

**ON THE USEFULNESS OF THE MODERN ANALOG APPROACH TO
INTERPRET HIGH RESOLUTION RECORDS: THE CASE OF VARVED
SEDIMENTS FROM LAKE MONTCORTÈS (CENTRAL PYRENEES)**

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Abstract

In the Quaternary paleosciences, the rationale behind analogical inference presupposes that former natural changes can be explained by causes operating now, although their intensity and rates can vary through time. In this paper we synthesise synthetize the results of different modern analog studies and discuss their value to obtain the best inferences from high resolution past records. This synthesis is based on the following:

- 1) The monthly monitoring of calcite precipitation reveals a strong connection with primary producers and between-years variability; this precipitation produces a seasonal signal with imprint on varve formation.
- 2) Clear pollen sedimentation peaks occur in spring/summer and fall/winter that coincide with temperature, precipitation, relative humidity and winds; this pattern converges with the two-layer coupled varves representing the same seasonality.
- 3) We assess the lake's contemporary oxygenation dynamics over a three- year period; a combination of sedimentary REDOX proxies revealed different scenarios of oxic/anoxic shifts since 1500 CE.
- 4) We investigate presence of seasonality in the production/distribution of glycerol dialkyl glycerol tetraethers and derived temperature estimates in soils and particulate matter. Branched glycerol dialkyl glycerol tetraethers signatures and some derived temperature estimates proxies appear to mainly depend on the non-seasonal shifts in soil properties.
- 5) Currently we examine relationships and similarities between extant phytoplankton and derived pigments in water and traps, and their correspondence with subfossil pigments; some preliminary results are presented here.

Keywords: high resolution, endogenic varves, calcite precipitation, pollen sedimentation, meromixis, freshwater glycerol dialkyl tetraether, subfossil pigments, long-term ecology.

1. Introduction

Analogy is a powerful form of reasoning that allows two domains to be considered similar, based on connected systems of relationships between them [1-3]. The analog approach permits the extension of knowledge of a base domain to a target past domain by virtue of their expected similarity and by means of analogical inference. This process involves reproducing structures and processes from the base to the target in which missing information is generated. Nonetheless, constraints must be placed on what information is to be carried over [4], in order to obtain inferences that are as realistic as possible. In the Quaternary paleosciences, the rationale behind analogical inference is based on the postulate that former natural changes can be explained by causes operating now (principle of uniformitarianism), but the intensity and rates at which processes take place vary through time [5]. In paleoecology, the direction and rate at which a community changes through time can be measured quantitatively by a wide array of numerical approaches and techniques [6]. On the other hand, it is clear that the reconstruction and derived inferences of any proxy demand a thorough comprehension of the spatial and temporal frame in which processes and environmental conditions leading to the existence and persistence of each particular proxy make sense.

Reconstructions of environmental variability at high resolution scales using varved sediments have provided an unprecedented level of detail that needs to be properly interpreted [7]. This implies knowledge of fine-tuned mechanisms that can only be observed today at the same temporal resolution at which they do occur [8]. Lakes with varved sediments are especially well suited for paleoecological and paleoclimatic research demanding high level of detail, as it is possible to downscale to annual and seasonal resolution [9] [7]. The interpretative power of such high-resolution paleoenvironmental reconstructions depend on the availability of suitable modern

analogs with the same temporal resolution [8]. With the advent of new techniques and proxies that allow acquiring an increasing level of detail, modern analog studies are gaining importance and, with this paper, we want to highlight this fact.

Specifically, to contribute to this purpose, we have taken advantage of the varved sediments of Mediterranean mountain Lake Montcortès (LM) and have conducted several independent modern analog studies. Our aim is to prove the potential of modern analog approach not only by determining how similar modern and past communities are, but also investigating different contemporary biological and physico-chemical processes that are expected to have occurred in the past, at annual and seasonal time frames. We also inquire which factors and relationships between modern and subfossil proxies can limit or hinder the applicability of modern analog approaches and can represent a potential source of error in analog analysis. Such errors may be foreseen and minimized [6].

The general project in which these studies are immersed seeks to investigate in detail the transition between natural climate variability since the late Little Ice Age (CE 1500) and the onset of Industrial Age, which marks the advent of an era in which climate variability also reflects the increase of greenhouse gases of anthropogenic origin, distorting natural climate signals. In this context, we examine how the different modern analog approaches can help extract important cause-effect relationships at seasonal/annual/subdecadal scale.

In this paper we synthesise, discuss and evaluate published results of four modern analog studies carried out in LM listed below:

- 1) We examine significant relationships between calcite precipitation, environmental variables and primary producers, and their influence on the thickness and patterns of calcareous varve sublayers as a high resolution paleoclimatic indicator [10].
- 2) Seasonal pollen sedimentation is compared with local meteorological variables to determine whether contemporary seasonal deposition is consistent with the two-layer coupled varves pattern found in the sediments of LM [11].
- 3) Contemporary shifts in dissolved O₂, Fe, Mn and anaerobic bacterial markers are assessed, in order to investigate whether mixing and oxygenation dynamics of LM have been the same over the last five centuries [12].
- 4) We examine the distribution of glycerol dialkyl glycerol tetraethers (GDGTs) in settling particulate matter and catchment soils, in order to determine whether Branched glycerol dialkyl tetraethers (brGDGT's) proxy estimates are seasonally biased in environments where annual brGDGT's production may not be constant [13].

Additionally, we provide and discuss preliminary results from an ongoing research that aims to relate contemporary phytoplankton taxa of LM with their corresponding marker pigments [14], and the latter with the subfossil pigment recorded at subdecadal scales.

General and common methods and environmental settings are provided in sections 2 and 3. Nonetheless, every study is presented separately in section 4 “Results and discussion” and relies on the corresponding published papers for methodological details, extended discussions and specific literature. Finally, general conclusions are provided in section 5.

2. Environmental settings

Lake Montcortès belongs to the southern flank of the Central Pyrenees (Catalonia, Spain) at 42° 19' N, 0° 59' E and 1027 m a.s.l. altitude (Figure 1). This karstic lake is emplaced on carbonated and evaporitic Mesozoic substrate that is chiefly characterised by Triassic limestones, marls and evaporites, as well as Oligocene-carbonate conglomerates [15][16]. Climatic data collected at La Pobla de Segur meteorological station show that annual average air temperature is 12.8 °C (2.9 °C in January to 23.2 °C in July). Total annual precipitation is 669 mm (33.4 mm in February and 88.4 mm in May). Maximum and minimum temperatures were 41 and -20 °C, respectively, during the period of study. The catchment's vegetation is evergreen with deciduous oak forests (*Quercus rotundifolia* and *Q.pubescens*), conifer forests (*Pinus nigra*), pastures and crops. The shore vegetation belt is dominated by hygrophite communities of *Typha domingensis* or *Cladium mariscus*, reed beds of *Phragmites australis* and communities of *Carex riparia*. Rush formations and grasslands grow on occasionally flooded soils [17]. The lake surface area is 0.14 km² and its maximum water depth 32 m [18]. No permanent inlet exists, and water is evacuated by an outlet brook located on the northern shore. The lake's sedimentary record shows thin, well preserved biogenic varves formed during the Late Holocene. These varve's structure alternates sublayers of endogenic calcite and organic detritus with additional detrital layers and turbidites embedded in the varve succession [15][19][20].

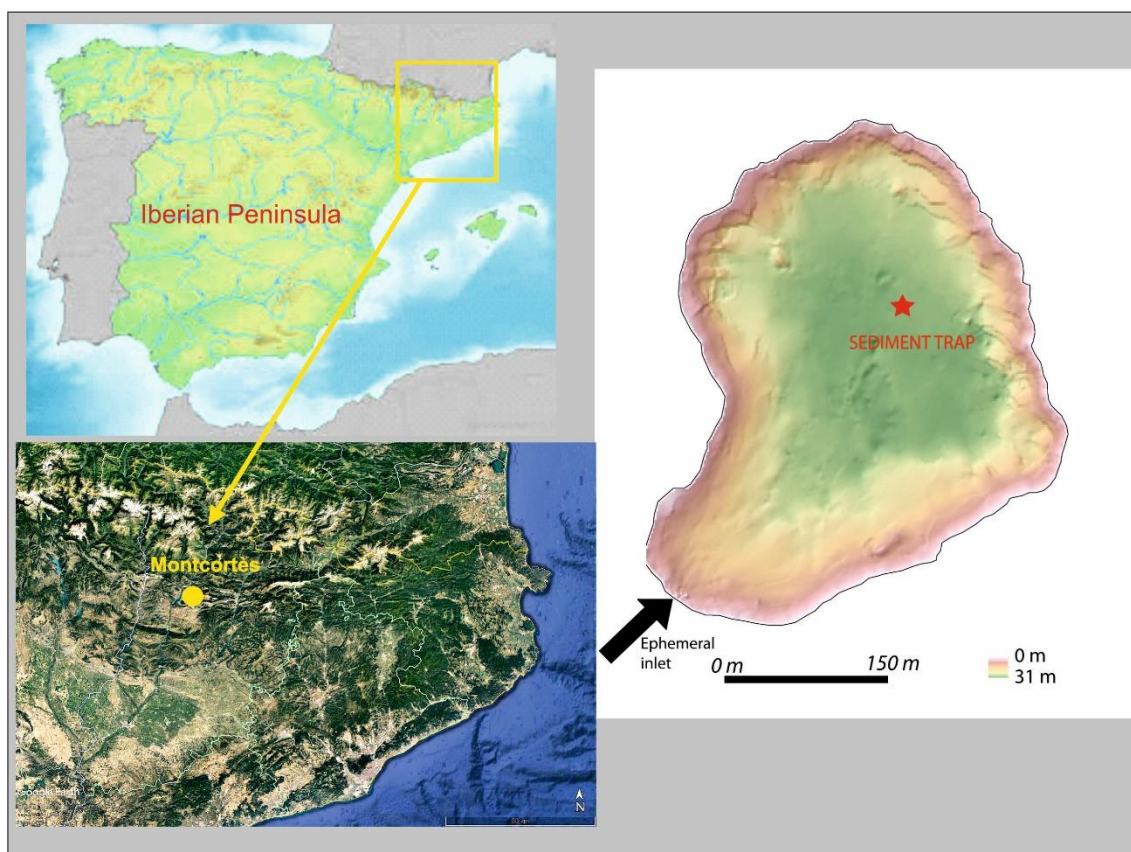


Figure 1: Geographic position of Lake Montcortès in the Pyrenean range (NE Iberian Peninsula), bathymetric map [18] and location of the sampling point and sediment traps within the lake.

3. Materials and methods

Here we provide a summary of the main field and laboratory methods used in each research study; detailed information can be found in the corresponding published papers and literature therein [10-13].

3.1. Field monitoring and coring

The limnological characteristics of LM were assessed monthly from October 2013 to October 2015. Detailed vertical profiles of temperature (T), dissolved oxygen (DO), electric conductivity (EC) and pH were obtained, as well as water samples at three

depths (epi-, meta- and hypolimnion) for analyses of alkalinity, cation, total phosphorus (TP), total nitrogen (TN), suspended solids, phytoplankton and molecular markers (pigments and GDGTs) analyses.

A set of sediment traps was deployed 20m from a floating platform located at the deepest part of the lake, coinciding with the point where the sediment coring was performed. Total suspended solids, calcite crystals [10], pollen and spores [11], marker pigments and settling particulate matter for GDGT extractions [12] were collected in these traps, with monthly or quarterly frequency depending on the variable of interest. Additionally, replicated 2-5 cm deep topsoil samples for GDGT analysis were collected monthly, in a 10 m radius area at the catchment.

A composite sedimentary sequence from three UWITEC gravity cores and a long Kullenberg core were retrieved from the deepest part of the lake basin (~30 m water depth) (Figure 1). The age-depth-model was based on two independent dating techniques: i) varve counting performed on calcite layers and ii) radiometric dating with ^{210}Pb and ^{14}C ; see [19] and [20] for further description.

3.2. Laboratory

The calcite saturation index (Ω) value was estimated from carbonate and calcium concentrations. Ca^{2+} was measured with inductively coupled plasma atomic emission spectroscopy, and CO_3^{2-} was calculated from alkalinity, pH and temperature with the CO2SYS_XLS v 2.1 program [21].

Additionally, to confirm the presence of calcite crystals and to assess seasonal variations of their size and shape, trapped material from quarterly traps was analysed by scanning electron microscope. Total suspended solids retained in the traps were

analysed for total particulate carbon (TPC) and total particulate organic carbon (POC) with a carbon analyser.

Diatom specimens from quarterly traps were prepared, identified and counted using standard methods [22]. At minimum 300 valves were counted per sample; identification was carried out following Krammer and Lange-Bertalot [23][24]. Chemical analyses of pollen trapped quarterly included analyses on exotic *Lycopodium* spores and followed standard methods [25]. Pollen was counted until a minimum of 300 grains; identification was made following previous, lower-resolution studies [26-28].

Phytoplankton was settled using sedimentation chambers; quantification was performed applying Utermöhl's technique for counting combined with biomass conversion.

Geochemical data to trace high resolution oxic/anoxic events at the hypolimnion were obtained on the core MON12-3A-1K by X-ray fluorescence (XRF) analysis (2000 A, 10–30 kV and 20–50 s measuring time) (0.2 mm resolution) using a XRF AVAATECH core scanner. We obtained subdecadal resolution time series data for Fe, Mn, Ca, Si, S, Ti and Br. The former five elements were normalised to Ti. The Fe/Mn ratio was used as the main redox tracer, together with sulfur bacteria marker pigments, such as okenone (oken) and isorenieratene (isore).

A second core, MONT-0713-G05, was used for pigment analyses; samples were obtained at sub-decadal resolution. Pigment analyses were also performed on epi-, meta- and hypolimnetic water sample and sediment trap material. In both cases, markers pigments were extracted from frozen filters in 5 mL of 90% acetone. The extract was centrifuged, filtered and analysed with ultrahigh-performance liquid chromatography. Pigments were identified by evaluating the retention times and absorption spectra against a library based on algae and photosynthetic bacterial cultures.

To isolate GDGTs from the different settings, soils samples and filters from sediment traps were extracted, evaporated and then fractionated over an aminopropyl silica column. The fractions containing the lipids were dried, re-dissolved and filtered. GDGTs were analysed in high-performance liquid chromatographs coupled with mass spectrometers and were detected in selected ion monitoring mode; prior to extraction, a synthetic tetraether lipid with a structure typical of neutral archaeal membrane lipids was added to the sample. Further analyses in soils were performed for water content and TOC.

4. Results and discussion

4.1. Examining the relationships between modern calcite precipitation processes and varve formation: origin and patterns.

Processes driving varve formation are remarkably variable and site-specific. Consequently, detailed knowledge of the local processes that promote and modulate particle flux dynamics to the lake's sediment in each site becomes essential. This knowledge is a pre-requisite for proper paleoenvironmental and paleoclimatic interpretations of the sedimentary imprint [29][9] and also for building reliable varve-based chronologies. The varves of LM are formed by couplets of white calcite and brownish organic layers. The white layer was assumed to have deposited during spring/summer, while the dark layer was assumed to have deposited in fall/winter [9][19]. Processes involved in biogenic varve formation are highly complex as they add the complexity of biological processes to the other processes (chemical, geochemical and physical) involved on varve formation [7].

Specifically, with this modern analog study several goals are pursued in LM: i) to identify the main limnologic and sedimentological mechanisms leading to varves formation; ii) to establish in which climatic season(s) the deposition of individual calcite sub-laminae occurs, and iii) to determine if the obtained results are coherent with varve structure and previously proposed mechanisms of formation [19].

Elevated calcite saturation index (Ω) values were recorded in the epi- and metalimnion of LM from late spring to early fall, suggesting that calcite precipitation mostly occurred taking place during this period. The deposition rates were highest in summer when photosynthetic organisms were the most active. Additionally, higher POC values were observed during this time lapse, indicating that the main origin of calcite crystals was newly formed autochthonous CaCO_3 precipitates.

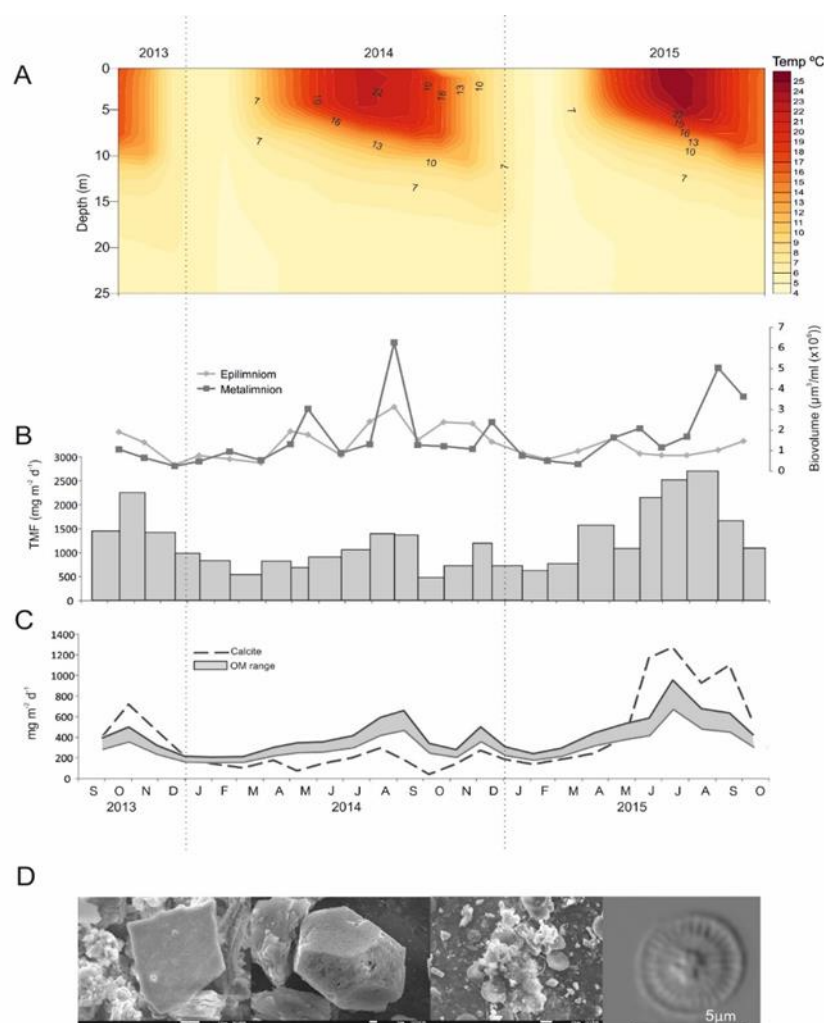


Figure 2. Summary of monthly column water and trap monitoring. A) Water column temperature at 1m resolution measured with a multi-parametric probe. B) Total mass fluxes found in monthly sediment traps compared with phytoplankton biovolumes found in the epi and metalimnion. C) Calcite fluxes versus organic matter fluxes. C) Well-formed blocky and rhombohedral calcite crystals, calcite crystals and diatom frustules and one of the small centric diatoms found on trapped material. Modified from [10].

In fact, the presence of blocky, polyhedral calcite crystals was confirmed in all quarterly trapped samples and showed a clear seasonal pattern, being smaller when formed in spring and summer. Deposition of smaller crystals was not only concurrent with Ω , but also with larger fluxes of very tiny diatoms ($\sim 10 \mu\text{m}$), suggesting that phytoplankton cells could be acting as calcite condensation nuclei under enhanced calcite Ω , pH and

productivity conditions. Other sources of calcite input, such as detrital carbonates from the catchment, were unimportant for the studied period.

Interestingly, the spring and the summer of 2015 were anomalously warm and this fact was well reflected in epi- and metalimnetic waters, which show higher temperatures and enhanced primary production. Coincidentally, calcite flux increased twofold when compared with the ones of the same season for the year 2014. These facts highlight an effect of atmospheric temperature increase on the mechanisms involving calcite precipitation. The latter will very likely leave a clearly related signal in the lake's sediment, most likely in the form of different amounts of deposited calcite between both years and therefore, on a thicker calcite layer as a signal of warmer years. Empirical support for this hypothesis may become available in coming years, with surface sediment diagenesis and compaction. This seasonal signal is also reflected on calcite crystal sizes. During fall and winter larger crystals were deposited, which progressively decreased in size, through spring and summer.

Larger crystals were deposited during fall and winter that progressively decreased in size, through spring and summer.

Other seasonal events are the contemporary alternation between calcite precipitation, during summer and fall, and organic matter deposition, during winter and spring. These results did not completely back previous paleolimnological inferences that assumed that the light calcite layer was formed in spring/summer and the organic layer was formed in fall/winter [19]. Because of lack of local or regional modern analog studies, the authors relied on available literature [9] to explain mechanisms of varve formation.

According to our results, varves in the process of forming during the period of study would probably match one of the varve patterns described in the sediments of LM, once

deposited [19], although this remains to be further confirmed. The seasonal calcite-crystal distribution observed in our quarterly traps would likely result in a coarsening upward calcite sub-layering, with fine grained calcite crystals deposited in summer and coarse-grained calcite crystals deposited in fall. This texture is frequently observed in the sedimentary sequence from CE ~1350 to 1850 CE and less frequently from then until the present day [19]. Therefore, we hypothesise that modern analog studies of crystal size of calcite sublayers may allow inferring environmental deposition conditions at seasonal resolution, which can be of obvious paleoclimatic interest.

4.2. *Is contemporary seasonal deposition of pollen consistent with the existence of a two-layer coupled varve pattern?*

The formation of annually laminated lake sediments relies on seasonal events linked to climate and on the related flux of biotic and abiotic materials to the sediment, coming from multiple autochthonous and allochthonous sources. In this way, pollen from the catchment and beyond arrives and is buried in the lake's sediments. Pollen data sets have been widely used to reconstruct past climate change at a supra annual or secular resolution and have provided insight into the influence of intra-annual weather conditions in pollination patterns. We now argue that combining pollen data from traps, varved lake sediments and modern meteorological data may produce long, high-resolution ecological time series. An example of what can be achieved with this approach are the continuous, long-term climate series obtained by associating paleoclimate data derived from tree rings and other similar proxies with instrumental climate measures, at annual and seasonal resolutions [30].

To empirically support this hypothesis, this study is primarily intended to identify seasonal pollen sedimentation patterns in a trap deployed in varved LM that would be useful for interpreting past records from the same lake. Hence, the interpretation of past

pollen records was beyond the scope of this work and has been addressed in another paper [31].

Seasonal pollen was collected quarterly from a trap located at a 20 m depth between fall 2013 and fall 2015. Pollen grains were taxonomically identified and specific and total pollen influxes to the traps were calculated. Cluster and Canonical Correspondence Analysis (CCA) were performed to obtain distinct pollen assemblages and their correlations with instrumental meteorological variables, i.e. average temperature (T_m), average relative humidity (H_m), average pressure (P_m), total precipitation (PPT), average wind velocity (W) and predominant wind direction (W_d).

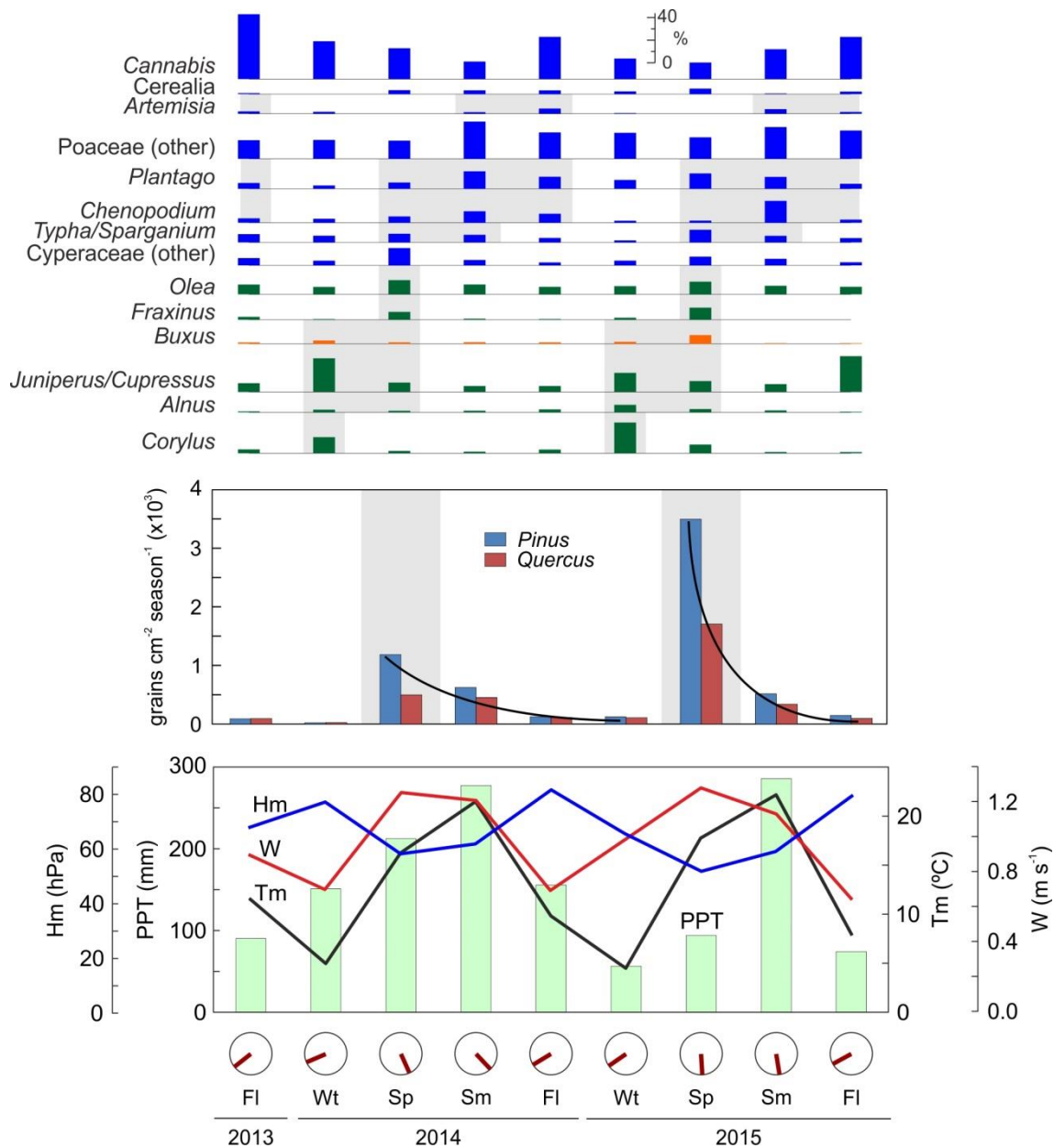


Figure 3. Summary of palynological results showing the relationship among the most relevant meteorological parameters (lower panel), the pollen influx of the superabundant pollen elements (middle panel) and the percentages of selected relevant pollen types (upper panel). Average temperature (Tm), relative humidity (Hm) and wind velocity (W) are represented by lines. Total precipitation (TP) is represented by bars. The predominant directions of the wind (Wd) are shown in circles. Sp – spring, Sm – summer, FI – fall, Wt – winter. Pollen percentages were calculated excluding the superabundant *Pinus* and *Quercus*. Taxa are ordered by their respective flowering seasons (grey bands) [32], from bottom to top and from left to right. The flowering season of all species of the different genera present in the Montcortès region [17] has been considered. Trees are in green, shrubs are in orange and herbs are in

blue. Cultivated plants, such as *Cerealia* and *Cannabis*, and families including many genera (Poaceae, Cyperaceae) are located based on their pollen patterns because of the difficulty of establishing a definite flowering season.

Pollen maxima were recorded during spring, concurrently with the flowering season of the involved taxa, and preceded the temperature and precipitation maxima (summer) by one month (Figure 3). Total pollen influx and taxonomic composition showed a strong seasonal signal over the study period, peaking positively during spring/summer and negatively during fall/winter. The major components of the pollen assemblages, *Pinus* (pine) and *Quercus* (oak), dominated pollen counts and approximately matched seasonal trends in temperature and precipitation, with *Pinus* almost tripling *Quercus* percentages in spring/ summer but falling to similar levels in fall/winter (<30%). As far as the remaining species are concerned, the most significant differences were observed in *Plantago*, *Chenopodium*, *Typha/Sparganium*, Cyperaceae, *Fraxinus* and *Juniperus/Cupressus*, which prevailed in the spring/summer assemblage, and *Cannabis* and *Corylus*, which were more abundant in the fall/winter assemblages (Figure 3). Pollen of *Cannabis* (hemp) prevailed during the fall. *Cannabis* is a cultivated plant whose pollen has been present and fairly abundant in the surroundings of Montcortès for the last 1200 years. Nonetheless, we still have not been able to locate the exact source of that pollen [27] [28].

Canonical correspondence analysis (CCA) was applied and showed the first two axes accounting for 70.74% of the total variance. The strongest gradient coincided with axis 1 (56.80% of the total variance), which was highly and positively correlated with relative humidity and pressure, and negatively correlated with wind velocity (Figure 4). Along this gradient, pollen samples became aligned from spring (left) to winter (right) suggesting a seasonal succession with summer and fall occupying intermediate

positions. Pollen taxa were ordered according to the same gradient. The spring/summer group was highly correlated with temperature, precipitation and wind velocity (SSE). The fall/winter group was also highly correlated with wind direction (WSW).

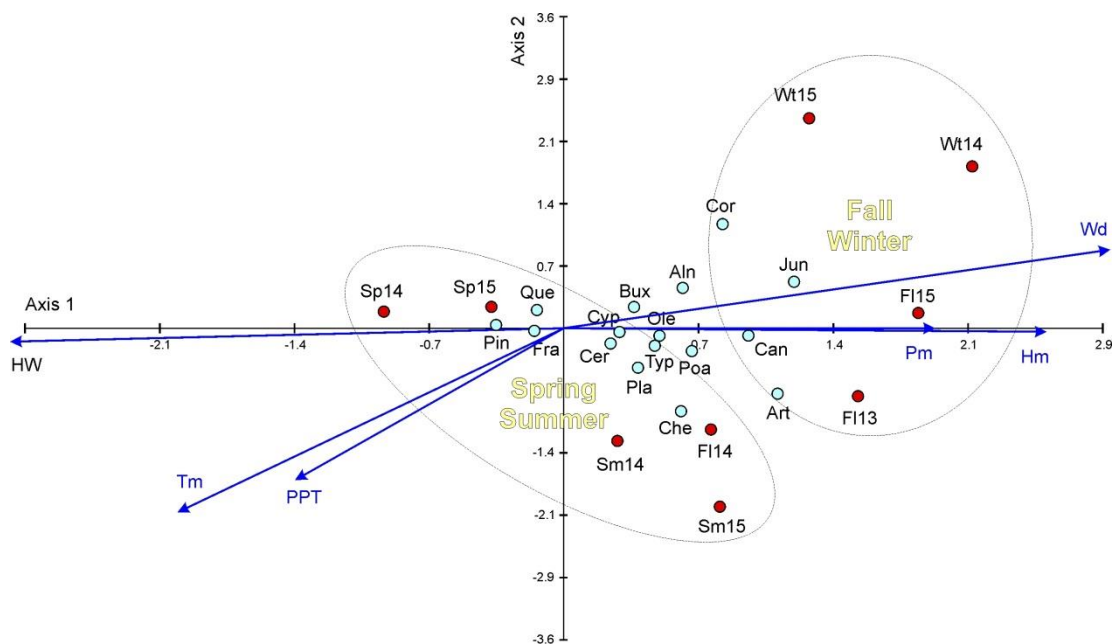


Figure 4. CCA biplot using the scores of the first two axes accounting for 70.74% of the total variance. Samples are represented by red dots and pollen taxa are represented by blue dots.

Specific aspects of the pollen sedimentation require further attention. For example, a lag in pollen sedimentation (PSL) was observed between production and deposition, throughout the year. This lag can obey several causes, for example the influence of internal water dynamics and resuspension, or the fact that soils, during the flowering season, can be washed into the lake along several months. With respect to the lake's internal processes, it is worth mentioning that strong thermal water column stratification occurred from March to November 2014 coinciding with the pollen production period. This steady structure could have slowed pollen sedimentation rate, whereas in winter

vertical mixing of water disrupted the lake's thermal stability in January and February 2015, facilitating release of pollen to the bottom layers several months after production.

The similarity in the sedimentation patterns between *Pinus* and *Quercus* pollen was unexpected, because their respective pollen grains show important morphological differences that make their capabilities for air suspension significantly distinct.

However, once the pollen is submerged in the waters of LM, the settling of both types of pollen was quite similar even during summer, when the lake's thermal stratification was very stable. This finding could suggest that internal lake dynamics neutralises the effect of such differences and that resuspension or catchment runoff may be more important with regard to pollen sedimentation. In general, the seasonal pattern in pollen deposition coincided well with the two-layered varved pattern of the sediments.

In summary, pollen analysis was able to identify two well differentiated modern assemblages, one corresponding to spring/summer and the other representing fall/winter, which match pretty well the same seasonal patterns identified in sedimentological (varve) studies [19], thus supporting the hypothesis that the combination of pollen data from traps, varved lake sediments and modern meteorological data may produce long, high-resolution ecological time series by utilising appropriate transfer functions.

4.3. Revisiting meromictic Lake Montcortès: has the mixing regime changed?

Oxygenation of lake water relies on oxygen diffusion from the atmosphere, addition from autochthonous primary production and external inputs of oxygenated water. Lake mixing is the main mechanism by which this oxygen becomes distributed throughout the water column and is therefore central for lacustrine health. Mixing regimes can shift with climate changes [33] and human activities, e.g. [34]. Recently, dissolved oxygen

depletion leading to hypoxia, or even to anoxia, has become a matter of concern around the world, and there is potential for worsening under current global warming [35].

Furthermore, it is suggested that climate change will favour cyanobacteria, which can form unpleasant blooms and produce toxins that harmful to humans [36].

At the same time, obtaining a series of instrumental oxygen records extending beyond a century is problematic, thus reducing the possibility of recording long-term changes in oxygen variations that can be associated with climate or human influence. One seminal exception is Lake Zürich, where 50 year long-series of temperature and oxygen profiles corroborated that the increase in the thermal stratification period tends to favour reduction in the homeothermal brake and consequently, the onset of hypoxia or anoxia in lakes, e.g. [37-39]. Where concurrent series of oxygen and temperature are not available, the combination of proxies of oxic/anoxic conditions with high-resolution paleolimnological records can result in a useful surrogate to assess long-term changes in oxic/anoxic shifts through time.

Interestingly, the varved sediment of LM appears to be highly suitable to provide such high-resolution data. Since redox processes depend on the biogeochemical conditions of lakes and the chemical characteristics of the involved compounds, we firstly conducted a modern analog study consisting of a monthly survey of main redox indicators, i.e. DO, Fe, Mn and marker pigments [40], between October 2013 to October 2016. The same type of marker pigments were examined in sediments: isorenieratene (Chlorobiaceae) as a tracer of euxinia, okenone (Chromatiaceae) as indicators of anoxia and oscillaxanthin (Oscillatoriales) as a general marker of cyanobacteria that often points to heavy eutrophy. Furthermore, we used selected elemental ratios of the sedimentary record to survey shifts in the oxic/anoxic conditions of the lake over the last 500 years: bromine (Br) as a tracer of organic content, e.g., [41], the Ca/Ti and Si/Ti ratios as indicators of

biologically mediated calcite and silica production, respectively, and the S/Ti and S/Fe ratios as indicators of the presence of sulfur compounds [42].

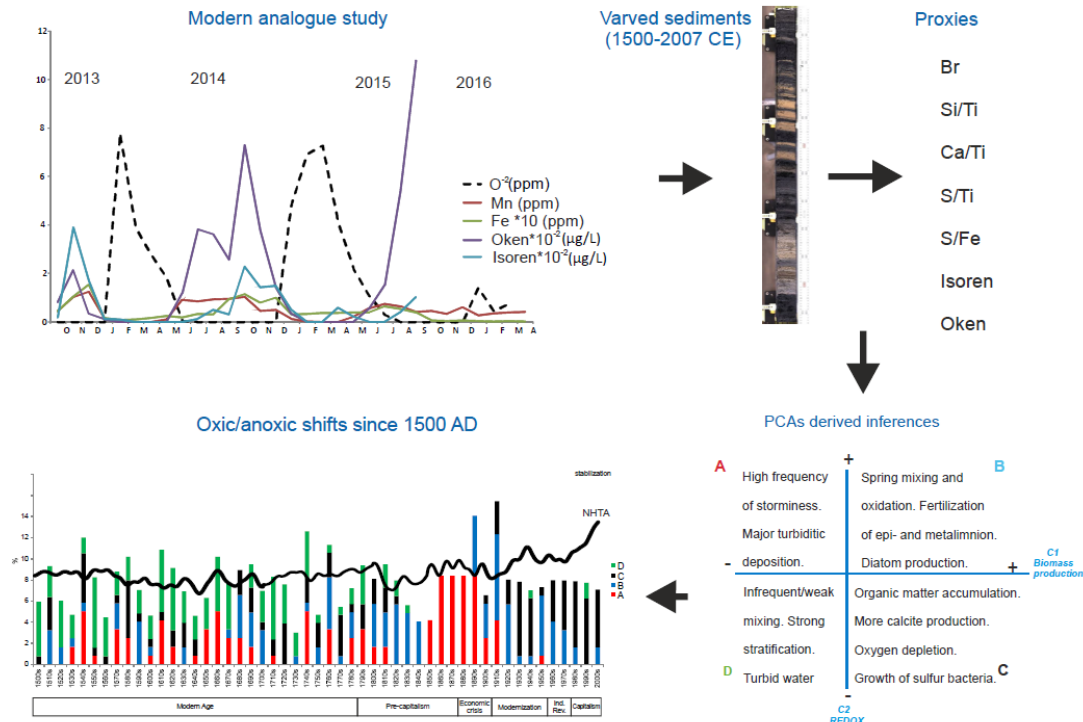


Figure 5. Graphical summary of the modern analog study about oxygen shifts and inferred results: seasonal variations of mean values of Fe, Mn, O₂ and bacterial pigments okenone (oken) and isorenieratene (isoren) in hypolimnetic waters of LM; varved sediments and proxies obtained; graphic synthesis of the inferences derived from PCA analyses; decadal evolution of the conditions represented by the subsets A,B, C and D, since 1500CE; the bars show the percentage of years of each subset (PC II) that fall within a particular decade.

LM had been reported to be meromictic for years [43], nonetheless we observed brief episodes of complete mixing during the winters of 2013 and 2014 that triggered precipitation of Fe and Mn oxides. With the onset of anoxic conditions, the precipitated Mn and Fe oxides started to redissolve and sulfur bacteria grew in the hypolimnion only under anoxic conditions, when Fe and Mn reached maximum concentrations. The

oscillaxanthin was absent during the sampling period. Such conditions endured over the year 2016, which was a non-mixing year. These results confirmed that redox proxies were working well and provided the information required to properly interpret the sedimentary record. Applying this knowledge to the results obtained from sediment analyses, we were able to distinguish four prevailing situations on the basis of the selected redox proxies: A) years with abrupt and substantial sediment input (turbidites); B) years with mixing and oxygenation of the water column; C) years with strong stratification, anoxia, intense activity of sulfur bacteria and increased biomass production; and D) years showing stratification and anoxia, but relatively less biomass production (Figure 5). We observed high supra-annual variability in the occurrence of oxygenation events over the last 500 years. Interestingly, approximately 45.3% of the years were monomictic years and mostly occurred arranged in groups of consecutive years and meromictic years showed a similar pattern. Broader time scale patterns were also identified. Most A years coincided with the climatic instability of the 1850 -1899 CE period. B years were rather scattered but were best represented between 1820-1849 CE. Most D years happened from 1500 to 1820 CE, when human activities were locally the most intense, over the studied period and before migrations to the cities. Almost all C years belonged to the 20th century and coincided with warmer and drier winters and abrupt land abandonment, especially after 1970 CE. From the obtained evidence it is clear that the oxic/anoxic shifts in the hypolimnion of the lake over the past 500 years were highly dynamic and resulted from multiple climate and anthropogenic factors.

4.4. Can glycerol dialkyl tetraethers in freshwaters be used as indicators of seasonal, temperature shifts?

Branched glycerol dialkyl tetraethers (brGDGTs) are bacteria derived, widespread lipids that can be found in terrestrial and aquatic environments. The global distribution of brGDGTs in soils and peats has been associated with past temperature, and are they therefore used as proxies of mean annual temperature (MAT proxies) in such environments [44]. However, in some regional studies brGDGTs indices appeared to be influenced by other variables as well (e.g. precipitation, humidity and soil properties) [45]. The brGDGTs paleoproxies were originally calibrated against annual averages of environmental variables, but a hypothesis has arisen that proxy estimates are biased towards particular seasons, because the bioproduction of brGDGTs is enhanced under the most favourable conditions, which occur mostly in summer. Nonetheless, no clear-cut seasonal pattern in their distribution has been found in soils, probably because of the slow turnover time, which is on timescales on decades or longer, in terrestrial environments.

To answer whether soil brGDGT proxy estimates are seasonally biased, the brGDGT distributions and the brGDGT-derived MAT estimates were examined in settling particulate matter (traps) and surface soil samples from around LM (MAT: -3.3 to 17.6 °C). No clear-cut seasonal pattern in brGDGT distribution has been found in soils, probably because of the slow turnover time in terrestrial environments, which is on timescales of decades or longer.

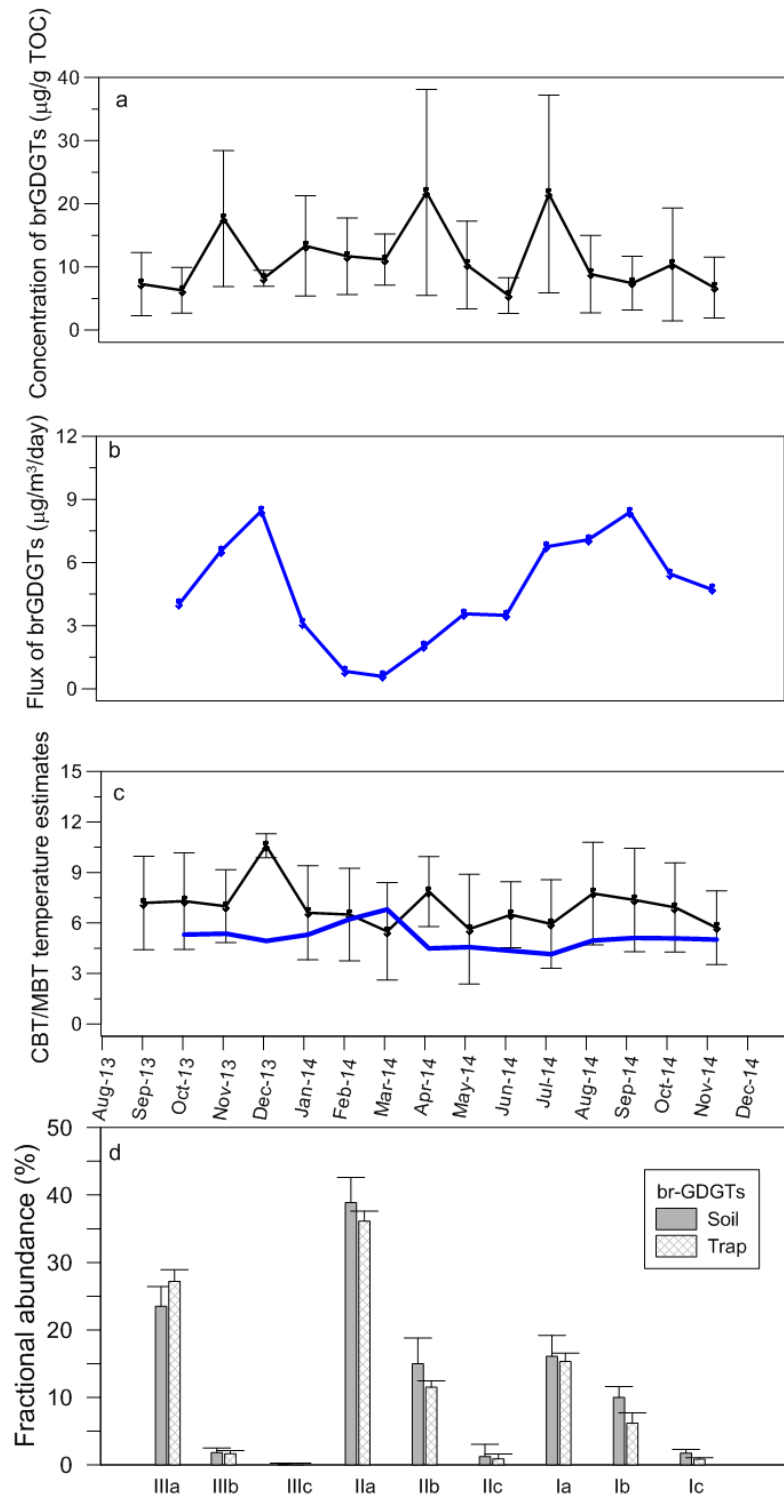


Figure 6. (a) the concentration of soil brGDGTs, (b) the brGDGT flux of settling particles in the sediment trap at 20 m depth in the lake, (c) brGDGT-based temperature estimates using the MBT/CBT proxy in soils (black line, average from three soils in the catchment around the lake) and settling particles (blue

line). (d) The fractional abundance of brGDGT distribution in soils and sediment trap. Modified from [13].

The results do not record any seasonal trends in the distribution of brGDGT, neither in soils nor in particulate matter coming from the same catchment. These results confirmed previous findings from mid-latitude soils showing that brGDGTs' distribution and some of the brGDGT-derived proxy measurements are relatively stable through the year [46]. However, we found that annual shifts in the abundances of brGDGTs were controlled by the variability of specific regional factors, i.e. soil humidity and pH in soils. In the case of particulate matter, heavy rain is the main factor influencing brGDGT abundance.

In terms of modern analogs, this study offers evidence that brGDGTs' signatures in depositional environments are representative of the soils of the catchment, and that the accuracy of the derived temperature estimates based on methylation/ cyclisation (MBT/CBT) proxies in soils will depend on soil properties that in turn depend on regional factors. Additionally, those brGDGT signatures in the catchment's soils probably represent average environmental conditions over decades or longer, and this observation means that any derived proxy reconstructions can be used only to infer variability in environmental variables over the same timescales. As to the absence of seasonality in the brGDGT proxies in the settling particulate matter, the main contribution to the LM (traps) is the lack of evidence of significant "in situ" production of brGDGTs within the lake. The most straightforward explanation for this is that the main source of brGDGTs is catchment soils, and that their non-seasonal signals are transferred into the settling particles, which have a distribution of brGDGTs that appear to be a weighted mean of the soil signals (Figure 6a). In addition, the similar pattern of brGDGT fractional abundance in the soils and settling particles confirmed soil-related sources to the sediment trap. It remains to be seen if at an annual scale, sediment

downcore variability in brGDGTs will capture changes derived from in situ production and sediment sources, and in such case, if derived brGDGT proxies will be suitable enough to build high resolution MAT reconstructions.

4.5. *From contemporary phytoplankton to subfossil pigments, what can we learn about community change?*

In general, the qualitative and quantitative relationships between modern climatic variables, aquatic primary producers, pigments of the water column and subfossil marker pigments of the sediment have received little attention, despite their potential to build reliable climatic proxies. To help fill this gap, we perform a modern analog study to determine how modern phytoplankton and marker pigment information compares with that obtained from the sedimentary record. For this purposes, we apply an analog matching technique (AM), which is concerned with identifying contemporary sites that most closely match the species assemblage identified in the past [47]. These sites are epi-, meta and hypolimnion in this study.

Here preliminary results of this research are presented. We assessed and compared the annual cycle of phytoplankton and marker pigments in the epi-, meta- and hypolimnion and in a sediment trap deployed at 20 m lake depth. Redundancy Analysis (RDA) was applied to identify potential associations between the phytoplankton taxa identified with microscopy and modern pigments. Then we performed a similarity analysis, in order to examine expected bias between modern and subfossil samples (1493-2013 CE), in terms of presence and absence of common pigment markers.

Phytoplankton total biovolume depicted a regular annual cycle peaking positively in early spring and summer and negatively in winter. Phytoplankton biovolume at the metalimnion was the highest in summer ($> 6 \times 10^6 \mu\text{m}^3/\text{mL}$). Central diatoms and

chlorophytes followed one another as the dominant taxa in 2013-2014, whereas smaller central diatoms took over during 2014-2015 (Figure 7).

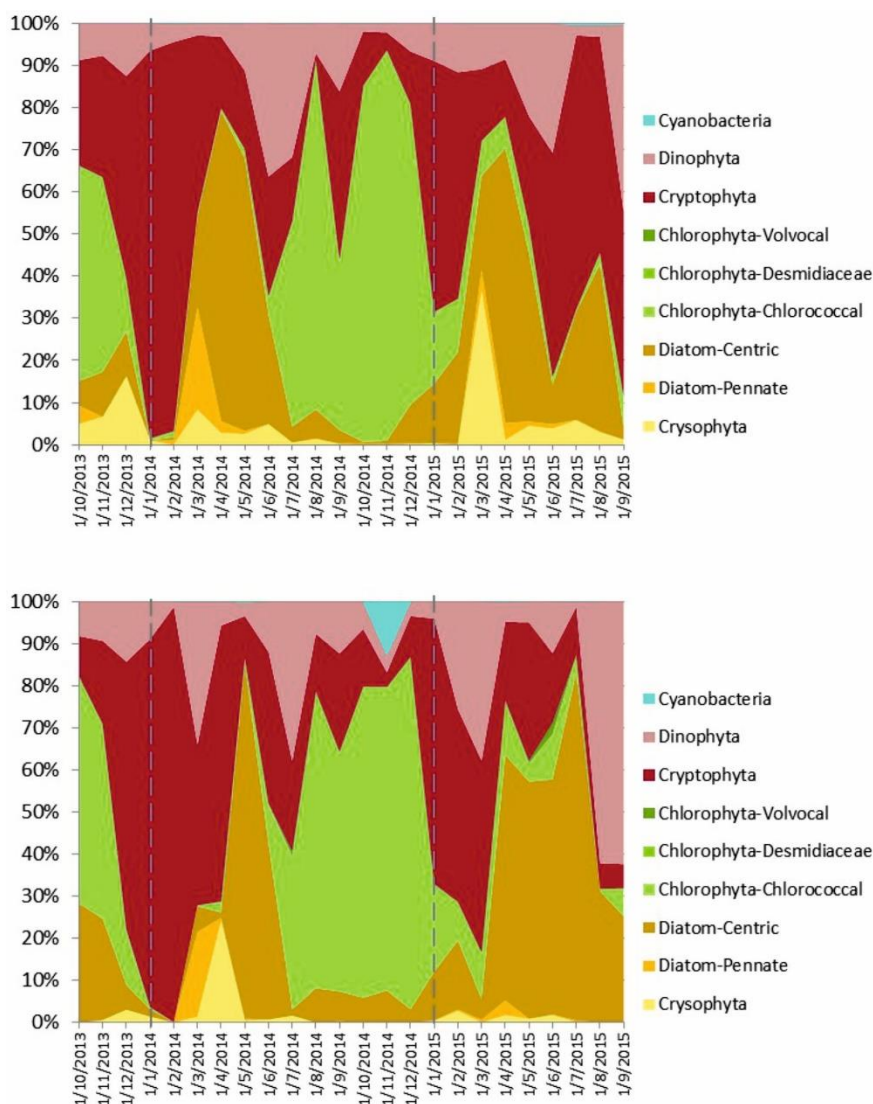


Figure 7. Succession of phytoplankton taxa in the epi- (upper) and the metalimnion (lower) at L (Oct 2013-Sept 2015)

The RDA biplot (Figure 8) shows the goodness of adjustment between phytoplankton taxa and the pigments in the water column that will be later used as their pigment markers in the sediment. The oxygen depletion of hypolimnetic waters towards the end of the stratification period allowed a significant development of sulfur bacteria [12], which is well reflected in the RDA biplot by the corresponding marker pigments

(okenone and isorenieratene) although sulfur bacteria have not been examined under microscope.

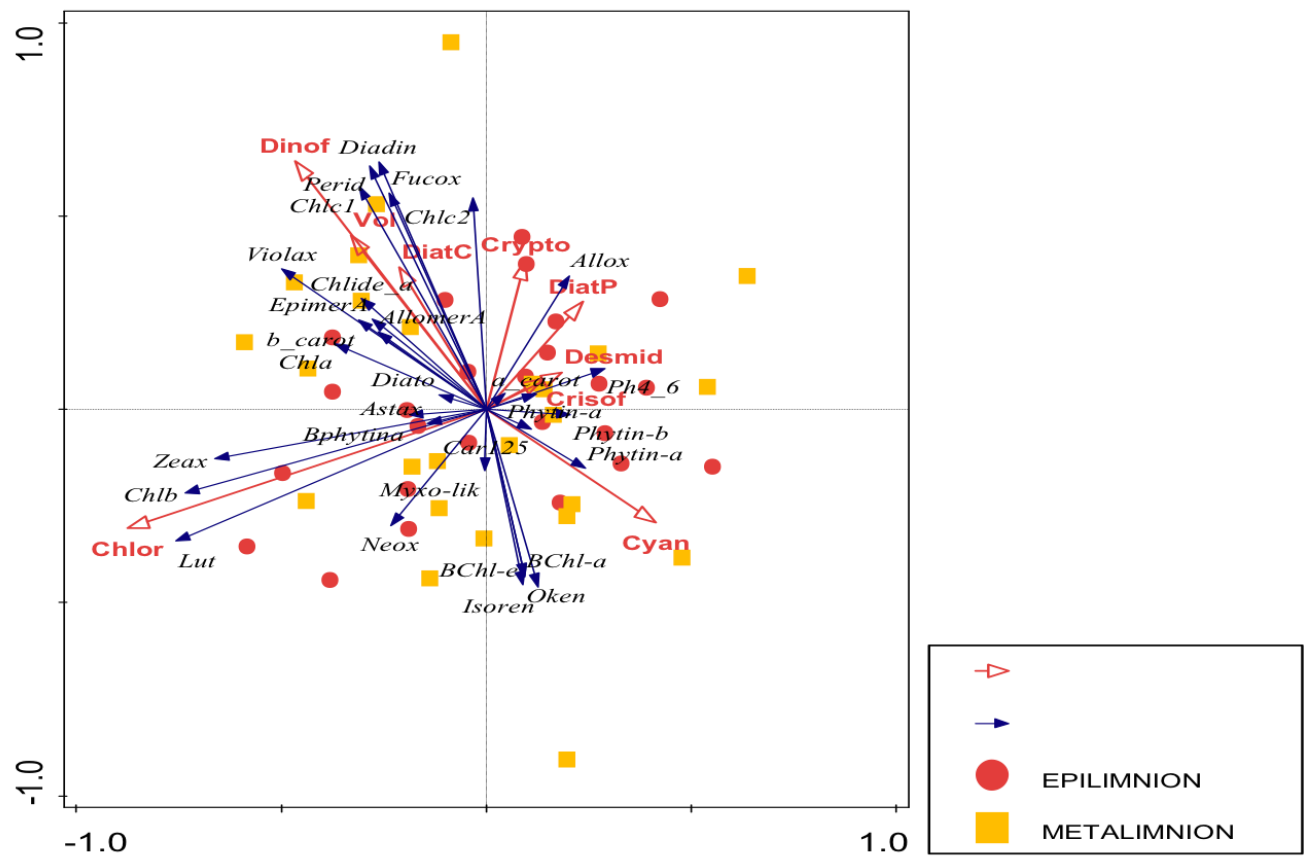


Figure 8. Redundancy analysis (RDA) between phytoplankton taxa and marker pigments. Red arrows: explanatory variables (47.5% of the variance in pigment composition was explained by phytoplankton taxa). Blue arrows: response variables.

Additionally the concentrations of the most representative marker pigments were calculated for the entire water sampling period (2013-2015), in order to compare their spatial variations between epi-, meta- and hypolimnion as well as with their deposition in the sediment trap (Figure 9). Marker pigments of Heterokontophytes (fucoxanthin) and Dinophytes (diadinoxanthin) were produced and deposited in higher amounts in spring and summer while Chlorophyta marker pigments (Chl-b, lutein and zeaxanthin) were produced mainly in fall and marker pigments of Cryptophytes (alloxanthin) were

produced throughout the year with the exception of fall. Zeaxanthin is a marker pigment also indicative of Cyanobacteria and decoupled from parent marker pigments of Chlorophyta during summer indicating a higher contribution of Cyanobacteria during summer months. Marker pigments of photosynthetic sulfur bacteria were only found in the hypolimnion during summer and fall.

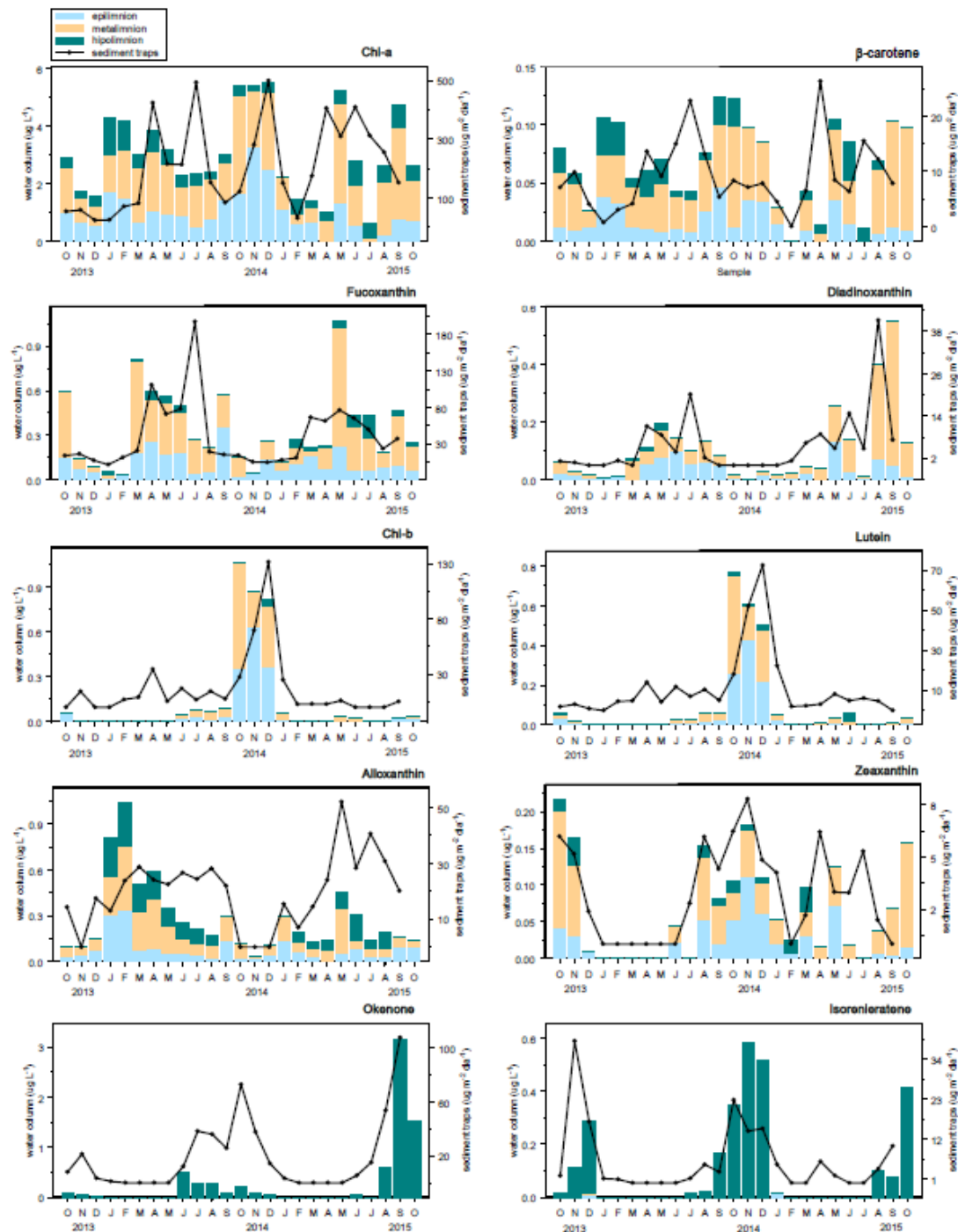


Figure 9. Comparison of most representative modern marker pigments from the epi-, meta- and hypolimnion as well as their deposition in the sediment trap, on the basis of annual values of the period 2013-2015.

The Sorensen index (S) was applied on a similarity matrix built with binary data. According to S, epi-, meta- and hypolimnion samples resembled each other $\geq 80\%$, whereas meta and hypolimnetic samples resembled each other relatively more (92%), likely because of the lower oxidation rates during anoxic conditions of the hypolimnion. Interestingly, when compared with sediment samples of the top of the core (2013-2007 CE), S diminished abruptly to 0.5, that is, the bias was 50% (Figure 10). This decrease was followed by a slower decrease of S, until values of 30% (c.a.1850) and its posterior stabilisation at values of 40- 60% that lasted c.a. two centuries. A Venn diagram highlights (Figure 9) that past communities only have a partial modern analog [5]. These results indicate that approximately 50% of the marker pigments of the water column were destroyed between deposition and permanent burying in the first centimetres of sediment, whereas the remaining 40 -60% was accurately represented over the period of study. This pattern is roughly consistent with the three phases of sediment loss proposed by [40]: 1) rapid oxidation, enzymatic metabolism and digestion by herbivores through the water column ($T_{1/2}$ = days) would account for breakdown while pigments sink; 2) slower post-depositional loss in surface sediments would result from structural rearrangements, release of labile compounds and further oxidation ($T_{1/2}$ = years) and 3) once buried pigment degradation would have continued at a very slow pace. ($T_{1/2}$ =centuries).

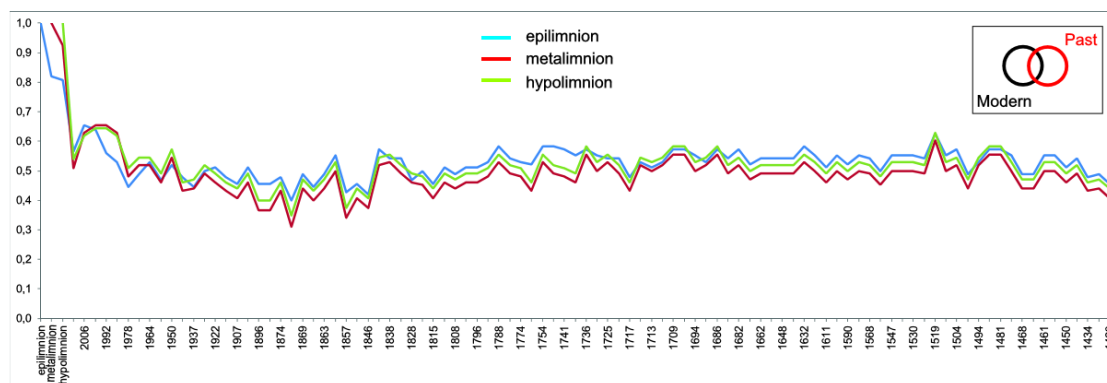


Figure 10: Comparison of Sorensen's similarity index (presence-absence) between modern and each subfossil samples. PD: present-day. Venn diagram comparing modern and past pigment assemblages.

Finally, table 1 shows which PBM were found to be exclusive from present-day samples and which PBM were found only in the subfossil samples. Some of these PBM unspecific chlorophyll derivatives that are produced by different mechanisms with time, chiefly through losses of Mg^{+2} , herbivory, viral attack and enzymatic catalysis, under distinct environmental conditions [40]. Noteworthy, however, is the presence of four cyanobacterial markers in the subfossil record that were not found in modern samples, suggesting either a detection limit for the identification of these pigments in the water column or that past lake conditions were favourable to a high diversity of cyanobacterial groups including N₂-fixing. In fact, massive blooms of filamentous *Planktothrix rubescens* De Candolle ex Gomont have been reported to have flourished in LM in the seventies [43]. However, based on microscopy counts or marker pigment detection, cyanobacteria seem to constitute only a low percentage of the phytoplankton currently thriving at LM (Figure 6), but this issue needs further confirmation.

Table 1

Marker pigments exclusively from the present-day

Peridinin	Dinophyta
Violaxanthin	Chlorophyta and
Neoxanthin	Chrysophyceae
	Chlorophyta
Chl-c1	Heterocontophyta
Allomer Chl-a	Chl-a derivative
Epimer Chl-a	Chl-a derivative

Marker pigments found only in the sediment record

Aphanizophyll-like	N ₂ -fixing Cyanobacteria
Canthaxanthin	Cyanobacteria and Cladocera tissues
Echinenone	Cyanobacteria
	Oscillatoriaceae
Oscillaxanthin	(Cyanobacteria
	Chlorobiaceae (brown
BChl-e homologues	groups)
Phaeophythin-b2	Chl-b senescence product
Phaeophorbide-a2	Chl-a grazing product
Phaeophorbide-a5	Chl-a grazing product

5. Conclusions

In this section we provide conclusions, raise some open questions and furnish guidelines for future research.

- i. There is evidence that a temperature increase enhanced the amount of calcite precipitation and flux to the lake's sediment. This relationship may be an important factor, when considering the role of calcite as a sink of atmospheric CO₂ in the context of global warming and needs further examination. On the other hand, if the size of endogenic calcite crystals varies seasonally, inferences at the subannual scale may be derived from the calcite crystals contained in the varve sublayers. These hypotheses

encourage the execution of further modern analog studies. Hence, three straightforward improvements to the present and other similar studies are suggested: 1) to increase the frequency and duration of sampling, in order to better document shifts in calcite precipitation rates as related with the current warming trend; 2) to install a meteorological station in the lake's catchment, in order to capture the local temperature and other variables that can condition water temperature; and 3) to perform a detailed survey of calcite crystal features retrieved from seasonal traps and from varve sublayers, to precisely determine their origin and diagnostic characteristics.

- ii. Two advantages of pollen studies are (i) the ability of identifying and characterizing seasonal layers even in the absence of evident sedimentary varves and (ii) the possibility of recording interannual variability and the associated meteorological drivers.
- iii. Even though our approach cannot provide estimations of past oxygen concentrations, it was a useful tool to connect and compare the dynamics of past and present oxygenation/anoxia events, at an annual resolution level. However, if past oxygen concentrations are to be inferred, a more specific modern analogue study is required. Perhaps a fruitful approach would be to monitor evolution of hypolimnetic oxygen concentrations and concurrently examine the content of Fe and Mn oxides in surface sediment samples, monthly and over several years, in order to catch mixing and meromictic years. It should then be possible to establish appropriate transfer functions to estimate dissolved oxygen from its mostly related proxies. If successful, truly long-term oxygen time series linking estimated and instrumental data could be built.

- iv. Cyanobacteria assemblages lack a modern analogue, since they are nearly absent today, whereas they were abundant, diverse and pervasive until, at least, the s1970s [43]. This observation is somewhat surprising because, to our knowledge, there have been few changes in land use or lake exploitation that could have affected LM's trophic conditions. In contrast, we expect cyanobacteria to play a prominent role during the coming warmer years, when LM becomes more intensely stratified. Cyanobacteria marker pigments (myxoxanthophyll and zeaxanthin) have been detected in the water column during monitoring, the lack of correspondence with microscopy counts may be due to the low cell size of the species involved, but this will need further study. Therefore, considering the known deleterious effects of cyanobacteria on aquatic ecosystems and human health, it would be advisable to continue present-day monitoring. The results would aid in obtaining a long-term picture of cyanobacterial succession and importance and allow deriving future scenarios, in the context of global warming.
- v. brGDGT production in the lake catchment soils show no seasonality, but in the settling particles, brGDGT flux presented a clear seasonality. However, the seasonal variations of flux confirmed the transport variation from the catchment rather than in situ production of brGDGT in the aquatic environment. The MBT/CBT temperature estimates indicate that brGDGT signatures in the settling particles are originated from surrounding soils, showing a mixed signature.
- vi. From a methodological point of view, cylindrical traps with seasonal or quarterly recovery of material yielded coherent results in all cases, i.e. total suspended solids, calcite crystals, pollen and spores, and settling particles for

GDGT extractions. However, it can be recommended that additional samples of the surface sediments at the end of the sampling years should be taken, to assess post-depositional transformations of the proxies. In our case, surface sediment samples would have provided complementary and relevant information, such as final material fluxes to the sediment and subsequent accumulation, interactions of proxies at the sediment-water interface, and early diagenesis. For example in the case of calcite precipitation as related to varve formation, initial organic matter content data may be needed to estimate the degree of sediment compaction with time [48], and for a full understanding of varve structuration and thickness of the different sublayers, at any depth. In the end, all this information allows a better connection and comparison between contemporary proxies and their modified version after years buried of being in the sediment, making the modern analogue technique more powerful.

- vii. Sometimes, modern analog studies performed at seasonal or annual frequency cannot be fully exploited to help infer sedimentary records, because of the mismatch in the resolution of present-day and past samples for a given proxy. This mismatch occurs when a relatively high weight or volume of sediment is needed to extract a sufficient amount of the targeted proxy, because contiguous varves must be joined into the same sample. In doing so, the resolution of the samples decreases. With time, this disadvantage will hopefully be overcome with the advent of new technologies. This disadvantage in our marker pigment and pollen samples. In the field of marker pigments, hyperspectral imaging spectroscopy is progressing quickly and offers a non-destructive, inexpensive approach that

permits high resolution. Hyperspectral imaging spectroscopy is being used in lake sediments, e.g. concentrations of sedimentary bacteriopheophytin “a” have been inferred in a sediment core, based upon diagnostic spectral properties and at high spatial and time resolution [49]. A good match of the resolutions of modern and past studies is indispensable to successfully connect past and present and to understand the ecosystem’s evolution with time.

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Figure legends

Figure 1: Geographic position of Lake Montcortès in the Pyrenean range (NE Iberian Peninsula), bathymetric map (Corella et al., 2019) and location of the sampling point and sediment traps within the lake.

Figure 2: Summary of monthly column water and trap monitoring. A) Water column temperature at 1m resolution measured with a multi-parametric probe. B) Total mass fluxes found in monthly sediment traps compared with phytoplankton biovolumes found in the epi and metalimnion. C) Calcite fluxes versus organic matter fluxes. C) Well-formed blocky and rhombohedral calcite crystals, calcite crystals and diatom frustules and one of the small centric diatoms found on trapped material. Modified from [10].

Figure 3: Summary of palynological results showing the relationship among the most relevant meteorological parameters (lower panel), the pollen influx of the superabundant pollen elements (middle panel) and the percentages of selected relevant pollen types (upper panel). Average temperature (Tm), relative humidity (Hm) and wind velocity (W) are represented by lines. Total precipitation (TP) is represented by bars. The predominant directions of the wind (Wd) are shown in circles. Sp – spring, Sm – summer, Fl – fall, Wt – winter. Pollen percentages were calculated excluding the

superabundant *Pinus* and *Quercus*. Taxa are ordered by their respective flowering seasons (grey bands) [32], from bottom to top and from left to right. The flowering season of all species of the different genera present in the Montcortès region [17] has been considered. Trees are in green, shrubs are in orange and herbs are in blue. Cultivated plants, such as *Cerealia* and *Cannabis*, and families including many genera (Poaceae, Cyperaceae) are located based on their pollen patterns because of the difficulty of establishing a definite flowering season.

Figure 4: CCA biplot using the scores of the first two axes accounting for 70.74% of the total variance. Samples are represented by red dots and pollen taxa are represented by blue dots.

Figure 5: Graphical summary of the modern analog study about oxygen shifts and inferred results: seasonal variations of mean values of Fe, Mn, O₂ and bacterial pigments okenone (oken) and isorenieratene (isoren) in hypolimnetic waters of LM; varved sediments and proxies obtained; graphic synthesis of the inferences derived from PCA analyses; decadal evolution of the conditions represented by the subsets A, B, C and D, since 1500CE; the bars show the percentage of years of each subset (PC II) that fall within a particular decade.

Figure 6: (a) the concentration of soil brGDGTs, (b) the brGDGT flux of settling particles in the sediment trap at 20 m depth in the lake, (c) brGDGT-based temperature estimates using the MBT/CBT proxy in soils (black line, average from three soils in the catchment around the lake) and settling particles (blue line). (d) The fractional abundance of brGDGT distribution in soils and sediment trap. Modified from [13].

Figure 7: Succession of phytoplankton taxa in the epi- (upper) and the metalimnion (lower) at LM (Oct 2013-Sept 2015).

Figure 8: Redundancy analysis (RDA) between phytoplankton taxa and marker pigment. Red arrows: explanatory variables (47.5% of the variance in pigment composition was explained by phytoplankton taxa). Blue arrows: response variables.

Figure 9: Comparison of most representative modern marker pigments from the epi- , meta- and hypolimnion as well as their deposition in the sediment trap, on the basis of annual values of the period 2013-2015.

Figure 10: Comparison of Sorensen's similarity index (presence-absence) between modern (epi-, meta-, hypolimnion) samples and each subfossil sample. PD: present-day. Venn diagram comparing modern and past pigment assemblages.