



Supplement of

Carbon sources of benthic fauna in temperate lakes across multiple trophic states

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Supplementary Information

Target	Primer	Sequence 5' - 3'	Reference	Standard
Archaeal	Arc915F_mod	AAT TGG CGG GGG AGC AC	Cadillo-Quiroz et al. (2006)	Thermoplasma
16S rRNA	Arc1059R	GCC ATG CAC CWC CTC T	Yu et al. (2005)	acidophilum
Bacterial	Bac908F_mod	AAC TCA AAK GAA TTG ACG GG	Lever et al. (2015)	Desolfotignum
16S rRNA	Bac1075R	CAC GAG CTG ACG ACA RCC	Ohkuma and Kudo (1998)	phosphitoxidans
pmoA	A189F	GGN GAC TGG GAC TTC TGG	Holmes et al. (1995)	Methylococcus
	Mb661R	CCG GMG CAA CGT CYT TAC C	Costello and Lidstrom (1999)	capsulatus
mcrA	Mlas_F	GGT GGT GTM GGD TTC ACM CAR TA	Steinberg and Regan (2009)	Methanocorpusculum
	mcrA-rev	CGT TCA TBG CGT AGT TVG GRT AGT	Steinberg and Regan (2009)	parvum

Table S1: qPCR primers and standards and their corresponding references

Table S2: qPCR protocols for each primer pair.

Primer target:		Arc	Bac	pmoA	mcrA
qPCR step	time (min:s)	primer	-specific t	emperatu	:e (°C)
1. Initial Activation	05:00	Always	95		
2. Denaturation	00:10	Always	95		
3. Annealing	00:30	55	60	(62) 52	56
4. Polymerization	00:15	Always	72		
5. Acquisition	00:05	81	82	80	80
Cycle repeats step	<i>25</i> .	40	45	(10+) 35	40
6. Denaturation	01:15	Always	95		
7. Acquisition	continuous	55-95	60-95	60-95	53-95
8. Cooling	x	Always	4		

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Table S3: Overview of chemicals used in the different master mix for PCR for one reaction (25 µl total reaction volume) (A). Temperate and time protocols used for each PCR during library preparation. Underlined are the steps which are repeated (cycle number), see main text for details (B).

(A)			
	Boost PCR	Tail PCR	Index PCR
Go Taq G2 DNA Polymerase (5 µl/ml)	0.125 µl	0.125 µl	0.125 µl
Go Taq Colorless reaction buffer (5x)	5 µl	5 µl	5 µl
PCR nucleotide mix (10 mM)	0.5 µl	0.5 μl	0.5 µl
primer 1	0.75 µl	0.75 µl (0.1875 of each: nex0-nex3)	2.5 μl
primer 2	0.75 µl	0.75 µl (0.1875 of each: nex0-nex3)	2.5 μl
BSA	1.25 µl	none	none
H ₂ O (mol. grade)	14.625 µl	16.875 μl	12.375 µl
Template	2 µl	1 µl	2 µl

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(B)

(D)	Temperatur	Temperature (°C) / Time (min:s)				
PCR	Boost	Tail	Index			
Activation	95 / 05:00	95 / 02:00	95 / 02:00			
Denaturation	98 / 00:20	95 / 00:20	95 / 00:20			
Annealing	49 / 00:30	49 / 00:30	55 / 00:40			
Polymerization	72 / 00:30	72 / 00:30	72 / 00:30			
Denaturation	72 / 05:00	72/05:00	72/05:00			
Cooling	4 / ∞	4 / ∞	4 / ∞			
0						

Table S4: ¹³C-C of specific sediment layers and phytoplankton samples from this study (A) and literature (B). For plankton samples, 'surface' indicates surface water samples that were obtained using plankton tows with different mesh sizes. Asterisks indicate samples that were not decarbonized.

(A)

Lake	Station	Туре	depth	size or feature	δ ¹³ C
Lucerne	shore	phytoplankton	surface	$> 50 \mu m$	-26.3*
Lucerne	shore	phytoplankton	surface	> 100 µm	-29.4*
Lucerne	shore	phytoplankton	surface	> 20 µm	-30.1
Lucerne	shore	phytoplankton	surface	$> 20 \ \mu m$	-19.5*
Lucerne	shore	phytoplankton	surface	> 20 µm	-31.2
Lucerne	shore	phytoplankton	surface	$> 20 \ \mu m$	-26.7*
Lucerne	shore	phytoplankton	surface	20-100 μm	-29.5*
Lucerne	shore	phytoplankton	surface	$> 50 \ \mu m$	-30.3
Lucerne	shore	phytoplankton	surface	$> 50 \ \mu m$	-21.1*
Baldegg	1	sediment	21 cm	layer	-35.4
Zurich	1	sediment	2-2.5 cm	algal bloom	-33.7
Greifen	1	sediment	13	layer	-34.1
Zurich	3	sediment	18.5 cm	sediment with plant material	-29.1
Zurich	3	sediment	18.5 cm	plant material (leaves, wood)	-29.2
Greifen	3	sediment	14-15 cm	layer	-35.7
Baldegg	1	sediment	21 cm	brighter layer	-35.6

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B)			
Target	δ ¹³ C range (‰)	Environment	Reference
Phytoplankton	-34.4 to -5.9	sub-arctic lakes	Vuorio et al. (2006)
Phytoplankton	-36.6 to -28.7	Lake Memphremagog, Quebec	Lazerte (1983)
Algae	-35 to -15	small water bodies Australia	Boon and Bunn (1994)
Floating plants	-32 to -25		
Emergent macrophytes	-31 to -25		
Submerged macrophytes	-33 to - 15		
Epiphytes	-32 to - 20		
Freshwater seston	-35.2 to -23.9	Sacramento–San Joaquin River Delta	Cloern et al. (2002)
sediment trap OM	-40 to -22	Lake Lugano	Bernasconi et al. (1997)
sediment trap POC	-39 to -27	Lake Greifen (yearly cycle)	Hollander et al. (1993)
POC	-60 to -20	Lake Lugano (yearly cycle)	Lehmann et al. (2004)

Table S5: Abundance of Oligochaetes and Chironomid Larvae per m² for each Lake station, indicated are averages of the three stations and the corresponding standard deviations (SD), please note for Lake Zurich averages and SD were calculated from station 2 and 3 only (*).

Lake	Oligochaetes (m ⁻²)	Chironomid Larvae (m ⁻²)	
Lucerne			
Station 1	170	1019	
Station 2	57	340	
Station 3	0	566	
Average $(\pm SD)$	75 (±86)	641 (±346)	
Zurich			
Station 1	0	0	
Station 2	906	962	15
Station 3	1302	736	
Average $(\pm SD)^*$	1104 (±280)	849 (±160)	
Zug			
Station 1	1132	57	
Station 2	1245	0	
Station 3	1641	57	
Average (±SD)	<i>1340 (±267)</i>	38 (±33)	
Greifen			
Station 1	3339	0	20
Station 2	962	0 20	
Station 3	3736	0	
Average (±SD)	2679 (±1500)	-	
Baldegg			
Station 1	9282	57	
Station 2	4868	0	
Station 3	396	170	
Average $(\pm SD)$	4849 (<u>+</u> 4443)	75 (±86)	

Table S6: (A): food sources and feeding modes as well as distributions of oligochaete worms, and (B): of chironomid larvae, where feeding modes are after Moog (2002).

(A)			
Таха	Food source and feeding mode	Distribution	Reference
Embolocephalus velutinus	<i>Naididae</i> : look for food at surface of sediment or other surfaces, most surface deposit feeders	oligo- und mesotrophic lakes, cold stenothermic species	Van Haaren and Soors (2013); (Martin et al. (2008);
Limnodrilus hoffmeisteri	all <i>Tubificidae</i> are thought to be subsurface deposit feeders that take in sediment: and mainly feed on bacteria (and algae) as main food source	in eu- to hypereutrophic lakes, very tolerant to oxygen deficiencies, omnipresent, wide ecological valence	Brinkhurst (1982) Brinkhurst and Chua (1969)
Limnodrilus profundicola			
Potamothrix hammoniensis		Mostly in bigger lakes, correlates with organic part in sediment originating from algae, omnipresent species with wide ecological valence	
Potamothrix veydovskyi		indicative for mid to high pollution, eutrophic conditions	
Tubifex tubifex		Widespread. Often dominant under eutrophic or highly oligotrophic conditions.	

(2)			
Taxa	Food sources and feeding mode	Distribution	Reference
Sergentia coracina	mainly detritus feeder (gathering collector), also filter feeding of sedimented fine particulate OM. Stretch out of tubes and feed from surrounding mud, mud dwellers.	mesotrophic systems	Pillot (2009)
Paracladopelma laminatum	mainly predators	Less Fe(II) tolerant < 0.2 mg Fe(II) /L, eutrophic lakes, rarely in oligotrophic lakes, tolerant of organic loading	Pillot (2009)
Procladius sp.	mainly predators, also detritus feeding of algae (gathering collector), prey are small crustaceans and later in life cycle <i>chironomidae</i> and oligochaetes.	in mineral and organic sediment, stagnant and slow flow water types, warm water chironomid, lower critical O ₂ concentration	Vallenduuk and Pillot (2007) Brodersen et al.
Tanytarsus norvegicus	mainly detritus feeders (gathering collector), also grazing (scrapers, raspers) and filter feeding of sedimented fine particulate OM, build tubes		(2004)
Macropelopia fehlmanni	mainly predacious, also detritus feeding, prey are mainly <i>chironomidae, plecoptera,</i> <i>copeopoda</i> , detritus		Vallenduuk and Pillot (2007)
Chironomus riparius/piger gr.	mainly filter feeder, also shredders and grazers, of suspended FPOM, CPOM, fallen leaves, plant tissue, terrestrial and algal OM (Goedkoop et al. 2006), but believed to switch from mainly surface deposit-feeding to microbial gardening under hypoxic conditions (Stief et al. 2005)	4-7 generations a year, emerging from march to november, one generation 34.8 days at 15 °C, prefer organic muddy substrate with characteristic for polluted flowing water, heavy load of OM, warm water chironomid, lower critical O ₂ concentrations	Pillot (2009) Stief et al. (2005) Goedkoop et al. (2006) Brodersen et al. (2004)
Ablabesmyia monilis	mainly predators, also detritus feeding, actively attacking <i>chironomidae</i> , oligochaetes and partially <i>cladocera</i> but also dead prey (diatoms, detritus)	warm water chironomid, lower critical O ₂ concentrations	Vallenduuk and Pillot (2007) Chaloner and
Tanytarsus sp.	mainly detritus feeding, but also grazing and filter feeding of sedimented fine particulate OM, build tubes	oligo to mesotrophic lakes (Saether, 1980)	Wotton (1996) Brodersen et al. (2004)
Micropsectra sp.	mainly detritus feeding, but also grazing and filter feeding of sedimented fine particulate OM	cold water chironomid	Saether (1980)
Stempellina bausei	grazing and detritus feeding, of epilithic algal tissue, biofilm, partially POM (endo and epilithic algal tissue, partially living plant tissue)		
Orthocladiinae gen. sp.	mainly algae		Stevenson et al. (1996)
Polypedilum nubeculosum	mainly detritus feeding, but also grazing and filter feeding of sedimented fine particulate OM, Bacteria seem to be most important food (Moore, 1979)	2-3 generations adults emerge from the end of April to early October when temp in spring reaches 8°C, bottom dwellers, make long tubes, density correlated with oxygen contents, organic sediment	Pillot (2009) Moore (1979)
Chironomus sp.	mainly filter feeding, also shredders and grazers of suspended FPOM, CPOM, prey, build tubes		
Chironomus commutatus	mainly filter feeding, also shredders and grazers of suspended FPOM, CPOM, prey, build tubes	More common in stagnant water, can stand low O2 conditions	Pillot (2009)

Table S7: Depth distribution of oligochaete (A) and chironomid (B) species in each lake

A: Oligochaetes

Lake Baldegg	# of individuals per species					Total # of individuals
Depth (cm)	Tubificidae (+bristles)	Tubificidae (- bristles)	Tubifex Tubifex	Limnodrilus hoffmeisteri	Limnodrilus profundicula	
0-1		2		1		3
1-2		5				5
2-3						
3-4						
4-6	1	9		11	10	31
6-8	2	19	1	26	4	52
8-10						
10-12		15		11		26

Lake Greifen	# of individuals per species					Total # of individuals
Depth (cm)	Tubificidae (+bristles)	Tubificidae (- bristles)	Tubifex Tubifex	Limnodrilus hoffmeisteri	Potamothrix hammoniensis	
0-1						
1-2	6					6
2-3	10	1			4	15
3-4	5					5
4-6	8		1		4	13
6-8	10		2	1		13
8-10	3		2			5
10-12						

Lake Zug	# of individuals per species					Total # of individuals
Depth (cm)	Tubificidae (+bristles)	Tubificidae (- bristles)	Tubifex Tubifex	Limnodrilus hoffmeisteri	Potamothrix hammoniensis	
0-1						
1-2	4					4
2-3		3				3
3-4						
4-6	2	2	2	1	3	10
6-8						
8-10						
10-12	3	1	1			5
12-14	3		2			5
14-16						
16-18	2					2

Lake Zurich	# of individuals per species						
Depth (cm)	Tubificidae (+bristles)	Tubificidae (- bristles)	Embolocephalus velutinus	Limnodrilus hoffmeisteri	Potamothrix hammoniensis		
0-1							
1-2							
2-3	1					1	
3-4	5				1	6	
4-6	1		1	1	2	5	
6-8	3	1			1	5	
8-10							
10-12		1				1	

Lake Lucerne	# of individuals per species	Total # of individuals
Depth	Potamothrix vejdoyski	
(cm)		
0-1		
1-2		
2-3	1	1
3-4		
4-6		
6-8		
8-10		
10-12		

B: Larvae

Lake Baldegg	# of individuals p	Total # of individuals		
Depth (cm)	Micropsectra sp.	Chironomus riparius/piger gr.	Orthocladiinae gen. Sp.	
0-1				
1-2	1			1
2-3				
3-4		1		1
4-6				
6-8				
8-10				
10-12				
12-14				
14-16				
16-18			1	1

Lake Zug	# of individuals p	Total # of individuals	
Depth (cm)	Sergentia coracina	Procladius sp.	
0-1			
1-2	1		1
2-3			
3-4		1	1

Laba	# of indivi	dualan	an an action *							Total # of
Zurich		duais p	er species *							individuals
Depth (cm)	S. cora- cina	P. sp.	T. norve- gicus	C. riparius /piger gr.	T. sp.	M. sp.	P. nube- culosum	C. sp.	C. commutatus	
0-1	1					2				3
1-2					1	2				3
2-3	1		1	2		2	1			7
3-4										
4-6	1			1						2
6-8	1	1		2						4
8-10									2	2
10-12						1		1		2
12-14								1		1
14-16					2					2
16-18					1					1
18-20										
20-24					1	1				2

Lake Lucerne	# of individuals per species *									Total # of individuals	
Depth (cm)	S. cora- cina	P. lami- natum	T. norve- gicus	C. riparius /piger gr.	T. sp.	M. sp.	A. monilis	P. sp.	M. fehl- manni	S. bausei	
0-1	1				3		1				5
1-2	1	1	2			5		3	2		14
2-3	1							1			2
3-4								2	1	1	4
4-6								5			5
6-8				1							1

*S. coracina = Sergentia coracina, P.laminatum= Paracladopelma laminatum, T. novergicus = Tanytarsus norvegicus, T. sp. = Tanytarsus sp., M. sp. = Micropsectra sp., A. monilis = Ablabesmyia monilis, P. sp. = Procladius sp., M, fehlmanni = Macropelopia fehlmanni, S. bausei = Stempellina bausei, C. xxxxx = Chironomus xxxxx, P. nubeculosum = Polypedilum nubeculosum.

Table S8: Overview of ZOTUs that were enriched (>5% of total reads) or highly enriched (>50% of total reads) in whole macrofaunal specimen (w), macrofaunal guts (g), or macrofaunal bodies after gut removal (b). Oligochaetes are shown in (A), chironomid larvae in (B). Classifications were done to the genus- or family-level via phylogenetic trees with manually optimized alignments in the ARB software (Ludwig et al. (2004), Supplementary Fig. S8). Fractions indicate the number of w, b, or g analyzed per lake in which a ZOTU was enriched (second column from right) or highly enriched (right column). LB = Lake Baldegg, LG = Lake Greifen, LZug = Lake Zug, LZ = Lake Zurich, LL = Lake Lucerne.

(A)

ZOTU#	Classification	# of fauna, where ZOTU enriched, broken down according to w, b, and g (range of % fraction of total reads)	# of total fauna where ZOTU highly enriched
Fusobacteria	ı		
ZOTU389	Fusobacterium Cl. I	LB: 1/11 w (7%)	LB: 5/14; LG: 5/5; LZug: 4/9;
ZOTU1	Fusobacterium Cl. I	LB: 4/11 w (44-79%), 1/3 b (87%), 1/3 g (91%); LG: 4/5 w (64-77%); LZug: 4/7 w (75-97%)	LL: 1/1
ZOTU7, 22	Fusobacterium Cl. I	LG: 1/1 b (62%), 1/1 g (86%)	
ZOTU5, 13	Fusobacterium Cl. I	LB: 4/11 w (5-24%); LG: 1/5 w (65%)	
Proteobacter	ia		
β -Proteobact	teria		
ZOTU6	Uncl. Subcl. (<i>Rhodocyclales</i>)	LZ: 1/1 w (59%)	LZ: 1/1
ZOTU8	Deefgea (Neisseriales)	LB: 1/3 g (93%)	LB: 1/14
E-Proteobact	eria	• · · ·	·
ZOTU18	Wolinella (Campylobacterales)	LZug: 1/7 w (55%)	LZug: 2/9
ZOTU9	Uncl. Cl.	LG: 1/5 w (31%); LZug: 1/2 b (5-69%)	
α -Proteobac	teria	· · ·	·
ZOTU4	Holosporaceae (Holosporales)	LB: 1/11 w (93%); LZug: 2/7 w (7-60%)	LB: 1/14; LZug: 1/9
Bacteroidete	S		-
ZOTU10	Flavobacterium	LB: 4/11 w (6-10%), 1/3 g (6%); LG: 3/5 w (10-17%);	-
	(Flavobacteriales)	LZug: 1/7 w (8%)	
Parcubacter	ia		· · · · · · · · · · · · · · · · · · ·
ZOTU199	Uncl. Cl. I	LB: 2/11 w (7%); 1/3 b, 60%; 1/3 g, 5%)	LB: 1/14
TOTAL			LB: 8/14; LG: 5/5; LZug: 7/9; LZ: 1/1; LL: 1/1

ZOTU#	Classification	# of fauna, where ZOTU <u>enriched</u> , broken down according to w, b, and g (range of % fraction of total reads)	# of total fauna where ZOTU <u>highly enriched</u>
Fusobacteria	a	I	
ZOTUI	Fusobacterium Cl. I	LZ (1/1 b) (8%)	LZ(3/7 > 5%; 1/7
ZOTU5, 13	Fusobacterium Cl. I	LZ (2/6 w) (6-57%)	_ >50%)
Proteobacter	ria	I	
γ-Proteobaci	teria		
ZOTU11	Serratia (Enteromonadales)	LL (1/6 b) (83%)	LL (5/10 >5%; 4/10
ZOTU21, 3	Aeromonas (Aeromonadales)	LL (1/4 w, 79%; 2/6 b, 11-78%; 2/6 g, 98-99%)	_ >30%)
ZOTU26	Uncl. Cl. (Pseudomonadales)	LL (1/4 w) (40%)	-
β-Proteobac	teria		
ZOTU6	Uncl. Subcl. (Rhodocyclales)	LL (1/4 w) (56%)	LL (3/10 >5%; 2/10
ZOTU12	Uncl. Subcl. (Burkholderiales)	<i>LL</i> (2/6 <i>b</i>) (22-56%)	_ >30%)
α -Proteobac	teria		
ZOTU2	Wolbachia (Rickettsiales)	LZ (2/6 w), LL (1/4 w, 3/6 b) (42-71%)	LZ (2/7 >5%; 2/7 >50%); LL (5/10 >5%; 4/10 >50%)
Bacteroidete	'S		
ZOTU28	Uncl. Wastewater & Gut Group (Bacteroidales)	<i>LZ</i> (1/6 w) (72%)	LZ (1/7 >50%)
Firmicutes	1	1	1
ZOTU19	"Insect Gut Cl." (<i>Clostridiales</i>)	LL (1/4 w, 2/6 g) (19-31%)	LL (3/10 >5%; 0/10 >50%)
TOTAL	1	1	LB: 0/2; LZ: 3/7; LL:



 N_{tax}= 18; N_{total}= 39
 N_{tax}= 29; N_{total}= 71
 N_{tax}= 57; N_{total}= 142
 N_{tax}= 117; N_{total}= 257

 ●Tubificidae (+bristles)
 ○Tubificidae (-bristles)
 ○Limnodrilus hoffmeisteri
 ●Potamothrix hammoniensis

 ●Potamothrix hammoniensis
 ●Tubifex Tubifex
 ○Limnodrilus profundicula
 ©Embolocephalus velutinus



5 Figure S1: Taxonomy results for selected individuals of the two main classes found (A: oligochaete worms, Ntax=222, Ntot = 513; B: chironomid larvae, Ntax=65, Ntot=70) for each lake and station individually. Numbers indicate the % abundance of taxonomically analyzed individuals. No chironomid larvae were found in Lake Greifen and at station 2 of Lake Baldegg (45 m) and Lake Zug (35 m). In Lake Lucerne only 4 oligochaetic worms were found of which 1 was taxonomically analyzed (Potamothrix vejdoyski).No Macrofauna was found at the deep station of Lake Zurich (137 m).



Figure S2: δ^{13} C of TOC, methane, specific sediment layers (water column phytoplankton and algal bloom sediment layers), oligochaetes and chironomid larvae vs. sediment depth (cmblf).



Figure S3: relative abundance of archaeal sequences (phylum level) for sediment, tubes, chironomid larvae and oligochaete samples.

Phylum



Family



5 Figure S4: PCoA analysis of the relative abundance of Bacteria on the phylum, class, family and genus level. Distances are calculated using Bray Curtis distances.



Figure S5: Total organic carbon (TOC) in [%], stable carbon isotopes of TOC (δ^{13} C-TOC) in [‰] and Chla concentrations [µg/g sedww] for each lake vs sediment age [AD]. Three stations per lake are plotted in one subplot. Light grey, open triangles= shallowest station, dark grey, closed circles = medium station, black, open circles = bottom station.



Figure S6: Profile of ²¹⁰Pb_{unsupported} and ¹³⁷Cs in Bq/kg, along sediment depth in centimetre below lake floor (cmblf) for each station. Blue arrow indicates the 137Cs peak due to the Chernobyl accident in 1986 and the red arrow indicates bomb testing in 1963. Please note different x-axes for Lake Lucerne.





0.10



Figure S7: Phylogenetic assignment for sequences of Fusobacteria (A), Proteobacteria (B), Bacteroidetes (C), Firmicutes (D) and Parcubacteria (E) performed in ARB (please see SI Table S8). The trees show IDs and source environments of the closest related environmental DNA sequences in black, the sequences detected in this study are marked in magenta.

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