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

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# 1 Abstract

2 Climate and human-induced environmental change promote biological regime shifts between 3 alternate stable states, with implications for ecosystem resilience, function, and services. 4 While these effects have been shown for present-day ecosystems, the long-term response of 5 microbial communities has not been investigated in detail. This study assessed the decadal 6 variations in phytoplankton communities in a *ca*. 150 year long sedimentary archive of Lake Nong Thale Prong (NTP), southern Thailand using a combination of bulk geochemical 7 8 analysis, quantitative polymerase chain reaction (qPCR) and lipid biomarkers techniques 9 including compound-specific hydrogen isotope analysis as a proxy for precipitation. Relatively drier and by inference warmer conditions from *ca.* 1857-1916 Common Era (CE) 10 11 coincided with a dominance of the green algae Botryococcus braunii, indicating lower nutrient levels in the oxic lake surface waters, possibly related to lake water stratification. A 12 13 change to higher silica (Si) input around 1916 CE was linked to increased rainfall and concurs 14 with an abrupt takeover by diatom blooms lasting for 50 years. These were increasingly outcompeted by cyanobacteria from the 1970s onwards, most likely because of increased 15 levels of anthropogenic phosphate and a reduction in rainfall. Our results showcase that the 16 multi-proxy approach applied here provides an efficient way to track centennial-scale 17 limnological, geochemical and microbial change, as influenced by hydroclimatic and 18 19 anthropogenic forcing.

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#### 1 **1 Introduction**

2 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 3 4 al., 2010). The resulting environmental effects easily observed today in plant and animal 5 communities (Scheffer et al., 2001) have been discussed as causing regime shifts (Folke et al., 6 2004) and/or introducing alternative ecosystem states (Dent et al., 2002). Changes in the 7 ecosystem due to climate dynamics, particularly for microbial communities, would likely 8 affect the capture and storage of CO<sub>2</sub> (Zeglin et al., 2013). However, predicting the effects of 9 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 10 11 difficulty of coupling micro-scale external and internal ecosystem variables to specific microbial communities over longer time scales. Consequently, most estimates are based on 12 13 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., 14 2013). Very little is therefore known about how natural microbial ecosystems respond to 15 16 climatic changes over longer time scales and whether the associated feedbacks due to climate shifts promote regime shifts and alternative stable states. Moreover, the impact of external 17 18 processes, such as weathering and runoff intensity, on carbon and nitrogen cycles still remain 19 relatively unknown.

20 Phytoplankton constitutes one of the most ecologically important groups of microorganisms in aquatic ecosystems (Yin et al., 2011) due to their vital position at the base of the food chain 21 22 (Carpenter et al., 1987; Young et al., 2013). As photoautotrophs, their activities are directly 23 coupled to climatic changes through CO<sub>2</sub> drawdown, organic matter availability and oxygen 24 production. Natural and/or human-induced disturbances may thus result in far-reaching consequences for the nutrient status of lakes, where phytoplankton is the primary producer 25 (Yin et al., 2011). For instance, the demise of the botryococcene lipid-producing green algae 26 27 Botryococcus braunii was linked to early eutrophication in a Norwegian fjord (Smittenberg et al., 2005). 28

Studies have shown that phytoplankton communities are sensitive to environmental stressors (Woodward et al., 2010; Häder and Gao, 2015) where variations in phytoplankton community structure, abundance and function have been related to changes in lake trophic status (Ravasi et al., 2012; Hou et al., 2014). Accordingly, the emerging paradigm suggests that the

structuring of phytoplankton communities, characterized by potential successional shifts in 1 2 population dynamics, may serve as a tracer for the trophic status in lakes (Mackay et al., 2003; De Senerpont Domis et al., 2007). However, reconstructing the trophic status of lakes 3 based on shifts in dominant phytoplankton communities over time are yet to be explored in 4 5 detail. Also, the broader linkages between different microbial groups and their response to 6 past environmental conditions are poorly understood. This is partly due to the lack of suitable 7 proxies that can capture and distinguish between the diverse parameters impacting microbial 8 ecosystem structure.

9 Lipid biomarkers specific for various types of microbes provide an important proxy of 10 microbial ecosystem structure and have therefore been employed in the reconstruction of past 11 ecosystems preserved in sedimentary records (Zimmerman and Canuel, 2000; Coolen et al., 2004; Smittenberg et al., 2005). Lipids are an integral part of the cell membranes of both 12 13 prokaryotes and eukaryotes. They aid as structural support and as storage compounds within 14 various microbial cells (Jungblut et al., 2009), and can be specific for certain groups or species. Moreover, lipid biomarkers can incorporate information of the chemical and physical 15 16 environment in which they have formed via their relative abundance or isotopic composition. 17 Because lipids are resistant to postmortem biodegradation, ecological variations through time 18 can be reconstructed (Zimmerman and Canuel, 2000; Coolen et al., 2004; Smittenberg et al., 2005) by tracking their occurrence and abundance over longer time scales. Moreover, when 19 20 coupled to hydroclimate variables such as hydrogen isotopes of leaf waxes ( $\delta D_{wax}$ ), a proxy 21 for precipitation (e.g. Konecky et al., 2013; Niedermeyer et al., 2014), microbial lipids may 22 help underpin the impact of past climates (wetter/drier) on microbial ecosystem changes (see 23 Supplement).

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

30 Here we explore the novel combination of biolipid analysis,  $\delta D_{wax}$ , qPCR, bulk isotopes of 31 carbon and nitrogen, and sedimentary geochemistry to reconstruct phytoplankton community 32 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.

#### 4 2 Materials and Methods

# 5 2.1 Study area, fieldwork, sediment sampling and dating

NTP (17° 11'N, 99° 23'E) is a shallow (<4 m water depth), small (~210 m<sup>2</sup>) sinkhole lake 6 located on the Thai-Malay Penninsula in southern Thailand (Fig. 1) at ~60 m above sea level 7 8 (Snansieng et al., 1976) (see Supplement). Prior to coring, a preliminary assessment of NTP 9 was conducted based on the catchment geology and topography, basin size and water depth. 10 Sounding in different parts of the lake showed that the deepest part (~3.7 m water depth) was 11 in the northeast and that there was little variability in the distribution of sedimentary materials throughout the lake. Two sediment cores (~74 cm length) were retrieved from the deepest part 12 of the lake in January 2012 using an HTH gravity corer (70 mm diameter, 1 m length). The 13 14 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 15 16 other core was archived. The sediment core was sliced onsite into 1 cm sub-samples, packed 17 in sterile plastics bags and chilled with ice. The samples were kept at temperature of about 18 4°C during transportation from Thailand to Stockholm. Such temperatures have been shown to be low enough to inactivate and preserve whole tropical communities in sediments without 19 20 the need for dramatic freezing because of a narrower temperature range of activity (e.g. 21 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 22 sediment sequence was dated using <sup>210</sup>Pb (46.51 keV) and <sup>226</sup>Rn (295.2 keV) on an EG&G 23 ORTEC® co-axial low energy photo spectrometer (LEPS) fitted with a high-purity 24 25 germanium crystal (see Supplement).

#### 26 **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 analysis. For the stable isotope measurements, the samples were pre-treated with HCl to 1 remove carbonate carbon before analysis on a Carlo Erba NC2500 elemental analyzer, 2 coupled to a Finnigan MAT Delta+ mass spectrometer.  $\delta^{13}C_{bulk org}$  and  $\delta^{15}N_{bulk org}$  values are 3 reported in parts per mille (‰) relative to the Vienna PeeDee Belemnite (VPDB, for C) and 4 standard air (for N), respectively, with an analytical error of ±0.15‰. The working standards 5 used for the analysis were: (1) acetanilide ( $\delta^{13}C$ =-27.07‰; %C=71.09%;  $\delta^{15}N$ =1‰; 6 %N=10.36%) and (2) methionine ( $\delta^{13}C$ =-26.23‰; %C= 40.25%;  $\delta^{15}N$ =-2.24‰; %N=9.39%). 7 These standards were calibrated against standards from IAEA.

8 Relative estimates of the chemical composition of the sediments were obtained by elemental 9 mapping using Environmental Scanning Electron Microscopy (FEI, Quanta FEG 650)-Electron Dispersive Spectroscopy (ESEM-EDS). An aliquot of the dried sediment was 10 11 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 12 elemental maps distributed over 15 samples across the entire core were acquired with the 13 14 AZtech software, at a horizontal field width of 2 mm, 512 pixels and an average frame count 15 of 5 with 100 µs pixel dwell time. The relative elemental abundance acquired was normalized 16 to 100%.

#### 17 2.3 Biomarker analysis

Description of the biomarker analysis follows that of Chawchai et al. (2015) and Yamoah et 18 19 al. (2016). Freeze-dried and powdered samples were extracted three times with a mixture of 20 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 21 22 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, 23 24 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 25 26 nitrogen gas, and placed on top of 15 g silica gel (deactivated with 5% (wt.) H<sub>2</sub>O) in 6-mL glass SPE tubes. Hydrocarbon (FI), ketone (F2) and polar (F3) fractions were recovered with 27 28 pure hexane, a hexane and DCM mixture (1:1) and DCM-MeOH (1:1), respectively. F2 and 29 F3 samples were stored in the freezer for later use. The F1 fraction was analyzed on a 30 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<sup>-1</sup> followed 3 by a ramp of 4°C min<sup>-1</sup> until 320°C where it was held for 20 min. MS operating conditions 4 were set to an ion source temperature of 200°C and 70eV ionization energy. Spectra were 5 6 collected using GC solution Workstation software (v2). n-alkanes, C<sub>25</sub> highly branched 7 isoprenoids (HBIs) and botryococcene compounds were identified by retention times and 8 comparison against mass spectra from the literature. Quantification of the *n*-alkanes, C<sub>25</sub> HBIs 9 and botryococcene compounds was done with an external standard consisting of a mixture of 10  $C_{20-40}$  *n*-alkanes of known concentration. Specifics on the mass spectra and retention times of 11 the *n*-alkanes, HBIs and Botryococcenes, including chromatograms as reference, are included 12 in Supplement (Fig. S1-S6).

#### 13 **2.4 δD analysis of leaf waxes**

14 The F1 fraction was further separated into three fractions (F1a, F1b and F1c) over a pipette column filled with 10% AgNO<sub>3</sub>-coated silica gel. F1a, which comprises *n*-alkanes, was eluted 15 16 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). 17 F1b and F1c were also stored in the freezer for further analysis. F1a was analyzed by gas 18 chromatography-isotope ratio monitoring-mass spectrometry (GC-IRMS) using a Thermo 19 20 Finnigan Delta V Plus mass spectrometer interfaced with a Thermo Trace GC 2000 using a 21 GC Isolink II and Conflo IV system. Helium was used as a carrier gas at constant flow mode. 22 A detailed description of the GC-IRMS program is described in Chawchai et al. (2015). 23 Instrumental performance and calibration of the reference gas (H<sub>2</sub>) was achieved by running a 24 standard mixture of *n*-alkanes with a known isotopic composition (reference mixture A4, provided by Arndt Schimmelmann, Indiana University, USA) several times daily. All 25 analyses performed follow that of Sessions et al., (1999, 2001). Results are reported as the 26 weighted mean of triplicate measurements with an average standard deviation of both 27 28 standards and samples around 4‰ (see Supplement).

#### 1 2.5 DNA extraction and qPCR

2 Freeze-dried samples were selected according to initial biomarker screening results, in order 3 to estimate the abundance of different groups of organisms related to: 1) the Prokarya, 4 Archaea and bacteria, cvanobacteria, and microorganisms involved in anaerobic methane cycling (quantification of the mcrA gene, e.g., Hallam et al., 2003, Hallam et al., 2004), 2) 5 6 Eukarya, diatoms, and *Botryococcus* sp. The samples were analyzed in order to specifically 7 reflect the sample conditions used for the biomarker analysis. Freeze-drving was not expected 8 to introduce significant biases but enhances cell breakage and the release of intracellular 9 DNA, following the freeze thaw method of DNA extraction (e.g. Tsai et al., 1991). This is 10 especially useful for soil and sediment samples (e.g. Tsai et al., 1991). Around 0.2 g (from 0.17 to 0.26 g) of freeze-dried sediment was extracted for DNA, using the MoBio PowerSoil<sup>®</sup> 11 DNA kit (Carlsbad, CA), following the manufacturer's instructions. About 500 µL of sterile 12 PBS 1X was also added to PowerSoil<sup>®</sup> Bead tube in order to enhance cell lysis efficiency. 13

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

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

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

The raw data of eukarya, diatoms and *Botryococcus* sp. were not further quantified into copy 3 4 numbers per g sediment, for two reasons: 1) there is high variability in 18S rRNA gene copies 5 per cell within the eukarya and diatoms (i.e. from 3 to more than 25000 copies per cell in the 6 plants ((Prokopowich et al., 2003, Zhu et al., 2005) and between 61 to 36,896 for the diatoms 7 (Godhe et al., 2008)); 2) the paucity of information related to the number of 18S rRNA gene 8 copy number in the Botryococcus sp. genome. Therefore, the results reported here are 9 indicative from the universal eukarya primer and should be considered as relative abundance 10 of the total eukarya due to the tendency of not detecting all eukaryotic groups. Yet, the data 11 are still useful to depict trends in the sediment record (see supplement for limitations of qPCR) 12 methods).

## 13 3 Results

The <sup>210</sup>Pb activity shows an exponential decay curve with depth, i.e. it shows a decreasing linear trend when plotted on a log-scale ( $\ln^{210}Pb_{unsupported}$  vs. depth;  $r^2 = 0.827$ ) (Fig. 2). The profile indicates minimal sediment bioturbation and is used to calculate an average sedimentation rate of about 4.7 mm yr-1.

Biogeochemical and biolipid screening of the sediment core, discussed further below, 18 19 demarcates three distinct periods: ca. 2010-1969 CE, ca. 1969-1916 CE and ca. 1916-1857 20 CE. The sedimentary deposits are highly organic with TOC contents between 30 and 40%. 21 TOC gradually increases from ca. 1857 to 1870 CE and then shows a decrease from ca. 1870 to 2008 CE (Fig. 3a). From *ca*. 1857-1970 CE both the  $\delta^{13}C_{\text{bulk org}}$  (Fig. 3b) and TN (Fig. 3c) 22 show a gradually increasing trend while  $\delta^{15}N_{org}$  values rise steadily between *ca*. 1857 and 23 24 1969 CE and then decreases from ca. 1970-2010 CE (Fig. 3d). The C/N ratio, on the other hand, decreases gradually from the bottom to the top of the sediment core (Fig. 3e). Si/Ti 25 26 ratio, a marker of silicon input into the lake from terrestrial sources, shows an increase between ca. 1916 and 1969 CE (Figs. 3f and g). Scanned photos using ESEM show higher 27 abundances and diversity of diatoms between ca. 1916 and 1969 CE (see Supplement; Fig. 28 29 S6). The P/Ti ratio, which can be used as a proxy for trophic conditions in lakes (Kirilova et 30 al., 2011), show an increase from ca. 1970-2010 CE (Fig. 3g).

Biomarkers and qPCR analysis targeting cyanobacteria, diatoms and botryococcus exhibited 1 2 similar trends, except ca. 1970-2010 CE where botryococcenes were not detected although the qPCR analysis detected the presence of Botryococcus sp. (Fig. 4) (see supplement for 3 detailed qPCR results). The botryococcene lipid concentrations show an increase between ca. 4 5 1857 and 1916 CE (Fig. 4a), followed by a decreasing trend from ca. 1916-2010 CE. The 6 qPCR data set also shows an increase in Botryococcus sp. abundances from ca. 1857-1916 7 CE, a decrease from ca. 1916-1969 CE (Fig. 4b), but then again an increase from ca. 1970-8 2010 CE. The HBIs exhibit minimum levels until 1916, after which they show much higher 9 abundance from ca. 1916-1969 CE, similar to the diatom abundance as detected by qPCR (Fig. 4c and d), and as seen by ESEM. After 1970 they decrease gradually. The  $C_{17}$  *n*-alkanes 10 11 concentrations show a close resemblance to the Cvanobacteria sp. detected by qPCR, with 12 rising concentrations and abundances between ca. 1970 and 2010 CE (Figs. 4e and f). The 13 hydrogen isotopic composition of leaf waxes ( $\delta D_{wax}$ ; weighted mean of  $\delta D C_{27-31}$  *n*-alkanes) shows a long-term oscillation over the entire 150-year record (Fig. 4g). δD<sub>wax</sub> values have 14 15 been used as a proxy for rainfall intensity such that lower and higher values indicate an increase and decrease in rainfall intensity, respectively (e.g., Konecky et al., 2013; 16 17 Niedermeyer et al., 2014). Reconstructed hydroclimatic conditions for southern Thailand over 18 the last 150 years based on  $\delta D_{wax}$  values show a relatively drier period from *ca*. 1857-1916 19 CE and ca. 1970-2010 CE and relatively wetter conditions from ca. 1916-1969 CE.

#### 20 4 Discussion

21 The bulk geochemical trends (Fig. 3) indicate that multiple processes control the organic matter (OM) input into Lake NTP. Decreasing C/N ratios with TOC contents of 22 23 approximately 30% from the bottom to the top of the core suggests organic matter diagenesis. which indicates an aquatic dominated system (Figs. 3a and b). The observed decreasing trend 24 of the C/N ratio, as well as increasing  $\delta^{13}C_{org}$  values, may be explained by a preferential 25 (anoxic) mineralization of nitrogen-rich OM, leading to residual OM with a higher C/N ratio 26 27 (Altabet, 1998; Sachs and Repeta, 1999; Emerson and Hedges, 2003). This explanation would 28 be consistent with the long-term diagenetic OM transformation (Sun et al., 2004). However, 29 successional deposition of different phytoplankton communities, botryococcus (ca. 1857-1916), diatoms (ca. 1916-1969) and cyanobacteria (ca. 1970-2010), with different C/N ratios, 30  $\delta^{13}$ Corg, and  $\delta^{15}$ N<sub>org</sub> can also influence the bulk profiles. 31

An alternate interpretation of the increasing  $\delta^{13}C_{org}$  values from the bottom to the top of the core lies in a successional shift of the trophic status of the lake (Brenner et al., 2000; Meyers and Teranes, 2001). Increasing nutrient levels promote primary productivity, thereby depleting the amount of dissolved inorganic carbon. This could lead to lower net fractionation against <sup>13</sup>C by the phytoplankton during photosynthesis, thereby increasing the  $\delta^{13}C_{org}$  values (Meyers and Teranes, 2001).

7 Observed variations of dominant phytoplankton community over time can be explained best 8 by changes in the trophic level of the lake. The period from *ca*. 1857 to 1916 CE is marked by 9 significant increases in both *Botrvococcus* sp. abundance (Fig. 4a) and botrvococcene lipids 10 (Fig. 4b), which also corresponds with slightly lower precipitation (Fig. 4g). Botryococcus 11 *braunii* has been suggested to thrive in relatively oligotrophic conditions (Souza et al., 2008) 12 and can, therefore, be used as a proxy for such conditions within the oxygenated epilimnion 13 (Waldmann et al., 2014). Although the highly organic sediment may suggest a productive 14 lake, at variance with oligotrophic lake water conditions, we argue that the final TOC content in lake sediments, especially in the tropics, is more determined by OM preservation than by 15 the incoming flux. In the tropics, the mean air temperature (MAT) is a direct result of 16 incoming solar radiation, and the relationship between the MAT and the amount of rainfall 17 18 are typically inversely proportional (Imboden and Wüest, 1995; Boehrer and Schultze, 2008). 19 Dry, cloudless and warmer conditions lead to stronger stratification in freshwater lakes 20 (Imboden and Wüest, 1995). We hypothesize that a long-term decrease in rainfall and slightly 21 higher temperatures would result in a thermal stratification of the lake. Under stratified 22 conditions reformed nutrients would remain in the anoxic hypolimnion. As a result, the 23 surface water remained relatively oligotrophic enabling Botryococcus braunii to thrive whereas the bottom of the lake was characterized by bacterial breakdown of organic N. This 24 argument is supported by increasing  $\delta^{15}$ N<sub>org</sub> values from *ca*. 1857 to 1916 CE, which suggests 25 extensive denitrification in the lake sediment, as denitrification typically leads to more 26 depleted  $\delta^{15}$ N values in the residual organic matter (Wada, 1980). 27

A stark difference in the dominant phytoplankton community is observed from *ca*. 1916-1969 CE. This period is marked by significant decrease in *Botryococcus* sp. gene abundance (Fig. 4a) and botryococcene lipid concentrations (Fig. 4b) while diatom abundance (Fig. 4c) and  $C_{25}$  HBIs concentrations (Fig. 4d), which is a useful indicator of diatom-derived OM inputs to sediments (McKirdya et al., 2013) increases. More directly, we observed a marked increase in

diatom diversity on the ESEM image scans of the sediments (Supplement; Fig. S6). Diatoms 1 2 dominate phytoplankton communities as long as there is abundant silica irrespective of changes in environmental conditions and nutrient levels (Egge and Aksnes, 1992). 3 Interestingly, the increase in diatom markers (Fig. 4c and d) coincides with an increase in 4 5 reconstructed rainfall intensity (Fig. 4g). Moreover, the increase in Si/Ti ratios (Fig. 3f), a run-off signal (Murphy et al., 2000), coincides with high diatom blooms, in particular 6 7 between ca. 1916 and 1969 CE. Since Ti is a highly immobile element, weathering and 8 transportation of Si is not accompanied by significant Ti delivery to aquatic basins. Therefore, 9 the Si/Ti ratio can serve as a proxy for nutrient dynamics linked to hydrological changes (e.g. 10 Cartapanis et al., 2014) and as an indicator for enhanced diatom production in lakes 11 (Wennrich et al., 2014). Altogether, it appears that the wetter conditions between ca. 1916 12 and 1969 CE increased catchment runoff into the lake, leading to elevated nutrient and silicate 13 mineral flux to the lake water (e.g. Paerl et al., 2006). In line with the argument above, it is 14 also well possible that an increase in precipitation was accompanied by cooler temperatures, 15 which then led to a decrease in thermal stratification and an increase in mixing between the epilimnion and hypolimnion, making reformed nutrients from the bottom waters available at 16 the lake water surface. 17

18 After *ca.* 1969 CE, the phytoplankton community structure changed again, with a diminishing 19 role for diatoms as evidenced by lower concentrations of C<sub>25</sub> HBIs (Fig. 4c) and the start of a marked increase in cyanobacteria gene numbers (Fig. 4e) and C<sub>17</sub> n-alkane concentrations 20 21 (Fig. 4f). C<sub>17</sub> *n*-alkanes are recognized biomarkers of aquatic algae and photosynthetic bacteria such as cyanobacteria (Meyers, 2003). Indeed, cyanobacteria gene numbers relative 22 23 to bacteria quantification based on the qPCR data covary strongly with  $C_{17}$  *n*-alkane 24 concentration, which indicates that the  $C_{17}$  *n*-alkanes were likely produced by cyanobacteria. Additionally, the decreasing  $\delta^{15}N_{\text{bulk org}}$  values during this period (Fig. 3d) also suggest 25 26 nitrogen fixation, a process strongly associated with cyanobacteria blooms (Vahtera et al., 27 2007). The most recent period (ca. 1969-2010 CE) is also characterized by somewhat lower 28 rainfall amounts (Fig. 4g). The amount of Si indeed decreased, and diatoms became less 29 abundant, allowing non-diatomaceous phytoplankton not dependent on Si to take over (Egge 30 and Aksnes, 1992).

The number of botryococcus genes increased again (Fig. 4a). However, this was not accompanied by the production of botryococcene lipids. This mismatch can be the result of

the primer used for the quantification of Botryococcus braunii not being specific enough. 1 2 Botryococcus braunii are classified into three races: A, B, and L, and it is only the B race that produces botryococcenes (Eroglu et al., 2011) whereas the A and L races produce long-chain 3 4 alkadienes and lycopadienes, respectively (Metzger and Largeau, 2005). A change in race 5 thus may explain the disappearance of botryococcenes from the upper part of the sediment 6 record. Since the qPCR primers for botryococcus were not specific enough, it is also possible 7 that it picked up closely related green algae that took over the ecological niche of *B. Braunii* 8 under different trophic conditions. The absence of long-chain alkadienes and lycopadienes in 9 the upper sediment layers supports this argument and indicate that conditions had indeed 10 become unfavorable to *B. Braunii*, in a similar way as described by Smittenberg et al. (2005).

11 Phosphorus bioavailability is one of the most important factors limiting aquatic cyanobacteria 12 blooms (Paerl and Fulton, 2006; Paerl and Valerie, 2012), and the shift to cyanobacterial 13 prevalence thus suggests eutrophic phosphorous-rich conditions, instead of the oligotrophic-14 like conditions that occurred a century earlier under otherwise similar climatic conditions. Indeed, higher levels of phosphorus were detected in the upper part of the sediment (Fig. 3g). 15 The source of the elevated phosphorus is unclear but has likely resulted from human 16 activities. Under a land development program in the 1990s, more than 20% of Thailand's 17 56,000 villages were located in forest reserves (Grav, 1991; Puri, 2006), which allowed the 18 19 expansion of land encroachment and agricultural activities. For instance, southern Thailand has seen an increase in the cultivation of rubber trees on small farms at rates above 7% yr<sup>-1</sup> 20 (Leturque and Swiggings, 2011). The use of fertilizers during farming activities. untreated 21 22 wastewater effluents and the use of detergents are likely sources of the elevated phosphorus 23 inputs into the lake (Litke, 1999; Chislock et al., 2013). These accelerated the eutrophic state of the lake beyond the natural rate of nutrient enrichment, which takes centuries to achieve 24 25 (Litke, 1999).

According to our analysis, photosynthetic primary production is at the base of internal organic matter production in the lake. However, changes in precipitation and anthropogenic nutrient input have produced fluctuations in dominant primary producer communities over the last 150 years, from botryococcene lipid-producing algae to diatoms to a dominance of cyanobacteria. Overall, we suggest that NTP is sensitive to environmental stressors and that multiple processes discussed above control the OM input and carbon storage. The bulk geochemical analysis was clearly inadequate to disentangle the processes that have been controlling the limnological, ecological and microbial dynamics in NTP. Instead, the combination of bulk
 geochemistry, lipid biomarkers, and molecular DNA analysis could appropriately constrain
 many of these processes.

#### 4 **5** Summary and conclusion

5 Validation of paleoenvironmental and paleoclimatic proxies is necessary to constrain 6 geochemical patterns through time. The coupled lipid biomarker and qPCR data allowed 7 different microbial groups in the lake sediments to be distinguished and quantified, leading to 8 the identification of meaningful biological relationships between the phytoplankton 9 community structure response to either anthropogenic or natural environmental changes over 10 the last 150 years.

11 Our results show that between ca. 1857 and 1916 CE, relatively drier climate in southern 12 Thailand coincided with relatively oligotrophic surface water conditions in Lake NTP, which allowed Botryococcus braunii species to bloom. From ca. 1916-1969 CE, an increase in 13 14 precipitation resulted in higher Si input into the lake, which led to a rapid takeover by diatoms 15 as primary producers. Since the 1970s, many aspects of the initial limnic state returned upon drier conditions, except that anthropogenic impact led to an increase in phosphorus thereby 16 17 allowing cyanobacteria to become a major contributor to primary productivity. Although the qPCR method did again detect genetic evidence for the presence of Botryococcus sp. over the 18 19 last decades, the specific lipid biomarkers for Botryococcus sp. were not found anymore, which can be due to limitations of either proxy. 20

We conclude that the combination of geochemical and lipid biomarker proxies together with qPCR analyses is a useful approach that has the potential to assist in tracking the effects of changing climate on primary producers and also to assess these effects on the carbon cycle. This multi-proxy approach may help refine the knowledge about the use and shortcomings of the different proxies, which is critical for their interpretation especially when used on a more stand-alone basis.

27

#### 28 Author contributions

29 This study was conceived and led by K. A. Yamoah, E. Chi Fru and R. H. Smittenberg. K. A.

30 Yamoah, N. Callac, A. Wiech and A. Chabangborn carried out laboratory analyses. K. A.

31 Yamoah, N. Callac, E. Chi Fru, B. Wohlfarth and R. H. Smittenberg wrote the manuscript.

1 All authors discussed the results and their implications and commented on the manuscript as it

2 progressed.

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# 2 Figures



southern Thailand and topography of Lake Nong Thale Prong (shaded blue). A red circle
shows the coring site. For interpretation of the references to color, the reader is referred to the
web version of this article.



Figure 2. Variations in <sup>210</sup>Pb down the sediment core, (a) Depth profile of total <sup>210</sup>Pb activity
 downcore and (b) Correlation between depth and ln <sup>210</sup>Pb<sub>excess</sub>.



Figure 3. Geochemical data from Lake Nong Thale Pron (NTP) plotted against depth and age (a) TOC (%), (b)  $\delta^{13}C_{\text{bulk org}}$ , (c) TN (%), (d)  $\delta^{15}N_{\text{bulk org}}$ , (e) C/N, (f) Si/Ti and (g) P/Ti. The dashed lines represent the transition between the different units I, II and III.



Figure 4. Depth and age profiles of: (a) *Botryococcus* sp. (18s rRNA genes copies  $g^{-1}$ sediment) (blue dashed), (b) botryococcenes, a proxy for Botryococcus braunii (B race) (blue solid), (c) diatoms (%) (purple dashed), (d) C<sub>25</sub> Highly branched Isoprenoid (HBIs), a proxy for diatoms (red solid), (e) cyanobacteria (%) (mustard dashed), (f) C<sub>17</sub> n-alkane, a proxy for cyanobacteria (brown solid) and (g)  $\delta D$  values of C<sub>27-29-31</sub> *n*-alkanes ( $\delta D_{wax}$ ), a proxy for rainfall amount (green). The black dashed lines represent the transitions between the different units. For interpretation of the references to color, the reader is referred to the web version of this article.