Validation of the MBT-CBT paleotemperature proxy: Effects of environmental and seasonal variability in soils and lacustrine sediments

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Abstract

Branched glycerol dialkyl glycerol tetraethers (GDGTs) are bacterial derived membrane lipids found ubiquitously in soils and lacustrine sediments (Weijers et al. 2006b, Blaga et al. 2009). The degree of methylation and cyclization of these lipids have been shown to be dependent on the temperature and pH of the growth environment (Weijers et al. 2007a). These relationships are the basis of the MBT-CBT proxy, which has been used to reconstruct paleotemperature from marine and lacustrine sediment archives (Weijers et al. 2007b, Blaga et al. 2010). Here, we aim to test the validity of the MBT proxy in terrestrial soils and lake sediments to determine whether branched GDGT distributions do reflect annual mean air temperature (MAT) of the watershed, as suggested by studies, and how other environmental factors, such as seasonality of growth and sub-environments within a watershed, might influence the preserved MBT-CBT temperature signal. GDGT-derived annual MAT was compared with instrumental temperature measurements at three sites in the continental United States. Watershed soils were collected monthly for one year under three different types of vegetative cover in Minnesota and Ohio. In Florida, soils were collected twice from an open field environment. Sediment cores were collected from corresponding lakes in each of the three states. We observed no significant differences in soil proxy-derived annual MAT or soil GDGT concentration with seasonal changes in temperature at any of the three sites. Concentrations of GDGTs in the soil were found to have a slight positive correlation with organic carbon content. The effects of vegetative cover on proxy estimates of annual MAT were minimal. Only under deciduous vegetation in Minnesota and Ohio, did proxy-derived annual MAT differ significantly from instrumental measurements. Soil GDGT concentration was unaffected by vegetation type in Minnesota, and in Ohio, pine soils had consistently higher concentrations, usually by an order of magnitude, than other vegetation types. The CBT proxy was found to be an accurate estimate of soil pH in some sub-environments, but in Minnesota and Ohio deciduous soils and Ohio open field soils, CBT-pH differed significantly from measured values. In the sediments of all three lakes studied, the MBT-CBT proxy provided a good estimate of measured annual MAT within the error of the proxy. Proxy-derived temperatures from the surface sediments of all lakes studied were cooler than corresponding instrumental measurements. These cooler surface temperatures could be attributed to in-situ production of branched GDGTs, but surface sediment MBT-MAT only differs significantly from instrumental measurements in Bath Pond, Ohio.

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Introduction

Recent trends of climate change and global warming have become popular topics of conversation in the scientific and political communities as well as among the general public. As people around the world experience tangible changes in climate, there is increasing demand for researchers to determine what the earth's climate has been like in the past in order to predict what it might be like in the future. Data from Greenland ice cores have shown that Earth has, in the recent past, experienced rapid shifts between warm and cold stable climate states (Severinghaus and Brook, 1999). Understanding this past climate variability is necessary to be able to predict future climate change (NRC Committee, 2006). In the development of predictive climate models, temperature is one of the most important parameters to understand because of its great impact on other climatic factors, such as hydrological variability (Schlesinger, 1997, NRC Committee, 2006). Despite the significance of temperature, it remains one of the most elusive parameters to describe. Observations from the instrumental record show that recent warming has occurred but confidence in older temperature reconstructions is lacking due to a dearth of reliable proxy data (NRC Committee, 2006).

There are a number of proxies for paleotemperature that can be applied to various environmental archives, such as marine and lacustrine sediments. However, paleotemperature is particularly difficult to reconstruct for continental systems. In the marine environment, preserved foraminiferal assemblages have been used to reconstruct sea-surface temperature (SST) based on species-specific growth requirements (Chapman et al. 1986). The oxygen isotope composition of foraminifera and corals and Mg/Ca ratio of biogenic carbonates in marine environments have also been used to reconstruct paleotemperature (McKenzie, 1985, Brassel et al. 1986, Lea et al. 2003). Long-chain alkenone biomarkers have successfully been used to reconstruct SST in marine systems, but the haptophyte algae that produce these biomarkers have rarely been identified in freshwater lakes (Brassel et al. 1986, D'Andrea et al. 2006). More recently, a microbial lipid-based proxy, the TEX_{86} (tetraether index of tetraethers with 86 carbon atoms), which uses isoprenoid GDGT (glycerol dialkyl glycerol tetraether) membrane lipids produced by marine Crenarchaeota, has been shown to provide accurate estimates of SST from marine sediment archives (Schouten et al. 2002).

In lacustrine environments the $\delta^{18}O$ value of authigenic and biogenic carbonates and diatom silica are commonly used as paleotemperature indicators (Leng and Marshall, 2004). In both marine and lacustrine systems, however, this proxy may be dependent on myriad environmental and biological factors, such as the $\delta^{18}O$ signal and carbonate concentration of the source water, the depth at which an organism lives, and any biological isotope fractionation effects (McKenzie, 1985, Hayes, 1993, Leng and Marshall, 2004). Carbonate-based proxies can also be unreliable in lacustrine systems due to inconsistent preservation (McKenzie, 1985, Hayes, 1993). Freshwater chironomid assemblages can be used to reconstruct recent paleotemperatures, but their use is limited to shallow, oxic systems that do not typically have long sediment records (Walker et al. 2001). Powers et al. (2004, 2005, 2010) showed that the microbial lipid-based TEX_{86} proxy is applicable in large lake systems, such as Lake Superior, but not in smaller lakes that receive large inputs of terrestrial organic matter. Few, if any, of the proxies discussed here provide estimates of paleotemperature that are independent of confounding factors, emphasizing the need for a reliable inland temperature proxy that can be applied to ancient lacustrine sediment archives.

In recent years, branched GDGT lipids, similar to the isoprenoid GDGTs that are the basis of the TEX_{86} proxy, have been found to be ubiquitous in peats, soils and lake sediments (Weijers et al. 2006a,b, Blaga et al. 2009). Weijers et al. (2007a) analyzed 134 soil samples from 90 globally distributed sites and found that the degree of cyclicity, defined as the number of cyclopentane groups, in branched GDGTs is related to the pH of the soil, and the degree of branching, or the number of methyl branches, in soil GDGTs was related to both temperature and pH of the growth environment. Using this information Weijers et al. (2007a) developed two molecular proxies, the MBT (methylation index of branched tetraethers) and the CBT (cyclization index of branched tetraethers). Together, these two proxies can be used to construct annual MAT and the pH of the soil environment that the GDGT-producing bacteria lived in. Documented findings of branched GDGTs in peat, soil, and marine and lacustrine sediments suggest that these microbial lipids may be transported fluvially from land to a depositional setting, raising the question of whether branched GDGTs preserved in lake sediments could be used as a continental proxy for paleotemperature (Schouten et al. 2000, Hopmans et al. 2004, Weijers et al. 2006a,b, Blaga et al. 2009).

Branched GDGTs found in near-shore marine sediments gave rise to the BIT (branched & isoprenoid tetraether) index, which compares the ratio of branched to isoprenoid tetraethers (Hopmans et al. 2004). Since branched GDGTs are thought to be primarily of terrestrial origin and isoprenoid tetraethers of aquatic origin, the BIT index therefore describes the relative contributions of terrestrial and aquatic organic matter to a depositional environment (Hopmans et al. 2004). The BIT index can also be used as a first screen to indicate whether there is enough organic matter of terrestrial or marine origin to apply the appropriate molecular proxy, TEX_{86} or MBT-CBT.

Isoprenoid and branched GDGTs are core membrane lipids that are produced by thermophilic and methanogenic euryarchaeota, nonthermophilic pelagic crenarchaeota, anaerobic terrestrial bacteria, and possibly sediment-dwelling aquatic bacteria as well (DeRosa and Gambacorta, 1988, Pancost et al. 2000, Schouten et al. 2002, Weijers et al. 2006a,b, Peterse et al. 2009b, Tierney and Russel, 2009). They serve as useful biomarkers because their chemical structure is particularly resistant to degradation by diagenetic processes (Van de Vossenberg et al. 2000, Schouten et al. 2004). Studies of thermophilic archaea in culture have yielded strong correlations between growth temperatures and the distribution of cyclopentane rings in archaeal GDGT lipids (Uda et al. 2001). Inclusion of more cyclopentane rings in a core lipid membrane with increasing temperatures helps the cell to maintain its fluidity (Gliozzi et al. 1983, Uda et al. 2001). The TEX_{86} SST proxy was developed based on the similarities between nonthermophilic crenarchaeotal and thermophilic archaeal membrane lipids. As seen in thermophilic archaeal membranes, Schouten et al. (2002) found a strong relationship between mean annual SST, analogous to growth temperature, and the distribution of cyclopentane rings in GDGT core lipids produced by marine crenarchaeota.

Unlike the archaeal isoprenoid lipids utilized by the TEX_{86} proxy, the GDGTs relevant to the MBT-CBT proxy are branched and believed to be of bacterial origin (Weijers et al. 2006a). Archaea typically produce ether-bonded, membrane spanning isoprenoid lipids while Bacteria produce ester-bonded, bi-layered, branched membrane lipids (Weijers et al. 2006a). The hybrid ether-bonded, membrane spanning, branched GDGTs were finally determined to be of bacterial origin by their taxonomic specific stereochemistry (Weijers et al. 2006a). The exact organism producing branched GDGTs in soils and peats is currently unknown. However, based on patterns of GDGT abundance in two peat cores from the Saxnas Mosse, Sweden and the Bolton Fell Moss, England they have been suggested to be anaerobes, potentially involved in the mineralization of organic matter (Weijers et al. 2006a). GDGT abundance in a set of 90 globally distributed soils was found to be greatest in soils with low pH, suggesting that GDGT producers may also be acidophiles (Weijers et al. 2007a).

In archaeal GDGTs, the degree of cyclicity is related to growth temperature (Uda et al. 2001). As previously mentioned, Weijers et al. (2007a) found that, in branched GDGTs of bacterial origin, the degree of methylation, i.e. the number of methyl branches on the glycerol backbone of the lipid structure, and not the degree of cyclicity, is related to the temperature of the growth environment. As growth temperatures decrease, a greater degree of branching prevents dense packing of the cell membrane and allows for the maintenance of fluidity that is necessary for survival (Sutaari and Laakso 1992). The pH of the growth environment affects the degree of cyclization, i.e. the addition of cyclopentyl moieties, of branched GDGTs as well as their distribution within the physical environment (Weijers et al. 2007a). Cyclization has been shown to affect membrane permeability, which is important in maintaining a proton gradient over the cell membrane to allow energy to be supplied to the cell (Mitchell et al. 1966). The relationship between GDGT cyclization and soil pH is the basis of the CBT proxy proposed by Weijers et al. (2007a).

Weijers et al. (2007a) also found the MBT index to be related to the pH of the growth environment as well as annual MAT. Similar to the addition of cyclopentyl moieties, methyl branches on the lipid backbone are thought to affect the proton permeability of the cell membrane (Van de Vossenberg et al. 1995). A three-dimensional calibration plot involving soil pH, annual MAT, and the MBT index, devised by Weijers et al. (2007a), removes the influence of pH on the MBT index and yields a set of equations that allow environmental pH and temperature to be calculated from measured MBT and CBT indices. Using these relationships it should theoretically be possible to reconstruct annual MAT and soil pH of a watershed based on the preserved GDGT assemblage in the corresponding sediment record.

Already the MBT-CBT and BIT proxies have been used in paleoclimate studies of ancient marine sediments and have produced reasonable results. Together, the BIT and MBT indices were used to analyze marine sediments near the Congo River outflow and produced a temperature record of the past 25 ky for continental Africa that is consistent with pollen and stable isotope-based reconstructions of paleotemperature for the region (Weijers et al. 2007b). The MBT proxy was also used in a study of arctic sediments from the Lomonosov Ridge in the Central Arctic Ocean (Weijers et al. 2007c). The results indicated an episode of warming similar in magnitude to a rise in SST that was documented by an earlier study. Schouten et al. (2008) compared MBT-constructed annual MAT to bio-climatic analyses of pollen assemblages for a marine sediment core from the Greenland Basin. Both proxies yielded comparable estimates of annual MAT, further confirming the usefulness of the MBT-CBT proxy in paleoclimate research (Schouten et al. 2008). Although the MBT-CBT proxy has been used successfully in marine environments, as discussed here, its applicability in the lacustrine setting remains to be demonstrated.

The MBT-CBT indices have the potential to be used as a much needed, independent continental temperature proxy. However little is known about the ecology of the organisms that produce GDGTs in soils. Subsequent research has begun to provide evidence that validates the use of the MBT-CBT proxy as well as to raise questions about its accuracy and applicability in different environments. A study of Mt. Kilimanjaro soils along an altitudinal gradient showed that branched GDGT assemblages varied with altitude and therefore, also with temperature (Sinninghe Damsté et al. 2009). Peterse et al. (2009a) confirmed that the MBT index is primarily dependent on temperature and the CBT index on pH. Soils sampled at varying distances from two California hot springs showed decreasing MBT-CBT constructed temperature and pH with distance from the springs (Peterse et al. 2009a). A study of arctic soils and near-shore sediments of Svalbard found that the soil MBT index yielded a calculated annual MAT that closely reflected the instrumentally measured annual MAT (Peterse et al. 2009b). However, the MBT signal recorded in near-shore sediments differed significantly from that of the soils and did not accurately reflect annual MAT. It is thought that low rates of soil organic matter deposition to coastal sediments off Svalbard and possible in-situ production of branched GDGTs in subaqueous sediments was the cause of this inconsistency (Peterse et al. 2009b). Rueda et al. (2008) compared instrumental and GDGT-based sea surface and air temperatures from marine sediments in the Skagerrak, off Southern Norway, and found that the estimates were similar for annual SST using the TEX_{86} proxy. However, the MBT-CBT estimate of annual MAT was most similar to summer air temperatures indicating a potential seasonal bias to the proxy (Rueda et al. 2008).

In addition to soils and marine sediments, branched and isoprenoid GDGTs have been found to be abundant in lacustrine sediments (Powers et al. 2004, Blaga et al. 2009). In large lakes with pelagic environments similar to marine systems, the TEX_{86} proxy has been calibrated and used to reconstruct mean annual lake surface temperatures (Powers et al. 2004, 2005, 2010). Smaller inland lakes often receive large amounts of terrestrial organic matter that render the TEX_{86} proxy useless (Weijers et al. 2006b). In small lakes, the MBT-CBT proxy may be able to provide long-term records of continental paleotemperature in place of the TEX_{86} proxy. In a study of 47 European lakes along a north-south transect, Blaga et al. (2009) found that in most systems, terrestrial-sourced branched GDGTs were greater than 40% of the total GDGT pool. Only 9 lakes in the study had GDGT distributions that would allow reliable application of the TEX_{86} proxy (Blaga et al. 2009). Blaga et al. (2010) analyzed surface sediments from 82 lakes for GDGTs in an attempt to justify the use of the MBT-CBT indices as a proxy for continental paleotemperature. Using the global soil calibration developed by Weijers et al. (2007a), they found that lacustrine MBT-constructed temperatures were consistently cooler than instrumentally measured annual MAT and suggest that the offset may be due to an unknown source of branched GDGTs to lake sediments in addition to GDGTs produced in watershed soils (Blaga et al. 2010).

Branched GDGTs in lake sediments are thought to be primarily of terrestrial origin, though the source organism is yet unknown. However, recent evidence suggests that *in situ* production of branched GDGTs may be occurring as well. In Lake Challa, Tanzania, the concentration of branched GDGTs in sedimenting particulate matter peaked during and shortly after rainy seasons and is thought to be primarily allocthonous in origin (Sinninghe Damsté et al. 2009). However, they also found high concentrations of branched GDGTs in anoxic bottom waters with a different distribution from that of catchment soils (Sinninghe Damsté et al. 2009). A study by Tierney and Russell (2009) also found evidence of potential *in situ* production of branched GDGTs in the sediments of Lake Towuti, Indonesia. Of the soil, river sediment and lake sediment samples they analyzed for the MBT and CBT indices, there were significant differences between the annual MAT recorded by terrestrial and aquatic GDGT assemblages. If *in situ* production of branched GDGTs is indeed occurring it could bias the temperature signal recorded in sediment archives.

Applications of the MBT-CBT proxy in soils and sediments have yielded varied results and raised new questions that need to be investigated before the proxy can be confidently used to reconstruct continental paleotemperature from lacustrine sediment archives. In soils, multiple studies have confirmed that the MBT is primarily dependent on temperature and that soil organic matter is transported to lake sediments through erosion, surface runoff, and river inputs (Peterse et al. 2009a, Sinninghe Damsté et al. 2009). What is still unknown is whether or not there is a seasonal bias contained within the terrestrial GDGT signal. The organisms that produce the branched GDGTs utilized in the MBT-CBT paleotemperature proxy have not been identified, and it is therefore difficult to predict what environmental factors will likely affect the proxy. Do soil microbes produce GDGTs throughout the entire year in cold climates, even when frost may reach several meters of depth in the soil, or do they slow down or completely stop growth? If the latter is true, the MBT proxy could not be expected to accurately reflect mean annual air temperature in higher latitude systems as it might in warmer systems where growth can occur throughout the year.

Another question is whether vegetation type, which may influence soil pH and soil temperature via canopy insulation and incident radiation, affects soil GDGT abundances and distributions throughout a watershed. In September 2006, samples were taken from wooded and open field environments at Chester Park in Duluth, Minnesota. These soils were analyzed with regards to the MBT-CBT proxy and the results showed that there was a difference of nearly 3°C in soil temperature between sites. The MBT-MAT for the shady, wooded site was very similar to the instrumentally-measured annual MAT for Duluth, while the open field soils yielded a warmer temperature estimate. These initial findings bring the accuracy of temperature reconstructions using the MBT-CBT proxy into question. Further research is needed to determine how problematic seasonal and sub-environmental effects may be with regards to the MBT proxy. If soil GDGT concentrations are biased by either seasonality or sub-environment, the archived sedimentary GDGT assemblage may be as well.

Other important questions facing the development of the MBT-CBT proxy are whether the core-top calibration is valid downcore, and whether the proxy is responsive to variation on rapid or long time scales. There are few ways to determine this absolutely, particularly as reconstructions go deeper into the past. The best approach at present is to carry out reconstructions with the proxy that can be compared to instrumental data on shorter time scales (e.g., the last 100 years). A preliminary study using a sediment core from the Valles Grande Caldera, which spans the mid-Pleistocene (~350-550 kybp), strongly suggests that the MBT index can be used to reconstruct temperatures of magnitudes and on timescales that are relevant to glacial/interglacial cycles (Weijers et al. 2009b, Werne et al., *unpublished*). Further support is given by a study of marine sediments from the Congo Fan, where a coherent 25 ky temperature record was reconstructed using the MBT-CBT proxy (Weijers et al., 2007b). However, neither of these studies compared instrumental measurements of temperature with the MBT reconstructions. Thus, it remains unclear whether the MBT-CBT proxy can accurately record higher resolution changes in temperature in the sediments. A recent study by Weijers et al. (2010) suggests a turnover time of ca. 17 years for branched GDGTs in the soil. Variation in MBT-derived temperature and relative accuracy at this resolution remains to be demonstrated in the sedimentary record.

The objective of my study is to test the validity of the MBT-CBT proxy in soils and lake sediments. I will determine whether branched GDGT distributions do reflect annual MAT of the watershed as they appear to in other studies, and how environmental factors, such as seasonality and sub-environments within a watershed, might influence the GDGT signal in soils and sediments. Of the many questions needing answers before the MBT-CBT proxy can be confidently used to reconstruct paleotemperature, my study investigates the following three. The first is whether there is a seasonal bias in soil GDGT assemblages. A second question is whether vegetation type (i.e. sub-environment) affects soil GDGT assemblages. Finally, I look at GDGTs from the sediments of 3 small, inland lakes to determine if they accurately reflect annual MAT over the past ~100 years of instrumental measurements.

A validation of the MBT-CBT paleotemperature proxy in lacustrine systems is much needed if it is to be of use to the paleoclimate community. Climate change has the potential to significantly affect both human populations and natural systems in the near future. An understanding of past and present climate systems is essential for future climate predictions. Molecular proxies such as the MBT and CBT indices can provide valuable information about paleoclimate but only if they are fully investigated to identify any biases that might hinder their successful application. The results of this study will help to clarify whether the MBT-CBT proxy can be successfully applied to inland lacustrine systems as a paleotemperature proxy. Furthermore, it will help to identify environmental factors that influence the MBT-CBT proxy so that future researchers will be able to better control for confounding factors in their own work.

Materials & Methods

Study Sites

Three sites were chosen over a climatic gradient in the continental United States (Figure 1). Elk Lake, located in Clearwater County in northwestern Minnesota, is a dimictic lake of glacial origin with a maximum depth of 29.6 m and a surface area of 124 hectares. It is surrounded by a mix of pine and northern hardwood forest (Dean et al. 2002). Northwestern Minnesota is considered to have a humid continental climate characterized by warm, humid summers and very cold winters (Peel et al. 2007). The average annual MAT in this part of the state is 3.8°C. The recent sediment record in Elk Lake was disturbed in the early 1900's by logging activity in the watershed (Rottsolk, 1960). To compare historical MBT-derived annual MAT with the instrumental temperature record in this region, sediments from nearby Deming Lake were also used in this study. Deming Lake is smaller than Elk Lake but it is situated in a similar landscape and is subject to similar climate conditions.

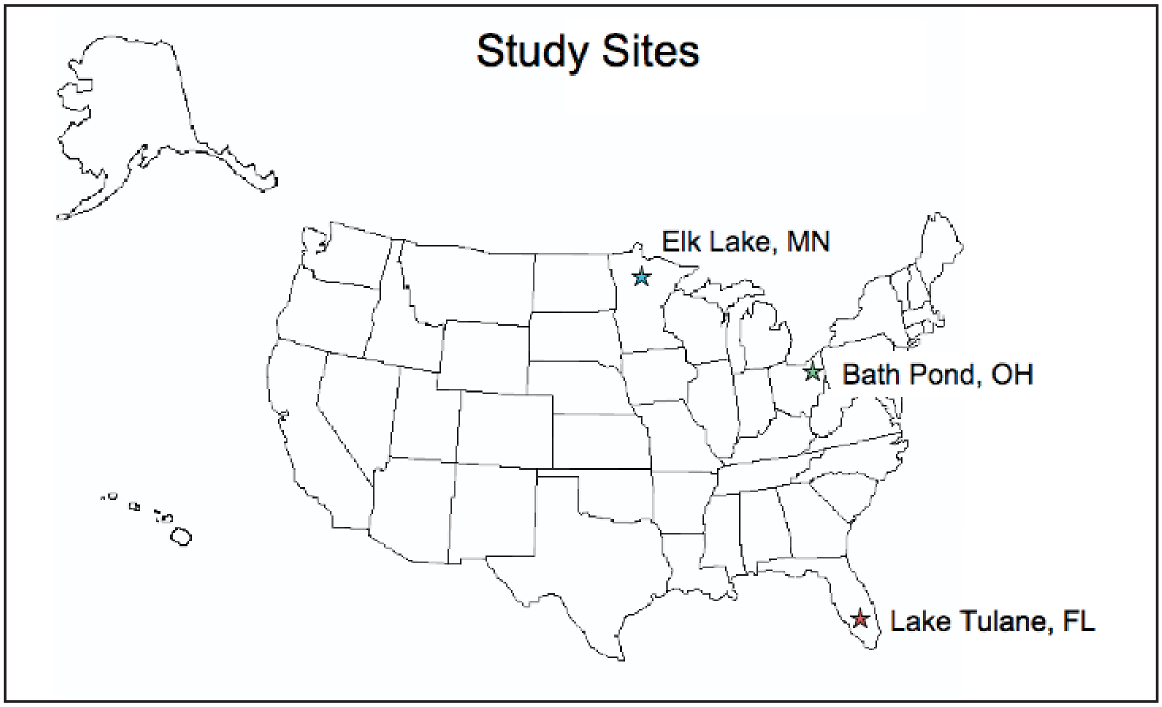

Figure 1: Map showing locations of the three study sites: 1. Itasca State Park, Minnesota, U.S.A.; 2. Bath Nature Preserve, Ohio, U.S.A; Lake Tulane, Avon Park, Florida, U.S.A.

Bath Pond is located within Bath Nature Preserve in Summit County, northeastern Ohio. The Nature Preserve is affiliated with the University of Akron and Bath Township Field Station. It has a surface area of 6.1 hectares and a maximum depth of 2.9 m. The surrounding vegetation is composed of mixed swamp and northern hardwood forest (Hood, 2002). The climate in northeastern Ohio is typical of humid continental regions with hot summers and cold winters (Peel et al. 2007). The 50-year average annual MAT in this region is 9.9^{o}C.

Lake Tulane, in Highlands County, south central Florida, is a sinkhole lake with a surface area of 36 hectares and a maximum depth of approximately 21.3 meters (Highlands SWCD, 2007). Unlike the other sites in this study, Lake Tulane is situated in an urban environment. The surrounding vegetation is mostly grass and is most similar to the open field type environments sampled at the other sites. The lake is unique, however, due to its unbroken sediment record that dates back approximately 62,000 years (Huang et al. 2006). The climate in south central Florida is subtropical with hot, humid summers and cool winters (Peel et al. 2007). The average annual MAT in this region is 22.5^{o}C.

Sampling

Soils in three different sub-environments were sampled monthly in Minnesota from September 2008 through August 2009. Square plots (3x3 m^2) were established in the Elk Lake watershed in areas dominated by pine, deciduous and open field vegetation. Duplicate soil cores were collected at each sampling plot using a hand auger during the growing season and a rock hammer, chisel and mallet when the soil was frozen. Three similar plots were established at Bath Nature Preserve in Ohio and sampled monthly from October 2008 to September 2009 by collaborators at the University of Akron. Florida soils were sampled in duplicate by collaborators at the University of South Florida in early April and late

October of 2009; minimal seasonality and relatively uniform vegetation throughout the Lake Tulane watershed did not justify monthly sampling as in more temperate sites. All soil samples were stored frozen in ashed, glass jars until processing.

Thermistors (NexSens micro-T temperature loggers) were buried at a depth of approximately 15 cm in each of the soil plots in Minnesota and Ohio. An additional thermistor was set to record ambient air temperature at these two sites at a height of ~1.5 meters. Thermistors recorded temperature approximately every 2 hours from September 2008 through October 2009.   Thermistors were initially deployed at the Florida sampling site, but were later lost during local construction around Lake Tulane.

Sediment cores were taken from Elk Lake in February 2009 using Bolivia and Livingstone piston corers. Sediment cores were taken in May and June 2008 from Lake Tulane and Bath Pond, respectively, using gravity corers with scuba diver assistance. Deming Lake sediments were collected in May 2006 using Bolivia and Livingstone piston corers. Sediment age in Deming Lake (Figure 2) was determined by varve counting (McLauchlan, unpublished). Bath Pond sediments were aged using ^{210}Pb analysis of sediments conducted at the St. Croix Watershed Research Station (Figure 3). Lake Tulane sediment ages were determined by ^{210}Pb and radiocarbon analyses conducted at the University of South Florida by collaborators, Julie Richey and Dave Hollander (Figure 4). Additional ^{210}Pb data for Bath Pond can be found in Appendix A.

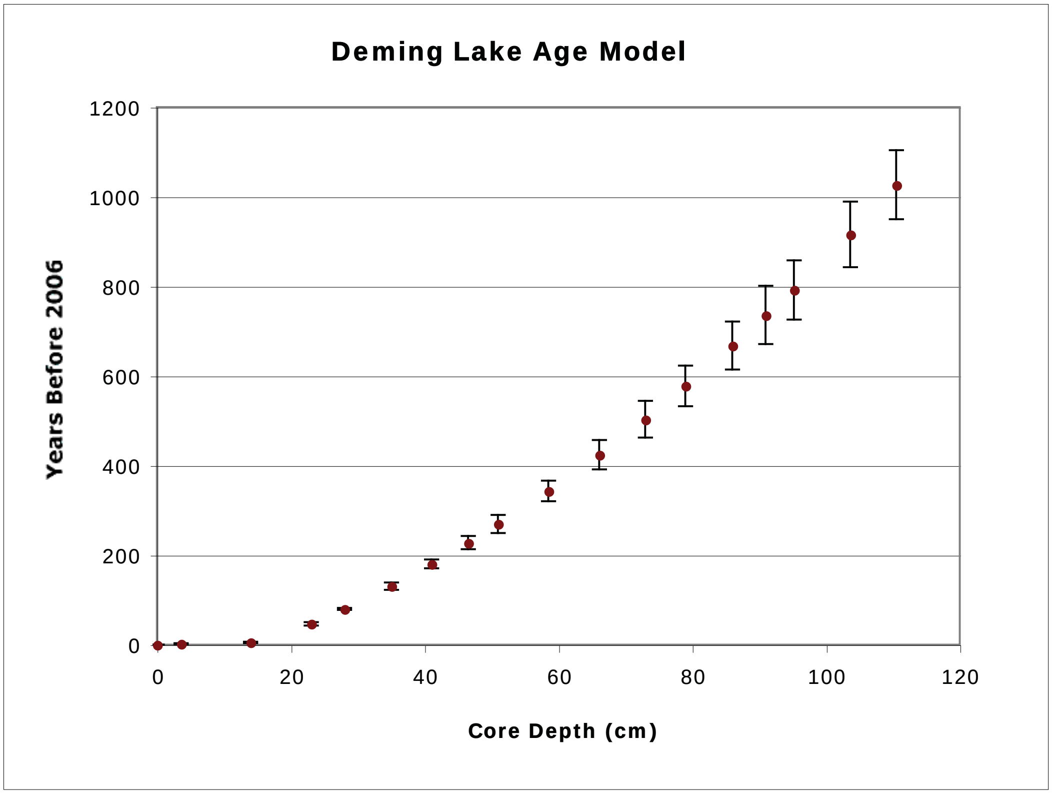

Figure 2: Age Model for Deming Lake in Itasca State Park, Minnesota (Lascu, personal communication). Ages before the date of sampling, represented by red dots, were determined by counting varves. Error bars represent the standard deviation of the age estimates.

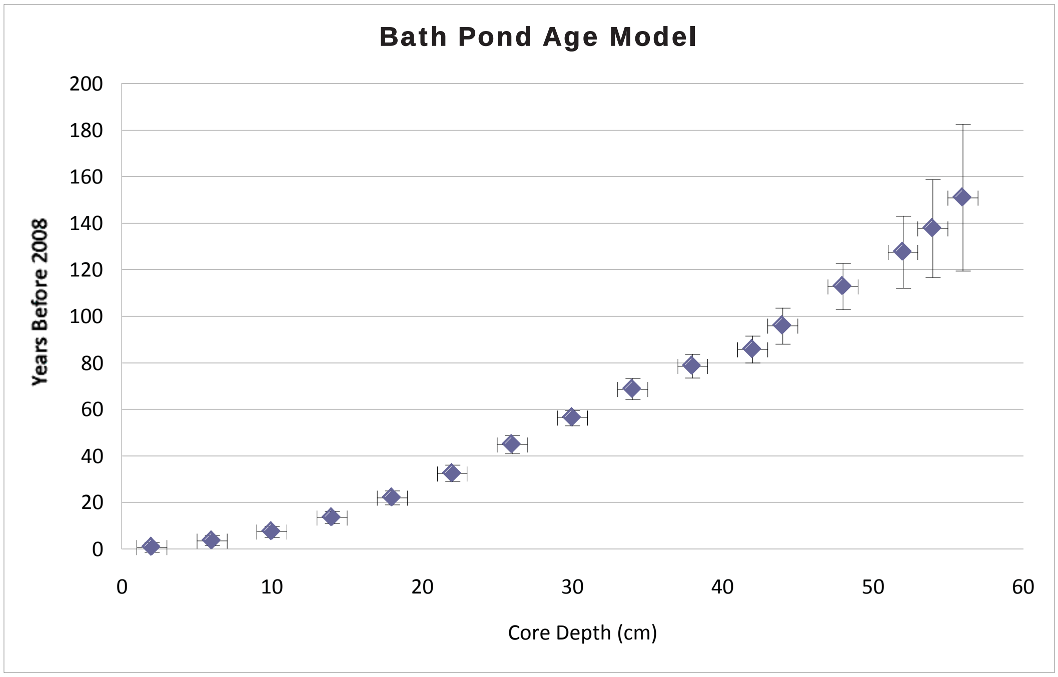

Figure 3: Age Model for Bath Pond in Bath Nature Preserve, Ohio. Ages before the date of sampling, represented by blue dots, were determined by ^{210}Pb analysis. Error bars represent the standard deviation of the age estimates.

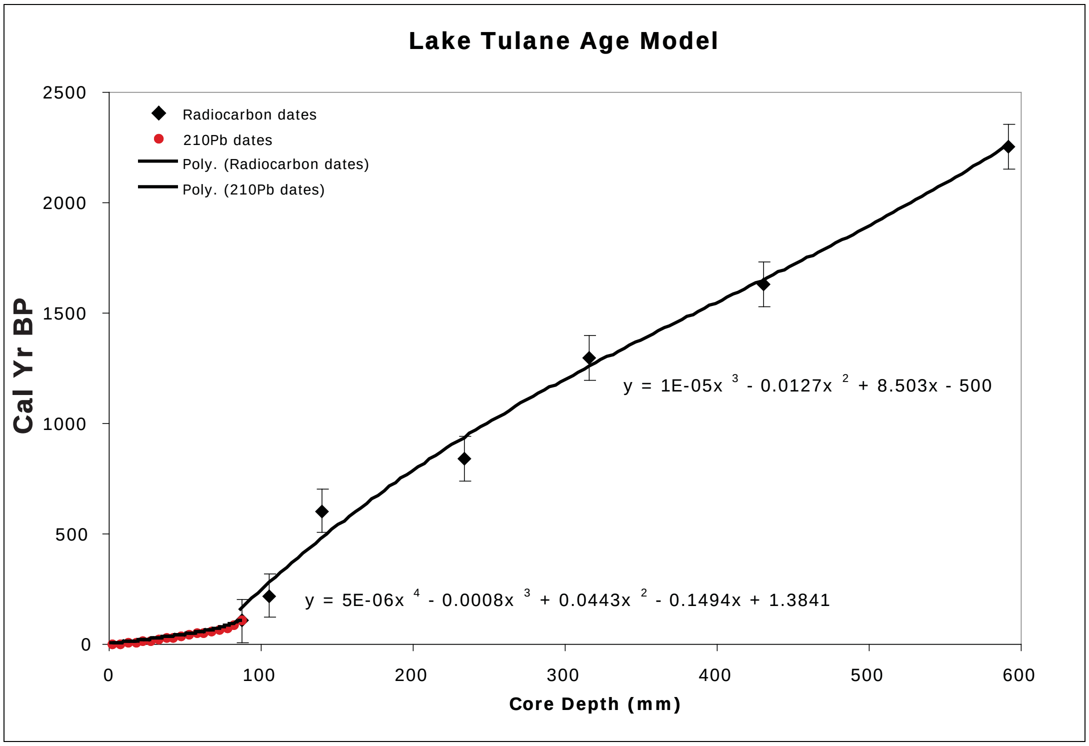

Figure 4: Age Model for Lake Tulane, Avon Park, Florida (Richey and Hollander, personal communication). Ages determined using ^{210}Pb analysis are represented by red circles and ages determined using radiocarbon are represented by black triangles. Error bars represent the standard deviation of the age estimates.

GDGT analysis

Sediment and soil samples were analyzed for GDGTs following methods modified from Schouten et al. (2007), Powers et al. (2004, 2005, 2010), and Weijers et al. (2007a). Soils and sediments were freeze-dried and homogenized with mortar and pestle after removal of root clumps and other large pieces of soil debris. Approximately 10 grams dry weight of soil, or 2 grams dry sediment, were solvent extracted using a DIONEX Accelerated Solvent Extractor (ASE) to obtain a total lipid extract (TLE). TLE aliquots were evaporated under nitrogen until dry, re-dissolved in hexane/DCM (9:1) and applied to an activated Al_2O_3 column. Apolar and polar phospholipid fractions were eluted with hexane/DCM (9:1) and DCM/MeOH (1:1), respectively. The polar fraction was again evaporated to dryness, re-dissolved in hexane/isopropanol (99:1) and passed through a PTFE 0.4 um filter to remove any remaining Al_2O_3 grains.

Prepared samples were analyzed using high performance liquid chromatography /atmospheric pressure chemical ionization/ mass spectrometry (HPLC/APCI-MS) at the Royal Netherlands Institute for Sea Research (NIOZ). An internal C_{46} GDGT standard was added in a known amount to allow for quantification of GDGT abundances in all samples. HPLC/MS analyses were carried out on an Agilent 1100 HPLC attached to a MSD SL mass detector. GDGTs were identified using Single Ion Monitoring (SIM) of $[M+H]^+$ ions and quantified by integrating peak areas following (Weijers et al. 2007a).

The following indices were calculated based on HPLC/MS measurements of relevant bacterial-sourced branched GDGTs:

CBT = - log {([Ib] + [IIb]) / ([I] + [II])},

MBT = {([I] + [Ib] + [Ic]) / ([I] + [Ib] + [Ic] + [II] + [IIb]+ [IIc] + [III] + [IIIb] + [IIIc])},

where I - III, b - c, refer to relevant GDGT structures (Figure 5).

The relationships between the MBT and CBT indices and temperature and pH are complex. Weijers et al. (2007a) performed a calibration that removes the influence of pH on the MBT index and yields a set of equations that allow environmental pH and temperature to be calculated from the GDGT based MBT and CBT indices. These equations are as follows:

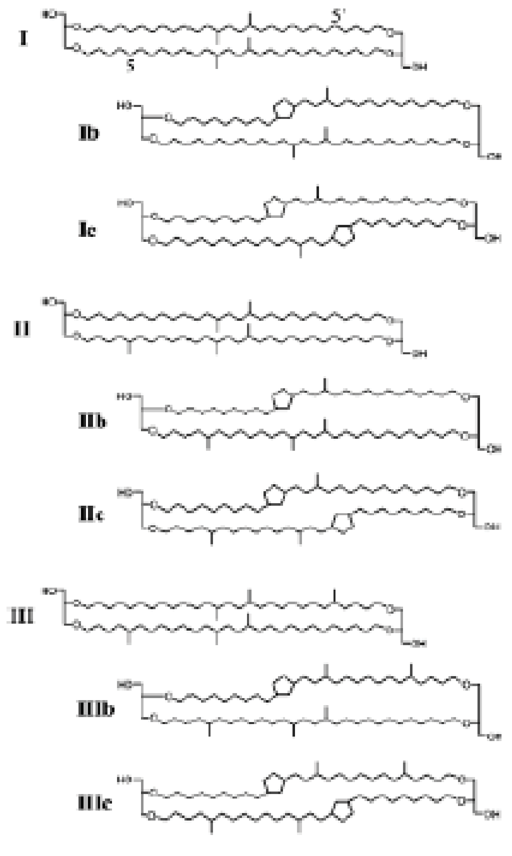

Figure 5: Chemical structures of branched GDGTs relevant to MBT-CBT proxy (Weijers et al. 2007a)

$$MBT = 0.122 + (0.187*CBT) + (0.020*MAAT) \textit{ or}$$

$$MBT = 0.867 – (0.096*pH) + (0.021*MAAT)$$

$$CBT = 3.33 – (0.38*pH)$$

pH analysis

Selected soil samples, taken for GDGT analysis from each sub-environment and sampling site, were analyzed for pH using standard electrode methods. Soil was mixed in a 1:1 ratio with distilled water and the pH of the supernatant was measured using a ThermoScientific Orion bench top pH meter.

Elemental Analysis

Freeze-dried and homogenized soils were acid fumigated, following Harris et al. (2001), to remove carbonates prior to analysis for total organic carbon. Oven-dried and cooled samples were placed in silver capsules in a microtiter tray and 50µl of MilliQ water was added to each sample. The tray was placed in a dessicator with a beaker of 12M HCL for 8 hours to allow release of carbonates. Dried and cooled acid-fumigated samples in silver capsules were then enclosed in 5x9 mm tin capsules and analyzed for total organic carbon on a Costech Elemental Analyzer.

Results - Soil Data

Thermistor Data

Thermistors measuring air and soil temperatures in Minnesota and Ohio recorded less variation and lower extremes in the soil than in the air (Figures 6.a. and 7.a.). All Florida thermistors were lost during construction around Lake Tulane during 2009. All temperature data for the Florida site was instead obtained online from the National Climate Data Center (NCDC).

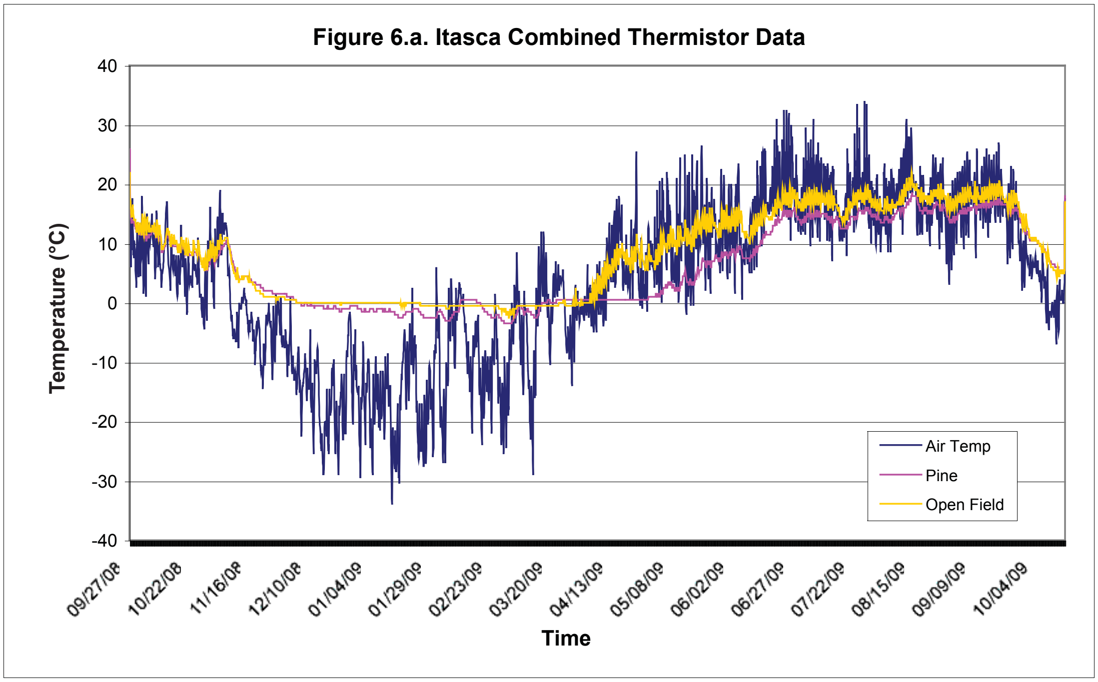

Figure 6.a: Air and soil thermistor data for one year at the Minnesota sampling site. The blue line represents air temperature, and the pink and yellow lines represent pine and open field soil temperatures at 15 cm depth, respectively.

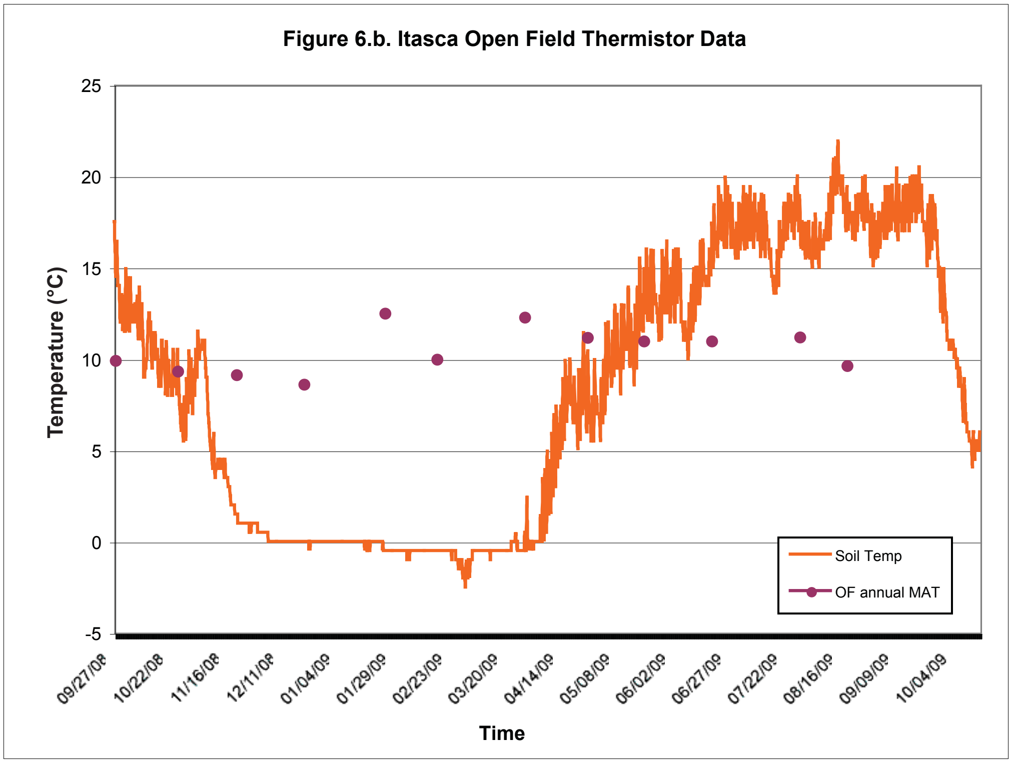

Figure 6.b: Soil thermistor and proxy temperature data from the open field plot in Minnesota. The orange line represents soil temperature at 15 cm of depth, measured every 2.5 hours, over the course of 1 year. The purple dots represent the MBT estimate of annual MAT, calculated using measured pH, of monthly soil samples.

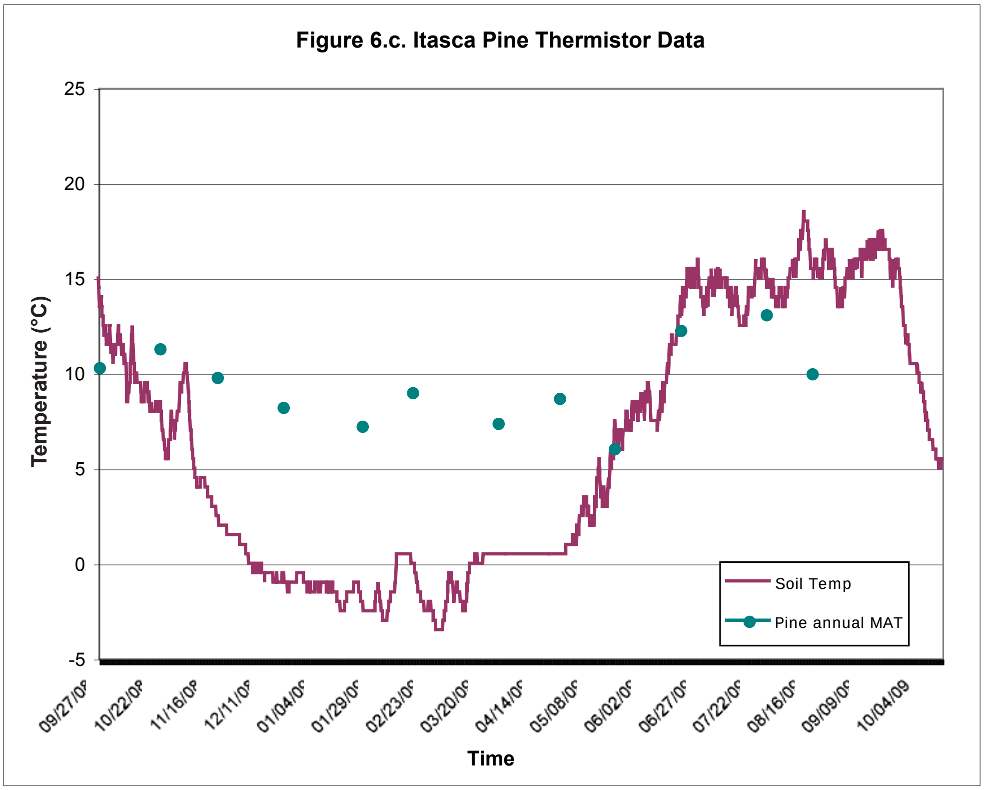

Figure 6.c: Soil thermistor and proxy temperature data from the pine plot in Minnesota. The purple line represents soil temperature at 15 cm of depth, measured every 2.5 hours, over the course of 1 year. The blue dots represent the MBT estimate of annual MAT, calculated using measured pH, of monthly soil samples.

In Minnesota, the annual MAT, calculated from thermistor measurements, was 3.8°C for the 2008 – 2009 sampling season. The mean annual soil temperature was 9.7°C in the Minnesota pine plot and 8.2°C in the open field plot (Figures 6.b and 7.c). The deciduous soil thermistor did not record any data over the course of the year.

In Ohio, the annual MAT, calculated from thermistor measurements, was 9.9°C. Open field soils were slightly warmer than pine soils with a mean annual soil temperature of 10.5°C (Figure 7.b). Ohio pine soils had a mean annual temperature of 10.1°C (Figure 6.c). The deciduous soil thermistor in Ohio was not recovered.

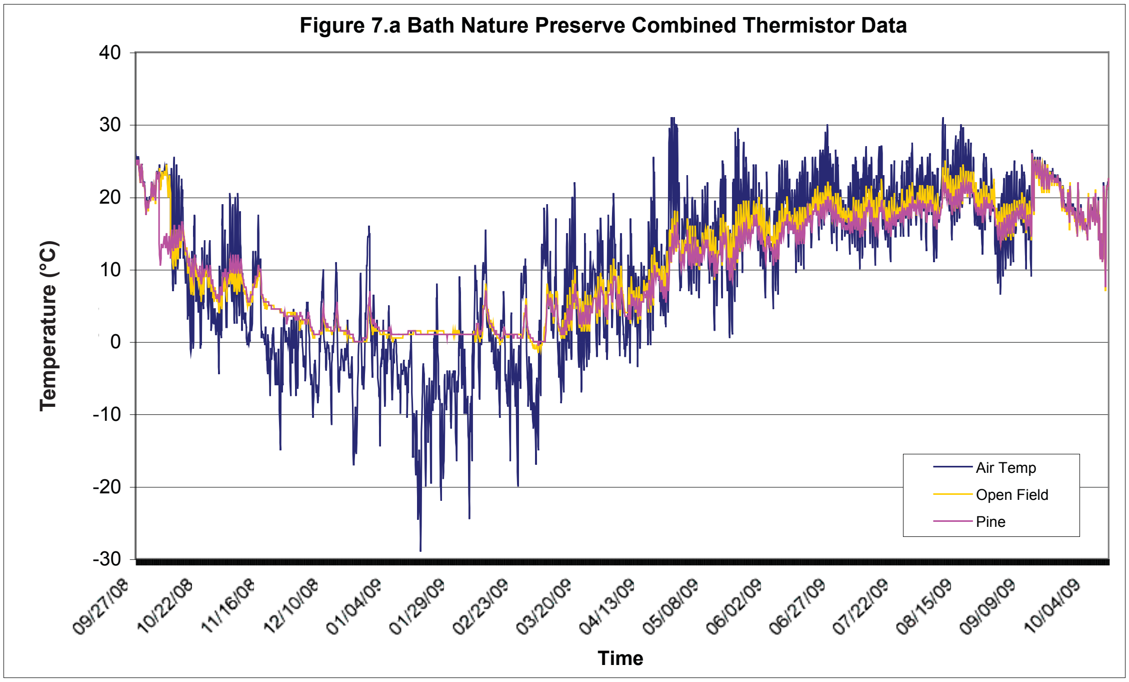

Figure 7.a: Air and soil thermistor data for one year at the Ohio sampling site. The blue line represents air temperature, and the pink and yellow lines represent pine and open field soil temperatures at 15 cm depth, respectively.

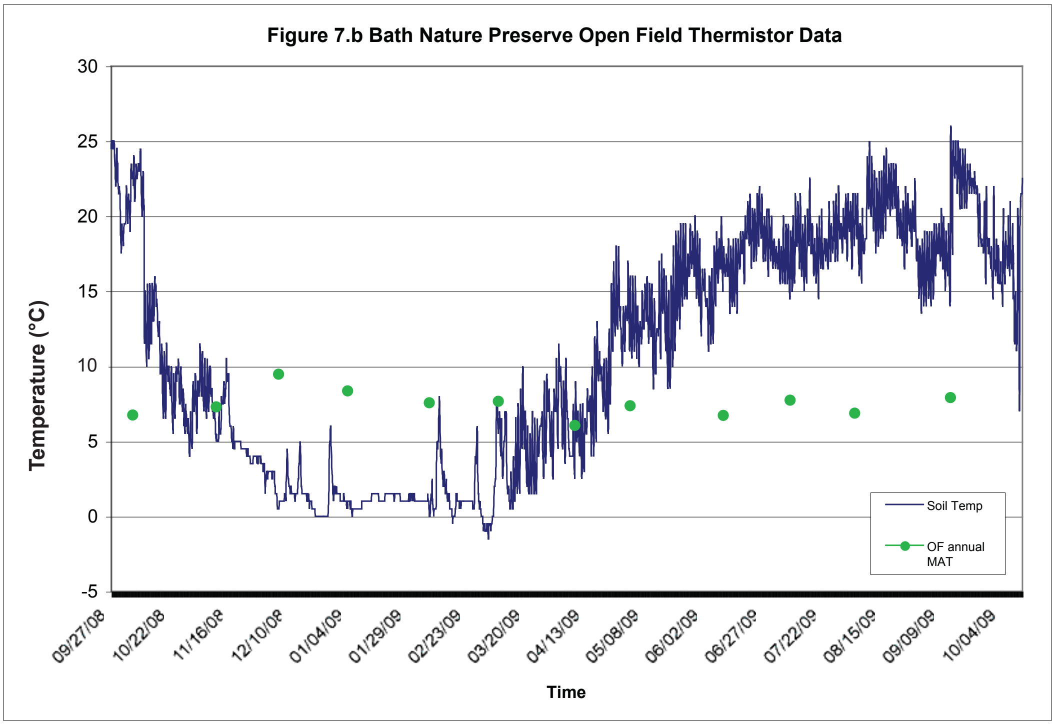

Figure 7.b: Soil thermistor and proxy temperature data from the open field plot in Ohio. The blue line represents soil temperature at 15 cm of depth, measured every 2.5 hours, over the course of 1 year. The green dots represent the MBT estimate of annual MAT, calculated using measured pH, of monthly soil samples.

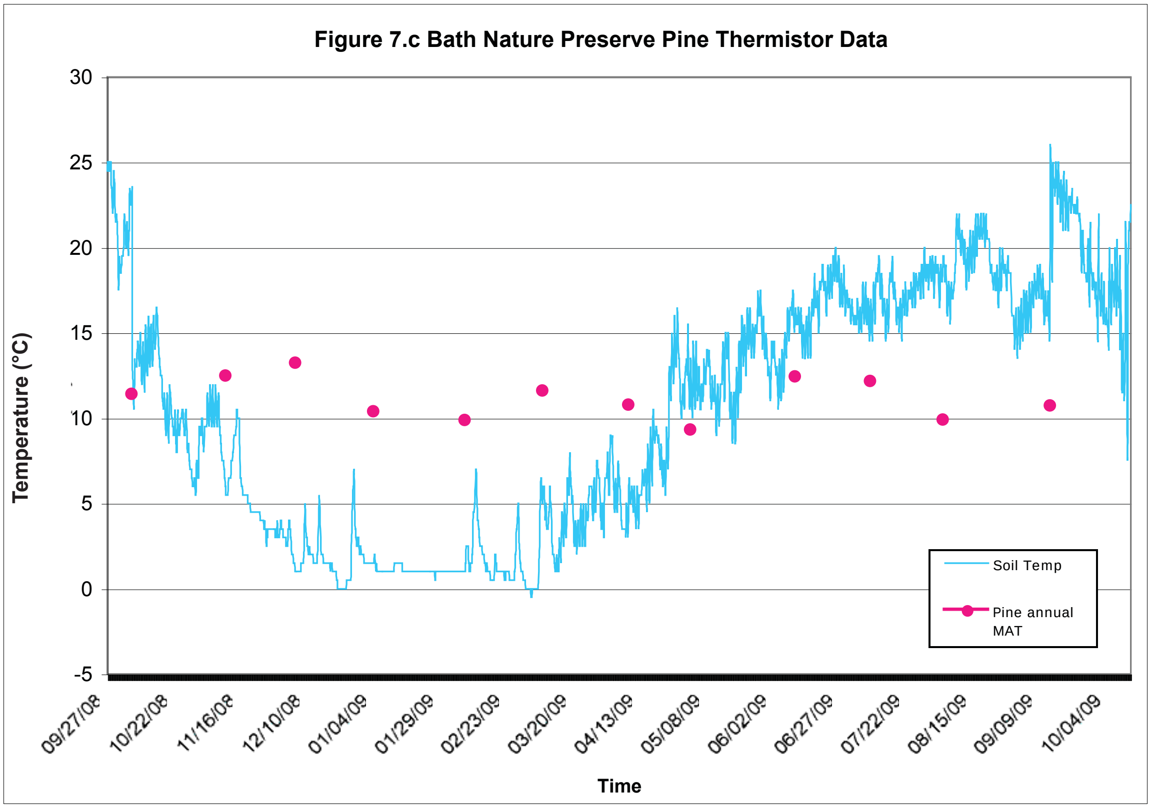

Figure 7.c: Soil thermistor and proxy temperature data from the pine plot in Ohio. The blue line represents soil temperature at 15 cm of depth, measured every 2.5 hours, over the course of 1 year. The pink dots represent the MBT estimate of annual MAT, calculated using measured pH, of monthly soil samples.

pH Comparisons

In Minnesota, pine soils were the most acidic with an instrumentally measured pH value of 5.1. Deciduous soils were closest to neutral with a pH of 6.6. Open field soils were measured to have a pH of 6. Measured soil pH was generally well-matched with proxy-derived pH in pine and open field plots within the error of the CBT index (Figure 8). The analytical error of the CBT proxy was determined to be ± 0.08 pH units and the standard error of the estimate to be ± 0.8 pH units. (J.W.H. Weijers, personal communication). The standard error of the estimate, calculated from the original calibration dataset, is a measure of the accuracy of proxy-based predictions. It is defined as the square root of the sum of squared differences between actual measurements and predicted measurements divided by the number of measured pairs. Measured pH for deciduous soils was significantly lower than the average CBT estimate of 7.8.

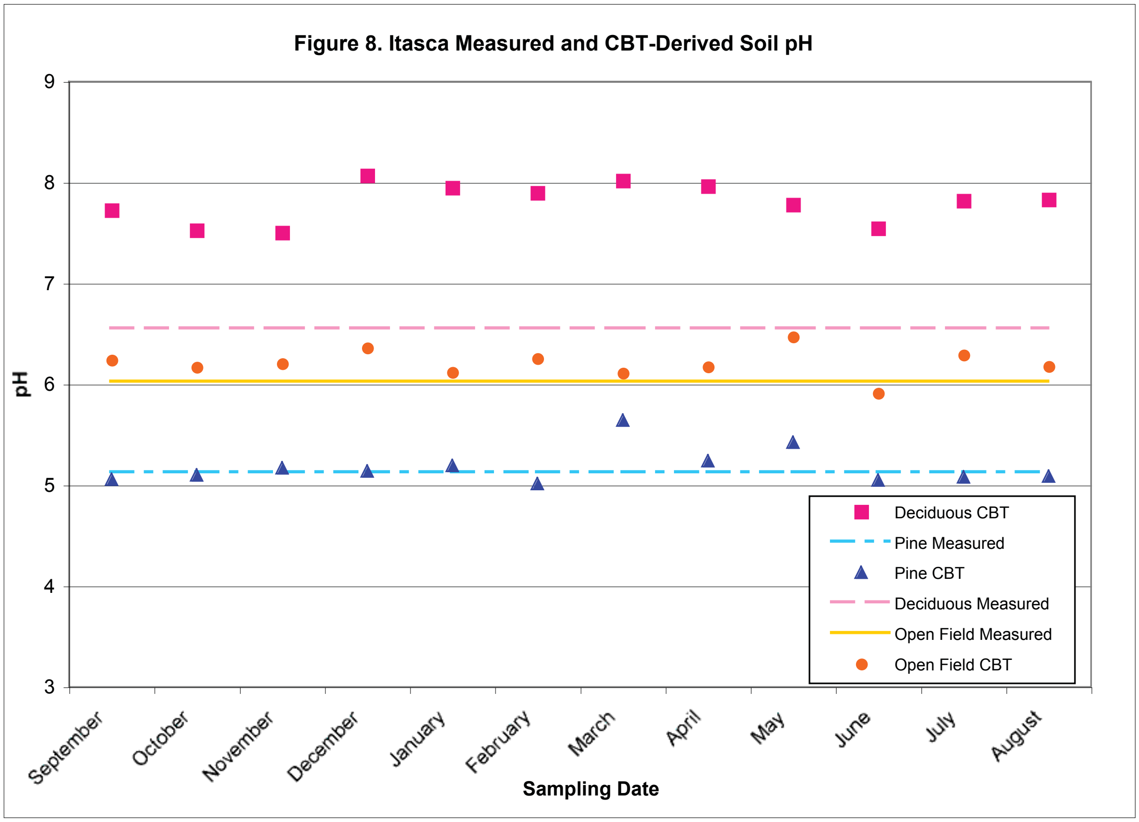

Figure 8: Measured and proxy-estimated pH for three soil types in Minnesota. The measured deciduous soil pH is shown by the dashed pink line, and the CBT estimates of soil pH from monthly soil samples are shown by pink squares. The measured open field soil pH is shown by a solid yellow line, and the CBT estimates of soil pH from monthly soil samples are shown by orange circles. Measured pine soil pH is shown by a dotted and dashed blue line, and the CBT estimates of soil pH from monthly soil samples are shown by blue triangles.

In Ohio, pine soils had the lowest measured pH of 4.8 and open field had the highest pH of 7.4. Deciduous soil pH was measured to be 6.1. Measured and proxy-derived pH values were well matched in the pine plot within the error of the CBT proxy (Figure 9). In both the deciduous and open field plots, average proxy pH values of 7.7 and 8.4 respectively, were significantly higher than instrumentally measured pH.

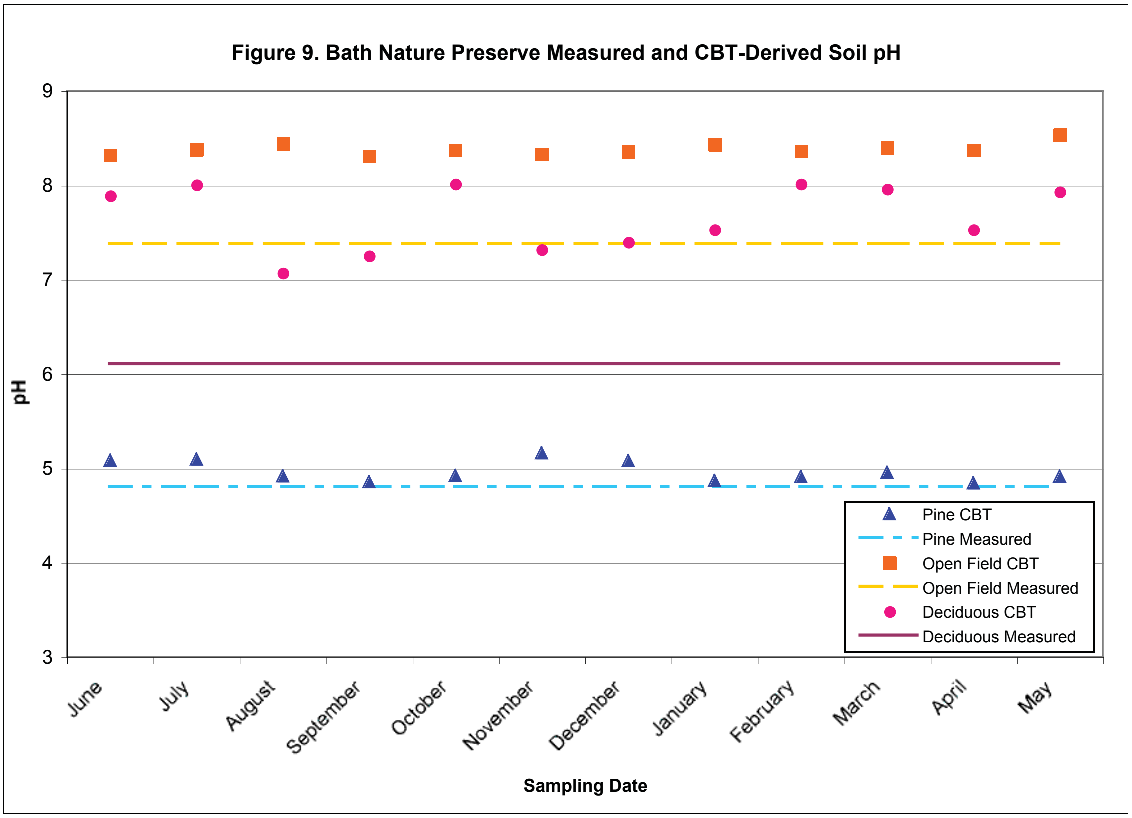

Figure 9: Measured and proxy-estimated pH for three soil types in Ohio. The measured deciduous soil pH is shown by the solid pink line, and the CBT estimates of soil pH from monthly soil samples are shown by pink circles. The measured open field soil pH is shown by a dashed yellow line, and the CBT estimates of soil pH from monthly soil samples are shown by orange squares. Measured pine soil pH is shown by a dotted and dashed blue line, and the CBT estimates of soil pH from monthly soil samples are shown by blue triangles.

In Florida, measured soil pH was 6.3 in April and 6.2 in October. CBT-pH ranged from 6.5 in April samples to 5.8 in October samples. Measured and CBT-derived pH were effectively the same between replicates with regards to the error inherent in the proxy (Figure 10). Overall, the measured and proxy-derived pH data from Florida soils are similar.

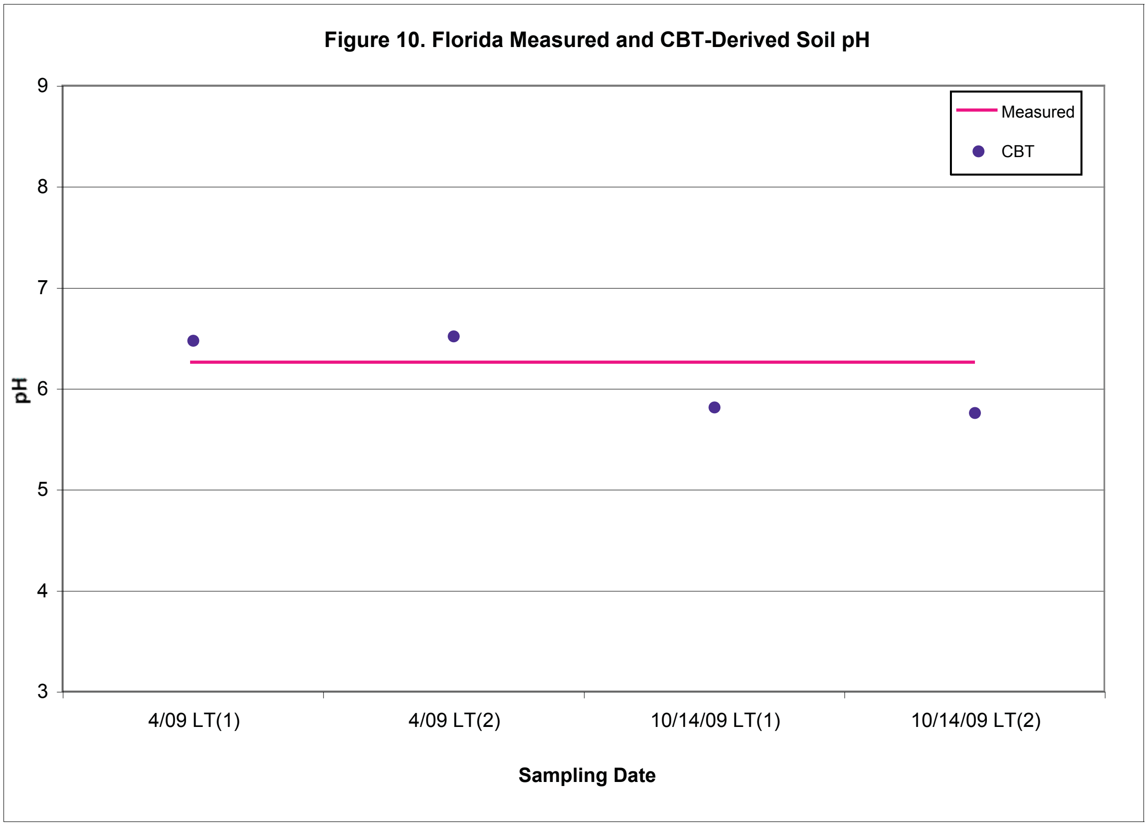

Figure 10: Measured and proxy-estimated pH for Florida soils. Measured pH is represented by a solid pink line, and replicate CBT estimates of soil pH from two sampling dates are represented by purple dots.

Annual MAT (MBT)

An incorrect CBT-derived estimate of pH has implications for the accuracy of the MBT proxy. The MBT can be calculated in two ways, with an equation that uses the CBT number or an equation that uses the actual measured pH. If the MBT-CBT proxies are being used for the purpose of paleoclimate reconstruction, the CBT estimate of pH is all that will be available. A higher CBT value will yield higher MBT temperatures, and vice versa. Considering the difference between the measured and proxy-derived pH values for certain soil types described above, soil MBT-annual MAT values were calculated both ways: using instrumentally measured soil pH values and CBT-derived soil pH values. The results of both calculations will be discussed. MBT-based estimates of annual MAT for all sediment samples were calculated using CBT-derived pH since instrumental pH measurements could not be made.

In Minnesota, the average MBT-annual MAT values of pine, open field and deciduous soils, calculated using measured pH values, were 9.6^{o}C (± 2.4^{o}C), 10.4^{o}C (± 1.6^{o}C) and 11.0^{o}C (± 1.2^{o}C), respectively. The analytical error of the MBT index was calculated to be ± 0.78^{o}C and the standard error of the estimate to be ± 5.5^{o}C (J.W.H Weijers, personal communication). In all three plots, soil MBT-annual MAT was significantly higher than the instrumental annual MAT of 3.8^{o}C, as calculated from thermistor measurements, (figure 11.a) but closer to annual mean soil temperatures of 6.0^{o}C in the pine plot and 8.2^{o}C in the open field plot. As previously mentioned, deciduous soil temperature was unavailable, but may be assumed to be similar to that of the pine environment. MBT data show no clear pattern of seasonality in any of the sub-environments and the average MBT-annual MAT, as calculated with measured pH, of the different vegetation types are the same within the error of the proxy.

When CBT-pH was used to calculate MBT-annual MAT, there was more spread among vegetation types with pine again recording the coolest average temperature of 10.0^{o}C (± 2.1^{o}C) and deciduous the warmest average temperature of 14.0^{o}C (± 1.0^{o}C) (Figure 11.b.). The average proxy-derived temperature of the open field plot was calculated to be 10.2^{o}C (± 1.8^{o}C). Using this calculation method, deciduous soil MBT-annual MAT differs significantly from instrumental annual MAT of 3.8^{o}C by 10.2^{o}C and measured mean annual soil temperature of the pine sub-environment by 8^{o}C.

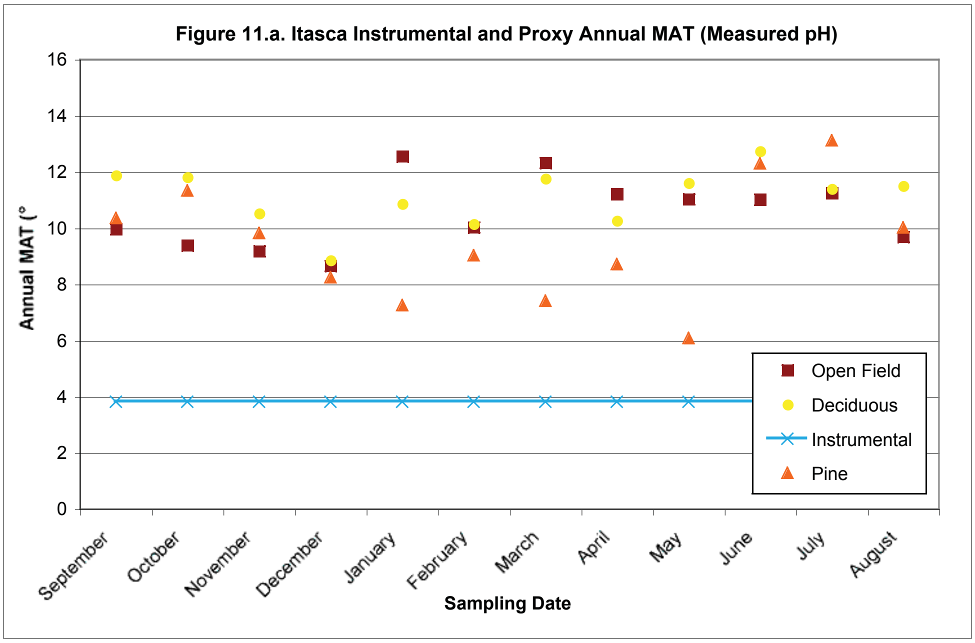

Figure 11.a: Instrumental annual MAT and proxy-estimated annual MAT from three soil types in Minnesota. MBT estimates of annual MAT are calculated using measured pH values and annual MAT is based on air thermistor measurements. Deciduous, open field and pine MBT-MATs are shown by yellow circles, dark red squares, and orange triangles, respectively. Instrumental annual MAT is shown by a solid blue line.

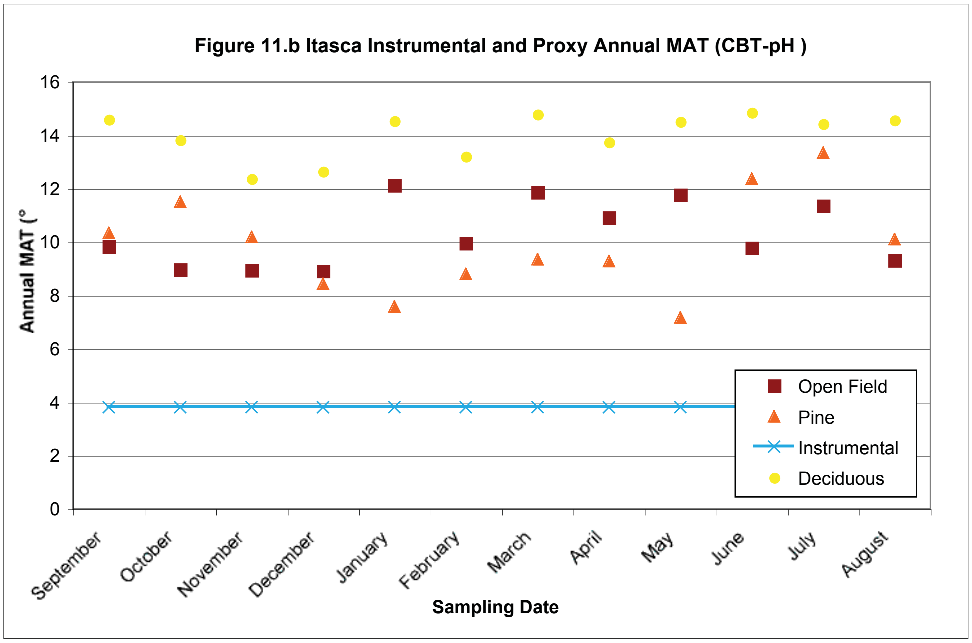

Figure 11.b: Instrumental annual MAT and proxy-estimated annual MAT from three soil types in Minnesota. MBT estimates of annual MAT are calculated using CBT-pH and annual MAT is based on air thermistor measurements. Deciduous, open field and pine MBT-MATs are shown by yellow circles, dark red squares, and orange triangles, respectively. Instrumental annual MAT is shown by a solid blue line.

In Ohio, the average MBT-annual MAT values of pine, open field and deciduous soils, calculated using measured pH, were 11.3°C (± 1.2°C), 7.5°C (± 0.9°C), and 14.5°C (± 0.9°C), respectively (figure 12.a). Considering proxy error, the MBT estimates for pine and open field plots, using measured pH, were the same as the instrumental annual MAT of 9.9°C and the annual mean soil temperatures of 10.0°C in pine soils and 10.4°C in open field soils, as calculated from thermistor measurements. The MBT-annual MAT of the deciduous soil was 4.6°C warmer than instrumental annual MAT but still within the error of the proxy.

When CBT-pH was used instead of instrumental pH measurements, Ohio MBT estimates of annual MAT were warmer in all sub-environments but still similar to the measured pH-MBT calculations within the range of proxy error. Using CBT-pH, annual MAT values were 12.6°C (± 1.6°C), 8.4°C (± 0.9°C), and 19.2°C (± 1.0°C) for pine, open field, and deciduous vegetation types, respectively (figure 12.b). Using this calculation method, the MBT estimates for pine and open field plots were still the same, within standard error of the proxy estimate, as the instrumental annual MAT of 9.9°C and the annual mean soil temperatures of 10.0°C in pine soils and 10.4°C in open field soils, as calculated from thermistor measurements. As in Minnesota, the Ohio deciduous soil MBT-annual MAT, when calculated using CBT-pH, differs significantly from measured annual MAT by 9.3°C and measured mean annual soil temperature of the pine sub-environment by 9.2°C.

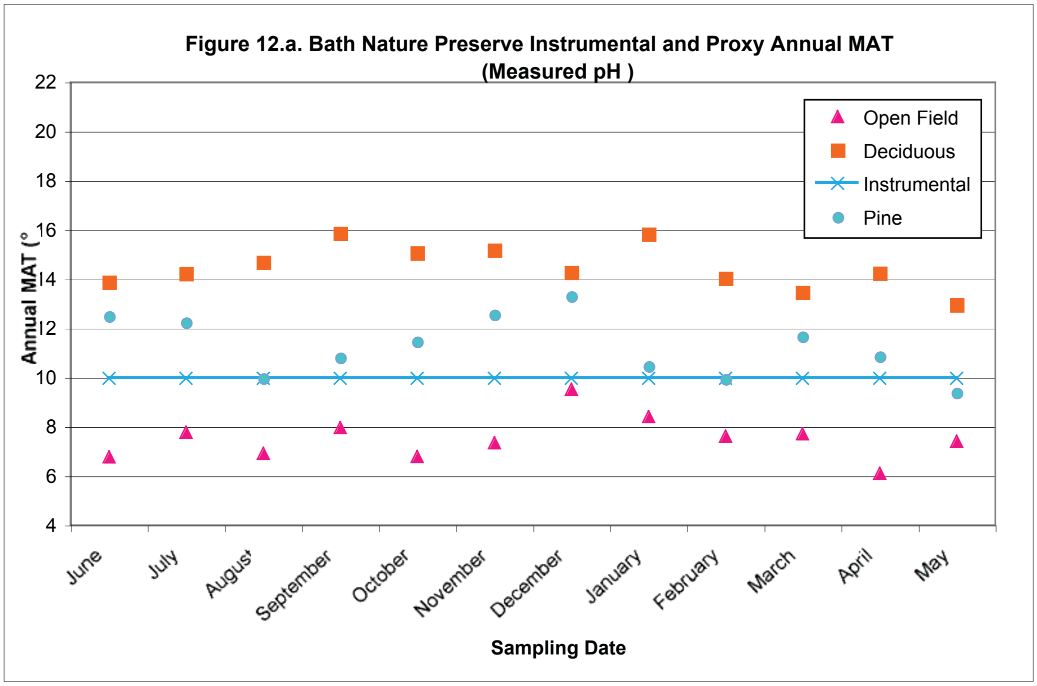

Figure 12.a: Instrumental annual MAT and proxy-estimated annual MAT from three soil types in Ohio. MBT estimates of annual MAT are calculated using measured pH values and annual MAT is based on air thermistor measurements. Deciduous, open field and pine MBT-MATs are shown by orange squares, pink triangles, and blue circles, respectively. Instrumental annual MAT is shown by a solid blue line.

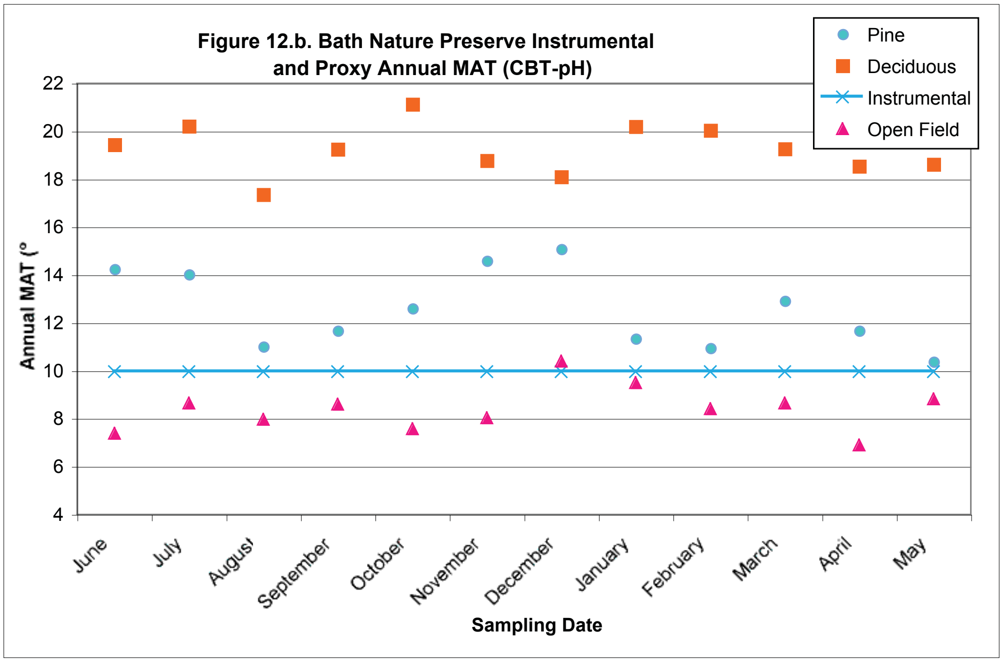

Figure 12.b: Instrumental annual MAT and proxy-estimated annual MAT from three soil types in Ohio. MBT estimates of annual MAT are calculated using CBT-pH and annual MAT is based on air thermistor measurements. Deciduous, open field and pine MBT-MATs are shown by orange squares, pink triangles, and blue circles, respectively. Instrumental annual MAT is shown by a solid blue line.

In Florida, average annual MAT, as calculated with measured pH values, was 29.3°C (± 0.3°C). Average annual MAT, calculated using CBT-pH, was the same within the range of proxy error at 28.8°C (± 1.8°C) (Figure 13). Both calculation methods yielded temperatures that were significantly warmer than the instrumentally measured annual MAT of 22.8°C, as recorded by the National Climate Data Center (NCDC(a)).

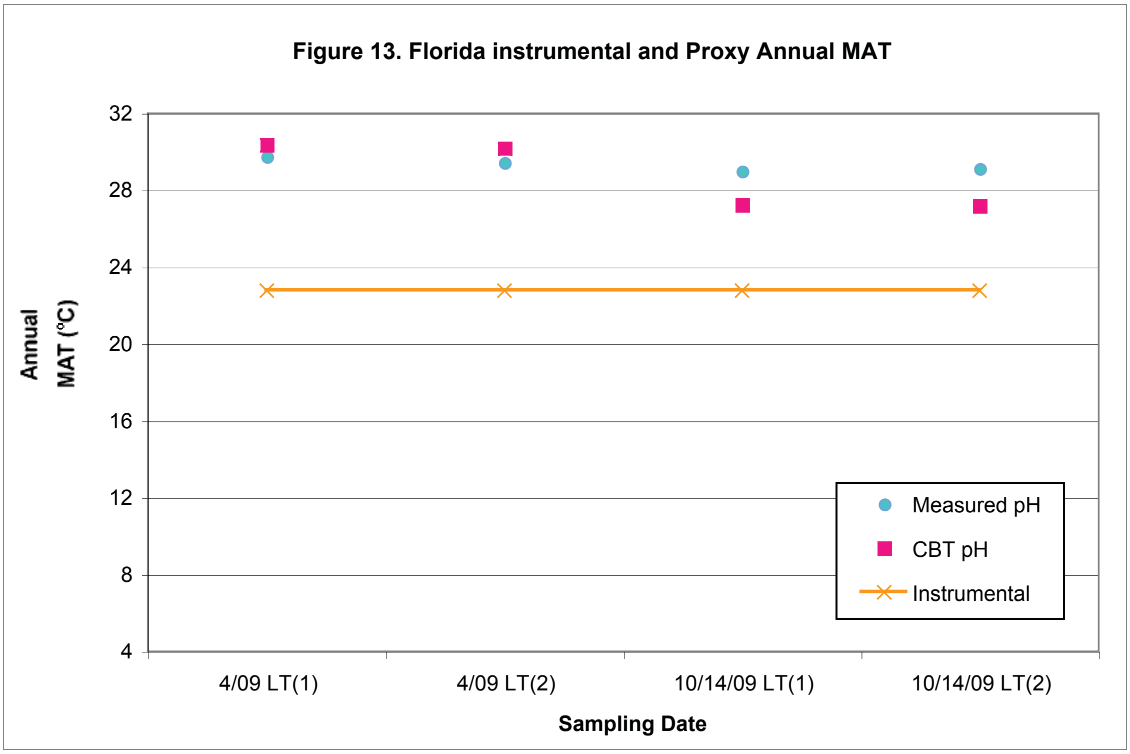

Figure 13: Instrumental annual MAT and proxy-estimates of annual MAT from Florida soils. Replicate MBT estimates of annual MAT from two sampling dates, calculated using measured pH, are shown by blue circles, and replicate MBT estimates of annual MAT from two sampling dates, calculated using CBT-pH are shown by pink squares. Instrumental annual MAT for the 2008-2009 sampling season was taken from NCDC climate records, and is shown by a solid yellow line.

Soil GDGT Abundance

GDGT abundance per gram soil organic carbon (OC) was variable in Minnesota samples (Figure 14). There does not appear to be a clear pattern of changing abundances over the course of an annual cycle or among vegetation types. Values ranged from 1.5 to 16.0 µg GDGT/g OC.

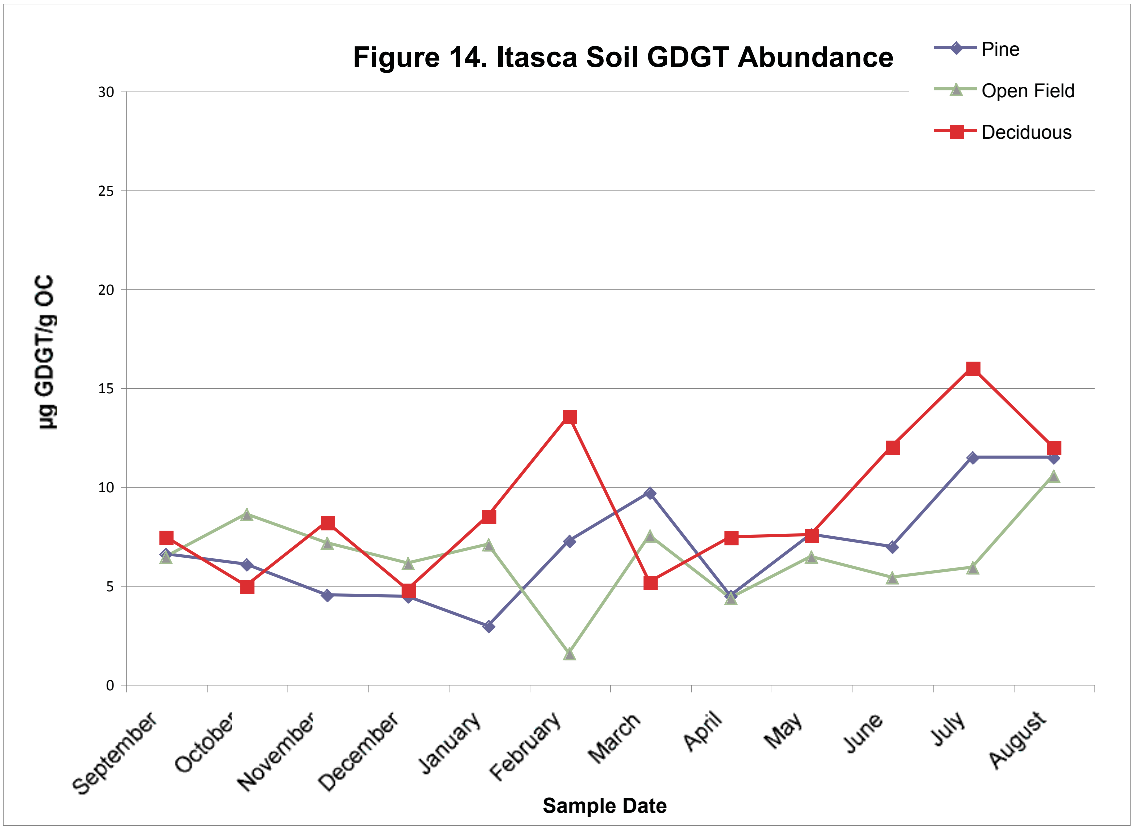

Figure 14: Mean monthly soil GDGT abundances per gram organic soil carbon, for three soil types in Minnesota. Pine, open field and deciduous soils are represented by blue diamonds, green triangles and red squares, respectively.

In Ohio, pine soils consistently had greater abundances of GDGTs than other vegetation types, usually by an order of magnitude (Figure 15). As in Minnesota, there was no clear seasonality to the pattern of GDGT abundances. GDGT concentrations in Ohio soils ranged from 1.3 to 55.8 µg GDGT/g OC.

GDGT concentrations in Florida soils ranged from 12.5 to 21.3 µg GDGTs/g OC. (Figure 16). October samples had a greater abundance of GDGTs than April. However, with only two sampling dates, the significance of the variability cannot be constrained.

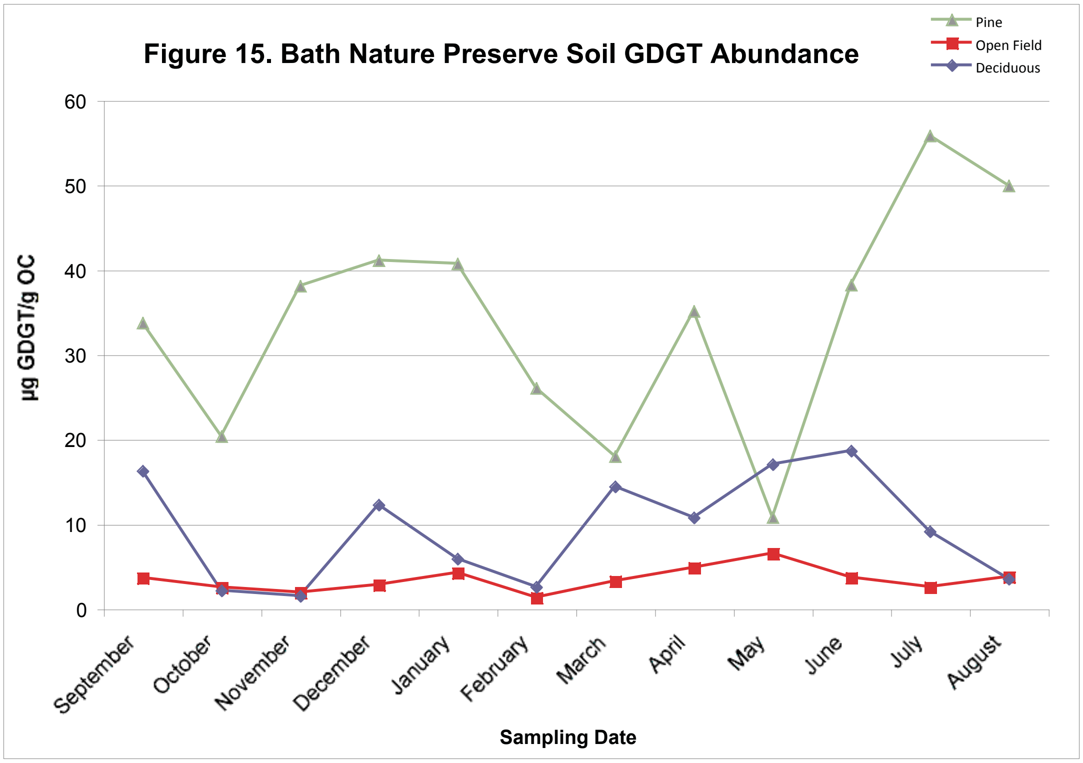

Figure 15: Mean monthly soil GDGT abundances per gram organic soil carbon, for three soil types in Ohio. Pine, open field and deciduous soils are represented by green triangles, red squares and blue diamonds, respectively.

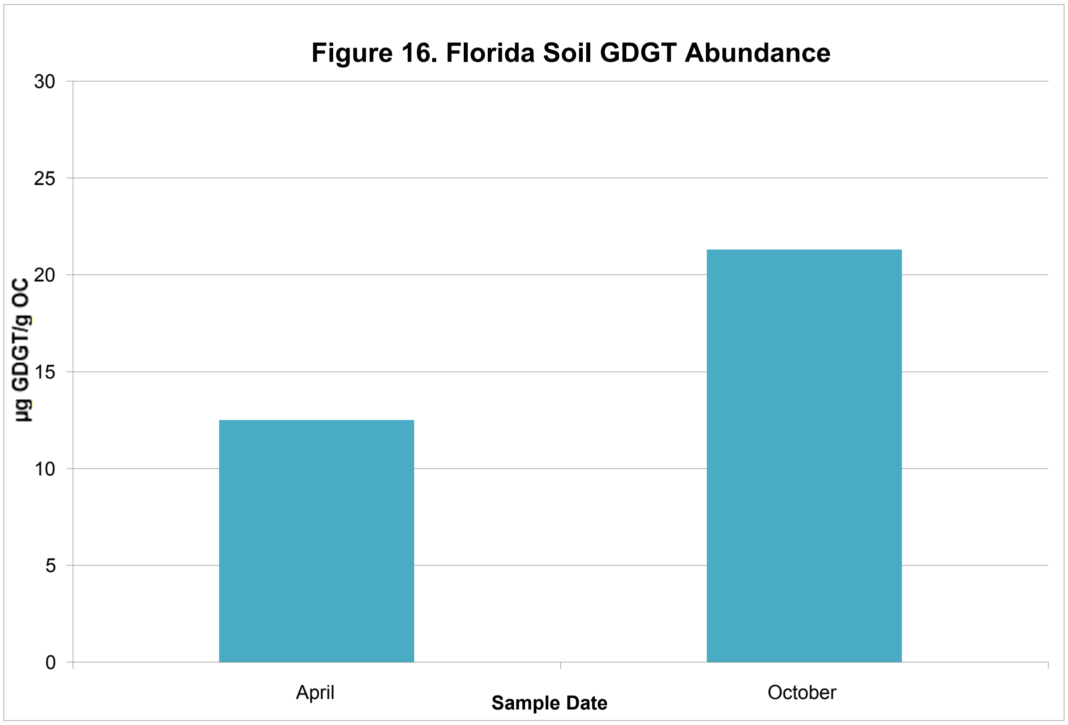

Figure 16: Mean GDGT abundance per gram organic soil carbon for April and October Florida soils.

Soil Organic Carbon

Similar to total GDGT abundances, there appears to be no clear pattern to bulk soil organic carbon concentrations among Minnesota soil plots (Figure 17). Values range from 2.8% to 9.2%. No vegetation type has consistently higher percentages of soil organic carbon, and values are relatively unchanged over the year except in a few samples.

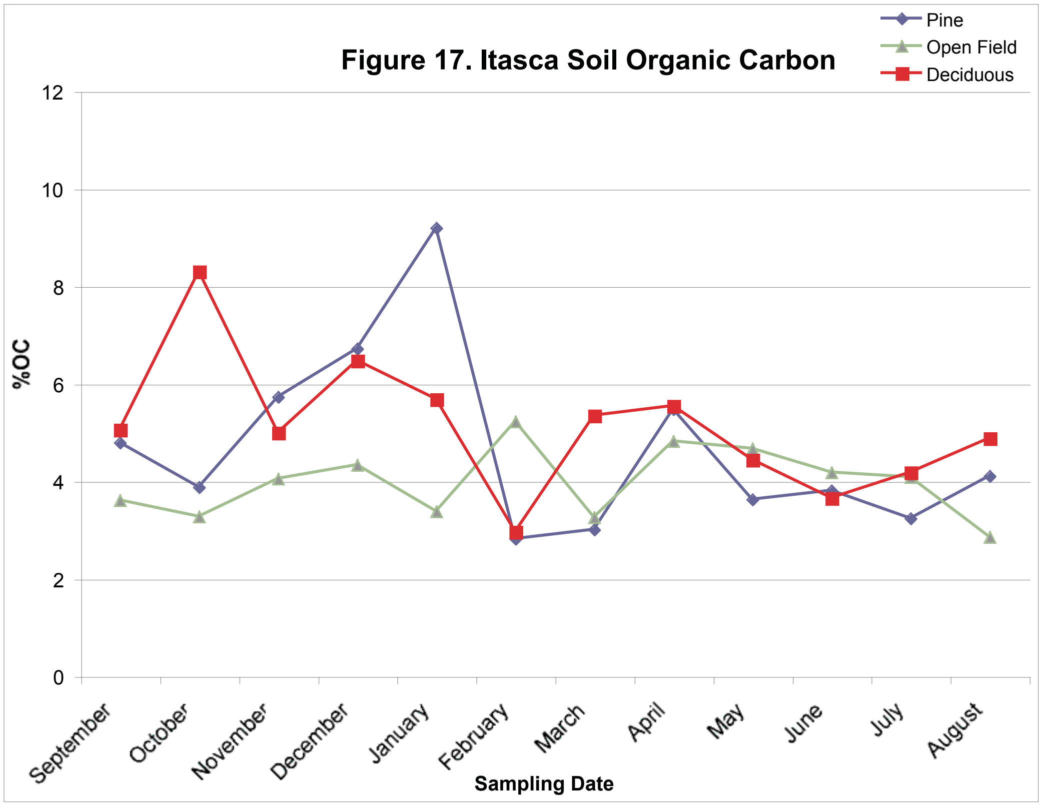

Figure 17: Monthly percent organic soil carbon for three vegetation types in Minnesota. Pine, open field and deciduous soils are represented by blue diamonds, green triangles and red squares, respectively.

In Ohio soils, pine plots had the highest percent organic carbon in all months but one (Figure 18). Open field soils had the next highest percent organic carbon over deciduous soils in eleven of twelve months of sampling. In Ohio, the high percent organic carbon in pine soils is mirrored by high GDGT abundances in these samples. However, despite the greater organic carbon content of open field soils over deciduous soils, deciduous soils generally had greater GDGT concentrations, suggesting that organic carbon is not the only control on GDGT abundances in soils. At this site, percent organic carbon ranged from 2.3 to 10.2.

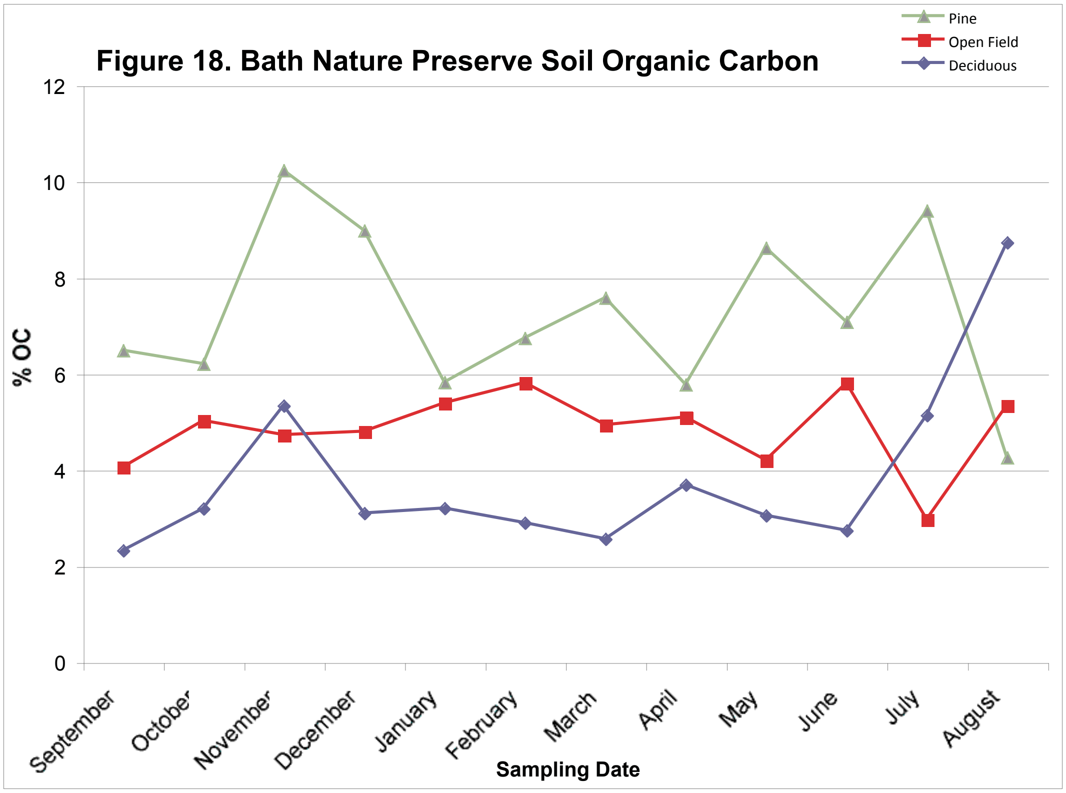

Figure 18: Monthly percent organic soil carbon for three vegetation types in Ohio. Pine, open field and deciduous soils are represented by green triangles, red squares and blue diamonds, respectively.

In Florida, April soils were 3.2% organic carbon and October soils were 1.0% organic carbon. Despite the difference in organic carbon content, October soils had higher GDGT concentrations than April samples (Figure 19).

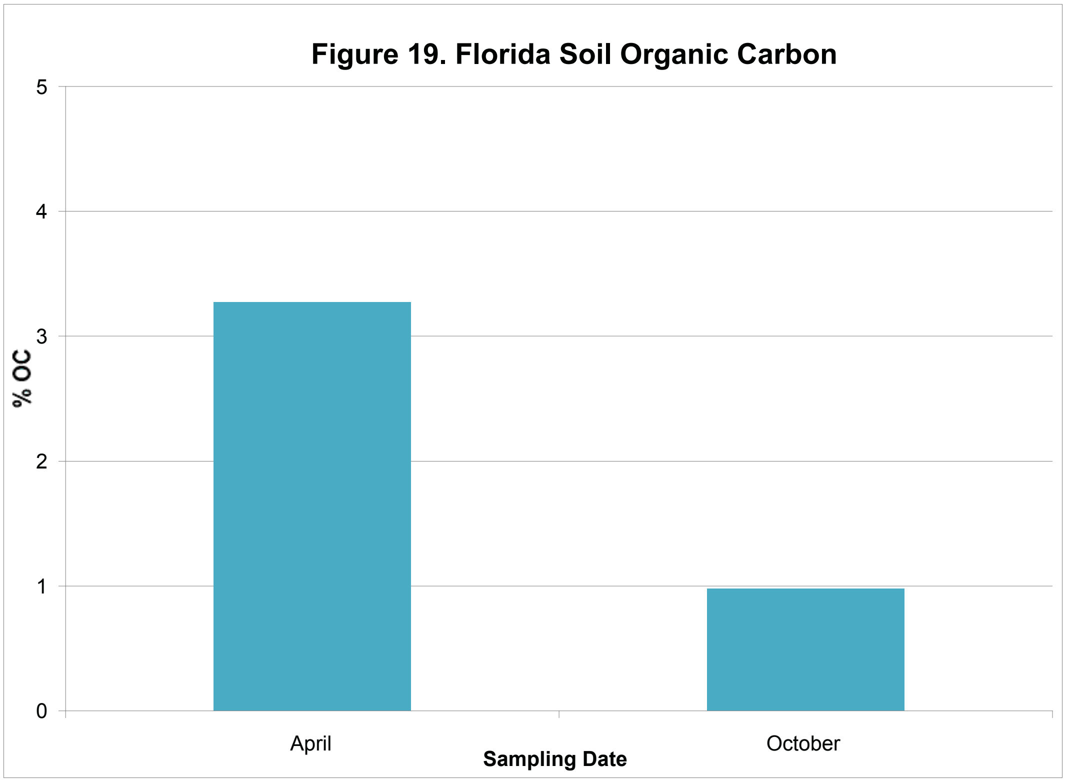

Figure 19: Percent organic soil carbon for April and October Florida soils.

To verify if there is any association between soil organic carbon and GDGT concentrations, the two parameters were plotted against each other for each soil sample analyzed. A trend line and r^2 value is displayed for each data set: Minnesota, Ohio and all data combined. Alone, Ohio soil samples show some correlation and the regression equation has an r^2 of 0.48 (Figure 20). Minnesota data shows no correlation ($r^2 = 0.006$) and the Florida data set has only two points. A regression line fitted to all data points, combined from three sites, had an r^2 value of 0.35 (Figure 21). There appears to be a slight positive correlation between GDGT abundances and soil organic carbon content, but the relationship is likely also dependent on other environmental parameters that affect bacterial abundance and growth rates, such as pH, soil moisture and nutrient availability.

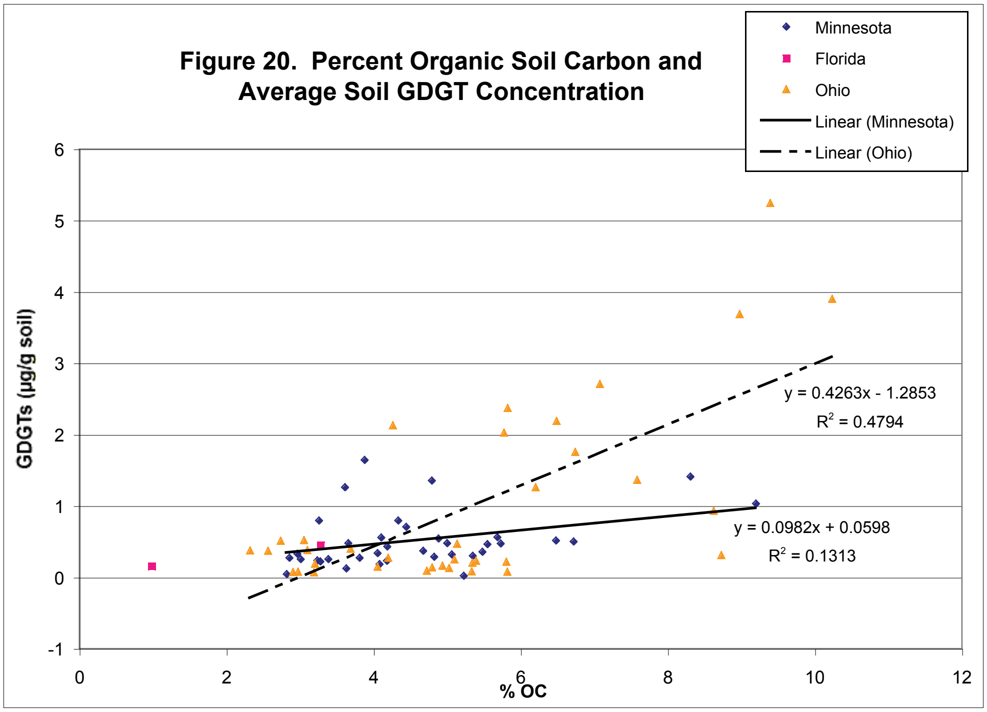

Figure 20: Average soil GDGT concentration, per gram soil, plotted against percent organic soil carbon for Minnesota, Ohio and Florida data sets. Minnesota, Ohio and Florida soils are represented by blue diamonds, yellow triangles and pink squares, respectively. The regression line for the Ohio data set is dashed and the regression line for Minnesota data set is solid. The Florida data set consists of two points.

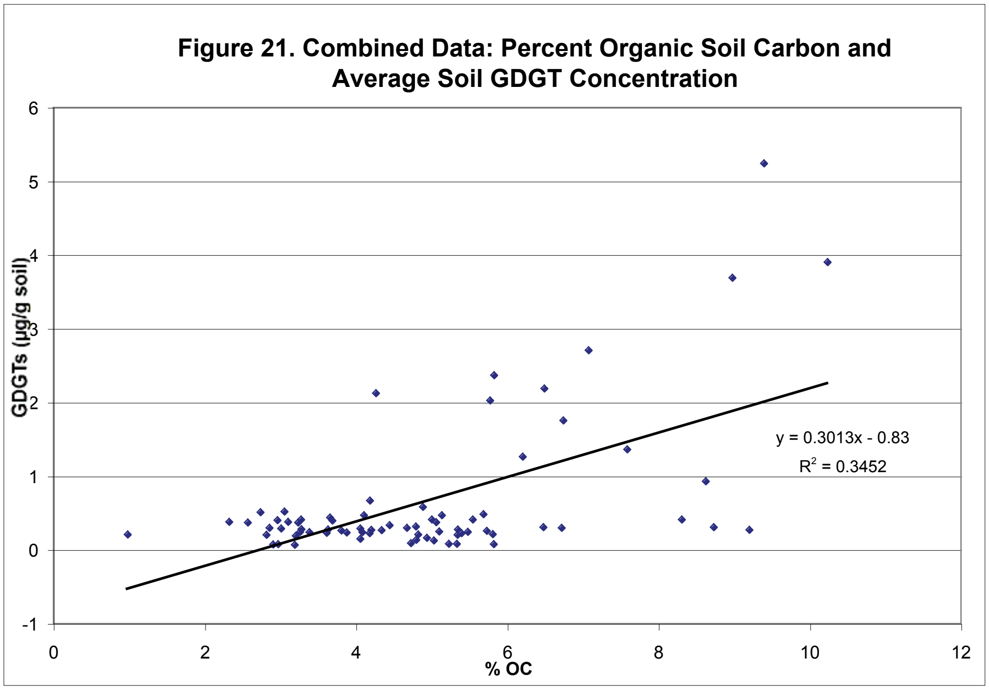

Figure 21: Average soil GDGT concentration, per gram soil, plotted against percent organic soil carbon for all data sets combined.

To investigate a possible relationship between soil GDGT concentration and pH, the two parameters were plotted against each other (Figure 22). The highest GDGT abundances were indeed found on the lower end of the pH range of all soil samples. However, it is worth noting that all of samples with very high GDGT concentrations are from the Ohio pine plot, and low GDGT abundances are also found in soil samples with low pH.

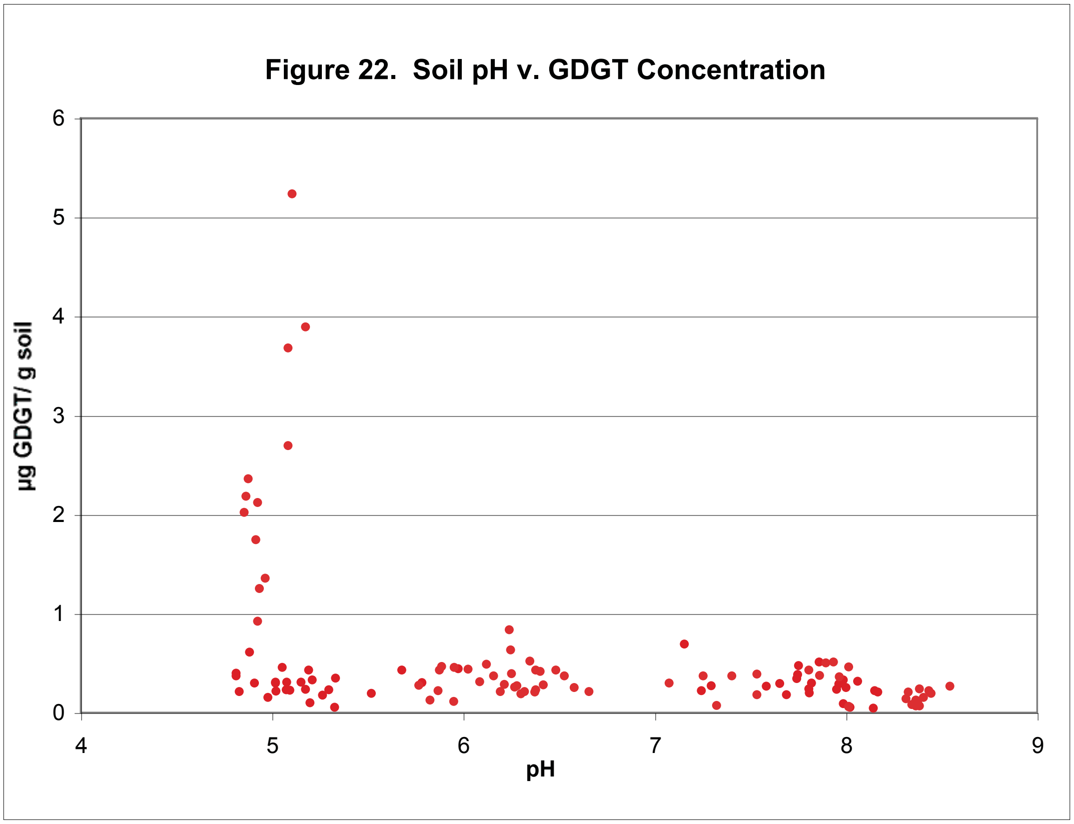

Figure 22: Soil pH plotted against GDGT concentration, per gram soil, from all study sites. The highest concentrations of soil GDGTs at low pH are from the Ohio pine plot.

Sediment Data

Surface Sediments

Surface sediments from Elk Lake, Minnesota were collected and analyzed for branched GDGTs in addition to the other 3 lakes in this study. A comparison of the surface sediment MBT-estimated annual MAT and the instrumental annual MAT of the year that each core was collected, according to National Climate Data Center records, shows that, in every system sampled, the instrumental annual MAT was warmer (NCDC(a)). However, MBT temperature estimates can still be considered the same as instrumental measurements within the standard error of the proxy estimate (Figure 23). These findings are similar to the results of other studies that have found cooler temperatures recorded in near-surface sediments than deeper in the sediment profile or in corresponding watershed soils (Sinninghe Damsté et al. 2009, Tierney and Russel 2009, Blaga et al. 2010). It is thought that *in situ* production of branched GDGTs may be the cause of the cooler, near-surface temperatures. Future work may benefit from a comparison of mean annual hypolimnion water temperature with the MBT-derived temperatures of surface sediments.

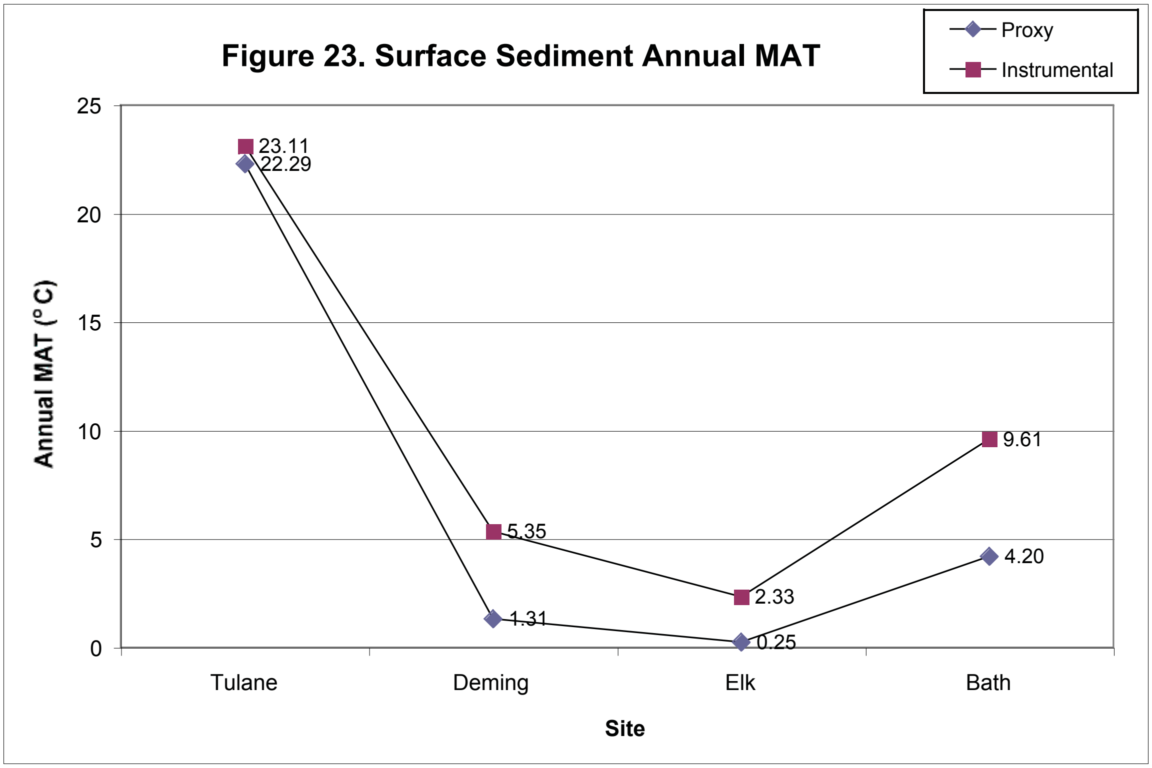

Figure 23: MBT estimate annual MAT and instrumentally measured annual MAT from the surface sediments of four study lakes. Instrumental averages from historical NCDC records for the year of core collection are represented by red squares. Proxy estimates of annual MAT are represented by blue diamonds.

MBT v. Instrumental Temperature Record

In Deming Lake, MN, historical instrumentally measured temperatures obtained from the NCDC, the University of Minnesota Lake Itasca Biological Field Station and Fort Snelling meteorological records, indicate a slight warming trend from 1820 to 2008 AD (Figure 24) (Baker et al. 1985). The MBT constructed temperature record, reaching back to ca. 980 AD, contains few points that are in range of instrumental measurements. Within the last two centuries, the MBT record is variable and seems to indicate cooling in the two most recent sediment samples (Figure 25). Throughout the sediment profile the MBT constructed annual MAT values are effectively the same as the instrumentally recorded temperatures within the range of error inherent to the proxy. Although there are very few data points available, they do not appear to reflect any short term variations in annual MAT.

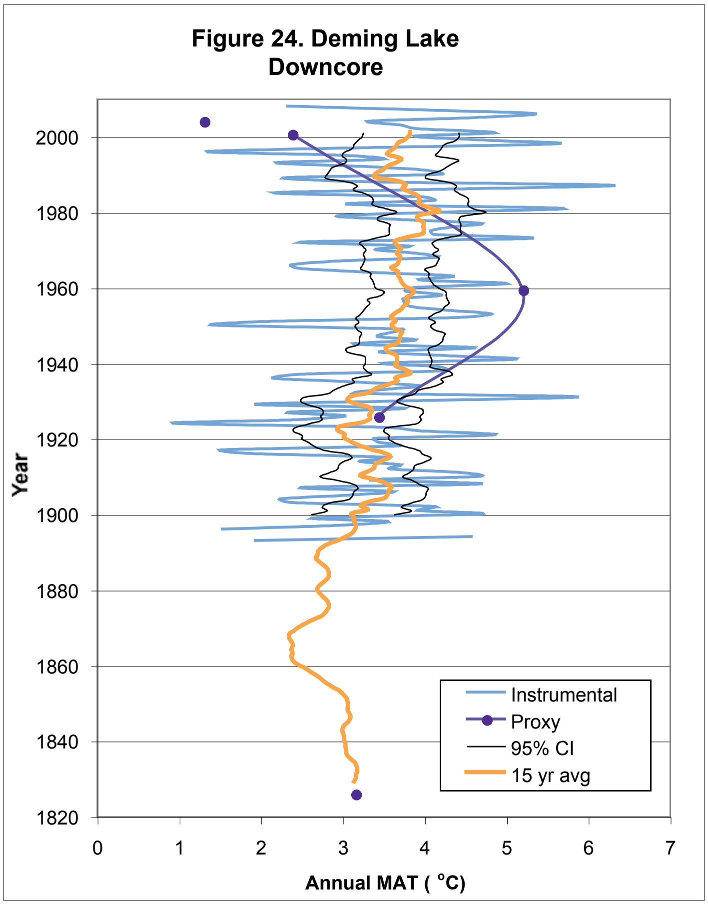

Figure 24: Proxy-derived and instrumentally measured annual MAT for the last ~190 years at Deming Lake, Minnesota. Instrumental annual MAT, shown by a solid blue line, from 1893 to the present is from Itasca State Park. The 15 year moving average of annual MAT is represented by a solid yellow line. Instrumental temperature data from before 1893 is adapted from annual MAT as measured at Fort Snelling, Minnesota and corrected to reflect temperatures in northwestern Minnesota. The 95% confidence interval, shown by thin black lines, is calculated based on the 15 year moving average of annual MAT and reflects the range of temperatures that 95% of all 15 year temperature averages fall within, generally ±1°C. MBT estimates of annual MAT from sediment samples are represented by purple dots.

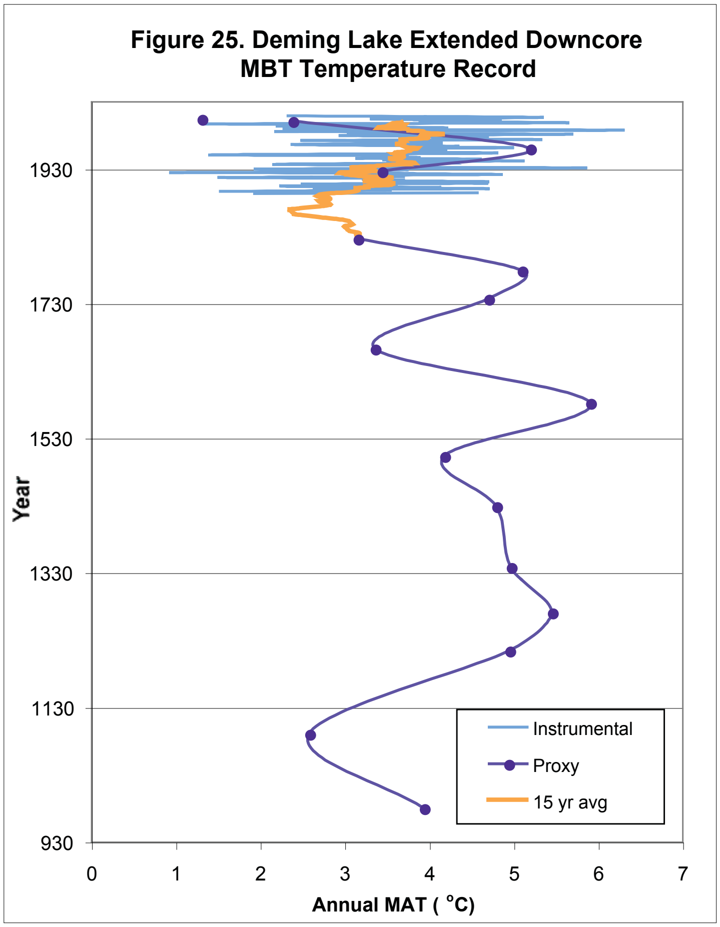

Figure 25: Extended MBT temperature record for Deming Lake, Minnesota. Instrumental annual MAT from 1893 to the present is from Itasca State Park. The 15 year moving average of annual MAT is represented by a solid yellow line. Instrumental temperature data from before 1893 is adapted from annual MAT as measured at Fort Snelling, Minnesota and corrected to reflect temperatures in northwestern Minnesota. MBT estimates of annual MAT from sediment samples are represented by purple dots.

The sedimentary and instrumental temperature records at Bath Pond, Ohio go back to the late 1800's (Figure 26). The instrumental temperature record, consisting of NCDC data, shows long-term averages of annual MAT of ca. 10^{o}C and no visible warming or cooling trend over the past 120 years (NCDC(a)). The sedimentary MBT temperature record shows apparent cooling in near-surface sediments, with the most recent sample recording an annual MAT that differs significantly from instrumental measurements. The rest of the sedimentary MBT record is variable but estimates of annual MAT are in the range of the instrumental measurements and, within the error of the MBT, the temperatures can be considered the same. The data seem to indicate that sedimentary GDGT assemblages reflect long term temperature averages, and variation in MBT estimated annual MAT over short time scales, e.g. 1-5 years, is not reflective of actual changes in annual MAT over similar time periods in Bath Pond.

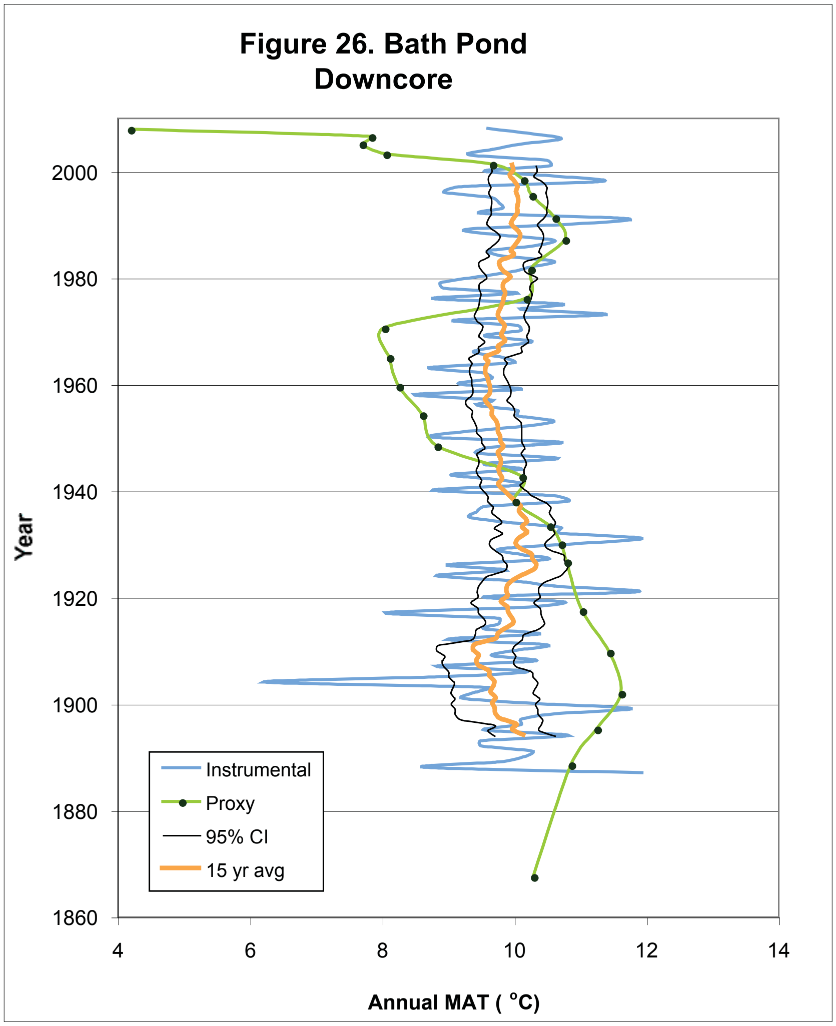

Figure 26: Proxy-derived and instrumentally measured annual MAT for the last ~120 years at Bath Pond, Ohio. Instrumental annual MAT, represented by a solid blue line, is from the NCDC, as measured in nearby Akron, Ohio. The 15 year moving average of annual MAT is represented by a solid yellow line. The 95% confidence interval, shown by thin black lines, is calculated based on 15 year averages of annual MAT and reflects the range of temperatures that 95% of all 15 year temperature averages fall within, generally ±0.5^{o}C. MBT estimates of annual MAT from sediment samples are represented by green dots.

In Lake Tulane, Florida the sediment record dates back approximately 300 years (Figure 27). The instrumental record, which is compiled from NCDC records and includes ~100 years of temperature data, shows a slight warming trend of approximately 1^{o}C over the past 50 years. The sedimentary GDGT record, on the other hand, displays a cooling trend during the same time period. The apparent cooling seen in the Lake Tulane sediments is rather prolonged in comparison with other sites in this study. Before 1950, the MBT temperature record is more stable and consistently indicates an annual MAT of ~25 °C that reaches as far back as the year 1700. Despite the two apparent phases of MBT-MAT in the Lake Tulane sediment profile, all reconstructed MBT temperatures are similar to the corresponding instrumental record within standard error of the estimate.

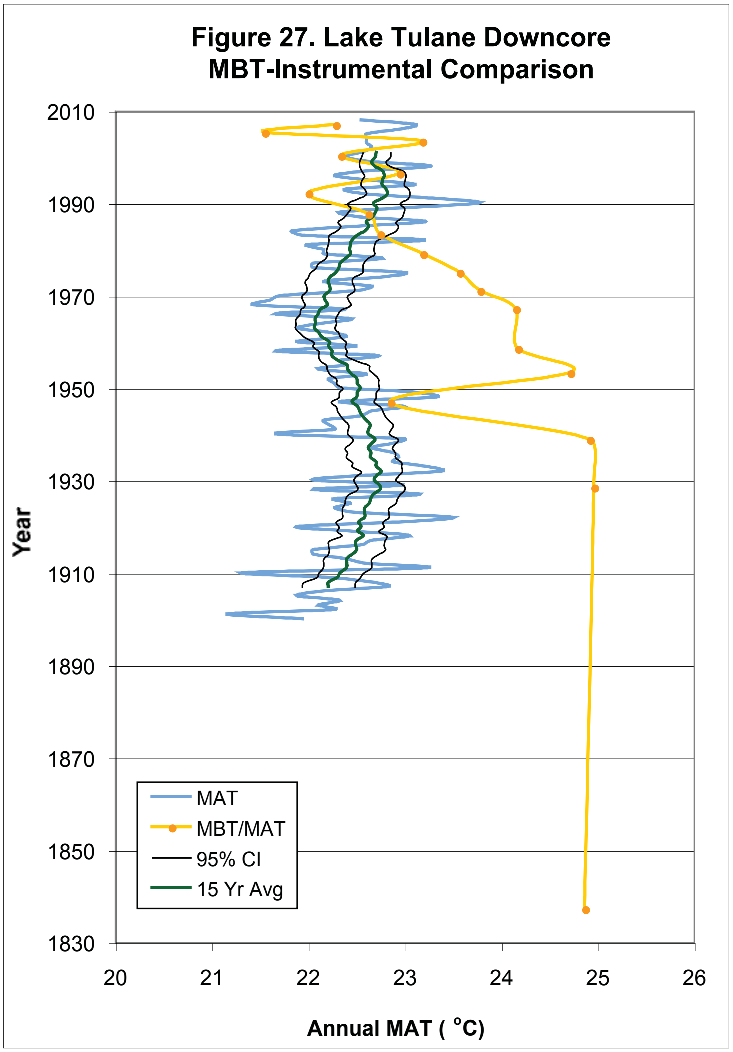

Figure 27: Proxy-derived and instrumentally measured annual MAT for the last ~110 years at Lake Tulane, Florida. Instrumental annual MAT, represented by a solid blue line, is from the NCDC, as measured in Avon Park and Orlando, Florida. The 15 year moving average of annual MAT is represented by a solid green line. The 95% confidence interval, shown by thin black lines, is calculated based on 15 year averages of annual MAT and reflects the range of temperatures that 95% of all 15 year temperature averages fall within, generally ±0.25°C. MBT estimates of annual MAT from sediment samples are represented by yellow dots.

GDGT abundances in the sediment profile

The abundance of GDGTs per gram of sediment in Deming Lake is variable throughout the record, ranging from 2.8 to 33.8 µg/g (Figure 28). In Bath Pond GDGT abundances range from 3.2 µg /g sediment to 2.1×10^3 µg/g sediment (Figure 29). Interestingly, there is a clear pattern in Bath Pond of very low sedimentary GDGT concentrations before 1950, and very high concentrations in more recent years. In Lake Tulane the concentration of sedimentary GDGTs ranges from 2.6 μg/g sediment to 4.4 μg/g sediment. Concentrations appear to be more variable over the last ~50 years and more stable in earlier sediments, though this observed pattern could be an artifact of a higher sampling resolution in more recent sediments than in older sediments (Figure 30).

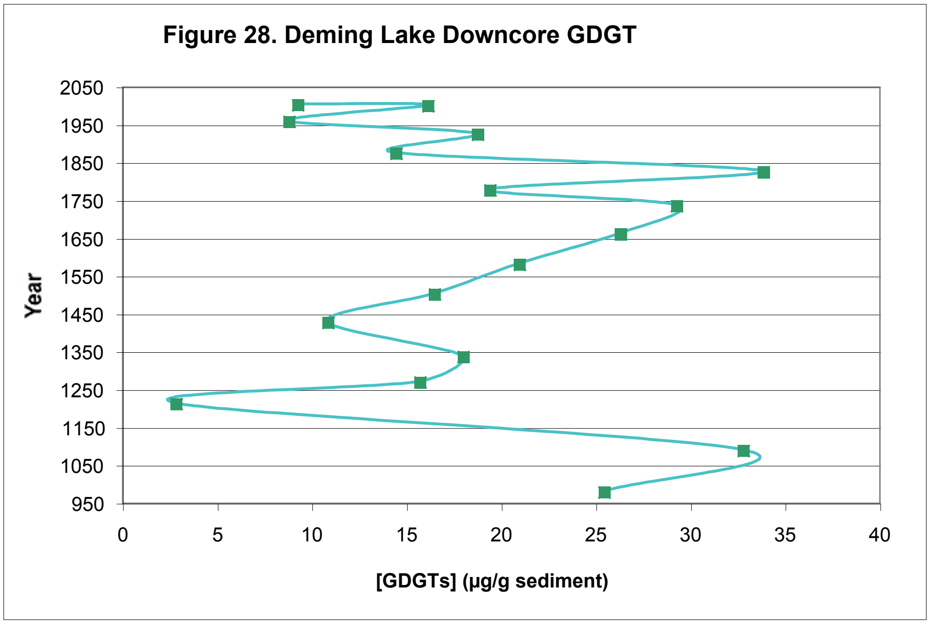

Figure 28: Downcore abundance of branched GDGTs, per gram sediment, in Deming Lake, Minnesota.

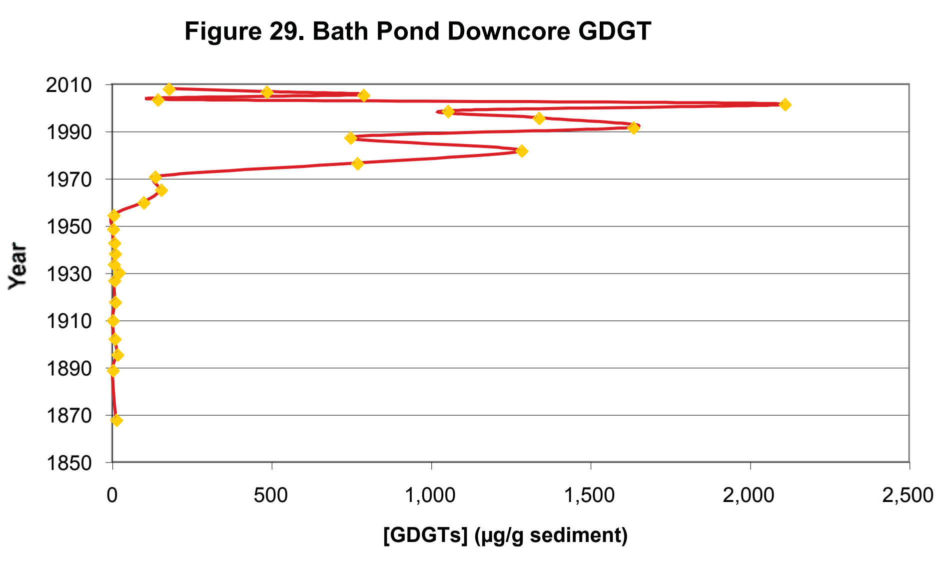

Figure 29: Downcore abundance of branched GDGTs, per gram sediment, in Bath Pond, Ohio.

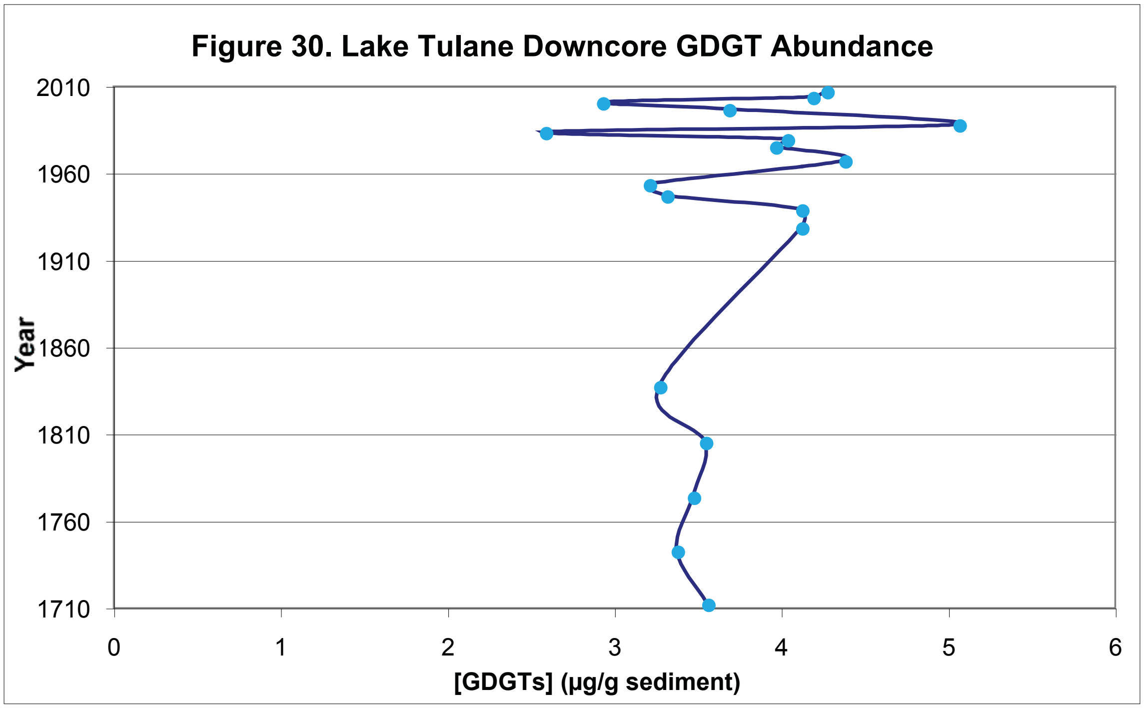

Figure 30: Downcore abundance of branched GDGTs, per gram sediment, in Lake Tulane, Florida.

Discussion - Branched GDGTs in Soils

Soil Thermistor Data

Measured soil temperatures are more stable over the course of a year than measured air temperatures in Minnesota and Ohio. This is due to the natural insulating capacity of the soil. Between sub-environments, the open field plot in Minnesota experienced warmer soil temperatures than the pine plot. These differences may be due to less dense canopy cover and therefore higher incident solar radiation in open field environments. In the spring, desynchronized snowmelt in open versus shaded areas is likely responsible for open field soil temperatures that are up to 10^{o}C warmer that pine soils at that same time.

In Ohio, the mean annual soil temperature was similar in the open field and pine plots. However, the open field plot did experience a greater amplitude of variation than the pine plot (Figure 7.a). Again, this is likely due to greater sun exposure and less canopy insulation in the open field environment than in the pine plot. In the deciduous plot in Ohio the thermistor could not be recovered and in Minnesota the thermistor did not record any data over the course of the year. The soil temperatures would be expected to be more similar to those of pine environments than open field because of the density of deciduous vegetation.

A comparison of measured soil temperatures with the MBT-derived annual MAT shows no response of the soil GDGT assemblage to seasonal changes in soil and air temperature. This is true for both vegetation types in Minnesota and Ohio. (Figures 6.b., 6.c., 7.b., and 7.c.).

CBT estimates of Soil pH

To verify the accuracy of the CBT proxy, electrode measurements of soil pH were compared with CBT-constructed values. The CBT proxy provided an accurate estimate of pH in pine and open field soils in Minnesota and in pine soils in Ohio (fig. 8, 9). Measurements of Florida soil pH were also similar to CBT estimates within the range of proxy error (fig. 10). The CBT proxy yielded higher pH values than those measured in Minnesota deciduous soils and Ohio deciduous and open field soils. The combined analytical and standard error of the proxy estimate is ± 0.88 pH units, but it does not account for the difference between measured and proxy pH in these plots. The CBT equation is a logarithmic representation of the exponential relationship between soil pH and the cyclization ratio (Weijers et al. 2007a). Both correlations between CBT and soil pH and cyclization ratio and soil pH have similar accuracy (r^2=0.70), but a cross plot of pH and cyclization ratio from Weijers et al. (2007a) clearly shows a greater degree of scatter in the relationship when soil pH is greater than 6. The inaccuracy of the CBT proxy in the deciduous soils and Ohio open field soil in this study may be attributed to their relative alkalinity, with soil pH ranging from 6.1 to 7.4, and the declining strength of the correlation between pH and cyclization ratio as pH increases.

The degree of cyclization, which refers to the inclusion of cyclopentyl moieties into the lipid membrane structure, is thought to be an adaptation to the pH of the growing environment (Weijers et al. 2007a). Maintenance of a proton gradient over the cell membrane is important for regular cell functioning. At higher pH, more abundant cyclopentyl groups loosen the packing of lipid membranes and allow entrapment of water molecules that allow for transport of protons across the cell wall. At low pH the membrane needs to be more impermeable for the cell to survive and there are therefore fewer cyclopentyl groups included in the lipid membrane. It appears that the cyclization ratio is less sensitive at the more alkaline end of the pH scale, perhaps because acid pH conditions are more stressful to bacterial cells than neutral or slightly alkaline conditions. Measured soil pH did not exceed 7.4 in this study and the most alkaline soils in the original calibration appear to have a pH of around 8.2 (Weijers et al. 2007a). An investigation of the cyclization ratio in more alkaline soils could provide important information on the sensitivity and accuracy of pH reconstructions using the CBT proxy.

An incorrect CBT estimate of pH has implications for the accuracy of the MBT proxy as well. The MBT can be calculated in two ways, with an equation that uses the CBT number or an equation that uses the actual measured pH. If the MBT-CBT proxy is being used for the purpose of paleoclimate reconstruction, the CBT estimate of pH is all that will be available. A higher pH or CBT value will yield higher MBT temperatures, and vice versa. In this study, the MBT proxy, using a CBT estimate of pH that is greater than the actual measured pH, significantly overestimates mean annual air temperatures in deciduous soils in Ohio and Minnesota by 9.3°C and 10.2°C, respectively. When the annual MAT is calculated using measured pH, the temperatures are more similar to the instrumentally measured value (fig. 11.a, 12.a).

MBT Estimates of Annual MAT – Effects of Seasonality and Sub-Environment (Seasonality)

Using the measured concentrations of branched GDGTs, the MBT-derived annual MAT was calculated for all soils using CBT-pH and measured pH values. Using both calculation methods the average MBT-derived temperatures for the Minnesota soils are significantly warmer than the annual MAT of 3.8°C but not as different from the annual mean soil temperature of 8.2°C in open field soils and 9.7^{o}C in pine soils. The large difference between annual MAT and annual mean soil temperature in Minnesota is due to the insulating capacity of the soil and snow cover during the winter. Thermistor data from our study show that soil temperatures at 15 cm of depth rarely dip below the freezing point even when the air temperature is much colder (fig. 6.a, 7.a). In Ohio, annual MAT and annual mean soil temperatures are within 0.5^{o}C of each other. Proxy-derived temperatures are similar to mean air and soil temperatures using both calculation methods and are generally within the range of proxy error.

The fact that MBT-estimated annual MAT is usually warmer than instrumentally measured values suggests that the microbes producing branched GDGTs in the soil are not growing throughout the entire year. Additionally, a comparison of MBT-constructed annual MAT with warmer mean growing season air temperatures of 14^{o}C in Minnesota and 16.5^{o}C in Ohio, as calculated from thermistor measurements, indicate that GDGT-producers are active for a greater part of the year than just the summer months. Growing season dates for Minnesota and Ohio were obtained from NCDC long-term climate records (NCDC(b)). It is likely that GDGT-producers can continue to grow and reproduce even when the soil and air temperatures are very cool and vascular plants have senesced or gone dormant. According to our soil thermistor data, in Minnesota in 2009, soil temperatures were consistently above freezing as early as March 21st in the Pine plot and as late as December 4th.

While it is likely that GDGT-producing microbes are growing at a variety of different temperatures throughout an annual cycle, there is no observable change in soil assemblages of branched GDGTs in response to seasonal variation in temperature. A possible explanation for the absence of a seasonal trend is that GDGT producers do not adapt to changing temperatures over the course of the year. This is unlikely; rather, it is more probable that branched GDGTs, considered to be ‘fossil’ lipids, do not accurately reflect seasonal changes in temperature but, instead, a long term average. GDGT lipids are initially bound to functional head groups that together form intact polar lipids (IPLs) (Weijers et al. 2009a, Liu et al. 2010).   IPLs quickly lose the functional head group during decomposition and are degraded to a core lipid (CL) upon cell death. Since IPLs are understood to be derived from live biomass, they are therefore assumed to better reflect small, short-term changes in growth temperature, while CLs represent past environmental conditions.

In this study we analyzed the ‘fossil’ soil GDGT pool, comprised of bacterial membrane lipids that have accumulated over long periods of time. While new production should hypothetically better reflect current environmental pH and temperature conditions, recently produced lipids will merely be incorporated into a much greater standing stock of fossil GDGTs. The CBT and MBT indices therefore represent long-term average environmental conditions in a particular micro-environment and do not reflect the small, short-term seasonal changes in temperature actually experienced by the microorganisms producing the branched GDGT compounds. Indeed, recent work by Weijers et al. (2010) has indicated that, over longer periods of time, the branched GDGT assemblage evolves to reflect new environmental conditions. The study used stable carbon isotope labeled branched alkanes, released from branched GDGTs, to define a turnover time of nearly 20 years in the soil. Another study by Peterse et al. (2010) showed that the branched GDGT assemblage in a grassland soil had fully adjusted to an experimental change in pH after 40 years, further supporting the idea of long-term GDGT accumulation in a soil.

These two studies have shown that, over decades, the standing stock of branched GDGTs will turn over and adjust to reflect new environmental conditions. The results of our seasonal survey of soil GDGT assemblages support these findings and clearly show that soil branched GDGT distributions do not reflect changes in growth temperature on time scales of less than one year. The turnover time of ~17 years, defined by Weijers et al. (2010), suggests that the soil MBT-estimate of annual MAT is a time-integrated signal comprised of nearly two decades of branched GDGT accumulation. Small differences between proxy estimates of annual MAT from month to month can likely be accounted for by natural soil heterogeneity, analytical error, and error inherent in the proxy calibration.

The turnover time of branched GDGTs in the soil has implications for the sensitivity of the proxy when applied to lacustrine sediment archives. Based on the suggested ~17 year time frame, an averaged MBT-CBT temperature signal means that the temporal resolution of paleotemperature reconstructions using the proxy is limited. Furthermore, with regards to paleo-applications of the MBT-CBT proxy, a potential confounding factor is in-situ production of branched GDGTs that may contribute a signal of its own with a different turn-over time than that of soil GDGTs.

(Sub-Environment)

Another question currently barring reliable application of the MBT/CBT proxy to inland paleoclimate reconstruction concerns the effect of vegetation type on soil GDGT assemblages. In Minnesota, the measured mean annual soil temperature within a stand of Red Pine was 2^{o}C cooler than that of an open field environment dominated by grass and small shrubs. In Ohio, pine soils had significantly greater abundances of branched GDGTs than other vegetation types. If these differences are reflected in the MBT-CBT proxy, the dominant vegetation cover within a watershed could have an effect on the sedimentary GDGT signal, potentially misrepresenting the actual annual MAT.

Branched GDGT assemblages from different sub-environments consistently yielded different annual MAT values in both Minnesota and Ohio soils. In Minnesota, the mean annual soil temperature of the pine and open field plots, as measured by soil thermistors, were 6.0 and 8.2^{o}C, respectively. Using measured pH values, pine, open field and deciduous soil GDGTs yielded annual MAT estimates of 9.5^{o}C, 10.5^{o}C and 11.1^{o}C, respectively. However, despite the clear differences in calculated annual MAT among vegetation types, all values are within 5.5^{o}C, the standard error of the proxy estimate, of measured soil temperatures.

When the CBT estimate of pH was used to calculate annual MAT instead of measured pH, the deciduous annual MAT increased to 14^{o}C. This temperature estimate is significantly greater than measurements of annual MAT and mean annual soil temperatures. In other vegetation types, the CBT proxy provided an accurate estimate of pH and the alternate calculation changed the MBT very little. Using either calculation method, all three vegetation types yielded annual MAT values that were significantly higher than the instrumentally measured annual MAT of 3.8^{o}C (fig. 11.a, 11.b) but not as different from the measured mean annual soil temperatures. Considering this data, the sedimentary GDGT signal at this site might be expected to record annual MAT values that are significantly higher than the instrumentally measured annual MAT. Considering the significant difference between soil MBT-MAT and instrumental MAT from the Minnesota site, it may be useful to develop local calibrations of the MBT-CBT proxy using soil temperature instead of annual MAT. Weijers et al. (2007a) used annual MAT in the original global soil calibration but acknowledged that soil temperatures would yield a better correlation if the data was widely available. While global soil temperature data may not be readily available, regional calibrations of the MBT index with soil temperature would likely allow for more accurate temperature reconstructions using the MBT-CBT proxy.

In Ohio, the differences among sub-environments, with regard to MBT-constructed annual MAT, are more distinct using both calculation methods (fig. 12.a, 12.b). Using measured pH values, pine, open field and deciduous soils had annual MATs of 11.3°C, 7.5°C, and 14.5°C respectively. Instrumental measurements indicate that pine soils were slightly cooler, with a mean annual soil temperature of 10.0°C, than open field soils, which had a mean annual soil temperature of 10.4°C. Mean annual soil temperatures in both sub-environments were very similar to the instrumental annual MAT of 10.0°C. Taking account of the error inherent in the proxy, pine and deciduous soil GDGT-based estimates of annual MAT are significantly different from each other by 7.0°C. However, none of the vegetation types differ significantly from measurements of annual MAT or mean annual soil temperature.

When the CBT pH estimate was used to calculate the MBT, the spread between sub-environments was increased but the general trends were the same. The MBT-MAT of the deciduous plot is significantly warmer than open field and pine vegetation types as well as measured soil and air temperatures. Factors that may account for differences in soil temperature among sub-environments in regions with temperate climates, such as Minnesota and Ohio, include canopy cover, exposure to wind, soil water content, and snow cover throughout the winter. If all three vegetation types are contributing equally to the sedimentary GDGT assemblage at this site, reconstructed MBT-MAT from lake sediments may be expected to be slightly warmer than actual air temperatures. However, if one sub-environment is more favorable for the growth of GDGT-producing microbes, the sedimentary GDGT record could be significantly skewed towards the soil temperature of that particular sub-environment. In the case of the deciduous sub-environment, the preserved MBT signal would yield temperatures that are significantly warmer than instrumentally measured annual MAT. GDGT abundance data will aid in the clarification of this uncertainty.

In Florida, there was no comparison of sub-environment type (fig. 13). However, using both calculation methods, the MBT indicated warmer annual MAT values of 29.3^{o}C using measured pH and 28.8^{o}C using CBT-pH, than instrumental measurements of 22.8^{o}C. Both MBT estimates are significantly warmer than the measured annual MAT. It is likely that the MBT-MAT would be more similar to the mean annual soil temperature than annual MAT, as was observed at the Minnesota and Ohio sampling sites. As previously mentioned, soil thermistor data is lacking for this site. The warm MBT-MAT, relative to instrumental MAT may be attributed to the sandy composition of the soil at this site. The lake is situated in an urban environment, and much of its shoreline is mowed grass. This vegetation type is most similar to the open field environment at the northern sites and likely receives high amounts of incident solar radiation. Additionally, the soil in the Lake Tulane watershed is mostly sand and may get hotter than a similarly situated mineral soil would. If the warmer soil MBT-MAT is indeed reflected in Lake Tulane sediments, the historical sedimentary archive could be expected to record temperatures that are ~7°C warmer than actual air temperatures.

Comparing the three sampling sites over a climatic gradient, the instrumental annual MAT was significantly lower than the MBT-constructions in all sub-environments sampled in Minnesota, where the climate is coldest. Further south, in Ohio, where the instrumental annual MAT was greater than that of Minnesota by ~6°C, the MBT proxy provided better estimates of annual MAT in all vegetation types. Finally, in Florida, where the climate is subtropical, the MBT again yielded temperature estimates that were significantly warmer than the instrumentally measured annual MAT. This result in Florida is somewhat unexpected. The subtropical climate at this southernmost sampling site means that the amplitude of variation and the extremes in air temperature over daily and annual cycles are much less than at the other sites. Therefore, we would expect the soil MBT signal in Florida to provide the most accurate estimate of annual MAT of any site. The nearly 7°C difference between instrumental air temperature and soil MBT-MAT could possibly be due to the sandy composition of the soil and the mowed grass vegetation at that particular sampling site as previously discussed.

GDGT Abundance

The concentrations of GDGTs in the soil in different sub-environments may affect the MBT temperature signal preserved in corresponding lake sediments. Branched GDGTs were present in all soils sampled, and measured concentrations were generally similar to those of soils sampled in other studies (Weijers et al. 2006b, Peterse et al. 2009b). The greatest amounts of GDGTs were observed in Ohio pine soils where concentrations reached 55.8 µg/g soil OC. Conversely, the lowest GDGT concentration measured at any sampling site was 1.3 μg/g soil OC from the Ohio open field soil. Microbial community composition and abundance in the soil may be related to the availability of macro and micronutrients, soil moisture, soil pH and the temperature of the growing environment (McArthur 2006). While it is likely that some of these factors may change throughout an annual cycle, a seasonal trend in branched GDGT concentrations was not observable in any of the soils (figures 14, 15). This finding supports the idea that soil GDGT assemblages are the product of long-term accumulation.

We measured the organic carbon content of monthly soil samples from each site and plotted the percentages against GDGT concentration in an attempt to understand the differences in soil GDGT abundance among sites and vegetation types (fig. 21). Based on patterns of occurrence in a Swedish peat bog, GDGT producing microbes have been hypothesized to be anaerobic heterotrophs using soil organic matter as a carbon source (Weijers et al. 2006a). If this assumption is correct, GDGT-producing microbes should be more abundant in organic rich soils. The correlation between the two factors for the combined data set was minimal (r^2 = .321). When the data sets from each state were plotted separately percent organic carbon was better correlated (r^2 = .479) with GDGT concentration in Ohio soils (fig. 20). Minnesota soils indicated no relationship between soil organic carbon and GDGT abundance, and the data set for Florida consisted of two points.

The large amount of scatter among points in the Minnesota data set is also likely due to natural soil heterogeneity. In Ohio, pine soils had the greatest % organic carbon and also the highest abundances of GDGTs. Factors that control litter decay and, subsequently, soil organic carbon content include temperature, moisture and the chemical composition of the plant matter (Schlesinger, 1997). Pine litter has been shown to have a greater C:N ratio than deciduous leaves and is therefore slower to decompose (Aber and Mellilo, 2001). Pine soils in Ohio were also measured to be the cooler than other sub-environments, and increased temperature has been shown to have a positive effect on microbial activity (Edwards, 1975). It is likely that both temperature and litter composition could be contributing to greater soil organic carbon content, and possibly greater abundance of GDGT producers, in pine plots than in other vegetation types. It is worth noting, however, that slow rates of litter decay by microbial decomposers due to cool temperatures is the same as slow rates of microbial metabolism, growth and reproduction. The high concentrations of GDGTs in pine soils may suggest that soil organic carbon content is a stronger control on populations of GDGT producers than temperature, or that the differences in soil temperature among vegetation types are small enough that they do not affect rates of growth and reproduction of GDGT-producing microbes.

It is also worth noting that pine soils, of all three sub-environments, were the most acidic, with a pH of 4.8 in Ohio and 5.1 in Minnesota. In very low pH environments, below 4, organic matter decay may be inhibited by low bacterial abundances, which could further lead to greater concentrations of soil organic matter (Aber and Mellilo, 2001). However, it is unlikely that pH is controlling soil organic carbon at our sites. Weijers et al. (2007a) examined a total of 134 soil samples from 90 globally distributed sites and found that soils with a lower pH generally had higher concentrations of GDGTs. They suggest that GDGT-producing microbes prefer more acidic growth environments. Our results from Ohio also seem to support this finding, however the relationship may be complicated by organic carbon availability (figure 22). In Minnesota, pine soils did record some of the highest concentrations of branched GDGTs at that site, but this result was not consistent among all monthly samples. Organic carbon data for Minnesota soils were also variable.

The observed differences in soil temperatures and soil GDGT concentrations among different vegetation types indicate the possibility of a biased sedimentary record of annual MAT. For example, in Ohio, where pine soils have consistently greater GDGT abundances than other vegetation types, the corresponding Bath Pond sediments may yield a MBT-constructed annual MAT that is more representative of the pine sub-environment than other vegetation types. In Ohio, the MBT-MAT for the pine plot is the most similar to instrumental annual MAT. However, if, for example, deciduous GDGTs were more greatly represented in the sediments, the temperature reconstruction could be significantly inaccurate. The use of a local calibration and additional paleoclimate proxies, such as pollen grain or lignin analyses may help to elucidate what type of vegetation was dominant in a watershed at a given time in history, and how well the sedimentary MBT-constructed MAT is reflective of actual air temperatures at the time.

Branched GDGTs in Sediments

Surface Sediments

Theoretically, terrestrially produced GDGTs are transported to lacustrine sediments via erosion, surface runoff and river inputs each year. Another major question facing the development of the MBT proxy is whether sedimentary GDGTs record an accurate temperature signal in surface and downcore sediments, and whether the proxy is responsive to variation on short or long time scales. To investigate this question we compare the sedimentary MBT record with the available historical instrumental record.

In all lakes sampled, the surface sediments record MBT temperatures that are noticeably cooler than the instrumentally measured annual MAT at the time of sampling (figure 23). While surface sediment MBT temperatures are the same as instrumental annual MAT within the range of proxy error in all systems, there appears to be clear, up-core cooling trends in the most recent sediments in Ohio and Florida (figures 26, 27). In Ohio, the surface MBT-MAT is ~6°C cooler than temperature estimates from more ancient sediments, which is a statistically significant departure from the rest of the sedimentary MBT temperature record. This finding is similar to the results of other studies that have found cooler temperatures recorded in near-surface sediments than concurrent instrumental air temperature measurements (Tierney and Russell, 2009, Blaga et al. 2010). It has been suggested that *in situ* production of branched GDGTs may be the cause of the cooler, surface temperatures (Tierney and Russel, 2009, Sinninghe Damste et al. 2009, Peterse et al. 2009b, Blaga et al. 2010). Recent work in African lake sediments lends further support to the *in situ* production hypothesis and has shown surface sediment GDGT distributions to be better correlated with mean lake water temperature than annual MAT in tropical regions (Tierney et al. 2010). Future work in temperate systems may also benefit from a similar comparison of mean lake water temperature with the surface sediment MBT index and could also help to describe this new potential source of branched GDGT lipids.

However, these previously mentioned studies have only examined the MBT in surface sediments. Downcore application of the MBT, shown to be successful in the marine environment, has yet to be demonstrated in lacustrine systems (Weijers et al. 2007b). The hypothesized *in situ* production of branched GDGTS does not fully explain the shift to cooler temperatures in recent sediments. If *in situ* production is occurring in surface sediments, why is the resulting cool MBT signal not preserved in deeper sediments as well? A possible explanation for the significant shift from cool, surface to warm, downcore MBT temperatures in the Bath Pond sediment profile and others could be a diagenetic effect, such as preferential decomposition of *in situ* GDGT production. Alternatively, *in situ* production, which appears to dominate the surface sediment GDGT assemblage, could be diluted downcore by the subsequent incorporation and preservation of terrestrially produced branched GDGTs over time, resulting in a mixed-source downcore MBT signal. Additional data is needed to constrain the sources of sedimentary branched GDGTs so the accuracy of temperature reconstructions using the MBT can be known.

Deming Lake, MN

In Deming Lake, the instrumental record reaches back to 1822 AD (figure 24). A fifteen-year moving average of annual mean air temperature, chosen to reflect the suggested ~17 year turnover time of GDGTs in soils, shows a slight warming trend of ~1^{o}C in this lake (Weijers et al. 2010). Though there are only a few samples from this lake that fall within the same period of time as the instrumental data, the available MBT data is consistent with instrumental measurements within the error of the proxy. Near the top of the core there appears to be the same cooling trend that has been observed in surface sediments at other sites (Tierney and Russell, 2009, Blaga et al. 2010). Despite increasing instrumental temperatures, surface MBT values are the coolest recorded temperatures of the entire sediment profile, which dates back to 980 AD (figure 25). While the surface sediment MBT is not significantly different from the instrumental average at that same time, it seems to follow a general trend of cooling in the sediments that begins around 1960 AD. However, due to the small number of samples from this site, it is difficult to make any conclusions about the sensitivity of the proxy or the degree of surface cooling that may actually be taking place in Deming Lake.

In the interpretation of downcore MBT data and comparisons with instrumental measurements, the significance of the temperature differences and the standard error of the proxy estimate may be slightly misleading. As stated earlier, the standard error of the proxy estimate is 5.5°C. While this may seem like a considerable amount of error with regards to paleoclimate reconstructions, it is important to recognize the large degree of natural variability inherent in the systems we are studying. In Deming Lake, 95% confidence intervals calculated for a 15-year moving average of instrumentally measured annual MAT were up to ±0.7°C. Instrumentally measured mean annual soil temperatures in Minnesota varied by up to 2.2°C among vegetation types, but were also seen to differ by 10°C during the spring snowmelt. Finally, there is also a degree of analytical error that is associated with sample preparation and instrumental reproducibility which was determine to be ±0.8°C (Weijers et al. 2007a). Combining all of these sources, yields an estimated 3.7°C of natural variability alone. Another reason the standard error of the proxy estimate may seem large is that the calibration uses annual MAT as an alternative for annual mean soil temperatures. As observed from our thermistor data, mean soil temperatures could differ from mean air temperatures by up to 4.4°C. Considering the natural sources of error that have potential to influence the proxy, the MBT temperature reconstructions for all lakes surveyed for this study are actually quite good.

Bath Pond, OH

In Bath Pond instrumental measurements are available as far back as 1887 AD (figure 26). The record does not indicate a clear warming or cooling trend over the past ~120 years. All sediment samples, excepting the most recent, yield a MBT-MAT that is the same as the corresponding instrumental average within the standard error of the proxy estimate. As seen in Deming Lake, the surface sediments record the coolest MBT-MAT. In Bath Pond, the differences between the surface MAT value and those of the rest of the sediment profile are statistically significant. Recent work in Lake Challa, Tanzania and Lake Towuti, Indonesia has shown that branched GDGTs found in hypolimnetic waters and shallow sediments yield MBT-MAT values that are considerably cooler than actual annual MAT (Sinninghe Damsté et al. 2009, Tierney and Russel, 2009). It has been suggested that the incorporation of in-situ produced branched GDGTs from the water column and shallow sediments may be the cause of lower MBT temperatures in more recent sediments. The relatively cool surface sediment MBT signal from Bath Pond seems to lend further support to this hypothesis. However, the relatively steep decline in surface sediment MBT-annual MAT in Bath Pond could indicate a diagenetic effect.

At first glance, MBT-MAT in Bath Pond appears to be steadily decreasing from ca. 1900 to 1970. However, this trend in the MBT is not mirrored by the instrumental record and could be due to natural sampling variability and error inherent in the proxy. Alternatively, if in-situ GDGT production is a significant contribution to the overall sedimentary GDGT pool, aquatic in-situ produced GDGTs must also be a portion of older sediments. Perhaps, in more ancient sediments, relatively small shifts in MBT temperatures, while instrumental averages remain constant, could be the product of changes in the rate of delivery of terrestrial material to the lake sediments. In rainy years, more runoff from the land would result in a greater influx of terrestrial branched GDGTs to the system and a warmer sedimentary MBT value. In dry years, in-situ production might contribute more greatly to the sedimentary GDGT assemblage resulting in cooler MBT temperatures. Additional geochemical data, such as sedimentary n-alkanes, bulk isotopes and C:N ratios, could be used to determine the source of organic matter, i.e. terrestrial to aquatic ratios, in sediments, potentially providing support of this hypothesis (Killops and Killops, 1993). Preserved plant lignins in lacustrine sediments could also provide information about the dominant vegetation present in a watershed at a given time in history (Killops and Killops, 1993). As seen in Ohio deciduous soils, vegetation type can have a significant effect on the MBT-CBT proxy estimate of annual MAT. The response of soil GDGTs to vegetation type is likely to differ by location and local soil calibrations may be needed, in addition to sedimentary lignin data, to determine what type of environmental temperature biases could be recorded in the sedimentary MBT record.

Lake Tulane, FL

The instrumental temperature record in Florida dates back to 1900 AD (figure 27). There appears to be a slight warming trend over the past century of ~1^{o}C. The MBT record, on the other hand indicates cooling from ca. 1938 to the present. Before 1938 there are few data points, but they consistently record an annual MAT of ~25^{o}C. All sedimentary MBT values, including surface sediments, are similar to the instrumental averages within the standard error of the proxy estimate. Considering the downcore MBT profiles in Minnesota and Ohio that indicate cooling in near-surface sediments, a decrease in MBT-constructed temperature near the top of the core is expected. However, in Lake Tulane, the cooling trend comprises the majority of the MBT temperature record, and instead of being much cooler than the recent annual MAT, the MBT of the surface sediments is well-matched with instrumental measurements. It is possible that, in Florida, where the climate is subtropical and the annual MAT is 22.5^{o}C, the temperature of the growth environment of aquatic GDGT producers is similar to the air temperature. Indeed, recent work in tropical African lakes has shown that MBT temperatures from shallow sediments are more similar to measurements of lake water temperature than air temperature (Tierney et al. 2010).

Additionally, in the Florida soils analyzed in this study, the MBT temperatures were found to be significantly warmer than the instrumentally measured annual MAT. Perhaps, *in situ* produced branched GDGTs dominate the surface sediments resulting in a relatively cool MBT signal, and subsequent incorporation and preservation of terrestrially produced branched GDGTs over time results in a mixed-source downcore MBT signal. In sub-tropical Florida, this proposed mixing model might result in downcore MBT temperatures that are warmer than annual MAT but cooler than soil temperatures and a surface sediment MBT-MAT that is close to instrumental measurements of annual MAT. Additional data to help constrain the sources of organic matter, i.e. terrestrial versus aquatic, in the sediments would be helpful in understanding sedimentary GDGT distributions. The BIT index might be also useful to test this hypothesis as it provides information about the relative contributions of terrestrial and aquatic sourced GDGTs in the sediment. If the BIT index is low in surface sediments, the branched GDGTs at the same depth could also be from an aquatic source.

Sedimentary GDGT Abundance

GDGT abundance in lacustrine sediments is likely a function of the rate of terrestrial sediment delivery to the lake as well as rates of potential *in situ* productivity. *In situ* productivity in sub-surface sediments will be most strongly controlled by temperature, oxygen and nutrient availability (McArthur, 2006). Rates of delivery of terrestrial material to lake sediments are primarily a function of erosion and surface runoff, most of which occurs during the spring of the year. In Deming Lake sediments, GDGT abundance is variable (Figure 28). A notable historical event that could have potentially affected the delivery of terrestrial produced GDGTs to Deming Lake sediments was a period of intense logging that lasted from ca. 1880 to 1920 AD in the area that is now Itasca State Park (Rottsolk,1960). However, peaks in GDGT abundance in the sediment record do not seem to correspond with this time period. The highest concentration of GDGTs occurs in 1826, at least 50 years before any logging activity began.

Alternate explanations for peaks in sedimentary GDGTs might include local forest fires in the watershed or a period of particularly wet spring seasons with increased rainfall or snowmelt. Another smaller peak in GDGT concentration occurred in 1925, not too long after the cessation of logging operations in Northwestern Minnesota and, in particular, Itasca State Park. It is possible that elevated GDGT concentrations at this point in history could be attributed to a relatively denuded landscape and correspondingly high remnant rates of terrestrial sediment delivery to the lake bottom, perhaps paired with some type of extreme weather event. It is also worth noting that in the Deming core, there are relatively few samples for the extensive time period covered. A higher sampling frequency, focused more heavily on recent history, would allow comparison with recorded historical events and help elucidate factors that may be controlling concentrations of branched GDGTs in sediments.

The GDGT profile in the sediments of Bath Pond is unlike that of the other lakes sampled in this study (Figure 29). GDGT concentrations are relatively low and constant from ca. 1867 to 1948 AD. During the next 50 years, concentrations increase by 1 to 3 orders of magnitude. It is possible that changes in land use within a watershed could substantially increase the delivery of terrestrial branched GDGTs to lake sediments. Alternately, extreme precipitation events could increase the GDGT load to a system through increased surface runoff and erosion. Supplemental geochemical and proxy data, such as n-alkanes, C:N ratios, stable isotopes and plant lignins, could help to explain the patterns of branched GDGT abundance in Bath Pond Sediments. In Ohio, pine soils had significantly greater concentrations of branched GDGTs than other vegetation types. The presence and concentration of gymnosperm-specific lignins in the sediments could be informative about the source of organic matter delivery to the sediments of Bath Pond over time.

Another possible explanation for patterns of sedimentary GDGT abundance is changing *in situ* production of branched GDGTs, likely caused by changes in the trophic status of the water body. In the case of increased *in situ* production, the sedimentary MBT signal could be expected to reflect the cooler growth environment of water column or sub-surface dwelling GDGT producers. The MBT record in Bath Pond does not show cooling, however, except in the most recent sediments (figure 29). Eutrophication would likely be the product of changes in land use in the watershed as well. Fertilizer from urban lawns and agricultural fields in surface runoff is a common source of limiting nutrients to nearby water bodies. Considering the multiple side effects of deforestation, agriculture, and suburban development, it is likely that changes in land use such as these could result in both increased in-situ production of branched GDGTs as well as increased delivery of terrestrially produced GDGTs to the sediment from the land.

However, the Bath Pond watershed is quite small, extending approximately two hundred meters beyond the perimeter of the pond itself. Situated in a nature preserve and at the high point its drainage, the pond experiences minimal human impact (Kemp, 2010). From the early 1940’s up until 1997, Bath Pond and the surrounding 411 acres that is now Bath Nature Preserve, was part of the Firestone Family Estate (Ohio Historical Society). Prior to 1940, the land around Bath Pond is thought to have been agricultural, kept mostly as hayfields and pasture (Michael Rorar, personal communication). As part of the Firestone Estate, the immediate perimeter of Bath Pond was subject to mowing three to four times per year and frequent wildlife removal. Beavers have historically been active in the area, and the pond, currently covering an area of ~15 acres, has been as large as 40 acres, occasionally encroaching upon neighboring properties. During the Firestone Era, beavers and their dams were frequently removed from Bath Pond resulting in a lowering of water levels and drainage of adjoining flooded areas. Since its purchase in 1997 by the Bath Township Parks Department, there has been one beaver dam removal in 2005 and mowing has ceased to allow native vegetation to reestablish around the perimeter of the pond (Michael Rorar, personal communication).

It is interesting that GDGT concentrations start to increase shortly after incorporation into the Firestone Family Estate in the 1940's, especially since the supposed historical land use on the property was agricultural. While there are no available records of wildlife removals on the property, it is possible that drainage events associated with beaver dam removals could be responsible for peaks in sedimentary GDGTs in Bath Pond in the last half century. In addition to increased delivery of terrestrial GDGTs, a drainage event, such as the one described, would also deliver large amounts of organic material and nutrients to the system, potentially fueling increased in-situ production of branched GDGTs immediately after the event and also into subsequent seasons. Again, additional geochemical and proxy data, as previously discussed, could help to explain the patterns of GDGT abundance in the Bath Pond sediments.

Similar to Bath Pond, concentrations of branched GDGTs in the sediments of Lake Tulane are relatively stable until the last half century. During the time period from ca. 1940 to the present, the amplitude of variation increases, but there is no great increase or decrease in GDGT concentrations as seen in Bath Pond. It is unlikely that climatic conditions in south central Florida have become significantly more variable over the past ~70 years as compared to the previous two centuries. Instead it is more likely that land use change in the watershed is responsible for greater variation in sedimentary branched GDGT concentrations in recent years. Increased GDGT concentration may be expected when sediment load to the water body is increased due to deforestation, agriculture and development, as previously discussed. Perhaps in Florida, development near the lake during the last 50 years added terrestrial soil and nutrients to the lake, boosting GDGT concentrations in the sediments. In contrast, decreases in GDGT concentration may signal revegetation or stabilization of habitat within a watershed. Although GDGT concentrations are more variable in the recent Lake Tulane sediments, it is worth noting that they are still an order of magnitude lower than the low background GDGT concentrations in older Bath Pond and Deming Lake sediments. The soils around Lake Tulane were very sandy and had the lowest percent organic carbon as well as the lowest concentrations of branched GDGTs of all three sites. These factors may account for the correspondingly low sedimentary GDGT concentrations found at Lake Tulane. The apparent increased variability in shallow sediment GDGT concentrations may be an artifact of an up core sampling resolution that is greater than deeper in the sediment profile, or it could alternately be attributed to natural sampling variability and error.

A close look at sedimentary GDGT concentrations in addition to the temperature signal they preserve may provide information about potential biases inherent in the proxy. For example, a peak in GDGT concentration, if terrestrially sourced, may result in warmer constructed temperatures than actual annual MAT. Alternately, if the increased concentrations are due to in-situ production, the temperature reconstruction may underestimate annual MAT. Unfortunately, little is known about the proposed in-situ production of branched GDGTs, and, at the moment, no work has been done to discern the aquatic or terrestrial source of GDGT lipids in lacustrine sediments. However, a significantly higher or lower sedimentary GDGT concentration could indicate time periods of potential inaccuracy in temperature reconstructions using the MBT-CBT proxy, where further investigation using additional proxy and geochemical methods would be useful.

Conclusions

The results of this study have answered some important questions about the MBT-CBT proxy. The seasonal study of soil GDGT assemblages has shown that branched GDGT core lipids do not appear to reflect changes in growth temperature on time scales of less than one year and are therefore thought to record a long-term average temperature. However, at all sites, the MBT generally yielded estimates of annual MAT that were warmer than the instrumentally measured air temperature but similar to the instrumentally measured soil temperature. This suggests that soil GDGTs are not necessarily reflecting a temperature bias due to a more favorable growth season so much as the warmer growth conditions in the insulated soil versus air. Future work with the MBT-CBT proxy may benefit from local calibrations using soil temperature instead of air.

Vegetation type did not significantly affect the soil MBT-MAT or soil concentration of GDGTs in Minnesota. Proxy-derived MAT under all vegetation types in Minnesota were significantly warmer than the instrumentally measured annual MAT, but similar, within the error of the proxy, to each other and the measured mean annual soil temperatures. In Ohio, pine soils were found to have the greatest abundance of branched GDGTs and there were significant differences in soil MBT-MAT between vegetation types. The observed differences in soil temperatures and soil GDGT concentrations among different vegetation types indicate the possibility of a biased sedimentary record of annual MAT.

The CBT proxy provided a good estimate of soil pH at most sites. However, in Minnesota deciduous soils and Ohio deciduous and open field soils, measured soil pH was significantly lower than the CBT estimate. An incorrect CBT estimate of pH has implications for the accuracy of the MBT proxy. For the purpose of palaeoclimate reconstructions, a higher pH or CBT value will yield a higher MBT temperature, and vice versa. It is unclear why the CBT is inaccurate in some soils and not others. An investigation of GDGTs and the cyclization ratio over a broad range of soil pH could provide important information about the sensitivity and accuracy of pH and temperature reconstructions using the MBT-CBT proxy as well as the ecology of the as yet unknown GDGT producing organisms.

In the sediments, relatively cool surface sediment MBT temperatures were apparent in all lakes. These results support the idea that in situ production of branched GDGTs in deep hypolimnetic waters or sub-surface sediments may be the cause of the cooler, near-surface temperatures. However, GDGTs from deeper in the sediment profile provide MBT estimates of MAT that are relatively accurate, with regards to the error inherent in the proxy, in comparison with instrumental measurements. It is unclear why the cool surface temperature signal is not apparent in deeper sediments though it is likely that historical GDGT assemblages are the product of both in situ and terrestrial GDGT production. Small downcore shifts from warmer to cooler MBT temperatures in some sediment profiles could also due to changes in the dominant source of GDGTs to the sediments during a certain time period and not to actual changes in annual MAT. Additional analyses are needed to constrain the sources of sedimentary branched GDGTs so that temperature reconstructions using the MBT can be accurately interpreted.

Finally, Patterns of GDGT abundance in lacustrine sediments are likely also a function of the rate of terrestrial sediment delivery to the lake as well as rates of potential in-situ productivity. Sedimentary GDGT abundances, used in addition to the temperature signal they preserve, could provide information about potential biases inherent in the proxy. Terrestrially sourced peaks in sedimentary GDGT concentration may result in warmer reconstructed MBT temperatures than instrumental measurements. Increased GDGT concentrations that are due to in-situ production may indicate an underestimate of annual MAT. To know if MBT-CBT based temperature reconstructions are accurate, the source of sedimentary branched GDGTs must be constrained. However, sedimentary GDGT concentration could still indicate time periods of potential inaccuracy in temperature reconstructions using the MBT-CBT proxy.

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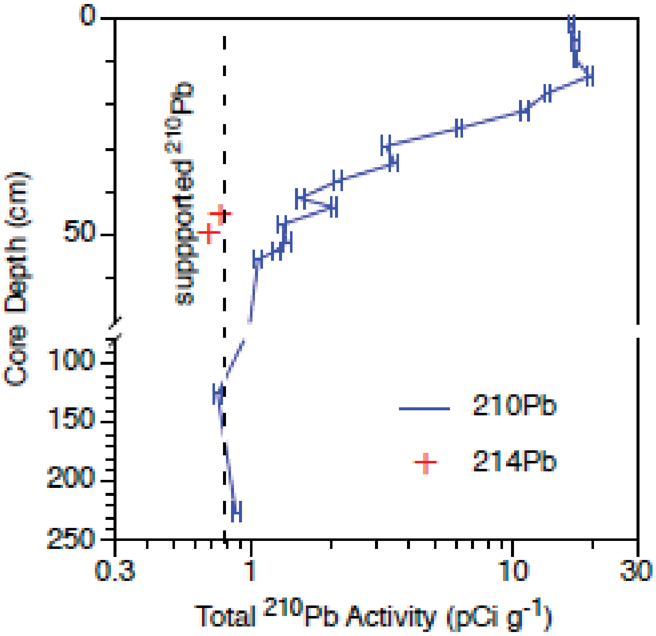
Appendix A



^{210}Pb Activity with depth in the sediment profile of Bath Pond, Ohio. The blue line represents the cumulative ^{210}Pb activity and the red crosses represent the supported ^{210}Pb activity.