PALEOENVIRONMENTAL VARIABILITY IN THE SOUTHEAST AFRICAN TROPICS SINCE THE LAST GLACIAL MAXIMUM: MOLECULAR AND ISOTOPIC RECORDS FROM LAKE MALAWI

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DEDICATION

This dissertation is dedicated to the memory of Geoffrey O. Seltzer, for introducing me to the subject of environmental geology and inspiring me to pursue a career in paleoclimatology, for his support and guidance throughout my undergraduate and graduate degrees, and for the fond memories of laughter during numerous cold, wet, and mud-filled field trips in central New York.
PALEOENVIRONMENTAL VARIABILITY IN THE SOUTHEAST AFRICAN TROPICS DURING THE PAST 23,000 YEARS: MOLECULAR AND ISOTOPIC RECORDS FROM LAKE MALAWI

ABSTRACT

This study utilizes molecular and isotopic techniques to examine past variability in terrestrial and aquatic tropical ecosystems from southeast Africa. Two different timescales are investigated, the past 23 cal ka to examine glacial-interglacial climate variability, and the past 730 years to examine decadal to centennial scale climate variability.

Carbon isotope measurements of plant leaf waxes provide a sensitive indicator of aridity and document dry conditions in southeast Africa during the Last Glacial Maximum, the Younger Dryas cold period, and during the Little Ice Age. Peak wet conditions are observed at 13.6 and 5 cal ka, and a shift to wetter conditions is also noted from 1800 AD to the present. Arid conditions in southeast Africa are associated with southward migrations of the mean latitudinal position of the Intertropical Convergence Zone (ITCZ) during Northern Hemisphere cold periods. In contrast to studies that have suggested Holocene climates were relatively stable, the Holocene in southeast Africa was characterized by extreme and abrupt changes in moisture availability, which likely affected human and faunal migrations as well as the development and collapse of human civilizations.

In addition to affecting aridity in southeast Africa, southward migrations of the ITCZ also influenced algal productivity in Lake Malawi. Lipids of aquatic algae indicate a major increase in the primary productivity at the Pleistocene/Holocene boundary, which is likely related to a northward migration of the ITCZ over Africa at this time. The Younger Dryas stands out as a major feature in the records of nearly all algal lipids and is
marked by an abrupt increase in algal productivity, which can be attributed to increased northerly winds over Lake Malawi. During the past 730 years there is also evidence for changes in algal productivity with decreasing abundances of diatom lipids and increasing abundances of dinoflagellate lipids noted over the past few centuries.
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OVERVIEW

The overall goal of this research is to examine the response of both terrestrial vegetation and aquatic algae of Lake Malawi, East Africa, to paleoenvironmental variability. Here, a combined molecular and isotopic geochemical approach is used to examine terrestrial and aquatic components of lacustrine organic matter separately, thus providing more accurate information on conditions in Lake Malawi and its catchment than previously obtained from bulk geochemical analyses alone. This study represents one of the first molecular studies of Lake Malawi, and is also one of the first molecular studies of the East African Rift Lakes.

This thesis contains four chapters, each of which will be submitted for publication as an individual paper. For this reason, it was necessary to repeat some of the background and methods information in multiple chapters.

Chapter 1, which is currently in review in *Geology*, stems from the vegetation study of Lake Malawi presented in Chapter 2, but focuses solely on the n-alkane carbon isotope record. This record presently provides the best indicator of past aridity in southeastern Africa, and confirms the results of previous studies that have provided evidence for arid conditions at Lake Malawi in the early Holocene. Thus, a noteworthy result of this research is that the frequently used term “African Humid Period”, which refers to the interval from 12 to 5.5 cal ka, cannot be used in reference to the climate of southeastern Africa. A comparison of the Lake Malawi aridity record to ice core records of atmospheric methane, an indicator of global tropical wetness, demonstrates that wet/arid conditions in the southeastern African tropics were in-phase with conditions in the equatorial and northern African tropics during the Late Pleistocene (23-11 cal ka) but out-of-phase during the Holocene. It is suggested that the switch from in-phase to out-of-phase conditions may be partly related to a shift in the mean latitudinal position of the Intertropical Convergence Zone (ITCZ). A significant finding of this research is that Holocene climates in southeastern Africa were as variable as Late Pleistocene climates, and were characterized by extreme and abrupt changes in water availability. Such
changes in water availability have important implications for both human and faunal migrations and histories.

Chapter 2, which will be submitted to *Palaeogeography, Palaeoclimatology, Palaeocology*, presents a 23 cal ka record of vegetation change from southeast Africa. This first continuous vegetation record from Lake Malawi demonstrates that major and sometimes abrupt shifts in the vegetation of southeast Africa occurred both during the Late Pleistocene and Holocene. The vegetation surrounding Lake Malawi consisted of a greater percentage of C4 plants (grasses) than at present during the Last Glacial Maximum, in the early Holocene (~10.5-7.7 cal ka) and during the Younger Dryas cold period, indicating arid conditions in southeast Africa. The greatest inputs of C3 vegetation (trees) are noted at ~13.6 and 4.5 cal ka, and indicate wetter conditions. Although early Holocene aridity in Lake Malawi has been previously suggested, the additional supporting evidence for a shift to increased C4 inputs is particularly important because the other East African rift lakes to the north experienced significantly wetter conditions at this time. Another important finding of this study is that both the lignin phenol and n-alkane carbon isotopic records demonstrate a good correlation with the carbon isotopic signature of bulk sediment. This suggests that the bulk carbon isotopic signature of Lake Malawi sediments is primarily a reflection of terrestrial inputs and does not mainly reflect changes in algal productivity, as previously thought.

Chapter 3, which will be submitted to *Limnology and Oceanography*, presents a 23 cal ka record of algal ecosystem variability from Lake Malawi. At the start of this study, the hypothesis was that biomarkers of each of the four main algal groups in Lake Malawi (diatoms, cyanobacteria, green algae and dinoflagellates) could be used to examine the response of the entire algal community to paleoenvironmental variability. Unfortunately, biomarkers of green algae were not found in Lake Malawi sediments, hampering this effort. However, the available molecular data provides valuable insights into past changes in both the primary productivity and algal community structure of Lake Malawi. Both algal biomarker and short-chain n-alkane carbon isotope records provide evidence for a major shift in algal productivity at the Pleistocene/Holocene boundary, which may be related to a northward shift of the ITCZ that occurred with the collapse of
Northern Hemisphere Ice Sheets. Low rates of primary productivity are noted during the Late Pleistocene, while the Holocene is characterized by higher rates of algal productivity. A particularly interesting feature of the record is a short-lived event centered at ~4.9 cal ka, which is characterized by the absence or near absence of almost all algal biomarkers. As significantly warmer surface waters were present in Lake Malawi at this time, it is hypothesized that stronger thermal stratification of the lake inhibited wind induced upwelling, and thus nutrient supply, to the surface waters. Another interesting finding of this study is the presence of the compound docosanyl 3-0-methylxylopyranoside in Lake Malawi, which presently has only been described from Ace Lake (Antarctica). This compound is believed to be produced by nitrogen-fixing cyanobacteria, and its presence in Lake Malawi sediments agrees well with bulk nitrogen isotope data, suggesting increased abundances of nitrogen-fixing cyanobacteria throughout the Holocene. This study also provides evidence for the presence of Eustigmatophyte algae in Lake Malawi, which have not been described in algal surveys of the lake.

Chapter 4 focuses on the past 730 years of the Lake Malawi sedimentary record and discusses both terrestrial and aquatic biomarker records. Results of this research support previous evidence for increased aridity in southeastern Africa during the Little Ice Age (~1270-1800 AD), which is attributed to a southward migration of the ITCZ over Lake Malawi. Following the Little Ice Age, a shift to wetter conditions is observed, and interestingly, the period from ~1800 AD to the present appears to be the wettest interval of the past few millennia. A number of changes are noted in both terrestrial and aquatic biomarker records since ~1900 AD and may be related to anthropogenic activities in the Lake Malawi watershed. These include increased abundances of the compound retene, which is thought to reflect increased soil erosion into the Malawi basin, and increased abundances of total algal biomarkers, which may be a reflection of higher nutrient loading to Lake Malawi. A shift in the algal community structure of Lake Malawi during the past few centuries is also suggested by increasing accumulation rates of dinoflagellate biomarkers, accompanied by decreasing accumulation rates of diatom biomarkers. This
research also provides evidence for links between solar forcing and climate variability in East Africa.

Finally, this thesis concludes with Chapter 5, a brief discussion of remaining outstanding questions and directions for future research. An appendix details the laboratory methods utilized for this research, as much time on this project was devoted to laboratory work and refining various laboratory procedures. The methods are presented as a laboratory manual, which future students will be able to utilize for similar types of research projects.
CHAPTER 1

ABRUPT SWITCHING OF THE DOMINANT MECHANISMS CONTROLLING ARIDITY IN THE SOUTHEASTERN AFRICAN TROPICS

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ABSTRACT

Plant leaf wax carbon isotopes provide a record of C\textsubscript{3} versus C\textsubscript{4} vegetation, a sensitive indicator of aridity, from the southeastern African tropics since the Last Glacial Maximum (LGM). This record shows southeastern African wet/arid phases closely paralleling high-latitude ice core methane records, indicative of global tropical wetness, from the LGM until \~11 ka. At the start of the Holocene this in-phase relationship abruptly deteriorates and an anti-phase relationship prevails, with dry conditions at Lake Malawi correlating with wet conditions in equatorial and northern Africa. The abrupt switching of the dominant mechanisms controlling aridity likely can be attributed to a change from the prevailing influence of extensive ice sheets on global climate from 23-11 ka, to precessional variability in summer insolation exerting a greater influence on southeastern African moisture availability following Northern Hemisphere ice sheet retreat.
INTRODUCTION

Changes in the hydrological cycle have a far greater impact on human welfare in tropical Africa than does the relatively small range of temperature variability. Therefore, understanding the timing of past arid and wet phases in low-latitude regions is critical to understanding human and faunal migrations as well as the role and response of the tropics in global climate change. Tropical vegetation is a sensitive indicator of aridity and its distribution is mainly controlled by precipitation, which, in tropical East Africa, is influenced by the convective intensity and seasonal migration of the Intertropical Convergence Zone (ITCZ; also known as the Meteorological Equator) (Leroux, 2001). The C₃ cycle (Calvin-Benson) and the C₄ cycle (Hatch-Slack) are the two main pathways of carbon fixation utilized by plants (O’Leary, 1981). C₄ plants are characterized by higher water use efficiency and are common today in tropical savannahs, temperate grasslands, and in semi-arid regions (Raven et al., 1999). Aridity is recognized as the dominant control on the large-scale distribution of C₃ versus C₄ vegetation in tropical Africa (Schefuβ et al., 2003). Thus, changes in aridity can be examined by determining the past distribution of C₃ versus C₄ vegetation.

Lake Malawi (9-14°S) is located just north of the southernmost extent of the ITCZ, which presently migrates to 15°S (Leroux, 2001). The lake's vast drainage basin is dominated by tree savannah, composed of mainly C₃ plants, but to both the north and south of the basin grass savannah is present, which is dominated by C₄ grasses (Schefuβ et al., 2003) (Fig. 1). The proximity of Lake Malawi to this ecosystem boundary makes it a sensitive location to examine past aridity driven vegetation shifts.

The sediments of Lake Malawi have provided one of the few high-resolution, continuous records of climate variability since the LGM on the African continent (Brown and Johnson, 2005; Filippi and Talbot, 2005; Powers et al., 2005; Gasse et al., 2002; Johnson et al., 2002; Gasse, 2000). To date, aridity in Lake Malawi has been examined by deducing lake level histories from seismic reflection data (Johnson and P. Ng'ang'a, 1990), benthic diatom assemblages (Gasse et al., 2002), and geochemical analyses (Ricketts and Johnson, 1996; Finney and Johnson, 1991). As Lake Malawi is undersaturated with respect to calcite at depths >200m, the presence of carbonate-bearing
minerals in sediments at these locations attests to periods of lower lake level (Ricketts and Johnson, 1996). While the evidence for a major low stand in lake level during the LGM is indisputable, the history of aridity in the region during the Holocene, particularly regarding hydrological conditions in the Early Holocene, has not been resolved. Seismic reflectors have not been well dated, the relative abundance of benthic diatoms can be influenced by preservation and sediment redeposition from shallow to greater depth, and the presence/absence of endogenic carbonates in lake sediments is not a particularly sensitive indicator of hydrological conditions in the lake basin. Moreover, given the tectonic setting of Lake Malawi within the East African Rift Valley, it is possible that lake level changes noted in seismic, diatom, or carbonate records could be tectonic, and not climatic, in origin.

In comparison, tropical vegetation is a sensitive indicator of aridity and the carbon isotopic composition of long-chain n-alkanes, used to distinguish between inputs of C3 and C4 vegetation, provides an additional method of examining aridity that is independent of lake level changes. Long-chain, odd numbered n-alkanes (C25-C35) are a major component of the epicuticular waxes of terrestrial plant leaves (Eglinton and Hamilton, 1967). These lipids are transported to lacustrine and marine sediments via erosion by wind and water and are generally well-preserved (Meyers, 1997). Carbon isotopic measurements of individual n-alkanes can be used to distinguish between plants utilizing C3 versus C4 carbon fixation as C3 plants tend to have average n-alkane carbon isotopic compositions of around -36‰ while C4 plants are isotopically heavier and have average n-alkane carbon isotopic compositions of around -21.5‰ (Collister et al., 1994).

METHODS

In this study, we examine the n-alkane record of piston core M98-1P, collected from the northern basin of Lake Malawi (10°15.9'S, 34°19.1'E, 403 m depth; Fig. 1). The age model for core M98-1P, which is based on radiocarbon dating, has been previously published (Johnson et al., 2002) and therefore is not discussed further here. All ages in this study are reported as calibrated years before present. Methods for n-alkane extraction, separation and identification are described by Werne et al. (2000), with
the exception that saponification was replaced by a bond elute column procedure (Russell and Werne, in press) to separate the neutral lipid, fatty acid, and phospholipid fatty acid fractions. The neutral lipids were further separated by silica gel column chromatography and the n-alkane containing fraction was passed through an Ag+ impregnated silica pipette column to separate the saturated and unsaturated hydrocarbons.

Molecular identification of compounds (n-alkanes) was performed on a Hewlett-Packard 6890 gas chromatograph (GC) coupled to an HP 5973 mass spectrometer (MS). An HP-1 capillary column (25m x 32µm x 0.5µm) was used with He flow rates set at 2mL/min. The GC/MS oven temperature program initiated at 50°C and increased at a rate of 10°C/min to 130°C, and next at a rate of 4°C to 320°C. The final temperature of 320°C was held for 10 minutes. Mass scans were made over the interval from 50 to 650. Compounds were identified by interpretation of characteristic mass spectra fragmentation patterns, gas chromatographic relative retention times, and by comparison with literature.

Compound-specific carbon isotopic analysis of n-alkanes was performed in the Department of Geological Sciences at Brown University using gas-chromatography-isotope ratio-mass spectrometry (GC-IRMS). An HP 6890 GC (DB-1 column: 60m, 0.32mm diameter, 0.1µm film thickness) was connected to a Finnigan MAT Delta+ XL mass spectrometer via a combustion interface. The GC temperature program initiated at 40°C and increased at a rate of 20°C/min to 220°C and next at a rate of 6°C/min to 315°C. The final temperature of 315°C was held for 10 minutes. Compounds (n-alkanes) separated by the GC column were oxidized at 940°C and converted to CO₂ for isotopic analysis. Each n-alkane sample was run in duplicate and the standard deviation of all samples is better than ± 0.5‰ (the standard deviation of a mixture of standard compounds, run multiple times daily, was less than ± 0.28‰). All δ^{13}C values are reported in standard per mil (‰) notation relative to the Vienna Pee Dee Belemnite (vPDB) standard.

RESULTS AND DISCUSSION

Lake Malawi samples are characterized by a moderate to high odd versus even carbon number predominance (average carbon preference index (CPI) values of 3.9) and
are thus mainly reflecting inputs from terrestrial plant leaf waxes (Eglinton and Hamilton, 1967). Likewise, the δ13C records of the C29-C33 n-alkanes display similar trends, reflecting a common higher plant source (Fig. 2a). We use the weighted mean of the δ13C values of the C29-C33 n-alkanes to examine vegetation shifts in East Africa and hereafter refer to this record as the δ13Calk record. The percentage of C4 grasses is estimated from a binary mixing model based on the δ13Calk record, assuming end member values of -36‰ and -21.5‰ for C3 and C4 vegetation, respectively (Collister et al., 1994).

The Lake Malawi record is characterized by heavy δ13Calk values during the LGM, corresponding to an estimated vegetation assemblage of 61% C4 grasses based on the binary mixing model (Fig. 2b). Following the LGM, a gradual transition to increased abundances of C3 vegetation occurred until 13.6 ka, when the vegetation assemblage consisted of 55% C3 plants (45% C4 grasses). The onset of the Younger Dryas is marked by a return to increased C4 inputs, with a vegetation assemblage of 60% C4 grasses. Fluctuating but generally heavier δ13Calk values are noted during the early Holocene from 11-7.7 ka and indicate a vegetation assemblage of 49-55% C4 grasses. From 7.7-4.9 ka a transition to increased inputs of C3 vegetation is noted, with C3 vegetation reaching its maximum abundance of 57% at 4.9 ka. During the past 4.9 ka, a return to increased C4 abundances occurs with a vegetation assemblage of 52% C4 grasses present at 0.2 ka.

As aridity is a main control on the distribution of C3 versus C4 vegetation in tropical Africa (Schefuß et al., 2003), we assume that the Lake Malawi δ13Calk record reflects moisture abundance in the southeastern African tropics, and compare it to the ice core methane records from Antarctica and Greenland (Brook et al., 2000). The record of atmospheric methane mainly reflects inputs from wetlands, of which the tropics are a major contributor in the pre-industrial period (Brook et al., 2000; Chappellaz et al., 1997). Methane emissions from the tropics versus the northern and southern high-latitudes have been distinguished by examining the interpolar gradient from ice cores in Greenland and Antarctica, in combination with 3-box source modeling, to distinguish between northern high-latitude, tropical, and southern high-latitude sources (Brook et al., 2000; Chappellaz et al., 1997). These records indicate the dominance of tropical methane sources from the LGM through the Younger Dryas (Chappellaz et al., 1997). Comparing
the Lake Malawi $\delta^{13}C_{\text{alk}}$ record with the atmospheric methane records, the two datasets closely parallel each other from the LGM until ~11 ka (Fig. 2b). The Younger Dryas is a major feature of both the atmospheric methane and $\delta^{13}C_{\text{alk}}$ records and is marked by an abrupt shift to more arid conditions, with a shift from 45 to 60% C$_4$ grasses registered at Lake Malawi. Ice core records indicate that tropical methane sources were also prevalent in the early Holocene from 11.5-9 ka (Chappellaz et al., 1997). At the start of the Holocene, the close correlation between the Lake Malawi $\delta^{13}C_{\text{alk}}$ record and atmospheric methane records abruptly ends and a more anti-phase relationship prevails (Fig. 2b). In contrast to an early wet Holocene found in the equatorial and northern tropics, arid conditions were present at Lake Malawi from 11-7.7 ka. Early Holocene aridity in Lake Malawi has been previously noted (Filippi and Talbot, 2005; Gasse et al., 2002; Johnson et al., 2002; Gasse, 2000; Finney and Johnson, 1991; Johnson and Ng'ang'a, 1990), but not universally accepted (Jolly et al., 1998), and is anomalous compared to lakes from equatorial and northern Africa, which experienced highstands or overflowing conditions at this time (Gasse, 2000). Out-of-phase relationships are also noted throughout the middle and late Holocene. While atmospheric methane records indicate drying of the tropics from 7-2.5 ka (Chappellaz et al., 1997), the Lake Malawi $\delta^{13}C_{\text{alk}}$ record displays the highest percentage of C$_3$ vegetation at 4.9 ka, attesting to wetter conditions in southeastern Africa at this time. During the past 2.5 ka, ice core records indicate a prevalence of tropical methane sources although anthropogenic methane contributions are also significant during this interval (Chappellaz et al., 1997; Ruddiman and Thomson, 2001). From 4.9 ka to the present, a return to more arid conditions occurs at Lake Malawi. A shift to more arid conditions during this time has been recognized at other locations in tropical Africa (Gasse, 2000) while records from the Amazon basin indicate a shift to wetter conditions over the past 3 ka (Mayle et al., 2000).

In addition to aridity, temperature and the concentration of atmospheric carbon dioxide ($pCO_2$) are the other main driving factors of C$_3$ versus C$_4$ variability (Schefuß et al., 2003) and their effects on the Lake Malawi record must be considered. Comparing the Lake Malawi $\delta^{13}C_{\text{alk}}$ record to the Taylor Dome $pCO_2$ record (Monnin et al., 2001), we note increased abundances of C$_4$ vegetation to occur at times of low (the LGM),
intermediate (the Younger Dryas) and high \( p\text{CO}_2 \) (the early and late Holocene) (Fig. 2c). Thus, \( p\text{CO}_2 \) fluctuations can be eliminated as a main driver of \( C_3 \) versus \( C_4 \) abundances in tropical East Africa. This result is consistent with other studies that have found \( p\text{CO}_2 \) changes alone are insufficient to drive vegetation change (Huang et al., 2001; Pagani et al., 1999). Comparing the \( \delta^{13}\text{C}_{\text{alk}} \) record to the Lake Malawi temperature record derived from \( \text{TEX}_{86} \) (Powers et al., 2005), a correlation is noted between cooler temperatures and heavier \( \delta^{13}\text{C}_{\text{alk}} \) values, and vice versa (Fig. 2b). While it then appears that temperature is the main control on the distribution of vegetation, we note that strong linkages between temperature and aridity exist in the Lake Malawi region with warm/wet and cool/dry conditions being associated (Powers et al., 2005; Brown and Johnson, 2005; Johnson et al., 2002). Furthermore, the observed relationship between \( \delta^{13}\text{C}_{\text{alk}} \) and temperature, with increased \( C_4 \) abundances noted at times of cooler temperatures, is contrary to previous studies that have found increased abundances of \( C_4 \) grasses associated with warmer temperatures (Ehleringer et al., 1997; Livingstone and Clayton, 1980; Teeri and Stowe, 1976). Thus, given the associations between warm/wet and cool/dry conditions in Africa, and the fact that temperature changes are operating in the opposing direction to conditions favoring the observed dominant vegetation type, we conclude that vegetation changes in East Africa are mainly driven by changes in aridity, consistent with previous studies of tropical Africa (Schefuß et al., 2003).

Taken together, the Lake Malawi \( \delta^{13}\text{C}_{\text{alk}} \) record and the records of atmospheric methane indicate that arid/wet phases at Lake Malawi were in-phase with conditions in the global tropics during the Late Pleistocene but were generally out-of-phase throughout the Holocene (Fig. 2b). Ties between Lake Malawi and high-latitude climates during the Late Pleistocene have been noted previously (Brown and Johnson, 2005; Filippi and Talbot, 2005; Powers et al., 2005; Gasse et al., 2002; Johnson et al., 2002; Gasse, 2000), and likely result from a combination of changes in global mean temperature and ITCZ variability. The latitudinal position of the ITCZ varies as a result of the interhemispheric temperature contrast, with southward displacements of the ITCZ occurring during northern hemisphere cold periods (Broccoli et al., 2006). These southward ITCZ displacements are thought to be accompanied by reduced convection in the low latitudes
Previous studies of Lake Malawi have provided evidence for southward shifts of the ITCZ during northern hemisphere cold periods, including the Younger Dryas and Little Ice Age (Brown and Johnson, 2005; Filippi and Talbot, 2005; Johnson et al., 2002). The $\delta^{13}C_{\text{alk}}$ record presented here is consistent with the results of these studies, attesting to dry conditions in southeastern Africa at times when southward ITCZ displacements have been noted. While changes in the mean latitudinal position of the ITCZ have been linked to climate variability in southeastern Africa on decadal to millennial timescales (Brown and Johnson, 2005; Filippi and Talbot 2005; Johnson et al., 2002; Nicholson, 1996), and also are thought to occur on longer glacial/interglacial timescales (Broccoli et al., 2006; Ivanochko et al., 2005), other climatic forcings are important and must be considered. Cooler global mean temperatures during the LGM would have led to decreased surface evaporation, resulting in a tendency for increased global aridity. Widespread aridity is noted in tropical Africa at this time (Gasse, 2000). Following the LGM, southeastern Africa gradually warmed until 13.8 cal ka (Powers et al., 2005) and this temperature increase was accompanied by increased precipitation throughout the African tropics (Gasse, 2000). We suggest that ITCZ variability may have played a role in maintaining the noted in-phase relationship between arid/wet conditions the southeastern African tropics with the global tropics during the Late Pleistocene. At this time, cool temperatures in the northern hemisphere may have caused a southward displacement of the ITCZ (Broccoli et al., 2006), shifting the meteorological equator farther south and thereby causing the Malawi basin to receive less rainfall, similar to the equatorial and northern tropics. With the collapse of the Northern Hemisphere ice sheets, ties between the southern African tropics and the northern high-latitudes were weakened, perhaps in part due to the meteorological equator shifting northwards over Africa. Following the retreat of Northern Hemisphere ice sheets in the early Holocene, the influence of summer insolation on moisture availability in the southern tropics became relatively more important, and anti-phased with the northern African tropics. This is apparent from a comparison of austral summer insolation at 10° S latitude (Berger and Loutre, 1991) with the $\delta^{13}C_{\text{alk}}$ record. The summer insolation minimum centered at 10 ka corresponds with arid conditions in southeastern Africa and
as insolation increases throughout the Holocene, wetter conditions are observed (Fig. 2c). We note that the uppermost data point at 0.2 ka, indicating a shift to more arid conditions despite high insolation values, occurs during the Little Ice Age, when there is independent evidence for cooler (Powers, 2005) and drier conditions over Malawi (Brown and Johnson, 2005), attributed to a southward shift of the ITCZ (Brown and Johnson, 2005). Other forcing mechanisms, such as Atlantic and Indian Ocean sea surface temperatures, have been linked to climatic variability in East Africa (Camberlin et al., 2001; Goddard and Graham, 1999), and variation in the intensity of austral summer insolation may influence SST distributions in a manner that promotes the history of moisture that we observe in this region of southern Africa. Our \( \delta^{13}C_{\text{alk}} \) profile, for example, tracks the meridional gradient in South Atlantic SST’s off West Africa reasonably well (Fig. 2b), inferring higher rainfall in the Malawi basin when the SST gradient is weak and, presumably, Southern Hemisphere trade winds are weak over Africa (Schefuß et al., 2005). This may result in an eastward shift in the north-south oriented portion of the ITCZ during austral summer, allowing for deeper penetration of the Atlantic moisture into East Africa associated with the West African monsoon and more intense rainfall over the Malawi basin.

**CONCLUSIONS**

Results of this study show that wet/arid phases in southeastern African tropics were in-phase with conditions in the equatorial and northern tropics during the Late Pleistocene (23-11 ka) but are out-of-phase during the Holocene. Arid conditions were present in southeastern Africa during the LGM, the Younger Dryas, in the Early Holocene, and during the Little Ice Age, whereas peak wet conditions are centered on 13.6 and 4.9 ka. The presence of arid conditions in southeastern Africa during the Early Holocene (11-7.7 ka) is significant and we note that the frequently used term, "African Humid Period," referring to 12-5.5 ka, is inappropriate when considering the climate of a broad expanse of southeastern Africa. We also note that both Holocene and Late Pleistocene (23-11 cal ka) climates in southeastern Africa quite variable, both in terms of aridity and temperature. Shifts of 43-56% C₄ grasses and a temperature range of 26-31°C
(Powers et al., 2005) occurred during the Holocene while the Late Pleistocene was characterized by 45-61% C4 grasses and a temperature range of 24-30°C (Powers et al., 2005). These extreme and abrupt changes in water availability undoubtedly played a significant role in human and faunal migrations and in the development and collapse of human civilizations.

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Figure 1  A: Vegetation zones of East Africa (Schefuß et al., 2003) and the summer and winter positions of the Intertropical Convergence Zone (the meteorological equator) and the Inter-Oceanic Confluence (IOC) (Leroux, 2001). The IOC separates Atlantic and Indian Ocean moisture. B: The location of L. Malawi in relation to Lakes Masoko and Rukwa. C: The location of sediment cores M98-1P (10°15.99S, 34°19.19E) and M98-2PG (9°58.6'S, 34°13.8'E) in the northern basin of L. Malawi.
Figure 2: Caption on next page.
Figure 2  African paleoclimate records. In all graphs, the Younger Dryas (YD) cold period is highlighted. A: Carbon isotope composition of the C_{29}-C_{33} n-alkanes. Solid data points indicate samples from core M98-1P while open data points indicate samples from core M98-2PG. Error bars indicate the standard deviation of duplicate runs. B: Atmospheric methane (CH_{4}) records from the GISP2 (Greenland) and Taylor Dome (TD, Antarctica) ice-cores (Brook et al., 2000) compared with the L. Malawi δ^{13}C_{alk} (open squares) and temperature (grey triangles; Powers et al., 2005) records, and the Atlantic meridional sea surface temperature gradient (ΔSST) from the coast of Angola (the difference between alkenone-derived SSTs from sediment cores GeoB 6518-1 (05°35.3′S, 11°13.3′E) and GeoB 1023-5 (17°09.5′S, 11°00.5′E); Schefuß et al., 2005). Note that the y-axis scales of the methane and temperature records are reversed. The vertical dashed line at 11 cal ka indicates the transition between in- and out-of-phase relationships between the δ^{13}C_{alk} and CH_{4} records. C: Atmospheric carbon dioxide concentrations from TD (Monnin et al., 2001) and January insolation at 10°S (Berger and Loutre, 1991).
CHAPTER 2

VARIABILITY IN TROPICAL EAST AFRICAN VEGETATION DURING LATE PLEISTOCENE AND HOLOCENE: MOLECULAR AND ISOTOPIC RECORDS FROM LAKE MALAWI

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ABSTRACT

Accurate reconstructions of past vegetation are needed to better understand the response of tropical terrestrial ecosystems to climate variability. The East African Rift Lakes are situated in a sensitive geographical location where the position and seasonal migration of the Intertropical Convergence Zone (ITCZ) is a major influence on (paleo)climate and numerous studies have documented the response of these lakes to global climate events, such as the Younger Dryas Cold Period. However, as relatively few continuous and well-dated vegetation records exist for East Africa that span from the Last Glacial Maximum (LGM) to the present, many outstanding questions remain regarding the response of East African vegetation to global and regional climatic forcings. The northern basin of Lake Malawi, characterized by a consistent basin-wide stratigraphy (Barry et al., 2002), contains both a continuous and high-resolution sedimentary record of the past 23 cal ka. In this study we examine a sediment core from the northern basin of Lake Malawi and use biomarkers of terrestrial plants and compound-specific carbon isotopes to examine vegetation change in East Africa since the LGM. We find that the vegetation surrounding Lake Malawi consisted of a greater percentage of C₄ plants (grasses) during the LGM and also note significant excursions to greater C₄ inputs during the Younger Dryas, in the early Holocene, and from ~4.5 cal ka to the present. A relationship is noted between the n-alkane average chain length (ACL) and temperature, with longer ACLs being associated with higher temperatures. Higher n-alkane carbon preference index (CPI) values correlate with higher mass accumulation rates of biogenic silica and may result from periodic increased northerly winds over Lake Malawi. The molecular data produced in this study demonstrate that the carbon isotopic signature of bulk sediment (δ¹³C_TOC) in Lake Malawi is primarily a reflection of terrestrial inputs (C₃ vs. C₄ vegetation) and does not mainly reflect changes in algal productivity, as previously thought.

1. INTRODUCTION

Understanding past distributions of vegetation on Earth is important as vegetation can impart important feedbacks on global carbon, water, and nutrient cycles. The C₃
(Calvin-Benson) cycle and the C₄ (Hatch-Slack) cycle are the two main pathways of carbon fixation utilized by plants (O’Leary, 1981). The C₃ pathway is the most common photosynthetic pathway and is used by almost all trees, shrubs, herbs, and cold-season grasses and sedges (Raven et al., 1999). The C₄ pathway involves a carbon-concentrating mechanism, which gives C₄ plants (mainly warm-season grasses and sedges) a competitive advantage in low atmospheric CO₂ conditions (Ehleringer et al., 1997). C₄ plants also have a higher water-use efficiency than C₃ plants, and thus are common today in tropical savannas, temperate grasslands, and in semi-arid regions (Raven et al., 1999). Major changes in the distribution of C₃ and C₄ plants have been documented on glacial-interglacial and longer timescales (Hughen et al., 2004; Schefuβ et al., 2003; Zhang et al., 2003; Huang et al., 2001; Pagani et al., 1999; Cerling et al., 1993), and have important implications for the global carbon cycle since woody vegetation is thought to store more carbon than grasslands (e.g., Jackson et al., 2002; Schimel et al., 2001; Scholes and Archer, 1997; Schlesinger et al., 1990). At present, the main forcing factors controlling the global distribution of C₃ and C₄ vegetation are debated (Schefuβ et al., 2003). Temperature, aridity, and atmospheric CO₂ concentrations (pCO₂) are known to be important drivers of vegetation change (Huang et al., 2001; Kuypers et al., 1999; Pagani et al., 1999; Collatz et al., 1998; Cerling et al., 1993); however, the relative importance of each of these factors is not well understood (Zhang et al., 2003).

In comparison with temperate and high-latitudes, tropical paleoclimate has not been studied in as much detail. Understanding the response of tropical ecosystems to natural climatic variability is particularly important as 50% of the Earth’s surface lies between 30°S and 30°N and this region is inhabited by over 75% of the world’s population (Thompson, 2000). Numerous studies have provided evidence for significant climatic variability in the low-latitudes since the Last Glacial Maximum (LGM). However, many questions still exist regarding low-latitude paleoenvironmental variability as well as the response of terrestrial and aquatic tropical ecosystems to these climatic fluctuations.

In this study we use molecular biomarkers and compound-specific carbon isotopes to examine the response of low-latitude tropical terrestrial vegetation to changes
in global climate since the LGM. At present, relatively few studies of vegetation change exist for the Lake Malawi region and the existing pollen studies (e.g. DeBusk, 1998; Meadows, 1984) have been hampered by poor chronology, or for certain time intervals, poor preservation of pollen grains. This has made it difficult to understand the timing of vegetation changes noted throughout the Late Pleistocene and Holocene. Here, we examine a Lake Malawi sediment core that contains a continuous sedimentary record of the past 23 cal ka and for which the chronology has been well-established (Barry et al., 2002; Johnson et al., 2002). Recent advances in organic geochemistry have provided valuable insight into the paleoclimatic history of East Africa by allowing for the reconstruction of a continental temperature record from Lake Malawi (Powers et al., 2005). This record, produced from the same sediment core that we examine in this study, has revealed significant temperature variability in East Africa both between the Last Glacial Maximum (LGM) and the present, as well as major temperature variations within the Holocene. Having a continuous and well-dated record from a site where temperature is known provides a unique opportunity to examine the response of tropical terrestrial vegetation to paleoenvironmental variability.

2. BACKGROUND

2.1 Study Location

Lake Malawi (9°S to 14°S) is situated between the countries of Malawi, Mozambique, and Tanzania, and is 560km long and up to 75km wide (Eccles, 1974) (Figure 1). Lake Malawi is at least 5 million years old (Finney et al., 1996), is underlain by over 4 km of sediment (Rosendahl, 1987), and has a maximum depth of over 700m (Johnson and Davis, 1989). The lake is permanently anoxic below ~200m (Eccles, 1974) and is characterized by relatively high sedimentation rates of 0.5-1.5mm/yr (Finney et al., 1996). The majority of water loss in Lake Malawi is by evaporation rather than outflow, making it extremely sensitive to minor changes in aridity (Spigel and Coulter, 1996). Lake levels in some of the East African lakes are known to have varied by hundreds of meters in the past (Gasse, 2000). However, even during times of severe drought when other African lakes were completely desiccated, the deepest basins of Lake Malawi
contained water and continued to accumulate sediment (Johnson, 1996; Scholz and Rosendahl, 1988).

In addition to having both a continuous and high-resolution sedimentary record, Lake Malawi is also a good site for paleoenvironmental reconstructions as the lake exhibits a strong response to changes in global climate. Lake Malawi is situated in a climatically sensitive geographical location that is heavily influenced by the intertropical convergence zone (ITCZ) (Figure 1a). The lake is located at the southern limit of the ITCZ (~13°S) and experiences one rainy season per year (November to March), while the rift lakes to the north experience two rainy seasons from the overhead passage of the ITCZ. The ITCZ is a major feature of tropical climates (Leroux, 2001; Nicholson, 1996) and is a main control on the distribution of tropical vegetation via its relationship to precipitation. Presently, Lake Malawi is surrounded by tree savanna, which mainly consists of C3 vegetation (Schefuβ et al., 2003). To both the north and south of Lake Malawi lies grass savanna, which is dominated by C4 grasses (Schefuβ et al., 2003). The proximity of Lake Malawi to this boundary in ecosystem type makes it a sensitive location to examine past shifts in C3 and C4 vegetation associated with changes in climate (Figure 1a).

Piston core M98-1P was collected from 403m water depth in the northern basin of Lake Malawi (10°15.99S, 34°19.19) in 1998 by an expedition of the International Decade for East African Lakes (IDEAL) (Figure 1b). The lithology and age model (based on ¹⁴C dating, varve counting, and ²¹⁰Pb dating) of this 7.8m piston core have been previously described in several studies (Barry et al., 2002; Johnson et al., 2002; Filippi and Talbot, 2005) and the age model is available at the NOAA web site for the World Data Center for Paleoclimatology (www.ncdc.noaa.gov/paleo/data.html). All ages in this study are reported as thousands of calibrated years before present (cal ka).

2.2 Biomarkers of terrestrial plants

The n-alkanes are straight-chain hydrocarbons that exhibit strong odd carbon number predominance in living organisms and are a major component of the epicuticular waxes of terrestrial plant leaves (Eglinton and Hamilton, 1967). Although n-alkanes are
produced by many organisms, carbon number distributions and isotopic compositions vary depending on the source organism. Terrestrial plants are dominated by the long-chain (C25-C33) n-alkanes while aquatic algae are dominated by the short-chain n-alkanes (C17-C21) (Giger et al., 1980; Cranwell et al., 1987). Distribution patterns of n-alkanes can be used to distinguish between different vegetation types (e.g. Schwark et al., 2002; Hasnich et al., 2003). The C31 n-alkane tends to be dominant in grasses while the C27 and C29 n-alkanes are dominant in deciduous trees (Cranwell, 1973). Carbon isotopes of the long-chain n-alkanes can be used to distinguish between vegetation utilizing different photosynthetic pathways. Plants utilizing the C3 photosynthetic pathway tend to have n-alkane carbon isotopic compositions of around -36%, while plants utilizing the C4 photosynthetic pathway have n-alkane carbon isotopic compositions of around -21.5% (Collister et al., 1994).

The carbon preference index (CPI) (Bray and Evans, 1961) of n-alkanes can provide important information regarding the origin of hydrocarbons. This parameter is defined as:

\[
CPI = \frac{1}{2} \left[ \frac{(C_{25}+C_{27}+C_{29}+C_{31}+C_{33})}{(C_{24}+C_{26}+C_{28}+C_{30}+C_{32})} + \frac{(C_{25}+C_{27}+C_{29}+C_{31}+C_{33})}{(C_{26}+C_{28}+C_{30}+C_{32}+C_{34})} \right],
\]

where \(C_x\) refers to the concentration of the n-alkane with \(x\) number of carbon atoms. The CPI is used to examine the odd over even carbon number predominance, which can be used to distinguish terrestrial plant from petroleum sources. Terrestrial plants are characterized by CPIs of >3 whereas mature hydrocarbons have CPIs of ~1 (Bray and Evans, 1961).

A second parameter that can be examined from n-alkanes is the average chain length (ACL), which has been related to aridity or temperature (e.g. Rommerskirchen et al., 2003; Peltzer and Gagosian, 1989). For higher plants this parameter is defined as:

\[
ACL_{23-33} = \frac{(23[C_{23}] + 25[C_{25}] + 27[C_{27}] + 29[C_{29}] + 31[C_{31}] + 33[C_{33}])}{([C_{25}] + [C_{27}] + [C_{29}] + [C_{31}] + [C_{33}])}
\]
where $C_x$ refers to the concentration of the $n$-alkane with $x$ number of carbon atoms.

Lignins are large and structurally complex phenolic polymers produced by vascular plants (Hedges and Mann, 1979a, 1979b; Hedges and Ertel, 1982) and have been used successfully in many studies to examine variability in terrestrial vegetation (e.g. Visser et al., 2004; Hu et al., 1999, Goñi et al., 1997; Huang et al., 1999). Lignin phenols are composed of several different structural units (p-hydroxyl, vanillyl, syringyl, cinnamyl) and differences in abundance between these structural units can be used to distinguish different plant and tissue types (Goñi and Hedges, 1992). The phenol vanillyl is produced only by vascular plants, and within the vascular plants syringyl phenols are produced by angiosperms while gymnosperms lack these compounds (Goñi et al., 1993; Hedges and Mann, 1979a, b). The phenol cinnamyl is present only in the non-woody tissues of gymnosperms and angiosperms. Therefore, the ratio of cinnamyl/vanillyl (C/V) phenols indicates contributions of woody vs. non-woody land plant tissues while the ratio of syringyl/vanillyl (S/V) phenols can be used to distinguish gymnosperm from angiosperm sources (Goñi and Hedges, 1992). Woody tissues are characterized by C/V values of <0.05 while non-woody tissues have values of 0.1 to 0.8 (Goñi, 1997). Angiosperms have S/V ratios of 0.6-4 while gymnosperms have S/V values of around 0 as they lack the syringyl phenol.

3. METHODS

**TOC and C/N ratios**

Total inorganic carbon (TIC) and total carbon (TC) measurements were determined on a UIC CO$_2$ Coulometer. TC is TOC because TIC was not present in any of the samples analyzed from core M98-1P. Carbon to nitrogen ratios (C/N) were determined on a Costech ECS 4010 elemental analyzer. Sediment samples did not receive acid pre-treatment prior to analysis on the elemental analyzer. Therefore, C/N ratios reported here reflect the ratio of total organic carbon to total nitrogen ($C_{org}/N_{tot}$).
Mass accumulation rates of TOC and biogenic silica (BSi) were calculated to account for the effects of sediment dilution using the formula:

\[ \text{MAR}_{\text{TOC or BSi}} = \% \text{TOC (or BSi)} \times \text{LSR} \times (1-\Phi) \times \rho \left( \frac{\text{g}}{\text{cm}^3} \right) \]

where \( \text{LSR} \) = linear sedimentation rate (cm yr\(^{-1}\)), \( \Phi \) = sediment porosity (determined from water content), and \( \rho \) = dry sediment density (g cm\(^{-3}\)), which is assumed to equal 2.54 g cm\(^{-3}\).

**Bulk \( \delta^{13}C \)**

Freeze dried sediment samples were treated with excess 0.1N hydrochloric acid for 3 hours to remove inorganic carbon. After acidification sediment samples were filtered through organic-free Whatman GF/F glass fiber filters (0.7µm pore size) and rinsed 4x with excess distilled and deionized water (Millipore filtration system). Sediment samples were then dried in an oven at 35°C and stored in a desiccator until they could be packed into tin capsules for isotopic analysis. Bulk carbon isotope (\( \delta^{13}C_{\text{TOC}} \)) samples were analyzed at the College of Marine Science at the University of South Florida on a Thermo-Finnigan Delta-Plus XL mass spectrometer coupled to a Carlo-Erba NA2500 Elemental Analyzer.

**Isolation of plant leaf waxes**

Sixty sediment samples were selected for molecular analysis at a sampling resolution of approximately one sample per 500 years. During time periods in which previous studies of Lake Malawi had documented significant changes, such as during the Younger Dryas cold period (Johnson et al., 2002), samples were taken at higher resolution. Sediment samples were soxhlet extracted in groups of five with an additional blank sample run with every batch. This extraction blank was then worked up in the same manner as the sediment samples to ensure that no contamination was introduced to the samples during any of the steps. Freeze dried sediment samples were soxhlet-extracted with 2:1 methylene chloride: methanol for 24 hours to obtain a total lipid
extract (TLE). The TLE was further separated into neutral lipid, fatty acid, and phospholipid fatty acid fractions using Alltech Ultra-Clean SPE Aminopropylsilyl bond elute columns. Prior to loading the sample, bond elute columns were pre-cleaned by running 10mL of methanol and 10mL of 1:1 methylene chloride: 2-propanol through the column. Eight mL each of 1:1 methylene chloride: 2-propanol, 4% glacial acetic acid in ethyl ether, and methanol were used to elute the neutral lipid, fatty acid, and phospholipid fatty acid fractions, respectively. The neutral lipid fraction is the only fraction examined in this study, and therefore the fatty acid and phospholipid fatty acid fractions are not discussed further here. Silica gel column chromatography was used to further separate compounds in the neutral fraction following the procedures outlined by Wakeham and Pease (1992), which are presented in Appendix 2. The n-alkanes were present in the first apolar fraction, which was eluted with hexane. This fraction was next passed through an Ag⁺ impregnated silica pipette column to separate the saturated and unsaturated hydrocarbons.

Mass accumulation rates of n-alkanes were calculated from the following formula:

\[ \text{MAR}_{\text{alkane}} = \text{LSR} \times \text{DBD} \times C, \]

where \( \text{MAR}_{\text{alkane}} \) is the MAR in ng cm\(^{-2}\) yr\(^{-1} \), \( \text{LSR} \) = linear sedimentation rate (cm yr\(^{-1} \)), \( \text{DBD} \) = dry bulk density (g cm\(^{-3} \)), and \( C \) = the mass of compound (ng g\(^{-1} \)) in dry sediment.

**Identification and quantification of compounds**

Molecular identification of compounds (n-alkanes) was performed on a Hewlett-Packard 6890 gas chromatograph (GC) coupled to an HP 5973 mass spectrometer (MS). An HP-1 capillary column (25m x 32mm x 0.5µm) was used with He flow rates set at 2mL/min. The GC/MS oven temperature program initiated at 50°C and increased at a rate of 10°C/min to 130°C, and then at a rate of 4°C to 320°C. The final temperature of 320°C was held for 10 minutes. Mass scans were made over the interval from 50 to 650.
Compounds were identified by interpretation of characteristic mass spectra fragmentation patterns, gas chromatographic relative retention times, and by comparison with literature. Quantification of compounds was performed on a Hewlett-Packard HP 6890 Gas Chromatograph with a FID detector using 5α-androstane as an internal standard. Compound concentrations were determined by relating chromatogram peak area to the concentration of the internal standard. Column type and the temperature program used for GC analysis are the same as described above for GC-MS except for that He flow rates were set at 2.6mL/min.

**Compound-specific carbon isotopes**

Twenty eight samples were selected for compound-specific carbon isotopic analysis, and were analyzed in the Department of Geological Sciences at Brown University. The carbon isotopic composition of n-alkanes was determined by gas-chromatography-isotope ratio-mass spectrometry (GC-IRMS). An HP 6890 GC (DB-1 column: 60m, 0.32mm internal diameter, 0.1µm film thickness) was connected to a Finnigan MAT Delta+ XL mass spectrometer via a combustion interface. The GC temperature program initiated at 40°C and increased at a rate of 20°C/min to 220°C and next at a rate of 6°C/min to 315°C. The final temperature of 315°C was held for 10 minutes. Compounds (n-alkanes) separated by the GC column were oxidized at 940°C and converted to CO₂. For calibration, six pulses of reference CO₂ gas with a known δ¹³C value were injected to the IRMS. A standard mixture consisting of four fatty acids with known δ¹³C values was measured multiple times daily to ensure accuracy. The standard deviation of all compounds in this standard mixture was less than ±0.28‰. Each n-alkane sample was run in duplicate and the standard deviation of the C₂₉, C₃₁ and C₃₃ n-alkanes is better than ±0.38‰, ±0.28 ‰, and ±0.5‰, respectively. All δ¹³C values are reported relative to the Vienna Pee Dee Belemnite (vPDB) standard using standard delta (per mil) notation.

*Lignin phenol isolation and quantification*
Twenty samples were selected for lignin analysis. Lignin phenols were analyzed in the Stable isotope Biogeochemistry Laboratory at Purdue University. Standard procedures for analysis of trimethylsilyl (TMS) derivatives of lignin phenol monomers by CuO oxidation were followed (Hedges and Mann, 1979a, 1979b). Laboratory-specific conditions and techniques for quantification of lignin phenol monomers are described by Dalzell et al. (2005). To be consistent with other lignin phenol studies, we follow standard convention and present the lignin phenol data in terms of abundance normalized to TOC (mg lignin/100g organic carbon (OC)). It should be noted that when converted to mass accumulation rates (mg lignin/cm²/yr), the overall trends do not differ significantly from the TOC-normalized abundance data.

4. RESULTS
4.1 Bulk Geochemical Data

Mass accumulation rates of total organic carbon (TOC_MAR) and the atomic ratio of organic carbon to total nitrogen (C_organic/N_total) in core M98-1P indicate considerable variability during both the Late Pleistocene and Holocene (Figure 2a,b). The lowest TOC_MAR values occur in the oldest part of the record, at ~22 cal ka. Low TOC_MAR values are also noted at 13.5 cal ka. The highest TOC_MAR values are noted at 17.5 cal ka, from 13 to 11.9 cal ka, at 8 cal ka, and from 1.8 to 1.7 cal ka. The C_organic/N_total record generally tracks changes in the TOC_MAR record (Figure 2a,c), with the highest C_organic/N_total values occurring when TOC_MAR values are the highest and vice versa. An exception to this observation occurs following the LGM when the TOC_MAR peaks at 17.5 cal ka while the C_organic/N_total ratio peaks later at 16.3 cal ka. A notable feature of both the TOC_MAR and C_organic/N_total records is the Younger Dryas Cold Period (11.7-13 cal ka; Broecker et al., 1989) which is marked by an abrupt increase in both TOC_MAR and C_organic/N_total.

Bulk sediment carbon isotopic values ($\delta^{13}C_{TOC}$) range from -22.5‰ to -25‰, with the heaviest values occurring from 20-18 cal ka (Figure 2d). Following the LGM, $\delta^{13}C_{TOC}$ values gradually become lighter until ~13 cal ka. Unlike the TOC_MAR and C_organic/N_total records, $\delta^{13}C_{TOC}$ values do not exhibit a significant excursion during the Younger Dryas, although a trend towards slightly lighter values is noted. Following the
Younger Dryas, \(\delta^{13}C_{\text{TOC}}\) values generally fluctuate between -25\(^\circ\) and -23.5\(^\circ\) for the remainder of the Holocene.

4.2 Plant Leaf Wax Biomarkers

Mass accumulation rates of the long chain (C\(_{25}\)-C\(_{33}\)) \(n\)-alkanes range from \(-0.33\) to 2.47 ng/cm\(^2\) yr (Figure 3). In most samples, the C\(_{27}\) or C\(_{29}\) \(n\)-alkane is the most abundant of the long chain \(n\)-alkanes (Figure 3b, c), and the C\(_{34}\) \(n\)-alkane is the longest homologue present. The average chain length (ACL) varies from a low value of \(-26\) at 19.2 cal ka to a high value of \(-28.5\) at 5.4 cal ka (Figure 4b). The carbon preference index (CPI) varies from a low value of 1.7 at 13.2 cal ka to a high value of 7.1 at 10.5 cal ka (Figure 4a). The average CPI value for these samples is 3.9.

The carbon isotopic composition of the C\(_{29}\), C\(_{31}\) and C\(_{33}\) \(n\)-alkanes display similar trends reflecting a common higher plant source. Following the approach of Zhang et al. (2003), we use the weighted mean \(\delta^{13}C\) of the C\(_{29}\), C\(_{31}\) and C\(_{33}\) \(n\)-alkanes to examine \(C_3\) vs. \(C_4\) variability in the Lake Malawi record:

\[
\text{Weighted mean } n\text{-alkane } \delta^{13}C = \frac{\delta^{13}C_{29} \times C_{29} + \delta^{13}C_{31} \times C_{31} + \delta^{13}C_{33} \times C_{33}}{C_{29} + C_{31} + C_{33}}
\]

where \(C_x\) refers to the abundance of the \(n\)-alkane with \(x\) number of carbon atoms.

Weighted mean \(n\)-alkane \(\delta^{13}C\) values (hereafter referred to as \(\delta^{13}C_{\text{alk}}\)) range from a low of -29.3\(^\circ\) at \(-4.9\) cal ka to a high of -26.5\(^\circ\) at \(-21\) cal ka (Figure 5). A gradual decrease from heavier to lighter \(\delta^{13}C_{\text{alk}}\) values is noted from 22 to 13.6 cal ka. From 13.6 to 7.7 cal ka \(\delta^{13}C_{\text{alk}}\) values fluctuate but display generally heavier values. From 7.7 cal ka until \(-4.9\) cal ka a trend towards lighter \(\delta^{13}C_{\text{alk}}\) values is noted followed by a return to heavier values from 5 cal ka to the present.

The percent contribution of \(C_4\) vegetation can be examined by using a simple binary mixing model with endpoints for \(C_3\) and \(C_4\) \(n\)-alkanes (Zhang et al., 2003; Schefuß et al. 2003; Boom et al. 2002; Huang et al., 2000). Here we use endpoint values of -36\(^\circ\)
for C₃ plant n-alkanes and -21.5% for C₄ plant n-alkanes (Collister et al., 1994). The following formula was used to calculate the percentage of C₄ plant contribution:

\[ \delta^{13}C_{\text{measured}} = -21.5\%o y + (1-y)(-36\%) \], where y represents percent C₄ contribution.

Over the past 23 cal ka in Lake Malawi, the estimated vegetation assemblage varies from 43 to 61% C₄ grasses, and indicates mixed C₃ and C₄ inputs throughout the length of the record.

4.3 Lignin Phenols

Lignin phenols are relatively resistant to diagenesis, however, the extent of oxygenic diagenetic alteration of lignin phenols can be examined from the ratios of vanillic acid to vanillin (Ad/Al)ᵥ and syringic acid to syringealdehyde (Ad/Al)ₛ (Ertel and Hedges, 1985; Hedges et al., 1982). (Ad/Al)ᵥ and (Ad/Al)ₛ ratios of greater than 0.6 are indicative of highly degraded lignin (Goñi, 1997). In core M98-1P, (Ad/Al)ᵥ values range from 0.27-0.81 while (Ad/Al)ₛ values range from 0.21-0.52 (Figure 6a). Only the sample at 4 cal ka had an (Ad/Al)ᵥ ratio greater than 0.6. Thus, with the exception of this sample, lignin in Lake Malawi sediments is not highly degraded with (Ad/Al)ᵥ values often plotting near the range of values for fresh plant tissue.

A plot of C/V vs. S/V ratios can be used to separate inputs of angiosperm leaves and needles, angiosperm woods, gymnosperm woods, and gymnosperm needles (Goñi, 1997). All of the Lake Malawi samples plot within the range of angiosperm woods and angiosperm leaves and grasses, clearly indicating the dominance of angiosperms in East Africa over the past 23 cal ka (Figure 7). In core M98-1P, vanillyl and syringyl phenols are present in greater abundance than cinnamyl phenols. The S/V ratio ranges from 0.97 to 1.23 with an average of 1.12 while the C/V ratio ranges from 0.10 to 0.37 with an average 0.20 (Figure 6). Throughout the length of the record, S/V ratios remain within the range of angiosperm tissues while C/V ratios remain within the range of non-woody tissues. Yields of total lignin (A₈; includes vanillin, acetovanillone, vanillic acid,
syringaldehyde, acetosyringone, syringic acid, p-hydroxycinnamic acid and ferulic acid) in core M98-1P range from ~0.2 to 0.4 mg/100g OC (Figure 6c).

5. DISCUSSION
5.1 Bulk vs. molecular geochemical records

The Lake Malawi TOC, C/N, and δ13C_TOC records have been examined previously and relationships between these parameters are complex with multiple scenarios existing to explain observed trends in the data (Filippi and Talbot, 2005). It is not always clear in parts of the Lake Malawi record whether changes observed in bulk organic geochemical parameters are reflecting changes in algal productivity, changes in the delivery of terrestrial organic matter to the lake, or a combination of both. Generally, observed trends in the bulk geochemical data are that higher TOC_MAR correlates with increased BSi_MAR, higher C-org/N_tot ratios, heavier δ13C_TOC values, and decreased temperature (Figure 2). The relationships between higher TOC_MAR, higher C-org/N_tot ratios and heavier δ13C_TOC values could be explained by variations in terrestrial inputs to Lake Malawi. Increased delivery of terrestrial organic matter to the lake can increase TOC_MAR values and C-org/N_tot ratios. In this scenario, if most of the organic matter is terrestrially derived, shifts noted in the δ13C_TOC record reflect changes in C_3 vs. C_4 vegetation in the watershed. However, the trends noted in the Lake Malawi bulk geochemical records can also be explained by variations in aquatic productivity. The observed relationship between high TOC_MAR or high BSi_MAR values and lower temperature may be a reflection of changes in the thermal stratification of Lake Malawi. Warming of surface waters would increase thermal stratification making it more difficult for wind-induced upwelling to occur whereas cooling of surface waters would weaken thermal stratification, facilitating wind-induced upwelling and increasing primary productivity. This relationship suggests that changes in algal productivity could be the dominant control on δ13C_TOC values.

The molecular data produced in this study, including C/V ratios and n-alkane δ13C, sheds light on the origin of organic matter in Lake Malawi. C/V ratios, indicative of changes in non-woody vs. woody vegetation, correlate closely with δ13C_TOC values (Figure 6b), suggesting a terrestrial control on the carbon isotopic signature of Lake
Malawi organic matter. Similarly, $\delta^{13}C_{\text{alk}}$ values also correlate closely with $\delta^{13}C_{\text{TOC}}$ values (Figure 5), which offers support to the idea that the Lake Malawi $\delta^{13}C_{\text{TOC}}$ record is primarily reflecting changes in C$_3$ vs. C$_4$ vegetation. An exception to this correlation between $\delta^{13}C_{\text{alk}}$ and $\delta^{13}C_{\text{TOC}}$ values occurs during the Younger Dryas, when the $\delta^{13}C_{\text{alk}}$ record indicates heavier values while $\delta^{13}C_{\text{TOC}}$ values become slightly more negative (Figure 5). The Younger Dryas clearly stands out in Lake Malawi TOCMAR and BSiMAR records (Figures 2, 8). This event has been previously described in Lake Malawi and is marked by a 2°C cooling of surface waters (Powers et al., 2005) and increased diatom productivity, which resulted from increased northerly winds enhancing upwelling in the northern basin of the lake (Johnson et al., 2002; Filippi and Talbot, 2005). It appears that during the Younger Dryas, algal productivity imparted a greater signature on the $\delta^{13}C_{\text{TOC}}$ record than did terrestrial inputs. However, for the majority of the record, changes in terrestrial vegetation appear to be the main driver of variability in the $\delta^{13}C_{\text{TOC}}$ record. These results are in contrast to the results of Filippi and Talbot (2005) who examined the hydrogen index (HI) of Lake Malawi sediments (measured on the same sediment core examined in this study) and concluded that the organic matter is primarily of algal origin, although no parts of the core contain purely algal remains. Although algal inputs may be an important source of organic matter to Lake Malawi they do not appear to be the main influence on $\delta^{13}C_{\text{TOC}}$ values. Thus, we conclude that the $\delta^{13}C_{\text{TOC}}$ signal in Lake Malawi primarily reflects changes in C$_3$ vs. C$_4$ vegetation and that terrestrial inputs to Lake Malawi are more important than previously reported (e.g. Filippi and Talbot, 2005).

5.2 n-alkane ACL and CPI

N-alkanes are transported to marine and lacustrine sediments via wind and runoff (Simoneit, 1977) although in arid environments, such as East Africa, wind can be the dominant transport mechanism (Schefuß et al., 2003b). In Lake Malawi, accumulation rates of n-alkanes likely reflect transport history rather than the amount of biomass in the watershed. Lake Malawi is situated at the southernmost extent of the ITCZ and normally the regional winds are from the south (Nicholson, 1996), but a strong diurnal component of onshore/offshore wind patterns is superimposed (Hamblin et al., 2003). Previous
studies of Lake Malawi have provided strong evidence for periods in the past when the ITCZ was located at a more southerly position than today (Johnson et al., 2002; Filippi and Talbot, 2005; Brown and Johnson, 2005). During these intervals, it is likely that ITCZ-driven and diurnal temperature-driven changes in the wind regime produced stronger or more frequent northerly winds over Lake Malawi, resulting in increased upwelling in the northern basin of the lake, and, in turn, increased diatom productivity (Johnson et al., 2002). It is likely that ITCZ driven changes in the wind regime over Lake Malawi have also affected the transport of plant leaf waxes to the lake because winds are a major feature of modern East African climate.

CPI values may provide some insight into wind transport history of \( n \)-alkanes. Plots of CPI values and the mass accumulation rate of biogenic silica (BSiMAR), a proxy for diatom productivity, display the same general trends although major differences in sampling resolution exist (796 BSi measurements compared to 61 \( n \)-alkane measurements). Higher CPI values are noted during times of increased BSiMAR values and vice versa (Figure 4a). A likely explanation for this relationship is that periodic increased northerly winds over Lake Malawi leads to increased upwelling and diatom productivity in the northern basin of the lake (Johnson et al., 2002), while concurrently enhancing wind erosion and transport of plant leaf waxes to the lake. The highest CPI values (5-7) observed in the Lake Malawi sedimentary record are found during intervals of increased BSiMAR, and clearly indicate a terrestrial plant source.

Lake Malawi samples have an average CPI value of 3.9, indicating a terrestrial plant source. However, in parts of the core CPI values are as low as 1.7, which may suggest an additional source of \( n \)-alkanes to Lake Malawi sediments (Figure 4a). Low CPI values (<3) can indicate hydrocarbon contamination by petroleum (Bray and Evans, 1961), and hydrocarbon seeps have been reported from nearby Lake Tanganyika (Simoneit et al., 2000). Although hydrocarbon seeps have not been reported from Lake Malawi, their presence cannot be ruled out. However, vegetation shifts indicated by the \( \delta^{13}C_{\text{alk}} \) record agree closely with the record of lignin phenols (discussed in detail below), and these trends would not be expected if Lake Malawi sediments contained significant amounts of \( n \)-alkanes from hydrocarbon sources. We note that wood burning is a
possible source of low CPI n-alkanes (Standley and Simoneit, 1987), and dry-season fires are a natural and common occurrence in East Africa.

An interesting feature of the Lake Malawi n-alkane record is the observed relationship between the ACL and temperature. In general, shorter ACLs are present during periods of lower temperature while longer ACLs are present during periods of higher temperature (Figure 4b), although some differences exist between the ACL and temperature curves. A relationship between ACL and temperature has been previously suggested (Gagosian and Peltzer, 1986; Kawamura et al., 2003; Zhang et al., 2006). Kawamura et al. (2003) found that trees growing in tropical zones had higher ACL values compared to trees growing in temperate or sub-temperate zones, although this observation was based on a limited data set. Zhang et al. (2006) note lower n-alkanol ACL values during colder periods and higher ACL values during warm periods in samples from the Chinese Loess Plateau that span the past 170 kyr. Rommerskirchen et al. (2003) suggest that leaf surface temperature, which is related to either mean daily maximum temperature or absolute maximum temperature, may be a key factor affecting the ACL. Increasing the ACL raises the melting point of protective leaf surface waxes and thus plants growing at higher temperatures may synthesize longer chain n-alkanes in order to maintain the protective waxy coating on their leaves (Rommerskirchen et al., 2003). The observed relationship between ACL and temperature, which was reconstructed from the same Lake Malawi sediment core that we examine for biomarkers in this study, offers further support for this idea.

Conversely, it has been suggested that a direct relationship exists between longer chain n-alkanes and increased aridity (Peltzer and Gagosian, 1989; Poynter et al., 1989; Schefuss, 2003b; Liu and Huang, 2005). However, this relationship does not appear to hold true for the Lake Malawi samples, especially in the Late Pleistocene. For example, it is known that Lake Malawi was cooler (4°C less than today; Powers et al., 2005) and significantly more arid during the LGM, with the lowest and most sustained lake level low-stand occurring from 22.8-16 cal ka, followed by a sharp rise in lake level from 15.7-15 cal ka, with a maximum highstand lasting until 13 cal ka (Gasse et al., 2002). Comparing this lake level (aridity) information data to ACL values, we note that ACL
values are the lowest at ~19 cal ka (Figure 4b), which centers on the lake-level low stand. ACL values gradually increase to higher values until ~15.5 cal ka and fluctuate but stay high until ~13 cal ka (Figure 4b). If a relationship exists between longer chain n-alkanes and aridity, we would expect to find higher ACL values during the LGM when it was more arid trending towards gradually lower ACL values from ~15-13 cal ka when more humid conditions were present, but in fact the opposite trends are observed. Thus, we conclude that in Lake Malawi a relationship between ACL values and temperature exists, with higher ACLs associated with higher temperatures.

Another interesting feature of the Lake Malawi record is that individual mass accumulation rates of C25, C27, C29 and C31 n-alkanes display maximum abundances at different times (Figure 3). For example, the C25 n-alkane reaches maximum abundance at 19.2 cal ka, the C27 n-alkane reaches maximum abundance at 10.5 cal ka, and the C29 and C31 n-alkanes reach maximum abundances at 12.3, 7.8 and 5.3 cal ka. These differences in the dominant homologue suggest varying sources of n-alkanes to Lake Malawi over the past 23 cal ka. As of present, no surveys of lipid distributions in modern East African vegetation have been conducted and this information is necessary in order to understand shifts in the dominant homologues. It should be noted that submerged and floating-leaf aquatic macrophytes have been found to contain increased abundances of C23 and C25 n-alkanes (Ficken et al., 2000). However, in Lake Malawi aquatic macrophytes are not believed to be an important source of n-alkanes as the extremely steep sides of the northern lake basin (Figure 1b) restrict the area of the littoral zone.

5.3 Lignin phenols

Studies have shown that lignin phenols are transported mainly via water/riverine transport (Goffi et al., 1997). Therefore, it is assumed that the lignin phenol record is representative of local vegetation present within the northern Lake Malawi watershed. In contrast, n-alkanes can be transported by both wind and water and long-range atmospheric transport of these compounds is known to occur (Schefuβ et al., 2003b; Simoneit, 1977). Lignin phenols provide a better record of local vegetation (Meyers, 1997) as n-alkane records may contain components of both local and distant vegetation.
However, C/V and $\delta^{13}$C$_{alk}$ records display the same general trends (Figure 8) suggesting that the C$_{29}$-C$_{33}$ n-alkane record of Lake Malawi primarily reflects changes in vegetation within the watershed.

A notable feature of the Lake Malawi lignin record is that increased $\Lambda_8$ (total lignin phenol) yields generally correlate with increased temperatures (Figure 6c, d). This relationship may be related to changes in precipitation (aridity) as warm/wet and cool/dry conditions are generally associated in East Africa (compare Brown and Johnson, 2005; Powers et al., 2005; Johnson et al., 2002). For example, during the LGM when conditions were cooler (Powers et al., 2005) and more arid (Gasse et al., 2002), low $\Lambda_8$ values are noted (Figure 6c, d). Following the LGM a trend of generally increasing $\Lambda_8$ values continues until ~13 cal ka. As water is the main transport mechanism for lignin phenols, the low $\Lambda_8$ values during the LGM are consistent with arid conditions and decreased lignin transport. Gradually increasing $\Lambda_8$ values from the LGM until ~13 cal ka are consistent with a transition to a more humid climate at this time (Gasse et al., 2002) as enhanced lignin transport would have occurred in response to increased runoff.

During the past 23 cal ka, C/V values fluctuate within the range of non-woody tissues (Figure 6b). Non-woody tissues are produced by both C$_3$ and C$_4$ plants. However, a close correlation is noted between C/V values and $\delta^{13}$C$_{alk}$ values in Lake Malawi (Figure 8). This correlation indicates that elevated C/V ratios and heavier $\delta^{13}$C$_{alk}$ values are reflecting inputs from C$_4$ grasses while lower C/V ratios and lighter $\delta^{13}$C$_{alk}$ values reflect increased input from C$_3$ trees. Details of these records and causes of these shifts in C$_3$ vs. C$_4$ vegetation are discussed in detail below.

5.4 Paleovegetation history of Lake Malawi

5.4.1 Previous studies

Previous studies of vegetation history from the Malawi region are limited and a major focus of these studies has been on addressing controversy regarding the origin of Afrotropical grasslands, which are characterized by small patches of forest surrounded by grasslands (Meadows and Linder, 1993). The debate focused over whether these grasslands existed as the result of recent anthropogenic land burning or if they occurred
naturally. Meadows (1984) examined pollen records from a series of peat cores from the Nyika Plateau in northern Malawi and concluded that small patches of montane forest surrounded by montane grasslands were present in Malawi for at least the past 5000 years and possibly to as far back as the past 12,000 years. These pollen records indicated significant variability in the vegetation assemblages of the Nyika Plateau during the past 5000 years but the limited chronology on the peat cores restricted comparisons to other East African paleoenvironmental proxies (Meadows, 1984). DeBusk (1998) examined the pollen records of two Lake Malawi sediment cores from the central and southern basins of the lake. These records extend back to 37,500 BP capturing the LGM in East Africa but this study is also hampered by uncertainties in the chronology. DeBusk (1998) reaches similar conclusions as Meadows (1984), that small patches of forest surrounded by montane grasslands were present throughout the Holocene and did not result from recent anthropogenic activities. To date, the most robust records of vegetation change from the Malawi region come from well-dated pollen records of two lakes that lie to the north of Lake Malawi, Lake Rukwa (Vincens et al., 2005) and Lake Masoko (Garcin et al., 2006; Vincens et al., 2003). These two records, located in close proximity to each other, also indicate considerable variability in East African vegetation during the Late Pleistocene and Holocene, and, for certain time intervals, reach differing conclusions regarding climatic conditions in East Africa. These records are discussed in more detail below where they are compared to the Lake Malawi record.

Many uncertainties exist regarding paleo-vegetation histories of East Africa presently exist due to the climatic and topographic complexity of the region. For example, Lake Malawi is presently situated within a tree savanna vegetation zone (C₃ dominated) but to both the north and south of the lake grass savanna vegetation zones (C₄ dominated) are present (Figure 1). Previous studies of tropical Africa have demonstrated that aridity is the main factor controlling the distribution of C₃ vs. C₄ vegetation (Castañeda et al., in review; Schefuß et al., 2003), and thus it is likely that the different vegetation zones have expanded/contracted and migrated during the past 23 cal ka in response to changes in precipitation. Superimposed on this regional variability, is local complexity that results from the steep topography surrounding Lake Malawi. Mountains
in the northern basin of Lake Malawi rise sharply from the lake and reach elevations of over 2500m. Vegetation along this gradient is zoned and ranges from types that are limited to the lakeshore, to closed-canopy (wetter) and open-canopy (drier) miombo woodlands at low elevations, to montane grasslands with patches of Afrotropical forests at high elevations (above 1500m) (DeBusk, 1998). These vegetation zones may have migrated to higher or lower elevations in response to climate variability.

5.4.2 Late Pleistocene-Holocene vegetation history of Lake Malawi

During the LGM, conditions in Lake Malawi were 3.5°C cooler (Powers et al., 2005) and significantly more arid than at present (Gasse et al., 2002), with an estimated lowering of lake level on the order of 100-200m (Filippi and Talbot, 2005; Gasse et al., 2002; Johnson and Ng’ang’a, 1990). Elevated C/V ratios and heavier δ13C values indicate that Lake Malawi was surrounded by a greater proportion of C4 grasses at this time (estimated C4 plant percentage of 61% based on the binary mixing model; Figure 8). In nearby Lake Rukwa, located to the north of Lake Malawi (Figure 1c; ~280 km between coring sites), a well-dated pollen record indicates lowering of montane taxa from 23 to 19 cal ka (Vincens et al., 2005). Similar changes have been noted during the LGM at other East African sites (Vincens et al., 1993; Livingstone, 1971; Van Zinderen Bakker, 1969; Kendall, 1969). In contrast, conditions at Lake Masoko, located between Lakes Malawi and Rukwa, were wetter during the LGM (Garcin et al., 2006).

Following the LGM, C/V ratios and δ13C values indicate a gradual transition from increased inputs of C4 vegetation to increased inputs of C3 vegetation until ~13.6 cal ka (Figure 8). Temperatures in Lake Malawi increased steadily from ~25.8°C at 23 cal ka to ~30.3°C at 13.8 cal ka (Powers et al., 2005) and precipitation also is known to have increased throughout this interval. Following low lake levels during the LGM, Lake Malawi refilled rapidly beginning at 15.7 cal ka and reached a highstand at ~13 cal ka (Gasse et al., 2002). This shift from C4 dominated to C3 dominated vegetation following the LGM is consistent with vegetation changes noted at other sites in East Africa (DeBusk, 1998; Vincens, 1991, 1993; Kendall, 1969). At Lake Rukwa, similar changes have been noted from ~17-11 cal ka and this interval is characterized by an increase of
arboreal taxa accompanied by a decrease of grasses (Vincens et al., 2005). Progressive reforestation is noted beginning after 15 cal ka at Lake Masoko (Garcin et al., 2006).

Following the post-LGM gradual warming period that lasted until 13.8 cal ka, temperatures in Lake Malawi decreased to 27°C at 12.5 cal ka (Powers et al., 2005). This 2°C cooling marks the Younger Dryas in Lake Malawi, which is clearly noted in the bulk geochemical records (Figure 2). At this time, δ13Calk values indicate a major shift back to heavier values, similar to those noted during the LGM. This shift reflects an increase in C₄ vegetation from 45 to 60% (Figure 8). The heaviest δ13Calk values are noted at ~12.5 cal ka, coincident with the maximum cooling observed during the Younger Dryas. Interestingly, pollen records from Lake Rukwa do not indicate any major excursions during the Younger Dryas (Vincens et al., 2005), while a decrease in herb pollen and a simultaneous increase in Zambezian tree pollen indicates wetter conditions at Lake Masoko (Garcin et al., 2006) (Figure 8).

The early Holocene in Lake Malawi (~11.6 cal ka until ~7.7 cal ka) is characterized by fluctuating but generally increased inputs of C₄ vegetation with variations of 48 and 55% C₄ vegetation indicated by the δ13Calk record (Figure 8). The sampling resolution of lignin phenols is lower than that of n-alkane δ¹³C throughout this interval, but C/V ratios display increased inputs of non-woody vegetation at ~9 cal ka. Temperature also fluctuates throughout this interval with slightly cooler temperatures of 26-28°C prevailing (Powers et al., 2005). Generally heavier δ¹³Calk values are consistent with previously noted Early Holocene arid conditions in Lake Malawi (Filippi and Talbot, 2005; Gasse et al., 2002; Finney et al., 1996). In Lake Masoko, more arid conditions are also noted beginning at ~11.7 cal ka (Garcin et al., 2006). Early Holocene aridity in Lake Malawi (and Lake Masoko) is anomalous compared to the other lakes farther north in the East African Rift Valley, which experienced lake level highstands or overflowing conditions at this time (Gasse, 2000). Humid conditions are noted from 12.1 to 5.5 cal ka at Lake Rukwa (Vincens et al., 2005) and a paleo-shoreline has been described from ~200m above present day lake level, which is dated to 11-10.7 cal ka (Delvaux et al., 1998).
From 7.7 until 5-4 cal ka, a shift towards greater inputs of C3 vegetation is noted in Lake Malawi. A low value of 43% C4 vegetation is noted in the δ13Calk record at 4.9 cal ka while low C/V values are noted at 4 cal ka. The temperature record indicates a gradual warming from ~27 °C at 7.7 cal ka to 31.4°C at 5 cal ka (Powers et al., 2005). Expansion of forests during the middle Holocene has been noted in many East African records (Bonnefille et al., 1995; Ssemmanda and Vincens, 2002; Beuning et al., 1997) and has been attributed to a strengthening of the African monsoon (DeMenocal et al., 2000). In Lake Rukwa humid conditions initiate 12.1 cal ka and continue until 5.5 cal ka, with the maximum abundance and diversity of arboreal taxa occurring during this time (Vincens et al., 2005).

The past 4 to 4.9 cal ka in Lake Malawi is marked by a return to greater inputs of C4 vegetation, indicating a shift back to increasingly arid conditions in East Africa (Figure 8). A gradual shift towards higher C/V values is noted from 4 cal ka to the present while heavier δ13Calk values are noted from 4.9 cal ka to the present. In Lake Rukwa, increasingly arid conditions and similar vegetation changes (decreased arboreal taxa and the expansion of grasses) are noted from 5.5 cal ka to the present (Vincens et al., 2005). A high-resolution pollen record from Lake Masoko, which extends back to 4.2 cal ka, indicates periods of dry conditions between 4.2 cal ka and the present (Vincens et al., 2003). However, the Lake Masoko record, which is of considerably higher resolution than the Lake Malawi lignin record, also indicates periods of wetter conditions occurring within the past 4.2 cal ka (Vincens et al., 2003).

With exception of the Younger Dryas and early Holocene (11.6 to 7.7 cal ka), the Lake Ruka pollen record and Lake Malawi molecular records parallel each other closely (Figure 8). The often out-of-phase behavior noted at Lake Masoko, which is situated between Lakes Malawi and Rukwa, is intriguing. It appears that when conditions in Lake Malawi were significantly more arid, such as during the LGM and during the Younger Dryas, Lake Masoko experienced significantly wetter conditions (Figure 8). Given the extremely short distances between these sites (<100km between the Lake Malawi M98-1P coring site and Lake Masoko and <200km between Lakes Masoko and Rukwa; Figure 1c) it is difficult to envision such dramatically different climate regimes. Considering the
small size of Lake Masoko (area of 0.38 km² compared with 22,490 km² for Lake Malawi) (Barker et al., 2003; Spigel and Coulter, 1996), and the fact that Lake Masoko is located at an elevation of 840 m in the Rungwe volcanic highlands surrounding northern Lake Malawi, it is likely that Lake Masoko is influenced more by local orographic effects whereas Lake Malawi responds to more regional climatic forcing. The often out-of-phase behavior noted between Lakes Malawi and Masoko, two sites located in close proximity to each other, highlights the complexity of East African (paleo)climates and demonstrates both the need to better understand modern climatic processes in this region and to obtain additional well-dated and high-resolution paleoclimatic records from East Africa.

6. CONCLUSIONS

1) Major vegetation changes have occurred in the Lake Malawi watershed since the LGM. The vegetation history of Lake Malawi can be summarized into four main periods: 1) increased abundances of C₄ grasses during the LGM followed by a gradual transition to increased abundances of C₃ vegetation until 13.6 cal ka, 2) fluctuating but generally elevated abundances of C₄ vegetation from 13.6 to 7.7 cal ka, including during the Younger Dryas, 3) a middle Holocene shift back towards greater C₃ abundances from 7.7 to ~4.5 cal ka, and, 4) a shift back towards increased C₄ abundances from ~4.5 cal ka until the present. Aridity is likely the main factor driving these vegetation changes.

2) A relationship is noted between ACL values and temperature in Lake Malawi samples with higher ACL values correlated with higher temperatures. Higher temperatures may cause plants to synthesize longer chain n-alkanes to increase the melting point of their leaf surface waxes, and thus maintain the protective waxy coating on their leaves.

3) Higher CPI values correlate with higher BSiMAR values. Increased BSiMAR values have been linked to upwelling associated with stronger or more frequent northerly winds over Lake Malawi, and therefore, the relationship between CPI and BSiMAR is likely
reflecting increased transport of higher plant leaf waxes during periods of increased northerly winds.

4) The $\delta^{13}$CTOC record in Lake Malawi mainly reflects terrestrial inputs and indicates shifts in C$_3$ vs. C$_4$ vegetation. Molecular data obtained in this study indicates that terrestrial inputs have greater control on $\delta^{13}$CTOC values in Lake Malawi than previously thought.

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Figure 1  

(a) Vegetation zones of East Africa (figure modified from Schefuß et al., 2003) and the January and July positions of the ITCZ (based on Leroux, 2001). Presently Lake Malawi is situated within the tree savanna vegetation zone, which is $C_3$ dominated. Note the southernmost limit of the ITCZ at the southern end of Lake Malawi.  

(b) Location of piston core M98-1P.  

(c) Locations of Lake Rukwa and Lake Masoko for comparison with Lake Malawi. The arrows indicate the approximate of sediment cores examined from Lake Rukwa (Vincens et al. 2005) and Lake Masoko (Garcin et al., 2006; Vincens et al., 2003).
Figure 2: Caption on next page.
Figure 2 (on previous page) Bulk geochemical records from core M98-1P. The interval of the Younger Dryas is highlighted. a) Mass accumulation rates of total organic carbon (TOC) plotted against age in calibrated years before present (cal yr BP) are shown by the grey dots while the black line represents the smoothed TOC$_{MAR}$ record (5 point running average). b) Mass accumulation rates of biogenic silica (BSi) are plotted in grey while the black line represents the smoothed BSi$_{MAR}$ data (BSi data are from Johnson et al., 2002 and were also measured on core M98-1P).

c) The ratio of total organic carbon to total nitrogen (Corg/Ntot) shown by the grey dots while the black line represents the smoothed Corg/Ntot data. d) Bulk δ$^{13}$C. e) Lake Malawi temperature reconstruction based on the TEX$_{86}$ paleotemperature proxy (data from Powers et al., 2005). The x-axis scale is reversed to facilitate comparisons with the bulk geochemical records.
Figure 3 Mass accumulation rates of a) the C27 \( n \)-alkane, b) the C27 \( n \)-alkane, c) the C29 \( n \)-alkane, d) the C31 \( n \)-alkane, e) the C33 \( n \)-alkane, and f) mass accumulation rates of total terrestrial \( n \)-alkanes (the sum of C25-C33 \( n \)-alkanes).
Figure 4  a) The Carbon Preference Index (CPI) plotted in black. BSi_{MAR} is plotted in gray. b) The Average Chain Length (ACL) of \textit{n}-alkanes is plotted in black. The Lake Malawi temperature record based on the TEX86 paleotemperature proxy (Powers et al., 2005) is plotted in gray.
Figure 5  

a) $\delta^{13}$C values for the C$_{29}$ n-alkane indicated by the black squares, b) $\delta^{13}$C values for the C$_{31}$ n-alkane indicated by the open diamonds, c) $\delta^{13}$C values for the C$_{33}$ n-alkane indicated by the black stars, d) weighted mean $\delta^{13}$C values for the C$_{29}$C$_{33}$ n-alkanes indicated by the open circles. The bulk $\delta^{13}$C record is plotted in the grey open triangles for comparison.
Figure 6: Caption on next page.
Figure 6 (on previous page) Lignin phenol data. a) Acid to aldehyde ratios of vanillyl and syringyl phenols. Values above 0.6 indicate highly degraded lignin. b) The C/V ratio, indicative of woody vs. non-woody inputs is plotted in black. The $\delta^{13}C_{\text{TOC}}$ record is plotted in grey on top of the C/V record for comparison. c) Total lignin (black dots) plotted next to d) the temperature data from Powers et al. (2005), indicated by the grey line.
A cross plot of $C/V$ and $S/V$ ratios. The shaded boxes indicate the range of values for angiosperm wood, angiosperm leaves and grasses, gymnosperm wood, and gymnosperm needles. All Lake Malawi samples plot within the range of angiosperm woods and angiosperm leaves and grasses.
Figure 8  Summary of records. a) The C/V ratio is indicated by the solid black circles and b) the weighted mean n-alkane δ¹³C record is shown with the open squares. The grey dashed lines behind the weighted mean n-alkane δ¹³C record indicate the calculated percentage of C₄ vegetation based on the binary mixing model. c) Percent Macaranga pollen, a tree that requires humid conditions and a short dry season (<3 months) for growth (data from Garcin et al., 2006). Note the x-axis scale is reversed. d) Magnetic susceptibility record from Lake Masoko (data from Garcin et al., 2006). High susceptibilities are interpreted to indicate lake-level lowstands while low susceptibilities are interpreted to indicate lake-level highstands. e) Summary of Lake Ruka pollen zones (data from Vincens et al., 2005). Zone IV displays a dominance of Afrotropical pollen (found above 1800m today) and increased herbaceous taxa, indicating arid conditions. Zone III is characterized by a decrease in Afrotropical taxa and an increase in woodland arboreal taxa, indicating a shift towards more humid conditions. Zone II is characterized by diverse woodland and bushland vegetation, indicating wet conditions. Zone I is characterized by scarce arboreal taxa and increased percentages of grass and sedge pollen, indicating a return to dry conditions.
CHAPTER 3

MOLECULAR AND ISOTOPIC EVIDENCE FOR CHANGES IN THE ALGAL COMMUNITY STRUCTURE AND PRIMARY PRODUCTIVITY OF LAKE MALAWI (EAST AFRICA) DURING THE PAST 23,000 YEARS

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ABSTRACT

Biomarkers of aquatic algae and compound-specific carbon isotopes are examined for changes in the algal community composition and primary productivity of tropical Lake Malawi. We find that a major change in primary productivity and algal community structure of Lake Malawi occurred at the Pleistocene/Holocene boundary. Low abundances of biogenic silica and diatom biomarkers are present from the Last Glacial Maximum (LGM) until ~11.8 cal ka, indicating that Lake Malawi was characterized by either low rates of primary productivity or algal productivity that was dominated by a group other than diatoms. At the start of the Holocene, conditions initiated that were similar to those observed in modern Lake Malawi, with diatoms and nitrogen-fixing cyanobacteria becoming more important contributors to primary productivity. As previous studies have suggested, this transition at ~11.8 cal ka is likely related to a shift in the dominant wind direction over Lake Malawi, resulting from a southward shift in the mean latitudinal position of the Intertropical Convergence Zone (ITCZ) during the last glacial. Throughout the entire 23 cal ka record, the effects of wind induced upwelling are important and may be the main control on the carbon isotopic composition of algal lipids through delivery of isotopically light CO$_2$ to the photic zone. Relationships are also noted between thermal stratification and primary productivity. High rates of primary productivity are noted during the Younger Dryas and at ~8 cal ka when cooler conditions, resulting in weaker thermal stratification, were present in East Africa. At 4.9 cal ka, the opposite situation is noted when significantly warmer temperatures lead to enhanced thermal stratification, resulting in decreased algal productivity.

Keywords: algae, diatoms, cyanobacteria, Eustigmatophyte algae, compound-specific carbon isotope

INTRODUCTION

Climate variability in tropical Africa is dominated by hydrological change (Gasse, 2000). The East African Rift Lakes experienced major and rapid hydrological fluctuations during the Late Pleistocene and Holocene, with lake level changes on the
order of hundreds of meters noted in Lakes Tanganyika and Malawi (Gasse, 2000; Finney et al., 1996; Johnson et al., 1996; Gasse et al., 1989). These massive shifts between relatively moist and arid conditions resulted in the complete desiccation of shallower lakes, such as Lake Victoria (Johnson, 1996), and undoubtedly played a major role in both human and faunal migrations.

At present, little is known regarding the impacts of major hydrological fluctuations on the algal ecosystems of these lakes. Did primary productivity increase or decrease with falling lake levels? Were there shifts in the dominant taxa present? Primary productivity in the deep East African Rift Lakes is strongly influenced by stratification, which affects wind-induced upwelling and influx of nutrient rich waters to the photic zone (Hecky and Kling, 1987). Previous studies of Lake Malawi have provided evidence for past changes in the dominant wind direction over the lake, which impacted diatom productivity (Brown and Johnson, 2005; Johnson et al. 2002; Johnson et al., 2001). How did such changes in wind regime impact the other main algal groups in the lake? East Africa also underwent significant temperature changes during the Late Pleistocene and Holocene (Powers et al., 2005), but these were much less severe than the massive hydrological fluctuations that occurred. However, temperature changes of a few degrees may have affected thermal stratification, which, in turn, would have affected wind-induced upwelling and primary productivity. In Lake Tanganyika, the recent surface water warming of ~0.7 °C relative to the deep-waters since the early 1900s is thought to have increased stratification and reduced primary productivity (O’Reilly et al., 2003; Verburg et al, 2003), possibly by as much as 20% (O’Reilly et al., 2003). What was the effect of the mid-Holocene warming when lake surface temperatures were ~3 °C warmer than at present (Powers et al., 2005)?

In this study, we investigate these questions by using molecular biomarkers and compound-specific carbon isotopes to examine past algal community composition and primary productivity in Lake Malawi. Diatom and biogenic silica records from Lake Malawi have provided evidence for major shifts in diatom productivity and the dominant species present since the Last Glacial Maximum (Johnson et al, 2002; Gasse et al., 2002). However, diatoms are only one of the major algal groups in Lake Malawi and the
response of the other algal groups, which lack hard parts that preserve well, is currently unknown. Understanding how tropical aquatic ecosystems respond to environmental variability is important as changes in algal productivity and community structure have important implications for higher organisms in the food chain, such as fish.

2. BACKGROUND

2.1 Study location

Lake Malawi, the southernmost of the East African Rift Lakes, is located between 9°S and 14°S, is 560km long, and up to 75km wide (Eccles, 1974) (Figure 1). Lake Malawi is at least 5 million years old (Finney et al., 1996), is underlain by over 4 km of sediment (Rosendahl, 1987), and has a maximum depth of over 700m (Johnson and Davis, 1989). The lake is permanently anoxic below ~200m (Eccles, 1974) and is characterized by relatively high sedimentation rates of 0.5-1.5mm/yr (Finney et al., 1996). The majority of water loss in Lake Malawi is by evaporation rather than outflow, making it extremely sensitive to minor changes in aridity (Spigel and Coulter, 1996). Lake levels in some of the East African lakes are known to have varied by hundreds of meters in the past (Gasse, 2000). However, even during times of severe drought when other African lakes were completely desiccated, the deepest basins of Lake Malawi contained water and continued to accumulate sediment (Johnson, 1996; Scholz and Rosendahl, 1988).

In addition to having both a continuous and high-resolution sedimentary record, Lake Malawi is also a favorable site for paleoenvironmental reconstructions as the lake exhibits a strong response to changes in global climate. Lake Malawi is situated in a climatically sensitive geographical location that is heavily influenced by the intertropical convergence zone (ITCZ) (Figure 1a). The lake is located at the southern limit of the annual transit of the intertropical convergence zone (ITCZ) (13-15°S), and therefore experiences one rainy season per year (November to March) while the rift lakes to the north experience two rainy seasons from the overhead passage of the ITCZ (Leroux, 2001; Nicholson, 1996) (Fig. 1a). During the rainy season, the main recharge period for lakes and rivers in southern Africa, the dominant winds are weak and northerly. Between
April and May the ITCZ moves northward towards the equator with strong southerly winds prevailing until September when winds become more easterly (Eccles, 1974). The distinct seasonal patterns of climate are reflected in the sedimentary record of Lake Malawi (Pilskaln and Johnson, 1991). During the windy season, phytoplankton blooms occur throughout the lake and provide autochthonous contributions to sedimentation, dominated by diatoms (Bootsma and Hecky, 1999; Patterson and Kachinjika, 1995). During the rainy season the combination of generally weak winds and increased runoff results in high allochthonous sedimentary contributions. This results in annual varve couplets, with a light layer representing the windy season and a dark layer representing the rainy season (Pilskaln and Johnson, 1991).

The strength of density stratification, which depends on water column gradients of temperature and dissolved solids, is important to algal productivity in tropical lakes as strong stratification can inhibit wind induced upwelling and the delivery of nutrients to the photic zone. Stratification is the main controlling factor of phytoplankton composition and succession in Lake Malawi (Hecky and Kling, 1987). The lake is permanently stratified and more strongly so during the warm and wet season (Nov. to April) when temperature differences between the surface and bottom waters are the greatest. During the dry and windy season, when temperature gradients between the surface and bottom waters are reduced, dissolved solids help to maintain stratification (Wuest et al., 1996). Today, surface water temperatures of Lake Malawi vary from 23°C to 29°C between austral winter and summer, and bottom waters are 22.5°C (Wuest et al., 1996).

The East African Rift lakes, dominated by a few but widespread algal taxa (Patterson and Kachinjika, 1995), provide an ideal location to examine past changes in algal productivity and community structure. Phytoplankton productivity in Lake Malawi is presently dominated by Bacillariophyta (diatoms), followed by contributions from Cyanophyta (cyanobacteria) and Chlorophyta (green algae), with minor contributions from Pyrrophyta (dinoflagellates) (Patterson and Kachinjika, 1995). From October to March, the rainy and non-windy season in Malawi, Cyanophyta and Chlorophyta are the
dominant phytoplankton (Hecky and Kling, 1987). Bacillariophyta dominates the rest of the year, when cool and windy conditions are present (Hecky and Kling, 1987).

In this study we use molecular biomarkers and compound-specific carbon isotopes to examine past changes in algal community structure and primary productivity in Lake Malawi. We examine the record of piston core M98-1P, collected from 403 m water depth in the northern basin of Lake Malawi (10°15.99S, 34°19.19) in 1998 by an expedition of the International Decade for East African Lakes (IDEAL) (Figure 1b). The lithology and age model (based on 14C dating, varve counting, and 210Pb dating) of this 7.8m piston core have been previously described in several studies (Barry et al., 2002; Johnson et al., 2002; Filippi and Talbot, 2005) and the age model is available at the NOAA web site for the World Data Center for Paleoclimatology (www.ncdc.noaa.gov/paleo/data.html). All ages in this study are reported in calibrated years before present (CAL BP).

2.2 Biomarkers of aquatic algae

A variety of different compound classes provide biomarkers for aquatic algae. The straight-chain n-alkanes, n-alkanoic acids, and n-alkanols, with 17 to 21 carbon atoms, are dominantly produced by aquatic algae, and are general biomarkers for aquatic algae (Giger et al., 1980; Cranwell et al., 1987). However, as terrestrial plants and bacteria also produce small amounts of these compounds, several other groups of compounds are often used as biomarkers for aquatic algae. Sterols, compounds that occur in all eukaryotes, are membrane rigidifiers and the specificity of these compounds for different phytoplankton groups is well known (Volkman et al., 1986, 1998). Sterols commonly provide biomarkers for diatoms and dinoflagellates, which are two of the four main algal groups present in Lake Malawi. The dominant sterol(s) in diatoms varies depending on the species present but brassicasterol (24-methylcholesta-5,22-dien-3β-ol), fucosterol (24-methylcholesta-5,24(28)-dien-3β-ol), and β-sitosterol (24-ethylcholesta-5-en-3β-ol) are common lipids of diatoms (Volkman et al., 1998; Barrett et al., 1995). The compound dinosterol (4α,23,24-trimethyl-5α-cholest-22-en-3β-ol) is found in many dinoflagellate species (Withers, 1983; Pirretti et al., 1997) and is commonly used as a
biomarker for these organisms (Boon et al., 1979; Robinson et al., 1984; Brassell et al., 1987; Volkman et al., 1998). The other two main algal groups in Lake Malawi, green algae and cyanobacteria, also have specific biomarkers. Biomarkers of green algae include botryococcane and botryococcene, which are produced by the green algae *Botryococcus braunii* (Volkman et al., 1998; Maxwell et al., 1968). Other biomarkers for green algae include the C25 and C27 n-alkenes and lycopadiene (Volkman et al., 1998). Many cyanobacteria have been shown to contain 7- and 8-methylheptadecanes (me-n-C17, Gelpi et al., 1970) or 2-methylhopanoids (Summons et al., 1999). Two new cyanobacterial markers recently have also been suggested from the glycolipids, docosanyl 3-O-methyl-α-rhamnopyranoside and docosanyl 3-O-methylxylopyranoside (Sinninghe Damsté et al., 2001).

3. METHODS

3.1 TOC and C/N ratios

Total inorganic carbon (TIC) and total carbon (TC) measurements were determined on a UIC CO2 Coulometer. TC is TOC because TIC was not present in any of the samples analyzed from core M98-1P. Carbon to nitrogen ratios (C/N) were determined on a Costech ECS 4010 elemental analyzer. Sediment samples did not receive acid pre-treatment prior to analysis on the elemental analyzer. Therefore, C/N ratios reported here reflect the ratio of total organic carbon to total nitrogen (Corg/Ntot). The TOC, Corg/Ntot and bulk carbon isotope data included here have been previously presented in Castañeda et al. (in prep).

Mass accumulation rates of TOC and biogenic silica (BSi) were calculated to account for the effects of sediment dilution using the formula:

\[
\text{MAR}_{\text{TOC or BSi}} = \% \text{TOC (or BSi)} \times \text{LSR} \times (1-\Phi) \times \rho \text{ (g/cm}^3)\],
\]

where LSR = linear sedimentation rate (cm yr\(^{-1}\)), \(\Phi\) = sediment porosity (determined from water content), and \(\rho\) = dry sediment density (g cm\(^{-3}\)), which is assumed to equal 2.54 g cm\(^{-3}\).
3.2 Bulk $\delta^{13}C$

Freeze dried sediment samples were treated with excess 0.1N hydrochloric acid for 3 hours to remove inorganic carbon. After acidification sediment samples were filtered through organic-free Whatman GF/F glass fiber filters (0.7µm pore size) and rinsed 4x with excess distilled and deionized water (Millipore filtration system). Sediment samples were then dried in an oven at 35°C and stored in a desiccator until they could be packed into tin capsules for isotopic analysis. Bulk carbon isotope ($\delta^{13}C_{\text{TOC}}$) samples were analyzed at the College of Marine Science at the University of South Florida on a Thermo-Finnigan Delta-Plus XL mass spectrometer coupled to a Carlo-Erba NA2500 Elemental Analyzer.

3.3 n-alkane isolation

Sixty sediment samples were selected for molecular analysis at a sampling resolution of approximately one sample per 500 years. During time periods in which previous studies of Lake Malawi had documented significant changes, such as during the Younger Dryas cold period (Johnson et al., 2002), samples were taken at higher resolution. Freeze dried sediment samples were soxhlet-extracted with 2:1 methylene chloride: methanol for 24 hours to obtain a total lipid extract (TLE). The TLE was further separated into neutral lipid, fatty acid, and phospholipid fatty acid fractions using Alltech Ultra-Clean SPE Aminopropylsilic bond elute columns. Prior to loading the sample, bond elute columns were pre-cleaned by running 10mL of methanol and 10mL of 1:1 methylene chloride: 2-propanol through the column. Eight mL each of 1:1 methylene chloride: 2-propanol, 4% glacial acetic acid in ethyl ether, and methanol were used to elute the neutral lipid, fatty acid, and phospholipid fatty acid fractions, respectively. As the neutral lipid fraction is the only fraction examined in this study, the fatty acid and phospholipid fatty acid fractions are not discussed further here. Silica gel column chromatography was used to further separate compounds in the neutral fraction on the basis of polarity following the procedures outlined by Wakeham and Pease (1992), which are presented in Appendix 2. The $n$-alkanes were present in the first apolar fraction,
which was eluted with hexane. This fraction was next passed through an Ag⁺
impregnated silica pipette column to separate the saturated and unsaturated hydrocarbons.
A second apolar fraction containing branched and cyclic hydrocarbons was eluted with
mixtures of toluene, hexane and ethyl acetate. Two polar fractions were collected and
were eluted with mixtures of hexane and ethyl acetate, which ranged from 15-75% ethyl
acetate. A final fraction, eluted with methanol, was used to collect any material
remaining on the column. All polar fractions were derivitized with bis-
(trimethylsilyl)trifluoroacetamide (BSTFA) immediately prior to GC analysis.

Sediment samples were soxhlet extracted in groups of five and an additional blank
to sample was run with every batch. This extraction blank was then worked up in the same
manner as the sediment samples to ensure that no contamination was introduced to the
samples during any of the steps.

3.4 Biomarker identification and quantification

Molecular identification of compounds (n-alkanes) was performed on a Hewlett-
Packard 6890 gas chromatograph (GC) coupled to an HP 5973 mass spectrometer (MS).
An HP-1 capillary column (25m x 32mm x 0.5µm) was used with He flow rates set at
2mL/min. The GC/MS oven temperature program initiated at 50°C and increased at a
rate of 10°C/min to 130°C, and next at a rate of 4°C to 320°C. The final temperature of
320°C was held for 10 minutes. Mass scans were made over the interval from 50 to 650.
Compounds were identified by interpretation of characteristic mass spectra fragmentation
patterns, gas chromatographic relative retention times, and by comparison with literature.

Quantification of compounds was performed on a Hewlett-Packard HP 6890 Gas
Chromatograph with a FID detector using 5α-androstane as an internal standard.
Compound concentrations were determined by relating chromatogram peak area to the
concentration of the internal standard. Column type and the temperature program used
for GC analysis are the same as described above for GC-MS except for that He flow rates
were set at 2.6mL/min.

Mass accumulation rates of individual compounds were calculated from the
following formula:
\[ \text{MAR}_{\text{compound}} = \text{LSR} \times \text{DBD} \times C, \]
where \( \text{MAR}_{\text{compound}} \) is the MAR in ng cm\(^{-2}\) yr\(^{-1}\), \( \text{LSR} \) = linear sedimentation rate (cm yr\(^{-1}\)), \( \text{DBD} \) = dry bulk density (g cm\(^{-3}\)), and \( C \) = the mass of compound (ng g\(^{-1}\)) in dry sediment.

3.5 Compound-specific carbon isotopes

Twenty eight samples were selected for compound-specific carbon isotopic analysis, and were analyzed in the Department of Geological Sciences at Brown University. The carbon isotopic composition of \( n \)-alkanes was determined through gas-chromatography-isotope ratio-mass spectrometry (GC-IRMS). An HP 6890 GC (DB-1 column: 60m, 0.32mm diameter, 0.1\( \mu \)m film thickness) was connected to a Finnigan MAT Delta+ XL mass spectrometer via a combustion interface. The GC temperature program initiated at 40°C and increased at a rate of 20°C/min to 220°C and next at a rate of 6°C/min to 315°C. The final temperature of 315°C was held for 10 minutes. Compounds (\( n \)-alkanes) separated by the GC column were oxidized at 940°C and converted to CO\(_2\). For calibration, six pulses of reference CO\(_2\) gas with a known \( \delta^{13} \)C value were injected to the IRMS. A standard mixture consisting of four fatty acids with known \( \delta^{13} \)C values was measured multiple times daily to ensure accuracy. The standard deviation of all compounds in this standard mixture was less than ±0.28‰. Each \( n \)-alkane sample was run in duplicate and the standard deviation of the C\(_{17}\), C\(_{19}\) and C\(_{21}\) \( n \)-alkanes is better than ±1.5‰, 0.9‰ and 2.0‰, respectively. All \( \delta^{13} \)C values are reported relative to the Vienna Pee Dee Belemnite (vPDB) standard using standard delta (per mil) notation.

4. Results and Discussion

4.1 bulk geochemical data

Mass accumulation rates of total organic carbon (TOC\(_{\text{MAR}}\)) and the atomic ratio of organic carbon to total nitrogen (\( C_{\text{org}}/N_{\text{Tot}} \)) in core M98-1P indicate considerable variability during both the Late Pleistocene and Holocene (Figure 2). TOC content indicates the abundance of organic matter in sediments while the \( C_{\text{org}}/N_{\text{Tot}} \) ratio provides a
proxy for terrestrial vs. aquatic input (Meyers, 1997). Aquatic algae generally have C/N ratios of 4-10 whereas higher land plants are characterized by C/N ratios of >20 (Meyers, 1994). The lowest TOC_{MAR} values occur in the oldest part of the record, at ~22 cal ka, and low TOC_{MAR} values are also noted at 13.5 cal ka. The highest TOC_{MAR} values are noted at 17.5 cal ka, from 13-11.9 cal ka, at 8 cal ka, and from 1.8-1.7 cal ka. The C_{org}/N_{tot} record generally tracks changes in the TOC_{MAR} record (Figure 2), with the highest C_{org}/N_{tot} values occurring when TOC_{MAR} values are the highest and vice versa. An exception to this observation occurs following the LGM when the TOC_{MAR} peaks at 17.5 cal ka while the C_{org}/N_{tot} ratio peaks later at 16.3 cal ka. A particularly striking feature of both the TOC_{MAR} and C_{org}/N_{tot} records is the Younger Dryas Cold Period (11.7-13 cal ka; Broecker et al., 1988) which is marked by an abrupt increase in both TOC_{MAR} and C_{org}/N_{tot} (Figure 2).

Bulk sediment carbon isotopic values ($\delta^{13}$C_{TOC}) range from -22.5%o to -25%o, with the heaviest values noted from 20-18 cal ka (Figure 2). Following the LGM, $\delta^{13}$C_{TOC} values gradually become lighter until ~13 cal ka. Unlike the TOC_{MAR} and C_{org}/N_{tot} records, $\delta^{13}$C_{TOC} values do not exhibit a significant excursion during the Younger Dryas, but a trend towards slightly lighter values is noted. Following the Younger Dryas, $\delta^{13}$C_{TOC} values generally fluctuate between -25%o and -23.5%o for the remainder of the Holocene.

4.2 Algal biomarkers

4.2.1 Biomarkers of Bacillariophyceae (diatoms)

Two of the common sterol biomarkers for diatoms, brassicasterol (24-methylcholesta-5,22-dien-3β-ol) and β-sitosterol (24-ethylcholesta-5-en-3β-ol) (Volkman, 1998), are found in low abundance in Lake Malawi sediments. However, only a very small number of Lake Malawi samples contained brassicasterol and while β-sitosterol was present in only about half of the samples analyzed. Thus, given that diatoms dominate algal productivity in Lake Malawi (Hecky and Kling, 1987; Patterson and Kachinjika, 1995) and that abundant diatoms are present throughout the entire core (Gasse et al., 2002), these sterols do not provide reliable biomarkers for diatoms in Lake
Malawi. These sterols can also be synthesized by land and emergent water plants
(Nishimura and Koyama, 1977), and given the proximity of the shore to the coring site
terrestrial sources of sterols are likely in Lake Malawi. However, Lake Malawi
sediments do contain the compound loliolide/isololiolide, which provides a reliable
diatom biomarker.

Loliolide and isololiolide are the anoxic degradation products of the pigment
fucoxanthin, the major carotenoid present in diatoms (Repeta, 1989; Klock et al., 1984)
and are abundant in Lake Malawi sediments (Figure 3). Although dinoflagellates and
haptophyte algae can also contain fucoxanthin (Klock et al., 1984; Jeffrey and Vesk,
1997), loliolide (or isololiolide) provides a reliable marker for diatoms since
dinoflagellates are only a minor contributor to algal productivity in Lake Malawi and
haptophyte algae are not present in the lake (Patterson and Kachinjika, 1995).

When the record of loliolide is compared to the record of biogenic silica
(Johnson et al., 2002), another proxy for diatom productivity, the two records correlate
closely (Figure 3). Biogenic silica and loliolide mass accumulation rates indicate low
values from 23-12.9 cal ka. A rapid increase in accumulation rates marks the start of the
Younger Dryas interval, with peak values being reached at ~12.5 cal ka. Following the
Younger Dryas, a return to lower values occurs until ~10 cal ka, when a rapid increase in
accumulation rates is again noted. Throughout the Holocene accumulation rates of
biogenic silica and loliolide fluctuate, but are generally higher than Late Pleistocene
accumulation rates.

4.2.2 Biomarkers of Chlorophyceae (green algae)

Common biomarkers of the green alga Botryococcus braunii include
botryococccenes and lycopadiene/lycopane derivatives (Adam et al., 2006; Metzger and
Largeau, 2005). Botryococcus braunii is found in fresh and brackish waters from alpine,
temperate and tropical regions, and is classified into three different chemical races: A, B,
and L. Algae of race A produce n-alkadienes and alkatrienes, those of race B produce
botryococccenes, while lycopadiene and lycopane derivatives are produced by race L
(Metzger and Largeau, 2005). Lake Malawi sediments do not contain any biomarkers of
B. braunii, although the species is included in lists of algal taxa present in the lake (Patterson and Kachinjika, 1995). However, it should be noted that B. braunii is not the most common type of green algae present in Lake Malawi and instead Closterium, Staurastrum and Mougeotia are the most abundant species (Hecky et al., 1998).

Furthermore, the presence of B. braunii in Lake Malawi, and in the other tropical lakes of East Africa, is currently under debate and it is more likely that the species present is actually Botryococcus terribilis Komarek and Marvan, a species that is primarily found in tropical lakes (Hedy Kling, pers. comm.). For these reasons, it is not surprising that biomarkers of Botryococcus braunii were not detected in Lake Malawi sediments. Lake Malawi samples also did not contain any lycopadiene/lycopane derivatives.

The C25 and C27 n-alkenes are recognized as biomarkers of Chlorophyceae (Volkman et al., 1998), and the C25-C29 n-alkenes are present in Lake Malawi sediments (Figure 4). However, these compounds can also be produced by diatoms (Volkman et al., 1998) and in Lake Malawi a mixed source from green algae and diatoms is likely given that diatoms are the dominant algal taxa in the lake. We note that accumulation rates of the C25 and C27 n-alkenes closely track each other whereas accumulation rates of the C29 n-alkene more closely track those of loliolide (Figures 3, 4). This may suggest that diatoms are the dominant source of the C29 n-alkene in Lake Malawi whereas green algae are the dominant producers of the C25 and C27 n-alkenes.

Mass accumulation rates of the C25 and C27 n-alkenes indicate a general trend of decreasing values from the LGM until 16 cal ka (Figure 4). From 16 to 11 cal ka, the C25 and C27 n-alkenes exhibit the lowest values of the record, with a very slight increase in accumulation rates noted during the Younger Dryas. Throughout the Holocene, accumulation rates are generally higher than in the Late Pleistocene, with peak accumulation rates of the C25 and C27 n-alkenes occurring at approximately 10, 8, 5.3, and 3 cal ka and a general decline in accumulation rates occurring over the past 3 cal ka. Like the C25 and C27 n-alkenes, the C29 n-alkenes display low accumulation rates from the LGM until 13 cal ka and increased accumulation rates during the Holocene. Peaks in the accumulation rate of the C29 n-alkene are centered at 12.5 (the Younger Dryas), 10.5, 7.8, 5.3 and 1.5 cal ka.
4.2.3 Biomarkers of Cyanophyceae (cyanobacteria)

Common cyanobacterial biomarkers such as the 7- and 8-methylheptadecanes (me-n-C\textsubscript{17}, Gelpi \textit{et al.}, 1970) and the 2-methylhopanoids (Summons \textit{et al.}, 1999) are not present in Lake Malawi sediments. However, the glycolipid docosanyl 3-\textit{O}-methylxylolpyranoside is present (Figure 3), which is believed to derive from a cyanobacterial source (Sinninghe Damsté \textit{et al.}, 2001). Docosanyl 3-\textit{O}-methylxylolpyranoside, hereafter referred to as methylxylolpyranoside, was first recognized in sediments from Ace Lake (Antarctica), a saline lake that is permanently anoxic at depth (Sinninghe Damsté \textit{et al.}, 2001). To our knowledge, Lake Malawi is presently the only location besides Ace Lake from which methylxylolpyranoside has been reported and, additionally, Lake Malawi presently represents the oldest samples from which methylxylolpyranoside has been reported (found in samples up to 12.5 cal ka). The presence of methylxylolpyranoside in Lake Malawi indicates that the compound can be produced by freshwater organisms, and thus is not derived solely from marine organisms as has previously been suggested (Sinninghe Damsté \textit{et al.}, 2001).

In the Lake Malawi record, methylxylolpyranoside is absent prior to 12.5 cal ka (Figure 3). The compound first appears at this time, coinciding with the Younger Dryas interval, and is present in varying abundances throughout most of the Holocene. At 11.8, 8 and 5.3 cal ka methylxylolpyranoside is present in maximum abundance (ignoring the peak at 10.5 cal ka that is defined by only one data point).

4.2.4 Biomarkers of Eustigmatophyte algae

Lake Malawi samples contain abundant long-chain 1,15-alkyl diols (Figure 3). These compounds are recognized as biomarkers of the algal class Eustigmatophyceae (yellow-green algae) (Versteegh \textit{et al.}, 1997; Volkman \textit{et al.}, 1992). Relatively little is known about Eustigmatophyceae, which is a small class that presently contains only 8 genera (Ott and Oldham-Ott, 2003). Eustigmatophytes have one or two flagella, contain the pigments violaxanthin and vaucheriaxanthin, and lack chlorophyll-c and fucoxanthin (the common pigment in diatoms and dinoflagellates) (Ott and Oldham-Ott, 2003).
Although Lake Malawi sediments contain abundant long-chain \( n \)-alkyl diols, it is interesting to note that Eustigmatophyte algae have never been identified in algal or sediment samples from the lake (Hedy Kling, pers. comm.). There are several possible explanations for the occurrence of these compounds in Lake Malawi sediments. First, it is possible that Eustigmatophyte algae are present in Lake Malawi but have been overlooked in previous algal surveys. Most species of Eustigmatophytes are very small (2-4 \( \mu \mathrm{m} \)) and can be easily confused with coccolid forms of Chlorophyceae or Xanthophyceae (Ott and Oldham-Ott, 2003; Gelin et al., 1999). In Lake Malawi the most abundant species of Chlorophytes are small chlorococcales (Hecky et al., 1998) so it may be possible that members of Eustigmatophyceae have been confused with members of Chlorophyceae. Second, the class Eustigmatophyceae includes members that inhabit terrestrial soils (Ott and Oldham-Ott, 2003) and therefore, it is possible that soil Eustigmatophytes are the source of the long-chain \( n \)-alkyl diols. However, as abundances of the long-chain \( n \)-alkyl diols closely track abundances of other aquatic biomarkers, such as the diatom biomarker loliolide, a solely terrestrial source of the long-chain \( n \)-alkyl diols to Lake Malawi sediments is unlikely. It is also possible that the long-chain \( n \)-alkyl diols are coming from another source besides Eustigmatophyte algae. It has been noted that the \( \mathrm{C}_{32} \) \( n \)-alkyl diol is dominant in Eustigmatophytes of the genus *Nannochloropsis* (Volkman et al., 1992) yet in marine sediments the dominant chain-lengths are \( \mathrm{C}_{28} \) or \( \mathrm{C}_{30} \), suggesting an additional source of these compounds. As Eustigmatophyte algae are not abundant in seawater, it has been suggested that eustigmatophytes are probably not the dominant source of long-chain \( n \)-alkyl diols in marine environments (Gelin et al., 1999). However, in Lake Malawi samples, the \( \mathrm{C}_{32} \) \( n \)-alkyl diol is more abundant than either the \( \mathrm{C}_{28} \) or \( \mathrm{C}_{30} \) \( n \)-alkyl diols, which may point to the presence of eustigmatophytes in the lake. Furthermore, a study of the phytoplankton of nearby Lake Tanganyika, based on PCR-amplified 18S rDNA, provides evidence for the presence of eustigmatophytes possessing a sequence similar to marine and freshwater members of *Nannochloropsis* (De Wever, 2006). At present, we are unable to resolve the issue of production of long chain \( n \)-alkyl diols by another group or eustigmatophytes in Lake Malawi having been mistaken for coccolid members of the chlorophytes. The close agreement between the \( n \)-alkyl diols
and other algal biomarkers strongly suggests an aquatic source for these compounds, and, thus, the record of long-chain \( n \)-alkyl diols provides important paleoenvironmental information although the exact biological source is presently unknown.

The C\(_{30}\) and C\(_{32}\) 1,15-diols display similar trends with abundances being low from 23 to \(~13\) cal ka (Figure 3). An initial increase in abundance is noted during the Younger Dryas and values remain high, but variable, throughout the Holocene. In contrast, the C\(_{34}\) 1,15-diol exhibits elevated abundances in the Late Pleistocene and lower abundances in the Holocene, although in the Holocene trends in accumulation rates are similar to the C\(_{30}\) and C\(_{32}\) 1,15-diols. This suggests that the C\(_{34}\) 1,15-diol is produced by a different source from the C\(_{30}\) and C\(_{32}\) 1,15-diols.

### 4.2.5 \( n \)-alkane carbon isotopes

The C\(_{11}\)-C\(_{21}\) \( n \)-alkanes are general algal biomarkers and variations in the carbon isotopic composition of these compounds can be used to examine changes in primary productivity. The C\(_{17}\), C\(_{19}\), and C\(_{21}\) \( n \)-alkanes exhibit similar trends in both accumulation rates (Figure 4) and isotopic composition (Figure 5), and thus reflect a common algal source. Here we use the weighted mean of the C\(_{17}\)-C\(_{21}\) \( n \)-alkanes (Figure 5) to examine changes in the primary productivity of Lake Malawi and hereafter refer to this record as the \( \delta^{13}C_{\text{algal}} \) record. The Lake Malawi \( \delta^{13}C_{\text{algal}} \) record exhibits an overall change of \(~8\)% with the lowest value of \(-34.2\)% noted at 12 cal ka and the heaviest value of \(-26.7\)% noted at 3 cal ka. The \( \delta^{13}C_{\text{algal}} \) record can be divided into three main intervals: the Late Pleistocene (23-13 cal ka), the Younger Dryas (12.9-11.6 cal ka), and the Holocene (11 cal ka to the present). Mean \( \delta^{13}C_{\text{algal}} \) values of \(-30.1\)% characterize the Late Pleistocene while the Younger Dryas is marked by a shift to lighter \( \delta^{13}C_{\text{algal}} \) values, which average \(-32.3\)% from 12.9 to 11.6 cal ka. During the Holocene, a shift occurs to heavier average \( \delta^{13}C_{\text{algal}} \) values of \(-27.8\)%.

### 4.3 Environmental history of Lake Malawi

The long-term algal history of Lake Malawi has been previously examined by Johnson et al. (2002), Gasse et al., (2002) and Filippi and Talbot (2005). The Johnson et
al. (2002) and Gasse et al. (2002) studies concentrated on the record of diatom productivity in Lake Malawi from biogenic silica and diatom assemblage data, respectively. A more recent study by Filippi and Talbot (2005) combined bulk geochemical analyses including %TOC, %biogenic silica, C/N ratios, bulk carbon and nitrogen isotopes, and Rock-Eval hydrogen index (HI) data, to examine changes in the type of organic matter preserved in Lake Malawi during the past 25 cal ka. Rock-eval HI is a measure of the amount of hydrocarbon-type compounds (mg hydrocarbons per gram of sediment) that is produced from the cracking of kerogen as a sample is heated to 600°C (Filippi and Talbot, 2005; Espitalié et al., 1977). Changes in the HI, which reflect the hydrogen content of bulk OM, can be used to distinguish terrestrial and aquatic sources of organic matter, as aquatic material is rich in hydrogen compared to terrestrial material (Talbot and Livingstone, 1989). The Rock-Eval HI is used to examine the kerogen (non-solvent extractable) fraction of organic matter while in this study the bitumen (solvent extractable) fraction of the organic matter is examined. Some of the results of our molecular study conflict with the HI index results of Filippi and Talbot (2005) and these differences will be presented following the discussion of results obtained in this study.

We note that Filippi and Talbot (2005) examined three piston cores from the northern basin of Lake Malawi, including core M98-1P, the core examined in this study. The other two cores, M98-2P and M98-3P, were collected from slightly shallower water depths of 363 and 392m, respectively. We also note that in this study we examine the TOC and biogenic silica records in terms of mass accumulation rates to account for the effects of sediment dilution whereas Filippi and Talbot (2005) examined these records in terms of weight percent. This causes the magnitude of peaks noted in the biogenic silica and TOC records to differ between the two studies.

4.3.1 The LGM (23-18 cal ka)

Nearly all algal biomarkers exhibit low accumulation rates in the interval from the LGM until ~14 cal ka (Figures 3, 4). Aridity in southeastern Africa during the LGM has been well documented (Castañeda et al., in review; Filippi and Talbot, 2005; Johnson et al., 2002; Gasse, 2002; Gasse, 2000; Johnson and Ng’ang’a, 1990) and although the
The magnitude of the lake level change during the LGM has not been precisely quantified, it is thought to have been on the order of 100-200 m (Filippi and Talbot, 2005; Gasse et al., 2002; Johnson and Ng’ang’a, 1990). A lake level change of 200 m would bring the coring site of M98-1P, located at 403 m depth, into the oxic zone. Therefore, one possibility is that the generally low accumulation rates of algal biomarkers observed prior to ~14 cal ka resulted from the oxic degradation of the organic matter. However, several independent lines of evidence suggest that the coring site remained anoxic throughout the past 23 cal ka. First, no TIC was detected in any of the samples examined from core M98-1P. In modern Lake Malawi, carbonates are not preserved in the sediments because the water column is undersaturated with respect to calcite below the upper few meters (Ricketts and Johnson, 1996). Carbonate minerals may have been preserved if the coring site was located within the oxic zone during the LGM. Second, a study of lignin phenols, compounds produced by higher plants, suggests that no major periods of oxic degradation have occurred in core M98-1P (Castañeda et al., in prep). The extent of oxygenic diagenetic alteration of lignin phenols can be examined from the ratios of vanillic acid to vanillin (Ad/Al), and syringic acid to syringealdehyde (Ad/Al), (Ertel and Hedges, 1985; Hedges et al., 1982). In core M98-1P these ratios are relatively constant throughout the past 23 cal ka, with values falling within a range that indicates the lignin is well-preserved (Castañeda et al., in prep). Third, mass accumulation rates of TOC (TOC) are high, and some of the highest values of the entire record are reached at 18 cal ka. If oxic conditions were present, it might be expected that TOC would be lower, due to increased degradation of organic matter. Finally, the compounds loliolide and isololiolide are present throughout core M98-1P. When fucoxanthin, the major pigment in diatoms, undergoes anoxic degradation, loliolide or isololiolide is produced on a mole-to-mole basis (Repeta, 1989). The formation of these compounds from fucoxanthin during oxic conditions is very unlikely because loliolide and isololiolide are anoxic degradation products (Menzel et al., 2003; Repeta, 1989). Thus, these independent lines of evidence do not provide support for the oxygenation of bottom waters at the coring site and suggest that the magnitude of the LGM lake level lowstand was <200 m.
If the coring site indeed remained anoxic during the past 23 cal ka, then lower accumulation rates of algal biomarkers during the LGM (Figures 3, 4) suggest reduced algal productivity in Lake Malawi. This idea is supported by both bulk and molecular geochemical records. Throughout this interval, $\text{C}_{\text{org}}/\text{N}_{\text{tot}}$ values indicate mixed terrestrial and aquatic inputs to sedimentary organic matter, but increase from 23 to 18 cal ka (Figure 2). Likewise, the terrestrial to aquatic ratio of $n$-alkanes ($T/A$ ratio), which is another proxy for terrestrial vs. aquatic input (Bourbonniere and Meyers, 1996), generally tracks changes noted in the $\text{C}_{\text{org}}/\text{N}_{\text{tot}}$ record and displays higher values during the Late Pleistocene than during the Holocene (Figure 4). These two records indicate either increased delivery of terrestrial organic matter or decreased algal inputs to Lake Malawi at this time. During the LGM and throughout the Late Pleistocene, $\delta^{13}$C$_{\text{alg}}$ values are low compared to Holocene values (Figure 5), suggesting lower rates of primary productivity in Lake Malawi. Furthermore, measurements of total phosphorus (TP) and inorganic phosphorus (IP) in nearby piston core M98-2P indicate that mass accumulation rates of TP and IP were significantly lower from ~23-13 cal ka than during the Holocene (Johnson et al., 2002). Low mass accumulation rates of phosphorous prior to ~13 cal ka may be responsible for low rates of algal productivity in Lake Malawi during the Late Pleistocene because phosphorous is a limiting nutrient for algal growth. Lower algal productivity in Lake Malawi is the simplest scenario to explain the observed trends in bulk geochemical and lipid biomarker records during the Late Pleistocene.

4.3.2 The Late Pleistocene (18 to 13 cal ka)

Following to the LGM, most algal biomarkers either continue to exhibit consistently low accumulation rates (loliolide and $n$-alkanes) or display a general decrease in accumulation rates ($n$-alkenes and $n$-alkyl diols) between 18 and 13 cal ka (Figures 3, 4). This period is also characterized by a decreasing trend in $\delta^{13}$C$_{\text{TOC}}$, which can be attributed to increasing abundances of $C_3$ vegetation in the Lake Malawi basin from 18 to 13.6 cal ka (Castañeda et al., in review). Lignin phenol $A_3$ values (a measure of the total lignin phenol input) also display a general increasing trend from the LGM to ~13 cal ka, and indicate increasing terrestrial input throughout this interval (Castañeda et
Thus, a likely explanation for the decrease in $\delta^{13}$C_{TOC} is from an increase in C$_3$ vegetation in the catchment, which was accompanied by elevated inputs of terrestrial organic matter to Lake Malawi. The switch from C$_4$ to C$_3$ vegetation is not surprising given that a rapid and major lake-level rise occurred from 15.7-15 cal ka (Gasse et al., 2002), reflecting increased moisture availability in the southeastern African tropics at this time. Similarly, Filippi and Talbot (2005) suggest that drowning of lake-shore vegetation and soils can account for the trend in $\delta^{13}$C_{TOC} as oxidation of terrestrial OM would lower $\delta^{13}$C$_{DIC}$ and also increase [CO$_2$]$_{aq}$, which would lower algal $\delta^{13}$C. A shift to wetter conditions would also release more nutrients from the drainage basin, leading to enhanced primary productivity. Given the strong evidence for both a major lake level rise (Gasse et al., 2002) and an increase in C$_3$ vegetation throughout this interval (Castafieda et al., in prep), both processes likely contributed to the overall decreasing trends in the $\delta^{13}$C$_{TOC}$ and $\delta^{13}$C$_{algal}$ records.

From 18-13 cal ka, algal biomarkers generally display increasing but still low accumulation rates (Figure 3, 4). There is firm evidence for a major rise in lake level at 15.7 cal ka (Gasse et al., 2002) and if the coring site had been oxygenated or periodically oxygenated at the LGM, rising lake levels would have led to the establishment of anoxic conditions thereby enhancing preservation of sedimentary organic matter. However, the low accumulation rates of algal biomarkers may be explained by increasing temperatures throughout this interval, which would have led to progressively stronger thermal stratification, possibly limiting upwelling and primary productivity. From 20-13 cal ka, temperatures in Lake Malawi steadily increased from ~24°C to 30°C (Powers et al., 2005). The general decline in TOCMAR values from 18 to ~13 cal ka is consistent with increasing stratification due to increasing temperature. Algal biomarkers are low throughout this interval, with the n-alkyl diols, n-alkanes and n-alkenes exhibiting the lowest accumulation rates of the entire record from ~16-13 cal ka.

4.3.3 The Younger Dryas (12.9 to 11.6 cal ka)

The onset of the Younger Dryas interval, at 12.9 cal ka, marks the start of a major increase in productivity in Lake Malawi. The Younger Dryas clearly stands out in mass
accumulation records of biogenic silica and TOC (Figure 2), and is also marked by high accumulation rates of most algal biomarkers (Figures 3, 4). During the Younger Dryas, increased northerly winds were present over Lake Malawi (Johnson et al., 2002) and a 2°C drop in temperature occurred (Powers et al., 2005) (Figure 2). At this time, diatom assemblages indicate increased diversity (Gasse et al., 2002), an abrupt shift to more arid conditions is noted by an increase in C4 vegetation (Castañeda et al., in review), and both \( \delta^{13}C_{\text{alg}} \) and \( \delta^{13}C_{\text{TOC}} \) values indicate a shift to more negative values. Cool and arid conditions are noted during the Younger Dryas, however, conditions were not as cool or as arid as during the LGM (Powers et al., 2005; Castañeda et al., in review).

The high rates of primary productivity are not surprising given the fact that cooling (Powers et al., 2005), in combination with the enhanced northerly winds that were present (Johnson et al., 2002), may have lead to a less strongly stratified water column. Both effects would facilitate upwelling in the northern basin of Lake Malawi and could lead to increased primary productivity, as is reflected in the biogenic silica, TOC, and biomarker records. The shift to more negative \( \delta^{13}C_{\text{alg}} \) and \( \delta^{13}C_{\text{TOC}} \) values is likely caused by increased amounts of isotopically depleted CO\(_2\) being upwelled to the surface waters and made available for primary productivity. It should be noted that the Younger Dryas is the only interval of the past 23 cal ka when terrestrial plant leaf wax \( \delta^{13}C \) does not track the \( \delta^{13}C_{\text{TOC}} \) signal. At this time, an abrupt shift to increased amounts of C4 vegetation occurred (Castañeda et al., in review); however, it appears that the increase in primary productivity was of great enough magnitude to counteract any effects of an increase in surface water DIC values from increased inputs of C4 vegetation.

Another notable feature of the Younger Dryas is that methylxylopyranoside (the cyanobacterial biomarker) makes its first appearance at this time and then remains present throughout the Holocene (Figure 3). It is not clear why cyanobacteria first appear at this time since in the modern lake cyanobacteria are dominant when the lake is stable and strongly stratified.

4.3.4 The Holocene
Following the Younger Dryas, an early Holocene dry spell occurred in the Malawi basin (Castañeda et al., in review) that may account for the observed low BSiMAR and TOCMAR, and low accumulation rates of the diatom biomarker loliolide (Figures 2, 3). All of these records display low values from the end of the Younger Dryas (~11.9 cal ka) until ~10 cal ka. The magnitude of this early Holocene aridity is not precisely known and the timing varies somewhat between the various records. However, numerous independent proxies all provide firm evidence for a significant fall in lake level in the early Holocene. For example, a lowstand is noted in seismic records (Owen et al., 1990; Johnson and Ng’ang’a, 1999) and is dated to ~10.7 cal ka (Owen et al., 1990). A study of redox metals from a sediment core collected from the central basin of Lake Malawi suggests that the water column was oxygenated at a site that is now at a depth of ~300m (Brown et al., 2000). Diatom assemblage records indicate poor preservation and suggest the lowstand was centered at 10.6 cal ka (Gasse et al., 2002), while the Rock-eval HI data indicates a shift from well-preserved to strongly oxidized organic matter occurring from 12-11.3 cal ka (Filippi and Talbot, 2005). Increased inputs of terrestrial organic matter are observed in smear slides (Filippi and Talbot, 2005) and molecular analyses indicate increased accumulation rates of terrestrial plant leaf waxes (long-chain n-alkanes) centered at ~10 cal ka (Castañeda et al., in prep). Additionally, the terrestrial to aquatic ratio of n-alkanes indicates an abrupt increase in terrestrial inputs in the early Holocene (Figure 4). Elevated, but fluctuating, inputs of C4 vegetation are also noted from ~11.5 to 8 cal ka, attesting to generally arid conditions throughout this interval (Castañeda et al., in review). Cyanobacterial and eustigmatophyte biomarkers exhibit major changes in accumulation rates throughout this interval, suggesting variable conditions.

From 10 cal ka to the present, algal biomarkers display fluctuating accumulation rates but are generally higher than during the Late Pleistocene (Figures 3, 4). Several of the algal biomarkers, including methylxylopyranoside, loliolide, n-alkanes and the n-alkenes, exhibit peaks in accumulation rates centered at 8 cal ka whereas a period of low accumulation rates of loliolide, n-alkyl diols and methylxylopyranoside occurs at 4.9 cal ka. It appears that these high and low peaks in accumulation rates of algal biomarkers may be at least partly related to changes in thermal stratification. Holocene temperatures
of Lake Malawi were highly variable with temperatures of ~28°C noted from 11-9 cal ka, a cooling of ~2°C occurs at ~8 cal ka (perhaps coinciding with the 8.2 cal ka cold event noted in the Northern Hemisphere), followed by a gradual warming until the warmest temperatures of the past 25 cal ka (~32°C) are reached at 4.9 cal ka (Powers et al., 2005). After this time a gradual cooling occurs, with temperatures reaching 27.7°C at 0.2 cal ka (Powers et al., 2005). Thus, increased accumulation rates of algal biomarkers at ~8 cal ka may be attributed to the cooler surface water temperatures, which could have reduced thermal stratification and facilitated wind-induced upwelling. The opposite situation may have occurred at 4.9 cal ka, when warmer surface water temperatures could have increased thermal stratification making it more difficult for upwelling to occur. Although temperatures began rising steadily at ~8 cal ka (Powers et al., 2005), the response of algal biomarkers did not gradually decrease and instead a sudden drop in accumulation rates of algal biomarkers is noted at 4.9 cal ka. This might suggest that either a threshold was reached where temperatures were warm enough to inhibit upwelling, or perhaps an interval of weaker winds combined with the more strongly stratified water column to impede upwelling and limit nutrient supply, thereby causing the abrupt decrease in primary productivity.

Increasing abundances of C₃ vegetation are noted from ~8 to 4.9 cal ka, attesting to the establishment of wetter conditions in southeast Africa, with the highest proportion of C₃ vegetation of the past 23 cal ka noted at 4.9 cal ka (Castañeda et al., in review). An elevated influx of lignin phenol $\lambda_8$ values also provides evidence for increased terrestrial input from runoff at ~5 cal ka (Castañeda et al., in prep). With the exception of the abrupt event at 4.9 cal ka, accumulation rates of algal biomarkers and biogenic silica are higher than during the Late Pleistocene (Figures 3, 4), suggesting a generally more productive lake. In addition, $\delta^{13}$C$_{algal}$ values are higher during the Holocene than in the Late Pleistocene (Figure 5), providing further support for increased productivity in Lake Malawi during this interval.

The later part of the Holocene, from 4.9 cal ka to the present, is characterized by a decline in C₃ vegetation and an increase in C₄ vegetation, indicating increasing aridity in southeastern Africa (Castañeda et al., in review). The vegetation shift is consistent with
diatom assemblage records, which also suggest lower lake levels throughout this interval (Gasse et al., 2002). Accumulation rates of algal biomarkers fluctuate throughout this interval but fall within the range of abundances noted during the earlier part of the Holocene. It appears that precipitation in the Lake Malawi basin is not the main control on primary productivity since accumulation rates of algal biomarkers are generally similar throughout the entire Holocene.

4.4 Molecular biomarker vs. HI records

In contrast to the results of this study which indicate generally low algal productivity from the LGM until the start of the Younger Dryas, the HI record provides a different picture of conditions in Lake Malawi. Filippi and Talbot (2005) find that higher HI values characterize the LGM, indicating that algal material comprised a greater portion of the organic matter. They suggest Lake Malawi was a significantly smaller but productive lake at this time, with bottom waters that may have been periodically oxygenated. Elevated $\delta^{13}$C$_{TOC}$ values characterize this interval (Figure 2), which Filippi and Talbot (2005) attribute to elevated primary productivity in the lake. However, a recent study of Lake Malawi found that changes in C$_3$ vs. C$_4$ vegetation are mainly responsible for driving changes in $\delta^{13}$C$_{TOC}$ values, and that the elevated $\delta^{13}$C$_{TOC}$ values noted during the LGM can be attributed to increased inputs of C$_4$ vegetation (Castañeda et al., in prep). However, terrestrial control of the $\delta^{13}$C$_{TOC}$ signal does not rule out the possibility of increased algal productivity at the LGM. Although the molecular biomarker and HI data results appear to be distinctly different, it is feasible that the two records are both accurately reflecting conditions in Lake Malawi during the Late Pleistocene. One possibility is that a change in depositional/preservation conditions occurred and affected the abundances of lipid biomarkers. First, even if the coring site was not oxygenated during the LGM, the oxic/anoxic boundary certainly would have been located in closer proximity to the coring site. Thus, it is likely that some portion of the organic matter would have been transported from the oxygenated shallow areas of the lake, which may explain the generally low abundances of algal biomarkers. Second, loliolide and isololiolide are anoxic degradation products but it may be possible that these
compounds were not formed in the water column but rather in the sediments after burial. Third, lower $\delta^{13}$C$_{\text{org}}$ values noted during the LGM do not rule out the possibility of increased algal productivity in the lake. In anoxic systems, anaerobic respiration of organic matter produces $^{13}$C-depleted methane and respired CO$_2$ (Whiticar et al., 1986; Woltemate et al., 1984), which can be incorporated into surface waters during upwelling events and produce algal biomass that is isotopically depleted in $^{13}$C during a time of enhanced productivity (Hollander and Smith, 2001). If at least part of the lake was anoxic at depth, upwelling of isotopically light CO$_2$ could lead to $^{13}$C depleted algal biomass.

A second possibility that may account for the differences between the lipid biomarker and HI records is that perhaps the type of algae that existed in Lake Malawi was rich in kerogen-type compounds, but not bitumen, and thus would be apparent from HI data but not from analysis of solvent-extractable lipids. For example, algaenans are compounds derived from algal material that are insoluble in organic solvents and are resistant to treatment with strong acids and bases. Members of Chlorophyceae, including Chlorella, Oocystus, Pediastrum, and Closterium, which are all present in Lake Malawi (Patterson and Kachinjika, 1995), are known to contain algaenans (Blokker et al., 2006; Gelin et al., 1999). Eustigmatophytes of Nannochloropsis and the dinoflagellate Gymnodinium catenatum also contain algaenans whereas diatoms lack these compounds (Gelin et al., 1999). Therefore, it is possible that a type of algae that produced algaenans was abundant in Lake Malawi at the LGM, which contributed to the HI signal but not to the lipid biomarker signal. Such compounds would also contribute to the TOC signal, and may explain the high TOC$_{\text{MAR}}$ values in this interval. Although not a highly specific biomarker, the C$_{25}$ and C$_{27}$ n-alkenes, which are biomarkers of green algae, exhibit slightly elevated abundances at this time compared to other algal biomarkers (Figure 4). A relative percent plot of diatom (loliolide), eustigmatophyte (C$_{32}$, 1-15 diol), green algae (C$_{27}$ n-alkene) and cyanobacteria (docosanyl 3-O-methyl-α-rhamnopyranoside) biomarkers also suggests that algal groups capable of producing algaenans comprised a greater portion of the algal taxa in Lake Malawi at the LGM, and indicates increased abundances of green and eustigmatophyte algae (Figure 6). Furthermore, the Younger
Dryas does not appear as a major event in the Rock-eval HI record although a fall in HI is observed, centered at 13 cal ka (Filippi and Talbot, 2005). Large increases in the mass accumulation rates of biogenic silica and loliolide provide firm evidence for a major increase in diatom productivity at this time (Figure 2, 3), which is not surprising as diatoms dominate modern Lake Malawi when cool and windy conditions are present. The fact the Younger Dryas does not appear as a major event in the HI record offers further support to the idea that HI data is mainly reflecting input from an algal group other than diatoms, which respond to a different set of environmental conditions. Given the multiple independent lines of evidence that suggest the coring site remained anoxic during the LGM, a shift to an algal group rich in algaenan-type compounds is presently the preferred explanation to account for the differences noted in Lake Malawi bitumen and kerogen records. However, the algaenan content of Lake Malawi organic matter must be examined in order to determine whether this is the case.

In contrast to the Late Pleistocene, the molecular biomarker and HI records exhibit more coherence in the Holocene. In the interval of 10 to 4.5 cal ka, Rock-eval HI data suggests that organic matter was relatively poor in algal material and that hydrogen-poor organic matter, indicating elevated inputs of terrestrial organic matter from high rates of runoff, was abundant (Filippi and Talbot, 2005). Like the HI data, the molecular data supports elevated inputs of terrestrial organic matter to Lake Malawi from the early Holocene until ~4.9 cal ka. However, molecular records also indicate higher levels of algal productivity compared to the Late Pleistocene. It might be possible that dilution effects are responsible for the apparent low abundances of algal material noted in the HI record because there is strong evidence for high terrestrial inputs in this interval. Similarly, in the later part of the Holocene, from 4.9 to 1.9 cal ka, the HI record suggests increased algal productivity while molecular records indicate similar levels of productivity compared with the early Holocene. Again, dilution effects from increased inputs of terrestrial OM during the early-middle Holocene wet phase may be responsible for the apparent increase in algal material noted in the HI record during the late Holocene.
4.5 Late Pleistocene vs. Holocene conditions

Overall, when geochemical records from the Holocene and Late Pleistocene are compared, several differences are readily apparent. First, algal biomarkers and biogenic silica records indicate generally low abundances in the Late Pleistocene and high abundances in the Holocene. Second, $\delta^{13}C_{\text{algal}}$ values are heavier in the Holocene compared to the Late Pleistocene. Third, HI values are lower in the Holocene than in the Late Pleistocene. Although the HI data may conflict with the lipid records, all three lines of evidence point to a major change in primary productivity occurring at the Pleistocene/Holocene boundary. There is evidence for a major change in the wind regime over Lake Malawi that occurred at $\sim$11.8-11 cal ka, due to a shift in the mean latitudinal position of the ITCZ, with northerly winds being more prevalent before this time and less so after this time (Filippi and Talbot, 2005; Johnson et al., 2002; Castañeda et al., in review). A switch in the mean latitudinal position of the ITCZ is also noted over Lake Masoko, a small crater lake located in the highlands at the northern end of Lake Malawi, at 11.7 cal ka (Garcin et al., in press). This switch in the dominant wind regime over the lake appears to have had a major impact on algal productivity in Lake Malawi.

The overall trends in the $\delta^{13}C_{\text{algal}}$ record (light values from 23-13 cal ka, lighter values during the Younger Dryas, and heavier values in the Holocene), can be explained by a switch in the dominant wind regime. Prior to the Holocene, longer annual exposure to northerly winds likely would have increased upwelling in the northern basin of the lake, thereby supplying isotopically depleted CO$_2$ to the surface waters and resulting in isotopically depleted algal biomass. During the Younger Dryas, when winds intensified, an increased amount of isotopically depleted CO$_2$ was supplied to the surface waters, producing algae that were further depleted in $^{13}$C. In the Holocene, when winds switched to a more southerly-dominated regime, upwelling and the supply of isotopically depleted bottom waters to the photic zone was reduced in the northern basin of Lake Malawi, resulting in increased competition for the available DIC and thus heavier $\delta^{13}C_{\text{algal}}$ values. Despite the more frequent or stronger northerly winds over the lake prior to $\sim$11.8 cal ka, the relatively cool, arid conditions retarded nutrient input to the lake, resulting in generally lower abundances of algal biomarkers and biogenic silica throughout this
interval. Although many questions remain regarding primary productivity prior to ~11.8 cal ka, it is clear that after this time, Lake Malawi operated in a mode similar to today.

One of the major features of the algal biomarker records is the presence of methylxylopyranoside (the cyanobacterial glycolipid) in the Holocene (Figure 3). As low $\delta^{15}N_{\text{OC}}$ values characterize the interval from 10-4.5 cal ka, it has been suggested that nitrogen fixing cyanobacteria were more abundant in this interval and thus, that Lake Malawi was prone to prolonged periods of stable stratification (Filippi and Talbot, 2005). The biomarker evidence presented here provides further support for the presence of nitrogen-fixing cyanobacteria in the Holocene. In modern Lake Malawi, diatoms dominate the algal taxa during the dry and windy season whereas cyanobacteria dominate in November-December, at the onset of the calm and rainy season that follows the period of deep mixing and maximum diatom productivity (Hecky et al., 1998; Hecky and Kling, 1987). Throughout the Holocene, wind induced upwelling was important in Lake Malawi; however, the presence of methylxylopyranoside also indicates that the lake experienced calm seasons, when cyanobacteria were abundant. As methylxylopyranoside is absent prior to ~12.5 cal ka, we speculate that before this time Lake Malawi may not have experienced as distinct calm seasons as it did during the Holocene. The sedimentary record offers support to this idea as sediments deposited prior to ~13 cal ka lack varve couplets that are present throughout much of the Lake Malawi record (Filippi and Talbot, 2005; Barry et al., 2002), and which reflect the distinct seasonal conditions of high diatom productivity in the windy season and high clastic input in the calm and wet season (Pilskaln and Johnson, 1991). Although lower lake levels may account for the lack of varves at around the time of the LGM, high lake levels had been reached by 15 cal ka (Gasse et al. 2002) and with the establishment of anoxic conditions (or more widespread anoxic conditions) in the lake, it is expected that varves would be preserved. The lack of varved sediments throughout this interval might be explained by a reduced seasonality in East Africa during the Late Pleistocene.

5. CONCLUSIONS
1) Results of this study provide additional support for a major change in wind regime that occurred at around ~11.8 cal ka, due to a change in the mean latitudinal position of the ITCZ (Filippi and Talbot, 2005; Johnson et al., 2002; Garcin et al., in press; Castañeda et al., in review). Prior to this time it appears that stronger or more frequent northerly winds promoted more frequent upwelling in the northern basin of the lake, bringing isotopically depleted CO₂ to the surface waters, and thereby producing algal biomass with light δ¹³C values. The degree of upwelling of hypolimnetic waters may be the main control on δ¹³Cₐ₉₉ throughout the entire record, as has previously been suggested (Filippi and Talbot, 2005). A shift in the dominant algal taxa of Lake Malawi appears to have accompanied the shift in wind regime over the lake. Diatoms were present in low abundance during the Late Pleistocene, and at this time it is possible that green algae, rich in algaenan-type compounds, were the dominant algal group in the lake. At the start of the Holocene, diatoms and cyanobacteria become more abundant suggesting conditions similar to those observed in modern Lake Malawi.

2) Wind induced upwelling appears to be a main control on algal productivity in the northern basin of Lake Malawi. However, the effects of thermal stratification are also apparent in lipid biomarker records. During the cool periods of the Younger Dryas and the early Holocene (cooling centered at ~8 cal ka), thermal stratification was weakened which likely facilitated wind-driven upwelling and nutrient supply to the surface waters, resulting in increased primary productivity. Productivity was especially high during the Younger Dryas when both cooler temperatures and stronger northerly winds were present in Lake Malawi. Conversely, the opposite situation occurred at 4.9 cal ka when maximum temperatures in Lake Malawi were reached. At this time, strong stratification, perhaps combined with weakened winds, likely either prevented or significantly reduced upwelling in the northern basin of Lake Malawi and caused an abrupt decline in algal productivity. This observation is significant as it is been suggested that global warming will lead to decreased algal productivity in tropical lakes, as has already been observed in recent records from Lake Tanganyika (O’Reilly et al., 2003; Verburg et al, 2003).
Significant changes in precipitation occurred during the Holocene but do not appear to be a main control on algal productivity in Lake Malawi.

4) The occurrence of abundant long chain \(n\)-alkyl diols in Lake Malawi sediments suggests that Eustigmatophyte algae are present in the lake. It is possible that this group has been mistaken for coccoid members of green algae in past algal surveys of Lake Malawi.

5) Nitrogen fixing cyanobacteria were present in Lake Malawi throughout the Holocene but are absent in the Late Pleistocene prior to 12.5 cal ka. It is suggested that during much of the Late Pleistocene, Lake Malawi may have experienced less seasonality than during the Holocene.

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Figure 1  Location of Lake Malawi in southeast Africa. The locations of Lakes Tanganyika and Victoria are shown for comparison, and coring site of piston core M98-1P is shown in the insert.
Figure 2: Caption on next page.
Figure 2 (previous page) Bulk geochemical data from core M98-1P. The interval of the Younger Dryas is highlighted. a) Mass accumulation rates of total organic carbon (TOC) plotted against age in calibrated years before present (cal yr BP) are shown by the grey dots while the black line represents the smoothed TOC$_{MAR}$ record (5 point running average). b) Mass accumulation rates of biogenic silica (BSi) are plotted in grey while the black line represents the smoothed BSi$_{MAR}$ data (BSi data are from Johnson et al., 2002 and were also measured on core M98-1P). c) The ratio of total organic carbon to total nitrogen (C$_{org}$/N$_{tot}$) shown by the grey dots while the black line represents the smoothed C$_{org}$/N$_{tot}$ data. d) The carbon isotopic composition of bulk sediment samples ($\delta^{13}$C$_{TOC}$). e) Lake Malawi temperature reconstruction based on the TEX$_{86}$ paleotemperature proxy (data from Powers et al., 2005). The x-axis scale is reversed to facilitate comparisons with the bulk geochemical records.
Figure 3 (previous page)  Mass accumulation rates of algal biomarkers.  a) Mass accumulation rates of isololiolide and loliolide, which are biomarkers of diatoms. The derivitized structure of loliolide is indicated near the left side of the graph. The mass accumulation rate of biogenic silica (BSi) is shown for comparison (BSi data from Johnson et al., 2002).  b) Mass accumulation rates of the C_{30}-C_{34} n-alkyl diols, which are biomarkers of eustigmatophyte algae.  c) Mass accumulation rates of the glycolipid docosanyl 3-O-methylxylopyranoside, which has been suggested as a biomarker for cyanobacteria. The derivitized structure of this compound is indicated below the graph.
Mass accumulation rates of general algal biomarkers. In all graphs, the interval of the Younger Dryas is highlighted. a) Mass accumulation rates of the C_{17}-C_{21} \textit{n}-alkanes. b) Mass accumulation rates of the C_{25}-C_{29} \textit{n}-alkenes.
Figure 5: Caption on next page.
Figure 5 (previous page)  Compound-specific carbon isotopes. The error bars represent the standard deviation of duplicate sample runs. In all graphs, the interval of the Younger Dryas is highlighted by the grey bar. a) Carbon isotopic composition of the C\textsubscript{17}-C\textsubscript{21} n-alkanes. b) The weighted mean carbon isotopic composition of the C\textsubscript{17}-C\textsubscript{21} n-alkanes. c) The carbon isotopic composition of the C\textsubscript{29}-C\textsubscript{31} n-alkanes, biomarkers of terrestrial plants, is shown for comparison.
Figure 6  Relative abundance plot of the main algal biomarkers in Lake Malawi samples. One compound was chosen are representative of each group and abundances were normalized to 100 percent. Loliolide was the compound used to represent diatom productivity, the C$_{32}$ n-alkyl diol was used to represent inputs from Eustigmatophyte algae, the C$_{27}$ n-alkene was used to represent inputs from green algae, and docosanyl 3-O-methylxylopyranoside was used to represent inputs from cyanobacteria.
CHAPTER 4

MOLECULAR AND ISOTOPIC EVIDENCE FOR RECENT TERRESTRIAL AND AQUATIC ECOSYSTEM VARIABILITY IN LAKE MALAWI (EAST AFRICA)

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ABSTRACT

Relatively few well-dated and high-resolution paleoclimate records spanning the past few millennia presently exist from tropical East Africa. Here we examine the bulk and molecular geochemical records of two varved sediment cores from Lake Malawi, which provide a continuous record of environmental variability in East Africa during past 730 years. We observe a number of changes in both the terrestrial and aquatic ecosystems of Lake Malawi, which are attributed to natural climatic forcing and anthropogenic activities. Biomarkers of terrestrial vegetation indicate increased abundances of C$_4$ vegetation from 1270-1800 AD, attesting to more arid conditions in southeast Africa during the Little Ice Age (LIA). From 1800 AD to the present, a shift to increased inputs of C$_3$ vegetation is noted, reflecting a return to relatively moister conditions during the past 200 years. Spectral analysis of the high-resolution TOC record indicates a strong periodicity of 204 years, similar to the 206 year cycle noted in $^{14}$C and $^{10}$Be records, suggesting links between East African climate and solar forcing. While such links may exist, arid conditions noted during the LIA (and throughout the Late Pleistocene and Holocene) can be attributed to southward migrations of the Intertropical Convergence Zone (ITCZ) over Lake Malawi. Increased accumulation rates of retene, a compound derived from conifers, are noted since 1900 AD and likely reflect increased soil erosion due to deforestation of the Lake Malawi watershed. During the past few centuries, accumulation rates of algal biomarkers suggest changes in the aquatic productivity of Lake Malawi. Biomarkers of dinoflagellates and bacterivorous ciliates display increased accumulation rates over this interval, while decreased accumulation rates of diatom biomarkers are observed. The record of total algal biomarkers indicates increased accumulation rates during the past century, suggesting increased algal productivity in Lake Malawi in response to increased nutrient input.

Keywords: Little Ice Age, solar forcing, biomarker, algae, C$_4$ vegetation
INTRODUCTION

The modern climate of East Africa is complex and is influenced by a number of factors including the position and seasonal migration of the Intertropical Convergence Zone (ITCZ) and Inter-Oceanic Confluence (IOC; also known as the Congo Air Boundary (CAB)), sea surface temperatures in the Atlantic and Indian Oceans, the presence of large lakes that create their own weather patterns through moisture recycling, and highly variable topography and related orographic effects (Camberlin et al., 2001; Leroux, 2001; Nicholson, 1996). The controls on modern East African climate are not fully understood, and thus it is perhaps not surprising that relatively little is known regarding decadal to century scale climate variability in tropical Africa. Over millennial timescales, variability in the mean latitudinal position of the ITCZ is recognized as a main factor controlling rainfall and can account for the large scale precipitation patterns observed in East Africa (Filippi and Talbot, 2005; Johnson et al., 2002; Castañeda et al., in review). However, on timescales of decades to centuries, ITCZ variability alone cannot account for the observed patterns of climatic variability and a number of other factors, including solar forcing (Garcín et al., in press; Cohen et al., 2006; Stager et al., 2005; Verschuren et al., 2000) and El Niño-Southern Oscillation (ENSO) events (Russell and Johnson, 2007; Conway, 2002; Nicholson, 2000), have been linked to rainfall in tropical Africa. At present, well-dated and high-resolution records of recent climatic variability in East Africa are relatively sparse but have documented major climatic shifts as well as significant decadal to century scale climatic variability since ~1000 AD (Garcín et al., in press; Russell and Johnson, 2007; Brown and Johnson, 2005; Stager et al., 2005; Cohen et al., 2005; Alin and Cohen, 2003; Johnson et al., 2001; Verschuren et al., 2000). Climate variability is typically expressed as hydrological fluctuations in tropical Africa (Gasse, 2000), and many historical records document numerous droughts that were severe enough to impact human populations (Nicholson et al., 1998; Owen et al., 1990). Unfortunately, many of these records are hampered by poor chronology and therefore many outstanding questions exist regarding the timing, severity, and regional extent of East African droughts.
The presence of large human populations in East Africa offers an additional complicating factor to examining climate variability during the past millennium. Superimposed on natural climatic fluctuations are the effects of anthropogenic activities such as deforestation and farming. Separating the effect of natural versus anthropogenic environmental changes is not always possible. However, as large human populations reside in East Africa, it is important to gain a better understanding of past environmental conditions and to understand the changes these environments are currently undergoing. Here, we use molecular biomarkers and compound-specific carbon isotopes to examine past variability in both the terrestrial and aquatic ecosystems of Lake Malawi during the past 730 years.

2. BACKGROUND
2.1 Study location

Lake Malawi (9°S to 14°S) is situated between the countries of Malawi, Mozambique, and Tanzania, and is 560km long and up to 75km wide (Eccles, 1974) (Figure 1). Lake Malawi has a maximum depth of over 700m (Johnson and Davis, 1989), is permanently anoxic below ~200m (Eccles, 1974), and is characterized by relatively high sedimentation rates of 0.5-1.5mm/yr (Finney et al., 1996). Lake Malawi is an excellent site for paleoenvironmental reconstructions as the lake contains both a continuous and high-resolution sedimentary record. In addition, Lake Malawi experiences its majority of water loss by evaporation rather than outflow making it extremely sensitive to minor changes in aridity (Spigel and Coulter, 1996). Moreover, Lake Malawi is situated in a climatically sensitive geographical location that is heavily influenced by the Intertropical Convergence Zone (ITCZ) (Figure 1a). The lake is located at the southern limit of the annual transit of the ITCZ (13-15°S) and experiences one rainy season per year from November to March (Leroux, 2001; Nicholson, 1996). During the rainy season, the main recharge system for lakes and rivers in southern Africa, the dominant winds are weak and northerly. Between April and May the ITCZ moves northward towards the equator with strong southerly winds prevailing until September when winds become more easterly (Eccles, 1974). The distinct seasonal patterns of
climate are reflected in the sedimentary record of Lake Malawi (Pilskaln and Johnson, 1991). During the windy season, phytoplankton blooms occur throughout the lake and provide autochthonous contributions to sedimentation, dominated by diatoms (Bootsma and Hecky, 1998; Patterson and Kachinjika, 1995). The combination of generally weak winds and increased runoff during the rainy season results in high allochthonous sedimentary contributions. This results in annual varve couplets, with a light layer representing the windy season and a dark layer representing the rainy season (Pilskaln and Johnson, 1991). The ITCZ is also a main control on the distribution of tropical vegetation via its relationship to precipitation. Presently, Lake Malawi is surrounded by tree savanna, which mainly consists of C3 vegetation (Schefuš et al., 2003a). To both the north and south of Lake Malawi lies grass savanna, which is dominated by C4 grasses (Schefuš et al., 2003a). The proximity of Lake Malawi to this ecosystem boundary makes it a sensitive location to examine past shifts in C3 and C4 vegetation associated with changes in climate (Figure 1a).

The East African Rift lakes, dominated by a few but widespread taxa (Patterson and Kachinjika, 1995), also provide an excellent location to examine past changes in algal productivity and community structure. Algal productivity in Lake Malawi is presently dominated by Bacillariophyta (diatoms), followed by contributions from Cyanophyta (cyanobacteria) and Chlorophyta (green algae), with minor contributions from Pyrrhophyta (dinoflagellates) (Patterson and Kachinjika, 1995). From October to March, the rainy and non-windy season in Malawi, Cyanophyta and Chlorophyta are the dominant phytoplankton (Hecky and Kling, 1987). Bacillariophyta dominates the rest of the year, when cool and windy conditions are present (Hecky and Kling, 1987). Phytoplankton composition and succession in Lake Malawi is mainly dependent on the strength of density stratification (Hecky and Kling, 1987), which is affected by water column gradients of temperature and dissolved solids (Wuest et al., 1996). Density stratification is important to algal productivity in tropical lakes as strong stratification can inhibit wind induced upwelling and the delivery of nutrients to the photic zone. Lake Malawi is permanently stratified and more strongly so during the warm and wet season (Nov. to April) when temperature differences between the surface and bottom waters are
the greatest. Dissolved solids help to maintain stratification during the dry and windy season, when temperature gradients between the surface and bottom waters are reduced (Wuest et al., 1996). Today, surface water temperatures of Lake Malawi vary from 23°C to 29°C between austral winter and summer, and bottom waters are 22.5°C (Wuest et al., 1996).

2.2 Biomarkers of terrestrial plants

The n-alkanes are straight-chained hydrocarbons that exhibit strong odd carbon number predominance in living organisms and are a major component of the epicuticular waxes of terrestrial plant leaves (Eglinton and Hamilton, 1967). Although n-alkanes are produced by many organisms, carbon number distributions and isotopic compositions vary depending on the source organism. Terrestrial plants are dominated by the long-chain (C$_{25}$-C$_{33}$) n-alkanes while aquatic algae are dominated by the short-chain n-alkanes (C$_{17}$-C$_{21}$) (Giger et al., 1980; Cranwell et al., 1987). Distribution patterns of n-alkanes can be used to distinguish between different vegetation types (e.g. Schwark et al., 2002; Hasnich et al., 2003). The C$_{31}$ n-alkane tends to be dominant in grasses while the C$_{27}$ and C$_{29}$ n-alkanes are dominant in deciduous trees (Cranwell, 1973). Additionally, carbon isotopes of the long-chain n-alkanes can be used to distinguish between vegetation utilizing different photosynthetic pathways. Plants utilizing the Calvin-Benson (C$_{3}$) photosynthetic pathway, typically trees and cold-season grasses and sedges, tend to have n-alkane carbon isotopic compositions of around $-36\%$ while plants utilizing the Hatch-Slack (C$_{4}$) photosynthetic pathway, typically warm-season grasses and sedges, have n-alkane carbon isotopic compositions of around $-21.5\%$ (Collister et al., 1994).

2.3 Biomarkers of aquatic algae

In addition to the short chain n-alkanes, which provide a general algal biomarker, a variety of different compound classes provide more specific biomarkers for aquatic algae. Sterols, compounds that occur in all eukaryotes, are membrane rigidifiers and the specificity of these compounds for different phytoplankton groups is well known (Volkman et al, 1986, 1998). Sterols commonly provide biomarkers for diatoms and
dinoflagellates, which are two of the four main algal groups present in Lake Malawi. The dominant sterol(s) in diatoms varies depending on the species, but brassicasterol (24-methylcholesta-5,22-dien-3β-ol), fucosterol (24-methylcholesta-5,24(28)-dien-3β-ol), and β-sitosterol (24-ethylcholesta-5-en-3β-ol) are common lipids of diatoms (Volkman et al., 1998; Barrett et al., 1995). The compound dinosterol (4α,23,24-trimethyl-5α-cholest-22-en-3β-ol) is found in many dinoflagellate species (Withers, 1983; Pirretti et al., 1997) and is commonly used as a biomarker for these organisms (Boon et al., 1979; Robinson et al., 1984; Volkman et al., 1998). The other two main algal groups in Lake Malawi, green algae and cyanobacteria, also have specific biomarkers. Biomarkers of green algae include the botryococcenes, which are produced by the green algae Botryococcus braunii (Volkman et al., 1998; Maxwell et al., 1968). Other biomarkers for green algae include the C_{25} and C_{27} n-alkanes and lycopadiene (Adam et al., 2006; Volkman et al., 1998). Many cyanobacteria have been shown to contain 7- and 8-methylheptadecanes (me-n-C_{17}, Gelpi et al., 1970) or 2-methylhopanoids (Summons et al., 1999). Additionally, new cyanobacterial markers recently have been suggested from two glycolipids, docosanyl 3-O-methyl-α-rhamnopyranoside and docosanyl 3-O-methylxylopyranoside (Sinninghe Damsté et al., 2001).

3. METHODS

Chronology

Multicore M98-11MC (10°0.2’S, 34°17.3’E) and gravity trigger core M98-2PG (9°58.6’S, 34°13.8’E) were collected from 403 and 363m water depth, respectively, in 1998 by an expedition of the International Decade for East African Lakes (IDeAL) (Figure 1c). The chronology of both cores has been previously published, and is based on varve counting and 210Pb dating (Johnson et al. 2001). Both cores consist entirely of varved sediments and possess several distinctive marker beds, including tephas and homogenites, which can be correlated between the two cores (Figure 2). Core M98-11MC contains a record spanning from 1998 to 1646 AD while core M98-2PG contains a record spanning from 1908 to 1270 AD. When plotted on their individual timescales, bulk geochemical records from cores M98-11MC and M98-2PG agree closely with each
other (Figure 3), attesting to the strength of the chronologies. As a high degree of correlation exists between the geochemical records of these two cores, we treat them as one continuous record spanning the past ~730 years. It is estimated that the uncertainty in absolute ages is ~5 years for the past century and ~20 years for older sediments (Johnason et al., 2001).

**TOC and C/N ratios**

Total inorganic carbon (TIC) and total carbon (TC) measurements were determined on a UIC CO₂ Coulometer. TC is TOC because TIC was not present in any of the samples analyzed from core cores M98-11MC and M98-2PG. Carbon to nitrogen ratios (C/N) were determined on a Costech ECS 4010 elemental analyzer. Sediment samples did not receive acid pre-treatment prior to analysis on the elemental analyzer. Therefore, C/N ratios reported here reflect the ratio of total organic carbon to total nitrogen (Corg/Ntot).

Mass accumulation rates of TOC and biogenic silica (BSi) were calculated to account for the effects of sediment dilution using the formula:

\[
\text{MAR}_\text{TOC or BSi} = \% \text{TOC (or BSi)} \times \text{LSR} \times (1-\Phi) \times \rho \ (\text{g/cm}^3),
\]

where LSR = linear sedimentation rate (cm yr⁻¹), \(\Phi\) = sediment porosity (determined from water content), and \(\rho\) = dry sediment density (g cm⁻³), which is assumed to equal 2.54 g cm⁻³.

**Bulk \(\delta^{13}C\)**

Freeze dried sediment samples were treated with excess 0.1N hydrochloric acid for 3 hours to remove inorganic carbon. After acidification, sediment samples were filtered through organic-free Whatman GF/F glass fiber filters (0.7μm pore size) and rinsed 4x with excess distilled and deionized water (Millipore filtration system). Sediment samples were then dried in an oven at 35°C and stored in a desiccator until they could be packed into tin capsules for isotopic analysis. Bulk carbon isotope (\(\delta^{13}C\)TOC)
samples from core M98-11MC were analyzed at the College of Marine Science at the University of South Florida on a Thermo-Finnigan Delta-Plus XL mass spectrometer coupled to a Carlo-Erba NA2500 Elemental Analyzer. For core M98-2PG, δ$_{^{13}}$C$_{TOC}$ samples were measured at the Stable Isotope Laboratory at the University of Saskatchewan using a Thermo Finnigan Flash 1112 EA coupled to a Thermo Finnigan Delta Plus XL mass spectrometer. To ensure consistency, 5 duplicate samples from M98-11MC were also run at the University of Saskatchewan. The δ$_{^{13}}$C$_{TOC}$ values of these replicate samples agree closely with the values obtained from the University of South Florida, and standard deviations are better than ±0.1%.

**Molecular biomarkers**

A total of 27 biomarker samples were analyzed; seventeen from core M98-11MCB, representing the period from 1645 AD to the present, and ten from core M98-2PG, representing the period from 1270-1645 AD. An overlapping sample of the same age (1645 AD) was analyzed from each core.

Sediment samples were soxhlet extracted in groups of five and an additional blank sample was run with every batch. This extraction blank was then worked up in the same manner as the sediment samples to ensure that no contamination was introduced to the samples during any of the steps. Freeze dried sediment samples were soxhlet-extracted with 2:1 methylene chloride: methanol for 24 hours to obtain a total lipid extract (TLE). The TLE was further separated into neutral lipid, fatty acid, and phospholipid fatty acid fractions using Alltech Ultra-Clean SPE Aminopropylsil 1 bond elute columns. Prior to loading the sample, bond elute columns were pre-cleaned by running 10mL of methanol and 10mL of 1:1 methylene chloride: 2-propanol through the column. Eight mL each of 1:1 methylene chloride: 2-propanol, 4% glacial acetic acid in ethyl ether, and methanol were used to elute the neutral lipid, fatty acid, and phospholipid fatty acid fractions, respectively. The neutral lipid fraction is the only fraction examined in this study and thus the fatty acid and phospholipid fatty acid fractions are not discussed further here. Silica gel column chromatography was used to further separate compounds in the neutral fraction following the procedures outlined by Wakeham and Pease (1992), which are
described in detail in Appendix 2. The first apolar fraction, which was eluted with hexane, was passed through an Ag⁺ impregnated silica pipette column to separate the saturated and unsaturated hydrocarbons. The polar fractions were derivitized with 100µL of bistrimethylsilyl trifluoroacetamide (BSTFA) and 100µL of acetonitrile for 2 hours at 60-70°C to convert compounds into trimethylsilyl-ethers.

**Biomarker identification and quantification**

Molecular identification of compounds was performed on a Hewlett-Packard 6890 gas chromatograph (GC) coupled to an HP 5973 mass spectrometer (MS). An HP-1 capillary column (25m x 32mm x 0.5µm) was used with He flow rates set at 2mL/min. The GC/MS oven temperature program initiated at 50°C and increased at a rate of 10°C/min to 130°C, and next at a rate of 4°C to 320°C. The final temperature of 320°C was held for 10 minutes. Mass scans were made over the interval from 50 to 650. Compounds were identified by interpretation of characteristic mass spectra fragmentation patterns, gas chromatographic relative retention times, and by comparison with literature.

Quantification of compounds was performed on a Hewlett-Packard HP 6890 Gas Chromatograph with a FID detector using 5α-androstane as an internal standard. Compound concentrations were determined by relating chromatogram peak area to the concentration of the internal standard. Column type and the temperature program used for GC analysis are the same as described above for GC-MS except for that He flow rates were set at 2.6mL/min.

Mass accumulation rates of individual compounds were calculated from the following formula:

\[ \text{MAR}_{\text{compound}} = \text{LSR} \times \text{DBD} \times C, \]

where \( \text{MAR}_{\text{compound}} \) is the MAR in ng cm\(^{-2}\) yr\(^{-1}\), LSR = linear sedimentation rate (cm yr\(^{-1}\)), DBD = dry bulk density (g cm\(^{-3}\)), and \( C \) = the mass of compound (ng g\(^{-1}\)) in dry sediment.
Compound-specific carbon isotopes

Twelve samples were selected for compound-specific carbon isotopic analysis, and were analyzed in the Department of Geological Sciences at Brown University. The carbon isotopic composition of n-alkanes was determined through gas chromatography-isotope ratio-mass spectrometry (GC-IRMS). An HP 6890 GC (DB-1 column: 60m, 0.32mm diameter, 0.1µm film thickness) was connected to a Finnigan MAT Delta XL mass spectrometer via a combustion interface. The GC temperature program initiated at 40°C and increased at a rate of 20°C/min to 220°C and next at a rate of 6°C/min to 315°C. The final temperature of 315°C was held for 10 minutes. Compounds (n-alkanes) separated by the GC column were oxidized at 940°C and converted to CO₂. For calibration, six pulses of reference CO₂ gas with a known δ¹³C value were injected to the IRMS. A standard mixture consisting of four fatty acids with known δ¹³C values was measured multiple times daily to ensure accuracy. The standard deviation of all compounds in this standard mixture was less than ±0.28‰. Each n-alkane sample was run in duplicate and the standard deviation of the long chain (C₂₉-C₃₃) n-alkanes is better than 0.4‰. Some samples contained very low accumulation rates of the short-chain (C₁₇-C₂₁) n-alkanes and thus the standard deviation is higher than for the terrestrial n-alkanes. The standard deviation of the C₁₉ and C₂₁ n-alkanes is better than 0.6‰ and 1‰, respectively. In most samples the C₁₇ n-alkane was not present in enough abundance to obtain an isotopic measurement. All δ¹³C values are reported relative to the Vienna Pee Dee Belemnite (vPDB) standard using standard delta (per mil) notation.

To account for the change in the δ¹³C of atmospheric CO₂ from anthropogenic fossil fuel burning (the Suess effect), it is necessary to correct bulk carbon and n-alkane δ¹³C values in the recent part of the Lake Malawi record. We corrected the Lake Malawi δ¹³C records using both the equations of Schleske and Hodell (1995) and Verburg (in press) and found that both methods produced nearly identical results. Here, we choose to apply the Verburg (in press) equation as it covers a slightly longer time period (2000 back to 1700 AD) than does the Schleske and Hodell (1995) method, which can be applied back to 1840 AD.
4. RESULTS

4.1 Bulk geochemical records

TOC values range from 4.86 to 1.95% in core M98-11MC and from 4.87 to 1.70% in core M98-2PG. The lowest TOC values are noted at 1675 AD in both cores while the highest values occur at ~1700 AD. Likewise, the lowest C$_{org}$/N$_{tot}$ values are noted at 1675 AD in both cores. Mean C$_{org}$/N$_{tot}$ values are 13.9 for core M98-2PG and 11.1 for core M98-11MC (Figure 3). We note that this offset in absolute C$_{org}$/N$_{tot}$ values between these two cores is not likely a real feature of the data. Our elemental analyzer was serviced between the time that samples from core M98-11MC and M98-2PG were analyzed and thus the offset in C$_{org}$/N$_{tot}$ values between the two cores appears to be an instrumental artifact. To facilitate comparison between the two records, a correction factor of +3 was applied to the values from core M98-11MC (Figure 3). Thus, we ignore absolute values and only examine overall trends in the C$_{org}$/N$_{tot}$ records, which are consistent between the two cores. The anomalously low C$_{org}$/N$_{tot}$ values noted at 1675 AD coincide with the presence of a thin (<1 cm thick) tephra layer (the marker bed “Y1” in Fig 2). This event is also marked by low TOC$_{MAR}$ values and following this event, a major increase in TOC$_{MAR}$ of ~1.4 to 3.4 ng/cm$^2$yr (or a change from 2 to 5% TOC) occurs from 1675-1710 AD (Figure 3).

The bulk carbon isotope ($\delta^{13}$C$_{TOC}$) record, corrected for the Suess effect, displays an overall change of 1.89‰ and ranges from a low value of ~24.74‰ in 1292 AD to a high value of ~22.85‰ in 1867 AD (Figure 3). From ~1275 AD until ~1650 AD, the $\delta^{13}$C$_{TOC}$ record indicates an overall trend toward heavier values. Between ~1650 and 1730 AD an excursion to lighter $\delta^{13}$C$_{TOC}$ values is noted, while the heaviest values of the record occur between 1750 AD and the present.

4.2 Molecular biomarkers

Mass accumulation rates of biomarkers are presented in Table 1 and Figures 4-7. Accumulation rates of most compounds are discussed in detail later in this manuscript and therefore only general observations are presented in this section.
Both the long (C29-C33) and short (C17-C21) chain \( n \)-alkanes are present in low abundance (Figures 4, 5). Average accumulation rates of the C17, C19 and C21 \( n \)-alkanes are 0.47, 0.87, 0.56 ng/cm\(^2\)/yr, respectively, throughout the length of the record. Average accumulation rates of the C29, C31 and C33 \( n \)-alkanes are also relatively low with values of 1.56, 0.89 and 0.31 ng/cm\(^2\)/yr, respectively. Significantly higher accumulation rates of both short and long chain \( n \)-alkanols are noted, with values of the C20 \( n \)-alkanol and C28 \( n \)-alkanols averaging 5.5 and 9.9 ng/cm\(^2\)/yr, respectively. The C25 \( n \)-alkanol (average accumulation rate of 16.3 ng/cm\(^2\)/yr) and tetrahymanol (average accumulation rate of 13.4 ng/cm\(^2\)/yr) are the most abundant compounds of the neutral lipids present in Lake Malawi sediments. Retene and docosanyl 3-O-methylxylopyranoside (Figure 6) have the lowest average accumulation rates as these compounds were not present in a significant number of samples. Of the sterols, \( \beta \)-sitosterol and dinosterol are the most abundant and have average accumulation rates of 3.1 and 2.8 ng/cm\(^2\)/yr, respectively (Figure 7).

4.3 \( n \)-alkane carbon isotopes

The Suess-corrected carbon isotopic values of the C29 \( n \)-alkane (the most abundant of the long-chain \( n \)-alkanes) vary from -28.2 to -29.1\%o (Figure 8) while values of the C19 \( n \)-alkane (the most abundant of the short-chain \( n \)-alkanes) vary from -26.6 to -28\%o (Figure 9). Both of these records exhibit a relatively small range of isotopic values with overall shifts of 0.9 and 1.4\%o observed for the C29 and C19 \( n \)-alkanes, respectively.

5. DISCUSSION

5.1 Bulk geochemical data

The \( C_{org}/N_{tot} \) ratio provides a proxy for terrestrial versus aquatic input as aquatic algae generally have C/N ratios of 4-10 whereas higher land plants are characterized by C/N ratios of >20 (Meyers, 1997). The \( C_{org}/N_{tot} \) record of the past 730 years displays relatively little variability and suggests combined terrestrial and aquatic inputs to Lake Malawi throughout the length of the record (Figure 3). It should be noted that \( C_{org}/N_{tot} \) ratios do not provide the best method for examining changes in terrestrial versus aquatic inputs as anomalously high C/N values have been observed in algal-dominated systems
with severe N-limitedness (Talbot and Lerdal, 2000), and periodic N-deficiency is known to occur in modern Lake Malawi (Guildford et al., 1999).

Bulk carbon isotopes ($\delta^{13}$C$_{TOC}$) can provide information regarding past primary productivity; however, terrestrial and bacterial inputs also contribute to the $\delta^{13}$C$_{TOC}$ signal. The $\delta^{13}$C$_{TOC}$ record indicates a shift in Lake Malawi since ~1750 AD, with generally heavier values noted after this time (Figure 3). In a recent study of a 23 cal ka sedimentary record from Lake Malawi, the $\delta^{13}$C$_{TOC}$ record was found to closely track changes in terrestrial $n$-alkane $\delta^{13}$C, suggesting a mainly terrestrial control on the $\delta^{13}$C$_{TOC}$ signal (Castañeda et al., in prep A). During the past 730 years, the low resolution of $n$-alkane carbon isotope measurements (of both the short and long-chain $n$-alkanes) makes comparison with the $\delta^{13}$C$_{TOC}$ record somewhat difficult. It appears that changes in the $\delta^{13}$C$_{TOC}$ record generally track changes in terrestrial $n$-alkane $\delta^{13}$C, although this relationship breaks down after ~1900 AD with terrestrial $n$-alkane $\delta^{13}$C values becoming lighter while the $\delta^{13}$C$_{TOC}$ record trends towards generally heavier values (compare Figures 3, 8, 9). It is possible that during the past century, controls on the $\delta^{13}$C$_{TOC}$ record have shifted and that algal productivity is presently driving changes in the $\delta^{13}$C$_{TOC}$ record. However, more data is needed to confirm this hypothesis.

5.2 Diagenesis of sedimentary organic matter

It is especially important to consider possible diagenetic effects on lipid biomarker distributions before making paleoenvironmental interpretations from the recent sedimentary record. The degradation of lipid biomarkers is strongly influenced by oxygen, with lipids being more rapidly degraded under oxic conditions than under anoxic conditions (Harvey and Macko, 1997; Sun et al., 1997; Sun and Wakeham, 1998; Teece et al., 1998). Lake Malawi is permanently anoxic below ~200 m water depth (Eccles, 1974) and the coring sites, located at 363 and 403 m water depth, are believed to have remained anoxic during the past 730 years. Thus, we can eliminate changes in bottom water oxygenation as a factor influencing the degradation of sedimentary organic matter. It has been shown that in the Cariaco basin, which like Lake Malawi contains oxygenated surface waters overlying anoxic bottom waters, organic matter is mainly altered in the
water column but does not change significantly upon reaching the sediments (Wakeham and Ertel, 1988). Constant sedimentation rates are noted in cores M98-11MC and M98-2PG and so the effects of enhanced preservation due to increased sediment accumulation rates (Canfield, 1989; Henrichs and Reeburgh, 1987) also can be eliminated as a factor affecting lipid preservation.

Differential degradation of lipids during diagenesis is another factor that must be considered when examining the sedimentary record. For example, in anoxic sediments polyunsaturated fatty acids are more labile than monounsaturated alkenes, which are more labile than alkanols, which are more labile than sterols (Grossi et al., 2001). Additionally, selective preservation is known to occur within the same class of lipids. For example, in anoxic sediments selective preservation between individual sterols has been noted, with cholesterol being more reactive than the C29 sterols (Grossi et al., 2001; Taylor et al., 1981). One method of separating the effects of differential degradation and early diagenesis from the paleoenvironmental signal is to apply a correction factor to the measured lipid concentrations (Zimmerman and Canuel, 2002). Downcore abundance profiles of some compounds in Lake Malawi sediments (i.e. cholesterol, brassicasterol, dinosterol and tetrahymanol) appear to resemble early diagenesis curves (Figure 7), however, we chose not to apply such a correction factor to the measured lipid abundances for several reasons. First, nearly all compounds are present in higher concentrations in older sediments. Piston core M98-1P was collected from a site immediately adjacent to core M98-11MC (examined in this study) and has a sedimentary record spanning from 0.2 to 23 cal ka (Castañeda et al., in prep). In this core, compounds including tetrahymanol, β-sitosterol, cholesterol, dinosterol, the long-chain n-alkyl diols, the long chain n-alkanols,isolololiolide and the glycolipid docosanyl 3-O-methylmethylxypyranside, are all present in higher concentrations than in cores M98-11MC or M98-2PG (Castañeda et al., in prep). Second, while some biomarker abundance profiles resemble early diagenesis curves, it should be noted that the uppermost data point of several of these compounds (i.e. cholesterol, brassicasterol, tetrahymanol) actually exhibit slightly lower abundances than the sample directly below it (Figure 7). This pattern is atypical of an early diagenesis curve, which displays a steady exponential
decrease from surface to deep sediments. Third, it has been observed that in anoxic sediments cholesterol is more reactive than the C29 sterols (Grossi et al., 2001; Taylor et al., 1981); however, the downcore profiles of cholesterol and other sterols are dissimilar, and the profile of cholesterol correlates well with downcore profiles of the long-chain n-alkanes, which are one of the most stable lipid classes (Figures 4, 7; Meyers, 1997). Thus, abundances of cholesterol, which is one of the most reactive compounds examined in this study, do not appear to be reflecting changes in abundance due to early diagenesis so it is reasonable to assume the same is true for more refractory classes of lipids. For these reasons, we assume that the Lake Malawi record mainly reflects changes in primary productivity. It is important to note that biomarker abundances cannot be directly correlated to biomass due to complications arising from degradation and heterotrophy, yet changes in accumulation rates of lipids can be used to examine past ecosystem structure and productivity.

5.3 Terrestrial ecosystem variability
5.3.1. C3 vs. C4 vegetation

Tropical vegetation is a sensitive indicator of aridity and its distribution is mainly controlled by precipitation (Castañeda et al., in review; Schefuß et al., 2003). The C3 cycle (Calvin-Benson) and the C4 cycle (Hatch-Slack) are the two main pathways of carbon fixation utilized by plants (O'Leary, 1981). C4 plants are characterized by higher water use efficiency and are common today in tropical savannahs, temperate grasslands, and in semi-arid regions (Raven et al., 1999). Aridity is recognized as the dominant control on the large-scale distribution of C3 vs. C4 vegetation in tropical Africa (Schefuß et al., 2003). The carbon isotopic composition of plant leaf waxes (C29-C33 n-alkanes) can be used to distinguish inputs of C3 and C4 vegetation, and thus to examine past changes in aridity.

In Lake Malawi sediments, the carbon isotopic composition of the C29, C31 and C33 n-alkanes displays similar trends reflecting a common higher plant source (Figure 8). Following the approach of Zhang et al. (2003), we use the weighted mean δ13C of the C29,
C$_{31}$ and C$_{33}$ n-alkanes, hereafter referred to as $\delta^{13}$C$_{alk}$, to examine C$_3$ vs. C$_4$ variability in the Lake Malawi record:

Weighted mean n-alkane $\delta^{13}$C = $\delta^{13}$C$_{29}$ * C$_{29}$ + $\delta^{13}$C$_{31}$ * C$_{31}$ + $\delta^{13}$C$_{33}$ * C$_{33}$,

\[ C_{29} + C_{31} + C_{33} \]

where C$_x$ refers to the abundance of the n-alkane with $x$ number of carbon atoms.

During the past 730 years, the $\delta^{13}$C$_{alk}$ record indicates a general trend towards elevated C$_4$ inputs from 1270 until 1800 AD (Figure 8). Since 1800 AD, a shift towards lighter $\delta^{13}$C$_{alk}$ values is noted reflecting an increase in the abundance of C$_3$ vegetation. This overall pattern of increasing C$_4$ abundances from 1270 to 1800 AD, indicating increasingly more arid conditions in southeast Africa, followed by a return to wetter conditions from 1880 to the present, agrees well with previous studies of Lake Malawi (Brown and Johnson, 2005; Johnson et al., 2001). Brown and Johnson (2005) inferred increasingly dry conditions during the LIA based on decreasing mass accumulation rates of terrigenous material (Figure 8), followed by wetter conditions since ~1800 AD. The $\delta^{13}$C$_{alk}$ record presented here offers independent evidence for generally increased aridity in southeast Africa between 1270 and 1800 AD. Aridity during the LIA is attributed to a southward shift in the mean latitudinal position of the ITCZ over Lake Malawi (Brown and Johnson, 2005). Southward displacements of the ITCZ occur during northern hemisphere cool periods (Broccoli et al., 2006), and such displacements are also observed during the Last Glacial Maximum and Younger Dryas at Lake Malawi (Castañeda et al., in review; Filippi and Talbot, 2005; Johnson et al., 2002).

In order to assess the severity of LIA aridity in southeastern Africa, we compare the $\delta^{13}$C$_{alk}$ record of the past 730 years to a 23 cal ka $\delta^{13}$C$_{alk}$ record from piston core M98-1P, which was collected from the same site as core M98-11MC. The percentage of C$_4$ grasses can be estimated from a simple binary mixing model based on the $\delta^{13}$C$_{alk}$ record (Zhang et al., 2003; Schefuß et al. 2003a; Boom et al. 2002; Huang et al., 2000), assuming end member values of -36%o and -21.5%o for C$_3$ and C$_4$ vegetation, respectively (Collister et al., 1994). Based on this binary mixing model, it is estimated that vegetation
in the Lake Malawi watershed consisted of 51.4% C4 grasses at ~1350 AD, with abundances of C4 grasses increasing until ~1805 AD when a high value of 57.5% C4 grasses is noted (Figure 8). A low value of 49.4% C4 grasses is noted in 1968 AD. It is clear that LIA aridity was not as severe as previous events when the records of cores M98-1MC/M98-2PG are compared to Lake Malawi piston core M98-1P, which spans the past 23 cal ka. The δ¹³Calk record of this core indicates shifts of 43-55% C4 grasses during the Holocene and shifts of 45-61% C4 grasses in the Late Pleistocene (23 to 11 cal ka) (Castañeda et al., in review). Thus, for the majority of the past 730 years, until ~1800 AD, conditions at Lake Malawi were significantly more arid than at present, although this aridity was not as severe as aridity during the Younger Dryas or the Last Glacial Maximum. It is also interesting to note that the trend toward increasing aridity in southeastern Africa appears to have begun prior to the LIA, at ~3 cal ka (Castañeda et al., in review). Therefore, the recent return to wetter conditions may be anomalous compared to conditions during the past few millennia.

Southward displacement of the ITCZ can account for arid conditions at Lake Malawi during the LIA; however, it does not account for the considerable spatial and temporal variability in wet/arid conditions noted across equatorial East Africa during the past millennium. While Lake Malawi experienced increasing aridity from 1270-1800 AD, conditions at nearby Lake Tanganyika were somewhat different as aridity is noted from ~1550-1850 AD but during the earlier part of the LIA, from ~1250-1550 AD, Lake Tanganyika experienced relatively high lake levels (Alin and Cohen, 2003). At Lake Masoko, a tiny crater lake located in the highlands at the northern end of Lake Malawi, aridity is also noted from 1550-1850 AD (Garcin et al., in press). Conditions during the earlier part of the LIA are presently unknown at Lake Masoko since the available record extends back to 1436 AD (Garcin et al., in press). Further to the north at Lake Edward, arid conditions were present from 1470-1750 AD (Russell and Johnson, 2007). In contrast, Lake Naivasha experienced relatively wet conditions from ~1270-1850 AD but this interval was punctuated by three droughts from 1380-1420 AD, 1560-1620 AD, and from 1760-1840 AD (Verschuren et al., 2000). Like Lake Naivasha, Lake Victoria also experienced relatively wet conditions during the LIA with high lake levels noted from
1400-1600 AD and from 1700-1750 AD, and with low lake levels noted from 1360-1380 AD, 1630-1660 AD, and 1780-1850 AD (Stager et al., 2005). Russell and Johnson (2007) suggest that shifts in the position of the Congo Air Boundary (CAB), a north-south trending convergence zone that separates Atlantic and Indian Ocean moisture (Nicholson, 1996), contributed to variability in East African precipitation patterns during the LIA, and suggest that migrations of the CAB are linked to ENSO variability.

Solar forcing is another mechanism that has been linked to climate variability in East Africa. During periods of low sunspot activity, including the Dalton (~1790-1820 AD), Maunder (1645-1715 AD), Spörer (~1420-1570 AD), and Wolf (~1280-1340 AD) minima, changes in lake level (precipitation) have been noted throughout East Africa, although the response is inconsistent. Solar minima correspond with precipitation maxima at Lake Naivasha (Figure 10; Verschuren et al., 2000). Links between solar minima and laminae thickness are noted at Lake Tanganyika (Figure 1b), reflecting changes in primary productivity (Cohen et al., 2006). However, at Lakes Tanganyika (Cohen et al., 2006) and Masoko (Garcin et al., in press) the trend is opposite to that observed at Lake Naivasha with drier conditions noted during solar minima. Ties between lake level and solar forcing have also been noted at Lake Victoria (Figure 1b) but the response varies. Lake Victoria rose during the Wolf, Spörer and Maunder sunspot minima but the relationship changed sign ~200 years ago and drought is noted during the Dalton minimum (Figure 10; Stager et al., 2005). In contrast, at Lake Edward no correlations between geochemical records and solar forcing are observed (Russell and Johnson, 2005).

To investigate possible ties between climate and solar forcing in southeastern Africa, we compare the high-resolution Lake Malawi bulk geochemical records (Figure 3) to the record of $^{14}$C production (Figure 10), which is thought to reflect solar forcing or a combination of solar forcing and oceanic response (Bard and Frank, 2006). High cosmogenic nuclide production is interpreted to reflect low solar forcing and vice versa (Bard and Frank, 2006). When the Lake Malawi TOC record is plotted as deviation from a linear fit, the record exhibits some coherence with $^{14}$C production and displays increased TOC values during solar minima (Figure 10). Moreover, spectral analysis of
the TOC record reveals a strong signal with a period of 204 years (Figure 11), similar to the 206 year cycle noted in $^{14}$C and $^{10}$Be records (Bard and Frank, 2006; Stuiver and Braziunas, 1993; Raisbeck et al., 1990). Thus, it appears that links between solar forcing and environmental variability exist at Lake Malawi, although it is not clear what is producing the response in TOC content. Relationships between solar forcing and lake level have been noted elsewhere in East Africa (Garcin et al., in press; Cohen et al., 2005; Stager et al., 2005; Verschuren et al., 2000), and also in Central America (Hodell et al., 2001), but it is not clear if changes in lake level occurred at Lake Malawi because no relationship is noted between TOC$_{MAR}$ data and historic lake level data, which extends back to 1930 AD (Vollmer et al., 2005). At present, the relationships between solar forcing and climate are largely unexplained as fluctuations in solar irradiance are thought to be too small to influence climate on their own (Bard and Frank, 2006; Foukal et al., 2004). However, a recent modeling study using a coupled ocean-atmospheric model has suggested that solar forcing can influence tropical hydrology through the combined influence of ozone and irradiance, which produces stratospheric heating (Shindell et al., 2006). Many outstanding questions exist regarding the physical mechanisms through which solar forcing influences tropical climate, however, the number of records demonstrating links between solar forcing and paleoclimate is substantial (i.e. Garcin et al., in press; Cohen et al., 2005; Stager et al., 2005; Hodell et al., 2001; Verschuren et al., 2000; Curtis et al., 1996; Peterson et al., 1991). In East Africa, the highly variable response, or lack of response, to solar forcing suggests that other climate forcings, including variability in the positions of the ITCZ and CAB, may locally enhance, diminish, or override the response to solar forcing. At Lake Malawi, southward migrations of the ITCZ appear to be a main factor controlling aridity, both during the LIA and throughout the Late Pleistocene-Holocene (Brown and Johnson, 2005; Filippi and Talbot, 2005; Castañeda et al., in review).

5.3.2 Higher plant biomarkers

Accumulation rates of terrestrial compounds offer further insight into the vegetation history of Malawi, but interpretation of these records is complex. For
example, higher accumulation rates of plant leaf waxes can indicate greater amounts of vegetation present in the watershed, increased input from erosion via increased wind or rainfall, or higher terrestrial input due to deforestation of the watershed. The long-chain $n$-alkanes exhibit a general trend of increasing accumulation rates from $\sim$1270 to 1550 AD, followed by decreasing accumulation rates from 1550 to 1700 AD. Increasing accumulation rates are again noted until $\sim$1775 AD, and are followed by decreasing accumulation rates until $\sim$1800 AD (Figure 4). Since $\sim$1800 AD, accumulation rates of $n$-alkanes remain relatively constant at low values or exhibit slightly decreasing values towards the present. While a dramatic increase in abundance is noted in the most recent sediments, this trend is ignored as it is only defined by one data point (Figure 4).

Accumulation rates of the long-chain $n$-alkanols display roughly the same overall trends as the long-chain $n$-alkanes, although the low peak and high peaks at $\sim$1675 AD and $\sim$1775 AD, respectively, are less well defined than in the $n$-alkane records.

Three other compounds, retene, de-A-lupane, and $\beta$-sitosterol are also derived from higher plants. Accumulation rates of these compounds display similar trends to each other but differ from those of the $n$-alkanes or $n$-alkanols (Figure 4). The compound de-A-lupane is a tetracyclic terpane thought to derive from angiosperms (Oung and Philip, 1994) while $\beta$-sitosterol derives from both higher plant and algal sources (Volkman et al., 1998; Nishimura and Koyama, 1977). In Lake Malawi it appears that $\beta$-sitosterol derives from a mainly higher plant source as accumulation rates of this compound closely track accumulation rates of retene and de-A-lupane but do not track accumulation rates of other algal biomarkers (Figures 4-7). The compound retene is formed by diageneis of diterpenoid abietic acid, which is found in conifer tree resin (Laflamme and Hites, 1978). Retene is commonly found in soils and can also be formed by combustion of coniferous wood (Ramdahl, 1983). In Lake Malawi sediments retene is present for much of the past 730 years, and displays increased accumulation rates since $\sim$1900 AD (Figure 4). At Lake Victoria, higher concentrations of retene are noted in sediments since 1960 AD, and are thought to derive from increased soil erosion due to deforestation and human activities (Lipiatou et al., 1996). Rapid deforestation is also occurring in the Lake Malawi basin (Calder et al., 1995) and thus may account for the
increased accumulation rates of retene noted in surface sediments. Alternatively, wood is a main fuel source for cooking in Malawi (Hudak and Wessman, 2000) and as human populations have grown, elevated concentrations of retene in surface sediments may also be attributed to increased biomass burning. As downcore accumulation rates of de-A-lupane and β-sitosterol resemble the downcore profile of retene (Figure 4), the generally higher accumulation rates noted since ~1900 AD may be the result of deforestation. However, it is not clear why accumulation rates of retene, de-A-lupane and β-sitosterol do not track accumulation rates of the n-alkanes and n-alkanols (Figure 4). It may be possible that that the n-alkanes are reflecting either changes in biomass or wind erosion while retene, de-A-lupane and β-sitosterol are reflecting soil erosion. Studies have shown that in arid environments, n-alkanes are mainly transported by wind (Schefuß et al., 2003b). The ratio of the trace metals niobium (Nb) to titanium (Ti) has been used as a proxy for the strength of northerly winds over Lake Malawi (Brown and Johnson, 2005; Johnson et al., 2002). When the Nb/Ti record is compared to the records of long-chain n-alkanes, some similarities are present. Both records show good agreement in the periods from 1270 to 1550 AD and from ~1750 AD to the present, with high Nb/Ti values associated with increased accumulation rates of long-chain n-alkanes and vice versa. However, from ~1550-1750 AD the records diverge with low accumulation rates of n-alkanes associated with the highest Nb/Ti values. Thus, it is unclear what is driving abundances of terrestrial biomarkers to Lake Malawi although it appears that some relationship may exist between the strength of northerly winds over Lake Malawi and accumulation rates of long-chain n-alkanes.

5.4 Aquatic ecosystem variability

5.4.1 Changes in algal productivity

The short-chain (C₁₇-C₂₁) n-alkanes and n-alkanols provide general biomarkers for aquatic productivity. Accumulation rates of the C₁₇-C₂₁ n-alkanes indicate a general increasing trend from ~1270-1525 AD, followed by an overall decreasing trend from 1525 until ~1900 AD, and slight increases in accumulation rates are noted since 1900 AD (Figure 5). Accumulation rates of the C₂₀ n-alkanol also display similar trends although
the increase in accumulation rate since 1900 AD is more dramatic. Increased algal productivity might be expected given the evidence for increased nutrient loading to Lake Malawi (Hecky et al., 2003). However, these observations do not appear to be reflected as strongly in the carbon isotope record.

To examine variability in the algal productivity of Lake Malawi, we use the weighted mean $\delta^{13}$C of the C$_{19}$ and C$_{21}$ n-alkanes, and hereafter refer to this record as the $\delta^{13}$C$_{algal}$ record. (In most samples abundances of C$_{17}$ n-alkanes were too low to obtain compound-specific $\delta^{13}$C measurements.) The $\delta^{13}$C$_{algal}$ record displays relatively constant values of around -27.5‰ from ~1350 until ~1800 AD, with a spike to heavier values of -26.3‰ noted at ~1690 AD (Figure 9). The lowest $\delta^{13}$C$_{algal}$ value of -28.1‰ is noted at 1861 AD, followed by a general trend toward heavier values from 1861 AD to 1941 AD. In piston core M98-1P, average Holocene $\delta^{13}$C$_{algal}$ values are -27.8‰, and range from -26.7‰ to -29.4‰, similar to the trends observed during the past 730 years (Castañeda et al., in prep B). This can also be seen by comparing box plots of the two $\delta^{13}$C$_{algal}$ records, which indicate similar median values (Figure 9c). While $\delta^{13}$C$_{algal}$ values from cores M98-11MC and M98-2PG are somewhat heavier than those from M98-1P, the data does not offer compelling evidence for a recent increase in primary productivity. We note that our $\delta^{13}$C$_{algal}$ record ends at ~1970 AD and thus may be insufficient for capturing recent changes in the primary productivity of Lake Malawi. However, accumulation rates of several different aquatic biomarkers provide evidence for recent ecosystem changes in Lake Malawi, as will be discussed in the following sections.

5.4.2 Diatoms

Diatoms are the dominant algal taxa in Lake Malawi and are present in highest abundance during the cool and windy season (Hecky and Kling, 1987). In Lake Malawi, the compound loliolide/isololiolide is relatively abundant and provides a biomarker for diatoms. Loliolide/isololiolide is the anoxic degradation product of the pigment fucoxanthin, the major carotenoid present in diatoms (Repeta, 1989; Klock et al., 1984). Dinoflagellates and haptophyte algae can also produce fucoxanthin (Klok et al., 1984; Jeffrey and Vesek, 1997), but in Lake Malawi dinoflagellates are only a minor contributor
to algal productivity and haptophyte algae are not present (Patterson and Kachinjika, 1995). Thus, loliolide/isololiolide provides a reliable marker for diatoms. When compared to the record of biogenic silica (Johnson et al., 2001), another proxy for diatom productivity, it is clear that accumulation rates of loliolide closely track abundances of biogenic silica (compare Figures 3 and 6), demonstrating that loliolide is a robust indicator of diatom productivity in Lake Malawi. The records of loliolide/isololiolide and biogenic silica indicate a general trend of increasing concentrations from 1270 AD until ~1750 AD, when maximum concentrations are noted, followed by a return to lower concentrations between 1750 AD and the present (Figure 6). Brown and Johnson (2005) interpreted the biogenic silica record, in combination with trace element data, as representing increased northerly winds over Lake Malawi during the LIA. The loliolide/isololiolide record is consistent with this idea.

The sterol brassicasterol (24-methylcholesta-5,22-dien-3β-ol), commonly used as a biomarker for diatoms, does not track accumulation rates of either loliolide/isololiolide or biogenic silica in Lake Malawi sediments (Figures 6, 7). These sterols can also be synthesized by land and emergent water plants (Nishimura and Koyama, 1977), and given the proximity of the shore to the coring site, terrestrial sources of sterols are likely in Lake Malawi. Moreover, downcore abundance profiles of β-sitosterol and cholesterol closely resemble that of de-A-lupane (compare Figures 4, 7), a compound produced by angiosperms. Thus, in Lake Malawi, these sterols appear to mainly reflect terrestrial inputs.

5.4.3 Cyanobacteria

In modern Lake Malawi, diatoms dominate the algal taxa during the dry and windy season whereas cyanobacteria dominate in November-December, at the onset of the calm and rainy season that follows the period of deep mixing and maximum diatom productivity (Hecky et al., 1998; Hecky and Kling, 1987). Common cyanobacterial biomarkers such as the 7- and 8-methylheptadecanes (me-n-C17, Gelpi et al., 1970) and the 2-methylhopanoids (Summons et al., 1999) are not present in Lake Malawi sediments. However, the glycolipid docosanyl 3-O-methylxylolpyranoside is present,
which is believed to derive from a cyanobacterial source (Sinninghe Damsté et al., 2001). Docosanyl 3-O-methylxylopyranoside, hereafter referred to as methylxylopyranoside, is present in Lake Malawi prior to 1750 AD but is absent in younger sediments (Figure 6). The highest concentration of methylxylopyranoside observed during the past 730 years is <0.1 ng/cm²/yr. When compared to the 23 cal ka record of Lake Malawi piston core M98-1P, these concentrations are low as methylxylopyranoside obtains maximum concentrations of ~2.5 ng/cm²/yr (Castañeda et al., in prep B). In Ace Lake, Antarctica, a trend of increasing concentration with increasing depth is also noted in the uppermost 25 cm of the sediments (Sinninghe Damsté et al., 2001). The absence of this compound in the youngest sediments is intriguing because cyanobacteria are one of the main algal groups present in modern day Lake Malawi. Presently, methylxylopyranoside has only been reported from Ace Lake and Lake Malawi and as both lakes display a similar pattern of increasing glycolipid abundance with depth in surface sediments, it may be possible that this compound forms or derives from another compound, and completion of this process may require a certain amount of time. Alternatively, it is possible that a different species of cyanobacteria is now present in Lake Malawi that does not produce methylxylopyranoside. Another possibility is that perhaps cyanobacteria were more abundant in the past and a certain amount of biomass may be required for a signal to be preserved in the sedimentary record. It is also possible that methylxylopyranoside is produced by a group other than, or in addition to, cyanobacteria. Lipid analysis of algae from Lake Malawi water column and surface sediment samples would be useful for resolving these issues and for gaining a better understanding of what ultimately is preserved in the sedimentary record.

5.4.4 Green Algae

Biomarkers of Chlorophycae (green algae) were not identified in Lake Malawi sediments, although the lake contains a number of green algae with *Closterium*, *Staurastrum* and *Mougeotia* being the most abundant species (Hecky et al., 1998). Many biomarker studies have reported on the green algae *Botryococcus braunii*, which has a number of specific biomarkers including botryococenes and lycopadiene/lycopane
derivatives (Adam et al., 2006; Metzger and Largeau, 2005). However, Lake Malawi sediments do not contain biomarkers of *B. braunii* and it is not clear if the species is present in the lake. *Botryococcus braunii* is included on lists on algal taxa present in Lake Malawi (Patterson and Kachinjika, 1995) but the presence of *B. braunii* in Lake Malawi, and in the other tropical lakes of East Africa, is currently under debate and it is more likely that the species present is actually *Botryococcus terribilis* Komarek and Marvan (Hedy Kling, pers. comm.). For these reasons, it is not surprising that botryococcenes and lycopadiene/lycopane derivatives are not present in Lake Malawi sediments. Other biomarkers of green algae include the C25 and C27 n-alkenes (Volkman et al., 1998), which are present in low abundance in a 23 cal ka record of Lake Malawi (Castañeda et al., in prep B) but are absent from the recent sedimentary record.

5.4.5 Eustigmatophyte algae

In contrast to Chlorophycae, which are present in Lake Malawi but are presently lacking biomarkers, Lake Malawi sediments were found to contain biomarkers of Eustigmatophyte algae, which have never been identified in the lake. The long-chain 1,15-alkyl diols are abundant in Lake Malawi sediments (Figure 6), and are recognized as biomarkers of the algal class Eustigmatophycae (yellow-green algae) (Versteegh et al., 1997; Volkman et al., 1992), an algal group that little is known about (Ott and Oldham-Ott, 2003). To date, Eustigmatophyte algae have never been identified in algal or sediment samples from the lake (Hedy Kling, pers. comm.) but evidence suggests they may be present. It is likely that Eustigmatophyte algae may have been overlooked in previous algal surveys of Lake Malawi as most species of Eustigmatophyces are very small (2-4µm), and can be easily confused with coccoid forms of Chlorophyceae or Xanthophyceae (Ott and Oldham-Ott, 2003; Gelin et al., 1999). In Lake Malawi the most abundant species of Chlorophytes are small chlorococcales (Hecky et al., 1998) so it may be possible that members of Eustigmatophycae have been misidentified. A study of the phytoplankton of nearby Lake Tanganyika, based on PCR-amplified 18S rDNA, provides evidence for the presence of eustigmatophytes possessing a sequence similar to marine and freshwater members of *Nannochloropsis* (De Wever, 2006). As Lakes Tanganyika
and Malawi are similar, eustigmatophyte algae may also be present in Lake Malawi. Alternatively, the class Eustigmatophyceae includes members that inhabit terrestrial soils (Ott and Oldham-Ott, 2003) and therefore, it is possible that soil Eustigmatophytes are the source of the long-chain \( n \)-alkyl diols. However, in the 23 cal ka Lake Malawi record, accumulation rates of the \( n \)-alkyl diols closely track accumulation rates of other aquatic biomarkers, such as the diatom biomarker loliolide, making a solely terrestrial source of the long-chain \( n \)-alkyl diols to Lake Malawi sediments unlikely (Castañeda et al., in prep B). If it is assumed that the long-chain \( n \)-alkyl diols derive from Eustigmatophyte algae, then they were relatively more important contributors to algal productivity in Lake Malawi prior to \(~1750\) AD (Figure 12).

5.4.6 Dinoflagellates and bacterivorous ciliates

The compound dinosterol is present in many Lake Malawi samples and provides a biomarker for dinoflagellates (Withers, 1983; Pirretti et al., 1997; Boon et al., 1979; Robinson et al., 1984; Brassell et al., 1987; Volkman, 1998). In contrast to many sterols that are produced by both aquatic algae and terrestrial plants, dinosterol is not synthesized by higher plants and is thus recognized as a robust biomarker for dinoflagellates (Volkman et al., 1999). The record of dinosterol indicates little variability from 1270 until \(~1700\) AD, with an overall trend to increasing accumulation rates noted after this time (Figure 7). Dinoflagellates are a minor contributor to algal productivity in Lake Malawi (Patterson and Kachinjika, 1995), but the recent increase in their abundance may be significant. Most dinoflagellates are warm-temperature organisms and exhibit maximum growth rates during summer (Carty, 2003). Stratification is another important factor influencing dinoflagellate abundance. Studies have suggested that dinoflagellate blooms are favored by a stable water column with minimal mixing (Pollingher, 1987). Since dinoflagellates possess a flagellum and are motile, they are capable of remaining in the surface waters of a stratified water column (Carty, 2003). It has also been shown that turbulent mixing can destroy or damage dinoflagellate cells (Pollingher, 1987).

The compound tetrahymanol (gammaceran-3\( \beta \)-ol) is one of the most abundant compounds in Lake Malawi sediments and displays a similar trend to dinosterol,
indicating an overall trend of increasing accumulation rates since ~1800 AD (Figure 7). The compound tetrahymanol is produced by bacterivores ciliates, such as the freshwater ciliate *Tetrahymena* (Harvey and McManus, 1991; Mallory et al., 1963), but can also produced by the anaerobic purple bacterium *Rhodopseudomonas palustris* (Kleeman et al., 1990), anaerobic rumen fungus (Kemp et al., 1984), and in small amounts by a fem (Zander et al., 1969). Sources from rumen fungus and ferns are unlikely in Lake Malawi sediments. Although production by anaerobic purple bacteria cannot be ruled out, in aquatic sediments tetrahymanol typically derives from ciliates (Harvey and McManus, 1991), and several genera of bacterivorous ciliates are present in Lake Malawi (Yasindi and Taylor, 2003). Previous studies have attributed the occurrence of tetrahymanol in sediments to the presence of a stratified water column (Hasnich et al., 2003) since bacterivores ciliates are typically found at the oxic-anoxic boundary where large bacterial populations are present (Sinninghe Damsté et al., 1995; Thiel, 1997). The published information on ciliates in Lake Malawi is presently limited to one study (Yasindi and Taylor, 2003) and thus controls on their abundance are not fully understood. However, in other systems, studies have indicated that ciliate biomass is controlled by food availability (Beaver and Crisman, 1989 and references therein). A plot of total algal biomarkers suggests an increase in the algal biomass of Lake Malawi since ~1825 AD (Figure 7), and therefore increased accumulation rates of bacterivorous ciliates may be a reflection of increased food availability.

Alternatively, both the trends of increasing accumulation rates of tetrahymanol and dinosterol can be interpreted as reflecting increased stratification. Increased stratification due to surface water warming has been observed over the past century in Lake Tanganyika (Verburg et al., 2003; O’Reilly et al., 2003). In Lake Malawi, a deep water warming trend is observed since ~1940, and the lake has remained stratified at depths below 100m (Vollmer et al., 2005). As Lake Malawi is believed to have remained stratified during the past 730 years, it seems more likely that other factors are more important controls on the accumulation rates of dinoflagellates and ciliates. One possibility is that the increase in dinosterol may be a reflection of generally less turbulent conditions in the northern basin of Lake Malawi during the past few centuries. While
historical wind measurements in Malawi are scarce, the Nb/Ti record displays its lowest values from ~1800 AD to the present, suggesting relatively weak northerly winds over Lake Malawi (Brown and Johnson, 2005), coincident with the increase in dinosterol. Decreased accumulation rates of loliolide/isololide and biogenic silica throughout this interval are also consistent with reduced windiness. Another possibility is that poorer light conditions from increased sediment input (Hecky et al., 1998) may be at least partly responsible for the increased accumulation rates of dinoflagellates, which are motile and can remain in the photic zone.

5.4.7 An unusual compound in Lake Malawi sediments

In cores M98-11MC and 2PG, one of the most abundant compounds is the C$_{25}$ n-alkanol (tetrahymanol is the only compound that exhibits higher accumulation rates) (Figure 7). The presence of this compound in Lake Malawi sediments is highly unusual as n-alkanols typically exhibit strong even-over-odd carbon number predominance in living organisms. In these samples, the C$_{25}$ n-alkanol is the only odd-carbon numbered n-alkanol present in measurable quantities, and is not a contaminant as the compound is not present in any of the blank samples. The C$_{25}$ n-alkanol is also present in high abundances in a 23 cal ka sedimentary record from Lake Malawi (Castañeda, unpublished data). While the source of the C$_{25}$ n-alkanol is unknown, its abundance in Lake Malawi sediments is noteworthy and requires more investigation.

5.5 Anthropogenic impacts vs. natural climate variability

A number of environmental changes are known to be occurring in Lake Malawi and its watershed. One of the most noticeable changes is the deforestation of the watershed. Various estimates of deforestation rates have been reported. Calder et al. (1995) report that forest cover in Malawi declined by 13% between 1967 and 1990, equivalent to a rate of 1.8% a year. Hudak and Wessman (2000) also report a deforestation rate of 1.8% a year, although their study area was limited to the Mwanza district of southern Malawi. The Office of the President and Cabinet (1988) reported a significantly higher annual deforestation rate of 3.5%. Estimates of deforestation vary
but significant deforestation is occurring in Malawi and is likely accelerating as populations increase. Yearly population growth in Malawi was estimated at 2.38% in 2006 (https://www.cia.gov/cia/publications/factbook/print/mi.html). The demand for wood is high, and exceeds supply in southern and central Malawi, as a great majority of the population relies on wood as a fuel source for cooking (Hudak and Wessman, 2000). The northern end of Lake Malawi is less densely populated than the southern end and contains a higher proportion of forested land, which is increasingly being converted to agriculture (Hecky et al., 2003).

Records of terrestrial plant biomarkers give some indication of recent environmental change in the Lake Malawi basin. As discussed earlier, an increase in the concentration of retene noted since ~1900 AD likely represents either increased soil erosion or wood burning in the Malawi basin. The δ^{13}C_{ak} record indicates greater inputs of C_3 vegetation from 1800 AD to the present (Figure 8). Likewise, at Lake Tanganyika, an increase in arboreal pollen, accompanied by a decrease in grass pollen, is noted over the past few centuries (Mskay et al., 2005). However, a simple interpretation of these records as reflecting a natural vegetation shift due to wetter conditions may be overly simplistic. For example, at Lake Tanganyika, where higher lake levels noted during the past few centuries than during the LIA, it is thought that a natural response of vegetation to wetter conditions is unlikely due to the large amount of human disturbance in the watershed (Mskay et al., 2005). Instead, it is thought that the trend towards increased arboreal pollen is either a reflection of increased soil erosion releasing previously trapped pollen or that a shift in pollen production has occurred due to changes in land use and agriculture. At Lake Tanganyika, it is thought that the increase in arboreal pollen results from wind-blown inputs from high-mountain forested areas in the region, as subsistence agriculture involves replacing low elevation Miombo woodlands with cassava, bananas and oil palm, which are not large pollen producers (Mskay et al., 2005). Another factor is thought to be important is the presence of goats, which continuously graze grasses before they can produce pollen, thereby reducing contributions of C_4 pollen to the lake. In contrast to agriculture around Lake Tanganyika, which is dominated by cassava (C_3 plant) production, maize (C_4 plant) is the dominant crop in Malawi. Thus it might be
expected that if an anthropogenic signal was overprinting the natural vegetation signal, increased C4 abundances would be noted in the δ13Calk record during the past few centuries in Lake Malawi, contrary to the observed trends. Given the evidence for widespread wetter conditions following LIA drought at Lakes Malawi (Brown and Johnson, 2005), Tanganyika (Alin and Cohen, 2003), Masoko (Garcin et al., in press) and Edward (Russell and Johnson, 2007), it is likely that the shift to increased abundances of C3 vegetation is a natural response to climate change. However, in some regions, human overprinting of the natural vegetation signal may be contributing to the elevated C3 signal observed during the past few centuries.

A number of recent changes have been observed within Lake Malawi in addition to watershed deforestation. These changes include a deep water warming of 0.7°C over the past 6 decades (Vollmer et al., 2005), which is accompanied by an estimated surface water warming of ~2°C since 1900 AD (Powers, 2005). A number of recent shifts in the algal taxa of Lake Malawi also have been noted, although the period of quantitative algal sampling is limited to the period since 1987 while qualitative studies extend back to the turn of the century (Hecky et al., 1998). Filamentous chlorophytes of the Mougoetia/Oedogonium complex have appeared in Lake Malawi since the 1960s, and at the south end of the lake diatoms indicative of higher nutrient availability and poorer light conditions have become dominant (Hecky et al., 1998). In addition, blooms of the cyanobacteria Anabaena have been noted in some areas of Lake Malawi and reflect elevated nutrient supply (Hecky et al., 1998). It is estimated that nutrient loading has increased by as much as 50% in areas of the lake where watersheds have been significantly disturbed by agricultural activities, in comparison to regions with forested watersheds (Hecky et al., 2003).

The most notable changes in the algal lipid records are the increase in dinoflagellate and bacterivorous ciliates over the past few centuries, accompanied by a decrease in diatoms (Figures 6, 7, 12). In addition, the record of total algal lipids also displays significantly higher accumulation rates throughout the past century (Figure 6d). Algal surveys offer support to the increased presence of dinoflagellates in Lake Malawi, and note recent blooms of the dinoflagellate Peridinium (Hecky et al., 1998). These
blooms are of concern since this group can produce phycotoxins (Hecky et al., 1998). A combination of natural and anthropogenic forcings is likely given evidence for surface (Powers, 2005) and deep water warming of Lake Malawi (Vollmer et al., 2005), generally decreased northerly winds (Brown and Johnson, 2005), rapid deforestation of the watershed (Hudak and Wessman, 2000; Calder et al., 1995) and increased nutrient and sediment loading (Hecky et al., 2003) during the past century. We note that the northern end of Lake Malawi, where this study was conducted, is the least disturbed and therefore it is likely that more significant impacts can be observed at the southern end of the lake.

6. CONCLUSIONS

1) Lake Malawi experienced arid conditions throughout the Little Ice Age with increased abundances of C₄ vegetation noted from 1270 to 1800 AD. From 1800 AD to the present, a shift to increased inputs of C₃ vegetation is observed in response to relatively wetter conditions in southeastern Africa. It is significant to note that much of the past millennium in southeastern Africa was drier than at present, as a return to these conditions would have major consequences for the large human populations of East Africa.

2) A number of changes are noted in records of terrestrial and algal biomarkers since ~1900 AD. An increase in the abundance of total algal biomarkers since ~1900 AD may reflect increased nutrient input to Lake Malawi. Dinoflagellate and bacterivorous ciliate biomarkers display increased accumulation rates during the past few centuries, while diatom biomarkers display decreased accumulation rates, suggesting recent aquatic ecosystem changes in Lake Malawi. During the past century, an increase in accumulation rates of the compound retene can likely be attributed to increased soil erosion due to deforestation, or from an increase in wood burning in the Lake Malawi basin.
3) Spectral analysis of the TOC record reveals a strong signal with a 204 year periodicity, suggesting links between climate and solar variability in southeastern Africa. However, variability in the mean latitudinal position of the ITCZ appears to be a more important factor influencing rainfall over Lake Malawi.

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Figure 1  

a) Vegetation zones of Africa based on Schefuß et al. (2003a). The July and January positions of the Intertropical Convergence Zone (ITCZ) are illustrated based on Leroux (2001). 

b) Location of Lakes Malawi, Masoko, Tanganyika, Edward, Naivasha and Victoria in the East African Rift Valley. 

c) The northern basin of Lake Malawi with coring sites M98-11MC and M98-2PG.
Figure 2  A) Lithology of cores M98-11MC and M98-2PG. Figure based on Johnson et al. (2001). Locations of homogenites (H1-H5) and marker beds (O1, R1, Y1) are indicated, and intervening sediments are varved. B) Age-depth model for core M98-11MC and for C) M98-2PG based on 210Pb dating and varve counting (Johnson et al., 2001).
Figure 3: Caption on next page.
Figure 3 (previous page)  Bulk geochemical data from cores M98-11MC and M98-2PG. In all graphs, data points from M98-11MC are represented by the open triangles while data points from M98-2PG are represented by the solid circles. a) Percent total organic carbon (TOC). b) Mass accumulation rates of TOC. As sedimentation rates vary slightly from site M98-11MC and M98-2PG the records do not exhibit as close a correlation as does the percent TOC data. c) Percent biogenic silica (BSi). Data from Johnson et al. (2001). d) Ratio of organic carbon to total nitrogen ($C_{org}/N_{tot}$). Note that values in M98-11MC were offset from values of M98-2PG so a correction factor of +3 was applied to all $C_{org}/N_{tot}$ values in M98-11MC. The uncorrected $C_{org}/N_{tot}$ values are illustrated by the grey triangles. e) Bulk carbon isotope ($\delta^{13}C_{TOC}$) data from cores M98-11MC and M98-2PG. Samples younger than 1700 AD were corrected for the Suess effect using the equation provided by Verburg (in press). The uncorrected $\delta^{13}C_{TOC}$ values are indicated by the grey data points.
Figure 4 Terrestrial plant biomarkers. a) Abundances of the C$_{29}$, C$_{31}$, and C$_{33}$ $n$-alkanes. b) Abundances of the C$_{28}$ and C$_{30}$ $n$-alkanols. c) Abundances of retene, de-A-lupane and $\beta$-sitosterol.
Figure 5  Mass accumulation rates of general algal biomarkers.  a) Mass accumulation rates of the C17, C19, and C21 n-alkanes.  b) Mass accumulation rates of the C20 n-alkanol.
Figure 6: Caption on next page.
Figure 6 (on previous page) Mass accumulation rates of a) isololiolide and loliolide, biomarkers of diatoms, b) the C_{30}-C_{34} 1,15-diols, biomarkers of eustigmatophyte algae, c) docosanyl 3-O-methylxylopyranoside, a suggested cyanobacterial biomarker. The structure of this compound is also shown. d) The sum of all algal biomarkers (C_{17}-C_{21} n-alkanes, C_{20}-C_{22} n-alkanols, C_{30}-C_{34} i,15-diols, docosanyl 3-O-methylxylopyranoside, and dinosterol).
Figure 7: Caption on next page.
Figure 7 (on previous page)  
a) Mass accumulation rates of the sterols cholesterol, brassicasterol and dinosterol.  
b) Mass accumulation rates of tetrahymanol. Note that tetrahymanol is one of the most abundant compounds present in Lake Malawi sediments.  
c) Mass accumulation rates of the C_{25} n-alkanol. The source of this compound is not known.
Figure 8: Caption on next page.

A) C_{31} n-alkane \delta^{13}C

B) weighted mean C_{29}-C_{33} n-alkanes \delta^{13}C

C) Terrigenous MAR (mg/cm²/yr)
Figure 8 (on previous page) Terrestrial n-alkane carbon isotopes. a) The carbon isotopic composition of the C$_{29}$, C$_{31}$, and C$_{33}$ n-alkanes. b) The weighted mean of the C$_{29}$-C$_{33}$ n-alkanes. All $\delta^{13}$C values displayed in this figure were corrected for the Suess effect (Verburg, in press). The uncorrected weighted mean $\delta^{13}$C$_{alk}$ values are shown in grey for comparison. The grey dashed lines indicate the estimated percentage of C$_4$ vegetation, based on a binary mixing model assuming end members of -36$\%$ and -21.5$\%$ for C$_3$ and C$_4$ vegetation, respectively (Collister et al., 1994). c) Lake Malawi terrigenous mass accumulation rates (data from Brown and Johnson, 2005) indicating periods of wetter and drier conditions. This record displays the same overall trends as the $\delta^{13}$C$_{alk}$ record.
Figure 9: Caption on next page.
Figure 9 (on previous page) Algal $n$-alkane carbon isotopes corrected for the Suess effect. a) The carbon isotopic composition of the $C_{19}$ and $C_{21}$ $n$-alkanes. Error bars representing the standard deviation are shown. b) The weighted mean of the $C_{19}$-$C_{21}$ $n$-alkanes. The uncorrected weighted mean $\delta^{13}C_{\text{algal}}$ values are shown in grey for comparison. c) Box plots of the $\delta^{13}C_{\text{algal}}$ records of core M98-11MC/2PG compared with the Holocene (0-10 cal ka) $\delta^{13}C_{\text{algal}}$ record of piston core M98-1P. The line in the middle of each box represents the median, the bottom and top of the box represent the first and third quartiles, respectively. The lines represent the highest and lowest values and outliers are indicated by the black dots.
Figure 10  Comparison of East African records indicating ties to solar forcing.

a) The Lake Malawi percent TOC record plotted as deviation from a linear fit. b) Atmospheric $^{14}$C residual series. Data from Stuiver et al. (1998). The Wolf, Spörer, and Maunder minima are shown by the letters W, S, and M, respectively. c) Lake level data from Lake Naivasha in Kenya (see Figure 1b for location; Verschuren et al., 2000). d) Percent salt water diatom (SWD) data for Lake Victoria (data from Stager et al., 2005). High percentages of SWD indicate periods of low lake level.
Figure 11  Power spectrum of the percent TOC record of cores M98-11MC and M98-2PG. The program Analyseries (Pillard et al., 1996) was used to calculate the power spectrum by interpolating the signal at constant time and depth increments and linear detrending. Estimates were made at the 90% confidence interval using a Bartlett window, 1/3 lag, and no prewhitening constant was applied. Significant peaks are noted at 204 and 58 years.
Figure 12: Relative percentage plot of the main algal biomarkers present in Lake Malawi sediments indicating an increase in dinoflagellates and a decrease in diatoms since ~1800 AD. One compound representative of diatoms (loliolide), eustigmatophyte algae (C₃₀ 1, 15-diol) and dinoflagellates (dinosterol) was chosen and the total normalized to 100%.
Table 1: Biomarker abundances of samples in cores M98-11MC and M98-2PG. The yellow highlighting indicates a sample of approximately the same age that was taken from each core. In most cases, the data agrees closely between the two cores.
Table 1 (continued): Biomarker abundances of samples in cores M98-11MC and M98-2PG. The yellow highlighting indicates a sample of approximately the same age that was taken from each core. In most cases, the data agrees closely between the two cores.
CHAPTER 5

DIRECTIONS FOR FUTURE RESEARCH

This first detailed molecular study of Lake Malawi has provided many insights into the response of both terrestrial vegetation and aquatic algae to climate variability. However, a number of outstanding questions remain that warrant more investigation. With regard to the terrestrial biomarker records, both n-alkane $\delta^{13}C$ and lignin phenol analyses were highly successful for examining past vegetation (and thus aridity) shifts in southeast Africa. This approach could be applied to other lacustrine records in order better understand the timing and regional extent of past arid phases in East Africa. In order to obtain more accurate information from the n-alkane $\delta^{13}C$ record, it would be useful to measure the carbon isotopic composition of modern C$_3$ and C$_4$ vegetation in the Lake Malawi watershed. For this project, past vegetation assemblages were estimated based on a binary mixing model that assumed endpoint members of -36% and -21.5% for C$_3$ and C$_4$ vegetation, respectively. However, these values represent average measurements of C$_3$ and C$_4$ vegetation, and thus it is likely that the model may need to be adjusted for vegetation specific to the Malawi watershed.

A main goal of this research was to examine the potential of using molecular analyses to obtain information on past primary productivity and algal community structure in Lake Malawi over longer timescales since the period of quantitative algal sampling on Lake Malawi is limited to the past few decades. While it appears that biomarkers can be successfully used to examine past algal ecosystem variability, interpretation of these records is presently limited by lack of information regarding the lipid composition of the main algal groups in modern Lake Malawi. For example, green algae are one of the major algal taxa in the lake, yet common biomarkers of green algae were not present in Lake Malawi samples. Inevitably, green algae must be producing lipids and therefore it is important to determine the molecular signature of this group. It is also necessary to determine if the source of the long-chain n-alkyl diols is from eustigmatophyte algae or from another group, and to determine if the glycolipid
docosanyl 3- O-methyl-α-rhamnopyranoside indeed derives from a cyanobacterial source. Furthermore, the source of the C25 n-alkanol needs to be determined as it is one of the most abundant compounds in Lake Malawi sediments. It would also be extremely useful to examine the lipid content of both water column and surface sediment samples, in order to gain a better understanding of what is ultimately preserved in the sedimentary record.

A remaining outstanding issue concerns conditions in Late Malawi during the Late Pleistocene and differences noted between the biomarker and hydrogen index (HI) records. To shed light on this issue, it would be particularly useful to examine the algaenan fraction of Lake Malawi sediments to gain more insights into the type and origin of material present in the kerogen (non-solvent extractable) fraction. Knowledge of the type of material present in the algaenan fraction is also important for determining the main sources of sedimentary organic matter to Lake Malawi. In addition, the record of bacterial biomarkers, such as hopanes and hopenes, should be examined. These compounds are present and abundant in all Lake Malawi samples examined, suggesting that bacterial inputs to sedimentary organic matter are important. Moreover, a number of hopanes/hopenes are present in the short cores spanning the past 730 years that are not present in the piston core spanning the past 23 cal ka, and thus these compounds may provide insights into recent environmental changes occurring at Lake Malawi.
APPENDIX 1: DESCRIPTION OF METHODS USED IN CHAPTER 1

METHODS:

Twenty-eight samples were examined from piston core M98-1P (10°15.99S, 34°19.19), covering the time interval from 0.2 to 23 cal ka, while three samples (at 0.46, 0.53 and 0.60 cal ka) were examined from gravity core M98-2PG (9°58.6’S, 34°13.8’E).

Compound extraction:

Freeze-dried sediment samples were soxhlet-extracted with 2:1 methylene chloride/methanol for 24 hours to obtain a total lipid extract (TLE). The TLE was further separated into neutral lipid, fatty acid, and phospholipid fatty acid fractions using aminopropylsilyle bond elute columns (Russell and Weme, 2007). Prior to loading the sample, bond elute columns were pre-cleaned by running 10mL of methanol and 10mL of 1:1 methylene chloride: 2-propanol through the column. Eight mL each of 1:1 methylene chloride: 2-propanol, 4% glacial acetic acid in ethyl ether, and methanol were used to elute the neutral lipid, fatty acid, and phospholipid fatty acid fractions, respectively. Silica-gel column chromatography was used to further separate compounds in the neutral fraction following the procedures outlined by Wakeham and Pease (1992). The n-alkanes were present in the first apolar fraction, which was eluted with hexane. This fraction was next passed through an Ag⁺ impregnated silica pipette column to separate the saturated and unsaturated hydrocarbons.

Sediment samples were soxhlet extracted in groups of five and an additional blank sample was run with every batch. This extraction blank was then worked up in the same manner as the sediment samples to ensure that no contamination was introduced to the samples during any of the steps.

Compound identification:

Molecular identification of compounds (n-alkanes) was performed on a Hewlett-Packard 6890 gas chromatograph (GC) coupled to an HP 5973 mass spectrometer (MS). An HP-1 capillary column (25m x 32mm x 0.5µm) was used with He flow rates set at
2mL/min. The GC/MS oven temperature program initiated at 50°C and increased at a rate of 10°C/min to 130°C, and next at a rate of 4°C to 320°C. The final temperature of 320°C was held for 10 minutes. Mass scans were made over the interval from 50 to 650. Compounds were identified by interpretation of characteristic mass spectra fragmentation patterns, gas chromatographic relative retention times, and by comparison with literature.

**Compound-specific carbon isotope analysis:**

The carbon isotopic composition of n-alkanes was determined by gas-chromatography-isotope ratio-mass spectrometry (GC-IRMS). An HP 6890 GC (DB-1 column: 60m, 32mm diameter, 0.1µm film thickness) was connected to a Finnigan MAT Delta+ XL mass spectrometer via a combustion interface. The GC temperature program initiated at 40°C and increased at a rate of 20°C/min to 220°C and next at a rate of 6°C/min to 315°C. The final temperature of 315°C was held for 10 minutes. Compounds separated by the GC column were oxidized at 940°C and converted to CO₂. A standard mixture consisting of four fatty acids with known δ¹³C values was measured multiple times daily to ensure accuracy. The standard deviation of all compounds in this standard mixture was less than ± 0.28 %. Each n-alkane sample was run in duplicate and the standard deviation of the C₂₉, C₃₁ and C₃₃ n-alkanes is better than ± 0.38‰, ± 0.28 ‰, and ± 0.5‰, respectively. All δ¹³C values are reported relative to the Vienna Pee Dee Belemnite (vPDB) standard using standard delta (per mil) notation:

\[
\delta^{13}C = \left( \frac{^{13}C}{^{12}C}_{\text{sample}} \right) - \left( \frac{^{13}C}{^{12}C}_{\text{standard}} \right) \times 1000
\]

\[
\frac{^{13}C}{^{12}C}_{\text{standard}}
\]

**REFERENCES:**


APPENDIX 2:

EXTRACTION OF NEUTRAL LIPIDS IN LACUSTRINE SEDIMENTS:
A COLLECTION OF LABORATORY PROCEDURES USED AT THE
LARGE LAKES OBSERVATORY

Compiled by Isla Castañeda
(Version 1: February, 2007)
Introduction:

The laboratory procedures outlined here are largely derived from the laboratory manual “Lipid Analysis in Marine Particle and Sediment Samples: A Laboratory Handbook” by S.G. Wakeham and T.K. Pease (1992). The procedure for the bond elute column is modified from a procedure used by Yongsong Huang at Brown University, and the Ag⁺ impregnated silica gel column procedure was derived from Schouten et al. (2001). This appendix contains several critical modifications to these procedures, laboratory-specific instructions for sample analysis at the Large Lakes Observatory, and general tips and suggestions for lipid extraction and analysis.

Outline of laboratory procedures used for analysis of neutral lipids:

Step 1: Cleaning of materials and reagents
Step 2: Soxhlet extraction of sediment samples
Step 3: Bond elute column procedure
Step 4: Preparation of deactivated silica gel
Step 5: Preparation of solvents for silica-gel chromatography
Step 6: Long silica-gel column chromatography procedure
Step 7: Derivitization of polar fractions with BSTFA
Step 8: Ag⁺ impregnated silica gel column of L1/L2 fraction.
Step 9: Addition of internal standard 5α-androstane for quantification on FID/FPD
Step 10: Run samples on GC for quantification. Check for elemental sulfur on FPD, remove with copper filled pipette column, if necessary.
Step 10: Run sulfur-free samples on GC-MS for compound identification.
Lab Procedure 1: Cleaning glassware and reagents for use

Proper cleaning is an essential part of obtaining contaminant-free samples! All procedures in this lab manual require that the following items are pre-cleaned.

Cleaning of Glassware:
All glassware that is going to be used should first be scrubbed with a brush and water, dried, covered loosely with aluminum foil, and ashed (baked in a muffle furnace) for at least 4 hours at 450°C. (The furnace takes a while to warm up so make sure the oven temperature is actually at 450°C for at least 4 hours.) Only glass should be put into the muffle furnace! Sample vials should also be ashed but it is not necessary to wash them first if they have not previously been used.

Pre-cleaning of metal items:
1. Spatulas and tweezers:
Use solvents in squirt bottles and rinse 3 times with methanol. Repeat using acetone and then DCM.

2. Nozzles for nitrogen blow-down apparatus:
Fill a clean 4mL vial with methanol. Clean nozzles by fully immersing into the solvent-filled vial. Repeat using acetone and then DCM. You can also use the solvent filled squirt bottles to rinse off the nozzles but using a vial is less messy and creates less waste.

3. Microliter syringes:
Fill 3 small beakers with methanol, acetone and hexane. Fill microliter syringe with methanol and then squirt out into a waste container. Repeat 10 times. Next clean the syringe 10 times with acetone and then 10 times with hexane.

Pre-cleaning of materials and reagents that cannot be ashed:
These items include: cotton wool, NaCl, Na2SO4 and cellulose thimbles. Silica gel also needs to be pre-cleaned before use and the instructions for that procedure are listed on page 189.
1. Place items to be extracted into a soxhlet apparatus. Cotton wool, NaCl and Na2SO4 should first be placed into a large cellulose thimble.
2. Fill a round bottom flask ~3/4 of the way full with 2:1 DCM/MeOH.
3. Turn on the cold water and make sure flow rate is high enough to keep soxhlet condensers cool to the touch.
4. Place the solvent-filled round bottom flask into a heating mantle and use a keck clip to connect the soxhlet apparatus to the round bottom flask. Set the temperature to setting “3”.
5. Let extract for 24 hours. Turn off heating mantle and let round bottom flask cool. Using solvent cleaned tweezers remove extracted item(s) from the soxhlet apparatus and place into an ashed beaker. Cover loosely with foil and let dry completely in the fume hood. Once completely dry, remove the beaker, tightly cover and label item(s) as “extracted”.

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Cleaning of plastics:
1. Vial caps
All vial caps should be pre-cleaned before use. The best way to do this is to fill a 4mL vial with methanol, cap, shake. Repeat process with acetone and hexane.
*Note: it is not good to fully immerse vial caps in solvent as the non-Teflon parts of the vial caps are a potential source of contaminants.*

2. Separatory funnel stopcocks
Fill 3 small beakers with methanol, acetone and hexane. Use tweezers to immerse the stopcock in methanol, acetone and then hexane. Also clean the white Teflon rings (but not the black rubber O-rings) in this manner.

3. Teflon adaptor for \( \text{N}_2 \) line
Soak in a small beaker of methanol. Rinse with clean methanol.

*Note: Plastics are only used in a few steps and have the potential to introduce many contaminants into the samples. For this reason, use of plastics is avoided whenever possible. Organic solvents should only be used with the Teflon squirt bottles.*

Extraction of water:
Materials needed: Milli-Q water, ring stand, separatory funnel, DCM
1. Use Milli-Q water for extraction. DO NOT use tap or distilled water as it contains too many contaminants!
2. Place separatory funnel in a ring stand and place a beaker below it. Fill a separatory funnel ~2/3 full of water.
3. Add DCM and fill up separatory funnel until nearly full (it is important to leave several inches of free space at the top as gasses will form).
4. Stopper the separatory funnel, pick it up and shake funnel vigorously. After ~15 seconds, invert and turn the stopcock to vent the gasses. Repeat shaking funnel and venting until no more gas is produced. Return separatory funnel to ringstand and let sit until the layers separate.
5. Slowly drain off the bottom layer (DCM) into the beaker. Add new DCM to the separatory funnel and repeat process 2x more. The extracted water is now ready for use.
Lab Procedure 2: Soxhlet extraction of sediment samples

Materials:
500mL round bottom flask (12 needed to work up 6 samples)
Solvent extracted boiling chips
Heating mantles (6)
Keck clips (6)
Solvent washed tweezers and spatulas
Ashed beakers (6)
Solvent extracted cellulose thimbles (6)
2:1 DCM-MeOH (~1.5 L needed)
Solvent extracted cotton
Ashed funnels (25mL)
Ashed or solvent extracted Na₂SO₄
4mL glass vials with solvent washed vial caps
Labeling tape

Procedure:
1. Place a few (~10) solvent extracted boiling chips into each 500mL round bottom flask using a solvent washed spatula. Fill up each round bottom flask ~3/4 of the way full with 2:1 DCM/MeOH.

2. Place a clean (ashed) beaker on scale and tare. Use solvent washed tweezers to place a solvent-extracted cellulose thimble into the beaker and record the weight of the empty cellulose thimble. Add the freeze-dried sediment to the cellulose thimble sample using a solvent washed spatula. (1-3g of sediment is sufficient for sediment with 2-5% TOC.) Record the weight of the sediment added. Use the solvent washed tweezers to place the cellulose thimble into the soxhlet extractor. Make sure each round bottom and soxhlet is labeled appropriately. An extraction blank should be run with every set of samples extracted.

3. Assemble each soxhlet apparatus using a keck clip to secure the round bottom flask to the soxhlet apparatus. Turn on cold water so that flow is fast enough to keep all condensers cool. Turn the heat on each heating mantle to setting 3.

4. Let sample extract for 24 hours. Check sample occasionally to make sure the solvent level in the round bottom flask is not dropping (if it is, add additional 2:1 DCM/MeOH through the top of the soxhlet apparatus) and that the water flow is sufficient to cool the condensers. (In the summer, it may be necessary to wrap the outside of the condensers with tissues to collect excess moisture.)

5. Turn off the heating mantles after 24 hours. It might be necessary to tilt the extractor to drain the solvent into the round bottom flask. When cool, remove the round bottom flasks and use 2:1 DCM/MeOH to rinse off the bottom of the soxhlet apparatus into the round bottom flask.
Note: if sediment sample contains salt (i.e. marine or evaporitic lacustrine sediments), it needs to be removed before proceeding on to the next step of removing water. Refer to Wakeham and Pease (1992, pg. 10, step 6) for instructions on this procedure.

6. Place each cellulose thimble into a beaker (and label). Loosely cover with foil and let dry in hood overnight. Once dry, weigh each thimble with dried sediment in it and record the weight. Save the dried residual sediment! It can be used for other procedures, such as lignin phenol analysis.

Removal of water from the total lipid extract:

7. For each sample, get a new 500mL round bottom flask and a small funnel. Using solvent extracted tweezers and a disposable glass pipette, place a small amount of extracted cotton wool into the base of each funnel.

8. Using a solvent extracted spatula, fill each funnel ~1/2 of the way full with solvent-extracted Na$_2$SO$_4$. Place filled funnel on top of the clean round bottom flask.

9. Carefully pour each sample through the Na$_2$SO$_4$ filled separatory funnel. Rise the round bottom flask 3 times with 2:1 DCM/MeOH to make sure the entire sample has been transferred. Next squirt some extra 2:1 DCM/MeOH through the funnel.

10. Rotavap each sample to near dryness. Use an ashed disposable glass pipette and 2:1 DCM/MeOH to transfer contents to a clean 4mL vial with a Teflon cap. Make sure each vial is labeled with the sample ID and “TLE”.

11. Store vials in the refrigerator or freezer.
Lab Procedure 3: Bond elute separation of compounds

Materials:
Ring stands and clamps (3)
Nitrogen line
Teflon adaptor for nitrogen line
Alltech® aminopropyl bond elute columns
2:1 DCM/isopropyl
Methanol
Distilled ethyl ether
Microliter syringe
25 mL graduated cylinders (3)
Disposable glass pipettes and bulbs
50 mL pear shaped flasks (12)
Cork rings (3)

Procedure:
1. Set up a ring stand and clamp (or 3 ring stands and clamps - it is relatively easy to set up and run 3 samples in the same batch). Attach the bond elute column to the clamp.

2. Soak a Teflon adaptor in 2:1 DCM:MeOH. Rinse with MeOH. Connect Teflon adaptor to the plastic adaptor, and the plastic adaptor to the N$_2$ line (Figure 1).

3. Next to, or in front of, each ring stand place a 25 mL graduated cylinder. Place a disposable glass pipette with bulb into each cylinder. It is helpful to take a marker and draw a dark line on the 8 mL level.

4. Mix solvents. For 3 samples, prepare 100mL of 2:1 DCM/isopropyl (2-propanol) and 100 mL of 4% acetic acid in ethyl ether. Note that the ethyl ether and the glacial acetic acid must be distilled before use!

5. If TLE's are in solvent, blow down under N$_2$ until completely dry. Determine the weight of the TLE. The maximum capacity of the bond elute columns is 25 mg. For each sample, determine what fraction of the TLE you will need to load onto the bond elute column (aim for 20-23mg). Quantitatively dissolve each sample in 2:1 DCM/isopropyl.

For example, if the TLE in your vial weighs 50 mg, you could dissolve the TLE in 200 µL of 2:1 DCM/isopropyl and then load 100µL onto the bond elute column (using a microliter syringe). Make sure you record the fraction of the sample loaded onto the bond elute column as well as the fraction remaining in the TLE vial in your lab notebook.

6. Next, the bond elute columns need to be pre-cleaned before use:
   a) First use a squirt bottle to wash the bottom of the bond elute column with MeOH. Be extremely careful to only wash the bottom of the BE column (it is
important to avoid getting solvent in contact with the writing on the side of the column, as the ink contains many contaminants).

b) Pour 8mL of MeOH into each graduated cylinder. Place a waste beaker under a bond elute column. Using a glass pipette, load some of the MeOH into the bond elute column (make sure not to fill the bond elute column entirely with solvent as there needs to be some space for the Teflon adaptor to fit in.

c) Start the N2 flow. Place the Teflon adaptor (connected to the N2 line) on the top of the bond elute column and blow the solvent through. Continue loading with solvent and blowing until all 8mL of MeOH have passed through. Next, blow the bond elute column dry.

d) Repeat the process using 2:1 DCM/isopropyl. Make sure to leave a meniscus of solvent at the end.

e) Rinse tip of bond elute column again with MeOH. The bond elute columns are now ready for use.

7. Place a clean 25 mL pear shaped flask under each bond elute column and label appropriately. Using a microliter syringe, quantitatively transfer the appropriate amount of the total lipid extract (dissolved in 2:1 DCM/isopropyl) to the bond elute column. Allow sample to soak into bond elute column for at least 1 minute.

8. Run 8 mL of 2:1 DCM/isopropyl through each bond elute column, using the same method as for cleaning the column. The flow rate should be slow, one drop of solvent at a time should be coming off the bond elute column. An appropriate flow rate is 2-3 mL per minute. Adjust the N2 flow as necessary. After 8mL of solvent have passed through the bond elute column, blow column dry. The pear shaped flask now contains the neutral/polar fraction.

9. Place a new 25 mL pear shaped flask under the bond elute column. Run 8 mL of 4% acetic acid in ethyl ether through the column. The pear shaped flask now contains the free fatty acids. Note: if you plan to examine the fatty acid fractions, it is useful to collect the sample in a 20mL centrifuge tube instead of a pear shaped flask as the sample needs to be in a centrifuge tube for the methylation step.

10. Place a new 25 mL pear shaped flask under the bond elute column. Run 8 mL of methanol through the column. The pear shaped flask now contains the phospholipid fatty acids (PLFA). Note: when this step is completed, it is normal for a lot of color to remain on the bond elute column.

11. Rotovap all samples in the pear shaped flasks. Use a new disposable glass pipette and either 2:1 DCM/isopropyl or 2:1 DCM/MeOH to transfer each sample to a clean 4mL vial. Label each vial as neutral, acids, of PLFA, appropriately.
12. Store samples in refrigerator or freezer.

Figure 1: Bond elute procedure setup. The nitrogen line is indicated by the number 1, the Teflon adaptor for the nitrogen line by the number 2, and the bond elute column by the number 3. The Teflon adaptor needs to be held into place while solvent is being pushed through the bond elute column.
Lab Procedure 4: Extraction and Deactivation of Silica Gel

Materials:
- Soxhlet apparatus
- Heating mantle
- 2:1 DCM/MeOH
- Large pair of solvent-washed tweezers
- 1000 mL beaker
- Cellulose thimble
- Silica gel (Whatman 60Å, 70-230 mesh)

Procedure:
1. Fill a large cellulose thimble with silica gel.

2. Use a large pair of tweezers to place the silica gel filled thimble into a soxhlet extractor.

3. Before setting up the soxhlet extraction, it is necessary to first moisten the silica gel. If this step is skipped, the silica gel will absorb all of the solvent in the round bottom flask and the extraction will not work. To moisten the silica gel, pour 2:1 DCM/MeOH into the soxhlet apparatus between the cellulose thimble and the glass. You can pour the solvent directly on to the silica gel, but be aware that it will spurt and make a mess. Once silica gel is fully saturated with solvent, proceed with the extraction as normal.

4. Extract for 24 hours.

5. Use solvent washed large tweezers to transfer the cellulose thimble filled with extracted silica gel into the 1000 mL beaker. Cover loosely with foil and let dry for 24 hours. When dry, pour silica gel out of the cellulose thimble into another clean beaker and cover loosely with aluminum foil.

6. Bake silica gel in the oven at 150°C for 2 days.

7. Remove silica gel from oven and let cool. When the gel is completely cooled, transfer to an ashd glass bottle with a tight-fitting stopper.

8. Weigh out 100 g of activated silica gel into the bottle. Deactivate the silica gel by adding 5mL of solvent extracted water (see lab procedure 1 for instructions). Use an ashed disposable pipette to add the water dropwise in 0.5 mL portions. After adding each 0.5 mL, stopper bottle and shake until clumps disappear. Label the bottle “deactivated silica gel” and record the date.

9. For the next 2-3 days, let silica gel sit in bottle and shake occasionally.

10. Deactivated silica gel is good to use for ~1 week after it is prepared.
Lab procedure 5: Preparation of Solvents for Si-gel Chromatography

Materials:
7 pint-size brown bottles with Teflon lined caps
200 mL graduated cylinder
50 mL graduated cylinder

Solvents needed:
1. Toluene
2. Hexane
3. Ethyl Acetate

Solvent recipes:
1. Hexane 200 mL
2. 25% toluene 50 mL
   75% hexane 150 mL
3. 50% toluene 100 mL
   50% hexane 100 mL
4. 5% ethyl acetate 10 mL
   95% hexane 190 mL
5. 10% ethyl acetate 20 mL
   90% hexane 180 mL
6. 15% ethyl acetate 30 mL
   85% hexane 170 mL
7. 20% ethyl acetate 40 mL
   80% hexane 160 mL
8. 25% ethyl acetate 50 mL
   75% hexane 150 mL
9. 75% ethyl acetate 150 mL
   25% hexane 50 mL

Make sure to date all bottles and cap tightly. Solvents are good for 1 week after mixing.
Note: Mixing 400 mL of solvent at once is not recommended as when the brown bottles are full, they are nearly impossible to pour from without making a huge mess.
Lab Procedure 6: Silica-gel column chromatography

Materials:
Chromatography columns (3)
50mL graduated cylinder (3)
9" disposable glass pipettes
5" disposable glass pipettes
Ring stands (3)
50mL pear shaped flask (9)
100mL pear shaped flask
Cork rings (3)
Deactivated silica gel (24 g)
Solvents prepared in Lab procedure 5
Solvent-extracted cotton
Long glass rod (1)
Clean 4mL vials with caps (15)
Ashed or solvent washed aluminum foil
Small beaker for weighing silica gel

Procedure:
Note: Before starting this procedure be aware that it can take the better part of a day to run 3 samples. Running 1 sample vs. 3 does not significantly change the amount of time required for the procedure, so running 3 samples at once is recommended. Materials listed above are for 3 samples. It is helpful to label all pear shaped flasks ahead of time.

1. Dry the TLE-neutral fraction under N2.

2. Set up 3 ring stands. Place a column in each ring stand.

3. Using solvent washed tweezers, place a small amount of cotton in the opening of each column.

4. Solvent rinse the long glass rod and use it to press the cotton into place at the bottom of the column.

5. Use a squirt bottle to fill column to the base of the bulb with hexane.

6. In a small beaker, weigh out 8 grams of deactivated silica gel.

7. Remove column from ring stand and hold at an angle. Carefully pour silica gel into column bulb so that it mainly collects in the bulb. Using a hexane squirt bottle, add enough solvent to fully cover the silica gel. At this point, it will start falling down the length of the column. Quickly rotate the column as silica gel fills it up. Tap the sides of the column to remove any bubbles.
8. Let silica gel settle for a few minutes. Slowly drain off hexane into a waste beaker leaving a meniscus of solvent. Do NOT let solvent drain off completely! If the silica column dries out (or has any air bubbles in it, it cannot be used). Once most of the solvent has drained off, it is usually necessary to squirt additional hexane into the bulb to loosen excess silica gel from the sides. When all excess hexane has been drained off, replace waste beaker underneath column with a 50mL pear shaped flask.

9. Fill a small graduated cylinder with 30mL of hexane. It is helpful to make a tray out of solvent-washed aluminum foil to rest the glass pipettes on. For each sample you will need both a 5" and a 9" glass pipette.

10. Draw up ~1/4 mL of hexane with a 9" glass pipette and add it to the TLE-neutral sample. Gently swirl solvent around in the sample vial. Using the glass pipette, draw up the sample and transfer the sample to the column by carefully touching pipette to the inside of the column. Drain the excess solvent from the column, leaving only the meniscus.

11. Repeat the previous step until 2mL of hexane has been transferred from the graduated cylinder onto the column. Lots of color will still remain in the sample vial, this is normal. Note: Make sure that the pipette that is used to draw up sample is not used to transfer new solvent from the graduated cylinder to the sample vial! It is recommended that a 9" pipette is used to transfer the sample onto the column while a 5" pipette (so you can tell the two apart) is used to transfer solvent from the graduated cylinder into the sample vial.

12. Transfer the remaining hexane from graduated cylinder directly onto the column. You can pour it carefully (the much faster method) or use a pipette to transfer the solvent.

13. Let solvent drain. While this sample is draining, load the next two samples. Be careful to watch the solvent level in the first column as it is important that the column does not dry out. You can turn the stopcock to stop the flow, if you need more time to get the other two columns loaded.

14. When only a meniscus of hexane is left on the column, stop the solvent flow. Remove the pear shaped flask and label it L1/L2. Next place a 100mL pear shaped flask under the column.

15. Measure out 20mL of the 25% toluene/75% ethyl acetate mixture into the graduated cylinder. Repeat steps 10-13.

16. Repeat steps 10-13 first with 50% toluene/50% ethyl acetate, then with 5% ethyl acetate/90% hexane, and finally with 10% ethyl acetate/80%hexane (refer to elution schedule table at end of this procedure). Collect these 4 fractions (A-L4B) in the same pear shaped flask.
17. Transfer a new 50mL pear shaped flask under the column. Repeat steps 10-13 with 15% ethyl acetate/hexane and then with 20% ethyl acetate/hexane. Collect these two fractions (L5/L6) together.

18. Transfer a new 100mL pear shaped flask under the column. Repeat steps 10-13 with 25% ethyl acetate/hexane, 75% ethyl acetate/hexane (Note: be very careful loading this solvent onto the column. It is significantly more dense than the other solvents and thus it is very easy to disturb the surface of the silica gel), and next with 100% ethyl acetate. Collect these three fractions (L7-L9) together.

19. Using 30mL of MeOH, collect the L10 fraction in a 50mL pear shaped flask. On this step, let the column drain completely.

20. Rotavap all fractions. Use hexane to transfer the L1/L2 and A-L4B fractions to 4mL vials. Use ethyl acetate to transfer the L5/L6 and L7-L9 fractions to 4mL vials. Use 2:1 DCM/MeOH to transfer the L10 fraction to a 4mL vial.

**Column elution schedule:**

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Volume</th>
<th>Solvent</th>
<th>Major compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1/L2</td>
<td>30 mL</td>
<td>hexane</td>
<td>alkanes, alkenes, PAHs</td>
</tr>
<tr>
<td>A</td>
<td>20 mL</td>
<td>25% toluene/hexane</td>
<td>alcohols, sterols</td>
</tr>
<tr>
<td>L3</td>
<td>20 mL</td>
<td>50% toluene/hexane</td>
<td>alcohols, sterols</td>
</tr>
<tr>
<td>L4A</td>
<td>20 mL</td>
<td>5% ethyl acetate/hexane</td>
<td>diols, TEX$_{86}$ compounds</td>
</tr>
<tr>
<td>L4B</td>
<td>20 mL</td>
<td>10% ethyl acetate/hexane</td>
<td>diols, TEX$_{86}$ compounds</td>
</tr>
<tr>
<td>L5</td>
<td>20 mL</td>
<td>15% ethyl acetate/hexane</td>
<td>glycolipids</td>
</tr>
<tr>
<td>L6</td>
<td>20 mL</td>
<td>20% ethyl acetate/hexane</td>
<td></td>
</tr>
<tr>
<td>L7</td>
<td>20 mL</td>
<td>25% ethyl acetate/hexane</td>
<td></td>
</tr>
<tr>
<td>L8</td>
<td>20 mL</td>
<td>75% ethyl acetate/hexane</td>
<td></td>
</tr>
<tr>
<td>L9</td>
<td>20 mL</td>
<td>ethyl acetate</td>
<td></td>
</tr>
<tr>
<td>L10</td>
<td>30 mL</td>
<td>methanol</td>
<td></td>
</tr>
</tbody>
</table>
Figure 2: Setup for silica gel column chromatography.

Note: it is recommended to make a tray out of aluminum foil (solvent washed or ashed) to rest glass pipette columns on, when not in use.
Lab procedure 8: Ag⁺ impregnated Si gel column
(Separation of saturated/unsaturated hydrocarbons and removal of elemental sulfur)

Materials:
AgNO₃
Silica gel
Methanol
1000 mL round bottom flask
Solvent-extracted water
Disposable glass pipettes
4mL vials and caps
Extracted cotton
Aluminum foil

Preparation of Ag⁺ impregnated silica gel:
1. In 1L roundbottom: dissolve 5g AgNO₃ in 125mL of solvent extracted Milli-Q water and 500mL MeOH.
2. Add 100 g of silica gel in small portions (Whatman 60Å, 70-230 mesh).
3. Evaporate solvents in rotavap, increase temperature slowly to 50°C. This step may take 30 minutes or more. Place a sheet of aluminum foil over the rotavap bath to help protect silica gel from the light. Note: This will almost certainly flash boil and there’s really no way to prevent it from happening. It will make a big mess in the rotavap, which should then be taken completely apart and thoroughly cleaned.
4. When powder is almost dry, transfer silica gel into a bowl and activate in an oven at 120°C for preferably 16h. (Make sure sample is kept out of light.)
5. Cool down in desiccator. Make sure to keep out of light. Note: Covering a beaker completely with aluminum foil is a good way to keep sample protected from the light.
6. Clean rotary evaporator very well.
7. Reactivate silica powder every time before use.

Note: Prepared Ag⁺ impregnated silica gel will eventually turn black. It is good to use as long as it is white or a very light shade of off-white/brown.

Ag⁺ pipette column procedure:
1. Set up a ring stand. Put some extracted cotton (or glass wool) in the bottom of a 5mL glass pipette. (Use a 9mL disposable pipette to pack the cotton into the bottom of the
5mL glass pipette.) Fill the 5mL pipette approximately 1/3 to 1/2 full of Ag⁺
impregnated silica gel. Secure pipette column to ring stand with a small clamp.

2. Blow down the L1/L2 fraction under N₂. Either leave a small amount (~100μL) of
solvent in the vial or dry completely and re-dissolve sample in a small amount of hexane.

4. Run some hexane through the column to moisten the silica gel.

5. Place a clean 4mL vial underneath the pipette column. Use a clean glass pipette to
transfer the sample onto the pipette column. Use a different glass pipette to transfer ~0.5
mL of clean hexane to the 4mL vial, cap and gently swirl, and then transfer the contents
onto the silica column. Repeat and keep eluting column with hexane until the 4mL
sample collection vial is nearly full. Label vial “saturated”.

6. Place a new 4mL vial underneath the pipette column. Elute the unsaturated/aromatic
hydrocarbons with 1:1 DCM/MeOH. If sample contains elemental sulfur, the silica gel
may turn brown or black during this step. This is normal.

7. Blow down the saturated and unsaturated fractions under N₂. The samples are ready
for GC or GC/MS analysis.

Note: This procedure is useful for separating n-alkanes and n-alkenes before running
compound-specific carbon isotopes. It also removes elemental sulfur.
Lab procedure 9: Derivitization of polar fractions

Materials:
- BSTFA
- Acetonitrile
- Na₂SO₄
- Nitrogen
- 200 µL syringe
- Heating block
- Hexane
- 4mL screw cap vial with Teflon-line cap
- Thermometer for heating block

Procedure:
1. The following fractions need to be derivitized prior to GC or GC/MS analysis:
   L5/L6 (sterol and alcohol fraction), L7-L9 (dial and TEX₈₆ fraction) and L10.
2. Dry sample under N₂. (Make sure to solvent wash the N₂ nozzles first to ensure that they are clean.)
3. Add 100µL of BSTFA and 100µL of acetonitrile (pour a small amount into a small beaker with Na₂SO₄ at the bottom) to each sample with a solvent-rinsed microliter syringe. Make sure to record the batch and lot number of BSTFA used.
4. Flush vial for a few seconds with N₂ and cap tightly.
5. Place vials in heating block and heat for 2 hours at 60-70°C. Check occasionally to make sure the vial caps are still secured tightly.
6. Place vials on a warm (~60-70°C) heating block and blow off the reagents under N₂. When dry, rinse each vial and cap with hexane and then dry under N₂.
7. Add “TMS’ed” to each vial label.

Notes:
1. It is best to derivitize the samples immediately prior to GC or GC/MS analysis. If samples have been sitting around for a while, it is best to re-derivitize them before analysis as resulting TMS-derivatives are susceptible to hydrolysis by traces of water.
2. If samples are going to be analyzed for compound-specific carbon isotopes, the isotopic composition of each batch of BSTFA must be determined. Thus, if you know you’re going to run isotopes on a batch of samples, it is best to ensure that all samples are derivitized using BSTFA from the same batch.
3. Water in the sample or reagents will inhibit the formation of TMS-ethers. It is best to use fresh reagents that are stored in a dessicator. Acetonitrile may be stored over Na₂SO₄.

4. If the vial is not kept warm when reagents are blown down, a silica precipitate can form on the vial as the hexane evaporates. If silica forms, dissolve sample in hexane and move to a new vial.

5. Make sure that temperature on the heating block has stabilized before walking away and letting samples reflux for 2 hours.
Lab procedure 10: Addition of internal standard for quantification

Materials:
5α-androstane
Microliter syringe
2 mL vials and clean vial caps
Hexane or ethyl acetate

Procedure:

1. Inject 1µL of either 20 or 40 ng/µL 5α-androstane on the GC. Determine the area of this peak.

2. Take one sample and quantitatively dissolve it in an appropriate amount of either hexane or ethyl acetate (use hexane for the apolar L1/L2 and A-L4B fractions, use ethyl acetate for derivitized L5/L6, L7-L9, and L10 fractions. (For samples that are 2-5% TOC, start with 500µL - you can always blow down the sample under N₂ and re-dissolve it in a lesser amount of solvent if it is too dilute.)

3. Inject 1µL of sample on GC. If the compounds appear to be abundant and the GC trace looks good, then determine the area of the largest peak. If compounds are present in too great or too little quantity, then re-dilute the sample appropriately and re-run.

4. Compare the area of the 5α-androstane peak to the area of the largest peak in the sample. Determine how much 5α-androstane should be added to the sample to get a peak equivalent to the largest peak in the sample.

Note: It is important to note that the solvent that the 5α-androstane is dissolved in will also dilute the compounds in your sample. For a large batch of samples from the same site, determine the size of the largest peak in several samples and use the average as a guideline for adding the correct amount of 5α-androstane.

Example:
1µL of 40 ng/µL 5α-androstane is injected on GC and the area of this peak = 320,000,000

1 µL of the sample (dissolved in 200µL solvent) is injected on the GC and the largest peak has an area = 160,000,000. Thus, the standard peak is twice as large as the sample peak.

Two options for obtaining the correct concentration of standard in the sample:
1) Dry the sample under N₂. Add 100µL of 40ng/µL 5α-androstane to the sample vial. Since the sample is 2x as concentrated, now the standard peak is in the correct range.
2) Dry sample and add 100μL of solvent plus 100μL of 40ng/μL 5α-androstane to the sample vial. The resulting concentration of 5α-androstane is 20ng/μL.

Note that hexane should be used for apolar fractions and ethyl acetate for polar fractions. Thus, it is useful to have prepared 40 ng/μL 5α-androstane in both hexane and ethyl acetate. Make sure you record the amount and concentration of standard (as well as additional solvent added, if necessary) in your lab notebook!
Lab procedure 11: Injection of samples on GC

Procedure:

1. Check all gas tanks to make sure that they are full. Gas tanks should not be used below 500psi.

2. Fill solvent wash vials with either hexane or ethyl acetate and place in autosampler. Make sure if you are running polar fractions that these wash bottles contain a polar solvent (or if running mixed apolar and polar samples in the same sequence fill the wash bottles with a mixture of 1:1 hexane, ethyl acetate).

3. To run the GC (FID/FPD): On the GC computer, pull down the “sequence” menu and go to “edit sample log table”. Enter sample, vial number, file number, and method information into the table. When done, hit “ok”. If a file number is entered that already exists, a warning message will appear.

4. Under the sequence method, hit “save” and give it a unique file name.

5. Under the sequence menu, hit print and select “brief format”. Add this to the lab notebook.

6. Load samples into autosampler tray making sure that the vial numbers match with the information entered into the sequence.

7. Under the sequence method, hit “run”. At this point the oven will light. The condensate tube should be disconnected while the ovens are igniting. Once both the front and back detectors indicate that the flame is “on” re-connect the condensate tube.

8. After samples are finished running, load the shutdown method.

Notes:
If running a very long sequence, check the solvent levels in the wash vials periodically as they might need to be refilled.

When labeling samples, be careful not to put the tape too high up on the vial or to put tape fully around the vial. If the vial is too snug in the tray, the autosampler will have problems.

Run a solvent blank at the beginning of each run and approximately every 10 samples.
Lab procedure 12: Injection of samples on GC/MS

Make sure that samples are sulfur-free before injecting on GC/MS!

Procedure:
1. Check all gas tanks to make sure that they are full. Gasses should not be used below 500psi.
2. Under the “method” menu, load the appropriate oven temperature program.
3. Under the “method” menu, select “run”.
4. Enter the 7 digit file name (first two digits are the year, the second two are the month, and the last three are the sample numbers). For example, the file number for the 10th sample run in Feb. 2007 would be: 0702010. Do not overwrite any files! Enter the operator name and the sample name. Record this information in the lab notebook along with how much was injected and the volume of solvent the sample was diluted in. Hit “run method”.
5. If the GC/MS has not been used for the day or if the oven temperature is too high, a message saying “waiting for GC ready will appear.” If it is the first run of the day, it can take ~20 minutes for this message to go away. When a message saying “press the prep run key...” appears, the instrument is ready for sample injection.
6. Press the “prep run” key on the GC. When the “not ready” light goes away and the “pre run” light stops blinking, the sample can be injected (Figure 3).
7. Inject sample in one quick motion, remove syringe immediately, press the “start” key on the GC.
8. On the computer and there will be a message about overriding solvent delay. Hit “no”.
9. When done running samples for the day load the shutdown method.

Figure 3. The prep-run, pre-run, not-ready, and start keys and lights are indicated by the numbered arrows 1-4, respectively.
REFERENCES