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1 # A 150-year record of phytoplankton community succession
2 # controlled by hydroclimatic variability in a tropical lake

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1 **Abstract**

2 Climate and human-induced environmental change promotes biological regime shifts between
3 alternate stable states, with implications for ecosystem resilience, function and services.
4 While this has been shown for recent microbial communities, the long-term response of
5 microbial communities has not been investigated in detail. This study investigated the decadal
6 variations in phytoplankton communities in a ~150 year long sedimentary archive of Lake
7 Nong Thale Prong (NTP), southern Thailand using a combination of DNA and lipid
8 biomarkers techniques. Reconstructed drier climate from ~1857-1916 Common Era (CE)
9 coincided with oligotrophic lake water conditions and dominance of the green algae
10 *Botryococcus braunii,* producing characteristic botryococcene lipids. A change to higher
11 silica (Si) input ~1916 CE, which was related to increased rainfall concurs with an abrupt
12 takeover by diatom blooms lasting for 50 years. Since the 1970s more eutrophic conditions
13 prevailed, which was likely caused by increased levels of anthropogenic phosphate (P), aided
14 by increased lake stratification caused by somewhat dryer conditions. The eutrophic
15 conditions led to increased primary productivity consisting again of a *Botryococcus sp.,*
16 though this time not producing the botryococcene lipids. Moreover, *Cyanobacteria* became
17 dominant. Our results indicate that a combined DNA and lipid biomarker approach provides
18 an efficient way to allow tracking centennial-scale hydroclimate and anthropogenic feedback
19 processes in lake ecosystems.

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1 Introduction

Natural and anthropogenically induced climatic change is often cited as the main factor controlling ecosystem dynamics (Scheffer et al., 2001; Malmqvist et al., 2008; Woodward et al., 2010). The resulting environmental effects easily observed today in large plant and animal communities (Scheffer et al., 2001) have been discussed as causing regime shifts (Folke et al., 2004) and/or introducing alternative ecosystem states (Dent et al., 2002). On longer scales, climate-induced changes in microbial communities might in effect affect the capture and storage of CO_2 (Zeglin et al., 2013). However, predicting the effects of changing climate on shifts in microbial community structures even on short-time scales is challenging (Lotter and Birks, 1997; Woodward et al., 2010). This is partially due to the difficulty of coupling micro-scale external and internal ecosystem variables to specific microbial communities on longer time scales.

Consequently, most estimates are based on small-scale and short-term experimental data and on easily manipulated ecosystems such as soils (Landesman and Dighton, 2010; Cregger et al., 2012; Kuffner et al., 2012; Zeglin et al., 2013). Very little is therefore known about how natural microbial ecosystems respond to climatic changes over longer time scales and whether the associated feedbacks due to climate shifts promote regime shifts and alternative stable states. Moreover, interactions between external and internal ecosystem regulators still remain relatively unknown.

Because of their vital position at the base of the food chain (Carpenter et al., 1987; Young et al., 2013), phytoplankton constitutes one of the most ecologically important groups of microorganisms in aquatic ecosystems (Yin et al., 2011). The activity of these microorganisms is directly coupled to climatic changes through CO_2 drawdown, organic matter availability and oxygen production, factors that are necessary for the functioning of an entire ecosystem. Since phytoplankton communities are sensitive to environmental stressors (Woodward et al., 2010; Häder and Gao, 2015), natural and/or human-induced disturbances may result in far-reaching consequences for the nutrient status of lakes, where phytoplankton is the primary producer (Yin et al., 2011). For instance, the demise of the botryococcene lipid-producing green algae *Botryococcus braunii* was linked to early eutrophication in a Norwegian fjord (Smittenberg *et al.,* 2005).

Other studies have related variations in phytoplankton community structure, abundance and function to changes in lake trophic status (Ravasi et al., 2012; Hou et al., 2014).

1 Consequently, the emerging paradigm suggests that the structuring of phytoplankton
2 communities, characterized by potential successional shifts in population dynamics, may
3 serve as a tracer of the trophic status in lakes (Mackay et al., 2003; De Senerpont Domis et al.,
4 2007). Yet, changes in phytoplankton communities and productivity are to a large extent also
5 influenced by complex internal and external controls (Kamenir et al., 2008; Wang et al.,
6 2015), which however still remain to be elucidated.

7 Although there is a growing understanding of the factors that influence microbial
8 communities in lake ecosystems (Thyssen et al., 2011; Wang et al., 2015), the broader
9 linkages between different microbial groups and their response to past environmental
10 conditions are poorly understood. This is partly due to the lack of suitable proxies that can
11 capture and distinguish between the diverse parameters impacting microbial ecosystem
12 structure.

13 Lipid biomarkers specific for various types of microbes provide an important proxy of
14 microbial ecosystem structure and have therefore been employed in the reconstruction of past
15 ecosystems preserved in sedimentary records (Zimmerman and Canuel, 2000; Coolen et al.,
16 2004; Smittenberg et al., 2005). Lipids are an integral part of the cell membranes of both
17 prokaryotes and eukaryotes. They aid as structural support and as storage compounds within
18 various microbial cells (Jungblut et al., 2009), and can be specific for certain groups or
19 species. Moreover, lipid biomarkers can incorporate information of the chemical environment
20 in which they have formed via their relative abundance or isotopic composition. Because
21 lipids are resistant to postmortem biodegradation, ecological variations through time can be
22 reconstructed (Zimmerman and Canuel, 2000; Coolen et al., 2004; Smittenberg et al., 2005)
23 by tracking their occurrence and abundance over longer time scales. Moreover, when coupled
24 to hydroclimate variables such as hydrogen isotopes of leaf waxes (δD_{wax}), a proxy for
25 precipitation (e.g. Niedermeyer et al., 2014), microbial lipids may help underpin the impact of
26 past climates (wetter/drier) on microbial ecosystem changes (see Supplement).

27 Quantitative polymerase chain reactions (qPCR) of specific DNA of living organisms and
28 well-preserved DNA in lake sediments are excellent tools to assess present and past microbial
29 ecosystem structures (Coolen and Gibson, 2009; Ravasi et al., 2012; Hou et al., 2014). These
30 analyses provide specific proof of recent and past biological processes by targeting specific
31 microbial taxa and key genes involved in various metabolic pathways (Takai and Horikoshi,
32 2000; Hou et al., 2014).

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Here we explore the novel combination of biolipid analysis, δD_{wax}, qPCR, bulk isotopes of C and N, and sedimentary geochemistry to reconstruct phytoplankton community dynamics over a 150-year history of Lake Nong Thale Prong (NTP), Southern Thailand. Together, these proxies allow unraveling how external forcing (hydroclimate and human impact) influences internal abiotic feedback processes, which in turn control phytoplankton regime shifts.

2 Materials and Methods

2.1 Study area, fieldwork, sediment sampling and dating

NTP (17˚ 11`N, 99˚ 23`E) is a shallow (<7 m water depth), small (~210 m^2) sinkhole lake located on the Thai-Malay Penninsula in southern Thailand (Fig. 1) at ~60 m above sea level (Snansieng et al., 1976) (see Supplement). Prior to coring, a preliminary assessment of NTP was conducted based on the catchment geology and topography, basin size and water depth. Sounding in different parts of the lake showed that the deepest part was in the northeast and that there was little variability in the distribution of sedimentary materials throughout the lake. The two sediment cores (~74 cm length) were retrieved from the deepest part of the lake in January 2012 using an HTH gravity corer (70 mm diameter, 1 m length). The sediment presented a strong sulphidic smell, suggesting anoxic conditions. Visually, the cores were lithologically similar. Therefore further analysis was performed on one core, while the other core was archived. The sediment core was sliced onsite into 1 cm sub-samples, packed in sterile plastics bags and chilled with ice. Such temperatures have been shown to be low enough to inactivate and preserve whole tropical communities in sediments without the need for dramatic freezing because of a narrower temperature range of activity (Robador et al., 2015). After arrival at the Department of Geological Sciences, Stockholm University the samples were immediately frozen (-18˚C) until further analysis. The sampled sediment sequence was dated using ^{210}Pb (46.51 keV) and ^{226}Rn (295.2 keV) on an EG&G ORTEC® co-axial low energy photo spectrometer (LEPS) fitted with a high-purity germanium crystal (see Supplement).

2.2 Bulk biogeochemical analysis

A total of 15 sub-samples were taken at different core depths for a low-resolution quantification of total organic carbon (TOC), total nitrogen (TN), and carbon and nitrogen isotopes ($\delta^{13}C_{bulk\ org}$ and $\delta^{15}N_{bulk\ org}$). Samples were freeze-dried and homogenized before

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analysis. For the stable isotope measurements, the samples were pre-treated with HCl to remove carbonate carbon before analysis on a Carlo Erba NC2500 elemental analyzer, coupled to a Finnigan MAT Delta+ mass spectrometer. $\delta^{13}C_{bulk\ org}$ and $\delta^{15}N_{bulk\ org}$ values are reported in parts per mille (‰) relative to the Vienna PeeDee Belemnite (VPDB, for C) and standard air (for N), respectively, with an analytical error of ±0.15‰.

Relative estimates of the chemical composition of the sediments were obtained by elemental mapping using Environmental Scanning Electron Microscopy (FEI, Quanta FEG 650)-Electron Dispersive Spectroscopy (ESEM-EDS). An aliquot of the dried sediment was mounted on aluminium stubs with carbon tape and imaged at 10 kV in low vacuum. Elemental analysis was conducted in low vacuum with EDS at 30 kV. Approximately 75 elemental maps distributed over 15 samples across the entire core were acquired with the AZtech software, at a horizontal field width of 2 mm, 512 pixels and an average frame count of 5 with 100 µs pixel dwell time. The relative elemental abundance acquired was normalized to 100%.

2.3 Biomarker analysis

After freeze-drying, powdered samples were extracted three times with a mixture of dichloromethane and methanol (DCM–MeOH, 9:1, v/v) to obtain a combined total lipid extract (TLE), using a microwave system (MILESTONE Ultra Wave Single Chamber Microwave Digestion System) fitted with a LABTECH smart H150-1000 Water Chiller. The TLE from the sediments was dried in a vacuum concentrator (Scanvac MaxiVac Beta, Labogene ApS, Denmark) before being re-dissolved in DCM and then adsorbed onto a small amount of silica gel. This was evaporated on a warm plate, under a very gentle stream of nitrogen gas, and placed on top of 15 g silica gel (deactivated with 5% (wt.) H_2O) in 6-mL glass SPE tubes. Hydrocarbon (FI), ketone (F2) and polar (F3) fractions were recovered with pure hexane, a hexane and DCM mixture (1:1) and DCM–MeOH (1:1), respectively. F2 and F3 samples were stored in the freezer for later use. The F1 fraction was analyzed on a Shimadzu GCMS-QP2010 Ultra gas chromatography–mass spectrometer (GC–MS), equipped with an AOC- 20i auto sampler and a split-splitless injector operated in splitless mode. A Zebron ZB-5HT Inferno GC column (30 m x 0.25 mm x 0.25µm) was used for separation.

The GC oven temperature was programmed from 60–180°C at a ramp of 20°C min^{-1} followed by a ramp of 4°C min^{-1} until 320°C where it was held for 20 min. MS operating conditions

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were set to an ion source temperature of 200°C and 70eV ionization energy. Spectra were collected using GC solution Workstation software (v2). *n*-alkanes, C_{25} highly branched isoprenoids (HBIs) and botryococcene compounds were identified by retention times and comparison against mass spectra from the literature. Quantification of the *n*-alkanes, C_{25} HBIs and botryococcene compounds was done with an external standard consisting of a mixture of C_{20-40} *n*-alkanes of known concentration. Specifics on the mass spectra and retention times of the *n*-alkanes, HBIs and Botryococcenes, including chromatograms as reference, are included in Supplement (Fig. S1-S6).

2.4 δD analysis of leaf waxes

The F1 fraction was further separated into three fractions (F1a, F1b and F1c) over a pipette column filled with 10% $AgNO_3$-coated silica gel. F1a, which comprises *n*-alkanes, was eluted with hexane; F1b, made up of a few unidentified compounds, was eluted with hexane-DCM (1:1); and F1c consisting of HBIs and botryococcenes, was eluted with DCM-Acetone (9:1). F1b and F1c were also stored in the freezer for further analysis. F1a was analyzed by gas chromatography–isotope ratio monitoring–mass spectrometry (GC-IRMS) using a Thermo Finnigan Delta V Plus mass spectrometer interfaced with a Thermo Trace GC 2000 using a GC Isolink II and Conflo IV system. Helium was used as a carrier gas at constant flow mode. The GC oven temperature was programmed from 100–250°C at a ramp of 15°C min^{-1} followed by a ramp of 10°C min^{-1} until 320°C where it was held for 9 min. A standard mixture of *n*-alkanes with a known isotopic composition (reference mixture A4, provided by Arndt Schimmelmann, Indiana University, USA) was run several times daily to check instrument performance and to calibrate the reference gas (H_2) against which the samples were measured. All analyses were performed in triplicate and results are reported as the weighted mean. The average standard deviation for standards and samples was around 4‰ (see Supplement).

2.5 DNA extraction and qPCR

Freeze-dried samples were selected according to initial biomarker screening results, in order to estimate the abundance of different groups of organisms related to: 1) the *Prokarya*, *Archaea* and *Bacteria*, *Cyanobacteria,* and microorganisms involved in anaerobic methane cycling (quantification of the *mcrA* gene, e.g., Hallam et al., 2003, Hallam et al., 2004), 2) *Eukarya*, diatoms, and *Botryococcus sp.* The samples were analyzed in order to specifically

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1 reflect the sample conditions used for the biomarker analysis. Freeze-drying was not expected
2 to introduce significant biases but enhances cell breakage and the release of intracellular
3 DNA, following the freeze thaw method of DNA extraction (e.g. Tsai et al., 1991). This is
4 especially useful for soil and sediment samples (e.g. Tsai et al., 1991). Around 0.2 g (from
5 0.17 to 0.26 g) of freeze-dried sediment was extracted for DNA, using the MoBio PowerSoil®
6 DNA kit (Carlsbad, CA), following the manufacturer's instructions. About 500 µL of sterile
7 PBS 1X was also added to PowerSoil® Bead tube in order to enhance cell lysis efficiency.

8 The qPCR amplifications were conducted in 96 well qPCR plates in a CFX96 Touch™ Real-
9 Time PCR Detection System Instrument (C1000 Touch™ Thermal, Cycler, Bio-Rad) and its
10 software. The reactions consisted of a final volume of 25 µL, using the SsoAdvancedTM
11 Univesal SYBR® Green Supermix (Bio-Rad) following the manufacturer's recommendations.
12 Reactions run in 35 cycles contained 5 µL of DNA template and specific primer sets at their
13 appropriate concentrations and annealing temperatures (see Supplement).

14 Standard curves were calibrated using ten-fold serial dilutions from pure cultures of each
15 representative target group (Supplement). The qPCR detection of 16S rRNA genes, 18S
16 rRNA gene as well as *mcrA* genes in all of the samples and in ten-fold serial dilutions used to
17 construct the standard curves was run in triplicates. For each qPCR, several negative controls
18 were performed in order to check for laboratory contamination. The efficiencies of the qPCR
19 analyses was up to 90% with a correlation to the standard calibration curve of up to R^2=0.996
20 (see Supplement).

21 A total of 16S rDNA, 18S rDNA and *mcrA* gene copy numbers per g of sediment were
22 calculated from the triplicate average of each sample as described by (Sylvan et al., 2013).
23 Overall Prokaryotic cell abundance per gram of sediment was estimated by taking into
24 account the average of the 16S rRNA gene per cell equivalent to 1.86 for *Archaea*, 4.1 for
25 Bacteria (Lee et al., 2009) as previously used by (Sylvan et al., 2013) and 2.18 for
26 *Cyanobacteria* (after calculation of the average using the data from (Schirrmeister et al.,
27 2012). Due to lack of references from lake sediments, one copy per cell of the *mcrA* gene was
28 used to quantify the population of organisms involved in anaerobic methane cycling.

29 The raw data of *Eukarya*, diatoms and *Botryococcus sp.* were not further quantified into copy
30 numbers per g sediment, for two reasons: 1) there is high variability in 18S rRNA gene copies
31 per cell within the *Eukarya* and diatoms (i.e. from 3 to more than 25000 copies per cell in the
32 plants ((Prokopowich et al., 2003, Zhu et al., 2005) and between 61 to 36,896 for the diatoms

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1 (Godhe et al., 2008)); 2) the paucity of information related to the number of 18S rRNA gene
2 copy number in the *Botryococcus sp*. genome. Therefore, the results reported here are
3 indicative from the universal *Eukarya* primer and should be considered as relative abundance
4 of the total *Eukarya* due to the tendency of not detecting all Eukaryotic groups. Yet, the data
5 are still useful to depict trends in the sediment record. The limitations of this method are
6 given in Supplement.

7 **3 Results**

8 The ^{210}Pb activity shows an exponential decay curve with depth, which shows a decreasing
9 linear trend when plotted on a log-scale (i.e. ln ($^{210}Pb_{unsupported}$ *vs* depth; $r^2 = 0.827$) (Fig. 2).
10 The profile indicates minimal sediment bioturbation, and is used to calculate an average
11 sedimentation rate of about 4.7 mm yr^{-1}, which is similar to that of estuarine sediments from
12 the eastern coast of Thailand (Cheevaporn and Mokkongpai, 1996).

13 Biogeochemical and biolipid screening of the sediment core, discussed further below,
14 demarcates three distinct units: unit I from the top to 20 cm depth, unit II between 20 and 45
15 cm depth and unit III between 45 and 74 cm depth. Increasing sediment depth is linearly
16 related to increasing sediment age, such that unit I corresponds to ~2008-1969 CE, unit II to
17 ~1969-1916 CE and unit III to ~1916-1857 CE.

18 The sediments are highly organic with TOC contents of between 30 and 40%. TOC (%)
19 gradually decreases with depth (units I and II), but increases sharply in the middle of unit III
20 and remains high until the bottom of the sequence (Fig. 3a). Both $\delta^{13}C_{bulk\ org}$ (Fig. 3b) and
21 total N (%) (Fig. 3c) show a gradually decreasing trend downcore while $\delta^{15}N_{org}$ values
22 increase in unit I and then steadily decrease through units II and III (Fig. 3d). The C/N ratio
23 on the other hand increases gradually from the top to the bottom of the sediment core (Fig.
24 3e). Si/Ti and O/Ti ratios, markers of nutrient input into the lake from terrestrial sources,
25 show strong co-variation and general decrease downcore, with the highest ratios recorded in
26 unit II (Figs. 3f and g). The covariation between Si/Ti and O/Ti ratios suggests that SiO_2
27 dominates among the silicate minerals in the catchment. Pictures taken using ESEM show
28 higher abundances and diversity of diatoms in unit II compared to units I and III (see
29 Supplement; Fig. S7). The P/Ti ratio, which can be used as a proxy for eutrophic conditions in
30 lakes (Kirilova et al., 2011), decreases with depth (Fig. 3h).

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1 Botryococcene lipid concentrations have low values in unit I, increase in relative abundance
2 in unit II and are most abundant in unit III, with a maximum at ~60 cm depth (~1880 CE)
3 (Fig. 4a). HBIs, which have very low relative abundances in unit I, sharply rise in unit II
4 where they maximize at ~30 cm (~1950s), and exhibit intermediate and slowly decreasing
5 concentrations in unit III (Fig. 4b). The relative abundance of C_{17} *n*-alkanes is markedly high
6 in especially the top of unit I, but is low throughout units II and III (Fig. 4c). The hydrogen
7 isotopic composition of leaf waxes (δD_{wax}; weighted mean of δD C_{27-31} *n*-alkanes) shows a
8 long-term oscillation over the entire 150-year record (Fig. 4d). The most recent decades
9 exhibit an increasing (drying) trend, when compared to the values of unit II. Minimum values
10 (wettest conditions) of δD_{wax} are reached in the middle of unit II. Relatively high values
11 (driest conditions) are found halfway through unit III (around 60 cm depth, ~1890 CE), after
12 which δD_{wax} values slowly decrease, suggesting a hydroclimatic trend towards relatively wet
13 conditions.

14 The qPCR data set shows generally identical trends for total Prokaryotes, *Eukarya* and
15 *Botryococcus sp.* (Figs. 5a-c). Their abundances decrease with depth (until about 43 cm) and
16 then increase gradually again in unit II, except for the numbers of the prokaryotes, which in
17 addition to the general trend also show a sharp spike in abundance at around 30 cm depth.
18 Bacterial abundance displays a generally decreasing trend without delineated structure (Fig.
19 5d), whereas the *Cyanobacteria* numbers (presented as a percentage of the Bacteria
20 quantified) decrease sharply with depth in unit I and then remain relatively low throughout the
21 entire sequence (Fig. 5e). Diatom abundance increases gradually in unit I and then sharply in
22 unit II before dropping significantly to a minimal level at the transition between unit II and III
23 (Fig. 5f). *Archaea* abundance only shows minimal variations (Fig. 5g). Bacterial communities
24 dominate among the prokaryotes throughout the whole sediment core (attaining more than
25 90% of the total prokaryotic abundance). *mcrA* genes were mostly detected in the upper part
26 of the sequence where they represent up to 8.5% of the *Archaea*, but also were found in
27 substantial amounts at depths of 26 and 61 cm. The *mcrA* gene has a maximum occurrence in
28 units I and III and relatively low abundance in unit II (Fig. 5h). The presence of *mcrA* genes
29 in the sediment likely indicates anaerobic methane cycling processes (anaerobic methane
30 oxidation and methanogenesis) (Hallam *et al.,* 2004) and this shows a significant correlation
31 with total *Eukarya* (Fig. 6; $r^2 = 0.85$).

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1 High proportions of *Cyanobacteria* (>8-26% of the total *Bacteria* numbers) and a low diatom
2 biomass (~1-1.4% of the total *Eukarya* abundances) characterize unit I. In contrast a dense
3 diatom signature (up to 1.7% of the total *Eukarya* abundances) and low proportions of
4 *Cyanobacteria* (~1% of the total bacteria numbers) is distinctive for unit II. *Cyanobacteria*
5 *and* diatom abundances are however relatively low in unit III. The transition between unit II
6 and III is marked by an important decrease in total biomass, which was characterized by low
7 estimates of eukaryotic and prokaryotic numbers (Fig. 5). *Botryococcus sp.* abundances show
8 an increase in unit I and unit III on a log scale, which correlates with the concentration of
9 botryococcenes, except for unit I, suggesting that in unit III most of the *Botryococcus sp.*
10 detected by qPCR could be *Botryococcus braunii.*

11 4 Discussion

12 4.1 Carbon cycle in NPK

13 The biogeochemical trends suggest that multiple processes control the organic matter (OM)
14 input into lake NTP, which in turn play a significant role in carbon storage. Increasing C/N
15 ratio with depth (Fig. 3e) may be explained by a preferential (anoxic) mineralization of OM
16 rich in N leading to residual OM with a higher C/N ratio, as observed in many other systems
17 (Emerson and Hedges, 2003). This explanation is also consistent with long-term diagenetic
18 OM transformation (Sun et al., 2004). Successional deposition of different phytoplankton
19 communities with different C/N ratios can also explain the C and N signal, which could
20 represent an original signal of deposition: replacement of lipid-rich *Botryococcus* with
21 particularly high C/N ratio in unit III by diatoms as the dominant plankton around 1920,
22 which were in turn replaced by cyanobacterial activity in the second half of the last century,
23 with low C/N ratio due to their capacity to fix N. Alternatively, the pattern of decreasing
24 $\delta^{13}C_{org}$ (Fig. 3b) while TOC increases (Figs. 3a) with depth could also be attributed to
25 successional shifts of trophic state of the lake (Brenner et al., 2000; Meyers and Teranes,
26 2001). Increase in nutrients from one trophic state to the other decreases the amount of
27 dissolved CO_2 available for use by the phytoplankton community. This leads to lower net
28 fractionation against ^{13}C by the phytoplankton during photosynthesis and thus increasing $\delta^{13}C$
29 values (Meyers and Teranes, 2001).

30 The presence of *mcrA* genes in the sediment can be a remnant signal of past methane cycle
31 activity (Hallam et al., 2004) in the upper sediment and/or the anoxic bottom waters of the

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1 lake but can also represent ongoing methanogenesis within the sediment (Stein et al., 2001;
2 Earl et al., 2003). During methanogenesis, degassing of ^{12}C-enriched methane could lead to
3 enriched ^{13}C in residual organics (Ogrinc et al., 2002). A strong correlation between *mcrA*
4 gene abundance and *Eukarya* (Fig. 6; $r^2 = 0.85$) could indicate that the depth profiles reflect a
5 concurrency of primary productivity and methane cycling in the anoxic lake bottom waters. It
6 is, however, also possible that methane cyclers in the lake sediments are living off the organic
7 matter deposited by the phytoplankton community in the lake surface.

8 ## 4.2 Climate influence on lake evolution and phytoplankton community
9 ## changes

10 The combination of all analyzed proxies (biolipids, δD_{wax}, qPCR, bulk CN isotopes, and
11 sedimentary geochemistry) allows discussing microbial community changes in NTP and
12 further constrains the parameter(s) that caused the shifts in lake status through time. Decadal
13 changes in NTP trophic states were accompanied by variations of dominant phytoplankton
14 community. The period from ~1857 to 1916 CE*,* is marked by significant increases of both
15 botryococcene lipids (Fig. 4a) and *Botryococcus sp*. abundance (Fig. 5c), which also
16 corresponds with lower precipitation (Fig. 4d). Several studies have shown that these algae
17 are tolerant to oligotrophic conditions (Souza et al., 2008) and can therefore be used as a
18 proxy for oligotrophic lake water conditions in the oxygenated epilimnion of the lake
19 (Waldmann et al., 2014). The presence of the *mcrA* gene in appreciable amounts indicates
20 substantial microbial activity by anaerobic microbial methane cyclers in the anoxic bottom
21 waters and/or sediment feeding off primary producers (*Botryococcus sp.*). Our interpretation
22 for this is one of a fairly strongly stratified lake where reformed nutrients stayed in the anoxic
23 hypolimnion thereby keeping the surface water oligotrophic. In the tropics, the mean air
24 temperature (MAT) is a direct result of incoming solar radiation and the relationship between
25 the MAT and the amount of rainfall are typically inversely proportional (Imboden and Wüest,
26 1995; Boehrer and Schultze, 2008). Dry, cloudless and warmer conditions lead to stronger
27 stratification in fresh water lakes (Imboden and Wüest, 1995).

28 A stark difference in the dominant phytoplankton community is observed from ~1916-1969
29 CE. This period is marked by significant decrease in botryococcene lipid concentrations (Fig.
30 4a) and *Botryococcus sp*. gene abundance (Fig. 5c), and an increase in both diatom abundance
31 (Fig. 5f) and C_{25} HBI concentrations (Fig. 4b), which is a useful indicator of diatom-derived
32 OM inputs to sediments (McKirdya et al., 2013). Diatoms dominate phytoplankton

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1 communities as long as there is abundant silica irrespective of changes in environmental
2 conditions and nutrient levels (Egge and Aksnes, 1992). Interestingly, the increase in diatom
3 markers (Fig. 4b and Fig. 5f) coincides with an increase in reconstructed rainfall intensity
4 (Fig. 4d). Moreover the increase in Si/Ti ratios (Fig. 3f), a run-off signal (Murphy et al.,
5 2000), coincides with high diatom blooms especially in unit II. Since Ti is a highly immobile
6 element, weathering and transportation of Si is not accompanied by significant Ti delivery to
7 aquatic basins. Therefore the Si/Ti ratio can serve as a proxy for nutrient dynamics linked to
8 hydrological changes (Cartapanis et al., 2014) and as an indicator for enhanced diatom
9 production in lakes (Wennrich et al., 2014). Altogether, it appears that the generally wetter
10 conditions between ~1916 and 1969 CE increased catchment runoff into the lake. The
11 catchment runoff in turn increased the nutrient and silicate mineral content of the lake water
12 (e.g. Paerl et al., 2006), changing it from oligotrophic to mesotrophic. Increased diatom
13 diversity and fluvial deposits observed from the image scans of the sediments further
14 substantiate the hydrologically driven diatom blooms (Supplement; Fig. S7). In addition, an
15 increase in precipitation was likely accompanied by cooler temperatures, as explained above,
16 which lead to a decrease in stratification and to an increase in mixing between the epilimnion
17 and hypolimnion. Risen nutrient levels available for the phytoplankton community may also
18 have been due to the mixing processes.

19 Between ~1969 and ~2008 CE, the phytoplankton community structure changed again, with a
20 diminishing role for diatoms as evidenced by lower concentrations of C_{25} HBIs (Fig. 4b) and
21 the start of a marked increase of *Cyanobacteria* gene numbers (Fig. 5e) and C_{17} *n*-alkanes
22 (Fig. 4c). C_{17} *n*-alkanes are recognized biomarkers of aquatic algae and photosynthetic
23 bacteria such as *Cyanobacteria* (Meyers, 2003). Indeed, *Cyanobacteria* gene numbers relative
24 to Bacteria quantification based on the qPCR data covary strongly with C_{17} *n*-alkane
25 concentration, which confirms that the C_{17} *n*-alkanes were produced mainly by
26 *Cyanobacteria.* Additionally, the decreasing $\delta^{15}N_{bulk\ org}$ values during this period (Fig. 3d) also
27 suggest intense nitrogen fixation, a process strongly associated with *Cyanobacteria* activity
28 (Vahtera et al., 2007). This time period (~1969-2008 CE) is also characterized by somewhat
29 lower rainfall amounts (Fig. 4d). The amount of Si indeed decreased and diatoms became less
30 abundant, allowing non-diatomaceous phytoplankton not dependent on Si to take over (Egge
31 and Aksnes, 1992), while the amount of *Botryococcus* genes increased again (Fig. 5c). The
32 amount of *mcrA* genes, indicating a stronger methane cycling, also showed higher levels (Fig.
33 5h). However, this was not accompanied by the production of botryococcene lipids,

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1 suggesting that another strain of these green algae became prevalent. Indeed, *Botryococcus*
2 *braunii* are classified into three main races: A, B and C and it is only the B race that produces
3 botryococcenes lipids (Eroglu et al., 2011).

4 The strong *Cyanobacteria* prevalence suggests a eutrophic phosphorous-rich condition
5 instead of the oligotrophic conditions occurring a century earlier and this notion is supported
6 by higher level of P in the sediment (Fig. 3h). P bioavailability is one of the most important
7 factors limiting aquatic *Cyanobacteria* blooms (Paerl and Fulton, 2006; Paerl and Valerie,
8 2012). The source of the elevated phosphorus is unclear, but likely results from human
9 activities. Under a land development program in the 1990s, more than 20% of Thailand's
10 56,000 villages were located within forest reserves (Gray, 1991; Puri, 2006), which allowed
11 the expansion of land encroachment and agricultural activities. For instance, southern
12 Thailand has seen an increase in the cultivation of rubber trees on small farms at rates above
13 7% yr^{-1} (Leturque and Swiggings, 2011). The use of fertilizers in farming activities, untreated
14 wastewater effluents and the use of detergents are likely sources of the elevated phosphorus
15 inputs into the lake (Litke, 1999; Chislock et al., 2013). These accelerated the eutrophic state
16 of the lake beyond the natural rate of nutrient enrichment, which takes centuries to achieve
17 (Litke, 1999).

18 According to our analysis, photosynthetic primary production is at the base of internal organic
19 matter production in the lake. However, changes in precipitation, anthropogenic forcing and
20 nutrient input have produced fluctuations in dominant primary producer communities over the
21 last 150 years, from botryococcene lipid-producing algae to diatoms and then currently to
22 *Cyanobacteria* predominance.

23 5 Summary and conclusion

24 The combination of geochemistry, lipid biomarker and qPCR analyses allowed distinguishing
25 and quantifying different microbial groups in the sediments of Lake Nong Thale Prong,
26 southern Thailand, and most importantly allowed the identification of biological relationships
27 between the phytoplankton community structure response to either natural environmental
28 changes or anthropogenic impact.

29 Between ~1857 and 1916 CE, relatively drier climate in southern Thailand coincided with
30 oligotrophic surface water conditions in Lake NTP, which was dominated by botryococcene
31 lipid-producing primary producers. These likely sustained anaerobic methane cycling in the

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1 anoxic bottom waters and sediment, as evidenced by the detection of *mcrA* genes. A change
2 to higher Si input into the lake could be linked to increased precipitation from ~1916-1969
3 CE, which led to a rapid takeover by diatoms as primary producers. The increase in
4 precipitation was likely accompanied by decreased stratification with a greater mixing of
5 reformed nutrients from the depth to the surface water and a decrease of methane cycling
6 related genes. Since the 1970s many aspects of the initial limnic state returned upon drier
7 conditions, except that anthropogenic impact led to an increase in P allowing cyanobacteria to
8 become an important contributor to primary productivity.

9 The change in TOC values and C/N ratios, which decrease between ~1857 CE and present,
10 suggest that the botryococcene lipid-producing *Botryococcus braunii* were a key part of an
11 alternate stable lake status that facilitated the most efficient capture and burial of C in the
12 sediments (~1857-1916 CE). The *mcrA* gene abundance suggests strong anaerobic methane
13 cycle dependence on the primary producers, phytoplankton community. However, processes
14 like lake stratification and mixing between the epilimnion and hypolimnion possibly affected
15 the *mcrA* gene abundance: strong stratification leads to increase in *mcrA* gene abundances
16 (units 1 and III) whereas mixing leads to decrease in *mcrA* gene abundances.

17 Our results show that the combinations of biolipid analysis, δD_{wax}, qPCR, bulk isotopes of C
18 and N, and sedimentary geochemistry are effective in unraveling how external forcing
19 (hydroclimate and human impact) influences internal abiotic feedback processes. The abiotic
20 feedback processes as a result of changing climate has implications for phytoplankton regime
21 shifts and their role in carbon capture storage and suggest that phytoplankton sedimentary
22 records may assist in tracking such changes over decadal to centennial timescales.

23 **Author contributions**

24 This study was conceived and led by K. A. Yamoah, E. Chi Fru and R. H. Smittenberg. K. A.
25 Yamoah, N. Callac, A. Wiech and A. Chabangborn carried out laboratory analyses. K. A.
26 Yamoah, N. Callac, E. Chi Fru, B. Wohlfarth and R. H. Smittenberg wrote the manuscript.
27 All authors discussed the results and their implications and commented on the manuscript as it
28 progressed.

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6 **References**

7 Boehrer, B. and Schultze, M.: Stratification of lakes, Rev. Geophys., 46, RG2005,
8 doi:10.1029/2006RG000210, 2008.

9 Brenner, M., Whitmore, T. J., Lasi, M. A., Cable, J. E. and Cable, P. H.: A multi-proxy
10 trophic state reconstruction for shallow Orange Lake, Florida, USA: possible influence
11 of macrophytes on limnetic nutrient concentrations, J. Paleolimno., 21, 215-233, 2000.

12 Carpenter, S. R., Kitchell, J. F., Hodgson, J. R., Cochran, P. A., Elser, J. J., Elser, M. M.,
13 Lodge, D. M., Kretchmer, D., He, X., and von Ende, C. N., Ecology, 68, 1863-1876,
14 1987.

15 Cartapanis. O., Tachikawa, K., Romero, O. E. and Bard, E.: Persistent millennial-scale link
16 between Greenland climate and northern Pacific Oxygen Minimum Zone under
17 interglacial conditions, Clim. Past., 10, 405-418, 2014.

18 Cheevaporn, V. and Mokkongpai, P.: Pb-210 radiometric dating of estuarine sediments from
19 the eastern coast of Thailand. Journal of Science Society, Thailand, 22, 313-324, 1996.

20 Chislock, M. F., Doster, E., Zitomer, R. A and Wilson, A.: Eutrophication: causes,
21 consequences, and controls in aquatic ecosystems, Nat. Edu. Knowledge, 4, 10, 2013.

22 Coolen, M. J. L. and Gibson, J. A. E.: Ancient DNA in lake sediment records, Pages News,
23 17, 104–106, 2009.

24 Coolen, M. J. L., Muyzer, G. Rijpstra, W. I. C. Schouten, S. Volkman, J. K. and Sinninghe
25 Damste J. S.: Combined DNA and lipid analyses of sediments reveal changes in
26 Holocene haptophyte and diatom populations in an Antarctic lake, Earth Planet Sci.
27 Lett., 223, 225–239, 2004.

28 Cregger, M. A., Schadt, C. W., McDowell, N. G., Pockman, W. T. and Classen, A. T.:
29 Response of the soil microbial community to changes in precipitation in a semiarid
30 ecosystem, Appl. Environ. Microbiol., 78, 8587–8594, 2012.

© Author(s) 2016. CC-BY 3.0 License.

1 De Senerpont Domis, L. N., Mooij, W. M. and Huisman, J.: Climate-induced shifts in an
2 experimental phytoplankton community: a mechanistic approach, Hydrobiologia, 584,
3 403-413, 2007.

4 Dent, C. L., Cummings, G. S. and Carpenter, S. R.: Multiple states in river and lake
5 ecosystems, Phil. Trans. R. Soc. B., 357, 635–645, 2002.

6 Egge, J. K and Aksnes, D. L.: Silicate as regulating nutrient in phytoplankton competition,
7 Mar Ecol Prog., 83, 281-289, 1992.

8 Emerson, S. and Hedges, J.: Sediment diagenesis and benthic flux, Treatise on Geochem 6,
9 293–319, 2003.

10 Eroglu, E., Okada, S. and Melis, A.: Hydrocarbon productivities in different Botryococcus
11 strains: comparative methods in product quantification, Journal of Applied Phycology,
12 23, 763-775, 2011.

13 Folke, C., Carpenter, S., Walker, B., Scheffer, M., Elmqvist, T., Gunderson, L. and Holling,
14 C. S.: Regime Shifts, Resilience, and Biodiversity in Ecosystem Management. Annu Rev
15 Ecol Evol Syst, 35, 557-581, 2004.

16 Godhe, A., Asplund, M. E., Härnström, K., Saravanan, V., Tyagi, A. and Karunasagar, I.:
17 Quantification of diatom and dinoflagellate biomasses in coastal marine seawater
18 samples by real-time PCR, Appl. Environ. Microbiol., 74, 7174-7182, 2008.

19 Gray, D., Piprell, C. and Mark, G.: National Parks of Thailand, Industrial Finance
20 Corporation of Thailand, 1991.

21 Häder, D. P. and Gao, K.: Interactions of anthropogenic stress factors on marine
22 phytoplankton, Front Environ. Sci, 3,14, 2015.

23 Hallam, S. J., Girguis, P. R., Preston, C. M., Richardson, P. M., DeLong, E. F.: Identification
24 of Methyl Coenzyme M Reductase A (mcrA) Genes Associated with Methane-Oxidizing
25 Archaea. Appl. Environ. Microbiol., 69, 5483-5491, 2003.

26 Hallam, S. J., Putnam, N., Preston, C. M., Detter, J. C., Rokhsar, D., Richardson, P. M. and
27 DeLong, E. F.: Reverse Methanogenesis: Testing the Hypothesis with Environmental
28 Genomics, Science, 305, 1457-1462, 2004.

29 Hou, W, Dong, H., Li, G., Yang, J., Coolen, M. J., Liu, X., Wang, S., Jiang, H., Wu, X., Xiao,
30 H., Lian, B. and Wan, Y.: Identification of photosynthetic plankton communities using

© Author(s) 2016. CC-BY 3.0 License.

1 sedimentary ancient DNA and their response to late-Holocene climate change on the
2 Tibetan Plateau, Sci. Rep., 4, 6648, 2014.

3 Imboden, D. M. and Wüest, A.: Mixing mechanisms in lakes, in Physics and Chemistry of
4 Lakes, edited by A, Lerman et al., pp. 83–138, Springer, Berlin, Germany, 1995.

5 Jungblut, A. D., Allen, M. A., Burns, B. P. and Neilan, B. A.: Lipid biomarker analysis of
6 cyanobacteria-dominated microbial mats in meltwater ponds on the McMurdo Ice Shelf,
7 Antarctica, Org. Geochem., 40, 258–269, 2009.

8 Kamenir, Y., Winder, M., Dubinsky, Z., Zohary, T. and Schladow, G.. Lake Tahoe vs. Lake
9 Kinneret phytoplankton: comparison of long-term taxonomic size structure consistency,
10 Aquat. Sci. Res. Across Boundaries, 70, 195–203, 2008.

11 Kirilova, E. P., Heiri, O., Bluszcz, P., Zolitschka, B. and Lotter, A. F.: Climate-driven shifts
12 in diatom assemblages recorded in annually laminated sediments of Sacrower See (NE
13 Germany), Aquat. Sci. 73, 201–210, 2011.

14 Kuffner, M., Hai, B., Rattei, T., Melodelima, C., Schloter, M., Zechmeister-Boltenstern, S.,
15 Jandl, R., Schindlbacher, A. and Sessits, A.: Effects of season and experimental warming
16 on the bacterial community in a temperate mountain forest soil assessed by 16S rRNA
17 gene pyrosequencing, FEMS Microbiol Ecol, 82, 551–562, 2012.

18 Landesman, W. J., and Dighton, J.: Response of soil microbial communities and the
19 production of plant-available nitrogen to a two-year rainfall manipulation in the New
20 Jersey Pinelands, Soil Biol Biochem, 42, 1751–1758, 2010.

21 Lee, Z. M-P., Bussema, C. and Schmidt, T. M.: rrnDB: documenting the number of rRNA and
22 tRNA genes in bacteria and archaea, Nucleic acids research, 37, D489-D493, 2009.

23 Leturque, H. and Wiggins, S.: Thailand's progress in agriculture: Transition and sustained
24 productivity growth, Overseas Development Institute, pp 11, 2011.

25 Litke, D. W.: Review of phosphorus control measures in the US and their effects on water
26 quality. National Water Quality Assessment Program: Water-Resources Investigations
27 Report, Report nr, 99-4007, 1999.

28 Lotter, A. F. and Birks, H. J. B.: The separation of the influence of nutrients and climate on
29 the varve time-series of Baldeggersee, Switzerland, Aquat. Sci., 59, 362–375, 1997.

1 Mackay, A. W., Jones, V. J. and Battarbee, R. W.: Approaches to Holocene climate
2 reconstruction using diatoms. In Mackay AW, Battarbee RW, Birks HJB, Oldfield F,
3 editors. Global change in the Holocene. Arnold, 294–309, 2003.

4 Malmqvist, B., Rundle, S. D., Covich, A. P., Hildrew, A. G., Robinson, C. T., Townsend, C.
5 R.: Prospects for streams and rivers: an ecological perspective. In Aquatic systems:
6 trends and global perspectives (ed. N. Polunin), pp. 19–29. Cambridge, UK, Cambridge
7 University Press, 2008.

8 McKirdy, D. M., Spiro, B., Kim, A. W., Brenchley, A. J., Hepplewhite, C. J. and Mazzoleni,
9 A.G.: Environmental significance of mid- to late Holocene sapropels in Old Man Lake,
10 Coorong coastal plain, South Australia: an isotopic, biomarker and palaeoecological
11 perspective, Org. Geochem., 58, 13-26, 2013.

12 Meyers, P. A. and Teranes, J. L.: Sediment organic matter. In: Last, W.M., Smol, J. (Eds.),
13 Tracking Environmental Change Using Lake Sediments. Physical and Geochemical
14 Methods, vol. 2. Kluwer Academic Publishers, Dordrecht, pp, 239-268, 2001.

15 Meyers, P. A.: Applications of organic geochemistry to paleolimnological reconstructions: a
16 summary of examples from the Laurentian Great Lakes, Org. Geochem., 34, 261–289,
17 2003.

18 Murphy, A. E., Sageman, B. B., Hollan-Der, D. J., Lyons, T. W. and Brett, C. E.: Black shale
19 deposition and faunal overturn in the Devonian Appalachian basin: Clastic starvation,
20 seasonal water-column mixing, and efficient biolimiting nutrient recycling,
21 Paleoceanography, 15, 280–291, 2000.

22 Niedermeyer, E. M., Sessions, A. L., Feakins, S. J. and Mohtadi, M.: Hydroclimate of the
23 Indo-Pacific Warm Pool during the past 24,000 years, PNAS, 111, 9402-9406, 2014.

24 Ogrinc, N., Lojen, S. and Faganeli, J.: A mass balance of carbon stable isotopes in an organic-
25 rich methane-producing lacustrine sediment (Lake Bled, Slovenia). Global Planet.
26 Change, 33, 57-72, 2002.

27 Paerl, H. W. and Valerie, J. P.: Climate change: Links to global expansion of harmful
28 Cyanobacteria. Water research, 46, 1349 -1363, 2012.

29 Paerl, H. W., Fulton III, R. S.: Ecology of Harmful Cyanobacteria, pp. 95e107. In: Graneli,
30 E., Turner, J. (Eds.), Ecology of Harmful Marine Algae. Springer-Verlag, Berlin, 2006.

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1 Prokopowich, C. D., Gregory, T. R., Crease, T. J.: The correlation between rDNA copy
2 number and genome size in eukaryotes, Genome, 46, 48-50, 2003.

3 Puri J.: Factors affecting agricultural expansion in forest reserves of Thailand: the role of
4 population and roads, dissertation submitted to the faculty of the graduate school of the
5 university of Maryland, college park, USA, 2006.

6 Ravasi, D. F., Peduzzi, S., Guidi, V., Peduzzi, R., Wirth, S. B., Gilli, A. and Tonolla, M.:
7 Development of a real-time PCR method for the detection of fossil 16S rDNA fragments
8 of phototrophic sulfur bacteria in the sediments of Lake Cadagno, Geobiology, 10, 196-
9 204, 2012.

10 Robador, A., Müller, A. L., Sawicka, J. E., Berry, D., Hubert, C. R. J., Loy, A., Jørgensen, B.
11 and B., Brüchert, V.: Activity and community structures of sulfate reducing
12 microorganisms in polar, temperate and tropical marine sediments, The ISME Journal,
13 1–14, doi:10.1038/ismej.2015.157, 2015.

14 Scheffer, M., Carpenter, S., Foley, J. A., Folke, C. and Walker, B.: Catastrophic shifts in
15 ecosystems, Nature, 413, 591–596, 2001.

16 Schirrmeister, B., Dalquen, D., Anisimova, M., Bagheri, H.: Gene copy number variation and
17 its significance in cyanobacterial phylogeny, BMC Microbiology, 12,177, 2012.

18 Smittenberg, R. H., Baas, M., Schouten, S. and Sinninghe Damsté, J. S., The demise of the
19 alga Botryococcus braunii from a Norwegian fjord was due to early eutrophication, The
20 Holocene, 15, 133-140, 2005.

21 Snansieng, S., Gitisan, N. and Sripongpan, P.: Geological map of Changwat Nakhon Si
22 Thammarat. NC47-15, GeolSurv Div, Dept Mineral Res, Thailand, 1976.

23 Souza, M. B. G., Barros, C. F. A., Barbosa, F., Hajnal, E. and Padisak, J.: Role of atelomixis
24 in replacement of phytoplankton assemblages in Dom Helvécio Lake, South-East Brazil,
25 Hydrobiologia, 607, 211-224, 2008.

26 Stein, L. Y., La Duc, M. T., Grundl, T. J. and Nealson, K. H,: Bacterial and archaeal
27 populations associated with freshwater ferromanganous micronodules and sediments,
28 Environ. Microbiol., 3, 10-18, 2001.

© Author(s) 2016. CC-BY 3.0 License.

1 Sun, M. Y., Zou, L., Dai, J., Ding, H., Culp, R. A. and Scranton, M. I.: Molecular carbon
2 isotopic fractionation of algal lipids during decomposition in natural oxic and anoxic
3 seawaters, Org. Geochem., 35, 895–908, 2004.

4 Sylvan, J. B., Sia, T. Y., Haddad, A. G., Briscoe, L. J., Toner, B. M., Girguis, P. R. and
5 Edwards, K. J.: Low temperature geomicrobiology follows host rock composition along
6 a geochemical gradient in Lau Basin. Front Microbiol., 4, 61, 2013.

7 Takai, K. and Horikoshi, K.: Rapid detection and quantification of members of the archaeal
8 community by quantitative PCR by using fluorogenic probes. Appl. Environ. Microbiol.,
9 66, 5066-5072, 2000.

10 Thyssen, M., Ferreyra, G., Moreau, S., Schloss, I., Denis, M. and Demers, S.: The combined
11 effect of ultraviolet B radiation and temperature increase on phytoplankton dynamics and
12 cell cycle using pulse shape recording flow cytometry, J. Exp. Mar. Biol. Ecol., 406, 95–
13 107, 2011.

14 Tsai, Y-L. and Olson, B. H.: Rapid method for direct extraction of DNA from soils and
15 sediments, Appl. Environ. Microbiol., 54, 1070-1074, 1991.

16 Vahtera, E., Conley, D. J., Gustafsson, B. G., Kuosa, H., Pitkanen, H., Savchuk, O. P.,
17 Tamminen, T., Viitasalo, M., Voss, M., Wasmund, N. and Wulff, F.: Internal ecosystem
18 feedbacks enhance nitrogen-fixing cyanobacteria blooms and complicate management in
19 the Baltic Sea, Royal Swedish Academy of Sciences, Ambio., 36, 2-3, 2007.

20 Waldmann, N., Borromei, A. M., Recasens, C., Olivera, D., Martínez, M. A., Maidana, N. I.,
21 Ariztegui, D., Austin, Jr. J. A., Anselmetti, F. S. and Moy, C. M.: Integrated
22 reconstruction of Holocene millennial-scale environmental changes in Tierra del Fuego,
23 southernmost South America, Palaeogeogr. Palaeoclimatol. Palaeoecol., 399, 294-309,
24 2014.

25 Wang, L., Wang, C., Deng, D., Zhao, X. and Zhou, Z.: Temporal and spatial variations in
26 phytoplankton: correlations with environmental factors in Shengjin Lake, China.
27 Environ. Sci. Pollut. Res., 22, 14144-56, 2015.

28 Wennrich, V., Minyuk, P. S., Borkhodoev, V., Francke, A., Ritter, B., Nowaczyk, N. R.,
29 Sauerbrey, M. A., Brigham-Grette, J. and Melles, M.: Pliocene to Pleistocene climate
30 and environmental history of Lake El'gygytgyn, Far East Russian Arctic, based on high-
31 resolution inorganic geochemistry data, Clim. Past, 10, 1381–1399, 2014.

Biogeosciences Discuss., doi:10.5194/bg-2015-633, 2016
Manuscript under review for journal Biogeosciences
Published: 18 January 2016
© Author(s) 2016. CC-BY 3.0 License.

1 Woodward, G., Perkins, D. M. and Brown, L. E. Climate change and freshwater ecosystems:
2 impacts across multiple levels of organization, Phil. Trans. R. Soc. B., 365, 2093-2106,
3 2010.

4 Yin, D. C., Zheng, L. L. and Song, L. R.: Spatiotemporal distribution of phytoplankton in the
5 Dan jiangkou Reservoir, a water source area for the South-to-North Water Diversion
6 Project (Middle Route), China, Chin. J. Oceanol. Limnol., 29, 531–540, 2011.

7 Young, H. S., Mccauley, D. J., Dunbar, R. B., Hutson, M. S., Ter-Kuile, A. M. and Dirzo, R.:
8 The roles of productivity and ecosystem size in determining food chain length in tropical
9 terrestrial ecosystems, Ecology 94, 692–701, 2013.

10 Zeglin, L. H., Bottomley, P. J., Jumpponen, A., Rice, C. W., Arango, M., Lindsley, A.,
11 McGowan, A., Mfombep, P. and Myrold, D. D.: Altered precipitation regime affects the
12 function and composition of soil microbial communities on multiple time scales,
13 Ecology, 94, 2334–2345, 2013.

14 Zhu, F., Massana, R., Not, F., Marie, D. and Vaulot, D.: Mapping of picoeucaryotes in marine
15 ecosystems with quantitative PCR of the 18S rRNA gene, FEMS Microbiol. Ecol., 52,
16 79, 92, 2005.

17 Zimmerman, A. R. and Canuel, E. A.: A geochemical record of eutrophication and anoxia in
18 Chesapeake Bay sediments: anthropogenic influence on organic matter composition. Mar
19 Chem. 69, 117-137, 2000.

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Biogeosciences Discuss., doi:10.5194/bg-2015-633, 2016
Manuscript under review for journal Biogeosciences
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1 **Figures**

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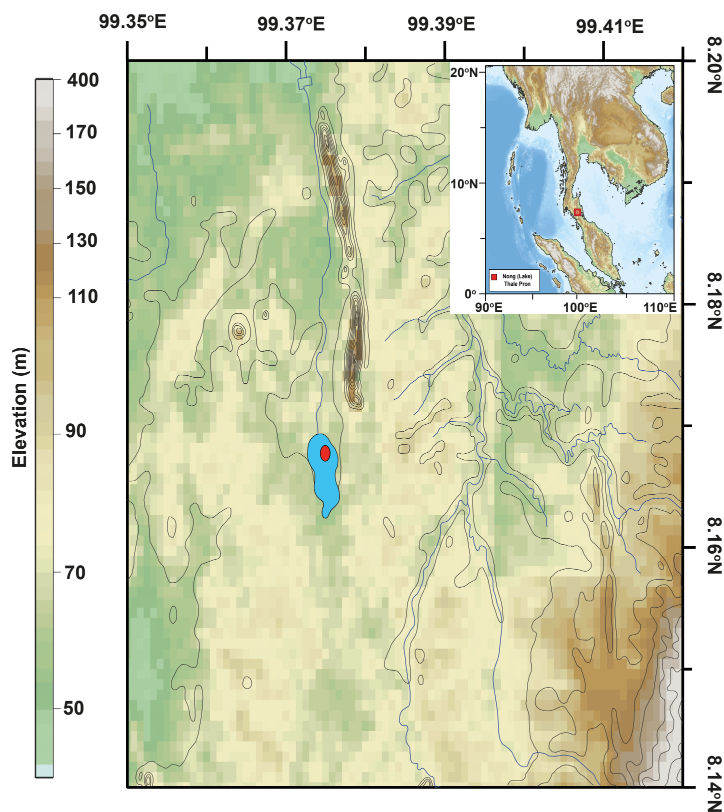

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7 Figure 1. Location of the study area in southern Thailand and topography of Lake Nong Thale
8 Prong (shaded blue). A red circle shows the coring site. For interpretation of the references to
9 color, the reader is referred to the web version of this article

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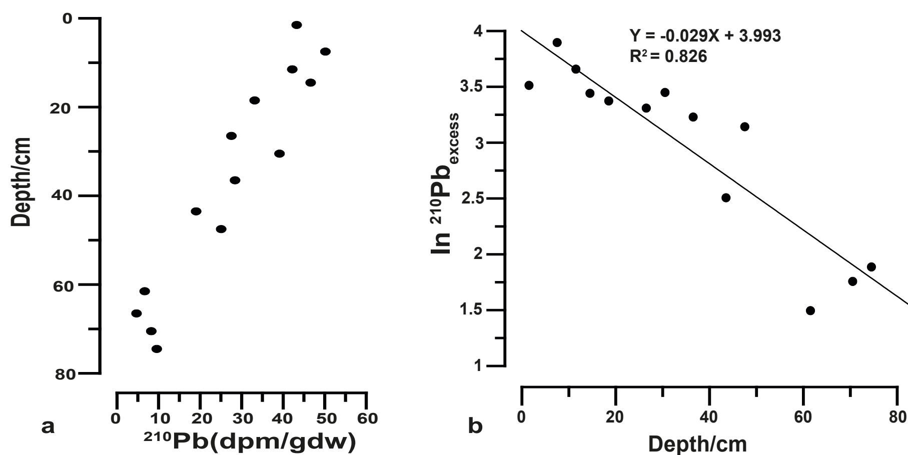

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11 Figure 2. Variations in ^{210}Pb down the sediment core, (a) Depth profile of total ^{210}Pb activity
12 downcore and (b) Correlation between depth and ln $^{210}Pb_{excess}$.

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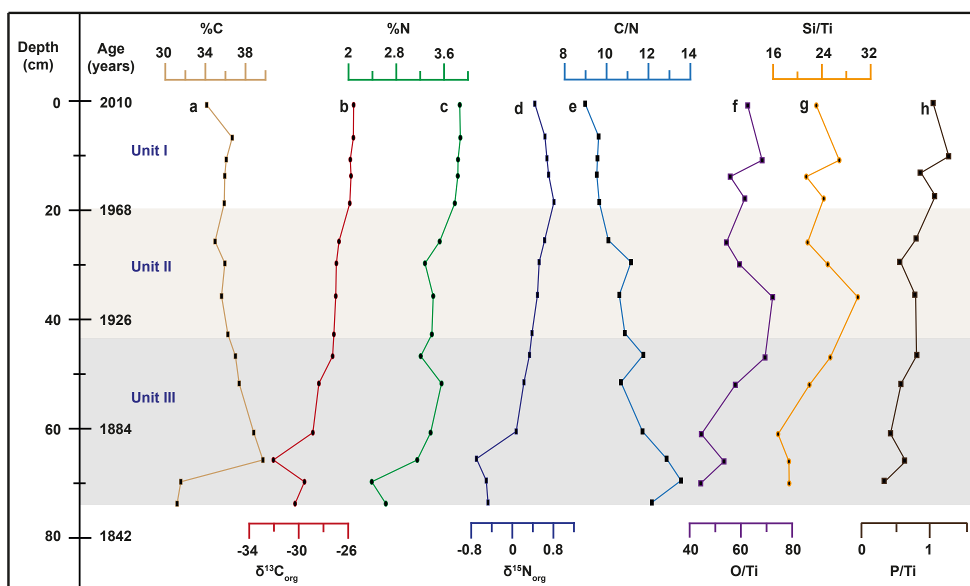

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10 Figure 3. Geochemical data from Lake Nong Thale Pron (NTP) plotted against depth and age
11 (a) TOC (%), (b) $\delta^{13}C_{bulk\ org}$, (c) TN (%), (d) $\delta^{15}N_{bulk\ org}$, (e) C/N, (f) Si/Ti, (g) O/Ti, (h) P/Ti.
12 The shaded boxes represent the transition between the different units I, II and III. For
13 interpretation of the references to color, the reader is referred to the web version of this
14 article.

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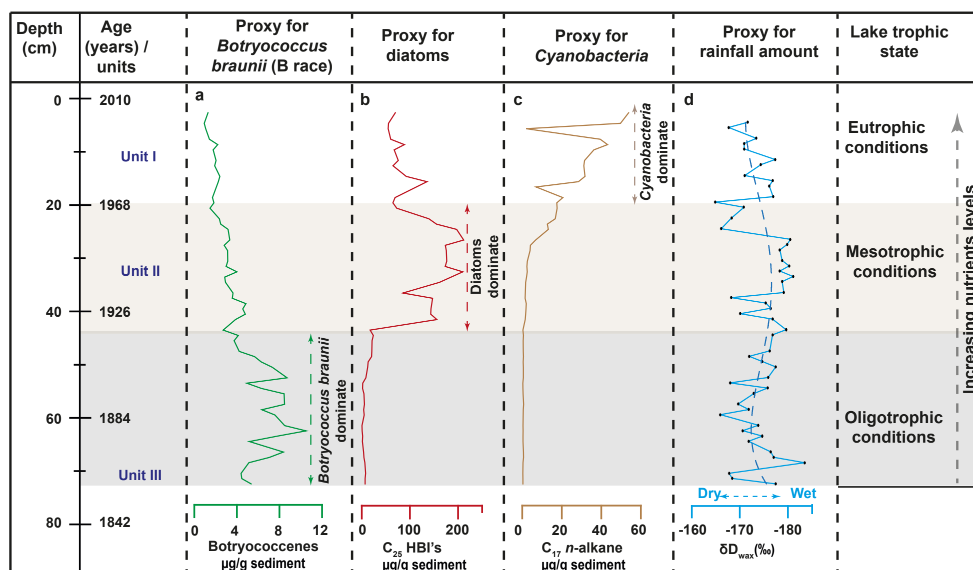

Figure 4. Depth and age profiles of lipid biomarkers, (a) botryococcenes, a proxy for *Botryococcus braunii* (B race) (b) C_{25} Highly branched Isoprenoid (HBIs), a proxy for diatoms (c) C_{17} *n*-alkane, a proxy for *Cyanobacteria* and (d) δD of $C_{27-29-31}$ *n*-alkanes (δD_{wax}), a proxy for rainfall amount. The blue line through the δD_{wax} data set represents a polynomial fitted trendline and the shaded boxes represent the transition between units I, II and III. The last column shows the different trophic changes with time.

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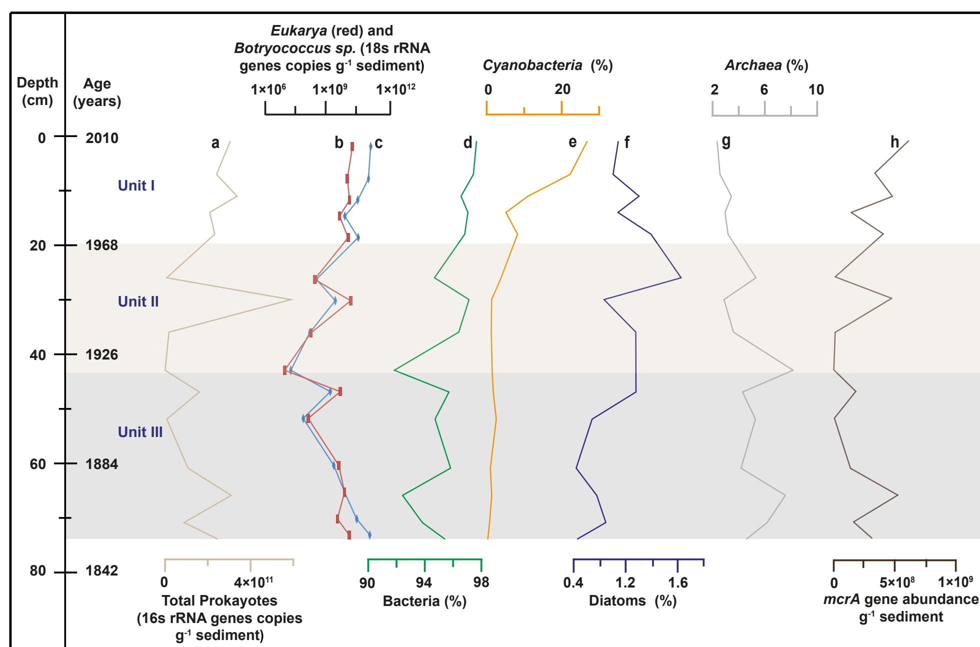

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9 Figure 5. Depth and age profiles for: (a) Total prokaryotes (16s rRNA genes copies g^{-1}
10 sediment), (b) *Eukarya* (18s rRNA genes copies g^{-1} sediment), (c) *Botryococcus sp.* (18s
11 rRNA genes copies g^{-1} sediment), (d) Bacteria (%), (e) *Cyanobacteria* (%), (f) Diatoms (%),
12 (g) *Archaea* (%), (h) *mcrA* gene abundance g^{-1} sediment. The shaded boxes represent the
13 transition between units I, II and III

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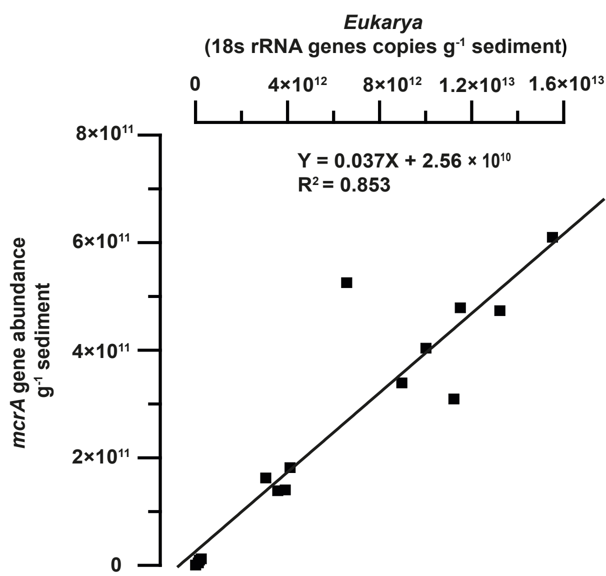
9 

10 Figure 6. Cross plot between *mcrA* gene abundance against *Eukarya* as a proxy for total
11 primary productivity

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