

Supplementary information for

Lake Microbiome and Trophic Fluctuations of the Ancient Hemp Rettery

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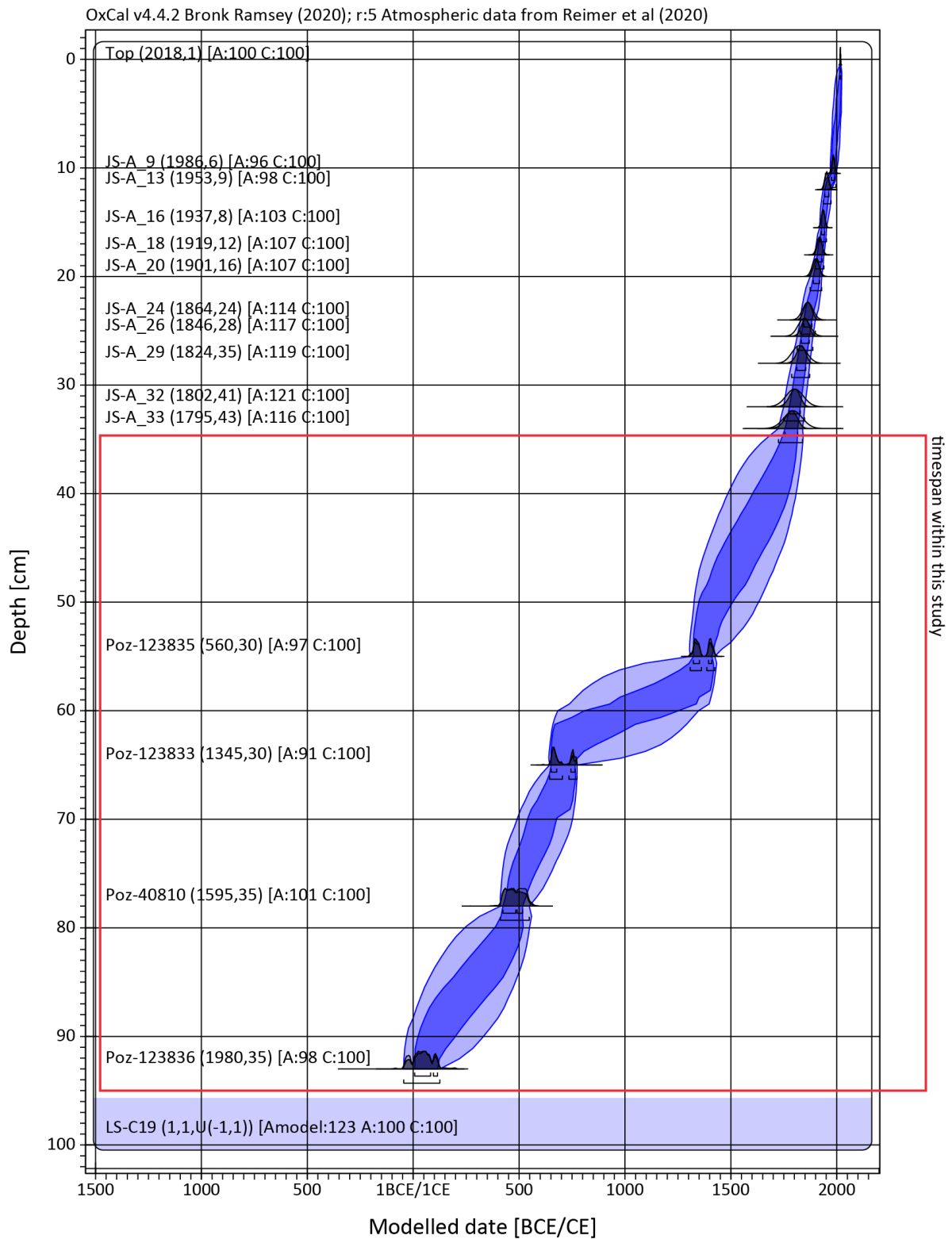
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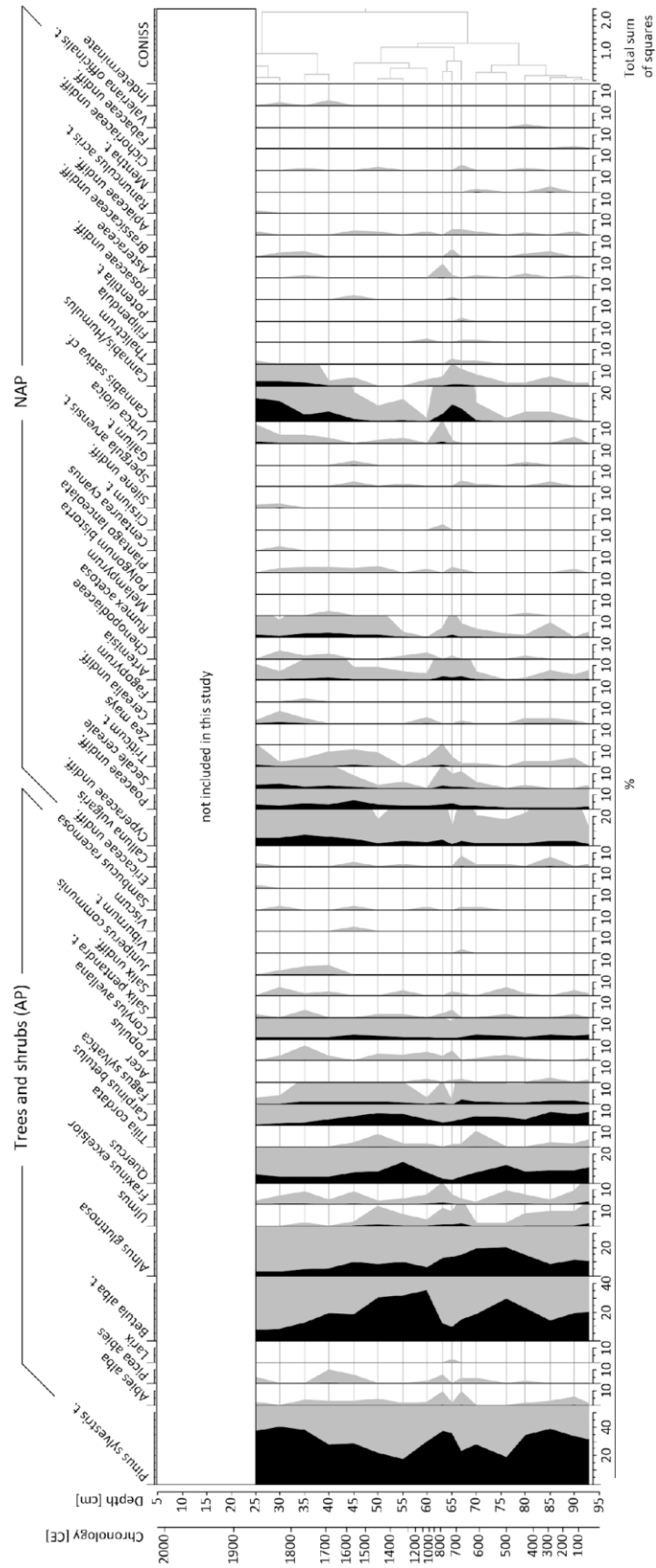
This supplemental material includes:

Supplemental Figure 1-6

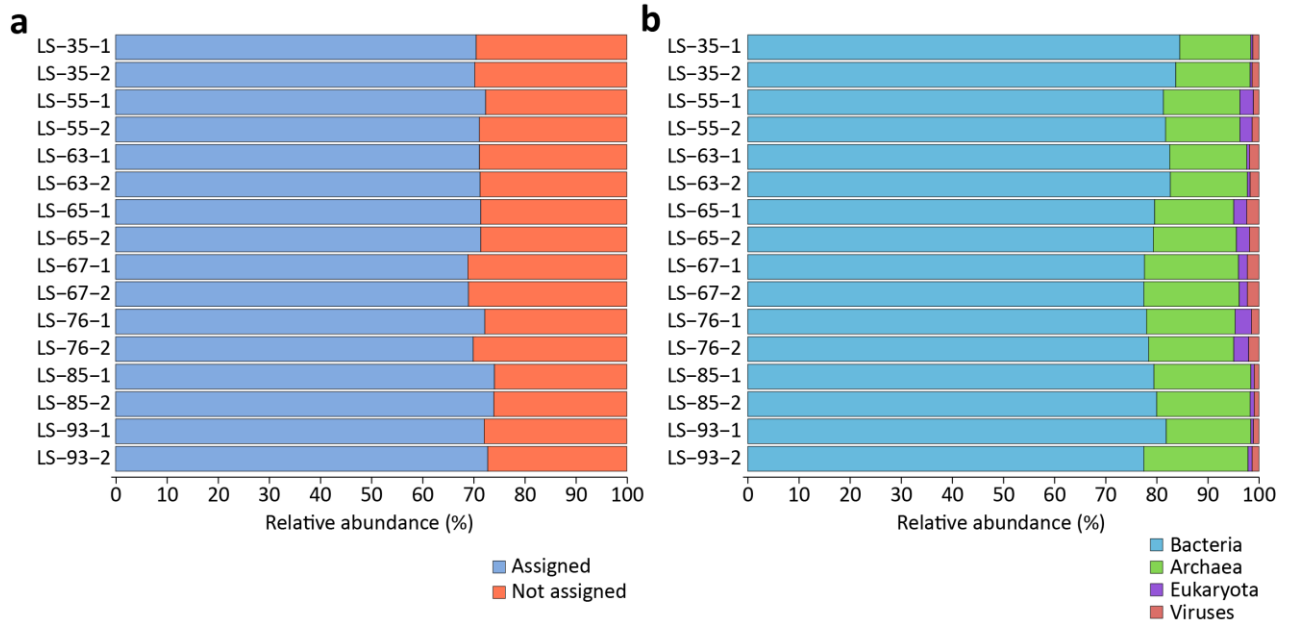
Supplemental Table 1-5



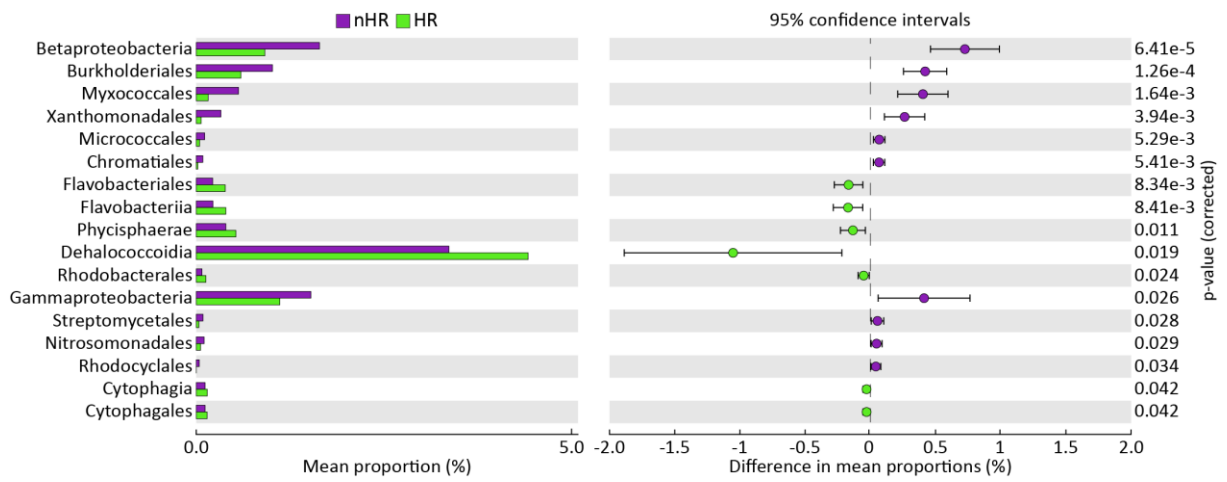
SuppFig 1. Age-depth model – constructed by MS with OxCal 4.4 software (<https://c14.arch.ox.ac.uk/oxcal.html>).



SuppFig 2. Pollen percentage diagram of LS-C19 core – drawn by MS with Tilia software (<https://www.tiliait.com>) based on data provided by IAP.



SuppFig 5. (a) Taxonomic assignment of sequences per sample. **(b)** Domain assignment per sample.



SuppFig 6. Extended error bar plot showing significant differences ($p < 0.05$) in the taxa abundances at the class-subclass level for 'HR' (green) vs 'nHR' (purple) groups, calculated with two-sided Welch's t-test (STAMP).

SuppTab 1. List of Cladocera taxa recorded in LS-C19 core and their ecological preferences according to Flössner (2000) and Bjerring *et al.* (2009), Błędzki and Rybak (2010).

taxa	habitat	
	planktonic/littoral	benthic/vegetation
<i>Leptodora kindti</i> (Focke, 1844)	planktonic	-
<i>Bosmina longirostris</i> (O.F. Muller 1785)	planktonic	-
<i>Eubosmina</i> (<i>E. coregoni</i> Baird, 1857 & <i>E. longispina</i> Leydig, 1860)	planktonic	-
<i>Daphnia longispina</i> -group (O.F. Muller 1776)	planktonic	-
<i>Ceriodaphnia sp.</i> (Dana, 1853)	planktonic/littoral	vegetation
<i>Sida crystallina</i> (O.F. Muller, 1776)	planktonic/littoral	vegetation
<i>Euryercus lamellatus</i> (O.F. Muller, 1776)	littoral	vegetation
<i>Acroperus harpae</i> (Baird, 1834)	littoral	vegetation
<i>Camptocercus rectirostris</i> (Schrödler, 1862)	littoral	vegetation
<i>Alona affinis</i> (Leydig, 1860)	littoral	vegetation
<i>Alona quadrangularis</i> (Baird, 1843)	littoral	benthic/vegetation
<i>Alonella excisa</i> (Fischer, 1854)	littoral	vegetation
<i>Alonella exigua</i> (Lilljeborg, 1853)	littoral	vegetation
<i>Chydorus sphaericus s.l.</i> (O.F. Muller, 1776)	littoral/planktonic (while blue-green algae bloom)	varied
<i>Alona costata</i> (Sars, 1862)	littoral	vegetation
<i>Alonella nana</i> (Baird, 1834)	littoral	vegetation
<i>Pleuroxus trigonellus</i> (O.F. Muller, 1776)	littoral	vegetation
<i>Pleuroxus uncinatus</i> (Baird, 1850)	littoral	vegetation
<i>Peracantha truncata</i> (O.F. Muller, 1785)	littoral	vegetation
<i>Pleuroxus laevis</i> (Sars, 1862)	littoral	vegetation
<i>Graptoleberis testudinaria</i> (Fischer, 1848)	littoral	vegetation
<i>Leydigia leydigi</i> (Schrödler, 1863)	littoral	benthic
<i>Leydigia acanthocercoides</i> (Fischer, 1854)	littoral	vegetation
<i>Alona guttata</i> (Sars 1862)	littoral	vegetation
<i>Alona rectangula</i> (Sars 1861)	littoral	vegetation
<i>Kurzia lattissima</i> (Kurz, 1875)	littoral	vegetation
<i>Disparalona rostrata</i> (Koch, 1841)	littoral	benthic
<i>Oxyurella tenuicaudis</i> (Sars, 1862)	littoral	vegetation
<i>Alona protzi</i> (Hartwig 1900)	littoral	benthic/phreatic
<i>Latona setifera</i> (O.F. Müller, 1776)	littoral	benthic
<i>Anchistropus emarginatus</i> (Sars, 1862)	littoral	vegetation
<i>Camptocercus lilljeborgi</i> (Schrodler, 1861)	littoral	vegetation
<i>Camptocercus fennicus</i> (Stenroos, 1898)	littoral	vegetation
<i>Poyphemus pediculus</i> (Linné, 1761)	littoral	vegetation

Cladocera taxa included in trophic inference as high trophic indicators (counted as the percentage among total littoral taxa): *Alona rectangula*, *Chydorus sphaericus*, *Pleuroxus uncinatus*, *Leydigia leydigi*, *Leydigia acanthocercoides*, *Disparalona rostrata*, *Oxyurella tenuicaudis*, *Camptocercus lilljeborgi* and *Graptoleberis testudinaria*.

SuppTab 2. List of dominant diatom taxa recorded in LS-C19 core and their ecological preferences; habitat system by Round (1981), pH system by Hustedt (1937–39), trophic system by Neumann (1932).

Taxa	habitat	pH	trophy
<i>Amphora libyca</i> Ehrenberg	benthic	alkaliphilous	oligotraphentic
<i>Asterionella formosa</i> Hassal	planktonic	alkaliphilous	eu-mesotraphentic
<i>Aulacoseira ambigua</i> (Grunow) Simonsen	planktonic	alkaliphilous- neutrophilic	eutraphentic
<i>Aulacoseira granulata</i> (Ehrenberg) Simonsen	planktonic	alkaliphilous	eutraphentic
<i>Aulacoseira italica</i> (Ehrenberg) Simonsen	planktonic	neutrophilic - alkaliphilous	eutraphentic
<i>Cyclostephanos dubius</i> (Fricke) Round	planktonic	alkaliphilous	eutraphentic
<i>Cyclotella cyclopuncta</i> Håkansson & J.R.Carter	planktonic	neutrophilic	oligotraphentic
<i>Cyclotella distinguenda</i> v. <i>unipunctata</i> (Hustedt) Håkansson & J.R.Carter	planktonic	neutrophilic	oligotraphentic
<i>Fragilaria nanana</i> Lange-Bertalot	benthic	neutrophilic	oligotraphentic
<i>Gomphonema acuminatum</i> Ehrenberg	benthic	alkaliphilous- neutrophilic	eu-dystraphentic
<i>Gomphonema angustum</i> Agardh	benthic	neutrophilic	eu-dystraphentic
<i>Gomphonella olivacea</i> (Hornemann) Rabenhorst	benthic	alkaliphilous	eu-dystraphentic
<i>Gomphonema parvulum</i> (Kützing) Kützing	benthic	alkaliphilous- neutrophilic	eu-mesotraphentic
<i>Gomphonema truncatum</i> Ehrenberg	benthic	alkaliphilous	eu-dystraphentic
<i>Lindavia comensis</i> (Pantocsek) Nakov et al.	planktonic	neutrophilic	oligotraphentic
<i>Lindavia ocellata</i> (Pantocsek) Nakov et al.	planktonic	alkaliphilous	oligotraphentic
<i>Lindavia radiosa</i> (Grunow, Lemmermann) De Toni & Forti	planktonic	alkaliphilous	mesotraphentic
<i>Pseudostaurosira parasitica</i> (Smith) Morales	benthic	alkaliphilous	eu-mesotraphentic
<i>Stephanodiscus hantzschii</i> Grunow	planktonic	alkalib- alkaliph	eutraphentic
<i>Stephanodiscus medius</i> Håkansson	planktonic	alkaliphilous	eutraphentic
<i>Stephanodiscus parvus</i> Stoermer & Håkansson	planktonic	alkalibiontic	eutraphentic
<i>Surirella bifrons</i> Ehrenberg	benthic	alkaliphilous	oligotraphentic
<i>Synedra ulna</i> (Nitzsch) Ehrenberg	benthic	alkaliphilous- neutrophilic	eutraphentic
<i>Ulnaria capitata</i> Ehrenberg Compère	benthic	alkaliphilous	eu-mesotraphentic
<i>Ulnaria contracta</i> (Østrup) Morales & Vis	benthic	alkaliphilous	eutraphentic

SuppTab 3. Investigated environmental factors with values for all samples.

Samples description				Environmental factors								
Name	Depth [cm]	Age [years]	Hemp retting [Yes/No]	Cereal* [%]	Weeds* [%]	Forestation* [%]	Pasture* [%]	Hemp pollen [%]	Carbonates (LOI 950°C × 2,274) [%]	Organic carbon (LOI 550°C) [%]	Fibres [per 1 g]	Trophy** [%]
LS-35-1	35	1754	N	7.6	9.9	71.4	0.4	7.9	24.73	20.19	400	19.05
LS-35-2	35	1754	N	7.6	9.9	71.4	0.4	7.9	24.73	20.19	400	19.05
LS-55-1	55	1367	N	1.9	5.1	89.4	0	1.5	35.8	11.49	200	10.53
LS-55-2	55	1367	N	1.9	5.1	89.4	0	1.5	35.8	11.49	200	10.53
LS-63-1	63	913	Y	9.4	8.4	76.5	0	6.9	31.1	23.26	5500	48.19
LS-63-2	63	913	Y	9.4	8.4	76.5	0	6.9	31.1	23.26	5500	48.19
LS-65-1	65	710	Y	14.2	10.1	71.8	0.3	14	25.9	30.95	5000	48.6
LS-65-2	65	710	Y	14.2	10.1	71.8	0.3	14	25.86	30.95	5000	48.6
LS-67-1	67	668	Y	10.9	7.8	74.8	0.2	10.6	29.5	22.57	1000	43.88
LS-67-2	67	668	Y	10.9	7.8	74.8	0.2	10.6	29.5	22.57	1000	43.88
LS-76-1	76	521	N	0.2	1.8	95.9	0	0.4	31.74	21.68	100	33.09
LS-76-2	76	521	N	0.2	1.8	95.9	0	0.4	31.74	21.68	100	33.09
LS-85-1	85	274	Y	1.3	4	89.8	0	1.2	32.57	18.85	4000	30.11
LS-85-2	85	274	Y	1.3	4	89.8	0	1.2	32.57	18.85	4000	30.11
LS-93-1	93	42	N	0.2	3.4	94.8	0	0.2	26.2	29.12	300	13.24
LS-93-2	93	42	N	0.2	3.4	94.8	0	0.2	26.2	29.12	300	13.24

* Cereal, weeds, forestation and pasture are calculated based on pollen percentages of the following species:

Cereal: *Secale cereale*, *Triticum* sp., *Fagopyrum* sp., *Linum usitatissimum*, *Cannabis sativa*, other (Cerealia undiff.)

Weeds: *Centaurea cyanus*, *Convolvulus arvensis*, *Artemisia* sp., *Chenopodiaceae*, *Urtica*, *Brassicaceae*, *Rumex acetosella* t., *Spergula arvensis*, *Scleranthus annuus*

Forestation: sum of all arboreal pollen

Pasture: *Plantago lanceolata*

**trophy inference was estimated based on the percentage abundance of the examined Cladocera taxa with ecological preferences towards high/low trophy (as listed in SuppTab. 1).

SuppTab 4. Selected carbon metabolic marker genes KOs involved in different pathways of the carbon cycle.

Cycle	KEGG Orthology (KO)	Gene
Anaerobic Carbon Fixation	K01648	ACLY; ATP citrate (pro-S)-lyase [EC:2.3.3.8]
	K00174	korA, oorA, oforA; 2-oxoglutarate/2-oxoacid ferredoxin oxidoreductase subunit alpha [EC:1.2.7.3 1.2.7.11]
	K00175	korB, oorB, oforB; 2-oxoglutarate/2-oxoacid ferredoxin oxidoreductase subunit beta [EC:1.2.7.3 1.2.7.11]
	K00244	frdA; fumarate reductase flavoprotein subunit [EC:1.3.5.4]
	K00194	cdhD, acsD; acetyl-CoA decarbonylase/synthase, CODH/ACS complex subunit delta [EC:2.1.1.245]
	K00197	cdhE, acsC; acetyl-CoA decarbonylase/synthase, CODH/ACS complex subunit gamma [EC:2.1.1.245]
Aerobic Carbon Fixation (Calvin cycle)	K00855	PRK, prkB; phosphoribulokinase [EC:2.7.1.19]
	K01602	rbcS, cbbS; ribulose-bisphosphate carboxylase small chain [EC:4.1.1.39]
Fermentation	K00016	LDH, ldh; L-lactate dehydrogenase [EC:1.1.1.27]
	K00169	porA; pyruvate ferredoxin oxidoreductase alpha subunit [EC:1.2.7.1]
	K00170	porB; pyruvate ferredoxin oxidoreductase beta subunit [EC:1.2.7.1]
Aerobic Respiration	K02274	coxA, ctaD; cytochrome c oxidase subunit I [EC:7.1.1.9]
	K02276	coxC, ctaE; cytochrome c oxidase subunit III [EC:7.1.1.9]
CO Oxidation	K03518	coxS; aerobic carbon-monoxide dehydrogenase small subunit [EC:1.2.5.3]
	K03519	coxM, cutM; aerobic carbon-monoxide dehydrogenase medium subunit [EC:1.2.5.3]
	K03520	coxL, cutL; aerobic carbon-monoxide dehydrogenase large subunit [EC:1.2.5.3]

SuppTab 5. The results of the radiocarbon dating of LS-C19 and JS-c core included in lake-depth modelling.

Depth bsl [cm]	Laborator y code	¹⁴ C date [¹⁴ C BP]	Calibrated age - 1σ range [cal BCE/CE] 68.3% probability	Calibrated age - 2σ range [cal BCE/CE] 95.4% probability	Dated material
LS-C19					
55	Poz-123835	560 ± 30 BP	1326 CE (34.9%) 1351 CE 1394 CE (33.4%) 1414 CE	1312 CE (48.6%) 1362 CE 1386 CE (46.9%) 1428 CE	Terrestrial plant macrofossil
65	Poz-123833	1345 ± 30 BP	650 CE (55.4%) 680 CE 747 CE (12.9%) 758 CE	643 CE (68.3%) 705 CE 738 CE (27.1%) 774 CE	Terrestrial plant macrofossil (hemp fibres)
93	Poz-123836	1980 ± 35 BP	33 BCE (10.7%) 16 BCE 6 CE (54.4%) 78 CE 101 CE (3.2%) 106 CE	45 BCE (95.4%) 122 CE	Terrestrial insect macrofossil
JS-c					
852 (depth bwl) 102 (depth bsl)	Poz-40810	1595 ± 35 BP	430 CE (10.1%) 442 CE 448 CE (25.0%) 480 CE 494 CE (33.1%) 536 CE	414 CE (95.4%) 552 CE	Terrestrial plant macrofossil

bwl – below water level

bsl – below sediment level

Comment:

Sediments of LS-C19 core taken with UWITEC sampler (with hammer application) were in the bottommost section more compacted than JS-c, sampled with Instorf corer. Both cores were correlated based on LOI, pollen and Cladocera data.

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