Distributions and sources of isoprenoidal GDGTs in Lake Lugano and other central European (peri-)alpine lakes: Lessons for their use as paleotemperature proxies

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ARTICLE INFO

ABSTRACT

Isoprenoidal glycerol dialkyl glycerol tetraether (isoGDGT) lipids occur ubiquitously in freshwater and marine environments. Since their distribution varies with temperature, sedimentary isoGDGTs have been used as proxies for the reconstruction of past continental climate for almost two decades. Yet, their application in lacustrine sediments is still not well constrained because the niches of isoGDGT-producing microorganisms in lakes are often ill-defined. Here, we study the distribution of isoGDGTs and their hydroxy derivatives (OH-isoGDGTs) in the water column of the deep (288 m), meromictic northern basin of Lake Lugano (Switzerland) using quantitative analysis of various pools of isoGDGTs and the stable carbon isotopic composition of isoGDGT-derived biphytanes. We provide strong evidence for archaeal water column sources of the isoGDGTs, based on comparison of lipid data with microbial diversity determined by 16S rRNA next generation sequencing. We find highest concentrations (i.e., 40 ng L^{-1}) of crenarchaeol, the isoGDGTs specific for thaumarchaea, in suspended particle matter (SPM) from deeper (30−100 m) waters below the thermocline. This correlates well with thaumarchaeal 16S rRNA gene abundances, comprised by a single thaumarchaeote of the order Nitrosopumilales. The concentrations of OH-isoGDGT with 0−2 cyclopentane rings follow this profile, suggesting an identical archaeal source. In the deeper anoxic waters, the archaeal community changes substantially and was comprised of various members of the Bathyarchaeota, Diapherotrites, Euryarchaeota, and Woesearchaeota. This change is accompanied by a changing distribution of isoGDGTs with a high contribution of GDGT-0 with a polar head group, and a more negative δ^{13}C value of the acyclic biphytane derived thereof. Comparison of the isoGDGT composition (distribution and δ^{13}C) in the surface sediment with that of the sinking particle flux studied over 1 year at three depths indicates substantial downward transport of isoGDGTs to the sediments from the waters between the thermocline and the anoxic hypolimnion, but not from the deeper (>100 m) waters. The relatively low value for the isoGDGT-based TEX$_{86}$ value (ca. 0.40) in the surface sediment is similar to that of the *in-situ* produced isoGDGTs in the waters below the thermocline, and is consistent with the year-around low water temperatures (<6 °C). We also analyzed the isoGDGT composition in surface sediments of 36 additional (peri-)alpine lakes in Central Europe; their isoGDGT concentration and distribution show a clear-cut difference based on lake size. In relatively large and deep lakes, like the Lake Lugano North Basin, the crenarchaeol concentration was much higher than in small-sized and shallow lakes, because Nitrosopumilales spp. have seemingly no niche in shallower waters (0 −~30 m depth), and production, and hence preservation in the sedimentary record, of crenarchaeol (and other thaumarchaeal isoGDGTs) is much lower than in large lakes. We argue that in the mid-latitudinal large and deep (peri-)alpine lakes Nitrosopumilales spp. generally thrive in cold waters below the thermocline, where temperature is largely not affected by fluctuations in mean annual air temperature. Therefore, the TEX$_{86}$ paleothermometer is relative insensitive to atmospheric temperature changes in

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these lakes. In the small-sized and shallow lakes and ponds crenarchaeol can still be present but is probably mainly derived from Nitrososphaerales spp. (either from *in-situ* production or soil erosion). These thaumarchaea are characterized by a higher relative content of the crenarchaeol isomer than the Nitrosopumilales spp. at the same temperature, which may compromise accurate paleotemperature estimations using the TEX$_{86}$ for small/shallow lakes.

1. Introduction

Lake sediments represent an important type of terrestrial climate archive for molecular fossils because they are widely distributed, often contain substantial amounts of well-preserved organic matter, and fast sedimentation allows for high temporal resolution studies (see Castañeda and Schouten, 2011 for a review). To generate long and continuous time series, however, lakes have to be sufficiently deep to not fall dry intermittently due to lake level variations. Molecular fossils such as glycerol dialkyl glycerol tetraether (GDGT) lipids provide promising records of environmental temperature, and are increasingly used for the assessment of past climate conditions in lakes (see Schouten et al., 2013 for a review). Isoprenoidal GDGTs (isoGDGTs) derived from a variety of archaea were first used to determine paleo sea surface temperature using the TEX$_{86}$ (TetraEther indeX applied to isoGDGTs with 86 carbon atoms; Schouten et al., 2002). This ratio reflects their degree of cyclization and is based on a ratio of four isoGDGTs: those with 1−3 cyclopentane moieties and an isomer of crenarchaeol. Crenarchaeol (Sinninghe Damsté et al., 2002; Holzheimer et al., 2021) is a structurally deviating isoGDGT in comparison to other archaeal isoGDGTs since it contains one six-membered ring and is produced by a special clade of Archaea, the Thaumarchaeota (see for a recent overview Bale et al., 2019). Crenarchaeol is always accompanied by an isomer, for which the exact stereochemical structure is not fully resolved (Liu et al., 2018; Sinninghe Damsté et al., 2018). A few years after the development of the TEX$_{86}$, isoGDGTs were also detected in the surface sediments of four lakes, and its relationship with lake surface temperature (LST) revealed that it followed the TEX$_{86}$ calibration in the marine environment (Powers et al., 2004). Subsequently, TEX$_{86}$ calibrations for lakes have been expanded (Blaga et al., 2009; Powers et al., 2010; Tierney et al., 2010a; Castañeda and Schouten, 2011), and successfully used to reconstruct the lake surface temperatures in Africa (e.g., Powers et al., 2005; Tierney et al., 2008, 2010a; Woltering et al., 2011; Johnson et al., 2016), Europe (Blaga et al., 2013) and elsewhere (Wang et al., 2015).

IsoGDGTs in lakes are typically produced by thaumarchaea, which vary their isoGDGT distribution as a response to temperature. However, apart from the crenarchaeol isomer, the other three isoGDGTs are also produced by some other archaeal clades (see Schouten et al., 2013 for a review). Additional sources of these isoGDGTs may thus affect the relationship between their distribution and temperature, hampering the successful application of the TEX$_{86}$ proxy in lakes (e.g., Sinninghe Damsté et al., 2012; Morissey et al., 2018; Yao et al., 2019; Daniels et al., 2021). An important additional source of isoGDGTs in lakes was initially thought to be eroded soil organic matter (Blaga et al., 2009). The distribution of isoGDGTs in soils is often quite different from that in lakes (e.g., Tierney et al., 2012), and the contribution of eroded soil organic matter could thus alter the TEX$_{86}$ value. Although terrestrial soils mainly contain another type of GDGTs, the so-called branched (br) GDGTs, isoGDGTs are also present, and their distribution often deviates from those found in thaumarchaea due to contributions from methanogenic and other archaea (Weijers et al., 2006).

Because soils often have high BIT ratios (i.e., the ratio of brGDGTs and crenarchaeol; Hopmans et al., 2004), Blaga et al. (2009) used this ratio in their survey of European lake sediments to determine for which lakes TEX$_{86}$ paleothermometry could be applied with confidence. However, it has subsequently been shown that brGDGT are, likely, predominantly produced *in situ* in the water column and sediments of lakes (e.g., Buckles et al., 2014; Weber et al., 2017, 2018; van Bree et al., 2020). Therefore, the BIT index can no longer be used to assess the contribution of soil organic matter to lake sediments, and thus, neither for assessing the applicability of TEX$_{86}$ in lake settings.

A second source of isoGDGT of non-thaumarchaeal origin may be formed by other isoGDGT-producing archaea residing in the water column or sediments of a lake. Blaga et al. (2009) cautioned that the production of isoGDGTs by methanogenic archaea in the water column or sediments could alter the distribution of isoGDGT, preventing reliable applications of the TEX$_{86}$ paleothermometer. Combined molecular ecological and lipid biomarker work in Lake Chala (a stratified anoxic crater lake on the flanks of the Kilimanjaro in equatorial Africa) revealed an active nitrifying population of thaumarchaea just above the chemocline (Buckles et al., 2013). The anoxic deep waters, however, contained a population of uncultured archaea classified as crenarchaeal groups 1.2 (52%) and MCG (41%). This archaeal population in deep waters produced predominantly GDGT-0, mainly in the form of an intact polar lipids (IPLs) containing one or more polar head group(s) attached to the glycerol group(s). A subsequent, time-resolved study of the water column of Lake Chala also identified GDGT-0 containing IPLs with phosphatidylglycerol, monohexose-phosphatidylglycerol and dihexose-phosphatidylglycerol head-groups in the deep waters of the lake (Baxter et al., 2021). Variable contributions of these archaea thriving in the deep waters may thus explain the large variations in the GDGT-0 over crenarchaeol ratio observed in the 25 kyr sedimentary record of Lake Chala (Sinninghe Damsté et al., 2012).

Another complicating factor for the application of the TEX$_{86}$ paleothermometer in lakes is the niche where thaumarchaea predominantly reside in the water column. Several studies (e.g., Sinninghe Damsté et al., 2009; Blaga et al., 2011; Schouten et al., 2012; Woltering et al., 2012; Zhang et al., 2016; Kumar et al., 2019; Yao et al., 2019) have indicated that thaumarchaea thrive in deeper waters and may thus record temperatures that are lower than LST. Furthermore, thaumarchaea may only occur during a specific period of the seasonal cycle and, hence, the temperature that their fossilized lipids record is not necessarily reflective of the mean annual air temperature (MAAT). A unique dataset comprising 1.5 year of high resolution suspended particulate matter (SPM) profiles, and a monthly-resolved sediment trap record of almost a decade in Lake Chala revealed only occasional "blooms" of thaumarchaea, followed by long periods when thaumarchaea were nearly absent, with obviously severe consequences for application of the TEX$_{86}$ paleothermometer (Baxter et al., 2021).

In order to unravel the sources and niches of isoGDGT-producing archaea in mid-latitude lakes, and their impact on the use of the TEX$_{86}$ paleothermometer, we investigated the distribution and stable carbon isotopic composition of isoGDGTs in SPM in the water

column of the meromictic northern basin of Lake Lugano (Switzerland). We compared the vertical distribution and abundance of archaeal 16S rRNA genes with that of individual isoGDGTs. Our results provide strong evidence for aquatic isoGDGT synthesis by spatially segregated microbial communities thriving under both oxic and microoxic/anoxic conditions. We, furthermore, show prevalent export of subsurface- but not deep water-derived isoGDGTs to sediments by examination of isoGDGT composition of the sinking particle flux at three positions in the water column of Lake Lugano over the annual cycle. Finally, we examined the distribution of isoGDGTs in surface sediments of 36 additional lakes in the European Alps. Our results in relationship with literature data provide important clues for the interpretation of TEX_{86} proxy records in lakes.

2. Materials and methods

2.1. Sampling

Suspended particulate matter (SPM) was collected from the northern basin of Lake Lugano at a site close to the depocenter (Fig. 1) by means of large-volume (~50–300 L) filtration of lake water using an in-situ pump in September 2014. We used a double layer of glass fiber filters (2.7 and 0.7 μm; Whatman GF/D and GF/F). The filters were frozen on dry ice immediately after sampling and subsequently stored at −80 °C until further processing (Weber et al., 2018). Settling particles were collected at the same location, using cylindrical sediment traps (diameter = 10 cm) deployed in 1990/1991 at three depths (20, 85, and 176 m), and were sampled every 10–30 days depending on the season.

Surface sediment from a variety of (peri-) alpine lakes from central Europe (Table 1; Fig. 1) were obtained by gravity coring during multiple field campaigns (2008–2014), mostly at the depocenters of the lakes. The obtained cores were then subsampled, and the core-tops (upper 0.5–5 cm) were analyzed. For several lakes, homogenized surface soils from a variety of locations in the catchment of the lake were also obtained (Weber et al., 2018). Samples were homogenized and freeze-dried prior to extraction.

2.2. Lipid extraction and isoGDGT analysis

A sequential solvent extraction and acid hydrolysis procedure to enable improved recovery of isoGDGTs from living cells (intact polar isoGDGTs) and to avoid losses and biases associated with modified Bligh-Dyer extraction was applied. For a detailed description see Weber et al. (2017). Briefly, the freeze-dried SPM was extracted with methanol and dichloromethane as solvents, and the residual material was subsequently subjected to acid-hydrolysis. To maximize lipid recovery for compound-specific stable C isotope analysis of isoGDGTs, SPM from selected depths (filtered from ~150 L of lake water) was subjected to acid hydrolysis, prior to ultrasonic solvent extraction. Sediments and soils were extracted three times by accelerated solvent extraction (ASE300; Thermo Fisher Scientific). IsoGDGT-containing lipid fractions were prepared as previously described (Weber et al., 2017), spiked with an internal standard (Huguet et al., 2006), and analyzed by ultra-high performance liquid chromatography - positive ion atmospheric pressure chemical ionization - mass spectrometry (UHPLC-APCI-MS) in selected ion monitoring mode (SIM). Analytical separation of GDGTs was achieved using two UHPLC silica columns in series (BEH HILIC columns, 2.1 × 150 mm, 1.7 μm; Waters) following the method of Hopmans et al. (2016). Concentrations

were determined by integration of peak areas of the MH^+ ion chromatograms of the internal standard and the GDGT of interest. Fractional abundances of isoGDGTs were calculated on basis of the sum of crenarchaeol and its isomer (Cren'), GDGT-0, -1, -2, and -3 (for structures see the overview of Schouten et al., 2013). The BIT index was calculated according to DeJonge et al. (204).

The TEX_{86} was calculated according to Schouten et al. (2002):

$$TEX_{86} = \{[GDGT-2] + [GDGT-3] + [Cren']\}/\{[GDGT-1] + [GDGT-2] + [GDGT-3] + [Cren']\}$$

(1)

The relative abundance of the isomer of crenarchaeol was calculated as follows:

$$\%Cren' = [Cren']/\{[crenarchaeol] + [Cren']\}*100 \quad (2)$$

Fractional abundances of OH-isoGDGTs were calculated based on the sum of the OH-GDGT-0, OH-GDGT-1, and OH-GDGT-2 (for structures see Liu et al., 2012). The RI-OH' index (representing the average number of cyclopentane moieties) was calculated according to Lü et al. (2015):

$$RI-OH' = \{[OH-GDGT-1] + 2*[OH-GDGT-2]\}/[OH-GDGTs]$$

(3)

Concentration- and depth-integrated values for proxy values in SPM were calculated by taking into account the concentration of the isoGDGTs of interest in a specific water mass and the thickness of that water mass.

2.3. Molecular stable carbon isotope analyses

Stable carbon isotope analysis of isoGDGTs was performed following procedures described earlier (Weber et al., 2015, 2018). Biphytanes released by ether cleavage with HI (Schouten et al., 1998) and converted to hydrocarbons by reduction with hydrogen of the formed iodides were dissolved in n-hexane and injected at 70 °C on a 50 m low-bleed Rxi®-5 ms GC column (0.2 mm iD, 0.33 μm df, Restek, USA). After 2 min hold time, the temperature was rapidly increased to 220 °C at 20 °C min⁻¹. Then this temperature was held for 45 min, and subsequently increased at 4 °C min⁻¹ to 320 °C (held for 35 min). The stable carbon isotopic composition of the biphytanes was calibrated by separate analysis of an n-alkane mixture with known isotopic compositions (mixture B3 provided by A. Schimmelmann, Indiana University, USA). GC/C/IRMS performance was monitored by regular analysis of this standard mixture.

2.4. Molecular biological methods

DNA was extracted using a modified phenol chloroform isoamyl alcohol protocol (Deiner et al., 2015) from parts of GF filters, equivalent to 2–7 L of filtered lake water as described earlier (Weber et al., 2018). Briefly, a two-step PCR approach was employed for DNA amplification of prokaryotes using the universal primers 515F–Y and 926R targeting the V4 and V5 regions of the 16S rRNA gene (Parada et al., 2015). The individual samples were indexed, pooled at equimolar concentrations, and sequenced on an Illumina MiSeq using the 2 × 250PE protocol. A total of 1,741,418 × 2 paired raw reads were obtained from 15 SPM samples. After initial quality control and bioinformatical processing (see
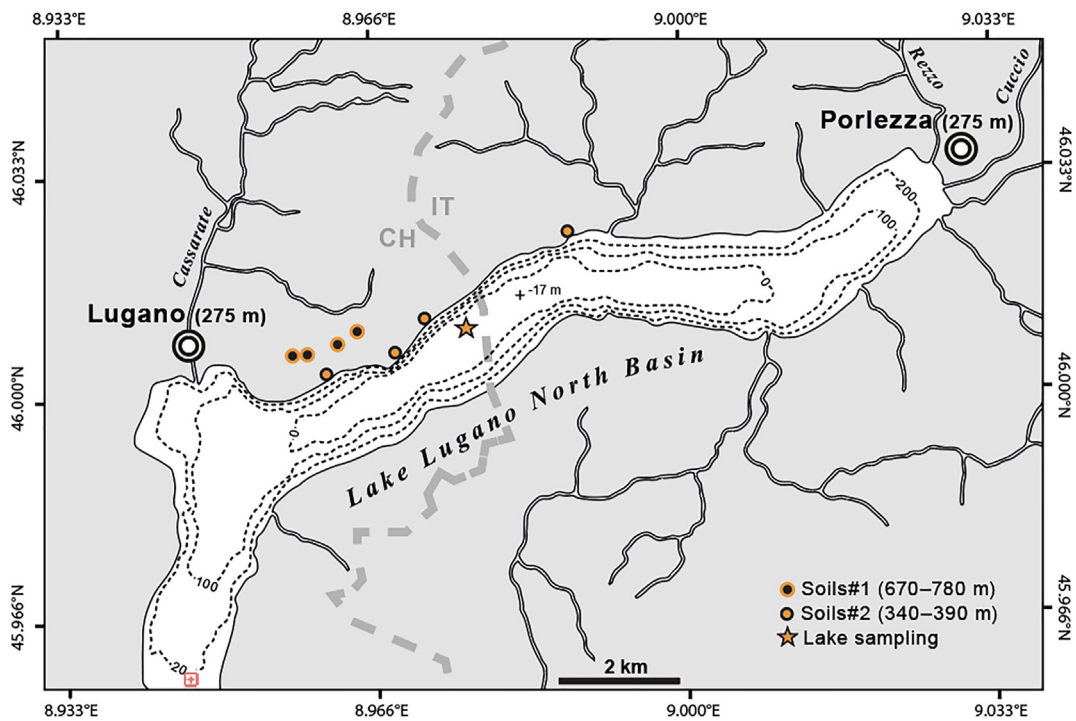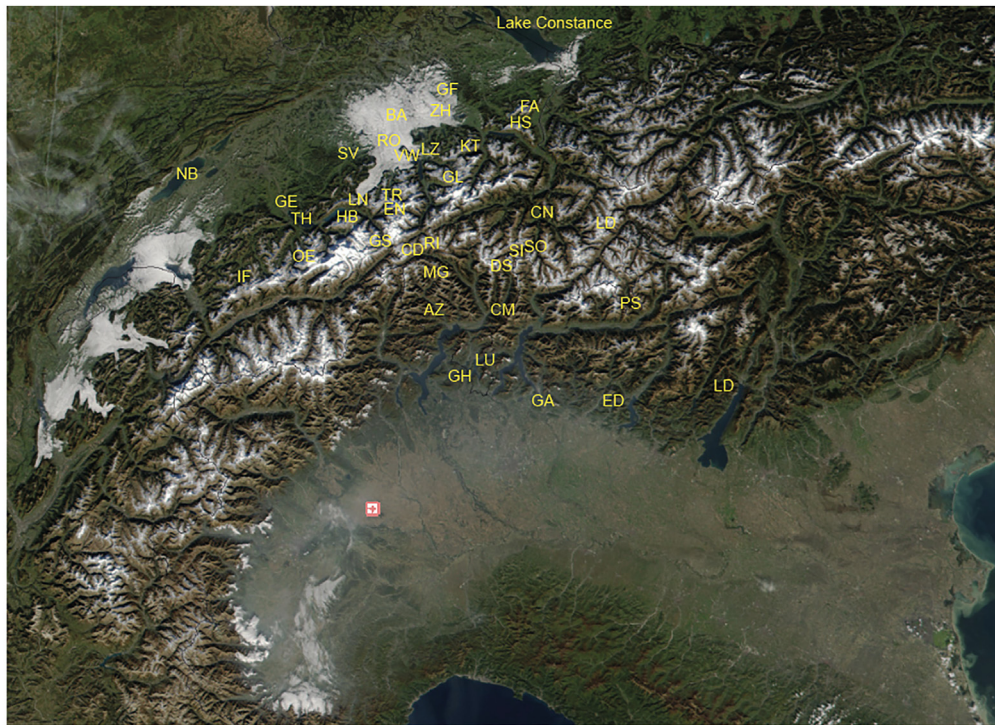
Fig. 1. Locations of (**a**) the Swiss and Italian (peri-) alpine lakes studied and (**b**) of the sampling station in Lake Lugano (star) used for the SPM and sediment trap sampling. The satellite image of the Alps was collected on November 3, 2001 by the MODIS (Moderate Resolution Imaging Spectroradiometer) aboard of the Terra satellite (https://earthobservatory.nasa.gov/).

Weber et al., 2018 for details), sequences were clustered into operational taxonomic units (OTUs) using a similarity threshold of 97%. The taxonomy assigned to OTUs is based on version 128 of the SILVA SSU reference database (Pruesse et al., 2012). In order to compare vertical trends of isoGDGTs and archaeal taxa, relative OTU abundances were converted to taxon-specific DNA concentrations (ng L^{-1}) by multiplication with the total amount of extracted DNA (see Weber et al., 2018 for details). Raw sequences have been deposited at NCBI under the Bioproject number PRJNA772618. Processed sequences of OTUs have been deposited in the GenBank database (accession nos. MH111698−MH113143 and OK687680−OK687682).

Table 1
Lake characteristics and GDGT data of the Swiss and Italian (peri-) alpine lakes studied.

Lake	Code	sample type	Depth max (m)	Area (km²)	Altitude (masf)	Crenarchaeol (μg·g⁻¹ TOC)	brGDGTs (μg·g⁻¹ TOC)	BIT index	TEX$_{86}$	% Cren'	GDGT-0/Cren ratio
Alzasca, Lago d'	AZ	Sed 0.5−1 cm	40	0.1	1857	0.8	157.5	0.99	0.42	3.2	37.9
		Soil				0.3	59.5	0.99		5.8	2.4
Baldeggersee	BA	Sed 0−1 cm	66	5.2	464			0.92	0.61	5.4	19.0
	BA2	Sed 0−3 cm				5.7	70.8	0.89	0.63	6.1	15.0
		Soil				7.0	25.4	0.65		5.1	0.5
Cadagno, Lago di	CD	Sed 0−1 cm	21	0.3	1926	0.2	48.1	0.99	0.40	3.0	28.9
		Soil				0.3	37.5	0.99		9.1	6.7
Cama, Lago di	CM	Sed 0−5 cm	16	0.1	1266	0.6	35.9	0.98	0.49	4.1	2.1
		Soil				0.3	33.2	0.99		14.0	1.8
Canovasee	CN	Sed 0−5 cm	10	0.0	777	0.2	33.7	0.99	0.29	3.7	145
		Soil				4.4	20.0	0.70		6.3	0.7
Dosso, Lago	DS	Sed 0−2 cm	6	0.0	1656	0.3	180	1.00			91.1
		Soil				0.5	21.2	0.97		5.8	0.8
Endine, Lago di	ED	Sed 0−1 cm	9	2.3	335	1.0	134	0.99	0.53	7.0	53.1
Engstlensee	EN	Sed 0−3 cm	45	0.5	1852	14.4	33.2	0.58	0.34	0.5	0.7
Fälensee	FA	Sed 0−5 cm	24	0.1	1455	1.5	47.1	0.96	0.52	4.0	16.8
		Soil				3.9	30.4	0.81		6.2	0.6
Garlate, Lake	GA	Sed 0−1 cm	34	4.6	198	23.3	52.0	0.57	0.34	0.6	1.7
Gerzensee	GE	Sed 0−2 cm	10	7.8	602	1.0	68.8	0.98	0.52	6.5	59.0
		Soil				8.2	23.3	0.59		6.1	1.7
Greifensee	GF	Sed 0−1 cm	32	0.4	452	2.0	42.0	0.92	0.57	6.9	58.2
Ghirla, Lago di	GH	Sed 0−1 cm	14	0.3	443	1.1	41.3	0.96	0.54	4.6	17.6
		Soil				1.8	20.7	0.89		6.2	0.6
Glattalpsee	GL	Sed 0−1 cm	23	0.2	1848	1.0	16.0	0.91	0.59	4.1	2.7
		Soil				0.9	28.7	0.95		5.7	1.3
Grimselsee	GS	Sed 0−5 cm	85	2.6	1909	1.8	63.8	0.97	0.49	2.1	2.0
Hinterburgsee	HB	Sed 0−1 cm	12	0.1	1523	0.3	36.0	0.99	0.46	5.7	110
		Soil				1.3	29.4	0.94		5.0	1.7
Hinterer Schwendisee	HS	Sed 0−0.5 cm	5	0.0	1160	0.3	45.4	0.99	0.33	2.4	40.9
		Soil				1.1	40.8	0.97		3.3	4.3
Iffigsee	IF	Sed 0−1 cm	24	0.1	2067	0.6	7.0	0.88	0.51	2.7	3.4
		Soil				0.8	22.3	0.95		4.3	1.8
Klöntalersee	KT	Sed 0−2 cm	46	3.2	847	2.8	27.8	0.84	0.49	2.7	1.9
Ledro, Lago di	LD	Sed 0−1 cm	38	2.2	654	3.9	22.9	0.77	0.45	2.0	8.6
Lungerensee	LN	Sed 0−1 cm	68	2.0	693	17.3	29.5	0.48	0.30	0.6	1.0
Lake Lugano	LU	Sed 0−1 cm	286	48.7	271	204	67.8	0.13	0.41	0.7	0.6
(Lugano, Lago di)		Soil				670−780	3.7	7.4	0.52	1.4	0.2
		Soil				340−390	2.8	8.5	0.64	9.2	0.2
Lauerzersee	LZ	Sed 0−1 cm	13	3.1	447	4.0	77.2	0.92	0.49	3.2	19.6
Mognola, Lago di	MG	Sed 0−1 cm	30	0.9	2005	0.3	75.0	0.99	0.47	2.4	7.1
		Soil				0.1	43.1	1.00		7.3	4.4
Lac de Neuchâtel	NB	Sed 0−5 cm	152	218	434			0.13	0.28	0.3	0.7
	NB2	Sed 0−6 cm				384	137	0.16	0.27	0.3	0.7
Oeschinensee	OE	Sed 0−2 cm	56	1.2	1580	21.6	13.4	0.23	0.38	0.6	0.7
Poschiavo, Lago di	PS	Sed 0−2 cm	85	2.0	963	33.6	64.3	0.59	0.28	0.5	1.0
Ritom, Lago	RI	Sed 0−2 cm	69	1.5	1855	2.3	90.0	0.97	0.48	2.7	4.1
Rotsee	RO	Sed 0−1 cm	16	0.5	428	3.4	51.1	0.91	0.43	2.1	16.9
		Soil				4.2	39.7	0.88		4.9	0.2
Silsersee	SI	Sed 0−2.5 cm	71	4.1	1800	16.7	116	0.84	0.28	0.6	0.9
		Soil				0.4	43.8	0.99		2.8	2.7
Silvaplana, Lago di	SO	Sed 0−0.5 cm	77	2.7	1791	129	110	0.38	0.28	0.4	0.8
Soppensee	SV	Sed top	27	3.2	596	0.3	34.9	0.99	0.36	4.3	52.8
		Soil				8.9	35.6	0.71		6.4	4.4
Thunersee	TH	Sed 0−2 cm	215	47.7	559	19.0	20.6	0.35	0.36	0.6	0.7
Trübsee	TR	Sed top	7	0.3	1767	1.0	16.4	0.91	0.41	1.7	1.8
		Soil				0.5	86.1	0.99		5.0	4.3
Vierwaldstättersee (Lake Lucerne)	VW	Sed 0−2 cm	112	114	599	719	77.7	0.06	0.31	0.3	0.6
Zürichsee	ZH	Sed 0−1 cm	135	88.2	406	202	33.1	0.09	0.31	0.4	0.8

3. Results

3.1. Profiles of physicochemical parameters in Lake Lugano

Water column parameters were measured in September 2014 (see Weber et al., 2018). The water column was thermally stratified: below 25 m, the water temperature was constant at 5 °C (Fig. 2a). Dissolved oxygen concentrations dropped with increasing water depth and reached zero at 125 m (Fig. 2a). Further below, waters were anoxic and the concentration of methane increased (Fig. 2a). The ammonium concentration was low in the surface waters and started to increase from 100 m depth to ca. 40 μM in the deepest
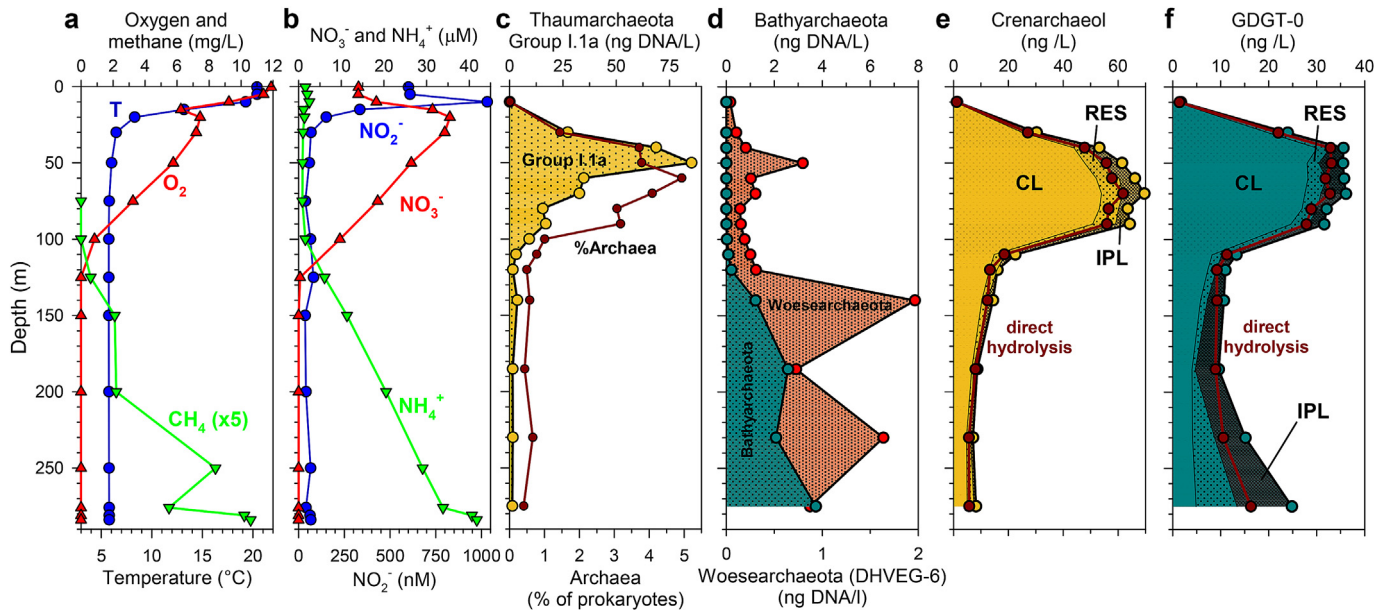
Fig. 2. Water column parameters and biological and chemical characteristics of SPM obtained at various water depths in the Northern basin of Lake Lugano on September 22, 2014. (**a**) Temperature (°C), dissolved oxygen (mg L^{-1}) and methane (mg L^{-1}) concentrations, (**b**) ammonium (μM), nitrate (μM) and nitrite (nM) concentrations. (**c**) Relative contribution of archaea to all prokaryotes (% of total 16R rRNA gene amplicons) and absolute abundance (in ng DNA L^{-1}) of Group 1.1a Thaumarchaeota. (**d**) Absolute abundance (in ng DNA L^{-1}) of the Bathyarchaeota and Woesearchaeota, the second and third most abundant groups of archaea. Note the different scales. (**e**) Absolute concentration of crenarchaeol (ng L^{-1}) and its speciation (CL = core lipids; IPL = intact polar lipids; RES = residually bound). The dark red line/symbols show the concentration of crenarchaeol but now after quantification of the CL after direct acid hydrolysis of the SPM (see experimental section). The depth trend follows that of the summed fractions quite well. (**f**) Same kind of depth-profile plot as in **e** for the GDGT-0 concentrations. Note the higher concentration and more intact (i.e.,"live") forms of GDGT-0 in the deep anoxic waters of the lake. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

water (Fig. 2b). The nitrate concentration was close to the detection limit in the deep waters, reached a maximum of ~35 μM at 20−30 m and then decreased again towards the surface. Nitrite levels were substantially lower and reached a distinct maximum in the surface water at 10 m (Fig. 2b).

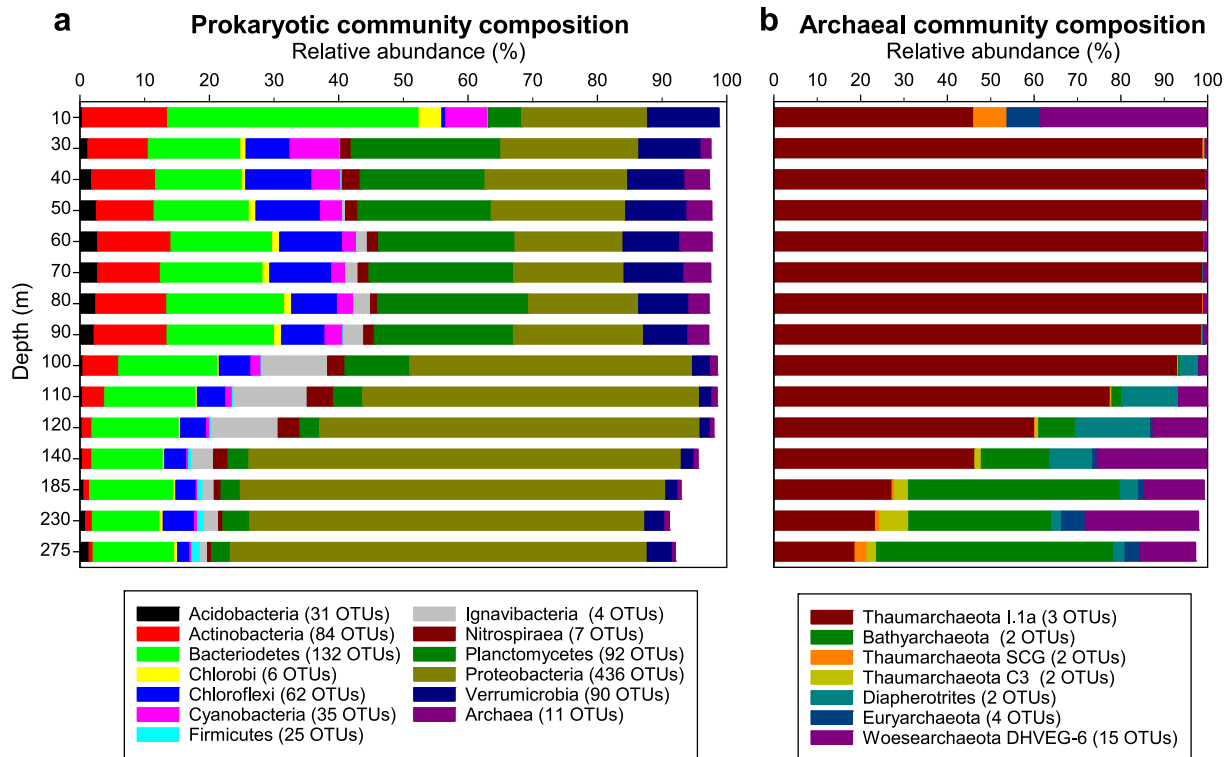### 3.2. Prokaryotic diversity in the water column of Lake Lugano

The prokaryotic diversity was determined by 16S rRNA gene amplification and sequencing, using SPM obtained from the water column of the northern basin of Lake Lugano close to the depocenter (Fig. 1) by in-situ filtration at high vertical resolution (15 different depths at 10−275 m) in September 2014. The total number of sequences obtained at each water depth varied from 1.8−3.5 × 10^5, and these were assigned to specific OTUs. The contribution of archaeal sequences relative to all recovered sequences varied from 0.01% at 10 m water depth to 4.9% at 60 m. After this maximum, it decreased with increasing water depth to ca. 0.5% in the deep waters (Figs. 2c and 3a). Generally, the archaeal community in the water column was dominated by Thaumarchaeota, especially in the waters above the oxycline (30−100 m), where thaumarchaeal sequences comprised 93−100% of all archaeal sequences (Fig. 3b). The diversity of the Thaumarchaeota was low; only three different OTUs were detected of which one (i.e., otu6, Table 2) was predominant: a Group I.1a thaumarchaeote (98.9% sequence similarity to various *Nitrosopumilus* spp.) represented 99.5−100% of all archaeal OTUs in the water depth range between 30 and 100 m. The partial 16S rRNA gene sequence detected in Lake Lugano was 100% identical to sequences recovered from other lake environments, e.g. from the southern basin of Lake Lugano (Frame et al., 2017), other Swiss lakes (Lake Lucerne, Vissers et al., 2013; Lago Maggiore, Coci et al., 2015), and a lake in France (Lake Bouchet; Hugoni et al., 2013). A single OTU from the Soil Crenarchaeotic group (otu1303) was clearly present in the surface

(14%) and, remarkably, the deepest anoxic waters (up to 12% of all archaeal sequences). Finally, otu704 related to Group C3 of the Thaumarchaeota was only detected in the anoxic waters and reached a maximum of 22% of all detected thaumarchaeal sequences. In the deeper anoxic waters, archaeal diversity increased and Bathyarchaeota (up to 55%) and the DHVEG-6 group of the Woesearchaeota (up to 26%) and Diapherotrites (up to 17%) formed a substantial part of the archaeal community (Fig. 3b).

Using the measured DNA concentration in the SPM (Weber et al., 2018) and the relative abundance of the recovered 16S rRNA genes, we estimated the absolute abundances of the most important archaeal groups. The Thaumarchaeota group I.1a had a maximum with almost 90 ng DNA L^{-1} at 50 m depth with gradually declining values up to the oxycline and low abundances in the anoxic deep waters and near absence in surface waters (i.e., 10 m) (Fig. 2c). Bathyarchaeota were absent in oxic surface waters but their absolute abundances increased to 4 ng DNA L^{-1} in deep anoxic waters (Fig. 2d). Woesearchaeota of the DHVEG-6 group were present throughout the water column at a lower absolute abundance (up to 2 ng DNA L^{-1}) and were also present in higher quantities in deep anoxic waters (Fig. 2d). Most OTUs of the Woesearchaeota of the DHVEG-6 group only occurred in anoxic water and there was only one of the more abundant OTUs that occurred in the oxic waters.

3.3. Depth distribution of isoGDGT in suspended particulate matter in Lake Lugano

The abundance and distribution of isoGDGTs in the same SPM samples that were analyzed for microbial diversity was analyzed using our earlier approach (Weber et al., 2017), which quantifies three different fractions of isoGDGTs, i.e., those occurring as free core lipids without a polar head group (labeled CLs), those bearing a polar head group (labeled as IPLs, referring to intact polar lipids), and those released by acid hydrolysis from the post-extraction SPM

Fig. 3. Prokaryotic (**a**) and archaeal (**b**) diversity at the phylum/class level based on sequenced 16S rRNA genes from SPM filtered at various depth in the water column of the northern basin of Lake Lugano on September 22, 2014. The number of unique OTUs detected for each phylum/class is specified with a minimum threshold of 100 (**a**) and 10 (**b**) copies in the total dataset, which provides an idea of the microbial diversity.

Table 2

Comparison of the abundance and sequence similarity between the abundant *Ca.* Nitrosopumilus oxyclinae OTU detected in Lake Lugano and Lake Constance and some apparently related bacteria that also occur in both lakes.

Taxon	Lake Lugano			Lake Constance[a]			16S rRNA gene sequence
	OTU[c]	Relative abundance (%)[d]	Co-correlation (R²)[e]	OTU[f]	Relative abundance (%)[g]	Co-correlation (R²)[h]	similarity (%)[b]
Ca. Nitrosopumilus oxyclinae	otu6 (OK687680)	0.00−4.9	1.00	OTU-3	0.01−21.4	1.00	100
Anaerolineaceae bacterium	otu2 (MH111865)	0.07−9.0	0.90	OTU-6	0.01−19.5	0.91	100
Nitrospiraceae bacterium	otu15 (MH111889)	0.01−2.7	0.83	OTU-86	0.00−1.1	0.89	98.8
Planktomycetes bacterium	otu19 (MH111928)	0.00−1.9	0.80	OTU-57	0.00−1.8	0.47	100
Ignavibacteriaceae bacterium	otu62 (MH112960)	0.00−0.62	0.83	OTU-42	0.00−2.7	0.92	100
Verrucomicrobiales bacterium	otu900 (MH112340)	0.00−0.05	0.61	OTU-152	0.00−1.3	0.63	100
Unclassified	otu80 (MH112339)	0.00−0.84	0.38	OTU-78	0.00−2.1	0.70	100
Verrucomicrobiales bacterium	otu31 (MH112386)	0.00−1.6	0.74	OUT-49	0.00−3.7	0.58	96.0

[a] Data from Herber et al. (2020).
[b] Sequence similarity of the specified OTUs detected in Lake Lugano and Lake Constance.
[c] NCBI accession number in brackets.
[d] Range of the abundance of this specific OTU relative to all prokaryotic sequences in the various SPM samples from different depths.
[e] R^2 of the correlation of the relative abundance of the OTU of *Ca.* Nitrosopumilus oxyclinae (otu6) with that of other bacterial OTUs.
[f] Genetic sequences of the OTUs are provided in the supplementary information of Herber et al. (2020).
[g] Range of the abundance of this specific OTU relative to all prokaryotic sequences in the various SPM samples from three different depths and at various sampling dates during the annual cycle.
[h] R^2 of the correlation of the relative abundance of the OTU of *Ca.* Nitrosopumilus oxyclinae (OTU-3) with that of other bacterial OTUs.

residue (labeled RES). These latter isoGDGTs are interpreted to derive from an unknown group of IPLs that escape solvent extraction by common methods, potentially due to the presence of large and hydrophilic head groups (Weber et al., 2017). The sum of the isoGDGTs in these three fractions represents the total amount of isoGDGTs. This sum was also independently determined by analyzing isoGDGTs in the total acid hydrolysate of the SPM, which typically showed good correspondence (Fig. 3e and f). The total concentration of isoGDGTs was low in the surface waters (3 ng L^{-1} at 10 m; two orders of magnitude lower than in deep waters), reached a maximum at 70 m at 140 ng L^{-1} and then declined to values of 30−40 ng L^{-1} in the deep, anoxic waters. Crenarchaeol

was the most abundant isoGDGT at most depths (30−140 m), representing 45−51% of total CL isoGDGTs (excluding SPM at 10 m; Fig. 4a). In contrast, the fractional abundance of CL GDGT-0 increased from 25 to 30% at 50−90 m to 40% in the deepest waters, and this increase was even more pronounced for the IPL and RES fractions (Fig. 4b). At the same time, the fraction of GDGT-0 containing a polar head group (i.e. %IPL) increased from 20 to 80% at depth, while %IPL for crenarchaeol changed much less (Fig. 4c). The less abundant isoGDGTs, i.e. GDGT-1, -2, -3 and the crenarchaeol isomer, individually showed a decrease in fractional abundance at all depths with the latter one typically only contributing <0.3% (Fig. 5a and b). The TEX_{86} values, which are derived from these four isoGDGTs, showed a variation with depth from 0.27 to 0.48 with a concentration- and depth-integrated value of 0.40 and 0.44 for the oxic and anoxic water masses, respectively, based on the sum of all isoGDGTs fractions (CL + IPL + RES). The peak in the concentration of crenarchaeol in the SPM at 30−100 m (Fig. 2e) in combination with the substantial in-situ production of brGDGTs in the anoxic waters (Weber et al., 2018) resulted in a large range of BIT index values with high values in the deep and shallow waters (Fig. 4f).

OH-isoGDGTs with 0−2 cyclopentane moieties were also encountered and their summed relative abundance amounts to 7−13% of the isoGDGTs in the CL fraction (Fig. 4d). Their concentration profile is most similar to that of crenarchaeol. They were almost exclusively found in the CL fraction, which is probably due to their partial loss of the labile hydroxyl group during the acid hydrolysis steps that were used to quantify isoGDGTs in the IPL and RES fractions. This is corroborated by the fact that direct acid hydrolysis of the SPM yielded much lower amounts of OH-isoGDGTs than detected in the CL fraction extracted from the unhydrolyzed SPM. In case of crenarchaeol and GDGT-0, however, the summed concentrations in the three fractions (CL, IPL and RES) agreed well with the concentrations obtained by direct acid hydrolysis (Fig. 3e and f). The distribution of the OH-isoGDGTs was relatively constant, the one without cyclopentane moieties (OH-isoGDGT-0) was typically slightly more abundant than the other members (Fig. 5a and b). Their average number of cyclopentane moieties (expressed with the RI-OH′ index for the CL fraction) varied with depth (Fig. 4e). The

concentration- and depth-integrated values for the oxic and anoxic waters were 0.87 and 0.98, respectively.

The stable carbon isotopic composition ($\delta^{13}C$) of the isoGDGTs in the SPM at seven different depths in the range 50−275 m and in the surface sediment (0−1 cm) was determined by ether cleavage with HI, followed by reduction to generate acyclic and cyclic biphytanes (Schouten et al., 1998). This was performed on the GDGTs generated by direct acid hydrolysis of SPM. The distribution of the generated biphytanes was dominated by the acyclic biphytane (fractional abundance 34−58%), predominantly derived from GDGT-0, OH-GDGT-0 and partly also from GDGT-1, -2, and OH-GDGT-1 and -2 (Fig. 6a). The tricyclic biphytane (13−24%), derived from crenarchaeol, and mono- (10−21%) and bicyclic (19−27%) biphytane, derived from mixed sources of (OH)-isoGDGTs, were also clearly present. The biphytane distribution in the surface sediment was most similar to that of the SPM from 50 m (Fig. 6a). The $\delta^{13}C$ values of the biphytanes in the SPM varied between −35 and −45‰ (Fig. 6b). Those of the tricyclic biphytane were the most constant throughout the water column and varied between −35.4 and −38.1‰. The acyclic, and, even more pronounced, the bicyclic biphytane displayed more negative values as low as −44.5‰ in the anoxic hypolimnion (140−275 m; Fig. 6b). The $\delta^{13}C$ values of the biphytanes in the surface sediment were quite uniform at −35.8 ± 0.6 (±SD) ‰ and matched those of the SPM at 50 m (−36.2 ± 0.1‰).

3.4. IsoGDGTs in settling particles and sediments of Lake Lugano

The fluxes and distribution of isoGDGTs in the water column were studied using sediment traps deployed at three different depths (20, 85, and 176 m) in the water column during the period March 1990−March 1991. This was done at the same location where the SPM sampling took place in September 2014. The physicochemical conditions of the lake have been monthly monitored over the period 1990−1991 (e.g., Barbieri and Simona, 2001). The LST changed from ca. 5.5 °C in winter to up to 25 °C at the end of summer (Fig. 7a). However, the thermocline in the summer was relatively shallow and temperatures in waters >30 m never exceeded 7 °C. During January and February, the temperature of the
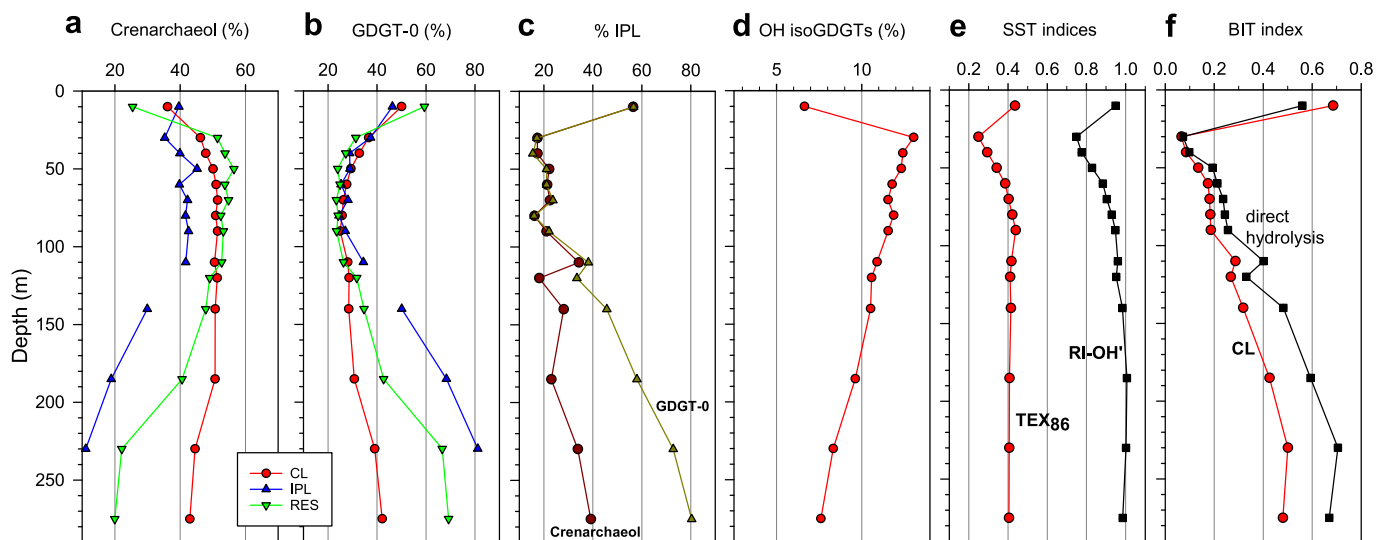
Fig. 4. Distribution of isoGDGTs and OH-isoGDGTs in the SPM at various levels in the water column of the northern basin of Lake Lugano in September 2014. (**a**) The fractional abundance of crenarchaeol in the CL, IPL, and RES fractions of the SPM. (**b**) The fractional abundance of GDGT-0 in the CL, IPL, and RES fractions of the SPM. (**c**) The fraction of crenarchaeol and GDGT-0 that is present as intact polar lipid (i.e., detected in the RES and IPL fractions) of the total. (**d**) The abundance of the total OH-isoGDGTs relative to that of the total isoGDGTs. (**e**) Depth variation of the LST indices, TEX_{86} and RI-OH′. (**f**) Depth variation of the BIT index, measured in the CL fraction and after acid hydrolysis.
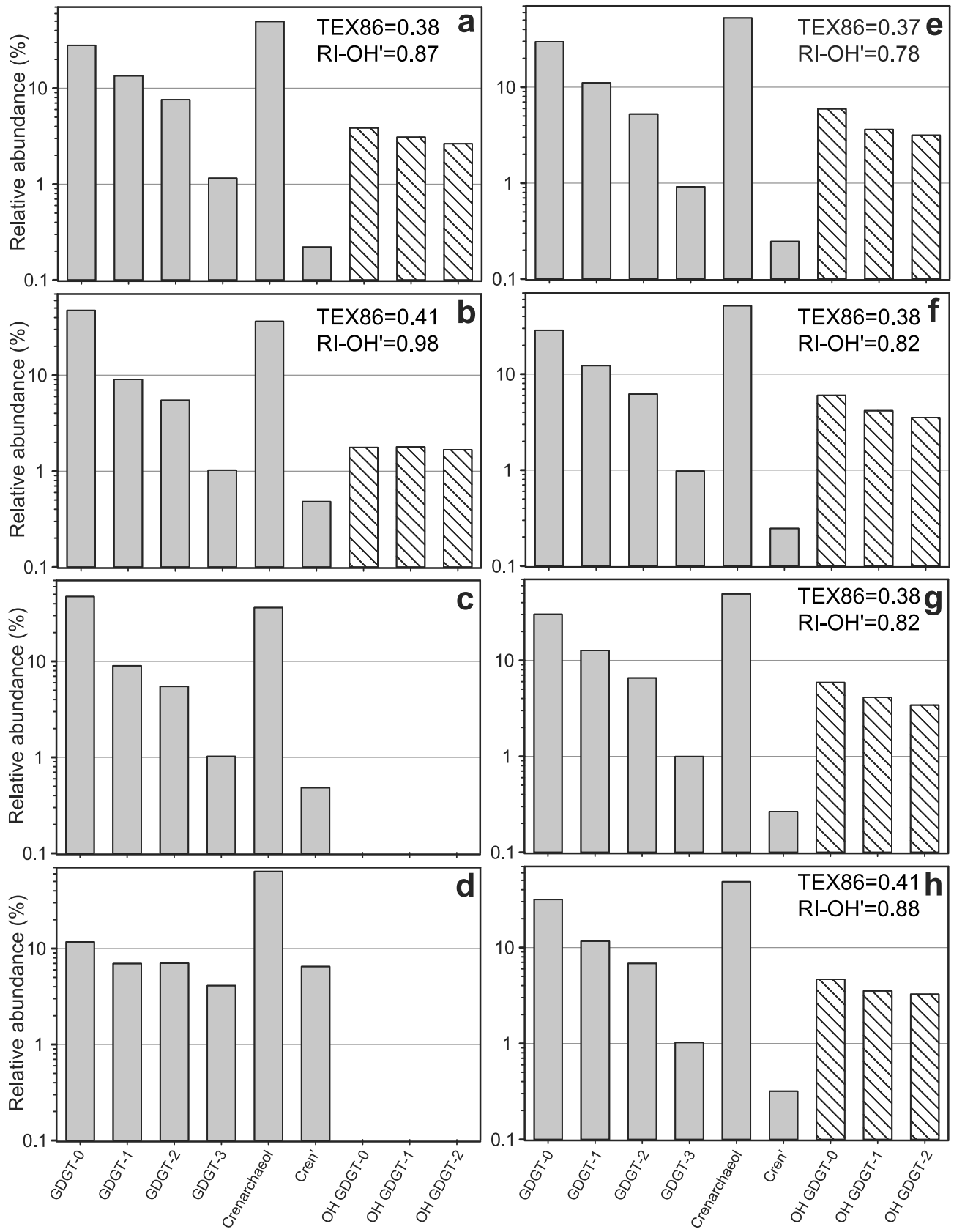
Fig. 5. Bar plots showing the distribution of isoGDGTs (grey bars) and OH-isoGDGT (hatched bars). Note the logarithmic scale used to depict abundances. Fractional abundances (i.e., normalized on their sum) are shown for the isoGDGTs, whereas the abundances of the OH iso-GDGTs are relative to the sum of all iso GDGTs. The depth and concentration-integrated distribution in the SPM from (**a**) the oxic (0−100 m) and (**b**) anoxic (100−285 m) water masses are shown and are based on the sum of the abundances in the CL, IPL and RES fractions (see text). The flux-weighted average distribution over the time period March 8, 1990−March 14, 1991 determined at (**e**) 20 m, (**f**) 85 m, and (**g**) 176 m for the CL fraction. Distributions in (**h**) the surface sediment (0−1 cm) of Lake Lugano and in composite soils from the watershed of Lake Lugano at (**c**) high elevation (670−780 masl) and (**d**) low elevation (340−390 masl) are also shown.

water column was uniform at ~5.5 °C. The oxycline remained relatively stable over the monitoring period, with oxygen concentrations below detection limit at ca. 100 m and deeper (Fig. 7b). The pH of the deeper (>30 m) waters was relatively constant at 7.4−7.7, while the pH of surface waters showed a clear seasonal cycle with higher values (up to 8.8) in spring and summer during times of highest phytoplankton production and CO_2 drawdown. The ammonium concentration was low (0.8−1.5 μM on average) in the upper 100 m and steadily increased with depth in the monolimnion; from, on average, ca. 11 μM at 100 m to 66 μM in the bottom waters (Fig. 7c). Nitrate was absent below the oxycline but showed a seasonal variation in the waters above 100 m (Fig. 7d). During May−September, the surface waters (0−15 m, with the exception of June 1991) are strongly depleted in nitrate due to primary production, while at other periods concentrations are uniformly high, especially during winter times (Fig. 7d). Traces of nitrite were detected in the water column with the highest concentrations (up to 1.3 μM) in the surface waters (0−15 m) during the summer season.

The fluxes of particulate organic carbon (POC) varied seasonally and followed the same trend at the three depths examined (Fig. 8a). They were highest in May−July, the main period of photosynthetic carbon fixation (Barbieri and Simona, 2001). The summed flux of isoGDGTs was more constant over time than that of POC with maxima in March and June 1990 (Fig. 8b), the latter coinciding with the maximum in the POC flux, and was more constant over time than that of POC. Most remarkably, the isoGDGT flux at the shallowest trap depth (20 m) was on average four times lower than those in the deeper waters, while the opposite was observed for the POC fluxes, that were slightly higher at 20 m than in the deeper waters. The annual, flux-weighted average isoGDGT distribution at the three trap locations is almost identical and quite close to that determined for the surface sediment (Fig. 5e−h). The sedimentation rate in the northern basin of Lake Lugano is estimated at ~0.6 cm yr^{-1} (Bechtel and Schubert, 2009), so the 0−1 surface sediment averages 2 years of sedimentation.

Over the annual cycle, some changes in the fractional abundances of individual isoGDGTs are evident in the trap series. The fractional abundances of GDGT-0 and crenarchaeol slightly varied with time, and especially the trends at 85 and 176 m follow each other closely. The fractional abundances of the less abundant isoGDGTs also show some minor changes with time. This leads to some variation in TEX$_{86}$ values with time, i.e. they range from 0.34 to 0.42 with a clear trend towards higher values at the end of the sampling interval in January−March 1991 (Fig. 8d). The distribution of the isoGDGTs in the surface sediment (Fig. 5h) is very similar to that of the SPM in the oxic waters (Fig. 5a) and the average distribution over the annual cycle at each trap deployment depth (Fig. 5e−g). The TEX$_{86}$ value for the surface sediment is 0.41, which is slightly higher than the flux-weighted average for the sinking particles collected with the three traps (0.37−0.38).

OH-isoGDGTs were also detected in the settling particles and were composed of the same three components as detected in the SPM (Fig. 5). Their flux was relatively constant at ca. 13% of the summed flux of isoGDGTs. Their distribution showed minor variations as illustrated by the slightly lower average number of cyclopentane moieties (RI-OH'), which on average was 0.05 lower at the shallow trap than in the deeper traps (Fig. 8d). Also in the surface sediment, the three OH-isoGDGTs were present and their abundance was 12% of the summed isoGDGTs, with a slightly higher RI-OH' value (0.88) than the flux-weighted average of the deeper traps (0.82).

3.5. Distribution of isoGDGTs in surface sediments and catchment soils from 34 (peri-) alpine lakes

Surface sediments from 34 other lakes from the (peri-)alpine area in Switzerland and Italy were analyzed for isoGDGTs in a follow-up of our study on brGDGT distributions in these lakes (Weber et al., 2018). The lakes varied in surface area, depth and altitude (Table 1) but generally fall into two major categories: a few large (>45 km^2) and deep (>110 m) lakes that are comparable to Lake Lugano (i.e., Lake Zürich, Lake Lucerne, Lake Neuchâtel, Lake Thun), and a more diverse group of smaller and shallower lakes (Table 1; Fig. 9a). IsoGDGTs were detected in all lake surface sediments but their distribution and absolute abundance varied widely. GDGT-0 was often the most abundant isoGDGT with a fractional abundance ranging between 0.29 and 0.98. Crenarchaeol also occurred abundantly, with fractional abundances varying between 0.07 and 0.54. Its isomer, however, was much less abundant: the % Cren' index falls between 0.4 and 7.0 with lower values in the larger and deeper lakes (Fig. 9c). GDGT-1, -2, -3 (in decreasing abundance) were minor contributors, with individual fractional abundances <0.12. The relative abundance of GDGT-0 and crenarchaeol (including its isomer) varied markedly in comparison to that of the total brGDGTs with the larger and deeper lakes displaying higher relative abundances as shown in the ternary diagram (Fig. 9a). This also resulted in a large variation in the BIT index (i.e., between 0.10 and 1.00; Table 1, Fig. 9b). The absolute concentration of crenarchaeol in the sediments (normalized to total organic carbon (TOC)) varied over more than three orders of magnitudes: i.e., between 0.2 and 720 μg g^{-1} TOC with the highest concentrations found in the surface sediments of the larger and deeper lakes (Fig. 9b−d). TEX$_{86}$ values ranged from 0.28 to 0.63. The deep lakes and some of the "medium-depth" lakes with higher crenarchaeol concentration (>10 μg g^{-1} TOC) showed lower TEX$_{86}$ values (<0.41) (Fig. 9d).

To assess soil erosion as a possible source for isoGDGTs in the lakes, composite soil samples from the drainage area for 19 of the 36 investigated lakes were also investigated for the distribution and absolute concentration of isoGDGTs. The fractional abundance of GDGT-0 ranged from 0.15 to 0.75. Crenarchaeol also occurred abundantly with fractional abundances ranging from 0.10 to 0.63. Its isomer was more abundant than in the lake sediments (i.e., % Cren' varied between 1.4 and 14.0; Fig. 9c). GDGT-1, -2, -3 represented minor isoGDGTs with individual fractional abundances <0.14, generally in decreasing order. The isoGDGT distributions of two composite soil samples from the drainage area of Lake Lugano at different altitude (Fig. 5c and d) serve as examples of the isoGDGT distributions in soils. The fractional abundance of the crenarchaeol isomer was quite variable between the two samples and OH-isoGDGTs were not detected, in contrast to the SPM and settling particles in the lake itself (Fig. 9). For most studied soils, the abundance of GDGT-0 and crenarchaeol (plus its isomer) relative to that of the total brGDGTs was low and consequently the soils plot in the lower left corner of the ternary diagram (Fig. 9a), and the BIT index is therefore often high (i.e., ranging from 0.52 to 1.00; Table 1, Fig. 9b; mean 0.86 ± 0.16 SD). The crenarchaeol concentration in the soils never exceeded 9 μg g^{-1} TOC (Fig. 9b and c) and was on average 2.6 μg g^{-1} TOC, much lower than those in the surface sediments of the large and deep lakes but in the same range as in the shallower lakes.
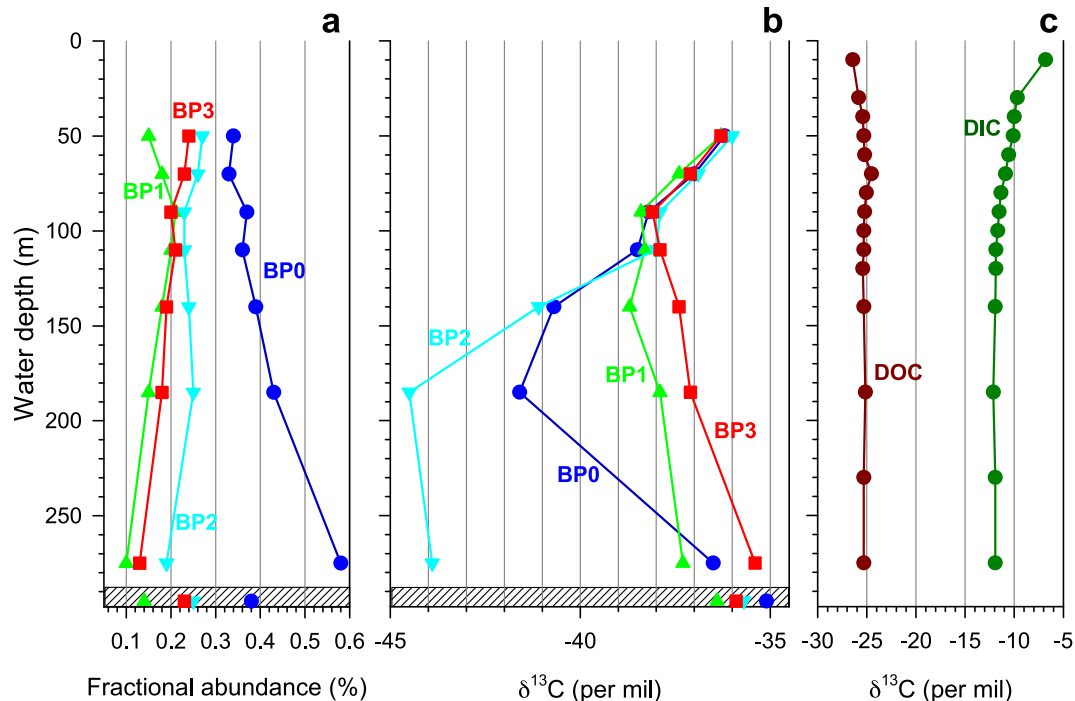
Fig. 6. (**a**) Fractional abundance (in %) and (**b**) stable carbon isotopic composition of biphytanes (in ‰) released by HI treatment and reduction from isoGDGT and OH isoGDGTs released by acid hydrolysis from the SPM filtered at various depths in the water column of the northern basin of Lake Lugano in September 2014 and the surface (0–1 cm) sediment. (**c**) Stable carbon isotopic composition (in ‰) of dissolved organic (DOC) and dissolved inorganic carbon (DIC) in the water column of Lake Lugano on November 1, 2014 (data from Weber et al., 2018). The hatched area represents the surface sediment of Lake Lugano. Key: BP0 = acyclic biphytane, BP1 = monocyclic biphytane, BP2 = bicyclic biphytane, BP3 = tricyclic biphytane (BP3 is specific for crenarchaeol).

4. Discussion

4.1. The niche of the Thaumarchaeota in the oxygenated waters of Lake Lugano

The high-resolution 16S rRNA gene profile (Fig. 2c) clearly defines a specific niche of thaumarchaea in Lake Lugano in the deeper waters above the oxycline (30–70 m), where ammonium is still present but at low concentrations (around 1 μM), and where oxygen levels range between 0.7 and 6 mg L^{-1}. This fits very well with their nitrifying physiology (Könneke et al., 2005; Wuchter et al., 2006). After their initial discovery in 500 m-deep waters of Crater Lake (Urbach et al., 2001), the presence of thaumarchaea as predominant ammonia oxidizers has commonly been reported in lakes (e.g. Hou et al., 2013; Hugoni et al., 2013; Vissers et al., 2013; Coci et al., 2015; Herber et al., 2020), including other large Swiss lakes like Lago Maggiore and Lake Lucerne. Commonly, their abundance was found to increase with increasing water depth, but no high-resolution profiles have been reported thus far.

A striking observation of our study is that one single thaumarchaeal OTU dominates the entire community of Archaea in the deep waters above the oxycline (Fig. 2c). A similar observation has been made at a 130 m-deep site in Lake Constance in southern Germany (Herber et al., 2020). There, a thaumarchaeal OTU, most closely affiliated to *Candidatus* 'Nitrosopumilus oxyclinae' (Qin et al., 2017a) and identical to the dominating OTU detected in Lake Lugano, represents 99% of all ammonia oxidizers in the hypolimnion throughout the year and is responsible for almost all turnover of ammonium in the hypolimnion. In other oligotrophic lakes, often a more diversified community of Thaumarchaeota has been reported (e.g. Hou et al., 2013; Hugoni et al., 2013; Vissers et al., 2013; Coci et al., 2015). They are typically affiliated with the Nitrosopumilales (Group 1.1a) and not with the Nitrososphaerales

(Group 1.1b). This is most likely related to the exceptionally high substrate affinity of the enzyme ammonia monooxygenase in the Nitrosopumilales (Martens-Habbena et al., 2009; Park et al., 2010; Kits et al., 2017), allowing them to outcompete other nitrifiers under oligotrophic conditions. Hence, the low ammonium concentrations in both Lake Constance (<1 μM; Herber et al., 2020) and Lake Lugano (0.8–1.0 μM; this study) are consistent with a thriving Nitrosopumilales community.

The similarity in the composition of the microbial communities of Lake Constance and Lake Lugano with respect to the positions of the single dominant thaumarchaeal OTUs goes even further. Using association network analysis, Herber et al. (2020) showed that the relative abundance of the "single species-level" OTU of Nitrosopumilales in Lake Constance co-varies with seven other OTUs (Table 2): a member of the CL500-11 clade within the Chloroflexi (OTU-6), a Nitrospira lineage II species (OTU-86), a member of the plaI-D clade within the Planctomycetaceae (OTU-57), an Ignavibacteria representative (OTU-42), and two members of the Verrucomicrobia (OTU-78 and -152). Some of these associations have also been observed in a number of Japanese deep freshwater lakes (Okazaki et al., 2017). The relative abundance of most of these species was often an order of magnitude lower than that of the thaumarchaeal OTU-3, which reached 21% in the bottom waters of Lake Constance. However, the Chloroflexi OTU-6 reached similarly high relative abundances (up to 19%; Table 2). Herber et al. (2020) hypothesized that its relationship with the thaumarchaeote may be due to the ability of members of the Chloroflexi group to either release ammonium by breaking down proteins or to oxidize the nitrite released by ammonium oxidation by the thaumarchaeote as a byproduct. The latter possibility is well consistent with the fact that the nitrite-oxidizer *Nitrolancea hollandica* falls within the Chloroflexi group (Sorokin et al., 2012). Remarkably, the oxygenated deeper water column of Lake Lugano
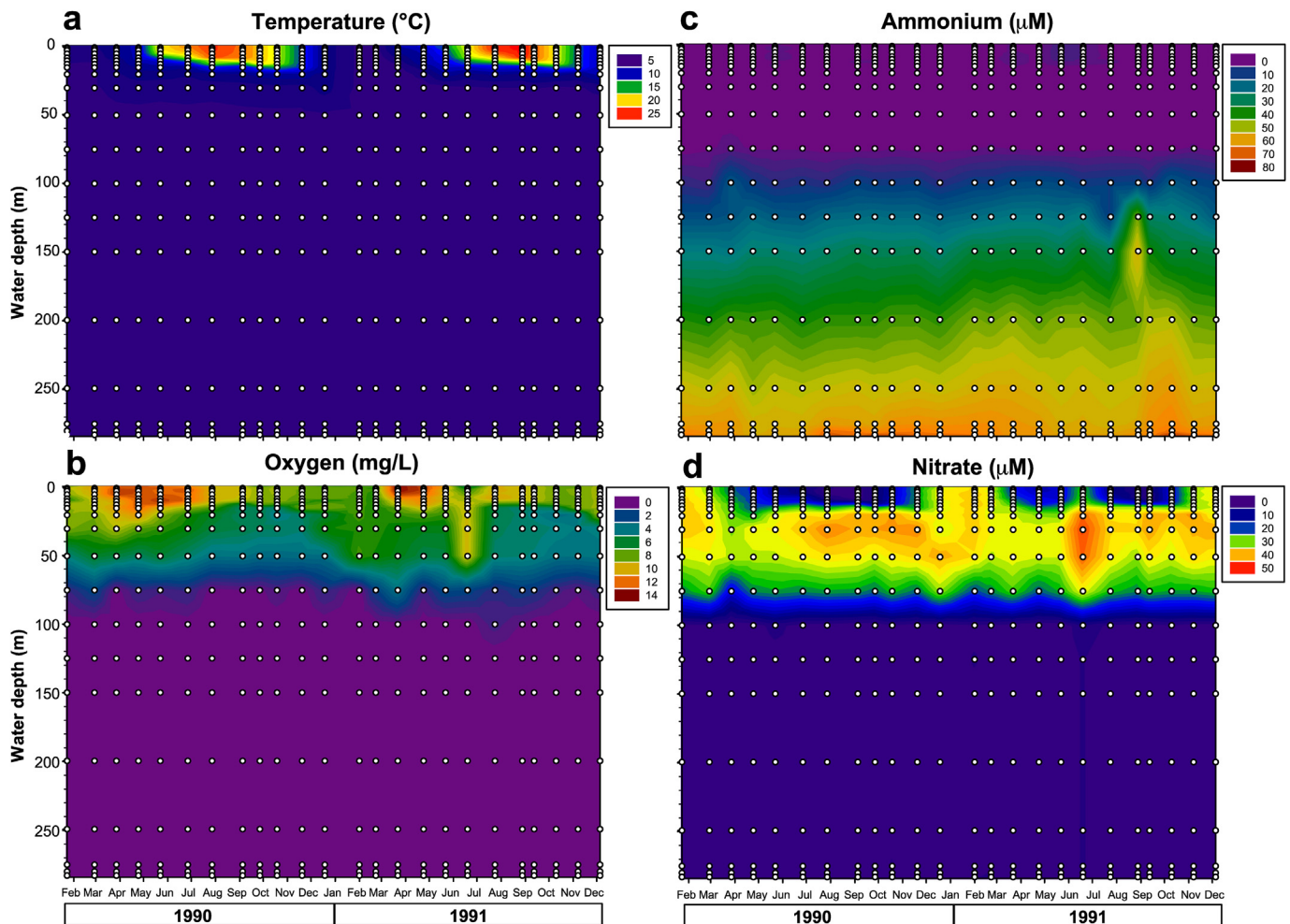
Fig. 7. Seasonal and spatial distribution of (**a**) temperature (°C), and concentrations of (**b**) oxygen (mg L^{-1}), (**c**) ammonium (μM), and (**d**) nitrate (μM) in the northern basin of Lage Lugano from January 1990 to December 1991 (a period, during which the sediment trap experiments were performed). Note the good correspondence between the water conditions in September 1990 and September 1991 and those in September 2014, when the SPM sampling took place (see Fig. 2).

contains the same seven bacterial species associated with the single species-level OTU of the Nitrosopumilales (i.e., otu6; Table 2) as in Lake Constance, often in relatively high abundances. BLAST searches revealed that the partial 16S rRNA gene fragments are highly similar, or even identical, between the two locations (Table 2). Furthermore, the relative abundances of these bacterial OTUs in Lake Lugano also correlate significantly with that of the thaumarchaeal OTU-6, as was observed for Lake Constance (Table 2; Herber et al., 2020). Hence, not only the phylogenetic identity but also the microbial "associations" with Nitrosopumilales seem highly comparable in both lakes.

Nevertheless, there are apparent differences between the lakes with respect to nitrification and the niche of the thaumarchaeote. Firstly, in Lake Constance nitrification was completely ascribed to archaeal nitrification (Herber et al., 2020). In Lake Lugano, however, a species (represented by otu60) was detected that is very closely related to *Nitrosospira briensis*, an ammonium-oxidizing bacterium of the Betaproteobacteria (Prosser, 2007). This bacterium has also been identified as an ammonium oxidizer in the seasonally stratified Upper Mystic Lake (USA; Preheim et al., 2016). Its highest relative abundance was at 10 m (representing 0.6% of the total community) and at 100 m at the oxycline (1.5%), where ammonium levels are slightly higher (1.6–2.5 μM) than in the waters where the thaumarchaeote represented by the abundant OTU (i.e., otu6) of the

Nitrosopumilales thrives (0.8–1.0 μM). It therefore seems that in Lake Lugano bacterial ammonium oxidizers are able to outcompete the thaumarchaea in areas where ammonium is more readily available. Secondly, the presence of an anoxic hypolimnion in Lake Lugano poses a restriction on the distribution of the thaumarchaea because they require oxygen to perform nitrification, albeit at low concentrations (Qin et al., 2017b). Hence, in contrast to Lake Constance, where the highest relative abundances of the thaumarchaea were actually detected in oxygen-replete deep waters (Herber et al., 2020), the anoxic deep waters of Lake Lugano consequently do not offer a proper niche for the nitrifying thaumarchaea. This interpretation seems to conflict with the detection of small amounts of thaumarchaeal DNA detected below the oxycline (Fig. 2c). However, this probably does not reflect the presence of living and active thaumarchaeal cells, but (remnant) DNA that originated from dead sedimenting or, perhaps, dormant cells, although in the Baltic Sea Berg et al. (2015) showed that thaumarchaea may still perform some nitrification under sulfidic conditions. The presence of 16S rRNA gene sequences of Thaumarchaeota in the upper euxinic waters of the Black Sea (Sollai et al., 2019) has also been explained by fossil DNA. Fossil DNA derived from phototrophic microorganisms such as haptophytes or green sulfur bacteria could indeed still be detected in surface sediments of anoxic deep basins (Coolen and Overmann, 2007; Coolen et al., 2006), attesting to the export of
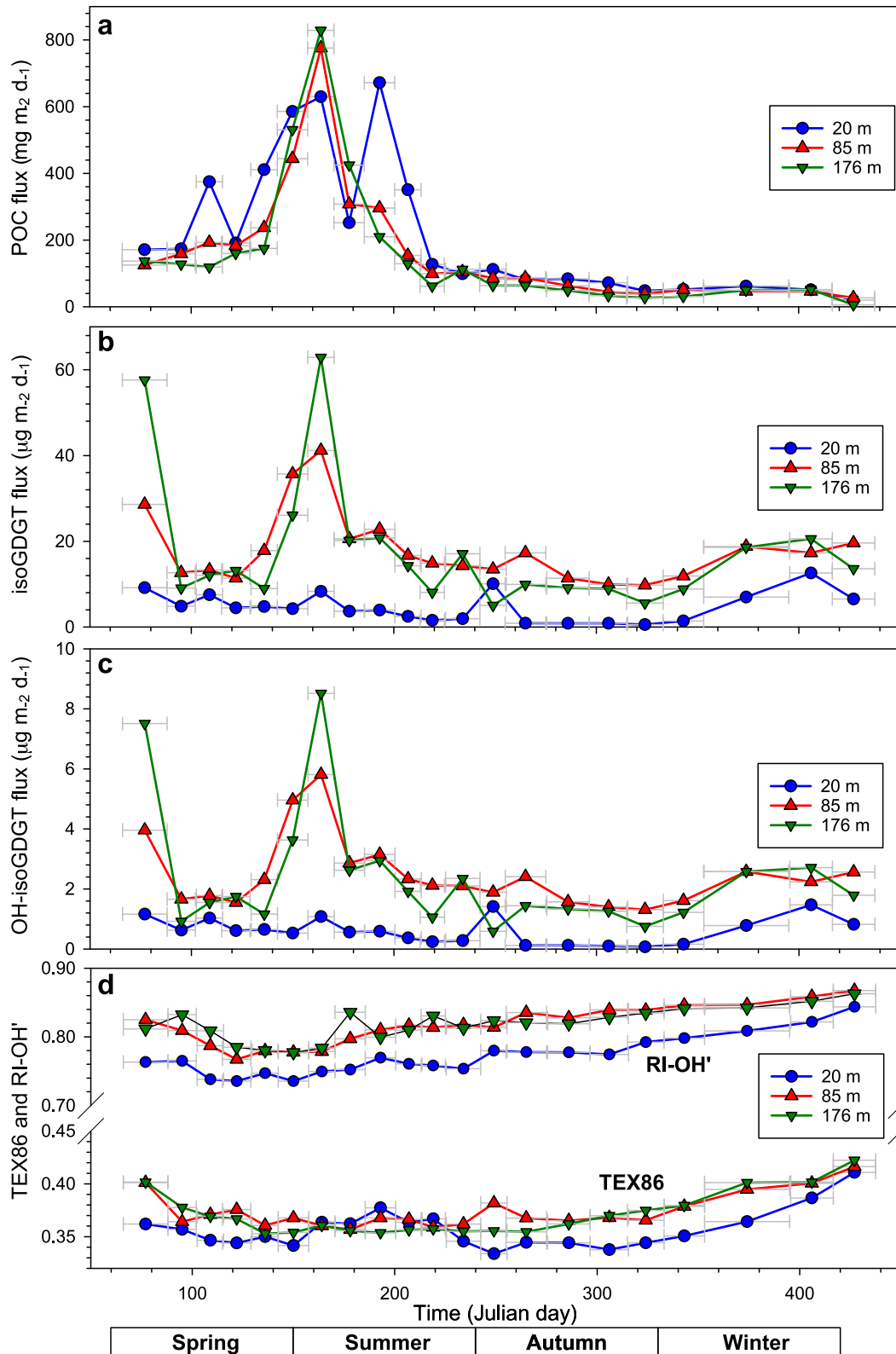
Fig. 8. Results from a trap experiment collecting sinking particles over an annual cycle (March 1990−March 1991) in Lake Lugano at three depths, 20, 85 and 176 m. (**a**) Fluxes (µg m^{-2} d^{-1}) of particulate organic matter (POC), and fluxes (ng m^{-2} d^{-1}) of (**b**) summed isoGDGTs and (**c**) summed OH isoGDGTs. The fourth panel (**d**) shows the variation in the values of the temperature proxies TEX$_{86}$ (lowermost values) and RI-OH' (uppermost values). Note the broken Y-axis for this panel. The time scale is given in Julian day with January 1, 1990 as the starting day. The grey symbols provide information on the duration of trap deployment, which varied from 13 to 42 days.
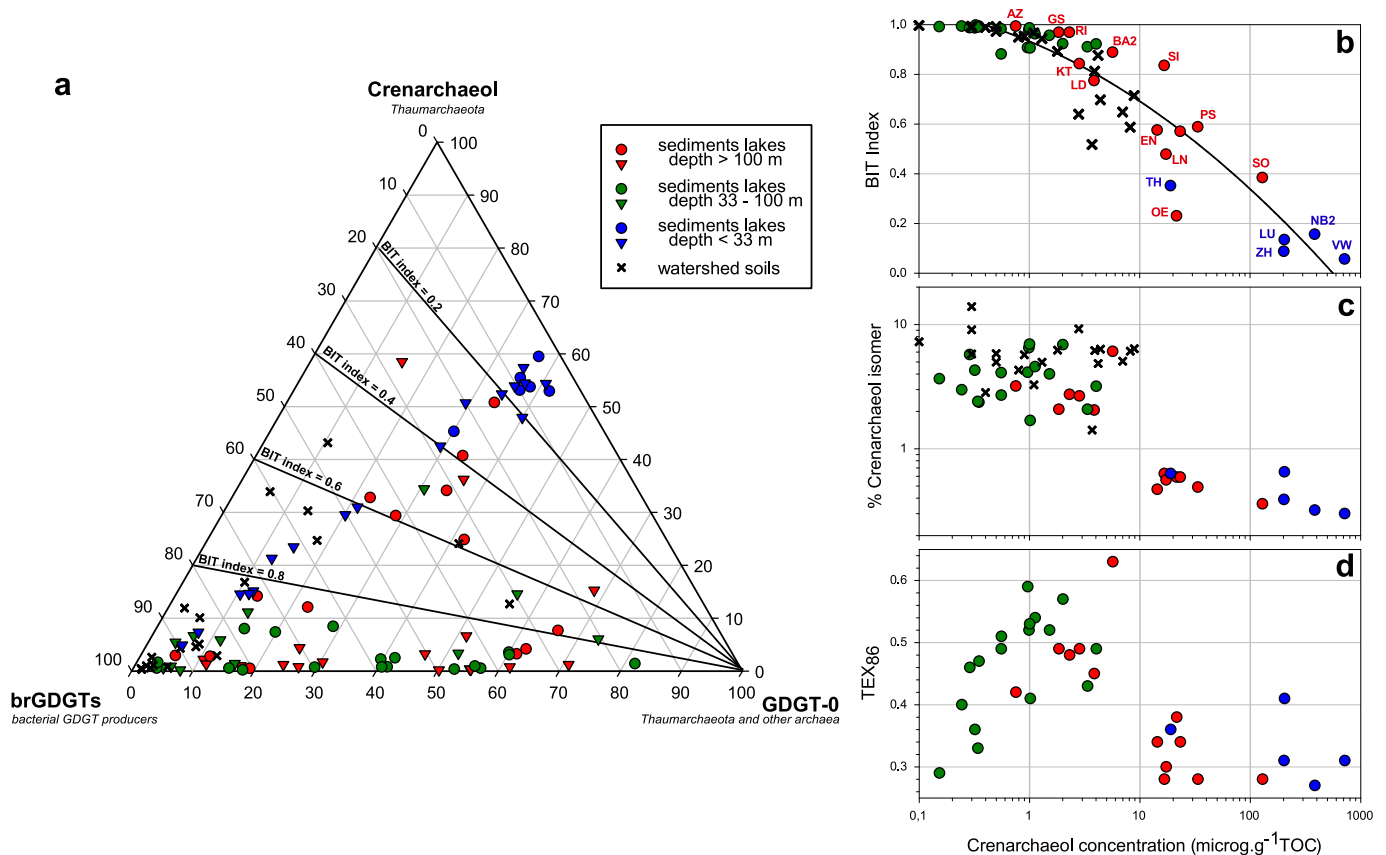
Fig. 9. IsoGDGTs in sediments of European lakes. (**a**) Ternary diagram showing the fractional abundances of GDGT-0, crenarchaeol (including its isomer), and summed brGDGTs in (peri-)alpine lakes (large filled circles; this study) and a set of 47 European lakes (filled lower triangles; data from Blaga et al., 2009). The color code classifies the depth of the lakes in three groups (see legend). The GDGT composition of soil composite samples of 15 of the studied (peri-)alpine lakes is indicated with crosses and reveals that these soils are dominated by crenarchaeol. The panels on the right show the concentration of crenarchaeol (including its isomer; in μg g^{-1} TOC) vs. (**b**) BIT index values, (**c**) %Cren' values, and (**d**) TEX$_{86}$ values for the (peri)alpine lake dataset and the 15 composite soil samples. Note the logarithmic axis in (**b-d**; i.e., y-axis in (**c**), and X-axes in (**b−d**)). The crenarchaeol concentration varies over almost four orders of magnitude. The two-letter codes of specific data points refer to the lakes depicted in Fig. 1 and listed in Table 1. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

microbial DNA from surface waters to the sediment.

Our data do not provide insight into seasonal changes in the distribution of the thaumarchaea in Lake Lugano (Fig. 2c) since we only analyzed one high-resolution water column SPM profile in September. In Lake Constance, which exhibits, in many ways, a comparable thaumarchaeal population, a year-round survey was undertaken (Herber et al., 2020), albeit at a depth resolution that was substantially less than in our study (i.e., SPM sampling only at 1, 15, and 85 m). The data from Lake Constance showed that (i) the same thaumarchaeote thrived year-round, (ii) the deep waters contained high relative abundances of Thaumarchaeota (13–21% of the prokaryotic gene sequences), and (iii) surface waters contained much lower relative abundances of Thaumarchaeota (except in January, most likely due to winter overturning of the water column of the lake). Low absolute and relative abundances of MG 1.1a Thaumarchaeota have been observed by combined molecular ecological and lipid analysis in surface waters of other deep lakes (e.g. Buckles et al., 2013; Kumar et al., 2019) and marine environments, too (e.g. Besseling et al., 2019; Sollai et al., 2019). Low thaumarchaeal abundances in shallow waters may be explained by a variety of reasons, e.g. the presence of reactive oxygen species (Kim et al., 2016; Tolar et al., 2016), preferential predation by mixotrophic flagellates (Ballen-Segura et al., 2017), and competition for ammonium with photoautotrophs (Small et al., 2013), but also growth inhibition by light seems to play an important role (Horak et al., 2018). Hence, it is reasonable to assume that the

specific niche of nitrifying thaumarchaea in the Lake Lugano water column (30−90 m) is constrained by the depth of light penetration of surface waters, the absence of oxygen in the deep waters, and the availability of ammonium. Overall, the unique situation where a single thaumarchaeal OTU dominates the archaeal community in the oxic hypolimnion allows studying the production, distribution and fate of the membrane lipids produced by this archaeon in a natural setting without too many confounding factors.

4.2. Sources of isoGDGTs in the oxygenated waters of Lake Lugano

Our high-resolution SPM sampling through the water column revealed a distinct maximum in the isoGDGT concentration at 60−70 m (see crenarchaeol and GDGT-0 profiles in Fig. 2e and f). This is quite different from lower-resolution profiles reported previously for the northern basin of Lake Lugano (Bechtel et al., 2010). These authors reported profiles for June and October 2007 at a site slightly west of the depocenter of the basin. Their results are similar to the data reported here with regards to the fact that GDGT-0 and crenarchaeol were also the most abundant isoGDGTs. However, maximum concentrations of isoGDGTs were almost two orders of magnitude lower (i.e., below 1.6 ng L^{-1}) and were reported for SPM from the uppermost (i.e., 10 and 40 m) water column. This substantial discrepancy in concentration cannot be explained by a potentially incomplete recovery of IPL-isoGDGTs in the earlier study, because our sequential extraction approach in which CL and

more polar forms of isoGDGTs were quantified separately showed that the majority of isoGDGTs (i.e., ca. 75%) was actually present as free CLs − the lipid fraction targeted by Bechtel et al. (2010). A reason for this apparent discrepancy might be that the Northern basin of Lake Lugano has undergone a complete mixing in winter 2005 and 2006, where large amounts of ammonium from the anoxic water mass below the chemocline have been mixed up to the surface (Holzner et al., 2009). Hence, the lower isoGDGT concentration in 2007 reported by Bechtel et al. (2010) could indicate that the nitrifying thaumarchaea have been i) diluted by the mixing and at the same time ii) outcompeted by bacterial nitrifiers adapted to higher ammonium concentration, and, therefore, have not reached again the post- and pre-mixing densities.

For our dataset, we obtained a good match between the depth profiles of the crenarchaeol concentration and the 16S rRNA gene abundance of Group I.1a Thaumarchaeota (cf. Fig. 2c and e). Thaumarchaeota are by far the most abundant archaea in the oxic water masses of Lake Lugano (Fig. 3b), are known producers of GDGT-0 and other cyclic isoGDGTs, and are, up to now, the only known biological producers for crenarchaeol and its isomer (Sinninghe Damsté et al., 2002; see Bale et al., 2019 for a recent overview). Especially the agreement in the upper part of the water column is striking: we found very low concentrations of both crenarchaeol and Group I.1a 16S rRNA genes at 10 m depth, followed by an almost two orders of magnitude increase in abundance with increasing water depth until 50 m. From this depth downwards, the two profiles somewhat deviate: the Group I.1a 16S rRNA gene abundances shows a rapid decline towards the oxycline, and further decrease to almost zero levels in bottom waters. Total concentrations of crenarchaeol (i.e., the sum of the three fractions quantified), on the other hand, increase slightly until 70 m before they decline further below, without, however, ever approaching zero. This difference in the two profiles is most likely due to different half-lives of the biomolecules: Nucleic acids are much more labile in the environment than lipids, especially isoGDGTs CLs, which represent ca. 75% of total crenarchaeol in the water column of Lake Lugano and can be preserved over millions of years. We therefore suggest that an active population of nitrifying thaumarchaea predominantly resides at 30−70 m depth, and upon cell death crenarchaeol is released to the water column, and is then subject to slow sinking with a good preservation potential, while thaumarchaeal DNA is readily degraded to trace amounts.

Supporting evidence for this hypothesis comes from cell density calculations. The genome size of ca. 1.6 Mbp (Walker et al., 2010) provides an estimated DNA content of 1.6 fg cell^{-1}, and an maximum thaumarchaeal DNA concentration of ca. 80 ng DNA L^{-1} at 50 m depth (Fig. 2c), corresponding to an thaumarchaeal cell density of approximately 5.10^6 cells L^{-1}. Thaumarchaeal cells contain ca. 1 fg of GDGT IPLs (see Besseling et al., 2020 for a compilation of data), so the estimated cell density would translate into a crenarchaeol IPL concentration of 2.5 ng L^{-1} (assuming that crenarchaeol IPL accounts for ca. 50% of all isoGDGTs in the cell). The concentrations we measured at this depth are, however, five times higher than that (i.e., 13.6 ng L^{-1}), which can be attributed to the different preservation potential of IPLs (higher) versus DNA (lower) after cell death. Hence, this comparison shows that (i) the lipids produced by the thaumarchaea in general follow the abundance profile as revealed by DNA-based methods, but (ii) due to their slower degradation than DNA, thaumarchaeal lipids accumulate predominantly in the waters below the main peak of thaumarchaeal DNA (cf. Fig. 2c and e). As a consequence, all of the CL crenarchaeol, and part of the IPL crenarchaeol, is 'fossil' (i.e. derived from dead/lysed cell material).

The interpretation that the isoGDGTs in the oxygenated water column are derived from the dominating thaumarchaeote is fully

supported by the measured $\delta^{13}C$ of the biphytanes derived from the isoGDGT. At 50 m (the uppermost depth where isotope measurements were possible) the four biphytanes have an identical $\delta^{13}C$ value of ca. −36‰ (Fig. 6b). Nitrifying thaumarchaea use bicarbonate (Wuchter et al., 2003) to fix carbon autotrophically using the 3−hydroxypropionate/4-hydroxybutyrate pathway (3HP/4HB) (Berg et al., 2007). Könneke et al. (2012) showed that in a culture of *Nitrosopumilus maritimus* the biphytanes released from the isoGDGTs displayed identical $\delta^{13}C$ values, which were independent of the concentration of dissolved inorganic carbon (DIC) in the 2−8 mM range. The measured isotopic fractionation (ε_{Ar} as defined in Pearson et al., 2019) was ~20‰. The $\delta^{13}C_{DIC}$ values in the water column in Lake Lugano vary depending on season but only in the upper 10−20 m, as a consequence of photoautotrophic carbon fixation (Lehmann et al., 2004). In the water below that depth, it is typically −10 to −12‰, due to a substantial contribution of remineralized organic matter. The $\delta^{13}C_{DIC}$ profile measured in the water column in October 2015 (Fig. 6c; Weber et al., 2018) − one month after the SPM sampling at the same sampling location − indeed shows a value of −10‰ at 50 m, which smoothly declined to −12‰ at depths >100 m. Matching this trend, we also observed a 2‰ decrease in the $\delta^{13}C$ values of the biphytanes from 50 to 100 m depth (Fig. 6b). The ε_{Ar} in the oxygenated hypolimnion of Lake Lugano thus seems to be robust at 26‰, which is higher than the ~20‰ reported for a culture of the *N. maritimus* (Könneke et al., 2012). Yet, when the reported $\delta^{13}C_{isoGDGT}$ values for SPM of the Western Atlantic Ocean (Hurley et al., 2019) are transformed into $\delta^{13}C_{biphytane}$ values (by adding 1‰ to correct for the ^{13}C-enriched glycerol units of the isoGDGTs; cf. Pearson et al., 2016), ε_{Ar} values between 20 and 24‰ are obtained, which gets fairly close to the Lake Lugano value of 26‰. On the other hand, ε_{Ar} has been reported to increase with increasing pH and decreasing CO_2 concentration (Hurley et al., 2019), implying that for an ε_{Ar} on the order of 26‰, a higher ambient pH, or a lower CO_2 concentration, would be required. The in-situ pH of 7.6−7.7 is, however, lower than that of the Western Atlantic Ocean and the Lake Lugano CO_2 concentration of ca. 140 µmol L^{-1} at this depth range (Lehmann et al., 2004) is substantially higher. Hence, the large ε_{Ar} of 26‰ in Lake Lugano seems to stand in disagreement with the existing marine data, suggesting that the theoretical model to predict ε_{Ar} (Pearson et al., 2019) may need to be revised for lacustrine settings. Nevertheless, the identical $\delta^{13}C$ values of all four biphytanes, in combination with the fact that they track $\delta^{13}C_{DIC}$ values in the oxygenated hypolimnion supports an exclusive nitrifying thaumarchaeal origin in the oxygenated hypolimnion.

OH-isoGDGTs have been reported in lakes much less commonly than in marine systems (e.g., Huguet et al., 2013). Wang et al. (2017) reported the presence of OH-isoGDGTs in sediments of Lake Qinghai, a very large but shallow, alkaline lake. In the sedimentary record, their abundance correlated with that of crenarchaeol, suggesting a potential thaumarchaeal source. Wang et al. (2019) reported the presence of OH-isoGDGTs in relatively low abundance (2% of isoGDGTs on average) in a set of surface sediments of 55 lakes from mid-latitude Asia, and only observed higher abundances (8−12%) in sediments of four lakes with surface sediment sampling depths of >10 m. To the best of our knowledge, the detection of the OH-isoGDGTs in Lake Lugano's SPM and sinking particles (Fig. 5) is the first time that these components were found in the water column of a lake. In the Lake Lugano water column, the concentration profiles of the total OH-isoGDGTs correlate well with that of crenarchaeol ($R^2 = 0.989$), strongly suggesting that they are also sourced by thaumarchaea. Indeed, in marine settings, OH-isoGDGTs have been attributed to thaumarchaea (e.g. Liu et al., 2012; Huguet et al., 2013; Sollai et al., 2019). OH-isoGDGTs have also been reported in cultures of most species of the Nitrosopumilales, but not

in those of the Nitrososphaerales (e.g., Elling et al., 2017; Bale et al., 2019). OH-isoGDGTs were not detected in the soils around Lake Lugano (Fig. 5c and d), indicating that they must be produced *in situ*, i.e., in the lake itself. Soils commonly contain thaumarchaea but these are typically dominated by members of Group 1.1b. Hence, all available evidence indicates that the OH-isoGDGTs are produced by thaumarchaea residing in the cold oxic waters well below the thermocline.

4.3. The anoxic hypolimnion of Lake Lugano: changes in isoGDGT distribution and archaeal community composition

Below the oxycline, our data show some marked changes in the isoGDGT distribution, which is most evident in the SPM from 275 m depth. The overall fractional abundance of GDGT-0 increases from 25 to 30% at 50−110 m to 68% at 275 m (Fig. 4). Similarly, the acyclic biphytane BP0, which is predominantly formed from GDGT-0 (generated by ether bond cleavage of GDGTs; Fig. 6a), showed an increase from 33 to almost 60%. Also, the summed concentration of GDGT-0 increased in deep waters (Fig. 2f). Remarkably, this increase is predominantly due to the much higher absolute abundance of GDGT-0 with polar head groups (Figs. 2f and 4c), suggesting that another group of archaea in the anoxic bottom waters of Lake Lugano is producing predominantly GDGT-0. This is supported by the deviating $\delta^{13}C$ value of the acyclic BP0 in the deep waters: in the anoxic hypolimnion at depths below 110 m the $\delta^{13}C$ value of BP0 deviates significantly from that of the tricyclic biphytane BP3 (−35.5 to −38.1‰), which is exclusively derived from crenarchaeol (Fig. 6b). The $\delta^{13}C$ values of BP0 decline with depth and reach −41.6‰ at 185 m, but are surprisingly similar to that of BP3 at 275 m, where GDGT-0 is most abundant. In addition to BP0, the bicyclic biphytane BP2 also becomes depleted in ^{13}C within the deeper waters, and reaches $\delta^{13}C$ values as low as −44.5‰ at 185−270 m depth (Fig. 6b).

These trends in the $\delta^{13}C$ values of biphytanes with depth show some resemblance to those of Black Sea, where the $\delta^{13}C$ values of BP-0, BP-1 and BP-2 released from isoGDGTs in the anoxic water column (down to −66‰), deviated substantially from those in the oxic/suboxic part of the water column and from BP-3, exclusively derived from thaumarchaea (Wakeham et al., 2003). The Black Sea deep euxinic waters also showed an almost equal abundance of GDGT-0, -1, and -2, a distribution that is typical for archaea involved in the anaerobic oxidation of methane (cf. Pancost et al., 2001). The assimilation of ^{13}C-depleted methane (−48‰ in the Black Sea) in the deep anoxic water column is thought to have resulted in this specific isotopic signature. Combined IPL and 16S rRNA gene analyses suggested that GDGT-0, -1, and -2 with two phosphatidyl-glycerol (PG) head groups were produced by Euryarchaeota belonging to the ANME-1b group (Sollai et al., 2019). A contribution from ANME archaea to the SPM-derived GDGT pool in Lake Lugano seems unlikely, as we could not detect ANMEs in the lake's SPM. Also, previous work could not find an active archaeal methanotrophic community in the anoxic water column (Blees et al., 2014). Furthermore, the ^{13}C-isotopic depletion of BP0 in the Lake Lugano anoxic hypolimnion is much less than that observed in the Black Sea, and is not accompanied by the specific isoGDGT distribution for ANMEs (i.e., comparable abundances of GDGT-0, -1, and -2).

The IPL fraction in the deep waters of Lake Lugano is strongly dominated by GDGT-0 (up to 90% at 275 m). This is quite comparable to the situation in Lake Chala: in this permanently stratified tropical lake, a high abundance (~90 ng L^{-1}) of an IPL with a GDGT-0 core in the anoxic hypolimnion dominated the GDGT distributions (Buckles et al., 2013; Baxter et al., 2021). The presence of 16S rRNA genes affiliated to the uncultured Crenarchaeota group 1.2 and the Bathyarchaeota, formerly known as the Miscellaneous

Crenarcheota Group (MCG; Kubo et al., 2012), in a clone library obtained from this depth suggested an origin of GDGT-0 from uncultured crenarchaea (Buckles et al., 2013). Subsequent studies of Lake Chala in which the GDGT composition and archaeal diversity was studies over an annual cycle (Baxter et al., 2021) showed that the deeper anoxic waters always contain a high abundance of isoGDGT-0, as well as archaea of the anaerobic heterotrophic group C3 MCG Bathyarchaea and specific euryarchaeal methanogens. Several novel isoGDGT-0 IPLs occurred in the deep anoxic water column, including those with a phosphatidylglycerol, monohexose-phosphatidylglycerol, and dihexose-phosphatidylglycerol head groups, which could not be assigned to a specific source. Although we did not determine the specific head groups of the GDGT-0 IPLs in Lake Lugano, the dominance of the (indirectly measured) GDGT-0 IPLs, especially at 275 m, suggests a similar origin as in Lake Chala. The 16S rRNA gene sequencing results show that Bathyarchaeota represented >50% of all archaeal sequences at this depth (Fig. 3), suggesting that these archaea may be the source of new production of GDGT-0 IPLs. In line with the Lake Chala data, the most abundant bathyarchaeal OTU, representing >90% of all sequences, falls in subgroup 6 of the Bathyarchaeota (e.g. Pan et al., 2019). Based on genomic analysis, Bathyarchaeota seemingly have the ability to anaerobically degrade complex carbohydrate polymers, potentially of photosynthetic origin (Lazar et al., 2016). Hence, the deep anoxic waters of Lake Lugano would represent a proper niche for the Bathyarchaeota subgroup 6. However, a similar dominance of bathyarchaea was evident for the deep anoxic waters of the Black Sea, where their 16S rRNA gene sequences also, at some depths, represented >50% of all archaeal sequences (Sollai et al., 2019) but comparison of the IPL and 16S rRNA gene profiles suggested that bathyarchaea likely produce archaeol with phospho-dihexose and hexose-glucuronic acid head groups but not GDGT-0 (Sollai et al., 2019). The DHVEG-6 group of the Woesearchaeota phylum forms the second most abundant group of archaea in Lake Lugano's deep anoxic water (Fig. 3), again similar to the Black Sea (Sollai et al., 2019). However, Woesearchaeota are also unlikely producers of the distinct GDGT-0 IPLs in the deep waters of Lake Lugano since they have only small genomes, lacking the genes required for isoGDGT biosynthesis (Castelle et al., 2015; Villanueva et al., 2017), and are thought to depend on recycling extracellular lipids to build their membranes (Lipsewers et al., 2018). Diaperotrites, also falling in the DPANN superphylum, represented 4−17% of the archaeal community and occurred between 100 and 275 m (Fig. 3). The Diaperotrites are dominated (>99%) by two OTUs, of which the most abundant one is phylogenetically closely (98.9%) related to a small (1.1 Mbp) metagenome (CP064981) of an archaeon isolated from activated sludge. This metagenome contains various genes encoding proteins involved in archaeal lipid synthesis (e.g., GGGP, CDP-archaeol synthase; see Villanueva et al., 2014 for a review) and hence these diapherotrites cannot be discounted as potential producers of isoGDGTs. However, they possess their maximum relative abundance at 120 m (Fig. 3), i.e., not at a position in the water column where the distribution of isoGDGT shows a larger shift, or where the changes in the $\delta^{13}C$ value of BP0 and BP2 occurred. This questions if they significantly affect the isoGDGT distribution. Euryarchaeota make up only a small part (up to 5%) of the archaeal community (Fig. 3) below the oxycline, and are dominated (ca. 50%) by one OTU that is closely (97.6−98.2%) related to *Methanoregula* sp., a methanogen that is producing GDGT-0 as the most important core membrane lipid (Bräuer et al., 2011). Thus, these methanogens could also be a source for the specific GDGT-0 IPLs detected in the deep anoxic waters of Lake Lugano.

4.4. Export of isoGDGTs to the sediment

To study the influence of water column sources on the isoGDGT distribution in the sedimentary record, we determined fluxes of isoGDGTs and OH-isoGDGTs at three different depths in the water column of the lake over one year. Though the trap deployment had already been performed almost three decades ago the characteristics of the water column at that time (Fig. 7) were almost identical as in the year of SPM sampling, which allows to compare these two datasets (see Lehmann et al., 2015 for a review on the lake's water column characteristics). The markedly higher fluxes of isoGDGTs and OH-isoGDGTs in the traps deployed at 85 and 176 m compared to the shallow trap at 20 m (Fig. 8) excellently fits the concentration profile measured for SPM (Fig. 2). The data show a predominant (OH)-isoGDGT production, most likely by thaumarchaea (see above), below 30 m (i.e., just below the shallowest trap) in the water column in September. No SPM concentration profiles have been obtained for other months than September, but a comparison with Lake Constance, where an identical archaeon thrives in the deeper water column, reveals that in that lake 16S rRNA gene copy numbers are high in the deep waters, and low in the surface waters throughout the year, except for the winter when mixing occurs and thaumarchaeal cells are brought to the surface (Herber et al., 2020). Winter mixing of the upper waters also occurs in Lake Lugano, as is evident from the temperature data (Fig. 7a), and this likely explains the somewhat higher fluxes of isoGDGTs at 20 m during winter as compared to summer and autumn (Fig. 8b).

There are some variations in the (OH)-isoGDGT fluxes at 85 and 176 m over the annual cycle. This co-varies, to some extent, with the differences in the POC flux (Fig. 8). These variations seem to follow clear seasonal dynamics, possibly due to (i) the effect that the higher particle flux during spring and early summer arising from the annual blooms of phytoplankton in the lake resulting in a higher degree of scavenging of thaumarchaeal cell material by settling particles, (ii) a higher degree of OM mineralization in the bloom period, resulting in a higher production rate of ammonium in the upper water column, enhancing the cell density of the thaumarchaea, and/or (iii) increased zooplankton grazing on thaumarchaeal cells at times of increased phytoplankton availability leading to enhanced (OH)-isoGDGT export from surface waters through fecal pellet formation. The difference between the peak and minimum values in the flux is, however, much larger for POC than for (OH)-isoGDGTs. This seems to be consistent with a more gradual export of the membrane lipids of the thaumarchaea residing below the thermocline during the year.

The flux-weighted distribution of the (OH)-isoGDGTs at the various sediment-trap depth levels is nearly identical to each other (Fig. 5e−g), and the distributions in the SPM of the oxic part of the water column (Fig. 5a), but different from that in the anoxic part of the water column, with its higher fractional abundance of GDGT-0 (Fig. 5b). The (OH)-isoGDGT distribution of the surface sediments of Lake Lugano (Fig. 5h) is strikingly similar to that of the SPM of the oxic waters (Fig. 5a), as well as to that of the sinking particles at all depths (Fig. 5e−g). This indicates that GDGTs present in the SPM from the anoxic part of the water column are not, or only to a minor extent, exported to the sediment. This is fully consistent with the $\delta^{13}C$ values of the biphytanes released from the (OH)-isoGDGTs, which in the surface sediment are similar to those in the SPM in the oxic waters, and which do not show any depletion in ^{13}C as observed for some of the biphytanes in the deeper, anoxic waters (Fig. 6b). Again, this situation is highly comparable to that observed for the stratified Black Sea, where the highly different distribution and deviating $\delta^{13}C$ signature of isoGDGTs in the deep, euxinic waters were not reflected in the surface sediments, indicating no substantial contribution of the highly deviating archaeal

community in the deep waters to the sedimentary lipid pool (Wakeham et al., 2003). This was attributed to the absence of microbial grazers in the anoxic waters that are most likely required for the vertical transportation of the GDGT of the tiny (<1 μm) archaeal cells through the packaging of isoGDGTs in fecal pellets, which sink much faster. This explanation most likely also applies to Lake Lugano. It should be noted that this situation is quite different for deep oxic water columns. For example, the Mediterranean Sea is characterized by a deep (>1000 m) water thaumarchaeal population that has a different isoGDGT distribution characterized by a substantially enhanced GDGT-2 content than that in shallow waters (Kim et al., 2015; Besseling et al., 2019). Despite the great depth in the water column of this specific thaumarchaeal population, their isoGDGT membrane lipids were transported to the surface sediments (Kim et al., 2015). Hence, the oxicity of the deep water column enabling the presence of grazers apparently plays a key role in the transport of deep water GDGTs and this likely explains why in the anoxic Lake Lugano the specific distribution of the isoGDGTs of the deep waters cannot be traced into its sedimentary record.

4.5. Implications for paleothermometry using TEX₈₆ and RI-OH′ in Lake Lugano

The ecological niche of the thaumarchaea, i.e., residing in year-round cold (ca. 6 °C) waters at 30−70 m depth (Figs. 2 and 7a), has important implications for the application of TEX_{86} and RI-OH′ in paleotemperature reconstructions. Sedimentary TEX_{86} values were originally thought to reflect LST (Powers et al., 2010) and since LST is primarily affected by air temperature, they could be used to provide an indication of continental climate changes. LST of Lake Lugano varies from 5 to 25 °C (Fig. 7a) over the seasonal cycle and the mean annual LST over the period 1986−2007 was ca. 14 °C (Bechtel et al., 2010). The thaumarchaea in the lake, however, never experience these temperature variations, because they reside below the steep thermocline, which builds up in late spring and ends in late autumn (Fig. 7a). In fact, only a small portion of Lake Lugano's total water mass is affected by the seasonal warming, and the nitrifying thaumarchaea reside in the continuously cold part of the lake. The measured TEX_{86} values of ~0.4 in the SPM in the water column, the sinking particles and the surface sediment are low, but in good agreement with those reported by Bechtel et al. (2010) for the same lake basin. These low TEX_{86} values translate into a temperature of ~8 °C when using the LST calibration of Powers et al. (2010). Hence, the ecological niche of the thaumarchaea in Lake Lugano results in a cold "bias" in the assessment of LST using TEX_{86} paleothermometry.

A similar observation has previously been made for Lake Lucerne (Blaga et al., 2011), although this study was based on much lower resolution sampling of SPM than the current study. In Lake Lucerne, concentrations of isoGDGTs in SPM and isoGDGT fluxes increased with depth, indicating of isoGDGTs production in the deep, aphotic zone of the lake. The flux-weighted average for TEX_{86} of 0.27 was even lower than that observed in Lake Lugano, but was in good agreement with the TEX_{86} value of 0.29 in the surface sediment of Lake Lucerne, indicating that the sedimentary TEX_{86} values record the annual mean temperature of deeper waters (4−6 °C) in Lake Lucerne but not LST, which increases in summer beyond 20 °C. These findings would imply that TEX_{86} paleothermometry of these and similar lakes is of limited use, as the paleothermometer would only record year-round cold bottom water temperatures of the lake. Yet, a decadal-resolution TEX_{86} record from Lake Lucerne reflecting the last deglaciation (~14,600−10,600 cal yr B.P.) revealed the typical oscillations known for the Late Glacial Interstadial, the abrupt cooling of 2 °C at the onset of Younger Dryas, and a rapid warming of 4 °C at the

beginning of the Holocene, within less than 350 years (Blaga et al., 2013). This record showed a remarkable resemblance with the Greenland and regional stable oxygen isotope records (e.g., $\delta^{18}O$ of ostracods shells from the Ammersee). This implies that TEX_{86} paleothermometry within these types of lakes still may yield valuable information, at least if climatic changes are such that also the deeper water column is affected by atmospheric temperature shifts.

In addition to isoGDGT, OH-isoGDGTs have also been applied in paleothermometry but so far only for marine settings (Huguet et al., 2013; Fietz et al., 2013, 2020; Lü et al., 2015). Recently, Davtian et al. (2021) showed that the OH-isoGDGTs-based temperature index RI-OH′ responded accurately to Dansgaard-Oeschger and Heinrich events in high-resolution 160−45 ka BP records from sites along the Iberian Margin in the Northern Atlantic. We were able to obtain RI-OH′ values for Lake Lugano. Remarkably, the changes in the RI-OH′ profiles in both the SPM as well as in the descending particles follow those in the TEX_{86} profiles quite well (Figs. 4e and 8d). The concentration- and depth-integrated value of RI-OH′ for the oxic SPM was 0.87, slightly higher than the flux-averaged values (0.82) for the two deeper sediment traps positioned below the waters where thaumarchaea produce these OH-isoGDGTs, but in line with the value obtained from the surface sediments (0.88). However, when the most recent marine calibration for RI-OH′ of Fietz et al. (2020) is used, these values translate into temperatures >20 °C, i.e. far beyond the *in-situ* temperatures at which these OH-isoGDGTs were produced. Hence, although our data clearly indicates that OH-isoGDGTs can be used as biomarkers for the presence of thaumarchaea in sediments of lakes, further research is required to assess the potential use of OH-isoGDGTs in the paleothermometry of lake deposits.

4.6. Comparison with isoGDGT distribution in surface sediments of other lakes and general implications for the application of the TEX_{86} in paleolimnological studies

A comparison of the isoGDGT distribution and abundance in the Lake Lugano surface sediments shows that, in many respects, it is comparable to that of other larger (i.e., >45 km^2) and deep (i.e., >100 m maximum depth) lakes (i.e., Lake Zürich, Lake Thun, Lake Neuchâtel, Lake Lucerne). The ternary diagram (Fig. 9a) shows that these lakes contain high abundances of crenarchaeol and GDGT-0 relative to the brGDGTs and, consequently, have relatively low BIT index values. This has been observed before for a variety of European large and/or deep lakes studied earlier by Blaga et al. (2009), including the (peri-)alpine lakes, Lake Maggiore, Lake Bourget, Lake Lucerne, Lake Zug, Lake Sil (Fig. 9a). For our dataset, where absolute abundances of isoGDGTs were determined, it is evident that the concentration of crenarchaeol (380 ± 240 SD μg g^{-1} TOC, with the exception of Lake Thun which has a lower concentration of 19 μg g^{-1} TOC) in the surface sediments of these five larger and deeper lakes is several orders of magnitude higher than the crenarchaeol concentration (~1 μg g^{-1} TOC; n = 17) in the sediments of small (<3.5 km^2) and shallow (<32 m) lakes (Fig. 9b−d). Summed brGDGT concentrations in surface sediments in our set of lakes range from 7 to 180 μg g^{-1} TOC, with an average of 60 ± 42 SD μg g^{-1} TOC (Weber et al., 2018) but, in contrast to crenarchaeol, do not show any correlation with water depth. Hence, the substantially increased crenarchaeol concentration is the predominant reason for the low values of the BIT index for the larger and deep lakes and the low concentrations of crenarchaeol for high values of the BIT index for the shallow lakes (Fig. 9b). A similar conclusion was reached on the basis of GDGT analysis of surface sediments of a small set of Chilean lakes (Kaiser et al., 2015) and 36 lakes in tropical Africa, including many shallow lakes (Tierney et al.,

2010b). Tierney et al. (2010b) reported TOC-normalized crenarchaeol concentration in 36 lakes in tropical Africa. The ones with the highest crenarchaeol concentration (>47 μg g^{-1} TOC) were indeed the largest and deepest lakes, i.e., Lake Tanganyika, Lake Victoria, and Lake Edward, in agreement with our data.

The straightforward explanation for the observation that the crenarchaeol concentration in the surface sediments depends on lake size is that our high-resolution SPM profile of thaumarchaeal abundance and crenarchaeol concentration in Lake Lugano shows that thaumarchaea do not thrive in shallow (~<25 m) lake water. Consequently, shallow lakes typically do not offer a proper niche for Group 1.1a Thaumarchaeota and only minor amounts of crenarchaeol are produced and are preserved in the lake sediments, resulting in low crenarchaeol concentrations. Conversely, in deeper lakes, a proper niche for the Group 1.1a Thaumarchaeota exists, and their GDGTs may be effectively exported to the surface sediments as our study has convincingly demonstrated. This is supported by lower resolution SPM profiles of the crenarchaeol concentration in Lake Lucerne (Blaga et al., 2011) and Lake Malawi (Kumar et al., 2019). Recently, high-resolution SPM profiles of the crenarchaeol concentration in the 90 m deep Lake Chala covering 17 consecutive months in 2013−2014 were reported (Baxter et al., 2021). In agreement with our observations, the crenarchaeol concentration in the SPM at 10 m in the surface waters never exceeded 0.2 ng L^{-1}. In contrast to Lake Lugano (Fig. 2e) and Lake Malawi (Kumar et al., 2019), however, no subsurface maxima in the crenarchaeol concentration profiles, which reach maxima of 60 ng L^{-1}, were observed. This is likely caused by the shallow boundary between oxic and anoxic water masses positioned at 20−40 m depth during the 17-month study period, apparently not creating a proper niche for nitrifying thaumarchaea, which require some oxygen (Baxter et al., 2021). However, the 2006−2015 trap record of Lake Chala revealed four short periods of relatively high crenarchaeol fluxes (up to 9 μg m^{-2} d^{-1}) and a concomitant substantial decrease in BIT values outside the SPM study period. This was attributed to increased wind mixing of the water column, resulting in a deepening of the oxycline, and, in turn, creating a niche for the Group 1.1a Thaumarchaeota (Baxter et al., 2021). This situation would be similar to Lake Lugano, where anoxic conditions only occur at a depth >100 m (Fig. 2a) and thaumarchaea thrive in the still oxygenated waters below the depth of light penetration. This illustrates that the depth of the oxycline in deep lakes, plays an essential role in creating a niche for the thaumarchaea. A number of intermediate-sized lakes (i.e., with a depth between 35 and 85 m; n = 14) in our dataset still displayed an elevated crenarchaeol concentration (range 15−130 μg g^{-1} TOC), although generally lower than in the deeper lakes. However, this does not hold for all "intermediate-depth" lakes; Lake Grimsel, Lake Ritom, Lake Baldegg, Lake Klöntal, Lake Alzasca all have low crenarchaeol concentrations that are at the high end of the range of the shallow lakes (e.g., Fig. 9b). It is plausible that for these lakes it depends on the specific environmental factors (e.g., depth of light penetration, turbidity, occurrence of a shallow oxycline), whether there exists a proper niche for Group 1.1a Thaumarchaeota.

Wang et al. (2019) reported for a large set of mid-latitude lakes in Asia a moderate ($R^2 = 0.58$) correlation between the fractional abundance of crenarchaeol and water depth of the lake, which they attributed to a preference of thaumarchaea for a niche in subsurface lake water. This would be in line with our reasoning above. However, it should be noted that (i) a large (>60%) fraction of the lakes in their dataset had a depth <10 m and even a substantial fraction <1 m (which may in fact not be called a "lake" anymore), and (ii) the fractional abundance of crenarchaeol is also strongly dependent on temperature (e.g. Schouten et al., 2002). Therefore, it remains questionable whether the fractional abundance of crenarchaeol

should be interpreted as a proxy for lake depth. Clearly, our data indicate that for the abundant presence of Group 1.1a Thaumarchaeota, the lake has to be sufficiently deep. In the sedimentary record, however, this will be more trustworthy recorded by the absolute concentration of crenarchaeol (normalized to TOC) or relative to another group of independent biomarkers, such as the brGDGTs (e.g. in the BIT index), than to the other isoGDGTs, as proposed by Wang et al. (2019). As discussed for the deep but highly stratified Lake Chala, shallowing of the oxycline may have an additional effect on the presence of thaumarchaea, complicating this proxy further.

Interestingly, our dataset shows that lake sediments with a relatively high crenarchaeol concentration (>10 µg g^{-1} TOC) also have a relatively low fraction of the crenarchaeol isomer (%Cren' <0.7; Fig. 9c). This holds true for all large lakes. In contrast, the lake sediments with a relatively low crenarchaeol concentration (<10 µg g^{-1} TOC) contain a higher fraction of the crenarchaeol isomer (%Cren' >1), comparable to those of the watershed soils (Fig. 9c). This is consistent with the fact that members of the Nitrosopumilales (Group 1.1a), which typically thrive in the larger and deeper lakes, contain less of the crenarchaeol isomer relative to crenarchaeol than the Nitrososphaerales (Group 1.1b) (see Bale et al., 2019, for an overview). This likely also affects the TEX$_{86}$ values (although these are also affected by the fractional abundances of GDGT-1, -2, and -3); the group of large lakes typically has low (i.e. <0.4) TEX$_{86}$ values, whereas those in the smaller and shallower lakes are higher and more variable (Fig. 9d). In our dataset of (peri-)alpine lakes, this is consistent with a population of Thaumarchaeota dominated by Group 1.1a members, residing below the thermocline in relatively cold waters, in large/deep lakes, while in small/shallow lakes the much smaller input of thaumarchaeal GDGTs is due to a mixed contribution of Nitrosopumilales spp. and, especially, Nitrososphaerales spp., perhaps partly due to soil erosion. Since TEX$_{86}$-temperature calibrations have been primarily performed in environments where Nitrosopumilales spp. thrive, such as oceans (e.g. Kim et al., 2010) and large lakes (Tierney et al., 2010a; Powers et al., 2010), the application of the TEX$_{86}$ paleothermometer in small/shallow lakes is not recommended, also because the variable soil input may have a biasing effect. Our detailed dataset of Lake Lugano and the supporting data from other large central European lakes indicates that TEX$_{86}$ paleothermometry in large/deep lakes would be possible because (i) the thaumarchaea form a substantial contribution to the microbial community of the lake, and hence their membrane lipids contribute substantially to the preserved sedimentary lipid pool, (ii) the thaumarchaeal community is dominated by Nitrosopumilales spp., and (iii) GDGTs of archaea thriving in the anoxic hypolimnion do not contribute markedly to the sedimentary GDGT pool, and therefore do not affect the TEX$_{86}$ signal. However, analysis of the dataset of (peri-)alpine large lakes revealed that the thaumarchaea predominantly reside in the waters below the thermocline of these lakes. Therefore, they record the low temperatures of these waters with concomitantly low TEX$_{86}$ values (Fig. 9d). In the mid-latitudinal area, where the investigated lakes are located, changes in MAAT will only minimally affect the temperatures of the deeper waters. Hence, the sensitivity of the TEX$_{86}$ paleothermometer towards changes in MAAT in these lakes is expected to be low. In large lakes from (peri-)tropical regions, the temperature of these deeper waters is much higher and they are more likely to be affected by changes in MAAT, for example during glacial/interglacial transitions. Indeed, TEX$_{86}$ records from these types of lakes do record climatic transitions (e.g., Tierney et al., 2008, 2010a; Woltering et al., 2011; Johnson et al., 2016). Nevertheless, even here, changing environmental conditions with time, such as changes in primary production affecting the depth of light penetration, as well as changes in redox conditions, may affect the optimal depth for thaumarchaea and, because of the steep thermocline in these systems, the temperature recorded by the TEX$_{86}$. The depth of the oxycline may play a particularly important role in defining a potential niche for Nitrosopumilales spp., as was clearly established by the long-time monitoring program of Lake Chala (Baxter et al., 2021), and, therefore, only part of the TEX$_{86}$ record may provide plausible data (Sinninghe Damsté et al., 2012; Baxter et al., 2021). The lesson to be learned is that, although TEX$_{86}$ values can be analytically produced for almost all lakes and ponds, they may often be difficult to interpret in a climatological context in a meaningful way. Whereas the study of TEX$_{86}$ sedimentary records of large/deep lakes in relatively warm areas seems to be the most promising, more detailed studies, like the one we performed for Lake Lugano, will be required to better understand the ecological niche of thaumarchaea before being able to provide fundamentally understood TEX$_{86}$-temperature relationships.

5. Summary and conclusions

1) The thaumarchaea in Lake Lugano, represented by one dominant OTU belonging to the Nitrosopumilales order, occur predominantly between 30 and 70 m water depth, below the thermocline in relatively cold water year-round. They are the predominant producers of isoGDGTs like crenarchaeol, which are transported to the surface sediment with only small changes in distribution and similarly low TEX$_{86}$ values (ca. 0.40), reflecting the low temperatures below the thermocline. The differential preservation potential rates of thaumarchaeal DNA and isoGDGTs explain the difference in their concentration profiles below 50 m. Cell death results in a relatively large fraction of the more refractory CL isoGDGTs.

2) OH-isoGDGTs with 0−2 cyclopentane moieties were detected for the first time in SPM from a lake. The depth distribution of their concentration in SPM, as well as the flux profiles determined with the sediment traps, closely followed those of crenarchaeol, indicating an origin from Nitrosopumilales spp., thriving at 30−70 m. Their distribution in the sediment was comparable, but the ring index based on the OH-isoGDGTs (RI-OH′ index) had such a high value that it would predict water temperatures of ca. ~20 °C using a recent marine RI-OH′-temperature calibration, clearly much higher than the *in-situ* temperatures of ca. 6 °C. Further research is required to assess the potential of the RI-OH′ index in lacustrine settings.

3) Below the oxycline in Lake Lugano archaea become much less dominant in the prokaryotic community and the archaeal community changes substantially. The detected Nitrosopumilales 16S rRNA gene sequences are considered to be 'fossil' (i.e., derived from dead cells) but Bathyarchaeota, Diapherotrites, Euryarchaeota, and Woesarchaeota are thought to be alive archaea in the deep anoxic waters. The change in archaeal community composition is also reflected by a change in the distribution of the isoGDGTs; a much higher contribution of IPLs with a GDGT-0 core is noted, as has also been observed in another anoxic lake. This different origin is also reflected by the more negative $\delta^{13}C$ value of the acyclic biphytane in the deep waters. Interestingly, the isoGDGTs produced by archaea in the deep anoxic water have no imprint on the sedimentary distribution of isoGDGTs, probably because of the absence of grazers that agglomerate archaeal debris into larger particles, which can then sink relatively fast to the bottom of the lake. In this way, Lake Lugano is comparable to the euxinic Black Sea, where the substantial production of anaerobic methane-oxidizing archaea in the euxinic waters, is not reflected by the isoGDGT composition of surface sediments.

4) Clear-cut differences in the isoGDGT concentration and distribution in surface sediments of 35 (peri-) alpine lakes in Switzerland and Italy were observed, seemingly constrained by the depth of the lake and the position of the oxycline. Generally, in deep (and usually large) lakes, like Lake Lugano, the crenarchaeol concentration is much higher than in small-sized and shallow lakes and ponds and are characterized by lower %Cren' values. This is can be explained by the fact that, for a number of reasons, Nitrosopumilales spp. have no niche in shallow waters (0−~30 m depth). Consequently, no stable community of Thaumarchaeota develops and production, and hence preservation in the sedimentary record, of crenarchaeol (and other thaumarchaeal isoGDGTs) is much lower than in large lakes (like Lake Lugano). In the small-sized and shallow lakes and ponds, crenarchaeol can still be present. There, it probably mainly derived from the Nitrososphaerales spp. (either from *in-situ* production or soil erosion), which are characterized by a higher relative content of the crenarchaeol isomer, disturbing accurate temperature estimations using the TEX$_{86}$. It is, therefore, recommended not to use the TEX$_{86}$ paleothermometer in these small lakes. Ironically, in the large and deep lakes Nitrosopumilales are found at high abundance, resulting in high concentrations of crenarchaeol (and other isoGDGTs). However, the Nitrosopumilales spp. in these mid-latitudinal lakes thrive in cold waters below the thermocline. The temperature of these waters will barely be affected by fluctuations in MAAT, in contrast to surface waters. Therefore, the TEX$_{86}$ paleothermometer is relative insensitive in these lakes. More promising results are expected for lakes in the (peri-)tropical regions, where also deeper waters are higher in temperature and more sensitive to fluctuations in MAAT, although on longer time scales. More detailed study programs, like the one we performed for Lake Lugano, of such lakes are required to develop and apply lacustrine TEX$_{86}$-temperature calibrations with a higher level of confidence.

Author contributions

J. S. Sinninghe Damsté; : Conceptualization, Methodology, Validation, Investigation, Formal analysis, Data curation, Writing − original draft, Writing − review & editing, Visualization, Supervision, Funding acquisition. Y. Weber: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing − review & editing. J. Zopfi: Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing − review & editing. M. F. Lehmann: Resources, Writing − review & editing, Supervision. H. Niemann: Conceptualization, Resources, Writing − review & editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.quascirev.2021.107352.

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