observed increase in NO_3^- abundance in January relative to June (Fig. 4.3). Conversely, in June, the absence of *napA* (periplasmic nitrate reductase) expression in the epilimnion was likely due to low concentrations of NO_3^-, which has mostly been consumed by algal production by June. Similarly, low epilimnetic concentrations of both NO_3^- and NH_4^+ align with absence of *hao* expression, which is responsible for the second step in nitrification, the oxidation of NH_2OH.

In the June hypolimnion, we saw an upregulation in *nrfA* relative to June epilimnetic samples. This distinct difference in gene expression across depths mirrors *nrfA* patterns previously identified by targeted qPCR in Lake Alchichica, Mexico (Pajares et al. 2017). In Lake Alchichica, another warm, monomictic lake, functional N gene abundances (*amoA*, *nirS*, *nirK*, *nrfA* and *hzsA*, anammox) were also driven by seasonal patterns in stratification. During the stratified season, there was an increased relative abundance of *nrfA*, as seen in Lake Yojoa. However, where *nrfA* was positively correlated with NH_4^+ in Lake Alchichica's hypolimnion, we identified a negative correlation between *nrfA* and NH_4^+ ($R^2 = 0.73$, $p = 0.003$) in Lake Yojoa's hypolimnion.

Gene expression is imperfectly correlated to protein expression (De Sousa Abreu et al. 2009; Vogel and Marcotte 2012) and rarely, with some exceptions, correlates with rate processes (Rocca et al. 2014), limiting the biological inference that can be concluded with regards to competition between pathways. Therefore, in the absence of rate measurements, we are unable to determine the relative proportion of NO_2^- reduced by DNRA versus denitrification. However, we can conclude that in the June hypolimnion, across all locations, DNRA pathway genes (*nrfA* and *nirB*) were enriched relative to denitrification pathway genes (*nirK*, *nirS*, *norB*, and *nosZ*). Despite the spatial heterogeneity in *nrfA* expression observed in other systems (Decleyre et al. 2015), the upregulation in all three sites, which have varying depths, local mixing dynamics and nutrient sources, suggests DNRA in the hypolimnion is a ubiquitous and perhaps dominant mechanism for NH_4^+ accumulation in Lake Yojoa. Further supporting our hypothesized DNRA activity is the presence of several lineages (such as *Anaerolineales*, *Burkholderiales*, and *Desulfobulbales*) whose transcriptomes contained *nrfA* expression (Fig. 4.7) and have been identified as performing DNRA in other ecosystems (Ishii et al. 2011; Murphy et al. 2020; Marzocchi et al. 2021) (Fig. 4.7). The majority of these lineages were absent in the oxic epilimnion June, though *nrfA* gene expression in a subset of those orders (i.e., *Burkholderiales*, *Phycisphaerales*, *Tepidisphaerales* and *UBA1135*), was present at both depths.

Organic carbon (C) supports fermentive DNRA (as the electron donor), while reduced inorganic substrates (such as sulfide) serve as the electron donor for respiratory DNRA. Due to the presence of the large aquaculture operation, fermentive DNRA would be expected due to the abundance of organic material supplied by fish waste (Christensen et al. 2000). However, of the 126 MAGs capable of DNRA, only 22 were identified as fermenters, suggesting that respiratory DNRA may be the more pervasive DNRA pathway in Lake Yojoa's anoxic hypolimnion. The putative dominance of chemolithoautotrophic DNRA (over fermentive DNRA) may be supported by abundant sulfide in Lake Yojoa. While water column sulfide measurements are unavailable, sediment samples from January 2020 likely contained elevated quantities of elemental S (2-11 mg g^{-1}), suggesting high concentrations of sulfide in the overlying water column. The hypothesized presence of sulfide is further supported by the upregulation of *nirK* (NO forming nitrite

reductase) and *norB/C* (nitric oxide reductase) relative to *nosZ* (nitrous-oxide reductase), the final step in denitrification, which can be inhibited by sulfide (Burgin and Hamilton 2007).

Organic C was an important regulator of DNRA activity in other systems (Song, Lisa and Tobias 2014). However, we did not identify any relationship between *nrfA* and DOC. This suggests DNRA in Lake Yojoa was unlikely to be C limited, owing perhaps to an abundance of non-fermenting (i.e., chemolithotrophic) DNRA. Interestingly, *nrfA* expression was negatively correlated to NO_3^- concentrations in the June hypolimnion (R^2= 0.84, p= 0.0004), as was *norB* (the most highly expressed NO_3^- reduction gene) (R^2= 0.53, p= 0.024), and *nirK* (NO forming NO_2^- reduction) (R^2= 0.632, p= 0.010). The expressions of these genes were all positively correlated with each other; *nirK* and *narB* (R^2= 0.96, p= 2.35e-06), *nrfA* and *narB* (R^2= 0.76, p= 0.002), and *nrfA* and *nirK* (R^2= 0.83, p= 0.0005). Given the multiple complicated interactions of co-varying resource gradients present in natural systems (Morrissey et al. 2013), combined with the variety of substrates capable of serving as electron donors in DNRA, it is likely that limitation of DNRA may not be constrained by the abundance of electron acceptors (NO_2^-). However, as none of our available data parameters were positively correlated with the most upregulated NO_3^- and NO_2^- reduction pathway genes, it is possible that our metadata failed to capture what limits NO_3^- and NO_2^- reduction in Lake Yojoa.

Despite yet unresolved mechanisms driving gene expression in Lake Yojoa, this metatranscriptomic analysis identified DNRA as a pathway that, in part, explains the accumulation of high levels of hypolimnetic NH_4^+. Additional pathways include sediment derived NH_4^+ flux (Fadum, Waters and Hall *in review*) and remineralization of waste from the industrial net-pen aquaculture, a likely contributor due to high nutrient recycling rates in the hypolimnion of tropical lakes compared to mid-latitude lakes (Sarmento 2012). Regardless of these other potential sources of NH_4^+, DNRA, because of its competition with inorganic N ecosystem loss pathways, remains a critical pathway for assessing annual N dynamics, particularly in systems, like Lake Yojoa, that experience seasonal N limitation (Fadum and Hall 2022).

While we were unable to determine rate measurements in this analysis, the upregulation of *nrfA* compared to *nirK* (NO-forming nitrite reductase) and the absence of evidence that anammox is occurring

suggests possible dominance of DNRA relative to competing NO_2^- reduction pathways. Although we are comparing only two sampling dates, the comparison of June 2021, when stratification was fully developed, juxtaposed against January 2022, when the water column was fully mixed, provides a clear example of the two seasons within Lake Yojoa. No disruption to stratification was identified prior to June sampling (Fadum, Waters and *Hall in review*) and lake microbial communities have been shown to be resilient (on the scale of days) to water column mixing disturbance (Shade et al. 2012). Thus, the samples taken in June and January are likely representative of the stratified and mixed water column seasons, respectively.

4.5 Conclusion

Identifying the exact mechanisms driving hypolimnetic N accumulation in Lake Yojoa is difficult owning to the multiplicity of possible biogeochemical paths and an ever-growing number of newly resolved mechanisms for NH_4^+ production and consumption (Kuypers, Marchant and Kartal 2018). However, by focusing on expression of fundamental N cycling genes, we conclude that Lake Yojoa supports a diverse and seasonally variable microbial community membership with the ability to conduct multiple NO_3^-/NO_2^- reduction pathways, across all seasons, but most prominently in the anoxic hypolimnion during stratified months. This work simultaneously provides insight into existing hypotheses (DNRA driven NH_4^+ accumulation, Fadum and Hall 2022) built on previous observation while identifying many new avenues of inquiry. One future research direction highlighted here is the importance of anaerobic metabolisms in oxic portions of the water column. The presence of strict anaerobes, such as *Desulfomonilia*, and the expression of genes associated with anaerobic N metabolisms in the June epilimnion and January water column suggest there may be ample anoxic microsites within the otherwise oxic water column as has been observed in other productive aquatic ecosystems (Angle et al. 2017). Another question this initial research raises is in regard to the prevalence and potential contributions of facultative DNRA performing microbes. In this analysis, 69 of all (126) identified DNRA capable MAGs were also aerobes suggesting prevalence of facultative DNRA. Finally, identifying limitations to NO_3^-/NO_2^- reduction in the hypolimnion might offer new insights into these ecologically critical pathways given the demonstrated unlikelihood of limitation by electron acceptor abundance or common electron donor (DOC and sulfide) abundance. Answering these questions

in Lake Yojoa and other low-latitude lakes, are critical steps in broadening our mechanistic understanding of microbially driven biogeochemical processes that influence the trophic state of tropical freshwater ecosystems. Only by establishing this fundamental ecological knowledge can we hope to understand why and how tropical lakes are responding to a changing world.

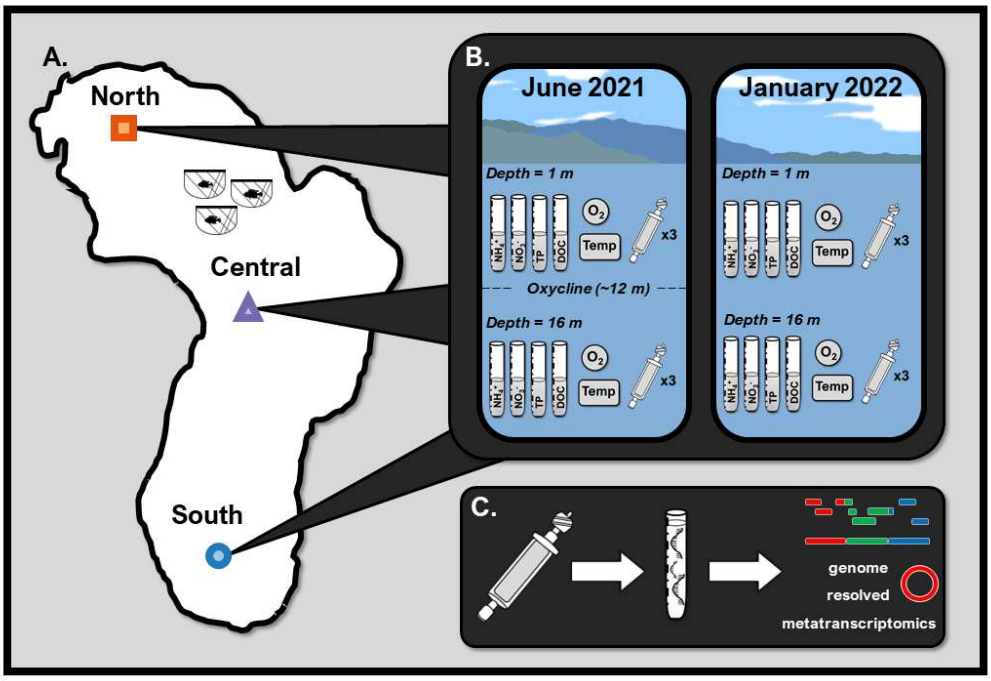

Figure 4.1. Data collection methods **(A)** Three pelagic sampling locations: North, Central and South. **(B)** Sampling (geochemical, thermophysical structure, and metatranscriptome- in triplicate) in two months, covering the rainy/ stratified water column season (June), and the dry/ mixed water column season (January). **(C)** Extraction, sequencing, genome assembly and metatranscriptomic mapping to assembled genome.

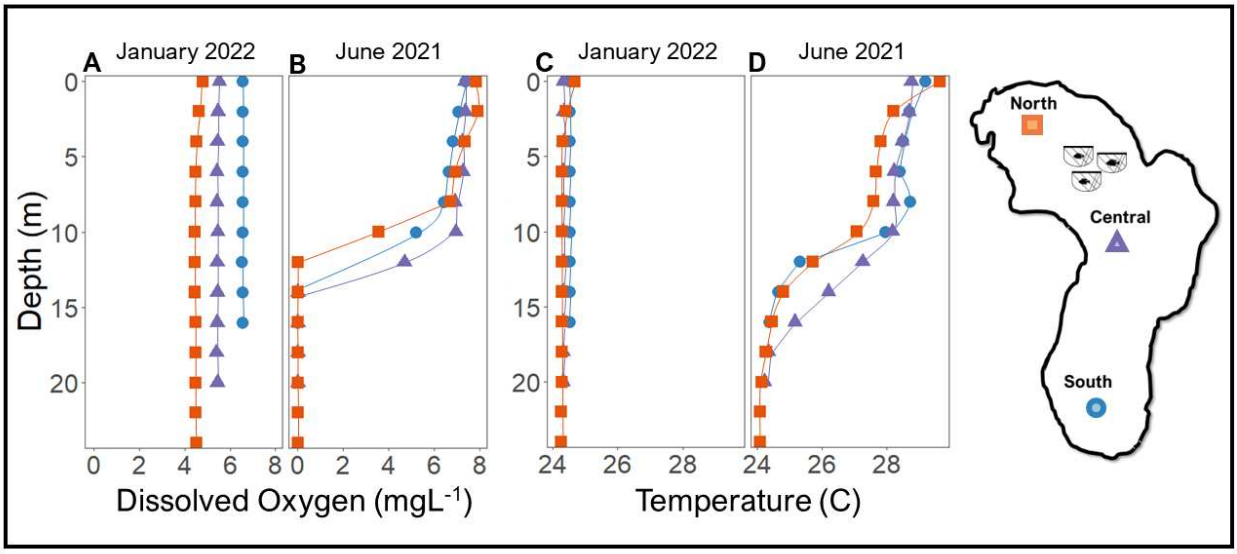

Figure 4.2. Thermophysical structure in Lake Yojoa. **(A)** Dissolved oxygen at 2 m intervals in January 2022 and June 2021**. (B)** Temperature at 2 m intervals in January 2022 and June 2021.

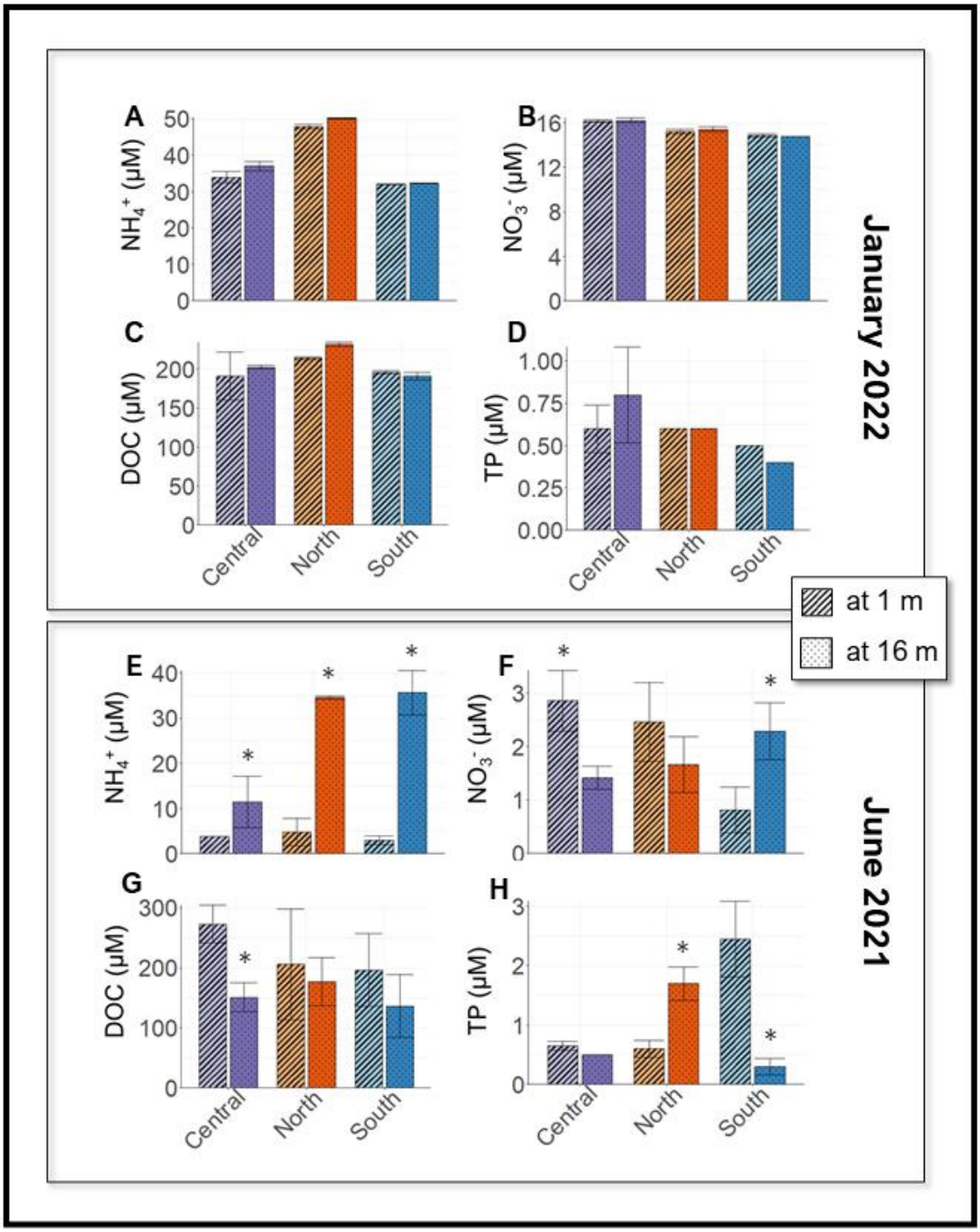

Figure 4.3. Nutrient concentrations in Lake Yojoa at 1 and 16 m across three sampling locations, mean ± standard deviation, in January 2022 **(A-D)** and June 2021 **(E-H).** Significant differences between 1 m and 16 m identified with an asterisk (*).

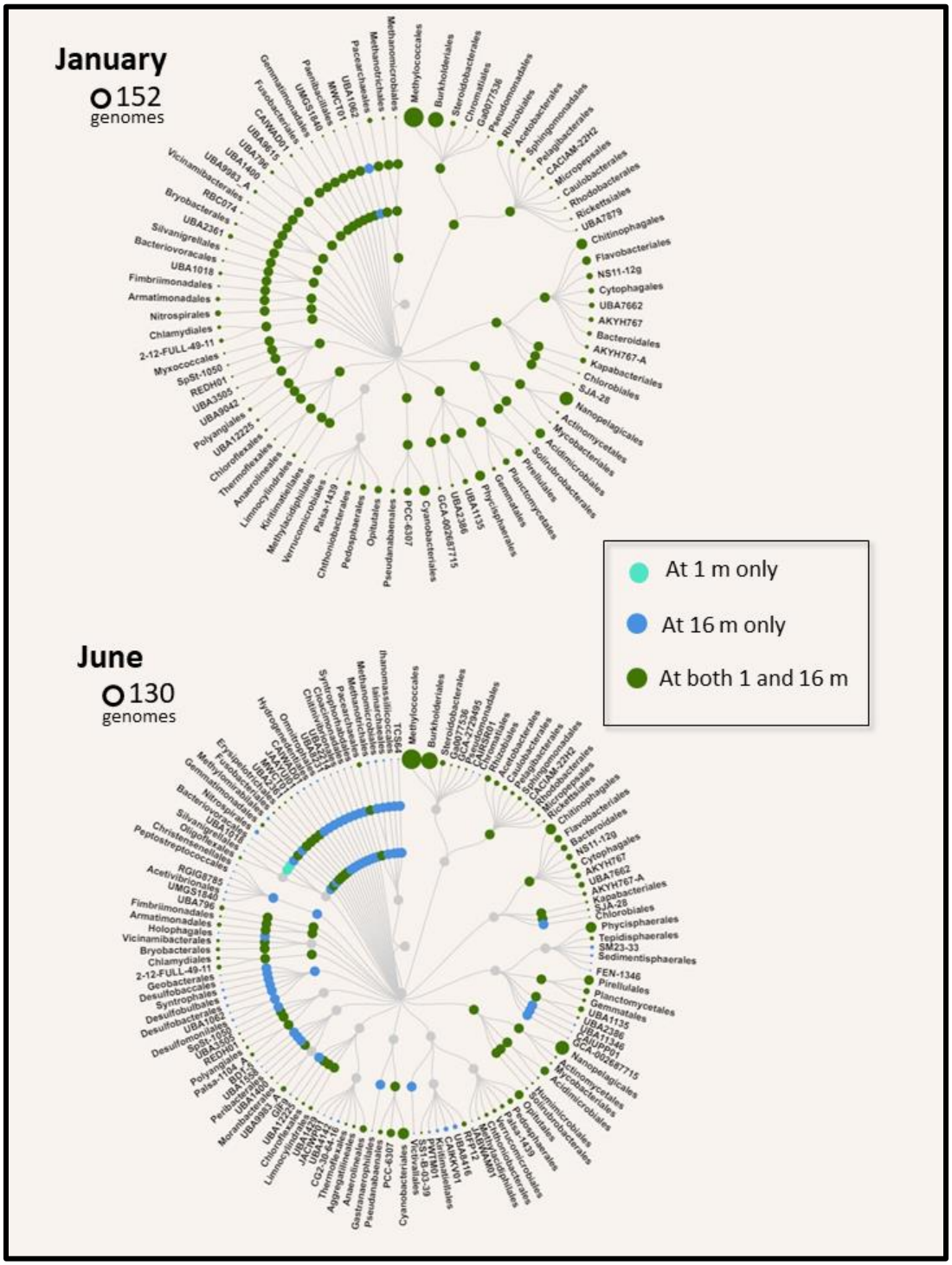

Figure 4.4. Taxonomic level (Domain, Phylum, Class, Order) assigned by GTDB-tk to genomes expressing N metabolism genes of interest (Table S4.1) in January (n= 1039) and June (n=977).

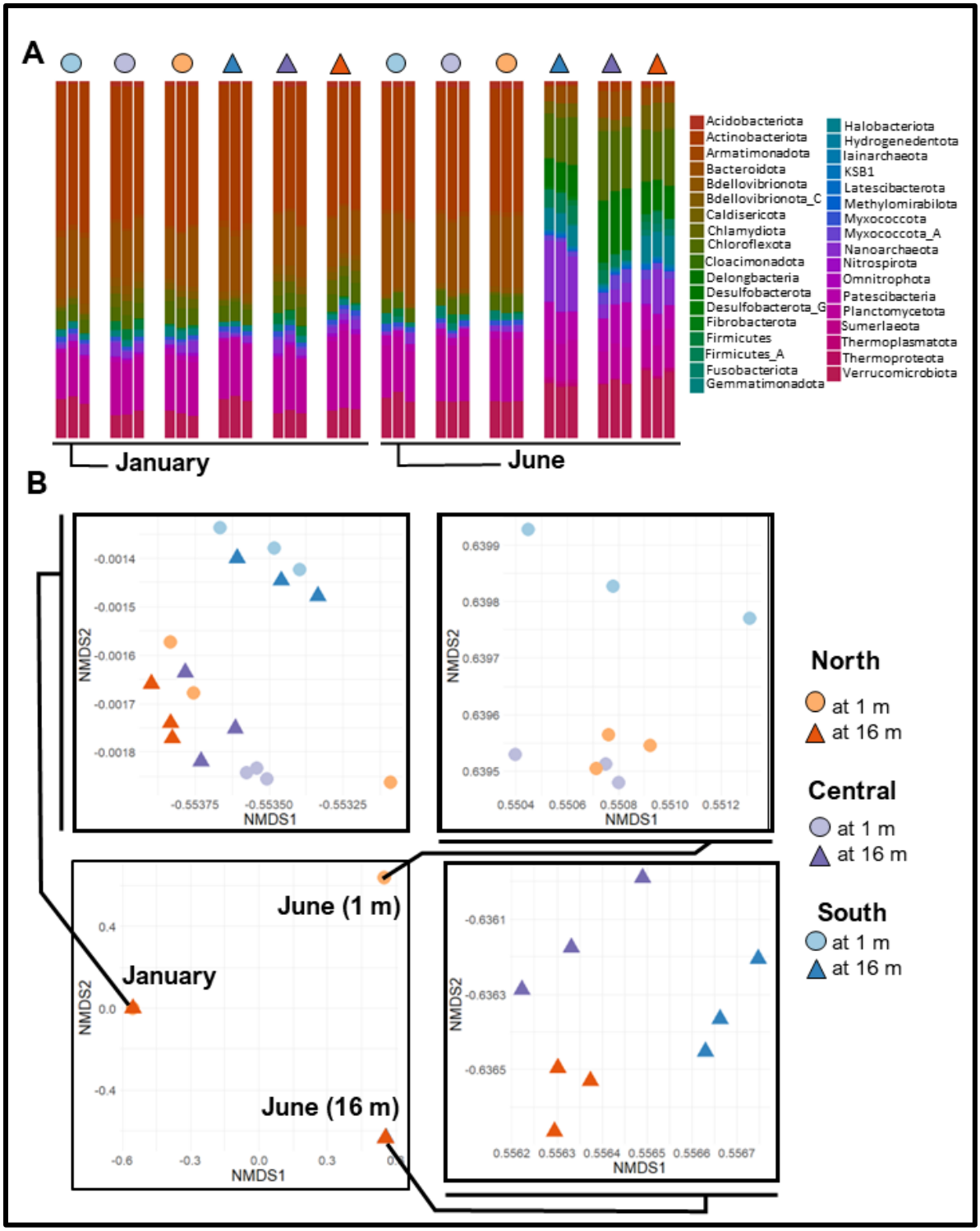

Figure 4.5 (A) Diversity of phyla among samples, with dominant phyla (*Cyanobacteria* and *Proteobacteria*) removed to more clearly reflect community differences between depths and across seasons. **(B)** Due to highly diverse community assemblages, ordination analysis of membership has been separated into January samples, June epilimnetic samples and June hypolimnetic samples.

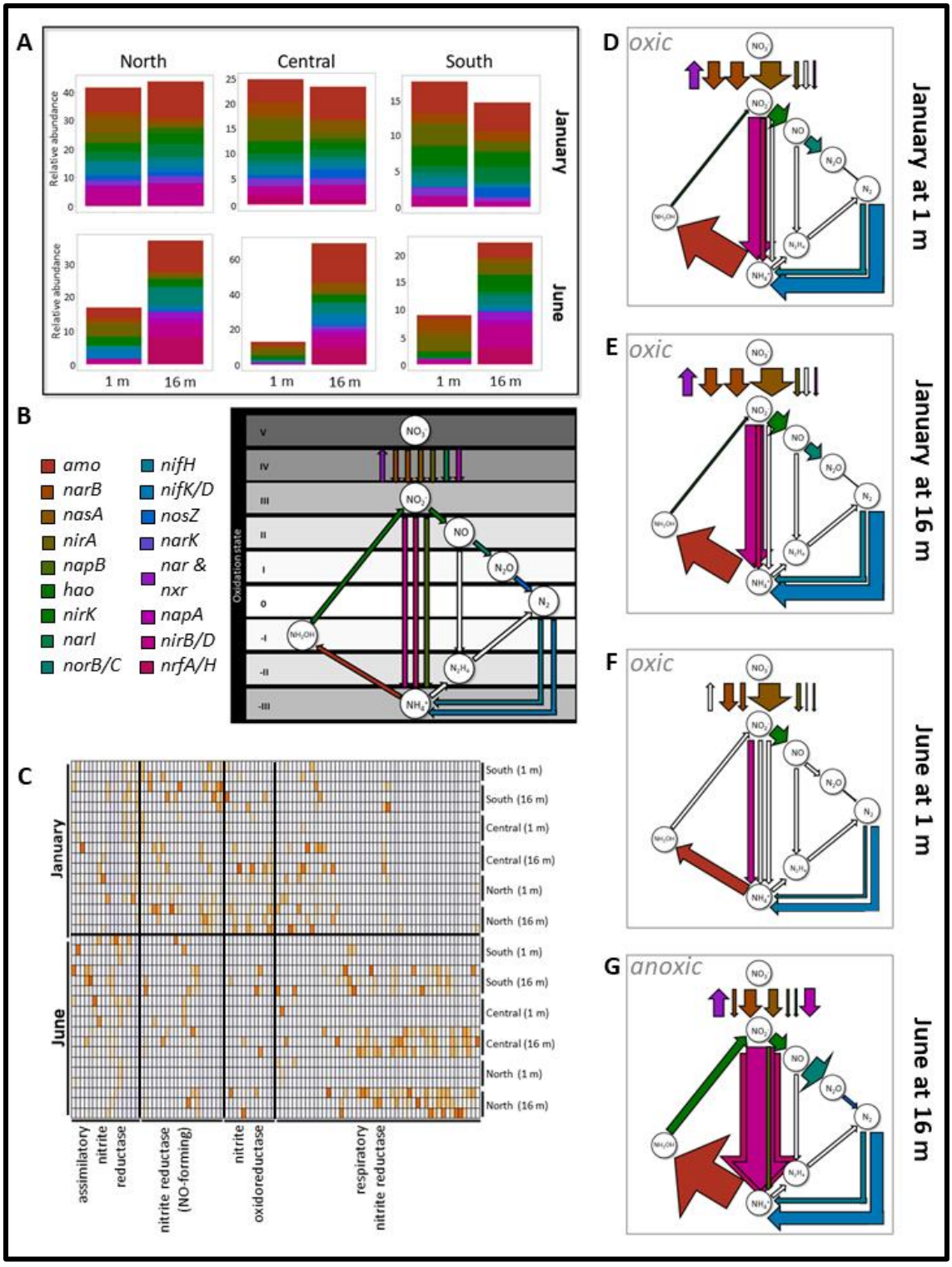

Figure 4.6 (A) N metabolism gene expression, grouped by function, across locations, depths and season. **(B)** N cycling pathways assessed in panels D-G. **(C)** Heatmap of gene expression of nitrite transformation pathways. **(D-G)** Conceptual diagram of dominant N transformation pathways across seasons and 1 m and 16 m, shown in arrows weighted by expression.

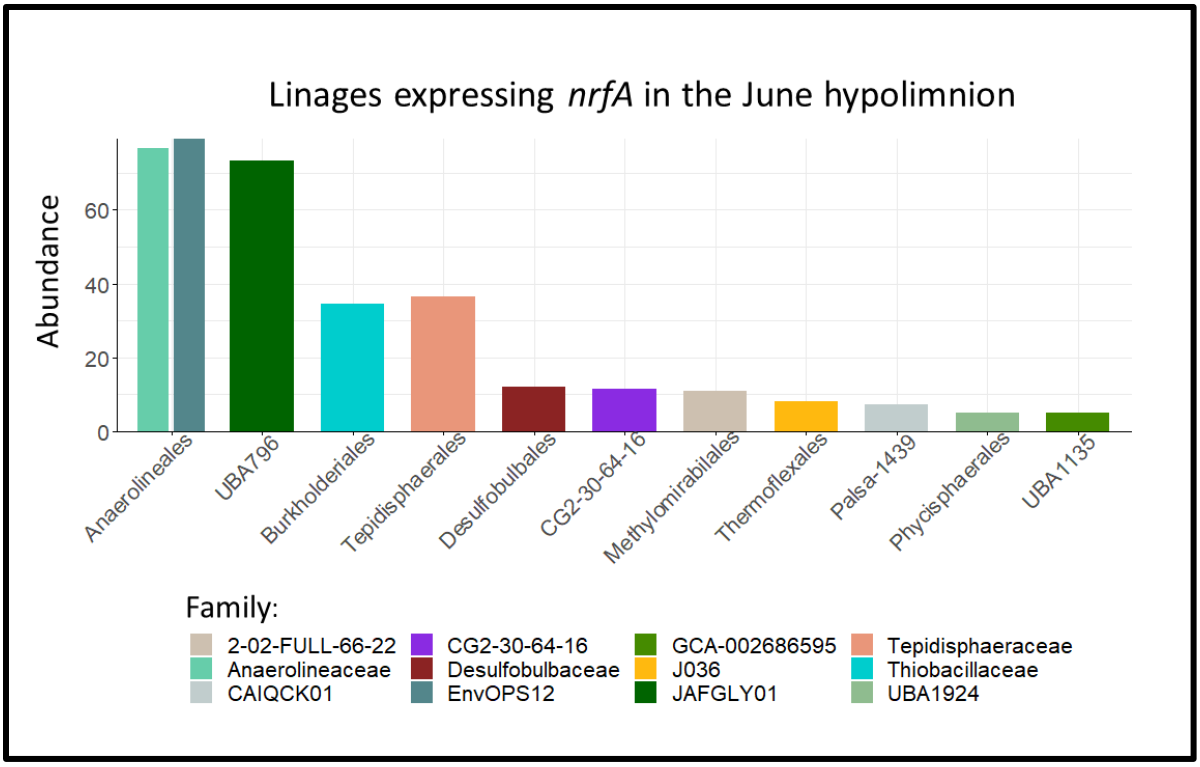

Figure 4.7 Lineage of genomes expressing *nrfA* in the June hypolimnion

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CHAPTER 5: REMOTE SENSING OF TROPHIC STATE IN LAKE YOJOA; A SUMMARY OF FUTURE OUTLOOKS AND OPPORTUNITIES[5]

5.1 Introduction

Passive remote sensing has numerable uses and advantages in the study of inland waters. Whereas the field has its roots in algorithm development, in recent years, there has been a shift towards model development aimed at assessing spatiotemporal trends and the impacts of changing water quality on ecosystems and communities (Topp et al. 2020). One of the advantages of remote sensing is that it provides information on lake ecology across remarkable spatial resolution, supporting research aimed at identifying regional (Oleksy et al. 2022) and global trends (Yang et al. 2022). Similarly, remote sensing can extend the temporal resolution of lake ecosystem research by providing information on historic conditions predating monitoring efforts (Hansen et al. 2020). These types of analyses broaden the temporal and/or spatial range of more traditional data collection techniques that consist of discrete sampling events. With global coverage and sampling frequencies ranging from days to weeks, and spatial resolution as low as < 1 m, satellite imagery provides a powerful tool not just for identifying inter-annual, directional changes in lake ecosystem characteristics but also has the spatial and temporal resolution required to assess intra-annual patterns. Changes in intra-annual variance is critical to predicting regime shifts (Gilarranza et al. 2022) in global lakes, as variance is a leading indicator of ecological thresholds and transition points, such as eutrophication (Carpenter and Brock 2006; Carpenter et al. 2011).

Remote sensing work in Latin America (Melack et al. 2009; Flores-Anderson et al. 2020; de Lucia Lobo et al. 2021, among others) demonstrates how using satellite imagery can aid in data collection in otherwise data-poor regions (Riveros-Iregui et al. 2018). The paucity of ecological data in tropical regions, and thereby significant portions of Latin America, is one of the significant contributing factors in the knowledge gap between temperate and tropical ecosystems. Because much of the data gap (and subsequent ecological knowledge gap) is maintained by the high cost of conducting research, remote sensing data

[5] This chapter is *in prep* with the following authors: Jemma Fadum, Matt Ross, Simon Topp, and Ed Hall.

acquisition techniques, based on free and publicly available imagery, may be one tool for increasing data richness in the Global South. However, to meaningfully narrow this data gap, produced data should be accessible to local scientists, practitioners, and stakeholders. Shared and accessible data is an important consideration in the effort to combat helicopter science and decolonize ecology (Baker et al. 2019; Trisos et al. 2021; Haelewaters et al. 2021). Towards this combined goal of conducting quantitatively rigorous science while promoting ethical international research practices, this chapter presents results from a project aimed at using surface reflectance values to estimate trophic state in Lake Yojoa, Honduras.

To test the efficacy of using remote sensing to expand the temporal resolution of our work in Lake Yojoa, we evaluated the ability of a machine learning algorithm, adapted from Topp et al. (2021), to estimate Secchi depth and chlorophyll a (Chl-a) concentrations in Lake Yojoa. The first aim of this work was to estimate trophic state in years when no *in situ* data is available in Lake Yojoa. We were particularly interested in estimating trophic state between 1984 and 2017, an unmonitored time period bookended by two multi-year continuous monitoring campaigns (Vaux and Goldman 1984; Fadum and Hall 2022). Our second aim was to use remotely sensed variables to monitor contemporary trophic state in Lake Yojoa, thereby ensuring continued data availability to local collaborators and stakeholders beyond the conclusion of funded monitoring efforts. To test our model's ability to identify seasonal variability and natural disturbance response, we evaluated if the model captured Lake Yojoa's trophic state response to Hurricanes Eta and Iota (Fadum, Waters and Hall in review).

5.2 Methods

5.2.1. Study site

Lake Yojoa is a large (~83 km2 surface area) oligotrophic, tropical lake in West-Central Honduras with a mean annual Secchi depth of 3.1 meters (Fadum and Hall 2022). As the subject of several recent and ongoing research efforts, Lake Yojoa has well documented intra-annual trophic state patterns (Fadum and Hall 2022), and a clearly characterized response to hurricane disturbance (Fadum, Waters and Hall in review). A contemporary dataset with semi-monthly *in situ* measurements aligned with Landsat satellite acquisition days (Fadum and Hall 2022, Fadum, Waters and Hall in review), and legacy data from the early

1980s (Vaux and Goldman 1984) make Lake Yojoa a relatively data-rich lake in a largely understudied region. Therefore, Lake Yojoa offers an unparalleled opportunity to assess remote monitoring practices in a tropical lake.

5.2.2 Dataset assembly

We collected Secchi depth and Chl-a data from five pelagic locations (B, E, F, P and R in Fadum and Hall 2022) within five days of satellite acquisition days and collated them with Landsat 7 and 8 imagery which was acquired using Google Earth Engine. Observations were removed if compromised by clouds or cloud shadows. All pixels classified as open water by the USGS Dynamic Surface Water Extent algorithm (DSWE) were retained (Jones 2019), leaving 282 pairs of data (Secchi depth and Chl-a) and useable imagery. We determined dominant wavelength (λd) by calculating visible color wavelength (nm) from reflectance values within the visible spectrum (blue, green, and red) (Topp et al. 2021). Additional information on atmospheric correction, sensor standardization and calculation of λd is presented in Topp et al. (2021).

5.2.3 Model training and testing

The machine learning approach we employed uses the caret package for R (Kuhn 2008), which employs XGBoost, a gradient-boosted decision trees based algorithm. Model parameters included surface reflectance values (near infrared (NIR), red, green and blue), surface reflectance ratios (NIR:red, blue:green, and blue:red) and λd. We trained the model using 80 percent of the available data from 2018-2020 and withheld 20 percent for testing. While the available data was randomly assigned to either the training or test datasets, we applied some selection criteria to ensure that the test data was pulled from distinct dates compared to the training dataset to ensure that validation was a test across sampling dates rather than locations due to the minimal difference across the five sampling locations within a given day. Without this 'paranoid' random sampling, our model was overfit (R^2= 0.83) to the data. We assessed model fit with simple linear regression. In an attempt to improve model fit, we experimented with supplementing the training set with data from AquaSat (Ross et al. 2019) but were unable to improve upon the original model.

5.2.4 Model evaluation

We evaluated model performance based on root mean square error (RMSE), mean absolute error (MAE), mean absolute percentage error (MAPE), bias, and symmetric mean absolute percentage error (SMAPE). We compared the performance of our model to other published models which similarly estimated Secchi depth or Chl-a using passive remote sensing (Majozi et al. 2014; Dlamini et al. 2016; Alikas and Kratzer 2017; Flores-Anderson et al. 2020; Grendaitė and Stonevičius 2022) (Table 5.1). To evaluate our models' ability to estimate Secchi depth and surface Chl-a concentrations in Lake Yojoa, we used our models to predict pelagic conditions in 2021.

5.3 Results

Our results suggest that passive remote sensing offers a viable tool for monitoring trophic state in Lake Yojoa, Honduras. The Secchi depth model performed well (RMSE= 0.56, MAE= 0.47, MAPE= 0.19, Bias= 0.03, SMAPE= 0.17) despite having a limited amount of training data available, particularly at the higher Secchi depth values (Fig. 5.1). While model validation was not particularly strong (R^2= 0.58), we achieved a comparable RMSE to other studies which used passive remote sensing to estimate Secchi Depth (Table 5.1). Most interesting and encouraging was that the Secchi depth model captured the post-hurricane clarity in January and February 2021 (Fig. 5.2) (Fadum, Waters and Hall *in review*). In contrast, the Chl-a model did not perform as well (RMSE= 3.21, MAE= 2.37, MAPE= 0.37, Bias= 1.06, SMAPE= 0.43). While the RMSE is comparable to other models (Table 6.1), the achieved R^2 suggest that the model had poor agreement with *in situ* observations. Though some seasonal dynamics were captured by the model, performance is currently too poor to be a reliable, stand-alone approach for monitoring of ecosystem state (Fig. 5.3).

5.4 Discussion

These initial results suggest that remote sensing may be a viable tool for monitoring Secchi depth in Lake Yojoa. While the Chl-a model is not currently sufficiently accurate to be used for monitoring purposes, changes in water clarity are largely caused by changes in algal abundance in Lake Yojoa as opposed to suspended sediment or browning (Fadum and Hall 2022, Appendix A). Therefore, Secchi depth

estimates are still a valuable metric for assessing contemporary trends in Lake Yojoa's trophic state. The Chl-a model's limited performance may have been due to seasonal variation in algal community structure, flocculent size, Chl-a degradation between sampling and analyses, or a poor relationship between Chl-a and algal abundance (Ramaraj et al. 2013).

Presently, we are unable to confidently estimate Secchi depth prior to the launch of Landsat 7 (1999), leaving a sizeable gap between the contemporary (2018-present) and legacy data (1979-1983). An aim of future work will be to assess different avenues for increasing the utility of Landsat 5 (1984-2013) and Landsat 4 (1982-1993) imagery. The recent launch of Landsat 9, combined with the additional *in situ* data collected since the completion of the presented analyses (including future monitoring planned through June 2023) will benefit this upcoming work through the availability of additional training data and therefore may improve the model in such a way as to more easily bridge the model's capabilities across satellites. While this inability to confidently estimate historical trophic state means that our first objective was not met, we were much more successful in our second aim, to use remote sensing as a monitoring tool for contemporary trophic state. The initial success of the Secchi depth model suggests that continued monitoring, using Landsat 8 and 9, will likely be equally if not more successful.

Beyond the primary objectives of this research, the data exploration involved in this project identified new inter-annual ecological trends that were not captured by the *in situ* monitoring. While the models were unable to completely fill the 40-year data gap, changes in surface reflectance values (i.e., color) hint at shifts in underlying ecological processes. The most interesting change in ecosystem state identified through remote sensing was an increase in surface water greenness in March and April in recent decades. March and April occur during peak annual temperature and act as a "shoulder season" in Lake Yojoa, bridging the last few weeks of the dry season with the early months of water column stratification.

This preliminary remote sensing work, combined with other recent investigations in Lake Yojoa, suggest future directions for more regionally robust analyses. For example, our Secchi depth model was able to capture the clear water phase following Hurricanes Eta and Iota, two Category 4 storms that directly crossed the lake (as tropical depression) and occurred during this monitoring period (Fadum, Waters and

Hall in review). Further refinement of this model or else identification of specific spectral signatures associated with hurricane disturbance response could not only allow us to assess ecosystem response to similar storms that predate *in situ* monitoring in Lake Yojoa, but also assess regional inland water response to tropical cyclones. Tropical storms can have devastating impacts on aquatic ecosystems and by extension, water security. Therefore, assessing the response of ecosystems to storms of varying intensities is of critical importance to local communities impacted by increasingly frequent and intense hurricane activity in the Atlantic basin (Morris et al. 2002; Bender et al. 2010; Knutson et al. 2010).

Future work could also test the ability of the derived models to estimate trophic state in similarly large Latin American lakes. While many lakes are data-poor, limiting model evaluation, Lake Atitlán, Guatemala would be a potential candidate location for assessing the model's efficacy outside of Lake Yojoa. Lake Atitlán is relatively data-rich for the region and has been assessed using remote sensing previously (Flores-Anderson et al. 2020). Additionally, we may expect Lakes Yojoa and Atitlán to have similar spectral characteristics during the stratified water column season (summer). *Lyngbya robusta*, a non-heterocyst forming cyanobacteria, is a dominant lineage in the June epilimnion (Fadum and Borton et al. in prep). Similarly, *L. robusta* reportedly dominates summer algal blooms in Lake Atitlán (Rejmánková et al. 2011; Komárek et al. 2013). This similarity highlights the degree to which tropical lakes may share key traits and functions though robust studies linking disperse research efforts are currently lacking. Therefore, broad remote sensing efforts in regionally constrained studies of tropical lakes may provide novel insights into intra-annual ecosystem dynamics and inter-annual trends.

5.5 Conclusion

In addition to expanding the temporal resolution of research efforts, this work demonstrates how existing remote sensing models can be applied to new systems and developed using data from short term continuous monitoring efforts in order to create longer term data products. Monitoring schedules that align with satellite acquisition days create an opportunity to invest in data availability beyond the duration of a single study while providing meaningful data to local communities, stakeholders, and managers. Such data availability efforts are important steps towards meaningful collaboration and transition away from

traditional, largely extractive data collection practices. Therefore, remote sensing not only has the potential to reduce the data gap between temperate and tropical ecosystems but also offers the opportunity to foster longer term data product investments in international collaboration. Possible outcomes of increasing remote sensing efforts and pairing remote sensing modeling with concurrent monitoring work in low-latitude lakes include increased tropical research, increased access to monitoring technologies, removal of barriers to data-driven management practices and increased environmental justice through data availability

Table 5.1. Comparison to other efforts to estimate Secchi depth and chlorophyll a from satellite data (best results listed if multiple approaches were compared in text)

Lake/Region	**Satellite(s)**	**Parameter estimated**	**R^2, RMSE**	**Reference**
Baltic Sea and Nordic inland waters	MERIS	Secchi depth	R^2 = 0.89, RMSE = 0.77 m	Alikas and Kratzer 2017
Lake Naivasha, Kenya	MERIS	Secchi depth	R^2 = 0.97, RMSE = 0.26 m	Majozi et al. 2014
Lake Chivero, Zimbabwe	MODIS	Chl-a	R^2 = 0.97, RMSE = 3 7 μg L^{-1}	Dlamini et al. 2016
Lithuania	Sentinel-2 MSI	Chl-a	R^2 = 0.77–0.79 RMSE = 7 μg L^{-1}	Grendaitė et al. 2018
Lake Atitlán, Guatemala	Hyperion	Chl-a	R^2 = *varied* RMSE = 2.0 μg L^{-1}	Flores-Anderson 2020

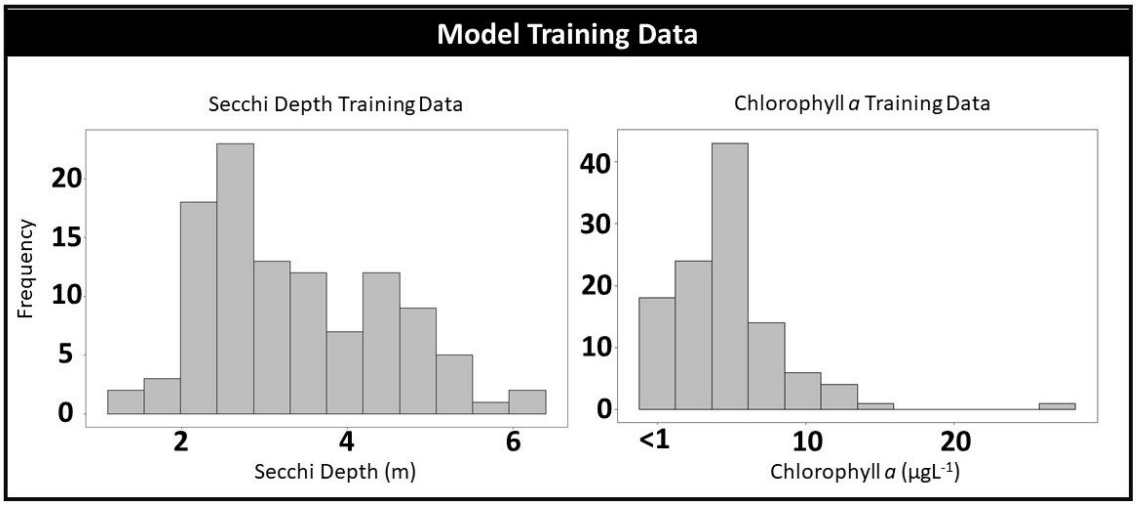

Figure 5.1. Distribution of randomly selected data used in model training

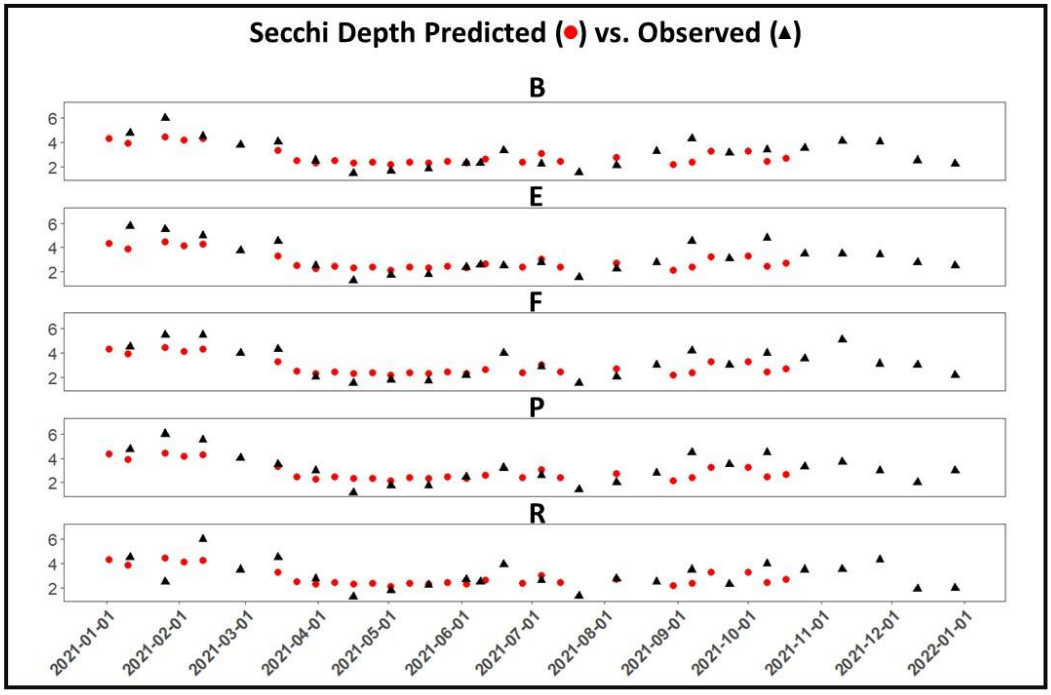

Figure 5.2. Secchi depth estimates compared to *in situ* estimates in 2021.

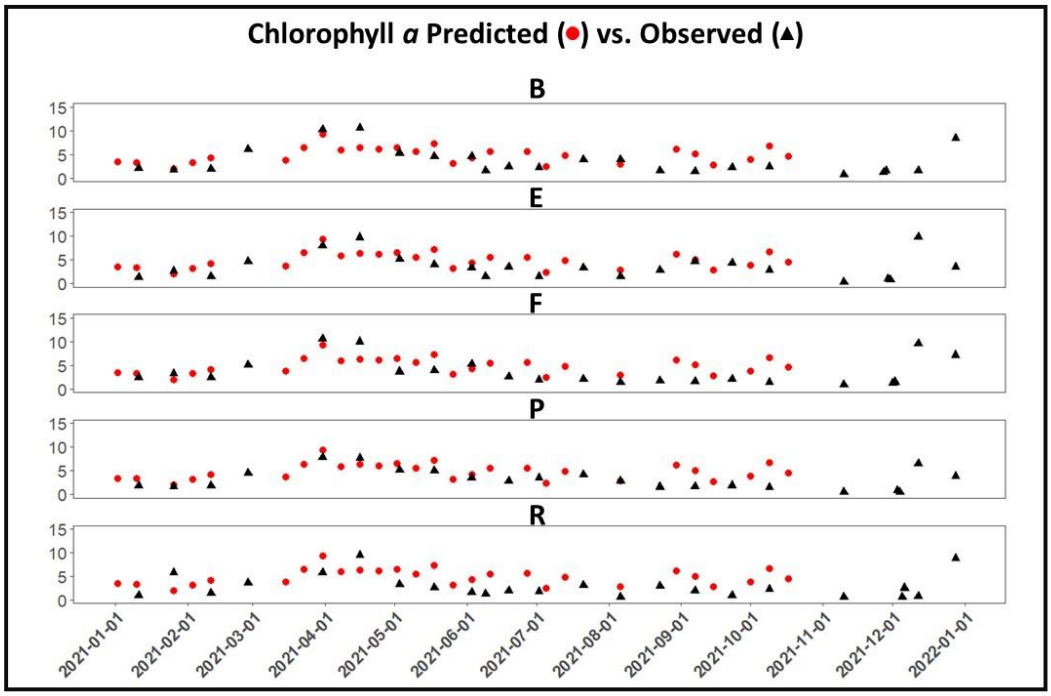

Figure 5.3. Chlorophyll *a* estimates compared to *in situ* estimates in 2021.

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CONCLUSION

This dissertation provides new perspective and understanding of biogeochemistry in tropical lakes by combining a literature review with an in-depth case study of Lake Yojoa, Honduras. Lake Yojoa typifies the interacting threats facing freshwater resources across the global tropics; a changing climate, increasing nutrient loading and the escalating demands of local and global economies. As a case study, Lake Yojoa could answer a multitude of ecological questions. For this body of research, the focus was on understanding the biogeochemistry underlying trophic state change by layering a diverse suite of approaches to ask questions at different spatial and temporal scales. Following a literature review (Chapter 1) aimed at more clearly defining one of the foremost foundational biogeochemical concepts in limnology, phytoplankton macronutrient limitation, the remaining chapters increased in specificity. Chapter 2 is a carefully designed comparative study that bridged 40 years of research in Lake Yojoa. This observational data paired with nutrient enrichment bioassay experiments provided a foundation for the remaining three chapters. A well-established baseline of contemporary conditions in Lake Yojoa made it possible to identify an ecosystem level response to Hurricanes Eta and Iota (Chapter 3) and unearthed hypotheses that were tested with an analysis of Lake Yojoa's microbial metatranscriptome (Chapter 4). The dissertation concludes with a look ahead to using the collected data from Chapters 2 and 3 to develop a remote monitoring model in order to continue to learn about ecosystem function in tropical lakes (Chapter 5).

The combination of these five chapters clearly demonstrates how stratification and the duration of warm, anoxic hypolimnions is a dominant control in monomictic lakes, both through physical mixing and in defining redox potential within the water column. These identified mechanisms of ecosystem scale processes are what lends applicability of this research to other tropical monomictic ecosystems, providing foundational knowledge with the goal of not only advancing the science of limnology but also reducing the knowledge gap between temperate and tropical lakes. Such basic science is required to ethically address (Colyvan et al. 2009) the most difficult and pressing environmental issues, such as the *wicked problems* in ecology (Rittel and Webber 1973; Head and Alford 2015).

Beyond the above results, this dissertation highlights the value of combining methodological approaches as well as multi and interdisciplinary collaborations. In recent years, there has been a rapid increase in the use of advanced research technologies such as multi-omics (Krassowski et al. 2020), microbiome assessment (Liwinski et al. 2021) and remote sensing, especially using hyperspectral imaging systems (Dierssen et al. 2021). These tools offer unparalleled opportunities to advance ecological understanding. However, this dissertation demonstrates how new technologies also benefit from basic monitoring, beyond the curation of metadata and ground truthing. By layering different assessment techniques, otherwise discrete datasets offer greater insight than any single tool provides alone. It is the emergent properties of these combined datasets which can offer a deeper understanding and appreciation of ecosystem function and a more wholistic sense of the underlying drivers. This works shows that, as ecologists, we need perhaps not a 30,000-foot view, but a 705 km view paired with a 120 nm view to truly derive new knowledge and formulate novel hypotheses.

One of the common threads throughout the varied research efforts included in this dissertation is the need to achieve a more balanced understanding of tropical lake ecosystems given that the ecological literature is disproportionately skewed towards temperate latitudes, leaving tropical regions vastly underrepresented by comparison (Nuñez et al. 2021). This is partially due to a lower abundance of lakes at tropical relative to temperate latitudes (McManamay et al. 2018). However, more limiting than the number of lakes available to study is a legacy of barriers to participation in ecological research including financial, infrastructural, and institutional constraints. Such barriers and associated challenges hinder the generation of long-term data acquisition, further widening the knowledge gap between temperate and predominantly tropical regions, such as Latin America (Riveros-Iregui et al. 2018). Long-term data collection and continuous monitoring work is not only critical to understanding directional ecosystem change but also necessary to understand the impact of abrupt disturbances and ephemeral events (such as hurricanes).

Stark funding differences between researchers from the Global North (i.e., wealthier nations in the northern hemisphere, and also including New Zealand and Australia) and the Global South (i.e., developing nations, largely situated in the tropics) create asymmetries in power and participation in ecology,

maintaining practices of *helicopter science* (Nuñez et al. 2021). Helicopter science, also referred to as *helicopter research* or *parachute research*, is the rightly criticized practice of researchers from the Global North collecting data and samples from the Global South before returning to their home institutions where they analyze and publish the results of their research without meaningful continued involvement with local collaborators (Haelewaters et al. 2021). As we, as a discipline, continue to work to decolonize science and curb practices which promote helicopter research and disadvantage local communities and collaborators in the Global South (Baker et al. 2019; Pettorelli and et al. 2021; Haelewaters et al. 2021), our discussions should include practical approaches for creating enduring and accessible data products that benefit local research participants beyond the limited duration of the typical grant funding cycle. The remote sensing work described in Chapter 5 offers one possible tool for narrowing the temperate-tropical monitoring and data collection gap while simultaneously investing in prolonged data access for local stakeholders.

Beyond the local level barriers to participation, global pressures are responsible for the inequity in tropical ecological research. This suggests that it is not a problem so succinctly solved by allocation of funding or development of infrastructure. It is not a pervasive disinterest in tropical regions or that they are, in any way, less deserving of scientific inquiry. Rather there are clearly identified barriers to tropical research that have resulted in the sizeable knowledge gap we see in the literature. Scientists from the Global South are underrepresented among top-publishing ecologists (Maas et al. 2021) and Asia, Africa and Latin America (combined) make up a diminutive portion of submitted and accepted manuscripts compared to Europe, North America, and Oceania (Nuñez et al. 2019). Large synthesis efforts in ecology often preclude studies published in non-English journals (Konno et al. 2020; Nuñez and Amano 2021). We have also seen that scientists from these underrepresented regions face greater barriers to participation in top international conferences. Taken together, it is evident that Ecology, as a discipline, systematically disadvantages and excludes tropical research to the detriment of the field. Therefore, to truly advance our collective understanding of tropical ecology, not only in lakes, but in all systems, we must not only pursue research aimed at foundational mechanistic understandings of ecosystem function but also work towards meaningful and equitable participation within our global scientific community.

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APPENDIX A

The relationship between Secchi depth and algal biomass in contemporary Lake Yojoa across all sampled months of the contemporary sampling period is statistically significant ($p < 0.05$) if not particularly strong ($R^2 = 0.49$) (Fig. S2.1). The lack of a stronger relationship is likely attributable to some of the well-documented challenges in using chlorophyll a as a direct proxy for algal biomass[6], coupled with high variability in algal flocculation we observed in Lake Yojoa (resulting in variability in estimates of volumetric chlorophyll concentrations for any given sampling event). Looking only at the months when the water column is mixed and when flocculation is uncommon (November-February), the relationship between Secchi depth and algal abundance is much stronger ($p < 0.000005$, $R^2 = 0.86$) (Fig. S2.2). Thus, in Lake Yojoa the vast majority of variance in Secchi depth can be explained by shifts in algal abundance.

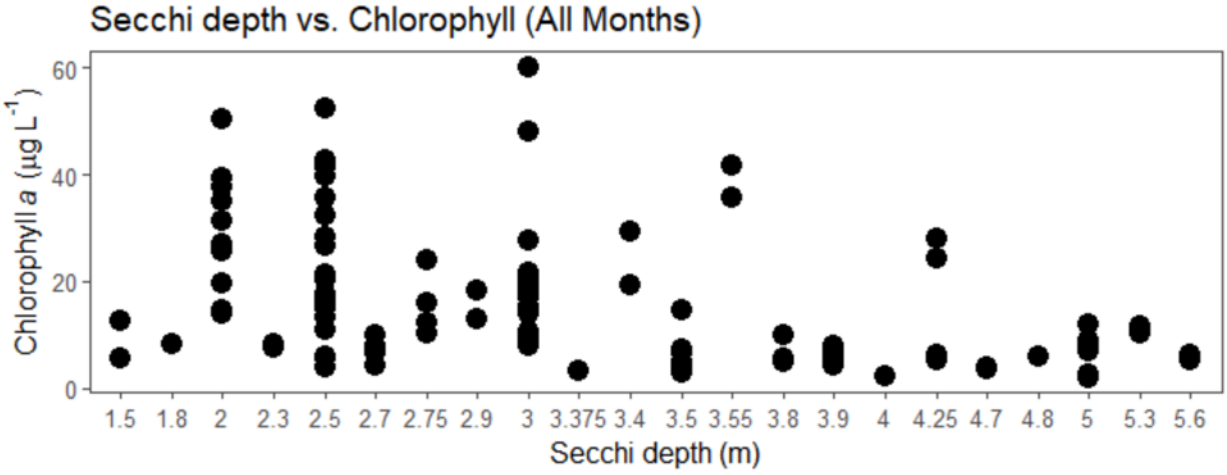

Figure S2.1. Secchi depth vs algal biomass, as estimated by chlorophyll a concentration, for all study months.

[6] Ramaraj, R et al. 2013. Chlorophyll is not accurate measurement for algal biomass. *Chiang Mai J. Sci.* **40**:1-9.

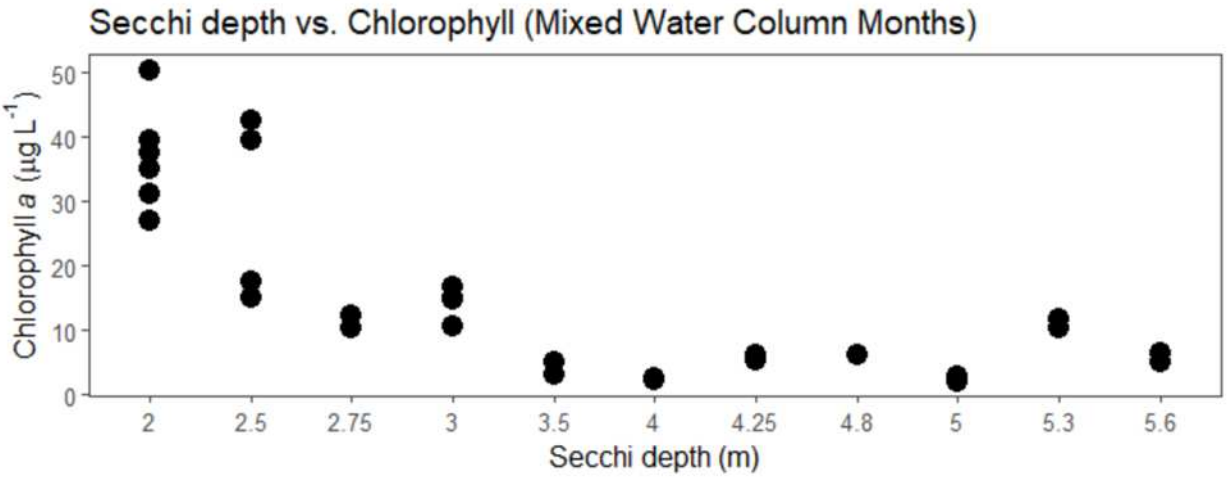

Figure S2.2. Secchi depth vs algal biomass, as estimated by chlorophyll a concentration, for months when the water column of Lake Yojoa is mixed (November-February).

CHAPTER 2 SUPPLEMENTARY TABLES AND FIGURES

Table S2.1. Physical characteristics of Lake Yojoa 1) Vaux and Goldman (1984) 2) Romero and Pineda (2007).

Lake Area (km^2)	88[1]
Lake Volume (km^3)	1.4[2]
Elevation (m above sea level)	638[1]
Catchment area (km^2)	337[1]
Mean depth (m)	14.6[2]
Maximum depth (m)	27.3[2]
1) Vaux and Goldman (1984) 2) Romero and Pineda (2007).	

Table S2.2. Mean hypolimnetic nutrient values for June 2018 and 2019 with corresponding Secchi depth.

	NH_4^+ (µM)	NO_3^- (µM)	TP (µM)	Secchi depth (m)
A	*Shallow, no hypolimnion*			
B	51.13	1.22	2.48	3.1
C	53.88	0.97	1.40	2.8
D	*Shallow, no hypolimnion*			
E	55.47	0.90	2.05	3.1
F	46.18	0.83	1.40	3.2
G	*Shallow, no hypolimnion*			2.8
H	35.15	0.67	1.95	2.7
I	57.24	1.09	2.35	2.6
J	*Shallow, no hypolimnion*			
K	59.36	1.50	3.22	2.5
L	*Shallow, no hypolimnion*			
M	66.95	2.47	1.68	2.5
N	6.31	1.61	0.79	2.2
O	16.88	2.99	0.62	2.6
P	55.47	1.00	3.42	3.2
Q	67.37	1.11	3.82	2.6
R	44.54	0.93	3.49	2.7

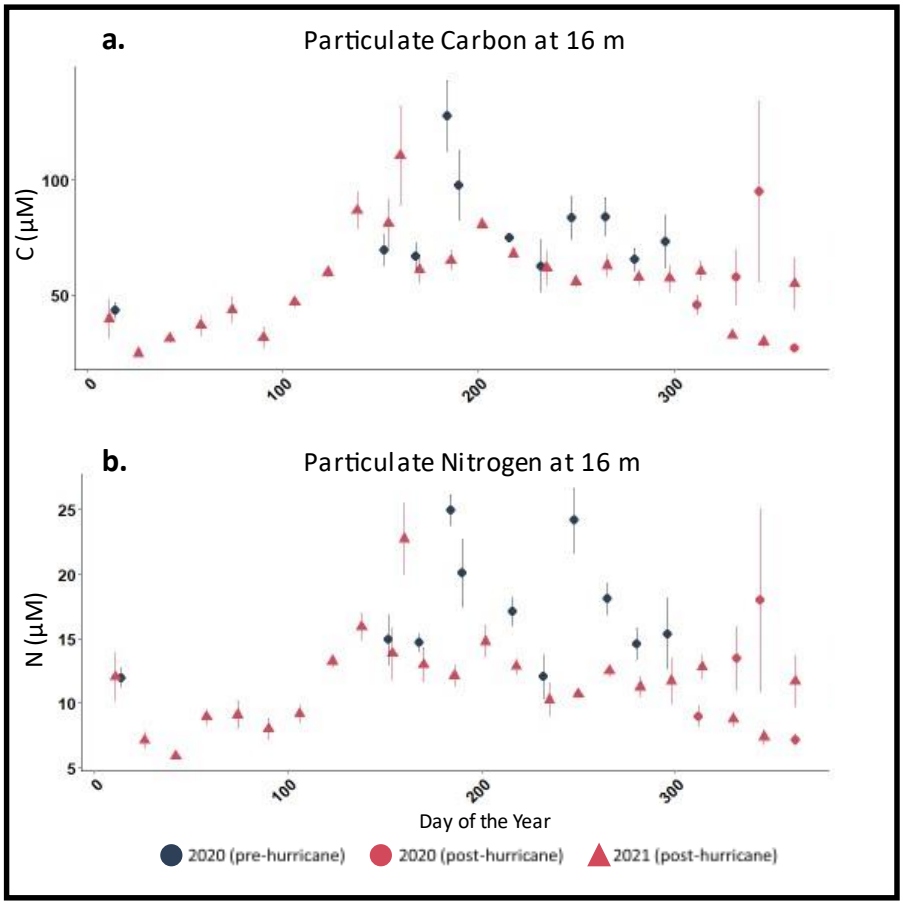

Figure S3.1. Particulate C (**a**) and N (**b**) at 16 m at continuously monitored in-lake locations. Data from February to May 2020 missing due to a combination of methodological errors (insufficient quantities of water filtered) and sampling disruption related to COVID-19.

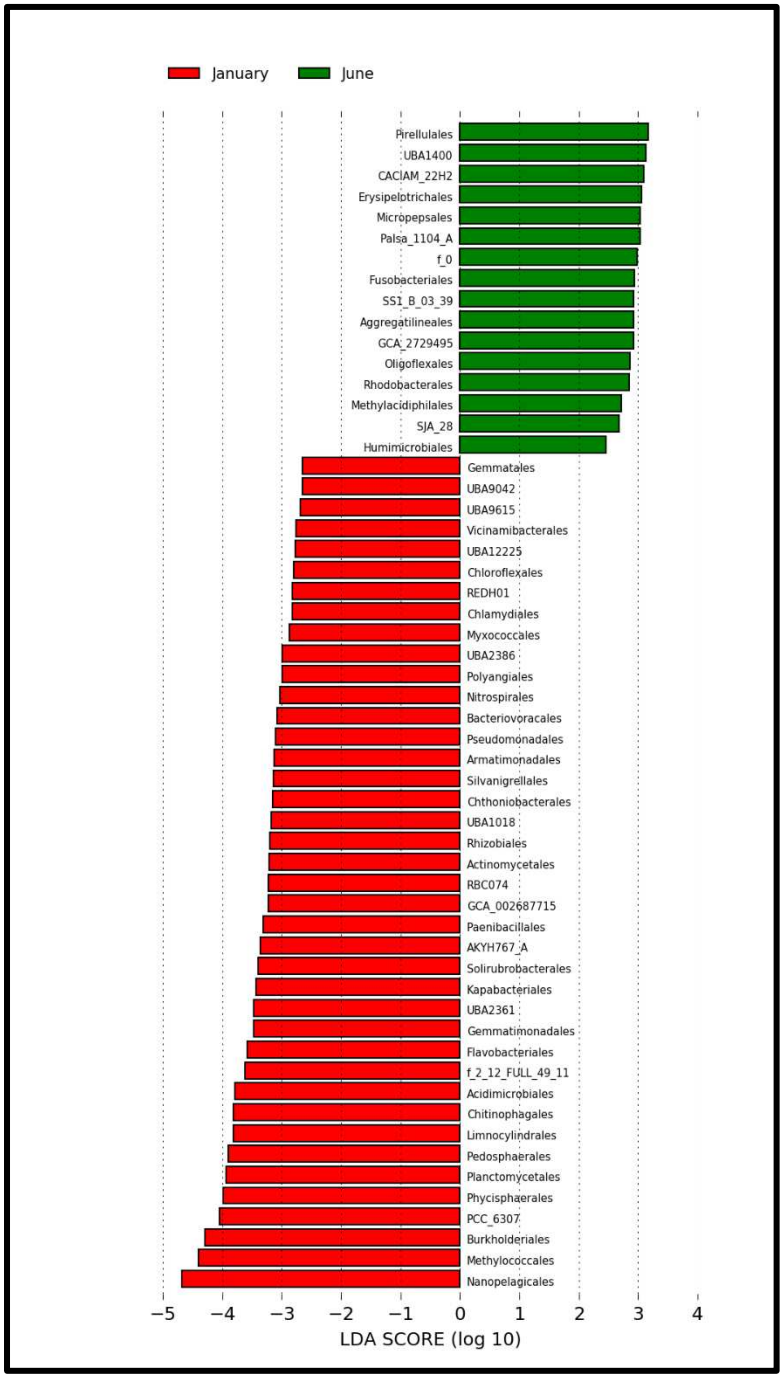

Figure S4.1. LEfSe analysis of seasonal dissimilarity at the order level.

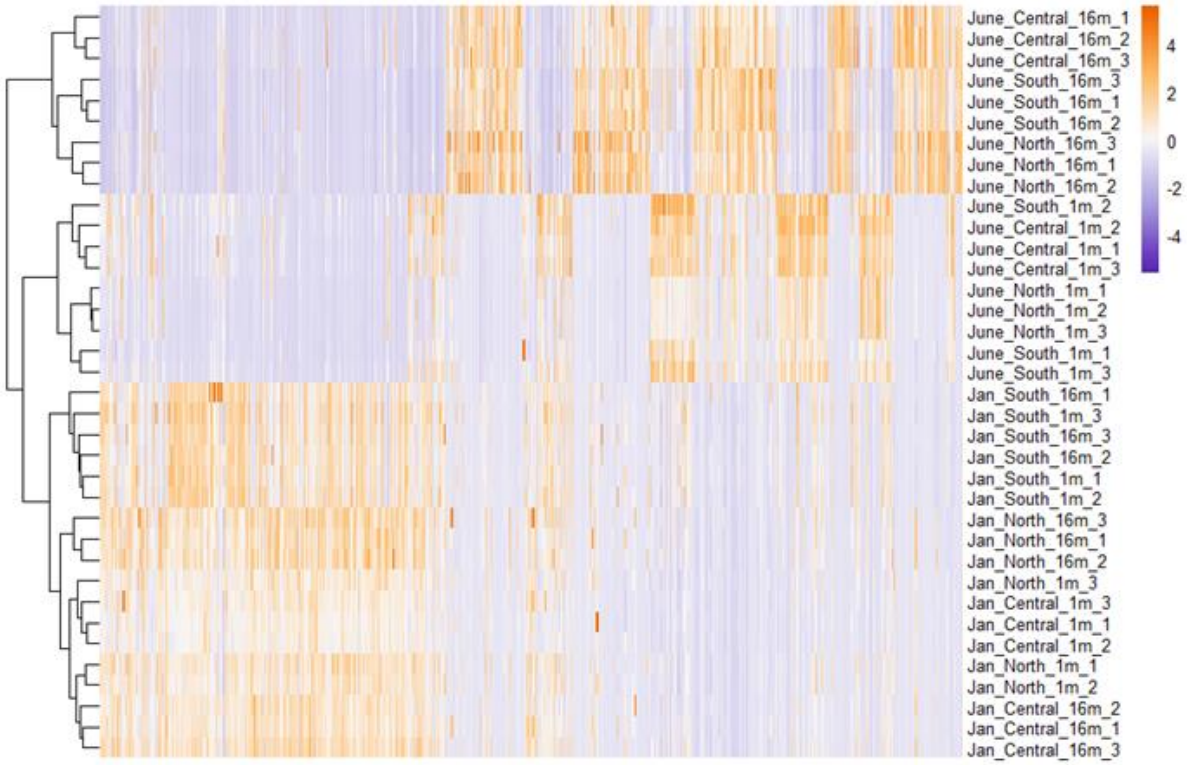

Figure S4.2. MAG relative abundance heatmap with hierarchical clustering.

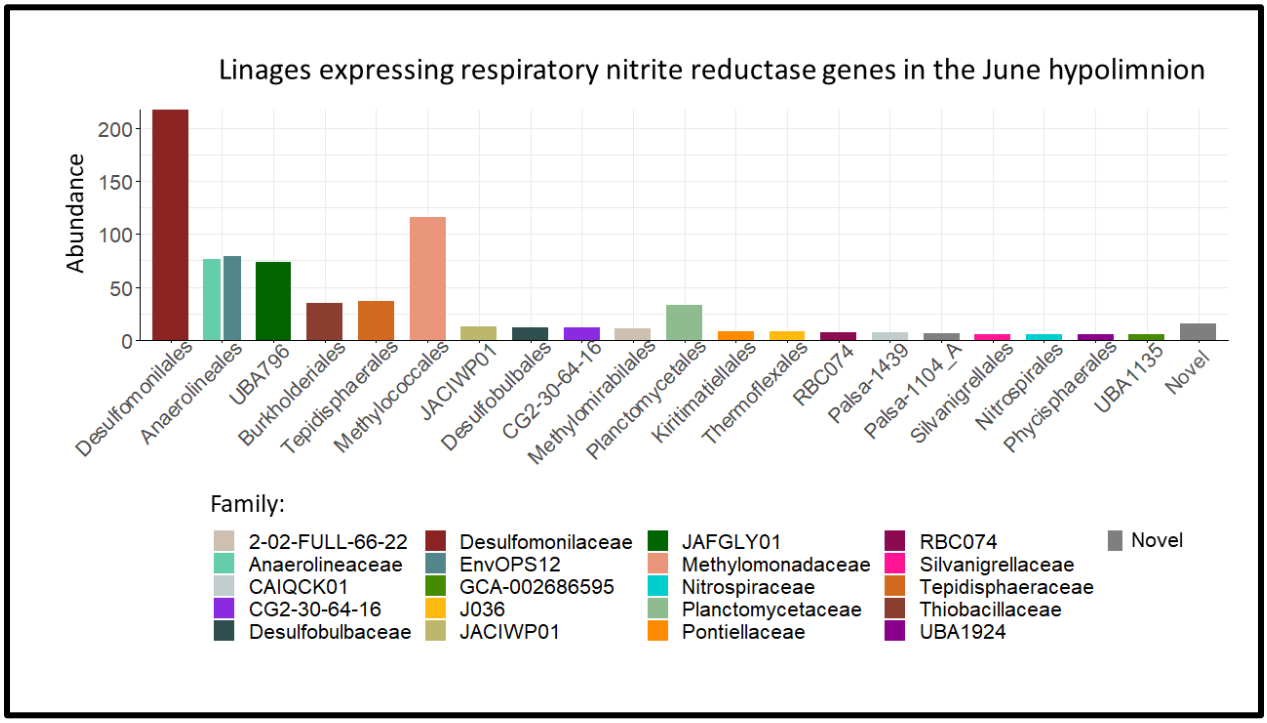

Figure S4.3. Lineages of genomes expressing genes related to respiratory nitrite reduction (Table S4.1)

Table S4.1 Nitrogen metabolism genes considered in this analysis.

Kegg ID	Gene	Description
K10944	*pmoA-amoA*	ammonia monooxygenase
K10945	*pmoB-amoB*	ammonia monooxygenase
K10946	*pmoC-amoC*	ammonia monooxygenase
K00367	*narB*	assimilatory nitrate reductase
K00372	*nasA*	assimilatory nitrate reductase
K00366	*nirA*	assimilatory nitrite reductase
K02568	*napB*	cytochrome c-type protein
K10535	*hao*	hox, hydroxylamine oxidase
K00368	*nirK*	nir, nitrite reductase (NO-forming)
K15864	*nirK*	nir, nitrite reductase (NO-forming)
K00374	*narI, narV*	nitrate reductase 1
K04561	*norB*	nitric oxide reductase
K02305	*norC*	nitric oxide reductase
K02588	*nifH*	nitrogenase iron protein
K02586	*nifD*	nitrogenase molybdenum-iron protein
K02591	*nifK*	nitrogenase molybdenum-iron protein
K00376	*nosZ*	nitrous-oxide reductase
K02575	*NRT, narK, nrtP, nasA*	NRT; MFS transporter, NNP family, nitrate/nitrite transporter
K00371	*narH, narY, nxrB*	nxr, nitrite oxidoreductase
K00370	*narG, narZ, nxrA*	nxr, nitrite oxidoreductase
K02567	*napA*	periplasmic nitrate reductase
K00362	*nirB*	respiratory nitrite reductase
K00363	*nirD*	respiratory nitrite reductase
K03385	*nrfA*	respiratory nitrite reductase
K15876	*nrfH*	respiratory nitrite reductase