LINKAGE BETWEEN BIOGEOCHEMICAL PROPERTIES AND MICROBIAL ACTIVITIES IN LAKE SEDIMENTS: BIOTIC CONTROL OF ORGANIC PHOSPHORUS DYNAMICS

By

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To those who fought, and still fight, so that women of my generation and the ones to come can have choices, opportunities, respect, and equal rights. There is still a long road towards respect and equality, hopefully it is soon to come.

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"We wrest secrets from nature by most unlikely routes. Societies will, of course wish to exercise prudence in deciding which applications of science are to be pursued and which not. But without funding of basic research, without supporting acquisition of knowledge for its own sake, our options become dangerously limited … Without vigorous, farsighted and continuing encouragement of fundamental scientific research, we are in the position of eating our seed corn: we may fend off starvation for one more winter, but we have removed the last hope of surviving the following winter." **CARL SAGAN** (The Dragons of Eden, p. 236, 1977)

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LINKAGE BETWEEN BIOGEOCHEMICAL PROPERTIES AND MICROBIAL ACTIVITIES IN LAKE SEDIMENTS: BIOTIC CONTROL OF ORGANIC PHOSPHORUS DYNAMICS

By

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Chair: K. Ramesh Reddy
Co-chair: Andrew Ogram
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In lakes, deposition of allochthonous and autochthonous particulate matter to sediments can alter the physico-chemical properties and associated biogeochemical processes. Coupling and feedback between sediment biogeochemistry and water column primary productivity often depends on biogeochemical processes within sediments and associated microbial communities. The current investigation was conducted to link biogeochemical properties of benthic sediments and microbial communities and their activities in sub-tropical lakes of different trophic state (Lake Annie - oligo-mesotrophic, Lake Okeechobee – eutrophic, and Lake Apopka - hypereutrophic). The central hypothesis of this study was that lakes with contrasting trophic states have sediments with different biogeochemical properties that have a selection pressure (i.e., C, N and P availability or limitation) on the microbial community that is reflected in their activities. Sediments sampled from sixteen different sites revealed that trophic state was not related to nutrient content of sediments. The relative abundance of phosphorus (P) forms in sediments was more important than total P concentration in characterizing the processes occurring in sediments. Laboratory batch incubation studies were conducted to determine the relationships between major sediment P forms, enzyme activity, heterotrophic microbial activity,

and nutrient limitation. Results showed that the concentrations of various P compounds changed with sediment depth, indicating that different processes were controlling P reactivity and mobility in these lakes. Also, P-associated enzyme activities were related to sediment microbial biomass and activity, as well as to the different P forms and availability in sediments. Microbial community biomass and activity, as well as incubation experiments, revealed that the Lake Annie sediment microbial community was carbon (C)-limited, while Lake Apopka was P-limited. Lake Okeechobee mud and sandy sediments were C and nitrogen (N) limited, whereas in the peat sediment a co-limitation of C and P was observed. Stable isotope analyses showed that, in each lake, different mechanisms control $\delta^{13}C$ and $\delta^{15}N$ signatures in these sediments, and were closely linked to lake physico-chemical properties, as well as the primary productivity in the water column. Isotopic signatures in the lake sediments showed a trend of enrichment in $\delta^{13}C$ and $\delta^{15}N$ with increasing trophic state. Oligo-mesotrophic Lake Annie sediment had the lowest values of $\delta^{13}C$ and $\delta^{15}N$. Eutrophic Lake Okeechobee mud sediments displayed intermediate values for both isotopes. And hypereutrophic Lake Apopka had the highest values for both $\delta^{13}C$ and $\delta^{15}N$. Catabolic response profiles of a wide variety of C-substrates added to sediments indicated that different microbial communities are present in these sediments. The microbial community of hypereutrophic lake sediments had higher efficiency use of energy and higher catabolic diversity. This study highlighted the relationships between sediment biogeochemical properties and the microbial community, how they differ among lakes with different trophic states, and how the physico-chemical conditions of lakes affect sediment properties and microbe-mediated processes. Results suggest that although the microbial community is C/energy limited, C, coupled with N and P availability had a strong influence on microbial communities in these lakes sediments.

CHAPTER 1
INTRODUCTION

In freshwater ecosystems an increase in external nutrient input resulting from anthropogenic activities is frequently the major cause of eutrophication (Krug 1993; Straskraba et al. 1995; Noges et al. 1998). Although some freshwater ecosystems can become eutrophic naturally, accelerated rate of eutrophication of many lakes is a direct consequence of high nutrient load from anthropogenic activities, such as agricultural practices and urban activities.

The main paths of anthropogenic eutrophication (also called cultural eutrophication) in lakes are: increase in input of nutrients (mainly nitrogen and phosphorus), increase of the phytoplankton biomass, loss of biological diversity, dominance by cyanobacteria, diatom, and unicellular green algae, occurrence of algae 'blooms' (high biomass production of certain species of algae at the water surface), reduction in light and oxygen availability, change in heterotroph community composition, death of fish. All these alterations will lead to an ecosystem change, loss of species diversity and decrease in water quality. Hence, lakes with different trophic states (oligotrophic: low productivity, mesotrophic: medium productivity, eutrophic: high productivity and hypereutrophic: very high productivity) will have distinctive physical, chemical and biological characteristics (i.e., pH, redox potential, and microbial community).

Sediment Organic Matter

Particulate matter that enters a lake (allochthonous) or is produced within a lake (autochthonous) is deposited and becomes an integral part of sediments. Consequently, lakes function as natural traps for particulate matter and associated nutrients. Accumulation and retention of particulate matter and nutrients in sediments depends on lake morphometry, water renewal, nutrient loading, edaphic characteristics of the drainage basin, among others (Boström et al. 1988) and can alter the physico-chemical properties of sediments and associated

biogeochemical processes (Rybak 1969). Organically bound nutrients in particulate matter supplied to the sediment are mineralized by heterotrophic decomposition, resulting in release of nutrients into the water column and stimulation of biological productivity (Capone and Kiene 1988; Gächter and Meyer1993; Brooks and Edgington 1994). Consequently benthic sediments play a critical role in nutrient cycling by acting as both sources and sinks of nutrients (Figure 1-1).

Lake sediments contain an archive of past environmental conditions in and around the water body (Smol 1992) and can be used to document anthropogenic impacts through time (Smeltzer and Swain 1985). Sediment organic matter (OM) provides information about past impacts and biogeochemical processes within lakes, and has been studied extensively using paleolimnological methods (Meyers 1997). The timing of past events in a basin is based on reliable dating of sediment cores. Sediment dating provides an age/depth relation from which bulk sediment accumulation rates can be calculated (Smeltzer and Swain 1985). The lead-210 (^{210}Pb) technique is used routinely to provide age/depth relations for the last 100-150 years (Appleby et al. 1986), and has been used widely in studies of Florida lake sediment cores (e.g., Binford and Brenner 1986; Brezonik and Engstrom 1998; Whitmore et al. 1996; Brenner et al. 2006; Schottler and Engstrom 2006). Bulk sediment accumulation rates in combination with analyses of sediment composition, can be used to calculate accumulation rates of sediment constituents such as OM and nutrients. Such measures provide insights into past changes in productivity and human impacts on the aquatic ecosystem.

Nutrient and OM accumulation rates in sediment have been studied in conjunction with stable isotope analyses (δ^{13}C and δ^{15}N) to infer past environmental impacts in marine (e.g., Gearing et al. 1991; Savage et al. 2004), lacustrine (e.g., Schelske and Hodell 1991; Gu et al.

1996; Bernasconi et al. 1997; Hodell and Schelske 1998; Ostrom et al. 1998; Brenner et al. 1999), and riverine ecosystems (e.g., McCallister et al. 2004; Anderson and Cabana 2004; Brunet et al. 2005). Measurements of $\delta^{13}C$ and $\delta^{15}N$ in several lake compartments, (i.e., dissolved and particulate matter in the water column and sediments) have been used to identify the origin of lacustrine OM (Filley et al. 2001; Griffths et al. 2002), infer past primary productivity (Schelske and Hodell 1991; Hodell and Schelske 1998; Bernasconi et al. 1997), document historical eutrophication (Gu et al. 1996; Ostrom et al. 1998; Brenner et al. 1999), elucidate biogeochemical cycles (Terranes and Bernasconi 2000; Jonsson et al. 2001; Lehmann et al. 2004), and shed light on microbial activity (Hollander and Smith 2001; Lehmann et al. 2002; Gu et al. 2004; Terranes and Bernasconi 2005; Kankaala et al. 2006).

Allochthonous OM usually has more negative $\delta^{13}C$ values than does autochthonous OM. Values of $\delta^{13}C$ can also be used to distinguish periods of high versus low primary productivity. Algae fractionate against the heavier isotope, ^{13}C. Consequently, under conditions of low to moderate primary productivity autochthonous OM displays high negative $\delta^{13}C$. During periods of very high primary productivity the preferred ^{12}C in the water column is exhausted and fractionation is diminished, yielding OM with higher $\delta^{13}C$ (Mizutani and Wada 1982; Raul et al. 1990). Hypereutrophic lakes with high rates of primary productivity have low concentrations of carbon dioxide (CO_2) in the water column. Moreover, in alkaline (high-pH) waters bicarbonate (HCO_3^-) dominates the dissolved inorganic carbon, and has a $\delta^{13}C$ that is 8‰ heavier than dissolved CO_2 (Fogel et al. 1992). High demand for inorganic carbon and low free CO_2 leads to utilization of HCO_3^- as a carbon source resulting in heavier $\delta^{13}C$ of OM (Goericke et al. 1994).

Stable isotope signatures of sediment OM can be used to identify impacts of anthropogenic activities. Sources of OM from wastewater and agricultural runoff can be identified because they

yield OM depleted in $\delta^{13}C$ and enriched in $\delta^{15}N$ (Gearing et al. 1991; Burnett and Schaffer 1980; Savage et al. 2004). Stable isotope $\delta^{15}N$ has also been used to study the nitrogen (N) biogeochemical cycle. Measurement of $\delta^{15}N$ in suspended and sedimented OM was used to address the source of N, as well as N limitation of, and utilization by the phytoplankton community in Lake Lugano (Terranes and Bernasconi 2000).

Sediment Phosphorus

Phosphorus (P) is often the limiting nutrient for primary productivity in freshwater ecosystems. Sources of P to lakes can be external (allochthonous) or internal (autochthonous). Allochthonous P input originates in the drainage basin, while autochthonous P originates from primary and secondary productivity within lakes. A major portion of P from these sources added to the water column accumulates in sediments. Sediment P is present in both inorganic and organic forms. Organic P and cellular constituents of the biota represent 90% of total P (TP) in freshwater ecosystems (Wetzel 1999), and in sediments 30-80% of TP is typically in organic form (Williams and Mayer 1972; Boström et al. 1982).

Although organic P is an important component of sediment P, it has been relatively under-studied as compared with the fate of inorganic P (Turner et al. 2005). The reason for this is that there is no direct way to measure organic P. It is usually estimated by difference (before and after ignition at high temperature) (Saunders and Williams 1955), or by sequential extraction or chemical fractionation (Condron et al. 2005; McKelvie 2005). These chemical fractionations are based on different solubilities of P forms in alkaline and acid extractions with different pH. Turner et al. (2006) compared two methodologies, chemical fractionation and phosphorus-31 nuclear magnetic resonance (^{31}P NMR) spectroscopy, to measure organic P, and showed that for wetland soils, alkaline extraction with molybdate colorimetry overestimated organic P by 30-54%. They concluded that alkaline extraction with ^{31}P NMR spectroscopy is a more accurate

method to quantify organic P. In recent years there have been many studies using this methodology to distinguish different organic P forms in lake sediments (Hupfer et al. 1995, 2004; Carman et al. 2002; Ahlgren et al. 2005; Ahlgren et al. 2006a, b; Reitzel et. al 2006a, b, 2007). Phosphorus-31 NMR spectroscopy can identify different P compounds, based on their binding properties, as orthophosphate, pyrophosphate (pyro-P), polyphosphate (poly-P), phosphate monoester, phosphate diester (e.g., DNA, lipids), and phosphonates (Newman and Tate 1980; Turner et al. 2003).

These different P compounds present in the sediment will be released to the water column (internal load) due to chemical, physical and biological processes (Figure 1-2). Therefore benthic sediments may play a critical role in P cycling by acting as sources or as sinks for P. With reduction and control of the external nutrient load, the internal load can become a major issue in regulating the trophic state and the time lag for recovery of lakes (Petterson 1998). Determination of the relative abundance of different P forms in sediments is important to understand sediment P processes and internal loading.

Organic P compounds present in sediments must be hydrolyzed before their uptake by microorganisms (Chrost 1991; Sinsabaugh et al. 1991). Organic P is hydrolyzed by enzymes produced by microbial communities (Gächter et al. 1988; Davelaar 1993; Gächter and Meyer 1993), and the product of enzymatic hydrolysis is orthophosphate that can be readily used by microorganisms (Barik et al. 2001) (Figure 1-2). Enzyme production can be induced by the presence of organic P and low levels of bioavailable inorganic P (Kuenzler 1965; Aaronson and Patni 1976). On the other hand, high levels of inorganic P inhibit the synthesis of enzymes (Torriani 1960; Lien and Knutsen 1973; Elser and Kimmel 1986; Jasson et. al. 1988; Barik et al. 2001).

Three main groups of hydrolytic enzymes are responsible for phosphate release: non specific and/or partially specific phosphoesterases (mono and diesterase), nucleotidases (mainly 5'-nucleotidase), and nucleases (exo and endonucleases) (Chrost and Siuda 2002). Phosphomonoesterases (PMEase) are nonspecific enzymes that hydrolyze phosphate monoester, and are reported to be produced by several microorganisms (e.g., bacteria, algae, fungi, and protozoa) that are found in the water column and sediment of lakes. Nonspecific PMEases are divided into two groups, depending on the pH at which they exhibit maximum activity, alkaline (pH 7.6-10) and acid (pH 2.6-6.8) (Siuda 1984). Both can be found inside or outside the cell, and the same cell can produce both alkaline and acid PMEase (Siuda 1984). Although both PMEase activities have been reported to be regulated by availability of orthophosphate, acid PMEase is usually regarded as a constitutive enzyme (Siuda 1984; Jasson et al. 1988).

The production of constitutive enzymes is not repressed nor stimulated by high or low orthophosphate availability in the environment. Its production is related to P concentration and demand inside the cell (Siuda 1984, Jasson et al. 1988). Jasson et al. (1981), however, suggested that in acidified lakes, acid PMEase may have a similar role to that of alkaline PMEase in neutral systems, as its production is also inhibited by orthophosphate. In aquatic systems, alkaline PMEase is by far the most studied enzyme, probably due to the high number of systems with neutral pH, that are inappropriate for preservation of extracellular acid PMEase (Siuda 1984).

Another important phosphatase is phosphodiesterase (PDEase) that hydrolyzes phosphate diester and is known to degrade phospholipids and nucleic acids (Hino 1989; Tabatai 1994; Pant and Warman 2000). It is the least studied enzyme in freshwater ecosystems. Few studies have reported on the occurrence and distribution of phosphatases or other organic P hydrolyzing

enzymes in sediments or their association with sediment bacteria (Wetzel 1991; Chrost and Siuda 2002).

The association of carbon (C), nitrogen (N) and P influences the structure, energetics and function of all life forms. The degradation of organic P is closely related to organic C degradation, as both are constituents of the OM. As an example, Siuda and Chrost (2001) demonstrated from controlled experiments that PMEase activity of bacteria is used for organic P hydrolysis and uptake of associated organic C moieties, concluding that bacterial PMEase contributes substantially to dissolved organic carbon (DOC) decomposition in lake water. Dissolved organic carbon is an important constituent of the C pool in an aquatic ecosystem, and due to the bacteria activity it can be converted to particulate organic C (POC) and thus become available to the upper levels of the aquatic food web (Søndergaard 1984, Azam 1998). As C is the major driver and basic constituent in all living forms, its cycle is strongly linked to the P cycle. As a result C:P ratios of the sediment-water column can influence P uptake by the bacteria community.

Nitrogen is also one of the major nutrients required for cell metabolism. Nitrogen is considered, together with P, to be responsible for the eutrophication process. In lakes where P is present in high concentrations, N can become the limiting nutrient for productivity (Wetzel 2001). The main difference between the P and N cycles is that the N cycle has an important gaseous phase that does not occur in the P cycle. The Redfield ratio, reported by Redfield et al. (1963) with respect to marine plankton, stated that there is a constancy in the molar C:N:P ratio = 106:16:1 (by weight 41:7.2:1). This ratio can be applied to different ecosystems and to processes such as decomposition of OM. The C:N:P ratio of materials is reflected in the composition of the

phytoplankton productivity (Wetzel 2001). Deviations in this ratio can indicate nutrient limitation as well as affect P uptake by microorganisms.

Microbial Communities

Coupling and feedback between sediment biogeochemistry and water column primary productivity often depends on biogeochemical processes within sediments and associated microbial communities. Heterotrophic bacteria play an important role in C and nutrient cycling in lakes. Phytoplankton and/or heterotrophic bacteria are the major drivers of C and nutrient cycling in the water column, while the heterotrophic bacteria dominate in sediments. Allochthonous and autochthonous particulate OM in the water column is deposited in the sediment, leading to high concentrations of nutrients and high microbial biomass. Lake depth affects the quality of organic material reaching the sediment. In deep lakes, sedimenting OM undergoes intense decomposition in the water column, due to the prolonged period of settling. Consequently low amounts of labile organic C reach the sediment (Suess 1980; Meyers 1997). In shallow lakes, the supply of labile C and nutrients can be higher than in deep lakes, and the latter often can have more refractory OM.

Organic matter deposition is an important source of C to sediments. Organic compounds and associated nutrients supplied to the sediment surface are mineralized through heterotrophic decomposition (Gächter and Meyer 1993; Capone and Kiene 1988; Megonigal et al. 2004) (Figure 1-3). The composition and activities of the microbial community are regulated by the quality and availability of C. In high depositional environments, such as eutrophic, or deep thermally stratified lakes, organic content in sediments is often high, oxygen (O_2) consumption occurs rapidly, and O_2 is depleted several millimeters below the sediment water interface (Jørgensen 1983; Jørgensen and Revsbrech 1983). In these systems, facultative and strict anaerobic communities dominate. Complete oxidation of a broad range of organic compounds in

these systems can occur, especially through the sequential activity of different groups of anaerobic bacteria (Capone and Kiene 1988).

In methanogenic habitats, i.e., in the absence of inorganic electron acceptors, different groups of microorganisms participate in decomposition of OM as no single anaerobic microorganism can completely degrade organic polymers (Zinder 1993, Megonigal et al. 2004). Cellulolytic bacteria hydrolyze organic polymers through extracellular enzyme production and further break down monomers to alcohols, fatty acids, and hydrogen (H_2) through fermentation. Alcohols and fatty acids are degraded by syntrophic bacteria (secondary fermenters) into acetate, H_2, and carbon dioxide (CO_2), which is used as a substrate by methanogens (Zinder 1993, Conrad 1999, Megonigal et al. 2004). The structures and functions of anaerobic microbial communities are therefore strongly affected by competition for fermentation products such as H_2 and acetate. Microorganisms derive energy by transferring electrons from an external source or donor to an external electron sink or terminal electron acceptor.

Organic electron donors vary from monomers that support fermentation to simple compounds such as acetate and methane (CH_4). Fermenting, syntrophic, methanogenic bacteria and most other anaerobic microorganisms (e.g., sulfate, iron reducers) are sensitive to the concentrations of substrates and products. Their activities can be inhibited by their end products and are dependent on the end product consumption by other organisms (Stams 1994; Megonigal et al. 2004). While fermenting bacteria shift their product formation to more oxidized products, syntrophic bacteria only metabolize compounds when methanogens or other anaerobic bacteria consume H_2 and formate efficiently (Stams 1994).

Microbial functional diversity includes a vast range of activities. One component of this diversity has been characterized by measuring catabolic response profiles, i.e., short-term

response of microbial communities to addition of a wide variety of C-substrates (Degens and Harris 1997; Degens 1998a). This has been widely applied in soil studies to address differences in microbial communities in different soil types, disturbance, and land use (Degens and Harris 1997; Lu et al 2000; Degens et al. 2000, 2001; Stevenson et al. 2004). Substrate induced respiration is often dependent on the size of the microbial biomass pool, however, response of microbial communities is also related to the catabolic diversity of soil microorganisms (Degens 1998). A greater relative catabolic response to a substrate in one system as compared with another indicates that the microbial community is more functionally adapted to use that resource as well as the presence of enzymes capable of their utilization, and previous exposure to different C-sources (Degens and Harris 1997; Degens 1998; Baldock et al. 2004; Stevenson et al. 2004).

Metabolic response of a microbial community in lake sediment may vary due to several factors that influence either the microbial community or due to physico-chemical characteristics of lakes, which include source and chemical composition of particulate matter and biogeochemical processes in the sediment and water column. Eutrophic and hypereutrophic lakes usually receive high external loads of nutrients and display high primary productivity and nutrient concentrations in the water column and these nutrients eventually reach the sediment, therefore sediments from eutrophic and hypereutrophic lakes are expected to have high concentrations of OM.

Binford and Brenner (1986) and Deevey et al. (1986) showed that net accumulation rates of OM and nutrients increase with trophic state for Florida lakes. In contrast small, oligotrophic lakes are expected to have relatively high proportions of allochthonous C input to their sediments (Gu et al. 1996). Sediments with different C-sources quality and quantity as well as nutrient concentration, will have different microbial communities. These communities can display

distinct a catabolic response, as the mineralization rates of a microbial community are dependent upon the metabolic capacity for a given substrate (Torien and Cavari 1982).

Several factors limit bacterial metabolism in sediments, i.e., temperature, C, and nutrient concentration. Most studies of microbial activity in sediments focus on C limitation and the effect of electron donors or acceptors in the production of CO_2 and/or CH_4 (Capone and Kiene 1988; Schulz and Conrad 1995; Maassen et al. 2003; Thomsen et al. 2004). Little work has been done relating production of CO_2 and CH_4 with biogeochemical properties of sediments such as nutrient availability. Studies in the water column of lakes have shown that several factors can limit bacterial metabolism (Gurung and Urabe 1999; Jasson et al. 2006).

Although it has been generally accepted that the heterotrophic community is C/energy limited, recent studies have shown that inorganic nutrients, especially P, can be the most limiting nutrient for the bacterial community (Gurung and Urabe 1999; Vadstein 2000; Olsen et al. 2002; Vadstein et. al. 2003; Smith and Prairie 2004; Jasson et al. 2006). Reviewing data from freshwater ecosystems, Vadstein (2000) showed that P limitation is a common phenomenon. Phosphorus limitation occurred in 86% of the cases, while N or C limitation was identified in 15% and 20%, respectively (percentages add up to more than 100% due to methodological aspects, cf. Vadstein 2000). Heterotrophic microbial metabolism can be limited by a single factor or multiple variables. Limitation varies among lakes and depends on lake characteristics and biogeochemical properties of the sediment.

Benthic sediments play a critical role in nutrient cycling by acting as sources or sinks for nutrients, and heterotrophic metabolism dominates in this compartment (Figure 1-3). It is, thus, important to study biogeochemical properties of sediments and how they relate to microbial community composition, growth, and activity to better understand processes that occur in

sediment. The primary goal of this study was to develop a linkage between the biogeochemical properties of benthic sediments and their bacterial communities in relation to their activities in sub-tropical lakes of different trophic states. The main focus of this study was on P compounds as it is the nutrient that in high concentration is reported to be responsible for eutrophication of freshwater ecosystems. The central hypothesis of this study was that lakes with contrasting trophic states will have sediments with different biogeochemical properties that will have a selection pressure (i.e., C, N and P availability or limitation) on the microbial community that will be reflected by their activities.

Site Descriptions

Three Florida lakes (USA) were selected for this study based on water quality variables and trophic status (Figure 1-4). Lake Annie, a small (0.37 km^2) oligo-mesotrophic lake, is located in south-central Florida (Highlands County) at the northern end of the Archbold Biological Station. Lake Annie is characterized by pristine water quality with little surface water input (most is ground water), and low anthropogenic impact due to the absence of development around the lake (Layne 1979). This lake has no natural surface streams but two shallow man made ditches allow surface water to flow into the lake and contribute to water and nutrient inputs during high rainfall periods (Battoe 1985). Benthic sediments vary from organic to sand in the littoral zone (Layne 1979) (Figure 1-4).

Lake Okeechobee is a large (1800 km^2) shallow lake located in south Florida. It is considered to be a eutrophic lake that has experienced cultural eutrophication over the last 50 years (Engstrom et al. 2006). Benthic sediments are characterized as: mud (representing 44% of the total lake surface area), sand and rock (28%), littoral (19%), dominated by macrophyte growth, and peat (9%) that refers to partially decomposed plant tissues (Fisher et al. 2001) (Figure 1-4).

Lake Apopka is also a shallow lake with 125 km^2 of surface area, located in central Florida. Once a clear-water macrophyte-dominated lake, Lake Apopka has changed to a turbid, algal-dominated lake since 1947 (Clugston 1963). This shift may have been caused by nutrient input from several sources, including agricultural drainage from adjacent vegetable farms (Baird and Bateman 1987, Schelske et al. 2000), although some suggest that the proximal 'trigger' for the switch was a hurricane or tornado (Bachmann et al. 1999). Even though these inputs were controlled and regulated to some degree, the eutrophication process continued and Lake Apopka is considered hypereutrophic. Benthic sediments are characterized by unconsolidated material, which mainly consists of algal deposits (Reddy and Graetz 1991) (Figure 1-4).

Objectives

The specific objectives of this study were to:

- Determine the biogeochemical properties of sediments and examine relationships among sediment biogeochemical properties (nutrient concentrations and availability) and microbial biomass and activity (Chapter 2).
- Determine relative distributions of P compounds in sediment profiles using two different techniques, ^{31}P NMR spectroscopy and a P chemical fractionation scheme. (Chapter 3).
- Characterize P-related enzyme activities in sediment profiles and determine relationships between different P compounds and enzyme activities (Chapter 4).
- Determine stratigraphic biogeochemical properties in sediment cores and evaluate how they are related to microbial biomass and activity; and establish whether there is nutrient limitation of the microbial community (Chapter 5).
- Determine the source and long-term accumulation of OM to sediments using $\delta^{13}C$ and $\delta^{15}N$ signatures. (Chapter 6).
- Evaluate the catabolic diversity of microbial communities in sediments (Chapter 7).
- Identify microbial communities that utilize acetate through RNA-stable isotope probing (Chapter 8).

A series of field sampling and laboratory studies were conducted to accomplish these objectives. Results of these studies provided insights into the relationships between sediment

biogeochemical properties and the microbial community, how they differ among lakes with different trophic states. Moreover, it demonstrated the importance of considereing several variables, such as C, N and P, to address questions related to microbial communities.

Dissertation Format

This dissertation begins with Chapter 1 in which a general introduction, main hypothesis and objectives are presented. Chapter 2 consists of a characterization of biogeochemical properties and microbial community activity of sediments (0-10 cm) from sixteen different sites from the three different lakes. The following four chapters (3, 4, 5 and 6) present data from the studies conducted in deep cores collected from selected sites. In Chapter 3, organic P compounds were characterized in sediment profile using two different techniques, ^{31}P NMR spectroscopy a and chemical P fractionation scheme. Chapter 4 focused on P-related enzyme activities and Chapter 5 focused on vertical distribution of microbial biomass and activity and addressed nutrient limitation in each sediment type. Chapter 6 investigated the long-term OM accumulation and stable isotope signatures in sediments of the three lakes. Microbial functional diversity of sediments (0-10 cm) of the lakes was investigated in Chapter 7 by measuring catabolic response to a wide variety of C-substrates. Chapter 8 presents the study of identification of microorganisms that utilize acetate in these sediments using RNA stable isotope probing. Chapter 9 is the summary and conclusions of the results of the dissertation.

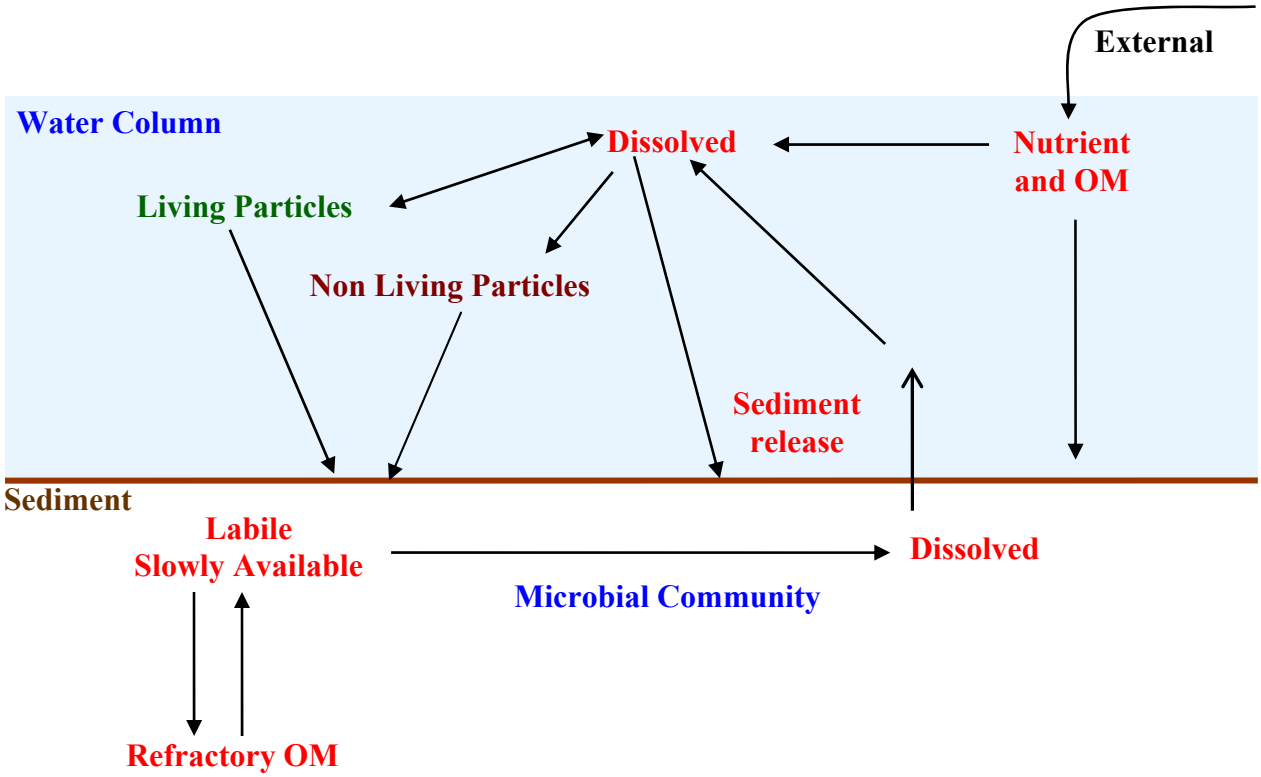


Figure 1-1. Schematic of major processes occurring in sediment and water column of lakes.

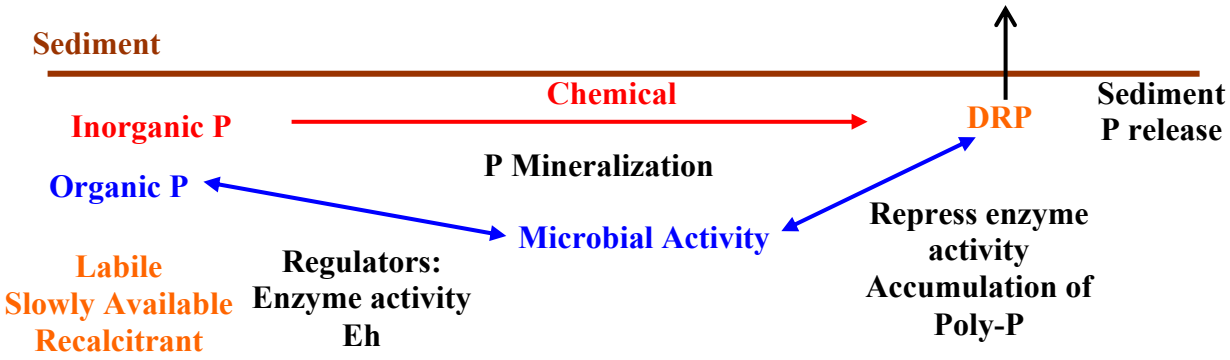


Figure 1-2. Schematic showing draw of chemical and biological P processes in lake sediments.

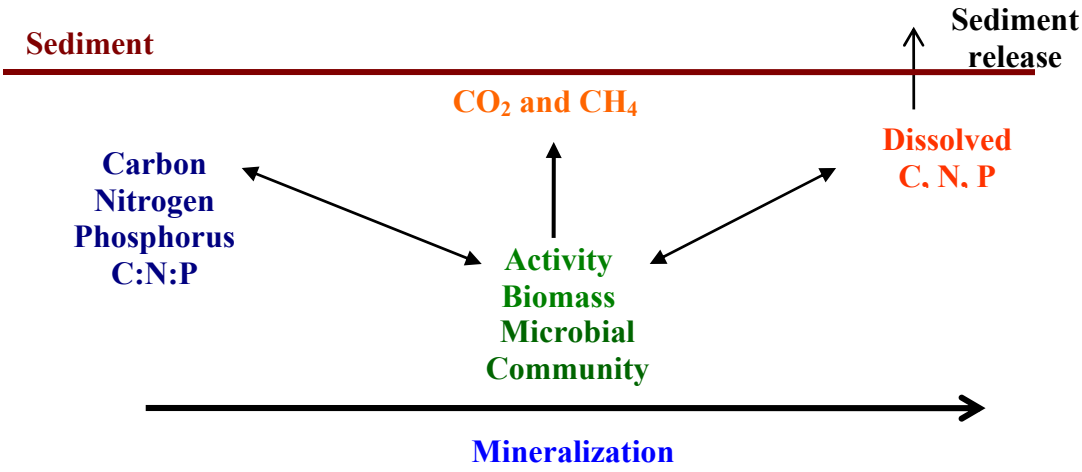


Figure 1-3. Schematic showing mineralization of organic matter through heterotrophic microbial activities in sediments.

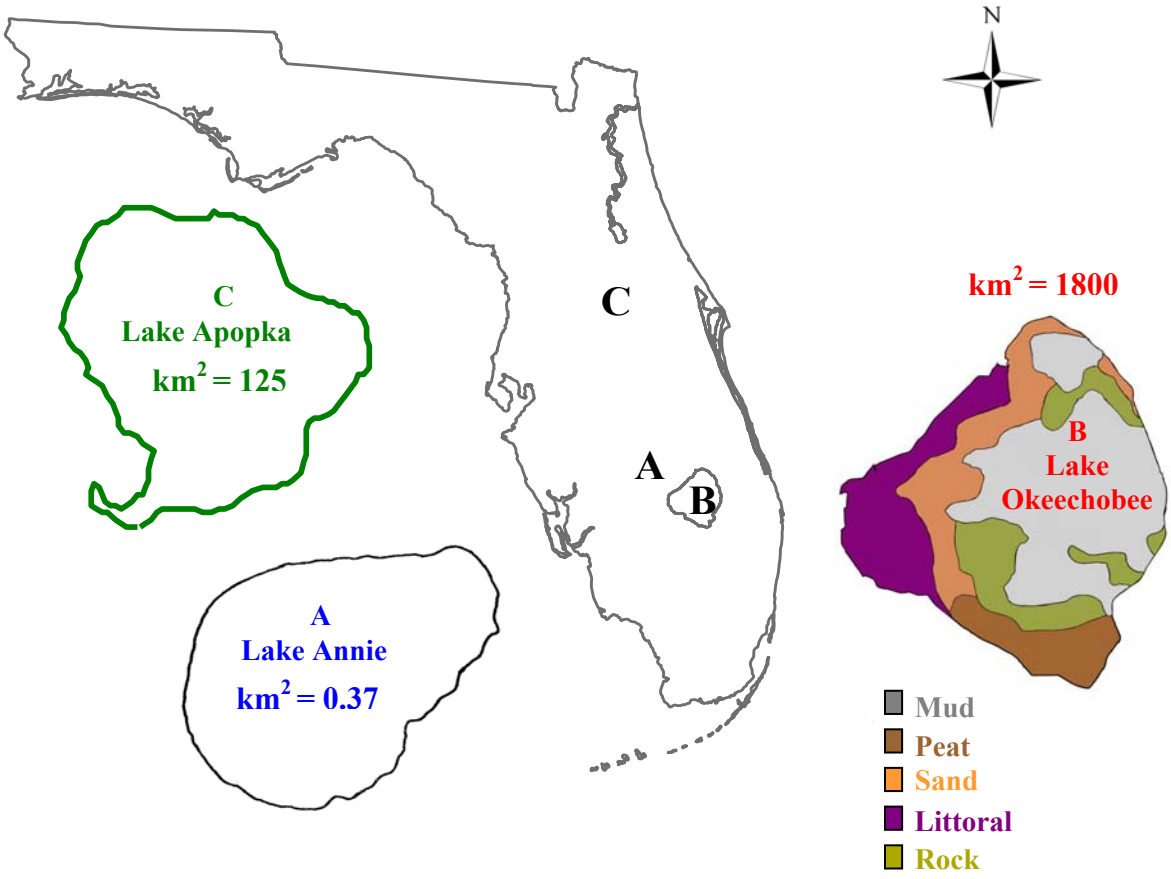


Figure 1-4. Map of Lake Annie, Lake Okeechobee, and Lake Apopka with their location in Florida State.

CHAPTER 2
BIOGEOCHEMICAL PROPERTIES AND MICROBIAL ACTIVITY OF BENTHIC SEDIMENTS OF SUBTROPICAL LAKES

Introduction

Particulate matter that enters a lake (allochthonous) or is produced within a lake (autochthonous) is deposited and becomes an integral part of sediments. Consequently, lakes function as natural traps for particulate matter and associated nutrients. Accumulation of particulate matter can alter the physico-chemical properties of sediments and associated biogeochemical processes in the sediment and water column (Rybak 1969). Accumulation and retention of particulate matter and nutrients in sediments depends on lake morphometry, water renewal, nutrient loading, edaphic characteristics of the drainage basin, among others (Boström et al. 1988). Lake sediment characteristics can provide evidence of anthropogenic impacts through time (Smeltzer and Swain 1985) as lake histories are archived in sediments (Smol 1992). Organically bound nutrients in particulate matter supplied to the sediment are mineralized by heterotrophic decomposition, resulting in release of nutrients into water column and potential for stimulation of biological productivity (Capone and Kiene 1988; Gächter and Meyer 1993; Brooks and Edgington 1994). Consequently benthic sediments may play a critical role in nutrient cycling by acting as both sources and sinks of nutrients.

Coupling and feedback between sediment biogeochemistry and water column primary productivity often depends on biogeochemical processes within sediments and associated microbial communities. Oxygen (O_2) availability in lake sediments typically is restricted to the uppermost few millimeters below the sediment-water interface due to limited O_2 diffusion and rapid O_2 consumption by the heterotrophic community (Charlton 1980; Boström et al. 1982). Facultative and strict anaerobic communities typically dominate the sediments. Anoxic sediments can be a good habitat for bacterial growth as they usually have high concentrations of

organic matter and inorganic nutrients (Fenchel et al. 1990; Pace and Funke 1991; Cole et al. 1993). In methanogenic habitats, i.e., in the absence of inorganic electron acceptors, different groups of microorganisms participate in decomposition of organic matter as no single anaerobic microorganism can completely degrade organic polymers (Zinder 1993, Megonigal et al. 2004). Fermenting bacteria hydrolyze organic polymers through enzyme production and further break down monomers to alcohols, fatty acids and hydrogen (H_2). Alcohols and fatty acids are degraded by syntrophic bacteria into acetate, H_2 and carbon dioxide (CO_2), which are used as substrates by methanogens (Zinder 1993; Conrad 1999; Megonigal et al. 2004). Consequently, carbon dioxide (CO_2) and methane (CH_4) are important end products in anaerobic decomposition of organic matter and their concentration can be used as a measure of microbial activity in sediments. The availability and quality of organic material can influence the microbial community, due to nutrient limitation for bacterial growth and competition for resources.

Several factors limit bacterial metabolism in sediments, i.e., temperature, biodegradable organic carbon, nutrients, and electron acceptors. Most studies of microbial activity in sediments focus on carbon (C) limitation and the effect of electron donors or acceptors in CO_2 and/or CH_4 production (e.g. Capone and Kiene 1988; Schulz and Conrad 1995; Thomsen et al 2004). Few studies have related production of CO_2 and CH_4 with biogeochemical properties of sediments and with nutrient availability or limitation. Benthic sediments play a critical role in nutrient cycling by acting as sources or sinks for nutrients, and heterotrophic metabolism dominates in this compartment. Thus, it is important to study biogeochemical properties of sediments and how they relate to microbial community composition, growth, and activity to better understand processes occurring in lake sediment.

The central hypothesis of this study was that lakes with contrasting trophic states will have sediments with different biogeochemical properties that will have a selection pressure (i.e., C, N and P availability or limitation) on the microbial community; that will be reflected in their activities. The specific objectives of this study were to: (i) determine the biogeochemical properties of benthic sediments in three subtropical Florida lakes with different trophic states (Lake Annie - oligo-mesotrophic, Lake Okeechobee – eutrophic, and Lake Apopka - hypereutrophic), and (ii) examine relationships among sediment biogeochemical properties (nutrient concentrations and availability) and microbial biomass and activity.

Materials and Methods

Study Sites

Three Florida lakes (USA) were selected for this study based on water quality variables and trophic status (Table 2-1, Figure 2-1). Lake Annie (Figure 2-1A), a small (0.37 km^2) oligo-mesotrophic lake, is located in south-central Florida (Highlands County) at the northern end of the Archbold Biological Station. Lake Annie is characterized by pristine water quality with little surface water input (most is ground water), and low anthropogenic impact due to the absence of development around the lake (Layne 1979). This lake has no natural surface streams but two shallow man made ditches allow surface water to flow into the lake and contribute to water and nutrient inputs during high rainfall periods (Battoe 1985). Benthic sediments vary from organic to sand in the littoral zone (Layne 1979). Lake Okeechobee (Figure 2-1B) is a large (1800 km^2) shallow lake located in south Florida. It is considered to be a eutrophic lake that has experienced cultural eutrophication over the last 50 years (Engstrom et al. 2006). Benthic sediments are characterized as: mud (representing 44% of the total lake surface area), sand and rock (28%), littoral (19%), dominated by macrophyte growth, and peat (9%) that refers to partially decomposed plant tissues (Fisher et al. 2001). Lake Apopka (Figure 2-1C) is also a shallow lake

with 125 km^2 surface area, located in central Florida. Once a clear-water macrophyte-dominated lake, Lake Apopka has changed to a turbid, algal-dominated lake since 1947 (Clugston 1963). This shift may have been caused by nutrient input from several sources, including agricultural drainage from adjacent vegetable farms (Baird and Bateman 1987, Schelske et al. 2000), although some suggest that the proximal 'trigger' for the switch was a hurricane or tornado (Bachmann et al. 1999). Even though these inputs were controlled and regulated to some degree, the eutrophication process continued and Lake Apopka is considered hypereutrophic. Benthic sediments are characterized by unconsolidated material, which mainly consists of algal deposits (Reddy and Graetz 1991).

Field Sampling

Three sites were sampled in Lake Annie on July 18, 2004 (North, South, and Central) (Figure 2-1A, Table 2-2). Nine sites representing four major sediment types (sites: M17 = peat; O11, M9 and K8 = mud; J7, KR and TC = sand, J5 and FC = littoral) in Lake Okeechobee were sampled on May 17 and 18, 2003 (Figure 2-1B, Table 2-2). Four sites were sampled in Lake Apopka on January 19, 2004 (North, South, Central and West) (Figure 2-1C, Table 2-2). Triplicate sediment cores were collected using a piston corer (Fisher et al. 1992) or by SCUBA divers. The topmost 10 cm of sediment were collected from each core for analyses. Results of all sediment variables are reported on a dry weight basis (dw). Measurements of water temperature (ºC), electrical conductivity ($\mu S\ cm^{-1}$) and dissolved oxygen ($mg\ L^{-1}$) were taken at 1 m water depth from each site during sampling, with a handheld YSI 85 (YSI Inc., Yellow Springs, OH).

Sediment Properties

Samples were transported on ice and stored in the dark at 4 ºC. Before each analysis, samples were homogenized and sub-samples taken. Sediment bulk density (BD) was determined on a dry weight basis (i.e., g of dry/cc wet) at 70 ºC for 72 hours, and pH was determined on wet

sediments (1:2 sediment-to-water ratio). Organic matter content (LOI-loss on ignition) was determined by weight loss at 550ºC. Total P was measured by ignition method, followed by acid digestion (6 M HCl) and measured colorimetrically with a Bran+Luebbe TechniconTM Autoanalyzer II (Anderson 1976; Method 365.1, EPA 1993). Total carbon (TC) and total nitrogen (TN) were determined on oven-dried samples using a Carlo Erba NA-1500 CNS Analyzer (Haal-Buchler Instruments, Saddlebrook, NJ). Measurements of TP, TC, and TN were conducted on sediment that was previously oven-dried (at 70 ºC for 72 hours), ground in a ball mill, and passed through a # 40 mesh sieve.

Sediment Phosphorus Fractionation

Organic phosphorus (P) pools were measured using a chemical fractionation scheme described by Ivanoff et al. (1998). The procedure involved sequential chemical extraction in a 1:50 dry sediment-to-solution ratio, with: 1) 0.5 M $NaHCO_3$ (pH = 8.5) representing labile inorganic and organic P; 2) 1 M HCl representing inorganic P bound to Ca, Mg, Fe, and Al; 3) 0.5 M NaOH representing organic P associated with fulvic and humic fractions (moderately and highly resistant organic P, respectively). Phosphorus remaining in the residual sediment after the sequential extraction was measured by the ignition method and is called residual P, non-reactive P that includes both organic and inorganic P. Extracts from each of these fractions were centrifuged at 10,000 x g for 10 min and filtered through a 0.45 µm membrane filter, and analyzed for SRP or digested for TP (with sulfuric acid and potassium persulfate). Solutions were analyzed by colorimetry, determined by reaction with molybdate using a Bran+Luebbe TechniconTM Autoanalyzer II (Murphy and Riley 1962; Method 365.1, EPA 1993). Residual P was determined using an ignition method (Anderson 1976), and analyzed as described previously for TP.

Microbial Biomass Carbon, Nitrogen, and Phosphorus

Microbial biomass carbon (MBC), nitrogen (MBN), and phosphorus (MBP) were measured through the chloroform fumigation-extraction method (Hedley and Stewart 1982; Brookes et al. 1985; Vance et al. 1987; Horwath and Paul 1994; Ivanoff et al. 1998). Briefly, sediment samples were split in two: one sample was treated with alcohol-free chloroform (0.5 mL) to lyse microbial cells, placed in a vacuum desiccator, and incubated for 24 hrs. The duplicate sample was left untreated. Both sets were extracted with 0.5 M K_2SO_4 for MBC and MBN, and with 0.5 M $NaHCO_3$ (pH = 8.5) for MBP, using a 1:50 dry sediment-to-solution ratio. Extracts from MBC and MBN samples were centrifuged at 10,000 x g for 10 min and filtered through Whatman # 42 filter paper, and 5 mL of the extracts were subjected to Kjeldahl nitrogen digestion (for MBN) and analyzed for total Kjeldahl-N colorimetrically using a Bran+Luebbe TechniconTM Autoanalyzer II (Method 351.2, EPA 1993). MBC extracts were acidified (pH < 2) and analyzed in an automated Shimadzu TOC 5050 analyzer (Method – 415.1, EPA 1993). Extracts from MBP samples were filtered using a 0.45 μm membrane filter and digested for TP with sulfuric acid and potassium persulfate, and analyzed as described previously. Microbial biomass (C, N and P) was determined by the difference between treated and non-treated samples. Non fumigated controls represent extractable organic carbon (Ext-C), extractable labile nitrogen (Ext-N), and extractable labile phosphorus (Ext-P).

Microbial Activity

Anaerobic microbial respiration and methanogenesis were quantified by incubating an amount of sediment (based on 0.5 g of dry weight) using methodology described by Wright and Reddy (2001). For microbial respiration experiments, sediments were incubated anaerobically in the dark at 30 ºC, and evolved CO_2 was trapped in vials containing 0.2 M NaOH. Trapped samples were periodically removed (2, 4, 7, and 10 days) and sealed. Samples were acidified

with 3 M HCl and CO_2 released was measured by gas chromatography using a Shimadzu 8A GC-TCD equipped with Poropak N column (Supelco Inc., Bellefonte, PA), using He as a carrier gas. For the methanogenesis experiment, samples were placed in a glass vial, closed with rubber stoppers and aluminum crimp seals, and incubated anaerobically at 30 ºC. Gas samples were obtained at 2, 4, 7, 10 days and analyzed on a Shimadzu gas chromatograph-8A fitted with flame ionization detector (110 ºC), N_2 as the carrier gas and a 0.3 cm by 2 m Carboxen 1000 column (Supelco Inc., Bellefonte, PA) at 160 ºC. Prior to measuring both CO_2 and methane (CH_4), headspace pressure was determined with a digital pressure indicator (DPI 705, Druck, New Fairfield, CT). Concentrations of CO_2 and CH_4 were determined by comparison with standard concentrations and production rates were calculated by linear regression ($r^2 > 0.95$).

Methane was not detected during the incubation period in Lake Okeechobee samples. Suspecting substrate limitation for methane production, additional experiments were conducted to evaluate the effect of naturally present electron donors acetate and hydrogen (H_2) on methane production in sediments. Wet sediment (based on 0.5 g of dry weight) was added to incubation bottles, sealed, and purged with N_2 gas. One control (no substrate addition) and three treatments were applied to each sediment type: 1) Acetate, 2) H_2, and 3) Acetate + H_2. Acetate (20 mM or 480 mg C kg^{-1} on a dry weight basis) was added from anaerobic sterile stock solution and H_2 addition was done by purging the headspace with 80:20 (vol/vol) H_2-CO_2 gas at 150 Kpa. Samples were incubated anaerobically in the dark at 30 ºC. Gas samples were obtained at 2, 4, 6, 8, 10 and 14 days after incubation and analyzed on a Shimadzu gas chromatograph-8A as described above.

Statistical Analysis

A regression analysis was conducted to compare microbial biomass C and anaerobic respiration. A Principal Component Analysis (PCA) was performed to address relationships

between variables. A one-way analysis of variance (ANOVA) was conducted to compare the effect of electron donors on methane production and also to compare the responses among sites in Lake Okeechobee. Pairwise comparisons of means were conducted using Tukey's HSD. All statistical analyses were conducted with Statistica 7.1 (StatSoft 2006) software.

Results

Electrical conductivity values reflected the trophic conditions of the three lakes, with lowest values for Lake Annie (43-45 μS cm^{-1}), and higher for both Lake Okeechobee (232-603 μS cm^{-1}), and Lake Apopka (370-418 μS cm^{-1}). Day-time dissolved oxygen concentrations were similar for all lakes (4.9-7.3 mg L^{-1}), with Lake Apopka (8.5-10.6 mg L^{-1}) presenting higher values, which is probably due to high algal biomass and lower (i.e. winter) temperatures (15.8-16.6 ºC). Surface water temperature in Lake Annie (29.9-30.1 ºC), and in Lake Okeechobee (28.2-30.7 ºC) were high, reflecting summer temperatures (Table A-1 Appendix).

Sediment Properties

Sediment pH varied from 5.7 to 8.1. Lake Annie sediment pH was lower than the other lakes. Both Lake Okeechobee and Lake Apopka sediment pH were around circum-neutral to slightly alkaline, reflecting eutrophic conditions of these lakes. Both Lake Apopka and Lake Annie (south and central) sediments had lower bulk density than Lake Okeechobee sediments (Table 2-3). Organic matter content (LOI %) and total carbon (TC) were highest in sediments from the peat zone in Lake Okeechobee (M17 site), followed by all sites in Lake Apopka, Lake Annie (south and central) (Table 2-3). The Lake Okeechobee peat zone is characterized by partially decomposed plant tissues (Fisher et al. 2001), with high organic matter content (72% LOI). High organic matter content in Lake Apopka sediments (>60%) was due to its algal origin. Total nitrogen (TN) was highest in Lake Apopka sediments followed by the peat zone in Lake

Okeechobee and Lake Annie (south and central). Lake Annie (south and central) sediments had higher TP concentrations than Lake Okeechobee and Lake Apopka sediments (Table 2-4).

Sediment Phosphorus Forms

Relative proportions of P pools varied among lakes and sediment type. Inorganic P (HCl-Pi) extracted with 1 M HCl (apatite and non-apatite P) was the major component of the P pool in all sediment types from Lake Okeechobee (38-91% of total P) (Table 2-4). Labile organic P (labile-Po) was low in all lakes, while labile inorganic P (labile-Pi) was higher in sediments from Lake Okeechobee (2.5-8.9% of total P) and Lake Annie sandy sediments (13% of total P). For Lake Apopka, the major fraction of the P was in microbial biomass (46-62% of total P) followed by HCl-Pi (13-35% of total P).

In Lake Annie mud sediments (south and central), major P forms included: HCl-Pi (36-41% of total P) and moderately and highly resistant organic P: fulvic acid P (26-28% of total P) and humic acid P (15-16% of total P) (Table 2-4). Residual P (Res.P) was low in Lake Annie mud sediments (0.3-0.6% of total P), with higher values for Lake Apopka (11-15% of total P) and Lake Okeechobee (4.5-18% of total P). Lake Annie sediments contained approximately equal proportions of inorganic and organic P pools, while Lake Okeechobee was dominated by inorganic P in all sediment types (≈ 46-94% of total P). Organic P was the major component of the TP in Lake Apopka sediments (≈ 70.5-86% of total P) (Table 2-4).

Microbial Biomass

Lake Apopka had the highest concentration of MBC, MBN and MBP, followed by Lake Annie (mud sediment) (Table 2-5). All sandy sediment types had low microbial biomass. Total C:N ratio (weight basis) was higher in Lake Okeechobee and Lake Annie, while C:P and N:P ratios were higher in Lake Apopka (Table 2-3, 2-4, A-2 Appendix). Extractable C:N ratio was similar in all sediments, however extractable C:P and N:P ratios were higher in Lake Apopka

(Table 2-5, A-2 Appendix). Extractable C:N:P represents the labile forms of these nutrients, and lower ratios could indicate nutrient limitation. Although microbial biomass C:N ratio was also similar among sediments, C:P and N:P ratios showed a different result, with Lake Apopka having the lowest ratios among the sediments (Table 2-5, A-2 Appendix).

Microbial Activity

Anaerobic respiration (CO_2-C mg $kg^{-1}d^{-1}$) rates were higher in Lake Apopka sediments followed by Lake Annie mud sediments, as compared to Lake Okeechobee sediments types. All sandy sediments had low anaerobic respiration rates (Table 2-6). Methane production rates (CH_4-C mg $kg^{-1}d^{-1}$) were higher in Lake Annie central site than south site in Lake Annie and all sites in Lake Apopka.

Addition of H_2 or acetate + H_2 to Lake Okeechobee sediments caused higher methane production rates (Table 2-6). Results of one-way ANOVA showed that methane production rates of the electron donor experiment with Lake Okeechobee sediments were significantly different among treatments (n = 27, df = 3, $F\text{-}test$ = 19.70, $p < 0.00001$). Tukey's pairwise multiple comparison method showed methane production rates were significantly different between control and H_2, and control and acetate + H_2 addition, but were not significantly different between control and acetate. Results were also significantly different when comparing acetate and H_2, and acetate and acetate + H_2 addition. However, results were not significantly different when comparing H_2 and acetate + H_2 addition. One-way ANOVA showed that there was significant difference in methane production rates among sediment types (n = 12, df = 8, $F\text{-}test$ = 5.10, $p < 0.00001$). Tukey's pairwise multiple comparison method showed that methane production in sites located in the mud zone of Lake Okeechobee were statistically different from methane production in all other sediment types, but were not different among each other. Methane production rates were not significantly different among peat, littoral, and sand deposits.

Because linear regression between MBC with MBN and MBP showed a strong positive significant relationship (Figure 2-2A, B) statistical analyses were performed using MBC as proxy for microbial biomass. Regression analysis of MBC and anaerobic respiration indicate that over a wide range of MBC represented by all three lakes there was a logarithmic relationship (Figure 2-3A). However, in the lower range of MBC, the relationship was linear, showing that anaerobic respiration increases rapidly with MBC (Figure2-3B). This regression analysis showed that the three lakes fall into distinct groups (Figure 2-3A). Although significant, the linear regression between anaerobic respiration and methanogenesis was weak (n = 47, $r^2 = 0.30$, $p = 0.0039$).

The first Principal Component Analysis (PCA-1) was performed using data from the three lakes to address relationships among biogeochemical properties. The second (PCA-2) used only Lake Okeechobee data and was conducted to verify how the results from the electron donor experiment relate to the biogeochemical data. The PCA-1 had 60.2% of the data variability explained by Axis 1 while Axis 2 explained 18.9% (Figure 2-4A). Inorganic P forms (labile-Pi and HCl-Pi) were the variables selected by Axis 2 while most variables were selected by Axis 1 (excluding CH_4, Res.P, extractable C:N, labile-Pi and HCl-Pi) and were plotted opposite to bulk density, showing an inverse relationship. Microbial biomass C was grouped with anaerobic respiration and ratios of extractable C:P and extractable N:P ratios. Methane production rates were plotted with most P forms measured in this study. The position of the sites in relation to the variables loadings in the first PCA showed that the three lakes are separated into different groups. Lake Apopka (all sites) placed in the position of microbial biomass, extractable C:P and extractable N:P ratios and anaerobic respiration. Lake Annie mud sediment type was placed in the position of methane production and P forms. Lake Okeechobee placed in a different position

from the other two lakes, and also displayed a separation of its sediment types. Mud sediment types (M9, O11, K8 sites) of Lake Okeechobee were placed closer to both forms of inorganic P (labile-Pi and HCl-Pi) with a gradient in relation to the three mud sites that were related to the KR site (sand sediment). The peat zone (M17) was placed in a different position with extractable C:N ratio, and was unrelated to any other site sampled. Sandy sediments from both Lake Okeechobee and Lake Annie were placed with bulk density (Figure 2-4B).

The PCA-2, using only Lake Okeechobee, corroborates the results from Pearson's correlation (Figure 2-5A, Appendix A-3). Methane production rates were placed with microbial biomass, showing that the stimulation of methane production was dependent on the original microbial biomass (MBC). Again, highest methane production rates were placed with P forms. Axis 1 explained 60.6% of the variability of the data and the variables selected were BD and in an opposite position all P forms, anaerobic respiration, methane production with electron donor addition, LOI and MBC. Axis 2 with 20.1% of the data variability explained selected extractable C:N, C:P and N:P ratios. The same distribution of Lake Okeechobee sites seen in PCA-1 was repeated in PCA-2. Peat zone position showed that this site had the highest concentration of the variables selected by Axis 2. Sandy sediments were placed with the bulk density and opposite to the other sites and parameters. Again the same distribution of the mud sediments with the KR site is seen and they were placed with P forms and microbial biomass and activity (Figure 2-5B).

Discussion

In this study commonly applied methods in soil science were used to measure microbial biomass in lake sediments. The chloroform fumigation-extraction method is a quick and simple procedure that has been used widely to measure microbial biomass in soils (e.g. Jenkinson et al. 2004). Soil microbial C, N, and P extraction by this method is largely dependent on soil characteristics and microbial community composition (Jenkinson et al. 2004). Therefore,

extraction efficiency is corrected by the k_{ec} factor. Reported k_{ec} values can vary from 0.2 to 0.45 for C, N and P (Bailey et al 2002). In this study, k_{ec} factor was not used, to avoid overestimation of the microbial biomass. For example, Lake Apopka sediments had MBP concentrations varying from 561 to 1031 mg kg^{-1}. If the reported k_{ec} factor of 0.37 for MBP (Hedley and Stewart 1982) was applied, the final MBP concentration in Lake Apopka sediments would be higher than the TP (1650-2786 mg kg^{-1}). These k_{ec} factors were determined for soils with lower microbial biomass than sediments like Lake Apopka. The efficiency of P extraction from samples with high microbial biomass is probably higher, thus resulting in low k_{ec} factors. Therefore the k_{ec} factors reported for typical soil samples are probably not suitable for use in samples containing high labile P in the microbial biomass.

Several studies, however, reported that MBC measured through the chloroform fumigation-extraction method (not corrected with the k_{ec} factor) yields similar results when compared with other alternative methods to measure microbial biomass in soils. Leckie et al. (2004), using humic soils, reported a strong positive linear relationship ($r^2 = 0.96$, $p = 0.007$) between microbial biomass C measured with chloroform fumigation-extraction (with no correction factor) and total phospholipids fatty acid analysis, a more accurate methodology to measure microbial biomass. Bailey et al. (2002), using mineral soils, also reported a strong linear relationship between these two measurements. Microbial biomass C concentration in eight different soils, including sewage sludge, were also strongly correlated ($r^2 = 0.96$) with DNA measurements (Marstorp et al. 2000). The use of microbial biomass concentrations not corrected by the k_{ec} factor is, therefore, a good measure of microbial biomass present in the samples. Although the chloroform fumigation-extraction has not been used widely in sediment studies

(Mcdowell 2003), the data enable comparisons among lakes in this study, and provide a good proxy for microbial biomass (Marstorp et al. 2000; Bailey et al. 2002; Leckie et al. 2004).

It is reasonable to expect that near-surface sediment variables will reflect recent lake trophic state conditions. Eutrophic and hypereutrophic lakes usually receive high external loads of nutrients. Eutrophic and hypereutrophic lakes also display high primary productivity and nutrient concentrations in the water column and these nutrients will eventually reach the sediment. Sediments from eutrophic and hypereutrophic lakes might be expected to have high concentrations of organic matter and nutrients. Binford and Brenner (1986) and Deevey et al. (1986) showed that net accumulation rates of organic matter and nutrients increase with trophic state for Florida lakes. Several other studies also have shown that there is a significant correlation between trophic condition (based on water measurements) and nutrient concentrations in sediments (Rybak 1969; Flanery et al. 1982; Wisniewski and Planter 1985; Maassen et al. 2003), while others have shown this is not always true, especially for P content (Brenner and Binford 1988; Lopez and Morgui 1993; Gonsiorczyk et al. 1998). The results from this study showed that organic matter, N and P concentrations were high in sediments with lower bulk density, and that trophic state conditions were not related to nutrient content of sediments. For example, Lake Annie, an oligo-mesotrophic lake, had higher sediment TP concentration compared to the other two lakes studied. Organic matter, TC, and TN in Lake Annie deposits were similar to values in Lake Okeechobee and Lake Apopka sediments (Table 2-3). Sediment composition reflects an integrative effect of trophic state conditions and diagenesis over a long period of time relative to water column physico-chemical variables. Moreover, the relative importance of P forms in sediments is more important than total P concentration and will depend

on sediment composition, sedimentation rate, and physicochemical conditions (Lopez and Morgui 1993; Gonsiorczyk et al. 1998; Kaiserli et al. 2002).

Lake Annie organic sediments contain high TP concentrations (south and central sites), with up to 45% of TP in moderate to highly resistant organic P pools (NaOH soluble), suggesting that organic P in this lake is old and stable (Table 2-4). The other major fraction is HCl-Pi, which makes up 40% of the total P, and represents total inorganic P bound to Ca, Mg, Fe and Al. Its solubility is controlled by either pH or redox potential (Moore and Reddy 1994). Being a deep lake that is thermically stratified from February through November or December (Battoe 1985), Lake Annie sediment P has little effect on P concentration of the water column during most of the year. In Lake Apopka, > 50% of the total P is in the microbial biomass in most of the sampled sites. This P form is highly available and P storage within microbial cells has been reported to contribute significantly to P release from sediments (Davelaar 1993; Gächter and Meyer 1993; Hupfer et al. 2004). Lake Apopka is shallow, and benthic sediments are subject to resuspension into the water column, potentially releasing soluble P (Reddy et al. 1991). Kenney et al. (2001) showed that polyphosphate (P storage within microbial cells) played an important role in the TP of Lake Apopka sediments, and suggested that between 25 and 90% of the sediment TP may be sequestered as polyphosphate. Lake Okeechobee is also shallow, with sediments frequently resuspended into the water column. In Lake Okeechobee, HCl-Pi constitutes approximately 60-91% of the total P, similar to values reported in other studies of Lake Okeechobee (Olila et al. 1995; Brezonik and Engstrom 1998).

Total C:N ratios (by weight) ranged from 6 to 19, similar to results reported by Brenner and Binford (1988). In both Lake Apopka and Lake Okeechobee sediment total C may include inorganic C (i.e. carbonates). Sediment C:N ratio can reflect varying contributions of

allochthonous (high ratio) versus autochthonous (low ratio) organic matter (Hutchinson 1957; Mackereth 1966). Terrestrial autotrophs have higher C:P and C:N ratios than does lacustrine particulate organic matter (Elser et al. 2000). Autochthonous organic matter has a C:N ratio around 12:1 (Wetzel 2001). Among all sediment types in this study, peat zone deposits from Lake Okeechobee had the highest total weight C:N and C:P ratios reflecting its higher plant origin. Deposits from other sediment types, especially Lake Apopka, with lower C:N, reflect algal origin.

Extractable nutrient ratios were low for Lake Annie, reflecting high concentrations of extractable labile nutrients relative to C. High availability of N and P may indicate C limitation in Lake Annie sediments. Carbon limitation may reflect the recalcitrant nature of C entering the lake and physical characteristics of this lake. Lake Annie has experienced an increase in color during the past decades, probably from high dissolved organic carbon (DOC) input to the lake from adjacent land (Swain and Gaiser 2005). Battoe (1985) reported high input of surface waters enriched in humic content to Lake Annie during high rainfall periods. This allochthonous DOC, of humic origin, will be utilized in the water column. Because Lake Annie is deep, the DOC will be mineralized during its descent to the sediment (Suess 1980). Consequently lower concentrations of DOC will reach the sediment (also being highly refractory) leading to low C:nutrient ratios.

Carbon and N limitation was observed in most Lake Okeechobee sediments, especially in the mud zone. Hence, there is low microbial biomass and activity. Crisman et al. (1995) reported that temperature and trophic state variables Secchi, total P, and total N, showed a weak correlation with bacterioplankton abundance (number of cells mL^{-1}) in a seasonal study in Lake Okeechobee. They concluded that the factors controlling bacterioplankton communities could be

related to grazing and/or C and nutrient availability. Work et al. (2005) reported high bacterioplankton production (mg L^{-1} h^{-1}) in Lake Okeechobee during summer. Also, several studies have shown that bacterioplankton is an important source of C to the food web in Lake Okeechobee (Havens and East 1997; Work and Havens 2003; Work et al. 2005). However, to my knowledge, there is no study addressing C or nutrient limitation of the bacterioplankton community in Lake Okeechobee. Nevertheless, Phlips et al. (1997) showed that in the central region of Lake Okeechobee (mud zone), phytoplankton abundance was high in the summer. Light is the most limiting factor of the phytoplankton community during most of the year in this area, however, during summer months, light limitation is relaxed and N becomes the limiting factor of the phytoplankton community (Aldridge et al. 1995). High labile inorganic P availability in mud zone sediments causes a high demand for C and N that is not met.

The opposite is seen for Lake Apopka with higher ratios for extractable C:P and N:P, but lower ratios in microbial biomass. Low nutrient ratios in microbial biomass strongly indicate P accumulation in cells. The bacterial community can have low C:P and N:P ratios by accumulating polyphosphate and reducing C and N content (Makino and Cotner 2004). Lake Apopka has high primary productivity (Carrick et al. 1993) and algae accumulate in surface sediments leading to high concentrations of labile C (Gale et al. 1992; Gale and Reddy 1994). The primary productivity of Lake Apopka is dominated by cyanobacteria and the dominant taxa are *Synechococcus* sp., *Synechocystis* sp. and *Microcystis incerta* (Carrick et al. 1993; Carrick and Schelske 1997). Approximately 1034 g C m^{-2} yr^{-1} from primary production is deposited in surface sediments of Lake Apopka (Gale and Reddy 1994). Schulz and Conrad (1995) showed that acetate concentrations increase drastically, from 100 μM to 1300 μM, in sediments of Lake Constance (Germany) after stimulation by greater algal deposition. High primary production in

Lake Apopka, with consequent sedimentation, is leading to high C concentration in sediments that is supporting high microbial biomass and activity.

The positions of extractable C:P and N:P ratios and microbial biomass or activity in PCA-1 (Figure 2-4), support the idea that P availability, more than any other nutrient, influences microbial community in these sediments. High P availability accompanied by relatively low C and N limits microbial community biomass and activity in these sediments. Both anaerobic respiration and CH_4 production rates reflect microbial activity in these sediments and C, N, P are required for microbial metabolism and growth. High availability of C and nutrients can support a larger microbial community that will be reflected in a higher turnover of organic substrates. Other studies have found the same relationship between nutrients and microbial activity. Drabkova (1990), in a study of bacterial production and respiration in lakes with different trophic conditions, reported that bacterial production correlates with P concentration, and that respiration increases with trophic state, but to some limit. Anaerobic respiration appears to approach an asymptote with increasing microbial biomass (Figure 2-3A).

Other studies in the water column of lakes have shown that CO_2 concentrations correlate positively with P and N concentrations (Kortelainen et al. 2000; Huttunen et al. 2003). Kortelainen et al. (2006) showed that highest CO_2 emissions from sediments to the water column were found in small shallow lakes with high total P and N and organic C. del Giorgio and Peters (1994) concluded that CO_2 flux from Quebec lakes was associated with TP concentration in the water column. Despite the fact that most investigators accept the idea that C availability is the major factor limiting heterotrophic microbial processes, in both aquatic and terrestrial ecosystems, nutrients other than C are likely limiting where detrital organic matter is nutrient poor (Grimm et al. 2003). Cimbleris and Kalff (1998) showed that for planktonic bacterial

respiration, the best predictor was TP, but also that higher respiration was observed with increasing C:N and C:P ratios, similar to the findings in my study.

Phosphorus control of microbial activity seems to be stronger for CH_4 production. In both statistical analyses (two PCAs), methane production had a strong relationship with P forms (Figures 2-4 and 2-5). Several studies have shown that methane production rates were higher in eutrophic than oligotrophic lakes. (Casper 1992; Rothfuss et al. 1997; Falz et al. 1999; Nüsslein and Conrad 2000; Huttunen et al. 2003; Dan et al. 2004). Other than these studies that reported higher CH_4 production in eutrophic lakes, there is no clear indication of how P availability affects methane production.

Methane was not detected in Lake Okeechobee sediments without the addition of electron donors. However, Fisher et al. (2005) reported CH_4 in sediment porewater of sites M9 and M17 in Lake Okeechobee. They also reported sulfate (SO_4^{-2}) in these sediment porewaters, and its decline with sediment depth was related to the use of SO_4^{-2} as a terminal electron acceptor in the oxidation of sediment organic matter. Iron (Fe) is important in controlling P solubility in Lake Okeechobee sediments (Moore and Reddy 1994) and Fe-reducers might also be present. Structure and function of anaerobic microbial communities are strongly affected by competition for fermentation products such as H_2 and acetate (e.g., Megonigal et al. 2004). Iron- and SO_4^{-2}-reducers outcompete methanogens for H_2/CO_2 and acetate, due to higher substrate affinities, and higher energy and growth yield (Lovley and Klug 1983; Lovley and Phillips 1986; Conrad et al. 1987; Bond and Lovley 2002), however, both processes can coexist (Mountfort and Asher 1981; Holmer and Kristensen 1994; Roy et al. 1997; Holmer et al. 2003; Roden and Wetzel 2003; Wand et al. 2006). Coexistence occurs because of spatial variation in the abundance of terminal electron acceptors or because the supply of electron donors is non-limiting (Roy et al. 1997;

Megonigal et al. 2004). Consequently low C availability with concomitant presence of Fe- and SO_4^{-2}-reducers is the probable explanation for lack of methanogenesis in Lake Okeechobee sediments.

Methanogens (archaebacteria) are obligate anaerobes and can be divided into two groups: H_2/CO_2 (hydrogenotrophic) and acetate (acetoclastic) consumers, CH_4 being the final product of both metabolisms (Deppenmeier 1996). Methanogens use a limited number of substrates, mainly acetate or H_2/CO_2. Theoretically H_2/CO_2 should account for 33% of total methanogenesis, although much higher contributions have been found (Conrad 1999). A ratio of 2:1 or higher of acetate and H_2/CO_2 contribution for methane production is usually expected (Conrad 1999). Although it has been reported that acetoclastic methanogenesis dominates freshwater ecosystems while hydrogenotrophic dominates marine systems (Whiticar 1999), results from the electron donor experiment in Lake Okeechobee show that in this freshwater ecosystem H_2/CO_2 is the major substrate for methane production. Other studies have reported that hydrogenotrophic methanogenesis can be dominant in freshwater ecosystems (Chauhan et al. 2004; Banning et al. 2005; Castro et al. 2005; Wand et al. 2006).

One explanation for higher methane production with H_2/CO_2 than acetate is temperature. Some studies in lakes have shown that acetoclastic methanogenesis is dominant at low temperatures, ≈ 10 ºC. Higher temperatures lead to an increased contribution of other fermentation pathways and H_2/CO_2-dependent methanogenesis (Schulz and Conrad 1996; Falz et al. 1999; Glissmann et al. 2004). In a study of rice paddy soil, Chin and Conrad (1995) reported that low temperatures led to a decrease in H_2-dependent methanogenesis that was caused by inhibition of H_2-production reactions (i.e. syntrophic bacteria) that seem to be sensitive to low temperatures. Lake Okeechobee lies in south-central Florida. It is subject to subtropical climate,

and the annual water column temperature ranges from 15-31 ºC (Rodusky et al. 2001). During sampling for this study, water temperature in Lake Okeechobee was around 28-31 ºC. Because the lake is shallow, sediment temperature is probably in this range. Another explanation for low methane production from acetate is the fact that high P availability inhibits acetotrophic methanogenesis (Conrad et al. 2000). Lake Okeechobee had high labile inorganic P availability (Table 2-4). Conrad et al. (2000) reported that high phosphate availability led to a 60% contribution of total methane production from H_2/CO_2.

In Figure 2-6, the major characteristics of sediments from the different lakes are summarized. Sediments from the central site were selected to represent Lake Annie data, while sediments from the mud zone were selected to represent Lake Okeechobee data. The three lakes, ranging in trophic state, had distinct sediment biogeochemical properties, however some similarities were present, such as high TP concentration in sediments from the different lakes. Sediments from the oligo-mesotrophic Lake Annie had the major P forms as HAP, FAP and HCl-Pi. Low extractable C:P and N:P ratios resulted from a high extractable labile P concentration (Figure 2-6). Lake Okeechobee mud sediments had similarities with Lake Annie sediments, such as low extractable C:P and N:P ratios due to a high extractable labile P concentration, and HCl-Pi as the major P form. Differences in sediments from this eutrophic lake included low microbial activity (CO_2 and CH_4 production rates), and high concentrations of labile Pi (Figure 2-6). The hypereutrophic Lake Apopka had high concentrations of microbial biomass P, N and C, as well as high extractable C:P and N:P ratios, and high microbial activity (CO_2 and CH_4 production rates) (Figure 2-6).

Conclusions

Eutrophic and hypereutrophic lakes usually receive high external loads of nutrients, and display high primary productivity and nutrient concentrations. Consequently, sediments from

these lakes might be expected to have higher concentrations of organic matter and nutrients than oligo-mesotrophic lakes. The results from this study, however, showed that trophic state conditions were not related to the nutrient content of sediments. Organic matter, N and P concentrations were higher in sediments with lower bulk density, independent of the trophic state of the lake. Sediment composition therefore reflects an integrative effect of trophic state conditions and diagenesis over a long period of time, relative to water column physico-chemical variables.

The relative importance of P forms present in the sediments seemed to be more important than total P concentration in characterizing the sediment of each of the studied lakes. The oligo-mesotrophic Lake Annie organic sediments contained major P forms in moderate to highly resistant organic P (NaOH soluble) and HCl-Pi, suggesting P in this lake is old and stable. The Lake Okeechobee sediment major P form was HCl-Pi, which constituted approximately 60-91% of the total P, while hypereutrophic Lake Apopka sediment had > 50% of the total P in the microbial biomass.

Extractable nutrient ratios seemed to have stronger influence on sediment microbial communities than total concentrations. Extractable nutrient ratios were low for Lake Annie, reflecting high concentrations of extractable labile nutrients relative to C, indicating C limitation in these sediments. High labile inorganic P availability resulted in low extractable C:P and N:P ratios, and C and N limitation in most Lake Okeechobee sediments, especially in the mud zone, followed by low microbial biomass and activity. Moreover, low C availability with concomitant presence of Fe- SO_4^{-2}-reducers appears to be inhibiting the methanogenic community in Lake Okeechobee sediments. Limitation of the methanogenic community in these sediments is supported by the positive effect of the addition of electron donors on methane production. The

results of electron donor addition also indicated that H_2/CO_2 is the major substrate for methane production in Lake Okeechobee sediments.

Hypereutrophic Lake Apopka sediments had higher ratios for extractable C:P and N:P, and the high C concentration in sediments is supporting high microbial biomass and activity. Lake Apopka sediments are highly influenced by the deposition of the primary production in the water column. The results from this study suggest that although the microbial community is C/energy limited, C, coupled with N and P availability has a strong influence in microbial communities in these lakes sediments. Therefore, studies of sediment heterotrophic microbial communities should take into account C as well as N and P availability.

Table 2-1. Morphometric and limnological variables of the three subtropical lakes.

	Lake		
	Annie[a,b]	Okeechobee[c]	Apopka[c]
Surface Area (km^2)	0.366	1800	125
Mean depth (m)	9.1	2.7	1.6
Maximum depth (m)	20.7		
Electrical Conductivity ($\mu S\ cm^{-1}$)	43.7	447.7	384
Chlorophyll-a ($\mu g\ L^{-1}$)	3.6	26	90
Total Nitrogen ($\mu g\ L^{-1}$)	373	1510	4890
Total Phosphorus ($\mu g\ L^{-1}$)	5.0	100	190
Secchi Transparency (m)	3.4	0.5	0.23
Trophic Classification	Oligo-mesotrophic	Eutrophic	Hypereutrophic

[a] Florida Lake Watch (2001), [b] Archbold Station (2005), [c] Havens et al. (1999)

Table 2-2. Location and sediment type of the sites sampled in the three different lakes.

Lake	Date	Sediment Type	Site	Latitude	Longitude
Annie	July/04	Mud/Clay	South	27º12'18"	81º21'40"
		Mud/Clay	Central	27º12'27"	81º21'44"
		Sand	North	27º12'32"	81º20'57"
Okeechobee	May/03	Peat	M17	26º45'24.4"	80º46'36.8"
		Mud	O11	26º55'14.8"	80º41'53.8"
		Mud	M9	26º58'17.6"	80º45'38.4"
		Mud	K8	27º00'16.6"	80º49'38.1"
		Littoral/Sand	FC	26º58'11.5"	80º00'51.8"
		Littoral/Sand	J5	27º05'28.1"	80º51'28.8"
		Sand	TC	27º11'55"	80º47'40"
		Sand	KR	27º58'11.5"	80º00'51.8"
		Sand	J7	27º02'11"	80º51'19.8"
Apopka	Jan/04	Organic	South	28º35'00"	81º36'22"
		Organic	Central	28º37'31"	81º37'24"
		Organic	West	28º38'01"	81º39'36"
		Organic	North	28º39'43"	81º37'25"

Table 2-3. pH, bulk density (BD), organic matter content (LOI - loss on ignition), total nitrogen, and total carbon concentration in sediments from three subtropical lakes. (mean ± standard deviation). Sediment depth 0-10 cm.

Lake	Site	pH	BD (g of dry cm^{-3} of wet)	LOI (%)	Total Nitrogen	Total Carbon
					(g kg^{-1} dw)	
Annie	South	5.7 ± 0.1	0.024 ± 0.003	53.8 ± 0.8	19.1 ± 1.6	263 ± 11
	Central	5.8 ± 0.01	0.026 ± 0.005	54.9 ± 0.5	20.2 ± 0.7	265 ± 10
	North	6.0 ± 0.1	1.64 ± 0.11	0.45 ± 0.3	0.26 ± 0.0	1.6 ± 0.1
Okeechobee	M17	7.4 ± 0.2	0.19 ± 0.02	72.2 ± 5.3	21.5 ± 2.8	403 ± 36
	O11	7.5 ± 0.03	0.16 ± 0.04	40.2 ± 2.6	11.9 ± 0.6	186 ± 6.5
	M9	7.6 ± 0.03	0.26± 0.02	28.5 ± 2.0	8.0 ± 0.6	146 ± 8.3
	K8	7.5 ± 0.02	0.16 ± 0.04	36.5 ± 1.7	11.4 ± 0.6	175 ± 3.2
	FC	7.1 ± 0.2	1.50 ± 0.07	2.6 ± 0.6	0.2 ± 0.1	1.3 ± 0.7
	J5	7.6 ± 0.1	1.43 ± 0.16	1.6 ± 0.3	0.3 ± 0.1	3.7 ± 1.7
	TC	7.2 ± 0.4	1.35 ± 0.12	2.4 ± 0.0	0.4 ± 0.0	5.1 ± 0.2
	KR	7.5 ± 0.1	0.47 ± 0.06	23.5 ± 3.5	6.4 ± 1.2	97 ± 15
	J7	8.1 ± 0.2	1.60 ± 0.14	2.2 ± 0.8	0.3 ± 0.0	4.4 ± 1.3
Apopka	South	7.5 ± 0.2	0.022 ± 0.005	64.2 ± 2.9	29.7 ± 1.6	335 ± 12
	Central	7.4 ± 0.2	0.016 ± 0.003	67.8 ± 1.9	31.5 ± 1.1	349 ± 1.7
	West	7.7 ± 0.1	0.016 ± 0.001	69.4 ± 2.7	30.5 ± 0.4	356 ± 5.1
	North	7.6 ± 0.03	0.015 ± 0.003	69.2 ± 0.2	32.9 ± 0.04	356 ± 6.1

Table 2-4. Phosphorus fractionation in sediments from Lake Annie, Lake Okeechobee, and Lake Apopka.

Lake	Site	Total P (mg kg^{-1})	Percentage (%) Total Phosphorus						
			Microbial Biomass P	Labile P		Inorganic P	Moderately Available Fulvic Acid-P	Highly Resistant Humic Acid-P	Residual P
				Organic	Inorganic				
Annie	South	1428	2.8	2.9	4.5	36.1	28.1	15.2	0.3
	Central	1435	3.7	2.4	5.5	41.4	26.5	16.6	0.6
	North	7.4	11.7	3.5	13.5	10.6	16.2	9.4	13.2
Okeechobee	M17	374	1.3	2.0	4.9	59.9	4.9	4.8	8.8
	O11	1166	1.8	1.7	7.7	66.0	9.5	3.0	17.8
	M9	922	0.4	1.1	8.4	79.6	2.2	0.6	15.0
	K8	1200	1.4	1.3	8.1	71.8	7.8	2.9	18.2
	FC	67	1.2	0.8	3.9	83.1	2.0	1.4	4.5
	J5	30	4.6	3.3	7.6	38.5	11.0	1.3	14.3
	TC	110	1.6	1.2	5.5	86.6	4.5	1.8	9.2
	KR	814	0.1	1.0	2.5	91.1	7.7	2.7	13.2
	J7	60	1.6	2.1	8.9	62.2	3.4	0.0	13.2
Apopka	South	1221	45.9	2.3	0.4	16.9	12.4	9.9	13.5
	Central	1417	52.6	2.5	0.2	20.3	15.0	9.1	13.4
	West	1215	51.9	3.2	0.7	35.2	15.5	14.6	15.3
	North	1635	61.9	1.6	0.1	12.9	15.4	6.9	11.3

Table 2-5. Extractable and microbial biomass C, N, and P concentrations in sediments from three subtropical lakes. (mean ± standard deviation). Extractable C and N non-fumigated 0.5 M K_2SO_4, Extractable P non-fumigated digested 0.5 M $NaHCO_3$.

Lake	Site	Extractable (mg kg^{-1} dw)			Microbial Biomass (mg kg^{-1} dw)		
		Carbon	Nitrogen	Phosphorus	Carbon	Nitrogen	Phosphorus
Annie	South	1642 ± 224	670 ± 84	107 ± 23	1526 ± 187	305 ± 41	40 ± 10
	Central	2619 ± 603	780 ± 139	108 ± 2	1705 ± 145	299 ± 24	53 ± 7.4
	North	51 ± 5	3 ± 2	1.3 ± 0.1	42 ± 7	3.7 ± 2	0.8 ± 0.2
Okeechobee	M17	1645 ± 221	179 ± 19	26 ± 7	249 ± 25	39 ± 6	4.9 ± 1.6
	O11	887 ± 99	196 ± 13	111 ± 37	655 ± 71	90 ± 26	21.2 ± 7.7
	M9	482 ± 44	104 ± 9	87 ± 7	338 ± 22	80 ± 18	3.7 ± 2.3
	K8	945 ± 171	156 ± 32	113 ± 20	579 ± 104	130 ± 20	17.0 ± 6
	FC	18 ± 9	10 ± 1	3.1 ± 0.1	18 ± 8	2.2 ± 2	0.8 ± 0.4
	J5	115 ± 41	21 ± 3	3.3 ± 0.8	56 ± 9	8 ± 7	1.4 ± 1.4
	TC	101 ± 5	24 ± 1	7.4 ± 1.3	44 ± 13	8 ± 3	1.8 ± 1.7
	KR	228 ± 59	71 ± 13	29 ± 6	125 ± 31	7 ± 6	0.9 ± 0.9
	J7	66 ± 9	20 ± 2	6.7 ± 0.2	34 ± 14	6 ± 4	0.9 ± 0.7
Apopka	South	3827 ± 827	1035 ± 224	33 ± 5	13182 ± 1524	2378 ± 278	561 ± 59
	Central	4169 ± 711	1331 ± 163	38 ± 1	20771 ± 2342	3516 ± 292	746 ± 48
	West	3711 ± 347	1070 ± 256	43 ± 5	18742 ± 830	2977 ± 130	632 ± 149
	North	4316 ± 655	1660 ± 309	29 ± 0.4	23244 ± 1327	4068 ± 254	1031 ± 83

Table 2-6. Anaerobic respiration and methane production rates in sediments from subtropical lakes. Control are values for basal methane production without substrate addition, and acetate*, hydrogen* and acetate + hydrogen* results from electron donor addition experiment only for Lake Okeechobee sediments. (mean ± standard deviation).

Lake	Site	Anaerobic Respiration (CO_2-C mg $kg^{-1}d^{-1}$ dw)	Methane Production (CH_4-C mg $kg^{-1}d^{-1}$ dw)			
			Control	Acetate*	Hydrogen*	Acetate + Hydrogen*
Annie	South	362 ± 48	48 ± 10			
	Central	283 ± 32	118 ± 17			
	North	3.8 ± 1.2	0.15 ± 0.02			
Okeechobee	M17	76 ± 17	N.D.	5.0 ± 4.5	3.3 ± 2.6	98 ± 42
	O11	117 ± 26	N.D.	27.3 ± 5.6	217 ± 64	127 ± 45
	M9	54 ± 14	N.D.	11.6 ± 5.2	130 ± 56	122 ± 7.1
	K8	98 ± 11	N.D.	24.8 ± 6.9	204 ± 60	230 ± 30
	FC	5.6 ± 0.5	N.D.	1.6 ± 1.5	41.6 ± 25	23.7 ± 14
	J5	15 ± 2.8	0.26	12.1 ± 10	21.0 ± 17	33.2 ± 13
	TC	13 ± 1.7	N.D.	0.6 ± 0.4	35.4 ± 10	43.5 ± 3.5
	KR	78 ± 19	N.D.	3.4 ± 1.0	38.6 ± 20	76.2 ± 22
	J7	11 ± 3.3	N.D.	0.5 ± 0.2	48.4 ± 7.8	55.2 ± 6.5
Apopka	South	563 ± 28	31 ± 4.5			
	Central	654 ± 87	40 ± 13			
	West	455 ± 54	34 ± 24			
	North	1170 ± 47	52 ± 19			

N.D. = Not Detected.

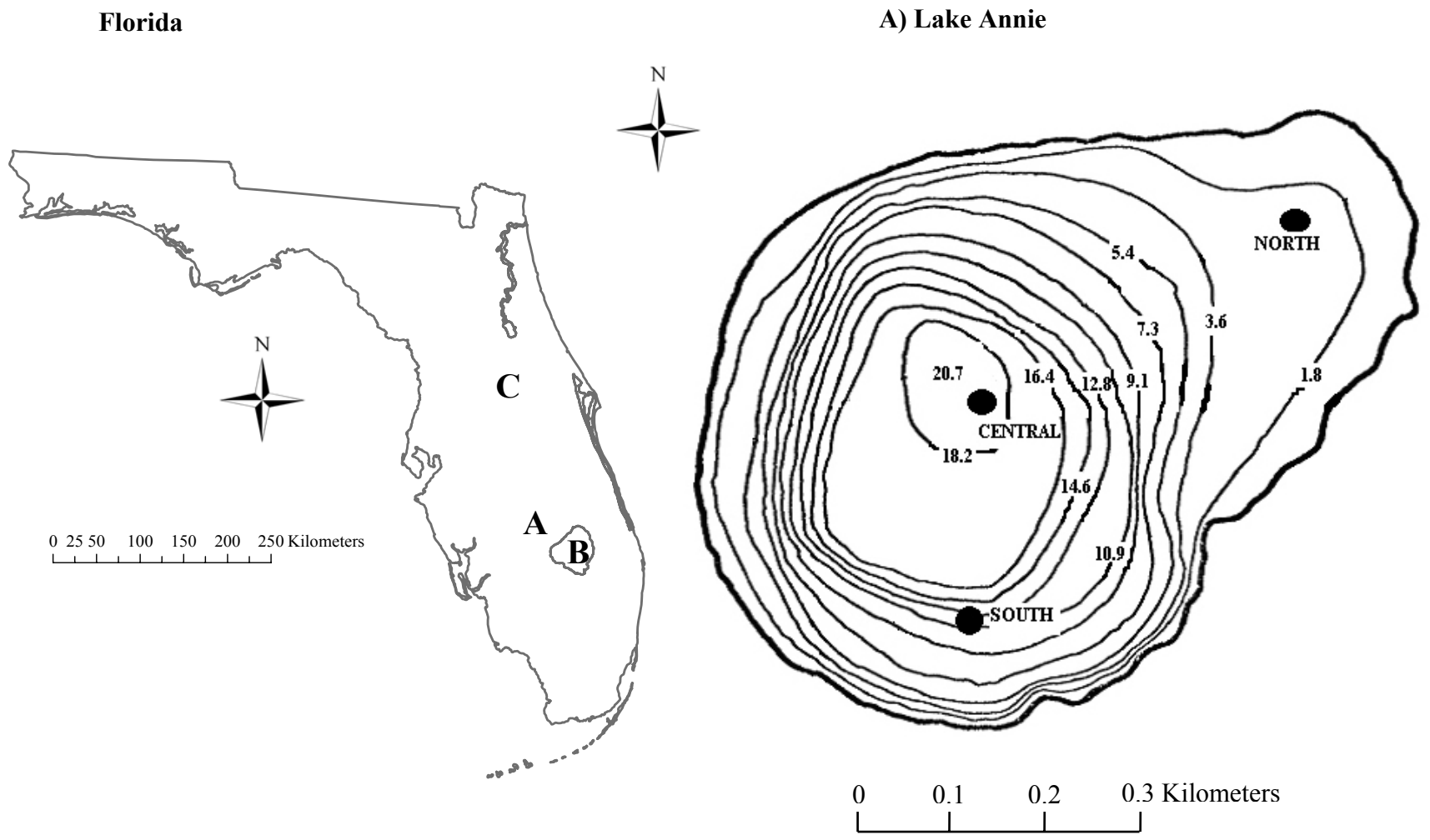


Figure 2-1. Map of the three subtropical lakes with sampled sites and their location in Florida State: A) Lake Annie (with water column depth in meters, modified from Layne 1979), B) Lake Okeechobee with different sediment types, and C) Lake Apopka.

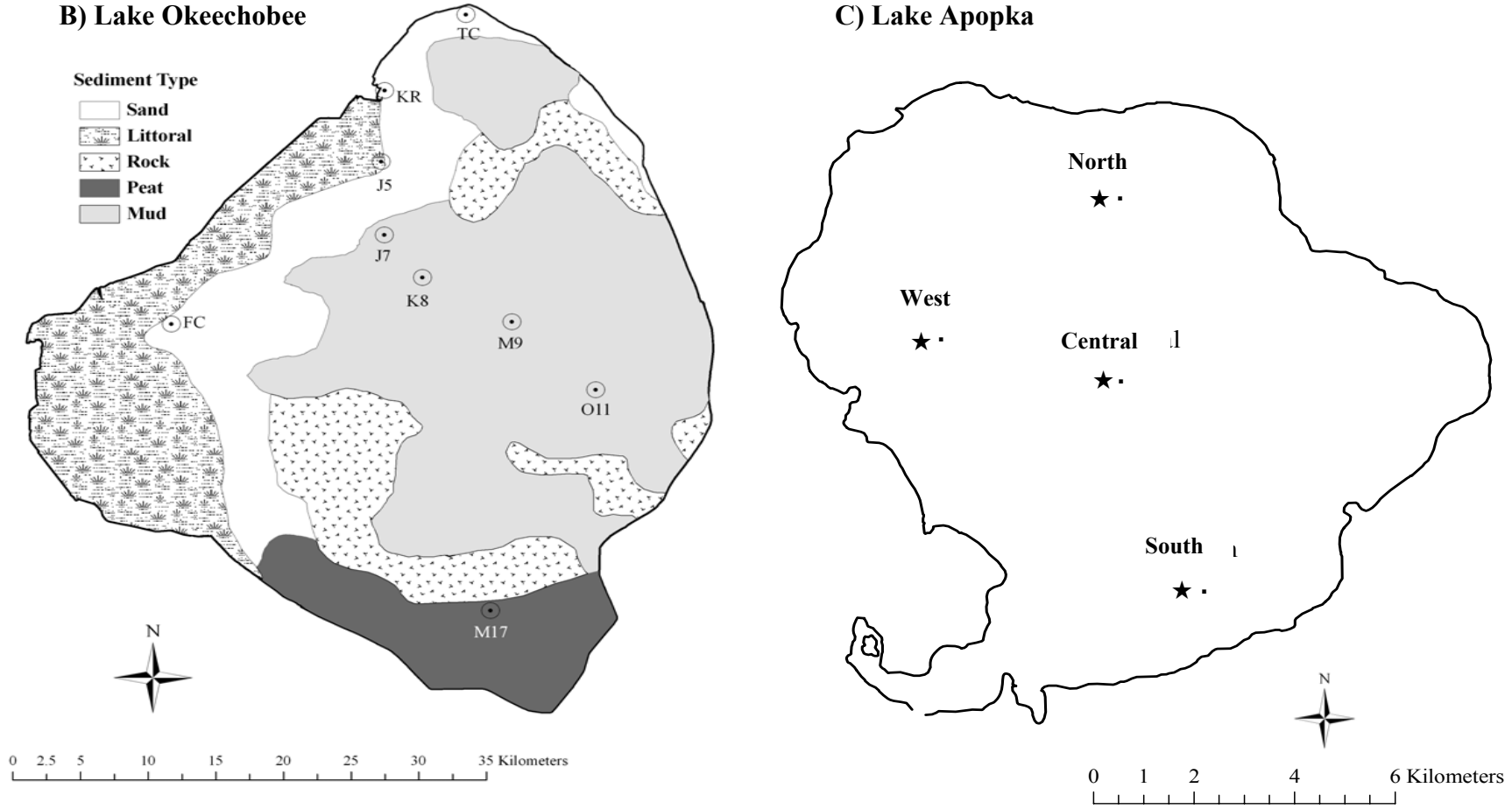


Figure 2-1. continued

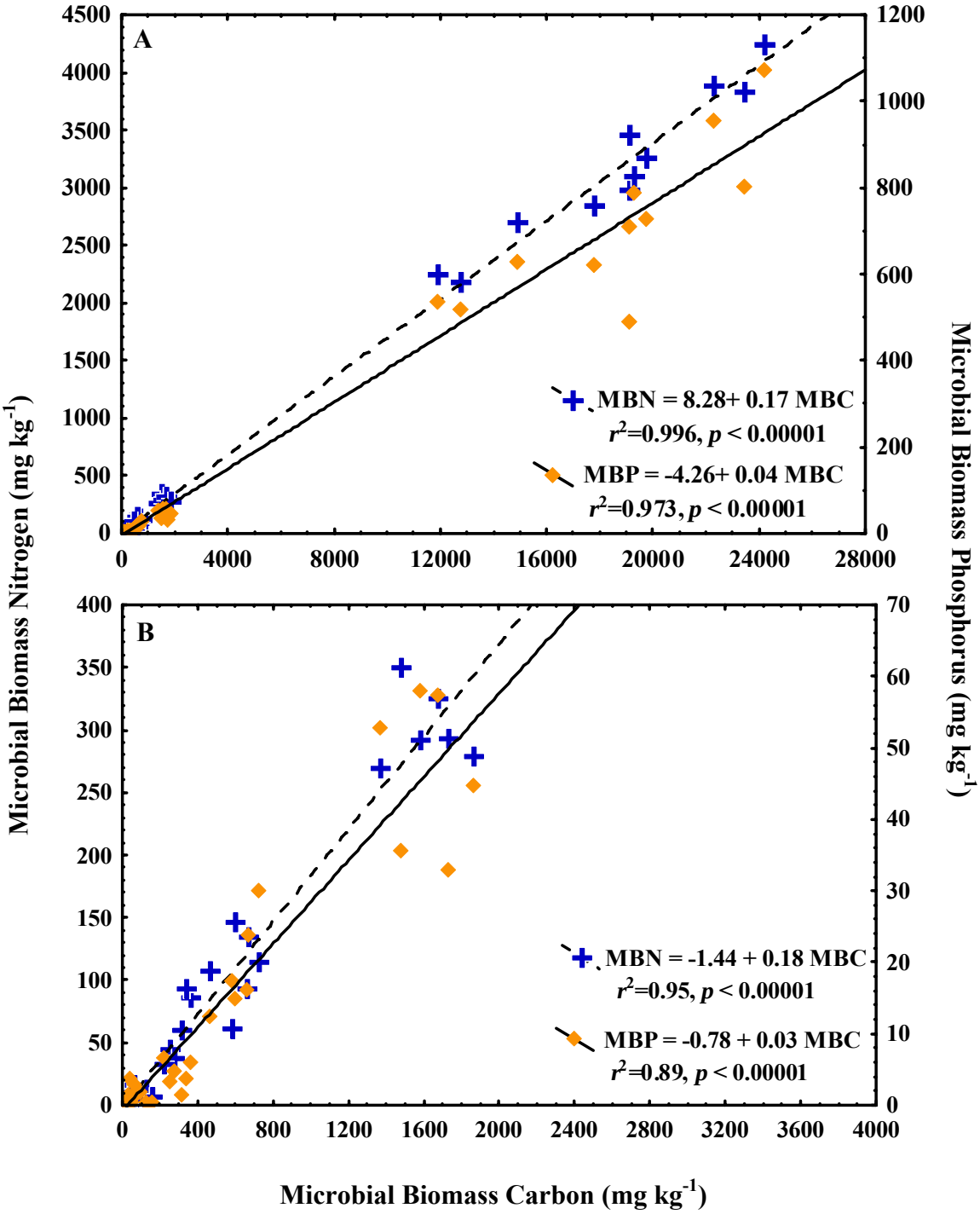


Figure 2-2. Linear regressions between 1) microbial biomass carbon and microbial biomass nitrogen, and 2) microbial biomass carbon and microbial biomass phosphorus of sediments from A) all lakes and B) data from Lake Annie and Lake Okeechobee only.

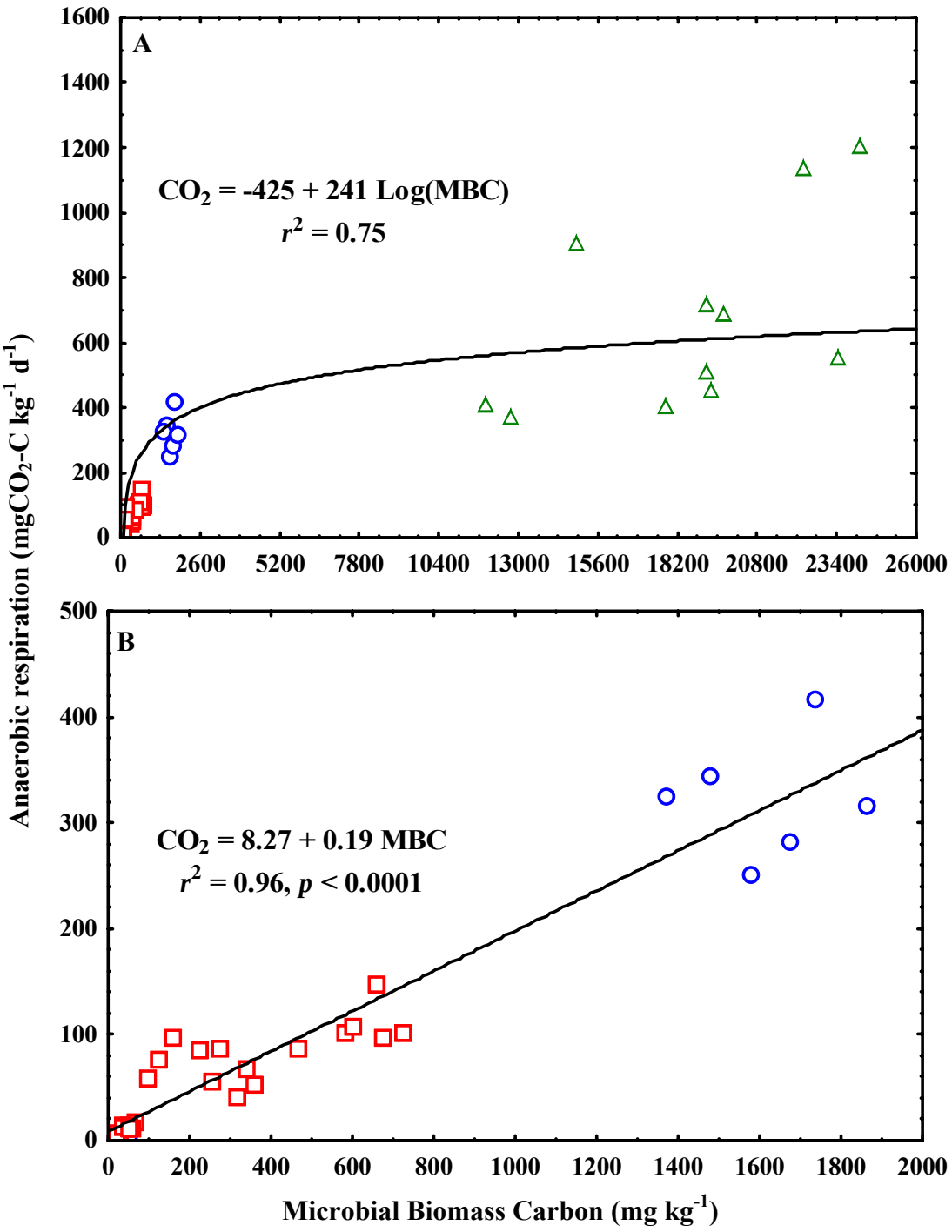


Figure 2-3. Relationship between anaerobic respiration and microbial biomass carbon of sediments from A) Lake Annie (blue circles), Lake Okeechobee (red squares), and Lake Apopka (green triangles) and B) data from Lake Annie and Lake Okeechobee only.

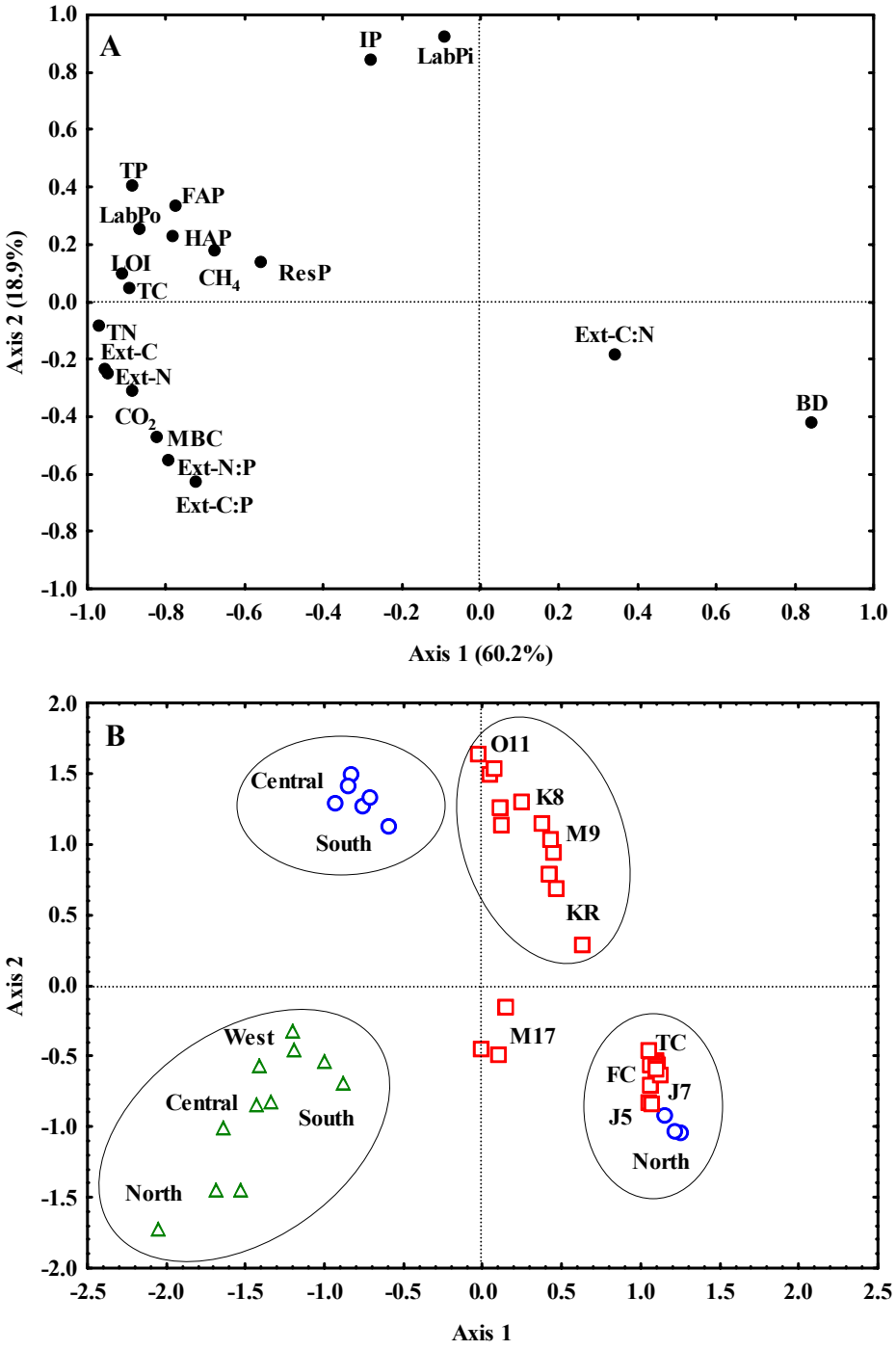


Figure 2-4. Results of the Principal Component Analysis (PCA-1), a) loadings (n = 47), and B) the plot of the scores of the sites from Lake Annie (blue circles), Lake Okeechobee (red squares), and Lake Apopka (green triangles).

BD: bulk density, LOI: loss on ignition, TC: total carbon, Ext-C: extractable organic carbon, TN: total nitrogen, Ext-N: extractable labile nitrogen, TP: total phosphorus, LabPi: labile inorganic phosphorus, LabPo: labile organic phosphorus, IP: HCl-Pi inorganic phosphorus, FAP: moderate labile organic phosphorus, HAP: highly resistant organic phosphorus, ResP: residual phosphorus, Ext-P: extractable labile phosphorus, MBC: microbial biomass carbon, CO_2: basal anaerobic respiration, CH_4: basal methane production rates.

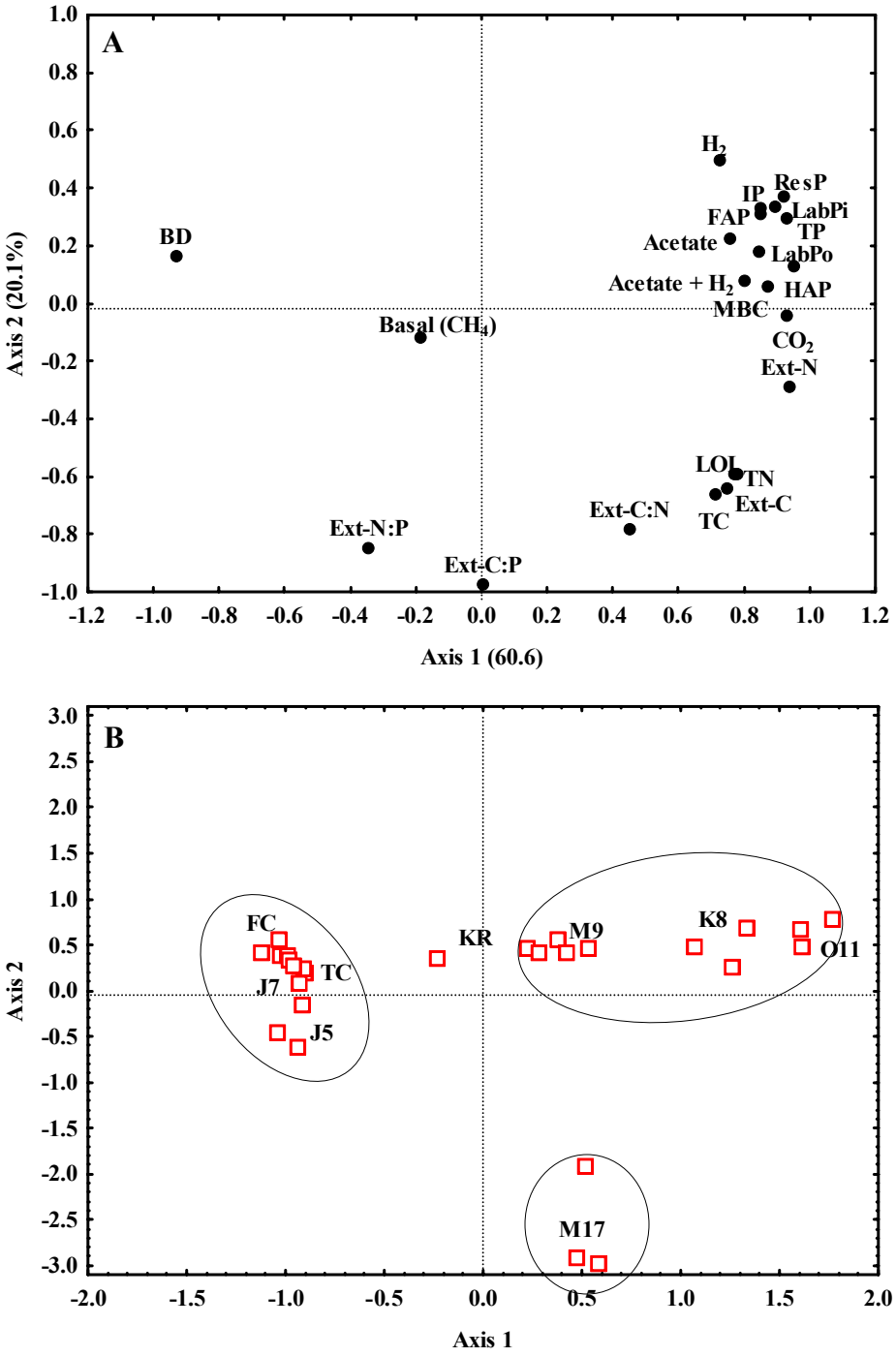


Figure 2-5. Results of the Principal Component Analysis (PCA-2), A) loadings of (n =27), and B) the plot of the scores of the sites of Lake Okeechobee.

BD: bulk density, LOI: loss on ignition, TC: total carbon, Ext-C: extractable organic carbon, TN: total nitrogen, Ext-N: extractable labile nitrogen, TP: total phosphorus, LabPi: labile inorganic phosphorus, LabPo: labile organic phosphorus, IP: HCl-Pi inorganic phosphorus, FAP: moderate labile organic phosphorus, HAP: highly resistant organic phosphorus, ResP: residual phosphorus, Ext-P: extractable labile phosphorus, MBC: microbial biomass carbon, CO_2: basal anaerobic respiration, Basal (CH_4): basal methane production rates, Acetate, H_2, and Acetate + H_2, methane production rates from electron donor addition.

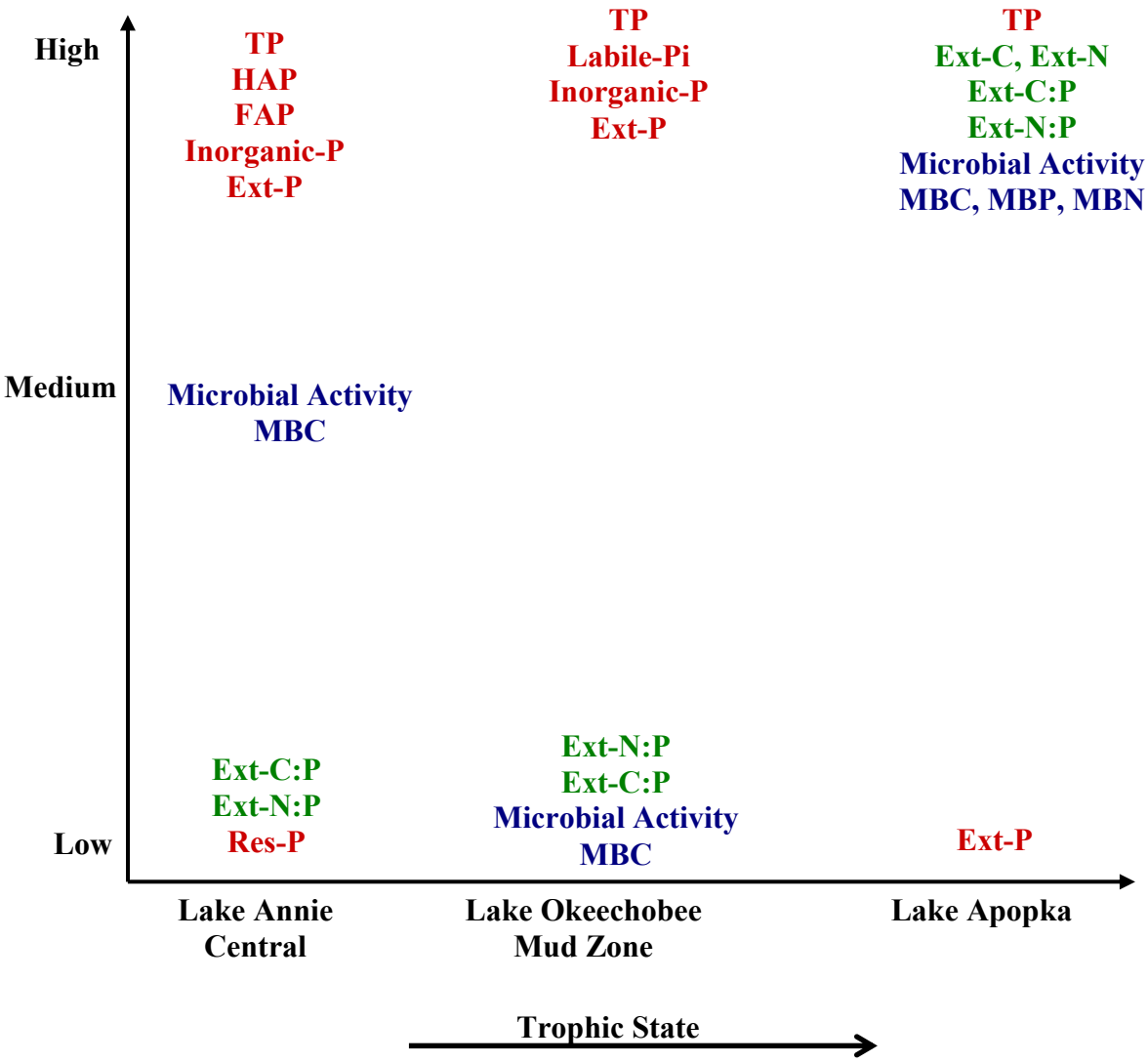


Figure 2-6. Graphic representation of sediment characteristics of three lakes in relation to their trophic state. Ext-C: extractable organic carbon, Ext-N: extractable labile nitrogen, TP: total phosphorus, Inorganic-P: HCl-Pi, FAP: moderate labile organic phosphorus, HAP: highly resistant organic phosphorus, Res-P: residual phosphorus, Ext-P: extractable labile phosphorus, MBC: microbial biomass carbon, MBP: microbial biomass phosphorus, MBN: microbial biomass nitrogen, and microbial activity: CO_2 and CH_4 production rates.

CHAPTER 3
SEDIMENT PHOSPHORUS FORMS IN SUBTROPICAL LAKES

Introduction

Phosphorus (P) is often the limiting nutrient for primary productivity in freshwater ecosystems. Sources of P to lakes can be external (allochthonous) or internal (autochthonous). Allochthonous P input originates in the drainage basin, while autochthonous P originates from primary and secondary productivity within lakes. A major portion of P from these sources added to the water column accumulates in sediments. Sediment P is present in both inorganic and organic forms. Organic P and cellular constituents of the biota represent 90% of total P (TP) in freshwater ecosystems (Wetzel 1999), and in sediments 30-80% of TP is typically in organic form (Williams and Mayer 1972; Boström et al. 1982).

Although organic P is an important component of sediment P, it has been relatively under-studied as compared with the fate of inorganic P (Turner et al. 2005). The reason for this is that there is no direct way to measure organic P. It is usually estimated by difference (before and after ignition at high temperature) (Saunders and Williams 1955), or by sequential extraction or chemical fractionation (Condron et al. 2005; McKelvie 2005). These chemical fractionations are based on different solubilities of P forms in alkaline and acid extractions with different pH. Turner et al. (2006) compared two methodologies, chemical fractionation and phosphorus-31 nuclear magnetic resonance (^{31}P NMR) spectroscopy, to measure organic P, and showed that for wetland soils, alkaline extraction with molybdate colorimetry overestimated organic P (between 30-54%). They concluded that alkaline extraction with ^{31}P NMR spectroscopy is a more accurate method to quantify organic P. In recent years there have been many studies using this methodology to distinguish different organic P forms in lake sediments (Hupfer et al. 1995, 2004; Carman et al. 2002; Ahlgren et al. 2005; Ahlgren et al. 2006*a*, *b*; Reitzel et. al 2006*a*, *b*,

2007). Phosphorus-31 NMR spectroscopy can identify different P compounds, based on their binding properties, as orthophosphate, pyrophosphate (pyro-P), polyphosphate (poly-P), phosphate monoester, phosphate diester (e.g., DNA, lipids), and phosphonates (Newman and Tate 1980; Turner et al. 2003).

These different P compounds present in the sediment will be released to the water column (internal load) due to chemical, physical and biological processes. Therefore benthic sediments may play a critical role in P cycling by acting as sources, or as sinks for P. With the reduction and control of external nutrient load, the internal load can become a major issue in regulating the trophic state and the time lag for recovery of lakes (Petterson 1998). Determination of the relative abundance of different P forms in sediments is important to understand sediment P processes and internal loading. In this study I characterized phosphorus compounds as a function of sediment depth using two different techniques, ^{31}P NMR spectroscopy and conventional organic P fractionation method. I hypothesized that surface sediments represent material accreted in recent years and chemically it will have different characteristics compared to subsurface older sediments. The specific objectives of this study were to: (i) to characterize organic P compounds in vertical sediment profiles using two different techniques, ^{31}P NMR spectroscopy and P fractionation extraction, (ii) address factors controlling P solubility in these sediments.

Materials and Methods

Study Sites

Three Florida (USA) lakes, ranging in trophic state, were selected. Lake characteristics were described in Chapter 2 (Table 3-1, Figure 3-1).

Field Sampling

Sediment sampling sites were selected based on previous spatial study conducted in all lakes (Chapter 2). Sediment –water interface cores of variable lengths were collected using a

piston corer (Fisher et al. 1992) or by SCUBA divers. One central site (80-cm core) was sampled in Lake Annie on June 25, 2005 (Figure 3-1A, Table 3-1). Cores were collected at three sites in Lake Okeechobee on July 16, 2005: M17 = peat (40-cm core), M9 = mud (70-cm core) and KR = sand (40-cm core) (Figs. 3-1B, Table 3-1). A western site (98-cm core) was sampled in Lake Apopka on May 28, 2005 (Figure 3-1C, Table 3-1). Cores were sectioned in the field at the following intervals: 0-5, 5-10, 10-15, 15-20, 20-30, 30-45, 45-60, 60-80, 80-100 cm. Samples were placed in plastic bags, sealed, and kept on ice. Nine cores were collected from each site. Three cores were used to make a composite core to obtain sufficient material for all measurements. The nine cores yielded three replicates of composite sediments from each site. All measured sediment variables are reported on a dry weight basis (dw). Water quality variables were described in Chapter 4, and in the present study values were used to characterize the lakes.

Sediment Properties

Samples were transported on ice and stored in the dark at 4 ºC. Before each analysis, samples were homogenized and sub-samples taken. Sediment bulk density (g dry cm^{-3} wet) was determined on a dry weight basis at 70 ºC for 72 hours, and pH was determined on wet sediments (1:2 sediment-to-water ratio). Sediment samples were ground in a ball mill and passed through a # 40 mesh sieve. Organic matter content (LOI-loss on ignition) was determined by weight loss at 550ºC. Total P was measured by ignition method, followed by acid digestion (6 M HCl) and measured colorimetrically with a Bran+Luebbe Technicon™ Autoanalyzer II (Anderson 1976; Method -365.1, EPA 1993).

Sediment Phosphorus Fractionation

Due to high water content of Lake Annie and Lake Apopka sediments, pore water was extracted (centrifuged at 10,000 x g for 10 min) prior to P fractionation. Pore water TP was measured after digestion with 11N H_2SO_4 and potassium persulfate (Method - 365.1, EPA 1993).

Organic P pools were measured using a chemical fractionation scheme described by Ivanoff et al. (1998). This procedure involved sequential chemical extraction in a 1:50 dry sediment-to-solution ratio, with (Figure 3-2): 1) 0.5 M $NaHCO_3$ (pH = 8.5) representing labile inorganic and organic P; 2) 1 M HCl representing inorganic P bound to Ca, Mg, Fe, and Al; 3) 0.5 M NaOH representing organic P associated with fulvic and humic fractions (moderately and highly resistant organic P, respectively). Phosphorus remaining in residual sediment after sequential extraction was measured by ignition method and is called residual P, non-reactive P that includes both organic and inorganic P. Extracts from each of these fractions were centrifuged at 10,000 x g for 10 min and filtered through a 0.45 μm membrane filter, and analyzed for SRP or digested for TP (with sulfuric acid and potassium persulfate). Solutions were analyzed by colorimetry, determined by reaction with molybdate using a Bran+Luebbe Technicon™ Autoanalyzer II (Murphy and Riley 1962; Method - 365.1, EPA 1993). Residual P was determined using an ignition method (Anderson 1976), and analyzed as described previously for TP.

Microbial biomass P (MBP) was measured by the chloroform fumigation-extraction method (Hedley and Stewart 1982; Horwath and Paul 1994; Ivanoff et al. 1998). Briefly, sediment samples were split into duplicates. One sample was treated with alcohol-free chloroform (0.5 mL) to lyse microbial cells, placed in a vacuum desiccator and incubated for 24 hrs. The other sample was left untreated. Both sample sets were extracted with 0.5 M $NaHCO_3$ (pH = 8.5) in a 1:50 dry sediment-to-solution ratio. Extracts from both sets were filtered through a 0.45 μm membrane filter and digested for TP with sulfuric acid and potassium persulfate, and analyzed as describe previously. Microbial biomass was calculated by the difference between treated (with chloroform) and non-treated samples.

^{31}P Nuclear Magnetic Resonance

Samples from each depth interval in triplicate cores from each site were combined and extracted using the methods described by Hupfer et al. (1995, 2004) and Turner et al. (2006). A pre-extraction with 100 mL of 0.067 M EDTA (1 hour shaking, centrifuged at 10,000 x g for 30 min) was conducted to reduce the influence of iron and calcium that can interfere with ^{31}P NMR spectroscopy (Hupfer et al. 1995, 2004). Samples were then extracted with 40 ml of 0.2 M NaOH/0.067 M EDTA (1:20 dry sediment-to-solution ratio), shaken for 2 hours and centrifuged at 10,000 x g for 30 min. A small aliquot of each extract (2 mL) was used to determine total P (NaOH-EDTA TP), digested and measured as described for organic P forms. The remaining sample was frozen immediately after centrifugation at -80 ºC and later lyophilized. Samples were analyzed by ^{31}P NMR spectroscopy as described by Turner et al. (2006). Each lyophilized extract (approx. 100 mg) was redissolved in 0.1 mL deuterium oxide (to provide a NMR signal lock) and 0.9 mL of a solution containing 1.0 M NaOH (to raise the pH to > 13 to ensure consistent chemical shifts and optimum spectral resolution) and 0.1 mL EDTA, and transferred to a 5 mm NMR tube. Solution ^{31}P NMR spectra were determined using a 6 µs pulse (45º), a delay time of 1.0 s and acquisition time of 0.2 s, with a Bruker Avance DRX 500 MHz spectrometer operating at 202.456 MHz for ^{31}P. Chemical shifts of signals were expressed in parts per million (ppm) relative to an external standard of 85% H_3PO_4. Signals were assigned to individual P compounds or functional groups based on literature (Makarov et al. 2002; Turner et al. 2003).

Statistical Analysis

A Pearson correlation and a Principal Component Analysis (PCA) were performed to determine relations among P forms measured with different methods. All statistical analyses were conducted with Statistica 7.1 (StatSoft 2006) software.

Results

Sediment Properties

Acidic pH conditions were observed in Lake Annie sediments and neutral to alkaline values in Lake Okeechobee and Lake Apopka deposits (Table 3-2). Sediment bulk density values were lowest in Lake Apopka, followed by Lake Annie reflecting their high fluid content relative to Lake Okeechobee sediments. Bulk density increased with depth in lakes Apopka, Annie and at Okeechobee site M9. Lake Okeechobee M17 and KR sediments, showed no clear trend. Organic matter content was highest at Lake Okeechobee M17 reflecting its high peat content, followed by Lake Apopka, Lake Annie, and Lake Okeechobee sites M9 and sandy KR (Table 3-2).

Sediment Phosphorus Forms

Total P decreased with depth in all lake cores (Table 3-3). Among surface sediment samples from all cores, Lake Annie had the highest TP concentrations as compared with other lakes. In Lake Okeechobee sites M9 and M17, and Lake Apopka sediments, the deepest layer had about half the TP concentration measured in surface sediments. The KR site in Lake Okeechobee displayed the most dramatic decrease in TP with depth (Table 3-3).

Surface sediment labile inorganic P (labile-Pi) concentrations were highest in Lake Okeechobee site M9 followed by Lake Annie (Table 3-3). Approximately 5% of the TP was present as labile-Pi in sediments of most sites. In sediments at site M9 in Lake Okeechobee, 7-15% of TP was present as labile-Pi. Labile-Pi decreased with sediment depth in Lake Annie and Lake Okeechobee sediments. Labile Pi increased with depth (0.1-9% of TP) in Lake Apopka.

Inorganic P (HCl-Pi) was highest at M9 in Lake Okeechobee, followed by Lake Annie and Lake Apopka sediments. There was no clear trend with depth in Lakes Annie and Apopka, but a slight decrease with depth in mud sediments of Lake Okeechobee (M9-site) was seen. Lake Okeechobee sites M17 and KR sediments showed a pronounced decrease of HCl-Pi with depth.

HCl-Pi accounted for 26-56 % of TP in Lake Apopka, 26-49% of TP in Lake Annie sediments, while in Lake Okeechobee site M9 sediments 64-89% of TP was present in inorganic P pool. In sediments of M17 and KR sites HCl-Pi contribution was 37-79% and 20-94% respectively, and decreased with depth.

Labile organic P (labile-Po) was highest in Lake Annie and Lake Apopka with lower concentrations in all Lake Okeechobee stations. Approximately 0.1-3% of the TP was present as labile-Po in sediments of most sites, with a general decrease with depth (Table 3-3). Microbial biomass P (MBP) decreased with depth in all cores except KR, where values were consistently low throughout. Highest MBP values were detected in Lake Apopka, where 47% of the TP was present as MBP in surface sediments. Moderately available organic P (FAP) was higher in Lake Annie and Lake Apopka, with lower values in Lake Okeechobee sediments. FAP displayed a general decrease with depth in the cores (Table 3-3). Similar results were detected for highly resistant organic P (HAP). Lake Annie had the highest values, followed by Lake Apopka, then Lake Okeechobee. Fulvic acid-P and HAP accounted for 17-26% and 12-19% of TP in Lake Annie, while in Lake Apopka was 5-17% and 4-10%, respectively. Residual P (Res-P) was higher in Lake Apopka and site M9 in Lake Okeechobee, and decreased with depth. In sediments at site M9 6-20% of TP was present as Res-P with a decreased with depth. In the other Lake Okeechobee sites, M17 and KR, 6-15% and 3-46% of TP was present as Res-P, respectively, and increased with depth. Lake Apopka Res-P (19-25% of TP) did not present a clear depth trend. In Lake Annie, the concentration of Res-P and its contribution to TP (0-0.5%) were low.

^{31}P Nuclear Magnetic Resonance

^{31}P NMR spectroscopy enabled the identification of discrete pools of organic P (Figure 3-3A, B, C, Table 3-4). Orthophosphate was the major P compound in sediments of Lake Okeechobee and none of the organic compounds were detected with this technique. Results of

^{31}P NMR spectroscopy were in agreement with the results of chemical P fractionation (Tables 3-3, 3-4). NMR analyses show that Lake Okeechobee sediments are dominated by inorganic P as orthophosphate (M9: 68-100%, M17: 100% and KR 100%), although in upper layers of mud sediments (site-M9) phosphate monoester (24-27%), and DNA-P (7-9%) were also detected (Figure 3-3B, Table 3-4). In Lake Annie, three P compounds were detected in all sediment depths: orthophosphate (51-71%), phosphate monoester (23-36%), and DNA-P (6-10%), but no clear trend was observed with depth (Figure 3-3A, Table 3-4). In Lake Apopka sediments, six different P compounds were detected: orthophosphate (28-85%), phosphate monoester (12-28%), DNA-P (15-31%), lipid-P (3-4%), pyro-P (3-10%), and poly-P (8-11%) (Figure 3-3C, Table 3-4). There was a general decrease in orthophosphate and organic P forms (phosphate monoester, lipid-P, DNA-P) with depth in Lake Apopka sediments.

Comparisons of P forms determined by the two different methods showed that orthophosphate (NMR) was correlated with HCl-Pi (r = 0.68), labile-Po (r = 0.73), FAP (r = 0.82), and HAP (r = 0.80) (chemical fractionation). Phosphate monoester (NMR) was strongly correlated with FAP (r = 0.94) and HAP (r = 0.68) (chemical fractionation). Lipid-P (r = 0.88) and DNA-P (r = 0.76) (NMR) showed positive correlation (r > 0.7) with MBP (chemical fractionation). To address relations between different P forms in sediments from the three different lakes a Principal Component Analysis (PCA) was conducted (Figure 3-4). The PCA had 40.5% of the data variability explained by Axis 1. Axis 2 explained 29.4% of the data variability and the selected variables were Lipid-P, MBP and Res-P (Figure 3-4A). Orthophosphate, phosphate monoester, DNA, labile-Po, FAP, HAP and TP were the variables selected by Axis 1. The position of the sites and sediment depth in relation to the variables loadings in the PCA showed that the three lakes are separated into different groups (Figure 3-

4B). Lake Apopka placed in the position of the parameters selected by Axis 2 and Lake Annie in the position of variables selected by Axis 1. Lake Okeechobee was placed in the position of inorganic P forms, i.e., HCl-Pi and labile-Pi (Figure 3-4B).

Discussion

Although an oligo-mesotrophic lake, Lake Annie contained more TP in sediments than both eutrophic Lake Okeechobee and hypereutrophic Lake Apopka. Lake Annie water inputs are from ground water (90%) and direct rainfall (10%), with negligible surface runoff. Anthropogenic impact is low (Swain and Gaiser 2005) and high TP concentration at all sediment depths is natural, not induced by anthropogenic activities. Schottler and Engstrom (2006) dated sediment cores from Lake Annie by ^{210}Pb and ^{137}Cs and reported that sediments at ~ 80 cm depth were approximately 125 years old. The results showed higher concentrations of TP at that depth in Lake Annie than in Lake Okeechobee and Lake Apopka sediments. Several studies in Lake Apopka and Lake Okeechobee indicate that the increase in TP concentration in upper sediment layers was due to cultural eutrophication (Brezonik and Engstrom 1998; Schelske et al. 2000; Kenney et al. 2002; Waters et al. 2005; Schottler and Engstrom 2006; Engstrom et al. 2006). Nevertheless, a decrease in TP with sediment depth has been observed in many lakes (Søndergaard et al. 1996; Gonsiorczyk et al 1998; Ahlgren 2005; Reitzel et al. 2006*a*, 2007).

The relative abundance of P forms in sediments is more important than the total concentration with respect to sediment P processes and internal loading, and was quite different among the study lakes. Also, concentrations of various P compounds changed with sediment depth, indicating different processes were controlling P reactivity and mobility in these lakes. The intrinsic difference of these P compounds in different sediments is highlighted by the PCA (Figure 3-4A, B).

Lake Annie had more stable P compounds with greater sediment depth. Dominant P forms were HCl-Pi, FAP, and HAP, as determined by chemical fractionation, and orthophosphate and phosphate monoester as determined by ^{31}PNMR. Inorganic P represents P bound to Ca, Mg, Fe, and Al, and its solubility is controlled by pH and/or redox potential. Lake Annie sediments (central site) were characterized as having high Fe (3640 mg kg^{-1}) and Al (34640 mg kg^{-1}) concentration, and its mineral particle size composition was clay (48%), silt (49%), and sand (2%) (Thompson 1981). Lake Annie sediment pH is low, and decreased with depth. Although redox potential was not measured in this study, these sediments are apparently highly reduced as they are under persistent anaerobic conditions. Consequently the influence of redox potential and pH in P solubility in this lake must be minimal, as physical and chemical conditions in Lake Annie already favor solubilization of inorganic P. There is no increase in labile-Pi with greater sediment depth, but there is an increase in HCl-Pi contribution to TP. Thus, it seems that total inorganic P is present in stable forms in deeper sediments. Also, inorganic P can be bound to clay minerals, in a stable form, as protonation of surface Fe and Al functional groups in clays increase the P binding capacity of non calcareous sediments (Edzwald et al. 1976). High labile-Pi in surface sediment of Lake Annie is probably caused by mineralization of organic P through enzyme activity (Chapter 4). High enzyme and microbial activities in Lake Annie, along with lake physico-chemical characteristics, and the major P forms found in the sediments, strongly indicate that biotic processes play an important role in P solubility in these mud sediments.

Lake Okeechobee sediments were dominated by HCl-Pi (chemical fractionation) and orthophosphate (^{31}P NMR). In Lake Okeechobee mud sediments, Fe-P precipitation controls the behavior of P under oxidizing conditions while Ca-P mineral precipitation governs P solubility under reducing conditions (Moore and Reddy 1994). Moore and Reddy (1994) reported that the

concentrations of soluble reactive P (SRP) in pore water increased under reduced conditions, and were low near neutral (pH 6.5 and 7.5), but higher under slightly acidic (pH 5.5) or basic (pH 8.5) conditions. Furthermore, Olila and Reddy (1997) reported that SRP increases exponentially with a decrease in redox potential in sediments from the mud zone of Lake Okeechobee. Consequently, P solubility in Lake Okeechobee mud sediments is controlled by abiotic processes, either pH, redox potential, or both (Moore and Reddy 1994; Olila and Reddy 1997).

The control of P solubility in other sediment types of Lake Okeechobee has not been studied. Nevertheless, the dominance of inorganic P in all Lake Okeechobee sites and lack of organic P found in ^{31}P NMR, suggests that pH and redox potential also regulate P solubility in M17 and KR sediments. Labile Pi follows the HCl-Pi distribution in sites of Lake Okeechobee (especially M9 and KR). Considering that the major P forms in Lake Okeechobee are HCl-Pi, as well as the fact that these sediments had low enzyme and microbial activities (Chapter 4), it is reasonable to speculate that abiotic processes control P solubility in these sediments.

In contrast to Lake Annie and Lake Okeechobee, in which either biotic or abiotic processes alone control P solubility respectively, in Lake Apopka sediments, P solubility is controlled by a combination of biotic and abiotic processes. Dominant P forms were MBP and HCl-Pi (chemical fractionation), and orthophosphate, phosphate monoester and DNA-P (^{31}P NMR). The high contribution of organic P forms in relation to total P in Lake Apopka results from deposition of algal primary producers to the sediment. Gale and Reddy (1994) reported gross primary productivity in Lake Apopka of 1400 g C m^{-2} yr^{-1} of which approximately 1034 g C m^{-2} yr^{-1} is deposited in sediments. The Lake Apopka phytoplankton community is dominated by cyanobacteria, (*Synechococcus* sp., *Synechocystis* sp., and *Microcystis incerta*), with little variation throughout the year (Carrick 1993; Carrick and Schelske 1997). Brunberg (1995)

simulated a *Microcystis* sedimentation event in a eutrophic lake deposit and found that organic P was the dominant fraction in the cells (74% of total P). After 15 days of incubation, most of the TP was transformed into total labile P and organic P (NaOH soluble).

The dominant P forms in Lake Apopka sediment reflect the high contribution of primary producers to sediment P. In Lake Apopka's calcareous sediments, abiotic phosphate uptake and solubility are controlled by pH (Olila and Reddy 1995, 1997). Phosphorus release is associated with dissolution of Ca-P, and a six-fold increase in pore water SRP concentration occurred with a 0.5 decrease in pH (Olila and Reddy 1995, 1997). Biotic P control is also important in Lake Apopka. Olila and Reddy (1997) reported a large increase in labile-Pi with highly reducing conditions and suggested it was caused by lysed microbial cells or degradation of stored poly-P. If the downcore decline in concentration of P forms measured with ^{31}P NMR is indicative of P degradation (Reitzel et al. 2007), then biotic processes are important in Lake Apopka. Almost 50% of the total P is in microbial biomass in surface sediments (Table 3-3). Presence of poly-P and pyro-P in sediments also indicates high activity of microorganisms involved in biological P cycling (Hupfer et al. 2004; Ahlgren et al. 2005; Reitzel et al. 2006a, 2007). High enzymatic activities found in Lake Apopka sediments strongly support the biological control of P solubility in these sediments (Chapter 4). Low concentrations of labile-Pi and its low percent contribution to total P in surface sediments in hypereutrophic Lake Apopka probably reflects a high P demand by the microbial community.

Some studies found a significant correlation between bacterial biomass and organic P extracted with NaOH, and suggested that organic P extracted with NaOH can be used as a proxy measure of bacterial P (poly-P) (Uhlman and Bauer 1988; Waara et al. 1993; Goedkoop and Petterson 2000). My results do not support this suggestion as Lake Annie had the highest

concentration and contribution to TP, of organic P extracted with NaOH (FAP and HAP), but poly-P was not detected with ^{31}P NMR (Tables 3-3 and 3-4). Moreover, pyro-P that can include degradation products of poly-P (Hupfer et al. 1995), was absent in Lake Annie. Several studies showed that microbial biomass correlates with enzyme activity (Davis and Goulder 1993; Massik and Cotello 1995; Barik et al. 2001). Since a high correlation between both FAP and HAP and PMEase activity (Chapter 4) exists, it seems that the correlation between NaOH-P and microbial biomass reflects the fact that these fractions are used as a P source by microorganisms through enzyme activity.

Lake Apopka was the only lake where poly-P was detected. Gächter and Meyer (1993) postulate that if sufficient organic carbon and PO_4^{-3} are available under aerobic conditions, bacteria can store poly-p. The occurrence of poly-P and the identification of phosphate-accumulating organisms come from studies in wastewater treatment plants with enhanced biological P removal (Seviour 2003). In lakes, the mechanism of poly-P formation is poorly understood, although poly-P has been detected in several recent studies (Hupfer et al. 1995, 2004; Carman et al. 2002; Reitzel et al. 2006*a*, *b*, 2007). Alternation of aerobic/anaerobic conditions, combined with available carbon and phosphate, leads to dominance of bacteria that can store poly-P (Mino et al. 1998; Seviour 2003). Khoshmanesh et al. (2001) used ^{31}P NMR and transmission electron microscopy, on a sediment spiked with acetate, and concluded that under aerobic conditions when acetate was available, microorganisms accumulated phosphate as poly-P.

Some sediments have ideal conditions for poly-P formation, such as oscillating aerobic/anaerobic conditions, labile dissolved organic carbon and labile phosphorus. These conditions exist in Lake Apopka (Chapter 4 and 5). Lake Annie, has available DOC, but low

DOC:P ratios (Chapter 4, Table 4-3). Perhaps more important its sediments are always anaerobic. Carman et al. (2002) reported similar results. They found high poly-P in Lake Gömmaren, with well-oxidized sediments and high organic matter content, some poly-P in Lake Långsjön, with oscillating oxic/anoxic conditions, and no poly-P in Lake Flaten, with constant anoxic conditions. Lake Okeechobee, though shallow, and probably subject to oscillating aerobic/anaerobic conditions, seems to be carbon limited for poly-P formation, with low ratios of DOC:P (Chapter 4 and 5). Perhaps more important is the high availability of labile-Pi in Lake Okeechobee. Poly-P does not accumulate under constant P-sufficient conditions (Vadstein 2000) as the enzyme responsible for poly-P formation (polyphosphate kinase) is a repressible enzyme that is derepressed under P starvation (Harold 1966).

Absence of poly-P in the uppermost 5 cm of sediment is surprising as Hupfer et al. (2004) reported poly-P in the top 0.5 cm of sediment for 22 European lakes, and Ahlgren et al. (2006*b*) found poly-P in the top 1 cm of sediment in two oligotrophic mountain lakes in Sweden. Hupfer et al. (2004) explained the presence of poly-P in surface sediments as coming from poly-P formed in the water column then deposited on the lake bottom. I found poly-P at 10, 15 and 20 cm depth in the sediment, and other recent studies reported similar results. Carman et al. (2002) reported distinct signals of poly-P in 0-7 and 8-16 cm in Lake Gömmaren. Reitzel et al. (2007) found poly-P in 0-10 cm sections of sediment from mesotrophic Lake Erken. In hypereutrophic Lake Sønderby (Denmark), Reitzel et al. (2006*a*) found poly-P in anoxic sediment layers up to 24 cm deep. Absence of poly-P in the uppermost first 5 cm of sediment in Lake Apopka may be related to the relatively lower DOC:P ratio, as the highest ratios were found in the sections where poly-P was detected (Chapter 4, Table 4-3). Also, presence of poly-P in deeper sections of the

sediment is a strong indication that the sediment microbial community is producing poly-P rather than its being produced in the water column and deposited in sediment.

Kenney et al. (2001), however, reported that 25-90% of the sediment TP in Lake Apopka may be sequestered in intact phytoplankton cells as poly-P, and found poly-P at 50 cm depth. Moreover Kenney et al. (2001) stated that poly-P could be used as an indicator of eutrophication, and suggested that poly-P is chemically inert. They used heat extraction and colorimetry, which may have overestimated poly-P. Phosphorus-31 NMR spectroscopy is a more accurate methodology, and chemical extraction and colorimetry have been shown to overestimate organic P compounds (Turner et al. 2006). My results contradict the findings of Kenney et al. (2001). Reitzel et al. (2007) also disagree with respect to the inert nature of poly-P. Several studies have shown that poly-P is highly labile and plays an important role in P release from sediment (Törnbon and Rydin 1998; Petterson 2001; Hupfer et al. 2004; Ahlgren et al. 2005; Reitzel et al. 2007).

Conclusions

All lakes had a decrease in TP concentration with sediment depth, and although oligo-mesotrophic, Lake Annie contained more TP in sediments than both eutrophic Lake Okeechobee and hypereutrophic Lake Apopka. The relative abundance of P forms in sediments, however, is more important than the total concentration with respect to sediment P processes and internal loading, and was quite different among the study lakes. Also, concentrations of various P compounds changed with sediment depth, indicating that different processes were controlling P reactivity and mobility in these lakes. Lake Annie had more stable compounds with greater sediment depth. Dominant forms of TP were inorganic P (HCl-Pi), FAP, and HAP, as determined by chemical fractionation, and orthophosphate and phosphate monoester as determined by ^{31}P NMR. Lake Annie physico-chemical characteristics, as well as the major P

forms found in the sediment, strongly indicated that biotic processes play an important role in P solubility in these mud sediments. Lake Okeechobee sediments were dominated by inorganic P (HCl-Pi) (chemical fractionation) and orthophosphate (^{31}P NMR), indicating abiotic processes control P solubility in these sediments. Dominant P forms in Lake Apopka were MBP and HCl-Pi (chemical fractionation), and orthophosphate, phosphate monoester and DNA-P (^{31}P NMR). Almost 50% of the total P was in microbial biomass in surface sediments. The presence of poly-P and pyro-P in these sediments also indicated high activity of microorganisms involved in biological P cycling. Low concentrations of labile-Pi, and its low percent contribution to total P in surface sediments in hypereutrophic Lake Apopka, probably reflects a high P demand by the microbial community. This study also showed that the results of ^{31}P NMR spectroscopy were in agreement with the results of chemical P fractionation, and that the determination of the relative abundance of different P forms in sediments is important to understand sediment P processes.

Table 3-1. Characteristics of sampled sites in the three different lakes with sampling date, location, sediment type and water quality parameters (measured at 1 m).

Parameters	Annie[a]	Lake Okeechobee[b]			Apopka[b]
	Central	M9	M17	KR	West
Sampling Date	June/2005		July/2005		May/2005
Sediment Type	Mud/Clay	Mud	Peat	Sand	Organic
Water Column Depth (m)	20	4.0	2.5	3.1	2.0
Secchi (m)	2.0	0.08	0.15	0.5	0.3
Latitude	27º12'27"	26º58'17.6"	26º45'24.4"	27º58'11.5"	28º38'01"
Longitude	81º21'44"	80º45'38.4"	80º46'36.8"	81º00'51.8"	81º39'36"
Temperature (ºC)	30.2	29.5	28.8	30.8	26.6
Electrical Conductivity ($\mu S\ cm^{-1}$)	41.9	385	320	143	443
pH	5.1	7.8	7.6	6.0	7.6
Dissolved Oxygen ($mg\ L^{-1}$)	6.4	6.5	6.3	1.8	8.7
Dissolved Organic Carbon ($\mu g\ L^{-1}$)*	13.8	14.5	17.9	19.8	31.1
Total Phosphorus ($\mu g\ L^{-1}$)*	33.2	255.9	263.2	146.4	69.7
Soluble Reactive Phosphorus ($\mu g\ L^{-1}$)*	7.4	90.4	113.1	62.5	11.1
Total Nitrogen ($\mu g\ L^{-1}$)*	1807	3439	3362	2957	11149
Ammonium - NH_4-N ($\mu g\ L^{-1}$)*	181.6	103.0	60.4	83.6	119.6

* Mean concentration in the water column. [a] Average depth: 0.5-1-2-5-10-20 (m) and [b] Average depth: 0.5-1-2 (m).

Table 3-2. pH, bulk density (BD), organic matter content (LOI - loss on ignition) in sediment profiles of the three lakes. (mean ± standard deviation - SD). ** No replicates for SD calculation.

Lake	Site	Depth (cm)	pH	BD (g of dry cm^{-3} of wet)	LOI (%)
Annie	Central	5	5.4 ± 0.15	0.04 ± 0.006	58 ± 0.4
		10	5.2 ± 0.05	0.04 ± 0.003	57 ± 1.2
		15	5.3 ± 0.09	0.06 ± 0.003	55 ± 1.0
		20	5.3 ± 0.11	0.07 ± 0.003	54 ± 1.6
		30	5.4 ± 0.03	0.07 ± 0.003	52 ± 0.8
		45	5.5 ± 0.09	0.08 ± 0.002	52 ± 0.1
		60	5.5 ± 0.06	0.10 ± 0.006	50 ± 0.9
		80	5.7 ± 0.06	0.11 ± 0.007	50 ± 1.0
Okeechobee	M9	5	7.7 ± 0.16	0.11 ± 0.005	36 ± 1.7
		10	7.7 ± 0.03	0.16 ± 0.003	37 ± 1.2
		15	7.8 ± 0.04	0.20 ± 0.020	21 ± 1.3
		20	7.8 ± 0.04	0.23 ± 0.020	26 ± 6.9
		30	7.9 ± 0.03	0.26 ± 0.056	16 ± 3.8
		45	7.9 ± 0.05	0.33 ± 0.040	25 ± 6.5
		60	8.0 ± 0.01	0.30 ± 0.009	29 ± 4.7
		70	8.0 ± 0.08	0.33 ± 0.043	35 ± 3.5
	M17	5	7.6 ± 0.20	0.14 ± 0.003	83 ± 0.9
		10	7.5 ± 0.08	0.13 ± 0.011	88 ± 0.9
		15	7.4 ± 0.06	0.13 ± 0.002	89 ± 0.3
		20	7.4 ± 0.10	0.12 ± 0.005	89 ± 0.5
		30	7.3 ± 0.15	0.12 ± 0.010	89 ± 0.2
		40	7.4 ± 0.17	0.13 ± 0.023	88 ± 0.4
	KR	5	7.4 ± 0.19	1.22 ± 0.314	1.9 ± 2.8
		10	7.5 ± 0.19	1.13 ± 0.272	3.9 ± 2.9
		15	7.7 ± 0.46	1.15 ± 0.323	4.9 ± 3.5
		20	7.4 ± 0.41	0.51 ± 0.085	18.1 ± 4.2
		30	7.2 ± 0.27	0.55 ± 0.096	16.6 ± 8.8
		40	6.9 ± **	1.07 ± **	6.4 ± **
Apopka	West	5	7.5 ± 0.07	0.01 ± 0.001	69 ± 0.7
		10	7.3 ± 0.06	0.02 ± 0.001	67 ± 3.2
		15	7.2 ± 0.02	0.02 ± 0.004	67 ± 1.1
		20	7.2 ± 0.06	0.03 ± 0.006	65 ± 2.7
		30	7.3 ± 0.04	0.03 ± 0.009	64 ± 1.0
		45	7.2 ± 0.05	0.04 ± 0.014	66 ± 1.9
		60	7.1 ± 0.10	0.06 ± 0.010	68 ± 1.3
		80	7.0 ± 0.06	0.07 ± 0.005	69 ± 0.8
		98	7.0 ± **	0.07 ± **	71 ± **

Table 3-3. Phosphorus fraction concentrations in sediment profiles. (mean ± SD). ** No replicates for SD calculation.

Lake	Site	Depth (cm)	Total P	TP Pore Water	MBP	Labile P		HCl-Pi	FAP	HAP	Res. P
						Inorg.	Org.				
						(mg kg^{-1} dw)					
Annie	Central	5	1439 ± 35	12 ± 3	78 ± 8	72 ± 13	50 ± 9	368 ± 61	371 ± 71	268 ± 71	6 ± 6
		10	1423 ± 28	7 ± 3	64 ± 18	67 ± 12	43 ± 7	444 ± 13	341 ± 37	255 ± 28	4 ± 4
		15	1459 ± 67	9 ± 3	44 ± 3	55 ± 6	40 ± 5	548 ± 58	378 ± 17	185 ± 18	0.0 ± 0
		20	1531 ± 80	15 ± 3	37 ± 5	52 ± 9	35 ± 6	600 ± 73	378 ± 7	183 ± 41	3 ± 3
		30	1484 ± 191	25 ± 8	30 ± 7	42 ± 8	28 ± 2	726 ± 78	338 ± 33	213 ± 17	0.5 ± 0.8
		45	1513 ± 258	24 ± 9	24 ± 10	34 ± 10	22 ± 3	665 ± 116	285 ± 29	180 ± 13	1 ± 1
		60	1133 ± 32	18 ± 4	22 ± 10	22 ± 0.3	19 ± 2	544 ± 33	195 ± 30	184 ± 64	0.0 ± 0
		80	1149 ± 51	8 ± 1	18 ± 6	15 ± 3.8	16 ± 1	540 ± 33	206 ± 1	153 ± 20	0.0 ± 0
Okeechobee	M9	5	1051 ± 39	ND	50 ± 2	110 ± 7	6 ± 4	670 ± 40	72 ± 8	34 ± 4	213 ± 12
		10	924 ± 24	ND	34 ± 5	87 ± 11	8 ± 1	582 ± 11	64 ± 3	35 ± 3	194 ± 9
		15	835 ±16	ND	28 ± 8	127 ± 33	7 ± 4	607 ± 35	2 ± 3	8 ± 7	229 ± 53
		20	732 ± 83	ND	18 ± 2	55 ± 7	4 ± 1	575 ± 7	4 ± 3	4 ± 3	127 ± 35
		30	644 ± 57	ND	11 ± 2	80 ± 6	2 ± 0	496 ± 16	0 ± 0	0 ± 0	132 ± 29
		45	575 ±48	ND	9 ± 1	55 ± 8	3 ± 1	510 ± 42	0 ± 0	0 ± 0	108 ± 10
		60	590 ± 3	ND	8 ± 0.4	46 ± 3	2 ± 1	458 ± 66	1 ± 1	2 ± 3	67 ± 10
		70	497 ± 155	ND	2 ± 1	36 ± 2	1 ± 1	423 ± 35	4 ± 3	5 ± 2	50 ± 5

Table 3-3. continued

Lake	Site	Depth (cm)	TP	TP Pore Water	MBP	Labile P		HCl-Pi	FAP	HAP	Res-P
						Inorg.	Org.				
						(mg kg^{-1} dw)					
Okeechobee	M17	5	270 ± 13	ND	6 ± 0.6	14 ± 6	5 ± 0.5	213 ± 40	5 ± 2	14 ± 2	16 ± 1
		10	138 ± 7	ND	5 ± 0.3	4 ± 0.3	4 ± 0.4	56 ± 3	8 ± 1	15 ± 2	16 ± 2
		15	129 ± 16	ND	3 ± 1	3 ± 1	3 ± 0.5	55 ± 28	5 ± 1	15 ± 4	17 ± 6
		20	121 ± 9	ND	3 ± 0.8	3 ± 0.4	4 ± 0.7	44 ± 3	5 ± 0.5	17 ± 3	18 ± 4
		30	112 ± 14	ND	2 ± 0.8	4 ± 0.3	4 ± 0.8	42 ± 3	5 ± 1	15 ± 2	16 ± 2
		40	144 ± 27	ND	2 ± 1	3 ± 0.4	3 ± 0.6	70 ± 12	4 ± 1	16 ± 7	17 ± 7
	KR	5	263 ± 20	ND	1 ± 0.5	4 ± 1	0.4 ± 0.3	234 ± 24	4 ± 2	1 ± 0.2	8 ± 6
		10	280 ± 50	ND	1 ± 0.2	7 ± 2	1 ± 0.5	263 ± 31	3 ± 2	1 ± 2	14 ± 6
		15	258 ± 83	ND	1 ± 0.6	3 ± 2	0.4 ± 0.3	211 ± 45	2 ± 2	1 ± 1	10 ± 2
		20	131 ± 29	ND	2 ± 0.3	3 ± 1	1 ± 0.4	85 ± 40	7 ± 1.6	2 ± 0.5	19 ± 2
		30	55 ± 12	ND	1 ± 0.9	2 ± 1	1 ± 0.2	22 ± 7	11 ± 6	3 ± 1	18 ± 2
		40	16 ± **	ND	1 ± **	1 ± **	0.3 ± **	3 ± **	2 ± **	1 ± **	7 ± **
Apopka	West	5	1264 ± 53	10 ± 3	598 ± 17	2 ± 0.4	15 ± 8	325 ± 13	214 ± 6	125 ± 12	240 ± 30
		10	1303 ± 73	7 ± 1	596 ± 85	1 ± 0.3	19 ± 7	347 ± 40	200 ± 36	118 ± 29	257 ± 10
		15	1350 ± 37	5 ± 1	616 ± 13	19 ± 2	25 ± 7	382 ± 48	219 ± 20	133 ± 32	262 ± 32
		20	1275 ± 122	5 ± 1	523 ± 115	20 ± 5	24 ± 13	361 ± 35	148 ± 26	95 ± 34	270 ± 8
		30	1234 ± 153	6 ± 2	399 ± 163	28 ± 8	15 ± 5	418 ± 6	120 ± 30	93 ± 38	284 ± 47
		45	990 ± 296	10 ± 2	267 ± 224	37 ± 3	11 ± 7	340 ± 31	93 ± 46	65 ± 30	198 ± 43
		60	795 ± 156	12 ± 4	106 ± 76	34 ± 15	16 ± 8	356 ± 71	63 ± 20	48 ± 18	188 ± 31
		80	615 ± 7.3	16 ± 4	31 ± 11	42 ± 8	7 ± 2	351 ± 44	37 ± 3.3	29 ± 7	154 ± 8
		98	694 ± **	7 ± **	52 ± **	61 ± **	1 ± **	386 ± **	34 ± **	27 ± **	139 ± **

Total P: total phosphorus, MBP: microbial biomass phosphorus, Inorg.: inorganic, Org.: organic, HCl-Pi: inorganic phosphorus, FAP: moderate labile organic phosphorus, HAP: highly resistant organic phosphorus, Res-P: residual phosphorus. ND = not determined

Table 3-4. Phosphorus composition of the sediment depth profile determined by ^{31}P NMR spectroscopy. (Percentage in relation to total extracted phosphorus with NaOH/EDTA in parenthesis)

Lake	Site	Depth (cm)	TP (NaOH/EDTA)	Phosphate	Monoester	Lipid	DNA	Pyro-P	Poly-P
					mg P kg^{-1} dw (%)				
Annie	Central	5	1559	788 (51)	552 (35)	TR	219 (10)	ND	ND
		10	1525	828 (54)	551 (36)	TR	146 (9)	TR	ND
		15	1468	852 (58)	483 (33)	TR	132 (9)	ND	ND
		20	1498	866 (58)	505 (34)	TR	127 (6)	ND	ND
		30	1584	1127 (71)	367 (23)	TR	94 (6)	TR	ND
		45	1296	777 (60)	438 (34)	ND	81 (8)	ND	ND
		60	1041	636 (61)	321 (31)	ND	82 (10)	ND	ND
		80	854	518 (61)	248 (29)	ND	88 (8)	ND	ND
Okeechobee	M9	5	896	604 (68)	210 (24)	ND	82 (9)	TR	ND
		10	547	363 (67)	147 (27)	ND	36 (7)	TR	ND
		15	405	405 (100)	ND	ND	ND	ND	ND
		30	174	174 (100)	ND	ND	ND	ND	ND
		60	228	228 (100)	ND	ND	ND	ND	ND
Apopka	West	5	1139	313 (28)	320 (28)	37.2 (3)	355 (31)	113 (10)	ND
		10	824	263 (32)	181 (22)	32.6 (4)	237 (29)	17 (2)	93 (11)
		15	670	231 (35)	136 (20)	20.1 (3)	204 (31)	18 (3)	61 (9)
		20	858	290 (33)	191 (22)	30.4 (4)	261 (30)	21 (3)	64 (8)
		30	349	165 (47)	87 (25)	TR	97 (28)	ND	ND
		45	224	141 (63)	31 (14)	TR	52 (23)	ND	ND
		60	150	95 (64)	18 (12)	TR	37 (25)	ND	ND
		80	135	107 (80)	ND	ND	28 (21)	ND	ND
		98	102	86 (85)	ND	ND	16(15)	ND	ND

ND: not detected, TR: trace (i.e., not quantifiable). TP NaOH/EDTA: total phosphorus in NaOH/EDTA extracts, Phosphate: orthophosphate, Monoester: phosphate monoester, Lipid: phospholipids, Pyro-P: pyrophosphate, and Poly-P: polyphosphate.

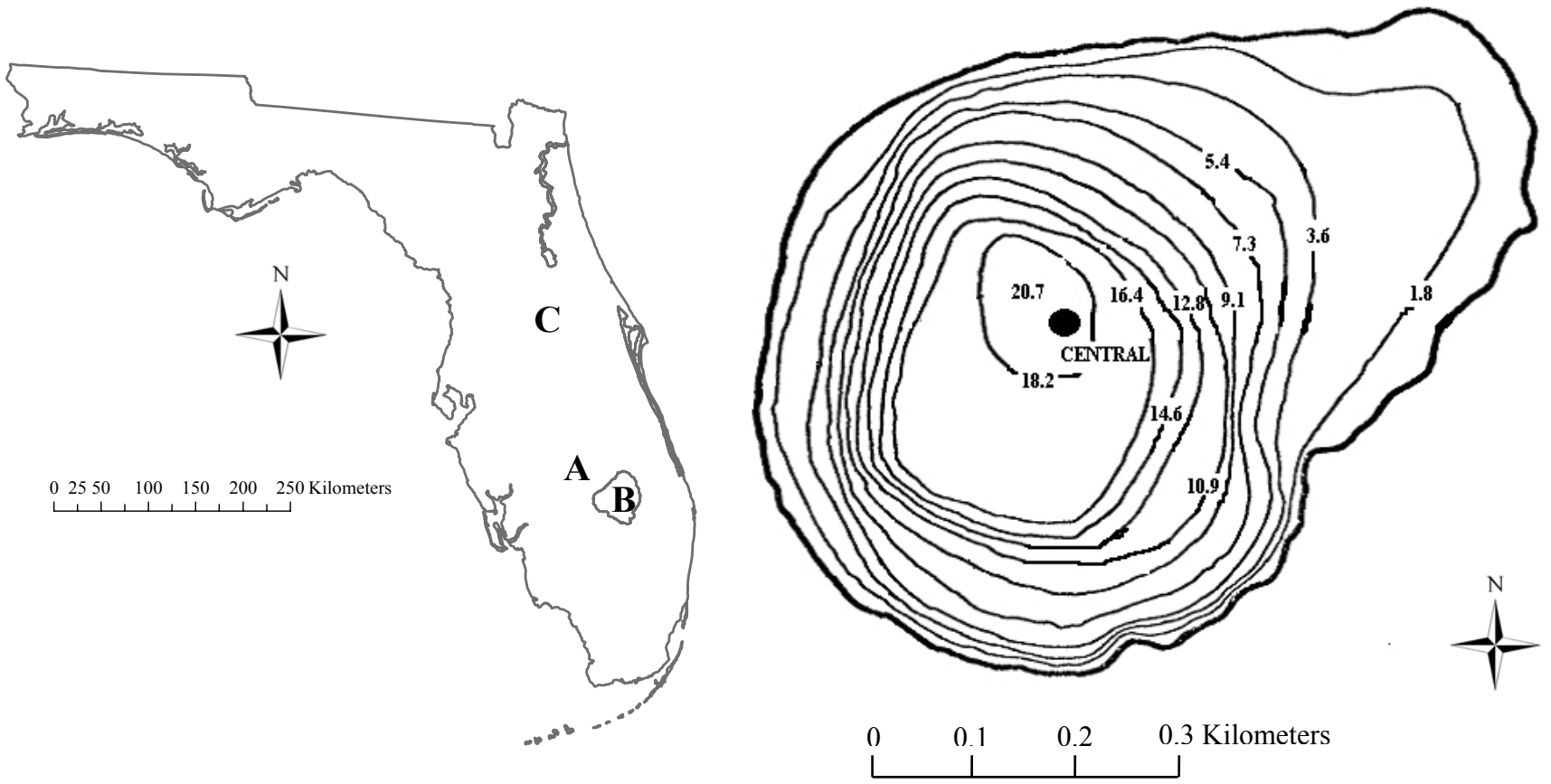


Figure 3-1. Map of the three subtropical lakes with sampled sites and their location in Florida State: A) Lake Annie (with water column depth in meters, modified from Layne 1979), B) Lake Okeechobee with different sediment types, and C) Lake Apopka.

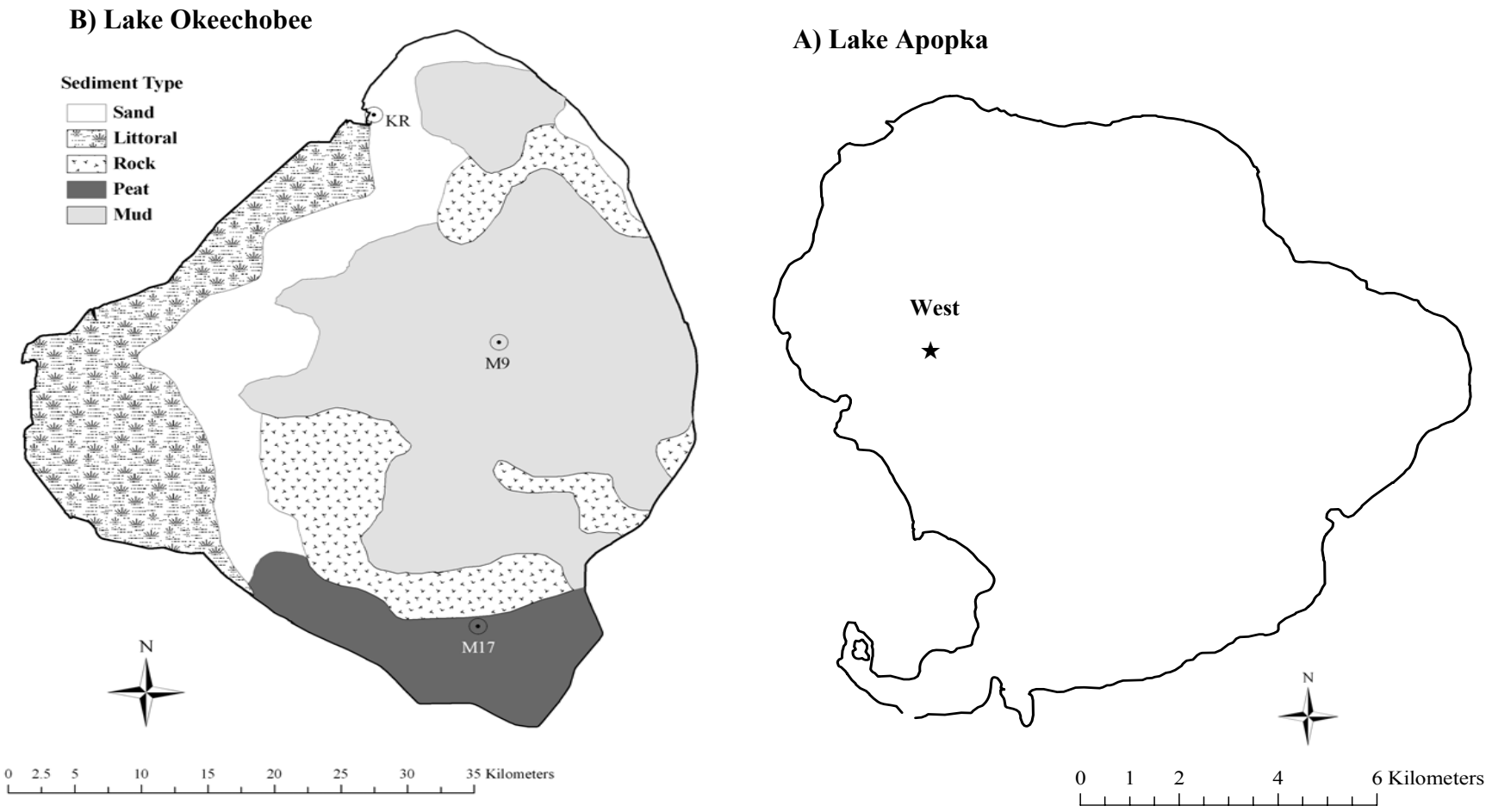


Figure 3-1. continued

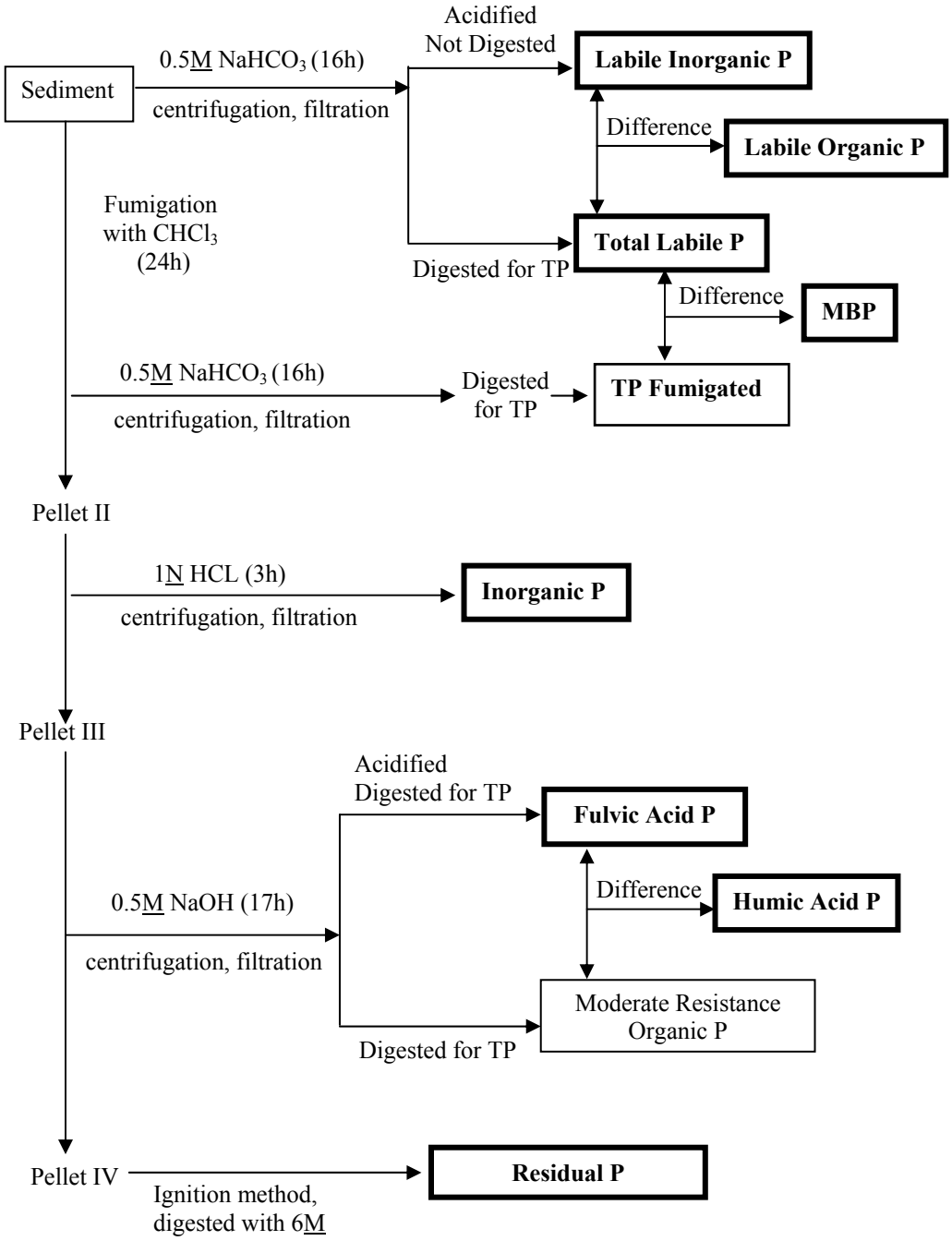


Figure 3-2. Fractionating scheme for the characterization of P organic forms (based on Ivanoff et al. 1998).

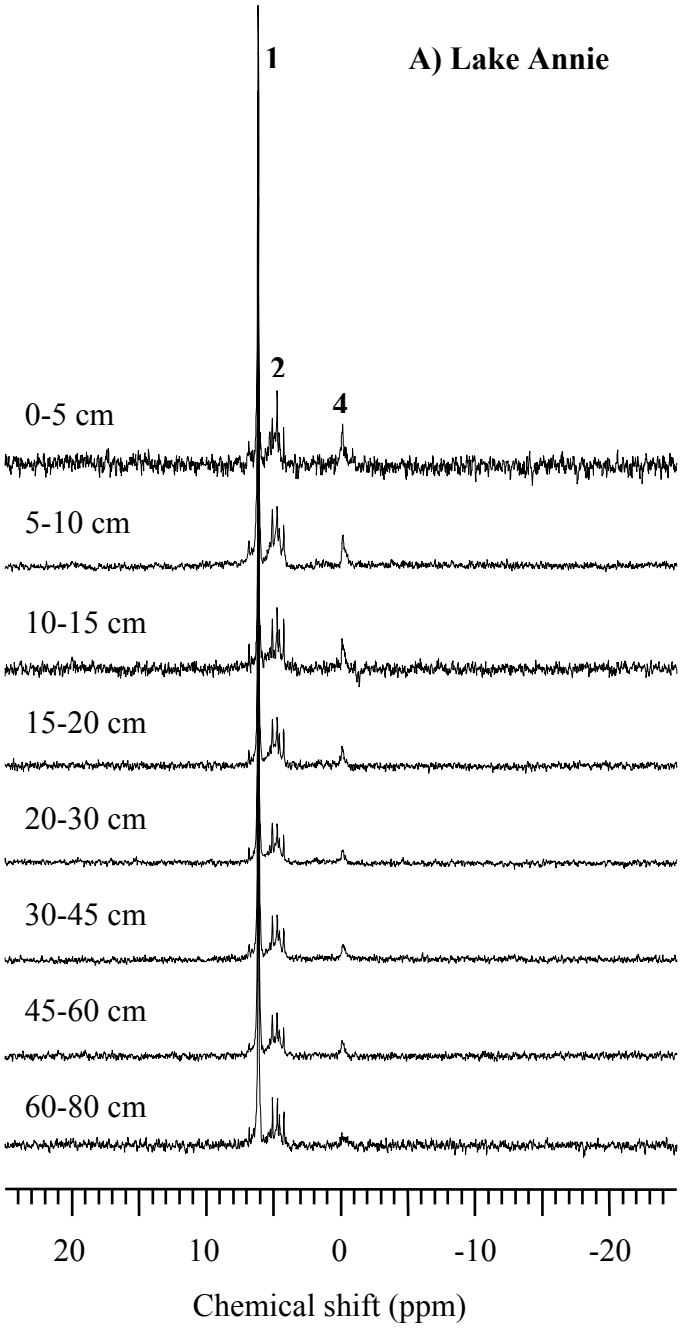


Figure 3-3. ^{31}PNMR spectra of the NAOH/EDTA extracts of sediment depth profile in Lake: A) Annie, B) Okeechobee – M9, and C) Apopka. 1 – Orthophosphate, 2 – Phosphate monoester, 3 – Lipids, 4 – DNA, 5 – Pyrophosphate and 6 – Polyphosphate

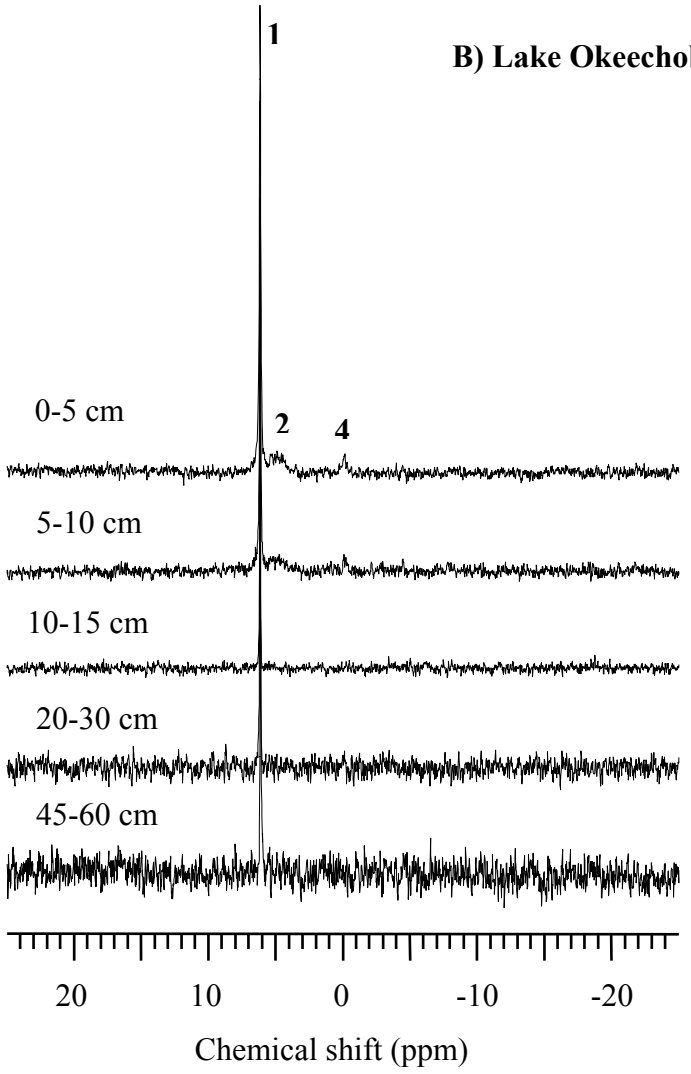

B) Lake Okeechobee – M9
1
2
4
0-5 cm
5-10 cm
10-15 cm
20-30 cm
45-60 cm
20
10
0
-10
-20
Chemical shift (ppm)

Figure 3-3B

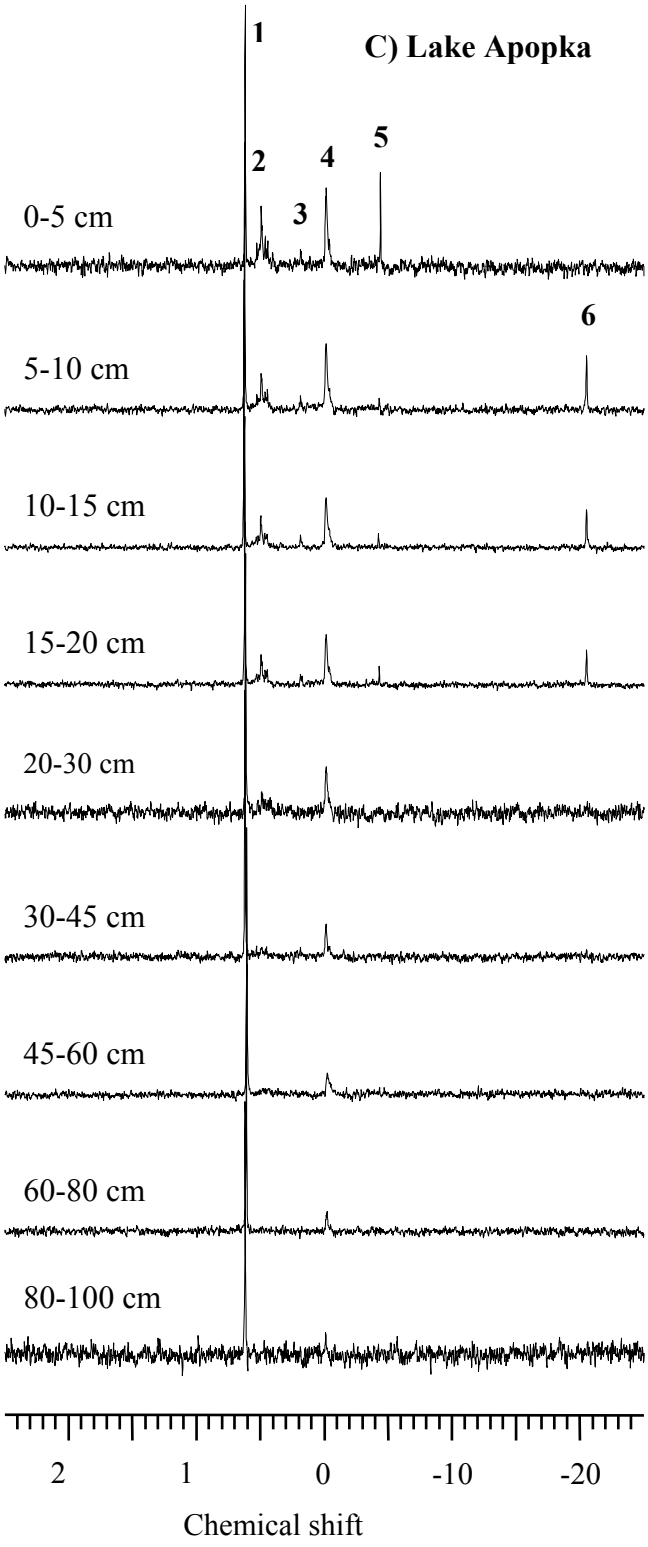

C) Lake Apopka
1
2
3
4
5
6
0-5 cm
5-10 cm
10-15 cm
15-20 cm
20-30 cm
30-45 cm
45-60 cm
60-80 cm
80-100 cm
2
1
0
-10
-20
Chemical shift

Figure 3-3C

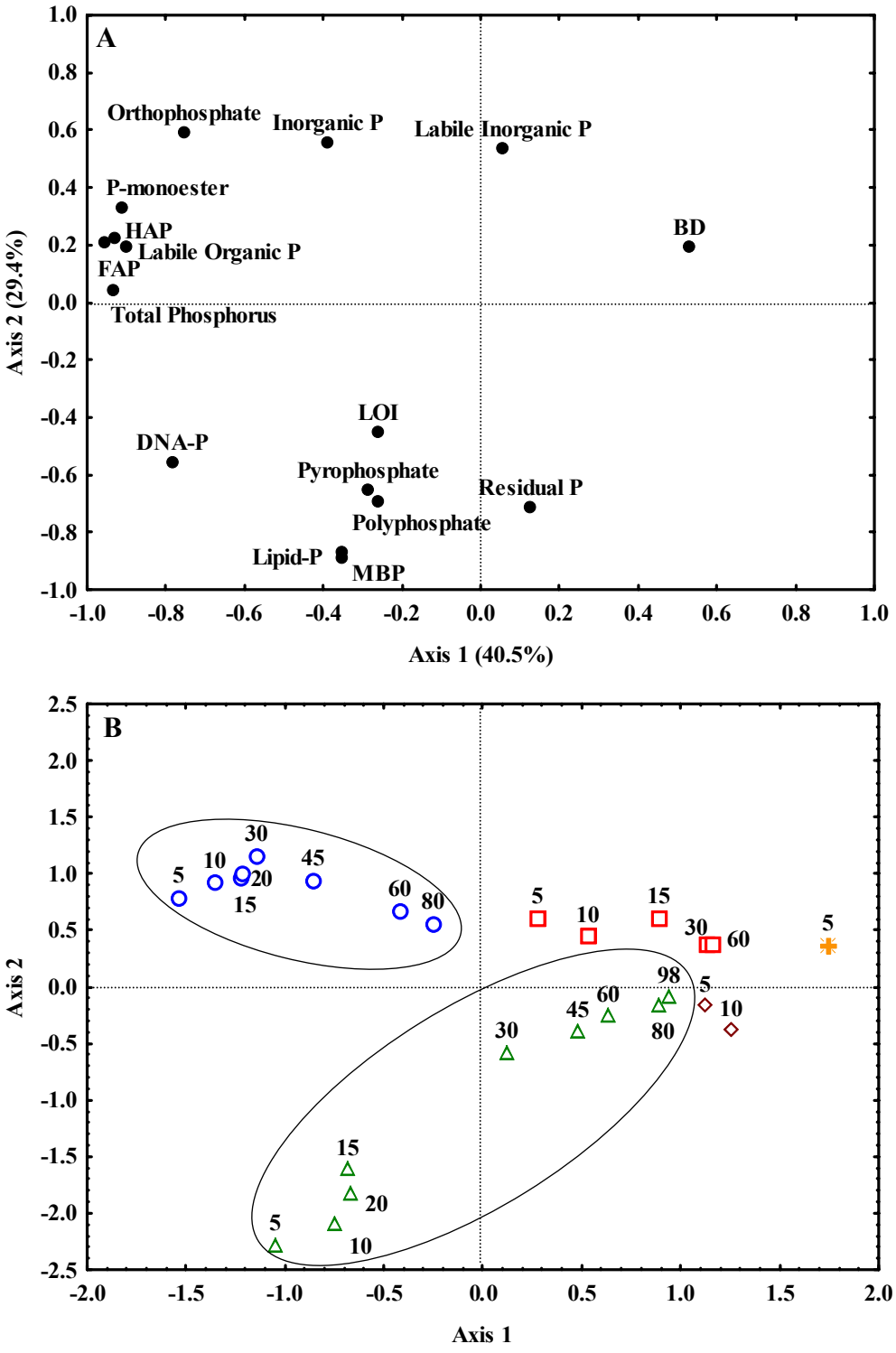


Figure 3-4. Results of the Principal Component Analysis, A) loadings of the different phosphorus compounds measured by ^{31}P NMR and P fractionation (n = 25), and B) the plot of the scores of the sites and sediment depth (numbers cm) from Lake Annie (blue circles), Lake Okeechobee: M9 (red squares), M17 (brown diamonds), KR (orange crosses), and Lake Apopka (green triangles).

CHAPTER 4
ENZYME ACTIVITIES IN SEDIMENTS OF SUBTROPICAL LAKES

Introduction

Sediment phosphorus (P) is present in both inorganic and organic forms. Organic P and cellular constituents of the biota represent 90% of total phosphorus (TP) in freshwater ecosystems (Wetzel 1999). These organic P compounds present in sediments must be hydrolyzed before their uptake by microorganisms (Chrost 1991; Sinsabaugh et al. 1991). Organic P is hydrolyzed by enzymes produced by microbial communities (Gächter et al. 1988; Davelaar 1993; Gächter and Meyer 1993), and the product of this enzymatic hydrolysis is orthophosphate which is readily used by microorganisms (Barik et al. 2001). Consequently the breakdown of organic P compounds through enzyme activity and release of labile inorganic P is an important component of P processing in sediments. Enzyme production can be induced by the presence of organic P and low levels of bioavailable inorganic P (Kuenzler 1965; Aaronson and Patni 1976). On the other hand, high levels of inorganic P inhibit the synthesis of enzymes (Torriani 1960; Lien and Knutsen 1973; Elser and Kimmel 1986; Jasson et. al. 1988; Barik et al. 2001).

Three main groups of hydrolytic enzymes are responsible for phosphate release: non specific and/or partially specific phosphoesterases (mono and diesterase), nucleotidases (mainly 5'-nucleotidase), and nucleases (exo and endonucleases) (Chrost and Siuda 2002). Phosphomonoesterases (PMEase) are nonspecific enzymes that hydrolyze phosphate monoester, and are reported to be produced by several microorganisms (e.g., bacteria, algae, fungi, and protozoan) that are found in the water column and sediment of lakes. Nonspecific PMEases are divided into two groups, depending on the pH at which they exhibit maximum activity, alkaline (pH 7.6-10) and acid (pH 2.6-6.8) (Siuda 1984). Both can be found inside or outside the cell, and the same cell can produce both alkaline and acid PMEase (Siuda 1984). Although both PMEase

activities have been reported to be regulated by availability of orthophosphate, acid PMEase is usually regarded as a constitutive enzyme (Siuda 1984; Jasson et al. 1988).

The production of constitutive enzymes is neither repressed nor stimulated by high or low orthophosphate availability in the environment. Its production is related to P concentration and demand inside the cell (Siuda 1984, Jasson et al. 1988). Jasson et al. (1981), however, suggested that in acidified lakes, acid PMEase may have a similar role to that of alkaline PMEase in neutral systems, as its production is also inhibited by orthophosphate. In aquatic systems, alkaline PMEase is by far the most studied enzyme, probably due to the high number of systems with neutral pH, that are inappropriate for preservation of extracellular acid PMEase (Siuda 1984). Another important phosphatase is phosphodiesterase (PDEase) that hydrolyzes phosphate diester and is known to degrade phospholipids and nucleic acids (Hino 1989; Tabatabai 1994; Pant and Warman 2000). It is the least studied enzyme in freshwater ecosystems. Few studies have reported on the occurrence and distribution of phosphatases or other organic P hydrolyzing enzymes in sediments or their association with sediment bacteria (Wetzel 1991; Chrost and Siuda 2002). As sediment P is important in P cycling in lakes, and it is well known that microorganisms can influence the organic P dynamics in sediments, the study of different P compounds and associated enzymes is important for understanding P cycling in sediments.

The primary hypothesis of this study is that enzyme activities will be higher in recently accreted sediments (surface) as compared to older sediments (sub-surface) and will be related to P forms and availability, as well as to microbial community activity. The specific objectives of this study were: (i) measure vertical distribution of PMEase and PDEase activities and relate them to microbial activity in sediments; and (ii) to explore relationships between different phosphorus compounds and enzyme activity.

Materials and Methods

Study Sites

Three Florida (USA) lakes ranging in trophic state were selected. Lake characteristics were described in Chapter 2. The characteristics and location of sampled sites and field sampling procedures were described in Chapter 3 (Table 3-1, Figure 3-1).

Water Characteristics

Water temperature (ºC), electrical conductivity (EC), pH, and dissolved oxygen (DO) were measured with a YSI 556 Multi-Probe Sensor (YSI Environmental, Yellow Springs OH) at different depths (Table 4-2). Greater depths in the Lake Annie water column, ºC, EC, and DO were measured using a handheld YSI 85 (YSI Inc., Yellow Springs, OH). Water samples were collected from various depths at each site using a Van Dorn bottle.

Water column nutrient concentrations were measured using U.S. EPA methods (EPA 1993). Total Kjeldahl nitrogen (TN) was measured by digestion with concentrated sulfuric acid (H_2SO_4) and Kjeldahl salt catalyst, and determined colorimetrically (Method - 351.2). Total phosphorus (TP) was digested with 11N H_2SO_4 and potassium persulfate (Method - 365.1). Water samples were filtered through a 0.45 µm membrane filter and filtrate was analyzed for dissolved reactive phosphorus (DRP) (Method - 365.1), ammonium-N (NH_4-N) (Method - 351.2), and dissolved organic carbon (DOC) (automated Shimadzu TOC 5050 analyzer (Method - 415.1).

Sediment Properties

Samples were transported on ice and stored in the dark at 4 ºC. Before each analysis, samples were homogenized and sub-samples taken. Sediment bulk density, pH, organic matter (LOI-loss on ignition), and total phosphorus were measured and described in a previous study (Chapter 3).

Water extracts were centrifuged at 10,000 x g for 10 min, filtered through a 0.45 µm membrane filter, and analyzed for dissolved reactive P (DRP) and DOC with the same method used for water samples.

Enzyme Activity

Enzyme activities including PMEase and PDEase were determined colorimetrically using as substrate *p*-nitrophenyl phosphate and bis-*p*-nitrophenyl phosphate, respectively (Tabatabai 1994; Alef et al. 1995), both from Sigma Chemical Co (St Louis, MO). Assays were conducted using three replicates and a control for each sample to account for non-enzymatic color development. As PMEase activity depends on pH range (Tabatabai 1994; Alef et al. 1995), alkaline phosphatase activity was measured in Lake Okeechobee and Lake Apopka; while the acid phosphatase activity was measured in Lake Annie sediments (see Table 3-2). A known amount of wet sample, 0.5 g for high organic sediment, and 1 g for mineral sediment, was added to polypropylene centrifuge bottles with the artificial substrate (1 ml of 0.05 M *p*-nitrophenyl phosphate for PMEase, and bis-*p*-nitrophenyl phosphate for PDEase), toluene (to inhibit microbial growth during measurement), a pH buffer (pH = 11 for alkaline, pH = 6.5 for acid phosphatase, and pH = 8 for PDEase) and incubated at 37 ºC for 1 hour. Enzymatic activity was stopped after incubation by addition of 1 mL of 0.5 M $CaCl_2$ and 4 mL 0.5 M NaOH (for PMEase) and 0.1 M/0.5 M THAM/NaOH (THAM: tris(hydroxymethyl)aminomethane) extractant solution (for PDEase). Samples were centrifuged and filtered through a Whatman # 1 paper filter and analyzed at 420 ηm using a UV-VIS spectrophotometer (Shimadzu Model UV – 160) (Tabatabai 1994; Alef et al. 1995). Absorbance was compared with standards. Control values were subtracted from sample values to account for non-enzymatic substrate hydrolysis.

Statistical Analysis

Sediment P compounds and anaerobic microbial respiration (microbial activity) methods and data was reported in other studies (Chapter 3 and 5 respectively). The combined data was used to explore relationships between these different variables and enzyme activity. A Pearson correlation analysis was performed to determine relations among P forms and enzyme activity. Regression analyses were conducted to compare sediment P forms and activities of enzymes and microbes. A Principal Component Analysis (PCA) was performed to address relations among variables, and how they relate to each lake and sediment depth. All statistical analyses were conducted with Statistica 7.1 (StatSoft 2006) software.

Results

Water Characteristics

Lake Annie displayed strong summer thermal stratification (Table 4-1). Electrical conductivity reflected trophic state conditions of the lakes, with higher values in Lake Apopka, followed by Lake Okeechobee and Lake Annie. Dissolved oxygen was highest in Lake Apopka, followed by Lake Annie and Lake Okeechobee sites M9 and M17. Lake Okeechobee site KR had the lowest values. Lake Annie's water column was anoxic below 5 m depth (Table 4-1). Lake Annie water column pH was lower than pH in the other lakes, and decreased with depth. Both Lake Okeechobee (except site KR) and Lake Apopka had pH values near neutral or alkaline (Table 4-1).

Highest DOC values were found at the sediment surface in Lake Apopka (53.9 mg L^{-1}), while all other DOC values in the lakes ranged from 12.3 to 25.1 mg L^{-1}. Surface water TP and DRP were highest in Lake Okeechobee, while TN was highest in Lake Apopka. Although Lake Annie displayed generally low TP, TN and NH_4-N concentrations in the water column, high values were registered at 20 m depth, just above the sediment surface (Table 4-2).

Sediment Properties

Sediment properties (i.e., pH, bulk density, and organic matter) and concentrations of TP and different P compounds were reported in a previous study (Chapter 3). Acidic pH conditions were observed in Lake Annie sediments and neutral to alkaline values in Lake Okeechobee and Lake Apopka deposits (Chapter 3). Water extractable organic C (expressed as DOC) displayed different distributions with sediment depth among lakes. In Lake Annie, sediment DOC increased with depth. A similar trend was also observed in Lake Okeechobee M17 sediments. However, DOC distribution in Lake Apopka sediments decreased with depth and no clear trend was noted at sites M9 and KR (Table 4-3). Concentration of water extractable DRP did not present a clear pattern with depth in the profile at any of the sites, although deeper sections in Lake Annie and Lake Apopka had higher concentrations than upper sections (Table 4-3).

Enzyme Activity

Lake Okeechobee sediments had very low enzyme activities for both PMEase (0.3-4.5 mg *p*-nitrophenol g^{-1} dw h^{-1}) and PDEase (0.4-5.7 mg bis-*p*-nitrophenol g^{-1} dw h^{-1}) (Figure 4-1A, B). Phosphomonoesterase (PMEase) and phosphodiesterase (PDEase) activities decreased with sediment depth at all sites (Figure 4-1A, B). Lake Annie sediments had the highest PMEase activity compared with sediments of the other lakes, while PDEase was higher in Lake Apopka sediments. Lake Okeechobee sediments had higher PDEase activity than PMEase, while Lake Annie and Lake Apopka had higher PMEase activity. Phosphomonoesterase activity was strongly correlated ($r > 0.7$) with phosphate monoester, labile organic P, FAP, and HAP, and increased linearly with phosphate monoester concentration (Figure 4-2). Phosphodiesterase activity showed strong correlation ($r > 0.7$) with MBP, lipid-P, DNA-P, and pyrophosphate, and increased linearly with phosphate diester (i.e., Lipid-P, DNA-P) concentration (Figure 4-3).

Pore water DOC and DRP concentrations in Lake Annie and Apopka were measured and described in another study (Chapter 5, Table 5-2), and the data were used to verify if there was a relation between enzyme production and DOC and DRP pore water concentration. In Lake Annie, there was no relationship between either acid PMEase or PDEase activities and pore water DOC and DRP. For Lake Apopka, however, there was an inverse relationship between DRP pore water concentration and both PMEase and PDEase (Figure 4-4A). Enzyme activities in Lake Apopka showed a strong linear relationship with pore water DOC (Figure 4-4B).

Anaerobic respiration (CO_2 production rates) data was described elsewhere (Chapter 5), and the values were used to address relations between anaerobic respiration and enzyme activity. Microbial activity had a positive relationship with both PMEase and PDEase activities. In Lake Annie, Lake Apopka, and Lake Okeechobee site-M9 sediments, microbial activity had a significant linear relationship with both enzyme activities (Figure 4-5A, B, E). The same relationship was observed between microbial activity and PDEase activity in site KR sediments, although no relationship was observed for PMEase activity (Figure 4-5D). In peat sediments (site-M17) no relationship between enzyme and microbial activities was observed (Figure 4-5C).

Two Principal Component Analyses (PCA) were conducted. One analysis used data from ^{31}P NMR, chemical fractionation (Chapter 3), and microbial (Chapter 5) and enzyme activities (n = 25) (Figure 4-6). The other used data from chemical P fractionation (Chapter 3), and microbial (Chapter 5) and enzyme activities (n = 107) (Figure 4-7). The first PCA had 38.6% of the data variability explained by Axis 1. Axis 2 explained 30.3% of the data variability (Figure 4-6A). Lipid-P, DNA-P, anaerobic respiration, microbial biomass P (MBP), fulvic acid P (FAP), PMEase and PDEase activity were the variables selected by Axis 1, while orthophosphate, phosphate monoester and residual P (Res. P) were selected by Axis 2. Phosphomonoesterase

activity was placed with labile organic P (labile-Po), FAP, humic acid P (HAP), and phosphate monoester. Phosphodiesterase activity was placed with anaerobic respiration, MBP, DNA-P and lipid-P. The position of the sites and sediment depth in relation to the variable loadings in the PCA showed that the three lakes are separated into different groups (Figure 4-6B). Lake Apopka placed in the PDEase cluster and Lake Annie in the PMEase cluster. Lake Okeechobee was further from any of these clusters (Figure 4-6B).

In the second PCA, using chemical P fractionation, Axis 1 explained 41.1% of the data variability and the variables selected were labile-Po, FAP, HAP, anaerobic respiration, PMEase, and PDEase activity. Axis 2, with 21.1% of the data variability explained, selected MBP (Figure 4-7A). Again FAP, HAP and labile-Po were placed with PMEase activity, while MBP was placed with PDEase activity and anaerobic respiration. The position of the sites and sediment depth in relation to the variables loadings in PCA-2 showed results similar to the first PCA-1 (Figure 4-7B). Samples from the three lakes were separated into different groups, and Lake Apopka placed in the PDEase cluster and Lake Annie in the PMEase cluster.

Discussion

There are few studies reporting on P related enzyme activity in freshwater sediments, and they focus on PMEase activity. The PMEase values found in the present study in Lake Annie and Lake Apopka are much higher than those observed in other freshwater systems. Lake Okeechobee sediment PMEase values, although being much smaller than the other lakes of this study, had similar or higher values than the ones reported for other freshwater sediments. In shallow eutrophic Lake Donghu (China), PMEase activity in surface sediments was much lower than the values detected in Lake Annie and Lake Apopka, but higher than the values detected in Lake Okeechobee sediments (17.6-44.05 mg *p*-nitrophenol g^{-1} dw h^{-1}) (Yiyong et al. 2001). Wobus et al. (2003) in a study of sediments of reservoirs of different trophic states in Germany

reported higher values for PMEase in oligotrophic Muldenberg, (17.2 mg *p*-nitrophenol g^{-1} dw h^{-1}), than mesotrophic Saidenbach (0.8 mg *p*-nitrophenol g^{-1} dw h^{-1}) and eutrophic Quitzdorf (0.17 mg *p*-nitrophenol g^{-1} dw h^{-1}). In a study of shallow, nutrient rich, freshwater sediments Boon and Sorrell (1991) reported values of PMEase that ranged from 1.35-1.75 mg *p*-nitrophenol g^{-1} dw h^{-1}). Small values of PMEase were reported by Barik et al. (2001) for 12 different nutrient rich fishpond sediments (25- 59 μg *p*-nitrophenol g^{-1} dw h^{-1}). Wright and Reddy (2001) reported PMEase values in soils of a freshwater wetland (Florida Everglades) ranging from ≤ 5.0 mg *p*-nitrophenol g^{-1} dw h^{-1} in P-impacted sites to 25 mg *p*-nitrophenol g^{-1} dw h^{-1} in non-impacted sites.

Enzyme activity decreased with greater sediment depth in all lakes, a result also reported in other studies. Wobus et al. (2003) found that PMEase activity declined with sediment depth in a mesotrophic reservoir in Germany. The same result was reported by Sinke et al. (1991) for Loosdretch Lake in the Netherlands. The decrease of enzyme activity with depth reflects lower microbial biomass (MBP) (Chapter 3), and is accompanied by decreased anaerobic respiration (Chapter 5). A positive correlation between PMEase activity and microbial density was found in several ecosystems (Reichart 1978; Kobori and Taga 1979; Davis and Goulder 1993; Massik and Cotello 1995; Barik et al. 2001). Enzymes can be produced by several organisms. Enzymes produced by algae predominate in the water column, while in sediments bacterial enzymes are dominant (Siuda 1984). Strong correlations between enzyme activities and anaerobic respiration indicate (PMEase $r = 0.65$, PDEase $r = 0.91$) that bacterial enzymes dominate these sediments (Figure 4-5). Only at sites M17 and KR was PMEase activity not related to anaerobic respiration (Figure 4-5C, D), indicating that in these sites other organisms (i.e., algae) are producing

hydrolytic enzymes. Moreover, the demand for available P can be low in these sediments, resulting in low enzyme activity.

Both enzyme activities, however, are related not only to microbial biomass and activity, but rather, they reflect different phosphorus composition and availability in these lakes. Wobus et al. (2003) reported higher activities of PMEase in an oligotrophic reservoir than in meso and eutrophic reservoirs. In my study, highest PMEase activity was found in the oligo-mesotrophic lake (Lake Annie). Phosphomonoesterase activity was lowest in eutrophic Lake Okeechobee, but displayed intermediate activity in hypereutrophic Lake Apopka. Lake Okeechobee had high concentrations of labile inorganic P (Chapter 3) and lowest activities for both PMEase and PDEase. Lake Annie had high concentrations of labile-Po, FAP and HAP fractions and phosphate monoester, and had high PMEase activity. Lake Apopka had high concentrations of MBP and phosphate diester (lipids and DNA), as well as PDEase activity. Statistical analyses support these results. There were higher correlation coefficients between PMEase and phosphate monoester ($r = 0.86$), labile-Po ($r = 0.83$), FAP ($r = 0.86$) and HAP($r = 0.89$), while PDEase had high correlations with phosphate diester (lipid-P $r = 0.89$, DNA-P $r = 0.93$) and MBP ($r = 0.88$). Linear regression analyses showed strong significant relations between PMEase and phosphate monoester, and between PDEase and phosphate diester concentrations (Figures 4-2, 4-3). These results were corroborated by the two PCAs positioning these P forms and their respective related enzymes as clusters (Figure 4-7, 4-8). Also in relation to the PCAs, if enzyme production were only a reflection of microbial biomass and activity, both enzymes, CO_2 production and MBP would all cluster together, but there is clear separation of these variables. These results show that although microbial activity (CO_2), microbial biomass (MBP) and enzyme activities are related, as expected, different P forms in sediments strongly influence enzyme production.

The lack of relationship between pore water SRP and enzyme activities in Lake Annie can be attributed to different mechanisms of enzyme production and sediment properties. Acid PMEase is usually regarded as a constitutive enzyme, and its production is not repressed by high orthophosphate availability (Siuda 1984; Jasson et al. 1988). In acidified lakes, however, acid PMEase seem to have a similar role to alkaline PMEase in neutral systems (Jasson et al. 1981). I measured acid instead of alkaline PMEase in Lake Annie sediments to evaluate the maximum potential enzyme activity. Measurement of alkaline PMEase activity would probably be underestimated in Lake Annie, as it would be influenced by pH rather than P availability (Chapter 3). In a study of acid PMEase in the water column of acid Lake Gårdsjön, Sweden, with high aluminum (Al) and iron concentration, Jasson (1981) showed that high acid PEMase activity was induced as a response of the plankton community to high Al concentration that blocks substrates by reacting with phosphate. Lake Annie sediments (central site) were characterized as having high Fe (3640 mg kg^{-1}) and Al (34640 mg kg^{-1}) concentration (Thompson 1981).

In Lake Annie, high PMEase activity, unrelated to P availability, can be a result of several factors: 1) high Al and Fe concentration in its sediment, 2) high P demand inside microorganism cells, 3) or presence of more stable phosphate monoester (i.e., inositol phosphate). Some phosphate monoesters (e.g., inositol phosphate) are more resistant to degradation than phosphate diester (Makarov et al. 2002), probably due to higher charge density, which enables the phosphate monoester to form strong complexes with cations, protecting them from degradation (Celi et al. 1999). Inositol phosphate, which is considered to be stable in soils, was present in Lake Annie spectra (Chapter 3, Figure 3-3A). Moreover, according to Turner and Haygarth (2005), both PDEase and PMEase are necessary for release of free phosphate from phosphate

diester. Initial hydrolysis by PDEase releases phosphate monoester and stimulates the production of PMEase.

Lake Apopka's PMEase production seems to be controlled by other mechanisms, perhaps related to both DOC and DRP availability. In a study of alkaline phosphatase activity in Lake Apopka sediments (topmost 30 cm, $n = 6$) Newman and Reddy (1993) reported different results. Phosphomonoesterase activity had an inverse correlation with labile-Po and HAP, and no correlation with DRP. My study found that there is an inverse relation between pore water DRP and PMEase activity, and a positive correlation with organic P forms (including HAP). I used the same method used by Newman and Reddy (1993) but my sample size was larger. Several studies have shown that there is an inverse correlation between PMEase activity and DRP in sediments (Jasson et. al. 1988; Barik et al. 2001; Wobus et al. 2003; Jin et al 2006; Rejmankova and Sirova 2007). However, Siuda and Chrost (2001) concluded from controlled experiments that even during periods of high concentration of orthophosphate in lake water, PMEase is still produced, and exhibits activity. They suggested that PMEase activity of bacteria is used for organic P hydrolysis and uptake of associated organic C moieties, concluding that bacterial PMEase contributes substantially to DOC decomposition in lake water. This seems to be the case of PMEase production in Lake Apopka, as I found a high correlation between enzyme activity and DOC concentration.

Conclusions

This study showed that PMEase and PDEase activities were related to sediment microbial biomass and activity, as well as to the different P composition and availability. Enzyme activity decreased with greater depth in all lakes, reflecting lower microbial biomass and activity. Strong correlations between enzyme activities and anaerobic respiration indicated that bacterial enzymes dominate these sediments. Different P forms in sediments were also affecting enzyme activity.

Highest PMEase activity was found in the oligo-mesotrophic lake (Lake Annie) with high concentrations of labile-Po, FAP and HAP. Lake Okeechobee had high concentrations of labile-Pi and lowest activities of both PMEase and PDEase. Lake Apopka had high concentrations of MBP and phosphate diester (lipids and DNA), as well as PDEase activity.

The mechanisms controlling PMEase activity, however, seemed to vary according to the difference in lake sediment. In Lake Annie, high PMEase activity was unrelated to DRP availability, and probably was controlled by factors such as high Al and Fe concentrations, high P demand inside microorganism cells, and/or presence of more stable phosphate monoester (i.e., inositol phosphate) in the sediment. Lake Apopka's PMEase production seemed to be controlled by both DOC and DRP availability. There was an inverse relation between pore water DRP and PMEase activity, and a positive relation between pore water DOC and PMEase activity. In Lake Apopka sediments production of PMEase by the microbial community was related to organic P hydrolysis, and uptake of associated organic C moieties.

Table 4-1. Measured parameters in the water column of Lake Annie, Lake Okeechobee, and Lake Apopka. ºC: water temperature in Celsius, EC: electrical conductivity, DO: dissolved oxygen.

Lake	Site	Depth (m)	ºC	EC ($\mu S\ cm^{-1}$)	pH	DO ($mg\ L^{1}$)
Annie	Central	0.5	30.4	42.0	6.2	6.3
		1	30.2	41.9	5.1	6.4
		2	29.2	40.3	4.8	4.9
		3	28.8	40.7	4.7	4.3
		4	27.2	40.3	4.6	2.4
		5	25.8	40.3	4.6	0.3
		6	22.3	38.6	4.4	0.2
		7	20.9	37.3	4.4	0.2
		8	19.4	36.3	4.2	0.2
		9	18.7	35.6	3.9	0.1
		10	18.2	35.3	3.7	0.1
		11	17.9	35.1	NM	0.1
		12	17.6	36.5	NM	0.1
		13	17.4	37.2	NM	0.1
		14	17.3	37.8	NM	0.1
Okeechobee	M9	0.5	29.8	385	7.9	6.4
		1	29.5	385	7.8	6.5
		1.5	29.1	385	7.8	6.6
		2	29.1	385	7.8	6.5
		3	29.1	385	7.7	6.4
		4	29.0	385	7.6	6.3
	M17	0.5	28.8	320	7.5	6.1
		1	28.8	320	7.6	6.3
		1.5	28.7	320	7.6	6.3
		2.5	28.7	320	7.6	5.1
	KR	0.5	31.0	144	6.0	1.8
		1	30.8	143	6.0	1.8
		1.5	30.7	143	5.9	1.9
		2	30.7	143	5.9	1.9
		3	30.7	142	5.9	1.7
Apopka	West	0.5	27.8	455	7.6	9.2
		1	26.6	443	7.6	8.7
		1.5	26.4	471	6.7	7.4
		2	26.3	652	6.4	3.0

NM: not measured

Table 4-2. Concentration of TP: total phosphorus, DRP: soluble reactive phosphorus, TN: total nitrogen, NH_4-N: ammonium-N and DOC: dissolved organic carbon in the water column of Lake Annie, Lake Okeechobee, and Lake Apopka.

Lake	Site	Depth (m)	TP	DRP	TN	NH_4-N	DOC
			($\mu g\ L^1$)				($mg\ L^1$)
Annie	Central	0.5	22.8	9.5	1484	51.8	14.3
		1	22.0	7.8	1374	102.5	15.2
		2	16.1	5.5	1264	66.7	15.3
		5	10.6	6.5	1154	48.2	12.3
		10	7.8	5.2	1099	111.3	12.4
		15	8.8	5.5	1319	183.4	13.1
		20	144.2	11.8	4955	707.6	14.1
Okeechobee	M9	0.5	211.3	90.5	3192	92.3	16.1
		1	258.3	93.4	3192	130.9	13.5
		4	298.0	87.4	3934	85.8	13.8
	M17	0.5	224.6	121.5	2938	53.5	20.2
		1	247.4	107.3	2883	69.4	16.0
		2	317.7	110.3	4266	58.3	17.6
	KR	0.5	113.9	64.3	2717	80.7	20.2
		1	118.4	59.9	2717	79.5	18.8
		3	206.9	63.5	3436	90.8	20.4
Apopka	West	0.5	60.0	15.3	5505	233.9	14.5
		1	72.6	10.2	6056	74.9	25.1
		2	76.3	7.8	21884	50.0	53.9

Table 4-3. Water extractable dissolved organic carbon (DOC), and dissolved reactive phosphorus (DRP). (mean ± SD). **No replicates for SD calculation.

Lake	Site	Depth (cm)	Water Extractable DOC	Water Extractable DRP	DOC:DRP (Weight)
			mg kg^{1} dw		
Annie	Central	5	499 ± 136	1.40 ± 0.5	378
		10	430 ± 284	0.66 ± 0.3	618
		15	1022 ± 413	1.5 ± 0.5	665
		20	1264 ± 441	2.9 ± 0.53	417
		30	3298 ± 1676	7.7 ± 2.8	411
		45	4268 ± 1105	10.0 ± 3.7	438
		60	4693 ± 617	7.6 ± 0.3	617
		80	4332 ± 930	9.1 ± 3.0	488
Okeechobee	M9	5	424 ± 276	2.4 ± 1.72	187
		10	262 ± 21	0.78 ± 0.1	351
		15	278 ± 86	0.78 ± 0.4	393
		20	204 ± 29	0.82 ± 0.4	306
		30	201 ± 52	0.52 ± 0.1	407
		45	279 ± 77	0.56 ± 0.4	615
		60	275 ± 138	1.4 ± 0.4	221
		70	447 ± 124	4.9 ± 0.2	91
	M17	5	459 ± 59	0.88 ± 0.3	605
		10	854 ± 86	0.25 ± 0.09	3843
		15	1219 ± 115	0.24 ± 0.04	5156
		20	1559 ± 249	0.31 ± 0.08	5275
		30	1648 ± 129	0.28 ± 0.02	5950
		40	2010 ± 895	0.34 ± 0.10	5878
	KR	5	34 ± 34	0.12 ± 0.03	288
		10	29 ± 18	0.07 ± 0.03	570
		15	53 ± 33	0.14 ± 0.08	586
		20	212 ± 4.3	0.06 ± 0.00	3364
		30	244 ± 65	0.05 ±0.03	5652
		40	109 ± **	0.01 ± **	10105
Apopka	West	5	2020 ± 199	1.3 ± 0.1	1624
		10	1422 ± 209	0.63 ± 0.02	2258
		15	1171 ± 240	0.49 ± 0.2	2628
		20	1072 ± 38	0.38 ± 0.2	3750
		30	1003 ± 240	2.8 ± 0.8	408
		45	819 ± 7.2	8.2 ± 5.4	127
		60	642 ± 65	12.2 ± 2.7	54
		80	733 ± 13	13.3 ± 0.8	55
		98	684 ± **	7.7 ± **	88

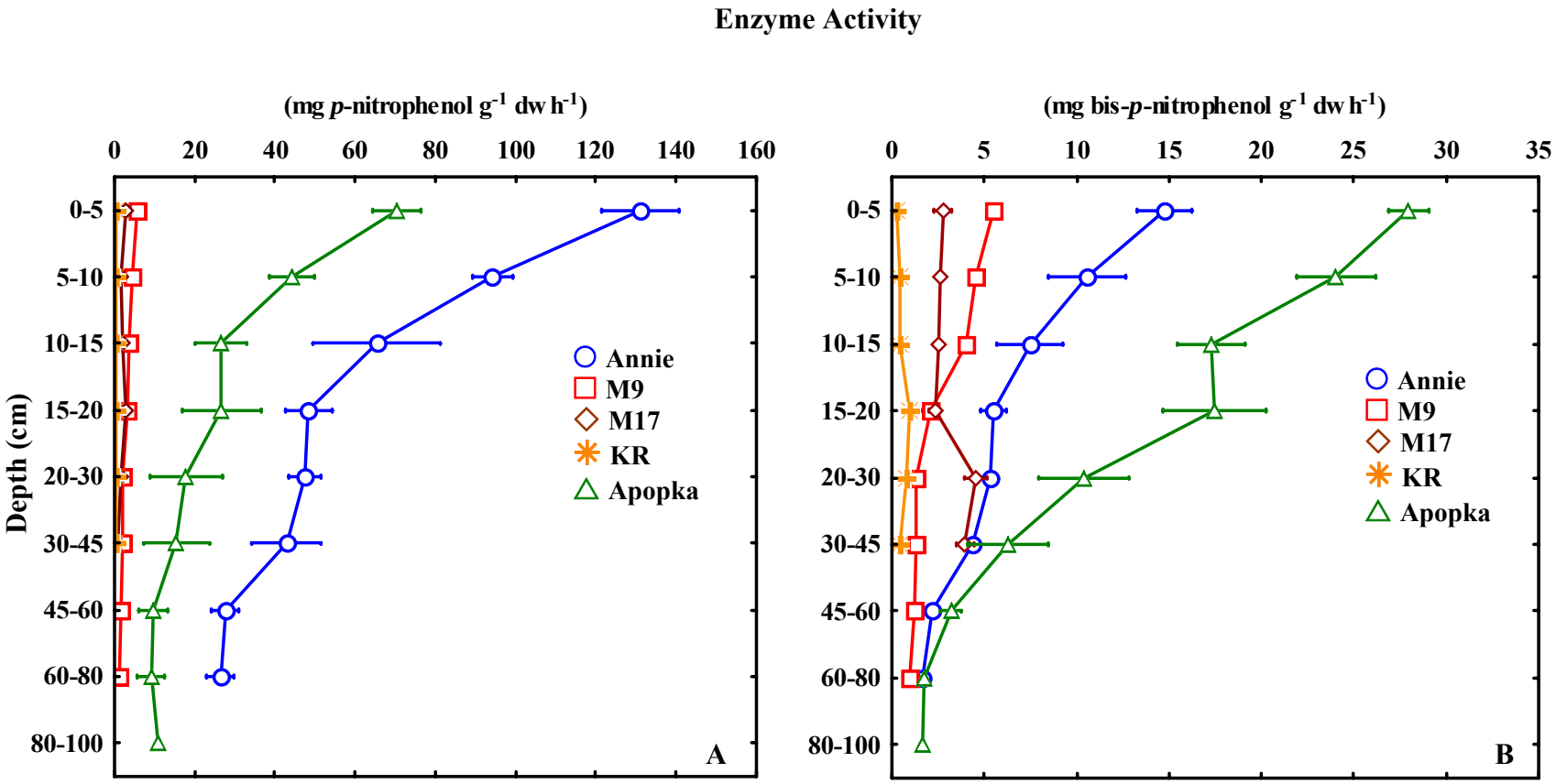


Figure 4-1. Enzyme activity of sediment depth profile in Lake Annie, Lake Okeechobee: M9, M17 and, KR, and Lake Apopka. A) Phosphomonoesterase and B) phosphodiesterase.

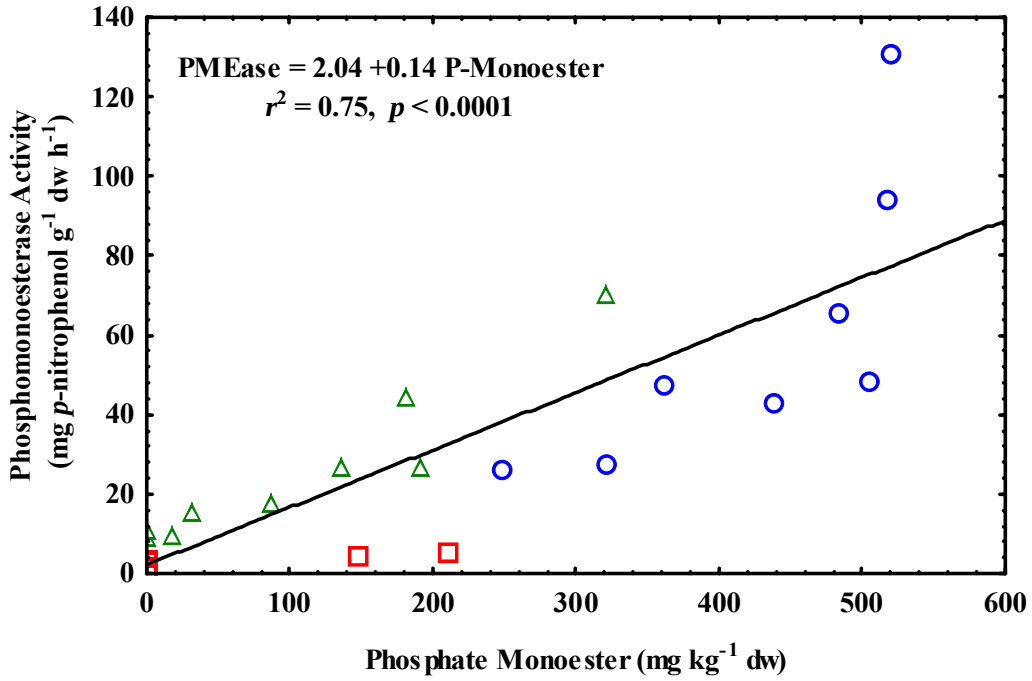


Figure 4-2. Relationship between phosphate monoester concentration and phosphomonoesterase activity in sediments from Lake Annie (blue circles), Lake Okeechobee - M9 (red squares), and Lake Apopka (green triangles).

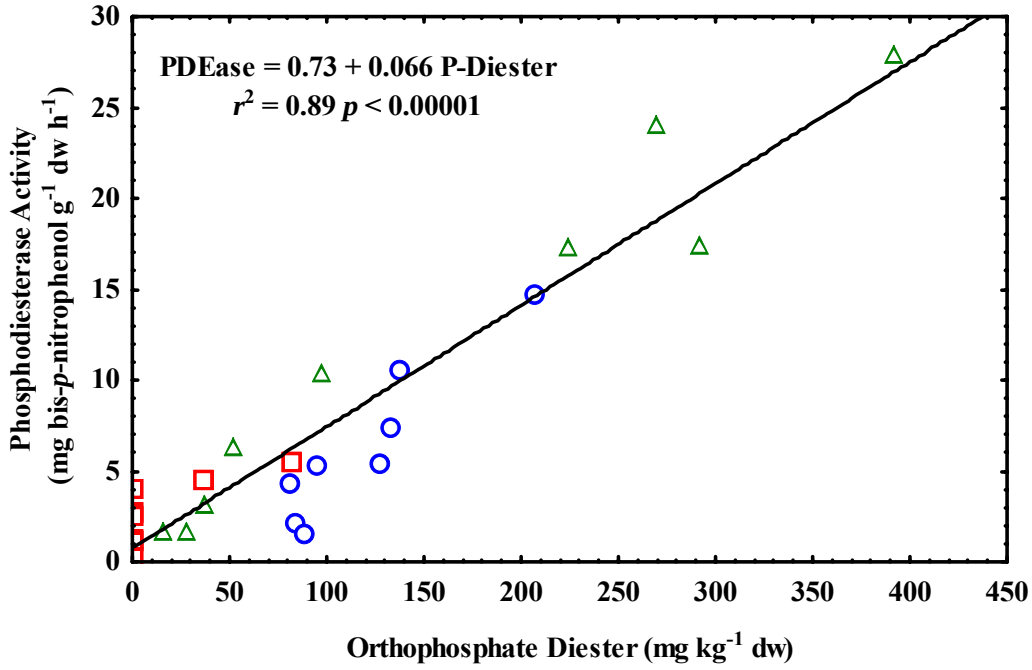


Figure 4-3. Relationship between phosphate diester concentration and phosphodiesterase activity in sediments from Lake Annie (blue circles), Lake Okeechobee - M9 (red squares), and Lake Apopka (green triangles).

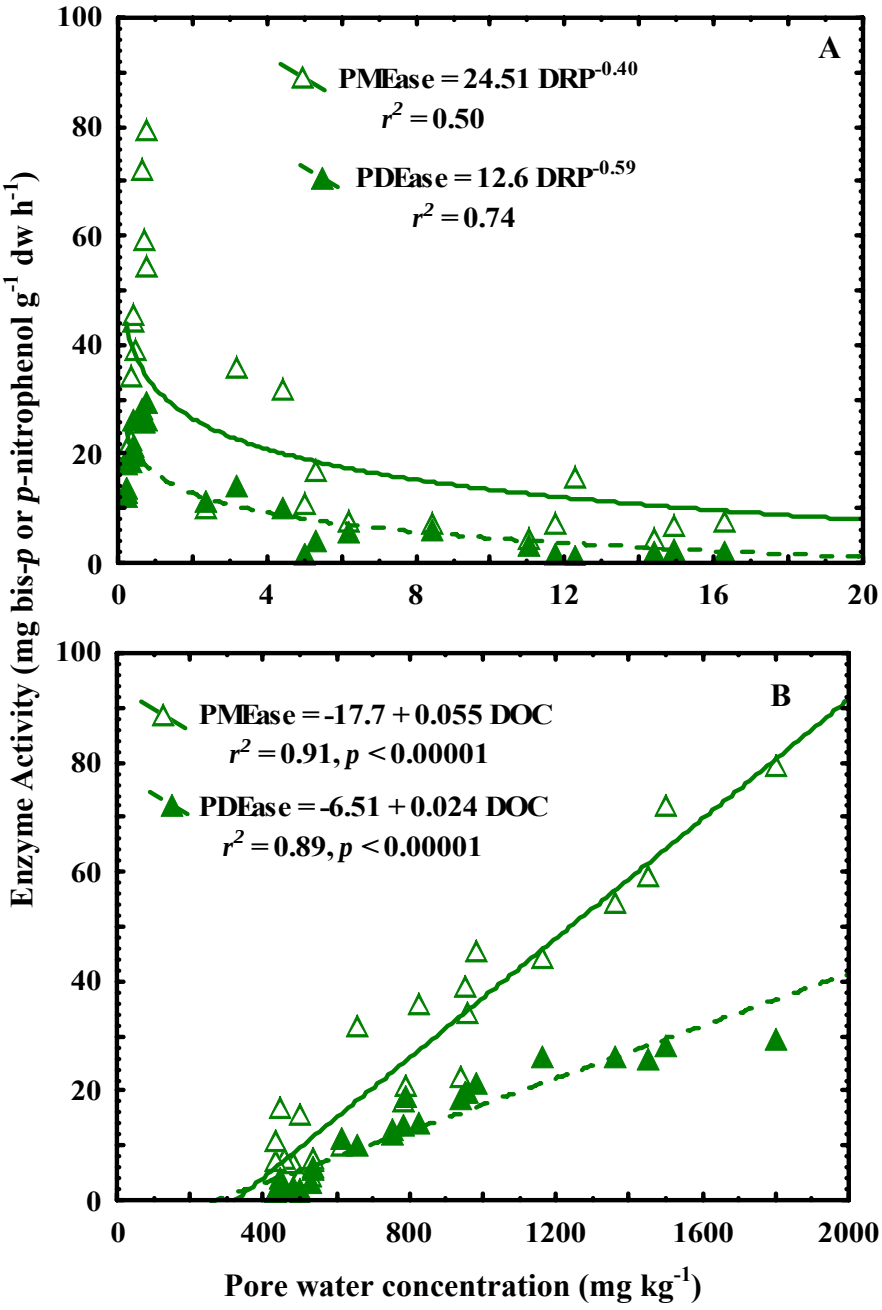


Figure 4-4. Relationship between enzyme activity, phosphomonoesterase (PMEase) and phosphodiesterase (PDEase) and A) pore water dissolved reactive phosphorus (DRP) and B) dissolved organic carbon (DOC) concentration in sediments from Lake Apopka. .

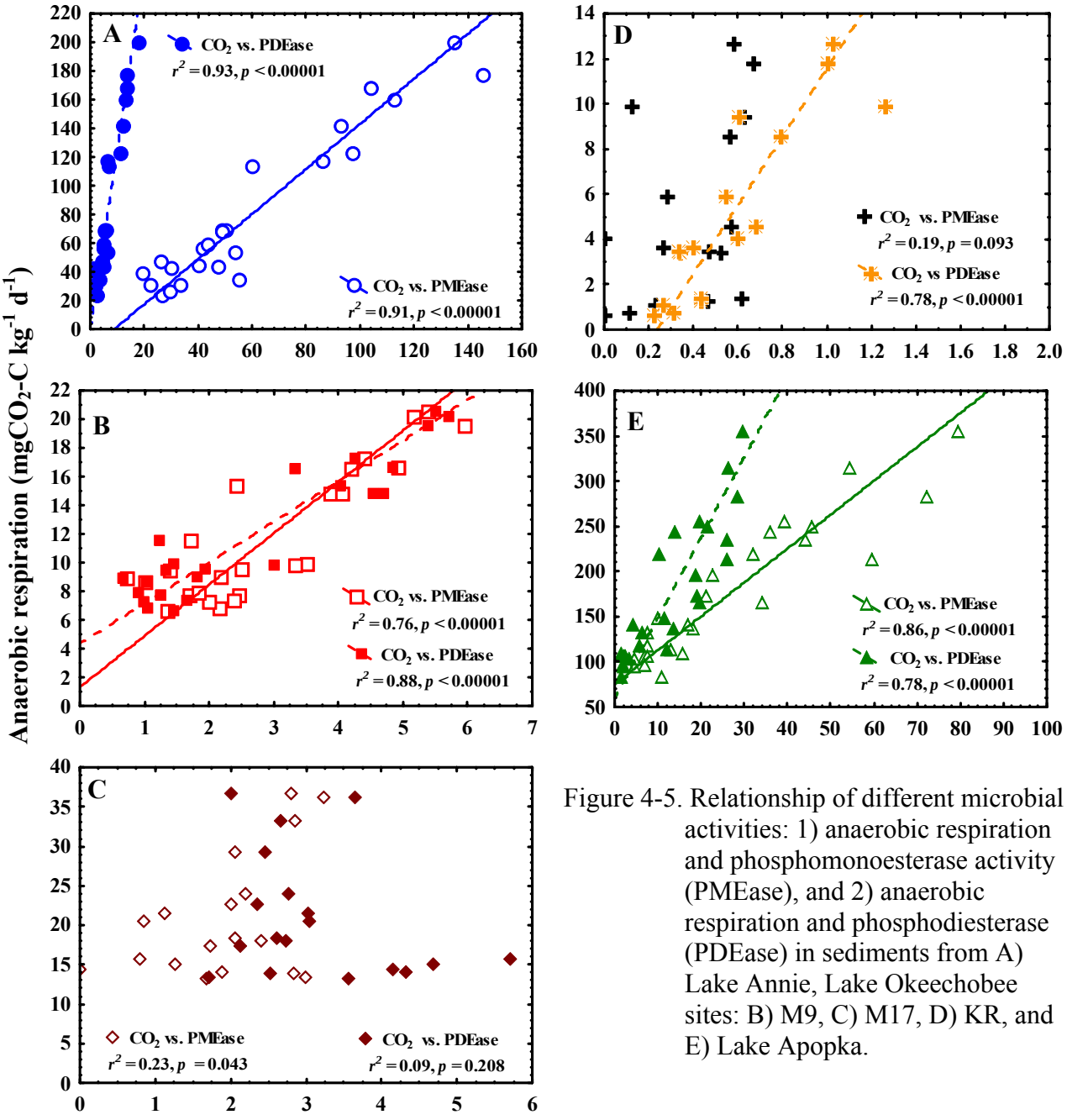


Figure 4-5. Relationship of different microbial activities: 1) anaerobic respiration and phosphomonoesterase activity (PMEase), and 2) anaerobic respiration and phosphodiesterase (PDEase) in sediments from A) Lake Annie, Lake Okeechobee sites: B) M9, C) M17, D) KR, and E) Lake Apopka.

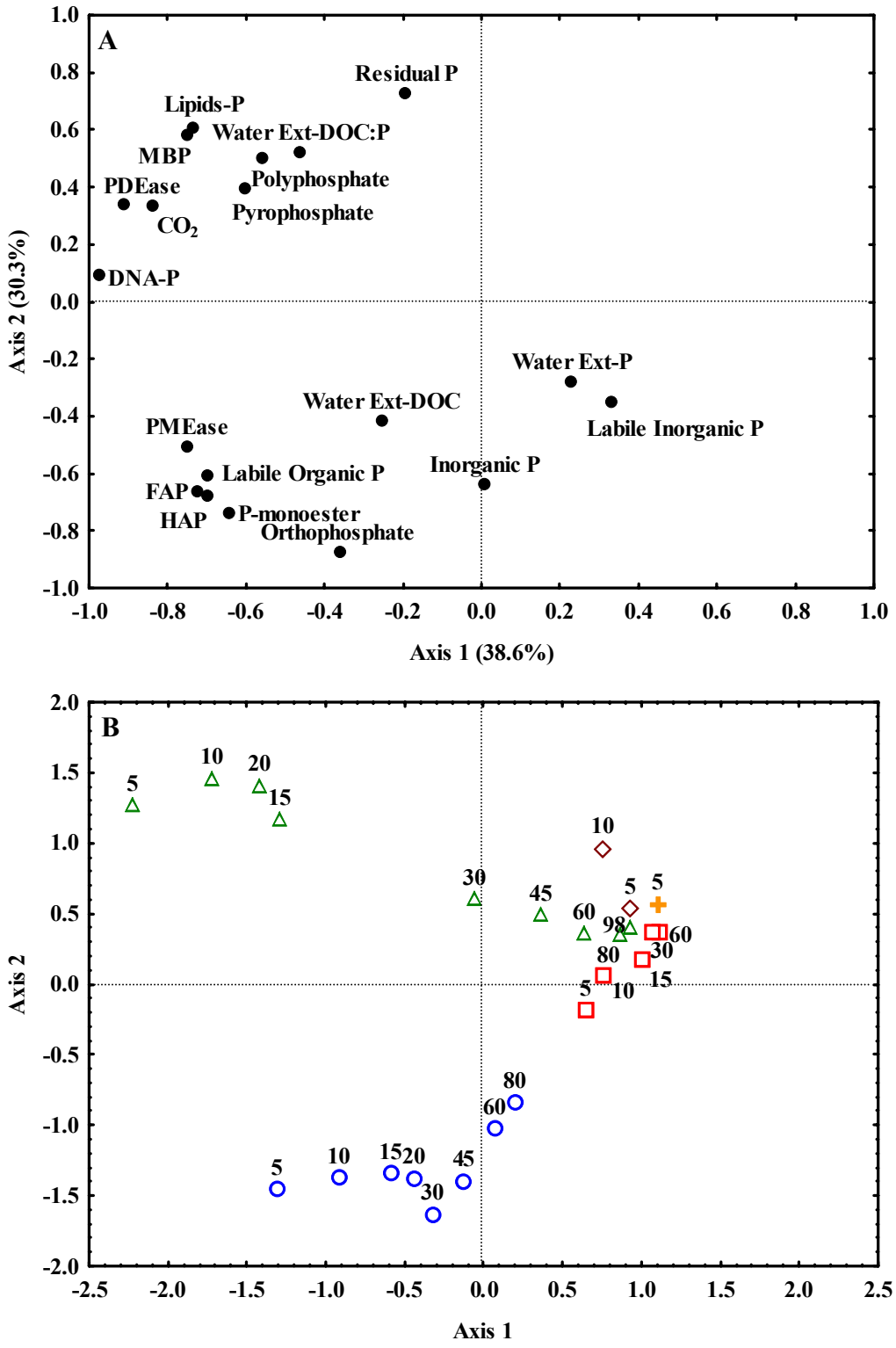


Figure 4-6. Results of the Principal Component Analysis, A) loadings of different phosphorus compounds measured by ^{31}P NMR and P fractionation, enzymes and microbial activities (n = 25), and B) the plot of the scores of the sites and sediment depth (numbers cm) from Lake Annie (circles), Lake Okeechobee: M9 (squares), M17 (diamonds), KR (crosses), and Lake Apopka (triangles).

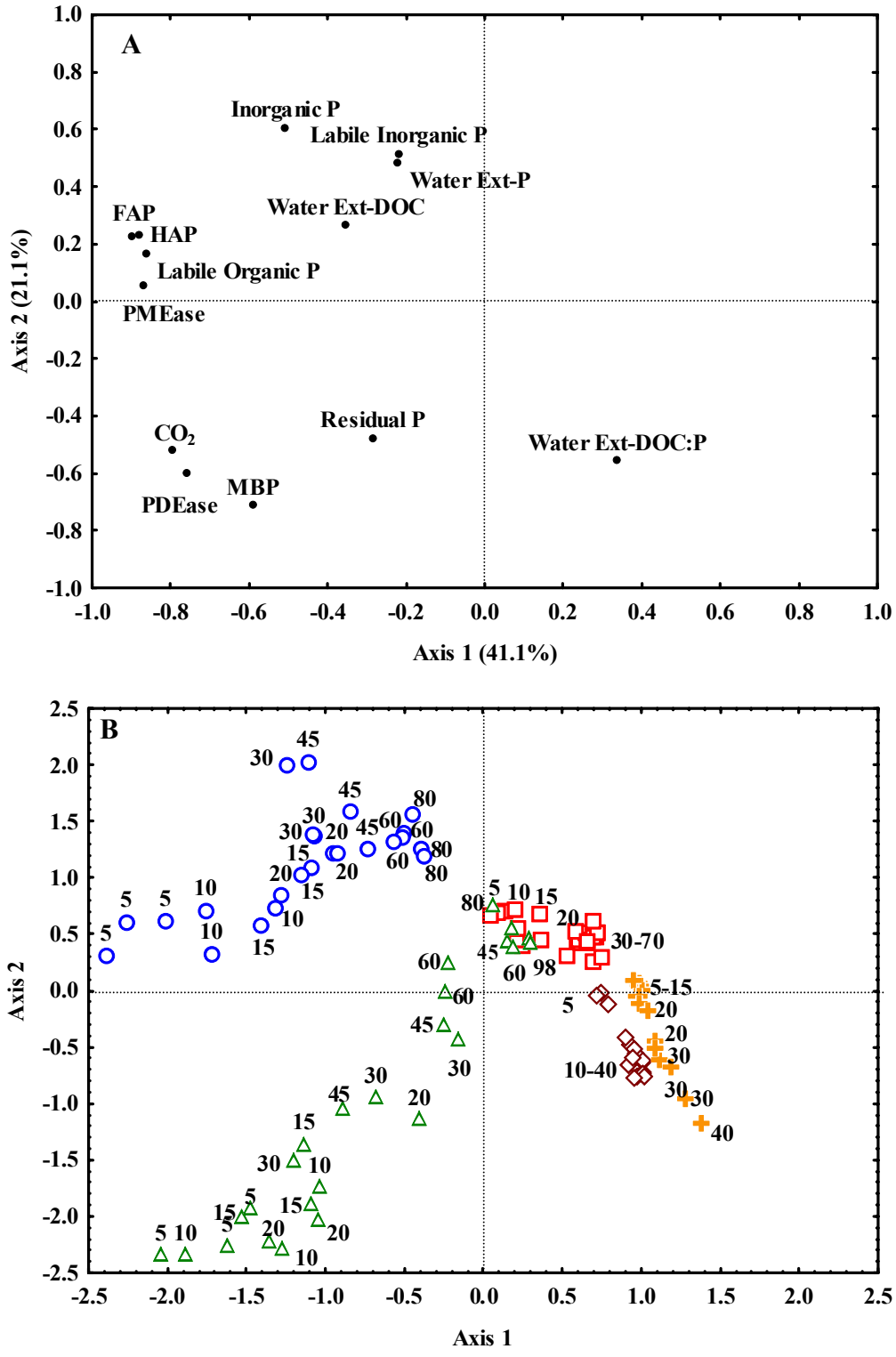


Figure 4-7. Results of the Principal Component Analysis, A) loadings of different phosphorus compounds measured by P fractionation, enzymes and microbial activities (n = 107), and B) the plot of scores for the sites and sediment depth (numbers cm) from Lake Annie (circles), Lake Okeechobee: M9 (squares), M17 (diamonds), KR (crosses), and Lake Apopka (triangles).

CHAPTER 5
MICROBIAL BIOMASS AND ACTIVITY IN SEDIMENTS OF SUBTROPICAL LAKES

Introduction

Phytoplankton and/or heterotrophic bacteria are the major drivers of carbon (C) and nutrient cycling in the water column of lakes, while the heterotrophic bacteria dominate in sediments. Allochthonous and autochthonous particulate organic matter in the water column is deposited in the sediment. Water column depth affects the quality of organic material reaching the sediment. In deep lakes, detrital organic matter undergoes intense decomposition in the water column, due to the prolonged period of settling. Consequently low amounts of labile organic C reach the sediment (Suess 1980; Meyers 1997). In shallow lakes, the supply of labile C and nutrients can be higher than in deep lakes, and the latter often can have more refractory organic matter (Suess 1980; Meyers 1997).

As bacteria are the dominant group in sediments, organic compounds and associated nutrients supplied to the sediment surface are mineralized through heterotrophic decomposition (Gächter and Meyer 1993; Capone and Kiene 1988). Complete oxidation of a broad range of organic compounds occurs through the sequential activity of a variety of anaerobic bacteria (Capone and Kiene 1988). In high depositional environments, such as eutrophic, or deep thermally stratified lakes, organic content in sediments is often high, oxygen (O_2) consumption occurs rapidly, and O_2 is depleted several millimeters below the sediment water interface (Jørgensen 1983; Jørgensen and Revsbrech 1983). In these systems, facultative and obligate anaerobic communities dominate. In methanogenic habitats, i.e., in the absence of inorganic electron acceptors, different groups of microorganisms participate in decomposition of organic matter as no single anaerobic microorganism can completely degrade organic polymers (Zinder 1993, Megonigal et al. 2004). Fermenting bacteria hydrolyze organic polymers through enzyme

production and further break down monomers to alcohols, fatty acids, and hydrogen (H_2). Alcohols and fatty acids are degraded by syntrophic bacteria into acetate, H_2, and carbon dioxide (CO_2), which is used as substrate by methanogens (Zinder 1993, Conrad 1999). Consequently, CO_2 and methane (CH_4) are important end products of anaerobic organic matter decomposition and such gas production can be used as a measure of microbial activity in sediments.

Several factors limit bacterial metabolism in sediments, i.e., temperature, biodegradable organic C, nutrients, and electron acceptors. Most studies of microbial activity in sediments focus on C limitation and the effect of electron donors or acceptors in production of CO_2 and/or CH_4 (Capone and Kiene 1988; Schulz and Conrad 1995; Maassen et al. 2003; Thomsen et al. 2004). Little work has been done relating production of CO_2 and CH_4 with biogeochemical properties of sediments such as nutrient availability. Studies in the water column of lakes have shown that several factors can limit bacterial metabolism (Gurung and Urabe 1999; Jasson et al. 2006). Although it has been generally accepted that the heterotrophic community is C/energy limited, recent studies have shown that inorganic nutrients, especially phosphorus (P) can be the most limiting nutrient for the bacterial community (Gurung and Urabe 1999; Vadstein 2000; Olsen et al. 2002; Vadstein et. al. 2003; Smith and Prairie 2004; Jasson et al. 2006). Reviewing data from freshwater ecosystems, Vadstein (2000) showed that P limitation is a common phenomenon. Phosphorus limitation occurred in 86% of the cases, while nitrogen or C limitation was identified in 15% and 20%, respectively (percentages add up to more than 100% due to methodological aspects, Vadstein 2000). Heterotrophic microbial metabolism can be limited by a single factor or multiple variables. Limitation varies among lakes and depends on lake characteristics and biogeochemical properties of sediments.

The primary hypothesis of this study is that microbial activities will be higher in recently accreted sediments (surface) as compared to older sediments (sub-surface). The second hypothesis is that nutrient limitation will vary among sediments from different lakes, and will be related to sediment biogeochemical properties (i.e., nutrient concentration and availability). The objectives of this study were to: (i) determine stratigraphic biogeochemical properties in sediments from three subtropical lakes of different trophic status and evaluate how they are related to microbial biomass and activity; (ii) measure microbial biomass at different depths in the sediment from the three different lakes, and test whether there is nutrient limitation; and, (iii) measure sediment microbial activity and establish relationships with nutrient concentration and availability.

Materials and Methods

Study Sites

Three Florida (USA) lakes ranging in trophic state were selected. Lake characteristics were described in Chapter 2. The characteristics and location of sampled sites and field sampling procedures were described in Chapter 3 (Table 3-1, Figure 3-1).

Sediment Properties

Samples were transported on ice and stored in the dark at 4 ºC. Before each analysis, samples were homogenized and sub-samples taken. Sediment bulk density, pH, organic matter (LOI-loss on ignition), and total phosphorus (TP) were measured and described in a previous study (Chapter 3). Total carbon (TC) and total nitrogen (TN) were determined using a Flash EA-1121 NC soil analyzer (Thermo Electron Corporation).

Extractable C, N and P

Due to high water content of Lake Annie and Lake Apopka sediments, pore water was extracted (centrifuged at 10,000 x g for 10 min) prior to C and nutrient extractions. Pore water

TP, dissolved reactive P (DRP), ammonium-N (NH_4-N), total nitrogen (TN), and dissolved organic carbon (DOC) were measured using U.S. EPA methods (EPA 1993). Total Kjeldahl nitrogen (TN) was measured by digestion with concentrated sulfuric acid (H_2SO_4) and Kjeldahl salt catalyst, and determined colorimetrically (Method - 351.2). Total P was digested with 11N H_2SO_4 and potassium persulfate (Method - 365.1). Water samples were filtered through a 0.45 µm membrane filter and filtrate was analyzed for DRP (Method - 365.1), NH_4-N (Method - 351.2), and DOC (automated Shimadzu TOC 5050 analyzer (Method - 415.1).

Microbial Biomass C, N and P

Microbial biomass carbon (MBC), nitrogen (MBN), and phosphorus (MBP) were measured with the chloroform fumigation-extraction method (Hedley and Stewart 1982; Brookes et al. 1985; Vance et al. 1987; Horwath and Paul 1994; Ivanoff et al. 1998). Briefly, sediment samples were split in two: one sample was treated with alcohol-free chloroform (0.5 mL) to lyse microbial cells, placed in a vacuum desiccator, and incubated for 24 hrs. The duplicate sample was left untreated. Both sets were extracted with 0.5 M K_2SO_4 for MBC and MBN, and with 0.5 M $NaHCO_3$ (pH = 8.5) for MBP, using a 1:50 dry sediment-to-solution ratio. Extracts from MBC and MBN samples were centrifuged at 10,000 x g for 10 min and filtered through Whatman # 42 filter paper, and 5 mL of the extracts were subjected to Kjeldahl nitrogen digestion (for MBN) and analyzed for total Kjeldahl-N colorimetrically using a Bran+Luebbe Technicon™ Autoanalyzer II (Method - 351.2, EPA 1993). MBC extracts were acidified (pH < 2) and analyzed in an automated Shimadzu TOC 5050 analyzer (Method - 415.1, EPA 1993). Extracts from MBP samples were filtered using a 0.45 µm membrane filter and digested for TP with sulfuric acid and potassium persulfate. Solutions were analyzed by colorimetry, determined by reaction with molybdate using a Bran+Luebbe Technicon™ Autoanalyzer II (Murphy and Riley 1962; Method - 365.1, EPA 1993). Microbial biomass (C, N and P) was determined by the

difference between treated (with chloroform) and non-treated samples. Untreated samples represent extractable organic carbon (Ext-C), extractable labile nitrogen (Ext-N), and extractable labile phosphorus (Ext-P).

Undigested N extracts were analyzed for ammonium-N (NH_4-N) (Method - 351.2, EPA 1993), and represent extractable ammonium-N (Ext-NH_4-N). The difference between Ext-N and Ext-NH_4-N represents extractable labile organic nitrogen (Ext-ON) (Mulvaney 1996). Undigested P extracts were analyzed for soluble P as described previously, and this fraction represents labile inorganic P (Ext-Pi). The difference between Ext-P and Ext-Pi, represents labile organic phosphorus (Ext-Po) (Ivanoff et al. 1998).

Microbial Activity

Anaerobic microbial respiration (CO_2) and methanogenesis (CH_4) were quantified by incubating a known amount of wet sediment equivalent to 0.5 g of dry weight, in 5 mL of DI water at 30 ºC under anaerobic conditions. Samples were placed in a glass vial and closed with rubber stoppers and aluminum crimp seals. Samples were purged with N_2 gas to achieve anaerobic conditions. Gas samples were obtained at 2, 4, 7, and 10 days and CO_2 released was measured by gas chromatography using a Shimadzu 8A GC-TCD equipped with Poropak N column (Supelco Inc., Bellefonte, PA), using He as a carrier gas. Methane was measured with the Shimadzu gas chromatograph-8A fitted with a flame ionization detector (110 ºC), N_2 as the carrier gas and a 0.3 cm by 2 m Carboxen 1000 column (Supelco Inc., Bellefonte, PA) at 160 ºC. Headspace pressure was determined with a digital pressure indicator (DPI 705, Druck, New Fairfield, CT). Concentrations of CO_2 and CH_4 were determined by comparison with standard concentrations and production rates were calculated by linear regression ($r^2 > 0.95$).

At time zero of the incubation experiment a sub-sample of sediment was extracted with 25 mL of DI water, shaken for 1 hour, centrifuged at 10,000 x g, and passed through a 0.45 µm

membrane filter. After the 10-day incubation period using the same extraction procedure, sediments were extracted. Dissolved organic carbon (DOC), NH_4-N and DRP were measured as described above.

Statistical Analysis

A *t* test was performed to evaluate whether there was a statistical difference in concentration (increase or decrease) of DOC, NH_4-N and DRP during incubation (time zero versus time ten) for each site and sediment depth. A Principal Component Analysis (PCA) was performed to address relations between the variables and in each lake and how they vary with sediment depth. All statistical analyses were conducted with Statistica 7.1 (StatSoft 2006) software.

Results

Sediment Properties

Sediment properties (i.e., pH, bulk density, and organic matter) and TP concentration were reported in a previous study (Chapter 3). Sediment TC was highest in Lake Okeechobee site M17, reflecting its peat nature. Next highest in TC was Lake Apopka, followed by Lake Annie, and sites M9 and KR in Lake Okeechobee (Table 5-1). Lake Annie alone displayed a decrease in TC with greater sediment depth. Total N also declined with depth in Lake Annie sediments and showed a generally similar trend to the core from Lake Okeechobee site M9. Total N was highest in Lake Apopka sediments, followed by Lake Okeechobee site M17. Neither core showed a clear trend in TN concentration with depth (Table 5-1). Total C:N ratios were similar in all sediments. Total C:P ratios were highest in M17 sediments. There was no clear trend in total C:P and N:P ratios in Lake Annie and Lake Okeechobee mud sediments (M9-site). Sites M17 and KR (Lake Okeechobee) and Lake Apopka showed an increase in total C:P and N:P ratios with depth (Table 5-1).Extractable and Microbial Biomass C, N and P

Lake Apopka had the highest concentration of DOC, NH_4-N and TN pore water concentration (Table 5-2). Lake Annie had low DRP pore water concentration (Table 5-2).

Extractable organic C was highest in Lake Apopka and decreased with sediment depth, followed by site M17 in Lake Okeechobee and Lake Annie (Table 5-3). In site M17 there was a general trend of increase of Ext-OC with sediment depth. The other sediments (Lake Annie, and Lake Okeechobee sites M9 and KR) showed no clear trend of Ext-OC distribution with sediment depth. Lake Apopka also had the highest concentration of Ext-NH_4-N and Ext-ON. There was a general increase in Ext-NH_4-N concentration with depth in Lake Apopka, Lake Annie and M17 site in Lake Okeechobee. Lake Okeechobee sand (KR and mud (M9) sediments were characterized by low Ext-NH_4^+-N concentration. While there was a trend of decrease in Ext-ON with depth in Lake Apopka and Lake Annie sediments, the same did not occur in Lake Okeechobee peat sediments (M17) where a general increase with depth was present (Table 5-3). In Lake Okeechobee mud and sand sediments there was no clear trend with depth. Surface sediment labile inorganic P (labile-Pi) concentrations were highest in Lake Okeechobee site M9 followed by Lake Annie (Table 5-3). There was a general decrease with sediment depth for Lake Annie and Lake Okeechobee mud sediments. Labile Pi increased with depth in Lake Apopka. Labile organic P (labile-Po) was highest in Lake Annie and Lake Apopka with lower concentrations in all Lake Okeechobee stations. There was a general decrease in labile-Po with depth in Lake Annie and mud sediments of Lake Okeechobee, while the other sediments did not present a clear trend (Table 5-3). Lake Apopka had the highest concentration of MBC, MBN and MBP, followed by Lake Annie. Among Lake Okeechobee sites, M9 had higher MBN and MBP, and M17 had the highest MBC. There was a general decrease in microbial biomass with depth in all sediments (Table 5-4).

Microbial Activity

Lake Apopka (Figure 5-1E) had the highest anaerobic CO_2 and CH_4 production rates, followed by Lake Annie (Figure 5-1A), and sites M17 (Figure 5-1C), M9 (Figure 5-1B) and KR (Figure 5-2D) in Lake Okeechobee. Both CO_2 and CH_4 production rates generally decrease with sediment depth at all sites except KR (Figure 5-1D). In KR sediments, there was a peak of CO_2 production at 15-20 cm of depth that coincided with an increase in organic matter content (Figure 5-1D, Table 3-2). In Lake Annie there was a sharp decrease in methane production below 10 cm depth, and CH_4 production was low in all sites from Lake Okeechobee.

Water extractable DOC, NH_4-N, and DRP concentrations before and after 10-day anaerobic incubation were different in the three lakes (Table 5-5). For each depth at each site, a *t*-test was run for each variable to test if there was a significant statistical difference in concentration with incubation time, and significant differences ($p < 0.05$) are in bold (Table 5-5). Dissolved organic C concentration increased during 10-day incubation at all depths of Lake Apopka and in the topmost 10 cm in Lake Annie (Table 5-5). In Lake Okeechobee M17 sediments, DOC decreased with incubation at all depths (Table 5-5). Sediments from sites M9 and KR showed variable effects of incubation on DOC concentration at different depths. Concentrations of NH_4-N were also higher after incubation in Lake Apopka sediments, and Lake Okeechobee M17 sediments (Table 5-5). The other sediments had variable trends for each depth although there was a general decrease in NH_4-N in M9 with time. Dissolved reactive P increased in site M17 and in some depths at site M9 in Lake Okeechobee. In Lake Annie there was a decrease with incubation time in deeper sediments (Table 5-5). In Lake Apopka sediments soluble P increased with incubation time in deeper sediments (Table 5-5).

As MBC, MBN and MBP are highly correlated (MBC x MBN $r = 0.97$, MBC x MBP $r = 0.96$, and MBN x MBP $r = 0.97$), MBC was chosen as a proxy of microbial biomass in statistical analyses. High correlations were seen between MBC and anaerobic CO_2 ($r = 0.88$) and CH_4 ($r = 0.85$) production rates. High correlations were seen between MBC and extractable N:P ($r = 0.74$), and extractable NH_4-N:Pi ratios ($r = 0.73$), while the other ratios had either low or not significant correlations with MBC. The same results were observed for microbial activity. Anaerobic CO_2 ($r = 0.63$) and CH_4 ($r = 0.77$) had significant correlations with extractable NH_4-N:Pi, and with extractable N:P ratios ($r = 0.62$ for both CO_2 and CH_4).

Principal Component Analysis was conducted to see how nutrient availability relates to microbial biomass and activities. Results showed that 48.6% of the data variability was explained by Axis 1 while Axis 2 explained 22.8% (Figure 5-2A). LOI, TN, Ext-C, Ext-NH_4-N, Ext-ON, Ext-N:Ext-P, MBC, CO_2, and CH_4, were the variables selected by Axis 1. While TP in the negative axis, and Ext-C:Ext-P and Ext-C:Ext-N in the positive axis were selected by Axis 2. The positions of the sites and sediment depths in relation to variable loadings in the PCA showed that the three lakes are separated into different groups (Figure 5-2B). Lake Apopka was positioned with variables selected by Axis 1 and had clear separation by sediment depth. Site M17 in Lake Okeechobee also presented some separation of sediment depth, but to a lower degree, and was placed in the positive axis of variables selected by Axis 2. Lake Annie sediments and site M9 were positioned with labile-Pi and KR with bulk density, and did not show clear separation by sediment depth (Figure 5-2B).

Discussion

Hypereutrophic Lake Apopka had the highest microbial biomass and activity (both CO_2 and CH_4) among the study lakes. Other studies that compared lakes of different trophic state conditions found that both bacterial production and respiration, and CH_4 production increased

with trophic state (Drabkova 1990, Casper 1992, Huttunen et al. 2003, Massen et al. 2003, Wobus et al. 2003). In this study, however, oligo-mesotrophic Lake Annie had higher biomass and activity than eutrophic Lake Okeechobee. Although Lake Annie is oligo-mesotrophic based on water column variables, organic matter content and nutrient concentrations are high in the sediment (Table 5-3, Chapter 3). High nutrient concentrations in sediments of this oligo-mesotrophic lake lead to high microbial biomass and activity. There was a decrease in microbial activity with sediment depth which also has been reported in other studies and is related to the decrease in easily degraded organic matter with sediment depth (Rothfuss et al. 1997, Falz et al. 1999, Kostka et al. 2002, Roden and Wetzel 2003; Dan et al. 2004).

Statistical analysis showed that nutrient (C, N and P) concentrations and nutrient ratios influenced the microbial community. This suggests that C, coupled with N and P availability has a strong influence in microbial communities in these lakes sediments. When sediments were incubated, there was a general increase in DOC and with time in Lake Apopka (all depths), and Lake Annie surface sediments (0-10 cm). Ammonium accumulation with time was detected in Lake Apopka and Lake Okeechobee peat sediments (site M17). Accumulation of DOC and NH_4-N can indicate high microbial activity. Maasen et al. (2003) and Wobus et al. (2003) studied sediments from reservoirs of different trophic states and concluded that high DOC and NH_4^+ in pore water was related to high microbial activity because both are end products of microbial decomposition. Falz et al. (1999) used NH_4^+ concentrations as evidence of high microbial activity that correlated with high CH_4 production in Lake Rotsee (Switzerland) sediments. Lake Annie sediments had higher microbial activities than peat sediments in Lake Okeechobee. Consequently, if DOC and NH_4-N accumulation were only a reflection of microbial activities they should have been higher in Lake Annie. In Lake Annie there is no accumulation of NH_4-N

and deeper sediment layers had a significant decrease in DOC concentration with time. This suggests that other factors are influencing accumulation of DOC and NH_4-N in these sediments.

Several studies have indicated that DOC accumulation is a reflection of P limitation in freshwater ecosystems. Gurung and Urabe (1999) concluded from controlled experiments on the bacterial planktonic community from eutrophic Lake Biwa (Japan) that DOC accumulation in surface water during summer is induced by the high bacterial growth rate and P limitation. Also, Olsen et al. (2002) studied nutrient limitation of aquatic food webs and showed that DOC accumulated in experiments where P was limiting, i.e., with high C:P ratios. Other studies also reported that in lakes where there is P limitation of heterotrophic bacteria, labile DOC accumulates (Vadstein et al. 2003). Jasson et al. (2006) did controlled experiments with bacterioplankton in subarctic Lake Diktar Erik, Sweden, and showed that growth of the heterotrophic community was controlled by DOC and inorganic nutrients. In their experiments, bacterial production was stimulated by the DOC supply, but the use of DOC for growth was dependent on the DOC:Pi ratio. Furthermore, DOC was used for growth under C-limited conditions, but used for respiration under Pi limitation, when bacterioplankton communities tend to respire large portions of assimilated C.

The increase in NH_4-N at the end of incubation is another indication of P limitation. Bacteria preferably utilize N in the form of amino acids over NH_4^+ (Kirchman 1990), and this preference has been reported to be stronger when bacterial growth is P-limited (Schweitzer and Simon 1995, Gurung and Urabe 1999). This can lead to an accumulation of NH_4^+ and other inorganic N forms in P limited systems (Gurung and Urabe 1999). In Lake Apopka sediments there was a general increase in DOC and NH_4-N with time, strongly indicating that there is P limitation during summer.

There is high P hydrolytic enzyme activity in surface sediments of Lake Apopka indicating high demand for labile inorganic P (Chapter 4). Lake Apopka sediments have high extractable C:P and N:P ratios, and the decrease of these ratios with depth is related to an increase in labile inorganic P concentration (Table 5-3). Although hypereutrophic lakes are characterized by high P concentration, P limitation can occur during summer due to high demand and competition for P (Gurung and Urabe 1999; Vadstein et al. 2003; Vrede 2005; Jasson et al. 2006).

Nitrogen is the primary limiting nutrient in Lake Apopka although co-limitation with P can occur (Aldridge et al. 1993). Phosphorus limitation of primary productivity, however, has been detected during summer in Lake Apopka (Newman et al. 1994). Even though this P limitation has been established for the phytoplankton community in the water column, Lake Apopka is shallow and there is high interaction between the water column and sediments. Moreover, bacteria have three times higher P requirements than do typical algae (Vadstein 2000). If the demand for P in the water column is high during summer, less P will reach the sediment, and there will be low labile P concentration in the sediments.

Summer temperatures in Lake Apopka sediments can be high, which could stimulate microbial activity and demand for P. High primary production and high labile C sedimentation (Gale et al. 1992, Gale and Reddy 1994) will lead to high demand for labile P in surface sediment that was reflected in high C:labile nutrient ratios. With decomposition and utilization of C by the heterotrophic community, C becomes increasingly refractory and there is a decrease in microbial biomass and activity with sediment depth, reducing the demand for P. Consequently labile P will accumulate in deeper sediments, yielding lower C:P ratios as seen for Lake Apopka deeper sediments.

The M17 sediment from Lake Okeechobee also had an increase in NH_4-N concentration during incubation and highest C:nutrient ratios. These sediments also seem to be P limited. The primary productivity in the southern region (peat zone) of Lake Okeechobee was reported to be limited by N and a high frequency of co-limitation by N + P occurs (Aldridge et al. 1995). A dual limitation by P and C, however, seems to be occurring in M17 sediments. M17 sediments are characterized by peat deposits formed by incomplete decomposition of higher plants (Reddy et al. 1991). Extractable C is probably rich in humic substances known to be refractory. Consequently, although the C:nutrient ratios are high for this site, available C is probably low. If DOC is not easily available, C can limit heterotrophic bacteria (Vadstein et al. 2003). Vrede (2005) showed that lakes with high concentrations of humic substances are usually limited by both C and P. The incubation experiment strongly indicates the refractory nature of C in these sediments. There was a decrease in DOC concentration during incubation, probably reflecting high demand for labile C by the microbial community. This site was the only one that showed a significant accumulation of DRP, and NH_4-N following incubation. The decrease in C and simultaneous increase in inorganic nutrients, N and P, indicates a high demand of these sediments for labile C.

High availability of P in Lake Okeechobee sites M9 and KR surface sediments is causing C limitation in the system. Moreover, in M9 sediments, low values of Ext-N:Ext-P indicate that N can be also limiting in this system. Crisman et al. (1995) reported that temperature and trophic state variables Secchi disk depth, total P, and total N, had a weak correlation with bacterioplankton abundance (number of cells mL^{-1}) in a seasonal study of Lake Okeechobee. They concluded bacterioplankton communities were probably controlled by grazing and/or C and nutrient availability. Work et al. (2005) reported high bacterioplankton production (mg L^{-1} h^{-}

[1]) in Lake Okeechobee during summer. Also, several studies have shown that bacterioplankton is an important source of C to the food web in Lake Okeechobee (Havens and East 1997; Work and Havens 2003; Work et al. 2005). However, to my knowledge, there is no study addressing C or nutrient limitation of the bacterioplankton community in Lake Okeechobee.

Nevertheless, Phlips et al. (1997) showed that in the central, mud zone region of Lake Okeechobee phytoplankton was dominated by small-celled species of cyanobacteria and diatoms. Light is the most limiting factor of the phytoplankton community during most of the year in this area, however, during summer months, light limitation is relaxed and N becomes the limiting factor of the phytoplankton community (Aldridge et al. 1995). Several studies have reported low chlorophyll-*a* and primary productivity in the mud zone is caused by light limitation (Aldridge et al. 1995, Phlips et al. 1993, 1995*c*; Gu et al. 1997). There are, however, no data for Lake Okeechobee reporting the contribution of primary productivity to sediment C. Also, although sites M9 and KR showed similar values of DOC in the water column, water extractable DOC was low in M9 sediments and even lower in KR deposits (Table 5-5). These low-DOC sediments had the lowest anaerobic respiration. There was no accumulation of DOC and NH_4-N, suggesting consumption of C and N with time. Also, site M9 had high labile inorganic P concentration, resulting in high demand for C and N. It is clear that C and N limit microbial biomass and activity at sites M9 and KR.

Lake Annie sediments appear to be C-limited, with low ratios of extractable C:P and N:P. Carbon limitation is probably a consequence of the C sources and physical characteristics of this lake. Lake Annie has experienced an increase in color in the water column during the past decade probably caused by recent high DOC input to the lake from the watershed (Swain and Gaiser 2005). Battoe (1985) reported high inputs of humic content to Lake Annie in surficial

runoff during high rainfall periods. This allochthonous DOC, from humic origin, is utilized in the water column. Because Lake Annie is deep, the DOC is highly mineralized before reaching the sediment (Meyer 1997). Thus low amounts of DOC reach the sediment and are highly refractory (Suess 1980), leading to low C:nutrient ratios and C limitation.

In Lake Annie, 36-56% of the total sediment P is bond to humic materials (Chapter 3), so much of the C in the sediments is probably in humic forms. High demand for C will lead to inorganic nutrient accumulation in this system, with consequently low C:nutrient ratios. Carbon limitation may be the main reason why there is a sharp decline in methane production with depth. Humic substances play an important role as electron sinks for anaerobic and fermentative bacteria, and high concentrations can inhibit methanogenesis as these compounds are used by better energetically competing organisms (Coates et al. 2002; Kappler et al. 2004; Karakashev et al. 2005).

The lack of methanogenesis in deeper sections of the sediments can also be due to competition between iron (Fe) or sulfate (SO_4^{-2}) -reducers for labile C. Lake Annie sediments were characterized by high Fe (3640 mg kg^{-1}) concentration (Thompson 1981), and dissolved SO_4^{-2} concentration (7.2 mg L^{-1}) in the water column (Swain and Gaiser 2005). High SO_4^{-2} reduction has also been reported to occur in the water column (Swain and Gaiser 2005). Sulfate reduction can be important in oligotrophic lake sediment where low organic matter input allows SO_4^{-2}-reducers to contribute to organic C oxidation (Lovley and Klug 1983). Although Fe oxides and SO_4^{-2} concentrations were not measured in this study it is probably safe to assume that both Fe- and SO_4^{-2}-reducers are active in the Lake Annie sediments. Structure and function of anaerobic microbial communities are strongly affected by competition for fermentation products such as H_2 and acetate (e.g., Megonigal et al. 2004). Iron and SO_4^{-2}-reducers outcompete

methanogens for H_2/CO_2 and aceteate, due to higher substrate affinities, and higher energy and growth yield (Lovley and Klug 1983; Lovley and Phillips 1986; Conrad et al. 1987; Bond and Lovley 2002), however, both processes can coexist (Mountfort and Asher 1981; Holmer and Kristensen 1994; Roy et al. 1997; Holmer et al. 2003; Roden and Wetzel 2003; Wand et al. 2006). Coexistence occurs because of spatial variation in the abundance of terminal electron acceptors or because the supply of electron donors is non-limiting (Roy et al. 1997; Megonigal et al. 2004). During the incubation experiment, DOC accumulated in Lake Annie surface sediments (Table 5-5). In the upper 10 cm of Lake Annie sediment, the concentration of electron donors must be sufficient for both methanogenesis and other anaerobic metabolic pathways to occur.

Lake Apopka displays high C availability and can thus support a more diverse community as reflected by anaerobic respiration and methanogenesis in the sediments. Algal deposition has been shown to increase acetate concentration, with a consequent increase in CH_4 production in sediments (Schulz and Conrad 1995). Several studies have shown that methane production rates are higher in eutrophic than oligotrophic lakes. (Casper 1992; Rothfuss et al. 1997; Falz et al. 1999; Nüusslein and Conrad 2000; Dan et al. 2004).

The negligible CH_4 production in Lake Okeechobee is clearly a consequence of electron donor limitation (Chapter 2). However, Fisher et al. (2005) reported CH_4 in sediment porewater of sites M9 and M17 in Lake Okeechobee. They also reported SO_4^{-2} in these sediment porewaters, and its decline with sediment depth was related to the use of SO_4^{-2} as a terminal electron acceptor in the oxidation of sediment organic matter. Iron is important in controlling P solubility in Lake Okeechobee sediments (Moore and Reddy 1994) and Fe-reducers might also be present. As discussed before, Fe- and SO_4^{-2}-reducers outcompete methanogens for substrates,

consequently low C availability with concomitant presence of Fe and SO_4^{-2}-reducers is the probable explanation for lack of methanogenesis in Lake Okeechobee sediments.

Conclusions

The results from this study showed that hypereutrophic Lake Apopka had the highest microbial biomass and activity (both CO_2 and CH_4) followed by oligo-mesotrophic Lake Annie. Microbial activity decreased with sediment depth and was related to decrease in easily degradable OM. Carbon, N and P concentrations, and especially nutrient ratios, had a strong influence on microbial communities in these sediments.

The sediment microbial community in each lake, or site, was limited by different variables. The Lake Apopka surface sediments appear to be P-limited. High primary production and high labile C sedimentation resulted in high demand for labile P in surface sediment, as reflected in high C:P ratio. Peat sediments of Lake Okeechobee were limited by both C and P. Nitrogen and C limitation were observed in mud and sand sediments of Lake Okeechobee. High availability of P in Lake Okeechobee mud and sand surface sediments resulted in C and N limitation. Lake Annie sediments seem to be C-limited, with low ratios of extractable nutrient ratios. Carbon limitation was probably a consequence of C sources (high humic content) and physical characteristics (deep) of this lake. The results showed that heterotrophic microbial metabolism can be limited by a single factor or multiple variables, and limitation varies among lakes depending on lake characteristics and biogeochemical properties of sediments.

Table 5-1. Total carbon (TC), total nitrogen (TN), and C:N:P ratios (weight) in sediment profiles of the three lakes. (mean ± standard deviation). ** No replicates for SD calculation.

Lake	Site	Depth (cm)	TC	TN	Ratios (weight)		
			($g\ kg^{-1}$)		C:N	C:P	N:P
Annie	Central	5	289 ± 9	21.6 ± 0.4	13	201	15
		10	280 ± 10	21.3 ± 0.8	13	197	15
		15	271 ± 6	20.5 ± 0.7	13	186	14
		20	266 ± 2	20.0 ± 0.2	13	174	13
		30	257 ± 5	18.4 ± 0.5	14	175	12
		45	257 ± 1	18.3 ± 0.4	14	173	12
		60	251 ± 3	16.6 ± 0.2	15	221	15
		80	245 ± 6	16.3 ± 0.3	15	213	14
Okeechobee	M9	5	187 ± 3	12.1 ± 0.2	15	178	11
		10	194 ± 1	12.6 ± 0.2	15	203	13
		15	131 ± 6	6.6 ± 0.4	20	157	8
		20	157 ± 4	8.5 ± 0.2	19	217	12
		30	113 ± 13	4.6 ± 1.1	25	176	7
		45	130 ± 8	5.0 ± 0.9	26	227	9
		60	155 ± 8	7.3 ± 0.7	21	263	12
		70	192 ± 9	10.6 ± 0.5	18	419	23
	M17	5	467 ± 13	26.7 ± 1.1	17	1735	99
		10	493 ± 7	27.7 ± 0.6	17	3589	201
		15	499 ± 3	27.4 ± 0.2	18	3913	215
		20	498 ± 3	26.7 ± 0.9	18	4144	222
		30	496 ± 2	25.5 ± 1.6	19	4488	229
		40	496 ± 5	24.9 ± 0.6	20	3526	176
	KR	5	11 ± 16	0.8 ± 0.9	10	44	3
		10	19 ± 18	1.2 ± 1.1	14	73	5
		15	31 ± 24	2.0 ± 1.4	14	130	8
		20	101 ± 27	6.8 ± 2.0	15	800	54
		30	106 ± 25	8.4 ± 1.8	13	1905	151
		40	35 ± **	2.6 ± **	13	2187	162
Apopka	West	5	353 ± 3	28.1 ± 0.6	13	280	22
		10	349 ± 6	28.0 ± 0.9	12	268	21
		15	349 ± 3	28.7 ± 1.0	12	258	21
		20	342 ± 1	28.1 ± 0.6	12	270	22
		30	343 ± 1	29.0 ± 0.8	12	281	24
		45	353 ± 11	28.8 ± 0.8	12	382	31
		60	357 ± 8	28.5 ± 0.2	13	461	36
		80	371 ± 5	29.4 ± 0.6	13	602	48
		98	379 ± **	28.8 ± **	13	542	41

Table 5-2. Pore water dissolved organic carbon (DOC), ammonium-N (NH_4-N), and dissolved reactive phosphorus (DRP), total nitrogen (TN), and total phosphorus (TP). (mean ± SD). **No replicates for SD calculation.

Lake	Site	Depth (cm)	Pore water (mg kg^1 dw) DOC	NH_4-N	DRP	TN	TP
Annie	Central	5	268 ± 163	62 ± 9	0.4 ± 0.1	384 ± 81	12 ± 3
		10	96 ± 11	94 ± 17	0.1 ± 0.03	374 ± 89	7 ± 3
		15	73 ± 24	107 ± 25	0.1 ± 0.05	402 ± 98	9 ± 3
		20	73 ± 4	114 ± 25	0.1 ± 0.03	423 ± 156	15 ± 3
		30	209 ± 168	83 ± 18	0.2 ± 0.1	506 ± 211	25 ± 8
		45	161 ± 44	77 ± 26	0.3 ± 0.1	484 ± 98	24 ± 9
		60	242 ± 135	76 ± 34	0.7 ± 0.1	437 ± 90	18 ± 4
		80	232 ± 163	116 ± 71	0.8 ± 0.1	382 ± 108	8 ± 1
Apopka	West	5	1586 ± 189	323 ± 140	0.7 ± 0.1	885 ± 303	10 ± 3
		10	1160 ± 201	541 ± 232	0.5 ±0.2	1353 ± 406	7 ± 1
		15	892 ± 96	617 ± 271	0.4 ± 0.1	1294 ± 535	5 ± 1
		20	842 ± 124	647 ± 248	0.3 ± 0.1	1581 ± 595	5 ± 1
		30	658 ± 149	617 ± 236	4 ± 2	1418 ± 536	6 ± 2
		45	557 ± 88	585 ± 191	8 ± 3	1280 ± 374	10 ± 2
		60	478 ± 45	460 ± 36	11 ± 5	1017 ± 44	12 ± 4
		80	479 ± 35	456 ± 31	13 ± 1	1020 ± 61	16 ± 4
		98	432 ± **	513 ± **	5 ± **	1093 ± **	7 ± **

Table 5-3. Extractable organic carbon, ammonium (NH_4-N), labile organic nitrogen (ON), labile inorganic phosphorus (LabilePi) and labile organic phosphorus (LabilePo) concentrations in sediment profiles of the three lakes.

Lake	Site	Depth (cm)	Extractable (mg kg^{-1} dw)				
			Carbon	NH_4-N	ON	LabilePi	LabilePo
Annie	Central	5	784 ± 111	66 ± 15	112 ± 3	72 ± 13	50 ± 9
		10	808 ± 133	108 ± 8	120 ± 22	67 ± 12	43 ± 7
		15	779 ± 76	169 ± 42	102 ± 13	55 ± 6	40 ± 5
		20	646 ± 51	200 ± 67	89 ± 17	52 ± 9	35 ± 6
		30	803 ± 66	262 ± 109	113 ± 9	42 ± 8	28 ± 2
		45	722 ± 58	284 ± 111	106 ± 23	34 ± 10	22 ± 3
		60	768 ± 119	363 ± 160	82 ± 22	22 ± 0.3	19 ± 2
		80	771 ± 66	485 ± 245	88 ± 12	15 ± 3.8	16 ± 1
Okeechobee	M9	5	322 ± 46	20 ± 11	92 ± 17	110 ± 7	6 ± 4
		10	241 ± 11	71 ± 11	75 ± 7	87 ± 11	8 ± 1
		15	296 ± 24	86 ± 5	91 ± 21	127 ± 33	7 ± 4
		20	236 ± 39	78 ± 13	68 ± 2	55 ± 7	4 ± 1
		30	249 ± 52	63 ± 7	86 ± 17	80 ± 6	2 ± 0
		45	259 ±36	64 ± 5	84 ± 14	55 ± 8	3 ± 1
		60	355 ± 21	66 ± 3	85 ± 16	46 ± 3	2 ± 1
		70	355 ± 84	51 ± 12	74 ± 9	36 ± 2	1 ± 1
	M17	5	714 ± 91	14 ± 2	118 ± 10	14 ± 6	5 ± 0.5
		10	1193 ± 199	27 ± 4	163 ± 16	4 ± 0.3	4 ± 0.4
		15	1337 ±80	35 ± 4	170 ± 25	3 ± 1	3 ± 0.5
		20	1620 ± 12	38 ± 2	187 ± 9	3 ± 0.4	4 ± 0.7
		30	1613 ± 231	48 ± 4	199 ± 18	4 ± 0.3	4 ± 0.8
		40	1657 ± 257	49 ± 2	194 ± 19	3 ± 0.4	3 ± 0.6
	KR	5	52 ± 27	6 ± 5	20 ± 9	4 ± 1	0.4 ± 0.3
		10	73 ± 22	10 ±5	18 ± 6	7 ± 2	1 ± 0.5
		15	79 ±31	8 ± 4	14 ± 2	3 ± 2	0.4 ± 0.3
		20	232 ± 27	28 ±13	34 ± 3	3 ± 1	1 ± 0.4
		30	206 ± 26	43 ± 7	40 ± 5	2 ± 1	1 ± 0.2
		40	89 ± **	14 ± **	15 ± **	1 ± **	0.3 ± **
Apopka	West	5	2670 ± 118	104 ± 41	419 ± 74	2 ± 0.4	15 ± 8
		10	2243 ± 311	234 ± 73	419 ± 106	1 ± 0.3	19 ± 7
		15	2024 ± 267	340 ± 89	384 ± 65	19 ± 2	25 ± 7
		20	1638 ± 164	377 ± 97	351 ± 58	20 ± 5	24 ± 13
		30	1804 ± 228	427 ± 74	349 ± 45	28 ± 8	15 ± 5
		45	1505 ± 216	449 ± 28	275 ± 29	37 ± 3	11 ± 7
		60	1587 ± 82	464 ± 15	243 ± 59	34 ± 15	16 ± 8
		80	1675 ± 188	534 ± 68	214 ± 22	42 ± 8	7 ± 2
		98	1300 ± **	665± **	228 ± **	61 ± **	1 ± **

Table 5-4. Microbial biomass carbon, nitrogen and phosphorus concentrations in sediment profiles of the three lakes. (mean ± SD). ** No replicates for SD calculation.

Lake	Site	Depth (cm)	Microbial Biomass (mg kg^{-1} dw)		
			Carbon	Nitrogen	Phosphorus
Annie	Central	5	5419 ± 195	282 ± 12	78 ± 9
		10	5089 ± 166	283 ± 38	64 ± 18
		15	4387 ± 218	172 ± 7	44 ± 3
		20	4205 ± 177	106 ± 19	37 ± 5
		30	3919 ± 405	65 ± 13	30 ± 7
		45	3652 ± 692	60 ± 3	24 ± 10
		60	3401 ± 624	41 ± 2	22 ± 10
		80	2740 ± 422	20 ± 7	18 ± 6
Okeechobee	M9	5	3821 ± 479	122 ± 2	50 ± 2
		10	3672 ± 187	115 ± 11	34 ± 5
		15	3465 ± 231	89 ± 14	28 ± 8
		20	2773± 205	64 ± 22	18 ± 2
		30	2616± 283	55 ± 10	11 ± 2
		45	2361± 164	42 ± 5	9 ± 1
		60	2177± 92	35 ± 8	8 ± 0.4
		70	1983 ± 437	33 ± 6	2 ± 1
	M17	5	3800 ± 437	75 ± 23	6 ± 0.6
		10	3811 ± 370	72 ± 5	5 ± 0.3
		15	3746 ± 873	66 ± 11	3 ± 1
		20	4667 ± 461	97 ± 21	3 ± 0.8
		30	5128 ± 447	100 ± 25	2 ± 0.8
		40	3354 ± 988	43 ± 11	2 ± 1
	KR	5	574 ± 249	4 ± 4	1 ± 0.5
		10	653 ± 227	12 ± 4	1 ± 0.2
		15	644 ± 220	15 ± 7	1 ± 0.6
		20	1446 ± 335	39 ± 14	2 ± 0.3
		30	1296 ± 113	26 ± 11	1 ± 0.9
		40	638 ± **	20 ± **	1 ± **
Apopka	West	5	36617 ± 3193	2630 ± 294	598 ± 17
		10	32926 ± 5437	2469 ± 278	596 ± 85
		15	30486 ± 3924	2284 ± 366	616 ± 13
		20	22265 ± 5640	1863 ± 55	523 ± 115
		30	19355 ± 4608	1731 ± 63	399 ± 163
		45	14725 ± 4586	804 ± 91	267 ± 224
		60	11037 ± 4291	479 ± 72	106 ± 76
		80	9584 ± 1273	85 ± 18	31 ± 11
		98	8011 ± **	67 ± **	52 ± **

Table 5-5. Water extractable dissolved organic carbon (DOC), dissolved reactive P (DRP), and ammonium-N (NH_4^+) concentrations at time 0 (before incubation) and time 10 (after incubation). Results of *t* test of incubation experiment, significant differences ($p < 0.05$) between T = 0 vs. T = 10 are in bold ($n = 3$ and $df = 2$ for all analysis).

Lake	Site	Depth (cm)	Water Extractable (mg kg^{-1})					
			DOC		DRP		NH_4-N	
			T=0	T=10	T=0	T=10	T=0	T=10
Annie	Central	5	**499**	**1225**	1.4	1.1	80	134
		10	430	875	0.7	1.5	111	119
		15	1022	370	1.5	1.0	135	137
		20	**1264**	**238**	2.9	1.4	141	154
		30	3298	1916	7.7	6.9	106	108
		45	**4268**	**1473**	**10.0**	**2.4**	100	93
		60	**4693**	**1545**	**7.5**	**1.6**	87	79
		80	**4332**	**382**	9.1	2.0	159	109
Okeechobee	M9	5	424	229	2.35	1.8	**28**	**43**
		10	262	258	**0.8**	**1.2**	40	40
		15	278	316	0.8	1.4	42	38
		20	204	291	0.8	1.7	35	27
		30	201	154	0.5	2.5	**28**	**25**
		45	279	225	0.6	1.0	29	25
		60	275	607	1.4	2.1	27	21
		70	**447**	**637**	4.9	4.3	**26**	**12**
	M17	5	**459**	**334**	**0.9**	**2.5**	**9**	**19**
		10	**854**	**458**	**0.3**	**1.4**	**10**	**17**
		15	**1219**	**723**	**0.2**	**1.1**	**12**	**16**
		20	1559	955	**0.3**	**1.1**	15	16
		30	1648	1376	**0.3**	**1.3**	**16**	**21**
		40	2010	1193	**0.3**	**0.8**	**17**	**20**
	KR	5	34	26	0.03	0.09	1	9
		10	29	35	0.03	0.07	3	3
		15	53	49	0.08	0.07	4	6
		20	212	208	0.01	0.07	10	10
		30	244	178	0.03	0.06	9	5
Apopka	West	5	**2020**	**2963**	1.3	2.4	**378**	**1042**
		10	1422	2267	0.6	1.3	**548**	**1159**
		15	**1171**	**1822**	0.5	0.9	**731**	**1178**
		20	**1072**	**1679**	0.4	0.7	**788**	**1120**
		30	**1003**	**1438**	2.8	2.2	**762**	**1041**
		45	819	1347	8.2	10.4	**636**	**865**
		60	**642**	**1412**	12.2	14.4	**457**	**667**
		80	**733**	**1509**	**13.3**	**25.0**	**466**	**666**

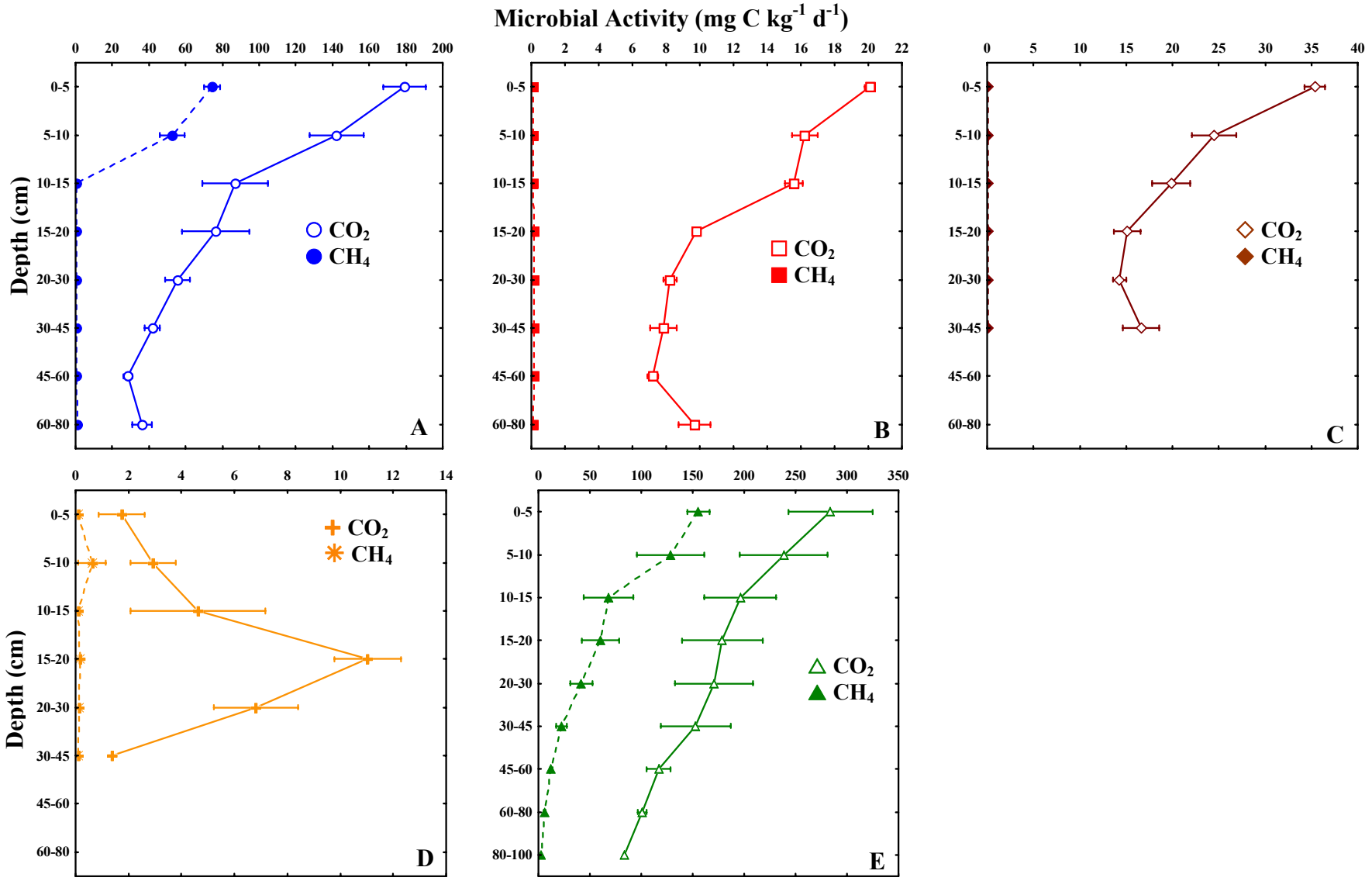


Figure 5-1. Microbial activity (CO_2 and CH_4 production rates) in sediments from: A) Lake Annie, B) Lake Okeechobee: M9, C) M17, D) KR, and E) Lake Apopka. Bars represent standard errors.

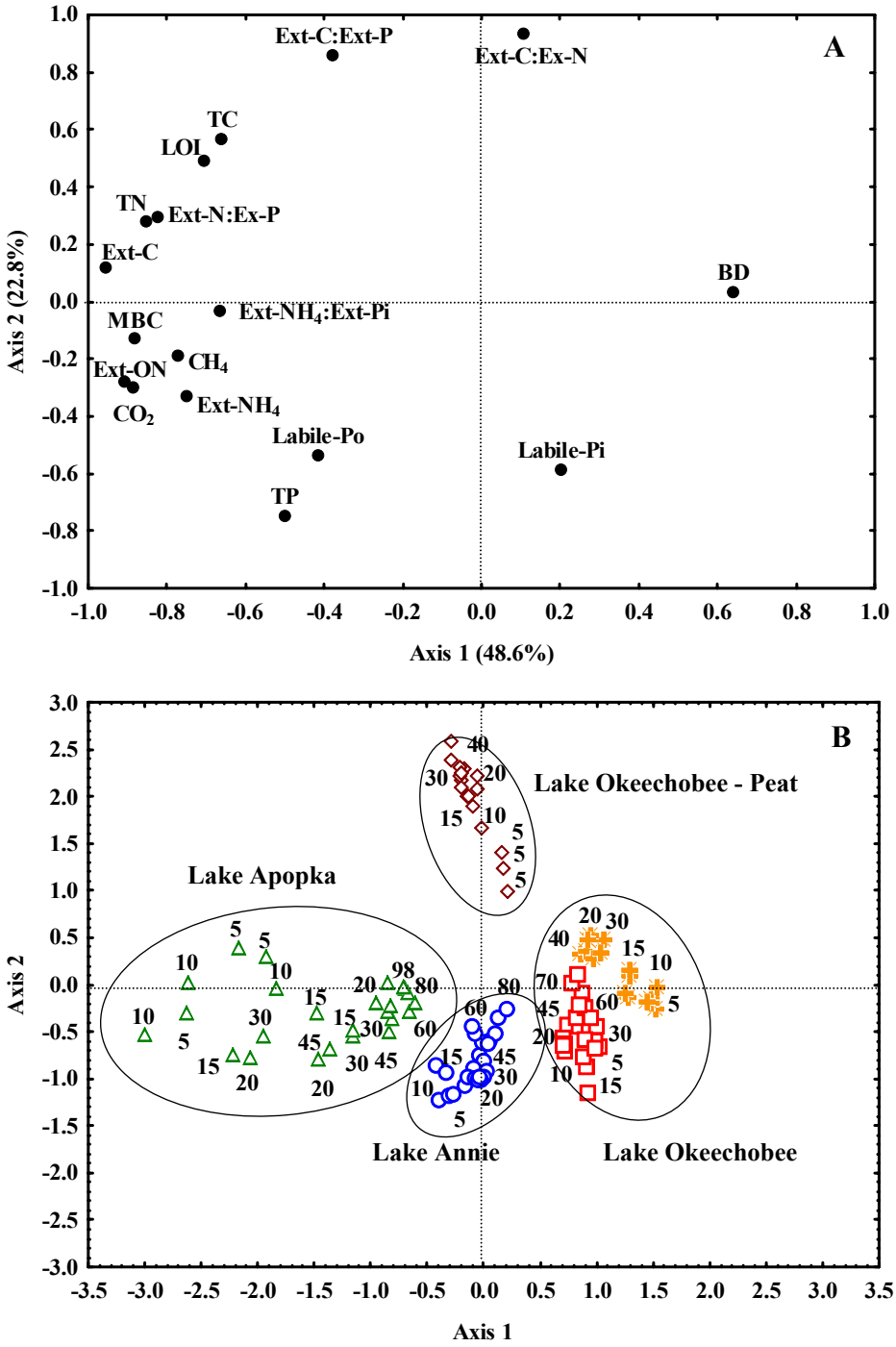


Figure 5-2. Results of the Principal Component Analysis: A) loadings of sediment variables (n = 107), and B) the plot of the scores of the sites and sediment depth (numbers cm) from Lake Annie (blue circles), Lake Okeechobee: M9 (red squares), M17 (brown diamonds), KR (orange crosses), and Lake Apopka (green triangles).BD: bulk density, LOI: loss on ignition, TC: total carbon, Ext-C: extractable organic carbon, TN: total nitrogen, Ext-N: extractable labile nitrogen, Ext-NH4-N: extractable ammonium, Ext-ON: extractable labile organic nitrogen, TP: total phosphorus, Ext-P: extractable labile phosphorus, Ext-Pi: labile inorganic phosphorus, Ext-Po: labile organic phosphorus, MBC: microbial biomass carbon, CO_2: anaerobic respiration, and CH_4: methane production rates.

CHAPTER 6
NUTRIENT ACCUMULATION AND STABLE ISOTOPE SIGNATURES IN SEDIMENTS OF SUBTROPICAL LAKES

Introduction

Organic matter (OM) that enters a lake from the watershed (allochthonous) or is produced within the lake (autochthonous) itself will be deposited on the lake bottom and incorporated into the sediments. Lakes function as natural traps for OM and associated nutrients. Lake sediments contain an archive of past environmental conditions in and around the water body (Smol 1992) and can be used to document anthropogenic impacts through time (Smeltzer and Swain 1985). Sediment OM provides information about past impacts and biogeochemical processes within lakes, and has been studied extensively using paleolimnological methods (Meyers 1997). The timing of past events in a basin is based on reliable dating of sediment cores. Sediment dating provides an age/depth relation from which bulk sediment accumulation rates can be calculated (Smeltzer and Swain 1985). The lead-210 (^{210}Pb) technique is used routinely to provide age/depth relations for the last 100-150 years (Appleby et al. 1986), and has been used widely in studies of Florida lake sediment cores (e.g., Binford and Brenner 1986; Brezonik and Engstrom 1998; Whitmore et al. 1996; Brenner et al. 2006; Schottler and Engstrom 2006). Bulk sediment accumulation rates in combination with analyses of sediment composition, can be used to calculate accumulation rates of sediment constituents such as OM and nutrients. Such measures provide insights into past changes in productivity and human impacts on the aquatic ecosystem.

Nutrient and OM accumulation rates in sediment have been studied in conjunction with stable isotope analyses (δ^{13}C and δ^{15}N) to infer past environmental impacts in marine (e.g., Gearing et al. 1991; Savage et al. 2004), lacustrine (e.g., Schelske and Hodell 1991; Gu et al. 1996; Bernasconi et al. 1997; Hodell and Schelske 1998; Ostrom et al. 1998; Brenner et al. 1999), and riverine ecosystems (e.g., McCallister et al. 2004; Anderson and Cabana 2004; Brunet

et al. 2005). Measurements of $\delta^{13}C$ and $\delta^{15}N$ in several lake compartments, (i.e., dissolved and particulate matter in the water column and sediments) have been used to identify the origin of lacustrine OM (Filley et al. 2001; Griffths et al. 2002), infer past primary productivity (Schelske and Hodell 1991; Hodell and Schelske 1998; Bernasconi et al. 1997), document historical eutrophication (Gu et al. 1996; Ostrom et al. 1998; Brenner et al. 1999), elucidate biogeochemical cycles (Terranes and Bernasconi 2000; Jonsson et al. 2001; Lehmann et al. 2004), and shed light on microbial activity (Hollander and Smith 2001; Lehmann et al. 2002; Gu et al. 2004; Terranes and Bernasconi 2005; Kankaala et al. 2006).

Allochthonous OM usually has more negative $\delta^{13}C$ values than does autochthonous OM. Values of $\delta^{13}C$ can also be used to distinguish periods of high versus low primary productivity. Algae fractionate against the heavier isotope, ^{13}C. Consequently, under conditions of low to moderate primary productivity autochthonous OM displays very negative $\delta^{13}C$. During periods of very high primary productivity the preferred ^{12}C in the water column is exhausted and fractionation is diminished, yielding OM with higher $\delta^{13}C$ (Mizutani and Wada 1982; Raul et al. 1990). Hypereutrophic lakes with high rates of primary productivity have low concentrations of carbon dioxide (CO_2) in the water column. Moreover, in alkaline (high-pH) waters bicarbonate (HCO_3^-) dominates the dissolved inorganic C, and has a $\delta^{13}C$ that is 8‰ heavier than dissolved CO_2 (Fogel et al. 1992). High demand for inorganic C and low free CO_2 leads to utilization of HCO_3^- as a C source resulting in heavier $\delta^{13}C$ of OM (Goericke et al. 1994).

Stable isotope signatures of sediment OM can sometimes be used to identify impacts of anthropogenic activities. Wastewater and agricultural runoff can be identified because they yield OM depleted in $\delta^{13}C$ and enriched in $\delta^{15}N$ (Gearing et al. 1991; Burnett and Schaffer 1980; Savage et al. 2004). Stable isotope $\delta^{15}N$ has also been used to study the nitrogen (N)

biogeochemical cycle. Measurement of $\delta^{15}N$ in suspended and sedimented OM was used to address the source of N, as well as N limitation of, and utilization by the phytoplankton community in Lake Lugano (Terranes and Bernasconi 2000).

In summary, sediment OM and nutrient content, along with $\delta^{13}C$ and $\delta^{15}N$ signatures, have proven useful to identify the origin of OM, infer past lake productivity, and understand mineralization processes in lakes. The objectives of this study were to: (i) determine sediment accumulation rates, and (ii) determine $\delta^{13}C$ and $\delta^{15}N$ signatures of OM in sediment cores from subtropical lakes of different trophic states.

Material and Methods

Study Sites

Three Florida (USA) lakes ranging in trophic state were selected. Lake characteristics were described in Chapter 2. The characteristics and location of sampled sites and field sampling procedures were described in Chapter 3 (Table 3-1, Figure 3-1). Sectioning of the cores (Set # 1) was described previously (Chapter 3).

One additional core was collected at each site for isotope analyses and ^{210}Pb dating (Set # 2). Sediment cores from Lake Annie (60 cm maximum depth), Lake Apopka (72 cm maximum depth) and Lake Okeechobee site M9 (73 cm maximum depth) were sectioned at 4-cm intervals. Sediment cores from Lake Okeechobee sites M17 (36 cm maximum depth) and KR (16 cm maximum depth) were sectioned at 2-cm intervals. All sediment variables are reported on a dry weight basis (dw). Water quality variables were described in a previous study (Chapter 4).

Sediment Properties

Samples were transported on ice and stored in the dark at 4 ºC. Total phosphorus (TP) total carbon (TC), and total nitrogen (TN) of core Set # 1 were measured and described in previous studies (Chapter 3, 5).

Sediment samples (Set # 2) for ^{210}Pb dating and isotopic analyses were dried in a Virtis Unitrap II freeze drier. Sediment bulk density (g dry cm^{-3} wet) was determined on a dry weight basis (weight before and after freeze-drying). Dried samples were ground in a mortar and pestle and passed through a 2.0-mm mesh sieve. Organic matter content (LOI-loss on ignition) was determined by weight loss at 550ºC.

Isotopic Analyses

Sediment samples for organic C isotope analysis were pretreated with acid to remove inorganic C (carbonates) (Harris et al. 2001). Samples were weighed in silver capsules, placed in the wells of a microtiter plate, and 50 µL of DI water was added to moisten the sediment. Plates were placed in a vacuum desiccator with 100 mL of concentrated HCl, and exposed to HCl vapor for 24 hours. Samples were dried at 60 ºC for 4 hours to remove any remaining HCl. Carbon (organic) and nitrogen (total) isotope values were determined using methodology described by Inglett and Reddy (2006). Isotope analyses were conducted using a Costech Model 4010 Elemental Analyzer (Costech Analytical Industries, Inc., Valencia, CA) coupled to a Finnigan MAT Delta^{Plus}XL Mass Spectrometer (CF-IRMS, Thermo Finnigan) via a Finnigan Conflo II interface. Stable isotope results are expressed in standard delta notation, with samples measured relative to the Pee Dee Belemnite for C and atmospheric N_2 for N. Analytical accuracy and precision were established using known isotopic standards (wheat flour, $\delta^{13}C$ = -26.43 ‰, $\delta^{15}N$ = 2.55 ‰, Iso-Analytical; IAEA-N1, $\delta^{15}N$ = 0.4 ‰; ANU-Sucrose, $\delta^{13}C$ = -10.5 ‰). Analytical precision for standards was less than ± 0.1‰ for $\delta^{13}C$ and ± 0.3‰ for $\delta^{15}N$.

^{210}Pb Dating

Lead-210 dating was done by gamma counting (Appleby et al. 1986; Schelske et al. 1994). Samples were placed in plastic Sarstedt^{TM} tubes to a height of ~ 30 cm. Sample mass was determined and tubes were sealed with epoxy glue and set aside for 3 weeks to allow ^{214}Bi and

^{214}Pb to equilibrate with *in situ* ^{226}Ra. Radioisotope activities were measured using ORTEC™ Intrinsic Germanium Detectors connected to a 4096 channel, multichannel analyzer. Total ^{210}Pb activity was obtained from the photopeak at 46.5 kilo electron volts (keV). Supported ^{210}Pb activity, expressed as ^{226}Radium activity, was estimated by averaging activities of ^{214}Pb (295.1 keV and 351.9 keV) and ^{214}Bi (609.3 keV). Cesium-137 activity was determined from the 662 keV photopeak. Unsupported ^{210}Pb activity was estimated by subtraction of supported activity from the total activity measured at each level. Activities are expressed as decays per minute per gram of dry sediment (dpm g^{-1}). Sediment ages and bulk sediment accumulation were calculated using the constant rate of supply (CRS) model (Appleby and Oldfield 1978, 1983). Lead-210 dates correspond to the base of each sediment section. In all cores but the one from Lake Annie, radioisotope activity was measured in all samples from the sediment water interface to the base of the section. The 2-4 cm portion of the Lake Annie core was lost during extrusion so interpolated values for bulk density and activities were used to compute dates. This is thought to have introduced negligible error as the topmost 12 cm have nearly identical bulk densities and activity values.

Results and Discussion

Core Chronology

Lake Annie

In Lake Annie, total ^{210}Pb activity declined with increasing sediment depth. ^{226}Ra activity, i.e., supported ^{210}Pb activity, varied from 2.9 ± 0.5 dpm g^{-1} in surface sediments to 2.1 ± 0.6 dpm g^{-1} in deeper sediments. Cesium-137 activity declined with sediment depth and showed no distinct peak (Figure 6-1A). Chronologies determined with the CRS model yield reasonably precise dates from c. 1900 (Figure 6-1B). The ^{210}Pb results of the current study are similar to those reported by Schottler and Engstrom (2006) for Lake Annie. The average sedimentation rate

(since c.1900) was 36.8 mg cm^{-2} yr^{-1} while Schottler and Engstrom (2006) reported a value of 34 mg cm^{-2} yr^{-1}. Lake Annie's sedimentation rate and organic matter accumulation rates varied slightly through time (Figure 6-1C). Sediment accumulation generally increased from late 1800 until ~ 1940, then decreased through 1970's. Over the past several decades, the sedimentation rate has increased. Lake Annie water inputs are from ground water (90%) and atmospheric deposition (10%) (Swain and Gaiser 2005). This lake has no natural surface streams but two shallow man made ditches flow into the lake along the south and southeast sides. Surface runoff from these ditches was reported to contribute to water and nutrient input to the lake during high rainfall periods (Battoe 1985). Shifts in historic sedimentation rates may reflect changing inputs of allochthonous OM and nutrients from the surrounding landscape. Water column characteristics in Lake Annie have experienced profound changes in the last 10 years. The lake has transformed from a clear-water system to a water body with appreciable dissolved color. The increase in color was probably due to high dissolved organic carbon (DOC) input to the lake from adjacent land (Swain and Gaiser 2005).

Most of the water input to the lake is from groundwater, and the source of DOC to this lake is allochthonous. The increase in color has been accompanied by a decrease in Secchi disk depth and dissolved oxygen, while pH has increased. No changes were recorded in electrical conductivity, and slight increases in N and P as well as chlorophyll-*a* have been detected in the past 20 years (Swain and Gleiser 2005). The increase of Lake Annie's sedimentation rate and OM accumulation rates are probably related to the increase in allochthonous DOC input.

Lake Okeechobee

In Lake Okeechobee site M9, activities of ^{210}Pb, ^{226}Ra, and ^{137}Cs could only be detected in near-surface sediments (Figure 6-2A). In sediments at sites M17 and KR, activity values were below detection limits suggesting the sites were non-depositional, which precluded dating.

Brezonik and Engstrom (1998) dated 11 cores from the Lake Okeechobee mud zone and two cores from the peat zone collected in 1998. In 2003, Schottler and Engstrom (2006) and Engstrom et al. (2006) re-sampled three sites in the mud zone that were previously dated (Brezonik and Engstrom 1998). Using ^{210}Pb dating models and ^{137}Cs, the authors concluded that Lake Okeechobee sediments preserve a reliable statigraphic history of the lake. Schottler and Engstrom (2006) concluded, however, that^{210}Pb dating of Okeechobee sediments is problematic, not very precise, with error terms for the last half century ca. ± 10 years. Furthermore, in September 2004, the eyes of two hurricanes, Frances and Jeanne, passed to the north of lake. Strong winds generated a large surface seiche in the lake (Chimmey 2005). Lake stage rose abruptly, by about 3.06 m during hurricane Frances, and 4.91 m during hurricane Jeanne. Peak wind velocity reached hurricane strength at platforms over the center of the lake (144 km h^{-1}) (Chimmey 2005).

Because Lake Okeechobee is large and shallow, its sediments are easily disturbed by wind, especially in the mud zone (Havens et al. 2007). Havens et al. (2007) reported the impact of Hurricane Irene on Lake Okeechobee. The storm passed 80 km south of the lake in October 1999 and produced maximal winds of 90 km h^{-1} over the center of the lake. Mean pelagic TP increased from 88 to 222 μg L^{-1}, and it is estimated that more than 10,000 metric tons of fine-grained mud sediment was resuspended during the storm (Havens et al. 2007). Considering that the 2004 hurricanes passed closer to the lake, and had higher winds than Hurricane Irene, it is very likely the storms caused substantial sediment resuspension and deposition. I sampled just 10 months after the storms. Extensive sediment translocation may explain why the cores I took were undatable. Alternatively, the sites I selected for samples may simply be inappropriate. Lake

Okeechobee displays heterogeneous sediment distribution, and not all locations yield datable cores (Schottler and Engstrom 2006).

Lake Apopka

Total ^{210}Pb activity remained relatively constant over the topmost 44 cm of the Lake Apopka core (Figure 6-2B). Furthermore, total ^{210}Pb values exceeded ^{226}Ra activities in the base of the section, suggesting there was still unsupported ^{210}Pb at the bottom of the core. The fairly constant total ^{210}Pb activity in the upper 44 cm of the core may be explained by two processes: 1) incoming unsupported ^{210}Pb is being diluted by higher and higher sediment accumulation rates, the increase in deposition keeping pace with the ^{210}Pb decay, or 2) uppermost sediments are mixed by physical or biological action, yielding fairly constant activities throughout the section. Waters et al. (2005) also reported failure to date Lake Apopka sediments. The CRS model assumes constant input of excess of ^{210}Pb through time. In some large, shallow lakes in Florida, resuspension and focusing of organic sediments may lead to violations of the dating model assumptions (Whitmore et al. 1996). Furthermore, land use and hydrological changes, including reduction of lake surface area as surrounding wetlands were cleared for agriculture, and construction of the Apopka-Beauclair Canal, both lowered lake stage by ~ 1 m and established a permanent outflow, which caused conditions that violated the assumptions of the CRS ^{210}Pb dating model (Schelske et al. 2005; Waters et al. 2005). Cesium-137 showed a slight peak at 48-52 cm depth (Figure 6-4B). A ^{137}Cs peak can sometimes be used to identify the period of maximum cesium fallout, from atomic bomb testing around 1963 (Krishnaswami and Lal 1978) and may be used to verify ^{210}Pb dates (Schelske and Hodell 1995). Neither ^{210}Pb nor ^{137}Cs yielded a reliable chronology. The 1963 ^{137}Cs peak is absent or preserved poorly in many Florida lakes as Cs is poorly bound and may move in the sediment column (Brenner et al. 1994, 2004; Schelske et al. 1994).

Carbon ($\delta^{13}C$) and Nitrogen ($\delta^{15}N$) Isotope Signatures

Lake Annie

Lake Annie sediment TC:TN ratios increased with sediment depth, while TN:TP decreased to ~ 50 cm and then increased again (Figure 6-3B, C). Both $\delta^{13}C$ and $\delta^{15}N$ values are depleted towards the sediment surface, varying from -28.2 ‰ to -29.4 ‰ and 2.0‰ to 0.9‰ (Figure 6-3D, E). Surface sediments are depleted relative to basal deposits by -1.24 ‰ ($\delta^{13}C$) and -1.13‰ ($\delta^{15}N$). Organic matter content (LOI %) decreased with sediment depth (Figure 6-3F). Statigraphic isotopic signatures of Lake Annie sediments indicate that this lake is going through changes in recent years. Statigraphic decrease in TC:TN ratios towards the sediment surface indicates contribution of autochthonous OM to sediments in Lake Annie. However, in recent years, lake productivity has slightly changed, but other changes, such as increase in DOC and color, occurred (Swain and Gleiser 2005).

Isotopic signatures of $\delta^{13}C$ and $\delta^{15}N$ of Lake Annie sediments probably resulted from a combination of several factors such as allochthonous OM input, primary productivity, and microbial biomass and activity. Small, oligotrophic lakes are expected to have relatively a high proportion of allochthonous C input to their sediments (Gu et al. 1996). Terrestrial C_3 plants discriminate against ^{13}C, and organic matter derived from land plants typically have $\delta^{13}C$ values of -27‰ to -29‰ (Bird et al. 1994, Meyers 1997).

Hammarlund et al. (1997) related successive depletion of ^{13}C in Lake Tibetanus (Sweden) with changes in the input of allochthonous material from surrounding vegetation. Moreover, Jonsson et al. (2001) reported very negative $\delta^{13}C$ values of dissolved C in a humic lake (Lake Örträsket, Sweden), resulting from the mineralization of allochthonous organic matter. Phytoplankton also discriminate against ^{13}C in the water column when CO_2 concentration is high, which is expected in this lake with low pH. Consequently, autochthonous organic matter is

also expected to be depleted in ^{13}C. The heterotrophic microbial community can also contribute to depleted values of $\delta^{13}C$.

Heterotrophic uptake of DOC preserves the C isotopic signature of the source, and biomass associated with chemoautrotrophic and methanotrophic microorganisms is generally depleted in ^{13}C (Conway et al. 1994; Kelley et al. 1998). The isotopic study on sediments from Lake Mendota (Wisconsin) illustrated that mineralization of C by the heterotrophic microbial community associated with expansion of anoxic conditions in the water column resulted in low $\delta^{13}C$ values in sediments (Hollander and Smith 2001). Moreover, seasonal and long term increases in contribution of depleted microbial biomass to sediments results in depleted values in the $\delta^{13}C$ (Hollander and Smith 2001). Lehmann et al. (2002) reported that ^{13}C depleted OM of sinking particles and sediments resulted from anaerobic decomposition in Lake Lugano (Swiss-Italian border). Terranes and Bernasconi (2005) associated the $\delta^{13}C$ of sedimentary OM in Lake Baldeggersee (Switzerland) to variation of relative inputs of eukaryotic biomass, which is enriched in ^{13}C and the contribution of microbial biomass, depleted in ^{13}C, which is produced in the expanding anoxic bottom waters. In Lake Annie the thermocline has been detected to be moving to shallower depths (i.e., higher) in the water column during thermal stratification, with anoxia below 5 m depth (Swain and Gaiser 2005). High sulfate reduction has been reported to occur in the anoxic layers of the water column (Swain and Gaiser 2005). Increased anoxia in the water column can be leading to increased anaerobic decomposition of already depleted suspended OM, and both depleted microbial biomass and OM will eventually reach the sediment.

The same processes are affecting N isotopic signatures. Plants tend to fractionate against ^{15}N during inorganic N uptake (Handley and Raven 1992). Allochthonous organic matter derived

from C_3 land plants has $\delta^{15}N$ is around +0.4‰ (Peterson and Howarth 1987). Autochthonous OM of aquatic ecosystems not dominated by cyanobacteria has a $\delta^{15}N$ signature of +8.6‰ (Peterson and Howarth 1987), while N_2 fixation by N-fixing algae yields values near 0‰ (Meyers 1997). Nitrogen fixation is also found in several microorganisms such as aerobic and anaerobic heterotrophic bacteria, methane oxidizing bacteria, sulfate reducers, among others (Siegee 2004). Nitrogen-fixing algae have not been detected in Lake Annie (Gaiser personal communication), however, N_2-fixation can be present and carried out by the heterotrophic microbial community. The N transformations can substantially modify organic matter signatures (Meyer 1997). High N availability in the Lake Annie water column (Table 4-2) can lead to autochthonous organic matter with depleted $\delta^{15}N$ as it will allow greater algae discrimination against ^{15}N (Meyers 1997). Nitrogen availability is also high in these sediments (Chapter 5). Allochthonous OM, primary productivity as well as heterotrophic microbial community in this lake can produce the $\delta^{13}C$ and $\delta^{15}N$ isotopic signatures seen in these sediments (Figure 6-9A).

The data presented here do not allow a clear separation of the main factors influencing isotopic signatures in Lake Annie sediments. Depleted allochthonous particulate and dissolved OM can be the major source of C in the lake, and is mineralized and utilized by the microbial community that will have depleted microbial biomass. Depleted end products, such as CO_2, NH_4^+ resulting from heterotrophic metabolism will be utilized by primary producers that will also produce a depleted autochthonous OM. A more detailed study of $\delta^{13}C$ and $\delta^{15}N$ isotopes in several compartments, i.e., dissolved C, different N compounds, phytoplankton biomass, bacteria biomass, particulate OM in the water column and sediment, of this lake, can elucidate major processes affecting the isotopic signatures in Lake Annie sediments.

Lake Okeechobee

Mud zone sediments (site M9) in Lake Okeechobee showed variable TC:TN ratios over the length of the core. From 70 cm depth TC:TN ratios rose until 50 cm, suggesting increased input of allochthonous material, or N loss to mineralization (Figure 6-4B). At 50 and 30 cm the ratio was similar, and from 30 cm to the surface there was a general decrease in TC:TN. Organic matter content was highly variable over the length of the core, however, there was an increase in OM content in surface sediments (Figure 6-4F). TN:TP showed a general decrease from the bottom of the core to the surface, reflecting more rapid increase of TP than TN concentration (Figure 6-4C). The same trend was reported by Engstrom et al. (2006) for the mud zone and was attributed to increase in TP content of these uppermost sediments, as a result of the eutrophication process. The $\delta^{13}C$ sediment profile showed a similar pattern of TC:TN ratios (Figure 6-4B, D). Delta ^{13}C values varied from -26.0‰ to -29.9‰ with surface sediments only slightly depleted (0.15‰) relative to bottom deposits (Figure 6-4D). Delta ^{15}N varied from 2.6‰ to 3.9‰ and showed ~ 1.3‰ enrichment in surface deposits relative to bottom deposits (Figure 6-4E). A similar pattern, i.e., depletion of $\delta^{13}C$ and enrichment of $\delta^{15}N$, was reported by Rosenmeier et al. (2004) in a study of recent eutrophication of Lake Petén Itzá, Guatemala, in which changes were related to sewage input (depleted in $\delta^{13}C$ and enriched in $\delta^{15}N$) and increased presence of cyanobacteria. Engstrom et al. (2006) also found $\delta^{15}N$ enrichment (1‰) in the mud zone, but did not discuss the mechanisms responsible.Stratigraphic changes in $\delta^{13}C$ and $\delta^{15}N$ in the mud zone are probably controlled by autochthonous OM, availability and demand for C and N and varying intensities of mineralization. Lake Okeechobee mud zone sediments are probably C and N limited and N demand is high in these sediments (Chapter 5). In the mud zone of Lake Okeechobee, light is the most limiting factor of the phytoplankton community during most of the year (Aldridge et al. 1995). During summer months, light limitation is relaxed and N

becomes the limiting factor for the phytoplankton community (Aldridge et al. 1995). The $\delta^{15}N$ of sediments in eutrophic and hypereutrophic lakes can be influenced by N_2-fixation by cyanobacteria (Gu et al. 1996; Rosenmeier et al. 2004). These cyanobacteria do not fractionate against ^{15}N and have $\delta^{15}N$ similar to atmospheric N (~0‰) (Peterson and Fry 1987). Non-N_2 fixing cyanobacteria, however, typically dominate the phytoplankton community and the N_2 fixation rate is low in the central area of Lake Okeechobee (Cichra et al. 1995; Gu et al. 1997; Phlips et al. 1997). Nitrogen limitation can lead to autochthonous organic matter with enriched $\delta^{15}N$ as algae discrimination against ^{15}N will be diminished (Meyers 1997). As a consequence autochthonous OM is expected to have an enriched $\delta^{15}N$ signature (Peterson and Howarth 1987).

Although some studies indicate that the isotopic signature of OM is resistant to alteration during water-column or post-burial diagenesis (Meyers and Eadie 1993; Schelske and Hodell 1995; Hodell and Schelske 1998; Terranes and Bernasconi 2000), others have shown that selective degradation of OM fractions change isotopic signatures (Bernasconi et al. 1997; Meyers 1997; Lehmann et al. 2002, 2004). Labile carbohydrates, proteins and amino acids are generally more enriched in ^{13}C, while lipids and cellulose are lighter (Meyers 1997). Selective loss of "heavy" amino acids, proteins and carbohydrates, which are particularly susceptible to microbial degradation, leaves residual (substrate) OM isotopically lighter, with respect to $\delta^{13}C$, than the original material (Hedges et al. 1988).

Loss of high $\delta^{15}N$ compounds (e.g., amino acids) can also occur, lowering the $\delta^{15}N$ in residual material. Nevertheless, decomposition of OM is generally thought to increase $\delta^{15}N$ through preferential loss of ^{14}N (Nadelhoffer and Fry 1988). Bernasconi et al. (1997) reported shifts in the $\delta^{13}C$ (depletion) and $\delta^{15}N$ (enrichment) of sinking OM in Lake Lugano, which they attributed to selective removal of C and N compounds during mineralization. Additionally,

sediment $\delta^{13}C$ in Lake Lugano indicates overall isotopic depletion during early sedimentary diagenesis (Lehmann et al. 2002). In the Lake Okeechobee mud zone, phytoplankton community and N limitation, high demand for C and N in sediments, and selective mineralization of OM probably influence $\delta^{13}C$ and $\delta^{15}N$ values in the sediments (Figure 6-9B).

Sediment OM content was highest in Lake Okeechobee site M17, reflecting its peat nature (Figure 6-5F). Sediment OM content decreased towards the sediment surface. Peat zone (site M17) TC:TN ratios increase downcore and are the highest values reported from sediment at all sites, reflecting their higher plant origin (Figure 6-5B). The TN:TP ratio declines above 30cm, reaching the lowest values at the sediment surface (Figure 6-5C). This pattern is driven by the high TP concentration in surface sediments (Figure 6-5A, C). The $\delta^{13}C$ of OM varied little in the core from site M17, from -26.7‰ to -26.3‰ (Figure 6-5D). With respect to $\delta^{15}N$, from 36 cm to 22 cm, values decline by about -0.6‰, but are followed by a period of enrichment of ~1.13‰ up to the sediment surface.

Stratigraphic changes in sediment in $\delta^{13}C$ and $\delta^{15}N$ from the peat zone probably reflect selective mineralization of OM. Small shifts in $\delta^{13}C$ of peat zone sediment may result from shifting intensity of mineralization. Sediments of the peat zone are probably C and P limited (Chapter 5). The demand for labile C is high in surface sediments (Chapter 5), and mineralization of C is reflected in the isotopic signature as well as in lower TC:TN ratios in surface sediments (Figure 6-5B, D). Values of $\delta^{15}N$ declined from about 1.4‰ to 0.7‰ from 36 to 22 cm, but rose again to a high of a little more than 1.8‰ at the surface. Contrary to the mud zone, the phytoplankton communities in the peat zone at the south end of the lake are dominated by large N_2-fixing cyanobacteria (Phlips et al. 1997). Nitrogen fixation rates can be high (Gu et al. 1997; Phlips et al. 1997), although NH_4^+ is the most important N source for phytoplankton

uptake (Gu et al. 1997). The depletion in $\delta^{15}N$ from 36 up to 22 cm may indicate high deposition of N_2-fixer biomass with low $\delta^{15}N$. With selective degradation of labile autochthonous OM, isotopically light $\delta^{15}N$ is removed and remaining material is enriched in ^{15}N. Ammonium-N concentration increases during anaerobic decomposition of OM (Chapter 5). Ammonium derived from OM decomposition is usually relatively depleted in ^{15}N (Terranes and Bernasconi 2000), while residual material is left relatively enriched. Isotopic signatures ($\delta^{13}C$ and $\delta^{15}N$) of sediment OM are related to several factors, including sediment origin (i.e., plant tissue), intensities of primary productivity and diagenesis (Figure 6-9C).

Sand zone (site KR) OM content and TC:TN ratios were low (< 16) (Figure 6-6B, F). Similar to the peat zone, TN:TP ratio increase with greater depth reflecting the decline in TP with depth in the core (Figure 6-6A, C). Sediment $\delta^{13}C$ values varied throughout the profile (-25.63‰ to -26.36‰) and were most depleted in ^{13}C near the sediment surface (Figure 6-6D). Nitrogen isotope values ($\delta^{15}N$) varied from ~ 0.63‰ to 4.22 ‰ and show general enrichment towards the sediment surface (Figure 6-6E). There are two periods where $\delta^{15}N$ declined (from 16-12 cm, and 4-0 cm), and where $\delta^{15}N$ increased (from 10-4 cm). Organic N mineralization is an important source of inorganic N in these sediments (Fisher et al. 2005).

The KR site differs from other sites in receiving greater influence from allochthonous material. The KR site is located near the location where the Kissimmee River flows into the lake. It is the largest inflow to Lake Okeechobee (31% of inflow), and carries a substantial nutrient load (Frederico et al. 1981; Aumen 1995). Agricultural activities, mainly dairies, are a principal non-point source of nutrients to the Kissimmee River, and are responsible for the nutrient enrichment of this lake (Aumen 1995; Reddy et al. 1995; Havens and Gawlik 2005). Other nutrient sources are sewage from treatment plants, septic tanks, urban runoff, and industrial

pollution (Aumen 1995; Whalen et al. 2002). The northern area of Lake Okeechobee is characterized by high intra-annual variability in chlorophyll-*a* concentration (Havens 1994; Phlips et al. 1995). Nitrogen limits primary productivity (Aldridge et al. 1995), and the phytoplankton community is dominated by large N_2-fixing cyanobacteria (Phlips et al. 1997), however, N_2-fixation is low (Gu et al. 1997; Phlips et al. 1997). Organic matter content in these sediments is low, and the contribution of autochthonous OM to isotopic signatures does not seem to be great at this site.

Wastewater and agricultural runoff are usually enriched in ^{15}N (Bedard-Haughn et al. 2003; Anderson and Cabana 2005), and sewage effluents are depleted in ^{13}C (Gearing et al. 1991). Other studies related the enrichment of $\delta^{15}N$ in Florida lakes sediments to agricultural runoff (Riedinger-Whitmore et al. 2005; Whitmore et al. 2006).

Depletion of ^{13}C and enrichment of ^{15}N, in sediments of Lake Petén Itzá (Guatemala) were related to sewage input (Rosenmeier et al. 2004). In a study of $\delta^{13}C$ distribution in sediments and food webs of estuaries, Gearing et al. (1991) reported that sewage C accumulated in sediments, and $\delta^{13}C$ value of impacted sites (-24.2‰) was significantly lower than the values from non-impacted sites (-21.6‰). In a study of a sewage dumpsite in the New York Bight, Burnett and Schaffer (1980) showed that OM from wastewater (-26.2‰) and from marine origin (-22.0‰) had distinct $\delta^{13}C$ signatures. Seasonality also can affect particulate organic carbon $\delta^{13}C$ values in rivers. During periods of high discharge in Sanaga River (Cameroon) $\delta^{13}C$ values are high, caused by an increase in the proportion of contribution of OM derived from C_4 plants from the further savanna region transported overland by wet season rains (Bird et al. 1994; Bird et al. 1998). In the dry season, when discharge is low, $\delta^{13}C$ values are low, reflecting OM derived primarily from C_3 plants growing close to the river bank (Bird et al. 1994; Bird et al. 1998).

Stratigraphic variation in $\delta^{13}C$ and $\delta^{15}N$ at the KR site probably reflects multiple factors as input of wastewater from anthropogenic activities, and variable contributions of river-borne allochthonous input, related to inter-annual rainfall variations (Figure 6-9D).

Lake Apopka

The Lake Apopka core displayed a narrow range of TC:TN values (~11.8-13.1). There was a general decrease from about 98-30 cm of depth, and then increase to the sediment surface (Figure 6-7B). The TN:TP ratio decreased toward the sediment surface is related to increase in TP concentration (Figure 6-7C). Organic matter $\delta^{13}C$ showed a general increase upward over the length of the core from -22.6‰ to -18.4‰ (Figure 6-7D). Nitrogen isotopic values also displayed an increasing trend upcore, from 3.9‰ (lower depths) to 4.7‰ (surface sediments) (Figure 6-7E). Organic matter content increased towards the sediment surface (Figure 6-7F).

Nitrogen availability is high in these sediments (Chapter 5). Nitrogen transformations in sediments of Lake Apopka include mineralization of organic N, NH_4^+ adsorption on sediment, nitrification, denitrification, and dissimilatory nitrate (NO_3^-) reduction (D'Angelo and Reddy 1993). In Lake Apopka sediments, nitrification is high in surface sediments with aerobic conditions, and dissimilatory NO_3^- reduction to NH_4^+, a respiratory process used by facultative and obligate anaerobic bacteria, is high in anaerobic sediments when the ratio of C/electron acceptors is high (D'Angelo and Reddy 1993). Lake Apopka had the highest $\delta^{13}C$ values and showed the greatest enrichment among studied sites. In a study of 83 Florida lakes that ranged in trophic state, Lake Apopka plankton had the highest $\delta^{13}C$ (Gu et al. 1996). Greater $\delta^{13}C$ towards the surface probably indicates increased in primary productivity reflecting greater nutrient concentration, i.e., eutrophication (Brenner et al. 1999).

The C isotopic signature of autochthonous OM is influenced by the $\delta^{13}C$ of the dissolved inorganic C pool in lake water. Hodell and Schelske (1998) related the seasonal pattern of $\delta^{13}C_{org}$

in Lake Ontario to seasonality of primary productivity. Gu et al. (2006) reported ^{13}C enrichment of particulate OM in Lake Wauberg (Florida) resulted from reduced isotopic fractionation due to C limitation and use of isotopically heavy inorganic C. Lehmann et al. (2004) concluded that the most important process controlling the C-isotopic signature of suspended particulate OM in Lake Lugano (Swiss-Italian border) is the concentration of CO_2 in surface water, which is a function of phytoplankton photosynthesis. Algae fractionate against ^{13}C, so autochthonous OM is depleted in $\delta^{13}C$. During periods of high primary productivity, however, this fractionation diminishes and more ^{13}C is incorporated into primary producer biomass (Mizutani and Wada 1982; Raul et al 1990).

Hypereutrophic lakes with high rates of primary productivity have depleted CO_2 (aq) concentrations in the water column. Moreover, in alkaline waters bicarbonate (HCO_3^-) is the dominant form of inorganic C, and is 8‰ heavier than C in dissolved CO_2 (Fogel et al. 1992). High demand for inorganic C and low free CO_2 leads to utilization of HCO_3^- as a C source resulting in heavier $\delta^{13}C$ (Goericke et al. 1994). Gu et al. (2004) reported high $\delta^{13}C$ of inorganic C in the water column of Lake Apopka. The authors showed that heavy $\delta^{13}C$ DIC in the water column was a result of isotopic fractionation from methanogenesis in the sediments. Methanogenesis produces ^{13}C-rich CO_2 and ^{13}C-poor methane (CH_4) (Games and Hayes 1976). Lake Apopka has low CO_2 partial pressure, high pH, and strong buffering capacity. Consequently isotopically heavy CO_2 is transferred from the sediments to the DIC of the water column (Gu et al. 2004). Lake Apopka sediments display high CH_4 production rates (Chapter 2 and 5). Furthermore, most of the primary productivity in this lake is deposited in its sediments (Gale and Reddy 1994). Primary productivity is dominated by cyanobacteria (*Synechococcus* sp., *Synechocystis* sp. and *Microcystis incerta*) (Carrick et al. 1993; Carrick and Schelske 1997).

Cyanobacteria are capable of active CO_2 transport (Miller et al. 1991) or utilizing HCO_3^- (Epsie et al. 1991), and both can result in enriched $\delta^{13}C$ in phytoplankton biomass. Jones et al. (2001) reported enrichment of phytoplankton $\delta^{13}C$ resulted from $\delta^{13}C$ DIC enrichment in Loch Ness (Scotland). Similar results were found in urban Lake Jyväskylä (Finland), where heavy $\delta^{13}C$ DIC resulted in enriched $\delta^{13}C$ of phytoplankton and zooplankton biomass (Syväranta et al. 2006). Heavy $\delta^{13}C$ DIC in the water column, with high demand for inorganic C due to high primary productivity, will produce autochthonous OM with enriched $\delta^{13}C$, which is then deposited in the sediments.

Recent ^{15}N enrichment in Lake Apopka sediments was surprising as it is generally expected that eutrophic and hypereutrophic lakes will have depleted $\delta^{15}N$, as a consequence of high rates of N_2 fixation (Fogel and Cifuentes 1993). Gu et al. (1996) also reported enriched $\delta^{15}N$ in Lake Apopka sediments. In this lake, N for phytoplankton assimilation is primarily supplied by transformation of organic N to NH_4^+ and then to NO_3^- by nitrification (D'Angelo and Reddy 1993). Although the phytoplankton community is dominated (> 90%) by cyanobacteria (Carrick et al. 1993), N_2 fixation is relatively unimportant in N dynamics (Schelske et al. 1992). High NO_3^- availability can lead to autochthonous OM with depleted $\delta^{15}N$ (Meyers 1997). However, if N incorporation uses a significant amount of the lake's NO_3^- pool, the residual NO_3^- will become enriched, ultimately leading to an increase in the $\delta^{15}N$ of newly produced OM (Terranes and Bernasconi 2000; Syväranta et al. 2006). Jones et al. (2004) reported heavier sediment $\delta^{15}N$ when inorganic N was low in the water column, reflecting reduced isotopic fractionation under N limitation. Nitrogen is the primary limiting nutrient in Lake Apopka although co-limitation with P can occur (Aldridge et al. 1993). Periods of N limitation in the water column can lead to

enrichment of autochthonous OM and stratigraphic variation in the $\delta^{15}N$ signature of sediments in Lake Apopka may indicate periods of N limitation.

Other mechanisms may also influence $\delta^{15}N$ of Lake Apopka sediments. The N isotopic signature of sediment integrates multiple fractionation processes that occur in the sediment and water column (Lehmann et al. 2004). Ammonium production through organic matter mineralization is high in these sediments (Chapter 5). Such mineralization processes can lead to isotopic enrichment of the remaining OM. Inglett et al. (2007) related $\delta^{15}N$ enrichment in the Everglades soil that is highly impacted with P, to an increase in microbial processes (i.e., respiration, mineralization rates). Moreover, denitrification discriminates against heavy ^{15}N and increases in denitrification rates have been related to enriched $\delta^{15}N$ signatures in sediments (Terranes and Bernasconi 2000; Savage et al. 2004). The N isotope signature in Lake Apopka sediments is generated by multiple factors including the isotopic signature of autochthonous N sources, the primary producer community, and N related processes in the water column and sediments (Figure 6-9E).

Samples from each core were plotted in isotope space, i.e., $\delta^{13}C$ vs. $\delta^{15}N$ (Figure 6-8). Each core occupies a distinct region in isotope space. Lake Apopka is relatively enriched in both $\delta^{13}C$ and $\delta^{15}N$. Mud zone site M9 in Lake Okeechobee displays intermediate values for both isotopes. Lake Annie is most depleted in $\delta^{13}C$, but similar in $\delta^{15}N$ to sites M17 and KR. Excluding the highly different sediment types, peat (M17) and sand (KR) from the figure, it seems that the remaining sediments show a gradient in relation to both $\delta^{13}C$ and $\delta^{15}N$. Oligo-mesotrophic Lake Annie is at the bottom of the graph with low values, followed by eutrophic Lake Okeechobee (mud sediments M9) with intermediate values, and then hypereutrophic Lake

Apopka with enriched values for both $\delta^{13}C$ and $\delta^{15}N$. This shows a trend of enrichment in $\delta^{13}C$ and $\delta^{15}N$ with increasing trophic state.

However, sediment $\delta^{13}C$ and $\delta^{15}N$ signatures in each site result from different mechanisms, and they might be more important in interpreting the data than solely trophic state (Figure 6-9). Difference in sediment isotope C and N can indicate variability on seasonal, inter-annual and century long-time scales of fractionation factors associated with allochthonous and autochthonous organic matter as well as mineralization processes occurring within lakes (Lehmann et al. 2004) (Figure 6-9).

Conclusions

In this study, the ^{210}Pb dating technique was used to provide an age/depth relation in the sampled sediments. Lake Annie sediments were the only datable samples, while sediments collected from Lake Okeechobee could not be dated reliably due to low or variable activities of ^{210}Pb and ^{226}Ra. In Lake Apopka, it is possible that uppermost sediments were mixed and it appears that the supported/unsupported boundary was not reached in the core. In Lake Annie, the bottom sediment layer of the core was estimated to date to the 1800s and the average sedimentation rate (since c.1900) was determined to be ≈ 36.8 mg cm^{-2} yr^{-1}. Isotopic signatures in Lake Annie sediments, depleted in $\delta^{13}C$ and $\delta^{15}N$, probably resulted from a combination of several factors such as allochthonous OM input, OM from primary productivity, and microbial biomass and activity. In the Lake Okeechobee mud zone, $\delta^{13}C$ values were slightly depleted while $\delta^{15}N$ values were enriched towards the sediment surface. These isotopic signatures resulted from several factors such as the phytoplankton community, high demand for C and N in sediments, and selective mineralization of OM. In the peat zone of Lake Okeechobee, the isotopic signatures (enrichment of $\delta^{13}C$ and $\delta^{15}N$ towards the sediment surface) of sediment OM

were related to several factors, including sediment origin (i.e., plant tissue), intensities of primary productivity and diagenesis. Stratigraphic variation in $\delta^{13}C$ and $\delta^{15}N$ at the KR site probably reflects an input of wastewater from anthropogenic activities, and variable contributions of river-borne allochthonous input, related to inter-annual rainfall variations. In Lake Apopka, heavy $\delta^{13}C$ DIC in the water column, with high demand for inorganic C due to high primary productivity, produced autochthonous OM with enriched $\delta^{13}C$. The enriched $\delta^{15}N$ signature in Lake Apopka sediments was generated by multiple factors including the isotopic signature of autochthonous N sources, the primary producer community, and N related processes in the water column and sediments. A more detailed study of $\delta^{13}C$ and $\delta^{15}N$ isotopes in several compartments, i.e., dissolved C, different N compounds, phytoplankton biomass, bacteria biomass, particulate OM in the water column and sediment, can confirm the major processes affecting the isotopic signatures of these sediments.

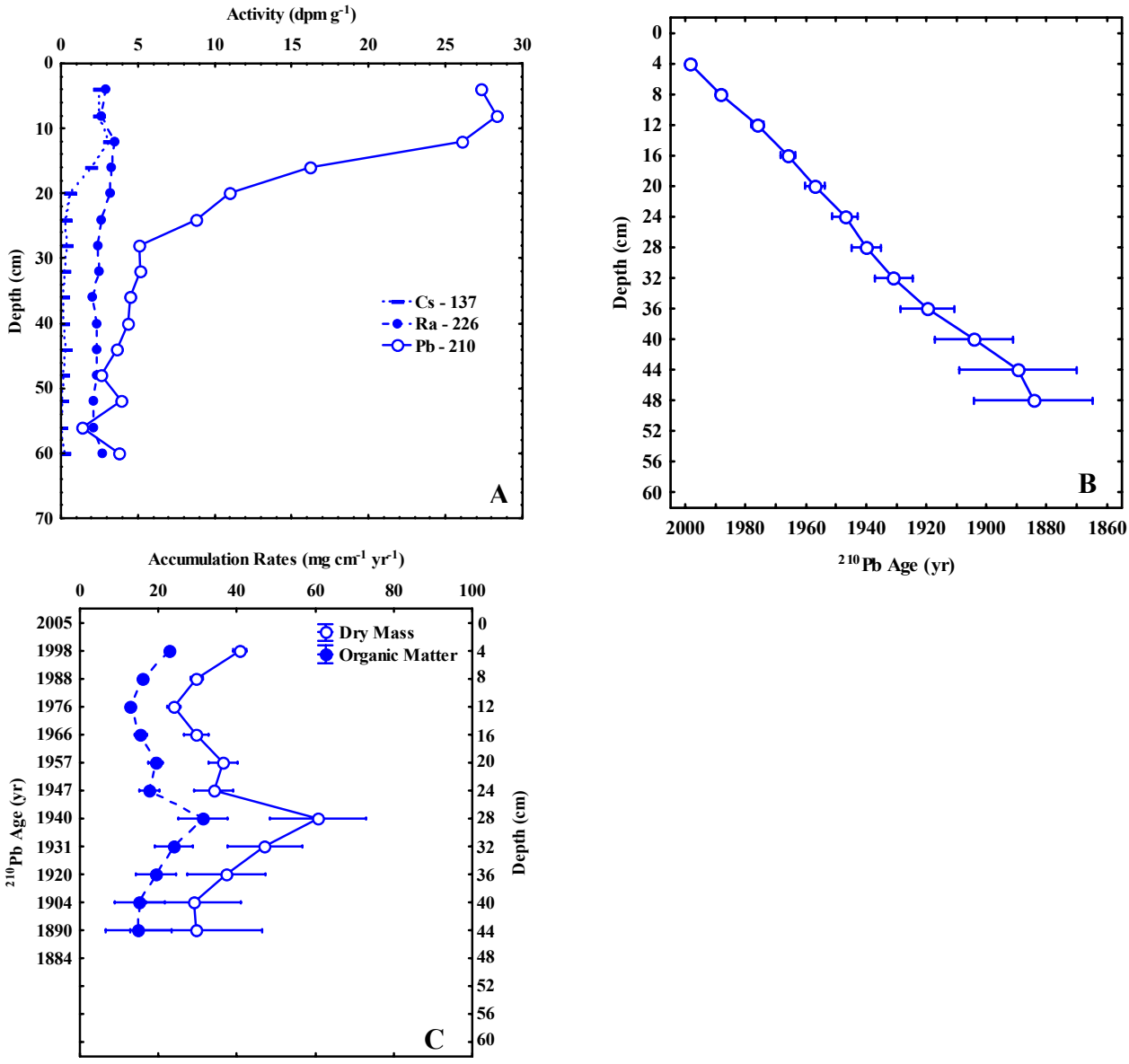


Figure 6-1. Results of ^{210}Pb dating of Lake Annie sediments: A) Radioisotope activities (total ^{210}Pb, ^{226}Ra, and ^{137}Cs) versus depth, B) sediment depth vs. age/date, and C) sediment and organic matter accumulation rates vs. age/year.

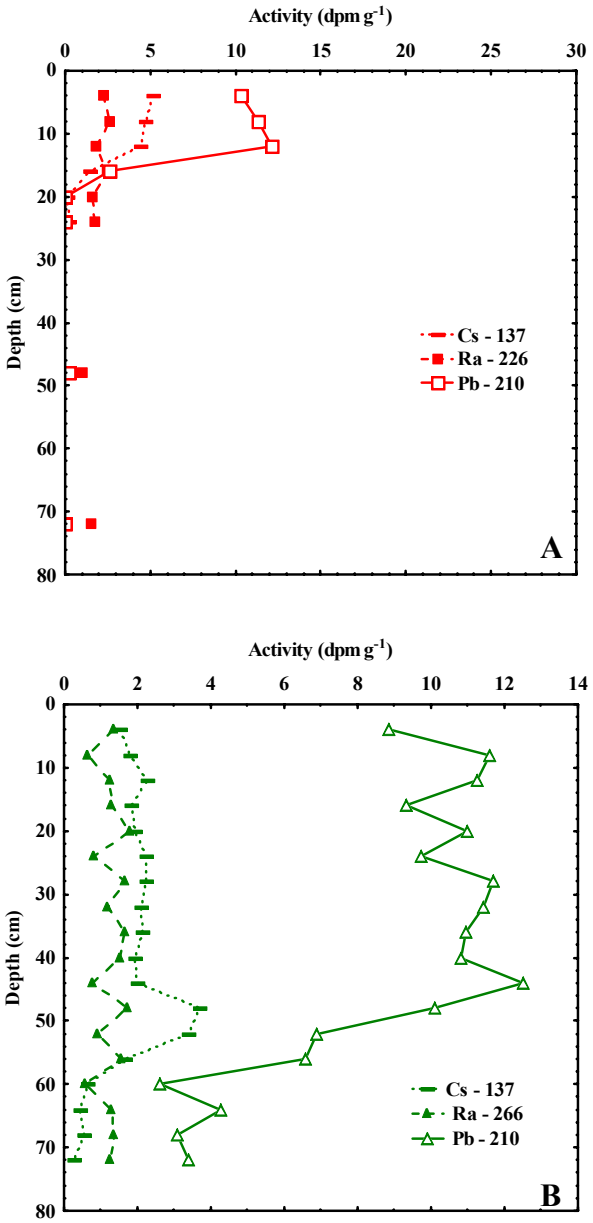


Figure 6-2. Radioisotope activities (total ^{210}Pb, ^{226}Ra, and ^{137}Cs) versus depth, in A) Lake Okeechobee, site M9 and, B) Lake Apopka.

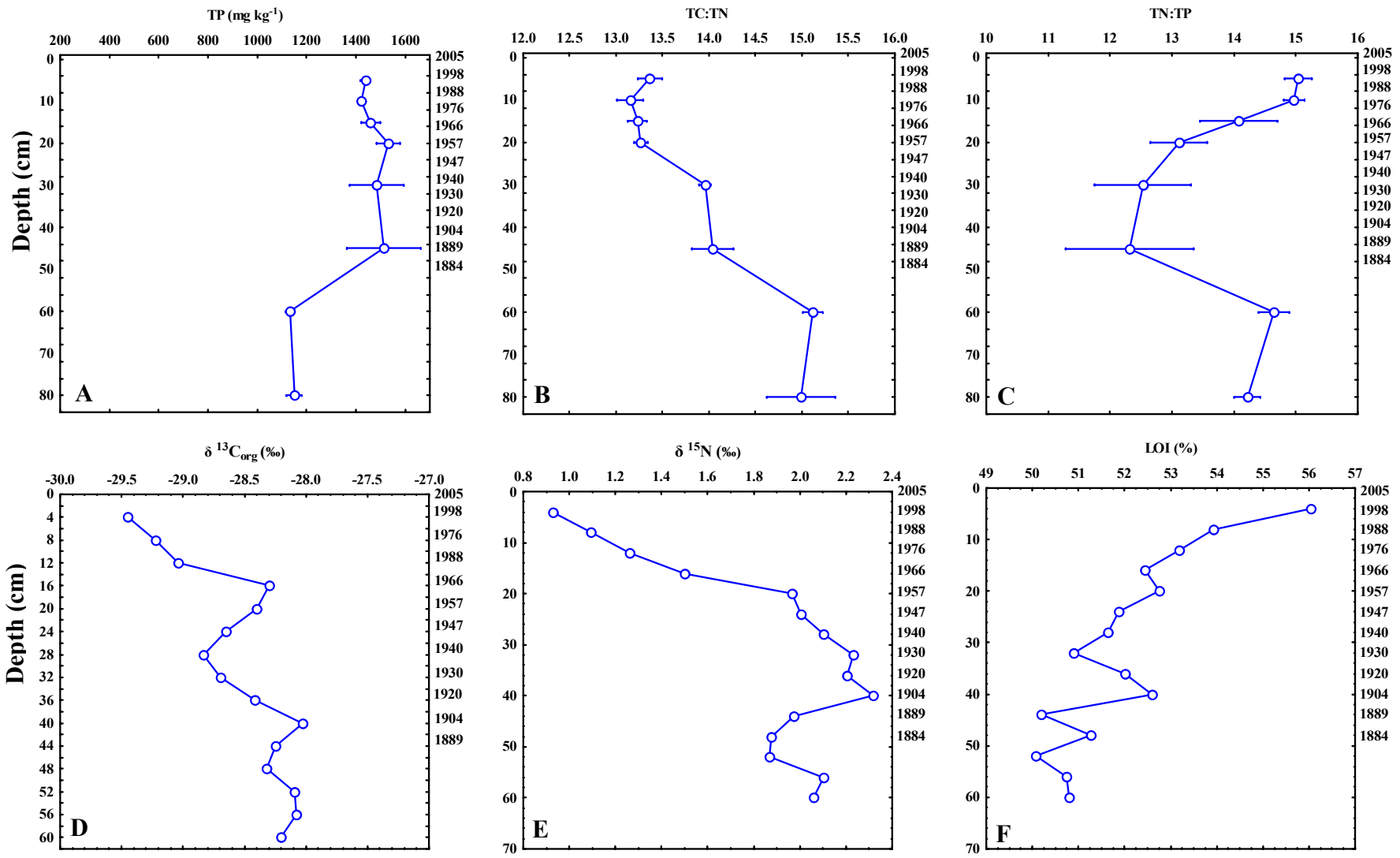


Figure 6-3. Lake Annie sediment depth profile of: A) Total phosphorus, B) TC:TN ratio, C) TN:TP ratio, D) $\delta^{13}C_{org}$ of sediment organic carbon, E) sediment $\delta^{15}N$, and F) organic matter content (LOI %).

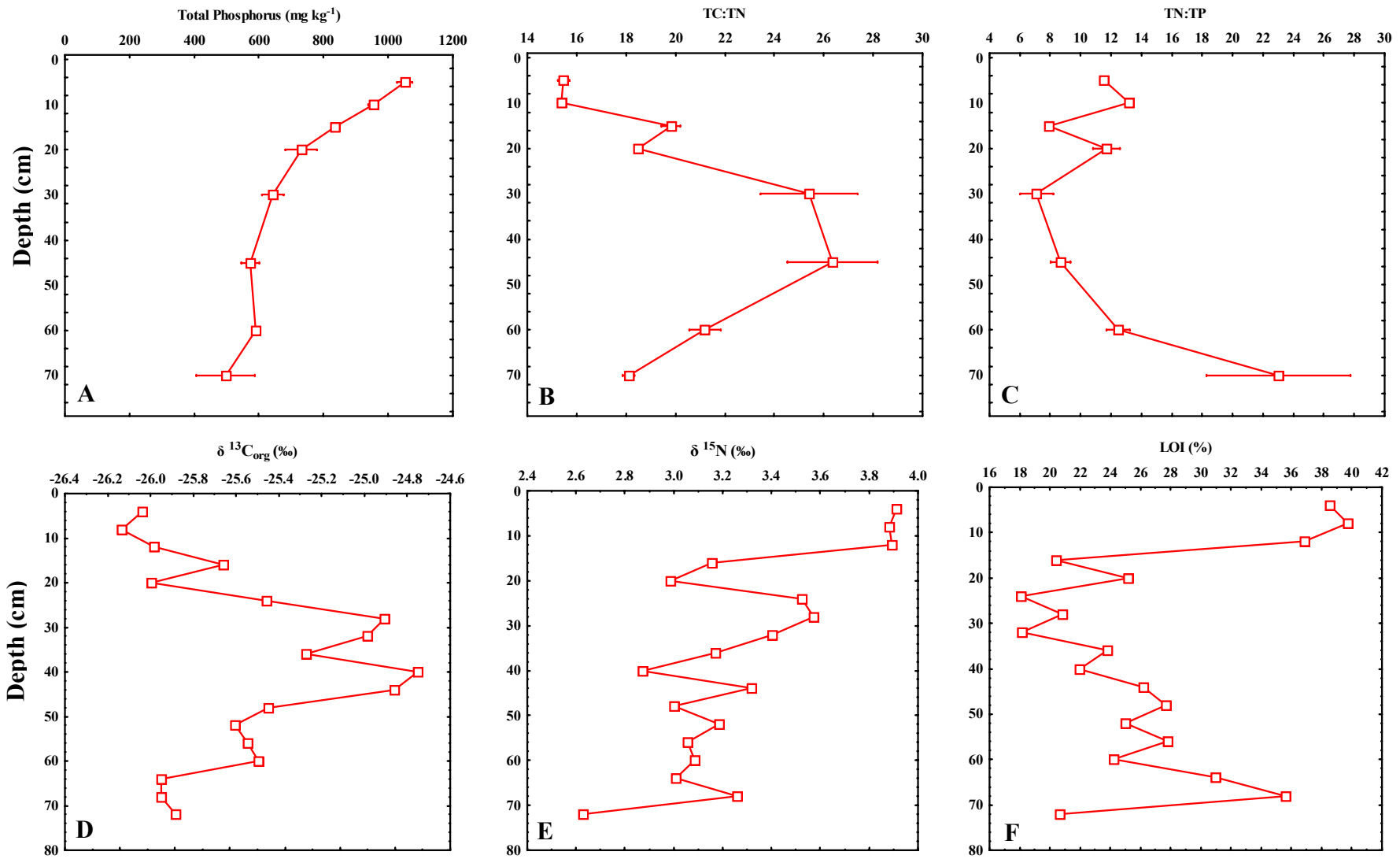


Figure 6-4. Lake Okeechobee mud zone (site M9) sediment depth profile of: A) Total phosphorus, B) TC:TN ratio, C) TN:TP ratio, D) $\delta^{13}C_{org}$ of sediment organic carbon, E) sediment $\delta^{15}N$, and F) organic matter content (LOI %).

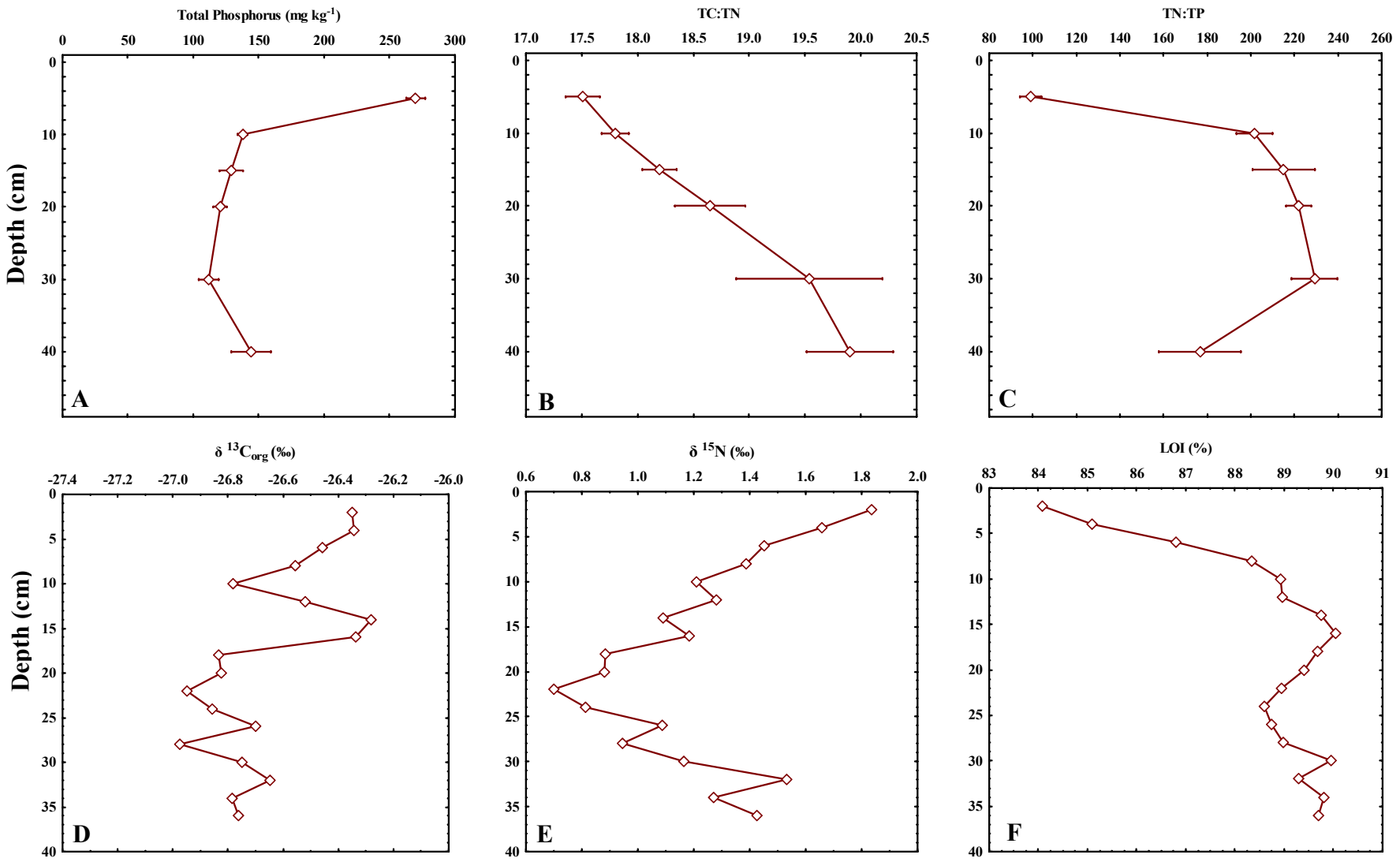


Figure 6-5. Lake Okeechobee peat zone (site M17) sediment depth profile of: A) Total phosphorus, B) TC:TN ratio, C) TN:TP ratio, D) $\delta^{13}C_{org}$ of sediment organic carbon, E) sediment $\delta^{15}N$, and F) organic matter content (LOI %).

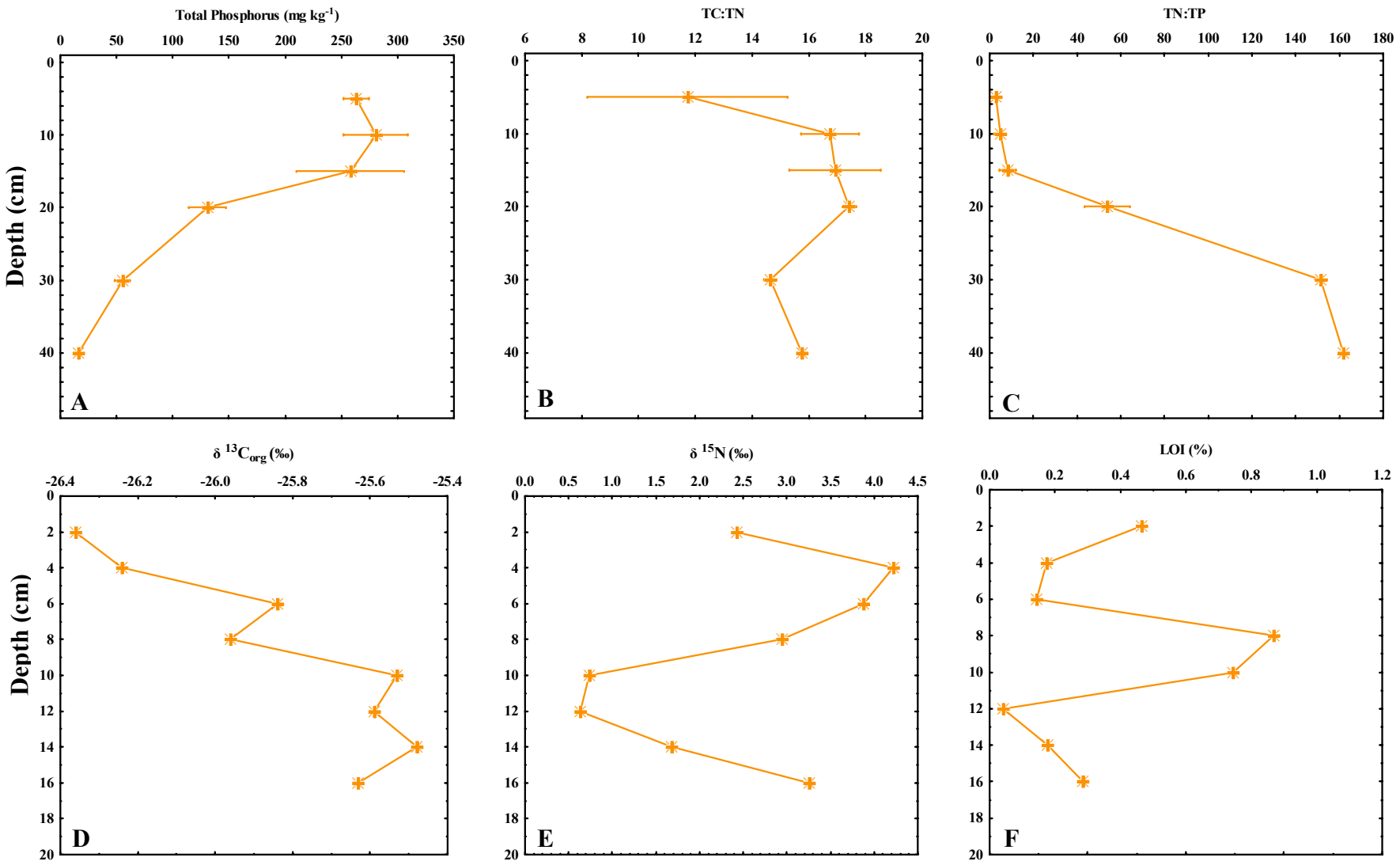


Figure 6-6. Lake Okeechobee sand zone (site KR) sediment depth profile A) Total phosphorus, B) TC:TN ratio, C) TN:TP ratio, D) $\delta^{13}C_{org}$ of sediment organic carbon, E) sediment $\delta^{15}N$, and F) organic matter content (LOI %).

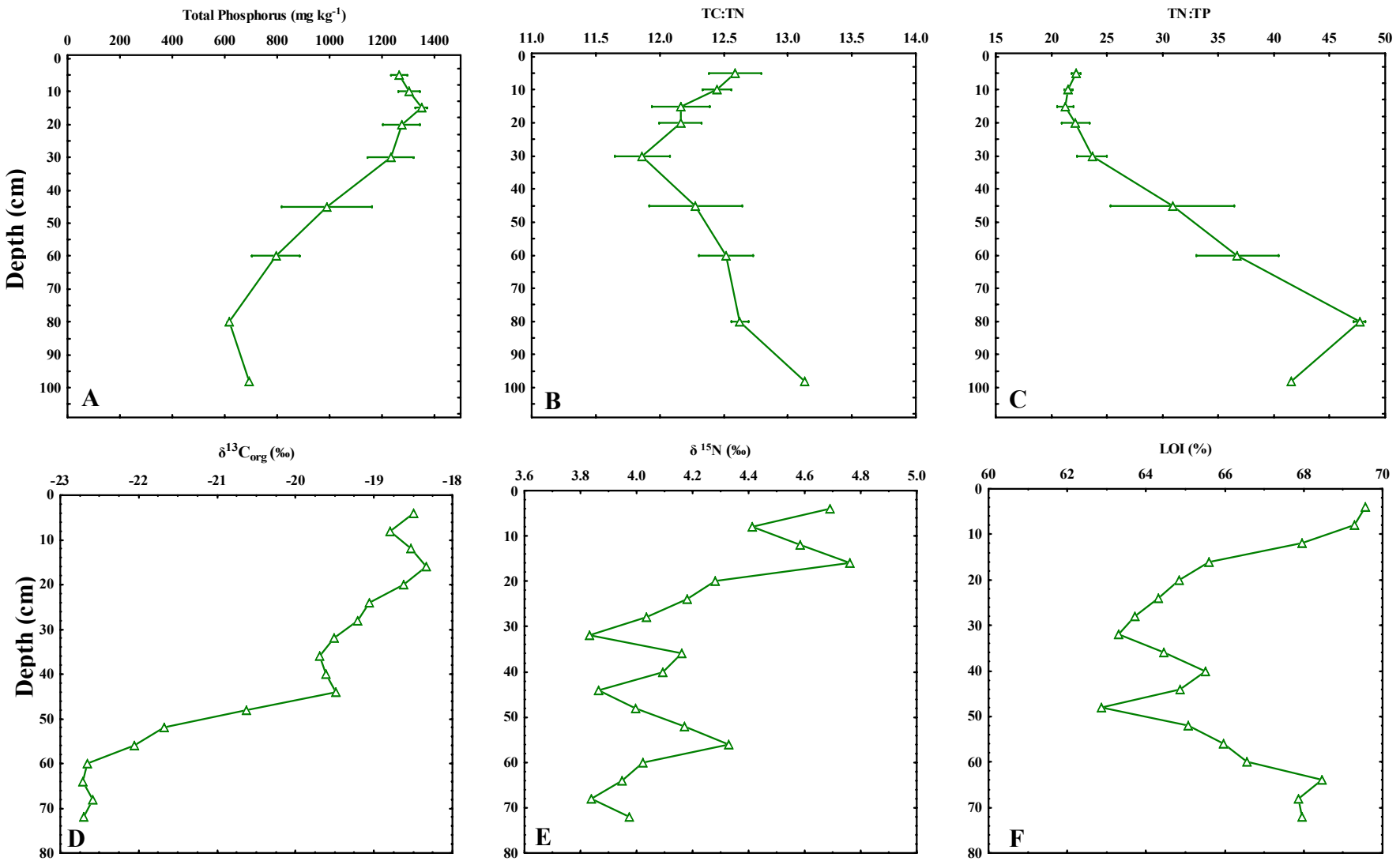


Figure 6-7. Lake Apopka sediment depth profile A) Total phosphorus, B) TC:TN ratio, C) TN:TP ratio, D) $\delta^{13}C_{org}$ of sediment organic carbon, E) sediment $\delta^{15}N$, and F) organic matter content (LOI %).

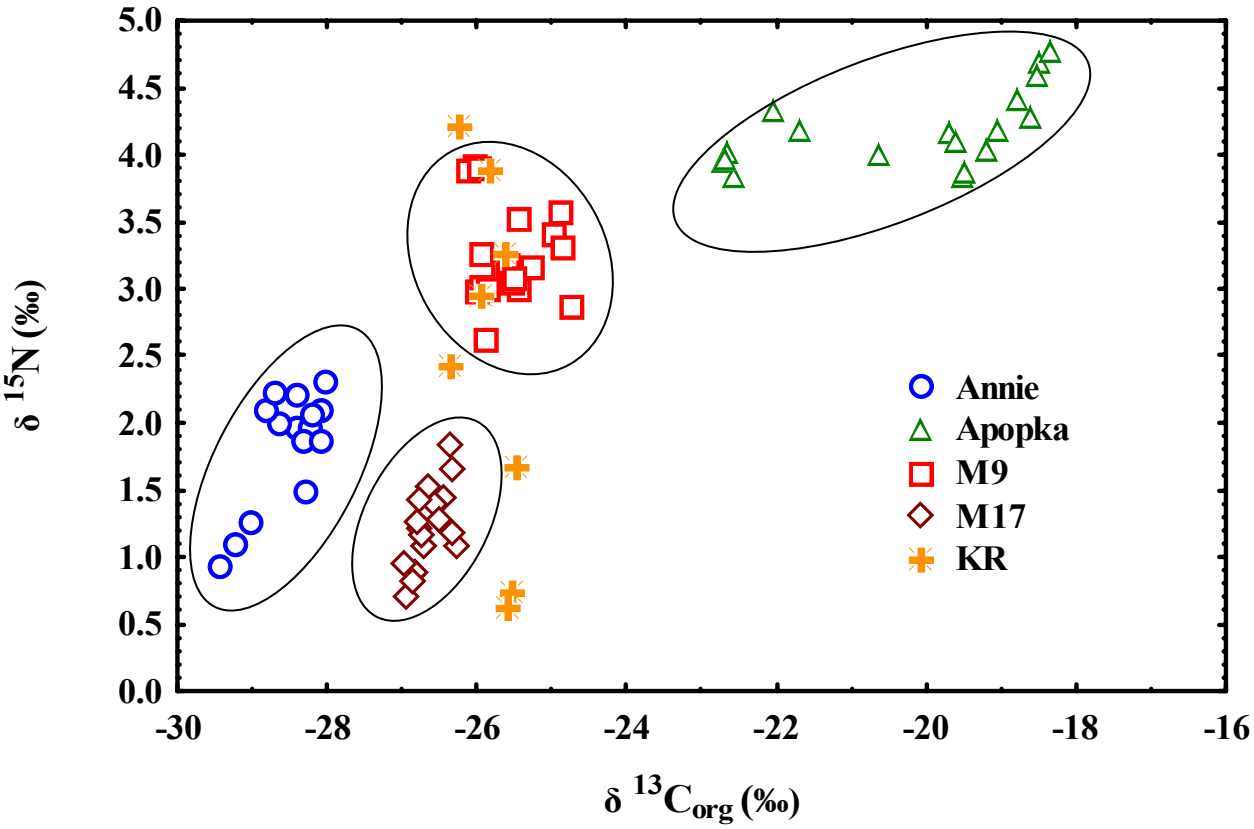
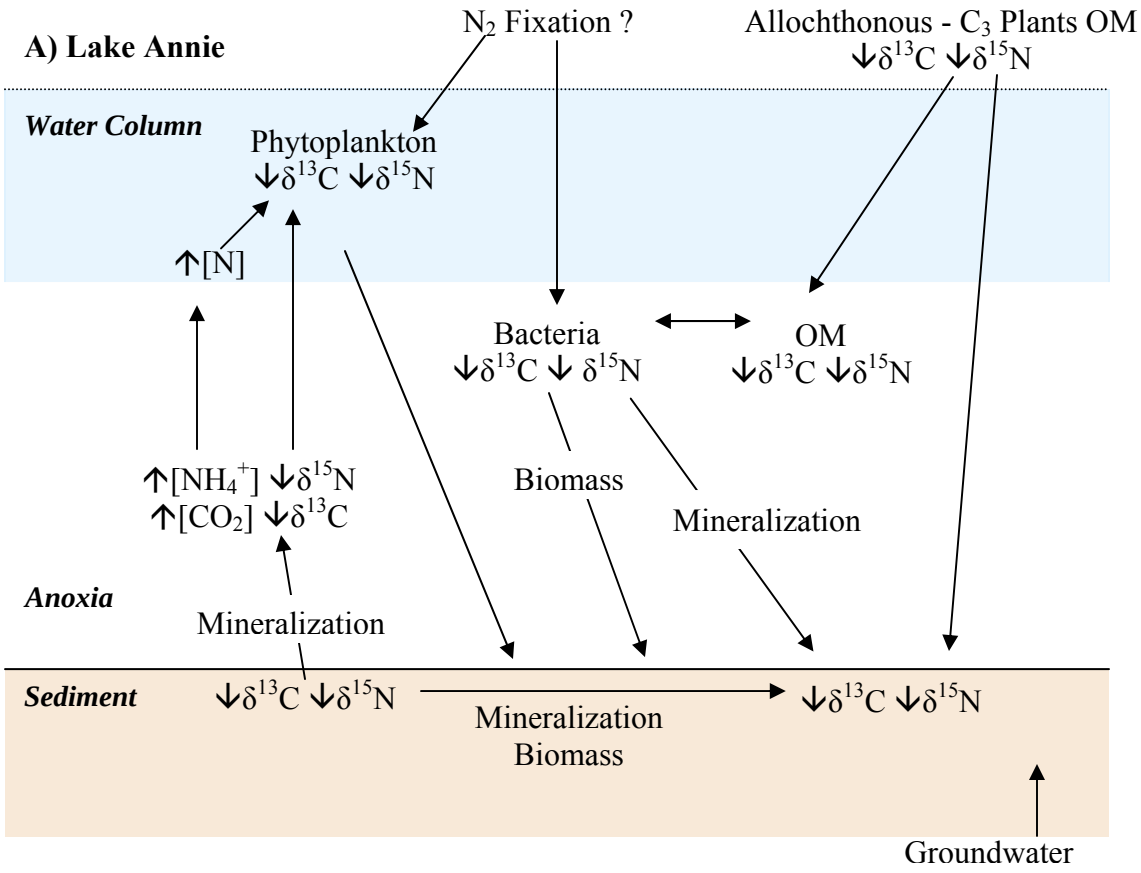


Figure 6-8. Carbon vs. nitrogen isotopic values of sediments in Lake Annie, Lake Okeechobee (sites M9, M17, and KR), and Lake Apopka.



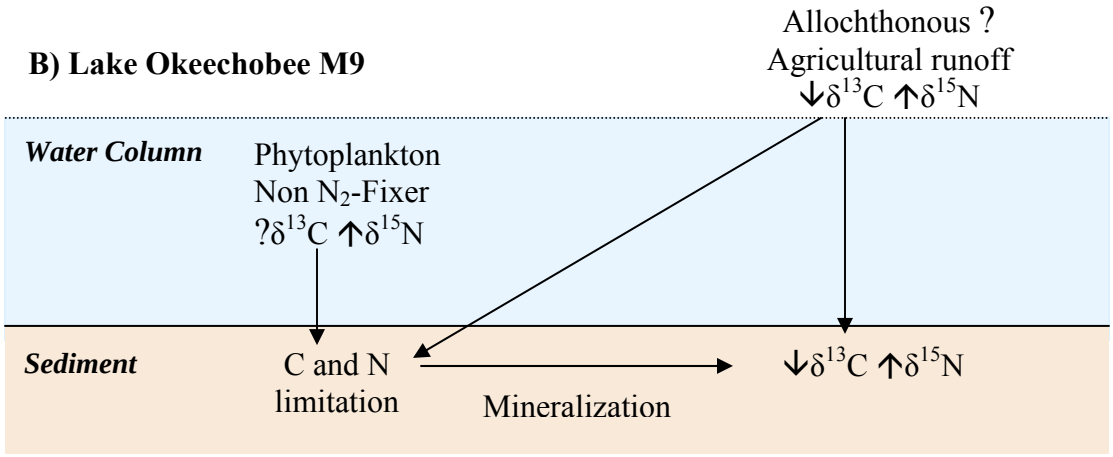
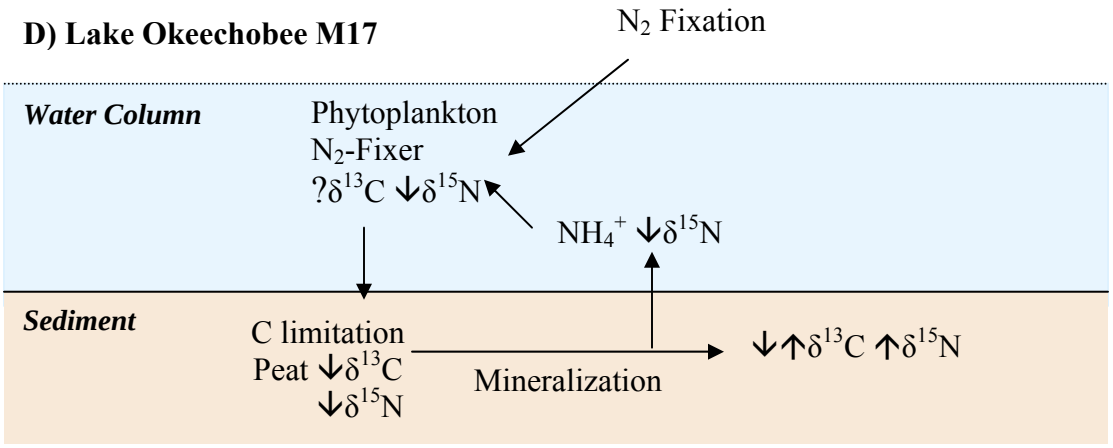
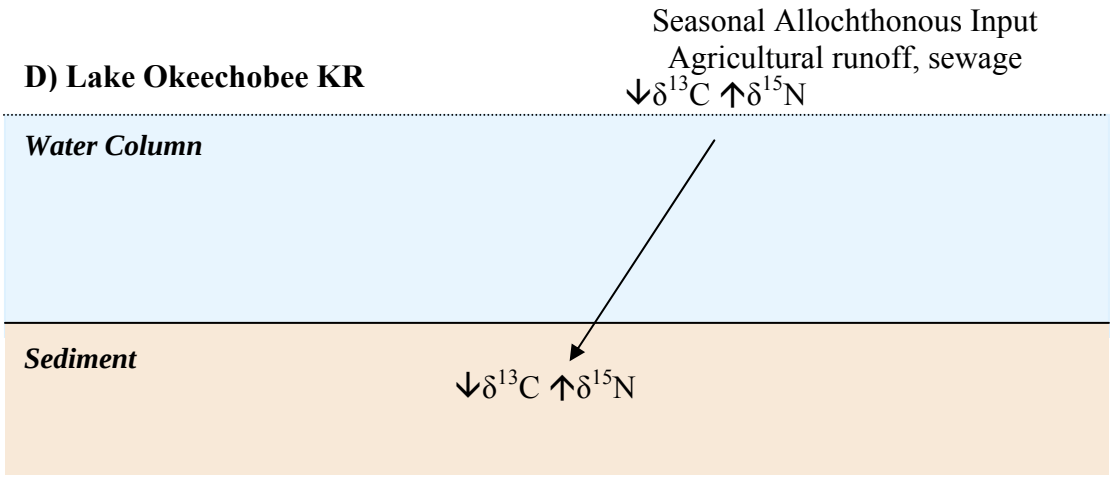


Figure 6-9. Major mechanisms affecting the sediment $\delta^{13}C$ and $\delta^{15}N$ signatures in: A) Lake Annie, Lake Okeechobee B) site M9, C) site M17 and D) site KR, and E) Lake Apopka.





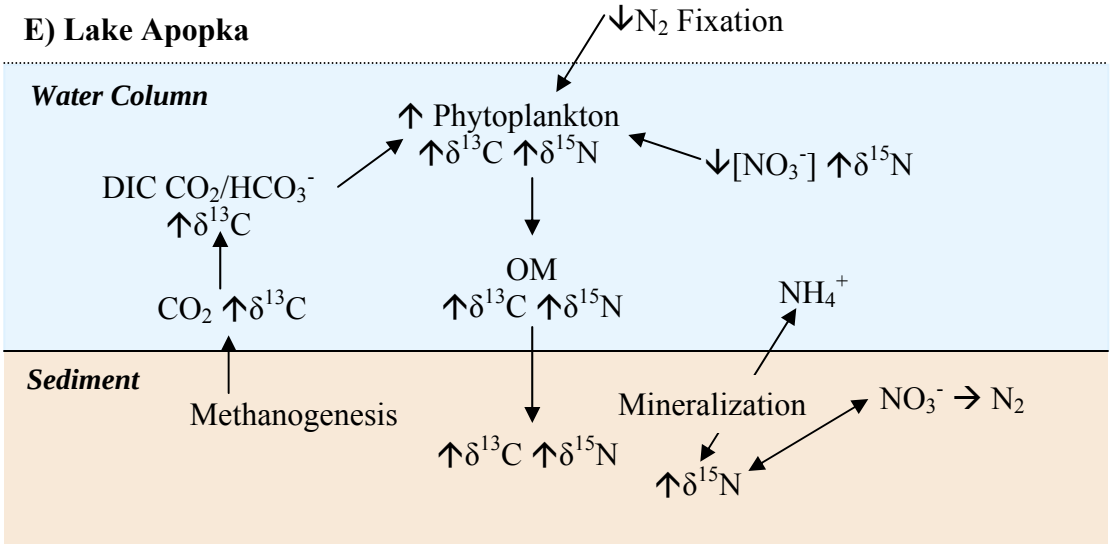


Figure 6-9. continued

CHAPTER 7
HETEROTROPHIC MICROBIAL ACTIVITY IN SEDIMENTS: EFFECTS OF ORGANIC ELECTRON DONORS

Introduction

Organic matter deposition is an important source of carbon (C) in lake sediments. Organic compounds and associated nutrients supplied to sediments are mineralized through heterotrophic decomposition (Gächter and Meyer 1993; Capone and Kiene 1988; Megonigal et al. 2004). The composition and activities of microbial communities are regulated by the quality and availability of carbon. In high depositional environments such as eutrophic or deep thermally stratified lakes, organic content in sediments is often high, such that oxygen (O_2) is rapidly consumed, and is depleted within several millimeters below the sediment water interface (Jørgensen 1983). In these systems, facultative and strict anaerobic communities dominate. Complete oxidation of a broad range of organic compounds in these systems can occur through the sequential activity of different groups of anaerobic bacteria (Capone and Kiene 1988).

In methanogenic habitats, i.e., in the absence of inorganic electron acceptors, different groups of microorganisms participate in decomposition of organic matter as no single anaerobic microorganism can completely degrade organic polymers (Zinder 1993, Megonigal et al. 2004). Cellulolytic bacteria hydrolyze organic polymers through extracellular enzyme production and further break down monomers to alcohols, fatty acids, and hydrogen (H_2) through fermentation. Alcohols and fatty acids are degraded by syntrophic bacteria (secondary fermenters) into acetate, H_2, and carbon dioxide (CO_2), which is used as substrate by methanogens (Zinder 1993, Conrad 1999, Megonigal et al. 2004). The structures and functions of anaerobic microbial communities are therefore strongly affected by competition for fermentation products such as H_2 and acetate. Microorganisms derive energy by transferring electrons from an external source or donor to an external electron sink or terminal electron acceptor. Organic electron donors vary from

monomers that support fermentation to simple compounds such as acetate and methane (CH_4). Fermenting, syntrophic, methanogenic bacteria and most other anaerobic microorganisms (e.g., sulfate, iron reducers) are sensitive to the concentrations of substrates and products. Their activities can be inhibited by their end products and are dependent on the end product consumption by other organisms (Stams 1994; Megonigal et al. 2004).

Microbial functional diversity includes a vast range of activities. One component of this diversity has been characterized by measuring catabolic response profile, i.e., short-term response of microbial communities to addition of a wide variety of C-substrates (Degens and Harris 1997; Degens 1998a). This has been widely applied in soil studies to address differences in microbial communities in different soil types, disturbance, and land use (Degens and Harris 1997; Lu et al. 2000; Degens et al. 2000, 2001; Stevenson et al. 2004). Substrate induced respiration is often dependent on the size of the microbial biomass pool; however, response of microbial communities is also related to the catabolic diversity of soil microorganisms (Degens 1998). A greater relative catabolic response to a substrate in one system as compared with another indicates that the microbial community is more functionally adapted to use that resource, and may indicate previous exposure to those C-sources (Degens and Harris 1997; Degens 1998; Baldock et al. 2004; Stevenson et al. 2004).

The metabolic response of microbial communities in lake sediments may vary due to several factors that either influence the microbial community or are due to physico-chemical characteristics of lakes, such as the source and chemical composition of particulate matter and biogeochemical processes in the sediment and water column. Accumulation and retention of particulate matter and nutrients in sediments depends on lake morphometry, water renewal, nutrient loading, edaphic characteristics of the drainage basin, among other factors (Boström et

al. 1988). Eutrophic and hypereutrophic lakes typically receive high external loads of nutrients and display high primary productivity and nutrient concentrations in the water column and these nutrients eventually reach the sediment, therefore sediments from eutrophic and hypereutrophic lakes are expected to have high concentrations of organic matter. Binford and Brenner (1986) and Deevey et al. (1986) showed that net accumulation rates of organic matter and nutrients increase with trophic state for Florida lakes. In contrast, small oligotrophic lakes are expected to exhibit a relatively high proportion of allochthonous carbon input to their sediments (Gu et al. 1996).

Lake depth can also affect the quality of organic material reaching the sediment. In deep lakes, sedimenting organic matter undergoes intense decomposition in the water column due to the prolonged period of settling. Consequently, low amounts of labile organic carbon reach the sediment (Suess 1980; Meyers 1997). In shallow lakes, however, the supply of labile carbon and nutrients can be higher in sediments than in deep lakes, and the latter often can have more refractory organic matter. Sediments with different C-sources and with different quality and quantity as well as nutrient concentration will have different microbial communities. These communities can display distinct catabolic responses as the mineralization rates of a microbial community are dependent upon the metabolic capacity for a given substrate (Torien and Cavari 1982). The objective of this study was to evaluate the short term catabolic response of the microbial communities in sediments of three subtropical lakes characterized by different trophic states. The central hypothesis is that sediments with higher C availability will have higher catabolic diversity.

Materials and Methods

Study Sites

Three Florida (USA) lakes ranging in trophic state were selected. Lake characteristics were described in Chapter 2. The characteristics and location of sampled sites were described in Chapter 3 (Table 3-1, Figure 3-1).

Field Sampling

Triplicate sediment cores were collected using a piston corer (Fisher et al. 1992) or by SCUBA divers. The topmost 10 cm of sediment were collected from one central site in Lake Annie on June 25, 2005 and a western site in Lake Apopka on May 28, 2005 (Figure 3-1C, Table 3-1). Cores were collected at three sites in Lake Okeechobee on July 16, 2005: M17 = peat, M9 = mud and KR = sand (Figure 3-1B, Table 3-1). Samples were placed in plastic bags, sealed, and kept on ice. All measured sediment variables are reported on a dry weight basis (dw). Water quality variables were described in a previous study (Chapter 4).

Sediment Properties

Samples were transported on ice and stored in the dark at 4 ºC. Before each analysis, samples were homogenized and sub-samples taken. Sediment bulk density (g dry wt cm^{-3} wet) was determined on a dry weight basis at 70 ºC for 72 hours, and pH was determined on wet sediments (1:2 sediment-to-water ratio). Sediment samples were ground in a ball mill and passed through a # 40 mesh sieve. Organic matter content (LOI-loss on ignition) was determined by weight loss at 550ºC. Total P was measured by ignition method, followed by acid digestion (6 M HCl) and measured colorimetrically with a Bran+Luebbe Technicon™ Autoanalyzer II (Anderson 1976; Method - 365.1, EPA 1993). Total carbon (TC) and total nitrogen (TN) were determined using a Flash EA-1121 NC soil analyzer (Thermo Electron Corporation).

Extractable Carbon (C), Nitrogen (N), and Phosphorus (P)

Sediment samples were extracted with 0.5 M K_2SO_4 for extractable N and with 0.5 M $NaHCO_3$ (pH = 8.5) for extractable P, using a 1:50 dry sediment-to-solution ratio (Mulvaney 1996; Ivanoff et al. 1998). Extracts from samples were centrifuged at 10,000 x g for 10 min and filtered through Whatman # 42 filter paper. For N analysis, 5 mL of the extracts were subjected to Kjeldahl nitrogen digestion and analyzed for total Kjeldahl-N colorimetrically using a Bran+Luebbe Technicon™ Autoanalyzer II (Method - 351.2, EPA 1993). Undigested N extracts were analyzed for ammonium (NH_4-N) (Method - 351.2, EPA 1993), and represent extractable ammonium (Ext-NH_4-N). The difference between Ext-N and Ext-NH_4-N represents extractable labile organic nitrogen (Ext-ON). Extracts from P samples were centrifuged at 10,000 x g for 10 min and filtered through a 0.45 μm membrane filter, and analyzed for soluble reactive P or digested for TP (with sulfuric acid and potassium persulfate). Solutions were analyzed by colorimetry, determined by reaction with molybdate using a Bran+Luebbe Technicon™ Autoanalyzer II (Murphy and Riley 1962; Method 365.1, EPA 1993). Undigested P extracts represents labile inorganic P (Ext-Pi). The difference between total extracted P and Ext-Pi represents labile organic phosphorus (Ext-Po).

Microbial Biomass Carbon

Microbial biomass carbon (MBC) was measured with the chloroform fumigation-extraction method (Hedley and Stewart 1982; Vance et al. 1987). Briefly, sediment samples were split in two: one sample was treated with alcohol-free chloroform (0.5 mL) to lyse microbial cells, placed in a vacuum desiccator, and incubated for 24 hrs. The duplicate sample was left untreated. Both sets were extracted with 0.5 M K_2SO_4 centrifuged at 10,000 x g for 10 min and filtered through Whatman # 42 filter paper. Carbon extracts were acidified (pH < 2) and analyzed in an automated Shimadzu TOC 5050 analyzer (Method - 415.1, EPA 1993). Microbial biomass

C was calculated by the difference between treated and non-treated samples. Extracts from the untreated samples represent extractable organic carbon (Ext-C).

Electron Donors

Basic catabolic response was characterized by increasing CO_2 and CH_4 production rates from sediment samples by addition of different electron donors. Eight different simple organic compounds (electron donors) were added to each sediment sample. They consisted of two amino acids (alanine and arginine), four carboxylic acids (acetate, formate, butyrate, and propionate), one polysaccharide (glucose), and lake suspended solids (Lake-SS). Wet sediment (based on 0.5 g of dry weight) was added to incubation bottles, sealed with rubber stoppers and aluminum crimp seals, and purged with N_2 gas. Alanine, arginine, acetate, formate, butyrate, propionate, and glucose were added from anaerobic sterile stock solutions to sediments on a C-equivalent basis, reaching a final concentration of 42 mM C (504 μg of C g^{-1} on a dry weight basis) (Degens 1998*a*). All stock solutions were adjusted to pH around 7.0 using either HCl or NaOH at the time of preparation to avoid any substrate-pH effects on microbial communities.

Lake-SS was obtained by centrifugation (10,000 x g for 30 min) of water samples collected at approximately 50 cm depth in the water column of each lake. Lake-SS was characterized for LOI, TC, TN, and TP as described previously, and was added on the same C-equivalent basis as the other electron donors. A sample from each Lake-SS was incubated to account for CO_2 and CH_4 production of the material. Values obtained were subtracted from CO_2 and CH_4 production rates of the Lake-SS treatment for each lake. Sediments from each site were also incubated with no substrate addition (control) to obtain values of basal anaerobic CO_2 and CH_4 production rates. Samples were incubated anaerobically at 30 ºC in the dark. Gas samples were taken at 1, 2, 4, 7, 10, and 14 days and quantified for CO_2 and CH_4. Gas samples from Lake Annie and Lake Okeechobee sediments were also taken at 20 days of incubation due to low CH_4 production

detected with some treatments at 14 days of the experiment. CO_2 was measured by gas chromatography using a Schimadzu (8A GC-TCD) and Poropak N column (Supelco Inc., Bellefonte, PA) with He as a carrier gas and CH_4 was analyzed on a Shimadzu gas chromatograph-8A fitted with flame ionization detector (110 ºC), N_2 as the carrier gas and a 0.3 cm by 2 m Carboxyn 1000 column (Supelco Inc., Bellefonte, PA) at 160 ºC. Prior to measurements of both CO_2 and CH_4, headspace pressure was determined with a digital pressure indicator (DPI 705, Druck, New Fairfield, CT). Concentrations of CO_2 and CH_4 were determined by comparison with standard concentrations and production rates were calculated by linear regression ($r^2 > 0.95$). Final production rates were determined after removing the lag phase (the time between substrate addition and quantifiable gas production) in each site. Turnover rates (d^{-1}) were determined by dividing the sum of CO_2 and CH_4 production rates by the amount of C added. Anaerobic CO_2 and CH_4 production rates were standardized by microbial biomass carbon of each sediment sample (CO_2 or CH_4 production divided by MBC).

Statistical Analysis

All statistical analyses were conducted with standardized values of anaerobic CO_2 and CH_4 production rates. One-way analysis of variance (ANOVA) with pairwise multiple comparisons Tukey's HSD test was used to assess the effect of different electron donor additions on CO_2 and CH_4 production and turnover rates. One-way ANOVA was performed separately on each site. A Principal Component Analysis (PCA) was performed to determine major patterns of CO_2 and CH_4 production rates with the addition different electron donors. All statistical analyses were conducted with Statistica 7.1 (StatSoft 2006) software.

Results

Sediment Properties

Sediment pH ranged from 5.9 to 7.8 (Table 7-1). Lake Annie sediment pH was lower than other lakes. Both Lake Okeechobee and Lake Apopka sediment pH were circum-neutral to alkaline (Table 7-1). Surface sediment bulk densities were lowest in Lake Apopka, followed by Lake Annie, and then Lake Okeechobee sites M9, M17, and KR, respectively (Table 7-1). Sediment organic matter content was highest at Lake Okeechobee site M17, reflecting high peat content. Next in order were Lake Apopka, Lake Annie, followed by site M9, and sandy KR in Lake Okeechobee (Table 7-1). Total C was highest in peat zone sediments of Lake Okeechobee, followed by sediments from Lake Apopka and Lake Annie. Lake Apopka and peat zone sediments of Lake Okeechobee exhibited similar values of TN (Table 7-1). Lake Annie sediments exhibited higher TP concentrations than Lake Okeechobee and Lake Apopka sediments (Table 7-1).

Extractable organic C and MBC were highest in Lake Apopka sediments (Table 7-1). Lake Apopka sediments also had the highest concentrations of Ext-ON and Ext-NH_4-N (Table 7-1). Lake Annie sediments, however, had the highest concentrations of labile inorganic P and labile organic P (Table 7-1). Sediment Ext-C:Ext-N ratio was similar in all lake sediments (Table 7-1). Lake Annie and sites M9 and KR in Lake Okeechobee exhibited low Ext-C:Ext-P and Ext-N:Ext-P ratios.

Electron Donors

Dry suspended material content of three lakes (Lake-SS) was characterized as: 34.2 % C and 2.9% N from Lake Annie; 15.6 % C and 1.5% N from Lake Okeechobee; and 33.6 % C and 3.7% N from Lake Apopka. Addition of electron donors to sediment microcosms stimulated heterotrophic microbial activity (Figures 7-1, 7-2, 7-3, 7-4, 7-5, Table 7-3). All sediments

showed a quick response by increasing CO_2 production after addition of electron donors (Figures 7-1, 7-2, 7-3, 7-4, 7-5B, C). Basal CO_2 and CH_4 production rates were highest in Lake Apopka sediments (Table 7-3). Sediments from Lake Okeechobee had the longest lag phase in CH_4 production (Figures 7-1, 7-2, 7-3, 7-4, 7-5D, E, F). Addition of different electron donors produced different response in each lake sediment (Table 7-3). Results from one-way ANOVAs were significantly different ($p < 0.05$, Table 7-2). Lake Annie sediments had the highest CO_2 production rates with both amino acids and formate addition. All Lake Okeechobee sediments had the highest CO_2 production rates with both amino acids and glucose addition. Lake Apopka sediments had the highest CO_2 production rates with alanine and formate (Table 7-3).

Higher CH_4 production rates in Lake Annie sediments were detected with the addition of both amino acids and acetate. In Lake Okeechobee mud (site M9) and sand (site KR) sediments, higher CH_4 production rates were detected in alanine, butyrate, and glucose treatments. In the peat zone sediments (site M17) of Lake Okeechobee, addition of the two amino acids, acetate, and butyrate produced higher CH_4 than other substrates. In Lake Apopka, highest CH_4 production rates were detected with the addition of alanine, glucose, and Lake-SS (Table 7-3). Turnover rates were highest for alanine, arginine, and acetate in Lake Annie sediments. In Lake Okeechobee M9 and KR sediments, turnover rates were higher for alanine and glucose. In the peat sediments the highest turnover rates were for alanine, arginine, and glucose. Lake Apopka had similar values of turnover rates for different C-sources and the highest was detected for alanine (Table 7-3). Lake Apopka sediments had the highest turnover rates of all C-sources when compared to the other sediments (Two-way ANOVA, $F = 3.88$, $d.f. = 28$, $p < 0.00001$).

The magnitude of CO_2 and CH_4 production following addition of different electron donors was strongly related to microbial biomass at each site. There was a strong significant positive correlation between MBC and CO_2, and MBC and CH_4 production rates (Figure 7-6A, B).

Two Principal Component Analyses (PCA) were conducted, PCA-1 was performed using the effect of electron donor additions on CO_2 production rates, and PCA-2 on CH_4 production rates. The PCA-1 indicated that 40.7% of the data variability was explained by Axis 1 while Axis 2 explained 20.1% (Figure 7-7A). Anaerobic respiration with the additions of acetate, butyrate, formate, and Lake-SS were the variables selected by Axis 1. Basal anaerobic CO_2 production was selected by Axis 2. The position of sites in relation to variable loadings in PCA-1 showed that sediments from each lake and site are separated into different groups (Figure 7-7B). Lake Annie sediments were plotted in the position of basal CO_2 production (Figure 7-7B). Lake Apopka sediments with Lake-SS cluster (Lake-SS, butyrate, acetate, formate, and propionate) opposite from Lake Annie. Lake Okeechobee site M17 was plotted close to Lake Apopka sediments, while the KR site was in the position with glucose and alanine additions. Lake Okeechobee mud zone (site M9) was not placed with any specific carbon addition (Figure 7-7B).

The PCA-2 had 33.6% of the data variability explained by Axis 1 while Axis 2 explained 27.4% (Figure 7-8A). Methane production rates with additions of alanine, butyrate, and glucose were the variables selected by Axis 1. Methane production rates from arginine, and basal production rate were selected by Axis 2. The position of the sites in relation to the variable loadings in PCA-2 showed a separation of sediments from each lake and site (Figure 7-8B). Lake Annie sediment was placed with the basal production, arginine, and acetate cluster. Lake Okeechobee M9 site was plotted in the position of propionate and formate and close to the KR

site that was positioned with glucose, alanine, and butyrate. Site M17 was plotted in opposite position of all C-sources. Lake Apopka sediments were placed with Lake-SS (Figure 7-8B).

Discussion

Addition of organic electron donors to sediment microcosms stimulated heterotrophic activity. Findlay et al. (2003) showed that the addition of different carbon sources, i.e., glucose, bovine serum albumin and natural leaf leachate to hyporheic biofilms enhanced microbial activities. Wang et al. (2007) showed that addition of electron donors (glucose, sucrose, potato starch, and sodium acetate) stimulated denitrification in Lake Taihu (China) sediments. In the study of benthic microbial response to the deposition of natural seston in Lake Erken (Sweden), Törnblom and Rydin (1998) showed that seston addition caused an immediate increase in bacterial production, activity, and total sediment metabolism.

The extent of response to electron donor addition was strongly related to microbial biomass. Most sediments responded rapidly to addition of most of electron donors by increasing their CO_2 production rates. Sediments from site KR in Lake Okeechobee with the lowest microbial biomass showed the longest lag phase before responding to electron donor addition (Figure 7-4A, B, C, Table 7-3). The turnover rates were also related to microbial biomass. Lake Apopka sediments with the largest microbial biomass exhibited the highest turnover rates (Table 7-1, 7-3). Statistical correlations suggest that observed rates of carbon source consumption are strongly a function of microbial biomass at each site (Figure 7-6A, B). These results are in accordance with other studies (Lu et al. 2000) that have shown that the response of soils to the addition of C sources is dependent on microbial biomass.

Although the magnitude of response to electron donor additions was related to microbial biomass, different responses in each sediment were related to the catabolic diversity of microorganisms. Principal Component Analysis 1 results showed that Lake Apopka had the

highest respiration per microbial biomass with most of the electron donor additions (propionate, Lake-SS, butyrate, acetate, and, formate), indicating that these sediments respired most of the C added (Figure 7-7A, B). This suggests that the catabolic diversity and activity in these sediments is higher than other sediments. Increased biogeochemical diversity can be present in environments with high organic matter content, with diversity in organic compounds as well as increased by-products diversity (Odum 1969). As an example, Castro et al. (2005) studied the distribution of sulfate (SO_4^{-2})-reducing prokaryotic assemblages in soils of the nutrient impacted regions of the Florida Everglades. The authors reported that complete oxidizing species, which are able to use a broader array of electron donors were dominant in eutrophic and transitional sites while incomplete oxidizers, which are more efficient at taking up low concentrations of a few substrates, were present in oligotrophic regions. The authors concluded that eutrophic regions with greater amount of carbon may select for generalists capable of taking advantage of a greater diversity of carbon substrates. Lake Apopka exhibits high primary production and high labile C sedimentation (Gale et al. 1992, Gale and Reddy 1994), supporting higher catabolic diversity.

Others studies, however, have reported that under P limitation heterotrophic bacteria tend to respire added C. In controlled experiments with bacterioplankton in subarctic Lake Diktar Erik, Sweden, Jasson et al. (2006) showed that addition of C was used for growth under C-limited conditions, but used for respiration under Pi limitation. They concluded that bacterioplankton communities tend to respire large portions of added C under P limitation, and high respiration rates of "excess C" was partly used to support growth and not only for maintenance. Lake Apopka exhibited the highest extractable C:P and N:P ratios (Table 7-1). In another study, I found that microorganisms in Lake Apopka surface sediments are P limited,

while the other sediments are limited by C alone (Lake Annie) or co-limited by C and N (Lake Okeechobee sites M9 and KR) or C and P (Lake Okeechobee site M17) (Chapter 5). The results from PCA-1 also placed peat sediments (site M17), which are also P limited, close to Lake Apopka sediments. These results indicated that sediments limited by P respired the C-added, while sediments limited by C might have used the C-added for growth (Jasson et al. 2006).

Addition of some electron donors did not stimulate heterotrophic microbial respiration, and with others the stimulation was not significantly different from basal activities (Table 7-3). This could indicate lack of organisms able to use the substrate as well as the assimilation of added C into microbial biomass rather than being released as CO_2 via respiratory pathways (Bremer and van Kessel 1990; Degens 1998a). Törnblom and Rydin (1998) found that after seston addition to sediment, bacterial biomass doubled indicating assimilation of C into microbial biomass. For forested soils, the partitioning between biomass-C incorporation and respiratory CO_2-C was determined to be substrate- rather than soil-dependent. van Hees et al. (2005) reported for forested soils that 60-90% of organic acid, 20-60% of monosaccharide, and 10-30% of amino acid is evolved as CO_2. Studies with different microorganisms reported that between 30 and 40% of glucose and up to 80% of formate of the C source supplied is immediately used for respiration and the remaining for biomass growth (Stouthamer 1976). King and Klug (1982) reported that the addition of glucose into microbial biomass was low (20%) in a eutrophic lake sediment (Wintergreen Lake). In this study, amino acids, glucose and formate were the C-sources that were used through respiratory pathways rather then added into biomass.

Lake Annie was positioned with basal CO_2 production rates indicating that this site had the highest anaerobic respiration per microbial biomass (Figure 7-7A, B). Lake Apopka was positioned on the opposite side, indicating the lowest basal anaerobic respiration per microbial

biomass. The metabolic quotient (qCO_2; proportion of basal respiration per microbial biomass) has been used in soil studies to indicate ecological efficiency of the soil microbial community (Anderson and Domsch 1990; Degens 1998b). This index is based on Odum's theory of ecosystem succession (1969), where during ecosystem succession towards maturity there is a trend of increasing efficiency in energy utilization concomitant with an increase in diversity. High qCO_2 indicates inefficient use of energy, while low qCO_2 indicates high efficiency and more carbon is utilized for biomass production (Anderson and Domsch 1990; Degens 1998b; Anderson 2003; Francaviglia 2004). Moreover, if the progression of lakes in time from less productive (oligotrophic) to more productive (eutrophic-hypereutrophic) can be viewed as a natural succession, higher qCO_2 should be detected in oligotrophic lakes. The trend of decreasing qCO_2 with increasing trophic state is clearly presented in Axis 2 of the PCA-1 (Figure 7-7A, and B). The same results were reported by Smith and Prairie (2004) in the study of bacterioplankton of lakes of different trophic states. These authors concluded that oligotrophy places high respiratory demands on bacterioplankton, with greater DOC flow to CO_2 rather than to biomass.

In Lake Annie sediments addition of propionate inhibited microbial activity (CO_2 and CH_4 production rates) (Table 7-3). Lake Annie sediments are characterized by high Fe (3640 mg kg^{-1}) (Thompson 1981), and dissolved SO_4^{-2}concentration (7.2 mg L^{-1}) in the water column (Swain and Gaiser 2005). High SO_4^{-2} reduction has also been reported to occur in the water column (Swain and Gaiser 2005). Although Fe oxides and SO_4^{-2} concentrations were not measured in this study it is probably safe to assume that both Fe- and SO_4^{-2}-reducers are present and/or active in the Lake Annie sediments.

Sulfate reducers are able to utilize a variety of organic compounds, including propionate and butyrate (Widell 1988). Propionate can also be oxidized by syntrophic and acetogenic

bacteria (Stams 1994; Schink 1997). These conversions, however, are often energetically unfavorable, and continuous removal of their products by methanogens is required so that these conversions become exergonic (Stams 1994; Schink 1997; Kleerebezem and Stams 2000). Presence of Fe- and SO_4^{-2}-reducers is thought to limit syntrophic bacteria as these are able to use products of primary fermentation more efficiently (Stams 1994; Schink 1997). In marine sediments, however, it has been shown that syntrophy occurs in sediments with high SO_4^{-2} concentration (Kendall et al. 2006). The oxidation of butyrate has a mechanism similar to that one described for propionate, although different syntrophic species are usually involved (Schink 1997; Kleebrebezem and Stams 2000). Addition of butyrate did not inhibit anaerobic respiration; however, anaerobic CO_2 production was not statistically different from basal respiration (Table 7-3). Holmer and Kristensen (1994) in the study of fish farm sediments amended with labile organic matter, reported accumulation of propionate due to SO_4^{-2} reducers inhibition. They concluded that this was an indication of suppression of H_2-sensitive fermentation reactions, as the formation of H_2 and acetate from propionate is thermodynamically more sensitive to H_2 inhibition than other reactions as with butyrate and ethanol. In the study of intermediary metabolism of organic matter in sediments of Wintergreen Lake (USA), Lovley and Klug (1982) reported that addition of H_2 inhibited the metabolism of propionate whereas the butyrate metabolism was only partially inhibited. The mechanism for inhibition of anaerobic respiration with propionate addition cannot be determined with the present data. However it can be speculated that it could have resulted from the absence of species able to use propionate, or H_2 was not efficiently removed by methanogens.

Basal CH_4 production rates were highest in hypereutrophic Lake Apopka. Several studies have shown similar results where methane production rates were higher in eutrophic than

oligotrophic lakes (Casper 1992; Rothfuss et al. 1997; Falz et al. 1999; Nüsslein and Conrad 2000; Huttunen et al. 2003; Dan et al. 2004). Extremely low basal CH_4 production rates in eutrophic Lake Okeechobee sediments may be explained by electron donor limitation. In a previous study (Chapter 2), basal CH_4 production was not detected, but was stimulated after the addition of acetate and/or H_2 in sediments of Lake Okeechobee. Although a lag phase for CH_4 production was observed in all sediments, CH_4 production was much delayed in sediments from sites M17 and KR in Lake Okeechobee (Figures 7-1, 7-2, 7-3, 7-4, 7-5D, E, F). Methanogens (Archaea) are obligate anaerobes and use a limited number of substrates, including H_2 plus CO_2, formate, acetate, methanol, and methylated amines (Oreland 1988). The most important substrates for methanogens are H_2/CO_2 and acetate, and they often depend on other anaerobic bacteria for these substrates (Conrad 1999).

Other anaerobic bacteria (i.e., Fe and SO_4^{-2} reducers) can outcompete methanogens for H_2/CO_2 and acetate due to higher substrate affinities and higher energy and growth yields (Lovley and Klug 1983; Lovley and Phillips 1986; Conrad et al. 1987; Bond and Lovley 2002); however, both processes can coexist (Mountfort and Asher 1981; Holmer and Kristensen 1994; Roy et al. 1997; Holmer et al. 2003; Roden and Wetzel 2003; Wand et al. 2006). Coexistence occurs because of spatial variation in the abundance of terminal electron acceptors or because the supply of electron donors is non-limiting (Roy et al. 1997; Megonigal et al. 2004). The lag phase observed for CH_4 production in all sediments can be explained by two mechanisms. First methanogenic activity was stimulated in the presence of their substrates that were produced by fermentative activity. Second, methanogens became active after other electron acceptors (FeIII, SO_4^{-2}) were consumed and depleted in sediment microcosms.

Most methanogenic species use H_2/CO_2 and a fewer number of species can use acetate (Garcia et al. 2000). The present data do not allow any conclusions about the major pathways of CH_4 production in these sediments. Formate can be used as a substitute for H_2/CO_2, however, only about half of the H_2-users are able to use formate for CH_4 production (Vogels et al. 1988). Methane production rates from acetate can also result from syntrophic acetate oxidation to CO_2 and H_2 coupled with methanogenesis from H_2/CO_2 (Zinder 1994).

In lake sediments the dominance of acetoclastic versus hydrogenotrophic methanogenesis has been reported to be related to sediment properties (i.e., pH and temperature). In acidic Lake Grosse Fuchskuhle (Germany), with high humic content, acetate users (*Methanosarcinaceae*) were the only detected methanogens (Casper et al. 2003). Phelps and Zeikus (1984) reported that acetoclastic methanogenesis was the major pathway for CH_4 production in a mildly acidic (pH 6.2) lake (Knaack Lake, Wisconsin). The increase in pH to neutral values enhanced total CH_4 production from H_2/CO_2, but did not affect the CH_4 produced from acetate (Zeikus 1984).

Acetoclastic methanogenesis is dominant at low temperatures. In mesotrophic Lake Rotsee (Switerland) sediments, it was reported that *Methanosaeta* (acetoclastic methanogen) was the major methanogenic population (91%), indicating that in cold sediments acetate is the main CH_4 precursor, and hydrogenotrophs were only found in the organic-rich, upper 2 cm of sediment (Falz et al. 1999). Nüsslein and Conrad (2000) reported that CH_4 was produced from acetate at low temperatures (4 ºC) but it was produced from both acetate and H_2/CO_2 at higher temperatures (25 ºC) in sediments of eutrophic Lake Pluβsee (Germany). Schulz and Conrad (1996, 1997) reported a change in the methanogenic degradation pathway of organic matter in sediments of mesotrophic Lake Constance (Germany). The authors showed that CH_4 production in these cold (4 ºC) sediments was exclusively from acetate, however, an increase in temperature

(20-25 ºC) lead to an increase in contribution of CH_4 production from H_2/CO_2 and probably from methanol. They hypothesized that at low temperatures hydrogenotrophs were unable to compete with H_2-utilizing homoacetogenic bacteria. Moreover, methanogenic degradation of organic matter should be dominated by homoacetogenesis plus acetoclastic methanogenesis at low temperatures versus fermentation, syntrophy, H_2 production and hydrogenotrophic methanogenesis at high temperatures. The same results were reported for sediments from eutrophic Lake Dagow (Germany), however, the change of dominance from acetoclastic to hydrogenotrophic methanogenesis with an increase in temperature was not followed by a change in community structure of the major phylogentic groups of methanogens (Glissmann et al. 2004).

Lake Annie sediments are acidic and probably maintain fairly constant low temperatures. Thermal stratification of the water column was detected during sampling in this lake with a temperature of 17.3 ºC below 14 m water column depth (Chapter 4). Sediment temperature is probably much lower in this deep (20 m) lake. Sediment acidic pH and low temperatures as well as the high CH_4 production rate with addition of acetate and the placement of Lake Annie with acetate cluster in the PCA-2 suggests that acetoclastic methanogenesis may be an important pathway for CH_4 production in these sediments (Table 7-3, Figure 7-8A, B). Lake Okeechobee and Lake Apopka had high temperatures at the sediment-water surface (26.3-30.7 ºC), and both lakes had circum-neutral to alkaline sediment pH (Table 7-1), good conditions for hydrogenotrophic methanogenesis. In Lake Okeechobee sediments it has been determined that hydrogenotrophic methanogenesis is the main pathway of CH_4 production (Chapter 2). The pathway for methane production in Lake Apopka cannot be determined with the current data, however, hydrogenotrophic methanogenesis might be an important pathway in this hypereutrophic lake. Algae deposition is an important source of C to methanogenic activity in

Lake Apopka sediments as high stimulation of CH_4 production with addition of Lake-SS was detected (Table 7-3, Figure 7-8A, B). Lake Apopka has high primary productivity (Carrick et al. 1993) and suspended solids in the water column are mainly composed of phytoplankton biomass (Phlips et al. 1995*c*; Havens et al. 1996). Although most sediments showed an increase in CO_2 and CH_4 with Lake-SS, in Lake Apopka the increase was highest. CH_4 production in Lake Apopka sediments was highly stimulated by the addition of Lake-SS and statistically higher than basal productions. This shows how well adapted methanogens in these sediments are to using algae derived-C. Molecular studies targeting the archaeal community are necessary to elucidate the major pathway for methane production in the present study lakes.

Conclusions

Addition of organic electron donors to sediments stimulated heterotrophic activity. The extent of the response, however, was strongly related to microbial biomass and catabolic diversity. Although the magnitude of the response to electron donor addition was related to microbial biomass, the different response in each sediment was related to the catabolic diversity of the sediment microbial community. The addition of some electron donors did not stimulate heterotrophic microbial respiration, and probably resulted in the incorporation of C into microbial biomass rather than release via respiratory pathways. Lake Apopka had the highest respiration per microbial biomass, indicating that these sediments respired most of the C added. This was probably caused by a P limitation. Lake Annie showed the highest qCO_2, indicating an inefficient use of energy. The low qCO_2 found in Lake Apopka's sediment indicates high efficiency. Lake Apopka's sediment catabolic diversity was higher than in the other sediments. In relation to methane production, acetoclastic methanogenesis is probably more important in Lake Annie sediments. The importance of hydrogentrophic methanogenesis in Lake Okeechobee sediments was determined in another study. The pathway for methane production in Lake

Apopka cannot be determined with the current data. These results showed that the sediments with different biogeochemical properties had different microbial communities with distinct catabolic responses to additions of C- sources.

Table 7-1. Sediment biogeochemical properties of Lake Annie, Lake Okeechobee, and Lake Apopka.

Variables	Annie	Lake Okeechobee			Apopka
	Central	M9	M17	KR	West
pH	5.9 ± 0.01	7.8 ± 0.07	7.7 ± 0.02	7.6 ± 0.2	7.5 ±0.06
BD	0.052 ± 0.002	0.137 ± 0.06	0.143 ± 0.018	1.183 ± 0.29	0.019 ± 0.003
LOI (%)	55.6 ± 1.0	37.5 ± 0.7	86.6 ± 2.0	4.6 ± 4.3	64.9 ± 1.8
Carbon					
TC (g kg^{1})	272 ± 6.2	193 ± 2.2	482 ± 8.9	25 ± 24.0	288 ± 9.2
Ext-C (mg kg^{1})	946 ±142	279 ± 8	894 ± 87	76 ± 19	4029 ± 719
MBC (mg kg^{1})	12116 ± 487	3910 ± 157	4081 ± 157	666 ± 231	42618 ± 6423
Nitrogen					
TN (g kg^{1})	20.3 ± 0.9	12.6 ± 0.3	27.7 ± 0.4	1.5 ± 1.5	27.3 ± 1.2
Ext-NH_4-N (mg kg^{1})	226 ± 96	48 ± 4	27 ± 1	8 ± 4	386 ± 32
Ext-Org. N (mg kg^{1})	147 ± 23	83 ± 7	141 ± 14	17 ± 1	859 ± 89
Phosphorus					
TP (mg kg^{1})	1427 ± 34	1018 ± 48	207 ± 12	366 ± 78	1185 ± 74
Lab. Pi (mg kg^{1})	124 ± 9	99 ± 6	4.8 ± 2	6.5 ± 2	1.8 ± 0.6
Lab. Po (mg kg^{1})	71 ± 19	8.3 ± 1.6	4.2 ± 1.1	1.2 ± 1.5	32.3 ± 6.5
Ratios					
Ext-C:Ext-N	3	2	5	3	3
Ext-C:Ext-P	5	3	102	10	119
Ext-N:Ext-P	2	1	19	3	37

BD: bulk density, LOI: loss on ignition, TC: total carbon, Ext-C: extractable organic carbon, MBC: microbial biomass carbon, TN: total nitrogen, Ext-N: extractable labile nitrogen, Ext-NH_4-N: extractable ammonium, Ext-ON: extractable labile organic nitrogen, TP: total phosphorus, Lab. Pi: extractable labile phosphorus, Lab. Po: labile inorganic phosphorus, Ext-Po: labile organic phosphorus, Ext-P: extractable labile phosphorus.

Table 7-2. One-way ANOVA statistics of the effect of the different carbon sources addition to sediment CO_2 and CH_4 production rates and turnover rates.

ANOVA	Annie	Lake Okeechobee			Apopka
	Central	M9	M17	KR	West
	Anaerobic Respiration (mg CO_2-C kg^{-1} d^{-1})				
n	27	27	27	27	27
d.f.	8	8	8	8	8
F	63.39	33.83	27.36	9.80	3.68
p	<0.00001	<0.00001	<0.00001	0.00003	0.0103
	Methanogenesis (mg CH_4-C kg^{-1} d^{-1})				
n	27	27	27	27	27
d.f.	8	8	8	8	8
F	91.14	124.30	43.94	4.32	24.34
p	<0.00001	<0.00001	<0.00001	0.00471	<0.00001
	Turnover Rates (d^{-1})				
n	27	27	27	27	27
d.f.	7	7	7	7	7
F	98.09	139.31	14.84	6.02	3.89
p	<0.00001	<0.00001	<0.00001	0.0014	0.0115

Table 7-3. Sediment CO_2 and CH_4 production, and turnover rates, with the addition of different carbon sources. Tukey's test was conducted within sites and different letters indicate significant statistical differences at $p<0.05$. (mean ± SD).

Treatment	Annie	Lake Okeechobee			Apopka
	Central	M9	M17	KR	West
	Anaerobic Respiration (mg CO_2-C kg^{-1} d^{-1})				
Basal	100 ± 9 **a**	26 ± 1 **a**	21 ± 4 **ae**	3 ± 2 **ac**	217 ± 23 **a**
Alanine	217 ± 9 **b**	62 ± 7 **bcd**	75 ± 10 **bcd**	18 ± 4 **bc**	874 ± 109 **b**
Arginine	212 ± 10 **b**	50 ± 6 **bc**	86 ± 8 **bcf**	10 ± 4 **abc**	623 ± 350 **ab**
Acetate	135 ± 16 **a**	33 ± 1 **a**	50 ± 10 **bde**	5 ± 5 **ac**	500 ± 259 **ab**
Butyrate	87 ± 3 **ac**	30 ± 2 **a**	33 ± 6 **ade**	4 ± 3 **ac**	307 ± 183 **ab**
Formate	209 ± 22 **b**	28 ± 2 **a**	51 ± 4 **bde**	3 ± 3 **ac**	773 ± 324 **b**
Propionate	55 ± 13 **c**	32 ± 1 **a**	42 ± 11 **ade**	4 ± 3 **ac**	629 ± 152 **ab**
Glucose	96 ± 12 **a**	66 ± 5 **bd**	104 ± 25 **cef**	13 ± 3 **bc**	559 ± 129 **ab**
Lake-SS	101 ± 12 **a**	29 ± 1 **a**	33 ± 3 **ade**	4 ± 2 **ac**	418 ± 160 **ab**
	Methanogenesis (mg CH_4-C kg^{-1} d^{-1})				
Basal	37 ± 8 **a**	0.09 ± 0.0 **a**	0.16 ± 0.02**a**	0.04 ± 0.01**a**	80 ± 7 **a**
Alanine	120 ± 15 **b**	45 ± 4 **be**	10 ± 4 **b**	12 ± 5 **b**	563 ± 20 **b**
Arginine	159 ± 7 **c**	19 ± 3 **cdf**	11 ± 3 **b**	2 ± 2 **a**	220 ± 24 **ace**
Acetate	155 ± 15 **c**	26 ± 1 **cd**	11 ± 1 **b**	5 ± 9 **a**	102 ± 23 **acd**
Butyrate	85 ± 4 **d**	41 ± 3 **b**	8 ± 1 **b**	5 ± 2 **a**	192 ± 16 **acd**
Formate	36 ± 15 **a**	5 ± 2 **a**	0.3 ± 0.08 **a**	3 ± 4 **a**	44 ± 8 **acd**
Propionate	3 ± 1 **e**	13 ± 1 **ce**	0.8 ± 0.4 **a**	0.4 ± 0.5 **a**	48 ± 10 **acd**
Glucose	44 ± 8 **a**	52 ± 5 **bf**	0.7 ± 0.4 **a**	13 ± 5 **b**	374 ± 22 **ce**
Lake-SS	47 ± 8 **a**	8 ± 2 **ae**	0.0 ± 0.0 **a**	2 ± 1 **a**	355 ± 14 **ce**
	Turnover Rates (d^{-1})				
Alanine	0.67 **ab**	0.21 **a**	0.17 **ab**	0.06 **a**	2.8 **a**
Arginine	0.74 **ab**	0.14 **b**	0.19 **ab**	0.03 **ab**	1.67 **ab**
Acetate	0.57 **ac**	0.12 **b**	0.12 **acd**	0.02 **b**	1.24 **b**
Butyrate	0.34 **d**	0.14 **b**	0.08 **cd**	0.02 **b**	0.99 **b**
Formate	0.49 **c**	0.07 **c**	0.10 **acd**	0.01 **b**	1.62 **ab**
Propionate	0.12 **e**	0.09 **c**	0.08 **acd**	0.01 **b**	1.34 **b**
Glucose	0.28 **d**	0.23 **a**	0.21 **b**	0.05 **a**	1.85 **ab**
Lake-SS	0.34 **d**	0.08 **c**	0.07 **cd**	0.01 **b**	1.81 **ab**

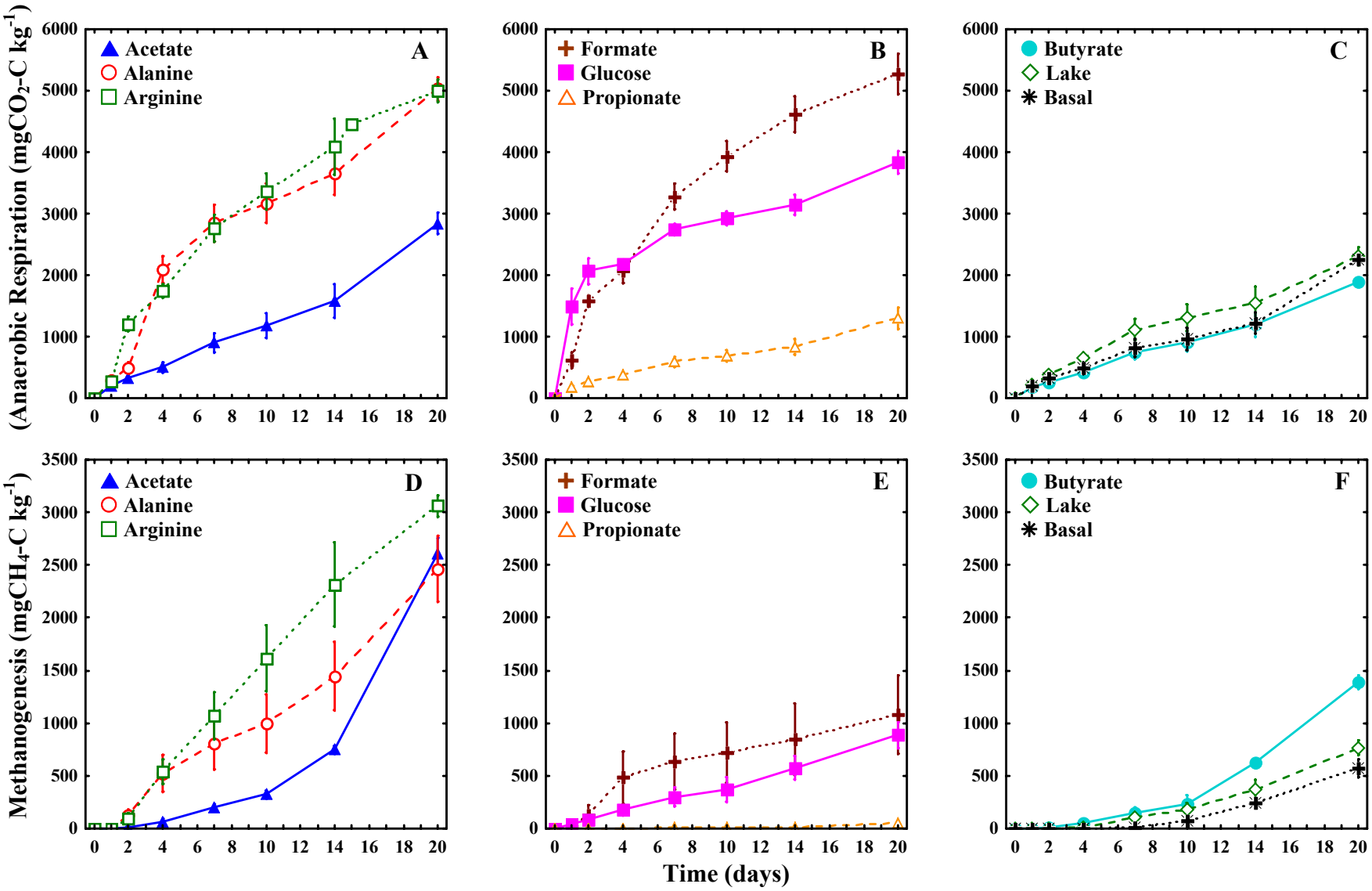


Figure 7-1. Microbial activity response to the different carbon source addition in Lake Annie sediments: A, B and C) Anaerobic respiration (mg CO_2-C kg^{-1}) vs. time (days), and D, E and F) methanogenesis (mg CH_4-C kg^{-1}) vs. time (days).

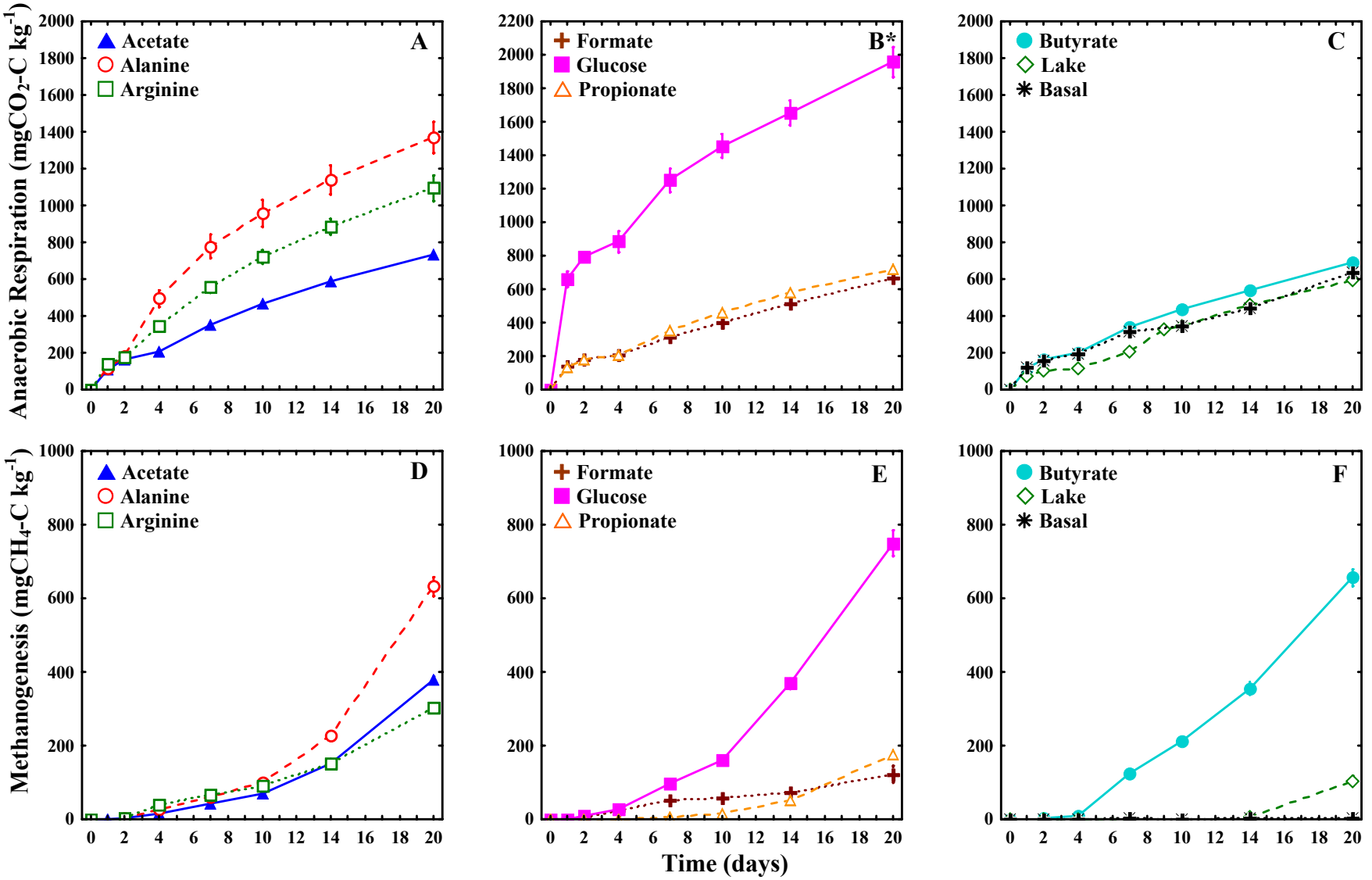


Figure 7-2. Microbial activity response to the different carbon source addition in the mud sediments (site M9) of Lake Okeechobee: A, B and C) Anaerobic respiration (mg CO_2-C kg^{-1}) vs. time (days), and D, E and F) methanogenesis (mg CH_4-C kg^{-1}) vs. time (days). *Different scales.

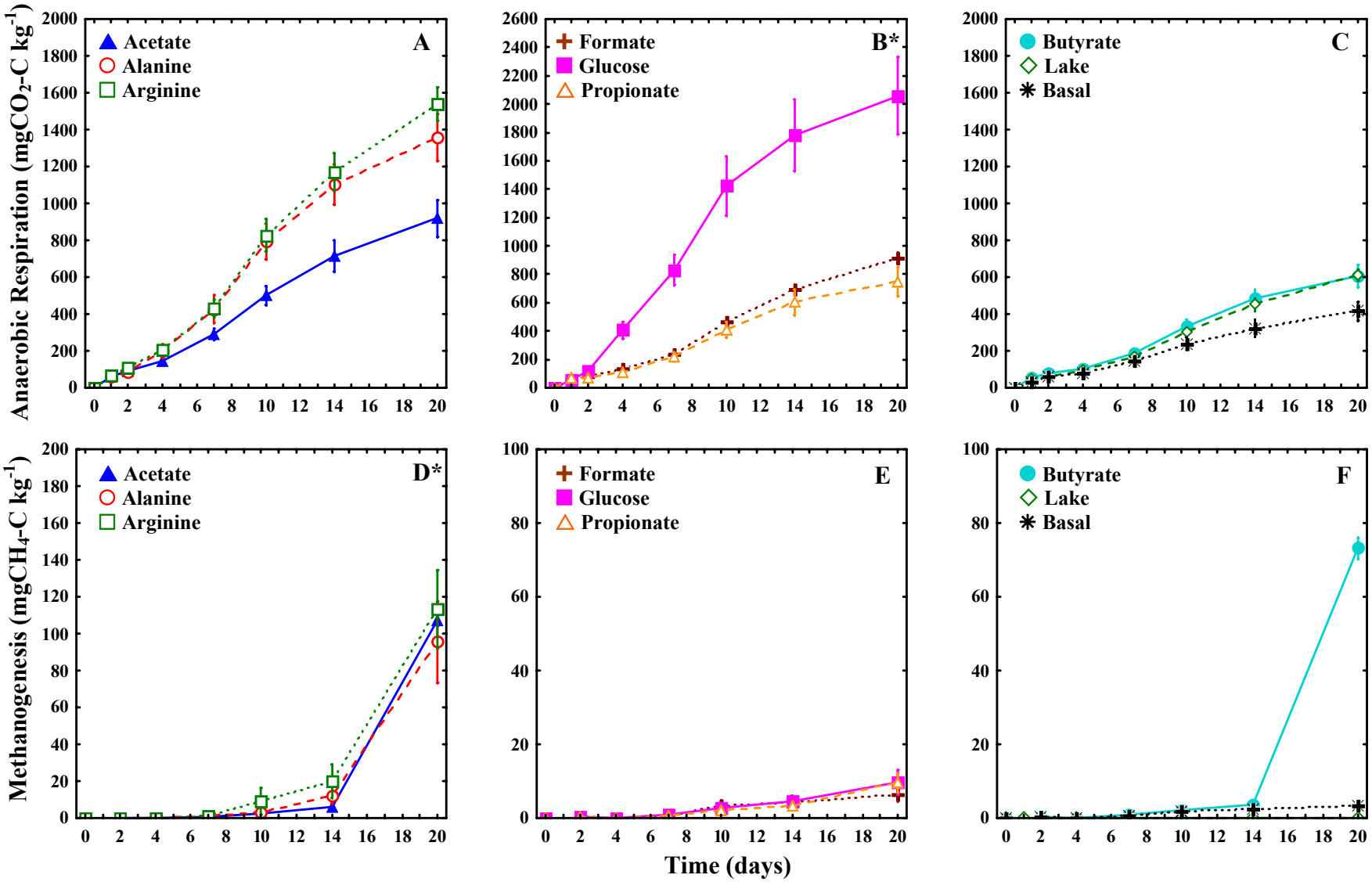


Figure 7-3. Microbial activity response to the different carbon source addition in the peat sediments (site M17) of Lake Okeechobee: A, B and C) Anaerobic respiration (mg CO_2-C kg^{-1}) vs. time (days), and D, E and F) methanogenesis (mg CH_4-C kg^{-1}) vs. time (days). *Different scales.

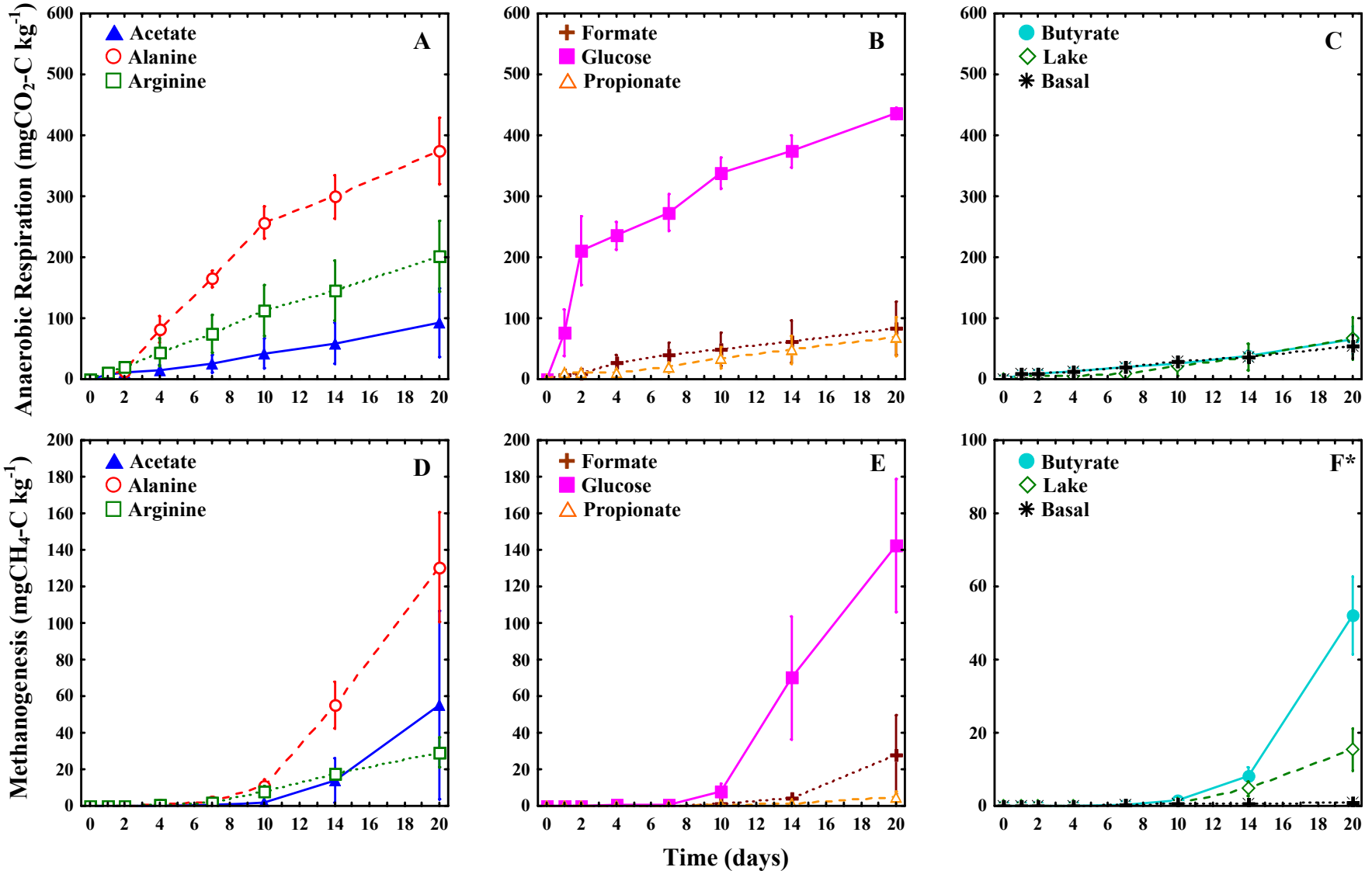


Figure 7-4. Microbial activity response to the different carbon source addition in the sand sediments (site KR) of Lake Okeechobee: A, B and C) Anaerobic respiration (mg CO_2-C kg^{-1}) vs. time (days), and D, E and F) methanogenesis (mg CH_4-C kg^{-1}) vs. time (days). *Different scales

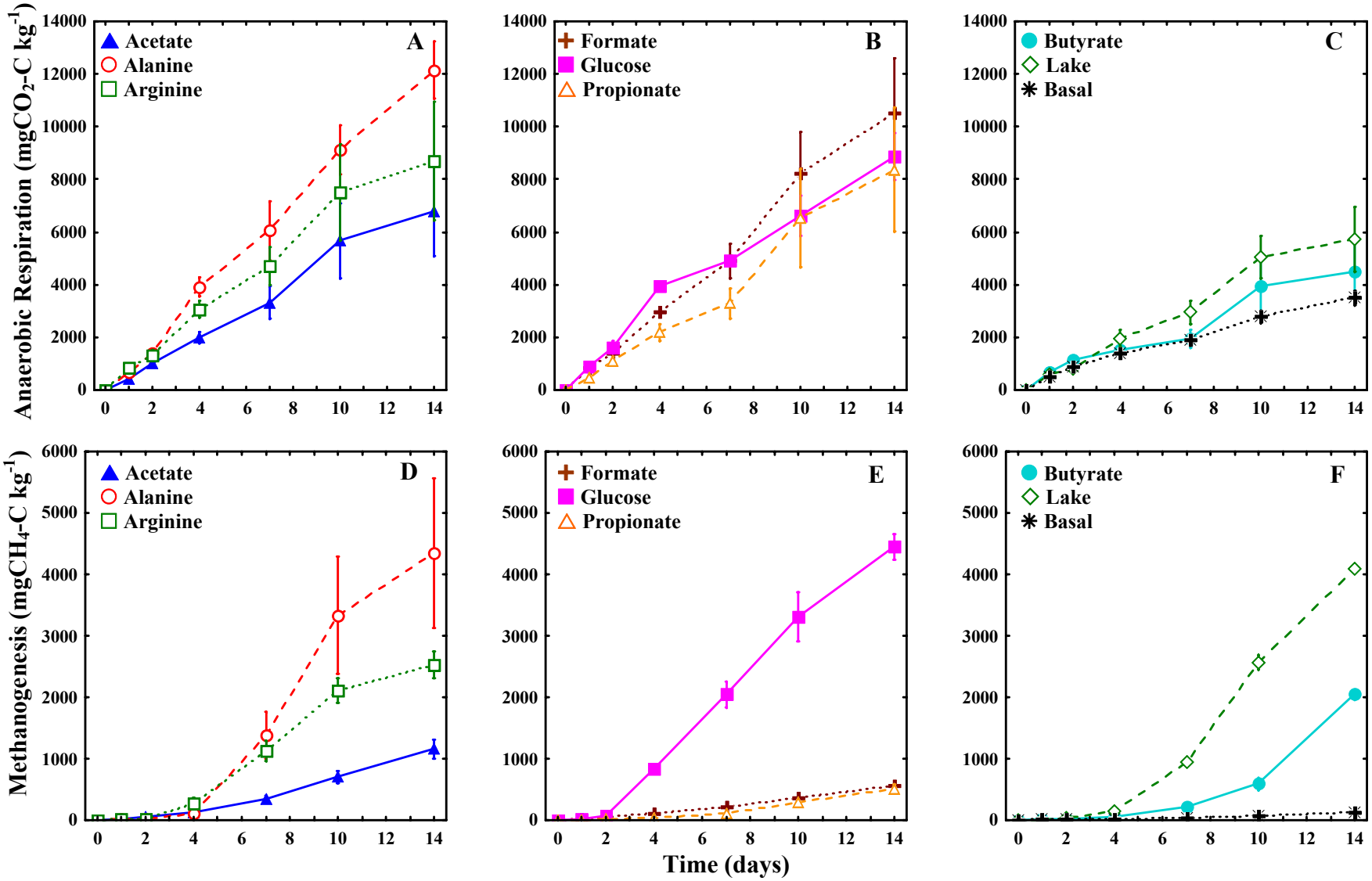


Figure 7-5. Microbial activity response to the different carbon source addition in Lake Apopka sediments: A, B and C) Anaerobic respiration (mg CO_2-C kg^{-1}) vs. time (days), and D, E and F) methanogenesis (mg CH_4-C kg^{-1}) vs. time (days).

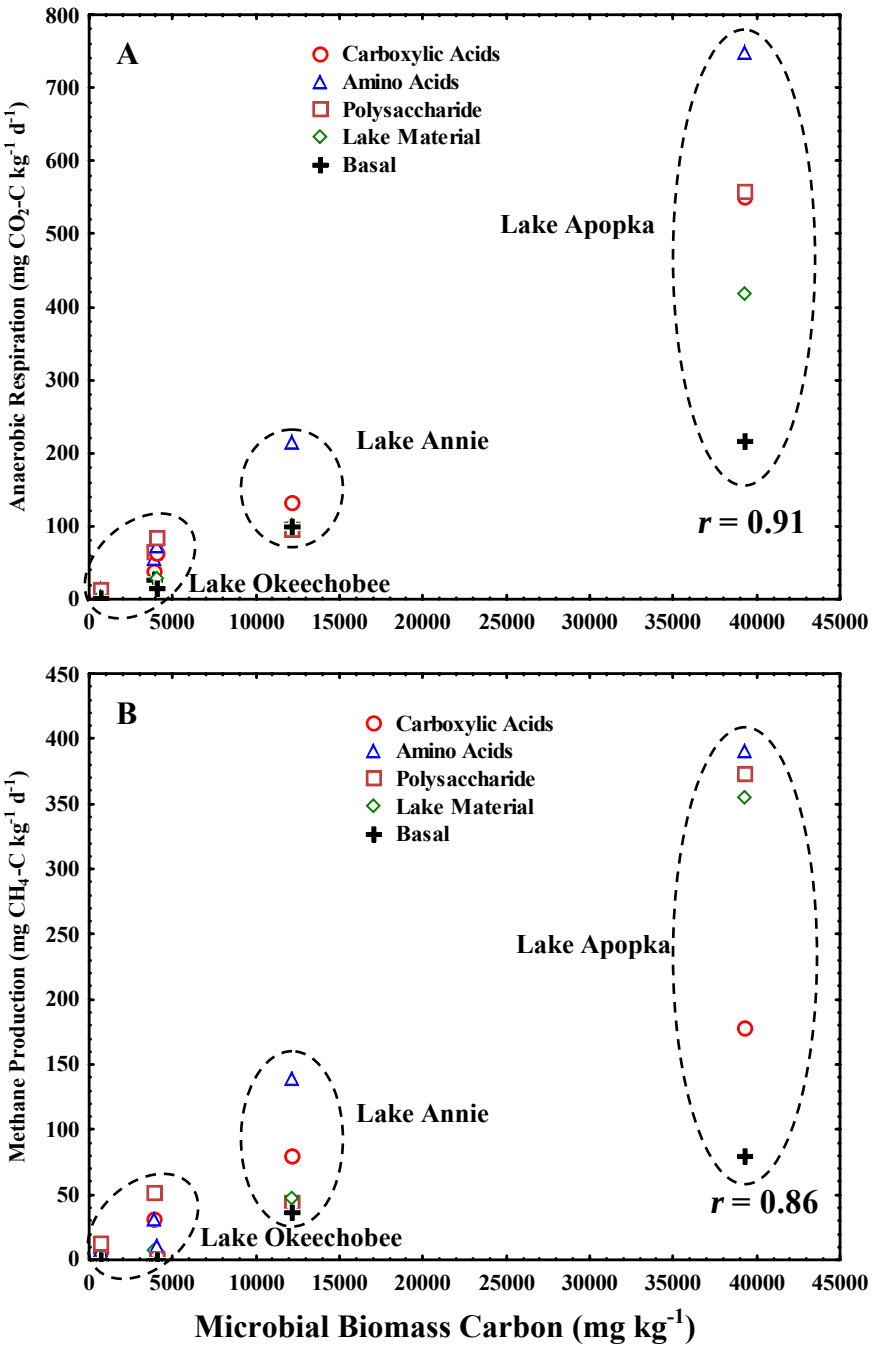


Figure 7-6. Relationship between microbial biomass carbon and activity: A) CO_2, and B) CH_4 production rates, with the different groups of carbon sources added to sediments from different lakes.

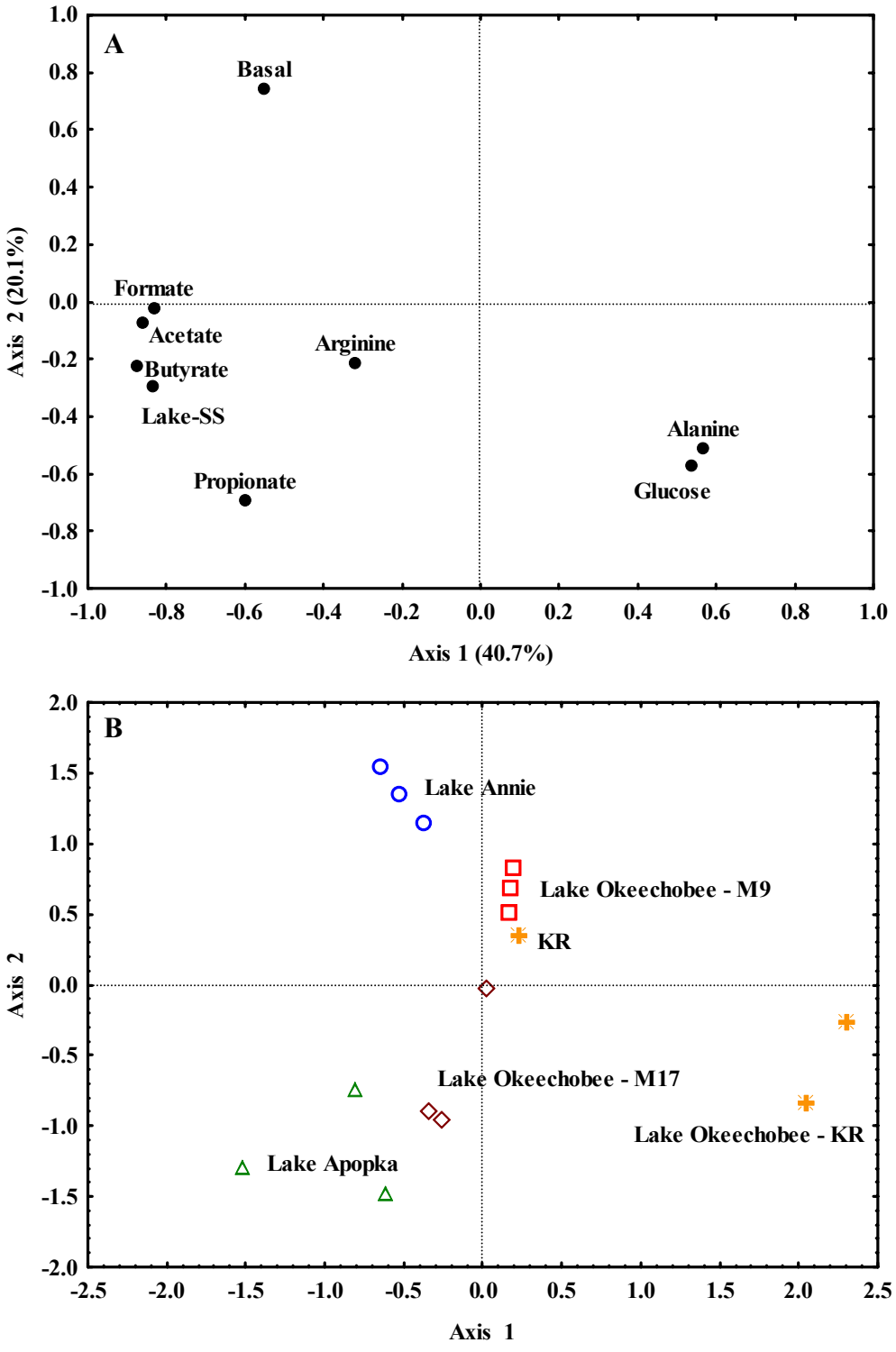


Figure 7-7. Results of the Principal Component Analysis (PCA-1): A) loadings of the effect of different carbon sources addition on sediment CO_2 production rates, and B) the plot of the scores of the sites from Lake Annie (blue circles), Lake Okeechobee: M9 (red squares), M17 (brown diamonds), KR (orange crosses), and Lake Apopka (green triangles). CO_2 production rates were normalized by microbial biomass carbon.

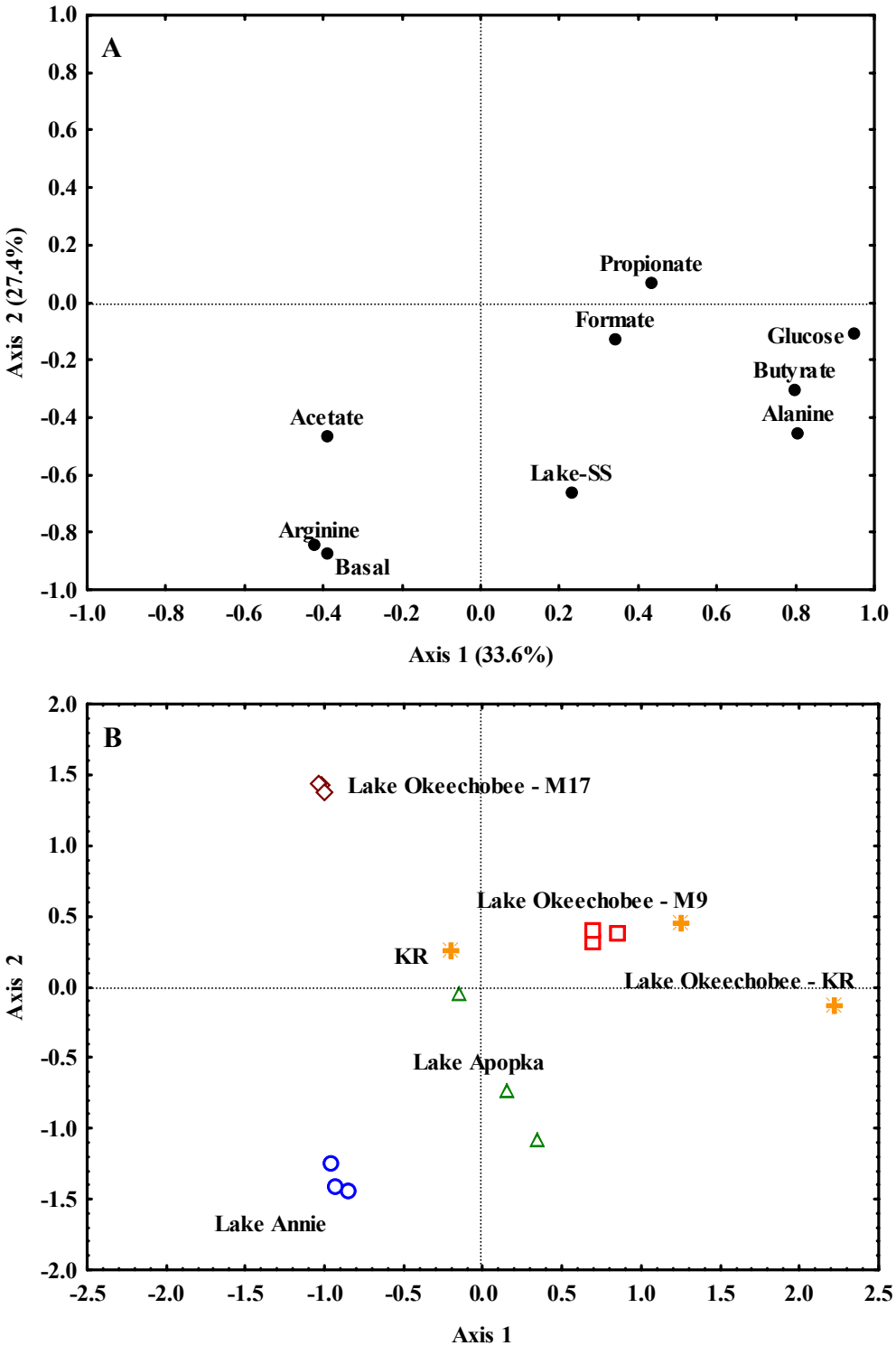


Figure 7-8. Results of the Principal Component Analysis (PCA-2): A) loadings of the effect of different carbon sources addition on sediment CH_4 production rates, and B) the plot of the scores of the sites from Lake Annie (blue circles), Lake Okeechobee: M9 (red squares), M17 (brown diamonds), KR (orange crosses), and Lake Apopka (green triangles). The CH_4 production rates were normalized by microbial biomass carbon.

CHAPTER 8
RNA-STABLE ISOTOPE PROBING OF ACETATE-UTILIZING MICROORGANISMS IN SEDIMENTS OF SUBTROPICAL LAKES

Introduction

In anoxic environments, different groups of microorganisms participate in anaerobic decomposition of organic matter as no single anaerobic microorganism can completely degrade organic polymers (Zinder 1993, Megonigal et al. 2004). Bacteria hydrolyze organic polymers through extracellular enzyme production, and under methanogenic conditions, ferment monomers to alcohols, fatty acids, and hydrogen (H_2). Alcohols and fatty acids are converted by syntrophic bacteria into acetate, H_2, and carbon dioxide (CO_2), which is used as substrate by methanogens (Zinder 1993, Conrad 1999, Megonigal et al. 2004). The structures and functions of anaerobic microbial communities are strongly affected by competition for fermentation products such as H_2 and acetate, and competition favors the following order of reduction processes, based on highest thermodynamic yield: NO_3^- > Mn(IV) > Fe(III) > SO_4^{-2} > HCO_3^- (i.e., methanogenesis) (e.g., Megonigal et al. 2004).

Several microorganisms use acetate as a carbon (C) source, making this compound the most important intermediate for microbial communities under anaerobic conditions. Acetate is assimilated into microorganism biomass and converted to methane (CH_4) and/or CO_2. In a previous study the addition of acetate enhanced both anaerobic CO_2 and CH_4 production rates in sediment microcosms of subtropical lakes with different trophic states (Chapter 7). However, the acetate-utilizing microorganisms in these lake sediments are not known. In a recent study, Schwarz et al. (2007) used RNA-based stable isotope probing to identify acetate-utilizing Bacteria and Archaea in sediments of Lake Kinneret (Israel). The authors concluded that acetate was predominantly consumed by acetoclastic methanogens and was also utilized by a small and heterogeneous community of anaerobic bacteria. In a previous study in Lake Kinneret sediments

Nüsslein et al. (2001), using addition labeled acetate, CH_4 production measurements and terminal restriction fragment polymorphism (T-RFLP), reported contrasting results from Schwarz et al. (2007). The authors concluded that hydrogenotrophic methanogenesis was the major pathway for CH_4 production, and acetate was used syntrophically by a consortium of acetate-oxidizing bacteria and hydrogenotrophs. Schwarz et al. (2007) related these different results to changes in biogeochemistry of CH_4 formation caused by environmental changes (i.e., unusually heavy rainfall, high input of C, phosphorus, nitrogen, and pollutants, and changes in biological and chemical variables). These different results, however, can be due to the use of a more sensitive technique, such as RNA-SIP, that can identify active microorganisms that constitute only a minor fraction of the total community (Schwarz et al. 2007).

Stable isotope probing (SIP) is an important tool to identify organisms utilizing a specific substrate (Radajewski et al. 2003; Whiteley et al. 2006; Neufeld et al. 2007). This procedure is based on the addition of a commercially prepared ^{13}C-labeled substrate into an environmental sample. The microorganisms that actively transform this substrate will incorporate ^{13}C into cellular biomarkers (Radajewski et al 2003; Whiteley et al. 2006; Neufeld et al. 2007). Originally SIP was applied to trace single C compounds into polar-lipid derived fatty acids of active microorganisms in sediments of Lake Loosdrecht (The Netherlands) (Boschker et al. 1998). Later this technique was extended to the use of DNA (DNA-SIP) (Radajewski et al. 2000) and RNA (RNA-SIP) (Manefield et al. 2002a) as labeled biomarkers.

DNA- and RNA-SIP are based on the principle that if an organism consumes the ^{13}C-labeled substrate, cell components will incorporate the 'heavy' isotope through anabolic processes (Radajewski et al 2003; Whiteley et al. 2006; Neufeld et al. 2007). The separation of labeled and non-labeled nucleic acids is accomplished by isopycnic centrifugation. In this

density gradient centrifugation, the 'heavier' ^{13}C migrates faster than the 'light' unlabeled ^{12}C nucleic acids. The DNA and RNA sequences present in the 'heavy' gradient fractions must be derived from organisms that have consumed the added ^{13}C labeled substrate. DNA-SIP is the least sensitive approach because it requires cell division to obtain sufficient label into DNA, thus requiring longer incubation times where cross-feeding and false results can occur (Wellington et al 2003; Neufeld et al. 2007). RNA-SIP has proven a more sensitive approach, since in active cells RNA synthesis occurs at higher rates, and labeling can occur without replication of the organism (Manefield et al. 2002a).

In the present study, RNA-SIP was used to identify microorganisms that utilize acetate in sediments of subtropical lakes with different trophic states. This approach, however, did not work. This chapter was written with the intent to document the methods used at every step and explore the source of error that contributed to the failure of the proposed study.

Materials and Methods

Study Sites and Field Sampling

Three Florida (USA) lakes ranging in trophic state were selected: Lake Annie (oligo-mesotrophic), Lake Okeechobee (eutrophic) and Lake Apopka (hypereutrophic). A map of the lakes with sampling locations as well as descriptions of the three lakes were reported previously (Chapter 2 and 3) Triplicate sediment cores were collected using a piston corer (Fisher et al. 1992) or by SCUBA divers. The topmost 10 cm of sediment were collected from one central site in Lake Annie on June 25, 2005 and a western site in Lake Apopka on May 28, 2005. Cores were collected at three sites in Lake Okeechobee on July 16, 2005: M17 = peat, M9 = mud and KR = sand.

Samples were transported on ice and stored in the dark at 4 ºC. Sub-samples were taken and frozen and kept at -80 ºC. These samples were also used in a previous study in which eight

different C sources were added to the sediment and microbial activity (anaerobic CO_2 and CH_4 production) was measured (Chapter 7).

RNA Extraction

Due to the high water content of sediments from Lake Annie and Lake Apopka and site M9 in Lake Okeechobee, pore water was removed (centrifuged at 10,000 x g for 10 min) prior to RNA extraction. Total RNA was extracted with the RNA PowerSoil isolation kit (Mo Bio Laboratories, Solana Beach, CA) using 1.0 g of sediment. Extracted RNA was evaluated by electrophoresis in agarose gel and ethidium bromide staining.

Pre-Experiment

Samples from sediments with high (site M9) and low (site KR) RNA yield from the first extraction were used to evaluate if the concentration of added [^{13}C]acetate and/or the length of the incubation would affect the concentration of the RNA extracted. Experiments were conducted in duplicate for both incubation time and concentration of substrate. Sediment (1:2 sediment to medium ratio, i.e., 1.0 g sediment:2 ml of medium) was added to anoxic BCYT-R medium (basal carbonate yeast extract trypticase-peptone containing 0.01 g L^{-1} trypticase-peptone) (Touzel and Albagnac 1983; Chauhan and Ogram 2006*a*) under N_2 stream to prevent exposure to oxygen and immediately crimped using butyl rubber septa and aluminum. Samples were reduced with cysteine (2%) to a final redox potential of approximately -110 to -200 mV (Chauhan and Ogram 2006*a*) and preincubated at 28 ºC in the dark for 1 week, prior to acetate addition. ^{13}C-labeled acetate (both carbon atoms labeled; Isotec, Miamisburg, OH) was added from anaerobic sterile stock solutions at two final concentrations (1 mM and 5 mM) and kept in the same incubation conditions. After 24 hours and 1 week of incubation, RNA was extracted as described previously.

RNA-SIP Experiment

Incubation and RNA extraction

Triplicate samples from Lake Apopka (core # 93, 94, 95), Lake Annie (core # 120, 121, 122), and Lake Okeechobee sites M9 (core # 147, 148, 149) and M17 (core # 168, 169, 170) were incubated for 1 week, as described previously. ^{13}C-labeled acetate was added from anaerobic sterile stock solutions to a final concentration of 1 mM. After 24 hours, total RNA was extracted as described previously.

Escherichia coli RNA

Escherichia coli (*E. coli*) RNA was extracted to be used as a RNA control (unlabeled ^{12}C RNA) to evaluate possible mixing of [^{12}C] RNA with ^{13}C labeled bands (Chauhan and Ogram 2006*a*). *E. coli* (strain TOP10F') was grown in 10 ml Luria-Bertani (LB) medium at 37ºC for 24h. The culture was then transferred to 260 ml LB medium and incubated for 2 hours in a shaker at 100 rpm at 37 ºC. Total RNA was extracted from *E. coli* culture with TRI Reagent (Ambion, Austin, TX) according to the manufacturer's instructions. *E. coli* RNA was resuspended in 1.0 ml of nuclease-free water, and the concentration was determined by spectrophotometry (GeneQuant, Biochem Ltd., Cambridge, UK).

Isopycnic centrifugation

Density gradient centrifugation was performed as described by Manefield et al. (2002*a*, *b*) and Lueders et al. (2004*a*). A total of four different centrifugations were performed and some modifications were made in each one to improve the density gradient.

First centrifugation. The gradient medium consisted of 2.56 ml of a 2.0 g ml^{-1} cesium trifluroacetate (CsTFA) (Amersham Pharmacia Biotech, Buckinghamshire, UK), 410 µl of nuclease-free water and 120 µl of formamide. Ten microliters of *E. coli* RNA (100 ng) and 100 µl of sample RNA were then added to the gradient medium. Gradient solutions were loaded in

Beckman polyallomer bell-top Quick-Seal centrifuge tubes (13 x 32 mm), sealed and centrifuged in a Beckman Coulter Optima TLX ultracentrifuge (TLA100.3 rotor) at 128,000 x g for 36 h at 20 ºC. Gradient solutions were fractionated via displacement by water (0.1% v/v DEPC-diethylpyrocarbonate treated) at the top of the tube and fractions were collected at the base of the tube. A controlled flow rate (0.2 ml min^{-1}) of water was used (LC-10AS Schimadzu HPLC pump) (Figure 8-1, 8-2). A total of 15 fractions of 200 µl each were collected from each centrifuge tube. The density of each fraction was determined by weighing 10µl of each fraction. RNA from each fraction was isolated by precipitation with isopropanol (Whiteley et al. 2007). Presence of RNA in each fraction was confirmed by standard agarose gel electrophoresis and ethidium bromide staining.

Second centrifugation A second centrifugation was performed with DNA-free RNA samples as described before. DNA was removed from samples as described below.

DNA removal. The triplicate incubations for each sediment core were combined to increase the amount of RNA. All RNA samples (sediment and *E. coli*) were re-purified with a PureLink micro-to-mid Total RNA Purification System (Invitrogen, Chicago, IL) according to the manufacturer's instructions for optimal DNAse in-column treatment. RNA concentration was determined by spectrophotometry (GeneQuant, Biochem Ltd., Cambridge, UK).

The presence of DNA in DNase-treated *E. coli* RNA samples was verified by PCR. *E. coli* RNA not treated with DNase (i.e, with DNA present) was used as a positive control to assure that the absence of *E. coli* DNA in cleaned samples resulted from successful removal of the DNA and not from PCR technical issues. PCR tubes were prepared as follows: 2.0 µl of nuclease free water, 1.0 µl of each primer, 10 µl of HotStarTaq Master Mix (QIAGEN, Valencia, CA) and 5.0 µl of *E. coli* RNA. Conditions of the PCR used were reported by Uz et al. (2003).

Third centrifugation. I contacted Dr. Andrew Whiteley (CEH Oxford, UK), co-author of Manefield et al. (2002a, b), to discuss potential troubleshooting of my experiments. Following Dr. Whiteley's suggestions and protocols (Whiteley et al. 2007), some modifications were done. First, two blank density gradients (without RNA) were prepared and centrifuged along with experimental samples to verify the distribution of the density gradients. The new gradient medium consisted of 2.64 mL of a 2.0 g/mL CsTFA (Amersham Pharmacia Biotech, Buckinghamshire, UK), 508 µl of nuclease free water, 109 µl of formamide. Prior to centrifugation, RNA samples were concentrated by precipitation with isopropanol and resuspended in 10 µl of nuclease free water (large volumes of water with RNA can affect the shape of the gradient - Whiteley pers. comm.). Then, 1.0 µl of *E. coli* RNA (100 ng) and 9.0 µl of sediment sample RNA (or 10 µl of nuclease-free water for the blank) were added to the gradient medium. A second modification consisted of preparing the density gradient for all tubes and later loading the specific amount to each centrifuge tube containing an RNA sample. Previous preparations were made separately for each tube and small differences could occur due to pipetting error. Density gradients were centrifuged as described previously with the following minor modification: an extended period of centrifugation of 42 h. Gradients were fractionated and a total of 30 fractions of 100 µl were collected from each tube. In addition, a loading dye (green) was mixed with the water used for displacement of the gradient to facilitate the visualization of mixing between water and the gradient solutions (Figure 8-2).

Fourth centrifugation. Gradient media were prepared as described in the third centrifugation. Three gradient density blanks and three samples containing different concentrations of *E. coli* RNA were used (1.0 ng, 10 ng and 100 ng). This new approach was performed to determine if different concentrations of *E. coli* RNA could improve the separation

of ‘heavy’ and ‘light’ RNA. Density gradients were centrifuged as described previously with an extended period of centrifugation of 46 h.

RT- PCR

RT- PCRs were conducted using an Access RT-PCR System (Promega, Madison, WI) following manufacturer instructions. *E. coli*-specific primers ECA75F (5’-GGAAGAAGCTTGCTTCTTTGCTGAC-3’) and ECR619R (5’-AGCCCGGGGATTTCACATCTGACTTA-3’) were used (Sabat et al. 2000). Bacterial genes were amplified with universal bacterial primers 16S rRNA gene sequences 27F (5’-AGAGTTTGATCMTGGCTCAG-3’) and 1492R (5’-TACGGYTACCTTGTTACGACTT-3’) (Lane 1991). Archaeal 16S rRNA genes were amplified with the universal primer 1492R and Archaea-specific primer 23F (5’-TGCAGAYCTGGTYGATYCTGCC-3’) (Burggraf et al. 1991). RT- PCRs were performed in an iCycler PCR system (Bio-Rad, Hercules, CA). RT-PCR products were analyzed by agarose electrophoresis.

Results

RNA Extraction

RNA extraction was successful for Lake Annie, Lake Apopka and Lake Okeechobee site M9 sediments but not for sediments from sites M17 and KR since RNA bands could not be visualized in agarose gels (Figure 8-3). All following experiments were conducted using 1.0 g of sediment from lakes Annie, Apopka and Okeechobee site M9. For sites KR and M17, the amount of sediment used was increased to 2.0 g to improve RNA extraction yield.

Pre-Experiment

Neither time nor concentration affected the quality of the RNA extracted from site M9 (Figure 8-4A). No visible RNA was extracted from sediments from site KR (Figure 8-4B).

Sediments from site KR are characterized by having low microbial biomass and activity (Chapter 7) and were excluded from further experiments.

RNA-SIP Experiment

Both RNA and DNA were observed in triplicate samples from sediments of Lake Apopka, Lake Annie, and Lake Okeechobee sites M9 and M17 (Figure 8-5).

Escherichia coli RNA

Concentration of extracted RNA from *E. coli* culture was 500 µg μl^{-1}, and DNA was observed in the samples (Figure 8-6).

Isopycnic centrifugation

First centrifugation. Six RNA samples were used in the first ultracentrifugation, and two samples were lost due to problems during piercing of the Beckman tube. The density of the gradient did not follow the expected linear distribution (Manefield et al. 2002*b*, Whiteley et al. 2007) thus no separation of 'heavy' and 'light RNA occurred (Figure 8-7, 8-8).

RNA from each fraction could not be visualized in agarose gel electrophoresis. Such phenomena could be due to the low concentration of RNA in each fraction. Reverse transcription polymerase chain reaction (RT-PCR) was conducted to verify if *E. coli* RNA was present in each fraction. Lake Apopka (core # 93) fractions were chosen as it was the best density distribution obtained for all samples. However, smears visualized in the agarose gel could be an indication of either primer contamination or RNA and/or primers degradation (Figure 8-9A). New primers were ordered and RT-PCR was conducted as described previously. Agarose (1%) gel electrophoresis showed the presence of *E. coli* in all fractions and confirmed that the ultracentrifugation failed to separate 'heavy' and 'light' RNA (Figure 8-9B).

The presence of DNA in both *E. coli* and sediment RNA samples (Figure 8-5A, B, 8-6), could be affecting the gradient medium and may be responsible for the detection of *E. coli* (DNA

or RNA) in all fractions. Thus, DNA was removed from all samples. *E. coli* DNA was not present in samples that were treated with DNase indicating a successful removal of DNA (Figure 8-10).

Second centrifugation. The results of the second centrifugation from two samples are as follows: there was some improvement of the expected linear distribution the density gradient (Figure 8-11A, B, 8-8A, B).

RT-PCR was performed with Lake Apopka fractions to detect *E. coli* RNA with the minor modification of 35 PCR cycles. *E. coli* RNA could be detected in all fractions confirming that the ultracentrifugation failed to separate the 'heavy' and 'light' RNA (Figure 8-12).

Third centrifugation. Four samples (two blanks and two sediment samples) were used in this ultracentrifugation. One of the blanks was lost due to problems during piercing of the Beckman tube. The expected linear distribution of the gradient improved considerably and it was similar to the one reported by Manefield et al. (2002*b*) and Whiteley et al. (2007) (Figure 8-13, 8-8A, B), indicating that modifications improved the methodology considerably. However, plateaus could be observed in all density gradient graphs. A slightly longer centrifugation may solve this problem (Whiteley pers.comm.).

If the new gradient fraction by density successfully separated the 'heavy' and 'light' RNA, fully labeled RNA was expected to have densities of approximately 1.79-1.81 g ml^{-1}, therefore around fraction numbers 5-7 (Whiteley pers. comm.). RT- PCR was conducted to check for Bacteria and Archaea RNA present in each fraction of Lake Apopka (core # 94). Expected products of RT-PCR were found in all fractions. The fractionation of 'heavy' and 'light' RNA was not achieved by the modified methodology (Figure 8-14). RT-PCR products were found in all fractions when universal bacterial primers were used, indicating the presence of 'light' RNA

from *E. coli* and/or native bacteria from sediments. Products of RT-PCR Archaea RNA were not found in any fractions (Figure 8-14).

Fourth centrifugation. Another attempt was made to verify if a density gradient fractionation with only 'light' RNA could be obtained. A longer centrifugation was performed in an attempt to remove the plateaus observed in the gradient fractions from the previous ultracentrifugation. One of the blanks and the 1.0 ng *E. coli* RNA was lost during fractionation due to problems during piercing of the Beckman tube. The distribution of the gradient density improved with longer centrifugation; however, plateaus could still be observed in some samples (Figure 8-15).

RT- PCR was done with the 10 ng and 100 ng *E. coli* RNA fractions. RT-PCR products for 10 ng *E. coli* RNA fractions were not found in any fraction, likely due to low RNA concentration (Figure 8-16A). RT-PCR products for 100 ng *E. coli* RNA fractions, however, were found in all fractions (Figure 8-16B). Although a linear distribution of density gradient could be observed, still 'light' *E. coli* RNA could be found throughout the gradient medium.

These experiments were conducted from February-November 2006. Collectively, the data indicated that improvements were still needed to obtain optimal density gradient fractioning of RNA samples. As all sediment samples from Lake Annie and Lake Apopka were used during the trial experiments, RNA-SIP experiments were discontinued.

Discussion, Conclusions and Recommendations

RNA-SIP has proven to be a sensitive approach to link microorganism function with phylogeny (Lueders et al. 2004*b*; Manefield et al. 2005; Haichar et al. 2007; Hatamoto et al. 2007; Hori et al. 2007; Schwarz et al 2007). RNA-SIP has been successfully applied to study functional diversity in several ecosystems. Manefield et al. (2005) used RNA-SIP to identify the

dominant phenol-degrading organisms from an industrial wastewater treatment plant. Schwarz et al (2007) used RNA-SIP to identify acetate-utilizing Bacteria and Archaea in sediments of Lake Kinneret (Israel). RNA-SIP was also used to identify acetate-assimilating organisms in an anoxic rice field (Hori et al. 2007) and cellulolytic bacteria in soils (Haichar et al. 2007). Organisms responsible for syntrophic oxidation in sludge (Hatamoto et al. 2007) and flooded soil (Lueders et al. 2004*b*) were also identified through RNA-SIP. The use of RNA-SIP has proven to be an effective method, because RNA is produced independently of cellular replication, and the activity of slow- and non-replicating cells can be detected (Manefield et al. 2007).

Fully labeling the target RNA with ^{13}C is essential to achieve an optimal separation of "heavy" and "light" RNA (Whiteley et al. 2005). A substantial amount of stable isotope atoms in the target RNA facilitates the density gradient separation by ultracentrifugation of labeled and unlabeled nucleic acids (Manefield et al. 2002*a*, 2007). High levels of labeled substrate beyond the naturally occurring concentrations and extended periods of incubation, however, can increase the chance of labeling non-target organisms through trophic interactions and cross feeding (Manefield et al. 2007). Thus, the [^{13}C]-acetate concentration and short incubation periods were chosen to avoid the above mentioned problems. However, RNA from the lake sediments were probably not fully labeled with ^{13}C. One week pre-incubation was chosen to exhaust naturally occurring C sources. Thus, when the [^{13}C]-acetate was added to the sediment microcosms, the microorganisms would assimilate it faster. To verify if the target RNA is fully labeled, measuring isotope ratio by mass spectrometry has been suggested (Manefield et al. 2002a). Different [^{13}C]-acetate concentrations must be run along with different periods with incubation to determine the optimal concentration and incubation period.

Another important issue that may have contributed to, or might be the main reason for the failure of this study, was the apparatus for collecting the gradient fractions (Figure 8-1, 8-2). After ultracentrifugation, tubes were placed in a holder. Then, needles (top and bottom) had to be introduced manually (Figure 8-2). To introduce the needle into the tube, a fair amount of force had to be used and sometimes it would make the needle go too deep in the tube and may disrupt the density gradient. If not enough force was used, the tip of the needle would be located too close to the hole and leaking would occur. The gradient then would be fractionated by the air, causing an improper fractionation. Of all procedures to fractionate the samples, the introduction of both needles proved to be the most difficult, unreliable, and consequently difficult to reproduce. Therefore, proper fractionation of the samples must be achieved. Samples were lost with every ultracentrifugation because of problems during puncture of the tube. Manual fractionation for small volume gradients is extremely difficult to control accurately (Whiteley et al. 2007). The use of a Beckman Fraction Recovery System, an apparatus developed to fractionate Quick-Seal tubes, is highly recommended (Whiteley et al. 2007).

The addition of control *E. coli* RNA to sediment RNA samples was not the reason for the failure of the experiment, since the density gradient was achieve in the third and fourth experimental centrifugation. However, it might not be suited for RNA-SIP experiments. Although this procedure has proven to be a successful control for DNA-SIP experiments (Chauhan and Ogram 2006*a*, *b*), for the RNA-SIP experiments it seems not to be adequate. DNA-SIP density gradient medium is prepared with CsCl (cesium chloride) and ethidium bromide and, typically, the labeled and unlabeled DNA can be visualized as two distinct bands, under UV light (Radajeski et al. 2000; Friedrich 2006). Chauhan and Ogram (2006*a*) reported that *E. coli* DNA was not detected in the denser [^{13}C]DNA fractions, but it was detected in all

lighter[^{12}C]DNA fractions, demonstrating that *E. coli* DNA was a successful control for the separation of the 'heavy' and 'light' DNA. In this study, although similar density gradients were obtained (Figure 8-13, 8-15) as described by (Manefield et al. (2002*b*); Whiteley et al. 2007) (Figure 8-8A, B), *E. coli* RNA was found throughout the density gradient, demonstrating that even after extended ultracentrifugation periods, 'heavy' RNA fractions can still contain 'light' RNA (Figure 8-14). Furthermore, the presence of *E. coli* RNA mixed with the 'heavy' RNA may be a further problem in the experiment. The creation of a 'heavy' RNA library is the goal of this method, thus the cross-contamination of 'heavy' and 'light' RNA may produce a large number of false-positive clones.

Small differences in buoyant densities are usually observed in RNA-SIP experiments. Typically, unlabeled RNA has a buoyant density of 1.755 g ml^{-1} while labeled RNA has a buoyant density of between 1.795 and 1.80 g ml^{-1}. However, several studies have shown overlapping of these two fractions (Manefield et al. 2002*a*; Lueders et al. 2004*a*). The detection of 'heavy' and 'light' RNA in the CsTFA density gradient fractions can also be caused by interactions of RNA molecules forming secondary structures (Lueders et al. 2004*a*). Recently, Lueders et al. (2004*a*) demonstrated that DNA- and RNA-SIP methodologies are distinguished in the fractioning of ^{12}C and ^{13}C-containing targets (Figures 8-17, 8-18). The authors compared the sensitivity of the two SIP methods with labeled (^{13}C) and unlabeled (^{12}C) pure cultures of *Methylobacterium extorquens* and *Methanosarcina barkeri*. DNA-SIP CsCl density gradient was effective to separate 'heavy' and 'light' DNA, either when samples were centrifuged separately (Figure 8-17A) or simultaneously in the same tube (Figure 8-17B).

However, separation of 'heavy' and 'light' RNA using RNA-SIP CsTFA density gradient could only be achieved when they were centrifuged separately (Figure 8-18A). An incomplete

separation was observed when samples were centrifuged in the same tube (Figure 8-18B). Several studies have shown overlap between 'heavy' and 'light' RNA in CsTFA density gradients (Manefield et al. 2002*a*; Lueders et al. 2004*a*, *b*; Haichar et al. 2007; Hatamoto et al. 2007; Schwarz et al. 2007). Although using *E. coli* RNA as control is a valid approach, it should not be added to the tube with the 'heavy' RNA sample, but centrifuged in a separate tube. Then, the density gradient of the *E. coli* RNA can be compared with the density gradient of the experimental sample.

Considering the problems that occurred during the RNA-SIP experiment several suggestions can be made. The first recommendation is to conduct several experiments with different concentrations and maybe pulses of [^{13}C]-acetate, with different incubation periods. Second, samples should be checked for amount of labeling by mass spectrometry, since it is necessary to assure that a sufficient amount of labeled RNA is present. Once this is determined, samples should be incubated in two different sets: one with [^{13}C]-acetate and another with [^{12}C]-acetate. RT-PCR of [^{12}C]-acetate density gradients can be used to compare with [^{13}C]-acetate density gradients. *E. coli* RNA can be used as a control as long as it is not added in the same tube of labeled target RNA. Blank density gradients should be used along with other samples during centrifugation, so the linear distribution of blank density gradients should be verified before fractionation of the samples. Finally, proper equipment to fractionate the samples after ultracentrifugation, such as a Beckman Fraction Recovery System, should be used for a more precise fractioning of the samples.

Figure 8-1. Picture of the apparatus for fractionating the gradients.

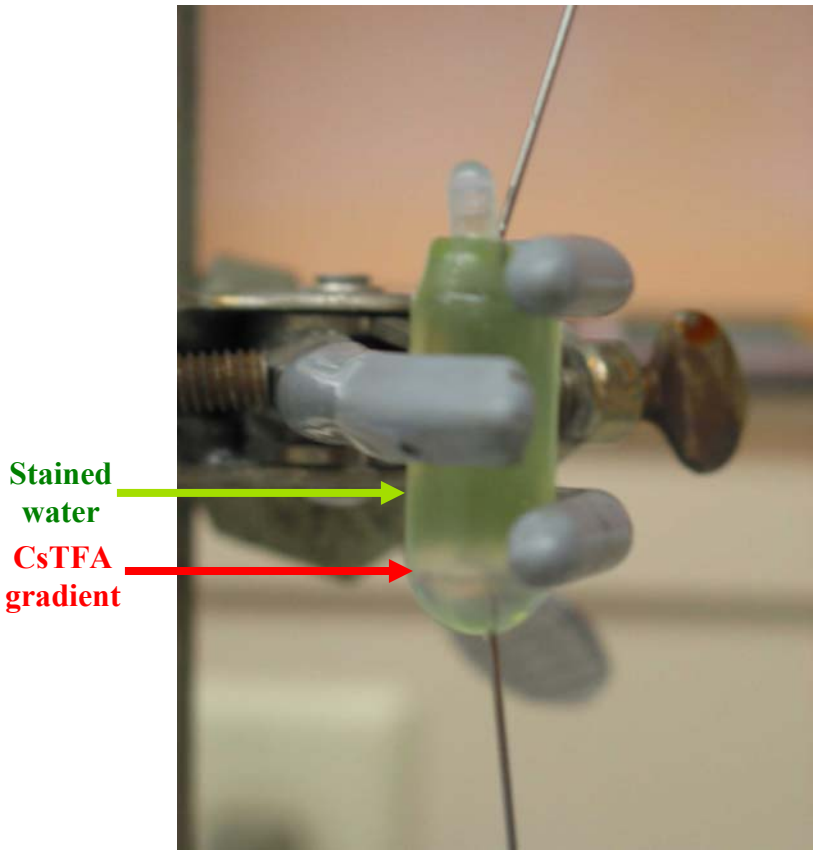


Figure 8-2. Photograph of gradient fractionation by displacement with stained water (green).

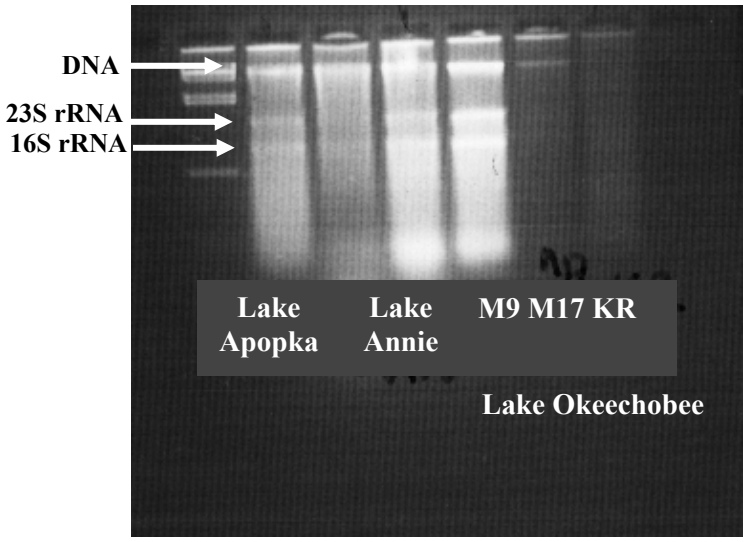


Figure 8-3. Agarose (2%) gel electrophoresis of RNA extracted from the three lakes sediments.

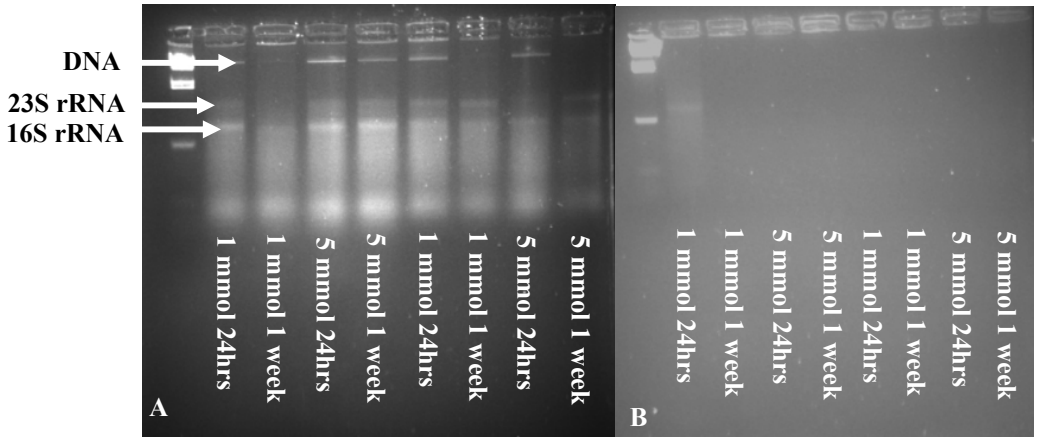


Figure 8-4. Agarose (2%) gel electrophoresis of RNA extracted from sediments of Lake Okeechobee sites M9 (A) and KR (B).

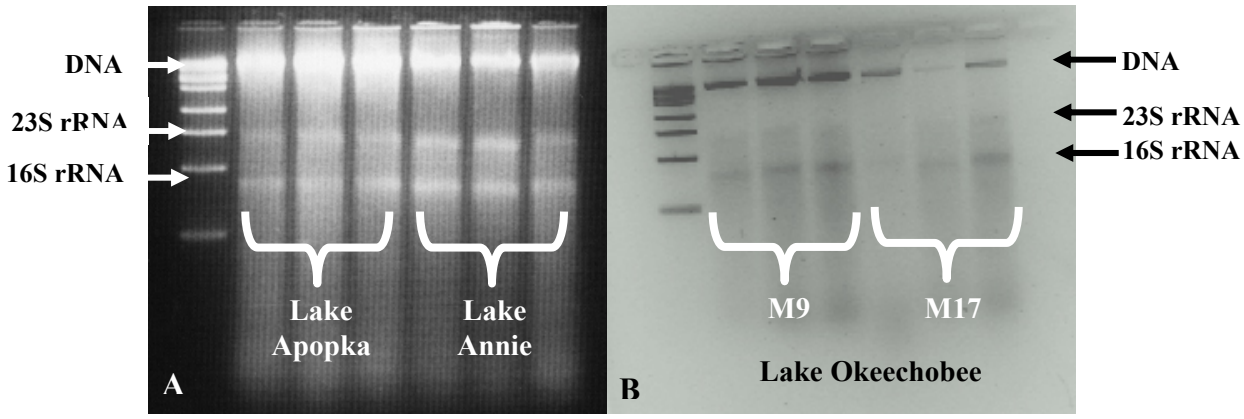


Figure 8-5. Agarose (2%) gel electrophoresis of RNA extracted of samples from A) Lake Annie, Lake Apopka, and B) Lake Okeechobee sites M9 and M17.

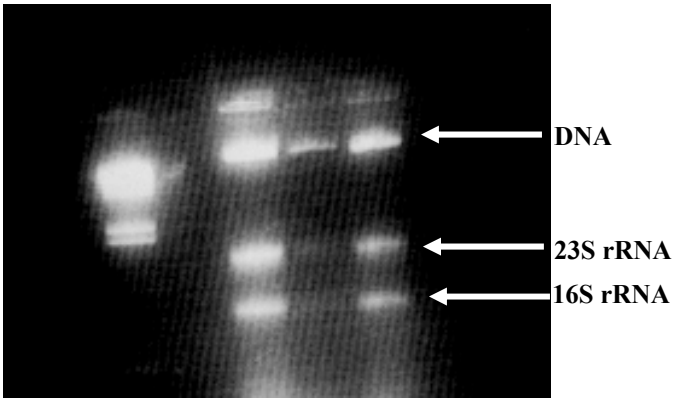


Figure 8-6. Agarose (2%) gel electrophoresis of RNA extracted from *E. coli* culture.

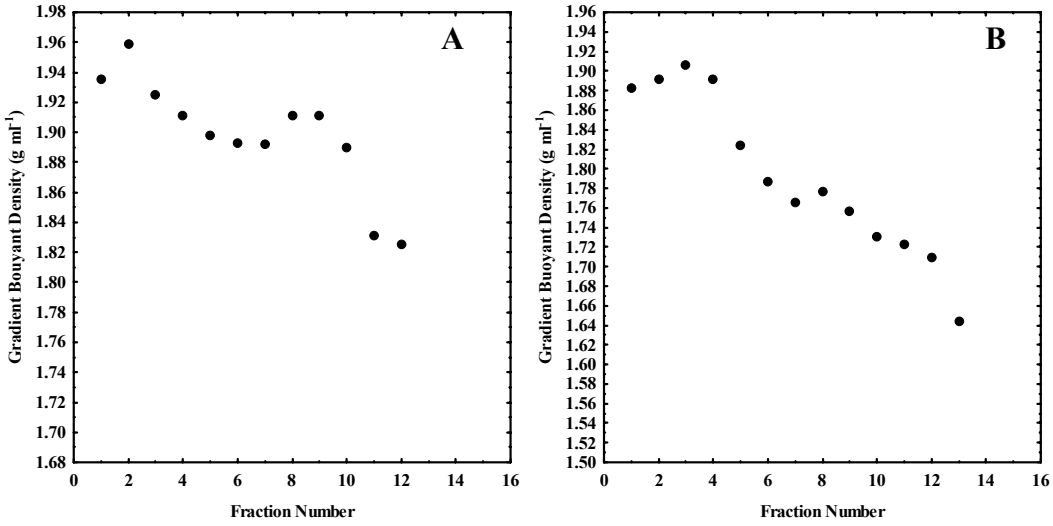


Figure 8-7. Graph illustrating the buoyant density of gradient fractions: (A) Lake Annie (core #120); (B) Lake Apopka (core # 93).

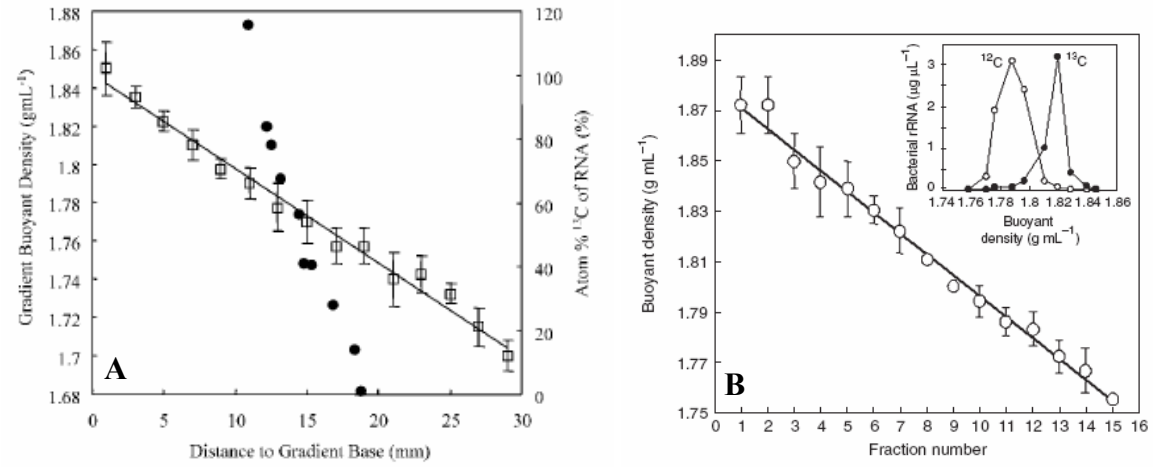


Figure 8-8. Graph illustrating the buoyant density of gradient fractions: (A) Manefield et al. (2002*b*); (B) Whiteley et al. (2007).

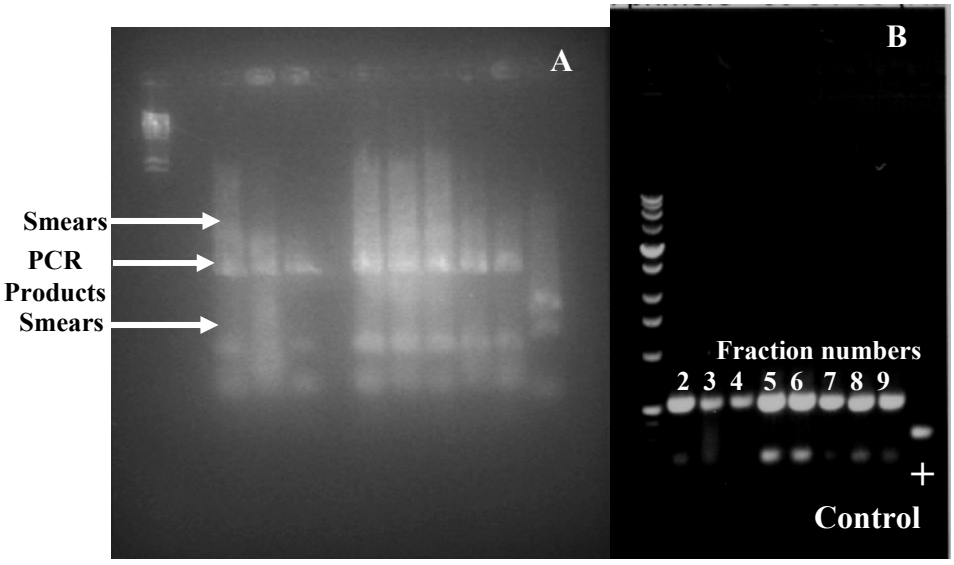


Figure 8-9. Agarose (2%) gel electrophoresis of RT-PCR of the *E.coli* added to Lake Apopka samples (core # 93). (A) old primers; (B) new primers.

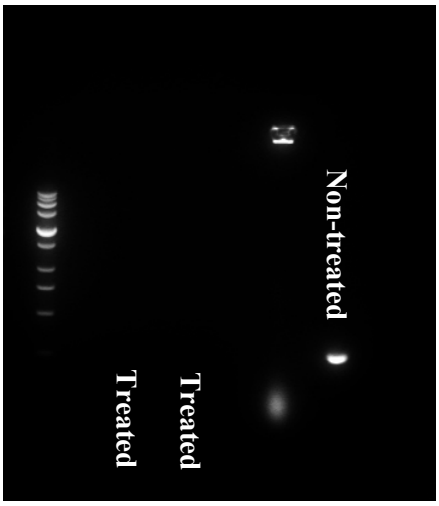


Figure 8-10. Agarose gel (1%) electrophoresis of PCR of *E. coli* RNA samples treated with DNase and not treated with DNase.

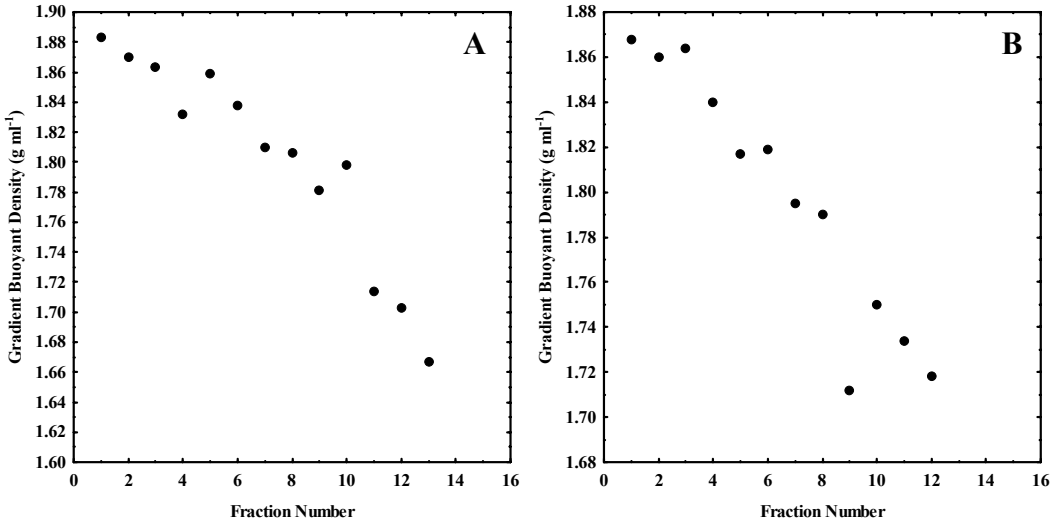


Figure 8-11. Graph illustrating the buoyant density of gradient fractions. (A) Lake Apopka (core # 95); (B) Lake Okeechobee-M9 (core # 148).

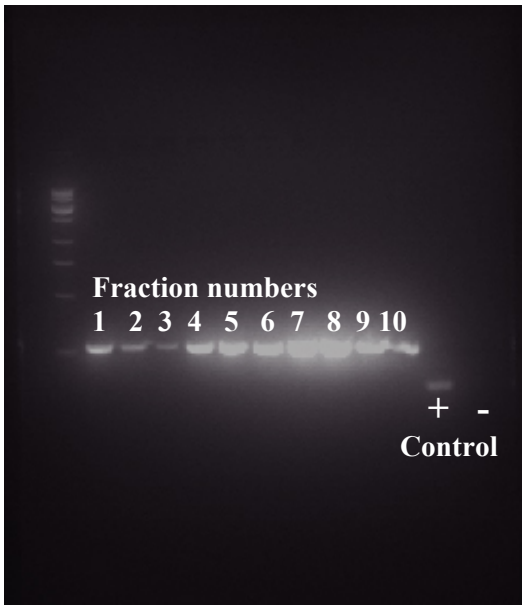


Figure 8-12. Agarose (2%) gel electrophoresis of RT-PCR of RNA extracted from Lake Apopka fractions (core # 95). *E. coli* specific primers were used.

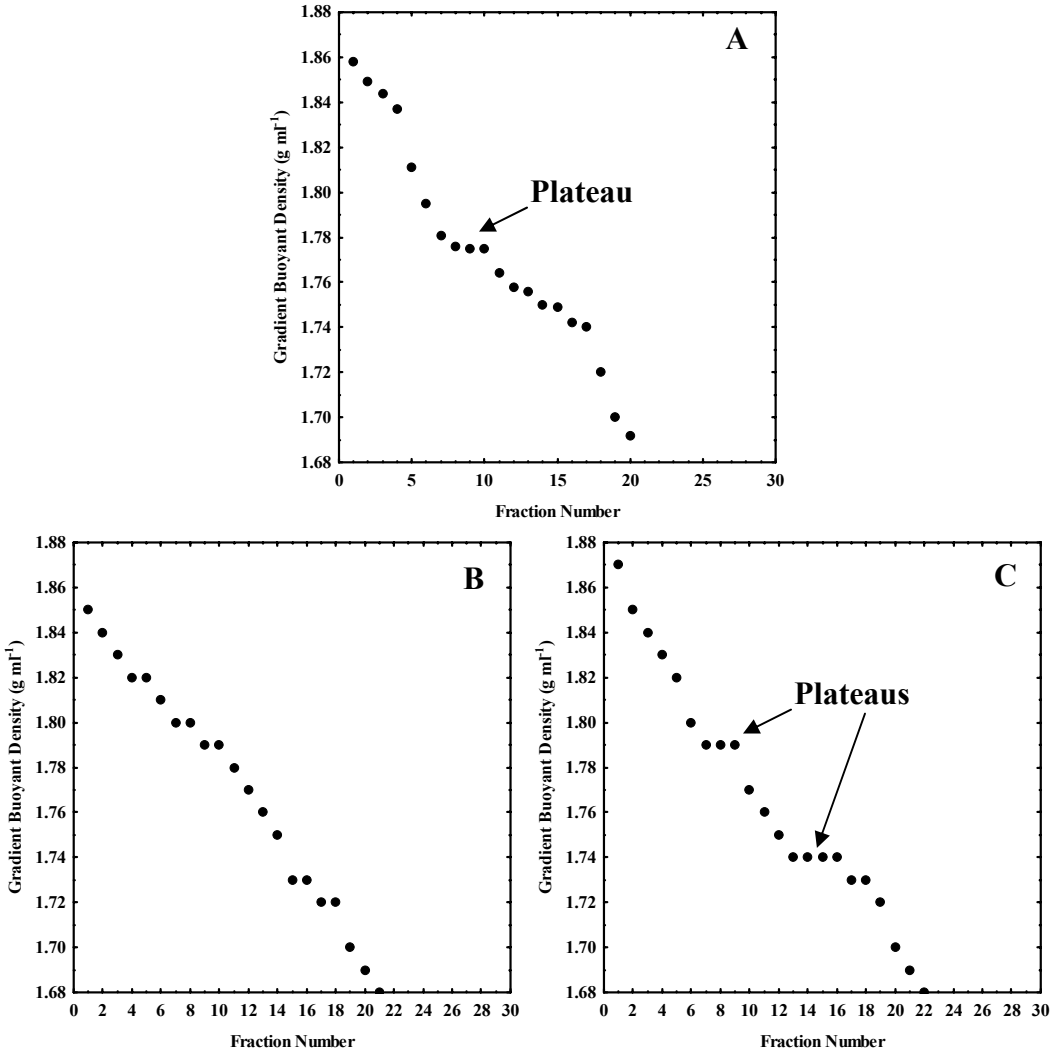


Figure 8-13. Graph illustrating the buoyant density of gradient fractions. (A) Blank (no RNA), (B) Lake Apopka (core #94) and (C) Lake Apopka (core # 95).

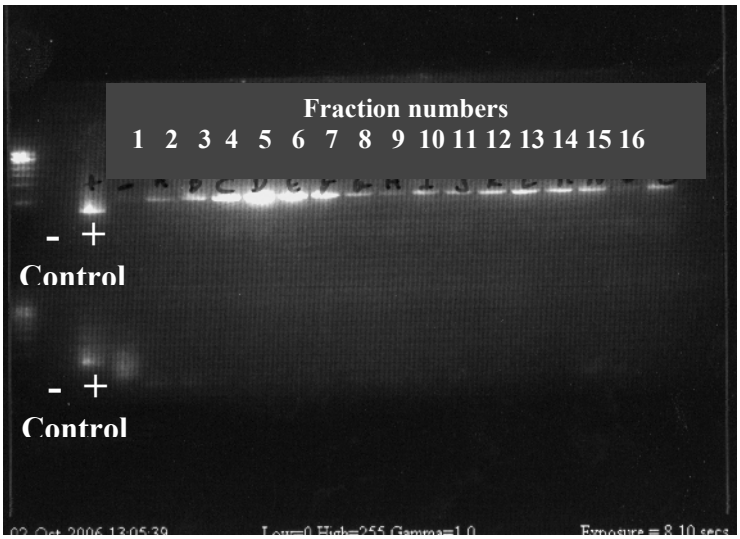


Figure 8-14. Agarose gel electrophoresis of RT-PCR of RNA extracted from Lake Apopka fractions (core # 94). Universal bacteria and Archaea primers were used.

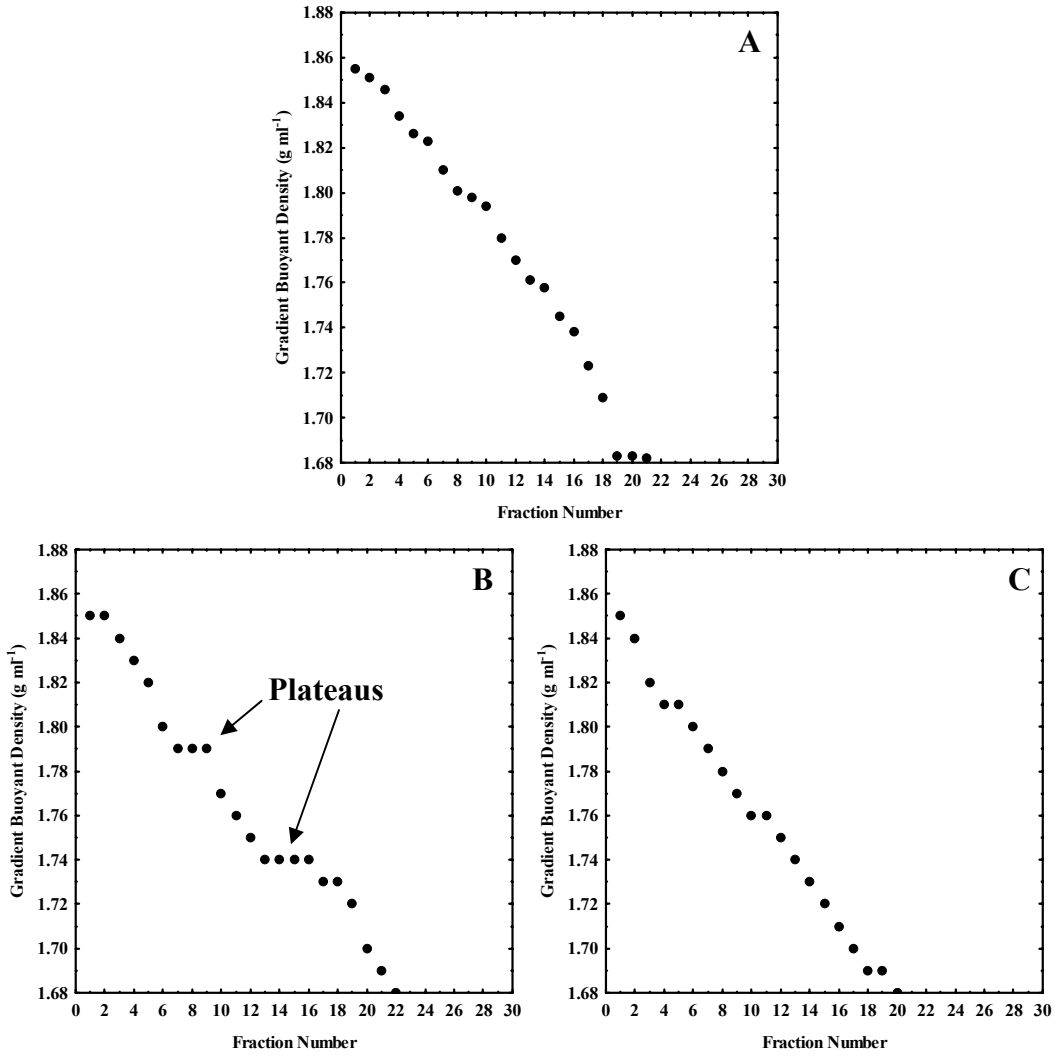


Figure 8-15. Graph illustrating the buoyant density of gradient fractions. (A) Blank (no RNA), (B) *E. coli* 10 ng and (C) *E. coli* 100 ng.

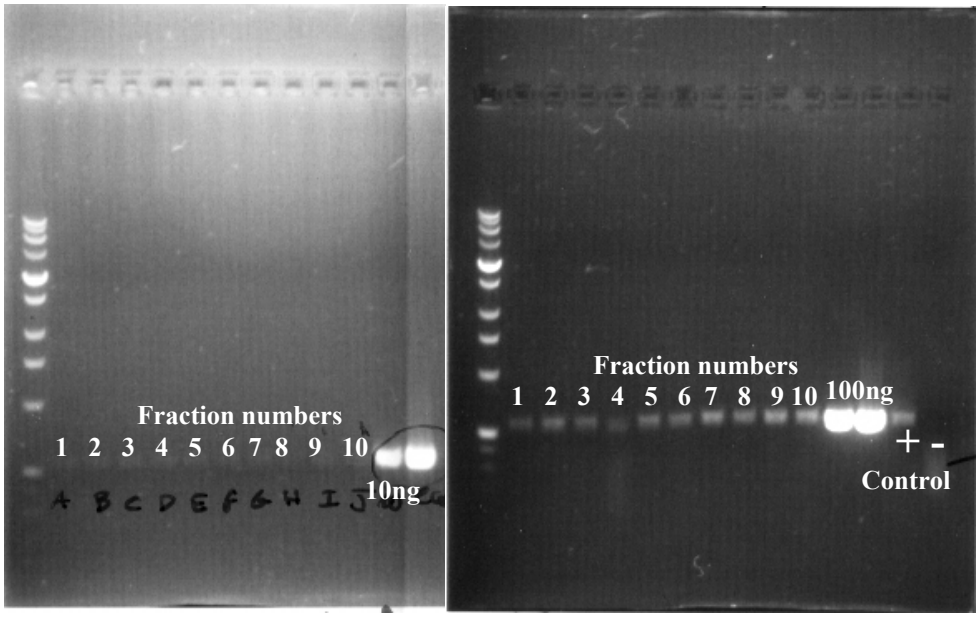


Figure 8-16. Agarose gel electrophoresis of RT-PCR of *E. coli* RNA extracted from gradient fractions. *E. coli*-specific primers were used. (A) *E. coli* 10 ng fractions and (B) *E. coli* 100 ng fractions.

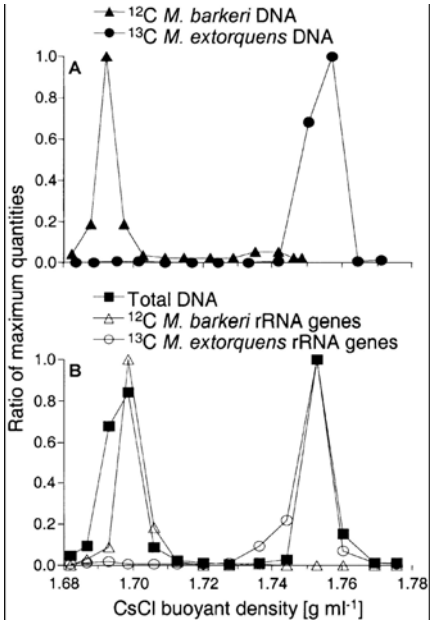


Figure 8-17. CsCl density gradient centrifugation of isotopically distinct DNA species and quantitative evaluation of nucleic acid distribution within gradient fractions. ^{12}C- and ^{13}C-DNA was centrifuged individually (A) or simultaneously (B) and detected fluorometrically (full symbols) or via domain-specific real-time PCR (empty symbols). Figures and captions are from Lueders et al. (2004*a*).

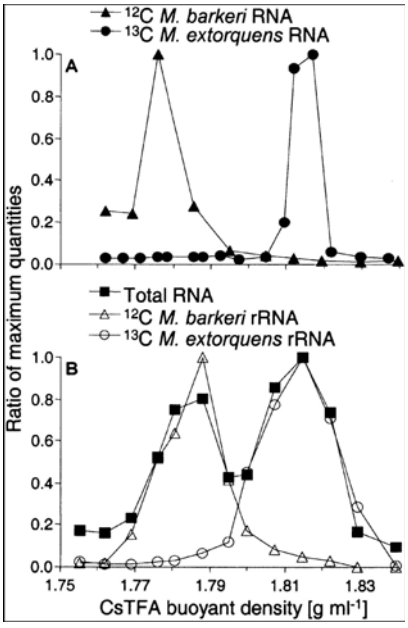


Figure 8-18. CsTFA density gradient centrifugation of isotopically distinct rRNA species and quantitative evaluation of nucleic acid distribution within gradient fractions. ^{12}C- and ^{13}C-rRNA was centrifuged individually (A) or simultaneously (B) and detected fluorometrically (full symbols) or via domain-specific real-time RT-PCR (empty symbols). Figures and captions are from Lueders et al. (2004*a*).

CHAPTER 9
SUMMARY AND CONCLUSIONS

In lakes, allochthonous and autochthonous particulate matter is deposited and becomes an integral part of sediments. Accumulation of particulate matter can alter the physico-chemical properties of sediments and associated biogeochemical processes in the sediment and water column. Coupling and feedback between sediment biogeochemistry and water column primary productivity often depends on biogeochemical processes within sediments and associated microbial communities. Benthic sediments may play a critical role in nutrient cycling by acting as sources or sinks for nutrients, and heterotrophic metabolism typically dominates in this compartment.

The primary goal of this study was to develop a linkage between the biogeochemical properties related to organic phosphorus dynamics of benthic sediments and the bacterial community in relation to their activities in sub-tropical lakes of different trophic states. The main focus of this study was on phosphorus (P) compounds as it is the nutrient that in high concentration is reported to be responsible for eutrophication of freshwater ecosystems. Eutrophic and hypereutrophic lakes usually receive high external loads of nutrients, display high primary productivity and nutrient concentrations, consequently sediments from these lakes might be expected to have higher concentrations of organic matter (OM) and nutrients than oligo-mesotrophic lakes. To accomplish the main goal of this research, a series of laboratory experiments were performed with sediments from three subtropical Florida lakes (Lake Annie - oligo-mesotrophic, Lake Okeechobee - eutrophic and Lake Apopka - hypereutrophic) with different trophic states. The specific objectives of this study were to:

- Determine the biogeochemical properties of surficial benthic sediments and examine relationships among sediment biogeochemical properties (nutrient concentrations and availability) and microbial biomass and activity.

- Determine relative distributions of P compounds in sediment profile using two different techniques, ^{31}P NMR spectroscopy and a P chemical fractionation scheme.
- Characterize P-related enzyme activities in sediment profiles and determine relationships between different P compounds and enzyme activity.
- Determine stratigraphic biogeochemical properties in sediment cores and evaluate how they are related to microbial biomass and activity; and establish whether there is nutrient limitation of the microbial community.
- Determine the source and long-term accumulation of OM and explore how they relate to sediment $\delta^{13}C$ and $\delta^{15}N$ signatures.
- Evaluate the short term catabolic response to the addition of different carbon (C) sources to existing microbial communities in sediments.
- Identity microorganisms that utilize acetate through RNA-stable isotope probing.

Key findings related to the above stated objectives are summarized below.

Biogeochemical properties and microbial activity of sediments (Objective 1)

This study consisted of a spatial study in which sediment was sampled from sixteen different sites from the three different lakes. This study revealed that trophic state conditions were not related to the nutrient content of sediments. Organic matter, nitrogen (N) and P concentrations were higher in sediments with lower bulk density, independent of the trophic state of the lake. The relative importance of P forms present in sediments seemed to be more important than total P concentration in characterizing and understanding the processes occurring in the sediment compartment of each of the studied lakes. The oligo-mesotrophic Lake Annie organic sediments contained P in moderate to highly resistant organic P forms (NaOH soluble), and inorganic P (HCl-Pi, Fe, Al, Ca and Mg bound-P) suggesting P in this lake is old and stable. In eutrophic Lake Okeechobee sediments, the major P form was HCl-Pi, which constituted approximately 60-91% of the total P, while hypereutrophic Lake Apopka sediment had > 50% of the total P in the microbial biomass (MBP).

Extractable nutrient ratios also seemed to have stronger influence on sediment microbial communities than total concentrations. Extractable C:P ratio was low for Lake Annie, reflecting high concentrations of extractable labile nutrients relative to C, indicating a C limitation in these sediments. High labile inorganic P availability resulted in low extractable C:P and N:P ratios, and C and N limitation in most Lake Okeechobee sediments, especially in the mud zone, along with low microbial biomass and activity. Moreover, low C availability appears to be inhibiting the methanogenic community in Lake Okeechobee sediments. Limitation of the methanogenic community in these sediments is supported by the positive effect of the addition of electron donors on methane (CH_4) production, which indicated that H_2/CO_2 is the major substrate for methane production in Lake Okeechobee sediments.

Hypereutrophic Lake Apopka sediments had higher ratios for extractable C:P and N:P, and the high C concentration in sediments is supporting high microbial biomass and activity. Lake Apopka sediments are highly influenced by the deposition of primary producers from the water column. The results from this study suggest that although the microbial community is C/energy limited, C, coupled with N and P availability has a strong influence in microbial communities in these lake sediments.

Sediment phosphorus forms (Objective 2)

Organic P compounds were characterized in sediment profiles using two different techniques, ^{31}P NMR spectroscopy and a chemical P fractionation scheme. In all study lakes TP concentration decreased with sediment depth, and although an oligo-mesotrophic lake, Lake Annie contained more TP in sediments than both eutrophic Lake Okeechobee and hypereutrophic Lake Apopka. This study showed that the concentrations of various P compounds changed with sediment depth, indicating that different processes were controlling P reactivity and mobility in these lakes.

Lake Annie had more stable compounds with greater sediment depth. Dominant forms of TP were HCl-Pi, fulvic acid P (FAP), and humic acid P (HAP), as determined by chemical fractionation, and orthophosphate and phosphate monoester as determined by ^{31}P NMR. Lake Annie physico-chemical characteristics, as well as the major P forms found in the sediment, strongly indicated that biotic processes play an important role in P solubility in these mud sediments.

Lake Okeechobee sediments were dominated by HCl-Pi (chemical fractionation) and orthophosphate (^{31}P NMR), indicating abiotic processes control P solubility in these sediments. Dominant P forms in Lake Apopka were MBP and HCl-Pi (chemical fractionation), and orthophosphate, phosphate monoester and DNA-P (^{31}P NMR). Almost 50% of the total P was in microbial biomass in surface sediments. The presence of poly-P and pyro-P in these sediments also indicated high activity of microorganisms involved in biological P cycling. This study also showed that the results of ^{31}P NMR spectroscopy were in agreement with the results of chemical P fractionation, and that the determination of the relative abundance of different P forms in sediments is important to understand sediment P processes.

Enzyme activities in sediments (Objective 3)

This study showed that phosphomonoesterase (PMEase) and phosphodiesterase (PDEase) activities were related to sediment microbial biomass and activity, as well as to the different P composition and availability. Enzyme activity decreased with sediment depth, reflecting lower microbial biomass and activity. Strong correlations between enzyme activities and anaerobic respiration indicated that bacterial enzymes dominate these sediments. Different P forms in sediments were also affecting enzyme activity. Highest PMEase activity was found in the oligo-mesotrophic lake (Lake Annie) with high concentrations of labile-Po, FAP and HAP. Lake Okeechobee had high concentrations of labile-Pi and lowest activities of both PMEase and

PDEase. Lake Apopka had high concentrations of MBP and phosphate diester (lipids and DNA), as well as PDEase activity.

The mechanisms controlling PMEase activity, however, seemed to vary among studied lakes. In Lake Annie, high PMEase activity was unrelated to dissolved reactive P (DRP) and dissolved organic C (DOC) concentration, and probably was controlled by factors such as high Al and Fe concentrations, high P demand inside microorganism cells, and/or presence of more stable phosphate monoester (i.e., inositol phosphate) in the sediment. Lake Apopka's PMEase production seemed to be controlled by both DOC and DRP availability. There was an inverse relation between pore water DRP and PMEase activity, and a positive relation between pore water DOC and PMEase activity. In Lake Apopka sediments production of PMEase by the microbial community was related to organic P hydrolysis, and uptake of associated organic C moieties.

Microbial biomass and activity in sediments (Objective 4)

The results from this study showed that hypereutrophic Lake Apopka had the highest microbial biomass and activity (both CO_2 and CH_4) followed by oligo-mesotrophic Lake Annie. Microbial activity decreased with sediment depth and was related to decrease in easily degradable OM. Carbon, N and P concentrations, and especially nutrient ratios, had a strong influence on microbial communities in these sediments.

The sediment microbial community in each lake, or site, was limited by different variables. The Lake Apopka's surface sediment heterotrophic community appears to be P-limited. High primary production and high labile C sedimentation resulted in high demand for labile P in surface sediment, as reflected in high C:P ratio. Peat sediments of Lake Okeechobee were limited by both C and P. Nitrogen and C limitation was observed in mud and sand sediments of Lake Okeechobee. High availability of P in Lake Okeechobee mud and sand surface sediments

resulted in C and N limitation. Lake Annie sediments seem to be C-limited, with low ratios of extractable nutrient ratios. Carbon limitation was probably a consequence of C sources (high humic content) and physical characteristics (deep) of this lake. The results showed that heterotrophic microbial metabolism can be limited by a single factor or multiple variables, and limitation varies among lakes depending on lake characteristics and biogeochemical properties of sediments.

Long-term OM accumulation and stable isotope signatures in sediments (Objective 5)

In this study, the ^{210}Pb technique was used to provide an age/depth relation in the sampled sediments. Lake Annie sediments were the only datable samples, while sediments collected from Lake Okeechobee could not be dated reliably due to low or variable activities of ^{210}Pb, and ^{226}Ra. Lake Apopka deposits were undatable due to possible mixing of the upper sediments and failure to reach the unsupported/supported ^{210}Pb boundary. In Lake Annie, the bottom sediment layer of the core was estimated to date to the 1800s and the average sedimentation rate (since c.1900) was determined to be $\approx$ 36.8 mg cm^{-2} yr^{-1}.

Lake Annie sediments were depleted in δ^{13}C and δ^{15}N, probably due to a combination of several factors such as allochthonous OM input, OM from primary productivity, and microbial biomass and activity. In mud sediments of Lake Okeechobee, δ^{13}C values were slightly depleted while δ^{15}N were enriched towards the sediment surface. These isotopic signatures resulted from several factors such as the phytoplankton community, high demand for C and N in sediments, and selective mineralization of OM. In the peat zone of Lake Okeechobee, the isotopic signatures of sediment OM (enriched in δ^{13}C and δ^{15}N towards the sediment surface) were related to several factors, including sediment origin (i.e., plant tissue), intensities of primary productivity, and diagenesis. Stratigraphic variation in δ^{13}C and δ^{15}N at the KR site probably reflects an input of

wastewater from anthropogenic activities, and variable contributions of river-borne allochthonous input, related to inter-annual rainfall variations.

In Lake Apopka, heavy $\delta^{13}C$ DIC in the water column, with high demand for inorganic C due to high primary productivity, produced autochthonous OM with enriched $\delta^{13}C$. The enriched $\delta^{15}N$ signature in Lake Apopka sediments was generated by multiple factors including the isotopic signature of autochthonous N sources, the primary producer community, and N related processes in the water column and sediments. A more detailed study of $\delta^{13}C$ and $\delta^{15}N$ isotopes in several compartments, i.e., dissolved carbon, different N compounds, phytoplankton biomass, bacteria biomass, particulate OM in the water column and sediment, can confirm the major processes affecting the isotopic signatures of these sediments.

Microbial activity in sediments: effects of organic electron donors (Objective 6)

Microbial functional diversity of surface sediments of the subtropical lakes was investigated by measuring catabolic response to a wide variety of C-substrates. Addition of organic electron donors to sediment microcosms from all lakes stimulated heterotrophic activity, however the extent of the response was strongly related to microbial biomass and catabolic diversity. Although the magnitude of the response to electron donor addition was related to microbial biomass, the different response in each sediment was related to the catabolic diversity of sediment microorganisms. The addition of some electron donors did not stimulate heterotrophic microbial respiration, and probably resulted in the addition of C into microbial biomass rather than release via respiratory pathways.

Lake Apopka had the highest respiration per microbial biomass, indicating that these sediments respired most of the C added, as a consequence of a P limitation. Lake Annie showed the highest metabolic quotient (qCO_2; proportion of basal respiration per microbial biomass) indicating inefficient use of energy. The low qCO_2 found in Lake Apopka's sediment indicates

high efficiency. Lake Apopka's sediment catabolic diversity was higher than in the other sediments. In relation to methane production, acetoclastic methanogenesis is probably important in Lake Annie sediments. Lake Okeechobee sediments were characterized by lower CO_2 production rates that the other sediments. The dominance of hydrogentrophic methanogenesis in Lake Okeechobee sediments was determined in another study (Chapter 2). The pathway for methane production in Lake Apopka cannot be determined with the current data. Molecular studies targeting the archaeal community are necessary to elucidate the major pathway for CH_4 production in these lakes sediment. These results showed that the sediments with different biogeochemical properties had different microbial communities with distinct catabolic responses to addition of the C sources.

RNA-stable isotope probing of acetate-utilizing microorganisms (Objective 7)

An attempt was made to identity microorganisms that utilize acetate in these sediments using RNA stable isotope probing. This approach, however, did not work. In this chapter the methods used at every step were documented and sources of error that contributed to the failure of the proposed study were discussed.

Synthesis

In Figure 9-1, the major characteristics of surface sediments (0-15 cm) in the different studies from the lakes are summarized. Sediments from the central site were selected to represent Lake Annie data, while sediments from the mud zone were selected to represent Lake Okeechobee data. The three lakes, ranging in trophic state, had distinct sediment biogeochemical properties despite some similarities were present.

All sediments (mud sediments from Lake Annie, Lake Okeechobee mud sites, and all sites of Lake Apopka) had high TP concentration. Sediments from the oligo-mesotrophic Lake Annie had the major P forms as HAP, FAP and HCl-Pi. These sediments were also characterized by

high PMEase activity and qCO_2. Low extractable C:P and N:P ratios resulted from a high availability of P. Isotope signatures of these sediments revealed low values of $\delta^{13}C$ and $\delta^{15}N$.

Lake Okeechobee mud sediments had similarities with Lake Annie sediments, such as low extractable C:P and N:P ratios due to a high extractable labile-Pi concentration, and HCl-Pi as the major P form. Differences in sediments from this eutrophic lake included low microbial activity (CO_2 and CH_4 production rates), and enzyme activities. Metabolic quotient (qCO_2) and $\delta^{13}C$ and $\delta^{15}N$ values were placed between the other lakes values (Figure 9-1).

Hypereutrophic Lake Apopka had high concentrations of microbial biomass P, N and C, as well as high extractable C:P and N:P ratios, and high microbial activity (CO_2 and CH_4 production rates). These sediments were also characterized by having high PDEase activity and high values of $\delta^{13}C$ and $\delta^{15}N$. Metabolic quotient (qCO_2) and labile-Pi concentrations were low in this lake (Figure 9-1). Among all variables, $\delta^{13}C$ and $\delta^{15}N$ values and qCO_2 were the ones that presented a gradient in relation to the trophic state of the lakes. Metabolic quotient was high in the oligo-mesotrophic lake and decreased with increasing trophic state. Isotopic signatures increased from the oligo-mesotrophic lake to the hypereutrophic lake (Figure 9-1). Although sharing some similarities, each lake had distinct sediment biogeochemical properties, and sediment processes which were a reflection of an integrative effect of trophic state conditions and diagenesis over a long period of time.

Lake Annie

Oligo-mesotrophic acidic Lake Annie, with high allochthonous OM input, had high TP concentration in its sediments, which is probably naturally occurring as the decrease of TP with sediment depth is not accentuated. The TP mainly consisted of organic bound-P with consequently high PMEase activity that indicates that P solubility in these sediments is mainly controlled by biotic processes (Figure 9-2). The production of PMEase is not controlled by P

availability in the sediments, rather it resulted from a combination of factors such as high Al and Fe concentrations, high P demand inside microorganism cells, and/or presence of more stable phosphate monoester in the sediment. High labile-P concentration in these sediments resulted in low extractable C:P and N:P ratios and a C limitation of the microbial heterotrophic community. Carbon limitation probably causes inefficient use of energy by the heterotrophic microbial community, where there is high respiration per microbial biomass. Heterotrophic microbial communities in these sediments probably have high respiratory demands, with greater C flow to CO_2 rather than to biomass.

Lake Okeechobee

Eutrophic Lake Okeechobee mud sediment had its TP pool dominated by inorganic P (HCl-Pi) (Figure 9-3). Sediments were characterized by having high labile-Pi concentration and low enzyme activity. High P availability in these sediments is repressing the production of P related enzyme activities. P solubility in these sediments is controlled by abiotic processes (Figure 9-3). High labile-Pi concentration in these sediments resulted in low extractable C:P and N:P ratios, and a C and N limitation of the microbial heterotrophic community. Carbon and N limitation is causing low microbial activities in these sediments. Methanogenesis was inhibited due to low electron donor availability with concomitant presence of iron and sulfate reducers. Moreover, it was established for these sediments that H_2/CO_2 is the major substrate for methane production.

Lake Apopka

Hypereutrophic Lake Apopka, with high autochthonous OM input and highly organic sediments, had the sediment TP pool dominated by diester P (i.e., MBP, DNA-P, Lipid-P) followed by inorganic P (HCl-Pi), orthophosphate, FAP/HAP and phosphate monoester. An intrinsic characteristic of these sediments was the presence of polyphosphate in some of the

sediment layers. P solubility in these sediments is controlled by a combination of abiotic (pH) and biotic processes (Figure 9-4). High concentration of diester P resulted in high PDEase activity. The activity of PMEase was also high and its production repressed by inorganic P availability, however, it seems that in these sediments PMEase is also related to C acquisition by the heterotrophic microbial community. Sediments were characterized by high extractable C and labile-N and low labile-Pi concentration, which resulted in high C:P and N:P ratios, and indicated P limitation in these sediments. Microbial biomass and activity were high in these sediments. High C availability in these sediments probably accounts for efficient use of energy that it is used for biomass (growth) as well as respiration. The heterotrophic microbial community in these sediments has high catabolic diversity.

Results from these studies demonstrated the mutual dependency of C, N and P transformations in regulating the sediment microbial community and nutrient bioavailability, especially P. Activity of the heterotrophic microbial community can be limited by a range of properties and will depend on limnological characteristics of lakes and sediment biogeochemical properties. The results also highlighted the significance of the relationships between sediment biogeochemical properties and microbial community activities in lakes with different trophic states, and showed how the physico-chemical conditions of lakes affect sediment properties and microbial mediated processes. Moreover, it illustrated the importance of measuring several variables, such as C, N and P, to address questions related to microbial communities.

Future studies should focus on identifying communities that regulate the OM turnover and nutrient mobilization. Controlled experiments addressing the effect of C, N and P addition to sediment microbial biomass and activity can strengthen the conclusions about nutrient limitation in each of these lake sediments. The study of other enzyme activities, such as C (i.e.,

glucosidase) and N (i.e. protease) related enzymes, would increase the knowledge of nutrient dynamics and microbial communities in these sediments. One important point that was not covered by the current study is the seasonal variation of nutrient limitation. Seasonal variation of nutrient availability occurs in the water column of lakes and can occur in sediments of shallow lakes like Lake Okeechobee and Lake Apopka. A study encompassing sampling of surficial sediments in different seasons (i.e. winter and summer) should also be conducted.

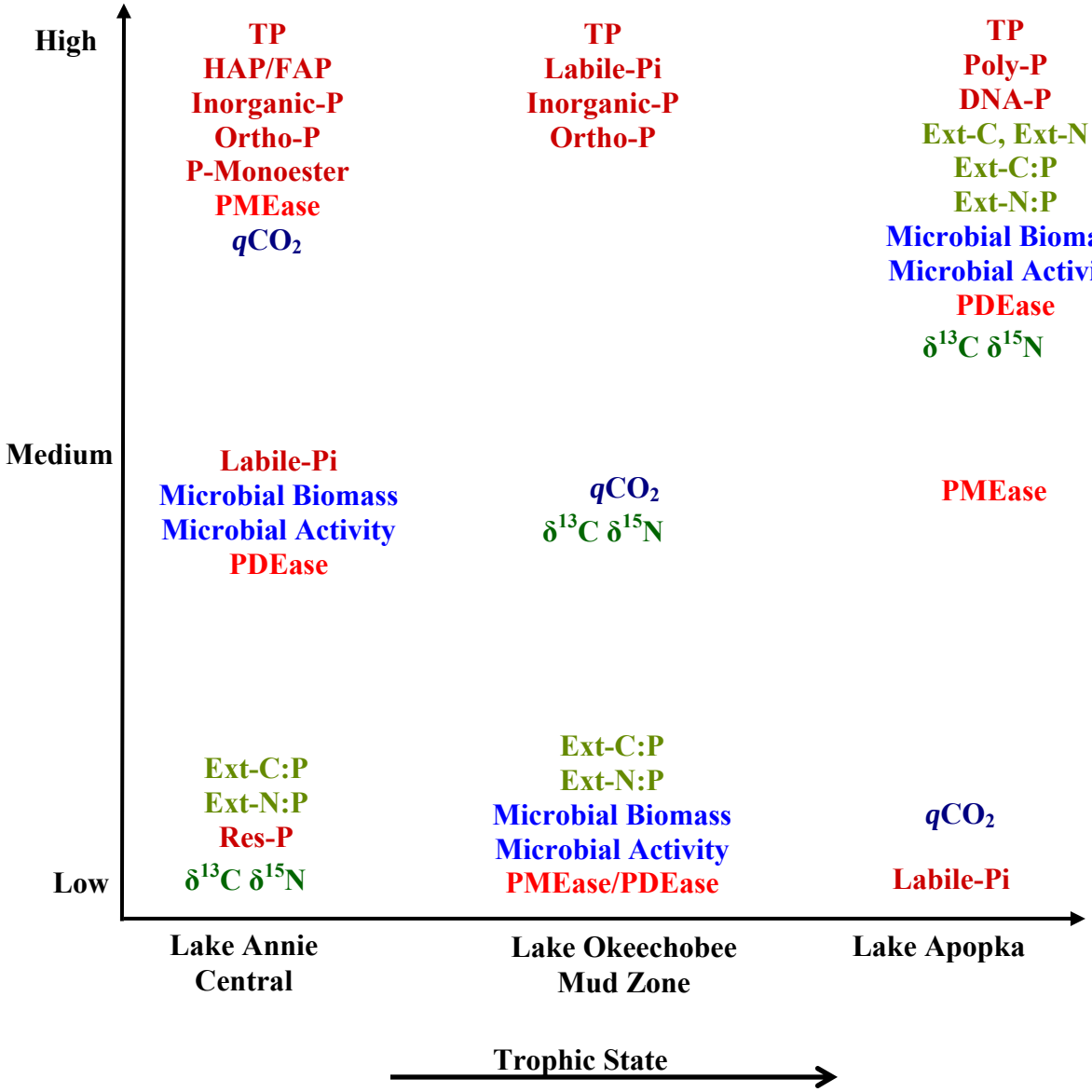


Figure 9-1. Graphic representation of main sediment characteristics of three lakes in relation to their trophic state. Ext-C: extractable organic carbon, Ext-N: extractable labile nitrogen, TP: total phosphorus, Inorganic-P: HCl-Pi, FAP: moderate labile organic phosphorus, HAP: highly resistant organic phosphorus, Res-P: residual phosphorus, Ext-P: extractable labile phosphorus, MBC: microbial biomass carbon, MBP: microbial biomass phosphorus, MBN: microbial biomass nitrogen, and microbial activity: CO_2 and CH_4 production rates.

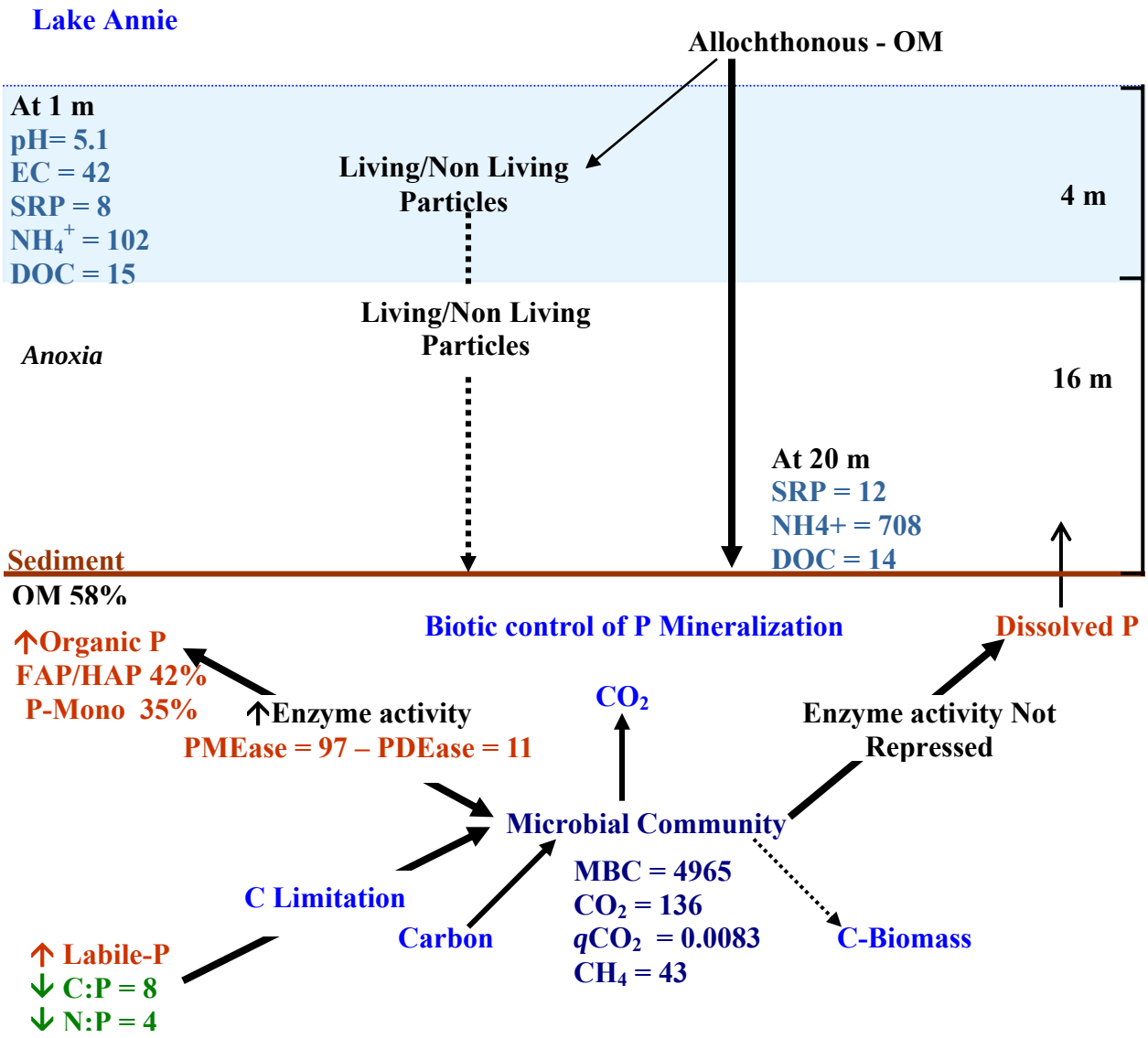


Figure 9-2. Summary of the main biogeochemical properties and processes occurring in Lake Annie water column and sediments. Numbers are mean of 0-15 cm sediment depth. EC: electrical conductivity (µS cm^{-1}); SRP: soluble reactive P (µg L^{-1}); NH_4^+: ammonium (µg L^{-1}); DOC: dissolved organic carbon (mg L^{-1}); C:N:P: ratios of extractable carbon, labile nitrogen and phosphorus. P forms % in relation to total phosphorus (P); HCl-Pi: inorganic P; FAP: moderate labile organic P; HAP: highly resistant organic P, Labile-Pi: extractable labile P; Ortho-P: orthophosphate (^{31}P NMR); P-Mono: phosphate monoester, Poly-P: polyphosphate; PMEase: phosphomonoesterase activity (mg g^{-1} dw h^{-1}); PDEase: phosphodiesterase activity(mg g^{-1} dw h^{-1}), MBC: microbial biomass carbon(mg kg^{-1}), qCO_2: metabolic quotient (basal respiration/microbial biomass); CO_2: anaerobic respiration(mg kg^{-1} dw d^{-1}); CH_4: methane production rates (mg kg^{-1} dw d^{-1}).

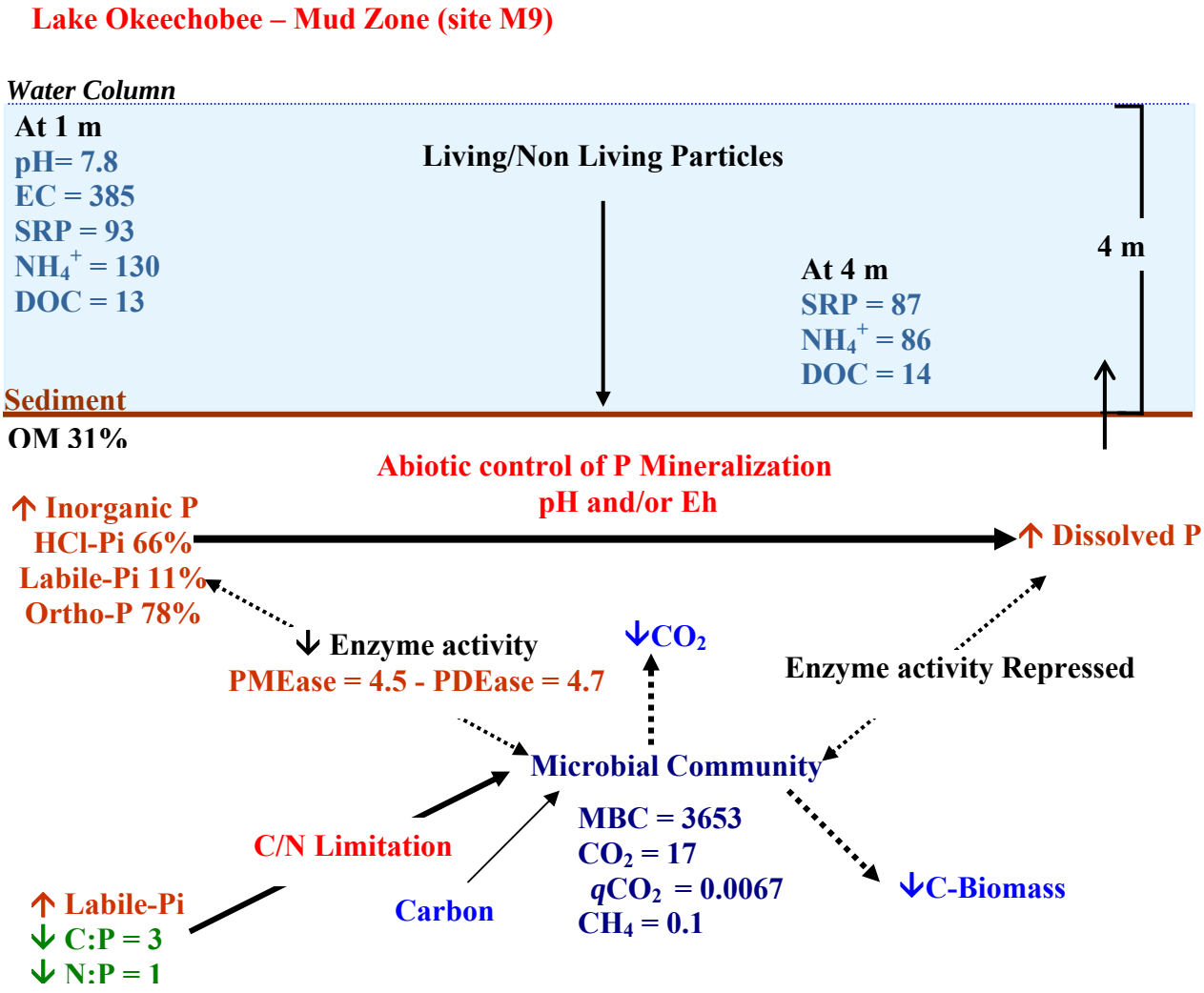


Figure 9-3. Summary of the main biogeochemical properties and processes occurring in the Lake Okeechobee site M9 water column and sediments. Numbers are mean of 0-15 cm sediment depth. EC: electrical conductivity (µS cm^{-1}); SRP: soluble reactive P (µg L^{-1}); NH_4^+: ammonium (µg L^{-1}); DOC: dissolved organic carbon (mg L^{-1}); C:N:P: ratios of extractable carbon, labile nitrogen and phosphorus. P forms % in relation to total phosphorus (P); HCl-Pi: inorganic P; FAP: moderate labile organic P; HAP: highly resistant organic P, Labile-Pi: extractable labile P; Ortho-P: orthophosphate (^{31}P NMR); P-Mono: phosphate monoester, Poly-P: polyphosphate; PMEase: phosphomonoesterase activity (mg g^{-1} dw h^{-1}); PDEase: phosphodiesterase activity(mg g^{-1} dw h^{-1}), MBC: microbial biomass carbon(mg kg^{-1}), qCO_2: metabolic quotient (basal respiration/microbial biomass); CO_2: anaerobic respiration(mg kg^{-1} dw d^{-1}); CH_4: methane production rates (mg kg^{-1} dw d^{-1}).

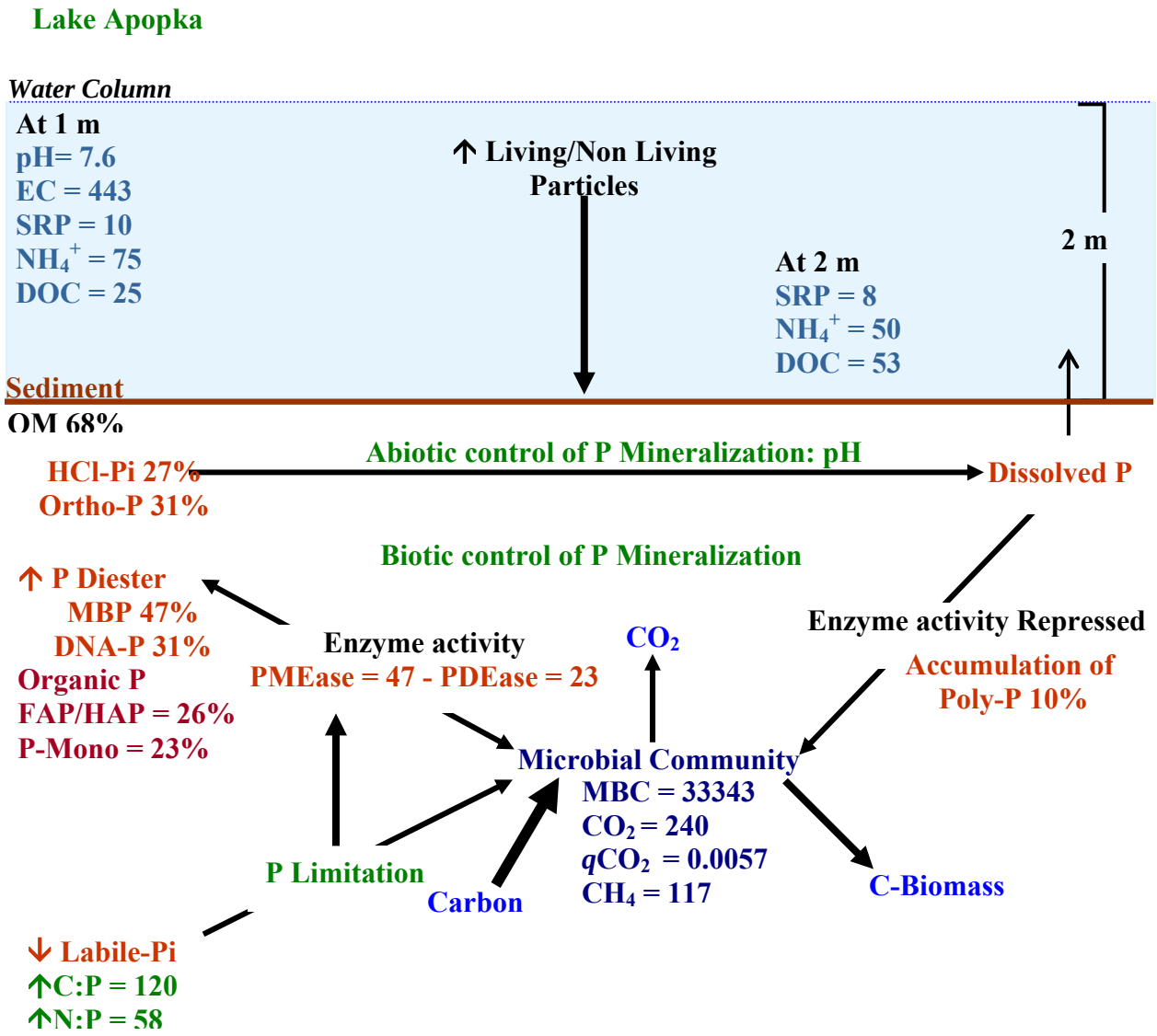


Figure 9-4. Summary of the main biogeochemical properties and processes occurring in the Lake Apopka water column and sediments. Numbers are mean of 0-15 cm sediment depth. EC: electrical conductivity (µS cm^{-1}); SRP: soluble reactive P (µg L^{-1}); NH_4^+: ammonium (µg L^{-1}); DOC: dissolved organic carbon (mg L^{-1}); C:N:P: ratios of extractable carbon, labile nitrogen and phosphorus. P forms % in relation to total phosphorus (P); HCl-Pi: inorganic P; FAP: moderate labile organic P; HAP: highly resistant organic P, Labile-Pi: extractable labile P; MBP: microbial biomass P; Ortho-P: orthophosphate (^{31}P NMR); P-Mono: phosphate monoester, Poly-P: polyphosphate; PMEase: phosphomonoesterase activity (mg g^{-1} dw h^{-1}); PDEase: phosphodiesterase activity(mg g^{-1} dw h^{-1}), MBC: microbial biomass carbon(mg kg^{-1}), qCO_2: metabolic quotient (basal respiration/microbial biomass); CO_2: anaerobic respiration(mg kg^{-1} dw d^{-1}); CH_4: methane production rates (mg kg^{-1} dw d^{-1}).

APPENDIX A
SUPPLEMENTAL TABLES

Table A-1. Water variables from Lake Annie, Lake Okeechobee and Lake Apopka (samples taken at 1 m depth).

Lake	Site	Temperature (ºC)	Electrical Conductivity ($\mu S\ cm^{-1}$)	Dissolved Oxygen ($mg\ L^{-1}$)
Annie	South	29.7	45	6.5
	Central	29.9	43	6.7
	North	30.1	43	7.0
Okeechobee	M17	29.3	465	5.8
	O11	30.1	467	6.4
	M9	28.7	471	6.4
	K8	28.5	512	6.2
	FC	30.7	393	1.2
	J5	28.2	603	0.3
	TC	29.6	362	7.3
	KR	29.1	232	4.9
	J7	29.3	524	6.3
Apopka	South	15.9	366	8.5
	Central	16.0	370	10.6
	West	15.8	418	9.7
	North	16.6	382	10.2

Table A-2. Total, extractable and microbial biomass carbon, nitrogen and phosphorus ratio (weight basis) measured in sediments from Lake Annie, Lake Okeechobee, and Lake Apopka.

Lake	Site	Total			Extractable			Microbial Biomass		
		C:N	C:P	N:P	C:N	C:P	N:P	C:N	C:P	N:P
Annie	South	14	185	13	2	16	8	5	40	8
	Central	13	185	14	3	24	6	6	33	6
	North	6	230	37	18	40	7	13	51	45
Okeechobee	M17	19	1079	58	9	68	3	6	55	9
	O11	16	160	10	5	9	7	8	33	4
	M9	18	159	9	5	6	2	4	128	28
	K8	15	147	10	6	8	1	5	35	8
	FC	7	19	3	2	6	1	9	23	3
	J5	12	123	10	5	35	3	11	76	7
	TC	13	46	1	4	14	6	6	49	8
	KR	15	123	1	3	8	3	26	250	17
	J7	16	72	1	3	10	3	6	122	25
Apopka	South	11	275	24	4	118	32	6	23	4
	Central	11	247	22	3	110	35	6	28	5
	West	12	293	25	4	87	25	6	31	5
	North	11	218	20	3	151	58	6	23	4

Table A-3. Pearson correlation coefficients of sediment biogeochemical properties significant at $p < 0.05$.

			Carbon		Nitrogen		Phosphorus						
	BD	LOI	TC	ExtC	TN	ExtN	TP	Pi	TIP	Po	FAP	HAP	Res
LOI	-0.91												
TC	-0.88	1.00											
Ext-C	-0.71	0.86	0.85										
TN	-0.80	0.91	0.91	0.92									
Ext-N	-0.65	0.78	0.77	0.96	0.87								
TP	-0.92	0.80	0.76	0.74	0.75	0.75							
Lab.Pi	-0.47	0.17*	0.13*	-0.11*	0.00*	-0.14*	0.47						
IP	-0.67	0.34	0.29	0.08*	0.21*	-0.04*	0.64	0.80					
Lab.Po	-0.77	0.77	0.74	0.77	0.76	0.78	0.85	0.29*	0.36				
FAP	-0.67	0.65	0.61	0.64	0.63	0.70	0.81	0.34	0.35	0.85			
HAP	-0.67	0.68	0.64	0.68	0.67	0.71	0.75	0.19*	0.29*	0.84	0.90		
Res.P	-0.64	0.52	0.50	0.52	0.52	0.47	0.68	0.28*	0.55	0.41	0.14*	0.12*	
Ratios													
Ext-C:Ext-N	0.30	-0.22*	-0.25*	-0.25*	-0.25*	-0.31	-0.38	-0.10*	-0.22*	-0.34	-0.34	-0.32	-0.19*
Ext-C:Ex-tP	-0.39	0.64	0.67	0.86	0.78	0.82	0.39	-0.47	-0.27*	0.41	0.29*	0.35	0.40
Ext-N:Ext-P	-0.43	0.62	0.63	0.87	0.78	0.91	0.52	-0.40	-0.19*	0.50	0.41	0.42	0.47

BD: bulk density, LOI: loss on ignition, TC: total carbon, Ext-C: extractable organic carbon, TN: total nitrogen, Ext-N: extractable labile nitrogen, TP: total phosphorus, Lab.Pi: labile inorganic phosphorus, Lab.Po: labile organic phosphorus, IP: inorganic phosphorus, FAP: moderate labile organic phosphorus, HAP: highly resistant organic phosphorus, Res.P: residual phosphorus, Ext-P: extractable labile phosphorus. *Not significant at $p < 0.05$.

Table A-4. Pearson's correlation coefficients of biogeochemical properties and microbial biomass and activity significant at $p < 0.05$.

	Microbial Biomass			Anaerobic	Methane Production			
	Carbon	Nitrogen	Phosphorus	Respiration	Control	Acetate*	Hydrogen*	Acetate + Hydrogen*
BD	-0.49	-0.49	-0.47	-0.59	-0.50	-0.59	-0.53	-0.69
LOI	0.65	0.66	0.64	0.71	0.53	0.36**	0.25**	0.52
TC	0.65	0.66	0.64	0.70	0.50	0.30**	0.18**	0.49
Ext-C	0.89	0.89	0.87	0.89	0.61	0.42	0.25**	0.57
TN	0.80	0.80	0.78	0.81	0.55	0.38	0.26**	0.40
Ext-N	0.91	0.92	0.90	0.95	0.66	0.67	0.55	0.68
TP	0.57	0.58	0.56	0.71	0.63	0.71	0.78	0.77
Lab.Pi	-0.36	-0.36	-0.38	-0.17**	0.19**	0.80	0.89	0.80
IP	-0.09**	-0.10**	-0.12**	-0.01**	-0.15**	0.62	0.68	0.71
Lab.Po	0.60	0.60	0.56	0.67	0.70	0.71	0.67	0.66
FAP	0.42	0.42	0.41	0.61	0.83	0.74	0.76	0.65
HAP	0.48	0.48	0.46	0.59	0.86	0.68	0.59	0.64
Res.P	0.57	0.57	0.55	0.46		0.79	0.89	0.79
MBC		1.00	0.99	0.88	0.38	0.86	0.81	0.78
MBN	1.00		0.99	0.90	0.39	0.83	0.78	0.84
MBP	0.99	0.99		0.90	0.36	0.84	0.76	0.64
Ratios								
Ext-C:Ext-N	-0.25**	-0.25**	-0.24**	-0.30	-0.27**	0.27**	-0.01**	0.39
Ext-C:Ext-P	0.87	0.88	0.88	0.83	0.31	-0.16**	-0.44	-0.01**
Ext-N:Ext-P	0.93	0.95	0.95	0.94	0.40	-0.36**	-0.63	-0.33**

BD: bulk density, LOI: loss on ignition, TC: total carbon, Ext-C: extractable organic carbon, TN: total nitrogen, Ext-N: extractable labile nitrogen, TP: total phosphorus, Lab.Pi: labile inorganic phosphorus, Lab.Po: labile organic phosphorus, IP: inorganic phosphorus, FAP: moderate labile organic phosphorus, HAP: highly resistant organic phosphorus, Res.P: residual phosphorus, Ext-P: extractable labile phosphorus. * Data from Lake Okeechobee sediments only. **Not significant at $p < 0.05$.

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BIOGRAPHICAL SKETCH

Isabela Claret Tôrres was born in Belo Horizonte (Minas Gerais State, Brazil). She grew up surrounded by mountains, water falls, Cerrado, and the remains of Atlantic Forest. She was always taken by her family to explore natural habitats, and taught to value and protect nature and animals. Before going to college she lived for one and a half years in Israel where she experienced the life of two different "Kibbutzim" near Haifa. In 1991, she started her studies in biology at the Federal University of Minas Gerais (UFMG). Her first experience with science was working with freshwater zooplankton species, and later she joined the Population Ecology Laboratory where she conducted research on a spider population in the beautiful mountain fields of Serra do Cipó (M.G.). During this study she had a unique opportunity to work with Dr. José E. C. Figueira, her advisor, a great ecologist who taught her a passion for ecology, science, and statistics. She graduated in 1995 with a B.S. degree in biology (major in ecology), and, knowing that lack of water would be the major issue that humanity would face in the near future, she decided to return to water research. In 1997, she joined the master's program in ecology, conservation and management of wildlife, at UFMG, where she studied mass balance of nutrients of a eutrophic reservoir, and graduated in 1999. In 2002 she joined the graduate program in soil and water science to pursue her Ph.D. degree. In this program, she felt she was becoming a more complete limnologist, as she was studying sediment processes. Studying lake sediment made her realize that she was following in the steps of her uncle and godfather, Dr. Geraldo Eustáquio Tôrres (*in memoriam*); a "mud" limnologist and benthos ecologist who left this world too soon and is truly missed by her. After graduating, she wants to become a professor so she can pass on to the next generation the same passion for nature, science, biology, ecology, and limnology that were passed to her by some of her professors and her family.